The 22nd European Carbohydrate Symposium

July 6-10, 2025

Gdańsk, Poland

Book of abstracts

Posters

CELLULOSE NANOCRYSTALS: A GREEN SOLUTION FOR ENHANCING PLANT GROWTH

Fatima-Zahra Ahchouch[a,b], Said Mouzeyar[a], Cédric Delattre[b,c], Jane Roche[a]

[a] Université Clermont Auvergne, INRAe, GDEC, F-63000 Clermont-Ferrand, France
fatima_zahra.ahchouch@doctorant.uca.fr
[b] Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, F-63000 Clermont-Ferrand, France
[c] Institut Universitaire de France (IUF), 1 Rue Descartes, 75005 Paris, France

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.

References:

1. FAO. 2024. La Situation mondiale de l'alimentation et de l'agriculture 2024. Rome, Italy: FAO.
2. Meena, Ram Swaroop, Sandeep Kumar, Rahul Datta, Rattan Lal, Vinod Vijayakumar, Martin Brtnicky, Mahaveer Prasad Sharma, et al. 2020. « Impact of Agrochemicals on Soil Microbiota and Management : A Review ». Land 9 (2): 34. https://doi.org/10.3390/land9020034.
3. Vinzant, Kari, Delaney E. Clouse, Sadat Kamal Amit, Ilia N. Ivanov, Virginia A. Davis, et Mariya V. Khodakovskaya. 2024. « Cellulose Nanocrystals Are a Renewable and Biocompatible Nanocarrier of Agrochemicals Directly to Plant Cells ». Advanced Sustainable Systems 8 (5): 2300511. https://doi.org/10.1002/adsu.202300511.
4. Squire, Henry J., Minjae Kim, Cerise Wong, Amanda Seng, Autumn Y. Lee, Natalie S. Goh, Jeffery Wei-Ting Wang, et Markita P. Landry. 2024. « Cellulose Nanocrystals Protect Plants from Pathogen Infection ». ACS Applied Nano Materials 7 (6): 6205-13. https://doi.org/10.1021/acsanm.3c06040.

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

Rahaf Akel[a], István Kacsir[a,b], Adrienn Sipos[c], Gábor Kardos[d], Péter Buglyó[b], Péter Bay[c], Sándor Kun[a], László Somsák[a]

[a] Department of Organic Chemistry, University of Debrecen, P.O. Box 400, H-4002 Debrecen, Hungary
rahaf.akel@science.unideb.hu
[b] Department of Inorganic & Analytical Chemistry, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary
[c] Department of Medical Chemistry, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary
[d] Department of Metagenomics, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary

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.

References:
1. Ranasinghe, R., et al. *Heliyon*, **2022**, 8, e10608.
2. Kacsir, I., et al. *Int. J. Mol. Sci.*, **2022**, 23, 813.
3. Kacsir, I., et al. *Int. J. Mol. Sci.*, **2021**, 22, 10454.

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

Rahaf Akel[a], Alshimaa I. Zaki[a], István Kacsir[a,b], Adrienn Sipos[c], György Attila Kiss[a], Gábor Kardos[d], Péter Buglyó[b], László Somsák[a], Péter Bay[c], Éva Bokor[a], Sándor Kun[a]

[a] Department of Organic Chemistry, University of Debrecen, P.O. Box 400, H-4002 Debrecen, Hungary
somsak.laszlo@science.unideb.hu
[b] Department of Inorganic & Analytical Chemistry, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary
[c] Department of Medical Chemistry, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary
[d] Department of Metagenomics, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary

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.

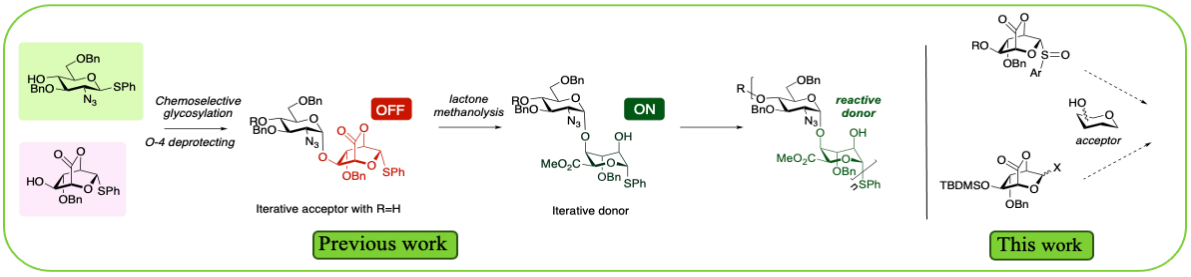
References:
1. Ranasinghe, R., et al. *Heliyon*, **2022**, 8, e10608.
2. Kacsir, I., et al. *Int. J. Mol. Sci.*, **2022**, 23, 813.
3. Kacsir, I., et al. *Int. J. Mol. Sci.*, **2021**, 22, 10454.

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

Rawan Alnufaie[a,b], Avantika Hasija[a], George Whitehead[a], John M. Gardiner[a]

[a] Department of Chemistry, University of Manchester, Oxford Road M13 9PL, United Kingdom
rawan.alnufaie@postgrad.manchester.ac.uk
[b] Collage of Science and Health Professions, King Saud bin Abdulaziz University for Health Sciences, Jeddah, Saudi Arabia

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.

References:

1. Jeanneret, R. A.; Dalton, C. E.; Gardiner, J. M.; *J. Org. Chem.,* **2019,** *84*, 15063-15078.
2. Hashimoto, Y.; Tanikawa, S.; Saito, R.; Sasaki, K.; *J. Am. Chem. Soc.* **2016,** *138*, 14840-14843.

RECONSTRUCTION OF CARBON ALLOCATION TOWARDS MULTIPLE PLANT CELL SINKS IN CYANOBACTERIA

Xinhui An, Vicente Ramírez, Markus Pauly

Institute for Plant Cell Biology and Biotechnology, Heinrich Heine University, 40225 Düsseldorf, Germany
xinhui.an@hhu.de

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

Chiara Andretta[a], Valentina Mocci[a], Simone Costagli[a], Giuseppe Stefanetti[b], Donata Medaglini[a], Elena Pettini[a]

[a] Laboratory of Molecular Microbiology and Biotechnology, Department of Medical Biotechnologies, University of Siena, Siena, Italy
c.andretta1@student.unisi.it
[b] Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy

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.

References:

1. Sorieul, C., Dolce, M., Romano, M. R., Codée, J. & Adamo, R. Glycoconjugate vaccines against antimicrobial resistant pathogens. *Expert Review of Vaccines* vol. 22 1055–1078 Preprint at https://doi.org/10.1080/14760584.2023.2274955 (2023).
2. Stefanetti, G., MacLennan, C. A. & Micoli, F. Impact and Control of Sugar Size in Glycoconjugate Vaccines. *Molecules* vol. 27 Preprint at https://doi.org/10.3390/molecules27196432 (2022).
3. Boonyaratanakornkit, J. & Taylor, J. J. Techniques to Study Antigen-Specific B Cell Responses. *Frontiers in immunology* vol. 10 1694 Preprint at https://doi.org/10.3389/fimmu.2019.01694 (2019).
4. Hoving, D. *et al.* Combinatorial multimer staining and spectral flow cytometry facilitate quantification and characterization of polysaccharide-specific B cell immunity. *Commun Biol* 6, (2023).
5. Micoli, F. *et al.* A scalable method for O-antigen purification applied to various Salmonella serovars. *Anal Biochem* 434, 136–145 (2013).

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

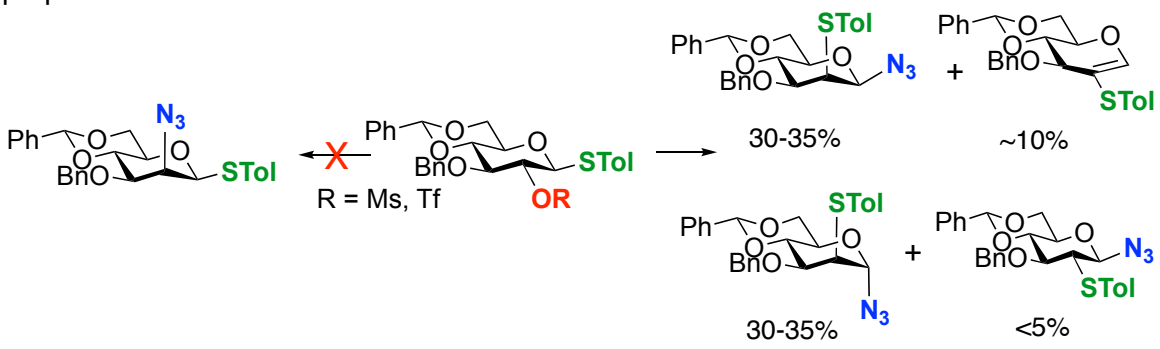
Zunaira Arshad, Avantika Hasija, John M. Gardiner

Department of Chemistry, University of Manchester, Oxford Road M13 9PL, United Kingdom
zunaira.arshad@postgrad.manchester.ac.uk

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.

References:
1. Lassfolk, R.; Leino, R.; *Chem. Eur. J.* **2023**, 29, 2023, 29, e202301489, 1-6.
2. Poláková, M.; Horákb, R.; Šestáka, S.; Holková, I.; *Carbohydr. Res.* **2016**, 428, 62-71.
3. Zhoua, Y.; Zhanga, X.; Rena, B.; Wua, B.; Peib, Z.; Dong, H.; *Tet. Lett.*, **2014**, 70, 5385-5390.

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

Milena Bartoloni[a], Sandra Delgado[a], Tammo Diercks[a], Jesús Jiménez-Barbero[a,b], Ana Ardá[a]

[a] CICbioGUNE, Basque Research and Technology Alliance, Bizkaia Technology Park, Ed. 800. E-48160, Derio, Spain
mbartoloni@cicbiogune.es
[b] Ikerbasque, Basque Foundation for Science, Euskadi Plaza 5, 48009 Bilbao, Bizkaia, Spain

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.

References:

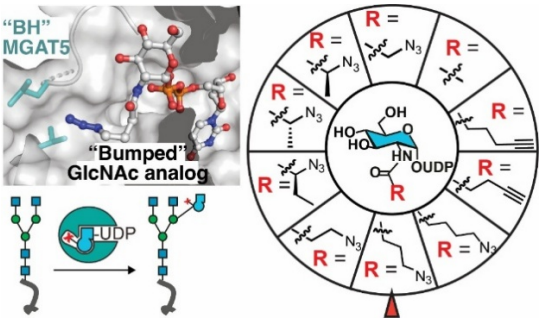
1. Yang R-Y, Rabinovich GA, Liu F-T. Galectins: structure, function and therapeutic potential. Expert Reviews in Molecular Medicine. 2008.
2. Cummings RD, Liu FT, Vasta GR. Galectins. 2017. In: Varki A, Cummings RD, Esko JD, et al., editors. Essentials of Glycobiology [Internet]. 3rd edition.,2015-2017, 36.
3. Lete MG, Franconetti A, Bertuzzi S, Delgado S, Azkargorta M, Elortza F, Millet O, Jiménez-Osés G, Arda A, Jiménez-Barbero J. NMR Investigation of Protein-Carbohydrate Interactions: The Recognition of Glycans by Galectins Engineered with Fluorotryptophan Residues. Chemistry. 2023
4. Gimenez D, Phelan A, Murphy CD, Cobb SL. 19F NMR as a tool in chemical biology. Beilstein J Org Chem. 2021,17:293-318.
5. Peter B. Crowley, Ciara Kyne, William B. Monteith, Simple and inexpensive incorporation of 19F-Tryptophan for protein NMR spectroscopy, Chem. Commun., 2012, 48, 10681–10683.

A BIOORTOGONAL PRECISION TOOL FOR HUMAN N-ACETYLGLUCOSAMINYLTRANSFERASE V

Ganka Bineva-Todd[b], Yu Liu[a,b], Richard W. Meek[c,d], Laura Mazo[e], Beatriz Piniello[e], Olga Moroz[c], Sean A. Burnap[i,j], Nadima Begum[a], André Ohara[g], Chloe Roustan[f], Sara Tomita[a], Svend Kjaer[f], Karen Polizzi[g], Weston B. Struwe[i,j], Carme Rovira[e,h], Gideon J. Davies[c], Benjamin Schumann[a,b]

[a] Department of Chemistry, Imperial College London, W12 0BZ, London, UK
[b] Chemical Glycobiology Laboratory, The Francis Crick Institute, NW1 1AT London, UK
ganka.bineva@crick.ac.uk

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.



References:
1. A. Varki *et.al.* in *Essentials of Glycobiology*, 4th edition, **2022**.
2. J.F. Darby *et al.*, *ACS Catal.* **2020,** 10, 8590-8596.
3. B. Schumann *et al., Mol. Cell* **2020**, 78, 824-834.
4. A. Cioce *et al.*, *Nat Commun.* **2022**, 3, 3341-3355.

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

Peter Black[a], Emma Hobbs[a,b], Terry Smith[a], Tracey Gloster[a]

[a]School of Biology, University of St Andrews, North Haugh, St Andrews KY16 9ST, UK
pmb9@st-andrews.ac.uk
[b]European Bioinformatics Institute, EMBL, Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK

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.

References:

1. Kaufer, A., Ellis, J., *et al.*, *Parasites Vectors* **2017**, *10*(1), 287.
2. Altamura, F., Rajesh, R., *et al.*, *Drug Development Research* **2022**, *83*(2), 225–252.
3. Veríssimo, C. M., Graeff-Teixeira, C *et al.*, *Parasitology* **2019**, *146*(10), 1217–1232.
4. Duncan, S. M., & Ferguson, M. A. J., *The Biochemical Journal* **2022**, *479*(17), 1743–1758.
5. Nardy AF, Freire-de-Lima CG, *et al.*, Front Microbiol. **2016**, *7*, 348.

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

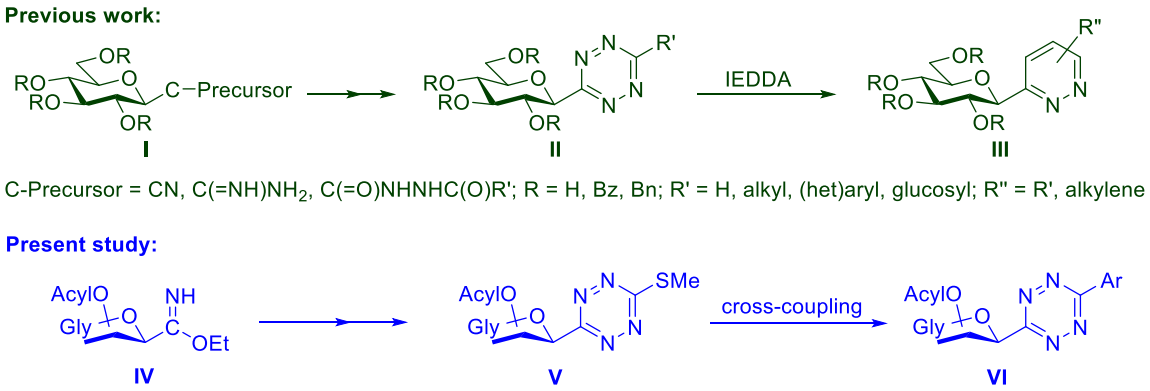
Éva Bokor, Eszter Kardos, Soma B. Sajbán, László Lázár, Akram Dabian, László Somsák

Department of Organic Chemistry, University of Debrecen, P.O. Box 400, H-4002 Debrecen
bokor.eva@science.unideb.hu

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.

References:

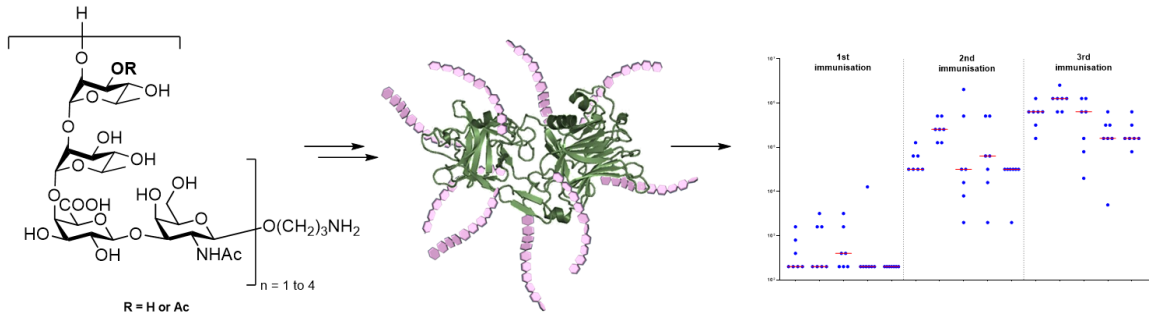
1. H. Neunhoeffer in *Comprehensive Heterocyclic Chemistry*, *Vol. 3* (Eds.: A. R. Katritzky, C. V. Rees), Pergamon, Oxford, **1984**, pp 531-572.
2. B. L. Oliveira, Z. Guo, G. J. L. Bernardes, *Chem. Soc. Rev.* **2017**, *46*, 4895-4950.
3. É. Bokor, D. T. Kecskés, F. Gombás, A. Fehér, E. Kardos, A. Dabian, Zs. Vonza, E. Szennyes, L. Somsák, *New J. Chem.* **2023**, *47*, 56-74.

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

Marion Bouchet, Olimpia Pitirollo, Catherine Guerreiro, Laurence A. Mulard

Chemistry of Biomolecules Unit, Department of Structural Biology and Chemistry, Institut Pasteur, Université Paris Cité, CNRS UMR3523, F-75015 Paris, France
marion.bouchet@pasteur.fr

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.



References:
1. K.L. Kotloff, et al., *The Lancet* 2013, 382, 209-222.
2. D. Cohen, et al., *Lancet Infect Dis* 2021, 21, 546-558.
3. S. Livio, et al., *CID* 2014, 59, 933-941.
4. Y.A. Knirel, et al., *Biochemistry Moscow* 2015, 80, 901-914.

SWEET-ANTIMICROBIALS

Jeroen Brzoskowski[a,b], Marta Artola[a], Zachary Armstrong[b]

[a] Medical Biochemistry Department, Leiden Institute of Chemistry, Leiden University, The Netherlands
[b] Bio-organic Synthesis Department, Leiden Institute of Chemistry, Leiden University, The Netherlands
brzoskowskijcr@vuw.leidenuniv.nl

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].

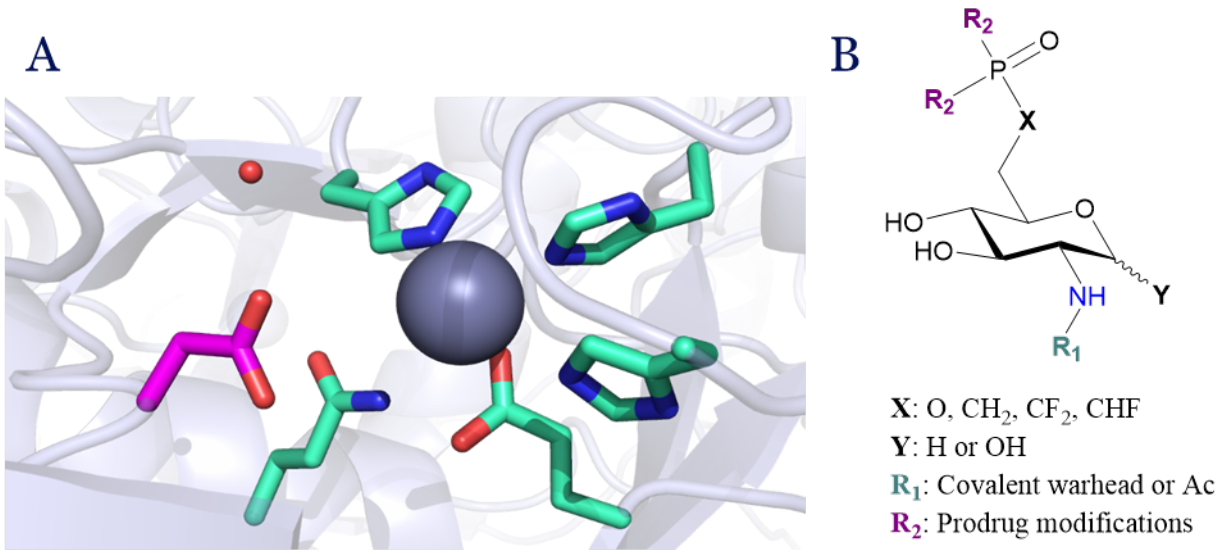


***Figure 1**. Active site residues of H.infNagA crystalstructure. B: Proposed natural substrat mimic inhibitor design.*

References:
1. Murray, C.J.L., et al., *The Lancet*, **2022**.
2. WHO., Antimicrobial resistance. **2021**.
3. Mayer, C., et al., Int J Med Microbiol, **2019**.
4. Sassetti, C.M., et al., *Mol Microbiol*, **2003**.
5. Ahangar, M.S., et al., *J Biol Chem,* **2018**.

SYNTHESIS OF *ACINETOBACTER BAUMANNII* LAC-4 POLYSACCHARIDE FRAGMENTS

Mohini Burnwal, Giuseppe D'Orazio, Luigi Lay

Department of Chemistry, University of Milan, Via Camillo Golgi 19 20133 Milan
mohini.burnwal@unimi.it

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.

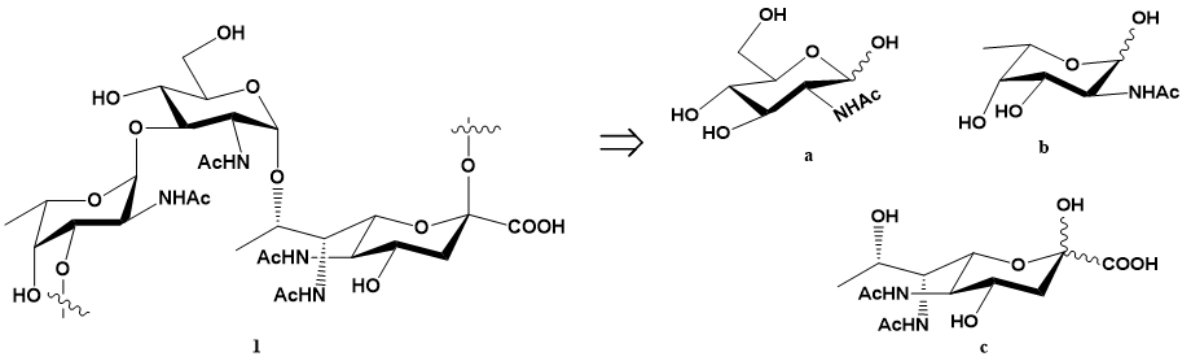


Figure 1 Retrosynthetic plan for LAC-4 trisaccharide

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.

References:

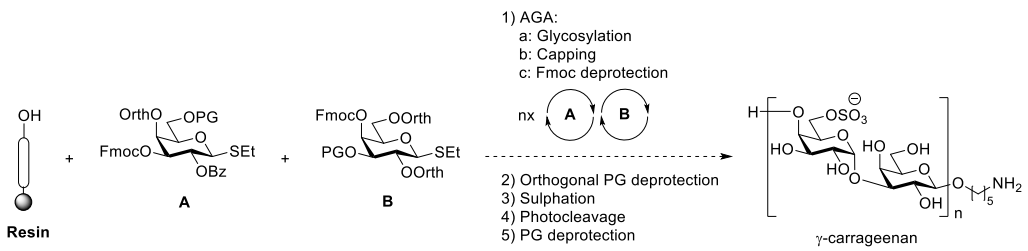
1. Evgeny Vinogradov, Leann MacLean, H. Howard Xu, Wangxue Chen, *Elsevier.* **2014***, 390,* 42-45.
2. Silvia Pires, Dane Parker, *Interferon & Cytokine Research,* **2019,** *39*, 441-449.
3. Greg Harris, Rhonda Kuo Lee, Christopher K. Lam, Gregory Kanzaki, Girishchandra B. Patel, H. Howard Xu, Wangxue Chena, *Antimicrobial Agents and Chemotherapy*, **2013***, 57*, 3601-3613.

CHEMICAL SYNTHESIS OF CARRAGEENAN OLIGOSACCHARIDES USING AUTOMATED GLYCAN ASSEMBLY

Brock Byers, Fabian Pfrengle

Department of Natural Sciences and Sustainable Resources, Institute of Organic Chemistry, BOKU University, Vienna 1190, Austria
brock.byers@boku.ac.at.

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.

References:

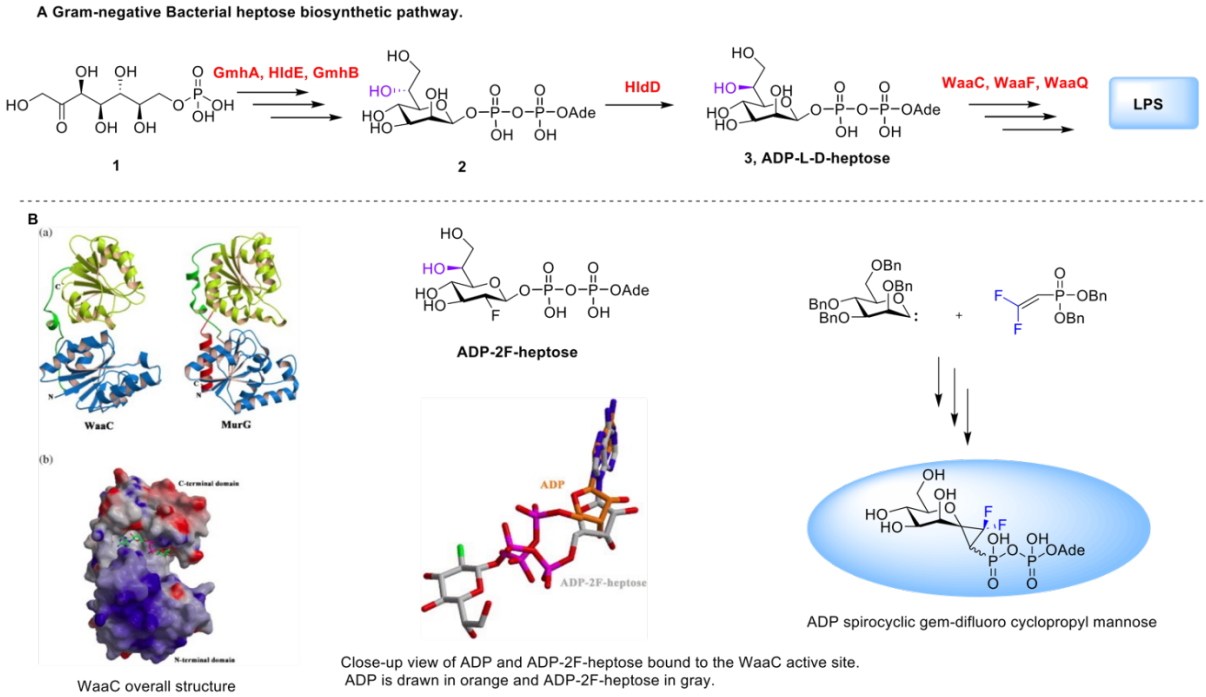
1. Berezhnaya, Y.D.; Kazachenko, A. S.; Kazachenko, A. S.; Malyar, Y. N.; Borovkova, V. S. *Chemistry*. **2024**, 6, 640–665.
2. Chevenier, A.; Jouanneau, D.; Ficko-Blean, E. *Cell Surf*. **2023**, 9, 100097.
3. Mirzaei, A.; Esmkhani, M.; Zallaghi, M.; Nezafat, Z.; Javanshir, S. *J. Environ. Polym. Degrad*. **2022**, 31, 1679–1705.
4. Ficko-Blean, E.; et al. *Nat. Commun*. **2017**, 8, 1685.
5. Liu, G-L.; Wu, S-L.; Sun, Z.; Xing, M-D.; Chi, Z-M.; Lui, Y-J. *Appl. Environ. Microbiol*. **2024**, 90, 7.

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

Jun Cao, Stéphane. P. Vincent

Department of Chemistry, Laboratory of Bio-Organic Chemistry – NAmur Research Institute for LIfe Sciences (NARILIS), University of Namur (UNamur), 5000 Namur, Belgium
jun.cao@unamur.be

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.



References:
1. Raetz C R H, Reynolds C M, Trent M S, et al. *Annu. Rev. Biochem.,* **2007**, 76(1): 295-329.
2. Grizot S, Salem M, Vongsouthi V, et al. *J. Mol. Biol.* **2006**, 363, 383-394.
3. Cao J, Vincent S P. *Org. Lett.* **2022**, 24(23): 4165-4169.

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

Zuzana Košťáľová, Iveta Uhliariková, Romana Vrzoňová, Peter Capek

Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK - 84538 Bratislava, Slovakia
chemcape@savba.sk

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.

References:

1. C. Laroche, *Mar. Drugs* **2022**, 20, 336. https://doi.org/10.3390/ md20050336.

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

Sara Capellato, Fabian Pfrengle

Department of Natural Sciences and Sustainable Resources, Institute of Organic Chemistry, BOKU University, Vienna, Austria
sara.capellato@boku.ac.at

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.

References:

1. E.J. Haining, D. Cherpokova, K. Wolf, I.C. Becker, S. Beck, J.A. Eble, D. Stegner, S.P. Watson, B. Nieswandt. *Blood* **2017**, 130(20), 224-2228.
2. M.T. Ale, A.S. Meyer, *RSC Advances* **2013**, 3 (22), 8131–8141.
3. H. Yu, S. Huang, H. Chokhawala, M. Sun, H. Zheng, X. Chen, *Angew. Chem. Int. Ed.* **2006**, 45 (24), 3938–3944.
4. H. Malekan, G. Fung, V. Thon, Z. Khedri, H. Yu, J. Qu, Y. Li, L. Ding, K. S. Lam, X. Chen, *Bioorganic & Medicinal Chemistry* **2013**, 21 (16), 4778–4785.
5.M.T. Ale, A.S. Meyer, *RSC Advances* **2013**, 3 (22), 8131–8141.

NEO-GLYCOPROTEINS: NATURE-LIKE LIGANDS WITH IMPRESSIVE AFFINITIES

Jakub Červený[a,b], Viktoria Heine[a], Michaela Hovorková[a], Sandra Delgado[c], Ana Ardá[c], Jesús Jiménez-Barbero[c], Pavla Bojarová[a]

[a] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, CZ-142 00, Prague 4, Czech Republic
jakub.cerveny@biomed.cas.cz
[b] Department of Analytical Chemistry, Faculty of Science, Charles University, Hlavova 8, CZ-128 43, Prague 2, Czech Republic
[c] CICbioGUNE, Basque Research and Technology Alliance, Derio, Spain

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.

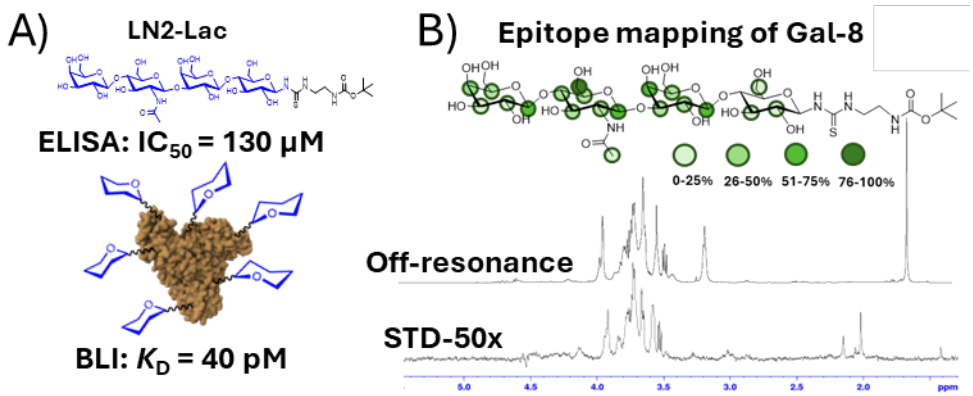


Figure [1]. Investigation of the interaction between LN2-Lac and Gal-8. A) Affinity determined using ELISA and BLI. B) Epitope mapping of Gal-8 using STD-NMR.

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

References:

1. T. Vašíček et al., *Chem. - Eur. J.*, **2020**, 26 (43), 9620-9631
2. D. Vrbata et al., *Bioorg. Chem.*, **2024**, 145, 10723

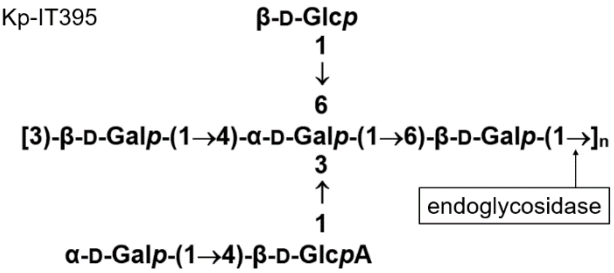
THE CAPSULAR POLYSACCHARIDE PRODUCED BY *KLEBSIELLA PNEUMONIAE* Kp-IT395 HAS A NOVEL PRIMARY STRUCTURE

Paola Cescutti[a], Michela Zaro[a], Marco M. D'Andrea[b], Neil Ravenscroft[c], Barbara Bellich[d], Francesca Nonne[e], Mariagrazia Molfetta[e], Carlo Giannelli[e], Francesca Micoli[e]

[a] Department of Life Sciences, University of Trieste, Italy
pcescutti@units.it
[b] Department of Biology, University of Rome - Tor Vergata, Italy
[c] Department of Chemistry, University of Cape Town, South Africa
[d] Department of Advanced Translational Diagnostics, IRCCS "Burlo Garofolo", Trieste, Italy
[e] GSK Vaccines Institute for Global Health (GVGH), Via Fiorentina 1, 53100 Siena, Italy

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



Acknowledgements: PRIN Project Prot. 20224T3X8K CUP J53D23001010006 realised with the contribution from Mission 4, Component 2, Investment 1.1 'Research Projects of Significant National Interest (PRIN) of the National Recovery and Resilience Plan (PNRR) funded by the European Union "Next Generation EU". This project was partially funded by a grant from the Gates Foundation (grant number INV-033019)

References:

1. K. Sands, M. J. Carvalho, E. Portal, *et al., Nat Microbiol*, **2021**, *6*, 512-523.

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

Paola Cescutti[a], Iztok Dogsa[b], Barbara Bellich[c], Mojca Blaznik[b], Cristina Lagatolla[a], Neil Ravenscroft[d], David Stopar[b]

[a] Department of Life Sciences, University of Trieste, Italy
pcescutti@units.it
[b] Department of Microbiology, University of Ljubljana, Slovenia
[c] Department of Advanced Translational Diagnostics, IRCCS "Burlo Garofolo", Trieste, Italy
[d] Department of Chemistry, University of Cape Town, South Africa

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.

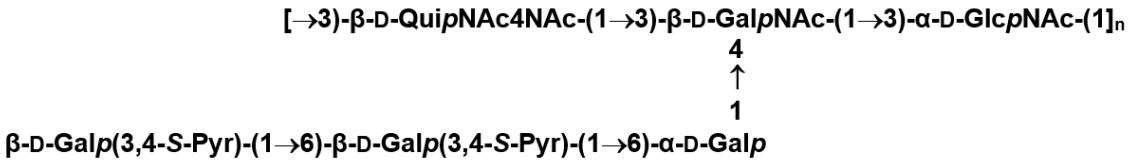


Figure 1. Structure of EpsA-O repeating unit

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.

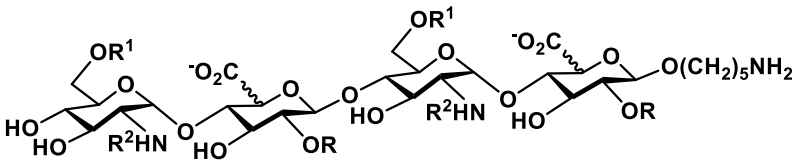
References:
1. M. Krajnc, P. Stefanic, R. Kostanjšek, I. Mandic-Mulec, I. Dogsa, D. Stopar, *npj Biofilms Microbiomes*, **2022**, *8*, (25) 1-11.
2. I. Dogsa, B. Bellich, M. Blaznik, C. Lagatolla, N. Ravenscroft, R. Rizzo, D. Stopar, P. Cescutti, *npj Biofilms Microbiomes*, **2024**, *10*, (98) 1-17.

PROBING SPECIFIC INTERACTION OF SYNTHETIC HEPARAN SULFATE TETRASACCHARIDES WITH MIDKINE

Cheng-Hsiu Chang, Zhi-Geng Chen, Yong-Qing Zhong, Anandaraju Bandaru, Chiao-Chu Ku, Shang-Cheng Hung

Genomics Research Center, Academia Sinica, Taipei 11529, Taiwan
d933426@oz.nthu.edu.tw

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.



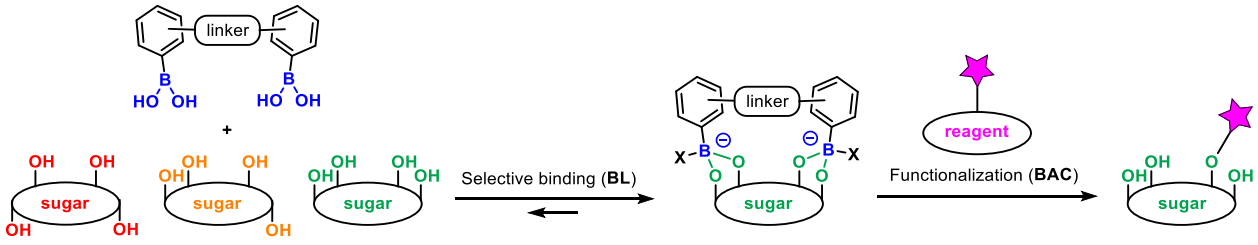
R = H or SO_3^-, R^1 = H or SO_3^-, R^2 = SO_3^- or Ac

DESIGN, SYNTHESIS AND REACTIVITY OF MANNOSE-SPECIFIC BORONOLECTINS

Mathieu Chaouat[a], Basile Parmelli[a], Edouard Brosset-Heckel[a], Paul Zheng[a], Pascal Bonnet[b], Tania Xavier[a], Patricia Busca[a]

[a]Université Paris Cité, LCBPT – UMR 8601 CNRS, 45 rue des Saints-Pères, 75006 Paris, France
[b]Université d'Orléans, ICOA – UMR 7311 CNRS, rue de Chartres, 54100 Orléans, France
mathieu.chaouat@u-paris.fr

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.

References:
1. Hart, G. W.; Varki, A. *Essentials of Glycobiology 4th edition*, Cold Spring Harbor (NY), **2022**.
2. Smith, B. A. H.; Bertozzi, C.R. *Nat. Rev. Drug. Discov.* **2021**, *20*, 217.
3. Palaniappan, K. K.; Bertozzi, C. R. *Chem. Rev.* **2016**, *116*, 14277.
4. Bian, Z.; Liu, A.; Li, Y.; Fang, G.; Yao, Q.; Zhang, G.; Wu, Z. *Analyst* **2019**, *145*, 719.
5. Graham, B. J.; Raines, R. T. *J. Org. Chem.* **2024**, *89*, 2069.

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

Adam Choma[a], Iwona Komaniecka[a], Anita Swatek[a], Zbigniew Kaczyński[b], Małgorzata Pawlik[c], Zofia Piotrowska-Seget[c]

[a] Department of Genetics and Microbiology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
adam.choma@mail.umcs.pl
[b] Laboratory of Structural Chemistry, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk, Poland
[c] Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Jagiellońska 28, 40-032 Katowice, Poland

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)

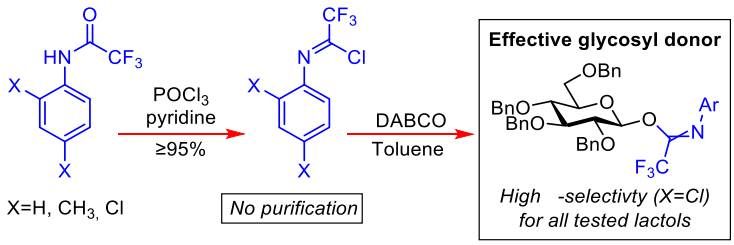
References:
1. D.M. Duell, A. Tobin, J.M. Box, V.S. Kolli, R.W. Carlson, K.D. Noel, *J. Bacteriol*. **2001**, 183(20), 6054-6064.

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

Nikolai Cordua, S. R. Steffensen, A. Mascherpa, L. G. Christensen, H. H. Jensen

Aarhus University, Department of chemistry, 8000 Aarhus, Denmark
hhj@chem.au.dk

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.

References:

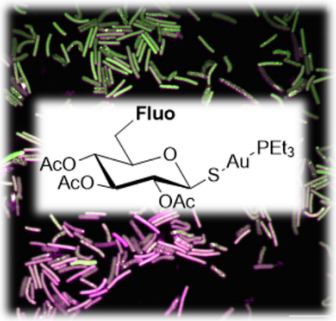
1) Yu, B.; Tao, H. Glycosyl Trifluoroacetimidates. Part 1: Preparation and Application as New Glycosyl Donors. *Tetrahedron Lett.* **2001**, *42*, 2405–2407.
2) Yu, B.; Tao, H. Glycosyl Trifluoroacetimidates. 2. Synthesis of Dioscin and Xiebai Saponin I. *J. Org. Chem.* **2002**, *67*, 9099–9102.

AURANOFIN PROBES TOWARDS A BETTER UNDERSTANDING OF ITS MECHANISM OF ACTION

Johan Cornil[a], Cyril Anjou[b], Isabelle Martin-Verstraete[b], Laurence A. Mulard[a]

[a] Chimie des Biomolécules, Department of Structural Biology and Chemistry, Institut Pasteur, Université Paris Cité, CNRS UMR3523 Chem4Life, F-75015 Paris, France
johan.cornil@pasteur.fr
[b] Laboratoire Pathogenèse des Bactéries Anaérobies, Université Paris Cité, UMR CNRS 6047, Institut Pasteur, F-75015 Paris, France

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.

References:

1. C. Bombardier, J. Ware, I.J. Russell, M. Larson, A. Chalmers, J.L. Read, *Am. J. Med.* **1986**, *4*, 565-578.
2. F. Cosciona, S. Zineddu, V. Vitali, M. Fondi, L. Messori, E. Perrin, *Antibiotics* **2024**, *13*, 652.
3. Y. Liu, Y. Lu, Z. Xu, X. Ma, X. Chen, W. Liu, *Drug Discovery Today* **2022**, *27*, 1961-1973.
4. S. Jackson-Rosario, *et al. Biol. Inorg. Chem.* **2009**, *14*, 507-519.
5. M. Yamashita, *International Immunopharmacology* **2021**, *101*, 108272.
6. L. Saujet, C.F. Pereira, A.O. Henriques, I. Martin-Verstraete, *FEMS Microbiol. Lett.* **2014**, *1*, 1-10.

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

Maria Michela Corsaro[a], Angela Casillo[a], Francesca Carella[b], Angelina Cordone[b], Silvia Fanina[a], Simonetta Fraschetti[b]

[a] Department of Chemical Sciences, University of Naples Federico II, Naples, Italy
corsaro@unina.it
[b] Department of Biology, University of Naples Federico II, Naples, Italy

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.

References:

1. Pruzzo, C., Gallo, G. *Environment. Microbiol.* **2005**, *7*(6), 761-772.
2. Destoumieux-Garzón, D., Canesi, L., et al. *Environment. Microbiol.* **2020**, *22*(10), 4323-4341.

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

Shane Coyle[a], Kyle Doherty[a], Marwa Alawi[b], Oliwier Dulawa[a], Fiona Walsh[b,c], Trinidad Velasco-Torrijos[a,c]

[a] Department of Chemistry Maynooth University, Maynooth, Co. Kildare, W23 F2H6, Ireland
shane.coyle.2020@mumail.ie
[b] Department of Biology Maynooth University, Maynooth, Co. Kildare, W23 F2H6, Ireland
[c] Kathleen Lonsdale Institute for Human Health Research, Maynooth University, Co. Kildare Ireland

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.

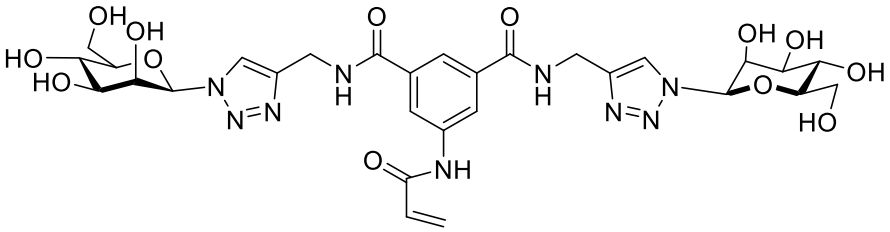


Figure 1. Chemical structure of a cross-linking divalent mannoside.

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

References:

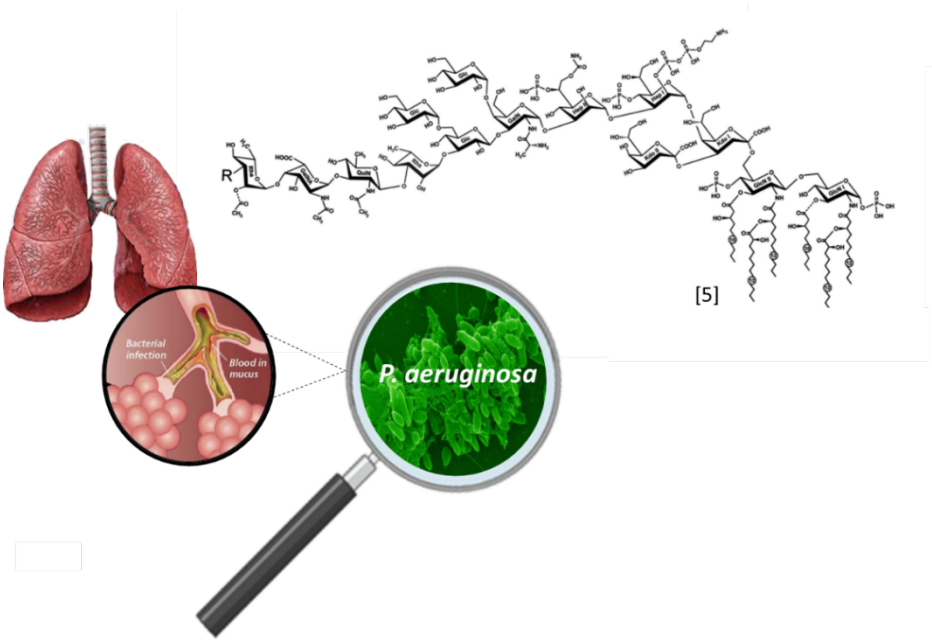
1. X.Yang et al., *Front. Public Health,* **2022,** 10.
2. L.mousavifar et al., *Pharmaceutics,* **2023**, 15, 527.
3. L.Boike et al., *Nat Rev Drug Discov* **2022,** 21,881-898.
4. K.doherty et al., *Bioorg. Med. Chem*, **2025,** 117, 118020.

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

Raffaele D'Amico[a], Angela Casillo[a], Wojciech Jachymek[b], Rosanna Papa[c], Anna Maciejewska[b], Marta Kaszowska[b], Jolanta Lukasiewicz[b], Maria Michela Corsaro[a]

[a] Department of Chemical Sciences, Federico II University, Complesso Universitario Monte S. Angelo, Via Cintia 4, 80126 Naples, Italy
raffaele.damico@unina.it
[b] Laboratory of Microbial Immunochemistry and Vaccines, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wroclaw, Poland
[c] Department of Public Health and Infectious Diseases, Sapienza University, Piazzale Aldo Moro 5, 00185 Rome, Italy

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].

References:

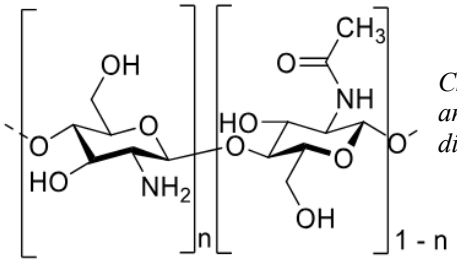
1. H.C. Flemming, J. Wingender, *Nat. Rev. Microbiol*. **2010**, *8*, 623–633.
2. R. Papa, E. Imperlini, *Antibiotics*, **2023**, *13*, 1
3. V. Deretic, M.J. Schurr, *Trends Microbiol*, **1995**, *3*, 351–356.
4. D.P. Speert, S.W. Farmer, *J. Clin. Microbiol*. **1990**, *28*, 188–194.
5. Y.A. Knirel, O.V. Bystrova, *J. Endotoxin. Res*. **2006**, *12*, 324-336.

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

Daphnée Brulé[a], Marie-Claire Héloir[a], Thibault Roudaire[a], Jérémy Villette[a], Silvère Bonnet[b], Yoann Pascal[b], Benoît Darblade[b], Philippe Crozier[c], Philippe Hugueney[d], Véronique Coma[e], Benoit Poinssot[a]

[a] UMR Agroécologie, INRAE, Institut Agro Dijon, Université de Bourgogne, Dijon, France
[b] Elicityl, Crolles, France
benoit.darblade@elicityl.fr
[c] SIPCAM, Puteaux, France
[d] UMR-A 1131 Santé de la Vigne et Qualité du Vin, Université de Strasbourg, INRAE, Colmar, France
[e] Laboratoire de Chimie des Polymères Organiques, Université de Bordeaux, CNRS, Bordeaux INP, UMR 5629, Pessac, France

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).

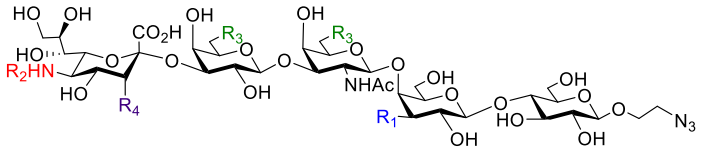
References:
1. Brulé D, Héloir MC, Roudaire T, Villette J, Bonnet S, Pascal Y, Darblade B, Crozier P, Hugueney P, Coma V, Poinssot B. Increasing vineyard sustainability: innovating a targeted chitosan-derived biocontrol solution to induce grapevine resistance against downy and powdery mildews. *Front Plant Sci*. **2024**,7;15:1360254
2. Huet G, Wang Y, Gardrat C, Brulé D, Vax A, Le Coz C, Pichavant F, Bonnet S, Poinssot B, Coma V. Deep Chemical and Physico-Chemical Characterization of Antifungal Industrial Chitosans-Biocontrol Applications. *Molecules*. **2023**, 18;28(3):966

DEVELOPMENT OF SIALOGLYCOMIMETICS FOR THE EVALUATION AS POTENTIAL SIGLEC INHIBITORS

Beatriz del Campo[a], Jon I. Quintana[a], Begoña Echeverría[a], Elisabete Elosegui[a], June Ereño[b], Niels C. Reichardt[a]

[a] Glycotechnology Laboratory, CIC BiomaGUNE, Paseo de Miramon 194, 20014, Donostia, Spain
bdelcampo@cicbiomagune.es
[b] Cancer Glycoimmunology Laboratory, CIC BioGune, Bizkaia Technology Park, 48160, Derio, Spain

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.



1

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

References:
1. M. Pia, U. Atxabal, I. Oyenarte, J. Jimenez-Barbero, J. Ereño-Orbea, *Cells,* **2024***, 62,* 1-9.
2. P. R. Crocker, J. C. Paulson, A. Varki, *Nat Rev Immunol,* **2007**, *7*, 255-266.
3. R. L. Schnaar, *Glycoconjugate Journal*, **2023**, *40*, 159-167.

SUGAR DERIVATIVES AS ALTERNATIVE RAW MATERIALS FOR QAS SYNTHESIS

Barbara Dmochowska, Justyna Samaszko-Fiertek, Janusz Madaj

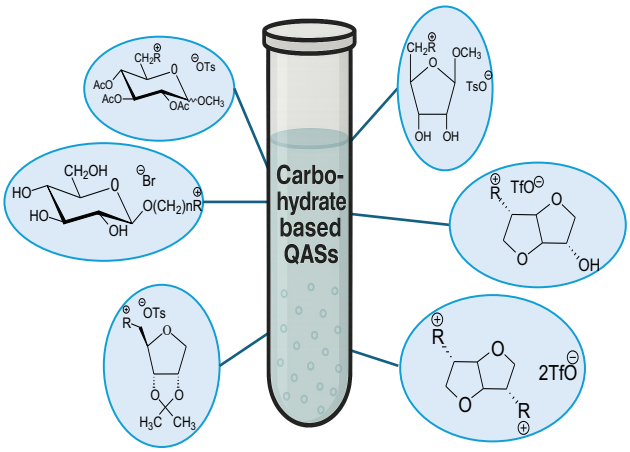
Department of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk
basia.dmochowska@ug.edu.pl

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].



Acknowledgements: This work was partially financed by grant DS. 531-T100-D501-25

References:

1. V. Zullo, A. Iuliano, L. Guazzelli, *Molecules* **2021**, *26*, 2052–2128.
2. S. Jopp, *Eur. J. Org. Chem.* **2020**, 6418–6428.

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

Patrícia Dudoňová[a], Kristína Kianičková[a], Lucia Pažitná[a], Peter Baráth[a], Tereza Kuracinová[b], Pavol Janega[b,c], Jaroslav Katrlík[a]

[a] Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 5807/9, 845 38, Bratislava, Slovakia
chempdud@savba.sk
[b] Faculty of Medicine, Comenius University Bratislava, Sasinkova 4, 814 99, Bratislava, Slovakia
[c] Medirex Group Academy, n.p.o., Novozámocká 67, 949 05 Nitra, Slovakia

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.*

References:

1. Mahema, S., et al., Cell Biochemistry and Biophysics, **2024,**82.4, 3367-3381.
2. H. Baker-Rand, et al., Cancers, **2024**, 16, 1028.
3. T. Shuang, et al., Molecules, **2024,** 29**,** 939.

ANALYSIS OF MOLASSES FOR ELECTROCHEMICAL CONVERSIONS

Annalena Erdmann[a], Ashin Paul Philip[b], Prof. Dr. Udo Kragl[a]

[a] Department of Chemistry, University of Rostock, Albert-Einstein-Straße 3a, annalena.erdmann@uni-rostock.de
[b] Leibniz Institute for Catalysis, Abert-Einstein-Straße 29a, 18059 Rostock

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]

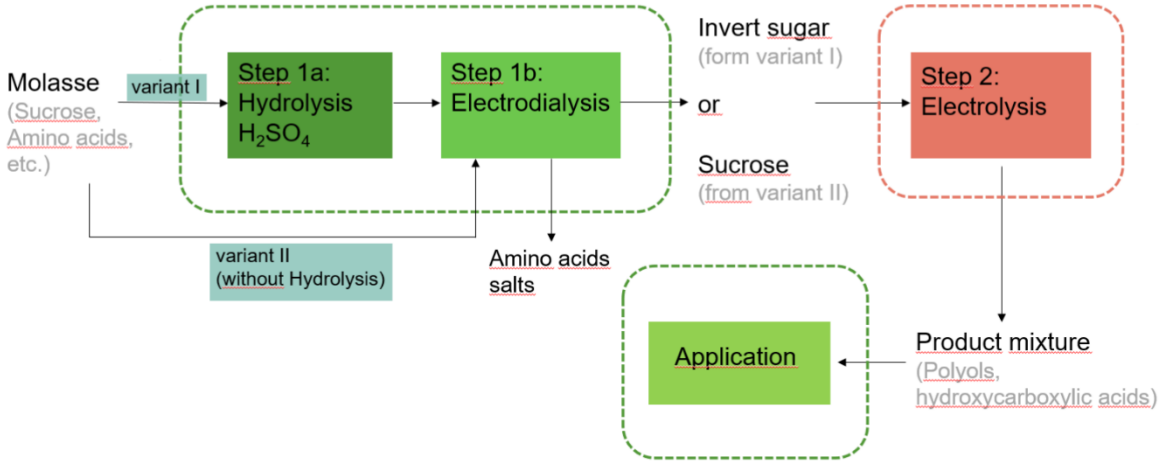


Figure 1. Flowsheet for the planned process for the electrochemical refinement of molasses.

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]

References:

1. A. Palmonari, D. Cavallini, C. J. Sniffen, L. Fernandes, P. Holder, L. Fagioli, I. Fusaro, G. Biagi, A. Formigoni, L. Mammi, *Journal of dairy science* 2020, 103, 6244.
2. W. Xu, L. Liang, M. Zhu, *International Journal of Food Properties* 2015, 18, 547.
3. H. Lou, Y. Yang, X. Bu, H. Fan, D. Weng, J. Zhang, W. Gao, D. Wen, *J. Mater. Chem.* A 2025, 13, 1067.

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

Silvia Fanina[a], Angela Casillo[a], Patrícia Concórdio-Reis[b,c], Filomena Freitas[b,c], Maria Michela Corsaro[a]

[a] Department of Chemical Sciences, Federico II University, Naples, Italy
silvia.fanina@unina.it
[b] Associate Laboratory i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, NOVA University of Lisbon, Caparica, Portugal
[c] Department of Chemistry, UCIBIO, Applied Molecular Biosciences Unit, NOVA School of Science and Technology, NOVA University of Lisbon, Caparica, Portugal

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.

References:

1. Macdonald J.F.H., Han Y., Astafyeva Y., et al. *Appl Microbiol Biotechnol*. **2025**;109(1):6.
2. Patrinou, V.; Patsialou, S.; Daskalaki, A.; Economou, C.N.; Aggelis, G.; Vayenas, D.V.; Tekerlekopoulou, A.G. *Life* **2023**, 13, 480.
3. Koletti A., Skliros D., Kalloniati C., et al. *Appl Microbiol Biotechnol*. **2024**;108(1):138.
4. Laroche C. *Mar Drugs*. **2022**;20(5):336.
5. Parra-Riofrio, G., Moreno, P., García-Rosado, E. et al. *Aquacult* **2023** Int 31, 3145–3157.
6.Parra-Riofrío G., García-Márquez J., Casas-Arrojo V., Uribe-Tapia E., Abdala-Díaz R.T. *Mar Drugs*. **2020**;18(11):534.

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

Aida García[a,b], Begoña Echeverría[a], Jon I. Quintana[a], Felipe Goñi de Cerio[b], Paloma Gómez Fernández[a] , Niels Reichardt[a]

[a] CICbiomaGUNE, Paseo de Miramón 182, 20009 Spain
[b] GAIKER Technology Centre, Bizkaia Technology Park 202, 48170, Spain
agarciapereira@cicbiomagune.es

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).

References:

1. X. Huang, X. Wang, J. Zhang, *et al*. *Vaccines*. **2017**, *2*, 3.
2. E. Strable, & M. G. Finn. *Current Topics in Microbiology and Immunology*. **2009**, 327, 1-21.
3. K. Stavenhagen, L. C. Laan *et al*. *CMLS*. **2021**, 78,14.
4. L. M. Kuijk, & I. van Die. *IUBMB life*. **2010**, 62(4), 303–312.
5. S. Holst, A.I. Belo, E. Giovannetti, *et al. Sci Rep*. **2017 7**, 16623.

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

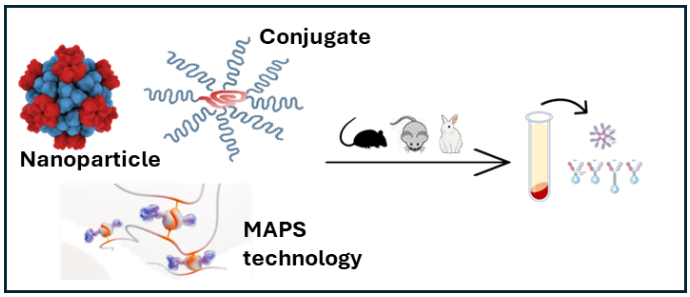
Gloria Gnoni[a], Martina Carducci[b], Renzo Alfini[b], Luisa Massai[b], Gianina Florentina Belciug[b], Francesca Nonne[b], Elena Palmieri[b], Rebecca Nappini[b], Vittorio Bordoni[b], Francesco Berti[c], Carlo Giannelli[b], Omar Rossi[b], Giuseppe Stefanetti[a], Francesca Micoli[b]

[a] Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy
gloria.x.gnoni@gsk.com; g.gnoni1@campus.uniurb.it
[b] GSK Vaccines Institute for Global Health, Siena, Italy
[c] GSK Vaccines, Siena, Italy

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.



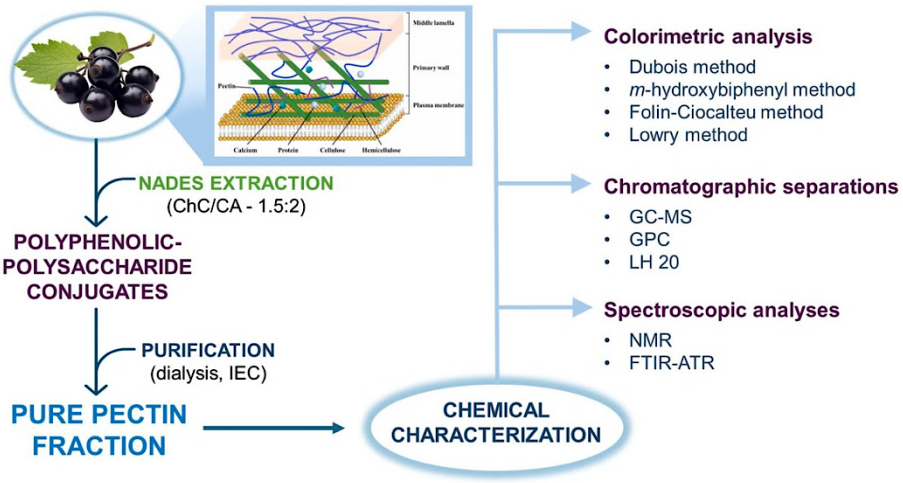
References:
1. Dangor Z, Benson N.,*Vaccine.* **2024** Jul 25;42(19S1):S125-S141.
2. La Guidara C, Adamo R., *Int J Mol Sci.* **2024** May 17;25(10):5487.
3. Micoli F, Del Bino L. *Expert Rev Vaccines.* **2019** Sep;18(9):881-895.
4. Olshefsky A, Richardson C, *Bioconjug Chem.* **2022** Nov 16;33(11):2018-2034.
5. Malley R, Lu YJ. *Expert Rev Vaccines.* **2024** Jan-Dec;23(1):196-204.
6. Nappini R, Alfini R., *Vaccines* (Basel). **2024** Jun 24;12(7):707.
7. Oldrini D, Di Benedetto R., *Vaccines* (Basel). **2023** Nov 28;11(12):1770.

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

Ewa Górska[a], Aleksandra Mazurek-Hołys[a], Zuzana Košťálová[b], Marta Tsirigotis-Maniecka[a], Peter Capek[b], Izabela Pawlaczyk-Graja[a]

[a] Laboratory of Bioproducts Technology, Faculty of Chemistry, Wroclaw University of Science and Technology, Wybrzeże Wyspiańskiego 29, 50-370 Wrocław, Poland
ewa.gorska@pwr.edu.pl
[b] Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 38, Bratislava, Slovakia

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.



Acknowledgements: This research was funded by the National Science Centre, Poland, under research project no UMO-2020/39/O/ST8/03514, and the Polish National Agency for Academic Exchange. The spectroscopic techniques were performed in collaboration with the Institute of Chemistry, Slovak Academy of Sciences (SAV).

References:

1. A. Belkheiri, A. Forouhar, A.V. Ursu, P. Dubessay, G. Pierre, C. Delattre, G. Djelveh, S. Abdelkafi, N. Hamdami, P. Michaud, *Appl. Sci.* **2021**, 11(14), 6596.
2. C.M.P. Freitas, J.S.R. Coimbra, V.G.L. Souza, R.C.S. Sousa, *Coatings*. **2021**, 11(8), 922.
3. J. Cui, Ch. Zhao, L. Feng, Y. Han, H. Du, H. Xiao, J. Zheng, *Trends Food Sci. Technol*. **2021**, 110: 39–54

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

Fruzsina Demeter, Anikó Borbás, Mihály Herczeg

Department of Pharmaceutical Chemistry, University of Debrecen, 4032 Debrecen, Egyetem tér 1, Hungary
herczeg.mihaly@pharm.unideb.hu

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.

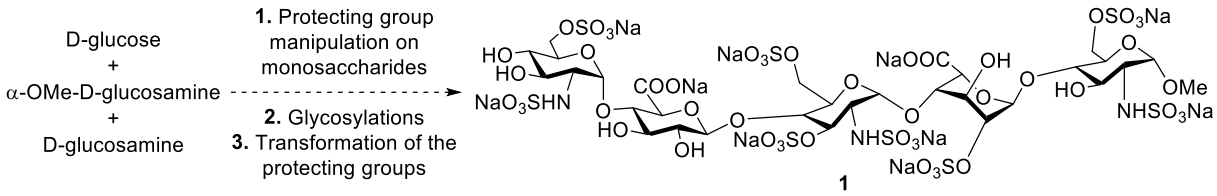


Figure 1. Schematic synthesis and structure of fondaparinux (**1**)

Acknowledgements: The authors gratefully acknowledge financial support for this research from the National Research, Development and Innovation Office of Hungary (NKFIH, FK 137924).

References:
1. M. Petitou *et* al. *Carbohydr. Res.*, **1987**, *167*, 67-75.
2. S. Dey, H.-J. Lo, C.-H. Wong, *Org. Lett.*, **2020**, *22,* (12), 4638-4642.
3. T. Li *et* al., *ChemMedChem*, **2014**, *9*, 1071-1080.
4. C.-H. Chang *et* al., *Angew. Chem. Int. Ed.*, **2014**, *53*, 1-5.

ENHANCING CYTOTOXICITY OF FLUORINATED SACCHARIDES BY ATTACHING ORGANOMETALLICS

Kateřina Hiršová[a,b], Vojtěch Hamala[a], Jindřich Karban[a], Roman Hrstka[c]

[a] Institute of Chemical Process Fundamentals of the CAS, Rozvojová 135, Prague 6, 165 02, Czech Republic
hirsova@icpf.cas.cz
[b] Faculty of Chemical Technology, University of Chemistry and Technology Prague, Technická 5, Prague 6, 166 28, Czech Republic
[c] Research Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Žlutý kopec 7, Brno, 656 63, Czech Republic

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].

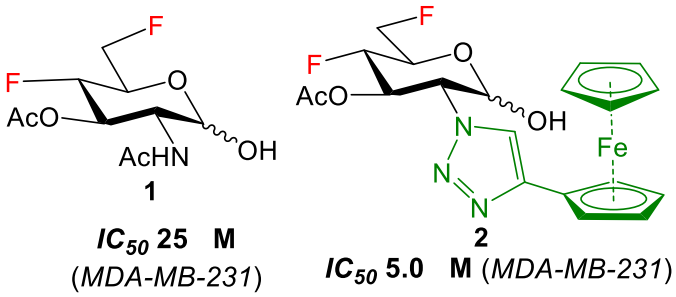


Figure 1. Difluorinated glucosamine (**1**) and its ferrocene conjugate (**2**) and their cytotoxicity to triple negative breast cancer cell line MDA-MB-231.

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

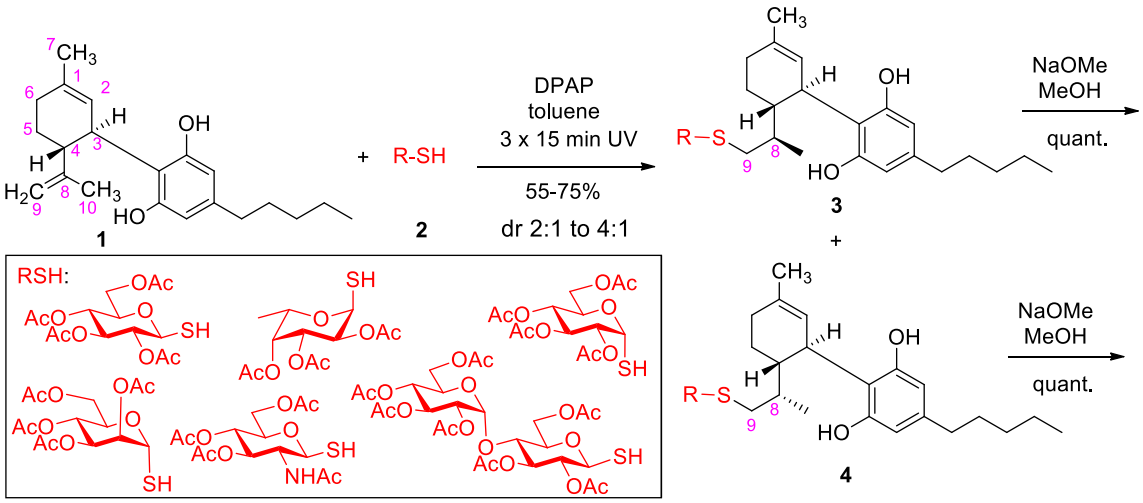
References:
1. Hamala, V.; Ondrášková, et al., *Appl. Organomet. Chem.* **2024**, *38* (5), e7399.

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

Fanni Hőgye[a], Viktor Kelemen[a,b], Tamás Huoranszki[a], Gréta Csordás[a], Nóra Debreczeni[a], Ilona Bereczki[a,b], Anikó Borbás[a]

[a] University of Debrecen, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 4032 Debrecen, Egyetem tér 1, Hungary
hogye.fanni@science.unideb.hu
[b] HUN-REN-UD Pharmamodul Research Group, 4032 Debrecen, Egyetem tér 1, Hungary

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.

References:

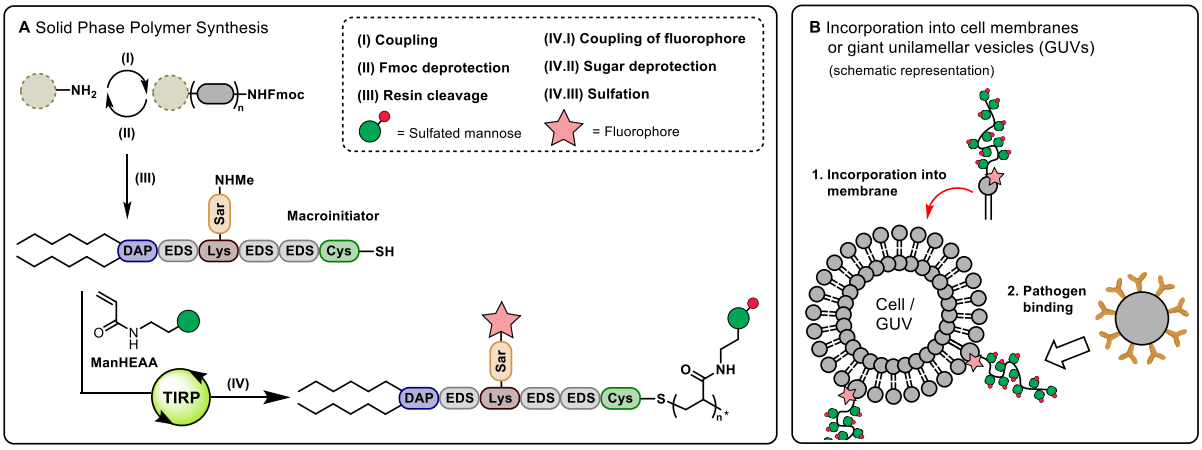
1. C. M. White, *J. Clin. Pharm.* **2019**, 59(7), 923-934.
2. A. Dondoni, *Angew. Chem. Int. Ed.* **2008**, *47*, 8995-8997.

AMPHIPHILIC GAG-MIMETICS FOR STUDYING PATHOGEN INTERACTIONS

Matthias Holzer[a,b], Luca-Cesare Blawitzki[a,b], Laura Hartmann[a,b]

[a] Institute for Macromolecular Chemistry, University Freiburg, Stefan-Meier-Strasse 31, 79104 Freiburg i. Br., Germany
matthias.holzer@makro.uni-freiburg.de
[b] Freiburg Materials Research Centre, University Freiburg, Stefan-Meier-Strasse 21, 79104 Freiburg i. Br., Germany

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]



References:
1. P. L. DeAngelis, *Anat. Rec.* **2002**, *268*, 317 – 326.
2. S. Ricard-Blum, S. Perez, *Curr. Opin. Struct. Biol.* **2022**. *74*, 102355.
3. L. Hartmann *et al.*, *J. Am. Chem. Soc.* **2020**, *142*, 5252 – 5265.
4. L. Hartmann *et al.*, *Macromolecules* **2023**, *56*, 5512 – 5523.
5. L. Hartmann *et al.*, *Biomacromolecules* **2024**, *25*, 5979 – 5994

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

Hosaka Yoshinao Z[a], Cahyadi Danang Dwi[b], Kochi Atsunosuke[a], Ojima Koichi[c], Takeda-Okuda Naoko[b], Tamura Jun-Ichi[b]

[a] Faculty of Agriculture, Kyushu University, Fukuoka, Japan
hosaka@agr.kyushu-u.ac.jp
[b] Faculty of Agriculture, Tottori University, Tottori, Japan
[c] Institute of Livestock and Grassland Science, NARO, Tsukuba, Japan

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.

Acknowledgement: This research was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number JP20K06411 and 23H02385 to YZH in a Grant-in-Aid for Scientific Research (C) and (B), respectively.

GLOBOTRIAOSYLCERAMIDE-TARGETED LECTIN-DEPENDENT CELLULAR CYTOTOXICITY WITH AURISTATIN E

Masahiro Hosono, Shigeki Sugawara, Takeo Tatsuta

Division of Cell Recognition, Institute of Molecular Biomembrane and Glycobiology, Tohoku Medical and Pharmaceutical University, 4-4-1, Komatsushima, Aoba-ku, Sendai, 981-8558, Japan
mhosono@tohoku-mpu.ac.jp

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.

References:
1. S. Sugawara, C. Im, *Glycoconj J*. **2017**, *34*, 127–138.
2. O. Kovbasnjuk, R. Mourtazina, *Proc. Natl. Acad. Sci.* **2005**, *102*, 19087–19092.
3. S. Sugawara, M. Takayanagi, *Glycobiol.* **2020**, *30*, 802–816.

DESIGN, SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF UNNATURAL GALLOTANNINS

Jana Hricovíniová[a], Renáta Vadkertiová[b], Zuzana Hricovíniová[b]

[a] Department of Cell and Molecular Biology of Drugs, Faculty of Pharmacy, Comenius University, 832 32 Bratislava, Slovakia
[b] Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia
zuzana.hricoviniova@savba.sk

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.

References:
1. K.T. Chung, T.Y. Wong et al. *Crit. Rev. Food Sci. Nutr.* **1998**, *38*, 421-464.
2. J. Zhang, L. Li, S.H. Kim et al. *Pharm. Res.* **2009**, *26*, 2066-2080.
3. L. Marquez, Y. Lee, D. Duncan et al. *ACS Infect. Dis.* **2023**, *9*, 1685-1694.

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

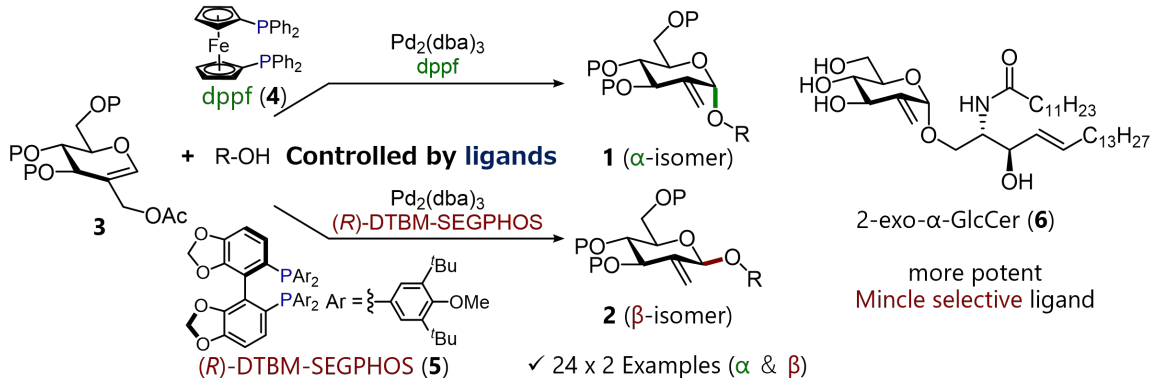
Takahiro Ikazaki[a], Eri Ishikawa[b], Hiroto Tamashima[a], Hisako Akiyama[c,d]
Yusuke Kimuro[a], Makoto Yoritate[a], Hiroaki Matoba[a], Akihiro Imamura[e]
Hideharu Ishida[e], Sho Yamasaki[b], Go Hirai[a]

[a] Grad. Sch. Pharm. Sci., Kyushu Univ., Fukuoka, Japan
ikazaki.takahiro.525@s.kyushu-u.ac.jp
[b] RIMD, Osaka Univ., Osaka, Japan
[c] JARIHES, Juntendo Univ., Tokyo, Japan
[d] RIKEN-CBS, Saitama, Japan
[e] iGCORE, Gifu Univ., Gifu, Japan

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.



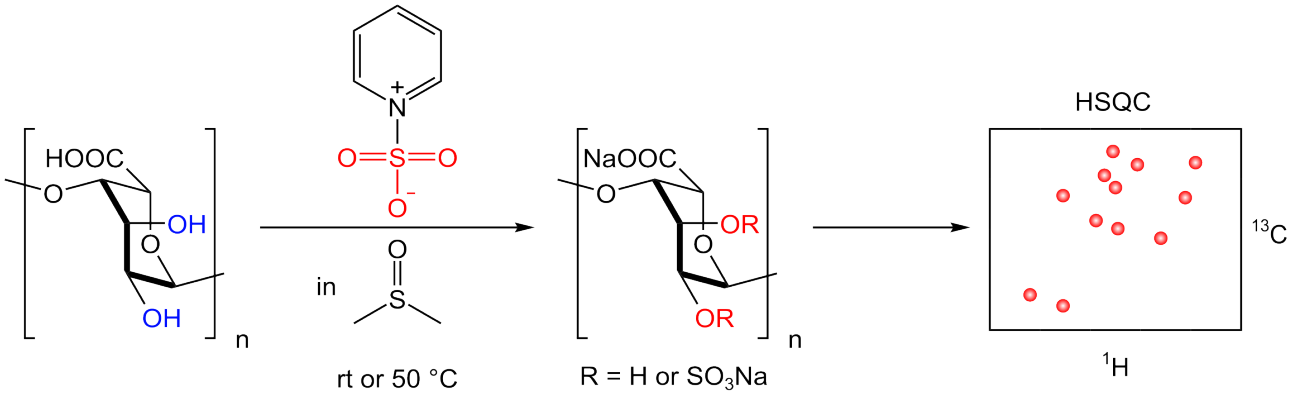
References:
1. Ikazaki, T.; Ishikawa, E.; Tamashima, H.; Akiyama, H.; Kimuro, Y.; Yoritate, M.; Matoba, H.; Imamura, A.; Ishida, H.; Yamasaki, S.; Hirai, G. Angew. Chem. Int. Ed. **2023** *62*, e202302569.

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

Karl M. Ingerma[a], Indrek Reile[b], Rando Tuvikene[a]

[a] Institute of Natural Sciences and Health, Tallinn University, Narva mnt 29, 10120, Tallinn, Estonia
karlmi@tlu.ee
[b] National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618, Tallinn, Estonia

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.



Poly-G acid form | **Sulfated poly-G** | **Structural analysis**

DS 0.49–1.62
Yields 28–83%

References:
1. X. Zhao, Y. Guangli; H. Guan; N, Yue; Z. Zhang; H., Li, *Carbohydr. Polym.* **2007**, *69*, 272–279.
2. D. Bi, J. Huang, J. Cao, L. Yao, W. Guo, Z. Zhang, Y. Wu, X. Hong, Z. Hu, X. Xu, *Carbohydr. Polym.* **2023**, 301B, 120370.
3. L. Wu, W. Wang, X. Zhang, X. Zhao, Y. Guangli, *Carbohydr. Polym.* **2016**, 143, 139–148.
4. M. Xin, Y. Sun, H. Chen, Q. Li, Y. Dun, H. Guan, J. Hao, C. Li, *Int. J. Biol. Macromol.* **2018**, 114, 26–34.
5. Ø. Arlov, F.L. Aachmann, A. Sundan, T. Espevik, G. Skjåk-Bræk, *Biomacromolecules*, **2014**, 15, 2744–2750.

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

Jun Iwaki, Hideki Ishida, Kenta Iino, Noriyuki Yuasa, Fumie Kimura, Masato Habu, Takahiro Tanji, Yasuki Kato, Yuji Matsuzaki

Tokyo Chemical Industry CO., LTD. Institute for Glycotechnology,
jun.iwaki@TCIchemicals.com

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.

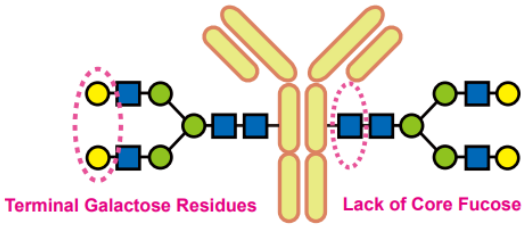


Figure 1. Non-fucosylated galactosyl glycoform

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

Andrius Jasilionis[a], Giang-Son Nguyen[b], Sebastian Kempengren[a], Anna Sofia Lewin[b], Eva Nordberg Karlsson[a]

[a] Division of Biotechnology and Applied Microbiology, Department of Process and Life Science Engineering, Lund University, PO Box 124, SE-221 00 Lund, Sweden
andrius.jasilionis@ple.lth.se
[b] Sustainable Biotechnology and Bioprospecting, Department of Biotechnology and Nanomedicine, SINTEF Industry, Richard Birkelands vei 3B, NO-7034 Trondheim, Norway

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

Line Juul-Madsen, Nikolai Cordua, Rasmus D. Greve, Kamilla Pedersen, Helle H. Trinderup, Elena Petersen, Henrik H. Jensen

Aarhus University, Department of Chemistry, Langelandsgade 140, 8000 Aarhus, Denmark
hhj@chem.au.dk

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.

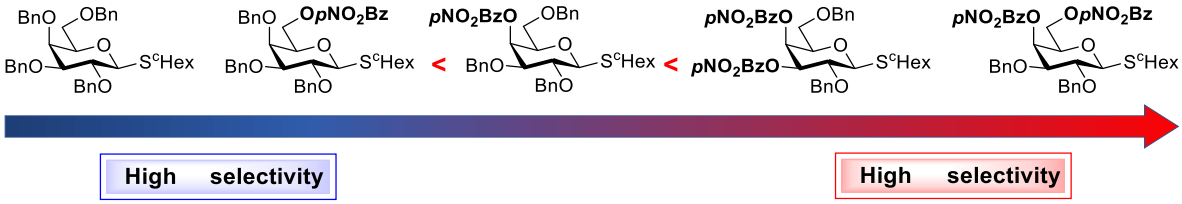


Figure 1. Graphical illustration of donor selectivity

References:
1. H. H. Trinderup, L. Juul-Madsen, L. Press, M. Madsen, H. H. Jensen, *J. Org. Chem.* **2022**, 87, 13763–13789

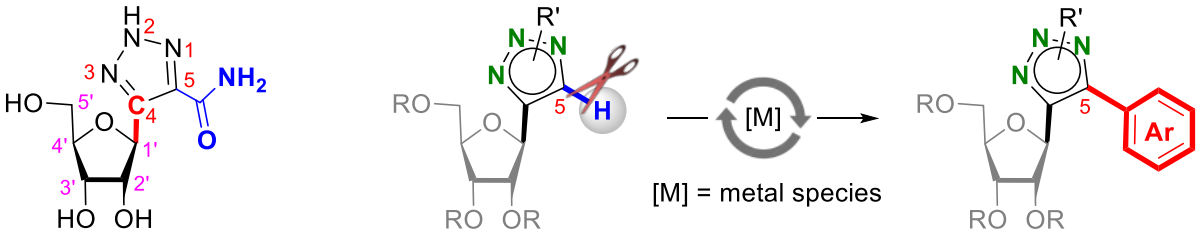
PALLADIUM-CATALYZED C-H ARYLATION OF 1,2,3-TRIAZOLYL-C-NUCLEOSIDE OF BIOLOGICAL INTEREST

Quentin Joachim[a,b,c], Nina Bozinovic[a,b], Bartosz Godlewski[a,b], Olivier Monasson[a,b], Nadège Lubin-Germain[a,b], Angélique Ferry[a,b,d]

[a] CY Cergy Paris Université, BioCIS, CNRS, 95000 Cergy-Pontoise cedex, France
quentin.joachim@cyu.fr
[b] Université Paris-Saclay, BioCIS, CNRS, Orsay, france
[c] Université de Poitiers, Organic Synthesis Team, IC2MP, CNRS, 86073 Poitiers Cedex 9, France
[d] Institut Universitaire de France (IUF)

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 :



References:
1. A.L. Garner, A. L. *ACS Pharmacol. Transl. Sci.* **2021**, 4, 1714.
2. (a) N. Sabat, E. Migianu-Griffoni, T. Tudela, M. Lecouvey, S. Kellouche, F. Carreiras, F. Gallier, J. Uziel, N. Lubin-Germain, *Eur. J. Med. Chem.* **2020**, *188*, 112009. (b) (b) N. Sabat, A. Ouarti, E. Migianu-Griffoni, M. Lecouvey, O. Ferraris, F. Gallier, C. Peyrefitte, N. Lubin-Germain, J. Uziel, *Bioorganic Chemistry*, **2022**, *122*, 105723.
3. (a) Z. Ruan, D. Ghorai, G. Zanoni L. Ackermann, *Chem. Commun.* **2017**, *53*, 9113. (b) Y. Liang, S.F. Wnuk, *Molecules* **2015**, *20*, 4874. (c) S. Allu, K.C.K. Swamy, *RSC Adv.* **2015**, *5*, 92045.
4. J. Ghouilem, M. de Robichon, F. Le Bideau, A. Ferry, S. Messaoudi, *Chem. Eur. J.* **2021**, 7, 491.

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

Natalia Kaczyńska[a,b], Karolina Ossowska[c], Agata Motyka-Pomagruk[a,b], Agnieszka M. Kowalczyk[c], Wojciech Śledź[a,b], Zbigniew Kaczyński[c], Ewa Lojkowska[a]

[a] Laboratory of Plant Protection and Biotechnology, Intercollegiate Faculty of Biotechnology UG&MUG, University of Gdansk, Abrahama 58, 80-309 Gdansk, Poland
natalia.kaczynska@ug.edu.pl
[b] Research and Development Laboratory, Intercollegiate Faculty of Biotechnology UG&MUG, University of Gdansk, Podwale Przedmiejskie 20, 80-824 Gdansk, Poland
[c] Department of Biomedical Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdansk, Poland

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.

References:

1. K. Ossowska et al., *IJMS.* **2022***, 23(4),* 2077

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

Marta Kalińska, Beata Liberek, Andrzej Nowacki

Department of Organic Chemistry, University of Gdańsk, Faculty of Chemistry,
63 Wita Stwosza str., 80-308 Gdańsk
marta.kalinska@phdstud.ug.edu.pl

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

Martyna Kapica[a], Sergey A. Samsonov[b], Ola Grabowska[a], Elżbieta Kamysz[c], Dariusz Wyrzykowski[a]

[a] Department of General and Inorganic Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk
martyna.kapica@phdstud.ug.edu.pl
[b] Department of Theoretical Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk
[c] Department of Molecular Biotechnology, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk

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.

References:

1. Esteso, M. A., & Romero, C. M. Cyclodextrins: Properties and applications. *International Journal of Molecular Sciences* **2024**,*25*(8), 4547.
2. Lakshmaiah Narayana, J.; Mechesso, A.F.; Rather, I.I.G.; Zarena, D.; Luo, J.; Xie, J.; Wang, G. Origami of KR-12 Designed Antimicrobial Peptides and Their Potential Applications. *Antibiotics* **2024**, *13*, 816.

DESIGN AND SYNTHESIS OF COVALENT LECTIN LIGANDS

Kevin Kappes, Valentin Wittmann

Department of Chemistry, University of Konstanz, 78457 Konstanz, Germany
kevin.kappes@uni-konstanz.de

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.

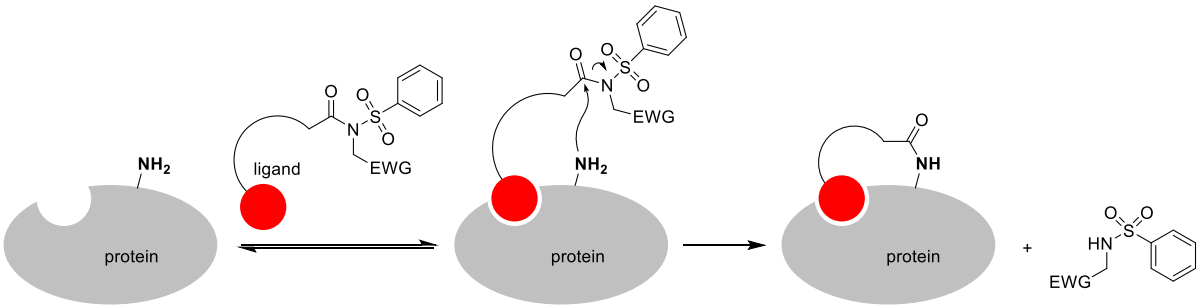


Figure1. Principle of covalent binding of a synthetic GM_1 mimic attached to a NASA warhead to a lysine side chain of a CT B-subunit. EWG: electron-withdrawing group

References:
1. V. Kumar, W. B. Turnbull, *Beilstein J. Org. Chem.* **2018**, *14*, 484-498.
2. A. Bernardi, L. Carrettoni, A. G. Ciponte, D. Monti, S. Sonnino, *Bioorganic Med. Chem. Lett.* **2000**, *10*, 2197-2200.
3. T. Tamura, T. Ueda, T. Goto, T. Tsukidate, Y. Shapira, Y. Nishikawa, A. Fujisawa, I. Hamachi, *Nat. Commun.* **2018**, *9*, 1870.

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

Anna Maciejewska[a], Krzysztof Mikołajczyk[b], Anna S. Świerzko[c], Paweł Link-Lenczowski[d], Dariusz Martynowski[e], Łukasz Sobala[b], Anna Bereznicka[b], Wojciech Jachymek[a], Marta Kaszowska[a], Gabriela Gajek[c], Marcin Czerwiński[b], Jolanta Lukasiewicz[a], Maciej Cedzyński[c]

[a] Laboratory of Microbial Immunochemistry and Vaccines, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
marta.kaszowska@hirszfeld.pl
[b] Laboratory of Glycoconjugates, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland
[c] Laboratory of Immunobiology of Infections, Institute of Medical Biology, Polish Academy of Sciences, Łódź, Poland
[d] Department of Medical Physiology and Center for the Development of Therapies for Civilization and Age-Related Diseases, Jagiellonian University Medical College, Krakow, Poland
[e] Laboratory of Genomics and Bioinformatics, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

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.*

References:
1. Y. Watanabe *et al.*, *Science* **2020**, *369*, 330-333.
2. P. Zhou P *et al.*, *Nature* **2020**, *579*, 270-273.
3. L.M. Parsons *et al. J Biol Chem* **2019**, *294*, 7797-809.

LECTIN-BASED MICROARRAY AS A TOOL FOR GLYCOSYLATION BIOMARKER RESEARCH

Lucia Pažitná, Patrícia Dudoňová, Muhammad Umair Khan, Kristína Kianičková, Jaroslav Katrlík

Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84538 Bratislava, Slovakia
chemjkat@savba.sk

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.

References:

1. O. Radojičić, L. Pažitná, Z. Dobrijević, P. Kundalia, K. Kianičková, J. Katrlík, V. Mandić Marković, Ž. Miković, O. Nedić, D. Robajac, *Biochemistry (Moscow).* **2024**, *89*, 148-158.
2. K. Kianičková, L. Pažitná, P. H. Kundalia, Z. Pakanová, M. Nemčovič, P. Baráth, E. Katrlíková, J. Šuba, J. Trebatická, J. Katrlík, *Int. J. Mol. Sci.* **2023**, *24*, 8745.

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

S. Kempengren[a], BIONEER consortium[b] O. Wallberg[c], E. Nordberg Karlsson[a]

[a] Division Biotechnology and Applied Microbiology, Department of Process and Life Science Engineering, Lund University, P.O. Box 124, Lund SE-221 00, Sweden
sebastian.kempengren@ple.lth.se
[b] Department of Biotechnology and Nanomedicine SINTEF Industry, Richard Birkelands vei 3B, NO-7034 Trondheim
[c] Division Chemical Engineering, Department of Process and Life Science Engineering Lund University, P.O. Box 124, Lund SE-221 00, Sweden

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.)

Iwona Komaniecka[a], Anita Swatek[a], Zbigniew Kaczyński[b], Małgorzata Pawlik[c], Agnieszka Tomczyk[d], Katarzyna Szewczuk-Karpisz[d], Zofia Piotrowska-Seget[c], Adam Choma[a]

[a] Department of Genetics and Microbiology, Institute of Biological Science, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland
iwona.komaniecka@mail.umcs.pl
[b] Laboratory of Structural Chemistry, Faculty of Chemistry, University of Gdansk, Wita Stwosza 63, 80-308 Gdańsk, Poland
[c] Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Jagiellońska 28, 40-032 Katowice, Poland
[d] Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland

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)

References:

1. S. Acosta-Jurado, F. Fuentes-Romero, J.-E. Ruiz-Sainz, M. Janczarek, J.-M. Vinardell, Rhizobial exopolysaccharides: genetic regulation of their synthesis and relevance in symbiosis with legumes. *Int. J. Mol. Sci.* **2021**, 22, 6233.

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

Natan Koraj[a], Ema Faganeli[a], Sushmaa Dangudubiyyam[b], Anne Harduin-Lepers[b], Marko Anderluh[a]

[a] University of Ljubljana, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Aškerčeva cesta 7, Ljubljana
natan.koraj@ffa.uni-lj.si
[b] Université de Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, F 59000 Lille, France

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.

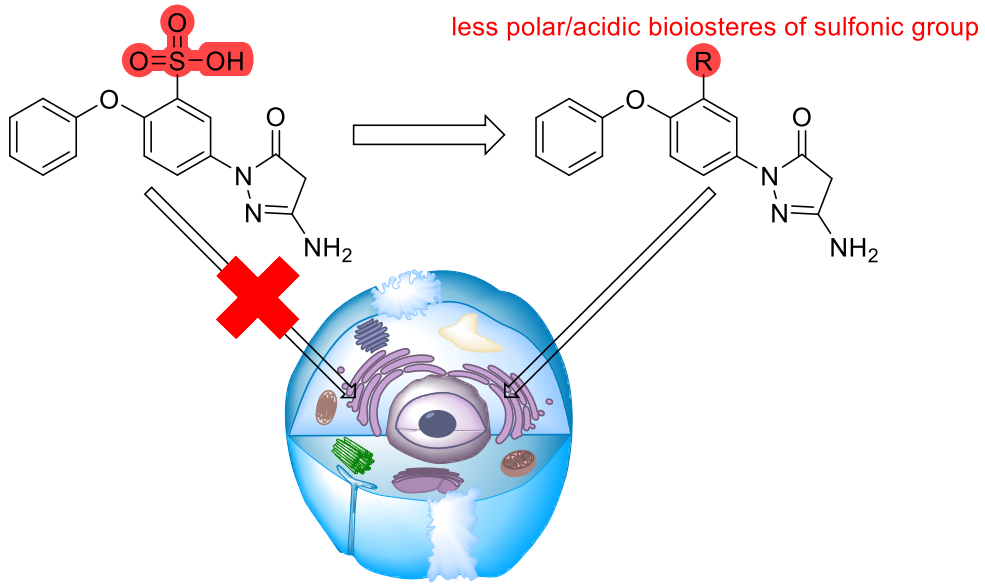


Figure 1. JFD00458 structure

References:
1. C. D. Rillahan, S. J. Brown, A. C. Register, H. Rosen, J. C. Paulson. High-Throughput Screening for Inhibitors of Sialyl- and Fucosyltransferases. *Angew Chem Int Ed Engl.* **2011**, *50(52), 12534-7*.
2. K. Clairene Filipe, S. Dangudubiyyam, C. Lion, M. Decloquement, R. Elin Teppa, C. Biot, A. Harduin-Lepers. A Rapid and Sensitive MicroPlate Assay (MPSA) Using an Alkyne-Modified CMP-Sialic Acid Donor to Evaluate Human Sialyltransferase Specificity. *Chembiochem*. **2024** Oct 29:e202400539.

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

Agnieszka M. Kowalczyk[a,b], Bradley R. Clarke[b], Chris Whitfield[b]

[a] Faculty of Chemistry, University of Gdańsk, Gdańsk, Poland
agnieszka.kowalczyk@ug.edu.pl
[b] Department of Molecular and Cellular Biology, University of Guelph, Guelph, Ontario, Canada

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

Yasir Ali, Monika Ďuračková, Barbora Stratilová, Stanislav Kozmon

Institute of Chemistry Slovak Academy of Sciences, Dúbravská cesta 5807/9,
845 38 Bratislava, Slovakia
chemsksa@savba.sk

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.

References:

1. P. Janoš, S. Kozmon, I. Tvaroška, J. Koča, *Chemistry. Eur. J.,* **2018**, 24, p.7051.
2. Fusani, L., Palmer, D.S., Somers, D.O. and Wall, I.D., J. *Chem. Inf. Model.,* **2020**, 60(3), pp.1528-1539.

FLUORINATED METHYL β-LACTOSIDES: SYNTHESIS AND AFFINITY TO GALECTINS

Aleš Krčil[a], Vojtěch Hamala[a], Jindřich Karban[a], Pavla Bojarová[b]

[a] Institute of Chemical Process Fundamentals of the Czech Academy of Sciences, Rozvojová 1/135, 165 00 Praha, Czech Republic
Krcil@icpf.cas.cz
[b] Institute of Microbiology of the Czech Academy of Sciences, Víděňská 1083, 142 20 Prague 4, Czech Republic

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**.

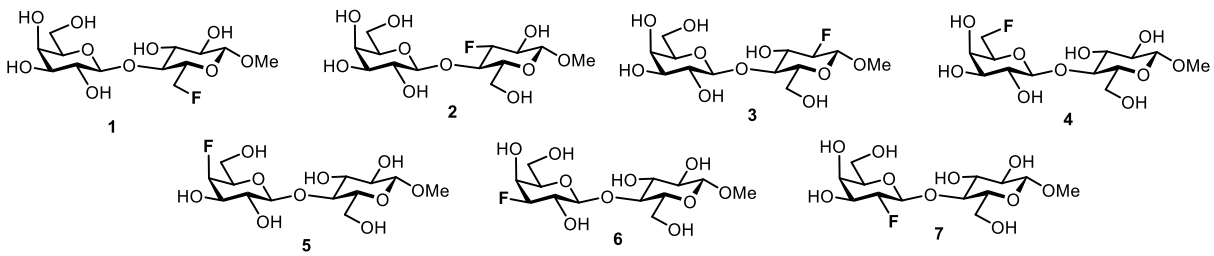


Figure 1. Structures of fluorinated methyl β-lactosides.

Acknowledgements: This work was supported by IGA and Czech Science Foundation (GA23-05146S). P.B. acknowledges support from mobility project LUC24024 by the Ministry of Education, Youth and Sports of the Czech Republic and COST Action CA22103 (Net4Brain).

References:

1. R. P. M. Dings, M. C. Miller, R. J. Griffin, K. H. Mayo, *Int. J. Mol. Sci.* **2018**, *19*, 905–926.
2. K. Wdowiak, T. Francuz, E. Gallego-Colon, N. Ruiz-Agamez, M. Kubeczko, I. Grochola, J. Wojnar, *Int. J. Mol. Sci.* **2018**, *19*, 1–21.
3. J. D. Martinéz, A. I. Manzano, E. Calvino, C. Romano, O. Millet, F. J. Canada, *J. Org. Chem.* **2020**, *85*, 16072–16081.
4. T. Diercks, A. S. Infantiono, L. Unione, J. Jiménez-Barbero, S. Oscarson, H. Gabius, *Chem. Eur. J.* **2018**, *24*, 15761–15765.

CHEMICAL SYNTHESIS OF OLIGOSACCHARIDES RELATED TO PLANT RHAMNOGALACTURONAN-I

Remya Kumbappilliyil Rajan, Fabian Pfrengle

Department of natural sciences and sustainable resources, Institute of Organic Chemistry, BOKU University, Vienna 1190, Austria
remya.kumbappilliyil-rajan@boku.ac.at

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.

References:

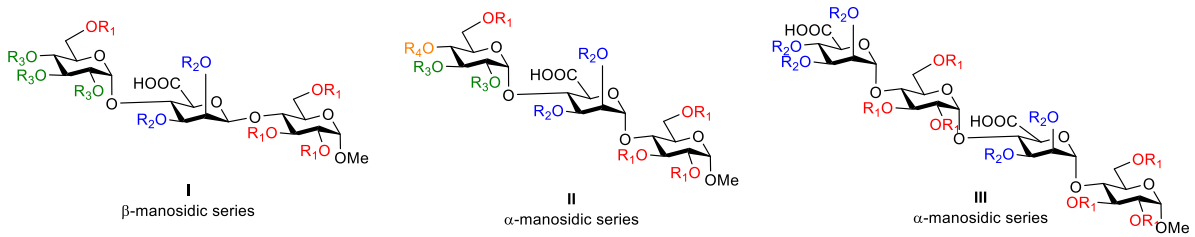
1. Witkamp, R. F. Biologically active compounds in food products and their effects on obesity and diabetes. Comprehensive Natural Products II,2010 509–545.
2. Keegstra, K. Plant Cell Walls. Plant Physiology, 154(2),2010 483–486.
3. Scanlan, E. M., Mackeen, M. M., Wormald, M. R., & Davis, B. G. Synthesis and solution-phase conformation of the RG-i fragment of the plant polysaccharide pectin reveals a modification-modulated assembly mechanism. *JACS*, **2010**,*132*(21), 7238–7239.
4. Zakharova, A. N., Madsen, R., & Clausen, M. H. Synthesis of a backbone hexasaccharide fragment of the pectic polysaccharide Rhamnogalacturonan I. Org. Letters,2013 15(8), 1826–1829

D-MANNURONIC ACID CONTAINING OLIGOSACCHARIDES: SYNTHESIS AND BIOLOGICAL ACTIVITIES

Katalin Kútvölgyi[a,b,c], János Hajkó[c], Anikó Borbás[a], Mihály Herczeg[a]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Debrecen H-4032 Debrecen, Egyetem tér 1., Hungary
kutvolgyi.katalin@pharm.unideb.hu
[b] Doctoral School of Pharmacy, Medical Science Doctoral Council, University of Debrecen, H-4032, Debrecen Egyetem tér 1., Hungary
[c] TAPI Hungary Industries Kft., 4042 Debrecen, Pallagi út 13., Hungary

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

Acknowledgements: The authors gratefully acknowledge financial support for this research from the National Research, Development and Innovation Office of Hungary (NKFIH, FK 137924), and by the EKÖP-KDP-2024 university research scholarship program – cooperative doctoral program of the ministry for culture and innovation from the source of the national research, development and innovation fund.

References:
1. Murata, K. et al. *Gastroenterology*, **1985** *9* 1248-1257
2. Haug, A. et al. *Acta Chem. Scand.*, **1967** *21* 691-704
3. Lisztes, E. et al. *ChemMedChem*, **2021** 1467-1476
4. Demeter, F. et al. *Biomolecules* **2024**, *14*, 1052. 10.3390/biom14091052

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

Jannis Langer[a], Nicole L. Snyder[b], Laura Hartmann[a]

[a] Institute for Molecular Chemistry, University Freiburg, Stefan-Meier-Straße 31, jannis.langer@makro.uni-freiburg.de
[b] Department of Chemistry, Davidson College, Davidson, NC 28035, USA

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).

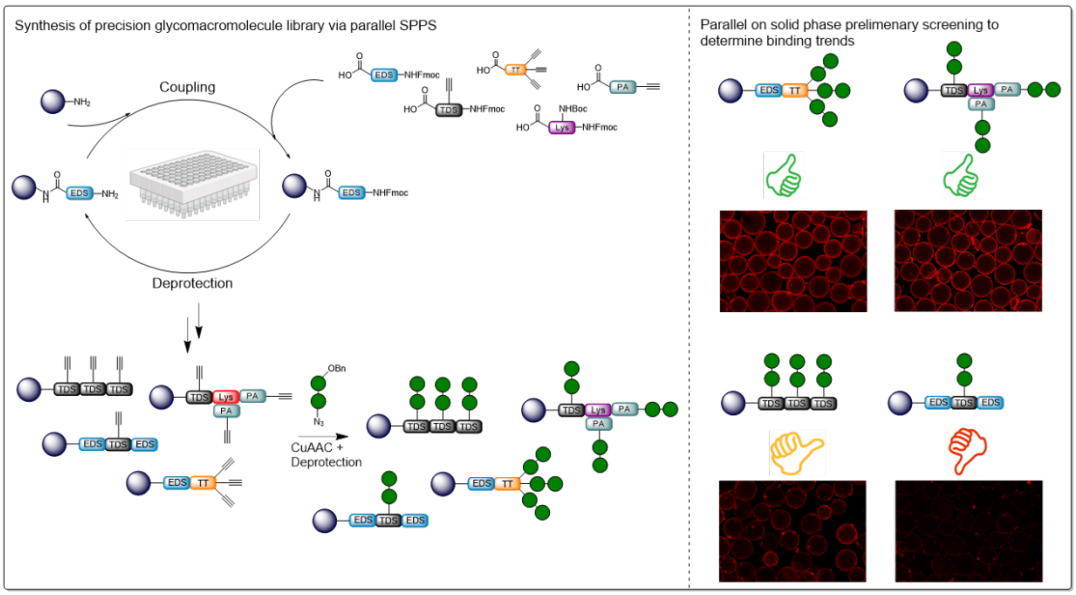


Figure 1. Parallel SPPS to obtain library of precision glycooligomers (left) and prelimenary on-resin screening of library (right).

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]

References:
1. B. Merrifield, Science. 1986, 232, 341.
2. D. Ponader, F. Wojcik, F. Beceren-Braun, J. Dernedde, L. Hartmann, Biomacromolecules. 2012, 13, 1845.
3. L. de Witte, A. Nabatov, M. Pion, D. Fluitsma, M. A. W. P. de Jong, T. de Gruijl, V. Piguet, Y. van Kooyk, T. B. H. Geijtenbeek, Nature Medicine. 2007, 13, 367.

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

George Lasisi[a], Laura O'Byrne[b], Ramón Hurtado Guerrero[b], Anne Harduin-Lepers[c], Erdmann Rapp[d], Faranak Nami[a], Luca Laraia[a], Mads H. Clausen[a]

[a] Department of Chemistry, Technical University of Denmark, Kemitorvet, 2800 Kgs. Lyngby, Denmark
ogrla@kemi.dtu.dk
[b] Institute of Biocomputation and Physics of Complex Systems, University of Zaragoza, 50018 Zaragoza, Spain
[c] Univ. Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, 59000 Lille, France
[d] Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg 39106, Germany

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.

References:
1. L. L. Lairson, B. Henrissat, G. J. Davies, and S. G. Withers, "Glycosyltransferases: Structures, Functions, and Mechanisms," *Annu Rev Biochem*, 77, **2008**, 521–555.
2. H. Shen, C.-Y. Lee, and C.-H. Chen, "Protein Glycosylation as Biomarkers in Gynecologic Cancers," *Diagnostics*, 12, **2022**.

ANALYSIS OF THE KDO BIOSYNTHETIC CLUSTER IN CroV

Antonio Lembo[a], Immacolata Speciale[a], Anna Notaro[a], Antonio Molinaro[a], Matthias Fischer[b], Hiroyuki Ogata[c], Cristina De Castro[a]

[a] Department of Chemical Sciences, University of Naples Federico II, Via Vicinale Cupa Cintia 26, Naples, Italy
antonio.lembo@unina.it
[b] Max Planck Institute for Marine Microbiology, Celsiusstr. 1, 28359 Bremen, Germany
[c] Bioinformatics Center, Institute for Chemical Research of Kyoto University, Ikenoura Gokasho, Uji, Kyoto 611-0011, Japan

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.

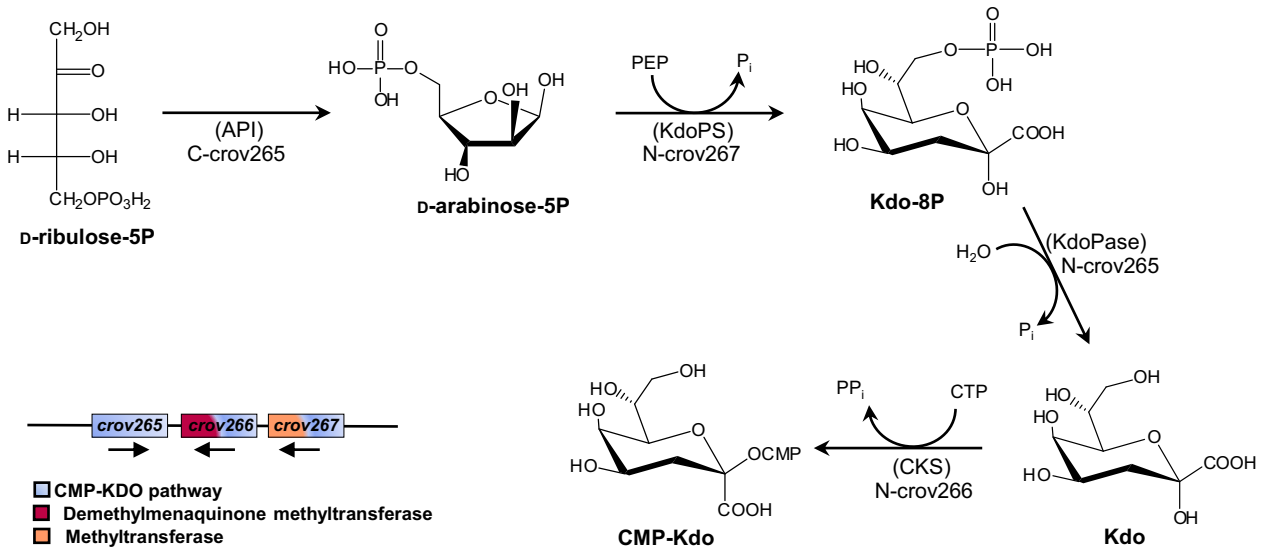


Figure 1. Putative Kdo biosynthetic pathway in CroV.

References:
1. M. G. Fischer et al., *PNAS*, **2010**, *107,* 19508–19513.
2. I. Speciale et al., *Chemical Reviews*, **2022**, *122,* 15717–15766.

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

Savvina Leontakianakou[a], Anders Sundin[b], Eva Nordberg Karlsson[a]

[a] Division of Biotechnology and Applied Microbiology, Department of Process and Life Science Engineering, Lund University, PO Box 124, Lund SE-22100, Sweden
savvina.leontakianakou@ple.lth.se
[b] Centre of Analysis and Synthesis, Department of Chemistry Lund University, PO Box 124, Lund SE-22100, Sweden

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

Larry S. Lico[a], Sheng-Kai Peng[a], Hung-Lin Chen[a,b], and Todd L. Lowary[a,c]

[a] Institute of Biological Chemistry, Academia Sinica, 128, Academia Rd., Sec. 2, Nangang District, Taipei City, Taiwan (R.O.C.) 11529
lslico@gate.sinica.edu.tw
[b] Master Program in Clinical Genomics and Proteomics, Taipei Medical University, 250, Wuxing St., Xinyi District, Taipei City, Taiwan (R.O.C) 11031
[c] Institute of Biochemical Sciences, National Taiwan University, 1, Roosevelt Rd., Sec. 4, Da'an District, Taipei City, Taiwan (R.O.C.) 10617

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.

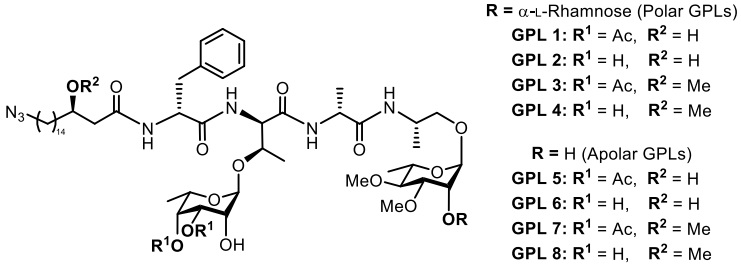


Figure 1. Structure of the target *Mycobacterium abscessus* glycopeptidolipids.

References:
1. Whang, J., *et al.*, *Cell Death Dis.* **2017**, *8*, e3012.
2. Daher, W., *et al.*, *ACS Infect. Dis.* **2020**, *6*, 2756–2770.
3. Daher, W., *et al.*, *Cell Chem. Biol.* **2022**, *29*, 910–924.
4. Mukherjee, R. and D. Chatterji, *IUBMB Life* **2012**, *4*, 215–225.
5. Gutiérrez, A.V., *et al.*, *Front. Microbiol.* **2018**, *9*, 1145.
6. Schorey, J.S. and L. Sweet, *Glycobiol.* **2008**, *18*, 832–841.

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

Po-Wei Lu[a,b], Cheng-Chung Wang[a]

[a] Institute of Chemistry, Academia Sinica, Taipei 115, Taiwan, o55249@gmail.com
[b] Institute of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, Taipei, Taiwan

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.

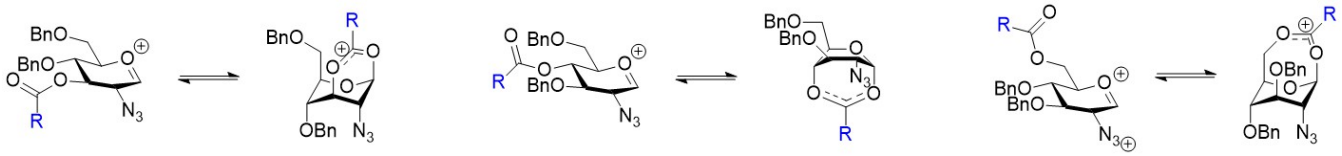


Figure 1. Possible dioxolenium ion intermediates of $GlcN_3$

References:
1. Chun-Wei Chang et al., *Angew. Chem., Int. Ed.* **2021**, *60*, 12413–12423

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

Lourriel Macale[a], Immacolata Speciale[a], Anna Notaro[a], James L. Van Etten[b], Irina Agarkova[b], Antonio Molinaro[a], and Cristina De Castro[a]

[a] Department of Chemical Sciences, University of Naples Federico II, lourriel.macale@unina.it
[b] Department of Plant Pathology, University of Nebraska-Lincoln

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.

Acknowledgement: We would like to acknowledge the Glyco-N Doctoral Network under Horizon Europe (HORIZON) and Marie Skłodowska-Curie Actions Doctoral Networks (MSCA-DN) funded by the European Union under Grant Agreement 101119499.

Reference:

1. Speciale I, Duncan GA, Unione L, Agarkova IV, Garozzo D, Jimenez-Barbero J, Lin S, Lowary TL, Molinaro A, Noel E, Laugieri ME, Tonetti MG, Van Etten JL, De Castro C. The *N*-glycan structures of the antigenic variants of chlorovirus PBCV-1 major capsid protein help to identify the virus-encoded glycosyltransferases. *J Biol Chem*. **2019**, 294(14), 5688-5699.

FUNCTIONAL CHARACTERIZATION OF BACTERIAL GALACTOSIDASES AND EXPLORATION OF THEIR SYNERGISTIC EFFECT

Vasiliki Makrygianni[a], Mads Hartvig Clausen[b], Birgitte Zeuner[a]

[a] DTU Bioengineering, Technical University of Denmark, Søltofts Plads Building 221. DK-2800 Kgs. Lyngby
vasimak@dtu.dk
[b] DTU Chemistry, Technical University of Denmark, Kemitorvet 207, 2800 Kgs. Lyngby

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

Annika Malmgren, Andrius Jasilionis, Erik Apelqvist, Pavithra Sivakumar, Eva Nordberg Karlsson

Division of Biotechnology and Applied Microbiology, Department of Process and Life Science Engineering, Lund University, PO Box 124, SE-221 00 Lund, Sweden
annika.malmgren@ple.lth.se

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.

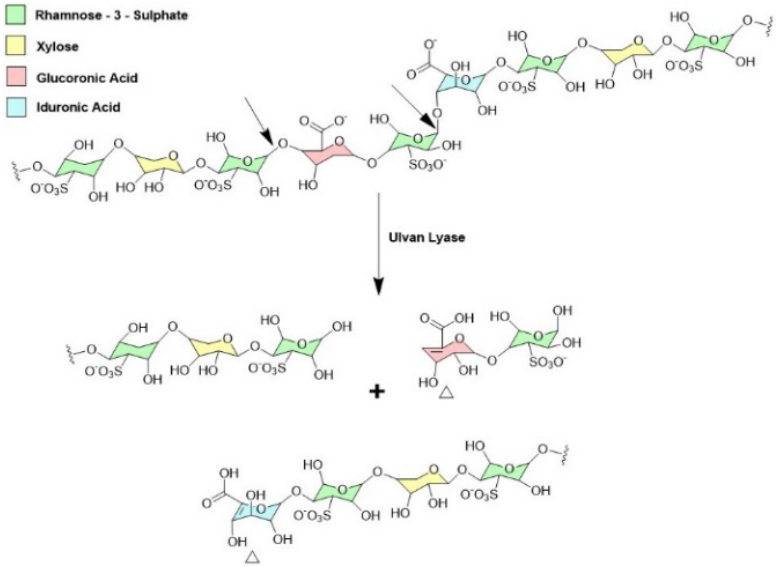


Figure 1. The currently known mechanism of ulvan lyases, which cleaves the ulvan between rhamnose-3-sulphate linked to either glucuronic acid or iduronic acid [1].

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).*

References:

1. V.R. Konasani, C. Jin, N.G. Karlsson, E. Albers, *Sci. Rep*. **2018**, *8,* 1-12.

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

Felista Manga, Marta Tsirigotis-Maniecka, Aleksandra Mazurek-Hołys, Izabela Pawlaczyk-Graja

Laboratory of Bioproducts Technology, Faculty of Chemistry Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 29, 50-370 Wrocław, Poland
282484@student.pwr.edu.pl

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

Roberta Marchetti

Department of Chemical Sciences, University of Naples Federico II, Naples, Italy
roberta.marchetti@unina.it

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.

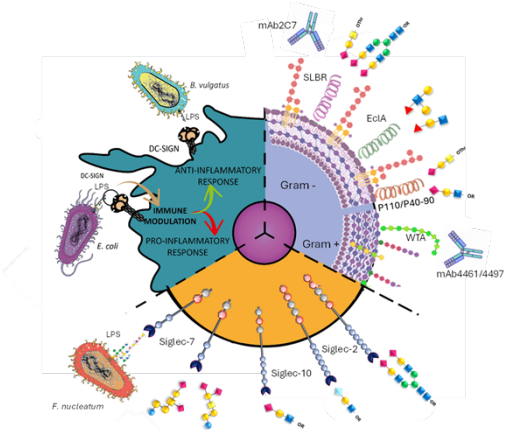


Figure 1. Schematic representation of studied protein-glycan interactions.

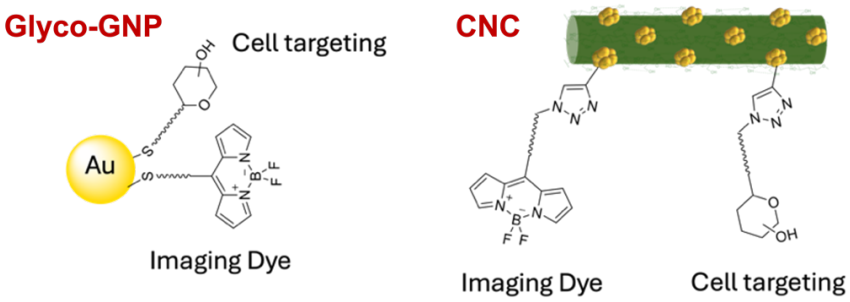
References:
1. a) C. Di Carluccio, F. Milanesi, M. Civera, C. Abreu, S. Sattin, O. Francesconi, A. Molinaro, O. Vaněk, R. Marchetti, A. Silipo, Eur. J. Org. Chem. 2023, 26, e202300644. b) D. Lamprinaki et al. Front. Immunol. 2021, 12:744184 c) C. Di Carluccio et al. iScience. 2020 29;24(1):101998. d) R.E. Forgione et al. ChemBioChem, 2022, e202200076.
2. a) F. Nieto-Fabregat, A. Marseglia, M. Thépaut, J.-P. Kleman, M. Abbas, A. L. Roy,..R. Marchetti. iScience. 2024, 27(2), 108792. b) F. Nieto-Fabregat et al. JACS Au 2024 4 (2), 697-712.
3. a) C. Di Carluccio et al., ACS Central Science 2024 10 (2), 447-459. b) C. Di Carluccio et al. RSC Chemical Biology 2021, 2 (6), 1618−1630.
4. A. Marseglia, MC. Forgione, .. K. Fukase, A.Molinaro, O. Q. Pich, D. Aparicio, A. Silipo, R. Marchetti, Int. Jour. of Biol.l Macromol. 2024, 279: 135277.
5. P.T. Beernink#, C. Di Carluccio#, R. Marchetti#, L. Cerofolini, S. Carillo, A. Cangiano, N. Cowieson, J. Bones, A. Molinaro, L. Paduano, M. Fragai, B. P. Beernink, S. Gulati, J. Shaughnessy, P. A. Rice, S. Ram, A. Silipo, JACS Au, 2024, 4: 2617−2629.
6. C. Di Carluccio, P. Soriano-Maldonado, F. Berni, C.J.C. de Haas, A. R. Temming, A. Hendriks, S. Ali, A. Molinaro, A. Silipo, N. M. van Sorge, M.J. van Raaij, J. D. C. Codee, R.Marchetti. ACS Central Science 2022 8 (10), 1383-1392.

MODULAR GLYCONANOMATERIALS FOR BIOMEDICAL APPLICATIONS

Marco Marradi[a], Jacopo Tricomi[a], Gianluca Cavazzoli[b], Giacomo Biagiotti[a], Laura Marongiu[c], Fabrizio Chiodo[d], Anna Bernardi[b], Barbara Richichi[a]

[a] Department of Chemistry "Ugo Schiff" (DICUS), University of Florence, via della Lastruccia, 3-13 - Sesto Fiorentino, Firenze, Italy
marco.marradi@unifi.it
[b] Department of Chemistry, University of Milan, via Golgi, 19 - Milano, Italy
[c] Department of Biotechnologies and Biosciences, University of Milano-Bicocca, Piazza dell'Ateneo Nuovo, 1 - Milano, Italy
[d] Institute of Biomolecular Chemistry, National Research Council (CNR), via Campi Flegrei, 34 – Pozzuoli, Napoli, Italy

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.

References:
1. M. Marradi, F. Chiodo, I. Garcia, S. Penades, *Chem. Soc. Rev*. **2013**, 42, 4728- 4745.
2. B. Richichi *et al.*, **2022** N°102022000011450, Appl. **2023** PCT/IB2023/055515.

TOWARDS DEVELOPING BACTERIAL ADHESION INHIBITORS USING ARYLAMIDE GLYCOFOLDAMERS

A. Farahat[a,b], L. Mingatos[a,b], M. Saraiva[a,b], M. Graça[c,d], P. Kis[a,b], R. Ventura[a,b], B. A. Pinheiro[c,d], A. L. Carvalho[c,d], A. S. Palma[c,d], E. Merlet[e], Y. Ferrand[e], P. Mateus[a,b]

[a] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
pmateus@itqb.unl.pt
[b] Associated Laboratory LS4FUTURE, ITQB NOVA, Universidade Nova de Lisboa, Oeiras, Portugal
[c] UCIBIO – Applied Molecular Biosciences Unit, Department of Chemistry, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
[d] Associate Laboratory i4HB - Institute for Health and Bioeconomy, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal
[d] Bordeaux, CNRS, UMR 5248, CBMN, France

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.

References:

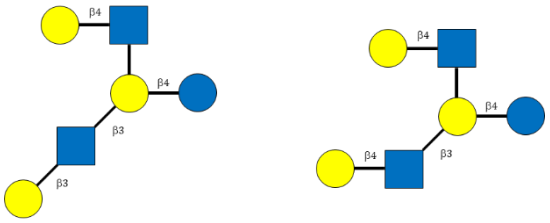
1. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance
2. S. W. Dickey, G. Y. C. Cheung, M. Otto, *Nat. Rev.* **2017**, *16*, 457-471
3. A. M. Krachler, K. Orth, *Virulence* **2013**, *4*, 284-294
4. S. Bhatia, L. Cuellar, C. R. Haag, *J. Am. Chem. Soc.* **2016**, *138*, 8654-8666
5. a) I. Huc, *Eur. J. Org. Chem.* **2004**, 17; b) D-W. Zhan, X. Zhao, J-L. Hou, Z-T. Li, *Chem. Rev.* **2012**, *112*, 5271-5316

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

Martin Matwiejuk

dsm-firmenich, Kogle Alle 4, 2970 Hørsholm, DK
martin.matwiejuk@dsm-firmenich.com

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).

References:
1. M.D. Bandara, K.J.Stine, A.V.Demchenko, *J. Org. Chem.* **2019***, 84,* 16192-16198.
2. M.D. Bandara, K.J.Stine, A.V.Demchenko, *Org. Biomol. Chem.* **2020***, 18(9),* 1747-1753.
3. I. Bajza et.al, *A method for preparation of the tetrasaccharide lacto-n-neotetraose (LNnT) containing n-acetyllactosamine*, **2011**, Patent: WO2011100980A1.
4. B. Soyyılmaz, M.H. Mikš, C.H. Röhrig, M. Matwiejuk, A. Meszaros-Matwiejuk, L.K. Vigsnæs, **2021**, *Nutrients 13(8)*, 2737-2759.

COMPARISON OF BOX-BEHNKEN AND I-OPTIMAL MODELS IN OPTIMIZATION OF NADES-BASED ULTRASOUND-ASSISTED EXTRACTION OF PECTINS FROM CARROT ROOT POMACE

Aleksandra Mazurek-Hołys, Ewa Górska, Marta Tsirigotis-Maniecka, Izabela Pawlaczyk-Graja

Laboratory of Bioproducts Technology, Department of Engineering and Technology of Chemical Processes, Faculty of Chemistry, Wrocław University of Science and Technology, wyb. Wyspiańskiego 29, 50-370 Wrocław, Poland
aleksandra.mazurek@pwr.edu.pl

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

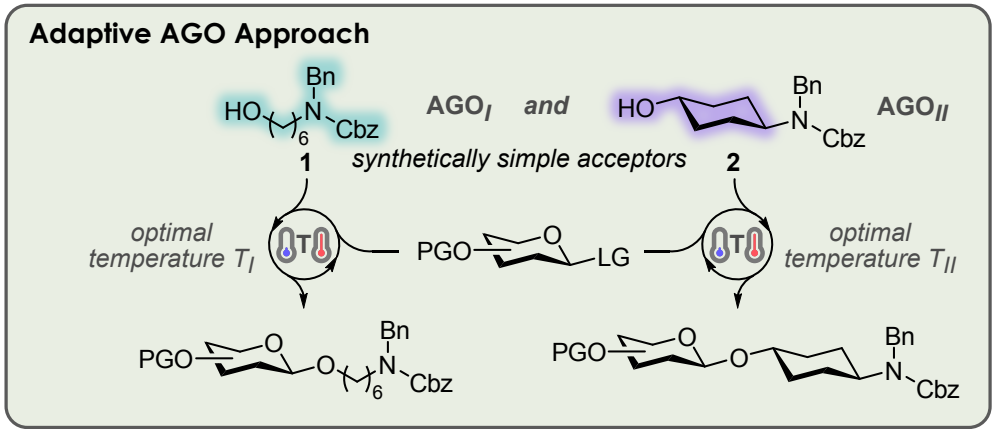
References:
1. C. M. P. Freitas, J.S.R. Coimbra, V. G. L. Souza, R. C. S. Sousa, *Coatings* **2021**, 11, 922.
2. J. S. Yang, T. H. Mu, M. M. Ma, *Food Chem.* **2018**, 244, 197–205.
3. M. M. A. N. Ranjha, S. Irfan, J. M. Lorenzo, B. Shafique, R. Kanwal, M. Pateiro, R. N. Arshad, L. Wang, G. A. Nayik, U. Roobab R. M. Aadil, *Processes* **2021**, 9, 1406.

ACCEPTOR-ADAPTIVE AUTOMATED GLYCOSYLATION OPTIMIZATION FOR AUTOMATED GLYCAN ASSEMBLY

Roey Gil Meir, Yasmeen Bakhtan, Mattan Hurevich

Department Of Chemistry, The Hebrew University of Jerusalem, Los Angeles Building
roey.meir@mail.huji.ac.il

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].



References:

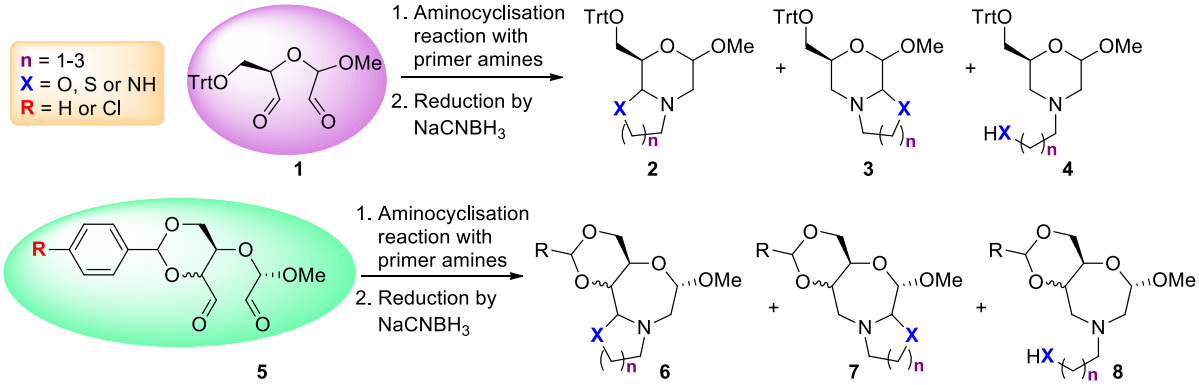
1. Yasmeen Bakhatan, Israel Alshanski, Chieh-Kai Chan, Wei-Chih Lo, Po-Wei Lu, Pin-Hsuan Liao, Cheng-Chung Wang, Dr. Mattan Hurevich, Chem. Eur. J. 2023, 29, e202300897
2. Yasmeen Bakhtan, Yonatan Sukhran, Luo-Sheng Tsa, Cheng-Chung Wang, Mattan Hurevich Acceptor-Adaptive Automated Glycosylation Optimization for Automated Glycan Assembly – submitted for publication

SYNTHESIS OF MORPHOLINE AND OXAZEPANE TYPE IMINOSUGAR ANALOGS

Erika Mező[a], Rasha Ghanem Kattoub[a], Anikó Borbás[a,b]

[a] Department of Pharmaceutical chemistry, Faculty of Pharmacy, University of Debrecen, 4032 Debrecen Egyetem tér 1, Hungary
mezo.erika@science.unideb.hu
[b] HUN-REN-UD Molecular Recognition and Interaction Research Group, University of Debrecen, Hungary

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.).



Acknowledgements: „The project has been implemented with support from the National Research, Development and Innovation Fund of the Ministry of Culture and Innovation and on the basis of a Certificate of Support issued by the NKFI Office (OTKA PD 146805, E.M.)”

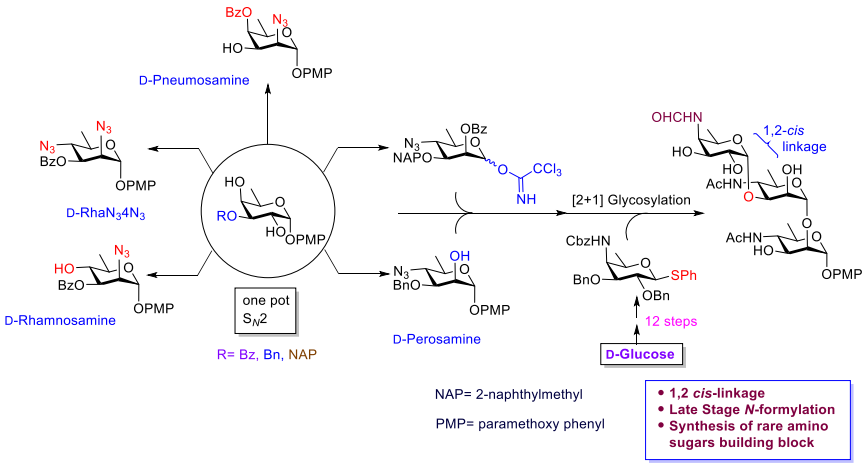
References:
1. A. E. Stütz, T. M. Wrodnigg; 2011, 66, 187-298; Chapter
2. Zamoner, L.O.B. *Pharmaceuticlas*, 2009, 12, 108–1220
3. Désiré, J. et al., *Org. Biomol. Chem.*, 2014, 12, 8977–8996
4. D'Alonzo, D. et al., *Curr. Med. Chem.* 2009, 16(4), 473–50

EXPANDING THE SCOPE OF A ONE-POT DOUBLE DISPLACEMENT PROTOCOL TO ACCESS THE ALL-RARE-SUGAR-CONTAINING TRISACCHARIDE UNIT OF *PSEUDOMONAS STUTZERI* OX1

Amar Kumar Mishra, Balasaheb K. Ghotekar, Chanchal K. Puley, Suvarn S. Kulkarni

Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai 400076, India
amarmishra@iitb.ac.in

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.



Acknowledgements: S.S.K. thanks the Science and Education Research Board (Grant SPR/2021/000328) for financial support. A.K.M. and B.K.G. thank CSIR and UGC, New Delhi, for research fellowships.

References:

1. Adibekian, A.; Stallforth, P.; Hecht, M.-L.; Werz, D. B.; Gagneux, P.; Seeberger, P. H. *Chem. Sci.* **2011**, *2*, 337−344.
2. Boltje, T. J.; Buskas, T.; Boons, G.-J. *Nat. Chem.* **2009**, *1*, 611−622.
3. Emmadi, M.; Kulkarni, S. S. *Nat. Protoc.* **2013**, *8*, 1870– 1879.
4. Sanapala, S. R.; Kulkarni, S. S. J. Am. Chem. Soc. **2016**, *138*, 4938− 4947.
5. Leone, S.; Izzo, V.; Lanzetta, R.; Molinaro, A.; Parrilli, M.; & Di Donato, *Carbohydr. Res.* **2005**, *340*, 651– 656.
6. Mishra, A. K.; Ghotekar, B. K.; Puley, C. K.; Kulkarni, S. S. *Org. Lett.* **2024**, *26* (46), 9955–9960.

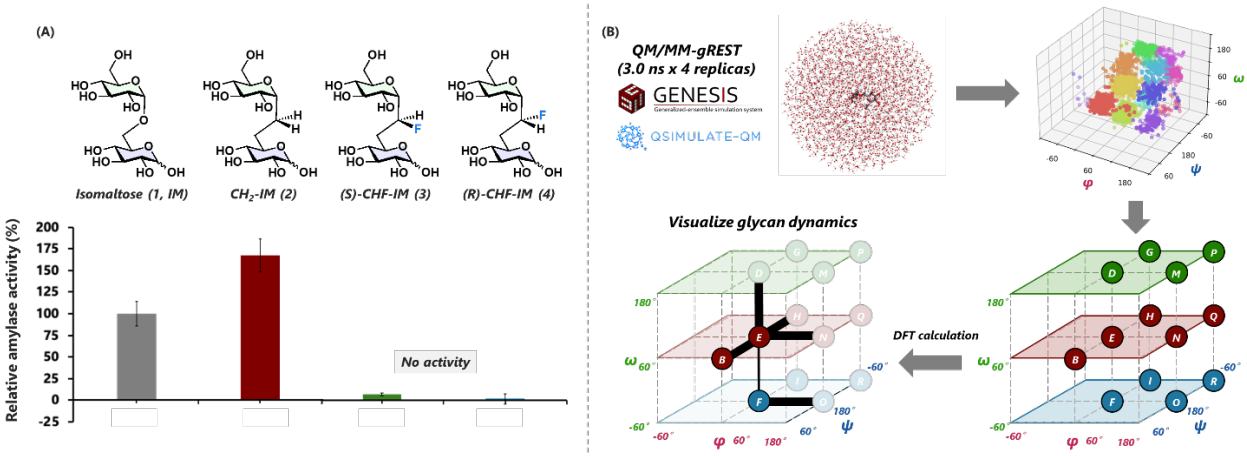
CONFORMATIONAL DISTRIBUTION AND DYNAMIC RANGE OF LINKAGE-EDITED PSEUDO-ISOMALTOSES WITH QM/MM-MD SIMULATIONS AND DFT CALCULATIONS

Taishi Miura[a], Takahiro Moriyama[a], Hiroyuki Koshino[b], Suyong Re[c], Makoto Yoritate[a], Go Hirai[a], Kiyoshi Yagi[d]

[a] Grad. Sch. Pharm. Sci., Kyushu University, Fukuoka, Japan
miura.taishi.079@s.kyushu-u.ac.jp
[b] RIKEN-CSRS, Saitama, Japan
[c] NIBIOHN ArCHER, Osaka, Japan
[d] Inst. Pure Appl. Sci., Univeristy of Tsukuba, Tsukuba, Japan

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.



References:
1. Kishi, Y. *et al.*, *Carbohydrate-Based Drug Discovery* (Eds.: Wong, C), Willey-VCH, Weinheim, **2003**, pp.305-340.
2. Kato, N. *et al.*, *Curr. Genet.* **2002**, *42*, 43-50.
3. Hirai, G. *et al.*, *J. Am. Chem. Soc.* **2024**, *146*, 2237-2247.

DESING AND SYNTHESIS OF NOVEL GLYCOMIMETICS AS GALECTIN ANTAGONISTS

Ben Mohan[a], Helen Blanchard[b], Paul V. Murphy[b]

[a]School of Biological and Chemical Sciences, University of Galway, Ireland
b.mohan2@universityofgalway.ie
[b]School of Biological and Chemical Sciences, University of Galway, Ireland

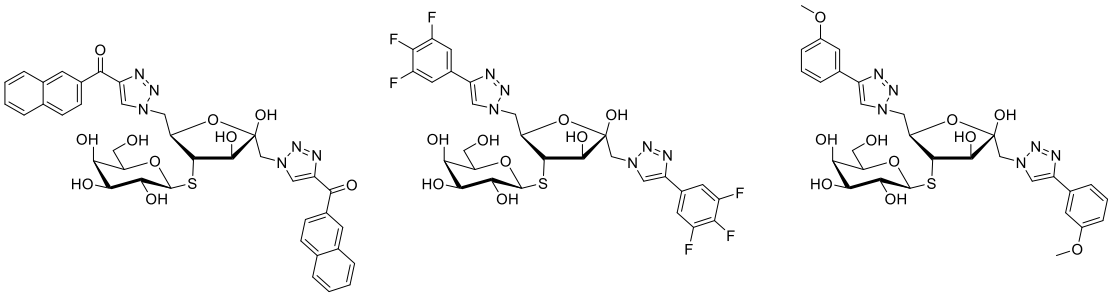


Figure 1. *Library of synthesised glycomimetics based on thiolactulose.*

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.

Acknowledgements: Funded by College of Science and Engineering Scholarship, University of Galway.

References:

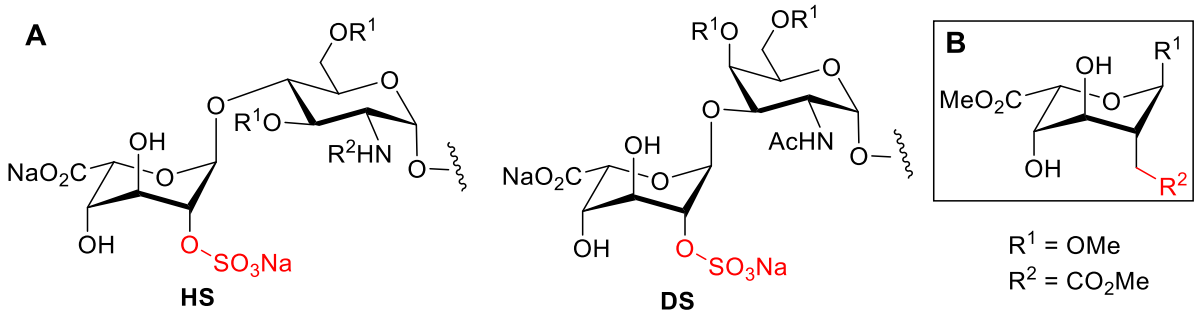
1. B. Patel et al. *Journal of Medicinal Chemistry*, **2020**, 63(*20*), 11573 – 11584.
2. F. T. Liu & S. Stowell. *Nature Reviews Immunology*, **2023**, 23, 479 – 494.
3. S. D. Sonkawade et al. *Circulation: Heart Failure*, **2021**, 14(*9*).
4. F.L. Oliveira et al. *Experimental Biology*, **2015**, 240(*8*), 1019 – 1028.
5. K. Bum – Erdene et al. *Journal of Medicinal Chemistry*, **2022**, 65(*8*), 5975 – 5989.
6. P. Compain. *Molecules*, **2018**, 23(7).

SYNTHESIS OF NOVEL PHARMACOLOGICAL CHAPERONES FOR THE TREATMENT OF MUCOPOLYSACCHARIDOSIS (MPS) II

Vaibhavi Nagendra, Shifaza Mohamed, Norbert Wimmer, Vito Ferro

School of Chemistry & Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia
uqvnagen@uq.edu.au

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

References:
1. Mohamed, S.; He, Q.; *Adv.Carb.Chem.Biochem.* **2020**, 77-117
2. Young, D., Harper, S.; *J. Med. Genet*. **1982**, 19, 408-411.
3. Keyzor, I., Shohet, S.; *Biomolecules* **2023**, 13, 1227.
4. Parenti, G.; *EMBO Mol. Med*. **2009**, 1, 268-279

LINKAGE-EDITED *C*-LINKED 6,6'-DIACYL NEOTREHALOSE ANALOGS

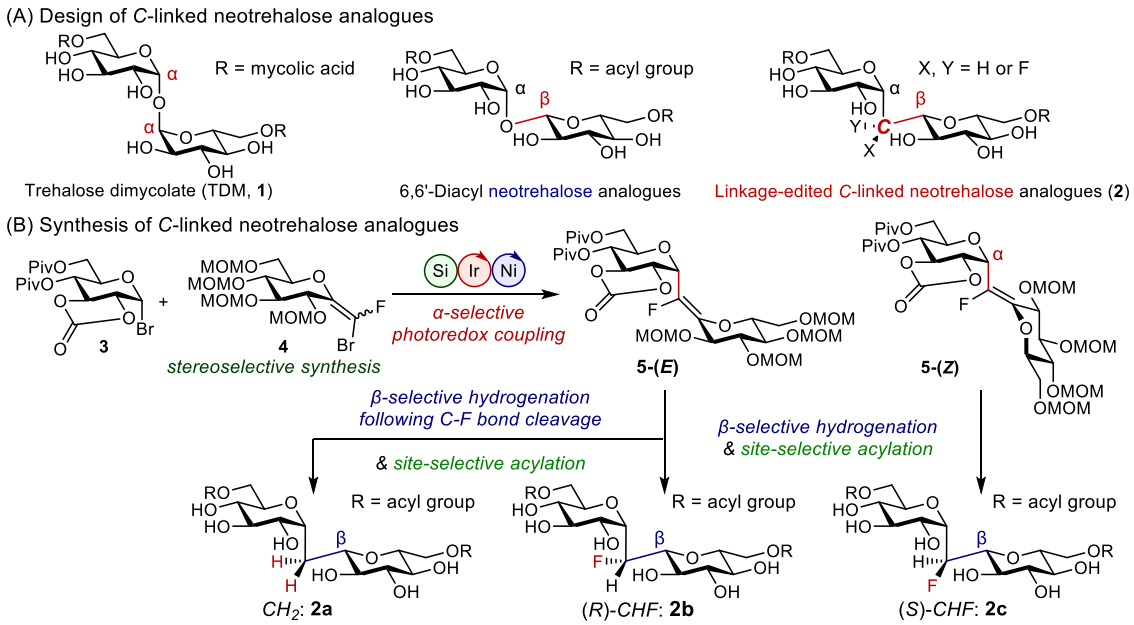
Rintaro Nakahashi[a], Yuki Sakai[b], Hiroaki Matoba[a], Takahiro Moriyama[a], Eri Ishikawa[b], Makoto Yoritate[a], Sho Yamasaki[b], Go Hirai[a]

[a] Grad. Sch. Pharm. Sci., Kyushu Univ., Fukuoka, Japan
nakahashi.rintaro.931@s.kyushu-u.ac.jp
[b] RIMD, Osaka Univ., Osaka, Japan

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.



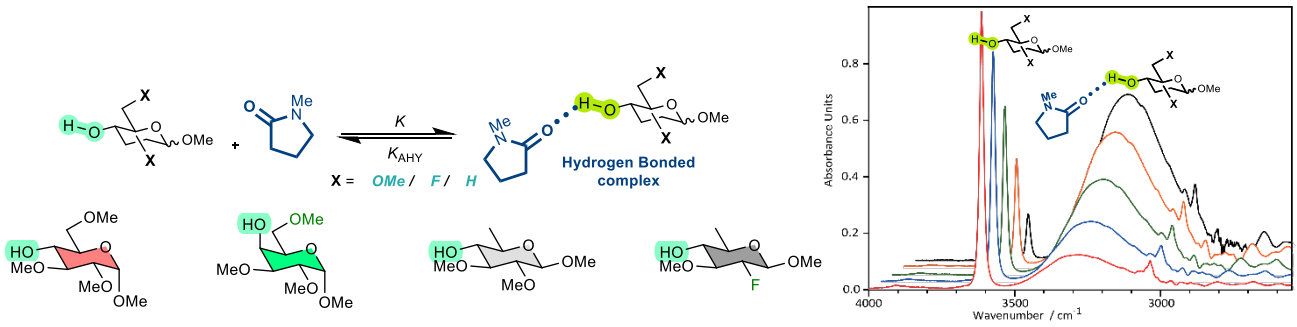
References:
1. Ryter K.T., *et al*. *Bioorg. Med. Chem.* **2020**, *28*, 115564.
2. Hirai, G. *et al*., *J. Am. Chem. Soc.* **2024**, *146*, 2237.

INVESTIGATING HYDROGEN BOND DONATING CAPACITY OF CARBOHYDRATES AND THE INFLUENCE OF DEOXYGENATION/DEOXYFLUORINATION

Mrinal Naskar[a], Jean-Yves Le Questel[b], Bruno Linclau[a]

[a] Department of Organic and Macromolecular Chemistry, Ghent University, S4, Krijgslaan 281, 9000 Ghent, Belgium
mrinal.naskar@ugent.be
[b] CEISAM UMR CNRS 6230, Université de Nantes, CNRS, CEISAM UMR 6230, 44000 Nantes, France

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 [1,2,3], 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.

References:

1. J. Graton, Z. Wang, A.-M. Brossard, D. Gonçalves Monteiro, J.-Y. Le Questel and B. Linclau , *Angew. Chem.*, **2012**, *124*, 6280-6284.
2. B. Linclau, F. Peron, E. Bogdan, N. Wells, Z. Wang, G. Compain, C. Q. Fontenelle, N. Galland, J.-Y. Le Questel, J. Graton, *Chem. Eur. J.* **2015**, *21*, 17808-17816.
3. J. Graton, G. Compain, F. Besseau, E. Bogdan, J. M. Watts, L. Mtashobya, Z. Wang, A. Weymouth-Wilson, N. Galland, J.-Y. Le Questel, B. Linclau, *Chem. Eur. J.* **2017**, *23*, 2811-2819.
4. C. Ouvrard, M. Berthelot, C. Laurence, *J. Chem. Soc. PerkinTrans*. **1999**, *2*, 1357 – 1362; J. Graton, J-Y Le Questel, *J. Phys. Chem. A.* **2013**, *117*, 13184.
5. Pedersen, C. M.; Olsen, J.; Brka, A. B.; Bols, M. Quantifying the Electronic Effects of Carbohydrate Hydroxy Groups by Using Aminosugar Models. *Chem. Eur. J.* **2011**, *17* (25), 7080-7086.

NOVEL FUNGAL α-L-FUCOSIDASE EXHIBITING TRANSFUCOSYLATION ACTIVITY

Pavlína Nekvasilová[a, b], Michaela Glozlová[a], Andrea Vopálenská[c], Vladimír Křen[a], Pavla Bojarová[a]

[a] Laboratory of Biotransformation, Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, CZ-14200 Prague, Czech Republic
pavlina.nekvasilova@biomed.cas.cz
[b] Department of Genetics and Microbiology, Faculty of Science, Charles University, Viničná 5, CZ-12843 Prague, Czech Republic
[c] Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 2030/8, CZ-12800 Prague, Czech Republic

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.

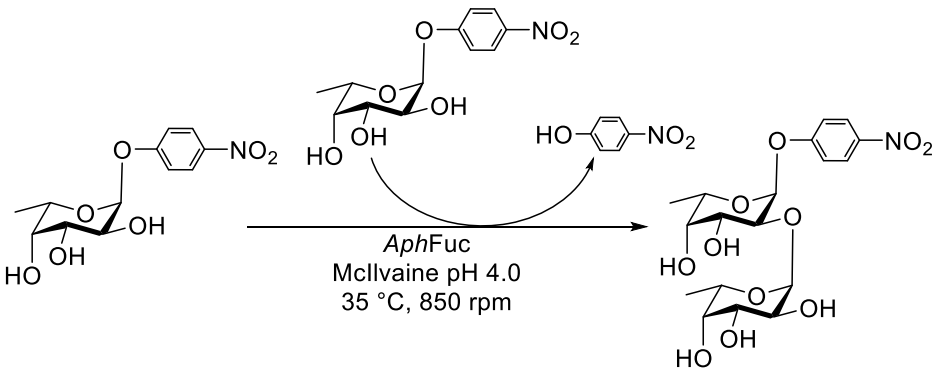


Figure 1. Transglycosylation reaction catalyzed by a novel fungal α-L-fucosidase.

Acknowledgements: This work was supported by the Ministry of Health of the Czech Republic (project NU23-08-00307), and by the Ministry of Education, Youth and Sports of the Czech Republic (mobility project LUC23148).

References:

1. L. Wan, Y. Zhu, W. Zhang, W. Mu, *Appl. Microbiol. Biotechnol.* **2020**, *104*, 5619-5631.
2. Z. Mészáros, P. Nekvasilová, P. Bojarová, V. Křen, K. Slámová, *Biotechnol. Adv.* **2021**, *49*, 107733.
3. H. Wu, C. D. Owen, N. Juge, *Essays in Biochem.* **2023**, *67*, 399-414.
4. H. Cao, J. D. Walton, P. Brumm, G. N. Phillips, *J. Biol. Chem.* **2014**, *289*, 25624-25638.

p53 AGGREGATION REGULATES EXPRESSION OF CL40-REACTIVE SIALYLATED GLYCANS IN OVARIAN CANCER

Midori Ikezaki[a], Naoyuki Iwahashi[b], Megumi Fujino[b], Masakazu Fujimoto[c], Noa Mori[a], Yoshiko Takeda-Uchimura[d], Kazuhiko Ino[b], Yoshito Ihara[a], Kenji Uchimura[d], Kazuchika Nishitsuji[a,d]

[a] Department of Biochemistry, School of Medicine, Wakayama Medical University, Wakayama 641-8509, Japan
ikezaki@wakayama-med.ac.jp
[b] Department of Obstetrics and Gynecology, School of Medicine, Wakayama Medical University, Wakayama 641-8509, Japan
[c] Department of Diagnostic Pathology, Kyoto University, Kyoto 606-8507, Japan
[d] Unité de Glycobiologie Structurale et Fonctionnelle, UMR 8576 CNRS, Université de Lille, Villeneuve d'Ascq 59655, France

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.

References:

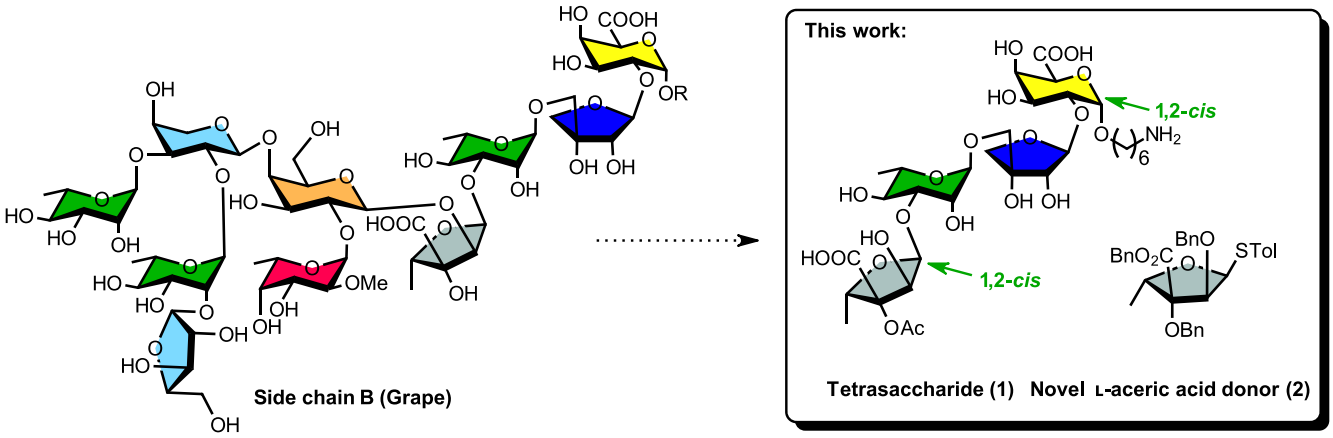
1. Iwahashi N, Ikezaki M, Nishikawa T, Namba N, Ohgita T, Saito H, Ihara Y, Shimanouchi T, Ino K, Uchimura K, Nishitsuji K. Sulfated glycosaminoglycans mediate prion-like behavior of p53 aggregates. Proc Natl Acad Sci USA. ***2020***, 117, 33225-34.
2. Iwahashi N, Ikezaki M, Komohara Y, Fujiwara Y, Noguchi T, Nishioka K, Sakai K, Nishio K, Ueda M, Ihara Y, Uchimura K, Ino K, Nishitsuji K. Cytoplasmic p53 aggregates accumulated in p53-mutated cancer correlate with poor prognosis. PNAS Nexus. ***2022***, 1, pgac128.
3. Nishitsuji K, Mito R, Ikezaki M, Yano H, Fujiwara Y, Matsubara E, Nishikawa T, Ihara Y, Uchimura K, Iwahashi N, Sakagami T, Suzuki M, Komohara Y: Impacts of cytoplasmic p53 aggregates on the prognosis and the transcriptome in lung squamous cell carcinoma. Cancer Science ***2024***, 115, 2947-60.
4. Takeda-Uchimura Y, Ikezaki M, Akama TO, Nishioka K, Ihara Y, Allain F, Nishitsuji K, Uchimura K. Complementary Role of GlcNAc6ST2 and GlcNAc6ST3 in Synthesis of CL40-Reactive Sialylated and Sulfated Glycans in the Mouse Pleural Mesothelium. Molecules. ***2022***, 27, 4543.

SYNTHESIS OF AN UNUSUAL TETRASACCHARIDE FROM RG-II

Katharina Obleser, Uwe Osswald, Fabian Pfrengle

Department of Natural Sciences and Sustainable Resources,
BOKU University Muthgasse 18, 1190 Vienna
katharina.obleser@boku.ac.at

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.



Acknowledgements: This research was funded in whole or in part by the Austrian Science Fund (FWF) [P35406].

References:

1. Ndeh, D., et al., Nature, 2017. 544(7648): p. 65-70.
2. Neill M. A. Ishii T., Alberheim P. G. Darvill, Annual Review of Plant Biology, 2004. Volume 55: pp. 109-139
3. Rao, Y. and G.J. Boons, Angew Chem Int Ed Engl, 2007. 46(32): p. 6148-51.

VCNA AS A BACTERIAL BIOMARKER: LABEL-FREE IMPEDIMETRIC DETECTION USING SIALOSIDE-MODIFIED ELECTRODES IN COMPLEX ENVIRONMENTS

Hen Okshtein, Isreal Alshanski, Shlomo Yitzchaik, Mattan Hurevich

Institute of Chemistry and Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Safra Campus, Givat Ram, Jerusalem, 91904, Israel
hen.okshtein@mail.huji.ac.il

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

Vicente Oliver[a], Juan Manuel Falcón[b], Niels Reichardt[a]

[a] Glycotechnology Lab, Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Parque Científico y Tecnológico de Gipuzkoa, Paseo Miramón 194, 20014 San Sebastián, Spain
voliver@cicbiomagune.es
[b] Exosomes Laboratory, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), Bizkaia Science & Technology Park, bld 801 A, 48160 Derio, Spain

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.

References:

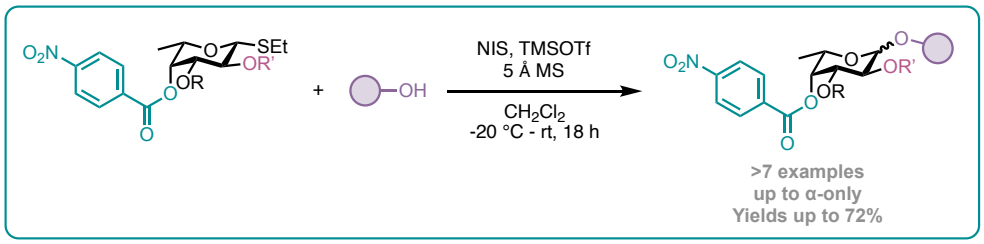
1. Lou G, Chen Z, Zheng M, Liu Y. *Exp Mol Med.*., **2017**, *49*(6), e346.
2. Williams C, Royo F, Aizpurua-Olaizola O, Pazos R, Boons GJ, Reichardt NC, Falcon-Perez JM, *J Extracell Vesicles*., **2018**, *7(1)*, 1442985.
3. Barthel SR, Gavino JD, Descheny L, Dimitroff CJ., *Expert Opin Ther Targets*., **2007**, *11(11),* 1473-91.

STEREOSELECTIVE SYNTHESIS OF α-FUCOSIDES

Maximilian G. O'Neill, Kate E. Donaghy, Eoghan M. McGarrigle

Centre for Synthesis and Chemical Biology, UCD School of Chemistry, University College Dublin, Belfield, Dublin 4, Ireland
maximilian.oneill@ucdconnect.ie

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.

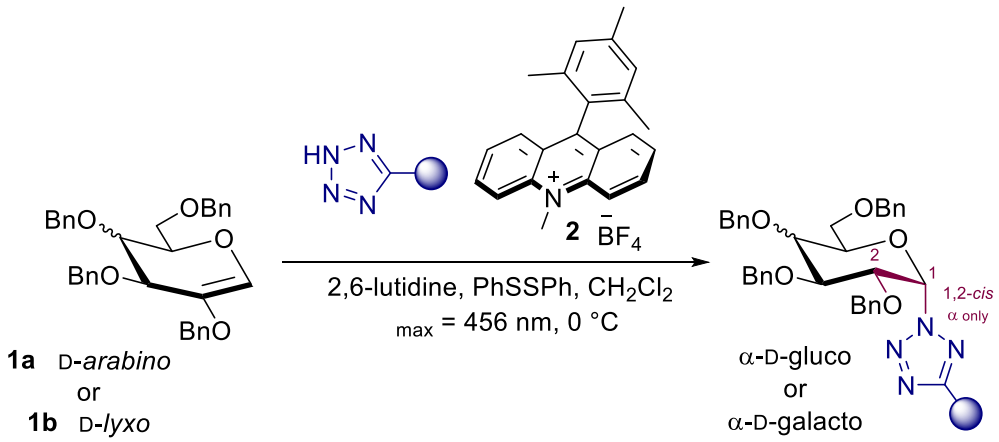
References:
1. M. Schneider, E. Al-Shareffi, R. S. Haltiwanger, *Glycobiology,* **2017**, 27, 601–618.
2. M. Dejter-Juszynski, H. M. Flowers, Carbohydr. Res. **1972**, 23, 41-45.
3. K. E. Donaghy, D. A. Pepe, J. J. Ruddy and E. M. McGarrigle, *manuscript submitted*.
4. K. E. Donaghy, M. G. O'Neill, E. M. McGarrigle, *manuscript in preparation*.

VISIBLE LIGHT-PROMOTED STEREOSELECTIVE SYNTHESIS OF A-D-GLYCOPYRANOSYL TETRAZOLE DERIVATIVES

Ondřej Orság, Jakub Zýka, Vít Prouza, Kamil Parkan

Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Technická 5, 166 28, Prague, Czech Republic
ondrej.orsag@vscht.cz

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 1**).



Scheme 1

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).

References:

1. Kulkarni, S. S.; Wang, C.-C., et al., *Chem. Rev.* **2018,** *118* (17), 8025-8104.
2. Cocinero, E. J.; Çarçabal, P., Carbohydrates. In *Gas-Phase IR Spectroscopy and Structure of Biological Molecules*, Rijs, A. M.; Oomens, J., Eds. Springer International Publishing: Cham, **2015**; pp 299-333.
3. Choutka, J.; Kratochvíl, M., et al., *Carbohydr. Res.* **2020,** *496*, 108086.
4. Kaur, S.; Zhao, G., et al., *Org. Biomol. Chem.* **2019,** *17* (7), 1955-1961.
5. Zhao, G. W., Ting, *Angew. Chem. Int. Ed.* **2018,** *57* (21), 6120-6124.
6. Joshi-Pangu, A.; Levesque, F., et al., *J. Org. Chem.* **2016,** *81* (16), 7244-7249.
7. Nguyen, T. M.; Manohar, N., et al., *Angew. Chem. Int. Ed.* **2014,** *53* (24), 6198-6201.

LONG-TERM MONITORING OF URINARY OLIGOSACCHARIDE BIOMARKERS IN GLYCOGEN STORAGE DISEASE TYPE II

Zuzana Pakanová[a], Iveta Uhliariková[b], Marek Nemčovič[a], Maroš Krchňák[a], Anna Šalingová[c], Anna Hlavatá[c], Katarína Juríčková[c], Peter Baráth[a], Mária Matulová[b]

[a] Department of Glycobiology, Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 84538 Bratislava, Slovakia
zuzana.pakanova@savba.sk
[b] Analytical Department, Institute of Chemistry, Slovak Academy of Sciences, Dubravska cesta 9, 84538 Bratislava, Slovakia
[c] Center for Inherited Metabolic Diseases, National Institute of Children´s Diseases, Limbova 1, 831 01 Bratislava, Slovakia

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.

Acknowledgements: This work was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00681.

References:

1. D. Güngör, et al. in *Orphanet J. Rare Dis.* **2013***, 8,* 49.
2. Z. Pakanová, et al. in *Chem. Papers.* **2019**, *73*, 701-711.

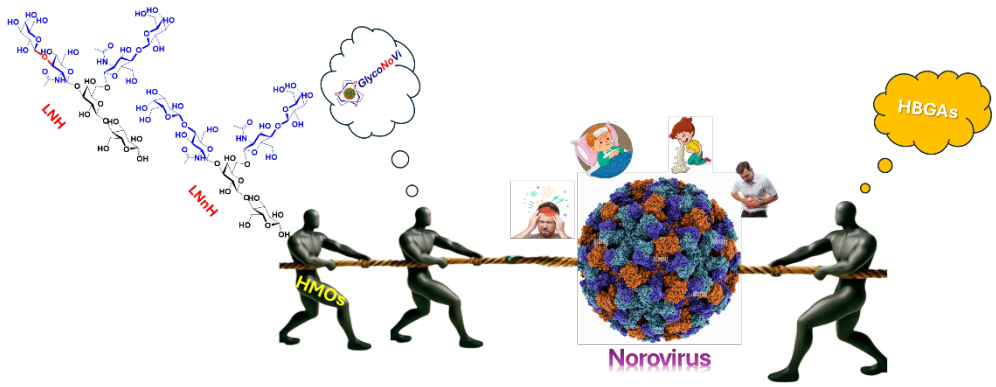
SYNTHESIS OF SELECTED HUMAN MILK OLIGOSACCHARIDES (HMOs)

Dileep Paladugu[a,b], Dora Molnar-Gabor[b], Mads H. Clausen[a], Nikolay Khanzhin[b]

[a] Technical University of Denmark, Department of Chemistry, 2800 Lyngby, Denmark
dilpal@kemi.dtu.dk
[b] Glycom A/S, Department of DSP & Analytics, 2970 Hørsholm, Denmark
dileep.paladugu@dsm-firmenich.com

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.



References:
1. Kirk, M. D., et al., PLoS Medicine. **2015**, *12*, e1001921.
2. Kapikian, A. Z., et al., *Journal of Virology*. **1972**, *10*, 1075–1081.
3. Harrington, P. R., et al., *Journal of Virology*. **2002**, *76*, 12335–12343.
4. Weichert S, et al., *Journal of Virology*.**2016**, *9*.
5. M. D. Bandara, et.al., *J. Org. Chem*., **2019**, *84*, 16192−16198; Imre Kovács et. al, Glycom A/S, US 2014/0235850 A1; M. D. Bandara et.al, *Org. Biomol. Chem.,* **2020***, 18,* 1747.

CHEMICAL TARGETING OF NGLY1: FROM INHIBITORS TO AFFINITY PROBES

Georgia Partalidou, Laura Hartmann, Ulla Gerling-Driessen

Institute for Molecular Chemistry, University Freiburg, Stefan-Meier-Straße 31, 79104 Freiburg, Germany
georgia.partalidou@makro.uni-freiburg.de

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.

References:

1. F. M. Tomlin, U. I. M. Gerling-Driessen, Y.-C. Liu, R. A. Flynn, J. R. Vangala, C. S. Lentz, S. Clauder-Muenster, P. Jakob, W. F. Mueller, D. Ordoñez-Rueda, M. Paulsen, N. Matsui, D. Foley, A. Rafalko, T. Suzuki, M. Bogyo, L. M. Steinmetz, S. K. Radhakrishnan, C. R. Bertozzi, *ACS Cent Sci* 2017, *3*, 1143–1155.
2. S. Walber, G. Partalidou, U. I. M. Gerling-Driessen, *Isr J Chem* 2023, *63*, DOI 10.1002/ijch.202200068.

GLUCOSE-CONTAINING MEMBRANE GLYCEROLIPIDS OF *CUTIBACTERIUM ACNES*

Chudzik Anna[a], Kaszowska Marta[b], Ciesielska Marzena[a], Kapczyńska Katarzyna[a], Smoliński Jakub[a], Gamian Andrzej[a], Mariola Paściak[a]

[a] Department Immunology of Infectious Diseases, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wroclaw, Poland
mariola.pasciak@hirszfeld.pl
[b] Department of Immunochemistry, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland

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.

References:

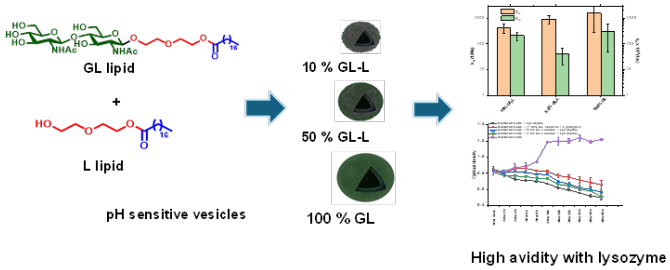
1. Chudzik A, Bromke MA, Gamian A, Paściak M., *mSphere*. **2024**, *29*, 9:e0005424
2. Chudzik A, Migdał P, Paściak M., *Int J Mol Sci*. **2022**, *23*, 5797

MULTIVALENT CHITOBIOSE-CONTAINING GLYCOSTRUCTURES AS LYSOZYME LIGANDS: IMPLICATIONS FOR BINDING AND INHIBITION

Bharat Singh Patel[a], Shivender Yadav[a], Avadhesha Surolia[b], Narayanaswamy Jayaraman[a]

[a] Department of Organic Chemistry, Indian Institute of Science, Bangalore, India
bharatsingh@iisc.ac.in
[b] Dr. Reddy's Institute of Life Sciences, Hyderabad, India

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]



Acknowledgements: We are grateful to Department of Biotechnology and Science and Engineering Research Board, New Delhi, India, for a research support. BSP acknowledges the Indian Institute of Science, Bangalore, for a research fellowship.

References:

1. S. Yadav, A. Surolia, *Sci. Transl. Med*. **2019**, *11*, eaav4176, 1-13.
2. B. S. Patel, S. Yadav, A. Surolia, N. Jayaraman, *Bioorganic Chemistry,* **2025**, *154,* 108009.

COMBINED GLYCOPROTEIN LECTIN-BASED MICROARRAY AND LC-MS ANALYSIS OF COLORECTAL CARCINOMA SAMPLES

Lucia Pažitná, Maroš Krchňák, Patrícia Dudoňová, Kristína Kianičková, Marek Nemčovič, Zuzana Pakanová, Peter Baráth, Jaroslav Katrlík

Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia
chemlpaz@savba.sk

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.

Acknowledgements: This work was supported by the grants APVV-21-0108 and VEGA 2/0120/22. Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V02-00049 and project No. 09I03-03-V04-00772. This publication is the result of the project implementation Centre for Advanced Therapies for Chronic Inflammatory Diseases of the Musculoskeletal System, ITMS2014+: 313011W410 supported by the Operational Programme Integrated Infrastructure funded by the ERDF.

References:

1. S. Holst, A.J.M. Deus et al., *Mol Cell Proteomics*. **2016**, *15*, 124-140.
2. D. Chandrasekar, C. Huerrier et al., *Clin Transl Gastroenterol.* **2023**, *14*, e00592.
3. D. Wang, V. Kuzyk et al., *Int J Mol Sci.* **2023**, *24,* 4842.
4. R. Li, S. He et al., *Sci. Rep.* **2025**, *15,* 798.

SYNTHESIS AND BIOLOGICAL PROFILING OF HEPARAN SULFATE ANALOGUE D-GLUCURONIC ACID-CONTAINING OLIGOSACCHARIDES

Zsófia Peleskei[a,b,c], István Bajza[c], Anikó Borbás[a], Mihály Herczeg[a]

[a] Department of Pharmaceutical Chemistry, University of Debrecen, 4032 Debrecen, Egyetem tér 1., Hungary
[b] Doctoral School of Pharmacy, Medical Science Doctoral Council, University of Debrecen, 4032 Debrecen, Egyetem tér 1., Hungary
[c] GlycOptim Kft., 4032 Debrecen Egyetem tér 1., Hungary
peleskei.zsofia@pharm.unideb.hu

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.

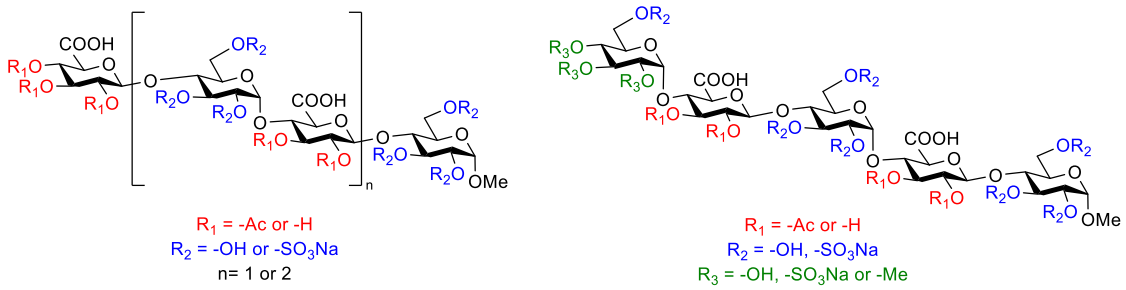


Figure 1. The structure of the synthesized D-glucuronic acid-containing oligosaccharides

Acknowledgements: The authors gratefully acknowledge financial support for this research from the National Research, Development and Innovation Office of Hungary (NKFIH, FK 137924), and by the EKÖP-KDP-2024 university research scholarship program – cooperative doctoral program of the ministry for culture and innovation from the source of the national research, development and innovation fund.

References:

1. Murata, K.; Ochiai, Y.; Akashio, K. *Gastroenterology*, **1985**, *9*, 1248-1257.
2. Lisztes, E.; Mező, E.; Demeter, F.; Bősze, Sz.; Tóth, B. I.; Borbás, A.; Herczeg, M. *ChemMedChem*, **2021**, *16* (9), 1467-1476.

CARBOHYDRATE CONJUGATES AS TROJAN HORSE TO IMPROVE ANTIBIOTIC IMPORT

Hanna Perius[a,b,c], Ronan Cochelin[d], Dirk Hauck[a,b,c], Aurelie Verneuil[d], Kevin Arnould[e], Charlotte Michaux[e], Axel Hartke[d], Florie Desriac[d], Vincent Cattoir[e], Nicolas Sauvageot[d], Alexander Titz[a,b,c]

[a] Chemical Biology of Carbohydrates (CBCH), Helmholtz Institution for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research, 66123 Saarbrücken, Germany
hanna.perius@helmholtz-hips.de
[b] Department of Chemistry, Saarland University, 66123 Saarbrücken
[c] Deutsches Zentrum für Infektionsforschung (DZIF), Standort Hannover-Braunschweig, Braunschweig, Germany
[d] Communication bactérienne & strategies anti-infectieuses (CBSA), University Caen Normandie, 14032 Caen, France
[e] Bacterial Regulatory RNAs & Medicine, University Rennes, 35042 Rennes Cedex, France

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.

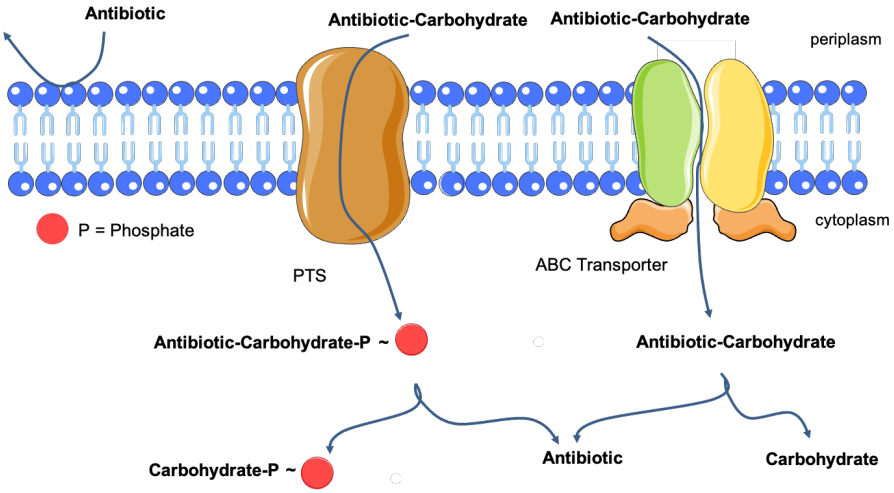


Figure 1. Schematic representation of the idea to hijack PTS and ABC transporter to actively pump carbohydrate-antibiotics into bacterial cells.

Acknowledgment: Financial support from Deutsche Forschungsgemeinschaft (Ti756/7-1) and Agence Nationale de Recherche for the DFG-ANR collaboration scheme is gratefully acknowledged.

References:

1. Naghavi M et al., Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050, The Lancet, Volume 404, Issue 10459, 2024, 1199-1226.
2. Grand M, Blancato VS, Espariz M, Deutscher J, Pikis A, Hartke A, Magni C, Sauvageot N. 2020. *Enterococcus faecalis* MalR acts as a repressor of the maltose operons and additionally mediates their catabolite repression via direct interaction with seryl-phosphorylated-HPr. Mol Microbiol 113:464-477.

DEFINED CHITOOLIGOSACCHARIDE CHAINS AS ALTERNATIVE CARRIERS OF BIOACTIVE GLYCANS

Lucie Petrásková, Michaela Sobotková, David Vrbata, Jan Zelený, Pavla Bojarová

Laboratory of Biotransformation, Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, Prague, Czech Republic
petraskova@biomed.cas.cz

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.

Acknowledgements: This research was funded by the Czech Science Foundation (project 25-16301K), and by the Ministry of Education, Youth, and Sports (OP JAK CZ.02.01.01/00/22_008/0004597 and mobility project LUC23097).

References:

1. G. Lodhi, Y.-K. Kim, J.-W. Hwang, S.-K. Kim, Y.-J. Jeon, J. Y. Je, C.-B- Ahn, S.-H- Moon, B.-T. Jeon, P.-J. Park, *Biomed. Res. Int.* **2014**, 13.
2. P.K. Dutta, (eds) Springer Series on Polymer and Composite Materials. Springer, New Delhi, **2016**.
3. C. Shi, Y. Zhu, X. Ran, M. Wang, Y. Su, T. Cheng, *J. Surg. Res.* **2006**, 133 (2), 185-192.
4. M.Prabaharan, P. R. Sivashankari, In: Dutta, P. (eds) Springer Series on Polymer and Composite Materials. Springer, New Delhi, **2016**.
5. S. Trombotto, C. Ladaviére, F. Delome, A. Domard, *Biomacromolecules* **2008**, 9, 1731–1738.

ITERATIVE BUMP-AND-HOLE ENGINEERING OF *N*-ACETYLGLUCOSAMINYLTRANSFERASE I

Saskia Pieters[a,b], Yu Liu[a,b], Ganka Bineva-Todd[a], Benjamin Schumann[a,b]

[a] Department of Chemistry, Imperial College London, W12 0BZ, London, United Kingdom
[b] Chemical Glycobiology Laboratory, The Francis Crick Institute, NW1 1AT London, United Kingdom

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.

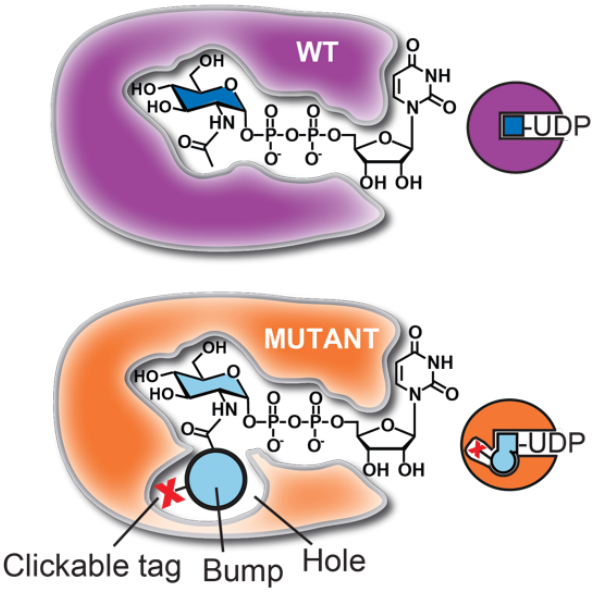


Figure 1. Bump-and-hole engineering of MGAT1.

References:
1. Stanley, P.; Moremen, K. W.; Lewis, N. E.; Taniguchi, N.; Aebi, M. N-Glycans. In *Essentials of Glycobiology*, 4th ed.; Cold Spring Harbor Laboratory Press, **2022**; pp. 487−494
2. E. Ioffe, P. Stanley, *Proc. Natl. Acad. Sci. USA.* **1994**, *91,* 728-732.

SWEET COCKTAILS FOR EVERYONE: PARAMETRIZATION OF FLEXIBLE CARBOHYDRATES FOR THE MARTINI 3 COARSE-GRAINED FORCE FIELD

Jakub Lisiecki[a], Wojciech Płaziński[a,b]

[a] Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Krakow, Poland
wojciech.plazinski@ikifp.edu.pl
[b] Department of Biopharmacy, Medical University of Lublin, Lublin, Poland

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.

Acknowledgements: This research was funded by the National Science Centre, Poland (contract financed in 2020–2024 under no. 2019/35/B/ST4/01149 OPUS 18).

References:
1. V. Lutsyk, P. Wolski, W. Plazinski, *J. Chem. Theory Comput.* **2022**, *18 (8), 5089–5107*.

GOLGI TARGETING: UNLOCKING NEW AVENUES IN CANCER THERAPY

Georgia-Myrto Prifti, Giacomo Biagiotti, Stefano Cicchi, Barbara Richichi, Marco Marradi

Department of Chemistry "Ugo Schiff", Università degli Studi di Firenze,
Via della Lastruccia 3-13, 50019-Sesto Fiorentino (Firenze), Italy
georgiamyrto.prifti@unifi.it

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.

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).

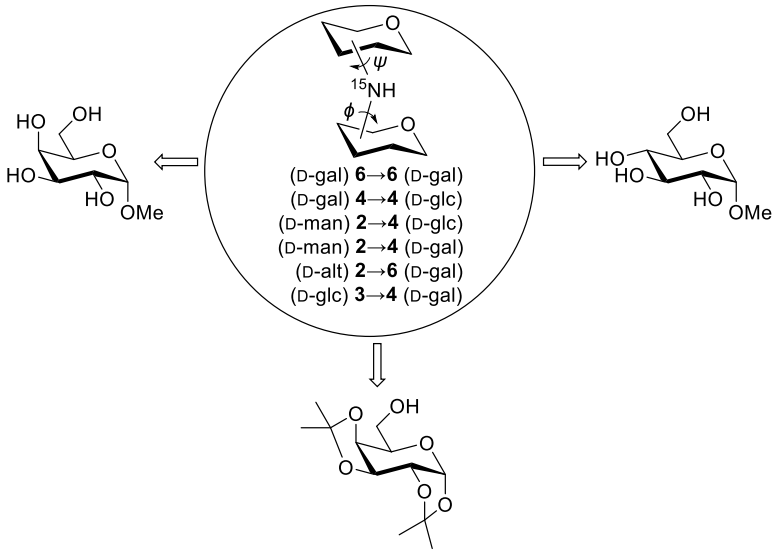
References:
1. S. Pinho, C. Reis, *Nat. Rev*. **2015***, 15,* 540-555.
2. S. Xu *et al.*, *Chem. Soc. Rev.* **2024**, *53*, 7590-7631.
3. G. Biagiotti *et al.*, *Bioorg. Chem.* **2021**, *109*, 104730.
4. E. Giacomazzo *et al.*, *RSC Adv*. **2019**, *9*, 30773-30777.

SYNTHESIS AND CONFORMATIONAL ANALYSIS OF ^{15}N-LABELED *N*-DISACCHARIDES

Vít Prouza[a,b], Adam Rybka[a], Nina Habanová[b], Radek Pohl[b], Kamil Parkan[a]

[a] UCT Prague, Technická 5, 166 28, Prague 6, Czech Republic
prouzav@vscht.cz
[b] IOCB Prague, Flemingovo náměstí 542/2, 160 00 Prague 6, Czech Republic

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.*

References:

1. Boulineau, F. P.; Wei, A., *Carbohydr. Res.* **2001,** *334* (4), 271-279.
2. Coxon, B., *Carbohydr. Res.* **2007,** *342* (8), 1044-1054.
3. Zhu, Y.; Pan, Q.; Thibaudeau, C.; Zhao, S.; Carmichael, I.; Serianni, A. S., *J. Org. Chem* **2006,** *71* (2), 466-479.

NANOMOLAR INHIBITOR OF THE GALECTIN-8 N-TERMINAL DOMAIN BINDS VIA A NON-CANONICAL CATION-Π INTERACTION

Edvin Purić[a], Tihomir Tomašič[a], Mojca Pevec[b], Jurij Lah[b], Hakon Leffler[c], Anders Sundin[d], Ulf J. Nilsson[d], Derek T. Logan[e], Marko Anderluh[a]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Aškerčeva cesta 7, 1000 Ljubljana
edvin.puric@ffa.uni-lj.si
[b] Department for Physical Chemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 13, 1000 Ljubljana, Slovenia
[c] Department of Laboratory Medicine, Section MIG, Lund University BMC-C1228b, Klinikgatan 28, 221 84, Lund, Sweden
[d] Department of Chemistry, Lund University, Box 124, SE-221 00, Lund, Sweden
[e] Biochemistry and Structural Biology, Centre for Molecular Protein Science, Dept. of Chemistry, Lund University, Lund, Sweden

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.

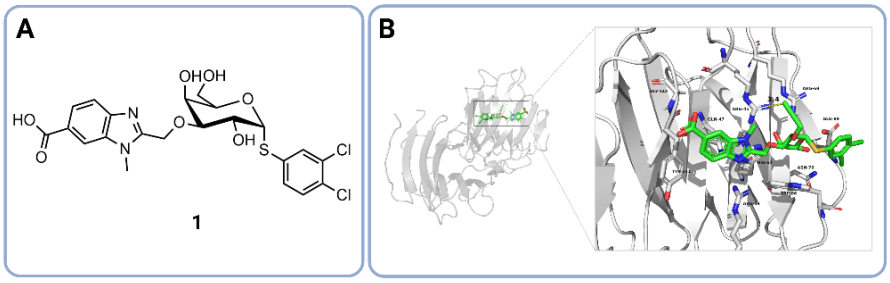


Figure 2. (**A**) The structure of lead **1**. (**B**) A crystal structure of **11** in complex with galectin-8N.

Acknowledgements: *The European Union's Horizon2020 program No. 765581 (project PhD4GlycoDrug; www.phd4glycodrug.eu), The Swedish Research Council (Grant no. 2020-03317), the Slovenian Research Agency (Grants P1-0208 and J1-50026) and COST actions CA18103 and CA18132 are gratefully acknowledged.*

References:

1. A. A. Klyosov et al. in Galectins. John Willey & Sons, Inc. **2008**.
2. E. Purić, U. J. Nilsson, M. Anderluh, *Med Res Rev* **2024**, *44(5)*, 2236-2265.
3. E. Purić, M. Hassan, F. Sjövall, T. Tomašič, M. Pevec, J. Lah, J. Adrover Forteza, A. Sundin, H. Leffler, U. J. Nilsson, D. T. Logan, M. Anderluh, *Commun. Chem.* **2025**, in Press.

AUTOMATED SYNTHESIS OF ACINETOBACTER *BAUMANNII* POLYSACCHARIDE FRAGMENTS

Martina Quaglia[a,b,c], Kim Le Mai Hoang[a], Peter H. Seeberger[b,c]

[a] GlycoUniverse GmbH&Co. KGaA, Am Mühlenberg 11, 14476 Potsdam, Germany
[b] Department of Biomolecular Systems, Max-Planck Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany
[c] Department of Chemistry and Biochemistry, Freie Universität Berlin, Arnimallee 22, 14195 Berlin, Germany

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], [2], [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].

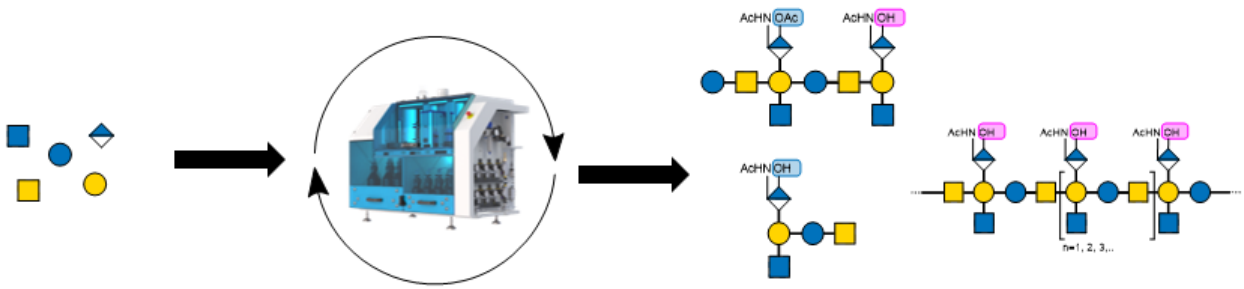


Figure 1. AGA scheme and products

Here I present the first progresses in the project with the design and synthesis of the building blocks and the AGA workflow.

References:
1. C. Ma and S. McClean, *Vaccines*, **2021**, 9, 570.
2. L. Dijkshoorn, A. Nemec and H. Seifert, *Nat. Rev. Microbiol.*, **2007**, 5, 939-951
3. WHO, Bacterial Priority Pathogen List 2024
4. J. K. Singh, F. G. Adams and M. H. Brown, *Front. Microbiol.*, **2019**, 9, 3301
5. J. Sianturi, P. Priegue, J. Hu, J. Yin and P. H. Seeberger, *Angew. Chem. Int. Ed.*, **2022**, 61, e202209556
6. D. Giguère, *Carbohydr. Res.*, **2015**, 418, 29-43

PHOTOSWITCHABLE GLYCOLIPIDS: ON SYNTHESIS AND MEMBRANE EXCITATION

Michael Röhrl[a], Svenja C. Hövelmann[b,c], Bridget M. Murphy[b,c], Thisbe K. Lindhorst[a]

[a] Otto Diels Institute of Organic Chemistry, Christiana Albertina University of Kiel, Otto-Hahn-Platz 3-4, 24118 Kiel, Germany
mroehrl@oc.uni-kiel.de
[b] Institute of Experimental and Applied Physics, Christiana Albertina University of Kiel, Leibnitzstraße 19, 24118 Kiel, Germany
[c] Ruprecht Haensel Laboratory, Deutsche Elektronen-Synchrotron DESY, Notkestraße 85, 22607 Hamburg, Germany

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.

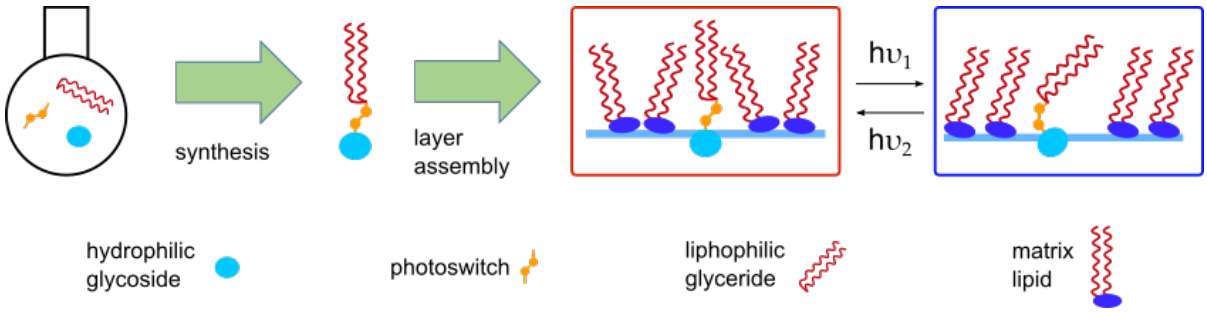


Figure 1. The synthesized photoswitchable amphiphiles are assembled in layer systems and used to emulate stimuli occurring in membranes causing rearrangements and phase transitions.

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.

References:
1. J. E. Warias, F. Reise, et al., *Sci. Rep.* **2023**, 13, 11480.
2. S: C. Hövelmann, E. Dieball, et al., *IUCrJ* **2024**, 11, 486–493.
3. M. Doroudgar, J. Morstein, et al., *JACS* **2021** 143 (25).
4. J. H. A. Folgering, J. M. Kuiper, et al., *Langmuir* **2004**, 20, 17, 6985–6987

UNVEILING THE MOLECULAR MECHANISM OF HUMAN LYSOSOMAL A-GLUCOSIDASE

Samanta Makurat[a,c], Marina Corbella[b,c], Carme Rovira[c]

[a] Faculty of Chemistry, University of Gdańsk, 80-308 Gdańsk, Poland
samanta.makurat@ug.edu.pl
[b] Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800, Kgs. Lyngby, Denmark
[c] Departament de Química Inorgànica i Orgànica (Secció de Química Orgànica) & Institut de Química Teòrica i Computacional (IQTCUB), Universitat de Barcelona, 08020 Barcelona, Spain

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.

References:
1. F. M. Platt, A. d'Azzo, B. L. Davidson, E. F. Neufeld, C. J. Tifft, *Nat Rev Dis Primers*, **2018**, 2018, 4(1), 27.
2. V. Roig-Zamboni, B. Cobucci-Ponzano, R. Iacono, *et al. Nat Commun*, **2017**, 8, 1111.

2,5-ANHYDROSUGARS AS SUBSTRATES IN THE REACTION WITH VANCOMYCIN

Justyna Samaszko-Fiertek[a], Monika Szulc[a], Barbara Dmochowska[a], Rafał Ślusarz[a], Maciej Jaśkiewicz[b], Wojciech Kamysz[b], Janusz Madaj[a]

[a] Sugar Chemistry Lab, Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, 80-308 Gdańsk, Poland
j.samaszko-fiertek@ug.edu.pl
[b] Faculty of Pharmacy, Medical University of Gdansk, Al. Gen. Hallera 107, 80-416 Gdansk, Poland

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.

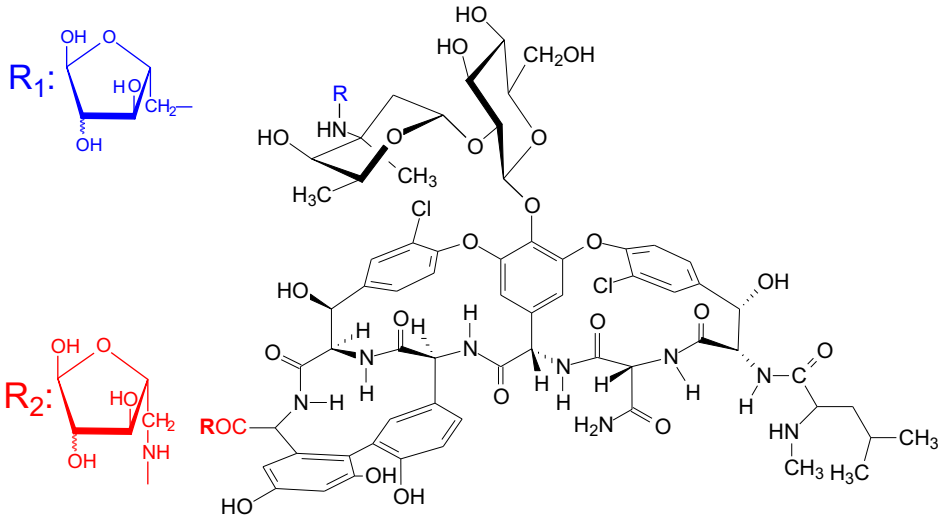


Figure 1

References:
1. Taylor, M. E.; Drickamer, K. *Intruduction to Glycobiology Third Edition*, Oxford, **2004**.
2. Trimble, R. B.; Tarentino, A. L. Identification of distinct endoglycosidase (endo) activities in Flavobacterium meningosepticum: endo F1, endo F2, and endo F3. Endo F1 and endo H hydrolyze only high mannose and hybrid glycans. *J. Biol. Chem.* **1991**, *266* (3), 1646–1651.

DISCOVERY OF ALTERNATIVE TECHNIQUES FOR QUANTIFICATION AND CHARACTERIZATION OF OLIGOSACCHARIDES

Giovanni Santostefano[a], Alessio Corrado[b], Carmine Malzone[b], Valeria Di Bussolo[c], Riccardo De Ricco[b]

[a] Department of Pharmacy, University of Pisa, 56126 Pisa, Italy
giovanni.santostefano@phd.unipi.it
[b] GSK, via Fiorentina 1, 53100 Siena, Italy,
[c] Department of Pharmacy, University of Pisa, 56126 Pisa, Italy

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.

References:

1. R. Rappuoli, Glycoconjugate vaccines: Principles and mechanisms, Sci Transl Med, 10 (2018).
2. F. Rech, R. De Ricco, S. Giannini, L. Nompari, M. Paludi, S. Berti, C. Parlati, S. Cianetti, F. Berti, Glycoconjugate content quantification to assess vaccine potency: A simplified approach, Biologicals, 76 (2022) 10-14.
3. R. Biemans, F. Micoli, M.R. Romano, Glycoconjugate vaccines, production and characterization, Recent Trends in Carbohydrate Chemistry2020, pp. 285-313.
4. A. Corrado, M. De Martino, V. Bordoni, S. Giannini, F. Rech, S. Cianetti, F. Berti, C. Magagnoli, R. De Ricco, A universal UHPLC-CAD platform for the quantification of polysaccharide antigens, Sci Rep, 13(1):10646 (2023).
5. H. Ahsan, Monoplex and multiplex immunoassays: approval, advancements, and alternatives, Comp Clin Path., 31 (2022) 333-345.

PHYSICOCHEMICAL CHARACTERIZATION OF *RHODOTHERMUS MARINUS* DSM 16675 EXOPOLYSACCHARIDES AS BIOEMULSIFIER

Roya R. R. Sardari, Milad Mohammadi, Lars Dahlgren, Eva Nordberg Karlsson

Division of Biotechnology and Applied Microbiology, Department of Process and Life science Engineering, Lund University, Naturvetarvägen 14, Sweden
roya.sardari@ple.lth.se

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

Aleksandra Sarnowicz[1,6], Bruno Steimbrüch[2,6], Keshab Bhattarai[1,6], Holger Heine[2,3,6], Susanne Krauss-Etschmann[2,4,5,6], Katarzyna A. Duda[1,6]

[1]Biofunctional Metabolites and Structures, Research Center Borstel, Leibniz Lung Center, Borstel, Germany
[2]DZL Laboratory – Experimental Microbiome Research, Research Center Borstel, Leibniz Lung Center, Borstel, Germany
[3]Division of Innate Immunity, Research Center Borstel, Leibniz Lung Center, Borstel, Germany
[4]Early Life Origins of CLD, Priority Area Asthma and Allergies, Research Center Borstel, Leibniz Lung Center, Borstel, Germany
[5]Institute for Experimental Medicine, Christian-Albrechts-Universität zu Kiel, Kiel, Germany.
[6]Member of the German Center for Lung Research (DZL), Germany
asarnowicz@fz-borstel.de

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.

References:

1. Clementi. 2011. *Frontiers in cellular and infection microbiology* 1.
2. Swords et al 2003. *Journal of endotoxin research* 9(3):131-44.
3. Bigot et al 2020. *The Journal of Infectious Diseases* 221, 1000-1005.
4. Jorgensen, et al 1987. *Journal of clinical microbiology* 25.11: 2105-2113.
5. Månsson et al. 2001. *European journal of biochemistry* 268.7: 2148-2159.

INTERACTIONS OF CD44 WITH TLR4-MD-2 COMPLEX AND IT'S INFLUENCE ON HYALURONAN BINDING

Mariia Savenko, Hector Martinez-Seara

Molecular modeling group, Institute of Organic Chemistry and Biochemistry of Czech Academy of Science,Flemingovo náměstí 542/2, Czech Republic
mariia.savenko@uochb.cas.cz

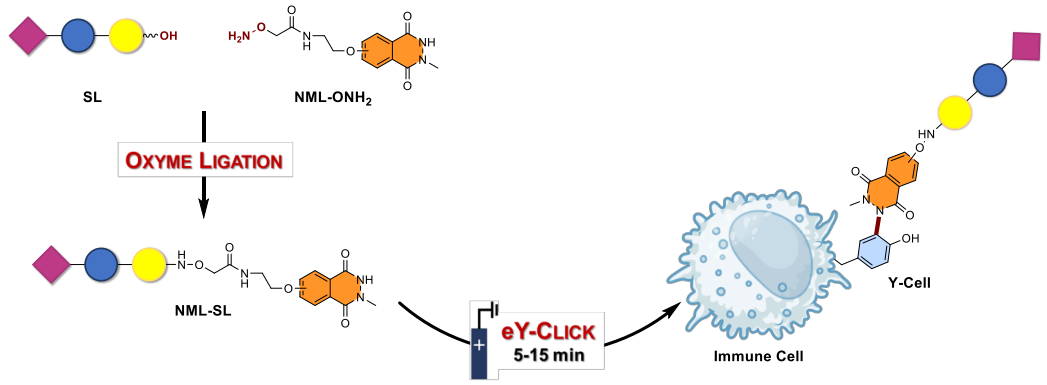
Toll-like receptors (TLRs) are widely distributed in the gastrointestinal tract as part of the innate immune system, making them relevant in the treatment of carcinomas of the duodenum, small intestine, colon, and rectum. Short hyaluronic acid (HA) oligosaccharides activate signaling cascades upon binding 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

Mathieu Scalabrini[a,b], Mohammed Bouzehla[b], Karine Pavageau[b], Emeline Richard Millot[c], Sébastien Fort[c], David Deniaud[a], Mathieu Mével[b], Sébastien Gouin[a]

[a] Nantes Université, CNRS, CEISAM, UMR 6230, F-44000 Nantes, France
mathieu.scalabrini@univ-nantes.fr
[b] Nantes Université, TaRGeT, Translational Research for Gene Therapies, CHU Nantes, INSERM, UMR 1089, F-44000 Nantes, France
[c] CNRS, CERMAV, Université Grenoble Alpes, France

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.



References:
1. Alvarez-Dorta, D.; Thobie-Gautier, C.; Croyal, M.; Bouzelha, M.; Mével, M.; Deniaud, D.; Boujtita, M.; Gouin, S. G. *J. Am. Chem. Soc.* **2018**, 140 (49), 17120–17126
2. Depienne, S.; Bouzelha, M.; Courtois, E.; Pavageau, K.; Lalys, P.-A.; Marchand, M.; Alvarez-Dorta, D.; Nedellec, S.; Marín-Fernández, L.; Grandjean, C.; Boujtita, M.; Deniaud, D.; Mével, M.; Gouin, S. G. *Nat. Commun*. **2023**, 14 (1), 5122

PARALLEL SYNTHESIS AND SCREENING OF SULFATED GLYCOSAMINOGLYCAN LIBRARIES

Marcel Schmitter[a], Merrit Wilig[a], Nicole Synder[b], Laura Hartmann[a]

[a] Institute for Macromolecular Chemistry, University Freiburg,
Stefan-Meier-Straße 31, Germany
marcel.schmitter@makro.uni-freiburg.de
[b] Department of Chemistry, Davidson College, Davidson, NC 28035, USA

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).

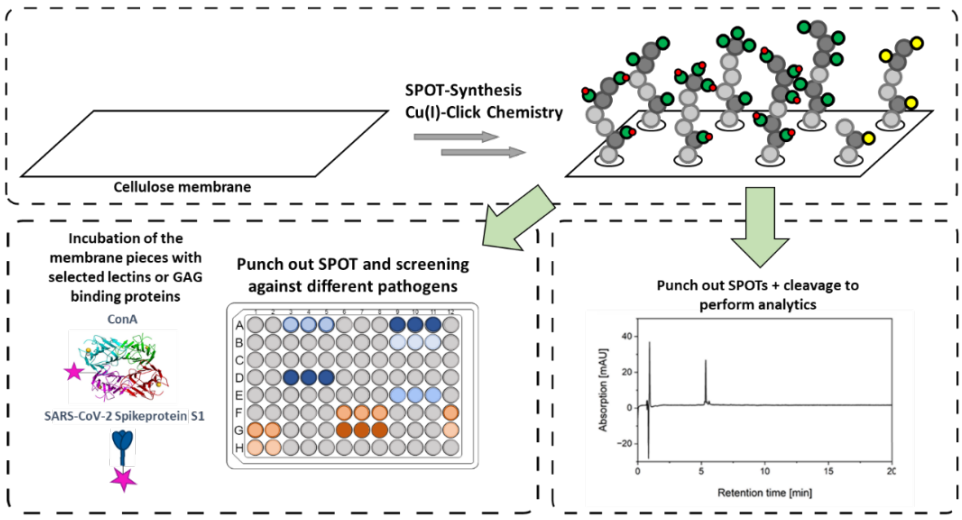


Figure 1. Parallel synthesis of sGAG mimetics on membrane support and screening against various pathogens.

The derived membrane-bound glycooligomer libraries are then screened for binding with various lectins and pathogens.

References:

1. M. Hoffmann, N. L. Snyder, L. Hartmann, *Biomacromolecules* **2022**, *23*, 4004–4014.
2. R. Frank, *J Immunol Methods* **2002**, *267*, 13–26.

DESIGN AND SYNTHESIS OF SIALOSIDES AS FICOLIN-1 ANTAGONISTS

Oliver Schwardt, Said Rabbani, Martin Smieško, Daniel Ricklin

Molecular Pharmacy Group, Department of Pharmaceutical Sciences,
University of Basel, Klingelbergstrasse 50, 4056 Basel, Switzerland
oliver.schwardt@unibas.ch

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.

References:

1. R. Hevey, R. B. Pouw, C. L. Harris, D. Ricklin, *Brit. J. Pharmacol.* **2020**, *178*, 2802-2822
2. T. Fujita, *Nat. Rev. Immunol.* **2002**, *2*, 346-353.
3. Y. Endo, M. Matsushita, T. Fujita, *Int. Rev. Cell. Mol. Biol.* **2015**, *316*, 49-110.
4. P. Wang, Q. Wu, Z. W. Shuai, *Pharmacol. Res.* **2021**, *163*, 105266.

OHMIC HEATING-ASSISTED EXTRACTION OF PECTINS FROM RASPBERRY POMACE

Nimra Shahid, Ewa Górska, Szymon Jarząbek, Izabela Pawlaczyk-Graja

Laboratory of Bioproducts Technology, Faculty of Chemistry, Wrocław University of Science and Technology, Wybrzeże Wyspiańskiego 29, 50-370 Wrocław, Poland
282360@student.pwr.edu.pl

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.

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

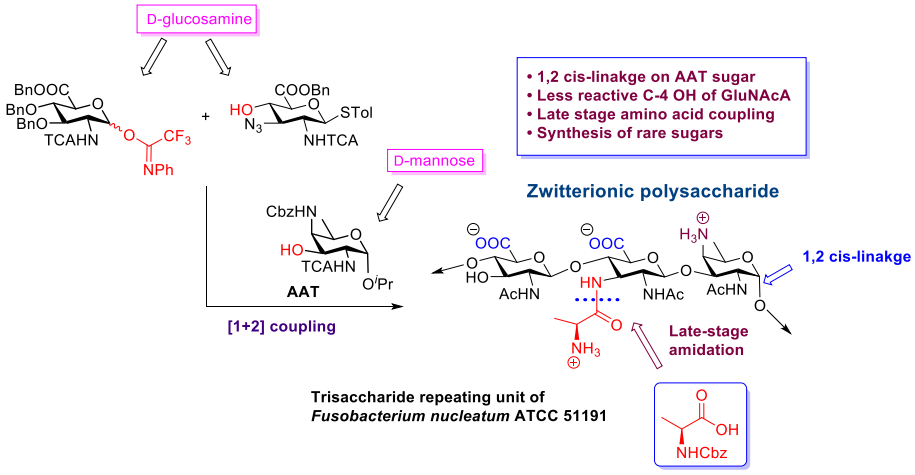
References:
1. Mohsen Gavahian, Girish N. Mathad, R. Pandiselvam, Jenshinn Lin, Da-Wen Sun, Trends in Food Science & Technology, 115, 2021, 42-54.
2. Cristina M.R. Rocha, Zlatina Genisheva, Pedro Ferreira-Santos, Rui Rodrigues, António A. Vicente, José A. Teixeira, Ricardo N. Pereira, Bioresource Technology, 254, 2018, 325-339.

TOTAL SYNTHESIS OF THE ZWITTERIONICTRISACCHARIDE REPEATING UNIT OF *FUSOBACTERIUM NUCLEATUM* ATCC51191 O-ANTIGEN

Aishwarya A. Shelke, Roberta Marchetti, Alba Silipo, Suvarn S. Kulkarni

Indian Institute of Technology, Bombay, India
shelkeaishwarya40@gmail.com

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%.



References:

1. Y. W. Han, *Curr. Opin. Microbiol*. 2015, *23*, 141– 147.
2. P. G Vello, F. D Lorenzo, D. Lamprinaki, A. Notaro, I. Speciale, A. Molinaro and N. Juge,; C. Castro, *ChemBioChem.* 2021, *22*, 1252–1260.

DESIGN AND SYNTHESIS OF INHIBITORS FOR SIALIC ACID ESTERASES

Foteini Skoulikopoulou, Zachary Armstrong

Bio-organic Synthesis Department, Leiden Institute of Chemistry LIC, Leiden University, Gorlaeus Laboratories, 2300 RA Leiden, The Netherlands
f.skoulikopoulou.3@lic.leidenuniv.nl

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.

References:

1. S. S. Pinho, C. A. Reis, Nat. Rev. Cancer **2015**, 15, 540–555.
2. A. Varki, Trends in Molecular Medicine **2008**, 14, 351-360.
3. T. Angata, A. Varki, Chem. Rev. **2002**, 102, 439–469.
4. A. M. T. Baumann, Nat. Commun. **2015**, 6, 1-12.
5. B. R. Wasik, K. N.Barnard, C. R. Parrish, Trends Microbiol. **2016**, 24, 991–1001.
6. M. Desforges, J. Desjardins, C. Zhang, P. J. Talbot, J. Virol. **2013**, 87, 3097–3107.

THREE-STEP ENZYMATIC SYNTHESIS OF BIOACTIVE CHITOOLIGOMERS

Kristýna Slámová, Lucie Petrásková, Natalia Kulik, Vladimír Křen

Laboratory of Biotransformation, Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, Praha 4, Czech Republic
slamova@biomed.cas.cz

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.

Acknowledgements: Financial support from the Ministry of Education, Youth, and Sports of the Czech Republic grant LUC23097 is gratefully acknowledged.

References:

1. F. Liaquat, R. Eltem, Carbohydr. Polym. **2018**, 184: 243-259.
2. Z. Mészáros, L. Petrásková, N. Kulik, H. Pelantová, P. Bojarová, V. Křen, K. Slámová, Adv. Synth. Catal. **2022**, 364: 2009-2022.
3. Z. Mészáros, N. Kulik, L. Petrásková, P. Bojarová, M. Texidó, A. Planas, V. Křen, K. Slámová, J. Agric. Food Chem. **2024**, 72, 15613-15623.

STRUCTURAL INSIGHTS INTO SIGLEC-10 INTERACTIONS WITH T-CELLS

Klaudia Sobczak[a], Asier Antoñana-Vildosola[a], Pablo Valverde[a], Eunate Valdaliso[a], Iker Oyenarte[a], Alexandre Bosch[a], Maria Jesús Moure[b], Mikel Azkargorta[c], Felix Elortza[c], Francisco Corzana[d], Asis Palazon[a,e], Ana Gimeno[b,e], Matthew Macauley[f,g], Jesús Jiménez-Barbero[b,e,h,i], June Ereño-Orbea[a,e]

[a] Cancer Glycoimmunology Lab, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), 48160 Derio, Bizkaia, Spain
ksobczak@cicbiogune.es
[b] Chemical Glycobiology lab, Center for Cooperative Research in Biosciences (CIC bioGUNE), Basque Research and Technology Alliance (BRTA), 48160 Derio, Bizkaia, Spain
[c] Proteomics Platform, CIC bioGUNE, CIBERehd, ProteoRed-ISCIII, Bizkaia Science and Technology Park, Derio, Spain
[d] Departament of Chemistry, Universidad de La Rioja, Centro de Investigación en Síntesis Química, E-26006 Logroño, Spain
[e] Ikerbasque, Basque Foundation for Science, Bilbao, Spain
[f] Department of Chemistry, University of Alberta, 11227 Saskatchewan Drive, Edmonton, Alberta T6G 2G2, Canada
[g] Department of Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta T6G 2E1, Canada
[h] Department of Organic & Inorganic Chemistry, Faculty of Science and Technology, University of the Basque Country, EHU-UPV, 48940 Leioa, Bizkaia, Spain
[i] Centro de Investigacion Biomedica en Red de Enfermedades Respiratorias, 28029 Madrid, Spain

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.

References:

1. Zhang, C., Zhang, J., Liang, F., Guo, F., Gao, S., Yang, F., Guo, H., *et al.* (**2022**) Innate immune checkpoint Siglec10 in cancers: mining of comprehensive omics data and validation in patient samples. *Frontiers in Medicine, 16*, 596-609.

FUNCTIONAL IMPACT OF *O*-GLYCANS ON SURFACE MANNOPROTEINS IN CRYPTOCOCCUS NEOFORMANS: INSIGHTS INTO THE SECRETION, STABILITY, AND IMMUNOGENICITY OF CDA1 AND MP88

Seon Woo Song[a], Tae Ho Lee[a], Eun Jung Thak[a,b], Hyun Ah Kang[a]

[a]Department of Life Science, Chung-Ang University, Seoul, South Korea
songsw6267@naver.com
[b]College of Pharmacy, Dongduk Women's University, Seoul, South Korea

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.

Acknowledgements: Supported by the Korean MSIT [NRF grant number, RS-2023-00212663].

References:

1. Eun Jung Thak, Ye Ji Son, *mBio*. 2022 Dec 20;13(6):e0211222.
2. Huang C, Nong SH, Mansour Mk, Specht CA, Levitz SM. *Infect Immun.* 2002 Oct;70(10):5485-93
3. Baker LG, Specht CA, Lodge JK. *Eukaryot Cell.* 2011 Sep;10(9):1264-8

STRUCTURAL INSIGHTS INTO GALECTIN-3 RECOGNITION OF A SELENOGLYCOMIMETIC

Alessia Stornaiuolo[a], Maria Pia Lenza[a,b], Antonio Molinaro[a], Alba Silipo[a]

[a] Department of Chemical Sciences, University of Naples "Federico II", Via Vicinale Cupa Cinthia 26, 80126, Naples, Italy
alessia.stornaiuolo@unina.it
[b] Department of Pharmacy, University of Naples "Federico II", Via Domenico Montesano 49, 80131, Naples, Italy

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.[1,2,3] 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.

References:

1. M. F. Troncoso, M. T. Elola, A. G. Blidner, L. Sarrias, M. V. Espelt, G. A. Rabinovich, *J. Biol. Chem.* **2023**, *299*.
2. S. Bouffette, I. Botez, F. De Ceuninck, *Trends Pharmacol. Sci.* **2023**, *44*, 519-531.
3. L. Song, J. Tang, L. Owusu, M.Z. Sun, J. Wu, J. Zhang, *Clin. Chim. Acta* **2014**, *431*, 185-191.
4. L. Johannes, R. Jacob, H. Leffler, *J. Cell Sci.* **2018**, *131*.
5. L. Pirone, F. Nieto-Fabregat, S. Di Gaetano, D. Capasso, R. Russo, S. Traboni, A. Molinaro, A. Iadonisi, M. Saviano, R. Marchetti, *Int. J. Mol. Sci.* **2022**, *23*, 8273.
6. S. Di Gaetano, L. Pirone, I. Galdadas, S. Traboni, A. Iadonisi, E. Pedone, M. Saviano, F. L. Gervasio, D. Capasso, *Int. J. Mol. Sci.* **2022**, *23*, 2581.
7. L. H. Duntas, *Horm. Metab. Res.* **2009**, *41*, 443-447.
8. M. P. Lenza, C. Di Carluccio, F. Nieto-Fabregat, L. Pirone, R. Russo, S. Di Gaetano, D. Capasso, A. Stornaiuolo, A. Iadonisi, M. Saviano, R. Marchetti, E. Pedone, A. Silipo, *ChemMedChem*. **2025**, e202401005.

SITE-SPECIFIC GLYCO-TAGGING OF ALLERGENS WITH HIGH AFFINITY SIGLEC-9 LIGANDS FOR IMMUNOTHERAPY

Bart F. Straten[a], Anna M. Ehlers[a], Bernd Stahl[a,b], Geert-Jan P.H. Boons[a,c]

[a] Chemical Biology and Drug Discovery, Utrecht Institute of Pharmaceutical Sciences, and Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, CG 3584, The Netherlands
b.f.straten@uu.nl
[b] Danone Nutricia Research, Utrecht, CT 3584, The Netherlands
[c] Complex Carbohydrate Research Center and Department of Chemistry, University of Georgia, Athens, Georgia 30602, United States

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.

References:

1. M. Perdicchio, J.M. Ilarregui, M.I. Verstege, L.A.M. Cornelissen, S.T.T. Schetters, S. Engels, M. Ambrosini, H. Kalay, H. Veninga, J.M.M. den Haan, L.A. van Berkel, J.N. Samsom, P.R. Crocker, T. Sparwasser, L. Berod, J.J. Garcia-Vallejo, Y. van Kooyk, W.W.J. Unger, *Proc. Natl. Acad. Sci. U.S.A.* **2016** ,113 (12), 3329-3334.
2. S. Duan, B. M. Arlian, C. M. Nycholat, Y. Wei, H. Tateno, S. A. Smith, M. S. Macauley, Z. Zhu, B. S. Bochner, J. C. Paulson. *J Immunol* **2021**; 206 (10), 2290–2300

STRUCTURAL ANALYSIS OF BACTERIAL ENVELOPE COMPONENTS

Maina Takahashi[a], Ferran Nieto-Fabregat[a], Yuxin Ma[b], Hanyingzi Fan[b], Stefania De Chiara[a], Antonio Molinaro[a], Flaviana Di Lorenzo[a], Guozhi Xiao[b], Alba Silipo[a]

[a] Department of Chemical Sciences, University of Naples Federico II,
Via Cinthia 4, 80126, Napoli, Italy
maina.takahashi@unina.it
[b] State Key Laboratory of Phytochemistry and Natural Medicines,
Kunming Institute of Botany, CAS, Kunming, China

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).

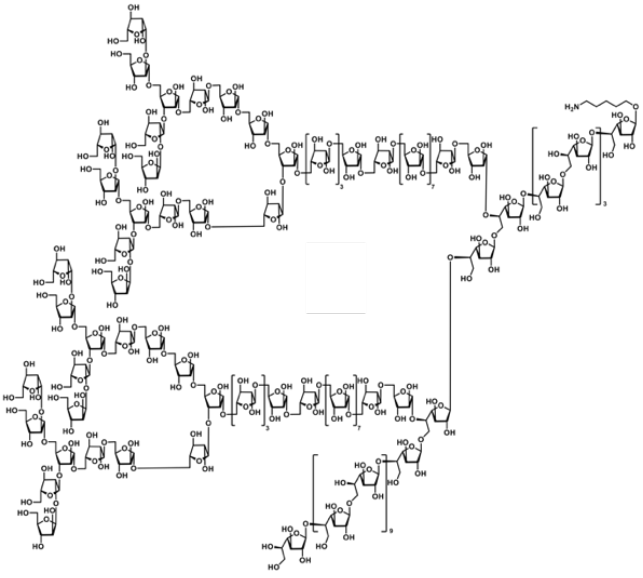


Figure 1. The structure of arabinogalactan 92-mer

References:
1. M. Daffé *et al. Adv. Microb. Physiol.* **1998**, *39*, 131–203.
2. D. A. Case *et al. J. Chem. Inf. Model.* **2023**, *63* (20), 6183–619.
3. C. D. Carluccio *et al. Carbohydr. Res.* **2021**, *503*, 108313.
4. F. Nieto-Fabregat *et al. Beilstein J. Org. Chem.* **2023**, *20*, 2084–2107.

ROLE OF LONG-RANGE INTERACTIONS IN STRUCTURE AND DYNAMICS OF CARBOHYDRATES

Beicer C. Tapia, Mateusz Marianski

Department of Chemistry and Biochemistry, Hunter College, The City University of New York, 695 Park Ave, New York, NY, 10065, USA
btapia@gradcenter.cuny.edu
The PhD Program in Biochemistry, The Graduate Center of The City University of New York, 365 5th Ave, New York, NY, 10016, USA

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.

References:

1. Gagneux P, Hennet T, Varki A. Essentials of Glycobiology: Biological Functions of Glycans. 4th edition, **2022**, pp. 127-143
2. Dwek, R.A., Glycobiology: Toward Understanding the Function of Sugars. Chem Rev, **1996**. 96(2): p. 683-720.
3. Fittolani, G., Tyrikos-Ergas, T., Poveda, A., Yu, Y., Yadav, N., Seeberger, P. H., Jimenez-Barbero, J., & Delbianco, M. Nat Chem, Synthesis of a glycan hairpin. **2023**, p. 1461-1469.

DEVELOPMENT OF PATHOBLOCKERS TARGETING ECLA: A NOVEL LECTIN FROM *ENTEROBACTER CLOACAE*

Beshoy Tawfik[a,b,c], Mario Fares[a,b,c], Markus Bischoff[d], Alexander Titz[a,b,c]

[a] Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Campus E8.1, 66123 Saarbrücken, Germany
[b] Deutsches Zentrum für Infektionsforschung (DZIF), Standort Hannover – Braunschweig, Germany
[c] Department of Chemistry, Saarland University, 66123 Saarbrücken, Germany
beshoy.tawfik@helmholtz-hips.de
[d] Institute of Medical Microbiology and Hygiene, Saarland University, 66421 Homburg, Germany

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].

References:
1. P. Venkateswaran, S. Vasudevan, H. David, A. Shaktivel, K. Shanmugam, P. Neelakantan, A. P. Solomon, *Front Cell Infect Microbiol* **2023***, 13,* 1159798.
2. G. Beshr, A. Sikandar, J. Gläser, M. Fares, R. Sommer, S. Wagner, J. Köhnke, A. Titz, *Journal of Biological Chemistry* **2025.** DOI: 10.1016/j.jbc.2024.108151
3. M. Fares et al, *unpublished*.
4. S. Leusmann, P. Ménová, E. Shanin, A. Titz, C. Rademacher, *Chem. Soc. Rev.* **2023**, *52* (11), 3663–3740.

DEVELOPMENT OF NEW ANALOGUES OF MANNOSE-6-PHOSPHATE

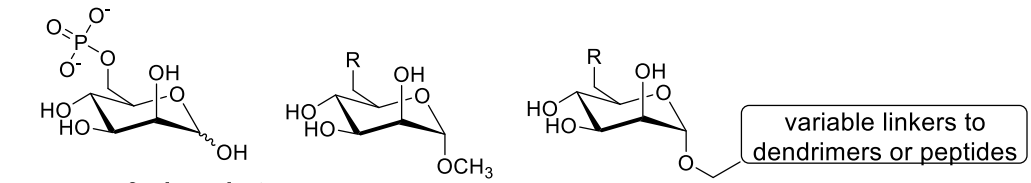
Barbora Toncarová[a], Jakub Zýka[a], Kamil Parkan[a], Klaudia Hladoníková[b], Jaroslav Kozák[b], Jiří Jiráček[b], Lenka Žáková[b]

[a] Department of Chemistry of Natural Compounds, UCT Prague, Technicka 5, 166 28, Prague, Czech Republic
toncarob@vscht.cz
[b] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Flemingovo nam. 2, 16000, Prague, Czech Republic

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).

References:

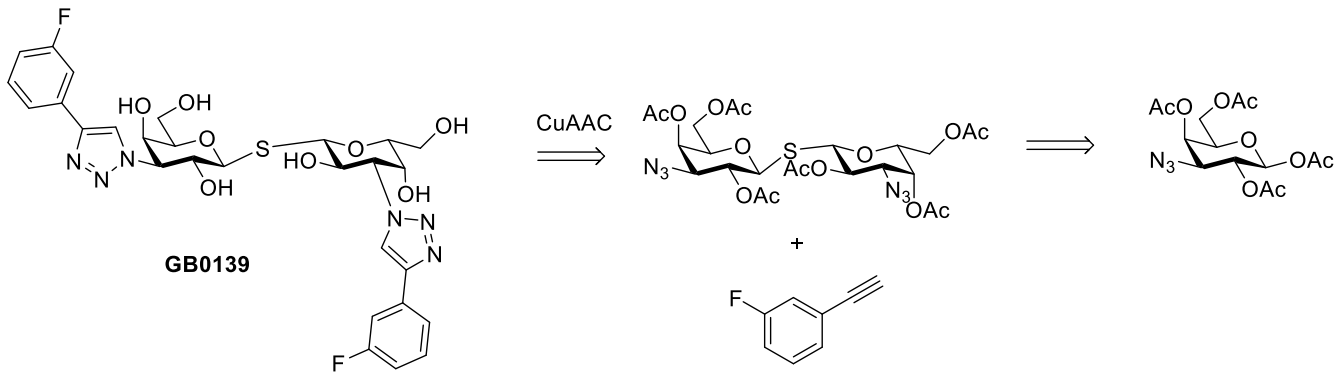
1. L. M. Pascual, S. Viana da Silva, et al. *EMBO Mol. Med.* **2014**, *6*, 1246-1262.
2. Roy, D. S.; Arons, A.; et al. *Nature* **2016**, *531*, 508-512.
3. Eggenschwiler, J.; Ludwig, T.; et al. *Genes Dev.* **1997**, *11*, 3128-3142.
4. Dynkevich, Y.; Rother, K. I.; et al. *Endocr. Rev.* **2013**, *34*, 798-826.
5. LeRoith, D.; Roberts, C. T. *Cancer Lett.* **2003**, *195*, 127-137.
6. Zavorka, M. E.; Connelly, C. M.; et al. *Oncotarget* **2016**, *7*)stránku.
7. Vidil, C.; Morère, A.; et al. *Eur. J. Org. Chem.* **1999**, *1999* (2), 447-450.
8. Vidal, S.; Vidil, C.; et al. *Eur. J. Org. Chem.* **2000**, *2000* (20), 3433-3437.
9. Ng, Y. Z.; Baldera-Aguayo, P. A.; et al. *Biochem.* **2017**, *56* (40), 5260-5268.

CASE STUDY: DEVELOPMENT OF GB0139 FOR IDIOPATHIC PULMONARY FIBROSIS

A. Tyrrell[a], R. Clarkson[a], K. Burns[a], C. Wallington-Smith[a], I. Davies[a], N. Blakiston[a], L. Morris[a], A. Weymouth-Wilson[a], U. J. Nilsson[b], L. Gravelle[b]

[a] Dextra Laboratories, A division of NZP UK Ltd., Thames Valley Science Park, The Gateway, 1 Collegiate Square, Reading, RG2 9LH, UK
andrew.tyrrell@dextrauk.com
[b] Galecto Biotech AB, Ole Maaloes Vej 3, DK-2200 Copenhagen, Denmark

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].

References:

1. A. C. MacKinnon *et al. Am. J. Respir. Crit. Care Med.* **2012**, *185* (5), 537–546.
2. T. Delaine *et al. ChemBioChem* **2016**, *17* (18), 1759–1770.
3. T-J. Hsieh *et al. Sci. Rep.* **2016**, *6* (1), 29457.
4. N. Hirani, A. C. MacKinnon, L. Nicol, P. Ford, H. Schambye, A. Pedersen, U. J. Nilsson, *et al. Eur. Respir. J.* **2021**, *57* (5) 2002559.
5. U. J. Nilsson *et al.* Galecto Biotech AB, WO2021198160A1

WOOD-DERIVED XYLOOLIGOSACCHARIDES STRUCTURE-FUNCTION RELATIONSHIP FOR PREBIOTIC FUNCTION AND GUT COLONISATION POTENTIAL FOR HUMAN AND BOVINE-ASSOCIATED GUT COMMENSALS

Uthesh Umapathy[a], Jessica Villegas-Moreno[a], Lokesh Joshi[a], Michelle Kilcoyne[a,b]

[a] Advanced Glycoscience Research Cluster, School of Biological and Chemical Sciences, University of Galway, Galway, Ireland
u.umapathy1@universityofgalway.ie
[b] Carbohydrate Signalling Group, Infectious Disease Section, School of Biological and Chemical Sciences, University of Galway, Galway, Ireland

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.

References:

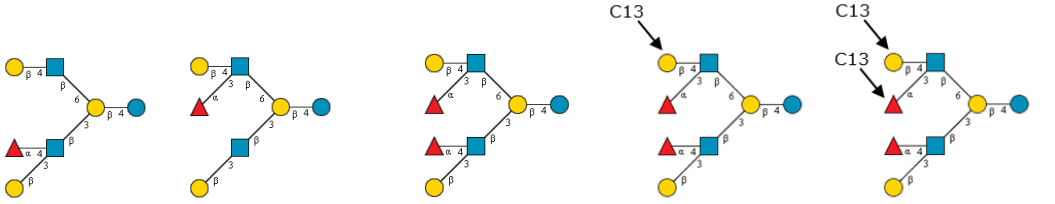
1. Environmental Protection Agency. (2024, September). Construction & Demolition Waste Statistics for Ireland.
2. Valladares-Diestra, K. K., de Souza Vandenberghe, L. P., Vieira, S., Goyzueta-Mamani, L. D., de Mattos, P. B. G., Manzoki, M. C., & Soccol, C. R. *Foods*. 2023,. *12*(14), 2681. The potential of xylooligosaccharides as prebiotics and their sustainable production from agro-industrial by-products.

ENZYMATIC FUCOSE TRANSFER ON C13 LABELED AND NON-LABELED LNH (BRANCHED TYPE I HUMAN MILK OLIGOSACCHARIDE STRUCTURE)

Elif Uslu[a], Patrycja Lenartowicz[a], Julia Vreugdenhil[a], Anthony Prudden[c], Bernd Stahl[d], Javier Sastre Toraño[a,b], Geert-Jan Boons[a,b,c]

[a] Department of Chemical Biology and Drug Discovery, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG, Utrecht, The Netherlands
e.uslu@uu.nl
[b] Bijvoet Center for Biomolecular Research, Utrecht University, 3584 CG, Utrecht, The Netherlands
[c] Complex Carbohydrate Research Center and Department of Chemistry, University of Georgia, 315 Riverbend Road, Athens, GA, 30602, USA
[d] Danone Research & Innovation, Uppsalalaan 12, Utrecht, The Netherlands

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 rearrangement 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 rearrangement products can be confidently identified as false positives via unique CCS values of the rearrangement 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*

Julia Van Bockstaele Fuentes[a], Josselin Lupette[a], Carole Plasson[a], Elodie Rivet[a], Patrice Lerouge[a], Narimane Mati-Baouche[a], Muriel Bardor[a,b]

[a] Université de Rouen Normandie (UNIROUEN), Normandie Univ, GlycoMEV UR 4358, SFR Normandie Végétal FED 4277, Innovation Chimie Carnot, IRIB, GDR CNRS Chemobiologie F-76000 Rouen, France
julia.van-bockstaele@univ-rouen.fr
[b] Alga Biologics, 25 rue Tesnières, 76821 Mont-Saint-Aignan Cedex, France

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.

References:
1. G. Walsh, E. Walsh.*Nat Biotechnol* **2022,** *40*, 1722–1760.
2. E. Mathieu-Rivet, M-C. Kiefer-Meyer, G.Vanier *et al. Frontiers in Plant Science* **2014** Vol 5.
3. R. Dumontier, C. Loutelier-Bourhis, M.L. Walet-Balieu *et al. Carbohydr. Polym*. **2021**, 259.
4. M. Bardor, C. Faveeuw, A-C Fitchette *et al. Glycobiology* **2003** 13, 427-434.
5. R. Strasser, J. Mucha, L. Mach *et al. FEBS Letters* **2000**, 471, 105-108.
6. R. Strasser, F. Altman, L. Mach *et al. FEBS Letters* **2004**, 561, 132-136.
7. K. Göritzer, M. Grandits, C. Grünwald-Gruber *et al*. *Front. Plant Sci.* **2022,** 13.

STRUCTURE DETERMINATION OF THE PHOSPHATE-CONTAINING POLYSACCHARIDE FROM *LACTOCOCCUS LACTIS* SUBSP. *LACTIS* NCIMB 700966

Marie-Rose Van Calsteren, Fleur Gagnon, Nancy Guertin

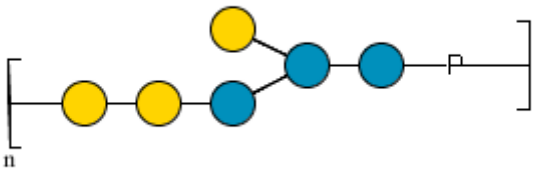
Saint-Hyacinthe Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Hyacinthe (Quebec), Canada
marie-rose.vancalsteren@agr.gc.ca

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:



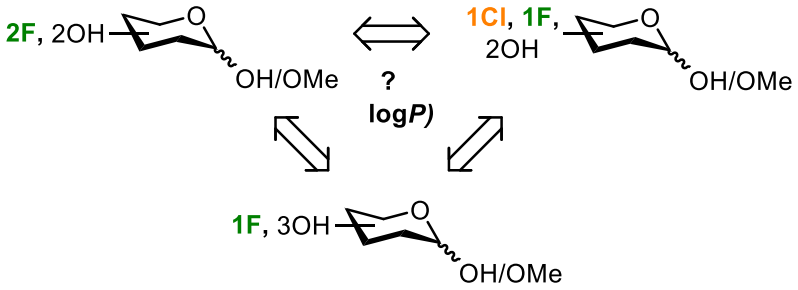
This structure is unique and was compared to that of other phosphate-containing polysaccharides from *Lactococcus* strains.

IN PURSUIT IF LARGER LIPOPHILICITY ENHANCEMENT: AN INVESTIGATION OF SUGAR DEOXYCHLORINATION

Jonas Van De Velde[a], Ariadna Calderón Rodríguez[a], Zhong Wang[b], David E. Wheatley[b], Bruno Linclau[a,b]

[a] Department of Organic and Macromolecular chemistry, Ghent University, Krijgslaan 281, S4, Ghent 9000, Belgium
jonasvdv.vandevelde@ugent.be
[b] School of Chemistry, University of Southampton, Southampton SO17 1BJ, UK

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.



References:
1. A. Varki, *Glycobiology,* **2017**, *27*, 3.
2. C. R. Bertozzi, Kiessling, L. Laura, *Science,* **2001**, *291*, 2357.
3. X. Cao, X. Du, H. Jiao, Q. An, R. Chen, P. Fang, J. Wang, B. Yu, *Acta Pharm. Sin. B,* **2022**, *12*, 3783.
4. B. Linclau, Z. Wang, G. Compain, V. Paumelle, C. Q. Fontenelle, N. Wells, A. Weymouth-Wilson, *Angew. Chem. Int. Ed.,* **2016**, *55*, 674.
5. R. Hevey, *Pharmaceuticals,* **2019**, *12*, 55.
6. R. Hevey, *Biomimetics (Basel),* **2019**, *4*, 53.
7. A. Tamburrini, C. Colombo, A. Bernardi, *Med. Res. Rev.,* **2020**, *40*, 495.

SYNTHESIS AND APPLICATION OF AI-2 DERIVED SUGAR PRODRUGS

Peter Kis[a,c], Miguel V. Rodrigues[a], Karina B. Xavier[b], M. Rita Ventura[a]

[a] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal
rventura@itqb.unl.pt
[b] Gulbenkian Institute for Molecular Medicine, 2781-901 Oeiras, Portugal
[c] Institute of Chemistry, Slovak Academy of Sciences, SK-845 38 Bratislava, Slovakia

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.[1,2,3] 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.

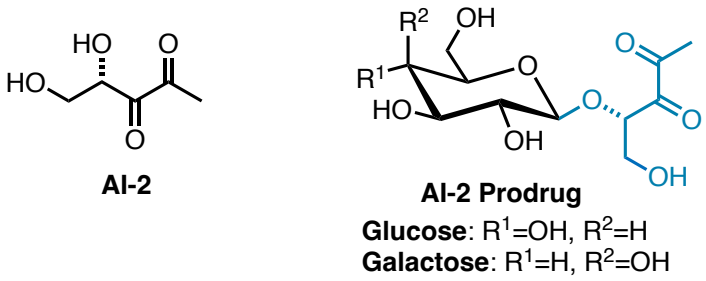


Figure 1.

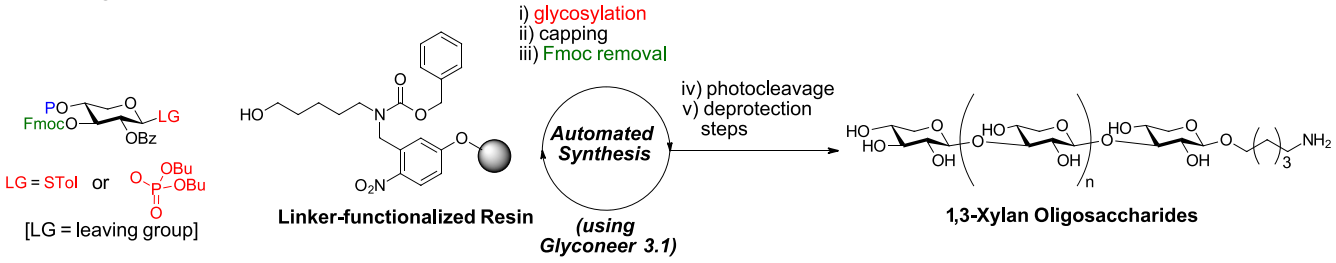
References:
1. Rodrigues, M. V.; Kis, P.; Xavier, K. B.; Ventura, M. R. *Is. J. Chem.* **2023**, *63*, e202200091.
2. Ascenso, O. S.; Marques, J. C.; Santos, A. R.; Xavier, K. B.; Ventura, M. R.; Maycock, C. D. *Bioorg. Med. Chem.* **2011**, *19*, 1236-1241.
3. Pereira, C.S.; Thompson, J.A.; Xavier, K.B. *FEMS Microbiology Reviews* **2013**, *37*, 156-181.
4. Chourasia, M. K.; Jain, S. K. *J. Pharm. Pharmaceut. Sci.*, **2003**, *6*, 33-66.

AUTOMATED GLYCAN ASSEMBLY OF β-1,3-XYLAN OLIGOSACCHARIDES

Nitish Verma, Uwe Osswald, Fabian Pfrengle

Department of Natural Sciences and Sustainable Resources, Institute of Organic Chemistry, BOKU University, Muthgasse 18, 1190 Vienna, Austria
nitish.verma@boku.ac.at

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).

References:

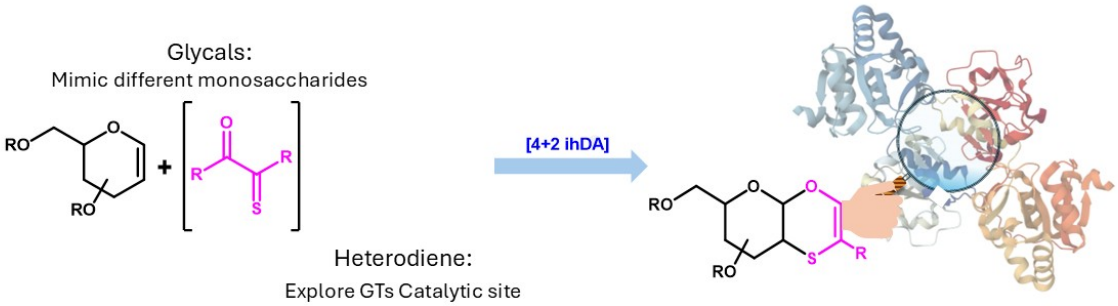
1. H-N Sun, C-M Yu, H-H Fu, P Wang, Z-G Fang, Y-Z Zhang, X-L Chen, F Zhao, *Front. Microbiol.* **2021**, 12:721422.
2. E. D. Goddard-Borger, K. Sakaguchi, S. Reitinger, N. Watanabe, M. Ito, S. G. Withers, *J. Am. Chem. Soc.* **2012**, *134*, 3895−3902.
3. D. Senf, C. Ruprecht, G. H. M. de Kruijff, S. O. Simonetti, F. Schuhmacher, P. H. Seeberger, F. Pfrengle, *Chem. Eur. J.* **2017**, *23*, 3197–3205.

UNVEILING INSIGHTS ON CANCER-LINKED GLYCOSYLTRANSFERASES USING GLYCOMIMETICS

Pedro M. Ascenso Vieira, Giacomo Biagiotti, Marco Marradi, Barbara Richichi

Department of Chemistry "Ugo Schiff", University of Firenze,Via della Lastruccia 3-13, 50019-Firenze, Italy
pedromiguel.ascensovieira@unifi.it

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)

References:
1. A. Costa, D. Campos, C. Reis, C. Gomes, *Trends Cancer*. **2020**, 6, 757-766
2. J. Magnani, B. Ernst*, Discov Med.* **2009**, 8, 247-252
3. K. Martin, J. Tricomi, […], B. Richichi, R. Sackstein, *Chem. Commun*. **2021**, 57, 1145-1148
4. B. Richichi, A. Imberty, […], C. Nativi, *Org Biomol. Chem*. **2013,** 11, 4086-4094
5. G. Biagiotti, L. Legnani, […], B. Richichi, *Eur. J. Org*. **2022**, 2022, 24-32

MULTIVALENT GLYCOCONJUGATES: GLYCOCLUSTERS AND GLYCOPOLYMERS FOR TARGETING GALECTIN-4

Andrea Vopálenská[a,b], David Vrbata[a], Michaela Hovorková[a], Vladimír Křen[a], Pavla Bojarová[a]

[a] Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, CZ-14200, Czech Republic
[b] Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 2030/8, CZ-128 43, Prague, Czech Republic
andrea.vopalenska@biomed.cas.cz

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.

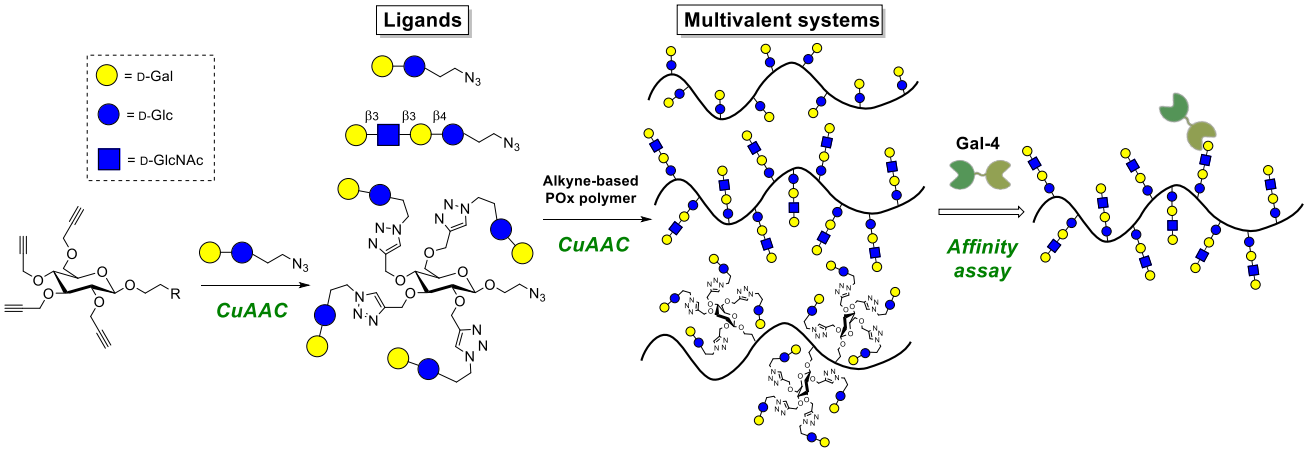


Figure 1. Synthetic pathway to multivalent glycoclusters and glycopolymers

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).

References:

1. M. Martínez-Bailén, J. Rojo, J. Ramos-Soriano, *Chem. Soc. Rev.* **2023**, *52*, 536-572.
2. K. Slámová, J. Červený, Z. Mészáros, T. Friede, D. Vrbata, V. Křen, P. Bojarová, *Molecules* **2023**, *28*, 4039.

GLYCOSYLATION OF 2-(2-PROPYLSULFINYL)BENZYL 1,2-ORTHOESTER GLYCOSIDES INITIATED BY SULFOXIDE ACTIVATION

Qian Wan

School of Pharmacy Huazhong University of Science and Technology, Wuhan 430030, China
wanqian@hust.edu.cn

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.

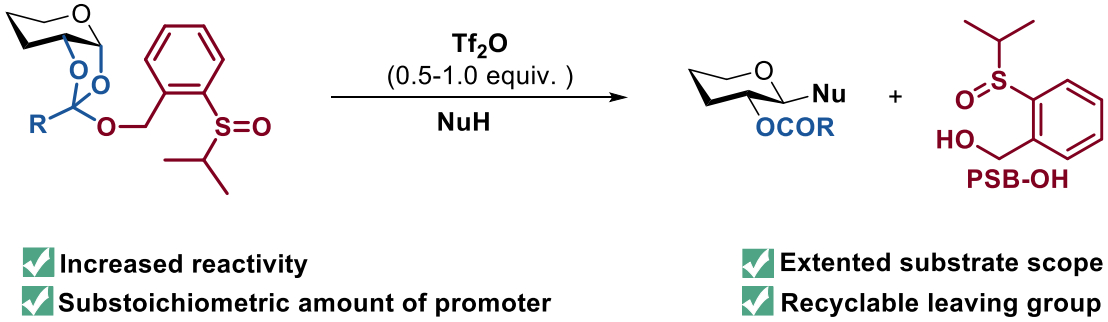


Figure 1. Glycosylation with OPSB glycosides and 1,2-orthoester glyco-sides.

Acknowledgements: We are thankful for the financial support from STI2030-Major Projects (2022ZD0211800) and The National Natural Science Foundation of China (22025102, 22077039, 22177034)

References:

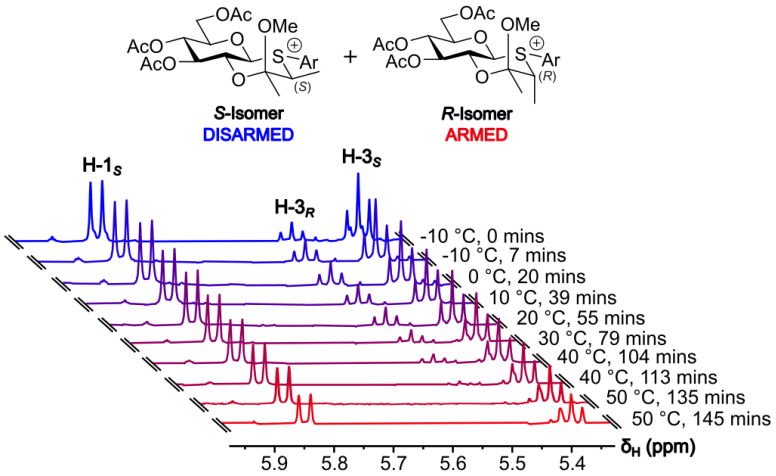
1. Shu, P.; Xiao, X.; Zhao, Y.; Tao, J.; Wang, H.; Lu, Z.; Yao, G.; Zeng, J.; Wan, Q. *Angew. Chem. Int. Ed.* **2015**, *54*, 14432.
2. Xiao, X.; Zhao, Y.; Shu, P.; Zhao, X.; Liu, Y.; Sun, J.; Zhang, Q.; Zeng, J.; Wan, Q. *J. Am. Chem. Soc.* **2016**, *138*, 13402.
3. Meng, L.; Wu, P.; Fang, J.; Xiao, Y.; Xiao, X.; Tu, G.; Ma, X.; Teng, S.; Zeng, J.; Wan, Q. *J. Am. Chem. Soc.* **2019**, *141*, 11775.
4. Fang, J.; Zeng, J.; Sun, J.; Zhang, S.; Xiao, X.; Lu, Z.; Meng, L.; Wan, Q. *Org. Lett.*, **2019**, *21*, 6213.
5. Xiao, X.; Zeng, J.; Fang, J.; Meng, L.; Wan, Q. *J. Am. Chem. Soc.* **2020**, 142, 5498.
6. Fang, J.; Li, T.; Ma, X.; Sun, J.; Cai, L.; Chen, Q.; Liao, Z; Meng, L.; Zeng, J.; Wan, Q. *Chin. Chem. Lett.*, **2022**, *33*, 282.
7. Cail, L.; Chen, Q.; Guo, J.; Liang, Z.; Fu, D.; Meng, L.; Zeng, J.; Wan, Q. *Chem. Sci.*, **2022**, *13*, 8759.
8. Chen, W.; Wu, P.; Fang, J.; Cai, L.; Xiao, X.; Wang, H.; Teng, S.; Zeng, J. Wan, Q. *Chin. J. Chem*. **2023**, *41*, 383.
9. Wu, P.; Zeng, J.; Meng, L.; Wan, Q. *Chem. Commun*. **2024**, *60*, 8670.
10. Wu, P.; Xiao, X.; Zhou, S.;Meng, L.; Zeng, J. Wan, Q. *Org. Lett.*, **2024**, *26,* 6053.

DISCERNING STRUCTURAL INFLUENCES ON STEREOSELECTIVITY AND KINETICS IN OXATHIANE GLYCOSYL DONORS

Matthew E. Warnes, Martin A. Fascione

Department of Chemistry, University of York, York, UK
mew547@york.ac.uk

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.



References:
1. M. A. Fascione, S. J. Adshead, S. A. Stalford, C. A. Kilner, A. G. Leach and W. B. Turnbull, *Chem. Commun.*, 2009
2. T. J. Boltje, T. Buskas and G.-J. Boons, *Nat. Chem.*, 2009, **1**, 611-622.
3. T. Fang, K.-F. Mo and G.-J. Boons, *J. Am. Chem. Soc.*, 2012, **134**, 7545-7552.
4. J. H. Kim, H. Yang, J. Park and G. J. Boons, *J. Am. Chem. Soc.*, 2005, **127**, 12090-12097.
5. M. A. Fascione, C. A. Kilner, A. G. Leach and W. B. Turnbull, *Chem. Eur. J.*, 2012, **18**, 321-333.

UNDERSTANDING THE DIVERSE MOLECULAR MECHANISMS DRIVING MPS-IIIA DEVELOPMENT AND DISEASE PROGRESSION AS A STEP TOWARDS PERSONALISED MEDICINE

Moli Williams, Dominic Byrne, Ed Yates, Urszula McClurg, Alan Cartmell

University of Liverpool, Institute of Systems, Molecular and Integrative Biology, Biosciences Building, Crown St, Liverpool L69 7ZB, UK
hlmwil10@liverpool.ac.uk

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.

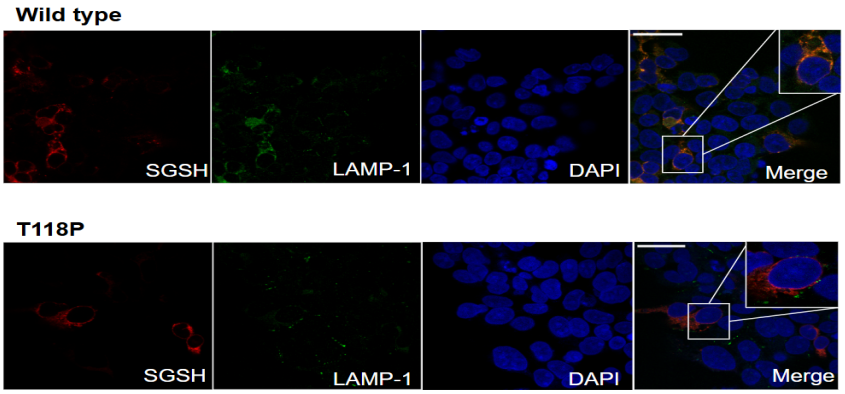


Figure 1. Fixed HEK293 SGSH-null cell line transfected with WT and T118 SGSH stained with Anti-SGSH (red), Anti-LAMP-1 (green) and DAPI. Scale bar 100 µM.

References:
1. *Mucopolysaccharidosis type III: Sanfilippo Syndrome: Nord* (2024) *National Organization for Rare Disorders*. Available at: https://rarediseases.org/rare-diseases/mucopolysaccharidosis-type-iii/ (A: 06 February 2025).
2. Coulson- Thomas, V.J. (2016) International Journal of Experimental Pathology, 97(3), 213–229.
3. Miguez, P.A. et al. (2024) *Periodontology* 2000, 94, 510–531.
4. Condomitti, G. and de Wit, J. (2018) *Frontiers in Molecular Neuroscience*, 11, 14.
5. Sidhu, N.S. *et al.* (2014) *Acta Crystallographica Section D Biological Crystallography*, 70(5), pp. 1321–1335.
6. Valstar, M.J. *et al.* (2010) *Annals of Neurology*, 68(6), 876–887.

TARGETING β-CYLCODEXTRIN TO THE LYSOSOMES

Flinta E.D. Wilson[a], Ayelen Tayagui[a], Matthieu Sollogoub[b], Antony J. Fairbanks[a]

[a] School of Physical and Chemical Sciences, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
fli83@uclive.ac.nz
[b] Sorbonne Université, CNRS Institut Parisien de Chimie Moléculaire (IPCM), UMR 8232, 4 place Jussieu, 75005, Paris, France

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.

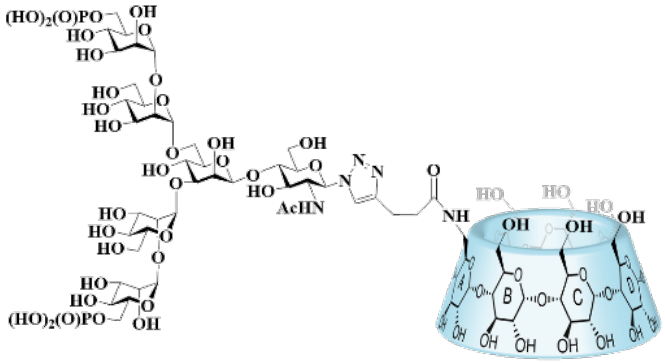


Figure 1. [2]

References:
1. M. M. Nociari, G. L. Lehmann, A. E. Perez Bay, R. A. Radu, Z. Jiang, S. Goicochea, R. Schreiner, J. D. Warren, J. Shan, S. Adam De Beaumais, M. Ménand, M. Sollogoub, F. R. Maxfield, E. Rodriguez-Boulan, *Proc. Natl. Acad. Sci.* **2014**, *111*, E1402–E1408.
2. A. Mascherpa, N. Ishii, A. Tayagui, J. Liu, M. Sollogoub, A. J. Fairbanks, *Chem. Eur. J.* **2023**, *29*, e202203252.

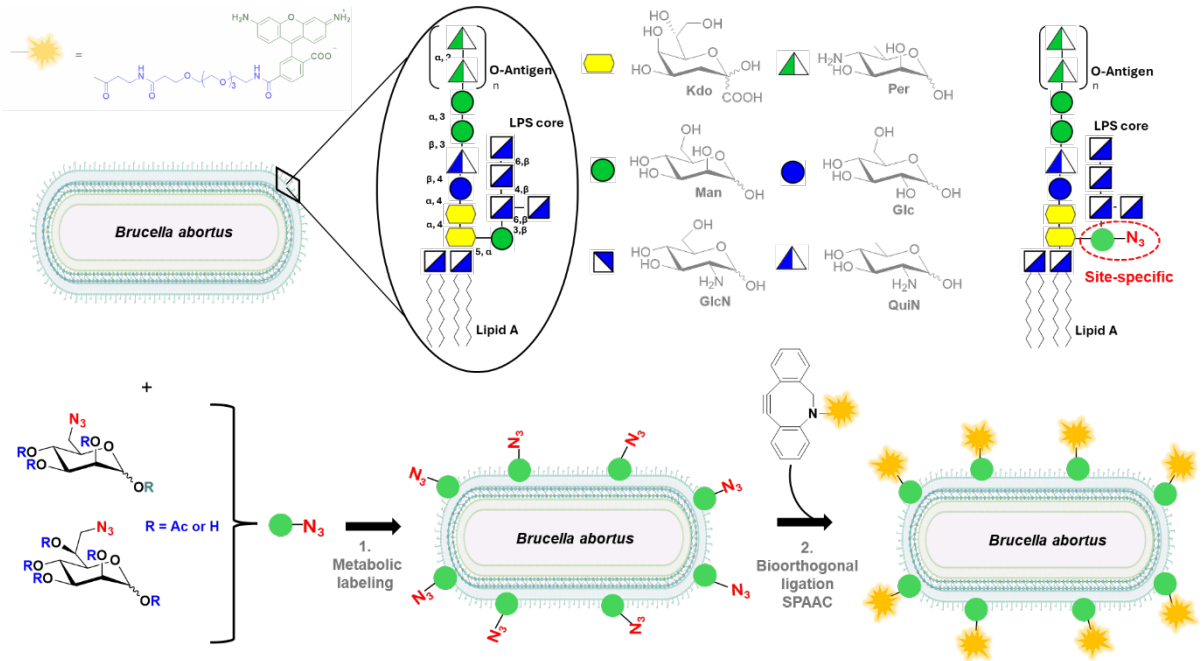
SITE-SPECIFIC INCORPORATION OF CLICKABLE D-MANNOSE DERIVATIVES IN THE LIPOPOLYSACCHARIDE CORE OF THE PATHOGEN *BRUCELLA ABORTUS*

Marine Lacritick, Angéline Reboul, Rym Yahia Boudhar, Xavier De Bolle, Stéphane P. Vincent

NARILIS (Namur Institute for Life Sciences), University of Namur, Namur, Belgium
rym.yahiaboudhar@unamur.be

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.



References:
1. Saxon, E., Bertozzi, C.R. *Science*. **2000**, 287, 2007-2010.
2. Vassen, V., Valotteau, C., Feuillie, C., Formosa-Dague, C., Dufrêne, Y.F., De Bolle, X. *EMBO J.* **2019**. 38, 1-15.

DECIPHERING THE FUCOSE MIGRATION PRODUCTS IN BLOOD GROUP EPITOPES

Murat Yaman[a,b], Kim Greis[c], Vasilis Kontodimas[a], Kevin Pagel[d,e], Mateusz Marianski[a,b]

[a] Department of Chemistry, Hunter College, The City University of New York, New York, NY 10065, USA
[b] Ph.D. Programs in Chemistry and Biochemistry, The Graduate Center, The City University of New York, New York, NY 10016, USA
myaman@gradcenter.cuny.edu
[c] Laboratory of Organic Chemistry, Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland
[d] Fritz-Haber-Institut der Max Planck Gesellschaft, 14195 Berlin, Germany
[e] Institute of Chemistry and Biochemistry, Freie Universita¨t Berlin, 14195 Berlin, Germany

Fucosylated glycans are involved in immune cell homeostasis[1], 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.

References:

1. Zhang NZ, Zhao LF, Zhang Q, Fang H, Song WL, Li WZ, Ge YS, Gao P. World J Gastrointest Oncol. 2023 Jul 15;15(7):1119-1134. doi: 10.4251/wjgo.v15.i7.1119. PMID: 37546555; PMCID: PMC10401475.
2. John B Lowe, Current Opinion in Cell Biology,Volume 15, Issue 5,2003,Pages 531-538, ISSN 0955-0674
3. Paulson JC, Blixt O, Collins BE. Nat Chem Biol. 2006;2:238–248. doi: 10.1038/nchembio785.
4. Wuhrer M, Deelder AM, van der Burgt YE. Mass Spectrom Rev. 2011 Jul-Aug;30(4):664-80. doi: 10.1002/mas.20337. Epub 2011 May 10. PMID: 21560141.
5. M. Lettow, K. Greis, E. Mucha, T. R. Lambeth, M. Yaman, V. Kontodimas, C. Manz, W. Hoffmann, G. Meijer, R. R. Julian, G. von Helden, M. Marianski, K. Pagel, *Angew. Chem. Int. Ed.* 2023, 62, e202302883.
6. Kontodimas, V.; Yaman, M.; Greis, K.; Lettow, M.; Pagel, K.; Marianski, M. *Phys. Chem. Chem. Phys.* **2024**, *26*, 14160–14170.

COMPARISON OF STREPTOCCOCUS PNEUMONIAE SEROGROUP 23 CAPSULAR POLYSACCHARIDES: A MOLECULAR MODELLING STUDY

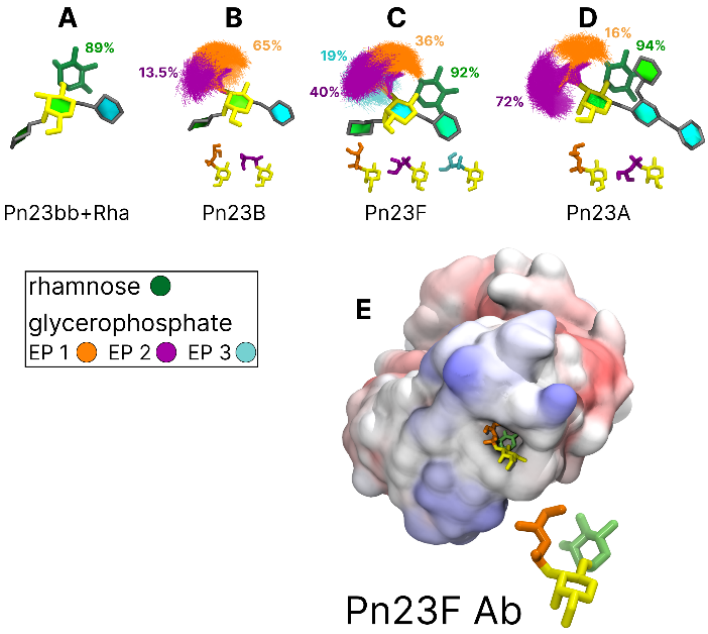
Nicholas Yerolemou[a], Michelle Kuttel[a], Neil Ravenscroft[b], Nicole Richardson[a]

[a] Department of Computer Science, University of Cape Town, Cape Town, South Africa, YRLNIC001@myuct.ac.za

[b] Department of Chemistry, University of Cape Town, Cape Town, South Africa

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

Dandan Yu[a], Antonio Molinaro[a], Anna Notaro[a], Zlata Vershinin[b], Marianna Zaretsky[b], Cristina De Castro[a], Jerry Eichler[b]

[a] Department of Chemical Sciences, University of Naples Federico II, Via Cinthia 4, 80126, Napoli, Italy
dandan.yu@unina.it
[b] Department of Life Sciences, Ben-Gurion University of the Negev, Beersheva, Israel

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.

References:

1. Taguchi, D. et al. (2016), *J. Biol. Chem.* 291 , 11042-11054.
2. Eichler, J. and Guan, Z. (2017) *Biochim. Biophys. Acta* 1862, 589-599.
3. Notaro, A. et al. (2022) *Carbohydr. Res.* 521, 108651.
4. Vershinin, Z. et al. (2024) *BBA Adv.* 6, 100120.

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

Alshimaa I. Zaki[a], István Kacsir[a,b], Adrienn Sipos[c], Péter Bay[c], Attila Bényei[d], Éva Bokor[a], László Somsák[a]

[a] Department of Organic Chemistry, University of Debrecen, P.O. Box 400, H-4002 Debrecen, Hungary
alshimaa.ibrahim@science.unideb.hu
[b] Department of Inorganic & Analytical Chemistry, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary
[c] Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary
[d] Department of Physical Chemistry, University of Debrecen, Debrecen, Egyetem tér 1., H-4032 Debrecen, Hungary

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.

Acknowledgements: Stipendium Hungaricum Scholarship and National Research, Development, and Innovation Office of Hungary, K146147.

References:

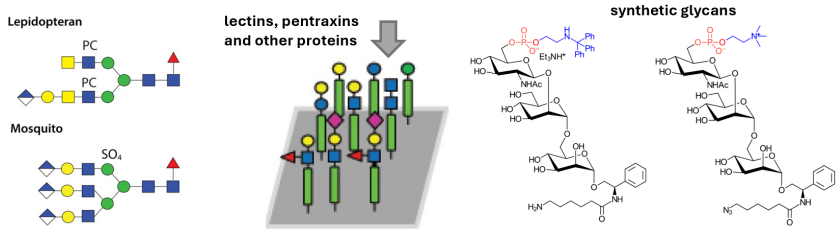
1. R. G. Kenny, C. J. Marmion, *Chem. Rev.* **2019**, 119, 1058-1137.
2. P. Štarha, Z. Trávníček, *Chem. Rev.* **2019**, 395, 130-145.
3. I. Kacsir et al., *Int. J. Mol. Sci.* **2022**, 23, 813.
4. I. Kacsir et al., *Front. Chem.* **2023**, 11, 1086267.

ZWITTERIONIC MODIFICATIONS OF SYNTHETIC GLYCAN EPITOPS DETERMINE PROTEIN BINDING IN A DIPTERAN GLYCAN ARRAY

Alessandro Monti[a], Madhuri Vangala[a], Prasad Kameshwar[a], Lena Nuschy[b], Iain Wilson[b], Alla Zamyatina[a]

[a] Institute of Organic Chemistry, Department of Natural Sciences and Sustainable Resources, BOKU University, Muthgasse 18, A-1190-Vienna
alla.zamyatina@boku.ac.at
[b] Institute of Biochemistry, Department of Natural Sciences and Sustainable Resources, BOKU University, Muthgasse 18, A-1190-Vienna

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.

Acknowledgements: financial support by FWF grant and WWTF grant LS21-017 is gratefully acknowledged

References:

1. Paschinger, K., Wilson, I. B. H. *Parasitology* **2019**, 10.1017/s0031182019000398, 1-10.
2. Smit, C. H., van Diepen, A., Nguyen, D. L., Wuhrer, M., Hoffmann, K. F., Deelder, A. M., Hokke, C. H. Glycomic *Mol Cell Proteomics* **2015**, *14*, 1750-1769.
3. Guzman, M. G., Harris, E. *Lancet* **2015**, *385*, 453–465.
4. Hykollari, A., Malzl, D., Eckmair, B., Vanbeselaere, J., Scheidl, P., Jin, C., Karlsson, N. G., Wilson, B. H., Paschinger, K. *Mol Cell Proteomics* **2018,** *17*, 2177-2196. 6736(14)60572-9
5. Paschinger, K., and Wilson, I. *Glycoconj J.* **2019**, 10.1007/s10719-019-09874-2

EXPLOITING BACTERIOPHAGE ENDOGLYCOSIDASE AGAINST THE CAPSULAR POLYSACCHARIDE OF *K. PNEUMONIAE* AS A TOOL FOR NOVEL ANTIMICROBIAL STRATEGIES

Michela Zaro[a], Dhanasri Jayamoorthi[a], Gaia Scaramella[a], Cristina Lagatolla[a], Sabrina Pacor[a], Barbara Bellich[b], Demi Vattovaz[c], Rita De Zorzi[c], Lucia Henrici De Angelis[d], Marco Maria D'Andrea[d], Paola Cescutti[a]

[a] Department of Life Science University of Trieste, Italy
michela.zaro@phd.units.it
[b] Department of Advanced Translational Diagnostics, IRCCS Burlo Garofolo, Trieste, Italy
[c] Department of Chemical and Pharmaceutical Sciences, University of Trieste, Italy
[d] Department of Biology, University of Rome Tor Vergata, Italy

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.

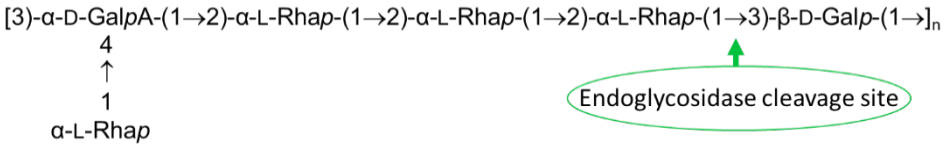


Figure 1. KpB-1 CPS repeating unit and EG cleavage site

Acknowledgements: PRIN Project Prot. 20224T3X8K 'Lipophagoids as innovative therapeutic tools of precision medicine against multi-drug resistant bacteria' - CUP J53D23001010006 realised with the contribution from Mission 4, Component 2, Investment 1.1 'Research Projects of Significant National Interest (PRIN) of the National Recovery and Resilience Plan (PNRR) funded by the European Union "Next Generation EU".

References:
1. B. Bellich, C. Lagatolla, R. Rizzo, M. M. D'Andrea, G. M. Rossolini, P. Cescutti, *Int. J. Biol. Macromol.* **2020**, *155*, 315-323.
2. M. M. D'Andrea, P. Marmo, L. Henrici De Angelis, M. Palmieri, N. Ciacci, G. Di Lallo, E. Demattè, E. Vannuccini, P. Lupetti, G. M. Rossolini, M.C. Thaller, *Sci. Rep.* **2017**, *7*:2614, 1-8.

A SIALOMIMETIC GLYCAN ARRAY FOR THE IDENTIFICATION OF VIRAL ENTRY INHIBITORS

Qiuyu Zhu[a,b,c], Thorsten Kinsinger[a,b,c], Omar Zareei[a,b,c], Christian Sieben[d], Alexander Titz[a,b,c]

[a] Helmholtz-Institute for Pharmaceutical Research Saarland, Helmholtz Centre for Infection Research, Saarbrücken, Germany
qiuyu.zhu@helmholtz-hips.de
[b] Department of Chemistry, Saarland University, Saarbrücken, Germany
[c] Deutsches Zentrum für Infektionsforschung (DZIF), Standort Hannover-Braunschweig, Germany
[d] Helmholtz Centre for Infection Research, Braunschweig, Germany

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.

Acknowledgements: Financial support from VolkswagenStiftung and DZIF for Scienion sciFLEXARRAYER S3 is gratefully acknowledged.

References:
1. De Clercq E, Li G. Clin. *Microbiol. Rev.* **2016**, *29*, 695-747.
2. Varki A. Trends. *Mol. Med.* **2008**, *14*, 351-60.
3. Heida R, Bhide YC, Gasbarri M, Kocabiyik Ö, Stellacci F, Huckriede AL, Hinrichs WL, Frijlink HW. *Drug Discov. Today.* **2021**, *26*, 122-37.
4. Leusmann S, Ménová P, Shanin E, Titz A, Rademacher C. *Chem. Soc. Rev.* **2023**, *52*, 3663-3740.