Enzymatic synthesis of UDP-GlcNAc/UDP-GalNAc analogs using *N*-acetylglucosamine 1-phosphate uridyltransferase (GlmU)†

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Reports the generation of a library composed of UDP-GlcNAc/UDP-GalNAc and investigates the substrate specificity of *Escherichia coli* GlcNAc-1-P uridyltransferase GlmU.

In glycobiology research, analogs of sugars and glycoconjugates equipped with functional groups that enable facile detection or further derivation are indispensable materials for deciphering structure–function relationships in carbohydrate-associated pathways[1] and discovering carbohydrate-based drugs.[2] These analogs can be produced chemically or enzymatically. Of the available enzymatic methods, glycosyltransferase-catalyzed oligosaccharide synthesis is generally preferred as the reactions are regio- and stereoselective and also eliminate the typical multiple protection/deprotection steps required for chemical synthesis. As a result, access to commercially unavailable unnatural sugar nucleotide substrates of Leloir-type glycosyltransferases is of considerable interest.

N-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc) are ubiquitous amino sugar components of glycoconjugates. GlcNAc is a component of peptidoglycans,[3] while both amino sugars play key roles in different glycosaminoglycans.[4] Moreover, they are prevalent in the core structures of glycans in glycoproteins,[5] and glycolipids,[6] affecting cell–cell interactions during various metabolic processes. Replacement of GlcNAc/GalNAc residues by GlcNAc/GalNAc analogs in a polysaccharide or glycoconjugate would thus be a good approach to understand the mechanism of GlcNAc/GalNAc-related pathways. GlmU, a GlcNAc-1-P uridyltransferase (pyrophosphorylase) from *Escherichia coli*,[7] was accordingly employed to obtain unnatural uridine 5′-diphosphate-GlcNAc/uridine 5′-diphosphate-GalNAc (UDP-GlcNAc/UDP-GalNAc) sugar donors. This enabled generation of a library composed of UDP-GlcNAc/UDP-GalNAc analogs and determination of the substrate specificity of GlmU towards GlcNAc-1-P, GalNAc-1-P and their corresponding analogs.

GlcNAc-1-P/GalNAc-1-P and their analogs used as GlmU substrates were prepared chemoenzymatically as previously described.[8] The corresponding sugar nucleotides were constructed by GlmU using sugar-1-P and UTP. In addition, yeast inorganic pyrophosphatase was added to drive the GlmU reaction forward by degrading the byproduct PPi. All sugar nucleotides produced here were purified by anion exchange chromatography followed by desalting with size exclusion chromatography.

Out of seventeen sugar-1-P compounds tested, eleven were accepted by GlmU (Table 1).[9] GlcNAc-1-P, its 4-epimer (GalNAc-1-P) and the 4-deoxy version were converted to sugar nucleotides at comparable levels, indicating the 4-hydroxyl group may not be necessary for enzyme recognition. In contrast, 4-azido GalNAc-1-P failed in the reaction, presumably resulting from the bulkiness of the azido group. The tolerance for *N*-acyl modifications was different for GlcNAc-1-P and GalNAc-1-P analogs. For the GlcNAc type, compounds with relatively small *N*-acyl groups (GlcNAz-1-P and GlcNPr-1-P) were good substrates, leading to generation of compounds **2** and **3**. Reaction efficiency decreased for compound **4**, however, with its bulky acyl group, while compound **5** was non-isolable and only detectable by MS, demonstrating poor acceptance for bulky substitutions. Interestingly, none of the *N*-acyl modified GalNAc-1-P analogs were accepted by GlmU. The changes at both the *N*-acyl and C-4 positions may enlarge these sugar-1-P compounds, thus causing them to fail to enter the uridyltransferase pocket.[10]

In contrast, the tolerance for 6-modified GlcNAc-1-P and GalNAc-1-P analogs was quite similar. The yields of smaller 6-deoxy UDP-sugars were comparable to that of UDP-GlcNAc and decreased dramatically for 6-azido compounds bearing a relatively large substituent. The 3-epimer of GlcNAc-1-P was also converted to the corresponding sugar donor but with lower yield than GlcNAc-1-P.

To probe whether these UDP-sugar analogs are prospective sugar donors for glycosyltransferases, 6-deoxy UDP-GlcNAc (**6**) and Lac-1-OBn were chosen as donor and acceptor respectively for LgtA (EC 2.4.1.56), which is a *N*-acetylglucosaminyltransferase. A trisaccharide with a terminal 6-deoxy-GlcNAc was produced (Scheme 1). In addition, a disaccharide (6-deoxy-GlcNAcβ1-3Gal*p*NO_2Ph) was detected by MS and TLC (Fig. S2 and S3†) when *p*-nitrophenyl α-D-galactopyranoside was used as another acceptor.

In summary, we have reported the enzymatic synthesis of a set of UDP-GlcNAc/UDP-GalNAc analogs. The substrate specificity of the pyrophosphorylase GlmU towards sugar-1-P compounds was also examined. The enzyme showed relaxed tolerance for modifications at *N*-acyl, C-3, C-4, and C-6 positions, with a preference for small substituent groups. The yields were low to moderate (10–65%) and some sugar-1-Ps failed to generate the corresponding UDP-sugars due to the

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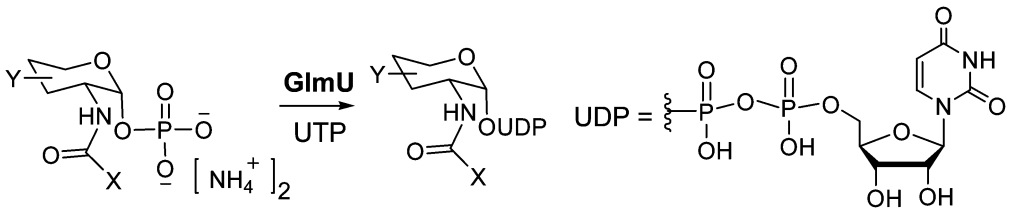
Table 1 Isolated yields for enzymatic pyrophosphorylation reactions



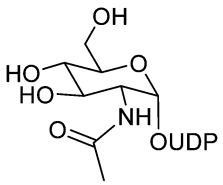
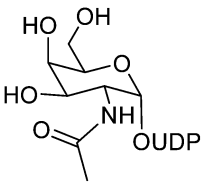
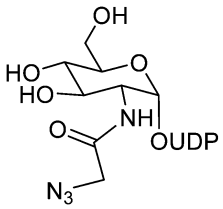
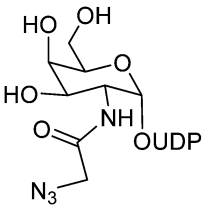
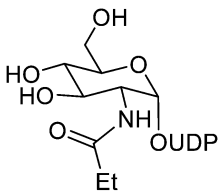
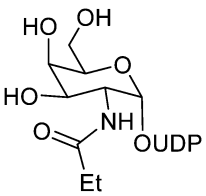
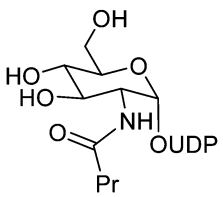
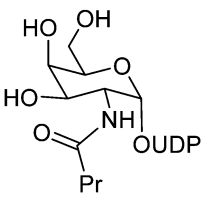
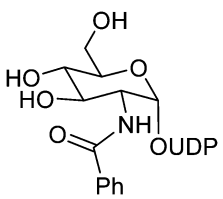
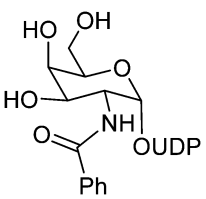
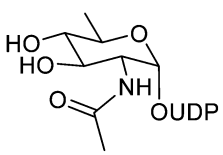
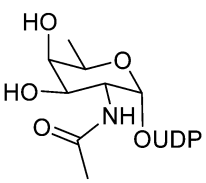
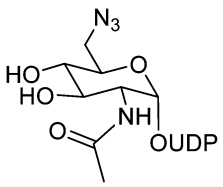
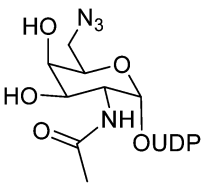
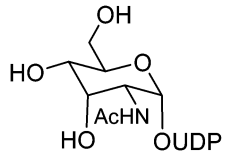
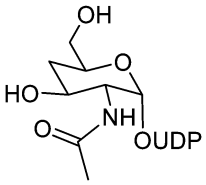
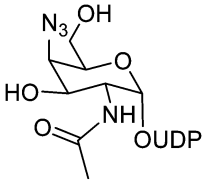
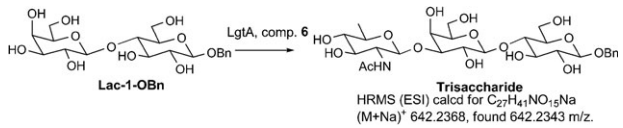
Entry	Product	Yield (%)[a]	Entry	Product	Yield (%)[a]
1		40	**9**		65
2		44	**10**		N/A[b]
3		57	**11**		N/A[b]
4		27	**12**		N/A[b]
5		N/A[b]	**13**		N/A[b]
6		50	**14**		55
7		20	**15**		10

Table 1 (*continued*)

Entry	Product	Yield (%)[a]	Entry	Product	Yield (%)[a]
8		20	**16**		59
			17		N/A[b]

[a] Isolated yield from DEAE cellulose and P-2 gel columns. [b] No product detected by MS or TLC, or product detected by MS but non-isolable due to very low yield, see TLC of reaction mixtures.



Scheme 1 Enzymatic synthesis of trisaccharide using glycosyltransferase LgtA.

steric problem. However, our approach still exceeds cumbersome chemical synthesis[9] and can quickly produce the desired UDP-sugar analogs in relatively large scale (30–50 mg). Mutants of GlmU or other pyrophosphorylases could be utilized to enrich the donor analog library. Nonetheless, the unnatural sugar donors produced here are applicable in carbohydrate/glycoconjugate analog synthesis, establishing a platform for developing new sugar-based therapeutics and unraveling mechanisms underlying sugar-related biological processes.

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