Electronic Supplementary Material (ESI) for RSC Advances.

Solid-phase assembly of glycosaminoglycan oligosaccharide precursors

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Supporting Information

1. **General Methods.** All anhydrous reactions were performed in flame-dried or oven-dried glassware under a positive pressure of dry Argon. Air- or moisture-sensitive reagents and anhydrous solvents were transferred with oven-dried syringes or cannulae. Purification of compounds was performed on a automated flash chromatography system or by conventional flash chromatography using silica gel 60 (63-200 mesh). Size exclusion chromatography was performed on Sephadex LH-20. All solution-phase reactions were monitored using analytical thin-layer chromatography (TLC) with 0.2 mm pre-coated silica gel 60 F254 aluminium plates. Components were visualized by illumination with a short-wavelength (254 nm) ultraviolet light and/or by charring with vanillin, ceric ammonium molybdate, potassium permanganate, or phosphomolybdate staining solution. All solvents used for anhydrous reactions were distilled. Tetrahydrofuran (THF) was distilled from sodium/benzophenone under Argon. Dichloromethane and acetonitrile were distilled from calcium hydride. Methanol was distilled from calcium sulfate. N,N-dimethylformamide (DMF) was stored over activated 4 Å molecular sieves under Argon. Solid-phase reactions were performed in a normal Schlenck tube under an Argon atmosphere.

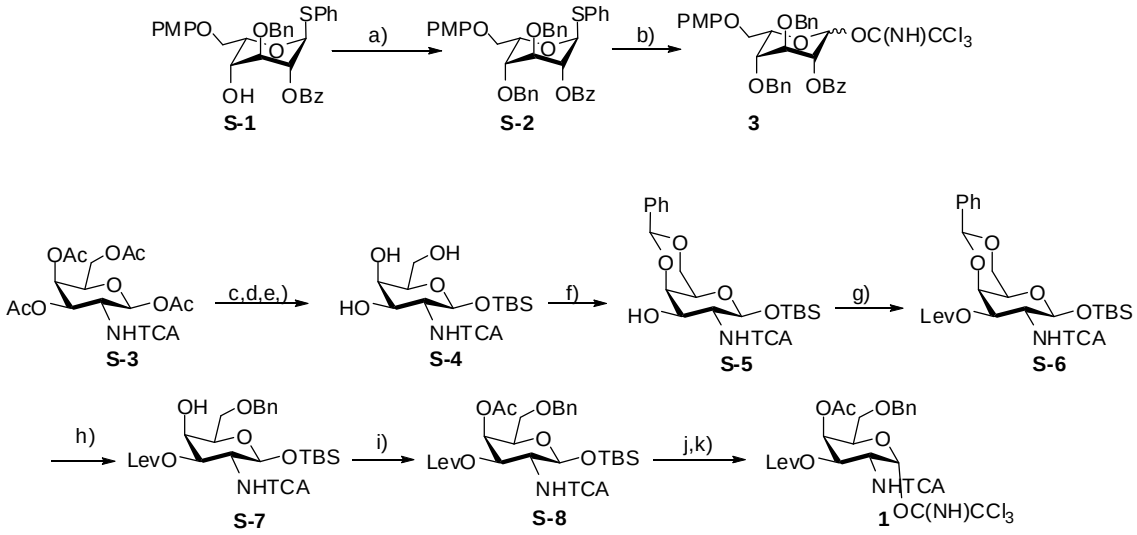
   H^1, DQF-COSY, HSQC and ^{13}C NMR spectra were recorded at ambient temperature on a 500 MHz NMR spectrometer to confirm the NMR peak assignments. Deuterated chloroform ($CDCl_3$), methanol (CD_3OD), or water (D_2O) was used as the solvent for NMR experiments, unless otherwise stated. Chemical shifts are reported in parts per million downfield from TMS and corrected using the solvent residual peak or TMS as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; dd, doublet of doublet; m, multiplet; b, broad. Low –resolution mass spectrometry (LRMS) was performed on a electrospray ionization time of flight mass spectrometer equipped with an electrospray source with a pump rate of 5µL/min using electrospray ionization (ESI) or a matrix-assisted desorption ionization time of flight (MALDI-TOF) mass spectrometer operated in the reflectron/positive ion mode with DHB in MeOH as the MALDI matrix. High-resolution mass spectrometry (HRMS) data were acquired on a time of flight mass spectrometer. Samples in CH_2Cl_2/ MeOH were mixed with ES tuning mix for internal calibration and infused into the mass spectrometer at µL/min. For microwave heating of reactions a monomode oven was used. LC-MS analysis were performed using Acquity UPLC coupled to a ESI-TOF LCT Premier XE (Waters, Milford, MA, US), column Acquity BEH 100x2.1 mm 1.7 um particle size (Waters); flow rate 300 uL/min; PDA wavelength range: 195 - 500 nm; eluents (A) Ammonium formate 10mM / (B) MeOH. grad. B (10 min: Isocratic 99% A – 0.5 min. / 99-25% A – 2.5 min / 25-1% A – 3 min / isocratic 1% A – 4 min) or grad. D (20 min: Isocratic 99% A – 0.5 min. / 99-25% A – 5.5 min / 25-75% A – 10.5 min / isocratic 1% A – 4 min).

Compound numbering:

Resin-bound compounds carry the prefix **SP-** (e.g. **SP-1**)

Intermediates in the building block synthesis, products from analytical resin cleavage and additional exploratory studies not discussed in the manuscript carry the prefix **S-** (e.g. **S-14**)

2. Synthesis of new glycosyl donors



Scheme S-1. **Synthesis of glycosyl donors used in the GAG assembly (DS and HS)**: a) TMSCl, pyridine; PhCHO, TES, TMSOTf (cat.), 70%; b) NBS, acetone/water, 72%; CCl_3CN, DBU, CH_2Cl_2, 76%; c) hydrazine acetate, DMF; d) TBDMSCl, imidazole, DMF, 86%; e) NaOMe, MeOH, quant; f) $PhCH(OMe)_2$, CSA (cat.), acetonitrile, 85%; g) levulinic acid, EDC·HCl, CH_2Cl_2, DMAP, 92%; h) triethylsilane, trifluoroacetic acid, CH_2Cl_2, 67%; i) Ac_2O, pyridine, 82%; j) TBAF 1M, AcOH, THF; k) CCl_3CN, DBU, CH_2Cl_2, **1**: 90%.;

Phenyl 2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-1-thio-α-L-idopyranoside (S-2): S-1 (0.637 g, 1.29 mmol) (as described in Guedes, N.; Czechura, P.; Echeverria, B.; Ruiz, A.; Michelena, O.; Martin-Lomas, M.; Reichardt, N.-C. *J. Org. Chem.* **2013**, 78, 6911–6934) was dissolved in pyridine (2 mL) and TMSCl (0.4 mL, 7.8 mmol) was added at 0 °C. After stirring for 2h at room temperature, the reaction mixture was diluted with ethyl acetate (50 mL) and was washed with water, saturated aqueous $CuSO_4$ solution, water and brine (10 mL each). The crude was concentrated, dried under vacuum and used in the next reaction without further purification. To a solution of this intermediate in dry dichloromethane (13 mL), molecular sieves (0.400 g) and benzaldehyde (0.16 mL, 1.55 mmol) were added and after stirring the suspension for 1h at room temperature. The reaction mixture was cooled at -78 °C, triethylsilane (0.25 mL, 1.55mmol) and TMSOTf (23µL, 0.13 mmol) were added via syringe. After stirred for 3h, a TLC analysis showed the presence of some starting material. Additional volume of TMSOTf (0.5eq.) was added and the reaction mixture was gradually warmed up. After stirred overnight, the reaction was diluted with dichloromethane (50 mL) and was washed with saturated aqueous $NaHCO_3$ solution, water and brine. The oily crude was purified by column chromatography (5-20 % ethyl acetate/ hexane) to obtain compound **S-2** (0.600 g, 70%). $[\alpha]_D^{20}$ - 43.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 – 8.01 (m, 2H, aromatic), 7.66 – 7.60 (m, 2H, aromatic), 7.56 – 7.46 (m, 3H, aromatic), 7.45 – 7.39 (m, 2H, aromatic), 7.38 – 7.18 (m, 9H, aromatic), 7.17 – 7.11 (m, 2H, aromatic), 6.91 – 6.83 (m, 4H, $aromatic_{PMP}$), 5.66 (s, 1H, H-1), 5.55 – 5.53 (m, 1H, H-2), 5.11 (td, *J* = 6.3, 2.0 Hz, 1H, H-5), 4.97 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.70 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.54 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.40 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.25 (d, *J* = 6.3 Hz 2H, H-6), 4.07 – 4.02 (m, 1H, H-3), 3.83 – 3.77 (s, 3H, CH_{3PMP}),

3.75 – 3.70 (m, 1H, H-4) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.8, 154.1, 153.0, 137.6, 137.5, 136.2, 133.3, 131.7, 130.1, 129.7, 129.0, 128.6, 128.4, 128.4, 128.1, 128.0, 127.9, 127.4, 115.6, 114.7, 86.1, 73.4, 72.6, 72.5, 71.1, 69.5, 67.4, 67.0, 55.8 ppm; HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd for $C_{40}H_{38}O_7SNa$ 685.2230; Found 685.2217.

2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-L-idopyranosyl trichloroacetimidate (3): Compound **S-2** (0.527 g, 0.79 mmol) was dissolved in acetone/ water (9/1; 11 mL) and freshly recristallyzed NBS (0.155 g, 0.87 mmol) was added in portions by spatula over 10 min at room temperature with vigorous stirring. After 45 minutes stirring, TLC analysis showed presence of some starting material and additional NBS (0.084 g, 0.47 mmol) was added. After 30 min, the reaction was diluted with ethyl acetate and was washed with 1M aqueous $Na_2S_2O_3$ solution, water and brine. The reaction crude was dried over $MgSO_4$, concentrated and purified by column chromatography (0-20% ethyl acetate/hexane) to obtain an α/β (1/1) mixture of the hemiacetal (0.324 g, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 – 7.94 (m, 2H, aromatic), 7.57 – 7.50 (m, 1H, aromatic), 7.42 – 7.29 (m, 7H, aromatic), 7.23 – 7.07 (m, 3H, aromatic), 7.06 – 7.03 (m, 1H, aromatic), 7.02 – 6.99 (m, 1H, aromatic), 6.89 – 6.81 (m, 4H, $aromatic_{PMP}$), 5.32 – 5.26 (m, 1H, H-1α, H-1β), 5.21 – 5.18 (m, 0.5H, H-2), 5.16 – 5.13 (m, 0.5H, H-2), 4.81 – 4.75 (m, 1H, CH_2Ph), 4.71 – 4.64 (m, 1H, CH_2Ph), 4.64 – 4.61 (m, 0.5H, H-5), 4.45 – 4.36 (m, 1.5H, H-5, CH_2Ph), 4.33 (d, *J* = 11.2 Hz, 0.5H, CH_2Ph), 4.28 (d, *J* = 11.2 Hz, 0.5H, CH_2Ph), 4.25 – 4.15 (m, 2H, H-6), 4.14 – 4.09 (m, 1H, H-3), 3.78 (2s, 3H, CH_{3PMP}), 3.68 (t, *J* = 2.7 Hz, 0.5H, H-4), 3.58 (t, *J* = 2.4 Hz, 0.5H, H-4). HRMS (ESI) *m/z:* $[M+Na]^+$ Calcd for $C_{34}H_{34}O_8$ 593.2151, Found 593.2185.The hemiacetal (0.360 g, 0.63 mmol) was dissolved in dry dichloromethane (6.3 mL), trichloroacetonitrile (0.95 mL, 9.46 mmol) and catalytic amount of DBU (0.019 mL, 0.126 mmol) were added at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred for 2h until TLC analysis showed disappearance of starting material. Then, the reaction crude was concentrated under vacuum and purified by column chromatography (10- 30% ethyl acetate/hexane using 5% of triethylamine) to obtain compound **3** as α/β (4/6) mixture in 76% of yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.65 (s, 0.4H, $OCNHCl_3$), 8.52 (s, 0.6H, $OCNHCl_3$), 8.05 – 8.00 (m, 2H, aromatic), 7.57 – 7.53 (m, 1H, aromatic), 7.39 – 7.09 (m, 12H, aromatic), 6.87 – 6.80 (m, 4H, $aromatic_{PMP}$), 6.51 (d, J = 2.7 Hz, 1H, H-1β), 6.43 (s, 1H, H-1α), 5.50 – 5.46 (m, 1H, H-2α, H-2β), 4.90 – 4.89 (d, J = 11.8 Hz, 1H, CH_2Ph), 4.83 – 4.81 (d, J = 11.5 Hz, 1H, CH_2Ph), 4.78 – 4.76 (m, 2H, H-5α, CH_2Ph), 4.66 – 4.64 (d, J = 11.8Hz, 1H, CH_2Phα), 4.63 – 4.52 (m, 5H, CH_2Ph, H-5β, CH_2Ph), 4.43 – 4.41 (m, 1H, CH_2Ph), 4.39 – 4.31 (m, 2H, H-6β), 4.27 – 4.17 (m, 3H, H-6α, H-3β), 4.04 - 4.03 (m, 1H, H-3α), 3.90 – 3.85 (m, 1H, H-4β), 3.80 – 3.75 (m, 4H, CH_{3PMP}, H-4α); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.9, 165.7, 160.8, 160.7, 154.2, 154.1, 152.8, 152.7, 137.7, 137.6, 137.5, 133.5, 133.3, 130.2, 130.1, 129.5, 129.4, 128.6, 128.5, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.9, 115.8, 115.6, 114.7, 97.8, 95.6, 95.3, 75.4, 74.5, 74.1, 73.7, 73.4, 72.7, 72.4, 72.1, 71.0, 69.3, 68.0, 67.3, 66.8, 66.3, 55.9.

1-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranose (S-4): Compound **S-3** (8.33 g, 16.90 mmol) (as described in Bartek, J.; Müller, R.; Kosma, P. *Carbohydr. Res.* **1998**, *308*, 259–273.) was dissolved in *N*,*N*-dimethylformamide (DMF, 180 mL) and hydrazine acetate (1.90 g, 20.63 mmol) was added. After stirring for 3 h at room temperature, the reaction mixture was diluted with ethyl acetate (400 mL) and washed with

1M HCl solution (50 mL). The aqueous phase was extracted with ethyl acetate (2x100 mL) and the combined organic layer (from all washings) were dried over $MgSO_4$, filtered and concentrated to give 3,4,6-tri-*O*-acetyl-2-deoxy-2-trichloroacetimido-D-galactopyranose as an oil. Without further purification the intermediate compound was dissolved in DMF (57 mL), imidazole (2.30 g, 33.72 mmol) and *tert*-butyldimethylsilyl chloride (3.05 g, 20.23 mmol) were added at room temperature. After the mixture was stirred for 3 h, water (60 mL) was added and the solution was diluted with ethyl acetate (300 mL). The aqueous phase was extracted with ethyl acetate (2x200 mL) and the combined organic extracts were washed with 1M HCl, water, saturated aqueous $NaHCO_3$ solution and brine (100 mL each). The organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude residue (8.20 g, 14.52 mmol) was dissolved in MeOH (35 mL) and 3.8 mL of a 0.25M methanolic sodium methoxide solution was added at room temperature. After 2 h, the reaction mixture was diluted with methanol (50 mL) and neutralized by addition of Amberlite IR120 (H+). The solution was filtered and concentrated *in vacuo* to yield **S-4** (6.35 g, 86 %) as a colorless foam. $[\alpha]_D^{20}$ + 11.1 (*c* 0.13, MeOH), ^{1}H NMR (500 MHz, MeOD) δ 4.80 (d, *J* = 8.1 Hz, H-1), 3.94 – 3.90 (m, 1H, H-2), 3.88 (d, *J* = 2.97 Hz,1H, H-4), 3.80 - 3.76 (m, 2H, H-6, H-3), 3.71 (dd, *J* = 6.2, 11.1 Hz, 1H, H-6), 3.51 (t, *J* = 6.2 Hz, 1H, H-5), 0.89 (s, 9H, CH_{3TBS}), 0.14 (s, 3H, $SiCH_{3TBS}$), 0.12 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, MeOD) δ 97.47, 76.60, 71.96, 69.65, 62.11, 58.50, 26.24, -3.83, -4.98 ppm. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd. for $C_{14}H_{26}Cl_3NO_6SiNa$ 460.0493, Found 460.0497.

4,6-Benzylidene-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranose (S-5): A solution of **S-4** (2.88 g, 6.57 mmol) in acetonitrile (45 mL) was treated with benzaldehyde dimethyl acetal (2.6 mL, 17.75 mmol) and catalytic amount of camphor sulfonic acid (305 mg, 1.31 mmol). After stirring for1 h at room temperature, the reaction mixture was diluted with ethyl acetate (100 mL) and washed with saturated aqueous $NaHCO_3$ solution, brine, and water (100 mL each). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by flash silica gel chromatography (30-50% ethyl acetate/ hexane) to yield **S-5** (2.56 g, 4.56 mmol, 93%). $[\alpha]_D^{20}$ +5.4 (*c* 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 – 7.49 (m, 2H, aromatic), 7.41 – 7.35 (m, 2H, aromatic), 6.83 (d, *J* = 7.6 Hz, 1H, NH), 5.57 (s, 1H, H_{acetal}), 4.97 (dd, *J* = 7.9, 1.7 Hz, 1H, H-1), 4.28 (dd, *J* = 12.4, 1.3 Hz, 1H, H-6), 4.21 (d, *J* = 3.3 Hz, 1H, H-4), 4.10 – 4.03 (m, 2H, H-6, H-3), 3.86 – 3.78 (m, 1H, H-2), 3.52 – 3.49 (m, 1H, H-5), 0.91 (s, 9H, CH_{3TBS}), 0.19 (s, 3H, $SiCH_{3TBS}$), 0.13 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.6, 137.6, 129.5, 128.5, 126.6, 101.5, 95.4, 75.1, 69.8, 69.4, 66.8, 58.7, 58.6, 25.9, 18.1, -3.8, -4.5 ppm. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd. for $C_{21}H_{30}Cl_3NO_6Na$ 548.0800; Found 548.0797.

4,6-Benzylidene-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (S-6): To a solution of **S-5** (2.56 g, 4.58 mmol) in dry CH_2Cl_2 (7.3 mL), EDC·HCl (1.39 g, 7.28 mmol), DMAP (415 mg, 3.39 mmol) and levulinic acid (0.74 mL, 7.28 mmol) were added at 0 °C and after stirring for 10 min was allowed to warm up to room temperature. After 3h, TLC analysis indicated full conversion of the starting material. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and was washed with saturated aqueous $NaHCO_3$ solution, water and brine (50 mL each). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude was purified by flash chromatography (40% ethyl acetate/ hexane) to obtain **S-6** (2.8 g, 92%). $[\alpha]_D^{20}$ +21.0 (*c* 1, $CHCl_3$), ^{1}H NMR (500 MHz, $CDCl_3$)

δ 7.54 –7.36 (m, 5H, aromatic), 6.69 (d, *J* = 8.5Hz, NH), 5.53 (s, 1H, PhCH), 5.28 (dd, *J* = 3.5Hz, 11.3Hz, 1H, H-3), 5.02 (d, *J* = 7.8Hz, 1H, H-1), 4.29 (m, 2H, H-4, H-6), 4.17 (m, 1H, H-2), 4.06 (dd, *J* = 1.6, 12.4Hz, 1H, H-6), 3.53 (d, *J* = 1Hz, 1H, H-5), 2.71 – 2.56 (m, 4H, CH_{2Lev}), 2.05 (s, 3H, CH_{3Lev}), 0.89 (s, 9H, CH_{3TBS}), 0.18 (s, 3H, $SiCH_{3TBS}$), 0.13 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.5, 172.6, 161.8, 137.8, 129.3, 128.4, 126. 6, 101.2, 95.8, 77.4, 77.2, 76.9, 73.3, 70.3, 69.4, 66.6, 55.0, 37.9, 29.8, 28.2, 25.9, 18.1, -3.8, -4.5 ppm. HRMS (ESI) *m/z*: $[M+NH_4]^+$ Calcd. for $C_{26}H_{36}Cl_3NO_8NH_4$ 641.1614; Found 641.1652.

6-*O*-Benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (S-7): To a solution of **S-6** (6.0 g, 9.6 mmol) in dry CH_2Cl_2 (35 mL) with molecular sieves, triethylsilane (7.6 mL, 48 mmol) and trifluoroacetic acid (3.7 mL, 48 mmol) were added at 0 °C and was stirred for 2h. The reaction mixture was then allowed to warm up to room temperature and stirred for 1h until disappearance of the starting material. The reaction was quenched by addition of triethylamine (6.7 mL) and concentrated. The oily residue was purified by flash column chromatography (20-40% ethyl acetate/ toluene) to obtain the desired product **S-7** as a colorless solid (4.2 g, 69%). $[\alpha]_D^{20}$ -0.8 (*c* 0.5, $CHCl_3$) ^1H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.27 (m, 5H, aromatic), 6.67 (d, *J* = 8.9 Hz, 1H, NH), 5. 08 (dd, *J* = 11.2, 3.0 Hz, 1H, H-3), 4.88 (d, *J* = 7.9 Hz, 1H, H-1), 4.58 (s, 2H, CH_2Ph), 4.19 – 4.11 (m, 2H, H-5, H-2), 3.81 – 3.76 (m, 1H, H-6), 3.75 – 3.69 (m, 2H, H-6, H-4), 2.77 – 2.72 (m, 2H, CH_{2Lev}), 2.65 – 2.51 (m, 2H, CH_{2Lev}), 2.19 (s, 3H, CH_{3Lev}), 0.87 (s, 9H, CH_{3TBS}), 0.14 (s, 3H, $SiCH_{3TBS}$), 0.10 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.6 (Cq), 172.4 (Cq), 161.9 (Cq), 137.9 (Cq), 128.6, 128.0, 127.8 (Caromatic), 96.1 (C-1), 92.7(Cq), 73.9 (CH_2Ph), 73.5 (C-4), 72.7 (C-3), 69.6 (C-6), 67.3 (C-5), 55.0 (C-2), 38.2 (CH_{2Lev}), 30.0 (Me), 28.3 (CH_{2Lev}), 25.8 (CH_{3TBS}), 18.0 (Cq), -3.9 (CH_{3TBS}), -5.0 (CH_{3TBS}) ppm. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd. for $C_{26}H_{38}Cl_3NO_8Na$ 648.1324; Found 648.1330.

4-*O*-Acetyl-6-*O*-benzyl-1-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (S-8): The compound **S-7** (0.136 g, 0.22 mmol) was dissolved in pyridine (0.5 mL, 6.12 mmol), acetic anhydride (0.29 mL, 3.07 mmol) was added at 0 °C and the reaction was allowed to warm up to room temperature stirring overnight. The solution was diluted with ethyl acetate (30 mL) and washed with 1M HCl solution, water, saturated aqueous $CuSO_4$ solution, and water (10 mL of each). The organic phase was dried over $MgSO_4$, concentrated under vacuum and purified by flash chromatography (20-40% ethyl acetate/ hexane) to obtain **S-8** (0.092 g, 82%). $[\alpha]_D^{20}$ -9.3 (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.25 (m, 5H, aromatic), 6.68 (d, *J* = 8.9 Hz, 1H, NH), 5.46 (dd, *J* = 3.1, 1.3 Hz, 1H, H-4), 5.20 (dd, *J* = 11.4, 3.3 Hz, 1H, H-3), 4.88 (d, *J* = 7.9 Hz, 1H, H-1), 4.54 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.43 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.14 – 3.98 (m, 1H, H-2), 3.91 – 3.79 (m, 1H, H-5), 3.61 – 3.42 (m, 2H, H-6), 2.83 – 2.70 (m, 1H, CH_{2Lev}), 2.67 – 2.37 (m, 3H, CH_{2Lev}), 2.16 (s, 3H, CH_{3Lev}), 2.08 (s, 3H, CH_{3Ac}), 0.88 (s, 9H, CH_{3TBS}), 0.14 (s, 3H, $SiCH_{3TBS}$), 0.11 (s, 3H, $SiCH_{3TBS}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.2 (Cq), 172.4 (Cq), 170.3 (Cq), 162.0 (Cq), 137.7 (Cq), 128.6, 128.0, 96.2 (C-1), 92.6 (Cq, TCA), 73.7 (C-6), 72.5 (C-5), 70.2 (C-3), 67.9 (C-6), 67.3 (C-4), 55.3 (C-2), 37.9 (CH_{2Lev}), 29.9 (CH_{3Lev}), 27.9 (CH_{2Lev}), 25.8 (TBS), 20.9 (CH_{3Ac}), 18.0 (TBS), -3.9 (TBS), -5.0 (TBS) ppm. HRMS (ESI) *m/z*: $[M+NH_4]^+$ calcd. for $C_{28}H_{40}Cl_3NO_9Si\ NH_4$ 685.1876; Found 685.1887.

4-*O*-Acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-α-D-galactopyranosyl trichloroacetimidate (1): Compound **S-8** (4.55 g, 6.8 mmol) was dissolved in THF (34 mL),

acetic acid (0.42 mL, 7.48 mmol) and TBAF (7.5 mL of 1M THF solution, 7.50 mmol) were added at 0 °C. The reaction mixture was allowed to warm up to room temperature and was stirred overnight. The solution was diluted with ethyl acetate (50 mL) and was washed with saturated aqueous $NaHCO_3$ solution. The aqueous phase was extracted with ethyl acetate (3x30 mL), the combined organic phases were washed with water, brine and dried over $MgSO_4$. The reaction crude was concentrated and purified by column chromatography (20-50% ethyl acetate/ hexane) to obtain 4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranose (3.4 g, 90%).^{1}H NMR (500 MHz, $CDCl_3$) as a mixture α/β (8.5/1.5) δ 7.37 – 7.26 (m, 5H, aromatic), 7.09 (d, J = 8.5 Hz, 0.15H, NHβ), 6.92 (d, *J* = 9.3 Hz, 0.85H, NHα), 5.44 – 5.42 (m, 0.15H, H-4β), 5.41 – 5.39 (m, 0.85H, H-4α), 5.38 (d, *J* = 2.7 Hz, 0.85H, H-1, H-1α), 5.29 (dd, *J* = 11.2, 3.2 Hz, 0.85H, H-3α), 5.11 (dd, J = 11.2, 3.3 Hz, 0.15H, H-3β), 4.70 (d, *J* = 8.2Hz, 0.15H, H-1β), 4.55 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.50 – 4.42 (m, 1.85H, CH_2Ph, H-2, H-2α), 4.41 – 4.36 (m, 0.85H, H-5α), 4.09 – 4.04 (m, 0.2H, H-2β), 3.82 – 3.80 (m, 0.15H, H-5β), 3.58 (dd, *J* = 9.5, 6.2 Hz, H-6$_a$β), 3.54 – 3.48 (m, 1H, H-6$_b$β, H-6$_a$α), 3.45 (dd, *J* = 9.8, 4.9 Hz, 0.85H, H-6$_b$α), 2.77 – 2.69 (m, 1H, CH_{2Lev}), 2.67 – 2.51 (m, 2H, CH_{2Lev}), 2.48 – 2.40 (m, 1H, CH_{2Lev}), 2.16 (s, 0.47H, $CH_{3Levβ}$), 2.15(s, 2.24H, $CH_{3Levα}$), 2.11 (s, 2.24H, $CH_{3Acα}$) 2.09 (s, 0.47H, $CH_{3Acβ}$) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.5, 172.5, 170.4, 162.2, 137.2, 128.6, 128.2, 128.1, 92.3, 91.6 (C-1), 73.7 (CH_2Ph), 68.7 (C-3), 68.6 (C-6), 68.1, 68.0, 50.3 (C-2), 37.8 (CH_{2Lev}), 29.8 (CH_{3Lev}), 27.9 (CH_{2Lev}), 27.9 (CH_{2Lev}), 20.8 (CH_{3Ac}) ppm. The hemiacetal (1.5 g, 2.7 mmol) was dissolved in dry dichloromethane (27 mL), trichloroacetonitrile (4.06 mL, 40.55 mmol) and catalytic amount of DBU (40 µL) were added at 0 °C. The reaction was gradually warmed up to room temperature. After the mixture had been stirred for 2h, TLC analysis showed complete consumption of the starting material. The reaction mixture was concentrated under vacuum and purified by column chromatography (10-30% ethyl acetate/ hexane with 5% of triethylamine) to obtain compound **1** (1.6 g, 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.78 (s, 1H, $OCNHCl_3$), 7.35 – 7.24 (m, 5H, aromatic), 6.91 (d, *J* = 8.7 Hz, 1H, NH), 6.50 (d, *J* = 3.5 Hz, 1H, H-1), 5.61 (dd, *J* = 3.1, 1.4 Hz, 1H, H-4), 5.40 (dd, *J* = 11.4, 3.1 Hz, 1H, H-3), 4.71 – 4.65 (m, 1H, H-2), 4.53 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.40 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 4.36 – 4.31 (m, 1H, H-5), 3.56 (dd, *J* = 9.6, 5.6 Hz, 1H, H-6$_a$), 3.48 (dd, *J* = 9.6, 7.4 Hz, 1H, H-6$_b$), 2.81 – 2.73 (m, 1H, CH_{2Lev}), 2.68 – 2.55 (m, 2H, CH_{2Lev}), 2.52 – 2.44 (m, 1H, CH_{2Lev}), 2.17 (s, 3H, CH_{3Lev}), 2.10 (s, 3H, CH_{3Ac}) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.1, 172.9, 170.0, 162.4, 160.3, 137.4, 128.6, 128.1, 128.0, 94.6, 92.0, 90.8, 73.6, 70.7, 68.5, 67.1, 67.1, 50.1, 37.8, 29.8, 27.9, 20.8 ppm. HRMS (ESI) *m/z*: $[M+Na]^+$ Calcd. for $C_{24}H_{26}Cl_6N_2O_9Na$ 718.9667; Found 718.9703.

3. General procedures for the Solid-Phase synthesis

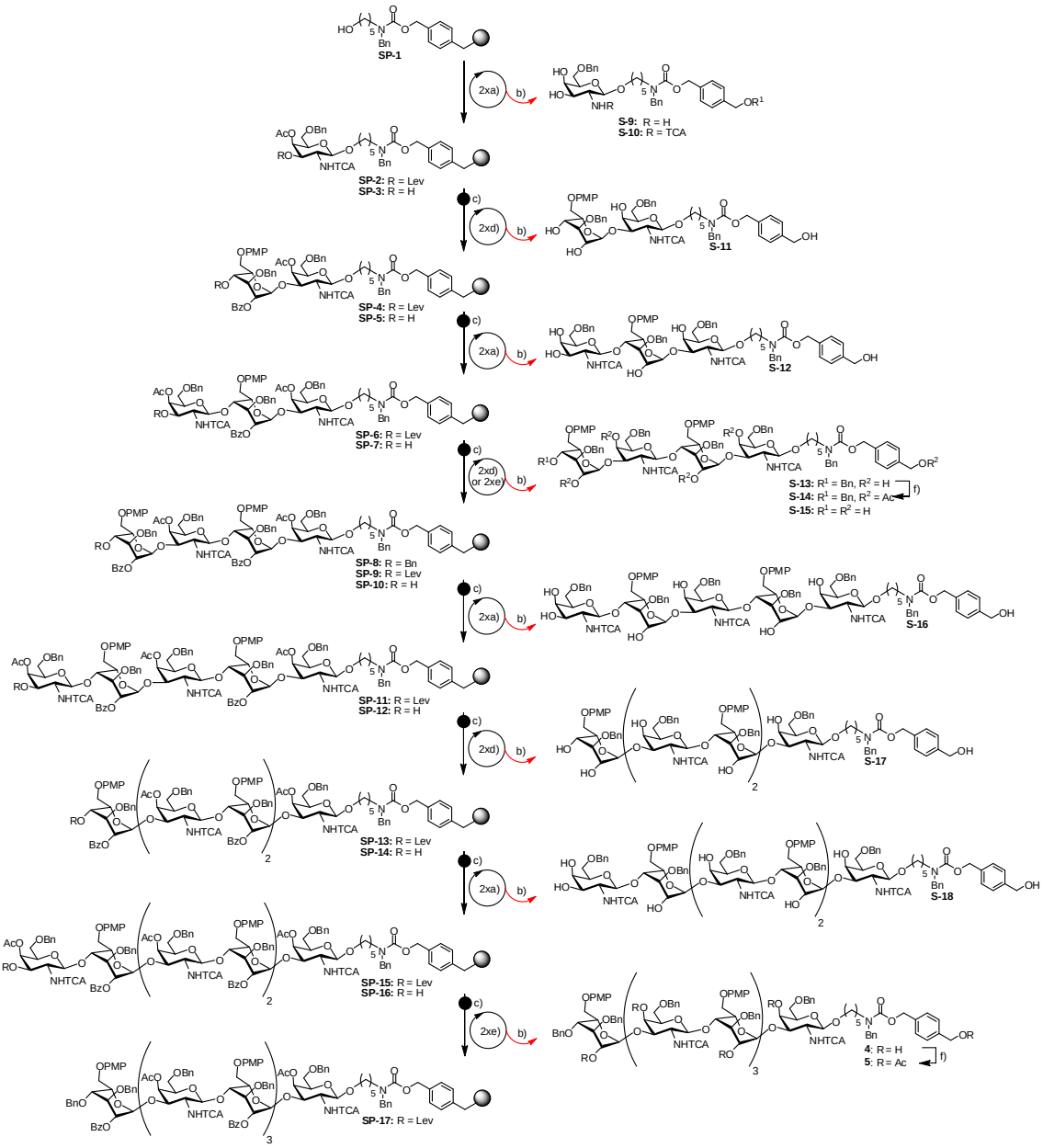
Procedure A (Solid phase glycosylation): Solid phase glycosylations were performed in either a Schlenk tube fitted with a cooling jacket or a normal Schlenk tube under Argon atmosphere. Unless otherwise noted the resin was swollen with the glycosyl donor (thioglycoside or trichloroacetimidate) in dry CH_2Cl_2 (750 µL/ 100 mg resin). The reaction mixture was shaken for 10 minutes on a vortex or an orbital shaker. Then, the Schlenk tube with cooling jacket was connected to a cryostat and cooled to the specified temperature. The normal Schlenk tube was

cooled Dewar containing acetone at the specified temperature. After additional shaking for 10 minutes the activator (NIS, TMSOTf/ TfOH for thioglycosides or TMSOTf for trichloroacetimidates) was added. The mixture was shaken for 10 minutes at the specified temperature. Then, the mixture was allowed to warm to room temperature and was shaking for 1 – 1.5 hours. The resin was washed with THF (5 x 3 mL/ 100 mg resin), CH_2Cl_2 (5 x 3 mL/ 100 mg resin) and dry diethyl ether (2 x 3 mL/ 100 mg resin) and dried in high vacuum. The THF washings were collected for possible recovery of the donor. The conversion for heparan sulfate derivatives was analyzed by LCMS after NaOMe cleavage: 5 mg of resin were placed into a small microwave vial (0.2 – 0.5 mL) equipped with magnetic stir bar. After swelling the resin with 250 µL of anhydrous CH_2Cl_2, 50 µL of a 0.2 M sodium methoxide solution was added. The mixture was irradiated in microwave oven for 5 min at 55 °C with pre-stirring of 30 sec. After cooling to room temperature the supernatant was transferred to an Eppendorf vial and the solution was concentrated to dryness by air stream. The residue was redissolved in 100 µL methanol (HPLC grade). A 1: 10 dilution of this solution was used for LCMS analysis. For dermatan sulfate derivatives the conversion was analyzed by LCMS after softer treatment with sodium methoxide solution: 5 mg of resin were placed into a small microwave vial (0.2 – 0.5 mL) equipped with magnetic stir bar. After swelling the resin with 200 µL of anhydrous CH_2Cl_2, 20 µL of a 0.2 M sodium methoxide solution was added. The mixture was irradiated in microwave oven for 5 min at 45 °C with pre-stirring of 30 sec. After cooling to room temperature the supernatant was transferred to an Eppendorf vial and the solution was concentrated to dryness by air stream. The residue was redissolved in 100 µL methanol (HPLC grade). A 1: 5 dilution of this solution was used for LCMS analysis.

Procedure B (Solid phase capping and delevulination): Solid phase synthesis was performed either in a Schlenk tube fitted with a cooling jacket or a normal Schlenk tube under an Argon atmosphere. The resin was swollen in dry CH_2Cl_2 (1 mL / 100 mg resin) for 10 minutes, followed by addition of pyridine (300 µL / 100 mg resin), acetic anhydride (300 µL / 100 mg resin) and a catalytic amount of DMAP. After 5 h at room temperature, the resin was washed with CH_2Cl_2 (5 x 3 mL/ 100 mg resin), MeOH (5 x 3 mL/ 100 mg resin) and dry diethyl ether (2 x 3 mL/ 100 mg resin) and dried in high vacuum. The resin was used without further characterization for the delevulination: the resin was swollen in dry CH_2Cl_2 (1 mL / 100 mg resin) for 10 minutes, followed by addition hydrazine acetate (5 eq. in 200 µL methanol). After 5 h at room temperature, the resin was washed with CH_2Cl_2 (5 x 3 mL/ 100 mg resin), methanol (5 x 3 mL/ 100 mg resin) and dry diethyl ether (2 x 3 mL/ 100 mg resin) and dried in high vacuum. The resin was used without further characterization for the subsequent glycosylation.

Procedure C (Quantitative cleavage from the resin using NaOMe solution): The resin was swollen in dry CH_2Cl_2 (0.8 - 1 mL/ 100 mg of resin) and was shaken for 10 min. Then was treated with 0.25 M NaOMe solution for 5 min at 55 °C (HS precursors) or for 5 min at 40 °C (DS precursors) until the TLC control (CH_2Cl_2: MeOH, 98: 2) showed no further cleavage from the resin. After each cycle the resin was washed with CH_2Cl_2/MeOH and MeOH. The washing solutions were pooled, neutralized with Amberlite® IR120 (H^+) and concentrated under vaccuum.

4. Solid-Phase synthesis of a DS octasaccharide precursor



Scheme S-2. Assembly of a DS octasaccharide precursor. a) **1**, 15% TMSOTf, -20 °C to r.t; b) NaOMe (cat), MeOH, 40 °C, MW; c) hydrazine acetate, CH_2Cl_2: MeOH (4:1); d) **2**, 20% TMSOTf, -20 °C to r.t; e) **3** 20% TMSOTf, -20 °C to r.t; f) Ac_2O, pyridine, 0 °C to r.t.

Resin-Bound 4-(hydroxymethyl)benzyl N-benzyl-N-[5-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-2): The reaction was performed according to general procedure A employing one cycle on resin **SP-1** (0.395 g, 0.22 mmol/g, 0.106 mmol) with trichloroacetimidate donor **1** (1 x 5 eq., 369 mg, 0.53 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined after analytical NaOMe cleavage. Conversion: 97%; LC-MS (ESI): 67% as compound **S-10** (*m/z*$[M+H]^+$ Calcd for $C_{36}H_{43}Cl_3N_2O_9H$ 753.20, found 752.91) and 30% as compound **S-9** (*m/z* $[M+H]^+$ Calcd for $C_{34}H_{44}N_2O_8H$ 609.30, found 609.25). The partial loss of trichloroacetamide could be avoided when the cleavage reaction was carried out at 40°C instead of 55°C.

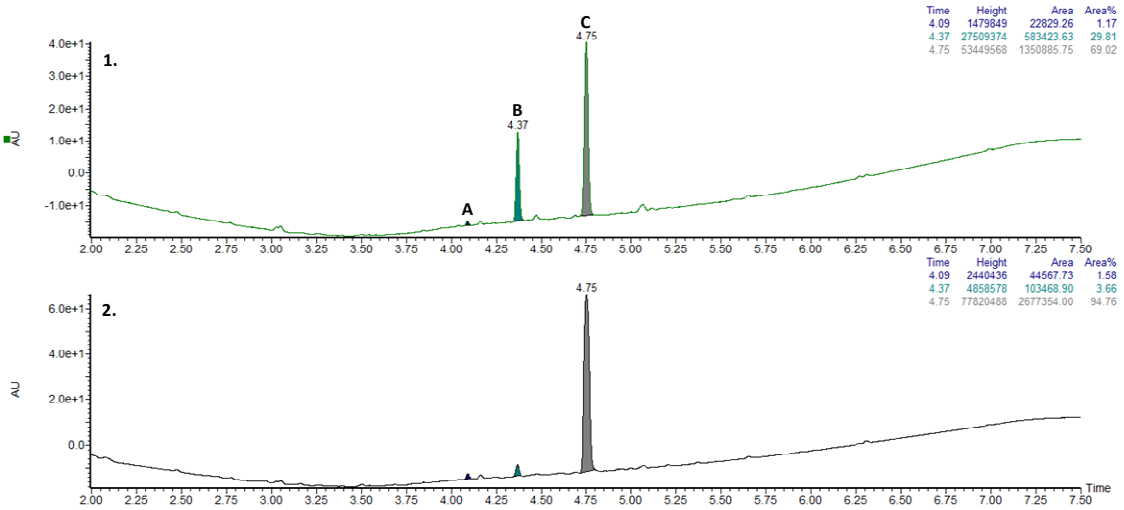


Figure SI-1. LC-MS chromatograms of analytical cleavage conversion of linker **SP-1** (retention time (t_r) at 4.09 min., peak **A** (*m/z* calcd for $C_{21}H_{27}NO_4[M+H]^+$ 358.19 found 358.24)) to monosaccharide **SP-2** (t_r at 4.37 min., peak **C** (**S-10**, *m/z* calcd for $C_{36}H_{43}Cl_3N_2O_9$ $[M+NH_4]^+$ 770.20 found 770.06); peak **B** (**S-9**, t_r at 4.75 min. *m/z* calcd for $C_{34}H_{44}N_2O_8[M+H]^+$ 609.30 found 609.25)). Trace 1: analytical cleavage at 55 °C; trace 2: analytical cleavage at 40 °C.

SP-3

The resin **SP-2** was transformed to resin-bound 4-(hydroxymethyl)benzyl N-benzyl-N-[5-(4-*O*-benzoyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyloxy)pentyl]carbamate **SP-3** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-4): Disaccharide formation was performed according to general procedure A using 2 cycles on resin **SP-3** (0.390 g, 0.087 mmol) with trichloroacetimidate donor **2** (5 equiv, 0.315 g, 0.44 mmol) and TMSOTf (3 µL, 0.017 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 99% as compound **S-11** (*m/z* $[M+NH_4]^+$ calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ NH_4 1128.34, found 1127.93.

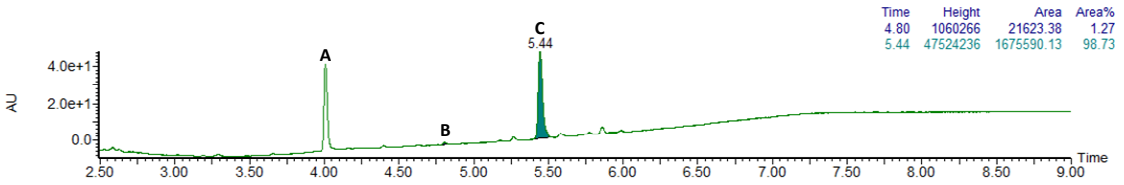


Figure SI-2. LC-MS chromatograms of analytical cleavage conversion of monosaccharide **SP-3** (t_r at 4.80 min. (peak **B**: compound **S-10**, *m/z* calcd for $C_{36}H_{43}Cl_3N_2O_9$ $[M+NH_4]^+$ 770.20 found 770.06)) to disaccharide **SP-4** (t_r at 5.44 min., peak **C** (**S-11**, *m/z* calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ $[M+NH_4]^+$ 1128.34 found 1128.27. Peak **A** (no mass detectable).

SP-5

The resin **SP-4** was transformed to resin-bound resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyloxy)pentyl]carbamate **SP-5** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-*O*-levulinoyl-2-trichloroacetamido-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyloxy)pentyl]carbamate (SP-6): Trisaccharide formation was performed according to general procedure A using 2 cycles on resin **SP-5** (0.378 g, 0.083 mmol) with trichloroacetimidate donor **1** (5 equiv, 0.290 g, 0.415 mmol) and TMSOTf (3 µL). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 97%, as compound **S-12** (*m/z* $[M+NH_4]^+$ calcd for $C_{71}H_{81}Cl_6N_3O_{20}NH_4$ 1523.35, found 1523.31).

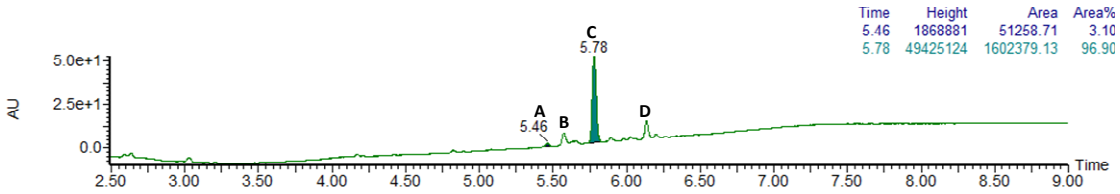


Figure SI-3. LC-MS chromatograms of analytical cleavage conversion of disaccharide **SP-5** (t_r at 5.46 min. (peak **A**, as **S-11**, *m/z* calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ $[M+NH_4]^+$ 1128.34 found 1128.31)) to trisaccharide **SP-6** (t_r at 5.78 min., peak **C** (as **S-12**, *m/z* calcd for $C_{71}H_{81}Cl_6N_3O_{20}$ $[M+NH_4]^+$ 1523.35 found 1523.31)). Peak **B** (as **S-12-TCA,** *m/z* calcd for $C_{69}H_{82}Cl_3N_3O_{19}$ $[M+H]^+$ 1362.42 found 1362.41), peak **D** (as **S-12+Bn**, *m/z* calcd for $C_{78}H_{87}Cl_6N_3O_{20}$ $[M+NH_4]^+$ 1613.44 found 1613.41).

SP-7

The resin **SP-6** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate **SP-7** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate (SP-8): Linker **SP-1** (0.5 g,

0.22 mmol/g, 0.11 mmol) was glycosylated in 1 cycle according to general procedure A with trichloroacetimidate donor **1** (5 equiv, 0.384 g 0.55 mmol) and TMSOTf (3 µL, 0.022 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) 98%, 94% as compound **S-10** (*m/z* for $C_{36}H_{43}Cl_3N_2O_9$ $[M+NH_4]^+$ 770.20, found 770.20 and 4% as compound **S-9** (*m/z* for $C_{34}H_{44}N_2O_8$ $[M+H]^+$ 609.30, found 609.25; (cycle 2) n.a (not analyzed). After capping and delevulination following procedure B, disaccharide formation **SP-4** was performed according to general A using 2 cycles on resin **SP-3** (0.490 g, 0.11 mmol) with trichloroacetimidate donor **2** (3 equiv, 0.521 g, 0.72 mmol) and TMSOTf (2 µL, 0.011 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 99% as compound **S-11** (*m/z* $[M+NH_4]^+$ calcd for $C_{56}H_{65}Cl_3N_2O_{15}NH_4$ 1128.34, found 1127.93. After capping and delevulination, trisaccharide formation **SP-6** was performed according to general procedure A using 2 cycles on resin **SP-5** (0.480 g, 0.11 mmol) with trichloroacetimidate donor **1** (3 equiv, 0.230 g, 0.33 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 95%, 74% as compound **S-12** (*m/z* for $[M+NH_4]^+$ calcd $C_{71}H_{81}Cl_6N_3O_{20}NH_4$ 1523.35, found 1523.31) and 21% as **S-12-TCA** (*m/z* $[M+H]^+$ calculd for $C_{69}H_{82}Cl_3N_3O_{19}H$ 1362.42, found 1362.35). After capping and delevulination, procedure A was applied consecutively to synthesized the tetrasaccharide **SP-8** on resin **SP-7** (0.4 g, 0.088 mmol) using trichloroacetimidate donor **3** (3 equiv, 0.186 g, 0.26 mmol) and TMSOTf (25 µL of 0.1M solution in CH_2Cl_2). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) n.a (not analyzed); (cycle 2) 97%, 71% as compound **S-13+Bz** with one remaining Bz group (*m/z* $[M+2NH_4]^{2+}$ calcd for $C_{105}H_{113}Cl_6N_3O_{27}(NH_4)_2$ 1046.78, found 1046.78), 19% as **S-13** (*m/z* $[M+NH_4]^+$ calcd for $C_{98}H_{109}Cl_6N_3O_{26}$ NH_4 1971.54, found 1971.47) and 6% as **S-13+Bz+Bn (***m/z* $[M+NH_4]^+$ calcd for $C_{98}H_{109}Cl_6N_3O_{26}$ NH_4 1971.54, found 1971.47).

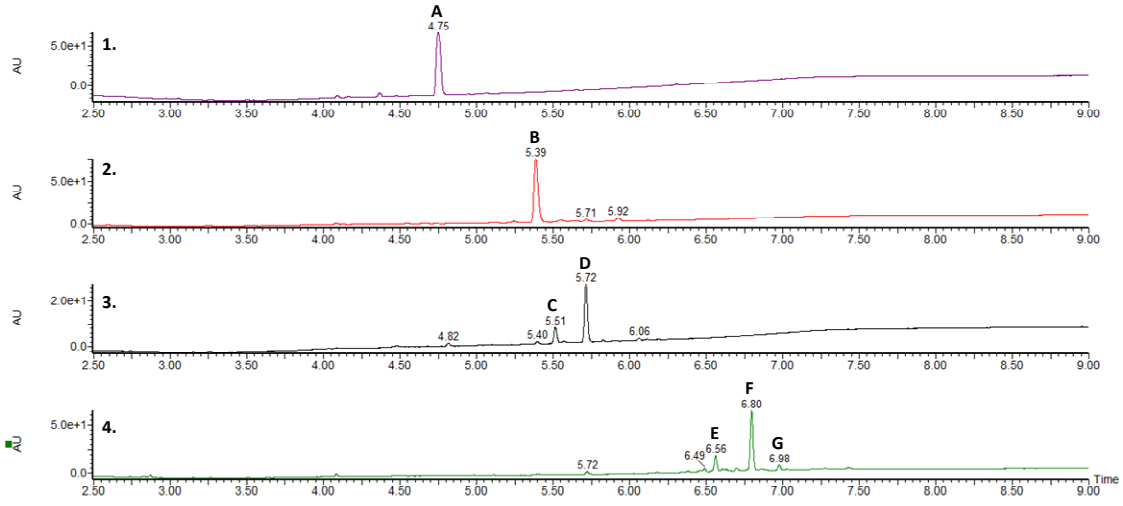


Figure SI-4. LC-MS analysis of tetrasaccharide **SP-8** synthesis. **(1)** LC-MS chromatogram from analytical cleavage of monosaccharide **SP-2** (t_r at 4.75 min., peak **A** (cleaved as **S-10**, *m/z* calcd for $C_{36}H_{43}Cl_3N_2O_9$ $[M+NH_4]^+$ 770.20 found 770.06). **(2)** LC-MS chromatogram from analytical cleavage of disaccharide **SP-4** (t_r at 5.39 min., peak **B** (cleaved as **S-11**, *m/z* calcd for $C_{56}H_{65}Cl_3N_2O_{15}$ $[M+NH_4]^+$ 1128.34 found 1128.27). **(3)** LC-MS chromatogram from analytical cleavage conversion of trisaccharide **SP-6** (t_r at 5.72 min., peak **D** (cleaved as **S-12,** *m/z* calcd

for $C_{71}H_{81}Cl_6N_3O_{20}$ $[M+NH_4]^+$ 1523.35 found 1523.24); t_r at 5.51 min., peak **C** (cleaved as **S-12-TCA**, *m/z* calcd for $C_{69}H_{82}Cl_3N_3O_{19}$ $[M+H]^+$ 1362.42 found 1362.35). **(4)** LC-MS chromatogram of analytical cleavage conversion of trisaccharide **SP-7** (cleaved as **S-12**, t_r at 5.72 min.) to tetrasaccharide **SP-8** (t_r at 6.56 min., peak **E** (cleaved as **S-13**, *m/z* calcd for $C_{98}H_{109}Cl_6N_3O_{26}$ $[M+NH_4]^+$ 1971.54 found 1971.47); t_r at 6.80 min., peak **F** (cleaved as **S-13+Bz**, *m/z* calcd for $C_{105}H_{113}Cl_6N_3O_{27}$ $[M+NH_4]^{2+}$ 1046.78 found 1046.78) and t_r at 6.98 min., peak **G** (cleaved as **S-13+Bz+Bn**, *m/z* calcd for $C_{112}H_{119}Cl_6N_3O_{27}$ $[M+2NH_4]^{2+}$ 1091.80 found 1091.81).

4-(Acetoxymethyl)benzyl N-benzyl-N-[5-((2-*O*-acetyl-3,4-di-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (S-14):

The resin **SP-8** (580 mg of resin) was swollen in dry CH_2Cl_2 (5 mL) and quantitative cleavage was performed according to general procedure C using 8 cycles of NaOMe/MeOH (500µL). The crude was treated with additional amount of 0.25M NaOMe solution until LC-MS showed the deprotection of all acyl groups **(S-13)**. Then, acetylation reaction was performed overnight at room temperature using acetic anhydride (0.25 mL) and catalytic amount of DMAP in pyridine (0.5 mL). The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with 1M HCl, saturated $CuSO_4$, water and brine. After concentration the crude was purified by column chromatography (hexane: acetone; 8:2 to 1:1) and preparative HPLC (C-18 (250x21.20 mm, 5 µm); flow rate 10 mL·min^{-1}; eluents: 20mM NH_4CO_3 in water/MeCN; gradient: initial 10% water/90% MeCN; 30 min: 1% water/99% MeCN to obtain compound **S-14** (71 mg, resin after cleavage 340 mg; 45% overall yield; 92% for each step). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.14 (m, 34H, aromatic), 7.10 (d, *J* = 7.5 Hz, 1H, NH), 6.92 (d, *J* = 7.4 Hz, 1H, NH), 6.89 (d, *J* = 9.0 Hz, 2H, aromatic$_{PMP}$), 6.84 – 6.70 (m, 6H, aromatic$_{PMP}$), 5.56 (dd, *J* = 16.1, 3.3 Hz, 2H, H-4$_{GalNAc}$), 5.21 – 5.14 (m, 2H, CH_2Ph_{carba}), 5.11 – 5.05 (m, 2H, CH_2Ph_{Ac}), 4.98 – 4.92 (m, 2H, 2xH-1$_{Ido}$), 4.92 – 4.89 (m, 1H, H-2$_{Ido}$), 4.86 – 4.80 (m, 3H, 2xH-1$_{GalN}$, H-2$_{Ido}$), 4.68 – 4.62 (m, 2H, H-5$_{Ido}$, CH_2Ph), 4.59 – 4.53 (m, 3H, H-5$_{Ido}$, CH_2Ph), 4.56 – 4.45 (m, 7H, CH_2Ph, CH_2PhN), 4.45 – 4.39 (m, 2H, CH_2Ph, H-3$_{GalNAc}$), 4.39 – 4.32 (m, 3H, CH_2Ph, H-3$_{GalNAc}$), 4.21 (dd, *J* = 10.2, 7.6 Hz, 1H, H-6$_{Ido}$), 4.18 – 4.12 (m, 2H, H-6$_{Ido}$, H-3$_{Idocap}$), 3.94 (dd, *J* = 10.2, 4.7 Hz, 1H, H-6 $_{Ido}$), 3.92 – 3.80 (m, 2H, H-6 $_{Ido}$, $OCH_{2Linker}$), 3.79 – 3.71 (m, 9H, H-5$_{GalNAc}$, 2xH-2$_{GalNAc}$, 2xCH_{3PMP}), 3.69 (t, *J* = 3.4 Hz, 1H, H-3$_{Ido}$), 3.64 (t, *J* = 6.0 Hz, 1H, H-5$_{GalNAc}$), 3.61 – 3.58 (m, 1H, H-4$_{Idocapp}$), 3.57 – 3.53 (m, 1H, H-4$_{Ido}$), 3.52 – 3.35 (m, 3H, H-6$_{GalNAc}$, $OCH_{2Linker}$), 3.29 (dd, *J* = 9.5, 5.7 Hz, 1H, H-6 $_{GalNAc}$), 3.26 – 3.09 (m, 3H, H-6$_{GalNAc}$, $NCH_{2Linker}$), 2.09 (s, 3H, CH_{3Ac}), 1.99 – 1.98 (2s, 6H, CH_{3Ac}), 1.75 (s, 3H, CH_{3Ac}), 1.70 (s, 3H, CH_{3Ac}), 1.58 – 1.43 (m, 4H, $CH_{2Linker}$), 1.35 – 1.26 (m, 2H, $CH_{2Linker}$) ppm. δ^{13}C (126 MHz, $CDCl_3$): 171.0, 170.1, 169.9, 169.7, 169.6, 162.0, 161.8, 154.2, 154.1, 153.2, 153.0, 138.3, 138.0, 138.0, 137.7, 137.6, 128.7, 128.6, 128.5, 128.5, 128.4, 128.3, 128.2, 128.0, 128.0, 127.9, 127.9, 127.8, 127.5, 127.3, 116.5, 115.8, 114.7, 101.1 (C-1$_{GalNAc}$), 100.2 (C-1$_{Ido}$), 100.1 (C-1$_{Ido}$), 99.6 (C-1$_{GalNAc}$), 92.7 (Cq, TCA), 92.3 (Cq, TCA), 76.3 (C-4$_{Idocapp}$), 73.8 (C-3$_{GalNAc}$), 73.7 (C$_{Bn}$), 73.6 (C$_{Bn}$), 73.3 (C-4$_{Ido}$), 73.3 (C-3$_{GalNAc}$), 72.9 (C-5$_{GalNAc}$), 72.6 (C-5$_{GalNAc}$), 72.6 (C$_{Bn}$), 72.3 (C-3$_{Ido}$), 70.0 ($OCH_{2Linker}$), 69.7, 69.7 (C-4$_{GalNAc}$), 68.9 (C-6$_{GalNAc}$),, 68.6 (C-6$_{GalNAc}$), 68.5 (C-2$_{Ido}$), 68.3 (C-6$_{Ido}$), 68.0 (C-2$_{Ido}$), 67.9 (C-6$_{Ido}$), 67.2 (C-5$_{Ido}$), 66.9 (CH_2Ph_{carba}), 66.1 (CH_2Ph_{Ac}), 66.0 (C-5$_{Ido}$), 56.5 (C-2$_{GalNAc}$),

56.4 (C-2_{GalNAc}), 55.9 (CH_{3PMP}), 50.7, 50.5 (CH_2PhN), 47.4, 46.3 ($NCH_{2Linker}$), 29.2, 29.1, 29.1, 28.0, 27.4 ($CH_{2Linker}$), 23.4, 22.8 ($CH_{2Linker}$), 21.2, 21.1, 21.1, 20.4, 20.3 (CH_{3Ac}) ppm. HRMS (ESI) *m/z*: $[M+NH_4]^+$ Calcd for $C_{108}H_{119}Cl_6N_3O_{31}NH_4$ 2181.6297; Found 2182.6685.

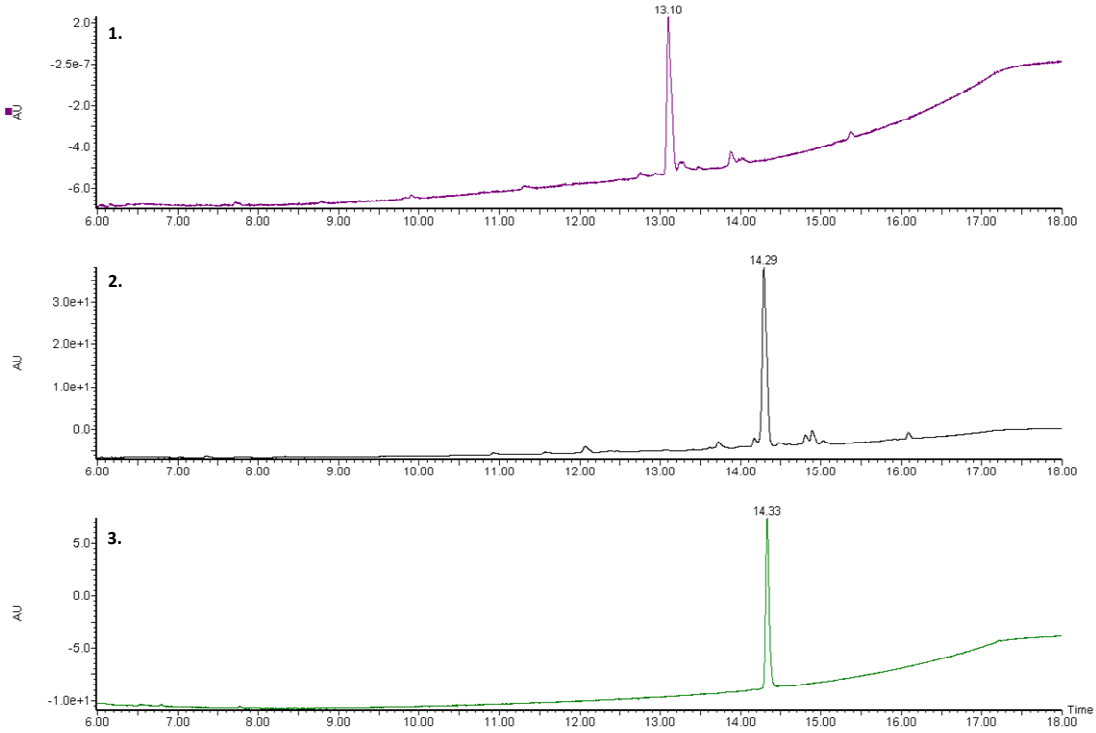


Figure SI-5. LC-MS data for the synthesis of tetrasaccharide **S-14**. (1) chromatogram of the crude **S-13** from preparative cleavage, (2) chromatogram after acetylation reaction **S-14**, (3) chromatogram after preparative HPLC purification of **S-14**.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*- *p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate (SP-9): Tetrasaccharide formation was performed according to general procedure A using 2 cycles on resin **SP-7** (0.372 g, 0.082 mmol) with trichloroacetimidate donor **2** (5 equiv, 0.296 g, 0.41 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) 86%; (cycle 2) 98% as compound **S-15** (*m/z* $[M+NH_4]^+$ calcd for $C_{91}H_{103}Cl_6N_3O_{26}$ NH_4 1881.49 found 1881.46).

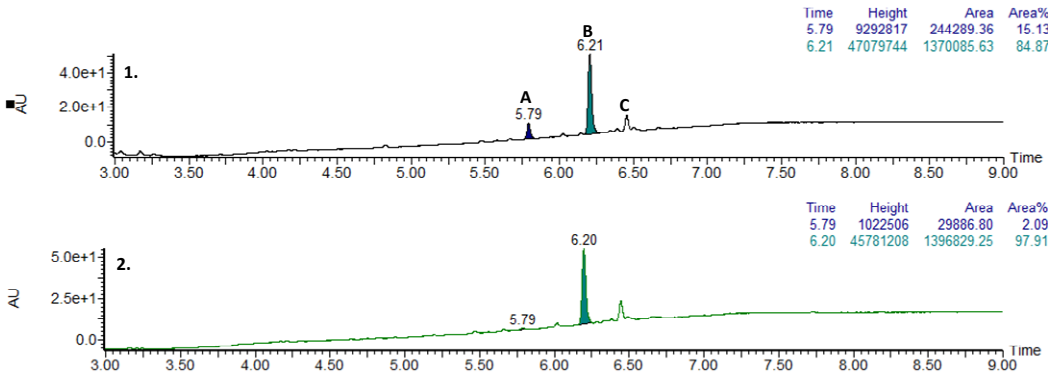


Figure SI-6. LC-MS chromatogram of analytical cleavage conversion of trisaccharide **SP-7** (cleaved as **S-12**, t_r at 5.79 min., peak **A** (*m/z* calcd for $C_{71}H_{81}Cl_6N_3O_{20}$ $[M+NH_4]^+$ 1523.35 found 1523.33)) to tetrasaccharide **SP-9** (cleaved as **S-15**; t_r at 6.20 min., peak **B** (*m/z* calcd for $C_{91}H_{103}Cl_6N_3O_{26}$ $[M+NH_4]^+$ 1881.49 found 1881.46)). Peak **C** (as **S-15+Bn**, t_r at 6.55 min. for *m/z* $C_{98}H_{109}Cl_6N_3O_{26}$ $[M+NH_4]^+$ 1971.54 found 1971.51).

SP-10

The resin **SP-9** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate **SP-10** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-levulinoyl-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-11): Pentasaccharide formation was performed according to general procedure A using 2 cycles on resin **SP-10** (0.370 g, 0.081 mmol) with trichloroacetimidate donor **1** (5 equiv, 0.283 g, 0.41 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) n.a; (cycle 2) full conversion as compound **S-16** (*m/z* $[M+2NH_4]^{2+}$ calcd for $C_{106}H_{119}Cl_9N_4O_3(NH4)_2$ 1147.25 found 1147.23).

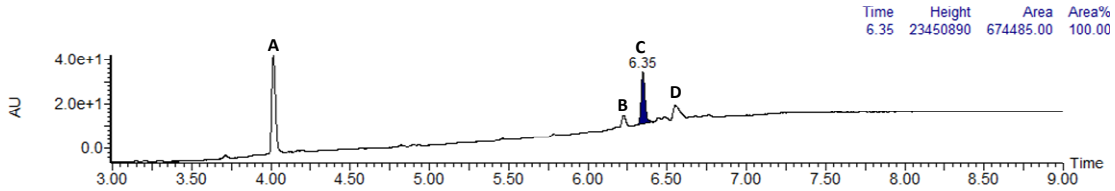


Figure SI-7. LC-MS chromatogram of analytical cleavage of pentasaccharide **SP-11** synthesis (t_r at 6.35 min., peak C (as **S-16**, t_r at 6.35 min *m/z* calcd for $C_{106}H_{119}Cl_9N_4O_{31}$ $[M+2NH_4]^{2+}$ 1147.25 found 1147.23)); peak B (cleaved as **S-16-TCA**, at 6.25 min *m/z* calcd for $C_{104}H_{120}Cl_6N_4O_{30}$ $[M+H+NH_4]^{2+}$ 1066.80 found 1066.78)); peak D (as **S-16+Bn**, t_r at 6.55 min. for *m/z* $C_{113}H_{125}Cl_9N_4O_{31}$ $[M+2NH_4]^{2+}$ 1192.27 found 1192.25). Unidentified: peak **A** (no mass detectable).

SP-12

The resin **SP-11** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate **SP-12** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-O-levulinoyl-6-*O*- *p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido- β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-13): Hexasaccharide formation was performed according to general procedure A using 2 cycles on resin **SP-12** (0.367 g, 0.081 mmol) with trichloroacetimidate donor **2** (5 equiv, 0.290 g, 0.40 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) n.a; (cycle 2) 90% as compound **S-17** *m/z* $[M+2NH_4]^{2+}$ calcd for $C_{126}H_{141}Cl_9N_4O_{37}(NH_4)_2$ 1326.32 found 1326.26).

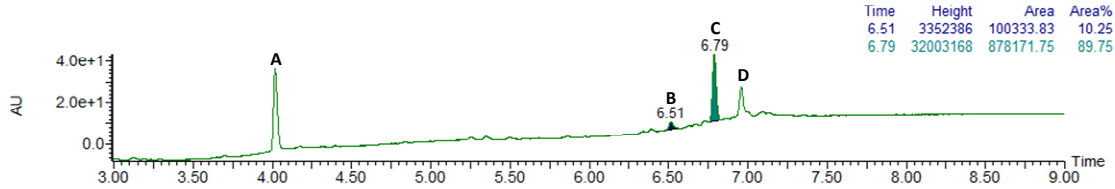


Figure SI-8. LC-MS chromatogram of analytical cleavage data. Conversion of pentasaccharide **SP-12** (t_r at 6.51 min., peak **B** (as **S-16**, m/z calcd for $C_{106}H_{119}Cl_9N_4O_{31}$ $[M+2NH_4]^{2+}$ 1147.25 found

1147.20) to the hexasaccharide **SP-13** (as **S-17,** t_r at 6.79 min., peak **C** (*m/z* calcd for $C_{126}H_{141}Cl_9N_4O_{37}$ $[M+2NH_4]^{2+}$ 1326.32 found 1326.26)); peak **D** (as **S-17+Bn**, t_r at 6.96 min. *m/z* calcd for $C_{133}H_{147}Cl_9N_4O_{37}$ $[M+2NH_4]^{2+}$ 1371.34 found 1371.28). Unidentified: peak **A** (no mass detectable).

SP-14

The resin **SP-13** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate **SP-14** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-3-levulinoyl 2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-15): Heptasaccharide formation was performed according to general procedure A using 2 cycles on resin **SP-14** (0.364 g, 0.080 mmol) with trichloroacetimidate donor **1** (5 equiv, 0.290 g, 0.40 mmol) and TMSOTf (3 µL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) 94%; (cycle 2) full conversion as compound **S-18** (*m/z*$[M+2NH_4]^{2+}$ calcd for $C_{141}H_{157}Cl_{12}N_5O_{42}(NH_4)_2$ 1523.82 found 1523.65).

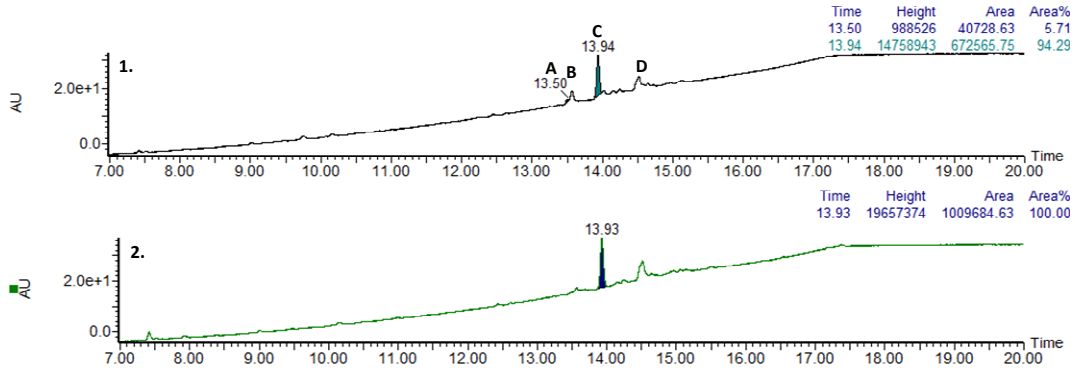


Figure SI-9. LC-MS chromatogram of analytical cleavage data conversion of hexasaccharide **SP-14** (t_r at 13.50 min., peak **A** (as **17**, m/z calcd for $C_{126}H_{141}Cl_9N_4O_{37}$ $[M+2NH_4]^{2+}$ 1326.32 found 1326.26) to the heptasaccharide **SP-15** (as **S-18**, t_r at 13.93 min., peak **C** (*m/z* calcd for $C_{141}H_{157}Cl_{12}N_5O_{42}$ $[M+2NH_4]^{2+}$ 1523.82 found 1523.65)); peak **B** (as **S-18-TCA**, t_r at 5.18 min. *m/z* calcd for $C_{139}H_{158}Cl_9N_5O_{41}$ $[M+NH_4+H]^{2+}$ 1443.40 found 1443.19), peak **D** (as **S-18+Bn**, t_r at 14.51

min. (*m/z* calcd for $C_{148}H_{163}Cl_{12}N_5O_{42}$ $[M+2NH_4]^{2+}$ 1568.85, found 1568.66). (1) cycle 1, (2) cycle 2.

SP-16

The resin **SP-15** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate **SP-16** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl]carbamate (SP-17): Octasaccharide formation was performed according to general procedure A using 2 cycles on resin **SP-16** (0.358 g, 0.080 mmol) with trichloroacetimidate donor **3** (5 equiv, 0.281 g, 0.40 mmol) and TMSOTf (2.8 µL, 0.016 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) n.a; (cycle 2) 97% as compound **5** (*m/z:* $[M+2NH_4]^{2+}$ calcd for $C_{141}H_{157}Cl_{12}N_5O_{42}$ NH_4 1523.82 found 1523.65).

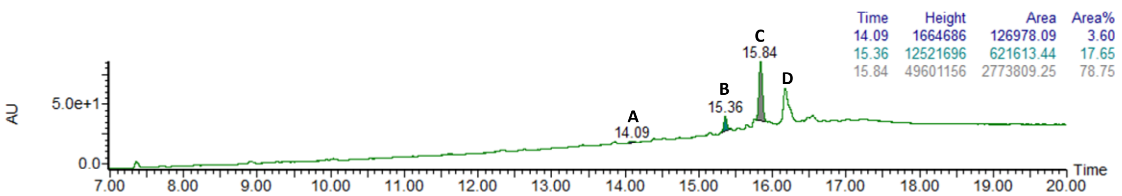


Figure SI-10. LC-MS analysis of analytical cleavage data conversion of heptasaccharide **SP-16** (**S-18**, t_r at 14.09 min., peak **A** (*m/z* calcd for $C_{141}H_{157}Cl_{12}N_5O_{42}$ $[M+2NH_4]^{2+}$ 1523.82 found 1523.65) to the octasaccharide **SP-17** (as **5**, t_r at 15.36 min., peak **B** (*m/z* calcd for $C_{168}H_{185}Cl_{12}N_5O_{48}$ $[M+2NH_4]^{2+}$ 1747.90 found 1747.98); peak **C** (as **5+Bz**, t_r at 15.84 min. *m/z* calcd for $C_{175}H_{189}Cl_{12}N_5O_{49}$ $[M+2NH_4]^{2+}$ 1443.40 found 1443.19), peak **D** (as **5+Bz+Bn**, t_r at 16.18 min. (*m/z* calcd for $C_{182}H_{195}Cl_{12}N_5O_{49}$ $[M+2NH_4]^{2+}$ 1844.96, found 1845.04).

4-(Acetoxymethyl)benzyl N-benzyl-N-[5-((2-*O*-acetyl-3,4-di-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→3)-(4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-p-methoxyphenyl-α-L-idopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-galactopyranosyloxy)pentyl] carbamate (5): The resin **SP-17** (570 mg of resin, resin after cleavage 340 mg; 0.075 mmol) was swollen in dry CH_2Cl_2 (5mL) and quantitative cleavage was performed according to general procedure C using 8 cycles of NaOMe/MeOH (500µL). The crude was treated with additional amount of 0.25M NaOMe solution until LC-MS showed the deprotection of all acyl groups affording intermediate **4** (see Figure SI-11). Acetylation was performed overnight at room temperature with acetic anhydride (0.25 mL) and a catalytic amount of DMAP in pyridine (0.5 mL). The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with 1M HCl, saturated $CuSO_4$, water and brine. After concentration the crude was purified by column chromatography (hexane: ethyl acetate; 7:3 to 1:1) and preparative HPLC (column: C-18 (21.2x250 mm 5 µm); flow rate 10 mL·min^{-1}; eluents: 20mM NH_4CO_3 in water/MeCN; gradient: initial 10% water/90% MeCN; 30 min: 1% water/99% MeCN to obtain compound **5** (27 mg, 9.4% overall yield) and **5+Bn** (16 mg, 5.6%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.13 (m, 59H, aromatic), 7.06 (bs, 1H, NH), 6.94 – 6.86 (m, 8H, aromatic$_{PMP}$, NH), 6.85 – 6.72 (m, 10H, aromatic$_{PMP}$), 5.59 – 5.52 (m, 4H, 4xH-4$_{GalNAc}$), 5.19 – 5.04 (m, 4H, CH_2-Ph$_{Carba}$, CH_2-Ph$_{Ac}$), 4.98 – 4.91 (m, 4H, 4xH-1$_{Ido}$), 4.91 – 4.88 (s, 1H, H-2$_{Ido}$), 4.84 – 4.76 (m, 7H, 4xH-1$_{GalNAc}$, 3x H-2$_{Ido}$), 4.67 – 4.60 (m, 4H, 2xH-5$_{Ido}$, CH_2Ph), 4.58 – 4.25 (m, 24 H, CH_2PhN, 8xCH_2Ph, 4xH-3$_{GalNAc}$, 2xH-5$_{Ido}$, CH_2Ph), 4.23 – 4.11 (m, 7H, 3xH-3$_{Ido}$, 2xH-6$_{Ido}$), 3.96 – 3.84 (m, 5H, 2xH-6$_{Ido}$, OCH$_{2Linker}$), 3.83 – 3.71 (m, 17H, 4CH$_{3PMP}$, 4xH-2$_{GalN}$, H-5$_{GalNAc}$), 3.69 (t, *J* = 3.4 Hz, 1H, H-3$_{Ido}$), 3.65 – 3.55 (m, 6H, 3xH-5$_{GalNAc}$, 3xH-4$_{Ido}$), 3.55 – 3.51 (bs, 1H, H-4$_{Idocapp}$), 3.51 – 3.33 (m, 3H, H-6$_{GalNAc}$, OCH$_{2Linker}$), 3.32 – 3.08 (m, 8H, CH_2N$_{Linker}$, 3xH-6$_{GalN}$), 2.12 (1s, 3H, CH$_{3Ac}$), 2.01 – 1.95 (s, 12H, CH$_{3Ac}$), 1.74 (s, 3H, CH$_{3Ac}$), 1.69 (s, 3H, CH$_{3Ac}$), 1.59 – 1.58 (2s, 6H, CH$_{3Ac}$), 1.55 – 1.44 (m, 4H, CH$_{2Linker}$), 1.35 – 1.28 (m, 2H, CH$_{2Linker}$) ppm; δ^{13}C (126 MHz, $CDCl_3$): 173.0, 170.1, 169.8, 169.7, 169.6, 169.5, 162.1, 162.0, 156.7, 156.2, 154.0, 153.9, 153.0, 152.9, 138.2, 138.2, 137.9, 137.6, 137.6, 137.4, 136.9, 135.7, 131.0, 128.6, 128.6, 128.5, 128.4, 128.4, 128.3, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.8, 127.4, 127.2, 116.2 (C$_{PMP}$), 115.5 (C$_{PMP}$), 114.6 (C$_{PMP}$), 114.5 (C$_{PMP}$), 101.6 (C-1$_{GalN}$, J_{CH} = 162.0 Hz), 101.4 (C-1$_{GalN}$, J_{CH} = 162.0 Hz) , 99.9 (4xC-1$_{Ido}$, J_{CH} = 172.0 Hz, C-1$_{GalNAc,}$ J_{CH} = 160.0 Hz), 92.8, 92.5 (2x), 92.5 (Cq, TCA), 76.5 (C-4$_{Ido}$), 73.7 (C$_{Bn}$, C-3$_{GalNAc}$), 73.6 (C-3$_{GalNAc}$), 73.3 (C-4$_{Ido}$), 73.1 (C-5$_{GalNAc}$, C-4$_{Ido}$), 72.8 (C-2$_{GalNAc}$), 72.5 (C$_{Bn}$), 72.3, 72.2 (C-3$_{Ido}$), 69.9 (OCH$_{2Linker}$), 69.6, 69.4 (C-4$_{GalNAc}$), 68.8, 68.5, 68.4, 68.3, 68.2 (C-6$_{GalNAc}$, C-2$_{Ido}$), 67.9 (C-6$_{Ido}$), 67.7 (C-2$_{Ido}$), 66.9 (CH_2Ph$_{Carba}$, C-5$_{Ido}$), 66.1 (CH_2Ph$_{Ac}$, C-2$_{Ido}$), 56.0, 55.9, 55.8 (CH$_{3PMP}$), 50.6, 50.4 (CH_2PhN), 47.3, 46.3 (NCH$_{2Linker}$), 29.4, 29.4, 29.2, 29.1, 28.0, 27.4, 23.3, 22.8, 21.1, 21.0, 20.4, 20.3, 20.2 ppm; HRMS (ESI) *m/z:* [M+2Na]$^{2+}$ calcd for $C_{186}H_{203}Cl_{12}N_5O_{57}Na_2$ 1941.9593, found 1941.9615.

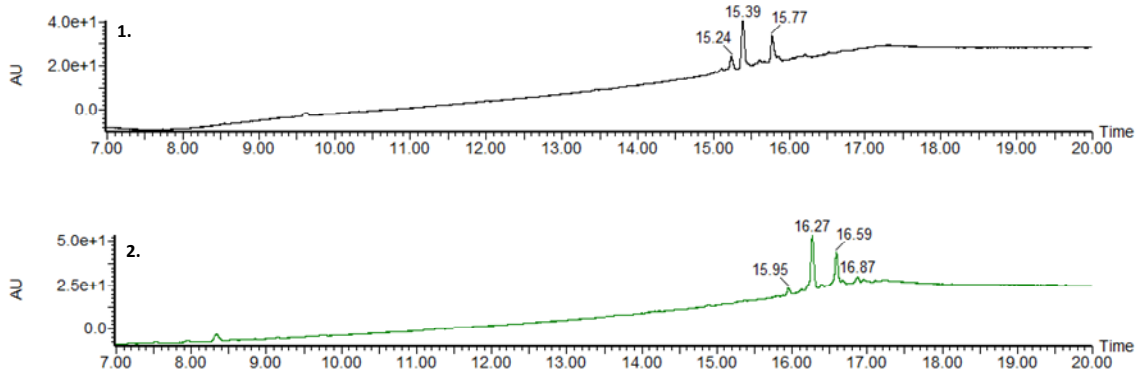


Figure SI-11. LC-MS data for the synthesis of octasaccharide **5**. **1)** chromatogram after quantitative cleavage (unidentified peak, t_r = 15.24 min, *m/z* 1808.47; as **4** (t_r = 15.39 min, *m/z* calcd for $C_{168}H_{185}Cl_{12}N_5O_{48}$ $[M+2NH_4]^{2+}$ 1747.95 found 1747.97); as **4+Bn** (t_r = 15.77 min, *m/z* calcd for $C_{175}H_{191}Cl_{12}N_5O_{48}$ $[M+2NH_4]^{2+}$ 1792.98 found 1792.95). **2)** chromatogram after acetylation (unidentified peak, t_r = 15.95 min, *m/z* 1885.64); as **5** (t_r = 16.27 min, *m/z* calcd for $C_{186}H_{203}Cl_{12}N_5O_{57}$ $[M+2NH_4]^{2+}$ 1937.01 found 1936.97); as **5+Bn** (t_r = 16.59 min, *m/z* calcd for $C_{193}H_{209}Cl_{12}N_5O_{57}$ $[M+2NH_4]^{2+}$ 1982.02 found 1981.97).

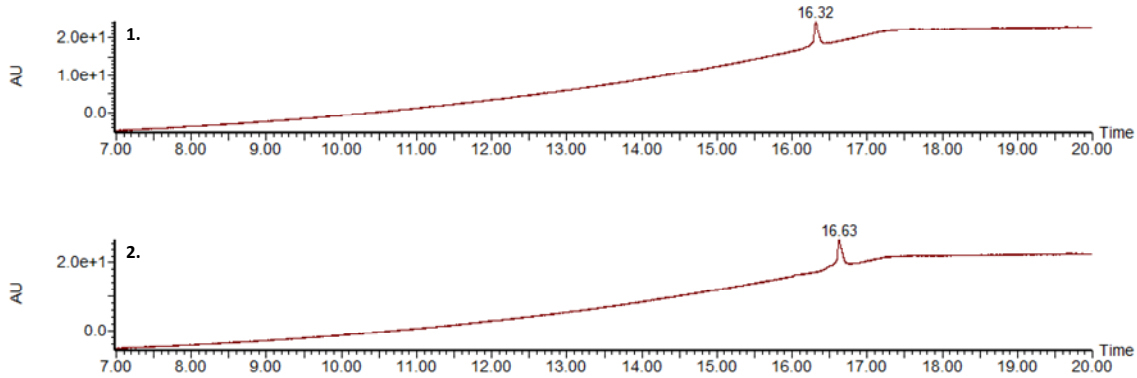
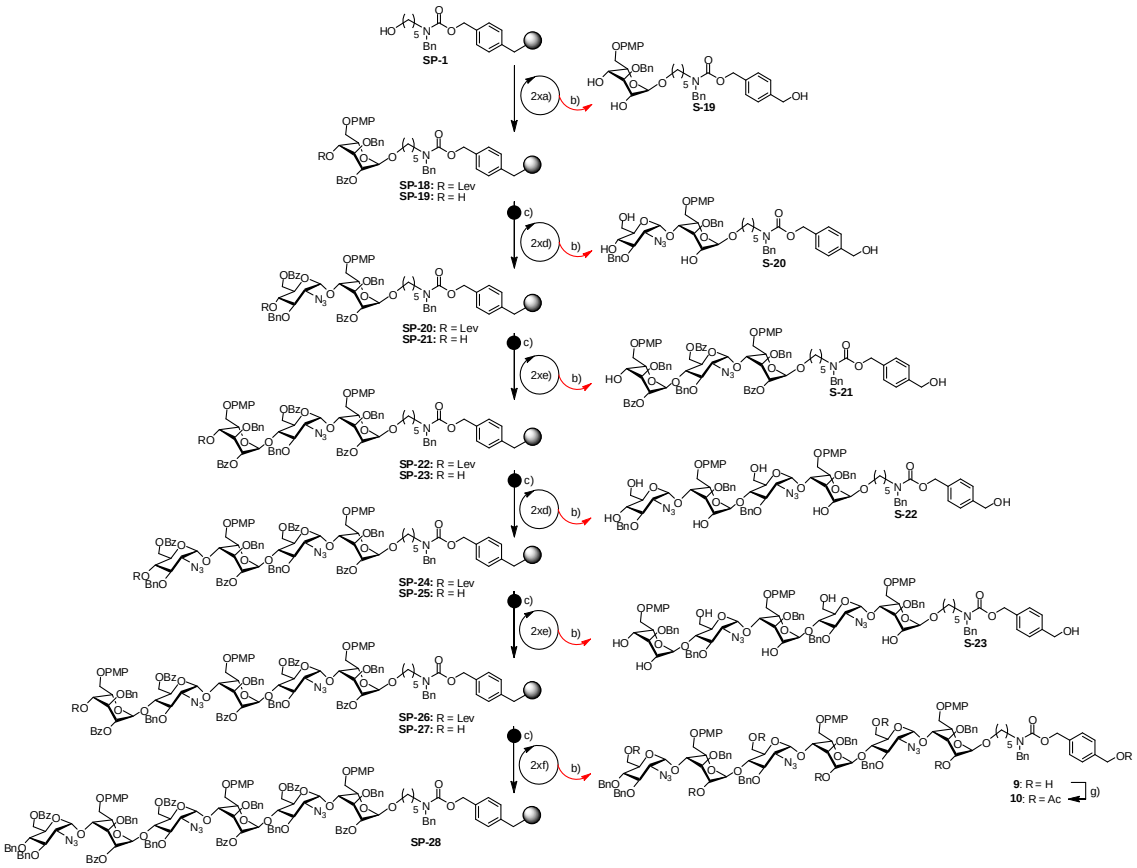


Figure SI-12. LCMS data for the synthesis of octasaccharide **5**. **1)** Chromatogram after preparative HPLC column of pure compound **5**. **2)** Chromatogram after preparative HPLC column of compound **5+Bn**.

5. Solid-phase synthesis of HS hexasaccharide precursor



Scheme S-3. **Solid-phase assembly of a heparin sulfate oligosaccharide precursor.** a) **6**, 20% TMSOTf, -20 °C to r.t; b) NaOMe (cat), MeOH, MW; c) hydrazine acetate, CH_2Cl_2: MeOH (4:1); d) **7**, 20% TMSOTf, -20 °C to r.t; e) **2**, 20% TMSOTf, -20 °C to r.t; f) **8**, 20% TMSOTf, -20 °C to r.t; g) Ac_2O, pyridine, 0 °C to r.t.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate (SP-18): Linker **SP-1** (340 mg, 0.22 mmol/g, 75 µmol) was glycosylated in one cycle with thioglycoside **6** (5 equiv, 0.251 g, 0.37 mmol), NIS (6 equiv, 0.101 g, 0.45 mmol) and TMSOTf (150 µL of 0.1M solution in dry CH_2Cl_2, 15 µmol) according to general procedure D. The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): 99% as compound **S-19** (*m/z:* $[M+Na]^+$ calcd for $C_{41}H_{49}NO_{10}Na$ 738.32, found 738.30).

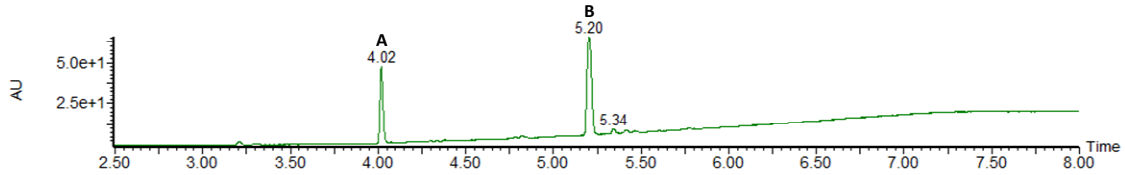


Figure SI-13. LC-MS chromatogram of analytical cleavage data conversion of linker **SP-1** to the monosaccharide **SP-18** (as **S-19**, retention time (t_r) at 5.20 min., peak **B** (*m/z* calcd for $C_{41}H_{49}NO_{10}$ $[M+Na]^+$ 738.32 found 738.30)), unidentified peak: peak **A** (no mass detectable).

SP-19

The resin **SP-18** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate **SP-19** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-(4-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-20): The reaction was performed according to general procedure A using two cycles on resin **SP-19** (0.340 g, 0.075 mmol) with thricloroacetimidate **7** (3 x 6 equiv, 289 mg, 0.45 mmol) and TMSOTf (150 µL of 0.1M solution in dry CH_2Cl_2, 15 µmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle1) ,n.a, (cycle 2) 85% as compound **S-20** (*m/z:* $[M+NH_4]^+$ calcd for $C_{54}H_{64}N_4O_{14}Na$ 1010.48 found, 1010.21).

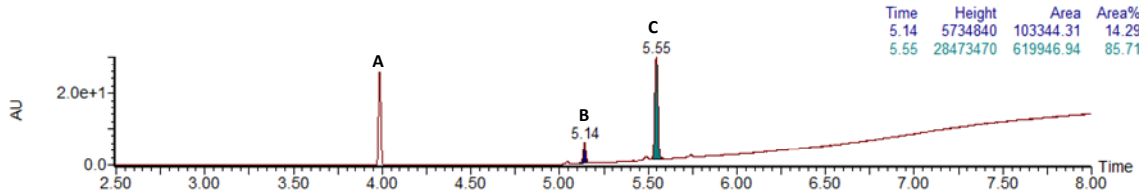


Figure SI-14. LC-MS chromatogram of analytical cleavage data conversion of monosaccharide **SP-19** (cleaved as **S-19** retention time (t_r) at 5.14 min., peak **B** (calcd for $C_{41}H_{49}NO_{10}$ $[M+Na]^+$ 738.32 found 738.29)) to the disaccharide **SP-20** (cleaved as **S-20** (t_r) at 5.55 min., peak **C** (*m/z* calcd for $C_{54}H_{64}NO_{14}$ $[M+Na]^+$ 1015.43 found), unidentified peak: peak **A** (no mass detectable).

SP-21

The resin **SP-20** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-(5-(4-O-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl) carbamate **SP-21** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-22): The reaction was performed according to general procedure A using three cycles on resin **SP-21** (0.335 g, 0.074 mmol) with trichloroacetimidate **2** (6 equiv, 0.320 g, 0.44 mmol) and TMSOTf (150 µL of 0.1M solution in dry CH_2Cl_2, 15 µmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle1) not analized, (cycle 2) 93% as compound **S-21** (*m/z:* $[M+NH_4]^+$ calcd for $C_{73}H_{84}N_4O_{20}NH_4$ 1368.62 found 1368.16).

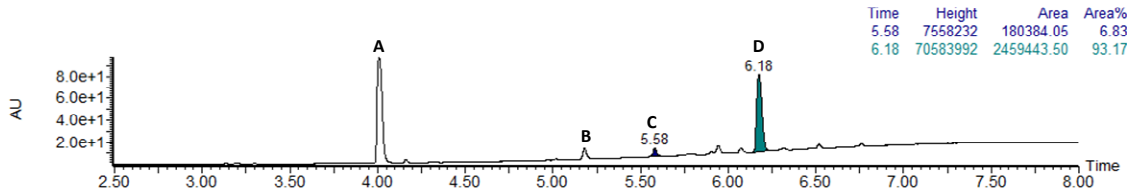


Figure SI-15. LC-MS chromatogram of analytical cleavage data conversion of disaccharide **SP-21** (t_r at 5.58 min., peak **C** (as **S-20**, m/z calcd for $C_{54}H_{64}NO_{14}$ $[M+NH_4]^+$ 1010.47 found 1010.69)) to the trisaccharide **SP-22** (as **S-21**, t_r at 6.18 min., peak **D** (*m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1368.62 found 1368.16)); **A** (no detectable mass), peak **B** (unreacted monosaccharide cleaved as **S-19,** t_r at 5.18 min. (calcd for $C_{41}H_{49}NO_{10}$ $[M+NH_4]^+$ 733.37 found 733.29).

SP-23

The resin **SP-22** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate **SP-23** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-4-levulinoyl-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-24): Tetrasaccharide formation was performed according to general procedure A using three cycles on resin **SP-23** (397 mg) with trichloroacetimidate **7** (3 x 6 equiv, 288 mg, 0.45 mmol) and TMSOTf (150 µL of 0.1M solution in dry CH_2Cl_2, 0.015 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) 28%; (cycle 2) 63%; (cycle 3) 78% as compound **S-22** (*m/z*: $[M+NH_4]^+$ calcd for $C_{73}H_{84}N_4O_{20}$ NH_4 1645.72 found 1645.57).

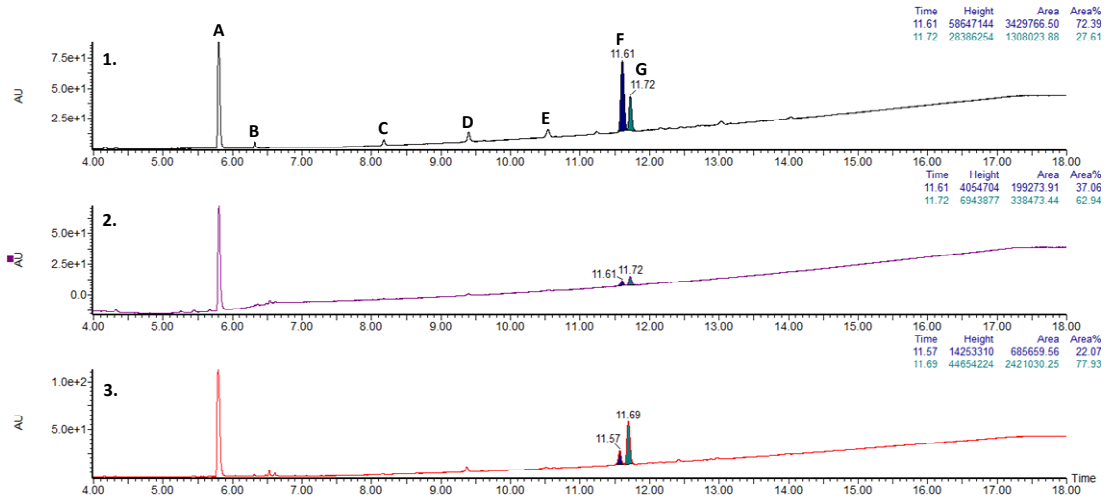


Figure SI-16. LC-MS chromatogram of analytical cleavage data conversion of trisaccharide **SP-23** (t_r at 11.61 min., peak **F** (as **S-21**, *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1368.62 found 1368.42)) to the tetrasaccharide **SP-24** (t_r at 11.72 min., peak **G** (as **S-22,** *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1645.72 found 1645.57)); **A** (no detectable mass), peak **B** (t_r = 4.17 min., *m/z* calcd for $C_{21}H_{27}NO_4$ $[M+Na]^+$ 380.18 found 380.14)), peak **C** (as **S-19**, *m/z* calcd for $C_{41}H_{49}NO_{10}$ $[M+NH_4]^+$ 733.37 found 733.25), peak **D** (as **S-20**, *m/z* calcd for $C_{54}H_{64}NO_{14}$ $[M+Na]^+$ 1015.43 found 1015.34), peak **E** (deletion sequence, t_r at 10.54 min. (*m/z* calcd for $C_{61}H_{71}NO_{16}$ $[M+NH_4]^+$ 1091.51 found 1091.36)). Traces 1-3 correspond to analytical cleavage after 1 ,2 and 3 cycles of glycosylation, respectively.

SP-25

The resin **SP-24** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate **SP-25** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-4-*O*-levulinoyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-26): Pentasaccharide synthesis was performed according to general procedure A using three cycles on resin **SP-25** with trichloroacetimidate **2** (3 x 6 equiv, 324 mg, 0.45 mmol) and TMSOTf (150 µL of 0.1M solution in dry CH_2Cl_2, 0.015 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) 62%; (cycle 2) 72%, (cycle 3) 76% as compound **S-23** (*m/z:* $[M+2NH_4]^{2+}$ calcd for $C_{107}H_{123}N_7O_{30}$ NH_4 1010.94 found 1010.84).

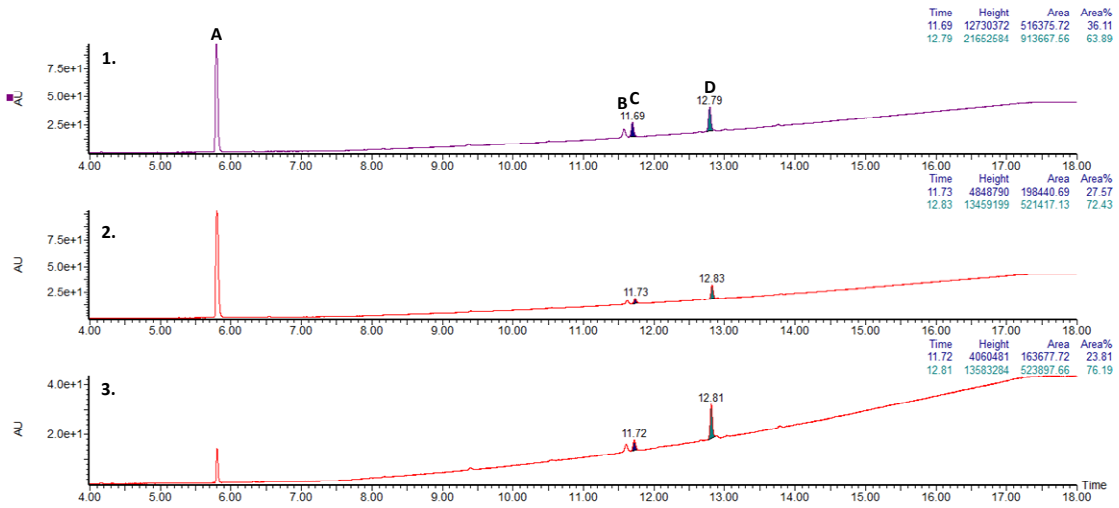


Figure SI-17. LC-MS chromatogram of analytical cleavage data conversion of tetrasaccharide **SP-25** (t_r at 11.69 min., peak **C** (as **S-22**, *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1645.72 found 1645.52)) to the pentasaccharide **SP-26** (as **S-23,** t_r at 12.79 min., peak **D** (*m/z* calcd for $C_{107}H_{123}N_7O_{30}$ $[M+2NH_4]^{2+}$ 1010.94 found 1010.84)); **A** (no detectable mass), peak **B** (as **S-21**, t_r = 11.57 min., *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1368.62 found 1368.42)).

SP-27

The resin **SP-26** was transformed to resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate **SP-27** using general procedure B.

Resin bound 4-(hydroxymethyl)benzyl *N*-benzyl *N*-[5-((2-azido-6-*O*-benzoyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl(-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-benzoyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (SP-28): Hexasaccharide synthesis was performed according to general procedure A using three cycles on resin **SP-27** with trichloroacetimidate **8** (3 x 6 equiv, 284 mg, 0.45 mmol) and TMSOTf (150 µL of 0.1M solution in dry CH_2Cl_2, 0.015 mmol). The conversion was determined by analytical NaOMe cleavage. LC-MS (ESI): (cycle 1) 46%; (cycle 2) 92%, (cycle 3) 92% as compound **10** (*m/z:* $[M+2NH_4]^{2+}$ calcd for $C_{127}H_{144}N_{10}O_{34}$ NH_4 1194.52, found 1194.48).

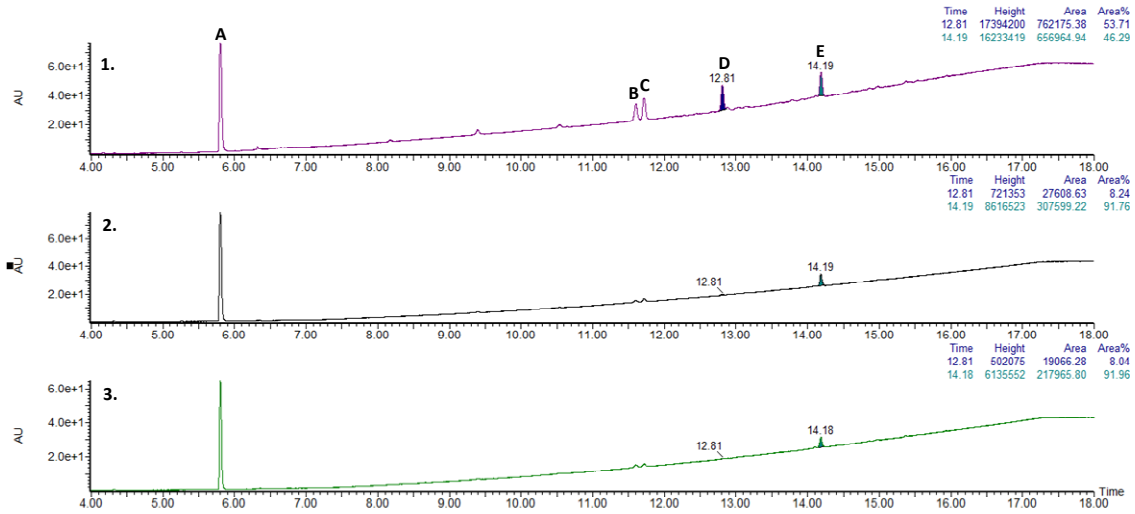


Figure SI-18. LC-MS chromatogram of analytical cleavage data conversion of pentasaccharide **SP-27** (t_r at 12.81 min., peak **D** (as **23**, *m/z* calcd for $C_{107}H_{123}N_7O_{30}$ $[M+2NH_4]^{2+}$ 1010.94 found 1010.92)) to the hexasaccharide **SP-28** (as **10**, t_r at 14.19 min., peak **E** (*m/z* calcd for $C_{127}H_{144}N_{10}O_{34}$ $[M+2NH_4]^{2+}$ 1194.52 found 1194.48)); peak **A** (no detectable mass), peak **B** (as **S-21**, *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1368.62 found 1368.60)) and peak **C** (as **S-22**, *m/z* calcd for $C_{73}H_{84}N_4O_{20}$ $[M+NH_4]^+$ 1645.72 found 1645.63).

4-(Acetoxymethyl)benzyl *N*-benzyl *N*-[5-((6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl(-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-(2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyl)-(1→4)-(6-*O*-acetyl-2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-2-*O*-acetyl-3-*O*-benzyl-6-*O*-*p*-methoxyphenyl-α-L-idopyranosyloxy)pentyl] carbamate (10):

The resin **SP-28** (392 mg of resin, resin after cleavage 257 mg; 0.056 mmol) was swollen in dry CH_2Cl_2 (4 mL) and quantitative cleavage was performed according to general procedure C using 12 cycles of NaOMe/MeOH (500µL). The crude was treated with additional amount of 0.25 M NaOMe solution until LC-MS shows complete deprotection to intermediate **9** (See Figure SI-18). Acetylation was performed overnight at room temperature using acetic anhydride (3 mL) and a catalytic amount of DMAP in pyridine (4 mL). The reaction mixture was diluted with CH_2Cl_2 (50 mL), and the organic layer was washed with 2x 1M HCl, saturated $CuSO_4$ (50 mL), water and brine. After concentration the crude (151 mg) was purified by column chromatography (hexane/ acetone; 9: 1 to 1:1) and by preparative HPLC (eluents: 20mM NH_4HCO_3/MeCN; gradient: 10% (5 min) - 99% (in 30 min) – 99% (isocratic) was obtained compound **10** (17 mg, 11% over 14 steps, 85% in each step). ^{1}H NMR (500 MHz, CDCl3) δ 7.40 – 7.27 (m, 27H, aromatic), 7.26 – 7.19 (m, 7H), 7.19 – 7.08 (m, 10H, aromatic), 6.86 – 6.64 (m, 12H, aromatic), 5.15 (d, J = 20.7 Hz, 1H, CH_2-Ph_{Carba}), 5.08 (s, 2H, CH_2-Ph_{Ac}), 5.00 – 4.87 (m, 7H, 2xH-1_{Ido}, 3xH-1_{Azido}, H-$2_{Ido,}$ CH_2Ph), 4.87 – 4.71 (m, 8H, 2xH-2_{Ido}, H-1_{Ido}, 5xCH_2Ph), 4.70 – 4.57 (m, 6H, 3xCH_2Ph), 4.57 – 4.52 (m, 2H, 1xCH_2Ph, H-5_{Ido}), 4.50 – 4.42 (m, 5H, CH_2Ph, 2xH-5_{Ido}, CH_2-PhN), 4.29 – 4.22 (m, 2H, 2x H-6_{Azido}), 4.19 – 4.14 (m, 1H, H-6_{Ido}), 4.13 – 4.04 (m, 4H, 3x H-6_{Azido} , H-6_{Ido}), 4.01 – 3.94 (m, 3H, 2x H-6_{Ido}, H-6_{Azido}), 3.94 – 3.73 (m, 16H, , 2x H-6_{Ido}, 3xH-5_{Azido}, 3xH-3_{Ido}, 3xH-3_{Azido}, 2xH-4_{Azido}, 3xH-4_{Ido}), 3.73 – 3.67 (m, 10H, CH_2O, 3x CH_{3PMP}), 3.47 (dd, J =

10.0, 8.7 Hz, 1H, H-4_{Azido}), 3.35 (bs, 1H, CH_2O), 3.32 (dd, J = 9.7, 3.7 Hz, 1H, H-2_{Azido}), 3.26 – 3.12 (m, 4H, 2xH-2_{Azido}, CH_2N), 2.11 (s, 3H, CH_{3Ac}), 2.09 (s, 3H, CH_{3Ac}), 2.07 (s, 3H, CH_{3Ac}), 2.04 (s, 3H, CH_{3Ac}), 1.96 (d, J = 1.9 Hz, 9H, CH_{3Ac}) 1.63 – 1.47 (m, 4H, $CH_{2Linker}$), 1.37 – 1.26 (m, 2H, $CH_{2Linker}$).^{13}C NMR (126 MHz, $CDCl_3$) from HSQC experiment δ = 128.2, 128.0, 127. 8, , 115.22, 115.15, 114.69, 97.96 (C-1_{Ido}, J_{CH} = 170.0 Hz), 97.95 (C-1_{Ido}, J_{CH} = 170.0 Hz), 97.9 (C-1_{Ido}, J_{CH} = 170.0 Hz), 95.8 (3xC-1_{Azido}, J_{CH} = 170.0 Hz), 81.2, 78.9, 78.4, 77.8, 75.3, 75.12, 75.05, 74.9, 74.8, 72.8, 72.1, 71.7, 70.5, 69.8, 69.6, 68.63 (C-2_{Ido}), 68.56 (C-2_{Ido}), 68.1 (C-2_{Ido}), 67.9(CH_2O_{Linker}), 66.77(CH_2Ph_{Carba}), 66.76 (C-6_{Ido}), 66.5 (C-5_{Ido}), 66.0 (CH_2Ph_{Ac}), 65.99(2xC-6_{Ido}), 65.3 (C-5_{Ido}), 63.7 (C-2_{Azido}), 63.2 (C-2_{Azido}), 62.5 (C-6_{Azido}), 62.2(2xC-6_{Azido}), 55.68 (CH_{3PMP}), 50.42 (CH_2PhN), 46.18 (CH_2N_{Linker}), 29.30($CH_{2Linker}$), 23.02($CH_{2Linker}$), 20.84(CH_{3Ac}), 20.80(CH_{3Ac}), 20.70(CH_{3Ac}); HRMS (ESI) *m/z*: $[M+2NH_4]^{2+}$ calcd. for $C_{141}H_{158}N_{10}O_{41}(NH_4)_2$ 1341.5631, found: 1341.5605.

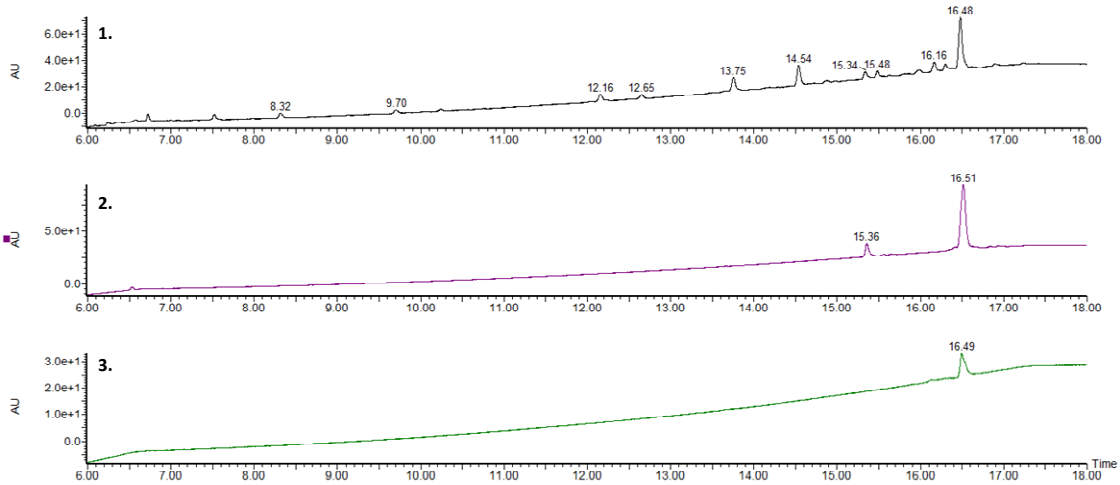
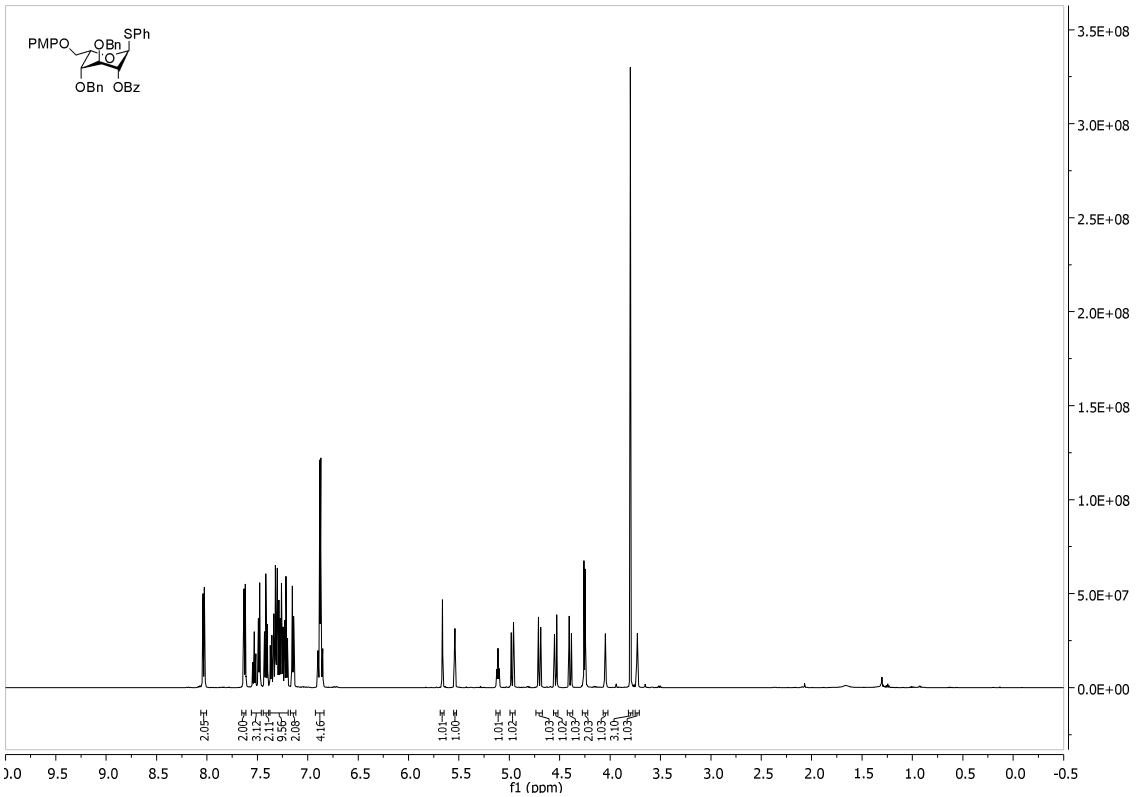


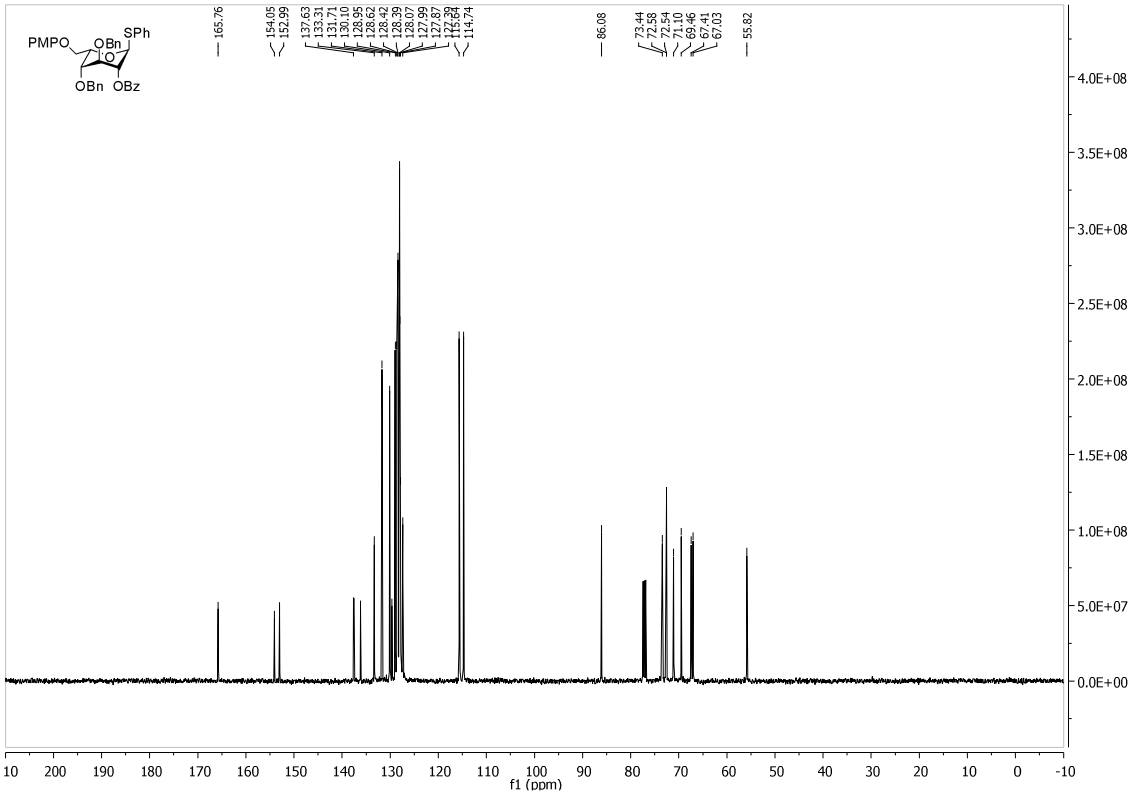
Figure SI-19. LC-MS data for the synthesis of hexasaccharide **10**. 1) Chromatogram of the reaction crude after acetylation; 2) chromatogram of **10** after column chromatography (t_r = 15.36 min, *m/z* calcd for $C_{121}H_{137}N_7O_{37}$ $[M+2NH_4]^{2+}$ 1157.98 found 1157.95, t_r = 16.51 min, *m/z* calcd for $C_{141}H_{158}N_{10}O_{41}$ $[M+2NH_4]^{2+}$ 1341.56 found 1341.55); 3) chromatogram of **10** after preparative HPLC purification (t_r = 16.49 min, *m/z* calcd for $C_{141}H_{158}N_{10}O_{41}$ $[M+2NH_4]^{2+}$ 1341.56 found 1341.55).

1. NMR spectra

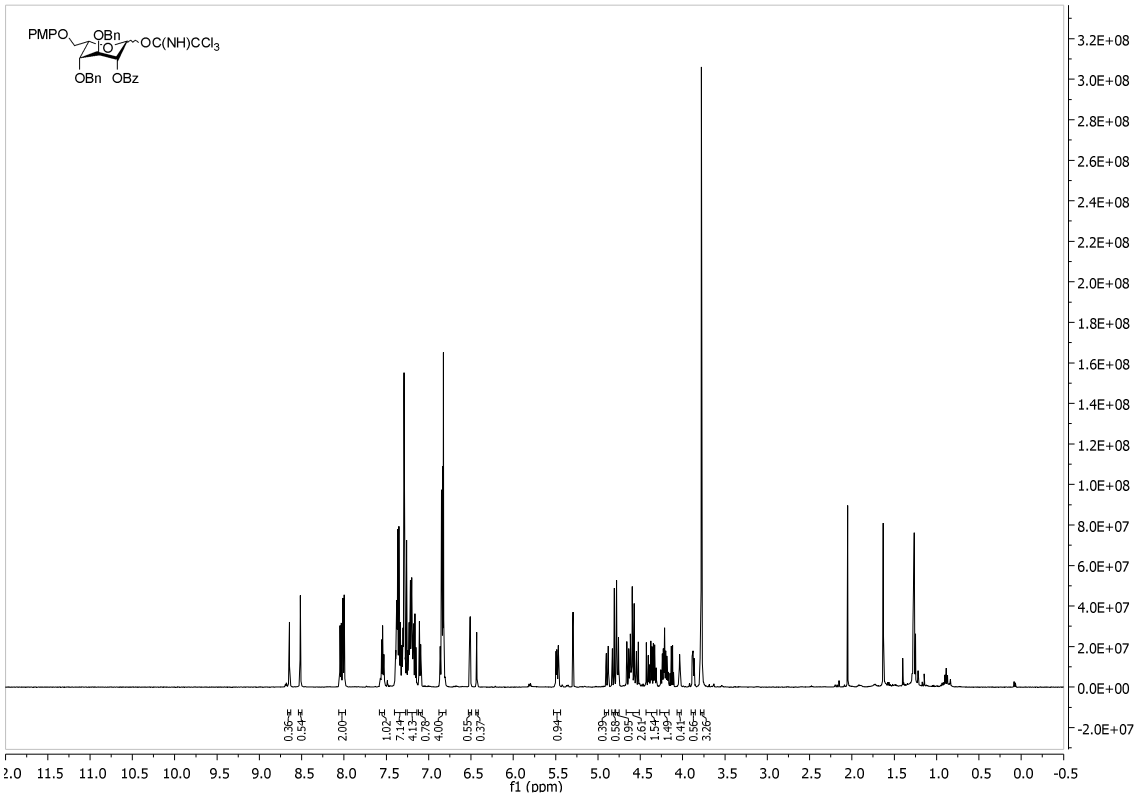
Compound S-2: ^{1}H NMR (500MHz, $CDCl_3$)



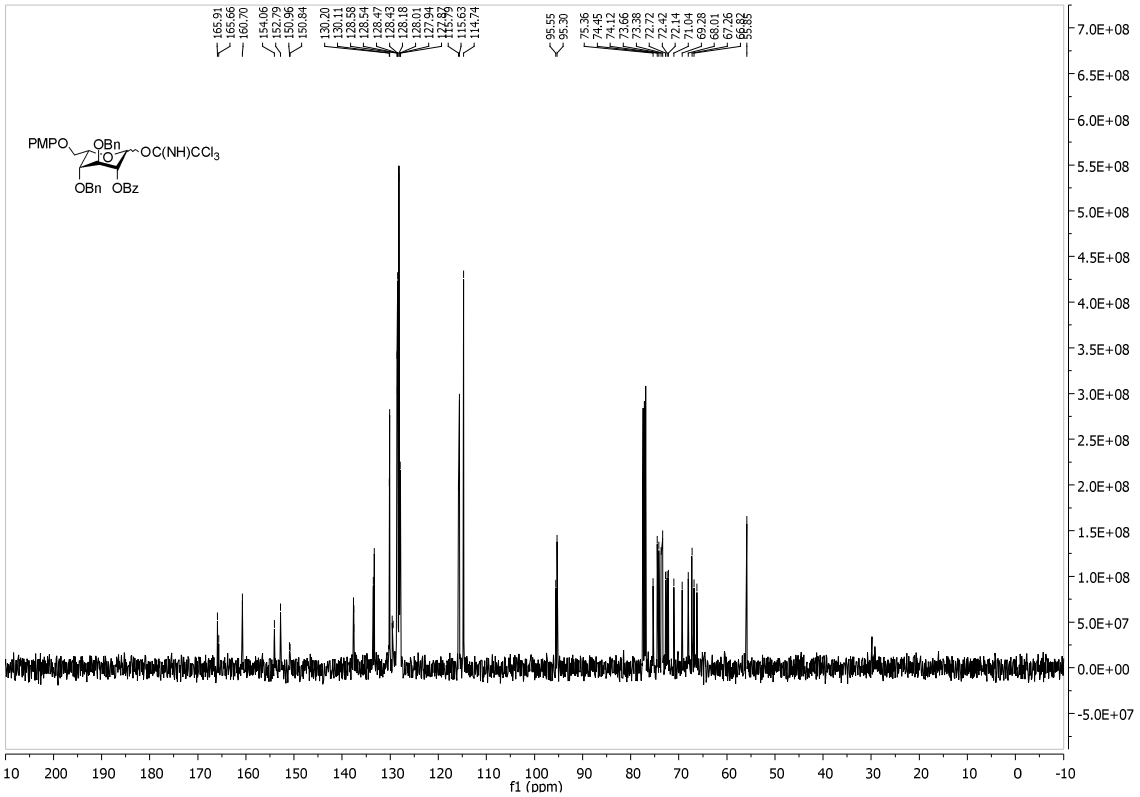
Compound S-2: ^{13}C NMR (125 MHz, $CDCl_3$)



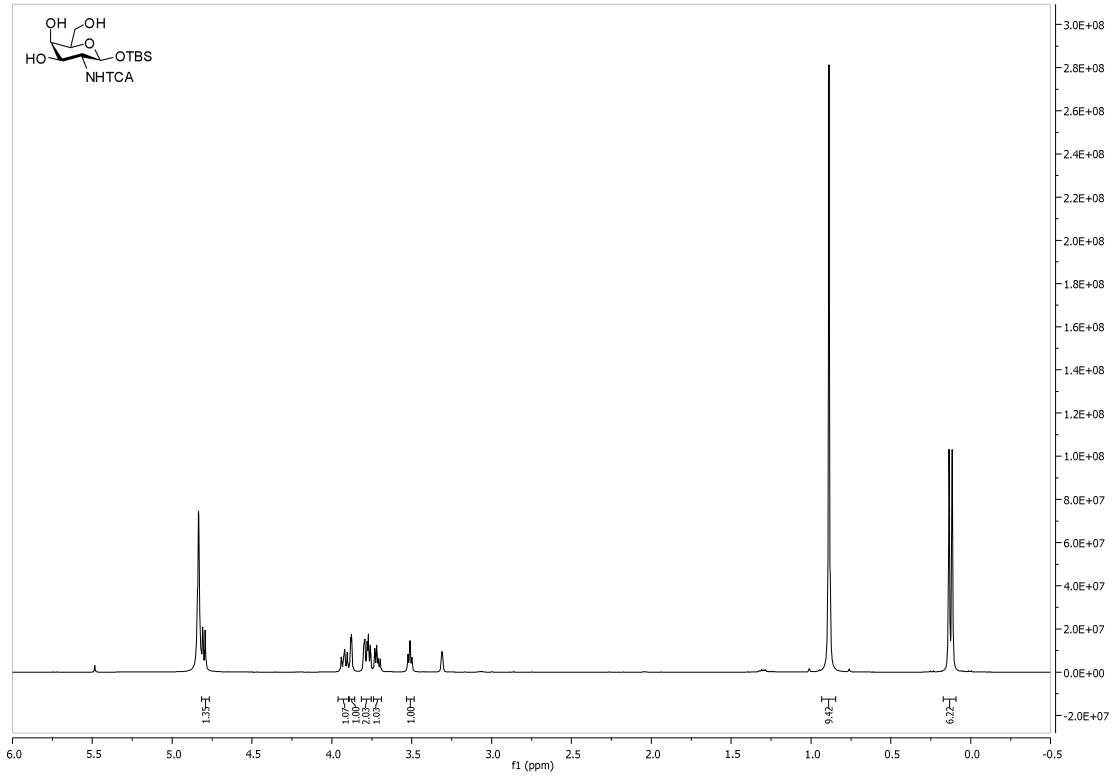
Compound 3: ^{1}H NMR (500MHz, $CDCl_3$)



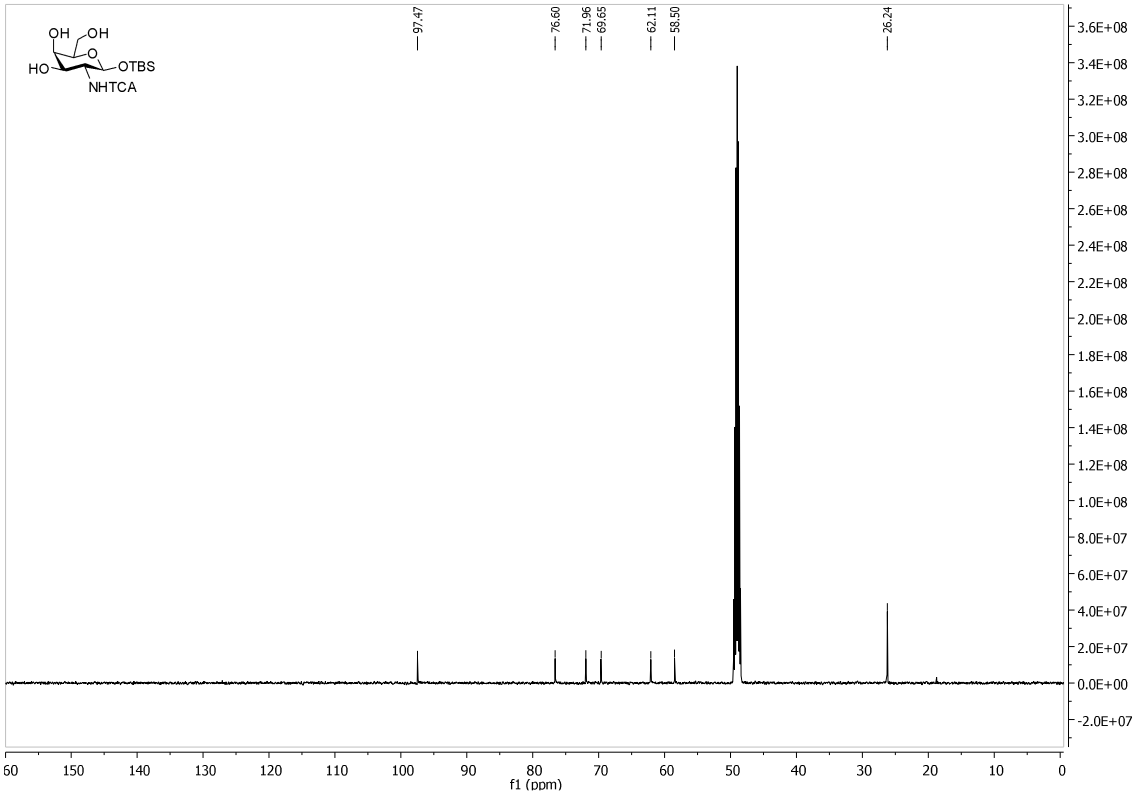
Compound 3: ^{13}C NMR (125 MHz, $CDCl_3$)



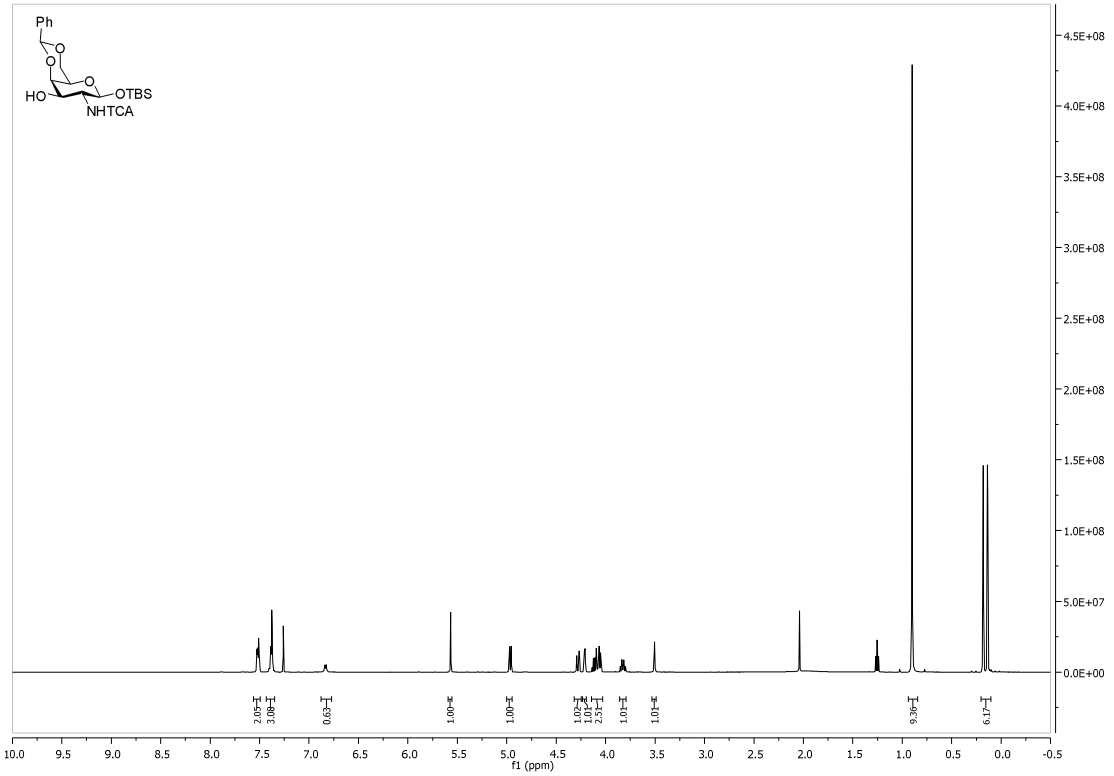
Compound S-4: ^{1}H NMR (500MHz, MeOH)



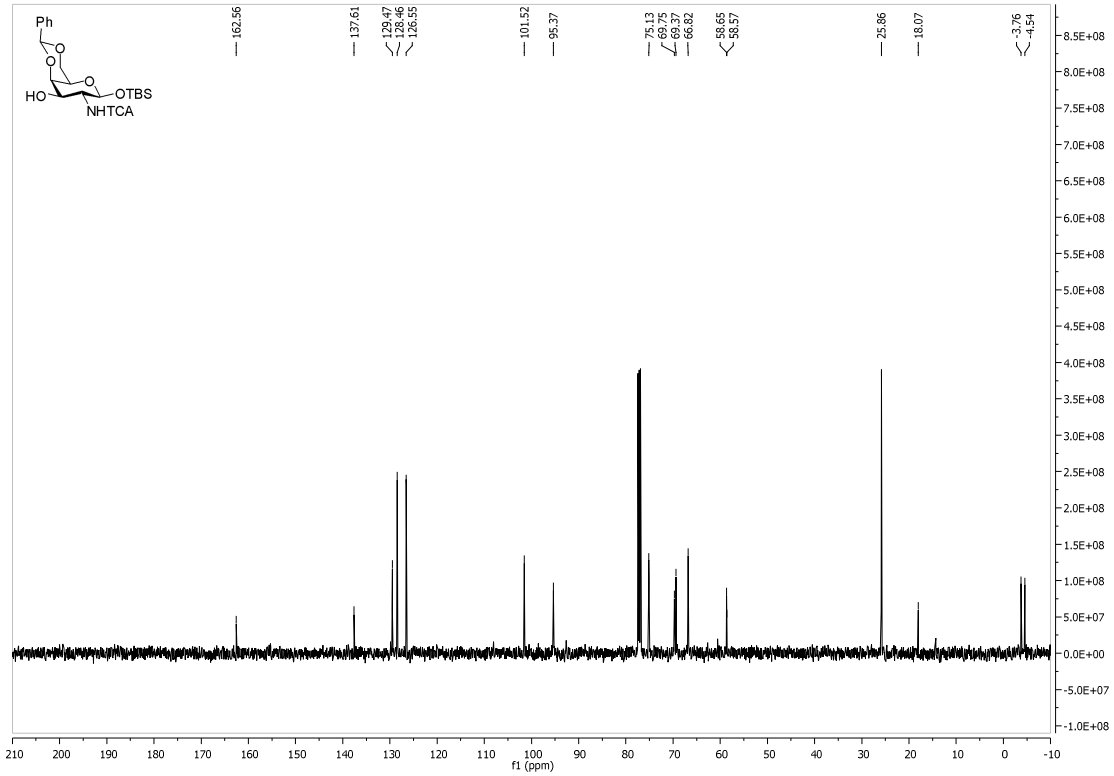
Compound S-4: ^{13}C NMR (125 MHz, $CDCl_3$)



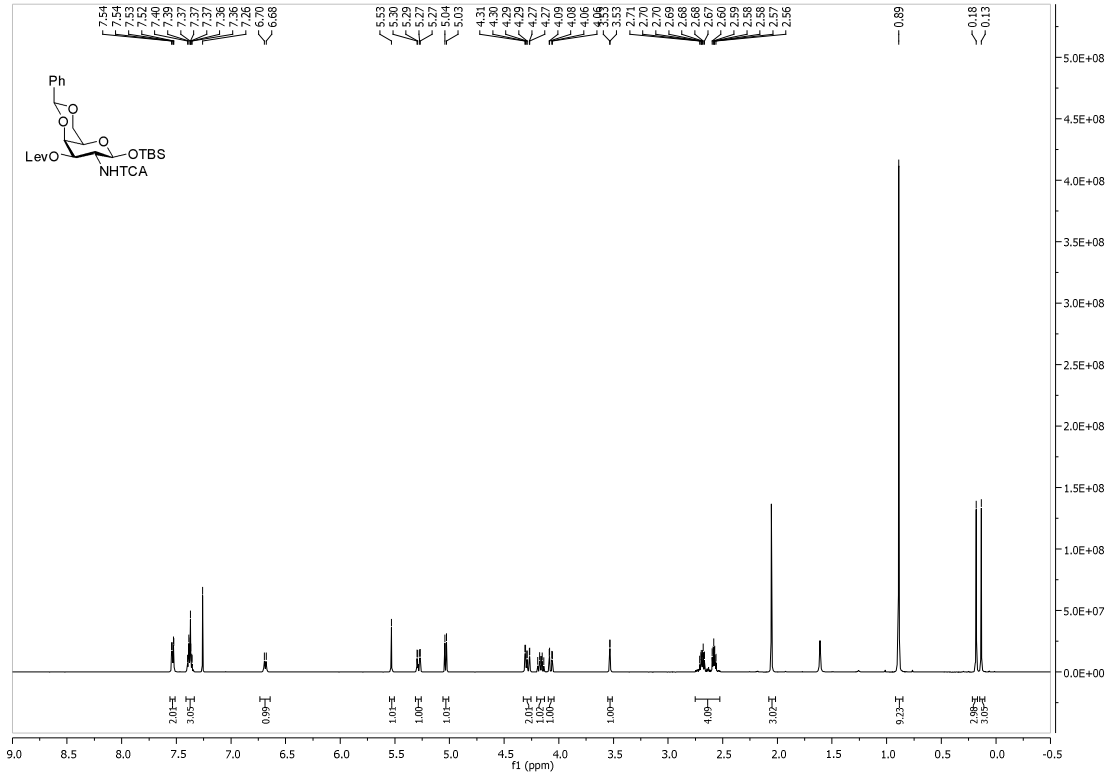
Compound S-5: ^{1}H NMR (500MHz, $CDCl_3$)



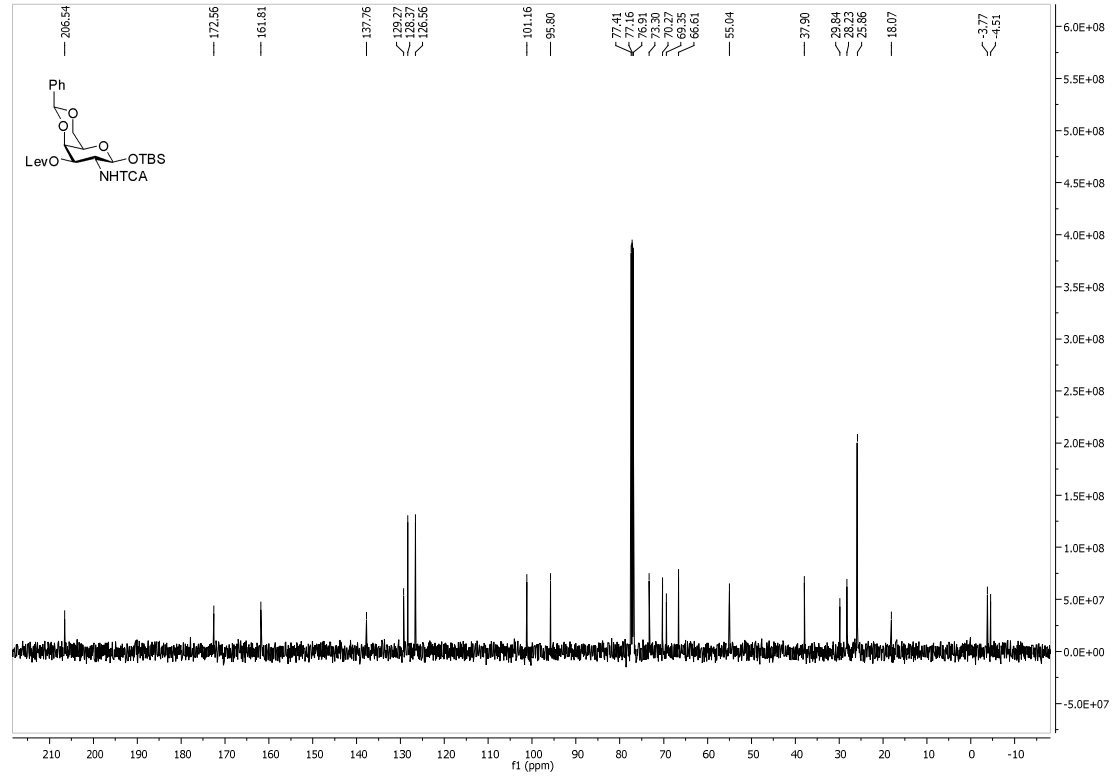
Compound S-5: ^{13}C NMR (125 MHz, $CDCl_3$)



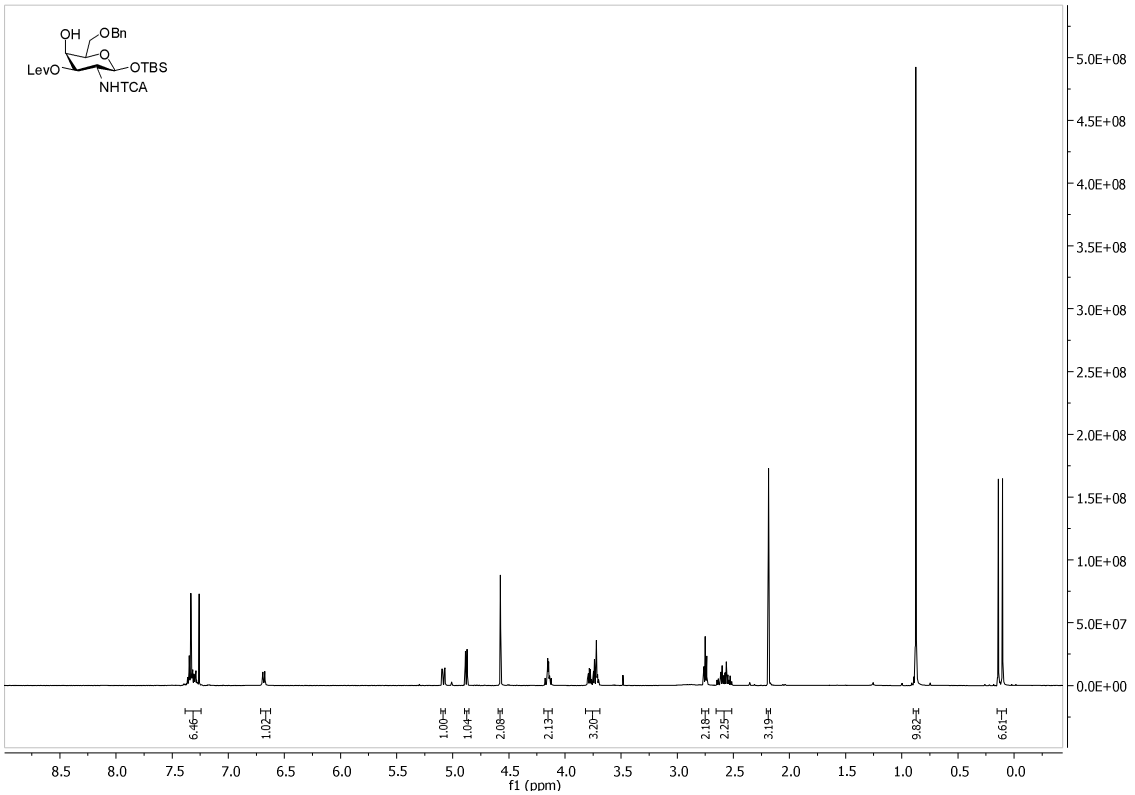
Compound S-6: ^{1}H NMR (500MHz, $CDCl_3$)



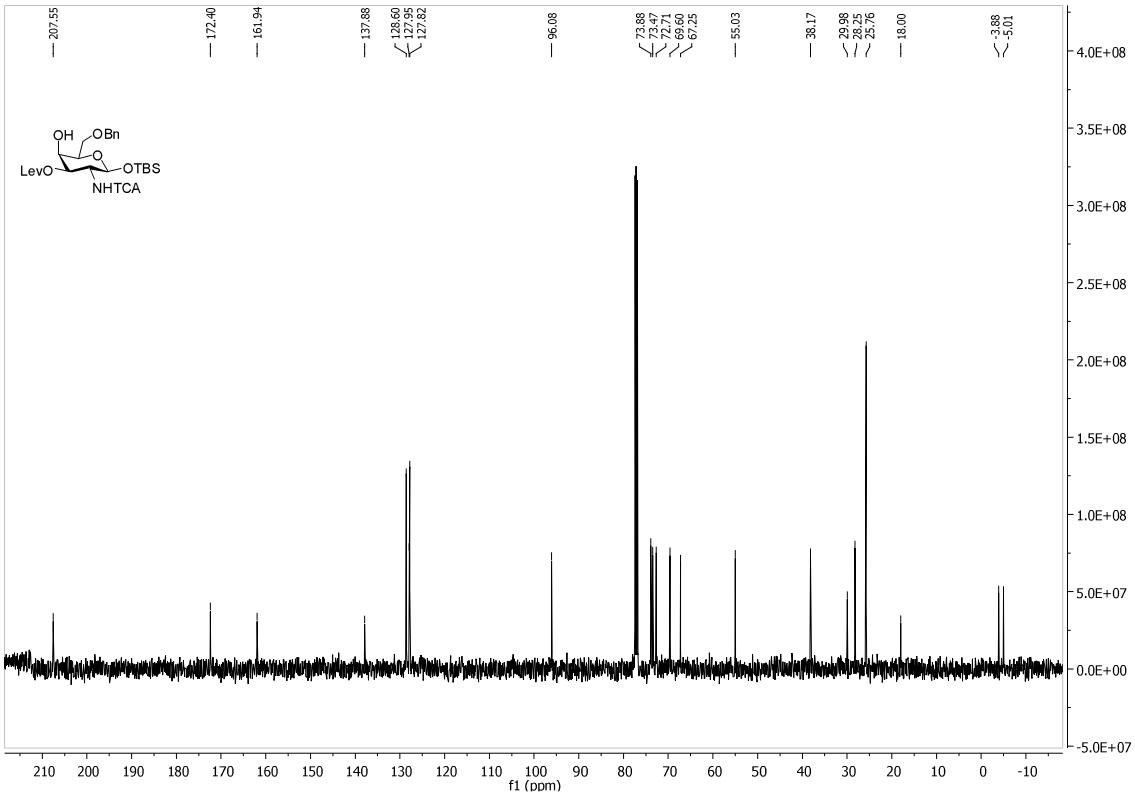
Compound S-6: ^{13}C NMR (125 MHz, $CDCl_3$)



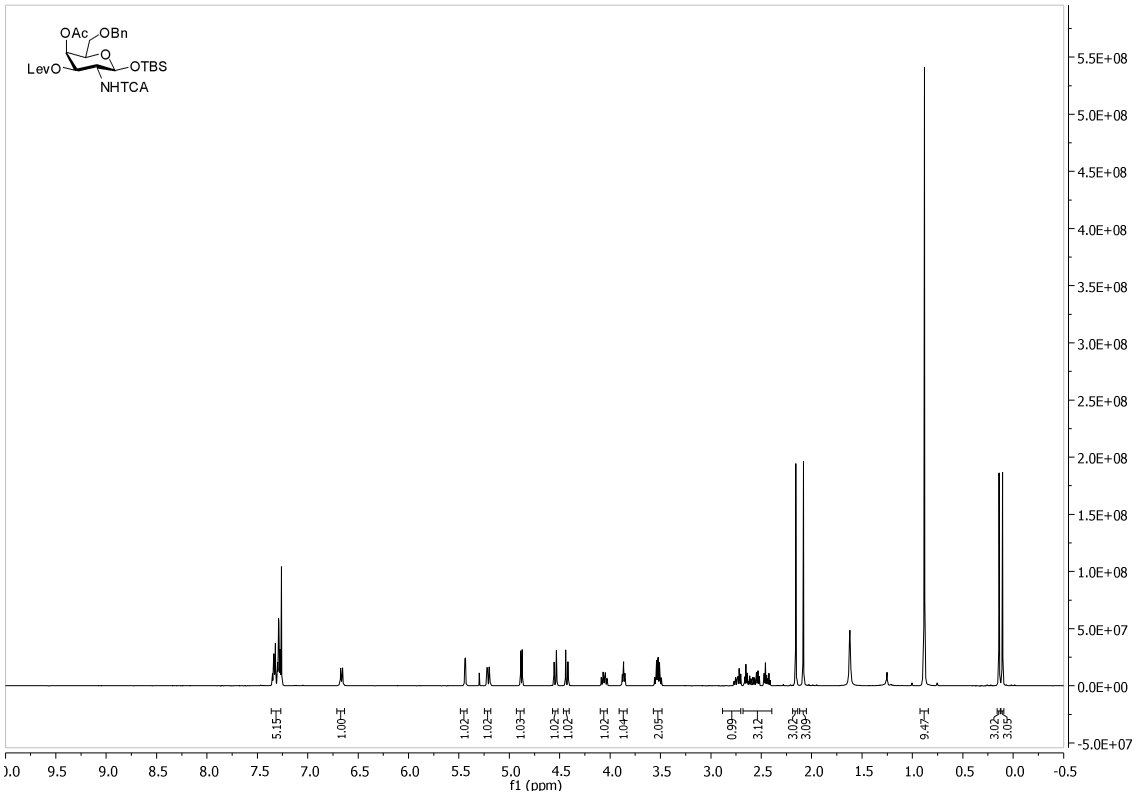
Compound S-7: ^{1}H NMR (500MHz, $CDCl_3$)



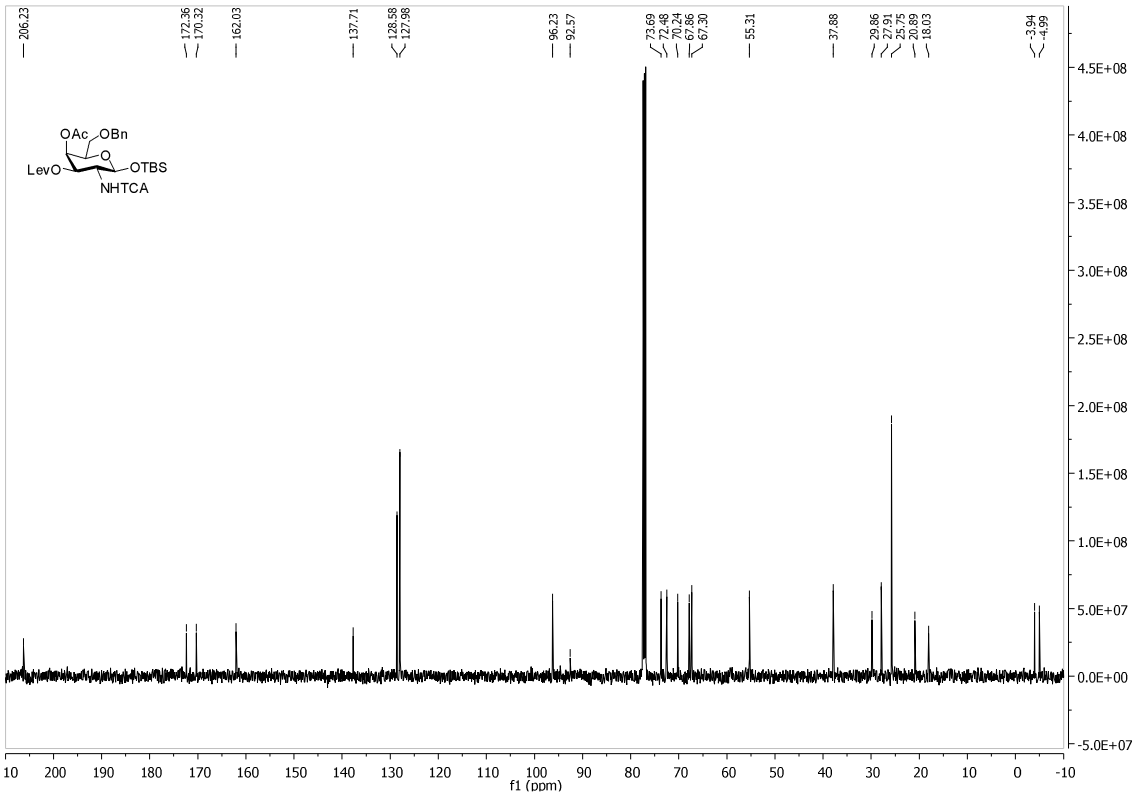
Compound S-7: ^{13}C NMR (125 MHz, $CDCl_3$)



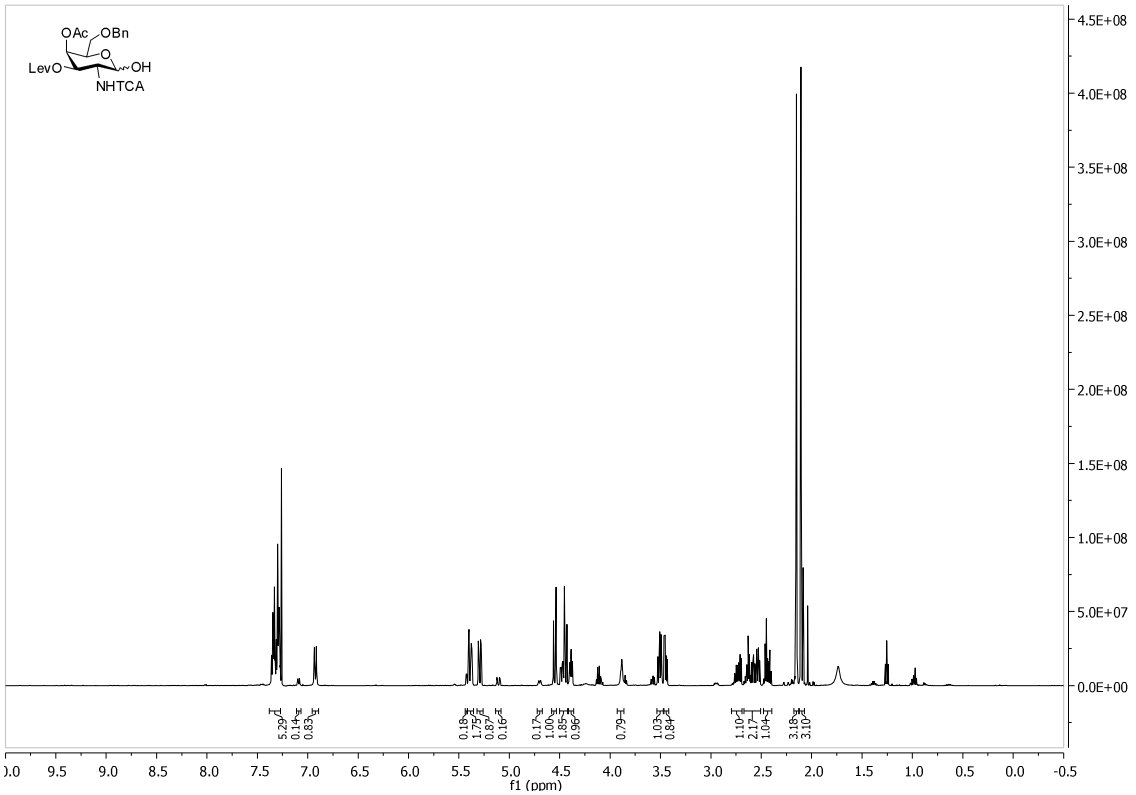
Compound S-8: ^{1}H NMR (500MHz, $CDCl_3$)



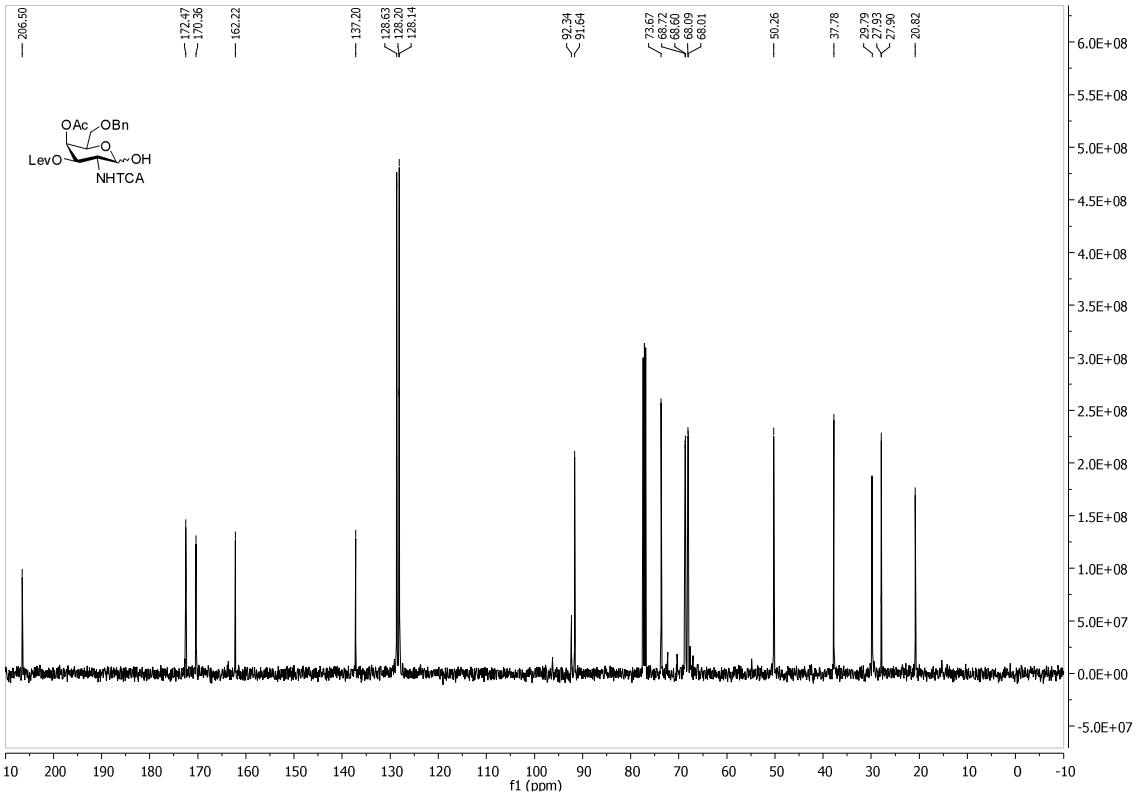
Compound S-8: ^{13}C NMR (125 MHz, $CDCl_3$)



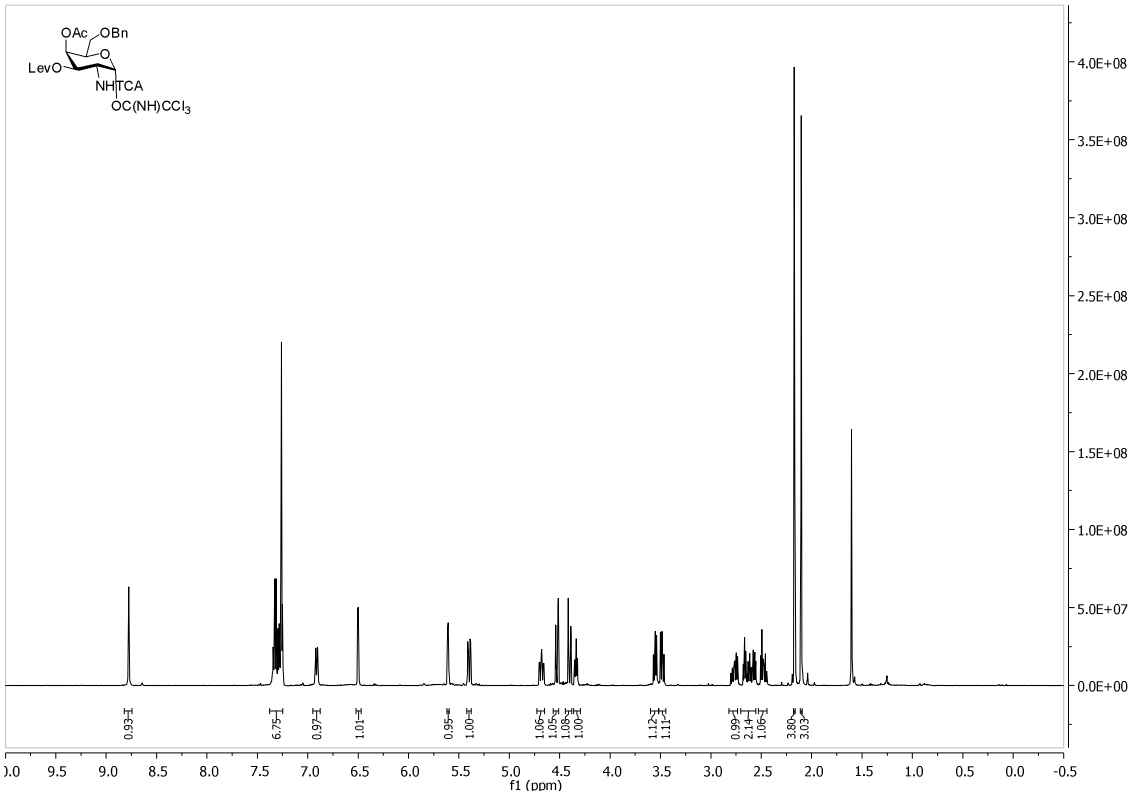
Intermediate compound 1: ^{1}H NMR (500MHz, $CDCl_3$)



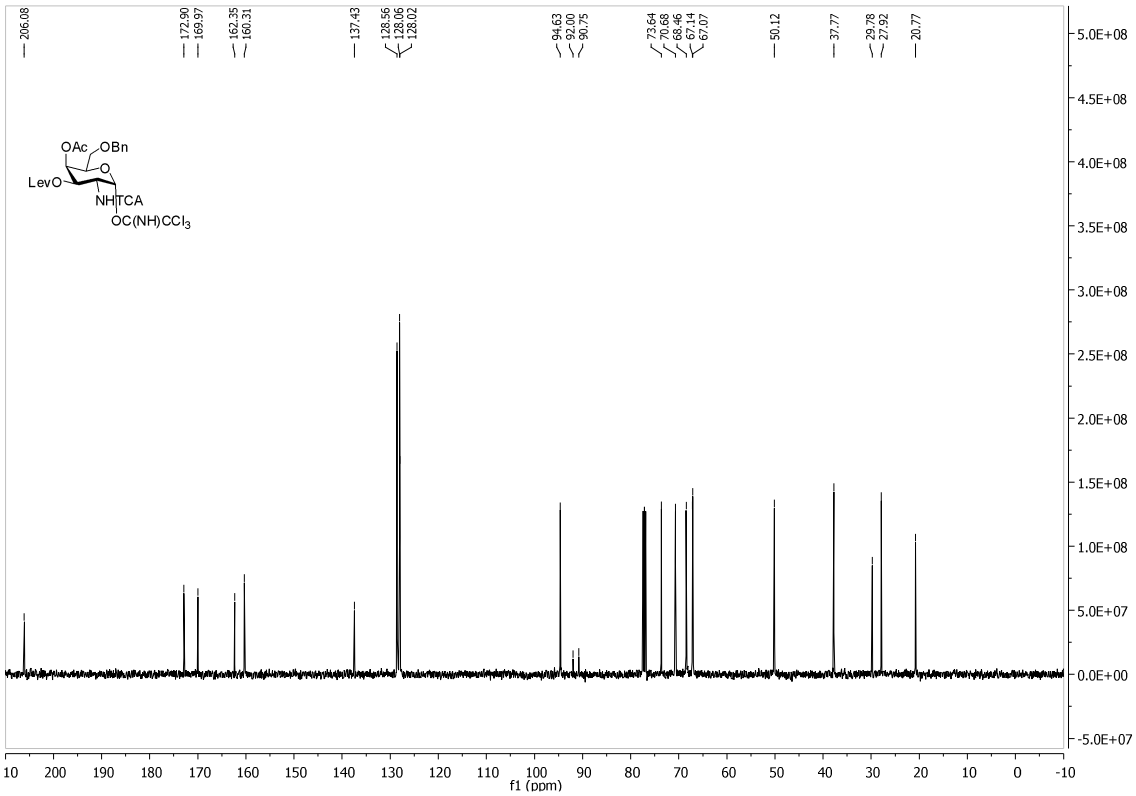
Intermediate compound 1: ^{13}C NMR (125 MHz, $CDCl_3$)



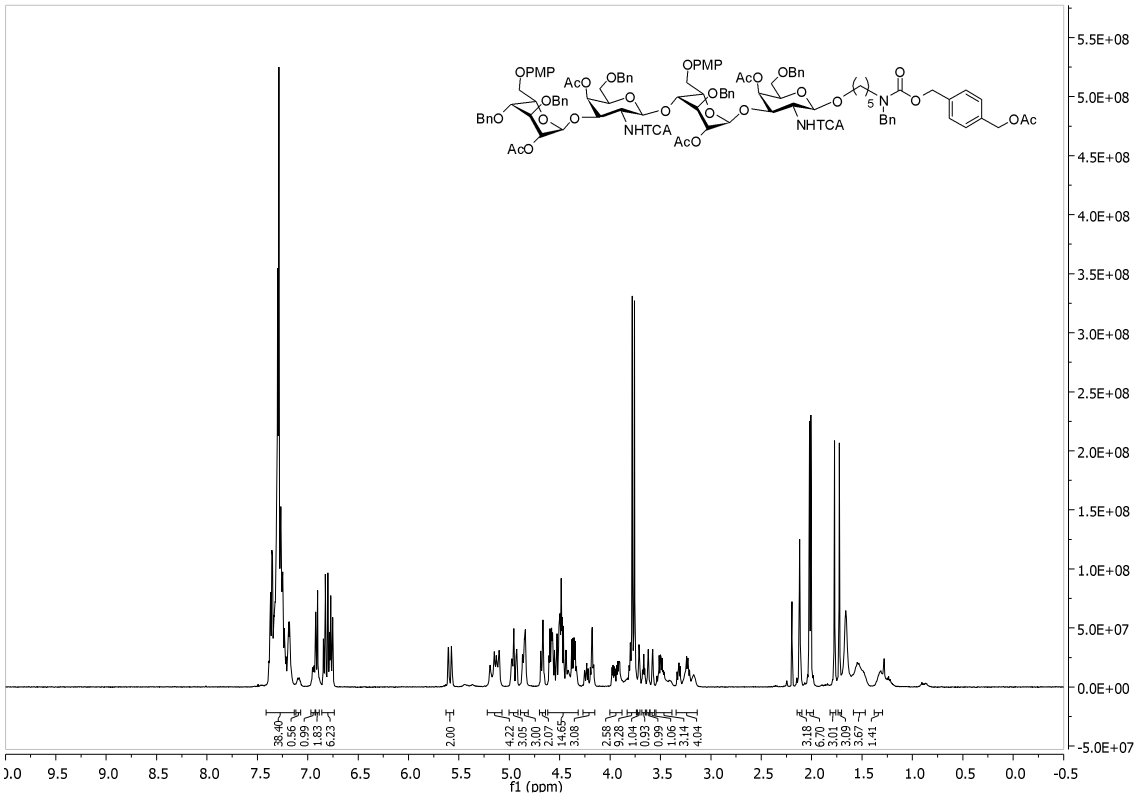
Compound 1: ^{1}H NMR (500MHz, $CDCl_3$)



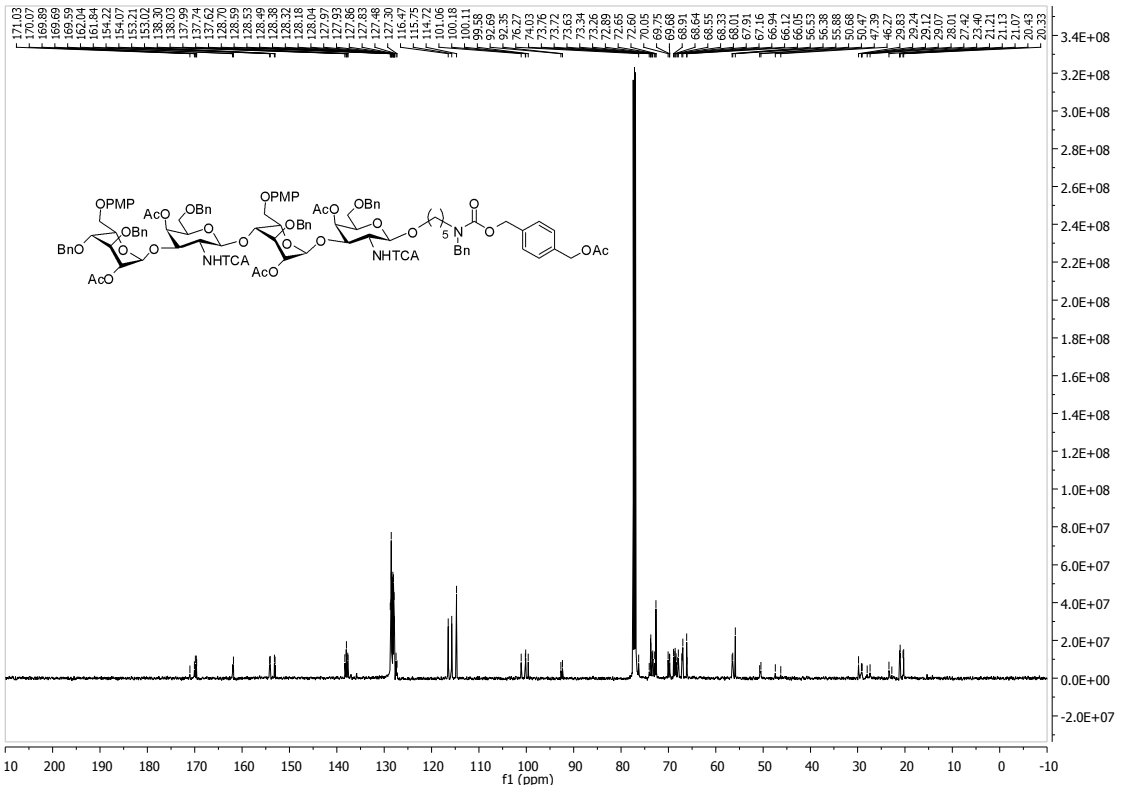
Compound 1: ^{13}C NMR (125 MHz, $CDCl_3$)



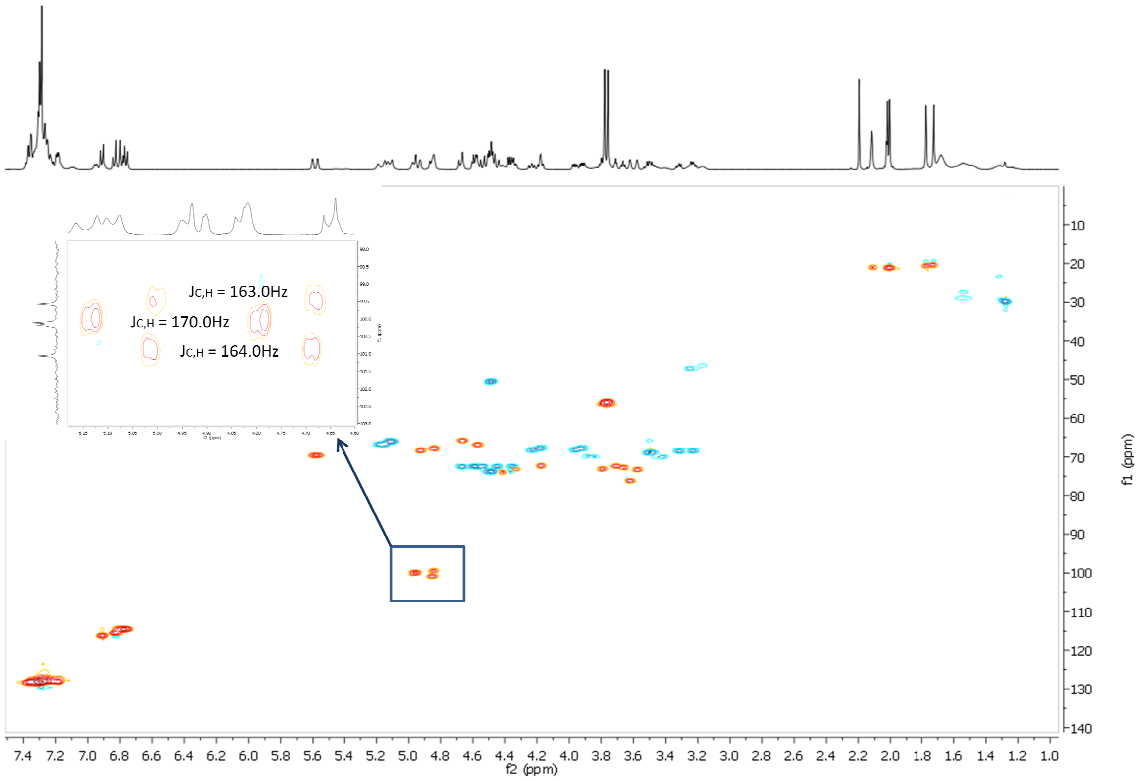
Compound S-14: ^{1}H NMR (500MHz, $CDCl_3$)



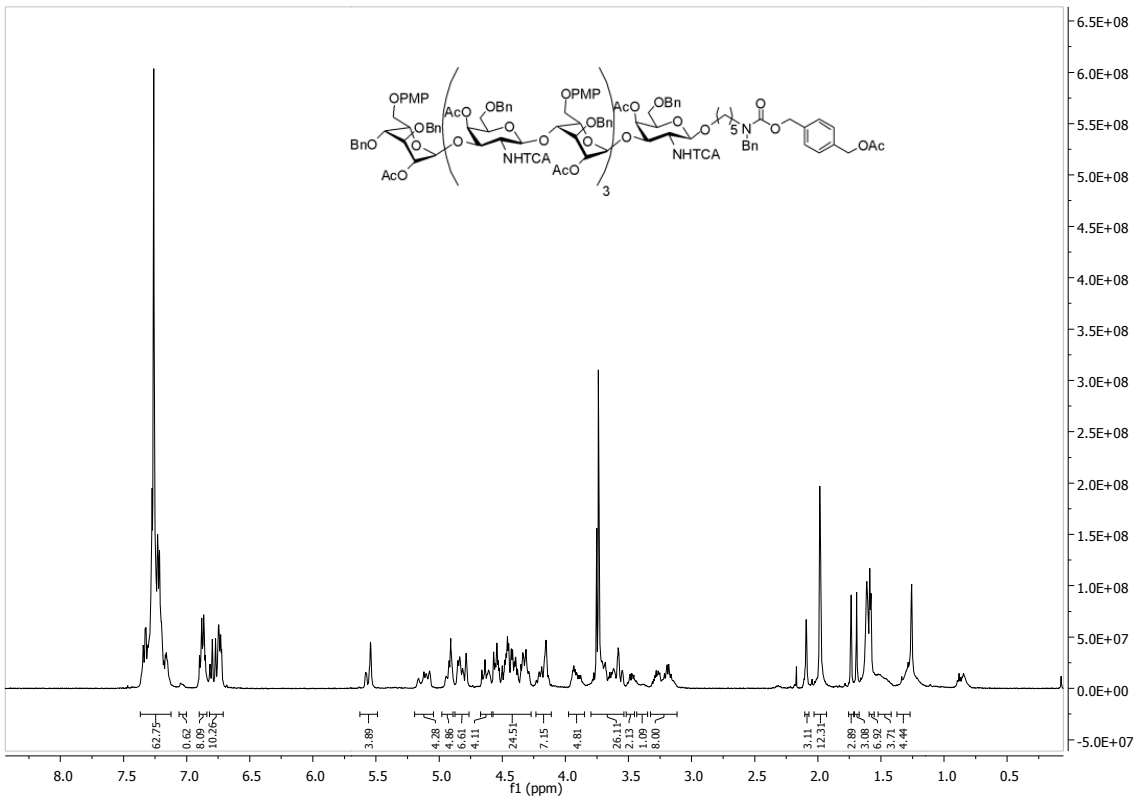
Compound S-14: ^{13}C NMR (125 MHz, $CDCl_3$)



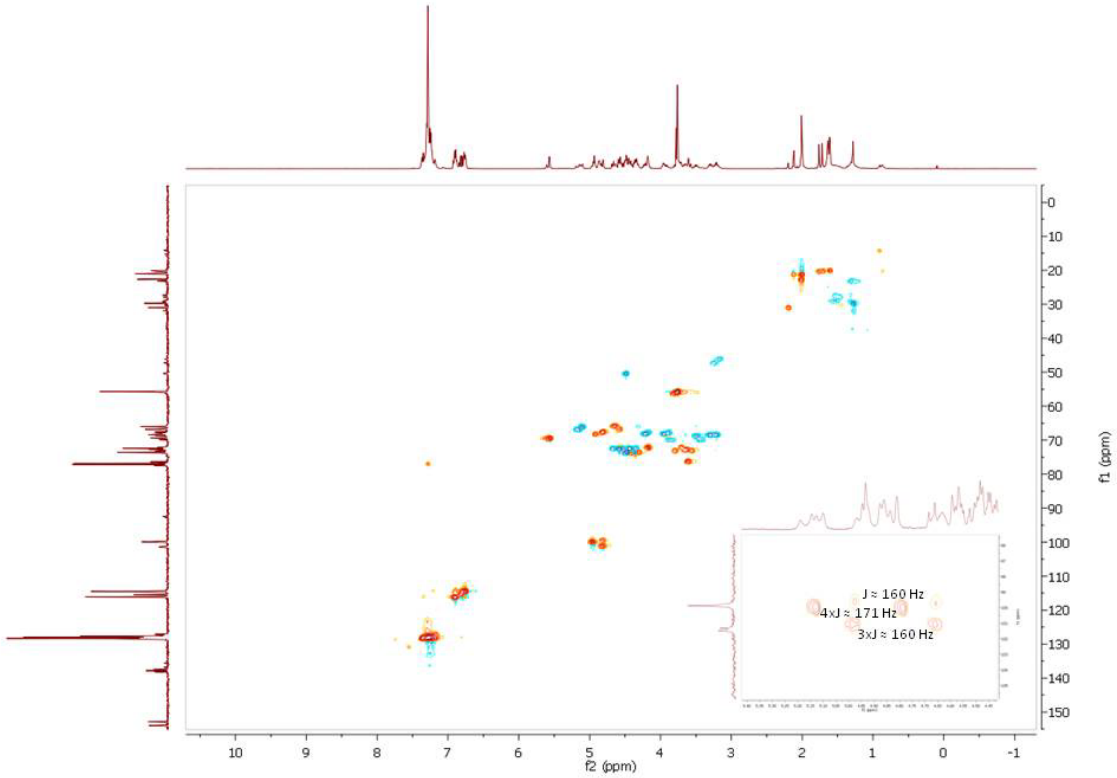
Compound S-14: HSQC edited and HSQC-J coupled NMR (500MHz, $CDCl_3$)



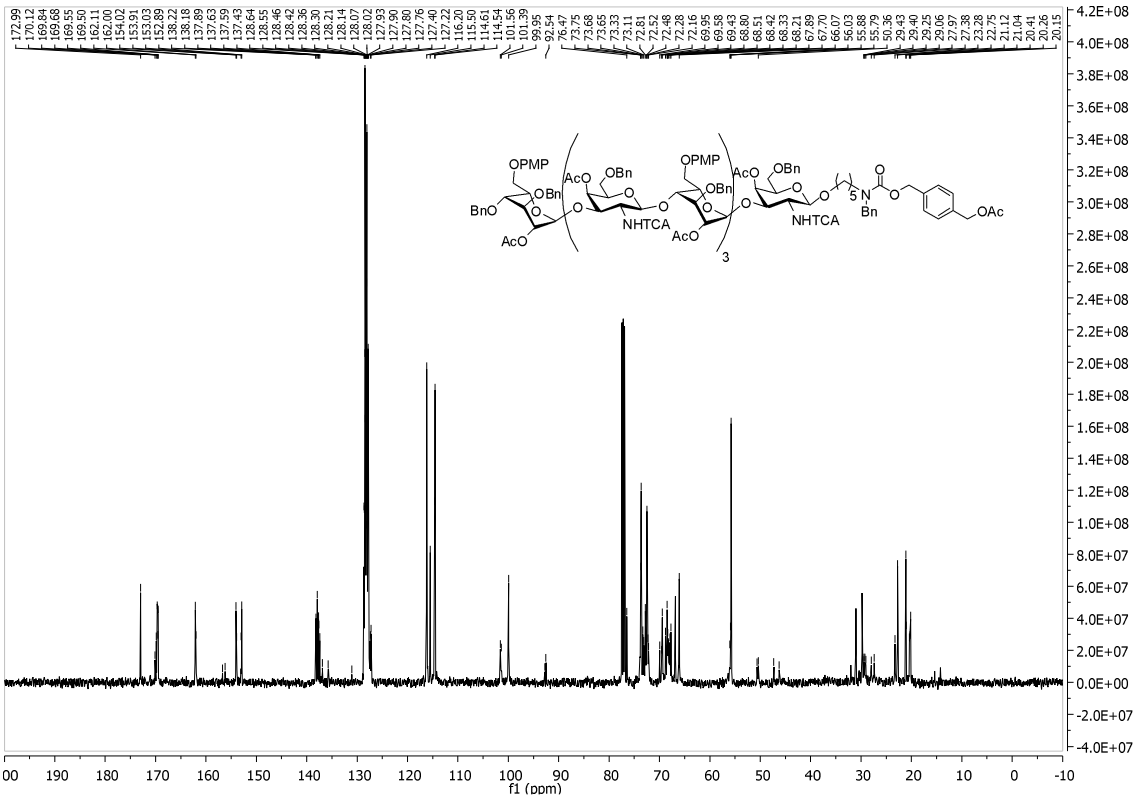
Compound 5: ^{1}H NMR (500MHz, $CDCl_3$)



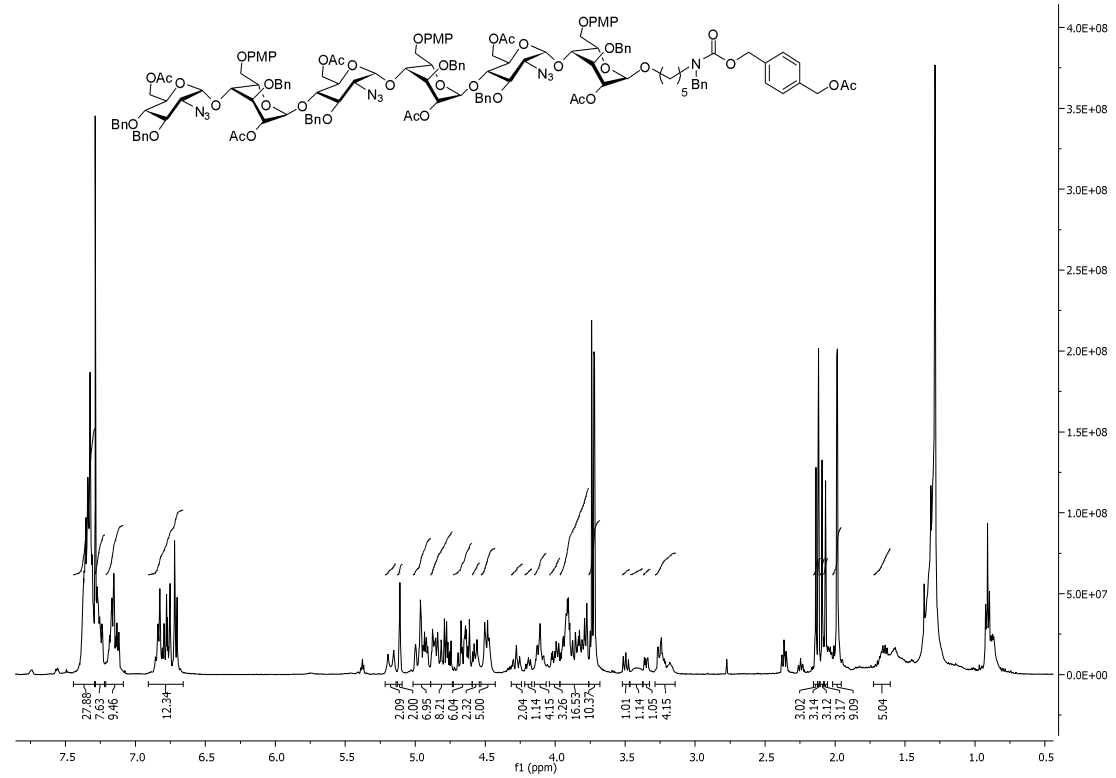
Compound 5: HSQC edited and HSQC-J coupled NMR (500MHz, $CDCl_3$)



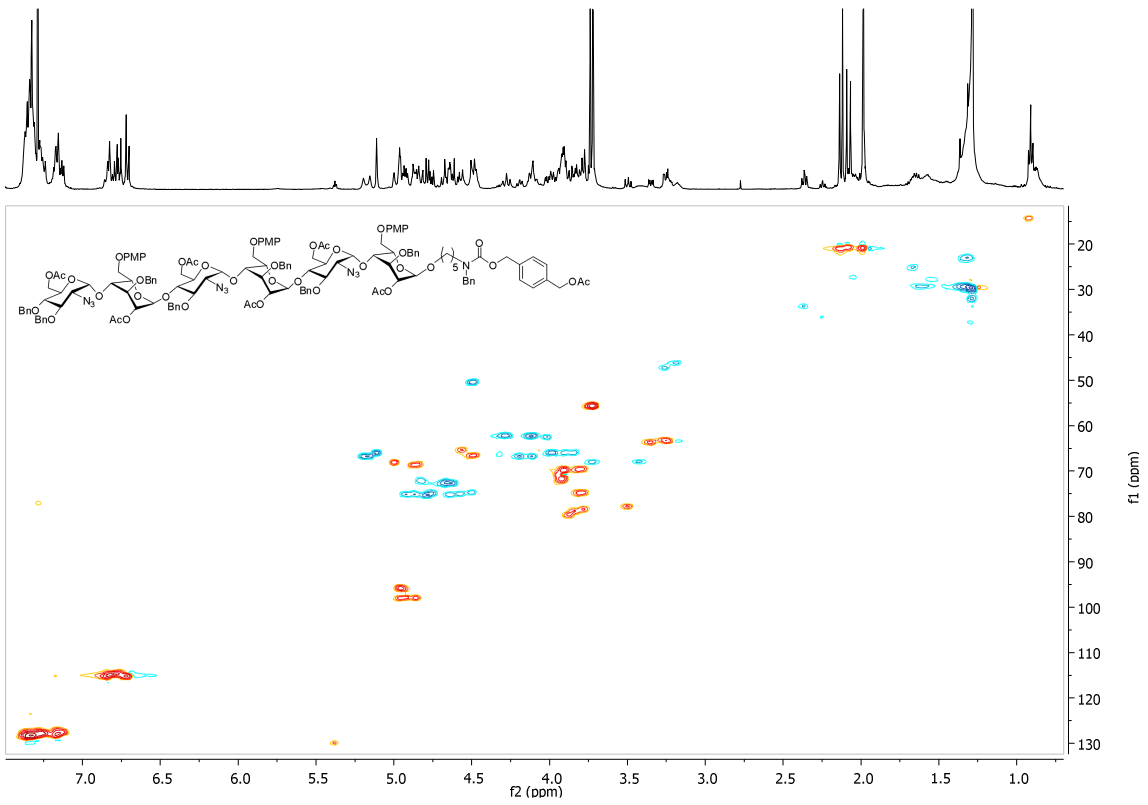
Compound 5: ^{13}C NMR (125MHz, $CDCl_3$)



Compound 10: ^{1}H NMR (500MHz, $CDCl_3$)



Compound 10: HSQC edited NMR (500MHz, $CDCl_3$)



Compound 10: HSQC edited and HSQC-J coupled NMR (500MHz, $CDCl_3$)