Bioorganic & Medicinal Chemistry Letters 19 (2009) 5433–5435

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Substrate specificity of *N*-acetylhexosamine kinase towards *N*-acetylgalactosamine derivatives

Li Cai [a,†], Wanyi Guan [a,b,†], Wenjun Wang [c], Wei Zhao [c], Motomitsu Kitaoka [d], Jie Shen [c], Crystal O'Neil [a], Peng George Wang [a,*]

[a] Departments of Chemistry and Biochemistry, The Ohio State University, 484 w. 12th Ave. Columbus, OH 43210, USA
[b] National Glycoengineering Research Center and The State Key Laboratory of Microbial Technology, Shandong University, Jinan, Shandong 250100, China
[c] College of Pharmacy, Nankai University, Tianjin 300071, China
[d] National Food Research Institute, National Agriculture and Food Research Organization, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

ARTICLE INFO

Article history:
Received 26 June 2009
Revised 21 July 2009
Accepted 22 July 2009
Available online 25 July 2009

Keywords:
Kinase
Phosphorylation
Specificity

ABSTRACT

We report herein a bacterial *N*-acetylhexosamine kinase, NahK, with broad substrate specificity towards structurally modified GalNAc analogues, and the production of a GalNAc-1-phosphate library using this kinase.

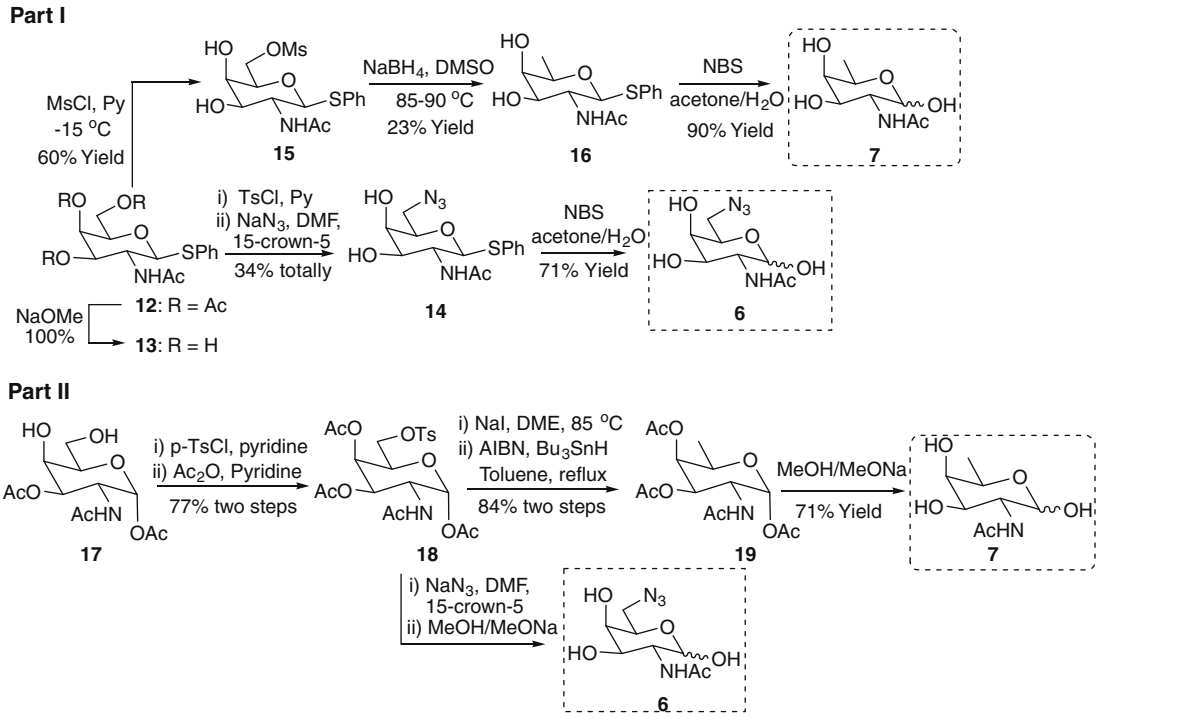
As an important sugar residue, *N*-acetylgalactosamine (GalNAc) widely exists in living systems and involves many biological processes: It is a main component of some glycosaminoglycans[1] and also exists as the initial sugar residues in mucin-type *O*-glycans.[2] Efficient construction of these clinically significant GalNAc-containing glycoconjugates enzymatically requires uridine 5′-diphospho-GalNAc (UDP-GalNAc) as a sugar donor by Leloir-type glycosyltransferases.[3] UDP-GalNAc can be obtained by enzymatic epimerization of UDP-GlcNAc, however the yield is limited,[4] restricting its use in biological areas. A salvage pathway, which reutilizes GalNAc released from degraded glycoconjugates, presents a more promising alternative: GalNAc is first activated by a GalNAc kinase to form GalNAc-1-P, then converted to UDP-GalNAc by a pyrophosphorylase.[5] Such GalNAc kinases have been discovered in mammals and for the past decade have been employed in in vitro synthesis[6] and in situ regeneration[7] of UDP-GalNAc; however, no bacterial GalNAc kinase has yet been reported. Our group has previously found that an *N*-acetylhexosamine kinase (NahK) from *Bifidobacterium longum* could well accept GalNAc as its substrate;[8] thereby GalNAc-1-P was synthesized on a gram scale.[9] Here, we report the investigation of the substrate specificity of this bacterial kinase toward structurally modified GalNAc analogues and the construction of a library of the corresponding GalNAc-1-P analogues.

To determine the substrate specificity of NahK behaving as a 'GalNAc kinase', we designed and synthesized eight GalNAc derivatives with modifications on the C-2, 4- or 6-positions. C-2 derivatives **2**–**5** were easily obtained through one step N-acylation as described before.[9] Synthesis of the 6-deoxy analogue **7** from intermediate **13** utilized the regioselective mesylation and reduction method as described by Bundle and co-workers[10] (Scheme 1, Part I). However, poor yield was observed for this reaction, contrary to our expectations based on the reasonable yield obtained with this method using GlcNAc as the substrate.[9] Anomeric deprotection using *N*-bromosuccinimide furnished 6-deoxy GalNAc **7**.[11] To improve the yield for preparing compound **7**, a second strategy was used as shown in Scheme 1, Part II. Starting from diol **17**,[12] 6-tosylated **18** was obtained by selective tosylation followed by acetylation. Deoxygenation at the 6-position of **18** was performed by iodination and subsequent radical reduction.[13] Deprotection of triacetate **19** by sodium methoxide afforded **7** in good overall yield. The 6-azido compound **6** was simply constructed either by selective tosylation of **13** followed by displacement by sodium azide to give intermediate **14** or direct substitution at C-6 of tosylated **18**. Similar deprotection procedures by NBS or sodium methoxide provided analogue **6**.

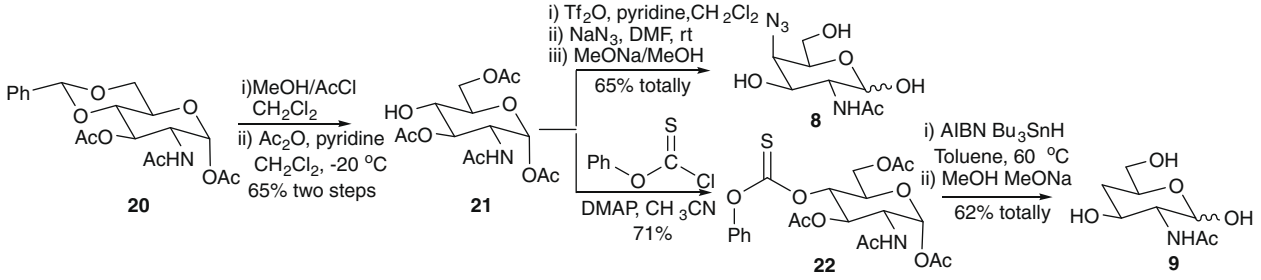
For the synthesis of 4-modified analogues (Scheme 2), 4,6-phenylmethylene protected *N*-acetyl glucosamine **20**[14] was deprotec-

* Corresponding author. Tel.: +1 614 292 9884; fax: +1 614 688 3106.
E-mail address: wang.892@osu.edu (P.G. Wang).
† Contributed equally to this work.

0960-894X/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2009.07.104



Scheme 1. Synthesis of 6-azido and 6-deoxy analogues.



Scheme 2. Synthesis of 4-azido and 4-deoxy analogues.

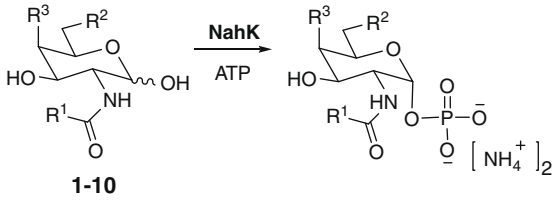
ted by treatment with acetyl chloride in methanol;[15] subsequent selective acetylation at C-6 position gave the 4-hydroxyl compound **21**.[16] Triflation of compound **21** followed by nucleophilic substitution using sodium azide gave the triacetate which was deprotected with sodium methoxide to afford the target 4-azido GalNAc **8**. Conversion of alcohol **21** to the corresponding thionocarbonate **22**, followed by radical deoxygenation[17] and deacetylation, yielded desired analogue **9**.[18]

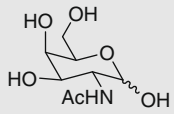
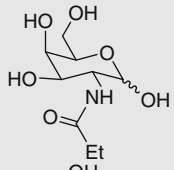
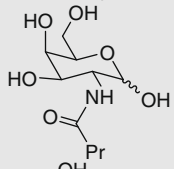
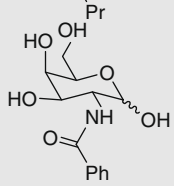
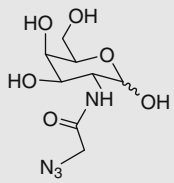
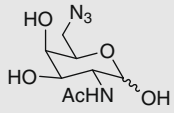
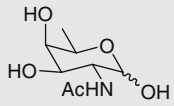
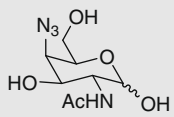
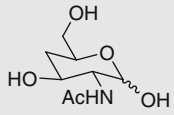
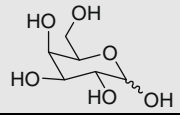
The recombinant NahK was expressed and purified as previously described.[9] The enzymatic production of GalNAc-1-P analogues was carried out in a mixture containing 40 mM GalNAc or its analogues, 50 mM ATP, 10 mM $MgCl_2$, and 1.5 mg/mL NahK in 100 mM Tris–HCl buffer (pH 9.0). After incubation at 37 °C for 19 h, the mixture was briefly boiled and centrifuged to remove protein. All GalNAc-1-P analogues synthesized here were purified by normal phase silica gel chromatography and isolated yields are shown in Table 1.[19]

The results clearly indicate that most GalNAc analogues can be phosphorylated in good yields (Table 1). We can conclude, from relatively high yields of 2-modified analogues **1–5** and almost unreactive galactose **10**,[8] that 2-amido group is necessary for enzyme activity. The bulkiness of R^1 group exhibited a slight influence on enzyme activity (compounds **4** and **5**). Significantly, the enzyme displayed a wide tolerance of 4-modification (R^3) including axial hydroxy (**1**), equatorial hydroxy (**GlcNAc**),[9] azido (**8**) and deoxy (**9**), indicating 4-hydroxyl group may not be necessary for enzyme recognition, a feature that could greatly expand our prospective library of UDP-GlcNAc and UDP-GalNAc sugar donor analogues. Conversely, the tolerance for 6-modification is still quite limited: the reactions of both 6-azido (**6**) and 6-deoxy (**7**) compounds produced dramatically decreased yields.

In summary, the bacterial *N*-acetylhexosamine kinase, NahK, has broad substrate specificity towards structurally modified GalNAc analogues. Tolerance for 2- and 4-modifications was high while tolerance for 6-modification was relatively limited. Enzymatic conversion of the sugar-1-P compounds produced here to a sugar donor library for glycosyltransferase-catalyzed reaction will be reported in due course.

Table 1
Isolated yields for enzymatic 1-phosphorylation reactions



Entry	Substrate structure	1-Phosphorylation yield (%)
1		78[a]
2		85[a]
3		86[a]
4		77[a]
5		65[a]
6		42[a]
7		37[a]
8		73[a]
9		70[a]
10		<5[b]

[a] Isolated yield from silica gel column chromatography.
[b] Detected by TLC.

Acknowledgements

P. G. Wang acknowledges National Cancer Institute (R01 CA118208), NSF (CHE-0616892) for financial support. W. Guan acknowledges China Scholarship Council for financial support. Mass spectra data were collected on a Bruker micrOTOF instrument provided by a grant from the Ohio BioProducts Innovation Center.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.104.

References and notes

1. Lindahl, U.; Hook, M. *Annu. Rev. Biochem.* **1978**, *47*, 385.
2. (a) Carraway, K. L.; Hull, S. R. *Glycobiology* **1991**, *1*, 131; (b) Hang, H. C.; Bertozzi, C. R. *Bioorg. Med. Chem.* **2005**, *13*, 5021.
3. Koeller, K. M.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465.
4. Piller, F.; Hanlon, M. H.; Hill, R. L. *J. Biol. Chem.* **1983**, *258*, 10774.
5. (a) Pastuszak, I.; Drake, R.; Elbein, A. D. *J. Biol. Chem.* **1996**, *271*, 20776; (b) Szumilo, T.; Zeng, Y.; Pastuszak, I.; Drake, R.; Szumilo, H.; Elbein, A. D. *J. Biol. Chem.* **1996**, *271*, 13147.
6. Bourgeaux, V.; Piller, F.; Piller, V. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5459.
7. Bourgeaux, V.; Cadene, M.; Piller, F.; Piller, V. *ChemBioChem* **2007**, *8*, 37.
8. Nishimoto, M.; Kitaoka, M. *Appl. Environ. Microbiol.* **2007**, *73*, 6444.
9. Cai, L.; Guan, W.; Kitaoka, M.; Shen, J.; Xia, C.; Chen, W.; Wang, P. G. *Chem. Commun.* **2009**, 2944.
10. Cai, Y.; Ling, C.-C.; Bundle, D. R. *J. Org. Chem.* **2009**, *74*, 580.
11. (a) Horton, D.; Rodemeyer, G.; Saeki, H. *Carbohydr. Res.* **1977**, *59*, 607; (b) Perry, M. B.; Daoust, V. *Can. J. Chem.* **1973**, *51*, 974.
12. Jiaang, W.-T.; Tseng, P.-H.; Chen, S.-T. *Synlett* **2000**, 797.
13. (a) Kajihara, Y.; Kodama, H.; Endo, T.; Hashimoto, H. *Carbohydr. Res.* **1998**, *306*, 361; (b) Jones, G. B.; Lin, Y.; Xiao, Z.; Kappen, L.; Goldberg, I. H. *Bioorg. Med. Chem.* **2007**, *15*, 784.
14. Yonehara, K.; Hashizume, T.; Mori, K.; Ohe, K.; Uemura, S. *J. Org. Chem.* **1999**, *64*, 9374.
15. Clinch, K.; Evans, G. B.; Furneaux, R. H.; Rendle, P. M.; Rhodes, P. L.; Roberton, A. M.; Rosendale, D. I.; Tyler, P. C.; Wright, D. P. *Carbohydr. Res.* **2002**, *337*, 1095.
16. Filice, M.; Palomo, J. M.; Bonomi, P.; Bavaro, T.; Fernandez-Lafuente, R.; Guisan, J. M.; Terreni, M. *Tetrahedron* **2008**, *64*, 9286.
17. Lin, F. L.; van Halbeek, H.; Bertozzi, C. R. *Carbohydr. Res.* **2007**, *342*, 2014.
18. Berkin, A.; Szarek, M. A.; Plenkiewicz, J.; Szarek, W. A.; Kisilevsky, R. *Carbohydr. Res.* **2000**, *325*, 30.
19. NMR data for the 1-phosphate derivatives of compound **2**, **3**, **7** and **9**: (a) Lazarevic, D.; Thiem, J. *Carbohydr. Res.* **2002**, *337*, 2187; (b) Illarionov, P. A.; Torgov, V. I.; Hancock, I. I.; Shibaev, V. N. *Russ. Chem. Bull.* **2001**, *50*, 1303; (c) Srivastava, G.; Alton, G.; Hindsgaul, O. *Carbohydr. Res.* **1990**, *207*, 259.