Poster session 2

Tuesday 11th

15h30 - 17h30

Odd numbers

Salle 151: P1 to P111

Salle 251: P113 to P221

Role of EPS in mitigation of plant abiotic stress: The case of *Methylobacterium extorquens* PA1

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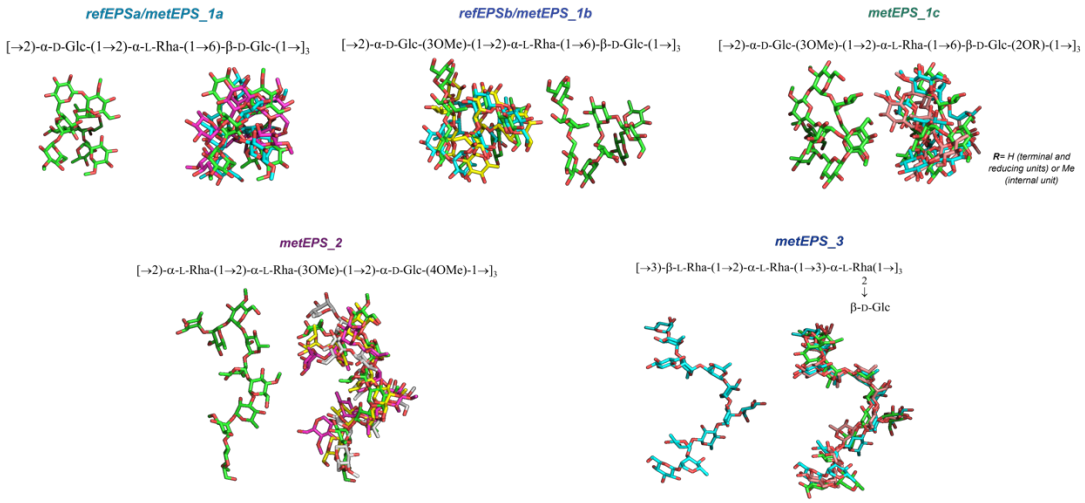
Methylobacterium extorquens is a facultative methylotrophic Gram-negative bacterium, often associated with plants [1], that exhibits a unique ability to grow in the presence of high methanol concentrations, which serves as a single carbon energy source [2].

We found that *M. extorquens strain PA1* secretes a mixture of different exopolysaccharides (EPSs) when grown in reference medium or in presence of methanol, that induces the secretion of a peculiar and heterogenous mixture of EPSs, with different structure, composition, repeating units, bulk and a variable degree of methylation [3]. These factors influenced 3D structure and supramolecular assets, diffusion properties and hydrodynamic radius, and likely contribute to increase methanol tolerance and cell stability.

No direct methanol involvement in the EPSs solvation shell was detected, indicating that the polymer exposure to methanol is water mediated. The presence of methanol induces no changes in size and shape of the polymer chains, highlighting how water-methanol mixtures are a good solvent for *refEPS* and *metEPS*.



M. extorquens PA1 RefEPS and MetEPS conformational behavior.

Bibliographic references:

[1] A. S. Ferreira Filho et al., (2012). World journal of microbiology & biotechnology, (28,4), 1475–1481.
[2] A. Sy et al. (2005). Applied and Environmental Microbiology, (71,11) 7245–7252.
[3] A. Vanacore, M. C. Forgione, D. Cavasso, H.N.A. Nguyen, A. Molinaro, J.P. Saenz, G. D'Errico, L. Paduano, R. Marchetti, A. Silipo, (2022). Carbohydrate polymers, (295), 119863

Palladium catalyzed Sonogashira and Heck coupling reactions of 2-iodo-1-C-substituted glycals

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Carbohydrates play an important role in the biological processes of living organisms [1], however the *O*-glycosidic bond of natural glycosides shows low stability against chemical and enzymatic hydrolysis. Glycomimetics can mimic the structure and biological function of natural carbohydrate derivatives and at the same time the stability of the molecule could be much higher [2].

C-2 branched carbohydrate derivatives and 1,2-annulated sugars can be potential antibiotics, or glycomimetics of 2-*N*-acetyl sugars and inhibitors of lipid biosynthesis [3].

1-C-Substituted glycals are unsaturated carbohydrate derivatives bearing endocyclic double bond and carbon substituents on the anomeric carbon. We have elaborated synthetic methods for the synthesis of 1-C-acceptor-substituted (CN, $CONH_2$, $COOCH_3$) glycals starting from anhydro-aldonic acid and (ulosylbromide)onic acid derivatives [3-5].

In this presentation we will represent the synthesis of 2-iodoglycals starting from glycals using *N*-iodoimides. During our research we optimized the Sonogashira cross coupling reaction between 2-iodoglycals and phenylacetylene or TMS-acetylene in details. From the TMS protected acetylene derivatives we synthetized 2-ethynylglycals, which were reacted with glycosyl azides under CuAAc conditions.

The 2-iodo 1-C-acceptor glycals could be the substrates for the Heck coupling reactions. The palladium catalyzed Heck coupling of 2-iodoglycal derivatives were studied in detail, we optimized the reaction conditions and examined the substrate scope of the coupling.

Acknowledgements

Research was supported by the Hungarian National Research, Development, and Innovation Office (FK 128766) and the Gedeon Richter Talentum Foundation.

Bibliographic references:

1. H. J. Gabius, Ed. The Sugar Code - Fundamentals of Glycosciences, Wiley-VCH: Weinheim, 2009.
2. C-H. Wong (1999), Acc. Chem. Res. (32) 376-385.
3. J. Yin, T. Linker (2012), Org. Biomol. Chem. (10) 2351-2362.
4. S. H. Mahmoud, L. Somsák, I. Farkas (1994), Carbohydr. Res. (254) 91-104.
5. L. Somsák (1989), Carbohydr. Res. (195) C1-C2.
6. L. Kiss, L. Somsák (1996), Carbohydr. Res. (291) 43-52.

Characterization of the host glycan specificity and recognition by Neisseria meningitadis T4P

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Neisseria meningitidis is a Gram-negative bacterium responsible for causing life-threatening meningococcal infections, which are traditionally treated with antibiotics. However, recent reports indicate the emergence of antibiotic-resistant strains, calling for new approaches to combat the infection. The bacterium\'s ability to adhere to host cells through long, thin protein assemblies called type IV pili (T4P) is a key factor to its virulence.

In this study, we aim to provide a molecular framework for PilE adhesion mechanism through the characterization of its glycan specificity and recognition using docking and molecular dynamics. We will also reconstruct a fully atomistic model of T4P to provide a 3D structural understanding of pili stability and dynamics within a substrate recognition context.

Our results will provide a rationale for glycan specificity and highlight essential information to screen and validate the binding poses identified through docking/MD. This work is expected to provide new insight into the mechanism of meningococcal infection and have a major impact on our understanding of Neisseria host-pathogen interactions.

Rendering of multi subunit T4P (pdb: 5KUA), visualised using Pymol

Bibliographic references:

Mubaiwa, T. D.; Semchenko, E. A.; Hartley-Tassell, L. E.; Day, C. J.; Jennings, M. P.; Seib, K. L., The sweet side of the pathogenic Neisseria: the role of glycan interactions in colonisation and disease. Pathog Dis 2017, 75 (5).

Craig, L.; Forest, K. T.; Maier, B., Type IV pili: dynamics, biophysics and functional consequences. Nat Rev Microbiol 2019, 17 (7), 429-440.

Unveiling the interaction siglecs-sialylated glycans to understand diseases of the immune system

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Carbohydrates are key mediators in fundamental biological events, taking an active part in cell-cell and cell-matrix interactions. Sialic acids are monosaccharides expressed on the terminal residues of glycolipids and glycoproteins that are extremely heterogeneous, counting more than 60 structural modifications [1]. Sialic acid can be overexpressed Cancer cells and on the cell envelope of feared microbes and pathogens. Sialylated glycans are recognized by several proteins, like Siglecs (Sialic acids immunoglobulin-like lectins).

By means of these interactions, they can evade the immune system and promote infection and cancer [2-3]. Siglecs recognise highly abundant sialylated glycans on the surface of tumor cells, inhibiting immunosurveillance and mediating immune evasion of tumor cells. Siglecs are also targets of human pathogens, such as viruses and bacteria, which are used to evade host immunity, promoting bacterial colonization [4-6]. For this reason, it is interesting to investigate the molecular basis involved in this process, to understand the progression of specific types of tumors.

To better understand the etiology of immune system diseases, it is certainly important to clarify how Siglecs and sialylated glycans interact, so that diagnostic/therapeutic tools for the treatment of these diseases can also be developed.

Bibliographic references:

[1]. M. Anderluh, S. van Vliet, (2021), FEBS J. 288: 4746-4772
[2]. Y. Chang, V. Nizet, (2014), Glycobiology. Volume 24, Issue 9, 818–825
[3]. A. Bärenwaldt, H. Läubli (2019), Expert Opinion on Therapeutic Targets, 23:10, 839-853.
[4]. S. Duan, JC. Paulson,(2020), Annu Rev Immunol. 26;38:365-395.
[5]. T. Angat, A. Varki. (2022), Molecular Aspects of Medicine. Volume 90,2023-101117.
[6]. J. Munkley, E. Scott, (2019), Medicines. 6-102.

Targeting the lectin LecA with high-affinity ligands enables the imaging of Biofilms and Infections

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Pseudomonas aeruginosa is one of the most common nosocomial pathogens and often causes life-threatening acute and chronic infections. To date, no fast and pathogen-specific diagnostic tools for *P. aeruginosa* infections are available. The two lectins of *P. aeruginosa,* LecA and LecB, are involved in biofilm formation, a hallmark of chronic infections. Here, we present the detection *of P. aeruginosa* by LecA- and LecB-targeted fluorescent probes as pathogen-specific diagnostic tools. To this end, several probes were developed to target these extracellular lectins and demonstrated to stain *P. aeruginosa* biofilms *in vitro*. For LecA an activity boost to low-nanomolar affinity could be achieved by multivalency. *In vitro*, the divalent LecA-targeting imaging probe accumulated effectively in biofilms under flow conditions. Investigation of divalent LecA- and monovalent LecB-targeting imaging probes in an *in vivo* murine lung infection model revealed elevated probe accumulation in lungs of infected animals. These findings demonstrate the use of LecA- and LecB-targeting probes for the imaging of *P. aeruginosa* infections and suggest their potential as pathogen-specific diagnostics.

Multivalent presentation of glycosidic antigen mimetics for the modulation of the immune system

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Mucins (MUCs) are heavily glycosylated proteins excreted by epithelial cells. Transmembrane mucin type 1 (MUC-1) is the most abundant MUC in humans. MUC-1 external domain is rich in serine (Ser) and threonine (Thr) residues. In tumor cells these residues are *O*-glycosylated with an *N*-acetyl galactosamine (GalNAc) through α-*O*-glycosidic bonds. This fragment is rarely exposed on healthy cell surfaces and it is known as Tn antigen. Indeed, in cancer cells, glycosylation mechanisms are limited and simple carbohydrate units are exposed to the immune system. These shortened residues, including Tn antigen, are overexpressed on many cancer cells and are known as Tumor-Associated Carbohydrate Antigens (TACAs).

In cancer events, immune response is mediated by Macrophages and Dendric Cells (DCs). Human Macrophage Galactose-type Lectin (MGL) is a C-type lectin expressed on macrophage and DCs surfaces and specifically recognize galactose and GalNAc. Lectins like MGL, poorly interact with carbohydrate monomers while multimeric sugar ligands generally confer much stronger binding and shall be preferred to the corresponding monomeric ligand.

We will discuss the effects of the multivalent presentation of sulfor-containing MUC1 mimetics on the binding *vs.* DCs and on immunogenicity.

Bibliographic references:

R. M. Wilson, S.l J. Danishefsky, R. M. Wilson. (2013), J. Am. Chem. Soc., (135) 14462–14472.

A. Diniz, H. Coelho, J. S. Dias, S. J. van Vliet, J. Jiménez-Barbero, F. Corzana, E. J. Cabrita, F. Marcelo. (2019), Chem. – A Eur. J., (25) 13945–13955.

F. Venturi, C. Venturi, F. Liguori, M. Cacciarini, M. Montalbano, C. Nativi (2004), Am. Chem. Soc. (69) 6153–6155.

Structural studies of *Plesiomonas shigelloides* CNCTC 70/89 lipopolysaccharide

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Plesiomonas shigelloides is a Gram-negative rod belonging to the *Enterobacteriaceae* family. *P. shigelloides* is associated with episodes of intestinal infections and outbreaks of diarrhoea in humans. Foreign travel, particularly to Latin America, the Caribbean, and South and Southeast Asia, is a second major risk factor associated with *Plesiomonas* infections in humans [1]. The extra-intestinal infections caused by this bacterium, *e.g.* meningitidis, bacteraemia and septicaemia, usually have gastrointestinal origin and serious course. Lipopolysaccharide (LPS, endotoxin), the main component of the outer membrane of the cell envelope of Gram-negative bacteria, is built of an O-specific polysaccharide and core oligosaccharide covalently linked to lipid A.

Despite the rising knowledge of *P. shigelloides* LPS structures over the past two decades, complete or partial LPS structures have been elucidated only for 15 strains out of 102 identified O-serotypes [2].

The structure of *P. shigelloides* O5 LPS was determined by chemical analysis, mass spectrometry and NMR spectroscopy. The O-specific polysaccharide of *P. shigelloides* O5 has the following structure: →4)-β-D-Man*p*NAc-(1→4)-α-D-Glc*p*NAc-(1→. The same structure was identified before in the O-specific polysaccharide of *Hafnia alvei* strain 38 [3]. Furthermore, a new core oligosaccharide was described, which shares *P. shigelloides* common feature, that is the presence of uronic acids. The lipid A of *P. shigelloides* O5 LPS is identical with lipid A of the *P. shigelloides* serotype O74 [4].

Bibliographic references:

[1] J. M. Janda, S.L. Abbott, C.J. McIver (2016), Clin. Microbiol. Rev. 29, 349–374.
[2] A. Maciejewska, B. Bednarczyk, C Lugowski, J. Lukasiewicz (2020), Int. J. Mol. Sci. 21(18), 6788.
[3] E. Katzenellenbogen, E. Romanowska, D. Witkowska D, A. S. Shashkov (1992), Carbohydr Res. 231:51-4.
[4] J. Lukasiewicz, T. Niedziela, W. Jachymek, Kenne L, C. Lugowski (2006), Glycobiology. 16(6):538-50.[1]

Synthesis of boronated nucleotidyl sugar analogues as glycosyltransferase inhibitors

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Nucleotide sugars are high-energy donor substrates that consist of a monosaccharide and a nucleoside mono- or diphosphate moiety. As glycosyl and phosphoglycosyl donors, nucleotide sugars are essential in the biosynthesis of glycans and glycoconjugates in all living organisms. These biosynthetic products play crucial roles in many biological processes, including cell growth and development, cell signaling, and host-pathogen interactions, while alterations in sugar nucleotide metabolism have been implicated in various diseases [1]. Hence, nucleotide sugar-utilizing enzymes are promising targets for drug development, and nucleotide sugar analogues present potential inhibitors. Replacement of the non-bridging oxygen of the phosphate with a borane group (BH_3) furnishes phosphate analogues closely resembling the natural (pyro)phosphate with regard to charge, bond angles, and acidity and may considerably increase lipophilicity and enzymatic stability. Preparation of boranophosphate analogues has been described in-depth for nucleoside (poly)phosphates containing the borane either in α, β, or in γ-position [2,3]. We herein describe the synthesis of various nucleotide-P-boranodiphospho sugars through a phosphoramidite approach. These boronated natural sugar nucleotide analogues could be employed as molecular probes to investigate the stereochemistry and mechanisms of relevant enzymes such as glycosyltransferases, phosphoglycosyltransferases, and nucleotide sugar pyrophosphorylases, or in therapeutic applications, including boron neutron capture therapy.

Bibliographic references:

[1] S. Mikkola (2020), Molecules (23) 5755.
[2] P. Li, Z.A. Sergueeva, M. Dobrikov, B.R. Shaw (2007), Chem. Rev. (107) 4746-4796.
[3] J. Lin, B.R. Shaw (2000), Tet. Lett. (41), 6701-6704.

A simplified method for the synthesis of robust oligomers of sialic acid (ROSAs)

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The COVID-19 pandemic was predicted by countless experts and generally accepted to be the first of many more viral outbreaks to come. Despite the quick response to develop a vaccine, there still exists a void in the development of antiviral prophylactics. The goal of the current project is the development of a new synthetic pathway for oligosaccharides that forgoes many of the traditional and challenging methods by which carbohydrate chemistry is executed, which includes the use of protecting groups.

The carbohydrate products presented here are derivatives of N-acetylneuraminic acid (sialic acid) which have been shown to be prime candidates as preventative agents against multiple viruses, such as HIV-1, SARS-CoV-2, and HPV, to name a few. While sialic acids are believed to be effective in preventing viral infections, they are naturally subjected to enzymatic and acidic degradation. Here we present the synthesis of robust oligomers of sialic acid (ROSAs) which are anticipated to be resistant in environments often destructive to sialic acid oligomers. In addition to this, we are using green chemistry methods that provide higher yields than those commonly seen in carbohydrate chemistry. This research has the potential to address the need for preventative measures against viral infection in addition to other possible applications in carbohydrate chemistry.

Use of glycans metabolic engineering for studying plant cell wall biosynthesis and functions

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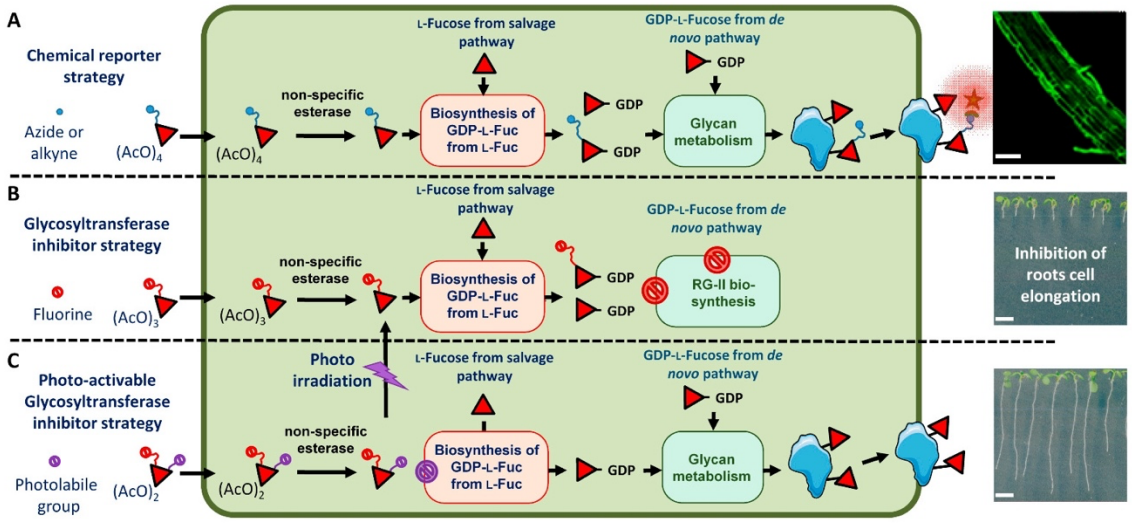
Glycans metabolic engineering is a powerful tool for studying the glycosylation of living cells. The use of modified monosaccharides, such as deoxy or fluorinated sugars, has been reported as a powerful pharmacological approach for studying carbohydrate metabolism and plant cell wall biosynthesis. Moreover, non-natural metabolite derivatives that carry functions enabling bio-orthogonal ligations are now widely used for glycomolecules imaging in living organism. In both cases, these derivatives must cross the cell membrane and be accepted by the biosynthetic machinery of the cell to produce nucleotide-sugars that will be taken in charge by the enzymatic machinery to build complex glycomolecule such as cell wall polysaccharides in plant.

Here, we illustrate the use of glycan metabolic engineering, photo release of caged monosaccharide derivative and click-chemistry, for studying the plant cell wall biosynthesis and function with an emphasis of the pectic domain rhamnogalacturonan II.



Glycan metabolic engineering in plant cells using fucose derivatives.

Synthesis of glycosylated schweinfurthins

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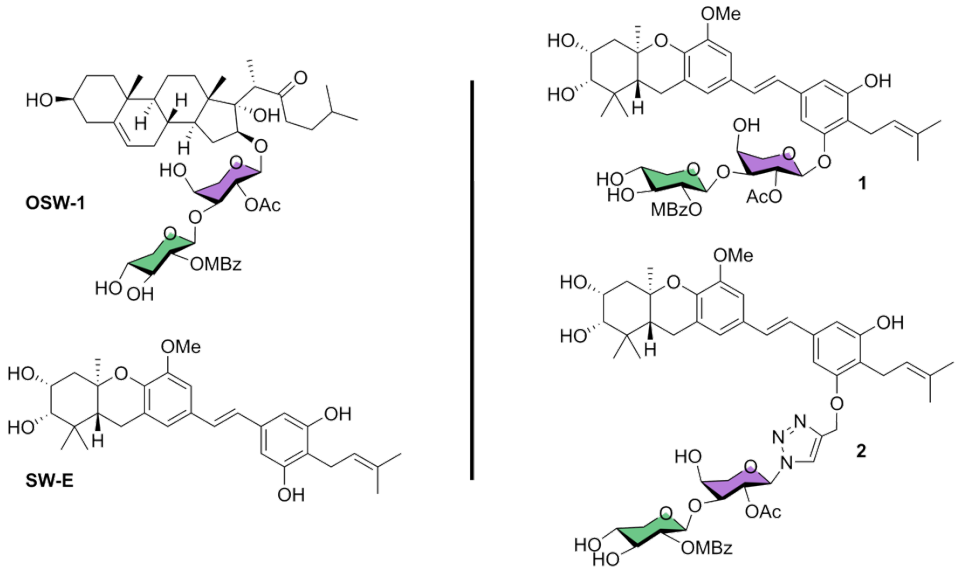
Schweinfurthins (SW) are a family of compounds comprising a stilbene motif associated to a hexahydroxanthene (HHX) moiety. The first derivative of this family was isolated from the leaves of Macaranga vedeliana in 1992.[1] These compounds were later found to exhibit a promising cytotoxicity preferentially towards cancer cells, based on screening on HL-60 cancer cells of NCI.[2] Even more remarkably, the pharmacological profile of these compounds bears no resemblance to the profiles of the compounds used so far in chemotherapy but is similar to other natural compounds (including OSW-1) which indicates that they act via a new common biological target. This common target is the protein OSBP which transports cholesterol in cells between the endoplasmic reticulum and the golgi. Our objective is to synthesize hybrid molecules between SW and OSW-1 while retaining the two pharmacophores essential to their biological activity: the HHX motif for SW and the glycosidic part for OSW-1.

As these two pharmacophores are positioned a priori in different places of the target protein, these hybrid molecules should have a better affinity. We are therefore interested in the preparation of compounds such as 1 and 2 which can be obtained either by glycosylation of SW-E, or by a Huigen-type cycloaddition reaction between a propargylated schweinfurthin and a disaccharide comprising an azide in the anomeric position (Figure 1).



OSW-1, SW-E and glycosylated SW-E hybrids

Bibliographic references:

[1] O. Thoison, E. Hnawia, F. Guéritte-Voegelein, T. Sévenet (1992), Phytochemistry (31), 1439.

[2] A. W. G. Burgett, T. B. Poulsen, K. Wangkanont, D. R. Anderson, C. Kikuchi, K. Shimada, S. Okubo, K. C. Fortner, Y. Mimaki, M. Kuroda, J. P. Murphy, D. J. Schwalb, E. C. Petrella, I. Cornella-Taracido, M. Schirle, J. A. Tallarico, M. D. Shair (2011), Nat. Chem. Biol. (7), 639–647.

Nucleation and growth of silver nanoparticles on reducing supramolecular glyco-nanofiber

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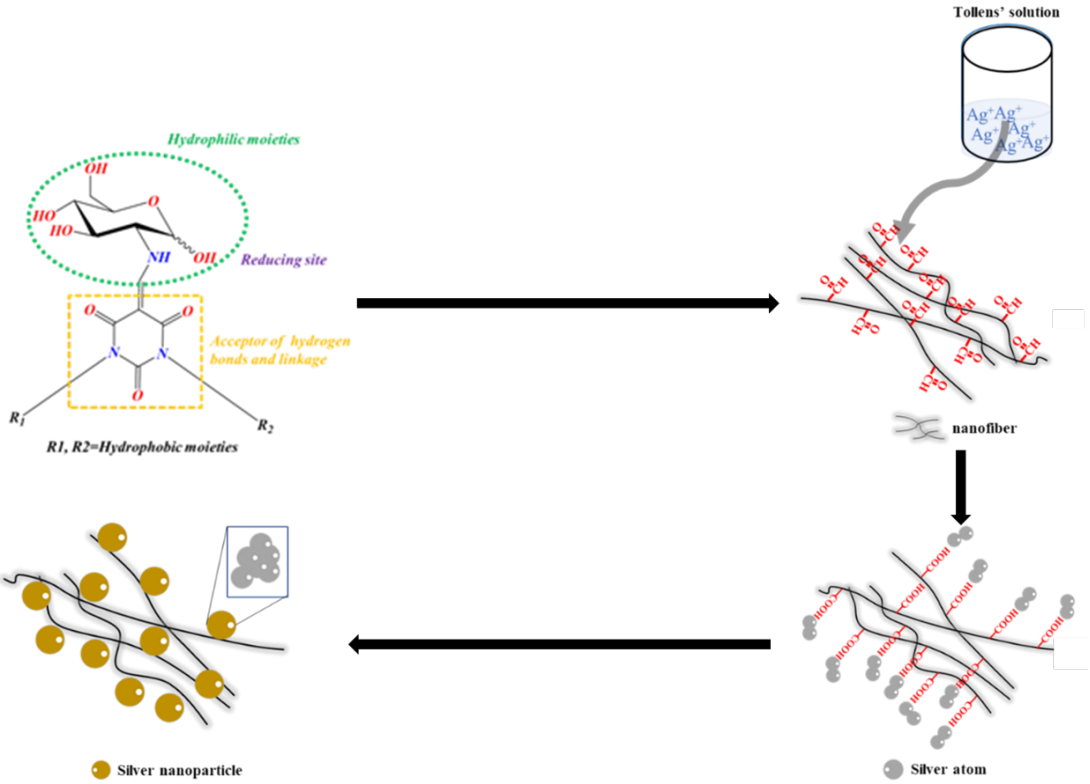
Among metallic nanoparticles, silver nanoparticles (AgNPs) are particularly interesting as a plasmonic nanoparticle for sensing and imaging, as well as for use as a catalyst or antimicrobial agent[1] . Carbohydrate hydrogel with the native reducing end aldehyde could form AgNPs, obviating the requirement for external reducing agents, stabilizers, or nucleation control measures.

The aim of this study is to devise a glyco-nanofiber consisting of reducing carbohydrate derivatives that exhibit intrinsic aldehyde functionality, thus facilitating the reduction of silver ions and subsequent formation of AgNPs on the surface of the glyco-nanofibers in aqueous media. This poster will present the synthesis of glyco-nanofibers by reacting between *N,N'*-substituted barbituric acid derivatives and the amino functionality of D-(+)-glucosamine hydrocholoride, allowing us to comprehensively assess its gelation properties. The formation of AgNPs on the surface of nanofiber has been studied using TEM and UV-vis spectrum.



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Bibliographic references:

1. Diehl, F.; Hageneder, S.; Fossati, S.; Auer, S. K.; Dostalek, J.; Jonas, U.(2022), Chem Soc Rev, (51), 3926-3963.

2. Yao, S.; Brahmi, R.; Bouschon, A.; Chen, J.; Halila, S.(2023),Green Chem., (25), 330–335

Stimuli responsive glycopolymers for the detection of bacterial lectins

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Prompt diagnosis of infectious disease is vital in combatting the spread of infection and improving patient prognosis. A potential biomarker for point of care (POC) testing is the carbohydrate binding proteins, or lectins, that are associated with a variety of pathogens such as bacteria and fungi. Lectins selectively bind to glycans displayed on almost all cell surfaces and play a key role in cellular recognition during pathogenesis. Inspired by natures glycocalyx, work toward a biosensor for the detection of bacterial lectins is reported. This biosensor will feature an array of fluorescently labelled glycopolymers on graphite electrodes that aims to rapidly identify bacterial lectins in complex samples via a thermal- and optical-detection dual sensing approach. These involve the monitoring of both thermal wave transport through functionalised interfaces and the fluorescent emission of the glycopolymers. [1]

Fluorescent glycopolymers that can mimic lectin-glycan interactions were synthesised; first, masked acyl hydrazide monomers were prepared and used to produce a library of polymers by reversible addition-fragmentation chain transfer (RAFT) polymerisation. *N*-Isopropylacrylamide (NIPAM) was also integrated into the polymer backbone to impart thermal responsiveness that will release a bound analyte when heated above the lower critical solution temperature (LCST) and facilitate the reuse of the sensor. [2] Two solvatochromic fluorophores were then conjugated to the polymers end groups using thiol-maleimide coupling, generating differing emission profiles based on their local environment. A variety of unmodified reducing sugars have been appended to the polymer backbone to produce glycopolymers. Graphite electrodes were also modified using electrochemical techniques to afford amine functionalised surfaces that were used to conjugate glycopolymers. Ongoing work aims to determine response and limits of detection using TWTA.

Bibliographic references:

[1] B. Van Grinsven, K. Eersels, M. Peeters, P. Losada-Pérez, T. Vandenryt, T. J. Cleij and P. Wagner, ACS Appl. Mater. Interfaces, 2014, 6, 13309-13318.

[2] C. S. Mahon, G. C. Wildsmith, D. Haksar, E. de Poel, J. M. Beekman, R. J. Pieters, M. E. Webb and W. B. Turnbull, Faraday Discuss., 2019, 219, 112-127.

Generation of galectin inhibitors from green sources: deglycosilación of lactoferrin

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Galectin 1 and 3 are over-expressed in cancer cells and are involved in tumour progression and metastasis. Thus they are an interesting target for cancer therapy and their inhibitors potential anti-tumour agents. Particularly, galactosides are potential inhibitors as they can bind to their carbohydrate recognition domain [1-2].

Lactoferrin (LF), one of the most abundant glycoproteins in serum milk, is rich in N-glycans with potential anti-galectin activity. Thus, their glycans could be an economic and green source of galactosides[3-4].

Here we describe the release of LF glycans by enzymatic and chemical approaches focusing on the comparison of these methodologies, both in terms of yield and N-glycan profile. Even though these strategies are used for sample preparation for analytical glycomics, they have not been applied for the generation of glycan at preparative scale.

Enzymatic deglycosylation (DG) was carried out using the enzyme PNGase F under denaturing conditions and chemical DG was achieved using NaClO solution. The released glycans were purified and their profile was evaluated by SDS-PAGE, HPLC and TLC. Then, graphitized carbon SPE-column was used to separate mannosylated from sialylated glycans and further characterized by NMR.

The N-glycan profile was slightly different for enzymatic and chemical release, nevertheless higher yields were obtained with chemical DG.

LF deglycosylation proved to be an interesting tool for high scale glycan generation. We are now working in their biological evaluation as potential galectin inhibitors both in vitro and in silico.

Bibliographic references:

[1] H. H. Freeze, L. Baum, A. Varki (2017), Essentials of Glycobiology Chapter 36.
[2] A. Varki (2017), Glycobiology (27) 3–49.
[3] N. O'Riordan, M. Kane, L. Joshi, R. M. Hickey, (2014) Glycobiology (24) 3, 220–236.
[4] A. J. Noll, J. P. Gourdine,Y. Yu, Y. Lasanajak, D.F. Smith, R. D. Cummings (2016) Glycobiology, (26) 6, 655–669.

Dimerization strategy towards higher carbon sugar alcohols as potential phase change materials

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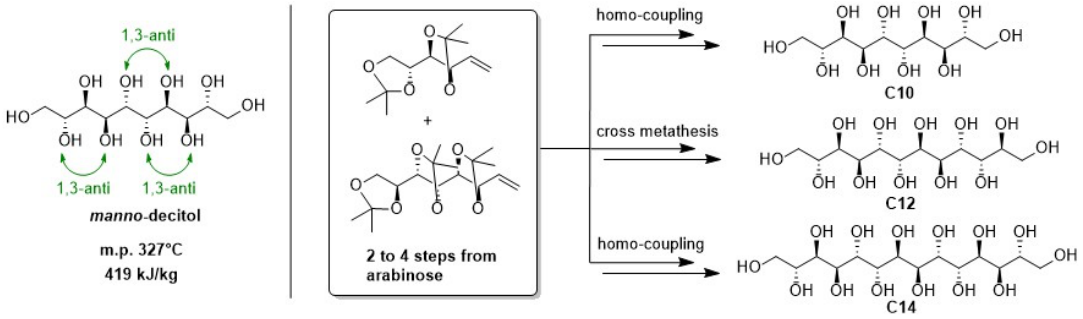
Sugar alcohols are interesting candidates for phase change materials (PCMs). Their strong hydrogen bonding tendency gives them high thermal storage potentials. Recently, specific sugar alcohols with extended chain length have been predicted by computational calculations to possess remarkably high values of latent heat making them promising PCMs [1] (Scheme left). An even number of carbon atoms, no branching and all hydroxy groups in 1,3-*anti* orientation have been identified as prerequisite. However, experimental validation of these predictions is yet lacking as these materials are not readily available.

We aim to make these PCM candidates accessible for thermal evaluation. In order to obtain higher sugar alcohols of C10 and longer chain length, we apply a dimerization approach [2]. Linking two sugar-derived building blocks by metathesis allows us to directly and efficiently translate the natural stereochemistry of the carbohydrates into our target materials.

We have already succeeded in the homo-coupling of a D-arabinose derived building block to the D-*manno*-D-*manno*-decitol (*manno*-decitol, C10). Next, by combining differently sized precursors even higher sugar alcohols (C12, C14) are accessible via (cross) metathesis. The synthesized compounds are being investigated for their thermal properties and compared to their computationally estimated potentials.



Bibliographic references:
[1] T. Inagaki, T. Ishida (2016), J. Am. Chem. Soc. (138) 11810–11819.
[2] M. Menzel, T. Ziegler (2014), Eur. J. Org. Chem. (34) 7658-7663.

Enzymatic synthesis of 0-, a-, b-, and c-series gangliosides

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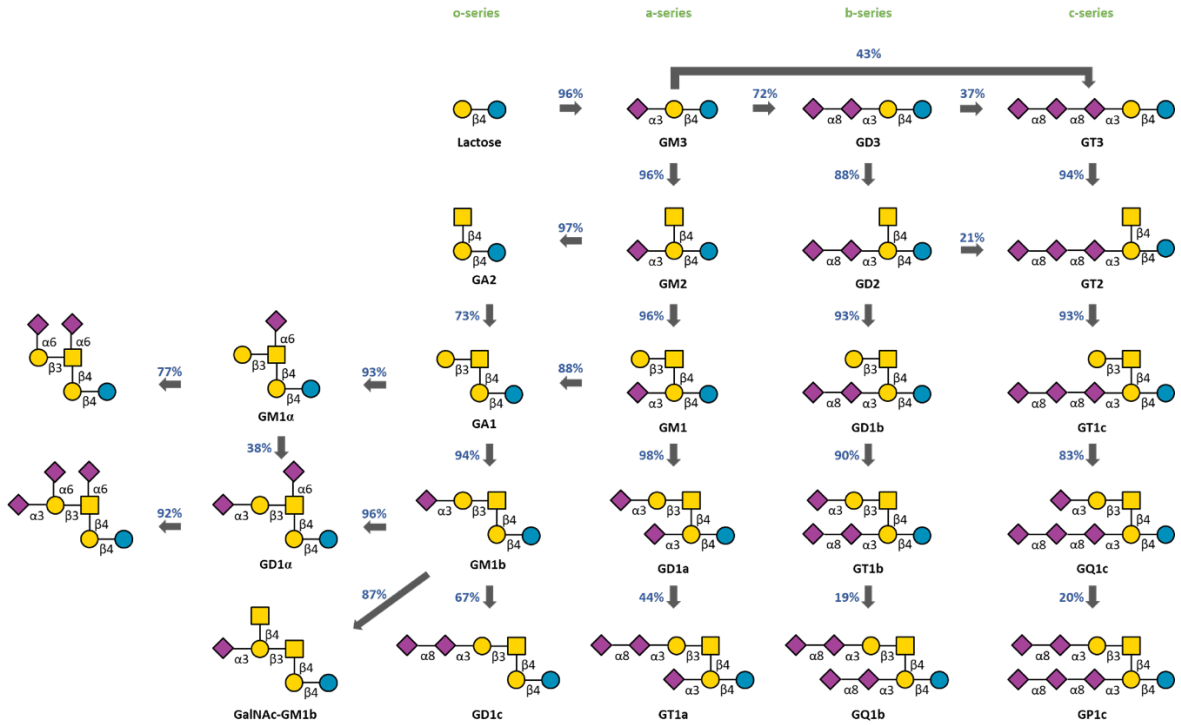
Gangliosides are sialic acid-containing glycosphingolipids. They are primarily localized in the outer leaflets of plasma membranes, participating in cell-cell recognition, adhesion, and signal transduction.[1]

In past decades, many groups had developed different chemical or enzymatic approaches to synthesize gangliosides. However, the chemical synthetic pathways encounter challenges not only precisely controlling regio- and stereo-selectivities in sialylation, but also complicated protection and deprotection are unavoidable. By contrast, enzymatic is more feasible to access these complicated glycans. In order to obtain pure and sufficient amount of these compounds, we started with the chemically synthesized lactose which contains an azide linker for further application in microarray.

Multiple enzymes were applied on the enzymatic synthesis of glycans of 0-, a-, b- and c-series gangliosides. Complicated gangliosides, including GT1a, GQ1b, and GP1c glycans, were synthesized by sequential one-pot enzymatic or regeneration synthesis.



Development of glycomimetic ligands blocking SARS-CoV-2 binding to C-type lectin receptor L-SIGN

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C-type lectin receptors (CLRs) are a class of Pathogen Recognition Receptors involved at the surface of dendritic cells in pathogen detection. They are dedicated to the recognition of carbohydrate-based molecular patterns associated to pathogens. [1]

Some of those pathogens have found strategies to bypass the role of CLRs in immunity activation and even hijack CLRs for their benefit during infection process. Thus, subversion of CLRs has been reported as alternate receptors or attachment factors, notably by HIV, Ebola virus as well as SARS-CoV virus, responsible of the severe acute respiratory syndrome (SARS) in 2002. [2]

In the context of the world-scale coronavirus outbreak, we demonstrated that two CLRs, DC-SIGN and L-SIGN, are used by SARS-CoV-2 to enhance its infection by trans-infection [3]. This enhancement can be blocked by glycomimetic. L-SIGN is present on airway epithelium cells while DC-SIGN is present on immune dendritic cells.

Our main issue focuses on the development of antagonists with a selectivity towards L-SIGN, as interesting tools to compete with SARS-CoV-2 anchoring. Being able to block virus attachment on airway epithelia (targeting L-SIGN) without activating immune systems (via DC-SIGN binding) is attractive in the SARS-CoV-2 physiopathological context.

Optimized and selected glycomimetic molecules through diverse biophysical studies will be presented as well as development of high affinity multivalent systems. Preliminary data of the use of these candidate-compounds in competition experiment within a cellular model of infection will also be presented.

Bibliographic references:

[1] S.J. van Vliet, J. J. Garcia-Vallejo, Y. van Kooyk (2008), Immunology and Cell Biology (86), 580-587.
[2] F. Zhang, S. Ren, Y. Zuo (2014), International Reviews of Immunology (33), 54-66.
[3] M. Thépaut, J. Luczkowiak, C. Vivès, N. Labiod, I. Bally, F. Lasala, Y. Grimoire, D. Fenel, S. Sattin, N. Thielens, G. Schoehn, A. Bernardi, R. Delgado, F. Fieschi (2021), PLoS Pathogens 17(5) e1009576

Oligogalacturonide (OG): new prospects for plant disease biocontrol and plant health

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The current work is part of the ECOPHYTO II+ project, to find safer alternatives to harmful phytosanitary products by developing bio-based molecules that can activate plant defenses. One promising approach is the use of oligogalacturonides (OGs) derived from plant cell wall pectins via the action of polygalacturonases (PGs). While OGs have been shown to elicit plant defenses, the diversity of tested OGs has been limited[1,2].

In this study, we investigated the structure, dynamics, enzymatic kinetics, hydrolysis products of various plant PGs. When, pectins from commercial sources or agriculture waste were digested by various plant PGs, to product differents OGs pools. LC-MS analysis of these OGs showed that the average degree of polymerization (DP) depended on the substrate's origin and the enzyme used.

We then applied concentrated OGs (5g/L) under controlled conditions to wheat two days before infection with *Blumeria graminis f.* sp. *Tritici (Bgt)*, the fungal causal pathogen of powdery mildew. The results showed that wheat protection against powdery mildew ranged from 25% to 55% depending on the pectin-enzyme pair used.

Our findings demonstrate the importance of characterizing OGs and highlight new ways of inducing defense mechanisms in wheat. By creating bio-based molecules with original structures, we can work towards gradually replacing harmful phytosanitary products and promoting a healthier and more sustainable environment.

OGs from sugar beet and digested by PGs were able to induce wheat resistance against powdery mildew

Acknowledgements

We would like to thank the Région Haut de France, the Université de Picardie Jules Verne and the Université du littoral Côt d'Opale for the funding.

Bibliographic references:

1. B. Randoux, D. Renard-Merlier, G . Mulard, S. Rossard, F. Duyme, J. Sanssené, J. Courtois, R. Durand, P. Reignault. Phytopathology 100 (2010) 1352-1363.

2. A. Silipo, G. Erbs, T. Shinya, M.J. Dow, M. Parrilli, R. Lanzetta, N. Shibuya, M.A. Newman, A. Molinaro. Glycobiology 20 (2010) 406-419.

Synthesis of sugar-functionalised micro-beads for *S. aureus* detection

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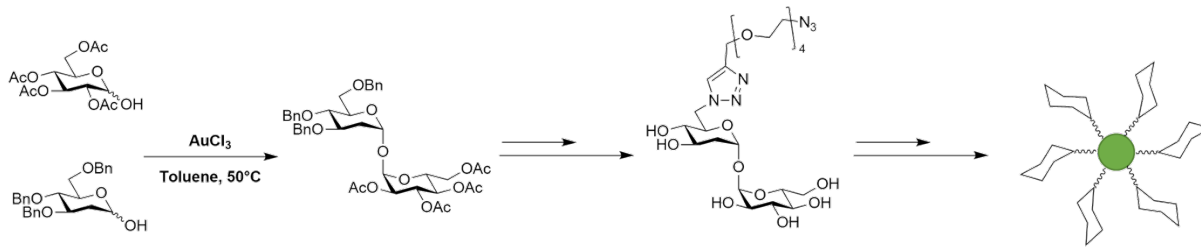
Staphylococcus aureus is a bacterial human pathogen responsible for a wide variety of diseases in both community and hospital-acquired settings. Its widespread occurrence, combined with the emergence of multi-drug resistant strains, requires the development of fast detection platforms for its early detection to maintaining effective antibiotic stewardship and avoiding the unnecessary prescription of broad-spectrum agents.

Bacteria surface ability to interact with specific sugars on the surface of cells can be exploited to specifically target bacteria[1]. Our team recently developed a mannose-based agglutination assay based on computer-aided cluster analysis for *E. coli* detection[2]. Herein, we expand the capabilities of this novel strategy and describe the preparation of trehalose-based microprobes and their evaluation for the cheap and fast detection of *S. aureus*. For this purpose, a small library of trehalose analogues was synthesised using a $AuCl_3$-catalysed glycosylation protocol previously established in our group[3]. The synthetic disaccharides were conjugated to polystyrene micro-beads via click chemistry, and the functionalised microparticles agglutination in the presence of bacteria was evaluated using cluster analysis revealing the key glycan features needed for selective binding to *S. aureus*. Using this platform, we could detect bacteria at 10^8 cfu/ml concentrations. Moreover, we further demonstrated this strategy can be used as a tool to investigate sugars-bacteria interactions which can aid the development of new detection probes and therapies.



Example of platform preparation

Bibliographic references:

1 M.D. Disney, P. H. Seeberger, (2004), Chemistry & Biology (12) 1701-1707

2 J-B. Vendeville, M. J. Kyriakides, Y. Takebayashi, S. Rama, J. Preece, J. Samphire, J. Ramos-Soriano, A. Mauleon Amieva, M. E. Holbrow-Wilshaw, H. R. Gordon Newman, S. Lou Kou, S. Medina-Villar, N. Dorh, J. N. Dorh, J. Spencer, M. C. Galan (2022), ACS Biomater. Sci. Eng. (1) 242–252

3 R. Jeanneret, C. Walz, M. van Meerbeek, S. Coppock, M. C. Galan (2022), Org. Lett. (34) 6304–6309

Kinetic *S*-glycosylation by photocatalytic thiol-ene reaction with high stereocontrol

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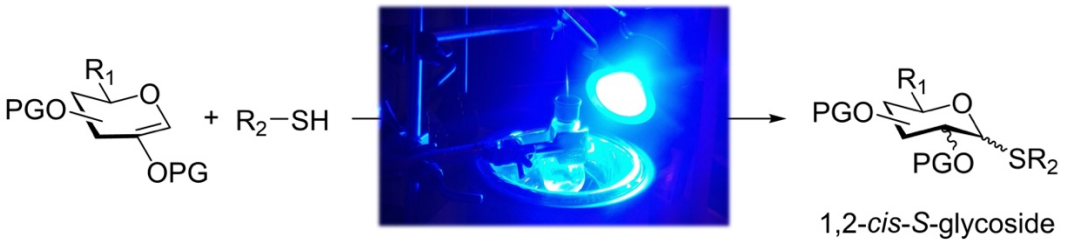
S-Glycosides, isosteres of the most common *O*-glycosides with higher hydrolytic stability, have become interesting for their potential in the development of novel therapeutics in recent decades [1]. However, most of the conventional methods for their synthesis lead to the formation of thermodynamic products with 1,2-*trans* configuration in particular. There are only a few approaches studying the kinetic *S*-glycosylation in the literature, but most of them suffer from use of expensive or toxic mediators composed of transition metals, or UV-light irradiation, which requires specialised laboratory equipment.

Therefore, the synthesis of *S*-glycosides with 1,2-*cis* configuration still remains challenging. In this work, we deal with the regio- and stereoselective synthesis of these *S*-glycosides by thiol-ene reaction, which has become popular in the last decades [2]. Our improved methodology utilises only a catalytic amount of a nontoxic organic initiator excited by environmentally and economically favourable blue visible light. This radical addition on 2-substituted glycals (1,2-unsaturated carbohydrates) provides a wide range of products in good yields with exclusive stereo- and regioselectivity within only 30 minutes.



PG = protecting group
R_1 = H, CH_3, CH_2OH
R_2 comes from EtSH, glycosyl thiols, 4-thioglycosides, 6-thioglycosides, or L-cysteine

Acknowledgements

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Bibliographic references:

[1] K. Ito, S. A. Scott, S. Cutler, L.-F. Dong, J. Neuzil, H. Blanchard, S. J. Ralph (2011), Angiogenesis (14), 293-307.
[2] D. Eszenyi, V. Kelemen, F. Balogh, M. Bege, M. Csávás, P. Herczegh, A. Borbás (2018), Chem. Eur. J. (24), 4532-4536.

Calixarene-based cluster for immunostimulation against *Streptococcus Pneumoniae* 19F

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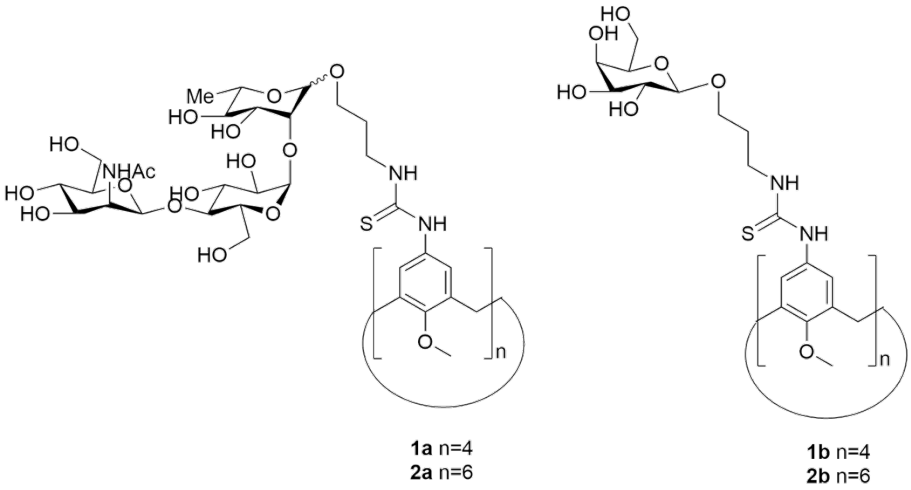
Streptococcus pneumoniae (SP) is one of the most diffused encapsulated bacteria and the principal cause of bacterial infections in young children [1]. Among virulence factors, capsular polysaccharide (CPS) is the most relevant. Serotype 19F is the most virulent. Its CPS structure is formed by β-D-ManpNAc-(1→4)-α-D-Glcp-(1→2)-α-L-Rhap repeating units linked through phosphodiester bridges. The chain length effect of the pneumococcal polysaccharide on immunogenicity has been demonstrated [2]. To trigger an immune response and, then, to develop, in perspective, potential vaccines, the use of molecularly well-defined and monodispersed polyglycosylated cores exposing multiple copies of the simple repeating unit could be exploited in alternative to disomogeneus poly(oligo)saccharide chains. Calixarenes are versatile platforms for the preparation of multivalent glycoclusters [3]. The possibility to regulate valency and geometry allowed to produce efficient and selective calixarene-based ligands, the glycocalixarenes, for carbohydrate recognition proteins (lectins) [3]. In a previous work, the different presentations of multiple copies of β-D-ManpNAc and 19F trisaccharide using calixarenes as scaffold were studied, evidencing the ability to interact with specific anti SP-19F antibodies [4]. Starting from these results, we selected the conformationally mobile methoxycalix[4]- and calix[6]arenes as scaffolds to continue our investigation in using them to develop immunostimulants. We will present the synthesis of the calixarenes and their functionalization with the SP-19F trisaccharide repeating unit (**1a** and **2a**) and with galactose as negative controls (**1b** and **2b**). The preliminary data relative to the biological properties and the immunization experiments will be also illustrated.



1a n=4
2a n=6

1b n=4
2b n=6

Glycocalixarenes respectively for immunostimulation and as negative control

Bibliographic references:

[1] N. Principi et all (2018) S. Curr. Infect. Dis. Rep. (20) 1
[2] C.T. Bishop, H.J Jennings, Immunology of polysaccharides. In "The Polysaccharides", Academic Press, New York (1982) pp 291-330
[3] F. Sansone, A. Casnati, (2013) Chem. Soc. Rev. (42) pp. 4623-4639
[4] M. Giuliani et all. (2019) Bioorg. Chem (93) pp. 103305

Gram scale synthesis of three regioisomeric tetrasaccharides composed of alpha-glucoside units

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Oligosaccharides are very common natural compounds composed of monosaccharides conjugated together via glycosidic bonds through exceptional diversity.[1] Their composition and structural diversity is as varied as their biological or physico-chemical properties. The tailor-made synthesis of complex glycans is an everlasting challenge of carbohydrate chemistry that can be tackled through multi-step organic synthesis[2]and/or chemo-enzymatic synthesis[3] in solution and/or through solid phase synthesis.[4] Stereoselective glycosylation is the key to such complex oligosaccharides synthesis, with a particular additional challenge to prepare 1,2-*cis* glycosides with high stereocontrol.[5] We focused here on the multi-step synthesis of three regioisomeric tetrasaccharides composed of glucose with exclusively 1,2-*cis* linkages, the four glucose units being connected through either alpha-1,4 or alpha-1,6 linkages, linear or branched (**Figure 1**). The first tetrasaccharides **A** was obtained in 9 steps from benzylated maltotriose with a key [3+1] glycosylation using a glucosyl *N*-phenyl trifluoroacetimidate donor according to Codée's protocol for primary alcohol acceptors.[6] Tetrasaccharide **B** was synthesized in a [2+2] glycosylation strategy from two maltose building blocks, using a *N*-phenyl trifluoroacetimidate donor. The third tetrasaccharides **C** could be accessed through two different [2+1+1] glycosylation strategies from (1) a maltosyl acceptor with either both 4'- and 6'-positions free to be glucosylated simultaneously or through (2) a sequential 6'- then 4'-glucosylation of a 4',6'-naphthylidene maltoside.

Figure 1

A detailed description of each strategy and synthetic steps will be provided with focus on the regio- and stereoselective access to each of the three tetrasaccharides in convergent and efficient synthetic schemes. Each tetrasaccharide could be obtained in a multi-gram scale from commercially available monosaccharides and commonly available reagents.

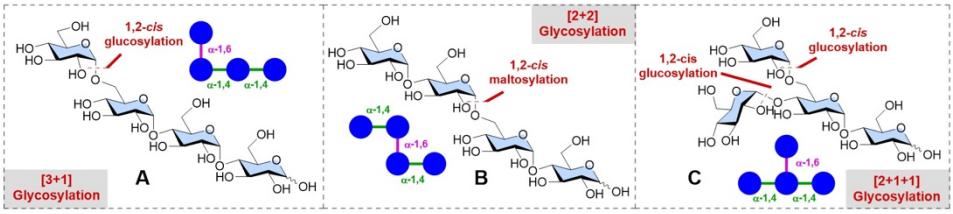

Figure 1 : Structure and general strategy towards the three regioisomeric tetrasaccharides

Bibliographic references:

1) Werz et al ACS Chem. Biol. 2007, 2, 685-691.

2) Comprehensive Glycosciences, 2nd Ed, Elsevier, 2021.

3) Overkleeft et al Chemoenzymatic Synthesis of Glycans and Glycoconjugates in Essentials of Glycobiology, 4th Ed, Cold Spring Harbor, 2022.

4) Ben Abba Amiel et al Automated Oligosaccharide Synthesis: Development of the Glyconeer® in Comprehensive Glycosciences, 2nd Ed, Elsevier, 2021.

5) Nigudkar et al Chem. Sci. 2015, 6, 2687-2704.

6) Wang et al Eur. J. Org. Chem. 2019, 1994-2003.

Epitope mapping of the PA surface polysaccharide Pel

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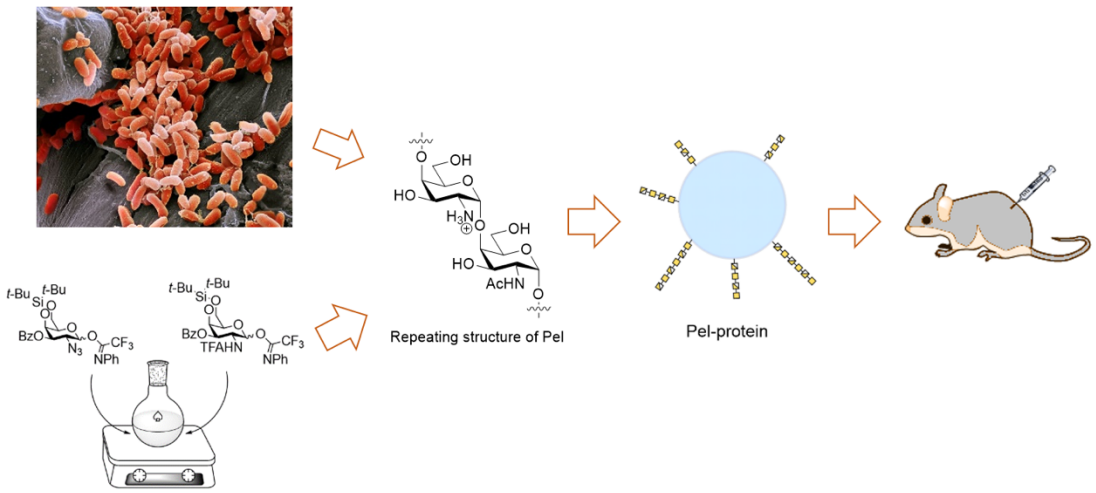
Pseudomonas aeruginosa (PA) is an antimicrobial resistant pathogen responsible for serious infections and it belongs to the ESKAPE pathogens, which are the main cause of nosocomial infections and a growing concern for antibiotic resistance. Developing a vaccine against *P. aeruginosa* is therefore a promising approach. Three exopolysaccharides, which are considered potential targets for bacterial vaccine development, have been identified in the *P. aeruginosa* biofilm: alginate, Psl, and Pel.

The structure of exopolysaccharide Pel has recently been elucidated and is thought to be composed of 1,4-alpha-linked galactosamine and N-acetyl galactosamine[1-2]. Well-defined Pel fragments have been synthesized and will be used as standards for structure elucidation studies, as well as being conjugated to CRM and BSA for use in *in vivo* studies. Meanwhile, natural Pel will be purified from the *P. aeruginosa* strain for vaccine generation and structural analysis experiments.



Acknowledgements

This project is funded by PAVax under the Marie Skłodowska-Curie grant agreement No 861194.

Bibliographic references:

1. L. K. Jennings, K. M. Storek, H. E. Ledvina, C. Coulon, L. S. Marmont, I. Sadovskaya, P. R. Secor, B. S. Tseng, M. Scian, A. Filloux (2015), Proc. Natl. Acad. Sci. (112), 11353-11358.

2. F. Le Mauff, E. Razvi, C. Reichhardt, P. Sivarajah, M. R. Parsek, P. L. Howell, D. C. Sheppard (2022), Commun. Biol. (5), 502.

The molecular basis for inhibition of heparanases and β-glucuronidases by Siastatin B.

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The sulfated glycosaminoglycan polysaccharide known as heparan sulfate (HS) is a highly heterogeneous molecule that's central to biological and pathological processes including its role as a ubiquitous component of the extracellular matrix [1] [2]. HS regulation is modulated by continual breakdown and biosynthesis. Heparanases are GH79 endo-b-glucuronidases that are involved in the breakdown of HS [2]. The human heparanase (HPSE) is involved in the cleavage of HS at GlcA residues. HPSE is a promising target for anti-cancer drugs due to its enzymatic; it is highly involved in cancer biology. Aberrant HPSE expression has been linked to the development of larger and highly metastatic tumours consequently leading to poor prognosis [3]. Multiple compounds that inhibit heparanase have significant effects in tumour models and have progressed to clinical trials [3].

The iminosugar siastatin B is a known inhibitor of neuraminidases, N-acetyl-glucosaminidases and b-D-glucuronidases [4]. However, structures of HPSE with inhibitors and substrates suggest that the active site would be unable to accommodate siastatin B due to the steric clashes of the N-acetyl group at the 2-position [5]. Here we show that the inhibition of HPSE (and related enzymes) reflects not the parent compound but a pair of breakdown products; a galacturonic-noeuromycin or 3-geminal diol derivative of galacturonic-isofagomine. This new class of glycosidase inhibitors have been subsequently re-synthesized and their co-crystal structures with the bacterial b-glucuronidase AcGH79 and HPSE were investigated.

Bibliographic references:

[1]Wu, L., et al. (2015), Nat Struct Mol Biol, 22(12) 1016-22.
[2]Wu, L., et al. (2020), Chemical Communications, 56(89) 13780-13783.
[3]Coombe,D.R. and N.S.Gandhi, (2019), Frontiers in Oncology, 9.
[4]Nishimura,Y.,et al.,(1988), JACS, 110(21) 7249-7250.
[5]de Boer, C., et al., (2022), PNAS, 119(31).

Synthesis of multivalent chondroitin sulfate (CS) oligosaccharides to study CS-Cat K interactions

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Chondroitin sulfates (CS) are polysaccharides which belong to a family of linear polyanionic polymers named glycosaminoglycans (GAGs). They consist of a repeating dimeric unit composed of a D-glucuronic acid (D-GlcA) linked to a 2-acetamido-2-deoxy-D-galactose (D-GalNAc) and contain sulfate groups at various positions. The most common sulfated positions are position 4 (CS-A) and/or position 6 (CS-E/CS-C) of the D-GalNAc moiety.[1] CS play an important role in different physiological and pathological processes[2] through their interaction with numerous proteins such as lectins, cathepsins or midkines. As an example, CS are involved in osteoporosis by forming a complex with the exosites of cathepsin K (Cat K) that is involved in the excessive degradation of collagen.

In order to better understand and to inhibit natural linear CS-Cat K interactions, we are particularly interested in the design and synthesis of variously sulfated multivalent oligosaccharides of CS. Indeed, multivalency is widely observed in Nature because it improves activity and affinity towards the ligands. Very few synthetic multivalent GAGs mimetics have been reported and the field of CS remains largely unexplored.[3]

Our strategy relies on the ligation of new CS oligosaccharides fragments bearing an azido group on the aglycone and various platforms with a terminal alkyne to build new multivalent CS oligosaccharides which will be assessed on Cat K. We will present here our recent results towards their synthesis.[4]

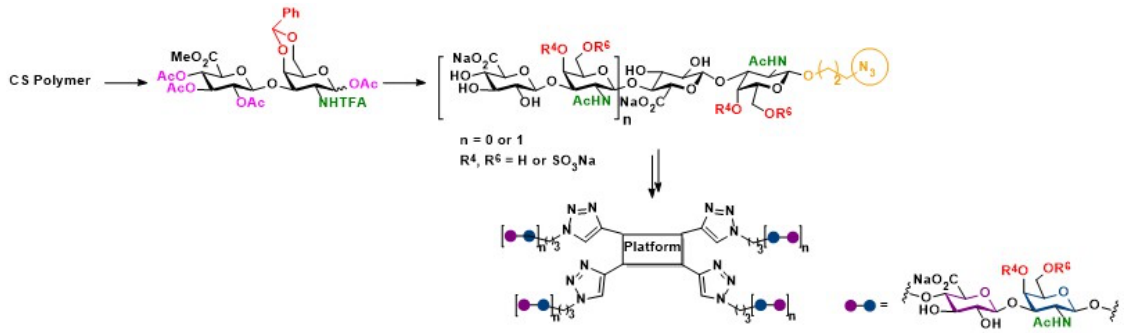

Figure 1: Synthesis of multivalent oligosaccharides

Acknowledgements

We gratefully acknowledge the University of Orleans, the ICOA and Labex Synorg for funding and SALSA platform.

Bibliographic references:

[1] U. Kjellen, et al. (1991) Annu. Rev. Biochem. (60) 443

[2] T. Mikami et al. (2013) Biochim. Biophys. Acta (1830) 4719; Soghi, H. et al. (2021) Biomolecules (11) 1

[3] Domínguez-Rodríguez, P. et al. (2017) Chem. Eur. J. (47) 11338; (2020) Biomacromolecules (21) 2726; Liu, H. et al. (2018) Carbohydr. Res. (467) 45

[4] Buisson P. et al. (2022) Carbohydr. Res. (512) 108514

Phosphates as traceless assisting groups in glycan synthesis

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Many new recent hardware and chemical developments in the instruments and protocols utilized for automated glycan assembly have expanded the possibilities of glycans achievable. Additionally, new protocols have been developed for the expedited synthesis of oligosaccharides containing either an anionic phosphate or sulfate moiety.

These phosphate motifs were then utilized to overcome glycan synthesis limitations including increasing solubility of cellulose oligosaccharide chains to ease purification and also used as a protecting group for regioselective enzymatic sialylation. The phosphate motifs could advantageously be removed in a traceless fashion in a controlled manner under mild conditions with phosphatase to allow for natural sialyated structures and controlled cellulose platelet formation.

Anti-toxin agents for the treatment of *Clostridium difficile* infections

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Clostridium difficile (*C. diff*) is the most common nosocomial infection in western countries with symptoms ranging from mild diarrhoea to a life-threatening inflammation of the bowel. *C. diff* is a spore-forming gram-positive bacteria, which usually lives harmlessly in the gut of about 1 in every 30 healthy adults. However, antibiotic use can unbalance the gut microbiota and facilitate *C. diff* proliferation. *C. diff* produces two toxins, **TcdA** and **TcdB**, which are responsible for the disease pathogenesis. TcdA and TcdB are glucosyltransferases that use UDP-glucose to glucosylate and inactivate host Rho GTPases, resulting in cytoskeletal changes causing cell rounding and loss of intestinal integrity (Figure 1). Developing a selective and potent anti-toxin agent would offer a new *C. diff* therapy while minimising damage to the colon. Importantly, the human gut microbiota barrier would be spared, which is critical to prevent *C. diff* recurrence.

Transition-state analysis of TcdA and TcdB has allowed the design of transition state analogue inhibitors of the toxins which demonstrate therapeutic potential.[a] Here we will present our latest results.

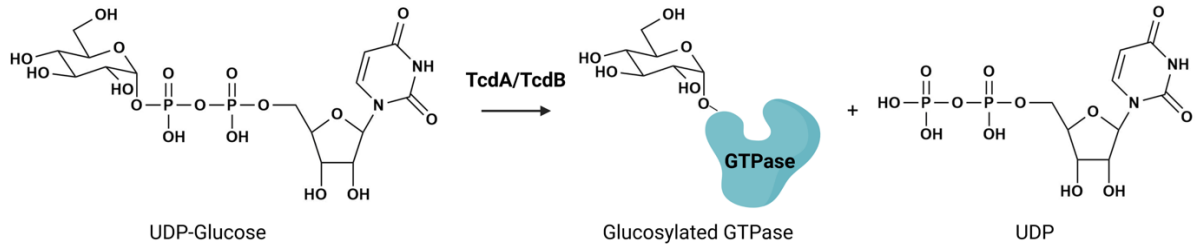

Figure 1: TcdA and TcdB catalysed glycosyltransferase reaction.

Bibliographic references:

a) A. S. Paparella, B. L. Aboulache, R. K. Harijan, K. S. Potts, P. C. Tyler and V. L. Schramm, Inhibition of Clostridium difficile TcdA and TcdB toxins with transition state analogues, Nature Communications, 2021, 12, 6285.

Bypassing cotton cellulose biosynthesis to design fibers with tailored properties

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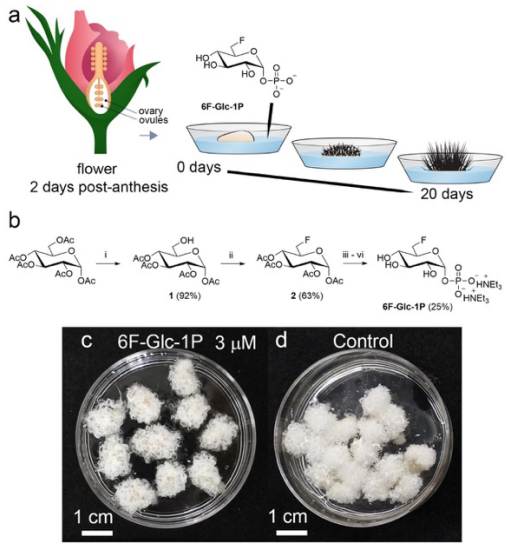
Current manufacturing strategies for cotton fiber post-processing generate large amounts of chemical waste, endangering the environment and the livelihoods of millions of people. Understanding and harnessing cotton's underlying mechanisms to produce fibers with tailored properties is a significant step toward future sustainable alternatives. In this study, we demonstrate that a chemosynthesized phosphorylated glucose derivative carrying a fluorine atom at position C6 of the glucose moiety - 6-deoxy-6-fluoro-glucose-1-phosphate - is biologically incorporated into cotton fibers in cotton ovule in vitro cultures. The resulting modified fibers showed increased tensile and water-retaining properties, as a result of an alteration of the cellulose structure. Finally, our study demonstrates yet another successful example of the advantages of biological fabrication of cellulose-based materials with novel emerging properties that surpass their natural counterparts toward the design of future sustainable materials and the implementation of a true bio-based economy and the concept of material farming.



Feeding cotton in vitro ovules with chemosynthesized 6F-Glc-1P to bypass cotton biosynthesis

Acknowledgements

This research was supported by the European Union, ERC Consolidator project" BIOMATFAB" (Project #101045466), GIF and MINERVA Stiftung.

Bibliographic references:

Natalio, F. et al. Biological fabrication of cellulose fibers with tailored properties. Science 357, 1118-1122 (2017).
O. Aharon-Kuperman, P. de Andrade, X. Sui, T. Terlier, J. J. K. Kirkensgaard, R. Field, F. Natalio, under review.

Molecular basis for substrate recognition and septum cleavage by AtlA from *Entercoccus faecalis*

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The cleavage of septal peptidoglycan at the end of cell division facilitates the separation of the two daughter cells. The hydrolases involved in this process (called autolysins) are potentially lethal enzymes that can cause cell death; their activity, therefore, must be tightly controlled during cell growth. In Enterococcus faecalis, the N-acetylglucosaminidase AtlA plays a predominant role in cell separation. atlA mutants form long cell chains and are significantly less virulent in the zebrafish infection model. The attenuated virulence of atlA mutants is underpinned by limited dissemination of bacterial chains in the host organism and more efficient uptake by phagocytes that clear the infection.

AtlA has structural homologs in other important pathogens, such as Listeria monocytogenes and Salmonella typhimurium, and therefore represents an attractive model for designing new inhibitors of bacterial pathogenesis. Here, we provide the crystal structure of the E. faecalis AtlA catalytic domain and a catalytic mechanism for the hydrolysis of the glycosidic bond. A model of the AtlA–substrate complex helped us identify key residues critical for substrate recognition and septum cleavage during bacterial growth. We propose this work will provide helpful information for the rational design of specific inhibitors targeting this enterococcal virulence factor and its orthologs in other pathogens.

Acknowledgements

We thank Laurent Hoffer (Cancer Research Center of Marseille, Marseille, France) for his help with the docking experiment and Gerlind Sulzenbacher fo

Bibliographic references:

Eckert, C., Lecerf, M., Dubost, L., Arthur, M., and Mesnage, S. (2006) J. Bacteriol. 188, 8513–8519
Mesnage, S., Chau, F., Dubost, L., and Arthur, M. (2008) J. Biol. Chem. 283, 19845–19853
Lipski, A., Herve, M., Lombard, V., Nurizzo, D., Mengin-Lecreulx, D., Bourne, Y., and Vincent, F. (2015) Glycobiology 25, 319-330

En route to the synthesis of mannosidase-resistant oligomannoside mimetics as anti-HIV vaccine

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Despite the development of highly efficient treatments preventing HIV contagion, 1.46 billion people still get infected every year. Some surface proteins of HIV-1 envelope are found under a glycosylated form, bonded with specific oligomannans. As theses oligomannans are targeted by several neutralizing antibodies, they present an interest in the development of anti-HIV vaccines.[1] Classic approaches of vaccination with mannosidic glycosides were unsuccessful. One hypothesis is that mannosidase-type enzymes present in blood trimmer oligomannosides into shorter chains during immunization process, making neutralizing antibodies non-specific to the molecules used for vaccination.[2] To validate our concept, we have focused on the synthesis of non-hydrolyzable mannans: thiomannans. We assume that a mannan bearing *S*- rather than *O*-glycosidic bonds could present a better tolerance towards blood mannosidases. Moreover, to make the mannan more distinct than others, our target will be an analog based on the LPS of *rhizobium radiobacter* Rv3 [3], which is recognized as a functional glycomimetic of the D1 arm of mannans expressed by HIV. To this end, we designed the synthesis of heptamannoside **1**. The retrosynthetic scheme presented in Fig. 1 consists in the synthesis of five building blocks. The synthesis of derivative **3** is crucial as the introduction of the thioacetyl moiety allows us to do stereocontrolled glycosylation (due the participation of the acetyl group). The most challenging reaction is the synthesis of the thioglycosidic bond as it represents the key of this project.

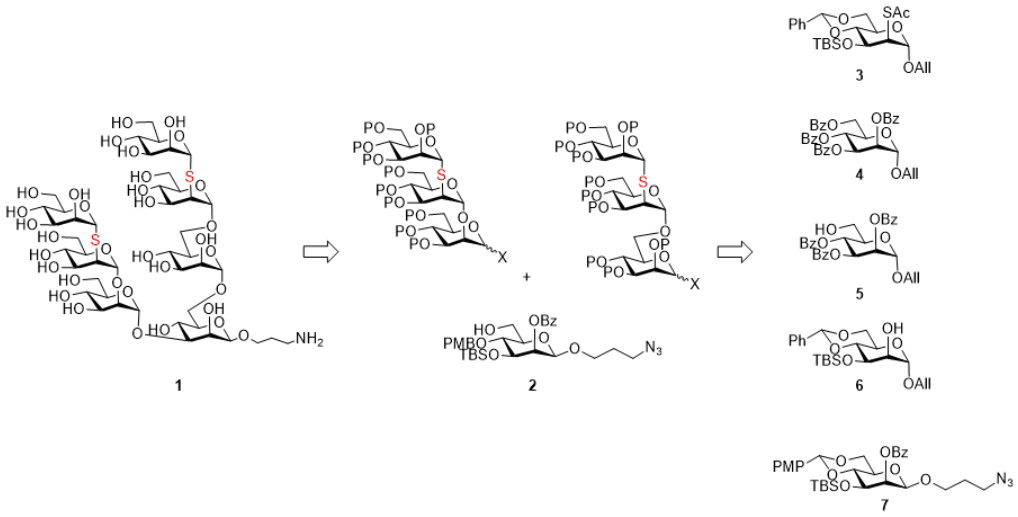

Figure 1: retrosynthetic scheme for the synthesis of 1

Acknowledgements

We thank NIH program R21 for the funding of this project.

Bibliographic references:

[1] Doores, K. J., FEBS J. 2015, 282, 4679-4691.
[2] Bruxelle, J.-F.; Kirilenko, T.; Qureshi, Q.; Liu, N.; Trattnig, N.; Kosma, P.; Pantophlet, R., Sci. Rep. 2020, 10, 7582.
[3] Pantophlet, R.; Trattnig, N.; Murrell, S.; Lu, N.; Chau, D.; Rempel, C.; Wilson, I. A.; Kosma, P., Nat. Commun. 2017, 8, 1601.

Characterization of a panel of isolates to inform vaccine design against *Klebsiella pneumoniae*

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Klebsiella pneumoniae (Kp) is a leading cause of neonatal sepsis in low- and middle-income countries (LMICs). In neonates, Kp is the major infectious disease killer globally and 87% of deaths are AMR-related[1].Two types of polysaccharides are expressed on Kp cell surface and have been proposed as key vaccine antigens: capsular polysaccharides or K-antigens (K-Ag) and O-antigens (O-Ag). Kp has been classified historically by capsule serotyping and of the 141 distinct K-Ag identified to date, only 79 have been classified[2]. In contrast, 11 distinct OAg serotypes have been described[2]. Considering their relevance for the design of Kp effective vaccines, the main goal of this work is to characterize 165 isolates enrolled to the BARNARDS study which aimed to identify the cause and burden of AMR in neonatal sepsis for LMICs.

Surface polysaccharides have been isolated from all the strains and both K- and O-Ag have been characterized in terms of expression levels, molecular weight and monosaccharide composition. Through a synergistic approach involving Nuclear Magnetic Resonance and Gas-Liquid Chromatography Mass Spectrometry, new molecular structures have been determined for unknown K-Ag belonging to the most epidemiological relevant Kp antigens associated neonatal sepsis.

Structural analysis/chemotyping of K- and O-Ag from clinical isolates is key to elucidate the sero-epidemiology specific to neonatal sepsis in LMICs and will contribute to define the design of an optimal vaccine composition for a maternal immunization strategy to prevent neonatal sepsis caused by *Kp* infections.

Bibliographic references:

1. Antimicrobial Resistance Collaborators, Lancet, 2022

2. Patro LPP, Frontiers, 2019

Insight of the molecular mechanisms of *A. oryzae* β-galactosidase transgalactosylation system

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The transgalactosylation system of Aspergillus oryzae's β-galactosidase has been successfully used for the synthesis of galactosides. To carry out this reaction it is necessary the presence of both a galactose donor (lactose/ONPG) and an acceptor molecule with a free hydroxyl group available for reaction. However, the chemical nature of the acceptor molecule determines the interactions with the enzyme's active site and therefore the specificity and efficiency of the reaction.

To provide a better understanding of the molecular mechanisms of this transgalactosylation system, we propose a combined approach of in silico and experimental studies, using acceptor molecules with differentiated structural characteristics.

Five molecules were selected: benzyl alcohol, tyrosine, dopamine (all with aromatic character), as well as lactic acid and ethyl lactate. The synthesis conditions (reaction time, temperature, acceptor, donor, and enzyme concentrations) were optimized, each galactoside was purified and its structure was determined by NMR. The enzymatic synthesis of the galactosides was successful only for benzyl- and ethyl lactate-galactosides, indicating that the phenolic hydroxyl groups are not appropriate acceptors, while the esterification of lactic acid is necessary for the reaction to occur. The galactosyl acceptors were docked into the active site of the enzyme covalently bound to galactose, followed by molecular dynamics experiments.

Results obtained with complementary in silico and experimental strategies, provide some insights to understand the transgalactosylation reaction.

Acknowledgements

This work was supported by Programa de Desarrollo de las Ciencias Básicas (PEDECIBA, Uruguay) and Agencia Nacional de Investigación (ANII, Uruguay)

Bibliographic references:

C. Porciúncula González, A. Cagnoni, C. Fontana, K. Mariño, P. Saenz Méndez, C. Giacomini, G. Irazoqui (2021) Bioorg. Med. Chem. 44(116309-19)

C. Porciúncula González, A. Cagnoni, K. Mariño, C. Fontana, P. Saenz Méndez, G. Irazoqui, C. Giacomini (2019) Carb.Res. 472(1-15)

C. Porciúncula González, C. Giacomini, G. Irazoqui. (2019) Editor: Nova Sci. Pub., Inc., NY, E. Kras (65-115)

Synthesis of photoswitchable glycosides as chiral dopants for liquid crystals,

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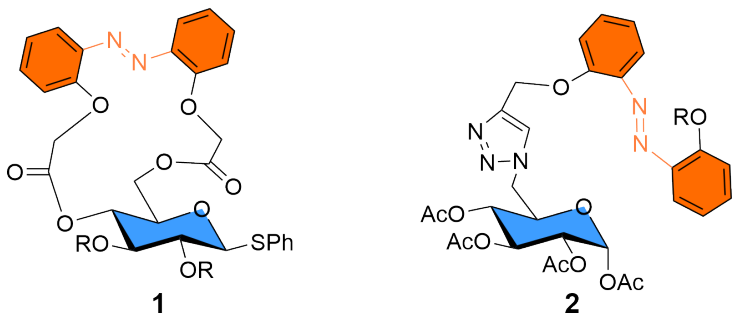
Carbohydrates and derivatives are of great interest because of their natural existence, their interesting biological and physicochemical properties, which offer potential applications in various fields.[1] One of the actual approaches to control molecular properties is to use the light as external stimulus by using photoswitchable species, especially photochromes such as azobenzene which can be reversibly isomerized by light into isomers featuring different structural and electronic properties.[2]

Since several years, we are developing in the group new photoresponsive molecules, such as photoswitchable glycomacrocycles and their acyclic analogues. Particularly for photoswitchable glycomacrocycles, we have shown their interesting photochemical and supramolecular properties like their ability to chirality transfer, organo-gel formation and their possibility to be used as chiral dopants for liquid crystals (LC).[3,4,5]

In this project, we designed and synthesized two new series (macrocyclic, acyclic) of photoswitchable glycosides, containing azobenzene and natural carbohydrates, in order to explore their potential as future chiral dopants for LC. Under light irradiation, it is possible to modulate or switch the orientation of LC molecules to achieve desired optical and mechanical properties of the system remotely and selectively. Also, adding a chiral dopant into a commercially available achiral nematic liquid crystal host, at the appropriate concentration, represents a convenient and economical approach to develop chiral LC. [5,6]

Macrocyclic serie **Acyclic serie**



1 **2**

R = C_6H_{13}, 4-CN-biphenyl-4'-OC_6H_{12}

Structure of target compounds.

Bibliographic references:

[1] J. Xie, N. Bogliotti (2014), Chem. Rev. (114) 7678-7739.
[2] A. A. Beharry, et al. (2011), Chem. Soc. Rev. (40) 4422-4437.
[3] C. Lin, et al. (2017), Chem. Eur. J. (23) 14996-15001.
[4] Y. Kim, et al. (2020), ACS Appl. Mater. Interfaces (12) 52146-52155.
[5] Y. Kim, N. Tamaoki (2019), ChemPhotoChem. (3) 284-303.
[6] L. Wang, Q. Li (2018), Chem. Soc. Rev. (47) 1044-1097.

Development of potential new vaccine adjuvants inspired by Lipid A structure of *Bacteroides fragilis*

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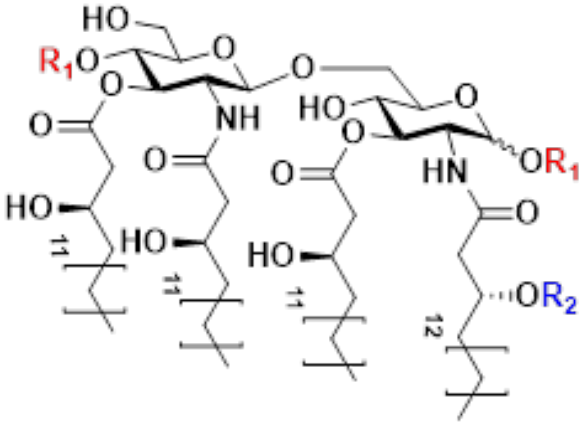
Adjuvants are components of vaccine formulations widely used to enhance their overall efficacy, providing an enhancement in the response towards antigens and allowing for the use of smaller doses of vaccine active ingredient[i]. Up to date, only few adjuvants are licensed and, only recently, their mechanism of action started to be understood. Hence, new efforts are needed for the research of novel chemical entities to be developed as adjuvants.

Bacteroides fragilis is a Gram-negative bacterium belonging to the human gastrointestinal microbiota, involved in the homeostasis of host immune system. Recently, new Lipid A moieties of this bacterium have been isolated, showing promising immunomodulatory activities[ii]. This feature, coupled with their probable low toxicity, makes them interesting candidates for the development of new vaccine adjuvants. In fact, some low toxicity Lipid A-based compounds can be used in vaccine formulations, as demonstrated by the authorization for the clinical use of "Monophosphoryl Lipid A", the first TLR-agonist vaccine adjuvant[iii].

The glycolipid mixture isolated from *B. fragilis* is only partially characterized and their exact structures is still not completely established. In this context, our project is focused on the chemical synthesis of a small library of Lipid A structures (Figure 1), based on the available information, with the aim to define and clarify their chemical structures and their immunological properties, as well as to investigate their potential application as vaccine adjuvants.



R_1 = H, $PO(OH)_2$ or $PO(ONa)_2$

R_2 = H or $CO(CH_2)_{13}CH_3$

Figure 1. Schematic representation of the designed lipid A library.

Bibliographic references:

[i] R. L. Coffman, A. Sher, R. A. Seder. (2010), Immunity (33).
[ii] D. Erturk-Hasdemir, et al. (2019), Proc National Acad Sci (116), 26157–26166.
[iii] R. N. Coler, S. Bertholet, M. Moutaftsi, J. A. Guderian, H. P. Windish, et al. (2011) PLOS ONE 6(1): e16333.

Chromatography-free deprotection of benzylidene acetals and p-methoxybenzyl ethers

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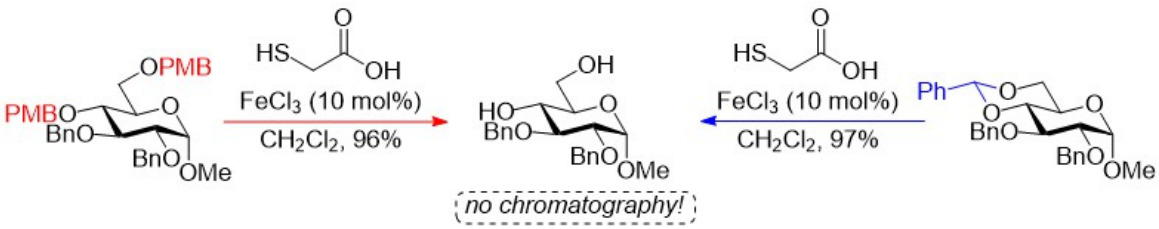
We demonstrate a new and high-yielding method for removal of benzylidene acetals and para-methoxybenzyl ethers under catalytic conditions with mercaptoacetic acid as a scavenger. The reaction co-products are converted to water-soluble molecules, which can be removed by aqueous extraction thereby bypassing the need for chromatographic purification. The reaction was demonstrated on both multi-milligram and multigram scale.



Acknowledgements

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Bibliographic references:

R. D. Greve, H. H. Jensen, (2023) Org. Lett. accepted for publication

Chemoenzymatic approach to produce rhamnoxylolipids

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Rhamnolipids (RLs) are a class of biosurfactants consisting of one or two rhamnose units linked to β-hydroxylated fatty acid chains. They are value-rich molecules because their chemical-physical properties and biological activities [1] are promising for the use in several fields, such as: detergents [2]; active agents in skin re-epithelialization; additives in the cosmetics industry [3]; as antibacterial, antifungal, antiviral and antitumoral agents in biomedicine. However, rhamnolipids are essentially obtained by fermentation of opportunistic bacteria, namely strains of *Burkholderia*, *Acinetobacter* or, mainly, of *Pseudomonas* [1]. Moreover, any microbial fermentation produces a mixture of rhamnolipid congeners with variations in the chain length and in the degree of unsaturation of the fatty acid chains, as well as differences in the number of rhamnose molecules, with the overall consequence that different production batches do not have the same composition. To overcome these troubles, new approaches have recently been pursued: a fully synthetic strategy [4] or their production in a cell-free (or in vitro) system [1]. Here, we propose a new chemoenzymatic strategy to produce a new class of RLs, the **rhamnoxylolipids** (RXL), that differ in the presence of a xylose monosaccharide as linker between rhamnose and lipids (Figure 1).

Therefore, the aim of our research activity is to create a library of compounds, obtained with a safe and targeted production, which could be excellent substitutes for rhamnolipids in dermatology industry.

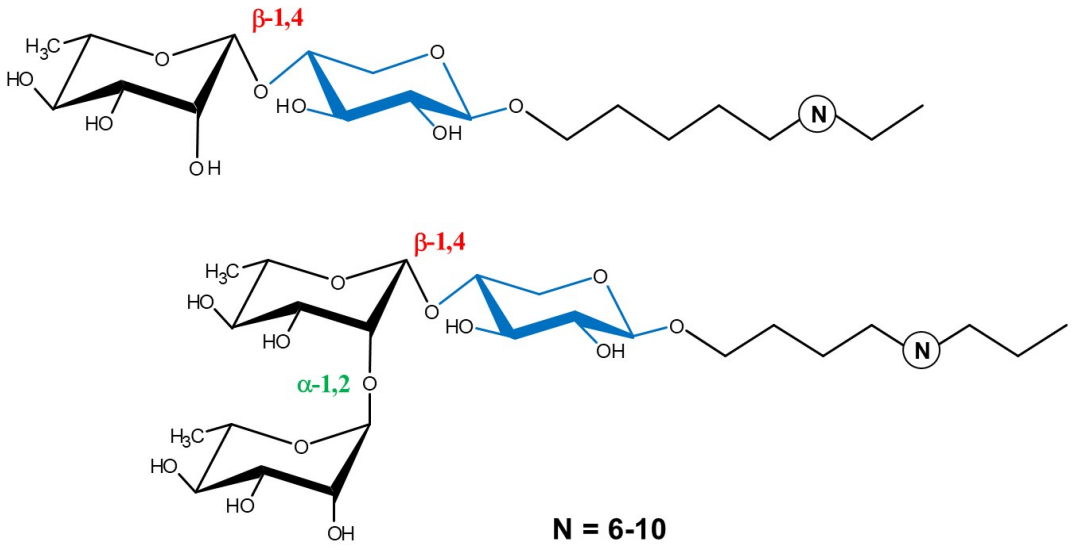

Figure 1. Examples of mono- and di-rhamnoxylolipids.

Bibliographic references:

[1] H. Chong, L. Qingxin (2017), Microbial Cell Factories (16), 137.
[2] A.M. Abdel-Mawgoud, F. Lépine, E. Déziel, (2010), Applied Microbiology and Biotechnology (86),1323-1336.
[3] T. Stipcevic, A. Piljac, G. Piljac., (2006), Burns (32), 24-34.
[4] F. Demeter, M.D.-T. Chang, Y.-C. Lee., T.-K. Fu, M. Herczeg, A. Borbàs (2020), Carbohydrate Research (496), 108102.

Rational design of picomolar sp^2-iminosugar-based mannobioside ligands of DC-sign

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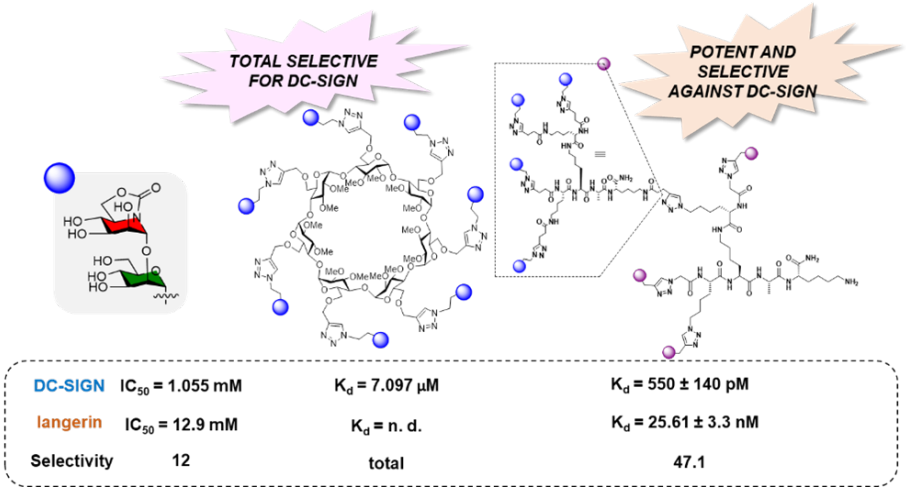
C-Type lectin receptors (CLRs) are present at the surface of antigen-presenting cells (APCs), notably in dendritic cells (DCs), allowing the recognition of carbohydrate-based PAMPS or DAMPS (pathogen- or danger-associated molecular patterns, respectively) and initiating adaptive immune responses. DC-SIGN and langerin are CLR expressed in DCs that recognize high mannose oligosaccharides (HMOs). Binding predominantly involves HMO terminal mannobioside residues (Manα1,2Man) for both lectins, making it difficult the design of ligands with discrimination capabilities [1]. Here we present a general strategy to target DC-SIGN preventing langerin binding that relies on manobioside mimetics where the reducing, non-reducing, or both mannose subunits have been replaced by sp^2-iminosugar motifs [2].

A comparative binding affinity study of monovalent ligands was first conducted using a range of techniques (tr-NOESY and STD NMR, computational methods and SPR) [3]. The most promising candidates were further "clicked" onto different scaffolds (β-cyclodextrin, RAFT cyclopeptides, polylysine dendron, and cyclotriphosphazene) to obtain 4-, 6-, 7-, 14-, 16- and 24-valent species. The impact of valency, structure, and size of the conjugates on DC-SIGN and langerin affinity have been investigated using SPR, and selective and potent multivalent ligands for DC-SIGN were identified. These results validate the hypothesis that sp^2-iminosugar-based glycoligand mimetics enable customizing the lectin binding profile in a structure- and valency-dependent manner. From a general perspective, these results open new opportunities to modulate the interaction with lectins involved in the immune response.



Bibliographic references:

[1] H. Feinberg et al (2011), Mol. Biol. (405,1027-39.
[2] E. M. Sánchez-Fernández et al., (2012), Chem. Eur. J., (18), 8527-39.
[3] I. Herrera-González et al., (2022), Chem. Commun., (58), 12086-89.

Development of glycomimetics as a novel herpesviral glycoprotein UL141 antagonists

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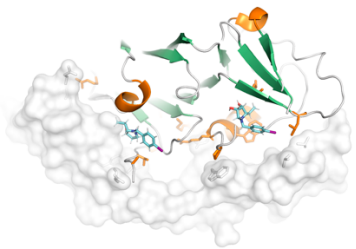
The worldwide seroprevalence of human cytomegalovirus (HCMV, a beta herpesvirus) is estimated to 83% (a global CMV seroprevalence) in the general population [1]. Usually, it is controlled by a vigorous immune response so that infections are asymptomatic or symptoms are mild. However, if the immune system is compromised, HCMV can replicate to high levels and cause serious end organ disease [2]. Although HCMV represents a complex target, the rounds of iterative studies could potentially bring this important and under-recognized human pathogen under control. Over four receptor-binding patches (RBP) of the glycosylated UL141 protein is capable of binding to human TRAIL death receptor 2 (TRAIL-R2) [3] and trigger the NK signaling pathway to benefit viral fitness [4, 5, 6]. Hence, it is rational to inhibit the RBP activity of the UL141 protein by blocking the RBP interaction with TRAIL-R2, which makes the glycosylated UL141 protein a potential target for designing and developing antiviral agents. In this study, the molecular features of the UL141 of HCMV are highlighted, such as the structure, functions, and interactions of the UL141 and TRAIL-R2. Furthermore, the development of glycomimetic structures by computational design and biochemical testing is reported. The aim is to develop the short peptide or synthetic compound (UL141 antagonist) based on our recent crystal structure and computational design that would specifically bind viral UL141 to block receptor binding thus prevent the viral action. This is relevant, as the UL141 is also the most abundant HCMV protein on plasma membrane and it is also a component of the virion. Based on our computational screening of iminosugars the 'hit' structure was selected. We test a small library of synthetized compounds (potential UL141 antagonists) that would block the receptor binding *in vitro*, on the cell or virion surface. Series of compounds that have been tested are of glycomimetics structures consisting of various saccharide units linked with non-saccharide. In particular; non-ionic glycolipids, 'click'-conjugates or iminosugars. The ELISA-like TMB assay has been used in combination with dynabeads™ coating to test whether the compound could block the TRAIL-R2 binding. Five most promising compounds out of 23 tested have proven the ability to block UL141/TRAIL-R2 complex formation. SPR kinetics analysis was then used to determine the binding constants (K_D). The affinities to UL141 were determined in low micromolar scale. The successful compounds will be further optimized by using *in silico* methods to target particular epitope on viral glycoprotein UL141 derived from our structural analysis and tested *in vivo* for HCMV inhibition.



UL141 (cartoon) and TRAIL-R2 (surface) in complex. The binding of UL141 antagonist (cyan molecules).

Acknowledgements

This research was funded by the Slovak Research and Development Agency (APVV-19-0376) and Scientific Grant Agency (VEGA 02/0026/22 and 02/0060/21).

Bibliographic references:

[1] M. Zuhair, et al. (2019), Rev. Med. Virol. (29) e2034; [2] G. Picarda, C. A. Benedict (2018), J. Immunol. (200) 3881. [3] I. Nemčovičová, C. A. Benedict, D. M. Zajonc (2013), PLoS Path. (9) 1003224; [4] I. Nemčovičová, D. M. Zajonc (2014), Acta Cryst. (70) 851; [5] W. Smith, et al. (2013), Cell Host Mic. (13) 324; [6] P. Tomašec, E. C. Y. Wang, et al. (2005), Nat. Immunol. (6) 181.

Biolayer interferometry – a soft technique for tough analysis of carbohydrate-lectin interactions

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Biolayer interferometry (BLI) is a label-free method used to detect biomolecular interactions by analyzing the interference pattern of reflected white light from two sensor surfaces (**Figure 1A**). Due to its speed, ability, and sensitivity, it enables to readily detect analytes even in complex matrices such as blood serum [1], as its response depends solely on the thickness of the bio-layer and not on the optical properties of the sample environment. BLI provides more reproducible data than other commonly used assays like ELISA [1], may require fewer samples and less specific antibodies for detection. Therefore, it is an emerging complementary method for characterizing protein-carbohydrate interactions, which is essential for designing better diagnostic and therapeutic tools.

This study demonstrates various aspects of using BLI for determining the kinetics of interactions between a library of galectins and various glycoconjugates. We compared two labels for protein immobilization: His-tag for complexation with nickel ions or *in vivo* monobiotinylated Avi-tag, which binds to streptavidin-coated sensors (**Figure 1B**). Advantageously, the biotin-streptavidin binding achieves a low equilibrium dissociation constant K_D and is highly selective for the biotin epitope. In addition, the blocking of the sensor surface with biocytin (lysine conjugated with biotin) reduced the non-specific interaction for assessing interactions in complex matrices such as blood serum. Thus measurement of high-affinity glycoconjugates [2] showed no significant effects between K_D values measured in blood serum and PBS buffer.

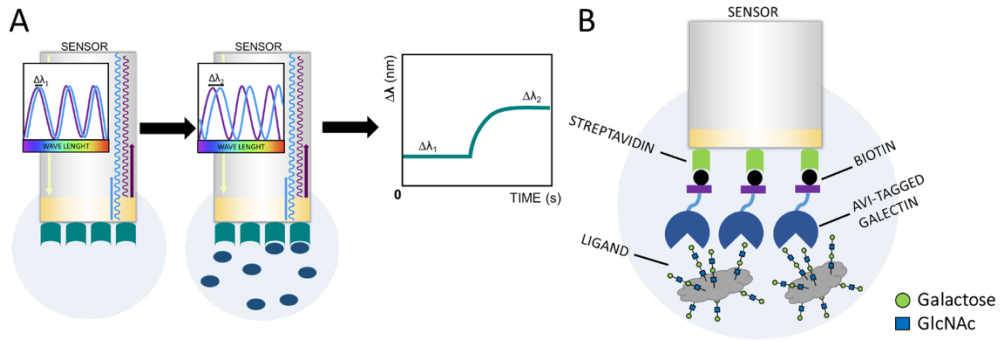

Figure 1. 1A – principle of biolayer interferometry; 1B Streptavidin coated sensor with Avi-tagged G

Acknowledgements

This study was supported by the project 22-00262S of the Czech Science Foundation.

Bibliographic references:

[1] A. Li et al., Fish Shellfish Immunol., 2021, 119, 231-237
[2] D. Vrbata et al., J. Med. Chem., 2022, 65 (5), 3866-3878

Probing aberrant glycosylation using lectin-based glycoprotein microarrays

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To detect aberrant glycosylation, various bioanalytical methods can be employed. We focus on the development and application of affinity techniques based on glycan-binding molecules on a microarray platform. Typically, lectins are used as biorecognition elements, which are proteins recognizing glycan structures enabling glycoprofiling of proteins, cells, and tissues. Our group develops lectin-based glycoprotein microarray assays in the reverse-phase format, the technique defined as a high-throughput approach enabling simultaneous and rapid analysis of a large cohort of samples by a set of lectins without the need for the separation of glycans from proteins [1,2]. The microarray biochip is prepared by spotting of tens to hundreds of samples on the microarray substrate which is then allowed to interact with a panel of lectins. This method provides effective glycoprofiling of samples and screening/analysis of glycan biomarkers having huge applications in biomedicine, biology, and biotechnology.

We have applied our microarray platform for the study of glycan changes in a number of various cases, e.g. cancer, gestational diabetes mellitus, kidney diseases, COVID-19, congenital disorder of glycosylation (CDG), attention-deficit hyperactivity disorder (ADHD), age-related glycosylation changes, or glycostructure of therapeutic proteins. Herein, we present our approach and some of the obtained results.

Acknowledgements

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Bibliographic references:

[1] D. Robajac, M. Križáková, M. Šunderić, G. Miljuš, P. Gemeiner, O. Nedić, J. Katrlík (2022), Methods in Molecular Biology. Glycan Microarrays: Methods and Protocols, 207-222.
[2] P. Damborský, M. Zámorová, J. Katrlík (2016), Proteomics (16), 3096-3104.

Sialic acid determinations by HPAE-PAD choosing the appropriate method

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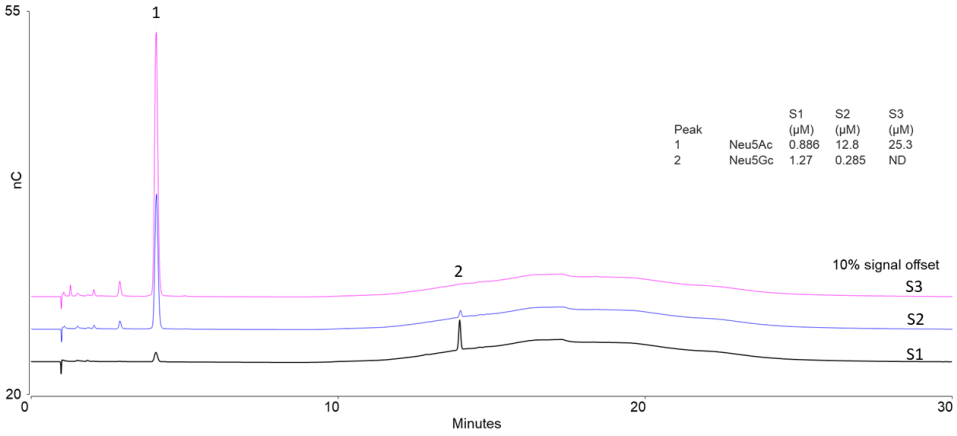
High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) was introduced over 40 years ago for the sensitive direct determination (no analyte derivatization) of carbohydrates. Typically, the carbohydrates are separated in high pH mobile phases which is also the alkaline environment needed for the electrochemical detection of carbohydrates. Shortly after the introduction of HPAE-PAD, it was applied to the determination of the sialic acids N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) from glycoproteins and glycolipids. Since then, multiple methods have been developed for a variety of samples and analytical goals.

These applications include the determination of sialic acids in glycoprotein drug products, the rapid determination of sialic acids to screen the efficacy of cell lines, the determination of sialic acids in milk and milk-containing products, the determination of sialic acids in serum, and the recent application of technology that enables online generation of the mobile phases needed for HPAE-PAD to sialic acid determinations. This poster will review these methods and include recommendations for choosing the method that is appropriate for the desired analytical outcome.



Determination of the Sialic Acid Content of Glycoproteins by HPAE-PAD with Eluent Generation

Acknowledgements

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Synthesis of potentially anticancer azido/guanidino nucleosides

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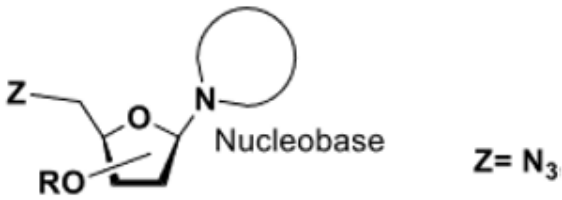
The synthesis of nucleosides, nucleotides as well as their analogs/mimetics have attracted much attention in organic and medicinal chemistry, due to their propensity to exhibit a variety of biological properties. Numerous examples of nucleoside and nucleotide analogs achieved clinical application as anticancer or antiviral drugs, acting through interference with nucleic acid biosynthesis [1].

The ability of these types of molecules to show antimicrobial effects [2] or to inhibit cholinesterases [3] has also been reported. The search for new structures of nucleos(t)ide analogs that may potentiate innovative mechanisms of action and open new therapeutic opportunities remain of interest.

In this context, in this communication we report on the synthesis of novel 5-azido/guanidino nucleosides based on a xylofuranose template. The synthetic pathway employed diacetone- D-glucose as precursor and involved the access to an acetylated 5-azidoglycosyl donor and its further N-glycosydation with uracil or with a purine derivative. Conversion of the azido nucleosides into their guanidino derivatives was then exploited. An interesting result during the synthesis of the glycosyl donor precursor was the access to an imino sugar via an intramolecular Boyer reaction. Herein our results will be presented and discussed.



General structure of the synthesized nucleosides.

Acknowledgements

We thank FCT for funding through grant CEECIND/03881/2018, project EXPL/MED-QUI/1017/2021, and project UIDB/00100/2020, UIDP/00100/2020.

Bibliographic references:

[1] Shelton, J.; Lu, X.; Hollenbaugh, J. A.; Cho, J. H.; Amblard, F.; Schinazi, R. F. Chem. Rev. 2016, 116, 14379. [2] M. Serpi, V. Ferrari, F. Pertusati, J. Med. Chem. 2016, 59, 10343–10382. [3] R. G. Pereira, M. P. Pereira, S. G. Serra, A. Loesche, R. Csuk, S. Silvestre, P. J. Costa, M. C. Oliveira, N. M. Xavier, Eur. J. Org. Chem. 2018, 2667 2681.

Stereoselective access to iminosugar *C,C*-glycosides

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Naturally occurring six-membered 1-deoxyiminosugars have been extensively used as non-covalent probes to decipher the mechanism of glycosidases by virtue of their mimicry of the oxocarbenium character of the enzyme transition state.[1,2] In the search for other therapeutic candidates, mimicking the aglycon moiety of the enzymatic substrate with the introduction of a pseudo-anomeric substituent has been pursued, leading to a vast array of so-called iminosugar *C*-glycosides.[3] Within this family, introduction of an extra carbon substituent at the pseudoanomeric position to yield iminosugar *C,C*-glycosides is worth investigating as it might lead to new potent and selective glycosidase inhibitors. In contrast with the broad palette of methods allowing access to iminosugar *C*-glycosides,[4] syntheses of iminosugar *C,C*-glycosides are scarce illustrating the challenging introduction of a quaternary carbon atom alpha to a nitrogen atom.

In this context, we have developped a robust route to original iminosugar *C,C*-glycosides exploiting a Staudinger aza-Wittig (SAW) reaction applied to 6-azidoketosugars.[5] The last developments of our strategy will be presented.

Target azepane and piperidine C,C-glycosides

Bibliographic references:

[1] R. J. Nash, A. Kato, C.-Y. Yu, G. W. Fleet (2011), Future Med. Chem. (3), 1513-1521.
[2] C.-H. Lin et al. (2010), ACS Chem. Biol. (5), 489-497.
[3] P. Compain, in Iminosugars; Compain, P., Martin, O. R. (2007), Eds.; John Wiley & Sons, Ltd: Chichester, UK, 63-86.
[4] P. Compain, V. Chagnault, O. R. Martin (2009), Tetrahedron Asymm. (20), 672-711.
[5] Z. Debbah, J. Marrot, N. Auberger, J. Désiré; Y. Blériot (2022), Org. Lett. (24), 4542-4546.

Clustering of mycobacterial ligands and DC-SIGN are key determinants for pathogen recognition

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The bacterial pathogen *Mycobacterium tuberculosis* binds to the C-type lectin DC-SIGN on dendritic cells to evade the immune system. While DC-SIGN glycoconjugate ligands are ubiquitous among mycobacterial species, the receptor selectively binds pathogenic species from the *M. tuberculosis* complex (*MTBC*). Here, we unravel the molecular mechanism behind this intriguing selective recognition by means of a multidisciplinary approach combining single-molecule atomic force microscopy with Förster resonance energy transfer and bioassays.

Molecular recognition imaging of mycobacteria demonstrates that the distribution of DC-SIGN ligands markedly differs between *Mycobacterium bovis* BCG (model *MTBC* species) and *Mycobacterium smegmatis* (non-*MTBC* species), the ligands being concentrated into dense nanodomains on *M. bovis* BCG. Upon bacteria-host cell adhesion, ligand nanodomains induce the recruitment and clustering of DC-SIGN. Our study highlights the key role of clustering of both ligands on *MTBC* species and DC-SIGN host receptors in pathogen recognition, a mechanism that might be widespread in host-pathogen interactions.

Synthesis of difluoromethylene bisphosphonate mimics as probes for bacterial capsular polysaccharide

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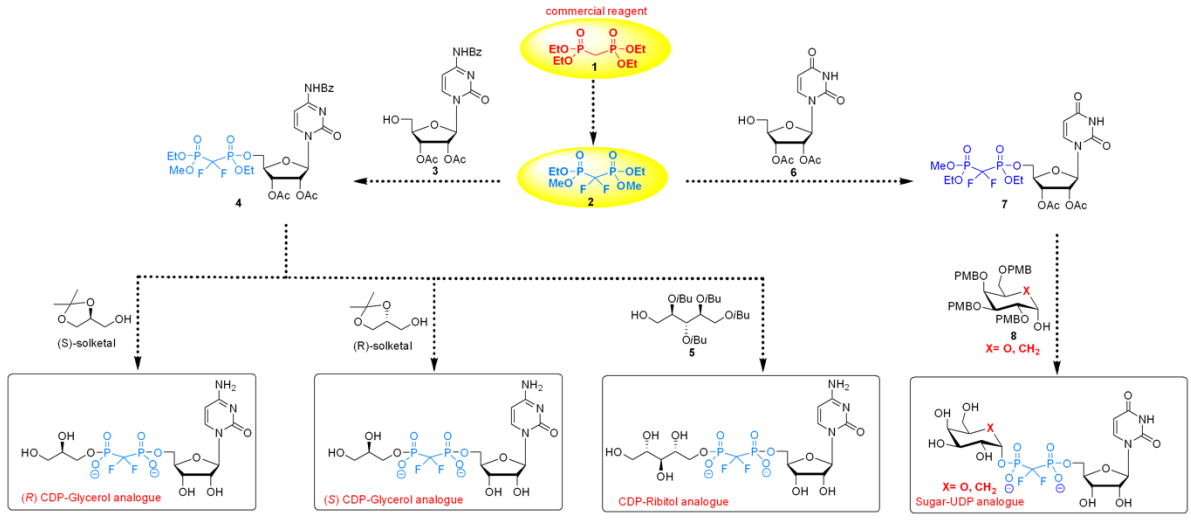
Poly(glycosylpolyol phosphate) polymerases and glycosyltransferases are significant enzymes for the biosynthesis of virulence-associated polysaccharides of several pathogenic Gram-negative bacteria. These enzymes construct polysaccharide chains using precursors that contain a pyrophosphate diester structural motif, such as CDP-glycerol, CDP-ribitol and Gal-UDP[1]. The sensitivity of the P-O-P for both enzymatic and chemical hydrolysis offers a unique opportunity for the design and synthesis of stable non-hydrolysable inhibitors. In particular, difluoromethylene bisphosphonate-linked (P-CF_2-P) analogues are highly sought after as versatile biochemical tools.

These analogues closely resemble the natural pyrophosphate, in terms of pKa-value[2] (as the electron-withdrawing effect of fluorine atoms increase the acidity of the phosphonates), as well as the bond angles and lengths. We will present a synthetic method to access P-CF_2-P-substituted analogues of several nucleotide phosphate donors, designed to study the interactions between these molecules and their cognate enzymes, to advance our understanding of the biosynthesis of components of bacterial cell wall and contribute to the development of novel therapeutic agents, and antimicrobial strategies.



Acknowledgements

This work was supported by the Chinese Scholarship Council (CSC grant to Jianyun Guo).

Bibliographic references:

(1) D. van der Es, W. F. J. Hogendorf, H. S. Overkleeft, G. A. van der Marel and J. D. C. Codée. Teichoic acids: synthesis and applications. (2017), Chem. Soc. Rev, (46), 1464-1482.

(2) G. M. Blackburn, D. A. England, F. Kolkmann. Monofluoro-and difluoro-methylenebisphosphonic acids: isopolar analogues of pyrophosphoric acid. (1981), J. Chem. Soc.,Chem. Commun. 930-932.

Substrate promiscuities of a bacterial galactokinase and a glucose-1-phosphate uridyltransferase enable xylose salvaging

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Galactokinases (GalKs) catalyse the transfer of a phosphate group from adenosine triphosphate (ATP) to galactose. Here we investigate the promiscuity of a bacterial galactokinase originating from the soil bacterium *Solitalea canadensis* (*Sc*GalK) for other monosaccharides than for the synthesis of diverse sugar-1-phosphates, which than can be further converted into sugar nucleotides to provide donors.

Together a previously uncharacterized glucose-1-phosphate uridyltransferase from the same soil bacterium (*Sc*GPUT), *Sc*GalK was able to convert D-xylose into uridine diphosphate xylose (UDP-Xylose) in the presence of ATP and uridine triphosphate (UTP), therefore showing the feasibility of a novel intrinsic salvage pathway for xylose in *Solitalea canadensis*.

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Bibliographic references:

*J.M. Shi, T.T. Wu, H. Zhou, Y.Y. Zhang, L. Liu, G. Widmalm, J. Voglmeir (**2022**), Green Chem. (24), 3717-3722.*
*L.P. Conway L.P., J. Voglmeir J. (**2016**) Carbohydr. Res. (432), 23-30.*

Efficient construction of homogenous glycoproteins

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Glycoproteins in cells are produced in a highly heterogeneous form. Thus, it is difficult to elucidate glycoprotein-mediated biological processes and to discover therapeutic agents. Therefore, general and efficient methods to obtain homogeneous glycoproteins are greatly demanded. We developed a general method for the efficient preparation of homogeneous glycoproteins that employs a combination of genetic code expansion and chemoselective ligation techniques. In this method, an alkyne tag-containing protein, that were produced by genetic encoding of an alkynylated unnatural amino acid, was quantitatively coupled via click chemistry to various azide-appended glycans.

The glycoproteins generated by this strategy were found to recognize mammalian cell-surface lectins and to enter the cells via the lectin-mediated endocytosis. In addition, the glycoprotein possessing multiple mannose-6-phosphate residues entered diseased cells lacking specific lysosomal glycosidases by binding to the cell-surface M6P receptor, and subsequently migrated to lysosomes for efficient degradation of stored glycosphingolipids.

Practical use of analytical tools for glycan structures for glycoproteins and cells

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We've tried new glycan engineering with the synergistic effects of chemical synthesis technology for oligosaccharides, development for anti-glycan antibodies, and practical use of endoglycosidase. As a technology related to the antibody drug field, we synthesized series of the structure-defined *N*-type glycans found on Fc region of antibody drug and achieved the separative production of 3-G1 and 6-G1 glycan isomers. We have also realized the development of antibodies for the non-human type of glycan epitopes using high-purity chemical synthetic glycans containing αGal and NeuGc which may cause anaphylactic symptoms as antigens, and the developed antibodies are capable of detection of antigenic antibody drugs using sandwich ELISA and western blotting. It was also confirmed that the antibody drug having this non-human type antigen can be captured by a recombinant lectin-agarose immobilized with high concentration by preferential affinity to αGal.

In addition, this lectin-immobilized resin can capture an artificial antibody possessing homogenous glycan structure produced by the transglycosylation reaction of endoglycosidase (Endo-M) with glycopeptides as a glycan donor. We have also succeeded in obtaining an antibody with a highly pharmacologically active glycosylation with a terminal galactose without core fucose.

Based on this technology base, we developed various types of glyco-conjugates in which chemically synthesized oligosaccharides are attached to proteins and chemically modified functional polysaccharides. Furthermore we attempt to develop unique tools and analytical techniques useful for functional research toward the glycocalyx of cells and microvesicles.

Transition-state analogue Inhibitors of *P. falciparum*, *T. Cruzi* and *T. Brucei* PPRTases

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The pathogenic protozoans such as *Plasmodium falciparum*, *Trypanosoma Cruzi* and *Trypanosoma Brucei* are responsible for malaria, Chagas disease and sleeping sickness respectively. These protozoan parasites are unable to synthesize purines *de novo* and rely on salvage of purine from the host. Purine salvage enzymes purine phosphoribosyltransferases (PPRTases) are critical for purine salvage and are potential targets for novel therapeutic development.

Transition states of N-ribosyl transferase enzymes have been characterized with all having some ribooxacarbenium ion characteristics. We have reported immucillin-H 5'-phosphate (ImmHP), immucillin-G 5'-phosphate (ImmGP), and some acyclic nucleoside phosphonates (AIP) as transition state analogue inhibitors of the *Plasmodium falciparum* PPRTase.

We have synthesised a range of cyclic and acyclic aza-*C*-nucleoside structural homologues (phosphates and phosphonates) as transition state analogue inhibitors of *Plasmodium* and *Trypanosoma* PPRTases and shown that some are selective and potent nanomolar inhibitors.

Bibliographic references:

1. Clinch, K.; Crump, D. R.; Evans, G. B.; Hazleton, K. Z.; Mason, J. M.; Schramm, V. L. Tyler, P. C.; Bioorg. Med. Chem. 2013 (21), 5629-5646.

2. Evans, G. B.; Furneaux, R. H.; Tyler, P. C.; Schramm, V. L. Org Lett 2003, 5 (20), 3639-3640.

3. Evans, G. B.; Furneaux, R. H.; Gainsford, G. J.; Schramm, V. L.; Tyler, P. C. Tetrahedron 2000, 56 (19), 3053-3062.

A chemoenzymatic approach to the synthesis of dolichol pyrophosphate linked oligosaccharide

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Dolichol pyrophosphate linked oligosaccharides (DLOs) are precursors of *N*-glycans. The DLOs are biosynthesized on the cytoplasmic side of the endoplasmic reticulum (ER) membrane with the construction of $Man_5GlcNAc_2$ structures. It is acknowledged that the $Man_5GlcNAc_2$-PP-Dol flips into the ER luminal side. However, the catalytic mechanism remains unclear [1,2].

Thus, we are aiming to reveal such a catalytic mechanism and develop a practical construction method of ER-mimicking liposomes having pure DLOs into the surfaces. In this research, we attempted to promote a chemoenzymatic approach by utilizing the activity of *N*-acetylglucosamine-phosphotransferase (DPAGT1) with the chemically synthesized UDP-GlcNAc probes, as well as endo-β-*N*-acetylglucosaminidase (ENGase) to the phosphorylated GlcNAc residues.

Bibliographic references:
[1] S. Sanyal, C. G. Frank, A. K. Menon (2008), Biochemistry (47), 7937-7946.
[2] A. Verchère, A. Cowton, A. Jenni, M. Rauch, R. Häner, J. Graumann, P. Bütikofer, A. K. Menon (2021), Sci. Rep. (11), 1411.

Towards the synthesis of bicyclic constrained carbacycles as competitive α-galactosidase inhibitors

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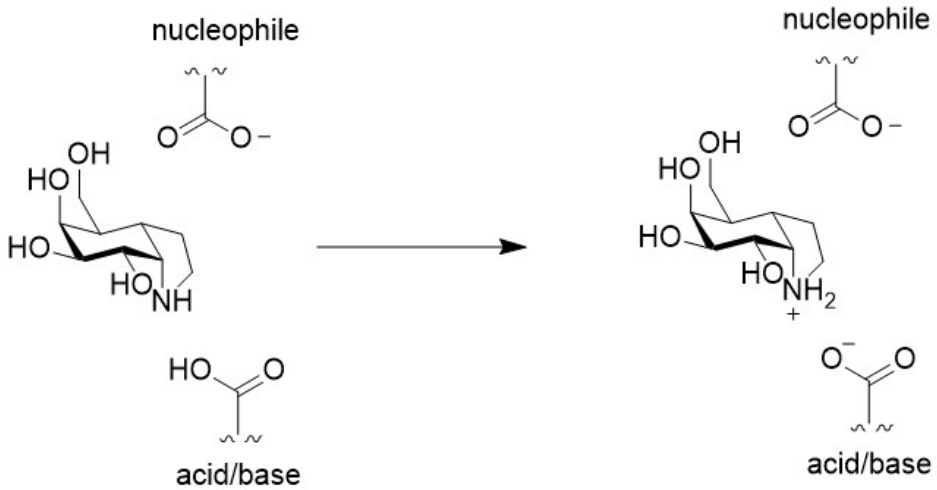
Fabry disease is an inherited lysosomal storage disorder caused by difficiency of α-galactosidase A (α-gal A). α-Gal A catalyses the hydrolysis of terminal, non-reducing α-galactose residues from globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). Mutations in the GLA gene encoding for α-gal A result in decreased or lack of activity of α-gal A leading to toxic accumulation of the glycosphingolipids Gb3 and lyso-Gb3. Current treatment is enzyme replacement therapy with a recombinant enzyme or pharmacological chaperone (PC) therapy, however both therapies have limited efficacy. Therefore, the joint administration of a recombinant enzyme with a pharmacological chaperone holds promise for a more effective treatment of Fabry disease.

Previous research for a new class of inhibitors showed that α-D-galactose-configured cyclosulfamidate stabilizes recombinant human a-D-galactosidase (agalsidase beta, Fabrazyme®) effectively. This cyclosulfamide is conformationally constrained and thereby mimics the Michaelis complex conformation. In a search for more potent and selective competitive inhibitors targeting α-gal A, it is thought that instead of the sulfamidate a nitrogen containing carbacycle, either exo- or endocyclic, might increase the potency through ionic interactions, while still keeping the selectivity. Here the synthesis towards these bicyclic contrained carbacycles is described.



Bibliographic references:

M. Artola, C. Hedberg, R. Rowland, et al. (2019) Chemical Science (10), 9233-9243.

Y. Harrak, C. Barra, A. Delgado, et al. (2011) Journal of the American Chemical Society (133), 12079-12084.

Synthesis and immunofunctional analysis of monoglycosphingolipids

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Monoglycosphingolipids such as GlcCer and GalCer are widely found in living organisms, and it has been reported that some of these glycosphingolipids modulate the immune system via innate immune receptors (eg. Mincle)[1] or lipid antigen-presenting molecules (CD1d).[2]There are many structural variations of lipid moiety as well as glycan part of glycosphingolipids, and the ratio of lipid length and structure varies depending on the type of organ or cell present,[3] as well as enzyme dysfunctions and other factors.[4] These structural transformations may affect the immunomodulatory function, but the details have not been clarified.

We therefore developed the synthetic strategy of diverse glycosphingolipids of GlcCers and GalCers having various lipids for building their compound library for further understanding of their immunomodulatory activities. We used allyl-type protecting group strategy to enable the synthesis of various GlcCers and GalCers containing lipid moieties with unsaturated bonds including regular sphingosine ceramide.[5] Firstly, allyl-protected glycosyl donor and sphingosine acceptor were prepared and used for the glycosylation to prepare the glycolipid backbone. After deprotection of the allyl-type protecting groups, subsequent selective amidation with various fatty acids gave GlcCers and GalcCers, containing a variety of lipids. We then evaluated biological activities of the synthesized glycosphingolipids, and characteristic immunological functions were observed.

Bibliographic references:

[1] S. Yamasaki et al. (2018), Int. Immunol. (30), 239.
[2] M. B. Brenner et al. (2011), Nat. Immunol. (12), 1202.
[3] Y. Ishibashi et al. (2013), Biochim. Biophys. Acta (1831), 1475.
[4] A. H. Futerman et al. (2011), J. Biol. Chem. (286), 30022.
[5a] Y. Fujimoto, H. Lotter et al. (2017), Sci. Rep. (7), 9472.
[5b] Y. Fujimoto et al. (2017), Chem. Eur. J. (23), 8304.

NMR Chemical Shift Prediction of Aglycone-linked Oligosaccharides using CASPER

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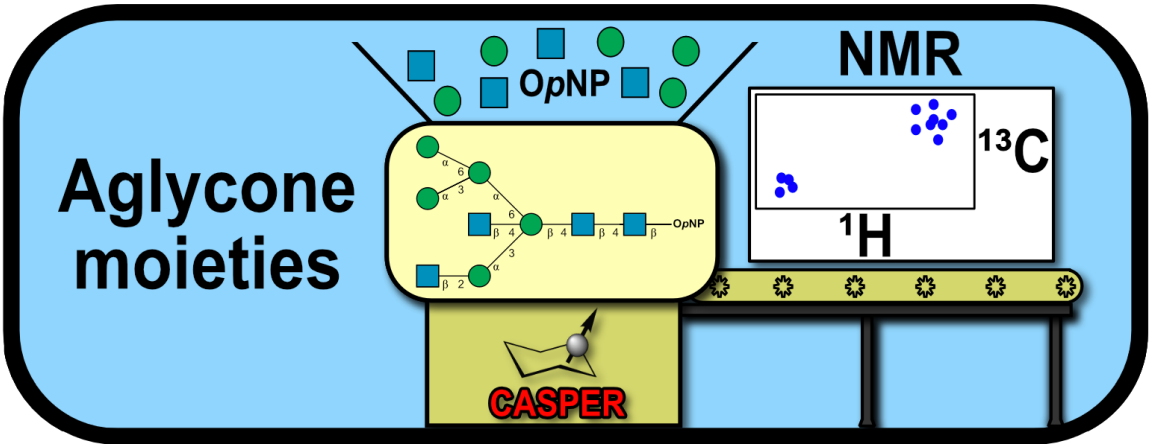
Advances in the structural characterization of carbohydrates: Carbohydrates play important roles in a plethora of biological processes.[1] Highly complex carbohydrate structures can be found in nature and isolated, though usually in small quantities. Elucidation of aforementioned structures with nuclear magnetic resonance (NMR) spectroscopy as the primary tool is a time-consuming and error-prone task.[2] In order to facilitate and partially automate this process dedicated software, such as CASPER (**C**omputer **A**ssisted **Sp**ectrum **E**valuation of **R**egular Polysaccharides), has been developed.[3]

With the developments of carbohydrate chemistry during the last decades, more complex structures can be obtained in a highly pure form via chemical/chemoenzymatic synthesis.[1,4] Often times, these synthetized structures are carrying an aglycone at the reducing end, making it suitable for e.g. conjugation to proteins or immobilization on microarrays. CASPER is now being developed to accommodate aglycones, such as aminoalkyl and p-nitrophenol substituents at the reducing end, both for NMR chemical shifts prediction of a user defined structure, as well as for automated structural elucidation of a structure based on unassigned NMR data.

This development provides a valuable tool not only for researchers focusing on elucidation of unknown structures and interaction studies by NMR, but furthermore for synthetic chemists seeking structural verification of their synthesized products, as well as tentative NMR chemical shift assignment.



Bibliographic references:

[1] Varki, A., et al. Eds. (2022) Essentials of Glycobiology, Fourth Edition.
[2] C. Fontana, G. Widmalm (2023) Chem. Rev. (3) 1040-1102.
[3] M. Lundborg, G. Widmalm (2011) Anal. Chem (83) 1514-1517.
[4] Demchenko, A. V. (2008) Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance. Wiley-VCH.

Biofunctional analysis of α-GalCer derivatives with CD1d-ligand complex stability modulation

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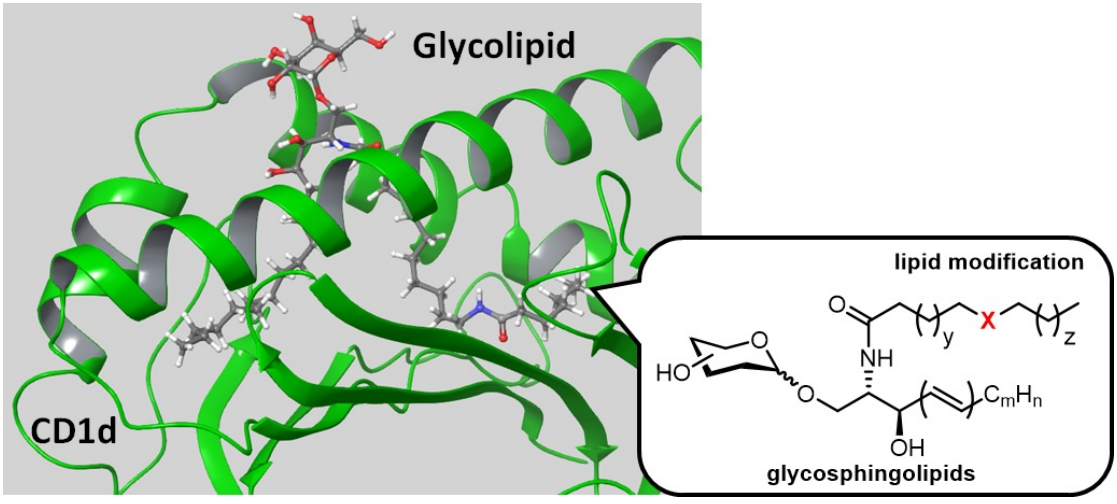
Glycolipid antigens play an important function in immune modulation by activating NKT cells through the formation of glycolipid-CD1d complexes on antigen presenting cells to secrete various cytokines. Examples of the antigens include glycolipids such as α-GalCer (KRN7000), bacterial α-GalCer$_{Bf}$, endogenous β-GalCer, and other complex lipids. We have previously shown that modification of the lipid moiety of α-GalCer (KRN7000) by polar functional groups strongly affects binding affinity and immune activity balance, but the mechanisms of these phenomena have been only partially investigated. Therefore, to elucidate the mechanism of immunomodulation by structural differences in CD1d ligands, we conducted synthesis and biofunctional evaluation of various glycolipids, and analysed the stability of CD1d-antigen complexes both experimentally and computationally. The synthesis of glycolipids used key intermediates to facilitate the introduction of various lipid structures. The desired glycolipids were obtained by global deprotection of these key intermediates followed by selective amidation. The biological functions of the obtained glycolipids were evaluated by measuring their binding affinity to CD1d. Modification of the lipid structure had a significant effect on the binding affinity. The thermal stability of the glycolipid-CD1d complex was also examined, combined with computational studies utilizing MD simulations. Details of the above experimental results will be further discussed in the poster presentation.



Bibliographic references:
a) Inuki, S.; Fujimoto, Y. et al. Angew. Chem. Int. Ed. 2018, 57, 9655. b) Inuki, S.; Fujimoto, Y. et al. ACS Chem. Biol. 2016, 11, 3132.

Rapid and stereoselective synthesis of novel azasugars as glycosyltransferase inhibitors

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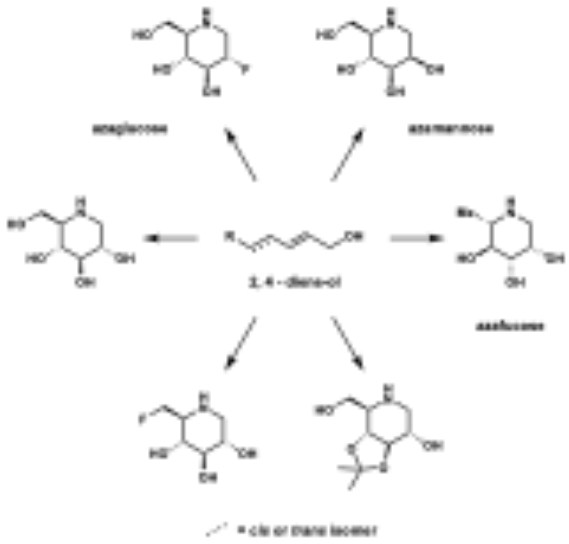
Glycosylation is known to play an essential role in cell signaling, immunity, and disease progression. Glycomimetics, such as azasugars, have proven to be effective therapeutics for both congenital disorders of glycosylation as well as viral infections. However, synthetic strategies for the synthesis of azasugars has remained extremely limited. We present a rapid, stereoselective, and diversifiable synthetic strategy to access a variety of valuable azasugar scaffolds.

Our collection of novel compounds are tested for biological efficacy as inhibitors of 1,2- and 1,3-fucosyltransferases, enzymes known to take part in essential cellular differentiation, gut cell health, and disease management. We utilize a uniquely synthesized FucT small molecule inhibitor in a novel assay to elucidate azasugars' ability to selectively target key glycoenzymes.



Diversification of an Azasugar Scaffold from A Common Starting Material

Combined experimental and computational study for the characterization of *Pseudoarthrobacter siccito*

Laura MASGRAU [1], André TABORDA [2], Tomás FRAZÃO [2], Miguel V. RODRIGUES [2], Xavier FERNÁNDEZ-LUENGO [1], Ferran SANCHO [3], Maria FÁTIMA LUCAS [3], Carlos FRAZÃO [2], M. Rita VENTURA [2], Patrícia T. BORGES [2] Lígia O. MARTINS [2]

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Here, we present the characterization of *Ps*P2Ox, a *C*-glycoside-3-oxidase from *Pseudoarthrobacter siccitolerans,* carried out by a combination of experimental and computational approaches [1].

C-glycosides are natural products that present important biological activities and chemical properties, such as high stability against degradation. Glycoside 3-oxidases (G3Oxs) are newly identified FAD-dependent bacterial enzymes capable of oxidizing *C*-glycosides with the concomitant reduction of O_2 to H_2O_2. Interestingly, soil and gut microorganisms have different oxidative enzymes. This oxidation is then followed by C-C acid/base-assisted bond cleavage in two-step *C*-deglycosylation pathways.

Our results show that *Ps*GO3x oxidizes the glucose moiety of mangiferin to 3-keto-mangiferin with 50,000-fold higher specificity (k_{cat}/K_m) than free D-glucose to 2-keto-glucose. Analysis of *Ps*G3Ox crystal structures (without and with substrates bound), combined with mutagenesis, molecular dockings and molecular dynamics simulations, reveal functional, structural and dynamical details that explain these enzymes' ability to bind and oxidize larger glycoside substrates (Fig 1).

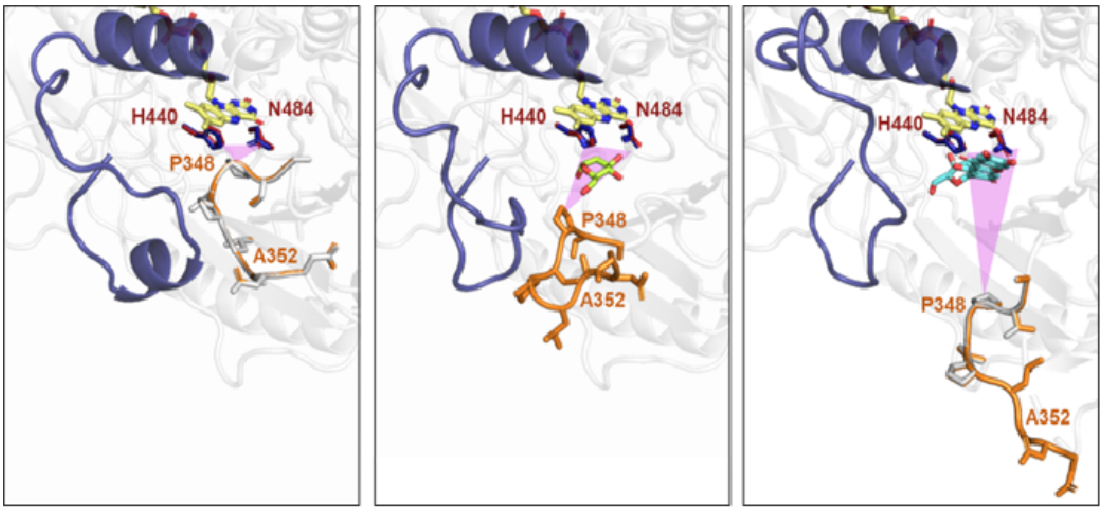

Fig 1. PsP2Ox modelled structure for the substrate free, the glucose-bound and the mangiferin bound

Acknowledgements

This work was supported by the Fundação para a Ciência e Tecnologia, Portugal, and the Spanish MCIN/AEI/10.13039/501100011033/ FEDER, UE.

Bibliographic references:

[1] A. Taborda et al. , Research Square, DOI: 10.21203/rs.3.rs-2662172/v1

Procainamide-labeled *N*-glycans and their structural role in mucin from bovine submaxillary glands

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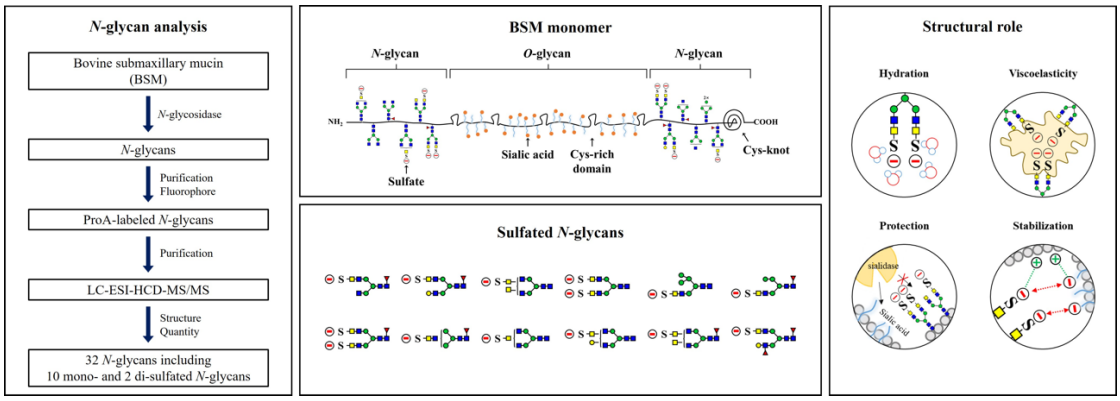
Bovine submaxillary mucin (BSM) is a heavily-glycosylated macromolecular (approximately 4MDa) protein that is used in various biomaterial applications in light of its high viscosity and biocompatibility, in addition to use as a biochemical substrate or inhibitor as a result of its abundant *O*-glycans. Although *N*-glycosylation has been reported to provides stability of human mucins, most BSM research has been focused on its *O*-glycans, while *N*-glycans have not been reported to date.

In this study, a common *N*-glycan core component was detected by monosaccharide analysis of BSM, and the structures and relative quantities of the *N*-glycans were determined by liquid chromatography–tandem mass spectrometry.

Seventeen *N*-glycans comprising ten complex-type [$Fucose_{0\sim2}Hexose_{3\sim4}$*N*-$acetylhexosamine_{1\sim6}Sulfate_{0\sim1}$; 61.1% (the sum of the relative quantities of each *N*-glycan out of the total *N*-glycans)], two high-mannose-type ($Hexose_{5\sim6}$*N*-$acetylhexosamine_2$; 12.0%), and five paucimannose type ($Fucose_{0\sim1}Hexose_{3\sim4}$*N*-$acetylhexosamine_{2\sim3}$; 26.9%) were identified, but no hybrid-type or sialylated *N*-glycans were found. Additionally, these are less-branched structures compared to human mucins. Of these, ten glycans (77.2%), including two sulfated glycans (8.0%), were core fucosylated, which confer unique biological functions to glycoproteins. These *N*-glycans are less-branched structures compared to human mucins. This is the first study to confirm *N*-glycosylation of BSM, and these results support further expansion of the biological function of non-human mucin.



Workflow of N-glycan analysis, BSM monomer, sulfated N-glycans, and their structural role.

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Investigation of anomeric selectivity in NIS/TfOH promoted glycosylation reactions

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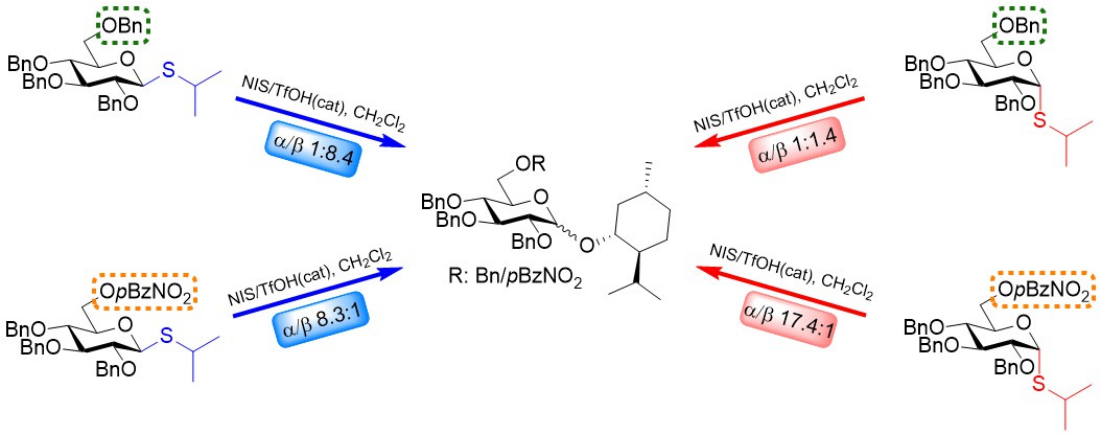
The reaction of a series of anomeric thioglycosides with various glycosyl acceptors and *N*-iodosuccinimide/triflic acid has been investigated with respect to reactivity and anomeric selectivity.[1] In general, beta-configured donors were found to give a more beta-selective reaction outcome compared to their alpha-configured counterparts.

Furthermore, a systematic study of various 6-*O*-acyls effect on anomeric selectivity in glucosylations with thioglucoside donors was conducted.[2] All eight different esters were found to induce moderate-to-high alpha-selectivity in glucosylation with L-Menthol as acceptor, with the best being 6-*O*-*p*-nitrobenzoyl. The alpha-directing effect was found to be general across various glucosyl acceptors, glucosyl donors, and modes of activation. No evidence was found in favor of distal participation.



Acknowledgements

We are grateful to Independent Research Fund Denmark and the Novo Nordisk Foundation for financial support.

Bibliographic references:

1) H. H. Trinderup, T. L. P. Sandgaard, L. Juul-Madsen, H. H. Jensen (2022), J. Org. Chem (87), 4154-4167.
2) H. H. Trinderup, L. Juul-Madsen, L. Press, M. Madsen. H. H. Jensen (2022), J. Org. Chem (87), 13763-13789.

Synthesis of open-chain carbohydrate amides, computational and bioactivity studies

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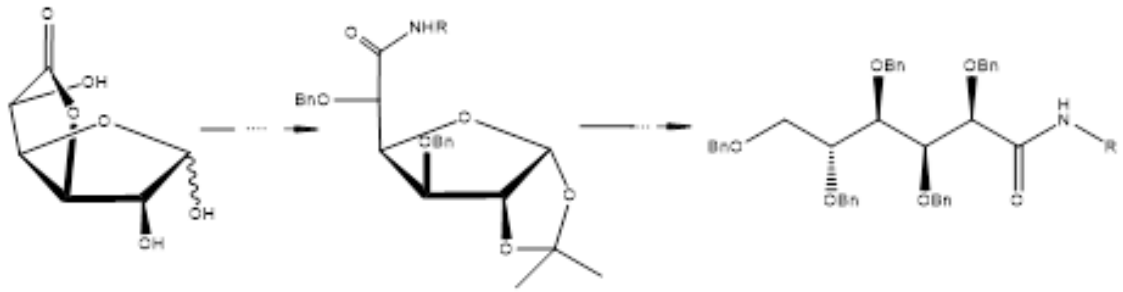
Alzheimer\'s disease (AD) is a severe neurodegenerative disorder characterized by progressive dementia with memory and cognition loss, ultimately leading to death [1,2]. It is considered an urgent public health problem [3,4], being the third leading cause of death after cancer and heart disease [2]. According to the 2020 update of the World Alzheimer Report 2015 [5,6], there are over 50 million people worldwide living with dementia. This number will almost double every 20 years, reaching 152 million in 2050. AChE inhibitors are the mainstay drugs for early disease stages [1].

Recently, we reported the synthesis of open-chain sugar amides which have some potential as AChE inhibitors [7]. Computational studies have shown that aromatic groups and the H-bond donor and acceptor groups, together with the molecule flexibility of these open-chain sugars, are responsible for their activity as the hydrophobic and H-bond interactions with the residues at the AChE gorge are mandatory for recognition and/or to block the action of the catalytic triad, and thus for the inhibitory activity. In this communication, we report the development of a new synthetic route to yield open-chain sugar amides from the commercially available glucuronolactone. The amide sugar derivatives were obtained in good yield (70 – 90%) (Scheme 1). The evaluation of acetylcholinesterase inhibition was carried out and the results will be disclosed.



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Bibliographic references:

[1] Przybylowska M,et all, J Enzy Inhib.Med Chem,2022,37,1,1012
[2] Vaz M,et all, European Journal of Pharmacology,2020,887,173553
[3] JSCristóvão, et all, Molecules,2021,26,440
[4] Association. Alzheimer's disease facts figures. Alzheimer's Dementia 2020,391
[5] Deture,MA,et all. Mol. Neurodegener,2019,14,1
[6] Guerchet,M,etall. World Alzheimer Report,2015,2020
[7] R.Gonçalves-Pereira,J.Figueiredo, SDLucas, MIGarcía-Moreno, COrtiz Mellet, APRauter, MIIsmael, Medicinal Chem2022,DOI:10.2174/157340641966

A novel enzyme collection as a potent tool for bacterial biofilm exopolysaccharides degradation

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Bacterial biofilm infections represent a major threat in medical care with conventional therapies failing due to low penetration of antibiotics into the biofilm. Biofilms are bacteria aggregates surrounded by a polysaccharide rich extracellular matrix. As such, enzymatic polysaccharide degradation is a potential strategy to enhance antibiotic biofilm penetration and bacteria eradication. Thus, a library of 135 carbohydrate active enzymes (CAZymes) with the potential to digest the main exopolysaccharides produced by *Staphylococcus aureus* (PNAG) and *Pseudomonas aeruginosa* (Pel, Psl and alginate) was explored [1,2].

The genes encoding the selected potential CAZymes were chemically synthesized, cloned, expressed and purified in a high-throughput platform. The enzymatic activity of the purified proteins was tested using chromogenic substrates and biofilm biomass evaluation.

All expressed enzymes were soluble and 30 presented high production yields. Four enzymes were able to efficiently depolymerize PNAG, Psl, Pel and alginate, respectively. One alginate lyase, in particular, was able to reduce the biofilm biomass of a mucoid *P. aeruginosa* strain by 70%, after 6.5h of incubation. Two family 20 glycoside hydrolases were also able to promote a 50-60% biofilm formation inhibition on a *S. aureus*strain after 6h of incubation. These enzymes will be further tested in combination with antibiotics to evaluate their synergistic potential towards bacterial elimination. Overall, this study allowed the identification of novel enzymes capable of *P. aeruginosa*and *S. aureus* biofilm degradation.

We acknowledge FCT, through the grants: UIDB/00276/2020 (CIISA); LA/P/0059/2020 (AL4AnimalS); and 2022.07903.PTDC. We also acknowledge ANI through the grant LISBOA-01-0247-FEDER-047033 [GlycoMed] and the Gilead GÉNESE program through the project 17805.

Bibliographic references:

[1] L.K. Jennings, K.M. Storek, H.E. Ledvina, C. Coulon, L.S. Marmont, I. Sadovskaya, P.R. Secor, B.S. Tseng, M. Scian, A. Filloux, D.J. Wozniak, P.L. Howell, M.R. Parsek (2015), Proc. Natl. Acad. Sci. U. S. A. (112) 11353–11358.

[2] S. Yu, T. Su, H. Wu, S. Liu, D. Wang, T. Zhao, Z. Jin, W. Du, M.J. Zhu, S.L. Chua, L. Yang, D. Zhu, L. Gu, L.Z. Ma (2015), Cell Res. 25 1352–1367.

Exploration of human gut microbiota enzymes targeting glycosaminoglycans

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The human gut microbiota is a complex community of microorganisms that inhabit the gastro intestinal tract and play major roles in our health. In particular, specific gut bacteria degrade the complex polysaccharides present at the surface of the epithelium, among which glycosaminoglycans (GAGs).

The catabolism of GAGs is an important process that leads to the production of short-chain fatty acids, which contribute locally to the health of colonocytes and also have systemic beneficial effects, especially on the central nervous system. To efficiently degrade GAGs, gut bacteria rely on Carbohydrate-Active enZymes (CAZymes) with complementary activities to completely break down the polysaccharides. These enzymes, primarily Glycoside Hydrolases and Polysaccharide Lyases, are classified in the CAZy database [1].

In the previous years, several new families of Polysaccharide Lyases have been identified which exhibited activities on GAGs [2]. Here, we present the characterization of several enzymes belonging to family PL35 and originating from gut bacteria, with specificities towards a variety of glycosaminoglycans.

Bibliographic references:

[1] E. Drula, M-L. Garron, S. Dogan, V. Lombard, B. Henrissat, N. Terrapon (2022), Nucleic Acids Res. 50(D1):D571-D577.

[2] W. Helbert, L. Poulet, S. Drouillard,S. Mathieu, M. Loiodice, M. Couturier, V. Lombard, N. Terrapon, J. Turchetto, R. Vincentelli, B. Henrissat (2019), Proc. Natl. Acad. Sci. U S A. 116(13):6063-6068.

Structural heterogeneity in *Streptococcus suis* serotype 9 capsular polysaccharide

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Streptococcus suis, an encapsulated bacterium, is an important swine pathogen and zoonotic agent. The capsular polysaccharide (CPS) of serotype 9 contains D-Gal, L-Rha, Glc-ol6*P*, and a labile keto sugar, D-6d*xyl*HexNAc-4-ulo [1]. The function of genes responsible for its biosynthesis by the Wzy pathway has been putatively assigned [1].

The CPS obtained from Ss2to9, a serotype-switched mutant from a serotype 2 strain that expresses the serotype 9 CPS, had different ^{1}H NMR data than published initially for *S. suis* serotype 9 [1,2]. First, doubling of signals was observed in the anomeric region, attributed to the *ribo*isomer of 2-acetamido-2,6-dideoxyhexos-4-ulose (Sug) formed by keto-enol tautomerization during the purification procedure. Second, signals corresponding to one residue were shifted from their original position, and indeed glucose instead of galactose was found in the side chain of the mutant CPS.

Molecular models of oligosaccharides comprising either the *xylo* or the *ribo* isomer of the keto sugar were constructed to support the structure of the new isomer and explain its formation.

Because the donor strain for the construction of the mutant was different from the strain used for CPS structure determination, glycosyltransferase genes in the different *S. suis* serotype 9 strains were examined to find mutations potentially responsible for the replacement of Gal by Glc.

Finally, a comparison was made with various CPS structures having a single Gal/Glc difference in serotypes of *Streptococcus pneumoniae*within the same serogroup, where their genetic basis has been examined.

$$\rightarrow 3)\text{-}\alpha\text{-L-Rha}p\text{-}(1\rightarrow 3)\text{-Glc-ol-}(6\rightarrow P\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Sug}p\text{-}(1\rightarrow \\ \alpha\text{-D-Glc}p\text{-}(1\rightarrow 2)\lrcorner$$

Ss2to9 mutant CPS repeating unit structure

Acknowledgements

This research was supported by the International Development Research Centre (IDRC) and GlycoNet.

Bibliographic references:

[1] E. Vinogradov, G. Goyette-Desjardins, M. Okura, D. Takamatsu, M. Gottschalk, M. Segura (2016), Carbohydr. Res. (433) 25-30.

[2] M. Okura, J.-P. Auger, T. Shibahara, G. Goyette-Desjardins, M.-R. Van Calsteren, F. Maruyama, M. Kawai, M. Osaki, M. Segura, M. Gottschalk, D. Takamatsu (2021), Sci. Rep. (11) 6513

Coupling of Surface Plasmon Resonance with Mass Spectrometry to study protein glycosylation

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Lectins are widely appreciated, in the fields of hematology and immunology, due to their specific binding to carbohydrate structures. Lectins are commonly present on the cell surface or elsewhere; they are also known as specific markers for membrane glycoprotein structures. This study is focused on the use of Jacalin lectin (Artocarpus integrifolia) (AIA), which belongs to the family of galactose-binding lectins and is used here as a model of lectin. The lack of sensitivity in MS detection hinders the development of approaches using Surface Plasmon Resonance (SPR) coupled with Mass Spectrometry (MS). Among the factors affecting sensitivity, the amount of ligands retained on the biochip surface is crucial [1]. Thanks to their smaller size and multimerism, lectins offer an attractive receptor alternative to usual antibodies for increasing ligand capture, which is particularly interesting in glycoconjugates analysis.

Our project aims to develop a multiplex SPR biochip with immobilized lectins to afford the coupling between SPR Imaging and MS (SPR_i-MS) to determine protein glycosylation and further study of unknown lectins and their interactions with *N*-glycans. This coupling allows the real-time monitoring of interactions and the determination of their kinetics and thermodynamics parameters, together with the structural identification of the glycoconjugates captured from a complex mixture [1], [2], [3].

Fetuin is a plasma glycoprotein containing *N*-acetylglucosamine (GlcNac) and *N*-acetylgalactosamine (GalNac) in its structure, which is commonly used as a model for *N*- and *O*-glycans analysis. Our SPR_i analysis confirmed significant interactions between AIA and fetuin. The sensorgrams showed considerable variations in reflectivity accompanied by the lighting of the spots upon the injection of increasing concentrations of fetuin. Detection of the captured fetuin was successfully achieved by MALDI-TOF MS directly on the lectin biochip.

Moreover, a protocol for the on-chip spraying of the MALDI matrix is currently in development in the laboratory. This mode of matrix deposit will be combined with a direct on-chip MALDI-TOF MS imaging (MS_i) experiment to evaluate the impact on both the sensitivity and the selectivity of detection of fetuin by a SPR_i-MS_i approach.

Bibliographic references:

[1] A. Halushkina, W. Buchmann, N. Jarroux, R. Daniel (2021), Methods in Molecular Biolog (2237), 55-67

[2] S. Bellon, W. Buchmann, F. Gonnet, N. Jarroux, M. Anger-Leroy, F. Guillonneau, R. Daniel (2009), Anal. Chem. (81), 7695–7702 [3] J. Musso, W. Buchmann, F. Gonnet, N. Jarroux, S. Bellon, C. Frydman, D-L. Brunet, R. Daniel (2015), Anal. Bioanal. Chem., (407),1285-1294

Synthesis of diversely *O*-acetylated segments from the *Shigella flexneri* 6 surface polysaccharide

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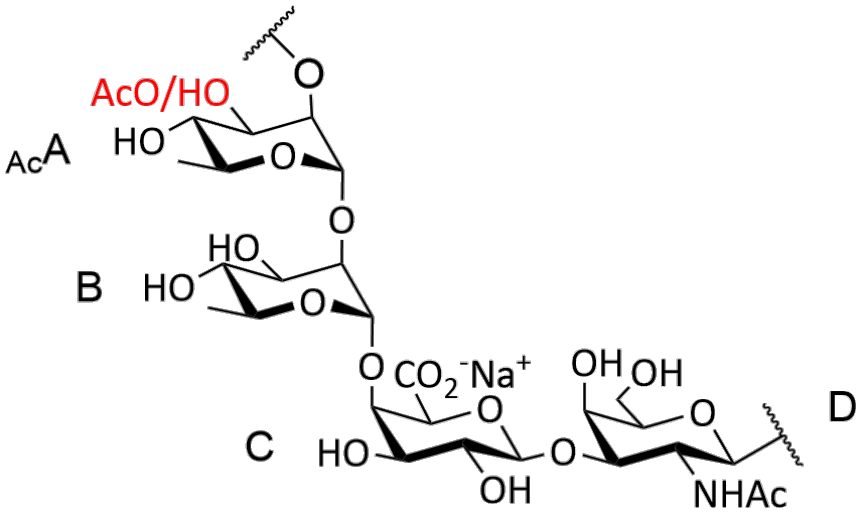
Shigella are gram-negative enteroinvasive bacteria causing shigellosis, a major diarrheal disease, especially in low and middle income countries. Shigellosis is responsible for a high burden and is a prime cause of mortality due to diarrhea in young children.[1]

The O-antigen (O-Ag) part of the *Shigella* lipopolysaccharide (LPS) is a major target of protection induced by natural infection. Numerous *Shigella* vaccine candidates aimed at inducing an immune response against the LPS were proposed. In this context, our group has investigated synthetic O-Ag surrogates as alternatives to antigens purified from biological extracts. A *S. flexneri* 2a synthetic glycan-based vaccine candidate was found immunogenic in a first-in-human phase 1 clinical trial.[2]

Focus is here on the identification of surrogates of the O-Ag from *S. flexneri* 6 (SF6), another prevalent serotype.[3] The SF6 O-Ag is defined by a partially *O*-acetylated linear tetrasaccharide repeat ($_{Ac}$ABCD).[4]

The strategy to ready-for-conjugation oligosaccharides (up to 4 repeats) acetylated in a non-stoichiometric fashion at OH-3_A is described. Emphasis is on the design and multi-step synthesis of tetrasaccharide blocks obtained in multi-gram amounts. Their combination and chemical diversification into sets of oligomers is discussed. Conditions enabling the final deprotection of the fully protected intermediates into fine-tuned linker-equipped SF6 O-Ag segments are presented.

These oligosaccharides will serve as molecular probes to decipher the importance of chain length and *O*-acetylation for O-Ag functional mimicry.



Bibliographic references:

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

Structure and properties of bacterial envelope components

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Human pathogens, including viruses and bacteria, have evolved their ability to activate or evade the host immune response by using the glycans of their envelopes. Bacterial lipopolysaccharides (LPSs) are the main components of the outer membranes of Gram-negative bacteria, they have an important role in the activation of the immune response as they act as microbe-associated molecular patterns" (MAMPs). They are microbes that mimic host glycans, to engage immune proteins and evade the host immune response, successfully promoting bacterial colonization and tolerance.[1] Some bacteria decorate their cell envelope with sialic acids, a group of 9-carbon backbone nonulosonic acids, found primarily at the termini of glycans displayed on vertebrate cell surfaces and therefore ideal candidates to be used as "self-associated molecular patterns" (SAMPs) for self/non-self discrimination by the host immune system. Siglecs (sialic acid-binding immunoglobulin-like lectins) are immune proteins with the ability to preferentially recognize and bind exposed sialylated glycans on cell surfaces, acting as regulators of a variety of critical cellular mechanisms.[2,3]

I will here present the structure and activity of bacteria envelope glycans (*Odoribacter splanchnicus*, *Acinetobacter baumannii…)* involved in the host immune recognition. Understanding the role of Glycans and glycosylation in immunity is critical to understanding the etiology and progression of immune-related diseases. Therefore, the structure and function of the bacterial envelope components will be evaluated.[4]

Bibliographic references:

1. F. Di Lorenzo, KA. Duda, R. Lanzetta, A. Silipo , C. De Castro, A. Molinaro (2022) Chem Rev. (20) 15767-158211.
2. Y.C. Chang, V. Nizet (2014) Glycobiology (24) 818-825.
3. S.S. Duan, J. Paulson (2020) Annu. Rev. Immunol. (38) 365-395.
4. T. Johannssen, B. Lepenies (2017) Trends in Biotechnology (35) 335-346.

Chemoselective enzymatic deacetylation of protected phenylpropanoid saccharides

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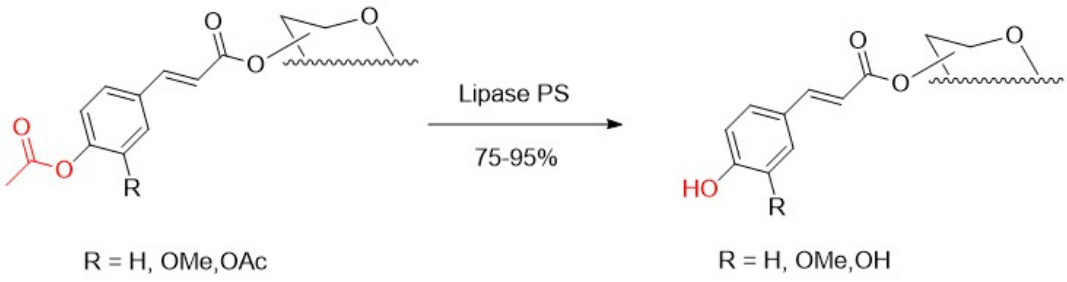
Glycophenolics represent a class of naturally occurring compounds with a wide range of biological activities, especially their antioxidant activity, from which they develop secondary anti-inflammatory, antiviral, cardioprotective, neuroprotective and anticancer properties [1]. These glycophenolics are divided into several subcategories, from which one of them are phenylpropanoid esters of carbohydrates, which consist of saccharide moiety decorated with hydroxycinnamic acids. Chemical synthesis of these compounds usually involves the use of acetyl protecting group on saccharides and on phenolic hydroxyls as well. However, free phenolic hydroxyl is the key factor in antioxidant activity of these glycophenolics and to evaluate biological activities of synthetic analouges of these natural products, chemoselective deacetylation of phenolic hydroxyls is essential. Common chemical methods of deacetylation usually lead to several different side products, and it is difficult to achieve desired chemoselectivity.

During our work on the synthesis of substrates for feruloyl esterases, we found that Lipase PS (lipase from *Burkholderia cepacia*) selectively deacetylates esters of 4-*O*-acetylferulic acid [2]. Therefore, we investigated the possibility of enzymatic deacetylation of protected phenylpropanoid saccharides with Lipase PS to deacetylate phenolic hydroxyls. Reaction proceeded in two-phase system at neutral pH and with only mild heating (37 °C). We achieved excellent chemoselectivity towards phenolic acetates with yields 75-95%, while other ester bonds remained intact.



Acknowledgements

Grant Agency for Science VEGA (grant 2/0111/22), COST Action CA18103 (INNOGLY), supported by COST (European Cooperation in Science and Technology)

Bibliographic references:

[1] Y. Tian, W. Liu, Y. Lu, Y. Wang, X. Chen, S. Bai, Y. Zhao, T. He, F. Lao, Y. Shang, Y. Guo, G. She (2016) Molecules (21) 1402.
[2] M. Mastihubová, V. Mastihuba L. Kremnický, J. L. Willet, G. L. Côté (2001), Synlett 1559.

Swainsonine and mannostation based analogues: targeting selective inhibition of Golgi α-mannosidase

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An obligatory step in the formation of complex *N*-glycans involves the removal of two mannosyl linkages in $GlcNAcMan_5GlcNAc_2$.[1] Golgi *α*-mannosidase (GMII) is a glycoside hydrolase that catalyses the removal of terminal *α*-1,3- and 1,6-linked mannoses from $GlcNAcMan_5GlcNAc_2$.[1] Dysregulated synthesis or alteration of *N*-glycans is often linked to the manifestation of various diseases.[2]

Therefore, GMII and its inhibition, is a promising candidate for the development of various therapeutics such as anticancer drugs. Swainsonine is a highly characterised inhibitor of GMII and can be used during the treatment of various cancers.[3] Nevertheless, swainsonine administration can elicit undesirable side effects such as mannosidosis due to its off-target inhibition of lysosomal *α*-mannosidase.[4]

As a result, there is a pressing need to find potent, yet selective, inhibitors towards GMII. Discussed herein, are the structural and biochemical based findings for the potential inhibition of GMII by employing a range of swainsonine and mannostatin based analogues.

Bibliographic references:

[1] N. Shah, D. A. Kuntz, D. R. Rose, (2008) Proc. Natl. Acad. Sci. U. S. A., 105 (28), 9570–9575.
[2] J. W. Dennis, S. Laferte, C. Waghorne, M. L. Breitman, R. S. Kerbel, (1987) Science, 236 (4801), 582– 585.
[3] S. M. Colegate, P. R. Dorling, C. R. Huxtable, (1979) Aust. J. Chem., 32 (10), 2257–2264.
[4] I. Cenci di Bello, P. Dorling, B. Winchester, (1983) Biochem. J., 215 (3), 693–696.

The role of substitution pattern on molecular structure and NMR parameters in sulphated saccharides

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Biological properties of sulphated saccharides are surprisingly large and include angiogenesis, anti-inflammatory, antiviral, anticoagulant properties, cell adhesion, growth and differentiation, etc. These manifold properties originate from the structural arrangement of these saccharide derivatives. The present contribution discusses applications of quantum chemical DFT methods, combined with NMR spectroscopy, for description of molecular properties of heparan sulphate, dermatan sulphate, chondroitin sulphate and iota-carrageenan. Theoretical methods have been used for examination of 3D molecular structure and NMR parameters taking into account explicit solvent molecules. The data showed that formation of a complex hydrogen bond network and strong ionic interactions influence the first hydration shell and play an important role in shaping the 3D saccharide molecules.

The DFT analysis of $^{3}J_{H–C–C–H}$ coupling constants indicated that the oxygen lone pairs of neighbouring oxygen atoms could significantly contribute to the magnitudes of coupling constants, mainly Fermi-contact terms. The computed $^{3}J_{H–C–C–H}$ data, together with theoretical analysis of molecular structure highlight the need of appropriate quantum-chemical calculations for detailed understanding NMR parameters as well as solution properties of sulphated oligosaccharides.

Acknowledgements

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New perspectives for aging research of an extremely long fertile life in a highly social termite

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Aging is characterized by a progressive loss of physiological integrity, leading to impaired function such as reduction of reproductive performance and increased physiological vulnerability to death. Over recent years, aging research has discovered that the rate of aging is controlled by genetic pathways and biochemical processes conserved in evolution of many species. I will present our results obtained from a novel natural aging system, the long-lived and highly fertile *Macrotermes natalensis* termite queen that overcomes simultaneously several well-known hallmarks of aging while receiving a prolonging carbohydrate-rich diet.

Open royal chamber with one physogastric queen, one king, and several workers and soldiers of Macrotermes natalensis. Credit: M. Vasseur-Cognet

Bibliographic references:

S. Séité, M. C. Harrison, D. Sillam-Dussès, R. Lupoli, T. J. M. Van Dooren, A. Robert, L-A. Poissonnier, A. Lemainque, D. Renault, S. Acket, M. Andrieu, J. Viscarra, H.S. Sul, Z. W. de Beer, E. Bornberg-Bauer and M. Vasseur-Cognet. Lifespan prolonging mechanisms and insulin upregulation without fat accumulation in long-lived reproductives of a higher termite. (2022), Communications Biology (5):44

Specificity of a MurNAc/GlcNAc peptidoglycan deacetylase acting on chitooligosaccharides

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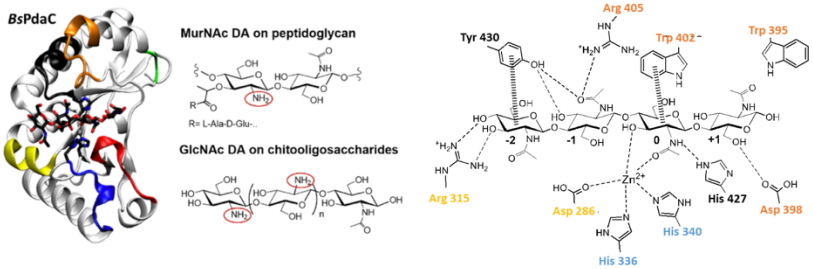
The bacterial cell wall peptidoglycan (PGN or murein) is an heteropolymer composed of a linear glycan chain of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) units linked by β-1,4 glycosidic bonds. Moreover, the lactyl group of the MurNAc units are substituted by short (stem) peptides that are crosslinked to form a mesh-like structure. In addition to variations in polymer length, many bacteria subsequently modify the sugar structures. One of the most common modification of the glycan backbone is the de-N-acetylation of either one or both sugars and pathogenic bacteria utilize those deacetylations to evade detection by the innate immune system. PGN deacetylases (PGN DAs) are members of family 4 carbohydrate esterases (CE4 enzymes) which operate by a metal-assisted general acid/base catalytic mechanism [1][2]. Whereas PGN GlcNAc DAs are specific for GlcNAc residues of the PGN glycan backbone and confer resistance to lysozyme hydrolysis, PGN MurNAc DAs are specific for MurNAc residues and are involved in sporulation. Both subfamilies have mutually exclusive specificities, but currently no sequence or structural signatures can be assigned to each enzyme class to predict specificity and function. PdaC from Bacillus subtilis was recently discovered as a novel MurNAc DA. It is unique in that it acts on intact PGN, it is not involved in sporulation and it is also active on chitooligosaccharide (COS), an activity that was thought to be restricted to GlcNAc deacetylases [3]. Moreover, BsPdaC shares higher sequence similarity with the peptidoglycan GlcNAc deacetylase SpPgdaA than with other MurNAc deacetylases.

Computational dockings with (GlcNAc)4 substrate confirmed the presence of two binding modes (BM) with equal energy which explain the experimental first deacetylation pattern at the two central GlcNAc units. The goal is to gain insight into the substrate specificity of BsPdaC by generating mutants in certain positions of the catalytic cleft and evaluate the changes in activity and specificity compared to the wild type enzyme. Thus, new information about the interactions of the residues surrounding the active site can be inferred, aimed at understanding the specificity of PGN DAs as therapeutic targets for the design of novel antimicrobials.



Acknowledgements

Work supported by Ministerio de Ciencia e Innovación. MINECO, Convocatoria 2019 Proyectos de I+D+i –RTI Tipo B (PRE2020-093759) and GQBB (2021SGR0053)

Bibliographic references:

[1] S. Pascual, A. Planas, Curr. Opin. Chem. Biol. 61 (2021) 9–18. [2] A. Planas, Curr. Med. Chem. 29 (2022) 1293–1312. [3] L. Grifoll-Romero, M.A. Sainz-Polo, D. Albesa-Jové, M.E. Guerin, X. Biarnés, A. Planas, J. Biol. Chem. 294 (2019) 19066–19080.

Photocleavable ABPs for a native enrichment assay

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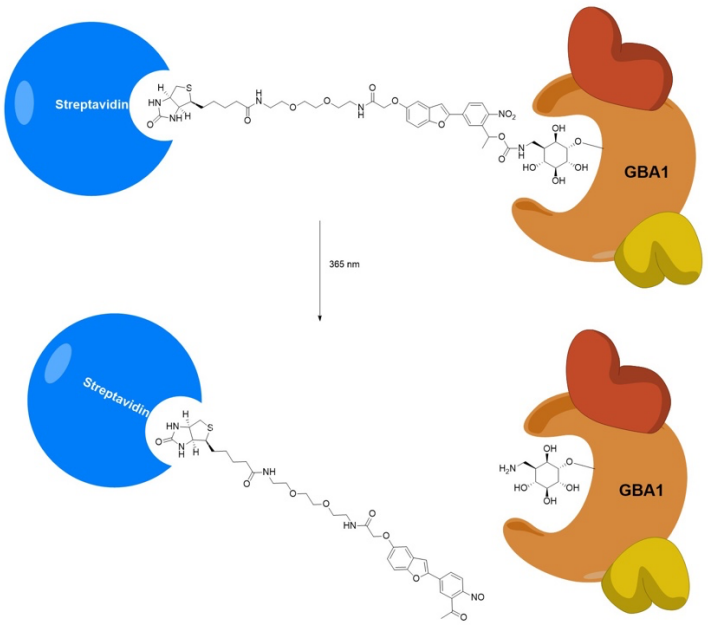
Lysosomal storage diseases Gaucher is caused by the accumulation of metabolite glucosylceramide in the lysosome due to impairment of its degrading enzyme Glucocerebrosidase. For a better understanding of the disease, the affected enzyme and its biological environment are investigated extensively. Identifying proteins that are interacting with the enzyme, for example activating or stabilizing it, could give insight in the enzyme's mechanisms and activated downstream pathways. In this work we aim to identify and characterize these interacting proteins via a native enrichment assay using photo-cleavable activity-based probes.



Photocleavable ABP bound to streptavidin-coated beads and GBA, before and after photocleavage.

Bibliographic references:

Aerts, J. M. et al. Lysosomal Storage Diseases. For Better or Worse: Adapting to Defective Lysosomal Glycosphingolipid Breakdown. eLS 1–13 (2017). doi:10.1002/9780470015902.a0027592

Niosomes functionalized with a synthetic carbohydrate binding agent for mannose-targeted doxorubicin

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Niosomes are self-assembled nanometer sized-vesicles, which represent a versatile drug delivery system (DDS) for several therapeutic applications.[1] Their excellent ability to encapsulate antitumoral drugs as well as the possibility to easily functionalize their surface, make these nanocarriers particularly suitable for the development of active targeted DDS in cancer therapy.

In the last decades, our research group has been involved in the development of synthetic carbohydrate binding agent (CBA), small-molecules selectively able to target saccharides of biological interest.[2] Recently, a diaminopyrrolic synthetic receptor has been developed, which is effectively able to recognize the mannosidic residues of high-mannose-type glycans, overexpressed on the surface of various cancer cells.

In this project doxorubicin-based niosomes were functionalized with the CBA effectively recognizing mannosides.[3] Several formulations and different preparation methods were taken into consideration to obtain functionalized nanovesicles suitable for parental administration, which resulted stable for over six months, and able to encapsulate up to 85% of antitumoral drug. Cell viability, evaluated on a triple-negative cancer cells (MDA-MB-231), showed an increase of apoptosis for the functionalized DOXO-based niosomes, whereas comparison studies, carried out with H9C2 normal cells, confirmed the protective role of niosomal formulations on rat cardiomyocytes by a reduction of cytotoxicity.

The encouraging results obtained may open the way to further in vivo investigations.

Bibliographic references:

1.Bragagni, M.; Mennini, N.; Ghelardini, C.; Mura, P.; J Pharm Pharm Sci, 2012, 15(1), 184.
2.Francesconi, O.; Roelens, S.; ChemBioChem, 2019, 20, 1329.
3.Burrini, N.; D'Ambrosio, M.; Gentili, M.; Giaquinto, R.; Settimelli, V.; Luceri, C.; Cirri, M.; Francesconi, O.; Pharmaceutics 2023, 15, 235.

Edition of Heparan sulfate by human endosulfatases monitored by several analytical techniques

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Heparan sulfate (HS) is a sulfated polysaccharide involved in cellular processes through its ability to bind and modulate various proteins. These interactions are controlled by the saccharide domains with rich sulfation patterns whose distribution is orchestrated by highly regulated biosynthetic machinery. In addition, at a different level of structural control, HS remodeling occurs at the cell surface. These modifications are ensured by editing enzymes involved in the post-synthetic sequencing of HS, including the extracellular sulfatases HSulf-1 and HSulf-2, which regioselectively remove 6-O-sulfate groups from HS. HSulf-1 and HSulf-2 are unique endosulfatases that target the functional sulfated domains of HS, alter ligand binding properties, and modulate a variety of signaling pathways [1]. Studies have shown that HSulfs are associated with many diseases, including cancer. Although they are closely related enzymes, HSulf-1 is most often largely reported as having a tumor-suppressive activity, whereas HSulf-2 has a pro-oncogenic activity and is consistently overexpressed in tumors.

Our work aims to characterize and understand these enzymes at the structural and functional levels [2]. Currently, conventional methods for monitoring and measuring the activity of these enzymes use synthetic sulfated substrates that are not carbohydrates in nature (e.g., p-catechol sulfate) and therefore do not accurately approach the appropriate true enzyme endolytic activity. To gain new insights into the structural features of the oligosaccharide substrates of these enzymes, we investigated the development of robust and resolving analytical methods that allow enzymatic monitoring of the natural sulfated substrates. First, kinetic studies of HSulf-2 using Hp oligosaccharides as substrates were performed by HILIC-MS [3]. We observed the sequential hydrolysis of 6-O-sulfate groups along the sulfated oligosaccharides; the activity of HSulf-2 was then compared with that of HSulf-1. We investigated whether these two enzymes also differ in their catalytic properties. Their desulfation reaction on various natural and synthetic Hp-oligosaccharide substrates was followed using several techniques. Overall, the use of IM-MS in combination with HILIC-MS, for example, can provide a powerful tool for the structural and functional characterization of HSulfs and their interactions with HS and other biomolecules.

Exploration of the functional specificities of each HSulf and their correlation to the structural differences will be discussed in detail.

Bibliographic references:

1. M. Bhattacharya, M. M. Malinen, P. Lauren, Y. R. Lou, S. W. Kuisma, L. Kanninen, M. Lille, A. Corlu, C. GuGuen-Guillouzo, O. Ikkala, A. Laukkanen, A. Urtti, M. Yliperttula (2012), Journal of controlled release (164) 291-298.

2. M. Assali, J. J. Cid, I. Fernández, N. Khiar (2013), Chemistry of Materials, (25) 4250-426.

Synthesis towards diversity of potential SGLT−2 inhibitors for the treatment of type 2 diabetes

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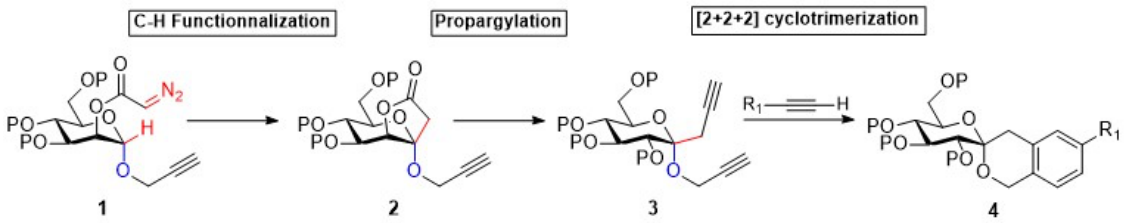
450 million people around the world are suffering from type 2 diabete, and its prevalence is increasing each year. In this context, gliflozines like Dapagliflozin have been marketed in 2013 as a new class of anti-diabetic agents acting as inhibitors of the sodium-glucose co-transporter SGLT−2. Among them, spiro-bicyclic compounds like Tofogliflozin showed great promises, but they have been underestimated because of synthetic approaches that only give rises to [6,5]-spiroacetals having an α configuration.

In this context, we wish to report herein a new approach towards potential inhibitors of SGLT-2, where a carbene-mediated functionalization of the anomeric C-H bond of carbohydrate would first give rise, on demand, to quaternary sugars of α- or β-configuration. Starting from mannose, the α-propargyl 2-diazoacetyl mannoside was first prepared and engaged in a Rh(II)-catalyzed 1,5-C-H insertion to yield γ-lactone. After conversion into the key *C,O*-bis propargyl glycoside, a [2+2+2] cyclotrimerization gave rise to [6,6]-spiroacetals fused to an aromatic ring.

This new approach allowing introduction of key pharmacophores in a late stage of the synthetic sequence provide a fast entry towards a large molecular diversity of unprecedented potential inhibitors of SGLT-2.



Divergent synthetic approach towards original [6,6] spirobicyclic potential inhibitors of SGLT-2.

Bibliographic references:

[1] K. Utsunomiya, N. Shimoto, M. Senda, Y. Kurihara, R. Gunji, S. Fuiji, S. Kakiuchi, H. Fujiwara, H. Kameda, M. Tamura, K.J. Kaku (2017), Diabetes Investig. (8), 766-775.

[2] F. E. McDonald, H. Y. H. Zhu, C. R. Holmquist (1995), J. Am. Chem. Soc. (117), 6605-6606.

[3] M. Boultadakis-Arapinis, C. Lescot, L. Micouin, T. Lecourt (2013), Synlett (24), 2477-2491.

Structure of the *Listeria innocua* ŽM39 cell wall teichoic acid and analysis of its biosynthesis gene

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Wall teichoic acids (WTA) make up the majority of cell wall carbohydrates of *Listeria*. They play vital physiological roles and are involved in the interaction with the hosts and the environment. They are composed of ribitol phosphate polymers that are differentially glycosylated. Most is known about the WTA of the foodborne pathogen *Listeria monocytogenes*. Since the non-pathogenic *Listeria innocua* is its close relative, it has been proposed as a surrogate organism for the foodborne opportunistic pathogen, especially for determining the efficacy of antimicrobial strategies against *L. monocytogenes* because it is safer to work with. Since there were few reports of WTA structures of *L. innocua*, we set out to determine the structure of native WTA, for the food isolate strain ŽM39, by 1D and 2D NMR spectroscopy. We report the complete structure of the WTA of *L. innocua* ŽM39, which consists of [→4)-β-GlcNAc*p*-(1→4)-Rbo-(1P→] backbone with an α-GlcNAc*p* bound to C3 of the β-GlcNAc*p* in stoichiometric amount. No acetyl groups were detected.

According to the current classification of *L. monocytogenes* WTA, the *L. innocua* ŽM39 WTA can be assigned to type II, since the β-GlcNAc residue is part of the backbone. This structure is similar to type II WTA serovar 6a which however contains a β-GlcNAc substituted in non-stoichiometric amounts with Gal, α-GlcNAc and acetyl groups. In addition, we sequenced the genome of this *L. innocua* strain and deciphered most of the putative WTA synthesis genes.

Acknowledgements

This work was supported by bilateral project BI-IT-18-20-006, and The Slovenian Research Agency with grants no. J4-9299, P4-0127, and J4-1771.

Bibliographic references:

B. Bellich, N. Janež , M. Sterniša, A. Klančnik, N. Ravenscroft, R. Rizzo, J. Sabotič, P. Cescutti (2022) Carbohydr Res. (511) 108499.

Evaluating the substrate specificity of ENGases using fluorogenic glycan probes

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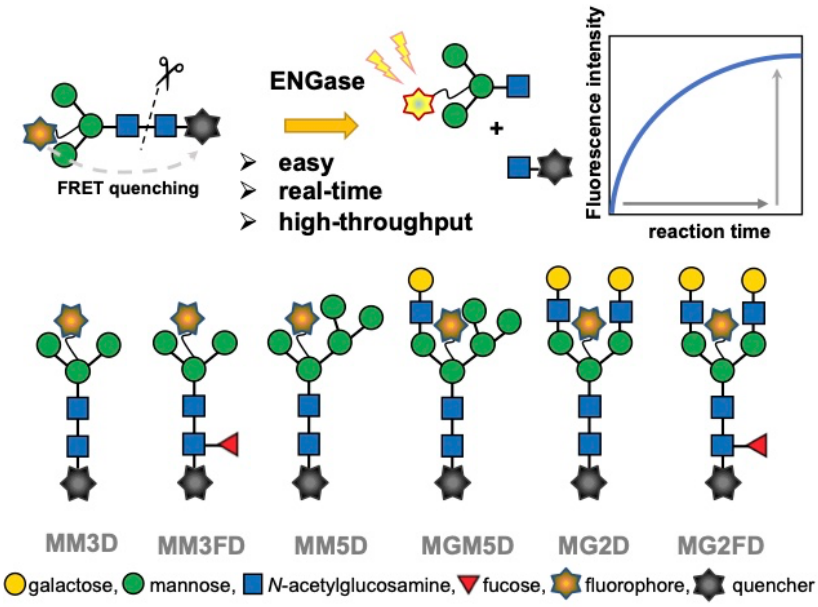
Endo-β-*N*-acetylglucosaminidases (ENGases) are glycosyl hydrolases widely used for glycan analysis of glycoproteins. ENGases hydrolase β1-4 glycosidic bond in the *N*,*N*-diacetylchitobiose core of *N*-glycan, release the glycan from glycoproteins and leave a single *N*-acetylglucosamine on proteins. ENGases are widely distributed in bacteria, fungi, and higher-order species, and each enzyme exhibits different substrate specificities.

To conveniently detect ENGase activity and evaluate the substrate specificity of ENGases, we have developed Förster resonance energy transfer (FRET) type substrates with *N*-glycans structure carrying a fluorescent group and a quenching group [1]. For a systematic understanding of substrate specificity of ENGases, we synthesized glycan probes with different structures of N-glycan, i.e., core-fucosylated hexasaccharide, biantennary asialocomplex type nonasaccharide, fucosylated complex type decasaccharide, oligomannose type heptasaccharide, and hybrid type nonasaccharide.

In this presentation, we will show the systematic synthesis of the FRET-based glycan probes and the results of hydrolytic reactions of these glycan probes by several ENGases and discuss the specificity of these ENGases.



Detection of ENGase activity using fluorogenic glycan probes

Bibliographic references:

[1] N. Ishii, C. Sunaga, K. Sano, C. Huang, K. Iino, Y. Matsuzaki, T. Suzuki, I. Matsuo (2018), Chembiochem (19) 660-663.
[2] N. Ishii, K. Sano, I. Matsuo (2019) Bioorg. Med. Chem. Lett. (29) 1643-1646.
[3] N. Ishii, H. Muto, M. Nagata, K. Sano, I. Sato, K. Iino, Y. Matsuzaki, T. Katoh, K. Yamamoto, I. Matsuo (2023) Carbohydr. Res. (523), 108724.

Complement C3 *N*-glycoprofiling in diagnosis of type 1 diabetes

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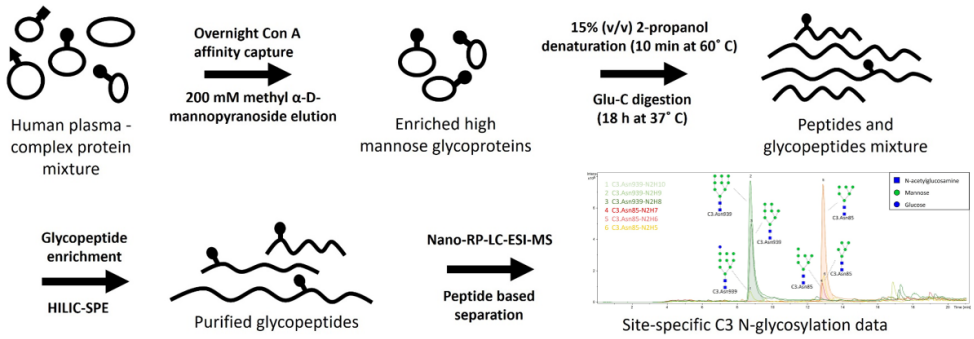
Recently, we showed that children at the onset of type 1 diabetes (T1D) have a higher proportion of oligomannose glycans on their total plasma proteins compared to their healthy siblings and identified these levels to be associated with the C3 gene. Complement component 3 (C3) contains two N-glycosylation sites occupied exclusively by oligomannose glycans. In order to evaluate the diagnostic potential of C3 N-glycosylation in T1D, we developed a high-throughput workflow for C3 concanavalin A lectin affinity enrichment and subsequent LC-MS glycopeptide analysis which enables protein-specific N-glycosylation profiling. Plasma samples of 61 children newly diagnosed with T1D and 84 of their unaffected siblings were analyzed. Significant changes in C3 N-glycome were found. T1D was associated with an increase in glycan structures with more mannose units. A regression model including C3 N-glycans showed considerable discriminative power (AUC = 0.879). Further, we analyzed serum samples from 189 adults with T1D concerning the most frequent complications of this disease. C3 N-glycome changed significantly among different stages of the albuminuria, as well as among hypertension status. All except one of the C3 glycopeptides proved to be associated with HbA1c levels. One of the glycoforms was also shown to be changed in retinopathy, in addition to elevated fasting glucose levels. Our study implies that the glycosylation of C3 could be a valuable diagnostic tool in assessing T1D risk in children as well as disease complications in adults.



F Schematic workflow for a high-throughput and site-specific C3 N-glycosylation LC-MS analysis

Bibliographic references:

N. Rudman, D. Kifer, S. Kaur, V. Simunović, A. Cvetko, F. Pociot F, G. Morahan, O. Gornik (2022), Diabetologia (65), 1315-1327.

D. Šolić, T. Keser, J. Štambuk, D. Kifer, F. Pociot, G. Lauc, G. Morahan, M. Novokmet, O. Gornik (2022), Mol Cell Proteomics (21), 100407.

The *N*-glycosylation of the SARS-CoV-2 SPIKE virus-like particles produced in tobacco plants

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The coronavirus SARS-CoV-2 emerged in China in late 2019 and led to the COVID-19 pandemic. Of the major structural proteins encoded by the SARS-CoV-2 genome, the SPIKE protein has attracted considerable research interest because of the central role it plays in entry into host cells. SARS-CoV-2 SPIKE is highly glycosylated with 22 predicted *N*-glycosylation sites, as well as numerous mucin-type *O*-glycosylation site. It is well documented that the protein glycans are mainly exposed at the surface and form a shield masking specific epitopes to escape the virus antigenic recognition.

Moreover, the analysis of SARS-CoV-2 SPIKE from coronavirus variants has revealed significant variations in their glycan profiles. Virus-like particles (VLPs) of the SARS-CoV-2 SPIKE were produced in *Nicotiana benthamiana* and its *N*-glycosylation was investigated by a glycoproteomic approach.

As demonstrated for the viral protein produced in mammalian cells, we show that 20 among the 22 *N*-glycosylation sites are dominated by complex plant *N*-glycans and one is occupied by oligomannosides. This suggests that the CoV-2 SPIKE protein produced in tobacco plants adopt an overall 3D structure similar to the one of recombinant homologues produced in mammalian cells [1].

Bibliographic references:

[1]: J. Balieu, J.W. Jung, P. Chan, G.P. Lomonossoff., P. Lerouge, M. Bardor (2022) Molecules 27(16), 5119-5131.

The dual-binding mode in cellulosome assembly: size over type and function

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The cellulosome is one of nature's most intricate macromolecular protein complexes, which centralizes the cellulolytic efforts of many anaerobic microorganisms, promoting enzyme synergy and stability. Its components are assembled through high-affinity protein-protein interactions between enzyme-borne dockerin (Doc) modules and repeated cohesin (Coh) modules present in non-catalytic scaffold proteins [1-3]. Typically, a sequence and function-based classification distinguishes between two types of Coh-Doc modules. Thus, type I Coh-Doc complexes are usually responsible for enzyme integration, while type II complexes tether the cellulosome to the bacterial wall [4]. Contrastingly, in *Bacteroides cellulosolvens,* Coh types are reversed in all scaffoldins, with type II Cohs located on the enzyme-recruiting primary scaffoldin and type I Cohs located on the anchoring scaffoldins [5]. It has been previously reported that type I *B. cellulosolvens* interactions possess a dual-binding mode (DBM) that adds flexibility to scaffoldin assembly.

Here, by combining structural characterization with affinity studies we have uncovered the mechanisms governing enzyme recruitment into *B. cellulosolvens'* cellulosome and identified the molecular determinants of its type II Coh-Doc interactions. The results indicate that, unlike type II complexes from other species, these possess a DBM, similarly to type I. Therefore, the plasticity of DBM interactions seems to play a pivotal role in the assembly of *B. cellulosolvens'* cellulosome, which is consistent with its unmatched complexity and size.

Acknowledgements

We thank: FCT for funding through grants UIDB/00276/2020; LA/P/0059/2020 and 2022.07903.PTDC; ANI for funding through grant LISBOA-01-0247-FEDER-047033 and the Gilead GÉNESE program for grant 17805

Bibliographic references:

[1] L. Artzi, E.A. Bayer, S. Moraïs (2017), Nat. Rev. Microbiol. (15) 83–95.
[2] C.M.G.A. Fontes, H.J. Gilbert (2010), Annu Rev Biochem. (79) 655–681.
[3] E.A. Bayer, R. Lamed, B.A. White, H.J. Flint (2008), Chem Rec. (8) 364–377.
[4] J.L.A. Brás et al. (2016), Sci Rep. (6) 38292.
[5] M. Duarte, A. Viegas, V.D. Alves, J.A.M. Prates, L.M.A. Ferreira, S. Najmudin, E.J. Cabrita, A.L. Carvalho, C.M.G.A. Fontes, P. Bule (2021), J. Biol. Chem. (296) 100552

Diglycosidases as a new synthetic tool in biocatalysis

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Diglycosidases are becoming a promissing group of endoglycosidases enabling fast and simple syntheses of oligosaccharides and their glycosides without formation of glycon isomers. Screening and application of diglycosidases is however hampered by limited availability of appropriate substrates. We have tested three types o diglycosidase activities of various origin in glycosylation of tyrosol.

1. Rutinosidase-comprising plant materials, flower buds from Japanese sophora (*Sophora japonica*) [1] and seed meal from tartary buckwheat (*Fagopyrum tartaricum*) rutinosylated tyrosol exclusively on the primary hydroxyl with conversions above 60 %, providing after isolation solely the 2-(4-hydroxyphenyl) ethyl β-rutinoside in 24 and 35 %, respectively. Both catalysts are highly specific towards rutin as the substrate.
2. On the other side, transrobinobiosylation from robinin to tyrosol catalyzed by seed meal from common buckthorm (*Rhamnus cathartica*) proceeded with lower chemoselectivity, providing after isolation 23 % of a mixture of both theoretical tyrosol β-robinobioside isomers 2-(4-hydroxyphenyl)ethyl β-robinobioside and 4-(2-hydroxyethyl)phenyl β-robinobioside in ratio ca. 8:1 in favor of glycosylation of the primary hydroxyl.
3. Aromase H2, a commercial mixture of glycosidases and diglycosidases, catalyzed transacuminosylation from 4-nitrophenyl β-acuminoside to tyrosol, providing after isolation approximately equal mixture of 2-(4-hydroxyphenyl)ethyl β-acuminoside and 4-(2-hydroxyethyl)phenyl β-robinobioside [2].

Acknowledgements

Grant Agency for Science VEGA (grant 2/0111/22), COST Action CA18103 (INNOGLY), supported by COST (European Cooperation in Science and Technology)

Bibliographic references:

[1] E. Karnišová Potocká, M. Mastihubová, V. Mastihuba (2021), Food Chem. (336) 127674.
[2] P. Haluz, M. Mastihubová, V. Mastihuba (2023), Int. J. Mol. Sci. (24) 5943.

Using the nitrogen dimension in NMR spectroscopy to resolve individual residues in chitin oligomers

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Chitin is the second most abundant biopolymer in nature after cellulose and is composed of *N*-acetylglucosamine (GlcNAc) connected via β(1→4)-glycosidic bonds. Due to its high abundance, the interaction with other biomolecules, such as enzymes during degradation or synthesis, is of a central role in the world´s carbohydrate turnover. Additionally, chitin is used as biomaterial in pharmaceutical applications.[1] Despite its prominence, there is still a need to develop methods to study structure and function of chitin and its corresponding oligomers and their interactions with other biomolecules. Efforts have been made to analyse chitin oligomers by NMR spectroscopy, but spectral overlap has prevented any differentiation between the interior residues. For glycosaminoglycans, such as hyaluronan, ^{15}N NMR has been utilized on amide and sulfamate groups to resolve individual residues in oligomers.[2-4] Herein, we present a similar approach on chitin oligomers.

Chitin oligomers up to hexaose with natural abundance of ^{15}N were analysed with NMR spectroscopy in H_2O:D_2O (9:1) solution. Under the present conditions ^{1}H,^{15}N-HSQC allowed for individual residues in the oligomer to be resolved in the nitrogen dimension. Additionally, all oligomers were analysed for the presence of the *cis* amide form. According to previous studies on GlcNAc, the *trans* conformation is predominant, but about 1% of *cis* form is present.[5] It is still unknown whether the *cis* amide form plays a significant role in the conformation of polysaccharides containing *N*-acetyl groups, or if it has any biological function.

Bibliographic references:

1 M. Rinaudo (2006), Progress in polymer science (31), 603-632
2 C. D. Blundell, P. L. DeAngelis, et al. (2004), Glycobiology (14), 999-1009
3 V. H. Pomin (2013), Analytical and bioanalytical chemistry (405), 3035-3048
4 D. J. Langeslay, C. N. Beecher, et al. (2013), Analytical Chemistry (85), 1247-1255
5 Y. Xue and G. Nestor (2022), ChemBioChem (23), e202200338

Mucosal glycans as novel antimicrobial agents

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

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

Therefore, through a multi-centre collaborative effort we have been actively: (i) characterizing complex mucin O-glycan pools to identify structures most likely to display biological activity; (ii) developing a synthetic approach to obtain individual mucin O-glycans in sufficient quantity for functional analysis [2]; and (iii) assessing the virulence attenuating capabilities of individual glycans in diverse pathogens [1,3]. Within this framework, we have successfully identified specific structures that suppress virulence phenotypes in the fungal pathogen *Candida albicans* (e.g., filamentation, biofilm formation), and regulate pathogenicity in *Vibrio cholerae* (e.g., reduced cholera toxin production), with potency comparable to native mucin glycan pools.

The authors gratefully acknowledge Innosuisse (104.462 IP-LS), the Swiss National Science Foundation (CRSK-3_196773), and the University of Basel for financial support.

Bibliographic references:

[1] J. Takagi, K. Aoki, B.S. Turner, S. Lamont, S. Lehoux, N. Kavanaugh, M. Gulati, A. Valle Arevalo, T.J. Lawrence, C.Y. Kim, B. Bakshi, M. Ishihara, C.J. Nobile, R.D. Cummings, D.J. Wozniak, M. Tiemeyer, R. Hevey, K. Ribbeck* (2020), Nat Chem Biol (18) 762-73.*
[2] G. Minzer, R. Hevey (2022), ChemistryOpen, e202200134.
[3] B.X. Wang, J. Takagi, A. McShane, J.H. Park, K. Aoki, C. Griffin, J. Teschler, G. Kitts, G. Minzer, M. Tiemeyer, R. Hevey, F. Yildiz, K. Ribbeck (2023), EMBO J (42) e111562.

A simplified analytical approach for Glycoconjugate content (potency) quantification

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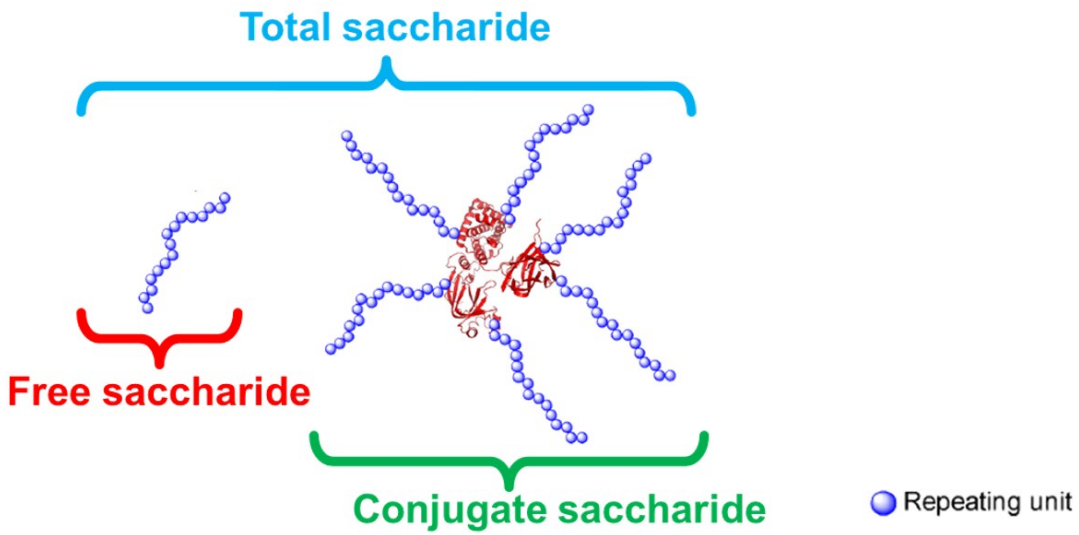
The use of glycoconjugate vaccines is a critical weapon in fighting bacterial infections, and several glycoconjugate vaccines have been licensed through times or are currently in clinical development. This work proposes a new approach to the glycoconjugates vaccine antigen quantification (vaccine potency). According to Pharmacopeia chapter <1234> to assess glycoconjugate vaccine potency, it is requested to measure both the Total amount of Saccharide content TS (conjugated and unconjugated saccharide) and the Free unconjugated amount of saccharide FS (which is not immunogenic), see Figure 1. With those two attributes (TS/FS), it is possible to estimate the amount of Conjugated Saccharide CS (which is the active ingredient). Here, we illustrate a new method to quantitatively purify the CS moiety from the sample matrix and from the FS, enabling, in one single test, the direct accurate quantification of the active ingredient[1]. The assay has been developed and validated for a quadrivalent conjugate vaccine containing meningococcal serogroup A, C, W and Y polysaccharides each coupled with CRM197 protein. Method validation results showed high linearity, precision, accuracy, repeatability, and specificity. In addition, the assay demonstrates to be stability indicating, meaning that if during drug product stability, a decrease of conjugated saccharide occurs it can be quantitatively and accurately measured. The CS test implementation as release test replacing the classical TS and FS assays has been agreed with FDA and has been successfully implemented in Quality control facility.



Total Free and Conjugated Saccharide graphical representation

Bibliographic references:

F. Rech, R De Ricco, S. Giannini, L. Nompari, M. Paludi, S. Berti, C. Parlati, S. Cianetti, F. Berti. (2022), Biologicals (76), 10-14.

PROTACs as new modality for targeting glycosidases and glycosyltransferases

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Glycosidases and glycosyltransferases are carbohydrate processing enzymes that are involved in a wide variety of diseases. Despite extensive research, discovery of effective drugs targeting glycosidases and especially glycosyltransferases has proven rather challenging. To overcome this problem, paradigm-shifting approaches are needed to target these enzymes in a clinical setting. We propose application of proteolysis-targeting chimeras (PROTACs) technology as a potential solution. PROTACs are bifunctional molecules that consist of a ligand of the protein of interest (POI), a linker and an E3 ligase ligand. Upon binding both the POI and the E3 ligase, the POI and E3 ligase are brought in close proximity causing ubiquitinylation of the POI, followed by downstream protein degradation. The mode of action of PROTACs provides several advantages over traditional occupancy-driven inhibitors, including a catalytic degradation mechanism and enhanced selectivity due to the requirement of efficient protein-protein (POI-E3) interactions. As model cytosolic glycosyltransferase and glycosidase targets we chose glycosylceramide synthase (GCS) and non-lysosomal glucocerebrosidase (GBA2) respectively, with implied relevance in Gaucher disease and Parkinson's disease. For this purpose, we selected a dual GCS- and GBA2 iminosugar ligand and functionalized the ligand both at the ring nitrogen atom and at the C1 position in β-configuration. Based on these designs, both GCS-selective and GBA2 selective PROTACs capitalizing on CRBN and VHL E3 ligands are currently under development.

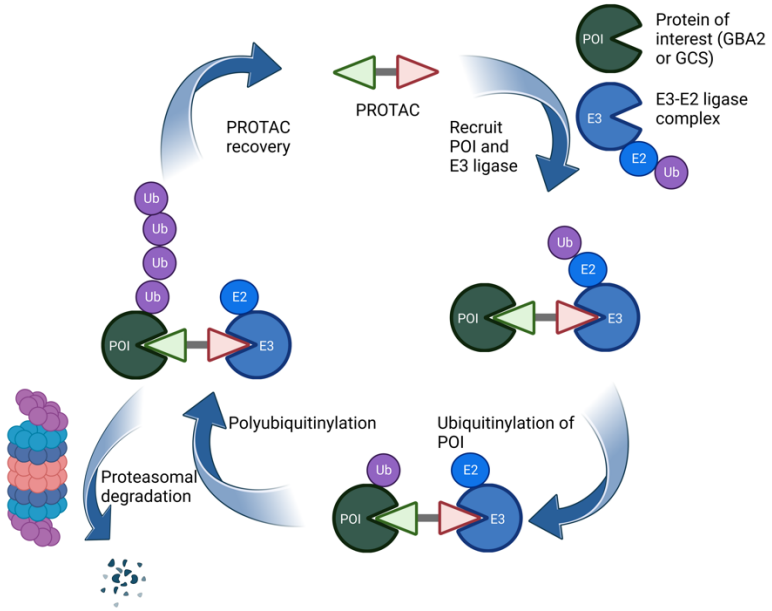

Figure 1. Mechanism of protein degradation caused by PROTACs. Created with BioRender.com.

Bibliographic references:

1. K. Li, C.M. Crews. PROTACs: past, present and future (2022), Chem. Soc. Rev. (51) 5214-5236.

C-glycosyl compounds, key intermediates for the synthesis of anti-proliferative [3.3.0]furofuranone

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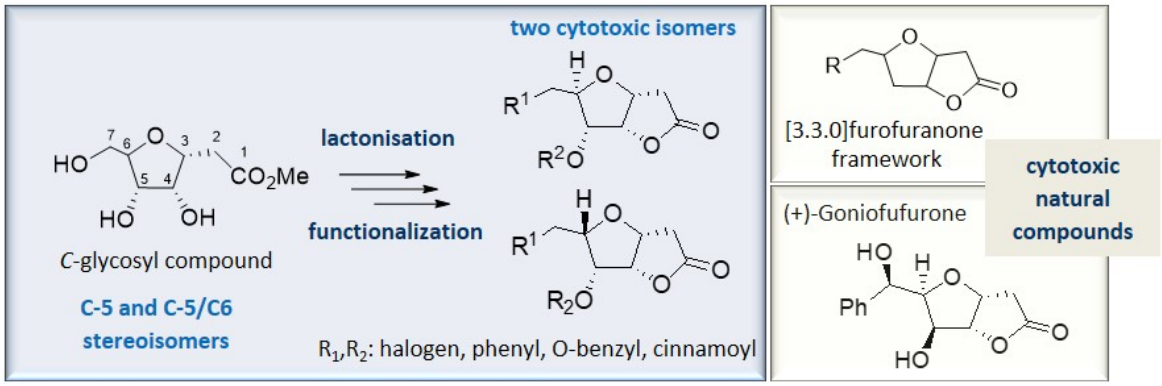
The [3.3.0]furofuranone structure is found in numerous families of biologically active natural products. We took advantage of the stereodiversity afforded by carbohydrate derivatives, and more precisely *C*-glycosyl compounds displaying chemical and biological stabilities,[1] to prepare several compounds structurally similar to goniofufurone and crassalactones which are natural cytotoxic agents.[2,3]

We designed and synthesized several stereoisomers of these natural compounds via lactonization of *C*-glycosyl compounds bearing a hydroxyl on position 4 and a methyl ester on the pseudo-anomeric position. The reactivity of this bicyclic moiety was explored through etherification of hydroxyls in position 5 and 7 and various substituents such as halogen, phenyl, benzyl and cinnamoyl were introduced. The anti-proliferative properties of these mimics were then evaluated on various cancer cell lines and the results highlighted that the stereochemistry of the furofuranone moiety is a crucial point to ensure cellular activity. Interestingly, two compounds demonstrated promising IC_{50} value of 1.34 µM (U251) and 7.60 µM (U87).[4]



Bibliographic references:

1 Y. Yang, B. Yu (2017), Chem. Rev. (117) 12281-12356.
2 V. Popsavin, G. Benedeković, B. Srećo, M. Popsavin, J. Francuz, V. Kojić, G. Bogdanović (2007), Org. Lett. (9) 4235-4238.
3 J. Francuz, I. Kovačević, M. Popsavin, et al. (2017), Eur. J. Med. Chem. (128) 13-24.
4 J. Ariztia, A. Chateau, C. Boura, C. Didierjean, S. Lamandé-Langle, N. Pellegrini Moïse (2021), Bioorg. Med. Chem. (45) 116313.

Towards the use of *Chlamydomonas reinhardtii* as biopharmaceuticals expression system[...]

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For the biopharmaceutical production, the green microalga *C. reinhardtii* benefits from numerous molecular and biochemical tools.. However, most of the biopharmaceutical products are glycoproteins and it is now well-established that glycans harbored by proteins are implicated in their life time, activity and immunogenicity (Lingg et al., 2012, van Beers and Bardor, 2012). To date, the genes encoding enzymes responsible for the *N*-glycosylation steps occurring in the endoplasmic reticulum and Golgi apparatus have been identified in *C. reinhardtii* (Mathieu-Rivet *et al*, 2013). This microalga synthesizes non canonical oligomannosidic *N*-glycans ranging from $Man_5GlcNAc_2$ to $Man_3GlcNAc_2$ and bearing specific residues such as β(1,2), β(1,4)-xylose and α(1,3)-fucose that are absent in mammalian glycoproteins (Lucas *et al*, 2020; Oltmanns *et al*, 2019; Schulze *et al*, 2018). Therefore, in the purpose to use *C. reinhardtii* for the expression of glycosylated biopharmaceuticals suitable for human therapy, the engineering of its *N*-glycosylation pathways represents a major issue. Crossings of insertional mutants for the genes encoding the characterised xylosyltransferases (XTA, XTB) and fucosyltransferase (FucT) lead to a glycoengineered strain with a higher amount of $Man_3GlcNAc_2$ *N*-glycan that represent the basic structure for further glycoengineering.

However, analysis of this strain suggests that an additional xylosyltransferase is involved in the xylosylation process in *C. reinhardtii*. Additional putative candidates have been identified and inactivated in this triple mutant by CRISPR/Cas9. On another hand, the expression level of recombinant proteins remains modest in Chlamydomonas current strains. Recently, it has been shown that mutations in a gene encoding for Sir2-like NAD(+) dependent protein deacetylase may allow a better insertion of DNA that newly enters the cells with no silencing and a better expression level (Neupert *et al*, 2020). The inactivation of this protein in a glycoengineered strain is also a step forward that we performed.The output of this work would help in a near future the heterologous expression of glycosylation enzymes to complement the N-glycosylation pathway in *C. reinhardtii* and increase the biologics production yield to compete with the current expression systems of biopharmaceuticals for human therapy.

Acknowledgements

The authors are thankful to Normandie University, the Graduate School of Research XL-Chem (ANR-18-EURE-0020 XL-Chem the Région Normandie [...]

Bibliographic references:

Lingg N, Zhang P, Song Z, Bardor M. (2012) Biotechnol J., Dec;7(12):1462-72.
Van Beers MM, Bardor M. (2012), Biotechnol J., Dec;7(12):1473-84
Mathieu-Rivet E, Scholz M, Arias C, Dardelle F, Schulze S, Le Mauff F, Teo G, Hochmal AK, Blanco-Rivero A, Loutelier-Bourhis C et al (2013), Molecular & cellular proteomics : MCP 12: 3160-3183
+4 others

Enzymatic transglycosylation for synthesis of novel glycosides from renewable galacto- and glucomann

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Hemicellulose, such as softwood galactoglucomannan is promising renewable bioresource [1]. Degradation is possible with a combination of Glycoside Hydrolases (GHs), e.g family GH27 α-galactosidase and GH5 β-mannanase in cooperation. These enzymes can also synthesize new glycosidic bonds during the catalysis in the presence of other acceptor molecules besides water, referred to as *transglycosylation*. In our work, we have demonstrated synergy between α-galactosidase and β-mannanase in formation of transglycosylation products using galactomannan as donor substrate through covalent fusion of mannose or galactose units to allyl alcohol. We have shown that using HPLC for product screening in conjunction with NMR for structural determination of novel glycosides is a powerful approach for monitoring this reaction. Coincubation of GHs with complimentary activities has potential to result in improved substrate conversion and increased synthesis yields of allyl glycosides [2]. These reactive glycosides could be utilized for downstream applications such as production of biomaterials and for surface modification [3]. Furthermore, an engineered subsite +1/+2 variant of *Trichoderma reesei* b-mannanase *Tr*Man5A showed enhanced allyl mannoside synthesis capacity [2], when compared to the wild-type. This application allows for generation of numerous different novel glycosides such as using allyl- or propargyl alcohol [4] as the acceptor, allowing for downstream application in thiol-ene or -yne click chemistry respectively [5].

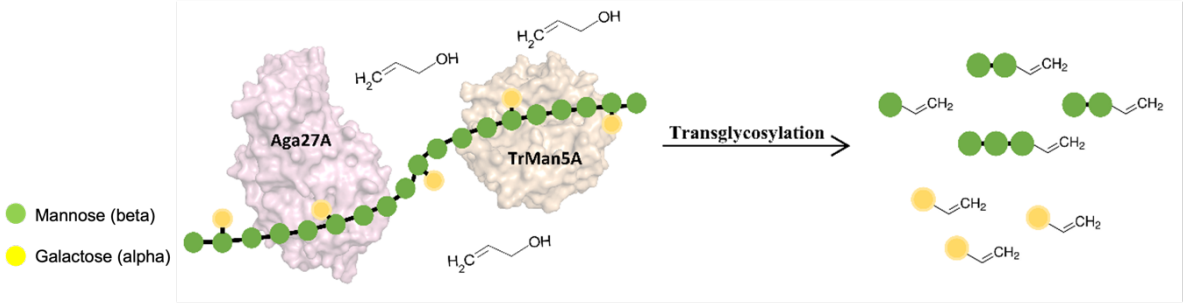

Figure 1. Schematic representation of synergistic approach to enzymatic synthesis of allyl-glycosides using locust bean gum galactomannan as substrate

Bibliographic references:

1. Rowell, Roger M., Roger Pettersen, in Handbook of Wood Chemistry and Wood Composites ed. Roger M. Rowell (2012)
2. Butler, S. J., Birgersson, S., Wiemann, M., Arcos-Hernandez, M., & Stålbrand, H. (2022). Process Biochemistry, 112, 154–166
3. McSweeney, L. et al. (2016). Chem. Eur. J. 2016(12), 2080–2095

Optimisation of a practical synthesis of Lactosamine from Lactulose using a Heyns Rearrangement

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Derivatives of *N*-acetyl-D-lactosamine play an important role in a variety of biological systems and are often found as a component of lipooligosaccharide in bacteria, as well as glycoconjugates. Literature suggests that one of the more practical synthetic methods for preparing *N*-acetyl-D-lactosamine is by employing a Heyns rearrangement on commercially available lactulose [1,2].

Following the published methods for converting lactulose into *N*-acetyl-D-lactosamine (Figure 1), we encountered challenges in the purification of *N*-benzyl-lactosamine, as well as poor yields due to a variety of side-reactions. This presentation will describe our modifications to the published methods in an attempt to improve the efficiency of compound purification and optimise the yield of *N*-acetyl-D-lactosamine from lactulose.

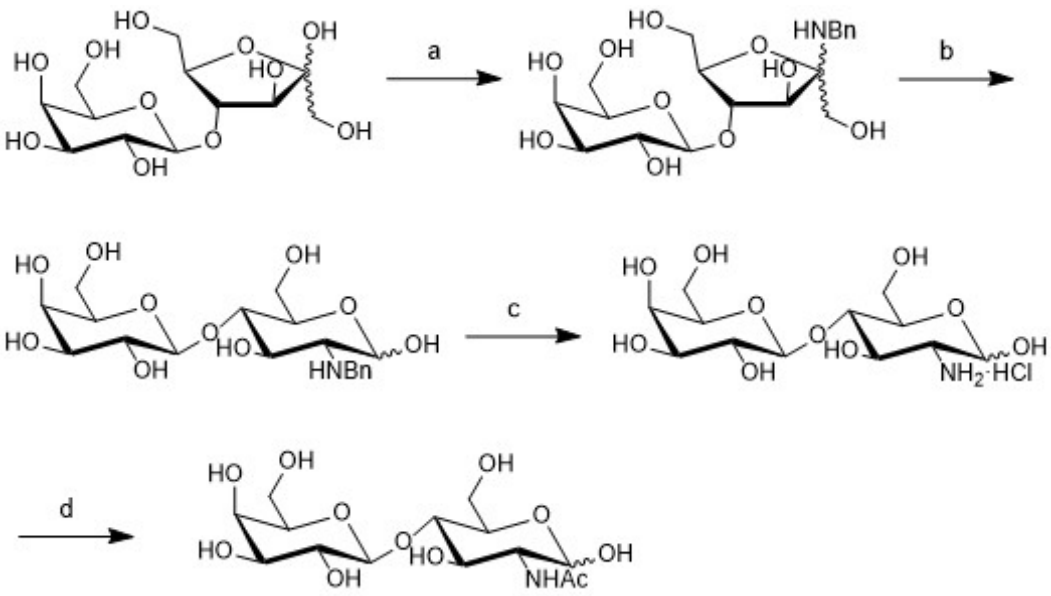

Figure 1. Conversion of lactulose into N-acetyl-D-lactosamine via a Heyns rearrangement

Bibliographic references:

[1] Y. Shan, F. Oulaidi, M. Lahmann (2013), Tetrahedron Lett. 54 (30) 3960–3961.

[2] K. Ágoston, G. Dékány, I. Bajza, M. Hederos (2016), Tetrahedron Lett. 57 (24), 22595–2597.

New 6-*O*-functionalized β-glucoside substrates

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Human cells and tissues contain retaining β-glucosidases that catalyze the removal of glucose from substrates, including the ubiquitous glycosphinglipid glucosylceramide (GlcCer).[1] The lysosomal β-glucosidase, aka glucocerebrosidase (GBA1), is deficient in Gaucher disease (GD), a common lysosomal storage disorder. Mutations in the GBA1 gene cause GD, a disorder manifesting with a great variation in severity and nature of symptoms. Disfunctional GBA1 leads to an accumulation of GlcCer within the macrophages of cell lysosomes, producing irregular and metabolically activated "Gaucher cells".

The various pathophysiological mechanisms are still poorly understood as is the increased risk for Parkinsonism in GD carriers.[2] In plants steryl-β-glucosides (sterolins) occur and some of these 6-*O*-acylated structures are also present.[3] The existence of steryl glycosides (SGs) in animals and humans is poorly understood and the sterol glycosyltransferases that are responsible for the biosynthesis of SGs in plants, fungi, and bacteria have not been identified in mammals. It has recently become apparent that lysosomal GBA1 and its cytosolic counterpart β-glucosidase named GBA2 not only cleave GlcCer to glucose, but also may act as a transglucosidase, by transferring a glucose to a metabolite acceptor.

The physiological relevance is well documented for glucosylated cholesterol (GlcChol).[4] This research aims to study the ability of human GBA1 to hydrolyze 6-*O*-acylated 4MU-β-glucose and close analogues and its ability to generate SGs. More stable variants of this ester linked compound towards esterases are desired as deacylation was observed in cell lysate samples. Five different analogues of this ester linked substrate were synthesized containing different chemical linkages to their acyl or alkyl chain. These substrates were tested for their activity against pure recombinant GBA1 (Cerezyme®) in a 4MU assay. Subsequently the transglucosylation of NBD-cholesterol, a fluorogenic analogue of cholesterol, with the most promising ether linked substrate was investigated by HPTLC analysis.

These results provide the first evidence that GBA1 is able to hydrolyse and transglucosylate 6-alkylated 4-MU-β-glc substrates to metabolite acceptors such as cholesterol. This new substrate toolbox will shed some light on the relevance and scope of glycosylation by GBA1 in humans.

Bibliographic references:

1 JMFG, Aerts, et al. Curr. Opin. Chem. Biol. 2019, 53, 204-215.
2 E. Aflaki, W. Westbroek, E. Sidransky. Neuron 2017, 93, 737–746.
3 M. Shimamura. Biochem. J. 2020, 477, 4243–4261.
4 AR, Marques. et al. J. Lipid Res. 2016, 57, 451-463 .

Synthesis, conformational analysis and GalNAc-Lectin interactions of constrained C-glycosidic analog

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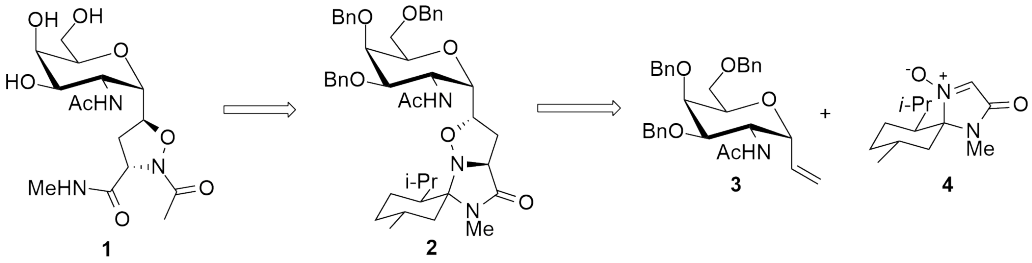
Tumor-associated carbohydrate antigens (TACAs) are highly present on cancer cells, but almost no detectable on normal cells. Such structures like T_N antigen represent interesting therapeutics targets, particularly by incorporating them in anticancer vaccines. However, glycoconjugate vaccines displaying *O*-glycoside could suffer from the lability of the *O*-glycosidic link [1] leading to a loss of the saccharidic moiety which would have a direct impact on first, the recognition and then on the immune response. The replacement of the glycosidic bond is well known to afford more stable analogues such as *C*-glycosidic ones. Incorporation of analogues containing non-natural carbohydrates in vaccine could also improve immune response of such vaccines. [2,3]

Recently, we focused our attention on the synthesis of constrained *C*-glycosidic analogues of Tn antigen, based on a [3+2] cycloaddition strategy between a *C*-vinyl-GalNAc **3** and a chiral cyclic nitrone **4** (Scheme 1). This key step provided access to cycloadduct **2** [4], precursor of analogue **1**. Conformational analysis of constrained analogue **1** and its molecular recognition with Gal-NAc-specific lectins have been studied and will be presented.



Scheme 1. Synthetic strategy of constrained C-glycoside analogue of Tn antigen

Bibliographic references:

[1] Dwek, R. A. Chem. Rev. 1996, 96, 683–720.
[2] Allen, J.; Harris, C.; Danishefsky, S. J. J. Am. Chem. Soc. 2001, 123, 1890-1897.
[3] Nativi, C.; Papi, F.; Roelens, S. Chem. Commun. 2019, 55, 7729–7736.
[4] Rouzier, F.; Sillé, R.; Montiège, O.; Tessier, A.; Pipelier, M.; Dujardin, G.; Martel, A.; Nourry, A.; Guillarme, S. Eur. J. Org. Chem. 2020, 6749–6757.

Synthesis of glycopolymers via Click2 Chemistry for Viral Inhibition

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Heparan sulfate proteoglycans, or HSPGs, are ubiquitous molecules found on host cell surfaces. They are implicated in the binding and fusion of many viruses such as HIV or SARS-CoV-2. The McReynolds lab is developing HSPG mimics to prevent the initial binding of the virus to the host cells, which could minimize transmission of viral pathogens, or lessen/shorten the severity or length of illness in those infected. My project focuses on synthesizing a sulfated carbohydrate compound that will be clickable to a polymer to make glycopolymers that will serve as viral-binding HSPG mimics.

A crucial step in my project is the attachment of a sulfated carbohydrate compound to a star-shaped polymer that utilizes the click chemical reaction SPAAC, or strain-promoted alkyne-azide cyclization, yielding the final sulfated glycopolymer. This reaction is under the scope of green chemistry, utilizing reagents and conditions that are environmentally friendly. Other important green aspects of this chemistry that make it attractive are the total atom economy, simple purification methods, and high resultant yields. The glycopolymer products will undergo binding affinity assays to the HIV glycoprotein, gp120, and the SARS-CoV-2 spike (S) protein. The overall goal of this project is the development of a broad spectrum anti-viral drug to combat a variety of viruses.

Synthesis and immunomodulatory functions of the glycolipid ligands of C-type lectin receptor Mincle

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The macrophage-inducible C-type lectin (Mincle, Clec4e, ClecSf9) recognizes various glycolipids, including trehalose dimycolate (TDM) from *Mycobacterium tuberculosis*, and activates the innate immune system.[1] Regarding the ligand recognition mechanism of Mincle, the polar head groups of the ligands (eg. trehalose moiety in TDM) are generally considered to interact with Ca ion /its proximal site around Mincle surface. Although several types of ligand structures have been reported, the detail recognition of the hydrophobic part of the ligands was not really understood. The intracellular behavior of Mincle and the ligands has not been explored either.

In order to understand the detailed structure-activity relationships, we firstly focused on b-mannosyloxymannitol glycolipid “44-2”, which consists of L-mannitol modified with b-mannosylated triacyl moieties at the 1,3,4-position showing a potent murine Mincle-mediated signaling activity. We established an efficient synthetic method for both enantiomers of 10-hydroxy stearic acid and achieved the synthesis of the diastereomeric pair of b-mannosyloxymannitol glycolipids and their analogues.[2] With using similarly modified lipid moiety, design and synthesis of trehalose diester analogues were achieved. The ligand structure–Mincle-mediated signaling activity relationships were then analyzed.[3] Based on the established synthetic method, we also synthesized molecular probes and utilized them for observation of the intracellular behavior of Mincle and the ligand.

Bibliographic references:

1) Yamasaki, S. et al. J. Exp. Med. 2009, 206, 2879.
2) Matsumaru, T.; Fujimoto, Y. et al. Eur. J. Org. Chem. 2022, 20, e202200109.
3) Matsumaru, T.; Fujimoto, Y. et al. Bioorg. Med. Chem. 2022, 75, 117045.

Synthesis of erythropoietin with tetraantennary *N*-Glycans

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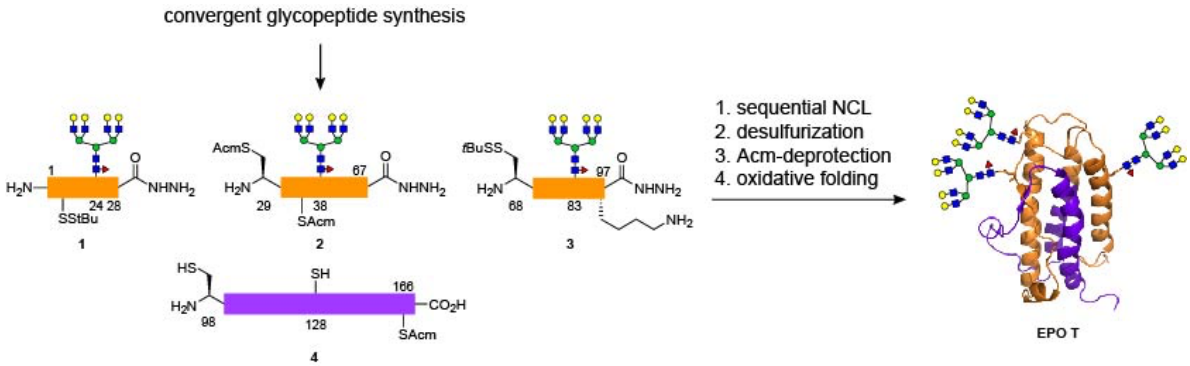
Erythropoietin (EPO) is an *N,O*-glycosylated cytokine that plays an important role in the formation of erythrocytes. Recombinantly expressed human EPO is thus used for the treatment of anaemia in kidney and cancer patients. EPO obtained from Chinese hamster ovary cells shows a broad spectrum of different glycoforms.[1] To determine glycan-related bioactivities of EPO, homogenous glycoforms are needed. This can be accomplished by chemical synthesis of glycoproteins and chemoenzymatic modification.[2,3]

Erythropoietin bearing three *N*-glycans can be synthesized by sequential native chemical ligation.[4,5] Here we show the synthesis of EPO carrying three of the typical tetraantennary core-fucosylated *N*-glycans.[1,6] The sialylation of the galactosylated tetraantennary glycopeptide segments **1**-**3** turned out to be difficult. Thus, the non-sialylated segments **1**-**4** were sequentially ligated followed by an intermediate desulfurization of the non-native cysteines to alanine and removal of the Acm groups. The homogenous glycoprotein **EPO T** was successfully obtained after oxidative folding (22 %). In contrast to the glycopeptide segments **1**-**3**, folded **EPO T** was more readily modified by α-2,6-sialyltransferase.[5]



Bibliographic references:

[1] Z. Szabo, ... C. Pohl (2018) J. Proteome Res., 17, 1559–1574.
[2] C. Unverzagt, Y. Kajihara (2013) Chem. Soc. Rev., 42, 4408–4420.
[3] C. Unverzagt, Y. Kajihara (2018), Curr. Opin. Chem. Biol., 46, 130–137.
[4] Y. Maki, ... Y. Kajihara(2020), J. Am. Chem. Soc., 142, 20671–20679.
[5] H. Hessefort, ... C. Unverzagt (2021), Angew. Chem. Int. Ed., 60, 25922–25932.
[6] T. Luber, ... C. Unverzagt (2018), Angew. Chem., 130, 14751–14757

Biochemical approach to the development of novel covalent inhibitors of viral 9-I-acetylesterases

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Sialic acids are a diverse family of nine carbon α-keto sugars, found at the termini of glycoproteins and glycolipids [1]. Many different pathogens use terminal sialic acids of sialoglycans as primary or accessory receptors, often showing specificity for a type of sialic acid [2]. The most prevalent post translational modification of sialic acids is the acetylation of the C9 hydroxyl group [3]. These 9-O-acetylated sialic acids are exploited by influenza C (ICV) and human coronaviruses (HCoV), specifically the HKU1 and OC43 strains [4,5]. Inhibition of haemagglutinin-esterase (HE) in recombinant HCoV-OC43 with a neutralizing antibody identified viral esterases as valid anti-viral targets [6].

The goal of this project is to identify and characterize small molecule inhibitors of viral sialic acid esterases, to generate a new type of anti-viral molecule. To achieve this goal, the HEs of HKU1, OC43, and the hemagglutinin esterase fusion (HEF) of ICV was purified. After obtaining pure protein samples, a high-throughput screen was developed to identify active small molecule inhibitor scaffolds. An active scaffold will lead to a hit-optimization study and eventually result in a novel viral esterase inhibitor.

Bibliographic references:

1]A.Varki (2008),Trends in Molecular Medicine(14)351-360
2]B.R.Wasik,K.N.Barnard,C.R.Parrish(2016),Trends in microbiology,24(12)991-1001
3]R.Gerardy-Schahn,...&R.Vlasak(2015),Sialoglyco chemistry and biology I,1-30
4]P.B.Rosenthal,...& D.C.Wiley(1998),Nature, 396(6706),92-96
5]R.Vlasak,...&P.Palese(1988),PNAS,85(12),4526-4529
6]M.Desforges,...&P.JTalbot (2013),Journal of virology, 87(6), 3097-3107

Characterization and engineering PsGalOx, a new bacterial galactose oxidase

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Galactose oxidases (GalOxs) are highly attractive enzymes because they show high specificity and regioselectivity toward carbohydrate substrates efficiently combined with reduction of oxygen to hydrogen peroxide.[1] These metalloenzymes have a copper ion as a cofactor and are secreted by various filamentous fungi, with GalOx from *Fusarium graminearum* being the best-studied representative. These enzymes' potential include biotechnological applications in small molecule synthesis, oxygen removal, biosensors, and cell surface glycoprotein modification.[2] While galactose oxidases from fungal origins have been vastly studied, there is a growing interest in GalOxs from bacterial origin due to its ease of production and engineering.

In this work we have investigated *Ps*GalOx, an enzyme from the bacterium *Pseudoarthrobacter siccitolerans*. We have unraveled its biophysical and kinetic characteristic while tailoring its catalytic properties through enzyme engineering approaches. To accomplish this, directed evolution was applied recurring to random mutagenesis through error-prone PCR (epPCR). The workflow was developed with two high-throughput screening approaches, 'activity-on-plate' and 96-well plate liquid screening, which were successfully optimized and validated. Multiple rounds of engineering led to the identification of a variant which shows close to 20-fold higher protein production yields and 10-fold increased catalytic efficiency (kcat/Km) for D-galactose as compared with the wild-type. We are currently evolving further *Ps*GalOx to enhance its catalytic activity for aromatic alcohols and further exploring its applications to carbohydrates. These findings will contribute to a better understanding of GalOx, revealing novel or improved properties to be explored in various biotechnological fields.

Acknowledgements

Fundação para a Ciência e Tecnologia (FCT), Portugal
B-Ligzymes (GA 824017) from the European Union's Horizon 2020 Research and Innovation Program.

Bibliographic references:

1 Savino, S. and Fraaije, M. W. (2021) The vast repertoire of carbohydrate oxidases: An overview. Biotechnol Adv. 51
2 Pedersen, A. T., Birmingham, W. R., Rehn, G., Charnock, S. J., Turner, N. J. and Woodley, J. M. (2015) Process Requirements of Galactose Oxidase Catalyzed Oxidation of Alcohols. Org Process Res Dev. 19, 1580-1589

Catching E. coli: targeting FimH with *C*-glycosidic α-mannoside modified cellulose

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C-Glycosides, where the glycosidic linkage consists of a carbon-carbon bond, are stable against most chemical reactions as well as hydrolysis in biological systems, compared to the naturally occurring *O*- and *N*-glycosides.[1] This stability makes *C*-glycosides interesting candidates for therapeutics against various diseases. Different ligation handles, such as azides, alkynes, amines or aldehydes, can be introduced, which makes the linkage to e.g. polysaccharides via orthogonal conjugation methods possible.[2] Thus, specific biological properties of the polysaccharide backbone can be introduced by target specific choice of the sugar based small molecule entity.

For example, uropathogenic *E. coli* (UPEC) uses lectin FimH to bind to D-mannose residues on the surface of human cells, causing urinary tract infections (**Figure 1A**). D-Mannose can therefore be used in an antiadhesion therapy against UPEC. To increase the effectivity of such therapies, different potent FimH inhibitors have already been synthesized.[3] In our approach, we synthesized *C*-glycosidic D-mannose derivatives followed by linkage to an azido modified cellulose matrix (**Figure 1B**). Synthetic details and results will be presented.

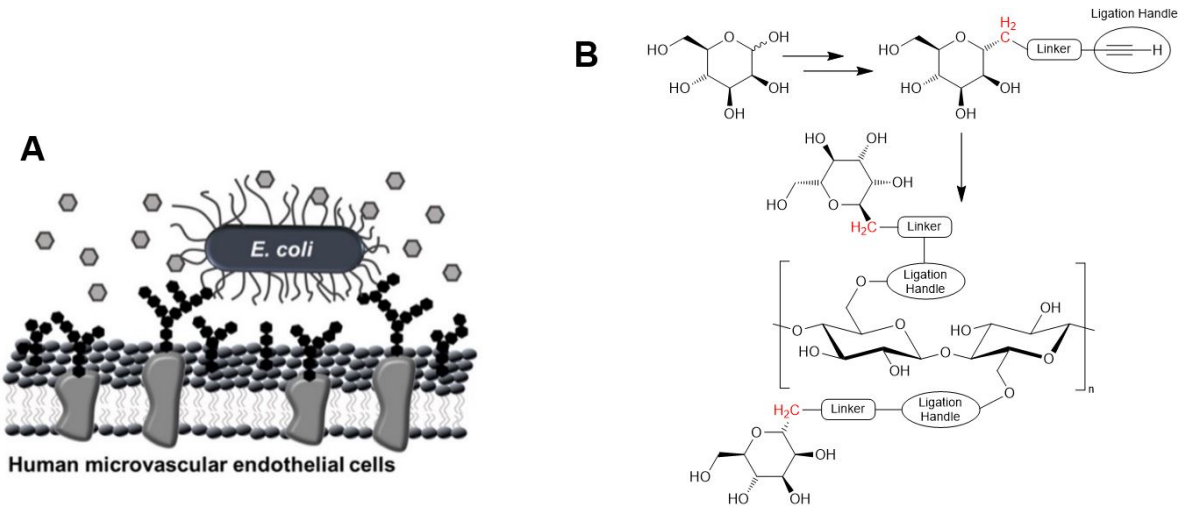

Figure 1: A) E. coli binds to human cells using FimH.[3] B) Synthesis of C-glycosidic D-mannose derivatives for a covalent linkage to cellulose.

Bibliographic references:

[1] J. Ati, P. Lafite, R. Daniellou (2017), Beilstein J. Org. Chem. (13), 1857–1865.
[2] A. Koschella, C.-Y. Chien, T. Iwata, M. S. Thonhofer, T. M. Wrodnigg, T. Heinze (2020), Macromol. Chem. Phys. (221), 1900343.
[3] L. Möckl, C. Fessele, G. Despras, C. Bräuchle, T. K. Lindhorst (2016), Biochimica et biophysica acta (1860), 2031-2036.

Synthesis of fully desymmetrized trehalose building blocks

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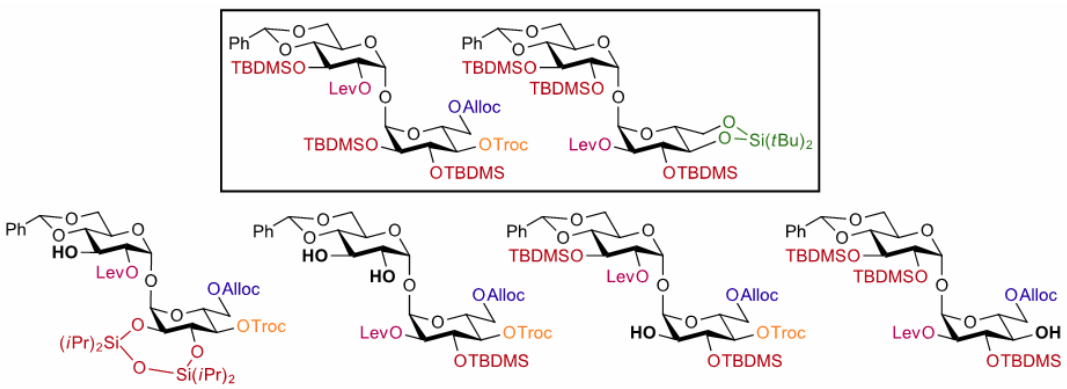
Trehalose-containing glycans found in bacteria (*Mycobacteria, Corynebacteria*), fungi (*Fusarium*) and worms (*Caenorhabditic elegans*) play an essential role in host-pathogen interaction, cell signaling and bacterial pathogenesis and are therefore important synthetic targets. The chemical synthesis of trehalose derivatives as part of glycoconjugates and parasitic glycans is challenging as it requires either stereoselective 1,1´-glycosylation or desymmetrization of eight hydroxyl groups in a C_2-symmetric nonreducing disaccharide with the aim of attaching different functionalities at a specific position. We aimed to produce a collection of differently and variably protected non-symmetrical trehalose building blocks ready for a final chemical derivatization with functional or reporter groups.

We developed an efficient and scalable (7-step, multigram scale) reaction sequence leading to a set of desymmetrized trehaloses as a starting point for a library of fully orthogonally protected trehalose derivatives. Using a site-specific protection strategy and regioselective functionalization, we created a pool of trehalose-derived building blocks with one or two free hydroxyl groups at specific positions. Such synthetically advanced trehalose derivatives can find wide application in the synthesis of sulfolipids[3], derivatized trehalose molecules ready for biorthogonal chemistry to track pathogenic *Mycobacterium* strains or to label the mycobacterial membrane; or for the development of probes to explore mycobacterial transporter proteins.[4]



Fully orthogonally protected trehalose building blocks (box), selection of desymmetrised trehaloses

Acknowledgements

Financial support by Austrian Science Fund FWF (grant P-32397-N28) is gratefully acknowledged.

Bibliographic references:

[1]S.-X.Yang, Y. Kuang et al., Org. Biomol. Chem. 2012, 10 (4),819.;B. M. Swarts, C. R. Bertozzi et al., J. Am. Chem. Soc., 2012, 134,16123
[2]S. Jana,; S. S.Kulkarni, Org.Biomol.Chem., 2020, 18 (11),2013
[3]D. Geerdink, A.J. Minnaard, Chem. Commun., 2014, 50,2286
[4]T. Dai, J. Rao et al., J. Am. Chem. Soc., 2020, 142 (36),15259;C.M. Furze, E. Fullam et al., J. Biol. Chem., 2021, 296,100307

Towards specific small molecule ligands for the C-type lectin-like receptor 2 (CLEC-2)

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Cardiovascular diseases are the primary cause of death worldwide [1]. One treatment option for acute coronary syndromes, stroke, and peripheral arterial disease are antiplatelet drugs that inhibit platelet aggregation and activation [2]. In this context, the glycoprotein receptor C-type lectin-like receptor 2 (CLEC-2) emerged as a promising therapeutic target due to its critical involvement in thrombocyte-associated pathologies and alongside predicted minimal disruption of homeostasis [3].

Here we present our efforts towards the development of specific ligands for CLEC-2 through a multifaceted, biophysical approach. Initially, we evaluated a repertoire of known small molecule ligands for CLEC-2 using a range of biophysical assays (thermal shift assay, nuclear magnetic resonance & grating-coupled interferometry). Further, we applied a fragment-based screening approach to discover novel ligands for CLEC-2.

Surprisingly, our experimental efforts to reproduce previously reported evidence of interaction between CLEC-2 and the available small molecule ligands yielded inconsistent results. This discrepancy emphasises the necessity for comprehensive and careful evaluation of ligand-receptor interactions. Ligands derived from the identified fragments hold the potential to modulate CLEC-2 and platelet activity, which could open up new avenues to tackle the significant burden imposed by cardiovascular diseases.

Acknowledgements

The project has been funded by the Vienna Science and Technology Fund [10.47379/LS21039] and Marie Skłodowska-Curie Grant Agreement 956314 ALLODD.

Bibliographic references:

[1] the CARDIoGRAMplusC4D Consortium (2015), Nat Genet (47) 1121–1130.
[2] M. H. Harbi, C. W. Smith, P. L. R. Nicolson, S. P. Watson & M. R. Thomas (2021), Platelets (32) 29-41.
[3] J. Rayes, S. P. Watson, B. Nieswandt (2019), J. Clin. Investig. (129)12–23.

Synthesis of oligosaccharides related to Rhamnogalacturonan-II

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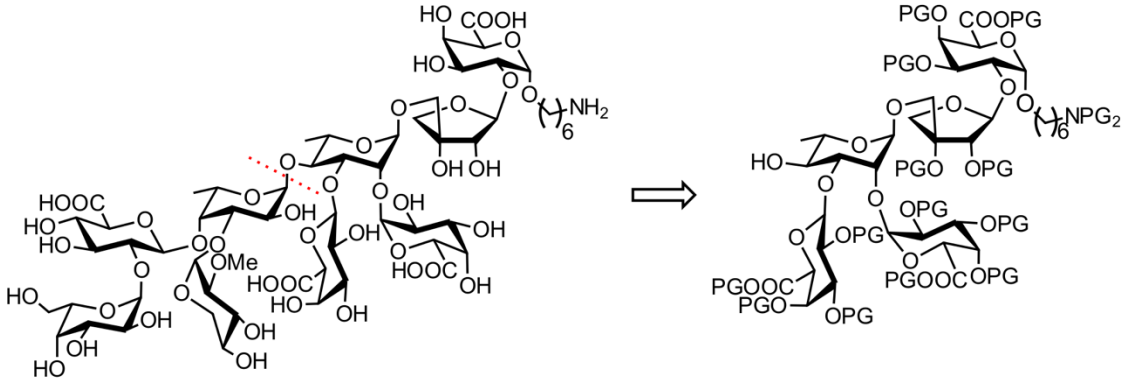
Pectin is one of the most complex, yet poorly understood polysaccharide structures in nature, with irreplaceable relevance for the human diet.[1] The pectin domain rhamnogalacturonan-II (RG-II) is the most heterogenic, structurally diverse part of pectin and crucial for vascular plant growth and survival.[2] We present here our work towards a chemical total synthesis of linker-functionalized RG-II side chain A, including the retrosynthesis and the synthesis of a pentasaccharide acceptor fragment.

Key features of the target structure are seven different monosaccharides including the rare sugar apiose, three branches, five 1,2-*cis*-glycosidic linkages, three galacturonic and one glucuronic acid and a methyl ether. The total synthetic approach relies on carefully chosen orthogonal protective group patterns and various glycosylation methods to tackle this highly branched and unusually complex nonasaccharide.



Acknowledgements

This work was supported by the Austrian Science Fund (FWF, grant P35406 to FP).

Bibliographic references:

[1] Ndeh, D. et al. (2017), Nature (544) 65-70.

[2] Sechet, J. et al. (2018), Plant J. (96) 1036-1050.

Active material from Bulgarian fenugreek polysaccharides

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Trigonella foenum graecum L. (Fenugreek) is a dicotyledonous medicinal plant which belongs to the Fabaceae family. Its seeds are used as a traditional remedy for decreasing blood sugar levels, lactation stimulant and kidney problems. These therapeutic applications are attributed to various bioactive compounds present in the seeds, such as saponins, flavonoids, alkaloids [1]. They also contain carbohydrates and, in particular, galactomannans which could be used for bone tissue engineering [2]. However, there is limited information on the determination of the composition of Bulgarian fenugreek polysaccharides and their use for hydrogel preparation. In the present study, isolation and purification of fenugreek seeds polysaccharides was carried out. Galactomannan composition and structural characterization were determined by GC-MS, HPAEC, SEC-MALLS, NMR and FTIR.

The obtained galactomannan was regio-selective oxidized using TEMPO chemistry to generate new family of polyglycuronic acid. Antioxidant and antimicrobial activities were investigated and compared with the natural Fenugreek galactomannan. The polyglycuronic acid derivative was investigated for reticulation using $CaCl_2$ as cross-linker to generate stable and homogenous hydrogels. This new anionic-biomolecule constitute an innovative active polysaccharides-based hydrogel as a bioink for 3D-bioprinting in the tissue engineering field.

Bibliographic references:

[1] T.Visuvanathan,L.Than,J.Stanslas,S.Chew,S.Vellasamy. Revisiting Trigonella foenum-graecum L.: Pharmacology and Therapeutic Potentialities (2022), Plants. 11(11):1–14.

[2] I. Zia,S.Mirza,R.Jolly,A.Rehman,R.Ullah,M.Shakir. Trigonella foenum graecum seed polysaccharide coupled nano hydroxyapatite-chitosan: A ternary nanocomposite for bone tissue engineering (2019), Int J Biol Macromol.124:88–101.

F-labelled 2-aminobenzamidoximes as probes for ^{19}F-NMR analysis of aldoses

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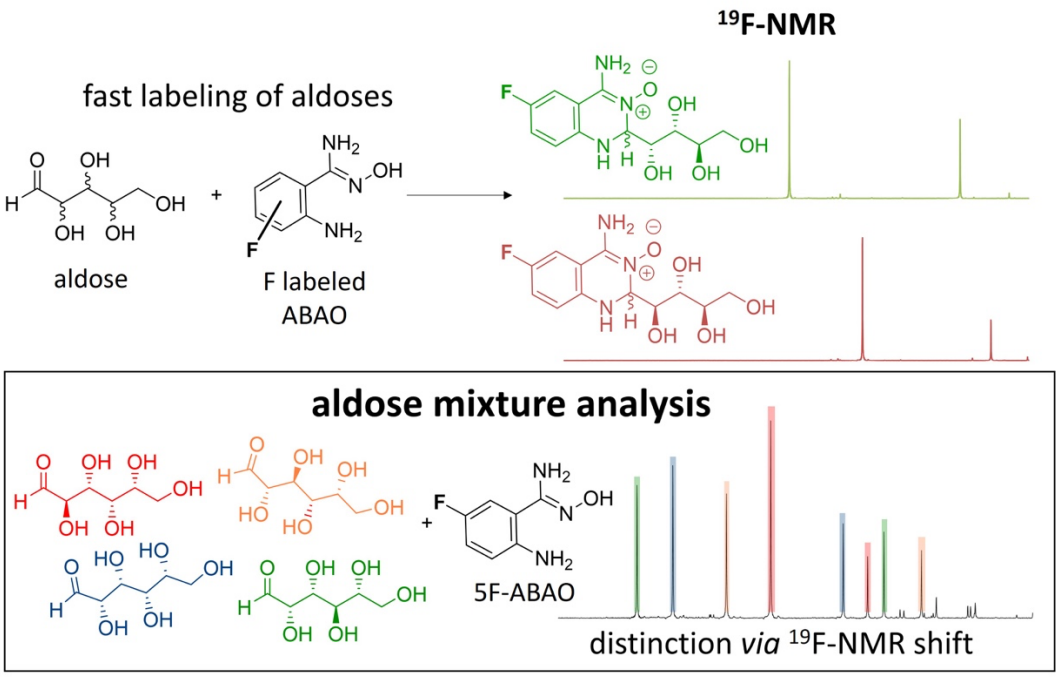
Aldoses are structurally composed of a reactive aldehyde group and multiple hydroxy groups. Each specific sugar exhibits a defined stereochemistry of these alcoholic moieties. Being constitutional isomers, the differentiation between sugars, especially in mixtures, is challenging. Previously, we have described a photometric kinetic assay to determine the open-chain content of aldoses utilizing the selective reaction with 2-aminobenzamidoxime (ABAO) [1].

Herein, we have taken further advantage of this aldehyde-selective reaction, using it for a qualitative aldose assay based on ^{19}F-NMR, a highly sensitive and fast method. The influence of the position of the F-label on the adduct formation rates was determined and derivatives of selected aldoses were prepared and the deviation of chemical shifts in ^{19}F-NMR of the products were compared. While all four tested F-ABAO derivatives showed differences in shifts between the tested aldoses, one derivative was especially promising for the application in a qualitative sugar assay and the scope was expanded to a larger set of aldoses for this derivative.



Acknowledgements

We thank Bianca Warton for technical support. The Austrian Science Foundation (FWF, P 29138-N34) is gratefully acknowledged for financial support.

Bibliographic references:

[1] H. Kalaus, A. Reichetseder, V. Scheibelreiter, F. Rudroff, C. Stanetty, M. D. Mihovilovic (2021), Eur. J. Org. Chem. (18) 2589-2593.

Libraries of Functionalized Human Milk Oligosaccharides

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Human milk oligosaccharides (HM-OS) were converted into glycine-spacered derivatives [1] to give rise to a library of over 50 amino functionalized oligosaccharides. These included core structures (lactose, N-acetyllactosamine, lacto-N-tetraose, lacto-N-neotetraose, lacto-N-hexaose, lacto-N-neohexaose, para-lacto-N-hexaose, lacto-N-iso-octaose), fucosylated (2'/3-fucosyllactoses, difucosyllactose, lacto-N-fucopentaoses I,II,III,IV,V, lacto-N-difucohexaoses I,II, lacto-N-neodifucohexaose I, monofucosyllacto-N-hexaoses I,II,III, monofucosyllacto-N-neohexaoses I,II, monofucosyl-para-lacto-N-hexaose I,IV, difucosyllacto-N-hexaoses a,b,c, difucosyllacto-N-neohexaose I,II, difucosyl-para-lacto-N-hexaose, difucosyl-para-lacto-N-neohexaose, trifucosyllacto-N-hexaoses I,II, monofucosyllacto-N-iso-octaoses I,II, difucosyllacto-N-iso-octaoses I,II, trifucosyllacto-N-iso-octaoses I,II, sialated (3'/6'-sialyllactoses, sialyllacto-N-tetraoses a,b,c, disialyllacto-N-tetraose, monosialyllacto-N-hexaose, monosialyllacto-N-neohexaose, disialyllacto-N-hexaose), and sialated/fucosylated (3'-sialyl,3-fucosyllactose, sialyllacto-N-fucopentaose, monosialyl,monofucosyllacto-N-hexaose, monosialyl,monofucosyllacto-N-neohexaose, disialyl,monofucosyllacto-N-hexaose) HM-OS.

These derivatives may be used for plasmon resonance studies [2], preparation of neoglycoconjugates [3] and nanoparticles [4] but most promising is their application for glycoarray preparation via amino [5] or aldehyde [6] group, or by click addition after modification with 4-pentinoic acid.

Acknowledgements

This work was supported by the Ministry of Science and Higher Education of the Russian Federation (Contract No. 075-03-2023-642)

Bibliographic references:

[1] L.M. Likhosherstov et al. (2012), Rus. Chem. Bull. (61)1816-1821.
[2] C. Li et al. (2021), Glycobiology (31) 931-946.
[3] J. Shang et al. (2013), Glycobiology (23) 1491-1498.
[4] N.A. Samoilova et al. (2017), J. Appl. Polymer Sci. (134) 1-12.
[5] M.A. Krayukhina et al. (2018), Biointerface Res. Appl. Chem. (8) 3095-3099.
[6] L.M. Likhosherstov et al. (2018), Rus. Chem. Bull. (67) 371-376.

Multiscale computational approaches to investigate the solution structure of saccharides

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Saccharides exhibit a high degree of conformational heterogeneity which makes studying their dynamic solution structure a demanding task. In addition to well-established experimental methods (e.g. the NMR spectroscopy) a series of theoretical approaches can be used either as independent or complementary techniques. One of the most popular one are the explicit-solvent, classical molecular dynamics (MD) simulations. However, the more advanced, quantum-mechanical (QM)-based techniques allow, at cost of reduced computational efficiency, to determine a series properties relying on electronic structure of molecule. There is a growing number of reports, indicating the QM-based calculations should not be limited to only a single saccharide structure (even if this structure has the lowest possible energy) but, instead, they should consider a broad set of diverse structures, accounting for conformational diversity of saccharides in solution systems.

We are going to present and discuss the computational approach that combines the most beneficial features of classical MD simulations and high-level QM computations. According to this method, designed, tested and validated in the context of small saccharide molecules, the structural data from classical MD simulations are rationally subsampled and passed to further, more advanced calculations, carried out at the QM level of theory. In particular, such approach can be used for studying the conformational properties of non-standard saccharides (e.g. conformers of glycosidic linkage and other rotatable groups), problematic degrees of freedom (e.g. ring distortions in both furanoses and pyranoses) as well as anomeric and tautomeric properties of monosaccharides. In particular, the use of QM-based methodology allows for eliminating the uncertainties related to the accuracy of the classical force fields, and, more importantly, for obtaining the spectroscopic data that can directly be referred to experiment (e.g. *J*-coupling constants or chemical shifts).

The applicability and generality of our approach will be demonstrated by presenting the calculation results related either to well-studied systems (D-glucopyranose, L-iduronate) or to rare sugars (tautomers of D-allosamine). Moreover, we will show how our theoretical predictions may contribute to obtaining valuable information on the saccharides that are either synthesized compounds or isolated from natural sources.

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

Semi-synthetic study of glycoprotein interleukin-6 (IL-6)

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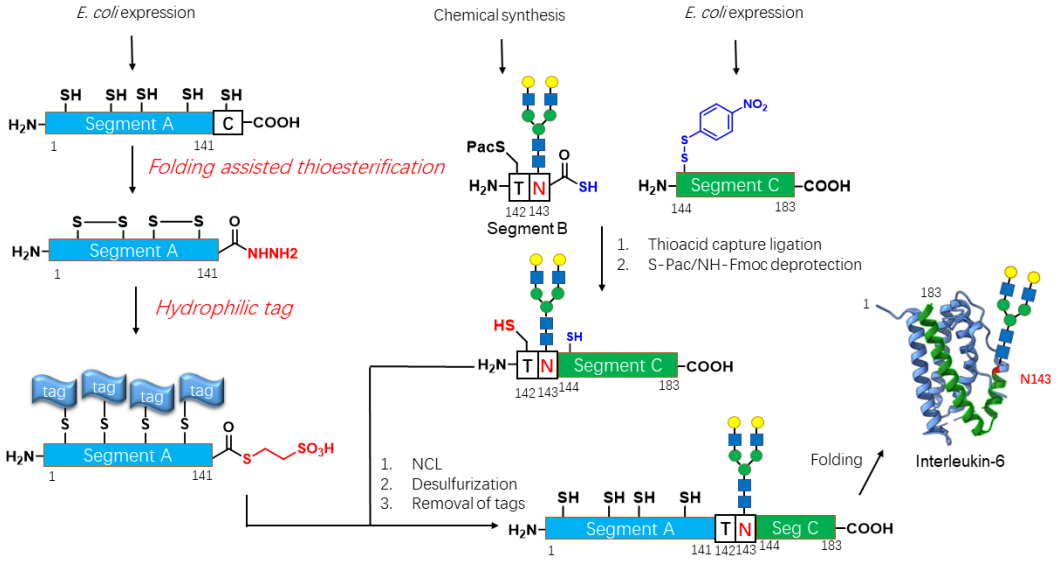
As a ubiquitous post-translational modification on protein, *N*-glycosylation performs various functions which are closely related with its structure. The elucidation of such structure-function relationship requires preparation of homogenous glycoprotein. However, mammalian cell expression only produce protein with heterogenous *N*-glycan structure, while total chemical synthesis using solid-phase peptide synthesis (SPPS) is usually laborious and time-consuming.

In this research, to investigate human interleukin-6 (IL-6) bearing *N*-glycan at N143, we established a novel semi-synthetic strategy to obtain homogeneous glycoprotein within few chemical conversion steps. In this strategy, a key building block: glycodipeptide (T142-N143) bearing asialo *N*-glycan was chemically synthesized, while non-glycosylated fragments were prepared by *E. coli* expression. Notably, the challenging expressed peptide thioesterification of N-terminal fragment containing 141 residues was achieved by utilizing a novel "folding-assisted thioesterification" method. In addition, the problems derived from hydrophobicity of IL-6 were solved by using a novel hydrophilic tag, which could improve the solubility of peptide and impede aggregation.

Finally, we succeeded in folding of N143-glycosylated IL-6 after two steps of ligation and removal of hydrophilic tag. We suppose that these newly developed methods could greatly facilitate semi-synthesis of homogeneous glycoprotein, especially challenging synthetic target, for elucidation of *N*-glycan functions.



Semi-synthetic strategy of N143-glycosylated interleukin-6

Analysis of selenoprotein F binding to UDP-glucose:glycoprotein glucosyltransferase (UGGT)

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UDP-glucose: Glycoprotein glucosyltransferase 1 (UGGT1) glycosylates non-glucosylated glycoproteins in an incompletely folded state. The mono-glucosylated glycans generated by the action of UGGT1 bind to the ER-resident lectin chaperones, calnexin and calreticulin, which in turn promote folding of the bound glycoproteins in collaboration with ERp57. UGGT1 is also known to form a 1:1 complex with selenoprotein F (SelenoF). However, the function of SelenoF associated with UGGT1 is not clear.

Furthermore, the binding position of SelenoF to UGGT1 has also been unclear. In this study, we aimed to investigate the binding region of human UGGT to recombinant SelenoF with Sec replaced by Cys by co-immunoprecipitation analysis using FLAG-tagged UGGT1 and c-myc-tagged SelenoF. The results show that SelenoF binds to the TRXL2 domain of UGGT1. In addition, a newly developed photoaffinity crosslinker was selectively introduced into the cysteine residues of SelenoF to determine the spatial orientation of SelenoF to UGGT1.

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Bibliographic references:

S. Higashi, Y. Imamura, T. Kikuma, T. Matoba, S. Orita, Y. Yamaguchi, Y. Ito, Y. Takeda (2023) ChemBioChem (24) e202200444.

Probing Glycosaminoglycan Interactome using a 'Designer' GAG oligosaccharide microarray

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Glycosaminoglycans (GAGs) have important roles in numerous biological systems through interactions with diverse proteins[1]. Due to their structural heterogeneity, defining the specificity of GAG–protein interactions is a challenge. GAG microarrays are much sought-after for detailed GAG recognition studies. A number of microarrays with synthetic heparan sulfate (HS) oligosaccharides have emerged as valuable tools in the field.[2,3] The GAG oligosaccharide microarray based on neoglycolipid (NGL) technology[4] is highly complementary to these, in that it covers size-defined oligosaccharides obtained from natural GAG polysaccharides, including hyaluronic acid, chondroitin sulfate (CS) A and C, dermatan sulfate, heparin, HS and keratan sulfate (KS). The NGL-based system has given insights into the GAG binding specificities of human papillomavirus 16[5], adenovirus 37[6], Chikungunya virus[7], Complement Factor H and Related Protein 5[8], and has led to the assignment of a KS antigen on induced pluripotent stem cells[9].

Here we describe the further development of the NGL-based system towards a 'Designer' GAG screening array platform. Among newly prepared probes are NGLs of CSD, HS, variously desulfated heparins, and heparins from different biological sources with distinct structural features as shown by HPLC disaccharide-compositional and NMR analyses. Binding profiles of a collection of GAG-specific antibodies, endogenous proteins, and pathogens will be presented. These contribute to our understanding of the GAG Interactome networks and potentially lead to novel therapeutic opportunities.

Acknowledgements

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Bibliographic references:

1. S.D. Vallet, et al. (2022) Am. J. Physiol. Cell Physiol. (322), C1271
2. M. Horton, et al. (2020) Glycobiology (31),188
3. L. Liu, et al. (2021) ACS Cent Sci. (7), 1009
4. S. Fukui, et al. (2002) Nat Biotechnol. (20), 1011
5. C. Cerqueira, et al. (2013) Cell Microbiol. (15), 1818
6. N. Chandra, et al. (2019) Viruses. (11), 247
7. N. McAllister, et al. (2020) J. Virol. (94), e01500
8. F. Gyapon-Quast, et al. (2021) J Immunol. (207), 534
9. N. Wu, et al. (2019) Mol Cell Proteomics. (18), 1981

Efficient synthesis of glycopeptides and glycoproteins by flow-based peptide synthesis

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Glycosylation of peptides and proteins is important for their stability and biological activity. For instance, the glycosylation pattern changes hematopoietic activity of erythropoietin [1] and the artificial glycosylation of insulin inhibits fibril formation.[2] Chemical synthesis of glycopeptides and glycoproteins has enabled us to investigate the functions of glycosylation. However, preparation of glycopeptides usually requires long synthetic time because the traditional solid-phase peptide synthesis (SPPS) is time-consuming and 30-60 minutes are needed for each amino acid elongation step.

In this work, we examined flow-based SPPS for the efficient synthesis of glycopeptides. The flow-based peptide synthesis enabled more rapid reactions at heated temperatures compared to the traditional SPPS, and each elongation step was performed within only 3 minutes. [3,4] The insulin B-chain was rapidly synthesized and the glycosyl asparagine was intentionally introduced at the N-terminal. The obtained glycosylated B-chain was ligated with the insulin A-chain by stepwise disulfide bond formation, resulting in the folded glycoinsulin. In addition, we have been investigating synthesis of native glycoproteins by the efficient flow-based peptide synthesis. In this presentation, we would like to present a detail of our efficient glycopeptide and glycoprotein synthesis.

Bibliographic references:

[1] Y. Maki, R. Okamoto, M. Izumi, Y. Kajihara (2020) J. Am. Chem. Soc. (142) 20671-20679.
[2] M. A. Hossain, Y. Kajihara, et al., (2020) J. Am. Chem. Soc. (142) 1164-1169.
[3] M. D. Simon, B. L. Pentelute, et al., (2014) Chem. Bio. Chem. (15) 713-720.
[4] N. Hartrampf, B. L. Pentelute, et al., (2020) Science, (368) 980-987.

Dual inhibitors of *Pseudomonas aeruginosa* virulence factors lectin LecA and elastase LasB

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The rise of antimicrobial resistance crisis has put us in a race against time to develop new antibiotics. Over the years, the antivirulence therapy and the polypharmacology research fields have made remarkable progress in drug discovery.[1] Extracellular virulence factors from *Pseudomonas aeruginosa*, lectin LecA and elastase LasB are considered as valuable targets to develop inhibitors.[2]

Combining these in multitarget polypharmacological drugs led to the selective and non-toxic thiol inhibitors.[3] Here, we present an expansion of the structure-activity relationship of these agents. Furthermore, the dimerization of thiol-based inhibitors under physiological conditions affords divalent inhibitors of LecA with 1000-fold increase in potency. The study will pave a way for a systematic exploration of polypharmacology in the development of anti-virulence agents in the aim of treating of resistant *Pseudomonas aeruginosa*-derived infections.

Bibliographic references:
[1] M. Totsika (2017), Future Med. Chem. (9) 267–269.
[2] S. Wagner, R. Sommer, S. Hinsberger, C. Lu, R.W. Hartmann, M. Empting, A. Titz (2016), J. Med. Chem. (59) 5929-5969.
[3] O. Metelkina, J. Konstantinović, A. Klein, S. Yahiaoui, W.A.M. Elgaher, J. Haupenthal, A. Titz, A.K.H. Hirsch. under review.

Stereoselective synthesis of 'superarmed' thioglycoside donors from 1,2-orthoesters

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Bovine submaxillary mucin (BSM) is a natural polymer used in biomaterial applications for its viscoelasticity, lubricity, biocompatibility, and biodegradability. *N*-glycans are important for the stability and function of mucins, but unlike *O*-glycans, their structures are not fully elucidated.

In this study, *N*-glycans labeled with procainamide of BSM were identified using UPLC and LC-ESI-HCD-MS/MS. The microheterogeneous structures of 32 *N*-glycans were identified, and the quantities (%) of each *N*-glycan relative to total *N*-glycans (100%) were obtained. The terminal *N*-acetylgalactosamines in 12 *N*-glycans (sum of relative quantities; 27.9%) were modified with mono- (10 glycans) and di- (2 glycans) sulfations. Total concentration of all sulfated *N*-glycans was 6.1 pmol in BSM (20 µg), corresponding to 25.3% of all negatively charged glycans (sum of present *N*-glycans and reported *O*-glycans). No *N*-glycans with sialylated or phosphorylated forms were identified, and sulfate modification ions were the only negative charges in BSM *N*-glycans. Mucin structures, including sulfated *N*-glycans located in the hydrophobic terminal regions, were indicated.

This is the first study to structurally characterize and quantify 12 microheterogeneous sulfate modifications in *N*-glycans of natural mucins. These sulfations play important structural roles in hydration, viscoelasticity control, protection from bacterial sialidases, and polymer stabilization to support the functionality of BSM via electrostatic interactions.

Acknowledgements

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Bibliographic references:

[1] A. C. Broussard, M. Boyce, (2019) Mol. Biol. Cell. (30) 525.
[2] M. Lahmann, S. Oscarson, (2002) Canad. J. Chem. (80) 889.
[3] R. Smith, H. Muller-Bunz, X. M. Zhu, (2016) Org. Lett. (18) 3578.
[4] N. L. Douglas, S. V. Ley, U. Lucking, S. L. Warriner, (1998) J. Chem. Soc. Perkin Trans. (1) 51.
[5] D. R. Mootoo, P. Konradsson, U. Udodong, B. Fraserreid (1988), J.Am. Chem. Soc. (110) 5583.

Unnatural gallotannins: powerful antioxidants, antibacterials and antibiofilm agents

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Gallotannins (GTs) are secondary metabolites of higher plants and represent a large class of biologically active polyphenols. GTs play an important role in protection of plants against free radicals, toxins, microbial pathogens and as response to various abiotic stress conditions [1]. Moreover, numerous plant polyphenols exhibited strong antibacterial and antibiofilm activity against staphylococci [2]. Among the staphylococci, *Staphylococcus aureus* is of most clinical concern. Undesirable bacterial *S. aureus* biofilm layers are formed on indwelling medical devices or food processing contact-surfaces, resulting in microbial community more resistant to the traditional disinfectants [3].

GTs from various species have been extensively studied as they exhibit multiple biological activities. As a general rule, the antioxidant activity of GTs is dependent on the number and position of galloyl groups, but the type of the sugar moiety also displays an important role. Many studies have been done with galloylated glucopyranose core, penta-1,2,3,4,6-*O*-galloyl-d-glucose (PGG) [5], but only few galloylated furanoses have been investigated. Thus, a series of new unnatural GTs derived from d-lyxose, d-ribose, l-rhamnose, d-mannose, d-glucose and d-fructose have been designed, synthesized and characterized in order to study the protective and antibacterial effects of synthetic polyphenols that are structurally related with plant-derived compounds. Based on the experimental results obtained in this study it can be concluded that studied GTs are excellent antioxidants and radical-scavenging agents.

Compounds exhibited only moderate activity against gram-positive pathogens *S. aureus* and *E. faecalis*. However, studied compounds proved to be potent inhibitors and disruptors of *S. aureus* biofilms at concentrations much lower than the MIC values. Moreover, they are non-toxic to eukaryotic cells, thus are promising candidates for further studies. Overall, these findings suggest that synthetic GTs could be considered as promising alternatives for biomedical, consumer products, and food industry applications.

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Bibliographic references:

[1] C. Manach, A. Scalbert, C. Morand, C. Remesy, L. Jimenez (2004) Am. J. Clin. Nutr. (79) 727-747.
[2] M. Daglia (2012) Curr. Opin. Biotechnol (23) 174-181.
[3] M. Takó, E.B. Kerekes, C. Zambrano (2020) Antioxidants (9)165.
[4] Y. Zheng, L. He, T. K. Asiamah, M. Otto (2018) Environm. Microbiol. (20) 3141-3153.
[5] Y. Cao, K. B. Himmeldirk, Y. Qian, Y. Ren, (2014) J. Nat. Med. (68) 465-472.

Metallic cation removal with quercus ilex acorn biosorbents

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Acorn pericarps of the Algerian holm oak (*Quercus ilex*) constitute a largely underexploited forestry co-product. In the aim of valorization, adsorption efficiencies of crude, parietal, and lignocellulosic fractions of acorn pericarps were evaluated towards lead, cadmium, nickel, and copper ions. The results were modeled using Langmuir and Freundlich isotherms. The best results were obtained with the lignocellulosic fraction towards lead and cadmium with q_{max} values of 370.37 and 303.03 mg.g^{-1}, respectively. On the contrary, crude and parietal fractions showed the highest capacities for nickel and copper, with q_{max} values of 200 and 303.03 mg.g^{-1}, respectively.

This work thus provides the first trial of acorn pericarps of oaks growing in northwest Algeria as an efficient biosorbent for the removal of metallic cations from aqueous solutions, whose adsorption capacities surpass most of the previously described biosorbents.