

Highly Efficient Synthesis of UDP-GalNAc/GlcNAc Analogues with Promiscuous Recombinant Human UDP-GalNAc Pyrophosphorylase AGX1**

Wanyi Guan,^[a, b] Li Cai,^[a] and Peng George Wang*^[a]

2-*N*-Acetamidoglycosaminos, ^[1] *N*-acetylgalactosamine (GalNAc) and *N*-acetylglucosamine (GlcNAc), are prevalent in living organisms. They are key building blocks of glycosaminoglycans,^[2] glycoproteins,^[3] and glycolipids,^[4] and are indispensable in glycoconjugate-involved cell communication and signaling.^[4,5] In this context, we presumed that the analogues of the above-mentioned biologically essential amino sugars are valuable tools, either to unveil the metabolic pathways endowed by bioorthogonal groups,^[6] or to expand the diversity of glycoconjugates with uncommon or non-natural sugars. Due to the structural complexity of glycans, chemical synthesis of glycoconjugates is an arduous task with tedious protection/deprotection, low yield and generally poor selectivity.^[7] Therefore, enzymatic approach, most commonly Leloir-type glycosyltransferases, which transfer monosaccharide from the corresponding sugar nucleotide donor to an acceptor with high efficiency and selectivity, is an attractive alternative.^[8] This means naturally occurring sugar nucleotides, as well as structural analogues are of paramount importance as substrates for enzymatic synthesis of oligosaccharides and glycoconjugates. Chemical synthesis of sugar nucleotides generally exploits the formation of the pyrophosphate linkage from smaller building blocks, however, it was normally plagued with long reaction time, low overall

yield and very strict reaction conditions.^[9] On the other hand, enzymatic approach following the biosynthetic pathways requires multiple enzyme systems and protein engineering which also restricts the production of sugar nucleotides.^[10] The natural donor molecules for GalNAc/GlcNAc are uridine 5'-diphospho-GalNAc/GlcNAc (UDP-GalNAc/GlcNAc), and previously, we have synthesized eight UDP-GlcNAc analogues and three UDP-GalNAc analogues using recombinant *Escherichia coli* *N*-acetylglucosamine 1-phosphate uridylyltransferase (GlmU)^[11] with moderate yields (10–65 %) in a relatively large scale. Unfortunately, some sugar 1-phosphate (sugar-1-P) analogues, mostly GalNAc-1-P analogues, were not accepted by this enzyme. In order to overcome the narrow substrate specificity of GlmU towards GalNAc-1-P analogues and also enrich our sugar nucleotide analogue library, we explored the potential of human UDP-GalNAc pyrophosphorylase (AGX1), which had been applied to synthesize UDP-*N*-azidoacetylglactosamine (UDP-GalNAz) in good yield but very small scale.^[12]

GalNAc-1-P/GlcNAc-1-P and their analogues used as substrates for sugar nucleotide preparation were produced chemoenzymatically as previously described.^[13] The sugar nucleotides were obtained through uridylyl transfer reactions catalyzed by recombinant AGX1, and yeast inorganic pyrophosphatase which was first employed in sugar nucleotide synthesis by Elling and Bülter^[14] was also used here to drive the coupling reaction forward by degrading the byproduct PP_i (Scheme 1). Reactions were monitored by thin-layer chromatography (TLC) and were terminated when complete consumption of sugar-1-Ps was observed. All sugar nucleotides were purified sequentially by anion exchange chromatography and, for desalting purpose, size exclusion chromatography (see Supporting Information).

Nine GalNAc-1-P analogues and five GlcNAc-1-P analogues (Table 1),^[15,16] many of which had low yield or no reaction with GlmU,^[11] were tested in this study. As shown in Table 1, AGX1 exhibited good tolerance towards both GalNAc- and GlcNAc-based structures. Eleven out of fourteen compounds were converted by AGX1 into the corre-

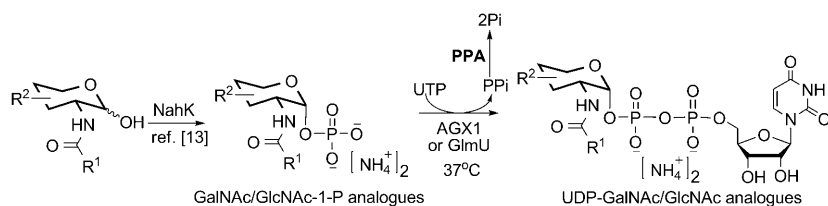
[a] W. Guan,⁺ L. Cai,⁺ Prof. Dr. P. G. Wang
Department of Chemistry and Biochemistry
The Ohio State University, 484 West 12th Avenue
Columbus, OH 43210 (USA)
Fax: (+1) 614-688-3106
E-mail: wang.892@osu.edu

[b] W. Guan⁺
National Glycoengineering Research Center and
the State Key Laboratory of Microbial Technology
Shandong University, Jinan, Shandong 250100 (China)

[⁺] Contributed equally to this work.

[**] UDP = uridine diphosphate, GalNAc = *N*-acetylglactosamine, GlcNAc = *N*-acetylglucosamine.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/chem.201002315>.



Scheme 1. General synthetic scheme of UDP-GalNAc/GlcNAc analogues; NahK = *N*-acetylhexosamine 1-kinase.

sponding sugar nucleotides in preparative scale. The natural substrates GalNAc-1-P, GlcNAc-1-P (entries 1 and 10), and the 4-deoxy version (entry 8) were recognized at similar level, excluding the role of 4-hydroxyl group in substrate binding. However, the axial-4-azido entry 9 was non-isolable and only detected by TLC and MS, probably resulting from the larger substituent than hydroxyl.

Unlike GlmU, which differentiated GalNAc-1-P and GlcNAc-1-P analogues with bigger *N*-acyl modifications, AGX1 was only slightly affected by the bulkiness of *N*-acyl groups in both GalNAc-1-P and GlcNAc-1-P analogues. Briefly, GalNPr/GlcNPr-1-P (entry 2 and 11) and GalNAz/GlcNAz-1-P (entry 4 and 12) with relatively smaller *N*-propionyl and *N*-azidoacetyl groups were accepted with good conversion yield (> 50%). GalNBu-1-P (entry 3) with a bulkier *N*-butyryl group had lower yield (44%) and longer reaction time, while a bulky *N*-benzoyl group prevents both GalNBz-1-P/GlcNBz-1-P to be accepted (entry 5 and 13).

Table 1. Synthesis of UDP-GalNAc/GlcNAc utilizing AGX1 and GlmU.^[11]

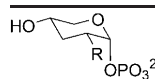
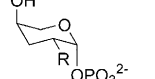
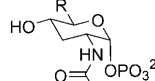
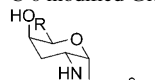
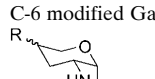
Entry	Product	AGX1 Yield [%] ^[a] <i>t</i> [h] Scale [mg] ^[d]	GlmU ^[11] Yield [%] ^[a] /12 h	Entry	Product	AGX1 Yield [%] ^[a] <i>t</i> [h] Scale [mg] ^[d]	GlmU ^[11] Yield [%] ^[a] /12 h
1		79 2 38	65	8		72 2 51.4	59
2		57 2 5.5	N/A ^[b]	9		< 5 ^[c] 24 0	N/A
3		44 24 9.5	N/A	10		75 2 46	40
4		64 0.5 29	N/A	11		51 2 8	57
5		N/A 24 0	N/A	12		55 2 26	44
6		77 2 66	55	13		N/A > 24 0	N/A
7		61 6 36.5	10	14		74 2 33	20

[a] Isolated yield from DEAE cellulose and P-2 gel columns. [b] N/A (not available): No product detected by MS or TLC. [c] Determined by TLC. [d] The mass of the product obtained (isolated).

AGX1 also shows good tolerance to 6-modified GalNAc/GlcNAc-1-P analogues. For example, not only 6-deoxy-GalNAc-1-P (entry 6) but also 6-azido-GalNAc-1-P and 6-azido-GlcNAc-1-P (entry 7 and 14) were taken by AGX1 to construct the corresponding UDP-sugars with good yields (>60%). This is another exciting result since GlmU only gave very poor yield for the 6-azido derivatives.

The respective substrate specificity of AGX1 and GlmU is summarized in Table 2: First, both enzymes had limited acceptance for C-4 modifications. However, the 4-OH configuration did not affect the enzyme activity. Secondly, although very broad specificity of GlmU towards C-2 modified GlcNAc-1-P analogues was observed, including 2-azido-Glc-1-P and 2-keto-Glc-1-P,^[17] it was not a good candidate for the preparation of C-2 and C-6 modified UDP-GalNAc analogues. Last but not least, we proved AGX1 as a better choice for synthesizing most UDP-GalNAc/GlcNAc analogues with C-2,4,6 modifications.

Table 2. Summarized substrate specificity of AGX1 and GlmU.

	AGX1	GlmU ^[11]
	broad specificity	very broad specificity ^[17]
C-2 modified GlcNAc-1-P		
	broad specificity	no activity
C-2 modified GalNAc-1-P		
	broad specificity	limited specificity
C-6 modified GlcNAc-1-P		
	broad specificity	limited specificity
C-6 modified GalNAc-1-P		
	both limited specificity not affected by the configuration of OH no acceptance for groups larger than OH	
C-4 modified		

In summary, owing to the promiscuity of recombinant human UDP-GalNAc pyrophosphorylase (AGX1), we have successfully prepared C-2,4,6 modified UDP-GalNAc/GlcNAc analogues on a preparative scale with good yields. We thus avoided having to design and obtain GlmU mutants to accommodate the unacceptable substrate structures. We believe our library of UDP-GalNAc/GlcNAc analogues could greatly facilitate our investigation into the substrate specificity of various glycosyltransferases as well as provide a significant step towards natural product glycodiversification.

Acknowledgements

P.G.W. acknowledges the NIH (R01 AI083754, R01 HD061935, and R01 GM085267) for financial support. W.G. acknowledges the China Scholarship Council for financial support.

Keywords: carbohydrates • enzymes • glycoconjugates • glycosyltransferases

- [1] H. C. Hang, C. R. Bertozzi, *J. Am. Chem. Soc.* **2001**, *123*, 1242–1243.
- [2] U. Lindahl, M. Hook, *Annu. Rev. Biochem.* **1978**, *47*, 385–417.
- [3] a) K. L. Carraway, S. R. Hull, *Glycobiology* **1991**, *1*, 131–138; b) Y. C. Lee, J. R. Scoocca, *J. Biol. Chem.* **1972**, *247*, 5753–5758; c) C. R. Torres, G. W. Hart, *J. Biol. Chem.* **1984**, *259*, 3308–3317.
- [4] S. Hakomori, *Annu. Rev. Biochem.* **1981**, *50*, 733–764.
- [5] a) T. Jensen, M. Nielsen, M. Gad, P. Hansen, S. Komba, M. Meldal, N. Odum, O. Werdelin, *Eur. J. Immunol.* **2001**, *31*, 3197–3206; b) G. W. Hart, M. P. Housley, C. Slawson, *Nature* **2007**, *446*, 1017–1022.
- [6] a) S. T. Laughlin, J. M. Baskin, S. L. Amacher, C. R. Bertozzi, *Science* **2008**, *320*, 664–667; b) H. C. Hang, C. Yu, D. L. Kato, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 14846–14851.
- [7] T. K. Lindhorst, *Essentials of Carbohydrate Chemistry and Biochemistry*, 2nd ed., Wiley-VCH, Weinheim, **2003**, pp. 39–118.
- [8] a) M. M. Palcic, O. Hindsgaul, *Trends Glycosci. Glycotechnol.* **1996**, *8*, 37–49; b) U. M. Ünlügil, J. M. Rini, *Curr. Opin. Struct. Biol.* **2000**, *10*, 510–517.
- [9] G. K. Wagner, T. Pesnot, R. A. Field, *Nat. Prod. Rep.* **2009**, *26*, 1172–1194.
- [10] C. J. Thibodeaux, C. E. Melancon III, H.-w. Liu, *Angew. Chem.* **2008**, *120*, 9960–10007; *Angew. Chem. Int. Ed.* **2008**, *47*, 9814–9859.
- [11] W. Guan, L. Cai, J. Fang, B. Wu, P. G. Wang, *Chem. Commun.* **2009**, 6976–6978.
- [12] V. Bourgeois, F. Pillier, V. Pillier, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5459–5462.
- [13] a) L. Cai, W. Guan, M. Kitaoka, J. Shen, C. Xia, W. Chen, P. G. Wang, *Chem. Commun.* **2009**, 2944–2946; b) L. Cai, W. Guan, W. Wang, W. Zhao, M. Kitaoka, J. Shen, C. O'Neil, P. G. Wang, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5433–5435; c) G. Zhao, W. Guan, L. Cai, P. G. Wang, *Nat. Protoc.* **2010**, *5*, 636–646.
- [14] T. Bülter, L. Elling, *Glycoconjugate J.* **1999**, *16*, 147–159.
- [15] Note: For reaction details, see the Supporting Information and reference [13c].
- [16] All ¹H NMR spectra are available in the Supporting Information as an evidence of their purity; for NMR data and characterization, see reference [12] (compound **1**); a) D. Lazarevic, J. Thiem, *Carbohydr. Res.* **2002**, *337*, 2187–2194 (compound **2**, **3**); b) H. C. Hang, C. Yu, M. R. Pratt, C. R. Bertozzi, *J. Am. Chem. Soc.* **2004**, *126*, 6–7 (compound **4**); c) P. Busca, O. R. Martin, *Tetrahedron Lett.* **2004**, *45*, 4433–4436 (compound **6**); reference [11] (compound **7**, **14**); d) G. Srivastava, G. Alton, O. Hindsgaul, *Carbohydr. Res.* **1990**, *207*, 259–276 (compound **8**); e) J. E. Heidlas, W. J. Lees, P. Pale, G. M. Whitesides, *J. Org. Chem.* **1992**, *57*, 146–151 (compound **10**); f) D. Lazarevic, J. Thiem, *Carbohydr. Res.* **2006**, *341*, 569–576 (compound **11**); g) D. J. Vocadlo, H. C. Hang, E.-J. Kim, J. A. Hanover, C. R. Bertozzi, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9116–9121 (compound **12**).
- [17] L. Cai, W. Guan, W. Chen, P. G. Wang, *J. Org. Chem.* **2010**, *75*, 3492–3494.

Received: August 12, 2010

Published online: October 28, 2010