The 22nd European Carbohydrate Symposium

July 6th–10th, 2025

Gdańsk, Poland

Book of abstracts

Welcome to EUROCARB 2025

It is a pleasure to welcome you to the **22nd EUROCARB Symposium**, which will be held in **Gdańsk, Poland, from July 6th to 10th, 2025**. The event is hosted by the **Faculty of Chemistry at the University of Gdańsk**, under the auspices of the **European Carbohydrate Organization**.

The first EUROCARB symposium took place in Austria in 1981. Since then, this biennial meeting has evolved from a forum focused on carbohydrate chemistry into a comprehensive glycoscience conference, encompassing **glycochemistry, glycobiology**, **biological chemistry**, and **applied glycosciences**. Today, EUROCARB stands as one of the leading symposia in glycoscience in Europe.

The conference will cover a wide range of topics reflecting the latest developments in glycosciences. It will provide a platform for discussion and networking, and will highlight current challenges and emerging trends in carbohydrate-related research and technologies.

EUROCARB 2025 will bring together **academia and industry** to drive innovation across diverse fields, including **medicine**, **food science**, **materials science**, **and biotechnology**.

The symposium also presents a wonderful opportunity to visit **Gdańsk**, a historic city on the Baltic coast of northern Poland. Whether before or after the symposium, you'll have the chance to enjoy the city's rich architecture, fascinating museums, and beautiful beaches.

On behalf of the Organizing Committee, I warmly welcome you to the 22nd EUROCARB in Gdańsk.

Zbigniew Kaczyński

Chairman of the Organizing Committee

Timeline of EUROCARB - International Symposium in Glycoscience

Since its inception in Austria in 1981, the EUROCARB symposium has travelled across Europe, marking its presence in numerous historic and scientific centres. Originally focused on carbohydrate chemistry, the meeting has grown over the decades into a key European event in glycoscience, encompassing glycobiology, chemical biology, and biomedical applications. Held every two years, EUROCARB has reflected the evolving landscape of the field while building a legacy of collaboration and innovation. The timeline below highlights the sequence of EUROCARB host cities, culminating in the upcoming edition in Gdańsk.

Venue of the EUROCARB22 Symposium

The 22nd EUROCARB Symposium will take place at the Polish Baltic Philharmonic in Gdańsk, Poland. Situated on the historic Ołowianka Island in the very heart of the city, the venue offers a unique blend of architectural heritage and modern conference facilities.

Originally built in the late 19th century as a municipal power plant, the building was transformed into a concert hall in the 1990s and today stands as one of the region's most prestigious cultural landmarks. The main auditorium, known for its remarkable acoustics and elegant design, provides a fitting setting for both plenary lectures and cultural events.

Overlooking the Motława River and surrounded by the vibrant landscape of the Old Town, the Polish Baltic Philharmonic creates an inspiring environment for scientific exchange and networking. Its central location offers easy access to hotels, restaurants, and historic attractions, further enriching the experience of EUROCARB participants.

Fryderyk Chopin Polish Baltic Philharmonic in Gdańsk

Plenary speakers

Emil Fischer Award 2025

Sabine FLITSCH
University of Manchester
UK

Emil Fischer Award 2025

Carmen GALAN
University of Bristol
UK

Carbohydrate Research Award 2025

Martina DELBIANCO
Max Planck Institute of Colloids and Interfaces, Potsdam
Germany

Hiromune ANDO
Gifu University
Japan

Cristina De CASTRO
University of Naples Federico II
Italy

Xuefei HUANG
Michigan State University
USA

Anne IMBERTY
ACERMAV - CNRS - Université Grenoble Alpes
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Suvarn S. KULKARNI
Indian Institute of Technology Bombay
Mumbai, India

Todd L. LOWARY
Academia Sinica, Taipei
Taiwan

Hermen OVERKLEEFT
Leiden University
Netherlands

Göran WIDMALM
Stockholm University
Sweden

Plenary Speakers

APPLICATION OF ENZYMES IN CARBOHYDRATE CHEMISTRY

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Carbohydrate active enzymes are well established tools for the synthesis and analysis of carbohydrates and glycoconjugates. When used for analytical purposes, their exquisite selectivity is highly valued. When used in synthesis, however, a much broader substrate tolerance can be useful, in particular for the generation of non-natural targets. Multiple protein engineering tools now provide us with opportunities to expand the scope of enzyme catalysis.This presentation will show how such biocatalysis have been applied the synthesis of a number of non-natural targets, including imino-sugars, oligosaccharides and glycoproteins.

FROM SYNTHESIS TO APPLICATIONS: SYNTHETIC GLYCO-TOOLS FOR EXPLORING AND EXPLOITING THE GLYCOME

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The stereoselective synthesis of glycosides remains one of the biggest challenges in carbohydrate chemistry.[1] The chemical synthesis of complex carbohydrates generally involves the coupling of a fully protected glycosyl donor bearing a leaving group at its anomeric centre, with a suitably protected glycosyl acceptor (R-OH). In many instances, these reactions lead to a mixture of two stereoisomers, which makes the process inneficient. Over the last few years, our group has endeavoured to develop expedient methods to address this important synthetic challenge.[1]

In this lecture, I will discuss the use of transition metal catalysis for the stereoselective synthesis of deoxyglycosides, including the α,α-stereoselective synthesis of trehalose analogues, as well as some of our recent work in organocatalyzed glycosylations with improved reactivity.[2] Moreover, I will also disclose the development of imidazolium-based MS labels and their applications to expedite oligosaccharide synthesis and enzyme discovery in glycobiology.[3] Examples of glycan-based probes and their application in the development of a rapid bacteria screening strategy will also be discussed.[4]

Acknowledgements: Thanks to all my past and present team members galanresearch.com for all their hard work and contributions to the work presented here.

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SYNTHESIS OF GLYAN PROBES FOR UNDERSTANDING THE ASSEMBLY AND FUNCTION OF MICROBIAL GLYCANS

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Synthetic glycoconjugates are important biological probes. This seminar will describe ongoing investigations from the group focused on synthesizing different classes of complex glycan probes and their use in downstream investigations. A particular focus is those from microbial systems.

AUTONOMOUS GLYCOSYLATION IN GIANT VIRUSES

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Giant viruses (GVs) differ from regular viruses in many ways. With few exceptions, their physical size is above 200 μm and it can be beyond 1 μm. The genome size is quite variable; it ranges from 0.150 in *Phaeovirus* (*Phycodnaviridae* family) to 2.5 Mbp in *Pandora salinus* (*Pandoraviridae* family). Despite these genomes range in size, all of them encode genes with functions commonly not found in human pathogenic viruses, and a common trait is the presence of genes able to manipulate carbohydrates at a different level. For this reason, GVs are gaining interest in the field of Glycobiology. Giant dsDNA viruses are catalogued in different families, with some yet to be classified. This lecture will focus on the experimental data collected for a few members of *Phycodnaviridae* and *Mimiviridae* families.
Regarding *Phycodnaviridae*, to date information is available for *Chloroviruses.* Chloroviruses are large (190 nm in diameter) icosahedral, plaque-forming viruses with an internal lipid membrane; they have genomes of 290 to 370 kb that contain up to 400 protein-encoding genes [1]. The prototype chlorovirus is *Paramecium bursaria chlorella virus* (PBCV-1), and its major capsid protein Vp54 is N-glycosylated by a complex oligosaccharide (Figure 1a) [2,3]. The glycobiology of PBCV-1 will be presented as well as information about its antigenic variants and other related chloroviruses [4-6]. As for *Mimiviridae*, these viruses infect *Acanthamoeba* sp. and were initially identified as bacteria because of their large size along with the heavily glycosylated fibrils of the capsid. Within *Mimiviridae*, information is available for *Mimivirus* and *Megavirus* genera, and this lecture will focus on the recent structural data available on APMV, the representative virus in *Mimivirus* genus (Figure 1b) [7].

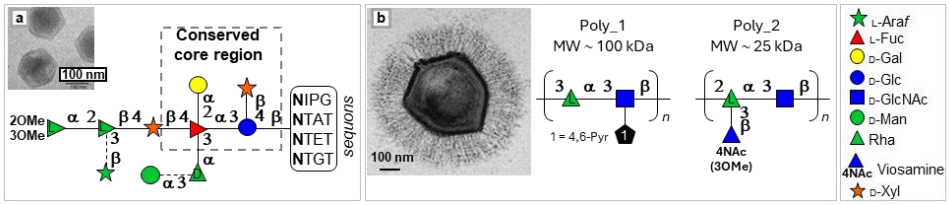

Figure 1. Electron microscopy images of: a) PBCV-1 and b) APMV along with the related glycans.

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ARE THERE SUGARS IN BACTERIA? WHAT CAN WE DO WITH THEM?

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Bacterial glycans are often comprised of rare D and L deoxy amino sugars, which are not present on the human cell surface. This peculiar structural difference allows discrimination between the pathogen and the host cell and offers avenues for target-specific drug discovery and carbohydrate-based vaccine development [1]. However, such complex glycans cannot be isolated with sufficient purity in acceptable amounts, and therefore chemical synthesis is a crucial step toward the development of these products [2,3]. We recently established short and convenient methodologies for the synthesis of orthogonally protected bacterial D and L-deoxy amino hexopyranoside and glycosamine building blocks starting from easily available D-mannose and L-rhamnose [4,5]. The one-pot protocols rely on highly regioselective nucleophilic displacements of triflates. These procedures have been applied to the synthesis of various conjugation-ready bacterial glycans [6-8] as well as zwitterionic [9] oligosaccharides. The azide containing sugars also enabled metabolic oligosaccharide engineering studies [10] that led to discovery of selective inhibitors of glycan biosynthesis [11]. In this talk I will present our recent results on the total synthesis of highly complex and densely functionalized bacterial glycans and the application of rare sugars in selective detection and disarming of pathogens. The synthetic oligosaccharides provide valuable epitopes for immunological studies aimed at vaccine development.

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GLYCAN STRUCTURE

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Characterization of the primary structure of glycans in detail is a requirement for further studies of three-dimensional aspects of carbohydrate molecules and glycoconjugates, their dynamics and the interactions with proteins in order to understand function and how to modify the molecules to obtain desired outcomes. In gram-negative bacteria the outer membrane is asymmetric with phospholipids in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet; the outer membrane is interspersed with proteins. Elucidation of the structure of these LPS is facilitated by chemical analysis, mass spectrometry and NMR spectroscopy. However, by including bioinformatics the structural elucidation process can proceed more rapidly and with higher confidence in the obtained results [1].

To expedite structural [2] as well conformational [3] analysis of carbohydrates by NMR spectroscopy the experiments and the following computer-assisted analysis [4] can be made more efficient by concatenation of NMR modules into a single 2D NMR experiment. In this way the efficiency is improved and results in time-saving due to the fact that two or more experiments share a common recovery delay prior to each subsequent scan of the 2D NMR experiment. This concept has been extended to parallel NOAH (NMR by Ordered Acquisition using ^{1}H-detection) supersequences utilizing sequential, parallel and time-shared acquisitions by which ten spectra can be acquired in a single measurement, referred to as a *p*-NOAH-10 [5]. A NOAH-5 measurement was tailored to produce NMR data for the computer program CASPER [4], which can be used to determine structure of oligo- and polysaccharides. Specifically, the supersequence (BS^{C}S^{J}T/S) consists of five NMR modules, viz., ^{1}H,^{13}C-HMBC, multiplicity-edited ^{1}H,^{13}C-HSQC-COSY, F_2-coupled ^{1}H,^{13}C-HSQC, ^{1}H,^{1}H-TOCSY, and a time-shared multiplicity-edited ^{1}H,^{13}C-HSQC module, covering most of the NMR data used as input to CASPER for a structural elucidation or NMR resonance assignments of an oligosaccharide.

Resonance overlap in NMR spectra of oligosaccharides can be greatly reduced, and resolution improved, by utilizing pure shift methods. Even though many correlations are resolved in ^{1}H,^{13}C-HSQC NMR spectra some may still remain, among other things, due to ^{1}H,^{1}H couplings, though these may be refocused and the resulting pure shift ^{1}H,^{13}C-HSQC NMR spectra are thus devoid of the homonuclear proton-proton couplings. However, peak-picking of cross-peaks in 2D NMR spectra is often time-consuming, limiting the potential of CASPER as an efficient analysis tool. Since pure shift methods aim to collapse multiplets into well-resolved singlets, pure shift data are ideal for use in conjunction with CASPER, allowing for efficient analysis by using automated peak-picking routines [6].

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CHALLENGE TO THE SYNTHESIS OF HIGHLY COMPLEX SIALO-GLYCANS

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Our research group has intensively explored robust chemistry for building a broad spectrum of sialic acid-containing molecules with a special focus on gangliosides and their functionalized probes. Gangliosides are a diverse family of sialic acid containing glycosphingolipids and serve as key players in the biological processes associated with cell membrane organization such as cell differentiation, cancer migration, and virus and toxin entries, while they are minor fractions of plasma membrane lipids. To elucidate the roles of gangliosides in the cell membrane organization, the production of the entire structure of gangliosides is of great importance. We have addressed two major issues in the chemical synthesis of gangliosides; sialylation and coupling of glycan and lipid (ceramide) moieties. We developed a promising strategy toward total synthesis of gangliosides using highly reactive synthetic units; *N*-Troc sialic acid donor, 1,5-lactamized sialic acid acceptors and glucosyl ceramide acceptors, thereby achieving the synthesis of highly complex gangliosides [1-3]. The strategy has also successfully served to create the fluorescently labeled ganglioside analogs, which allowed us to observe specific interactions with membrane molecules in live cell membrane by single molecule tracking techniques [4]. The observation revealed that gangliosides were frequently colocalized with a nano-sized cluster of GPI-anchored protein receptors as a lipid raft model with six times stronger affinity than an unsaturated phospholipid, DOPC. Moreover, we have found that gangliosides frequently underwent homo-dimerization rather than hetero-dimerization at the resting state of the cell membrane.

Meanwhile, we have attempted in parallel to develop a fully α-selective sialylation. In 2019, we reported that macrobicyclic sialyl donors, which were tethered at the anomeric carboxyl group and the C5 amino group to generate a bridgehead oxocarbenium cation, ensured the fully α-selective glycosidation by the bicyclic system without being affected by substrate structures or reaction conditions [5]. This method enabled the direct sialylation of oligosaccharides and glycolipids in high yields. Our new sialylation method has been used for the synthesis of fluorescent ganglioside analogs [6,7], highly complex ganglioside and polysialic acids. Recently, we demonstrated that macrobicyclic Kdo donors with α-configuration enabled the full stereocontrol in the α-glycosidation [8]. This method facilitated the stereoselective synthesis of the dimeric and trimeric Kdos found in lipopolysaccharide of pathogenic bacteria.

In this lecture, I will share our recent results on the α-glycosidations of sialic acid and Kdo using bicyclic donors and their application to the synthesis of highly complex glycans and functionalized probes.

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LECTINS AND LECTOMES FOR DECIPHERING THE GLYCOCODE

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A large number of pathogenic microorganisms display receptors for specific recognition and adhesion to the glycoconjugates present on human tissues. In addition to membrane-bound adhesins, soluble lectins are involved in lung infections caused by the *bacteria Pseudomonas aeruginosa* and *Burkholderia cepacia* and by the fungus *Aspergillus fumigatus* that are responsible for hospital-acquired diseases. The multivalency of lectin is proposed to play a role in their strong avidity for glycosylated cell surfaces, in their specific binding to targeted human tissues, and also in their ability to affect membrane dynamics by clustering glycosphingolipids, resulting in some cases in internalization of intracellular pathogens.
Accumulated knowledge about the structures of the lectins and the interactions with host glycoconjugates has led to the design of powerful glyco-derived inhibitors that can serve as antimicrobial therapeutic agents, as a complement to or an alternative to antibiotic therapy. Several strategies are developed with development of glycoderivatives and/or multivalent glycostructures. The structural role of calcium present in the binding site of fucose and galactose specific lectins has been investigated through x-ray and neutron crystallography [1] and novel inhibition strategy with using carbohydrate glycomimetics and non-carbohydrate glycomimetics are being developed [2,3].
Structural information on lectins is now organized in databases with possibility for datamining of lectin sequences in genomes [4]. This opens the possibility to develop tools from bacterial lectins, that can be used for purification and labelling glycoconjugates, vectorisation or as glue for creating artificial tissue. Synthetic glycobiology offers innovative methods for building super-lectins as novel architectures [5].

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ACTIVITY-BASED GLYCOSIDASE PROFILING

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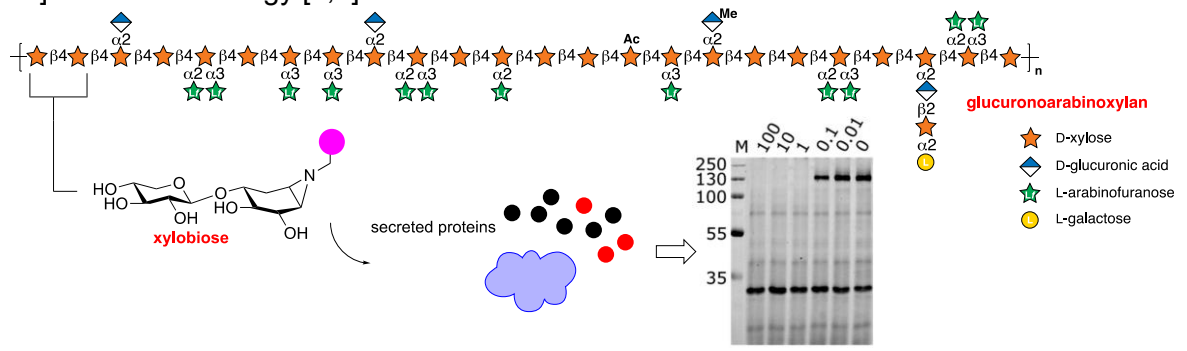
Activity-based protein profiling (ABPP) is a rapidly emerging field in chemical biology research. Enzymes that employ a mechanism in processing their substrate that involves formation of a covalent enzyme-intermediate adduct can be blocked by mechanism-based suicide inhibitors: compounds that react within the enzyme active site to form a covalent and irreversible adduct. Introduction of a reporter moiety (the purple bulb in the below picture) yields an activity-based probe (ABP) through which enzyme activities are discovered (comparative ABPP) and the efficacy of enzyme inhibitors analysed (competitive ABPP). Our work on ABPP development focuses on retaining glycosidases: hydrolytic enzymes able to cleave interglycosidic linkages and that do so through the formation of covalent enzyme-substrate intermediates [1]. Configurational and functional analogues of the natural product and mechanism-based retaining beta-glucosidase inhibitor, cyclophellitol, prove to be highly versatile tools to study retaining glycosidases of various nature and origin in relation to human health and disease [2-4] and biotechnology [5,6].



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DEVELOPMENT OF SIALYL LEWIS^A-BASED ANTI-CANCER IMMUNOTHERAPY

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Sialyl Lewis^a (sLe^a), also known as cancer antigen 19-9 (CA19-9), is a tumor associated carbohydrate antigen. The overexpression of sLe^a on the surface of a variety of cancer cells makes it an attractive target for anti-cancer immunotherapy. However, sLe^a based anti-cancer vaccines and monoclonal antibodies have been under-explored. In this presentation, the development of immunotherapy targeting sLe^a will be discussed.
To develop sLe^a based immunotherapy, sLe^a in a conjugable form is needed. Furthermore, to boost the antibody responses against sLe^a, it needs to be conjugated to an immunogenic carrier. We have developed an efficient stereoselective synthesis of sLe^a with an amine bearing linker by overcoming challenges related to low reactivities of sialic acid donor and stereochemical controls of sialylation. The synthetic sLe^a was conjugated with a powerful carrier bacteriophage Qβ. Mouse immunization with the Qβ-sLe^a conjugate generated strong and long-lasting anti-sLe^a IgG antibody responses, which were superior to those induced by the corresponding conjugate of sLe^a with the benchmark carrier keyhole limpet hemocyanin. Antibodies elicited by Qβ-sLe^a were highly selective toward sLe^a structure, could bind strongly with sLe^a expressing cancer cells and human pancreatic cancer tissues, and kill tumor cells via complement mediated cytotoxicity. Furthermore, vaccination with Qβ-sLe^a or treatment with the anti-sLe^a monoclonal antibodies significantly protected the mice from tumor development in a metastatic cancer model. This is the first time that tumor protection was observed from a sLe^a based vaccine. These results highlight the significant potential of sLe^a as a promising cancer antigen for immunotherapy development.

CARBOHYDRATES FOLDAMERS AND ASSEMBLIES

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Natural biopolymers have inspired the development of synthetic analogues – i.e. foldamers – capable of adopting defined conformations and forming programmable three-dimensional architectures. These compounds are mainly based on peptides and nucleic acids, that are well understood at the molecular level. In contrast, the complexity of carbohydrate synthesis and structural analysis have prevented access to synthetic carbohydrates capable of adopting defined geometries. In the Delbianco group, we synthesize well-defined carbohydrates to understand how the primary sequence affects conformation and aggregation [1,2].
Building on this fundamental knowledge, we present the rational design and synthesis of glycans adopting stable secondary structures, challenging the common belief that glycans are not capable of folding due to their flexibility. For example, by combining natural glycan motifs, we created a glycan hairpin, a secondary structure not present in nature [3]. Moreover, we designed glycan sequences that assemble into programmable supramolecular architectures, from fibers and particles to hydrogels [4]. Analogous to how the discovery of peptide-based foldamers launched a new field, we anticipate that carbohydrate foldamers and assemblies may find applications in areas across materials science, biology, and catalysis [5,6].

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Keynote Speakers

THE DIVERSITY AND FUNCTIONALITY OF THE CANCER GLYCOME: MOLECULAR MECHANISMS AND CLINICAL IMPLICATIONS

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Alterations of glycosylation in the tumour and its microenvironment are common molecular alterations with major biological implications for disease progression [1]. Cancer is a heterogeneous and complex disease that requires the understanding of the different components underlying the biology of the tumour. Alterations of glycosylation, such as the overexpression of sialylated glycans are common molecular features during carcinogenesis with major biological implications during cancer progression [1]. This presentation will report on the basis of alterations of glycosylation that occur in gastric cancer (GC). Recent results applying glycomic and glycoproteomic strategies have provided key information regarding the alterations of glycosylation occurring in cancer cells and their impact the activation of oncogenic receptors tyrosine kinase (RTK) in tumour samples, such as EGFR and HER2 (ErbB2) [2,3]. We demonstrate that ErbB2 is modified with both α2,6- and α2,3-sialylated glycan structures in GC. Glycomic and glycoproteomic of ErbB2's ectodomain disclosed a site-specific glycosylation profile in GC cells, in which the sialyltransferase ST6Gal1 specifically targets ErbB2 N-glycosylation sites occurring within the receptor's binding domain of the therapeutic antibody used in the clinics [2]. Abrogation of ST6Gal1 reshaped the cellular and ErbB2-specific glycosylation, expanded the cellular half-life of the ErbB2 receptor, and sensitized ErbB2-dependent GC cells to therapeutic antibody-induced cytotoxicity through the stabilization of ErbB dimers at the cell membrane, and the decreased activation of both ErbB2 and EGFR RTKs [2]. These results highlight the functional aspects of sialylated glycoforms occurring in cancer and supports their potential application of glycans as biomarkers for patient stratification [1,3,4,5]. Recent advances in cancer patient-derived organoids and their glycosylation will also be presented as models for cancer targeted therapies.

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INVESTIGATING CURTIN-HAMMETT SCENARIOS IN GLYCOSYLATION REACTIONS: THE HIGH IMPACT OF LOW-ABUNDANCE REACTION INTERMEDIATES

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The stereoselective introduction of glycosidic bonds (glycosylation) is one of the main challenges in the chemical synthesis of carbohydrates. Glycosylation reactions can be difficult to control because in many cases the exact reactive species driving product formation cannot be detected and the product outcome cannot be explained by the primary reaction intermediate observed. In these cases, reactions are expected to take place *via* other, low-abundance, reaction intermediates that are in rapid equilibrium with the primary reaction intermediate according to a Curtin-Hammett scenario. Despite this principle being well-known in organic synthesis, mechanistic studies investigating this model in glycosylation reactions are complicated by the challenge of detecting the extremely short-lived reactive species responsible for product formation. I will discuss the utilization of the chemical equilibrium between low abundance reaction intermediates and the stable, readily observed, axial-glycosyl triflate intermediate to infer the structure of the former species by employing various forms of exchange NMR. Using these techniques, we enabled the detection of elusive reaction intermediates such as equatorial-glycosyl triflates and glycosyl dioxanium ions [1-4]. This demonstrates the power of exchange NMR to unravel reaction mechanisms and to build a catalogue of kinetic parameters allowing for the understanding and the eventual prediction of glycosylation reactions that are under Curtin-Hammett control.

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DIVERSITY AND EVOLUTION OF THE VERTEBRATE SIALYLTRANSFERASE REPERTOIRE

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Sialic acids (SA) are commonly found on cell surface glycoproteins and glycolipids. Biosynthesis of sialylated molecules is an essential pathway that controls cellular functions like embryonic development or host-pathogen interactions. It involves biosynthetic enzymes known as sialyltransferases (STs), that catalyze the stereo- and regio-specific transfer of SA from activated sugar donors (CMP-SA) to a variety of acceptor substrates [1].
We used bioinformatics to identify and predict the function of animal sialyltransferase genes of the CAZy GT#29. Four ST families (ST3GAL, ST6GAL, ST6GALNAC and ST8SIA) are identified in Metazoa [2]. Phylogenomic and protein sequence analyses shed light into the origin ST and sialic acid pathway in Eukaryota and we showed that the major actors of the sialic acid pathway were already present in the Last Common Ancestor of Eukaryotes (LECA) [3].
We focused on the ST8SIA family comprised of mono-, oligo- and poly-α2,8-sialyltransferases and we showed that while the mammalian ST8SIA family is comprised of six subfamilies, nine vertebrate ST8SIA subfamilies originated from genome duplication events that occurred at the base of vertebrates. Comparative genomics analyses highlighted the diversity and heterogeneous distribution of the *st8sia* genes among teleost fish and sequence-based analyses unveiled potential changes of function after gene or genome duplications [4]. We developed a glyco-tool box with engineered recombinant enzymes and chemo-enzymatically synthetized donor substrates (both natural and functionalized) and we set cell-free enzymatic assays that enabled us to characterize novel fish ST8SIA activities [5]. Computational approaches and molecular dynamic simulations were used to investigate the structural and biochemical determinants of the donor substrate specificity in vertebrate ST8Sia IV [6]. Collectively, our data underscore molecular and functional evolution of vertebrates ST8SIA that account for the wide diversity of vertebrate sialoglycoconjugates.

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N-GLYCOSYLATION IN ARCHAEA – EXTREMELY SWEET

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Today, it is clear that N-glycosylation takes place across evolution. Still, N-glycosylation in Archaea remains far less well characterized than the parallel processes in Eukarya and Bacteria. Indeed, not only have few pathways of archaeal N-glycosylation been delineated, the reasons for the enormous diversity seen in archaeal N-linked glycan composition and architecture also remain unclear. At the same time, the importance of N-glycosylation in Archaea is only starting to be understood. As with numerous aspects of archaeal biology, roles currently assigned to N-glycosylation in Archaea may be unique to this domain of life or have yet to be reported elsewhere. Moreover, the relation between archaeal N-linked glycan composition and the impact this has on the different aspects of archaeal cell physiology affected by N-glycosylation remains poorly defined. Better understanding of the roles played by N-glycosylation in Archaea and how changing the makeup of N-linked glycans decorating archaeal glycoproteins impacts these roles requires a model species for which the pathway used for such protein processing is known and for which an appropriate molecular toolkit exists. With a sequenced genome, developed genetic and biochemical tools, simple growth conditions and a relatively well-defined N-glycosylation pathway, the halophile (“salt-loving”) *Halobacterium salinarum* is one such species. In my talk, I will consider N-glycosylation in *Hbt. salinarum* and how modifying the composition of an N-linked glycan decorating *Hbt. salinarum* glycoproteins impacts different roles served by this post-translational modification, an emerging aspect of glycobiology and cell biology research.

TAILORED IMINOSUGARS TO TACKLE COMPLEX DISEASES

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Iminosugars are a fascinating class of glycomimetics and are more and more investigated as potential new drugs, as they offer the opportunity to mimic the biological action of carbohydrates, often with a higher activity than sugar themselves, while circumventing their inherent drawbacks. Their synthesis is highly challenging due to the presence of several chirality centers, which in turn give the synthetic organic chemists the opportunity to investigate new stereoselective reactions, with the chiral pool approach from carbohydrates being certainly the most straightforward route. Precision-engineered modifications and decoration of iminosugars with task-specific moieties can not only enhance affinity and selectivity towards the biological target, but also improve their drug-like properties, which are important for their biologic applications.
Widely known as inhibitors of glycosidases and glycosyl transferases, iminosugars have recently gained attention in the treatment of complex and multisystemic diseases, such as lysosomal storage disorders (e.g. Gaucher disease) and neurodegenerative diseases (e.g. Parkinson’s disease).
In this Keynote lecture, I will illustrate our recent synthetic efforts to access new task-specific iminosugars based on a trihydroxypiperidine *core* derived from D-mannose. The decoration of the iminosugar *core* with specific moieties allowed not only to selectively bind a specific lysosomal enzyme, but also to impart additional features such as antioxidant properties, important in multisystemic neuronopathic diseases, or the capacity to bind intrinsically disordered proteins, whose aggregation is responsible for neurodegenerative diseases (i.e. α-synuclein in Parkinson’s disease) [1,2].

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GLYCOSAMINOGLYCANS: WHAT REMAINS TO BE SOLVED?

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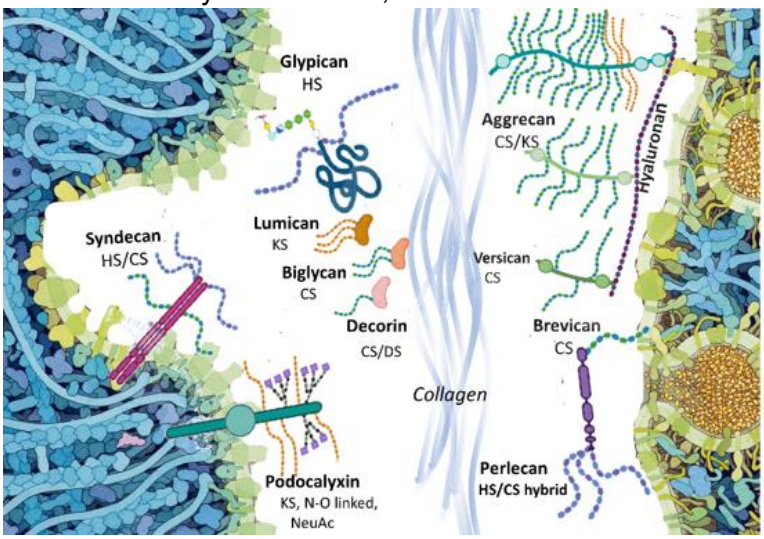
On behalf of all the authors cited in References 1 and 2.

Within the context of a European Cooperation in Science and Technology Action (INNOGLY), scientists of the Glycosaminoglycans (GAG) research community addressed the questions of what remains to be solved to understand the structure and function of GAGs fully and address their role in proteo-glycans (PG) and further in the glycocalyx and peri- and extra-cellular matrix.
They identified those pending issues that will benefit from the development of new approaches, namely in chemistry and biology, with emphasis on

(i) The synthesis of GAG oligosaccharides to build large and diverse GAG libraries,
(ii) GAG analysis and sequencing by mass spectrometry (*e.g.*, ion mobility-mass spectrometry), gas-phase infrared spectroscopy, recognition tunnelling nanopores,
(iii) Biophysical methods to investigate binding interfaces
(iv) Molecular modelling to identify bioactive GAG sequences and to expand our knowledge and understanding of glycocode governing GAG molecular recognition
(v) Artificial intelligence for in-depth investigation of GAGomic data sets and their integration with proteomics.
(vi) The functional characterisation of the new PGs recently identified by glycoproteomics,
(vii) The selectivity of interactions mediated by GAG chains,
(viii) The display of GAG chains and PGs at the cell surface and their impact on the availability and activity of soluble ligands and on their move through the glycocalyx layer to reach their receptors,
(ix) the human GAG profile in health and disease,
(x) the roles of GAGs and particular PGs involved in cancer, inflammation, and fibrosis.



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POLYSACCHARIDES VS. ALLERGIES: THE SWEETEST BATTLE YOU DIDN'T KNOW YOU NEEDED

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New research is challenging the traditional view that live bacteria are essential for health benefits. Experimental studies show that inactivated bacteria, their surface components (such as peptidoglycan, polysaccharides, lipoteichoic acid, glycolipids) and secreted outer membrane vesicles can also exert therapeutic effects. These structured bioactive compounds, now referred to as postbiotics, offer distinct advantages over live probiotics in preventive and therapeutic applications, including improved safety profiles, well-defined chemical structures and extended shelf life.
Despite these advantages, the precise mechanisms linking postbiotic composition to their biological functions remain poorly understood. I will present the current evidence on the potential of Bifidobacterium- and Lactobacillus-derived polysaccharides for the treatment of allergy, synthesising findings from recent investigations [1-6]. I will focus on their immunomodulating properties and the relationship between structure and biological function.

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DECIPHERING *IN VIVO* GLYCOBIOLOGY WITH GENETICALLY-ENCODED, MULTIVALENT LIQUID GLYCAN ARRAY (LiGA) AND LIQUID LECTIN ARRAY (LiLA)

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A major barrier to studying the role of glycans *in vivo* is the fundamental lack of one-to-one correspondence between sequence of DNA and structures of carbohydrates. Investigations of carbohydrates, thus, cannot rely on DNA sequencing directly. To solve this challenge, we introduce genetically-encoded platform technologies termed Liquid Glycan Array (LiGA) [1-4] and Liquid Lectin Array (LiLA) [5-6]. Glycan arrays, made by ligation of carbohydrates to glass or bead surfaces and complementary array of lectins on glass slides are the workhorse tools in glycobiology. These technologies cannot measure interactions of glycans and GBPs in their natural environment on the surface of cells in tissues *in vivo*. In contrast, Liquid arrays introduce one-to-one correspondence between DNA sequence and carbohydrate structure of glycan or glycan binding protein displayed on phage. They enable unsupervised profiling of interactions of glycans with receptors on the surface of cells *ex vivo* and *in vivo*.
LiGA is produced by chemical [1] and chemoenzymatic ligation [2] of carbohydrates to bacteriophage (phage) particles. Genetically encoded, monodisperse carriers based on 700 mm long M13 phage particles display 50-1000 copes of glycans on their surface. The identity and presentation (density) of glycans are encoded by the DNA barcode inside the phage genome. LiLA in turn is produced by enzymatic ligation of lectins to bacteriophage using SpyCatcher-SpyTag technology [5-6]. LiGA and LiLA uncovered an optimal structure/density combination for recognition for a wide collection of lectins and immune lectin proteins (DC-SIGN and Siglec family proteins) expressed on live cells [1,2,4,5]. LiLA can detect presence of specific glycoisoforms on live cells. Injection of the LiGA or LiLA into mice identified glycan:GBP interactions necessary for homing to specific organs. This work provides an unprecedented quantitative evaluation of the interaction of complex glycans with GBPs *in vitro* and *in vivo*.

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WEAVING NONCOVALENT INTERACTIONS INTO CARBOHYDRATE SYNTHESIS: AN EMERGING FRONTIER IN GLYCOSYLATION STEREOCONTROL

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The exploitation of unconventional noncovalent interactions (NCIs) has emerged as a powerful strategy to access difficult-to-synthesize glycosides [1,2]. In my talk, I will describe our group's efforts in developing mild and robust *σ*-hole based noncovalent catalyzed methods for selective carbohydrate synthesis – an approach our laboratory now defines as the "*σ-hole glycosylation strategy*". These methods have facilitated the access of elusive glycosidic chemical space (Figure 1), with selectivity and scope that was not possible with alternative means.

I will offer an overview of our early discovery efforts in the development of exclusively halogen bonding (XB) catalyzed strain-release glycosylation [3] and 2-deoxyglycosylation [4]. These strategies had unraveled unique advantages, such as the elevation of anomeric selectivity as well as expanded substrate tolerance compared to classical catalytic methods.

Next, I will introduce an emerging concept from my laboratory, which is the use of the highly modular phosphonochalcogenide (PCH) catalyst [5] for chalcogen bonding (ChB) catalyzed glycosylations and glycomimetic synthesis. We recently demonstrated in a series of studies that the ChB activation imparted by PCH catalyst performed exceptionally on glycosyl substrates. As a consequence, we developed versatile strategies that enabled access into biologically relevant 7-membered ring sugars known as septanosides [6,7], β-indolyl glycosides [8] as well as the underexplored iminoglycosides [9]. I will also touch on recent efforts to exploit NCIs for carbohydrate stereocontrol in the context of asymmetric transition metal catalysis [10].

Benefiting from insights gained through experiment and theory, we discovered that a blend of unconventional NCIs is synergistically dictating the stereoselectivity determining steps in many glycosylations. By bridging supramolecular chemistry, physical organic chemistry, catalytic method development and carbohydrate chemistry, we are optimistic that innovative solutions into highly desired glycosidic chemical space will be discovered at the interface of these fields.

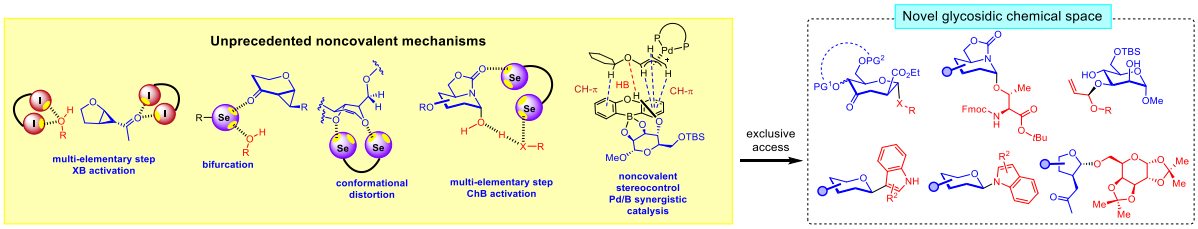

Figure 1. Emerging noncovalent strategy in stereoselective carbohydrate synthesis

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SYNTHESIS AND FUNCTIONS OF BACTERIAL LIPID A FOR SAFE VACCINE ADJUVANT DEVELOPMENT

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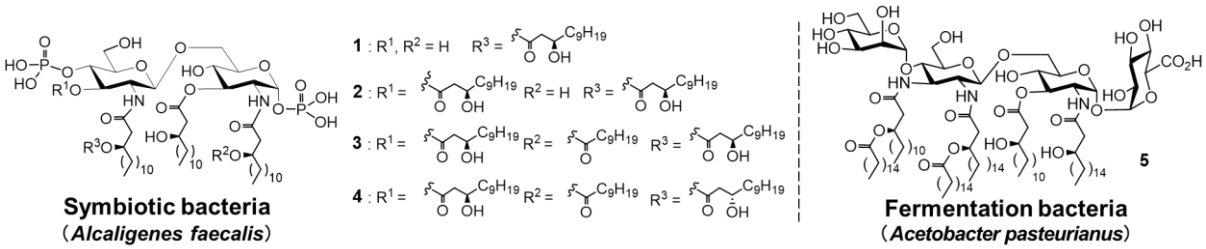
Lipopolysaccharide (LPS) is a major glycoconjugate in outer membrane of Gram-negative bacteria and canonical *Escherichia coli* LPS activate innate immunity to induce lethal strong inflammation. The terminal glycolipid lipid A is the active principle of LPS. Low inflammatory lipid A have been expected as vaccine adjuvants.

We hypothesized that co-evolved parasitic and symbiotic bacterial components should modulate host immunity moderately with low toxicity. We synthesized parasitic [1] and symbiotic [2] bacterial lipid A and elucidated the molecular basis of immunoregulation, and developed safe and useful adjuvants. In this presentation, we introduce chemical synthesis and functions of lipid A from *Alcaligenes faecalis* inhabiting gut-associated lymphoid-tissue (GALT) that is responsible for the mucosal immunity regulation.

We synthesized *A. faecalis* lipids A **1**-**3** with diverse acyl group patterns and identified the active center as hexa-acylated **3** [2]. Lipid A **3** was confirmed to exhibit non-toxic but useful adjuvant function (enhancing antigen-specific IgA and IgG production) [3-5], and the vaccine model using **3** was found to be significantly protective against bacterial infection [4]. Since IgA is responsible for mucosal immune homeostasis, we found a promising adjuvant that can safely regulate mucosal immunity by focusing on GALT symbiotic bacteria. Furthermore, lipid A **4**, which reversed the stereochemistry of the acyl side chain hydroxy group, was found to be more active than **3**, and the molecular basis of the adjuvant function is also becoming clear.

Acetobacter pasteurianus is a Gram-negative bacteria used for the fermentation process of traditional Japanese black rice vinegar (kurozu). *A. pasteurianus* LPS, which is a candidate of immunostimulatory component of kurozu, contains lipid A with a distinctive structure [6]. Here, we considered *A. pasteurianus* lipid A as a pool of acid resistant and safe immunostimulants. We achieved the systematic synthesis of three kinds of *A. pasteurianus* lipid A and identified the active center as lipid A **5** [7]. Structure-activity relationship studies revealed that the glucuronic acid residue, a characteristic structure of *A. pasteurianus* lipid A, is important for both immune function and acid resistance ability.



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THE HYDROGEN BOND DONATING CAPACITY OF INDIVIDUAL ALCOHOL GROUPS IN CARBOHYDRATES

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Alcohol groups play a crucial role in determining the chemical and physical properties of carbohydrates. The extensive hydrogen bonding with water results in their excellent aqueous solubility and low lipophilicity, and the hydrogen bonding with protein receptors is one of the main determinants for binding selectivity and affinity. Intramolecular hydrogen bonding, and – in glycans – interresidue hydrogen bonding contributes to sugar/glycan conformation. Hence, sugar hydrogen bonding and hydration has been extensively investigated.
Knowledge of innate sugar alcohol hydrogen bond donating capacities and how these are influenced by modification elsewhere will aid glycomimetic development as well as the interpretation of binding data of carbohydrate probes in chemical biology.
Here we report the first efforts to map the hydrogen bond donating capacity of individual alcohol groups in carbohydrates onto a scale relevant to medicinal chemistry (pK_{AHY} scale). For this purpose, model compounds were designed that not only allow measurement (by IR), but also exclude any cooperative effects. The results show that the hydrogen bond donating capacities of alcohol groups in carbohydrates strongly depends on the position in the ring, and on the relative stereochemistry even of remote substituents, and a rationalisation of the data is proposed. We also show the considerable effect of alcohol deoxygenation and deoxyfluorination.

EXPLORATION OF UNFREQUENTED REGIONS OF GLYCOCHEMICAL SPACE USING NOVEL SYNTHETIC METHODOLOGIES

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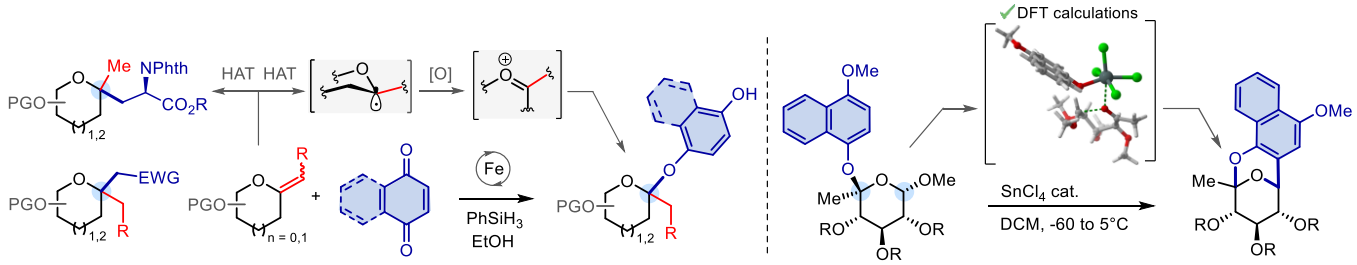
Despite recent advances, harnessing the vast therapeutic potential of carbohydrates remains a formidable challenge in glycoscience. One promising approach involves the development of glycomimetics [1] – molecules that mimic the structure and function of natural carbohydrates while circumventing their common pharmacological limitations. In addition to their potential as drug candidates, glycomimetics offer enhanced stability and serve as powerful mechanistic probes for dissecting carbohydrate-mediated biological processes. The synergy between glycomimetics and advanced synthetic methodologies holds great promise for driving major progress in glycobiology and addressing urgent medical needs. In our recent work [2], we have combined novel synthetic strategies with non-classical molecular architectures to explore uncharted areas of chemical and intellectual space. We have developed efficient methodologies for constructing quaternary (pseudo)anomeric centers through iron-hydride hydrogen atom transfer (HAT) and gold-catalyzed processes, facilitating the creation of new glycomimetic scaffolds [3]. Additionally, we are investigating the synthetic potential of bifunctional glycosides – an underexplored class of intermediates with two reactive (pseudo)anomeric centers [3a,4]. These "superglycosides" exhibit unique reactivity profiles, particularly in cascade reactions [4], and have the potential to significantly expand the glycoscience toolbox for therapeutic discovery.



The key aspects and implications of this work will be presented and discussed in more detail during the keynote lecture.

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CARBOHYDRATES AS KEYWORDS IN THE MOLECULAR DIALOGUE

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The evaluation of the structure of biomolecules built up of carbohydrates is a very challenging task due to the inherent complexity of sugar chemistry, which also impairs any computerized/automated approach. Nevertheless, this is a fundamental mission devoted to understanding interaction events at atomic level, including host-guest cross-talk. The combined use of complementary, biophysical approaches, including NMR spectroscopy, computational and biophysical techniques, native MS, together with immunological experiments is essential to unravel structure, properties, functions of glycans and understanding the mechanisms at the basis of recognition of the sugar code. In this talk, I will give a special focus to the description of bacterial glycocode, either as beneficial mediator of host homeostasis and immune system development or when harmful to the host. I will describe the chemical glyco-features located on bacterial cell surface able to tune eukaryotic immune responses.

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STEREOSELECTIVE SYNTHESIS OF α-GLYCOSYL-THIOLS AND THEIR CONJUGATES BY CRYO THIOL-ENE PHOTOCOUPLING

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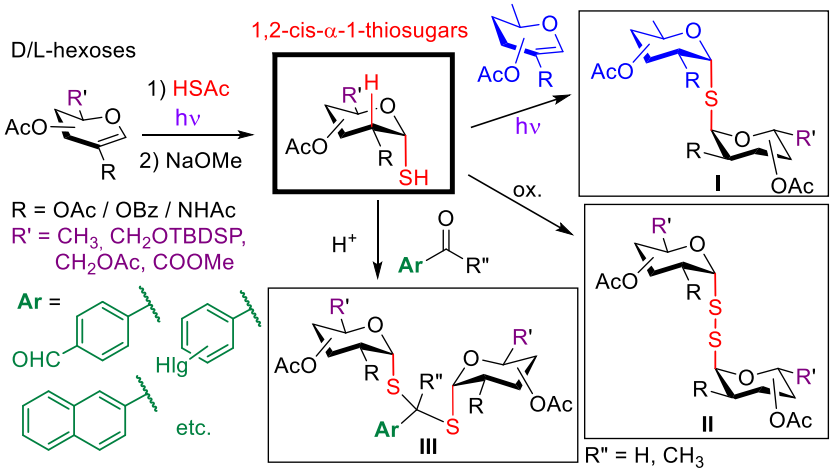
Thio-linked oligosaccharides, due to their high biological stability, are widely utilized as tools for elucidating carbohydrate function in living organisms, and several thioglycosides are currently under investigation as therapeutics. α-Glycosyl thiols can serve as key building blocks for the synthesis of stable thioglycoside mimetics of widespread and biologically relevant α-O-glycosides, however, the stereocontrolled synthesis of 1,2-cis-α-glycosyl thiols is notoriously difficult using classical synthetic methods. To address this problem, we turned to the photocatalyzed thiol-ene reaction, which has been shown to be an efficient method for the synthesis of thioglycosides containing challenging glycosidic bonds [1].
Here, it will be presented that the photoinitiated radical-mediated addition of thioacetic acid to 2-substituted glycals followed by selective S-deacetylation is a general and fully stereoselective method for the synthesis of 1,2-cis-α-glycosyl thiols [2]. The low reactivity of thioacetic acid in the radical reaction was overcome by carrying out the reaction in AcOH or in neat HSAc at -80 °C with UV irradiation, resulting in high yields regardless of the sugar configurations. A self-made spiral vessel reactor was used to achieve efficient irradiation and simultaneous cooling, which allows for large-scale synthesis. By subjecting 1,2-cis-α-1-thiosugars to a second thiol-ene coupling reaction with 2-substituted glycals, trehalose-type symmetrical and unsymmetrical α,α'-thiodi- and oligosaccharides (**I**) were obtained with complete stereoselectivity. Oxidation of α-1-thiosugars provided an easy access to α,α'-diglycosyl disulfides (**II**), while dithioacetal-linked thiodisaccharide mimetics (**III**) were formed by reaction with oxo compounds.



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CHEMOENZYMATIC SYNTHESIS AND BIOLOGICAL FUNCTIONS OF COMPLEX GLYCANS AND GLYCOCONJUGATES

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Carbohydrates are involved in a wide range of physiological and pathological processes. Due to the complexity and diversity of carbohydrates, it is difficult to isolate a large number of structurally well-defined glycans and glycoconjugates in high purity and sufficient quantities from natural sources, which results in poor understanding of their biological roles and structure-function relationships. To address the issue, we have developed an innovative diversity-oriented chemoenzymatic platform by integrating chemical synthesis and enzyme-catalyzed diversification. This platform enables the efficient synthesis of a wide variety of complex carbohydrates, including sulfated ganglioside glycans [1], glycosphingolipids [2,3], and *O*-glycopeptides [4,5]. Furthermore, we have employed high-throughput glycan microarray and surface plasmon resonance technologies to investigate the structure-binding relationships of various carbohydrates. Our research has unveiled the specific recognition modes of different glycan sequences towards disease-related proteins, thereby providing new opportunities for biomedical discovery and the development of carbohydrate-based therapeutics.

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STREAMLINE CARBOHYDRATES SYNTHESIS THROUGH NEW METHODS AND STRATEGIES

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Carbohydrates are the essential and most abundant biomolecules with pivotal roles in numerous biological processes. However, in comparison with proteins and DNA, biosynthesis of carbohydrates is not a template-driven but stepwise process, which results in heterogenous and complex carbohydrates structures. The accessibility to well-defined, pure and sufficient glycans remains a bottleneck in carbohydrates chemistry, impeding the in-depth biological and functional studies and development of carbohydrates-based therapeutics. To address this issue, we have developed new glycosylation reactions with glycosyl *ortho*-(1-phenylvinyl)benzoates (PVB) [1] as versatile donors and new one-pot glycans assembly strategies on the basis of PVB glycosylation [2] for streamlined synthesis of carbohydrates from oligosaccharides to polysaccharides (Figure 1), including plants glycans such as the undecasaccharide from *Dendrobium Huoshanense* [3] and tridecasaccharide from *Angelica Sinensis* [4], fungi glycans such as nona-decasaccharide from *Ganoderma sinense* [5] and tetradecasaccharide from *Lentinus giganteus* [6], nucleosides such as capuramycin [2], mucin-related tumor associated carbohydrate antigens [7], bacterial glycans such as lipopolysaccharide from *Bacteroides vulgatus* [8] and mannose capped lipoarabinomannan up to a 101-mer [9] from *Mycobacterium tuberculosis* [10].

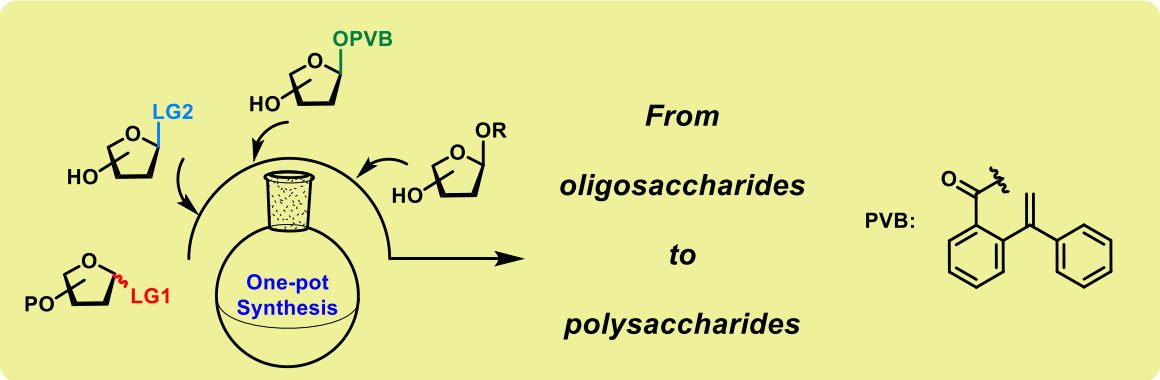

Figure 1. One-pot glycans assembly strategies on the basis of PVB glycosylation

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SIGLEC-GLYCAN INTERACTIONS IN IMMUNE REGULATION

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Hypersialylation, an aberrant increase in the expression of sialic acid, profoundly impacts tumor cell interactions with their microenvironment. Siglecs, immune receptors that recognize cell surface sialic acids, play a pivotal role in immune surveillance within the tumor microenvironment. Our group has studied the interaction between glycans containing sialic acids (sialoglycans) and Siglec receptors on immune cells, demonstrating that this pathway can be targeted to regulate immune responses and control tumor growth. Leveraging structural biology techniques, including X-ray crystallography, NMR, and molecular dynamics, we have scrutinized the molecular details governing their specificities for sialoglycans [1-5]. This information offers opportunities for developing novel molecules targeting Siglecs through modified sialic acids. Our research also focuses on uncovering structural insights on anti-Siglec antibodies to refine antibody-based therapies.

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WHAT'S THE FUZZ ABOUT MICROBIAL SLIME?

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Microbes secrete polysaccharides that play multiple roles in biology, such as increasing viscosity, enhancing water-holding capacity, masking identity, forming biofilms, and potentially many more. These polysaccharides, which exhibit tremendous structural variation, are associated with microbial cell walls. Although many microbial exopolysaccharides and cell wall polysaccharides have been documented, their immense structural variation remains largely undiscovered.

Notably, the production of these carbohydrates is costly for microbes, requiring 10+ proteins to synthesize. Since most microbes live in complex communities, it is likely that some form of recycling occurs, where various community members utilize these polysaccharides as carbon sources. However, very little is known about how microbes degrade and catabolize exo- and cell wall- polysaccharides.

I will present examples of novel, unpublished, bacterial exopolysaccharides and cell wall polysaccharides, discuss strategies related to their production, purification, and analysis, and identify key challenges in understanding the polysaccharide utilization systems that bacteria use for specific degradation.

Some microbial polysaccharides are already used in a number of applications, but with the structural variation present, microbial polysaccharides have a large untapped potential. Relevant applications include viscosity modifications, prebiotics, and their use as elicitors in biological systems and immune stimulants.

MULTIVALENT AND SUPERSELECTIVE BINDING BY BACTERIAL TOXINS

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AB_5 toxins, such as cholera toxin (CTx) and shiga toxin (STx), exploit multivalent interactions with cell surface glycans to gain entry into host cells. While glycolipid receptors like GM1 and Gb_3 have long been identified as key ligands for CTx and STx respectively [1], recent evidence implicates fucosylated glycans, such as Lewis^x, in CTx binding [2,3]. This suggests a broader role for non-glycolipid components of the glycocalyx in toxin recognition, but these interactions remain poorly characterized. To investigate these complex interactions, we have developed defined valency neoglycoproteins [4], and biomimetic glycocalyx models with mucin-like glycopolymers displayed on supported lipid bilayers (Figure) [5]. These glycoconjugates present defined densities of glycans, including GM1, Gb_3, and Lewis^x, on protein and polysaccharide backbones, with high control over glycan presentation. Using quartz crystal microbalance with dissipation monitoring and spectroscopic ellipsometry, we have quantified toxin binding processes in a tunable artificial glycocalyx environment. The non-toxic B-subunits of CTx andB and STx showed significantly enhanced binding to their respective ligands in the polymer-grafted glycocalyx, with super-linear ("superselective") responses to increasing glycan density, highlighting how the physical and chemical architecture of the glycocalyx modulates multivalent interactions. Together, these approaches allow us to dissect the rules governing multivalent toxin-glycan recognition and provide a modular platform for the design of glycan-based inhibitors and probes. Our results offer new insights into host-pathogen interactions and tools for glycocalyx engineering.

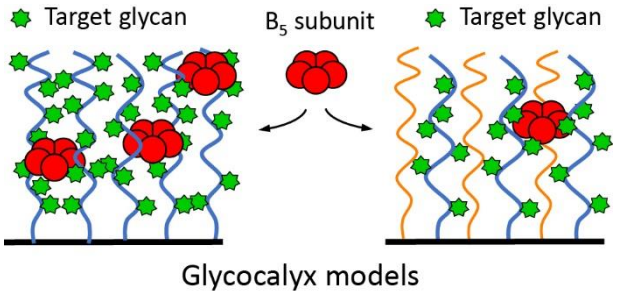

Figure 1. Biomimetic glycocalyx models binding to bacterial toxins [5].

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TAILOR-MADE GLYCOPOLYMERS AS PROSPECTIVE THERAPEUTIC TOOLS

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Galectins are a family of soluble human β-galactoside-binding lectins. Their overexpression accompanies a range of pathologies. In cancerogenesis, they promote tumour growth, angiogenesis, tumour cell migration, and evasion from the host immune system [1]. They are also associated with pathological fibrotisation related to the understudied disease of pulmonary hypertension [2]. Synthetic glycopolymers are especially suitable for interacting with biomedical targets like galectins *in vivo* due to their biocompatibility, bioavailability, prolonged half-life and ability of intracellular penetration. Simultaneous presentation of multiple carbohydrates in various architectures [3] on a polymer carrier enhances the biological potency of glycopolymers by many orders of magnitude, reaching outstanding affinity and selectivity in galectin binding and inhibition, which makes these glycopolymers prospective for biomedical research and clinical application.
Here we will present our new results in the synthesis of tailor-made glycopolymers based on *N*-(2-hydroxypropyl)methacrylamide and polyoxazoline carrying various glycomimetic ligands. The glycopolymers efficiently protected immune cells and immune effectors like IFNγ against the detrimental effects of galectin-3. They also decreased the expression of markers of pulmonary hypertension. In a biodistribution and pharmacokinetics study in rat and mouse models, we identified crucial parameters influencing *in vivo* half-life, which further enhances the potential of carbohydrate-loaded glycopolymers as therapeutics of galectin-associated pathologies.

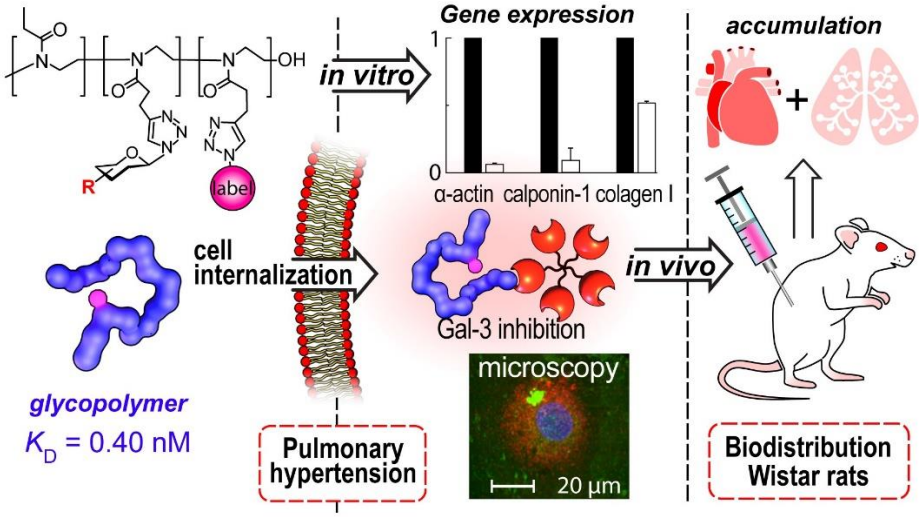

Figure 1. Synthetic glycopolymers as agents prospective for treating pulmonary hypertension.

Acknowledgements: We acknowledge support from the Ministry of Health of the Czech Republic (project NU23-08-00307), and the Ministry of Education, Youth and Sports of the Czech Republic (mobility project LUC23148).

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THE ENZYMATIC ORIGIN OF PENTAFURANOSES IN BACTERIAL POLYSACCHARIDES

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The remarkable diversity of carbohydrate backbone structures in bacterial polysaccharides results from the activities of glycosyltransferase enzymes with precise donor and linkage specificities. The most prevalent glycosyltransferases are classical Leloir enzymes that use sugar nucleotide donors to assemble lipid-linked oligo- and polysaccharides. These enzymes can often be identified (and activities sometimes assigned) by sequence and predicted protein structure data. However, examination of genetic loci encoding enzymes required for the biosynthesis of ribofuranose (Rib*f*)-containing glycans identified no obvious candidate glycosyltransferase for incorporating this sugar, suggesting it may require an enzyme with different type of donor or mechanism. Using two lipopolysaccharide (LPS) O antigen polysaccharides from *Klebsiella pneumoniae* as prototypes, we discovered cytoplasmic dual-catalytic domain enzymes that incorporate β-linked Rib*f* into glycan backbones [1]. These enzymes use phosphoribosyl-5′-phospho-D ribosyl-α-1-phosphate (PRPP) as the donor substrate in a 2-step Rib*f*-transferase reaction. In the first step, a *glycosyl*-phosphoribosyltransferase (*g*PRT) module transfers Rib*f*-5-phosphate to a specific acceptor. The reaction sequence is completed by removal of the 5′-phosphate by a promiscuous phosphoribosylphosphate (PRP) module. We solved the crystal structure of a homolog from a thermophilic bacterium and found the *g*PRT domain represents a new glycosyltransferase fold that diverges substantially from previously known PRTs, while the PRP module is structurally related to phosphatases belonging the haloacid dehalogenase family. This characterization allowed assignment of *g*PRT-PRP pairs involved in biosynthesis of diverse β-Rib*f*-containing polysaccharides, from a wide range of bacteria with different lifestyles. However, this mechanism did not explain the presence of α-linked Rib*f* in some glycans. Investigation of prototypes from *Citrobacter youngae* revealed a more complex extracytoplasmic mechanism for incorporating α-Rib*f* or α-Xyl*f* [2]. In these systems, PRPP is used by a polyprenylphosphoribose synthase enzyme to generate polyprenol phosphate-linked Rib*f*, which can be further epimerized to produce the Xyl*f* derivative at the cytosol-membrane interface. These lipid-linked sugars are then flipped across the cytoplasmic membrane by a dedicated exporter, where they provide the direct glycosylation donor substrate for an integral membrane GT-C-fold glycosyltransferase. The overall process resembles glycosylation pathways that incorporate arabinofuranose and hexose residues into glycostructures from mycobacteria and some Gram-negative bacteria, respectively. In summary, investigation of Rib*f* incorporation has provided fundamental new insight into the biochemical foundation of microbial glycobiology and antigenic diversification, and provides a structural and bioinformatic framework to discover new enzyme representatives from diverse bacterial species.

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THEORETICAL INSIGHTS INTO THE STRUCTURE AND NMR SPIN-SPIN COUPLING CONSTANTS IN BIOLOGICALLY ACTIVE SACCHARIDES

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High-resolution NMR spectroscopy, combined with theoretical analysis is one of the most important tools for the determination of the 3D structure and dynamics of carbohydrate molecules in solution. The 3D structural NMR analysis relies heavily on indirect spin-spin coupling constants and NOEs. However, the correct interpretation of these spectroscopic parameters also depends also on the theoretical analysis of the carbohydrate molecular structures. Density Functional Theory (DFT) method is currently the method of choice for the determination of molecular structures and the calculation of NMR parameters.
This paper presents several examples of theoretical analyses and calculations of spin-spin coupling constants on important biological carbohydrates – including dermatan sulphate, chondroitin sulphate, keratan sulphate and heparin. These saccharides are highly charged molecules and their structures are strongly influenced by complex electronic properties. Determining the correct molecular structures in aqueous solutions is therefore a challenge for computational methods. The same is true for the spin-spin coupling constants – the data presented show that direct calculations of the three-bond coupling constants ($^3J_{H-H}$ or $^3J_{C-H}$) by DFT often give better data than the application of the parametrized relationships between the $^3J_{X-H}$ magnitudes and the torsion angles. It is shown that geometry optimization using DFT with hybrid functionals and the 6–311++G(2d,2p) basis set, together with proper evaluation of the solvent effect, can yield sufficiently accurate structural and NMR data for biologically active saccharides.

Acknowledgements: This work was financially supported by Slovak grant agency VEGA 2/0071/22.

DUO Keynote Speakers

CHEMICAL SYNTHESIS OF RHAMNOGALACTURONAN-I OLIGOSACCHARIDES AND THEIR ROLE IN PLANT IMMUNITY

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The plant cell wall not only serves as a physical barrier against pathogens but, when damaged, also functions as a source of cell wall-derived molecules that play crucial roles in plant immunity. These elicitors of pattern-triggered immunity in plants may be derived from cell wall polysaccharides such as cellulose, hemicellulose and pectin. Rhamnogalacturonan-I (RG-I) is a structurally diverse and functionally significant domain of pectin, predominantly found in the primary cell walls of terrestrial plants. Its backbone consists of alternating α-1,2-linked rhamnose and α-1,4-linked galacturonic acid residues, frequently substituted at the rhamnose positions with side chains rich in arabinose and galactose. Given the structural complexity of this polysaccharide, well-defined synthetic RG-I related oligosaccharides are necessary for investigating enzymes involved in pectin biosynthesis and degradation, as well as its role in plant immunity.

We present here chemical syntheses of oligosaccharides related to RG-I and their role in activating immune responses in plants. The synthetic strategy relies on assembling the RG-I backbone using galactose building blocks, which are oxidized to the corresponding galacturonic acids before side chain installation. A [3+4] glycosylation procures the protected backbone, which after oxidation can be deprotected or is functionalized with mono-, di-, and trigalactan side chains [1].

Using a glycan array equipped with synthetic and natural glycans, we discovered a group of plant receptor kinases named ARMs (AWARENESS of RG-I MAINTENANCES) that interact with RG-I. When plants were treated with RG-I, pattern-triggered immunity responses were induced. We identified RG-I oligosaccharide structures required for interaction with ARM receptors and immune activation and found that ARM receptors are redundantly involved in plant immunity. The application of synthetic carbohydrate chemistry for discovering glycan-receptor pairs in plants thus provides new opportunities in plant immunity research [2].

Acknowledgements: This work was funded by the Novo Nordisk Foundation (NNF18OC0053048 and NNF20OC0065094), the Danish Council for Independent Research (grant no. 9041-00080B), the Austrian Science Fund (FWF) (P 35404), the Austrian Academy of Science through the Gregor Mendel Institute, the Vienna Science and Technology Fund Project (LS17-047), and by a grant from the National Research Foundation of Korea (RS-2024–00338015).

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DOCKING LONG GLYCOSAMIGLYCANS WITH ADVANCED SAMPLING MOLECULAR DYNAMICS-BASED APPROACHES

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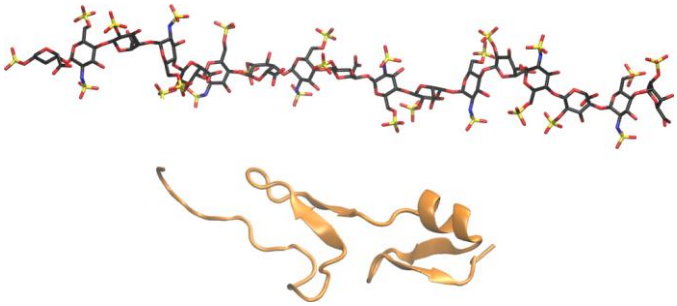
Due to their unique intrinsic properties, glycosaminoglycans (GAGs), long anionic periodic polysaccharides, are challenging molecules to analyze experimentally. Consequently, computational methods are particularly promising for uncovering the structural and dynamic principles underlying the biologically relevant interactions of these molecules with their protein targets. Conventional molecular docking approaches, which predict the structures of protein-ligand complexes, encounter substantial difficulties when applied to long GAG ligands because of their flexibility and high number of degrees of freedom. To address these challenges, we have developed and successfully applied the Repulsive Scaling - Replica Exchange Molecular Dynamics (RS-REMD) approach to dock GAGs up to 50-mers in length. Currently, RS-REMD is the only efficient method for docking long GAGs that does not require prior knowledge of the GAG binding region, incorporates explicit solvent, which is a key for modeling protein-GAG interactions, and its performance is independent of GAG length within a protein-GAG complex. The application of this method enables the modeling of large tertiary complexes involving multiple proteins and a long GAG chains, thereby advancing the fundamental understanding of the biochemical processes mediated by GAGs.



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Oral Lectures

ENHANCING GLYCOPROTEOME PROFILING: EVALUATING AND COMBINING ENRICHMENT TECHNIQUES FOR COMPLEX BIOLOGICAL SAMPLES

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Glycosylation is a fundamental and widespread post-translational modification where sugars are covalently attached to proteins, typically through asparagine (N-glycan) or serine/threonine (O-glycan) side chains. Unlike proteins, glycans are not directly encoded by the genome but are dynamically regulated by glycosyltransferases and glycosidases. This complexity makes glycosylation analysis particularly challenging using mass spectrometry, as glycosylated peptides exhibit structural and occupancy heterogeneity and often suffer from reduced ionization efficiency. Consequently, glycopeptide enrichment has become an indispensable strategy to improve the depth and accuracy of glycosylation characterization in biological research.

This study systematically compares glycopeptide enrichment techniques for complex biological samples, including cells and biofluids, addressing practical considerations like sample volume and time. We evaluate iSPE®-HILIC, a widely used ZIC-HILIC resin, and a phosphorylcholine enrichment (PCE) strategy, which utilizes a related resin. While iSPE®-HILIC has shown promise in serum glycoproteomics, its application to cell samples requires further investigation. Similarly, the glycoproteomic potential of PCE, primarily used for CRP enrichment, remains unexplored. By combining iSPE®-HILIC and PCE in a sequential enrichment workflow, we demonstrate a significant improvement in glycoproteome coverage in both HEK293 cells and plasma, using high-performance Orbitrap Eclipse mass spectrometer. Our results show that the iSPE-first followed by PCE strategy increased the detection of unique N-glycopeptides by more than fourfold compared to unenriched samples, even in unfractionated digests. This study underscores the importance of optimizing glycopeptide enrichment strategies to achieve comprehensive, unbiased profiling of the glycoproteome, thereby enabling more precise and detailed insights into the roles of glycosylation in biological systems. Furthermore, we explore a novel HILIC-based material, BioSPE™ GlycaClean, showing a ~5-fold increase in glycopeptide identification in single analyses, highlighting its potential for targeted glycan enrichment.

This work not only expands the toolkit for glycoproteomic analysis but also provides substantial improvements in the sensitivity, breadth, and accuracy of glycopeptide identification. It sets the stage for more robust and expansive glycoproteomic studies, with potential applications in biomarker discovery, disease mechanism exploration, and therapeutic development.

Acknowledgements: The work was supported in part by the National Institutes of Health (NIH)-funded R24GM137782 and by GlycoMIP, a National Science Foundation Materials Innovation Platform DMR-1933525 and BioF:GREAT, a National Science Foundation BioFoundry funded through Cooperative Agreement DBI-2400220.

STRUCTURAL IDENTIFICATION OF N-GLYCANS AND N-GLYCOPEPTIDES USING LOGICALLY DERIVED SEQUENCE TANDEM MASS SPECTROMETRY

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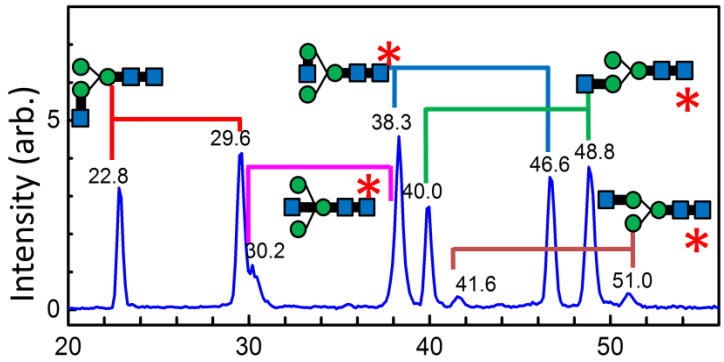
The *N*-linked glycosylation is an important post-translational modification of proteins. Current knowledge of multicellular eukaryote *N*-glycan biosynthetic pathways suggests N-glycans are produced in endoplasmic reticulum and Golgi apparatus through conserved biosynthesis. According to these biosynthetic pathways, there is only isomer of *N*-glycan with the composition of GlcNAc($Man_3GlcNAc_2$) and two isomers of *N*-glycan with composition of $GlcNAc_2(Man_3GlcNAc_2)$. In this study, we applied our newly developed mass spectrometry method, i.e., logically derived sequence tandem mass spectrometry (LODES/MS^n), and enzyme digestion to re-examine the structures of *N*-glycan extracted from various samples, including human milk, human saliva, bovine milk, HEK 293, HeLa cell, hen egg, duck egg, squid, and *Drosophila melanogaster*. Many isomers not predicted by the biosynthesis were identified, and many samples show these unusual isomers are the dominant isomers, indicating additional biosynthetic pathways are involved in these *N*-glycan generation. The complex *N*-glycans extracted from fused lobes, MGATII, MGATIVa, or MGATIVb knock out of *Drosophila melanogaster* confirm some additional biosynthetic pathways. We also applied LODES/MS^n to *N*-glycopeptide analysis. We show that LODES/MS^n provides detailed information on glycosylation sites as well as resolving isomeric N-glycan structures. Using this approach, we have successfully characterized N-glycopeptides from a variety of samples.



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SHEDDING LIGHT ON *O*-GLYCANS: DIFFERENTIATING GLYCAN ISOMERS BY THEIR GAS-PHASE STRUCTURE

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O-glycosylation is a common post-translational modification that is essential for the defensive properties of mucus barriers. Incomplete and altered *O*-glycosylation is often linked to severe diseases, such as cancer, cystic fibrosis, and chronic obstructive pulmonary disease. However, *O*-glycans are often present as complex mixtures containing multiple isomers that can be difficult to distinguish. Chromatographic separation of isomers is time-consuming and assignment by tandem-mass spectrometry remains challenging.
Here, we develop a glycomics workflow for separating and identifying mucin-type *O*-glycans based on trapped ion mobility mass spectrometry. Compared to LC-MS, the acquisition time is reduced from an hour to two minutes. To test the validity, the workflow was applied on sputum samples from cystic fibrosis patients to map *O*-glycosylation features associated with disease. However, separation because more challenging with increasing glycan complexity. To address this problem, we investigate the use of cryogenic infrared spectroscopy and provide the first data on *O*-glycans. Here, we uncover highly diagnostic features of negative ion mode spectra of glycans that help elucidate the structural motifs. A future perspective for the integration of cryogenic infrared spectroscopy in *O*-glycomics is provided.

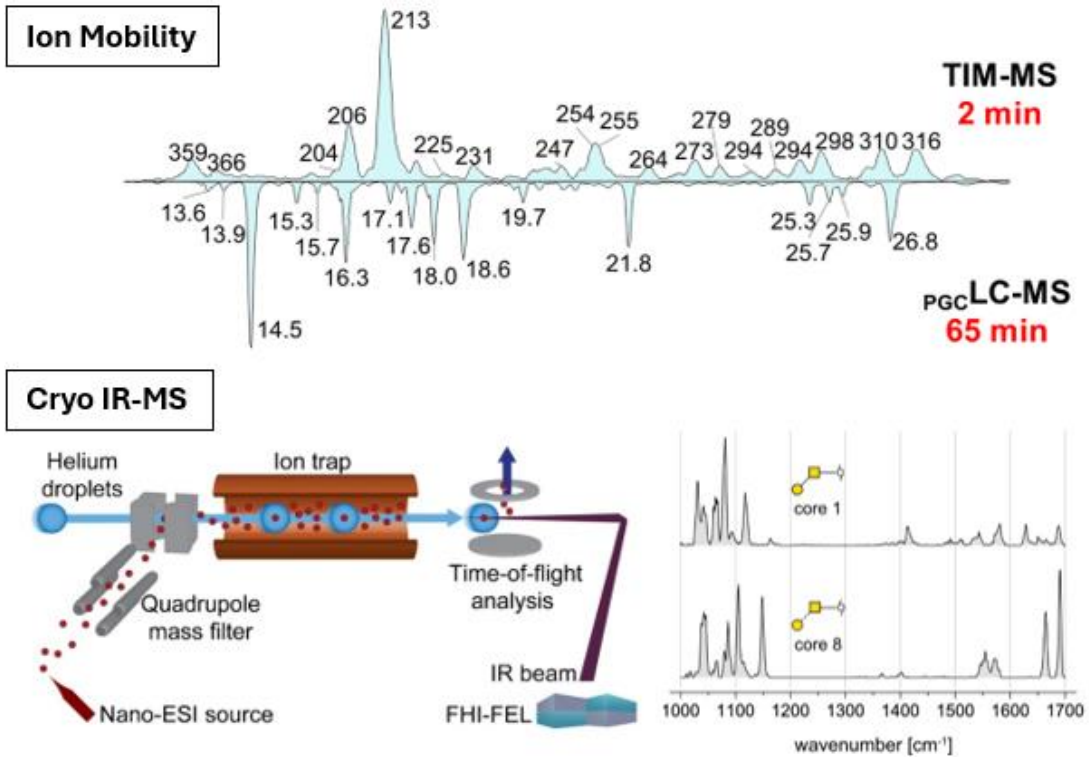

Figure 1. Top) Spectra of Trapped Ion Mobility Spectroscopy compared with traditional PGCLC. Bottom) Set-up for Cryogenic Infrared Spectroscopy and spectra for O-glycan core structures.

QUANTITATIVE ANALYSIS OF SITE-SPECIFIC N-GLYCAN STRUCTURES IN HUMAN IMMUNOGLOBULINS

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Immunoglobulins (Igs), are crucial to the immune system and are classified into five classes: IgG, IgM, IgA, IgE, and IgD, each with distinct functions and locations in the body. All Igs share a characteristic Y-shaped structure, with Fc domains containing one or more N-glycosylation sites. These sites, harboring diverse glycan structures, play key roles in Igs' functionalities, such as interacting with Fc receptors or maintaining stability. The functionality of glycans is determined by their precise structures, including branches and linkages. Although site-specific glycan compositions have been studied by mass spectrometry, the structural details remain largely unexplored. Our work offers a comprehensive analysis of glycan structures at specific sites across Igs, providing new insights into their role in immune modulation.
Briefly, We developed an advanced HILIC-LC-MS/MS method [1] that enables site-specific quantitative structural analysis of glycans on immunoglobulin-specific glycopeptides, allowing for comprehensive analysis across all immunoglobulins.
Key findings from our study include:
1. Recombinant Igs display a wider variety of glycan structures compared to those from human biofluids, with distinct quantitative glycan signatures characteristic of particular Ig classes and subclasses, irrespective of source.
2. Igs from the same donor show unique glycan structural signatures differing across various biofluids.
3. Within a single serum sample, different molecular forms of Igs present distinctive glycan structural signatures.
4. The glycan structures of Igs exhibit several conserved features: most remain stable over extended periods, far exceeding the half-lives of the Igs themselves. Unlike other glycoproteins in human serum, which are predominantly produced by liver cells and show monosialylated glycans on both 6-branch and 3-branch structures, Igs consistently display a primarily 3-branch monosialylated glycan structure.
Our research offers pioneering insights into the quantitative glycan structural patterns at specific sites of endogenous immunoglobulins, enhancing our understanding of their function in the human body and offering a glycosylation blueprint for the production of therapeutic antibodies.

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MECHANISTIC AND SYNTHETIC STUDIES IN DEOXY SUGAR SYNTHESIS

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The oligosaccharides found on many bioactive natural products and the O-antigens of Gram-negative bacteria are often composed of so-called rare sugars. Assembling these rare sugars into oligosaccharides presents a challenge as they often do not contain functionality at the C2 and C6 positions classically used to control the stereochemical outcome of glycosylation reactions. As a consequence, chemistries developed for the synthesis of C2 substituted sugars cannot be used for the construction of deoxy sugars. Our lab has demonstrated that activating hemiacetals with sulfonyl chlorides leads to the in situ formation of α-linked glycosyl sulfonates. When treated with a nucleophile, these latter species undergo highly selective glycosylation reactions to afford β-linked products, provided that the reactivity of the sulfonate is matched to the reactivity of the sugar donor. Primary kinetic isotope effect studies demonstrate that the selective reactions proceed through an S_N2-like manifold. Importantly, while different sugars require different sulfonate promoters, preliminary data form our lab is beginning to demonstrate that it should be possible to predict which sulfonate use for a particular reaction based off of the sugar donor's relative reactivity value (RRV). The scope of this reaction, mechanistic studies, and its application to synthesis will be discussed.

INSIGHT INTO THE COURSE OF THE FERRIER REARRANGEMENT

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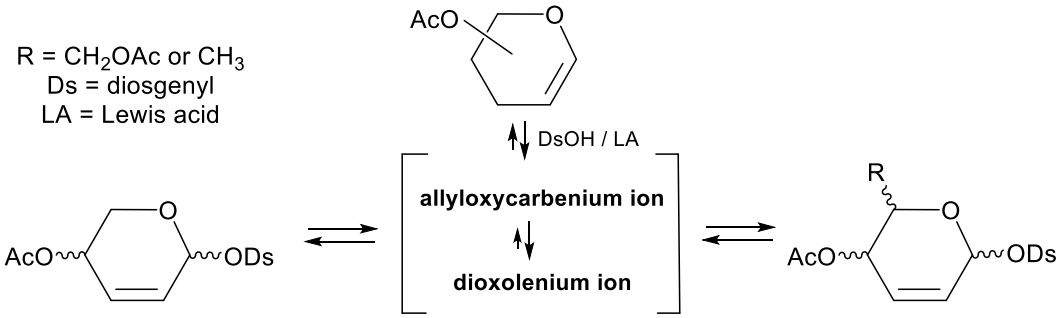
The Ferrier rearrangement is a widely explored reaction in which 2,3-unsaturated glycosides are obtained from per-*O*-acetylglycals (Figure) [1-4]. It is known for its efficiency and high α stereoselectivity characteristic of hexoses.
We applied the Ferrier rearrangement to get 2,3-unsaturated diosgenyl glycosides [5]. The diversity of the glycals used in these syntheses allowed us to discuss thoroughly the Ferrier rearrangement mechanism and its stereoselectivity. The DFT calculations were performed to compare stability of the dioxolenium and allyloxycarbenium ions, considered intermediates of the reaction. Presented results indicate that the thermodynamic equilibrium between them is shifted toward the former ion. However, it is the allyloxycarbenium ion which determines the reaction regioselectivity. In turn, stereoselectivity of the Ferrier rearrangement is closely related to the stability of the formed 2,3-unsaturated glycosides associated with the adopted conformation. Factors influencing this stability in the case of hexoses are ranked as the equatorial orientation of the terminal R group > anomeric effect > allylic effect. In the case of pentoses only the last two factors influence the stability of the Ferrier rearrangement products. This causes 2,3-unsaturated hexopyranosides and 2,3-unsaturated pentopyranosides to have different preferences for the anomeric configuration. Presented findings may contribute to the rational design of glycosylation strategies for bioactive glycosides.



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EXPLOITING CHALCOGENONIUM CATALYSIS TO ACHIEVE STEREOSELECTIVE SYNTHESIS OF DEOXYGLYCOSIDES FROM GLYCALS

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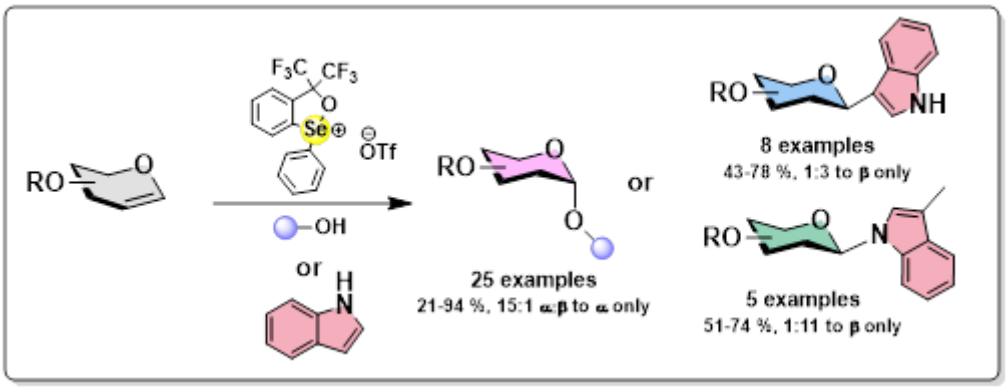
The development of stereoselective glycosylation methods is vital for enabling the synthesis of biologically relevant carbohydrate-based molecules [1]. Often, glycosylation reactions result in a mixture of anomers, leading to purification problems and lower enantiomeric yields. Additionally, achieving stereoselectivity is more challenging when using 2-deoxy sugars which lack substituents at C-2 that can direct the nucleophilic attack. Our group is interested in the development of novel catalytic methods for the synthesis of deoxyglycosides to address this challenge through the activation of both glycals and hemiacetals as glycosyl donors [2,3].
In this work, we demonstrate our latest effort to apply group 16 elements in transition metal-free catalysis for carbohydrate synthesis. We demonstrate the direct activation of glycal donors via hypervalent chalcogenonium catalysis to synthesise 2-deoxy glycosides in good to high yields and stereoselectivity. The glycosylation strategy is mild, practical and compatible with a range of orthogonally protected glycal substrates and both primary and secondary OH nucleophiles, as well as indolyl C- and N- nucleophiles. High α-stereocontrol is observed with alcohol nucleophiles, whilst β-stereocontrol is obtained in the case of indolyl nucleophiles. ^{1}H-NMR and kinetic isotope studies provided insights towards the reaction mechanism pathways to unravel the key steps in the activation process [4].



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FRONTSIDE S_N2 GLYCOSYLATION REACTIONS

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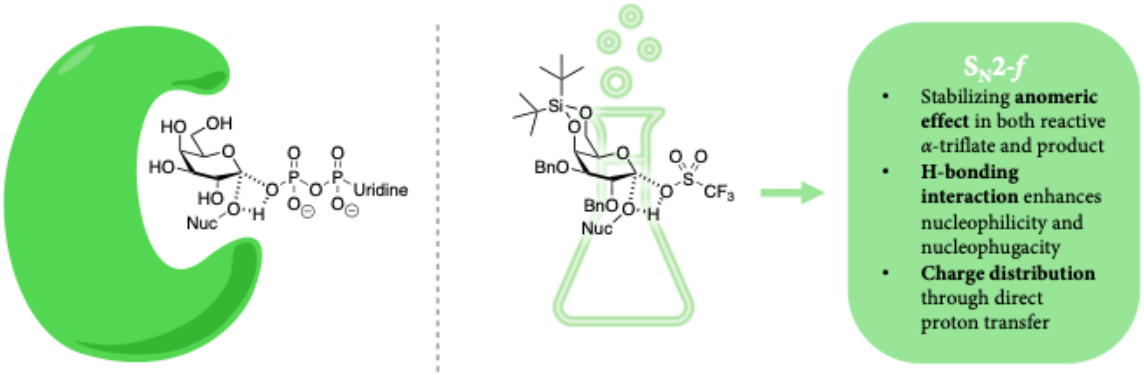
Controlling the stereochemical outcome of glycosylation reactions remains one of the fundamental challenges in carbohydrate chemistry. Mechanistic models generally describe chemical glycosylation reactions as proceeding via S_N1 or backside S_N2 (S_N2-*b*) pathways [1]. Notably, retaining glycosyl transferases operate via a different mechanism, and substitution reactions on nucleotide diphosphate sugar donors can take place through a front face substitution mechanism (termed S_Ni) [2]. This mechanism has been largely ignored to play a role in chemical glycosylation reactions, but we here provide evidence for a concerted frontside S_N2-like (S_N2-*f*) displacement mechanism in glycosylations of 4,6-*O*-di-*tert*-butylsilylene (DTBS)-protected galactosyl donors.
Through ^{19}F EXSY and ^{1}H CEST NMR spectroscopy, we demonstrate that steric hindrance introduced by the DTBS group suppresses triflate exchange, restricting glycosylation reactions to the α-triflate and α-face of the donor. Acceptor concentration-dependent kinetics and primary ^{13}C KIE values support an associative mechanism featuring an “exploded” transition state with retention of stereochemistry. Density functional theory (DFT) calculations further reveal that strong hydrogen bonding between the nucleophile and the triflate leaving group stabilizes the transition state, making this frontside displacement pathway favour more acidic nucleophiles. This was substantiated by acceptor competition experiments, in which the more electron poor *O*-nucleophiles provided faster glycosylation reactions.
Our findings establish a new paradigm in chemical glycosylation chemistry, demonstrating that triflates can be substituted in a stereoretentive manner by exploiting the S_N2-*f* reaction pathway. This work provides new insights to understand the outcome of glycosylation reactions and offers new perspectives for future mechanistic exploration.



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RECOGNITION BY SIGLECS AND GALECTINS OF DISTINCT CARBOHYDRATE STRUCTURES ON *KLEBSIELLA PNEUMONIAE* CELLS

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The innate immune system is equipped with a battery of lectins that recognize carbohydrate structures displayed on bacterial surfaces. Siglecs (immunomodulatory sialic acid-binding immunoglobulin-like lectins) and galectins are two main families of innate immune lectins. In this work, we have comparatively examined the binding patterns of different Siglecs and galectins to a collection of *Klebsiella pneumoniae* clinical isolates exhibiting or not a hypermucoviscous (HMV) phenotype [1]. To this aim, we have used an application of the microarray technology developed in our group [2,3], which consists in the generation of bacteria microarrays and subsequent lectin binding assays to the array-printed bacterial cells. No significant differences among isolates in sialic acid content or recognition by Siglecs were observed, irrespective of their HMV/non-HMV phenotype and capsular or O-antigen serotype. Siglec binding to most of the isolates points to the recognition of a common epitope, capsular glucuronic acid appearing as possible candidate. In contrast, clearly different isolate-selective binding patterns were observed for the galectins tested. Indeed, galectin- and Siglec-binding patterns did not correlate, with some isolates showing almost exclusive binding of Siglecs, others displaying predominant binding of galectins, and a third set of isolates exhibiting moderate to intense binding of several Siglecs and galectins. Galectin recognition correlated with the binding of galactose-specific model lectins and detection of the O1 serotype. Moreover, galectin binding to isolates recognized by mannose-specific lectins was very weak or even negligible, particularly for isolates with mannose-based O3 typed chains. Altogether, the results pointed to the lipopolysaccharide O-chain as a ligand candidate for galectins. In addition, protein bands recognized by galectin-9, but not by Siglec-10, examined as representative lectins, were detected by Western blot analysis in the outer membrane of a selected isolate. Thus, Siglecs and galectins apparently target different carbohydrate structures on *K. pneumoniae* surfaces, thereby behaving as non-redundant complementary tools of the innate immune system.

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EXPLORING THE MOLECULAR RECOGNITION OF MUCIN O-GLYCANS BY *SALMONELLA* SIIE ADHESIN

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Mucin O-glycan domains play a key role in the gastrointestinal tract, acting as recognition sites for bacterial adhesins. While they normally protect against pathogens, some bacteria, like *Salmonella*, use mucin O-glycans for adherence and invasion [1]. This poses a significant public health issue, especially with the rise of antibiotic-resistant strains. Understanding how *Salmonella* interacts with mucin O-glycans is critical for developing anti-adhesion strategies [1].
This research investigates the interaction between *Salmonella* and host cells, focusing on the role of the non-fibrillar SiiE adhesin in targeting Mucin-1 (MUC1) via sialic acid-containing O-glycans [2]. Understanding the specificity and molecular interactions of SiiE with mucin O-glycans is crucial for developing new anti-adhesion molecules to combat bacterial resistance. Our approach combines advanced techniques, including glycan microarrays, mucin cell-based arrays, and Nuclear Magnetic Resonance (NMR) spectroscopy, providing both high-throughput screening and detailed insights into molecular structure and dynamics. This methodology is key to revealing the molecular specificity of SiiE and advancing the development of innovative therapies against Salmonella infections.

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ENGINEERING THE INVERTING MECHANISM OF A HIGHLY SPECIFIC SIALIDASE TO RETAINING

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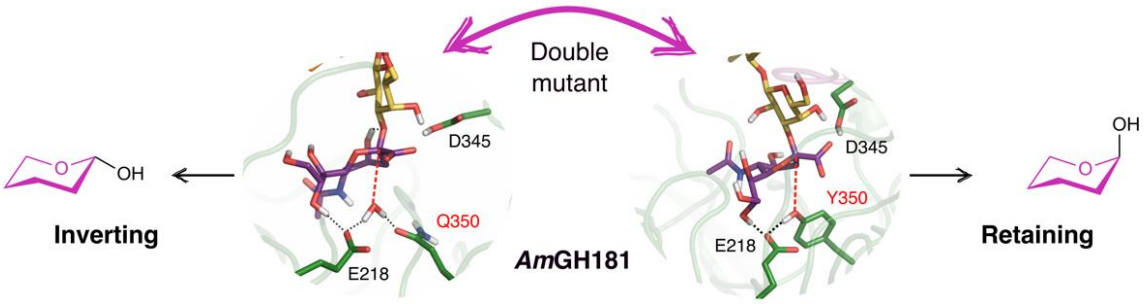
Akkermansia muciniphila is a dedicated mucin degrading gut symbiont, which is associated to beneficial impacts on host metabolism and body weight. This bacterium possess a battery of enzymes that collectively degrade mucin, the main constituents of the mucosal layer. This process starts with the action of exoglycosidases that remove terminal caps from muicn *O*-glcyans. Uniqely, the sialidase *Am*GH181 from *A. Muciniphila*, the founding memebr of a recently discovered family, exhbits high selectivty to the sialyl T-antigen [1]. Combining mutagenesis, X-ray crystallography and QM/MM metadynamics simulations [2,3], we show that specificity is established via a flexible tryptophan-histidine pair, forming a “sugar tang” that positions the sialyl T-antigen for catalysis. Hydrolysis of the sialyl-T antigen proceeds via a single-step S_N2 reaction via a $^{3,6}B/^{3}S_O \rightarrow [^{3,6}B]^{\ddagger} \rightarrow {}^{3,6}B/^{3}S_O \rightarrow {}^{2}C_5$ conformational itinerary of the sialyl unit at subsite *-1*, resulting in inversion of anomeric configuration. For the first time, we altered the reaction stereochemistry by a double mutation, introducing a tyrosine residue to perform nucleophilic attack on the C2 of the sialyl unit at subsite *-1* [4]. The retaining mutant acquired trans-sialidase activity and performed synthesis of sialyl-oligosaccharides at remarkably high yields, pioneering an innovative strategy for harnessing inverting glycosidases for oligosaccharide synthesis.



Acknowledgements:
This study has been funded from the Independent Research Fund Denmark FNU (1026-00386B), the Novo Nordic Foundation (NNF22OC0077684), the Spanish Ministry of Science, Innovation and Universities (MICIU/AEI, PID2023-147939NB-I00 and CEX2021-001202-M), the Agency for Management of University and Research Grants of Catalonia (AGAUR, 2021-SGR-00680) and the European Research Council (ERC-2020-SyG-951231).

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DECODING THE MOLECULAR BASIS OF THE SPECIFICITY OF AN ANTI-STN ANTIBODY

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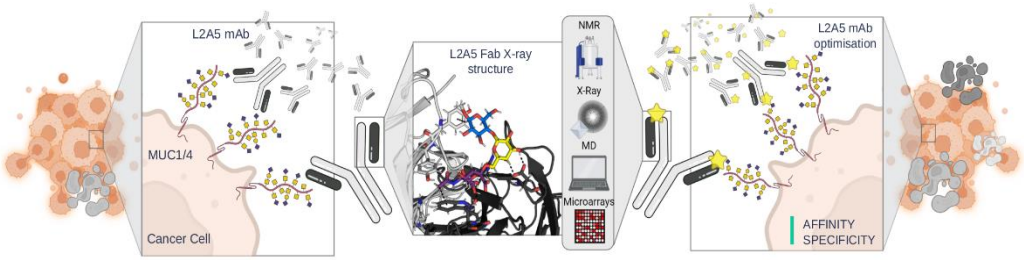

The mucin O-glycan sialyl Tn antigen is a cancer exposed truncated O-glycan, linked to Ser or Thr residues. It has been related with different types of cancers, such as breast, colorectal, and bladder cancer, often leading to a high risk of metastasis and poor prognosis. Anti-glycans antibodies (Abs) with high specificity and affinity have been developed for diagnostic and therapeutic purposes. However, these Abs are challenging to generate and have limited effectiveness, resulting in low titers and short protection durations. For these reasons, experimental structural insights are needed to study anti-glycan Ab specificities. In this study, through a multidisciplinary approach, we combined X-ray crystallography, NMR spectroscopy, computational methods, glycan/glycopeptide microarrays, and biophysical techniques, to thoroughly investigate the L2A5 sTn recognition mode [1], a novel preclinical anti-sTn Ab [1]. The X-Ray data, in line with the NMR experiments, demonstrate that the L2A5 fragment antigen-binding (Fab) specifically binds to core sTn moieties, with a similar binding pose for the sTn linked to Ser or Thr. The Neu5Ac establishes key interactions with the receptor and the GalNAc moiety provides additional contacts. Furthermore, L2A5 exhibits specificity toward cancer-related MUC1 and MUC4 mucin-derived sTn glycopeptides, contributing to its selective targeting against tumor cells. This newfound knowledge holds promise for the rational improvement and potential application of this anti-sTn Ab in diagnosis and targeted therapy against sTn expressing cancers, improving patient care.

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PROBING ALTERED RECEPTOR SPECIFICITIES OF ANTIGENICALLY DRIFTING HUMAN H3N2 VIRUSES BY CHEMOENZYMATIC SYNTHESIS, NMR, AND MODELING

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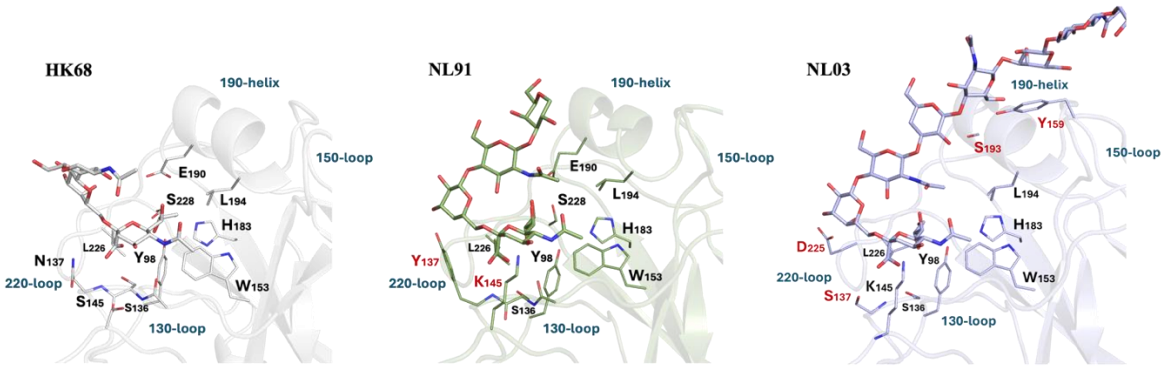
Human H3N2 influenza A viruses (IAVs) have continuously evolved evading neutralization by antibodies elicited by prior infections or vaccination. Through this process, known as antigenic drift, the viral hemagglutinin (HA) protein undergoes substantial sequence divergence while preserving structure and function. Most of the accumulated mutations take place in the most exposed parts of the globular head of HA, where binding occurs with sialic acid-containing receptors of host cells. Receptor binding modes of IAVs usually co-evolve by orchestrated mutations of several amino acids that allow immune evasion but maintain glycan binding capabilities. As a result, A/H3N2 viruses which are the leading cause of severe seasonal influenza illness resulted in altered receptor binding properties. Predicting influenza evolution requires an understanding, at a molecular level, of how mutational changes shape glycan recognition. However, comprehensive analysis of such epistatic networks across HA proteins remains challenging. Our work contributes to examine the binding modes of such drifted hemagglutinins. We employ a multidisciplinary approach which combines chemical synthesis, protein expression, and high-resolution binding studies to detail viral proteins/receptors interactions. The results contribute to understand viral proteins evolution which is important for vaccine design and for the development of predictive models of IAV variants.



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TARGETING VIRAL GLYCANS TO STOP VIRAL INFECTIONS

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Viruses use glycans as an attachment factor during a viral docking to the host cell. Understanding and disrupting these binding processes could lead to new and desperately needed antiviral agents to counter the threat of viral outbreaks, especially those posed by enveloped viruses (EnV). In this contribution, we apply methods of molecular dynamics (MD) simulations to explain the mechanism how synthetic carbohydrate receptors (SCRs) - small molecules designed to bind carbohydrates - are able to bind selectively to EnV glycans and disrupt the viral docking.
First, the MD simulations of SCRs and *N*-glycans common to the surfaces of EnV reveal that SCRs would consistently bind to the trimannosylchitobiose core, the only conserved structural motif present in all *N*-glycans. Importantly, the binding affinity between SCRs and a specific glycan would depend on strength of interactions between the recognition element of the SCR and the *N*-glycan antennae. Systematic variations in the SCR and glycan composition helps to spell out the relationship to design more efficient SCRs which attain affinity and selectivity towards a chosen glycan target on a viral surface. Next, the MD techniques were used to investigate the binding of SCRs to the SARS-CoV-2 spike and NipaH Fusion glycoproteins. The simulations reveal that the SCRs associate on the glycan portion of the glycoprotein, and the resulting distribution of receptors on the protein surface depends on the type of SCRs and the glycan. The observed selectivity follows prediction made for free *N*-glycans. The reported simulations, in addition to experimental NMR, glycan microarray, *in vivo*, and animal studies, suggest that glycans can be selectively targeted by SCRs which could lead to reclassification of glycans from “undruggable” to a viable antiviral target and open new avenues for developing novel treatments, diagnostics and sensors.

EXPLORING THE N-GLYCOSYLATION MACHINERY IN *CHLOROVIRUSES*

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N-glycosylation has been extended from living forms to non-living forms with the discovery that giant viruses have an autonomous glycosylation system[1]. The complex mechanism by which giant viruses produce N-glycans is completely unknown and represents a new frontier to be crossed. In this study, we investigate how the N-glycosylation process occurs in Paramecium Bursaria Chlorella Virus-1 (PBCV-1), the prototype of the family Phycodnaviridae, for which the structure of the N-glycans and some of the Glycosyl transferases (GTs) involved in the assembly of the oligosaccharide are known [1]. In detail, we want to address three fundamental questions: 1) Does the virus follow the same pathway as eukaryotes and prokaryotes? 2) Does the virus have a soluble GT that binds the first sugar directly to the protein moiety? 3) Is there a mechanism other than those currently known? A comprehensive bioinformatics study was carried out to identify potential GTs involved in this process. PBCV-1 encodes 416 proteins, of which 60 are of unknown function, forming the narrow set for GT identification. This set of proteins was screened using several approaches: 1) refinement of the annotation of protein functions using the HHpred tool; 2) evaluation of the degree of conservation of the target protein in chloroviruses using tBlastn (https://blast.ncbi.nlm.nih.gov); 3) phylogenetic relationships with proteins known to be involved in the N-glycosylation process. As results, three putative glucosyltransferases have emerged: A473L, A219/222/226R and A301L. Among these, A473L represents a very good candidate for several reasons: 1) it is conserved in all chloroviruses sharing the same core region of the N-glycan; 2) it is very well expressed at the early stage of the infection cycle; 3) all residues involved in UDP-glucose binding and catalysis are conserved, as demonstrated by modelling (alpha fold) and structural multiple alignment studies. From the structural point of view, A473L is predicted to be a GT-2 membrane protein by alpha fold and it resembles the cellulose synthase of *Rhodobacter sphaeroides-4HG6* (Dali server comparison). Indeed, like 4HG6, A473L has an extramembrane catalytic domain with the typical GT-A fold (98-346 aa) linked to three membrane interface helices (IFH), and a transmembrane domain (TM) that, unlike 4HG6, consists of 6 TMHs instead of 8. In addition, A473L has a structural similarity to the dolichol phosphate (Dol-P) mannosyltransferase from *Pyrobaculum calidifontis* (6YV8), which has a minimal cellulose synthase-like fold. In addition to the GT-A domains, the IFHs of A473L overlaps well with that of 6YV8. These data are of great interest because it has been known that a common feature of the enzymes that recognise the Dol-P-like is the presence of two or three IFHs rich in hydrophobic residues that can interact with the dolichol molecules. A473L could therefore be involved in the binding of UDP-Glc to a Dol-P-like. Based on the above results, work is in progress the express A473L in order to experimentally validate its function.

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COMPARATIVE GLYCOMICS REVEALS CHANGES IN N-GLYCOME PATTERNS IN MULTICELLULARITY AND EARLY ANIMAL EVOLUTION

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Extensive self and non-self cell recognition and division of labor between groups of cells are key requirements of animal multicellularity. In mammals, N-glycosylation is tissue-specific and cancer cells are known to have altered glycosylation patterns. However, little N-glycosylation data from early branching animals or their closest unicellular relatives is available, leaving the evolutionary patterns in the dark.

To overcome this gap, we conducted a systematic, comparative N-glycomics study that includes representatives of early branching animal groups (ctenophores, sponges, placozoans and cnidarians), as well as their closest protist relatives (filastereans, choanoflagellates, ichthyosporeans and corallochytreans) and additional eukaryotic outgroups.

Here, we report a huge variety of N-glycan structures, including novel compositions. The data suggest that N-glycan complexity is positively correlated with organismal complexity and linked to lifestyle. Ichthyosporeans, which have a complex life cycle and often are animal parasites, synthesize a wide variety of N-glycan structures, similar to animals. In contrast, facultatively multicellular protists (*C. owczarzaki*, *S. rosetta*) synthesize simpler oligomannose N-glycans, despite possessing the genes encoding for glycan branching. Our results indicate that the N-glycan biosynthetic pathway became more important for obligate multicellularity, both as a mechanism of protein quality control and a way to synthesize recognition tags. This study provides a foundation for future work on non-canonical species by establishing several reference stage-specific glycomes. Further, single species-focused studies are needed to unravel the significance of the observed structures for each organism.

Acknowledgements: Iñaki Ruiz-Trillo, Michelle Leger, Institut de Biologia Evolutiva (CSIC–Universitat Pompeu Fabra), Barcelona, Spain; Pawel Burkhardt, Michael Sars Centre, University of Bergen, Bergen, Norway; Harald Gruber-Vodicka, Zoological Institute, Christian-Albrechts-University Kiel, Kiel, Germany; Iain Wilson, Jorick Vanbeselaere, Barbara Eckmair, Department für Chemie, Universität für Bodenkultur, Wien, Austria; Sebastián Najle, Arnau Sebé-Pedrós, Centre for Genomic Regulation (CRG), Barcelona, Spain; Zoological gardens of Wrocław and Łódź, Poland

SHELF-STABLE 3-THIOCRESYL-PROP-1-ENYL (TCP) GLYCOSIDES IN GLYCOSYLATIONS

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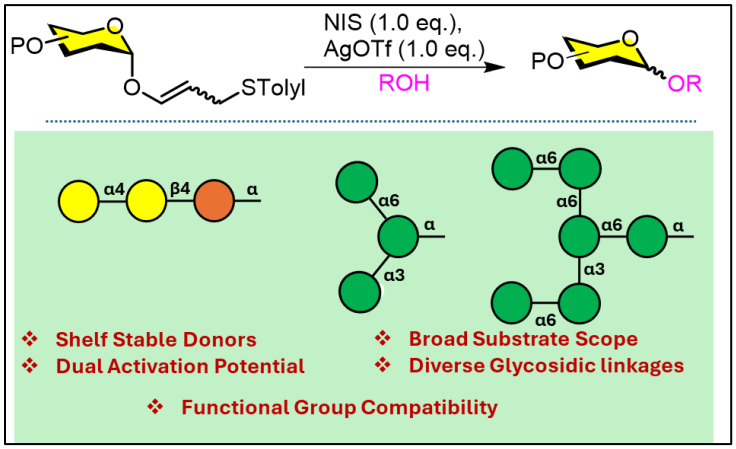
Well-known allyl glycoside activation in glycosylations involve transition metal-catalyzed isomerization to a glycosylation-active vinyl glycoside. In an effort to develop a shelf-stable, active vinyl glycoside, we present a method to transform a of latent allyl glycoside to the corresponding vinyl glycoside, as a TCP glycoside. The TCP glycoside is synthesized by allylic bromination of allyl glycoside, followed by reaction with sodium salt of thiocresol. The TCP glycosides are shelf-stable for more than a year. Utilizing *N*-iodosuccinimide / silver triflate reagents, a remote electrophilic activation occurs, leading to the formation of glycosylation-active intermediate, reaction of which with a glycosyl acceptor completes the glycosylation. The effectiveness of TCP glycoside donors in glycosylations is demonstrated through the synthesis of a number of di- to hhexasaccharides of varying constitutions, in pyranosides and furanosides, including the globo-trioside Gb3 antigen. The reactions also confirm the transformation of disarmed-TCP glycosides to armed-TCP glycosyl donors, illustrating the compatibility with protective group variations. The synthetic utility of TCP glycoside donors is validated through the synthesis of biologically relevant tri- and hexasaccharide oligomannans, establishing the potential of these new donors for oligosaccharide synthesis. Mechanistic studies indicate the remote activation of the thioether moiety by promoters, as evidenced by the identification of thioether and acrolein by-products.



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SWEET TARGETS: A BIOCATALYTIC METHOD TO GLYCOSYLATE NUCLEOSIDE ANALOGUES

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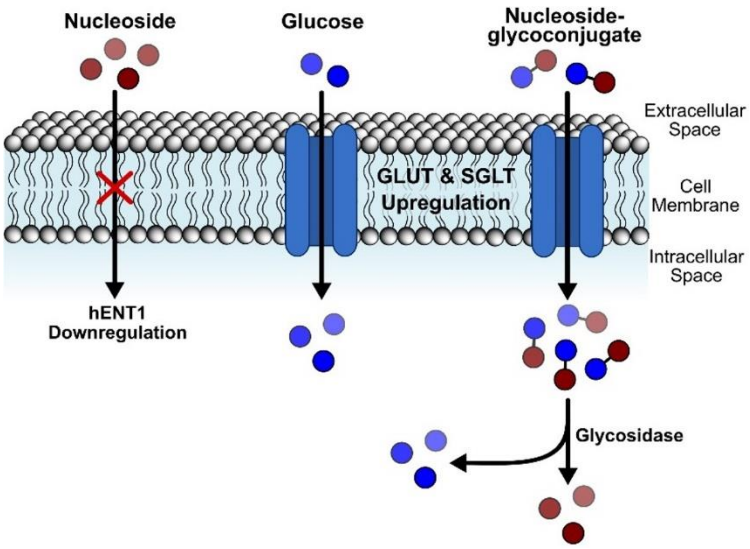
Nucleoside analogue therapeutics have a proven capability within drug discovery as antiviral and anticancer agents [1,2]. However, their efficacy can be limited by poor cellular uptake caused by the down regulation of nucleoside transporters [e.g., human equilibrative nucleoside transporter 1 (hENT1)], off target toxicity and poor bioavailability. Prodrugs of such analogues contribute to an improved pharmacokinetic profile, exemplified by the ProTide therapeutics Sofosbuvir and Tenofovir. Herein, we explore biocatalytic glycosylation of nucleoside analogues as a means towards an alternative prodrug strategy, highjacking glucose transport. In cancerous tissues, an upregulation of hexose transporters (GLUTs) is observed, leading to enhanced glucose uptake and this has led to targeting GLUTs as a method of increasing the selectivity of antitumour therapeutics [3].



Building upon our previous work targeting biocatalytic synthesis of nucleoside analogues and novel gemcitabine glycoconjugates for GLUT1,[4,5] the activity of the nucleoside-specific 3'-*O*-glycosyltransferase AvpGT from *Streptomyces sp. AVP053U2* is investigated against a panel of both natural and clinically relevant purine and pyrimidine nucleoside analogues. AvpGT demonstrates broad substrate promiscuity, with 16 of 22 nucleosides tested showing glycosylation by HILIC-MS. Of these, 13 nucleosides were successfully glycosylated on 25 µmol scale in 39-91% yields, including four current nucleoside analogue therapeutics. The resulting conjugates were screened for their antitumor activity and selectivity against metastatic PC3, LNCaP & AML cell lines.[6] Furthermore, a novel β-glucosidase, AvpGS, was identified from the same *Streptomyces sp.* strain, heterologously expressed, purified and shown to display high substrate promiscuity in subsequently removing glucose from the glycoconjugates. Notably, the human cytosolic glucosidase, GBA3, was inactive here and such orthogonal activity of AvpGS to GBA3 posits an opportunity to explore target specific drug delivery systems, similar to those reported for lectin- and antibody-directed prodrug therapies.

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DEVELOPMENT OF A FLUORESCEIN-BASED LABEL FOR SIMPLE AND SELECTIVE LABELLING OF OLIGOSACCHARIDES

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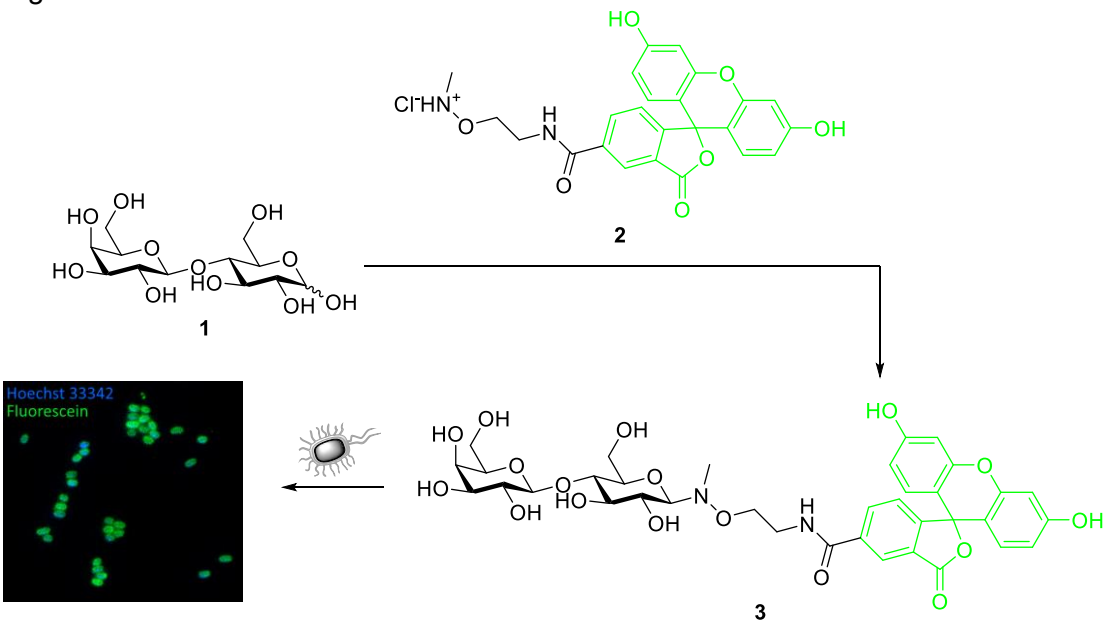
Fluorescence labelling is a useful tool for investigating carbohydrate-cell interactions, such as metabolic uptake or carbohydrate-lectin binding. Two approaches are mostly used to generate labeled carbohydrates: (1) Statistical labeling of a random hydroxyl-group, leading to complex product mixtures and difficult purification and (2) reductive amination of the reducing end, leading to linearization which may impact the interaction with probed organism. To minimize the loss of precious oligosaccharides, we developed a fluorescein-based label **2**, which can be ligated selectively to the reducing end of the oligosaccharide in a single step without requiring any protective groups to form non-linearized labeled oligosaccharides. The synthesis of the labels as well as the labeling of oligosaccharides can be done on gram-scale, allowing for a wide range of screening. Fluorescence microscopy was then used to examine Gram-positive and Gram-negative bacterial strains after they were exposed to the labeled oligosaccharides.



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DIAMINOCYCLOPENTANE GLYCOMIMETICS AS SELECTIVE *O*-GlcNAcASE INHIBITORS: NEW APPROACH TO ALZHEIMER DISEASE TREATMENT

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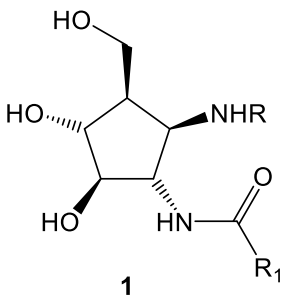
Alzheimer's disease (AD) is the most prevalent form of senile dementia, accounting for 60-80% of cases in people over the age of 65. Amidst the intricate tapestry of AD, two cardinal pathological features predominate: the formation of neurotoxic amyloid β-plaques and the emergence of neurofibrillary tangles. The latter arises from the **abnormal hyperphosphorylation of tau protein**. This **pathological phosphorylation can be prevented by selective inhibition of *O*-GlcNAcase** (*O*-*N*-acetyl-β-D-glucosaminidase, GH84), which already advanced to clinical trials. **A new class of compounds**, namely highly **substituted diaminocyclopentanes** including L-lysine adducts (**1**), have been discovered as potent inhibitors of human *O*-GlcNAcase, an enzyme crucial for protein de-*O*-glycosylation [1-3]. These inhibitors with K_i **at low nanomolar concentrations** exhibit exceptional selectivity and reversibility and are the first example of human *O*-GlcNAcase inhibitors that are structurally related to the transition state (coined as “extended substrate mimetics”) of the rate-limiting step with the “aglycone” still in bond-length proximity. The new inhibitors proved a high selectivity over human β-hexosaminidase B (100 000 ×), negligent toxicity up to 1 mM (HepG2; Balb/3T3 cells), and **increased protein *O*-GlcNAcylation in murine neuronal astrocyte cell** culture. Selected compounds were shown to pass the blood-brain-barrier model of brain microvascular cells (hCMEC/D3) and human astrocytes (P10251-IM-HA). Tests on **human brain organoids** (over)producing human β-amyloid protein bearing trisomy at chromosome 21 clearly proved increasing GlcNAc-ylation and a decrease in phosphorylation of the tau protein. Pilot experiments with the **transgenic mouse model P301S** (PS19), which carries the T34 isoform of the microtubule-associated protein tau, showed a decrease in tau protein levels in the brain after intracranial administration of the compound.



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Acknowledgements: Czech-Croatian project (GAČR 25-15587K & HRZZ Weave-2024-6631), Croatian National Recovery and Resilience Programme BrainClock (NPOO.C3.2.R3-I1.04.0089) and joint Austrian-Czech project (FWF 88525 & GAČR 25-18949L).

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MONO- AND MULTIVALENT GLYCOMIMETICS TARGETING DENDRITIC CELLS AS NOVEL TOOLS FOR MEDICINAL CHEMISTRY

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The recognition of complex carbohydrate epitopes on the surface of pathogens or aberrant cells is a hallmark of the innate immune system. Dendritic cells (DCs) are equipped with a variety of lectin receptors that bind and internalize glycosylated cells in a context dependent manner.[1] This event is often coupled with the induction of specific immune responses tailored to the encountered stimuli. Carbohydrate-based drugs that mimic the structure of certain glycan epitopes represent an underappreciated tool for the modulation of innate immune functions in a disease-related context. In this context, the C-type lectin receptor dendritic cell-specific intermolecular adhesion molecule 3 grabbing non-integrin (DC-SIGN) represents a particularly attractive target. DC-SIGN acts as a pathological host factor in viral infections, enabling either direct infection of DCs, or virus dissemination through the lymphatic system.[2] In addition, DC-SIGN is highly expressed on competent antigen-presenting immature DCs, as well as on tumor associated DCs and macrophages, thus making it a prime target for immune modulation and targeted delivery.
Here, the development of DC-SIGN-targeting glycomimetics and carbohydrate-based multivalent compounds is presented. A thermodynamics-guided ligand optimization campaign is described that resulted in the discovery of a potent mannose-mimetic compound.[3,4] Key insights about the formation of a cooperative interaction network in the DC-SIGN binding site were obtained by X-ray crystallography combined with a thorough analysis of the interaction thermodynamics. The combination of a stereospecific hydrogen bond and an electrostatic polarization of the interaction network was identified as the origin for a non-additive enhancement of binding enthalpy. In addition, it is shown how the complex interplay of design choices for multivalent DC-SIGN ligands gave rise to compounds with unexpected supramolecular properties that successfully interacted with DC-SIGN-expressing cells and efficiently enabled cell-specific intracellular delivery.
These insights pave the way for a further development of DC-SIGN-targeting mono- and multivalent compounds as multi-purpose tools in the context of infectious diseases and cancer.

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TARGETING GLYCAN-MEDIATED ADHESION IN FUNGAL INFECTION

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Chronic fungal infections affect over 150 million individuals. These infections can have a huge impact on immunocompromised and vulnerable populations [1]. Yeasts from the *Candida* spp. are opportunistic fungal pathogens, some of which like *C. albicans* and *C. auris* have been classified as critical priority pathogens by the World Health Organization. Adherence to host tissue is critical to their ability to colonise and infect the host. We have designed and evaluated carbohydrate-based anti-adhesion compounds as inhibitors of *C. albicans* adherence to exfoliated buccal epithelial cells (BECs). A small library of aromatic glycoconjugates were synthesised using synthetic carbohydrate chemistry and Copper-Catalyzed Azide-Alkyne Cycloaddtion (CuAAC) chemistry. These were evaluated as anti-adhesion ligands and it was found that a divalent galactoside (Figure 1) showed the best anti-adhesive properties, capable of displacing over 50% of yeast cells already attached to the BECs [2].
To optimise the activity of this lead compound, several strategies have been explored: we have carried out Structure-Activity Relationship (SAR) studies, where we have investigated alternative scaffolds and glycomimetic compounds. We have also considered different multivalent presentations, where the lead compound was graphed numerous times onto a common scaffold to enhance binding affinity [3,4]. Our results pave the way for the identification of new carbohydrate-binding adhesins involved in fungal pathogenesis.

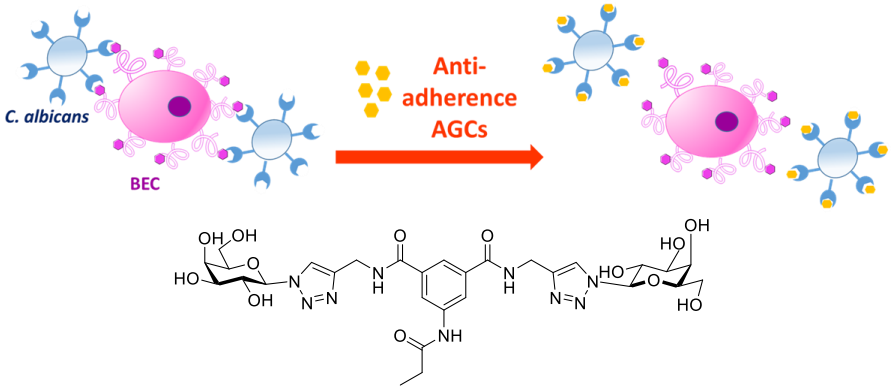

Figure 1. Graphical representation of the anti-adhesion approach (top) and the structure of lead compound divalent galactoside (bottom).

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LIGAND-DIRECTED PROFILING OF GLYCOSIDASES WITH GLYCOMIMETIC-BASED PROBES BASED

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Activity based protein profiling (ABPP) is a versatile tool for evaluating enzyme activity rather than their abundance in living systems and complex environments [1]. With respect to carbohydrate processing enzymes (CPE), several versatile and efficient ABPP strategies have been introduced [2]. One cachet of the majority of these strategies is that the respective enzyme undergoes labelling by covalent binding of the probe to the active site. We are interested in a complementary method by applying the ligand-directed chemistry (LDC) for protein labelling of CPEs. This strategy, introduced by Hamachi and coworkers, allows for labelling of the respective enzyme in a certain proximity to the active site [3].
We have designed and synthesised glycomimetic-based probes featuring the respective components for LDC labelling [4]. Biological evaluations of these probes have been conducted with a selected panels of glycoside hydrolases.
Details about the synthesis of glycomimetic-based probes and biological evaluation for LCD labelling of CPEs will be presented.

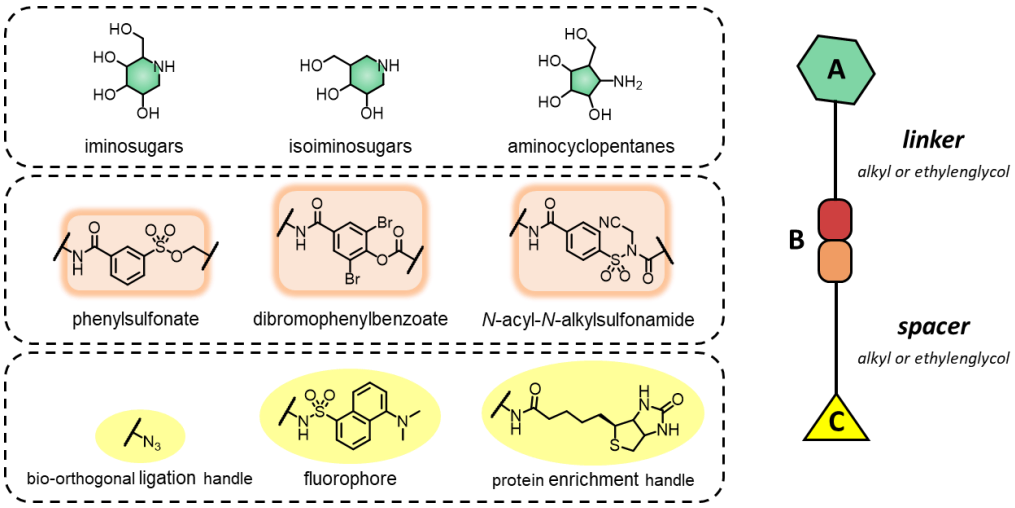

Figure 1. Building block concept for ligand directed chemistry (LDC) probes targeting glycoside hydrolases. (A) reversible inhibitor as ligand; (B) linker with electrophilic reactive group; (C) reporter tag.

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ACTIVITY-BASED PROBES TARGETING MARINE FUCOIDAN HYDROLASES

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Marine glycans are vital biomolecules that play a pivotal role in the global carbon cycle, contributing to the fixation of approximately 50 gigatons of carbon by the oceans each year [1]. Glycans derived from marine organisms have gained significant attention due to their diverse biological activities and their potential applications across various industries. Among these, fucoidans – sulfated polysaccharides primarily composed of L-fucose residues – are of particular interest. Fucoidans display a high degree of structural heterogeneity, especially in their sulfation patterns, making it challenging to establish clear structure-function relationships [2]. This knowledge gap could potentially be narrowed by studying the enzymes that degrade these complex biopolymers, which may also serve as valuable tools for the consistent enzymatic extraction of well-defined fucoidan structures [3]. Fucoidans are typically broken down by marine microbes, yet their degradation pathways remain poorly understood and involve a wide array of enzymes classes, including sulfatases and glycosidases [4]. Among these are fucoidan hydrolases, or fucoidanases, which cleave the glycosidic linkages between sulfated fucose moieties [5]. Despite limited knowledge about these enzymes, studies suggest that they could operate *via* a retaining catalytic mechanism [6]. As this mechanism proceeds through a covalent enzyme-glycosyl intermediate, it presents the opportunity for covalent trapping of the active site residues using mechanism-based inhibitors and probes [7]. In this work, we designed, synthesized and applied new activity-based probes to selectively target fucoidan hydrolases from marine organisms.

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DESIGN AND SYNTHESIS OF SACCHARIDE-BASED MOLECULAR TOOLS TO PROBE OSP AND fOS TRAFFICKING

Michaël Bosco[a], Laia Saltor Núñez[a], Su-Jin Paik[b], Asad Rabee[b], Isabelle Chantret[b], Stéphanie Pradeau[c], Sébastien Fort[c], Stuart E. H. Moore[b], Patricia Busca[a], Christine Gravier-Pelletier[a]

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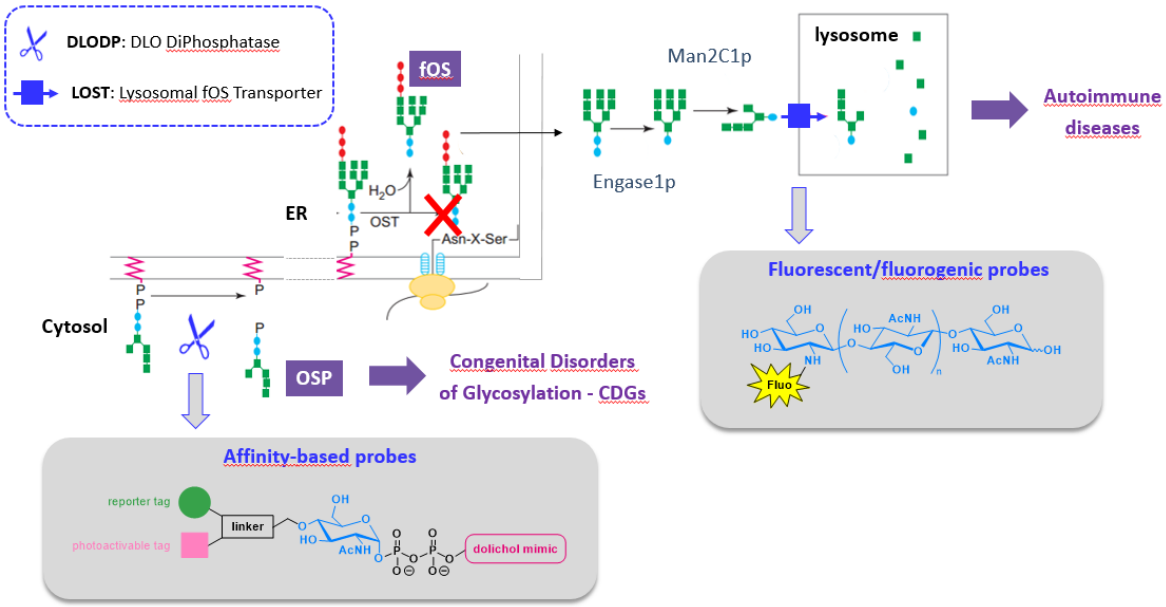
Phosphorylated oligosaccharides (OSP) and free oligosaccharides (fOS) are associated with severe pathologies of two different types: Congenital Disorders of Glycosylation (CDGs) [1] and autoimmune diseases [2]. These OSP and fOS are generated during protein *N*-glycosylation and two key players are involved in their production and/or regulation: Dolichol-Linked Oligosaccharide Diphosphatase (DLODP) [3], and Lysosomal Oligosaccharide Transporter (LOST) [4] (see figure key). Although these activities are partially characterized at the biochemical level, their corresponding proteins/genes are still unknown. In order to study, isolate and identify DLODP and LOST associated proteins, several probes are needed.



In this communication, we will describe our recent results regarding the design, synthesis and biological activity of these saccharide-based molecular tools.

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PHOTOACTIVE CHITOOLIGOSACCHARIDE (COS) PROBES TO IDENTIFY AND ISOLATE LYSOSOMAL OLIGOSACCHARIDE TRANSPORT (LOST)

Laia Saltor Núñez[a], Michäel Bosco[a], Asad Rabee[b], Stéphanie Pradeau[c], Sébastien Fort[c], Stuart E. H. Moore[b], Patricia Busca[a], Christine Gravier-Pelletier[a]

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Small polymannose-type oligosaccharides (fOS) that are generated during protein *N*-glycosylation are transported from the cytosol into lysosomes to be degraded [1]. Presently, lysosomal oligosaccharide transport (LOST) remains to be identified at the molecular level. In terms of its activity, *in vitro* studies using rat liver lysosomes revealed that radioactive [^{3}H]Man$_5$-GlcNAc ([^{3}H]M$_5$) import is blocked by GlcNAc but not by mannose. Moreover, [^{3}H]M$_5$ transport is blocked efficiently by chitooligosaccharides containing 2 to 4 residues of β1-4 linked GlcNAc (COS2-4) with intact reducing end [2]. These data indicate that LOST may have a wider tolerance for the substrate than initially imagined and suggest that it could potentially have diverse transport functions. However, characterising LOST faces important obstacles as the protein, or protein complex, involved has not been identified. To have a better understanding of this process, we are developing chemical tools to characterize and identify LOST proteins.

Useful probes to study LOST were obtained after enzymatic de-*N*-acetylation of the non-reducing end GlcNAc residue of COS2-4. The resulting free amine allowed incorporation of a fluorophore, rhodamine B, and the resulting fluorescent probe was shown to be transported into lysosomes by LOST or a similar process [3,4]. Following this strategy, we synthesized a new generation of COS-derivatized probes containing a photoreactive moiety, a benzophenone, as well as a precursor for a reporter tag, norbornene, allowing the incorporation of tetrazine derivatized compounds, including biotin or fluorophores, after the photo-crosslinking with LOST [Figure 1] . We aim to track fluorescent probes in semi-intact cells using confocal microscopy and to isolate covalently biotinylated proteins for further study by proteomics.

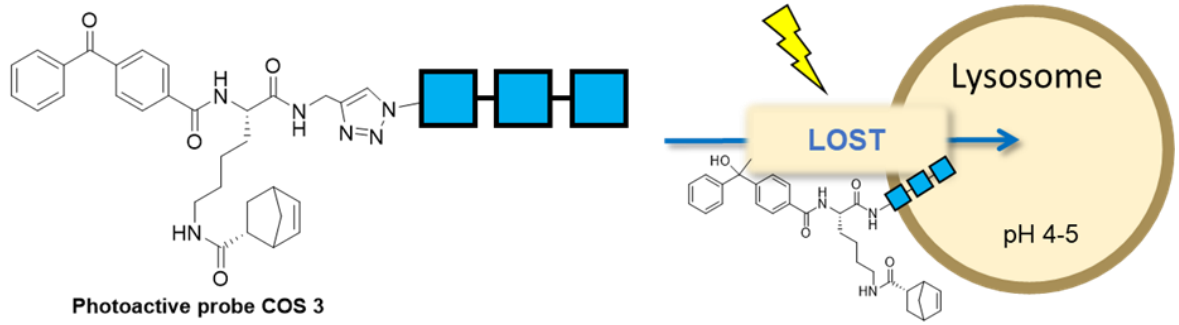

Figure 1. *Photo-cross linking of the COS-derivatized probes with LOST*

The synthesis of the probes will be presented as well as their use in rat liver lysosomes or lysosomes membranes for LOST labelling.

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SYNTHESIS AND SCREENING OF RIBITOL-5-PHOSPHATE METABOLIC LABELLING TOOLS TO STUDY MUSCULAR DYSTROPHIES

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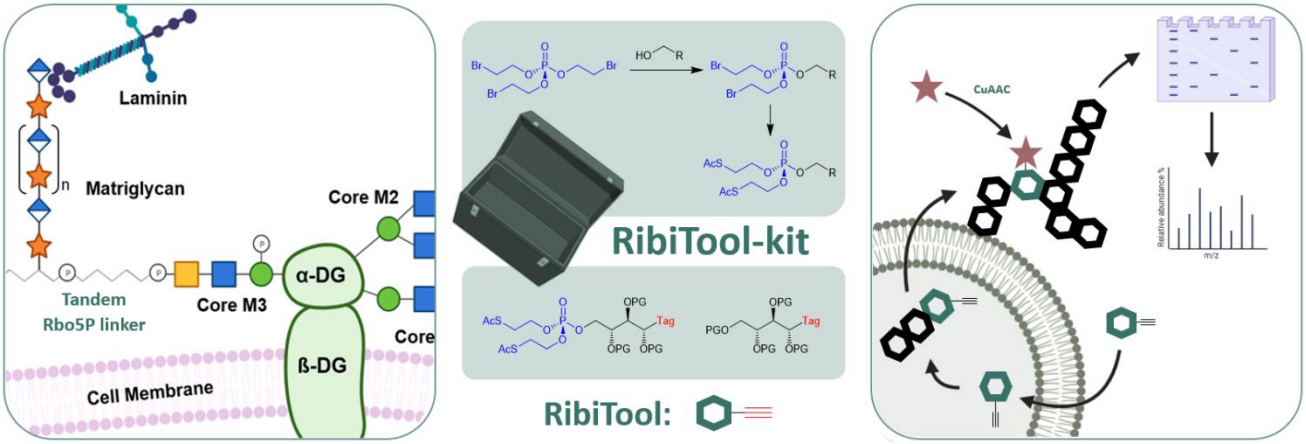
α-Dystroglycan (*α*-DG) is a highly glycosylated cell surface protein essential for binding interactions with laminin within the extracellular matrix, playing a critical role in skeletal muscle, peripheral nerve, and brain tissues. A key glycan, core M3, contains a unique tandem ribitol-5-phosphate (Rbo5P) linker, first identified in mammalian cells in 2016 [1,2]. Disruptions in Rbo5P biosynthesis are linked to congenital muscular dystrophies, highlighting the need for new tools to study its presence and function.

This work describes the development of novel alkyne-tagged ribitol and Rbo5P metabolic labeling tools, synthesised using a newly established methodology for installing cell-labile protected phosphates without the use of hazardous phosphoramidite intermediates [3]. The scope of this approach was explored, enabling the efficient synthesis of multiple probes. These metabolic tools were applied to mammalian cells, and their biological activity was assessed via bioorthogonal reactions with azide-containing reporter groups, followed by in-gel fluorescence and mass spectrometry analysis.

This study presents the first chemical tool capable of fluorescently labeling overexpressed α-DG, allowing for direct probing of mammalian cells. Expanding the scope of this methodology will facilitate broader applications in glycoprotein research and metabolic labeling strategies.



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NEAR ATOMIC-RESOLUTION CRYO-EM STUCTURE OF THE *ZYMOMONAS MOBILIS* GH68 LEVANSUCRASE FIBRILS

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Levansucrases (LS) are retaining glycoside hydrolases (GH) that hydrolyze sucrose and release glucose and a high molecular weight β-(2-6)-fructose chain termed levan. Previously, we demonstrated that the GH68 LS from *Zymomonas mobilis* exhibits two active forms depending on the pH. At pH above 7, the enzyme is soluble as either a monomer or a dimer, primarily hydrolyzing sucrose to glucose and fructose and synthesizing levantriose. At pH below 6, the enzyme self-assembles into stable fibrils, predominantly synthesizing high molecular weight levan from sucrose [1]. The transition of the enzyme between the two forms is completely reversible, simply by changing the pH. Attempts to obtain high-resolution crystal structures of both soluble and fibrils forms were unsuccessful since the protein was not amended for crystallization. Recently, utilizing high-resolution Cryo-Electron Microscopy (Cryo-EM), we achieved a near atomic resolution structure of the *Z. mobilis* LS fibrils, at 2.1 Å resolution (Figure 1). The helical parameters are twist -163.328 degrees (left-handed helix) and rise 23.144 Å. The basic unit is a dimer which is stabilized by six salt bridges and twelve hydrogen bonds. Dimer units assemble into helical structure and interact with each other via three hydrophobic interfaces, highest along the 2-start (~523 Å^2), followed by 3-start (~408 Å^2) and 1-start (~228 Å^2) helical interfaces, consistent with our previous observation that fibril formation is driven by hydrophobic interactions [1]. To further understand the pH-dependent behavior, we computed the electrostatic potential of the levansucrase dimeric structure at different pH values using the PDB2PQR web server, which includes the APBS algorithm. At pH above 7, the surface potential was highly negative, while at pH 5.5, it was nearly neutral, explaining the pH-dependent assembly and disassembly of the fibrils.

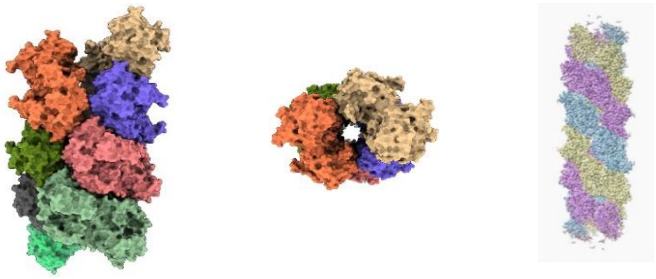

Figure 1. Cryo-EM helical fibril structure of *Z. mobilis* GH68 levansucrase. Each dimer appears in a different color. On the right LS fibril colored as a 3-start helix.

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ENZYMES FROM THE GUT SYMBIONT *AKKERMANSIA MUCINIPHILA* CATALYSE EFFICIENT REMOVAL OF EXTENDED A AND B BLOOD ANTIGENS

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Akkermansia muciniphila is a mucin degrading specialist and the sole representative of the Verrucomicrobiota phylum in the human gut. Recently we characterised the battery decapping sialidases and fucosidases from this bacterium, which revealed the activity of two α-1,2-fucosidases of glycosidase hydrolase family 95 on H antigens, found as terminal epitopes of mucin *O*-glycans [1]. These finding inspired us to revisit the enzymatic conversion of group A and B red blood cells (RBCs) to the universal donor type O (ECO concept). The genesis of this vision dates more than four decades ago, using a native *exo*-glycosidase from coffee bean, which was followed by the discovery of several more efficient bacterial enzymes [2]. All these studies aimed at removing the canonical textbook A and B antigens, but extended forms of both the A and B antigens are known to be displayed on RBC glyco-lipids.
We harnessed *A. muciniphila* to discover enzymes that efficiently remove both the canonical and all known extended A and B motifs, which led to significant improvement of compatibility with group O plasmas. Our work establishes for the first time the known extended A and B motifs as true antigens associated with the A and B RBC phenotypes [3]. We will present this study with focus on the molecular signatures of the blood antigen removing enzymes.

Acknowledgements: This study has been funded from the Independent Research Fund Denmark (grants 1026-00386B, 1026-00386B) and the Novo Nordic Foundation (NNF22OC0077684), Swedish Research Council (2019-01683), the Knut and Alice Wallenberg Foundation (2020.234) and the Swedish government and county councils (grant no. ALFSKANE-446521 and 2022-Projekt0287).

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FINE MOLECULAR STRUCTURE OF GLYCOGEN PARTICLES IN *ESCHERICHIA COLI*

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Glycogen in *Escherichia coli* exhibits dynamic structural alterations, switching between fragile and stable states, yet the molecular mechanisms underlying these changes remain unclear. Recent studies suggest that glycogen degradation enzymes may play a crucial role in the structural regulation of glycogen particles. However, the specific contributions of glycogen phosphorylase (GlgP) and glycogen debranching enzyme (GlgX) have not been thoroughly investigated. This study aims to explore the roles of GlgP and GlgX in glycogen structural stability and fragility. In particular, the fine molecular structure of glycogen particles in *E. coli* wild-type and three mutant strains, *ΔglgP*, *ΔglgX*, and *ΔglgP/ΔglgX*, were explored. Using a combination of biochemical assays and advanced imaging techniques, glycogen particle fragility and stability across these strains were compared. Our findings reveal that glycogen in *E. coli ΔglgP* and *ΔglgP/ΔglgX* mutants was consistently in a fragile state, whereas glycogen in the *ΔglgX* mutant exhibited a stable conformation. These observations suggest that GlgP plays a dominant role in maintaining glycogen structural stability. The absence of *glgP* leads to a significant disruption in the structural stability of glycogen particles, while *glgX* deletion alone does not appear to affect glycogen stability. In sum, this study highlights the essential role of glycogen phosphorylase in regulating glycogen structural stability in *E. coli*. Our findings provide important molecular insights into the mechanisms governing glycogen structural stability, offering a deeper understanding of glycogen metabolism and its regulation in prokaryotic systems.

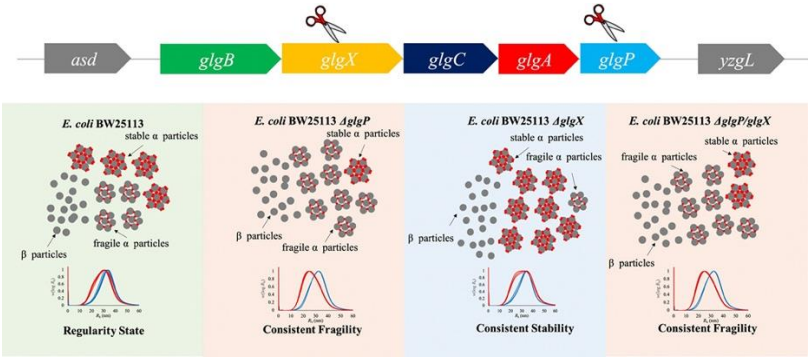

Figure 1. Schematic illustration of the influences of key genes on glycogen structural stability and fragility in *Escherichia coli* [1].

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EVOLUTION OF GLYCOSIDE HYDROLASES AND THEIR ELECTROSTATIC PROPERTIES

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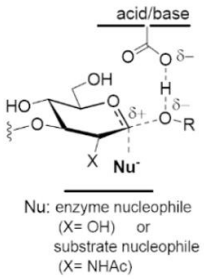
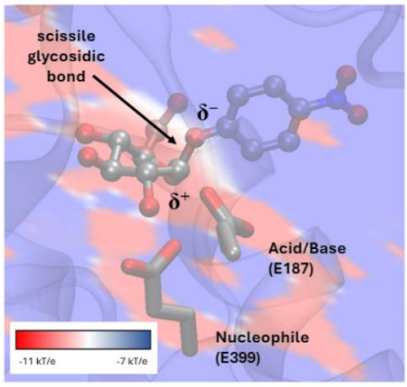
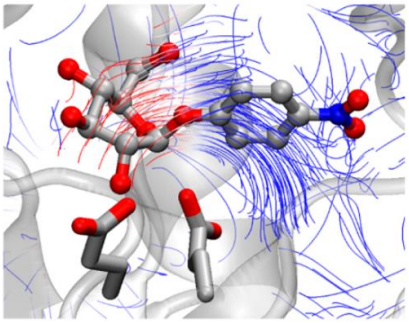
The high catalytic efficiency of enzymes is attained, in part, by their capacity to stabilize electrostatically the transition state of the chemical reaction. In glycoside hydrolases, the oxocarbenium ion-like nature of the transition state has been well characterized both experimentally and computationally [1]. However, limited attention has been paid to how this oxocarbenium-ion gets stabilized by glycoside hydrolases. Here we will present how the structure of this large family of enzymes has evolved to generate an electrostatic potential gradient at the active site complementary to the charge separation that is formed along the scissile glycosidic bond during the catalytic reaction [2]. These electrostatic properties have never been analysed before from the perspective of the glycoside hydrolase enzyme. We will show how such electrostatic potential gradient at the active site is an evolutionary constrained feature present across all clans of the glycoside hydrolases families. We have translated this property into an easy to compute metric that can be used to guide protein engineering. We will present examples of how this metric is able to predict mutation sites aimed at modulating the hydrolytic activity of glycosidases, for example, for the development of efficient transglycosidases [2].







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EXPLORING NEW PATHWAYS OF SULFOQUINOVOSE BIODEGRADATION

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Sulfoquinovose (SQ), a sulfosugar that forms polar head group of plant sulfolipid SQDG, plays a critical role in the global sulfur cycle with an estimated 10 billion tonnes production per year [1]. We present a desulfonation pathway in *Agrobacterium tumefaciens* that can grow solely on SQDG as carbon source, through complete SQ catabolism to glucose and sulfite [2]. This pathway involves a 'gateway' GH31 glycoside hydrolase [3] and a central two-component FMN-dependent SQ monooxygenase that recognize the distinguishing 'sulfonate' group in SQ and facilitate the direct cleavage of C–S bond, releasing inorganic sulfite and glucose, allowing direct entry into glycolysis (**Figure 1**). Structural and biochemical analyses provide insights into enzyme-substrate interactions and specific sulfonate-binding motifs. Our recent work also uncovered the founding members of new GH188 family in the CAZy database that utilize alternative mechanisms to hyrolyse SQ from the sulfolipids [4]. Strikingly different to GH31 SQases, these NAD^+-dependent SQases from GH188 CAZy family operate through transient oxidation at C3. We will present bioinformatic data that show that this pathway is widespread among alpha- and beta-proteobacteria, particularly within the Rhizobiales order and marine Roseobacter clades, suggesting a broad ecological significance of SQ biology.

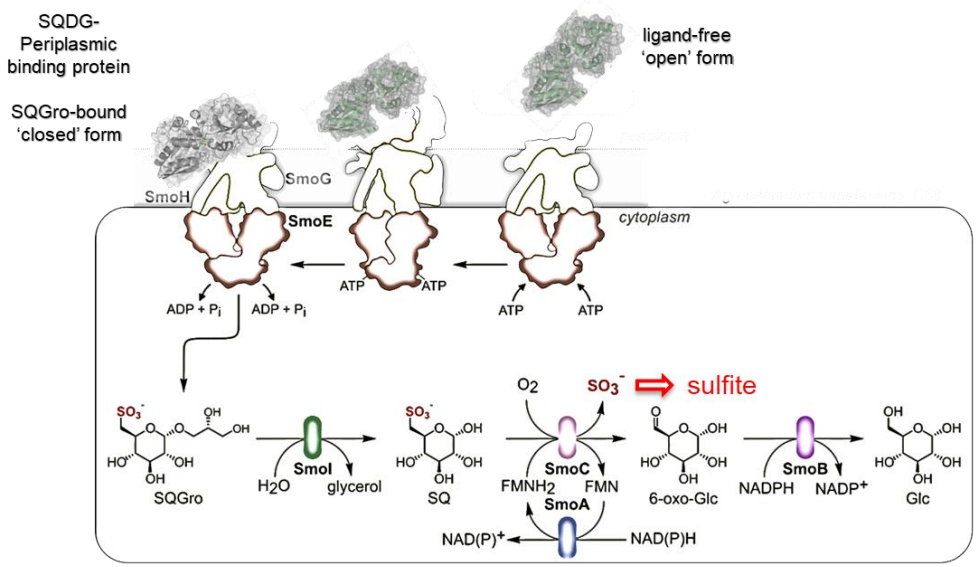

Figure 1. Schematic summary of SMO pathway in A. tumefaciens. SQGro, a metabolite of SQDG, is imported into the cell and hydrolysed to liberate SQ, which is further metabolized to release sulfite and glucose.

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KEY STRUCTURAL IDENTIFICATION OF CHITOOLIGOSACCHARIDES RESPONSIBLE FOR THEIR ANTI-INFLAMMATORY ACTIVITY

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Endotoxemia is mainly caused by a massive burst of inflammatory cytokines as a result of lipo-polysaccharide (LPS) invasion. Chitooligosaccharides (COS) has attracted wide attention for relieving endotoxemia due to its anti-inflammatory properties. However, the structural parameters of COS are often ambiguous, and the structural basis of COS for its anti-inflammatory remains unknown. In this study, some specific COSs with well-defined structure were successfully obtained. Their structures and sequences were confirmed by ^{1}H NMR and MS analysis. Then, the effect of DA and PA (degree and pattern of acetylation) on the anti-inflammatory activity and relieving endotoxemia potential of COS was researched. The results revealed that COS with a DA of 12% had better anti-inflammatory activity than COSs with other DAs, mainly in inhibiting LPS-induced inflammatory cytokines burst, down-regulating its mRNA expression and reducing phosphorylation of IκBα. The KEGG results showed that COS induces the pleiotropic modulation of classical inflammatory pathways and shows an obviously protective effect on endotoxemia mice, such as inhibiting the increase in inflammatory cytokines and transaminases, alleviating the injury of liver and intestinal tissue. Furthermore, the key sequence responsible for the anti-inflammatory activity of COS was identified. This study explored the relationship between the structure of chitooligosaccharides and their anti-inflammatory activity and lays the foundation for the development of COS as an anti-inflammatory drug against endotoxemia.

FUNGAL POLYSACCHARIDES FROM *INONOTUS OBLIQUUS* INDUCE MACROPHAGE ANTI-CANCER ACTIVITY

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Plant and fungal polysaccharides have been reported to interact with the immune system in multiple ways and the effects on macrophages have been studied extensively. The polysaccharides can bind pattern recognition receptors (PRRs) on the surface of macrophages and initiate an immune response [1,2]. Within cancer immunotherapy there is an increasing awareness on the role of macrophages, and the therapeutic potential of switching tumor-associated macrophages (TAMs) from a pro-tumor to an anti-tumor state [2,3]. When properly activated, macrophages can kill cancer cells directly, secrete cytokines for immune cell recruitment to tumors, and stimulate T cells to fight metastases [4]. As such, macrophages are attractive targets for novel anti-tumor immunotherapy drugs. We have evaluated six polysaccharides isolated from the medicinal fungus *Inonotus obliquus* for their ability to activate mouse and human macrophages. Of these, two water-soluble polysaccharides, AcF1 and AcF3, were able to trigger anti-tumor functions of macrophages [5]. Macrophages were activated by AcF1 and AcF3 to secrete nitric oxide and the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin (IL)-6. The polysaccharides also triggered production of IL-12p70 when combined with interferon-γ, a central cytokine for anti-tumor immunity, and induced macrophage-mediated inhibition of cancer cell growth *in vitro* and *in vivo*. AcF1 and AcF3 were shown to interact with the PRRs Toll-like receptor 2 (TLR2) and TLR4, and dectin-1a. The polysaccharides were complex and highly branched, consisting of a (1,3/1,6)-β-glucose (Glc) region, in addition to monomers such as (1,6)-α-galactose (Gal) and (1,4)-α-galacturonic acid (GalA) [6]. We have shown in this study that the polysaccharides AcF1 and AcF3 from *I. obliquus* have a strong potential for cancer immunotherapy by binding to multiple PRRs and by inducing potent anti-cancer activity of macrophages.

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DISCOVERY OF NON-TOXIC ANTIBACTERIAL GLYCOSIDES FROM *DROSERA GIGANTEA*

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The rise of antibiotic resistance necessitates the discovery of novel, safe antimicrobials. Carnivorous plants accumulate phenolic acids and flavonoids with modest antibacterial activity, while their more potent naphthoquinones (e.g., plumbagin) are cytotoxic [1]. Our previous study showed that antibacterial potency among selected carnivorous plant species did not correlate with plumbagin content [2], suggesting the presence of alternative bioactive metabolites. We hypothesized that carnivorous plants could be a source of novel, non-toxic antibacterial compounds effective against antibiotic-resistant pathogens.

Extracts from twelve *in vitro*-propagated Drosera species were analysed for plumbagin content using HPLC and screened for activity against a plumbagin-resistant *Pseudomonas aeruginosa* [2] strain by broth microdilution. Five species, representing high, moderate, and low plumbagin producers, were further evaluated for antibacterial activity and *in vivo* toxicity in *Caenorhabditis elegans* (LC_{50} assay). The most potent extract was fractionated by TLC, and its constituents were identified using HPLC-MS and NMR. TLC-MTT bioautography was used to pinpoint the compound responsible for antibacterial activity. Additionally, due to its exceptional biological activity, the extract's systemic and dermal toxicity was assessed in a murine model.

Drosera gigantea exhibited strong antibacterial activity despite having the lowest plumbagin content among the tested Drosera species. Fractionation yielded nine metabolites; the analysis of mass spectra (HPLC-MS, Compound Discoverer) allowed preliminary identification of four metabolites. Three compounds with the highest yield were characterized by NMR, revealing two known droserone glycosides [3] and one novel glycoside. Bioautography confirmed that the novel glycoside co-localized with the antibacterial zone. The systemic and dermal safety of *D. gigantea* extract was confirmed in mice.

We report the first non-toxic antibacterial glycoside from *D. gigantea*, demonstrating that carnivorous plants can provide glycosidic antibiotics that avoid naphthoquinone-associated cytotoxicity. This discovery expands the chemical space available for combating antibiotic resistance.

Acknowledgements: This work was financially supported by the Ministry of Science and Higher Education of Poland as a part of a research subsidy to the University of Gdansk from 2019 to 2023 (DS 531-N104-D800-25), and by a mobility grant from the Foreign Internship Program for Young Researchers at the University of Gdańsk, awarded to MKM.

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ALGINATE-AMPHOTERICIN B BIFUNCTIONAL CONJUGATES FOR RESISTANT AND RECURRENT FUNGAL INFECTIONS

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Antimicrobial resistance (AMR) is among the top threats to global human health [1]. The progressive increase in AMR supersedes the rate of development of new antibiotics [2]. Moreover, fungal biofilm infections are up to 1000-fold more tolerant to antifungal agents than fungal cells in planktonic growth [3]. This intrinsic resistance illustrates the need to develop improved anti-fungal drugs with greater efficacy against biofilm growth, while also reducing the potential for drug toxicity, resistance and tolerance. Amphotericin B (AmpB) is a polyene macrolide antibiotic with broad specificity and has been the gold standard for antifungal treatment since the 1950s for the most severe fungal infections. Unfortunately, AmpB has poor solubility and is limited by infusion-related reactions and nephrotoxicity [4]. Although new formulations such as liposomal AmpB have shown significant reductions in nephrotoxicity [5], there is still considerable scope for improvement for less toxic formulations.

Alginates are a family of anionic and linear polysaccharides produced by brown algae and some bacteria and consist of 1→4 linked β-D-mannuronic acid (M), and its C5-epimer α-L-guluronic acid (G) (Fig. 1). Research has shown that low molecular weight alginate oligosaccharides possess biofilm disruption properties [6], which combined with their high solubility and low toxicity represent ideal candidates for creating novel bifunctional anti-fungal compounds. Using click chemistry, we have developed a novel conjugation at the reducing end that retains the intrinsic biofilm disruption properties of the low molecular weight alginate oligosaccharides while also maintaining the fungal cell-wall disrupting role of the AmpB component. Preliminary data shows the conjugates have reduced toxicity and improved solubility characteristics, while maintaining the AmpB anti-fungal properties.

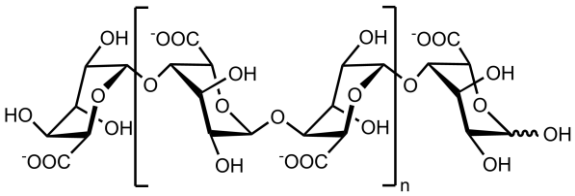

Figure 1. The structure of alginate consisting of α-L-guluronic acid (G) residues.

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STRUCTURE-ACTIVITY RELATIONSHIP OF MODIFIED AMPHIPHILIC CATIONIC CYCLODEXTRINS FOR RNA DELIVERY

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The success of mRNA vaccines has validated RNA-based therapies for non-infectious diseases as well like neurodegenerative and inflammatory disorders. While viral vectors are common, safety and scalability concerns drive the search for non-viral alternatives. Lipid nanoparticles excel in liver delivery, but broader use demands novel biomaterials for RNA stability and efficient targeting [1].

Cyclodextrins (CDs), cyclic oligosaccharides with tunable chemical properties, offer a promising platform for RNA delivery (Figure 1). A library of amphiphilic CDs was synthesized and systematically modified to enhance stability and gene delivery efficiency. Structural variations including modifications in lipid tail length, branching, ionizable group substitution (primary vs. tertiary amines), and linker chemistry. The resulting compounds were characterized using nuclear magnetic resonance spectroscopy and mass spectrometry, confirming successful synthesis.

As part of the GENEGUT project, these modified CDs were screened for physicochemical properties and gene silencing efficiency in undifferentiated and differentiated Caco-2 cell models. The four most effective candidates were selected for further evaluation in biorelevant media, assessing their resistance to enzymatic degradation and potential for oral RNA delivery.

Our findings establish amphiphilic CDs as a viable alternative to conventional nanocarriers, with implications for non-viral gene therapy applications.

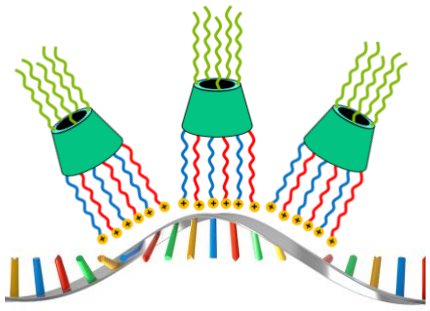

Figure 1. Cyclodextrin-Nucleic Acid complex.

Acknowledgements: Funded by the European Union (GENEGUT, GA 101057491).

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GLYCOMICS MICROARRAYS REVEAL HIGHER CD64 BINDING AND GREATER PHAGOCYTOSIS ASSOCIATED WITH SERUM IGG FUCOSYLATION IN SEVERE COVID-19 DISEASE

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the global coronavirus disease 2019 (COVID-19) pandemic. SARS-CoV-2 infection typically stimulates production of serum antibodies against various viral antigens (Ag) over time. We previously demonstrated that there was a significant association between disease severity and serum antibody binding intensity for specific viral protein antigens including spike protein fragment S1 [1]. Serum antibody level is also associated with neutralisation and clearance as Fc receptors (FcR) and effector functions are engaged. However antibody-Ag immune complex receptor binding has not yet been associated with COVID-19 disease severity and phagocytosis.

Serum IgG core fucosylation was revealed in the severe COVID-19 disease cohort by lectin microarray profiling and HPLC analysis, which was absent in the mild and moderate disease and pre-pandemic healthy donor cohorts. The affinity (Kd) of IgG from severe patients was highest by 3 weeks post-infection. Serum IgG from the different disease severity and healthy cohorts were also complexed with various viral protein Ags of differing glycosylation and incubated with Fc receptors (FcR) in a custom microarray format. Serum IgG-Ag immune complexes modulated FcR interactions and Kd compared to Ag alone, and Ag glycosylation also impacted binding. Phagocytosis of bead bound S1 Ag was assessed in the presence and absence of serum IgG from the different cohorts. Overall, higher binding to CD64 and greater phagocytosis was associated with severe disease serum IgG and fucosylation. Further, custom glycomics microarray platforms provide a novel and rapid methodology to identify and measure immune interactions, which can contribute to disease severity prognostics and treatment modality decisions.

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GLYCAN MICROARRAYS – ESSENTIAL TOOLS FOR GLYCAN RECEPTOR TYPING OF EMERGING INFLUENZA VIRUSES

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The binding of influenza virus haemagglutinin (HA) to sialic acid (Sia)-terminating glycans on host respiratory epithelia is a critical step in viral infection and a key determinant of virus transmissibility and pathogenicity. While HA specificity for sialic acid linkage (α2-3 for avian-adapted viruses and α2-6 for human-adapted viruses) is widely recognized as a major factor in cross-species transmission, increasing evidence suggests that additional glycan structural features significantly influence viral binding, affecting species, tissue, and cellular tropism. Glycan microarrays have proven to be powerful tools for characterizing receptor-binding specificities of influenza viruses. The neoglycolipid-based microarray system has been instrumental in defining the binding characteristics of the 2009 H1N1 pandemic virus [1,2] and highly pathogenic H5N1 viruses isolated from human patients in Vietnam [3].
This communication highlights recent advancements in expanding the sialyl glycan library via chemoenzymatic synthesis, coupled with the Fmoc-Amino-Azido (FAA) glycan derivatization strategy [4]. The newly constructed sialyl glycan microarrays have been used to screen H5 influenza strains of clade 2.3.4.4b, responsible for recent outbreaks in avian and mammalian species, including cattle influenza strains from the US, and severe human infections. Robust microarray data were obtained using both live and inactivated viruses, as well as recombinant HA proteins [5]. Moreover, the FAA-glycan probes can be readily converted into biotinylated probes for label-free kinetic analysis of virus-glycan interactions in the Biolayer Interferometry (BLI) system. This integrated workflow, combining high-throughput microarray analyses with detailed kinetic measurements in BLI, holds great potential to deepen our understanding of glycan-mediated influenza virus interactions and support surveillance efforts.

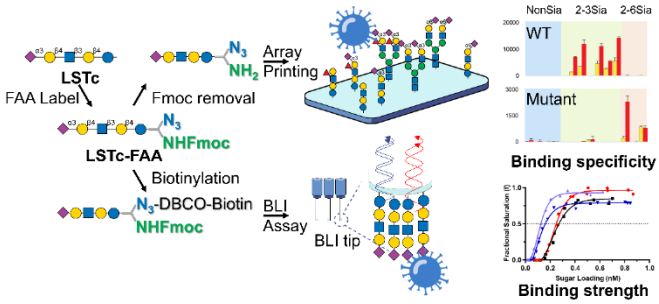

Figure 1. Multifunctional glycan probes for analyzing glycan-mediated influenza virus interactions using glycan microarray and biolayer interferometry platforms.

Acknowledgements: This work is supported by Wellcome Trust Biomedical Resource Grants and the BBSRC/MRC/Defra jointly-funded UK FluTrialMap/FluTrailMap:One Health consortia.

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ELECTROCHEMICL AND SURFACE ANALYSES FOR STUDYING AND UTILIZING GLYCAN INTERACTIONS

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Modifications of complex glycans govern their binding preferences and biological activities. Evaluating the effect of modifications, e.g. sulfation and sialylation, on interaction preferences is not straightforward. First, obtaining pure modified complex glycans in sufficient quantity is not easy. Second, many interactions are too weak to study using standard bioanalytical tools. We use electrochemical and surface analysis for studying the effect of modifications on complex glycans interaction preferences. These alternative approaches have seveal advantages. They are label-free and utilize minute quantities of complex glycans for elucidating a variety of interaction. The resulting electrochemical signal provide sensing even of weak interactions. The signal is the outcome of the glycan monolayer reorganization thereby reflect on interaction of the analyte with an ensemble rather than from an interaction of a single entity.
The talk will highlight the empowering combination between synthetic glycans and electrochemical methods for analyzing huge variety of glycans-derived interactions. I will explain how surface characterization provide atomic level evidence that connects between glycan sulfation patterns, metal-ion glycan binding preference and metal-ion mediated glycan-protein interactions [1]. I will present a new multiparameteric diagnostic tool to profile neuraminidase and sialyl transferases activities and inhibition utilizing both the surface properties and glycan structural features [2]. An electrochemical gas phase biosensors based on systematically modified synthetic monosaccharides will be presented. Enantiomer discrimination of volatile organic molecules using the monosacchride-based biosensor will be described.

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LECTINS IN HOST-PATHOGEN INTERACTIONS: STRUCTURE, FUNCTION, AND THEIR ANALYTICAL POTENTIAL

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Lectins are carbohydrate-binding proteins of non-immune origin. While some play a role in immune system processes, they are not primary immune response products and lack catalytic activity toward their ligands. Microbial lectins recognize carbohydrates on host cells (glycoproteins or glycolipids), facilitating adhesion to host cells or mucosal surfaces, making them important virulence factors. Our long-term aim is an identification, isolation and characterization of lectins from bacteria and fungi, especially from pathogens affecting humans, plants and insects.
Contribution is focused on structure-function studies of several examples of microbial lectins participating in the host-pathogen interaction as well as lectins from bacteria that may be involved in nematobacterial complexes highly pathogenic for a broad range of insects.
As a demonstration of an importance of lectin research, several examples of lectins recently discovered and structurally and functionally characterized in our group will be presented. A novel calcium dependent lectin able to bind glycosaminoglycans will be shown and structural features that are responsible for different specificities among all homologous proteins will be discussed.

FROM STEREOCONTROLLED GLYCOSYLATION TO AUTOMATED CHEMICAL SYNTHESIS

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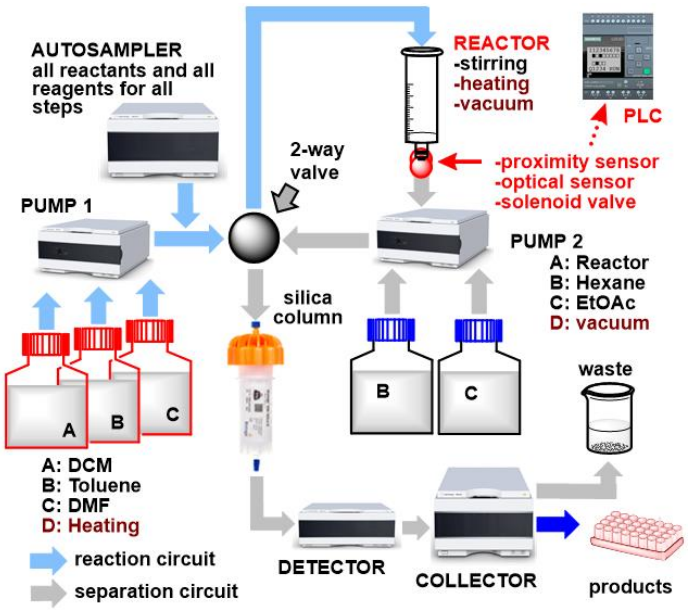
From the building blocks of nature to disease-battling pharmaceuticals, carbohydrates have had a profound impact on evolution, society, economy, and human health. Numerous applications of these essential biomolecules in many areas of science and technology exist, most of which can be found at the forefront of therapeutic agent and diagnostic platform development. Although carbohydrates are desirable for the pharmaceutical and biomedical communities, these molecules are very challenging targets for chemists because of the need for functionalization, protecting and leaving group manipulations, controlling anomeric stereoselectivity, separation, and analysis. The development of practical methods for the synthesis of building blocks, chemical glycosylation, and glycan assembly represent demanding areas of research.

At the core of this presentation is the development of new methods, strategies, and technologies for the chemical synthesis of glycans. These tools will be discussed in light of recent results related to the development of new glycosylation reactions [1], methods for controlling the stereoselectivity [2], and HPLC-based automated synthesis [3]. The effectiveness of methods developed will be illustrated by the synthesis of glycopharmaceuticals. This work has been generously supported by the National Institutes of Health and the National Science Foundation.



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STEREOSELECTIVE GLYCOSYLATIONS

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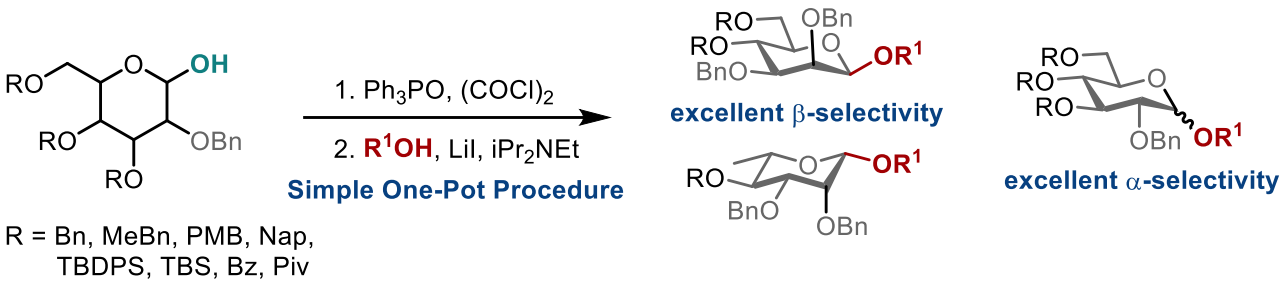
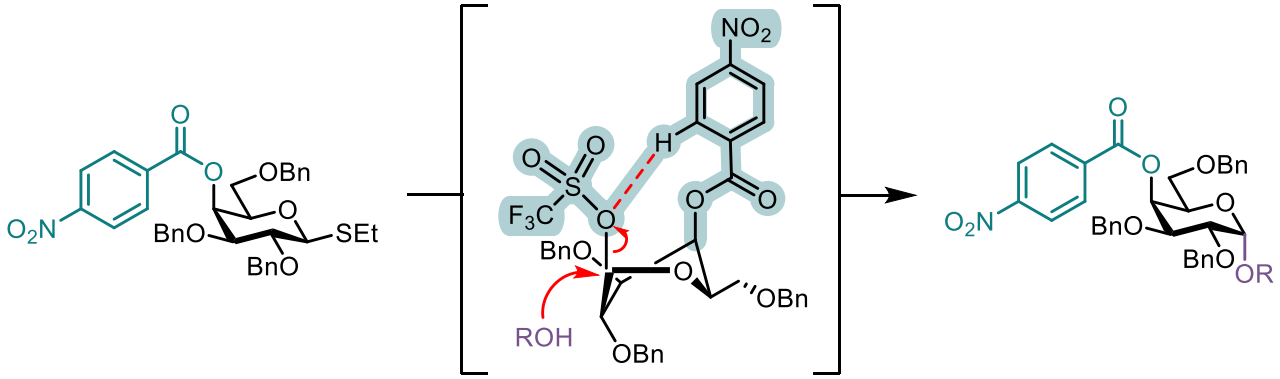
We will describe our efforts to develop stereoselective glycosylation reactions. We have adapted Denton's catalytic Appel methodology for the synthesis of glycosyl chlorides. The chlorides are first transformed *in situ* into iodides and then glycosides in a novel one-pot transformation of glycosyl hemiacetals to difficult-to-make β-mannosides and rhamnosides [1]. In contrast, the same protocol gives α-glucosides in high selectivity [2]. This selectivity switch will be discussed.

We have also investigated the role of remote substituents at the 4-position of galactosides and fucosides in influencing α/β-selectivity in glycosylations [3]. *para*-Nitrobenzoates gave high to excellent α-selectivity. The results of our mechanistic studies (experimental and computational) led us to propose that a non-classical hydrogen bond from the *ortho*-CH of benzoates to the β-triflate intermediate is important for the selectivity of the reactions of galactosides.





If time permits, results of our efforts to automate the synthesis of monosaccharide building blocks might be described.

Acknowledgements: We thank Research Ireland for funding (CDA/15/3625, GOIPG/2019/2747, GOIPG/2020/1129, GOIPD/2019/538).

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THE GLYCOSYL 8-ALKYNYL NAPHTHOATE DONOR STREAMLINES THE SYNTHESES OF CARBOHYDRATES

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We present a structurally unique and highly effective leaving group, 8-alkynyl naphthoate, which can be efficiently activated under mild gold(I) catalysis. Unlike traditional anomeric ester groups, this ester-type leaving group is base-stable, making it compatible with a wide range of chemical transformations and facilitating the rapid preparation of unprotected ester-type glycosyl donors. Notably, the glycosyl 8-alkynyl naphthoate donor enables the successful execution of challenging glycosylation reactions, including unprotected (or minimally protected) glycosylation, as well as the versatile synthesis of complex phenylethanoid glycosides (Figure 1.).

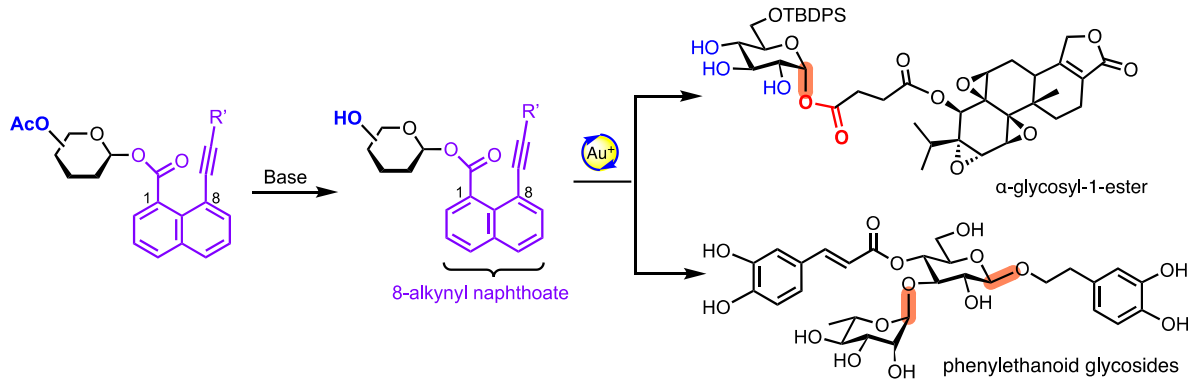

Figure 1. The leaving group of 8-alkynyl naphthoate and its applications.

Acknowledgements: The authors acknowledge the financial support from the National Natural Science Foundation of China.

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ELUCIDATING REACTIVE SUGAR-INTERMEDIATES BY CRYOGENIC INFRARED SPECTROSCOPY

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Carbohydrates are ubiquitous in nature. The stereochemistry (α/β configuration) of glycosidic bonds plays a crucial role in glycan function within biological systems. However, it remains a long-standing challenge to precisely control glycosidic bond formation. Particularly in carbohydrate chemistry, it relies on a centenary reaction: glycosylation. During this reaction, glycosyl cations act as key intermediates. These intermediate structures directly influence the stereochemical outcomes of the glycosylation reaction. Despite their importance, the role of glycosyl cations is still not fully understood. This, in turn, makes it difficult to fully control the stereoselectivity of glycosidic bonds in the synthesis of complex glycans.

Studying the structure of the glycosyl cation is particularly challenging. The glycosyl cation is a cationic S_N1-type intermediate. It has an extremely short lifetime, making it inaccessible to traditional analytical techniques. Here, we report the first high-resolution structure of a glycosyl cation (Figure 1). Technically, this is achieved by isolating the intermediates in the gas phase of a mass spectrometer. Using cryogenic infrared spectroscopy, we probe the vibrational patterns of glycosyl cations. The high-resolution IR spectra enable the reconstruction of their three-dimensional structure using computational calculation.

Gas-phase research provides a snapshot of the S_N1-glycosylation reaction. The intermediate structure in the gas-phase holds great potential to disclose the origins of the stereoselective outcome in this reaction. Besides, it provides evidence for many key concepts in organic synthesis, including neighbouring group effect [1], remote participation [2], and benzylidene- directed glycosylation [3]. Consequently, these discoveries establish a platform for designing building blocks and optimizing reaction conditions.

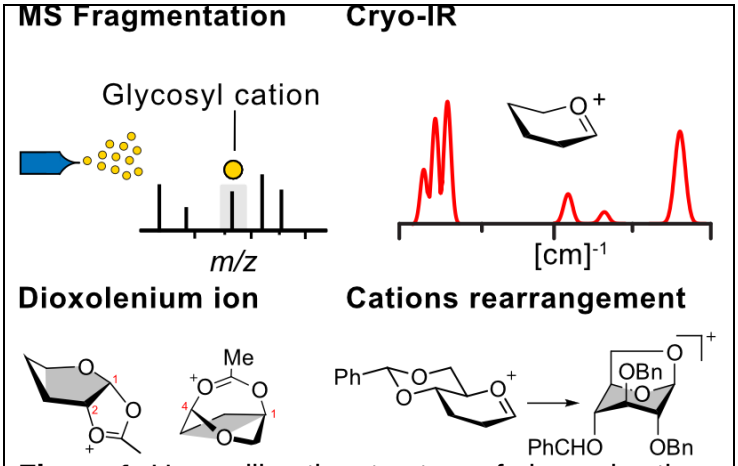

Figure 1. Unravelling the structure of glycosyl cations through gas-phase and synthetic approaches.

Acknowledgements: This work was funded by the European Research Council, ERC-2019-CoG-863934-GlycoSpec and the European Union's Horizon 2020 Researc.

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OPTIMIZING CONJUGATION OF PATHOGEN ASSOCIATED PROTEINS FOR NOVEL GLYCOCONJUGATE VACCINES

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Increasing resistance to antibiotics renders the development of vaccines against the main pathogens involved on this silent pandemic an urgency [1]. Glycoconjugate vaccines are well established tools to prevent bacterial infections and can play a pivotal role in combating antimicrobial resistance (AMR) [2].
The articulated escape mechanisms of some antimicrobial resistant bacteria will require complex vaccine formulations for the prevention of infections from AMR pathogens. Use of proteins as carrier and antigens can support targeting multiple pathogenic mechanisms and simplify the vaccine composition.
Recently, methods for site-selective glycoconjugation have emerged, enabling to control the directionality of the conjugation step and to better preserve protein epitopes. Novel vaccine candidates based on these technologies are advancing at clinical level [2].
Carbohydrate to protein ratio, number of linked glycan moieties and directionality of the conjugation are important interconnected parameters to be considered in vaccine design.
Herein, we conceived a conjugate vaccine to target simultaneously two AMR pathogens by designing a multimeric fusion of two cytotoxins, Hla and PcrV, two potent cytotoxins from S. aureus and P. aeruginosa, respectively. A conjugation strategy based on selective targeting of a histidine tag introduced on purpose, was developed to preserve the structure and antigenicity of epitopes from the two proteins, leveraging their dual role as carrier and antigen. By comparing in animal models multimeric constructs obtained through site selective and random conjugation, we identified a lead candidate with a single attachment site inducing a robust immune response against the protein and the glycan component. These findings can guide the future design of site selective glycoconjugate vaccines.

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GSK Vaccines Institute for Global Health is an affiliate of GlaxoSmithKline Biologicals SA

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MUTAGENESIS AND NON-CANONICAL AMINO ACID INCORPORATION FOR THE PREPARATION OF HOMOGENEOUS GLYCOCONJUGATE VACCINES

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Glycoconjugate vaccines have proven their efficacy and safety in combating bacterial infections, particularly in young children. They are composed of a carbohydrate antigen covalently linked to a protein referred to as a carrier protein [1]. Mutagenesis and non-canonical amino acid incorporation strategies, applied alone or in combination, offer a unique opportunity to control the sites at which the carbohydrate antigen is linked to the carrier. These approaches lead to easily-characterized, homogeneous glycoconjugates (in comparison with ill-defined, randomly synthesized current glycoconjugate vaccine generation). Most importantly, these methodologies prove invaluable to master the shape and the structure-immunogenicity relationships of the conjugates [2,3].

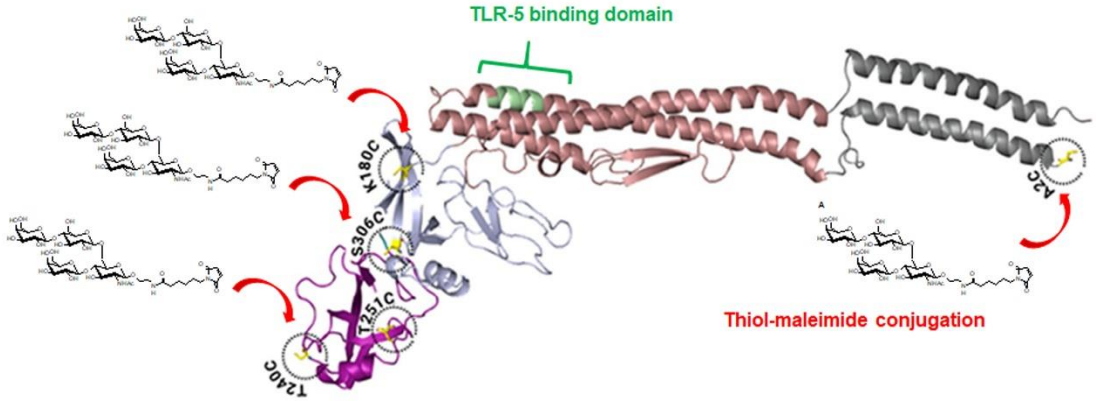

Figure 1. Cysteine mutagenesis is used to orient carbohydrate hapten conjugation and preserve adjuvant properties of flagellin used as a carrier protein [3].

Examples illustrating these aspects will be given based on pneumococcal infection as model disease.

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STRAIN-LEVEL MICROBIOTA VARIATION SHAPES IMMUNE RESPONSES TO GLYCOCONJUGATE VACCINATION

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Glycoconjugate vaccines are widely used to induce protective immune responses, primarily against encapsulated bacteria. However, inter-individual variability in vaccine immunogenicity remains poorly understood. Growing evidence suggests that gut microbiota plays a critical role in shaping host immune responses [1,2], but the contribution of specific microbial taxa and strain-level variation remains underexplored. This study investigates how distinct microbiota compositions influence humoral and cellular responses to glycoconjugate vaccination.
We vaccinated 6-week-old Swiss Webster (SW) (outbred) and C57BL/6 (inbred) specific pathogen–free (SPF), antibiotic-treated, and germ-free (GF) mice with a panel of glycoconjugate antigens two times at a two-week interval using alum as an adjuvant. Serum antigen-specific IgG responses were measured by ELISA, and cellular immune responses were characterized via flow cytometry in draining lymph nodes and spleens. To identify microbial taxa linked to enhanced IgG responses, we performed 16S rRNA sequencing and applied differential abundance analysis (ANCOM). GF mice were selectively colonized with single bacterial strains or complex microbial communities via oral gavage.
Bioinformatics analysis identified microbiota compositional patterns associated with increased IgG responses to vaccination, which was further confirmed by targeted colonization experiments. Notably, strain-level variation within gut microbiota significantly influenced humoral immune responses, with specific strains enhancing IgG production. Additionally, microbiota composition influenced germinal center B cell responses and regulatory/inhibitory immune environments, shaping the overall immune landscape. Colonization experiments in both SW and C57BL/6 mice demonstrated that host genetic background modulates the immunogenic impact of microbiota, with outbred mice displaying greater sensitivity to microbiota-driven effects.
These findings underscore the importance of specific microbial taxa in modulating immune responses to glycoconjugate vaccines and highlight the interplay between host genetics, microbial strain diversity, and carbohydrate immunogenicity. Understanding how microbiota shape antibody responses to carbohydrate antigens could open new avenues for microbiota-targeted vaccine strategies and optimize the efficacy of glycan-based therapeutics.

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MANNOSE-FUNCTIONALIZED NANOPARTICLES AS MRNA VACCINES SELECTIVELY TARGETED TO ANTIGEN-PRESENTING CELLS

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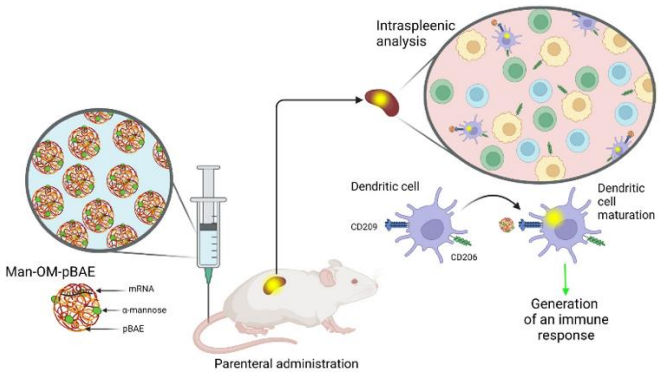
Cancer represents one of the most devastating diseases without an effective treatment, with lung cancer ranking among the most lethal. Immunotherapies, especially therapeutic vaccines that use mRNA as an active compound, stand as the most promising solutions [1]. Poly b-(amino esters) (pBAE) have been demonstrated to efficiently encapsulate mRNA generating small polymeric nanoparticles, the protection and controlled release of the genetic material, their biocompatibility; and their ability to promote cellular internalization and transfection [2,3]. However, their selectivity is not fully controlled due to the lack of an active targeting moiety.
It is of utmost importance to design selective vectors to selectively target them toward antigen presenting cells (APCs), and specifically, to dendritic cells (DCs). Knowing the significance of glycobiology [4], in which the carbohydrates exposed on the nanoparticles interact with the targeted cells surface proteins, this project aims at engineering α-mannose-nanoparticles to enhance cell dendritic targeting presenting lectins like DC-SIGN and MMR.
Three different polymer candidates were synthesized with variable-length mannose functionalization [5,6]. Fine formulation of these polymers with mRNA resulted in nanoparticles decorated with surface-exposed α-mannoses with sizes around 180 nm and positive surface charge (see figure: Man-OM-pBAE). Notably, these particles maintained their properties after freeze-drying and subsequent redispersion. Finally, our mRNA carriers preferentially targeted and transfected APCs in vitro and in vivo. In conclusion [5, 6], we demonstrated, at a preclinical level, that the mannose functionalization enables more selective targeting of APCs and, thus, these polymer and nanoparticles are candidates for a new generation of mRNA immunotherapy vaccines.



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TOWARDS THE SEMI-SYNTHESIS OF PHOSPHORYLATED GLYCOSAMINOGLYCANS

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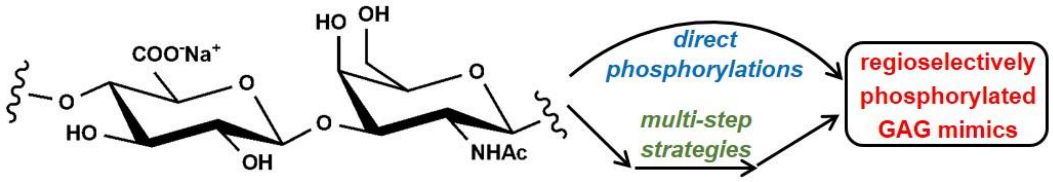
Glycosaminoglycans (GAGs) are highly negatively charged polysaccharides found in both vertebrates and invertebrates. They are typically composed of disaccharide repeating units, that are very often extensively decorated with sulfate groups. GAG sulfation is a dynamic, complex post-translational modification process that seems to be a result of evolution in order to let sulfated GAGs play key roles in many physiological and pathological processes in animals [1].
In the frame of developing artificial GAG mimics [2], an interesting topic concerns phosphorylated GAGs. Indeed, phosphate *vs.* sulfate differences in size, polarity, acid-base and chelation properties could lend unreported activities to phosphorylated GAGs, as indicated by an *in silico* study comparing the structural flexibility and intra- and intermolecular interaction patterns of native GAGs with their phosphorylated counterparts [3]. Actually, this theoretical investigation suggested that phosphorylated GAGs could bind proteins generally with a stronger affinity than their sulfated counterparts and the differences in the binding modes might be highly protein target-dependent. This would propose phosphorylated GAGs as promising, new species to specifically control biochemical processes where the mediating role of sulfated GAGs is crucial. Nonetheless, the preparation of phosphorylated GAGs is still rather underdeveloped [3-5]. In this communication we present the results of a screening of either multi-step strategies or direct phosphorylation methods – relying upon both standard and innovative phosphorylation reactions – applied on unsulfated GAG-like polysaccharides from microbial sources [6].



The final aim is to open a general, semi-synthetic access to phosphorylated GAG mimics, overcoming the several concerns regarding the phosphorylation of polysaccharides, *i.e.* harsh reaction conditions, poor yields and degree of phosphorylation, no regiochemistry control [7].

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GLYCOSAMINOGLYCAN-MIMETIC INFERNAN AS THERMORESPONSIVE POLYSACCHARIDE FOR TISSUE ENGINEERING APPLICATIONS

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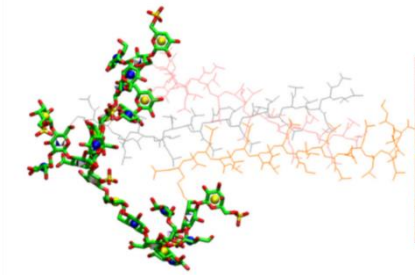
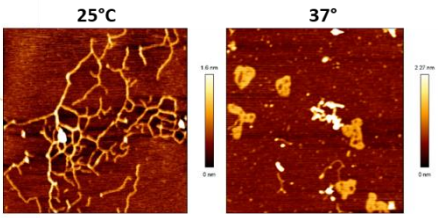
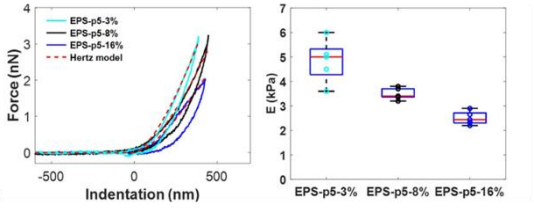
Bacteria have developed a unique strategy to survive in extreme conditions through a synthesis of an extracellular polymeric matrix conferring to the cells a protecting microenvironment. The main structural component of this complex network constitutes exopolysaccharides (EPS), high-molecular weight hydrophilic macromolecules. Deep-sea hydrothermal vent bacteria have been shown to produce EPS rich in hexosamines and uronic acids, frequently substituted by sulfate groups. Such a particular composition ensures interesting functional properties, including biological activities mimicking those known for glycosaminoglycans (GAGs) of mammalian tissues [1]. The aim of the present study was to exploit GAG-mimetic properties of infernan, a high-molecular weight slightly sulfated EPS produced by the deep-sea hydrothermal vent bacterium *Alteromonas infernus* from Ifremer collection, and develop EPS-based hydrogels for cartilage tissue engineering. For this purpose, infernan was grafted with poly(N-isopropylacrylamide) (pNIPAM), thus leading to a thermoresponsive polysaccharide [2]. The molecular characteristics of grafted polysaccharide (conformation, molecular weight, degree of grafting) and its thermosensitive properties were firstly determined at molecular scale using a multi-technique approach. Then, mechanical properties of EPS-pNIPAM hydrogels were assessed in their fully hydrated state by compression experiments using Atomic Force Microscopy. Finally, cytocompatibility of hydrogels was evaluated by incorporating model cells inside. The full characterization of the grafted EPS at both molecular and macromolecular scales remains crucial for its further optimal use as hydrogels in cartilage tissue engineering [3].



MD simulations of infernan partially grafted at GlcA residue in the side chain with three pNIPAM chains.



AFM imaging of infernan chains grafted with pNIPAM, showing the polysaccharide folding due to pNIPAM-pNIPAM interactions at 37°C.



AFM force-indentation curves measured in DMEM at 37°C on three infernan-pNIPAM hydrogels displaying three grafting densities.

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SEMI-SYNTHETIC PATHWAYS TO OBTAIN GLYCOSAMINOGLYCANS MIMETICS FROM SUSTAINABLE SOURCES

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Sulfated glycosaminoglycans (GAGs) are highly complex, anionic, linear polysaccharides extracted from extracellular matrix of animals cells. Some of them are exploited in already approved therapeutic treatments, and a significant number of novel drugs are currently under development [1]. Nonetheless, naturally occurring GAGs exhibit variable chemical compositions and biological activities, which could cause unpredictable results during applications (*e.g.* heparin crisis in 2007). However, sulfated polysaccharides can also be obtained in a semi-synthetic way: the introduction of sulfate groups into the backbones of natural unsulfated polysaccharides allows to endow them with bioactivities similar to sulfated GAGs but without risks derived from their typical animal sources [2]. This work is focused on the development of semi-synthetic strategies for the regioselective modification of polysaccharides to obtain new polysaccharide-based products, which can be proposed as substitutes for GAG-based drugs already existing but obtained from less eco-sustainable sources.
The regioselective derivatizations that are carried out aim at the insertion of negatively charged functionalities (sulfate or phosphate groups, Figure 1), in order to mimic the structural characteristics of natural GAGs. The starting materials are polysaccharides extracted from bacterial or algal sources. In particular, the attention is focused on the development of suitable multi-step sequences all relying upon protection-derivatization-deprotection sequences [3,4].

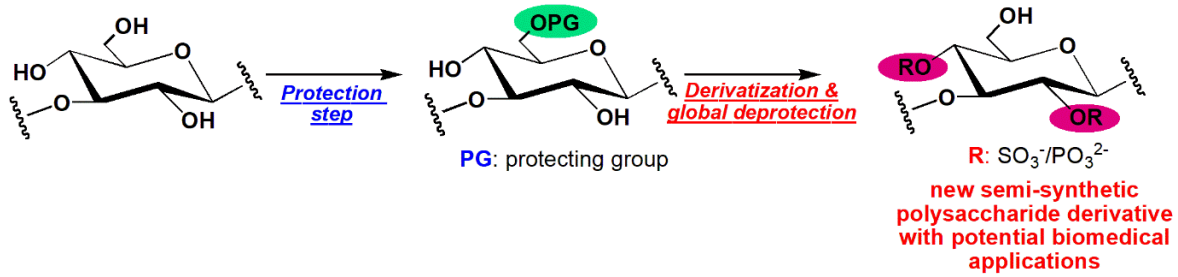

Figure 1

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MIMICKING THE BARRIER: SULFATED GLYCOSAMINOGLYCAN MIMETICS TO PROBE VIRAL INTERACTIONS

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Eukaryotic cells are covered by a dense layer of glycans, commonly referred to as the glycocalyx. While the glycocalyx is involved in manifold vital biological processes, such as cellular communication, protection and signalling, many pathogenic microorganisms, such as viruses, have evolved to exploit distinct components of the glycocalyx in order to infect host cells. Native glycans recognized by viruses are diverse and complex, most often containing terminating sialic acids (Neu5Ac) and glycosaminoglycans (GAGs) such as heparan sulfates (HS), making them hard to access and analyse properly.
Previous work from our lab has shown that globally sulfated glycomacromolecules as mimetics of native heparan sulfates (sGAG mimetics) can act as broadband inhibitors for viral adhesion and infection, rendering this class of synthetic sGAG mimetics a suitable tool to study virus-glycan interactions in a more controlled setting [1]. In our ongoing studies, we employ Solid-Phase Polymer Synthesis (SPPoS) and TIRP (thiol-induced, light-activated controlled radical polymerization) to prepare highly tailorable sGAG mimetics featuring key structural parameters such as site-specific glycosylation, valency, sulfation patterns as well as site-selective functional handles, e.g. lipid moieties for membrane tethering and fluorophores for probing [2,3].
Intercalation of the thusly prepared sGAG mimetics into phospholipid bilayers (e.g. GUVs) then allows us to construct highly defined models of native glycocalyces presenting sGAGs, yielding a versatile platform to study the fundamental mechanisms of viral interactions with sGAG-decorated membrane surfaces regarding e.g. ligand density, valency and spatial distribution.

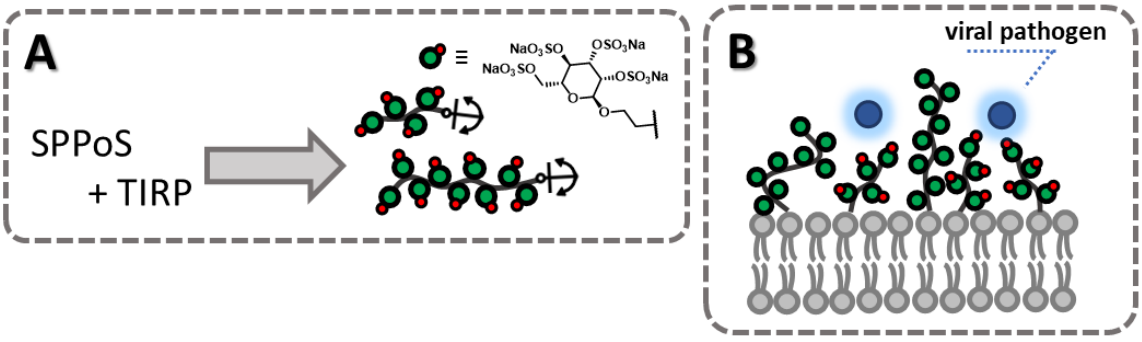

Figure 1.
A: A combination of SPPoS and TIRP yields highly tailorable sGAG mimetics, enabling precise editing of key structural parameters e.g. valency, glycosylation and lipid anchor.
B: Incorporation of sGAG mimetics into phospholipid bilayers enables the construction of glycocalyx models to study viral interactions with sGAGs.

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ILLUMINATING A SOLVENT-DEPENDENT HIERARCHY FOR AROMATIC CH/π COMPLEXES WITH DYNAMIC COVALENT GLYCO-BALANCES

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CH/π interactions are prevalent among aromatic complexes and represent invaluable tools for stabilizing well-defined molecular architectures. Their energy contributions are exceptionally sensitive to various structural and environmental factors, resulting in a context-dependent nature that has led to conflicting findings in the scientific literature. Consequently, a universally accepted hierarchy for aromatic CH/π interactions has remained elusive. Herein, we present a comprehensive experimental investigation of aromatic CH/π complexes, employing a novel approach that involves isotopically-labeled glyco-balances generated in situ (*Figure 1*). This innovative strategy not only allows us to uncover thermodynamic insights but also delves into the often less-accessible domain of kinetic information. Our analyses have yielded more than 180 new free energy values, while considering key factors such as solvent properties, the interaction geometry, and the presence and nature of accompanying counterions. Remarkably, the obtained results challenge the conventional wisdom regarding the stability order of common aromatic complexes. While it was believed that cationic CH/π interactions held the highest strength, followed by polarized CH/π, non-polarized CH/π, and finally anionic CH/π interactions, our study reveals that this hierarchy can be subverted depending on the environment. Indeed, the performance of polarized CH/π interactions can match, or even outcompete that of cationic CH/π interactions making them a more reliable stabilization strategy across the entire spectrum of solvent polarity. Overall, our results provide valuable guidelines for the selection of optimal interacting partners in every chemical environment, allowing the design of tailored aromatic complexes with applications in supramolecular chemistry, organocatalysis and/or material sciences.

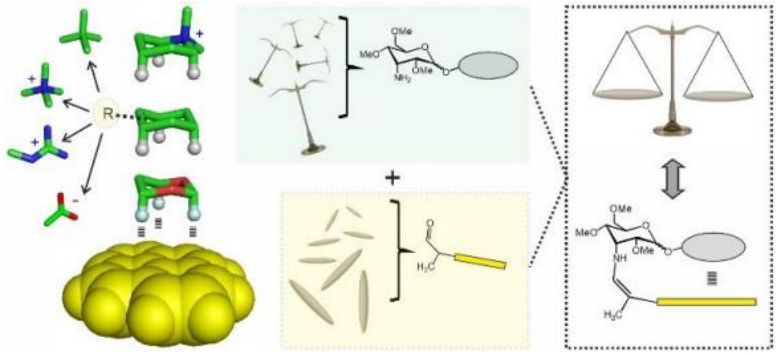

Figure 1. Aromatic X-CH/π complexes analyzed in this study and schematic representation of the in situ generated glyco-balances employed in this work.

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RECENT DEVELOPMENTS AND CHALLENGES IN AUTOMATED GLYCAN ASSEMBLY

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Automated reactors are indispensable tools to meet the growing demand for complex synthetic molecules. Specially biooligomers synthesis benefits from devices performing iterative operations [1-3]. Up to now Automated Glycan Assembly (AGA) has offered access to well defined glycans for custom-made research tools in glycosciences [4-6]. However the initial platforms left room for significant improvements in terms of cost and operability. The instrument sets the limits as to the types of chemistries that can be executed. We developed a the new generation of AGA instruments, smaller, and more economical, allowing quick temperature adjustments between -40 and +90°C based on an energy-efficient local temperature-controlled (LTC) reactor. The power of the new instrument is illustrated in the context of several syntheses of complex oligosaccharides. At the same time, we identify remaining challenges to be address for wide spreading this technology.

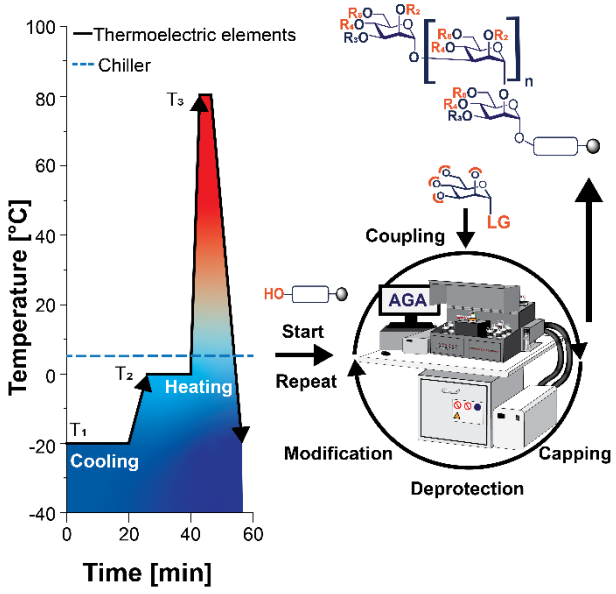

Figure 1. Optimized automated glycan assembly device.

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BREAKING THE REACTIVITY TREND WITH COOPERATIVELY-CATALYZED (4K) REACTION

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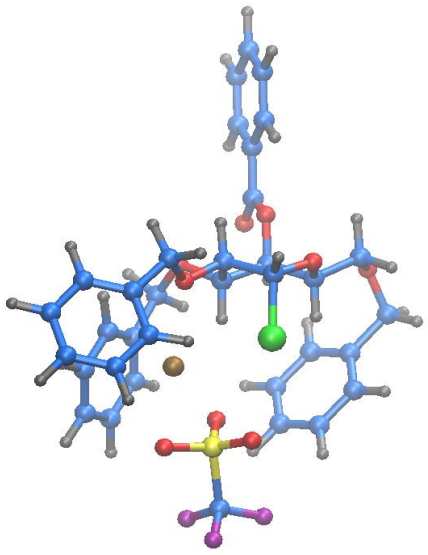
The classical Koenigs-Knorr glycosidation of bromides or chlorides promoted with Ag_2O or Ag_2CO_3 works well only with reactive substrates, ideally both reactive (armed) donor and reactive acceptor [1]. This reaction was found to be practically ineffective with unreactive (disarmed) per-*O*-benzoylated halides and sugar alcohols. Recently, it was discovered that the addition of catalytic (Lewis) acids to a silver salt-promoted reaction has a dramatic effect on the reaction rate and yield [2]. A tentative mechanism for this cooperatively-catalyzed glycosylation reaction has been proposed, and the improved understanding of the reaction led to more efficient protocols and broader applications to a variety of glycosidic linkages [3]. Since Ag_2O-mediated activation was introduced by German chemists Koenigs and Knorr, and "cooperatively catalyzed" is *Kooperativ Katalysiert* in German, we refer to this new reaction as "the 4K reaction"[4].

Remarkably, the reactivity order of differentially substituted glycosyl halides changes dramatically under these cooperatively catalyzed reaction conditions [3]. For instance, we have observed reactivity trends wherein the reaction rates of the expectedly more reactive armed glycosyl halides were surpassed by their expectedly less reactive disarmed counterparts. Described herein is our investigation into the peculiar reactivity trends of the 4K reaction through synthetic, computational, and theoretical reasoning.



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GLYCOCONJUGATE METAL COMPLEXES AS LECTIN-TARGETING ANTI-ADHESIVES AGAINST PATHOGENS AND SENSORS

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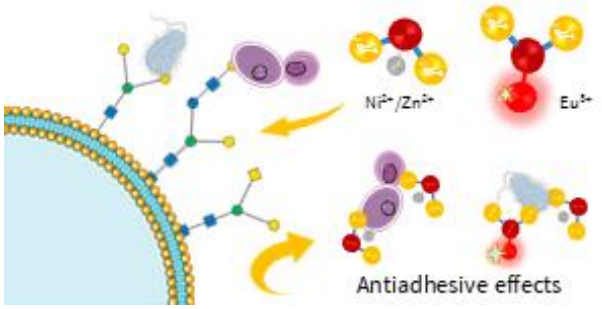
Carbohydrate–lectin interactions are key to the pathology of many bacterial and fungal infections, including by *P. aeruginosa (PA)* [1]. PA is categorised a WHO critical-priority pathogen, due to lack of new treatments and diagnostics, and rising antimicrobial ressistance. Targeting the lectins of *PA* has become an area of increasing interest in glycoconjugate chemistry [2]. While various multivalent glyconconjugate approaches are reported, use of coordination chemistry in designing lectin-targeting compounds is not widely explored. Metal complexes confer glycoconjugates with properties include well-defined coordination geometry, metal-centred charge and redox behaviour and potential additional medicinal effects. Carbohydrate-functionalised coordination-complexes can exploit properties of both carbohydrates and metals to address healthcare challenges.

We synthesised Ru^{2+}-centred glycoclusters, whose ability to inhibit *PA* biofilm formation depended on the identity and presentation of the carbohydrate motif [3a]. We demonstrated galactoside ligands can bind *PA*'s LecA lectin with micromolar affinity and both its Tb^{3+} complex and a boronic acid analogues can act as sensors for galactophilic lectins [3b]. Several examples of glycoconjugate complexes with *d*- and *f*-metal ions also inhibit fungal adhesion by *C. albicans* to human buccal epithelial cells, or demonstrate *in vitro* anti-biofilm activity against *PA*, further demonstrating therapeutic promise of these systems [3c]. Ligand strcuture modifies biological activity, but moreover the identity of metal ion and stroichiometry of the complex modulate biological activity not seen for ligands alone, even though activity is carbohydrate-mediated. This presents an impactful role for coordination chemistry in such targeted antimicrobial systems.



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HISTIDINE-RICH GLYCOPROTEIN SIALYLATION AFFECTS ITS INHIBITION OF FXII AUTOACTIVATION

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Histidine-rich glycoprotein (HRG) is a multifunctional plasma protein modulating many biological processes, including coagulation and pathogen clearance. HRG is highly polymorphic, with five variations having minor allele frequencies (MAF) exceeding 10%, varying across populations. Additionally, HRG has 3 *N*-glycosylation sites (Asn63, Asn125, Asn344) and the Pro204Ser substitution introduces a potential *N*-glycosite at Asn202.
The autoactivation of Factor XII (FXII) plays a crucial role in initiating the contact pathway of coagulation. Recent studies have indicated that HRG might inhibit FXII autoactivation into FXIIa, which is the first step of the contact pathway of coagulation. Given the critical roles and connections of HRG and FXII in coagulation, we hypothesized that changes in sialylation and specific variations of HRG could affect FXII autoactivation.
We used LC-MS/MS-based (glyco)proteomics with HCD and stepped HCD fragmentation to characterize samples after IMAC and AEX chromatography purification. FXII autoactivation was measured using a S-2302 chromogenic assay, with OD at 405nm recorded continuously. MS results revealed that HRG was not the dominant protein in any fraction, as indicated by intensity analysis using Byonic software after purification. In each fraction, alpha-2-macroglobulin, albumin, hemopexin and other proteins were identified as the most abundant proteins. However, to the best of our knowledge, after consideration of the available literature, none of these proteins are expected to interfere with FXII autoactivation. We compared the relative abundance and profiling of HRG among all these fractions to select the fraction with the highest relative purity of HRG.
A kinetic FXII conversion bioassay showed that, the inhibition of autoactivation increased with higher HRG concentrations. Independent *t*-tests were performed to determine whether IC_{50} values from the samples of HRG with sialic acids differed significantly(p=0.0004).
We reveal that sialylation is an important component of HRG's role in inhibiting FXII autoactivation.

PHOSPHORYLATED BACTERIAL GLYCOLIPIDS AND MIMETICS WITH A ROLE IN INNATE IMMUNITY

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Innate immunity-related factors play an important role in the development of various malignancies, and several components are currently being investigated as potential therapeutic targets or adjuvants. 100 years ago, Dr. William B. Coley achieved remarkable success in the treatment of advanced solid cancers by using mixtures of killed bacteria to induce local and systemic acute inflammation [1, 2]. It is now understood that the anti-tumour effects of the 'Coley vaccine' were related to the activation of pattern recognition receptor (PRR)-mediated innate immune responses, including NF-κB-mediated cytokine release and NLRP3 inflammasome activation, triggered by bacterial pathogen-associated molecular patterns (PAMPs). In recent years, considerable efforts have been made to revive bacterial-mediated cancer therapy [3,4]. However, identifying a strain that achieves the optimal balance between effective immune stimulation and safety remains a challenge, as overly attenuated bacterial strains may not sufficiently activate the immune system—an essential factor for tumor regression and healing, according to W. Coley. Preclinical development of LPS-based cancer therapies has also been hampered by adverse effects or lack of clinical efficacy due to LPS microheterogeneity and detoxification [5].
Identifying the structural factors characteristic of certain bacterial PAMPs that contribute to antitumor effects could open new therapeutic avenues for cancer adjuvant treatment. Synthetic bacterial glycolipids and glycan fragments are perfect candidates for studying the involvement of bacteria-sensing PRRs in cancer biology [6,7]. The library of synthetic phosphorylated bacterial glycans, glycolipids, and their mimetics was assessed for interaction with several PRRs in vitro with particular emphasis on structural aspects and immunomodulatory effects, while selected molecules showed therapeutic potential in targeting experimental tumours. The structural basis for ligand-protein interactions of several glycolipids with picomolar activity was studied using Cryo-EM. Providing in-depth insight into the recognition of structurally different glycolipids by innate immune receptors, including species-specific sensing and correlation with biological activity, paves the way for the design and development of novel adjuvants and immunotherapeutics.

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SECONDARY ALKOXYAMINE: ADVANTAGES AND CAVEATS IN THE PREPARATION OF GLYCOCONJUGATES

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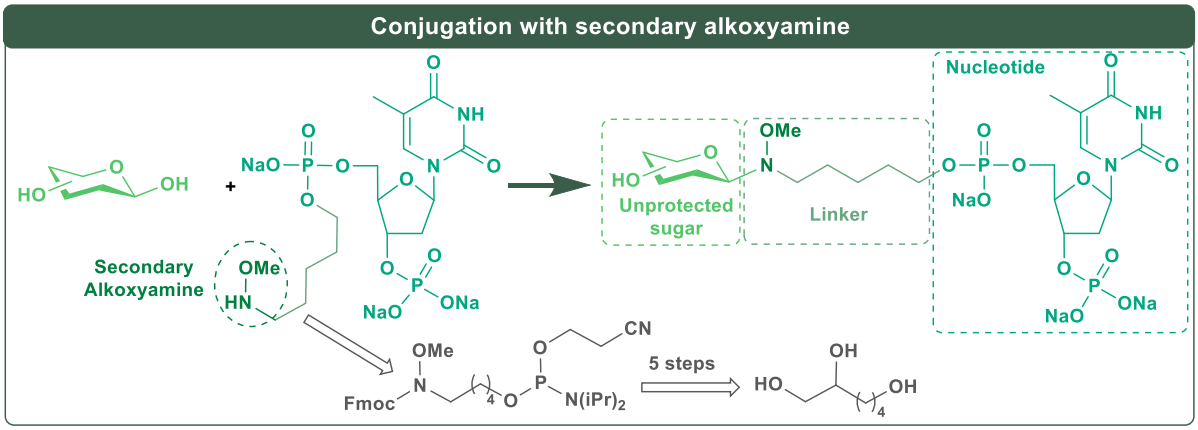
Due to their inherent functional diversity, unprotected carbohydrates offer an interesting challenge for conjugation reactions. In this regard, the reducing-end hemiacetal allows relatively straightforward chemoselective reactions, such as reductive amination or hydrazides and oximes formation [1]. However, these methods give acyclic products or a mixture of open and closed forms, leading at best to a loss of conformational rigidity and at worst to intractable mixtures.
In 1998, Peri, Dumy and Mutter introduced secondary alkoxyamine nucleophiles as a new method to prepare neoglycoconjugates. Advantageously, this reaction leads to the sole formation of cyclic conjugates with pyrano/furano and high anomeric selectivity [2]. In this communication, we will disclose our results on the coupling of different reducing carbohydrates with an alkoxyamine-functionalized nucleotide. They illustrate the main advantages of this method but also shed light on its major disadvantages.
Part of the presentation will focus on the preparation and introduction of the alkoxyamine linker onto the nucleotide, then on the optimisation of the conditions on a model, using an experimental design and how these conditions hold when applied to a variety of sugars.



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ADVANCING THE ISOMERIC SEPARATION OF GLYCAN AND GLYCOPEPTIDE ISOMERS BY LC-MS/MSTITLE IN CAPITAL LETTERS

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Mesoporous graphitized carbon (MGC) has emerged as a versatile and robust stationary phase, enabling high-resolution separations of structurally diverse glycan and glycopeptide mixtures—a challenge central to glycochemistry and glycobiology. This work analyzed permethylated glycans derived from model glycoproteins (e.g., bovine fetuin, RNase B) and biological specimens (human blood serum and cancer cell lines) using a custom-packed, 1 cm MGC nano-column. By fine-tuning mobile-phase composition—combining isopropanol and acetonitrile—and operating at elevated temperature (75 °C), highly branched and sialylated N-glycan isomers were baseline-resolved, underscoring the utility of MGC in addressing critical chemical and structural questions in carbohydrate research.

In a complementary approach, the same MGC platform was used to separate N- and O-glycopeptides from bovine fetuin, asialofetuin, alpha-1 acid glycoprotein, and human serum, effectively highlighting the interplay between glycan linkage microheterogeneity and protein context. Subtly different glycoforms—such as α2,3- versus α2,6-linked sialic acids—were resolved and reliably detected in a single run. Moreover, O-glycopeptide isomers were separated with minimal carryover, reflecting both the strong chromatographic performance and the method's adaptability to a range of glycoproteins. Notably, robust reproducibility across three months of continuous use reaffirms the potential of MGC-based separations for collaborative research efforts that bring together multiple facets of glycan-focused studies and applied technologies.

These findings emphasize MGC's effectiveness in supporting high-throughput glycomic and glycoproteomic investigations. By integrating advanced chromatographic selectivity, reliable quantitation, and detailed structural analysis, MGC columns can drive innovation in disease biomarker identification, therapeutic development, and fundamental glycoscience research.

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COMPREHENSIVE CHARACTERIZATION OF CELL-SURFACE GLYCOPROTEINS AND THEIR DYNAMICS USING MASS SPECTROMETRY-BASED PROTEOMICS

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The cell surface contains many important proteins, such as receptors and transporters, and almost all of them are glycosylated. These surface glycoproteins regulate nearly every extracellular activity, including cell-cell communication and cell immune response. Systematic characterization of surface glycoproteins and their dynamics will advance our understanding of glycoprotein functions, cellular activities, and disease mechanisms. In our lab, we have worked on developing novel and effective methods to globally analyze surface glycoproteins. Integrating metabolic labeling and bioorthogonal chemistry, we can selectively separate and enrich glycoproteins only from the cell surface, enabling us to comprehensively and site-specifically analyze surface glycoproteins [1-2]. The method was applied to systematically analyze surface glycoproteins in different types of human cells. The results revealed that besides cell-specific glycoproteins, the uniqueness of each cell type further arises from differential expression of surface glycoproteins [3].
One big advantage of this method is that the experimental conditions are very mild, which allows for studying the dynamics of surface glycoproteins. In combination with multiplex proteomics, we systematically quantified the degradation of surface glycoproteins in human cells and the dynamics of surface glycoproteins during cell immune response [4-5]. For the dynamics of surface proteins in monocytes and macrophages with the infection, it was found that the surface glycoproteomes were remodeled in cells during the bacterial infection, including the expression of new glycoproteins to the surface and the removal/internalization of existing surface glycoproteins [5]. A comparison of the immune responses between monocytes and macrophages showed the similarities and differences between their surface glycoproteomes, and the priming of monocytes for the response during the differentiation process. Besides reported markers, the results revealed dramatic changes of other surface glycoproteins that have never been reported to play a role in the immune system. Furthermore, we systematically investigated the trafficking of glycoproteins on the cell surface [6]. The results demonstrated that protein folding, N-glycosylation, and N-glycan maturity have distinct impacts on the trafficking of surface glycoproteins. Considering the importance of cell-surface glycoproteins, systematic and quantitative analysis of these glycoproteins will allow us to discover novel biomarkers for disease detection and identify drug targets for disease treatment.

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COMPREHENSIVE CHARACTERIZATION OF ANTIBODY QUANTITIES, ISOTYPES, SUBCLASS AND GLYCOSYALTION

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Antibodies are a key element of adaptive immunity, critical to its role in protection against pathogens. As glycoproteins, antibodies are a prime example of the diversity of attributes that are regulated by glycosylation, such as effector functions, immune tolerance, structural integrity and half-life. Antibody glycosylation is also a critical element in the efficacy and safety of various therapies, including vaccination. Antibody responses have multiple structural and functional layers interacting in a complex way to achieve an efficient, but appropriate immune response. Levels, class, subclass, and glycosylation of antibodies are structural features that interact extensively to determine antibody effector functions, such as cellular cytotoxicity, phagocytosis, and complement activation.
Over more than a decade, we have developed and refined an LC-MS platform for subclass-specific glycosylation analysis of antigen-specific antibodies which we recently termed GlycoLISA [1]. It combines the efficiency and wide availability of ELISA-type immunosorbent assays with the superior structural resolution of LC-MS. We will showcase the latest continuous development of GlycoLISA for clinical and (bio)pharmaceutical bioanalysis. We can now simultaneously analyze IgG, IgA and IgM glycosylation in a protein- and site-specific manner. Through the implementation of stable isotope labeled protein standards, quantity, subclass, and glycosylation of an immunoglobulin G (IgG) response can now be determined in a single measurement [2]. This allowed us to comprehensively compare vaccination responses, for example between healthy controls and patients with inborn errors of immunity in the context SARS-CoV-2.
In conclusion, a comprehensive coverage of levels, class, subclass, and glycosylation has great potential to further our understanding of dynamics, regulation, and impact of antibody-mediated immune responses. This knowledge can in turn guide therapeutic interventions and help to assess their efficacy.

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INFRARED SPECTROSCOPY IN A MASS SPECTROMETER – MOLECULAR FINGERPRINTS FOR GLYCOMICS

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Tandem mass spectrometry is currently the gold standard in biomolecular analysis. The combination of robust and sensitive ionization techniques such as electrospray ionization with efficient fragmentation techniques and subsequent detection with high mass resolution enables the rapid identification of hundreds of proteins within hours. Likewise, highly complex maps of lipids and small molecule metabolites can be identified reliably from complex biological samples. However, differentiating isomeric species remains challenging through conventional mass spectrometry. In metabolomics, multiple structural candidates often exist for a given *m/z*, complicating precise identification. Similarly, in glycomics, isomeric glycan structures differing only in regio- or stereochemistry of a single glycosidic bond often coexist, which represents a significant analytical challenge. Recently, advances in commercially available ion mobility–mass spectrometers, gas-phase ion spectroscopy, and computational chemistry have opened new avenues to solve the isomer problem in mass spectrometry [1]. Here we illustrate examples how isomeric molecules can be unambiguously identified using a novel combination of ion mobility mass spectrometry and cryogenic gas-phase spectroscopy. Particular focus will be put on challenging glycoconjugates such as mucin-type *O*-glycans and glycosaminoglycans.

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CATALYTIC GLYCOSYLATIONS USING GLYCOSYL FLUORIDE BY BORATE COMPLEX

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Glycosyl fluorides offer effective approaches for glycan synthesis because they are easy to handle and provide access to diverse glycosidic linkages. Various methods for the activation of glycosyl fluorides have been developed. However, catalytic activation of glycosyl fluorides remains a challenging, as highly fluorophilic reagents that activate stable C-F bonds are generally deactivated upon fluoride attachment. In particular, low-reactive disarmed glycosyl fluorides, protected with electron-withdrawing groups (e.g., acyl), have not been catalytically activated without trapping in-situ-generated HF.

Herein, we present $BF_3{\cdot}Et_2O$-catalyzed glycosylation of glycosyl fluorides with alcohol acceptors (unsilylated nucleophiles) using 1 mol% $BF_3{\cdot}Et_2O$ under nitrogen-filled glovebox conditions, without any HF-trapping agents.[1] The present conditions smoothly activate both armed and disarmed glycosyl fluorides, thereby affording the desired glycoside products in high yields. Notably, this reaction exhibits reaction vessel dependency, proceeding catalytically only in a glass flask. The formation of SiF_4 has been confirmed, suggesting that a mixed Lewis acid species of BF_3 and SiF_4 serves as the active catalytic species.

However, glycosylation exhibits significant variations in reactivity depending on the structure of substrates, making it difficult to develop a universal glycosylation method applicable to a wide range of substrates. To address this issue, we harnessed the tunable Lewis acidity of cage-shaped borates to develop practical glycosylation methods tailored to the reactivities of individual substrates. After optimizing the reaction conditions for the efficient catalytic (1–10 mol%) activation of glycosyl fluorides using cage-shaped borates, we systematically explored a range of glycosylation reactions employing various borates. As anticipated, each borate exhibited distinctive reactivity; thus, systematic screening of borate catalysts enabled the efficient activation of glycosyl fluorides under mild conditions. Glycosylation was successfully achieved by fine-tuning the Lewis acidity to match the substrate reactivity. This glycosylation methodology addresses the fundamental challenges of glycosylation and the highly structure-dependent nature of reactivity, thereby paving the way for efficient glycan synthesis.

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UNEXPECTED BINDING OF FLUORINATED DISACCHARIDES TO WGA

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Fluorinated carbohydrates exhibit improved metabolic stability and higher lipophilicity than natural glycans. However, deoxyfluorination of glycans often results in reduced or abrogated binding affinity to their cognate lectins due to the removal of hydrogen bonds with the binding site. We synthesized the complete series of methyl β-glycosides of *N*,*N*′-diacetylchitobiose (GlcNAcβ1–4GlcNAcβ1-OMe) and LacdiNAc (GalNAcβ1–4GlcNAcβ1-OMe) systematically monofluorinated at all hydroxyl positions and determined the binding affinity of the fluoroanalogues to wheat germ agglutinin (WGA), a plant defense lectin. For the first time, the binding profile of a previously unexplored WGA ligand LacdiNAc was investigated. Unexpectedly, fluorination at the 4-position of the GalNAc moiety in LacdiNAc resulted in a stronger binder than the unmodified LacdiNAc. Even more surprisingly, fluorination at the 6-position of the non-reducing end GlcNAc moiety in chitobiose increased binding to WGA by an order of magnitude (Figure 1). These results demonstrate that systematic deoxyfluorination of carbohydrate lectin ligands can identify analogues that exceed the binding affinity of unmodified parent oligosaccharides [1].

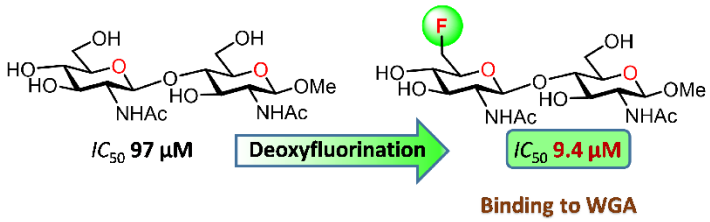

Figure 1. Deoxyfluorination of chitobiose increased binding to WGA

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DEOXYFLUORINATED SYNTHESIS OF GLYCOSYL FLUORIDES

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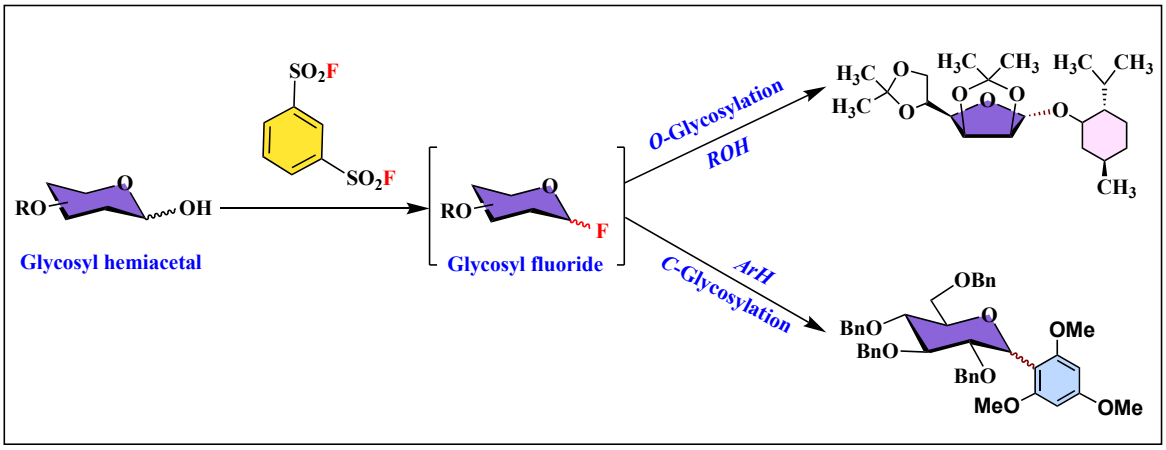
Glycosyl halides are classical glycosylation donors widely employed by carbohydrate chemists. Among these, glycosyl fluorides are particularly notable as versatile donors due to their exceptional thermal and moisture stability. The discovery of glycosyl fluoride-based glycosylation has driven significant advancements in glycoscience, allowing the investigation of the molecular roles of glycans in health and disease. However, the synthesis of fluoro-glycosides under mild conditions remains challenging and is in high demand. We have developed benzene-1,5-disulfonyl fluoride as a deoxyfluorinating reagent to synthesize glycosyl fluorides from glycosyl hemiacetals under mild conditions. This approach enables the efficient synthesis of various fluoro-glycosides from glycosyl hemiacetals, including disaccharides and 2-deoxy sugar derivatives. This method is compatible with various functional groups and provides a simple and effective route to produce a straightforward synthesis of both *O*-glycosides and *C*-glycosides.



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SYNTHESIS OF MANNOSIDES AS INHIBITORS OF PIMs BIOSYNTHESIS

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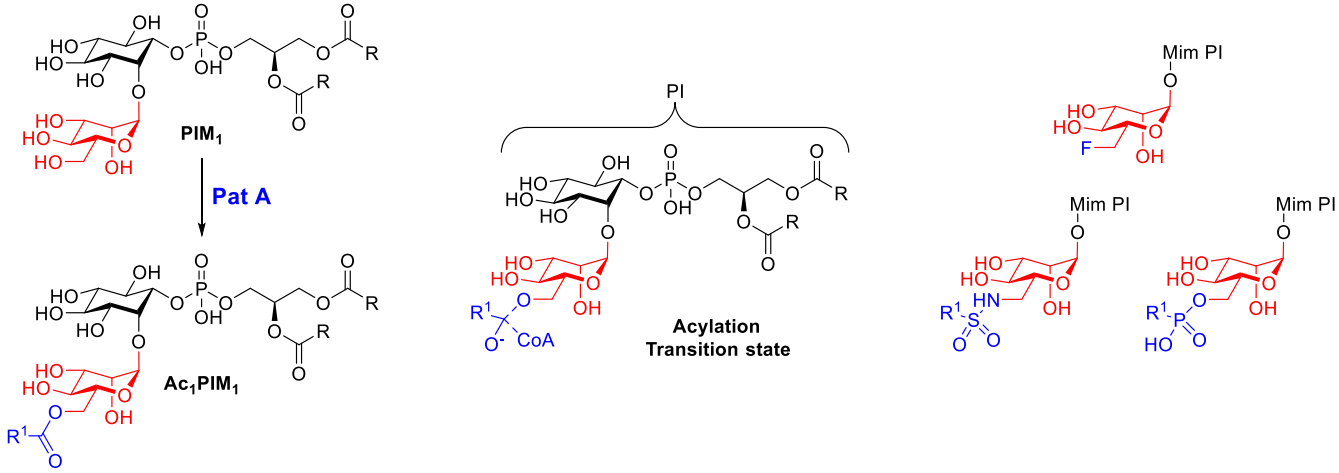
Mycobacterium tuberculosis, the causative pathogen of tuberculosis (TB), is the second most deadly infectious agent in the world, affecting several million people and causing around one million deaths every year. In addition, the World Health Organization is warning of the emergence and proliferation of multidrug-resistant strains, on which current treatments have little or no effect. It is therefore crucial to find new therapeutic targets and develop new treatments. Current anti-TB drugs are targeting diverse biological processes [1]. But no molecules are designed to target PIMs biosynthesis. PIMs (Phosphatidyl-*myo*-Inositol Mannosides) are essential components of mycobacterial cell wall and the precursors of two major lipoglycans implicated in host-pathogen interactions. According to the currently accepted model, the biosynthesis starts with the transfer of mannopyranosyl residues to the inositol ring of PI leading to PIM_1 and PIM_2 [2]. Then, the membrane-associated acyltransferase PatA catalyzes the transfer of a palmitoyl moiety from palmitoyl coenzyme A to the 6-position of the first transferred mannose in PIM_1/PIM_2 to give Ac_1PIM_1/Ac_1PIM_2.This enzyme was recently found to be essential for mycobacteria growth *in vitro* and *in vivo* making it a novel therapeutic target for drug discovery [3].



Therefore, we currently focus on the synthesis of a panel of molecules with mannopyranosyl scaffold with the aim to develop PatA inhibitors. Structures present different aglycones to mimic the PI part and different groups at the 6-position of mannose as a fluorine or a group mimicking the acylation tetrahedral transition state. The synthesized molecules will be tested on PatA as well as on *Mycobacterium tuberculosis*.

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SYNTHESIS OF BIO-SOURCED ANTIOXIDANTS BY PHOTOINDUCED THIOL-ENE COUPLING UNDER CONTINUOUS-FLOW CONDITIONS

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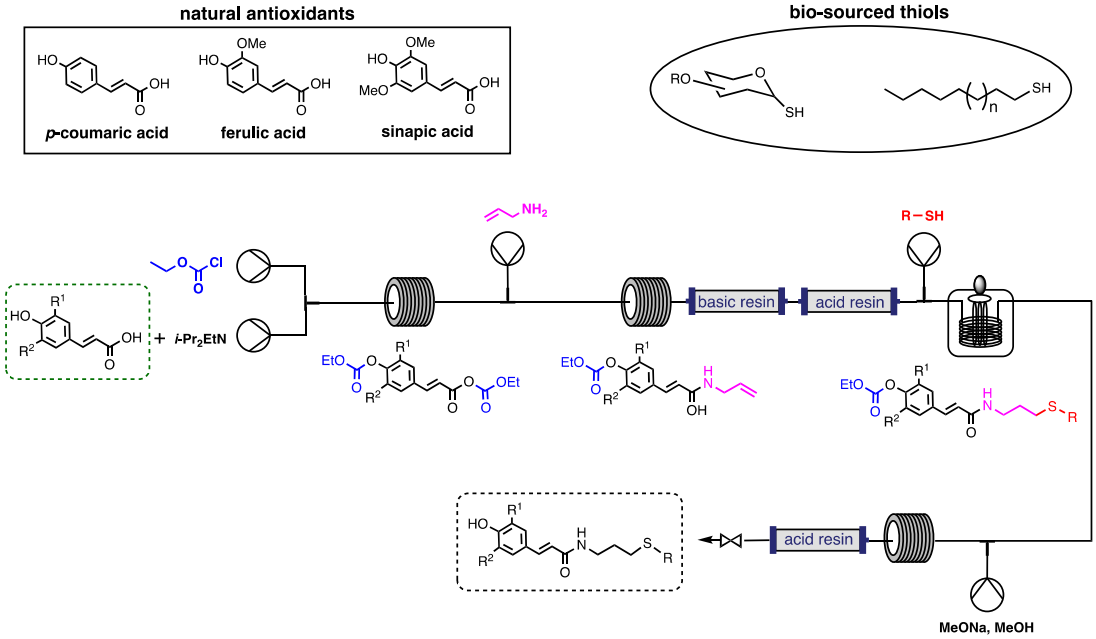
The use of antioxidants as additives to maintain quality and durability of products is indispensable in a considerable range of industries (from fuels to food), thus, there is a true potential for the development of processes for the large-scale synthesis of new antioxidants from bio-sourced molecules. Technical lignins are by-products recovered from the industrial fractionation of lignocellulosic biomass, either from the paper industry or from biorefinery processes developed to produce bioethanol. These by-products contain phenolic compounds, which can be extracted by organic solvents, mainly constituted of *p*-coumaric, ferulic, and sinapic acids, well-known antioxidants. The tuning of the polarity of these acids can be achieved by introducing polar or apolar residues at their carboxylic group to preserve the functions involved in the antioxidant activity, i.e. the phenolic OH and the conjugated double bond.

The reaction of the acid with 2 equiv. of ethyl chloroformate gave the mixed anhydride, *O*-protected as ethyl carbonate, that was coupled with allyl amine to afford the allyl amide. The totally chemo- and regio-selective photoinduced (UV-A, 365 nm) radical addition of a thiol to the terminal alkene (Thiol-Ene Coupling, TEC), in the presence of a photoinitiator, produced the stable sulfide that was then *O*-deprotected by transesterification (see Figure). Bio-sourced thiols, either hydrophilic (anomeric sugar thiols) or hydrophobic (aliphatic thiols derived from fatty acids) were employed in the thiol-ene coupling. The new antioxidants may be exploited for skin care applications (anti-aging formulations).



SOLVENT-FREE SYNTHESIS OF GLYCOSIDIC, ESTER AND PEPTIDE BONDS: STREAMLINED ACCESS TO VALUABLE GLYCOCONJUGATES

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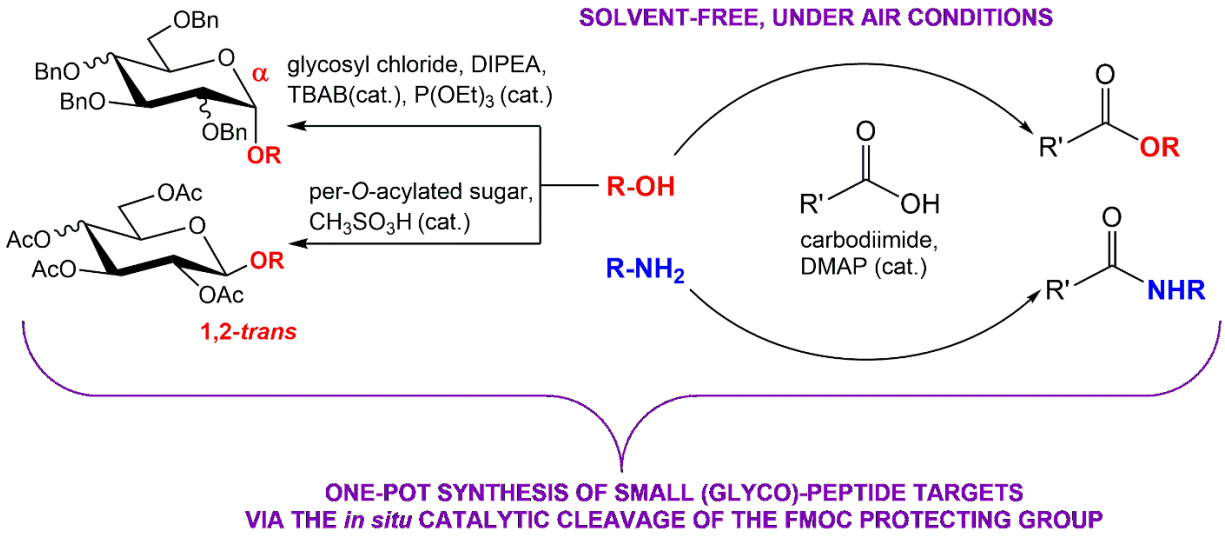
The need for more sustainable methods in organic synthesis calls for the ongoing search of novel approaches relying on greener and safer alternatives to classic organic solvents. In this frame, solvent-free protocols featuring enhanced reaction efficiency and a low economic/environmental impact, have also been addressed to several organic transformations [1], but their target scope is often limited by their hard applicability to complex compounds. Our research has been recently aimed at revisiting the chemistry of carbohydrates, highly functionalized and abundant natural compounds, through the development of streamlined solvent-free synthetic methodologies [2]; these turned out to be more practical and convenient alternatives to routinely adopted protocols for a large number of glyco-synthetic transformations, which spurred the extension of this research-line to further organic compounds. Herein is presented the application of original solvent-free, under air procedures to the synthesis of key structural motifs embedded in natural organic products, namely the glycosidic, ester and amide bonds. Two solvent-free glycosylation methods, respectively addressing the construction of α and 1,2-*trans* glycosides under air, are firstly discussed; on the other hand, it is presented the first solvent-free revisiting of the carbodiimide coupling chemistry [3] and its versatile targeting to both ester and amide conjugation of non-trivial precursors, including the synthesis of small model peptides. Lastly, it is also shown the first catalytic, solvent-free protocol for the cleavage of the widely popular Fmoc protecting group [4], and the overall merging of these approaches to the one-pot synthesis of carbohydrate and glyco-peptide targets.



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COMPARISON OF THE PHYSICAL AND CHEMICAL FEATURES OF COMPOSITES PRODUCED FROM TEXTILE WASTE AND CELLULOSES PLANTS

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One of the most concerning unresolved problems is the massive amount of textile waste that is not recycled. Our current studies have shown that reinforced textile recycling materials by plant cellulose fibers are suitable for the production of functional composites. We try to find out a plant polysaccharide fibers that will maintain the functionality of composites while being ecologically friendly at the same time. We tested following fibers plants: sisal, cotton, linen, coconut, wood, abaca, jute, and hemp,. Both fiber plants and textiles were grinding, the plant materials to fragments with lengths ranging from 2 to 15 mm. Plant-textile composites were produced in extruder at temperatures up to 195℃, it allowed plasticizing polymers of textiles .Fiber plants were added to textile recycling material from 3 to 6%. Total 245 different plant-textile composite samples were tested for their mechanical properties: tensile strength, bending, and compression. Fiber glass additive exhibited significantly higher stiffness (Et) than all tested natural fibers, as confirmed by ANOVA results ($p < 0.0001$ modulus values compared to fibers glass, as demonstrated by the Tukey HSD results). Fibers glass samples demonstrated significantly higher **flexural modulus (Ef)** ($F = 13.99$, $p < 0.0001$) and **maximum flexural stress (σfм)** ($F = 8.68$, $p < 0.0001$) values compared to natural, cellulose fibers, confirming their superior stiffness and strength. However, natural fibers like 3% **abaca** and 3% **sisal** showed comparative values. With regard to **strain at break (εfb)**, the performance of natural fibers such as **abaca, hemp**, and **sisal** was superior to that of fiberglass ($F = 5.60$, $p < 0.0001$. Although **breaking stress (σfb)** ($F = 2.53$, $p = 0.0033$) values were higher for fiberglass. FTIR spectra of all cellulose fibers displayed the characteristic cellulose bands, but with notable differences in intensity and distribution. The O-H stretching band (3600–3200 cm^{-1}), which is indicative of hydrogen bonding within cellulose, varied significantly between materials. The C-H stretching band (~2900 cm^{-1}), which is associated with aliphatic chains in cellulose and hemicellulose, was present across all fibers but was particularly strong in cotton and flax, suggesting these fibers have a high degree of purity and structural uniformity in their polysaccharide matrix. Clear differences were detected in lignin content between fibers, as shown by the C=O stretching band (1740–1600 cm^{-1}), which was particularly intense in coniferous fibers, In contrast, cotton and flax showed weaker signals in this region, indicating lower lignin content and a higher proportion of pure cellulose. The C=C stretching band (~1600–1500 cm^{-1}), indicative of aromatic groups in lignin, was similarly more pronounced in coniferous fibers, further confirming their higher lignin content. The SEM microphotographs surface of the sisal-reinforced composite was relatively smooth and even, with a regular structure, while the fiberglass composite had a rough and crinkled surface texture. In conclusion textile – celluloses fibers plants composites, offer a new, eco-friendly textile recycling method.

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SITE-SELECTIVE PHOTOBROMINATION OF CARBOHYDRATES

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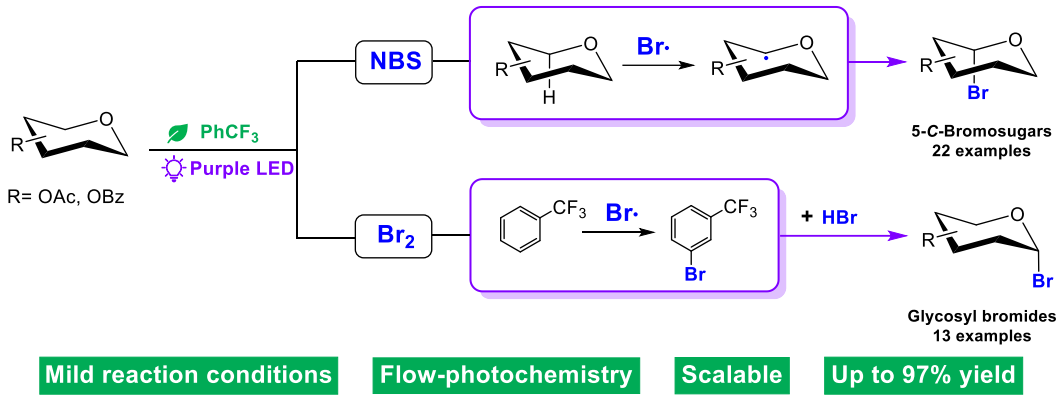
The Ferrier photobromination provides direct synthetic access to valuable 5-*C*-bromosugars [1]. However, its broader application is constrained by the use of energy-inefficient heat lamps for irradiation and the reliance on highly toxic (and banned) CCl_4 under reflux conditions. Herein, we demonstrate that the reaction proceeds rapidly and efficiently under mild conditions (≤40 °C) using benzotrifluoride ($PhCF_3$) as a safe and environmentally benign alternative to CCl_4, with irradiation by a compact photoreactor fitted with purple LEDs (405 nm), and NBS serving as the bromine source.



Furthermore, the introduction of 2.5 mol% bromine significantly enhances substrate conversion rates and reaction efficiency [2]. However, excessive bromine results in the formation of the glycosyl bromide, which gradually becomes the dominant product and eventually the sole product as bromine equivalents increase. We have thus optimized this latter pathway for the efficient photochemical synthesis of glycosyl bromides. The proposed mechanism is attributed to the *in situ* generation of HBr from bromine and $PhCF_3$ under light irradiation [3], followed by substrate bromination to form glycosyl bromide in accordance with the classical bromination pathway. Introducing a base neutralizes the HBr, resulting in only trace amounts of 5-*C*-bromosugar being formed via the radical pathway.
This strategy demonstrates excellent substrate generality for the synthesis of both 5-*C*-bromo sugars and glycosyl bromides, depending on the conditions. Notably, this strategy is compatible with continuous production via the use of a continuous-flow photoreactor.

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ADVANCING MOLECULAR DYNAMICS SIMULATIONS OF GLYCANS THROUGH CHARGE SCALING

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Electrostatic interactions are fundamental to biomolecular processes, particularly in glycan-mediated recognition and binding. However, traditional nonpolarizable molecular dynamics (MD) force fields struggle to accurately model these interactions, often leading to overbinding and unrealistic ion pairing. We designed the prosECCo75 force field to overcome this limitation, a charge-scaled variant of the widely used CHARMM36 force field [1]. Our newly developed force field incorporates the missing electronic polarization in a mean-field manner, refining the description of charged and polar biomolecules such as glycans, lipids, and proteins without additional computational cost.

Glycosaminoglycans (GAGs), highly charged polysaccharide polymers forming the extracellular matrix, interact with ions and other biomolecules such as proteins primarily through electrostatics. Due to their numerous carboxyl and sulfate/sulfamate groups, the accuracy of MD simulations in describing these electrostatic interactions is critical for understanding their biological roles. Using charge-scaled models, we demonstrate substantial improvements in simulating glycan-ion [1-2] and glycan-peptide interactions [3], revealing, for example, that solvent-shared ion pairing is the dominant binding mode of calcium to sulfated GAGs [2]. Our approach outperforms conventional force fields, mitigating overestimated contact ion pairing and improving agreement with experimental data.

Extensive validation across diverse biomolecular systems highlights the broader applicability of charge scaling in improving force field accuracy. By refining partial charges without significantly modifying molecular topologies, our method aligns better with experimental observations while maintaining the same computational efficiency. These advancements enhance the reliability of large-scale biomolecular simulations involving glycans, enabling more precise insights into glycan-mediated interactions.

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SELECTIVE BINDING OF IL-1α TO ISLET HEPARAN SULFATE ENHANCES IL-1 RECEPTOR ACTIVATION

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Interleukin-1α (IL-1α) and interleukin-1β (IL-1β) are key mediators of islet inflammation and β-cell dysfunction. While both cytokines signal through the IL-1 receptor type I (IL-1RI), IL-1α functions as an alarmin and is actively involved in early inflammatory responses [1]. Heparan sulfate, a sulfated glycosaminoglycan (GAG) present on the cell surface and in the extracellular matrix, is known to regulate protein activity e.g. by acting as co-receptor or protein binding partner [2]. Given its crucial role in insulin secretion, we investigated whether heparan sulfate modulates IL-1α activity in pancreatic islets.
Using a GAG binding assay, we observed a concentration- and sulfate-dependent binding of IL-1α to immobilized heparin but not to the non-sulfated GAG hyaluronan. Surface plasmon resonance analysis confirmed that the GAG heparin binds IL-1α with high affinity and low dissociation, indicating stable complex formation, whereas IL-1β shows almost no detectable interaction. Molecular docking of heparin (tetra- and hexa-) to IL-1α predicted two main recognition sites surrounding its N-terminal region, which displays a localized positive electrostatic potential patch. Furthermore, MD-refinement of the obtained complex structures enabled the identification of key IL-1α residues involved in heparin recognition. Competitive ELISA experiments revealed that heparin selectively enhances IL-1α binding to IL-1RI, while having no effect on IL-1 receptor accessory protein.
Immunohistochemical staining of murine and human pancreatic islets further shows the abundance of heparan sulfate at the islet surface. The enzymatic degradation of heparan sulfate via heparanase treatment increased IL-1α levels but did not affect IL-1β levels in hypoxic human islets. Removal of cell surface heparan sulfate impaired glucose-stimulated insulin secretion of islets. The heparan sulfate antagonist surfen disrupted IL-1α interactions, promoting insulin secretion in IL-1β-/- islets compared to IL-1α-/- islets.
Functionally, both IL-1α and IL-1β suppress glucose-stimulated insulin secretion in β-cells, yet heparan sulfate enhances IL-1α-mediated effects, amplifying its inhibitory impact. These findings suggest that heparan sulfate fine-tunes IL-1α but not IL-1β signaling. Targeting the IL-1α–heparan sulfate axis may offer novel therapeutic strategies to mitigate inflammation and improve islet transplantation success.

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1-AMINO-1-DEOXY-LACTITOL-GRAFTED HYALURONIC ACID: STRUCTURAL AND CONFORMATIONAL CHARACTERIZATION BY MULTIPLE MOLECULAR DYNAMIC SIMULATION

Stefano Elli[a], Tommaso Sisto[a], Sofia Nizzolo[a], Nadia Freato[b], Laura Bertocchi[b], Giulio Bianchini[c], Edwin A. Yates[d], Marco Guerrini[a]

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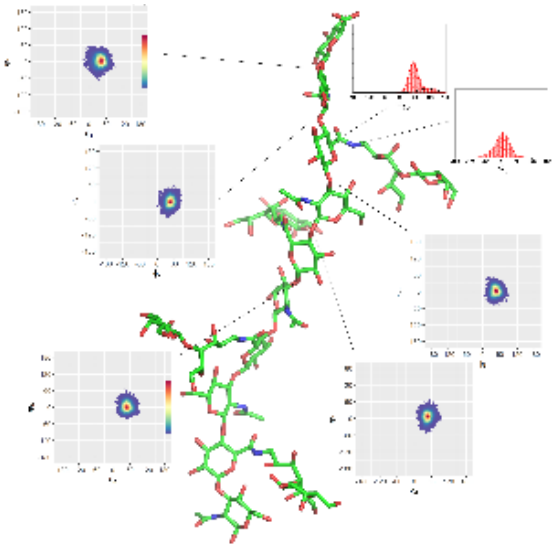
Hyaluronic acid (HA) is a linear non sulfated polysaccharide that is part of the extracellular matrix of animal cells. HA is widely used in cosmetics as an 'anti-age' treatment, combining physical filling, hydration, antioxidant, and degradable capabilities [1]. In addition, HA presents anti-inflammatory properties and modulates cellular activities [2]. Considering these aspects, and the possibility of functionalization with pharmacophores and/or vectorizable groups, HA is a perfect scaffold for drug-delivery polymers. This study focuses on the structural and conformational characterization of the 1-amino-1-deoxy-lactitol-grafted HA in water solution, a new polysaccharide known by the registered name HYLACH® [3], and designed as a broad range therapy for fibrotic disease [4], through the inactivation of galectin-3. In fact, the grafted groups of HYLACH®, that terminate with the β-D-Gal moiety, are known to bind galectin-3. Decasaccharides were constructed *in silico*, repeating the structural units of HA: β(1-3)-D-GlcNAc (1-4) β-D-GlcA and this unit was then grafted with the 1-amino-1-deoxy-lactitol at the carboxyl group of selected GlcA residues, to obtain degrees of grafting (DG) 0%, 50% and 100%. To improve the efficiency of the sampling of the phase space, two independent MD simulations, characterized by different initial backbone conformations, were run for each model. The state-of-the-art GLYCAM06 force field for carbohydrate was used; the 1-amino-1-deoxy-lactitol grafted GlcA residue was parametrized according to preliminary conformational analysis. The backbone conformation, the hydrogen bond network of these oligosaccharides, as well as the size, and conformation of the grafted groups, that are crucial properties for development of these as drug delivery polymers, were investigated. 1-amino-1-deoxy-lactitol grafted HA presents conformation, secondary structure, and hydrogen bond network that slightly but, significantly, differs from that of HA [5] and, furthermore, grafted groups exercise an attractive rather than repulsive force toward the backbone [6].



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DYNAMIC HYALURONAN-ARGININE INTERACTIONS MODULATE SOLUTION PROPERTIES AT THE MOLECULAR LEVEL

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Glycosaminoglycans (GAGs) are complex polysaccharides found in the extracellular matrix. They are vital for regulating cellular processes, such as cell-cell communication and molecular recognition. All GAGs have high negative charge, which facilitates interactions with positively charged regions of proteins. These interactions are of both industrial and biological interest, particularly for tuning hydrogel properties and GAG-protein binding.
In this study, we focus on the interactions between hyaluronan (HA), one of the most prevalent GAGs, and polyarginines or polylysines—positively charged peptides that serve as simplified interaction models, while also being of medical interest as cell-penetrating drug carriers. First, we investigated small HA molecules of well-defined length (octasaccharides) interacting with short (tetra-) peptides to identify characteristic interaction fingerprints. Using a combination of nuclear magnetic resonance (NMR) experiments and molecular dynamics (MD) simulations, we demonstrate that electrostaticsalone are insufficient to justify their different interaction patterns and strength. Polyarginines are favored over polylysines due to the dual polar/hydrophobic nature of the arginine side chain, unlike the only polar lysine. Furthermore, we identify a highly dynamic interaction pattern characterized by short-lived HA-peptide contacts, contrasting with the conventional view of stable complex formation [1].
We extended our study to larger, polydisperse HA and longer (nona-) peptides. In this case, the formation of insoluble molecular clusters hindered the straightforward use of NMR methods. To overcome this, we developed a novel approach combining MD simulations with second harmonic scattering (SHS), an experimental technique that probes solvent structure and is effective at much lower concentrations than NMR. This enabled us to study interactions at biologically relevant submicromolar concentrations where molecular complexes remain in solution. Using SHS, we confirmed polypeptide-HA interactions and aggregation even at micromolar concentrations. Extensive MD simulations provide an atomistic view of these interactions, revealing that the clusters are maintained through multivalent, dynamic contacts rather than single, strong binding events.
Overall, our study characterizes the interactions of industrially relevant polymers, potentially improving their applications, and introduces a new methodology for investigating such interactions at physiologically relevant concentrations. Furthermore, we provide a consistent molecular picture of the interactions, comprehensively addressing arginine selectivity and the effects of HA polymer length.

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REINFORCEMENT OF PROTEIN-PROTEIN INTERACTION BY GLYCOSYLATION

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Proteins are ubiquitously glycosylated and play essential roles in a variety of biological phenomena. The unique hydration properties of glycans facilitate interactions between glycoproteins and water molecules. However, little is known about the correlation between glycan hydration and protein function. To address this fundamental question, we carried out the total chemical synthesis of homogeneous glycoproteins and their functional analyses. We synthesized several glycoprotein derivatives incorporating different glycans. To investigate the interaction between water and glycoproteins, we conducted hydrogen-deuterium exchange (HDX) experiments using homogeneous glycoproteins. The HDX experiments indicated that the glycan moiety of glycoproteins affected the dynamic behavior of surrounding water molecules. Using isothermal titration calorimetry (ITC), we measured the binding affinity between small glycoproteins and their receptor. These data indicated that glycans enthalpically enhance protein-protein binding affinity. In addition to these experiments, using a new NMR technique, we also analyzed which sugar hydroxyl groups interact with water molecules. In this presentation, I will discuss how glycans regulate protein-protein binding events and interact with water.

CHEMICAL SYNTHESIS AND FUNCTIONAL STUDY OF COMPLEX GLYCOPROTEINS

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Protein glycosylation plays a key role in all aspects of life activities. Glycosylation is controlled by a variety of glycosyltransferases and glycosidases, resulting in its high heterogeneity. Chemical synthesis of glycoprotein can provide homogeneous glycoproteins. However, the process is complicated and usually provides a small amount of glycoprotein. We developed an efficient method for the preparation of thiolated amino acids and selective protection of thiol group using quinoline protecting group. These methods provide powerful solutions for the synthesis of large glycoproteins.

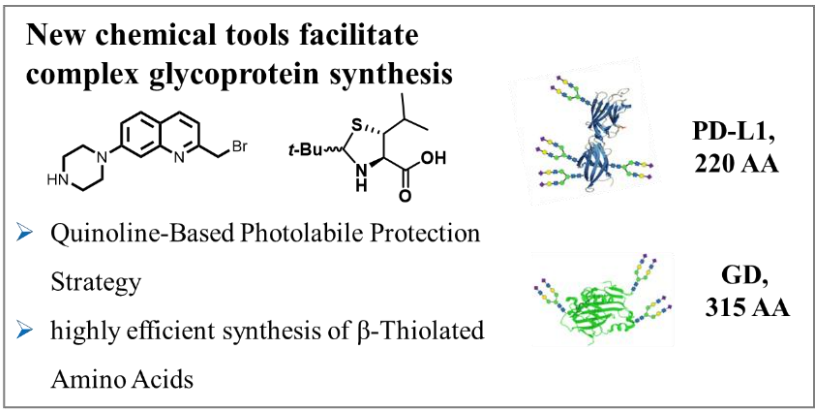

Figure. 1 Chemical synthesis of complex glycoproteins

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EXPEDITIOUS SYNTHESIS OF MULTIPLY GLYCOSYLATED PEPTIDES

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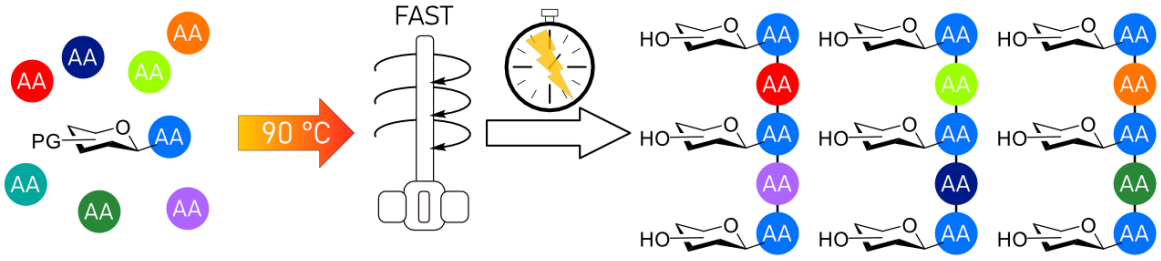
Glycopeptide libraries with a high degree of purity are indispensable tools for investigating the effects of glycosylation pattern on interactions and function. Glycopeptides are prepared *via* a combination of solution- and solid-phase techniques, which require large amounts of protected glycosylated amino acids that are very difficult to obtain. The assembly of glycopeptides is very slow and inefficient due to the increased steric hindrance of glycosylated amino acids and their tendency to undergo substantial racemization. Finally, the deprotection of glycopeptides requires harsh conditions that are not compatible with the SPPS process. The above difficulties increase when peptides with multiple glycosylations are synthesized. These limitations make the state-of-the-art glycopeptide synthesis unattractive and the preparation of libraries hardly achievable.
We developed a new accelerated method for the efficient synthesis of a diverse range of peptides. The method utilizes a combination of high temperature and overhead stirring to assemble peptides within minutes [1]. Adaptation of this method provided an accelerated, racemization-free assembly of singly glycosylated peptides using equimolar quantities of glycosylated amino acids [2]. The method was further optimized to enable the rapid deprotection of the glycan on the solid support, thereby averting post-cleavage manipulations. We show that the accelerated method also prevails over the increased steric hindrance associated with multiglycosylated peptides and allows the simultaneous deprotection of multiple glycans. Thus, we are able to prepare high-purity glycoconjugates within minutes, while still using equimolar quantities of glycosylated amino acids. This presents a new path for obtaining a variety of glycopeptides in high purity and large quantities, which will allow for a better understanding of the role of glycosylation in biological systems.



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NEXT-GENERATION CHEMICAL TOOLS FOR IMPROVED GLYCOPROTEIN ANALYSIS

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Glycoproteins are involved in all essential cellular processes ranging from intercellular communication to pathogen recognition and immune modulation. Aberrant glycosylation has been recognized as a hallmark of many diseases including cancer, neurodegenerative, or metabolic diseases. However, the glycobiology underlying the pathology in these diseases is fragmented at best. In particular, a correlation between altered glycan structures and resulting consequences for protein function are mostly missing. The structural complexity and heterogeneity of glycans complicates a detailed analysis with currently available methods.
Our research applies chemical biology to elucidate how aberrant glycosylation can modulate the structure and function of proteins and thereby contribute to the pathogenesis of certain diseases. To this end, we develop new chemical tools and apply novel concepts for improved glycoprotein analysis. This includes the synthesis of various next-generation chemical probes. Using established concepts of solid phase synthesis, we customize complex multi-functional probes that carry bioorthogonal handles, such as azides, alkynes or tetrazines for covalent attachment to chemically engineered glycoproteins. These probes allow efficient enrichment, isolation and multi-modal analysis of glycoproteins in multiple coordinated experiments. We apply these tools in state-of-the-art technologies, such as live cell imaging and intact chemical glycoproteomics to decipher aberrant protein glycosylation in a class of rare genetic glycosylation defects; so-called Congenital Disorders of Glycosylation as well as in some types of malignant cancers.
Other probes are equipped with diazo groups or sulfated and phosphorylated glyco-oligomers and can be used to study glycan-mediated pathogen infections. Our main focus is to contribute to a better understanding of the role that glycans play in disease development as a basis for designing suitable diagnostic and therapeutic concepts.

FROM A UNIQUE TETRASACCHARIDE SCAFFOLD TO A BROAD SEROTYPE COVERAGE *SHIGELLA FLEXNERI* VACCINE CANDIDATE

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Shigella flexneri are Gram-negative enterobacteria and the main causative agent of endemic shigellosis, a major diarrheal disease especially in children under five from low- and middle-income countries. Disease burden calls for a *Shigella* vaccine that would induce broad serotype protection in the population most at risk. Protective immunity is believed to be achieved to a large extent by antibodies directed at the *Shigella* O-antigen (O-Ag), making it a prime target for vaccine development. Most *S. flexneri* serotypes exhibit closely related O-Ags built from the same backbone. Structural diversity reflecting serotype specificity derives from site-selective substitutions on a tetrasaccharide core (Figure 1) [1].

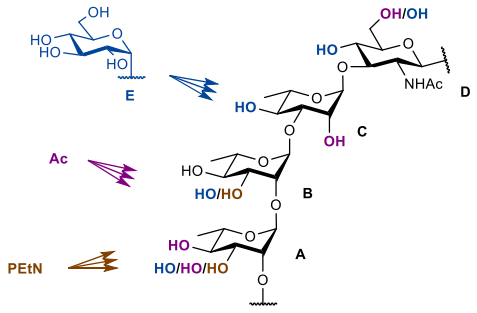

Figure 1. Backbone repeating unit (RU) from most *S. flexneri* O-Ags and type-specific substitutions thereof [1].

A semi-synthetic glycoconjugate was designed to help protect against *S. flexneri* serotype 2a. Promising data in phase 1 and phase 2a clinical trials support the development of novel strategies enabling serotype broadening to answer the need in the field [2,3].
This presentation illustrates the concept of synthetic glycan-based vaccines in the context of *S. flexneri*. Focus is on the design of functional oligosaccharide mimics of O-Ags representing the most prevalent serotypes. Going beyond original achievement [2,3], we report a concept whereby key RU building blocks featuring type-specific substitutions are built from a single fine-tuned tetrasaccharide scaffold by means of controlled 1,2-*cis* glucosylation of suitable acceptors. Chain elongation at either end of the glucosylated bricks and full deprotection delivered the required panel of linker-equipped type-specific oligosaccharides. The subsequent conjugation of the latter onto a protein carrier provided sets of potential immunogens representative of three different *S. flexneri* serotypes.
Immunogenicity data in mice will be discussed. The proof-of-concept for a broad coverage synthetic glycan-based *S. flexneri* conjugate vaccine will be illustrated.

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TOTAL SYNTHESIS OF CAMPYLOBACTER JEJUNI NCTC11168 CPS

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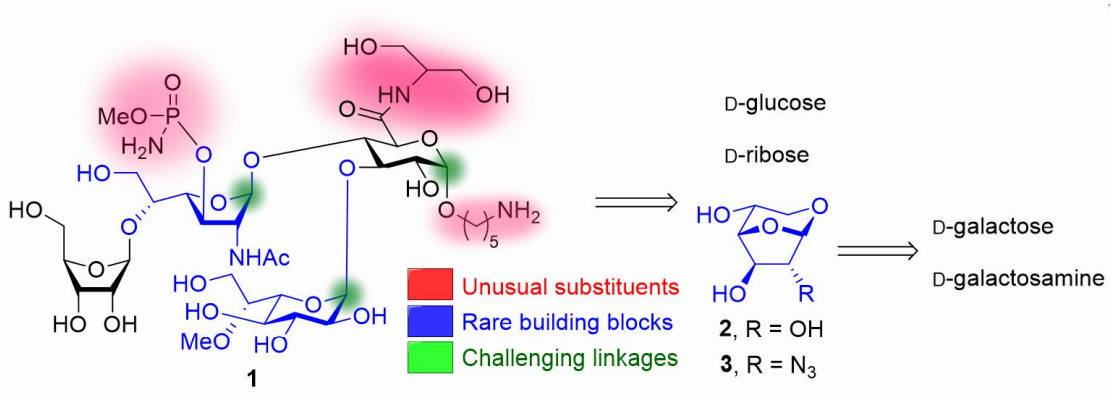
Campylobacter jejuni is a typical foodborne pathogen, and its infection results in a significant diarrhea disease, which is highly fatal to young children in unindustrialized countries. Herein, we described a total synthesis of a *C. jejuni* NCTC11168 capsular polysaccharide repeating unit containing a linker moiety via an intramolecular anomeric protection (iMAP) strategy [1]. Applying the iMAP strategy efficiency reduces the reaction steps of configuration modification to furanoside structure and the following regioselectivity protection. Therefore, we successfully concisely synthesize a rare sugar, heptose, in a six-steps manner from D-galactose, and generate the desired furanosyl galactosamine building block after 2 steps from a commercial galactosamine. Accordingly, we construct the target tetrasaccharide in merely 28 steps using [2 + 1 + 1] glycosylation strategy and accomplish the required substitutive modification and global deprotection, including the preparation of all the building blocks. Our research provides the first synthesis study on this complex tetrasaccharide structure, and the amine of linker moiety is capably conjugated with carrier protein for further vaccination study.



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TOTAL SYNTHESIS OF PECTIC POLYSACCARIDE RG-II SIDE CHAIN A

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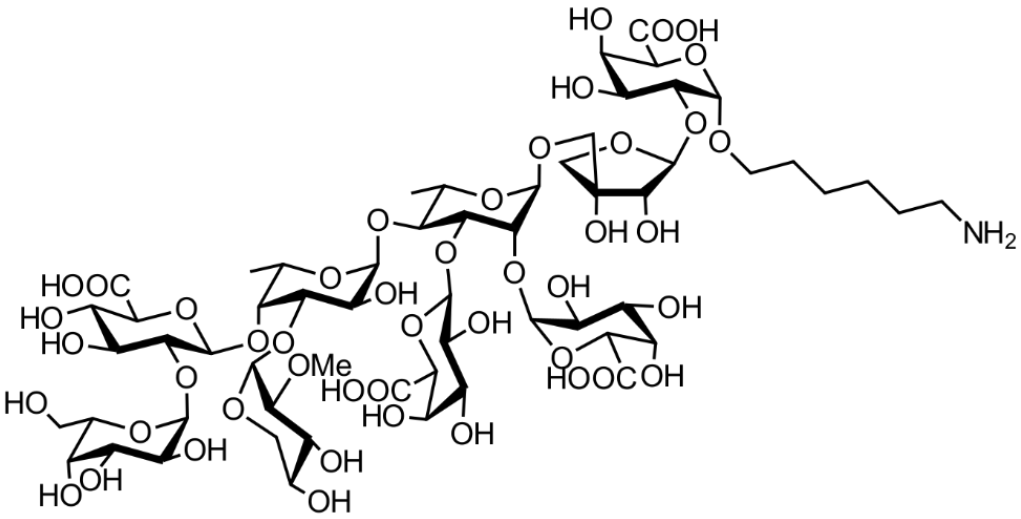
The pectin domain rhamnogalacturonan-II (RG-II) is one of the most complex, yet poorly understood polysaccharide structures in nature with irreplaceable relevance for plant growth and human diet [1]. RG-II evolved very early in plant evolution and its unique structure has been conserved in all vascular plants to this day [2]. It is hypothesized that side chain A self-assembles with borate to a chiral borate diester in the plant cell wall [3], which is essential for cell wall stability and plant growth [4]. Despite its importance its three-dimensional structure and biosynthesis remain elusive. In this work we report the successful convergent synthesis of RG-II side chain A in 74 total steps, with the longest linear sequence comprising 19 steps.



Its composition of seven different monosaccharides including the rare sugar apiose, the presence of five different 1,2-*cis*-glycosidic linkages, a dense branching pattern, and the unique reactivity of the four uronic acids pose the major challenges for the synthesis of this highly branched and unusually complex nonasaccharide. The presented approach is based on a carefully designed set of orthogonal protecting groups and various advanced glycosylation methods and is assisted by analytical tools such as advanced NMR techniques and x-ray crystallography.

Acknowledgements:
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SYNTHETIC PYROPHOSPHATE - CONTAINING ZWITTERIONIC LPS/LIPID A VARIANTS

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Gram-negative bacteria exploit various mechanisms to adapt their cell wall membranes by altering LPS composition to evade the host immune responses. Bacterial LPS-remodelling, modulated by the PhoP-PhoQ regulatory system and the activation of PmrA-PmrB leads to the decoration of the lipid A phosphate groups with amino group–containing appendages (i.e., ethanolamine, phosphoethanolamine or β-L-Ara4N) which are essential for LPS/lipid A recognition by the mammalian innate immune receptors. On the other hand, modifications of the LPS phosphate groups can shield bacteria from recognition by host cationic antimicrobial peptides, contributing to bacterial virulence. Due to intrinsic microheterogeneity and inherent instability of glycosyl phosphodiester and pyrophosphate modifications in LPS, LPS preparations from bacterial cultures exhibit significant structural variability making a consistent immunobiological assessment of the consequences of LPS remodelling difficult.
To study the impact of LPS phosphate groups modification on the host innate immune responses and on the LPS-protein interactions, we synthesised a collection of immunomodulatory LPS/lipid A motifs possessing various appendages on their phosphate groups. We developed an efficient and robust methodology to synthesize phosphoethanolamine (PE)-modified lipid A variants, comparing the use of phosphoramidite and H-phosphonate chemistries. As regarding to the synthesis of glycosyl pyrophosphodiesters, as found in the LPS of several bacterial species such *Salmonella* or *Neisseria,* this is challenged by the intrinsic instability of glycosyl pyrophosphates under different conditions, as well by the complex control of stereoselectivity required. To tackle this challenge, we exploited the application of P(V)-P(III) coupling which provided a more efficient and rapid coupling step in comparison to the classical P(V)-P(V) approaches.

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STRUCTURAL INSIGHTS INTO CARBOHYDRATE-LECTIN INTERACTIONS

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Carbohydrates represent a crucial class of molecules that are generally exhibited on cell's surface, covalently attached on proteins, lipid or RNA [1]. The presence of these carbohydrates plays an essential role in cellular communication and regulate the immune responses. As example, they enable immune system to distinguish between self and non-self, such as pathogens or tumour cells [2].
The interaction between glycans and immune cells is mediated by a class of proteins called lectins, which are capable of binding to specific carbohydrate structures. Lectins, including Siglecs (Sialic acid-binding Ig-like lectins) and Galectins, play a pivotal role in recognizing and interpreting the information encoded in surface carbohydrates. These lectins bind to specific carbohydrate motifs, triggering downstream signalling events that modulate immune function [3]. For instance, Siglecs can bind to sialic acid-containing glycans on the surface of cells, regulating immune cell activation and cytokine production [4].
Herein, we combined Nuclear Magnetic Resonance (NMR) spectroscopy, computational and biophysical techniques to gain structural insights into the molecular recognition processes between modified carbohydrates and lectins. One notable example is Siglec-8, which plays a key role in regulating allergic responses [5]. We elucidated the binding mechanism of four high-affinity analogues of Sialoglycans, demonstrating that sialic acid binds to the classic sialyl binding pocket of the Siglec receptor family. Notably, compounds with sulfonate groups and 9-naphthyl sulfonate exhibited the highest affinity, as determined by NMR and biophysical experiments.
These findings provide valuable insights for the rational design of the next generation of Siglec-8 inhibitors. By understanding the structural determinants of carbohydrate-lectin interactions, we can develop novel therapeutic strategies that target these interactions to modulate immune responses and treat diseases associated with dysregulated immune function.

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NEW NMR STRATEGIES TO INVESTIGATE GLYCAN-LECTIN INTERACTIONS OF COMPLEX GLYCOCONJUGATES

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The molecular recognition of glycans is at the heart of numerous biological processes within living organisms and plays crucial roles in health and disease. Understanding these interactions at the molecular/atomic level is of paramount importance for developing novel therapeutic approaches, improving disease diagnostics and/or advancing in biotechnological applications [1].

Given the dynamic nature of glycans, NMR has been extensively used to study glycan-lectin interactions and remains at the forefront of existing approaches [2]. In this context, ^{19}F-labelled glycan probes have been exploited in diverse applications [3] and fluorine NMR has emerged as a powerful strategy to interrogate biological systems in complex environments closely resembling native conditions [4].

Herein, we describe the NMR analysis of the molecular recognition of complex glycoconjugates, even in complex environments such as the cell, by the exploitation of ^{19}F-NMR spectroscopy. This novel approach allowed easily detecting complex glycans, monitoring binding events and/or profiling the glycosylation of intact cells.

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NMR INVESTIGATION OF RUTHENIUM-BASED SELECTIVE GALECTIN-1 INHIBITOR

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Human galectin-1 is a carbohydrate-binding protein that has emerged as a promising target for therapeutic intervention in cancer [1]. However, the development of bioactive compounds with high selectivity and inhibitory potency towards human galectin-1 is quite challenging due to its structural similarity with other human galectins. This study focuses on the NMR investigation of novel hybrid *N*-acetyllactosamine-based ruthenium-containing compound **1** (Figure 1A), developed in our laboratory at the Institute of Chemical Process Fundamentals. Inhibitor **1** showed surprisingly high selectivity to human galectin-1 over galectin-3. To understand the origin of this selectivity, we investigated the molecular recognition process using established NMR methods such as ^{1}H-^{1}H saturation transfer difference spectroscopy (STD), ^{1}H-^{1}H transferred NOESY, or ^{1}H-^{15}N chemical shift perturbation analysis [2]. This investigation uncovered that selectivity to galectin-1 is most likely attributable to the unique “piano-stool” geometry of the ruthenium complex, which fits well into the binding site of galectin-1 but discriminates binding to galectin-3 (Figure 1B).

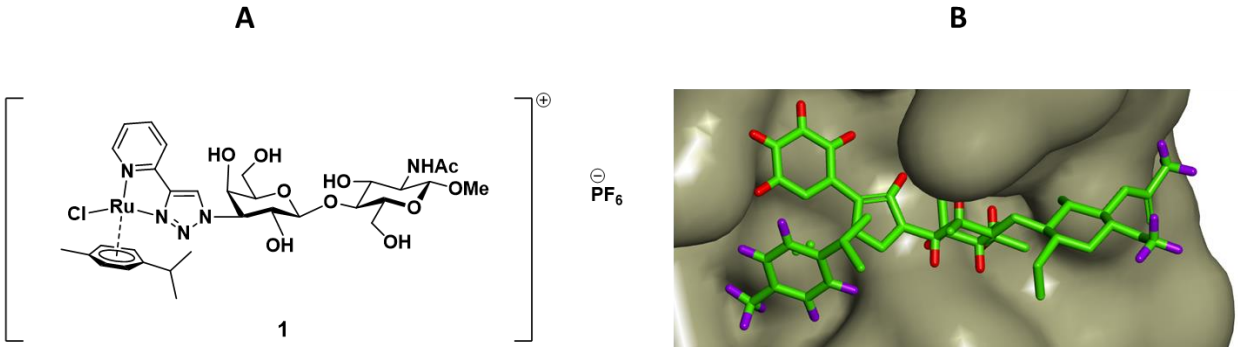

Figure 1. A) Structure of ruthenium-containing galectin inhibitor **1**. B) Conformation of compound **1** in the binding site of human galectin-1, refined on the basis of ^{1}H-^{1}H STD measurement (red protons = high STD saturations, purple protons = low STD saturations).

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STRUCTURAL AND CONFORMATIONAL INSIGHTS INTO BACTERIAL LECTIN RECOGNITION OF HOST RECEPTORS

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Lectins play a crucial role in bacterial colonization of host tissues and have emerged as promising therapeutic targets due to their involvement in host-pathogen interactions [1]. The increasing threat of antimicrobial resistance (AMR) is rendering standard antibiotics ineffective, necessitating the development of novel treatment strategies against multidrug-resistant pathogens, particularly Gram-negative ESKAPE pathogens. Targeting bacterial lectins, such as LecA and LecB in *Pseudomonas aeruginosa*, has been shown to effectively interfere with bacterial virulence and biofilm formation, offering a promising alternative to traditional antibiotics [2].

In this context, an orthologue of LecA, named EclA, has been identified in *Enterobacter cloacae*, a member of the human gut microbiota that can also act as an opportunistic pathogen in immunocompromised patients.

The crystal structure of EclA, in complex with methyl α-L-selenofucoside, revealed a unique two-domain architecture, consisting of a dimeric N-terminal LecA-like domain and a novel dimeric C-terminal carbohydrate-binding domain. This domain adopts an unprecedented intertwined β-sheet dimeric structure, suggesting a binding mode distinct from previously characterized bacterial lectins and indicating a potential role for EclA as a cross-linker for specific host glycans. Glycan array analysis demonstrated high specificity of EclA for fucosylated blood group antigens, particularly LewisA and H-type II [3], in contrast to LecA, which binds galactose-containing sugars.

Despite these findings, the molecular mechanisms underlying EclA's interaction with fucosylated ligands remained unresolved. To address this gap, a multidisciplinary approach was employed, using saturation transfer difference (STD) NMR to obtain ligand epitope mapping, trNOESY for the identification of the bioactive conformation, and computational studies to generate a three-dimensional model of the complexes. Additionally, isothermal titration calorimetry (ITC) was used to quantify binding thermodynamics and affinities, providing a comprehensive understanding of EclA-ligand interactions. These findings not only deepen insights into *E. cloacae* adhesion mechanisms but also lay the groundwork for developing anti-adhesion therapies targeting bacterial lectins in drug-resistant infections.

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FUNCTIONALIZATION OF TAMARIND (*TAMARINDUS INDICA* L.) SEED POLYSACCHARIDE WITH 1,4-BUTANEDIOL DIGLYCIDYL ETHER (BDDE)

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Natural polysaccharides had increased their interest as a biomaterial in different applications due to their properties such as biocompatibility, non-toxicity, and biodegradability [1]. Tamarind seed polysaccharide (TSP) is a galactoxyloglucan, which is already extensively used for biomedical applications, due to its physical, chemical, and biological properties. However, TSP has certain limitations due to its molecular structure, such as low water solubility, the lack of charged groups on the sugar chain and the fast biodegradability [2]. To overcome these challenges and expand its potential applications, a functionalization of the TSP was obtained using 1,4-Butanediol diglycidyl ether (BDDE), well-established epoxy crosslinking agent used primarily for the formation of hyaluronic acid (HA) hydrogel. Different concentrations of the reagent were used to obtain both gels and soft gels. Characterization of the chemical-physical properties of the synthetized products were conducted by different analytical approaches, including viscosity, swelling capacity, morphology chemical structure, and molecular weight distribution. The degree of crosslinking and the evaluation of the stability of the products were obtained by NMR spectroscopy and LC-MS spectrometry after the enzymatic hydrolysis of the hydrogels.

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ENCAPSULATION PROCESS OF MUSCLE STEM CELLS IN ALGINATE MATRICES TO ENHANCE THEIR *IN VIVO* POST-TRANSPLANT PROPERTIES

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Identification of a stem cell population derived from human muscle (hMuStem) and the demonstration of its regenerative potential have led to new cell therapy proposals for muscle diseases [1]. However, the ability of these adult stem cells to survive and integrate into the host tissue is a limiting factor with regard to their overall therapeutic impact. Emergence of tissue biomimetic approaches based on encapsulating cells in biocompatible and biodegradable biomaterials offers new opportunities to overcome the limitations of cell therapy in terms of viability and potentiation of effects. Initial *in vitro* results have enabled us to validate the possibility of encapsulating hMuStem cells in alginate matrices without altering the biological properties associated with their commitment in the myogenic program [2]. It was shown that macroscopic hydrogels obtained by molding methods possess mechanical, structural and diffusion properties compatible with hMuStem cells. However, their large size limits their therapeutic applications to subcutaneous implants. For this reason, transposition of the encapsulation method to microfluidic approaches seems essential to achieve micrometric matrices more suitable with transplantation protocols [3]. To improve the efficiency of hMuStem cell delivery, we have developed a microfluidic chip enabling the efficient encapsulation of cells in micrometer-scale alginate matrices. In parallel, we assessed the impact of this encapsulation process on the morphology and viability of hMuStem cells.

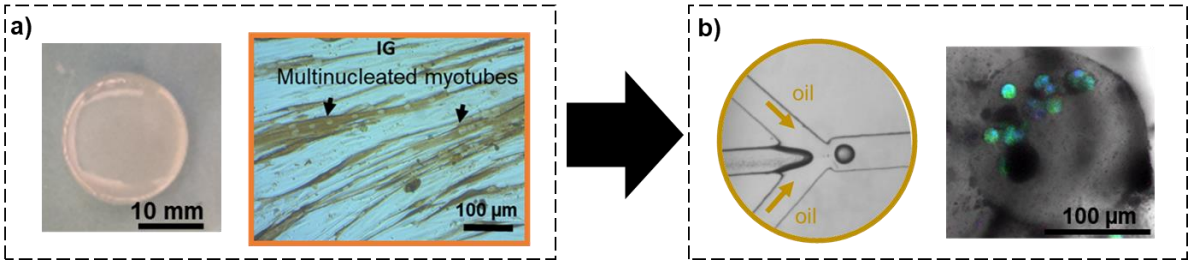

Figure 1. (a) Conservation of biological activities of hMuStem cells inside calcium-alginate macrogels mimicking muscle elastic modulus (12 kPa). (b) Scaling up by transposition to droplet microfluidic approaches to open up application for cell transplantation-based therapy.

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OLIVE STONES: AN ATTRACTIVE SOURCE OF BIOPOLYMERS (PECTIN, HEMICELLULOSE, AND CELLULOSE) FOR FOOD AND BIOMEDICAL APPLICATIONS

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Olive stones (OS), a major byproduct of the olive oil industry, are rich in lignocellulosic material, comprising approximately 32% cellulose, 26% lignin and 22% hemicellulose, making them a promising and sustainable source of valuable biopolymers [1,2]. These biopolymers offer significant potential for applications in food, pharmaceuticals, and biomaterials. This study focuses on the eco-friendly extraction, characterization, and functional assessment of cellulose, hemicellulose, and pectin from OS. Conventional (acid/base hydrolysis) and emerging green extraction techniques (hydrothermal, ultrasound and microwave-assisted methods) were compared to optimize the recovery of high-purity fractions (Figure 1). The extracted biopolymers were analyzed using Fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM) to assess their structural, thermal and morphological properties. The results revealed high purity and functionality, supporting their potential in biodegradable packaging, neutraceuticals, and biomedical applications. This study underscores the valorization of OS as a renewable resource, aligning with circular economy principles and sustainable development goals. By leveraging innovative extraction techniques, olive stones can be transformed from agricultural waste into high-value biopolymers, fostering eco-friendly alternatives for industrial applications.

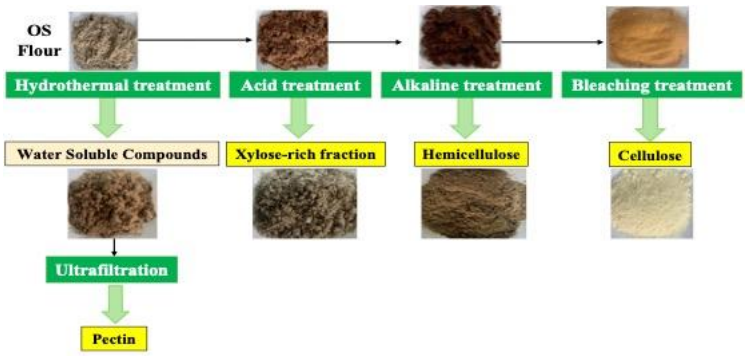

Figure 1: Biopolymers extraction procedure.

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THE SMART APPLE-BASED FOIL: THE ROLE OF PECTIN-GLYCEROL-LIPID INTERACTIONS ON THERMORESPONSIVE MECHANISM

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Natural polymer films represent a class of materials which primary advantages include a vast scope for modifying mechanical properties, conductivity, and a high degree of sensitivity to external stimuli, like temperature. The possibility of applying them to various substrates, makes them ideal for applications such as flexible wearable electronics, artificial skin, or biomedical devices. Such systems respond to external stimuli by changing electrical parameters [1]. The most commonly used natural polymers are cellulose, chitosan, or gelatin. However, these polymers have intrinsically, poor electrical conductivity. Given the abundance of carboxylic acid within macromolecules and its remarkable capacity to form hydrogels, pectin emerges as a promising candidate for this propose. In this studies pectin films (containing lipid and glycerol) were developed that changed their shape and electrical parameters (Fig. 1) under the influence of temperature. I found that the conduction occurs according to the Grothuss mechanism. The observed changes are strongly correlated with thermal phase transitions of the pectin films, such as the polymer glass transition and the lower critical dissolution temperature (LCST) [2].

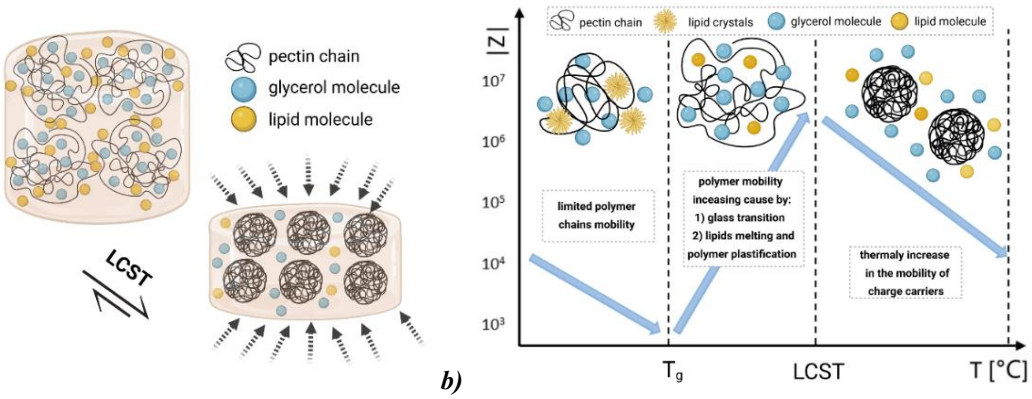

Figure 1. Scheme of pectin film thermal: a) shape change, b) conductivity mechanism [2]

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LECTIN LIGANDS: FROM MULTIVALENCY TO PHOTOSWITCHING

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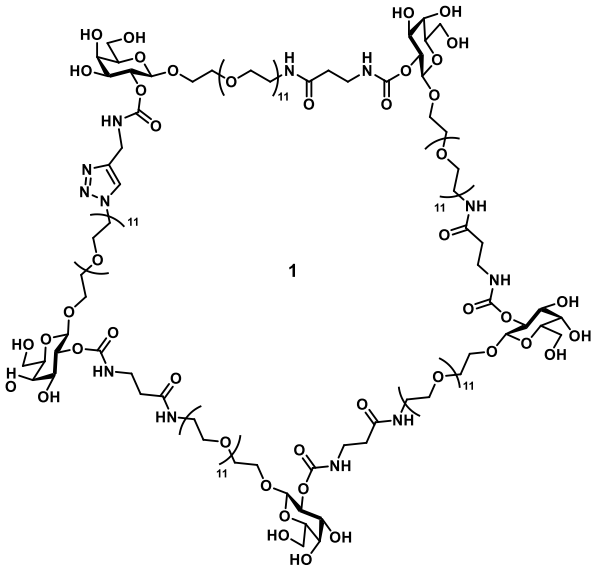
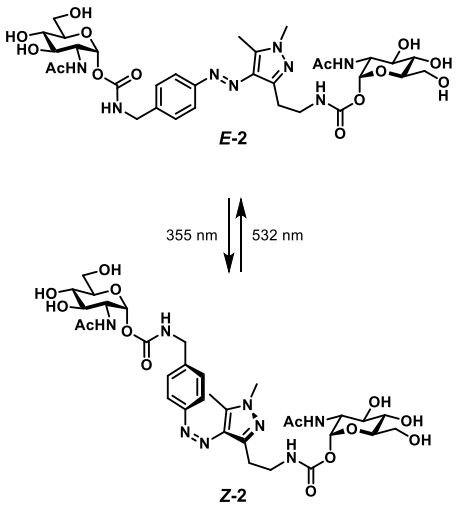
Carbohydrates are involved in a myriad of cellular recognition processes. High-affinity lectin ligands are of high medicinal interest for diagnostic and therapeutic applications. However, the binding affinities between individual carbohydrate epitopes and carbohydrate-binding proteins (lectins) are usually low. Multivalency can drastically enhance binding affinities between the interacting species [1]. In the past, we developed a new design of multivalent lectin ligands, termed inline lectin ligands (iLecs) [2]. iLecs lead to exceptionally high binding affinities without concurrent precipitation of proteins due to crosslinking.
In this communication, I will present recent efforts in the development of iLecs for bacterial AB_5-type toxins. We designed macrocyclic ligand **1** that can bind to five binding site of the B_5-subunit of Shiga toxin simultaneously. Binding affinities were determined with microscale thermophoreses (MST) and showed an increased affinity of the cyclic ligand over its linear precursor.





Furthermore, we developed divalent ligand **2** for the plant lectin wheat germ agglutinin (WGA) that contains an arylazopyrazole photoswitch [3]. The ligand was designed in a way that only the (*E*)-isomer is able to bridge adjacent binding sites of WGA leading to a chelating binding mode. Photoswitching induces an unprecedentedly high change in lectin binding affinity as determined by isothermal titration calorimetry (ITC). Furthermore, we observe a change of the binding mode of the ligand from chelating binding of *E*-**2** to crosslinking binding of *Z*-**2**.

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HIGH-AFFINITY LECTIN LIGANDS ENABLE THE DETECTION OF PATHOGENIC *PSEUDOMONAS AERUGINOSA* BIOFILMS: IMPLICATIONS FOR DIAGNOSTICS AND THERAPY

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Pseudomonas aeruginosa is a critical priority I pathogen and causes life-threatening acute and biofilm-associated chronic infections. The choice of a suitable treatment for complicated infections requires lengthy culturing for species identification after swabs or invasive biopsy. To date, no fast and pathogen-specific diagnostic tools for *P. aeruginosa* infections are available. Here, we present the non-invasive pathogen-specific detection of *P. aeruginosa* using novel fluorescent probes that target the bacterial biofilm-associated lectins LecA and LecB. Several glycomimetic probes were developed to target these extracellular lectins and demonstrated to stain *P. aeruginosa* biofilms in vitro. Importantly, for the targeting of LecA an activity boost to low-nanomolar affinity could be achieved which is essential for in vivo application. In vitro, the nanomolar divalent LecA-targeted imaging probe accumulated effectively in biofilms under flow conditions, independent of the identity of the fluorophore. Investigation of these glycomimetic imaging probes in a murine lung infection model and fluorescence imaging revealed accumulation at the infection site. These findings demonstrate the use of LecA- and LecB-targeting probes for the imaging of *P. aeruginosa* infections and suggest their potential as pathogen-specific diagnostics to accelerate the start of the appropriate treatment [1].

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ENHANCING ANTIBODY THERAPEUTICS VIA GLYCAN ENGINEERING AND INTRACELLULAR DYNAMICS CONTROL

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The development of antibody therapeutics has revolutionized cancer treatment, offering high target specificity and potent immune-inducing activities such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). Enhancing these activities remains a significant focus in both academic and industrial research.
In our recent study, we aimed to control antibody dynamics and enhance immune-inducing activity by utilizing glycan-lectin interactions. Specifically, we introduced galactose-containing glycans to anti-HER2 antibodies—therapeutic agents for breast cancer—and successfully suppressed antibody internalization through binding to galectin-3. This led to a significant enhancement in CDC activity [1]. Further live-cell imaging analysis revealed that interactions between glycan-modified antibodies and the galectin lattice on the cell surface are crucial in inhibiting internalization [2].
In addition, we explored nuclear medicine therapy using the alpha-emitting isotope astatine (^{211}At), which has shown promise due to its high cancer-killing efficacy and limited side effects. We developed radioimmunotherapy complexes (RICs) by labeling anti-glypican-1 antibodies with ^{211}At for targeted therapy in pancreatic cancer models.[3] By incorporating intracellular enzyme cleavage sites and nuclear translocation signal peptides into the antibody linker region, we significantly enhanced the cytotoxic effect of ^{211}At.[4]
These findings demonstrate that controlling antibody dynamics through glycan engineering and advanced radioisotope conjugation strategies is a promising approach for improving the therapeutic efficacy of antibody-based cancer treatments. Our ongoing research strives to translate these innovations into clinical applications, with the ultimate goal of developing next-generation antibody therapeutics.

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GLYCAN MEDIATED FUNCTION OF OSTEOPONTIN

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Osteopontin (OSTP) is a highly phosphorylated and glycosylated extracellular matrix protein that plays a pivotal role in bone remodeling and wound healing, also in inflammation and tumor progression [1]. The specific glycosylation pattern of OSTP tips the balance of those processes through interactions with glycan-binding proteins [2]. Yet, the precise regulation mechanism remains not fully understood at molecular level.
We herein present a synergic combination of NMR and LC-MS/MS to precisely characterize the glycoprofile of OSTP, recombinantly produced in HEK293 cells [3]. State-of-the-art NMR binding experiments revealed, at atomic resolution, the glycan mediated interactions between OSTP and key members of the glycan-binding protein family of Galectins, known to play a role in cancer progression. These findings offer new insights into the structural basis of OSTP's elusive glycan recognition mode and its regulatory role in biochemical processes.
We have further explored the interaction of OSTP with the CD44 receptor, which is involved in the activation of the PI3K/AKT signaling pathway in non-small cell lung carcinoma and promotes tumor cell migration. We have finally examined the interplay with Galectin-8 (Gal8), a known binding partner of CD44 [4].
Our structural insights have been validated *in cellulo* by biological assays, demonstrating the formation of the OSTP/CD44/Gal8 ternary complex and its effects on downstream signaling events, including cell apoptosis and survival pathways.
Our findings pave the way to illuminate previously uncharted glycan-mediated interactions, advancing the structural and functional understanding of OSTP's role in cancer biology and paving the way for potential therapeutic interventions targeting glycan recognition pathways.

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S-GALACTOSIDE INHIBITORS OF GALECTIN-1 AND -3: FROM MOLECULAR DESIGN TO HIGHLY SELECTIVE NANOMOLAR INHIBITORS

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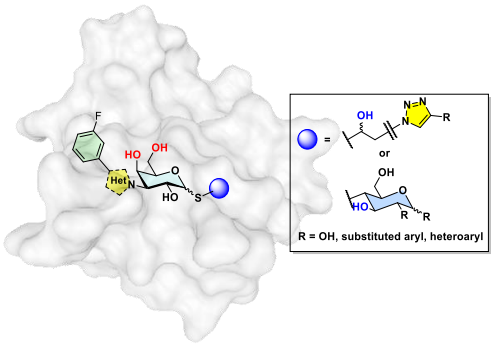
Galectins-1 and -3 play crucial roles in cell adhesion, apoptosis, and immune responses, with their regulation linked to cancer progression. This makes them attractive therapeutic targets.[1] In our laboratory, we have developed *S*-galactoside inhibitors of galectin-1 and -3 with high selectivity and affinity. Through structural optimization, we successfully designed nanomolar selective inhibitors of galectin-3, exhibiting remarkable specificity. Their development involved the synthesis and modification of *S*-galactoside derivatives, detailed biochemical characterization, and biological evaluation.

This presentation focuses on new methods and strategies for the chemical synthesis of these *S*-galactosides, discussing novel glycosylation reactions and stereoselectivity control.[2-3] The effectiveness of these methods will be illustrated by the synthesis of relevant galectin inhibitors. Our findings enhance the understanding of structural and functional requirements for selective galectin inhibition[4] and open new possibilities for developing carbohydrate-binding protein inhibitors with therapeutic potential.



Acknowledgements: This work was supported by the Czech Science Foundation (grant No. 23-05805S) and the grant Specific University Research (grant No. A1_FPBT_2024_003).

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ORGANORUTHENIUM GLYCOMIMETICS AS NEXT-GENERATION GALECTIN-1 INHIBITORS

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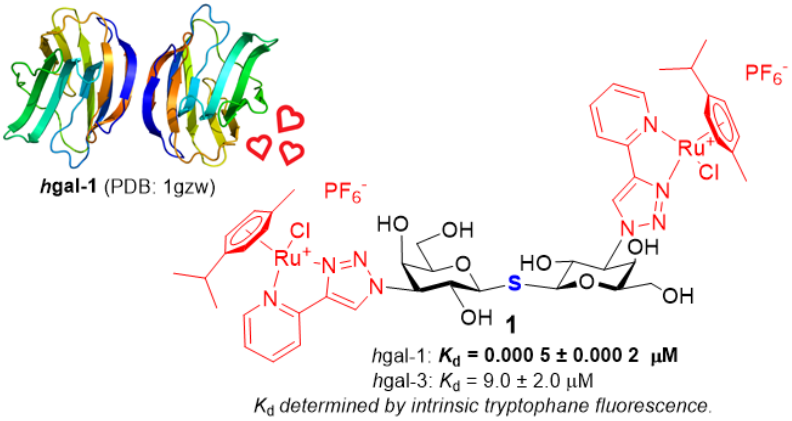
Galectins, a family of β-galactoside-binding lectins, play key roles in various biological processes, including tumor development and progression. Human galectin-1 (*h*gal-1) has been implicated in immune evasion, cell migration, and regulation of apoptosis, making it an attractive target for anticancer drug development [1]. Here, we introduce a novel class of ruthenium-based glycomimetic inhibitors designed to selectively target *h*gal-1. These inhibitors are based on *N*-acetyllactosamine and thiodigalactoside scaffolds, functionalized with organoruthenium piano-stool complexes.

Our ruthenium-functionalized glycomimetics (such as **1**) show nanomolar binding affinities for both human and mouse galectin-1, while exhibiting unprecedented selectivity—over 1000-fold preference for *h*gal-1 compared to human galectin-3 (*h*gal-3). Biological studies revealed that these inhibitors prevent *h*gal-1-induced apoptosis in Jurkat cells, a mechanism that may contribute to suppressing immune escape in certain cancers. Additionally, they selectively reduce the viability of *h*gal-1-expressing MDA-MB-231 breast cancer cells at low micromolar concentrations while displaying minimal toxicity toward galectin-1 null HEK-293 noncancerous cells. Furthermore, they effectively scavenge extracellular *h*gal-1, preventing its association with the cancer cell surface—a crucial property for disrupting *h*gal-1-mediated signaling pathways.



Acknowledgements: The financial support of the Czech Science Foundation is gratefully acknowledged (23-06115S). P.B. acknowledges support from the Ministry of Education, Youth and Sport of the Czech Republic, project no. LUC23149, and COST Action CA21116 - Identification of biological markers for prevention and translational medicine in pancreatic cancer (TRANSPAN).

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BICYCLIC IMINOSUGARS AS POTENTIAL GLYCOSIDASE INHIBITORS

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Glycosidases (or glycoside hydrolases) play a crucial role in breaking down glyosidic bonds, making them essential for the catabolism of carbohydrates in all living organisms. Given their fundamental biological significance and the therapeutic advantages associated with targeting these enzymes pharmacologically, there is a considerable motivation for the development of new inhibitor classes. Compounds capable of selectively inhibiting these enzymes are the conformationally locked sugar analogues cyclophellitol (**1**), cyclophellitol aziridine (**2**), α-cyclosulfate (**3**), β-cyclosulfate (**4**) [1] or basic sugar analogues such as the iminosugar 1-deoxynojirimycin (**5**) and its numerous derivatives [2,3]. Motivated by the compelling biological prospects offered by these two substance classes, this presentation will showcase the design, synthesis, and potential biological applications of new bicylic iminosugars, exemplified by compounds **6** and **7** (Figure 1.).

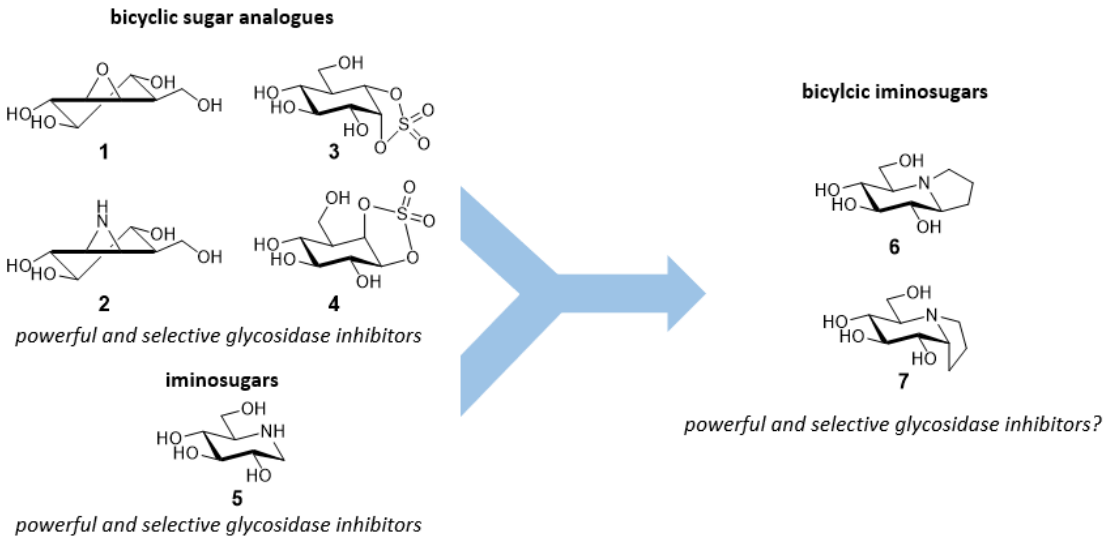

Figure1. Bicyclic iminosugar as potential glycosidase inhibitors.

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DESIGN, SYNTHESIS AND EVALUATION OF BICYCLIC AND SPIRO COMPOUNDS AS GLYCOMIMETIC INHIBITORS OF GALECTINS

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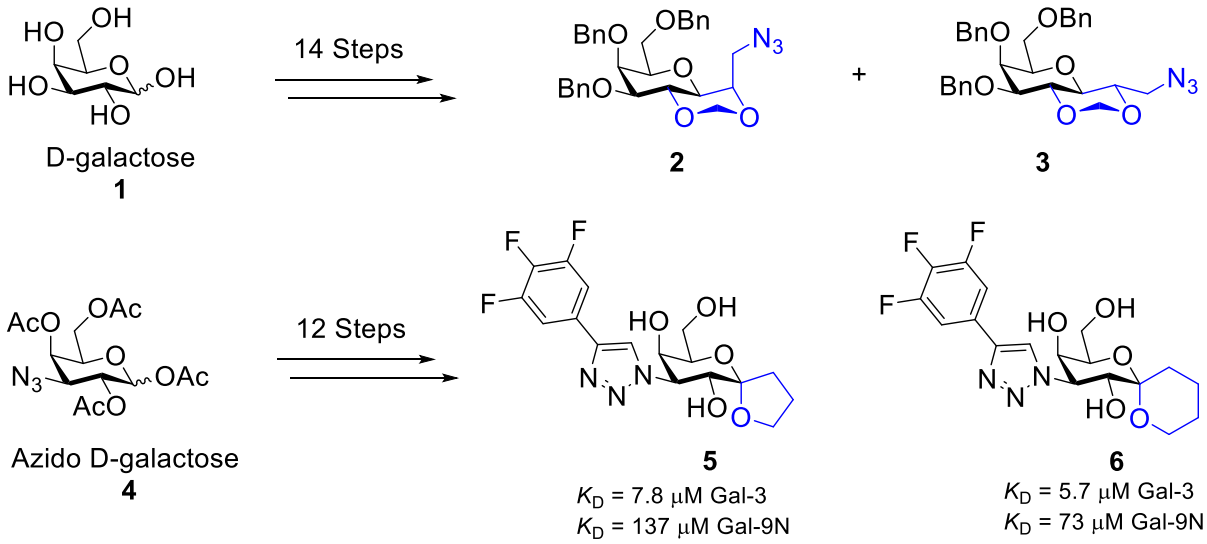
Galectins are soluble β-galactopyranoside binding lectins [1] expressed in numerous cells and play multiple roles in various physiological and cellular processes. Galectin-1 (Gal-1), galectin-3 (Gal-3), galectin-8 (Gal-8), and galectin-9 (Gal-9) were found as the most predominant galectins reported to participate in inflammation, cancer and virus infection.(5) Galectins are classified by the number and structure of CRDs into three major groups: prototype (e.g. galectins-1,7), chimeric (galectin-3) and tandem repeat (e.g. galectin-4,8,9). Tandem repeat galectins have two unique CRDs, referred to as the N and C-terminal domain, separated by a covalently bound peptide linker [2]. Their involvement in disease processes have made galectins interesting targets for inhibitor development.
In this work, both constrained bicyclic compounds and spiro galactopyranosides have been synthesised and evaluated as inhibitors of galectins. The synthetic routes and affinities of various compounds derived from **2** and **3** and spiro compounds **5** and **6** will be presented.



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PEPTIDOGLYCAN DEACETYLASES FROM *BACILLUS SUBTILIS*. SPECIFICITY AND STRUCTURAL INSIGHTS FOR MurNAc AND GlcNAc DEACETYLATION

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Peptidoglycan (PG) is a net-like structure in the bacterial cell wall that envelops the cytoplasmatic membrane with a fundamental role to preserve and protect cell integrity. It is composed of a glycan chain of alternating β-1,4-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) units that are cross-linked by short peptides. PG is constantly remodeled during cell growth and division to incorporate new material. After PG biosynthesis, post-synthetic modifications tightly control the action of autolysins. Among them, de-*N*-acetylation of either GlcNAc or MurNAc residues regulate autolysis and provide resistance to host lysozymes to evade detection by the innate immune system.

PG deacetylases (PG DAs) are members of family 4 carbohydrate esterases (CE4) in the CAZY database, which operate by metal-assisted general acid/base catalysis [1]. Our objective is to understand the different specificities (GlcNAc *vs.* MurNAc PG deacetylases) and evaluate them as novel therapeutic targets for antimicrobials.

Here we analyze the six PG DAs annotated in the genome of *Bacillus subtilis*. PdaA was the first characterized MurNAc deacetylase and its X-ray structure solved [2], being the prototype of a canonical MurNAc deacetylase involved in sporulation. PdaB was isolated later, but with yet unknown substrate [3]. We characterized and solved the X-ray structure of PdaC [4], being a novel subclass of MurNAc deacetylases with dual activity, acting on MurNAc residues of PG but also on GlcNAc residues of chitin oligosaccharides (GlcNAc oligomers). We will report its mode of action and a mutational analysis to modulate specificity and catalytic activity. We also recombinantly expressed and preliminary characterized the other three annotated putative PG deacetylases, YheN, YxkH and YlxY, in the *B. subtilis* genome. YheN and YxkH also show activity on chitin oligosaccharides, whereas YlxY is inactive because it lacks the catalytic base and one metal-coordinating residue in the conserved MT1 motif (Figure 1). Their mode of action and structural and mutational analyses will be discussed.

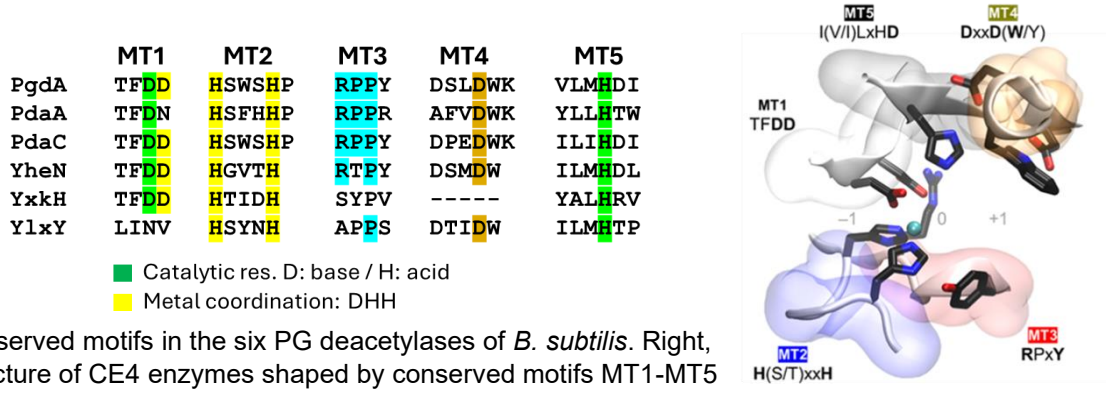

Figure 1. Conserved motifs in the six PG deacetylases of *B. subtilis*. Right, active site structure of CE4 enzymes shaped by conserved motifs MT1-MT5

Acknowledgements: Work supported by grant PID2022-138252OB-I00, MINECO, Spain.

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ENZYMATIC INITIATION AND POLYMERIZATION OF GROUP 2 BACTERIAL CAPSULES

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Capsule polysaccharides (CPS) and wall teichoic acid (WTA) are the most important surface glycans in Gram-negative and Gram-positive bacteria, respectively, and are essential for survival during host colonization [1,2]. CPS present structurally diverse antigens key for the development of glycoconjugate vaccines against pathogenic bacteria such as *Streptococcus pneumoniae, Neisseria meningitidis* and *Haemophilus influenzae* [3]. The enzymes catalyzing capsule assembly are potential drug targets and valuable biotechnological tools to synthesize vaccine antigens. It remains unknown how structurally variable capsule polymers of Gram-negative pathogens are linked to the conserved glycolipid that anchors these virulence factors to the bacterial membrane. Here, we identify two enzymes that synthesize this linker in a large group of Gram-negative pathogens. Using chemically synthesized analogs of the glycolipid, we reconstructed the entire biosynthesis of the capsule polymer, demonstrating that the two enzymes not only produce the linker between the glycolipid and capsule polymer, but also stimulate the capsule polymerase to produce more and longer polymers. We identify the linker as a wall teichoic acid (WTA) type I homolog, demonstrating similarity between the biosynthesis of Gram-positive WTA and Gram-negative capsules. Moreover, our X-ray crystallographic structure of the capsule polymerase highlights two catalytic sites, CgaT and CgoT, for the processive galactose and glycerol-3-P addition of the repeating unit respectively, and a tetratricopeptide repeat (TPR) domain as central element (**Figure 1**), crucial for recognizing the linker and, in turn, mediating processive elongation and modulating the activity of the three enzymes.

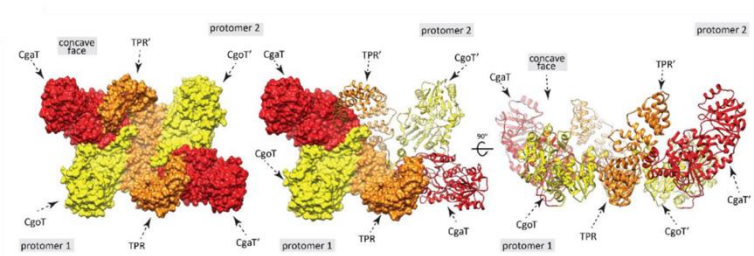

Figure 1. Overall structure of Cps3D surface and ribbon representation of the multimodular architecture of Cps3D as observed in the crystal structure. Each protomer of the homodimer es colored as follos: CgaT red, CgoT yellow, TPR orange.

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REGULATED ASSEMBLY OF LIPOPOLYSACCHARIDE AND ITS BALANCE WITH PHOSPHOLIPIDS IN *ESCHERICHIA COLI* REQUIRED A NEW THIOESTERASE TESD

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The outer membrane (OM) is essential for the viability of Gram-negative bacteria. This OM is asymmetric in nature due to the location of lipopolysaccharide (LPS) in its outer leaflet and phospholipids directed inwards. Maintenance of this asymmetry is crucial for bacterial cell envelope integrity. Bacteria keep a tight balance between the amounts of LPS and phospholipids because they use *R*-3-hydroxymyristate-ACP as the common metabolic precursor. This balance is achieved by regulating amounts of LpxC, which catalyzes the first committed step in LPS biosynthesis. The LpxC enzyme is unstable, and its amounts are determined by its FtsH-mediated proteolysis and several poorly defined signals such as bacterial growth rate, fatty acid composition, FabZ activity and accumulation of lipid A precursors. As FtsH cannot carry LpxC proteolysis by itself, our work discovered key players that either positively regulate FtsH-dependent proteolysis by identifying LapB as an adaptor or negatively by LapC. Based on our genetic and biochemical data, we show that the interaction between FtsH-LapB directs LpxC to the degradation pathway, while LapB-LapC complex formation inhibits this reaction. While addressing how bacteria balance these two pathways and couple them with phospholipid biosynthesis, we discovered additional factors that contribute to LpxC stability and potentially couple lipid A and fatty acid biosynthesis via LapD, the acyl carrier protein and a novel thioesterase TesD. We further show that LapD becomes essential for bacterial viability in the absence of either cardiolipin synthase A or myristoyl transferase LpxM. We show that this lethality can be overcome by mutations in different subunits of acetyl-CoA carboxylase enzyme or by overexpression of the *tesD* gene, due to reduction in fatty acid biosynthesis. Thus, these results reveal a pathway by which bacteria such as *Escherichia coli* balance phospholipids and LPS to maintain outer membrane homeostasis.

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MECHANISM OF RECOGNITION OF LPS BY LAPC AND A REQUIREMENT FOR NEW REGULATORS INCLUDING POLYPHOSPHATE KINASE PPK

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A balance between lipopolysaccharide (LPS) and phospholipids, the two key components of outer membrane, requires a regulated control of the amount of the essential LpxC enzyme that mediates the first committed step in LPS biosynthesis. This is achieved in *Escherichia coli* by proteolytic control of LpxC amounts by the FtsH-LapB complex, which is inhibited by the activity of essential inner membrane protein LapC, whose mode of action is not completely understood. *lapC* mutant bacteria expressing only the essential transmembrane domain of LapC exhibit a temperature-sensitive phenotype, which can be overcome by various suppressors, including overproduction of GnsA, whose function remains unknown. The identification of factors that abrogate GnsA-mediated suppression revealed a requirement for PhoU, a protein involved in the regulation of PhoB/R two-component system, polyphosphate kinase and specific processes involved in cell envelope homeostasis. Examination of LPS of *lapC* mutant bacteria revealed preponderance of glycoforms with a third Kdo and rhamnose in the inner core and the lipid A part, exhibiting non-stoichiometric incorporation of the palmitoyl chain. Overexpression of the *gnsA* gene suppressed the incorporation of palmitoyl chain, PhoB/R-mediated GlcUA incorporation into the LPS inner core and increased the ratio of unsaturated vs saturated fatty acids, revealing a role in balancing LPS composition and membrane fluidity. Using mutagenesis, purification of various LapC mutant proteins and isothermal titration calorimetry (ITC), we show that LapC recognizes LPS using its N-terminal anchor TM1 domain, revealing the mechanism of LapC function in the regulated assembly of LPS.

Acknowledgements: This research was funded by National Science CEnter (NCN) Grant 2023/49/B/NZ1/01986 to S.R.

SUBTLE BUT SYSTEMATIC ANOMERIC PREFERENCES IN SACCHARIDE INTERACTIONS

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Saccharides, particularly hexoses, are essential to a plethora of biochemical processes and find applications in diverse fields. In aqueous solutions, hexoses predominantly exist in two rapidly interconverting cyclic forms (α and β anomers, respectively), with negligible contribution from the linear form. Although interactions of different anomers play crucial roles in processes such as enzymatic recognition and receptor binding, the relatively weak nature of saccharide interactions and the dynamic α/β equilibrium make it challenging to systematically study anomeric binding preferences.
In this work, we address this gap by examining the interactions of several hexoses, including a subset of their derivatives that are methylated at anomeric carbon, which prevents mutarotation and locks the saccharide in a single anomeric state. We explored saccharides' binding to various amino acids (representing nearly every chemical subtype) as well as their self-association. Combining experimental osmotic coefficient measurements with extensive all-atom molecular dynamics simulations (including free energy calculations and corresponding osmotic coefficient predictions), we observed a consistent pattern: α-anomers exhibit slightly stronger interactions with amino acids and with themselves than corresponding β-anomers. These differences, while small and sometimes near the limits of experimental uncertainty, emerged consistently across dozens of saccharide–saccharide and saccharide–amino acid combinations tested in our work. Our simulation analyses suggest that subtle differences in the solvation shell are responsible for this trend. Specifically, the axial orientation of the anomeric hydroxyl group in α-anomers (a consistent feature in the hexoses studied) leads to a less structured saccharide's solvation shell compared to β-anomers, where the hydroxyl group adopts an equatorial position. This eventually favors stronger solute–solute contacts for α-anomers. Furthermore, molecular dynamics simulations show that this trend is even more pronounced in short oligosaccharide sequences composed exclusively of either α or β units. Overall, our findings underscore how minor structural differences can have detectable impact on carbohydrate interactions in aqueous environments. These insights not only deepen our understanding of carbohydrate chemistry but also offer valuable guidance for studying protein–carbohydrate and ligand–receptor interactions.

Acknowledgements: This work was supported by the Czech Science Foundation (grant 24-11274S).

EXPLORING THE POTENTIAL OF COARSE-GRAINED SIMULATIONS: NOVEL MODELS AND BROADER APPLICATIONS FOR SACCHARIDES

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The molecular simulation of saccharides presents a significant challenge due to their structural complexity and conformational heterogeneity. Coarse-grained (CG) models offer a promising solution by enabling the simulation of longer polysaccharide chains at a reduced computational cost. In this work, we present advancements in CG modeling for saccharides, focusing on three main aspects: the development of a CG model based on the Martini 3 force field, efforts to extend it for glycosaminoglycans, and the development of an alternative Monte Carlo-based coarse-grained model for polysaccharides.

Regarding the development, refinement, and validation of the Martini 3 force field for saccharides, we designed a model for glucopyranose-based saccharides and hyaluronic acid. The model, parameterized based on atomistic MD simulations and experimental data, successfully captures the structural and conformational features of saccharides of interest, as well as their interactions with other biomolecules. Most notably, the developed model accurately predicts the location of binding sites for saccharides on the surface of carbohydrate-binding proteins, highlighting its potential for large-scale simulations of carbohydrate-protein-containing biomolecular systems.

As an alternative to molecular dynamics-based simulations, we developed a Monte Carlo-based coarse-grained model (referred to as CG MC) that focuses on the conformational properties of polysaccharides. The CG MC model is designed for rapid conformational sampling of long carbohydrate chains of realistic length (hundreds of monomers). This model uses data from atomistic simulations of short saccharide chains to extrapolate their behavior to longer polysaccharides, incorporating the effects of glycosidic linkage conformations and local structural features. We demonstrate the ability of the CG MC model to predict the persistence length and other polymer properties, showing that even small discrepancies in force fields or local conformational properties can lead to significant differences in the predicted conformations of longer polysaccharide chains. The model's flexibility allows for the simulation of polysaccharides of arbitrary length, making it a valuable tool for large-scale simulations and comparative analysis of different force fields.

Together, these two types of models provide new insights into the behavior of saccharides and enable large-scale simulations of complex carbohydrate systems.

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A COMPARATIVE STUDY OF DIFFERENCES IN BINDING AND CONFORMATIONAL FLEXIBLITY OF THE VANCOMYCIN, DALBAVANCIN AND TEICOPLANIN COMPLEXES WITH THE PEPTIDOGLYCAN FRAGMENT

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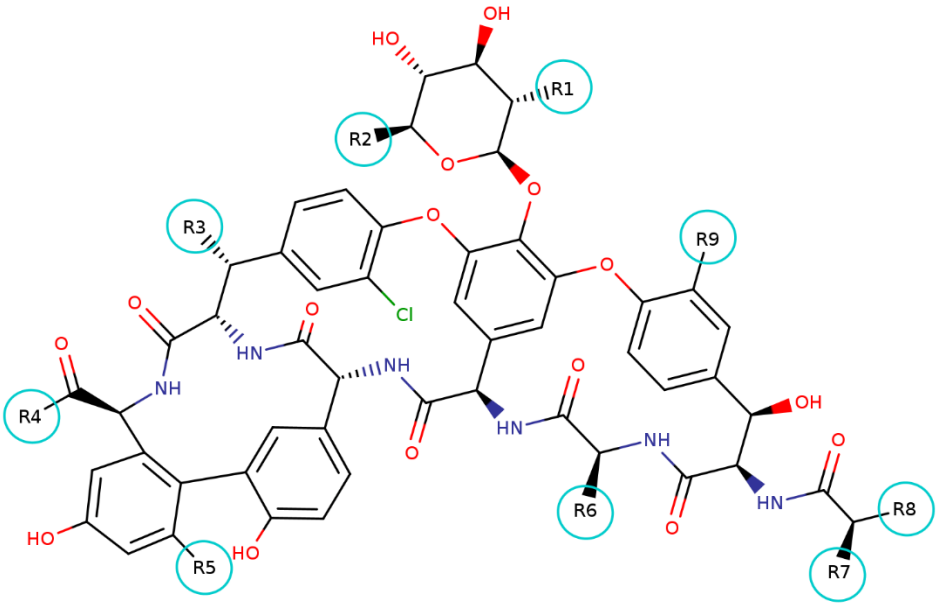
Vancomycin is a glycopeptide antibiotic used in the treatment of serious and multidrug resistant infections caused by Gram-positive bacteria. Vancomycin is composed of a disaccharide (D-glucose and vancosamine) and a heptapeptide core. Dalbavancin and teicoplanin are semisynthetic derivatives of vancomycin.



General structure of antibiotics modeled.

These three antibiotics bind to D-alanyl-D-alanine stem terminus on the bacterial cell wall peptidoglycan precursor. This binding inhibits cross-linking between stem peptides which prevents bacterial cell wall synthesis.
In this study full atom, unrestricted molecular dynamics (MD) in explicit water run on complexes of selected antibiotics (vancomycin; dalbavancin A_0, A_1, B_0, B_1, B_2; teicoplanin A_2-1, A_2-2, A_2-3, A_2-4, A_2-5) with natural bacterial peptidoglycan representative (Ala-D-iGlu-Lys-D-Ala-D-Ala pentapeptide) was done. The sugar moieties (N-acetylglucosamine or a-D-mannose) attached to aglycon (present as R5 in dalbavancin or as R3 and R5 in teicoplanin) show high conformational flexibility. Some conformational freedom is present in the macrocyclic rings within the heptapeptide core as well. The greatest differences occur within the hydrophobic carbon chains/additional sugar moieties (R1 in teicoplanin and dalbavancin). MD trajectories and resultant structures were analyzed in detail and advantages and disadvantages of conformational restrictions resulting from structural modifications were discussed.

Acknowledgements: The computational time in the Academic Computer Center in Gdańsk (CI TASK), Poland is acknowledged.

TOWARD COMPUTER-AIDED DESIGN OF CATALYTIC AMINOGLYCOSIDE – "A WOLF IN SHEEP'S CLOTHING"

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The growing resistance of bacteria to traditional antibiotics has driven the search for innovative strategies to combat pathogenic bacteria. One promising approach to address this issue is the development of catalytic antibiotics. Catalytic antibiotics bind specifically to their biological target, like conventional antibiotics, but also inactivate it through catalytic degradation. This approach would decrease the required drug dosage, thereby reducing side effects, overcome existing resistance mechanisms, and mitigate the emergence of new resistance.
We present two classes of antibiotics for which we have attempted to create a catalytic drug: aminoglycosides and fluoroquinolones. Aminoglycosides target bacterial ribosomes, while fluoroquinolones inhibit DNA topoisomerases, both through reversible non-covalent binding in their standard modes of action. The catalytic mechanism of these antibiotics is based on the hydrolysis of RNA and DNA phosphodiester bonds at their respective binding sites, leading to the irreversible deactivation of the molecular target.
So far, developing an effective ribonuclease among aminoglycoside derivatives has proven challenging [1]. To better understand these difficulties, we solved the cryo-EM structure of the lead aminoglycoside-warhead compound bound to the *E. coli* ribosome and performed molecular dynamics simulations of this complex. We found that the catalytic warhead was unable to induce the in-line conformation of the rRNA backbone required in the classical mechanism of rRNA hydrolysis.
In parallel research on fluoroquinolones [2-4], we found that the 1,4,7-triazacyclononane (TACN) moiety with a guanidinoethyl sidechain in its metal-free form efficiently cleaves DNA under physiological conditions. Since the mechanism of DNA cleavage by TACN warhead does not require a significant conformational change of the DNA backbone, we hypothesized that integrating the TACN-guanidinoethyl moiety within an aminoglycoside scaffold might enable hydrolytic cleavage of rRNA in a similar manner. In addition, by employing a metal-free catalytic warhead, the proposed strategy circumvents the issues linked to metallic compounds, such as loss of nuclease activity under physiological conditions [2-4].
We discuss how modern computational methods and tools can facilitate the rational design of aminoglycosides with ribonuclease capabilities, presenting a promising direction for future research in combating antibiotic resistance.

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DIBLOCK CO-POLYSACCHARIDES: SYNTHESIS, CATION-INDUCED SELF-ASSEMBLY, AND BINDING AND DELIVERY OF RADIONUCLIDES

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The conjugation at chain termini of two different polysaccharides provides diblock co-polysaccharides, a new class of precisely engineered polysaccharides. This architecture provides on one hand new solution and stimuli-responsive self-assembly properties, while retaining key properties such as biodegradability on the other.
The first part of the presentation will focus on the preparation of blocks through dioxyamine linkers [1–3]. The second part will focus on diblocks containing Ca-reactive oligoguluronates [4] (derived from alginates) and their Ca-induced self-assembly to nanoparticles using static and dynamic light scattering, AFM, SEC-MALLS, SANS and SAXS for their characterization [5].
The final part describes the binding and release of radionuclides and related cations in a dialysis assay.

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SELF-ASSEMBLED COMBINATORIAL SURFACES MIMICKING HEPARAN SULFATE BIOACTIVIY: FROM 2D SCREENING TO IFN-γ INHIBITION OR DELIVERY IN CELLULAR CONTEXT

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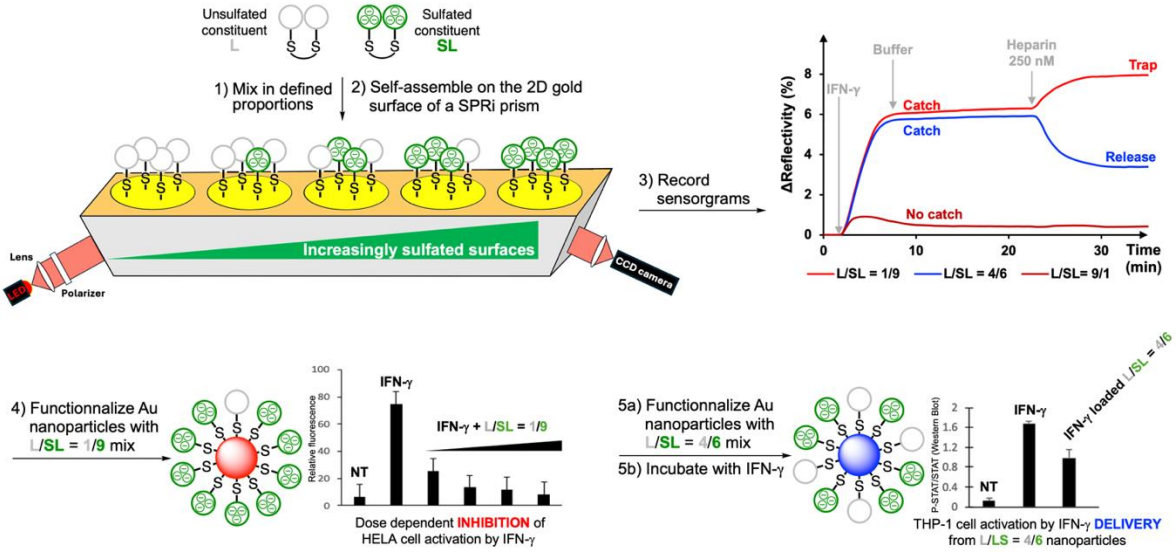
We have previously shown that 2D combinatorial surfaces, prepared by self-assembly on a gold flat (2D) surface of mixtures with controlled composition of sulfated or non-sulfated disulfide building-blocks derived from lactose, were able to mimic some biological properties of heparan sulfate.[1,2] Here we present, taking IFN-γ as model, the achievement of a new milestone by showing that the binding properties of combinatorial 2D surfaces of a given composition can be transferred to soluble 3D nanoparticles. In this way we generated water-stable 3D devices able in cellular context to either inhibit the cytokine, by catching and trapping it, or serve as delivery device, by catching and releasing it, these behaviors being surface composition dependent. We will also disclose a general synthetic methodology for the preparation of disulfide building-blocks suitable for gold nanoparticle functionalization.



This approach offers an easy implementable screening assay that can be used to engineer complex water-stable 3D device harboring distinct properties and pave the way for the discovery of new hybrid glyco-materials with potential for biomedical applications.

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GLYCO-GOLD NANOPARTICLES DECORATED WITH IMINOSUGARS AS MULTIVALENT ENHANCERS OF GCASE ENZYME

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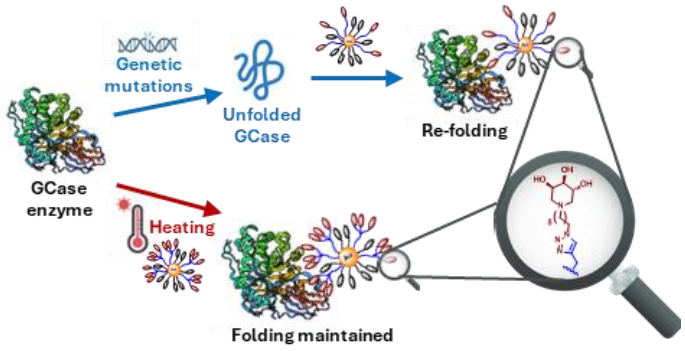
The lysosomal enzyme β-glucocerebrosidase (GCase) hydrolyses the glucosyl moiety of glucosylceramide and glucosylsphingosine. GCase defective activity is involved in the metabolic disorder Gaucher Disease and, more recently, emerged to be correlated also with Parkinson's disease [1]. Therefore, functional GCase enhancers may act as new therapeutics for such pathologies, both lacking disease-modifying drugs.
In this context we report the first multivalent iminosugars (namely, *N*-heterocyclic sugar analogues) built onto a glyco-gold nanoparticle core (glyco-AuNPs) capable of stabilizing or enhancing the activity of GCase. An *N*-nonyltrihydroxypiperidine was selected as the bioactive iminosugar unit and further functionalized, via copper catalysed alkyne-azide cycloaddition, with a thiol-ending linker that allowed the conjugation to the gold core. The bioactive ligands were obtained with either a linear monomeric or a dendritic trimeric arrangement of the iminosugar. The concentration of the bioactive iminosugar on the gold surface was modulated with different amounts of a glucoside bearing a short thiol-ending spacer as inner ligand. The new mixed-ligand coated glyco-AuNPs were fully characterized with different techniques (UV-vis, TEM, ^{1}H-NMR). The biological evaluation suggested a different behaviour in stabilizing wild-type recombinant GCase or mutant GCase from Gaucher Disease patients' cells depending on the iminosugar presentation (e.g. linear vs branched ligand) [2].



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LOOKING INTO NEW DIRECTIONS FOR GLYCONANOMATERIALS: GLYCO-NANORADIOSENSITIZERS FOR PRECISION RADIOTHERAPY IN CANCER

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Glyconanomaterials clearly emerged as appealing tools with an immense potential in nanomedicine applications. Their implications in a wide range of biomedical investigations (i.e. studies of glycans-lectins interactions, development of cancer/pathogens vaccine prototypes and precision drug delivery systems, to mention some) stem for their unique properties [1]. The list of glyconanomaterials available to date is quite extensive and the huge amount of knowledge collected so far in this field has the potential to open new perspectives for such tools.

In this framework, we have recently reported on a modular, and functional glyconanomaterial that combines the complementary properties of a polysaccharide-based nanomaterial, the cellulose nanocrystal (CNC), with small-sized gold nanoparticles (AuNPs) which are stably embedded into the CNC matrix. It is a versatile glyconanomaterial that provides a conceptual advance in the field: it can be prepared on a scale of grams, and its surface is easily engineered with structurally different bioactive headgroups and with high batch-to-batch reproducibility [2-4].

In this presentation, we describe how the peculiar structure of our CNC-AuNPs allowed us to investigate its versatility in either conventional or more advanced applications. In particular, we will discuss in detail one of our *business cases*: its implication as nanoradiosensitizer in precision radiotherapy in oncology. The combination of X-rays and the CNC-AuNPs sensitizes radioresistant tumors to radiotherapy treatment and, notably, allows us to use half a dose of radiation with the same therapeutic effect [4].

Acknowledgements: Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3277 of 30 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code ECS_00000017, Concession Decree No. 1055 of 23 June 2022 adopted by the Italian Ministry of University and Research, CUP B83C22003930001, project title "Tuscany Health Ecosystem – THE".

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PATTERNED PRECISION: CONSTRUCTING BIOACTIVE SUPRAMOLECULAR CARBOHYDRATE-BASED NANOMATERIALS

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The precise patterning of nanostructure surfaces with ligands offers the potential to create materials with unique physical and chemical properties. Such materials could be particularly powerful in biological signaling contexts due to the multivalent presentation of epitopes. The "bottom-up" approach based on self-assembly offers a promising pathway to develop modular and tunable supramolecular materials with patterned surfaces. However, a significant challenge remains: most scaffolds struggle to incorporate bulky or hydrophilic ligands, such as carbohydrates.

In our recent work, we demonstrated the utility of cellulose oligomers in creating nanomaterials with tunable shapes and properties [1-3]. The antiparallel chain orientation of these oligomers makes cellulose-based scaffolds particularly promising for constructing nanomaterials capable of presenting a precise pattern of various carbohydrate ligands on the surface of self-assembled nanocrystallites. To demonstrate the potential of this system, we developed glycan-functionalized nanocrystals and expanded the approach to create supramolecular carbohydrate-based hydrogels. Furthermore, we combined these concepts to produce densely glycosylated hydrogels with diverse glycan epitopes (Figure 1).

We explored the bioactivity of these materials, which serve as simplified mimics of complex extracellular matrices due to their dense glycan presentation and material properties. Notably, we observed a significant impact of carbohydrate presentation from the patterned surface on the morphology of *Candida albicans*, providing insights into host-fungi interactions. This work highlights the versatility and potential applications of these novel materials in biological contexts.

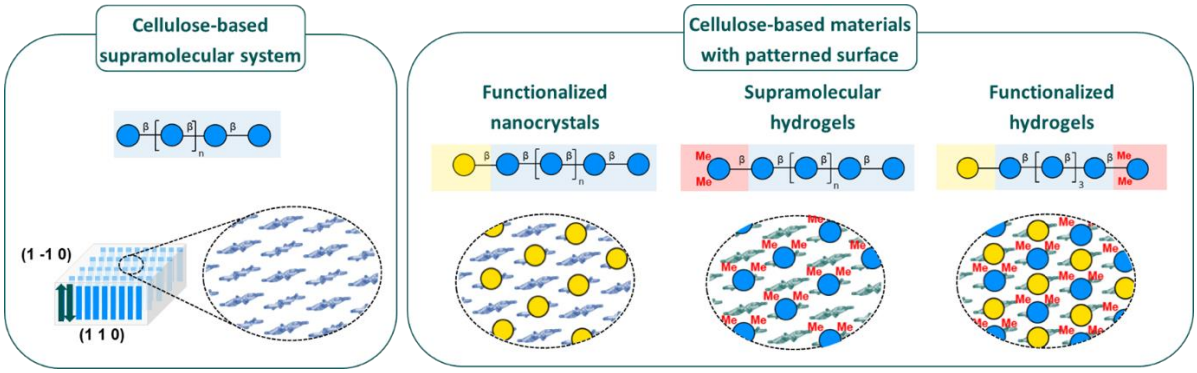

Figure 1. Cellulose-based supramolecular materials with patterned surface.

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INVESTIGATING THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF CAPSULAR POLYSACCHARIDE FROM *PSYCHROBACTER SP.* TAE2020

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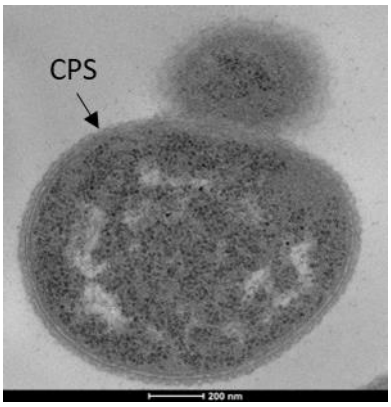
The extracellular polysaccharides produced by extremophilic bacteria stand out for their structural complexity, often characterized by the presence of rare sugars [1]. *Psychrobacter* sp. TAE2020 is a vesiculating γ-proteobacterium isolated from an Antarctic coastal seawater sample [2].



TEM images of thin sections of *Psychrobacter sp.* TAE 2020.

This marine bacterium can produce and secrete molecules endowed with surfactant and emulsifying properties. We recently isolated and purified the anti-adhesive and emulsifying polysaccharide-protein complex, CATASAN. The complex can reduce biofilm formation and the detachment of biofilm of the nosocomial bacterium *Staphylococcus epidermidis* [3]. Here we reported the purification and the characterization of the polysaccharide portion of CATASAN produced from *Psychrobacter* sp. TAE2020. The polysaccharide was found to be associated with cells as a capsule (CPS). The purified polymer was analysed using chemical analyses, and NMR spectroscopy technique and consists of a tetrasaccharide repeating unit containing all aminosugars. Anti-biofilm assay and confocal laser scanning microscopy suggested that CPS reduces *S. epidermidis* biofilm formation without affecting cell viability and leads to a more unstructured and heterogeneous biofilm. Finally, the adhesion of CPS on liposomes has been evaluated.

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MONOGLYCOSYLCERAMIDE LIPID STRUCTURE-DEPENDENT CD1D FUNCTIONAL STABILIZATION AND SELECTIVE IMMUNOMODULATION

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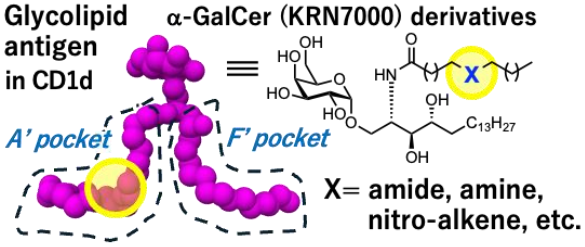
On antigen-presenting cells, the lipid antigen-presenting molecule CD1d recognizes glycolipid antigens and forms complexes that activate NKT cells, leading to the secretion of various cytokines that regulate immune responses. Recently, we have shown that endogenous monoglycosylceramides, such as GlcCer and GalCer, strongly bind to CD1d, stabilizing its structure while partially inhibiting its function. Among these, endogenous sphingosine-type β-GlcCer exhibited stronger binding affinity to CD1d than α-and β-GalCer [1]. Additionally, it was recently identified that dihydrosphingosine-based saturated α-GalCer serves as an endogenous active lipid antigen in mammals [2]. Meanwhile, through the exploration of lipid-modified KRN7000 type α-GalCer glycolipid antigens of CD1d, we also demonstrated that the binding affinity, cytokine induction activity and selectivity can be modulated by modifying the acyl group with polar functional groups [3], including covalent-ligand-type reactive group modification [3b].

To further investigate CD1d-ligand interactions, we developed highly Th2-biased glycolipids, performing synthesis and biofunctional evaluation of the novel α-GalCer derivatives [4], along with computational analysis to visualize the dynamics of the CD1d-ligand complex. Namely, for the synthesis of the novel α-GalCer derivatives with modified acyl groups with nitro group, we developed a novel synthetic method to introduce functional groups to the acyl groups, and achieved a highly efficient synthesis. The biological activities and functions of these derivatives were evaluated by binding affinity assays with the CD1d protein and cytokine induction assays using mouse splenocytes. Comparisons with previously reported amide- and amine-modified Th2-biased α-GalCer derivatives revealed that the newly synthesized compounds exhibit enhanced CD1d binding affinity and a significantly higher Th2-selective cytokine induction profile. Molecular dynamics (MD) simulations were also conducted to analyze the binding modes of these glycolipids, further elucidating their interactions with CD1d.



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CHEMICAL EXTENSION AND GLYCODENDRIMER FORMATION OF THE MATRIGLYCAN DECASACCHARIDE, (-3Xylα1-3GlcAβ1-)$_5$ AND ITS AFFINITY FOR LAMININ

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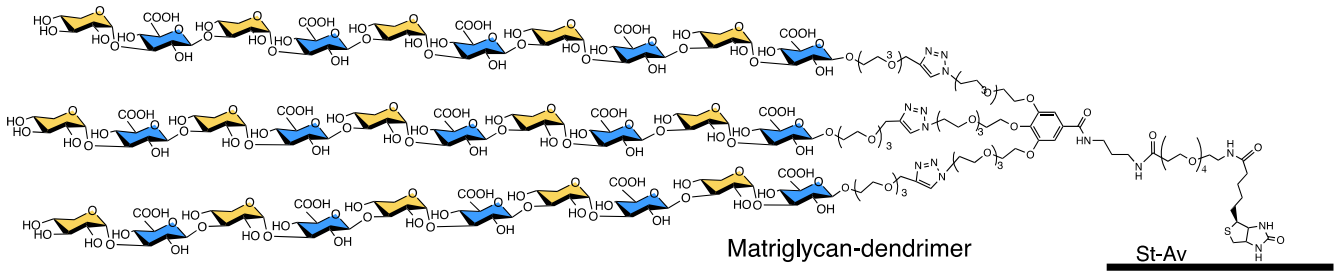
Muscle tissue is stabilized by the strong interaction between laminin and matriglycan. Matriglycan is a polysaccharide composed of the repeating disaccharide, -3Xylα1-3GlcAβ1-, and is a pivotal part of the core M3 *O*-mannosyl glycan. Patients with muscular dystrophy cannot synthesize matriglycan or the core M3 *O*-mannosyl glycan due to a defect in or the lack of glycosyltransferases owing to glycan synthesis. We herein report the synthesis of a matriglycan-repeating decasaccharide and a dendrimer comprising three branches of the decasaccharide. The glycan was regio- and stereoselectively synthesized by the stepwise addition of the corresponding disaccharide unit. The immobilized decasaccharide and glycodendrimer on the sensor tip via biotin and streptavidin bound to laminin-G-like domains 4 and 5 of laminin-α2. The dissociation constants of the decasaccharide and dendrimer obtained from bio-layer interferometry (BLI) were estimated to be 4.4×10^{-8} M and 6.8×10^{-8} M, respectively, showing higher affinity than those of a matriglycan-repeating hexasaccharide (1.6×10^{-7} M) and the dendrimer (1.8×10^{-7} M) [1].



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TRITERPENOID SAPONINS BEARING LEWIS-X AND QS-21 EPITOPES: ANTIVIRAL, TOXICOLOGICAL, AND IMMUNOLOGICAL EVALUATION

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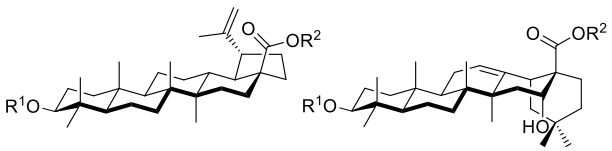
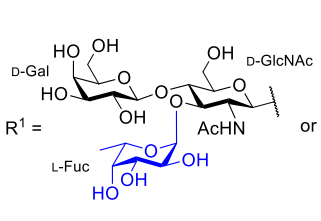
The syntheses of betulinic acid and echinocystic acid saponins bearing either a Lewis-X trisaccharide [1] or the minimal QS-21 trisaccharide epitopes required for adjuvant activity [2] are described (Figure 1). These chimeric triterpenoid saponins were synthesized using convergent, stereoselective, and efficient glycosylation strategies involving various glycosyl donors. We demonstrate that Lewis-X-containing triterpenoid saponins are among the most potent monovalent inhibitors reported to date of dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) and liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN)-mediated transfer of human immunodeficiency virus 1 (HIV-1) infection to CD4-positive cells, with IC_{50} values in the low micromolar range (21-50 μM). These triterpenoid saponins, along with their rhamnose-modified analogs [3], were evaluated *in vivo* for their toxicological and immunological potential in both C57BL/6 and hDC-SIGN transgenic mice. Our findings reveal that, while the synthetic saponins exhibit low toxicity, replacing echinocystic acid with betulinic acid negatively impacts their immunogenicity profiles. This work provides a valuable foundation for the development of saponin-based antiviral agents and highlights the potential of these glycosylation strategies for synthesizing complex and unnatural glycoconjugates for therapeutic and prophylactic applications.

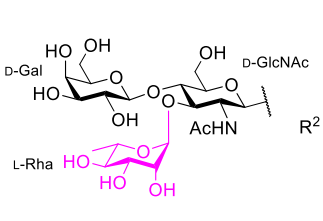


betulinic acid: $R^1 = R^2 = H$
R^1 = Lewis-X; R^2 = H
R^1 = RhaLewis-X; R^2 = H
R^1 = H; R^2 = QS-21 epitope

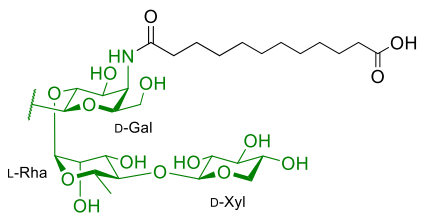
echinocystic acid: $R^1 = R^2 = H$
R^1 = Lewis-X; R^2 = H
R^1 = RhaLewis-X; R^2 = H
R^1 = H; R^2 = QS-21 epitope



Lewis-X
(*ref. 1*)



RhaLewis-X
(*ref. 2*)



QS-21 epitope
(*ref. 3*)

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INVESTIGATION TOWARDS THE SYNTHESIS OF ALKYL *C*-GALACTOFURANOSIDES DERIVATIVES WITH POTENTIAL ANTILEISHMANIAL PROPERTIES

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Leishmaniasis, a disease caused by protozoan parasites of the genus *Leishmania* and transmitted by the bite of infected sandflies, poses a significant global health challenge. Although this infection is a treatable and curable disease, its treatment faces many challenges. Current drugs suffer from limited availability, side effects, long treatment durations, high costs, and drug resistance issues. Therefore, there is a critical need for the development of new antileishmanials that are not only more effective but also safe and affordable. The abundance of rare carbohydrates assembly with β-D-galactofuranoside linkage within the glycocalyx of these microorganisms [1] led to the exploration of their biochemical pathways as promising antimicrobial targets. Inspired by antimicrobial-active octyl β-D-galactofuranoses [2], hydrolytically resistant alkyl *C*-galactofuranosides were designed as promising antimicrobial agents. The main synthetic approach to access β-D-galactofuranosyl lipids and other analogs relied on the C-1 allylation of glycosyl acetates, followed by cross-metathesis with various α-olefines and hydrogenation (Figure 1). 22 derivatives were isolated and their biological evaluation against both *Leishmania major* promastigote and *L. mexicana* amastigote revealed antileishmanial activity of 1-(glycosyl)heptadecanes, exhibiting IC_{50} values of ~50 µM [3]. Notably, 1-(β-d-arabinofuranosyl)heptadecane and its enantiomer showed promising antimicrobial efficacy with IC_{50} values and selectivity index comparable to the conventional antileishmanial drug miltefosine, highlighting their promising therapeutic efficacy.

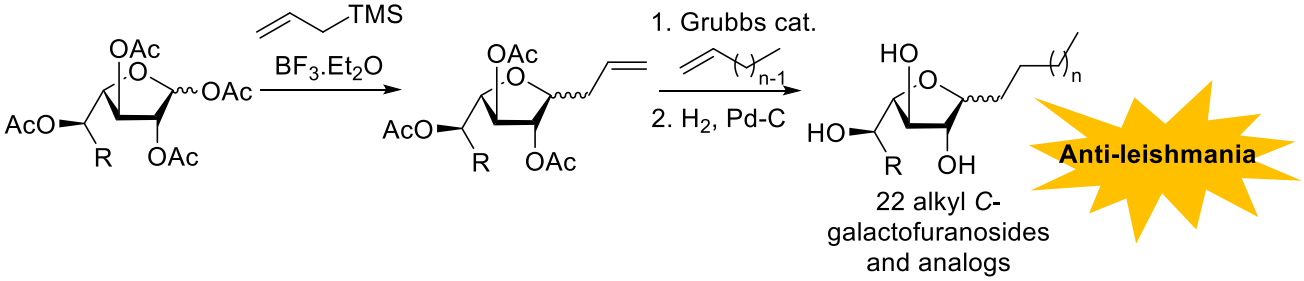

Figure 1

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FAST AND EFFECTIVE PREPARATION OF HIGHLY CYTOTOXIC HYBRID MOLECULES OF SCHWEINFURTHIN E AND OSW-1

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We present the first synthesis of hybrid molecules combining two biologically active compounds: Schweinfurthin E (SW-E) and OSW-1, both exhibiting very powerful antiproliferative properties. Schweinfurthins (SWs) are a family of natural prenylated stilbenes isolated from Macaranga. Some of them contain a hexahydroxanthene (HHX) moiety, which is essential for their biological properties [1]. SW's pharmacological profile is distinct from traditional chemotherapy agents, suggesting it targets a novel biological pathway, specifically the OSBP protein [2], which regulates cholesterol transport between the endoplasmic reticulum and the Golgi apparatus. OSW-1, on the other hand, is a steroid with a disaccharide moiety crucial for its cytotoxicity. The integrity of this disaccharide unit is essential for maintaining its activity, and it likely contributes to hydrophobic clustering that enhances the compound's potency [3]. The preparation of these hybrids was achieved through a CuAAC reaction involving a polyfunctionalized alkyne derived from SW-E and various azido sugars. Additionally, we developed a novel and efficient method for preparing the disaccharide of OSW-1 through sequential functionalization of L-arabinose, facilitated by a boronic ester as a switchable protective/activating group [4]. This study allowed us to improve the existing synthesis routes of OSW-1 disaccharide by reducing the number of steps and purifications, thereby saving time and simplifying manipulation. The cytotoxicity of the hybrids was also evaluated, with some being much more cytotoxic than SW-E on a glioblastoma cancer cell line [5]. Finally, a molecular modeling study was carried out to rationalize the biological results obtained.

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DEVELOPMENT OF A SYNTHETIC METHOD OF *C*-ACYL GLYCOSIDE VIA PHOTOREDOX COUPLING

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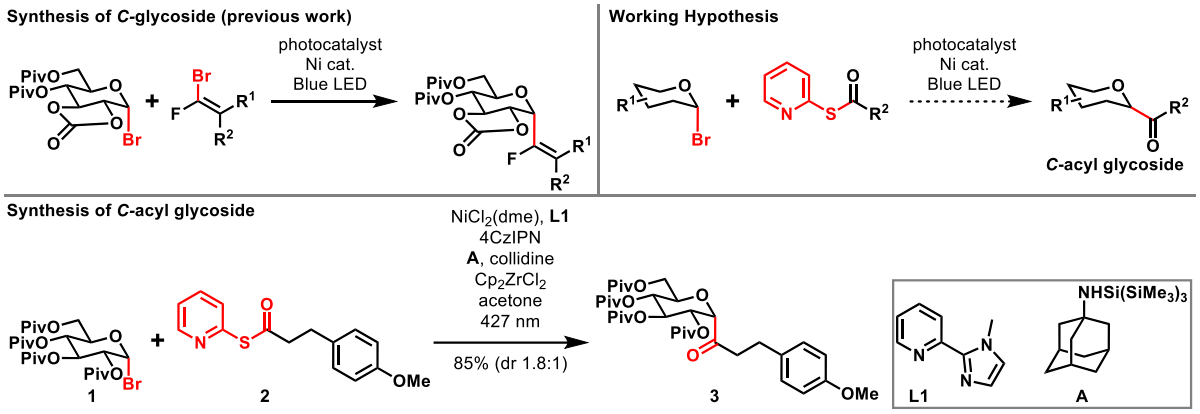
Glycoconjugates are biologically important molecules, yet their precise roles in many biological processes remain poorly understood due to their instability under conditions such as acidic and enzymatic hydrolysis. *C*-glycosides, which feature a *C*-glycosidic bond in place of the *O*-glycosidic bond, are attractive analogs of native glycans because they are resistant to hydrolysis. Recently, we demonstrated that *C*-glycosides can enhance or modify the biological activities of their parent molecules [1]. While numerous synthetic methods for *C*-glycosides have been developed, reports on *C*-acyl glycosides remain scarce. To address this, we developed a novel method for the synthesis of *C*-acyl glycosides.

We have developed a photoredox/Ni-catalyzed reductive cross-coupling strategy to synthesize fluorovinyl *C*-glycosides from Br-sugars [1]. We hypothesized that replacing the alkenyl bromide with a suitable acyl donor could produce *C*-acyl glycosides.

Inspired by Weix and Kishi's reports [2,3], we explored the cross-coupling reaction between Br-sugar and pyridyl thioester. Through optimization, we found that Br-sugar **1** and thioester **2** gave desired coupling adduct **3** under photoirradiation with blue LED in the presence of $NiCl_2$(dme), **L1**, 4CzIPN, aminosilane **A**, collidine, and Cp_2ZrCl_2. This protocol proved to be broadly applicable for various Br-sugars and thioesters, enableing the synthesis of *C*-acyl glycosides.



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YERSINIA ENTEROCOLITICA O:3 OUTER MEMBRANE VESICLES – CHARACTERISTICS AND MOLECULAR BASICS FOR INTERACTION WITH COMPLEMENT SYSTEM

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Originating from the outer membrane, outer membrane vesicles (OMVs) contain cell surface structures, including lipopolysaccharide (LPS, endotoxin). Even after eliminating living pathogen cells, those small vesicles are still capable of reaching distant organs and triggering immune response [1].
We investigated OMVs secreted by *Yersinia enterocolitica* O:3 (*Ye* O:3) and their interaction with human complement. The species is responsible for yersiniosis, intestinal disorders and has capacity to survive and multiply in a wide range of temperature (4-40°C) [2]. OMVs were isolated form bacteria grown at 4, 22, 37°C; and from four strains characterised by S, Ra, Rd1 and Re chemotypes of LPS [3]. Their concentrations and size were analysed by DLS, NTA, and TEM. Regardless of the growth temperature and LPS chemotype, OMVs were capable of complement activation, including classical, alternative, and lectin pathways. *Ye* O:3 OMV were recognised by human mannose binding lectin (MBL), but not by ficolins. We have identified the EPS, [→2)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→]$_n$, as the ligand recognised by MBL. Proteins, DNA and mannans derived from LB culture medium were excluded as ligands. OMVs activated the THP-1 cells to express mRNA for the IL-8, IL-10, IL-6 and TNF-α genes, what was dependent on lipid A structure and inhibited the killing activity of NHS serum against *Ye* O:3. Treatment of mice with OMVs caused some symptoms of SIRS, that were partially dependent of the complement.
The obtained results confirm the hypothesis that *Ye* O:3 OMVs may serve as a "shield" for the bacteria protecting from the complement system and constitute a strong activator of the inflammatory response, which is important for a better understanding of the development of infections and sepsis.

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STRUCTURAL CHARACTERIZATION AND IMMUNE RECOGNITION OF PAENALCALIGENES HOMINIS LIPOPOLYSACCHARIDES: EXPLORING POTENTIAL HOST-PATHOGEN INTERACTIONS

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The human gut microbiota plays a fundamental role in maintaining immune homeostasis and protecting against pathogenic invasions [1]. However, shifts in microbial composition, particularly in ageing populations, have been linked to inflammatory and neurodegenerative disorders. *Paenalcaligenes hominis*, a Gram-negative bacterium increasingly detected in the gut microbiota of elderly individuals, has been implicated in cognitive decline and gut-brain axis dysfunction. Despite this growing evidence, little is known about its molecular components and their role in host-pathogen interactions [2-3].
In this study, we focus on the structural and immunological characterisation of *P. hominis* lipopolysaccharide (LPS), a key mediator of bacterial-host communication. Using a multidisciplinary approach that combines chemical analyses, spectroscopy, computational modelling, and biophysical assays, we define the structural features of *P. hominis* LPS and its interaction with the immune system. Our findings reveal a distinct O-antigen composition enriched in rhamnose and glucosamine residues, with a non-stoichiometric O-acetylation pattern that could modulate immune recognition. Lipid A analysis identified a penta-acylated structure, correlating with reduced Toll-like receptor 4 (TLR4) activation compared to *Escherichia coli* LPS. Notably, microarray binding assays demonstrated recognition of *P. hominis* LPS by key immune lectins, including Ficolin-3 and Galectin-4, suggesting alternative immune pathways that may compensate for its weak TLR4 stimulation.
These findings provide the first detailed characterisation of *P. hominis* LPS and its immunomodulatory properties, offering insights into its potential role in microbiota-driven inflammation and neurodegeneration. Given its increasing prevalence in ageing individuals, further research is warranted to elucidate its contribution to gut dysbiosis and its broader implications in host immune regulation.

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ESCHERICHIA COLI P2B POLYSACCHARIDE AS A LIGAND FOR COMPLEMENT LECTIN PATHWAY ACTIVATING FICOLIN-2 AND 3

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Ficolin-2 and ficolin-3 are important factors of innate immunity. They are able to participate in elimination of pathogens through opsonisation of their cells (contributing to phagocytosis) or complement activation *via* the lectin pathway (thanks to co-operation with serine proteases of the MASP family), leading to the direct cell lysis. They may form heterocomplexes, called ficolin-23. While ficolin-3 specificity is restricted to very few microorganisms, ficolin-2 recognises numerous bacteria, fungi, parasites, and viruses. Among its ligands, capsular polysaccharides, lipoteichoic acids, mycobacterial lipoarabinomannan and antigen 85 complex, *Streptococcus pneumoniae* pneumolysin, 1,3-β-glucans, and hemagglutinin of influenza A virus are mentioned. Moreover, artificial ligands like acetylated albumin (Ac-BSA) or N-acetylglucosamine pentamer (GN5-DPPE) were previously used to detect active ficolin-2 molecules (although no ficolin-2-dependent complement activation by Ac-BSA was observed) [1,2].

Here we report interaction of ficolin-2 and ficolin-3 with *Escherichia coli* P2b (serotype O15) bacteria, isolated from blood of preterm neonate, born with signs of intrauterine infection. Both ficolin-2 and ficolin-3 (as well as murine ficolin A, but not human ficolin-1 or mannose-binding lectin) were found to recognise high molecular mass fraction of supernatant obtained after ultracentrifugation of *E. coli* P2b lipopolysaccharide (LPS). Upon recognition of that fraction by ficolin-2/ficolin-3, MASP-1 enzyme became activated leading to complement lectin pathway activation.

Structural analyses of composition of the supernatant indicated the presence of O15 LPS, enterobacterial common antigen (ECA_{PG} and ECA_{CYCLIC}), and capsular polysaccharide (CPS, antigen K) built up of the $[\rightarrow 3)$-α-D-Glc*p*NAc-$(1\rightarrow P]_n$. Immunoblotting and ELISA analyses indicated CPS as a functional ligand for ficolin-2 and 3, and excluded O15 LPS and ECA. Identified *E. coli* P2b CPS represents previously described K51 antigen reported as $[\rightarrow 3)$-α-D-Glc*p*NAc-$(1\rightarrow P]_n$ with *O*-acetyl groups at 4 and 6 position [3], however contrary to the published structure, identified CPS was devoid of *O*-acetyl groups.

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MODELLING INSIGHTS INTO GROUP B STREPTOCOCCUS CPS: USEFULLY INFORMING VACCINE DESIGN AND PROCESSING

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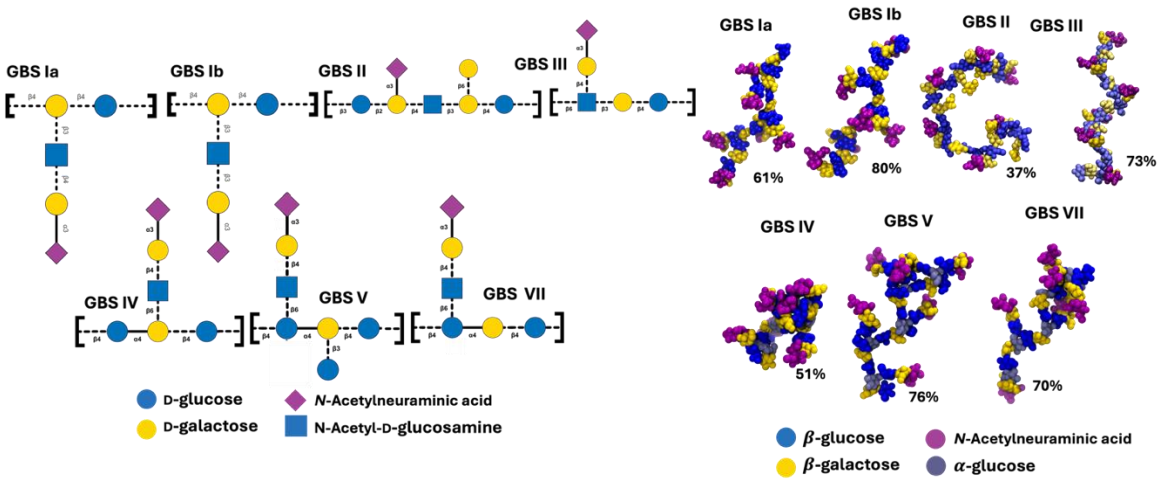
Group B Streptococcus (GBS) is a bacterial pathogen causing significant morbidity and mortality in pregnant women and infants especially in resource-limited settings [1]. Vaccination is a promising approach to reducing disease burden and overcoming antimicrobial resistance [2]. As such, hexavalent vaccine candidates are in development, incorporating the capsular polysaccharides (CPSs) of the most prevalent serotypes (Ia, Ib, II, III, V and IV or VII) as CPS [3]. Such a vaccine is predicted to have a significant impact on reducing disease burden through both direct and cross-protection [4].

Cross-reactivity and resulting cross-protection against different antigens occurs when common conformational epitopes are present. Polysaccharide conformation is difficult to study with laboratory techniques, thus we performed molecular modelling studies comparing the conformation and dynamics of GBS CPSs. Our comparative analysis reveals key conformational differences that provide mechanistic rationale for observed vaccine processing challenges as well as immunologic differences that could account for cross-protection or lack thereof. Ultimately this work links CPS antigen structure to conformation and function providing deeper mechanistic understanding of the immunologic aspects of antigen selection supporting multivalent vaccine development to reduce GBS-related infant and maternal mortality.



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GENERATION OF GLYCOSYLIUM IONS IN SUPERACID: ENZYMATIC RELEVANCE AND SYNTHETIC POTENTIAL

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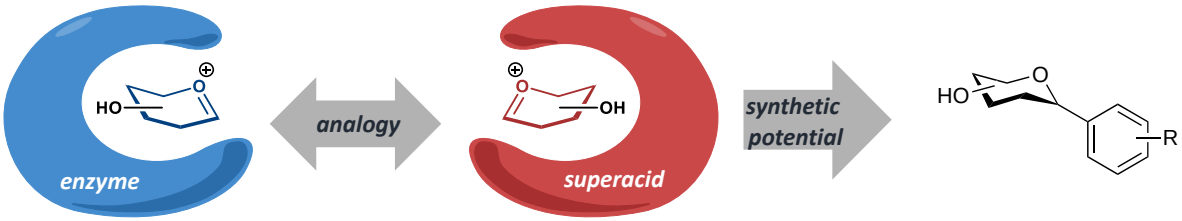
The hydrolysis of glycosides is a biochemical transformation that occurs in all living organisms, catalyzed by a broad group of enzymes, including glycoside hydrolases. These enzymes cleave the glycosidic bond via a transition state with substantial oxocarbenium ion character, resulting in short-lived oxocarbenium ion-like species [1]. While such transient species have been inferred through theoretical studies [2] and kinetic isotope effect measurements [3], their direct spectroscopic characterization remains challenging [4]. Here, we exploit a superacid environment to generate, accumulate, and fully characterize nonprotected glycosylium ions using low-temperature NMR spectroscopy, supported by DFT calculations. Additionally, QM/MM MD simulations reveal that the properties of these glycosyl cations in superacid closely resemble those within the active sites of glycosidase enzymes, particularly in terms of conformation and anomeric charge distribution [5]. Finally, the synthetic potential of these glycosylium ions is illustrated through the one step synthesis of *C*-aryl glycosides starting from simple glycosides.



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CO-FACTOR PROSTHESIS FACILITATES THE BIOSYNTHESIS OF PSEUDAMINIC ACID DERIVATIVES

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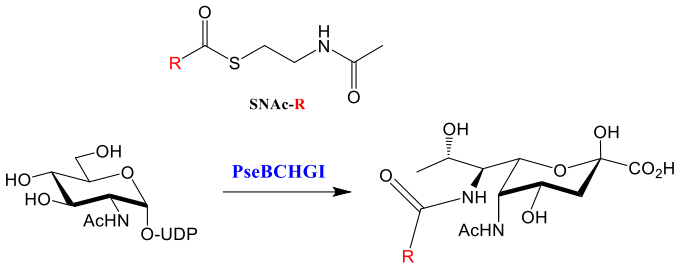

5,7-Diacetyl pseudaminic acid (Pse5Ac7Ac) and its analogues are rare non-mammalian sugars, belonging to the nonulosonic acid family, that are widely distributed in nature and found in diverse cell-surface glycoconjugates. Pse sugars are particularly prevalent in pathogenic bacteria, including many bacteria on the WHO global priority pathogens list, and have proposed roles in flagellar assembly, immune evasion and biofilm formation. Considering the importance of these sugars in bacterial virulence, understanding their biosynthesis is of great interest in the development of new chemical probes to study the sugar, as well as in the identification of new drug targets. We have previously reconstituted the biosynthesis of the nucleotide activated form of the sugar, CMP-Pse5Ac7Ac in vitro, starting from UDP-GlcNAc and using enzymes from *C. jejuni* (PseBCHGI) and *A. caviae* (PseF) [1,2]. The third enzyme in the pathway, PseH is an aminoglycoside *N*-acetyltransferase that catalyses acetyl transfer to C4 of UDP-4-amino-4,6-dideoxy-β-ι-AltNAc, using acetyl-CoA (Ac-CoA) as a co factor. In this work we explore the use of the truncated thioester *N*,*S*-diacetyl cysteamine (SNAc) as a PseH co factor mimic, in an approach we denominate “co-factor prosthesis” [3]. Using this approach, we demonstrate the utility of SNAc as an alternative co factor to the costly Ac-CoA, in the biosynthesis of Pse5Ac7Ac. Additionally, we explore the use of SNAc analogues to facilitate the introduction of both natural and unnatural functional groups into the N7-position of the pseudaminic acid backbone.

Acknowledgements: This work was supported by The University of York, the EPSRC (PhD award to E. K. F. P.), the Rosetrees Trust (PhD award to H. C.), and a Horizon Europe Guarantee Consolidator award to M. A. F. (selected by the ERC, funded by UKRI; EP/X023680/1).

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STRUCTURAL INSIGHTS INTO MUCIN O-GLYCOSYLATION AND RECOGNITION

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Mucin glycoproteins, involved in fundamental biological processes in health and disease, have multiple sites of glycosylation decorated with complex *O*-GalNAc glycans [1]. Altered regulation of several glycosyltransferases (GTs) is a hallmark feature of cancer, which yields mucin-based tumour-associated glycans key players in cancer cell growth and tumour immune surveillance [2]. Specifically, lectins from immune system interact with mucin-based tumour-associated glycans strongly modulating immune responses in cancer [3].
In this communication, new structural insights into the molecular mechanism of mucin-1 (MUC1) O-glycosylation will be reported. Through the concerted integration of biophysical and structural biology protocols with focus on NMR spectroscopy the MUC1 O-glycosylation by GalNAcTs, C1GaT1 and ST6GalNAc-I will be described [4,5,6]. In addition, the molecular basis of tumour-associated these MUC1 O-glycans recognition by lectins from immune system will be highlighted [7].

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MUCIN GLYCANS AS REGULATORS OF PATHOGEN VIRULENCE

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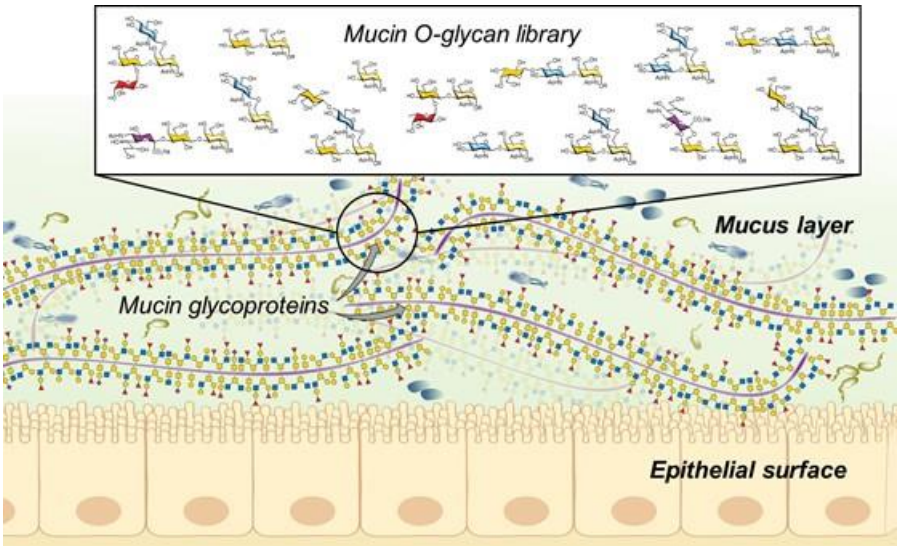
The mucosal barrier is well-established to play an important role in microbiome development and as a first line of host defense. Although this has traditionally been attributed to the physicochemical properties of mucus, several recent reports indicate that mucin glycoproteins and their associated glycans can regulate gene expression and are capable of attenuating virulence in diverse, cross-kingdom pathogens, including Gram-positive bacteria, Gram-negative bacteria, and fungi.

With mucins displaying several hundred distinct glycan structures, we sought to identify specific glycans responsible for this novel gene regulation. Individual mucin O-glycan structures are not commercially available, are not amenable to automated synthesis, and given their overlapping physical and chemical properties cannot be isolated as pure compounds from natural sources using current technologies.

Therefore, we have been developing routes [1,2] to obtain a library of individual mucin glycan structures in sufficient quantity (>30 mg) for extensive functional analysis (see Figure; adapted from [2]). With a diverse mucin glycan library in-hand, we have been working with collaborators to identify specific structures with anti-virulence activity [3,4], and in more recent work have begun to elucidate their discrete mechanisms-of-action.



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CLASSICAL INHIBITORS VS. PROTACs TARGETING GALECTIN-8

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Galectin-8 (Gal-8), a tandem-repeat galectin, is involved in a number of physiological processes such as cell adhesion, proliferation, cell signalling, autophagy, and differentiation, as well as in a number of diseases, including fibrosis, cancer, and heart disease, making it a target of interest for highly selective and potent Gal-8 inhibitors [1]. These inhibitors follow a rather traditional principle of occupying one of the carbohydrate binding sites of Gal-8, which directly inhibits the protein's function by a competitive binding mechanism. The current issue with these classical Gal-8 inhibitors is their rather modest potency, with inhibitory or binding constants that mostly reach the low micromolar concentration range, although our group just revealed inhibitors that reach high nanomolar K_d values [2].
Proteolysis targeting chimeras (PROTACs) have recently emerged as small-molecule modalities that hijack the cell's degradation pathway (ubiquitin–proteasome system) to selectively induce degradation of a specific protein of interest [3]. Most importantly, they control the pathological effects of proteins by lowering their intracellular concentration rather than by classical inhibition, and as a consequence, PROTACs do not follow the classical dose-response pharmacodynamic effect. The current state-of-the-art in the design of PROTACs focuses mostly on intracellular proteins, yet Gal-8 (like many other galectins) exists both as an intracellular and an extracellular protein, which makes it a potential target for PROTACs-induced degradation. We have designed and synthesized a series of PROTACs designed to target Gal-8 and CRBN or VHL E3 ligases. These molecules were assayed for their Gal-8 and Gal-3 degradation in MD-MB-231 and HUVEC cell lines. PROTACs were further compared with classical inhibitors by using a tube formation assay [4] as a phenotypic assay to screen molecules for their anti-angiogenic effect. To the best of our knowledge, there is no PROTAC developed to trigger proteolysis of a protein that exists both in extracellular and intracellular space by depleting its intracellular pool. Therefore, this important question was addressed for the first time and the results of our work will be shared at EUROCARB22.

Acknowledgements: *The European Union's Horizon2020 program No. 765581 (project PhD4GlycoDrug; www.phd4glycodrug.eu), the Slovenian Research Agency (Grants P1-0208) and COST actions CA18103 and CA18132 are gratefully acknowledged.*

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PURINE NUCLEOSIDES SECRET - METAL CHELATION AND CHOLINERGIC EFFECTS

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Nucleosides are well known for their antiviral and anticancer activities, with some of them being clinically used as antiviral agents and in chemotherapeutics. Along the course of our studies on the synthesis of purine nucleosides, we uncovered their usefulness as inhibitors of acetylcholinesterase and of butyrylcholinesterase, enzymes relevant for the control of cholinergic effects in neurodegenerative diseases.
Nucleosides potential to chelate biological relevant metals can lead to another promising therapy as disproportion of metal ion homeostasis is common in neurodegenerative disorders and in cardiovascular diseases, contributing to disease progression.
Aiming at the discovery of new dual target compounds based on purine nucleosides, we now report our recent findings on their activity as cholinesterase inhibitors and metal chelators, covering synthetic approaches, and structure/activity relationships to access selectivity for the inhibition of each of the enzymes, and metal chelation.

Acknowledgements: Fundação para a Ciência e a Tecnologia is gratefully acknowledged for the support of the Research Unit Centro de Quimica Estrutural (UIDB/00100/2020 and UIDP/00100/2020) and of the Associate Laboratory Institute of Molecular Sciences (LA/P/0056/2020)

GLYCAN EDITING *vs* GLYCAN-MOTIF EDITING: CHEMICAL TOOLS FOR THE PRECISION REMODELLING OF THE GLYCOCALYX

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The design of glycan-based therapeutics that aim to remodel the glycosylation pattern of the glycocalyx requires an in-depth understanding of the processes that regulate the biosynthesis of glycans [1-2]. For the case of cancer, the precision editing of cancer-associated glycan motifs has the immense potential to target the 'heart' of cancer, thus providing highly effective therapeutic interventions that will profoundly improve outcomes for cancer patients [3-4].
In this presentation, strategies to disrupt the expression of glycan motifs within the glycocalyx will be discussed, comparing and contrasting the broad "glycan editing" approach with the more discriminatory "glycan-motif editing" approach [5]. These strategies profoundly differ in the scope, investigative goals, applications, and precision.
With a specific focus on the prevention of cellular expression of sialyl Lewis X (sLe^X), a driver of carcinogenesis and metastasis, the presentation will focus on how agents that selectively disrupt the activity of specific fucosyltransferases [6-7] yield precision "glycan-motif editing", ensuring the achievement of the preferred custom-modification of the glycocalyx and of the intended therapeutic aim.

Acknowledgements: "This work has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie Actions Grant Agreement n. 101119601 (GLYCANDRUG)"

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GLYCOCALYX ENGINEERING TO IMPROVE THE METABOLISM AND GLUCOSE CLEARANCE CAPACITY OF ADIPOCYTES

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Glycocalyx engineering with synthetic heparan sulfate (HS) mimetics has been emerging as an approach to tune cellular interactions with HS-dependent growth factors to influence downstream signalling pathways and, ultimately, differentiation. This presentation will describe our recent discovery of a crucial role for cell-surface HS during adipogenesis in establishing glucose uptake capacity and metabolic utilization in differentiated adipocytes. Specifically, we uncovered that HS attenuates Wnt signaling during the first three days of adipogenesis by limiting Wnt ligand availability and primes adipocytes after differentiation toward glycolysis and away from fatty acid metabolism. Augmenting the glycocalyx of preadipocytes with synthetic HS mimetics further enhanced basal levels of glucose uptake in adipocytes, thus paving a new possible strategy for managing plasma glucose levels in Type 2 Diabetic patients with limited insulin sensitivity.

SWEET SPARKS: REMODELLING OF CELL SURFACES WITH CARBOHYDRATES VIA CLICK ELECTROCHEMISTRY

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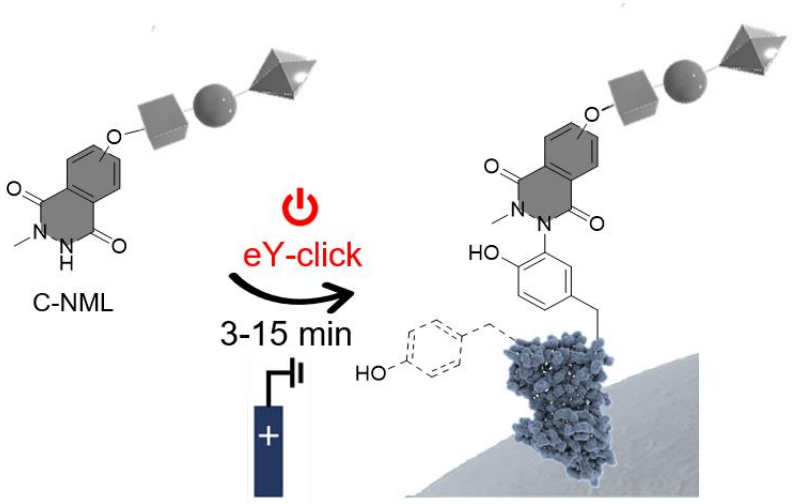
The chemo-selective modification of native proteins is crucial in chemical biology and for the development of therapeutic conjugates. Recently, we introduced eY-click, the first electrochemical method designed to functionalize tyrosine (Y) residues in proteins within biocompatible media [1]. Using a three-electrode system, this approach enables the selective oxidation (activation) of a functionalized diazodicarboxamide anchor in situ, allowing the labeling of peptides, enzymes, and antibodies in aqueous buffers.
In this study, we employed N-methylluminol (NML) [2], a highly selective Y-anchoring group upon one-electron oxidation [3], for the electro-bioconjugation of cell surfaces from viruses, living bacteria, and eukaryotic cells [4]. The click-electrochemistry method was successfully applied to therapeutic adeno-associated viruses (AAV2), *E. coli* (Gram-negative), S. epidermidis (Gram-positive), and eukaryotic cell lines. Within minutes, biologically relevant carbohydrates were grafted onto cell surfaces, providing a versatile alternative to metabolic engineering for vectorized biotherapies and the study of cell surface glycans.



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VECTOR GLYCOENGINEERING

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Adeno-associated viruses (AAVs) are among the most common vectors used for in vivo gene therapy for gene cargos below 5 kb due to their high transduction efficiency, a good safety profile, broad tropism, low immunogenicity, and general ease of production. Depending on the serotype AAVs bind to cell surface glycans via heparan sulfate, galactose or sialic acid residues, as their primary binding receptor As AAVs show only a low degree of surface glycosylation in contrast to most enveloped viruses which are heavily glycosylated, we were interested in exploring the effect of a pronounced AAV surface glycosylation to modulate vector tropism, improve vector uptake, or avoid immune recognition. To this end, we have prepared a collection of structurally varied synthetic glycans for attachment to the capsid and studied the influence of glycan structure, attachment site, and glycan density on vector internalization, gene transduction, and immune recognition both in vitro and in vivo. We found a strong dependency for the gene transduction capacity of the modified vectors, particularly on glycan capsid density and glycan structure, examples and trends will be given in the talk. As non-viral vector, data on exosome glycoengineering by metabolic and chemical glycoengineering to change exosome tropism in vivo will be presented.

AZALEVOGLUCOSAN, A USEFUL SCAFFOLD TO REACH IMINOSUGARS WITH HIGH MOLECULAR DIVERSITY

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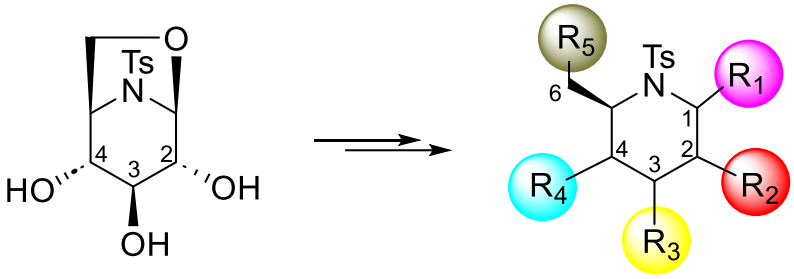
Iminosugars, notably six-membered polyhydroxylated piperidines, are one of the most promising classes of carbohydrate analogs for therapeutic purposes [1]. While structural diversity has been extensively introduced at N, C1 and C6 positions to identify and validate new biological leads [2,3], C2, C3 and C4 positions have been poorly scrutinized according to their more difficult chemical access.
In this context, we have developed a robust synthesis of an azalevoglucosan scaffold [4,5], a bicyclic 1,6-anhydro iminosugar in which the trans diaxial arrangement of the secondary hydroxyl groups allows unprecedented introduction of structural diversity in a regio- and stereoselective manner at C2, C3 and C4 positions. Further bicycle ring opening allows additional decoration of the C1 and C6 positions to generate highly substituted piperidines.
The synthesis of the key azalevoglucosan scaffold, its decoration at C2, C3 and C4 positions and its ring opening to access polyfunctionalized piperidines will be presented [6].



Azalevoglucosan scaffold

Polyfunctionalized piperidines

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DEVELOPMENT OF NEW TYPE OF THIO-GLYCOMIMETICS USING TANDEM KNOEVENAGEL-HETERO-DIELS-ALDER REACTIONS

Hanna Zinchenko[a,b], Omar Lahna[a], Stefan Leusmann[c,d], Mario Fares[c,d], Alina Sotskova[a], Magdalena Ceborska[e], Alexander Titz[c,d], Mykhaylo A. Potopnyk[a,b]

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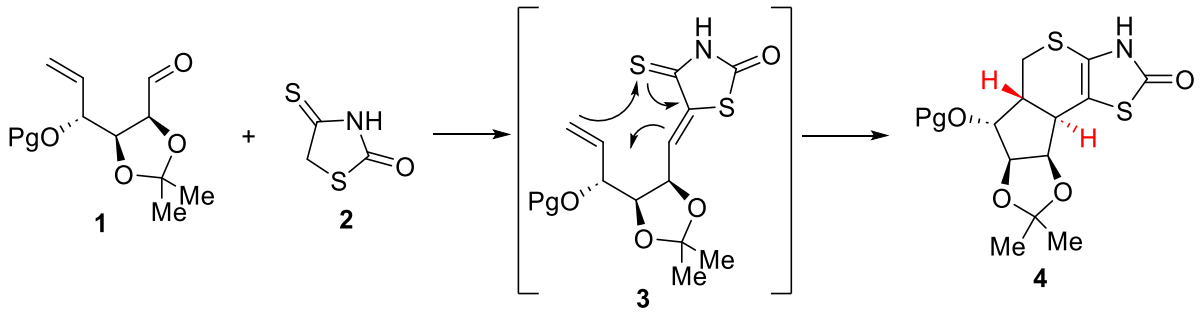
Glycomimetics are among the most important compounds with biological activity. Many iminosugars, as such, are potent inhibitors of glycosidases and glycosyltransferases [1], and have been highlighted as lead candidates for the treatment of various diseases, including diabetes, cancer, viral infections, and lysosomal storage disorders (e.g., Gaucher and Fabry diseases) [1-3]. Based on this, the development of new types of compounds, based on carbohydrate fragments, is highly desired.

In our work, we propose the synthesis of new thio-glycomimetics using a tandem Knoevenagel-*hetero*-Diels-Alder reaction. Olefin-aldehydes **1** and isorhodanine **2** were used as substrates. The condensation of these two compounds results in the formation of olefino-thio-diene **3**, which simultaneously undergoes conversion into the tricyclic product **4**. This reaction occurs in a highly stereoselective manner, giving only one isomeric product.



After the removal of the protecting groups, the biological activity of the final compounds was tested. The obtained thio-glycomimetics exhibit inhibitory activity against LecA and LecB proteins.

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MODIFICATION OF NON-POLAR POLYMERS WITH CARBOHYDRATES: ACCESS THROUGH MALEIC ANHYDRIDE

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Non-polar polymers are a class of resistant molecules which do not undergo many chemical reactions, e.g., polyethylene, poly(iso)propylene. Their molecular structure allows for a narrow range of modifications, which limits the number of applications. Thus, the presence of maleic anhydride in their structure could provide a perfect target for chemical modification. In particular, carbohydrates seem to be perfect modifiers for anhydride rings due to forming reactive alkoxides/oxyanions in different conditions, e.g., solvents (DMF, DMSO), deprotonating agents (K_2CO_3, NaH, LiH) [1]. In addition, the application of different carbohydrates based on size and chemical character could provide tailor-made properties in a wide range of technologies. Our previous studies indicated cyclodextrin oxyanions as efficient reagents for chemically resistant dianhydrides based on biphenyl [2] or perylene [3]. Thus, the aim of our research is to evaluate carbohydrates as chemical modifiers for non-polar polymers containing maleic anhydride. Herein, we have applied carbohydrates (e.g., glucose, lactose, cyclodextrin, cellulose) for the modification of non-polar polymers which were copolymers or grafted with maleic anhydride e.g. poly(ethylene-alt-maleic anhydride), polypropylene-graft-maleic anhydride. The reactions were carried out under mild conditions in dry DMF with a strong base as NaH. Depending on the conditions and the polymers, the cross-linking or grafting resulted depending on stoichiometry of carbohydrates and a deprotonating agent. The molecular structure was confirmed with spectral methods like NMR, FT-IR and chromatographic methods like GPC-SEC. Based on the results, we believe that the modified polymers with carbohydrates are promising tools for drug delivery and environmental protection.

Acknowledgements: This project is funded by „Regional Excellence Initiative" project no. RID/SP/0035/2024/01 as Microgrant no. RID_MG/1/2025.

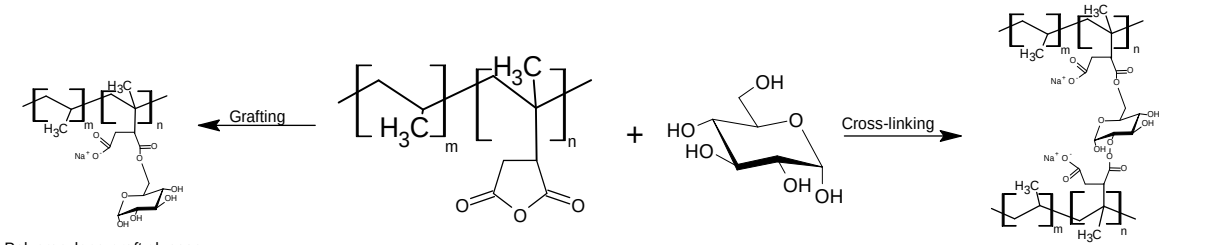

Figure 1. Reaction ways of polypropylene-*graft*-maleic anhydride with glucose.

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LIPOPOLYSACCHARIDES FROM MICROBIOTA: A JOURNEY FROM THE STRUCTURE TO THE FUNCTION

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The gut microbiota plays a fundamental role in modern health concepts, influencing various physiological and pathological processes in the human body, including immune system regulation. A key aspect of host immune surveillance involves the recognition of microbial-associated molecular patterns, such as lipopolysaccharides (LPS), which are characteristic of Gram-negative bacteria. These molecules are present not only in pathogenic species but also in commensal and mutualistic bacteria residing in the intestines [1,2].
Due to its chemical composition, LPS is widely recognized as a powerful inducer of inflammatory responses in mammals and is often linked to harmful bacteria and adverse health effects. However, LPS is also a structural component of the outer membrane of beneficial Gram-negative bacteria within the gut microbiota. The mechanisms that allow LPS from these commensal bacteria to be tolerated without triggering overt immune activation remain largely unexplored, representing a key frontier in our understanding of innate immunity [3]. Unraveling the chemical structure and immunological properties of LPS from gut microbes, particularly those maintaining a neutral or beneficial relationship with the human host, is of critical importance for both fundamental biology and clinical research. A thorough investigation of LPS from the gut microbiota will shed light on host-microbe interactions at both intestinal and systemic levels. This, in turn, will enhance our understanding of how gut bacteria influence immune responses through their LPS structures, ultimately expanding our knowledge of immune system dynamics. By analyzing microbiota-derived LPS, it will be possible to generate novel structural and functional insights that may drive innovation in biomedical research. These findings have the potential to contribute to the development of new immunotherapies and facilitate the identification of biomarkers for diagnosing, prognosticating, and predicting immune-mediated diseases.
In this communication, I will share recent and unprecedented findings on the structure and distinctive immunological characteristics of LPS from specific commensals of the human gut. Additionally, I will show some important advancements in structure-to-function studies on gut microbiota LPS, building on our previously published work.

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A PUZZLING BORDETELLA PETRII O-ANTIGEN
THE CORE OLIGOSACCHARIDE OF B. PETRII TYPE STRAINS
AND AN EPHEMERAL PRESENCE OF THE O-SPECIFIC POLYSACCHARIDE

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Bordetella petrii is the only environmental species found among host-restricted and pathogenic members of the genus *Bordetella. It* was first isolated from an anaerobic, dechlorinating bioreactor culture enriched from river sediment [1] Later it was linked to jaw bone infection [2], ear infection [3] and chronic pulmonary disease [4]. *V*irulence factors of *B. petrii comprise* a lipopolysaccharide, *i.e.* the major outer-membrane component of Gram-negative bacteria. Data on the structural features of the lipopolysaccharide (LPS) of *B. petrii* are scarce and confounding. To date only the structures of the *B. petrii* lipid A have been characterized [5] Moreover, Zelazny *et al*. [6] showed that in the environmental isolate of *B. petrii* strain (ATCC BAA-461) the O-antigen is present, but neither the O-antigen nor the core OS were recognized by serum of a patient with *B. petrii* infection. Despite reports to the contrary, our preliminary structural data indicates the lack of O-specific polysaccharide segment in the O-antigen. This observation has been further confronted with the O-antigen structural features of the primary clinical isolate *B. petrii* strain (NCTC 13363).
The components released by mild acidic hydrolysis of the LPS were separated and investigated by ^{1}H and ^{13}C NMR spectroscopy. The structure of the core oligosaccharide of *B. petrii* has been identified for the first time. The main oligosaccharide fraction contained an octasaccharide. The NMR data of *B. petrii* core oligosaccharide were compared with these of the core OS among respiratory pathogens from the genus Bordetella. The analysis indicated that the oligosaccharide conforms to the conserved structural features of the Bordetella spp. LPS core with some variability in relation to the terminal GlcN. Furthermore, the serological cross-reactivities of the *B. petrii* LPS also confirmed the similarity between the core oligosaccharides of *B. petrii* and other Bordetellae.

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AGAINST THE TREND: INSIGHTS INTO LIPOPOLYSACCHARIDE STRUCTURE AND OUTER MEMBRANE DYNAMICS IN COLD-ADAPTED PSEUDOMONAS ISOLATED FROM ENIGMA LAKE, ANTARTICA

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Pseudomonas is one of the most versatile genus able to adapt to extreme environmental conditions [1]. Therefore, *Pseudomonas* is an ideal research model for studying how environmental factors affect the chemical and physical properties of the cell envelope in order to maintain proper membrane integrity even in the most extreme habitats [2]. Numerous studies have shown that the biosynthesis of lipopolysaccharide (LPS), a key component of the outer membrane in Gram-negative bacteria, can be altered to ensure the preservation and optimal functioning of the membrane [3-5]. LPS properties in fact are strictly related to its chemical structure, which typically comprises a glycolipid anchor (lipid A), a core oligosaccharide (OS), and an O-antigen polysaccharide chain [3].
This communication [6] presents a study on the LPS structure of the cold-adapted *Pseudomonas* strain ESM#7, isolated from Enigma Lake in Antarctica's Northern foothills, a lake characterized by unique conditions including strong alkalinity, oxygen oversaturation, and near-zero temperatures. To study how the very low temperature affects the LPS membrane properties, LPS was isolated from this bacterium grown at 0.4 °C (*in situ* temperature of collected Enigma Lake water) and compared with the one isolated from the same bacterium grown at 20 °C. The chemical structure of both LPSs was investigated by chemical analyses, 1D and 2D NMR and MALDI TOF MS experiments that showed key structural differences in the lipid A region. This structural study was then complemented by morphological analysis of the LPS assemblies in water by Cryo-EM and SAXS which together with molecular coarse-grained MD simulations provided insights into the viscoelastic properties of the membrane and the role of the lipid A component in membrane stability.

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TUMOR HEMORRHAGIC POLYSACCHARIDES FROM STREPTOCOCCUS AND SERRATIA ARE THE API'S OF COLEY'S TOXIN: PS1, THE SERRATIA CONSTITUENT

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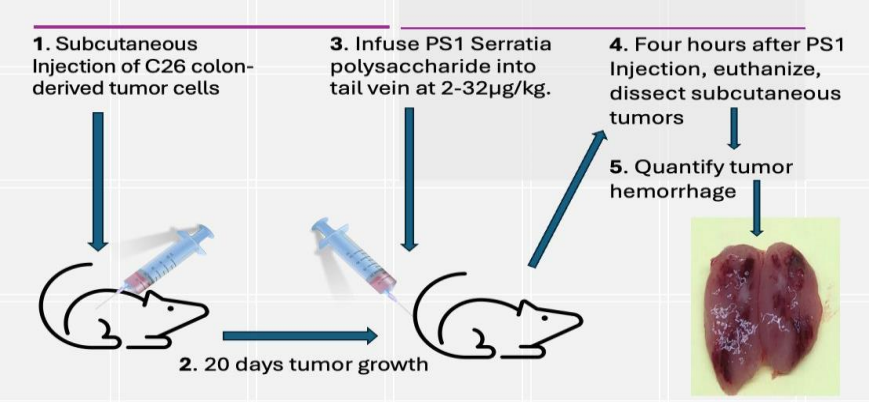
Abstract: Serratia PS1 polysaccharide causes tumor hemorrhage within 4 hours.



Coley's Toxin comprised a mixture of cell-free, heat-treated culture media from *Streptococcus pyogenes* (originally *Streptococus erysipelatos)* and *Serratia marcescens (originally Bacillus prodigiosus)*. A 250kDa tumor hemorrhage-inducing polysaccharide "PS1" is reported here secreted into culture medium by *Serratia marcescens*. Four hours after PS1 is injected at 32µg/kg (10*p*M) into the tail vein of Balb/C mice bearing C26 subcutaneous colon-derived tumors, tumor-specific capillary hemorrhage is exhibited in 90% of tumors. As a positive control, CM101, a similar tumor hemorrhagic polysaccharide from *Streptococcus agalactiae* caused tumor hemorrhage in 75% of tumors in the Balb/C-C26 model at 7.5µg/kg(2.5pM).
In 1866-1868, Busch observed that a sarcoma patient with nosocomial **erysipelas** experienced profound regression of tumors, results published in German, 1866. Busch infected other sarcoma patients by cauterizing the tumor and hospitalizing them in the same, unwashed hospital bed sheets with an open wound to induce **erysipelas (**at the time, of unknown etiology). Patients who contracted erysipelas showed tumor regression, Busch, 1868. In 1881, Fehleisen isolated "*Streptococcus erysipelatos"* from erysipelas patients (now christened *S. pyogenes*, Lancefield Group A). Following Busch' observations, Fehleisen deliberately infected cancer patients with cultured live bacteria, and observed tumor regression. In 1890, Wm B Coley Independently found tumor regression in patients upon erysipelas infections. He mixed cell-free heat-treated culture media from Streptococcus and Serratia, known as "Coley's Toxin, and in 1910 reported more than 800 cases with 50% 5 year survival rates. Shear, et al. 1943, showed that an active fraction from Serratia was polysaccharide, peptide and LPS free. We report PS1, from Serratia marcescens, as a 250Kda tumor hemorrhagic polysaccharide isolated from Shear's fraction.

COMPARISON OF MILK OLIGOSACCHARIDES IN HOLSTEIN-FRIESIAN COWS AND WATER BUFFALOS DURING EARLY LACTATION

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Holstein-Friesian cows and water buffaloes are important livestock in the dairy industry. In addition to important nutrients, their milk contains numerous bioactive molecules, such as lactoferrin and immunoglobulins. Milk oligosaccharides (MOs) are another important group of biomolecules supporting numerous beneficial processes in the offspring. For example, MOs positively influence the maturation of the microbiome and immune system. Furthermore, MOs are able to inhibit the adhesion of various pathogens to epithelial cells. In addition to the benefits for the offspring, MOs play an important role, when milk is used for the production of functional food and infant formula.
Due to these beneficial effects, we characterized in detail the structure and distribution of MOs in Holstein-Friesian cows and water buffaloes during early lactation. The MOs of colostrum (the first milk after parturition), transitional (first week of lactation) and mature milk were analysed by LC-MS. In colostrum of both livestock, the majority of MOs were sialylated. However, the amount of sialylated MOs rapidly decrease during transition phase in Holstein-Friesian cows and water buffaloes. Interestingly, only fucosyllactose was detectable in bovine milk, whereas several different fucosylated MOs were present in buffalo milk. In general, there was a higher structural diversity of MOs in the milk of water buffaloes. Thus, we observed distinct differences in the composition and distribution of MOs between the two livestock.

ROLE OF SIALIC ACID AND GLYCOSYLATIONS AS BIOMARKERS FOR SEVERITY STRATIFICATION IN POST-VIRAL INFECTION SYNDROME

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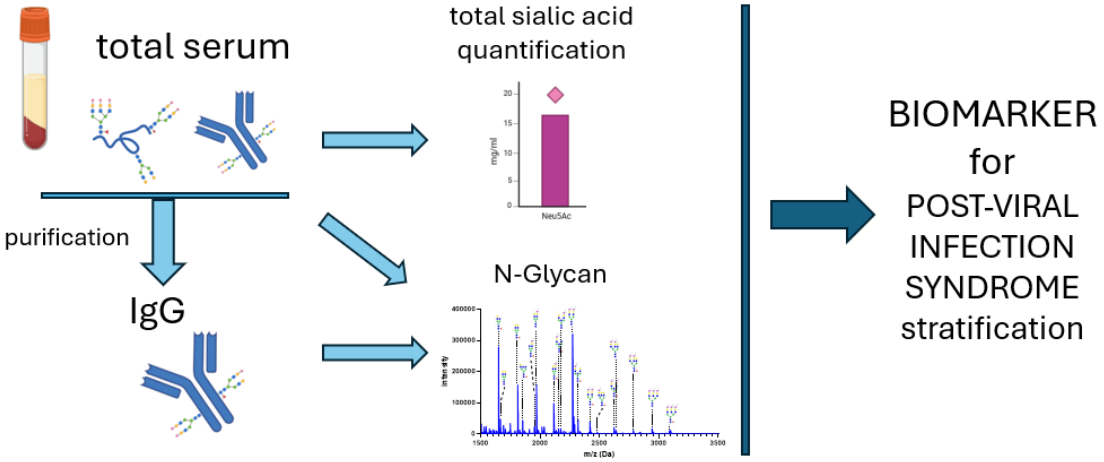
Our group focuses on the study of sialic acid and sialylated glycans in inflammatory diseases. In this work, we aim to find better biomarkers for severity stratification in post-viral syndrome using a glycomics approach [1]. Glycomics is the communication language of organisms, based on a sugar code (glycans) imprinted on tissues, proteins, and lipids. Many diseases, such as autoimmune disorders and pathogenic infections, has an alterered sugar code in the organism, leading to inflammation.



N-glycome was characterized in serum and purified IgG by MALDI mass spectrometry in positive mode after purification and derivatization with methylamine [2]. After labelling with DMBA, total sialic acid content was quantified by HPLC-RP-FL.
We observed variations in N-glycome and sialic acid concentration between patients and controls, suggesting the involvement of sialic acid in the severity of post-viral fatigue syndrome.

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ANALYSIS OF SPECIFIC INTERACTIONS BETWEEN GLYCANS AND WATER BY NUCLEAR MAGNETIC RESONANCE

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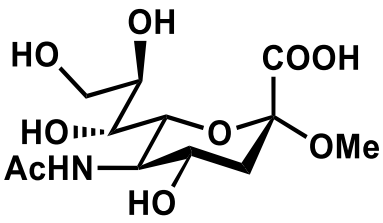
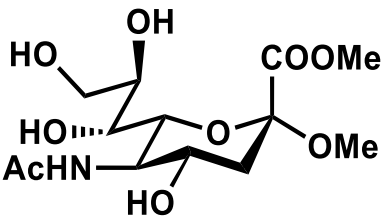
Glycans of glycoproteins regulate stability and functions of proteins. However, the detailed mechanisms remain unclear. We have hypothesized that glycans regulate water behavior around glycoproteins. To analyze the interaction between glycan and water, we succeeded in the development of a unique sampling method for NMR measurement.
After dissolving the sugar in dried deuterium-d6-DMSO, we added a further drying agent to reduce the water amount that was less than 1 equivalent of the sugar. From this state, proton NMR and the T1 and T2 relaxation times of the water were measured while the amount of water was gradually increased to about 100 equivalents. The resultant spectra revealed that sialic acid at the end of the glycans lower the mobility of water molecules around glycans.
Then, we especially focused on sialic acid which is found at the end of the glycan. Sialic acid has a unique fonctional group, the carboxylic acid. Therefore, methyl α-sialoside and its methyl ester form were analyzed to investigate how the carboxylic acid influences the water behavior. The contributions of each hydroxy group were also analyzed through synthesizing each deoxyzed analog.
In this presentation, we will present the details of the our experiment and discuss the specific interactions between glycans and water.





CONFORMATIONAL ANALYSIS OF D-ALTROPYRANOSIDE DERIVATIVES

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Due to their low ring inversion barrier, D-altrose derivatives often occur as conformational mixtures of $^4C_1$, $^1C_4$ and other conformations. In addition to NMR spectroscopic and theoretical analysis [1], we have employed vibrational circular dichroism (VCD) [2], which has the potential to serve as a spectroscopic technique for assigning dynamic equilibria of monosaccharide conformations.
We have employed VCD to explore the conformational properties of altrobiosides and of both anomers of *O*-(D-altropyranosyl) trichloroacetimidate showing different conformations [2]. We have been inspired by the work of Taniguchi and Monde who demonstrated that VCD is suitable for distinguishing anomers and moreover for studying carbohydrate conformations [3]. Here, we further demonstrate the potential of VCD for the analysis of sugar conformations, hence complementing other spectroscopic techniques such as NMR.
The VCD of the anomeric *O*-(D-altropyranosyl) trichloroacetimidates are depicted in Figure 1 together with the calculated reference spectra (using the Jaguar software). Weighting of the spectra led to a remarkable agreement between the experimental and calculated spectra. It could be deduced from this data that the α-anomer mainly adopted a $^4C_1$ conformation while the β-anomer showed an equally weighted conformational dynamic between the complementary chairs $^4C_1$ and $^1C_4$.

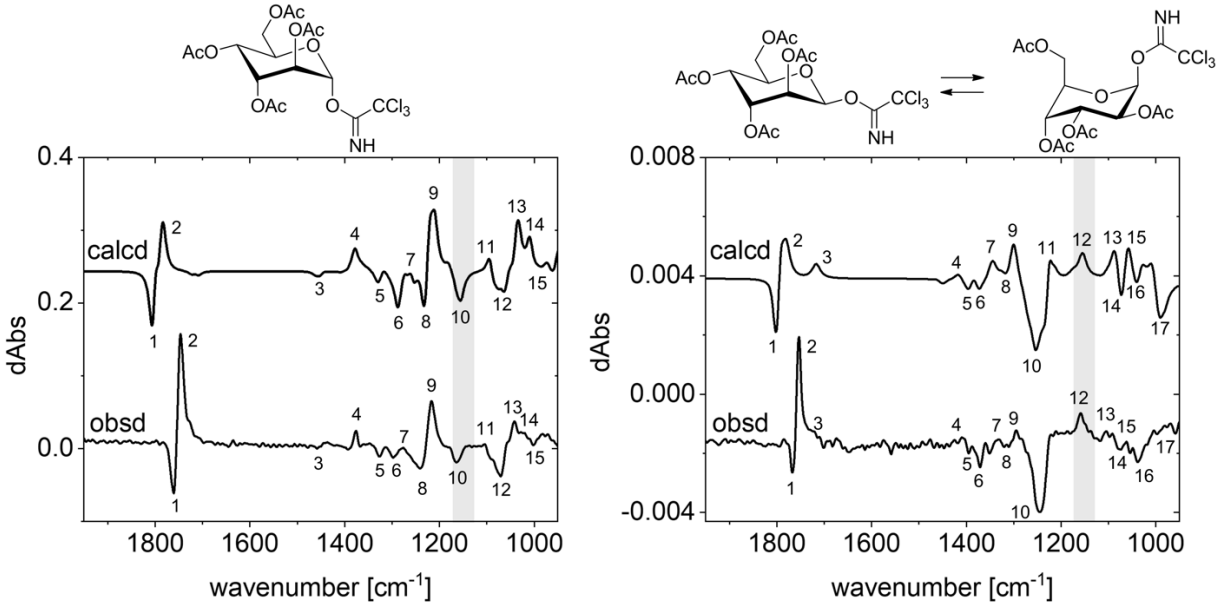

Figure 1. VCD spectra of the anomeric *O*-(D-altropyranosyl) trichloroacetimidates.[2] The VCD bands were numbered accordingly and the glycoside band is highlighted in grey.

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DUO Oral Lectures

UNRAVELLING THE MOLECULAR BASIS OF A4GALT CATALYSIS AND INHIBITION: TOWARDS A NEW THERAPEUTIC APPROACH FOR FABRY DISEASE

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Fabry disease is a rare and severe genetic lysosomal disorder caused by a deficiency of α-galactosidase A. This deficiency leads to the accumulation of the sphingolipid globotriaosylceramide (Gb3) and its deacylated form globotriaosylsphingosine (LysoGb3), resulting in progressive heart and kidney dysfunction as well as chronic pain. Current treatments, including enzyme replacement therapy (Fabrazyme® or Replagal®) [1] and the pharmacological chaperone Galafold® [2], have limited efficacy, as patients often continue to suffer disease progression. An alternative therapeutic approach is the inhibition of lactosylceramide 4-α-galactosyltransferase (A4GALT), the enzyme responsible for synthesizing Gb3. However, no A4GALT inhibitors have been reported to date.

In this multidisciplinary project, we have elucidated the enzyme's substrates recognition motifs and catalytic mechanism, enabling the rational design of novel inhibitors. Using molecular dynamics simulations with an Alphafold-derived A4GALT model, we identified key interactions governing substrate binding, which were experimentally validated through mutagenesis and lipidomics. Additionally, we modelled the enzyme's reaction mechanism using QM/MM metadynamics, revealing that α4GalT follows an S_Ni-type reaction mechanism [3], where the UDP-galactose phosphate group acts as a general base. Based on these insights, we designed, synthesised and experimentally validated several acceptor analogues [4].

This duo speaker presentation will highlight how an integrative approach –combining metadynamics, cell biology, organic chemistry and biochemistry– advances our understanding of galactosyltransferases and facilitates the rational design of mechanism-based inhibitors for therapeutic applications.

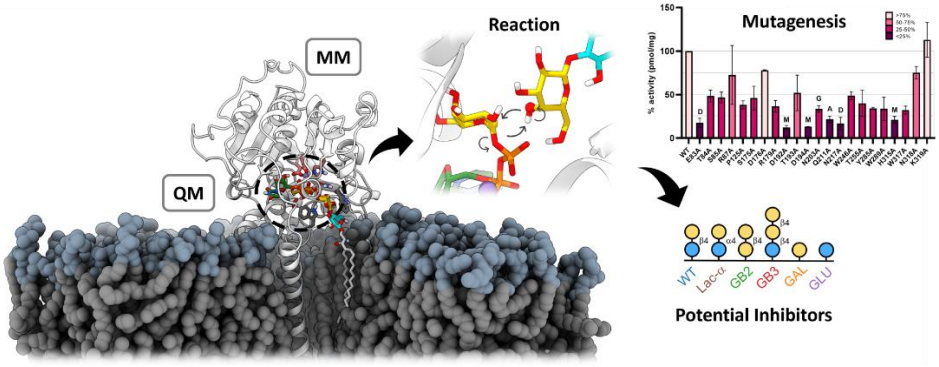

Figure 1. Structure of the Michaelis complex of the A4GALT enzyme and the delimited QM and MM regions, reaction mechanism, A4GALT mutagenesis and potential inhibitors.

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SUGARDB: USER-CENTRED DESIGN OF A CARBOHYDRATE STRUCTURAL DATABASE FOR *KLEBSIELLA PNEUMONIAE* AND OTHER PATHOGENS

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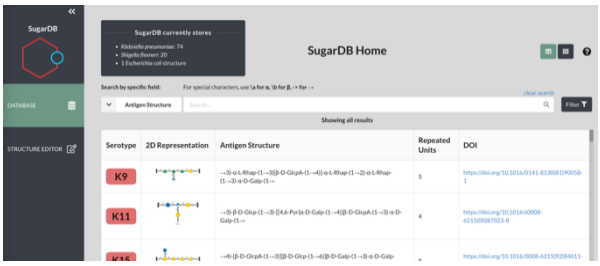
With the increase in anti-microbial resistance (AMR), vaccination is becoming the first line of defense against bacterial pathogens. For example, *Klebsiella pneumoniae* (*Kp*) is a leading cause of neonatal sepsis and hence a major killer of newborns globally, with 87% of deaths AMR-related [1,2]. Two surface carbohydrates are potential vaccine targets: capsular polysaccharides (K-Ags) and O-antigens (O-Ags). The characterization of a large collection of emerging *Kp* strains has identified 32 distinct K-Ag and 8 O-Ag types [3]. Detailed structural elucidation (chemotyping) of the K-Ags recognised may enable identification of common structural features for potential cross-reactivity and thus the development of a simplified multivalent vaccine against neonatal *Kp* infections.

Validation and comparison of K-Ags across *Kp* strains could be facilitated by an effective database of *Kp* structures. Several web-based carbohydrate structure databases have been developed, including the Carbohydrate Structure Database curated collection of glycomics data [4], and the more focussed K-PAM (exclusively *Kp*) [5] and ECODAB (primarily *Escherichia coli* some *Streptococcus pneumoniae)* [6]. However, in the design and development of these tools, typically little attention has been to important software development principles such as usability (the ease with which users can interact with software to accomplish tasks) and extensibility (the ability to extend a system and the level of effort required to implement the extension).

We developed SugarDB (https://sugardb.cs.uct.ac.za/) as a prototype repository for the primary structure of Kp K-Ags using an iterative user-centred design process with user evaluation. SugarDB has a carbohydrate-specific query system for rapid searches, an intuitive interface to provide an instant overview of the contents of the database and to facilitate data access, automatic generation of Symbol Nomenclature for Glycans primary structure graphs from CASPER text and the ability to rapidly add new structures. SugarDB adheres to standardised usability principles and evaluation heuristics, with novel features to support research into bacterial carbohydrates. The SugarDB prototype can be extended to cover a wide range of other pathogens.



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Flash Presentations

THE DEVELOPMENT OF CHEMICAL TOOLS FOR THE INVESTIGATION OF CARRAGEENAN BIOCHEMISTRY

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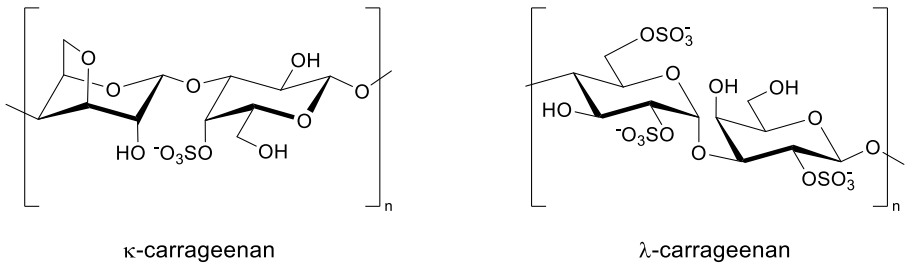
Carrageenans (Figure 1) are marine carbohydrates that are found in the extracellular matrix of red macroalgae [1]. Red seaweeds produce these sulfated linear polysaccharides for structural purposes, with these carbohydrates providing flexible support to the macroalgae in the dynamic marine environment [2]. Industrially, carrageenans are known for their gelation properties and are therefore used extensively as thickening agents or for formulation purposes [3]. These polysaccharides also constitute a large biomass and are thus a precious carbon source for marine bacteria. To investigate both the biosynthetic and degradation pathways of carrageenan [4,5], chemical tools are needed to probe the putative enzymes involved. Outlined here are approaches undertaken to develop a series of chemical tools to elucidate the structure and function of putative carrageenan-active enzymes. This will lead to a deeper understanding of both carrageenan biosynthesis and degradation as it occurs in the natural environment.



κ-carrageenan λ-carrageenan

Figure 1. Two common subtypes of carrageenan, kappa (κ) and lambda (λ).

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REGIO- AND STEREOSELECTIVE SYNTHESIS OF THE BIOTIN-CONJUGATED KERATAN SULFATE OLIGOSACCHARIDE

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Keratan sulfate (KS), an acidic linear polysaccharide, is one of the members of glycosaminoglycan (GAG). KS is composed of repeating disaccharide, -4)βGlcNAc(1-3)βGal(1- having different sulfation patterns at the primary hydroxyl groups. L4 is one of the subclasses of KS, sulfated at all primary hydroxyl groups. Together with other GAG members, KS also acts as hydrating role in cornea and cartilage tissues, and exhibits various bioactivities. Imagama et al. demonstrated the inhibitory effect of KS towards axonal growth. Although the sulfation patterns were unclear, they suggested the glycan length might be 6-8 saccharide stretch [1]. KS oligosaccharides with defined structure including sulfated positions can be a powerful tool to investigate the role of KS at a molecular level. We herein report the effective synthesis of KS L4 oligosaccharide as biotin-conjugate. The target oligosaccharide was attributed to form the glycan-backbone without protection at some hydroxyl groups during the 2+2n glycosylation procedures. We employed the unprotected glycosyl acceptors at 2,3,4- and 2,4-positions of galactose (Gal) residues at the non-reducing terminal and the inside residues, respectively, for the coupling reaction. *O*-3 of the non-reducing terminal predominantly reacted to give the desired 1-3-linkage. The primary hydroxyl groups of Gal and *N*-acetylglucosamine (GlcNAc) moieties were regiospecifically protected with orthogonally removable NAP (2-naphthylmethyl) and TBDPS (*tert*-butyldiphenylsilyl) groups, respectively. Formation of all type of sulfation patterns may be available with the versatile protection. In addition, our oligosaccharide was formed as 2-aminoethyl glycoside. The short linker made it possible to connect the appropriate functional group. Complete sulfation and the subsequent deprotection were performed for the oligosaccharide substrates to form the biotinylated KS-L4 oligosaccharide (Fig. 1).

Figure 1.

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SYNTHESIS OF MULTIVALENT CHONDROITIN SULFATE OLIGOSACCHARIDES TO EXPLORE CS-PROTEIN INTERACTIONS

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Chondroitin sulfate (CS) are heteropolysaccharides that belong to a family of complex polyanionic polymers called glycosaminoglycans (GAGs). They consist of a repeating disaccharide unit composed of a D-glucuronic acid (D-GlcA) and 2-acetamindo-2-deoxy-D-galactose (D-GalNAc) and bearing sulfate groups primarily at the position 4 (CS-A) and/or position 6 (CS-E/CS-C) of the D-GalNAc [1]. Their structural diversity and sulfation patterns determine their biological functions, including neuronal development, morphogenesis, and cell-cell recognition, through interactions with various proteins [2]. Additionally, CS are major structural components of connective tissues, particularly in cartilage, skin, and bone [3]. Multivalency is a key feature in glycan-protein interactions, enhancing both affinity and specificity while enabling supramolecular organization at the cell surface [4]. Despite its importance, multivalent CS constructs remain scarce [5]. Here, we present our approach, for the synthesis of well-defined CS oligosaccharides with controlled sulfation [6] patterns and various linkage systems and platforms to promote multivalency.

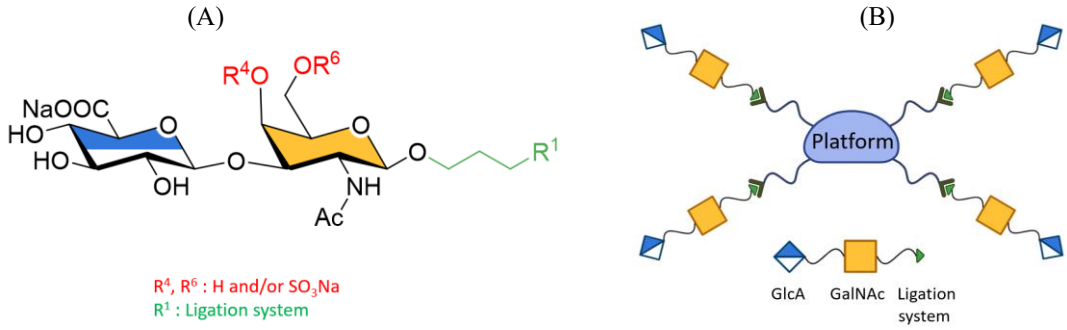

Figure 1. (A) Structure of CS disaccharide, (B) Multivalent CS oligosaccharides

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CHEMICAL SYNTHESIS OF UDP-SUGAR DONORS FOR THE IDENTIFICATION AND CHARACTERIZATION OF PLANT GLYCOSYLTRANSFERASES

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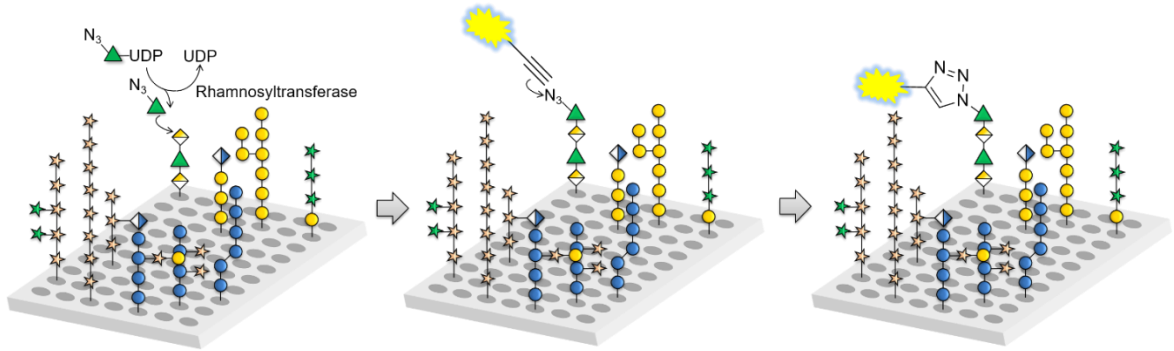
As plant cell walls represent the most abundant renewable resource on Earth, understanding the structure, function and biosynthesis of plant glycans is critically important [1]. Although the identification and characterization of plant glycosyltransferases (GTs) is vital to elucidate these biosynthetic pathways, only a few GTs have been biochemically validated so far [2]. Effective tools for GT analysis are therefore crucial in plant cell wall research.
GTs facilitate the transfer of monosaccharides from activated donors to acceptor substrates, making the availability of such probes critical for GT analysis, for instance using glycan microarrays. These enable the immobilization of hundreds of acceptors, allowing for high-throughput GT substrate specificity studies [3].
We report the chemical synthesis of two uridine diphosphate-activated sugar (UDP-sugar) donors involved in plant cell-wall biosynthesis. We describe the syntheses of UDP-rhamnopyranose and UDP-arabinofuranose, along with their azido-modified analogues, which enable direct detection via click chemistry on glycan microarrays [4]. Efforts towards the synthesis of UDP-apiofuranose and its azido-modified analogue are also reported [5].
Finally, a glycan microarray-based study of GTs using the synthesized UDP-sugars is presented [4]. Analysed GTs include xylan arabinosyltransferase XAT3, involved in the biosynthesis of a hemicellulosic glycan, and rhamnosyltransferases RRT4 and RRT5, involved in the biosynthesis of a pectic glycan.



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ARTIFICIAL GLUCOSINOLATES AS MASKED ISOTHIOCYANATES FOR ENZYMATICALLY PROMOTED *IN SITU* BIOCONJUGATION

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Glucosinolates are sulfur-containing secondary metabolites whose structures are based on a *β*-D-glucopyranose unit linked through an *O*-sulfated (Z)-thiohydroximate function to a variable aglycon. They are part of the defence mechanism [1] of cruciferous vegetables, such as broccoli, mustard, or wasabi. Myrosinase, a specific *β*-thioglucohydrolase, hydrolyses the anomeric C-S bond, leading to the formation of an isothiocyanate species (ITC). This hydrolysis allows the shift from stable non-toxic and water-soluble precursors to a toxic electrophile, highly reactive, difficult to prepare, to store in most cases and also water insoluble [2]. This unique system can be explored as an alternative bioconjugation tool for labelling with diverse applications such as grafting carbohydrates onto carrier protein [3] to generate neoglycoproteins, or selective protein labelling with fluorophores for target detection (Figure 1).

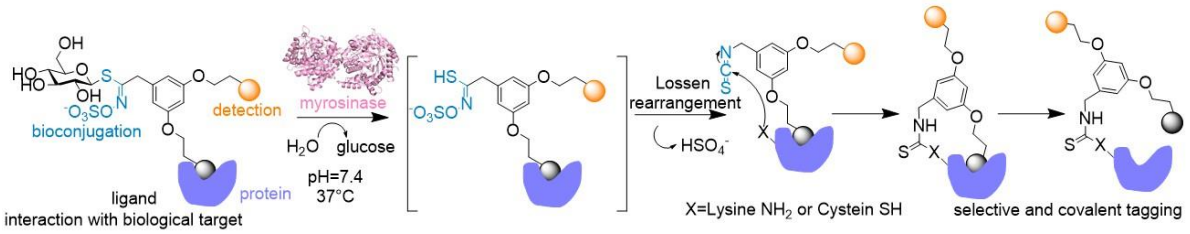

Figure 1. Myrosinase-Glucosinolates system for selective protein labelling

In this context, we describe our recent results on the design, synthesis and reactivity of artificial glucosinolates. We show that those compounds are substrates of recombinant myrosinase and can be efficiently hydrolysed into their corresponding ITCs. In addition, we will describe our currently developed approaches to the synthesis of pluri-functional glucosinolates, specifically designed to selectively label proteins such as lectins, with the use of a recombinant myrosinase.

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SYNTHESIS AND STRUCTURAL ANALYSIS OF ANTIPARALLEL GLYCAN HAIRPINS

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Natural biopolymers have inspired the development of synthetic analogues – i.e. foldamers – capable of adopting defined conformations and forming programmable three-dimensional architectures. These compounds are mainly based on peptides and nucleic acids, that are well understood at the molecular level. Following these examples, we recently designed a glycan adopting a stable secondary structure, challenging the common belief that glycans are not capable of folding due to their flexibility [1].
Herein, we present new design principles to access a linear glycan capable of spontaneously folding into an antiparallel arrangement, establishing a new class of synthetic oligosaccharide foldamers. We have rationally designed a rigid turn unit sustained by a non-conventional hydrogen bond. This turn unit allowed for the construction of a glycan antiparallel hairpin, stable secondary structures not identified in nature. Automated glycan assembly facilitated rapid access to synthetic analogues of different lengths. Nuclear magnetic resonance (NMR) and SAXS conformational analysis provided definitive evidence of the folded conformation of the synthetic glycan hairpin. These results offer a basis for designing new glycan architectures, with potential applications in glycobiology and material sciences.

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MILD AND EFFECTIVE METHOD FOR THE CROSS-COUPLING OF GLYCOSYL THIOLS IN AQUEOUS SURFACTANT SOLUTION

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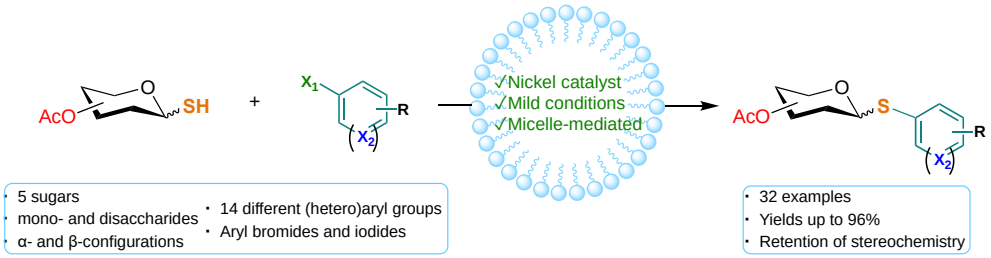
Thioglycosides have become universal in carbohydrate chemistry not only as oligosaccharide building blocks, but also as enzymatically stable glycomimetics [1]. In both cases, the identity of the aglycon and the anomeric configuration can greatly affect the properties of the glycoside [2]. Traditionally, aryl thioglycosides have been synthesised by reaction of a glycosyl acetate with an aryl thiol in the presence of excess Lewis acid, but this method can have drawbacks including necessity of foul-smelling thiol reagents, limitation of available thiols, and difficulty in obtaining 1,2-*cis* stereochemistry. Conversely, by using glycosyl thiols as starting material, aryl thioglycosides can be generated through transition metal catalysed cross coupling with a breadth of aryl halides while retaining anomeric stereochemistry [3].
Recently, Lipschutz and coworkers reported a surfactant-mediated, nickel catalysed C-S cross coupling which can be carried out in aqueous solution [4]. Using *bis*-phenanthrolino Ni (II) catalyst and surfactant TPGS-750-m, we demonstrate an analogous cross coupling reaction for glycosyl thiols, achieving arylation with inexpensive and abundant nickel, and avoiding the need for toxic or environmentally harsh organic solvents. We have shown this methodology to be effective in the coupling of multiple mono- and disaccharide 1-glycosyl thiols with aryl- and heteroaryl halides bearing both electron-donating and electron-withdrawing substituents. The reaction proceeds under mild conditions in yields of up to 96% with retention of native anomeric stereochemistry [5].



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DEVELOPMENT OF NEW MULTIVALENT GLYCOCONJUGATES AGAINST INFLUENZA VIRUSES

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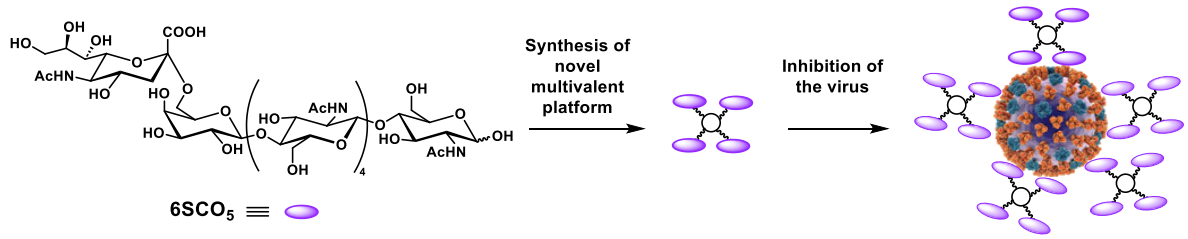
Oligosaccharides, present on the surface of human cells, play crucial biological roles as mediators of various cellular interactions [1]. In particular, they serve as entry points for different pathogens, which recognize them through specific surface proteins [2]. This is notably the case for influenza viruses, which cause human flu. The infection process is indeed triggered by the interaction between viral hemagglutinins and α-2,6-sialylated oligosaccharides covering our respiratory epithelial cells. Despite current ways of protecting against or treating influenza, between 290,000 and 650,000 deaths per year are recorded by the World Health Organization [3], highlighting the need to develop new therapies. In this context, we have discovered an oligosaccharide ($6SCO_5$) [4], capable of effectively blocking influenza viruses in an anti-adhesive therapy (*Scheme 1*). AIS biotech company has been founded to further develop and market a first drug candidate from these findings. Here, we report the advances in the design and biological evaluation of library of multivalent glycoclusters with varying valencies derived from $6SCO_5$.



Scheme 1. Strategy towards new anti-adhesive glycoclusters against Influenza viruses based on $6SCO_5$ oligosaccharide

Acknowledgements: This work is funded by AIS Biotech

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GLUCURONIDATION OF DIOSGENIN: THE STRATEGY FOR ENHANCING ANTIMICROBIAL ACTIVITY

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Diosgenin, a steroidal sapogenin recognized as a natural precursor to numerous pharmacologically significant compounds [1-3], continues to reveal its potential. *In vivo*, it undergoes metabolic transformations, including glucuronidation.
This study presents the synthesis and structural characterization of diosgenyl glucuronides, covering both the α and β anomers (Fig.). While the β anomer is a naturally occurring metabolite, the α anomer has not yet been synthesized or isolated. Through a precisely designed synthetic strategy, both anomers were obtained, and their structures were confirmed using X-ray crystallography.
Subsequent investigations were aimed to enhance the biological properties of the synthesized compounds. To achieve this, the carboxyl group was modified through conjugation with L- and D-alanine. The obtained derivatives were subjected to microbiological assays, which demonstrated diverse antimicrobial activity. Moreover, selected compounds were subjected to an in-depth evaluation of their effects on *Staphylococcus aureus* biofilm, a structure that represents a significant challenge in contemporary medicine. Notably, certain derivatives exhibited biofilm-disrupting activity, which is rare among structurally related compounds.
The research findings indicate a potential correlation between molecular structure and biological activity, offering valuable insights for further exploration of steroidal glucuronides as prospective antimicrobial agents.

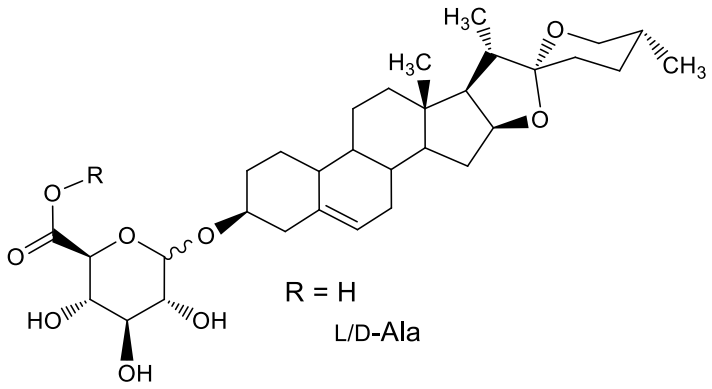

Figure 1. Chemical structure of diosgenyl glucuronides and their derivatives.

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OVERCOMING BACTERIAL RESISTANCE: GLYCOSYLATED THIAMPHENICOL AS A NEXT-GENERATION ANTIMICROBIAL PRODRUG

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Antimicrobial resistance (AMR) remains a global health challenge, demanding innovative approaches to enhance antibiotic efficacy. Thiamphenicol, a broad-spectrum antibiotic structurally related to chloramphenicol, has seen limited application due to resistance mechanisms developed by bacteria CAT (chloramphenicol acetyltransferase). Inspired by previous studies [1] demonstrating that glycosylation effectively protects antibiotics from CAT enzymatic inactivation, this work presents a glycosylated derivative of thiamphenicol as a promising antimicrobial prodrug. Through strategic conjugation with glucose, the modified thiamphenicol aims to circumvent bacterial defense systems, enhance aqueous solubility, and reduce toxicity compared to the parent compound. This study outlines a novel approach, highlighting the potential of glycosylation to transform existing antibiotics into more effective and safer therapeutic options in the fight against bacterial infections.

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DESIGNING A NEW SIALIDASE-ANTIBODY CONJUGATE FOR CANCER: LOOKING FOR NEW THERAPEUTIC STRATEGY

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Despite advances in cancer immunotherapies, many patients remain unresponsive, highlighting the need for new therapeutic targets. Aberrant glycosylation in cancer has emerged as a key mediator of immune evasion, with the overexpression of sialic acid-capped glycans being a common cancer associated alteration. These tumor-associated sialoglycans can engage sialic-acid-binding Ig-like lectin (Siglec) receptors on immune cells, triggering inhibitory pathways that suppress antitumor immunity. In human breast cancer (HBC), multiple Siglec receptors have been implicated in its ability to evade immune surveillance. Given the similarities between HBC and feline mammary carcinoma (FMC), cats serve as a valuable model for developing immunotherapeutic strategies. As such, we sought to develop a new therapeutic approach for FMC with potential translation to HBC, based on the design of a sialidase – single chain fragment variable (scFv) antibody conjugate with the ability to promote a targeted tumour cell desialylation, blocking Siglec activation and promoting cell mediated tumour clearance.

A panel of putative neuraminidases was designed and expressed in *E. coli*. Sialidase activity was assessed using a fluorometric assay, while flow cytometry was used to evaluate the capacity of the sialidases to remove cell surface sialic acids from tumour cells. The most promising sialidase, a feline neuraminidase, was conjugated to an anti-Trop2 scFv antibody. The resulting conjugate (fNeu-scFv) was characterized for its ability to desialylate tumour cells and bind Trop2 using flow cytometry, fluorescence microscopy, and western blot. fNeu-scFv retained sialolytic activity comparable to the isolated neuraminidase while exhibiting specific Trop2 binding in both HBC and FMC cell lines.

These findings suggest that targeted tumor desialylation via a sialidase-antibody conjugate represents a promising immunotherapeutic strategy for FMC, with potential translational applications in HBC. This approach aligns with the “One Health” concept, emphasizing its translational potential. Future studies will assess the conjugate’s ability to promote anti-tumoral FMC clearance by PBMCs and macrophage *in vitro*, as well as its capacity to reduce disease progression in mouse models of FMC.

Acknowledgements:
This research was funded by the Portuguese Foundation for Science and Technology (FCT), under projects UIDB/00276/2020 (CIISA), LA/P/0059/2020 (AL4AnimalS) and the PhD studentship UI/BD/153068/2022 (A. Couto Oliveira). We acknowledge Agência Nacional de Inovação through grant LISBOA-01-0247-FEDER-047033 (Glycomed), the Gilead GENESE program through the project 17805, the project grant 2022.07903.PTDC and La Caixa through the Junior Leader Fellowship LCF/BQ/PR23/11980039.

THE SYNTHESIS OF MULTIVALENT GLYCOMIMETICS FOR THE IMPROVED TREATMENT OF GLIOBLASTOMA MULTIFORME

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Glioblastoma Multiforme (GBM) is a fatal form of malignant brain tumour that is complicated to treat due to its heterogeneity and therapy resistance [1]. The standard treatment is to surgically remove as much of the tumour as safely possible and place a chemotherapy directly into the resection site; undoubtedly an invasive and potentially damaging procedure. Activation of complement system (CS) following brain injury is a major contributor to secondary brain damage [2]. Studies have shown that inhibition of Mannose Binding Lectin (MBL), an activator of the CS, can reduce the loss of sensorimotor capabilities after traumatic brain injury or stroke [3]. This project seeks to improve treatment outcomes by taking advantage of this cerebral protective effect, via the formulation of biodegradable chemotherapeutic hydrogels with MBL inhibitors. To prepare the hydrogels, the multivalent pseudo-dimannoside MBL inhibitor (Fig. 1-A) was synthesised at half gram scale, alongside a new generation of potentially more stable glycomimetic inhibitors (Fig.1-B) [4]. Their tetravalent scaffold linked to a pseudodisaccharide is believed to be a key factor in affinity for the MBL's oligomeric binding site (Fig 1-C). The goal of this work is to reduce treatment-related complications of GBM tumour removal and establish MBL's potential as a therapeutic target to minimize surgical brain damage.

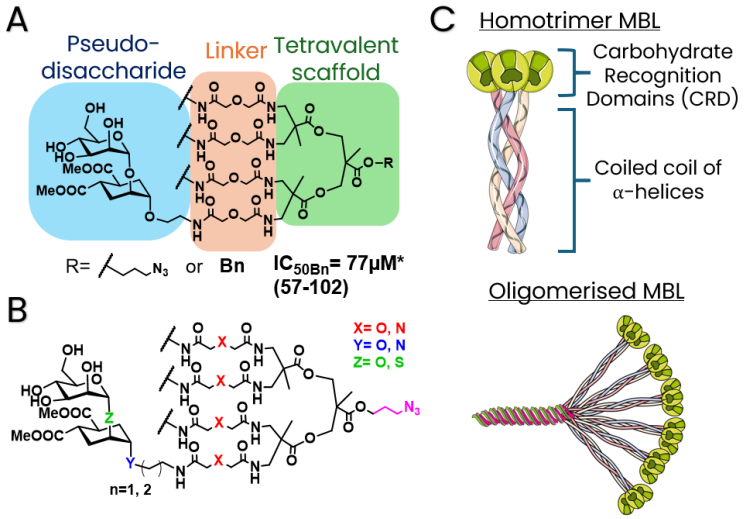

Figure 1. A: Structure of the tetravalent psedo-dimannoside inhibitor with its building block approach. *Inhibition of murine MBL-C binding to Man-BSA immobilised on a SPR sensor.[4] **B**: Novel inhibitors which will contain various combinations of heteroatoms applicable due to its interchangable synthetic route. **C**: Diagram of the MBL in it's monomeric (homotrimer) and oligomerised forms.

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UNSATURATED BETULIN GLYCOSIDES, THEIR HYDROGENATION AND ANTICANCER ACTIVITY

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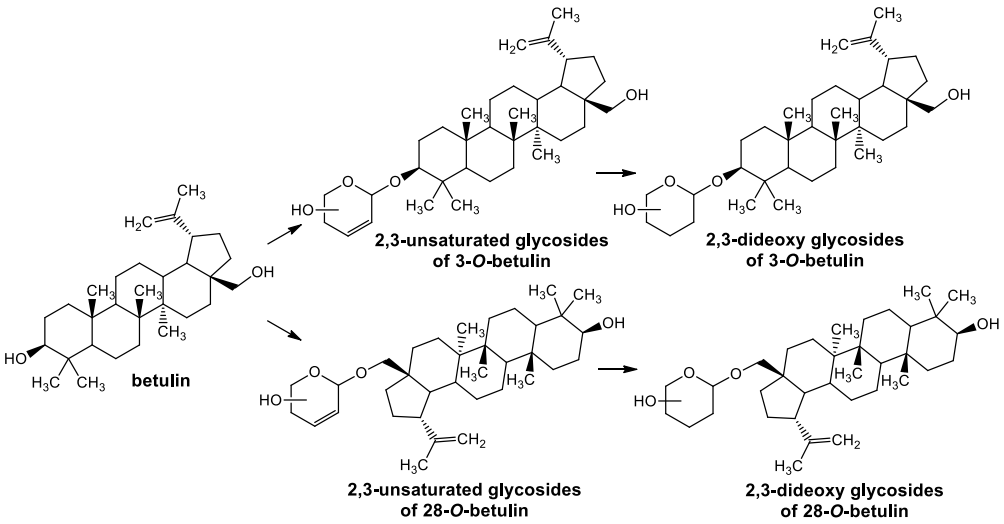
Betulin and its derivatives exhibit a wide range of biological activities, including anticancer, anti-inflammatory, antiviral and antiparasitic properties [1]. Glycosylation of betulin introduces a biorecognition fragment while maintaining its biological activity [2-4].
In search of betulin derivatives with improved anticancer properties, we synthesized a series of 2,3-unsaturated glycosides of 3-*O*- and 28-*O*-betulin (Figure). An adapted Ferrier rearrangement was used for this purpose [5]. Subsequently, obtained glycosides were treated with *in situ* generated diazene, leading to the formation of 2,3-dideoxy glycosides. Reaction conditions used enable hydrogenation exclusively within the sugar moiety while preserving the double bond in aglycone.
The anticancer activities of both 2,3-unsaturated and 2,3-dideoxy betulin glycosides were evaluated on prostate cancer (PC3) and breast cancer (MCF-7) cells and compared to the activity of betulin. Additionally, the selectivity of the tested glycosides was assessed in relation to non-cancerous keratinocyte (HaCaT) cells.



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β-D-ARABINOFURANOSYL CYCLITOL AZIRIDINES AS FIRST-IN-CLASS COVALENT AND SELECTIVE GBA2-BINDING SCAFFOLD

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Non-lysosomal glucocerebrosidase (GBA2) is a retaining β-glucosidase involved in glucosylceramide metabolism and cellular homeostasis. Although it is known that homozygous mutations in the GBA1 gene coding for the closely related lysosomal glucocerebrosidase GBA1 causes Gaucher disease, the relation between GBA2 and this disease remains poorly understood. In an attempt to find the first selective covalent GBA2 inhibitors, we screened our cyclitol aziridine-based ABP library as conformationally restricted cyclitols, armed with an electrophilic warhead suitable to intercept the Koshland double-displacement mechanism of GBA2. To our delight, we found that β-D-arabinofuranosyl cyclitol aziridines are selective and covalent GBA2 inhibitors. We harnessed this β-D-arabinofuranosyl cyclitol aziridine scaffold to develop new selective probes for visualizing GBA2 using confocal microscopy in cellular environments (Figure 1A) [1]. By connecting these cyclitol aziridines to lipophilic aglycons, we generated several potent and selective mechanism-based GBA2 inhibitors (Figure 1B). Finally, we connected several E3 ligase ligands to this scaffold using different linker lengths aiming to cause degradation of GBA2, a technique called proteolysis-targeting chimeras (Figure 1C). In summary, the new research tools presented herein hold promise for advancing our understanding of this enigmatic enzyme and exploring potential clinical applications.

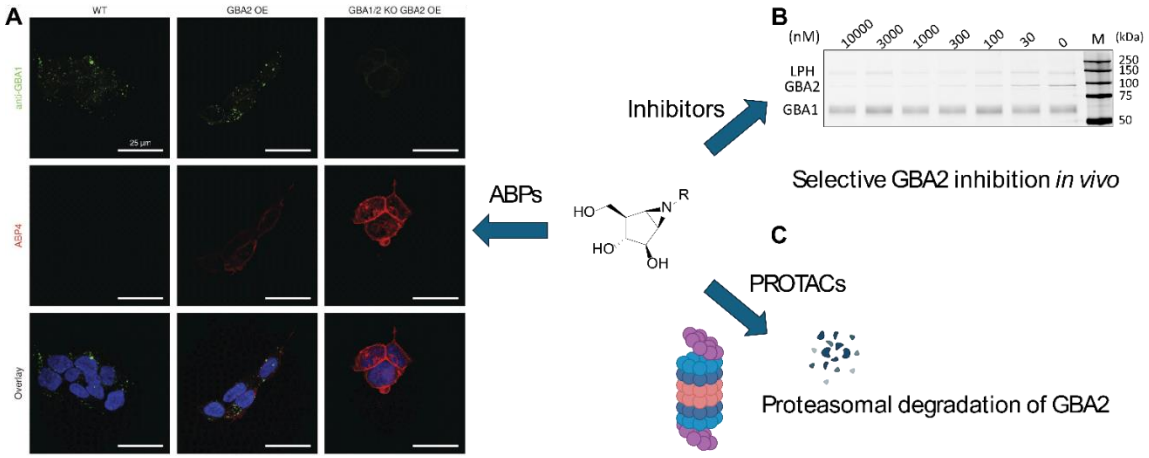

Figure 1. Exploitation of β-D-arabinofuranosyl cyclitol aziridine scaffold. **A**: Cellular imaging of GBA2 using ABPs. **B**: Selective inhibition of GBA2 in zebrafish embryos. **C**: PROTACs.

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FLUORINATED TRIHYDROXYPIPERIDINES AS POTENTIAL PHARMACOLOGICAL CHAPERONES FOR GLUCOCEREBROSIDASE

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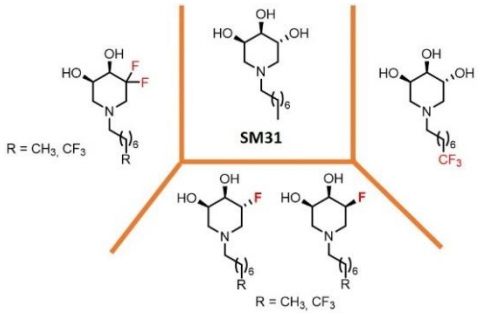
Glucocerebrosidase (Glucosylceramidase Beta or GCase) is a lysosomal enzyme that hydrolyses glucocerebroside to glucose and ceramide. *GBA1* mutations impair GCase function, leading to substrate accumulation in macrophages. This underlies Gaucher disease, one of the most common lysosomal storage disorders (LSDs) [1], and is a major genetic risk factor for α-synucleinopathies, including Parkinson's Disease [2]. Pharmacological chaperones (PCs) have emerged as a promising therapeutic strategy to restore GCase function. These small molecules bind and stabilize misfolded enzymes, enhancing residual activity at sub-inhibitory concentrations. Glycomimetics, particularly iminosugar derivatives, are the most widely studied PC class for LSDs [3]. Various substitutions and modifications in heterocyclic carbon configurations have been explored. Of these, the incorporation of fluorine has garnered significant interest due to its ability to influence conformation, pKa, potency, membrane permeability, metabolic pathways and pharmacokinetic characteristics [4].
Our current research explores substituted trihydroxypiperidines for GCase function restoration [5]. In this communication, I will outline our synthetic efforts to integrate fluorine into the iminosugar core of one of our best-in-class PC (SM31) [6], aiming to explore the fluorine contribution on the physicochemical properties and the efficacy of the system.



Scheme 1. Fluorine integration on our best-in-class PC (SM31)

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GLYCOSYLTRANSFERASES GT-B DOMAIN SWAPPING: HOW TO ENHANCE VERSATILITY FOR SUGARS DONORS AND ACCEPTORS

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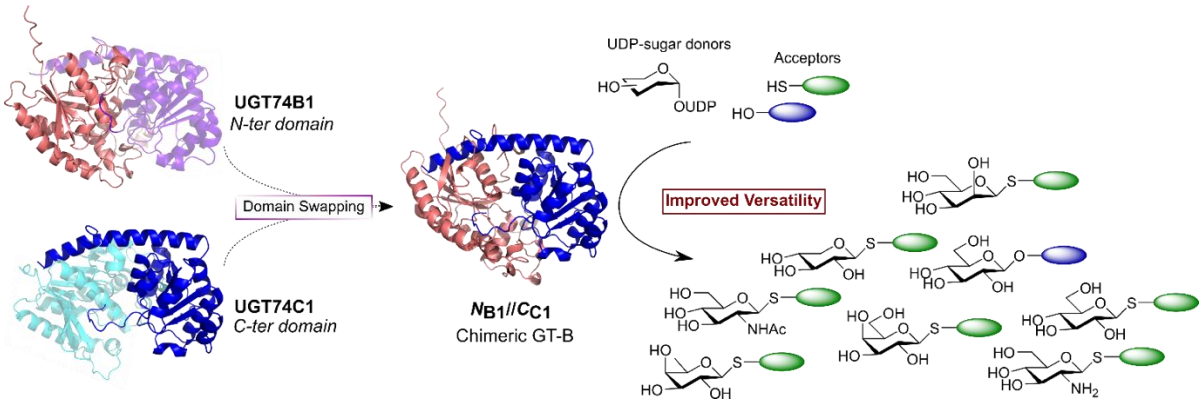
In glycochemistry, a wide range of sugar-metabolising enzymes such as **glycosyltransferases** (GTs) have been developed and used for the chemo-enzymatic synthesis of glycosides, providing an alternative to the chemical synthesis of glycosides. GTs generally catalyse glycosidic bond formation using **sugar donors containing a phosphate-leaving group** to specific acceptors [1]. However, GTs commonly **lack the modularity** required to tailor the nature of the transferred sugar or the acceptor. As a result, the identification of a new GT, followed by its genetic engineering, is required to graft a new sugar onto a given acceptor. In this context, we have **recently developed an engineering approach based on the chimerization of GT domains**, belonging to GT-B structural GT family [2]. This family is constituted of GTs with two domains facing each other: the acceptor recognition domain (*N*-terminus) and the donor binding domain (*C*-terminus), linked by an unstructured loop [3].



We have demonstrated the possibility to **exchange and assemble domains** originating from two GT-B enzymes to **generate a dimeric enzyme** [2]. This resulting chimera was found to exhibit **higher acceptor promiscuity** compared to parent GT-B enzymes, able to generate both *O*- and *S*-glucosides with diverse structures, which was correlated to higher flexibility of domains interface. In addition, we recently investigated the ability of this chimera to transfer a wider range of sugars than the native enzyme. As we observed for acceptor promiscuity, the chimeric enzyme allowed the efficient **transfer of a much broader range of sugars** compared to the native GT-B from UDP-sugars. This approach makes the GT-B domain swapping strategy particularly attractive for the development of a versatile biocatalytic tool for glycoside synthesis.

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COMPREHENSIVE INVESTIGATIONS OF MUC1 O-GLYCOSYLATION PROCESS REVEAL INITIAL SITE PREFERENCE BY THE POLYPEPTIDE GALNAC TRANSFERASES

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Mucin1 (MUC1) is an attractive target for anticancer vaccines, due to its overexpression and highly aberrant O-GalNAc glycosylation in many prevalent cancers. The distribution and pattern of O-GalNAc glycosylation on MUC1 are essential for its biological activity, which is regulated by 20 members of the polypeptide N-acetyl-α-galactosaminyltransferase (GalNAc-T) family in human cells. However, the site-specific O-glycosylation process of MUC1 by each GalNAc-T isoform is still incompletely understood.

In this study, we successfully obtained 14 members of the human GalNAc-T family with high catalytic activity based on a simple bacterial expression system. Using MUC1-derived peptides as substrates, we comprehensively investigated the substrate specificity and site selectivity of GalNAc-Ts through chromatographic and mass spectrometric analyses. The results reveal that based on their initial acceptor sites, GalNAc-Ts can be grouped into two clusters: cluster1 enzymes preferentially initiate O-glycosylation at the GVTS motif on MUC1, and cluster2 enzymes initiate at the GSTA motif, leading to the high O-glycosylation occupancy of both motifs. Furthermore, molecular dynamics simulations and site-directed mutagenesis revealed that the initial O-glycosite preferences of GalNAc-Ts are governed by two key residues located within the catalytic flexible loop, which plays a critical role in regulating peptide substrate binding. Swapping these residues between representative members of the two clusters not only influences the initial glycosylation of MUC1, but also affects the O-glycosite selectivity of interferon alpha-2b (IFNα2b) and granulocyte colony-stimulating factor (G-CSF). In addition, we identified that the lectin domain of GalNAc-Ts is involved in the cooperative regulation of substrate selectivity. Taken together, these results establish a classification of the GalNAc-T family based on initial site selectivity and uncover the enzymatic mechanism underlying MUC1 O-glycosylation, providing insights into its aberrant O-glycosylation and roles in cancer immunomodulation.

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DISCOVERY OF NEW BACTERIAL A2,3- AND A2,6-SIALYLTRANSFERASES WITHOUT UNDESIRED HYDROLYTIC ACTIVITIES FOR EFFICIENT GLYCAN SYNTHESIS

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The development of efficient enzymatic tools for sialylated glycan synthesis remains a critical challenge in glycobiotechnology. This study addresses longstanding limitations in sialyltransferase (SiaT) applications by identifying novel bacterial enzymes with superior catalytic properties. While bacterial SiaTs are generally preferred over mammalian orthologues due to simpler recombinant production and broader substrate specificity [1,2], their utility has been constrained by undesirable hydrolytic side activities toward CMP-Neu5Ac donors and sialylated products [3,4]. Previous engineering attempts to suppress these activities resulted in compromised catalytic efficiency or thermal stability [5]. Here, we report the discovery of previously uncharacterized bacterial α2,3- and α2,6-SiaTs that maintain native transferase activity while exhibiting negligible hydrolase, sialidase, and trans-sialidase activities. The absence of hydrolytic side reactions enables unprecedented reaction yields (>95%) in gram-scale syntheses of complex glycans, including sialylated human milk oligosaccharides. Here, we demonstrate the synthesis of sialyllactoses. This breakthrough establishes a new generation of biocatalysts for industrial glycoconjugate production while providing insights into the molecular determinants of sialyltransferase promiscuity.

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OPTIMISED CHEMOENZYMATIC SYNTHESIS OF 3-*(EQ)*-*F*-PSE5AC7AC, A POTENTIAL PRODRUG INACTIVATOR OF PSEUDAMINYL TRANSFERASES, UTILISING RATIONALLY ENGINEERED MUTANTS OF PSEUDAMINIC ACID SYNTHASE PSEI

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5,7-di-*N*-acetyl-pseudaminic acid (Pse5Ac7Ac) is a non-mammalian nonulosonic acid sugar found on bacterial cell surfaces as well as directly *O*-glycosylated on flagella [1]. Notably Pse5Ac7Ac plays a key role in the virulence of various multidrug resistant bacteria including *Pseudomonas aeruginosa* and *Acinetobacter Baumannii* [2]. The discovery of the biosynthetic pathway of Pse5Ac7Ac in recent years has allowed for the chemoenzymatic synthesis of unnatural derivatives including potential inactivators of the pathway. Here we present a strategy, utilising rationally designed mutants of *Campylobacter jejuni* Pse5Ac7Ac synthase PseI, to increase the activity of PseI with unnatural cofactor 3-*F*-PEP to afford 3-(*eq*)-*F*-Pse5Ac7Ac **2** (Figure 1). Through motility assays, we have shown that 3-(*eq*)-*F*-Pse5Ac7Ac can decrease motility of *C. jejuni* 81116 which is tentatively predicted to be a result of *in vivo* metabolism of 3-(*eq*)-*F*-Pse5Ac7Ac **2** by PseF to active CMP-3-(*eq*)-*F*-Pse5Ac7Ac **3**. We propose that CMP-3-(*eq*)-*F*-Pse5Ac7Ac inactivates dedicated pseudaminyl transferases (PseTs) namely the motility associated factor proteins (Mafs) blocking transfer of Pse5Ac7Ac onto the flagella [3].

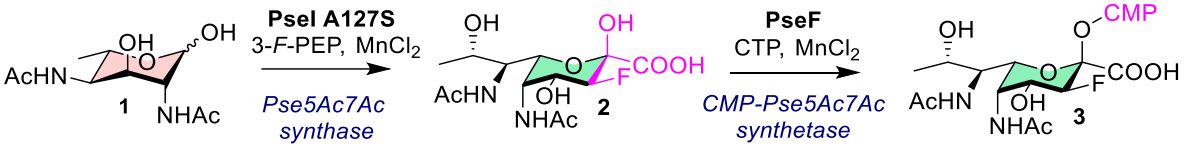

Figure 1. PseI A127S reacting 6-deoxy-Alt-diNAc **1** with 3-*F*-PEP to afford 3-(*eq*)-*F*-Pse5Ac7Ac **2** which in turn, through PseF, can react with CTP to give potential Maf inactivator CMP-3-(*eq*)-*F*-Pse5Ac7Ac **3**.

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A SCREENING METHODOLOGY FOR THE EVALUATION OF NATIVE STARCH-DEGRADING AMYLASES IN CEREAL-BASED PROCESSING

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Enzymes are increasingly used in industrial processes to tackle challenges like reducing energy consumption, promoting sustainability and enhancing waste valorisation. In cereal-based processes, there is a growing interest in native starch-degrading amylases because of their ability to degrade starch in its native crystalline granular structure, eliminating the need for gelatinisation which typically requires high processing temperatures. Although several native starch-degrading amylases have been identified in the literature, the variety of methods and analytical conditions makes it difficult to compare their effectiveness. We therefore propose a screening methodology to evaluate the potential of native starch-degrading amylases under relevant conditions.

Nine promising native starch-degrading amylases were selected after an extensive literature review for a comparative study. These enzymes, all endo-amylases, originated from both bacterial and fungal sources. The first element of the screening methodology was to monitor starch degradation over time by measuring the production of reducing sugars. As the scope was set on cereal-based processes, the screening was performed at pH 5.0 and 40°C. Reducing sugar concentrations were determined by a colourimetric assay, namely the BCA assay. With this approach, different native starch-degrading amylases with potential for cereal-based processing conditions could be identified. Complete starch degradation was defined as the amount of reducing sugars produced when starch is fully hydrolysed to glucose. Starch degradation levels between 15% and 44% were measured after an incubation of 24h with 0.08 Ceralpha Units amylase (as defined with the Ceralpha assay from Megazyme, Bray, Ireland). By measuring starch degradation at multiple time points, a kinetic study was performed to determine the degradation rates, which ranged from 0.05/s to 0.94/s.

The second element of the screening methodology was to assess the capacity of the amylases to bind to native starch. The binding capacity ranged from 0% to 70% with 100% binding capacity indicating complete binding of all amylases to the native starch granules. Strong differences were observed between the selected amylases. The results showed that amylases with higher binding capacity tended to show higher degradation rates and/or higher native starch degradation levels. This binding assay could thus provide initial insights into the capabilities of the amylases to degrade native starch, without the need for extensive experimental work. The two elements of the screening methodology allowed us to compare the ability of the selected amylases to degrade native starch in the context of cereal-based processes.

PRODUCTION OF UNSULFATED CHONDROITIN AND ASSOCIATED CHONDRO-OLIGOSACCHARIDES IN RECOMBINANT *ESCHERICHIA COLI*

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Glycosaminoglycans (GAGs) are key components of vertebrate extracellular matrices. Among them, chondroitins are distinguished by a polysaccharide backbone composed of repeating disaccharide units of β4-glucuronic acid (β4GlcUA) and β3-N-acetylgalactosamine (β3GalNAc). Chondroitin is typically sulfated, which defines the family of chondroitin sulfates, although unsulfated forms of chondroitin can be found in certain bacteria [1]. Given their involvement in various biological processes, chondroitin oligosaccharides are promising molecules for chemical or enzymatic modifications, such as sulfation, to yield well-defined biologically active compounds [2]. The bacterial production of oligosaccharides presents a viable alternative to in vitro synthesis, as it allows for the integrated synthesis of all polysaccharide substrates, nucleotide sugars, and enzymes in the recombinant host, effectively functioning as a living enzymatic reactor. This method has already been successfully applied to produce heparosan oligosaccharides [3] and, more recently, di- and tetrasaccharides of chondroitin [4]. In this study, we engineered non-pathogenic strains of *Escherichia coli* to produce unsulfated chondroitin, both with and without chondroitin lyase, to generate either the chondroitin polymer or its associated oligosaccharides. Chondroitin was synthesized using chondroitin synthase KfoC from *E. coli K4*, and degradation of chondroitin was achieved with chondroitin lyase from *Victivallis vadensis* ATCC BAA-548 [5], acting as a true endo-enzyme to produce a broad range of oligosaccharides, from trimers to 18-mers. This is the first report of the microbial production of large chondro-oligosaccharides.

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EXPLORING PROTEIN GLYCOSYLATION *VIA* CHEMICAL TAGGING OF METHIONINES

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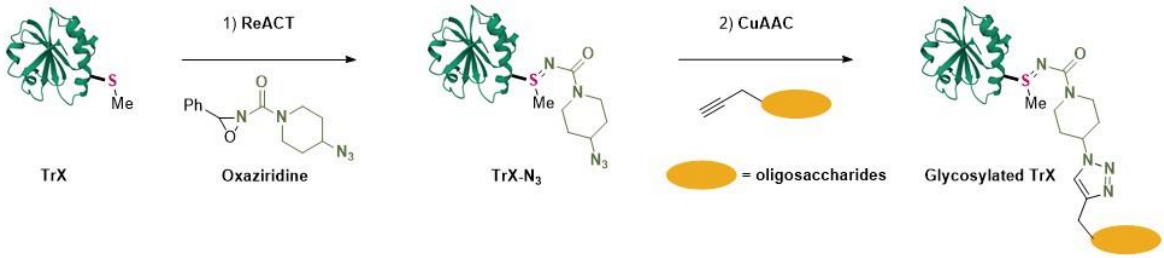
Glycosylation is a major post-translational modification of proteins that significantly influences their folding, distribution, stability and activity [1]. Despite significant advancements in protein expression systems, mixtures of glycoforms are often produced and glycosylation profiles are specific to each host organism. Controlling protein glycosylation is thus a major challenge, particularly when therapeutic proteins are targeted. Chemical conjugation is an interesting alternative method to this purpose. Redox Activated Chemical Tagging (ReACT) has recently emerged as an efficient bioconjugation method for protein modification. This click reaction consists of the addition of methionine sulfur atom to an electrophilic oxaziridine leading to a sulfimide adduct [2]. In the present work, we combine ReACT and CuAAC click reactions in a one pot sequence as a general strategy to access glycosylated proteins. As a proof of concept, the methodology was first applied to a pentapeptide derived from the sequence of thioredoxin, a model protein, before extending its application to the entire protein (Scheme 1). To go further, the impact of glycosylation on the activity and stability of a target enzyme was assessed.



Scheme 1. General strategy towards protein glycosylation based on one-pot sequential ReACT-CuAAC reactions

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OHMIC HEATING AND ITS POTENTIAL APPLICATION IN POLYSACCHARIDE EXTRACTION FROM AGRICULTURAL WASTE

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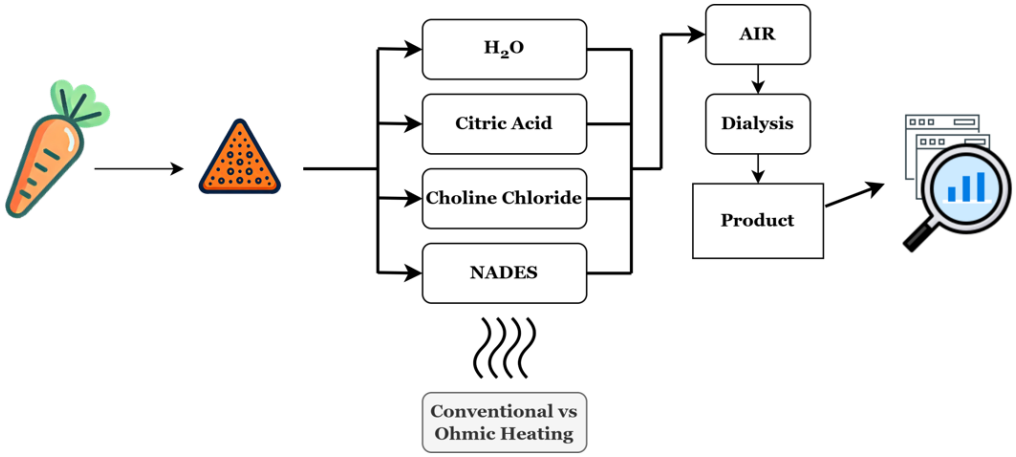
Ohmic heating is a well-known and eco-friendly technique that consists in passing an electric current through food materials, generating internal heat due to their electrical resistance. In this way, food components as well as the final products can be pasteurized. The advantage of this method is the rapid and uniform heating of the environment, which can be used in a new way, i.e. to increase the efficiency of the extraction process of natural products, including pectins from plant biomass, moreover in a shorter time with lower energy consumption [1].
In the presented studies, Ohmic heating was used as a factor supporting the extraction of pectins from carrot root pomace. The biomass selected for the experiments is a by-product of juice extraction and, as is known, is rich in polysaccharides, which are also present as fiber. Unfortunately, it is most often used as an additive to animal feed [2], and the potential for producing pectins from these pomace, which have a much higher market value, is not used. The aim of the experiments was to assess the potential of using Ohmic heating to obtain pectins from carrot root, when the process will be examinated in Natural Deep Eutectic Solvents (NADES), eco-friendly, biodegradable solvents made from natural compounds, offering an effective, low-toxicity solution for extracting wide range of bioactive compounds from various sources [3]. The results of experiments were compared to those conducted in water and in the water solutions of each of NADES ingredients, and when the heating source was conventional.



Acknowledgements: The work was supported by the National Science Centre, Poland, under the research project „ Processes of obtaining polyanionic saccharides from native agricultural waste, including Ohmic heating-assisted extraction in environmentally safe solvents.”, no UMO-2023/51/B/ST8/02851

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STRUCTURAL ELUCIDATION OF A CAPSULAR POLYSACCHARIDE FROM *LACTIPLANTIBACILLUS PLANTARUM*

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In recent times, nutrition has become one of the main health patterns due to disorders related to sedentary lifestyles, and therefore, consumers consider functional foods an attractive solution [1]. Among the main ingredients of functional foods are probiotics, that are live microbes that have beneficial effects on the host [2]. Among probiotics, lactic acid bacteria (LAB) have several scientifically proven effects on human health, such as antimicrobial activity, immune enhancement, and anti-cancer activity. Their activities derive from the molecules they produce, including polysaccharides, which are used not only as ingredients but also especially as food additives. The wide spectrum of applications of microbial polysaccharides is due both to their properties as thickeners and, above all, to their immunomodulatory properties, i.e., anti-cancer, anti-inflammatory or antimicrobial [3].
Based on the above, the present work focuses on the study of cell wall polysaccharides produced by a strain of *Lactiplantibacillus plantarum*, a Gram-positive, mesophilic bacterium belonging to the LAB group, which colonises the human and animal gastrointestinal tract. *L. plantarum* can produce antimicrobial and antioxidant molecules, modulate the immune system, and strengthen the intestinal microflora [4]. However, the identity of the glycans produced by this bacterium is unknown, even though they are supposed to be key players in the activities reported. This communication will describe the structural characterisation of the capsular polysaccharide produced by *L. plantarum*, paving the way for studies aimed at determining its beneficial functions.

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DESIGN OF GLYCOLIPID-FUNCTIONALIZED EXTRACELLULAR VESICLES FOR THEIR SELECTIVE TARGETING TO DENDRITIC CELLS

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Despite progress in cancer treatment, many new drugs developed through conventional approaches still cause significant side effects and are limited by tumour resistance to chemotherapy [1]. As a result, immunotherapy has gained attention as an alternative strategy that works by activating the immune system to recognise and attack cancer cells. Among the different types of immunotherapies, therapeutic vaccines aim to stimulate the patient's immune response by delivering tumour antigens after the disease has developed. This is intended to activate cytotoxic lymphocytes, which are responsible for killing cancer cells [2]. Using nucleic acids to encode antigens has several advantages, including their ability to act as natural adjuvants and the flexibility to modify the enconded antigens without major changes to the formulation. However, nucleic acids are unstable and do not easily enter cells, so they need to be delivered by a carrier that can both protect them and target antigen-presenting cells specifically. Thus, the aim of the present work is to develop new nano-vectors based on lipid systems that encapsulate the antigenic genetic material and are selectively vectorised towards the antigen presenting cells.

Among the various existing vectors, extracellular vesicles (EVs), nanosystems naturally released by all human cells that already contain genetic material and have intercellular communication functions through gene material and proteins transference, have been chosen [3]. It has been designed a new type of vectorization of EVs, using glycolipids, which are expected to integrate into their lipid membrane and expose the sugar moiety on the surface, enabling selectively targeted to antigen presenting cells. Five different compounds have been developed, which differ in the presence, length or absence of a linker between the lipid and sugar units, monosaccharide or disaccharide and the inclusion of a peptide moiety for comparison. The method of incorporation of these glycolipids into the vesicles has been established and their integration has been confirmed by confocal microscopy and flow cytometry. Furthermore, preliminary results were also obtained from in vitro and in vivo assays. In conclusion, this study demonstrates the successful functionalization of EVs, resulting in altered biodistribution to lymph nodes and modified uptake patterns by immune cells—supporting their potential as a promising platform for nucleic acid-based therapeutic vaccines.

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RECOMBINANT PRODUCTION OF HUMAN MILK OLIGOSACCHARIDES AND THEIR DERIVATIVES FOR GLYCOCONJUGATION

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Human milk oligosaccharides (HMOs) are the third largest group of human breast milk compounds, with less nutritional, rather than bioactive, functions such as prebiotics, immunomodulators, or neurostimulators [1]. Research into their bioactivity is limited by the complex synthesis, and therefore the high cost of HMOs. We were able to solve this problem by using genetically engineered bacterial cell factories, creating recombinant production strains able to produce selected HMOs *in vivo* [2]. By cultivating the strains in a fed-batch bioreactor, followed by tailored isolation procedures, we are able to achieve gram-scale production of HMOs at a favourable price. Fast and efficient biotechnological synthesis unlocks the opportunity for glycoconjugation of isolated HMOs on various carriers. Multivalent presentation can amplify the interaction of HMOs with gut microbiota or cells of the immune system and facilitate the detection and visualization of these interactions [3]. Suitable carriers can also be used for high-throughput screening of HMO interactions with various compounds such as lectins, cytokines, interleukins, drugs, etc. The interdisciplinary approach of engineered recombinant strains offers a suitable platform for carbohydrate synthesis, which can speed up the research on these highly valuable compounds.

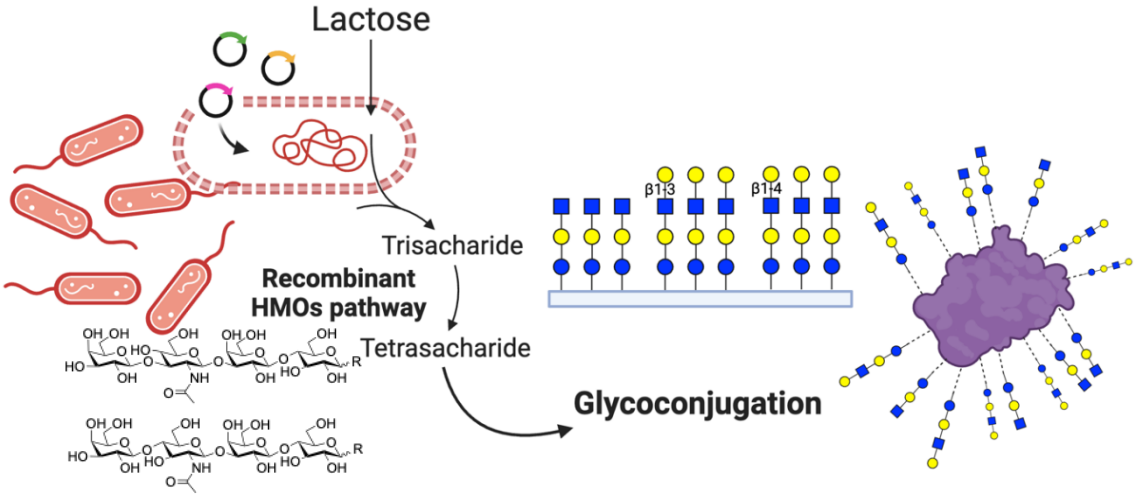

Figure 1. Schematic representation of HMOs recombinant synthesis with its possible glycoconjugation on various carriers.

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TARGETING THE DIFFERENT BINDING SITES ON SARS-CoV-2 WITH SELF-ASSEMBLING β-CYCLODEXTRINS

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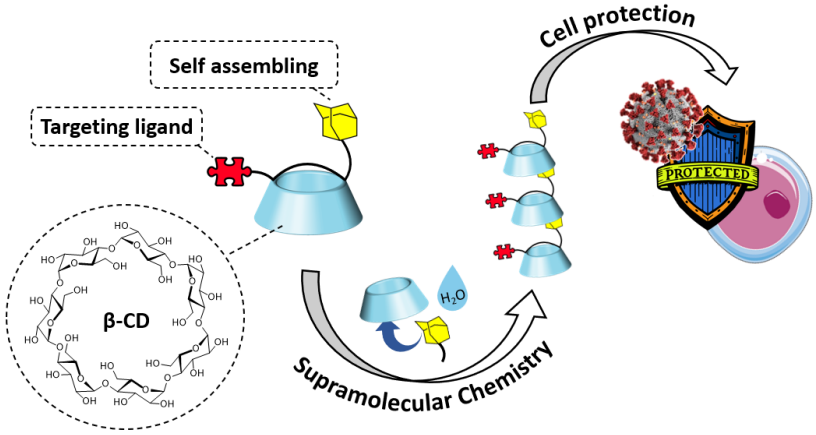
Despite the awareness risen by the global pandemic caused by SARS-CoV-2, there is still a lack of over-the-counter tools to prevent the transmission of this and other respiratory viruses. To fight the infection caused by SARS-CoV-2, the main approach is to target its spike protein, which serves as a point of attachment to the host cells through two binding domains: the glycan- and the receptor-binding domain (GBD and RBD, respectively). Literature has shown examples of both GBD- and RBD-targeting inhibitors to prevent the infection by SARS-CoV-2, taking advantage of their affinity for different carbohydrates [1,2] or peptides [3]. In our laboratory, we have synthesised a system based on β-cyclodextrins decorated with an adamantane unit to promote a thermodynamically favoured self-assembly in water [4]. Now, we have designed and synthesised a library of ligands targeting both the GBD and RBD of SARS-CoV-2, which provides specificity to the scaffold to protect Vero E6 cells against viral infection. This research has provided encouraging results on the development of antiviral therapies against SARS-CoV-2 targeting either or both of its binding domains, informing a toolset of self-assembling cyclodextrins with potential to inhibit the infection not only by SARS-CoV-2, but also by other respiratory viruses.



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GLYCONANOCAPSULES WITH pH RESPONSIVENESS FOR ENCAPSULATION AND SMART DELIVERY OF THERAPEUTICS

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The integration of carbohydrates in nanomaterials is key to develop water-dispersible, biocompatible and targeted delivery systems. In particular, smart glyconanomaterials have received attention due to their responsiveness to specific stimuli, making them promising candidates for precise and programmable drug delivery.
Our research group has expertise in the self-assembly of poly(allylamine) hydrochloride (PAH) polymers into nanoparticles (NPs) in phosphate buffer (PB). These NPs are stable at pH 6-8 and reversibly disassemble at pH<5, making them suitable for intracellular use [1]. However, the positive charges of the protonated primary amines limit their use for biological applications. In the present work, to reduce toxicity and improve targeted release, the polysaccharide dextran (DEX) has been introduced in different molar ratios to the PAH backbone *via* chemically controlled reductive amination approach. In the case of 1:1 PAH/DEX ratio, the polymer assembles as glyconanocapsules (glyco-NCs) at PB concentrations above 5 mM [2]. As a proof of concept, the application of these novel glyco-NCs has been initially investigated for the pH-controlled delivery of bovine serum albumin (BSA) as a model protein. Ongoing studies are exploring the encapsulation of therapeutic proteins and dextran-driven cell targeting, paving the way for advanced smart drug release glyconanosystems.

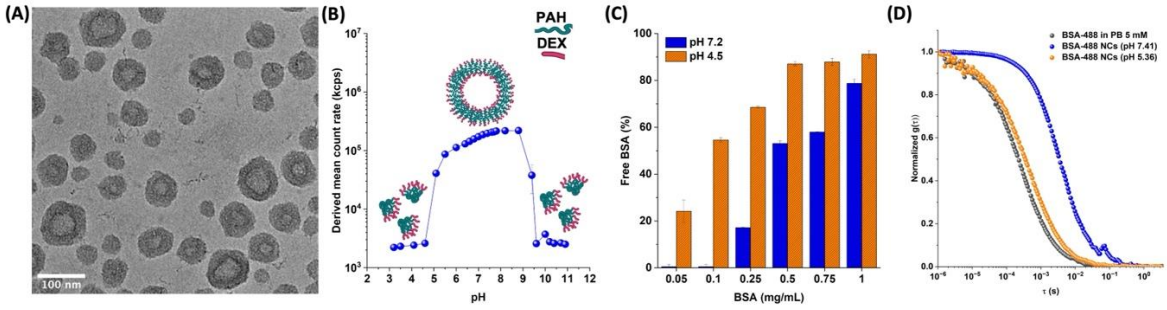

Figure 1. Glyco-NC analysis: (A) Cryo-EM showing morphology and size; (B) pH-responsiveness; (C) Bicinchoninic acid assay and (D) Fluorescence Correlation Spectroscopy for characterization of BSA encapsulation and release.

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NATURALLY DERIVED POLYSACCHARIDES INTERACTING WITH PATTERN RECOGNITION RECEPTORS

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Naturally derived polysaccharides have the potential to trigger pattern recognition receptors (PRRs) on the surface of immune cells, thereby initiating an immune response [1]. Therefore, polysaccharides could represent an attractive alternative for novel immunotherapeutic drugs. In this study, water-soluble polysaccharides isolated from selected medicinal plants and a fungus from Norway and Brazil were screened for their ability to activate an immune response by interacting with PRRs. The polysaccharides included, among others, pectins and arabinogalactan-proteins, and their structures are previously characterized. To identify polysaccharides of potential interest for immunotherapy, human embryonic kidney (HEK293) blue reporter cell lines transfected with specific human PRRs (TLR2-TLR1, TLR2-TLR6, TLR3, TLR4, TLR5 and Dectin-1a) were utilized. Additionally, THP1-Blue™ cells were used to monitor NF-κB activation in a monocyte-like environment. Several of the polysaccharides showed the ability to activate both TLR2-TLR6 and TLR4, while some also activated TLR2-TLR1 and the classical β-glucan receptor Dectin-1a. Furthermore, the same polysaccharides activating multiple PRRs showed a potent activation of THP1-Blue™ cells, comparable to the known immunogenic crude Zymosan [2]. The most active polysaccharides were pectins isolated from *Opuntia ficus-indica* (L.) Mill. and *Daphne mezereum* L., as well as arabinogalactan proteins isolated from *Persea americana* Mill. Interestingly, of the most potent stimulants of THP1-Blue™ cells, only the pectic polysaccharide from *D. mezereum* showed interaction with Dectin-1a. This indicates a lesser importance of this receptor for immune stimulation in THP1-Blue™ cells. In conclusion, this research shows the ability of purified water-soluble polysaccharides to stimulate multiple PRRs, possibly contributing to a synergistic effect on stimulation of immune cells.

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CYANOBACTERIA AS AN ATTRACTIVE SOURCE OF POLYSACCHARIDES FOR FOOD AND COSMETIC APPLICATIONS

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Biopolymers produced by microalgae or cyanobacteria, as well as those extracted directly from cell biomass, such as polysaccharides,have been discussed and created a lot of interest, especially for food packaging, medical product, and other areas that, despite recent successful developments in bio-based polymers, continue to require improvement [1,2].
In the present work, extracellular polymeric substances (EPS) produced by the rod-shaped and filamentous cyanobacteria identified as cyanobacterium aponinum (PP663238). and persinema sp. (PP662646), respectively, were isolated. The EPS structure was investigated by chemical and instrumental analysis including Size Exclusion Chromatography (SEC), gas chromatography mass spectrometry (GC-MS), Fourier transform infrared (FTIR) spectroscopy, and nuclear magnetic spectroscopy (1H-NMR). The morphological, antioxidant, cytotoxic, and thermal properties of the EPS were studied. Chemical results revealed a rich composition in total sugars for both EPS with a heterogeneous polysaccharides. The structure and composition of the polysaccharides influence their thermal properties. In fact, the thermal resistance was equivalent for the EPS isolated from both strains with a difference in thermal stability. The scanning electron microscopy (SEM) images highlighted the difference of the EPSs in their surface structure. The EPS isolated from c. aponinum has a filamentary structure, leading to faster and rougher degradation and mass reduction compared to the EPS extracted from persinema sp which has a smooth plate structure. Both EPS displayed an effective antioxidant activity with tolerable cytotoxic effects. The outcomes of this study demonstrate the possible potential use of c. aponinum and persinema sp. EPS in several food and pharmaceutical applications.

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MULTIVALENT, MULTIFUNCTIONALIZED, AND MULTICHIRAL GLYCOSIDE RECEPTORS IN CHIRAL GAS SENSING

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Volatile organic compounds (VOCs) exhaled, excreted, and released through the skin reflect underlying physiological activity and pathological states. However, this valuable information remains mostly inaccessible due to the inherent challenges associated with sensing of VOCs: 1) detection of sub-ppm concentrations of weakly interacting small molecules that lack prominent or universal functional groups and 2) discrimination between structurally similar molecules and enantiomer pairs while maintaining sensitivity toward diverse families of biorelevant VOCs. Despite the growing demand for biosensors that could enable rapid and non-invasive breath and body odor analyses, there is a shortage of bioactive recognition layers with high sensitivity, selectivity, and capability for chiral discrimination.
Glycans are natural multichiral and multivalent interaction mediators that can be incorporated into functional sensing layers [1]. Exploitation of the innumerous possible combinations of glycan scaffolds and site-specific modifications opens a route toward versatile sensing applications. Selectively substituted monosaccharides (sMS) modified with aryl or benzyl esters, ethers, and carbonates are commonly used as glycosyl donors for the synthesis of complex glycans. The combination of a chiral multivalent monosaccharide scaffold with diversely functionalized substitutions can facilitate discrimination of weakly interacting, structurally similar or enantiomeric VOCs through the formation of a unique docking site. A meticulous rational design of the identity and arrangement of substituents can enable the functionalization of nanomaterial surfaces and the tuning of selectivity toward VOCs.
In this work we demonstrate enantioselective gas sensing using devices based on semiconducting single-walled carbon nanotubes (sc-SWCNTs) functionalized with substituted thioglycoside receptors as interaction mediators [2]. This represents the first instance of glycans that function in an ensemble with single sc-SWCNTs for chiral discrimination in the gas phase. This novel strategy prompts the exploration of the endless combinations of chiral scaffolds and modification patterns with various VOCs, and empowers future sensing applications.

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GLYCOSIDASE INHIBITORS ON POLYSACCHARIDES: COMBINING TWO WORLDS

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Glycoconjugates, like glycoproteins and glycolipids, make up an essential part of all cell surfaces and are taking part in many biological processes, for example in cell-cell communication events. Additionally, cells are covered in a glycan layer [1]. The solid liquid interface of such oligo- and polysaccharides is therefore of importance in biology and medicine, for example for cell adhesion and recognition processes [2]. Semi-synthetic polysaccharide interfaces can be used to mimic such surfaces for interaction studies for e.g. protein/enzyme binding.

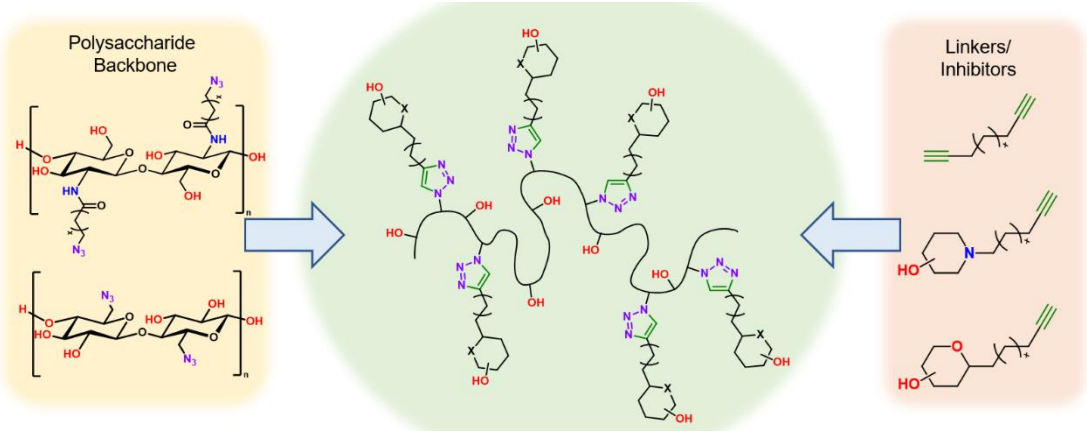

Figure 1. Linking azide-containing polysaccharides and alkyne-modified small molecules by azide-alkyne CLICK chemistry.

We are interested in the interactions between glycosidases and glycomimetic-modified polysaccharides. Therefore, small molecules, like imino- or isoiminosugars, are attached to e.g. chitosan or cellulose [3], which contain respective azido-groups, by azide-alkyne CLICK chemistry (Figure **1**). This can be done on thin-film surfaces [4], which enables analysis techniques like AFM, QCM-D or SPR, as well as in bulk-material. Potential applications are interaction with respective biological targets e.g. lectins from bacteria like uropathogenic *E. coli* [5] or protein purification by affinity chromatography. Synthetic and analytical details as well as biological evaluations will be presented.

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SYNTHESIS AND STRUCTURE-AFFINITY RELATIONSHIP OF A NEW CLASS OF LACTOSYLAMIDE GALECTIN INHIBITORS

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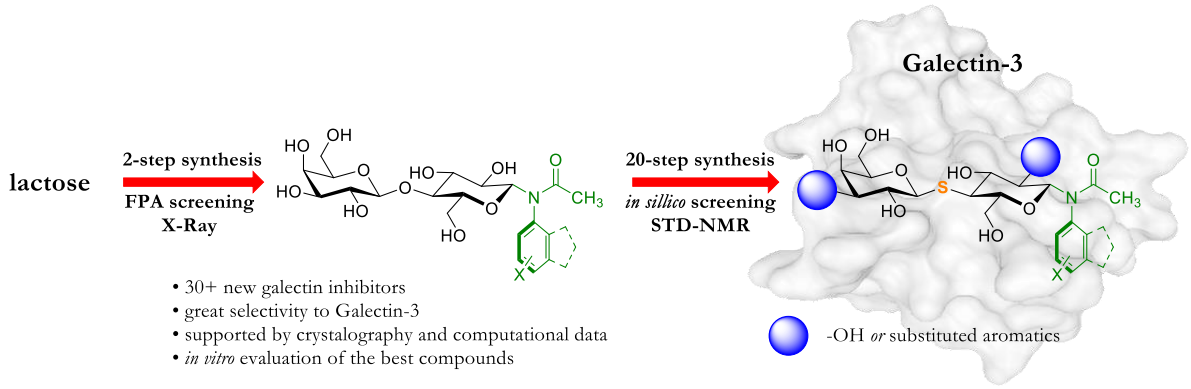
Galectins are proteins from the lectin family that selectively bind D-galactosides, such as glycosylated proteins bearing terminal lactose or LacNAc units. Over the years, interest in their inhibition has been growing, as they are responsible for several key cell functions [1] such as cell adhesion, communication and other functions, which influence pathological processes like cancer [2] and virus cell entry [3]. Recently, there have been several attempts to bring a glycomimetic drug targeting galectins to the market [4].
Building on previously developed synthesis of glycosylamines and amides [5], we employed a new two-step procedure to obtain a series of *N*-lactosylanilides. A new binding motif was identified based on fluorescence polarization/anisotropy (FPA) affinity measurements and protein-ligand crystal structure with galectin-3. Further investigation by thorough *in silico* screening and saturation transfer difference NMR provided valuable insight into the structure-affinity relationships between the newly prepared compounds and binding to galectins-3 and -1. These findings culminated in a multistep synthesis of highly decorated glycomimetics with single digit nanomolar affinity and high selectivity towards galectin-3. Further evaluation by means of cell-based assays showed promising results.



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DEVELOPMENT OF POTENT *AF*KDNASE INHIBITORS AS POTENTIAL ANTIVIRULENCE AGENTS FOR *ASPERGILLUS FUMIGATUS*

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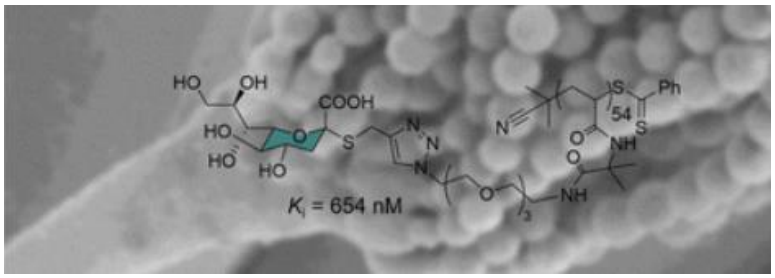
Aspergillus fumigatus is a deadly opportunistic pathogen responsible for severe infections in immunocompromised individuals. Recently, *Af*KDNAse, an exoglycosidase that hydrolyzes the rare sugar 3-deoxy-D-galacto-D-glycero-nonulosonic acid (KDN), was identified as a key player in fungal cell wall morphology and virulence [1]. However, the precise function of *Af*KDNAse remains unclear, necessitating the development of potent inhibitors to better understand its biological role and its potential as a target for antivirulence strategies.
In this work, we report the design and synthesis of a novel set of *Af*KDNAse inhibitors based on thio-KDN motifs, which are enzymatically stable and capable of fitting into the unique KDN-binding pockets of the enzyme. Two classes of inhibitors, C2- and C9-linked heterodi-KDN, were developed, alongside a polymeric compound containing an average of 54 KDN motifs, synthesized using click chemistry. Enzymatic assays demonstrated that these compounds inhibited AfKDNAse with moderate to strong potency, with the poly-KDN showing a remarkable more than 900-fold improvement in inhibitory activity (IC_{50}= 1.52 ± 0.37 µM, 17-fold on a KDN molar basis) over a monovalent KDN reference.
Our findings suggest that multivalency is a key strategy for enhancing KDNase inhibition. Importantly, poly-KDN demonstrated a strong reduction in *A. fumigatus* filamentation when co-cultured at micromolar concentrations, offering promising prospects for the development of novel antivirulence agents targeting fungal pathogenesis [2].



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DESIGN AND SYNTHESIS OF BIVALENT AND TETRAVALENT THIOGLYCOSIDES AS POTENTIAL INHIBITORS OF *PSEUDOMONAS AERUGINOSA* LECTINS LECA (PA-IL) AND LECB (PA-IIL)

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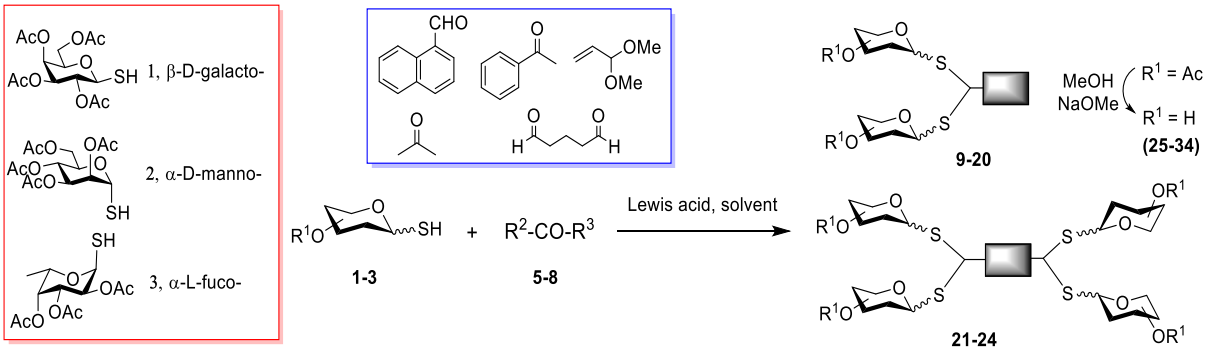
Pseudomonas aeruginosa is a Gram-negative bacterium currently recognized as the most critical bacterial pathogen on the WHO's priority pathogen list. Its ability to form abundant biofilms significantly contributes to its antimicrobial resistance. As a result, various strategies are being explored to identify and develop new antibacterial agents that can effectively inhibit biofilm formation. Two soluble lectins, LecA (PA-IL) and LecB (PA-IIL), play crucial roles in the initial adhesion to host tissues and biofilm formation, and act as key virulence factors [1]. LecA specifically binds to D-galactose (Gal), while LecB shows a strong preference for L-fucose (Fuc) but can also attach to other sugars like D-mannose. In pursuit of effective antibacterial agents, we report the synthesis of novel hydrolytically stable bivalent and tetravalent thioglycosides.

The construction of these analogs involved the formation of the 1-thiomonosaccharides derivatives **1-3**. 1,2-*trans*-glycosyl thiols such as **1** and **2** were prepared from the corresponding bromo sugar through reaction with thiocarbamide, followed by the cleavage with sodium bisulfite, whereas 1,2-*cis*-α-configured L-fucose **3** was produced through the thiol-ene coupling reaction of glycals and thioacetic acid (HSAc), using our recently published optimized conditions, followed by selective *S*-deacetylation [1]. The 1-thiomonosaccharides obtained were then reacted with aliphatic/aromatic oxo-compounds or their dialkyl acetals in the presence of a Lewis acid catalyst to produce the bivalent **9-20** or tetravalent **21-24** thiosugars [2]. This reaction was initially investigated using various solvents, including DMF, MeCN, and DCM, as well as several acidic catalysts such as pTSA, CSA, $BF_3.Et_2O$, TMSOTf, and TfOH. Finally, Zemplén deacetylations were performed to yield the free thioglycosides **25-34** (**Scheme 1**).



Scheme 1. Synthesis of bivalent and tetravalent thioglycosides

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STEREOSELECTIVE SYNTHESIS OF CARBOHYDRATE-FUSED ISOCHROMANS BY OXA-PICTET-SPENGLER REACTION AS POTENTIAL INHIBITORS OF SGLT-2 AND GLYCOGEN PHOSPHORYLASE

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Diabetes mellitus (DM) is a chronic multireasonal metabolic disorder associated with high morbidity and mortality, and its incidence is increasing despite available treatments [1]. The search for antidiabetic compounds with an optimal pharmacological profile, despite the challenges, has led to safer, more effective oral therapies. C-aryl glucoside SGLT2 inhibitors (gliflozins), featuring an aryl or heteroaryl aglycone, represent a promising advancement in diabetes treatment [2]. Additionally, inhibition of glycogen phosphorylase, a key liver enzyme regulating blood glucose levels, has become a validated target for the treatment of type 2 diabetes [3]. Our research aims to synthesize novel gliflozin analog molecules as potential inhibitors of SGLT2 and glycogen phosphorylase by conducting oxa-Pictet-Spengler reaction in which aromatic alcohol derivatives were reacted with sugar aldehydes starting from enantiopure chiral alcohol derivatives, and commercially available monosaccharides (Fig 1). The effect of the reaction conditions, the protecting groups and the configuration of the starting materials were studied on the yield and the stereochemical outcome of the reaction. It was found that the amount and the type of the applied acid catalyst, the configuration of the alcohol, and the sugar (glucose, galactose) aldehyde influence the stereochemistry of the cyclization. Preliminary studies indicate that some of our synthesized compounds exhibit moderate activity as glycogen phosphorylase inhibitors, while their potential as SGLT-2 inhibitors is currently under investigation, highlighting their dual therapeutic promise in diabetes management.

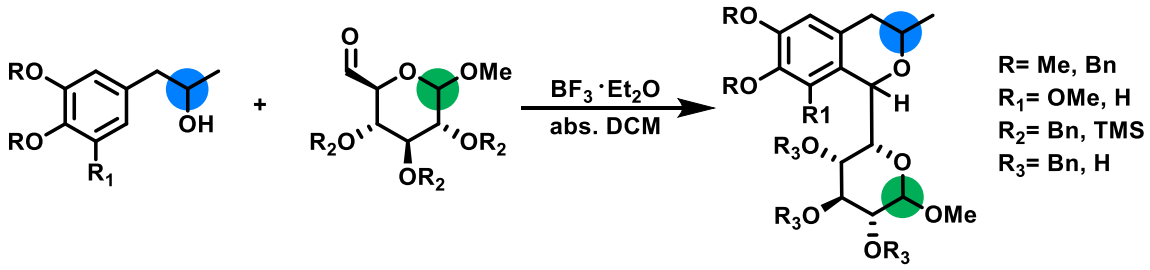

Figure 1. Synthesis of isochroman-sugar hybrids with benyzl and silyl protecting groups

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SOLVENT MODELS AND CHARGE SCALING: BENCHMARKING FOR MOLECULAR DYNAMICS SIMULATIONS OF GLYCOSAMINOGLYCANS

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Glycosaminoglycans (GAGs) are highly negatively charged polysaccharides found extensively on the surface of cells and in the extracellular matrix. They possess a vast range of biological functions involved in cell signaling, cardiovascular health, tissue regeneration, inflammation and neurodegeneration [1]. The intrinsic properties of GAGs makes them difficult to study effectively but, recent efforts to quantify the efficacy of various forcefields and water models within GAG simulations has highlighted promising routes to more accurate modelling of GAGs. Regarding forcefields, a novel approach of implicitly polarisable forcefields for GAGs has been demonstrated to overcome the limits of nonpolarisable forcefields and the computational demand of explicitly polarisable forcefields [2]. For the choice of water models, recent work has demonstrated the limits of the conventional TIP3P water model used and highlighted more appropriate alternatives [3]. By combining and comparing these approaches we have implemented the “charge-scaled” method for various implicit and explicit water models to identify the best compromise in terms of both computational efficiency and efficacy in simulating GAGs. In this work, both unbound heparin (HP) and heparin bound with basic fibroblast growth factor (FGF) were simulated with five implicit and six explicit water models to mirror previous work whilst also implementing charge scaling developed for the GLYCAM-ECC75 forcefield. From these simulations, we aim to identify the best approach for simulating GAGs within the context of both solvent model and forcefield choice.

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DEVELOPING A COMPREHENSIVE DATABASE OF CATHEPSIN-GAG IN SILICO INTERACTIONS

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Glycosaminoglycans (GAGs) represent a class of linear anionic periodic polysaccharides known to regulate enzymatic activity in various biological processes. Among the enzymes influenced by GAGs are cysteine cathepsins, a family of proteases primarily involved in protein degradation [1]. While cathepsins have been extensively studied for their proteolytic functions, their interactions with GAGs remain poorly understood due to the limited availability of experimental structures [2, 3].
To address this research gap, we are developing a web-based database designed to provide a comprehensive analysis of GAG-cathepsin interactions. This work is focused on the entire family of 11 cathepsins and 6 classes of GAGs, resulting in over 300 unique complexes. Advanced *in silico* approaches, including all-atom and coarse-grained molecular dynamics simulations, along with Hamiltonian replica exchange molecular dynamics techniques, are employed to investigate these interactions. With a total of over 5000 simulations, this resource aims to generate novel insights into the role of GAGs in modulating cathepsin function, offering a comparative computational framework to support the rational design of GAG-based therapeutic strategies.

Acknowledgements: This work was funded by the National Science Centre (grant number UMO-2023/49/B/ST4/0004).

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AUTOMATED SYNTHESIS OF MONOSACCHARIDE BUILDING BLOCKS AND APPLICATIONS IN OLIGOSACCHARIDE SYNTHESIS

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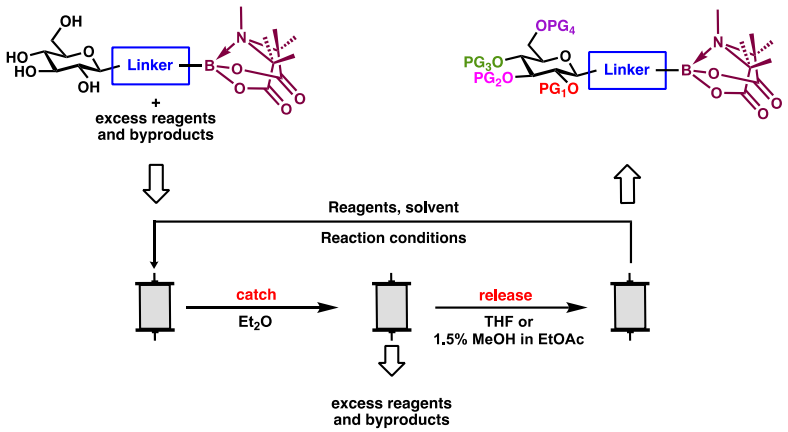
Despite significant progress in the synthesis of oligosaccharides, the synthesis of targets featuring complex glycosidic linkages of monosaccharide building blocks remains a challenge. These compounds are present in a wide range of biologically relevant compounds.
While much of the emphasis in the development of automated platforms for carbohydrate synthesis has been on the construction of oligosaccharides, manual syntheses of monosaccharide building blocks can represent up to 90% of the synthetic effort and thus constrain throughput [1]. This is often laborious and time-consuming. Furthermore, excess amounts of glycosyl donor building blocks are frequently used in glycosylations, presenting a pressing need to develop methods for streamlining the acquisition of monosaccharides.
This work aims to improve the purification of monosaccharides, which is often a bottleneck in the preparation of important carbohydrates. By using a purification tag, TIDA [2], the process of purifying monosaccharides is made simpler and more efficient. One of the key findings of this research is that the silica binary affinity properties of the TIDA tag can be extended to monosaccharides bearing a variety of protecting groups (>22 examples) [3]. This characteristic proved beneficial during the synthesis of the tagged molecules as it simplified purification and eliminated the need for arduous column chromatography. As a result, this process is potentially amenable to automation. This process significantly reduces the amount of silica and solvent used to isolate the products in each step and makes the synthesis more environment-friendly.



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STEREOSELECTIVE 1,2-*CIS*-GLUCOSYLATIONS

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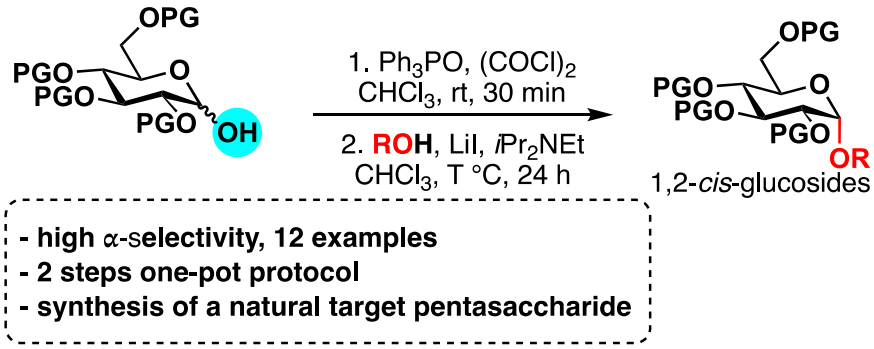
Oligosaccharide synthesis, consisting of multiple glycosylation steps, poses many difficulties with respect to regio- and stereoselectivity [1]. Depending on the reaction conditions, 1,2-*cis*- or 1,2-*trans*-glycosides can be obtained, of which the former are usually more difficult to synthesize. Previously, the McGarrigle group reported access to 1,2-*cis*-glycosides, by treatment of the glycosyl hemiacetal donor with Denton's catalytic Appel conditions [2,3], followed by reaction with LiI, *i*Pr$_2$NEt and the acceptor [4]. This procedure was successfully applied to the stereoselective synthesis of β-mannosides and β-rhamnosides.
In contrast to β-mannosides and β-rhamnosides, we will describe how glucosyl hemiacetal donors give α-glucosides under the same protocol (Scheme 1). A range of glucosyl hemiacetal donors and acceptors have been tested. Optimization studies were required to prevent unwanted elimination of the glycosyl iodide intermediate to form the corresponding glucal side product. Changing the rate of addition of base *i*Pr$_2$NEt was found to limit the formation of the side product, affording an increase in the acceptor conversion, and still with an excellent α selectivity. To demonstrate the usefulness of the method, a target pentasaccharide containing four α-linkages was also synthesized using these conditions [5].



Scheme 1. Reaction conditions for the synthesis of α-glucosides.

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A CONCISE SYNTHESIS OF AN L-ALTRURONIC ACID GLYCOSYL DONOR

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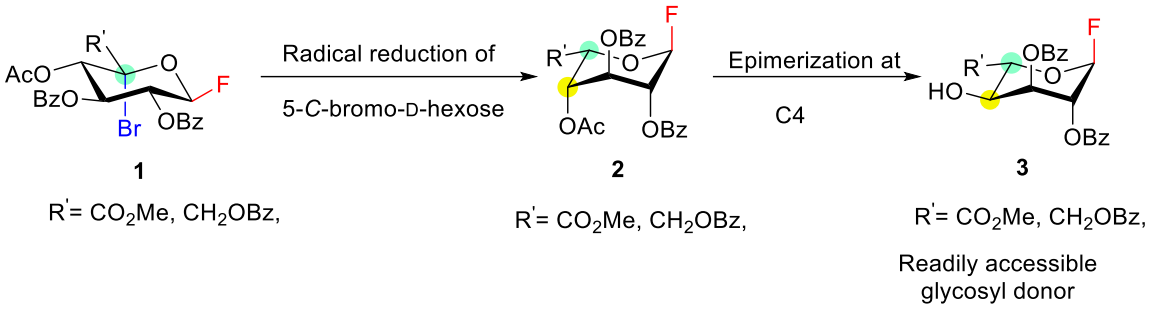
L-Altruronic acid (L-AltA) is a rare hexose that manifests in the capsular polysaccharides of numerous pathogenic bacteria, including Enterococcus faecium. Encouraging evidence has been provided for the efficacy of glycoconjugates as vaccine candidates against E. faecium. Of those examined, the highest-performing structure contained L-AltA as a signature sugar. However, further investigation in this area has been limited by poor synthetic access to appropriate L-AltA building blocks. We now report the straightforward preparation of a glycosyl donor-functionalized derivative of L-AltA from inexpensive starting materials. Central to this synthesis is the fluorine-directed C-5 epimerization, which has previously been instrumental in accessing other rare L-hexoses [1-3].



1

R'= CO_2Me, CH_2OBz,

2

R'= CO_2Me, CH_2OBz,

3

R'= CO_2Me, CH_2OBz,

Readily accessible glycosyl donor

References:
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THE USE OF PSE-GLUCAL AS A VERSATILE INTERMEDIATE TO ACCESS NOVEL DEOXY-, GLUCO-, AND MANNO-PYRANOSIDE DERIVATIVES

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Protecting groups are commonly used in carbohydrate chemistry as intermediate agents to ensure selective and specific reactions, and also for stereoisomeric control, so that they play a key role in the reactivity of carbohydrates. A few years ago, our research group introduced a protecting group in carbohydrate chemistry, the phenylsulfonylethylidene (PSE) acetal, which can easily be prepared through a Michael-type reaction of unprotected sugars under basic conditions [1,2]. Unlike most of cyclic acetal protecting groups, PSE acetal is stable under acidic conditions [2], and in contrast, can easily deprotect under basic conditions [3].They can also selectively be opened under strongly reductive or basic conditions [2], or desulfonylated to produce ethylidene acetals or vinyl ethers. PSE-glucal remains a new and unexplored intermediate, which leads us to observe that the double bond between C-1 and C-2 could be more reactive in addition, glycosylation, and epoxidation reactions under acidic conditions than the classical Ferrier rearrangement. The core of this work is to further extend the reactivity of PSE-glucal toward the development of new *N*,*O*-glycosides [4,5], 2-deoxy-glycosides [6], and 2-halo-mannopyranosides [7,8] (Figure 1).

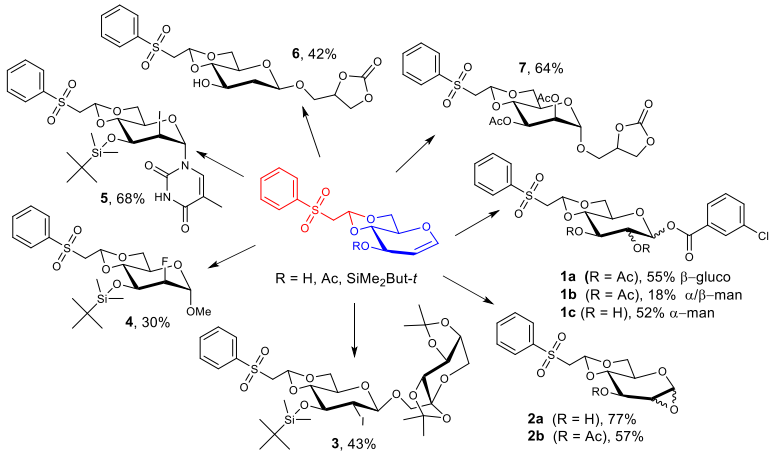

Figure 1. Synthetic strategy of this work.

Acknowledgements: Eiffel fellowship (CJOS), FACEPE, CAPES, CNPq.

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FOUR OF A KIND: CHEMICAL SYNTHESIS AND GLYCOSYLATION OF BACTERIAL NONULOSONIC ACIDS

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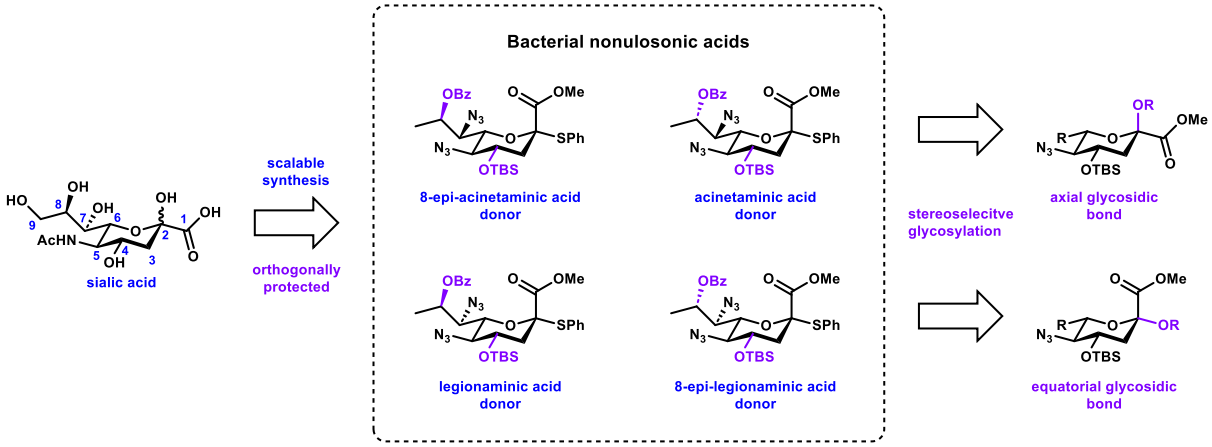
The development of novel antimicrobial therapies is increasingly aided by synthetic glycans, which can be used as probes to unravel biological interactions, as glycoconjugate vaccines, as diagnostics, and carbohydrate-based drugs. As attractive therapeutic targets, bacterial surface glycans, including capsular polysaccharides (CPS) and lipopolysaccharides (LPS), feature an incredible diversity of different monosaccharide types. A particularly intriguing class of monosaccharides, nonulosonic acids (NulOs), are nine-carbon sugars that consist of an α-keto acid pyranose ring connected to a three-carbon exocyclic side chain. In contrast to sialic acid, bacterial analogues are typically deoxygenated at the terminus of the side chain (C-9) and usually contain an additional amino group at C-7. Bacterial NulOs display a wide structural diversity, as five of the six stereocenters can vary in configuration, resulting in numerous possible stereochemical combinations. The structural complexity is further increased by the presence of various *O*/*N*-substituents, including acetyl, acetimidoyl, formyl, and 3-hydroxybutyryl groups. Additionally, these bacterial NulOs are both α- and β-linked to a wide variety of different sugar types within bacterial glycans.
The availability of bacterial NulOs has been hampered by difficulties in the synthesis of these structurally complex sugars [1]. Furthermore, directing NulO glycosylation is challenging due to facile elimination of reactive intermediates into a 2,3-glycal side product, the absence of neighboring group participation, and the deactivating effect of the C-1 carbonyl [2].
In this talk, a divergent gram-scale synthesis route to bacterial NulO donors will be presented, which provides these building blocks in sufficient amounts for mapping their glycosylation reactivity, as well as for synthesizing CPS fragments (K-Units) present on the surface of *Acinetobacter baumannii*.



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SYNTHESIS OF ATCC 17961 AND ATCC 17978 *ACINETOBACTER BAUMANNII* POLYSACCHARIDE FRAGMENTS

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The Gram-negative pathogen *Acinetobacter baumannii*, associated with numerous nosocomial infections, poses a significant threat to human health due to its multi-drug resistant nature. In 2017, the World Health Organization classified the bacterium as a critical priority for the development of new treatments[1]. To date, over twenty distinct surface saccharides of *A. baumannii* have been structurally elucidated, including those from the virulent ATCC 17978 and ATCC 17961 strains.[2] Both strains share the same pentasaccharide repeating unit, with the only structural difference being a single O-acetylation found in ATCC 17978 (**Figure 1**).

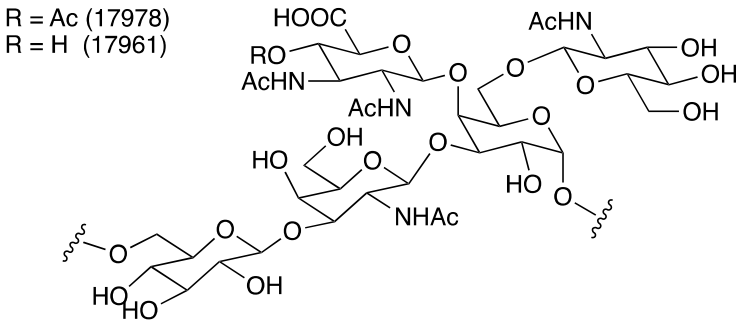

Figure 1. Structure of pentasaccharide repeating unit of ATCC 17978 and ATCC 17961 strains.

As part of the EU-funded MSCA-DN ACINETWORK, our research aims to synthesise the pentasaccharide repeating unit of both strains, along with disaccharide and trisaccharide fragments. The conjugation of the synthesised ligands to various carriers will be targeted for immunology testing, with the goal to investigate the design and development of a semi-synthetic carbohydrate-based vaccine, a therapeutic strategy that has already shown efficacy against other Gram-negative bacteria.[3] In this communication, we will introduce this ongoing project's preliminary findings, highlighting the modulable synthetic approach employed and the synthesis of monosaccharide units of each strain.

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SYNTHESIS OF *CAMPYLOBACTER JEJUNI* HS:23/36 FRAME-SHIFTED TRISACCHARIDES

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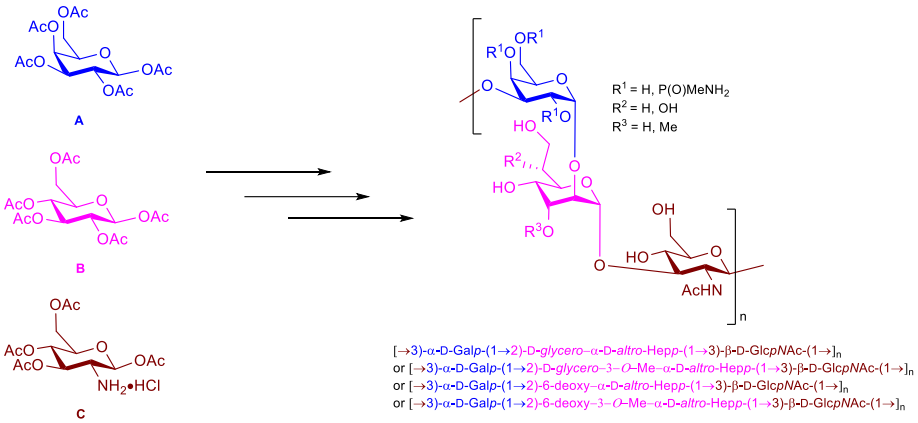
With 400 000 deaths annually, diarrhoeal diseases are the third cause of death in children under the age of 5 worldwide [1]. *Campylobacter* falls within the four most common causes of diarrhoeal diseases [2]. Infections, especially frequent in young children in developping countries, are often attributed to *C. jejuni* [3]. Considering the threat of anti-microbial resistance (AMR), alternatives are to be developped, among which prevention through vaccination. Conjugate vaccines based on capsular polysaccharides (CPS) make an attractive strategy to mitigate the burden of campylobacteriosis [4]. This approach has been successfully applied to the developpement of a semi-synthetic glycoconjugate vaccine candidate against *Shigella flexneri* 2a by our group [5].



Here, focus is on one of the common *C. Jejuni* serotypes, HS:23/36, the CPS repeating unit of which is a trisaccharide composed of a D-galactose (A) bearing a non-stoechimoetric methyl phospharamidate (MeOPN) moiety, a rare D-*altro*-heptose that can be D-*glycero* or 6-deoxy, and/or 3-*O*-Me (B) and an *N*-acetyl D-glucosamine residue (C). Synthetic studies of orthogonally protected monosaccharides prepared on a large scale (up to 50 g) and their assembly into trisaccharides ready for elongation at both ends will be presented.

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UNRAVELLING THE GLYCAN RECOGNITION PROPERTIES OF HUMAN GALECTIN-9

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Galectin-9 is a tandem-repeat galectin that has been reported to have a major role in many biological processes such as cell growth, differentiation, communication, and death [1]. Moreover, it has been proposed as a possible biomarker for many pathologies due to its immunomodulatory capacity [2]. In view of the biological importance, its molecular recognition properties have been analyzed during this work. As a tandem-repeat galectin, Galectin-9 is composed of two carbohydrate recognition domains (CRDs) covalently linked by a peptide linker. Herein, the NMR backbone assignment of both the C-domain and N-domain of Galectin-9 has been achieved, instrumental for obtaining site-specific information by defining the protein residues implicated in oligosaccharide binding. Additionally, ligand-observed techniques such as STD and STD-HSQC experiments have been performed. In particular, the binding to Lactosamine (LacNAc), the B- and the A-antigen tetrasaccharide type 2 (B-type 2 and A-type 2) [3], 3-Sialyl Lactosamine (3-S'LacNAc), and polylactosamine (PLNA) [4] oligosaccharides has been compared. Specifically PLNA structures have been found in both glycoproteins and glycolipids acting as elongated scaffolds to provide recognition by glycan binding proteins (GBPs). Besides, they have also been associated to key biological processes, mainly related to immune regulation [5], making it important the study of the interaction between Galectin-9 and PLNA structures. Finally, affinity values were confirmed by isothermal titration calorimetry (ITC), and molecular dynamics (MD) simulations were performed to obtain 3D atomic resolution complexes, providing the impetus for disentangling the glycan-lectin interaction.

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MOLECULAR BASIS FOR SPECIFICITY OF NOROVIRUS TOWARDS HUMAN GLYCANS

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Noroviruses (NoVs) are a leading cause of viral gastroenteritis worldwide and are responsible for a significant public health and economic burden [1]. These highly infectious pathogens bind to human glycans, such as histo-blood group antigens (HBGAs), to attach to the host cell and initiate infection [2]. NoV strains, with potentially unique glycan specificities, pose a growing challenge, particularly in the absence of an effective vaccine [3]. However, is known in literature that Human milk oligosaccharides (HMOs), which structurally mimic HBGAs, are able to act as natural decoys that inhibit norovirus binding, thereby reducing the risk of infection [4]. This project aims to investigate the molecular determinants of glycan specificity in predominant (GII.4), rare (GII.2) and emerging (GII.17) NoV strains to assist the development of glycomimetics for anti-adhesion therapy (AAT).
To achieve this, a novel and efficient protocol for the expression and purification of the P domain in *Escherichia coli* for these three NoV strains was successfully developed, yielding to 10-15 mg of highly pure protein per litre of culture. The dimerization necessary for carbohydrate binding was confirmed by SEC-MALLS and SEC-SAXS on the purified P-domains. Those were then subjected to crystallisation trials, resulting in the identification of successful crystallisation conditions for the three strains. In particular, new co-crystals were obtained with new HMOs for the GII.4 strain, and a high-resolution structure was obtained for the GII.2 apo protein in an unreported space group. Diffractions tests are underway for GII.17 in complex with HMOs and HGBAs. Comparative structural and biophysical analyses of different NoV genotypes will reveal both conserved and variable binding motifs, providing a deeper understanding of the determinants for NoV specificity.
The knowledge gained from this study will facilitate the rational design of glycan-based inhibitors capable of blocking NoV-host interactions, providing a promising avenue for the development of novel therapeutic strategies against norovirus infections.

Acknowledgements: HMOs were donated by Glycom A/S - part of dsm-firmenich as a collaboration partner within GlycoNovi project

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STRUCTURAL BASIS OF CAPSULE POLYMERASES IN PATHOGEN BACTERIA

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Bacterial capsules are long-chain carbohydrate polymers that form a protective layer around the cell surface. They have critical roles in host-pathogen interactions and provide a protective envelope against host recognition, leading to immune evasion and bacterial survival. Capsule biosynthesis enzymes are potential drug targets and valuable biotechnological tools for generating vaccine antigens [1]. In this work we define the capsule biosynthesis pathway of *Haemophilus influenzae* serotype b (Hib), a Gram-negative bacterium that causes severe infections in infants and children [2]. Reconstitution of this pathway enabled the fermentation-free production of Hib vaccine antigens starting from widely available precursors and detailed characterization of the enzymatic machinery. The X-ray crystal structure of the capsule polymerase Bcs3 reveals a multi-enzyme machine adopting a basket-like shape that creates a protected environment for the synthesis of the complex Hib polymer. This architecture is commonly exploited for surface glycan synthesis by both Gram-negative and Gram-positive pathogens. Supported by biochemical studies and comprehensive 2D nuclear magnetic resonance, our data explain how the ribofuranosyltransferase CriT, the phosphatase CrpP, the ribitol-phosphate transferase CroT and a polymer-binding domain function as a unique multi-enzyme assembly (Figure 1).

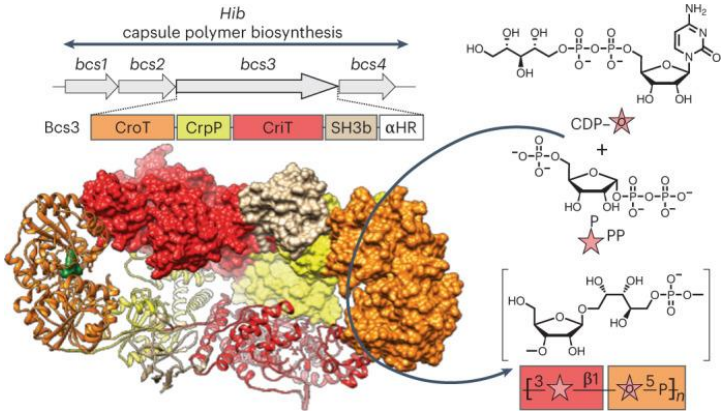

Figure 1. Overall structure of the Bcs3 dimer in complex with CMP, with both protomers shown in surface representation (left) and one protomer shown in cartoon representation (right) to visualize the secondary structural organization and the CMP (green). Each protomer of the homodimer is composed of (1) the ribofuranosyltransferase CriT (red), the phosphatase CrpP (yellow), (3) the ribitol-phosphate transferase CroT (orange) and (4) an SH3b domain (tan).

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IDENTIFICATION OF RECEPTOR BINDING-DOMAIN OF BACTEROIDALES SECRETED ANTIMICROBIAL PROTEIN-3 (BSAP-3)

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Bacteroidales, a dominant order of Gram-negative bacteria, are highly efficient long-term colonizers of the human intestinal microbiota [1], playing a pivotal role in host physiology through their immunomodulatory and anti-inflammatory functions [2,3]. Their ability to persist and thrive in this competitive ecosystem is largely attributed to sophisticated antimicrobial strategies, including the production of Bacteroidales Secreted Antimicrobial Proteins (BSAPs) [4-6]. BSAPs represent a recently identified class of bactericidal pore-forming toxins with an unprecedented level of receptor specificity. Unlike other members of the membrane attack complex/perforin (MACPF) and cholesterol-dependent cytolysin (CDC) superfamily, BSAPs exhibit an extraordinary degree of selectivity, targeting only a single outer membrane β-barrel protein [7] or lipopolysaccharide [4-6], thus restricting their function predominantly to intra-species competition. This unique mechanism distinguishes BSAPs as the first known bacterial MACPF proteins with bactericidal activity. Their highly specific mode of action suggests significant potential as next-generation antimicrobial agents, offering precise bacterial targeting with minimal perturbation to the commensal microbiota and a lower propensity for inducing antibiotic resistance. Despite their therapeutic promise, the molecular determinants governing BSAP receptor specificity remain poorly understood.
In this study, we employed a structural biology approach to characterize the protein BSAP-3, aiming to delineate the specific domains responsible for receptor recognition and interaction. Our findings provide critical insights into the molecular basis of BSAP-3 specificity, advancing our understanding of bacterial mechanisms and paving the way for the potential development of BSAP-inspired antimicrobial therapeutics.

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MOLECULAR EXPLOITATION OF *NEISSERIA GONORRHOEAE* LOS RECOGNITION BY THE THERAPEUTIC MONOCLONAL ANTIBODY 2C7

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Glycan–protein interactions play a pivotal role in immunomodulation and offer promising therapeutic avenues for bacterial infections, tumors, and viral diseases. In this study, we employed a multidisciplinary approach to investigate both exogenous and endogenous ligands, underscoring the crucial role of glycans in immunotherapy [1,2].
On one hand, we characterized the lipooligosaccharide (LOS) of *Neisseria gonorrhoeae strain 15253*, focusing on the conserved 2C7 epitope, which is specifically recognized by the monoclonal antibody 2C7[3]. Biophysical, computational and spectroscopical (NMR) binding studies [4] revealed that the recognition is predominantly mediated by the β-chain, with a specific epitope involving the terminal β-Gal residue of the core region. MD (Molecular dynamics) simulations and ITC (isothermal titration calorimetry) experiments proved stability and property of the 3D complex and the energetics of the interaction. Moreover, the synthetic terminal tetrasaccharide from *N. gonorrhoeae* core LOS further demonstrated recognition by 2C7, paving the way for new strategies to combat infections in an era of increasing antibiotic resistance [5].
Overall, our results remark the importance of studying glycans as a transversal immunotherapeutic target, providing a solid foundation for the development of interventions that exploit glycan–protein interactions—both exogenous and endogenous—for the treatment of bacterial infections and cancer diseases.

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ELUCIDATION OF NOVEL GLYCAN FUNCTIONS THAT PROMOTE THE α-HELIX FORMATION OF PEPTIDE

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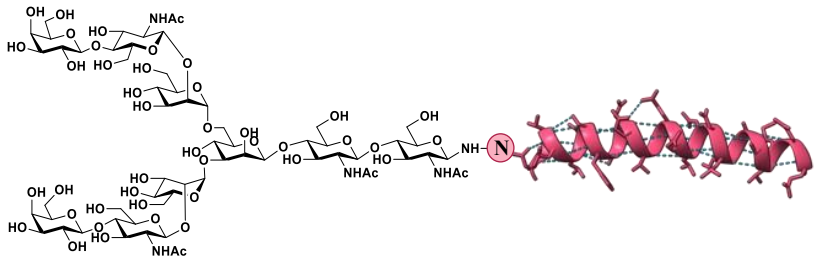
Among protein modifications, glycosylation is one of the most abundant modifications in nature. Several glycan functions have been widely reported, particularly in relation to the stabilities, activities, and properties of proteins. Previously, Kajihara's group found that glycan can enhance the α-helix formation of glucagon and exenatide [1,2]. However, the underlying mechanisms of this phenomena remain unclear. Here, we conducted a detail study on the role of glycan in promoting the secondary structure of peptides, specifically focusing on the α-helix formation.

We have been investigating the glycan functions on glycopeptide by using homogeneous glycopeptides obtained through the chemical synthesis. For this purpose, several peptide fragments of proteins and their glycosylated forms consisting of less than 30 amino acid residues were chemically synthesized by using Fmoc Solid-Phase Synthesis (Fmoc-SPPS) protocol. Using these synthesized peptides and glycosylated peptides, the peptide secondary structures were evaluated by Circular Dichroism (CD) spectroscopy and Nuclear Magnetic Resonance (NMR), including 1-Dimensional and 2-Dimensional NMR. Particularly, the detailed comparisons were examined between peptides with glycan and without glycan. As a results, we found that glycans influence the peptide secondary structure formation. In this presentation, we present a comprehensive discussion of this glycan function.



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CONTROLLING SIALYLATION LEVELS WITH A NOVEL α-2,6-SIALYLATRANSFERASE

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α2,6 sialylation enhances the immunomodulatory role of IgG-type monoclonal antibodies (mAbs), ultimately increasing therapeutic efficacy and reducing intrinsic side effects compared to their asialylated counterparts [1]. Current mAb manufacturing platform is unable to incorporate sialic acid at the α2,6 positions, but this can be achieved through *in-vitro* glycoengineering (IVGE). Prior to the α2,6-sialylation, enzymatic IVGE requires a galactosylation step using human ß1,4-galactosyltransferase and UDP-galactose. For α2,6-sialylation, human α2,6-sialyltransferase (α2,6SiaT) is preferred due to its high efficiency in generating di-sialylated (S2) glycoforms and low CMP-Neu5Ac hydrolytic and sialidase activities [2]. However, its limited availability and its high costs hinder its commercial applicability. In contrast, bacterial α2,6-sialyltransferases are a more economical and readily available alternative, but exhibit high CMP-Neu5Ac hydrolytic and sialidase activities that are difficult to counteract with alkaline phosphatase or other additives to achieve high levels of sialylation [2-4]. Another significant hurdle is the cost of CMP-Neu5Ac.
This study introduces a novel bacterial non-hydrolytic α2,6-sialyltransferase that lacks hydrolytic activity towards CMP-Neu5Ac, thus, eliminating the need for alkaline phosphatase. Coupled with low-cost CMP-Neu5Ac, and following a preliminary galactosylation step, Rituximab from two sources underwent α2,6-sialylation in the absence of alkaline phosphatase. Approximately, 35% S2 and 40% mono-sialylated (S1) glycoforms were achieved for Rituximab produced in CHO cells, while around 90% S2 and 5% S1 were achieved for Rituximab produced in High Five™ insect cells. Overall, the presented approach constitutes a feasible and practical alternative for the *in-vitro* enzymatic α2,6 sialylation of monoclonal antibodies.

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SYNTHESIS AND INCORPORATION OF MODIFIED SIALIC ACIDS FOR CELL LABELLING

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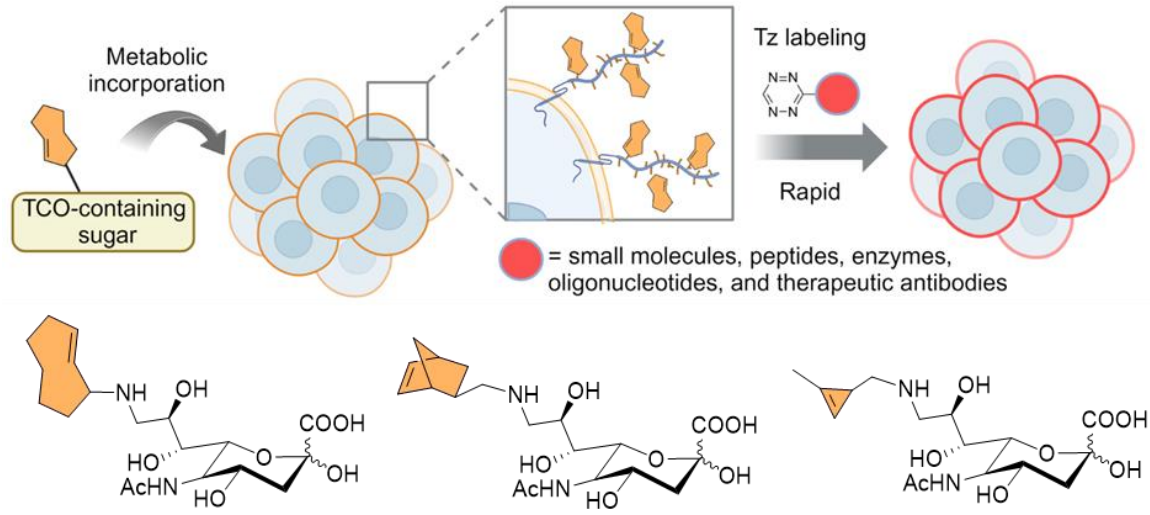
Sialic acid is uniquely suited for cell labeling, as it is typically the terminal unit of cell surface polysaccharides. When modified with a reactive dienophile moiety, it undergoes a rapid bioorthogonal inverse-electron-demand Diels–Alder (IEDDA) reaction with tetrazines. We utilize chemical synthesis in tandem with metabolic glycoengineering (MGE) to incorporate modified sialic acids into cell structures. The engineered cells are then labeled with tetrazine-containing molecules. This method is effective for conjugating both small and large molecules. Compared to established protocols, it demonstrates superior efficiency in ligating large active molecules, such as antibodies, at lower concentrations.



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SENSING SIALIC ACIDS WITH MULTI-FUNCTIONAL ELECTROCHEMICAL PROBES

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Sialic acids (Sias) are carbohydrates found on the surface of cells or in bodily fluids. They play important roles in the human nervous/immune system, and altered levels can reflect multiple disorders. Therefore, the design of new analytical methodologies for this family of monosaccharides may be useful for disease treatment and diagnosis [1]. Boronic acids are well-known carbohydrate receptors which form pH-dependent covalent interactions with diol-containing target molecules, leading to boronate ester species; at acidic pH values, they bind α-hydroxy acids, which are present in Sias. Electrochemical sensing of Sias using commercially available electroactive boronic acids is limited by their structural simplicity and can result in lactic acid interference, but it can be easily implemented compared to other methods and may avoid interference from neutral saccharides [2].
In this work, a series of novel electroactive ferrocene phenylboronic acids were synthesised and characterised; their ability to bind with the model sialic acid *N*-acetyl-D-neuraminic acid (Neu5Ac) was examined. Neu5Ac is the most common Sia naturally found in healthy humans, and it may be found free in solutions such as the cerebrospinal fluid of people with pyogenic meningitis [1]. The presence of an α-hydroxy acid group, carbohydrate structure and the anionic nature of Neu5Ac (p*K*a = 2.6) [1] was exploited for selectivity purposes, providing rationale for probe design and binding conditions employed. For example, a phenyl ring may allow for CH-π interactions [3]. The redox properties of the synthetic probes were established (cyclic voltammetry and differential pulse voltammetry), which led to the most promising compound for Neu5Ac recognition (see crystal structure in Figure 1). Qualitative and quantitative Neu5Ac interactions were examined by monitoring the altered redox properties of this chemoreceptor (Fc-LC) both in solution and via confinement within a carbon paste matrix. Neu5Ac binding *vs.* competing interferents (lactic acid and co-existing biological mono/disaccharides) establishes the potential of this methodology as a portable, rapid and s elective biodiagnostic tool. Fc-LC apparently recognises Sialic acid with reduced interference from lactic acid.

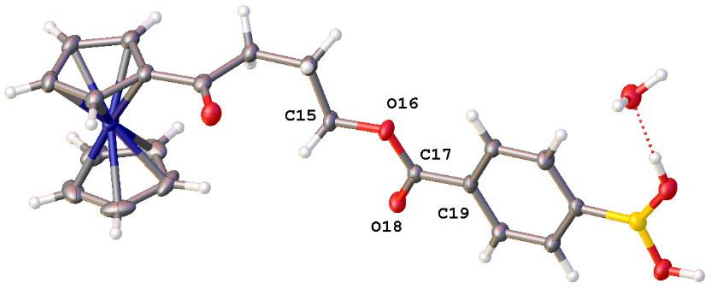

Figure 1.

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O-GLYCOSYLATION PATTERNS IN POST-VIRAL FATIGUE SYNDROME: SIALIC ACID-PRESERVING CHEMICAL RELEASE

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Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) is a complex inflammatory condition characterized by chronic fatigue, post-exertional malaise, and immune dysregulation whose underlying mechanisms remain poorly understood. Glycosylation, the process of attaching glycans to proteins and lipids, plays a crucial role in immune cell communication and inflammation [1]. As sialic acid has great importance in autoimmune and inflammatory diseases, the focus was directed towards a controlled release and labelling reaction with all conditions avoiding acidic hydrolysis of sialic acid [Figure 1.].
O-glycan from blood sera and purified antibodies where methyl amidated to stabilize sialic acid [2]. The release reaction proceeds via non-reductive β-elimination and subsequent labelling with a fluorescent compound in conditions able to conserve sialic acid in antennary position. The O-glycan profiles are analyzed by HPLC with fluorescence and MALDI mass spectrometry.
O-glycosylation profiles with intact sialylation of ME/CFS patients and healthy controls reveal an altered O-Glycan pattern which may contribute to the chronic inflammatory state observed in ME/CFS.

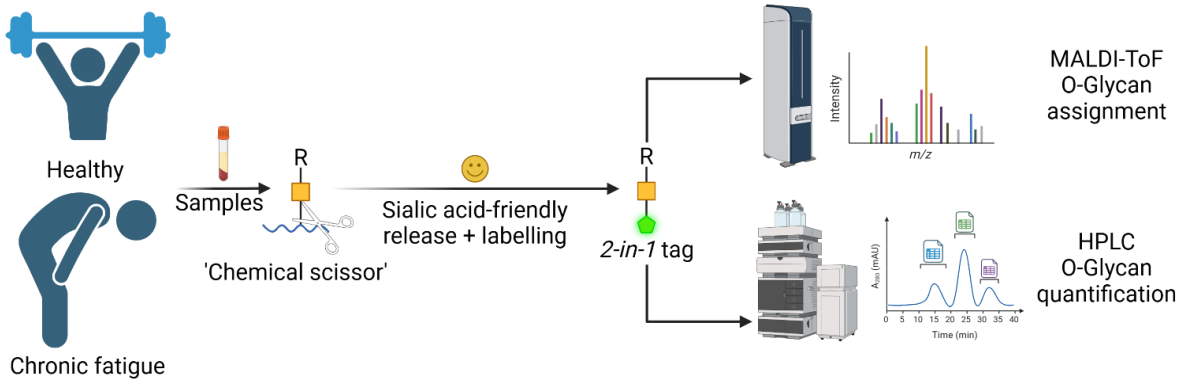

Figure 1. Graphical abstract. O-Glycans from blood serum and antibodies were analyzed from patients and healthy controls using a novel release and labelling method. Image created with BioRender.

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DETERMINATION OF GALACTOSYLLACTOSES IN GALACTOOLIGOSACCHARIDE MIXTURES

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The galactosyllactoses 3'-galactosyllactose (3'GL), 4'-galactosyllactose (4'GL) and 6'-galactosyllactose (6'GL) have all been identified as components of human milk and bovine milk [1]. They are also typically components of galactooligosaccharide (GOS) ingredients [2]. As interest in human milk oligosaccharides continues to increase there is also interest in knowing how much of these galactosyllactoses are present in GOS ingredients and the products to which they are added.
We have applied different methods for the analysis of 3'GL, 4'GL and 6'GL in GOS ingredients using liquid chromatography coupled to fluorescence detection and to mass spectrometric detection. The biggest challenge for this analysis is large number of isobaric components that are present in GOS ingredients. In order to assess if the chromatographic separation was sufficient for accurate quantification it was necessary to use ion mobility separation as well as mass spectrometry.

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SPECIFIC NANOPARTICLE FORMATION VIA AGGREGATION IN CASE OF GLYCOSAMINOGLYCANS AND PROTEOGLYCANS

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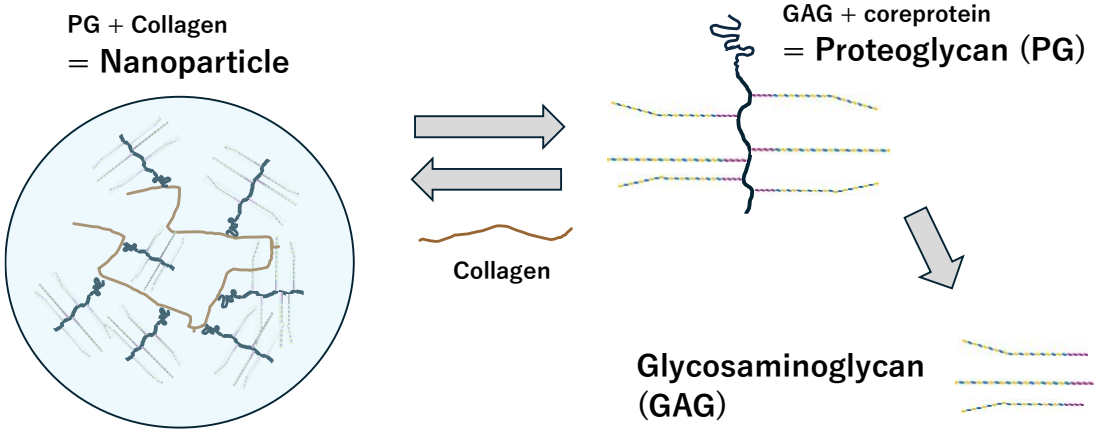
Proteoglycans (PGs) are glycoconjugate composed of glycosaminoglycans (GAGs) and core protein which are covalently bound to each other. PGs interact with collagen and hyaluronan in cartilage and skin to form extracellular matrix. Generally, the molecular weight of GAGs are several tens of kDa. Recently, a GAG with a molecular weight of approximately 696 kDa was isolated from a cartilage of *Dosidicus gigas* [1]. We also isolated PGs and GAGs from specific tissues of animals besides squid with molecular weight than expected.
We chose inedible parts of squid, salmon and cattle (such as skin, fins, alimentally canal) which were minced, defatted, dried, and enzymatically digested by protease to get GAGs [2]. On the other hands, PGs were extracted from these tissues with 4 M guanidine HCl. We observed decreased molecular weights after ion exchange treatment. We also checked nanoparticle formation of the PGs, and measured the particle size by applying Dynamic Light Scattering method. These results suggest that some PGs and GAGs exist as aggregated nanoparticle.



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Posters

CELLULOSE NANOCRYSTALS: A GREEN SOLUTION FOR ENHANCING PLANT GROWTH

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Nowadays, global agriculture faces three major challenges: (1) climate change, (2) the growth of the world population, and (3) the excessive use of synthetic agrochemicals [1]. These chemical inputs pose a danger to both human health and environmental sustainability. It contributes to the degradation of agro-systems, soil toxicity, and the emergence of pathogens resistance [2]. As a result, the search for renewable and organic alternatives has become important to safeguard the environmental quality and to ensure long-term food security. In this context, the use of eco-friendly polymers in the formulation of smart fertilizers appears as a promising approach. Due to their biodegradability, nontoxicity, and eco-compatibility, these polymers present an innovative and sustainable strategy for promoting alternative methods to achieve the concept of durable agriculture. Cellulose, a naturally abundant biopolymer, makes up 40 to 60% of plant biomass and has been extensively utilized in modern agriculture, particularly in fertilizer production.

CNC (plant-derived cellulose nanocrystals) have been applied as fertilizers through foliar spraying. Studies have confirmed the potential of CNC to penetrate and deliver bioactive compounds, such as growth regulators, to leaves and plant cells, without causing any negative effects, including phytotoxicity or genotoxicity [3]. Furthermore, the role of CNC as a plant elicitor has been well confirmed, with studies approving that these biopolymers can trigger an immune response, which confers disease resistance to plants against pathogens [4]. Relying on this potential, our study aims to explore the biostimulant and agronomic properties of cellulose nanocrystals (CNC) and TEMPO-oxidized nanocellulose (TO-CNC) on plants. The oxidation process was performed using the TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) method, which selectively converts the primary hydroxyl groups in cellulose into the negatively charged carboxylic groups, transforming CNC into a polyglucuronic acid derivative. This modification enhances the surface charge of nanocelluloses, improving its physicochemical properties and introducing new functionalities for biostimulation, making it a promising candidate for agriculture applications. To assess their potential, CNC and the synthesized TO-CNC were characterized using various analytical techniques, including FTIR, RMN and conductimetry, and tested at different concentrations on plants, to determine the most effective biostimulant molecule.

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PLATINUM-GROUP METAL COMPLEXES OF ISOXAZOL(IN)E GLYCOCONJUGATES AS PROMISING ANTICANCER AND ANTIBACTERIAL AGENTS

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Platinum-based compounds such as cisplatin spearheads the anticancer chemotherapeutics, but their use is limited by resistance and toxicity. Based on this, extensive research is conducted to find drug candidates as effective or surpassing the benefits of cisplatin [1,2].
In our previuos studies half-sandwich type complexes of platinum-group metal ions (Ru(II), Os(II), Rh(III) and Ir(III)) with *O*-protected *C*- and *N*-glycosyl heterocyclic N,N-bidentate ligands (pyridin-2-yl or quinolin-2-yl substituted 1,2,4- and 1,3,4-oxadiazoles, 1,2,3-triazole) were developed and showed promising antitumor and antimicrobial effects [2][3]. Since the azole moiety of the above ligands proved to be decisive for the biological activity, in this work our aim is to investigate the effect of a new heterocycles, namely isoxazol(in)es coupled to various sugar forms on the biological efficiency.
We synthesized a library of half-sandwich complexes with N,N-bidentate monosaccharide ligands.
Per-*O*-acetylated or benzoylated spirocyclic glycoconjugates of isoxazolines were prepared in cycloaddition reactions, and then these compounds were transformed into per-*O*-benzoylated polyhydroxyalkyl isoxazoles.
Half-sandwich type metal complexes with bidentate *O*-unprotected monosaccharide ligands were also prepared to investigate the effect of protecting groups on the biological activity.
The distribution coefficient values (logD) of the synthesized complexes were determined and the compounds were evaluated as anticancer and antibacterial agents. Details of the syntheses and biological activity of the new compounds will be shown on the poster.

Acknowledgements: This work received financial support from the National Research, Development and Innovation Office of Hungary (Grants FK132222 and K146147), as well as the Stipendium Hungaricum scholarship.

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PLATINUM-GROUP METAL HALF SANDWICH COMPLEXES WITH *C*-GLYCOPYRANOSYL 1,2,3-TRIAZOLES AND ISOXAZOLES AS LIGANDS: SYNTHESIS AND EVALUATION AS ANTINEOPLASTIC AND ANTIMICROBIAL AGENTS

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Due to the toxicity and the development of platinum resistance over time of platinum-based chemotherapeutics such as cisplatin, oxaliplatin, and carboplatin, extensive research is being conducted to develop new compounds that could provide greater therapeutic benefits [1,2].
In our previous publications, we provided a detailed description of the synthesis of half-sandwich type complexes involving platinum-group metal ions (Ru(II), Os(II), Rh(III), and Ir(III)) with *O*-protected *C*- and *N*-glycosyl heterocyclic N,N-bidentate ligands (pyridin-2-yl or quinolin-2-yl substituted 1,2,4- and 1,3,4-oxadiazoles, and 1,2,3-triazoles). These complexes demonstrated promising anticancer and antimicrobial effects, which were found to be strongly correlated with the tested azole moieties [2,3]. In the current work, our goal was to modify the conjugation mode of the previously prepared *N*-heterocycle-derived sugars into *C*-glycosyl analogues, using 1,2,3-triazole as the azole moiety. Additionally, we aimed to investigate the impact of a new heterocycle, namely isoxazole, on the biological activity. To achieve this, we synthesized a library of half-sandwich complexes with new heterocyclic N,N-bidentate ligands, represented by *O*-perbenzylated and *O*-perbenzoylated pyridin-2-yl substituted *C*-glucopyranosyl 1,2,3-triazoles and isoxazoles to form a five-membered chelate ring with the platinum-group metals.
The target ligands were prepared in 1,3-dipolar cycloaddition reactions starting with *C*-glucosyl acetylene, followed by protecting group exchange from benzyl to benzoyl to explore the effect of different protecting group on the biological activity. The prepared ligands underwent a complexation reaction to afford the target half sandwich complexes of Ru(II), Os(II), Rh(III) and Ir(III).
The logD values were determined and the anticancer and antimicrobial effects of the compounds were also tested. Details of the syntheses and biological activity of the new compounds will be presented on the poster.

Acknowledgements: This work received financial support from the National Research, Development and Innovation Office of Hungary (Grants FK132222 and K146147), as well as the Stipendium Hungaricum scholarship.

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STRUCTURAL AND REACTIVITY STUDIES OF [2.2.2]-IDURONIC ACID LACTONES

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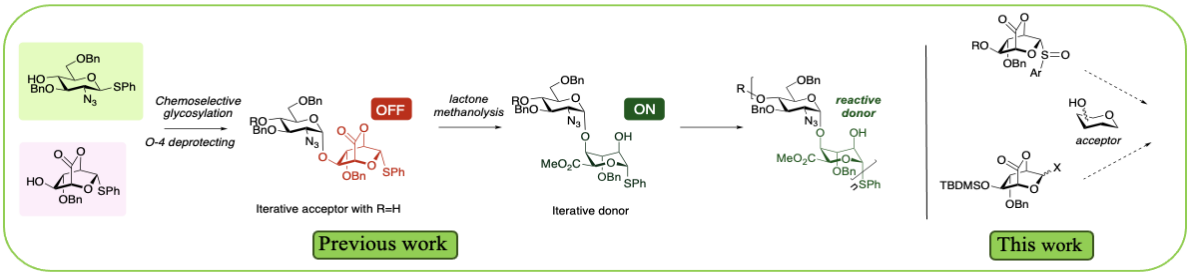
Glycosaminoglycans (GAGs) are sulfated, unbranched polysaccharides found on cell surfaces, within cells, and surrounding the extracellular matrix (ECM). They contain partially *N*-sulfated and/or *O*-sulfated disaccharide units, which facilitate involvement in a variety of biological processes, including cellular recognition and signaling processes. GAGs are critically implicated in diseases such as cancer, viral infections, and Alzheimer's disease. Due to their highly heterogeneous structure and varying sulfation patterns, studying GAG-protein interactions has been challenging. Chemical synthesis of structurally-defined oligosaccharides offers the ability to control the sequence length, monosaccharide order, and sulfation patterns, enabling detailed investigation of GAG-protein interactions.
Heparin (HP), heparan sulfate (HS), and dermatan sulfate (DS) contain the (biologically rare) L-iduronic acid (IdoA) unit. The flexibility of IdoA, particularly the low energy barrier between its confirmed chair conformations (e.g., 4C1 and 1C4), may be critical in many interactions. However, IdoA and its derivatives are not commercially available and are difficult to isolate from natural sources, thus synthesis of L-ido pyranose building blocks is required.



Previously, our group has reported the synthesis of IdoA-lactone (IdoA-lac) as a super-disarmed glycosyl donor. The use of this for lactone-based glycosylation requires no anomeric manipulation steps between glycosylations, and opening reactivates the donor capacity of the IdoA terminus. In contrast to our IdoA-lactone, Hashimoto et al reported a structurally similar [2.2.2]-manno lactone to function effectively as a glycosyl donor. Here we report our ongoing work to evaluate reactivity of IdoA-[2.2.2] lactones. Synthesis and X-ray crystallographic analysis of new glycosyl sulfoxide derivatives of IdoA-[2.2.2.] lactones, including both S-disastereoisomers of a single lactone glycoside the comparative unreactivity of IdoA-[2.2.2.] lactones and new perspectives on their potential applications in glycosylation chemistry.

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RECONSTRUCTION OF CARBON ALLOCATION TOWARDS MULTIPLE PLANT CELL SINKS IN CYANOBACTERIA

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Lignocellulosic cell walls of plants composed of cellulose, hemicellulose, pectin, and lignin serve as the major carbon sink for photosynthetically fixed carbon on Earth. While recent studies have extensively explored polymer synthesis pathways, the regulatory mechanisms governing carbon allocation toward wall polysaccharides at the cellular level remain poorly understood. Investigating these mechanisms in multicellular plants is challenging due to their multicellularity and genetic redundancy. To address these limitations, we aim to reconstruct cellulose biosynthesis in cyanobacteria, CO_2-fixing unicellular photosynthetically active cells. In this study, putative cellulose synthase genes (*XcsA, XcsB, XcsC, BcsA1,* and *XcsB2*) potentially representing two cellulose synthesis systems from *Synechococcus elongatus* PCC 7942, a cellulose-producing cyanobacterium, have been cloned and introduced into *Synechocystis* sp. PCC 6803, a cyanobacterium lacking endogenous cellulose production. Cellulose synthesis has been assessed through histological staining, monosaccharide composition and glycosidic linkage analysis. Additionally, metabolic analysis will be conducted by measuring precursor concentrations for polymer synthesis, and epitope labeling will be further employed to identify key factors influencing carbon allocation toward cell wall polysaccharides. This study is expected to offer a quantitative framework for understanding carbon flow from photosynthesis to extracellular polymers, an approach not feasible in multicellular plants.

SPECTRAL FLOW CYTOMETRY FOR PROFILING B-CELL RESPONSES TO A *SALMONELLA* GLYCOCONJUGATE VACCINE

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Polysaccharides (PS), widely used in licensed vaccines due to their surface exposure and strain specificity, are key antigens for inducing protective immunity against encapsulated bacteria [1]. While PS can elicit specific B-cell responses and antibody production, they do not generate durable memory B cells, as they activate the immune system in a T-cell–independent manner. This limitation has been addressed by covalently linking PS to carrier proteins to generate glycoconjugate vaccines [2]. Although anti-PS antibodies have been extensively studied, the cellular mechanisms underlying their induction - particularly the identity and dynamics of PS-specific B-cell subsets - remain largely unexplored. Conventional ELISpot assays quantify PS-specific B cells but do not enable phenotypic profiling or isolation of specific subsets [3]. In contrast, spectral flow cytometry using fluorescently-labelled PS probes, allows direct identification, characterization, and sorting of PS-specific B-cell populations [4].
To investigate PS-specific B-cell responses elicited by *Salmonella* Typhimurium glycoconjugate vaccines in mice, we first purified and characterized the *S.* Typhimurium O-polysaccharide (O-PS) [5]. The O-PS was then biotinylated and tetramerized with streptavidin to generate fluorescent probes for flow cytometry. Prior to use in immune profiling, O-PS tetramers were validated though bead-based assays coated with anti-O-PS antibodies, confirming their specificity and structural identity. These validated probes were incorporated into a spectral flow cytometry panel alongside antibodies markers for the identification of B-cells response following vaccination. The panel enabled the characterisation of different B-cells populations including plasma cells, germinal centre B cells and memory B cells.
This strategy offers a robust, high-resolution platform for dissecting PS-specific B-cell responses and supports the rational design of next-generation glycoconjugate vaccines with enhanced immunogenicity.

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AZIDO SUBSTITUTIONS AT O-2 OF THIOGLUCOSIDES AND GLUCO-GLUCOAZIDE DISACCHARIDES

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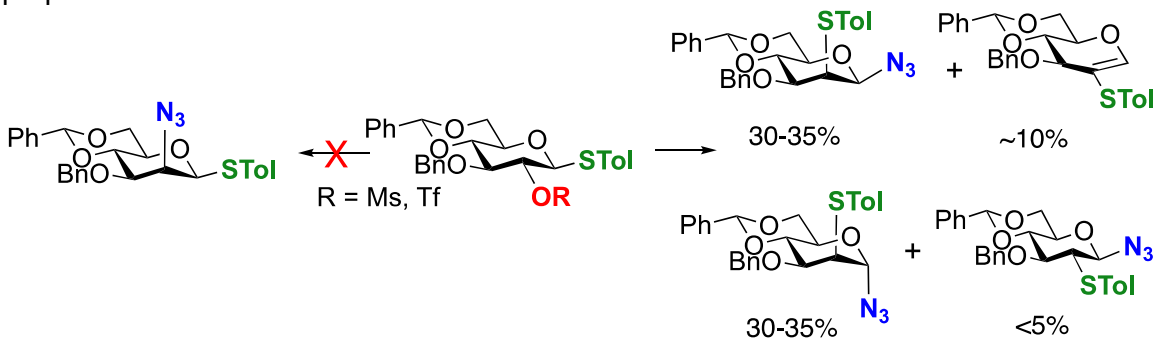
The introduction of azide groups to carbohydrates is widely deployed to provide a surrogate for amine, or, for use in Click reactions. 2-Azido functionality is typically introduced through SN2 substitutions of leaving groups (with inversion) or using azido transfer reagents (azidation with retention). Here we report evaluating using this approach towards preparation of mannosamine-glucosamine dissacharides by azide introduction at the disaccharide or monosaccharide donor precursor stages.

With thioglycoside substrates for 2-azidation, there are prior examples of 1,2-thio migration during this substation process, but with retention of the thio-face of attachment. This is rationalised via a thiiranium mechanism, with additional outcomes from a ring-opened oxonium pathways to account for some reports of anomeric mixtures. Other rearrangement products do not appear to have been reported.

Here we report that using 2-OMs or 2-OTf as leaving group in thioglycosides, no direct azide C2 substitution occurs, and similar amounts of the α– and β–1-azido products with β–thio at C2 are obtained. However, these two major products - consistent with mechanism through thiiranium and opened oxonium-type intermediates - are accompanied with by-products not reported previously; a 2-thio-ene elimination product and the β–1-azide with β–2-thio product. These provides new insight into mechanisms of such carbohydrate migrations, with the latter product suggesting a (reversible) pathway via the elimination. X-ray structures of the three main isomers will be presented. Separable multigram amounts of these isomers have been prepared.



Comparative work on azide substation on gluco-glucoazide disaccharides is also described, which directly provides expected substations and access to mannosamine-glucosamine dissacharides.

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^{19}F-TAGS IN GALECTINS TO MONITOR GLYCAN-MEDIATED MOLECULAR RECOGNITION PROCESSES

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Galectins are glycan binding proteins involved in diverse physiological and pathological processes, including homeostasis, inflammation, and tumour development [1]. 16 Galectin members have been described so far in mammals, sharing the ability to bind to ubiquitous β-galactose containing epitopes on the surface of human cells, such as polylactosamine and 3´syalyl lactosamine [2,3]. Binding studies carried out under reductionist conditions show that different Galectins bind to these epitopes with different affinities. Herein, we aim to explore how these selectivities manifest in a complex scenario. To achieve this, we have introduced an NMR-observable tag into the protein to provide a unique and simple NMR signal per Galectin CRD, enabling the monitoring of competing Galectin binding.
Due to the high NMR sensitivity of the ^{19}F [4,5] and the key role of Trp in glycan binding in Galectins, ^{19}F-Trp was chosen for incorporation into the protein structure as NMR-observable tag without altering the binding affinity [3]. In this work, we optimized the expression of different ^{19}F-Trp-containing Galectins and the acquisition ^{19}F-NMR experiments to enhance their performance in monitoring molecular recognition in complex environments.

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A BIOORTOGONAL PRECISION TOOL FOR HUMAN N-ACETYLGLUCOSAMINYLTRANSFERASE V

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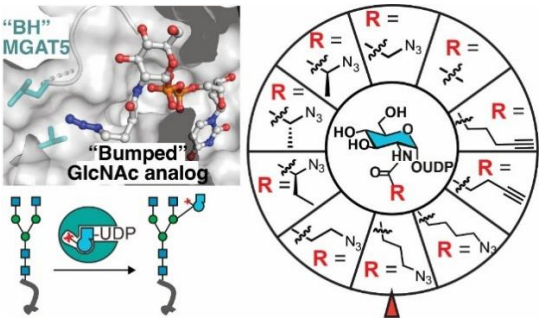
N-linked glycans play an important role in a plethora of biological processes. Despite their structural heterogeneity, the presence of the correct N-glycoforms at key sites is essential for glycoprotein integrity. The biosynthesis of N-glycans follows an assembly-line principle and involves a series of elongation and trimming events, carried by the combinatorial activity of glycosyltransferases (GT's) and glycosidases [1]. GlcNAc transferase V (MGAT5) has an important role in N-glycan elaboration as it primes the biosynthesis of an N-glycan antenna that is known to be heavily upregulated in cancer [2]. Yet, despite its functional relevance, the rules governing the substrate choices of MGAT5 are not well defined.
To address this, we develop a tool to report on the activity of MGAT5 [3,4]. We demonstrated that chemo-enzymatic synthesis is an efficient method to generate UDP-GlcNAc analogues with extended and branched acylamides. The engineered MGAT5 mutant was designed to lose its activity with the native sugar, but displays a substantial increase of activity toward the 4-azidobutyramide-containing substrate analogue UDP-GlcNButAz. We demonstrated that this orthogonal enzyme-substrate pair is suitable for biorthogonal tagging of glycoproteins. Structural insights from X-ray crystallography and molecular dynamics simulations revealed the structural basis of this biorthogonal pair, providing guidance for the development of similar precision chemical tools for other GT's.



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ELUCIDATION OF THE CORE CAZOME AND IDENTIFICATION OF CAZYME DRUG TARGETS FROM THE KINETOPLASTID PARASITE FAMILY *TRYPANOSOMATIDAE*

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The family *Trypanosomatidae* comprises eukaryotic, single-celled, flagellate kinetoplastid parasites. All members of this family parasitize insects, with most associated genera exhibiting a monoxenous (single-host) lifecycle. However, some species within the genera *Trypanosoma* and *Leishmania* have adapted to a dixenous (two-host) lifecycle, parasitizing various mammalian hosts [1].

Several species infect humans and cause severe diseases, including African trypanosomiasis (*Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*), American trypanosomiasis (*Trypanosoma cruzi*), and leishmaniasis (*Leishmania* spp). Without appropriate treatment, these infections can become chronic, leading to progressive tissue atrophy and/or eventual death [1].

While effective treatments exist for the acute stages of human trypanosomiasis and leishmaniasis, they are often less effective against chronic infections. Moreover, these treatments are associated with severe adverse effects due to their mechanism of action, which target conserved eukaryotic metabolic and functional pathways. Therefore, developing new drugs with fewer side effects, either as a replacement or as a complementary therapy, is crucial. Such treatments should target essential cellular processes or virulence factors unique to these early divergent eukaryotes [2].

Carbohydrate-active enzymes (CAZymes) represent promising drug targets due to the parasites' heavy reliance on glycan production for virulence, immune evasion, and enzymatic function [3]. While various CAZymes have been individually studied in these organisms [4], their collective interplay in regulating the distinct glycan compositions observed across different lifecycle stages remains poorly understood.

This project aims to use a variety of bioinformatics tools to mine and classify CAZymes from currently available trypanosomatid genomes, allowing identification of their core CAZyme repertoire (the CAZome) across the *Trypanosomatidae* family, hence elucidating CAZymes unique to dixenous species which may serve as novel drug targets against these parasites. Current analysis has identified variations among strains of an expanded group of sialidases in *Trypanosoma cruzi*. These sialidases serve as key virulence factors, modifying extracellular glycans to help the parasite evade their host's immune system [5]. Additionally, the GT67 family, a unique CAZy family known for catalyzing diverse glycosylation activities in trypanosomes [4], has been found across the entire family *Trypanosomatidae*, not just in infectious species.

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PREPARATION OF NEW *C*-GLYCOSYL-1,2,4,5-TETRAZINES

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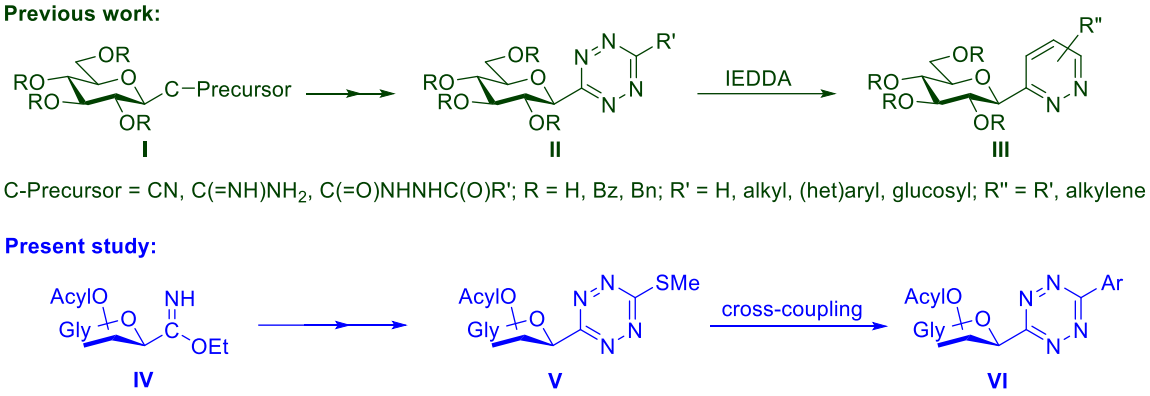
1,2,4,5-Tetrazines (*s*-tetrazines) are commonly used azadienes, whose [4+1] and [4+2] cycloadditions pave the way for constructing further N-heterocycles, such as pyrazoles, triazines and pyridazines [1]. Among these ring-transformations, the inverse electron-demand Diels-Alder reactions (IEDDA) promoted with strained cyclic dienophiles have become the most intensively studied ones in recent years. This type of transformations has emerged as one of the most powerful bioorthogonal reactions, providing possibilities for exploring physiological processes by labeling of biomolecules [2].

In a recent paper [3], we reported the first representatives of *C*-glycosyl-1,2,4,5-tetrazines, which were completely unknown in the literature prior to our work. A set of 3-β-D-glucopyranosyl-1,2,4,5-tetrazines (Scheme 1, **II**) were prepared by ring-closing reactions of different *C*-β-D-glucopyranosyl precursors (**I**). In addition, the synthetic applicability of these heterocyclic monosaccharides by their conversions into 3-β-D-glucopyranosyl pyridazines (**III**) was demonstrated [3].

As a continuation of this study, a new synthetic route to get additional *C*-glycosyl-1,2,4,5-tetrazines (**V**), suitable for functionalization (even with fluorescent moiety) by cross-couplings (**V**→**VI**), have been examined. Furthermore, the IEDDA reactions of tetrazines **II** and **VI** with strained cycloalkynes have also been under investigation to test their possible use as sugar-based bioorthogonal labeling agents.



Scheme 1.

In the presentation the details of route **IV**→**V**→**VI** as well as the first results of the strain-promoted IEDDA reactions of compounds **II** and **VI** will be reported.

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SYNTHESIS AND IN VIVO EVALUATION OF GLYCOCONJUGATES COMPRISING SURROGATES OF THE *SHIGELLA FLEXNERI* 6 O-ANTIGEN

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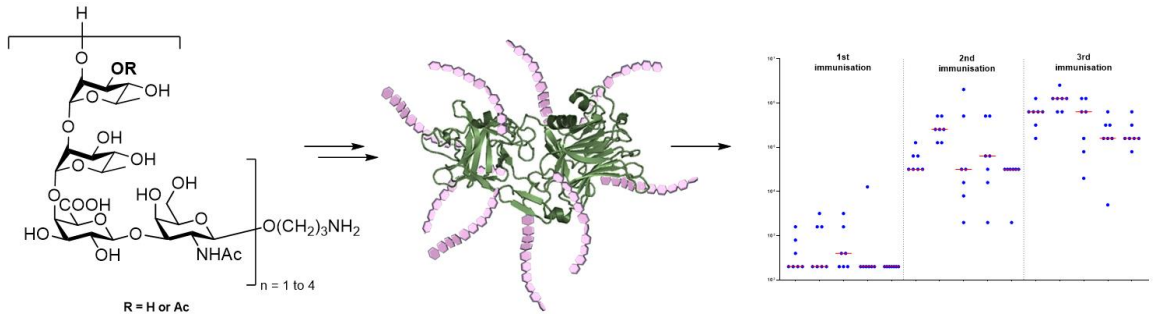
Shigella are gram-negative enteroinvasive bacteria causing shigellosis, a major diarrheal disease, especially in low-and-middle income countries. Shigellosis is responsible for a high burden and is a prime cause of mortality due to diarrhea in young children [1].
The O-antigen (O-Ag) part of the Shigella lipopolysaccharide (LPS) is a major target of protection induced by natural infection. Numerous Shigella vaccine candidates aimed at inducing an immune response against the LPS were proposed. In this context, our group has investigated synthetic O-Ag surrogates as alternatives to antigens purified from biological extracts. A S. flexneri 2a synthetic glycan-based vaccine candidate was found well-tolerated, safe and immunogenic in a first-in-human phase 1 and phase 2a clinical trials [2].
Aiming at a Shigella vaccine providing broad serotype coverage in the field, interest here is on the identification of a semi-synthetic glycoconjugate as vaccine candidate targeting S. flexneri serotype 6 (SF6), another prevalent serotype [3]. The SF6 O-Ag is defined by a partially O-acetylated linear tetrasaccharide repeat (AcABCD) [4].
To this end, we report the synthesis of a panel of glycoconjugates obtained by site-selective conjugation of diversely site-specifically O-acetylated SF6 oligosaccharides (up-to 4 repeats) onto a protein carrier. We also describe the immunogenicity of the obtained semi-synthetic glycoconjugates in mice and discuss the most valuable O-Ag mimics and conjugates thereof, as governed by a promising balance between synthesis efficiency and immunogenicity.



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SWEET-ANTIMICROBIALS

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Antibiotic-resistant microbials are on the rise and are classified as a top ten global health threat to humanity by the WHO [1,2]. Therefore, the development of new antibiotic treatment strategies is required [2]. This research project focuses on the inhibition of the carbohydrate amidase enzyme class as a novel approach to combat bacterial infections. One such enzyme is N-acetylglucosamine-6-phosphate deacetylase (NagA), that is responsible for the recycling of N-acetylglucosamine-6-phosphate (GlcNAc-6P), an essential component for the biosynthesis of peptidoglycans and viability of pathogenic bacteria [3,4]. Selective inhibition of NagA could prove a valuable addition to the anti-microbial infection toolbox and is therefore the target of this project.
E.Coli, B. Subtillis and *H.influenza* NagA homologs have been overexpressed, purified and crystalized. We were the first to solve the structure for NagA from the pathogen *H.influenza* (Figure 1A), giving us a clinically relevant target as model protein for our sweet-antimicrobials. A fluorescamine based activity assay has been adapted from literature which allows for the screening of libraries of compounds as well the biological characterization of new mechanism-based inhibitors (Figure 1B) that are currently been synthesized using the organic synthesis that has been developed for these carbohydrate analogues [5].

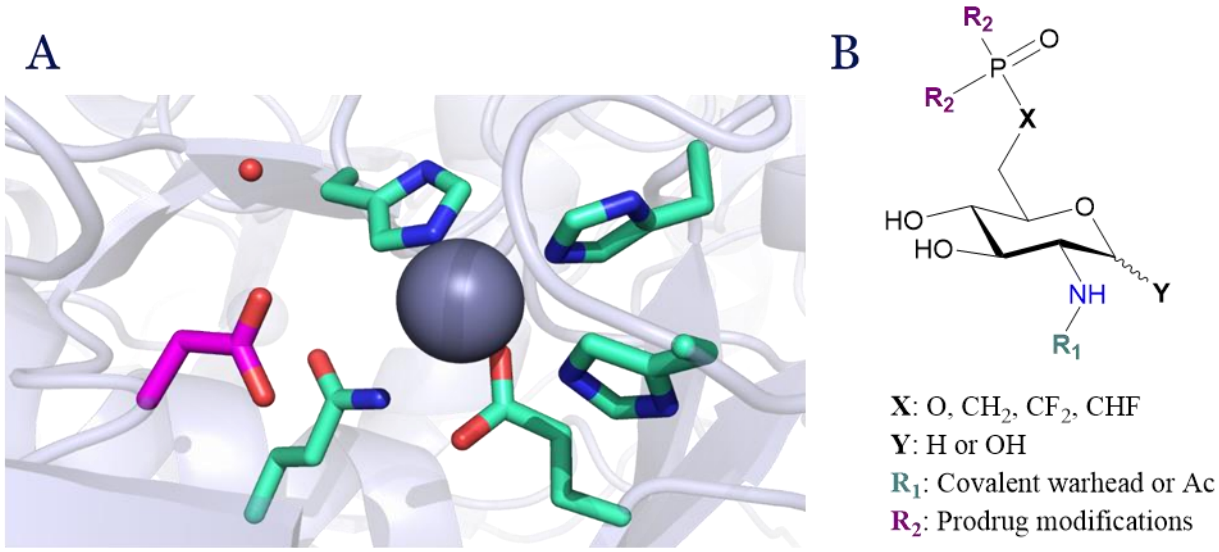

Figure 1. A: Active site residues of H.infNagA crystalstructure. B: Proposed natural substrat mimic inhibitor design.

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SYNTHESIS OF *ACINETOBACTER BAUMANNII* LAC-4 POLYSACCHARIDE FRAGMENTS

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Acinetobacter baumannii (Ab) is a highly adaptable opportunistic pathogen known for its multidrug resistance and increasing prevalence in nosocomial and community-acquired infections, including pneumonia and severe urinary tract infections. Its ability to persist on abiotic surfaces for extended period of time facilitates its survival in hospital environments, making it a serious global health threat[1,2]. Hence, investigating its virulence factors is crucial for developing novel therapeutics and vaccines. In particular LAC-4 was identified as hypervirulent strain in a mouse model of intranasal infection in comparison to other clinical isolates and laboratory strains of Ab. Importantly, the LAC-4 strain exhibits high serum resistance and reliably reproduces the most relevant features of human pulmonary Ab infection, including significant extrapulmonary dissemination and bacteremia[3]. LAC-4 polysaccharide is composed of trisaccharide repeating units (**1**, **Figure 1**) containing *N*-acetyl-α-D-glucosamine (a), *N*-acetyl-α-L-fucosamine (b) and α-8-*epi*-legionaminic acid (c). Synthetic fragments of Ab bacterial polysaccharides can be valuable tools to understand how the polysaccharide interacts with receptors of the immune system and to be used for antigen mapping studies, with the aim of investigating the possible development of semisynthetic glycoconjugate vaccines capable of conferring protection against Ab infections. Hence, our group embarked on the synthesis of LAC-4 polysaccharide fragments, including the trisaccharide repeating unit and longer oligomers. As a first step of this endeavour, in this communication we will describe the design and synthesis of 8epiLegionaminic acid building block, and its incorporation into the full trisaccharide repeating unit of LAC-4 polysaccharide. The synthetic glycans will be chemically conjugated to different carriers (immunogenic proteins and nanoparticles) for immunoevaluation.

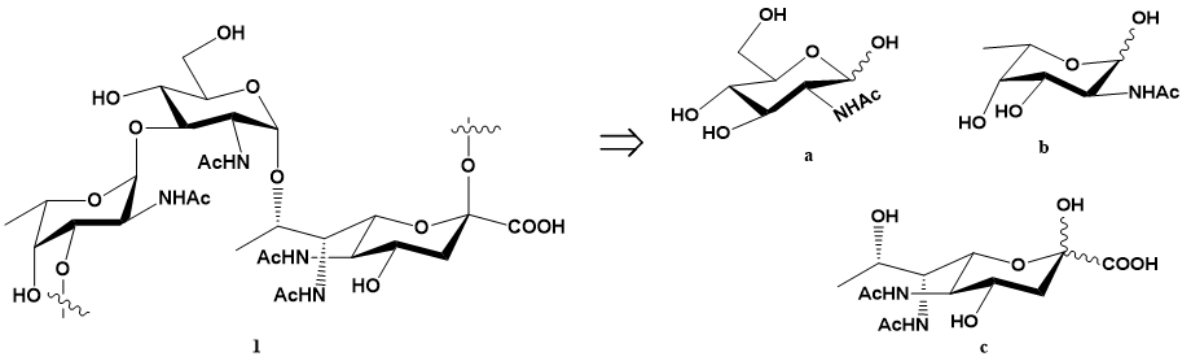

Figure 1 Retrosynthetic plan for LAC-4 trisaccharide

Acknowledgements: This project has receiving funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie Grant Agreement no. 101119575.

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CHEMICAL SYNTHESIS OF CARRAGEENAN OLIGOSACCHARIDES USING AUTOMATED GLYCAN ASSEMBLY

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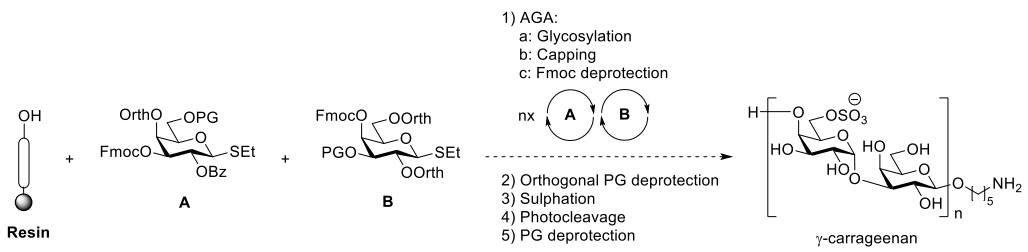
Carrageenan is a linear, sulphated polysaccharide consisting of repeating D-galactose residues, with alternating α-1,3-/β-1,4- glycosidic linkages [1]. Variations in sulphation patterns, and in some instances, the inclusion of 3,6-anhydrogalactose residues result in a range of structurally diverse galactans. These polysaccharides are primarily produced by red macroalgae and constitute a major component of their cell walls and extracellular matrices [2]. Several forms of carrageenan have found widespread industrial and commercial applications due to their abilities to form gels and viscous solutions [2,3]. However, carrageenan biosynthesis and catabolism remains largely uncharacterized, and only a handful of enzymes that act on the sulphated galactans have been described, evoking further investigation [4,5].
To alleviate the time commitment involved in oligosaccharide synthesis while maintaining structural specificity and purity of the final product, automation offers an attractive solution. Automated glycan assembly involves subsequent attachment of individual monosaccharide building blocks to a solid support resin via a cleavable linker. Iterative capping and deprotection cycles are performed to prepare the growing oligosaccharide for the attachment of the next monosaccharide residue, all the while being done using a computer-controlled delivery system in a fully automated fashion.
Herein this poster, our current synthetic efforts toward sulphated carrageenan oligosaccharides– using automated glycan assembly – will be discussed, highlighting accomplishments and challenges in using such an approach (***scheme 1***).



Scheme 1. Automated glycan assembly (AGA) for the synthesis of carrageenan oligosaccharide fragments. PG = Protecting Group, Ortho = Orthogonal Protecting Group.

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SYNTHESIS OF ADP SPIROCYCLIC GEM-DIFLUORO CYCLOPROPYL MANNOSE DESIGNED AS HEPTOSYLTRANSFERASE INHIBITORS

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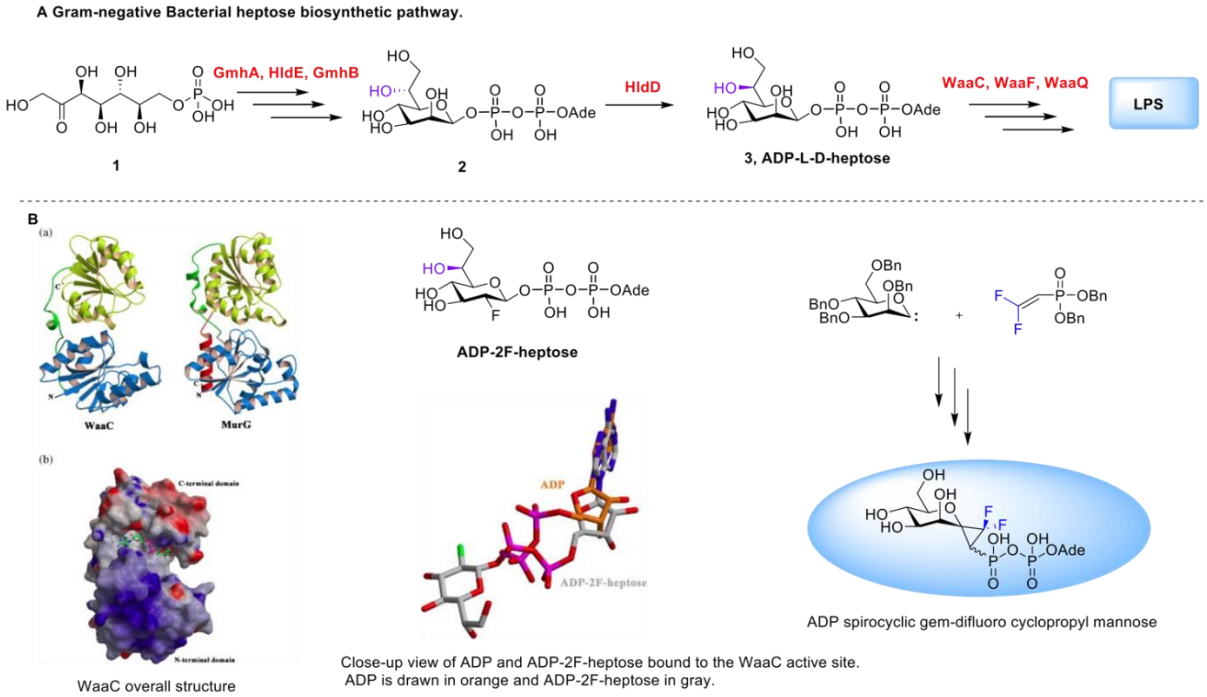
Antibiotic resistance remains a major threat to public health worldwide, the development of new types of antibacterial agents provides an interesting challenge. The absence of heptose in the LPS core domain of Gram-negative bacteria results in a truncated lipopolysaccharide associated with the deep rough phenotype causing a greater susceptibility to antibiotics and an attenuated virulence for pathogenic Gram-negative bacteria [1]. Therefore, heptosyltransferase WaaC is a validated antibacterial drug target. According to previous studies, when bound to WaaC, substrate analogs adopt remarkably distinct conformations inside the active site compared to ADP [2]. Thus, introducing a cyclopropyl moiety [3] to lock the conformation of the ADP-heptose analogs to mimic the binding model of WaaC may give good inhibition results to develop new antibacterial drugs.



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EXTRACELLULAR POLYSACCHARIDE PRODUCED BY CYANOBACTERIUM NOSTOC CALCICOLA - STRUCTURAL FEATURES AND PREBIOTIC ACTIVITY

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Secondary metabolites of microscopic organisms (microalgae and cyanobacteria) are the subject of research due to their wide range of biological effects, which are interesting for industrial applications [1]. In our systematic work aimed at elucidating the structural features of an unknown EPS, the cyanobacterial strain *N. calcicola* was investigated for EPS production and characterization of its composition and structural features. Preliminary results showed very low solubility (~20%) of the crude biopolymer recovered after lyophilization, a complex composition consisting of up to eight sugar units, and the presence of a protein indicating the proteoglycan type of the biopolymer. Treatment of EPS with pronase to remove proteins increased its solubility to 80%. The complexity of the EPS structure is indicated by the seven neutral sugar units that participate in its composition, however, of these, only Glc and Fuc were dominant (~55%), followed by Xyl, Man and Ara (~40%) and the remainder Gal and Rha (~5%) residues. In addition, uronic acids was found (~13%), indicating an acidic nature of this biopolymer. Due to the complex structure of *N. calcicola* EPS, it was subjected to ion-exchange chromatography, yielding five fractions, however, the fractions obtained were viscous and NMR measurements provided little information only. Since the high molecular weight and viscosity make structural analysis difficult, EPS was partially degraded to obtain lower fragments which were analysed by chemical and spectroscopic methods. The prebiotic activity test of EPS from *N. calcicola* confirmed the ability of the biopolymer to selectively promote the growth of probiotic bacteria.

Acknowledgements: This work was supported by the Slovak Grant Agency VEGA, Slovakia (Grant No. 2/0054/22) and COST Actions CA22134 FoodWaStop and CA18238 Ocean4Biotech.

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TOWARDS THE SYNTHESIS OF DEFINED CARBOHYDRATE-BASED LIGANDS FOR THE C-TYPE LECTIN CLEC-2

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C-type lectin-like receptor 2 (CLEC-2) is a receptor expressed on human platelets. Platelets play an important role in several biological events such as hemostasis, inflammation, infection and immunity. CLEC-2 is activated by podoplanin, a mucin-type protein, causing platelet aggregation [1]. Another ligand that was reported to act as an agonist of CLEC-2 is fucoidan, a sulfated polysaccharide from the algae *Fucus vesiculosus* [2].
We report on our research towards the chemical synthesis of podoplanin- and fucoidan-based ligands for studying CLEC-2 biology. The sialyl Tn (STn) disaccharide moiety of the glycoprotein ligand podoplanin interacts most closely with CLEC-2 [3]. Therefore, aminoalkyl-linker-functionalized STn was prepared by enzymatic 2,6-selective sialylation of linker-functionalized *N*-acetyl-galactosamine with CMP-sialic acid. Later, analogs with different hydrophobic groups at the position 9 of the sialic acid were synthesized following the same approach. Podoplanin-based glycopeptide-ligands were synthesized as well, starting from a GalNAc threonine building block. In this case, both the sugar and the peptide moiety will contribute to binding to the receptor [4]. Fucoidan-based hexasaccharide ligands with alternating α1,3- and α1,4-linkages are prepared through several rounds of glycosylation reactions using four different L-fucose building blocks, followed by selective sulfation. The choice of the positions for sulfation are guided by the sulfation pattern of the natural fucoidan ligand for CLEC-2 from *Fucus vesiculosus* [5]. The binding between the ligands and CLEC-2 will be studied using biophysical techniques.

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NEO-GLYCOPROTEINS: NATURE-LIKE LIGANDS WITH IMPRESSIVE AFFINITIES

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Galectins play key roles in immune regulation, inflammation, and cancer progression [1]. While Gal-1 and Gal-3 have been extensively studied, the tandem-repeat galectins (Gal-4, Gal-8, and Gal-9) remain underexplored despite their involvement in cancer-related processes [2]. These galectins possess two carbohydrate recognition domains (CRDs) with distinct glycan specificities, enabling complex ligand recognition. To address the need for high-affinity ligands targeting tandem-repeat galectins, we investigated the binding of multivalent glycoconjugates, including poly-LacNAc-based neo-glycoproteins and human milk oligosaccharide derivatives such as lacto-*N*-tetraose, lacto-*N*-neotetraose.
We employed biolayer interferometry (BLI) and ELISA-based assays to quantify binding affinities (see Figure [1]), complemented by nuclear magnetic resonance (NMR) for detailed epitope mapping. Using ^{15}N-labeled galectins and ^{1}H-STD-NMR (see Figure [1]), we identified both lectin and ligand binding regions. Additionally, molecular docking and molecular dynamics simulations provided structural insights into galectin-ligand recognition, revealing key amino acid residues involved in binding. Compared to single-domain galectins, tandem-repeat galectins exhibited distinct multivalent binding properties, emphasizing the importance of studying full-length proteins rather than isolated CRDs. Our findings provide novel insights into the molecular basis of tandem-repeat galectin recognition of complex glycoconjugates and highlight their potential as targets for diagnostic and therapeutic applications.

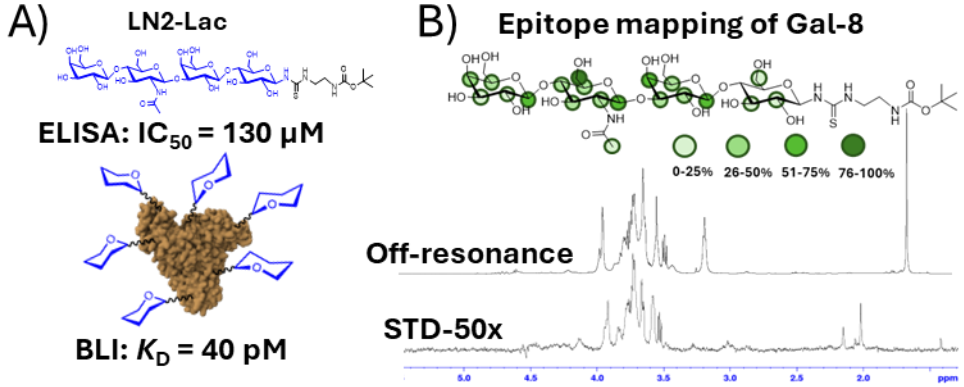

Figure 1. Investigation of the interaction between LN2-Lac and Gal-8. A) Affinity determined using ELISA and BLI. B) Epitope mapping of Gal-8 using STD-NMR.

Acknowledgements: This study was supported by the project LUC23149 by the Ministry of Education, Youth and Sports of the Czech Republic.

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THE CAPSULAR POLYSACCHARIDE PRODUCED BY *KLEBSIELLA PNEUMONIAE* Kp-IT395 HAS A NOVEL PRIMARY STRUCTURE

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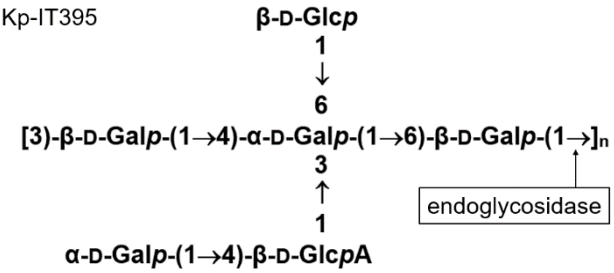
Klebsiella pneumoniae strain Kp-IT395 belongs to ST307, a clone that was first identified in the early 1990s and has spread widely in healthcare settings, particularly in the United States and Europe. Strains of ST307 are multidrug-resistant (MDR) and responsible for high levels of morbidity and mortality rates in hospitalized patients because they cannot be effectively controlled by any available antibiotic. As reported by several international organizations, there is therefore an urgent need to find new alternative strategies to solve or at least reduce the burden of MDR bacterial infections. In addition to vaccination, a promising approach is phage therapy, i.e. the use of bacteriophages, i.e. viruses that are able to selectively kill bacteria.
Since the capsular polysaccharide (CPS) together with the ability to form biofilms are important virulence factors, the CPS primary structure of Kp-IT395 was determined by chemical analysis and NMR spectroscopy. The results showed that the CPS structure is novel and identical to that of *K. pneumoniae* strain KL102 CPS, which was also characterized in our laboratories (Fig. 1). *K. pneumoniae* strain KL102 was isolated as part of the BARNARDS study [1], which aimed to generate important data on the impact of antibiotic resistance in neonatal sepsis in several low- and middle-income countries. With the aim of exploiting the use of phages against MDR bacteria, a phage capable of lysing the Kp-IT395 strain and possessing an endoglycosidase specific for the Kp-IT395 CPS was isolated. Afterwards, whole phage particles were used to depolymerize the CPS and the site of polysaccharide cleavage was determined by NMR spectroscopy.

Figure 1: structure of Kp-IT395 CPS repeating unit



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BACILLUS SUBTILIS EPSA-O: A NOVEL POLYSACCHARIDE IMPORTANT FOR BIOFILM ARCHITECTURE

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Bacillus subtilis is one of the best-studied Gram-positive organisms and a key model for the study of bacterial biofilms. Bacterial biofilms are complex microbial communities encased in a self-produced extracellular polymeric matrix that facilitates cell adhesion to biotic and abiotic surfaces. *B. subtilis* is also used as a model system for the in-depth study of the many aspects involved in biofilm formation and stability, such as extracellular matrix production, biofilm development and differentiation [1]. It is well known that exopolysaccharides are crucial components of the biofilm matrix. They are usually involved in the colonization of surfaces, form a hydrated network that mediates the mechanical stability of the biofilm and prevents bacterial desiccation, confer resistance to host defenses during infection and can be a source of nutrients. In *B. subtilis* biofilms, the most important exopolysaccharide (EpsA-O) not only provides structural support, but is also involved in the colonization of plant roots, the binding of metals and protection against reactive oxygen species.
Due to its importance, the structure of EpsA-O was determined by chemical analysis, Smith degradation and 1D and 2D NMR spectroscopy. The collected data revealed that EpsA-O has a novel structure [2], which is shown in Figure 1.

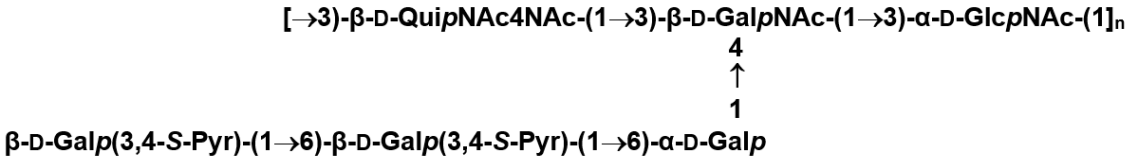

Figure 1. Structure of EpsA-O repeating unit

In addition, attribution of the glycosyltransferases of the *epsA–O* operon, devoted to the synthesis of the EpsA-O, to specific catalyzed reactions was achieved by *in silico* analysis with a good degree of homology.

Acknowledgements: The work was supported by the Slovenian Research and Innovation Agency (ARIS) J1-3021 research grant and National program grant P4-0116.

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PROBING SPECIFIC INTERACTION OF SYNTHETIC HEPARAN SULFATE TETRASACCHARIDES WITH MIDKINE

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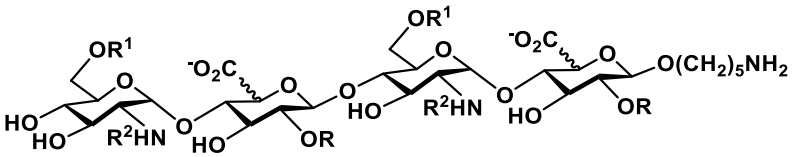
Heparan sulfate (HS) is a biomolecule of the glycosaminoglycan (GAG) family, widely distributed on cell surfaces and within the extracellular matrix. It plays vital roles in biological processes such as viral infection, cell growth regulation, blood coagulation, inflammatory responses, tumor metastasis, and neuro-related diseases. However, deciphering the HS code remains highly challenging due to its structural diversity. Therefore, well-defined HS oligosaccharides are essential for evaluating structure–activity relationships and understanding HS functions in physiological processes. In this study, a divergent strategy was employed to synthesize 32 HS tetrasaccharides with specific sulfation patterns—2-*O*-sulfate, 6-*O*-sulfate, 2,6-di-*O*-sulfate, and either *N*-sulfate or *N*-Ac—using two orthogonally protected disaccharide building blocks: D-glucosamine-α1→4-D-glucose and D-glucosamine-α1→4-1,6-anhydro-L-idose. The resulting tetrasaccharide library was employed to assess binding affinity with Midkine, revealing the structural requirements and sulfation patterns crucial for HS interactions.



R = H or SO_3^-, R^1 = H or SO_3^-, R^2 = SO_3^- or Ac

DESIGN, SYNTHESIS AND REACTIVITY OF MANNOSE-SPECIFIC BORONOLECTINS

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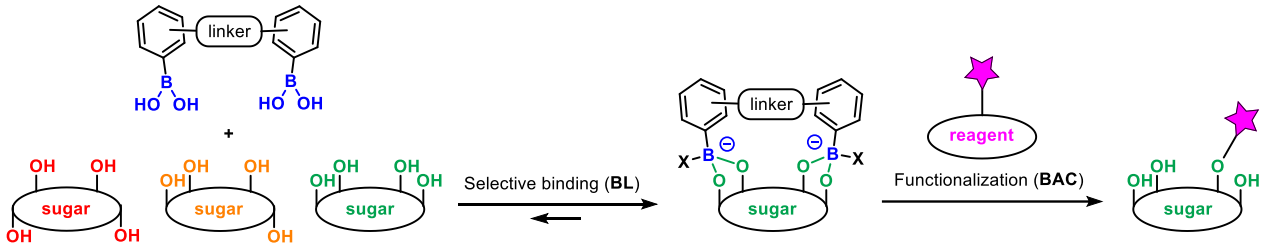
Glycoproteins practically cover all eukaryotic cells and are responsible for numerous cellular communication and recognition events. Glycobiology underwent significant development at the end of the last century but remains hampered by the high structural complexity of glycans [1]. The need for new tools to study the structure and function of glycoproteins is therefore crucial, given that certain diseases result from glycosylation defects or are marked by specific surface oligosaccharides [2]. The recent development of Metabolic Oligosaccharide Engineering (MOE) by Carolyn Bertozzi and coworkers represents a significant breakthrough allowing the study of native glycans in living cells [3]. Our goal is to develop an alternative chemical-based bioconjugation method. To specifically functionalize saccharides of interest, we aim to use boronolectins (BL) for their selectivity [4] coupled with Boronic Acid Catalytic methods (BAC) to ensure reactivity [5].



In this communication, we will disclose our latest results regarding the design, the synthesis and the reactivity of mannose-specific boronolectins.

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STUDIES ON THE CORE OLIGOSACCHARIDE FROM LPS OF *RHIZOBIUM* SP. CAS 24 THE WHITE SPANISH BROOM ENDOPHYTE

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Rhizobium sp. CAS 24 is a soil bacterium belonging to *Rhizobiaceae* family. This bacterial endophyte was originally isolated from roots of *Chamaecytisus albus*. Similarly to the majority of Gram-negative rods, the strain CAS 24 produces lipopolysaccharide (LPS), which is a main component of an outer leaflet of the outer membrane. There are three structural regions in LPS molecule: a lipid A, a core oligosaccharide (core OS), and an external part called an O-specific polysaccharide (O-PS). The LPS composed of these three parts is called the smooth (S) form, in comparison to the rough (R) form without the O-PS region. The SDS-PAGE profile of the LPS from *Rhizobium* sp. CAS 24 indicated that the majority of the material migrated as the R-LPS. The core oligosaccharide was isolated from the LPS by mild-acid hydrolysis and purified by gel permeation chromatography on a Sephadex G50 fine column. The obtained preparation was subjected to chemical analyses (sugar composition, linkage analysis), as well as ^{1}H and ^{13}C Nuclear Magnetic Resonance spectroscopy and mass spectrometry techniques (MALDI-TOF-MS, ESI-MS, and MS/MS). The molecular weight of oligosaccharides from the core OS fraction ranged from 1300 Da to 1400 Da. The chemical studies revealed that *Rhizobium* sp. CAS 24 core OS contains 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo), glucose, galacturonic acid and its 3-*O*-methyl derivative, as well as is deprived of heptoses and phosphate residues. The linkage analysis showed that the Kdo is substituted at C-4 position, similarly as it was described prevoiusly in the *Rhizobium etli* CE3 core oligosaccharide [1].

Acknowledgements: This work was financially supported by the grant from the National Science Centre in Poland (grant OPUS no. 2020/37/B/NZ8/00855)

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PURIFICATION-FREE PREPARATION OF TRIFLUOROACETIMIDOYL CHLORIDE AND THEIR USE IN CATALYTICAL CHEMICAL GLYCOSYLATION

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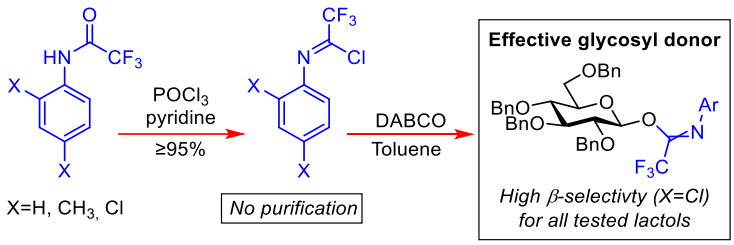
This project presents an enhanced alternative to the *N*-(phenyl)trifluoroacetimidate glycosyl donor, first introduced by Yu and colleagues [1,2]. While this donor has become widely used, several challenges persist. The synthesis of trifluoro-N-phenylacetimidoyl chloride, a key reagent for donor preparation, involves the use of the highly toxic CCl_4. Additionally, the stereochemistry of the synthesized donors remains uncontrollable, leading to complex α/β mixtures, further complicated by the formation of E/Z isomeric mixtures of the acetimidates.



This project has developed a method for synthesizing trifluoro-*N*-phenylacetimidoyl chloride and similar compounds in high yields by reacting the amide, pyridine, and $POCl_3$ at 160 °C for 4 hours in microwave reactor glass vials. Additionally, a supplementary method that does not require special glassware was developed, using 2,4,6-collidine instead of pyridine. The dichloro trifluoro-*N*-phenylacetimidoyl chloride reagent was found to be less volatile than trifluoro-*N*-phenylacetimidoyl chloride. It was also possible to synthesize N-(dichlorophenyl)trifluoroacetimidate donors in good yields with high β-selectivity using DABCO in toluene, and in some cases, high α-selectivity using TEA and DMAP in toluene. The synthesized donors were succefully used in several glycosylation reactions.

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AURANOFIN PROBES TOWARDS A BETTER UNDERSTANDING OF ITS MECHANISM OF ACTION

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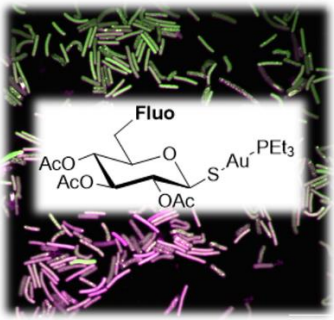
Auranofin (AF) is a therapeutic compound consisting of a peracetylated thioglucose moiety linked to a triethylphosphine-chelated gold. It has been primarily used for over 40 years, receiving FDA approval in 1985, as an oral treatment for rheumatoid arthritis (RA) in patients who have not responded well to conventional therapies such as nonsteroidal anti-inflammatory drugs (NSAIDs) or disease-modifying antirheumatic drugs (DMARDs) [1]. AF exhibits anti-inflammatory properties by inhibiting the NF-κB-IL-6-STAT3 pathway and increases cellular oxidative stress through the inhibition of thioredoxin reductase.
Although AF has been gradually replaced by other drugs for RA, ongoing research continues to explore its potential in broader therapeutic areas, including cancer, autoimmune disorders, and antibiotic treatments. Its well-characterized toxicological profile makes it particularly valuable for drug repurposing [2]. In the context of antimicrobial resistance (AMR), the search for new antibiotics [3] or combination therapies is crucial, and recent studies have demonstrated AF's antimicrobial efficacy against bacteria such as *S. aureus* (including MRSA) and *C. difficile* [4].



While most of the modes of action are well understood at molecular level, the mechanisms governing AF transport in and out of cells, as well as influence of AF on the absorption and elimination of other drugs, remain unknown [5].
As part of the investigation of the control of *C. difficile* growth and sporulation [6], we aimed to explore this question by the use of a suitable novel auranofin probe. The synthesis of the selected fluorescent auranofin conjugate will be described.

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ANALYSIS OF GLYCANS FROM *VIBRIO CRASSOSTREAE* ISOLATED DURING AN EPISODE OF MORTALITY OF THE MEDITERRANEAN SEA URCHIN *PARACENTROTUS LIVIDUS*

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Vibrios are among the most abundant microorganisms found either free-living in aquatic environments or associated with several marine organisms. Due to their enormous biodiversity and pathogenicity, controlling diseases in the aquaculture industry can be challenging [1,2].
Vibrio crassostreae is a Gram-negative bacterium known to be pathogenic to shellfish, mostly oysters and reported in other marine species during episodes of morbidity and mortality, causing significant losses in the aquaculture industry. Following an episode of sea urchin mortality (*P. lividus*) in Procida Island, tissues and spines of diseased animals were analysed for microbiology. The study of the bacterium's surface glycans aims to establish a baseline for future research to determine whether *Vibrio crassostreae* may also be pathogenic to sea urchins, specifically investigating its potential contribution to “bald sea urchin disease,” a condition resulting in spine loss of these animals. To isolate *Vibrio* spp., soft tissues (oesophagus/intestines) and spines from *P. lividus*, were inoculated into 10 ml of Marine Broth (MB) for 48 h at 30°C. Serial dilutions were performed for each sample and 100 µl of 10-2 and 10-3 of diluted samples were plated on the selective medium Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar. Plates were incubated for 24-48 hours at 30°C to allow the growth of Vibrio spp.
The bacterium has been shown to produce a rough lipopolysaccharide (LPS) and a capsular polysaccharide (CPS). In this study, we analysed both glycans' sugar composition together with a full characterisization of the capsular polysaccharide's structure.

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COMBATTING THE VIRULENCE OF *E. COLI* THROUGH COVALENT CROSSLINKING OF FIMH

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The type 1 fimbrial adhesin (FimH) is a mannose specific binding lectin found on *Escherichia coli* (*E. coli*). Notably, it is found on uropathogenic *E. coli* (UPEC) which caused 400 million urinary tract infections in 2019 [1]. FimH has been extensively studied for its properties as a virulence factor for UPEC. A leading research area is the use of mannosides in the anti-adhesion approach to *E. coli*-host cell interactions [2]. Covalent drugs are gaining renewed interest due to their increase potency and selectivity [3]. In our research group, we are interested in developing glycoconjugates as new covalent antimicrobial agents [4]. The presence of electrophilic groups or warheads in covalent ligands allows for their crosslinking to nucleophilic side chains in the binding site. Preliminary testing of the divalent mannoside in Figure 1, which features acrylamide moiety, has shown it inhibits biofilm inhibitor in laboratory and environmental *E. coli* strains. We aim to further explore the ability of mannoside ligands designed as covalent antagonist of FimH in the crosslinking and biofilm formation inhibition in a range of *E. coli* strains, including UPEC.

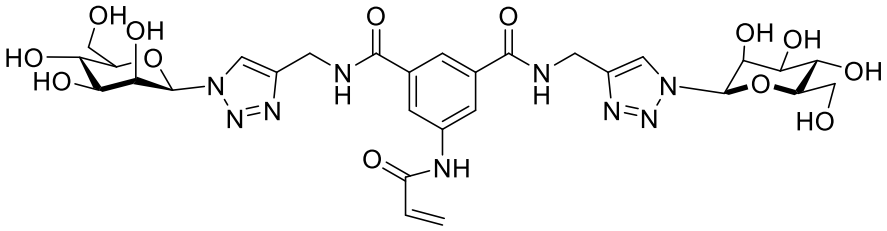

Figure 1. Chemical structure of a cross-linking divalent mannoside.

Acknowledgements: We would like to thank the Research Ireland for awarding the Government of Ireland Postgraduate award to Shane Coyle.

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PSEUDOMONAS AERUGINOSA LPS FROM CYSTIC FIBROSIS PATIENTS: IMPLICATIONS OF STRUCTURE IN ANTIBIOTIC RESISTANCE

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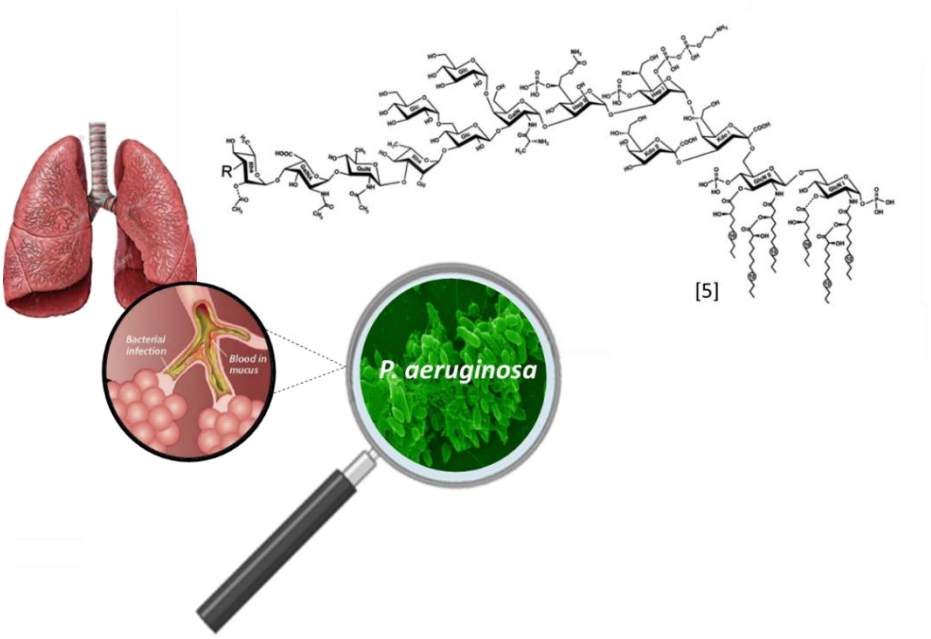
Biofilm-associated infections pose a major healthcare challenge, particularly in chronic and nosocomial diseases, where antibiotic resistance complicates the treatment. *Pseudomonas aeruginosa*, a key pathogen in cystic fibrosis (CF), forms biofilms and modifies its lipopolysaccharide (LPS) structure, contributing to persistence and immune evasion. Understanding these modifications is crucial for developing targeted therapeutic strategies [1]. Here we analyzed LPS and Lipid A structural variations in *P. aeruginosa* clinical isolates from CF patients at different stages of disease, by comparing antibiotic-sensitive clinical strain (WT2), two multi-drug resistant (MDR1 and MDR5) and pan-drug-resistant (PDR7) strains with the antibiotic-sensitive reference strain PA14 [2]. The isolates were grown under planktonic and biofilm conditions to mimic the CF pulmonary environment.



Structural variations in Lipid A across these strains were evaluated concerning the degree of antibiotic resistance and chronic infection. Furthermore, we will assess the effects of LPSs and Lipids A on inflammatory pathway activation [3,4].

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DEVELOPMENT OF A CHITOSAN-BASED BIOCONTROL PRODUCT TO INDUCE GRAPEVINE RESISTANCE AGAINST DOWNY AND POWDERY MILDEWS

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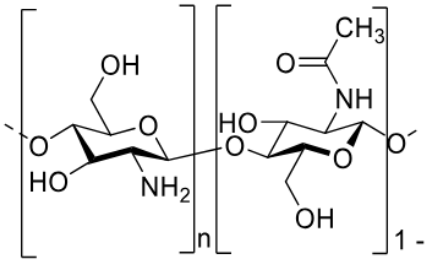
The European Green Deal promotes reducing pesticide use by encouraging biocontrol solutions that are safer for the environment and human health. Grapevine (*Vitis vinifera*), due to its economic importance and reliance on fungicides to combat diseases like downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*), and grey mold (*Botrytis cinerea*), was identified as a priority crop.
Chitosan, a biopolymer derived from crustacean shells, has shown promise as a biocontrol agent against these fungal pathogens, though its exact mode of action - whether direct antifungal activity or immune system stimulation - remains unclear.



Chemical structure of chitosan with glucosamine and acetyl-glucosamine units randomly distributed along the chain

In this study, six chitosans with varying degrees of polymerization (DP12 to DP470) were tested for both antifungal activity and their ability to trigger grapevine immune responses.
Results revealed that low DP chitosans, especially DP12, were the most effective in both direct pathogen inhibition and activating plant defences. Field trials over three years demonstrated that chitosan DP12 could provide adequate protection under low disease pressure and, when combined with just two fungicide treatments, maintained good disease control throughout the season. Overall, a chitosan-based biocontrol product presents a promising alternative to reduce chemical inputs in sustainable viticulture.

Acknowledgements: This work has been financially supported by Agence Nationale de la Recherche (ANR) and Office Français de la Biodiversité (OFB).

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DEVELOPMENT OF SIALOGLYCOMIMETICS FOR THE EVALUATION AS POTENTIAL SIGLEC INHIBITORS

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The sialic acid-binding immunoglobulin-like lectins (Siglecs) are a family of I-type lectin receptors predominantly expressed on immune cells, where they play key roles in immune regulation through their interactions with sialylated glycans [1]. These interactions, mediated by the regulatory motifs of Siglecs, can influence several immune processes [2]. Given that gangliosides serve as natural ligands for Siglecs, this project focuses on the design and synthesis of novel glycomimetics derived from the ganglioside core structure **1**, with several positions for tuning affinity through additional substituents. (Figure 1) [3]. By synthesizing smaller ganglioside fragments, which can be considered as new pharmacophores, this approach will enable the development of a diverse library of compounds for screening against different Siglecs to assess their affinity and selectivity. Compounds exhibiting the strongest binding in microarray assays will be further analyzed using competitive STD-NMR experiments to identify key interaction sites between the ligand and the protein. These insights will guide the rational design of glycomimetics with enhanced affinity, selectivity, and modulatory effects for Siglecs. This methodology aims to provide lead compounds for the advancement of glycan-based immunotherapies.

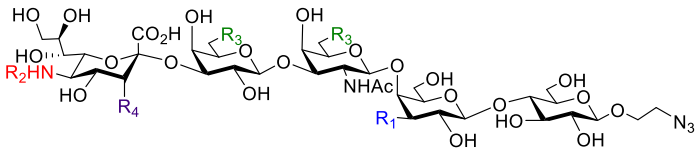

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Figure 1. Structure of ganglioside **1.**

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SUGAR DERIVATIVES AS ALTERNATIVE RAW MATERIALS FOR QAS SYNTHESIS

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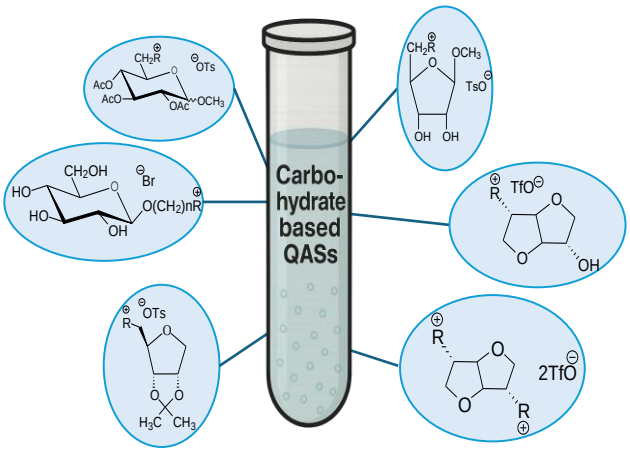
Quaternary ammonium salts (QASs) are a class of cationic surfactants with a typical head-and-tail structure, composed of a positively charged nitrogen atom and four organic substituents or derivatives of heterocyclic amines. The positive charge of the cation is balanced by the presence of the anion (e.g., halide ions or organic acid derivatives). Due to their different properties, QASs are used in many areas of modern life: medicine, industry, everyday life.

Basing solely on the properties of QAS structure leads to an inadequate assessment of their harmfulness and a mistaken inference regarding the safety of the group as a whole. Studies have shown differences in toxicity between QASs that are structurally similar but differ by a few carbon atoms. Therefore, each QAS with potential applicability should be tested for toxicity.

Limitations in the use of QASs, along with the increasing resistance of microorganisms motivate chemists to search for new, more active and environmentally friendly QASs that can replace currently used, harmful chemicals. This is why the synthesis of new quaternary ammonium salts of sugar derivatives, alditols and anhydroalditols is so important [1].

Although sugars are economically favorable starting materials, the process of producing their ionic derivatives is complicated by the required multi-step synthesis method. Therefore, recent research work aims to simplify the obtaining of QASs by introducing procedures that reduce the number of synthesis steps [2].



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THE USE OF LECTIN-BASED GLYCOPROTEIN MICROARRAY TECHNOLOGY FOR PLASMA GLYCOME PROFILING IN PATIENTS WITH ENDOMETRIAL CARCINOMA

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Endometrial carcinoma (EC) is a malignancy of the uterus originating from the epithelium of the endometrium. Its incidence is increasing, particularly among postmenopausal women, with several etiological risk factors implicated, including hyperestrogenism, metabolic syndrome, and genetic predispositions [1]. Although most EC cases are diagnosed at an early stage allowing for a favorable prognosis and high curability approximately 20% of patients present with extrauterine disease at diagnosis. The prognosis for advanced-stage disease (stage IV) remains poor, with a five-year survival rate of only around 15%. Therefore, early detection remains a key factor influencing treatment success. The development of non-invasive diagnostic approaches aimed at effectively excluding EC in women with postmenopausal bleeding has significantly progressed in recent years and offers a potentially more acceptable alternative to invasive procedures such as endometrial biopsy [2]. Despite available treatments, there remains a strong need for more accurate risk stratification and personalized therapy, leading to intensified research into molecular biomarkers, including changes in protein glycosylation [3]. Glycosylation plays a crucial role in regulating cell signaling, interactions with the extracellular environment, and immune recognition. Aberrant glycosylation is a hallmark of malignant transformation and may serve as a sensitive indicator of tumor presence or therapeutic response. In this study, we employed a lectin-based glycoprotein microarray platform to comprehensively profile the plasma glycome in EC patients. The array consisted of 21 lectins with various glycan specificities. We analyzed 66 pairs of plasma samples (preoperative and postoperative) to identify systemic changes associated with tumor presence. Our results revealed significant alterations in the binding profiles of several lectins following surgical tumor removal, suggesting the presence of dynamic changes in systemic glycosylation associated with the disease. These findings support the hypothesis that plasma glycosylation monitoring may serve as a non-invasive tool for assessing tumor status and potentially as a prognostic or diagnostic marker in EC. Lectin-based glycoprotein microarray technology thus appears to be a promising platform for identifying novel glycan-related biomarkers in tumor glycobiology, with potential applications in the development of personalized therapeutic strategies in oncogynecology.

Acknowledgements: *Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V02-00049. This work was supported by the grant VEGA 2/0120/22. This publication is the result of the project implementation CEMBAM – Centre for Medical Bio-Additive Manufacturing and Research, ITMS2014+: 313011V358 supported by the Operational Programme Integrated Infrastructure funded by the European Regional Development Fund.*

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ANALYSIS OF MOLASSES FOR ELECTROCHEMICAL CONVERSIONS

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The use of molasses as a feedstock for electrochemical conversions offers a sustainable way to produce valuable chemical compounds. In this work, the chemical analysis of molasses is investigated with regard to its suitability for electrochemical processes that have the potential to generate higher value products. The exact clarification of the molasses composition is of great importance, as possible secondary components such as organic acids or other impurities could negatively influence the electrochemical reactions or generate undesirable by-products [1].

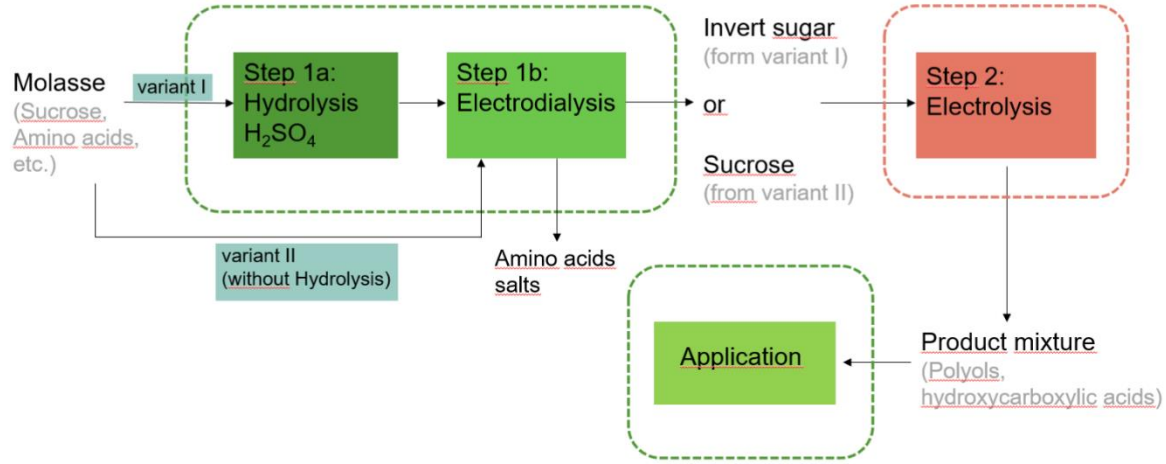

Figure 1. Flowsheet for the planned process for the electrochemical refinement of molasses.

Initial analyses of the sugar composition were carried out using high-performance liquid chromatography (HPLC) to identify and quantify the main types of sugar, which are of particular interest for electrochemical conversions [2]. In this study, two different types of molasses, sugar beet and sugar cane molasses, were investigated to characterize their composition and evaluate their suitability for electrochemical conversions.
For future work, other analytical methods such as LC-MS and GC-MS will be used to obtain a more precise characterization of the molasses and the electrochemically generated products. These methods will provide more precise information on the structure and identity of the products and thus promote the optimization of electrochemical conversions. The results of this study could provide important impact for the development of efficient and sustainable processes for converting molasses into valuable chemical compounds [3].

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STRUCTURAL CHARACTERIZATION OF EPS PRODUCED BY THREE MARINE MICROALGAE SPECIES BELONGING TO *TETRASELMIS* GENUS

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The genus *Tetraselmis*, a green marine microalga of the order Chlorodendrales, has garnered significant attention in various applications. These species are widely employed in nutraceuticals for their antioxidant properties [1] and serve as a valuable feedstock in aquaculture due to their high Eicosapentaenoic acid (EPA) content [2]. Furthermore, the substantial lipid content of *Tetraselmis* species makes them a promising biofuel source [3]. Finally, several *Tetraselmis* species have been observed to produce extracellular polysaccharides (EPS) [4]. Studies have revealed that certain cultivation conditions, particularly heterotrophic growth, enhance EPS production in this genus [5]. These EPSs have substantially shown different biological activities, such as the EPS derived from *Tetraselmis suecica*, which has cytotoxic effects on tumor cells and exhibits antioxidant properties [6].
Here we present the production of EPS from three distinct marine strains of the genus *Tetraselmis*: *T. chuii*, *T. suecica*, and *T. suecica* 22D. These strains were cultivated under autotrophic, heterotrophic, and mixotrophic conditions to maximize EPS yields. The obtained supernatants were extracted and purified. The polysaccharide material was analysed using chemical analyses and NMR spectroscopy.

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Qβ VIRUS-LIKE PARTICLES AS A PLATFORM TECHNOLOGY FOR GLYCOCONJUGATE VACCINES

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Virus-like particles (VLPs) are composed of viral structural proteins but lack viral genetic material, which prevents them from replicating in cells. Their uniform size and geometric configuration facilitate consistent conjugation of molecules [1]. The objective of this project is to functionalize bacteriophage Qβ VLPs with glycans at varying densities and conjugation chemistries in order to evaluate their potential to elicit a robust and selective immune response. The selected glycans -Globo H, Man3 and LDNF- have distinct immunological relevance. Globo H is a tumor-associated carbohydrate antigen (TACA) that is overexpressed in cancer and has been used in the development of anti-cancer vaccines [2]. Man3, a paucimannosidic glycan, is emerging as a promising target for cancer research, with potential roles as a contributor to immune evasion or recognition, and a target for immunotherapy or glycan-based treatments [3]. LDNF, a glycan moiety found in helminth parasites (e.g. *Schistosoma mansoni*), appears to be involved in host defense, suggesting potential as an immunomodulator in autoimmune diseases [4]. Moreover, aberrant expression of N-glycans containing LDNF has been reported in certain cancers, highlighting its possible role in immune evasion and tumor progression [5]. Functionalization of VLPs is analyzed using MALDI-TOF and SDS-PAGE, while internalization assays are performed by High-Content Screening (HCS).

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COMPARING DIFFERENT TECHNOLOGIES FOR THE DEVELOPMENT OF A CAPSULAR-BASED VACCINE AGAINST *KLEBSIELLA PNEUMONIAE*

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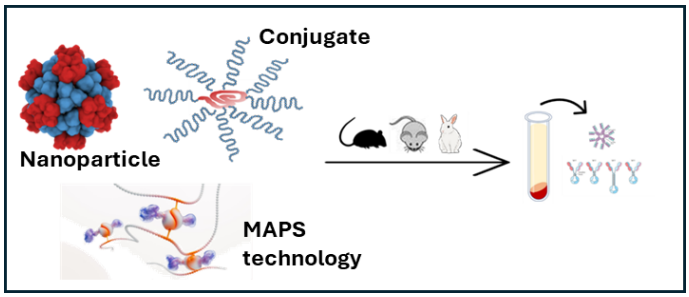
Klebsiella pneumoniae is a Gram-negative and anti-microbial resistant bacterium, leading cause of neonatal sepsis in low- and middle-income countries [1]. Despite the urgent need, no licensed vaccines are currently available. Capsular polysaccharides, also known as K-antigens (KAg), are key virulence factors and promising vaccine targets. However, the high diversity of KAg serotypes poses a significant challenge for vaccine development [2]. This study evaluates multiple technology platforms to accelerate the development of a broadly protective, multivalent KAg-based vaccine. Herein, a KAg capsular polysaccharide, one of the most prevalent serotypes associated with neonatal sepsis, has been selected as model antigen.

We compared traditional conjugation [3], nanoparticle-based delivery [4], and MAPS technology [5]. For traditional conjugation, an improved 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) chemistry [6] was used and two carrier proteins, CRM_{197} and recombinant tetanus toxoid (8M-TT)[7], were evaluated. Nanoparticles offer advantages such as enhanced antigen uptake by antigen-presenting cells due to their optimal size (10-150nm), symmetrical and organized structure, with the possibility to accommodate multiple antigens on their surface. MAPS technology utilizes a high-affinity, noncovalent interaction between biotin-tagged polysaccharides and rhizavidine-fused proteins, potentially allowing for broader immune responses by combining KAg with conserved pathogen proteins. The resulting vaccine candidates were characterized for purity, saccharide-to-protein ratio and size. Immunogenicity of the vaccine candidates was assessed both in mice and rabbits by measuring the level of antigen specific antibodies classes and IgG subclasses, their ability to kill *Klebsiella* strains *in vitro*, as well as by evaluating the cellular immune response. All constructs elicited a significant anti-KAg specific immune response with some differences among the platforms used.

This study provides valuable insights into the development of a multivalent KAg-based vaccine against *Klebsiella pneumoniae*. The comparative evaluation of these technology platforms in different animal models, looking both at humoral and cellular responses, offers data that can inform the development of polysaccharide-based vaccines against other bacterial pathogens too.



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PECTIN FROM BLACK CURRANT POMACE - EXTRACTION BY NADES AND CHEMICAL CHARACTERIZATION

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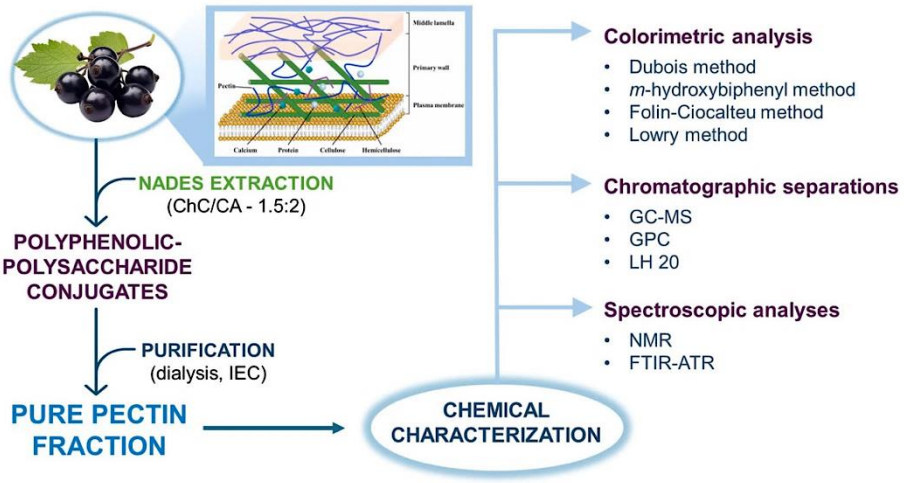
The growing interest in green technologies has significantly influenced the development of environmentally friendly extraction methods that can be used to recover valuable compounds from fruit industry waste. Blackcurrant pomace, the main waste product in juice production in Poland, is rich in polysaccharides, especially pectins, which can have many applications [1,2]. Traditional pectin extraction on an industrial scale is mainly based on the use of strong mineral acids, which creates significant environmental problems. A promising alternative is the use of natural deep eutectic solvents (NADES) [1,3].
In the presented research work, optimal conditions of the extraction process were developed to obtain pectin from blackcurrant pomace in a NADES environment. Polyphenol-polysaccharide conjugates were obtained, which were purified by dialysis and ion exchange chromatography (IEC). In order to characterize the pure pectin fraction, a series of analyses were performed, including the use of chromatographic and spectroscopic methods.



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SYNTHESIS OF THE ANTICOAGULANT PENTASACCHARIDE FONDAPARINUX VIA A NOVEL PROTECTING GROUP STRATEGY

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Heparin is an invaluable drug in the prevention and treatment of thromboembolic diseases due to its anticoagulant effect. However, its use can face several limitations due to its polyanionic and heterogeneous nature. A major milestone in research was the development of a synthetic heparin analogue pentasaccharide drug, Arixtra (**1**, fondaparinux), which successfully minimized the side effects of anticoagulant therapy. Fondaparinux can be used to treat deep vein thrombosis and acute pulmonary embolism. It has a faster anticoagulant effect, higher and more predictable anti-Xa activity, a longer duration of action, and less biological risk compared to LMWH. This modified analogue of the antithrombin-binding pentasaccharide part of heparin was first prepared by French and Dutch researchers using a 55-step chemical synthesis [1]. The development of the compound into a drug was achieved in collaboration with the pharmaceutical companies Sanofi and Organon, and it was marketed under the name Arixtra in 2001. Although the pentasaccharide has some clinical shortcomings (e.g. short half-life) and is extremely complex to synthesize, it is still the only commercially available synthetic heparin analogue used in medicine. Over the years, several synthetic routes have been developed for the preparation of fondaparinux. In a recent publication, Dey *et* al. developed a novel, programmable, one-pot synthesis [2]. In another work, Li *et* al. constructed fondaparinux (**1**) using a [3+2] block synthesis [3]. At about the same time, Chang *et* al. also reported a synthetic route for the economical preparation of the fully protected pentasaccharide and then fondaparinux [4]. Despite all this, the efficient synthesis of the pentasaccharide has not been solved to date. The common problem in all these works was the economical synthesis of the L-iduronic acid moiety.

In our research, we will attempt to synthesize fondaparinux using an L-idose donor prepared by an efficient synthetic route developed by us. Thanks to the new L-idose donor, we have had the opportunity to test a new protecting group strategy and the way has also been opened to reduce the number of reaction steps.

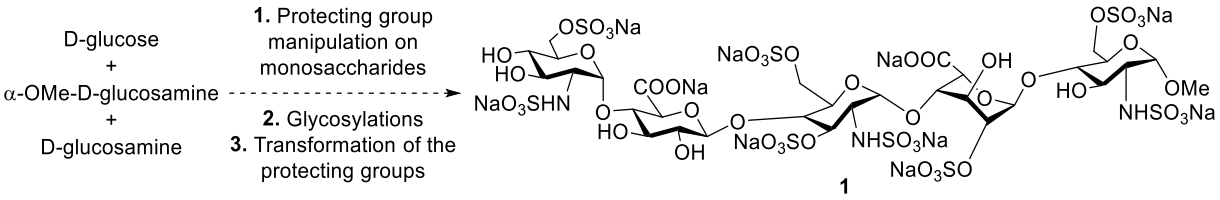

Figure 1. Schematic synthesis and structure of fondaparinux (**1**)

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ENHANCING CYTOTOXICITY OF FLUORINATED SACCHARIDES BY ATTACHING ORGANOMETALLICS

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Conjugates of simple sugars with organometallic fragments are being explored for cancer treatment because the carbohydrate component can potentiate the effect of the organometallic part. In addition, organometallic complexes, especially ruthenium- and ferrocene-based compounds, are emerging as promising alternatives to platinum-based therapies, offering lower general toxicity and favorable kinetics.

In this study, we synthesized and investigated the effects of fluorination at the 4- and/or 6-positions on the cytotoxicity of D-gluco- and D-galactosamine modified by attachment of a ferrocene or ruthenium(II) complex at the 1- or 2-position. The *in vitro* cytotoxicity of these compounds was evaluated against three different cancer cell lines and one noncancerous cell line.The cytotoxicity values, expressed as IC_{50}, of the 4-fluorinated and 4,6-difluorinated precursors were compared with the values of their organometallic conjugates. Fluorinated hexosamines alone (e.g. **1**, Figure 1) exhibit moderate cytotoxicity. A further increase in cytotoxicity was observed after conjugation with organometallics. Interestingly, while ruthenium half-sandwich complexes were found to be nontoxic, ferrocene conjugates(e.g. complex **2**) and ruthenium tetrazene glycoconjugates exhibited significant cytotoxicity that often depended on the substituent at the anomeric position [1].

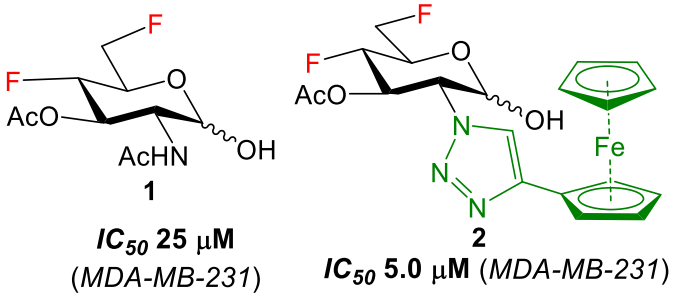

Figure 1. Difluorinated glucosamine (**1**) and its ferrocene conjugate (**2**) and their cytotoxicity to triple negative breast cancer cell line MDA-MB-231.

Acknowledgements: The financial support of the Czech Science Foundation is gratefully acknowledged (23-06115S)

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Synthesis of cannabidiol-thioglycoside conjugates by chemoselective thiol-ene reactions

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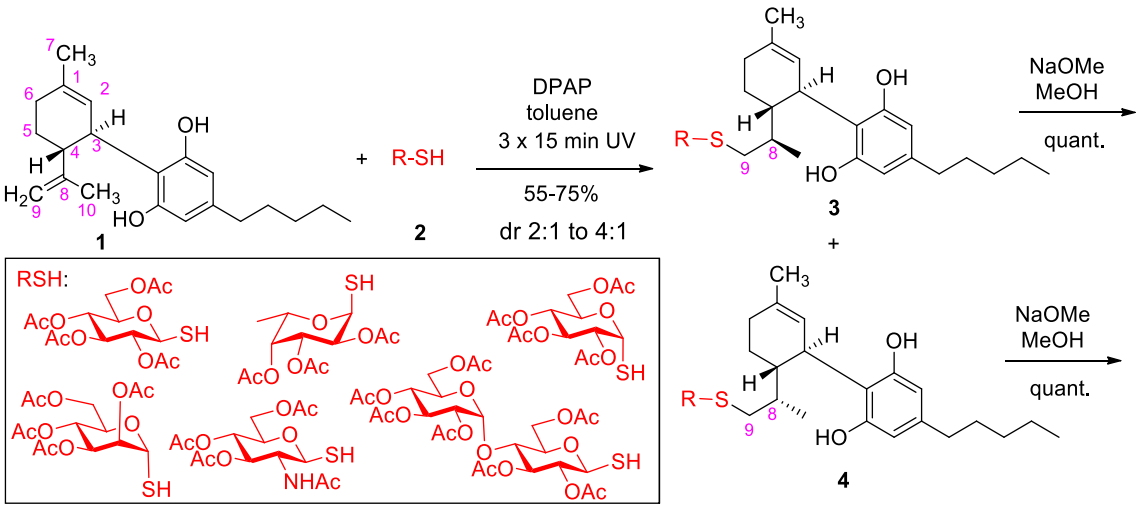
Cannabidiol (CBD) is a non-psychotropic cannabinoid isolated from *Cannabis sativa* that has several promising biological effects.[1] However, its low water solubility and first-pass metabolism limit its oral bioavailability, which hinders the medicinal use of CBD. Therefore, there is a great need for appropriate chemical modifications to improve its physicochemical and biological properties. Our aim was to conjugate various carbohydrates to CBD to increase the water solubility and possibly enhance the biological effects of the parent molecule.



Scheme 1. Photoinitiated chemoselecteive hydrothiolation of CBD at Δ^8 with glycosyl thiols

In this work, we chose the photoinduced thiol-ene reaction which is a widely applied atom-economic click-ligation method [2] to couple protected 1-thiosugars to CBD (Scheme 1). Using an equimolar amount of thiol (1-thio-D-Gluc, -Man and -GlcNAc, and 1-thio-L-Fuc, etc.), the reactions proceeded with complete chemoselectivity in all cases, due to the higher reactivity of the exocyclic C8 double bond in the radical addition. The thiol-ene reaction produced the anti-Markovnikov addition product with full regioselectivity, but the stereoselectivity was variable and depended on the sugar configuration. After separation of the two diastereoisomers, Zemplén deacylation was performed to produce several novel carbohydrate-cannabidiol thioconjugates that will be potential candidates for a wide range of biological studies.

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AMPHIPHILIC GAG-MIMETICS FOR STUDYING PATHOGEN INTERACTIONS

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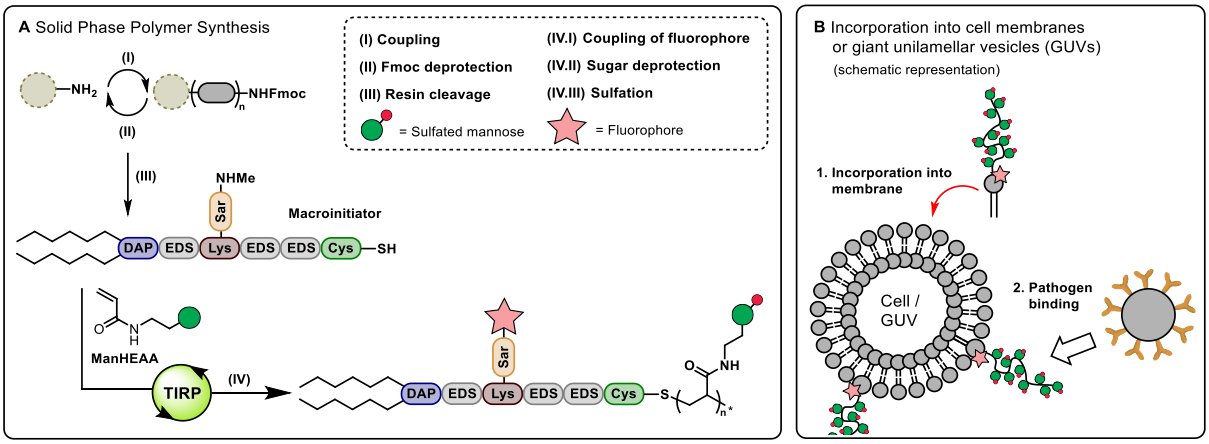
Glycosaminoglycans (GAGs) are linear polysaccharides attached to a core protein to form proteoglycans, which are located at the extracellular membrane of eukaryotic cells [1]. As these highly sulfated polymers promote protein binding and therefore enable intercellular interactions, GAGs also play a crucial role in cell infection process by mediating pathogen adhesion and invasion [2]. In context of novel therapeutic strategies tackling this issue, the design of synthetic GAG-mimetics aims for a deeper elucidation of GAG-mediated cell infection processes [3].
The systematic synthesis of GAG-mimetics shall enable the generation of a library of diverse polymeric structures which are to be evaluated by structural differences, binding affinities and other characteristics. We deploy mannose-based acrylamide monomers (ManHEAA), which can be polymerized by a method, recently developed by our group: The thiol-induced, light-activated controlled radical polymerization (TIRP) allows the straightforward synthesis of low-dispersity polymers with specific end-groups [4]. Incorporation of the polymers into membranes and study of pathogen interactions can be conducted by additional functionalization with a membrane anchor and a fluorophore, enabled through the synthesis of a new TIRP macroinitiator [5].



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DYNAMICS OF SULFATED GLYCOSAMINOGLYCANS DURING ADIPOCYTE DIFFERENTIATION: COMPREHENSIVE PROFILING OF CHONDROITIN/DERMATAN SULFATE

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Chondroitin sulfate/dermatan sulfate (CS/DS), a glycosaminoglycan (GAG) that regulates cell proliferation and differentiation, exists in cell surfaces and extracellular matrices. Studies on myoblast cells have shown that CS reduction promotes differentiation, suggesting the complex role of CS/DS in cellular processes. While CS/DS has been implicated in the differentiation processes of various cell types, the molecular details of CS/DS profiles during adipocyte differentiation remain largely unexplored. To bridge this knowledge gap and provide a more comprehensive understanding of CS/DS in tissue formation, this study aimed to analyze CS/DS structural changes and gene expression in mouse 3T3-L1 cells to elucidate molecular mechanisms underlying adipose tissue formation.

Using high-performance liquid chromatography (HPLC) and reverse transcription real-time PCR, we comprehensively analyzed CS/DS dynamics during 3T3-L1 adipocyte differentiation. Differentiated cells exhibited a significant decrease in total CS/DS content alongside reduced expression of biosynthesis genes, including chondroitin sulfate N-acetylgalactosaminyltransferase 1 and 2 (Csgalnact1/2) and chondroitin polymerization factor. Moreover, CS/DS chains showed reduced molecular weight, suggesting structural remodeling associated with cellular differentiation.

Furthermore, A-units (CS/DS chains) predominated in both undifferentiated and differentiated cells, with a notable increase in CS-A proportion during differentiation. This shift correlated with altered sulfotransferase activity. To complement these findings, we conducted additional experiments using rat mesenchymal progenitor cells (MPCs). Intriguingly, inhibiting Csgalnact1/2 unexpectedly enhanced adipocyte differentiation, indicating complex regulatory mechanisms of these enzymes.

Ultimately, this study shed light on the dynamic remodeling of CS/DS during adipocyte differentiation, providing new insights into the mechanisms of cellular development. The downregulation of CS chain elongation enzymes highlights the complex regulatory processes underlying cell differentiation. By revealing the unexpected impact of Csgalnact1/2 on adipocyte differentiation, this research deepens our understanding of molecular controls in adipogenesis, potentially paving the way for new avenues in lipid metabolism research and the understanding of adipose tissue development.

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GLOBOTRIAOSYLCERAMIDE-TARGETED LECTIN-DEPENDENT CELLULAR CYTOTOXICITY WITH AURISTATIN E

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In recent years, antibody-drug conjugates (ADCs) have attracted attention as a new modality of antibody-based therapies that leverage antibody-dependent cellular cytotoxicity (ADCC). However, some concerns have been raised regarding antibody-specific drawbacks, such as the depletion of target antigens and toxicity associated with the antibodies themselves. Lectins are gaining interest as potential probes for cancer-associated carbohydrate antigens, serving as alternatives to antibodies. Several examples of lectin-drug conjugates (LDCs) have been reported to date.
The rhamnose –binding lectin from catfish (*Silurus asotus*) eggs (SAL) can bind and internalize into globotriaosyl ceramide (Gb3) expressing cancer cells, and induces cell growth retardation[1]. Gb3 is known as a cancer-related carbohydrate marker overexpressing in such as cervical, breast and colon cancer, and associating with their malignancy[2]. Interestingly, we observed that SAL enhanced influx rate of anticancer drugs in human Burkitt's lymphoma Raji cells and cervical carcinoma HeLa cells[3]. Here, we prepared two SAL/LDCs, SM30 and SM50, which were conjugated with monomethyl auristatin E (MMAE) as a payload toxin with binding capacities of 30 and 50%, respectively. Cytotoxicity of SM30 and SM50 for Gb3 expressing Raji cells, HeLa cells, and human testicular seminoma JKT-1 cells were evaluated by trypan-blue exclusion test. Both LDCs exhibited concentration- and binding capacity-dependent cytotoxicity. The IC50 value of SM30 against Raji, HeLa and JKT-1cells were 17.33 μg/mL, 1.21 μg/mL, and 0.72 μg/mL, and those of SM50 were 6.14 μg/mL, 1.02 μg/mL, and 0.48 μg/mL, respectively. To confirm the importance of Gb3, we established Gb3 removed (KO) cells by editing of Gb3 synthase A4GALT gene. As expected, SM30/50 had no effects for KO-Raji, KO-Hela, and KO-JKT-1 cells. To further determine whether SM30/50 can selectively recognize Gb3 positive cells, we prepared co-culture and detection system of Gb3 non-expressing K562 and A4GALT-transfected and GFP-labeled K562 cells (Gb3-K562). Even in the co-culture medium, SM30/50 killed only Gb3-K562 but not K562 cells.
Although the internalization rates of SAL into respective cell lines could be different, these results suggest that SAL/LDC is promising as a probe for the selective delivery of drugs to Gb3 expressing cancer cells.

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DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF UNNATURAL GALLOTANNINS

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Phytochemicals play an important role in drug discovery. In this context, there is a renewed interest in natural products and their structural analogues to address antimicrobial resistance. The diverse biological activities of natural polyphenols are responsible for their significant therapeutic properties (e.g. antioxidant, anti-inflammatory, antibacterial, anticancer, antimutagenic) and many of them are considered as potential therapeutic agents against neurodegenerative diseases, cardiovascular disorders, diabetes, cancer and aging [1]. In addition to antioxidant activity, many representatives of this group exhibit significant antimicrobial activity, which makes them promising antibacterial and antifungal agents. In line with this concept, natural and synthetic gallotannins have been a subject of considerable interest. Gallotannins (GTs) are generally composed of a central carbohydrate core esterified with gallic acid. Among various naturally occurring GTs, 1,2,3,4,6-penta-*O*-galloyl-D-glucose (PGG) was most widely studied, due to its diverse pharmacological effects [2]. Moreover, natural PGG exhibited potent antifungal activity against drug-resistant *Candida albicans, Candida auris* and *Candida glabrata* [3].
Presented research is focused on the synthesis and biological activity evaluation of non-natural PGG analogues. The molecular structures of GTs derived from D-mannose, D-glucose and L-rhamnose were characterized by various spectroscopic methods. Their genotoxic effect and DNA-damaging/protective potential on human blood lymphocytes were evaluated. Furthermore, the compounds were screened for antifungal activity against a panel of pathogenic *Candida* species. It was shown that GTs with a number of hydroxyl and phenolic groups are able to inhibit or slow down yeast growth. However, there are differences between the GTs types in terms of efficacy, as well as differences resulting from their chemical structure. The results suggest that synthetic GTs could be considered as non-toxic agents for the design of new antimicrobials with potential applications in biomedicine and consumer products.

Acknowledgements: This work was financially supported by Slovak grant agency VEGA 2/0071/22 and VEGA 2/0151/22.

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STEREOSELECTIVE SYNTHESIS OF PSEUDO-GLYCOCONJUGATES WITH 2-EXOMETHYLENE GROUP AND BIOLOGICAL ACTIVITY OF PSEUDO-GLUCOSYLCERAMIDES

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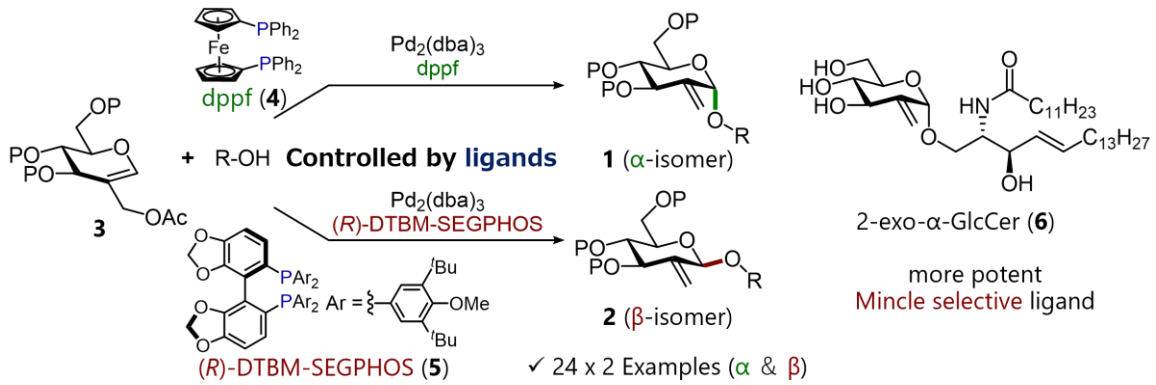
Our group is investigating the development of “pseudo-glycoconjugates”, in which the original activity or function are altered by slight structural modification of the native glycoconjugates. We recently developed novel pseudo-glycoconjugates by replacing the functional group at C2, which characterizes the function of glycans, to the exomethylene functionality. The introduction of an exomethylene group results in the loss of hydrogen bonding ability at functional group at C2, but the π-bond can cause different intermolecular interaction. In addition, the ring strain caused by the introduction of sp^2 carbon results in a unique conformational property from that of native glycoconjugates. We expected that these steric and electronic property of this pseudo-glycoconjugates would lead to finding new biological-active glycan analogues.

Firstly, we challenged to develop a methodology for the stereoselective synthesis of various 2-exomethylene-type α-glucosides **1** and β-glucosides **2**. In this study, we investigated the Tsuji-Trost-type reaction of glucal derivative **3** as a common precursor of glycosides. We anticipated that the stereoselectivity could be controlled by a ligand of the Pd catalyst. Treatment of **3** and an acceptor and catalytic amount of $Pd_2(dba)_3$ and dppf (**4**) under heated conditions gave the **1** in a stereoselective manner. Further examination of the ligand revealed that the use of (*R*)-DTBM-SEGPHOS (**5**) reversed the stereoselectivity and selectively gave **2**. These methods were applicable to 24 species of primary or secondary alcohols [1].

We synthesized the pseudo-glucosylceramide (pseudo-GlcCer) **6** and evaluated its function as a ligand for immune receptor. Finally, we found that it has different property from that of native GlcCers as expected. We will report the development of synthetic methodology and the biological activities of pseudo-GlcCers in detail.



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HOMOGENEOUS SULFATION OF POLYGULURONATES (POLY-G) AND THEIR CHARACTERIZATION

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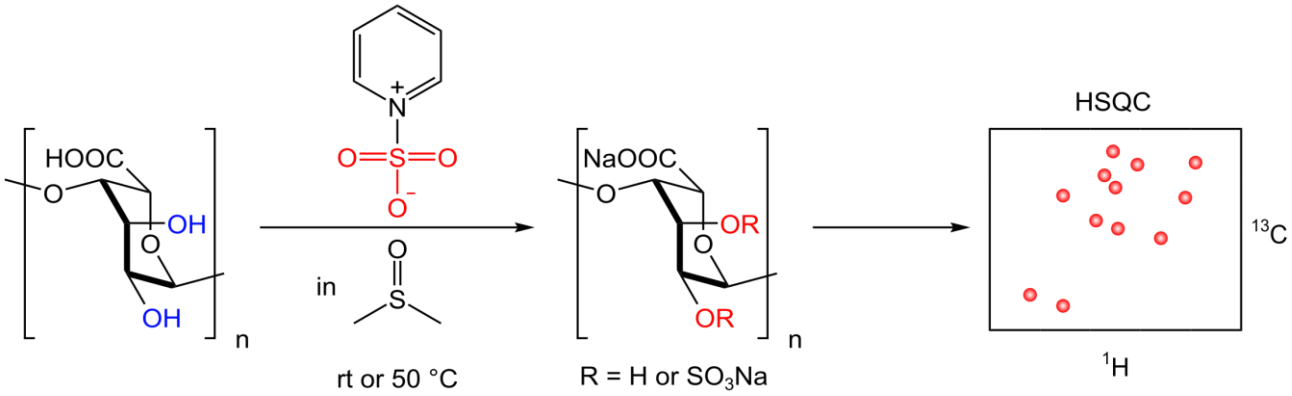
Polyguluronates (poly-G) are alginates consisting exclusively of α-L-guluronic acid residues. Sulfated poly-G-s have shown promising applications, found to have an anticoagulative, anti-inflammatory [1], immunomodulatory [2], antiviral [3] and lipid-lowering activities [4]. Despite useful properties, they have seen only limited structural characterization [5], complicating determination of their structure-activity relationship for applications development. Therefore poly-G-s were sulfated homogeneously in DMSO with Py·SO_3, followed by comprehensive analysis with NMR-spectroscopy (^{1}H, HSQC and COSY) to assign the spectra of starting materials and reaction products in order to determine sulfation patterns of poly-G sulfates. It was found that at lower concentrations of sulfating reagent, G-3 sulfation was slightly preferred, while at higher concentrations, G-2 sulfation alongside G-2,3 disulfation were prominent. Protecting groups (PG) were used to direct regioselectivity by protecting one hydroxyl group, leaving other open for sulfation, thus aiding the structural characterization. By using TBDMS-ether as PG, poly-G sulfated primarily at G-2, while with Bz-ester, G-3 selectivity was found.



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HOMOGENOUS REARRANGEMENT AND RISK ANALYSIS FOR *N*-GLYCAN OF THERAPEUTIC ANTIBODY

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The *N*-glycans attached to the Fc region of therapeutic antibodies significantly influence their pharmacological efficacy. It has been reported that the absence of core fucose and the addition of terminal galactose enhances the affinity of therapeutic antibodies for Fc receptors (FcR), thereby improving antibody-dependent cellular cytotoxicity (ADCC) activity (Fig. 1). Utilizing sialylglycopeptide (SGP) and asialo-glycoprotein (G2P) as homogeneous glycan donors, we developed glycan remodeling technology employing endoglycosidase (Endo-M) and its mutant variant (Glycosynthase: Endo-M N175Q). This approach enabled the resolution of glycan heterogeneity in therapeutic antibodies and demonstrated enhanced binding affinity to FcR.

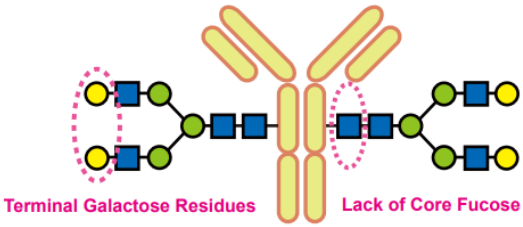

Figure 1. Non-fucosylated galactosyl glycoform

While the glycan structures of therapeutic antibodies contribute to pharmacological efficacy, the presence of non-human type glyco-antigens raises concerns about the risk of anaphylactic reactions. To address this, we synthesized non-human type *N*-glycan antigens using chemical glycan synthesis technology to create reference standards for quantitative analysis. Furthermore, we developed anti-NeuGc and anti-αGal antibodies utilizing chemically synthesized glycan epitopes, establishing a simplified analytical method. We anticipate that these advancements will contribute to the enhanced functionality and quality of antibody drugs.

MINING AND PRIORITIZATION OF NOVEL GH51 FAMILY XYLAN-DEBRACHING ARABINOFURANOSIDASES

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Xylan is a highly abundant biopolymer with potential to be modified obtaining outstanding chemical and physicochemical properties. Utilization of xylan remains limited as processing of the biopolymer remains inefficient due to shortage of substituent-modifying enzymes acting on the polymeric material. Efficient enzymatic xylan-debranching is desirable to reduce water solubility of the biopolymer for successful introduction to biobased consumer products. GH51 family α-L-arabinofuranosidases liberating L-arabinose directly from branched xylan are required for effective conversion of the raw biopolymer to a high-value product component. Manual as well as programmatical sequence curated mining was implemented for candidate arabinofuranosidase prioritization for production and characterization aiming subsequent exploitation in xylan debranching. Candidate sequences (~11000) indexed in CAZy database as attributed to GH51 family were analyzed in respect of domain organisation, conservative motif patterns and sequence identity to characterized xylan-debranching enzymes resulting in prioritized sequences (3) of manual mining. SINTEF data mining pipeline based on the HMMER suite and the dbCAN3 meta server with the profile Hidden Markov Model (HMM) of GH51 family was used for programmatical sequence mining. Candidate sequences indexed in Marine Metagenomics databases of University of Tromsø (Norway) and proprietary databases of SINTEF Industry (Norway) were analyzed resulting in prioritized sequences (8) of programmatical mining. Prioritized sequences were selected considering soluble expression predictions from a shortlist of sequences (35) curated by Multiple Sequence Alignment and Sequence Similarity Network from HMM hits (~4200).

Acknowledgements: H2020 EnXylaScope (Mining microbes and developing advanced production platforms for novel enzymes to rapidly unleash xylans' potential in a scope of products for the consumer market) project (grant no. 101000831) and H2023 BIONEER (Scaled-up production of next-generation carbohydrate-derived building blocks to enhance the competitiveness of a sustainable European chemicals industry) project (grant no. 10157779).

THE STEREOCHEMICAL OUTCOME OF GALACTOSYLATIONS IS INFLUENCED BY BOTH THE POSITION AND ELECTRON WITHDRAWING POWER OF DISTAL ACYL PROTECTING GROUPS

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We have previously studied the influence of 6-*O*-acyls on anomeric selectivity in glucosylations with thioglycoside donors.[1] Our findings showed that the most electron withdrawing esters promoted the highest a-selectivity in glucosylation reactions.
In this study, we extended our investigations to the stereodirecting effect of various distal benzoyl esters on anomeric selectivity in galactosylation reactions. We found that esters at O6 of thiogalactosyl donors had a negligible influence on anomeric selectivity. Instead, a-selective galactosylations were observed with 4-*O*-benzoyl, 3,4-di-*O*-benzoyl and 4,6-di-*O*-benzoyl protected galactosyl donors, with the highly electron withdrawing *p*-nitrobenzoyl protecting group providing the most a-selective galactosylations. Furthermore, a-selectivity was enhanced by replacing the thiophenyl aglycon functionality with the highly reactive cyclohexyl aglycon functionality. These findings enabled the successful synthesis of the biological relevant a-D-Gal(1→4)Gal linkage.

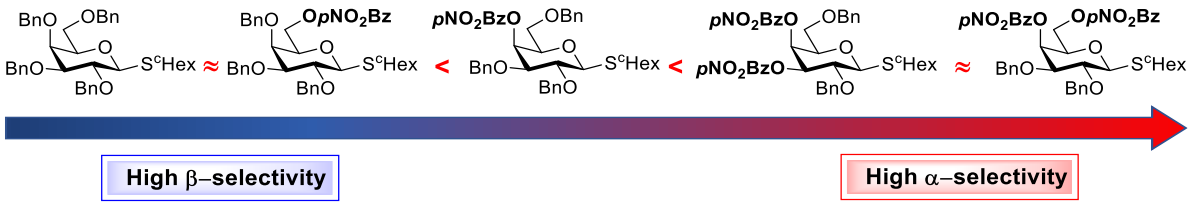

Figure 1. Graphical illustration of donor selectivity

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PALLADIUM-CATALYZED C-H ARYLATION OF 1,2,3-TRIAZOLYL-C-NUCLEOSIDE OF BIOLOGICAL INTEREST

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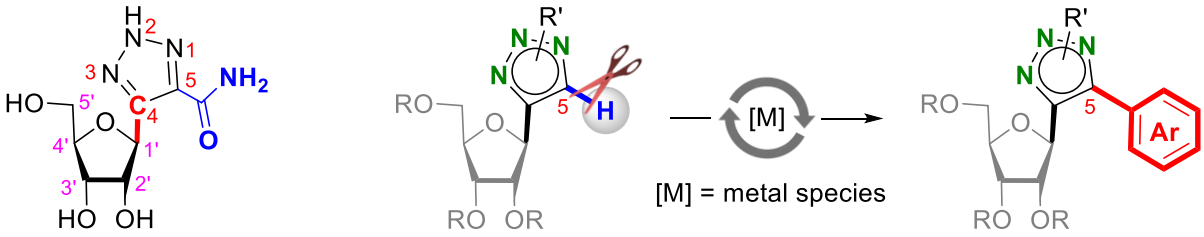
Nucleosides represent a family of biomolecules involved in numerous crucial life processes in every living organism. Regarding their importance, it is not surprising that this class of substances emerged as a prototype for the discovery of bioactive compounds. Nowadays, many unnatural nucleosides and their analogues (Gemcitabine, Remdesivir, Sofosbuvir) are clinically used for the treatment of different types of cancer and viral infections (HIV, Hepatitis C and B) [1]. In the past few years, our group has investigated the biological activities of an unnatural 1,2,3-triazolyl-*C*-nucleoside family (SRO-91 family) in relation to various viruses and cancer cell lines.[2] SRO-91 family possesses an amide function on position 5 of the triazole ring, whose derivatization showed the importance of functionalizing this position for the antiviral and anticancer activities.[2] Thus, the objective of this project is to incorporate molecular diversity on this particular position via a versatile methodology.
Even if examples dealing with nucleosides are underrepresented [3] metal-catalyzed C-H functionalization (MCF) of carbohydrates has very recently gained attention.[4] Our strategy consists thereby in using a non-prefunctionalized SRO-91-like structure, bearing a C-H bond in the targeted position 5, to form unnatural C-C bonds *via* MCF reactions. Herein we will present the introduction of aryl moieties via a direct palladium-catalyzed process.

Direct C-H functionalization of the nucleobase :



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STRUCTURAL DIVERSITY OF O-ANTIGENS IN *PECTOBACTERIUM PARMENTIERI* LIPOPOLYSACCHARIDES

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Lipopolysaccharides (LPS) are crucial virulence factors in plant pathogenic bacteria, serving as pathogen-associated molecular patterns (PAMPs) that mediate plant colonization and trigger host defence responses. The structural diversity of O-polysaccharides (OPS) within LPS molecules from different strains of *Pectobacterium parmentieri*, an economically significant phytopathogen causing soft rot and blackleg diseases in potato crops worldwide, was investigated.

The chemical structures of OPS from five *P. parmentieri* strains were elucidated using comprehensive NMR spectroscopy and chemical methods. The analysis revealed remarkable heterogeneity, with three distinct OPS molecular structures identified among the strains. Notably, strains SCC3193 and IFB5432, isolated from different countries, possess identical OPS structures containing the rare sugar residue – pseudaminic acid [1]. Strains IFB5427 and IFB5408 exhibited a second, different OPS structure, while strain IFB5441 possessed a third unique OPS molecule, further demonstrating the structural diversity within this species.

Comparative genomic analyses revealed that strains sharing identical OPS structures exhibited the highest genome-wide similarity based on average nucleotide identity. Furthermore, these strains demonstrated superior plant tissue maceration capabilities compared to others. Strain IFB5441, despite having a distinct OPS structure, showed comparable virulence in chicory leaf maceration assay. The observed OPS heterogeneity appears to correlate with both genomic diversity and phenotypic traits, suggesting that LPS structural variation may contribute to the remarkable adaptability and virulence potential of *P. parmentieri* in diverse environmental conditions.

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SYNTHESIS OF NOVEL 1,4-DIHYDROPYRIDINE DERIVATIVES CONTAINING SUGAR-BASED STRUCTURAL ELEMENTS

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Heterocyclic compounds are among the most extensively studied groups of bioactive molecules. One particularly significant class is 1,4-dihydropyridine (1,4-DHP) derivatives, which possess a six-membered ring with a nitrogen atom at the first position. These compounds play a crucial role in the pharmaceutical industry, particularly in treating hypertension and cardiovascular diseases. Their pharmacological activity stems from interactions with calcium channels and other metabolic pathways, leading to antihypertensive, antioxidant, anticancer, neuroprotective, and cardioprotective effects. However, despite their therapeutic benefits, 1,4-DHP derivatives often exhibit adverse side effects, limiting their clinical applications.

To address this issue, structural modifications are being explored to enhance their solubility, selectivity, and reduce toxicity. One promising approach involves introducing sugar-based structural elements into the 1,4-DHP ring. This modification is expected to improve the compounds' pharmacokinetic properties by increasing water solubility, preventing bioaccumulation, and enhancing selectivity toward specific cellular receptors.

This study focuses on the synthesis of new sugar-modified 1,4-DHP derivatives. The synthetic strategy involves a multicomponent reaction to obtain core 1,4-DHP derivatives, followed by monosaccharide modification and *N*-alkylation to incorporate the sugar moiety. The results of this study will contribute to evaluating the potential of sugar-functionalized 1,4-DHP derivatives as safer and more effective therapeutic agents.

BINDING AFFINITY AND PUTATIVE ENTRANCE MECHANISMS OF OCTANOYLATED, LAURYLATED AND MYRISTOYLATED KR12 PEPTIDES INTO α-, β- AND γ-CYCLODEXTRIN CAVITIES

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Cyclodextrins (CDs) belong to a class of cyclic oligosaccharides, the most common ones being α-, β- and γ-CDs formed by six, seven and eight α-(1–4) D-glucopyranoses units, respectively. They are one of the most widely used macrocyclic host molecules in the food, pharmaceutical and cosmetic industries due to their ability to form inclusion complexes with a variety of lipophilic guest ligands. Host-guest (cyclodextrin-ligand) inclusion complexes are formed through non-covalent interactions, such as van der Waals forces, hydrogen bonding and dipole-dipole interactions, between host and guest molecules. The formation of these complexes alters the physicochemical properties of the guest molecules, including their solubility, which may be enhanced or reduced [1]. Furthermore, complexation within cyclodextrin cavities can influence the chemical reactivity of the guest molecule and enhance its stability, protecting it against degradation processes such as hydrolysis, photolysis, and thermal stress.

In this contribution, we present the physicochemical principles underlying the interactions between cyclodextrins (α-, ß- and γ-) and three lipopeptides, namely the octanoylated (C8-KR12-NH_2), laurylated (C12-KR12-NH_2) and myristoylated (C14-KR12-NH_2) KR12 peptide (K18R19IVQR23IK25DFLR29-NH_2), the smallest antimicrobial peptide derived from human cathelicidin LL-37 [2]. Isothermal titration calorimetry (ITC), supported by circular dichroism spectroscopy data, allowed for the determination of the stoichiometry of the resulting complexes, the binding constants and the thermodynamic parameters of the interactions (ΔG, ΔH, TΔS). This approach enables the description of the investigated interactions on the molecular level. To further gain better insight into the physicochemical nature of the interactions, the experimental findings were complemented by molecular dynamics simulations. Particular attention has been paid to the correlation between the strength of the interactions and the structural features of the reactants, namely the differences in the hydrophobic chain lengths of fatty acid residues and cyclodextrin (α-, ß- and γ-) cavity size. Finally, the putative entrance mechanisms of the lipopeptides into the cyclodextrin cavities were proposed and discussed. The proven affinity of cyclodextrins for lipopeptides and their ability to form relatively stable host-guest complexes may protect lipopeptides from environmental degradation. This process may also alter their biological activity, including antimicrobial, antiviral and other therapeutic effects.

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DESIGN AND SYNTHESIS OF COVALENT LECTIN LIGANDS

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AB_5-type toxins are bacterial enterotoxins released during severe infectious diseases, such as cholera and traveler's diarrhea. Whereas the A-subunit is responsible for the toxic catalytic activity, the B_5-subunit is necessary for cell surface adhesion. Neutralization of the toxin with a suitable ligand with a high binding affinity offers new therapeutic options to fight these threats. The pentameric B_5-subunit with its five-fold symmetry suggests the development of multivalent inhibitors. This strategy has been followed by many groups and multivalent ligands with spectacular affinities have been developed [1]. However, these high-affinity ligands have complex structures and are synthetically demanding.
Here, we present a ligand-directed (LD) approach to effective cholera toxin (CT) ligands. The natural ligand of CT is the GM_1 oligosaccharide. We designed a GM_1 mimic based on a published structure [2] to which we attached an electrophilic *N*-alkyl-*N*-acyl sulfonamide (NASA)-type [3] warhead *via* a suitable linker. Using this approach, a lysine residue near the binding site is targeted and covalently modified. Upon reversible binding of this ligand to a CT B-subunit, the NASA warhead is positioned near a close by lysine residue (**Figure 1**). A proximity-driven acylation reaction leads to covalent attachment of the ligand to the protein. Covalent attachment of the ligand to the protein increases the effective molarity of the ligand at the binding site, similar to a multivalent interaction resulting in an effective inhibition of the protein. We present the ligand design, its synthesis, and the covalent protein modification that we monitored by mass spectrometry.

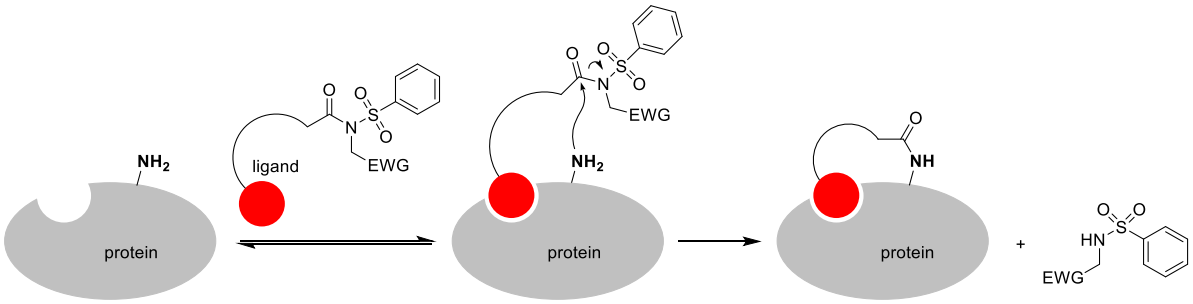

Figure1. Principle of covalent binding of a synthetic GM_1 mimic attached to a NASA warhead to a lysine side chain of a CT B-subunit. EWG: electron-withdrawing group

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INFLUENCE OF N-GLYCOSYLATION OF THE SARS-COV-2 SPIKE PROTEIN ON ITS INTERACTION WITH COLLECTINS AND COMPLEMENT

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The Spike glycoprotein (S-gp) of SARS-CoV-2, a virus responsible for the COVID-19 pandemic, contributes to the virus entry into host cells and is the major target of host's humoral immune response [1-2]. It contains two highly glycosylated subunits, S1 and S2, mediating attachment and membrane fusion, respectively [3]. To study the role of the complement system in SARS-CoV-2 infection, we investigated interactions between S-gp N-glycosylation variants and complement-activating collectins (MBL, CL-10, CL-11), ficolins (ficolin-1, -2, -3), as well as a non-activating collectin SP-D. Recombinant SARS-CoV-2 B.1.617.2 (Δ) S-gp with wild type N-glycans (wt), a variant with high-mannose N-glycans (GnTI-), as well as N234 (S1), N343 (S1), N801 (S2) N-glycan knockouts were used. The N-glycosylation patterns were confirmed by mass spectrometry. The wt and GnTI- variants were shown to activate C3 in pooled sera collected before the pandemic. In contrast to ficolins, all tested collectins (both recombinant and present in sera) recognized the S-gp. Western blot analysis demonstrated that collectins bind only intact S-gp, not separate S1 or S2. With the exception for SP-D, this interaction led to the activation of complement. The S-gp glycosylation variants were similarly recognized by SP-D, but the complement-activating collectins showed the highest affinity to the GnTI- variant. Moreover, the N-glycan at position 801 affected the MBL recognition. In silico studies confirmed that the presence of N-glycan at position 801 is required for interactions with MBL. The intramolecular interactions between S-gp variants and human recombinant MBL and SP-D will be examined by surface plasmon resonance (SPR).

Acknowledgements: *Funding: National Science Centre (NCN, Poland), grant 2020/39/B/NZ6/01660.*

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LECTIN-BASED MICROARRAY AS A TOOL FOR GLYCOSYLATION BIOMARKER RESEARCH

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Protein glycosylation plays a crucial role in cellular communication, influencing numerous biological processes. Alterations in glycan structures of glycoproteins and other glycoconjugates are associated with key physiological interactions, such as pathogen-host recognition, immune responses, stem cell function, and fertilization, as well as various diseases, including cancer, inflammatory and neurological disorders, and psychiatric conditions. Microarrays have emerged as a powerful high-throughput tool for analyzing glycosylation changes, enabling precise identification of target glycans among diverse molecular structures. Since the introduction of microarray technology for high-throughput glycotyping, numerous platforms with distinct customizations have been developed for glycomic applications. We have designed and implemented reverse-phase lectin-based glycoprotein microarray biochips, a technique that allows for the simultaneous and rapid analysis of a large cohort of samples. The method involves printing multiple samples onto a microarray substrate, which is then incubated with biotinylated lectins, followed by fluorescent detection via streptavidin conjugation. This approach enables efficient glycotyping and biomarker screening with potential applications in biomedicine, biotechnology, and molecular biology. Although lectin interactions do not provide precise structural identification of glycans, they serve as a valuable tool for detecting glycosylation changes. To achieve structural validation, we complement microarray analysis with mass spectrometry techniques, allowing for a more comprehensive characterization of glycosylation patterns. We focus on serum/plasma glycosylation and also pay attention to extracellular vesicle (EV) glycosylation as a potential source of biomarkers, as EVs carry glycan structures reflecting the glycosylation patterns of their cell of origin, making them valuable biomarkers for disease detection and monitoring. We have recently applied the described microarray approach to study and determine glycosylation changes in, for example, endometrial cancer (EC), gestational diabetes mellitus (GDM), attention-deficit hyperactivity disorder (ADHD), and congenital disorders of glycosylation (CDG) [1,2].

Acknowledgements: This work was supported by the grants VEGA 2/0120/22, APP0620, APVV-21-0108, APVV SK-SRB-23-0048 and COST CA20117. Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V02-00049 and the project No. 09I03-03-V04-00772.

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TECHNO-ECONOMIC ANALYSIS OF SECOND-GENERATION CARBOHYDRATE UTILIZATION FOR SUSTAINABLE PRODUCTION OF BIONEER BUILDING BLOCKS

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The BIONEER Project, led by SINTEF Industry (Norway), focuses on the valorization of second-generation carbohydrate sources, specifically non-food lignocellulosic biomass, for the sustainable production of bioactive building blocks. By leveraging underutilized feedstocks such as agricultural residues and forestry waste, the project aims to overcome the limitations of first-generation biomass, which often competes with food systems and contributes to land-use challenges. A particular focus of the project is the enzymatic conversion of xylan, a major hemicellulose polysaccharide, into high-value, bio-based building blocks. These xylan-derived intermediates have the potential to serve as versatile platform chemicals, targeting a range of market applications including furniture coating and personal care products.
The project will explore innovative enzymatic and process technologies to optimize the conversion of xylan and other carbohydrate fractions, improving yield and selectivity. A critical component of the BIONEER Project is comprehensive techno-economic analysis, which will evaluate the economic viability and scalability of the proposed bioconversion processes. This analysis will consider factors such as feedstock availability, processing costs, energy efficiency, and environmental impact, and relevant examples will be presented. By integrating biotechnological innovation with economic assessment, the BIONEER Project aims to develop cost-effective and sustainable solutions for future biorefineries. Ultimately, this work will support the transition to a circular bioeconomy in Europe, promoting innovation, resource efficiency by reducing reliance on fossil resources and enhancing the value of renewable biomass.

Acknowledgements: BIONEER (grant no. 10157779) and H2020 EnXylaScope (Mining Microbes and Developing Advanced Production Platforms for Novel Enzymes To Rapidly Unleash Xylans' Potential In a Scope Of Products For the Consumer Market) project (grant no. 101000831. BIONEER is supported by the Circular Bio-Based Europe Joint Undertaking (CBE JU) and its members and is funded by the European Union.

STRUCTURAL STUDIES ON THE EGZOPOLYSACCHARIDE OF *RHIZOBIUM* SP. CAS 24 THE ENDOPHYTE ISOLATED FROM ROOTS OF WHITE SPANISH BROOM (*CHEMAECYTISUS ALBUS* (HACQ.) ROTHM.)

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Rhizobium sp. CAS 24 is a soil bacterium belonging to *Rhizobiaceae* family grouping symbiotic and plant endophytic strains. This endophytic bacterium was originally isolated from roots of *Chemaecytisus albus.* Similarly to the other Gram-negative soil bacteria, the strain CAS 24 is able to produce an abundant amounts of egzopolysaccharides (EPSs), covering the bacteria, loosely attached to the cell, and acting as protective agent in environmental stressing conditions. Large amounts of EPS were obtained from *R. giardini* CAS 24 post-culture medium and subjected to chemical characterisation procedures and SEC, together with structural analysis of the polymer using FT-IR and NMR spectroscopies, as well as MALD-TOF mass spectrometry. The EPS is composed of an α-(1→4)-glucan backbone decorated occasionally (nonstoichiometric) at positions C-2, C-3 and C-6 (mainly) with hexoses or very short oligosaccharides residues. The molecular weight was determined to be above 1,000 kDa. The polymer is scanty decorated with non-sugar residues (only traces of acetyl moieties were detected by NMR). Moreover, absence of substituents with a negative or positive electrical charges indicates the neutral nature of this polymer. To our knowledge, among bacteria that fix atmospheric nitrogen in symbiotic systems with legumes, no bacteria that produce EPS with a starch-like structure have been so far described [1].

Acknowledgements: This work was financially supported by the grant from the National Science Centre in Poland (grant OPUS no. 2020/37/B/NZ8/00855)

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DESIGN AND SYNTHESIS OF NON-CARBOHYDRATE CELL-PERMEABLE POTENT INHIBITORS OF α-2,6-SIALYLTRANSFERASE

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Sialyltransferases transfer sialic acid to glycoproteins and glycolipids in the Golgi apparatus. α-2,6-sialyltransferase (ST6Gal I) does so via an α-2,6-linkage and plays an important role in cancer progression. Rillahan et al. discovered a series of glycosyltransferase inhibitors using high-throughput screening, of which the compound JFD00458 (Figure 1) showed the strongest inhibitory activity against ST6Gal I with an IC_{50} of 10.8 μM. However, this inhibitor is not expected to cross the cell membrane(s) and reach the Golgi apparatus *in vitro* or *in vivo* due to its high polarity [1]. In this work, we designed and synthesized analogues of JFD00458 to increase cell permeability and improve compound potency. We also used a recently developed biochemical assay to evaluate ST6Gal I inhibitors [2]. Our structure-activity study demonstrates that potent inhibition can be maintained with simultaneous cellular permeation.

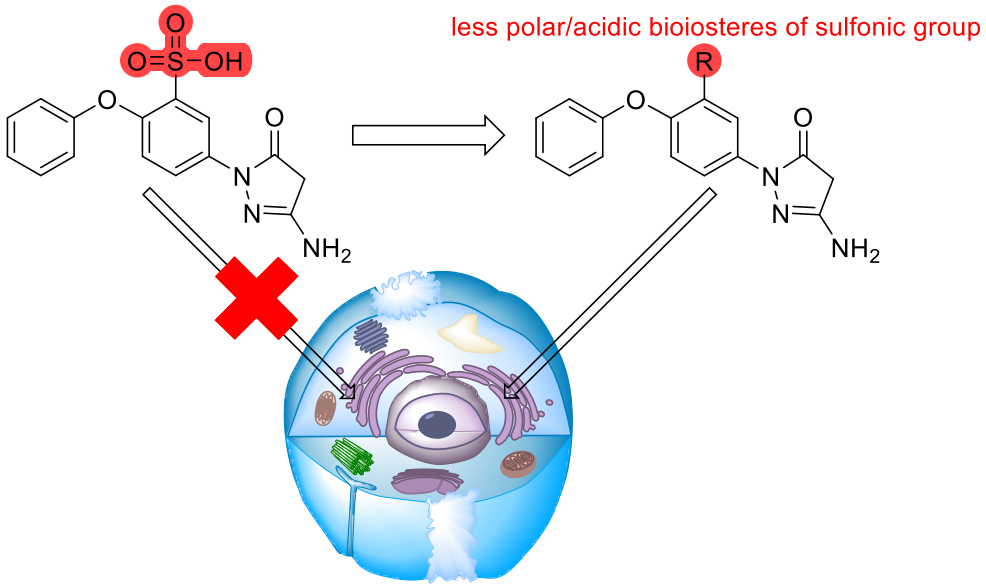

Figure 1. JFD00458 structure

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DIVERSIFICATION OF O ANTIGEN POLYSACCHARIDES IN *KLEBSIELLA PNEUMONIAE*: THE MOLECULAR BASIS FOR O-ACETYLATION IN SOME SEROGROUPO2 SUBTYPES

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Klebsiella pneumoniae is one of the most prevalent human pathogens associated with global human deaths and a leading cause of deaths linked to antimicrobial resistant pathogens. It appears at top of the “critical” group of bacteria in the 2024 Bacterial Pathogen Priority List published by the World Health Organization. It is the subject of intense investigation aimed at developing alternative therapeutic approaches to antibiotics, including vaccination and immunoprophylaxis. Surface polysaccharides (capsular K antigens and lipopolysaccharide (LPS) O antigens) are potential targets and are being pursued.

The structural diversity of O antigens in clinical isolates of *K. pneumoniae* is relatively limited, compared to K antigens and our research group is interested in the molecular basis of O-antigen diversity in this species. Several O antigens belong to serogroup O2, and are based on decoration of a backbone with a disaccharide repeat unit designated the O2α (formerly O2a) antigen: [→3)-β-D-Gal*f*-(1→3)-α-D-Gal*p*-(1→]

Modifications to this backbone may influence its antigenicity and affect epitopes recognized by therapeutic antibodies, so the structures need to be considered in immune-based therapies. The enzymology of O2α-biosynthesis and the addition of sugar-based modifications is well understood, but the process for O-acetylation to create the O2γ antigen is not. Prior structural characterization of O-acetylated O2 subtypes indicated non-stochiometric modifications of ~40% of Gal*f* residues divided almost equally between positions 2 and 6.

We have identified the gene (*orf8*) responsible for O-acetylation, located adjacent to the chromosomal genetic locus for the O2α antigen. The DNA sequence suggests an acetyl-CoA-dependent membrane-embedded O-acetyltransferase. Coexpression of compatible plasmids carrying *orf8* and the O2α locus in an *E. coli* K-12 host resulted in a significant change in the physical properties of the lipopolysaccharide molecules and their behaviour in conventional hot aqueous-phenol extraction. While O2α-containing LPS molecules partition predominantly in the aqueous phase of extracts, LPS from cells expressing both plasmids was almost exclusively contained from the phenol phase and most LPS molecules in the aqueous phase lacked O antigen.

These observations highlight a need to consider both phases in hot aqueous-phenol extracts in O-antigen structural characterization. They create uncertainty about the original analyses of O-acetylated O2 subtypes, which were obtained from the aqueous phase of extracts. potentially those results reflect an under-O-acetylated portion of the total LPS population. Alternatively, the higher gene dosage of orf8 in the recombinant strain may drive levels of O acetylation not observed in wildtype bacteria. Reinvestigation of these structures is in progress.

IN-SILICO DESIGN OF GLFT2 INHIBTORS USING SCAFFOLD HOPPING, VIRTUAL SCREENING AND BINDING POSE METADYNAMICS

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Galactofuranosyltransferase (GlfT2) is an essential enzyme that transfers galactofuranose (Galf) subunits from donor UDP onto the acceptor galactan chain during *Mycobacterium tuberculosis* cell wall assembly. As tuberculosis to be a major global health threat, with 1.25 million fatalities reported in 2023, targeting GlfT2 presents a promising strategy for the development of new therapeutic agents. In this study, we employed two distinct drug design approaches to identify potential inhibitors of GlfT2. First, we utilized the transition state of UDP-Galf from a previous QM/MM study [1] to design transition state mimetics using iterative R-group Enumeration, Bio-isostere expansion and core hopping. Second, we performed virtual screening of the Asinex database to identify lead compounds against GlfT2's active site. Our hierarchical approach started with initial filtration based on molecule weight < 1000 da and number of rotatable < 10 followed by high throughput virtual screening and molecular docking. This process led to the identification of several lead candidates with better binding affinities than UDP-Galf. With the subsequent geometry optimization using B3LYP-D3 and 6-31G+* basis sets, re-docking and Binding pose metadynamics, we were able to show six compounds with comparable stability to UDP-Galf across the metadynamics simulations. These compounds exhibited PoseScore values < 2.0 and PerScore values > 0.4, a threshold often attributed to X-ray crystalized poses [2]. Principal component analysis indicated that the proposed compounds are well within the chemical space of FDA approved drugs. Taken together, our results provide a strong rationale for the experimental testing of these compounds to validate their potential as GlfT2 inhibitors.

Acknowledgements: This work was financially supported by APVV-20-0230, APVV-21-0346 grants.

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FLUORINATED METHYL β-LACTOSIDES: SYNTHESIS AND AFFINITY TO GALECTINS

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Human galectins (hGals) are a family of β-galactoside-binding lectins that play a key role in regulating biological processes linked to cancer progression [1]. Given their involvement in these pathways, there is a growing interest in the development of selective galectin inhibitors. However, this effort is complicated by the fact that there are 12 hGals with similar substrate specificities. A deeper understanding of the structural and functional differences among individual hGals is essential for the design of more selective inhibitors [2].
Deoxyfluorinated carbohydrates, in which at least one hydroxyl group is replaced by fluorine, effectively mimic natural carbohydrate ligands [3]. The systematic substitution of hydroxyl groups with fluorine in galectin ligands allows us to evaluate the role of each hydroxyl in galectin binding and to identify subtle differences between individual galectins. In addition, ^{19}F NMR spectroscopy provides insight into the molecular mechanisms of galectin-ligand interactions [4].
This study focuses on the synthesis of a complete series of mono-deoxyfluorinated methyl β-lactosides (**1**–**7**) and their binding affinity towards selected hGals, assessed using an ELISA assay. Furthermore, the enzymatic glycosylation of the fluorinated β-lactosides has been explored. The synthetic approach involves the use of monosaccharide building blocks, which undergo glycosylation and subsequent deprotection to afford the final compounds **1**–**7**.

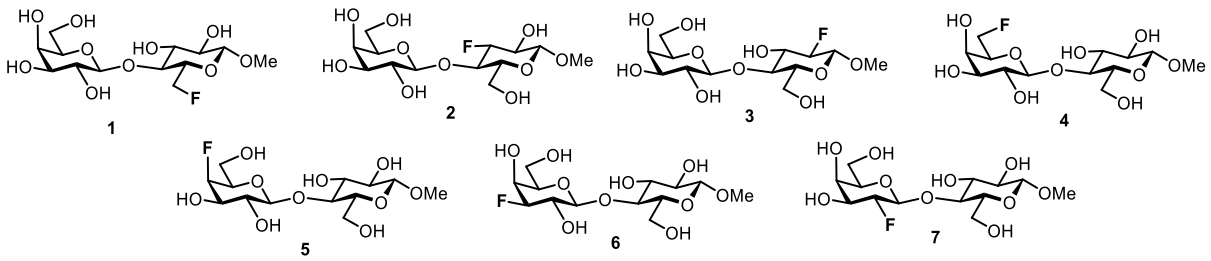

Figure 1. Structures of fluorinated methyl β-lactosides.

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CHEMICAL SYNTHESIS OF OLIGOSACCHARIDES RELATED TO PLANT RHAMNOGALACTURONAN-I

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The plant cell wall is the outer fibre composite layer which plays an important role in providing support to cells as well as in microbe interactions, such as the defence response against pathogens,which is a complex network to a large part composed of structural polysaccharides such as cellulose, hemicellulose and pectin.[1] Pectin is comprised of the domains homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II). The RG-I backbone is made up of alternating units of (1→2)-α-L-rhamnopyranose and (1→4)-α-D-galactopyranuronic acid, substituted with various L-arabinan, D-galactan, and arabinogalactan side chains.[2] Oligosaccharide fragments of RG-I represent important research tools for studying RG-I biosynthesis as well as its potential involvement in plant immune responses such as Danger Associated Molecular Patterns (DAMPs).
Towards the chemical synthesis of RG-I oligosaccharides, we employ a post-assembly-oxidation strategy, transforming the galactose residues to the corresponding galacturonic acids.[3] The backbone is constructed using thioglycoside donors activated with NIS and TMSOTf. Fluorenylmethoxycarbonyl (Fmoc) serves as a temporary protecting group for chain elongation, and benzoyl (Bz) protecting groups at C-6 of the galactose units provide remote participation to facilitate α-selective glycosylations before they are selectively removed and oxidized after backbone assembly.[4] The aim of the experiment is to synthesis RG-I fragments in varying length with a goal of probing plant immune response.

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D-MANNURONIC ACID CONTAINING OLIGOSACCHARIDES: SYNTHESIS AND BIOLOGICAL ACTIVITIES

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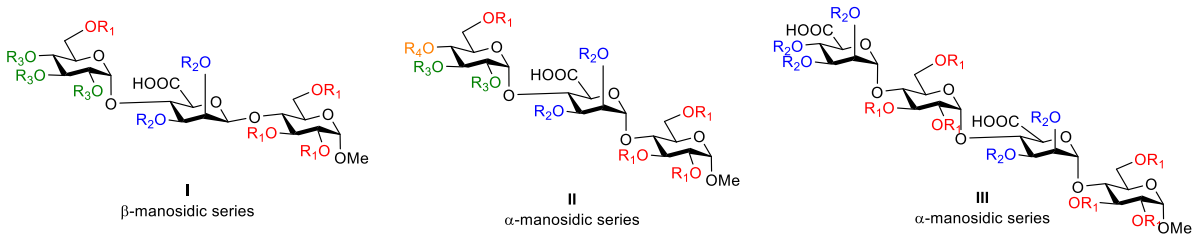
In the nature, the negatively charged oligo- and polysaccharides are playing an important role. One of their main classes is glycosaminoglycans (GAGs), e.g.: heparin, heparan sulphate. GAGs are built from repeating disaccharide units, have high molecular weight and are linear in structure. In addition to anticoagulant effects, they also have several other effects, including inhibiting cell growth [1]. Alginates are another significant group of compounds of this type, which also include linear anionic polysaccharides composed of 1,4-linked β-D-mannuronic acid and α-L-guluronic acid alternately or randomly [2]. These compounds are found in cell wall, on the cell-surfaces or in the extracellular matrix. Alginates also have a number of biological effects, including antioxidant and anti-inflammatory activity.
Our research group has been engaged in the synthesis of oligosaccharides for decades, and for several years we have been investigating the possibility of producing new, synthetic anticoagulant pentasaccharides. Based on our previous results, we managed to produce three heparin-analogue trisaccharide derivatives that selectively inhibited the proliferation of tumour cells without any harmful effects on healthy cells [3,4].
With this knowledge and results, tri- and tetrasaccharides were synthesized, which are built up alternately from D-glucose and D-mannuronic acid units (Figure 1.). We formed acetyl-, methyl-, -OH groups in addition to sulphate-ester groups in different patterns, examining the effect of these patterns, the type of uronic acids and the number of units on antioxidant, anti-inflammatory and cell growth inhibiting effects.



R_1 = H or SO_3Na; R_2 = Ac or H; R_3 = SO_3Na or Me or H; R_4= H or Ac or Me

Figure 1. The structure of the synthesized heparan sulphate analogue derivatives

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FROM SYNTHESIS TO SCREENING: A PARALLEL APPROACH TO PRECISION GLYCOOLIGOMERS FOR IMMUNE LECTIN TARGETING

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Inspired by the precision and control of natural macromolecules such as DNA, saccharides, and proteins, we have applied the well-established technique of Solid Phase Peptide Synthesis (SPPS), originally developed by Bruce Merrifield, along with a growing toolbox of synthetic amino acids to synthesize precision oligomers [1,2]. These oligomers serve as scaffolds for the precise presentation of carbohydrate motifs in regard to valency and spatial arrangement [2].
Our research focuses on synthesizing a library of glycooligomers via SPPS to study the interaction of carbohydrate motifs on these precise scaffolds with lectins of the immune system. We employ 96-well plates for synthesis, drastically enhancing efficiency through parallel coupling, washing, and deprotection steps (see Figure 1).

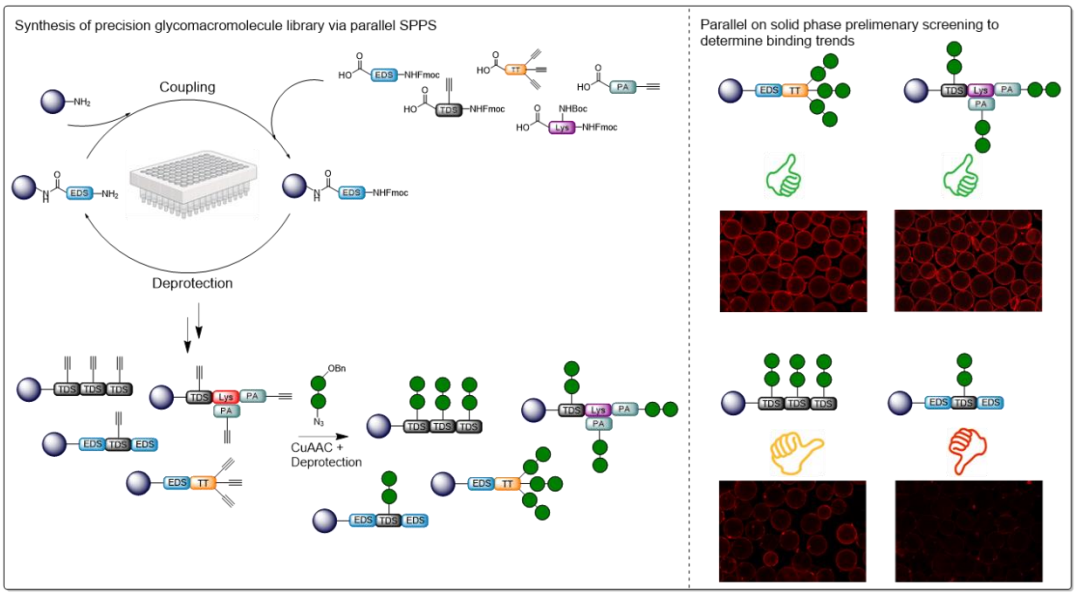

Figure 1. Parallel SPPS to obtain library of precision glycooligomers (left) and prelimenary on-resin screening of library (right).

Additionally, we have developed a rapid on-resin fluorescent screening method, allowing preliminary binding analysis before resin cleavage (see Figure 1). This innovative approach minimizes labor-intensive steps, streamlining the identification of promising glycooligomer candidates for detailed binding assays e.g., with surface plasmon resonance. Currently, we are validating this screening method using the model lectin Concanavalin A (Con-A) to ensure its consistency with traditional off-bead assays. Following validation, we will target lectins involved in HIV infection, specifically aiming for selective binding to langerin over DC-SIGN [3].

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ASSAY DEVELOPMENT AND ACTIVITY SCREENING OF INHIBITORS OF SIALYL- AND FUCOSYLTRANSFERASES

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Glycosyltransferases are a class of enzymes that are responsible for building a cell's glycans. They transfer a glycosyl donor (typically a nucleoside mono- or diphosphate sugar) onto a glycosyl acceptor (typically a specific hydroxy group on a mono- or oligosaccharide being extended) [1].
During tumor development, preneoplastic cells undergo various cellular changes, including alterations in glycosylation, which are significant in malignant processes and are associated with oncogenic transformation. These glycosylation changes, which include biomarkers used for cancer diagnosis such as CA125, CA19-9, and CA72-4 play a functional role in tumor progression and affect processes like invasion, angiogenesis, metastasis and therapy response. Specifically, changes such as increased sialylation, overexpression of sialylated Lewis antigens, and unique expression of O-truncated glycans, are catalyzed by glycosyltransferases (GTs) like fucosyltransferases (FTs) and sialyltransferases (STs), which are key in the biosynthesis of cancer-associated glycoforms [2].
In this project, we will identify novel compounds as leads for inhibiting glycosyl transferases, such as FTs and STs. We will also develop a fluorescence-polarization (FP) based assay for GT binding by putative inhibitors.

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ANALYSIS OF THE KDO BIOSYNTHETIC CLUSTER IN CroV

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Cafeteria roenbergensis virus (CroV) is a double-stranded DNA giant virus (692 kb) that infects the marine unicellular heterotrophic nanoflagellate *C. burkhardae* and it is the first giant virus reported to infect zooplankton[1]. Viruses should be seen as integral components of any ecosystem where they contribute to the maintenance of the balance between species and resources. Indeed, bacterivorous nanoflagellates such as *C. burkhardae*, make up a significant portion of the ocean's protozoan communities. Unlike mammalian viruses (e.g. HIV-1, SARS-CoV-2), which exploit host-encoded enzymes to build glycans that echo those of the host, most giant viruses encode their own enzymes with the result that their polysaccharides differ from those produced by their hosts[2]. Gene annotation suggests that CroV encodes a partial Kdo biosynthetic pathway (Fig. 1); each of the three viral proteins, crov265, crov266, crov267, presents a dual activity that in some cases (C-termini of crov266 and crov267) seem not to be related to CMP-Kdo production (Fig. 1). This study aims to provide new insights into the CroV Kdo biosynthetic pathway by analysing the features of the encoded proteins by bioinformatic analysis.

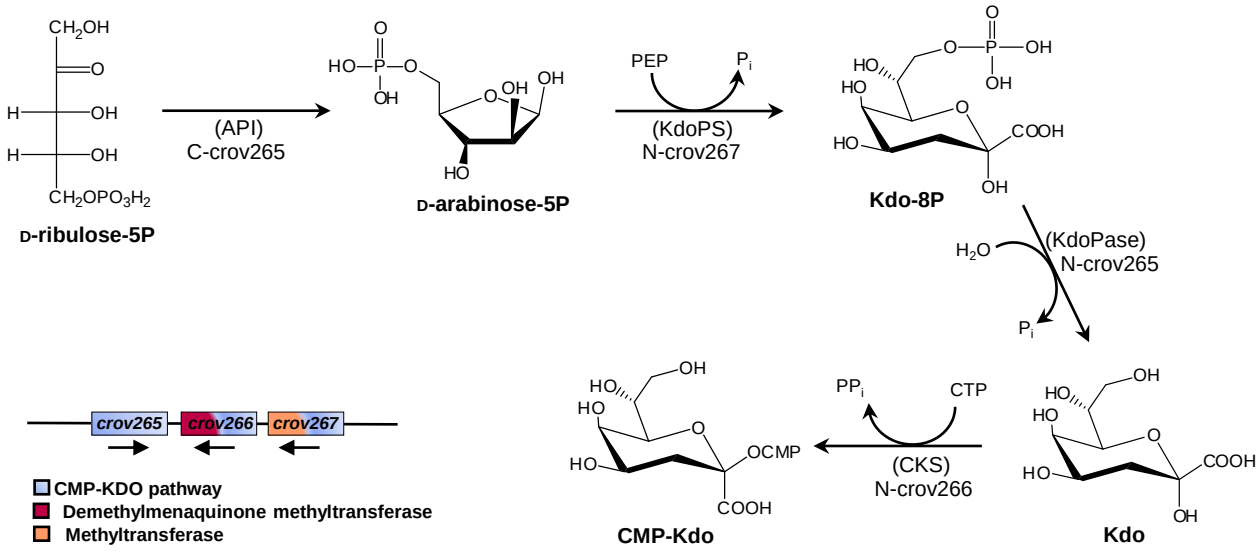

Figure 1. Putative Kdo biosynthetic pathway in CroV.

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INVASTIGATION OF THE REACTION MECHANISM OF A NOVEL GH115 α-GLUCURONIDASE FROM *FLAVOBACTERIACEAE*.

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Xylan is a major component of the complex network of the plant cell wall. It consists of xylose units connected by β-(1,4) glycosidic bonds, with α-D-glucuronic acid (GlcAp) or 4-O-methyl GlcAp (MeGlcAp) commonly substituting at the O-2 position. The GH115 family includes α-glucuronidases that can remove this substituent.
FAgu115A, a novel α-glucuronidase from the unclassified Flavobacteriaceae bacterium strain 3519-10, is encoded within a polysaccharide utilization locus (PUL) for xylan degradation. FAgu115A, exhibits activity on both polymeric and oligomeric xylan. Key catalytic residues have been identified, and Molecular Dynamics simulations suggest that water is responsible for the nucleophilic attack. Additionally, the C-terminal domain determines the dimeric status of the protein, ensuring its activity and polymeric substrate binding, through the presence of a critical tryptophan residues.

SYNTHESIS OF *M. ABSCESSUS* GLYCOPEPTIDOLIPIDS AND THEIR EVALUATION OF BIOACTIVITY

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Glycopeptidolipids (GPLs) isolated from the smooth (*S*)-morphotype of *M. abscessus* (*Mab*) are reported to inhibit macrophage apoptosis by binding to mitochondrial Cyclophilin D protein [1]. The reduced amount or complete absence of these compounds in rough-morphotype *Mab* is linked to the more proapoptotic activity and high virulence of the strain compared to the smooth morphotype [2–6]. These *Mab* GPLs generally contain a 24–33-carbon lipidated tetrapeptide core of D-Phe-D-*allo*-Thr-D-Ala-L-alaninol sequence, where the lipopeptide is glycosylated at the D-*allo*-Thr and L-alaninol moieties with 6-deoxy-L-talose and L-rhamnose, respectively [5–6]. Modification such as acylation, methylation, or further glycosylation at the sugar units have also been reported. With these diverse structures, the exact GPL sequence responsible for the reported bioactivity has yet to be identified. Thus, we chemically synthesized eight GPLs that mimic those found in *S*-type *Mab*. We then used the synthesized compounds to study their macrophage apoptotic activity and their possible role in *Mab* virulence. We report herein our chemical synthetic route for these eight GPL mimics and our findings on how these synthesized GPLs interact with macrophage cells compared to what was reported in the literature.

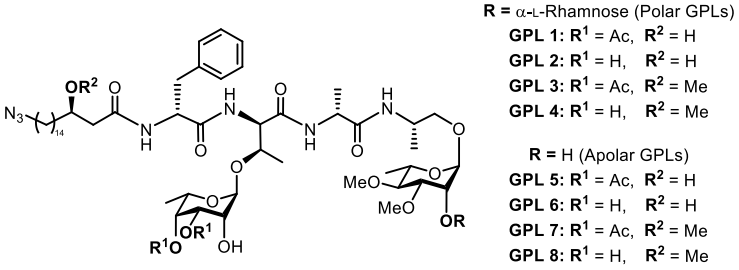

Figure 1. Structure of the target *Mycobacterium abscessus* glycopeptidolipids.

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EVALUATION OF REMOTE GROUP PARTICIPATION EFFECT IN 2-AZIDO-2-DEOXY-GLUCOSIDE DONORS WITH ACYL GROUPS

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Carbohydrates, presented on the cell surface, play an important role in cell-cell communication, and pathogen infection. Obtaining carbohydrate molecules is paramount to investigating the interaction between carbohydrate molecules and proteins. However, those glycans are heterogeneous and usually hard to isolate from natural sources. Therefore, chemical synthesis is a promising way to obtain those molecules while building the glycosylic bond via glycosylation reaction needs to face the challenges of stereoselectivities. Recently, remote participation, similar to the neighboring group effect, has drawn more attention. During the activation, the acyl group was suspected to interact with the anomeric position and form the dioxolenium ion (figure 1), which can enhance the stereoselectivity by constraining the direction of acceptor attack. However, the existence of this effect has been debated in the literature for more than two decades.

Recently, our group has built a GlycoComputer system that introduces two indicators, RRV and Aka, for the reactivity quantitation of both the donors and acceptors respectively [1]. The stereoselectivities of the glycosylation reactions can be predicted in a precise manner. Numerous environmental effects, such as solvent, promoter, and temperature, can be effectively analyzed. Herein, we synthesized a series of 2-azido-2-deoxy-1-thioglucoside donors with an acetyl (Ac) or benzoyl (Bz) group at positions 3, 4, or 6 and carried out their glycosylation reactions with four different acceptors under the NIS/TfOH promotor system. The stereoselectivity of glycosylation was studied systematically, and by comparing these results with our GlycoComputer platform, we confirmed the presence of this long-range neighboring group participation effect in 2-azido-2-deoxy-1-thioglucoside donors, and Bz shows a higher participation effect than Ac. Therefore, among all the donors, *p*-tolyl 2-azido-3,6-di-O-benezoyl-4-O-benzyl-2-deoxy-1-thio-β-D-glucopyranoside provided the highest α-selectivity.

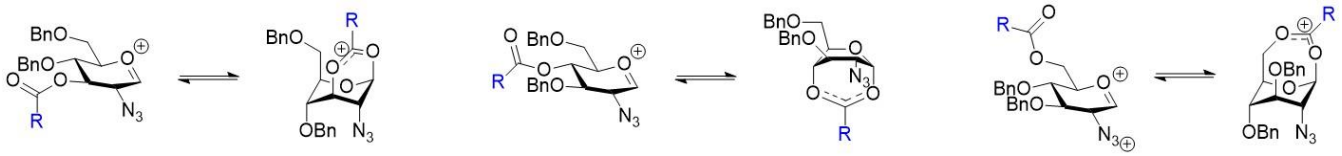

Figure 1. Possible dioxolenium ion intermediates of $GlcN_3$

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DIFFERENTIAL METHYLATION OF VIRAL CAPSID N-GLYCANS IN CHLOROVIRUSES, MT325 AND FR483

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N-glycosylation is an essential post-translational protein modification in all forms of life including viruses. Chloroviruses, however, exhibit a unique glycosylation pattern with a distinct N-glycan core structure not found in other species. Diversity among chloroviruses, occurs due to differences in methylation and non-stereoisomeric substituents attached to the core glycans. In this study, we reported the differential modifications of N-glycans from two strains of chloroviruses, MT325 and FR483. NMR analysis of the capsid N-glycans of both viruses showed the presence of the core glycan structure containing an N-linked β-glucose, a hyperbranched fucose, a proximal D-xylose and a terminal D-galactose. NMR spectra of MT325 also showed methylation at the α-rhamnose linked to the core fucose residue via α-1,3 linkage. However, this modification was not found in FR483. The different glycoforms can be attributed to the virus-encoded glycosyltransferases.

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FUNCTIONAL CHARACTERIZATION OF BACTERIAL GALACTOSIDASES AND EXPLORATION OF THEIR SYNERGISTIC EFFECT

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Glycoside hydrolases (GHs) constitute a diverse family of enzymes that catalyse the hydrolysis of glycosidic bonds in carbohydrates. Under certain conditions, the hydrolytic water molecule can be replaced by a saccharide acceptor, facilitating the formation of a new glycosidic bond. Galactose is a highly abundant monosaccharide in nature, and its derivatives, galactosides, hold pivotal roles across biological kingdoms and hold significant industrial relevance. Consequently, galactosidases, the enzymes that hydrolyse or form galactosides, are of significant interest.

In our comprehensive genomic analysis of a representative bacterial strain, we identified a plethora of galactosidases, each potentially exhibiting unique or synergistic functions. Notably, we aim to investigate a conserved dual-domain enzyme comprising GH36 (α-galactosidase) and GH173 (β-galactosidase) domains, frequently located within polysaccharide utilization loci (PULs) across various bacterial taxa. Additionally, our genomic exploration was extended to multiple pathogenic bacterial strains, predominantly respiratory pathogens, and revealed the presence of numerous retaining α- and β-galactosidases, including yet another dual GH domain enzyme.

Our research endeavours to elucidate the synergistic interactions among these galactosidases by expressing them and characterizing their substrate specificities against a comprehensive panel of substrates, both commercially available and synthesized in-house. The overarching objective is to uncover the functional significance of these galactosidases, identify novel enzymes for diverse applications, perform comparative analyses of their enzymatic activities and substrate specificities as well as their capacity for regioselective glycan synthesis.

INVESTIGATION OF PL24, PL28 AND PL37 ULVAN LYASES ANNOTATED IN *WENYINGZHUANGIA FUCANILYTICA* GENOME

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Green macroalgae are found globally and thrive in eutrophic water. Their abundance makes them a valuable source of polysaccharides, with ulvan being the most abundant polysaccharide in green macroalgae. Ulvan is a sulphated polysaccharide which possesses promising applications for food, biofuels and demonstrating bioactive properties. Enzymatic degradation [Figure] of ulvan using polysaccharide lyases is an effective method to generate oligosaccharides while still preserving the intricate bioactive structure. Enzymes acting as ulvan lyases have been identified in families PL24, PL25, PL28, PL37 and PL40, but have just been sporadically investigated, making it important to further investigate putative members of these families.

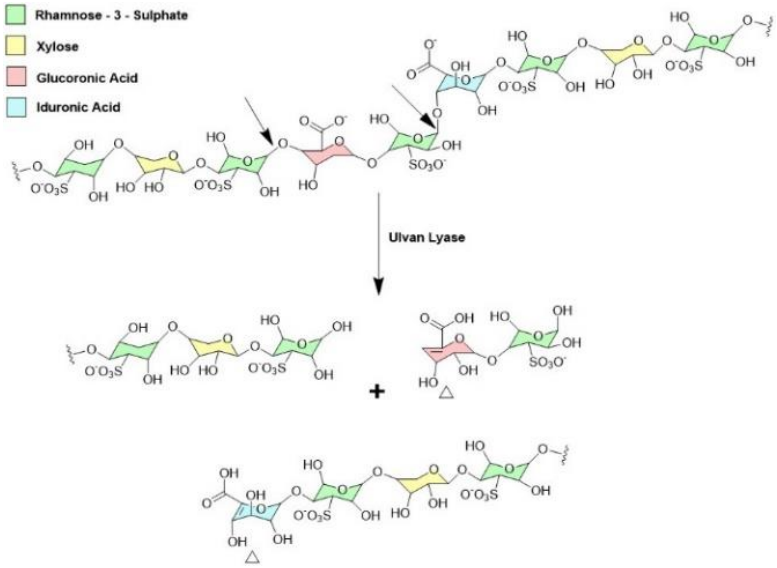

Figure 1. The currently known mechanism of ulvan lyases, which cleaves the ulvan between rhamnose-3-sulphate linked to either glucuronic acid or iduronic acid [1].

To address this issue, putative ulvan polysaccharide lyases from families PL24, PL28 and PL37 were selected from *Wenyingzhuangia fucanilytica* CZ1127 through genome mining. PL40 enzymes were already mined from the same genome. Bioinformatic analysis was performed to determine the evolutionary relationship between the investigated ulvan lyases from different families and the genomic organization of the genes. These enzymes were cloned and heterologous overexpressed in *Escherichia coli* and through affinity chromatography. The enzymes activity was evaluated using different ulvans as the substrate and was determined spectrophotometrically and products were visualized using thin layer chromatography.

Acknowledgements: *H2022 SeaMark (Seaweed-based market applications) project (grant no. 101060379).*

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PHYSICOCHEMICAL PROPERTIES AND SUITABILITY OF CARROT ROOT POMACE PECTINS AS BUILDING MATERIALS FOR ORAL DELIVERY SYSTEMS

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A comprehensive approach to the valorization of agrowastes, such as peels, pulp, roots, shells, and leaves, focuses on utilizing these materials as sources of industrially valuable compounds. The aim of the study was to verify whether pectins extracted from carrot root pomace using natural deep eutectic solvents (NADES), optimized according to the Box-Behnken design, are suitable materials for the construction of oral delivery systems for biologically active substances. The physicochemical properties of pectins were analyzed under varying conditions of pH, ionic strength, temperature, and the presence of polyvalent ions. The viscosity and gelling capacity of the pectins were determined *in situ* using a rotational viscometer, while the zeta potential was measured by means of the electrophoretic light scattering (ELS) technique. The behavior of pectins in a four-stage gastrointestinal model (saliva, small intestinal fluid, and large intestinal fluid, with regard to pH, ions, enzymes, bile salts, temperature and residence time) was monitored using ATR-FTIR and UV-Vis spectroscopy techniques. Finally, the pectins were used as building materials for hydrogel microcarriers. The impact of pectins on the morphology (size and shape) and structural integrity of the carrier system was assessed using scanning electron microscopy (SEM) and optical microscopy. The loading capacity of the microcarriers was determined by UV-vis spectroscopy. The physical stability and release behavior of the pectin-hydrogel microcarriers were evaluated in an *in vitro* human gastrointestinal tract.

Acknowledgments: The work was supported by the National Science Centre, Poland, under the research project „ Processes of obtaining polyanionic saccharides from native agricultural waste, including Ohmic heating-assisted extraction in environmentally safe solvents.”, no UMO-2023/51/B/ST8/02851

THE POWER OF NMR AND MD IN THE ANALYSIS OF MOLECULAR RECOGNITION EVENTS

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Protein-glycan interactions play pivotal roles in numerous biological processes, ranging from cellular recognition to immune response modulation. Understanding the intricate details of these interactions is crucial for deciphering the molecular mechanisms underlying various physiological and pathological conditions.
In the last years, by the means of a combined and integrated approach including synthetic, spectroscopic, biophysical and computational methods, we dissected the molecular basis of the binding between host proteins, including Siglecs [1] and C-type lectins such as DC-SIGN [2], and both endogenous and exogenous glycans (Figure 1).
In addition, we unveiled the recognition of complex glycans, typically exposed on host cells' surface, by microbial receptor proteins, such as streptococcal Siglec-like adhesins [3], mycoplasma cytoahesins [4] (Figure 1) and other bacterial lectins.
Finally, we investigated the action of selected monoclonal antibodies against multidrug-resistant strains of *Neisseria gonorrhoeae* [5] and *Staphylococcus aureus* [6] (Figure 1).
Overall, our outcomes contributed to the fields of structural glycobiology and molecular recognition studies.

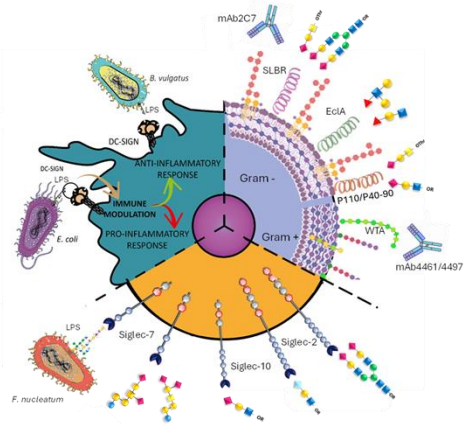

Figure 1. Schematic representation of studied protein-glycan interactions.

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MODULAR GLYCONANOMATERIALS FOR BIOMEDICAL APPLICATIONS

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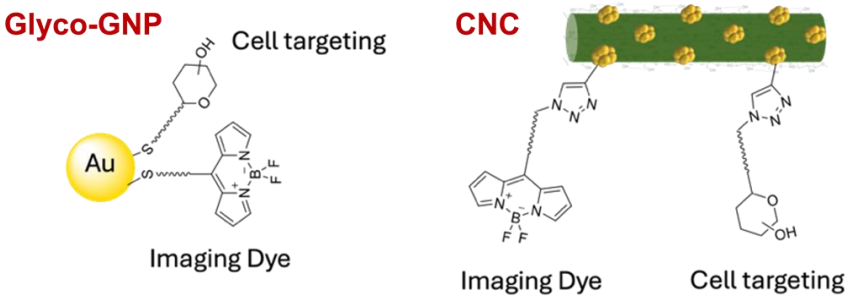
Multifunctional nanoplatforms that present in a multivalent way different active components have emerged as appealing candidates for solving biological problems. Size and shape control, water dispersibility, colloidal stability, controlled surface functionalization with multiple (bio)active molecules, multivalency, and batch-to-batch reproducibility, are among the key challenges to be addressed toward the construction of advanced nanomaterials for biomedical applications.
In this contribution, we show the progresses of our research on the development of easily programmable nanoplatforms, *i.e.* glyco-gold nanoparticles (glyco-GNPs) [1] and glyco-GNPs embedded in submicrometric cellulose nanocrystals (CNC) [2]. The ability to fine tune the surface of these glyconanomaterials by loading structurally different (bio)active headgroups under strictly controlled reaction conditions will be presented. In particular, the presence of sugar heads ensures cell homing ability thus allowing to employ our glyconanomaterials as a new generation of precision delivery systems and chemical tools to intervene in carbohydrate-mediated interactions. Furthermore, the incorporation of fluorescent dyes allows to track their intracellular fate as a function of the nanomaterial size and degree of functionalization.



Our results foreshadow a substantial step forward in the design of precision tools for medical intervention in the field of vaccination and cancer treatment.

Acknowledgements: Financial support under the National Recovery and Resilience Plan (NRRP), Mission 4, Comp. 2, Inv. 1.1, Call for tender No. 104 publ. 2.2.2022 by the Italian Ministry of University and Research (MUR), funded by the European Union – NextGenerationEU – Project 20224LLK82 – GLYCONANOVACS – CUP B53D23015080006 – Grant Assignment Decree No. 1064 adopted on 18/07/2023 by the MUR.

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TOWARDS DEVELOPING BACTERIAL ADHESION INHIBITORS USING ARYLAMIDE GLYCOFOLDAMERS

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The WHO has placed antimicrobial resistance among the top ten global public health threats [1]. Antivirulence agents promise to circumvent resistance by disarming the pathogen as opposed to affecting growth or viability [2]. A common strategy consists in interfering with adhesion, which is frequently mediated by proteins that bind multiple carbohydrates displayed on the host cell surface [3]. To inhibit such interactions, many molecular scaffolds have been devised for the multivalent presentation of carbohydrates [4]. However, few allow a precise control of number, orientation, and distance between the sugars, which is fundamental to maximize biological activity.

Arylamide foldamers are bioinspired synthetic oligomers that, like peptides or nucleic acids, fold into well-defined conformations [5]. They feature key properties which make them ideal to build materials for multivalent presentation of carbohydrates: they adopt stable helical conformations in solution, whose predictability, tunability, and ease of synthesis render them particularly suitable to allow precise control of number, nature, and orientation of carbohydrate ligands. In addition, they can feature proteinogenic side chains to mimic protein surfaces.

Herein we describe our current efforts towards developing arylamide glycofoldamers as mimics of naturally occurring glycoproteins that decorate host cell surfaces, aiming to competitively interfere with the recognition processes that takes place between host cells and pathogens.

Acknowledgements: This work is supported by FCT - Fundação para a Ciência e a Tecnologia, I.P., through MOSTMICRO-ITQB R&D Unit (UIDB/04612/2020, UIDP/04612/2020), LS4FUTURE Associated Laboratory (LA/P/0087/2020), UCIBIO research unit (UIDB/04378/2020), i4HB Associated Laboratory (LA/P/0140/2020) and project 2022.03561.PTDC. The latter supported A.F., L.M. and M.S.. P.M. acknowledges FCT for research contract 2021.02532.CEECIND. The National NMR Facility is supported by CERMAX through Project 022162.

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CHEMICAL SYNTHESIS OF LACTO-*N*-HEXAOSE (LNH) AND LACTO-*N*-NEOHEXAOSE (LNnH)

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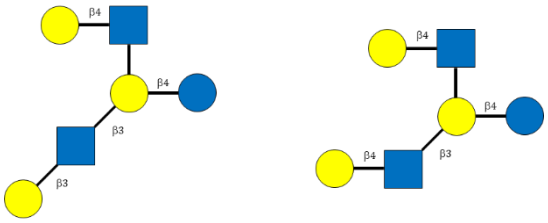
The chemical synthesis of the branched human milk oligosaccharides Lacto-N-hexaose (LNH) and Lacto-*N*-neohexaose (LNnH) is described [1,2]. All donor and acceptor building blocks are crystalline and were obtained by synthetic routes without any chromatographic purification steps [3]. Additionally, the discovery of crystalline tetra- and hexasaccharide intermediates further minimized the use of tedious purification steps. The reported synthetic routes give access to LNH and LNnH with high purity and in large quantities. LNH, LNnH and their fucosylated and/or sialylated derivatives are highly abundant in human milk and of interest for biological studies [4].



LNH LNnH

Figure 1. Lacto-*N*-hexaose (LNH) and Lacto-*N*-neohexaose (LNnH).

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COMPARISON OF BOX-BEHNKEN AND I-OPTIMAL MODELS IN OPTIMIZATION OF NADES-BASED ULTRASOUND-ASSISTED EXTRACTION OF PECTINS FROM CARROT ROOT POMACE

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Pectins, in addition to cellulose and hemicelluloses, are key elements of plant cell walls [1]. Plant biomass, which is waste from juice production, is a good raw material for obtaining pectins with different architecture, and consequently with different physicochemical properties. The approach based on the management of this type of food industry waste, in order to obtain products with high market value, fits well with the principles of sustainable development and circular economy. On an industrial scale, pectins are extracted in high temperature conditions, in an environment of highly corrosive mineral acids [2].
The research work presents the effectiveness of using an extraction medium based on compounds of natural origin, able to form natural deep eutectic solvents (NADES), i.e. choline chloride, glucose, and citric acid, in various proportions, to obtain pectins from carrot root pomace. In order to increase the efficiency of the extraction process, ultrasounds as a physical supporting factor, facilitating the transport of mass from the raw material particles to the extraction medium, were used [3].
In order to optimize the extraction process parameters, which would provide a satisfactory degree of fit of the mathematical model to the actual experimental results, two different design of experiment (DoE) models were used, i.e. Box-Behnken design and I-optimal design. The independent variables in the experimental matrix were components of NADES in different proportions, and the dependent variable was the extraction efficiency, maximizing the total amount of polysaccharides, especially those rich in uronic acids. The results of the obtained predictions were analysed using the response surface methodology (RSM). The pectin-rich products obtained under optimal extraction conditions were analysed by GC-MS for their saccharide composition in the form of alditol acetates. These analyses provided insight into the structure of the obtained products, although further detailed studies are necessary to be able to propose the chemical structure of these polysaccharides.

Acknowledgements: The work was supported by the National Science Centre, Poland, under the research project „ Processes of obtaining polyanionic saccharides from native agricultural waste, including Ohmic heating-assisted extraction in environmentally safe solvents.”, no UMO-2023/51/B/ST8/02851

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ACCEPTOR-ADAPTIVE AUTOMATED GLYCOSYLATION OPTIMIZATION FOR AUTOMATED GLYCAN ASSEMBLY

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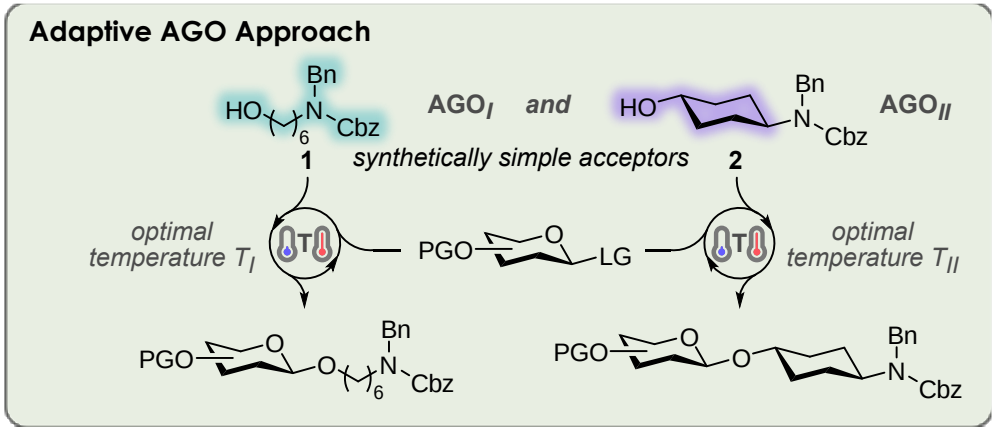
Carbohydrates form some of the most important biopolymers on earth. The study and implementation of carbohydrates is therefore invaluable for biological and biotechnological studies. Obtaining pure carbohydrates from natural sources is time-consuming, costly and inefficient, resulting in impure heterogeneous mixtures. Synthetic assembly of oligosaccharides offers an alternative for the procurement of pure homogenous carbohydrates. Advances in synthetic approaches have led to the implementation of automated solid-phase glycan assembly (AGA). This technique relies on the precise and iterative addition of each monomeric building block. The strategy highly benefits from optimized glycosylation conditions. A reliable optimization system (AGO) is thereby extremely valuable for AGA [1]. Such optimization must mimic key reaction components to increase atom economy, reproducibility and efficiency. While ideal in theory, the use of real glycosyl acceptors for optimization studies is impractical. Simple model acceptors can surrogate real glycosyl acceptors for optimization as long as they retain key structural features. We developed an automatic screening of glycosylation conditions at different temperatures on the same platform on which the solid phase assembly takes place. this ensures an efficient translation to AGA. Here we show that while using only *n*-alkyl acceptor provides rough optimization trends, co-optimization with a 4-aminocyclohexanol acceptor mimics the optimized glycosylation conditions for glycosyl acceptors more reliably. Implementation of the acceptor-derived optimization principles enabled precise evaluation of glycosylation conditions which were validated by AGA for several oligosaccharides. Adoption of this optimization process would increase the predictability of AGA [2].



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SYNTHESIS OF MORPHOLINE AND OXAZEPANE TYPE IMINOSUGAR ANALOGS

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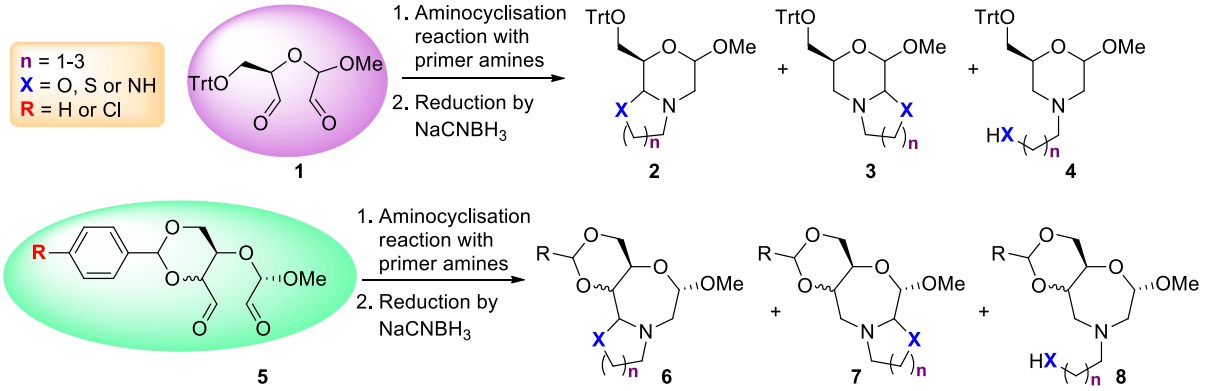
Among the compounds related to carbohydrates, iminosugars play a prominent role [1,2]. Since their discovery, several advantageous properties have been revealed. Among others their effectiveness has been proven in the treatment of many diseases, including antiviral infections, metastatic processes but some of them has antidiabetic effect too [3]. It was proven as well that their ability has also been linked to their glycosidase inhibitory effects. Thus, glycosidase enzymes as regulators of carbohydrate metabolism can become potential targets for the treatment of these diseases [4].
The nitrogen atom of iminosugars in the ring plays a significant role in the development of the glycosidase inhibitory effect, because through its protonation it can form a very similar structure to the transition state of carbohydrates, the oxocarbenium ion, and thus bind to the active site of the enzyme [3].
Our goal is the synthesis of a new family of iminosugar type molecules, which can be potential glycosidase enzyme inhibitors in the future. For the preparation of these monocyclic and condensed bicycle oxazepane and morpholine iminosugar derivatives, the reaction route is short and cost-efficient (Figure 1.).



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EXPANDING THE SCOPE OF A ONE-POT DOUBLE DISPLACEMENT PROTOCOL TO ACCESS THE ALL-RARE-SUGAR-CONTAINING TRISACCHARIDE UNIT OF *PSEUDOMONAS STUTZERI* OX1

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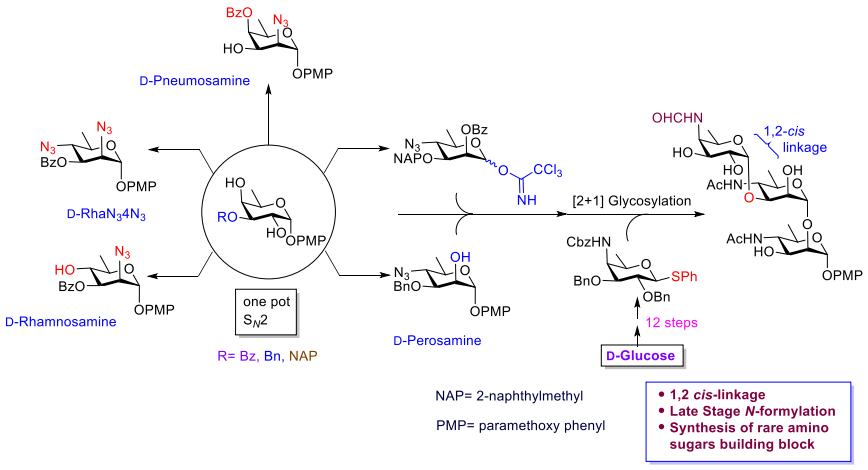
Bacterial glycoproteins and oligosaccharides contain several rare deoxy amino sugars virtually absent in human cells [1]. This structural difference between the bacterial and host cell surface glycans can be exploited to develop carbohydrate-based vaccines and target specific drugs [2]. Herein, we extend strategic use of one-pot bis-triflation and double serial displacement protocol [3] on D-fucose to access various less explored 6-deoxy rare amino D-sugars i.e. D-perosamine, 2,4-diazido-2,4,6-trideoxy-D-mannose, D-pneumosamine, and D-rhamonsamine. All these azide-bearing rare sugars offer a path to access new bacterial glycoproteins, target specific drugs, and can be used as metabolic incorporation of glycans [4]. This strategy applies to the first total synthesis of the trisaccharide unit of *Pseudomonas stutzeri* OX1 strain [5] containing rare sugar, D-perosamine [6]. Installation of 1,2 *cis*-linkage and late-stage *N*-formylation are the key challenges in total synthesis, accomplished via longest linear sequence of 21 steps with 1.4% overall yield.



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CONFORMATIONAL DISTRIBUTION AND DYNAMIC RANGE OF LINKAGE-EDITED PSEUDO-ISOMALTOSES WITH QM/MM-MD SIMULATIONS AND DFT CALCULATIONS

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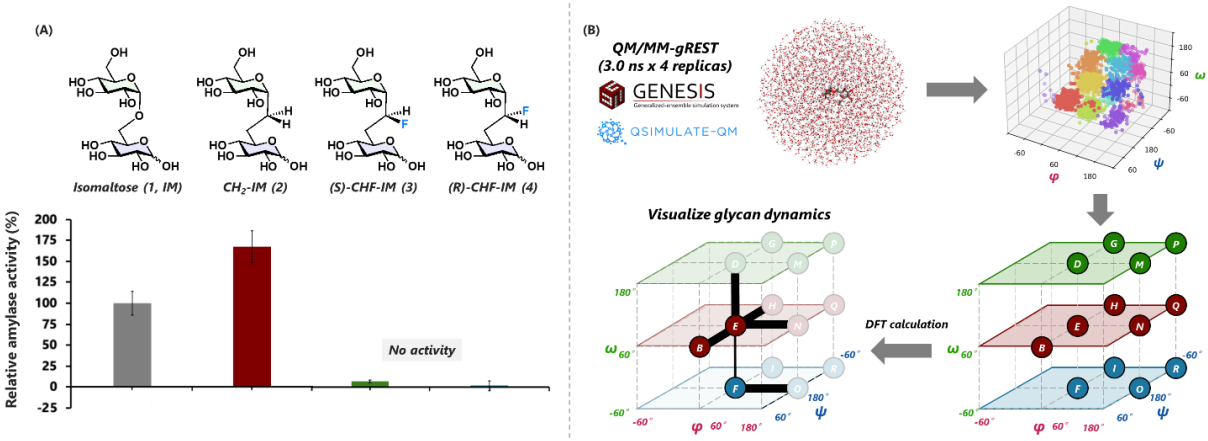
Glycans are important biomolecules involved in various biological phenomena, including inflammation control, through precise molecular recognition. The biological functions of glycans originate from their interactions with other biomolecules. However, the glycosidic bonds are flexible and can adopt multiple conformations. As a prevailing assumption, sterically and stereoelectronically stable conformations have been regarded as “active conformations” for biological functions of glycans, a view supported by X-ray crystallography and NMR analysis [1].

Isomaltose (IM, **1**) is a disaccharide that induces amylase production in *A. nidulans* [2]. Our group designed and synthesized its analogs (pseudo-IM) by editing the *O*-glycosidic bond to CH_2 (**2**), (*S*)-CHF (**3**), and (*R*)-CHF (**4**) linkage to modify steric and stereoelectric effects, aiming to find molecules that reproduce the active conformation of **1** (linkage-editing strategy) [3]. Biological evaluation revealed that **2** induced prolonged amylase production compared to **1**, while **3** and **4** showed negligible activity (Figure A). These results suggested that **2** can reproduce the active conformation, while neither **3** nor **4** can. However, NMR analysis **1**-**4** suggested that both **3** and **4** also shared stable conformations that are also found in **1** and **2**. Thus, we hypothesized that several different conformations of isomaltose can be involved in the amylase induction process. Here, we discuss the conformational distribution and dynamic range of (pseudo-)IM, computed by QM/MM-gREST simulations and DFT calculations (Figure B). We revealed that linkage-editing affected glycan dynamics, with biologically active compounds **1** and **2** displaying common specific motions.



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DESING AND SYNTHESIS OF NOVEL GLYCOMIMETICS AS GALECTIN ANTAGONISTS

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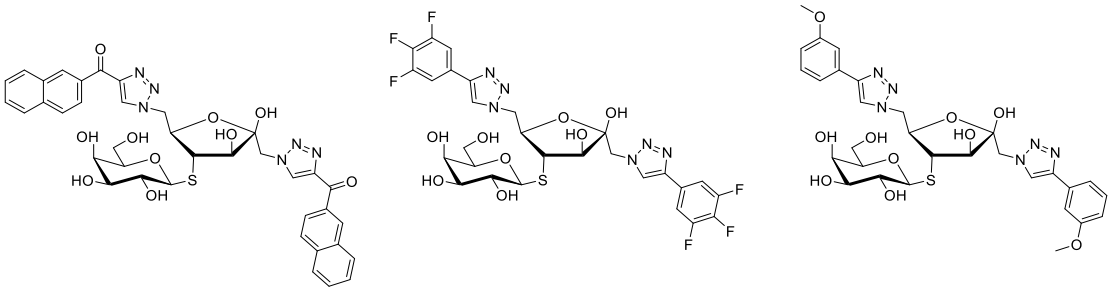

Figure 1. *Library of synthesised glycomimetics based on thiolactulose.*

Galectins are carbohydrate-binding proteins that contain at least one carbohydrate recognition domain (CRD) with an affinity for β-galactoside containing carbohydrates [1]. Galectins play key roles in regular physiological functions including modulating the immune system and in inflammation, but are also implicated in pathophysiological processes including cancer progression and metastasis [2], heart failure [3] and rheumatoid arthritis [4]. Although the highly conserved nature of the galectin CRDs enables the specificity for β-galactosides, it simultaneously makes the design of selective antagonists challenging [5]. A promising strategy to target these proteins is using glycomimetics.

Glycomimetics are compounds that mimic the structure and function of endogenous carbohydrates with strategic modifications to improve binding affinity and other drug – like properties such as metabolic stability and oral bioavailability [6]. PyMOL and AutoDock Vina were used to explore the structure of the gal - 3 CRD and to evaluate the binding affinities and protein-ligand interactions of potential compounds using molecular docking. These computational studies guided the structure-based design and optimisation of target compounds. A library of these compounds (Figure.1) have been synthesised using established synthetic methodologies such as amide coupling and copper-catalysed azide-alkyne cycloaddition (CuAAC), and characterised through NMR and mass spectroscopy. The binding affinities of the synthesised compounds will be assessed against a panel of galectins, and protein-ligand interactions will be investigated using techniques such as STD-NMR and X-ray crystallography. The resulting data will inform the structure-based design of the next generation of ligands.

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SYNTHESIS OF NOVEL PHARMACOLOGICAL CHAPERONES FOR THE TREATMENT OF MUCOPOLYSACCHARIDOSIS (MPS) II

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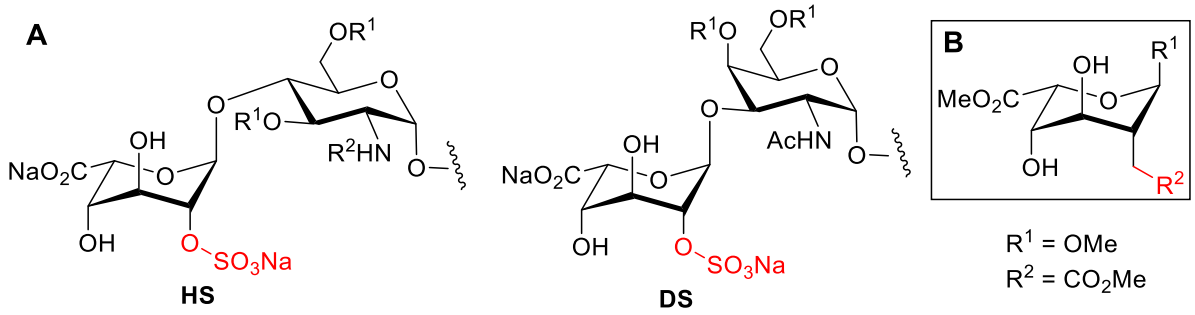
Mucopolysaccharidosis type II (or MPS II), also known as Hunter syndrome, is a rare X-linked recessive disorder caused by a deficiency of the enzyme iduronate 2-sulfatase (IDS) in the lysosomes. IDS is responsible for the degradation of the GAGs heparan sulfate (HS) and dermatan sulfate (DS) [1]. Accumulation of these undegraded sugars underpins MPS II. In severe cases, progressive and debilitating neurodegeneration occurs, thereby reducing the life expectancy of the patients to 10 – 20 years of age [2]. In view of the current absence of effective therapies to treat neurological symptoms, we set out to develop new pharmacological chaperones (PCs) as treatments for MPS II. These small molecules selectively bind to the target mutant protein in the neutral pH environment of the endoplasmic reticulum (ER). Most pharmacological chaperones act as competitive inhibitors by binding to the active site of their target enzyme [3]. In association with the target protein, these small molecules can correct the three-dimensional conformation of the protein to confer stability and facilitate trafficking of the correctly folded enzyme through the Golgi apparatus to the lysosome [4]. Our strategy for synthesizing small molecule PCs in the form of prodrugs is based on mimicking the structure of natural substrates for the IDS enzyme (i.e., HS and DS). L-Iduronic acid (L-IdoA) is a crucial component of HS and DS. This approach aims to replace the sulfate functional group at C-2 of L-IdoA derivatives with non-hydrolyzable groups such as carboxymethyl and various analogues with the remainder of the natural structure largely retained.



R^1 = SO_3Na or H; R^2 = Ac, SO_3Na or H

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LINKAGE-EDITED *C*-LINKED 6,6'-DIACYL NEOTREHALOSE ANALOGS

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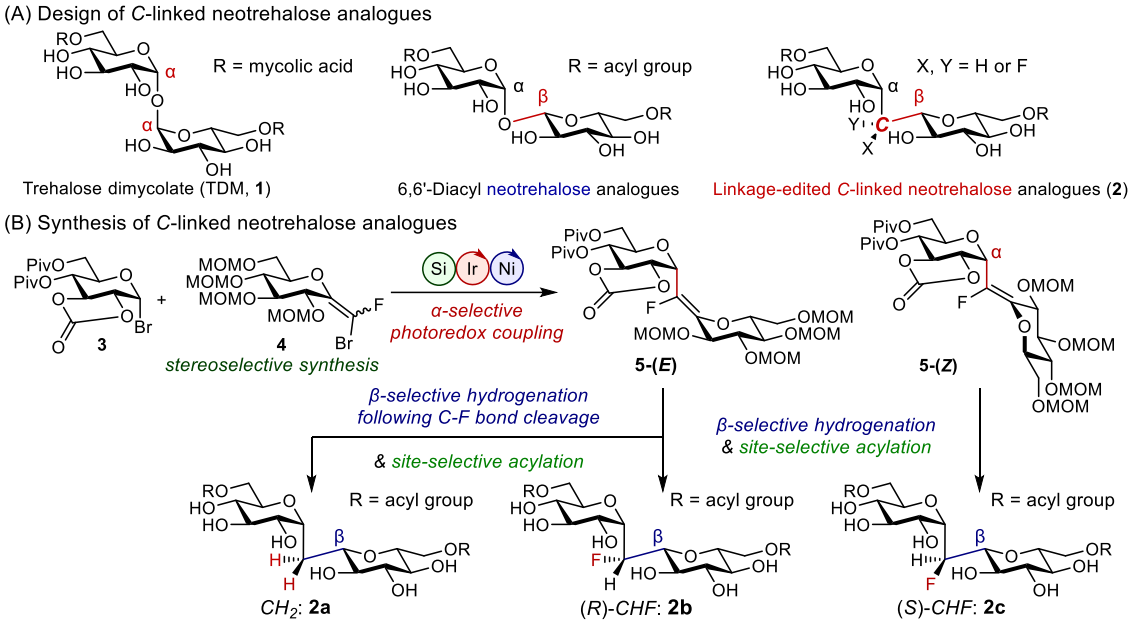
6,6'-Diacyltrehaloses, such as trehalose dimycolate (TDM, **1**), are known as potent immune-stimulating agents and the high-affinity ligands for Mincle, making their structure-activity relationship a focus of considerable interest. By contrast, the corresponding neotrehalose analogs, a stereoisomer of trehalose, exhibit significantly lower affinity for Mincle than trehalose derivatives [1]. The difference in affinity is thought to result from the distinct spatial positioning of the two glucose units.

We recently have developed glycan analogs based on linkage-editing strategy [2]. Our hypothesis is that linkage-editing alters the conformational distribution of glycans, resulting in change to their biological activities. Here, we applied this concept to the design of neotrehalose analogs with the aim of developing potent immune-stimulating agents based on the neotrehalose structure. Specially, we have designed and synthesized three neotrehalose analogs with *CH*$_2$, (*R*)-*CHF*, and (*S*)-*CHF* linkages.

Glycosyl bromide **3** was coupled with each stereoisomer of **4** under reductive cross-coupling conditions, stereoselectively affording the desired disaccharide **5** [2]. Three types of *C*-linked analogs **2** were synthesized via stereoselective hydrogenation followed by site-selective acylation with each stereoisomer of **5**. Starting from **5-(*E*)**, the selective synthesis of **2a** and **2b** was achieved by using appropriate hydrogenation catalysts, whereas the same procedure applied to **5-(*Z*)** afforded **2c**. Ultimately, the biological activity of these analogs was found to be dependent on the glycan linkage.



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INVESTIGATING HYDROGEN BOND DONATING CAPACITY OF CARBOHYDRATES AND THE INFLUENCE OF DEOXYGENATION/DEOXYFLUORINATION

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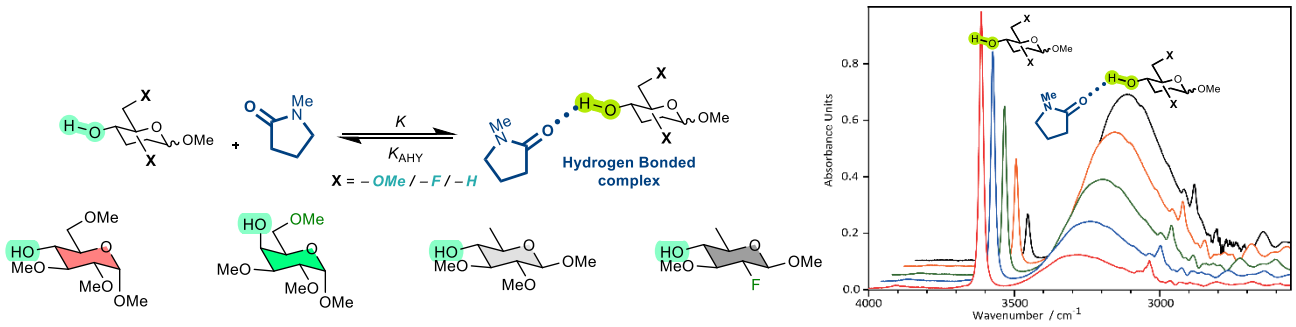
The hydrogen bond (HB) is one of the main interactions between carbohydrates and proteins. This is certainly the case with the excessively hydroxylated carbohydrates. There is little detailed knowledge of HB properties of individual alcohol groups in carbohydrates. Based on our previous work on HB donating (HBD) properties of alcohol groups in model compounds [1-3], this work aims to chart the HBD properties of alcohols in important sugars, and how this is influenced by substitutions such as deoxygenations and deoxyfluorinations.
The HB donating capacity of sugars are measured on the pK_{AHY} scale, which is defined as the equilibrium constant of the complexation between the substrate and a standard acceptor (*N*-methyl pyrrolidinone, NMP) in an apolar solvent (CCl_4) [4].



The data show that the hydrogen bond donating capacity of alcohol groups of carbohydrates display marked differences depending on relative stereochemistry and introduction of remote deoxygenation and deoxyfluorinaton, which will be explained in detail. Furthermore, the comparison of the selected sugar HB acidities with the Bronsted acidity values [5] of the corresponding gluco- and galactosamines will be discussed in detail. The mapping of sugar alcohol groups on the medicinal chemistry relevant pK_{AHY} scale allowed to establish a number of parameters that influence sugar alcohol HB acidity. These results will be valuable for the interpretation of binding data of carbohydrates and glycomimetics to proteins.

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NOVEL FUNGAL α-L-FUCOSIDASE EXHIBITING TRANSFUCOSYLATION ACTIVITY

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α-L-Fucosidases of glycoside hydrolase family 29 (GH29; CAZy; http://www.cazy.org/) are retaining *exo*-glycosidases that typically hydrolyze α-linked fucose from non-reducing end of polysaccharides and glycoconjugates [1,2]. GH29 α-L-fucosidases have a broad substrate specificity, hydrolyzing α-(1→2), α-(1→3), α-(1→4) and α-(1→6) linked fucosyl residues from glycans [3]. Of the two GH29 subfamilies with distinct sequence homology and substrate specificity, GH29A enzymes are more suitable for transfucosylation because they utilize chromogenic substrates such as 4-nitrophenyl α-L-fucopyranoside (*p*NP-Fuc) or 2-chloro-4-nitrophenyl α-L-fucopyranoside (CNP-Fuc). To date, the CAZy database contains 14 527 annotated GH29 α-L-fucosidases, only four of which belong to the fungal kingdom and one of them is fully characterized [4].

We present here the screening of selected fungi potentially producing proteins with α-L-fucosidase activity under the induction by six carbohydrate inducers. We have identified new fungal α-L-fucosidases and produced them heterologously in a *Pichia pastoris* expression system. We identified their transglycosylation capabilities with *p*NP-Fuc donor.

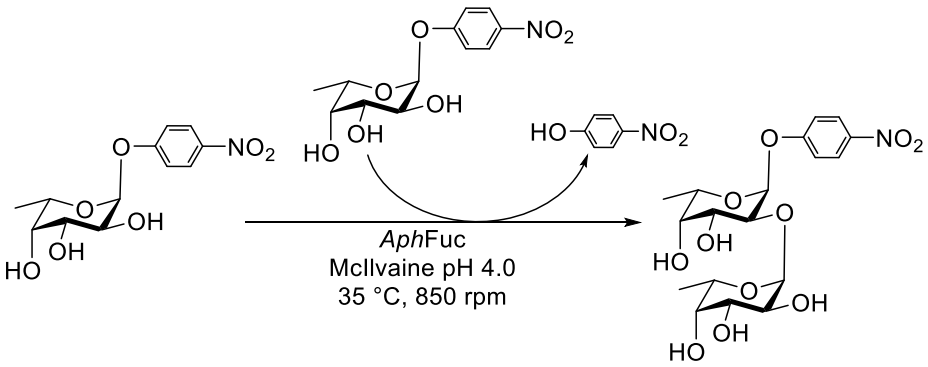

Figure 1. Transglycosylation reaction catalyzed by a novel fungal α-L-fucosidase.

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p53 AGGREGATION REGULATES EXPRESSION OF CL40-REACTIVE SIALYLATED GLYCANS IN OVARIAN CANCER

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TP53 is the most frequently mutated gene in human cancers. The vast majority of cases of high-grade serous ovarian cancer (HGSOC) display abnormalities in the *TP53* gene. We showed that the presence of cytoplasmic p53 aggregates correlated with poor prognoses in several cancers [1,2]. We also reported that cytoplasmic p53 aggregates suppressed apoptosis induction by cisplatin or ultraviolet irradiation by interfering with the p53 apoptotic function [1-3]. Here, we investigated the relationship between p53 aggregation and expression of aberrant glycan structures, another characteristic of many cancers. By using the CL40 antibody, which recognizes 6-sulfo sialyl Lewis X and 6-sulfo sialyl LacNAc with an absolute dependency on GlcNAc 6-O-sulfation [4], we found that the presence of CL40-positive cancer cells and p53 aggregates in peritoneal- or omentum-disseminated HGSOC tissues. In the cell-based assay with HGSOC-derived OVCAR-3 cells harboring p53 aggregates, the ReACp53 p53 aggregates inhibitor suppressed the expression of CL40-reactive glycans. The mRNA expressions of GlcNAc-6-*O*-sulfotransferase genes were reduced by ReACp53 treatment. Finally, pretreatment of OVCAR-3 cells with CL40 reduced Siglec-9 binding to the cell surface. Overall, our results suggest a novel function of p53 aggregation in regulating synthesis of CL40-reactive sialylated glycans in HGSOC. Future work will clarify the roles of CL40-reactive glycans in HGSOC pathology.

Acknowledgements: This work was partly supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (MEXT)/Japan Society for the Promotion of Science (JSPS). This study was also supported by the Suzuken Memorial Foundation, the Mochida Memorial Foundation for Medical and Pharmaceutical Research, the Princess Takamatsu Cancer Research Fund (23-255025), and the Foundation for Promotion of Cancer Research.

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SYNTHESIS OF AN UNUSUAL TETRASACCHARIDE FROM RG-II

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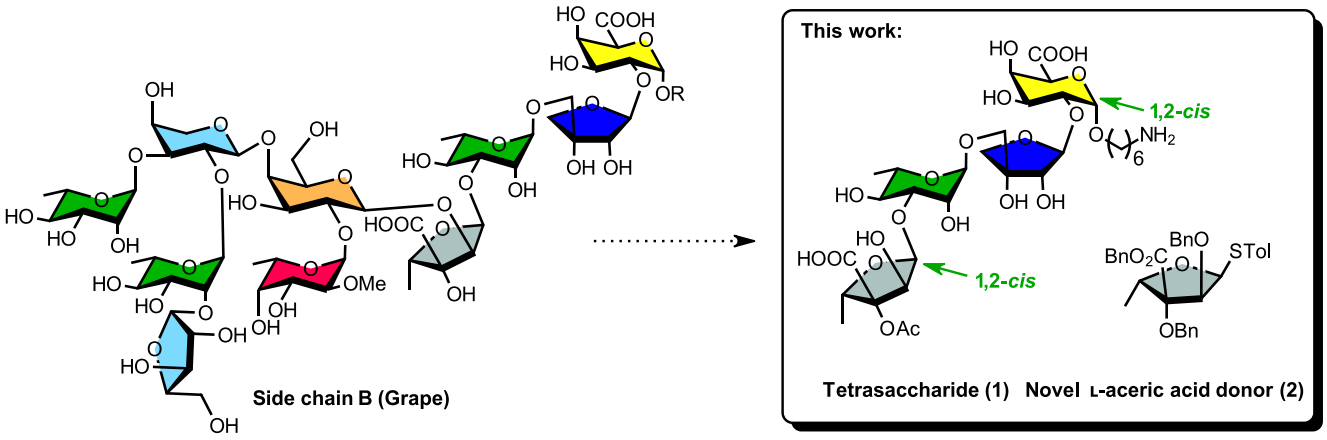
Pectin is an integral component of the plant cell wall. The most complex part of pectin is rhamnogalacturonan II (RG-II), a highly branched polysaccharide with various side chains A-F [1]. While the structure of RG-II is highly conserved throughout the plant kingdom, slight variations have been reported solely on side chain B. Depending on different plant species, the size of side chain B can span from a penta- to a decasaccharide. However, the structural motif of the first five glycosidic linkages between D-galacturonic acid, D-apiose, L-rhamnose, L-aceric acid and D-galactose is preserved [2]. While there are reports of the synthesis of varying parts of side chain B [3]. starting from the D-galactose, to our knowledge, the synthesis of oligosaccharides derived from the conserved inner structure has not been attempted yet. Herein, we report the synthesis of a tetrasaccharide (**1**) comprised of the first four sugars found in RG-II side chain B. The synthesis of this tetrasaccharide poses a significant synthetic challenge, as there are two challenging 1,2-*cis* glycosidic linkages to be made in this molecule. Moreover, the rare sugar donors derived from L-aceric acid and D-apiose need to be synthesized from commercially available starting materials in multiple steps. Notably, we report the synthesis and first successful use of an L-aceric acid donor (**2**) in chemical glycosylation. This novel glycosylation reaction was thoroughly analysed and we were able to obtain either 1,2-*trans* and the desired 1,2-*cis* linkage with partial selectivity by varying the reaction temperature and activator system. We utilized NOESY experiments to tackle the significant challenge of determining the anomeric configuration of disaccharides comprising L-aceric acid.



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VCNA AS A BACTERIAL BIOMARKER: LABEL-FREE IMPEDIMETRIC DETECTION USING SIALOSIDE-MODIFIED ELECTRODES IN COMPLEX ENVIRONMENTS

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Cholera is a severe infectious disease caused by Vibrio cholerae. Cholera primarily spreads through contaminated food and water sources. is a significant global health concern. The pathogenesis of V. cholerae is facilitated by a secreted neuraminidase (VCNA). The cleavage of host cell surface sialic acids is pivotal for Cholera colonization and infection. While the interaction of VCNA with sialosides holds promise as a bacterial biomarker, evaluating these interactions in complex food environments presents a challenge. We developed an electrochemical biosensor based on impedance spectroscopy (EIS) to detect VCNA. We utilized a variety of synthetic sialosides as molecular probes. These sialosides were immobilized on gold electrodes to form a self-assembled monolayer. The changes in EIS signals were observed upon the interaction between VCNA and the immobilized sialosides. This work demonstrates the quantitative measurement of these interactions under varying environmental conditions, including different media and pH values. Our selective detection of VCNA activity is demonstrated through distinct impedance variations corresponding to the enzymatic cleavage of sialosides. This approach provides insights into the development of robust biosensing platforms for bacterial detection, offering potential applications in various diagnostic.

GLYCOENGINEERING OF EXTRACELLULAR VESICLE FOR TARGETING TO ENDOTHELIA LECTINS AS A POTENTIAL CANCER AND INFLAMMATION THERAPY

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Exosomes are 40-160nm sized vesicles secreted by most types of cells as vehicles for intercellular trafficking of cellular components including miRNA, mRNA, proteins, lipids, metabolites, etc. Their ability to connect distant tissues has stimulated a broad interest in exosomes as a natural non-immunogenic drug and gene delivery vehicle [1]. Considering the importance of glycans for exosome targeting capacity [2], we aim to develop exosomes, as a tool for drug delivery in cancer therapy by glycoengineering their surface glycans. To this end we will functionalize mesenchymal stem cell derived exosomes with covalently attached sialyl Lewis^X tetrasaccharide, a glycan with high affinity to the human lectin E-selectin, which is overexpressed in the endothelia of inflamed tissues and also tumors [3]. To this end , we have synthesized sialyl Lewis^X with an anomeric PEG linker (sialylLewisX-PEG-NH_2) via a combination of chemical and chemo-enzymatic reactions starting from lactosamine. For the chemo-enzymatic synthesis , both an α-2,3-sialyltransferase and an α-1,3 fucosyltransferase were expressed and purified. After each purification, a protein electrophoresis, and an activity assay by UPLC were performed. As a first approximation, the activation of lactosamine-PEG-NH_2 has been already carried out and different functionalization trials on purified exosomes have been performed. We will evaluate the effects of reaction time, buffer pH and reagent excess on the degree of surface functionalization with the help of mainly anti-sialyl Lewis X antibodies, Lectin-probed western blot, MALDI-TOF MS and Z-potential. Moreover, we will employ lectin-array technology for analyzing surface glycosylation before and after every glycoengineering approach. Our recent advances towards this intermediate goal will be presented.

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STEREOSELECTIVE SYNTHESIS OF α-FUCOSIDES

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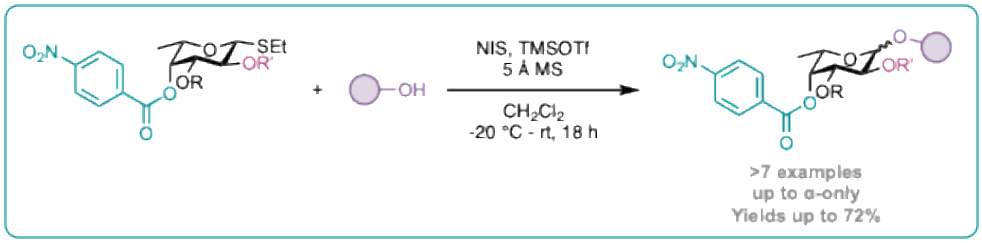
α-Fucoside units are found in many biologically important compounds, and have interesting implications in cancer biology [1]. As glycosidic linkages are the key to diversification of saccharide function and biological properties, controlling the stereochemistry of fucosylation reactions is of great importance within the field of carbohydrate chemistry. However, existing methods for the α-selective synthesis of fucosides are limited by a lack of generality in terms of stereoselectivity, as well as a requirement for the use of 3,4-di-*O*-benzoate-protected donors. This limits the potential for orthogonal deprotection towards saccharide diversification. [2].
This work is concerned with stereochemical control in α-fucosylation reactions. Recently, the McGarrigle group has employed an α-selective methodology for galactosylation using an orthogonal *para*-nitrobenzoate protecting group at position four of galactosyl donors [3]. This poster will describe the application of this methodology with the analogous fucose donor [4].



A model 4-*O*-nitrobenzoate-protected donor was prepared and gave access to fucosyl α-1,3-, -1,4- and -1,6-linkages in exclusive α-selectivities and up to 72% isolated yield.
Two orthogonally protected 4-*O*-nitrobenzoate fucosyl donors were also prepared over 8 steps to examine functional group tolerance and were found to maintain exclusive α-selectivity in the presence of an orthogonal allyl group and using a 2-*O*-(*para*-methylbenzyl)-3-*O*-benzyl donor in up to 69% isolated yield. Work towards the synthesis of a Lewis antigen derivative trisaccharide using this methodology will also be reported.

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VISIBLE LIGHT-PROMOTED STEREOSELECTIVE SYNTHESIS OF A-D-GLYCOPYRANOSYL TETRAZOLE DERIVATIVES

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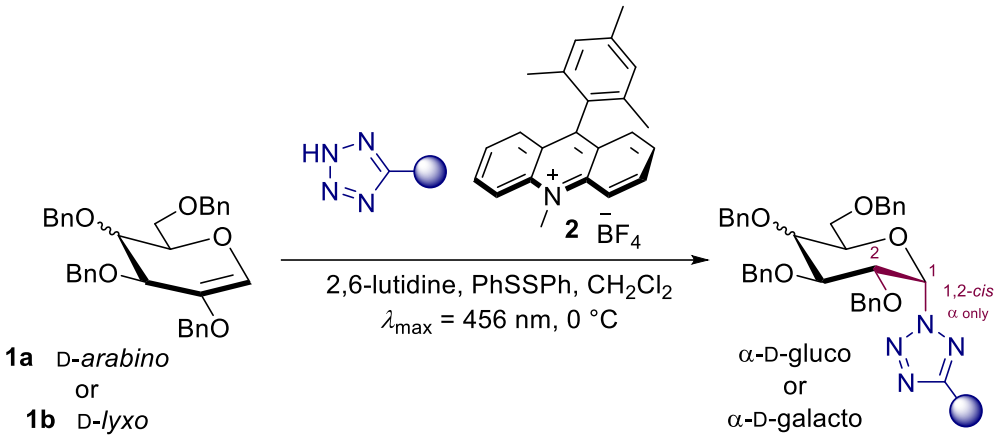
Carbohydrates are essential to numerous biological processes, making them a common focus of research and synthesis, including the development of their diverse derivatives. A crucial aspect of carbohydrate synthesis is the stereoselective formation of the glycosidic bond. [1,2] In this study, we introduce a novel approach to the stereoselective and regioselective synthesis of *N*-glycopyranosyl tetrazoles using a visible light-promoted photocatalytic reaction. This method employs readily available glycal donors (**1a** or **1b**) [3] and tetrazole derivatives, obtaining α-d-glycopyranosyl tetrazoles in exclusive 1,2-*cis* stereoselectivity.
This photocatalytic radical reaction is facilitated by a commercially available acridinium organocatalyst (**2**), which is re-oxidized in the presence of diphenyldisulfide [4-7] (**Scheme**).



Scheme 1

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LONG-TERM MONITORING OF URINARY OLIGOSACCHARIDE BIOMARKERS IN GLYCOGEN STORAGE DISEASE TYPE II

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Glycogen Storage Disease Type II (GSD II) is a rare, autosomal recessive disorder caused by mutations in the *GAA* gene, leading to a deficiency of acid α-glucosidase (GAA). This enzymatic deficiency results in glycogen accumulation, primarily affecting muscle tissues. Enzyme replacement therapy (ERT) with recombinant human GAA has significantly improved survival and quality of life [1]; however, individual immune responses to treatment can lead to varying therapeutic effectiveness among patients. To address this variability, non-invasive monitoring of free oligosaccharide (FOS) biomarkers contributes to the reliable assessment of therapy efficacy in individual patients [2]. In Slovakia, more than 20 GSD II patients have been diagnosed, with most receiving ERT. For over five years, we have been systematically monitoring these patients, utilizing ^{1}H NMR analysis, which offers high specificity and reproducibility for precise quantification of FOS. The levels of αGlc(1→6)αGlc(1→4)αGlc(1→4)Glc tetrasaccharide (Glc4) ranged from 3.4 μM/mM creatinine in late-onset patient receiving ERT to 882.5 μM/mM creatinine in infantile-onset case prior to ERT initiation. Long-term monitoring has provided valuable insights into treatment response and disease progression, reinforcing the role of Glc4 as a key biomarker for individualized patient management.

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SYNTHESIS OF SELECTED HUMAN MILK OLIGOSACCHARIDES (HMOs)

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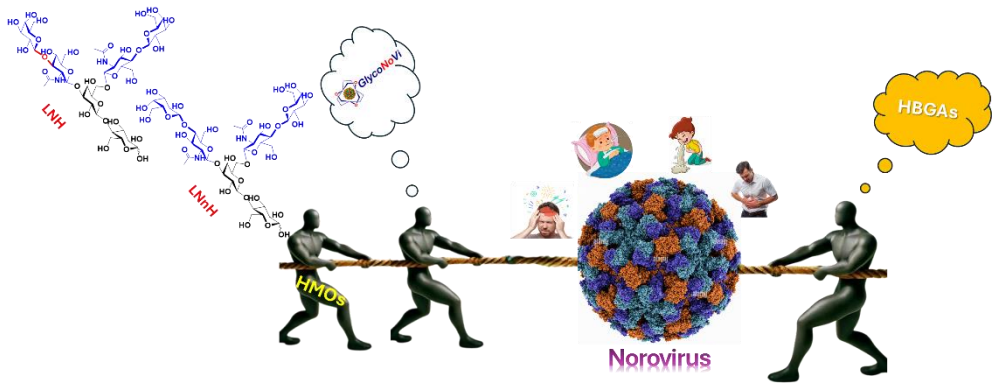
Norovirus, a highly contagious virus causing acute gastroenteritis, affects millions globally each year [1]. Despite extensive research, no approved antivirals or vaccines exist [2]. Human noroviruses bind to histo-blood group antigens (HBGAs) [3], but structurally similar fucosylated Human Milk Oligosaccharides (HMOs) act as natural inhibitors [4]. This work focuses on synthesizing rare, branched HMOs like LNH, LNnH, and their fucosylated derivatives to explore their potential in norovirus inhibition. Limitations in (chemo)enzymatic methods, along with complex chemical synthetic routes [5], inspire new strategies for LNnH and LNH synthesis.

The chemical synthesis of LNnH & LNH were accomplished in 19 and 27 steps, respectively, starting from lactulose, lactose, glucosamine, and galactose. A key tetrasaccharide intermediate is reached in 15 steps before the pathways diverge, with the final four steps selectively yielding LNH or LNnH. This common synthetic approach enhances efficiency, scalability, and economy. Key transformations include regioselective silyl protection using tin chemistry, [2+2] and [2+4] glycosylations, and orthogonal protection/deprotection strategies.



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CHEMICAL TARGETING OF NGLY1: FROM INHIBITORS TO AFFINITY PROBES

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N-Glycanase 1 (NGLY1) is an enzyme that removes N-Glycans from misfolded glycoproteins that are targeted to ER-associated degradation (ERAD). Loss of NGLY1 function as a result of heterozygous inactivating mutations in the ngly1 gene causes a rare genetic disorder, referred to as NGLY1 deficiency. Previous studies have confirmed that de-N-glycosylation by NGLY1 is a critical processing step in activating the transcription factor Nuclear Factor Erythroid 2 Like 1 (NFE2L1) [1]. Misglycosylation of NFE2L1 is contributing to the severe presented phenotype in NGLY1 deficiency. In addition, NFE2L1 represents an important, but inaccessible target in oncology as it is associated with acquired resistance mechanisms in certain cancers, including multiple myeloma or mantle cell lymphoma [2]. Genetic deletion as well as chemical inhibition of NGLY1 has been shown to successfully inactivate the transcription factor and sensitizes cancer cells to proteasome inhibitor cytotoxicity. A targeted screen of thiol-reactive compounds identified a selective inhibitor (WRR139) for NGLY1. However, high concentrations are required for efficient inhibition, which limit the clinical applications of WRR139.
In ongoing studies, we aim to convert the established inhibitor into affinity probes for NGLY1. By modification of the established synthesis route, we successfully introduced a hydroxyl group into the benzene ring that serves as basis for further functionalization. Attachment of photo-inducible crosslinkers will yield NGLY1-specific probes for proto-affinity labelling (PAL). We will use these probes to identify interaction partners of NGLY1. We further show the attachment of the structurally modified WRR139 to a ligand for an E3 ligase via several linkers of varying length and flexibility, giving a set of first-generation PROTACs targeting NGLY1. Such an NGLY1 degrader will also indirectly target NFE2L1 activity and could thus potentially serve as a powerful therapeutic strategy for the treatment of cancers associated with these proteins.

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GLUCOSE-CONTAINING MEMBRANE GLYCEROLIPIDS OF *CUTIBACTERIUM ACNES*

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Cutibacterium (formerly known as *Propionibacterium*) comprises representatives of the human skin microbiome. These bacteria are associated with various infections, including prostheses, sarcoidosis, soft tissue infections, and acne lesions. Within *Cutibacterium acnes*, three major phylotypes — I, II, and III — have been identified, each linked to different disease associations. In 2024, a comprehensive comparative analysis of the lipidome across various species within the *Cutibacterium* genus was published [1] revealing distinct profiles of lipid metabolites that enable differentiation between species of the *Cutibacterium* genus and the phylotypes of *C. acnes*. Additionally, our preliminary studies on extracellular vesicles (EVs) released by *C. acnes* indicated that these EVs' lipid and protein profiles vary between the different phylotypes of *C. acnes* [2].

The study aims to perform a comparative structural analysis of the cellular membrane glycolipids from four strains of *C. acnes*, which represent different phylotypes.

Glycolipids found in the *C. acnes* (phylotypes IA1, IB, II, and III) were isolated and obtained in pure form. Based on their chromatographic behaviour, the glycolipids were designated as GL1, GL2, GL3, and GL4. These compounds were analysed using various techniques, including thin-layer chromatography (TLC), gas-liquid chromatography-mass spectrometry (GLC/MS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and nuclear magnetic resonance (NMR). *In vitro* tests were conducted with PWR-1E human prostate cells and MSU1.1 fibroblasts to evaluate the toxicity of the major glycolipids.

All four glycolipids were identified in lipid extracts from *C. acnes* phylotype IB cells. In contrast, lipid extracts from the other phylotypes contained only glycolipids GL1 and GL3. Notably, glycolipid GL1 was present in lipid extracts from *C. acnes* extracellular vesicles (EVs) across all phylotypes, while glycolipid GL3 was found only in phylotypes IA1 and III.

GLC-MS and MALDI-TOF MS analyses revealed that glycolipid GL1 consists of two glucose residues attached to a glycerol molecule, along with two fatty acid chains (C15:0/C17:0). Glycolipid GL3 shares the same sugar-glycerol backbone but includes three fatty acid residues (C15:0/C17:0). Glycolipids GL2 and GL4 were structural modifications of GL1 and GL3. Additionally, both glycolipids GL1 and GL3 were shown to be non-toxic to selected human cells *in vitro*.

The chemical and biological characteristics of *C. acnes* glycolipids serve as a basis for further research into their antigenic properties. This research could lead to the development of new vaccine components and diagnostic tools. Additionally, exploring the role of glycolipids in bacterial extracellular vesicles (EVs) presents an exciting opportunity for future studies.

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MULTIVALENT CHITOBIOSE-CONTAINING GLYCOSTRUCTURES AS LYSOZYME LIGANDS: IMPLICATIONS FOR BINDING AND INHIBITION

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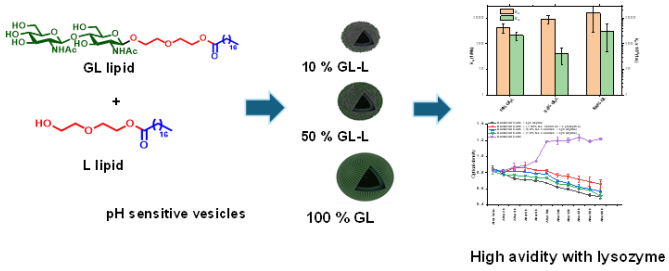
Lysozyme is an important immune enzyme, possessing antimicrobial and immune-modulating functions. However, excess production of lysozyme leads to abnormalities such as sarcoidosis, neuropathic pain and hematologic malignancies [1]. This study explores the interaction of lysozyme with multivalent chitobiose-containing self-assembled glycostructures, in order to uncover the roles of lysozyme. Chitobiose-containing glycolipid (**GL**) and aglycon lipid (**L**) were synthesised for this purpose. Glycovesicles were formed by varying the molar ratios of **GL** and **L** and characterized in solution and in the solid state. Spherical morphologies with varied sizes were observed for these glycovesicles. The glycovesicles, possessing pH-sensitive ester linkages, allow the disassembly under varying pH of the solutions. Steady-state fluorescence spectroscopy and surface plasmon resonance techniques were used to study the ligand-receptor interactions, in this instance, the binding of glycovesicles with lysozyme was evaluated. The binding of glycovesicles having chitobiose molar ratio of ~50% **GL**-**L** with the enzyme occurred significantly higher than vesicles with other **GL**/**L** ratios. The multivalent glycovesicles demonstrated faster association and slower dissociation kinetics, with equilibrium binding constants (K_a) 2-4 orders of magnitude higher than that for the monomeric chitobiose-lysozyme complex. Further the complexation of lysozyme with multivalent glycovesicles delayed the antimicrobial lytic activity of the enzyme, as compared to lysozyme alone. These findings offer new insights into lysozyme inhibition and will be presented in detail [2].



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COMBINED GLYCOPROTEIN LECTIN-BASED MICROARRAY AND LC-MS ANALYSIS OF COLORECTAL CARCINOMA SAMPLES

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Colorectal carcinoma (CRC) is currently the third most common cancer in men and the second most common in women, with highly variable disease progression and clinical outcomes among patients [1]. Although CRC is preventable and treatable when detected early, it remains a leading cause of cancer-related deaths [2,3], highlighting the urgent need for improved early detection strategies. One promising approach involves studying changes in glycosylation. Altered glycan structures, key players in processes such as cell adhesion, invasion, and signaling are commonly associated with cancer progression [1]. Aberrant glycosylation enhances the immunosuppressive capacity of tumor cells and promotes immune escape, which contributes to tumor recurrence, metastasis, and drug resistance [4]. To gain a better understanding of glycan changes during CRC, we conducted N-glycan profiling using lectin-based microarray method and LC-MS. Lectin-based microarray technique has already shown its great potential in detecting glycan changes in analysis of samples related to various diseases such as GDM, ADHD, and SARS-CoV-2 infection. Using lectin-based microarray, we analyzed plasma samples of patients with CRC and healthy plasma controls. Samples were spotted on epoxy-coated slides in triplicates (1 spot = 0.5 nL) using non-contact robotic spotter. Slides with immobilized samples were then blocked, incubated with a panel of 17 biotinylated lectins with various glycan specificities and labeled with fluorescent dye conjugated with streptavidin. For LC-MS analysis of unlabelled reduced N-glycans, plasma samples were dot-blotted onto a PVDF membrane. N-glycans from immobilized glycoproteins were enzymatically released with PNGase F, reduced and desalted. Samples were analysed using PGC-ESI-MS/MS in negative ion mode. Results from lectin-based microarray and LC-MS were statistically analyzed, and compared to identify glycan changes associated with CRC. Our findings demonstrate that the glycoprotein lectin-based microarray is not only an effective tool for glycan profiling but, when combined with LC-MS, can sensitively detect glycan alterations occurring during CRC progression.

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SYNTHESIS AND BIOLOGICAL PROFILING OF HEPARAN SULFATE ANALOGUE D-GLUCURONIC ACID-CONTAINING OLIGOSACCHARIDES

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In recent decades, researchers have paid increasing attention to the highly negatively charged polysaccharides and higher oligosaccharides on the cell, on the cell surfaces or in the extracellular matrix. An important class of these structures are glycosaminoglycans. Heparin and heparan sulphate (HS) are very important molecules belonging to the family of GAGs, which are built up from alternating α-D-glucosamine and hexuronic acid units. These derivatives and its analogues have a wide range of biological effects [1]. Our research group has long been working on the synthesis of heparin and heparan sulphate analogue oligosaccharides. We already have synthesized some of non-glycosaminoglycan-type, heparin-analogue oligosaccharides (di-, tri- and pentasaccharides), in which the glucosamine unit was replaced by a simpler glucose moiety. Moreover, in our department three D-glucuronate-containing trisaccharides were synthesized, which showed significant and selective inhibitory effects on the growth of tumour cells [2]. We supposed that the newly synthesized tetra-, penta- and hexasaccharide fragments of heparan sulphate might also display biological activity.
Based on the mentioned results we synthesized oligosaccharides that are composed of alternating D-glucose and D-glucuronic acid units with various sulphation and acetylation patterns. We planned of our compounds to investigate *in vitro* studies on cell proliferation, anti-inflammatory effects and cytotoxicity both healthy and tumour cell lines.

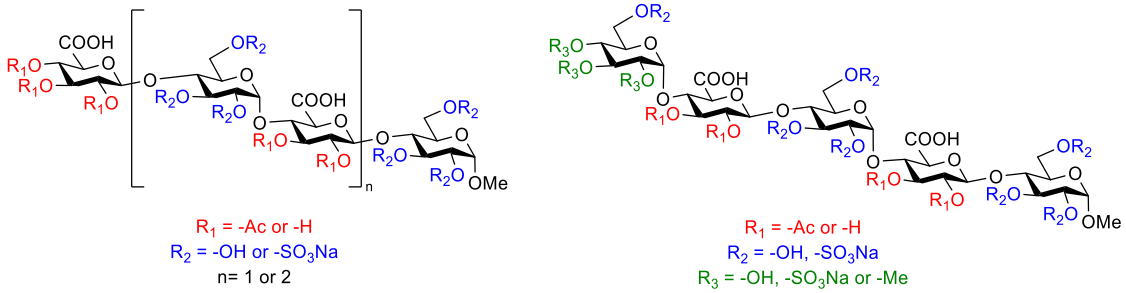

Figure 1. The structure of the synthesized D-glucuronic acid-containing oligosaccharides

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CARBOHYDRATE CONJUGATES AS TROJAN HORSE TO IMPROVE ANTIBIOTIC IMPORT

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Antimicrobial resistance (AMR) is a major threat for individual and public health [1]. AMR is often induced by limited permeability and uptake. In this project, we aim to overcome antimicrobial resistance by hijacking energy-driven carbohydrate uptake systems (PTS and ABC transporters) to actively transport carbohydrate-antibiotic conjugates into bacteria and break AMR (Figure 1). These transporters are able to accumulate mM intracellular concentrations of their substrates [2]. After uptake, the carbohydrate-antibiotic conjugates will be hydrolyzed by cytosolic glycosidases to release the antibiotics. To date, we have synthesized carbohydrate conjugates attached with levofloxacin with different linker lengths or linezolid. Their antimicrobial activities were evaluated by MIC determination.

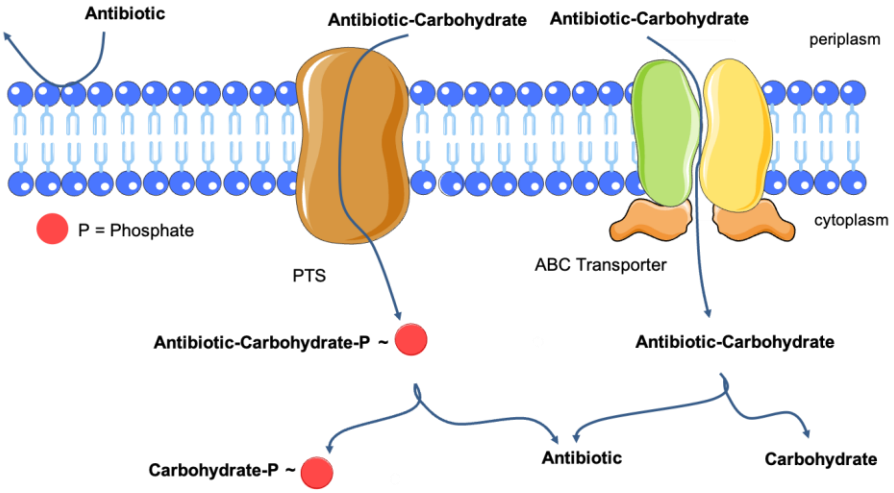

Figure 1. Schematic representation of the idea to hijack PTS and ABC transporter to actively pump carbohydrate-antibiotics into bacterial cells.

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DEFINED CHITOOLIGOSACCHARIDE CHAINS AS ALTERNATIVE CARRIERS OF BIOACTIVE GLYCANS

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Chitosan is a widely used linear biopolymer composed mainly of glucosamine and to a lesser extent of *N*-acetylglucosamine units [1] . Many biological activities of chitosan are attributed to its shorter oligomeric chains (COS with degree of polymerization DP 10-25) [2-4]. However, the extraction of such chains, which are well defined in terms of the degree of acetylation and polymerization, is very problematic on a preparative scale (tens of mg) [5]. The first step to obtain shorter oligomeric chains from polymeric chitosan is usually acid hydrolysis, which can be steered by the choice of a suitable acid. Therefore, we compared the kinetics of chitosan hydrolysis by various (in)organic acids, investigating the distribution of the polymer chain lengths in time. Hydrolyzed mixtures of shorter chitooligosaccharide chains were subsequently separated by ion exchange gel chromatography. The prepared individual COS were used as an ideal alternative scaffold for the multivalent presentation of bioactive carbohydrates such as galactoside-based glycomimetics with affinity to galectins. To test the feasibility of chemical functionalization of COS, we conjugated pentynoic acid to the glucosamine monomer. The introduction of this functional group for the biocompatible method of CuAAC click chemistry into the chitosan scaffolds enabled the conjugation of galactoside ligands. The resulting multivalent chitosan-galactoside conjugates were then tested in competitive ELISA-type assays with recombinant galectind to assess the application potential of these glyco-nanomaterials in galectin-associated pathologies.

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ITERATIVE BUMP-AND-HOLE ENGINEERING OF *N*-ACETYLGLUCOSAMINYLTRANSFERASE I

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N-glycosylation, is important for cell-cell adhesion, cell signalling and protein stability [1]. N-linked glycosylation starts from a common precursor, from which more complex structures are built up through sequential action of mannosidase and glycosyltransferase enzymes. MGAT1, the first enzyme in the pathway for generating complex and hybrid N-glycans, is crucial for proper development, as MGAT1 knockouts are embryonically lethal in mice [2]. Here, we employ a bump-and-hole approach to develop a bioorthogonal precision tool for MGAT1. In a structure-guided process, we replaced so-called gatekeeper residues in the MGAT1 active site to smaller amino acids, thereby allowing the enzyme to accept a modified artificial UDP-GlcNAc analogue (Figure 1). We generated a series of these artificial substrates containing bulky alkyl groups and an bioorthogonal handles. A library of MGAT1 mutants was screened against a series of chemically modified substrate analogues, from which the most compatible enzyme-substrate pair was selected and characterized kinetically. Iterative, optimization using molecular dynamic simulations provided an optimized enzyme-substrate pair with greater selectivity and fewer background incorporation by other glycosyltransferases. After establishing cellular biosynthesis of the modified UDP-GlcNAc analogue, we employed the MGAT1 bump-and-hole pair for selective chemical tagging of N-linked glycans on proteins in living mammalian cells. Thereby, our work expands the toolbox for glycan-based reporter compounds.

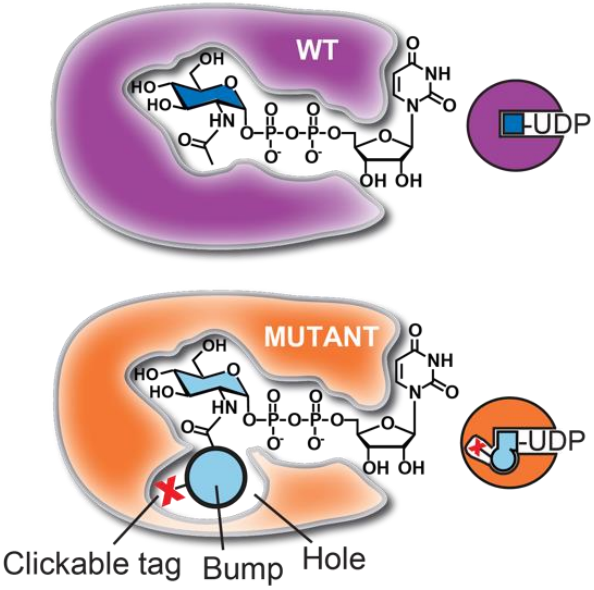

Figure 1. Bump-and-hole engineering of MGAT1.

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SWEET COCKTAILS FOR EVERYONE: PARAMETRIZATION OF FLEXIBLE CARBOHYDRATES FOR THE MARTINI 3 COARSE-GRAINED FORCE FIELD

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Molecular dynamics simulations are a popular tool used to predict the behaviour and properties of particles. However, the all-atom representation of complex systems, especially long polysaccharide chains or large biological systems containing carbohydrates, suffers from high computational costs. To alleviate this problem, coarse-grained (CG) force fields, e.g. Martini 3, are used, where groups of atoms are mapped to beads [1]. Unfortunately, this simplification can cause the loss of information regarding selected conformational properties, like natural flexibility of glycosidic linkages leading to the formation of *anti-* conformers or pyranose ring distortions. In this poster, we present the strategy of parametrization of conformationally flexible glycans. The bonded interactions for glucopyranose polysaccharides (model compounds for flexible linkages) and non-sulfated dermatan (model compound for flexible rings) were optimised with respect to their possible conformation (accordingly *syn* and *anti-*ϕ/ψ glycosidic linkage conformers, and regular chairs, inverted chairs, boats and skew-boats of L-iduronic acid ring conformers). Obtained parameters were then mixed into tablulated potentials, considering the relative energies of all considered forms. This approach increases the accuracy of CG-MD simulations and allows for partial recovery of behaviour inherently lost in the process of all-atom-to-CG model mapping.

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GOLGI TARGETING: UNLOCKING NEW AVENUES IN CANCER THERAPY

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The altered expression of glycans within the glycocalyx is recognized as a cancer hallmark. These altered glycans are the products of the aberrant activity of specific enzymes known as glycosyltransferases (GTs) which are mainly resident in the Golgi apparatus and endoplasmic reticulum [1]. Accordingly, the targeting of the Golgi apparatus is considered a primary goal for the future development of molecules able to control the aberrant activity of GTs.
Despite this, only a few Golgi-targeting probes have been reported to date. Their identification has long been hampered by poor understanding of the targeting mechanisms and even though key related aspects are gradually being elucidated, Golgi-targeting strategies are still in their infancy [2]. Consequently, a systematic study on Golgi-targeting moieties is missing.
Taking advantage of BODIPY probes developed in our laboratories [3,4], we will report on a systematic study aimed at providing advancements in the knowledge on Golgi-targeting strategies. With this in mind, we will provide details on the chemical strategies to fluorescently-label structurally different Golgi-targeting moieties, with the aim to track their subcellular fate and to unveil insights on their ability to reach and target the Golgi apparatus. The identification of the most efficient compounds capable of targeting the Golgi apparatus will pave the way to the precision subcellular delivery of GT modulators and thus improve their therapeutic effect against cancer.

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SYNTHESIS AND CONFORMATIONAL ANALYSIS OF ^{15}N-LABELED *N*-DISACCHARIDES

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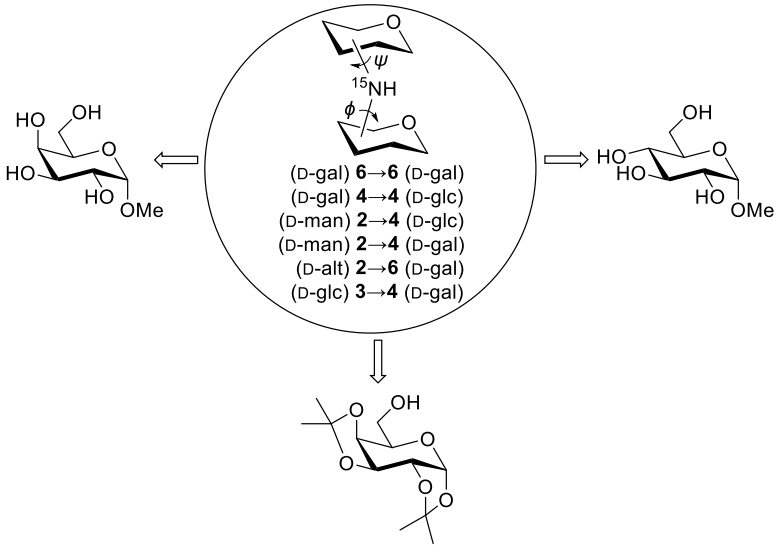
This work focuses on the synthesis of isotopically ^{15}N-labeled *N*-disaccharides via three different approaches: reductive amination, substitution, and epoxide ring opening, and their application in NMR conformational analysis. The ^{15}N-labeled bridge enables the measurement of NMR parameters, such as ^{15}N-^{13}C and ^{15}N-^{1}H *J*-couplings, which are not accessible at the natural abundance [1]. The conformation of ^{15}*N*-disaccharides is defined by torsional angles ϕ and ψ, influenced by hydrogen bonding, steric effects, and electronic interactions, which describe the mutual orientation of the two connected monosaccharide units [2]. *N*-disaccharides are flexible molecules and can exist in multiple conformations and presented labeling method could help to estimate preferred conformers and their population in solution [3]. Additionally, ^{15}N-labeled *N*-disaccharides could be valuable in NMR binding studies with lectins, where changes in ^{15}N chemical shift, signal shape, or *J*-coupling can provide key insights into binding events, interaction strength, and the structural details of the *N*-disaccharide-lectin complex.



Acknowledgements: *This work was supported by the Czech Science Foundation, project* No. *23-05805S and project* No. *22-17586S.*

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NANOMOLAR INHIBITOR OF THE GALECTIN-8 N-TERMINAL DOMAIN BINDS VIA A NON-CANONICAL CATION-Π INTERACTION

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Galectin-8 (Gal-8) plays an important role in innate and adaptive immune responses as well as in the regulation of cancer growth and metastasis [1], making it a target of interest for highly selective and potent Gal-8 inhibitors. We have designed focused libraries of 2-*O*-substituted D-galactosides that bind to the N-terminal domain of Gal-8, starting from lead **1** (***Figure 1A***) [2,3]. All designed compounds were docked to the selected Gal-8N crystal structure (PDB ID: 7AEN) using Glide to explore the possibility of 2-O-substituents that can make favourable contacts with the protein and help select candidates for synthesis and biochemical evaluation. We evaluated compound binding by a competitive fluorescence polarisation assay (FP) and isothermal titration calorimetry (ITC). This strategy led us to selective nanomolar inhibitors of Gal-8N. We solved a co-crystallized structure of a selected galectin-8 inhibitor **11** (***Figure 1B***) in complex with the protein (PDB ID: 9FYJ), which helped us to decipher the nature of the interaction of the substituents at position 2. A detailed thermodynamic analysis revealed important differences in enthalpic and/or entropic contributions to binding. Finally, an energy decomposition analysis was performed, which showed that a bonding interaction between Gal-8 Arg45 and acetylene of **11** occurs via a new molecular orbital, indicating the presence of an unanticipated non-canonical cation-π interaction.

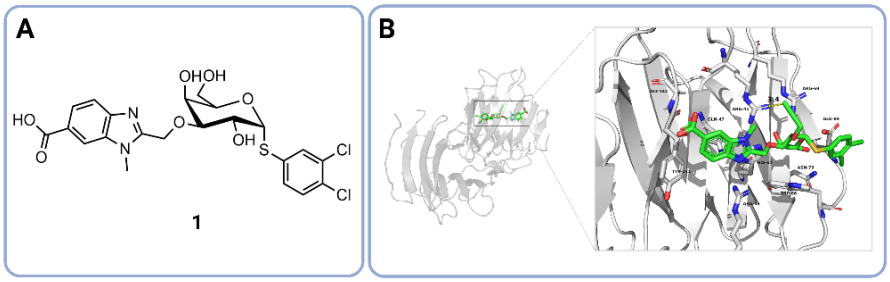

Figure 1. (**A**) The structure of lead **1**. (**B**) A crystal structure of **11** in complex with galectin-8N.

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AUTOMATED SYNTHESIS OF ACINETOBACTER *BAUMANNII* POLYSACCHARIDE FRAGMENTS

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Acinetobacter *baumannii* is a Gram-negative opportunistic pathogen responsible for hospital-acquired infections such as pneumonia, meningitis, urinary trait infections, etc. In the Bacterial Priority Pathogen List 2024 compiled by the WHO, the bacterium has been classified as critical: high mortality rates associated with an alarming spread of multi-drug resistant strains make its treatment quite challenging [1-3].
To fight infections, research is focusing on developing alternative therapies including vaccines based on A. *baumannii* capsular polysaccharide (CPS): surface polysaccharide consisting of tightly repeating oligosaccharide subunits that protects the bacterium against variuous external insults [4].
This project, funded by the European Union, focuses on the automated-glycan assembly (AGA) of different fragments from A. *baumannii* ATCC17961 and ATCC17978 CPS. This automated approach, deviating from the one in solution reported so far [5], will enable the synthesis of oligosaccharide fragments with varying repeating units but also lenghts since multiple consecutive units will be assembled on solid-phase. Additionally, AGA will allow us to explore, quickly and effortlessly, structures with different acetylation patterns, since the presence or absence of an acetate group is a key difference between the two reported strains **(Fig.1)** [6].

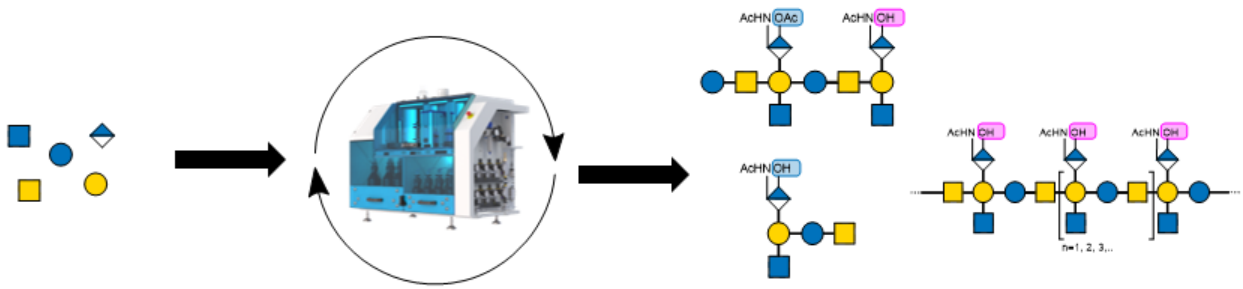

Figure 1. AGA scheme and products

Here I present the first progresses in the project with the design and synthesis of the building blocks and the AGA workflow.

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PHOTOSWITCHABLE GLYCOLIPIDS: ON SYNTHESIS AND MEMBRANE EXCITATION

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Photoswitchable surfactants have proven themselves to be an invaluable tool for membrane research. Employing these artificial lipids allows for the introduction of membrane internal stimuli, yielding profound structural rearrangement while minimizing external system perturbation for highly precise measurements. The field ranges from research on the inner workings of membranes, such as bidimensional phase transitions [1] and changes to lipid mesophases [2], to evaluating the influence of membrane states on the function of membrane-embedded proteins [3,4]. As a non-ionic and spatially demanding head group, carbohydrates provide a unique range of properties to these surfactants and present, with their unmatched potential for derivatizations, a promising foundation for tailoring membrane properties.

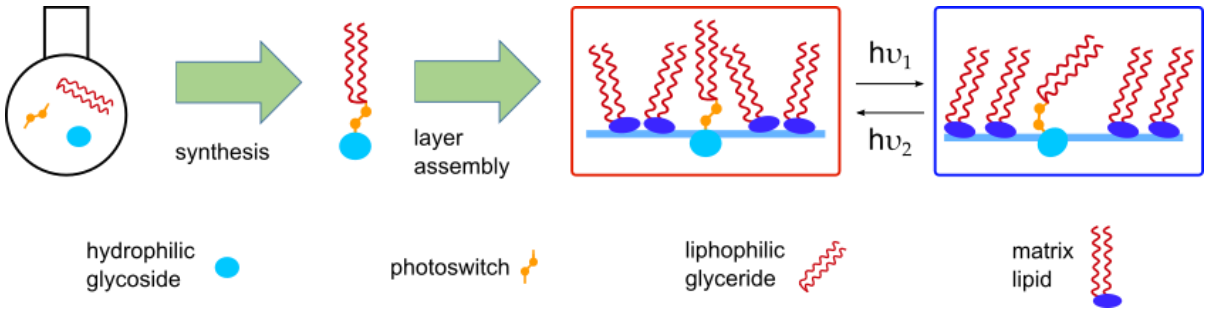

Figure 1. The synthesized photoswitchable amphiphiles are assembled in layer systems and used to emulate stimuli occurring in membranes causing rearrangements and phase transitions.

The synthetic combination of an azobenzene-based photoswitch between the polarity-wise strongly opposing head and tail group puts severe limitations on protecting group-based carbohydrate chemistry. Considering additional requirements defined by the model system, like miscibility and molecular rigidity, these multifunctional compounds make for an interesting synthetic challenge. To cover a broad range of lipid geometries, the synthesized surfactants include single-chain, sterol and glycerol-based lipids. Glucose was used as the default head group, which was optionally replaced by maltose or maltotriose. The photoswitchable glycolipids were employed in the reversible switching of mesophases in multilamellar arrangements and analyzed by small angle X-ray scattering. The synthesis of the photoswitchable surfactants will be presented alongside an elucidation of the glycobiophysical context.

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UNVEILING THE MOLECULAR MECHANISM OF HUMAN LYSOSOMAL A-GLUCOSIDASE

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Lysosomal storage diseases count more than 70 different diseases, collectively affecting more than 1 in 5,000 live births. One of the most prevalent ones is Pompe disease (PD, glycogen storage disease type 2), where the mutated lysosomal α-glucosidase (α-GAA) can not catalyze the breakdown of glycogen and other large glycosides into glucose units. Only 12% of patients with a so-called classic infantile form of PD live longer than 18 months if not treated. Nonetheless, PD has no cure yet – only enzyme replacement therapy, with a limited clinical efficiacy, is employed for treating the sympthoms [1,2].
In this work, by means of computational chemistry methods, we employ molecular dynamics and QM/MM metadynamics to unravel the molecular mechanism of the α-GAA enzyme, describe the the conformational space of sugar substrate during the catalysis and the most important protein-ligand interactions. The enzyme, as a retaining hydolase follows a double displacement Koshland mechanism, with characteristic conformational changes in the leaving sugar conformations. The results obtained are used to propose new pharmacological chaperones, that could be used either with an enzyme replacement therapy or to stabilize selected versions of the defective enzyme.

Acknowledgements: This work was supported by Polish National Agency for Academic Exchange (NAWA) Bekker Programme.

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2,5-ANHYDROSUGARS AS SUBSTRATES IN THE REACTION WITH VANCOMYCIN

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Vancomycin is a glycopeptide antibiotic used to fight infections with Gram-positive bacteria. It is often the antibiotic of last resort in the treatment of methicillin-resistant (MRSA) or vancomycin-resistant (VRSA) strains of *Staphylococcus aureus* [1].
In order to increase the efficacy of vancomycin and at the same time reduce its toxicity, many of its modifications are synthesized. Typical modification sites are the aglycone, the amino group of vancosamine and the side carboxyl group of the cyclic heptapeptide [2].
Here we present the synthesis of four vancomycin analogues (Figure 1). Two modifications involved reductive amination of the vancosamine amino group with 2,5-anhydro-D-mannose or 2,5-anhydro-D-tallose. The other two involved attachment of 1-amino-2,5-anhydro-1-deoxy-D-mannitol or 1-amino-2,5-anhydro-1-deoxy-D-tallititol to the carboxyl group of the cyclic heptapeptide. It is worth emphasizing that due to the addition of 2,5-anhydroalditol derivatives to vancomycin, the analogues obtained in this way showed better solubility in water than vancomycin.

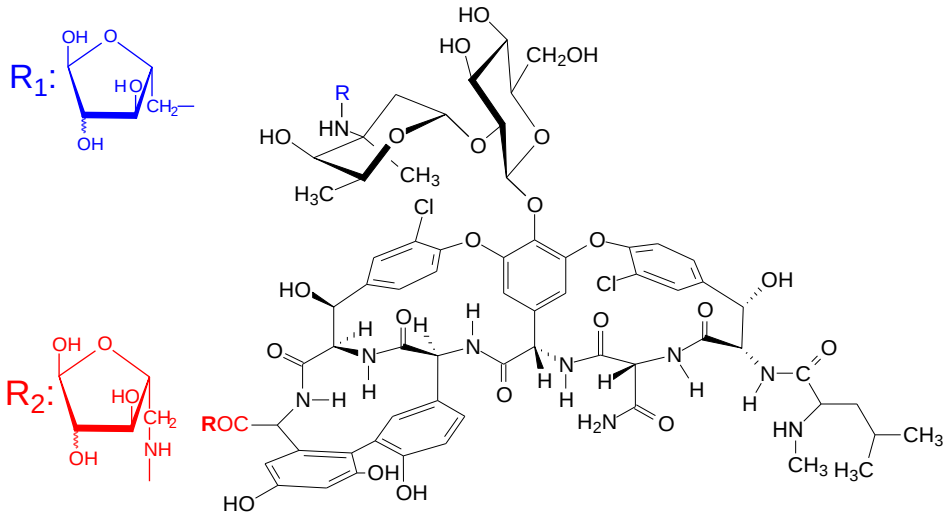

Figure 1

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A MULTIPLEX METHOD FOR HIGH-THROUGHPUT QUANTIFICATION OF CONJUGATED SACCHARIDE IN GLYCOCONJUGATE VACCINES: MENACWY AS KEY STUDY

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Advancements in vaccine research have significantly enhanced human health by preventing many bacterial and viral infections. In this context glycoconjugate vaccines, which combine a polysaccharide (PS) or oligosaccharide (OS) antigen with a carrier protein, have been developed and commercialized [1]. Since only the conjugated portion is highly immunogenic, a key challenge in their production is the accurate monitoring of saccharide content throughout the entire process. Moreover, distinguishing between free and conjugated (or total) saccharides is essential to ensure regulatory compliance [2,3]. Therefore, a robust analytical platform is essential to quantify PS/OS during various production stages, including the drug substance (DS) and the final drug product (DP). Current methods, such as HPAEC-PAD and colorimetric techniques require multiple sample manipulation steps (conjugate precipitation, ultrafiltration, and solid phase extraction), which are time-consuming and material-intensive [4]. This project aims to develop new, faster, and more accurate analytical platforms for saccharide quantification in multivalent glycoconjugate vaccines. To achieve this goal, various analytical tools were explored, incorporating both physico-chemical and immune-based assays, with MenACWY vaccines as case studies. The first attempt to analyze all four antigens in a one-shot analysis was performed using capillary electrophoresis (CE) techniques. Micellar electrokinetic chromatography with UV-Vis detection (MEKC-UV) showed good results in the oligosaccharides and their respective free saccharides separation at DS level. However, it showed significant limitations when all four antigens were analyzed simultaneously. Moreover, the use of UV-Vis detection did not allow for accurate quantification of low levels of free saccharide, which lack suitable chromophores. To increase the sensitivity of our tools, we shifted our focus to immuno-based approaches, evaluating a multiplex bioassay. This method enables the selective and sensitive detection of all serotypes simultaneously using color-coded magnetic beads coupled with serotype-specific antibodies and a phycoerythrin conjugate species as reporter [5]. To this end, several configurations of the sandwich assay were designed and developed, aiming to create a sensitive method capable of detecting all four antigens simultaneously. Very promising results were obtained for the conjugate attribute, while the quantification of free and total saccharides together is still ongoing.

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PHYSICOCHEMICAL CHARACTERIZATION OF *RHODOTHERMUS MARINUS* DSM 16675 EXOPOLYSACCHARIDES AS BIOEMULSIFIER

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The exopolysaccharides (EPSs) produced by *Rhodothermus marinus* DSM 16675 were studied for their emulsifying capabilities and chemical composition under various cultivation conditions. Both batch and single-cycle fed-batch fermentations were assessed, with the highest EPS yield (20 mg/L) obtained during fed-batch cultivation. The EPSs showed strong emulsifying activity, achieving an emulsification index (E24) of 64.3%. Chemical analysis identified the EPS as a heteropolysaccharide mainly composed of rhamnose, arabinose, and mannose, with a protein content of 19.22 ± 0.29%, indicating a glycoprotein nature. Fatty acid profiling revealed the presence of both covalently bound and encapsulated lipids, with a total lipid content of 10% (w/w). Optimal emulsifying performance was observed at a concentration of 5 g/L, within a pH range of 7–9, and temperatures between 4°C and 25°C. However, emulsifying stability declined under high salinity and extreme pH conditions. Dynamic light scattering (DLS) analysis revealed a bimodal particle size distribution with a Z-average of 260 nm and a moderately negative ζ-potential of –26.88 mV, indicating good colloidal stability. The EPSs maintained emulsifying activity over time and under moderate conditions, demonstrating their promise as natural emulsifiers for use in pharmaceutical and biotechnological applications.

Acknowledgement: This work was supported by funding from the European Community's Horizon 2020 Programme (Grant Agreement No. 101000794), for which we are sincerely grateful.

BIO-CHEMICAL INVESTIGATIONS OF LIPOOLIGOSACCHARIDE OF CLINICAL ISOLATE OF NTHi RELEVANT FOR THE CHRONIC LUNG DISEASES

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Non-typeable *Haemophilus influenzae* (NTHi) is a common commensal of the human nasopharynx and an opportunistic pathogen associated with infections of both the upper and lower airways. Accumulating evidence suggests that NTHi is also a common driver of asthma and chronic obstructive pulmonary disease (COPD) exacerbations. With no vaccines in development, NTHi remains a major epidemiologic concern [1]. This pathogen displays a variety of virulence factors, such as lipooligosaccharide (LOS) which are involved in pathogenesis, immune evasion, and adherence to host cells [2]. Our preliminary data showed that NTHi infections can induce tolerance in primary bronchial epithelial cells (NHBE), which could drive chronic NTHi infections. However, the enhanced CXCL-8 levels after *Escherichia coli* LPS and Pam3CSK4 stimulation in NHBE previously treated with inactivated NTHi suggest that some bacterial ligands, such as LOS, may induce a memory in epithelial cells [3]. Nevertheless, the specific role of NTHi LOS in trained immunity and its relationship with the development of asthma and COPD remains unknown.

To elucidate the biological and chemical properties of LOS in the context of chronic lung diseases we have cultivated NTHi strain H-189, a clinical isolate from pharyngeal smear of patient with pneumonia (kindly provided by Prof. Rupp, UKSH, Lübeck) in HTM medium [4] and isolated LOS [5]. Pure LOS was subjected to SDS/PAGE which indicated the presence of a longer LOS molecule in NTHi H-189 than typically expressed for the NTHi rough LPS [2]. Thus, in the next steps detailed chemical analysis via GC and GC/MS of LOS and its fractions will be performed. In parallel, we aim to uncover the host immune mechanisms regulated by NTHi LOS. To achieve this goal, NHBE will be treated with LOS for 24 hours followed by a 7-day resting period. Afterwards, the cells will be subjected to a secondary stimulus using LOS or Pam3CSK4 for the same time. The immune mechanisms will be evaluated by performing a whole transcriptomic analysis from each stimulation point.

In our presentation we will discuss the obtained structure-function results on LOS of NTHi H-189 in the context of chronic lung infections.

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INTERACTIONS OF CD44 WITH TLR4-MD-2 COMPLEX AND IT'S INFLUENCE ON HYALURONAN BINDING

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Toll-like receptors (TLRs) are widely distributed in the gastrointestinal tract as part of the innate immune system, making them relevant in the treatment of carcinomas of the duodenum, small intestine, colon, and rectum. Short hyaluronic acid (HA) oligosaccharides activate signaling cascades upon binding to TLR4-MD2 complex, leading to the production of angiogenic cytokines IL-1β and IL-8. HA plays a key role in drug resistance mechanisms, as cancerous cells exhibit a higher proportion of low molecular weight hyaluronan (LMW-HA) in the extracellular matrix (ECM). This triggers pathways responsible for ECM remodeling and promotes tumor cell aggressiveness.
Immunoprecipitation studies have shown that CD44 and TLR4-MD2 complex can physically associate. Interestingly, CD44-knockout cells still exhibit HA binding to TLR4, indicating that TLR4-induced pathways are CD44-independent but can benefit from CD44 presence. We study three hypotheses regarding how CD44 assists HA binding, which can be tested using molecular dynamics simulations: (1) CD44 blocks HA from interacting with off-target regions of the TLR4-MD-2 complex, (2) CD44 actively binds HA, limiting its degrees of freedom and directing it to the TLR4-MD-2 binding site, and (3) CD44 increases the local effective concentration of HA around the TLR4-MD-2 complex.
In this work, we use enhanced sampling molecular dynamics simulations to find optimal structure of the CD44-TLR4-MD-2 complex. Our model incorporates existing glycosylation data for these proteins, which limits their interaction surface-accessible area. The placement of HA-binding residues of CD44 in proximity to TLR4-MD-2 found binding motive suggests that CD44 acts as a facilitator by maintaining HA close to the complex, thereby increasing the likelihood of cascade activation. Found complex structure is used to assess differences in binding HA by TLR4-MD-2 with and without presence of CD44.

ELECTROCLICK-DRIVEN CARBOHYDRATE VECTORIZATION: A PROMISING GLYCOCALYX EDITING STRATEGY

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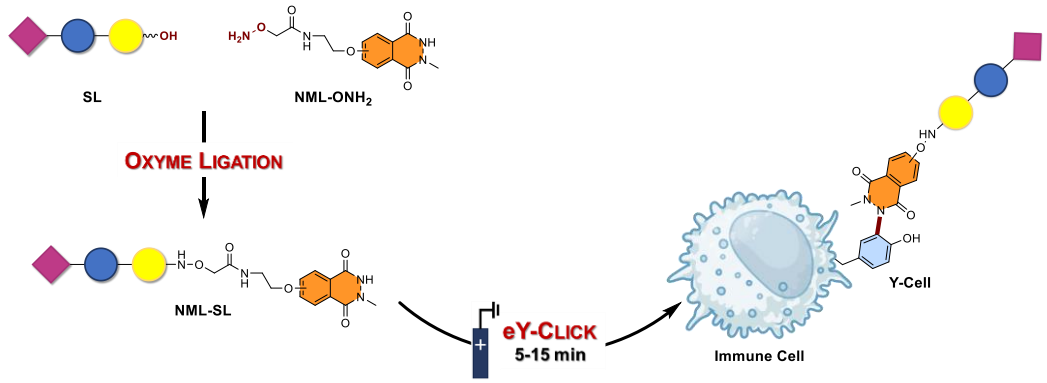
Genetic or chemical modifications of cell membranes are revolutionary for advancing diverse therapeutic approaches and gaining deeper insights into biological processes. We recently introduced eY-Click, a biocompatible electrochemical method to functionalize native proteins [1]. Employing a *N*-methylluminol (NML), a fully tyrosine-selective protein anchoring group, we were able to functionalized cell surfaces from viruses, living bacteria and eukaryotic cells models, with simple molecules in just minutes [2].
In this study, we extended this strategy to apply it to more complex biomolecules, especially by taking advantage of biological relevant oligosaccharides. To achieve this, we synthesized NML-glycoconjugates via oxime ligation between unprotected sugar and an aminoxy-functionalized NML. Our electro-bioconjugation method was successfully applied to a protein model, paving the way for the development of glycoproteins, which are widely explored for the study of biological process and vaccine applications. Finally, we vectorized the membrane surface of immune cells with NML-SialylLactose (NML-SL) conjugates, aiming to enhance their ability to recognize and target B-cell lymphoma, known to overexpress the siglec-2 receptor (CD22). This innovative solution offers a promising new strategy for advancing cancer immunotherapy beyond CAR-T or metabolic engineering, providing a streamlined and versatile approach to immune cell functionalization.



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PARALLEL SYNTHESIS AND SCREENING OF SULFATED GLYCOSAMINOGLYCAN LIBRARIES

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Eukaryotic cells are enveloped by a dense carbohydrate layer that plays a crucial role in interactions such as pathogen attachment to host cells. A key class of these carbohydrates are sulfated glycosaminoglycans (sGAGs), such as heparan. While traditionally recognized for their anticoagulant properties, sGAGs are increasingly being studied for their antiviral properties. However, research on these molecules is hindered by the limited availability of well-defined sGAG structures from biological sources.
Through solid-phase polymer synthesis, we can precisely generate sequence-defined, monodisperse, oligomeric sGAG mimetics with tailored properties [1] to study their binding affinity and selectivity against different pathogens. In addition to a stepwise assembly approach, we are working on a parallel synthesis strategy to create diverse libraries of sGAG mimetics using solid-phase techniques. By integrating this parallel synthesis with high-throughput screening, we aim to accelerate the discovery and optimization of sGAG mimetics. As a basis for establishing this parallel synthesis approach, we utilize the "SPOT synthesis" developed by Frank et al. [2] Using solid-phase synthesis on cellulose membranes and copper(I)-catalyzed click chemistry, we can generate a diverse range of glycooligomers in good purities. Furthermore, by using specialized cellulose membranes, we are able to sulfate the synthesized glycooligomers directly on the membrane and thus generate the sGAG mimetics (see Figure 1).

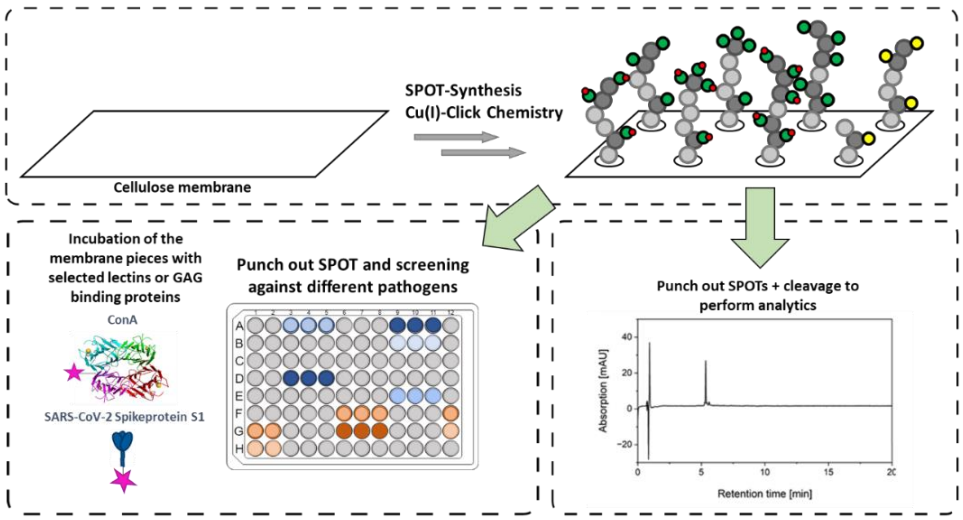

Figure 1. Parallel synthesis of sGAG mimetics on membrane support and screening against various pathogens.

The derived membrane-bound glycooligomer libraries are then screened for binding with various lectins and pathogens.

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DESIGN AND SYNTHESIS OF SIALOSIDES AS FICOLIN-1 ANTAGONISTS

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As integral part of the host's antimicrobial defense, the complement system can mount a quick and effective response by causing inflammation and cell lysis and by inducing downstream immune processes. The recognition of microbial carbohydrate signatures by pattern recognition receptors (PRRs) of the complement system's lectin pathway, including mannose-binding lectin (MBL), collectins, and ficolins plays a critical role in this process [1,2]. However, as observed during severe cases of COVID-19, a fulminant activation of complement can cause adverse thromboinflammatory states. Therefore, there is an unmet need to elucidate this interaction network on a molecular level and to develop glycomimetic entities to inhibit (or enhance) such recognition events.

Ficolins are oligomeric lectins that have been identified as PRRs for a wide range of disease-triggering pathogens, including eukaryotic protozoa, bacteria, and viruses, and also play a major role in the pathogenesis of several autoimmune diseases. They mainly bind to *N*-acetylated glycans such as Glc*N*Ac, Gal*N*Ac and Neu*N*Ac, although with distinct specificity [3,4].

Based on available 3D structures, glycomimetic compounds were designed, synthesized and evaluated for their antagonistic activity to the recombinantly expressed CRD of ficolin-1. Several of the newly designed sialosides showed improved binding affinity and inhibitory efficacy when compared to Neu*N*Ac. Therefore, our initial structure-activity relationship experiments for neuraminic acid and derivatives thereof show promising activity profiles for further development.

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OHMIC HEATING-ASSISTED EXTRACTION OF PECTINS FROM RASPBERRY POMACE

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Pectins can be commercially extracted from several agricultural byproducts. However, conventional extraction has several limitations such as thermal degradation, undesirable physicochemical and functional properties, and low degrees of esterification due to prolonged, direct heating than the emerging processing technologies. Emerging thermal and non-thermal technologies, as well as their combination, have been explored [1]. The extensively promising novel techniques for extraction and isolation of bioactive compounds appears to be microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), pressurized hot water extraction (PHWE), pressurized liquid extraction (PLE), pulsed electric field assisted extraction (PEFAE), ohmic heated assisted extraction (OHAE) [2]. Due to the internal thermal energy generation mechanism, OH could significantly reduce energy consumption compared to conventional extraction, which is beneficial.
The aim of this study was to find the most convenient process parameters for extraction of pectin from waste pomace after blackcurrant juice production, supported by Ohmic heating (OH), and then to compare the physicochemical properties of this product with pectin obtained from the same raw material, but extracted by the conventional extraction method. In these experiments, the extraction system was allowed to reach a maximum temperature, close to the boiling point of the extraction medium (~90°C), regardless of the heating source. The effect of the heating agent on the yield and quality of the obtained pectin was investigated, also taking into account different types of extraction medium, i.e. aqueous acidic solution, alkaline solution, and NADES.

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TOTAL SYNTHESIS OF THE ZWITTERIONICTRISACCHARIDE REPEATING UNIT OF *FUSOBACTERIUM NUCLEATUM* ATCC51191 O-ANTIGEN

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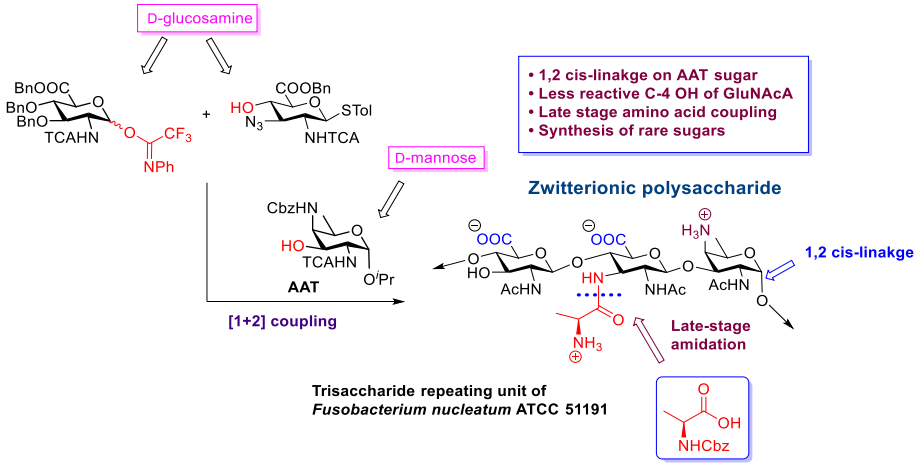
Fusobacterium nucleatum, an oral anaerobe linked to various cancers and Alzheimer's disease, which has gained attention as a potential antibiotic target and vaccine candidate [1]. This work builds on prior research focused on bacterial glycans and their roles in host-pathogen interaction. Herein, we report details an efficient total synthesis of the zwitterionic trisaccharide repeating unit from *Fusobacterium nucleatum* ATCC 51191. Remarkable features of the target molecule are the presence of rare sugars and multiple nitrogen atoms across three sugar units, along with carboxylic acids and an amide linkage [2]. Key challenges associated with the synthesis are: 1, 2-cis Glycosidic linkage (achieving the desired linkage in the AAT sugar), glycosylation (using GlcNAcA donors with the less reactive 4-OH group), late-Stage Coupling (conducting amino acid coupling in a sterically hindered environment), rare Amino Sugar Synthesis (synthesis of orthogonally protected rare sugars). The synthesis contains a Cbz protecting group in the AAT building block, which act as to temporarily mask the C4-amino function and ensure 1, 2-cis selectivity with isopropanol. The strategy includes late-stage amide bond formation and early-stage oxidation at the monosaccharide level, promote efficient assembly of the target molecule. This synthetic approach also useful for the total synthesis of other frame shift trisaccharides and longer oligomers. The total synthesis involved a 22-step linear process, yielding 2.3%.



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DESIGN AND SYNTHESIS OF INHIBITORS FOR SIALIC ACID ESTERASES

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Human cells are coated with a dense array of glycoproteins and glycolipids called the glycocalyx [1]. Sialic acids are nine-carbon monosaccharides that are mostly found as the terminal residues of glycans forming the glycocalyx with N-acetyl-5-neuraminic acid (Neu5Ac) being the most common member of this family [2]. A common post translational modification of glycoproteins, is the O-acetylation of the hydroxyl at C-4, 7, 8, and/or 9 on Neu5Ac which is important in cell-cell signaling, autoimmunity and viral infection [3,4]. O-acetylated-Neu5Ac is a receptor for Influenza C and type 2a coronaviruses and toroviruses [5]. These viruses bind host cell surface O-acetylated-Neu5Ac-glycoproteins via hemagglutinin-esterases (HEs) or a spike protein followed by cleavage of the acetyl-group, leading to the destruction of the receptor. Viruses bearing non-functional HEs have reduced infectivity, highlighting their potential anti-viral targets [6]. The goal of this project is to design and synthesize covalent inhibitors and non-hydrolysable substrate mimics, targeting viral sialic acid esterases (SAEs). These compounds expand the chemical space available for sialic acid modifications and will be tested for their efficacy against recombinant 9-O-SAEs.

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THREE-STEP ENZYMATIC SYNTHESIS OF BIOACTIVE CHITOOLIGOMERS

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Chitooligomers (COS, β-1-4-linked oligomers of *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcN)) are bioactive compounds with diverse beneficial properties, such as antitumor, immunomodulatory, antiangiogenic, antioxidant, and antimicrobial. Importantly, they can activate plant defense mechanisms and protect plants from diseases indirectly as elicitors of resistance against bacterial, fungal, and insect pathogens [1]. The biological activity of COS depends on their degree of polymerization (DP) and the degree of acetylation (DA). Longer COS of DP 6-8 exhibit higher antifungal activity and stronger affinity to the respective plant receptors than shorter COS of DP 4-5, inducing thus chitin-responsive genes more effectively. Unfortunately, enzymatic synthesis of COS with higher DP faces many challenges and the development of a robust and scalable method for the synthesis of COS with DP ≥ 6 is of eminent importance.
Here we describe a pioneering enzymatic approach for the efficient transformation of low-DP COS obtained from chitinase-hydrolyzed chitin, waste material of the food industry, into valuable chitooligomers with a degree of polymerization ranging from 6 to 11. In the first step, chitin was hydrolyzed using engineered variants of the novel fungal chitinase from *Talaromyces flavus* with increased hydrolytic activity to generate low-DP chitooligomers, followed by an extension to the desired DP using the high-yielding variants of β-*N*-acetylhexosaminidase from *Aspergillus oryzae* [2], achieving yields of insoluble COS (DP ≥ 6) up to 57% [3]. Subsequently, the prepared chitooligomers can be partially deacetylated using novel fungal chitin deacetylases. This innovative enzymatic route demonstrates sustainability and feasibility for the transformation of waste chitin into unavailable bioactive chitooligomers, providing a potential application of the prepared COS in zero-pesticide organic agriculture.

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STRUCTURAL INSIGHTS INTO SIGLEC-10 INTERACTIONS WITH T-CELLS

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Sialic acid-binding Ig-like lectin 10 (Siglec-10) is an immune modulator and emerging cancer immunotherapy target [1]. However, limited understanding of its structure and mechanism of action hinders the development of drug candidates and hence preventing the unleash of its full therapeutic potential. In this study, we elucidate the crystal structure of Siglec-10 and its binding epitope for an anti-Siglec-10 antibody (Ab). Interestingly, besides the conserved canonical arginine (R119) located on β strand F of the carbohydrate recognition domain, we have identified a second arginine residue (R127) on β strand G. Furthermore, we demonstrate the importance of both, the canonical R119 and R127 residues in the interactions of Siglec-10 with T cells. Additionally, we showcase that binding of Siglec-10 to T cells depends on the presence of α(2,3)- and α(2,6)-linked sialoglycans, which can be blocked with anti-Siglec-10 Ab. Collectively, our findings provide an integrated understanding of the structural features of Siglec-10 and emphasize glycosylation as a crucial factor in controlling T cell responses.

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FUNCTIONAL IMPACT OF *O*-GLYCANS ON SURFACE MANNOPROTEINS IN CRYPTOCOCCUS NEOFORMANS: INSIGHTS INTO THE SECRETION, STABILITY, AND IMMUNOGENICITY OF CDA1 AND MP88

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The human pathogenic yeast *Cyptococcus neoformans* assembles two types of *O*-glycans on its proteins; major *O*-glycans without xylose and minor *O*-glycans containing xylose, which are mediated independently by two mannosyltransferases encoded by *KTR3* and *CAP6* [1]. In this study, we investigated the effect of altered *O*-glycan structures of glycosylphosphatidylinositol-anchored mannoproteins, MP88 and chitin deacetylase I (Cda1) [2,3], on proteolytic cleavage and host-cell interactions. *KTR3* deletion (*ktr3*Δ) led to the accumulation of truncated *O*-glycans with single-mannose residues and the increased proportion of xylose-containing *O*-glycans. Notably, the absence of extended major *O*-glycans caused aberrant cleavage of the Cda1 protein, resulting in the loss of its glycosylphosphatidylinositol-anchor. The host cells incubated with the purified MP88 and Cda1 proteins from *cap6*Δ showed decreased IL-6 secretion compared to those exposed to the mannoproteins from *ktr3*Δ. Moreover, mice immunized with Cda1 produced by *ktr3*Δ showed higher anti-Cda1 antibody production compared to those immunized with wild type-Cda1, consistent with the enhanced IL-6 secretion. These results highlight the contribution of xylose residues in *O*-glycans to the induced host immune response. Understanding the structure and roles of *O*-glycans assembled on immunogenic proteins, particularly their roles in protein stability and host-cell interactions, would be useful in designing vaccines against fungal infections.

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STRUCTURAL INSIGHTS INTO GALECTIN-3 RECOGNITION OF A SELENOGLYCOMIMETIC

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Galectins, a family of carbohydrate-binding proteins, are critically involved in various physiological and pathological processes, including fibrosis and carcinogenesis, where Galectin-3 (Gal-3) is often up-regulated, making it a promising therapeutic target [1-3]. Galectin-3 is defined by a single carbohydrate-recognition domain (CRD) of approximately 130 amino acids, which displays a particular attraction for galactosides [4]. Selenoglycomimetics, particularly seleno-digalactoside (SeDG) and its benzyl-modified derivative SeDG-Bn, have shown potential as Gal-3 inhibitors [5,6], especially in the context of treating cancerous conditions. Selenium can modulate inflammation [7], potentially affecting galectins. Studies show selenium may reduce inflammatory markers in conditions like arthritis and heart disease, possibly influencing galectin pathways. We employed ligand-based and protein-based Nuclear Magnetic Resonance (NMR) spectroscopy to elucidate the molecular details of the interaction between SeDG-Bn and Gal-3 [8]. Specifically, we utilized saturation transfer difference (STD)-NMR to probe ligand binding, and chemical shift perturbation (CSP) experiments to identify the binding site on Gal-3. Our NMR studies revealed significant binding affinity of SeDG-Bn for Gal-3, with specific residues within the canonical binding site, including the β-sheets S4-S5-S6, comparable to SeDG, showing important chemical shift perturbations upon ligand binding. Furthermore, notable distinctions were observed, illustrating the effect of the benzyl modification on how the ligand is recognized. Specifically, SeDG-Bn induced changes in additional amino acids within the S2 and S3 β-sheets. CSPs were analyzed to determine the binding affinity (K_D) of hGal-3 (CRD) for SeDG and SeDG-Bn using key perturbed residues. Also, STD-NMR revealed the SeDG-Bn binding epitope, showing strong interaction with aromatic protons and some involment of galactose protons. These findings provide valuable insights into the molecular recognition of Gal-3 by SeDG-Bn, supporting its potential as a therapeutic agent for conditions associated with Gal-3 overexpression. The detailed binding information obtained through NMR spectroscopy can further guide the rational design of more potent and selective Gal-3 inhibitors.

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SITE-SPECIFIC GLYCO-TAGGING OF ALLERGENS WITH HIGH AFFINITY SIGLEC-9 LIGANDS FOR IMMUNOTHERAPY

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Food allergies are a major health concern that in the most severe cases can result in life-threatening anaphylaxis. Strict avoidance of an allergen is the most common approach to avoid severe disease but in daily practice difficult to achieve. Thus, there is a need to develop approaches to prevent or manage food allergies. There is data to support that modification of an allergen with a ligand for a sialic acid binding immunoglobulin-like lectin (Siglec) can provide possible immunotherapies for severe allergies [1,2]. Reported approaches to attach a Siglec ligand to an allergen are based on random conjugation to for example side chains of lysine residues. This approach does not provide control over the number of attached Siglec ligands and may result in masking or modifying of specific epitopes. To address these limitations and gain a better understanding of structure-function relationships of the sialic acid-Siglec axis, we describe here a methodology to attach in a controlled manner between 1 and 9 Siglec-9 ligands to a protein by peptidoligase catalyzed modification of the *N*-terminus with a synthetic glycopeptide ester having a different number of azides. Next, strain-promoted azide-alkyne cycloaddition (SPAAC), allowed the introduction of (high affinity) Siglec-9 ligands or clusters thereof. GFP was used as a model protein and β-lactoglobulin as allergen. Cell-based studies established the importance of valency and the affinity of the monovalent ligand for biological activity.

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STRUCTURAL ANALYSIS OF BACTERIAL ENVELOPE COMPONENTS

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Bacterial cells express structural diverse glycans on the surface. Based on the type of cell envelope, bacterial species are classified into two large groups: Gram-negative bacteria and Gram-positive bacteria. Gram-negative bacteria have an outer membrane and present lipopolysaccharides (LPSs), amphiphilic molecules composed of three different moieties namely lipid A, core oligosaccharide and O-antigen polysaccharide. Depending on chemical structure of LPSs, the host innate immune system is elicited. On the other hand, Gram-positive bacteria lack an outer membrane but are protected by thick peptidoglycan layers. Some Gram-positive bacteria have unique polysaccharides such as arabinogalactans (AGs) from *Mycobacterium tuberculosis* (Mtb). AGs have long and highly branched chain containing Ara*f* and Gal*f* residues. AGs are known to perform important roles in infectivity and pathogenicity of tuberculosis, which is one of the most serious diseases [1].
To elucidate the role of bacterial glycans in immune system and provide insights for development of new biomedical therapies, we focused on isolation, characterization and molecular recognition at the atomic level. In this context, a multidisciplinary approach combining wet lab with synthesis, NMR experiments and computational studies [2–4], was followed in order to extract, purify, characterize and define the 3D structure of the LPSs from Gram-negative strains, like *Bacteroides uniformis*. A similar approach was applied to the synthetic AGs, from 14-mer up to 92-mer, from Mtb (Gram-positive, Figure.1).

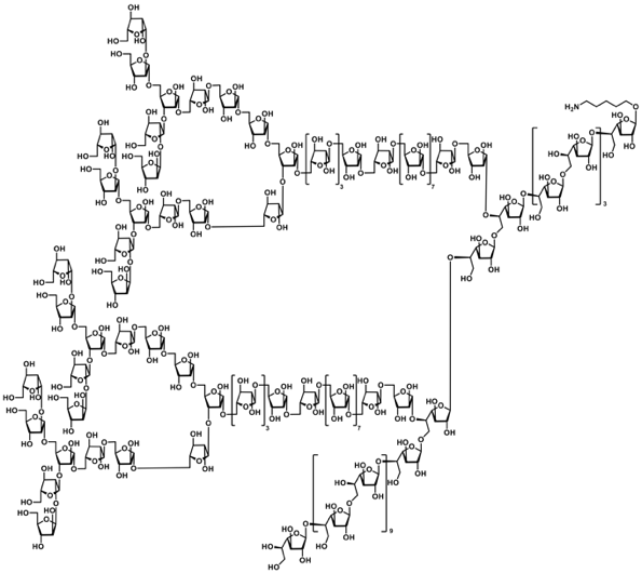

Figure 1. The structure of arabinogalactan 92-mer

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ROLE OF LONG-RANGE INTERACTIONS IN STRUCTURE AND DYNAMICS OF CARBOHYDRATES

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Carbohydrate oligomers play a significant role in various biological processes, such as energy storage, intercellular communication, and structural polymer formation [1,2]. To be able to convey these diverse functions, carbohydrate structure and dynamics are mainly governed by interactions between adjacent monomers of the polymer sequence and feature very little interactions between units far away in sequence. Herein, we use molecular dynamics simulations to examine the effect of interactions between non-adjacent monomers on the overall structure of small oligosaccharides and *N*-glycans. The difference in conformational dynamics between a full glycan and its disaccharide constituents can be attributed to interactions between non-adjacent monomers. First, we observe that among all possible trimes of Glucose, Galactose, and Mannose, only those with branched structures and adjacent glycosidic bonds exhibit non-negligible differences in equilibrium distributions. Next, we investigated the impact of long-range interactions between non-adjacent sugars in 33 common *N*-glycans. We observe that only a few glycosidic bonds such as alpha 1-3 and alpha 1-6 glycosidic bonds between mannose monosaccharides within the $GlcNAc_2Man_3$ pentasaccharide core, core fucosylation, exhibit different dynamics that the disaccharide fragments. These findings confirms that carbohydrate's structural behavior is mainly dominated by short-range interactions between adjacent monomers, but for few specific sequences, such as those present in Lewis antigens [3], the effect of non-adjacent interactions becomes non-negligible. A better understanding of the role long-range interactions play in carbohydrates structure formation and dynamics will result in new simulation techniques being developed, force-field designs being developed, as well as practical applications involving the discovery of new biomaterials and the design of novel carbohydrate-binding drugs.

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DEVELOPMENT OF PATHOBLOCKERS TARGETING ECLA: A NOVEL LECTIN FROM *ENTEROBACTER CLOACAE*

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Enterobacter cloacae, a Gram-negative opportunistic human bacterium, is one of the highly virulent and antibiotic resistant ESKAPE pathogens and classified by the WHO (2024) as 'critical priority' for the development of new antibiotics [1]. EclA, a newly discovered lectin from *E. cloacae*, is a key player in cell attachment, biofilm formation [2,3]. The inhibition of lectins with glycomimetics can hinder the infection process and effectively attenuate pathogenesis while minimizing the risk of development antimicrobial resistance [4].
In previous studies, glycan array analysis indicated a high specificity of EclA for the Lewis^a antigen and the type II H-antigen (blood group O) over Lewis^x, Lewis^y, and Lewis^b antigens as well as blood group A or B. In competitive binding assay including several monosaccharides, EclA showed affinity to L-fucose, methyl α-L-fucoside and methyl β-L-fucoside in the millimolar range. Moreover, EclA hemagglutinated blood group O red blood cells. The addition of fucose resulted in disruption of the hemagglutination activity [3].
We hypothesize that fucomimetics that bind with higher affinity to EclA would contribute to a reduced virulence in *E. cloacae* infections. In this work, we screened a number of fucosides, fucosyl amides and fucomimetic derivatives through a fluorescence polarization-based competitive binding assay. Results showed 10-fold increased affinity compared to methyl L-fucosides and 40-fold than L-fucose. Synthesis of C-4 modified fucose derivatives is currently performed, evaluating their activity as inhibitors of the lectin EclA and exploring the structure-activity relationship for further optimization.

Acknowledgements: This work was supported by the TALENTS Marie Sklodowska-Curie COFUND-Action of the European Commission [101081463].

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DEVELOPMENT OF NEW ANALOGUES OF MANNOSE-6-PHOSPHATE

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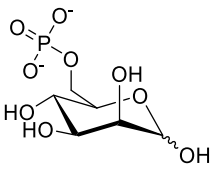
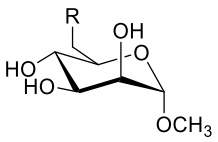
Our research focuses on developing mannose-6-phosphate (M6P) analogues designed to regulate the binding of insulin-like growth factor 2 (IGF2) to the insulin-like growth factor receptor 2 (IGF2R/M6P). IGF2 and IGF2R, along with insulin and IGF1, belong to the insulin superfamily of proteins and play key roles in regulating blood sugar levels, embryonic growth, and overall metabolic homeostasis. Although the exact role of IGF2 remains incompletely understood, its abnormal expression has been linked to severe neurodegenerative diseases, including Alzheimer's disease, Angelman syndrome, autism spectrum disorders, and various types of cancer [1-5].

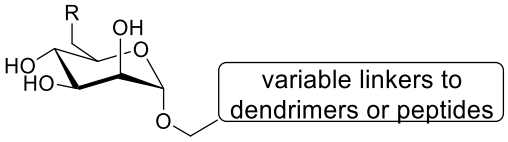
Our goal is to develop isosteric M6P analogues that are resistant to hydrolysis by phosphatases and exhibit higher affinity for IGF2R, thereby modulating IGF2 binding and internalization [6-8]. To further enhance the affinity of M6P analogues, the prepared monosaccharide analogues of M6P were covalently attached to peptides of varying lengths. The affinity of these M6P monosaccharide analogues and glycopeptide dendrimers for IGF2R was determined using fluorescence polarization anisotropy (FPA) [9].

This research may provide new therapeutic strategies for modulating IGF2 levels, with potential applications in treating neurodegenerative diseases and cancers associated with aberrant IGF2 expression.



mannose-6-phosphate (M6P)





R = variable substituents
phosphonate, azole, carboxylic acid

Acknowledgements: This work was supported by the Czech Science Foundation (grant No. 23-05805S) and the grant Specific University Research (grant No. A1_FPBT_2024_003).

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CASE STUDY: DEVELOPMENT OF GB0139 FOR IDIOPATHIC PULMONARY FIBROSIS

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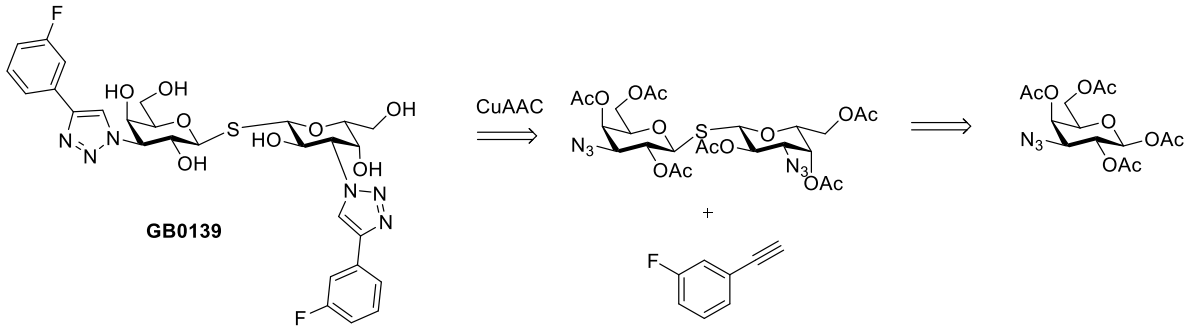
GB0139 is an investigational drug which, until recently, was being developed for treatment of Idiopathic Pulmonary Fibrosis (IPF) by Galecto [1-4]. GB0139 is a specific inhibitor of the galactoside binding pocket of galectin-3.
Dextra collaborated with Galecto to select, and develop, a drug candidate and bring it to the clinic. Following a medicinal chemistry programme, GB0139 was chosen as the candidate for development. Chemistry and manufacturing protocols were developed to allow the provision of material for GLP toxicological studies and subsequent clinical trials. The synthesis of GB0139 uses click methodology to install the aryl triazole via a copper catalysed azide-alkyne Huisgen cycloaddition (CuAAC).



We report our findings from the evaluation of the medicinal chemistry route and present a scalable route to GB0139, which was used to produce 1 kg for IND enabling toxicological studies and 0.5 kg to support Phase 1 and 2a clinical trials (GMP manufacture). A new approach to the regulatory starting material for GMP manufacture, 1,2,4,6-Tetra-*O*-acetyl-3-azido-3-deoxy-D-galactopyranoside, provided IP and reduced the number of steps required [5].

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WOOD-DERIVED XYLOOLIGOSACCHARIDES STRUCTURE-FUNCTION RELATIONSHIP FOR PREBIOTIC FUNCTION AND GUT COLONISATION POTENTIAL FOR HUMAN AND BOVINE-ASSOCIATED GUT COMMENSALS

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In Ireland, 11% of the total land area is covered by forest, and around 8.2 million tonnes of wood waste are produced annually from construction, demolition, and deforestation [1]. Wood waste can provide a sustainable source of wood-derived xylan and xylooligosaccharides (XOS), proposed as next-generation prebiotics for human health and animal feed supplements [2]. However, whether human- and bovine-associated commensals demonstrate different preferences and utilisation of these prebiotics and impact on gut colonisation potential to enhance gut health is unknown. In this study, four lactobacilli species, human-associated (*Lacticaseibacillus rhamnosus*, *Lacticaseibacillus paracasei*) and human- and bovine-associated *(Lactobacillus acidophilus*, *Levilactobacillus brevis*), were investigated for their utilisation mechanisms of xylans and XOS of varying lengths. XOS improved the growth of lactobacilli strains by 5-30%, particularly by mixing different concentrations and lengths of XOS (cocktail mix, CTM). Degradation profiles of xylan and XOS CTM indicated that bacterial utilisation mechanisms were not restricted by host species, but were strain-specific, and *L. brevis* xylanase was produced within 2 h of supplementation. Xylan and XOS also impacted on commensal ability to form biofilms which affects colonisation in the gut. This research provides key insights into the different utilisation mechanisms in human and bovine digestion. Therefore, tailored combinations of valorised xylan and XOS of different lengths could be targeted for different outcomes in animal and human health.

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ENZYMATIC FUCOSE TRANSFER ON C13 LABELED AND NON-LABELED LNH (BRANCHED TYPE I HUMAN MILK OLIGOSACCHARIDE STRUCTURE)

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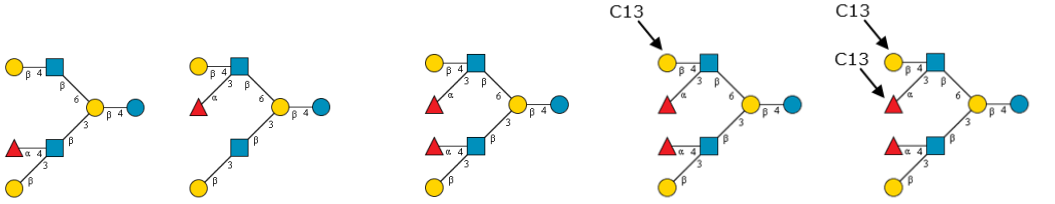
Human milk offers nutrients to promote infant growth and development in the early stages of life. Human milk consists of bioactive substances such as lipids, proteins, and human milk oligosaccharides (HMOs). These HMOs are crucial for protection against infections and for the development of the intestinal microbiome. To establish structure-function relationships and exploit glycoscience for the development of future nutraceuticals and therapeutics, it is essential to determine exact HMO structures in human milk. The development of an Ion Mobility – Mass Spectrometry (IMS-MS) technology provided elucidation of HMO structures with undefined carbohydrate sequences and linkages in Human milk donors. Fragments of HMOs were identified by their accurate mass and CCS values, using fragment ion entries from the reference database, which were used for *de novo* sequence assembly to elucidate the HMO structures. However, in a specific case, the resolution of the IMS system was insufficient to provide a distinction between two CCS values of the fragment ions needed for isomer confirmation of the LNH isomers with a fucose linkage either of α1-3 on the 6-arm (F-LNH-III) or α1-4 on the 3-arm (F-LNH-II). Therefore, enzymatic synthesis was necessary to identify the CCS values of the intact structures of the fucosylated LNH isomers. Furthermore, a hurdle for identification in the positive ionization IMS-MS method was the practical implication of observed fucose rearrangements in branched HMO structures, which complicated the *de novo* sequencing of unknown HMOs with fucose linkages.



F-LNH-II F-LNH-III DF-LNH-II DF-LNH-II* DF-LNH-II**

This study presents the enzymatic synthesis of DF-LNH-II, F-LNH-II, and F-LNH-III HMO structures for elucidating the unique intact CCS values for confident identification via IMS-IMS. Furthermore, *novel* practical insights were obtained for the fucose rearangement process via C13 labeled branched DF-LNH structures, which will greatly improve the attractiveness of developing positive ionization methods for fluorescently labeled and free reducing end HMOs. Now, fucose rearangement products can be confidently identified as false positives via unique CCS values of the rearangement products. It will allow the glyco-workfield to rapidly and unambiguously identify exact structures in biological samples, independently of reducing end labels, without a need for more synthetic standards.

BIOCHEMICAL CHARACTERISATION OF A XYLOSYLTRANSFERASE FROM THE MICROALGAE *PHAEODACTYLUM TRICORNUTUM*

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In 2024, the biopharmaceutical market value reached above $469 billion. This market includes not only recombinant proteins such as monoclonal antibodies, but also biosimilars, hormones, nucleic acid and engineered cell-based products. Currently, most of the monoclonal antibodies are produced in mammalian cell expression systems, such as Chinese Hamster Ovary (CHO) cells [1]. However, production in CHO cells is expensive. This leads to a growing interest for the development of cheaper and effective expression systems, such as microalgae. Since they are photosynthetic eukaryotic cells, the cultivation of these microorganisms in photobioreactors is inexpensive. In addition, microalgae perform efficient folding and *N*-glycosylation of proteins [2]. For instance, it has been shown that the diatom *Phaeodactylum tricornutum* introduces on its proteins oligomannoside *N*-glycans that are identical to that of plants and mammals [3]. Moreover, microalgae are classified as Generally Recognized as Safe (GRAS) strains and, therefore, microalgae have emerged as alternative cell factories for the production of biopharmaceuticals.
N-glycan xylosylation of plant-derived biologics has been demonstrated to induce immune responses [4]. As a consequence, the present research project aims at characterizing a xylosyltransferase (XylT) in the microalgae *P. tricornutum* because the xylosylation of *N*-glycans attached to microalgae-derived biologics may impact their use in human therapy. While plant XylTs, such as *Arabidopsis thaliana* XylT [5], have been well-studied, involvement of XylTs in the *N*-glycosylation pathway of microalgae proteins remains poorly understood. In this context, experiments to first identify xylosylated *N*-glycans on *P. tricornutum* proteins by mass spectrometry are performed. Moreover, bioinformatic approaches allowed the identification of a putative sequence coding for a xylosyltransferase in the *P. tricornutum* genome. Functional complementation of plant mutants impaired in XylT activity [6, 7] with the gene coding for this putative XylT from *P. tricornutum* will help unravelling its substrate. *In vitro* enzymatic bioassays, using recombinant XylTs produced in heterologous systems, will be also performed to better define the substrate specificity, reaction kinetics and optimal parameters (pH, temperature).

Acknowledgements: This work is funded by the European Union under Grant Agreement 101119499 through Glyco-N project HORIZON TMA MSCA Doctoral Networks.

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STRUCTURE DETERMINATION OF THE PHOSPHATE-CONTAINING POLYSACCHARIDE FROM *LACTOCOCCUS LACTIS* SUBSP. *LACTIS* NCIMB 700966

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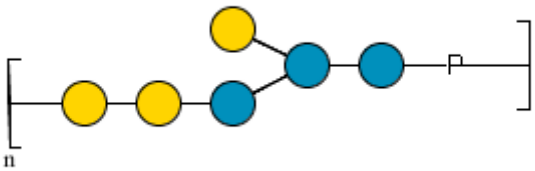
Strain NCIMB 700966 of *Lactococcus lactis* subsp. *lactis* has originally been isolated from Swedish long milk, a Scandinavian soured milk which is viscous because of 'ropiness', a feature usually associated with the production of exopolysaccharides by lactic acid bacteria. In this study, the purified polysaccharide was characterized by physicochemical methods, and its primary structure was elucidated by chemical, chromatographic, and spectroscopic methods.

The bacterium was grown in milk, and its polysaccharide was isolated from the whole culture and purified by trichloroacetic acid and acetone precipitations, solvent extractions, dialysis, and freeze-drying. Yields ranging between 62 and 76 mg were obtained for 2 L cultures.

Sugar and absolute configuration analyses gave D-Glc and D-Gal in equimolar proportions. The following partially methylated alditol acetates were obtained by methylation analysis: 1,5-Ac_2-2,3,4,6-Me_4-Gal, 1,4,5-Ac_3-2,3,6-Me_3-Glc, 1,3,5-Ac_3-2,4,6-Me_3-Gal, 1,5,6-Ac_3-2,3,4-Me_3-Glc, and 1,4,5,6-Ac_4-2,3-Me_2-Glc.

1D and 2D ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy of the native polysaccharide and a chemically modified oligosaccharide alditol obtained by autolysis followed by reduction led to the following hexasaccharide structure for the repeating unit:



This structure is unique and was compared to that of other phosphate-containing polysaccharides from *Lactococcus* strains.

IN PURSUIT IF LARGER LIPOPHILICITY ENHANCEMENT: AN INVESTIGATION OF SUGAR DEOXYCHLORINATION

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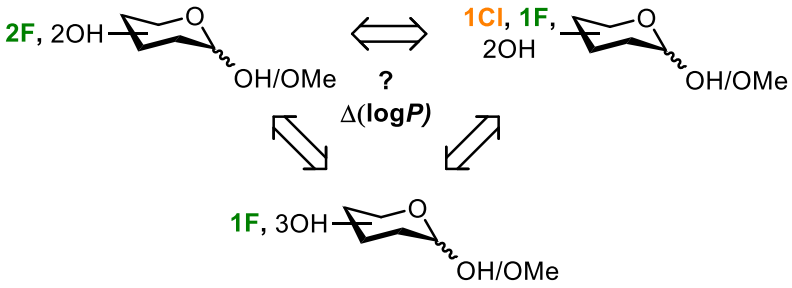
Carbohydrates are implicated in many important biological processes [1]. Hence, there is much interest in manipulating protein-carbohydrate interactions or activities of carbohydrate-processing enzymes [2]. Despite this, there are not many carbohydrate-based drugs (apart from nucleoside analogues) [3], and this is due to their excessive hydrophilicity. Deoxyfluorination is one of the validated strategies to increase sugar lipophilicity [4-7]. However, lipophilicities of dideoxy-difluorinated monosaccharides are still well below the desired range for oral drug candidates. Little is known about the potential of other halogens, such as chlorine. Here we investigate the power of deoxychlorination to increase sugar lipophilicities. A series of dideoxygenated chloro-fluorosugars was synthesized and for these substrates it was shown, by means of a ^{19}F NMR method [4], that deoxychlorination increased the log*P* significantly compared to analogous deoxyfluorination. This shows the potential of deoxychlorination of carbohydrates to increase lipophilicity while limiting the number of potentially important hydrogen bond donating groups to be sacrificed and will be of interest for glycomimetic development.



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SYNTHESIS AND APPLICATION OF AI-2 DERIVED SUGAR PRODRUGS

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Bacteria are able to coordinate the behaviour of cell population by secreting and sensing small molecules called autoinducers. This phenomenon is known as quorum sensing (QS). Among the QS compounds, autoinducer-2 (AI-2, Figure 1) stands out as a potential "universal" bacterial signalling molecule for inter-species communication [1-3]. Understanding the molecular mechanisms that bacteria use to communicate and therefore regulate their group behaviours can lead to the development of new therapies to control bacterial infections.
AI-2 plays a crucial role in controlling the colonisation and homeostasis of the gut microflora. It has been shown that AI-2 can be used to mitigate the adverse effects caused by antibiotic-induced microbiota imbalances in the gut [3]. Therefore, our hypothesis is that synthetic AI-2 can aid in restoring a healthy bacterial phyla ratio after antibiotic treatment.
We will present the synthetic strategies towards the preparation of AI-2 prodrugs to orally deliver intact AI-2 to the gut based in colon-specific drug delivery systems.[4] The AI-2 prodrugs consist of AI-2 linked to beta-glycosides (Figure 1) that will be specifically hydrolysed in the gut taking advantage of beta-glucosidases and beta-galactosidases produced by the gut microbiota. The enzymatic release of AI-2 from the new AI-2 prodrugs was demonstrated using commercial beta-glycosidases and mice gut extracts. Quantification of AI-2 released from the prodrugs was performed using a new GC-MS AI-2 quantification method developed by our group.

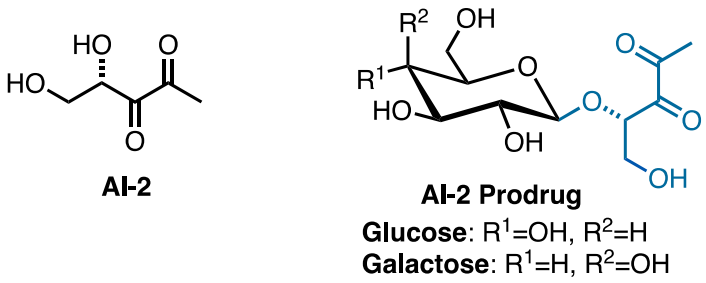

Figure 1.

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AUTOMATED GLYCAN ASSEMBLY OF β-1,3-XYLAN OLIGOSACCHARIDES

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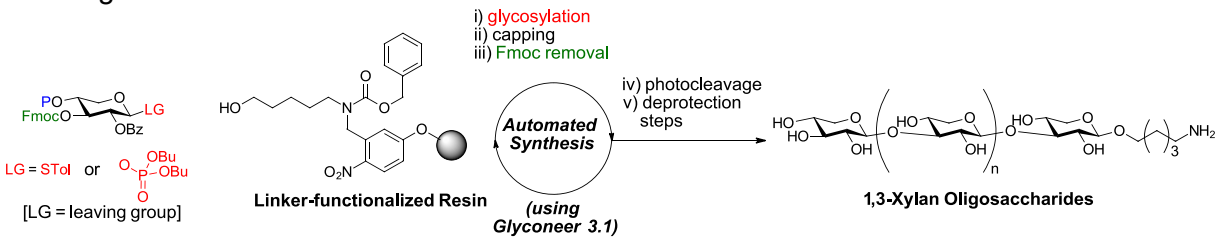
β-1,3-xylan is a linear homopolysaccharide in which D-xylose units are linked through β-1,3-glycosidic bonds. It occurs as the main xylan structure in the cell walls of marine algae, such as green and red algae [1]. Algal biomass containing 1,3-xylan can serve as a feedstock to produce renewable chemical commodities. Rapid syntheses of β-1,3-xylan oligosaccharides of defined lengths would pave the way for studies of β-1,3-xylan-degrading bacteria [2]. In this work, we use an automated glycan synthesizer [3] to develop a solid-phase synthesis of β-1,3-xylan oligosaccharides. First, we developed an efficient route for multigram scale synthesis of disarmed and armed xylose-thiotolyl and -phosphate donors, which are protected with Fmoc as a temporary protecting group for chain elongation. Then, through systematic screening of glycosylation temperature and reactions times as well as reactivity control through the choice of protecting groups, we achieved high yields in the automated glycan assembly of 1,3-xylan oligosaccharides (27% for decasaccharide in 21 steps). After UV-induced cleavage from the resin, the protected 1,3-xylan oligosaccharides were subjected to global deprotection to obtain the final products equipped with an amino-alkyl-linker at the reducing end.



Acknowledgements: This work was supported by an ERC Consolidator Grant (ASAP, 771349 to FP).

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UNVEILING INSIGHTS ON CANCER-LINKED GLYCOSYLTRANSFERASES USING GLYCOMIMETICS

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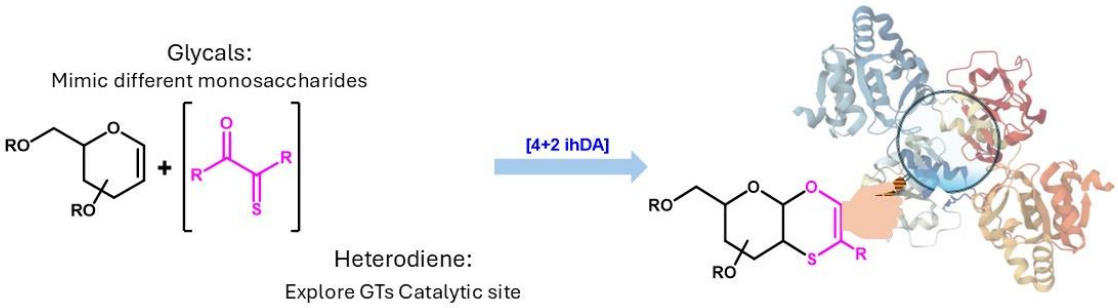
Cancer-associated glycosylations are the results of aberrant activity of specific glycosyltransferases (GTs) [1]. Such alterations on the meshwork of glycans within the glycocalyx of cancer cells are non-random and are essential for cancer survival. For this reason, they are emerging as critical therapeutic targets [1]. However, drug discovery in this field is mainly hampered by the lack of crucial information on the structure of key cancer-associated GTs. Thus, some of the cancer-linked GTs remain severely unappreciated as potential investigation focal points. For this reason, developing chemical tools that can help scientists to unveil insights on the structure of these enzymes is a sought-after goal. In this context, glycomimetics,[2] can serve as functional or structural mimics thus can have the potential to be recognized by carbohydrate-processing enzymes, enabling the study of how the enzymes identify and engage with the substrate [3].
With this in mind, our group is approaching the creation of glycomimetics by taking advantage of an original version (Figure) of the inverse electron demand [4+2] hetero Diels-Alder (ihDA) [3-5]. In this communication, we aim to highlight the versatility of our ihDA in addressing the goal of an easy access to a large library of glycomimetics, provided with the mandatory structural variability to explore the catalytic site of specific GTs. Moreover, this approach can ensure improved interactions with the pertinent GTs and precision in the cancer-associated glycan-motif editing of the glycocalyx. As a proof of the strength of this approach, the synthesis of fucose and sialic acid mimetics is discussed.



Acknowledgements: This work has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie Actions Grant Agreement n. 101119601 (GLYCANDRUG)

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MULTIVALENT GLYCOCONJUGATES: GLYCOCLUSTERS AND GLYCOPOLYMERS FOR TARGETING GALECTIN-4

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Galectins (Gal-) are β-D-galactoside-binding proteins involved in cell adhesion, proliferation, and apoptosis. Tandem-repeat galectin-4 (Gal-4) is highly expressed in gastrointestinal tissues and plays a key role in epithelial glycoprotein transport. Due to the weak affinity of carbohydrate-lectin interactions, nature employs multivalency, known as the glycoside cluster effect. Inspired by this concept, synthetic multivalent glycoconjugates like glycopeptides, glycodendrimers, and glycopolymers have been designed to enhance galectin binding [1]. While Gal-1 and Gal-3 have been extensively studied, research on tandem-repeat galectins like Gal-4 remains limited due to their complex carbohydrate recognition domains. Recent studies show that valency and ligand presentation critically affect Gal-4 binding, emphasizing the usefulness of tailored multivalent synthetic glycomimetics for galectin research [2]. In this work, we present the synthesis of a library of glycoconjugates: (*i*) tetravalent glycoclusters based on D-glucose core with lactose ligands of varying spacer lengths, and (*ii*) polyoxazoline (POx)-based glycopolymers featuring different multivalent presentations of lactose and a previously identified high-affinity tetrasaccharide ligand of Gal-4 (Fig.1). The impact of ligand presentation on Gal-4 affinity was systematically evaluated using ELISA competitive assay and biolayer interferometry. Our findings contribute to a better understanding of Gal-4 recognition and provide new insights into the rational design of synthetic glycomimetics for targeting tandem-repeat galectins.

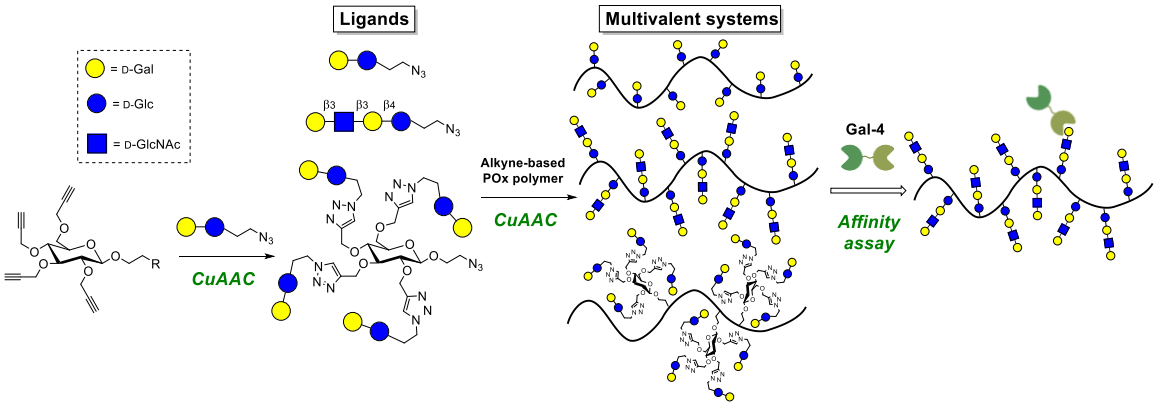

Figure 1. Synthetic pathway to multivalent glycoclusters and glycopolymers

Acknowledgements: This work was supported by the Czech Science Foundation (projects 22-00197K, and 25-15587K), and by the Ministry of Education, Youth and Sports of the Czech Republic (mobility projects LUC23148 and LUC24024).

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GLYCOSYLATION OF 2-(2-PROPYLSULFINYL)BENZYL 1,2-ORTHOESTER GLYCOSIDES INITIATED BY SULFOXIDE ACTIVATION

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We have developed a highly effective glycosylation method that involves the activation of 2-(2-propylsulfinyl)benzyl 1,2-orthoester glycosides using triflic anhydride (Tf2O). Our research indicates that half of the glycosyl donor is activated through Tf2O via an interrupted Pummerer reaction mechanism, while the remaining portion is activated by triflic acid (TfOH) generated in situ. As a result, as little as 0.5 equiv of Tf2O is adequate for activating the orthoester glycoside donors. This glycosylation procedure offers several benefits, such as high efficiency, wide applicability, and the utilization of a recyclable leaving group.

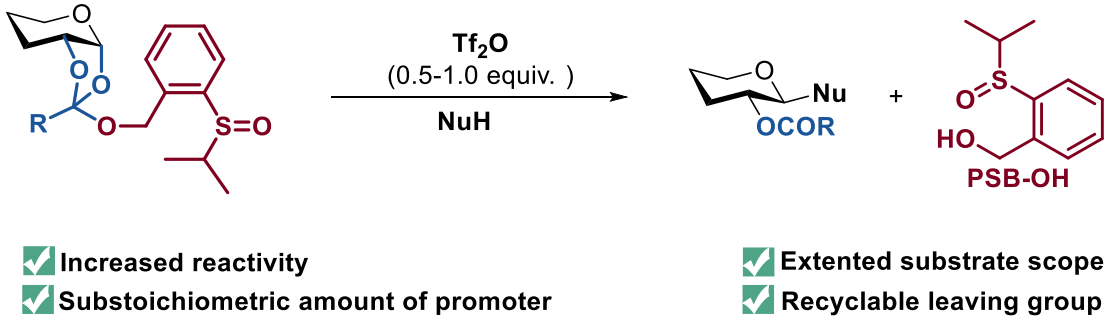

Figure 1. Glycosylation with OPSB glycosides and 1,2-orthoester glyco-sides.

Acknowledgements: We are thankful for the financial support from STI2030-Major Projects (2022ZD0211800) and The National Natural Science Foundation of China (22025102, 22077039, 22177034)

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DISCERNING STRUCTURAL INFLUENCES ON STEREOSELECTIVITY AND KINETICS IN OXATHIANE GLYCOSYL DONORS

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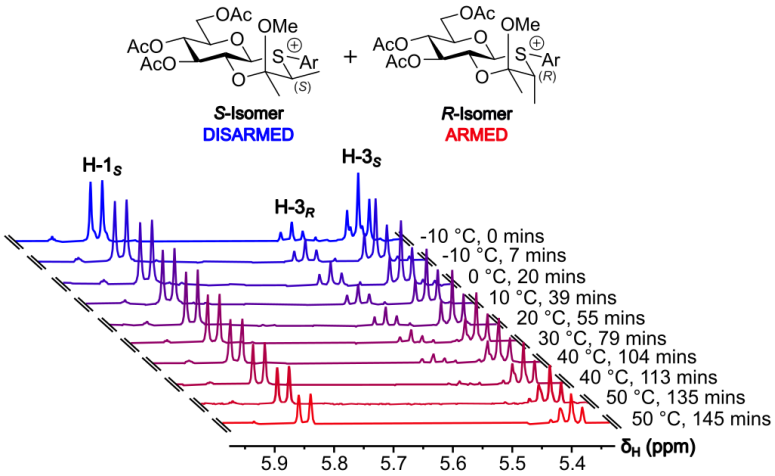
Trans-decalin like sulfonium oxathiane glycosyl donors have been previously shown as a viable route to access 1,2-*cis*-glycosides [1-3], though these reports have indicated donor structure has a large influence on glycosylation outcome [4,5]. This work describes the synthesis of an array of methyl oxathiane donors and their subsequent use in discerning the effect of structural differences on stereoselectivity and reactivity in glycosylations. In particular, introduction of 3-methyl groups in the oxathiane heterocycle allowed kinetic tuning of the glycosyl donor without diminishing α-selectivity, and even improved the poor selectivity often observed in armed oxathiane 2-*O*-ether donors.



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UNDERSTANDING THE DIVERSE MOLECULAR MECHANISMS DRIVING MPS-IIIA DEVELOPMENT AND DISEASE PROGRESSION AS A STEP TOWARDS PERSONALISED MEDICINE

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Mucopolysaccharidosis Type 3A (MPS IIIA, also called Sanfilippo Syndrome) is a rare autosomal recessive lysosomal storage disorder. Although the disease is systemic, it is primarily characterised by severe developmental defects in the brain and the central nervous system which present as a form of childhood dementia [1]. MPS IIIA is caused by a mutation in the lysosomal carbohydrate sulfatase (SGSH), which disrupts heparan sulphate degradation. HS is the major glycosaminoglycan component of the extracellular matrix (ECM), and is a linear polysaccharide composed of alternating glucosamine and uronic acid repeats. HS is essential in functions in development and homeostasis [2,3] with significant roles in neuronal circuits, and a key regulator of connectivity and synapse function and development [4]. SGSH removes sulfate groups from the glucosamine residues, which is a critical step for HS recycling. However, the precise substrate specificities of SGSH, and the mechanisms through which many MPS IIIA-associated mutations disrupt SGSH functions remain unknown. This project aims to functionally characterise a panel of SGSH mutations, some of which are predicted to indirectly modulate SGSH activity by inducing enzyme mislocalisation or destabilisation [5]. There are significant variations in disease phenotype dependent on the causative mutation, showing phenotype-genotype correlations [6]. Preliminary data has revealed variable localization profiles of SGSH mutants, including T118P which is no longer appropriately trafficked to the lysosome (Fig 1) and variants that disproportionately accumulate in aggresome-like structures. We will analyse subcellular localization, expression profiles, metabolic activity and structurally characterise 76 clinically relevant SGSH mutants to better understand the pathophysiological processes that drive MPSIIIA.

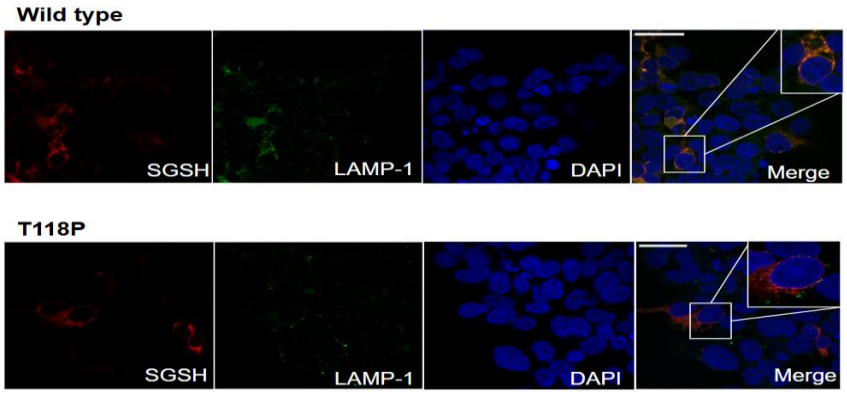

Figure 1. Fixed HEK293 SGSH-null cell line transfected with WT and T118 SGSH stained with Anti-SGSH (red), Anti-LAMP-1 (green) and DAPI. Scale bar 100 µM.

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TARGETING β-CYLCODEXTRIN TO THE LYSOSOMES

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Lysosomal storage diseases (LSDs) are a group of inherited genetic disorders that result from deficiencies in lysosomal enzymes or transport proteins, leading to the accumulation of undegraded macromolecules within the lysosome. The ability of β-cyclodextrin (β-CD) to form inclusion complexes with cholesterol, or with lipids such as lipofuscin bisretinoids, makes it a useful chaperone for either reducing cholesterol accumulation in disorders such as Niemann-Pick type-C (NPC), or for the treatment of Stargardt disease [1].
Previously, our group has developed a method for lysosomal targeting by covalently attaching a biantennary N-glycan hexasaccharide containing two mannose-6-phosphate (M6P) residues to β-CD (figure a) [2]. In this second-generation approach reported here, we detail the synthesis of several other M6P-containing oligosaccharide and their attachment to β-cyclodextrin. Lysosomal targeting of β-CD may in the future provide the basis for the development of new therapies to treat Niemann-Pick C and other metabolic disorders, such as Stargardt disease.

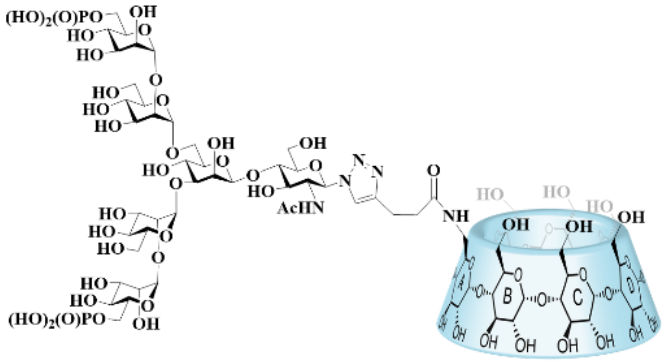

Figure 1. [2]

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SITE-SPECIFIC INCORPORATION OF CLICKABLE D-MANNOSE DERIVATIVES IN THE LIPOPOLYSACCHARIDE CORE OF THE PATHOGEN *BRUCELLA ABORTUS*

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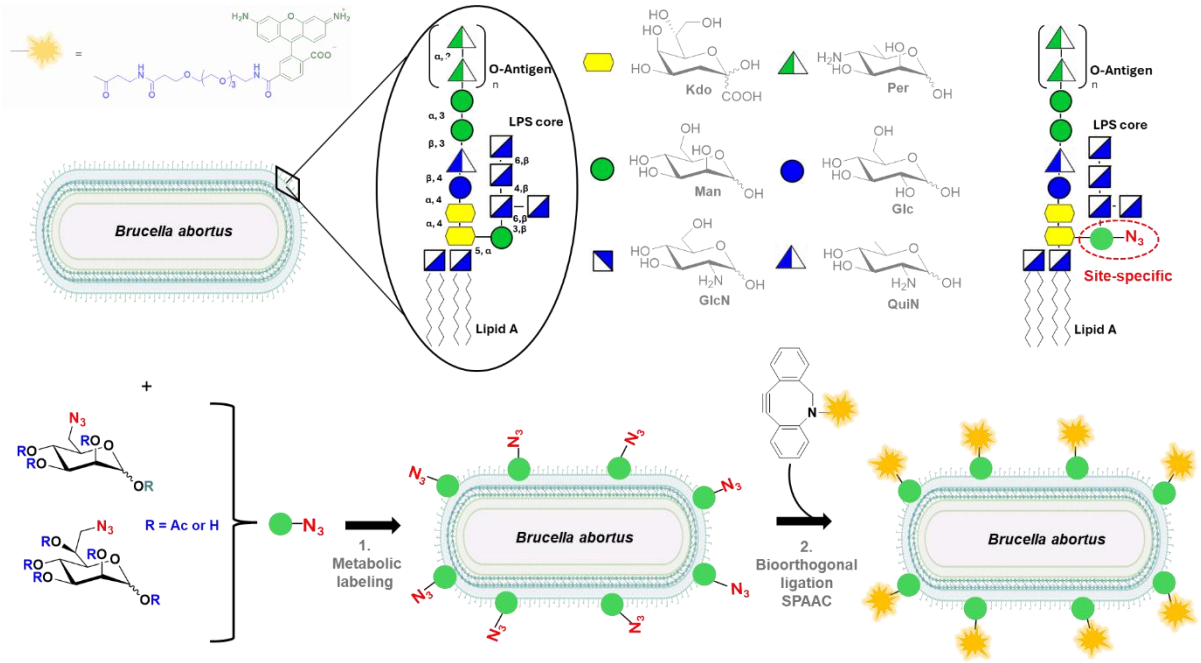
Metabolic Glycoengineering (MGE) is a powerful tool to manipulate the glycan metabolism in living prokaryotic or eukaryotic systems [1,2]. In this study, we explore the D-mannose metabolism using clickable D-mannose analogues. We show that 6-azido-D-mannose and 7-azido-heptose can selectively be incorporated in the cell wall of *Brucella abortus* but not in *Escherichia coli* or *Sinorhizobium meliloti*. Using a series of mutants, we demonstrate that the clickable monosaccharides are exclusively incorporated into the lateral branch of the core LPS glycan, but not in the O-chain or any other cell wall component. (Figure 1) The metabolic route followed by the mannose analogues was also evidenced using bacterial mutants and involves, as a first step, the anomeric phosphorylation of the azido-sugars by phosphomannomutase ManB. The XRD 3D-structure of ManB was obtained and docking of clickable azido-sugars in ManB allowed the rationalization of their phosphorylation. GDP-N_3-sugars are then generated and selectively used by mannosyltransferase WadC to transfer N_3-sugars on the LPS inner core.

Site-specific incorporation of mannose in the lateral chain of the LPS core opens new perspectives such as the identification of macromolecules binding this important structure for the host-pathogen interactions, highlighting a strategy that could be applied to many other bacterial pathogens.



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DECIPHERING THE FUCOSE MIGRATION PRODUCTS IN BLOOD GROUP EPITOPES

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Fucosylated glycans are involved in immune cell homeostasis [1,2], selectin-dependent leukocyte adhesion, and intercellular communication [3]. However, structural analysis of fucosylated glycans by mass spectrometry (MS) often shows non-native peaks that originate from a fucose migration along the glycan sequence which can lead to incorrect structure assignment of a glycan [4]. In this study, we used computational techniques to investigate the fucose rearrangement mechanisms that occurs during MS analysis of blood group antigens. Energetics, vibrational frequencies, and collision cross section (CCS) values of blood group antigens and all their possible isomers were calculated by using density functional theory (DFT) and the results were compared with the available experimental cryogenic IR spectroscopy and CCS data. Computationally predicted IR spectra and CCS values of Lewis x and BG-H2 antigens were inconsistent with the experimental measurements. Instead, the theory predicts that the isomer which has fucose linked to the galactose via an α1-6 glycosidic bond provides the best match with the experiment [5]. Similar analysis was then performed on the protonated ions of Lewis a and BG-H1 antigens resulted in the assignment of the experimental spectra to the structures of the respective parent ions [6]. By comparing these two pairs of antigens, we determine that the internal rearrangement reaction is triggered by the proximity of the protonated amide group to the glycosidic bond and the mobility of the proton: the Lewis a and BG-H1 structures lacks the mobile proton to initiate the migration, whereas a loosely bound proton in Lewis x and BG-H2 was able to protonate the fucose glycosidic bond. These findings show that the migration of fucose moiety is a sequence-dependent phenomenon and most likely occurs following in-source activation of intact ions during the nanoESI process.

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COMPARISON OF STREPTOCCOCUS PNEUMONIAE SEROGROUP 23 CAPSULAR POLYSACCHARIDES: A MOLECULAR MODELLING STUDY

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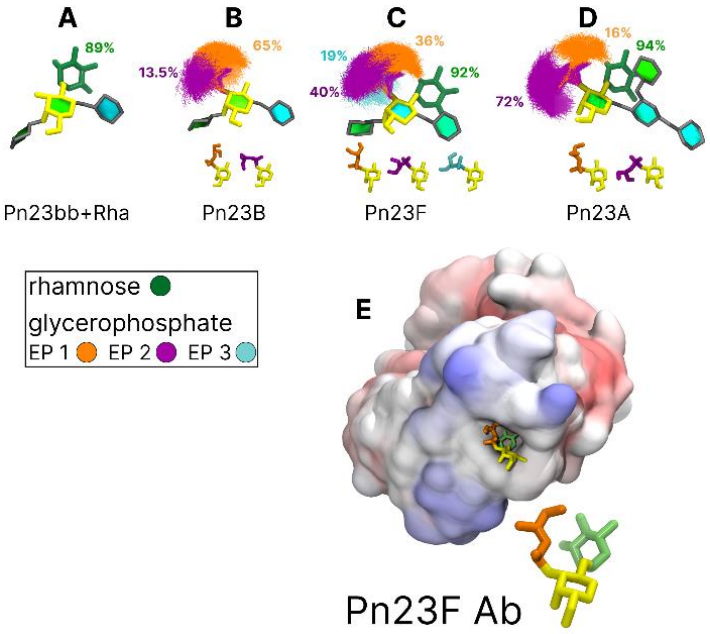
Streptococcus pneumoniae is a bacterial pathogen and a frequent cause of disease in humans. The bacterial capsular polysaccharide (CPS) is a key virulence factor and the primary target of pneumococcal conjugate vaccines (PCVs). Although PCVs have greatly reduced the disease burden, the prevalence of non-vaccine serotypes, such as 23A and 23B, has increased, necessitating the development of higher valency vaccines. For serogroup 23, preliminary studies have indicated a lack of cross protection between vaccine serotype 23F and structurally similar 23A and 23B, the causes of which remain unclear.
Here we employ molecular modeling to investigate and compare the conformation and exposed epitopes of pneumococcal serotypes 23A, 23B and 23F, and hence provide insight into cross-reaction within serogroup 23. We find that all three CPS adopt stable helical conformations, with 23A the most well-defined. While both the rhamnose and glycerol-phosphate (Gro2P) side chains are exposed on the exterior of the helix, steric crowding limits their accessibility in 23A. Notably, the immunodominant rhamnose side chain is well defined and adopts an identical orientation in both 23F and 23A. In contrast, the Gro2P side chain adopts three distinct conformational epitopes (EP1–EP3). Serotype 23B favors EP1, 23F EP1 and 2 and 23A EP2. Structural analysis of a 23F antibody-binding fragment suggests that recognition requires both the rhamnose side chain in the orientation presented and the EP1 Gro2P conformation. These findings provide a potential explanation for the lack of cross-protection between 23F and non-vaccine serotypes 23A/23B. Justifying their inclusion in next generation vaccines.



IDENTIFICATION OF THE D-GLUCURONYL C5-EPIMERASE RESPONSIBLE FOR THE APPEARANCE OF IDURONIC ACID IN N-LINKED GLYCANS DECORATING ARCHAEAL GLYCOPROTEINS

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N-glycosylation is a conserved post-translational modification found across all domains of life, i.e., Eukarya, Bacteria, and Archaea. *Halobacterium salinarum*, a halophilic archaeon and the first non-eukaryotic organism shown to perform N-glycosylation, decorates its glycoproteins with sulfated oligosaccharides assembled on dolichol phosphate or pyrophosphate carriers [1-2]. While some components of these glycosylation pathways have been identified, the enzymes responsible for introducing certain sugars—such as L-iduronic acid (L-IdoA)—remain unknown.

L-IdoA, the C-5 epimer of D-glucuronic acid (D-GlcA), is a well-documented constituent of glycosaminoglycans (GAGs) in animals and appears in some bacterial polysaccharides. *Hbt. salinarum* offers the only known example of L-IdoA being incorporated into a N-linked glycan, specifically a tetrasaccharide. However, the pathway used for IdoA biosynthesis in this domain of life has not been established [3,4]. In this study, we produced a deletion strain lacking a putative D-glucuronyl C5-epimerase predicted to be responsible for the generation of L-IdoA at the third position of the tetrasaccharide. We subsequently determined the function of the gene product by assessing the N-glycan assembled in the mutant. Our findings represent the first identification of such an enzyme in Archaea and provide new insight into the complexity and evolutionary diversity of archaeal N-glycosylation systems.

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SYNTHESIS AND ANTICANCER APPLICATIONS OF NEW HALF-SANDWICH PLATINUM-GROUP METAL COMPLEXES WITH HETARYL SUBSTITUTED *N*-GLUCOPYRANOSYL-1,2,3-TRIAZOLE N,N-CHELATING LIGANDS

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Platinum-based compounds, including cisplatin, oxaliplatin, and carboplatin, play a crucial role in anticancer chemotherapy [1], however, their clinical utility is often constrained by poor selectivity for tumor cells and diminishing effectiveness with prolonged treatment [1,2]. This underscores the need for novel cytostatic agents. Half-sandwich complexes of platinum-group metal ions, such as Ru(II), Os(II), Ir(III), and Rh(III), have emerged a promising alternatives to platinum-based drugs. In this context, our research group has synthesized a library of half-sandwich platinum-group metal complexes incorporating monosaccharide-derived heterocyclic N,N-bidentate ligands. Sevaral of them demonstrated superior antineoplastic activity against different cancer cell lines and also exhibited bacteriostatic effect on multiresistant Gram-positive bacteria [3,4]. A *p*-cymene containing Os(II) complex with *O*-perbenzoylated 1-*N*-(β-D-glucopyranosyl)-4-(2-quinolyl)-1,2,3-triazole ligand was the most efficient complex in the series.

The aim of the current study is to further modify the sugar derived ligand by changing the anomeric configuration from β to α. Accordingly, a series of the targeted half-sandwich type complexes containing 1-*N*-(α-D-glucopyranosyl)-4-hetaryl-1,2,3-triazoles as N,N-chelators (hetaryl = 2-pyridyl, 2-quinolyl) were synthesized in both *O*-peracylated and *O*-unprotected forms as a mixture of diastereomers. To assess the hydrophilic/lipophilic feature of these complexes, their distribution coefficient (logD) was determined. Moreover, the anti-cancer activity of the newly synthesized complexes was evaluated, these findings will be detailed in the poster presentation.

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ZWITTERIONIC MODIFICATIONS OF SYNTHETIC GLYCAN EPITOPS DETERMINE PROTEIN BINDING IN A DIPTERAN GLYCAN ARRAY

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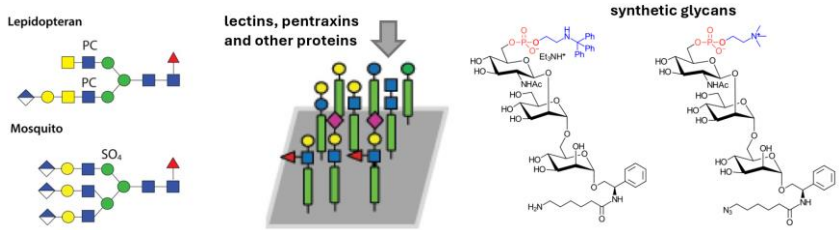
Glycan structures exhibit remarkable variability also in the non-mammalian systems, in particular, in insects, displaying a wide range of glycan diversity. Some insects serve as intermediate hosts for parasites or viruses, posing public health challenges, while others are significant due to their roles in allergies or as agricultural pests [1, 2]. Despite their relevance, the roles of insect glycans in disease transmission and pest control remain largely unexplored. Mosquitoes are key vectors for various human and animal pathogens in tropical regions, including the malaria parasite *Plasmodium* and flaviviruses such as Dengue; additionally, tsetse flies (*Glossina morsitans*) transmit African trypanosomes, which cause sleeping sickness in humans [3]. Climate change is expanding the geographical range of these insect-borne diseases, whose transmission often relies on glycan-mediated interactions. Insects exhibit a diverse array of modifications on non-vertebrate N-glycans, including the presence of multiantennary structures and highly modified cores featuring zwitterionic modifications such as phosphoethanolamine (PE) and phosphorylcholine (PC) on their N-glycans [4, 5]. Sulphate groups have also been detected on the mannose residues of N-glycan cores in *Drosophila*, mosquitoes, and lepidopteran species.



Since PC-, PE-, and sulphate-modified insect glycan fragments are not available in sufficient homogeneity or quantity, and the corresponding enzymes are lacking, chemical synthesis represents a state-of-the-art approach for creating a library of molecularly defined PE/PC-modified glycan epitopes. We have chemically synthesized PC-, PE-, and sulphate-modified trisaccharide motifs equipped with amino group-terminated linkers as probes for glycan array studies and investigated their binding to antibodies, pentraxins, and other proteins of the mammalian immune system.

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EXPLOITING BACTERIOPHAGE ENDOGLYCOSIDASE AGAINST THE CAPSULAR POLYSACCHARIDE OF *K. PNEUMONIAE* AS A TOOL FOR NOVEL ANTIMICROBIAL STRATEGIES

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Klebsiella pneumoniae is a Gram-negative opportunistic pathogen and is considered by several international health organizations to be one of the most critical bacterial species. Indeed, infections caused by multidrug-resistant *K. pneumoniae* (MDR-Kp) represent a major clinical challenge due to their massive spread in hospitals and the lack of effective drugs. The bacterial capsule (CPS) is an important virulence factor as it protects the cells from the host's immune defences and from available antibiotics, making it an interesting target for the development of alternative antimicrobial strategies. In recent years, bacteriophage-derived enzymes have emerged as promising alternatives to conventional antimicrobial molecules.
The aim of this study is to characterize the activity of bacteriophage-derived endoglycosidases (EG) specific for the CPS of *K. pneumoniae* strain KpB-1, whose structure was determined in our laboratory (Fig. 1) [1]. To test phage-associated EG activity, phage particles [2] were incubated with pure CPS; characterization of the products by NMR spectroscopy and ESI-MS led to the recognition of the EG cleavage site in the α-L-Rhap-(1→3)-β-D-Galp linkage. Subsequently, a recombinant version of phage EG was obtained and its activity against pure CPS and bacterial cells was evaluated. The hydrolytic activity of the enzyme was monitored using a colorimetric assay specific for the reducing ends. Microbiological methods and flow cytometry analysis confirmed that the enzyme alone has no effect on cell viability, but it effectively cleaves CPS and enhances the antibacterial effect of colistin. In addition, the possibility of using EG to functionalize drug carriers to target specific bacteria, e.g. Kp-MDR, is being investigated.

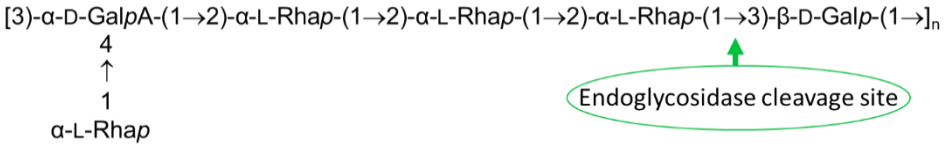

Figure 1. KpB-1 CPS repeating unit and EG cleavage site

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A SIALOMIMETIC GLYCAN ARRAY FOR THE IDENTIFICATION OF VIRAL ENTRY INHIBITORS

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Zoonotic viruses can rapidly emerge from animals and transmit to humans, causing pandemics that lead to millions of deaths and a massive economic burden for humanity. Some widely recognized respiratory viruses such as the Spanish flu, Swine flu, MERS-CoV, and SARS-CoV-2, have resulted in significant human casualties due to the lack of effective drugs and vaccines [1]. Those pandemic influenza and corona viruses exploit animal and human sialic acid receptors for transmission and infection.
Sialic acids are a family of monosaccharides composed of a nine-carbon backbone. They are typically located at the terminal end of carbohydrate chains, attached to several glycoproteins and glycolipids on the cell membrane and thus serve as primary receptors by many viruses for establishing infection [2]. These receptors therefore constitute an important target for drug research against these viruses [3].
Our research aims to develop molecules that perfectly lock the virus sialic acid binding proteins and thereby block viral infection. To this end, we systematically varied the sialic acid motif through chemical synthesis, ensuring ongoing enrichment of a sialomimetic library. These synthetic sialomimetics were immobilized onto glass slides in an array format [4]. These arrays were subsequently exposed to recombinant sialic acid binding lectins (SNA, CD-22 and CD-33) for array validation. Then Influenza A viruses H1N1 and H3N2 were tested to evaluate receptor-specific binding. Additional viruses will be examined as more compounds become available. The information obtained will guide the development of antiviral drugs employing multivalency to enhance viral scavenging efficiency. Within the context of pandemic preparedness, this pipeline prepares for potential future outbreaks of unknown sialic acid-binding viruses.

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RESURRECTING THE ANCIENT GLYCOME TO EXPLORE THE NATURAL ANTI-NEU5GC ANTIBODY RESPONSE

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N-glycolylneuraminic acid (Neu5Gc) is a non-human sialic acid which is taken up from dietary sources and presented on the human glycocalyx. This makes Neu5Gc a unique example of a 'xeno-autoantigen'- a non-human, immunogenic molecule found on the surface of human cells [1]. Neu5Gc-reactive antibodies have implications for many aspects of human health [2]. However, current methods to detect and study anti-Neu5Gc antibodies do not represent the complex mixture of glycans present on human cells, and require access to animal handling expertise, expensive reagents, and specialist equipment [3]. Here, we outline a simple workflow to enrich and detect natural anti-Neu5Gc antibodies from small volume human samples. This strategy uses human cells engineered to produce Neu5Gc by 'resurrecting' the enzyme required to synthesise it- CMAH. This strategy successfully enriched Neu5Gc-specific antibodies from individual plasma specimens from healthy donors. Anti-Neu5Gc antibodies were detected in all donors regardless of age or sex, and were predominantly of the IgG isotype. While reproducible individual differences in Neu5Gc-reactivity were observed between the donors, in all cases anti-Neu5Gc antibodies were capable of binding to Neu5Gc-containing glycans on whole human cells, supporting the hypothesis that they may play a physiologically relevant role in human health.

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LIPID-LINKED ARABINOGALACTAN SUBSTRATE OF MYCOBATERIAL CELL WALL LIGASES – INITIAL CHARACTERIZATION OF THE POLYSACCHARIDE

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Mycobacterium tuberculosis is the causative agent of tuberculosis, an infectious disease responsible for over a million deaths annually. A typical feature of this bacterium is the complex cell wall, which largely contributes to its pathogenicity. The core of mycobacterial cell wall is formed by the mycolyl-arabinogalactan-peptidoglycan complex (mAGP). Enzymes from the LCP (LytR-CpsA-Psr) ligase family, which are responsible for the covalent attachment of arabinogalactan to peptidoglycan through a phosphodiester bond, play a key role in the construction of mAGP. However, the native substrates of LCP ligases in mycobacteria are unknown. Lipid-linked galactan or its arabinosylated version could serve as a donor substrate, an acceptor substrate might be the mature peptidoglycan or its precursors. The challenge in addressing this task is a redundancy of LCP proteins in mycobacteria [1,2], which precludes simple repression of the target gene and search for the accumulated precursors. Recently, we found conditions for the inhibition of the LCP ligase reaction in mycobacteria by transcriptional repression of multiple LCP ligases by CRISPR interference. The constructed strain allowed us to isolate the accumulated substrate and to perform its preliminary characterization by MALDI-TOF and GC-MS analyses. We show that the polysaccharide part of the LCP ligase donor substrate is about ~10 kDa polymer composed of multiple arabinoses and galactoses, but its detailed characterization will require application of state-of-art analytical techniques.

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INNOVATIVE 3D-PRINTED SKIN REGENERATION MODEL BASED ON CHITOSAN HYDROGEL FUNCTIONALIZED WITH BIOACTIVE PEPTIDE

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More than 20 million people worldwide suffer from difficult-to-heal skin wounds, which is a growing global health problem that is further exacerbated by lifestyle diseases such as diabetes. The skin, as the largest organ in the human body, performs crucial protective functions, and its damage can lead to complications such as chronic inflammation, infection, or necrosis [1]. Given the limitations of current treatment methods, innovative approaches in tissue engineering, in particular 3D bioprinting, are gaining increasing importance [2].
The aim of ours project is to develop a multilayer, dynamic model of a skin substitute. This model will be an ECM imitation, to create which we will use a chitosan and agarose composite enriched with collagen proteins and/or the QHREDGS peptide. We will try to use the peptide and/or collagen, both in free form and covalently bound to chitosan using a mild, patented cross-linking agent - maleimidoglycine. The innovative method of using carbon dioxide to saturate the chitosan solution allows for the creation of a biocompatible hydrogel without the need for chemical modification, which is a significant advantage over the methods currently described in the literature [3,4]. The chitosan-agarose composite, thanks to the rapid sol-gel phase transition, supports the reproduction of natural tissue architecture, while maintaining appropriate mechanical and biological properties.
Nevertheless, one of the most important stages in determining the biological functionality of the composite will be the synthesis of a bioactive peptide in the solid phase in the Fmoc strategy. The QHREDGS peptide, whose biological properties are associated with the induction of primary skin keratinocyte migration, is able to significantly accelerate wound healing and increase the formation of granular tissue. This peptide will be used to create a chitosan-based structure using a bifunctional linker derived from the NHS ester of maleimidoglycine.
The results of this project could have a significant impact on the field of regenerative medicine, offering new tools for treating extensive skin lesions, modeling skin diseases, testing drug toxicity, and research related to cancer and light damage [5].

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GLYCOSYLATION AS A MODULATOR OF TNF AND LTA BINDING TO TNFRS: BIOPHYSICAL INSIGHTS INTO CYTOKINE-RECEPTOR INTERACTION

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Tumor necrosis factor (TNF) and lymphotoxin alpha (LTα) are central cytokines in inflammatory and immune responses, exerting their effects primarily through binding to TNFR1 and TNFR2. Although their signaling pathways have been extensively studied, the role of glycosylation in modulating their receptor interactions remains underexplored. Recent findings suggest that glycan structures, both *N*- and *O*-linked, may significantly influence cytokine receptor affinity and specificity [1-4].

In this study, we investigate the impact of glycosylation on the binding of TNF and LTα binding to TNFR1 and TNFR2 using molecular dynamics. Additionally, we also used the nanoDSF technique to measure the thermal stability of proteins and their complexes by analysing their fluorescence during heating for both glycosylated and sugar-free proteins. Our results demonstrate that glycosylation may have a significant effect on the binding kinetics of TNF and LTα binding kinetics, suggesting a regulatory role of glycosylation in modulating cytokine activity.

These findings provide new insight into the glycan-dependent regulation of TNF superfamily signaling and may open novel avenues for the design of glyco-optimized therapeutics targeting chronic inflammation and autoimmune diseases.

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DESIGN AND SYNTHESIS OF INHIBITORS AND PROBES TARGETING FUCOSE-PROCESSING ENZYMES

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Fucose, an unusual 6-deoxy L-configured sugar, is a highly abundant component of many important oligosaccharides and glycoconjugates in both pro- and eukaryotic organisms. Accordingly, fucosylation plays a key role in numerous biological and pathological pathways [1,2]. Therefore, studying the enzymes that process and regulate these sugar moieties is crucial. The enzymes that transfer L-fucose residues to acceptor molecules (e.g., oligosaccharides, glycoproteins and glycolipids) are known as fucosyltransferases, while those responsible for cleaving fucose moieties from glycoconjugates are referred to as fucose hydrolases, or fucosidases. The biological and biomedical relevance of fucose-processing enzymes, combined with the limited crystallographic data and poor understanding of their catalytic itineraries warrants the development of inhibitors and probes [3,4]. In the past, our group has been successful at targeting α-L-fucosidases using cyclophellitol-based glycomimetics [5]. Capitalizing on this work, we have designed and synthesized a library of putative inhibitors and probes to target new classes of fucose-processing enzymes.

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BACTERIA LECTIN RECOGNITION TOWARDS SYNTHETIC MUCIN TANDEM REPEAT GLYCOPEPTIDES

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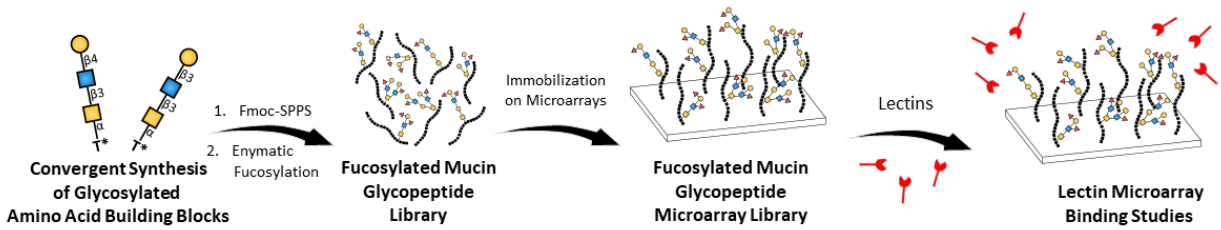
Mucins are densely *O*-glycosylated membrane-bound and secreted proteins ubiquitously found on the epithelial cell surface [1]. In the intestines the membrane-bound mucins are coated with an inner and outer mucus layer. The mucus consist to a large extend of secreted mucins and serve as a protective barrier, which keeps bacteria on distance from the epithelial tissue [2]. However, the microbiota have co-evolved with the human host and developed strategies to feed, degrade and penetrate the mucus barrier and thereby enable adhesion to carbohydrate ligands on the host cell-surface using lectins. In order to improve our understanding of the pathogenic adhesion processes on a molecular level, we study the interactions between bacteria lectins and carbohydrate ligands presented on mucin peptide backbones. For instance binding specificities will be presented of fucose binding lectins from the toxin A of *Clostridium difficile* (TcdA), a bacterium that causes gastrointestinal disorders.[3] Selected synthetic mucin core and extended core tandem repeat glycopeptides were enzymatically modified with Lewis a, Lewis x, or H-type motifs as well as bi-fucosylated Lewis b and Lewis y structures [4]. Then the glycopeptides were immobilized on microarray slides and applied to evaluate TcdA binding preferences. Binding preferences of bacteria CBMs from mucin degrading enzymes “mucinases” will additionally be presented.



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STEREOSELECTIVE RING CONTRACTIONS IN GLYCOPYRANOSIDES AS KEY STEP *EN ROUTE* TO NITROGEN CONTAINING GLYCOMIMETICS. ACCESS TO ISOIMINOSUGRAS AND BEYOND

Martin Thonhofer[a], A. Culum [a], T. Dorn[a], W. Festl[a], R. Fischer[b], Luttenberger[a], H. Prasch[a], A. Luttenberger[a], F. Schmutz[a], E. Spari[a], A.E. Stütz[a], P. Weber[a], T.M. Wrodnigg[a]

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Isoiminosugars (**I**) are glycomimetics in which a nitrogen atom is located at the anomeric position and the ring oxygen is replaced by a methylene group [1]. These compounds are selective and highly potent inhibitors of their corresponding glycoside hydrolases [2-4]. Moreover, C-5a-chain elongated derivatives (**2**) of 4-*epi*-isofagomine (4-*epi*-IFG, **1**) have been proven as highly potent pharmacological chaperones for the treatment of G_{M1} gangliosidosis [2,3]. As a matter of fact, the indicated structural characteristics of isoiminosugars (**I**) remain synthetically challenging. However, valuable synthetic strategies towards this compound class have been reported [2-4]. In context with our interest in the design and synthesis of such structures, we have found an efficient and concise synthetic approach towards isoiminosugars (A). This strategy relies on a $LiAlH_4$ triggered 1,2-shift in O-2 tosylated glycopyranoses (**A**) leading to corresponding C-2 carbon chain branched glycofuranosides (**B**) [5]. We applied this stereoselective ring contraction for the synthesis of isoiminosugars (**I**) [6]. Employing different configurations of **A** and variations in the reaction sequence open the avenue to various modifications in the substitution pattern. Herein, synthetic and mechanistic details, scope and limitations of this approach as well as their biological evaluation will be presented.

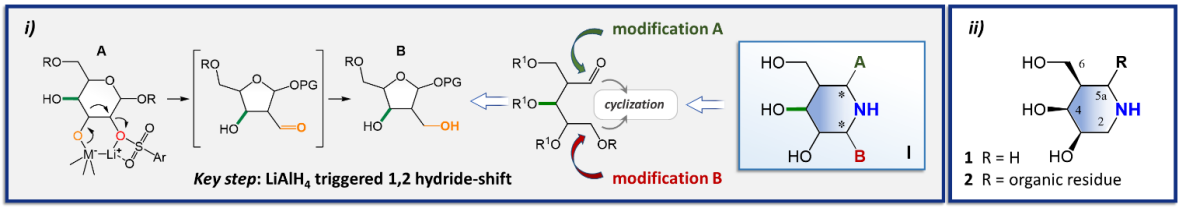

Figure 1. i) Schematic overview of a $LiAlH_4$ triggered 1,2-shift in O-2 tosylated pyranosides (**I**) as key-step in our general (retro-) synthetic concept for isoiminosugars (**I**, R, R^1 = protecting groups). **ii**) Structures of 4-*epi*-IFG (**1**) and C-5a carbon chain elongated derivatives (**2**), R = alkyl + reporter group, ligation handle and the like.

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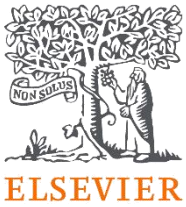
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