

## Supplementary information

### Modified minimal-size fragments of Heparan Sulfate as inhibitors of endosulfatase-2 (Sulf-2)

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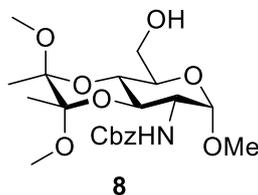
## General experimental

All reactions involving organometallic or other moisture-sensitive reagents were carried out under a nitrogen or argon atmosphere using standard vacuum line techniques and glassware that was flame dried and cooled under nitrogen before use. Solvents were dried over a MBRAUN MB SPS-800 solvent purification system. Water was purified by an Elix® UV-10 system. All other reagents were used as supplied (analytical or HPLC grade) without prior purification. Organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>. Thin layer chromatography was performed on aluminium plates coated with 60 F254 silica. Plates were visualised using UV light (254 nm), 1% aq KMnO<sub>4</sub>, 10% ethanolic phosphomolybdic acid or 10% ethanolic *p*-anisaldehyde. Flash column chromatography was performed either on Kieselgel 60 silica on a glass column, or on a Biotage Isolera Four automated flash column chromatography platform. Melting points were recorded on SRS MPA120 EZ-Melt Melting Point Apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a water-jacketed 10 cm cell. Specific rotations are reported in 10<sup>-1</sup> deg cm<sup>2</sup> g<sup>-1</sup> and concentrations in g/100 mL. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer as a thin film. Selected characteristic peaks are reported in cm<sup>-1</sup>. NMR spectra were recorded on Bruker Avance spectrometers in the deuterated solvent stated. Spectra were recorded at rt unless otherwise stated. The field was locked by external referencing to the relevant deuterium resonance. Low-resolution mass spectra were recorded on either an Agilent 6120 Single Quadrupole or a Waters LCT Premier spectrometer. Accurate mass measurements were run on either a Bruker MicroTOF internally calibrated with polyalanine.

Inhibitors **1**, **1β** and **2** were prepared following reported protocols and characterisation data were consistent with the literature.<sup>1,2</sup>

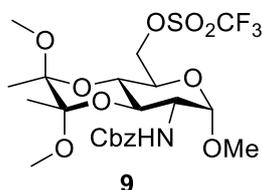
## Compound preparations

### Benzyl ((2*S*,3*S*,4*aS*,5*R*,7*S*,8*R*,8*aR*)-5-(hydroxymethyl)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-*b*][1,4]dioxin-8-yl)carbamate (**8**)



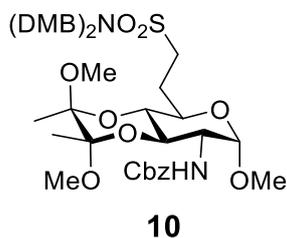
**7**<sup>2</sup> (1.75 g, 5.35 mmol), (1*S*)-(+)-10-camphorsulfonic acid (190 mg, 0.535 mmol), trimethyl orthoformate (1.75 mL, 16.05 mmol) and 2,3-butanedione (516  $\mu$ L, 5.89 mmol) were suspended in CH<sub>3</sub>OH (20 mL) and the resulting solution was heated at 75 °C for 72 h. The solvent was removed *in vacuo* and the residue was partitioned between EtOAc (20 mL) and satd aq NaHCO<sub>3</sub> (20 mL). The organic layer was washed with brine (10 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification via column chromatography (eluent pentanes/EtOAc 1:1) gave **8** as a white solid (1.95 g, 83%); mp 65–68 °C;  $[\alpha]_{\text{D}}^{25} + 194$  (c 0.2 in CH<sub>3</sub>OH);  $\nu_{\text{max}}$  (film) 2948 br (O–H), 2160 (C–H), 2034 (C–H), 1977 (C–H), 1707 (C=O), 1512 (C=O);  $\delta_{\text{H}}$  (400 MHz, CDCl<sub>3</sub>) 1.27 (s, 3H, C(11)H<sub>3</sub> or C(12)H<sub>3</sub>), 1.27 (s, 3H, C(11)H<sub>3</sub> or C(12)H<sub>3</sub>), 3.19 (s, 3H, C(9)H<sub>3</sub> or C(10)H<sub>3</sub>), 3.25 (s, 3H, C(9)H<sub>3</sub> or C(10)H<sub>3</sub>), 3.34 (s, 3H, C(8)H<sub>3</sub>), 3.69 – 3.78 (m, 3H, C(4)H, C(5)H, C(6)H<sub>A</sub>), 3.79 – 3.88 (m, 2H, C(3)H, C(6)H<sub>B</sub>), 3.89 – 3.99 (m, 1H, C(2)H), 4.79 (d, *J* = 3.0 Hz, 1H, C(1)H), 4.87 (d, *J* = 8.5 Hz, 1H, NH), 5.03 – 5.21 (m, 2H, C(7)H<sub>2</sub>), 7.28 – 7.45 (m, 5H, *Ph*);  $\delta_{\text{C}}$  (101 MHz, CDCl<sub>3</sub>) 17.8, 17.9 (C(11), C(12)), 48.0 (C(9), C(10)), 53.0 (C(2)), 55.3 (C(8)), 61.5 (C(6)), 66.9 (C(4)), 67.0 (C(7)), 68.0 (C(3)), 69.9 (C(5)), 99.0 (C(1)), 99.8, 100.0 (C(13), C(14)), 128.3, 128.6 (*o,m,p-Ph*), 136.6 (*i-Ph*), 156.2 (C=O); *m/z* (ESI<sup>+</sup>) 464 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>21</sub>H<sub>31</sub>NNaO<sub>9</sub><sup>+</sup> ([M+Na]<sup>+</sup>) requires 464.1891; found 464.1887.

**((2S,3S,4aS,5R,7S,8R,8aR)-8-(((Benzyloxy)carbonyl)amino)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5H-pyrano[3,4-b][1,4]dioxin-5-yl)methyl trifluoromethanesulfonate (9)**



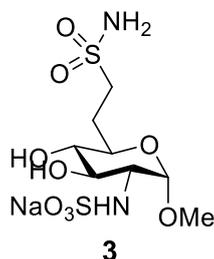
Trifluoromethanesulfonic anhydride (46  $\mu$ L, 0.272 mmol) was added to a solution of **8** (100 mg, 0.227 mmol) and *N,N*-diisopropylethylamine (47  $\mu$ L, 0.272 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) at 0 °C. The resulting solution was stirred at 0 °C for 1 h. The reaction was retreated with *N,N*-diisopropylethylamine (47  $\mu$ L, 0.272 mmol) and trifluoromethanesulfonic anhydride (46  $\mu$ L, 0.272 mmol) and the reaction was stirred at rt for an additional 1 h. The reaction was quenched with H<sub>2</sub>O (1 mL) and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3  $\times$  3 mL). The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0% $\rightarrow$ 20% EtOAc in pentanes) gave **9** as a pale yellow solid (75 mg, 58%); mp 58–61 °C;  $[\alpha]_D^{25}$  +82.7 (c 0.03 in CH<sub>3</sub>OH);  $\nu_{\max}$  (film) 1717 (C=O), 1514 (C=O), 1515 (S=O), 1246 (S=O);  $\delta_H$  (400 MHz, CDCl<sub>3</sub>) 1.27 (s, 3H, C(11)H<sub>3</sub> or C(12)H<sub>3</sub>), 1.28 (s, 3H, C(11)H<sub>3</sub> or C(12)H<sub>3</sub>), 3.19 (s, 3H, C(9)H<sub>3</sub> or C(10)H<sub>3</sub>), 3.22 (s, 3H, C(9)H<sub>3</sub> or C(10)H<sub>3</sub>), 3.36 (s, 3H, C(8)H<sub>3</sub>), 3.69 (t, *J* = 9.8 Hz, 1H, C(4)H), 3.83 (dd, *J* = 10.8, 9.5 Hz, 1H, C(3)H), 3.98 (ddd, *J* = 10.1, 5.3, 1.9 Hz, 2H, C(2)H, C(5)H), 4.60 (dd, *J* = 10.7, 5.4 Hz, 1H, C(6)H<sub>A</sub>), 4.65 – 4.75 (m, 1H, C(6)H<sub>B</sub>), 4.84 (d, *J* = 8.9 Hz, 1H, NH), 5.02 – 5.20 (m, 2H, C(7)H<sub>2</sub>), 7.28 – 7.45 (m, 5H, *Ph*);  $\delta_C$  (126 MHz, CDCl<sub>3</sub>) 17.5, 17.7 (C(11), C(12)), 48.0, 48.1, (C(9), C(10)), 52.46 (C(3)), 55.48 (C(8)), 66.32 (C(4)), 66.99 (C(7)), 67.34 (C(5)), 67.81 (C(3)), 73.83 (C(6)), 98.95 (C(1)), 99.96, 100.05 (C(13), C(14)), 118.61 (q, *J* = 319.5 Hz, CF<sub>3</sub>), 128.26, 128.51 (*o,m,p-Ph*), 136.38 (*i-Ph*), 155.88 (CO);  $\delta_F$  (376 MHz, CDCl<sub>3</sub>) –74.53; *m/z* (ESI<sup>+</sup>) 596 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>22</sub>H<sub>30</sub>F<sub>3</sub>NNaO<sub>11</sub>S<sup>+</sup> ([M+Na]<sup>+</sup>) requires 596.1384; found 596.1386.

**Benzyl ((2S,3S,4aR,5R,7S,8R,8aR)-5-(2-(N,N-bis(2,4-dimethoxybenzyl)sulfamoyl)ethyl)-2,3,7-trimethoxy-2,3 dimethylhexa-hydro-5H-pyrano[3,4-b][1,4]dioxin-8-yl)carbamate (10)**



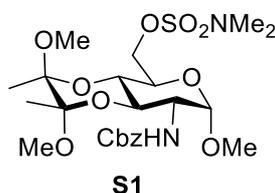
*n*-BuLi (1.6 M in hexanes, 445  $\mu$ L, 0.712 mmol) was added dropwise to a solution of CH<sub>3</sub>SO<sub>2</sub>(DMB)<sub>2</sub> (268 mg, 0.678 mmol) in THF (3 mL) at  $-78$  °C. The resulting solution was stirred for 15 min before a solution of **9** (194 mg, 0.339 mmol) in THF (2 mL) was added dropwise and the reaction was stirred for 5 h; the temperature was maintained at  $-78$  °C throughout. The reaction was quenched with H<sub>2</sub>O (5 mL) and extracted with EtOAc (3  $\times$  5 mL). The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification via column chromatography (eluent EtOAc/pentanes 4:6) gave **10** as a white solid (180 mg, 65%); mp 85–91 °C;  $[\alpha]_D^{25} +113$  (*c* 0.1 in CH<sub>3</sub>OH);  $\nu_{\max}$  (film) 2927 (C–H), 2836 (C–H), 1721, 1613 (C=O), 1588, 1507 (C=O), 1456, 1328, 1293, 1261, 1208 (S=O), 1115, 1032 (S=O);  $\delta_H$  (500 MHz, CDCl<sub>3</sub>) 1.25 (s, 6H, C(13)H<sub>3</sub>, C(14)H<sub>3</sub>), 1.73 – 1.84 (m, 1H, C(6)H<sub>A</sub>), 2.24 – 2.34 (m, 1H, C(6)H<sub>B</sub>), 2.73 – 2.83 (m, 1H, C(7)H<sub>A</sub>), 2.95 – 3.05 (m, 1H, C(7)H<sub>B</sub>), 3.16 (s, 3H, C(15)H<sub>3</sub> or C(16)H<sub>3</sub>), 3.21 (s, 6H, C(9)H<sub>3</sub>, C(15)H<sub>3</sub> or C(16)H<sub>3</sub>), 3.31 – 3.42 (m, 1H, C(4)H), 3.55 (t, *J* = 8.7 Hz, 1H, C(5)H), 3.71 – 3.78 (m, 7H, C(3)H, 2  $\times$  C(11)H<sub>3</sub>), 3.80 (s, 6H, 2  $\times$  C(12)H<sub>3</sub>), 3.84 – 3.94 (m, 1H, C(2)H), 4.33 – 4.42 (m, 4H, 2  $\times$  C(10)H<sub>2</sub>), 4.66 (d, *J* = 2.8 Hz, 1H, C(1)H), 4.83 (d, *J* = 9.0 Hz, 1H, NH), 5.03 – 5.21 (m, 2H, C(8)H<sub>2</sub>), 6.37 – 6.42 (m, 2H, 2  $\times$  C(19)H), 6.44 (dd, *J* = 8.3, 2.3 Hz, 2H, 2  $\times$  C(21)H), 7.22 (d, *J* = 8.3 Hz, 2H, 2  $\times$  C(20)H), 7.28 – 7.42 (m, 5H, *Ph*);  $\delta_C$  (126 MHz, CDCl<sub>3</sub>) 17.6, 17.7 (C(13), C(14)), 24.9 (C(6)), 45.5 (C(10)), 47.8, 47.9 (C(15), C(16)), 49.5 (C(13)), 52.8 (C(2)), 54.9 (C(9)), 55.1, 55.4 (2  $\times$  C(11), 2  $\times$  C(12)), 66.9 (C(8)), 67.8 (C(5)), 67.9 (C(3)), 70.4 (C(4)), 98.3 (2  $\times$  C(19)), 98.5 (C(1)), 99.7, 99.8 (C(17), C(18)), 103.9 (2  $\times$  C(21)), 117.1 (2  $\times$  PMB-*i-Ph*), 128.2, 128.5 (*o,m,p-Ph*), 131.0 (2  $\times$  C(20)), 136.5 (*i-Ph*), 156.0 (CO), 158.4 (2  $\times$  PMB *m-Ph*), 160.5 (2  $\times$  PMB *o-Ph*); *m/z* (ESI<sup>+</sup>) 841 (100%, [M+Na]<sup>+</sup>); HRMS (ESI<sup>+</sup>) C<sub>40</sub>H<sub>54</sub>N<sub>2</sub>NaO<sub>14</sub>S<sup>+</sup> ([M+Na]<sup>+</sup>) requires 841.3188; found 841.3187.

**((2S,3R,4R,5S,6R)-4,5-Dihydroxy-2-methoxy-6-(2-sulfamoyl)tetrahydro-2H-pyran-3-yl)sulfamic acid (3)**



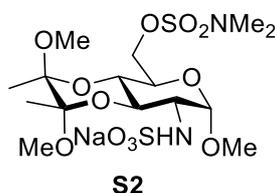
**(1) Cbz-deprotection.** Pd/C (10% wt, 8 mg) was added to a solution of **9** (170 mg, 0.210 mmol) in CH<sub>3</sub>OH (3 mL) under Ar. The reaction was evacuated and filled with Ar three times before being placed under an atmosphere of H<sub>2</sub>. The reaction was stirred at rt for 16 h, filtered through Celite and concentrated *in vacuo*. **(2) N-sulfation.** Pyridine-sulfur trioxide complex (17.0 mg, 107 μmol) was added portion-wise to a solution of the above amine in H<sub>2</sub>O (1 mL) at pH 9–10. The pH was re-adjusted after each addition by addition of 1 M aq NaOH. The reaction was stirred at rt for 16 h. The solvent was removed *in vacuo*, and the residue was triturated in chloroform, filtered and the filtrate was concentrated *in vacuo*. **(3) Bis-acetal hydrolysis.** F<sub>3</sub>CCO<sub>2</sub>H (1 mL) was added to the intermediate bisacetal in H<sub>2</sub>O (1 mL) and the resulting solution was stirred at rt for 2 h. The solvent was removed *in vacuo* and purification via column chromatography (eluent CH<sub>3</sub>OH:CH<sub>2</sub>Cl<sub>2</sub>:NH<sub>4</sub>OH 3:7:0.01), followed by elution from a Dowex® 50WX8 Na<sup>+</sup>-form column using water as the eluent gave **3** as a white gum (5 mg, 7% over 3 steps) [ $\alpha$ ]<sub>D</sub><sup>25</sup> +12.5 (c 0.4 in H<sub>2</sub>O);  $\delta$ <sub>H</sub> (500 MHz, D<sub>2</sub>O) 1.85 – 1.94 (m, 1H, C(6)H<sub>A</sub>), 2.26 – 2.34 (m, 1H, C(6)H<sub>B</sub>), 3.17 (dd, *J* = 10.4, 3.7 Hz, 1H, C(2)H), 3.20 – 3.27 (m, 1H, C(4)H), 3.28 – 3.41 (m, 5H, C(7)H<sub>2</sub>, C(8)H<sub>3</sub>), 3.46 (dd, *J* = 10.3, 9.1 Hz, 1H, C(3)H), 3.64 (td, *J* = 9.6, 2.7 Hz, 1H, C(5)H), 4.92 (d, *J* = 3.6 Hz, 1H, C(1)H);  $\delta$ <sub>C</sub> (126 MHz, D<sub>2</sub>O) 25.38 (C(6)), 50.6 (C(7)), 55.4 (C(8)), 57.8 (C(2)), 69.0 (C(5)), 71.2 (C(3)), 73.5 (C(4)), 98.5 (C(1)); *m/z* (ESI<sup>-</sup>) 349 (100%, [M-H]<sup>-</sup>); HRMS (ESI<sup>-</sup>) C<sub>8</sub>H<sub>17</sub>N<sub>2</sub>O<sub>9</sub>S<sub>2</sub><sup>-</sup> ([M-NH<sub>4</sub>]<sup>-</sup>) requires 349.0381; found 349.0384.

**((2S,3S,4aS,5R,7S,8R,8aR)-8-(((benzyloxy)carbonyl)amino)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5H-pyrano[3,4-b][1,4]dioxin-5-yl)methyl dimethylsulfamate (S1)**



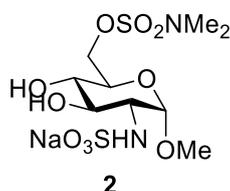
**7** (500 mg, 1.13 mmol) in THF (5 mL) was added to NaH (60% wt in mineral oil, 100 mg, 2.49 mmol) under N<sub>2</sub> at 0 °C and the resulting suspension was stirred at rt for 1 h. *N,N'*-Dimethylsulfamoyl chloride (146 μL, 1.36 mmol) was added and the resulting solution was stirred at rt for 16 h. The reaction was retreated with NaH (60% in mineral oil, 100 mg, 2.49 mmol) and *N,N'*-dimethylsulfamoyl chloride (146 μL, 1.36 mmol) and the resulting solution was stirred at rt for 16 h. The reaction was quenched with satd aq NaHCO<sub>3</sub> (5 mL) and extracted with EtOAc (2 × 5 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→55% EtOAc in pentanes) gave **S1** as a white solid (351 mg, 57%); mp 63–73 °C;  $[\alpha]_D^{25} + 180$  (c 0.1 in CH<sub>3</sub>OH);  $\nu_{\max}$  (film) 2951 (C–H), 2181 (C–H), 1716 (C=O), 1512 (C=O), 1455 (S=O), 1358 (S=O);  $\delta_H$  (500 MHz, (CD<sub>3</sub>)<sub>2</sub>CO) 1.22, 1.25 (2 × s, 2 × 3H, C(12)H<sub>3</sub>, C(13)H<sub>3</sub>), 2.91 (s, 6H, 2 × C(9)H<sub>3</sub>), 3.22, 3.25 (2 × s, 2 × 3H, C(10)H<sub>3</sub>, C(11)H<sub>3</sub>), 3.36 (s, 3H, C(8)H<sub>3</sub>), 3.64 (t, *J* = 9.4 Hz, 1H, C(4)H), 3.83 – 3.97 (m, 3H, C(2)H, C(3)H, C(5)H), 4.27 (dd, *J* = 10.9, 5.4 Hz, 1H, C(6)H<sub>A</sub>), 4.39 (dd, *J* = 10.9, 2.0 Hz, 1H, C(6)H<sub>B</sub>), 4.75 (d, *J* = 3.0 Hz, 1H, C(1)H), 4.99 – 5.15 (dd, *J* = 34.5, 12.6 Hz, 2H, C(7)H<sub>2</sub>), 6.25 (d, *J* = 8.5 Hz, 1H, NH), 7.27 – 7.45 (m, 5H, *Ph*);  $\delta_C$  (126 MHz, (CD<sub>3</sub>)<sub>2</sub>CO) 18.0, 18.1 (C(12), C(13)), 38.7 (C(9)), 48.2, 48.3 (C(10), C(11)), 53.9 (C(2)), 55.6 (C(8)), 66.6 (C(7)), 67.7 (C(4)), 68.3 (C(3)), 68.7 (C(6)), 68.8 (C(5)), 100.1 (C(1)), 100.5, 100.6 (C(14), C(15)), 128.55, 128.60, 129.18 (*o,m,p-Ph*), 138.35 (*i-Ph*), 157.07 (CO); *m/z* (ESI<sup>+</sup>) 571 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>23</sub>H<sub>36</sub>N<sub>2</sub>NaO<sub>11</sub>S<sup>+</sup> ([M+Na]<sup>+</sup>) requires 571.1932; found 571.1934.

**Sodium((2*S*,3*S*,4*aS*,5*R*,7*S*,8*R*,8*aR*)-5-(((*N,N*-dimethylsulfamoyl)oxy)methyl)-2,3,7-trimethoxy-2,3-dimethylhexahydro-5*H*-pyrano[3,4-*b*][1,4]dioxin-8-yl)sulfamate (**S2**)**



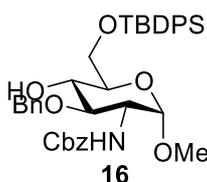
**(1) Cbz-deprotection.** Pd/C (10% wt, 35 mg) was added to a solution of **S1** (350 mg, 0.638 mmol) in CH<sub>3</sub>OH (4 mL) under Ar. The reaction was evacuated and filled with Ar three times before being placed under an atmosphere of H<sub>2</sub>. The reaction was stirred at rt for 16 h, filtered through Celite and concentrated *in vacuo* to give the intermediate amine which was used without further purification (233 mg, 88%). **(2) N-sulfation.** Pyridine-sulfur trioxide complex (42.0 mg, 0.266 mmol) was added portion-wise to a solution of the abovementioned amine (100 mg, 0.241 mmol) in H<sub>2</sub>O (1 mL) at pH 9–10. The pH was re-adjusted after each addition by addition of 1 M aq NaOH. The reaction was stirred at rt for 16 h. The solvent was removed *in vacuo* and the residue was triturated in CH<sub>3</sub>OH, filtered and the filtrate was concentrated *in vacuo* to give **S2** as a white gum (118 mg, 96%);  $[\alpha]_{\text{D}}^{25} + 9$  (c 0.1 in CH<sub>3</sub>OH);  $\delta_{\text{H}}$  (500 MHz, D<sub>2</sub>O) 1.35, 1.36 (2 × s, 2 × 3H, C(11)H<sub>3</sub>, C(12)H<sub>3</sub>), 2.95 (s, 6H, 2 × C(8)H<sub>3</sub>), 3.30, 3.33 (2 × s, 2 × 3H, C(9)H<sub>3</sub>, C(10)H<sub>3</sub>), 3.41 – 3.48 (m, 4H, C(2)H, C(7)H<sub>3</sub>), 3.74 (t, *J* = 9.9 Hz, 1H, C(4)H), 3.85 (t, *J* = 10.3 Hz, 1H, C(3)H), 4.02 (dt, *J* = 10.0, 3.0 Hz, 1H, C(5)H), 4.47 (d, *J* = 2.7 Hz, 2H, C(6)H<sub>2</sub>), 5.14 (d, *J* = 3.5 Hz, 1H, C(1)H);  $\delta_{\text{C}}$  (126 MHz, D<sub>2</sub>O) 16.75, 16.80, (C(11), C(12)), 37.88(C(8)), 47.90, 48.10 (C(9), C(10)), 54.93 (C(2)), 55.71 (C(7)), 66.43 (C(4)), 67.18, 67.19 (C(3), C(5)), 68.08 (C(6)), 98.87 (C(1)), 100.21, 100.53 (C(13), C(14)); *m/z* (ESI<sup>+</sup>) 539 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>15</sub>H<sub>29</sub>N<sub>2</sub>Na<sub>2</sub>O<sub>12</sub>S<sub>2</sub><sup>+</sup> ([M+Na]<sup>+</sup>) requires 539.0976; found 539.0953. Carbon NMR contains TFA as an impurity.

**Ammonium ((2*S*,3*R*,4*R*,5*S*,6*R*)-6-(((*N,N*-dimethylsulfonyl)oxy)methyl)-4,5-dihydroxy-2-methoxytetrahydro-2*H*-pyran-3-yl)sulfamate (2)**



F<sub>3</sub>CCO<sub>2</sub>H (0.8 mL) was added to a solution of **2** (118 mg, 0.23 mmol) in H<sub>2</sub>O (0.8 mL) and the resulting solution was stirred at rt for 2 h. The solvent was removed *in vacuo* and purification via column chromatography (eluent CH<sub>3</sub>OH:CH<sub>2</sub>Cl<sub>2</sub>:NH<sub>4</sub>OH 3:7:0.01) gave **2** as a white solid (10 mg, 11%); mp 95–109 °C;  $[\alpha]_D^{25} + 108$  (c 0.1 in CH<sub>3</sub>OH);  $\delta_H$  (500 MHz, D<sub>2</sub>O) 2.86 (s, 6H, 2 × C(8)H<sub>3</sub>), 3.18 (dd, *J* = 10.2, 3.6 Hz, 1H, C(2)H), 3.35 (s, 3H, C(7)H<sub>3</sub>), 3.41 – 3.48 (m, 1H, C(4)H), 3.48 – 3.56 (m, 1H, C(3)H), 3.81 (ddd, *J* = 10.0, 4.8, 2.1 Hz, 1H, C(5)H), 4.37 (dd, *J* = 11.3, 4.9 Hz, 1H, C(6)H<sub>A</sub>), 4.41 (dd, *J* = 11.2, 2.1 Hz, 1H, C(6)H<sub>B</sub>), 4.96 (d, *J* = 3.6 Hz, 1H, C(1)H);  $\delta_C$  (126 MHz, D<sub>2</sub>O) 37.9 (C(8)), 55.5 (C(7)), 57.5 (C(2)), 69.2 (C(5)), 69.4 (C(6)), 69.5 (C(4)), 71.3 (C(3)), 98.6 (C(1)); *m/z* (ESI<sup>+</sup>) 781 (7%), 759 ([2M-H]<sup>+</sup>, 18%) 379 ([M-H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>9</sub>H<sub>19</sub>N<sub>2</sub>O<sub>10</sub>S<sub>2</sub><sup>+</sup> ([M-Na]<sup>+</sup>) requires 379.0487; found 379.0483.

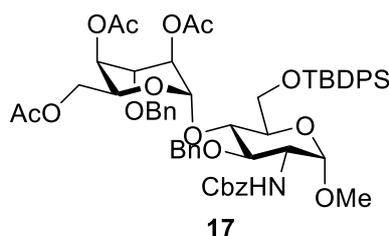
**Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4-(benzyloxy)-6-(((*tert*-butyldiphenylsilyl)oxy)methyl)-5-hydroxy-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (16)**



TBDPSCI (1.17 mL, 4.51 mmol) was added to a solution of **15** (synthesised according to literature procedures)<sup>3,4</sup> (1.34 g, 3.22 mmol), DMAP (78 mg, 0.639 mmol) and Et<sub>3</sub>N (2.80 mL, 20.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (16 mL) and the reaction was stirred at rt for 16 h. The reaction was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with H<sub>2</sub>O (50 mL). The organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→20% EtOAc in pentane) gave **16** as a colourless viscous oil (1.82 g, 86%);  $[\alpha]_D^{25} + 32.3$  (c 1.0 in CHCl<sub>3</sub>);  $\nu_{\max}$  (film) 3439 (O-H), 2930 (C-H), 2897 (C-H), 2855 (C-H), 2837 (C-H), 1707 (C=O);  $\delta_H$  (400 MHz, CDCl<sub>3</sub>) 1.07 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 3.31 (s, 3H, C(7)H<sub>3</sub>), 3.53 (dd, *J* =

10.4, 8.7 Hz, 1H, C(3)*H*), 3.62 (dt, *J* = 9.4, 4.4 Hz, 1H, C(5)*H*), 3.76 (t, *J* = 9.2 Hz, 1H, C(4)*H*), 3.88 (d, *J* = 4.4 Hz, 2H, C(6)*H*<sub>2</sub>), 3.96 (td, *J* = 10.1, 3.4 Hz, 1H, C(2)*H*), 4.67 (d, *J* = 3.6 Hz, 1H, C(1)*H*), 4.73 (d, *J* = 2.0 Hz, 2H, C(9)*H*<sub>2</sub>), 4.92 (d, *J* = 9.9 Hz, 1H, *NH*), 5.08 (d, *J* = 12.2 Hz, 1H, C(10)*H*<sub>A</sub>), 5.15 (d, *J* = 12.2 Hz, 1H, C(10)*H*<sub>B</sub>), 7.27 – 7.48 (m, 16H, *Ph*), 7.70 (ddt, *J* = 5.9, 4.2, 1.7 Hz, 4H, *Ph*);  $\delta_c$  (101 MHz, CDCl<sub>3</sub>) 19.4 (C(CH<sub>3</sub>)<sub>3</sub>), 27.0 (C(CH<sub>3</sub>)<sub>3</sub>), 54.4 (C(2)), 55.1 (C(7)), 64.6 (C(6)), 67.1 (C(9)), 71.2 (C(5)), 72.2 (C(4)), 74.5 (C(10)), 80.8 (C(3)), 99.1 (C(1)), 127.9 (*Ph*), 127.9 (*Ph*), 128.1 (*Ph*), 128.3 (*Ph*), 128.4 (*Ph*), 128.6 (*Ph*), 128.7 (*Ph*), 129.9 (*Ph*), 129.9 (*Ph*), 133.2 (*Ph*), 133.3 (*Ph*), 135.8 (*Ph*), 135.8 (*Ph*), 136.6 (*Ph*), 138.6 (*Ph*), 156.1 (C=O); *m/z* (ESI<sup>+</sup>) 678 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>38</sub>H<sub>45</sub>NNaO<sub>7</sub>Si<sup>+</sup> ([M+Na]<sup>+</sup>) requires 678.2858; found 678.2854.

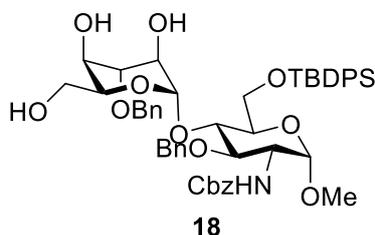
**(2*S*,3*R*,4*S*,5*R*,6*S*)-2-(acetoxymethyl)-4-(benzyloxy)-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-(benzyloxy)-5-(((benzyloxy)carbonyl)amino)-2-(((tert-butyl)dimethylsilyloxy)methyl)-6-methoxytetrahydro-2*H*-pyran-3-yl)oxy)tetrahydro-2*H*-pyran-3,5-diyl diacetate (**17**)**



**16** (2.35 g, 3.58 mmol) and **12** (synthesised according to literature procedures)<sup>5</sup> (1.50 g, 2.98 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (60 mL), under N<sub>2</sub>, and dried over 4 Å molecular sieves (2.0 g, freshly activated) for 1 h at rt. The reaction was cooled to –60 °C and NIS (873 mg, 3.88 mmol) and TfOH (1 M in Et<sub>2</sub>O, 895  $\mu$ L, 0.895 mmol) were added in quick succession. The reaction was allowed to warm to –20 °C over 3 h. The reaction was quenched by addition of NaHCO<sub>3</sub> (30 mL) and filtered through Celite<sup>®</sup>. The organic layer was washed with satd aq Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (30 mL), brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. Purification via column chromatography (gradient elution, 10%→30% EtOAc in pentane) gave **17** as a white solid (2.60 g, 84%); mp 64–66 °C;  $[\alpha]_D^{25} +13.7$  (c 1.0 in CHCl<sub>3</sub>);  $\nu_{max}$  (film) 2933 (C–H), 2900 (C–H), 2858 (C–H), 1739 (C=O);  $\delta_H$  (400 MHz, CDCl<sub>3</sub>) 1.07 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.93 (s, 3H, Ac), 1.94 (s, 3H, Ac), 2.04 (s, 3H, Ac), 3.25 (s, 3H, C(7)*H*<sub>3</sub>), 3.51 (dd, *J* = 10.3, 9.2 Hz, 1H, C(3)*H*), 3.62 (ddd, *J* = 9.7, 3.5, 1.9 Hz, 1H, C(5)*H*), 3.69 (td, *J* = 2.9, 1.0 Hz, 1H, C(3)*H*), 3.81 – 3.92 (m, 3H, C(6)*H*<sub>2</sub>, C(6')*H*<sub>A</sub>), 3.96 (dq, *J* = 10.3, 4.3 Hz, 2H, C(6')*H*<sub>B</sub>, C(2)*H*), 4.13 (t, *J* = 9.5 Hz, 1H, C(4)*H*), 4.43 (d, *J* = 11.3 Hz, 1H, C(3')OCH<sub>A</sub>Ph), 4.58 – 4.64 (m, 2H, C(1)*H*, C(5')*H*), 4.65 – 4.69 (m, 2H, C(3')OCH<sub>B</sub>, C(3)OCH<sub>A</sub>),

4.72 (d,  $J = 11.5$  Hz, 1H, C(3)OCH<sub>B</sub>), 4.75 (t,  $J = 2.5$  Hz, 1H, C(4')H), 4.79 (d,  $J = 10.0$  Hz, 1H, NH), 4.87 – 4.94 (m, 1H, C(2')H), 5.01 (d,  $J = 2.6$  Hz, 2H, C(9)H<sub>2</sub>), 5.18 (s, 1H, C(1')H), 7.10 (dd,  $J = 7.3, 2.3$  Hz, 2H, Ph), 7.19 (dd,  $J = 5.6, 1.8$  Hz, 3H, Ph), 7.28 (td,  $J = 8.9, 5.7$  Hz, 5H, Ph), 7.31 – 7.45 (m, 11H, Ph), 7.67 – 7.71 (m, 2H, Ph), 7.71 – 7.75 (m, 2H, Ph);  $\delta_c$  (101 MHz, CDCl<sub>3</sub>) 19.6 (C(CH<sub>3</sub>)<sub>3</sub>), 20.9 (Ac), 20.9 (Ac), 21.0 (Ac), 26.9 (C(CH<sub>3</sub>)<sub>3</sub>), 55.0 (C(7)), 55.5 (C(2)), 62.4 (C(6')), 62.9 (C(6)), 63.6 (C(5')), 67.0 (C(9)), 67.2 (C(4')), 67.5 (C(2')), 72.2 (C(5)), 72.5 (C(3')OCH<sub>2</sub>Ph), 72.9 (C(3')), 73.1 (C(4)), 75.3 (C(3)OCH<sub>2</sub>Ph), 79.3 (C(3)), 96.6 (C(1')), 98.8 (C(1)), 127.4 (Ph), 127.6 (Ph), 127.7 (Ph), 128.1 (Ph), 128.3 (Ph), 128.3 (Ph), 128.5 (Ph), 128.6 (Ph), 129.6 (Ph), 129.7 (Ph), 133.5 (Ph), 134.1 (Ph), 135.8 (Ph), 136.1 (Ph), 136.4 (Ph), 137.5 (Ph), 138.4 (Ph), 156.0 (C<sub>Cbz</sub>=O), 169.7 (C<sub>Ac</sub>=O), 170.4 (C<sub>Ac</sub>=O), 170.8 (C<sub>Ac</sub>=O);  $m/z$  (ESI<sup>+</sup>) 1056 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>57</sub>H<sub>68</sub>NO<sub>15</sub>Si<sup>+</sup> ([M+H]<sup>+</sup>) requires 1034.4353; found 1034.4347.

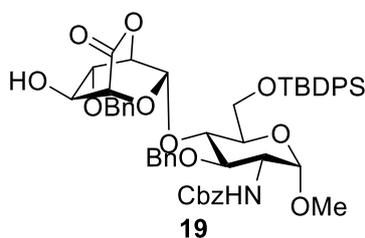
**Benzyl ((2S,3R,4R,5S,6R)-4-(benzyloxy)-5-(((2S,3R,4S,5R,6S)-4-(benzyloxy)-3,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-6-(((tert-butyldiphenylsilyl)oxy)methyl)-2-methoxytetrahydro-2H-pyran-3-yl)carbamate (18)**



CH<sub>3</sub>ONa (126 mg, 2.34 mmol) was added to a solution of **17** (4.85 g, 4.69 mmol) in CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> (1:1, 60 mL) and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of 1 M HCl, and concentrated *in vacuo*. The residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O (1:1, 60 mL), the organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to give **18** as a white solid (4.18 g, 98%); mp 74–77 °C;  $[\alpha]_D^{25} +23.1$  (c 1.0 in CHCl<sub>3</sub>);  $\nu_{\max}$  (film) 3437 (O–H), 2932 (C–H), 2895 (C–H), 2858 (C–H), 1713 (C=O);  $\delta_H$  (400 MHz, CDCl<sub>3</sub>) 1.07 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 2.99 (dd,  $J = 12.5, 2.2$  Hz, 1H, C(6')H<sub>A</sub>), 3.32 (s, 3H, C(7)H<sub>3</sub>), 3.42 – 3.53 (m, 2H, C(3)H, C(6')H<sub>B</sub>), 3.60 – 3.73 (m, 3H, C(5)H, C(2')H, C(3')H), 3.82 (d,  $J = 3.4$  Hz, 1H, C(4')H), 3.87 (d,  $J = 2.8$  Hz, 2H, C(6)H<sub>2</sub>), 4.02 – 4.11 (m, 3H, C(2)H, C(4)H, C(5')H), 4.41 – 4.50 (m, 2H, C(3)OCH<sub>2</sub>Ph), 4.55 (d,  $J = 11.3$  Hz, 1H, C(3')OCH<sub>A</sub>Ph), 4.63 – 4.74 (m, 2H, C(1)H, C(3')OCH<sub>B</sub>Ph), 4.96 (d,  $J = 10.1$  Hz, 1H, NH), 5.06 (s, 2H, C(9)H<sub>2</sub>), 5.19 (s, 1H, C(1')H), 7.05 – 7.15 (m, 2H, Ph), 7.22 (dd,  $J = 5.1, 2.0$  Hz, 3H, Ph), 7.28 – 7.46 (m, 16H, Ph), 7.75

(ddt,  $J = 14.1, 6.4, 1.8$  Hz, 4H, *Ph*);  $\delta_c$  (101 MHz,  $CDCl_3$ ) 19.5 ( $C(CH_3)_3$ ), 26.9 ( $C(CH_3)_3$ ), 55.1 ( $C(7)$ ), 55.5 ( $C(2)$ ), 62.9 ( $C(6)$ ), 64.8 ( $C(5')$ ), 65.2 ( $C(6')$ ), 66.4 ( $C(2')$ ), 67.1 ( $C(9)$ ), 71.5 ( $C(4')$ ), 72.0 ( $C(3')OCH_2Ph$ ), 72.4 ( $C(5)$ ), 73.5 ( $C(4)$ ), 75.2 ( $C(3)OCH_2Ph$ ), 75.6 ( $C(3')$ ), 79.8 ( $C(3)$ ), 98.9 ( $C(1)$ ), 101.1 ( $C(1')$ ), 127.5 (*Ph*), 127.7 (*Ph*), 127.8 (*Ph*), 128.0 (*Ph*), 128.2 (*Ph*), 128.3 (*Ph*), 128.3 (*Ph*), 128.3 (*Ph*), 128.5 (*Ph*), 128.6 (*Ph*), 129.8 (*Ph*), 129.9 (*Ph*), 133.2 (*Ph*), 133.5 (*Ph*), 135.9 (*Ph*), 136.1 (*Ph*), 136.5 (*Ph*), 138.5 (*Ph*), 156.1 ( $C=O$ );  $m/z$  ( $ESI^+$ ) 930 ( $[M+Na]^+$ , 100%); HRMS ( $ESI^+$ )  $C_{51}H_{61}NNaO_{12}Si^+$  ( $[M+Na]^+$ ) requires 930.3855; found 930.3857.

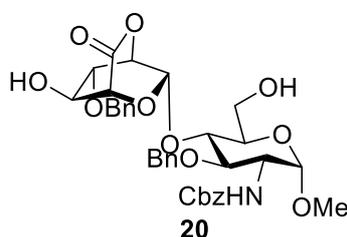
**Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4-(benzyloxy)-5-(((1*R*,3*R*,4*R*,7*S*,8*S*)-8-(benzyloxy)-7-hydroxy-6-oxo-2,5-dioxabicyclo[2.2.2]octan-3-yl)oxy)-6-(((tert-butyldiphenylsilyl)oxy)methyl)-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (**19**)**



TEMPO (28 mg, 179  $\mu$ mol) and (diacetoxyiodo)benzene (567 mg, 1.76 mmol) were added in quick succession to a solution of **18** (800 mg, 882  $\mu$ mol) in  $CH_2Cl_2$  (8.8 mL) and the reaction was stirred at rt for 16 h. The reaction was diluted with  $CH_2Cl_2$  (10 mL), washed with satd aq  $NaHCO_3$  (10 mL), 10%  $Na_2S_2O_3$  (10 mL) and brine, dried over  $Na_2SO_4$  and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→30% EtOAc in pentane) gave **19** as a colourless glass (485 mg, 61%); mp 77–81 °C;  $[\alpha]_D^{25} +13.6$  (c 1.0 in  $CHCl_3$ );  $\nu_{max}$  (film) 3437 (O–H), 2932 (C–H), 2895 (C–H), 2858 (C–H), 1792 ( $C_{Lactone}=O$ ), 1715 ( $C_{Cbz}=O$ );  $\delta_H$  (400 MHz,  $CDCl_3$ ) 1.06 (s, 9H,  $C(CH_3)_3$ ), 3.30 (s, 3H,  $C(7)H_3$ ), 3.55 (dt,  $J = 9.7, 2.4$  Hz, 1H,  $C(5)H$ ), 3.59–3.67 (m, 2H,  $C(3)H, C(3')H$ ), 3.71 (dd,  $J = 11.5, 3.1$  Hz, 1H,  $C(6)H_A$ ), 3.79 (dd,  $J = 11.7, 1.8$  Hz, 1H,  $C(6)H_B$ ), 3.98 (d,  $J = 3.3$  Hz, 1H,  $C(5')H$ ), 3.99–4.10 (m, 2H,  $C(2)H, C(4)H$ ), 4.14 (dd,  $J = 5.0, 3.0$  Hz, 1H,  $C(4')H$ ), 4.27 (d,  $J = 2.8$  Hz, 1H,  $C(2')H$ ), 4.48 (s, 2H,  $C(3)OCH_2Ph$ ), 4.65 (d,  $J = 11.2$  Hz, 1H,  $C(3')OCH_APh$ ), 4.71 (d,  $J = 3.7$  Hz, 1H,  $C(1)H$ ), 4.83 (t,  $J = 11.5$  Hz, 2H, NH,  $C(3')OCH_BPh$ ), 5.08 (q,  $J = 12.2$  Hz, 2H,  $C(9)H_2$ ), 5.41 (dd,  $J = 2.6, 1.3$  Hz, 1H,  $C(1')H$ ), 7.26–7.34 (m, 12H, *Ph*), 7.41 (dtd,  $J = 16.5, 7.6, 6.0$  Hz, 8H, *Ph*), 7.67 (dp,  $J = 9.1, 2.1$  Hz, 5H, *Ph*);  $\delta_c$  (101 MHz,  $CDCl_3$ ) 19.3 ( $C(CH_3)_3$ ), 27.0 ( $C(CH_3)_3$ ), 54.8 ( $C(2)$ ), 55.2 ( $C(7)$ ), 62.4 ( $C(6)$ ), 67.1 ( $C(9)$ ), 71.3 ( $C(5')$ ), 71.3 ( $C(5)$ ), 71.5 ( $C(4')$ ), 72.3 ( $C(3)OCH_2Ph$ ), 72.5 ( $C(2')$ ), 74.2 ( $C(3')OCH_2Ph$ ), 77.4 ( $C(4)$ ),

79.1 (C(3)), 81.3 (C(3')), 97.6 (C(1')), 99.0 (C(1)), 127.7 (Ph), 127.8 (Ph), 127.9 (Ph), 127.9 (Ph), 128.0 (Ph), 128.2 (Ph), 128.4 (Ph), 128.6 (Ph), 128.6 (Ph), 129.9 (Ph), 130.1 (Ph), 132.9 (Ph), 135.8 (Ph), 136.1 (Ph), 136.5 (Ph), 137.5 (Ph), 138.7 (Ph), 156.0 (C<sub>Cbz</sub>=O), 168.0 (C<sub>Lactone</sub>=O); *m/z* (ESI<sup>+</sup>) 926 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>51</sub>H<sub>58</sub>NO<sub>12</sub>Si<sup>+</sup> ([M+H]<sup>+</sup>) requires 904.3723; found 904.3717.

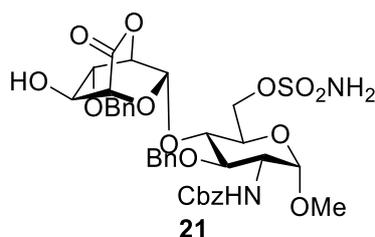
**Benzyl ((2*S*,3*R*,4*R*,5*S*,6*R*)-4-(benzyloxy)-5-(((1*R*,3*R*,4*R*,7*S*,8*S*)-8-(benzyloxy)-7-hydroxy-6-oxo-2,5-dioxabicyclo[2.2]octan-3-yl)oxy)-6-(hydroxymethyl)-2-methoxytetrahydro-2*H*-pyran-3-yl)carbamate (**20**)**



Tris(dimethylamino)sulfonium difluorotrimethylsilicate (TASF, 110 mg, 399  $\mu$ mol) was added to a solution of **19** (380 mg, 421  $\mu$ mol) in DMF (4 mL) under N<sub>2</sub> and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of satd aq NH<sub>4</sub>Cl (5 mL) and extracted with EtOAc (2  $\times$  10 mL). The combined organic layers were washed with  $\frac{1}{2}$  sat brine (5  $\times$  5 mL), brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo. Purification via column chromatography (gradient elution, 0% $\rightarrow$ 60% EtOAc in pentane) gave **20** as a white solid (130 mg, 75%); mp 86–88  $^{\circ}$ C;  $[\alpha]_D^{25} +24.7$  (c 1.0 in CHCl<sub>3</sub>);  $\nu_{\max}$  (film) 3424 (O–H), 2936 (C–H), 2922 (C–H), 2865 (C–H), 2844 (C–H), 1790 (C<sub>Lactone</sub>=O), 1702 (C<sub>Cbz</sub>=O);  $\delta_H$  (400 MHz, CDCl<sub>3</sub>) 3.28 (s, 3H, C(7)H<sub>3</sub>), 3.48 – 3.59 (m, 1H, C(5)H), 3.59 – 3.71 (m, 4H, C(3)H, C(3')H, C(6)H<sub>2</sub>), 3.79 (t, J = 9.4 Hz, 1H, C(4)H), 3.93 (d, J = 3.4 Hz, 2H, C(2)H, C(5')H), 4.13 (s, 1H, C(4)H), 4.46 (t, J = 2.7 Hz, 1H, C(2')H), 4.61 (s, 4H, C(1)H, C(3)OCH<sub>2</sub>Ph, C(3')OCH<sub>A</sub>Ph), 4.71 (d, J = 10.8 Hz, 1H, C(3')OCH<sub>B</sub>Ph), 4.81 (d, J = 9.9 Hz, 1H, NH), 5.00 (q, J = 12.2 Hz, 2H, C(9)H<sub>2</sub>), 5.34 (dd, J = 2.6, 1.3 Hz, 1H, C(1')H), 7.14 – 7.33 (m, 15H, Ph);  $\delta_C$  (151 MHz, CDCl<sub>3</sub>) 54.9 (C(2)), 55.4 (C(7)), 61.9 (C(6)), 67.2 (C(8)), 70.9 (C(5)), 71.3 (C(5')), 71.5 (C(4')), 72.7 (C(2')), 74.0, 74.2 (C(3)OCH<sub>2</sub>Ph and C(3')OCH<sub>2</sub>Ph), 77.5 (C(4)), 79.1 (C(3)), 81.2 (C(3')), 97.5 (C(1')), 99.1 (C(1)), 127.8 (Ph), 127.9 (Ph), 128.2 (Ph), 128.4 (Ph), 128.4 (Ph), 128.5 (Ph), 128.7 (Ph), 128.8 (Ph), 136.4 (Ph), 137.3 (Ph), 138.5 (Ph), 156.0

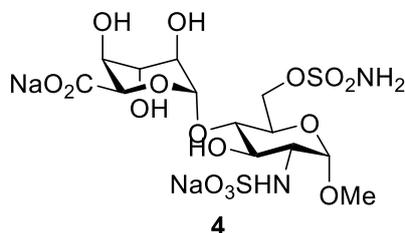
( $C_{\text{Cbz}}=\text{O}$ ), 167.7 ( $C_{\text{Lactone}}=\text{O}$ );  $m/z$  (ESI<sup>+</sup>) 688 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $\text{C}_{35}\text{H}_{40}\text{NO}_{12}^+$  ([M+H]<sup>+</sup>) requires 666.2545; found 666.2544.

**((2R,3S,4R,5R,6S)-4-(benzyloxy)-3-(((1R,3R,4R,7S,8S)-8-(benzyloxy)-7-hydroxy-6-oxo-2,5-dioxabicyclo[2.2.2]octan-3-yl)oxy)-5-(((benzyloxy)carbonyl)amino)-6-methoxytetrahydro-2H-pyran-2-yl)methyl sulfamate (21)**



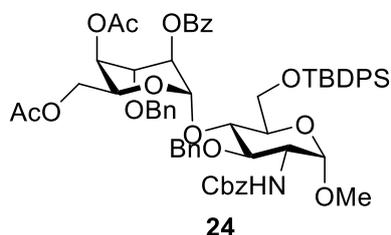
$\text{ClSO}_2\text{NH}_2$  (52.0 mg, 401  $\mu\text{mol}$ ) in  $\text{PhCH}_3$  (220  $\mu\text{L}$ ) was added to a solution of **20** (150 mg, 225  $\mu\text{mol}$ ) in DMF (1.35 mL) at  $-20^\circ\text{C}$  under  $\text{N}_2$  and the reaction was stirred at  $-20^\circ\text{C}$  for 16 h. The reaction was quenched by addition of satd aq  $\text{NaHCO}_3$  (5 mL) and extracted with EtOAc (3  $\times$  5 mL). The combined organic layers were washed with  $\frac{1}{2}$  sat brine (5  $\times$  5 mL), brine, dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%  $\rightarrow$  60% EtOAc in pentane) gave **21** as a white solid (111 mg, 66%); mp 164-167  $^\circ\text{C}$ ;  $[\alpha]_{\text{D}}^{25} -3.5$  (c 1.0 in  $\text{CH}_3\text{OH}$ );  $\nu_{\text{max}}$  (film) 3378 (O-H), 2918 (C-H), 2877 (C-H), 2851 (C-H), 2838 (C-H), 1787 ( $C_{\text{Lactone}}=\text{O}$ ), 1702 ( $C_{\text{Cbz}}=\text{O}$ );  $\delta_{\text{H}}$  (400 MHz, MeOD) 3.4 (s, 3H,  $\text{C}(7)\text{H}_3$ ), 3.7 – 3.8 (m, 5H,  $\text{C}(2)\text{H}$ ,  $\text{C}(3)\text{H}$ ,  $\text{C}(4)\text{H}$ ,  $\text{C}(5)\text{H}$ ,  $\text{C}(3')\text{H}$ ), 4.0 (d,  $J = 3.5$  Hz, 1H,  $\text{C}(2')\text{H}$ ), 4.2 (dd,  $J = 10.9, 1.7$  Hz, 1H,  $\text{C}(6)\text{H}_A$ ), 4.3 (dd,  $J = 4.6, 3.5$  Hz, 1H,  $\text{C}(5')\text{H}$ ), 4.3 (dd,  $J = 11.0, 3.3$  Hz, 1H,  $\text{C}(6)\text{H}_B$ ), 4.6 (d,  $J = 3.5$  Hz, 2H,  $\text{C}(1)\text{H}$ ,  $\text{C}(3')\text{OCH}_A$ ), 4.7 (s, 2H,  $\text{C}(3)\text{OCH}_2$ ), 4.9 – 4.9 (m, 2H,  $\text{C}(5')\text{H}$ ,  $\text{C}(3')\text{OCH}_B$ ), 5.0 – 5.1 (m, 2H,  $\text{NHCOOCH}_2\text{Ph}$ ), 5.4 (dd,  $J = 2.7, 1.3$  Hz, 1H,  $\text{C}(1')\text{H}$ ), 7.2 – 7.5 (m, 15H, 3  $\times$  Ph);  $\delta_{\text{C}}$  (101 MHz, MeOD) 55.8 ( $\text{C}(7)$ ), 56.6 ( $\text{C}(2)$ ), 67.7 ( $\text{NHCOOCH}_2\text{Ph}$ ), 68.8 ( $\text{C}(6)$ ), 69.9 ( $\text{C}(5)$ ), 72.3 ( $\text{C}(4')$ ), 72.9 ( $\text{C}(2')$ ), 73.0 ( $\text{C}(3)\text{OCH}_2\text{Ph}$ ), 73.6 ( $\text{C}(5')$ ), 76.0 ( $\text{C}(3')\text{OCH}_2\text{Ph}$ ), 80.2, 80.5 ( $\text{C}(3)$  and  $\text{C}(4)$ ), 82.5 ( $\text{C}(3')$ ), 100.2 (Ar), 100.5 (Ar), 128.5 (Ar), 128.9 (Ar), 129.0 (Ar), 129.0 (Ar), 129.2 (Ar), 129.4 (Ar), 129.5 (Ar), 139.2 (Ar), 139.9 (Ar), 158.6 ( $C_{\text{Cbz}}=\text{O}$ ), 170.1 ( $C_{\text{Lactone}}=\text{O}$ );  $m/z$  (ESI<sup>+</sup>) 767 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $\text{C}_{35}\text{H}_{41}\text{N}_2\text{O}_{14}\text{S}^+$  ([M+H]<sup>+</sup>) requires 745.2273; found 745.2273.

**Sodium (2R,3S,4S,5R,6R)-3,4,5-trihydroxy-6-(((2R,3S,4R,5R,6S)-4-hydroxy-6-methoxy-2-((sulfamoyloxy)methyl)-5-(sulfonatoamino)tetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-2-carboxylate (4)**



**(1) Hydrogenation.** A suspension of **21** (100 mg, 0.134 mmol), Pd(OH)<sub>2</sub>/C (20% wt, 200 mg) and cyclohexene (4 mL) in CH<sub>3</sub>OH (10 mL) and H<sub>2</sub>O (0.75 mL) was heated at 80 °C for 16 h. The reaction was filtered through a PTFE membrane filter and concentrated *in vacuo* to give **22** as a colourless gum (36 mg, 58%). **(2) N-sulfation.** SO<sub>3</sub>-Py (12.0 mg, 71.0 μmol) was added portion-wise to a solution of **22** (30.0 mg, 65.0 μmol) in H<sub>2</sub>O (0.5 mL, pH 9-10) at rt and pH was readjusted after each portion by addition of 1 M NaOH. The reaction was stirred at rt for 16 h, concentrated *in vacuo* and purification via ion-paired reversed phase chromatography (gradient elution, 0→100% MeCN in triethylammonium bicarbonate (1 M)). The product containing fractions were concentrated *in vacuo* then eluted from a Dowex® 50X8 Na<sup>+</sup>-form column (eluent H<sub>2</sub>O) and lyophilised to give **4** as a white solid (8 mg, 22%); mp 248–254 °C (dec.); [α]<sub>D</sub><sup>25</sup> +36.1 (c 1.0 in CH<sub>3</sub>OH); ν<sub>max</sub> (film) 3347 (br., O–H), 3066 (C–H), 3032 (C–H), 2917 (C–H), 2852 (C–H), 1675 (C=O); δ<sub>H</sub> (600 MHz, D<sub>2</sub>O) 3.35 (ddd, J = 10.4, 3.7, 1.3 Hz, 1H, C(2)H), 3.47 (d, J = 1.4 Hz, 3H, CH<sub>3</sub>), 3.49 (ddd, J = 7.6, 6.1, 1.3 Hz, 1H, C(2')H), 3.66 – 3.70 (m, 1H, C(3')H), 3.72 (ddd, J = 10.4, 8.8, 1.3 Hz, 1H, C(3)H), 3.82 – 3.89 (m, 2H, C(4)H, C(4')H), 4.05 – 4.10 (m, 1H, C(5)H), 4.51 – 4.59 (m, 3H, C(6)H<sub>2</sub>, C(5')H), 4.86 (dd, J = 6.2, 1.4 Hz, 1H, C(1')H), 5.09 (d, J = 3.4 Hz, 1H, C(1)H); δ<sub>C</sub> (151 MHz, D<sub>2</sub>O) 55.6 (CH<sub>3</sub>), 57.6 (C(2)), 68.1 (C(6)), 68.2 (C(5)), 69.5 (C(3)), 71.1 (C(4)), 71.7 (C(5')), 71.8 (C(2')), 72.7 (C(3')), 77.5 (C(4')), 98.4 (C(1)), 100.8 (C(1')), 176.0 (C=O); HRMS (ESI<sup>-</sup>) C<sub>13</sub>H<sub>23</sub>N<sub>2</sub>O<sub>16</sub>S<sub>2</sub><sup>-</sup> ([M–2Na+H]<sup>-</sup>) requires Exact Mass: 527.0494; found 527.0494.

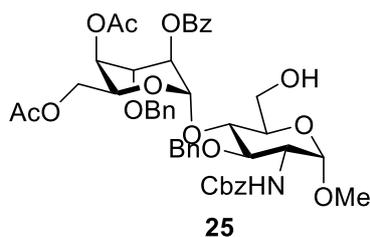
**(2S,3R,4S,5R,6S)-6-(acetoxymethyl)-4,5-bis(benzyloxy)-2-(((2R,3S,4R,5R,6S)-4-(benzyloxy)-5-(((benzyloxy)carbonyl)amino)-2-(((tert-butylidiphenylsilyl)oxy)methyl)-6-methoxytetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3-yl) benzoate (**24**)**



**16** (875 mg, 1.04 mmol) and **23** (synthesised according to literature procedures)<sup>6</sup> (450 mg, 0.690 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/Et<sub>2</sub>O (1:4, 7 mL) were dried over 4 Å molecular sieves (700 mg) under N<sub>2</sub> for 1 h and the reaction was cooled to -4 °C and TMSOTf (50 µL, 270 µmol) was added dropwise. The reaction was stirred at 0 °C for 1 h before being quenched by addition of satd aq NaHCO<sub>3</sub> (4 mL). The reaction was filtered through Celite<sup>®</sup> and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 5 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification via column chromatography (eluent acetone/pentane, 1:4) gave **24** as a white solid (757 mg, 96%); mp 59–61 °C;  $[\alpha]_{25}^D +35.8$  (c 1.0 in CHCl<sub>3</sub>);  $\nu_{\max}$  (film) 3066 (C–H), 3032 (C–H), 2931 (C–H), 2894 (C–H), 2858 (C–H), 1718 (C=O);  $\delta_H$  (400 MHz, CDCl<sub>3</sub>) 0.96 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.90 (s, 3H, C(7')H<sub>3</sub>), 3.20 (s, 3H, C(7)H<sub>3</sub>), 3.37 (t, J = 2.9 Hz, 1H, C(3')H), 3.50 (t, J = 9.7 Hz, 1H, C(3)H), 3.54 – 3.59 (m, 1H, C(5)H), 3.79 (d, J = 11.6 Hz, 1H, C(6)H<sub>A</sub>), 3.85 – 3.89 (m, 2H, C(6)H<sub>B</sub>, C(4')H), 3.90 (td, J = 10.1, 3.5 Hz, 1H, C(2)H), 4.07 (d, J = 6.3 Hz, 2H, C(6')H<sub>2</sub>), 4.15 (t, J = 9.4 Hz, 1H, C(4)H), 4.26 (d, J = 11.5 Hz, 1H, C(3')OH<sub>A</sub>Ph), 4.40 – 4.47 (m, 2H, C(3)OH<sub>A</sub>Ph C(3')OH<sub>B</sub>Ph), 4.50 (dt, J = 7.9, 3.8 Hz, 1H, C(5')H), 4.56 – 4.60 (m, 2H, C(1)H, C(4')OH<sub>A</sub>Ph), 4.69 (d, J = 9.9 Hz, 1H, NH), 4.71 – 4.79 (m, 2H, C(3)OH<sub>A</sub>Ph, C(4')OH<sub>B</sub>Ph), 4.93 – 5.03 (m, 2H, C(8)H<sub>2</sub>), 5.16 (s, 1H, C(2')H), 5.28 (s, 1H, C(1')H), 7.08 – 7.16 (m, 9H, Ar), 7.17 – 7.33 (m, 18H, Ar), 7.48 (td, J = 7.4, 1.4 Hz, 1H, Ar), 7.57 (d, J = 7.8 Hz, 2H, Ar), 7.65 (d, J = 7.3 Hz, 2H, Ar), 7.88 (d, J = 8.2 Hz, 2H, Ar);  $\delta_C$  (176 MHz, CDCl<sub>3</sub>) 19.5 (C(CH<sub>3</sub>)<sub>3</sub>), 21.0 (C(7')), 26.9 (C(CH<sub>3</sub>)<sub>3</sub>), 55.0 (C(7)), 55.3 (C(2)), 62.9 (C(6)), 63.4 (C(6')), 65.6 (C(5')), 67.0 (C(8)), 68.4 (C(2')), 72.1 (C(4')), 72.2 (C(3')OCH<sub>2</sub>Ph), 72.2 (C(5)), 72.5 (C(4')OCH<sub>2</sub>Ph), 73.2 (C(4)), 73.6 (C(3')), 75.2 (C(3)OCH<sub>2</sub>Ph), 79.2 (C(3)), 96.8 (C(1')), 98.8 (C(1)), 127.3 (Ar), 127.5 (Ar), 127.6 (Ar), 127.8 (Ar), 127.9 (Ar), 128.0 (Ar), 128.1 (Ar), 128.2 (Ar), 128.3 (Ar), 128.4 (Ar), 128.4 (Ar), 128.4 (Ar), 128.5 (Ar), 128.6 (Ar), 129.6 (Ar), 129.6 (Ar), 129.7 (Ar), 130.1 (Ar), 133.3 (Ar), 133.4

(Ar), 133.8 (Ar), 135.8 (Ar), 136.1 (Ar), 136.5 (Ar), 137.7 (Ar), 137.7 (Ar), 137.8 (Ar), 138.7 (Ar), 156.0 (C<sub>Cbz</sub>=O), 165.8 (C<sub>Bz</sub>=O), 171.0 (C<sub>Ac</sub>=O); *m/z* (ESI<sup>+</sup>) 1145 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>67</sub>H<sub>73</sub>NO<sub>14</sub>NaSi<sup>+</sup> ([M+Na]<sup>+</sup>) requires 1166.4693; found 1166.4638.

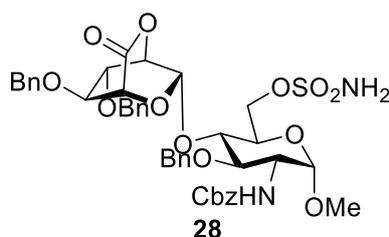
**(2S,3R,4S,5R,6S)-6-(Acetoxymethyl)-4,5-bis(benzyloxy)-2-(((2R,3S,4R,5R,6S)-4-(benzyloxy)-5-(((benzyloxy)carbonyl)amino)-2-(hydroxymethyl)-6-methoxytetrahydro-2H-pyran-3-yl)oxy)tetrahydro-2H-pyran-3-yl benzoate (25)**



Acetic acid (100  $\mu$ L, 1.75 mmol) and TBAF (1 M in THF, 1.65 mL, 1.65 mmol) were sequentially added to a solution of **24** (940 mg, 822  $\mu$ mol) in THF (23.5 mL) at rt and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of satd aq NaHCO<sub>3</sub> (20 mL) and extracted with EtOAc (3  $\times$  20 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0% $\rightarrow$ 30% acetone in pentane) gave **25** as a white solid (475 mg, 64%); mp 117–120  $^{\circ}$ C;  $[\alpha]_{25}^D$  +32.0 (*c* 1.0 in CHCl<sub>3</sub>);  $\nu_{\max}$  (film) 3443 (OH), 3064 (CH), 3031 (CH), 2952 (CH), 2924 (CH), 1718 (C=O);  $\delta_{\text{H}}$  (400 MHz, CDCl<sub>3</sub>) 1.96 (s, 3H, C(7')H<sub>3</sub>), 3.31 (s, 3H, C(7)H<sub>3</sub>), 3.51 (t, *J* = 3.5 Hz, 1H, C(4')H), 3.56 (dd, *J* = 10.4, 9.1 Hz, 1H, C(3)H), 3.64 (dt, *J* = 10.0, 3.2 Hz, 1H, C(5)H), 3.82 (d, *J* = 3.4 Hz, 2H, C(6)H<sub>2</sub>), 3.87 – 3.97 (m, 3H, C(2)H, C(4)H, C(3')H), 4.14 (dd, *J* = 11.5, 4.9 Hz, 1H), 4.22 (dd, *J* = 11.5, 7.6 Hz, 1H), 4.37 (d, *J* = 11.6 Hz, 1H, C(4')OCH<sub>A</sub>Ph), 4.43 – 4.52 (m, 2H, C(5')H, C(3)OCH<sub>A</sub>Ph), 4.55 (d, *J* = 11.5 Hz, 1H, C(4')OCH<sub>B</sub>Ph), 4.61 – 4.67 (m, 2H, C(1)H, C(3')OCH<sub>A</sub>Ph), 4.69 (d, *J* = 9.6 Hz, 1H), 4.75 – 4.87 (m, 2H, C(3)OCH<sub>B</sub>Ph, C(3')OCH<sub>B</sub>Ph), 5.01 (s, 2H, C(8)H<sub>2</sub>), 5.20 (d, *J* = 3.1 Hz, 2H, C(1')H, C(2')H), 7.13 – 7.39 (m, 22H, 4  $\times$  Ph, 2  $\times$  C(9')H), 7.49 – 7.57 (m, 1H, C(10')H), 7.89 – 8.00 (m, 2H, 2  $\times$  C(8')H);  $\delta_{\text{C}}$  (151 MHz, CDCl<sub>3</sub>) 21.1 (C(7')), 55.1 (C(2)), 55.3 (C(7)), 61.7 (C(6)), 63.0 (C(6')), 66.7 (C(5')), 67.0 (C(8)), 69.2 (C(5)), 71.7 (C(2')), 72.5 (C(4')OCH<sub>2</sub>Ph), 73.0 (C(3')OCH<sub>2</sub>Ph), 73.0 (C(3')), 74.1 (C(4')), 74.7 (C(4)), 75.1 (C(3)OCH<sub>2</sub>Ph), 78.9 (C(3)), 97.4 (C(1')), 99.1 (C(1)), 127.5 (Ar), 128.0 (Ar), 128.0 (Ar), 128.1 (Ar), 128.2 (Ar), 128.2 (Ar), 128.3 (Ar), 128.3 (Ar), 128.4 (Ar), 128.5 (Ar), 128.5 (Ar), 128.6 (Ar), 128.6 (Ar), 129.6 (Ar), 130.1 (2  $\times$  C(8')), 133.4 (C(10')), 136.5 (*i*-Ph), 137.6 (*i*-Ph), 137.7 (*i*-Ph), 138.5

(*i-Ph*), 155.9 ( $C_{\text{Cbz}}=\text{O}$ ), 165.9 ( $C_{\text{Bz}}=\text{O}$ ), 171.1 ( $C_{\text{Ac}}=\text{O}$ );  $m/z$  (ESI<sup>+</sup>) 928 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>)  $\text{C}_{51}\text{H}_{55}\text{NNaO}_{14}^+$  ([M+Na]<sup>+</sup>) requires 928.3515; found 928.3509.

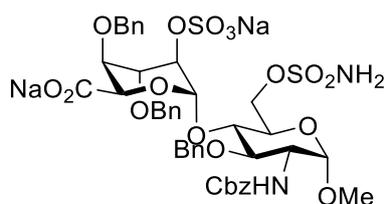
**((2R,3S,4R,5R,6S)-4-(Benzyloxy)-5-(((benzyloxy)carbonyl)amino)-3-(((2S,3R,4R,5R,6S)-4,5-bis(benzyloxy)-3-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-6-methoxytetrahydro-2H-pyran-2-yl)methyl sulfamate (28)**



**(1) 6O-sulfamoylation.** Sulfamoyl chloride (490 mg, 4.28 mmol) was added to a solution of **25** (2.30 g, 2.14 mmol) in DMF (10 mL) at 0 °C and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of ½ sat brine (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with ½ sat brine (5 × 5 mL), brine, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification via column chromatography (gradient elution, 0%→30% acetone in pentane) gave **26** as a white solid (2.12 g, 85%). **(2) Ester deprotection.** NaOCH<sub>3</sub> (125 mg, 2.27 mmol) was added to a solution of **26** (1.33 g, 1.36 mmol) in CH<sub>3</sub>OH (11 mL) at 0 °C and the reaction was stirred at rt for 16 h. The reaction was quenched by addition of Amberlite IR-120 (2 g), filtered and concentrated *in vacuo* to give **27** that was used without further purification (1.07 g, 94%). **(3) Lactone formation.** TEMPO (29 mg, 187 μmol) and (bisacetoxyl)iodobenzene (723 mg, 2.25 mmol) were consecutively added to a solution of **27** (960 mg, 936 μmol) in CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O (2:1, 15.6 mL) at rt and the reaction was stirred vigorously for 16 h. The reaction was diluted with EtOAc (20 mL) and washed with 10% aq Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (20 mL), satd aq NaHCO<sub>3</sub> (20 mL), brine, then dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification via column chromatography (eluent acetone/pentane, 3:7) gave **28** as a white solid (925 mg, 96%); mp 80–82 °C;  $[\alpha]_{25}^{\text{D}}$  +2.5 (*c* 1.0 in CHCl<sub>3</sub>);  $\nu_{\text{max}}$  (film) 3370 (br., N–H), 3033 (C–H), 2936 (C–H), 1789 (C=O), 1715 (C=O), 1518 (N–H), 1454 (S=O), 1369 (S=O);  $\delta_{\text{H}}$  (400 MHz, CDCl<sub>3</sub>) 3.33 (s, 3H, C(7)H<sub>3</sub>), 3.66 – 3.79 (m, 2H, C(3)H, C(5)H), 3.81 (d, *J* = 9.4 Hz, 1H, C(4)H), 3.87 (ddd, *J* = 4.4, 3.0, 1.2 Hz, 1H, C(3')H), 3.99 (td, *J* = 9.9, 3.5 Hz, 1H, C(2)H), 4.07 (t, *J* = 4.0 Hz, 1H, C(4')H), 4.22 (d, *J* = 11.1 Hz, 1H, C(6)H<sub>A</sub>), 4.29 (d, *J* = 3.3 Hz, 1H, C(5')H), 4.35 – 4.42 (m, 1H, C(6)H<sub>B</sub>), 4.43 (d, *J* = 11.8 Hz, H, C(4')OH<sub>A</sub>Ph), 4.55 (d, *J* = 1.7 Hz, 1H, C(4')OH<sub>B</sub>Ph), 4.57 –

4.61 (m, 3H, C(3)OH<sub>A</sub>Ph, C(3')OH<sub>2</sub>Ph), 4.62 – 4.64 (m, 1H, C(2')H), 4.67 (d, J = 3.5 Hz, 1H, C(1)H), 4.76 (d, J = 10.7 Hz, 1H, C(3)OH<sub>B</sub>Ph), 4.89 (d, J = 9.9 Hz, 1H, NH), 4.97 (s, 2H, SO<sub>2</sub>NH<sub>2</sub>), 5.01 – 5.11 (m, 2H, C(8)H<sub>2</sub>), 5.42 (s, 1H, C(1')H), 7.13 – 7.43 (m, 20H, 4 × Ph); δ<sub>C</sub> (151 MHz, CDCl<sub>3</sub>) 54.7 (C(2)), 55.7 (C(7)), 67.3 (C(8)), 68.7 (C(5')), 68.8 (C(5)), 68.9 (C(6)), 71.8 (C(4')), 72.2 (C(2')), 72.6 (C(3')OCH<sub>2</sub>Ph), 74.5 (C(3)OCH<sub>2</sub>Ph), 77.2 (C(4)), 77.9 (C(4')), 78.7 (C(3)), 80.0 (C(3')), 97.9 (C(1')), 99.1 (C(1)), 128.0 (Ph), 128.0 (Ph), 128.1 (Ph), 128.3 (Ph), 128.4 (Ph), 128.4 (Ph), 128.4 (Ph), 128.6 (Ph), 128.7 (Ph), 128.7 (Ph), 136.2 (*i*-Ph), 137.0 (*i*-Ph), 137.1 (*i*-Ph), 137.9 (*i*-Ph), 156.0 (C<sub>Cbz</sub>=O), 167.5 (C<sub>lactone</sub>=O); *m/z* (ESI<sup>+</sup>) 835 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>) C<sub>42</sub>H<sub>47</sub>N<sub>2</sub>O<sub>14</sub>S<sup>+</sup> ([M+H]<sup>+</sup>) requires 835.2720; found 835.2738.

**Sodium (2*R*,3*R*,4*S*,5*S*,6*R*)-4,5-bis(benzyloxy)-2-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-(benzyloxy)-5-(((benzyloxy)carbonyl)amino)-6-methoxy-2-((sulfamoyloxy)methyl)tetrahydro-2*H*-pyran-3-yl)oxy)-6-carboxytetrahydro-2*H*-pyran-3-yl sulfate (29)**

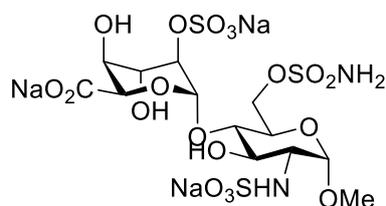


**29**

**(1) Lactone hydrolysis.** LiOH (1 M aq, 1.65 mL, 1.65 mmol) was added to **28** (230 mg, 276 μmol) in THF (15 mL) and stirred at rt for 1 h. The reaction was neutralised by addition of Dowex® 50WX4-200R acidic resin, filtered and concentrated *in vacuo*. **(2) O-Sulfation.** The residue was dissolved in DMF (3.45 mL) under N<sub>2</sub> and sulfur trioxide-pyridine complex (263 mg, 1.65 mmol) was added. The reaction was heated at 60 °C for 18 h. The reaction was quenched by addition of NaHCO<sub>3</sub> (800 mg) and H<sub>2</sub>O (1 mL), and was stirred at rt for 1 h. The reaction was concentrated *in vacuo* and the residue was azeotroped with H<sub>2</sub>O (2 × 15 mL), then PhMe (1 × 15 mL). Purification via column chromatography (eluent, EtOAc/IPA/H<sub>2</sub>O = 82:16:2) gave **29** as a white solid (178 mg, 66% over two steps); mp 87-89 °C; [α]<sub>25</sub><sup>D</sup> +23.2 (c 1.0 in CH<sub>3</sub>OH); ν<sub>max</sub> (film) 3437 (O–H), 3064 (C–H), 3033 (C–H), 2954 (C–H), 1714 (C<sub>carbamate</sub>=O), 1615 (C<sub>carboxylate</sub>=O), 1517 (C<sub>carbamate</sub>=O), 1455 (S<sub>sulfate</sub>=O), 1368 (S<sub>sulfamate</sub>=O); δ<sub>H</sub> (400 MHz, MeOD) 3.38 (s, 3H, C(7)H<sub>3</sub>), 3.64 – 3.84 (m, 3H, C(2)H, C(3)H, C(4)H), 3.88 – 3.96 (m, 1H, C(5)H), 4.05 (s, 1H, C(3')H), 4.20 (t, J = 2.5 Hz, 1H, C(4')H), 4.33 (dd, J = 11.1, 5.9 Hz, 1H, C(6)H<sub>A</sub>), 4.44 – 4.56 (m, 3H, C(6)H<sub>B</sub>, C(6')H<sub>A</sub>, C(9)H<sub>A</sub>), 4.57 (d, J = 3.5 Hz, 1H, C(1)H), 4.62 – 4.71 (m, 3H,

C(2')H, C(6')H<sub>B</sub>, C(7')H<sub>A</sub>), 4.78 (t, J = 11.4 Hz, 2H, C(7')H<sub>B</sub>, C(9')H<sub>B</sub>), 4.90 (d, J = 1.9 Hz, 1H, C(5')H), 4.92 – 5.03 (m, 2H, C(8)H<sub>2</sub>), 5.36 (s, 1H, C(1')H), 7.11 – 7.37 (m, 16H, Ar), 7.37 – 7.47 (m, 4H, Ar);  $\delta_c$  (126 MHz, MeOD) 55.7 (C(7)), 56.9 (C(2)), 67.7 (C(8)), 69.6 (C(6)), 71.1 (C(5')), 71.2 (C(5)), 71.8 (C(2')), 72.6 (C(4')), 72.9 (C(7')), 73.6 (C(6')), 76.1 (C(3')), 77.2 (C(9)), 78.6 (C(3)), 80.4 (C(4)), 100.1 (C(1)), 101.0 (C(1')), 128.6 (Ar), 128.8 (Ar), 128.9 (Ar), 129.0 (Ar), 129.0 (Ar), 129.3 (Ar), 129.3 (Ar), 129.4 (Ar), 129.4 (Ar), 129.6 (Ar), 130.0 (Ar), 138.0 (*i-Ph*), 138.7 (*i-Ph*), 139.0 (*i-Ph*), 139.3 (*i-Ph*), 158.4 (C<sub>cbz</sub>=O), 176.3 (C<sub>acid</sub>=O); *m/z* (ESI<sup>-</sup>) 931 (100%, [M-Na]<sup>-</sup>); HRMS (ESI<sup>-</sup>) C<sub>42</sub>H<sub>47</sub>N<sub>2</sub>O<sub>18</sub>S<sub>2</sub><sup>-</sup> ([M-Na]<sup>-</sup>) requires 931.2271; found 931.2266.

**Sodium (2*R*,3*S*,4*S*,5*R*,6*R*)-3,4-dihydroxy-6-(((2*R*,3*S*,4*R*,5*R*,6*S*)-4-hydroxy-6-methoxy-2-((sulfamoyloxy)methyl)-5-(sulfonatoamino)tetrahydro-2*H*-pyran-3-yl)oxy)-5-(sulfonatooxy)tetrahydro-2*H*-pyran-2-carboxylate (5)**

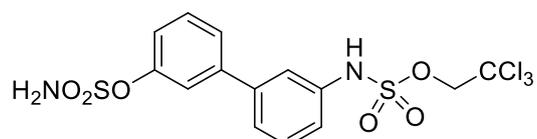


5

**(1) Global deprotection.** Pd(OH)<sub>2</sub>/C (120 mg) was added to a solution of **29** (160 mg, 171  $\mu$ mol) in phosphate buffer (20 mM, pH = 7.0)/CH<sub>3</sub>OH (17 mL, 1:9) under N<sub>2</sub>. The reaction flask was evacuated and back filled with N<sub>2</sub> ( $\times$  3) before being put under an atmosphere of H<sub>2</sub> and the reaction was stirred at rt for 16 h. The reaction was filtered through celite and concentrated *in vacuo*. **(2) N-Sulfation.** The crude residue was dissolved in H<sub>2</sub>O (3 mL), and the solution was adjusted to pH 9-10 by addition of 2 N NaOH (aq). Sulfur trioxide-pyridine complex (40.0 mg, 257  $\mu$ mol) was added in four equal portions in half-hour intervals at rt, and the pH value was re-adjusted to pH 9-10 using of 2 N NaOH(aq) after each addition. The reaction was stirred at rt for 3 h, then concentrated *in vacuo* and purification via column chromatography on Sephadex G-25 (eluent H<sub>2</sub>O), followed by a column of DOWEX 50WX8-Na<sup>+</sup> (eluent H<sub>2</sub>O) gave **5** as a white solid (45 mg, 39%); mp 269–277 °C (dec.);  $[\alpha]_D^{25} + 13.2$  (c 1.0 in H<sub>2</sub>O);  $\nu_{\max}$  (film) 3348 (br. O–H), 2918 (C–H), 2852 (C–H), 1676 (C<sub>carboxylate</sub>=O), 1365 (S=O), 1180 (S=O);  $\delta_H$  (400 MHz, D<sub>2</sub>O) 3.28 (dd, J = 10.2, 3.6 Hz, 1H, C(2)H), 3.42 (s, 3H, C(7)H<sub>3</sub>), 3.68 (dd, J = 10.2, 8.8 Hz, 1H, C(3)H), 3.75 (t, J = 9.4 Hz, 1H, C(4)H), 3.97 (t, J = 3.7 Hz, 2H, C(5)H,

C(4')H), 4.00 – 4.07 (m, 1H, C(3')H), 4.25 (ddd, J = 4.2, 2.6, 0.8 Hz, 1H, C(2')H), 4.36 (d, J = 3.1 Hz, 2H, C(6)H<sub>2</sub>), 4.6 – 4.7 (m, 1H, C(5')H) 5.02 (d, J = 3.6 Hz, 1H, C(1)H), 5.15 (d, J = 2.6 Hz, 1H, C(1')H);  $\delta_c$  13C NMR (151 MHz, D<sub>2</sub>O) 55.5 (C(7)), 57.8 (C(2)), 67.1 (C(6)), 68.4 (C(5)), 69.3 (C(5')), 69.3 (C(4')), 69.5 (C(3')), 69.8 (C(3)), 75.0 (C(2')), 77.8 (C(4)), 98.3 (C(1)), 99.3 (C(1')), 176.1 (C=O); *m/z* (ESI<sup>+</sup>) 675 (100%, [M-H]<sup>-</sup>); HRMS (ESI<sup>-</sup>) C<sub>13</sub>H<sub>23</sub>N<sub>2</sub>O<sub>19</sub>S<sub>3</sub><sup>-</sup> ([M-3Na+2H]<sup>-</sup>) requires 607.0063; found 607.0055.

### 2,2,2-trichloroethyl (3'-(sulfamoyloxy)-[1,1'-biphenyl]-3-yl)sulfamate (11)



Compound **11** was synthesised according to the route described by Reuillon et al;  $\delta_H$  (400 MHz, CDCl<sub>3</sub>)  $\delta$  7.54 – 7.40 (m, 6H), 7.37 – 7.32 (m, 1H), 7.30 – 7.26 (m, 1H), 6.85 (s, 1H), 4.99 (s, 2H), 4.69 (s, 2H); *m/z* (ESI<sup>-</sup>) 473 (95%, [M(<sup>35</sup>Cl)<sub>3</sub>-H]<sup>-</sup>), 475 (100%, [M(<sup>35</sup>Cl)(<sup>37</sup>Cl)<sub>2</sub>-H]<sup>-</sup>), 479 (9%, [M(<sup>39</sup>Cl)<sub>2</sub>-H]<sup>-</sup>). Characterisation data are consistent with the literature.<sup>7</sup>

## Biological assay protocols

### HSulf-2 activity assay

HSulf-2 was expressed, isolated and purified following a protocol by Seffouh et al.<sup>8</sup> Sulfatase activity was monitored by the hydrolysis of 4-MUS to fluorescent methylumbelliferone (4MU,  $\lambda_{\text{Ex}}$  360 nm,  $\lambda_{\text{Em}}$  460 nm), and compounds were screened for the inhibition of 4-MUS hydrolysis in the presence of HSulf-2 according to a protocol described by Seffouh et al.<sup>8</sup> Assays were performed in white 96-well plates (Corning). HSulf-2 (160 ng/ $\mu\text{L}$ ) activity was measured by using 4-MUS (10 mM) in assay buffer (50 mM Tris base, 10 mM  $\text{MgCl}_2$ , (pH 7.5)) in the absence or presence of inhibitors. Experiments were incubated at 37 °C for 4 h, then quenched by addition of an equal volume of 1M Tris base (pH 11.5) and analysed on a fluorescence plate reader (Fusion, Perkin–Elmer). Inhibition is reported as a percentage of the uninhibited control: % activity=[rate with inhibitor/rate control $\times$ 100]; and % Inhibition=[100–% activity]. All values represent an average of two or more independent measurements.

### Sulfatase from *A. aerogenes* activity assay

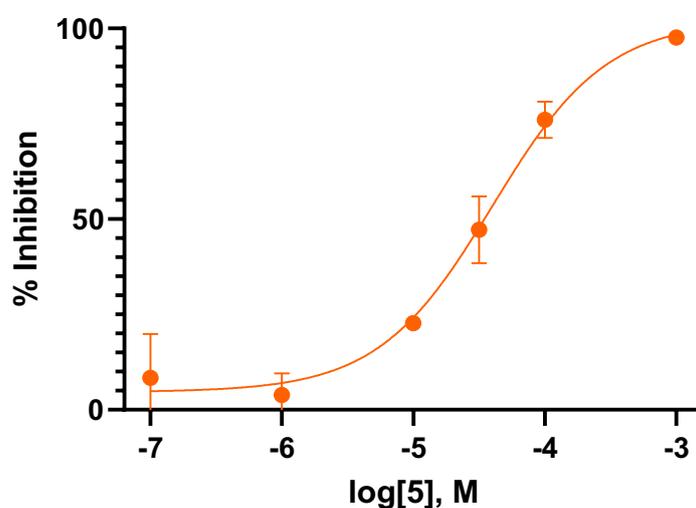
Compounds were screened in a 96-well white plate (Corning) using 4-MUS as a substrate. Assays were performed in white 96-well plates (Corning). Sulfatase from *Aerobacter aerogenes* (Sigma Aldrich, S1629) (172 ng/ $\mu\text{L}$ ) activity was measured by using 4-MUS (10 mM) in assay buffer (50 mM Tris base, 10 mM  $\text{MgCl}_2$ , (pH 7.5)) in the absence or presence of inhibitors. Experiments were incubated at 37 °C for 1 h, then quenched by addition of an equal volume of 1M Tris base (pH 11.5) and analysed on a fluorescence plate reader (Fusion, Perkin–Elmer). Inhibition is reported as a percentage of the uninhibited control: % activity=[rate with inhibitor/rate control $\times$ 100]; and % Inhibition=[100–% activity]. All values represent an average of two or more independent measurements.

### Determination of IC<sub>50</sub> value for compound 5 against HSulf-2

Percentage inhibition was plotted against logarithmic inhibitor concentration (M) using the non-linear sigmoidal dose-response curve described by the equation below using Prism 4 software (GraphPad), with constraining curve fit parameters set to reflect minimum and maximum inhibition values (set to 0 and 100 % inhibition, respectively).

$$Y = Bottom + (Top - Bottom)/(1 + 10^{((X - LogIC50))})$$

A



B

X	Group A		
log[5], M	% inhibition		
X	Mean	SD	N
-3.0	97.60500	0.58200	2
-4.0	76.06145	3.35845	2
-4.5	47.24400	6.21600	2
-5.0	23.61690	0.89990	2
-6.0	3.87600	4.02100	2
-7.0	8.40500	8.10300	2

C

Best-fit values	
Bottom	102.1
Top	4.667
LogIC50	-4.400
IC50	3.976e-005
Span	-97.44
95% CI (profile likelihood)	
Bottom	92.20 to 112.6
Top	-2.119 to 11.30
LogIC50	-4.588 to -4.215
IC50	2.582e-005 to 6.099e-005

**Figure S1.** Determination of IC<sub>50</sub> value for compound 5 against HSulf-2. **(A)** IC<sub>50</sub> curve for compound 5. **(B)** Experimental data given as the mean of %Inhibition at different concentrations of compound 5 (given as log[5] (M)) and standard deviation (SD) based on two replicates (N). **(C)** Best -fit values and 95% confidence intervals.

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# NMR spectra

