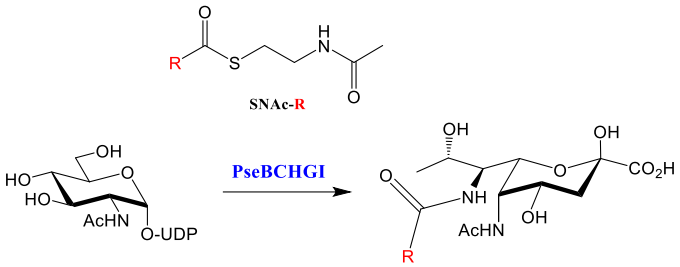
CO-FACTOR PROSTHESIS FACILITATES THE BIOSYNTHESIS OF PSEUDAMINIC ACID DERIVATIVES

Tessa Keenan[a], Harriet Chidwick[a], Matthew Best[a], Emily K. P. Flack[ab], Nicholas D. J. Yates[a], Natasha E. Hatton[a], Matthew Warnes[a], Martin Fascione[a]

[a] Department of Chemistry, University of York, York, UK
tessa.keenan@york.ac.uk
[b] Department of Biology, University of York, York, UK



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

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

References:

1. H. S. Chidwick, E. K. Flack, T. Keenan, J. Walton, G. H. Thomas and M. A. Fascione, *Scientific Reports*, 2021, **11**, 4756.
2. E. K. Flack, H. S. Chidwick, G. Guchhait, T. Keenan, D. Budhadev, K. Huang, P. Both, J. Mas Pons, H. Ledru and S. Rui, *ACS Catalysis*, 2020, **10**, 9986-9993.
3. T. Keenan, H. S. Chidwick, M. Best, E. K. Flack, N. D. Yates, N. E. Hatton, M. E. Warnes and M. A. Fascione, *Chemical communications*, 2024, **60**, 1428-1431.