Supplementary Material (ESI) for Chemical Communications

Surporting Information

Library assembly of mono-, di- and tri-*O*-sulfated β-D-xylopyranosides; Effect of *O*-sulfation on pyranose ring conformation

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Experimental

Sulfatases from snail (*Helix pomatia*, 14.2 units/mg), abalone entrails (not specified, 29 units/mg), limpet (*Patella vulgata*, 37 units /mg) and aerobacter aerogenes (2.5 units/mg) were purchased from Sigma and used without further purification. All other reagents were obtained from Aldrich or Sigma and used as received. Enzymatic reactions were monitored by HPLC-MS (SHIMADZU LC-MS 2010A) with a column of TSKgel Super-ODS (4.6 × 100 mm) eluted gradually with 5% → 100 % methanol in water containing 0.1% formic acid at a flow rate of 0.2 mL/min and with a UV detector monitored at 200 and 300 nm. Flash column chromatography was performed on Silica Gel 60 RP-18 (ODS-C18, E. Merck or Yamazen, Japan, 40-63μm). Optical rotations were measured with a JASCO DIP-1000 digital polarimeter at ambient temperature, using a 10-cm micro cell. ^{1}H- and ^{13}C-NMR spectra were recorded

on a JEOL LA-600 or a Varian INOVA 400 spectrometer for solutions in D_2O. Chemical shifts are given in ppm and referenced to *tert*-butyl alcohol (δ_H 1.23 in D_2O or δ_C 31.2 in D_2O) as an internal reference. All data are assumed to be first order with apparent doublet and triplet reported as d and t, respectively. Resonances that appear broad are designated br. FAB mass spectra (FAB-MS) were recorded using a JEOL DX 303 mass spectrometer with 3-nitrobenzyl alcohol as the matrix.

Synthesis of *p*NP 2,3,4-tri-*O*-sulfo-β-D-xylopyranoside 1 and *p*NP 2,4-di-*O*-sulfo-β-D-xylopyranoside 7

A mixture of *p*NP β-D-xylopyranoside (200 mg, 0.74 mmol) and SO_3-NMe_3 (436 mg, 3.1 mmol) was dissolved in DMF at 80°C. After 2 h, the reaction mixture was diluted with MeOH (5 ml) and concentrated *in vacuo*. The residue was purified on an ODS C-18 column and treated with ion-exchange resin (Dowex Na^+) to afford **1** (173 mg, 41%) and **7** (11 mg, 3%).

***p*NP 2,3,4-tri-*O*-sulfo-β-D-xylopyranoside 1**: $[\alpha]_D$ -64° (*c* 0.86, H_2O). ^{1}H-NMR (600MHz): δ 8.273 (d, aromatic, 9.1Hz), 7.312 (d, aromatic, 9.1Hz), 5.917 (br s, H1), 4.912 (br s, H3), 4.746 (H2, overlapped with DOH), 4.581 (br s, H4), 4.254 (br d, H5, 13.0Hz), 3.927 (br d, H5, 13.0Hz). ^{13}C-NMR (100MHz) : δ 162.4, 144.1, 127.7, 118.5, 96.8 (C-1), 72.2 (overlapped), 60.2. FAB-MS (negative): 553 $[M-Na-H]^-$.

***p*NP 2,4-di-*O*-sulfo-β-D-xylopyranoside 7**: $[\alpha]_D$ -106° (*c* 0.25, H_2O). ^{1}H-NMR (600MHz): δ 8.261 (d, aromatic, 9.5 Hz), 7.260 (d, aromatic, 9.5 Hz), 5.555 (d, H1, $J_{1,2}$ 5.8 Hz), 4.464 (dd, H2, $J_{1,2}$ 5.8 Hz, $J_{2,3}$ 7.6 Hz), 4.405 (ddd, H4, $J_{3,4}$ 7.4 Hz, $J_{4,5}$ 4.5 Hz, $J_{4,5'}$ 7.7 Hz), 4.325 (dd, H5, $J_{4,5}$ 4.5 Hz, $J_{5,5'}$ 12.2 Hz), 4.038 (dd, H3, $J_{2,3}$ 7.6 Hz, $J_{3,4}$ 7.4 Hz), 3.779 (dd, H5', $J_{4,5'}$ 7.7 Hz, $J_{5,5'}$ 12.2 Hz).

Synthesis of *p*NP 2,3-di-*O*-sulfo-β-D-xylopyranoside 2

A mixture of **1** (18.1 mg, 31.3 μmol) and *Helix pomatia* sulfatase (18 mg, 256 U) was dissolved in 0.25 M acetic acid-sodium acetate buffer (pH 6.8, 0.36 ml) at 37°C for 2 days. The reaction mixture was then purified by reverse phase preparative HPLC with a column of TSK-gel ODS-80Ts (21.5 mm I.D. × 30cm; TOSOH, Japan) eluted with methanol at a flow-rate of 5mL/min and treated with ion-exchange resin (Dowex Na^+) to afford **2** (4.0 mg, 25%). $[\alpha]_D$ -49° (*c* 0.08, H_2O). ^{1}H-NMR (600MHz): δ 8.269 (d, aromatic, 9.3Hz), 7.295 (d, aromatic, 9.3Hz), 5.835 (br d, H1, 2.0Hz), 4.686 (br t, H2), 4.638 (br t, H3), 4.174 (dd, H5, 2.5Hz, 12.5Hz), 3.982 (m, H4), 3.678 (br dd, H5, 3.4Hz, 12.5Hz). ^{13}C-NMR (100MHz) : δ 162.6, 144.1, 127.7, 118.5, 97.6 (C-1), 75.5, 73.7, 66.9, 62.6. FAB-MS (negative): 429 $[M-2Na]^-$. FAB-MS (positive): 497 $[M+Na-H]^+$.

Synthesis of *p*NP 3,4-di-*O*-sulfo-β-D-xylopyranoside 3, *p*NP 3-*O*-sulfo-β-D-xylopyranoside 5 and *p*NP 4-*O*-sulfo-β-D-xylopyranoside 6

A mixture of **1** (20.6 mg, 35.7 μmol) and abalone sulfatase (5 mg, 145 U) was dissolved in 0.25 M acetic acid-sodium acetate buffer (pH 6.8, 1.0 ml) at 37°C for 2 days. The reaction mixture was then purified by reverse phase HPLC with a column of TSK-gel ODS-80Ts (21.5 mm I.D. × 30cm; TOSOH, Japan) eluted with methanol at a flow-rate of 5mL/min and treated with ion-exchange resin (Dowex Na^+) to afford **3** (1.6 mg, 9%), **5** (0.3 mg, 7%) and **6** (1.8 mg, 18%).

***p*NP 3,4-di-*O*-sulfo-β-D-xylopyranoside 3**: $[\alpha]_D$ -84° (*c* 0.11, H_2O). ^{1}H-NMR (600MHz): δ 8.270 (d, aromatic, 9.4Hz), 7.273 (d, aromatic, 9.4Hz), 5.502 (d, H1, 5.3Hz), 4.605 (br t, H3, 6.7Hz), 4.511 (ddd, H4, 4.1Hz, 6.7Hz, 7.0Hz), 4.315 (dd, H5, 4.1Hz, 12.5Hz), 4.009 (br dd, H2, 5.3Hz, 6.7Hz), 3.822 (dd, H5, 7.0Hz, 12.5Hz). ^{13}C-NMR (150MHz): δ 162.9, 144.2, 127.7, 118.3, 100.0 (C-1), 78.7, 74.1, 71.1, 63.0. FAB-MS (negative): 451 $[M\text{-}Na\text{-}H]^-$.

***p*NP 3-*O*-sulfo-β-D-xylopyranoside 5**: $[\alpha]_D$ -31° (*c* 0.02, H_2O). ^{1}H-NMR (600MHz): δ 8.269 (d, aromatic, 9.5Hz), 7.255 (d, aromatic, 9.5Hz), 5.376 (d, H1, 7.0Hz), 4.391 (br t, H3, 8.4Hz), 4.101 (dd, H5, 5.5Hz, 11.7Hz), 3.934 (ddd, H4, 5.5Hz, 8.4Hz, 9.5Hz), 3.854 (dd, H2, 7.0Hz, 8.4Hz), 3.620 (dd, H5, 9.5Hz, 11.7Hz). FAB-MS (negative): 350 $[M\text{-}Na]^-$. FAB-MS (positive): 413 $[M+K]^+$.

***p*NP 4-*O*-sulfo-β-D-xylopyranoside 6:** $[\alpha]_D$ -84° (*c* 0.05, H_2O). ^{1}H-NMR (600MHz): δ 8.266 (d, aromatic, 9.3Hz), 7.249 (d, aromatic, 9.3Hz), 5.263 (d, H1, 7.4Hz), 4.361 – 4.288 (m, H4, H5), 3.773 (br t, H3, 8.2Hz, 9.4Hz), 3.718 (dd, H2, 7.4Hz, 9.4Hz), 3.740 – 3.676 (m, H5, overlapped). ^{13}C-NMR (150MHz): δ 163.1, 137.4, 127.7, 118.1, 101.4 (C-1), 77.3, 74.6, 73.9, 64.9. FAB-MS (negative): 372 $[M\text{- }H]^-$, 350 $[M\text{-}Na]^-$. FAB-MS (positive): 413 $[M+K]^+$, 395 $[M+Na\text{-}H]^+$.

Synthesis of *p*NP 2-*O*-sulfo-β-D-xylopyranoside 4

A mixture of **1** (10.0 mg, 17.3 μmol) and *Patella vulgata* sulfatase (2.5 mg, 92.5 U) was dissolved in 0.25 M acetic acid-sodium acetate buffer (pH 6.8, 1.0 ml) at 37°C for 2 days. The reaction mixture was then purified by reverse phase HPLC with a column of TSK-gel ODS-80Ts (21.5 mm I.D. × 30cm; TOSOH, Japan) eluted with methanol at a flow-rate of 5mL/min and treated with ion-exchange resin (Dowex Na^+) to afford **2** (1.0 mg, 11%) and **4** (1.7 mg, 27%).

***p*NP 2-*O*-sulfo-β-D-xylopyranoside 4**: $[\alpha]_D$ -59° (*c* 0.25, H_2O). ^{1}H-NMR (600MHz): δ 8.259 (d, aromatic, 9.2Hz), 7.245 (d, aromatic, 9.2Hz), 5.452 (d, H1, 6.9Hz), 4.368 (br dd, H2, 6.9Hz, 8.0Hz), 4.086 (dd, H5, 4.5Hz, 12.0Hz), 3.838 – 3.769 (m, H3, H4), 3.568 (dd, H5, 9.3Hz, 12.0Hz). FAB-MS (negative): 372 $[M\text{- }H]^-$, 350 $[M\text{-}Na]^-$. FAB-MS (positive): 395 $[M+Na\text{-}H]^+$.

Time courses of the enzymatic reactions by HPLC-MS analyses

The reaction mixture was monitored by HPLC-MS. Samples (1 μL) were taken out from reaction mixture at intervals (0, 6, 10, 30, 45, 61, 70, 78 and 104 hr) during the incubation, and were heated over water bath at 95^{o}C for 5 min to stop each enzyme reaction. Every sample was then analyzed by HPLC-MS. Abalone (0.22 mg), snail (1.0 mg) and limpet (0.34 mg) sulfatases with **1** (1 mg) were used in this study.