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One-pot three-enzyme synthesis of UDP-GlcNAc derivatives†

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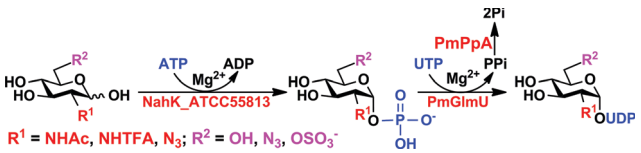
A *Pasteurella multocida* *N*-acetylglucosamine 1-phosphate uridylyltransferase (PmGlmU) was cloned and used efficiently with an *N*-acetylhexosamine 1-kinase (NahK_ATCC55813) and an inorganic pyrophosphatase (PmPpA) for one-pot three-enzyme synthesis of UDP-GlcNAc derivatives with or without further chemical diversification.

Glycosyltransferases are key enzymes for the formation of oligosaccharides and glycoconjugates in nature.[1] Most glycosyltransferases require sugar nucleotides as donor substrates and catalyze the transfer of monosaccharides from sugar nucleotides to acceptors in a high regio- and stereoselective manner. Some carbohydrate structures contain post-glycosylational modifications (modifications on carbohydrates and glycoconjugates which take place after the formation of glycosidic bonds).[2] One strategy to obtain naturally existing oligosaccharides and glycoconjugates with modified sugar moieties is to develop novel chemoenzymatic methods using structurally modified monosaccharides as starting materials and carbohydrate biosynthetic enzymes (the simplest carbohydrate biosynthetic route usually involves a monosaccharide kinase, a nucleotidyltransferase, and a glycosyltransferase[3,4]) with substrate promiscuities.[5,6] Carbohydrates with non-natural modifications can be synthesized similarly. Some of these compounds are potential drug candidates[7–9] as they can effectively interfere with carbohydrate-dependent biological processes.

Glycosaminoglycans including keratan sulfate, heparan sulfate, and heparin are *N*-acetylglucosamine (GlcNAc)-containing polysaccharides[2,10–12] with post-glycosylational modifications.[2] While GlcNAc and 6-*O*-sulfo-GlcNAc are commonly found in keratan sulfate, additional modified GlcNAc forms such as *N*-sulfo-glucosamine and 3-*O*-sulfo-GlcNAc are common for heparan sulfate and heparin. In addition, 6-*O*-sulfation on GlcNAc is also common in Lewis x and sialyl Lewis x structures[13] and has been shown to affect the binding affinity of the related carbohydrate-binding proteins such as Selectins[14,15] and Siglecs.[16,17] In attempts to synthesizing glycans containing naturally modified GlcNAc and their non-natural derivatives using glycosyltransferase-catalyzed reactions, we applied an efficient one-pot three-enzyme approach to synthesize UDP-GlcNAc derivatives including UDP-6-*O*-sulfo-GlcNAc, UDP-GlcNTFA, and azido-containing UDP-GlcNAc derivatives. Additional UDP-GlcNAc derivatives, including UDP-*N*-sulfo-glucosamine, were also produced by chemical diversification from enzymatically produced UDP-GlcNAc derivatives. These compounds will be tested as potential donor substrates for GlcNAc-glycosyltransferases.

As shown in Scheme 1, three enzymes were used in one-pot to synthesize UDP-GlcNAc and derivatives. The first enzyme was an *N*-acetylhexosamine 1-kinase cloned from *Bifidobacterium longum* strain ATCC55813 (NahK_ATCC55813) which showed promiscuous substrate specificity and was able to use *N*-sulfated, 3-*O*-sulfated, or 6-*O*-sulfated GlcNAc and derivatives as substrates for the formation of GlcNAcα1-phosphate derivatives.[18] The second enzyme was an *N*-acetylglucosamine-1-phosphate uridylyltransferase that we cloned from *Pasteurella multocida* strain P-1059 (ATCC15742) (PmGlmU) (see ESI† for details about cloning, expression, and purification of PmGlmU). It catalyzes the reversible formation of UDP-GlcNAc and pyrophosphate from UTP and GlcNAcα1-phosphate with tolerance on some substrate modifications. The third enzyme was an inorganic pyrophosphatase also cloned from *Pasteurella multocida* strain P-1059 (PmPpA)[6] for hydrolyzing the pyrophosphate by-product formed to drive the reaction towards the formation of UDP-GlcNAc and derivatives. A recombinant NahK cloned from another strain of *Bifidobacterium longum* (NahK_JCM1217)[19] was used in the synthesis of GlcNAc-1-phosphate, GalNAc-1-phosphate, and their derivatives.[20,21] The purified HexNAc-1-phosphates were then used in a one-pot two-enzyme system containing a commercially



Scheme 1 One-pot three-enzyme synthesis of UDP-GlcNAc and derivatives. NahK_ATCC55813, *N*-acetylhexosamine 1-kinase cloned from *Bifidobacterium longum* ATCC55813;[18] PmGlmU, *Pasteurella multocida* *N*-acetylglucosamine-1-phosphate uridylyltransferase; PmPpA, *Pasteurella multocida* inorganic pyrophosphatase.[6]

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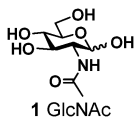
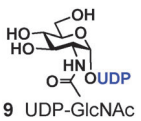
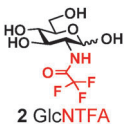
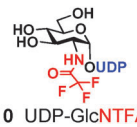
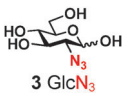
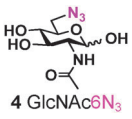
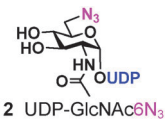
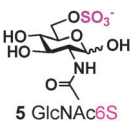
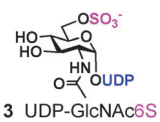
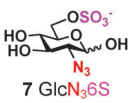
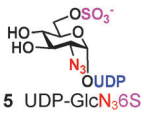
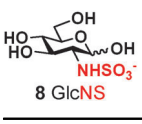
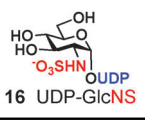
† Electronic supplementary information (ESI) available: Experimental details for cloning, expression, and purification of PmGlmU, chemical and enzymatic synthesis, NMR and HRMS data. See DOI: 10.1039/c1cc14034e

available inorganic pyrophosphatase (PpA) and a GlmU cloned from *E. coli* (EcGlmU)[22–26] or an AGX1 cloned from human[24] for the synthesis of UDP-GlcNAc, dNDP-GlcNAc, dNDP-Glc, UDP-GalNAc, and derivatives. Nevertheless, chemoenzymatic synthesis of UDP-GlcNAc derivatives using all three enzymes in one-pot has not been reported. In addition, UDP-GlcNAc derivatives containing *N*-sulfated glucosamine or *O*-sulfated GlcNAc have not been synthesized using the combination of these three enzymes.

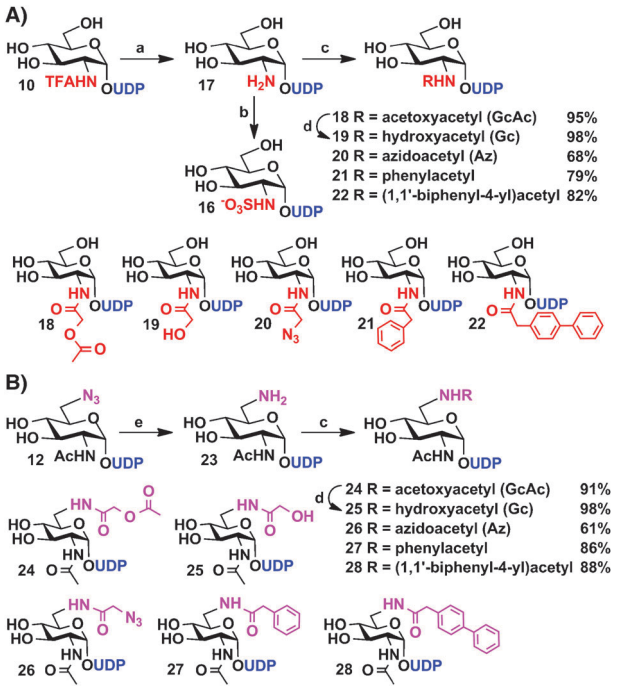
As shown in Table 1, the one-pot three-enzyme system was quite efficient in synthesizing UDP-GlcNAc (**9**, 81%), its C-2 derivatives such as UDP-*N*-trifluoroacetylglucosamine (UDP-GlcNTFA, **10**, 97%) and UDP-2-azido-2-deoxy-glucose (UDP-$GlcN_3$, **11**, 54%), as well as its C-6 derivatives including UDP-*N*-acetyl-6-azido-6-deoxy-glucosamine (UDP-$GlcNAc6N_3$, **12**, 72%) and UDP-*N*-acetyl-6-*O*-sulfo-glucosamine (UDP-GlcNAc6S, **13**, 62%) from GlcNAc (**1**) and derivatives (**2–5**). An interesting observation was that the yield of the one-pot three-enzyme reaction was improved from 81% to 97% when the *N*-acetyl group of GlcNAc was substituted by an *N*-trifluoroacetyl group in GlcNTFA (**2**). However, while 6-*O*-sulfated GlcNAc (GlcNAc6S, **5**) was used as a substrate to produce UDP-GlcNAc6S (**13**) in 62% yield, the synthesis of its *N*-trifluoroacetyl analogue UDP-6-*O*-sulfo-GlcNTFA (UDP-GlcNTFA6S, **14**) from 6-*O*-sulfo-GlcNTFA (GlcNTFA6S, **6**) was not successful. In addition, although both 2-azido-2-deoxy-glucose (**3**) and 6-*O*-sulfo-GlcNAc (**5**) could be used for the synthesis of the corresponding UDP-GlcNAc derivatives UDP-$GlcN_3$ **11** and UDP-GlcNAc6S **13** in 54% and 62% yields, respectively, the synthesis of UDP-2-azido-2-deoxy-6-*O*-sulfo-glucose (UDP-$GlcN_3$6S, **15**) from $GlcN_3$6S (**7**) with the combined modifications at C-2 and C-6 was not successful. Furthermore, the one-pot three-enzyme synthesis of UDP-*N*-sulfo-glucosamine (UDP-GlcNS, **16**) from *N*-sulfo-glucosamine (GlcNS, **8**) was not achieved. As compounds **3–8** have all been shown to be weak substrates for NahK_ATCC55813,[18] the successful synthesis of compounds **11–13** and the unsuccessful synthesis of compounds **14–16** by the one-pot three-enzyme system indicate that the substrate specificity of PmGlmU is most likely the limiting factor.

Table 1 Synthesis of UDP-GlcNAc and its derivatives using the one-pot three-enzyme system shown in Scheme 1. ND, not detected

Substrates	Products	Yields (%)
 **1** GlcNAc	 **9** UDP-GlcNAc	81
 **2** GlcNTFA	 **10** UDP-GlcNTFA	97
 **3** $GlcN_3$	 **11** UDP-$GlcN_3$	54
 **4** $GlcNAc6N_3$	 **12** UDP-$GlcNAc6N_3$	72
 **5** GlcNAc6S	 **13** UDP-GlcNAc6S	62
 **6** GlcNTFA6S	 **14** UDP-GlcNTFA6S	ND
 **7** $GlcN_3$6S	 **15** UDP-$GlcN_3$6S	ND
 **8** GlcNS	 **16** UDP-GlcNS	ND

Taking advantage of the substrate promiscuity of NahK_ATCC55813 and PmGlmU, UDP-GlcNAc and a number of its natural and non-natural derivatives were synthesized efficiently by the one-pot three-enzyme system illustrated in Scheme 1. However, the success of the approach relied on the substrate promiscuity of all enzymes used. In order to increase the size of the library of UDP-GlcNAc derivatives with various modifications that can be used to test the activity of diverse GlcNAc-transferases, we further carried out chemical diversification of chemoenzymatically-produced UDP-GlcNAc derivatives.

The *N*-TFA group in UDP-GlcNTFA (**10**) as well as the N_3 group in UDP-$GlcN_3$ (**11**) and UDP-$GlcNAc6N_3$ (**12**) can be easily converted to a free amine, allowing further modifications to generate a diverse array of *N*-substituted UDP-GlcNAc derivatives. As shown in Scheme 2A, the *N*-TFA group at C2 of UDP-GlcNTFA **10** was removed under mild basic conditions to produce UDP-glucosamine (UDP-$GlcNH_2$, **17**) in 98% yield. Selective acylation of the free amine group in **17** using various acyl chlorides produced C-2 modified UDP-GlcNAc derivatives UDP-*N*-acetoxyacetylglucosamine (UDP-GlcNGcAc, **18**), UDP-*N*-azidoacetylglucosamine (UDP-GlcNAz, **20**), UDP-*N*-phenylacetylglucosamine (UDP-GlcNPhAc, **21**), and UDP-*N*-(1,1′-biphenyl-4-yl)-acetylglucosamine (UDP-$GlcNPh_2Ac$, **22**) in 68–95% yields. Deacetylation of compound **18** using catalytic amounts of NaOMe in MeOH provided UDP-*N*-hydroxyacetylglucosamine (UDP-GlcNGc, **19**) in 98% yield. In addition, although UDP-GlcNS **16** was unable to be prepared from GlcNS (**8**) (Table 1) in the one-pot three-enzyme system, it was readily obtained by *N*-sulfation of compound **17** with Py·SO_3 in 2 M of NaOH aqueous solution in a very good yield (86%) (Scheme 2A). Similarly as shown in Scheme 2B, catalytic hydrogenation of the azido group at the C-6 of UDP-$GlcNAc6N_3$ **12** generated UDP-6-amino-6-deoxyl-*N*-acetyl-glucosamine (UDP-$GlcNAc6NH_2$, **23**) with an excellent yield (96%). Selective acylation of the free amino group of



Scheme 2 Chemical diversification at (A) the C-2 of glucosamine and (B) the C-6 of *N*-acetylglucosamine in UDP-sugar nucleotides. Reagents and conditions: (a) K_2CO_3, CH_3OH, H_2O, 20 °C, overnight, 98%; (b) Py·SO_3, 2 M NaOH, H_2O, overnight, 86%; (c) RCOCl, $NaHCO_3$, CH_3CN, H_2O; (d) NaOMe, MeOH; (e) H_2, Pd/C, MeOH, H_2O, 1 h, 96%.

23 using various acyl chlorides produced C-6 modified UDP-GlcNAc derivatives including UDP-6-acetoxyacetamido-*N*-acetylglucosamine (UDP-GlcNAc6NGcAc, **24**), UDP-6-azidoacetamido-*N*-acetylglucosamine (UDP-GlcNAc6NAz, **26**), UDP-6-phenylacetamido-*N*-acetylglucosamine (UDP-GlcNAc6NPhAc, **27**), and UDP-*N*-(1,1′-biphenyl-4-yl)-acetamido-*N*-acetylglucosamine (UDP-GlcNAc6NPh$_2$Ac, **28**) in 61–91% yields. Finally, C-6 modified derivative UDP-*N*-hydroxyacetamido-*N*-acetylglucosamine (UDP-GlcNAc6NGc, **25**) was obtained in 98% yield by treating compound **24** in NaOMe and methanol.

In summary, we have developed a highly efficient chemo-enzymatic approach for producing a series of UDP-GlcNAc derivatives containing diverse natural and non-natural modification at C-2 or C-6 of the glucosamine moiety using a combination of one-pot three-enzyme system followed by parallel chemical diversification. The obtained UDP-glucosamine derivatives containing natural occurring GlcNAc, 6-*O*-sulfo-GlcNAc, *N*-sulfo-glucosamine, and non-natural C2- or C6-modified GlcNAc analogues are excellent compounds for testing the donor substrate specificity of diverse GlcNAc-transferases.

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Notes and references

1 H. J. M. Gijsen, L. Qiao, W. Fitz and C.-H. Wong, *Chem. Rev.*, 1996, **96**, 443–474.
2 H. Yu and X. Chen, *Org. Biomol. Chem.*, 2007, **5**, 865–872.
3 X. Chen, *ACS Chem. Biol.*, 2011, **6**, 14–17.
4 J. Yang, X. Fu, J. Liao, L. Liu and J. S. Thorson, *Chem. Biol.*, 2005, **12**, 657–664.
5 H. Yu, S. Huang, H. Chokhawala, M. Sun, H. Zheng and X. Chen, *Angew. Chem., Int. Ed.*, 2006, **45**, 3938–3944.
6 K. Lau, V. Thon, H. Yu, L. Ding, Y. Chen, M. M. Muthana, D. Wong, R. Huang and X. Chen, *Chem. Commun.*, 2010, **46**, 6066–6068.
7 S. J. Danishefsky and M. T. Bilodeau, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1380–1419.
8 M. Oberthür, C. Leimkuhler and D. Kahne, *Org. Lett.*, 2004, **6**, 2873–2876.
9 K. Toshima and K. Tatsuta, *Chem. Rev.*, 1993, **93**, 1503–1531.
10 U. Lindahl and M. Hook, *Annu. Rev. Biochem.*, 1978, **47**, 385–417.
11 K. Honke and N. Taniguchi, *Med. Res. Rev.*, 2002, **22**, 637–654.
12 J. L. Funderburgh, *Glycobiology*, 2000, **10**, 951–958.
13 C. Mitsuoka, M. Sawada-Kasugai, K. Ando-Furui, M. Izawa, H. Nakanishi, S. Nakamura, H. Ishida, M. Kiso and R. Kannagi, *J. Biol. Chem.*, 1998, **273**, 11225–11233.
14 N. Kimura, C. Mitsuoka, A. Kanamori, N. Hiraiwa, K. Uchimura, T. Muramatsu, T. Tamatani, G. S. Kansas and R. Kannagi, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 4530–4535.
15 K. Uchimura, J. M. Gauguet, M. S. Singer, D. Tsay, R. Kannagi, T. Muramatsu, U. H. von Andrian and S. D. Rosen, *Nat. Immunol.*, 2005, **6**, 1105–1113.
16 B. S. Bochner, R. A. Alvarez, P. Mehta, N. V. Bovin, O. Blixt, J. R. White and R. L. Schnaar, *J. Biol. Chem.*, 2005, **280**, 4307–4312.
17 H. Tateno, P. R. Crocker and J. C. Paulson, *Glycobiology*, 2005, **15**, 1125–1135.
18 Y. Li, H. Yu, Y. Chen, K. Lau, L. Cai, H. Cao, V. K. Tiwari, J. Qu, V. Thon, P. G. Wang and X. Chen, *Molecules*, 2011, **16**, 6396–6407.
19 M. Nishimoto and M. Kitaoka, *Appl. Environ. Microbiol.*, 2007, **73**, 6444–6449.
20 L. Cai, W. Guan, M. Kitaoka, J. Shen, C. Xia, W. Chen and P. G. Wang, *Chem. Commun.*, 2009, 2944–2946.
21 L. Cai, W. Guan, W. Wang, W. Zhao, M. Kitaoka, J. Shen, C. O'Neil and P. G. Wang, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 5433–5435.
22 J. Fang, W. Guan, L. Cai, G. Gu, X. Liu and P. G. Wang, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 6429–6432.
23 G. Zhao, W. Guan, L. Cai and P. G. Wang, *Nat. Protoc.*, 2010, **5**, 636–646.
24 W. Guan, L. Cai and P. G. Wang, *Chemistry*, 2010, **16**, 13343–13345.
25 L. Cai, L. Ban, W. Guan, M. Mrksich and P. G. Wang, *Carbohydr. Res.*, 2011, **346**, 1576–1580.
26 L. Cai, W. Guan, W. Chen and P. G. Wang, *J. Org. Chem.*, 2010, **75**, 3492–3494.