Discovery of Rare Sulfated N-Unsubstituted Glucosamine Based Heparan Sulfate Analog Analogs Selectively Activating Chemokines.

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1. General information

All chemicals were reagent grade and used as supplied except where noted. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates. Compounds were visualized by UV irradiation or dipping the plate in CAM/ninhydrin solution followed by heating. Column chromatography was carried out using force flow of the indicated solvent on Fluka Kieselgel 60 (230–400 mesh). $^1$H and $^{13}$C NMR spectra of all compounds were recorded on Jeol 400 MHz, and Bruker 600 MHz with cryo probe using residual solvents signals as an internal reference (CDCl$_3$ δH, 7.26 ppm, δC 77.3 ppm, CD$_3$OD δH 3.31 ppm, δC 49.0 ppm and D$_2$O δH 4.79 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (J) in Hz.

2. Synthesis of HS tetrasaccharide (1-13):

2.1 Synthesis of HS tetrasaccharide precursor:

![Chemical structure diagram]

4-Methylphenyl 2-azido-4-O-chloroacetyl-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-1-thio-α/β-D-glucopyranoside (16)

To a solution of compound 15 (1.5 g, 2.78 mmol) in CH$_2$Cl$_2$ (5 mL) and MeOH (10 mL) was added PTSA (0.95 g, 5.56 mmol) and stirred for 6 h. Upon completion, the reaction mixture was quenched using Et$_3$N and extracted with ethyl acetate and brine. The organic layer was collected, dried over Na$_2$SO$_4$, filtered, concentrated and purified through flash silica gel column chromatography (ethyl acetate/
Next, the obtained compound (1 g, 2.21 mmol) was dissolved in CH$_2$Cl$_2$ (10 mL) and stirred at ice cold temperature under N$_2$ atmosphere followed by the addition of imidazole (0.45 g, 6.64 mmol) and DMAP (0.10 g, 0.88 mmol). Finally, TBDPS-Cl was added dropwise and reaction mixture was left for stirring. After 12 h, the reaction was quenched using MeOH and extracted with brine. The combined organic layer was collected, dried over Na$_2$SO$_4$, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/10, v/v). Further, the solution of 6-O-TBDPS compound (1 g, 1.45 mmol) in CH$_2$Cl$_2$ (8 mL) and pyridine (2 mL) was stirred at ice cold temperature for 15 minutes under N$_2$ atmosphere before the addition of chloroacetic anhydride (0.32 g, 1.85 mmol). Reaction completion was monitored by TLC (approx. 20 min). After the completion of reaction, the mixture was washed with 1 N HCl and brine. The organic layer was collected, dried over Na$_2$SO$_4$, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/20, v/v) to obtain compound 16 in 95 % yield. ¹H NMR (400 MHz, Chloroform-d) (α anomer) δ 7.87 – 7.83 (m, 3H), 7.78 (s, 1H), 7.64 – 7.61 (m, 4H), 7.51 – 7.48 (m, 2H), 7.43 – 7.30 (m, 9H), 7.06 (d, J = 7.9 Hz, 2H), 5.59 (d, J = 5.4 Hz, 1H), 5.28 – 5.23 (m, 1H), 5.07 (d, J = 11.6 Hz, 1H), 4.80 (d, J = 11.6 Hz, 1H), 4.37 (dd, J = 10.1, 4.2, 2.3 Hz, 1H), 4.03 (dd, J = 10.2, 5.4 Hz, 1H), 3.86 – 3.81 (m, 1H), 3.67 (qd, J = 11.7, 3.3 Hz, 2H), 3.53 – 3.44 (m, 2H), 2.33 (s, 3H), 1.02 (s, 9H). ¹³C NMR (101 MHz, Chloroform-d) δ 165.85, 138.06, 135.81, 135.67, 134.92, 134.89, 133.28, 133.12, 133.11, 132.96, 132.43, 130.01, 129.81, 129.72, 129.65, 128.47, 128.13, 127.79, 127.70, 127.17, 126.40, 126.31, 126.15, 87.46, 79.35, 75.46, 71.68, 71.56, 64.24, 62.35, 40.43, 26.77, 21.24, 19.28. (β anomer) ¹H NMR (400 MHz, Chloroform-d) δ 7.87- 7.85 (m, 3H), 7.78 – 7.74 (m, 3H), 7.70 (dd, J = 7.7, 1.4 Hz, 2H), 7.57 (d, J = 8.1 Hz, 2H), 7.54 – 7.51 (m, 2H), 7.47 – 7.41 (m, 7H), 7.09 (d, J = 8.0 Hz, 2H), 5.21 (t, J = 6.8 Hz, 1H), 5.03 (d, J = 11.5 Hz, 1H), 4.80 (d, J = 11.6 Hz, 1H), 4.43 (d, J = 10.0 Hz, 1H), 3.80 (dd, J = 11.6, 1.9 Hz, 1H), 3.68 – 3.64 (m, 1H), 3.58 (dd, J = 13.2, 5.4 Hz, 1H), 3.51 – 3.45 (m, 4H), 2.36 (s, 3H), 1.10 (s, 9H). ¹³C NMR (101 MHz, Chloroform-d) δ 165.67, 139.07, 135.79, 135.78, 134.83, 134.54, 133.25, 133.12, 132.99, 132.92, 130.01, 129.90, 129.81, 128.48, 128.08, 127.83, 127.78, 127.25, 126.51, 126.40, 126.33, 126.16, 86.05, 82.60, 78.65, 75.49, 70.67, 64.75, 62.20, 26.80, 21.29, 19.26. HR-ESI-MS (m/z): [M+Na]$^+$ calcd for C$_{42}$H$_{44}$ClN$_3$O$_5$SSiNa, 788.2357; found, 788.2358.
1,6-Anhydro-[2-azido-4-O-chloroacetyl-6-O-tert-butyldiphenylsilyl-3-O-(2 naphthylmethyl)-
2-deoxy-α-D-glucopyranosyl](1→4)-O-2-O-benzoyl-3-O-benzyl-β-L-idopyranose (17)

A solution of donor 16 (1 g, 1.30 mmol) and acceptor 14 (0.41 g, 1.17 mmol) in CH₂Cl₂ (20 mL) was stirred under N₂ atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 2 h. The mixture solution was cooled down to -78 °C followed by the addition of NIS (0.46 g, 2.08 mmol) and TMSOTf (71 µL, 0.39 mmol). The temperature was gradually increased to -20 °C and reaction mixture was left for stirring for another 15 minutes. After 15 minutes reaction completion was monitored by TLC and quenched using few drops of Et₃N. Molecular sieves were filtered using celite and organic layer was washed with aqueous Na₂S₂O₃ and brine. The collected organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/12, v/v) to obtain compound 17 in 65 % yield.

¹H NMR (400 MHz, Chloroform-d) δ 8.08 – 8.06 (m, 2H), 7.85 (td, J = 7.0, 6.4, 2.9 Hz, 3H), 7.78 (s, 1H), 7.64 (dt, J = 8.0, 1.4 Hz, 4H), 7.61 – 7.58 (m, 1H), 7.30 – 7.27 (m, 2H), 7.26 – 7.22 (m, 2H), 5.55 (d, J = 1.7 Hz, 1H), 5.36 (d, J = 3.8 Hz, 1H), 5.16 – 5.09 (m, 2H), 5.06 (d, J = 11.5 Hz, 1H), 4.91 (d, J = 10.8 Hz, 1H), 4.80 (dd, J = 11.0, 9.5 Hz, 2H), 4.65 (t, J = 4.5 Hz, 1H), 4.19 (d, J = 7.6 Hz, 1H), 4.12 (t, J = 8.0 Hz, 1H), 4.07 (dd, J = 11.4, 5.3 Hz, 1H), 3.87 – 3.79 (m, 1H), 3.60 – 3.50 (m, 1H), 1.06 (s, 9H).

¹³C NMR (101 MHz, Chloroform-d) δ 165.84, 165.80, 137.92, 135.73, 135.68, 134.87, 133.55, 133.28, 133.14, 132.86, 132.74, 129.97, 129.47, 128.60, 128.52, 128.50, 128.07, 127.93, 127.89, 127.87, 127.82, 127.01, 126.45, 126.34, 125.96, 99.48, 99.37, 79.51, 78.94, 75.07, 74.96, 74.24, 71.75, 71.41, 65.77, 63.36, 62.64, 40.41, 26.82, 19.22. HR-ESI-MS (m/z): [M+Na]⁺ calcd for C₅₅H₅₆ClN₃O₁₁SiNa, 1020.3270; found, 1020.3271.

1,6-Anhydro-[2-azido-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-
glucopyranosyl](1→4)-O-2-O-benzoyl-3-O-benzyl-β-L-idopyranose (18)

To a solution of compound 17 (0.70 g, 0.7 mmol) in MeOH (5 mL) and pyridine (5 mL) was added thiourea (0.10 g, 1.4 mmol) and refluxed at 80 °C. After 1 h, volatiles were evaporated and residue was extracted with ethyl acetate, 1 N HCl and brine. The collected organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/8, v/v) to obtain compound 18 in 90 % yield. ¹H NMR (400 MHz, Chloroform-d) δ 8.06 (dd, J = 8.3, 1.2 Hz, 2H), 7.86 (dq, J = 9.6, 4.2 Hz, 4H), 7.68 (td, J = 7.6, 7.0, 1.5 Hz, 4H), 7.62 – 7.55
Phenyl-[2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl]-(1→4)-O-2-O-benzoyl-3-O-benzyl-6-O-acetyl-1-thio-α-L-idopyranoside (21)

A solution of compound 20 (1 g, 0.98 mmol) in Ac₂O (10 mL) was stirred at ice cold temperature for 15 minutes before the addition of Cu(OTf)₂ (0.035 g, 0.098 mmol). After 16 h, the reaction mixture was concentrated under reduced pressure and the residue was extracted with ethyl acetate, NaHCO₃ and washed with brine. The combined organic layer was dried over Na₂SO₄, filtered, concentrated and preceded further without any purification. Next, the as obtained acetylated solution of compound (0.98 g, 0.88 mmol), ZnI₂ (0.59 g, 1.84 mmol) and phenyl trimethylsilyl sulphide (0.50 g, 2.72 mmol) in CH₂Cl₂ (15 mL) was stirred under N₂ atmosphere for 2 h. Upon completion, the reaction mixture was filtered through celite, evaporated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/5, v/v) to obtain compound 21 in 85 % yield. ¹H NMR (400 MHz, Chloroform-d) δ 8.20 (dd, J = 6.8, 2.9 Hz, 2H), 7.87 – 7.77 (m, 3H), 7.72 (s, 1H), 7.71 – 7.68 (m, 2H), 7.68 – 7.66 (m, 1H), 7.61 – 7.58 (m, 3H), 7.54 – 7.48 (m, 4H), 7.44- 7.41 (m, 4H), 7.40 (s, 1H), 7.38 (d, J = 2.2 Hz, 4H), 7.37 – 7.35 (m, 4H), 7.34- 7.32 (m, 4H), 7.31- 7.29 (m, 2H), 7.21- 7.19 (m, 2H), 5.64 (s, 1H), 5.42 (s, 1H), 5.01 (d, J = 11.6 Hz, 1H), 4.92 (dt, J = 6.9, 4.0 Hz, 1H), 4.83 (d, J = 10.9 Hz, 1H), 4.78 (d, J = 11.7 Hz, 1H), 4.72 (d, J = 10.8 Hz, 1H), 4.65 (d, J = 3.6 Hz, 1H), 4.57 (d, J = 10.6 Hz, 1H), 4.44 (dd, J = 11.5, 8.1 Hz, 1H), 4.26 (d, J = 10.6 Hz, 1H), 4.20 (dd, J = 10.7, 3.6 Hz, 2H), 4.02 (dd, J = 11.6, 2.1 Hz, 1H), 3.91 (d, J = 11.5 Hz, 1H), 3.81 (s, 1H), 3.79 – 3.76 (m, 1H), 3.73 (d, J = 9.8 Hz, 1H), 3.68 (s, 1H), 3.38 (dd, J = 9.9, 3.6 Hz, 1H), 1.93 (s, 3H), 1.08 (s, 9H). ¹³C NMR (101 MHz, Chloroform-d) δ 170.36, 165.76, 138.04, 137.34, 135.88, 135.61, 135.54, 135.09, 133.48, 133.27, 133.05, 133.03, 131.71, 129.88,
129.70, 129.69, 128.90, 128.48, 128.43, 128.24, 128.12, 128.07, 127.98, 127.77, 127.73, 127.67, 127.59, 127.49, 127.00, 126.11, 126.06, 125.98, 98.96, 85.87, 80.76, 77.80, 75.34, 75.12, 75.05, 73.17, 72.83, 71.95, 69.74, 66.42, 64.12, 63.11, 62.18, 26.88, 20.72, 19.36.

HR-ESI-MS (m/z): [M+Na]⁺ calcd for C₆₈H₆₉N₅O₁₁SiNa, 1186.4320; found, 1186.4319

1,6-Anhydro-[(2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(2-benzoyl-3-O-benzyl-6-O-acetyl-α-L-idopyranosyl)-(1→4)-O-(2-azido-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-2-O-benzoyl-3-O-benzyl-β-L-idopyranose (22)

A solution of donor 21 (0.87 g, 0.74 mmol) and acceptor 18 (0.55 g, 0.60 mmol) in CH₂Cl₂ (15 mL) was stirred under N₂ atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 2 h. The mixture solution was cooled down to -10 °C followed by the addition of NIS (0.26 g, 1.18 mmol) and TMSOTf (26 µL, 0.148 mmol). After 15 minutes reaction completion was monitored by TLC and quenched using few drops of Et₃N. Molecular sieves were filtered using celite and organic layer was washed with aqueous Na₂S₂O₃ and brine. The collected organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/6, v/v) to obtain compound 22 in 95 % yield. ¹H NMR (400 MHz, Chloroform-d) δ 8.03 (dd, J = 6.5, 1.4 Hz, 4H), 7.83 – 7.81 (m, 1H), 7.78 – 7.75 (m, 1H), 7.71–7.58 (m, 15H), 7.48 – 7.27 (m, 29H), 7.22-7.19 (m, 9H), 5.51 (d, J = 1.7 Hz, 1H), 5.36 (s, 1H), 5.22 (s, 1H), 5.18 (d, J = 3.9 Hz, 1H), 5.13 (d, J = 11.3 Hz, 1H), 5.04 (dd, J = 8.2, 1.7 Hz, 1H), 4.87 (d, J = 11.3 Hz, 1H), 4.83 (d, J = 4.2 Hz, 1H), 4.81 – 4.80 (m, 1H), 4.75 (s, 1H), 4.73 – 4.67 (m, 3H), 4.63 (d, J = 10.6 Hz, 1H), 4.50 (dt, J = 6.8, 3.5 Hz, 1H), 4.41-4.38 (m, 1H), 4.36 (d, J = 4.78 Hz, 1H), 4.18 – 4.06 (m, 4H), 4.01 (tt, J = 7.0, 3.9 Hz, 2H), 3.93 – 3.86 (m, 4H), 3.83 (dd, J = 9.4, 2.9 Hz, 2H), 3.74 (t, J = 9.8 Hz, 3H), 3.66 – 3.61 (m, 2H), 3.59 – 3.51 (m, 2H), 3.38 (dd, J = 10.4, 3.8 Hz, 1H), 3.31 (dd, J = 10.1, 3.5 Hz, 1H), 1.41 (s, 3H), 1.03 (s, 9H), 1.00 (s, 9H). ¹³C NMR (101 MHz, Chloroform-d) δ 170.07, 165.75, 138.11, 137.91, 137.44, 135.87, 135.82, 135.66, 135.59, 135.45, 135.21, 133.43, 133.37, 133.29, 133.21, 133.15, 133.03, 132.98, 132.86, 129.89, 129.82, 129.76, 129.72, 129.55, 129.43, 128.66, 128.55, 128.48, 128.44, 128.40, 128.36, 128.21, 128.15, 127.98, 127.84, 127.79, 127.76, 127.71, 127.67, 127.62, 127.53, 127.00, 126.94, 126.30, 126.08, 126.06, 125.97, 125.76, 125.72, 99.35, 99.21, 98.32, 97.35, 80.38, 79.21, 79.04, 78.45, 77.70, 77.25, 76.95, 75.35, 75.30, 75.10, 74.95, 74.13, 73.46, 73.34, 73.00, 72.95, 72.90, 72.83, 68.87, 65.52, 65.34, 64.04,
Acetyl-O-[(2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(2-O-benzoyl-3-O-benzyl-6-O-acetyl-α-L-idopyranosyl)-(1→4)-O-(2-azido-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-2-O-benzoyl-3-O-benzyl-6-O-acetyl-α/β-L-idopyranoside (23)

A solution of compound 22 (1 g, 0.98 mmol) in Ac₂O (10 mL) was stirred at ice cold temperature for 15 minutes before the addition of Cu(OTf)₂ (0.035 g, 0.098 mmol). After 16 h, the reaction mixture was concentrated under reduced pressure and the residue was extracted with ethyl acetate, NaHCO₃ and washed with brine. The combined organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/5, v/v) to obtain compound 23 in 90% yield.

1H NMR (400 MHz, Chloroform-d) δ 8.16 – 8.11 (m, 3H), 8.05 (d, J = 7.7 Hz, 3H), 7.86 – 7.77 (m, 4H), 7.64 (ddq, J = 22.1, 13.3, 7.6, 6.4 Hz, 21H), 7.56 – 7.29 (m, 50H), 7.28 – 7.14 (m, 17H), 6.24 (d, J = 2.3 Hz, 1H), 5.42 (s, 1H), 5.25 – 5.16 (m, 3H), 4.95 – 4.67 (m, 13H), 4.61 (dd, J = 7.2, 3.4 Hz, 2H), 4.41 (dq, J = 16.1, 6.1, 5.4 Hz, 5H), 4.30 – 4.10 (m, 8H), 4.09 – 4.01 (m, 3H), 3.93 – 3.63 (m, 18H), 3.52 (s, 1H), 3.35 – 3.30 (m, 3H), 2.12 (d, J = 3.9 Hz, 2H), 1.97 (s, 2H), 1.90 (s, 3H), 1.28 (s, 2H), 1.25 (s, 3H), 1.05 (s, 15H), 1.00 (s, 13H).

13C NMR (101 MHz, Chloroform-d) δ 170.44, 170.31, 169.98, 169.97, 169.04, 168.91, 166.05, 165.76, 165.71, 138.18, 137.52, 137.51, 137.24, 135.90, 135.87, 135.64, 135.60, 135.49, 135.45, 135.23, 133.46, 133.40, 133.36, 133.34, 133.28, 133.25, 133.22, 133.17, 133.16, 133.02, 132.99, 132.74, 132.71, 129.90, 129.80, 129.76, 129.72, 129.65, 129.63, 129.50, 128.72, 128.69, 128.63, 128.60, 128.52, 128.47, 128.44, 128.36, 128.23, 128.18, 128.15, 128.13, 128.09, 128.05, 128.00, 127.98, 127.84, 127.76, 127.75, 127.71, 127.66, 127.63, 127.53, 127.43, 127.41, 127.37, 126.94, 126.10, 126.04, 126.00, 125.94, 125.88, 125.86, 125.69, 125.67, 125.61, 125.58, 98.52, 98.13, 97.91, 97.00, 96.98, 91.74, 90.56, 80.36, 79.11, 78.91, 77.64, 77.27, 75.31, 75.04, 73.97, 73.88, 73.83, 73.33, 73.29, 73.26, 72.93, 72.80, 72.68, 72.55, 72.46, 71.98, 68.63, 68.54, 67.80, 67.25, 64.65, 64.37, 64.29, 64.06, 62.48, 62.33, 62.26, 61.94, 26.87, 26.79, 21.01, 20.73, 20.66, 20.01, 19.95, 19.44, 19.34. HR-ESI-MS (m/z): [M+Na]⁺ calcd for C₁₁₉H₁₂₅N₆O₂₅Si₂Na, 2100.8137; found, 2100.8171.
Phenyl-[(2-azido-4-O-benzyl-6-O-tert-butylidiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-\(\beta\)-D-glucopyranosyl)-(1→4)-O-(2-O-benzoyl-3-O-benzyl-6-O-acetyl-\(\alpha\)-L-idopyranosyl)-(1→4)-O-(2-azo-6-O-tert-butylidiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl)](1→4)-O-2-O-benzoyl-3-O-benzyl-6-O-acetyl-1-thio-\(\alpha\)-L-idopyranoside (24)

A solution of compound 23 (0.98 g, 0.88 mmol), ZnI\(_2\) (0.59 g, 1.84 mmol) and phenyl trimethylsilyl sulphide (0.50 g, 2.72 mmol) in CH\(_2\)Cl\(_2\) (15 mL) was stirred under N\(_2\) atmosphere for 2 h. Upon completion, the reaction mixture was filtered through celite, evaporated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/5, v/v) to obtain compound 24 in 85 % yield. \(^1\)H NMR (400 MHz, Chloroform-d) \(\delta\) 8.12 (ddd, \(J = 5.7, 3.0, 1.6\) Hz, 2H), 8.03 (s, 1H), 8.01 (d, \(J = 1.3\) Hz, 1H), 7.82 – 7.79 (m, 1H), 7.77 – 7.74 (m, 1H), 7.67 – 7.56 (m, 15H), 7.48 – 7.43 (m, 7H), 7.40 – 7.37 (m, 7H), 7.35 – 7.33 (m, 4H), 7.31 – 7.28 (m, 14H), 7.25- 7.21 (m, 5H), 7.17 (d, \(J = 7.5\) Hz, 2H), 7.14 – 7.10 (m, 4H), 5.62 (s, 1H), 5.38 (s, 1H), 5.34 (s, 1H), 5.18 (s, 1H), 4.94 – 4.83 (m, 3H), 4.79 (d, \(J = 10.8\) Hz, 1H), 4.75 (d, \(J = 4.0\) Hz, 1H), 4.72 (d, \(J = 4.1\) Hz, 1H), 4.70 (d, \(J = 2.4\) Hz, 1H), 4.67 (d, \(J = 3.2\) Hz, 1H), 4.64 (d, \(J = 3.5\) Hz, 1H), 4.56 (d, \(J = 10.6\) Hz, 1H), 4.47 (d, \(J = 3.8\) Hz, 1H), 4.38 (dd, \(J = 5.5, 3.2\) Hz, 1H), 4.36 – 4.32 (m, 2H), 4.14 – 4.08 (m, 2H), 4.05 – 4.02 (m, 3H), 3.99 (t, \(J = 5.4\) Hz, 1H), 3.91 (dd, \(J = 5.6, 1.9\) Hz, 1H), 3.88 – 3.85 (m, 2H), 3.83 (t, \(J = 3.4\) Hz, 1H), 3.79 (d, \(J = 10.2\) Hz, 1H), 3.74 (d, \(J = 9.9\) Hz, 1H), 3.71 – 3.66 (m, 2H), 3.63 – 3.57 (m, 2H), 3.54 – 3.51 (m, 1H), 3.47 (s, 1H), 3.28 (d, \(J = 3.5\) Hz, 1H), 3.26 (d, \(J = 3.6\) Hz, 1H), 1.86 (s, 3H), 1.20 (s, 3H), 1.02 (s, 9H), 0.97 (s, 9H). \(^13\)C NMR (101 MHz, Chloroform-d) \(\delta\) 170.26, 169.96, 165.86, 165.70, 138.18, 137.54, 137.31, 135.90, 135.87, 135.65, 135.59, 135.46, 135.45, 135.23, 133.46, 133.39, 133.28, 133.26, 133.21, 133.14, 133.02, 132.70, 131.73, 129.90, 129.82, 129.76, 129.71, 129.66, 129.63, 128.91, 128.69, 128.60, 128.47, 128.43, 128.36, 128.25, 128.18, 128.12, 128.09, 127.98, 127.85, 127.82, 127.76, 127.75, 127.70, 127.66, 127.63, 127.52, 127.40, 127.34, 126.93, 126.10, 126.03, 125.99, 125.94, 125.85, 125.69, 125.57, 98.16, 98.03, 96.96, 96.84, 85.84, 80.36, 79.15, 77.63, 77.25, 75.30, 75.04, 74.97, 74.22, 73.28, 72.91, 72.83, 72.80, 72.59, 72.42, 71.56, 69.80, 68.54, 66.30, 64.62, 64.46, 64.06, 62.89, 62.27, 61.93, 26.87, 26.80, 20.65, 19.95, 19.44, 19.33. HR-ESI-MS (m/z): [M+N\(^+\)]

calcd for C\(_{123}\)H\(_{126}\)N\(_6\)O\(_{22}\)SSi\(_2\)Na, 2149.8080; found, 2149.8088.

N-benzyloxycarbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-6-O-tert-butylidiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-\(\beta\)-D-glucopyranosyl)-(1→4)-O-(2-O-benzoyl-3-O-benzyl-6-O-acetyl-\(\alpha\)-L-idopyranosyl)-(1→4)-O-(2-azo-6-O-tert-butylidiphenylsilyl-3-O-(2-
naphthylmethyl)-2-deoxy-α-D-glucopyranosyl]-{(1→4)-O-2-O-benzoyl-3-O-benzyl-6-O-acetyl-α-L-idopyranoside (25)

A solution of donor 24 (0.90 g, 0.42 mmol) and linker benzyl (3-hydroxypropyl)carbamate (0.097 g, 0.46 mmol) in CH₂Cl₂ (15 mL) was stirred under N₂ atmosphere in round bottom flask containing freshly dried 4 Å molecular sieves for 2 h. Next, NIS (0.15 g, 0.67 mmol) and TfOH (7.4 µL, 0.084 mmol) was added at room temperature and reaction completion was monitored by TLC, quenched using few drops of Et₃N. Molecular sieves were filtered using celite and organic layer was washed with aqueous Na₂S₂O₃ and brine. The collected organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/3, v/v) to obtain compound 25 in 90 % yield. ¹H NMR (400 MHz, Chloroform-d) δ 8.09 (dd, J = 6.4, 3.1 Hz, 2H), 8.01 – 7.99 (m, 1H), 7.82 – 7.78 (m, 1H), 7.76 – 7.74 (m, 1H), 7.67 – 7.54 (m, 14H), 7.51 (s, 1H), 7.47 – 7.45 (m, 2H), 7.43 (s, 1H), 7.40 (s, 1H), 7.39 (s, 2H), 7.37 (s, 2H), 7.34 – 7.27 (m, 24H), 7.24 – 7.20 (m, 6H), 7.17 – 7.11 (m, 6H), 5.63 (s, 1H), 5.37 (s, 1H), 5.18 (s, 1H), 5.08 (s, 2H), 5.03 (s, 1H), 4.96 (s, 1H), 4.88 (dd, J = 11.4, 4.5 Hz, 1H), 4.78 (d, J = 10.7 Hz, 2H), 4.74 (d, J = 11.3 Hz, 2H), 4.68 (d, J = 10.8 Hz, 1H), 4.64 (d, J = 3.6 Hz, 1H), 4.58 (dd, J = 17.4, 11.3 Hz, 2H), 4.39 (dt, J = 8.6, 4.4 Hz, 1H), 4.33 (dt, J = 11.2, 3.9 Hz, 2H), 4.27 – 4.24 (m, 2H), 4.10 (t, J = 9.4 Hz, 2H), 4.03 (d, J = 12.5 Hz, 1H), 3.99 – 3.93 (m, 3H), 3.89 (s, 1H), 3.87 – 3.80 (m, 5H), 3.74 (d, J = 11.9 Hz, 1H), 3.68 (t, J = 9.7 Hz, 2H), 3.61 (d, J = 10.3 Hz, 2H), 3.57 – 3.54 (m, 3H), 3.47 (s, 1H), 3.39 (s, 1H), 3.28 (d, J = 3.6 Hz, 1H), 3.25 (d, J = 3.3 Hz, 1H), 3.24 – 3.18 (m, 2H), 1.86 (s, 3H), 1.18 (s, 3H), 1.01 (s, 9H), 0.97 (s, 9H). ¹³C NMR (101 MHz, Chloroform-d) δ 170.37, 169.91, 165.79, 165.68, 156.55, 138.18, 137.54, 137.50, 136.72, 135.88, 135.86, 135.61, 135.58, 135.47, 135.23, 133.46, 133.42, 133.28, 133.23, 133.19, 133.14, 133.01, 132.70, 129.88, 129.84, 129.74, 129.69, 129.64, 129.63, 129.61, 129.59, 128.70, 128.66, 128.58, 128.54, 128.45, 128.38, 128.34, 128.30, 128.25, 128.17, 128.13, 128.10, 128.01, 127.97, 127.82, 127.74, 127.72, 127.68, 127.64, 127.61, 127.58, 127.51, 127.40, 127.34, 126.91, 126.08, 126.01, 125.95, 125.92, 125.84, 125.65, 125.56, 98.15, 98.11, 97.84, 96.93, 80.36, 79.09, 77.63, 77.24, 75.28, 75.02, 74.97, 73.82, 73.25, 73.16, 72.89, 72.80, 72.56, 72.50, 72.40, 72.38, 71.63, 68.79, 68.56, 67.01, 66.57, 65.12, 64.59, 64.42, 64.07, 63.04, 62.34, 62.21, 61.94, 39.64, 29.41, 26.86, 26.78, 20.63, 19.90, 19.44, 19.32. HR-ESI-MS (m/z): [M+Na]⁺ calcd for C₁₂₈H₁₃₆N₇O₂₅Si₂Na, 2248.8944; found, 2248.9016.
N-benzyloxycarbonyl-3-aminopropyl-\(O\)\{(2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-O-(3-O-benzyl-\(\alpha\)-L-idopyranosylurono-6,2-lactone)-(1\(\rightarrow\)4)-O-(2-azido-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl)\}-(1\(\rightarrow\)4)-O-3-O-benzyl-\(\alpha\)-L-idopyranoside (26)

To a solution of compound 25 (0.84 g, 0.37 mmol) in \(\text{CH}_2\text{Cl}_2\) (7 mL) and MeOH (7 mL) was added NaOMe (0.06g, 1.11 mmol) and stirred at room temperature. After 12 h, reaction mixture was quenched using Amberlite IR 120H\(^+\) resin, filtered, evaporated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/2.5, v/v) to obtain compound 26 in 95% yield. \(^1\)H NMR (400 MHz, Chloroform-d) \(\delta\) 7.81 – 7.68 (m, 13H), 7.57 (d, \(J = 8.5\) Hz, 2H), 7.51 – 7.44 (m, 15H), 7.41 – 7.35 (m, 11H), 7.33 – 7.30 (m, 4H), 7.29 – 7.27 (m, 6H), 7.17 – 7.14 (m, 3H), 5.72 (s, 1H), 5.10 (s, 1H), 5.05 (s, 3H), 5.01 – 4.94 (m, 2H), 4.88 – 4.84 (m, 2H), 4.80 (dd, \(J = 10.6, 4.7\) Hz, 2H), 4.73 (d, \(J = 11.3\) Hz, 2H), 4.68 (dd, \(J = 10.9, 3.7\) Hz, 2H), 4.63 – 4.51 (m, 2H), 4.25 (dt, \(J = 14.0, 6.9\) Hz, 2H), 3.99 (t, \(J = 9.5\) Hz, 1H), 3.89 – 3.83 (m, 7H), 3.81 – 3.71 (m, 8H), 3.61 – 3.54 (m, 8H), 3.48 (d, \(J = 11.5\) Hz, 1H), 3.36 (d, \(J = 8.3\) Hz, 1H), 3.22 (ddt, \(J = 18.0, 13.6, 5.6\) Hz, 2H), 2.70 (dt, \(J = 10.7, 5.2\) Hz, 1H), 2.16 (s, 1H), 1.85 (s, 2H), 1.10 (s, 9H), 1.05 (s, 9H). \(^1\)C NMR (101 MHz, Chloroform-d) \(\delta\) 156.68, 138.02, 137.50, 137.48, 136.61, 136.00, 135.84, 135.79, 135.60, 135.10, 134.86, 133.44, 133.32, 133.30, 133.15, 133.14, 133.08, 132.84, 132.64, 129.87, 129.82, 129.79, 129.69, 128.72, 128.51, 128.48, 128.45, 128.43, 128.27, 128.07, 128.04, 128.02, 127.90, 127.84, 127.79, 127.70, 127.67, 127.60, 127.56, 127.52, 127.08, 126.61, 126.32, 126.07, 126.00, 125.59, 100.99, 100.07, 94.79, 94.36, 81.25, 79.86, 77.75, 77.26, 76.02, 75.52, 75.06, 73.64, 72.94, 72.47, 72.32, 72.02, 71.69, 70.87, 70.30, 69.56, 66.99, 66.95, 66.88, 66.86, 66.85, 66.63, 66.51, 66.36, 64.61, 63.99, 62.76, 62.49, 61.79, 61.44, 39.68, 29.72, 26.93, 26.83, 19.46, 19.31. HR-ESI-MS (m/z): [M+Na]\(^+\) calcd for C\(_{110}\)H\(_{123}\)N\(_3\)O\(_{21}\)Si\(_2\)Na, 1957.8242; found, 1957.8326.

N-benzyloxycarbonyl-3-aminopropyl-\(O\)\{(2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-O-(3-O-benzyl-\(\alpha\)-L-idopyranosylurono-6,2-lactone)-(1\(\rightarrow\)4)-O-(2-azido-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl)\}-(1\(\rightarrow\)4)-O-3-O-benzyl-\(\alpha\)-L-idopyranosidurono-6,2-lactone (27)

To a solution of compound 26 (0.67 g, 0.34 mmol) in \(\text{CH}_2\text{Cl}_2\) (5 mL) and \(\text{H}_2\text{O}\) (5 mL) was added TEMPO (0.01g, 0.07 mmol), BAIB (0.54 g, 1.70
mmol) and stirred at room temperature. After 16 h, the reaction mixture was extracted using saturated aqueous NH₄Cl. The collected organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/3, v/v) to obtain compound 27 in 65 % yield. ¹H NMR (400 MHz, Chloroform-d) δ 7.89 – 7.78 (m, 7H), 7.71 – 7.68 (m, 4H), 7.65 – 7.61 (m, 4H), 7.51 – 7.29 (m, 36H), 7.22 – 7.17 (m, 3H), 5.84 (s, 1H), 5.47 (s, 1H), 5.25 (d, J = 12.0 Hz, 1H), 5.20 – 5.05 (m, 4H), 4.99 (d, J = 10.7 Hz, 1H), 4.95 (s, 1H), 4.92 (s, 1H), 4.87 (d, J = 11.6 Hz, 1H), 4.82 (d, J = 11.5 Hz, 1H), 4.76 (d, J = 8.9 Hz, 1H), 4.70 (d, J = 20.3 Hz, 1H), 4.65 – 4.62 (m, 1H), 4.49 (d, J = 12.4 Hz, 2H), 4.32 (d, J = 2.8 Hz, 1H), 4.24 (s, 1H), 4.21 – 4.14 (m, 3H), 4.01 – 3.85 (m, 5H), 3.82 – 3.76 (m, 3H), 3.71 (dt, J = 8.0, 3.3 Hz, 3H), 3.65 – 3.59 (m, 3H), 3.53 (dd, J = 14.1, 3.7 Hz, 1H), 3.48 – 3.45 (m, 2H), 3.34 (dt, J = 13.6, 6.6 Hz, 1H), 2.77 (dd, J = 9.4, 4.4 Hz, 1H), 1.89 – 1.87 (m, 2H), 1.07 (s, 18H). ¹³C NMR (101 MHz, Chloroform-d) δ 167.43, 167.04, 156.60, 138.13, 137.02, 136.86, 136.70, 136.21, 136.00, 135.88, 135.58, 135.56, 135.19, 133.65, 133.34, 133.30, 133.14, 133.01, 132.77, 132.74, 130.02, 129.85, 129.73, 129.70, 129.64, 128.52, 128.49, 128.42, 128.32, 128.23, 128.21, 128.19, 128.11, 128.08, 128.03, 127.93, 127.81, 127.70, 127.65, 127.53, 127.52, 127.19, 126.49, 126.19, 126.09, 126.02, 125.91, 124.87, 124.68, 99.84, 98.98, 96.78, 96.74, 80.99, 80.27, 80.00, 78.32, 78.06, 77.87, 77.77, 77.24, 76.10, 75.82, 74.95, 74.34, 72.65, 72.57, 72.49, 72.35, 72.25, 71.82, 69.42, 69.37, 69.11, 66.64, 63.34, 61.69, 61.47, 39.89, 29.48, 26.91, 19.36, 19.28. HR-ESI-MS (m/z): [M+Na]⁺ calcd for C₁₁₀H₁₁₅N₇O₂₁Si₂Na, 1949.7616; found, 1949.7651.

N-benzyloxy carbonyl-3-aminopropyl-O-[(2-acetamido-4-O-benzyl-6-O-tert-butyl diphenylisilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyloxy carbonyl-α-L-idopyranoslyuronono-6,2-lactone)-(1→4)-O-(2-acetamido-6-O-tert-butyl diphenylisilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyloxy carbonyl-α-L-idopyranosiduronono-6,2-lactone (28)

To a solution of compound 27 (0.40 g, 0.20 mmol) in THF (3 mL), AcOH (2 mL) and Ac₂O (2 mL) was added Zn dust (0.52 g, 8 mmol) and stirred for 12 h at room temperature. Upon completion, the reaction mixture was filtered through celite and volatiles were evaporated. The remaining residue was extracted with ethyl acetate, saturated NaHCO₃ and washed with brine. The collected organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography (ethyl acetate/ hexane= 1/2.5, v/v) to obtain compound 28 in 70 % yield. ¹H NMR (400 MHz, Chloroform-d) δ 7.92 – 7.86 (m, 3H), 7.82- 7.81 (m, 2H), 7.78 (d, J = 8.5 Hz, 1H), 7.76 – 7.74 (m, 1H), 7.71- 7.70 (m, 2H),
7.69 – 7.68 (m, 3H), 7.68 – 7.66 (m, 2H), 7.65 (d, J = 1.3 Hz, 1H), 7.58 (s, 1H), 7.53 (dt, J = 6.1, 2.7 Hz, 2H), 7.51 – 7.49 (m, 1H), 7.48 – 7.46 (m, 2H), 7.45 – 7.42 (m, 3H), 7.41 (s, 2H), 7.39 – 7.38 (m, 2H), 7.38 – 7.37 (m, 1H), 7.35- 7.31 (m, 7H), 7.29 (s, 1H), 7.28 – 7.27 (m, 7H), 7.26 – 7.24 (m, 3H), 7.23 – 7.20 (m, 3H), 7.18 – 7.16 (m, 2H), 7.05 (d, J = 7.3 Hz, 2H), 5.50 (s, 1H), 5.42 (t, J = 5.2 Hz, 1H), 5.16 (d, J = 3.6 Hz, 1H), 5.07 – 5.03 (m, 4H), 5.00 (s, 1H), 4.93 – 4.90 (m, 3H), 4.80 (d, J = 3.5 Hz, 1H), 4.77 (d, J = 2.1 Hz, 1H), 4.73 (d, J = 6.9 Hz, 1H), 4.63 (d, J = 12.4 Hz, 1H), 4.48 (s, 1H), 4.42 (d, J = 3.4 Hz, 1H), 4.37 (s, 1H), 4.33 (d, J = 2.5 Hz, 2H), 4.30 (d, J = 5.4 Hz, 1H), 4.27 – 4.21 (m, 1H), 4.09 (qd, J = 6.2, 4.2, 3.7 Hz, 3H), 4.02 – 3.89 (m, 7H), 3.86 – 3.70 (m, 2H), 3.68 – 3.59 (m, 5H), 3.53 (q, J = 10.8, 9.0 Hz, 2H), 3.36 (dt, J = 11.3, 6.0 Hz, 1H), 3.27 (dt, J = 13.8, 6.7 Hz, 1H), 1.83 – 1.80 (m, 2H), 1.59 (s, 3H), 1.39 (s, 3H), 1.09 (s, 9H), 1.06 (s, 9H). ^13C NMR (101 MHz, Chloroform- d ) δ 169.88, 169.77, 167.57, 167.18, 156.46, 138.08, 136.62, 136.59, 135.92, 135.89, 135.78, 135.64, 135.57, 133.55, 133.28, 133.20, 133.08, 133.02, 132.94, 132.85, 132.54, 129.96, 129.83, 129.63, 128.64, 128.50, 128.46, 128.33, 128.29, 128.22, 128.09, 128.04, 127.93, 127.90, 127.78, 127.71, 127.66, 127.52, 127.16, 126.58, 126.44, 126.38, 126.34, 126.28, 126.26, 98.94, 98.73, 98.38, 96.61, 79.70, 79.39, 79.08, 79.00, 78.65, 78.28, 78.04, 77.29, 76.88, 75.09, 74.98, 74.79, 72.84, 72.28, 72.22, 72.13, 71.74, 69.35, 68.57, 66.60, 61.94, 61.70, 52.92, 52.80, 39.16, 29.61, 26.94, 26.87, 23.17, 22.87, 19.26, 19.20. HR-ESI-MS (m/z): [M+H]^+ calcd for C_{114}H_{124}N_{3}O_{23}Si_{2}, 1958.8164; found, 1958.8163.

2.2 General procedure for benzyl ester formation:

To a solution of starting material (1 mmol) in THF/ H₂O (1/ 1) was added LiOH·H₂O (3 mmol) and stirred at room temperature for 2 h. Upon completion, the reaction mixture was quenched using Amberlite IR 120H⁺ resin, filtered, evaporated and dried. Next, the residue was dissolved in DMF and added BnBr (4 mmol), TBAI (1.4 mmol), NaHCO₃ (5 mmol) and stirred at 60 °C under N₂ atmosphere. After 2 h, the reaction mixture was extracted with ethyl acetate and washed with brine. The collected organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography.
N-benzyloxycarbonyl-3-aminopropyl-O-[(benzyl (2-amidom-4-O-benzyl-6-O-tert-butylidiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-(3-0-benzyl-α-L-idopyranosyluronate)-(1→4)-O-benzyl (2-amidom-6-O-tert-butylidiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosiduronic acid (39)

\begin{align*}
\text{H NMR} (400 \text{ MHz, Chloroform-}d) \delta \, & \text{7.74} – 7.66 (m, 3H), 7.65 – 7.41 (m, 13H), 7.38 – 7.35 (m, 2H), 7.33 – 7.11 (m, 37H), 7.07 (dd, J = 13.9, 6.6, 2.9 Hz, 4H), 6.94 \,(ddt, J = 8.6, 3.3, 1.7 \text{ Hz}, 1H), 6.85 \,(t, J = 7.5 \text{ Hz}, 2H), 6.77 \,(d, J = 7.2 \text{ Hz}, 2H), 5.44 – 5.36 (m, 2H), 5.04 \,(d, J = 12.4 \text{ Hz}, 1H), 4.96 \,(d, J = 3.6 \text{ Hz}, 1H), 4.93 – 4.88 (m, 4H), 4.77 – 4.73 (m, 4H), 4.70 – 4.67 (m, 2H), 4.61 \,(dd, J = 12.2, 6.2 \text{ Hz}, 2H), 4.56 \,(d, J = 2.7 \text{ Hz}, 2H), 4.53 \,(d, J = 4.4 \text{ Hz}, 1H), 4.51 – 4.39 (m, 3H), 4.08 \,(t, J = 9.5 \text{ Hz}, 1H), 3.95 – 3.93 (m, 2H), 3.85 \,(t, J = 3.9 \text{ Hz}, 1H), 3.76 \,(ddd, J = 22.1, 12.9, 7.5 \text{ Hz}, 5H), 3.68 \,(d, J = 4.5 \text{ Hz}, 1H), 3.64 – 3.57 (m, 4H), 3.53 – 3.47 (m, 3H), 3.43 – 3.30 (m, 5H), 3.19 \,(d, J = 10.6 \text{ Hz}, 1H), 3.16 – 3.11 (m, 1H), 1.78 – 1.65 (m, 2H), 0.98 (s, 9H), 0.94 (s, 9H).
\text{C NMR} (101 \text{ MHz, Chloroform-}d) \delta \, & 169.02, 168.76, 156.46, 138.24, 137.35, 137.32, 136.57, 135.99, 135.88, 135.86, 135.60, 135.19, 135.18, 134.81, 133.47, 133.34, 133.30, 133.26, 133.15, 133.05, 132.75, 129.74, 129.69, 129.67, 128.68, 128.62, 128.51, 128.41, 128.33, 128.27, 128.24, 128.21, 128.12, 128.01, 127.94, 127.92, 127.81, 127.77, 127.72, 127.65, 127.62, 127.55, 127.53, 127.50, 126.95, 126.30, 126.25, 126.05, 126.02, 125.96, 125.71, 125.51, 101.48, 100.99, 95.43, 80.83, 79.48, 79.46, 77.59, 77.24, 75.79, 75.17, 74.93, 74.29, 73.33, 72.96, 72.55, 72.22, 71.56, 71.39, 71.24, 68.44, 68.22, 68.00, 67.99, 67.21, 67.09, 67.08, 67.00, 66.64, 66.61, 64.15, 63.81, 62.13, 61.68, 39.83, 29.45, 26.94, 26.88. HR-ESI-MS (m/z): [M+Na]^+ calcd for C_{124}H_{131}N_{7}O_{23}Si_{2}Na, 2165.8766; found, 2165.8772.

N-benzyloxycarbonyl-3-aminopropyl-O-[(benzyl (2-acetamido-4-O-benzyl-6-O-tert-butylidiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-(3-0-benzyl-α-L-idopyranosyluronate)-(1→4)-O-benzyl (2-acetamido-6-O-tert-butylidiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosiduronic acid (42)

\begin{align*}
\text{H NMR} (400 \text{ MHz, Chloroform-}d) \delta \, & 7.82 – 7.77 (m, 3H), 7.76 – 7.74 (m, 2H), 7.73 – 7.72 (m, 2H), 7.71 \,(d, J = 1.6 \text{ Hz}, 1H), 7.70 \,(d, J = 1.6 \text{ Hz}, 2H), 7.69 – 7.68 (m, 2H), 7.67 – 7.66 (m, 1H), 7.65 \,(d, J = 1.3 \text{ Hz}, 1H), 7.63 \,(d, J = 1.4 \text{ Hz}, 1H), 7.55 \,(s,
1H), 7.45 (dd, J = 6.2, 3.3 Hz, 2H), 7.42 (s, 1H), 7.41 – 7.40 (m, 2H), 7.39 (d, J = 1.5 Hz, 2H), 7.37 (d, J = 2.1 Hz, 2H), 7.36- 7.35 (m, 2H), 7.35 (s, 4H), 7.32 – 7.30 (m, 6H), 7.29 (s, 1H), 7.28 – 7.22 (m, 17H), 7.20- 7.16 (m, 5H), 6.95 (t, J = 7.7 Hz, 2H), 6.86 (d, J = 7.9 Hz, 2H), 5.77 – 5.75 (m, 1H), 5.45 (t, J = 5.3 Hz, 1H), 5.37 (s, 1H), 5.16 (s, 1H), 5.14 – 5.09 (m, 2H), 5.03 – 5.00 (m, 1H), 5.00 – 4.93 (m, 1H), 4.91 (s, 1H), 4.88 (s, 2H), 4.85 (s, 1H), 4.81 (s, 1H), 4.77 – 4.74 (m, 2H), 4.70 (dd, J = 11.7, 3.6 Hz, 2H), 4.62 (d, J = 12.3 Hz, 1H), 4.56 (d, J = 11.4 Hz, 1H), 4.48 (d, J = 5.4 Hz, 1H), 4.46 – 4.42 (m, 2H), 4.30 (td, J = 10.7, 3.0 Hz, 1H), 4.26 – 4.15 (m, 2H), 4.05 (s, 1H), 3.95 (d, J = 9.5 Hz, 1H), 3.92 – 3.84 (m, 3H), 3.84 – 3.81 (m, 1H), 3.78 (d, J = 12.1 Hz, 2H), 3.73 (dd, J = 5.9, 1.8 Hz, 1H), 3.71 – 3.65 (m, 3H), 3.65 – 3.62 (m, 3H), 3.58 – 3.53 (m, 2H), 3.53 – 3.50 (m, 1H), 3.39 (dd, J = 16.5, 7.9 Hz, 2H), 3.30 – 3.21 (m, 1H), 2.90 – 2.86 (m, 1H), 2.74 (s, 1H), 1.80 – 1.79 (m, 2H), 1.67 (s, 3H), 1.29 (s, 3H), 1.07 (s, 9H), 1.04 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) δ 170.65, 170.42, 169.27, 168.97, 156.50, 138.43, 137.24, 137.15, 136.47, 135.96, 135.89, 135.70, 135.60, 134.57, 134.36, 133.54, 133.43, 133.27, 133.18, 133.09, 132.91, 132.80, 129.82, 129.62, 129.61, 128.66, 128.63, 128.55, 128.44, 128.33, 128.29, 128.18, 128.09, 128.00, 127.93, 127.87, 127.85, 127.79, 127.70, 127.62, 127.55, 126.73, 126.51, 126.17, 126.12, 125.97, 125.93, 101.13, 100.61, 96.80, 94.89, 79.93, 78.26, 77.73, 75.17, 75.13, 74.48, 74.26, 73.00, 72.73, 72.53, 72.45, 72.08, 71.87, 71.55, 69.35, 68.01, 67.60, 67.33, 67.18, 67.07, 66.67, 62.15, 62.05, 52.64, 52.41, 39.54, 29.73, 29.48, 26.93, 26.86, 22.83, 22.56, 19.47, 19.29. HR-ESI-MS (m/z): [M+Na]$^+$ calcd for C$_{128}$H$_{139}$N$_3$O$_{25}$Si$_2$Na, 2196.9134; found, 2196.9077.

2.3 General procedure for selective NAP deprotection:

To a solution of starting material (1 mmol) in CH$_2$Cl$_2$ (6 mL) and H$_2$O (340 µL) was added DDQ (5 mmol) portion wise over the interval of 20 min. After 1 h, the reaction mixture was quenched using NaHCO$_3$, extracted with CH$_2$Cl$_2$ and washed with brine. The collected organic layer was
dried over Na$_2$SO$_4$, filtered, concentrated and purified through silica gel column chromatography.

N-benzyloxy carbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosyluronono-6,2-lactone)-(1→4)-O-[(2-azido-6-O-tert-butyldiphenylsilyl-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosidurono-6,2-lactone (31)

$^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.72 (t, J = 1.3 Hz, 1H), 7.70 – 7.67 (m, 3H), 7.63 – 7.61 (m, 4H), 7.46 – 7.42 (m, 3H), 7.41 – 7.34 (m, 12H), 7.33 – 7.29 (m, 13H), 7.26 – 7.23 (m, 4H), 5.82 (t, J = 5.4 Hz, 1H), 5.12 – 5.08 (m, 3H), 5.04 (dd, J = 2.3, 1.0 Hz, 1H), 4.99 (d, J = 3.8 Hz, 1H), 4.91 (d, J = 3.6 Hz, 1H), 4.81 – 4.69 (m, 3H), 4.62 (d, J = 12.4 Hz, 1H), 4.54 (d, J = 11.6 Hz, 1H), 4.45 – 4.42 (m, 2H), 4.36 (dd, J = 5.9, 3.3 Hz, 2H), 4.22 – 4.19 (m, 1H), 4.13 – 4.11 (m, 1H), 4.03 (t, J = 2.6 Hz, 1H), 4.00 – 3.92 (m, 5H), 3.90 (dd, J = 12.0, 1.8 Hz, 1H), 3.81 – 3.79 (m, 2H), 3.77 – 3.75 (m, 2H), 3.72 (s, 1H), 3.70 – 3.68 (m, 2H), 3.65 – 3.59 (m, 2H), 3.49 – 3.41 (m, 1H), 3.35 – 3.28 (m, 1H), 3.24 (dd, J = 10.4, 3.7 Hz, 1H), 3.18 (dd, J = 10.5, 3.7 Hz, 1H), 2.46 (s, 1H), 1.86 (s, 1H), 1.09 (s, 9H), 1.06 (s, 9H). 13C NMR (101 MHz, Chloroform-d) $\delta$ 167.28, 166.35, 156.61, 137.96, 136.92, 136.69, 136.53, 135.95, 135.79, 135.67, 135.57, 135.32, 134.02, 132.78, 130.02, 129.89, 129.82, 129.76, 128.69, 128.65, 128.54, 128.48, 128.32, 128.23, 128.18, 128.16, 128.10, 128.09, 128.00, 127.93, 127.86, 127.72, 127.70, 127.60, 100.17, 99.91, 97.84, 96.72, 81.04, 80.48, 79.81, 79.06, 78.70, 78.06, 77.23, 74.89, 72.67, 72.55, 72.27, 71.67, 71.17, 71.04, 70.29, 69.61, 69.46, 69.29, 66.63, 62.68, 62.43, 62.26, 61.72, 39.87, 29.44, 26.94. HR-ESI-MS (m/z): [M+Na]$^+$ calcd for C$_{88}$H$_{99}$N$_{22}$O$_{24}$Si$_2$Na, 1668.6330; found, 1668.6327.

N-benzyloxy carbonyl-3-aminopropyl-O-[(benzyl (2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosyluronate)-(1→4)-O-(benzyl (2-azido-6-O-tert-butyldiphenylsilyl-2-deoxy-α-D-glucopyranosyl))-(1→4)-O-3-O-benzyl-α-L-idopyranosiduronate (47)

$^1$H NMR (400 MHz, Chloroform-d) $\delta$ 7.67 (tdd, J = 8.0, 4.1, 1.8 Hz, 8H), 7.42 – 7.28 (m, 19H), 7.25 – 7.20 (m, 15H), 7.19 – 7.15 (m, 6H), 7.10 (dd, J = 7.2, 2.5 Hz, 2H), 5.52 (t, J = 5.2 Hz, 1H), 5.16 (d, J = 3.5 Hz, 1H), 5.11 (d, J = 12.1 Hz, 1H), 5.04 – 4.94 (m, 6H), 4.81 (d, J = 3.3 Hz, 2H), 4.75 – 4.70 (m, 4H), 4.52 (d, J = 8.7 Hz, 3H), 4.30 (d, J = 2.0 Hz, 1H), 4.10 – 4.08 (m, 1H), 4.03 (s, 1H), 3.88 – 3.70 (m, 11H), 3.65 – 3.56 (m, 3H),
3.55 – 3.46 (m, 4H), 3.40 (td, J = 10.9, 10.4, 4.7 Hz, 2H), 3.34 (dd, J = 10.2, 3.7 Hz, 1H), 3.20 (ddt, J = 12.6, 7.0, 4.5 Hz, 1H), 2.95 (d, J = 9.3 Hz, 1H), 1.78 (s, 2H), 1.08 (s, 9H), 1.06 (s, 9H). $^{13}$C NMR (101 MHz, Chloroform-d) $\delta$ 169.04, 168.53, 156.46, 138.23, 137.34, 137.30, 136.56, 135.84, 135.74, 135.79, 135.74, 135.64, 134.78, 134.55, 134.55, 133.41, 133.27, 133.25, 133.12, 129.83, 129.77, 129.74, 129.70, 128.70, 128.64, 128.62, 128.59, 128.51, 128.50, 128.40, 128.27, 128.11, 128.08, 128.05, 128.00, 127.97, 127.78, 127.75, 127.61, 102.91, 101.42, 96.09, 94.99, 80.84, 77.92, 77.24, 74.96, 74.24, 73.00, 72.65, 72.41, 72.19, 71.94, 71.80, 71.25, 71.09, 69.65, 69.53, 68.00, 67.66, 67.24, 67.13, 66.95, 66.59, 63.27, 63.02, 62.19, 61.96, 39.84, 29.41, 26.93.

2.4 General procedure for desilylation pre O- sulfation:

A solution of starting material (1 mmol) in pyridine (1 mL) was stirred at ice cold temperature under $N_2$ atmosphere. After 15 minutes, 70 % HF.py complex (5 mmol) was added drop wise and reaction mixture was stirred for 12 h. Upon completion, the mixture was extracted with ethyl acetate and washed with 1 N HCl and brine. The combined organic layer was dried over Na$_2$SO$_4$, filtered, concentrated and purified through silica gel column chromatography.

N-benzyloxycarbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-3-O-(2-naphthylmethyl)-2-deoxy-$\alpha$-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-$\alpha$-L-idopyranosyluronono-6,2-lactone)-(1→4)-O-(2-azido-3-O-(2-naphthylmethyl)-2-deoxy-$\alpha$-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-$\alpha$-L-idopyranosidurono-6,2-lactone (29)
$^1$H NMR (400 MHz, Chloroform-d) δ 7.92 (s, 1H), 7.89 – 7.76 (m, 6H), 7.72 (s, 1H), 7.53 – 7.50 (m, 2H), 7.49 – 7.45 (m, 3H), 7.44 – 7.40 (m, 2H), 7.38 (s, 2H), 7.37 – 7.31 (m, 8H), 7.30 – 7.29 (m, 7H), 7.24 (dd, J = 7.5, 1.9 Hz, 2H), 5.75 (t, J = 5.1 Hz, 1H), 5.35 (s, 1H), 5.16 (d, J = 12.1 Hz, 1H), 5.09 – 5.07 (m, 3H), 5.01 (s, 1H), 4.98 – 4.93 (m, 3H), 4.79 – 4.70 (m, 3H), 4.67 – 4.64 (m, 1H), 4.62 – 4.56 (m, 2H), 4.52 (t, J = 3.4 Hz, 2H), 4.46 (t, J = 2.6 Hz, 1H), 4.30 – 4.28 (m, 2H), 3.95 – 3.85 (m, 5H), 3.82 – 3.76 (m, 2H), 3.73 (td, J = 3.1, 1.5 Hz, 1H), 3.71 – 3.68 (m, 3H), 3.66 – 3.61 (m, 2H), 3.57 (d, J = 3.8 Hz, 1H), 3.51 – 3.27 (m, 6H), 2.83 (dd, J = 10.4, 3.7 Hz, 1H), 2.05 (s, 1H), 1.85 (s, 2H).

$^{13}$C NMR (101 MHz, Chloroform-d) δ 167.92, 167.82, 156.62, 137.78, 137.02, 136.76, 136.67, 135.98, 135.15, 133.50, 133.28, 133.06, 132.84, 129.71, 128.57, 128.54, 128.48, 128.46, 128.27, 128.24, 128.22, 128.18, 128.09, 128.00, 127.99, 127.84, 127.79, 127.71, 127.70, 127.68, 127.61, 126.87, 126.22, 126.10, 126.00, 125.94, 125.89, 125.04, 124.64, 99.68, 99.50, 97.00, 96.69, 80.78, 80.67, 79.57, 78.47, 78.22, 77.97, 77.67, 77.23, 76.49, 75.54, 74.97, 73.94, 72.66, 72.63, 72.30, 72.26, 72.08, 72.03, 69.47, 69.23, 69.18, 66.63, 63.23, 63.16, 61.09, 39.76, 29.44. HR-ESI-MS (m/z): [M+Na]$^+$ calcd for C$_{78}$H$_{79}$N$_7$O$_{21}$Na, 1472.5227; found, 1472.5231.

N-benzyloxy carbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosylurono-6,2-lactone)-(1→4)-O-(2-azido-2-deoxy-α-D-glucopyranosyl)]-{(1→4)-O-3-O-benzyl-α-L-idopyranosidurono-6,2-lactone (34)
N-benzyloxy carbonyl-3-aminopropyl-O-[(2-acetamido-4-O-benzyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosyluronate-6,2-lactone)-(1→4)-O-(2-acetamido-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosiduronate-6,2-lactone (36)

\[^1\text{H}\text{ NMR (400 MHz, Chloroform-d)} \delta 7.8 (s, 1H), 7.84 (d, J = 10.4 Hz, 5H), 7.77 (s, 1H), 7.67 (s, 1H), 7.51 (dd, J = 7.8, 4.9 Hz, 4H), 7.45 (dd, J = 8.4, 1.5 Hz, 1H), 7.36 – 7.30 (m, 14H), 7.24 (d, J = 8.4 Hz, 3H), 7.20 – 7.17 (m, 2H), 7.08 (d, J = 6.7 Hz, 2H), 5.50 (t, J = 5.1 Hz, 1H), 5.41 (d, J = 0.8 Hz, 1H), 5.11 (d, J = 3.7 Hz, 1H), 5.08 – 5.03 (m, 3H), 5.01 – 4.88 (m, 4H), 4.85 (d, J = 11.0 Hz, 1H), 4.75 – 4.67 (m, 4H), 4.59 (s, 1H), 4.53 – 4.52 (m, 1H), 4.47 (d, J = 2.8 Hz, 1H), 4.42 – 4.31 (m, 5H), 4.18 (d, J = 3.4 Hz, 1H), 4.13 – 4.07 (m, 2H), 3.95 – 3.87 (m, 3H), 3.78 (dd, J = 25.8, 10.9 Hz, 3H), 3.68 – 3.53 (m, 11H), 3.44 – 3.36 (m, 1H), 3.23 (ddd, J = 10.4, 9.5, 5.3 Hz, 1H), 1.81 (s, 2H), 1.52 (d, J = 9.8 Hz, 6H). \[^1\text{C NMR (101 MHz, Chloroform-d)} \delta 170.00, 169.88, 167.96, 167.82, 156.58, 137.84, 136.61, 136.48, 135.58, 133.28, 133.22, 132.99, 132.95, 128.67, 128.56, 128.51, 128.48, 128.46, 128.39, 128.21, 128.09, 128.04, 128.01, 127.97, 127.74, 127.69, 127.28, 126.70, 126.52, 126.41, 126.35, 126.21, 125.86, 98.80, 98.69, 97.72, 96.52, 79.65, 79.29, 78.94, 78.81, 78.28, 77.97, 77.26, 76.64, 75.08, 74.77, 73.37, 72.48, 72.22, 72.11, 72.02, 71.89, 69.41, 69.34, 68.53, 66.62, 61.28, 61.02, 52.73, 52.32, 39.22, 29.71, 29.52, 23.03, 22.92. HR-ESI-MS (m/z): [M+Na]^+ \text{calcd for } C_{82}H_{67}N_{13}O_{23}Na, 1504.5628; \text{found, 1504.5608.}

N-benzyloxy carbonyl-3-aminopropyl-O-[(benzyl (2-azido-4-O-benzyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosyluronate-6,2-lactone)-(1→4)-O-(2-azido-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosiduronate (45)

\[^1\text{H NMR (400 MHz, Chloroform-d)} \delta 7.82 – 7.78 (m, 2H), 7.75 (dd, J = 6.1, 3.5 Hz, 1H), 7.71 (s, 4H), 7.67 (d, J = 8.5 Hz, 1H), 7.46 (dd, J = 6.2, 3.3 Hz, 2H), 7.42 (dd, J = 8.5, 1.6 Hz, 1H), 7.38 – 7.29 (m, 21H), 7.25 – 7.16 (m, 8H), 7.14 – 7.10 (m, 2H), 7.04 – 7.02 (m, 2H), 5.54 (t, J = 5.0 Hz, 1H), 5.32 – 5.29 (m, 2H), 5.13 (d, J = 12.4 Hz, 1H), 5.03 (s, 2H), 4.99 (s, 1H), 4.97 (d, J = 3.7 Hz, 1H), 4.90 – 4.87 (m, 3H), 4.84 (t, J = 2.6 Hz, 2H), 4.81 (s, 1H), 4.78 (d, J = 3.2 Hz, 1H), 4.74 (s, 1H), 4.72 – 4.59 (m, 5H), 4.48 (d, J = 12.2 Hz, 1H), 4.08 – 4.06 (m, 1H), 4.04 (s, 1H), 3.93 – 3.85 (m, 3H), 3.81 – 3.69 (m, 7H), 3.62 – 3.57 (m, 2H), 3.47 (ddt, J = 22.2, 15.5, 7.5 Hz, 9H), 3.28 – 3.20 (m, 1H), 2.02 (s, 1H), 1.83 (s, 2H), 1.44
\[ \text{(s, 1H).} \] \(^{13}\text{C NMR (101 MHz, Chloroform-d)} \delta 169.31, 169.21, 156.48, 137.79, 137.23, 137.18, 136.55, 135.22, 135.07, 134.92, 134.70, 133.27, 133.02, 132.85, 128.74, 128.71, 128.65, 128.52, 128.48, 128.43, 128.41, 128.30, 128.26, 128.24, 128.21, 128.13, 128.08, 128.07, 128.04, 127.98, 127.97, 127.92, 127.85, 127.78, 127.76, 127.65, 127.61, 126.81, 126.34, 126.09, 125.99, 125.89, 125.85, 125.68, 125.63, 101.63, 101.07, 95.96, 95.53, 80.66, 79.37, 77.24, 75.69, 75.26, 75.05, 74.98, 74.48, 73.15, 72.54, 72.38, 72.30, 72.11, 71.54, 69.45, 69.28, 68.07, 67.31, 67.10, 67.03, 66.96, 66.65, 63.99, 63.64, 61.18, 60.98, 39.90, 29.45. \]

\[ \text{HR-ESI-MS (m/z): [M+Na]}^+ \text{ calcd for C}_{92}\text{H}_{95}\text{N}_{7}\text{O}_{23}\text{Na}, 1689.6411; \text{found, 1689.6372}. \]

\( \text{N-benzyloxycarbonyl-3-aminopropyl-O-[(benzyl (2-acetamido-4-O-benzyl-3-O-(2-naphthymethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl)])-(1→4)-O-(3-O-benzyl-\(\alpha\)-L-idopyranosyluronate)-(1→4)-O-(benzyl (2-acetamido-3-O-(2-naphthymethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl))]-1-(1→4)-O-3-O-benzyl-\(\alpha\)-L-idopyranosiduronate (50) } \]

\[ \text{1H NMR (400 MHz, Chloroform-d) } \delta 7.81-7.75 (m, 4H), 7.75 – 7.70 (m, 2H), 7.66 (d, J = 6.9 Hz, 2H), 7.47 (dd, J = 6.2, 3.3 Hz, 2H), 7.44 – 7.39 (m, 3H), 7.39 – 7.36 (m, 2H), 7.35 – 7.33 (m, 4H), 7.32-7.29 (m, 9H), 7.27-7.22 (m, 9H), 7.21 – 7.13 (m, 5H), 7.07 (d, J = 7.5 Hz, 2H), 5.48 (t, J = 5.4 Hz, 1H), 5.31 – 5.25 (m, 2H), 5.06- 5.04 (m, 4H), 4.99 – 4.96 (m, 2H), 4.93 – 4.87 (m, 4H), 4.87 – 4.82 (m, 3H), 4.75 (d, J = 11.7 Hz, 1H), 4.65 (dd, J = 11.3, 8.1 Hz, 2H), 4.58 (d, J = 12.2 Hz, 1H), 4.52 (d, J = 11.6 Hz, 2H), 4.43 (t, J = 12.2 Hz, 1H), 4.23 – 4.15 (m, 2H), 4.11 – 4.09 (m, 1H), 4.01 – 3.94 (m, 2H), 3.88 – 3.84 (m, 2H), 3.79 – 3.62 (m, 9H), 3.61 – 3.48 (m, 6H), 3.42 – 3.37 (m, 1H), 3.31-3.25 (m, 1H), 3.08 (s, 1H), 2.63 (s, 1H), 1.81 (s, 2H), 1.67 (s, 3H), 1.44 (s, 3H). \]

\[ \text{^{13}C NMR (101 MHz, Chloroform-d) } \delta 170.93, 170.65, 169.45, 169.26, 156.53, 138.08, 137.19, 136.45, 135.79, 135.67, 134.75, 134.58, 133.22, 133.20, 132.90, 132.83, 128.75, 128.72, 128.65, 128.52, 128.46, 128.45, 128.42, 128.34, 128.26, 128.22, 128.18, 128.15, 128.10, 128.01, 127.97, 127.84, 127.62, 126.62, 126.17, 126.04, 125.98, 101.15, 96.81, 96.07, 79.79, 77.92, 77.70, 75.51, 75.07, 74.03, 73.70, 72.84, 72.66, 72.44, 72.35, 71.98, 71.32, 69.22, 68.66, 67.92, 67.48, 67.34, 67.30, 67.11, 66.69, 61.31, 61.10, 52.76, 52.55, 39.55, 29.72, 29.47. \]

\[ \text{HR-ESI-MS (m/z): [M+H]}^+ \text{ calcd for C}_{96}\text{H}_{104}\text{N}_{25}\text{O}_{25}, 1698.6959; \text{found, 1698.6862}. \]

2.5 General procedure for O-sulfation:

To a solution of starting material (1 mmol) in DMF (2 mL) was added \(\text{SO}_3\text{.NEt}_3\) complex (10 mmol per -OH) under \(\text{N}_2\) atmosphere and stirred at 60 °C for 3 days. Upon completion of
reaction, aqueous NaHCO₃ (20 mmol) was added to reaction mixture and stirred for another 16 h. Next, the reaction mixture was concentrated under reduced pressure and the resulting residue was filtered with MeOH through whatman filter paper. The filtrate was concentrated and purified using silica column chromatography.

N-benzylxocarbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-6-O-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosylurono-6,2-lactone)-(1→4)-O-(2-azido-6-O-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosidurono-6,2-lactone (30)

1H NMR (400 MHz, Methanol-d₄) δ 7.85 – 7.80 (m, 4H), 7.79 (d, J = 2.2 Hz, 1H), 7.76 – 7.74 (m, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.58 – 7.54 (m, 2H), 7.52 – 7.50 (m, 1H), 7.49 (d, J = 2.1 Hz, 1H), 7.48 – 7.39 (m, 8H), 7.38 – 7.26 (m, 13H), 5.29 (s, 1H), 5.18 (d, J = 3.2 Hz, 1H), 5.15 (d, J = 3.5 Hz, 1H), 5.07 (s, 2H), 4.99 (d, J = 2.9 Hz, 1H), 4.93 (s, 2H), 4.82 (d, J = 3.1 Hz, 2H), 4.80 – 4.79 (m, 2H), 4.76 – 4.75 (m, 5H), 4.34 – 4.26 (m, 2H), 4.23 (dt, J = 4.9, 2.4 Hz, 1H), 4.20 – 4.18 (m, 1H), 4.12 (d, J = 9.4 Hz, 1H), 4.04 (t, J = 3.8 Hz, 1H), 3.98 – 3.93 (m, 3H), 3.91 – 3.84 (m, 3H), 3.78 – 3.68 (m, 4H), 3.67 – 3.58 (m, 3H), 3.55 (dd, J = 9.9, 3.5 Hz, 1H), 3.45 (d, J = 9.5 Hz, 1H), 3.26 (tt, J = 13.0, 6.5 Hz, 2H), 1.86 – 1.79 (m, 2H). 13C NMR (101 MHz, Methanol-d₄) δ 170.23, 170.06, 157.43, 138.27, 138.00, 137.89, 136.95, 135.52, 135.13, 133.42, 133.31, 133.05, 133.01, 131.01, 128.47, 128.31, 128.09, 128.04,
128.01, 127.83, 127.76, 127.68, 127.65, 127.60, 127.55, 127.50, 127.48, 127.45, 127.38, 127.30, 127.25, 127.15, 126.35, 126.22, 125.91, 125.65, 125.59, 125.49, 101.46, 100.56, 96.18, 95.58, 80.06, 78.87, 77.24, 74.86, 74.67, 74.40, 74.03, 72.77, 72.58, 72.36, 71.69, 70.72, 70.38, 68.65, 68.51, 68.36, 67.99, 67.71, 66.45, 66.03, 65.64, 65.33, 64.09, 63.48, 51.72, 50.83, 38.77, 38.03, 33.50, 31.67, 30.76, 29.07, 28.70, 23.55, 22.63, 22.34. HR-ESI-MS (m/z): [M-2H]²⁻ calcd for C₇₈H₁₁₁N₇O₁₁₂S₂₂, 803.7160; found, 803.7170.

N-benzyloxy carbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-6-O-tert-butyldiphenylisilyl-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosylurono-6,2-lactone)-(1→4)-O-(2-azido-6-O-tert-butyldiphenylisilyl-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosidurono-6,2-lactone (32)

1H NMR (400 MHz, Methanol-d₄) δ 7.68 – 7.67 (m, 1H), 7.66 (d, J = 2.0 Hz, 1H), 7.65 – 7.61 (m, 4H), 7.60 (s, 1H), 7.58 (d, J = 1.3 Hz, 1H), 7.41 (d, J = 7.4 Hz, 2H), 7.39 – 7.34 (m, 4H), 7.32 – 7.29 (m, 9H), 7.29 – 7.22 (m, 13H), 7.20 – 7.13 (m, 4H), 5.49 (s, 1H), 5.18 (d, J = 10.6 Hz, 1H), 5.13 (d, J = 3.4 Hz, 1H), 5.06 (s, 1H), 5.02-5.00 (m, 2H), 4.98 – 4.87 (m, 2H), 4.76 – 4.70 (m, 5H), 4.56 (dd, J = 18.2, 11.0 Hz, 2H), 4.43 – 4.42 (m, 1H), 4.41- 4.39 (m, 2H), 4.35 – 4.32 (m, 1H), 4.07 – 3.94 (m, 3H), 3.87- 3.83 (m, 3H), 3.80- 3.65 (m, 5H), 3.55 (dt, J = 9.3, 5.5 Hz, 1H), 3.26 – 3.16 (m, 3H), 1.78 – 1.74 (m, 2H), 0.99 (s, 9H), 0.96 (s, 9H). 13C NMR (101 MHz, Methanol-d₄) δ 168.67, 167.84, 157.33, 138.44, 137.64, 137.34, 136.96, 135.65, 135.56, 135.43, 135.40, 133.36, 133.05, 132.95, 132.75, 129.53, 129.50, 129.39, 128.37, 128.18, 128.13, 128.08, 128.02, 127.98, 127.72, 127.67, 127.62, 127.59, 127.41, 127.38, 127.36, 127.29, 127.07, 100.08, 99.67, 97.02, 94.52, 79.87, 79.56, 79.41, 79.12, 78.50, 78.07, 76.48, 75.53, 74.72, 73.99, 71.92, 71.81, 71.51, 71.32, 69.49, 69.39, 67.63, 66.07, 62.84, 62.52, 62.40, 62.10, 38.36, 29.23, 26.05. HR-ESI-MS (m/z): [M-2H]²⁻ calcd for C₈₈H₁₂₁N₁₀O₁₂₂S₂₂Si₂, 901.7711; found 901.7706.

N-benzyloxy carbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-6-O-sulfonato-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosylurono-6,2-lactone)-(1→4)-O-(2-azido-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosidurono-6,2-lactone (35)

1H NMR (400 MHz, Methanol-d₄) δ 7.53 (d, J = 7.0 Hz, 2H), 7.46 (d, J = 7.4 Hz, 2H), 7.42 – 7.22 (m, 16H), 5.53 (s, 1H), 5.21 – 5.14 (m, 4H), 5.08 – 5.02 (m, 2H), 4.83 (d, J = 11.8 Hz, 2H), 4.78 – 4.76 (m, 1H), 4.70 (d, J = 11.7 Hz, 2H), 4.64 (t, J = 9.1 Hz, 2H), 4.58 – 4.52 (m, 1H), 4.32 (td, J = 10.4, 9.7, 3.0 Hz, 1H), 4.27 (d, J = 3.2 Hz, 1H), 4.24 –
4.18 (m, 3H), 4.11 (t, J= 4.6 Hz, 1H), 3.94 – 3.80 (m, 6H), 3.71 – 3.67 (m, 2H), 3.63 – 3.55 (m, 2H), 3.55 – 3.44 (m, 2H), 3.31 – 3.22 (m, 2H), 1.85 – 1.76 (m, 2H). $^{13}$C NMR (101 MHz, Methanol-\(d_4\)) $\delta$ 171.28, 170.46, 157.45, 138.41, 138.10, 137.97, 136.94, 128.91, 128.10, 128.08, 128.00, 127.80, 127.69, 127.59, 127.45, 127.37, 127.11, 126.99, 102.93, 101.09, 96.85, 95.66, 78.94, 78.08, 76.76, 76.60, 75.76, 74.57, 73.85, 72.82, 72.31, 72.09, 70.61, 70.20, 69.48, 69.24, 69.04, 67.90, 66.56, 66.02, 65.71, 65.54, 63.01, 62.67, 37.93, 29.35. HR-ESI-MS (m/z): [M-4H]$^+$ calcd for C$_{56}$H$_{69}$N$_7$O$_{35}$S$_4$; 371.3015; found, 371.3012.

**N-benzyloxy carbonyl-3-aminopropyl-O-[(2-acetamido-4-O-benzyl-6-O-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-3-O-benzyl-α-L-idopyranosyluronato-6,2-lactone (37)***

$^{1}$H NMR (400 MHz, Methanol-\(d_4\)) $\delta$ 7.91 – 7.70 (m, 9H), 7.51 – 7.37 (m, 14H), 7.33 – 7.22 (m, 11H), 5.54 (s, 1H), 5.13 (d, J = 7.0 Hz, 2H), 5.09 (s, 1H), 5.06 (s, 2H), 4.81 – 4.52 (m, 9H), 4.46 (d, J = 8.9 Hz, 1H), 4.39 (d, J = 8.3 Hz, 2H), 4.32 – 4.27 (m, 3H), 4.25 – 4.16 (m, 3H), 4.10 – 3.89 (m, 8H), 3.83 – 3.54 (m, 6H), 3.26 – 3.15 (m, 2H), 1.84 – 1.75 (m, 8H). $^{13}$C NMR (101 MHz, Methanol-\(d_4\)) $\delta$ 171.75, 171.66, 168.63, 168.43, 157.34, 138.24, 137.55, 137.08, 136.22, 136.19, 133.39, 133.35, 133.33, 133.02, 132.99, 132.91, 128.29, 128.26, 128.11, 128.03, 127.90, 127.81, 127.64, 127.60, 127.51, 127.46, 127.43, 127.29, 126.00, 125.76, 125.60, 125.51, 125.46, 125.41, 125.28, 125.04, 99.43, 99.29, 98.51, 96.93, 80.13, 80.10, 79.78, 79.62, 78.24, 78.08, 77.74, 77.66, 74.58, 72.13, 72.10, 71.96, 71.92, 71.87, 70.26, 69.65, 69.62, 66.93, 65.94, 65.52, 52.93, 52.82, 37.71, 29.29, 21.50, 21.42. HR-ESI-MS (m/z): [M-2H]$^{2+}$ calcd for C$_{82}$H$_{88}$N$_7$O$_{29}$S$_2$; 819.7369; found, 819.7369.

**N-benzyloxy carbonyl-3-aminopropyl-O-[(2-azido-4-O-benzyl-6-O-tert-butyldiphenylsilyle-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(benzyl-2-azido-6-O-tert-butyldiphenylsilyle-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]- (1→4)-O-3-O-benzyl-2-O-sulfonato-α-L-idopyranosiduronate (40)***

$^{1}$H NMR (400 MHz, Methanol-\(d_4\)) $\delta$ 7.79 – 7.76 (m, 7H), 7.74 – 7.70 (m, 3H), 7.61 – 7.41 (m, 13H), 7.38 – 7.30 (m, 14H), 7.29 – 7.15 (m, 23H), 7.04 (t, J = 6.6 Hz, 2H), 6.96 (t, J = 7.5 Hz, 2H), 6.65 (d, J = 7.7 Hz, 1H), 5.71 (s, 1H), 5.37 (d, J = 12.1 Hz, 2H).
N-benzyloxy carbonyl-3-aminopropyl-O-[(benzyl (2-acetamido-4-O-benzyl-6-O-tert-butyldiphenylsilyl)-3-O-(2-naphthylmethy1)-2-deoxy-α-D-glucopyranosyl)](1→4)-O-(3-O-benzyl-2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(benzyl (2-acetamido-6-O-tert-butyldiphenylsilyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-2-O-sulfonato-α-L-idopyranosiduronate (43)

1H NMR (400 MHz, Methanol-d4) δ 7.83 (s, 1H), 7.82 - 7.80 (m, 2H), 7.79 (s, 2H), 7.76 - 7.73 (m, 3H), 7.71 (s, 1H), 7.68 (d, J = 7.6 Hz, 3H), 7.63 (s, 1H), 7.61 (d, J = 5.1 Hz, 2H), 7.58 (d, J = 6.1 Hz, 3H), 7.48 (dd, J = 16.8, 8.0 Hz, 4H), 7.43 - 7.38 (m, 9H), 7.36 - 7.30 (m, 16H), 7.25 - 7.20 (m, 7H), 7.19 - 7.15 (m, 7H), 6.98 (d, J = 7.4 Hz, 2H), 5.71 (s, 1H), 5.20 (s, 4H), 4.98 (s, 3H), 4.83 (s, 1H), 4.81 - 4.75 (m, 3H), 4.69 (d, J = 12.0 Hz, 2H), 4.57 - 4.50 (m, 5H), 4.41 - 4.34 (m, 5H), 4.24 - 4.03 (m, 5H), 3.90 (d, J = 15.9 Hz, 2H), 3.81 - 3.74 (m, 6H), 3.65 – 3.56 (m, 5H), 3.29 – 3.25 (m, 1H), 3.20 - 3.13 (m, 1H), 2.01 (s, 3H), 1.84 - 1.75 (m, 5H), 1.07 (s, 9H), 0.98 (s, 9H). 13C NMR (101 MHz, Methanol-d4) δ 169.56, 169.49, 169.38, 157.31, 137.61, 137.59, 136.82, 136.31, 135.95, 135.79, 135.75, 135.66, 135.38, 135.15, 134.79, 134.55, 134.31, 134.25, 133.42, 133.33, 133.32, 133.29, 133.20, 133.01, 132.93, 132.63, 129.45, 129.28, 129.24, 128.45, 128.33, 128.28, 128.23, 128.21, 128.18, 128.10, 128.05, 127.97, 127.92, 127.83, 127.74, 127.71, 127.67, 127.63, 127.61, 127.57, 127.45, 127.35, 127.28, 127.26, 127.23, 127.21, 127.18, 126.98, 126.38, 125.90, 125.63, 125.58, 125.47, 125.41, 99.99, 99.54, 98.82, 80.73, 79.13, 78.20, 77.87, 77.70, 77.67, 77.55, 75.38, 75.34, 74.51, 73.19, 72.75, 72.48, 72.13, 72.07, 72.03, 70.76, 70.49, 70.36, 67.09, 66.53, 66.31, 66.01, 62.40, 62.09, 53.23, 38.23, 34.06, 31.66, 30.78, 29.33, 26.37, 26.05. HR-ESI-MS (m/z): [M-2H]2+ calcd for C128H137N3O31S2Si22+, 1166.4130; found, 1166.4142.
N-benzylxocarbonyl-3-aminopropyl-O-[(benzyl (2-azido-4-O-benzyl-6-O-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)→(1→4)-O-(3-O-benzyl-2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(benzyl (2-azido-6-O-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]]-(1→4)-O-3-O-benzyl-2-O-sulfonato-α-L-idopyranosiduronate (46)

\[\text{H NMR (400 MHz, Methanol-}d_4\text{)} \delta 7.84 - 7.75 (m, 4H), 7.71 - 7.68 (m, 3H), 7.62 (d, J = 8.5 Hz, 1H), 7.55 - 7.42 (m, 9H), 7.40 (d, J = 8.5 Hz, 5H), 7.36 - 7.31 (m, 10H), 7.28 - 7.23 (m, 7H), 7.15 - 7.09 (m, 1H), 7.02 (t, J = 7.3 Hz, 2H), 6.59 (d, J = 7.5 Hz, 2H), 5.53 (s, 1H), 5.44 (d, J = 12.1 Hz, 1H), 5.22 - 5.15 (m, 3H), 5.11 (s, 1H), 5.07 - 5.02 (m, 3H), 5.01 - 4.94 (m, 3H), 4.82- 4.74 (m, 4H), 4.72- 4.65 (m, 4H), 4.62 - 4.57 (m, 2H), 4.53-4.50 (m, 2H), 4.40 - 4.34 (m, 4H), 4.17- 4.07 (m, 4H), 4.01 - 3.80 (m, 6H), 3.67- 3.58 (m, 3H), 3.41 - 3.36 (m, 1H), 3.31 - 3.16 (m, 2H), 1.87- 1.75 (m, 2H). \text{13C NMR (101 MHz, Methanol-}d_4\text{)} \delta 170.01, 169.88, 157.39, 138.37, 137.81, 137.42, 136.92, 135.91, 135.57, 135.31, 134.50, 133.35, 133.33, 133.03, 132.92, 128.59, 128.49, 128.40, 128.36, 128.29, 128.16, 128.15, 128.08, 127.99, 127.83, 127.57, 127.44, 127.34, 127.26, 127.15, 126.22, 125.75, 125.65, 125.63, 125.60, 125.46, 125.35, 99.56, 97.94, 96.62, 80.01, 78.48, 77.41, 74.92, 74.68, 74.57, 72.35, 72.08, 71.84, 70.68, 70.48, 67.73, 67.28, 67.04, 66.95, 66.40, 66.01, 56.38, 64.16, 63.91, 63.89, 38.09, 31.67, 30.77, 29.33, 29.19, 29.06, 28.72. \text{HR-ESI-MS (m/z): [M-4H]^- calcld for C}_{92}H_{91}N_{7}O_{35}S_{4}, 495.3615; found, 495.3588.

N-benzylxocarbonyl-3-aminopropyl-O-[(benzyl (2-azido-4-O-benzyl-6-O-tert-butylidiphenylsilyl-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)→(1→4)-O-(3-O-benzyl-2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(benzyl (2-azido-6-O-tert-butylidiphenylsilyl-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]]-(1→4)-O-3-O-benzyl-2-O-sulfonato-α-L-idopyranosiduronate (48)

\[\text{H NMR (400 MHz, Methanol-}d_4\text{)} \delta 7.61 (dd, J = 6.4, 3.0 Hz, 3H), 7.58 (dd, J = 3.0, 1.4 Hz, 2H), 7.56 (d, J = 1.6 Hz, 1H), 7.53 (d, J = 1.3 Hz, 1H), 7.51 (d, J = 1.4 Hz, 1H), 7.31 (t, J = 2.1 Hz, 1H), 7.29 (dd, J = 2.6, 1.2 Hz, 1H), 7.27 (s, 2H), 7.25 (s, 2H), 7.24 (s, 2H), 7.22- 7.20 (m, 7H), 7.18 - 7.15 (m, 7H), 7.13 - 7.11 (m, 5H), 7.07 (d, J = 1.5 Hz, 2H), 7.05 - 7.03 (m, 6H), 7.02 (s, 4H), 7.01 - 6.99 (m, 3H), 5.35 (s, 2H), 5.09 (d, J = 10.1 Hz, 1H), 5.04 (d, J = 2.6 Hz, 1H), 5.03- 4.99 (m, 3H), 4.96 (s, 1H), 4.90 (d, J = 12.1 Hz, 2H), 4.84 (s, 2H), 4.61 (d, J = 9.4 Hz, 2H), 4.55 (d, J = 12.3 Hz, 2H), 4.44 (d, J = 6.3 Hz, 1H), 4.33 (s, 1H), 4.28 (d, J = 12.2 Hz, 2H), 4.16 (d, J = 11.7 Hz, 2H), 3.94 (s, 1H), 3.90 (s, 1H), 3.84 (d, J =
8.2 Hz, 2H), 3.77 (dd, J = 19.4, 9.5 Hz, 3H), 3.71 - 3.69 (m, 3H), 3.56 - 3.50 (m, 3H), 3.44 - 3.41 (m, 2H), 3.29 (dd, J = 10.2, 3.3 Hz, 1H), 3.10 (d, J = 6.4 Hz, 1H), 3.05 - 3.00 (m, 1H), 2.92 (dd, J = 9.7, 3.5 Hz, 1H), 1.66 - 1.60 (m, 2H), 0.91 (s, 9H), 0.87 (s, 9H). $^{13}$C NMR (151 MHz, Methanol-$d_4$) δ 170.11, 169.28, 157.33, 138.09, 137.94, 137.46, 136.89, 135.80, 135.65, 135.62, 135.45, 134.95, 134.76, 133.52, 133.39, 133.16, 132.94, 129.54, 129.50, 129.30, 129.20, 128.54, 128.43, 128.28, 128.11, 128.08, 128.03, 127.80, 127.77, 127.49, 127.43, 127.41, 127.36, 127.31, 127.25, 127.15, 99.53, 99.36, 94.65, 79.20, 76.45, 75.07, 72.15, 72.10, 72.06, 71.95, 71.55, 70.56, 69.92, 69.39, 67.90, 67.23, 67.21, 66.91, 66.21, 65.90, 65.61, 63.16, 62.32, 62.05, 37.89, 29.37, 29.08, 26.26, 26.06. HR-ESI-MS (m/z): [M-4H]$^+$ calcd for C$_{102}$H$_{111}$N$_2$O$_{33}$S$_4$Si$_2$$^+$, 544.6399; found, 544.6364.

N-benzyloxycarbonyl-3-aminopropyl-O-[(benzyl (2-acetamido-4-O-benzyl-6-O-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(benzyl (2-acetamido-6-O-sulfonato-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl))-(1→4)-O-3-O-benzyl-2-O-sulfonato-α-L-idopyranosiduronate (51)

$^1$H NMR (400 MHz, Methanol-$d_4$) δ 7.78 - 7.62 (m, 8H), 7.53 (d, J = 8.5 Hz, 1H), 7.43 - 7.25 (m, 20H), 7.25 - 7.13 (m, 11H), 7.11 - 7.07 (m, 1H), 6.98 (t, J = 7.6 Hz, 2H), 6.60 (d, J = 7.5 Hz, 1H), 5.47 (s, 1H), 5.35 (d, J = 12.1 Hz, 1H), 5.16 (d, J = 12.9 Hz, 3H), 5.03 - 4.94 (m, 4H), 4.83 - 4.76 (m, 2H), 4.74 - 4.68 (m, 4H), 4.67 - 4.46 (m, 8H), 4.36 (d, J = 11.0 Hz, 1H), 4.28 (d, J = 14.2 Hz, 2H), 4.23 - 4.17 (m, 2H), 4.13 - 4.11 (m, 2H), 4.07 - 4.02 (m, 2H), 3.89 - 3.87 (m, 2H), 3.83 - 3.72 (m, 5H), 3.63 - 3.55 (m, 2H), 3.19 - 3.12 (m, 1H), 1.92 (s, 3H), 1.83 - 1.74 (m, 2H), 1.69 (s, 3H). $^{13}$C NMR (126 MHz, Methanol-$d_4$) δ 173.34, 170.81, 170.57, 158.07, 139.19, 138.14, 138.04, 137.53, 137.05, 137.02, 135.96, 135.16, 134.06, 133.67, 133.47, 129.10, 129.07, 129.05, 128.98, 128.81, 128.77, 128.65, 128.55, 128.50, 128.45, 128.32, 128.29, 128.26, 128.24, 128.17, 128.13, 127.94, 127.89, 127.76, 126.73, 126.44, 126.23, 126.03, 125.89, 125.80, 125.73, 100.33, 99.07, 80.98, 79.68, 78.24, 75.91, 75.42, 75.31, 73.50, 72.95, 72.47, 71.73, 71.46, 71.40, 71.31, 71.18, 68.13, 67.81, 67.34, 67.29, 66.78, 66.38, 66.11, 54.01, 53.95, 39.07, 29.98, 29.87. HR-ESI-MS (m/z): [M-4H]$^+$ calcd for C$_{96}$H$_{99}$N$_3$O$_{37}$S$_4$$^+$, 503.6224; found, 503.6204.
2.6 General procedure for O-phosphorylation:

To a solution of starting material (1 mmol) in CH₂Cl₂ (1 mL) and pyridine (1 mL) was added NEt₃ (10 mmol), DMAP (0.3 mmol) and Diphenyl phosphoryl chloride (5 mmol for 38 and 10 mmol for 52) at 0 °C. After 12 h, the reaction mixture was diluted with CH₂Cl₂ and extracted with 1 N HCl and brine. The combined organic layer was dried over Na₂SO₄, filtered, concentrated and purified through silica gel column chromatography.

N-benzyloxycarbonyl-3-aminopropyl-O-[(2-acetamido-4-O-benzyl-6-O-diphenylphosphoryl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-α-L-idopyranosyluronono-6,2-lactone)-(1→4)-O-(2-acetamido-6-O-diphenylphosphoryl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosiduronono-6,2-lactone (38)

¹H NMR (400 MHz, Chloroform-d) δ 7.84 (dd, J = 12.9, 8.5 Hz, 6H), 7.73 (d, J = 20.7 Hz, 2H), 7.54 – 7.46 (m, 4H), 7.44 – 7.31 (m, 16H), 7.28 – 7.21 (m, 19H), 7.19 – 7.08 (m, 7H), 5.35 – 5.29 (m, 2H), 5.05 – 4.97 (m, 3H), 4.89 (d, J = 11.6 Hz, 2H), 4.78 – 4.69 (m, 3H), 4.63 (t, J = 2.6 Hz, 1H), 4.56 (dd, J = 15.6, 7.7 Hz, 3H), 4.52 – 4.42 (m, 4H), 4.41 – 4.33 (m, 4H), 4.31 – 4.27 (m, 2H), 4.21 (dd, J = 4.9, 3.3 Hz, 1H), 4.11 – 4.07 (m, 1H), 4.06 – 3.88 (m, 3H), 3.76 – 3.69 (m, 2H), 3.66 – 3.52 (m, 7H), 3.39 – 3.30 (m, 1H), 3.23 (dt, J = 12.9, 5.5 Hz, 1H), 1.81 (s, 2H), 1.51 (s, 3H), 1.42 (s, 3H). 13C NMR (101 MHz, Chloroform-d) δ 169.99, 169.79, 167.70, 167.68, 156.42, 156.41, 156.05, 150.55, 150.49, 150.43, 150.36, 150.21, 150.14, 137.59, 136.71, 136.63, 136.45, 135.57, 135.34, 133.21, 133.00, 132.96, 130.06, 129.92, 129.84, 129.81, 128.68, 128.51, 128.46, 128.40, 128.33, 128.29, 128.16, 128.07, 128.02, 127.95, 127.85, 127.70, 127.28, 127.00, 126.59, 126.46, 126.41, 126.25,
126.22, 125.93, 125.62, 125.54, 125.48, 120.34, 120.29, 120.09, 120.07, 120.05, 120.03, 99.12, 98.65, 98.28, 96.58, 80.09, 79.95, 79.02, 78.28, 77.92, 77.70, 77.24, 75.06, 74.82, 73.61, 72.65, 72.25, 72.20, 71.59, 70.70, 70.62, 69.99, 69.92, 69.48, 69.26, 68.38, 66.56, 52.50, 52.21, 39.01, 29.59, 23.02, 22.73. \(^{31}\)P NMR (162 MHz, Chloroform-d) \(\delta\) -10.81, -11.15, -14.13, -14.74. HR-ESI-MS (m/z): [M+Na]+ calcld for C\(_{106}\)H\(_{106}\)N\(_5\)O\(_{26}\)P\(_2\), 1947.6421; found, 1947.5958.

N-benzyloxycarbonyl-3-aminopropyl-O-[(benzyl(2-acetamido-4-O-benzyl-6-O-diphenylphosphoryl-3-O-(2-naphthylmethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-O-(3-O-benzyl-2-O-diphenylphosphoryl-\(\alpha\)-L-idopyranosyluronate)-(1\(\rightarrow\)4)-O-(benzyl(2-acetamido-6-O-diphenylphosphoryl-3-O-(2-naphthylmethyl)-2-deoxy-\(\alpha\)-D-glucopyranosyl))-(1\(\rightarrow\)4)-O-3-O-benzyl-2-O-diphenylphosphoryl-\(\alpha\)-L-idopyranosiduronate (52)

\(^{1}\)H NMR (400 MHz, Chloroform-d) \(\delta\) 7.78 – 7.74 (m, 1H), 7.67 (t, \(J = 7.7\) Hz, 3H), 7.65 – 7.60 (m, 4H), 7.51 (s, 1H), 7.43 – 7.39 (m, 2H), 7.36 (s, 1H), 7.35 – 7.34 (m, 1H), 7.33 – 7.32 (m, 3H), 7.31 (dd, \(J = 3.2, 1.1\) Hz, 2H), 7.29 (d, \(J = 1.9\) Hz, 1H), 7.27 (s, 2H), 7.26- 7.25 (m, 2H), 7.23 (t, \(J = 2.3\) Hz, 3H), 7.22- 7.21 (m, 3H), 7.20 (d, \(J = 2.1\) Hz, 2H), 7.19 (s, 6H), 7.17 – 7.16 (m, 5H), 7.15- 7.14 (m, 10H), 7.13 (s, 5H), 7.12 (s, 6H), 7.10 (d, \(J = 4.1\) Hz, 3H), 7.08 (s, 3H), 7.05 (s, 3H), 7.04 (s, 2H), 7.02 (s, 1H), 7.00- 6.95 (m, 3H), 6.91- 6.87 (m, 4H), 6.80 (d, \(J = 7.3\) Hz, 2H), 5.49 (t, \(J = 5.4\) Hz, 1H), 5.46 (s, 1H), 5.27 (d, \(J = 12.0\) Hz, 1H), 5.06 (d, \(J = 12.0\) Hz, 1H), 4.99 (s, 2H), 4.92 (s, 2H), 4.84 (dd, \(J = 23.1, 11.8\) Hz, 3H), 4.78 – 4.74 (m, 1H), 4.71 (s, 1H), 4.69 – 4.64 (m, 2H), 4.64 – 4.61 (m, 2H), 4.61 – 4.57 (m, 4H), 4.56- 4.49 (m, 4H), 4.44 (s, 2H), 4.41- 4.40 (m, 3H), 4.37 (d, \(J = 2.0\) Hz, 1H), 4.24 – 4.13 (m, 4H), 4.01 (d, \(J = 8.8\) Hz, 1H), 3.94 – 3.81 (m, 6H), 3.78 – 3.71 (m, 1H), 3.59 (d, \(J = 9.5\) Hz, 1H), 3.56 – 3.50 (m, 1H), 3.46- 3.40 (m, 1H), 3.25 – 3.17 (m, 1H), 1.80 (m, 2H), 1.75 (s, 3H), 1.68 (s, 3H).

\(^{13}\)C NMR (101 MHz, Chloroform-d) \(\delta\) 171.23, 170.89, 168.76, 168.18, 156.40, 150.52, 150.52, 150.45, 150.44, 150.42, 150.34, 150.26, 150.24, 150.20, 150.17, 150.03, 149.95, 138.27, 136.47, 136.39, 136.35, 136.32, 136.28, 134.92, 134.82, 133.29, 133.22, 132.77, 132.74, 130.10, 130.07, 130.02, 129.80, 129.77, 129.72, 128.97, 128.70, 128.65, 128.60, 128.42, 128.37, 128.31, 128.20, 128.10, 128.07, 127.99, 127.92, 127.90, 127.78, 127.72, 127.60, 127.57, 127.49, 127.43, 126.14, 125.99, 125.83, 125.77, 125.64, 125.58, 125.54, 125.44, 125.38, 125.35, 125.32, 102.44, 100.52, 99.12, 98.97, 98.77, 98.64, 80.34, 78.62, 74.77, 74.72, 73.43, 72.75, 72.64, 72.54, 72.42, 72.29, 72.22, 72.16, 70.67, 70.65, 70.61, 70.57, 68.21, 67.98, 67.13, 66.96, 66.73, 66.55, 53.12, 52.41, 39.83, 29.38, 22.65. \(^{31}\)P NMR (162 MHz, Chloroform-d) \(\delta\) -10.81, -11.15, -14.13, -14.74. HR-ESI-MS (m/z): [M+Na]+ calcld for C\(_{144}\)H\(_{138}\)N\(_5\)O\(_{37}\)P\(_4\)Na, 2649.7969; found, 2649.4785.
2.7 General procedure for desilylation post O- sulfation:

To a solution of starting material (1 mmol) in pyridine (1 mL) was added HF.py (5 mmol) at ice cold temperature. After stirring for 12 h the volatiles were evaporated and the residue was purified using Sephadex LH 20 column using MeOH as an eluent.

N-benzyloxycarbonyl-3-aminopropyl-O-[2-azido-4-O-benzyl-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl](1→4)-O-(3-O-benzyl-α-L-idopyranosylurono-6,2-lactone)-(1→4)-O-(2-azido-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-3-O-benzyl-α-L-idopyranosidurono-6,2-lactone (33)

1H NMR (400 MHz, Methanol-d4) δ 7.50 – 7.41 (m, 7H), 7.38- 7.37 (m, 3H), 7.34 – 7.25 (m, 10H), 5.40 (dd, J = 7.5, 2.8 Hz, 2H), 5.24 – 5.13 (m, 4H), 5.07 (s, 2H), 4.94 (dd, J = 5.1, 3.0 Hz, 1H), 4.83 – 4.78 (m, 3H), 4.76 (d, J = 3.7 Hz, 4H), 4.61 (d, J = 3.0 Hz, 1H), 4.57 (d, J = 10.5 Hz, 1H), 4.50 (d, J = 3.2 Hz, 1H), 4.41 – 4.37 (m, 1H), 3.95 – 3.88 (m, 3H), 3.83 – 3.57 (m, 2H), 3.26 (dt, J = 12.8, 6.1 Hz, 2H), 1.82 – 1.79 (m, 2H). 13C NMR (101 MHz, Methanol-d4) δ 169.02, 168.19, 157.38, 138.44, 137.74, 137.4, 137.37, 137.00, 128.48, 128.29, 128.10, 128.06, 128.01, 127.73, 127.63, 127.57, 127.53, 127.39, 127.19, 100.09, 100.02, 97.04, 96.02, 80.05, 79.93, 79.30, 78.82, 78.46, 78.07, 76.40, 76.10, 75.34, 74.56, 72.12, 72.08, 71.82, 71.77, 71.57, 69.71, 69.54, 67.44, 66.02, 62.71, 62.32, 60.35, 60.02, 38.16. HR-ESI-MS (m/z): [M-2H]^2– calcd for C_{56}H_{61}N_{7}O_{27}S_{2}^{2–}, 663.6534; found 663.6534.
N-benzyloxy carbonyl-3-aminopropyl-O-[(benzyl (2-acetamido-4-O-benzyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(benzyl (2-azido-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)])-(1→4)-O-3-O-benzyl-2-O-sulfonato-α-L-idopyranosiduronate (41)

$^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.82 – 7.78 (m, 1H), 7.76-7.74 (m, 2H), 7.72-7.70 (m, 2H), 7.63 (dd, $J = 16.5, 8.2$ Hz, 2H), 7.47-7.39 (m, 10H), 7.36-7.34 (m, 5H), 7.32-7.27 (m, 10H), 7.26 – 7.24 (m, 5H), 7.17 – 7.10 (m, 3H), 7.02 (t, $J = 7.5$ Hz, 2H), 6.62 (d, $J = 7.5$ Hz, 2H), 5.51 (s, 1H), 5.41 (d, $J = 12.1$ Hz, 1H), 5.27 – 5.26 (m, 1H), 5.20 (s, 1H), 5.15 (d, $J = 3.0$ Hz, 1H), 5.09 – 5.06 (m, 2H), 5.02 – 4.97 (m, 4H), 4.94 – 4.89 (m, 2H), 4.79 – 4.69 (m, 3H), 4.64 – 4.62 (m, 1H), 4.62 – 4.50 (m, 4H), 4.39- 4.35 (m, 3H), 4.18 – 4.14 (m, 1H), 4.12 – 4.03 (m, 2H), 3.99 – 3.73 (m, 7H), 3.61-3.51 (m, 3H), 3.48 – 3.38 (m, 3H), 3.31 – 3.13 (m, 4H), 1.84- 1.76 (m, 2H). $^{13}$C NMR (101 MHz, Methanol-$d_4$) $\delta$ 174.15, 169.81, 157.39, 138.37, 137.87, 137.71, 136.94, 135.77, 135.69, 135.07, 134.51, 133.33, 133.31, 133.04, 128.85, 128.51, 128.41, 128.27, 128.25, 128.20, 128.13, 128.06, 127.95, 127.89, 127.82, 127.75, 127.68, 127.60, 127.58, 127.55, 127.54, 127.39, 127.37, 127.31, 127.24, 127.18, 126.39, 125.86, 125.62, 125.49, 125.45, 125.22, 125.09, 99.55, 98.87, 95.46, 95.27, 79.55, 78.42, 77.42, 74.73, 74.36, 74.06, 73.69, 72.81, 72.32, 72.19, 72.08, 71.28, 71.25, 70.76, 70.26, 69.98, 69.74, 68.03, 67.49, 67.26, 66.50, 66.39, 65.96, 65.07, 63.71, 62.66, 59.83, 59.72, 37.99, 33.56, 31.67, 29.37, 29.35, 29.31, 29.20, 29.07, 29.01, 28.81, 24.60, 22.34, 13.07, 7.89. HR-ESI-MS (m/z): [M-2H]$^2^+$ calcd for C$_{92}$H$_{93}$N$_7$O$_{29}$S$_2$: 911.7735; found, 911.7748.

N-benzyloxy carbonyl-3-aminopropyl-O-[(benzyl (2-acetamido-4-O-benzyl-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(3-O-benzyl-2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(benzyl (2-azido-3-O-(2-naphthylmethyl)-2-deoxy-α-D-glucopyranosyl)])-(1→4)-O-3-O-benzyl-2-O-sulfonato-α-L-idopyranosiduronate (44)

$^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.68 – 7.65 (m, 2H), 7.63-7.60 (m, 3H), 7.53 – 7.45 (m, 4H), 7.34 (d, $J = 3.3$ Hz, 2H), 7.32 – 7.31 (m, 4H), 7.28-7.25 (m, 7H), 7.20- 7.18 (m, 7H), 7.16 – 7.12 (m, 5H), 7.08 (dd, $J = 5.0, 1.9$ Hz, 3H), 7.05 (dd, $J = 8.5, 1.5$ Hz, 2H), 6.99 (dd, $J = 6.6, 3.1$ Hz, 2H), 6.95 (d, $J = 7.4$ Hz, 1H), 6.81 (t, $J = 7.7$ Hz, 2H), 5.43 (s, 1H), 5.21 (d, $J = 12.0$ Hz, 1H), 5.07 (d, $J = 9.6$ Hz, 2H), 4.97- 4.94 (m, 2H), 4.87 (d, $J = 2.9$ Hz, 1H), 4.68 – 4.66 (m, 2H), 4.61 (dd, $J = 11.1, 4.7$ Hz, 2H).
Hz, 3H), 4.48 (d, J = 5.3 Hz, 3H), 4.44 (dd, J = 9.1, 3.0 Hz, 3H), 4.39 (d, J = 9.3 Hz, 2H), 4.16 (s, 3H), 4.04 – 3.98 (m, 4H), 3.85 (t, J = 12.0 Hz, 4H), 3.67– 3.60 (m, 4H), 3.55 – 3.41 (m, 5H), 3.30– 3.26 (m, 2H), 3.11 – 3.05 (m, 2H), 1.93 (s, 3H), 1.74 – 1.68 (m, 2H), 1.61 (s, 3H). 13C NMR (151 MHz, Methanol-d4) δ 169.82, 169.70, 157.37, 138.45, 137.52, 137.49, 136.90, 136.37, 136.00, 134.89, 134.19, 133.34, 133.01, 132.71, 128.57, 128.46, 128.33, 128.08, 128.06, 128.04, 127.83, 127.75, 127.70, 127.61, 127.55, 127.54, 127.48, 127.45, 127.36, 127.29, 127.28, 127.21, 127.10, 126.34, 126.13, 125.89, 125.59, 125.51, 125.43, 125.10, 124.47, 99.56, 98.88, 80.32, 78.98, 77.33, 75.18, 74.47, 73.33, 72.75, 72.09, 71.64, 70.55, 70.13, 67.37, 66.78, 66.68, 66.51, 66.00, 59.78, 59.45, 53.23, 53.14, 38.22, 29.19, 22.04, 21.76. HR-ESI-MS (m/z): [M-2H]2– calcld for C96H101N3O31S22, 928.2952; found, 928.2969.

2.8 Global Deprotection:

To a solution of starting material (1 mmol for 1-7) in THF (1 mL) and H2O (1 mL) was added LiOH·H2O (10 mmol) and stirred at room temperature for 2 h. Upon completion, the reaction mixture was diluted with MeOH and quenched using Dowex 50WX8 H+ resin. The mixture was filtered, concentrated and eluted through Bond Elute C-18 column (H2O/ACN= 1/5, v/v). The combined fraction was concentrated under reduced pressure and dissolved in MeOH for hydrogenolysis. The reaction mixture along with Pd(OH)2 was stirred under H2 atm. After 36 h,
the mixture was filtered, concentrated and eluted through Bond Elute C-18 column using H$_2$O as eluent. The combined H$_2$O fraction were pooled and lyophilized to yield fully deprotected O-sulfated tetrasaccharides. For 7 & 13 after Pd(OH)$_2$ hydrogenolysis the as obtained product was again kept for hydrogenolysis using PtO$_2$ as a catalyst under H$_2$ atm for 24 h. Finally, the reaction mixture was filtered, concentrated under reduced pressure and eluted through Bond Elute C-18 column using H$_2$O as eluent. The combined H$_2$O fraction were pooled and lyophilized to yield fully deprotected O-sulfated tetrasaccharides 7 & 13. For 8-12 hydrogenation was carried out by the above described method.

3-aminopropyl-O-[(2-amino-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(α-L-idopyranosyluronate)-(1→4)-O-(2-amino-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-α-L-idopyranosiduronate (1)

\[
\begin{align*}
&\text{1H NMR (400 MHz, Deuterium Oxide) } \delta 5.37 (d, J = 3.6 Hz, 1H), 5.29 (d, J = 3.7 Hz, 1H), 4.72 (d, J = 4.2 Hz, 1H), 4.59 (d, J = 3.2 Hz, 1H), 4.36 (d, J = 2.7 Hz, 1H), \\
&4.03 - 4.01 (m, 1H), 3.96 (t, J = 3.1 Hz, 1H), 3.91 - 3.88 (m, 1H), 3.79 (dd, J = 7.0, 3.8 Hz, 1H), \\
&3.76- 3.72 (m, 2H), 3.70 (s, 3H), 3.68- 3.66 (m, 3H), 3.65 - 3.63 (m, 1H), 3.61 (d, J = 9.4 Hz, \\
&1H), 3.57- 3.56 (m, 1H), 3.55- 3.51 (m, 3H), 3.32 (t, J = 9.4 Hz, 1H), 3.19 (ddd, J = 12.6, 10.6, \\
&3.6 Hz, 2H), 2.97 (t, J = 6.7 Hz, 2H), 1.87 - 1.78 (m, 2H). \\
&\text{13C NMR (101 MHz, Deuterium Oxide) } \delta 174.73, 174.51, 101.85, 100.65, 92.25, 92.22, 75.58, 74.41, 73.27, 72.32, 71.49, 70.12, 70.10, \\
&69.42, 69.23, 68.69, 68.41, 68.12, 67.59, 66.35, 59.88, 59.30, 54.23, 53.97, 38.03, 26.36. \\
&\text{HR-ESI-MS (m/z): } [M+H]^+ \text{ calcd for C}_{27}H_{47}N_3O_{21}, 750.2775; \text{ found, 750.2780.}
\end{align*}
\]

3-aminopropyl-O-[(2-amino-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(α-L-idopyranosyluronate)-(1→4)-O-(2-amino-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-α-L-idopyranosiduronate (2)

\[
\begin{align*}
&\text{1H NMR (600 MHz, Deuterium Oxide) } \delta 5.41 (d, J = 3.7 Hz, 1H), 5.39 (d, J = 3.6 Hz, 1H), 5.04 - 5.03 (m, 3H), \\
&4.15 - 4.11 (m, 1H), 4.07 - 3.97 (m, 1H), 3.85 - 3.81 (m, 2H), 3.80- 3.72 (m, 5H), 3.70 - 3.59 (m, 5H), 3.50 - 3.39 (m, 2H), 3.30 (td, J = 10.7, \\
&10.0, 3.6 Hz, 1H), 3.08- 3.05 (m, 2H), 2.76 (d, J = 5.2 Hz, 1H), 1.96- 1.88 (m, 2H). \\
&\text{13C NMR (151 MHz, Deuterium Oxide) } \delta 171.27, 101.97, 100.86, 93.16, 92.70, 76.71, 73.37, 73.00, 72.55,
\end{align*}
\]
3-aminopropyl-O-[(2-amino-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(α-L-idopyranosyluronate)-(1→4)-O-[(2-amino-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-α-L-idopyranosiduronate (3)

\[
\text{HR-ESI-MS (m/z): } [M-2H]^2^- \text{ calcd for } C_{27}H_{45}N_3O_{27}S_2^2-, 453.5846; \text{ found, 453.5981.}
\]

\[
3\text{-aminopropyl-O-[(2-amino-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(α-L-idopyranosyluronate)-(1→4)-O-[(2-amino-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-α-L-idopyranosiduronate (4)}
\]

\[
\text{HR-ESI-MS (m/z): } [M+H]^+ \text{ calcd for } C_{27}H_{48}N_3O_{33}S_4^+, 1070.1048; \text{ found, 1070.1050.}
\]

\[
\text{1H NMR (600 MHz, Deuterium Oxide) } \delta 5.51 \text{ (d, J = 3.5 Hz, 1H)}, 5.41 - 5.39 \text{ (m, 1H)}, 4.95 \text{ (d, J = 2.8 Hz, 1H)}, 4.87 \text{ (d, J = 4.0 Hz, 1H)}, 4.56 - 4.50 \text{ (m, 1H)}, 4.48 - 4.42 \text{ (m, 2H)}, 4.19 \text{ (t, J = 3.6 Hz, 1H)}, 4.08 - 4.05 \text{ (m, 1H)}, 3.99 \text{ (t, J = 3.8 Hz, 1H)}, 3.93 \text{ (t, J = 9.6 Hz, 1H)}, 3.89 \text{ (s, 1H)}, 3.88 - 3.84 \text{ (m, 2H)}, 3.82 - 3.77 \text{ (m, 6H)}, 3.69 - 3.67 \text{ (m, 1H)}, 3.64 - 3.59 \text{ (m, 4H)}, 3.55 \text{ (dd, J = 10.4, 3.6 Hz, 1H)}, 3.06 - 3.04 \text{ (m, 2H)}, 1.96 - 1.87 \text{ (m, 2H)}.\]

\[
\text{13C NMR (151 MHz, Deuterium Oxide) } \delta 174.57, 100.92, 100.59, 92.63, 92.42, 77.31, 74.86, 74.78, 73.72, 72.07, 71.46, 71.42, 69.98, 69.80, 69.50, 68.57, 68.15, 67.39, 67.29, 66.43, 59.78, 59.17, 53.63, 53.12, 38.06.\]

\[
\text{HR-ESI-MS (m/z): } [M+H]^+ \text{ calcd for } C_{27}H_{48}N_3O_{33}S_4^+, 1070.1048; \text{ found, 1070.1050.}
\]

\[
3\text{-aminopropyl-O-[(2-amino-6-O-sulfonato-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(α-L-idopyranosyluronate)-(1→4)-O-[(2-amino-6-O-sulfonato-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-α-L-idopyranosiduronate (4)}
\]

\[
\text{1H NMR (600 MHz, Deuterium Oxide) } \delta 5.26 \text{ (d, J = 3.2 Hz, 1H)}, 5.09 \text{ (d, J = 3.5 Hz, 1H)}, 4.87 \text{ (d, J = 4.2 Hz, 1H)}, 4.80 - 4.78 \text{ (m, 2H)}, 4.42 \text{ (d, J = 2.7 Hz, 1H)}, 4.31 - 4.24 \text{ (m, 4H)}, 4.20 - 4.17 \text{ (m, 1H)}, 4.13 - 4.08 \text{ (m, 2H)}, 4.02 - 3.99 \text{ (m, 3H)}, 3.96 - 3.95 \text{ (m, 2H)}, 3.88 \text{ (t, J = 9.7 Hz, 1H)}, 3.84 - 3.79 \text{ (m, 2H)}, 3.67 - 3.55 \text{ (m, 4H)}, 3.08 - 3.04 \text{ (m, 4H)}, 2.99 \text{ (dd, J = 10.2, 3.5 Hz, 1H)}, 1.94-1.87 \text{ (m, 2H)}.\]

\[
\text{13C NMR (151 MHz, Deuterium Oxide) } \delta 174.98, 171.04, 100.49, 100.43, 96.66, 95.49, 79.53, 75.67, 74.77, 71.37, 70.91, 70.40, 70.14, 70.03, 69.95, 68.98, 68.79, 68.40, 67.47, 66.48, 66.29, 66.13, 54.46, 53.86, 38.14, 26.39.\]

\[
\text{HR-ESI-MS (m/z): } [M+H]^+ \text{ calcd for } C_{27}H_{48}N_3O_{33}S_4^+, 1070.1048; \text{ found, 1070.1050.}
\]
3-aminopropyl-O-[(2-acetamido-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(α-L-idopyranosyluronate)-(1→4)-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-α-L-idopyranosiduronate (5)

$^1$H NMR (400 MHz, Deuterium Oxide) δ 5.08 (d, J = 3.7 Hz, 1H), 5.05 (d, J = 3.8 Hz, 1H), 5.00 (d, J = 2.7 Hz, 1H), 4.90 (d, J = 2.7 Hz, 1H), 4.84 (d, J = 1.8 Hz, 1H), 4.67 (d, J = 2.4 Hz, 1H), 4.02 (t, J = 3.1 Hz, 1H), 3.99 (t, J = 2.6 Hz, 1H), 3.94 (t, J = 3.6 Hz, 1H), 3.91 – 3.79 (m, 4H), 3.75- 3.71 (m, 4H), 3.67 – 3.65 (m, 3H), 3.64 – 3.58 (m, 3H), 3.58 – 3.52 (m, 2H), 3.45 (d, J = 9.3 Hz, 1H), 3.06 (t, J = 6.6 Hz, 2H), 1.95 – 1.88 (m, 8H). $^{13}$C NMR (101 MHz, Deuterium Oxide) δ 174.43, 174.40, 173.41, 173.02, 101.63, 100.57, 95.15, 95.00, 76.85, 73.76, 73.26, 72.45, 71.49, 71.08, 69.77, 69.29, 69.03, 68.07, 68.04, 67.96, 67.16, 66.63, 60.03, 59.55, 53.64, 53.39, 38.13, 26.24, 21.93. HR-ESI-MS (m/z): [M-H] calcd for C$_{31}$H$_{50}$N$_3$O$_{25}$, 832.2840; found, 832.2835.

3-aminopropyl-O-[(2-acetamido-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(α-L-idopyranosyluronate)-(1→4)-O-(2-acetamido-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-α-L-idopyranosiduronate (6)

$^1$H NMR (400 MHz, Deuterium Oxide) δ 4.99 (t, J = 4.2 Hz, 2H), 4.95 (d, J = 2.4 Hz, 1H), 4.87 (s, 1H), 4.77 (s, 1H), 4.60 (d, J = 1.9 Hz, 1H), 4.16 (ddd, J = 11.0, 8.0, 3.3 Hz, 2H), 4.06 (ddd, J = 10.9, 6.7, 1.8 Hz, 2H), 3.94 (dt, J = 4.8, 2.8 Hz, 2H), 3.89 – 3.72 (m, 6H), 3.64- 3.62 (m, 2H), 3.60 – 3.53 (m, 4H), 3.50 (d, J = 10.4 Hz, 1H), 3.42 (d, J = 9.7 Hz, 1H), 2.99 (t, J = 6.6 Hz, 2H), 1.88- 1.80 (m, 8H). $^{13}$C NMR (101 MHz, Deuterium Oxide ) δ 174.43, 174.33, 173.41, 101.86, 100.50, 95.24, 94.96, 77.25, 73.66, 73.34, 71.03, 70.60, 69.91, 69.54, 68.99, 68.58, 68.06, 67.55, 67.42, 67.15, 66.67, 66.58, 66.53, 66.13, 53.49, 53.22, 38.18, 26.21, 21.96. HR-ESI-MS (m/z): [M-2H]²⁻ calcd for C$_{30}$H$_{49}$N$_3$O$_{22}$S$_2$²⁻, 495.5952; found, 495.5974.

3-aminopropyl-O-[(2-acetamido-6-O-phosphonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(α-L-idopyranosyluronate)-(1→4)-O-(2-acetamido-6-O-phosphonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-α-L-idopyranosiduronate (7)

$^1$H NMR (400 MHz, Deuterium Oxide ) δ 4.98 (d, J = 3.1 Hz, 2H), 4.89 (s, 1H), 4.78 (s, 1H), 3.99- 3.70 (m, 6H), 3.85 (ddd, J = 14.1, 8.1, 3.2 Hz, 4H), 3.80 – 3.73 (m, 1H), 3.69 – 3.49 (m, 9H), 3.48 – 3.40 (m, 1H), 2.99 (t, J = 6.4 Hz, 2H), 1.92- 1.82 (m, 8H). $^{13}$C NMR (151 MHz, Deuterium Oxide ) δ 174.65, 174.46, 174.34, 101.85, 100.44, 95.06, 94.58, 81.76, 77.10, 74.05, 73.37, 71.07, 70.33, 70.28, 69.87, 69.15, 68.97, 68.52, 68.46, 68.13, 67.97,
$^{31}$P NMR (162 MHz, D$_2$O) $\delta$ 1.35, 0.85. HR-ESI-MS ($m/z$): [M-2H]$^{2-}$ calcd for C$_{31}$H$_{53}$N$_3$O$_{29}$P$_2$$^{2-}$, 495.6047; found, 495.6060.

3-aminopropyl-O-[(2-amino-2-deoxy-\(\alpha\)-D-glucopyranosyl)-(1→4)-O-(2-O-sulfonato-\(\alpha\)-L-idopyranosyluronate)-(1→4)-O-(2-amino-2-deoxy-\(\alpha\)-D-glucopyranosyl)]-(1→4)-O-2-O-sulfonato-\(\alpha\)-L-idopyranosiduronate (8)

$^1$H NMR (600 MHz, Deuterium Oxide) $\delta$ 5.33 (s, 1H), 5.15 (s, 1H), 5.05 (s, 1H), 4.84 (s, 1H), 4.46 (s, 1H), 4.28 – 4.25 (m, 3H), 4.17 (s, 1H), 4.06 (d, $J$ = 14.2 Hz, 2H), 3.88 (t, $J$ = 9.9 Hz, 1H), 3.81 – 3.77 (m, 5H), 3.73 (s, 2H), 3.68 - 3.67 (m, 2H), 3.64 – 3.62 (m, 1H), 3.40 (t, $J$ = 9.6 Hz, 1H), 3.29 (d, $J$ = 10.7 Hz, 1H), 3.26 – 3.24 (m, 1H), 3.09 – 3.05 (m, 2H), 1.96 - 1.87 (m, 2H). $^{13}$C NMR (151 MHz, Deuterium Oxide) $\delta$ 175.38, 174.85, 98.74, 97.78, 91.06, 90.96, 76.07, 72.53, 72.42, 72.01, 71.44, 70.06, 69.95, 69.33, 69.06, 67.99, 66.87, 66.59, 66.43, 62.40, 62.37, 59.71, 59.27, 54.50, 54.14, 38.32, 26.06. HR-ESI-MS ($m/z$): [M-2H]$^{2-}$ calcd for C$_{27}$H$_{45}$N$_3$O$_{27}$S$_2$$^{2-}$, 453.5846; found, 453.5911.

3-aminopropyl-O-[(2-acetamido-2-deoxy-\(\alpha\)-D-glucopyranosyl)-(1→4)-O-(2-O-sulfonato-\(\alpha\)-L-idopyranosyluronate)-(1→4)-O-(2-acetamido-2-deoxy-\(\alpha\)-D-glucopyranosyl)]-(1→4)-O-2-O-sulfonato-\(\alpha\)-L-idopyranosiduronate (9)

$^1$H NMR (600 MHz, Deuterium Oxide) $\delta$ 5.12 (s, 2H), 5.06 (s, 1H), 5.03 (d, $J$ = 3.4 Hz, 1H), 5.01 (d, $J$ = 3.4 Hz, 1H), 4.76 (d, $J$ = 1.8 Hz, 1H), 4.21 (d, $J$ = 6.5 Hz, 2H), 4.17 (d, $J$ = 13.4 Hz, 2H), 3.97 (d, $J$ = 11.1 Hz, 2H), 3.90 (ddd, $J$ = 18.5, 10.5, 3.4 Hz, 2H), 3.85 – 3.81 (m, 1H), 3.76 (s, 2H), 3.71 (d, $J$ = 2.8 Hz, 2H), 3.65 – 3.59 (m, 4H), 3.56 – 3.50 (m, 2H), 3.42 (d, $J$ = 9.2 Hz, 1H), 3.06 (t, $J$ = 6.3 Hz, 2H), 1.98 (s, 3H), 1.96 (s, 3H), 1.93 – 1.88 (m, 2H). $^{13}$C NMR (151 MHz, Deuterium Oxide) $\delta$ 174.80, 173.03, 172.79, 99.17, 98.34, 94.86, 94.32, 76.95, 73.19, 73.15, 72.36, 71.60, 71.36, 71.05, 70.94, 69.70, 69.34, 66.72, 66.19, 63.97, 63.42, 60.02, 59.47, 53.51, 53.22, 38.15, 26.02, 22.14. HR-ESI-MS ($m/z$): [M-2H]$^{2-}$ calcd for C$_{31}$H$_{49}$N$_3$O$_{29}$S$_2$$^{2-}$, 495.5952; found, 495.5922.
3-aminopropyl-O-[(2-amino-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(2-amino-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-2-O-sulfonato-α-L-idopyranosiduronate (10)

1H NMR (400 MHz, Deuterium Oxide) δ 5.29 (dd, J = 7.0, 3.7 Hz, 2H), 5.10 (s, 1H), 5.01 (s, 1H), 4.83 (s, 1H), 4.47 (s, 1H), 4.25 – 4.23 (m, 2H), 4.22 (d, J = 2.0 Hz, 1H), 4.19 (d, J = 3.0 Hz, 1H), 4.17 – 4.14 (m, 2H), 4.13 – 4.11 (m, 1H), 4.09 (s, 1H), 4.06 (s, 1H), 4.04 (s, 1H), 3.87 (d, J = 9.6 Hz, 1H), 3.82 (d, J = 10.4 Hz, 1H), 3.80 – 3.75 (m, 2H), 3.74 – 3.69 (m, 1H), 3.66 (d, J = 9.7 Hz, 1H), 3.61 - 3.55 (m, 1H), 3.43 (t, J = 9.7 Hz, 1H), 3.31 – 3.22 (m, 2H), 3.02 (q, J = 6.7, 5.6 Hz, 2H), 1.91- 1.82 (m, 2H). 13C NMR (151 MHz, Deuterium Oxide) δ 174.95, 98.71, 97.82, 91.30, 90.92, 75.55, 72.67, 72.48, 70.32, 70.14, 69.59, 69.23, 68.64, 68.10, 67.05, 66.76, 66.38, 66.13, 62.72, 62.30, 54.27, 53.99, 38.43, 26.08. HR-ESI-MS (m/z): [M+H]⁺ calcd for C_{27}H_{48}N_{3}O_{33}S_{4}⁺, 1070.1048; found, 1070.1051.

3-aminopropyl-O-[(2-amino-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(2-amino-3-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-2-O-sulfonato-α-L-idopyranosiduronate (11)

1H NMR (600 MHz, Deuterium Oxide) δ 5.44 (d, J = 3.6 Hz, 1H), 5.37 (d, J = 3.7 Hz, 1H), 4.83 (d, J = 3.7 Hz, 1H), 4.80 (d, J = 2.4 Hz, 1H), 4.77 (d, J = 3.1 Hz, 1H), 4.52 (d, J = 2.6 Hz, 1H), 4.12 – 4.11 (m, 1H), 4.06 – 4.05 (m, 1H), 4.00 – 3.97 (m, 1H), 3.90 (dd, J = 6.8, 4.1 Hz, 1H), 3.85- 3.81 (m, 2H), 3.77- 3.72 (m, 6H), 3.70 – 3.67 (m, 2H), 3.66 – 3.62 (m, 3H), 3.41 (t, J = 9.6 Hz, 1H), 3.31 – 3.26 (m, 2H), 3.07 – 3.05 (m, 2H), 1.94 – 1.87 (m, 2H). 13C NMR (151 MHz, Deuterium Oxide) δ 174.23, 173.94, 101.86, 100.69, 92.45, 92.34, 75.87, 74.15, 73.16, 72.48, 69.81, 69.45, 69.40, 69.17, 68.49, 68.09, 68.03, 67.22, 66.45, 59.90, 59.32, 54.23, 53.96, 38.06, 26.35 HR-ESI-MS (m/z): [M+H]⁺ calcd for C_{27}H_{48}N_{3}O_{33}S_{4}⁺, 1070.1048; found, 1070.1052.

3-aminopropyl-O-[(2-acetamido-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)-(1→4)-O-(2-O-sulfonato-α-L-idopyranosyluronate)-(1→4)-O-(2-acetamido-6-O-sulfonato-2-deoxy-α-D-glucopyranosyl)]-(1→4)-O-2-O-sulfonato-α-L-idopyranosiduronate (12)

1H NMR (400 MHz, Deuterium Oxide) δ 5.14 (s, 1H), 5.10 (d, J = 1.7 Hz, 1H), 5.07 (d, J = 3.7 Hz, 3H), 4.76 (d, J = 2.0 Hz, 1H), 4.28 – 4.13 (m, 8H), 4.04 – 4.03 (m,
2H), 3.96 (ddd, J = 16.3, 10.3, 3.5 Hz, 2H), 3.91- 3.83 (m, 2H), 3.77 – 3.62 (m, 4H), 3.61 – 3.55 (m, 1H), 3.50 (d, J = 9.6 Hz, 1H), 3.09 (t, J = 6.4 Hz, 2H), 2.00 (s, 3H), 1.98 (s, 3H), 1.96- 1.91 (m, 2H). \(^{13}\)C NMR (101 MHz, Deuterium Oxide) δ 174.82, 173.03, 99.15, 98.35, 95.05, 93.90, 76.67, 73.68, 73.24, 72.06, 70.91, 70.83, 70.52, 69.88, 69.62, 69.12, 66.82, 66.56, 66.50, 64.65, 63.24, 53.30, 53.15, 38.30, 26.05, 22.19. HR-ESI-MS (m/z): [M-3H]- calcd for C\textsubscript{31}H\textsubscript{48}N\textsubscript{3}O\textsubscript{35}S\textsubscript{3}, 383.3656; found, 383.3639.

3-aminopropyl-O-[(2-acetamido-6-O-phosphonato-2-deoxy-\(\alpha\)-D-glucopyranosyl)-(1\(\rightarrow\)4)-O-(2-O-phosphonato-\(\alpha\)-L-idopyranosyluronate)-(1\(\rightarrow\)4)-O-(2-acetamido-6-O-phosphonato-2-deoxy-\(\alpha\)-D-glucopyranosyl)]-(1\(\rightarrow\)4)-O-2-O-phosphonato-\(\alpha\)-L-idopyranosiduronate (13)

\[\begin{align*}
&\text{HO} &\text{HO} &\text{HO} &\text{HO} &\text{HO} &\text{HO} &\text{HO} &\text{NH}_2 \\
&\text{OP}_3\text{O}^2- &\text{OP}_3\text{O}^2- &\text{OP}_3\text{O}^2- &\text{OP}_3\text{O}^2- &\text{OP}_3\text{O}^2- &\text{OP}_3\text{O}^2- &\text{OP}_3\text{O}^2- &\text{OP}_3\text{O}^2- \\
&\text{13} &\text{13} &\text{13} &\text{13} &\text{13} &\text{13} &\text{13} &\text{13}
\end{align*}\]

\(^{1}\)H NMR (400 MHz, Deuterium Oxide) δ 5.13 (d, J = 10.1 Hz, 1H), 5.05 (dd, J = 10.2, 6.5 Hz, 2H), 4.79 (s, 1H), 4.15 – 4.02 (m, 10H), 3.94 (td, J = 10.5, 3.4 Hz, 2H), 3.88 – 3.75 (m, 4H), 3.72 – 3.62 (m, 4H), 3.59 – 3.57 (m, 1H), 3.51 (d, J = 9.2 Hz, 1H), 3.08 (t, J = 6.4 Hz, 1H), 2.02 – 1.90 (m, 8H). \(^{13}\)C NMR (151 MHz, Deuterium Oxide) δ 174.81, 172.75, 172.64, 100.08, 99.35, 95.88, 95.61, 77.05, 73.30, 73.24, 71.62, 71.33, 70.66, 69.65, 69.12, 66.86, 66.79, 66.61, 63.79, 53.58, 53.34, 38.27, 26.07, 22.21, 22.15. \(^{31}\)P NMR (162 MHz, Deuterium Oxide) δ 0.75, 0.15, -0.10, -0.27. HR-ESI-MS (m/z): [M-2H]\(^{2-}\) calcd for C\textsubscript{31}H\textsubscript{53}N\textsubscript{3}O\textsubscript{35}P\textsubscript{4}, 575.5710; found, 575.5759.

3. Glycan microarray

Materials. PBSx10 was purchased from Hy-labs, ethanolamine from Fisher, ovalbumin (Grade V), sodium phosphate monobasic monohydrate, sodium phosphate dibasic heptahydrate, Tween-20 and Tris/HCl were purchased from Sigma-Aldrich. Antibodies were purchased from Peprotech: Human SD1α CXCL12, human IL8 CXCL8 72 aa, human exodus-2 CCL21, human MCP-3 CCL7, human MCP-4 CCL13, human IP-10 CXCL10, human MEC CCL28, human RANTES CCL5, human MCP-1/MCAF CCL2, biotinylated antigen affinity-purified goat-anti-murine SDF-1α, biotinylated-rabbit-anti-human IL8 CXCL8, biotinylated-rabbit-anti-human Exodus-2 CCL21, biotinylated goat-anti-human MCP-3, biotinylated anti-H-MCP-4, biotinylated rabbit-anti-human IP-10, biotinylated anti-human MEC, biotinylated-rabbit-anti-human MCAF/MCP-1. Biotinylated anti-Rantes was purchased from R&D. Cy3-streptavidin (Cy3-SA) was purchased from Jackson ImmunoResearch. IVIG GammaGard 10% (Baxter, USA) were a kind gift from Adriana Tremolot, Childrens hospital UCSD.
**Heparin tetrasaccharide microarray fabrication.** Arrays were fabricated with NanoPrint LM-60 Microarray Printer (Arrayit) on epoxide-derivatized slides (PolyAn 2D) with 16 sub-array blocks on each slide. Glycoconjugates were distributed into 384-well source plates using 4 replicate wells per sample and 7 µl per well. Each glycoconjugate diluted into 50 and 100 µM in an optimized printing buffer (300 mM phosphate buffer, pH 8.4 Version VrHI.01. To monitor printing Alexaflour-555-hydraside (Invitrogen, at 1 ng/µl in 178 mM phosphate buffer, pH 5.5) was used for each printing run. The arrays were printed with four 946MP3 pins (5 µm tip, 0.25 µl sample channel, ~100 µm spot diameter; Arrayit). The humidity level in the arraying chamber was maintained at about 70% during printing. Printed slides were left on arrayer deck overnight, allowing humidity to drop to ambient levels (40-45%). Next, slides were packed, vacuum-sealed and stored at room temperature (RT) until used.

**HS tetrasaccharide microarray binding assay.** Slides were developed and analyzed as previously described with some modifications. Slides were rehydrated with dH2O and incubated for 30 min in a staining dish with 50°C pre-warmed ethanolamine (0.05 M) in Tris-HCl (0.1 M, pH 9.0) to block the remaining reactive epoxy groups on the slide surface, then washed with 50 °C pre-warmed dH2O. Slides were centrifuged at 200×g for three min then fitted with ProPlate™ Multi-Array 16-well slide module (Grace Bio-lab P37001) to divide into the sub-arrays (blocks). Slides were washed with PBST (0.1% Tween 20), aspirated and blocked with 200 µl/sub-array of blocking buffer (PBS pH 7.3 + 1% w/v ovalbumin) for 1 hour at RT with gentle shaking. Next, the blocking solution was aspirated and 100 µl/block of primary detection proteins (for each detection, 3 serially decreasing concentrations were used, see Table S1) diluted in PBS pH 7.3 + 1% w/v ovalbumin, were incubated with gentle shaking for 2 hours at RT. Slides were washed 4 times with PBST, then with PBS (without Tween-20) for 2 min. Bound antibodies were detected by incubating with biotinylated secondary detections (1 ng/µl, see Table S1) diluted in PBS, 200 µl/block at RT for 1 hour. Slides were washed 4 times with PBST, then with PBS (without Tween-20) for 2 min and biotinylated antibodies were detected with Cy3-SA (1.2µg/ml). Slides were washed 4 times with PBST, then with PBS for 10 min followed by removal from ProPlate™ Multi-Array slide module and immediately dipping in a staining dish with dH2O for 10 min with shaking. Slide then were centrifuged at 200×g for 3 min. and the dry slides immediately scanned.

**Array slide processing.** Processed slides were scanned and analyzed as described at 10 µm resolution with a Genepix 4000B microarray scanner (Molecular Devices) using 350 gain. Image analysis was carried out with Genepix Pro 4.0 analysis software (Molecular Devices). Spots
were defined as circular features with a variable radius as determined by the Genepix scanning software. Local background subtraction was performed. RFU from each spot was calculated and Ranking (per printed glycan per concentration, per each chemokine dilution, per printed block) was used to compare the data between detections; since each glycans was printed at 2 concentration, 100% binding was set separately for each concentration. Then, binding to all the other glycans at the same concentration was ranked in comparison to the maximal binding, and the average rank binding and SEM for each glycan across the two glycan concentrations and three examined dilutions of each chemokine was calculated (n=6, 2 glycan concentrations across 3 chemokine dilutions).\(^1\)

Table S1. Primary detection concentrations used on the array and Secondary Biotinylated antibodies.

| Primary Detection          | Concentrations used (ng/µl) | Secondary Detection                                      |
|----------------------------|-----------------------------|----------------------------------------------------------|
| Human SD1α (CXCL12)        | 20, 10, 5                   | Biotinylated antigen affinity-purified goat-anti-murine SDF-1α |
| Human IL8 (CXCL8) 72 aa    | 20, 10, 5                   | Biotinylated-rabbit-anti-human IL8 (CXCL8)               |
| Human Exodus-2 (CCL21)     | 20, 10, 5                   | Biotinylated-rabbit-anti-human Exodus-2 (CCL21)         |
| Human MCP-3 (CCL7)         | 20, 10, 5                   | Biotinylated-goat-anti-human MCP-3                       |
| Human MCP-4 (CCL13)        | 20, 10, 5                   | Biotinylated anti-human MCP-4                           |
| Human IP-10 (CXCL10)       | 10, 5, 2.5                  | Biotinylated-rabbit-anti-human IP-10                     |
| Human BCA-1 (CXCL13)       | 10, 5, 2.5                  | Biotinylated-rabbit-anti-human BCA-1                     |
| Human MEC (CCL28)          | 10, 5, 2.5                  | Biotinylated-anti-human MEC                              |
| Human RANTES (CCL5)        | 2, 1, 0.5                   | Biotinylated anti-Rantes                                 |
| Human MCP-1/MCAF (CCL2)    | 2, 1, 0.5                   | Biotinylated-rabbit-anti-human MCAF/MCP-1               |
Figure S1. Chemokine glycan microarray binding assay. Binding was tested at 3 serial dilutions across 2 glycan concentrations (High, medium and low chemokine concentration at 50µM and 100µM glycan concentration on the array), then detected with the relevant biotinylated secondary antibody (1 µg/ml) followed by Cy3-Strepavidin (1.5 µg/ml) (Table S1). Arrays were scanned, relative fluorescent units (RFU) obtained, and maximum RFU determined and set as 100% binding. Then rank binding (per printed glycan per concentration, per each chemokine dilution, per printed block) was determined. Since each glycans was printed at 2 concentration, 100% binding was set separately for each concentration. Then, binding to all the other glycans at the same concentration was ranked in comparison to the maximal binding, and the average rank binding and SEM for each glycan across the two glycan concentrations and three examined dilutions of each chemokine was calculated (n=6, 2 glycan concentrations across 3 chemokine dilutions). This analysis allowed to compare the glycan binding profiles of the different chemokines and dissect their binding preferences. The mean rank is shown as a heatmap of all the examined binding assays together (red highest, blue lowest and white 50th percentile of ranking).

4. Surface Plasmon Resonance binding kinetics.

HS tetrasaccharides (HT-2,6S-NH or HT-3,6S-NH) were immobilized on CM5 sensor surface using a coupling reaction. Briefly, CM5 chip was activated with N-hydroxysuccimide (0.05 mM) and EDC (0.1mM) in water before injecting 0.05 mM of HS oligosaccharides at a flow rate of 100 µl/min until 500 RU is achieved. Next, remaining active esters were quenched with ethanolamine (0.1 mM) in HBS-EP buffer. Similarly, the control flow cell was active with NHS and EDC and neutralized with ethanolamine. A known concentration of chemokines was injected in HBS-EP buffer at a flow rate of 50 µl/min at 25 °C for 200 sec followed by running buffer (HBS-EP without chemokine) was then flowed for another 100 sec to enable dissociation. The CM5 chip was regenerated by washing with 0.1% SDS and 0.085% H₃PO₄ injected for 3 min at a flow rate of 100 µL/min. Kinetic analysis was executed using the BLAevaluation software for T100. K_{off} and K_{on} phase was globally fitted to a simple 1:1 interaction model.
Figure S2. SPR analysis of chemokine binding profile on sensor chip having HT-2,6S-NH and HT-3,6S-NH ligands: (a) (b) & (c) SPR binding analysis of the interaction between HT-2,6S-NH with CXCL8, CXCL10 and CCL7 respectively. Concentrations of chemokine were 0.05–2 μM. A global fit according to a 1:1 binding model was applied (black curves); (d), (e) & (f) SPR binding analysis of the interaction between HT-3,6S-NH with CXCL8, CXCL10 and CCL7 respectively.

Table S2: SPR analysis of kinetic rate constants and equilibrium affinities for HS ligands binding to chemokines from Fig S2 and Fig 3.

| Substrate   | Chemokines | $K_D$ (μM) | $K_{on}$ (M$^{-1}$S$^{-1}$) | $K_{off}$ (s$^{-1}$) |
|-------------|------------|------------|-----------------------------|---------------------|
| HT-2,6S-NH  | CXCL8      | 15.93 ± 0.23 | 1.23 ± 0.17 × 10$^4$     | 1.96 ± 0.29 × 10$^{-1}$ |
|             | CXCL10     | 10.7 ± 0.37  | 2.07 ± 0.26 × 10$^4$     | 2.24 ± 0.35 × 10$^{-1}$ |
|             | CCL7       | 11.51 ± 0.17 | 1.96 ± 0.22 × 10$^4$     | 2.25 ± 0.29 × 10$^{-1}$ |
|             | CCL5       | 2.34 ± 0.44  | 9.15 ± 0.34 × 10$^4$     | 2.14 ± 0.48 × 10$^{-1}$ |
|             | CCL2       | 9.82 ± 0.18  | 2.07 ± 0.13 × 10$^4$     | 2.03 ± 0.17 × 10$^{-1}$ |
| HT-3,6S-NH  | CXCL8      | 11.67 ± 0.17 | 1.87 ± 0.22 × 10$^4$     | 2.18 ± 0.28 × 10$^{-1}$ |
|             | CXCL10     | 11.02 ± 0.37 | 1.53 ± 0.26 × 10$^4$     | 1.69 ± 0.33 × 10$^{-1}$ |
|             | CCL7       | 11.3 ± 0.18  | 2.53 ± 0.15 × 10$^4$     | 2.86 ± 0.21 × 10$^{-1}$ |
|             | CCL5       | 6.81 ± 0.33  | 4.37 ± 0.31 × 10$^4$     | 2.98 ± 0.31 × 10$^{-1}$ |
|             | CCL2       | 1.89 ± 0.24  | 11.17 ± 0.32 × 10$^4$    | 2.11 ± 0.34 × 10$^{-1}$ |
5. **CCR2 expression level.**

MCF-7 cells were seeded on 6 well plate in RPMI-1640 medium in 1% FBS. Approximately $10^4$ cells were transferred to FACS tubes and incubated with primary murine Anti-CCR2 antibody (2 µl) for half hour, followed by FITC tagged secondary antibody. The fluorescent channel FL-2 was used to quantify CCR2 expression level on MCF-7 cells.

6. **Cell proliferation assay**

Approximately $10^4$ MCF-7 were feeded on 96-well plates in a RPMI-1640 medium in 1% FBS. Then, HS tetrasaccharide (HT-3,6S-NH, 10 or 50 µg/ml) and native heparin (10 or 50 µg/ml) were mixed with CCL2 (50 ng) and added to the cells. After 72 h of incubation, cell were fixed with paraformaldehyde and stained with 4% sulforhodamine B in 1% acetic acid for 30 min. Cell proliferation was quantified with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt at 450 nm.

7. **Cell-division cycle analysis**

Chemokines and HT-3,6S-NH treated MCF-7 cells were harvested and fixed with 70 % ethanol at -20 °C for 12 h. The fixed cells were incubated with propidium iodide (5 µg/mL) and RNase (10 µg/mL) for 30 min at RT. Using flow cytometer stained cells were quantified. The DNA content in the cell phases was quantified by using ModFitLT version 3.0 software.

8. **Wound healing assay**

MCF-7 cells were seeded on 24-well plat and cell monolayer was generated. Then, by using 1000 µl pipette tip wound was created. Cells were treated with native heparin and HT-3,6S-NH (50 µg/ml) with CCL2 (50 ng). It was observed that CCL2 treated monolayer showed complete wound healing after 8 h. At that point, percentage of cell migration distance of HT-3,6S-NH, heparin and control cells were quantified.

9. **Cell invasion assay.**

Boyden chamber cell invasion assay was performed in as reported in the protocol. Briefly, the upper chamber of transwell inserts were coated with Matrigel and the bottom chamber contained RPMI-1640 medium supplemented with 1 % FBS and CCL2 (50 ng/ml) with HT-3,6S-NH (50 µg/ml) and heparin. MCF-7 cells were feeded to the upper chamber. After 24 h of
incubation, non-invading cells were removed and lower chamber cells were fixed and stained with 0.5 % crystal violet for 30 min and cell invasion was quantified by bright field imaging.

10. Western Blot Analysis

MCF-7 cells were seeded on Petri dishes and CCL2 (50 ng) was added with or without HT-3,6S-NH (50 µg./ml) for half hr. The cells were transferred into falcon tube and pelleted. Then cell pellet was treated with lysis buffer containing 150 mM NaCl, 1% NP-40, 0.25% sodium dodecyl sulfate (SDS), 1 mM ethylenediamine tetraacetic acid (EDTA) and protease inhibitor, and 1 mM phenylmethane sulfonyl fluoride (PMSF) in 50 mM Tris-Cl (pH 7.4). After 1 h, mixture was centrifuge and supernatant was collected. Using Brandford method, the protein concentration was determined. Approximately 35 µg of protein was loaded on SDS-polyacrylamide gel electrophoresis (10%) and transferred onto a polyvinylidene fluoride (PVDF) membrane. Using specific antibodies corresponding to MAPK blot was developed. Finally, The membrane was incubated with horse radish peroxidase (HRP)-conjugated secondary antibody and visualization by using an Immobilon Western Chemiluminescent HRP substrate kit (Millipore Corporation, MA, USA) with GAPDH as internal standard.

11. References:

1. a) V. Padler-Karavani, X. Song, H. Yu, N. Hurtado-Ziola, S. Huang, S. Muthana, H. A. Chokhawala, J. Cheng, A. Verhagen and M. A. Langereis, J. Biol. Chem., 2012, 287, 22593-22608; b) S. L. Ben-Arye, H. Yu, X. Chen and V. Padler-Karavani, J. Vis. Exp., 2017, e56094.
12. NMR Spectra

α-anomer

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β- anomer
