Convenient synthetic approach towards regioselectively sulfated sugars using limpet and abalone sulfatase-catalyzed desulfation

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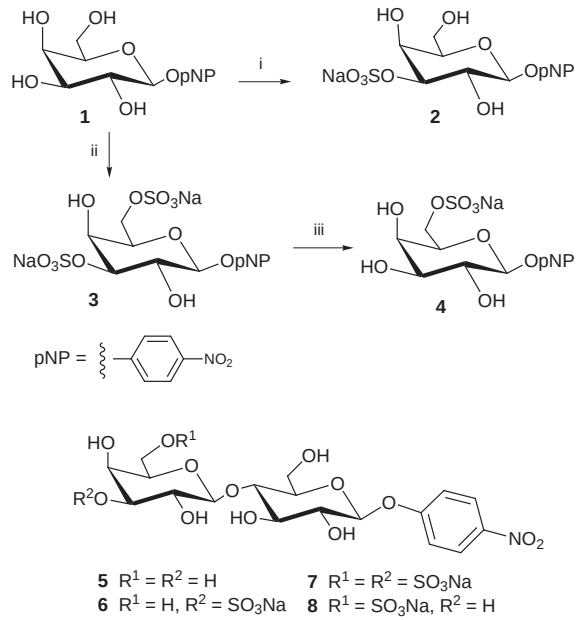
3,6-Disulfated *p*-nitrophenyl β-D-galactopyranoside 3 and 3′,6′-disulfated *p*-nitrophenyl β-lactoside 7, which were regioselectively obtained *via* organotin methodology, were subjected to enzymatic desulfation: limpet and abalone sulfatases (EC 3.1.6.1) hydrolyzed regioselectively the 3- and 3′-sulfate moieties of 3 and 7 to afford 6-monosulfated galactopyranoside 4 and 6′-monosulfated lactoside 8, respectively.

Various sulfated analogues of sialyl Le[a] and Le[x] have been prepared by different groups[1–9] as artificial ligands of the selectin families, which include artificial glycoconjugate polymers and dendrimers carrying sulfated carbohydrates.[10–13] As part of our project to develop biofunctional glycoconjugate polymers able to adsorb certain pathogenic bacteria and their toxins,[14,15] our interests have been directed to regioselectively sulfated sugars as substitutes for natural sialyl oligosaccharides and sulfatides known to bind with various viruses and pathogenic bacteria.[16] Here we report a new chemoenzymatic synthetic approach towards regioselectively sulfated *p*-nitrophenyl (pNP) β-D-galactopyranosides and lactosides.

Regioselective sulfations of carbohydrate molecules reported in recent years may be divided into the following types; chemical methods using stannylated sugars[17] and enzymatic approaches using sulfotransferase for non-sulfated sugar acceptors[18] or glycosyltransferases for sulfated acceptors.[19] Here we describe a convenient synthetic approach using *sulfatases*. Although various types of sulfatases are commercially available at low cost, their utility for chemoenzymatic syntheses has not yet been fully explored. Now we propose a new practical approach which involves introduction of plural sulfates into sugars *via* organotin methodology and then regioselective desulfation with sulfatases. pNP β-D-Galactopyranoside **1** and pNP β-lactoside **5** (Scheme 1) were employed as model compounds to demonstrate our approach. The pNP group is used due to its ready conversion to a *p*-*N*-acryloylaminophenyl group ready for subsequent polymerization.[20,21] Moreover, sulfated galactoses and lactoses are promising ligand candidates for influenza viruses and the other microbes.

The initial chemical approach to **1** and **5** was as follows. Reaction of **1** with Bu_2SnO (1 equiv.) and then SO_3NMe_3 (1 equiv.) gave 3-monosulfated galactoside **2** in 77% yield, while the use of $(Bu_3Sn)_2O$ (0.75 equiv.) and an excess of SO_3NMe_3 (5 equiv.) gave 3,6-disulfated derivative **3** in 97% yield.§ Thus, these chemical methods afforded 3-mono- and 3,6-di-sulfated galactosides in a regioselective manner, while not affording directly the 6-monosulfated galactose **4**. A similar process for **5** using Bu_2SnO (1 equiv.) and then SO_3NMe_3 (1 equiv.) gave selectively 3′-monosulfated pNP β-lactoside **6** (57% isolated yield).¶ Use of Bu_2SnO (3 equiv.) and SO_3NMe_3 (3 equiv.) afforded 3′,6′-disulfated lactoside **7** as the main product (40% yield).‖ Similar reaction of **5** using $(Bu_3Sn)_2O$ instead of Bu_2SnO gave a complex mixture of sulfated products.

The location of sulfate groups in sulfated lactosides **6** and **7** was determined by 2D NMR experiments (^{1}H-^{1}H COSY). The



Scheme 1 *Reagents and conditions*: i, Bu_2SnO (azeotropic), SO_3NMe_3, then Dowex Na^+; ii, $(Bu_3Sn)_2O$, (azeotropic), SO_3NMe_3, then Dowex Na^+, 97%; iii, sulfatase, 0.25 M NaOAc–AcOH buffer (pH 6.8), 37 °C, 80%.

signal of H-3′ of a galactose residue (δ 4.32–4.38, dd, *J* 3.0–3.3 and 9.5–9.8 Hz) of **6** and **7** showed downfield shift due to the sulfation at the geminal O-3′, while the H-3 of glucose (δ 3.796 and 3.805, br t, *J* 9.5–9.9 Hz) did not show the corresponding downfield shift. These results showed that the sulfate group was located at O-3′ in the galactosyl residues for both **6** and **7**.

3,6-Disulfated galactoside **3** and 3′,6′-disulfated lactoside **7** were subjected to enzyme-catalyzed desulfation with sulfatases (EC 3.1.6.1) (Table 1).** Three types of commercially available sulfatases were tested, and each of the desulfated products was analyzed by ^{1}H NMR spectroscopy. The enzyme reaction of **3** with snail sulfatase in NaOAc–AcOH buffer (pH 6.8, 37 °C) was completed in 2 days to afford a less polar product. The

Table 1 Regioselectivity of sulfatase-catalyzed desulfation

Substrates	Sulfatases origins[a]	*t*/days[b]	Conversion (%)	Product
3	snail	2	94[c]	**4**
3	abalone	2	> 95[d]	**4**
3	limpet	2	> 95[d]	**4**
7	snail	3	0	—
7	abalone	3	40[c]	**8**
7	limpet	3	> 95[d]	**8**

[a] Commercially available sulfatases from snail (*Helix pomatia*), abalone and limpet (*Patella vulgata*) were used. [b] Typical procedure is shown in footnote ††. [c] Isolated yield. [d] Determined by ^{1}H NMR analysis or TLC.

product was isolated in >90% yield and identified as 6-monosulfated galactoside **4**.†† The 6-sulfated structure could be determined by ^{1}H NMR analysis, *via* comparison of the spectral data with those of **3** and pNP β-D-galactopyranoside **1**. The downfield shift of H-3 (δ 4.484, dd, J = 3.3 and 9.6 Hz for **3**) due to the 3-sulfate could not be observed for **4**, while the downfield shift of the H-6 signals remained. The structure of **4** was further confirmed by an alternative synthesis *via* multiple protections and deprotections. A reference reaction of **3** without the enzyme gave no product. This eliminated the possibility of non-enzymatic hydrolysis. The other two sulfatases (abalone and limpet) showed similar reactivity and regioselectivity. This is the first regioselective desulfation by sulfatases of sulfated sugars bearing more than two sulfate groups in one molecule. 3′,6′-Disulfated lactose **7** showed characteristic behavior towards each of the three enzymes as shown in Table 1; this compound is not a substrate for the snail enzyme, but is an excellent substarte for the limpet enzyme and an acceptable one for the abalone enzyme. The desulfated product of both enzymes could be identified as the 6′-monosulfated lactoside **8**.‡‡ Thus, the sulfatases studied here showed a clear tendency to hydrolyze the 3-sulfate group of galactose and the 3′-sulfate of lactose. This activity can be ascribed to the ability of arylsulfatase A to catalyze desulfation for sulfatides.[22]

In conclusion, we have presented a convenient, regioselective, chemoenzymatic method of sulfating pNP-β-D-galactosides **2**–**4** and pNP-lactosides **6**–**8**. Synthesis and application of glycoconjugate polymers carrying each of these sulfated sugars are in progress and will be reported in due courses.

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§ *Synthesis* of **3**. A mixture of **1** (600 mg, 1.99 mmol) and $(Bu_3Sn)_2O$ (0.764 ml, 1.5 mmol) was refluxed in THF–benzene (1:1, 50 ml) for 3 h with continuous azeotropic removal of water. The reaction mixture was concentrated *in vacuo* and the stannylene acetal intermediate treated with SO_3NMe_3 (1.4 g, 0.01 mol) in DMF at 60 °C for 3 h. The reaction mixture was diluted with BnOH (8 ml) and concentrated *in vacuo*. The residue was then purified by sequential column chromatography with sephadex LH-20, ODS and anionic ion exchange resin (Dowex Na^+) to afford 980 mg of **3** (97%). *Selected data* for **3**: δ_H(300 MHz; D_2O) 5.33 (H-1, d, *J* 7.8), 4.48 (H-3, dd, *J* 3.3 and 9.6), 4.43 (H-4, d, *J* 3.3), 4.34–3.93 (m, H-6 and H-6′), 4.01 (H-2, dd, *J* 7.8 and 9.6).

¶ *Synthesis* of **6**. A procedure similar to that used for **1** was performed using **5** (79 mg, 0.17 mmol), Bu_2SnO (43 mg, 0.17 mmol) and SO_3NMe_3 (24 mg, 0.17 mmol) to give **6** (56 mg, 57%). *Selected data* for **6**: δ_H(600 MHz, D_2O) 5.283 (H-1, *J* 7.8), 4.596 (H-1′, *J* 8.1), 4.342 (H-3′, *J* 3.0 and 9.8), 4.297 (H-4′, *J* 3.0), 4.03–3.97 (br d, H-6 of glucose residue, *J* 11.0), 3.89–3.74 (m, H-6, H-6′ and H-6″ of glucose and galactose residues), 3.796 (br t, H-3, *J* 9.5).

‖ *Synthesis* of **7**. A procedure similar to that used for **1** was performed using **5** (79 mg, 0.17 mmol), Bu_2SnO (128 mg, 0.51 mmol) and SO_3NMe_3 (71 mg, 0.51 mmol) to give **7** (53 mg, 40%). *Selected data* for **7**: δ_H(600 MHz, D_2O) 5.313 (H-1, *J* 8.1), 4.629 (H-1′, *J* 7.7), 4.38–4.32 (H-3′, *J* 3.3 and 9.5 and H-4′, *J* 3.3), 4.27–4.20 (H-6 and H-6′ of galactose residue), 4.07–4.02 (br t, H-5′, *J* 6.2), 4.02–3.97 (br d, H-6 of glucose residue, *J* 12.0), 3.805 (br t, H-3, *J* 9.9), 3.90–3.77 (m, H-5).

** Enzymes available from Sigma Co. Ltd. were used without purification. The activities and sources were as followings; snail (*Helix pomatia*), 16.1 units mg^{-1}; abalone (not specified), 23 units mg^{-1}; limpet (*Patella vulgata*), 7.6 units mg^{-1}.

†† *Synthesis* of **4**. A mixture of **3** (80 mg) and sulfatase (from *Helix pomatia*, 5 mg) was dissolved in 0.25 M NaOAc–AcOH buffer (pH 6.8, 2 ml) at 37 °C for 2 days. The reaction mixture was purified by sequential column chromatography with Sephadex LH-20, ODS and anionic ion exchange resin (Dowex Na^+) to give 60 mg (94%) of **4**. *Selected data* for **4**: δ_H(300 MHz, D_2O) 5.17 (H-1, d, *J* 7.3), 4.29–4.17 (H-6 and H-6′, m), 4.07 (H-4, br d, *J* 3.0), 3.87 (H-2, dd, *J* 7.3 and 9.9), 3.82 (H-3, dd, *J* 3.0 and 9.9).

‡‡ *Selected data* for **8**: δ_H(600 MHz, D_2O) 5.304 (H-1, *J* 8.1), 4.506 (H-1′, *J* 7.8), 4.25–4.20 (m, H-6 and H-6′ of galactose residue), 4.03–3.96 [H-4′ (*J* 3.3), H-5′ and H-6 (glucose residue), *J* 12.0], 3.90–3.74 (m, H-5), 3.756 (br t, H-3, *J* 9.0), 3.73–3.66 (m, H-3′ and H-2).

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