Appendix A

Expedient Synthesis of Chondroitin Sulfate Glycomimetic Polymers

A.1 Rationale

The activity of sulfated glycosaminoglycans (GAGs) depends on protein binding, which occurs through multiple protein-carbohydrate interactions along the length of an oligosaccharide. However, the chemical synthesis of defined oligosaccharides is extremely difficult, necessitating alternative approaches to obtain higher order, chemically pure carbohydrates. One method used by our lab is the production of glycomimetic polymers based on ring-opening metathesis polymerization (ROMP). In this procedure, disaccharide units of known sulfation pattern are appended to a ROMP monomer.[1-4] After polymerization with a ruthenium-based catalyst, the formed structure displays a number of disaccharide units on a single backbone as controlled by the catalyst loading, providing a macromolecular scaffold that mimics the natural multivalent presentation of GAG sulfation patterns. Unfortunately, production of the disaccharide starting material was still an arduous task, requiring nine and eight steps for the glucuronic acid (GlcA) and galactosamine (GalNAc) monomers, respectively, followed by a coupling step to reach the fully protected dissacharide building block for further sulfation steps.[1, 2, 5] Here, we describe the adaptation of an efficient hydrolysis of natural chondroitin sulfate (CS) polysaccharides[6] to produce an equivalent disaccharide building block in only four steps on a multi-gram scale and its elaboration into CS sulfation epitope monomers and the respective polymers.

A.2 General Chemical Methods

Unless otherwise noted, reactions were performed under Ar in sealed, flame-dried glassware using dry, deoxygenated solvents by passing through a column of activated alumina with Ar. Commercially obtained reagents were used as received. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm) and visualized by UV fluorescence quenching or staining with potassium permanganate, ceric ammonium molybdate, *p*-anisaldehyde, or ninhydrin. Flash column chromatography was performed using ICN silica gel (particle size 0.032 – 0.063 mm). Ion exchange chromatography was performed using Amberlite IR-120 H^+ form (Sigma Aldrich, washed with 1 M HCl, ddH_2O, MeOH, then Et_2O) or Na^+ form (Sigma Aldrich). For sulfated compounds, gel filtration chromatography was used with Sephadex LH-20 gel resin (GE Healthcare Life Sciences) preswollen in 1:1 CH_2Cl_2/MeOH. Polymers were purified using gel filtration chromatography with Sephadex G-25 Fine gel resin (GE Healthcare Life Sciences) preswollen in ddH_2O.

^{1}H NMR spectra were recorded on either a Varian Inova 500 (500 MHz) or a Varian Mercury 300 (300 MHz) and are reported relative to the solvent residual peak ($CDCl_3$ at δ 7.26, CD_3OD at 3.31, and D_2O at 4.79). Data for NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad), coupling constant in Hz, and integration. HRMS were acquired using an Agilent 6200 Series TOF with an Agilent G1978A Multimode source in electrospray or mixed mode ionization.

A.3 Norbornenyl Linker Synthesis

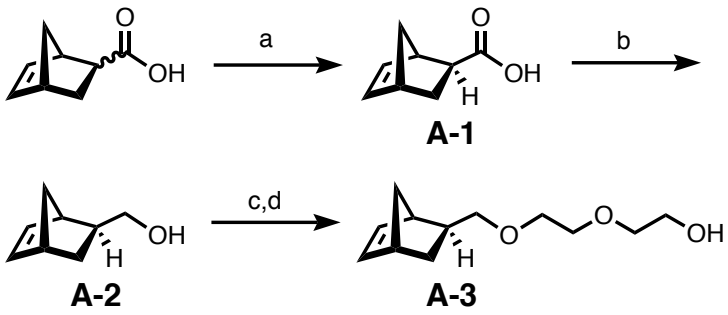

Figure A-1. Synthesis of norbornene linker.[2] (a) KI (3 eq), I_2 (1 eq), 0.75 M $NaHCO_3$, 1 h, RT, 90% recovery of *exo* isomer; (b) $LiAlH_4$ (3 eq), Et_2O, 0° to 50°C, 97% yield; (c) NaH (1.33 eq), 2-(2-((*tert*-butyldimethylsilyl)oxy)ethoxy) ethyl methanesulfonate (0.83 eq), 18-crown-6 (0.17 eq), 4 Å mol. sieves, THF, 0° to RT, 3 h, then 0.5 M TBAF, THF, 0° to RT, 3 h, 43% over two steps.

(1R, 2S, 4R)-Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (**A-1**). A 4:1 mixture of *endo*- and *exo*-5-norbornene-2-carboxylic acid (25.0 g, 181 mmol) was dissolved in 820 mL 0.75 M $NaHCO_3$. KI (90.12 g, 543 mmol, 3 eq) and I_2 (45.94 g, 181 mmol, 1 eq) were dissolved separately in 115 mL H_2O. This brown solution was added dropwise to the stirring solution of the carboxylic acid using an addition funnel. Once completely added, the solution was extracted with Et_2O (5 x 150 mL), and the organic layers were discarded. After decolorizing with 10% Na_2SO_3, the pH of the aqueous layer was lowered to 2 using 1 N H_2SO_4, and the solution was extracted again with Et_2O (5 x 150 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated to give the pure *exo* enantiomer (4.52 g, 90%) as a clear oil. If desired, the product could be further purified by precipitating in pentane at -78 °C; however, the product was

generally used directly in the next step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.14 (tt, *J* = 7.1, 4.1 Hz, 2H), 3.12 (d, *J* = 2.3 Hz, 1H), 2.95 (s, 1H), 2.28 (ddt, *J* = 8.4, 4.2, 1.7 Hz, 1H), 2.03 – 1.89 (m, 1H), 1.54 (dd, *J* = 8.3, 1.7 Hz, 1H), 1.41 (td, *J* = 9.4, 1.9 Hz, 2H).

(1R, 2S, 4R)-Bicyclo[2.2.1]hept-5-ene-2-ylmethanol (**A-2**). *Exo*-5-norbornene-2-carboxylic acid **A-1** (4.52 g, 32.7 mmol) was dissolved in dry Et_2O (80 mL) and cooled to 0 °C. Lithium aluminum hydride (3.75 g, 98.8 mmol, 3 eq) was then added portionwise with vigorous stirring between additions. The resulting suspension was refluxed at 50 °C. After 5 h, the suspension was cooled down to 0 °C and excess lithium aluminum hydride was quenched (4 mL H_2O, 8 mL 10% NaOH, then 12 mL H_2O). The white aluminum salts were filtered washed, and the solution was concentrated. Column chromatography (5:1 to 3:1 hexanes/EtOAc) gave the product (3.93 g, 97%) as a volatile, clear oil. R_f 0.24 (5:1 hexanes/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.09 (qd, *J* = 5.6, 2.8 Hz, 2H), 3.70 (dd, *J* = 10.6, 6.4 Hz, 1H), 3.54 (dd, *J* = 10.6, 8.8 Hz, 1H), 2.82 (s, 1H), 2.75 (s, 1H), 1.68 – 1.55 (m, 1H), 1.38 – 1.19 (m, 3H), 1.11 (ddd, *J* = 11.7, 4.5, 3.5 Hz, 1H).

2-(2-((1R,2S,4R)-Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethanol (**A-3**). *Exo*-5-norbornenyl-2-methanol **A-2** (11.10 g, 89 mmol, 1.2 eq) was added to dry THF (80 mL) with 4 Å molecular sieves under Ar. The mixture was stirred at RT for 30 minutes then cooled to 0 °C. NaH (2.83 g, 118 mmol, 1.6 eq) was added portionwise, and the resulting mixture was stirred for 30 minutes at 0 °C. 2-(2-

((*tert*-butyldimethylsilyl)oxy)ethoxy) ethyl methanesulfonate (22.23 g, 74 mmol) and 18-crown-6 (3.94 g, 15 mmol, 0.2 eq) were then added. The reaction was allowed to warm to RT while stirring for 3 h. After quenching with a minimum of H_2O, the mixture was diluted with CH_2Cl_2 and filtered through Celite. The organic layer was washed with saturated $NaHCO_3$, dried by $MgSO_4$, filtered, and concentrated. The crude was then redissolved in dry THF (120 mL) and cooled to 0 °C. 1 M TBAF in THF (150 mL) was added dropwise, and the reaction was stirred for 3 hours while warming to RT. The mixture was diluted with EtOAc and washed with H_2O. The organic layer was dried ($MgSO_4$), filtered, and concentrated. Column chromatography (1:1 hexanes/EtOAc) yielded the pure product (6.78 g, 43% over two steps) as a yellow oil. R_f 0.26 (1:1 hexanes/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.10 (dd, *J* = 5.7, 3.1 Hz, 1H), 6.05 (dd, *J* = 5.7, 2.9 Hz, 1H), 3.76 – 3.72 (m, 2H), 3.70 (t, *J* = 4.7 Hz, 2H), 3.66 – 3.57 (m, 4H), 3.53 (dd, *J* = 9.4, 6.3 Hz, 1H), 3.39 (t, *J* = 9.2 Hz, 1H), 2.80 (s, 1H), 2.74 (s, 1H), 2.17 (bs, 1H) 1.75 – 1.67 (m, 1H), 1.36 – 1.21 (m, 3H), 1.10 (ddd, *J* = 11.7, 4.3, 3.4 Hz, 1H); HRMS: calculated $[M+H]^+$ 213.1491, determined: 213.1473.

A.4 Production of the CS Disaccharide Building Block

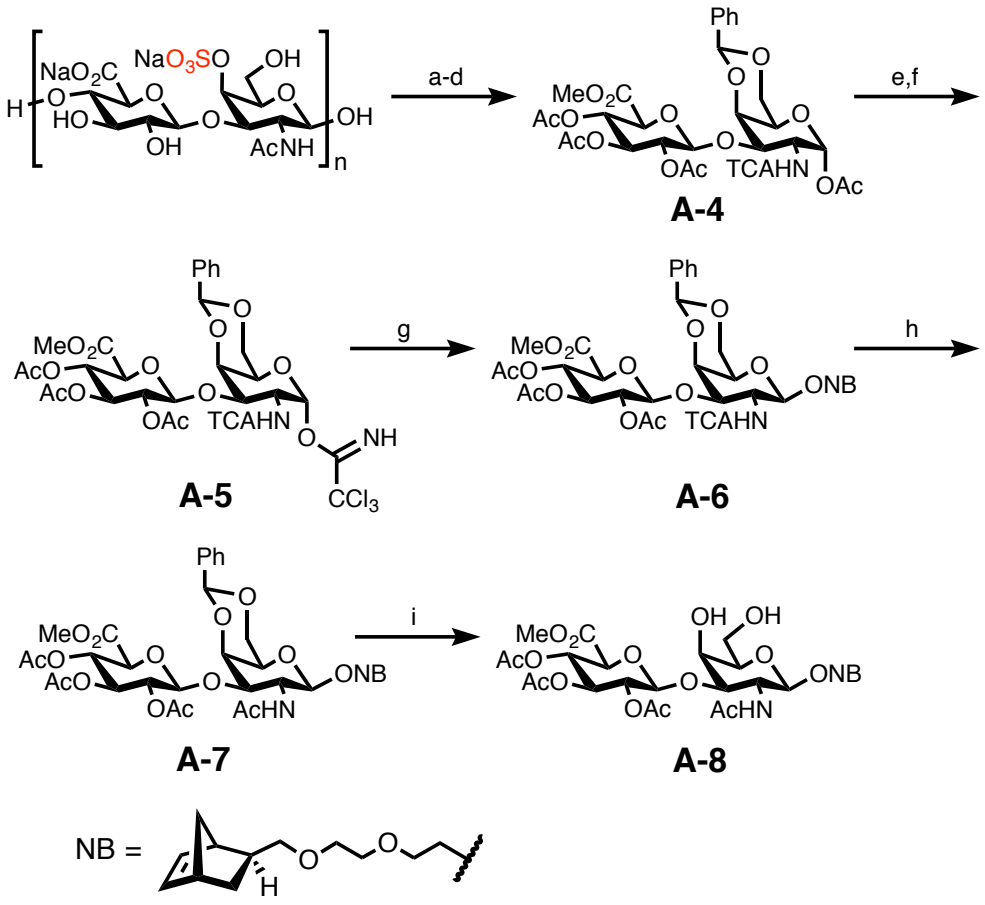

Figure A-2. Synthesis of core CS monomer building block. (a) 0.5 M H_2SO_4, H_2O, reflux, 6 h; (b) 0.02 M HCl, MeOH, 0 °C, 4 d; (c) TCACl (11.3 eq), pyridine, 0 °C, 1 h, then MeOH, DCM, pyridine, RT, 4 h; (d) benzaldehyde (36.5 eq), TFA (2.64 eq), neat, RT, 1 h, then Ac_2O (27.3 eq), NaOAc (3.9 eq), pyridine, RT, 16 h, 11.5% yield over four steps; (e) hydrazine acetate (1.5 eq), DMF, RT, 1 h; (f) trichloroacetonitrile (8 eq), DBU (0.6 eq), DCM, RT, 1 h, 56% yield over two steps; (g) **A-3** (1.6 eq), 0.5 M TMSOTf (0.1 eq), 4 Å mol. sieves, DCM, -65 to -40 °C, 30 min, 95% yield; (h) Bu_3SnH (6 eq), AIBN (0.17 eq), benzene, reflux, 3 h, 76% yield; (i) 80% AcOH, 80 °C, 99% yield.

O-(Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-1-O-acetyl-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-α-D-galactopyranose (**A-4**).[6] 100.0 g of CS-A polymer (Federal Laboratories, Corp., Alden, NY, USA) was dissolved in 1

L deionized H_2O, and the pH of the solution was lowered to 1.6 by addition of Amberlite IR-120 H^+ resin. The solution was filtered, and the resin was washed with deionized H_2O (4x100 mL). The final volume was adjusted to 1.94 L and 55.6 mL conc. H_2SO_4 was added. The solution was boiled at 100 °C for 6 h. $Ba(OH)_2{\cdot}8H_2O$ was added while vigorously stirring until the pH reached 3.5. The solids were allowed to settle overnight, then the solution was filtered with Celite. The solid was washed with deionized H_2O until the filtrate was clear. The solution was concentrated to 1 L then applied to a column of Amberlite IR-120 H^+ resin (1 L settled volume). The column was flushed with H_2O (2 L), 3:1 AcOH/H_2O (2 L), and 1 M HCl (6 L). The positively staining fractions via ninhydrin were concentrated and then coconcentrated with H_2O (2x500 mL). The crude solid was dried overnight *in vacuo*, then dissolved in 1 L 0.02 M methanolic HCl, and stirred at 0 °C for 4 d. This solution was then concentrated and co-concentrated with EtOH (2x100 mL) to give the crude disaccharide (55.93 g) as the hydrochloride salt. A portion of this crude disaccharide (20.0 g, 49.3 mmol) was then dissolved in 220 mL pyridine and cooled to -30 °C. Trichloroacetyl chloride (62 mL, 0.555 mol, 11.3 eq) was added dropwise. Once the addition was complete, the reaction was stirred at 0 °C for 1 hour then cooled to -30 °C again. Deionized H_2O (4.2 mL) was added very slowly while maintaining temperature. This was then diluted with CH_2Cl_2, washed with deionized water, brine then deionized water, dried over $MgSO_4$, and concentrated. The residue was redissolved in 150 mL 1:1:1

CH_2Cl_2/MeOH/pyridine and stirred for 4 hours at RT. The solution was concentrated and dried *in vacuo*. A silica plug (4:1 CH_2Cl_2/MeOH) was used to yield the crude trichloroacetamide (10.54 g), which was again used directly as well. The crude trichloroacetamide (10.0 g, 19.4 mmol) was dissolved in benzaldehyde (72 mL, 709 mmol, 36.5 eq) along with TFA (3.8 mL, 51.2 mmol, 2.64 eq). This mixture was for 24 hours at RT. Directly to the mixture, NaOAc (6.20 g, 75.6 mmol, 3.90 eq), pyridine (80 mL, 989 mmol, 51.0 eq), and acetic anhydride (50 mL, 529 mmol, 27.3 eq) were added sequentially. This was then stirred for 16 hours at RT. The solution was then added to ice-cold deionized H_2O and stirred for an additional 2 h. This was then extracted with CH_2Cl_2 (2x150 mL). The organic layers were combined, washed with deionized H_2O, saturated $NaHCO_3$ (aq.), then deionized H_2O, dried over $MgSO_4$, and concentrated. Column chromatography (100% CH_2Cl_2 →15:1 CH_2Cl_2/acetone) afforded the pure alpha anomer (6.10 g, 11.5% yield from polymer) as a white powder. R_f 0.44 (15:1 CH_2Cl_2/acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 – 7.49 (m, 2H, Ph*H*), 7.42 – 7.31 (m, 3H, Ph*H*), 6.77 (d, *J* = 7.7 Hz, 1H, GalN N*H*), 6.49 (d, *J* = 3.4 Hz, 1H, GalN H-1), 5.55 (s, 1H, PhC*H*), 5.30 – 5.19 (m, 2H, GlcA H-3, H-4), 5.10 (dd, *J* = 8.7, 7.8 Hz, 1H, GlcA H-2), 4.95 (d, *J* = 7.8 Hz, 1H, GlcA H-1), 4.65 (ddd, *J* = 11.1, 7.7, 3.4 Hz, 1H, GalN H-2), 4.51 (dd, *J* = 3.3, 1.2 Hz, 1H, GalN H-4), 4.47 (dd, *J* = 11.1, 3.3 Hz, 1H, GalN H-3), 4.30 (dd, *J* = 12.6, 1.6 Hz, 1H, GalN H-6a), 4.12 (d, *J* = 9.7 Hz, 1H, GlcA H-5), 4.08 (dd, *J* = 12.6, 1.8

Hz, 1H, GalN H-6b), 3.85 (m, 1H, GalN H-5), 3.74 (s, 3H, CO_2CH_3), 2.19 (s, 3H, OCH_3), 2.04 (m, 9H, OCH_3).

O-(Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-1-O-trichloroacetimidoyl-α-D-galactopyranose (**A-5**). Disaccharide **A-4** (8.10 g, 10.51 mmol) was dissolved in 65 mL dry DMF. To this solution, hydrazine acetate (1.48 g, 16.07 mmol, 1.5 eq) was added. The reaction was stirred at RT for 2 hours then diluted with EtOAc and washed with deionized H_2O, brine, then deionized H_2O. The organic layer was dried over $MgSO_4$ and concentrated. The residue was redissolved in 65 mL dry CH_2Cl_2, and DBU (0.25 mL, 1.67 mmol, 0.16 eq) and trichloroacetonitrile (8.51 mL, 84.87 mmol, 8 eq) were added. Upon addition of DBU, the reaction turned bright yellow and slowly darkened to brown. After stirring for 1 hour at RT, the reaction was concentrated to afford an orange solid. This solid was passed through a silica plug (15:1 CH_2Cl_2/acetone). The crude product was then recrystallized with Et_2O to afford the pure imidate (5.32 g, 56%) as white crystals. R_f 0.78 (15:1 CH_2Cl_2/acetone); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.76 (s, 1H, =N*H*), 7.57 – 7.50 (m, 2H, Ph*H*), 7.42 – 7.32 (m, 3H, Ph*H*), 6.79 (d, *J* = 8.1 Hz, 1H, -N*H*), 6.65 (d, *J* = 3.4 Hz, 1H, GalN H-1), 5.57 (s, 1H, C*H*Ph), 5.25 (t, *J* = 9.4 Hz, 1H, GlcA H-3), 5.20 (t, *J* = 8.8 Hz, 1H, GlcA H-4), 5.09 (dd, *J* = 8.6, 7.6 Hz, 1H, GlcA H-2), 4.95 (d, *J* = 7.6 Hz, 1H, GlcA H-1), 4.77 (ddd, *J* = 11.4, 8.1, 3.4 Hz, 1H, GalN H-2), 4.56 (d, *J* = 4.1 Hz, 1H, GalN H-4), 4.40 (dd, *J* = 11.0, 3.4 Hz, 1H, GalN H-3), 4.34 (dd, *J* = 12.6, 1.6 Hz, 1H, GalN H-6a), 4.17 – 4.05 (m,

2H, GalN H-6b, GlcA H-5), 3.93 (s, 1H, GalN H-5), 3.72 (s, 3H, -CO_2CH_3), 2.05 – 1.98 (m, 9H, -OCH_3); HRMS: calculated $[M+Na]^+$ 894.9802, determined: 894.9742.

2-(2-((1R,2S,4R)-Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-4,6-O-benzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranose (**A-6**). Disaccharide **A-5** (1.84 g, 2.11 mmol, 1 eq) was dissolved in 30 mL dry CH_2Cl_2 with 4 Å molecular sieves under Ar, and the solution was cooled to –60 °C. To this was added the norbornenyl linker **3** (0.70 g, 3.30 mmol, 1.6 eq) dissolved in 7 mL dry CH_2Cl_2. The mixture was stirred at –60 °C for 1 h, then 440 μL 0.5 M TMSOTf in CH_2Cl_2 (0.1 eq) was injected by syringe. This was then stirred at –60 °C for 30 minutes then allowed to warm to –40 °C over 45 m. The reaction was subsequently quenched with NEt_3, filtered using Celite, and concentrated. Column chromatography (2:1 to 1:2 hexanes/EtOAc) yielded the final product as a white solid (1.82 g, 95%). R_f 0.95 (1:2 hexanes/EtOAc); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 – 7.50 (m, 2H, Ph*H*), 7.40 – 7.30 (m, 3H, Ph*H*), 7.08 (dd, *J* = 6.7, 1.4 Hz, 1H, N*H*), 6.07 (ddd, *J* = 22.4, 5.7, 2.9 Hz, 2H, C*H*=C*H*), 5.59 (s, 1H, C*H*Ph), 5.23 (dd, *J* = 9.8, 9.3 Hz, 1H, GlcA H-3), 5.16 (t, *J* = 9.0 Hz, 1H, GlcA H-4), 5.09 (d, *J* = 8.3 Hz, 1H, GalN H-1), 5.04 (dd, *J* = 8.8, 7.6 Hz, 1H, GlcA H-2), 4.91 (d, *J* = 7.5 Hz, 1H, GlcA H-1), 4.67 (ddd, *J* = 11.0, 3.5, 1.8 Hz, 1H, GalN H-3), 4.43 (d, *J* = 3.6 Hz, 1H, GalN H-4), 4.32 (dd, *J* = 12.3, 1.6 Hz, 1H, GalN H-6a), 4.09 (d, *J* = 10.9 Hz, 1H, GalN H-6b), 4.02 (d, *J* = 9.9 Hz, 1H, GlcA H-5), 4.02 – 3.96 (m, 1H, -OC*H*), 3.85 – 3.78

(m, 1H, GalN H-2), 3.79 – 3.70 (m, 1H, OC*H*), 3.72 (s, 3H, CO_2CH_3), 3.65 (dd, *J* = 5.5, 4.3 Hz, 2H, 2 OC*H*), 3.63 – 3.60 (m, 2H, 2 OC*H*), 3.59 – 3.54 (m, 2H, 2 OC*H*), 3.52 – 3.50 (m, 1H, GalN H-5), 3.50 – 3.48 (m, 1H, OC*H*), 3.34 (td, *J* = 9.3, 1.1 Hz, 1H, OC*H*), 2.79 (bs, 1H, NB-*H*), 2.73 (bs, 1H, NB-*H*), 2.06 – 1.96 (m, 9H, 3 C(O)CH_3), 1.68 (tt, *J* = 8.8, 4.7 Hz, 1H, NB-*H*), 1.33 – 1.18 (m, 3H, NB-*H*), 1.12 – 1.04 (m, 1H, NB-*H*); HRMS: calculated $[M-H]^-$ 920.2072, determined: 920.2090.

2-(2-((1R,2S,4R)-Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-4,6-O-benzylidene-2-deoxy-2-acetamido-β-D-galactopyranose (**A-7**). To a solution of disaccharide glycoside **A-6** (0.405 g, 0.439 mmol, 1 eq) in 13 mL dry benzene, Bu_3SnH (0.72 mL, 2.86 mmol, 6 eq) and AIBN (12 mg, 0.073 mmol, 0.17 eq) were added. The mixture was stirred at RT for 1 hour with Ar bubbling through the solution. The reaction was then refluxed at 80 °C under Ar for 3 hours then quenched with NEt_3. Column chromatography (100% EtOAc to 100:1 to 20:1 EtOAc/MeOH) afforded the product as a white solid (0.310 g, 86%). R_f 0.45 (9:1 EtOAc/MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 – 7.52 (m, 2H, Ph*H*), 7.41 – 7.29 (m, 3H, Ph*H*), 6.14 – 6.03 (m, 2H, C*H*=C*H*), 5.98 (s, 1H, N*H*), 5.55 (s, 1H, C*H*Ph), 5.32 – 5.17 (m, 2H, GlcA H-3, GlcA H-4), 5.09 (dd, *J* = 8.2, 1.6 Hz, 1H, GalN H-1), 5.00 (t, *J* = 8.1 Hz, 1H, GlcA H-2), 4.92 (d, *J* = 7.6 Hz, 1H, GlcA H-1), 4.71 (dt, *J* = 11.2, 3.0 Hz, 1H, GalN H-3), 4.34 (d, *J* = 3.4 Hz, 1H, GalN H-4), 4.30 (dd, *J* = 12.4, 1.6 Hz, 1H, GalN H-6a), 4.06 (dd, *J* = 12.5, 1.8 Hz, 1H, GalN H-6b), 4.02 (d, *J* = 8.3 Hz, 1H,

GlcA H-5), 4.00 – 3.96 (m, 1H, OC*H*), 3.76 – 3.70 (m, 1H, OC*H*), 3.69 (s, 3H, CO_2CH_3), 3.67 – 3.61 (m, 4H, 4 OC*H*), 3.61 – 3.55 (m, 2H, 2 OC*H*), 3.54 – 3.46 (m, 3H, OC*H*, GalN H-2, GalN H-5), 3.36 (td, *J* = 9.2, 3.7 Hz, 1H, OC*H*), 2.79 (bs, 1H, NB-*H*), 2.73 (bs, 1H, NB-*H*), 2.05 – 1.93 (m, 12H, 4 C(O)CH_3), 1.72 – 1.65 (m, 1H, NB-*H*), 1.33 – 1.19 (m, 3H, NB-*H*), 1.09 (dtd, *J* = 11.7, 3.8, 2.3 Hz, 1H, NB-*H*); HRMS: calculated $[M-H]^-$ 820.3386, determined: 820.3384.

2-(2-((1R,2S,4R)-Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-2-deoxy-2-acetamido-β-D-galactopyranose (**A-8**). Acetamide **A-7** (0.310 g, 0.378 mmol) was suspended in 9 mL 80% AcOH. The suspension was stirred at 80 °C for 30 minutes, during which the compound dissolved. The reaction was concentrated, and column chromatography (9:1 EtOAc/MeOH) afforded the product as a white solid (0.273 g, 99%). R_f 0.18 (9:1 EtOAc/MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.69 (t, *J* = 7.0 Hz, 1H, N*H*), 6.15 – 6.04 (m, 2H, C*H*=C*H*), 5.26 (td, *J* = 9.4, 1.9 Hz, 1H, GlcA H-3), 5.16 (t, *J* = 9.6 Hz, 1H, GlcA H-4), 5.01 (ddd, *J* = 9.3, 7.9, 1.2 Hz, 1H, GlcA H-2), 4.78 (dd, *J* = 8.5, 2.9 Hz, 1H, GalN H-1), 4.74 (d, *J* = 8.0 Hz, 1H, GlcA H-1), 4.25 – 4.02 (m, 3H, GlcA H-5, GalN H-3, H-4), 3.98 – 3.84 (m, 2H, OC*H*, GalN H-2), 3.86 – 3.67 (m, 4H, OC*H*, CO_2CH_3), 3.70 – 3.44 (m, 8H, OC*H*, GalN H-5), 3.42 (bs, 1H, GalN O*H*), 3.43 – 3.29 (m, 1H, OC*H*), 3.28 (bs, 1H, GalN O*H*), 2.81 (bs, 1H, NB-*H*), 2.72 (bd, *J* = 6.8 Hz, 1H, NB-*H*), 2.09 – 1.93 (m, 12H, C(O)CH_3), 1.72 – 1.61 (m, 1H, NB-*H*), 1.37 – 1.15 (m, 3H, NB-*H*), 1.17 – 1.04 (m, 1H, NB-*H*); HRMS: calculated $[M-H]^-$ 730.2928, determined: 730.2945.

A.5 Elaboration to CS Sulfation Epitope Monomers

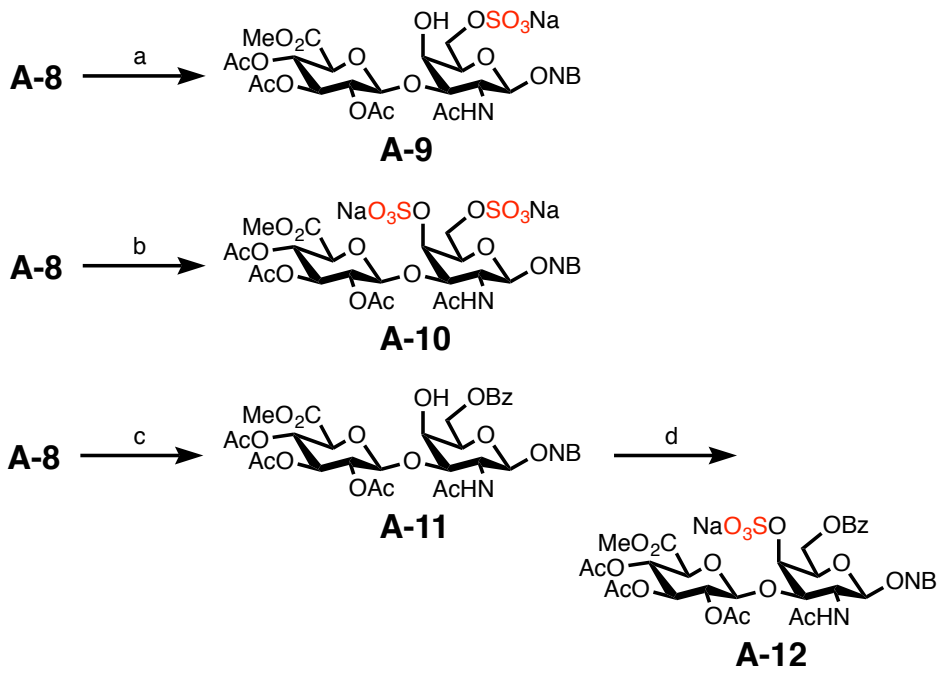

Figure A-3. Synthesis of CS sulfation epitope monomers.[5] (a) SO_3·TMA (2.7 eq), DMF, 50 °C, 40 min, 90% yield; (b) SO_3·TMA (25 eq), DMF, 50 °C, 1 d, 43% yield; (c) BzCN (2 eq), pyridine, RT, 16 h, 80% yield; (d) SO_3·TMA (6 eq), DMF, 50 °C, 1.5 d, 36% yield.

CS-C monomer (**A-9**). Diol **A-8** (100 mg, 0.137 mmol) was dissolved in 3.8 mL dry DMF under Ar. To this, sulfur trioxide trimethylamine complex (56 mg, 0.369 mmol, 2.7 eq) was added. The reaction was stirred at 50 °C for 40 m then cooled and added directly to an LH-20 column. After eluting the product from the LH-20 column, the crude mixture was subjected to cation exchange chromatography (Amberlite IR-120 Na^+ form) followed by silica column chromatography (10:2:1 EtOAc/MeOH/H_2O), which gave the product as a white solid (103 mg, 90%). R_f 0.37 (10:2:1 EtOAc/MeOH/H_2O); ^{1}H NMR (500

MHz, MeOD-d_4) δ 6.10 (ddd, *J* = 18.7, 6.3, 3.5 Hz, 2H, C*H*=C*H*), 5.32 (td, *J* = 9.5, 1.6 Hz, 1H, GlcA H-3), 5.11 (td, *J* = 9.8, 1.6 Hz, 1H, GlcA H-4), 4.99 (ddd, *J* = 9.6, 7.8, 1.6 Hz, 1H, GlcA H-2), 4.91 (dd, *J* = 7.9, 1.5 Hz, 1H, GlcA H-1), 4.48 (dd, *J* = 8.5, 1.6 Hz, 1H, GalN H-1), 4.30 (dt, *J* = 10.0, 1.7 Hz, 1H, GlcA H-5), 4.19 (d, *J* = 5.8 Hz, 2H, GalN H-6a, H-6b), 4.12 – 4.05 (m, 2H, GalN H-3, H-5), 4.01 – 3.91 (m, 1H, OC*H*), 3.87 – 3.76 (m, 2H, GalN H-2, H-4), 3.78 – 3.67 (m, 4H, OC*H*, CO_2CH_3), 3.70 – 3.51 (m, 7H, OC*H*), 3.43 (td, *J* = 9.3, 1.6 Hz, 1H, OC*H*), 2.81 (bs, 1H, NB-*H*), 2.74 (bs, 1H, NB-*H*), 2.14 – 1.93 (m, 12H, C(O)CH_3), 1.74 – 1.65 (m, 1H, NB-*H*), 1.39 – 1.21 (m, 3H, NB-*H*), 1.21 – 1.13 (m, 1H, NB-*H*); HRMS: calculated $[M-H]^-$ 810.2496, determined: 810.2511.

CS-E monomer (**A-10**). Diol **A-8** (250 mg, 0.343 mmol) was dissolved in 12 mL dry DMF under Ar. To this, sulfur trioxide trimethylamine complex (1.19 g, 8.55 mmol, 25 eq) was added. The reaction was stirred at 50 °C for 24 hours then cooled and added directly to an LH-20 column. After eluting the product from the column, the crude mixture was subjected to cation exchange chromatography (Amberlite IR-120 Na^+ form) followed by silica column chromatography (5:2:1 EtOAc/MeOH/H_2O), which yielded the product as a white solid (139 mg, 43%). R_f 0.18 (10:2:1 EtOAc/MeOH/H_2O); ^{1}H NMR (500 MHz, MeOD-d_4) δ 6.09 (ddd, *J* = 19.9, 6.0, 3.2 Hz, 2H, C*H*=C*H*), 5.31 (t, *J* = 9.4 Hz, 1H, GlcA H-3), 5.20 (t, *J* = 9.9 Hz, 1H, GlcA H-4), 5.07 (t, *J* = 8.7 Hz, 1H, GlcA H-2), 4.95 (d, *J* = 8.1 Hz, 1H, GlcA H-1), 4.86 (hidden under HDO peak, 1H, GalN H-4), 4.51 (d, *J* = 8.3 Hz, 1H, GalN H-1), 4.38 (dd, *J* = 11.8, 3.4 Hz, 1H,

GalN H-6a), 4.31 – 4.24 (m, 2H, GalN H-6b, GlcA H-5), 4.06 (t, *J* = 9.6 Hz, 1H, GalN H-2), 4.03 – 3.88 (m, 3H, OC*H*, GalN H-3, H-5), 3.75 (s, 3H, CO_2CH_3), 3.72 – 3.51 (m, 8H, OC*H*), 3.42 (t, *J* = 9.2 Hz, 1H, OC*H*), 2.79 (bs, 1H, NB-*H*), 2.73 (bs, 1H, NB-*H*), 2.11 – 1.95 (m, 12H, $C(O)CH_3$), 1.74 – 1.63 (m, 1H, NB-*H*), 1.38 – 1.20 (m, 3H, NB-*H*), 1.15 (dt, *J* = 11.7, 3.9 Hz, 1H, NB-*H*); HRMS: calculated $[M\text{-}Na]^-$ 912.1883; determined: 912.1905.

2-(2-((1R,2S,4R)-Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-(1→3)-6-O-benzoyl-2-deoxy-2-acetamido-β-D-galactopyranose (**A-11**). Diol **A-8** (200 mg, 0.273 mmol) was dissolved in 5.0 mL dry pyridine along with benzoyl cyanide (72 mg, 0.547 mmol, 2 eq) under Ar. The reaction was stirred at RT for 16 h. MeOH (2 mL) was added to quench the reaction, after which the solution was concentrated. Column chromatography (100% EtOAc) afforded the product as a white solid (0.251 g, 80%). R_f 0.16 (100% EtOAc); ^1H NMR (500 MHz, $CDCl_3$) δ 8.07 – 8.01 (m, 2H, Ph*H*), 7.60 – 7.53 (m, 1H, Ph*H*), 7.48 – 7.41 (m, 2H, Ph*H*), 6.14 – 6.06 (m, 2H, C*H*=C*H*), 6.00 – 5.91 (m, 1H, N*H*), 5.28 – 5.15 (m, 2H, GlcA H-3, H-4), 5.02 (dd, *J* = 9.0, 7.7 Hz, 1H, GlcA H-2), 4.93 (dd, *J* = 8.4, 4.1 Hz, 1H, GalN H-1), 4.74 (d, *J* = 7.7 Hz, 1H, GlcA H-1), 4.60 (dd, *J* = 11.4, 5.6 Hz, 1H, GalN H-6a), 4.56 (dd, *J* = 11.4, 6.9 Hz, 1H, GalN H-6b), 4.49 (td, *J* = 11.5, 3.2 Hz, 1H, GalN H-3), 4.15 – 4.11 (m, 1H, GalN H-4), 4.03 (dd, *J* = 9.4, 7.0 Hz, 1H, GlcA H-5), 3.94 (dt, *J* = 11.6, 4.1 Hz, 1H, OC*H*), 3.88 (t, *J* = 6.3 Hz, 1H, GalN H-5), 3.74 (dt, *J* = 11.2, 5.4 Hz, 1H, OC*H*), 3.66 (s, 3H, CO_2CH_3), 3.66 – 3.46 (m, 8H, GalN H-2,

OC*H*), 3.37 (dt, *J* = 16.0, 9.2 Hz, 1H, OC*H*), 2.81 (bs, 1H, NB-*H*), 2.73 (bd, *J* = 7.4 Hz, 1H, GalN 4-O*H*), 2.67 (bs, 1H, NB-*H*), 2.09 – 1.95 (m, 12H, C(O)CH_3), 1.74 – 1.64 (m, 1H, NB-*H*), 1.42 – 1.20 (m, 3H, NB-*H*), 1.11 (ddt, *J* = 11.3, 7.4, 3.9 Hz, 1H, NB-*H*); HRMS: calculated $[M+Cl]^-$ 870.2951, determined: 870.2966.

CS-A monomer (**A-12**). Disaccharide **A-11** (250 mg, 0.299 mmol) was dissolved in 15 mL dry DMF under Ar. To this, sulfur trioxide trimethylamine complex (250 mg, 1.79 mmol, 6 eq) was added. The reaction was stirred at 50 °C for 36 hours then cooled and added directly to an LH-20 column. After eluting the product from the LH-20 column, the crude mixture was subjected to cation exchange chromatography (Amberlite IR-120 Na^+ form) followed by silica column chromatography (10:2:1 EtOAc/MeOH/H_2O), which gave the product as a white solid (101 mg, 36%). R_f 0.55 (10:2:1 EtOAc/MeOH/H_2O); ^{1}H NMR (500 MHz, MeOD-d_4) δ 8.06 (dt, *J* = 9.0, 1.7 Hz, 2H, Ph*H*), 7.63 – 7.55 (m, 1H, Ph*H*), 7.47 (td, *J* = 7.6, 1.5 Hz, 2H, Ph*H*), 6.12 – 6.02 (m, 2H, C*H*=C*H*), 5.31 (td, *J* = 9.4, 2.4 Hz, 1H, GlcA H-3), 5.23 (td, *J* = 9.6, 2.3 Hz, 1H, GlcA H-4), 5.07 (ddd, *J* = 10.5, 8.4, 2.4 Hz, 1H, GlcA H-2), 5.02 – 4.90 (m, 2H, GlcA H-1, GalN H-3), 4.65 (dd, *J* = 6.5, 2.4 Hz, 2H, GalN H-6a, H-6b), 4.61 (d, *J* = 8.1 Hz, 1H, GalN H-1), 4.23 (dq, *J* = 9.9, 1.9 Hz, 1H, GlcA H-5), 4.10 (d, *J* = 11.0 Hz, 1H, GalN H-4), 4.04 – 3.96 (m, 2H, GalN H-2, H-5), 3.87 (dt, *J* = 11.4, 4.3 Hz, 1H, OC*H*), 3.77 – 3.66 (m, 4H, OC*H*, CO_2CH_3), 3.66 – 3.46 (m, 7H, OC*H*), 3.37 (ddt, *J* = 11.6, 9.4, 1.7 Hz, 1H, OC*H*), 2.78 (bs, 1H, NB-*H*), 2.71 (bs, 1H, NB-*H*), 2.06 – 1.93 (m, 12H, C(O)CH_3), 1.70 – 1.61 (m, 1H, NB-*H*), 1.36 – 1.26 (m, 2H, NB-*H*), 1.22 (ddd, *J* = 11.1, 8.4, 2.4

Hz, 1H, NB-*H*), 1.13 (dt, *J* = 11.7, 3.9 Hz, 1H, NB-*H*); HRMS: calculated $[M\text{-}Na]^-$ 914.2758, determined: 914.2770.

A.6 CS Polymerization

General polymerization procedure.[1, 2] A vial with a rubber septum was charged with disaccharide monomers (60.0 mg) under Ar. To this dry, degassed MeOH (0.25 mL) and DCE (1.25 mL) were added. The solution was heated to 55 °C, and then an appropriate amount (mol % to determine polymer size) of the fast-activating Grubbs 3rd generation catalyst (2.0 mg/mL in DCE) was rapidly added by syringe injection. This was stirred for 1 hours at 55 °C. The reaction was quenched with ethyl vinyl ether (0.30 mL) with stirring for 30 minutes at 55 °C and concentrated. The polymer residue was dissolved in a minimum of 1:1 DCM/MeOH and precipitated by adding dropwise to a vortexing solution of 1:1 hexanes/diethyl ether (50 mL). The precipitate was centrifuged down and the solvent decanted. This was repeated three times to give a white pellet.

Deprotection steps. The precipitated pellet was redissolved in 2:1 THF/H_2O (3 mL). The solution was cooled to 0 °C, and LiOH (1 M, 0.50 mL) and H_2O_2 (30%, 0.25 mL) were added. The reaction was stirred at 0 °C for 1 hour then allowed to warm to RT while stirring for another 12 h. To this, NaOH (4 M, 0.8 mL) and MeOH (2.0 mL) were added directly. The reaction was stirred at RT for 24 hours then neutralized with Amberlite IR-120 H^+. The solution was filtered through a 0.2 μm syringe filter, and organic solvents were removed. The remaining H_2O was removed by lyophilization. Gel filtration chromatography (G-25 Fine, ddH_2O) and subsequent lyophilization affording the deprotected polymers as a white solid. For long-term storage, polymers were subjected to

cation exchange with tributylammonium containing Amberlite IR-120. Amberlite IR-120 H^+ resin was loaded into a column and then washed with ten column volumes of 1 M TBACl, and excess reagent as removed by washing with ten column volumes of ddH_2O. Next, the CS polymers were dissolved at 1 mg/mL in ddH_2O and flowed over the column by gravity. The flowthrough was then combined, lyophilized, and subjected to gel filtration chromatography as previously described. Polymers were stored as the lyophilized powder at -80 °C.

A.7 References

1. M. Rawat, C. I. Gama, J. B. Matson, L. C. Hsieh-Wilson. Neuroactive chondroitin sulfate glycomimetics. *J Am Chem Soc*. **2008**, *130*: 2959-2961.
2. S.-G. Lee *et al.* End-functionalized glycopolymers as mimetics of chondroitin sulfate proteoglycans. *Chem Sci*. **2010**, *1*: 322-325.
3. G. J. Sheng, Y. I. Oh, S.-K. Chang, L. C. Hsieh-Wilson. Tunable heparan sulfate mimetics for modulating chemokine activity. *J Am Chem Soc*. **2013**, *135*: 10898-10901.
4. Y. I. Oh, G. J. Sheng, S.-K. Chang, L. C. Hsieh-Wilson. Tailored glycopolymers as anticoagulant heparin mimetics. *Angew Chem Int Ed*. **2013**, *52*: 11796-11799.
5. C. I. Gama *et al.* Sulfation patterns of glycosaminoglycans encode molecular recognition and activity. *Nat Chem Biol*. **2006**, *2*: 467-473.
6. C. Lopin, J.-C. Jacquinet. From polymer to size-defined oligomers: an expeditious route for the preparation of chondroitin oligosaccharides. *Angew Chem Int Ed*. **2006**, *45*: 2574-2578.