Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 1597–1602
Carbohydrate RESEARCH

Sulfatase-catalyzed assembly of regioselectively O-sulfonated *p*-nitrophenyl α-D-gluco- and α-D-mannopyranosides

Hirotaka Uzawa,[a,*] Yoshihiro Nishida,[b] Kenji Sasaki,[b] Takehiro Nagatsuka,[c] Hideo Hiramatsu[c] and Kazukiyo Kobayashi[b]

[a]*Research Center of Advanced Bionics, National Institute of Advanced Industrial Science and Technology (AIST), Central 5, 1-1-1 Higashi, Tsukuba 305-8565, Japan*
[b]*Department of Molecular Design and Engineering, Graduate School of Engineering, Nagoya University, Chikusa, Nagoya 464-8603, Japan*
[c]*College of Industrial Technology, Nihon University, 1-2-2 Izumichou, Narashino, Chiba 275-8575, Japan*

Received 17 December 2003; accepted 21 March 2004
Available online 10 May 2004

Abstract—A chemoenzymic methodology is extended to the library synthesis of regioselectively O-sulfonated *p*NP D-gluco and D-mannopyranosides. The method involves the sequential reactions of chemical O-sulfonation and sulfatase-catalyzed O-desulfonation. *p*NP 2,6-di-*O*-sulfo-α-D-glucopyranoside and *p*NP 3,6-di-*O*-sulfo-α-D-mannopyranoside were obtained as sodium salts using chemical methods by way of dibutylstannylene acetals or tributylstannyl ethers. They were then applied to enzyme reactions using three molluscan enzymes (snail, limpet, and abalone). The sulfatase reactions cleaved a sulfate group at the secondary O-2 or O-3 position to yield the corresponding *p*NP 6-*O*-sulfo sugars. Neither *p*NP 6-*O*-sulfo-α-D-glucopyranoside nor 6-*O*-sulfo-α-D-mannopyranoside became the enzyme substrate. Evidently, the molluscan sulfatases have a tendency to cleave the secondary *O*-sulfo group with assistance from the 6-*O*-sulfo group.

Keywords: O-sulfonation; Sulfatase; Regioselectivity; Library synthesis

1. Introduction

The O- and N-sulfonated oligosaccharides occurring widely in mammalian glycoproteins and lipids are thought to have many biological roles.[1,2] Among them, O-sulfonated D-galacto sugars involved in GlyCAM-1 and glycosaminoglycans have received particular attention[3] because they are considered to serve as informational molecules associated with cell–cell recognition and differentiation events. Some of the synthetic sulfo sugars, after being converted to cluster models, work as selectin antagonists that inhibit the binding of selectins to cell-surface sialyl Lewis^x (or Lewis^a) that may lead to malignant transformations.[4] Although the sulfo sugars reported for mammalian sources possess either a D-galacto- or an *N*-acetyl-D-glucosamino backbone,[2] variable sugar species are known to occur in nature like L-fucoses in algae and D-glucoses in microbial systems. For example, 2-*O*-sulfotrehalose was found in mycobacteria and archebacteria,[5,6] and 6-*O*-sulfo-D-mannose was identified in *Dictyostelium discoideum* as a component of secreted glycoproteins.[7,8] These situations indicate that the assembly of various types of sulfo sugars provides a key step to elucidate the biological roles of these natural and synthetic sulfo sugars. On the other hand, synthesis and handling of the sulfo sugars are believed to require special techniques and care different from those for neutral sugars. This may be linked also to the fact that little is known about the biological potential of sulfo sugars in applications to carbohydrate-based drugs and materials.

* Corresponding author. Tel.: +81-298-61-4431; fax: +81-298-61-4680; e-mail: h.uzawa@aist.go.jp

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.03.026

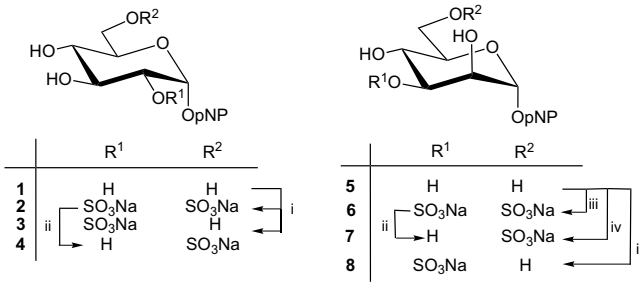
Regarding chemical syntheses, regioselective O-sulfonation methods assisted by Bu_2SnO and $(Bu_3Sn)_2O$ have been proposed and applied to the synthesis, for example, of 3-*O*-sulfo Lewis[a], 3-*O*-sulfo Lewis[x], and their cluster models like glycopolymers and dendrimers.[9–12] Recently, chemoenzymatic approaches have received increasing attention because of their efficiency and green processes. A 6-sulfotransferase using 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as the donor substrate was applied to the construction of the 6-*O*-sulfo-D-GlcNAc residue in chitobiose, chitotriose, and chitotetraose.[13,14] A bovine UDP-galactosyltransferase was used for the synthesis of 6-*O*-sulfo-*N*-acetyllactosamines.[15] Recently, a novel methodology based on glycosidase-catalyzed transglycosylation was proposed for the assembly of 6′-*O*-sulfo-disaccharides carrying the D-GlcNAc residue at the nonreducing moiety.[16] Though these chemical and enzymatic methods may provide practical ways for the synthesis of natural sulfo sugars, there is no established way for the library assembly of sulfo sugars and their cluster models.

In our preceding studies, we have searched for a chemoenzymic pathway leading to the library assembly of *p*NP[†] *O*-sulfo-glycosides, and we have disclosed the high potential of molluscan sulfatases (E.C. 3.1.6.1, abalone, snail, and limpet).[17,18] Though the sulfatases are still thought to hold rigid specificity for 3-*O*-sulfo-β-D-galactopyranoside in natural sulfatides,[19] we recently found that they can also accept multiply sulfated *p*NP D-galactopyranosides. For example, a snail sulfatase cleaves selectively the 3-*O*-sulfo group in *p*NP 3,6-di-*O*-sulfo-galactosides to afford 6-*O*-sulfo-galctosides.[17,18] The results have allowed us to propose a synthetic methodology involving sequential reactions of chemical O-sulfonation and sulfatase-catalyzed O-desulfonation. The combinatorial method has allowed us to assemble the molecular library of regioselectively O-sulfonated *p*NP D-galactosides, *N*-acetyl D-galactosaminides, and lactosides.[17,18] The *p*NP group can be reduced to *p*-aminophenyl group, which is also extendable to multivalent models.[20–22] In the present study, we examined the potential utility of our approach for the assembly of regioselectively *O*-sulfated *p*NP D-gluco- and D-mannopyranosides as non D-galacto-types of hexopyranosides.

2. Results and discussion

2.1. Chemical syntheses of *p*NP *O*-sulfo-α-D-gluco- and α-D-mannopyranosides as substrates of molluscan sulfatases

Five enzyme substrates of *O*-sulfo-α-D-Glc-*Op*NP (**2** and **3**) and *O*-sulfo-α-D-Man-*Op*NP (**6**, **7**, and **8**) (Scheme 1) were prepared by chemical methods as summarized in



Scheme 1. Reagents and conditions: (i) Bu_2SnO, THF–benzene (50:50 → 2:98, v/v), 4 h, reflux, then SO_3NMe_3, DMF, 50 °C, 16 h; (ii) Sulfatases from snail (*Helix pomatia*), abalone or limpet (*Patella vulgata*), 0.25 M HOAc–NaOAc buffer (pH 6.8), 37 °C, 75 h.; (iii) $(Bu_3Sn)_2O$, THF–benzene (1/1(v/v)), 4 h, then SO_3NMe_3, DMF, 40 °C, 11 h; (iv) SO_3NMe_3, DMF, 40 °C, 48 h.

Table 1. Conditions and products in the chemical O-sulfonation of **1** and **5**

Starting materials	Reagents	Solvents	Main products (yields[b])
1	Bu_2SnO	THF–benzene (50:50 → 2:98, v/v)	**2** (35%) and **3** (21%)
1	Bu_2SnO	MeOH	—[c]
5	$(Bu_3Sn)_2O$	THF–benzene (1:1)	**6** (48%)
5	—[a]	—[a]	**7** (37%)
5	Bu_2SnO	THF–benzene (1:1)	**8** (54%)

[a] Without the tin activation using SO_3NMe_3 (3.0 mol equiv) in DMF.
[b] Isolated yields.
[c] Unidentified complicated products.

Table 1. Compound **2** was obtained in 35% yield by applying a conventional dibutylstannylene acetal method to *p*NP α-D-glucopyranoside (**1**). The product **2** was obtained together with 2-*O*-sulfo-α-D-Glc-*Op*NP (**3**), which were, however, separable with an ODS column. The products **2** and **3** were characterized by ^{1}H NMR and FAB mass spectroscopy. The signals of H-2 (δ 4.42) and H-6 and H-6′ (δ 4.28–4.19) in **2** were largely deshielded in comparison with the corresponding signals of **1**. In the ^{1}H NMR spectrum of **3**, a similar downfield shift was observed selectively for H-2 (δ 4.39), supporting the 2-*O*-sulfo-α-D-Glc structure. The tin-activation method, when carried out in MeOH for the activation followed in DMF for the sulfation, gave a complicated mixture of nonselectively O-sulfonated products (Table 1). The observed solvent effect was in good accordance with the results for D-galacto sugars.[17,18]

In the same way, 3,6-*O*-SO_3-α-D-Man-*Op*NP (**6**) was derived from α-D-Man-*Op*NP (**5**) in 48% yield using $(Bu_3Sn)_2O$ (ca. 1.5 mol equiv) in THF–benzene, followed by O-sulfonation with SO_3Me_3N in DMF. For the transformation of **5** to 3-*O*-sulfo-α-D-Man-*Op*NP (**8**), Bu_2SnO was applied instead of $(Bu_3Sn)_2O$ for the tin activation. These products were assigned by ^{1}H NMR and FAB mass data in the same way as mentioned above. Moreover, as a reference compound to assign

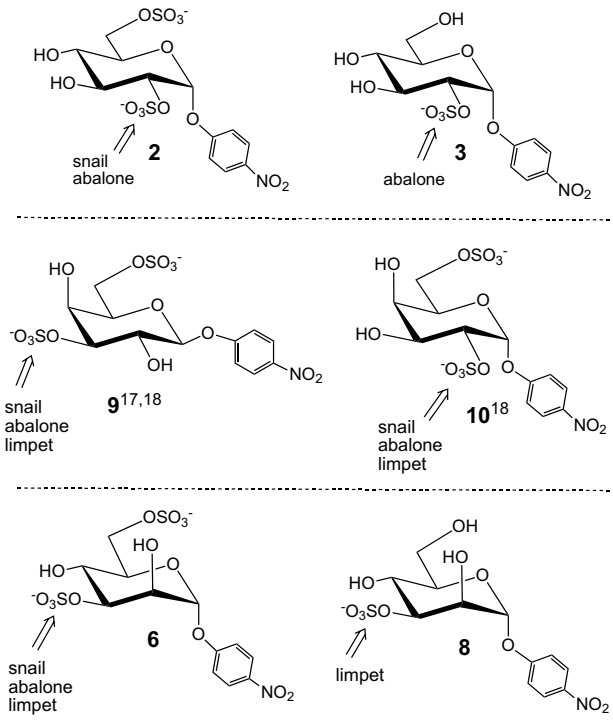
[†] *p*NP = *p*-nitrophenyl.

products in following sulfatase reactions, 6-*O*-sulfo-α-D-Man-*Op*NP (**7**) was prepared from **5** in a random sulfonation with SO_3Me_3N.

2.2. Substrate- and regioselectivity in sulfatase reactions for *O*-sulfo-α-D-Glc-O*p*NP and *O*-sulfo-α-D-Man-O*p*NP

Each of the synthetic substrates was applied to enzyme reactions with three sulfatases from snail (*Helix pomatia*), abalone (not specified), and limpet (*Patella vulgata*) (Table 2). When **2** was treated with the snail sulfatase under the conditions established previously [see (ii) in Scheme 1], a less polar compound appeared on a silica gel TLC plate (2:1 $CHCl_3$–MeOH, v/v, twice developed). The product was isolated in 32% yield after chromatographic purification with Sephadex LH-20, ODS C-18, and ion-exchange columns (Dowex Na^+). In the ^1H NMR spectroscopic study, the doublet of doublet (dd) signals of H-2 appeared at δ 3.80. The chemical shift indicates that the product is 6-*O*-sulfo-α-D-Glc-*Op*NP **4** carrying no 2-*O*-sulfo group. No other product was isolated from the enzyme reaction, indicating that the snail enzyme regiospecifically cleaved the 2-*O*-sulfo group. The abalone sulfatase also gave **4** as a single product (40% yield) in the reaction with **2**. In contrast, the limpet sulfatase gave the same product but in poor yields (<5%, determined by TLC) showing a considerable decrease in the reactivity. The results summarized in Scheme 2 and Table 2 show that no α-D-Glc-*Op*NP (**1**) was produced in the reactions of **2** examined here. Thus, 6-*O*-sulfo-α-D-Glc-*Op*NP (**4**) becomes the final product. Previously,[17,18] we showed that 6-*O*-sulfo-α-D-Gal-*Op*NP and its β-isomer become the final products in the sulfatase reactions of 2,6-di-*O*-sulfo-α-D-Gal-*Op*NP (**10**) and 3,6-di-*O*-sulfo-β-D-Gal (**9**), respectively (Scheme 2). Thus, the enzyme reactions are selective for the secondary sulfates in both the D-gluco- and D-galacto-type of hexopyranosides.

Here, it is of interest to note that the snail and abalone enzymes accepted both 2,6-di-*O*-sulfo-α-D-glucopyranoside (**2**) and 2,6-di-*O*-sulfo-α-D-galactopyranoside



Scheme 2.

(**10**) as the substrates (Scheme 2). This means that the stereochemistry at position C-4 is not of crucial importance for the enzyme reactions as long as both the O-2 and O-6 positions are sulfated. For 2-*O*-sulfo-α-D-Glc-*Op*NP (**3**), only the abalone enzyme showed reactivity to produce α-D-Glc-*Op*NP (**1**). Comparing the enzyme reactions between **2** and **3**, it is obvious that the snail enzyme cleaves the O-2 sulfo group in **2** with assistance from the O-6 sulfo group. In our preceding studies for D-galacto-type substrates,[17,18] the three enzymes showed a common tendency. The presence of a 6-*O*-sulfo group promotes the hydrolysis of an *O*-sulfo group at secondary positions O-2 and O-3. In the present case, the snail enzyme showed a similar tendency. On the other hand, the abalone enzyme required no O-6 sulfo group essentially to cleave the O-2 sulfo group in **3**.

Table 2. The results of sulfatase reactions with mono- and di-*O*-sulfonated *p*NP α-D-glycopyranosides[a]

Substrates	Location of de-*O*-sulfation[b]			Products (yields[c])		
	Snail	Abalone	Limpet	Snail	Abalone	Limpet
2	O-2	O-2	O-2	**4** (32%)	**4** (40%)	—[d]
3	—	O-2	—	—[e]	**1** (28%)	—[e]
6	O-3	O-3	O-3	**7** (21%)	**7** (60%)	**7** (10%)
8	—	—	O-3	—[e]	—[e]	**5** (21%)[f]

[a] Sulfatases from snail (*Helix pomatia*), abalone, and limpet (*Patella vulgata*) are commercially available.
[b] Determined with ^1H NMR analyses.
[c] Isolated yields.
[d] The yield was less than 5%.
[e] The starting material was recovered.
[f] Determined with HPLC analyses. Chromatographic conditions: temperature, 40 °C; flow rate, 1.0 mL min^{-1}; detection, 300 nm; mobile phase, 10% MeOH–H_2O containing 0.1% TFA. Retention time (RT): 22.8 min (compound **8**) and 45.9 min (compound **5**).

For D-manno-type substrates (Scheme 2 and Table 2), every sulfatase cleaved the O-3 sulfo group of 3,6-di-*O*-sulfo-α-D-Man-*Op*NP (**6**) to afford 6-*O*-sulfo-α-D-Man-*Op*NP (**7**) exclusively. On the other hand, only a limpet sulfatase recognized 3-*O*-sulfo-α-D-Man-*Op*NP (**8**) as the substrate to give a nonsulfated α-D-Man-*Op*NP (**5**). These results are in accordance with the tendency as mentioned before as well as the assisting role of the 6-*O*-sulfo group.

As can be seen in product yields, however, the enzyme reaction for the 3,6-di-*O*-sulfo-α-D-Man-*Op*NP (**6**) was obviously slower than that for **2**, **9**, and **10**. Although the reactivity varies also among the three enzymes, the overall relative reactivity among the three D-hexopyranoside substrates can be summarized in the order of 3,6-di-*O*-sulfo-β-D-Gal-*Op*NP (**9**) = 2,6-di-*O*-sulfo-α-D-Gal-*Op*NP (**10**) > 2,6-di-*O*-sulfo-α-D-Glc-*Op*NP (**2**) > 3,6-di-*O*-sulfo-α-D-Man-*Op*NP (**6**). Apparently, the D-manno- and D-gluco-configurations carrying an axial OH-2 and an equatorial OH-4 group, respectively, provide poorer substrates than the D-*galacto*-configuration can do. Most of the 2,3- and 3,6-di-*O*-sulfo-derivatives, however, can satisfy the minimal substrate structure required for the binding and reaction with the three molluscan sulfatases.

In conclusion, we have demonstrated the utility of our chemoenzymatic method for the library assembly of regioselectively O-sulfonated *p*NP D-gluco- and D-mannopyranosides. The method allowed us to assemble not only 2,6-di-*O*-sulfo-α-D-Glc-*Op*NP (**2**) and 3,6-di-*O*-sulfo-α-D-Man-*Op*NP (**6**) by chemical tin-activation methods and but also 6-*O*-sulfo-α-D-Glc-*Op*NP (**4**) and 6-*O*-sulfo-α-D-Man-*Op*NP (**7**) in the following enzymic sulfatase reactions. We assembled also 2-*O*-sulfo-α-D-Glc-*Op*NP (**3**) and 3-*O*-sulfo-α-D-Man-*Op*NP (**8**) along the chemical way. The *p*NP group can be converted to the 4-aminophenyl function by catalytic hydrogenation with $Pd(OH)_2$ irrespectively of the *O*-sulfo group.[20,21] Thus, these *p*NP-based O-sulfonated glycosides provide a useful tool for analytical studies to determine carbohydrate–protein interactions with surface plasmon resonance, a quartz crystal microbalance, and other sensing.[23,24] In addition, they can be applied as the key carbohydrate modules[20,21] for the assembly of multivalent glycoconjugates having higher potential in biological and medicinal applications. These application studies presently in progress will be reported elsewhere.

3. Experimental

3.1. General methods

Sulfatases (E.C. 3.1.6.1) from snail (*Helix pomatia*, 16.1 units/mg), abalone entrails (not specified, 23 units/mg) and limpet (*Patella vulgata*, 7.6 units/mg) were purchased from Sigma Chemical Co. and used without further purification. All other reagents were obtained from Aldrich or Sigma Chemical Co. and used as received. Reactions were monitored by thin-layer chromatography (TLC) on Silica Gel 60 F_{254} (E. Merck), which were visualized by UV light and by spraying with 20% H_2SO_4 in EtOH followed by charring at 180 °C. Flash column chromatography was performed on Silica Gel 60 RP-18 (ODS-C_{18}, E. Merck, 40–63 μm). Gel-filtration chromatography was performed on Sephadex LH-20 (Amersham Pharmacia Biotech). Optical rotations were measured with JASCO DIP-1000 digital polarimeter at ambient temperature, using a 10-cm micro cell. ^1H and ^{13}C NMR spectra were recorded on a Varian G300, a Varian INOVA 400 MHz or a Bruker AVANCE-500 spectrometer for solutions in D_2O. Chemical shifts are given in ppm and referenced to internal *tert*-butyl alcohol (δ_H 1.23 in D_2O or δ_C 31.2 in D_2O). All data are assumed to be first order with apparent doublet and triplets reported as 'd' and 't', respectively. Resonances that appear broad are designated 'b'. FAB mass spectra (FABMS) were recorded using a JEOL DX 303 mass spectrometer, and high-resolution mass spectra (HRMS) were recorded using a Hitachi M 80 mass spectrometer. Elemental analyses were performed with a Carlo Elba EA-1108 or Perkin–Elmer EA-2400 instrument. The HPLC measurements were carried out on a TOSOH HPLC system with a PD-8020 photodiode-array detector from TOSOH Corporation, Tokyo, Japan. The chromatographic system was equipped with an autosampler AS-8020 (TOSOH Corporation, Tokyo, Japan). The column used was TSKgel ODS-80Ts QA (ODS-C_{18} column) with 5-μm particle size, 250 mm × 4.6 mm i.d. (TOSOH Corporation, Tokyo, Japan).

3.2. *p*NP 2,6-di-*O*-sulfo-α-D-glucopyranoside, disodium salts (2) and *p*NP 2-*O*-sulfo-α-D-glucopyranoside, sodium salt (3)

A mixture of *p*NP α-D-glucopyranoside (**1**) (500 mg, 1.7 mmol) and Bu_2SnO (992 mg, 4.0 mmol) was refluxed in THF–benzene (changed from 50:50 gradually to 2:98, v/v, 500 mL) for 4 h with continuous azeotropic removal of water. After the evaporation of the solvents, the residue was treated with SO_3NMe_3 (554 mg, 4.0 mmol) in DMF at 50 °C for 16 h. The reaction mixture was diluted with MeOH (10 mL) and concentrated in vacuo. The crude product was subjected to reversed-phase chromatography on a column of ODS C_{18}, and the column was eluted with H_2O. Each of fractions containing the desired di-*O*-sulfonated **2** and mono-*O*-sulfonated **3** was collected, combined, and concentrated. Each of the products (**2** and **3**) was treated with ion exchange resin (Dowex Na^+ form) to obtain di-*O*-sulfonated **2** (297 mg, 35%) and mono-*O*-sulfonated **3**

(141 mg, 21%), respectively. **2**: $[\alpha]_D$ +114 (*c* 0.7, H_2O). ^1H NMR (300 MHz): δ 8.23 and 7.30 (d, *J* 9.3 Hz, *p*NP), 6.09 (d, $J_{1,2}$ 3.6 Hz, H-1), 4.42 (dd, $J_{2,3}$ 9.9 Hz, H-2), 4.28–4.19 (m, H-6 and H-6′), 4.08 (t, $J_{3,4}$ 9.9 Hz, H-3), 4.01–3.93 (m, H-5), 3.71 (t, $J_{4,5}$ 9.9 Hz, H-4). ^{13}C NMR (75 MHz): δ 163.0, 144.4, 127.8, 118.9, 96.7, 78.3, 72.5, 72.3, 70.5, 68.3. Anal. Calcd for $C_{12}H_{13}NNa_2O_{14}S_2{\cdot}H_2O$: C, 27.54; H, 2.89; N, 2.68. Found: C, 27.97; H, 3.00; N, 2.43. FABMS (positive-ion): 505 $[M]^+$, 528 $[M+Na]^+$. HRMS: Calcd for $C_{12}H_{14}NNa_2O_{14}S_2$ $[M+H]^+$: 505.9651. Found: 505.9560. **3**: $[\alpha]_D$ +158 (*c* 0.7, H_2O). ^1H NMR (300 MHz): δ 8.16 and 7.25 (d, *J* 9.3 Hz, *p*NP), 6.08 (d, $J_{1,2}$ 3.6 Hz, H-1), 4.39 (dd, $J_{2,3}$ 9.9 Hz, H-2), 4.08 (t, $J_{3,4}$ 9.9 Hz, H-3), 3.82–3.59 (m, H-4, H-5 H-6, H-6′). ^{13}C NMR (75 MHz): δ 163.1, 144.2, 127.7, 118.7, 96.6, 78.5, 74.5, 72.4, 70.7, 61.8. FABMS (positive-ion): 426 $[M+Na]^+$.

3.3. *p*NP 6-*O*-sulfo-α-D-glucopyranoside, sodium salt (4)

A mixture of **2** (50 mg, 0.10 mmol) and snail sulfatase (25 mg, 402 U) was dissolved in 0.25 M HOAc–NaOAc buffer (pH 6.8, 2 mL) at 37 °C for 75 h. The reaction mixture was purified in the same way as described above to give **4** (13 mg, 32%) with recovered **2** (18 mg, 36%). $[\alpha]_D$ +93 (*c* 0.2, H_2O). ^1H NMR (300 MHz): δ 8.27 and 7.30 (d, *J* 9.3 Hz, *p*NP), 5.80 (d, $J_{1,2}$ 3.6 Hz, H-1), 4.25–3.95 (m, H-6, H-6′), 3.95 (t, $J_{2,3}$ 9.6 Hz, H-3), 3.80 (dd, H-2), 3.59 (t, $J_{3,4}$ 9.6 and $J_{4,5}$ 9.6 Hz, H-4). ^{13}C NMR (75 MHz): δ 138.7, 127.8, 118.6, 98.4, 74.4, 72.6, 72.5, 70.5, 68.3. Anal. Calcd for $C_{12}H_{14}NNaO_{11}S{\cdot}H_2O$: C, 34.21; H, 3.83; N, 3.33. Found C, 34.55; H, 3.70; N, 3.17. FABMS (positive-ion): 404 $[M+H]^+$, 426 $[M+Na]^+$. HRMS: Calcd for $C_{12}H_{14}NNaO_{11}S$ $[M]^+$: 403.2955. Found: 402.9916.

3.4. *p*NP 3,6-di-*O*-sulfo-α-D-mannopyranoside, disodium salts (6)

A mixture of *p*NP α-D-mannopyranoside (**5**) (80 mg, 0.27 mmol) and $(Bu_3Sn)_2O$ (203 μL, 0.40 mmol) was refluxed in THF–benzene (1/1(v/v), 500 mL) for 4 h with continuous azeotropic removal of water. After the evaporation of the solvents, the residue was treated with SO_3NMe_3 (222 mg, 1.6 mmol) in DMF at 40 °C for 11 h. The reaction mixture was diluted with MeOH (10 mL) and concentrated in vacuo. The residue was then purified in the same way as described above to afford O-disulfonated **6** (65 mg, 48%). $[\alpha]_D$ +84 (*c* 0.36, H_2O). ^1H NMR (500 MHz): δ 8.27 and 7.31 (d, *J* 9.3 Hz, *p*NP), 5.79 (bd, $J_{1,2}$ 1.9 Hz, H-1), 4.73 (dd, $J_{2,3}$ 3.0 and $J_{3,4}$ 8.9 Hz, H-3), 4.54 (bdd, H-2), 4.23 (m, H-6, H-6′), 3.97 (t, $J_{4,5}$ 8.9 Hz, H-4). ^{13}C NMR (125 MHz): δ 162.3, 144.0, 127.6, 118.4, 99.2, 79.9, 73.3, 69.6, 68.4, 65.6. Anal. Calcd for $C_{12}H_{13}NNa_2O_{14}S_2{\cdot}H_2O$: C, 27.54; H, 2.89; N, 2.68. Found C, 27.11; H, 3.10; N, 2.86. FABMS (positive-ion): 528 $[M+Na]^+$.

3.5. *p*NP 6-*O*-sulfo-α-D-mannopyranoside, sodium salt (7)

3.5.1. By chemical synthesis. A mixture of *p*NP α-D-mannopyranoside (**5**) (98 mg, 0.33 mmol) and SO_3NMe_3 (138 mg, 1.0 mmol) was dissolved in DMF at 40 °C. After 48 h, the reaction mixture was diluted with MeOH (5 mL) and concentrated in vacuo. The residue was then purified in the same way as described above to afford **7** (48 mg, 37%). $[\alpha]_D$ +109 (*c* 0.3, H_2O). ^1H NMR (500 MHz): δ 8.25 and 7.28 (d, *J* 9.2 Hz, *p*NP), 5.74 (bd, $J_{1,2}$ 1.5 Hz, H-1), 4.25–4.15 (m, H-2, H-6, H-6′), 4.06 (dd, $J_{2,3}$ 3.4 and $J_{3,4}$ 8.9 Hz, H-3), 3.82 (t, $J_{4,5}$ 10.1 Hz, H-4). Anal. Calcd for $C_{12}H_{14}NNaO_{11}S{\cdot}H_2O$: C, 34.21; H, 3.83; N, 3.33. Found C, 34.64; H, 3.82; N, 3.49. FABMS (positive-ion): 426 $[M+Na]^+$.

3.5.2. By enzymatic synthesis. A mixture of **6** (3.2 mg, 6.3 μmol) and abalone sulfatase (3 mg, 69 U) was dissolved in 0.25 M HOAc–NaOAc buffer (pH 6.8, 0.5 mL) at 37 °C for 14 days. The reaction mixture was purified in the same way as described above to give **7** (1.5 mg, 60%). The physical data were consistent with the procedure 3.5.1.

3.6. *p*NP 3-*O*-sulfo-α-D-mannopyranoside, sodium salt (8)

A mixture of *p*NP α-D-mannopyranoside (**5**) (70 mg, 0.23 mmol) and Bu_2SnO (69 mg, 0.28 mmol) was refluxed in THF–toluene (1/1(v/v), 500 mL) for 3 h with continuous azeotropic removal of water. After the evaporation of the solvents, the residue was treated with SO_3NMe_3 (53 mg, 0.38 mmol) in DMF at room temperature for 4 h 40 min. The reaction mixture was diluted with MeOH (10 mL) and concentrated in vacuo. The residue was then purified in the same way as described above to afford 3-mono-O-sulfonated **8** (51 mg, 54 %). $[\alpha]_D$ +99 (*c* 2.1, H_2O). ^1H NMR (400 MHz): δ 8.20 and 7.26 (d, *J* 9.2 Hz, *p*NP), 5.76 (bs, H-1), 4.72 (bdd, $J_{2,3}$ 3.2 and $J_{3,4}$ 9.6 Hz, H-3), 4.52 (bdd, H-2), 3.92 (t, $J_{4,5}$ 9.6 Hz, H-4), 3.83-3.65 (m, H-5, H-6, H-6′). ^{13}C NMR (100 MHz): δ 162.3, 143.8, 127.6, 118.2, 99.1, 80.2, 75.3, 69.6, 65.9, 62.1. FABMS (negative-ion): 401 $[M-2H]^-$.

Acknowledgements

The authors gratefully acknowledge Dr. Yukishige Ito, RIKEN (The Institute of Physical and Chemical Research) for his suggestions.

References

1. David, B. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3215–3237, and references cited therein.
2. Rosen, S. D.; Bistrup, A.; Hemmerich, S. Carbohydrate Sulfotransferases. In *Carbohydrates in Chemistry and Biology*; Ernst, B.; Hart, G. W.; Sinaÿ, P. Eds.; VCH-Verlag GmbH: Weinheim, Germany, 2000; Vol. 3, pp 245–260.
3. Cummings, R. D.; Lowe, J. B. Selectin. In *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory: Spring Harbor, NY, 1999; pp 391–415.
4. Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.- H. *Chem. Rev.* **1998**, *98*, 833–862.
5. Mougous, J. D.; Green, R. E.; Williams, S. J.; Brenner, S. E.; Bertozzi, C. R. *Chem. Biol.* **2002**, *9*, 767–776.
6. Desmarais, D. D.; Jablonski, P. E.; Fedarko, N. S.; Roberts, M. F. *J. Bacteriol.* **1997**, *179*, 3146–3153.
7. Freeze, H. H.; Wolgast, D. *J. Biol. Chem.* **1986**, *261*, 127–134.
8. Parthasarathy, N.; Gotow, L. F.; Bottoms, J. D.; Kute, T. E.; Wagner, W. D.; Mulloy, B. *J. Biol. Chem.* **1998**, *273*, 21111–21114.
9. Manning, D. D.; Hu, X.; Beck, P.; Kiessling, L. L. *J. Am. Chem. Soc.* **1997**, *119*, 3161–3162.
10. Gordon, E. J.; Sanders, W. J.; Kiessling, L. L. *Nature* **1998**, *392*, 30–31.
11. Bruning, J.; Kiessling, L. L. *Tetrahedron Lett.* **1996**, *37*, 2907–2910.
12. Roy, R.; Park, W. K. C.; Zanini, D.; Foxall, C.; Srivastava, O. M. *Carbohydr. Lett.* **1997**, *2*, 259–266.
13. Liu, C.-H.; Shen, G.-J.; Garcia-Junceda, E.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 8031–8032.
14. Burkart, M. D.; Izumi, M.; Chapman, E.; Lin, C.-H.; Wong, C.-H. *J. Org. Chem.* **2000**, *65*, 5565–5574.
15. Lubineau, A.; Auge, C.; Le Goff, N.; Le Narvor, C. *Carbohydr. Res.* **1998**, *305*, 501–509.
16. Uzawa, H.; Zeng, X.; Minoura, N. *Chem. Commun. (Cambridge)* **2003**, 100–101.
17. Uzawa, H.; Toba, T.; Nishida, Y.; Kobayashi, K.; Minoura, N.; Hiratani, K. *Chem. Commun. (Cambridge)* **1998**, 2311–2312.
18. Uzawa, H.; Nishida, Y.; Sasaki, K.; Minoura, N.; Kobayashi, K. *ChemBioChem.* **2003**, *4*, 640–647.
19. Van der Pal, R. H. M.; Klein, W.; van Golde, L. M. G.; Lopes-Cardozo, M. *Biochim. Biophys. Acta* **1990**, *1043*, 91–96.
20. Nishida, Y.; Uzawa, H.; Toba, T.; Sasaki, K.; Kondo, H.; Koboyashi, K. *Biomacromolecules* **2000**, *1*, 68–74.
21. Sasaki, K.; Nishida, Y.; Tsurumi, T.; Uzawa, H.; Kondo, H.; Kobayashi, K. *Angew. Chem. Int. Ed.* **2002**, *41*, 4463–4467.
22. Sasaki, K.; Nishida, Y.; Uzawa, H.; Kobayashi, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2821–2823.
23. Uzawa, H.; Kamiya, S.; Minoura, N.; Dohi, H.; Nishida, Y.; Taguchi, K.; Yokoyama, S.; Mori, H.; Shimizu, T.; Kobayashi, K. *Biomacromolecules* **2002**, *3*, 411–414.
24. Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555–578.