Supporting information

Protecting Group-Free Synthesis of Chondroitin 6-Sulfate (CS-C) Disaccharide and Tetrasaccharide

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General Procedure: All commercially available reagents were purchased from Sigma Aldrich and Alfa Aesar and used without further purification. Solvents such as water, methanol, N,N-dimethylformamide were pre-distilled prior to usage. Thin layer chromatography (TLC) was performed using Merck silica gel 60 F$_{254}$ pre-coated glass plates and visualised with charring using CAM stain (Hanessian’s Stain; Cerium Ammonium Molybdate Stain). Gel filtration chromatography was conducted using Sephadex® LH-20, G-10 and C-25. Flash column chromatography was conducted using silica gel (Merck, 230-400 mesh). $^1$H and $^{13}$C NMR spectrums were recorded on Bruker Avance 400 (AV400), Bruker Avance 500 (AV500) and Bruker Avance 500 (AVNEO500) at 298K. All J-values are reported in Hz and chemical shift (δ) reported in parts per million (ppm) relative to tetramethylsilane (TMS). Mass spectra were determined by high resolution mass spectrometry (HRMS) electrospray ionization (ESI) using micrOTOF-Q II. High Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS) was conducted using Agilent Technologies 1100 Series – Thermo Scientific LCQ Fleet and Dionex Ultimate 3000 RS – Bruker Amazon X. Optical rotation values were achieved using Anton Paar’s MCP 100 Modular Circular Polarimeter at 298K with a wavelength (λ) of 589.44 nm in vacuum as well as a cell length (l) of 100 mm.

Acid Hydrolysis of CS-A Mixture from Bovine Trachea: Chondroitin sulfate A sodium salt from bovine trachea (CS-A) (5 g) was dissolved in water (50 mL) and brought to pH 1.6 with Amberlite IR-120 [H+] resin by pH meter monitoring. The amberlite was then filtered and the resin was washed with water (4 x 10 mL). The volume of the filtrate was adjusted to 97 mL with water. Concentrated H$_2$SO$_4$ (18 M, 2.78 mL, 0.5 M final concentration) was added to the filtrate and the mixture was stirred at 100 °C for 6 h and then cooled. Solid Ba(OH)$_2$.8H$_2$O was added under vigorous stirring and brought to pH 3.5 by pH meter monitoring. The slurry that formed was allowed to settle overnight. The solids were then filtered off through a Celite pad, washed with water, and the combined filtrate and washing was concentrated to approximately 50mL and added slowly to an Amberlite IR-120 [H$^+$] resin column (50 mL, settled volume). The column was washed with water (100 mL), AcOH/water (3:1, 100 mL) and aqueous HCl (1 M, 300 mL). The fractions which were ninhydrin-positive were collected, concentrated and evaporated with water (2 x 500 mL) and dried under vacuum to give a crude mixture of 5 and 6 (3.6 g, brown solid) which was purified via HPLC using a Luna 3 μm HILIC 200 Å LC column (150 x 4.6 mm) and a gradient eluent system (-10 to 10 min: 10%A:90%B → 11 to 20min: 40%A:60%B → 21 to 25 min: 10%A:90%B where A is pure water with 5 mM ammonium acetate and B is 90% CH$_3$CN(aq) with 5mM ammonium acetate) to give 1.585 g and 1.567 g of 5 and 6 respectively.
Disaccharide 5: C_{12}H_{20}NO_{11}. [\alpha]^{25}_D = -3.0 (c = 0.1 in deionized water). HRMS: m/z 354.1042. Measured m/z 354.1044. \(^1\)H NMR (500 MHz, Deuterium Oxide) \(\delta\) 5.38 (d, \(J = 3.8\) Hz, 2H), 4.80 (d, \(J = 8.5\) Hz, 1H), 4.59 (dd, \(J = 7.9, 3.1\) Hz, 1H), 4.22 (d, \(J = 1.9\) Hz, 1H), 4.20 – 4.11 (m, 3H), 4.04 (t, \(J = 6.6\) Hz, 1H), 3.97 (dd, \(J = 10.9, 3.1\) Hz, 1H), 3.78 – 3.71 (m, 3H), 3.69 – 3.59 (m, 7H), 3.56 (dd, \(J = 10.8, 3.7\) Hz, 2H), 3.47 – 3.41 (m, 6H), 3.35 (qd, \(J = 7.7, 2.7\) Hz, 3H), 3.24 (dd, \(J = 10.9, 8.4\) Hz, 1H), 1.98 (s, 1H). \(^{13}\)C NMR (126 MHz, Deuterium Oxide) \(\delta\) 174.65, 103.40, 103.17, 92.95, 89.40, 79.21, 77.12, 75.78, 75.67, 75.17, 75.12, 72.79, 72.69, 71.46, 71.43, 70.30, 67.37, 66.74, 61.08, 60.83, 53.01, 49.91.

**Figure S1:** HRMS of 5

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Figure S2: HRMS (with expanded X-axis) of 5
Figure S3: $^1$H NMR Data of 5
Figure S4: $^{13}$C NMR data of 5
Tetrasaccharide 6: C_{24}H_{39}N_{2}O_{21}. \([\alpha]^{25}_D = -13.0\) (c = 0.1 in deionized water). HRMS: m/z 691.2056. Measured m/z 691.2056. \(^1\)H NMR (500 MHz, Deuterium Oxide) \(\delta 5.38\) (d, \(J = 3.7\) Hz, 3H), 4.81 (d, \(J = 8.5\) Hz, 1H), 4.65 – 4.55 (m, 2H), 4.24 – 4.11 (m, 10H), 4.07 – 3.95 (m, 8H), 3.93 – 3.80 (m, 10H), 3.74 – 3.61 (m, 16H), 3.61 – 3.52 (m, 7H), 3.52 – 3.42 (m, 13H), 3.42 – 3.31 (m, 8H), 3.29 – 3.20 (m, 2H), 1.98 (s, 1H), 1.89 (s, 1H). \(^{13}\)C NMR (126 MHz, Deuterium Oxide) \(\delta 173.31, 173.29, 103.48, 103.24, 92.95, 89.40, 79.40, 77.29, 75.08, 75.04, 74.90, 72.70, 72.61, 71.25, 70.37, 67.43, 61.04, 60.80, 52.98, 52.96, 49.88, 49.84, 36.30.

**Figure S5:** HRMS data of 6

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Figure S6: HRMS (with expanded X-axis) data of 6
Figure S7: $^1$H NMR Data of 6
Figure S8: $^{13}$C NMR Data of 6
**Figure S9:** Extracted-Ion Chromatogram (XIC) of Compound 5 from HPLC-MS Analysis of the Crude Mixture Obtained from the Acidic Hydrolysis of CS-A Polymer Mixture

RT: 0.00 - 19.99

Time (min)

Intensity

Intensity

NL: 4.52E3
m/z = 353.74-354.74
F: ITMS - p ESI Full
ms [100.00-2000.00] MS
Ys096-
COOH_18121816164
8
**Figure S10: Mass Spectrometry Analysis of 5**

F: ITMS - p ESI Full ms [100.00-2000.00]
**Figure S11**: Extracted-Ion Chromatogram (XIC) of Compound 5 (Non-Covalent Dimer, [2M-H]) from HPLC-MS Analysis of the Crude Mixture Obtained from the Acidic Hydrolysis of CS-A Polymer Mixture
Figure S12: Mass Spectrometry Analysis of 5 (Non-Covalent Dimer, [2M-H])
Figure S13: Extracted-Ion Chromatogram (XIC) of Compound 6 from HPLC-MS Analysis of the Crude Mixture Obtained from the Acidic Hydrolysis of CS-A Polymer Mixture
Figure S14: Mass Spectrometry Analysis of 6

Ys096-COOH_181218161648 #632-655

RT: 10.85-11.19 AV: 24 SB: 49 2.29-3.12 NL: 3.00E3

F: ITMS - p ESI Full ms [100.00-2000.00]

691.42 693.33 694.25 695.25 689.92 685.50 689.08 698.25 699.25 686.17 701.25 684.50 680.75 682.17 703.25 696.83 704.75

m/z
Selective N-Acetylation: A crude mixture of 5 and 6 (400mg) was dissolved in methanol (100 mL) before adding NaHCO₃ (260 mg, 3 equiv) and stirring the reaction mixture for 15 minutes. Ac₂O (1238 µl, 26 equiv) was then added and the reaction mixture was stirred overnight at room temperature. When the reaction has completed, water was added followed by Amberlyst 15 (1.3 g). The reaction mixture was then filtered and concentrated on a rotary evaporator to give crude CS-O 3 and 4 which was purified via HPLC-MS using a Luna 3 µm HILIC 200 Å LC column (150 x 4.6 mm) and a gradient eluent system (-10 to 10 min: 10%A:90%B → 11 to 20 min: 40%A:60%B → 21 to 25 min: 10%A:90%B where A is pure water with 5 mM ammonium acetate and B is 90% CH₃CN(aq) with 5mM ammonium acetate) to give 3 (143 mg, 0.782 mmol, 68%, white solid, over 2 steps) and 4 in (51 mg, 0.373 mmol, 26%, white solid, over 2 steps) respectively.
Disaccharide 3: C_{14}H_{22}NO_{12} \ [\alpha]^{25}_{D} = -16.0 \ (c = 0.1 \ in \ deionized \ water). \ HRMS: \ m/z \ 396.1147. \ Measured \ m/z \ 396.1146. \ \textsuperscript{1}H \ NMR \ (500 \ MHz, \ Deuterium \ Oxide) \ \delta \ 5.12 \ (d, \ J = 3.7 \ Hz, \ 2H), \ 4.60 \ (d, \ J = 8.4 \ Hz, \ 1H), \ 4.50 \ (dd, \ J = 27.8, \ 7.9 \ Hz, \ 3H), \ 4.22 \ (dd, \ J = 11.2, \ 3.8 \ Hz, \ 3H), \ 4.10 \ (d, \ J = 3.1 \ Hz, \ 2H), \ 4.06 - 3.99 \ (m, \ 4H), \ 3.91 \ (qd, \ J = 6.8, \ 6.2, \ 2.3 \ Hz, \ 8H), \ 3.74 \ (dd, \ J = 10.9, \ 3.3 \ Hz, \ 3H), \ 3.71 - 3.62 \ (m, \ 8H), \ 3.59 \ (dd, \ J = 8.3, \ 4.3 \ Hz, \ 3H), \ 3.52 - 3.38 \ (m, \ 9H), \ 3.33 - 3.23 \ (m, \ 5H), \ 1.98 - 1.89 \ (m, \ 14H). \ \textsuperscript{13}C \ NMR \ (126 \ MHz, \ Deuterium \ Oxide) \ \delta \ 174.65, \ 172.49, \ 104.23, \ 104.06, \ 95.11, \ 91.21, \ 80.65, \ 77.65, \ 75.11, \ 75.08, \ 74.86, \ 74.39, \ 72.50, \ 72.45, \ 71.28, \ 70.27, \ 68.59, \ 67.87, \ 61.18, \ 60.96, \ 52.34, \ 48.83, \ 22.25, \ 22.03.

**Figure S15**: HRMS data of 3
Figure S16: HRMS (with expanded X-axis) data of 3

[Diagram showing HRMS data with peaks and m/z values]
Figure S17: $^1$H NMR Data of 3
Figure S18: $^{13}$C NMR Data of 3
Tetrasaccharide 4: \( \text{C}_{28}\text{H}_{43}\text{N}_2\text{O}_{23} \). \([\alpha]_{D}^{25} = -16.0 \) (c = 0.1 in deionized water). HRMS: m/z 775.2262. Measured m/z 775.2258. \(^1\)H NMR (500 MHz, Deuterium Oxide) \( \delta 5.13 \) (s, 1H), 4.49 (dd, \( J = 14.6, 7.8 \) Hz, 2H), 4.42 (d, \( J = 7.2 \) Hz, 1H), 4.33 (d, \( J = 4.4 \) Hz, 1H), 4.25 – 4.19 (m, 2H), 4.16 (q, \( J = 4.4 \) Hz, 2H), 4.09 (s, 1H), 4.05 – 3.96 (m, 3H), 3.96 – 3.89 (m, 3H), 3.74 (s, 2H), 3.71 – 3.58 (m, 10H), 3.42 (td, \( J = 7.9, 6.7, 2.5 \) Hz, 4H), 3.32 – 3.23 (m, 3H), 2.04 (s, 1H), 2.01 – 1.89 (m, 12H), 1.87 (s, 3H). \(^{13}\)C NMR (126 MHz, Deuterium Oxide) \( \delta 175.98, 175.95, 174.97, 174.66, 104.09, 103.90, 95.20, 91.22, 80.31, 77.31, 76.16, 75.42, 75.37, 74.99, 74.41, 72.79, 72.73, 72.45, 71.81, 71.29, 70.34, 68.46, 61.28, 61.06, 52.33, 48.97, 22.26, 22.04.

**Figure S19: HRMS Data of 4**

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Figure S20: HRMS (with expanded X-axis) Data of 4
Figure S21: $^1$H NMR Data of 4
Figure S22: $^{13}$C NMR Data of 4
**Sulfation Reaction**: Tetrasaccharide 4 (22 mg, 0.0284 mmol) was dissolved in DMF (0.4 mL) before adding sulfur trioxide trimethylamine complex (40 mg, 10 equiv). The mixture was stirred at 50°C for 5 h. After the reaction has completed, the reaction mixture was quenched with methanol (0.2 mL), concentrated and dried under vacuum to give crude CS-C 2 which was purified via HPLC-MS using a Luna 3 μm HILIC 200 Å LC column (150 x 4.6 mm) and a gradient eluent system (-10 to 10 min: 10%A:90%B → 11 to 20min: 40%A:60%B → 21 to 25 min: 10%A:90%B where A is pure water with 5 mM ammonium acetate and B is 90% CH₃CN(aq) with 5mM ammonium acetate) to give pure 2 (10.5 mg, 0.0112 mmol, 39 %, white solid).
Tetrasaccharide 2 \( \text{C}_{28}\text{H}_{44}\text{N}_{2}\text{O}_{29}\text{S}_{2} \). \([\alpha]^{25}_{\text{D}} = -21.0 \) (c = 0.1 in deionized water). HRMS: m/z 935.1398. Measured m/z 935.1381. \(^1\)H NMR (500 MHz, Deuterium Oxide) \( \delta \) 5.14 – 5.10 (m, 1H), 4.61 – 4.38 (m, 2H), 4.28 – 4.22 (m, 1H), 4.18 (tdd, \( J = 10.1, 5.0, 2.7 \) Hz, 1H), 4.15 – 4.03 (m, 3H), 4.03 – 3.86 (m, 2H), 3.86 – 3.81 (m, 1H), 3.77 – 3.69 (m, 1H), 3.68 – 3.62 (m, 2H), 3.59 (ddd, \( J = 8.5, 6.0, 3.1 \) Hz, 1H), 3.44 – 3.35 (m, 6H), 3.34 – 3.27 (m, 1H), 3.27 – 3.19 (m, 4H), 1.92 (t, \( J = 1.9 \) Hz, 6H). \(^{13}\)C NMR (126 MHz, Deuterium Oxide) \( \delta \) 175.96, 175.94, 174.66, 171.05, 104.05, 103.87, 95.88, 95.15, 91.26, 83.72, 80.02, 77.01, 76.22, 76.12, 75.56, 75.35, 74.98, 74.01, 72.74, 71.81, 71.31, 70.34, 68.36, 68.31, 61.07, 48.86, 22.26, 22.04.

**Figure S23:** HRMS Data of 2
Figure S24: HRMS (with expanded X-axis) Data of 2
Figure S25: $^1$H NMR Data of 2
Figure S26: $^{13}$C NMR Data of 2

ys0202.1.fid
13C AMX500
Ys132-Tet 13C
Disaccharide 1 was prepared in a similar manner from disaccharide 3 (20 mg, 0.0504 mmol) to give pure 1 (8 mg, 0.0168 mmol, 33 %, white solid). C_{14}H_{22}NO_{15}S. [α]_{25}^{D} = -16.0 (c = 0.1 in deionized water).

HRMS: m/z 476.0716. Measured m/z 476.0715. $^1$H NMR (500 MHz, Deuterium Oxide) δ 5.43 (dd, J = 3.8, 1.7 Hz, 1H), 5.38 – 5.25 (m, 2H), 5.20 – 5.13 (m, 2H), 4.64 – 4.54 (m, 2H), 4.54 – 4.42 (m, 4H), 4.28 – 4.17 (m, 10H), 4.17 – 4.12 (m, 5H), 4.12 – 4.07 (m, 4H), 4.07 – 3.99 (m, 6H), 3.99 – 3.90 (m, 6H), 3.87 (d, J = 3.2 Hz, 3H), 3.82 – 3.74 (m, 5H), 3.72 – 3.60 (m, 15H), 3.50 – 3.39 (m, 10H), 3.27 (d, J = 7.5 Hz, 3H), 1.97 (d, J = 11.0 Hz, 13H). $^{13}$C NMR (126 MHz, Deuterium Oxide) δ 176.01, 174.72, 104.07, 103.89, 95.90, 76.19, 75.47, 72.80, 71.86, 70.36, 68.33, 55.57, 48.98, 22.14.

**Figure S27:** HRMS Data of 1

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*MS, 2.46 min #147, Background Subtracted*
Figure S28: HRMS (with expanded X-axis) Data of 1
Figure S29: $^1$H NMR Data of 1
Figure S30: $^{13}$C NMR Data of 1

[Chemical structure and NMR spectrum image]
Figure S31: HPLC-MS of 7 (Extracted Ion Chromatogram) C_{13}H_{23}NO_{11}. Exact m/z: 369.13. Measured m/z: 370.2. [M+1]