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Synthesis of 6′-sulfodisaccharides by β-*N*-acetylhexosaminidase-catalyzed transglycosylation

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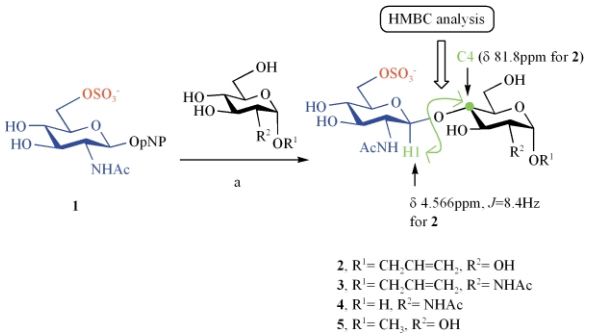
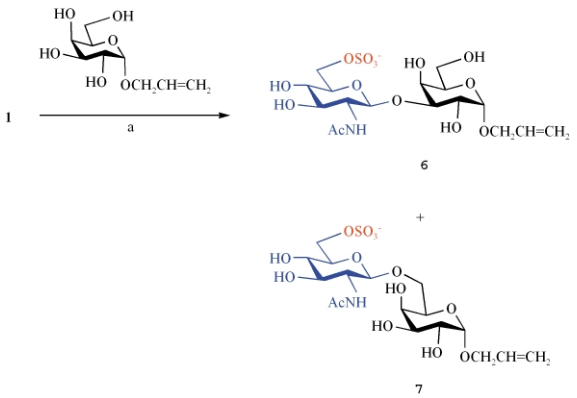
Presulfated *N*-acetylglucosaminyl donor (*p*NP β-D-6-SO_3-GlcNAc) was applied for the synthesis of sulfosugars using the β-*N*-acetylhexosaminidase-catalyzed transglycosylation, to afford the critically stereocontrolled sulfodisaccharides carrying the 6-sulfo GlcNAc residue at the non-reducing sides in one step.

Among a large number of oligosaccharide structures found in naturally occurring glycoconjugates, sulfated sugars have attracted special attention.[1] This is mainly because the sulfosugars construct key recognition structures of natural ligands of selectins,[2] pathogenic bacteria and viruses,[3] and other carbohydrate receptor proteins. In the exploration of new drug designs associated with chronic inflammation and cancer metastasis,[4] numerous artificial sulfosugars mimicking natural ligands such as sulfosialyl Le^x and Le^a are investigated.[5] Most of the syntheses have been addressed chemically by using either a stannylene acetal technology or conventional chemical transformation.[5] Recently, more extensive interests have been directed to the use of enzymes like sulfotransferases[6] and glycosyltransferase[7] in connection with the chemoenzymatic synthesis of cell surface oligosaccharides. For instance, *Rhizobium* Nod factor sulfotransferases[6] with 3′-phosphoadenosine-5′-phosphosulfate as the donor were utilized for the synthesis of chitooligosaccharides which have the 6-sulfo GlcNAc moiety at the reducing terminal. We recently reported a convenient use of sulfatases[8] for the synthesis of sulfated sugars and extended the way to prepare biofunctional glycoconjugate polymers serving as potent selectin blockers.[9] Described herein is a synthetic pathway clearly distinct from these previously reported ones, in which β-*N*-acetylhexosaminidase (E.C.3.2.1.51) is applied to the synthesis of various disaccharides carrying a 6-sulfo GlcNAc residue at the non-reducing sides.

We selected *p*-nitrophenyl (*p*NP) 6-sulfo-β-D-*N*-acetylglucosaminide **1**[10] as a 6-sulfo GlcNAc donor, because the 6-sulfo GlcNAc is an essential component of 6-sulfosialyl Le^x or Le^a bound to GlyCAM-1[2] as well as of glycosaminoglycans linked to proteoglycans. We examined several glycosyl acceptors with α-allyl, α/β-hydroxy, and α-methyl groups (Schemes 1 and 2, Table 1).



Scheme 1 *Reagents and conditions*: (a) β-*N*-acetylhexosaminidase from *Aspergillus oryzae*, 50 mM sodium phosphate buffer (pH 6.0), 35 °C.



Scheme 2 *Reagents and conditions*: (a) β-*N*-acetylhexosaminidase from *Aspergillus oryzae*, 50 mM sodium phosphate buffer (pH 6.0), 35 °C.

The enzyme reactions with β-*N*-acetylhexosamdinidase from *Aspergillus oryzae*[11] in sodium phosphate buffer (pH 6.0, 35 °C) were monitored by HPLC (silica gel, MeOH–H_2O = 1:9), and each of the products was purified successively on ODS, BioGel P-2, and ion-exchange columns. When allyl α-D-Glc was employed as an acceptor, the enzymatic transfer was achieved regioselectively at O-4 to afford sulfodisaccharide **2**† ‡ carrying a 6-sulfo-GlcNAcβ1–4Glc linkage [74 h, 38% yield based on the donor added (Table 1)]. The structure of **2** was determined by NMR spectroscopy and mass spectroscopy. The doublet signal at δ 4.566 ppm (H-1′, J = 8.4 Hz) showed the β-coupling of the GlcNAc residue. Long-range hetero coupling between H-1′ (δ 4.566 ppm) of GlcNAc and C-4 (δ 81.8 ppm) of Glc was clearly observed by the HMBC technique (Scheme 1). These results indicated that 6-sulfo GlcNAc and Glc are connected *via* a β1–4 linkage. FAB mass spectra also supported the transfer product to be disaccharide **2** ($[M + Na]^+$ 548). Other D-*gluco*-type substrates showed similar results regarding the regioselectivity to afford **3**,§ **4**¶ and **5**.‖ However, the enzymatic reaction with GlcNAc as an acceptor was quite sluggish (370 h) compared with allyl α-D-GlcNAc (66 h), allyl α-D-Glc (74 h), and methyl α-D-Glc (71 h). It may be possible that free GlcNAc shows an inhibitory effect on the enzyme reaction.[12] Allyl α-D-GlcNAc showed the highest reactivity to afford 6′-sulfo-*N*,*N*′-diacetylchitobioside **3** exclusively (92%).

Table 1 Summary of transfer products and yields catalyzed by β-*N*-acetylhexosaminidase from *Aspergillus oryzae*

Donor	Acceptor	Specificity of transglycosylation	Product	Isolated yield[a] (%)
1	Glcα-OAll	O-4	**2**	38
1	GlcNAcα-OAll	O-4	**3**	92
1	GlcNAc	O-4	**4**	38
1	Glcα-OMe	O-4	**5**	17
1	Galα-OAll	O-3/O-6	**6** + **7**	25 (**6**:**7** = *ca.*1:7)

[a] Based on the donor added.

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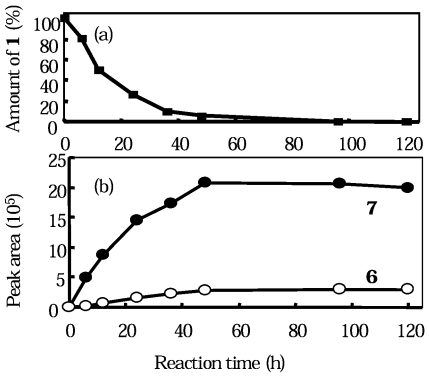

Fig. 1 Time course of β-*N*-acetylhexosaminidase catalyzed- (a) hydrolysis of **1**, and (b) formation of transfer products **6** and **7** with **1** and allyl α-D-Gal. Each amount of **1**, **6** and **7** was determined by HPLC (ODS C-18, 1 ml min^{-1}, 10% MeOH containing 0.05% THF, UV 210 nm).

The enzyme had also a potent transfer activity even to a D-galactopyranose structure to afford two 6′-sulfodisaccharides, the transferred product **6**** at O-3 of the acceptor together with its regioisomer **7**†† ‡‡ that was transferred at O-6 in a molar ratio of *ca*. 1:7 (Scheme 2 and Table 1). The time course reactions show that the enzyme transferred 6-SO_3 GlcNAc residue mainly at O-6 of the acceptor rather than at O-3, to afford **6** and **7**, respectively (Fig.1). After 48 h, the amount of **6** and **7** reached apparent equilibrium, while 95% of the donor **1** was hydrolyzed under the conditions. In this experiment, none of oligomerized products such as sulfated tri- or tetrasaccharides was detected. Thus, the sulfo donor **1** could be utilized as a good substrate, supplying sulfodisaccharides that have the 6-SO_3 GlcNAc unit at the non-reducing moiety.

In summary, we have first demonstrated that β-*N*-acetylhexosaminidase from *A. oryzae* could catalyze the transfer of 6-sulfo GlcNAc from the sulfo donor **1** to monosaccharide acceptors, which is applied to the synthesis of sulfated disaccharides with 6-sulfo GlcNAc core elements at the non-reducing moiety. To the best of our knowledge, this is the first report that β-*N*-acetylhexosaminidase mediates the transglycosylation of 6-sulfo GlcNAc for the synthesis of sulfated sugars.

The enzymatic strategy described here provides a practical and convenient entry to various sulfosugars, which may find many applications in the study on biological roles of sulfated oligosaccharides in carbohydrate-mediated biological phenomena. As can be seen from the products derived in this study, the enzyme reaction seems to permit various aglycon structures. This will be of high practical values for subsequent polymerization or immobilization[9,14,15] actually at the stage to evaluate carbohydrate/receptor protein interactions[16] with a surface plasmon resonance (SPR) technique,[17] a quartz crystal microbalance (QCM) method,[18] and other cell-free assay systems as well as *in vivo* systems.

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Notes and references

† Typical procedure for enzymatic transglycosylation. *Synthesis of 2*; **1** (60 mg, 0.14 mmol) and allyl α-D-Glc (300 mg, 1.36 mmol) dissolved in 50 mM sodium phosphate buffer (pH 6.0, 0.8 mL) were incubated with β-*N*-acetylhexosaminidase (*A. oryzae*, 23 U) at 35 °C for 74 h to afford **2** (27 mg, 38%) after usual work-up. The β-*N*-acetylhexosaminidase from *A. oryzae* purchased from Sigma may contain a trace amount of β-galactosidase and α-glucosidase activities that have no hydrolytic and transfer activity toward **1**.

‡ *Selected data for* **2**: δ_H(400 MHz, D_2O) 4.926 (H-1, d, *J* 4.0), 4.566 (H-1′, d, *J* 8.4), 3.506 (H-4, m), 2.031 (3 H, Ac). δ_C (100 MHz, D_2O) 103.2 (C-1′), 98.6 (C-1), 81.8 (C-4). $[\alpha]_D$ +67.4° (*c* 1.76, H_2O). FAB MS: 548 $[M + Na]^+$.

§ *Selected data for* **3**: δ_H (400 MHz, D_2O) 4.915 (H-1,d, *J* 2.4), 4.621 (H-1′, d, *J* 8.4), 3.571 (H-4, m), 2.055 and 2.018 (2Ac). δ_C (100 MHz, D_2O) 103.2 (C-1′), 97.3 (C-1), 81.9 (C-4). FAB-MS: 589 $[M + Na]^+$. $[\alpha]_D$ +66.8° (*c* 2.4, H_2O).

¶ *Selected data for* **4**: δ_H (400 MHz, D_2O) 5.174 (H-1α, d, *J* 3.2), 4.673 (H-1β, d, *J* 8.4), 4.608 (H-1′, d, *J* 8.4), 2.037 and 2.005 (2Ac). δ_C (100 MHz, D_2O) 103.2 (C-1′), 96.5 (C-1β), 92.0 (C-1α), 82.0 (C-4α), 81.5 (C-4β). FAB-MS: 549 $[M + Na]^+$. $[\alpha]_D$ +11.7° (*c* 1.29, H_2O).

‖ *Selected data for* **5**: δ_H (400 MHz, D_2O) 5.187 (H-1, d, *J* 3.2,), 4.704 (H-1′, d, *J* 8.4), 3.403 (OMe, s), 2.032 (Ac). FAB-MS: 522 $[M + Na]^+$. $[\alpha]_D$ +27.5° (*c* 0.47, H_2O).

** *Selected data for* **6**: δ_H (400 MHz, D_2O) 4.954 (H-1, d, *J* 3.6), 4.522 (H-1′, d, *J* 8.4), 2.012 (Ac). FAB-MS: 548 $[M + Na]^+$. $[\alpha]_D$ +69.0° (*c* 0.24, H_2O).

†† *Selected data for* **7**: δ_H (400 MHz, D_2O) 4.933 (H-1, d, *J* 3.6), 4.502 (H-1′, d, *J* 8.4), 2.004 (Ac). FAB-MS: 548 $[M + Na]^+$. $[\alpha]_D$ +56.1° (*c* 0.58, H_2O).

‡‡ The core unit 6-sulfoGlcNAcβ1–6GalNAc is found naturally in L-selectin ligand glycans; the 6′-sulfosugar **7** is related to this structure but with the GalNAc unit replaced by Gal. Some structures carrying the 6-sulfoGlcNAcβ1–6 sequence have recently been studied in connection with the biosynthesis of L-selectin ligands.[13]

1 A. Varki, *Glycobiology*, 1993, **3**, 97–130.
2 S. Hemmerich, H. Leffler and S. D. Rosen, *J. Biol. Chem.*, 1995, **270**, 12035; S. Hemmerich and S. D. Rosen, *Biochemistry*, 1994, **33**, 4830.
3 T. Suzuki, A. Sometani, Y. Yamazaki, G. Horiike, Y. Mizutani, H. Masuda, M. Yamada, H. Tahara, G. Y. Xu, D. Miyamoto, N. Oku, S. Okada, M. Kiso, A. Hasegawa, T. Ito, Y. Kawaoka and Y. Suzuki, *Biochem. J.*, 1996, **318**, 389.
4 K. G. Bowman and C. R. Bertozzi, *Chem. Biol.*, 1999, **6**, R9–R22; S. Hemmerich, *Drug Discov. Today*, 2001, **6**, 27.
5 E. E. Simanek, G. J. McGarvey, J. A. Jablonowski and C.-H. Wong, *Chem. Rev.*, 1998, **98**, 833; D. D. Manning, X. Hu, P. Beck and L. L. Kiessling, *J. Am. Chem. Soc.*, 1997, **119**, 3161; B. Guilbert, N. J. Davis, M. Pearce, R. T. Aplin and S. L. Flitsch, *Tetrahedron: Asymmetry*, 1994, **5**, 2163.
6 C.-H. Lin, G.-J. Shen, E. G. -Junceda and C.-H. Wong, *J. Am. Chem. Soc.*, 1995, **117**, 8031–8032; M. D. Burkart, M. Izumi and C.-H. Wong, *Angew. Chem., Int. Ed.*, 1999, **38**, 2747.
7 A. Lubineau, C. Auge, N. L. Goff and C. L. Narvor, *Carbohydr. Res.*, 1998, **305**, 501.
8 H. Uzawa, T. Toda, Y. Nishida, K. Kobayashi, N. Minoura and K. Hiratani, *Chem. Commun.*, 1998, 2311.
9 Y. Nishida, H. Uzawa, T. Toba, K. Sasaki, H. Kondo and K. Kobayashi, *Biomacromolecules*, 2000, **1**, 68.
10 H. Kresse, E. Paschke, K. Figura, W. Gilberg and W. Fuchs, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 6822.
11 S. Singh, M. Scigelova and D. H. G. Crout, *Chem. Commun.*, 1996, 993.
12 E. Rajnochová, J. Dvořáková, Z. Hunková and V. Křen, *Biotechnol. Lett.*, 1997, **19**, 869.
13 K. G. Bowman, B. N. Cook, C. L. Graffenried and C. R. Bertozzi, *Biochemistry*, 2001, **40**, 5382.
14 K. Kobayashi, E. Tawada, T. Akaike and T. Usui, *Biochim. Biophys. Acta*, 1997, **1336**, 117.
15 Y. Nishida, H. Dohi, H. Uzawa and K. Kobayashi, *Tetrahedron Lett.*, 1998, **39**, 8681; H. Dohi, Y. Nishida, M. Mizuno, M. Shinkai, T. Kobayashi, T. Takeda, H. Uzawa and K. Kobayashi, *Bioorg. Med. Chem.*, 1999, **7**, 2053.
16 J. J. Lundquist and E. J. Toone, *Chem. Rev.*, 2002, **102**, 555.
17 X. Zeng, T. Murata, H. Kawagishi, T. Usui and K. Kobayashi, *Carbohydr. Res.*, 1998, **312**, 209.
18 H. Uzawa, S. Kamiya, N. Minoura, H. Dohi, Y. Nishida, K. Taguchi, S. Yokoyama, H. Mori, T. Shimizu and K. Kobayashi, *Biomacromolecules*, 2002, **3**, 411.