**Sweet targets: sugar nucleotide biosynthesis inhibitors**

**Introduction:**

Sugar nucleotides represent the cornerstone building blocks of glycoconjugate biosynthesis and are essential to carbohydrate metabolism. In structural terms, such biomolecules are generally composed of a pyranose sugar attached to a nucleotide diphosphate leaving group. This energetic phosphate component enables transfer of the sugar to an appropriate acceptor, for example, a protein or carbohydrate chain. In mammalian systems there are nine common sugar nucleotide building blocks that serve the Leloir glycosyltranferases responsible for glycan biosynthesis

 The biosynthesis of sugar nucleotides is achieved *via* two pathways: (i) *de novo* synthesis, where the components are assembled from simple carbohydrate precursors (e.g., UDP-glucose from glucose) or other sugar nucleotides (e.g., UDP-glucose from UDP-galactose *via* an epimerase) and (ii) salvage pathways, which generally utilise fewer biosynthetic steps and convert free sugars through the anomeric monophosphate to the sugar nucleotide donor using a final pyrophophorylative step.

Beyond mammalian systems, bacteria and fungi also utilise sugar nucleotides within their metabolic processes and for glycan synthesis. Because the enzymes that biosynthesise and interconvert these non-mammalian sugar donors do not exist in humans, they represent an attractive prospect for drug development,[1] particularly at a time when identifying novel antibacterial and antifungal targets for medicinal chemistry is crucial.[2,3] Underpinning to this prospect is the development of targeted chemical biology tools and substrate-based inhibitors that can interrogate key biosynthetic enzyme function and lay a blueprint for wider inhibitor development. In this Editorial we summarise recent achievements around the construction of targeted sugar nucleotide biosynthesis inhibitors and discuss requirements for the continued development of this exciting and essential division of glycoscience.

**Antifungal Targets:** Fungi are encapsulated by a cell wall that is composed mainly of the glycopolymers glucan, chitin and mannoproteins. Chitin is formed using UDP-GlcNAc as the sugar donor and within its biosynthesis UDP-GlcNAc pyrophosphorylase (UAP1) performs a key transformation, catalysing the ultimate step of combining uridine triphosphate (UTP) and GlcNAc 1-phosphate, to form UDP-GlcNAc. The proposed mechanism here suggests direct S*N*2 attack of the anomeric phosphate group onto a UTP α,β-phosphodiester bond. An α,β-methylenebisphosphonate analogue of UTP (*me*UTP), in which the scissile α,β-pyrophosphate bond is replaced with a methylenebisphosphonate, was recently developed by van Aalten and coworkers and demonstrated the first mechanism-based inhibitor of UAP1 from *Aspergillus fumigatus* (A*f*UAP1).[4] Inhibition of A*f*UAP1 with *me*UTP as substrate was studied using an HPAEC (high-performance anion-exchange chromatography) assay, following the conversion of UTP to UDP-GlcNAc. Accordingly, *me*UTP was shown to be an A*f*UAP1 inhibitor with an IC50 of 115 µm (GlcNAc 1-P Km = 34 mM, UTP Km = 21 mM).

**UDP-Galactopyranose mutase (UGM):** UGM plays a key role in the cell wall biosynthesis of many pathogenic microorganisms, including *Mycobacterium tuberculosis*. The enzyme catalyses a reversible isomerization of UDP-Gal*p* to UDP-Gal*f*, the biosynthetic precursor of all galactofuranose-containing eukaryotic and prokaryotic glycoconjugates. An absence of Gal*f* in humans makes UGM a potential target for developing novel antibacterial, antifungal and antiparasitic agents. Several exciting concepts surrounding sugar nucleotide probes for UGM have been developed in recent years,[5,6] Following such studies, and supported by virtual screening,[7] small molecule inhibition strategies continue to develop against UGM. As important complexities, such as the conformational dynamics surrounding enzyme inhibition and cofactor oxidation state (UGD is flavin dependent) are questioned,[8] an exciting prospect to develop new generations of highly efficacious inhibitors presents itself.

**Antibacterial targets:** Over the lifetime of a cystic fibrosis patient one bacterial strain in particular, *Pseudomonas aeruginosa* (*PA*), becomes the dominant pathogen and causes a chronic respiratory infection that infects over 80% of CF patients.[9] Strains chronically infecting the respiratory tract of CF patients undergo a phenomenon known as mucoid conversion and, by age 16, over 90% of CF patients have infections of mucoid *PA*.[9] Mucoid *PA* secretes copious amounts of alginate (a carbohydrate exopolysaccharide), which confers bacterial resistance to antibiotics on the host immune system.[10] Alginate is therefore a major virulence factor for CF lung infections and strategies that can stop the production of alginate in mucoid *PA* are of paramount importance.

The foundation to produce alginate lies in the biosynthesis of GDP-d-mannuronic acid (GDP-d-ManA). This sugar nucleotide is sourced from the cytosolic metabolic pool, starting from fructose 6-phosphate and consecutive biosynthetic modifications culminate at the *limiting step in alginate precursor biosynthesis*, the action of GDP-d-mannose dehydrogenase (GMD), which oxidises GDP-d-Man **1** to GDP-d-ManA (*Figure 1a*). GMD is a member of a small group of NAD+-dependent four-electron-transfer dehydrogenases, which includes UDP-d-glucose dehydrogenase (UGD) and UDP-*N*-acetyl-d-mannosamine dehydrogenase (U*N*AcMD). Analogous to UGD, the GMD catalysed oxidation is suggested to have four discrete steps and indicates a key mechanistic role for Cys268 (*Figure 1a*). Mutation of Cys268 to Ser reduced GMD activity by 95%[11] and penicillic acid (*Figure 1b*), which contains a conjugated Michael acceptor, has been shown by HRMS analysis to alkylate Cys268, acting as an irreversible inactivator of GMD *in vitro*, but with low selectivity for the active site of the protein.[12]



***Figure 1.*** *a) Suggested mechanism of action for GMD b) A non-specific inhibitor, pencillinic acid c) Initial strucutre to function probes for GMD and d) Idenitification of a first sugar nucleotide inhibitor and overlay of this with the native substrate* ***1*** *in the GMD active site.*

The first series of targted sugar nucleotide probes for GMD were recently disclosed.[13,14] Utilising a chemoenzymatic approach,[15] to enable synthetic pyranose modification of the GDP-sugar, alongside a calorimetric measurement of NADH produced during the oxidation, it was demonstrated that a C6-methyl analogue was oxidised by GMD (*Figure 1c*).[13] Direct evidence of a ketone product from this oxidation was obtained alongside a reduction in NADH production. Whilst this product could clearly not be oxidised further, it was evident that the ketone was not tightly bound, as spiking 60 min incubations with **1** led to renewed NADH production. Furthermore, introduction of a C6-amide (*Figure 1d*) identiﬁed the ﬁrst example of a micromolar inhibitor of GMD (IC50 =112 mM).[14] Access to these ligands sets a scene for the development of further generations of probes (including to target the active site Cys268), alongside devloping small molecule inhibition stratgeies against GMD. Progress in this area compliments other recent work targetting alternative sections of the alginate biosythetic machinery (c-di-GMP-Alg4 axis) using small molecule inhibitors.[16]

**Outlook:** The development of inhibitory strategies for non-mammalian sugar nucleotide processing enzymes remains an attractive prospect, yet challenges endure; perhaps most notably surrounding delivery of large, negatively charged species into cells, either as probes or putative inhibitors. Prospects here are supported by the development of new, uncharged chemotypes for glycosyltransferases (which utilise sugar nucleotides),[17] alongside classical covalent strategies, typified by inhibitors of the bacterial glycosyltransferase LgtC.[18] The value of ligandability assessments arising from small molecule screening processes is also vital to further such processes and recently informed strategy in targeting GDP-mannose pyrophosphorylase within Chagas’ disease.[19]

 Consideration of these concepts must remain at the forefront of thinking, as new, emerging target areas are contemplated, illustrated through the recent structural disclosure of a key fungal sugar nucleotide transporting enzyme,[20] and the regulatory importance of UDP-glucose pyrophosphorylase in pancreatic cancer. Inhibiting the biosynthesis of sugar nucleotides offers an exciting, selective pathway to future pathogenic drug discovery programs. Compounded by recent research in the connecting realm of chemical glycobiology a bright translational future emanates from this underpinning facet of glycoscience.

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