Supplementary Methods for

Sulfation Patterns of Glycosaminoglycans Encode Molecular Recognition and Activity

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Supporting Online Material

General Methods

Unless stated otherwise, reactions were performed in flame-dried glassware under a nitrogen or an argon environment, using freshly distilled solvents. Acetonitrile used for LC/MS was HPLC grade, and all aqueous solutions were made from nanopure water. All other 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). Visualization of the developed chromatogram was performed by fluorescence quenching, cerium ammonium molybdate stain, or ninhydrin stain as necessary. ICN silica gel (particle size 0.032 - 0.063 mm) was used for flash chromatography. Gel filtration chromatography (Sephadex® LH-20, G-10 and G-25 ultrafine) and ion exchange chromatography [Sephadex® C-25 (Na^+)] were used in order to achieve purification of the final products.

^1H NMR and proton decoupling spectra were recorded on Varian Mercury 300 (300 MHz) and Varian Mercury 600 (600 MHz) spectrometers and the ^1H NMR spectra are reported in parts per million (δ) relative to the residual solvent peak. Data for ^1H are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in Hz, and integration. ^{13}C NMR spectra were obtained on a Varian Mercury 300 (75 MHz) spectrometer and are reported in parts per million (δ) relative to the residual solvent peak. IR spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer and are reported in terms of frequency of absorption (cm^{-1}). A JASCO P-1010 was used to measure optical rotation. Mass spectra were obtained from the Protein/Peptide MicroAnalytical Laboratory and the Mass Spectrometry Facility at the California Institute of Technology.

Synthetic Methods

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (5), methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranosyluronate trichloroacetimidate (6), allyl 2-deoxy-2-trichloroacetamido-4,6-*O*-*p*-methoxybenzylidene-β-D-galactopyranoside (7), methyl (2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-trichloroacetamido-α-D-galactopyranoside trichloroacetimidate (8), allyl (methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (9) were synthesized as described previously [1].

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-(4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-trichloroacetamido-β-D-galactopyranoside (10). 8 (0.10 g, 0.088 mmol) and **9** (0.065 g, 0.074 mmol) were combined and azeotroped by co-evaporation with toluene (3x) and placed under high vacuum overnight to dry. The mixture was dissolved in CH_2Cl_2 (3.0 mL) and 4Å powdered molecular sieves added. The mixture was stirred for 1 h at rt and then cooled to –75 °C. Trimethylsilyl trifluoromethanesulfonate (0.*5 N* solution in CH_2Cl_2, 0.0033 g, 0.015 mmol, 30 μL) was cooled to –75 °C and added dropwise to the reaction mixture. After 10 min, the reaction mixture was warmed to –20 °C, stirred for 30 min and then quenched with TEA. The mixture was filtered and concentrated to afford a yellow oil. Purification of this oil by flash chromatography (30 → 40% EtOAc:hexanes containing 0.1% TEA) afforded **10** (59 mg, 44%) as a white solid. R_f 0.43 (60% EtOAc:hexanes). $[\alpha]_D^{25}$ = +13.4 (*c* = 0.5, CH_2Cl_2); IR (thin film on NaCl): υ = 3424, 2956, 2361, 1732, 1638, 1519, 1452, 1368, 1251, 1173, 1093, 1173, 1093, 1070, 1028; ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.88 – 7.80 (m, 8H, Ar*H*), 7.49 – 7.45 (m, 4H, Ar*H*), 7.38 – 7.28 (m, 8H, Ar*H*), 7.22 – 7.20 (m, 2H, Ar*H*), 7.06 (d, *J* = 8.4 Hz, 2H, C_6H_4OMe), 6.93 (d, *J* = 8.4 Hz, 2H, C_6H_4OMe), 6.85 (d, *J* = 6.6 Hz, 1H, N*H*), 6.74 (d, *J* = 8.4 Hz, 2H, Ph), 6.66 (d, *J* = 7.2 Hz, 1H, N*H*), 5.87 – 5.81 (m, 1H,

$OCH_2CH{=}CH_2$), 5.58 (dd, *J* = 7.8, 7.8 Hz, 1H, H-3 GlcA), 5.49 (s, 1H, MeOPhC*H*), 5.44 (dd, *J* = 8.7, 8.7 Hz, 1H, H-3 GlcA), 5.35 (m, 2H, H-2 GlcA, H-2 GlcA), 5.23 (d, *J* = 18.0 Hz, 1H, $OCH_2CH{=}CH_2$), 5.20 (s, 1H, MeOPhC*H*), 5.15 (m, 2H, $OCH_2CH{=}CH_2$, H-1 GlcA), 5.11 (d, *J* = 7.8 Hz, 1H, H-1 GalNAc), 5.03 (d, *J* = 7.2 Hz, 1H, H-1GlcA), 5.00 (d, *J* = 8.4 Hz, 1H, H-1 GalNAc), 4.68 (dd, *J* = 3.6, 10.8 Hz, 1H, H-3 GalNAc), 4.58 (dd, *J* = 9.0, 9.0 Hz, 1H, H-4 GlcA), 4.39 – 4.30 (m, 5H, $OCH_2CH{=}CH_2$, H-3 GalNAc, H-4 GalNAc, H-4 GlcA, H-6 GalNAc), 4.14 (m, 2H, H-4 GalNAc, H-5 GlcA), 4.06 (m, 3H, $OCH_2CH{=}CH_2$, H-5 GlcA, H-6 GalNAc), 3.83 (s, 3H, $PhOCH_3$), 3.81 – 3.68 (m, 4H, H-2 GalNAc, H-2 GalNAc, H-6 GalNAc, H-6 GalNAc), 3.80 (s, 3H, $PhOCH_3$), 3.80 (s, 3H, CO_2CH_3), 3.79 (s, 3H, CO_2CH_3), 3.48 (s, 1H, H-5 GalNAc), 3.10 (s, 1H, H-5 GalNAc), 0.72 (s, 9H, $(CH_3)_3CSi$), -0.09 (s, 3H, CH_3Si), -0.24 (s, 3H, CH_3Si); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 168.8, 168.4, 165.7, 165.4, 165.2, 165.1, 162.2, 161.9, 160.0, 159.8, 133.8, 133.4, 133.3, 133.1, 130.5, 130.4, 130.2, 130.1, 130.0, 129.9, 129.6, 129.5, 129.2, 129.1, 128.6, 128.5, 128.4, 127.9, 127.8, 118.2, 113.7, 113.4, 100.8, 100.5, 100.4, 100.2, 98.6, 97.7, 77.4, 76.4, 75.9, 75.8, 75.3, 75.0, 74.2, 74.1, 73.5, 73.4, 72.1, 71.9, 70.8, 70.6, 69.3, 68.4, 66.9, 55.7, 55.6, 54.8, 53.5, 52.8, 25.7, 18.1, -4.1, -4.8. ESI MS: *m/z*: calcd for $C_{83}H_{89}Cl_6N_2O_{29}Si$: 1819.4; found 1819.5 $[M + H]^+$.

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-(2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-2-deoxy-2-acetamido-β-D-galactopyranoside (11). Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-(4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-acetamido-β-D-galactopyranoside was prepared using a procedure modified from Bélot and coworkers [2]. To a solution of **10** (98 mg, 0.054 mmol) in benzene (1.7 mL) and *N*,*N*-dimethylacetamide (0.43 mL) were added tributylstannane (0.20 mL, 0.97 mmol) and 2,2'-azobisisobutyronitrile (5.2 mg). The reaction mixture was stirred at rt for 30 min and then heated at 80 °C. After 5 h, it was cooled to rt, concentrated to afford a yellow-white solid, and purified by flash chromatography (80% → 100% EtOAc:hexanes) to yield the

product as a white solid (80 mg, 92%). R_f 0.69 (100% EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.95 – 7.84 (m, 8H, Ar*H*), 7.52 – 7.43 (m, 6H, Ar*H*), 7.38 – 7.27 (m, 8H, Ar*H*), 7.21 (d, *J* = 9.0 Hz, 2H, C_6H_4OMe), 6.86 (d, *J* = 8.7 Hz, 2H, C_6H_4OMe), 6.80 (d, *J* = 9.0 Hz, 2H, Ph C_6H_4OMe), 5.89 – 5.76 (m, 1H, OCH_2CH=CH_2), 5.61 (dd, *J* = 7.2, 8.1 Hz, 1H, H-3 GlcA), 5.51 (s, 1H, MeOPhC*H*), 5.44 (dd, *J* = 8.7, 9.0 Hz, 1H, H-3 GlcA), 5.42 (d, *J* = 6.6 Hz, 1H, N*H*), 5.31 (dd, *J* = 6.6, 7.2 Hz, 1H, H-2 GlcA), 5.28 (dd, *J* = 7.2, 8.7 Hz, 1H, H-2 GlcA), 5.20 (dd, *J* = 0.9, 17.3 Hz, 1H, OCH_2CH=CH_2), 5.18 (s, 1H, MeOPhC*H*), 5.13 (d, *J* = 11.4 Hz, 1H, OCH_2CH=CH_2), 5.11 (d, *J* = 8.1 Hz, 1H, H-1 GlcA), 5.05 (d, *J* = 7.2 Hz, 1H, H-1 GalNAc), 4.98 (d, *J* = 6.6 Hz, 1H, N*H*), 4.89 (d, *J* = 7.5 Hz, 1H, H-1 GalNAc), 4.86 (d, *J* = 9.0 Hz, 1H, H-1GlcA), 4.75 (dd, *J* = 3.3, 10.8 Hz, 1H, H-3 GalNAc), 4.51 (dd, *J* = 8.1, 9.3 Hz, 1H, H-4 GlcA), 4.37 – 4.25 (m, 5H, OCH_2CH=CH_2, H-3 GalNAc, H-4 GalNAc, H-4 GlcA, H-6 GalNAc), 4.16 (d, *J* = 9.3 Hz, 1H, H-5 GlcA), 4.06 – 3.98 (m, 4H, OCH_2CH=CH_2, H-4 GalNAc, H-5 GlcA, H-6 GalNAc), 3.77 – 3.73 (m, 1H, H-6 GalNAc), 3.80 (s, 3H, PhOCH_3), 3.79 (s, 3H, PhOCH_3), 3.73 (s, 3H, CO_2CH_3), 3.70 (s, 3H, CO_2CH_3), 3.56 – 3.52 (m, 1H, H-6 GalNAc), 3.46 (s, 1H, H-5 GalNAc), 3.35 – 3.26 (m, 2H, H-2 GalNAc, H-2 GalNAc), 2.84 (s, 1H, H-5 GalNAc), 1.54 (s, 3H, HNC(O)CH_3), 1.50 (s, 3H, HNC(O)CH_3), 0.70 (s, 9H, $(CH_3)_3$CSi), -0.10 (s, 3H, CH_3Si), -0.25 (s, 3H, CH_3Si). ESI MS: *m/z*: calcd for $C_{83}H_{94}N_2O_{29}Si$: 1645.5; found 1645.4 $[M + Cl]^-$.

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-acetamido-*β*-D-galactopyranoside (42 mg, 0.026 mmol) was dissolved in CH_3CN (840 μL) and H_2O (90 μL), and the reaction was covered with aluminum foil and stirred in the dark. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (24 mg, 0.10 mmol) was added and the reaction stirred for 2 h at rt. The reaction mixture was subjected to Sephadex LH-20 (50% CH_2Cl_2:MeOH) to afford **11** as a pale pink solid (34 mg, 93%). R_f 0.2 (100% EtOAc). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.85 – 7.76 (m, 8H, Ar*H*), 7.47 – 7.42 (m, 4H, Ar*H*), 7.36 – 7.27 (m, 8H, Ar*H*), 5.79 – 5.66 (m, 1H, OCH_2CH=CH_2), 5.52 (dd, *J* = 8.4, 8.4 Hz, 1H, H-3

GlcA), 5.51 (dd, J = 8.4, 9.9 Hz, 1H, H-3 GlcA), 5.27 – 5.19 (m, 3H), 5.12 (dd, J = 1.6 Hz, 17.3 Hz, 1H, $OCH_2CH=CH_2$), 5.00 – 4.96 (m, 4H), 4.43 – 4.42 (m, 1H), 4.32 – 4.26 (m, 2H), 4.20 – 4.10 (m, 5H), 4.00 (d, J = 2.4 Hz, 1H), 3.96 – 3.88 (m, 3H), 3.70 (s, 3H, CO_2CH_3), 3.69 (s, 3H, CO_2CH_3), 3.41 – 3.35 (m, 2H), 3.17 – 3.10 (m, 3H), 3.04 – 3.00 (m, 1H), 1.20 (s, 3H, $HNC(O)CH_3$), 1.18 (s, 3H, $HNC(O)CH_3$), 0.66 (s, 9H, $(CH_3)_3CSi$), -0.10 (s, 3H, CH_3Si), -0.26 (s, 3H, CH_3Si). ESI MS: *m/z*: calcd for $C_{67}H_{82}N_2NaO_{27}Si$: 1397.5; found 1397.6 $[M + Na]^+$.

Allyl (sodium β-D-glucopyranosyluronate)-(1 → 3)-(4,6-di-*O*-sodium sulfonato-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(sodium β-D-glucopyranosyluronate)-(1 → 3)-4,6-di-*O*-sodium sulfonato-2-deoxy-2-acetamido-β-D-galactopyranoside (1: CS-E). To a solution of **11** (23 mg, 0.017 mmol) in DMF (600 μL) was added SO_3•TMA (90 mg, 0.64 mmol). The reaction stirred at 50 °C for 2 d, after which time additional SO_3•TMA (50 mg, 0.36 mmol) was added, and the reaction continued at 50 °C for 1 d. It was quenched with MeOH, concentrated to afford a yellow solid, and purified on Sephadex LH-20 (50% CH_2Cl_2:MeOH). The resulting crude product was purified by flash chromatography (6:2:1 EtOAc:MeOH:H_2O) to afford allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-(4,6-di-*O*-sodium sulfonato-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-4,6-di-*O*-sodium sulfonato-2-deoxy-2-acetamido-β-D-galactopyranoside as a white solid (24 mg, 84%). R_f 0.51 (6:2:1 EtOAc:MeOH:H_2O). ^{1}H NMR (300 MHz, CD_3OD): δ = 7.92 – 7.81 (m, 8H, Ar*H*), 7.55 – 7.45 (m, 4H, Ar*H*), 7.43 – 7.33 (m, 8H, Ar*H*), 5.87 – 5.73 (m, 1H, $OCH_2CH=CH_2$), 5.67 (dd, J = 9.0, 9.0 Hz, 1H, H-3 GlcA), 5.61 (dd, J = 9.3, 9.3 Hz, 1H, H-3 GlcA), 5.42 – 5.32 (m, 3H), 5.19 (dd, J = 1.6, 17.3 Hz, 1H, $OCH_2CH=CH_2$), 4.93 – 4.79 (m, 4H, H-4 GalNAc, H-4 GalNAc), 4.54 – 4.52 (m, 1H), 4.49 (dd, J = 9.0, 9.6 Hz, 1H, H-4 GlcA), 4.40 – 4.33 (m, 5H), 4.28 – 4.22 (m, 3H), 4.18 (d, J = 9.3 Hz, 1H, H-5 GlcA), 4.08 – 3.98 (m, 4H), 3.90 – 3.89 (m, 1H), 3.87 (s, 3H, CO_2CH_3), 3.86 – 3.85 (m, 2H), 3.83 (s, 3H, CO_2CH_3), 1.20 (s, 3H, $HNC(O)CH_3$), 1.18 (s, 3H, $HNC(O)CH_3$), 0.73 (s, 9H, $(CH_3)_3CSi$), -0.03 (s, 3H, CH_3Si), -0.19 (s, 3H, CH_3Si). ESI MS: *m/z*: calcd for $C_{67}H_{78}N_2Na_3O_{39}S_4Si$: 1759.3; found 1759.8 $[M - Na]^-$.

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(4,6-di-*O*-sodium sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranosyluronate)-(1 → 3)-4,6-di-*O*-sodium sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranoside (32 mg, 0.019 mmol) in a plastic centrifuge tube was dissolved in pyridine (585 μL) and THF (585 μL). The reaction was cooled to 0 °C and to this was added HF•pyridine (94 μL, 5.2 mmol). After stirring at 0 °C for 1 h and at rt overnight, the reaction mixture was loaded onto a Sephadex LH-20 column (50% CH_2Cl_2:MeOH). The product was concentrated, taken up in H_2O, and lyophilized to afford a white solid (45 mg, 91%) that was immediately used in the next reaction. R_f 0.36 (6:2:1 EtOAc:MeOH:H_2O). ESI MS: *m/z*: calcd for $C_{61}H_{67}N_2O_{39}S_4$: 1579.2; found 1579.4 $[M - H]^-$.

The alcohol was deprotected in a manner similar to a procedure from Lucas and coworkers [3]. The alcohol (45 mg, 0.028 mmol) was dissolved in THF (2.3 mL) and H_2O (1.7 mL) and cooled to 0 °C. To this were added 1 M aq. LiOH (330 μL) and 30% H_2O_2 (170 μL, 0.0015 mmol). The reaction stirred at 0 °C for 1 h and at rt for 12 h. At this time, 4 M NaOH (230 μL) and MeOH (1.7 mL) were added and the reaction stirred for another 12 h. It was then neutralized with Amberlyst IR-120 resin, filtered, and lyophilized to afford an orange solid. The product was purified by Sephadex G-25 UF (0.9 % NaCl in H_2O) and desalted with Sephadex G-25 UF (100% H_2O) to afford **1: CS-E** as a white solid upon lyophilization (23 mg, 70%). ^{1}H NMR (600 MHz, D_2O): δ = 5.94 – 5.88 (m, 1H, OCH_2CH=CH_2), 5.33 (dd, *J* = 1.5, 17.1 Hz, 1H, OCH_2CH=CH_2), 5.27 (d, *J* = 10.2 Hz, 1H, OCH_2CH=CH_2), 4.85 (s, 1H, H-4 GalNAc), 4.79 (d, *J* = 1.2 Hz, 1H, H-4 GalNAc), 4.61 (dd, *J* = 3.9, 7.8 Hz, 1H, H-1 GalNAc), 4.59 (d, *J* = 7.8 Hz, 1H, H-1 GalNAc), 4.49 (d, *J* = 7.8 Hz, 1H, H-1 GlcA), 4.47 (d, *J* = 7.8 Hz, 1H, H-1 GlcA), 4.35 (dd, *J* = 5.4, 13.2 Hz, 1H, OCH_2CH=CH_2), 4.29 (dd, *J* = 3.0, 11.4 Hz, 2H, H-6 GalNAc), 4.24 – 4.18 (m, 4H, OCH_2CH=CH_2, H-2 GalNAc, H-5 GalNAc), 4.13 (dd, *J* = 2.7, 8.7 Hz, 1H, H-3 GalNAc), 4.09 – 4.05 (m, 4H, H-6 GalNAc, H-2 GalNAc, H-3 GalNAc), 3.77 (dd, *J* = 9.6, 9.6 Hz, 1H, H-4 GlcA), 3.69 (d, *J* = 9.6 Hz, 1H, H-5 GlcA), 3.67 (d, *J* = 9.6 Hz, 1H, H-5 GlcA), 3.61 (dd, *J* = 9.0, 9.6 Hz, 1H, H-3 GlcA), 3.52 (dd, *J* = 9.0, 9.0 Hz, 1H, H-4

GlcA), 3.47 (dd, *J* = 9.0, 9.6 Hz, 1H, H-3 GlcA), 3.41 (dd, *J* = 8.4, 9.0 Hz, 1H, H-2 GlcA), 3.34 (dd, *J* = 7.8, 9.0 Hz, 1H, H-2 GlcA), 2.04 (s, 3H, HNC(O)CH_3), 2.01 (s, 3H, HNC(O)CH_3). ESI MS: *m/z*: calcd for $C_{31}H_{42}N_2Na_5O_{35}S_4$: 1245.0; found 1245.0 [*M* - Na]$^-$.

Allyl (*β*-D-glucopyranosyluronate)-(1 → 3)-(6-*O*-sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(*β*-D-glucopyranosyluronate)-(1 → 3)-6-*O*-sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranoside (2: CS-C). To a solution of **11** (25 mg, 0.018 mmol) in DMF (1.0 mL) was added SO_3•TMA (17 mg, 0.12 mmol). The reaction was stirred at 50 °C for 3 h, then cooled to rt, and eluted through a Sephadex LH-20 column (50% CH_2Cl_2:MeOH). The resulting crude product was purified by flash chromatography (6:1:1 EtOAc:MeOH:H_2O) to afford allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(6-*O*-sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranosyluronate)-(1 → 3)-6-*O*-sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranoside as a white solid (17 mg, 61%). R_f 0.32 (6:1:1 EtOAc:MeOH:H_2O). Also observed was allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(6-*O*-sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranosyluronate)-(1 → 3)-4,6-di-*O*-sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranoside (8.6 mg, 31%) which could be used to generate **1: CS-E** upon further sulfation. R_f 0.16 (6:1:1 EtOAc:MeOH:H_2O). Desired product ^{1}H NMR (600 MHz, CD_3OD): δ = 7.88 (dd, *J* = 6.0, 6.6 Hz, 4H, Ar*H*), 7.82 (dd, *J* = 6.6, 7.2 Hz, 4H, Ar*H*), 7.53 – 7.49 (m, 4H, Ar*H*), 7.40 – 7.33 (m, 8H, Ar*H*), 5.80 – 5.73 (m, 1H, OCH_2CH=CH_2), 5.60 (dd, *J* = 8.4, 9.0 Hz, 1H, H-3 GlcA), 5.56 (dd, *J* = 9.0, 9.6 Hz, 1H, H-3 GlcA), 5.27 (dd, *J* = 8.4, 8.4 Hz, 2H, H-2 GlcA), 5.16 (d, *J* = 16.8 Hz, 1H, OCH_2CH=CH_2), 5.04 – 5.00 (m, 3H), 4.48 (d, *J* = 7.8 Hz, 1H, H-1 GalNAc), 4.32 – 4.29 (m, 2H, H-1 GlcA, H-1 GalNAc), 4.25 – 4.20 (m, 3H), 4.17 – 4.08 (m, 5H), 3.98 – 3.95 (m, 3H), 3.84 – 3.72 (m, 9H, CO_2CH_3), 3.69 – 3.60 (m, 2H), 3.52 (dd, *J* = 5.4, 6.0 Hz, 1H), 1.19 (s, 6H, HNC(O)CH_3), 0.70 (s, 9H, $(CH_3)_3$CSi), -0.05 (s, 3H, CH_3Si), -0.21 (s, 3H, CH_3Si). ESI MS: *m/z*: calcd for $C_{67}H_{81}N_2O_{33}S_2Si$: 1533.4; found 1533.6 [*M* - H]$^-$.

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(6-*O*-sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranosyluronate)-(1 → 3)-6-*O*-sulfonato-2-deoxy-2-acetamido-*β*-D-galactopyranoside (4.6 mg, 0.0030 mmol) in a plastic centrifuge tube was dissolved in pyridine (55 μL) and THF (55 μL). The reaction was cooled to 0 °C and to this was added HF•pyridine (15 μL, 0.83 mmol). After stirring at 0 °C for 1 h and at rt overnight, the reaction mixture was loaded onto a Sephadex LH-20 column (50% CH_2Cl_2:MeOH). The product was concentrated, dissolved in H_2O, and lyophilized to afford a white solid (3.8 mg, 89%) that was immediately used in the next reaction. R_f 0.44 (6:2:1 EtOAc:MeOH:H_2O). ESI MS: *m/z*: calcd for $C_{61}H_{67}N_2O_{33}S_2$: 1419.3; found 1419.6 [*M* - H]$^-$.

The alcohol (1.2 mg, 0.00084 mmol) was dissolved in THF (70 μL) and H_2O (50 μL) and cooled to 0 °C. To this were added 1 M aq. LiOH (10.0 μL) and 30% H_2O_2 (5.0 μL, 0.044 μmol). The reaction was stirred at 0 °C for 1 h and at rt for 12 h. At this time, 4 M NaOH (7.0 μL) and MeOH (50 μL) were added and the reaction stirred for another 12 h. It was then neutralized with Amberlyst IR-120 resin, filtered, and lyophilized to afford an orange solid. The product was purified by Sephadex G-25 UF (0.9 % NaCl in H_2O) and desalted with Sephadex G-25 UF (100% H_2O) to afford **2: CS-C** as a white solid upon lyophilization (0.8 mg, 100%). ^{1}H NMR (600 MHz, D_2O): δ = 5.93 – 5.86 (m, 1H, OCH_2CH=CH_2), 5.31 (d, *J* = 17.4 Hz, 1H, OCH_2CH=CH_2), 5.26 (d, *J* = 10.8 Hz, 1H, OCH_2CH=CH_2), 4.53 (d, *J* = 8.4 Hz, 2H, H-1 GalNAc), 4.50 (d, *J* = 7.2 Hz, 1H, H-1 GlcA), 4.49 (d, *J* = 7.2 Hz, 1H, H-1 GlcA), 4.34 (dd, *J* = 5.1, 13.5 Hz, 1H, OCH_2CH=CH_2), 4.23 – 4.16 (m, 7H, OCH_2CH=CH_2, H-4 GalNAc, H-6 GalNAc), 4.04 – 4.00 (m, 2H, H-2 GalNAc), 3.99 (dd, *J* = 6.0, 6.0 Hz, 1H, H-5 GalNAc), 3.92 – 3.90 (m, 1H, H-5 GalNAc), 3.83 (d, *J* = 11.4 Hz, 2H, H-3 GalNAc), 3.72 – 3.67 (m, 3H, H-4 GlcA, H-5 GlcA), 3.58 (dd, *J* = 8.4, 9.0 Hz, 1H, H-3 GlcA), 3.49 (dd, *J* = 8.4, 9.0 Hz, 2H, H-3 GlcA, H-4 GlcA), 3.38 (dd, *J* = 8.4, 9.0 Hz, 1H, H-2 GlcA), 3.31 (dd, *J* = 8.4, 8.4 Hz, 1H, H-2 GlcA), 2.02 (s, 3H, HNC(O)CH_3), 2.00 (s, 3H, HNC(O)CH_3). ESI MS: *m/z*: calcd for $C_{31}H_{46}N_2NaO_{29}S_2$: 997.2; found 997.2 [*M* + Na - 2H]$^-$.

Allyl (β-D-glucopyranosyluronate)-(1 → 3)-(4-*O*-sulfonato-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(β-D-glucopyranosyluronate)-(1 → 3)-4-*O*-sulfonato-2-deoxy-2-acetamido-β-D-galactopyranoside (3: CS-A). 11 (60 mg, 0.44 mmol) was dissolved in pyridine (3.0 mL) and to this were added benzoyl cyanide (29 mg, 0.22 mmol) and 4-(dimethylamino)pyridine (13 mg, 0.11 mmol). The reaction stirred at rt for 12 h and was then concentrated to afford a yellow solid. The resulting crude product was purified by flash chromatography (100% EtOAc) to afford allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-(6-*O*-benzoyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-6-*O*-benzoyl-2-deoxy-2-acetamido-β-D-galactopyranoside (64 mg, 93%). R_f 0.86 (100% EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.02 – 7.98 (m, 3H, Ar*H*), 7.95 – 7.86 (m, 5H, Ar*H*), 7.58 – 7.30 (m, 22H, Ar*H*), 5.86 – 5.70 (m, 1H, OCH_2CH=CH_2), 5.55 (dd, *J* = 9.0, 9.0 Hz, 2H, H-3 GlcA), 5.47 (d, *J* = 6.9 Hz, 1H, N*H*), 5.40 (dd, *J* = 8.4, 8.4 Hz, 2H, H-2 GlcA, N*H*), 5.32 (dd, *J* = 8.4, 8.7 Hz, 1H, H-2 GlcA), 5.15 (dd, *J* = 1.4, 17.3 Hz, 1H, OCH_2CH=CH_2), 5.08 (dd, *J* = 1.4, 10.4 Hz, 1H, OCH_2CH=CH_2), 5.02 (d, *J* = 8.1 Hz, 1H, H-1 GalNAc), 4.94 (d, *J* = 8.4 Hz, 1H, H-1 GalNAc), 4.88 (d, *J* = 7.5 Hz, 1H, H-1 GlcA), 4.83 (d, *J* = 7.8 Hz, 1H, H-1 GlcA), 4.68 (dd, *J* = 3.2, 10.7 Hz, 1H, H-3 GalNAc), 4.61 (dd, *J* = 3.2, 10.7 Hz, 1H, H-3 GalNAc), 4.56 – 4.54 (m, 2H), 4.30 – 4.23 (m, 3H), 4.11 – 4.01 (m, 5H), 3.92 – 3.84 (m, 2H), 3.68 – 3.59 (m, 8H, CO_2CH_3), 3.20 – 3.12 (m, 1H, H-5 GalNAc), 3.06 – 2.97 (m, 1H, H-5 GalNAc), 1.33 (s, 3H, HNC(O)CH_3), 1.33 (s, 3H, HNC(O)CH_3), 0.70 (s, 9H, $(CH_3)_3$CSi), -0.07 (s, 3H, CH_3Si), -0.21 (s, 3H, CH_3Si). ESI MS: *m/z*: calcd for $C_{81}H_{91}N_2O_{29}Si$: 1583.6; found 1583.2 $[M + H]^+$.

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-(6-*O*-benzoyl-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-6-*O*-benzoyl-2-deoxy-2-acetamido-β-D-galactopyranoside (64 mg, 0.040 mmol) was dissolved in DMF (3.2 mL) and to this was added SO_3•TMA (170 mg, 1.2 mmol). The reaction mixture was stirred at 50 °C for 2 d, then cooled to rt, and loaded onto a Sephadex LH-20 column (50% CH_2Cl_2:MeOH). The resulting crude product was purified by flash chromatography (6:0.5:0.5

EtOAc:MeOH:H_2O) to afford allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(4-*O*-sulfonato-6-*O*-benzoyl-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranosyluronate)-(1 → 3)-4-*O*-sulfonato-6-*O*-benzoyl-2-deoxy-2-acetamido-*β*-D-galactopyranoside as a white solid (59 mg, 84%). R_f 0.40 (6:0.5:0.5 EtOAc:MeOH:H_2O). ^{1}H NMR (300 MHz, CD_3OD): δ = 8.06 (d, *J* = 7.5 Hz, 2H, Ar*H*), 7.99 (d, *J* = 7.5 Hz, 2H, Ar*H*), 7.96 – 7.92 (m, 4H, Ar*H*), 7.87 (d, *J* = 7.5 Hz, 2H, Ar*H*), 7.80 (d, *J* = 7.5 Hz, 2H, Ar*H*), 7.62 – 7.32 (m, 16H, Ar*H*), 7.26 (dd, *J* = 7.5, 7.5 Hz, 2H, Ar*H*), 5.84 – 5.71 (m, 1H, OCH_2CH=CH_2), 5.55 (dd, *J* = 9.0, 9.0 Hz, 1H, H-3 GlcA), 5.49 (dd, *J* = 9.0, 9.3 Hz, 1H, H-3 GlcA), 5.34 (dd, *J* = 7.8, 9.3 Hz, 1H, H-2 GlcA), 5.29 (dd, *J* = 8.1, 8.1 Hz, 1H, H-2 GlcA), 5.16 – 5.14 (m, 3H), 5.06 (d, *J* = 13.5 Hz, 1H, OCH_2CH=CH_2), 4.98 (d, *J* = 10.2 Hz, 1H, OCH_2CH=CH_2), 4.93 (s, 1H, H-4 GalNAc), 4.78 (s, 1H, H-4 GalNAc), 4.75 – 4.67 (m, 3H, H-1 GlcA, H-1 GalNAc) 4.51 (d, *J* = 7.8 Hz, 1H, H-1 GlcA), 4.43 (dd, *J* = 9.0, 9.0 Hz, 1H, H-4 GlcA), 4.29 (dd, *J* = 7.5, 9.3 Hz, 1H), 4.21 – 4.10 (m, 4H), 4.02 – 3.89 (m, 5H), 3.74 – 3.66 (m, 7H, CO_2CH_3), 3.47 (dd, *J* = 6.3, 6.3 Hz, 1H), 1.65 (s, 3H, HNC(O)CH_3), 1.54 (s, 3H, HNC(O)CH_3), 0.71 (s, 9H, $(CH_3)_3CSi$), -0.02 (s, 3H, CH_3Si), -0.19 (s, 3H, CH_3Si). ESI MS: *m/z*: calcd for $C_{81}H_{89}N_2O_{35}S_2Si$: 1741.5; found 1742.0 [*M* - H]$^-$.

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(4-*O*-sulfonato-6-*O*-benzoyl-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranosyluronate)-(1 → 3)-4-*O*-sulfonato-6-*O*-benzoyl-2-deoxy-2-acetamido-*β*-D-galactopyranoside (59 mg, 0.034 mmol) in a plastic centrifuge tube was dissolved in pyridine (1.3 mL) and THF (1.3 mL). The reaction was cooled to 0 °C and to this was added HF•pyridine (210 μL, 11.6 mmol). After stirring at 0 °C for 1 h and at rt overnight, the reaction mixture was loaded onto a Sephadex LH-20 column (50% CH_2Cl_2:MeOH). The product was concentrated, dissolved in H_2O, and lyophilized to afford a white solid (50 mg, 90%) that was immediately used in the next reaction. R_f 0.46 (6:1:0.5 EtOAc:MeOH:H_2O). ESI MS: *m/z*: calcd for $C_{75}H_{75}N_2O_{35}S_2$: 1627.4; found 1627.6 [*M* - H]$^-$.

The alcohol (50 mg, 0.031 mmol) was dissolved in THF (3.3 mL) and H_2O (2.5 mL) and cooled to 0 °C. To this were added 1 M aq. LiOH (370 μL) and 30% H_2O_2 (250 μL, 0.0022 mmol). The reaction stirred at 0 °C for 1 h and at rt for 12 h. At this time, 4 M NaOH (330 μL) and MeOH (2.5 mL) were added and the reaction stirred for another 12 h. It was then neutralized with Amberlyst IR-120 resin, filtered, and lyophilized to afford an orange solid. The product was purified by Sephadex G-25 UF (0.9 % NaCl in H_2O) and desalted with Sephadex G-25 UF (100% H_2O) to afford **3: CS-A** as a white solid upon lyophilization (21 mg, 70%). ^{1}H NMR (600 MHz, D_2O): δ = 5.94 – 5.86 (m, 1H, OCH_2CH=CH_2), 5.31 (dd, *J* = 1.5, 17.7 Hz, 1H, OCH_2CH=CH_2), 5.27 (dd, *J* = 1.2, 10.2 Hz, 1H, OCH_2CH=CH_2), 4.79 (d, *J* = 3.0 Hz, 1H, H-4 GalNAc), 4.74 (d, *J* = 3.0 Hz, 1H, H-4 GalNAc), 4.58 (d, *J* = 7.8 Hz, 1H, H-1 GalNAc), 4.56 (d, *J* = 7.8 Hz, 1H, H-1 GalNAc), 4.47 (d, *J* = 7.8 Hz, 1H, H-1 GlcA), 4.46 (d, *J* = 7.8 Hz, 1H, H-1 GlcA), 4.34 (dd, *J* = 5.1, 13.5 Hz, 1H, OCH_2CH=CH_2), 4.18 (dd, *J* = 3.3, 13.2 Hz, 1H, OCH_2CH=CH_2), 4.07 – 4.00 (m, 4H, H-2 GalNAc, H-3 GalNAc), 3.85 – 3.77 (m, 7H, H-4 GlcA, H-6 GalNAc, H-5 GalNAc), 3.66 (d, *J* = 9.6 Hz, 1H, H-5 GlcA), 3.66 (d, *J* = 10.2 Hz, 1H, H-5 GlcA), 3.58 (dd, *J* = 9.0, 9.0 Hz, 1H, H-3 GlcA), 3.52 (dd, *J* = 9.0, 9.6 Hz, 1H, H-3 GlcA), 3.46 (dd, *J* = 9.0, 9.0 Hz, 1H, H-4 GlcA), 3.39 (dd, *J* = 8.4, 8.4 Hz, 1H, H-2 GlcA), 3.34 (dd, *J* = 8.4, 8.4 Hz, 1H, H-2 GlcA), 2.04 (s, 3H, HNC(O)CH_3), 2.01 (s, 3H, HNC(O)CH_3). ESI MS: *m/z*: calcd for $C_{31}H_{46}N_2NaO_{29}S_2$: 997.2; found 997.2 [*M* + Na - 2H]$^-$.

Allyl (2,3-di-*O*-sulfonato-*β*-D-glucopyranosyluronate)-(1 → 3)-(2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(2,3-di-*O*-sulfonato-*β*-D-glucopyranosyluronate)-(1 → 3)-2-deoxy-2-acetamido-*β*-D-galactopyranoside (4: CS-R). 11 (22 mg, 0.016 mmol) was dissolved in CH_3CN (1.2 mL), and to this were added benzaldehyde dimethyl acetal (120 μL, 0.78 mmol) and DL-10-camphorsulfonic acid (3 mg). The reaction was stirred at rt for 12 h, quenched with TEA, and concentrated to afford an orange solid. The product was purified by flash chromatography (80% → 100% EtOAc:hexanes) to afford allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(4,6-*O*-benzylidene-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-*β*-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-benzylidene-2-deoxy-2-acetamido-

β-D-galactopyranoside (23 mg, 96%) as a white crystalline solid. R_f 0.71 (100% EtOAc). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.94 – 7.85 (m, 4H, Ar*H*), 7.86 (d, *J* = 8.1 Hz, 4H, Ar*H*), 7.58 – 7.42 (m, 7H, Ar*H*), 7.38 – 7.27 (m, 15H, Ar*H*), 5.89 – 5.76 (m, 1H, OCH_2CH=CH_2), 5.62 (dd, *J* = 7.8, 8.1 Hz, 1H, H-3 GlcA), 5.56 (s, 1H, MeOPhC*H*), 5.49 (dd, *J* = 8.7, 8.7 Hz, 1H, H-3 GlcA), 5.43 (d, *J* = 6.9 Hz, 1H, N*H*), 5.34 – 5.23 (m, 4H, H-2 GlcA, H-1 GalNAc, OCH_2CH=CH_2, MeOPhC*H*), 5.17 – 5.06 (m, 3H, H-2 GlcA, H-1 GalNAc, OCH_2CH=CH_2), 4.98 (d, *J* = 6.6 Hz, 1H, N*H*), 4.91 (d, *J* = 8.4 Hz, 1H, H-1 GlcA), 4.90 (d, *J* = 7.2 Hz, 1H, H-1GlcA), 4.77 (dd, *J* = 3.6, 11.1 Hz, 1H, H-3 GalNAc), 4.51 (dd, *J* = 8.4, 9.3 Hz, 1H, H-4 GlcA), 4.39 – 4.28 (m, 5H, OCH_2CH=CH_2, H-3 GalNAc, H-4 GalNAc, H-4 GlcA, H-6 GalNAc), 4.16 (d, *J* = 9.6 Hz, 1H, H-5 GlcA), 4.07 – 3.99 (m, 4H, OCH_2CH=CH_2, H-5 GlcA, H-6 GalNAc), 3.79 – 3.70 (m, 7H, PhOCH_3), 3.57 (d, *J* = 11.4 Hz, 1H), 3.48 (s, 1H, H-5 GalNAc), 3.35 – 3.29 (m, 2H), 2.87 (s, 1H, H-5 GalNAc), 1.54 (s, 3H, HNC(O)CH_3), 1.51 (s, 3H, HNC(O)CH_3), 0.70 (s, 9H, $(CH_3)_3$CSi), -0.11 (s, 3H, CH_3Si), -0.25 (s, 3H, CH_3Si). ESI MS: *m/z*: calcd for $C_{81}H_{90}N_2NaO_{27}Si$: 1573.5; found 1573.6 $[M + Na]^+$.

Allyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-(4,6-*O*-benzylidene-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(methyl 2,3-di-*O*-benzoyl-β-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-benzylidene-2-deoxy-2-acetamido-β-D-galactopyranoside (23 mg, 0.015 mmol) was dissolved in THF (2.0 mL) and H_2O (1.0 mL) and cooled to 0 °C. To this were added 1 M aq. LiOH (175 μL) and 30% H_2O_2 (120 μL, 1.1 μmol). The reaction was stirred at 0 °C for 1 h and at rt for 12 h. At this time, 4 M NaOH (1.5 mL) and MeOH (1.2 mL) were added and the reaction stirred for another 12 h. It was then neutralized with Amberlyst IR-120 resin, filtered, and lyophilized to afford an orange solid. The product was purified by flash chromatography (6:2:1 EtOAc:MeOH:H_2O) to afford allyl (methyl 4-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyluronate)-(1 → 3)-(4,6-*O*-benzylidene-2-deoxy-2-acetamido-β-D-galactopyranosyl)-(1 → 4)-(methyl β-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-benzylidene-2-deoxy-2-acetamido-β-D-galactopyranoside (9.3 mg, 56%). The yield was reduced due to partial migration of the *tert*-butyldimethylsilyl protecting group during the reaction. R_f 0.75 (6:2:1 EtOAc:MeOH:H_2O). ^{1}H NMR (600 MHz, CD_3OD): δ = 7.50 – 7.49 (m, 4H, Ar*H*), 7.35 – 7.31 (m, 6H,

Ar*H*), 5.92 – 5.86 (m, 1H, OCH_2CH=CH_2), 5.66 (s, 1H, MeOPhC*H*), 5.58 (s, 1H, MeOPhC*H*), 5.28 (d, *J* = 17.4 Hz, 1H, OCH_2CH=CH_2), 5.14 (d, *J* = 11.4 Hz, 1H, OCH_2CH=CH_2), 4.66 (d, *J* = 8.4 Hz, 1H, H-1 GalNAc), 4.58 (d, *J* = 7.8 Hz, 1H, H-1 GlcA), 4.52 (d, *J* = 8.4 Hz, 1H, H-1 GalNAc), 4.42 – 4.32 (m, 4H), 4.27 – 4.22 (m, 1H), 4.17 – 4.07 (m, 5H), 3.88 – 3.84 (m, 2H), 3.70 (dd, *J* = 9.0, 9.6 Hz, 1H), 3.64 – 3.61 (m, 3H), 3.53 – 3.52 (m, 3H), 3.45 (dd, *J* = 8.4, 9.0 Hz, 1H, H-3 GlcA), 3.43 (dd, *J* = 7.2, 9.0 Hz, 1H, H-3 GlcA), 3.37 (dd, *J* = 9.0, 9.0 Hz, 1H, H-2 GlcA), 3.16 (dd, *J* = 7.8, 8.4 Hz, 1H, H-2 GlcA), 2.01 (s, 3H, HNC(O)CH_3), 1.93 (s, 3H, HNC(O)CH_3), 0.87 (s, 9H, $(CH_3)_3CSi$), 0.09 (s, 3H, CH_3Si), 0.08 (s, 3H, CH_3Si). ESI MS: *m/z*: calcd for $C_{51}H_{69}N_2O_{23}Si$: 1105.4; found 1105.6 $[M - H]^-$.

Allyl (methyl 4-*O*-*tert*-butyldimethylsilyl-*β*-D-glucopyranosyluronate)-(1 → 3)-(4,6-*O*-benzylidene-2-deoxy-2-acetamido-*β*-D-galactopyranosyl)-(1 → 4)-(methyl *β*-D-glucopyranosyluronate)-(1 → 3)-4,6-*O*-benzylidene-2-deoxy-2-acetamido-*β*-D-galactopyranoside (9.3 mg, 0.0084 mmol) was dissolved in DMF (500 μL) and to this was added SO_3•TEA (30 mg, 0.17 mmol). The reaction mixture was stirred at 65 °C for 36 h, then cooled to rt, and loaded onto a Sephadex LH-20 column (50% CH_2Cl_2:MeOH). The product was concentrated, dissolved in H_2O, and lyophilized to afford a yellow solid (7.5 mg) that was immediately used in the next reaction. Prolonged reaction times or greater amounts of sulfating reagent led to degradation of the product during the reaction to afford a mixture of the desired product, various sulfated disaccharides, and loss of the *tert*-butyldimethylsilyl protecting group to afford the pentasulfated product. ESI MS: *m/z*: calcd for $C_{57}H_{83}N_3O_{35}S_4Si$: 1526.6; found 1526.4 $[M + TEA - 2H]^-$.

The tetrasulfated product (7.5 mg, 0.053 mmol) was dissolved in 0.01 M AcOH, pH 3.0 (500 μL) and the reaction stirred at rt for 4 d. During this time, the pH was carefully monitored to prevent loss of the allyl group. The reaction was then quenched with TEA and concentrated to afford a yellow syrup. The product was purified by Sephadex G-25 UF (H_2O) and Sephadex C-25 (Na^+) (H_2O) chromatography. **4: CS-R** was afforded as a white solid upon lyophilization (1.6 mg, 17%, 2 steps). ^{1}H NMR (600 MHz, D_2O): δ = 5.90 – 5.83 (m, 1H, OCH_2CH=CH_2), 5.28 (d, *J* = 17.4 Hz, 1H, OCH_2CH=CH_2), 5.22 (d, *J* = 10.8 Hz, 1H,

$OCH_2CH=CH_2$), 4.96 (d, J = 5.4 Hz, 1H, H-1 GlcA), 4.93 (d, J = 6.0 Hz, 1H, H-1 GlcA), 4.58 (d, J = 7.8 Hz, 1H, H-1 GalNAc), 4.52 (d, J = 8.4 Hz, 1H, H-1 GalNAc), 4.45 – 4.44 (m, 2H, H-2 GlcA), 4.38 (dd, J = 9.0, 9.0 Hz, 1H, H-4 GlcA), 4.30 (dd, J = 5.4, 13.2 Hz, 1H, $OCH_2CH=CH_2$), 4.18 – 4.10 (m, 4H, $OCH_2CH=CH_2$, H-4 GlcA, H-3 GlcA), 4.02 – 3.97 (m, 2H, H-2 GalNAc), 3.94 (dd, J = 8.7, 10.2 Hz, 2H, H-5 GlcA), 3.89 – 3.82 (m, 3H, H-3 GalNAc, H-4 GalNAc), 3.77 –3.73 (m, 3H, H-3 GalNAc, H-6 GalNAc), 3.67 – 3.64 (m, 4H, H-5 GalNAc, H-6 GalNAc), 2.02 (s, 6H, $HNC(O)CH_3$). ESI MS: *m/z*: calcd for $C_{31}H_{46}N_2O_{35}S_4$: 567.0; found 567.0 $[M - 2H]^{2-}$. In addition to the previously described conditions, CS-R could also be purified by liquid chromatography/mass spectrometry (LC/MS). Separations were performed on a Hewlett Packard (Agilent) 1100 LC-MSD with an Agilent Zorbax Stable Bond C18 column (80 Å pore, 4.6 x 250 mm). Eluent A was water/acetonitrile (80:20) and eluent B was water/acetonitrile (35:65). Tributylamine (15 mM) and ammonium acetate (50 mM) were added to both eluents. The mobile phase pH was adjusted to 7.0 with acetic acid [4]. Sample injection sizes were 40 μL, and a linear gradient (from 0 to 100% eluent B in 120 min) at a flow rate of 0.5 mL/min was used for elution. ESI mass spectra were obtained with the electrospray interface set in negative ionization mode with a fragmentor voltage of 60 V, a capillary voltage of –3500 V, and a source temperature of 350 °C. Nitrogen was used as a drying (12 L/min) and nebulizing gas (60 p.s.i.). Total ion chromatograms and mass spectra were processed using Agilent Chemstation. Optimization of the method was performed with pure CS-A (22 min), CS-E (34 min), and mixtures of CS-A and CS-E tetrasaccharides. CS-R eluted at 28 min.

Conjugation of CS Oligosaccharides to 1,2-(Bisaminooxy)ethane for Microarray Production

Ozonolysis of the anomeric allyl group and linkage of CS compounds **1**-**4** to 1,2-(bisaminooxy)ethane[5] proceeded as follows: oligosaccharide (0.51 μmol) was dissolved in MeOH (500 μL) and cooled to –78 °C. O_3 was bubbled through the reaction until a blue color persisted (1 min). The reaction was then purged with N_2 until colorless, quenched with Ph_3P beads (3 mg), and gradually warmed to rt over 12 h. It was filtered and the product concentrated to afford the desired aldehyde as a white solid. The aldehyde (0.51 μmol) was then reacted for 14 h at rt with 1,2-(bisaminooxy)ethane hydrochloride (1.4 mg,

15 μmol) that had been dissolved in H_2O (100 μL) and pH adjusted to 5.0 with 1 M NaOH. The resulting oxime product was purified using a SepPak C18 column (500 mg, H_2O) and Sephadex G-10 (CS-E disaccharide, H_2O) or Sephadex G-25 (tetrasaccharides, H_2O) to afford a white solid in quantitative yield (0.51 μmol). **CS-A aminooxy:** ESI MS: *m/z*: calcd for $C_{32}H_{48}N_4Na_3O_{31}S_2$: 1117.1; found 1117.0. **CS-C aminooxy:** ESI MS: *m/z*: calcd for $C_{32}H_{48}N_4Na_3O_{31}S_2$: 1117.1; found 1117.0. **CS-E aminooxy:** ESI MS: *m/z*: calcd for $C_{32}H_{46}N_4Na_5O_{37}S_4$: 1321.0; found 1321.0. **CS-R amminooxy:** ESI MS: *m/z*: calcd for $C_{32}H_{46}N_4Na_5O_{37}S_4$: 1321.0; found 1321.0.

The relative concentrations of the aminooxy oligosaccharides were calibrated to one another using the carbazole assay for uronic acid residues[6]. Briefly, the acid borate reagent (1.5 mL of 0.80 g sodium tetraborate, 16.6 mL H_2O, and 83.3 mL H_2SO_4) was added to 20-mL glass vials with Teflon caps. The aminooxy oligosaccharides (50 μL of a 0.2 mg/mL stock in H_2O) were added and the solution placed in a boiling H_2O bath for 10 min. Following addition of the carbazole reagent (50 μL of 0.1% *w/v* carbazole in 100% EtOH), the solution was boiled for 15 min. The absorbance was read at 530 nm and compared to a D-glucuronolactone standard in H_2O.

CS-C Antibody Development

The CS-C monoclonal antibody was generated according to standard immunological techniques, using the CS-C tetrasaccharide conjugated to keyhole limpet hemocyanin (KLH) as the antigen. The conjugation of tetrasaccharide **2** to KLH was performed as follows. Ozonolysis of the anomeric allyl group of the tetrasaccharide (0.51 μmol) as described above was followed by treatment with KLH (0.44 mg, 0.0063 μmol) and $NaCNBH_3$ (0.5 mg) in H_2O (pH'd with 5% K_2CO_3 to pH 9.5) for 2 d at rt. The product was then exhaustively dialyzed against 0.01 M Na_2HPO_4, 0.15 M NaCl, pH 7.4 at 4 °C and the protein concentration determined by BCA assay (Pierce). The epitope density was determined by comparing the conjugated proteins to the unconjugated proteins using the Habeeb assay[7]. In short, to the protein solution (10 μL) in PBS (40 μL) were added 0.1% trinitrobenzenesulfonic acid (50 μL) and 4% $NaHCO_3$, pH 9.5

(50 μL). The mixture was incubated at 40 °C for 2 h, quenched with 10% SDS (50 μL), 1 M HCl (25 μL), and H_2O (500 μL), and the absorbance at 363 nm was measured to afford an epitope density of 15.

Three Balb/c female mice, 4-6 weeks old, were primed and boosted at 2-week intervals for a total of 5 intraperitoneal injections (5 μg per injection). The CS-C-KLH conjugate was mixed with RIBI adjuvant (RIBI Immunochem) for the first two injections, and a final series of 3 boosts was performed without adjuvant. Bleeds were taken 1 week after each injection and monitored by dot blot analysis. The most responsive mouse was boosted and sacrificed after three days. Spleen cells were fused with HL-1 murine myeloma cells (Ventrex) using polyethylene glycol (PEG 1500, Boehringer-Mannheim) as described previously by Lebron *et al*.[8] Multiclonal and monoclonal cell lines were screened via ELISA and dot blot analysis.

ELISA Analysis

CS tetrasaccharides **1**-**3** were conjugated to bovine serum albumin (BSA) as follows. Ozonolysis of the anomeric allyl group of the CS-A, -C, and -E tetrasaccharides (0.51 μmol) as described above was followed by treatment of each compound with BSA (0.34 mg, 0.0051 μmol) and $NaCNBH_3$ (0.5 mg) in H_2O (pH 9.5 using K_2CO_3) for 2 d at rt. The CS-BSA conjugates were then exhaustively dialyzed against 0.01 M Na_2HPO_4, 0.15 M NaCl, pH 7.4 at 4 °C, and the protein concentrations were determined using the BCA assay (Pierce). The epitope densities were measured by comparing the conjugated proteins to the unconjugated proteins using the Habeeb assay. The epitope densities were as follows: CS-A conjugate = 14, CS-C conjugate = 16, CS-E conjugate = 14.

The BSA conjugates (1 μg/mL in 50 mM Na_2CO_3, pH 9.6) were added to a 384-well NUNC Maxisorp clear plate (25 μL per well), and the plate was sealed and incubated for 12 h at 4 °C. The wells were aspirated, washed four times with PBS containing 0.05% Tween-20 (PBST, 75 μL/wash), and blocked for 2 h at rt with 10% horse serum (Gibco) in PBS (75 μL). After the blocking step, the plate was washed four times with PBST, and the supernatants from the CS-C antibody producing cultures (25 μL) were added to the wells and incubated at rt for 2 h. Following aspiration, the wells were washed four times

with PBST and treated with a horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (Pierce; 1:10,000, 25 μL/well) in blocking buffer for 1 h at rt. The wells were again aspirated, washed four times with PBST, and then developed with ABTS liquid substrate solution (Sigma; 25 μL/well, solution at rt) for 30 min at rt. Color development was monitored on a Victor plate reader (PerkinElmer) at 405 nm. Only clones specific for the CS-C tetrasaccharide and with absorbance values greater than 1.0 were kept for subsequent dot blot screening.

Dot Blot Analysis

Immunoblotting analysis was performed by spotting solutions of the BSA conjugates (relative epitope density adjusted, 1-100 ng, 1 μL/spot) in 10 mM Tris•HCl, 0.02% Nonidet P-40, pH 7.5 onto 0.45 μm nitrocellulose, allowing the spots to air dry, and fixing the blots with 40% MeOH, 10% AcOH, 50% H_2O for 15 min at rt. The dot blots were then blocked for 30 min in 5% non-fat milk containing 50 mM Tris•HCl pH 7.4, 150 mM NaCl, 0.05% Tween-20 (TBST) followed by treatment with appropriate dilutions (1:500, 1:1000, and 1:2000) of the antibody serum in blocking buffer or supernatant from the monoclonal cell cultures for 2 h at rt. The blots were then washed with TBST three times for 10 min and treated with an HRP-conjugated goat anti-mouse antibody (Pierce; 1:10,000) in blocking buffer for 1 h at rt. The dot blots were washed with TBST three times for 10 min and visualized by chemiluminescence (SuperSignal West Pico, Pierce).

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