Supplementary Information for

Identification of Carbohydrate Anomers Using Ion Mobility-Mass Spectrometry

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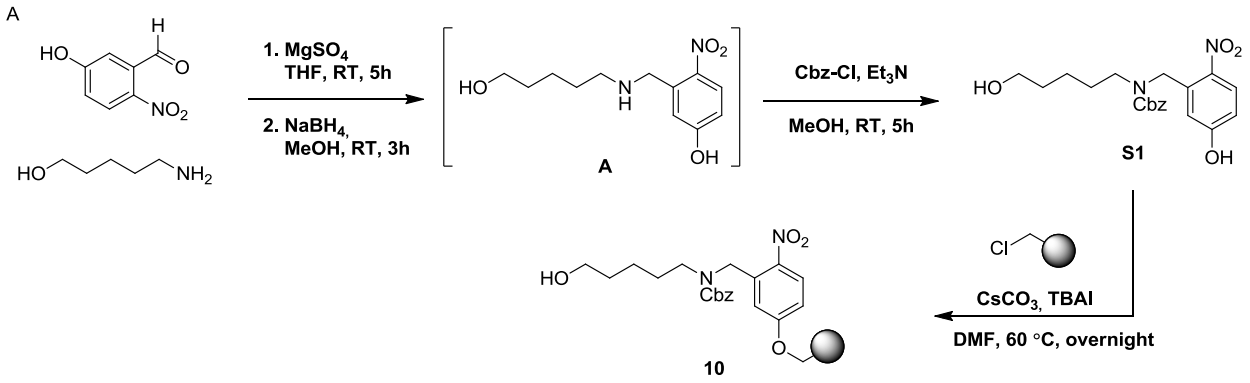
1 General Information

All chemicals used were reagent grade and used as supplied, exceptions are noted. All reactions were performed in oven-dried glassware under an argon atmosphere, unless noted otherwise. Prior to use, molecular sieves were activated by heating under high vacuum. *N*,*N*-dimethylformamide, (DMF) dichloromethane (DCM), toluene and tetrahydrofuran (THF) were purified in a Cycle-Tainer Solvent Delivery System. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV-irradiation, or by dipping the plate either in a cerium sulfate ammonium molybdate (CAM) solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Fluka silica gel 60 (230-400 mesh). All automated glycosylations were performed on a automated oligosaccharide synthesizer demonstrator unit using anhydrous solvents of the Cycle-Tainer Solvent Delivery System. LCMS chromatograms were recorded on an Agilent 1100 Series spectrometer. Preparative HPLC purifications were performed on an Agilent 1200 Series. Loading determination of functionalized resins was obtained using a Shimadzu UV-MINI-1240 UV spectrometer. ^{1}H, ^{13}C spectra were recorded on a Varian Mercury 400 (400 MHz), 600 (600 MHz) or a Bruker AVIII 700 (700 MHz) spectrometer in $CDCl_3$ or CD_3OD with chemical shifts (δ) referenced to internal standards ($CDCl_3$: 7.26 ppm ^{1}H, 77.16 ppm ^{13}C; CD_3OD: 4.87 or 3.31 ppm ^{1}H, 49.0 ppm ^{13}C; D_2O: 4.79 ppm ^{1}H) unless stated otherwise. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; brs, broad singlet for ^{1}H-NMR data. NMR chemical shifts (δ) are reported in ppm and coupling constants (*J*) are reported in Hz. High resolution mass spectrometry (HRMS) analyses were performed by the MS-service in the Department of Organic Chemistry at Free University Berlin using an Agilent 6210 ESI-TOF (Agilent Technologies, Santa Clara, CA, USA). IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured with a UniPol L 1000 polarimeter (Schmidt & Haensch, Berlin, Germany), with concentrations expressed in g per 100 mL.

2 Pre-Automation Steps

2.1 Polystyrene resin equipped with a photolabile linker 10

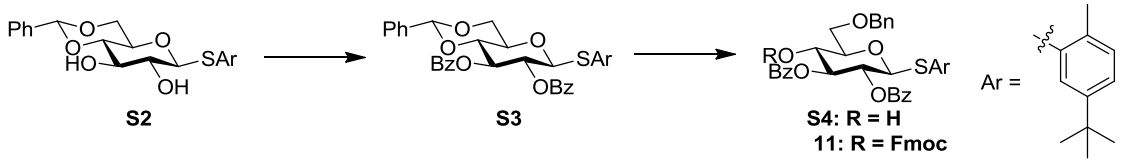


5-Aminopentanol (10 g, 97.0 mmol, 1.0 eq.), 5-hydroxy-2-nitrobenzaldehyde (15.4 g, 92 mmol, 0.95 eq.) and $MgSO_4$ (19.0 g, 160 mmol, 2.1 eq.) were stirred in anhydrous THF (242 mL) at room temperature. After 5 h, the suspension was filtered and concentrated *in vacuo*. The crude was dissolved in MeOH (280 mL) and cooled to 0°C. $NaBH_4$ (3.67 g, 97 mmol, 1.0 eq.) was added portion-wise to the mixture and allowed to warm to room temperature. After 3 h, the mixture was quenched by addition of acetone (15 mL) and the

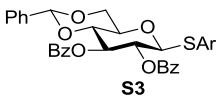
solvents were evaporated to yield crude compound **A**. To a solution of the crude in MeOH (280 mL) was added triethylamine (41 mL, 291 mmol, 3.0 eq.) and benzyl chloroformate (Cbz-Cl, 34.6 mL, 243 mmol, 2.5 eq.) at room temperature. After 5 h, K_2CO_3 (40.0 g) was added to the reaction mixture and stirred for an hour. The reaction mixture was then filtered through celite and the solvents evaporated *in vacuo*. The crude was extracted with DCM. The organic layer was dried over $MgSO_4$, filtered, concentrated and subjected to flash chromatography (Silica/EtOAc:hexane) to obtain photo-cleavable linker **S1** in 73% yield (17 g, 71.1 mmol).

To a solution of the photolabile linker **S1** (6.21 g, 16.0 mmol, 4.0 eq.) in DCM was added Merrifield resin (8.0 g, 4.0 mmol, loading 0.50 mmol/g). This suspension was carefully evaporated *in vacuo*, and redissolved in the minimum amount of DMF (4 mL DMF / 1 g resin). The suspension was then degassed by placing the flask under high vacuum for a couple of minutes, followed by refilling the evacuated flask with argon. After repeating this degassing procedure twice more, Cs_2CO_3 (5.21 g, 16 mmol, 4.0 eq.) and tetrabutylammonium iodide (TBAI) (1.48 g, 4.0 mmol, 1.0 eq.) were added to the flask and the entire suspension rotated on a rotovap at 60°C and atmospheric pressure overnight. The next morning, water was added to the resin to dissolve all solids and the resin was subsequently washed with THF/water (1/1), THF, DMF, MeOH, DCM, MeOH, and finally DCM (six times each) to remove the yellow color. The resin was transferred again to a round bottom flask, swollen in a minimal amount of DMF (~4 mL DMF/g resin) and the flask degassed as above. Afterwards, CsOAc (1.54 g, 8.0 mmol) was added and the entire suspension rotated on a rotovap at 60 °C and atmospheric pressure overnight. The next morning, the resin was washed with THF/water (1/1), THF, DMF, MeOH, DCM, MeOH, and finally DCM (six times each) to remove the yellow color. The resin was then dried under high vacuum overnight and stored in the dark to obtain the photolabile linker bound resin **7 (Scheme S1)**. Loading (0.392 mmol/g) was determined as reported previously[31].

2.2 Building Block Preparation



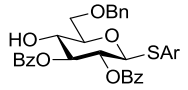
(2-Methyl-5-tert-butylphenyl)2,3-di-O-benzoyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside: S3



To a solution of compound **S2**[27] (10 g, 23 mmol, 1 eq.) in anhydrous DCM (116 mL, 0.2 M) were added benzoic anhydride (15.8 g, 70 mmol, 3.0 eq.), triethylamine (Et_3N) (13 mL, 93 mmol, 4.0 eq.), and a catalytic amount of DMAP (0.57 g, 4.65 mmol, 0.2 eq.) at 0°C, and then stirred for 2 h at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **S3** (13.9 g, 21.7 mmol, 93%). R_f : 0.25

(Hexane/EtOAc/DCM : 1/9/0.5). $[\alpha]_D^{25}$ = 78.73 (C= 3.33, $CHCl_3$). IR (thin film): υ = 2962, 1727, 1270, 1095 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.97 (m, 4H), 7.58 (d, J = 2.1 Hz, 1H), 7.56 – 7.45 (m, 2H), 7.45 – 7.28 (m, 9H), 7.24 (dd, *J* = 8.0, 2.1 Hz, 2H), 7.11 (d, *J* = 8.0 Hz, 2H), 5.81 (t, *J* = 9.4 Hz, 1H, H-2), 5.64 – 5.46 (m, 2H, H-3, C*H*Ph), 4.99 (d, *J* = 10.1 Hz, 1H, H-1), 4.44 (dd, *J* = 10.5, 4.9 Hz, 1H, H-6), 3.96 (t, *J* = 9.5 Hz, 1H, H-4), 3.93 (t, *J* = 10.3 Hz, 1H, H-6), 3.74 (td, *J* = 9.8, 5.0 Hz, 1H, H-5), 2.22 (s, 3H, Me), 1.29 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 165.54 (OBz), 165.20 (OBz), 149.64, 137.10, 136.66, 133.28, 133.08, 131.79, 130.10, 130.03, 129.83, 129.78, 129.35, 129.16, 129.02, 128.36, 128.27, 128.17, 126.11, 126.06, 125.50 (Ar), 101.46 (*C*Ph), 87.96 (C-1), 78.64 (C-4), 73.30 (C-2), 71.13 (C-3), 70.81 (C-5), 68.58 (C-6), 34.43 (Cq, *t*-Bu), 31.24 (Me, *t*-Bu), 20.24 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{38}O_7S$ 661,2230, found 661.2255.

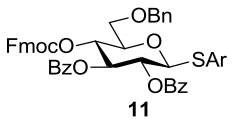
(2-Methyl-5-tert-butylphenyl) 2,3-di-O-benzoyl-6-O-benzyl-1-thio-β-D-glucopyranoside: S4



S4

Compound **S3** (4.7 g, 7.36 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (41 mL, 0.18 M). To a solution of compound **S3** (4.7 g, 7.36 mmol, 1.0 eq.) was added triethylsilane (7.05 mL, 44.1 mmol, 6.0 eq.) and trifluoroacetic anhydride (0.520 mL, 3.68 mmol) at 0 °C. After 30 min, trifluoroacetic acid (2.83 mL, 36.8 mmol, 5.0 eq.) was added dropwise, and the mixture was stirred and allowed to warm to room temperature. Afterwards, the mixture was quenched with saturated aqueous $NaHCO_3$ and diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **S4** (4.44 g, 6.94 mmol, 94%). R_f : 0.21 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = 93.96 (C= 3.50, $CHCl_3$). IR (thin film): υ = 3458, 2961, 1729, 1274 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* 8.07 – 7.82 (m, 4H), 7.59 (d, *J* = 2.0 Hz, 1H), 7.51 (ddd, *J* = 8.7, 2.5, 1.3 Hz, 2H), 7.40 – 7.27 (m, 8H), 7.22 (dd, *J* = 8.0, 2.1 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 5.63 – 5.21 (m, 2H, H-2, H-3), 4.88 (d, *J* = 9.7 Hz, 1H, H-1), 4.62 (q, *J* = 12.0 Hz, 2H, CH_2Ph), 4.00 (td, *J* = 9.3, 3.1 Hz, 1H, H-4), 3.86 (d, *J* = 4.6 Hz, 2H, H-6), 3.71 (dt, *J* = 9.3, 4.5 Hz, 1H, H-5), 3.23 (br, *J* = 3.4 Hz, 1H, OH), 2.23 (s, 3H, Me), 1.26 (s, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 167.29 (OBz), 165.41 (OBz), 149.79, 137.71, 137.22, 133.56, 133.38, 132.33, 130.20, 130.10, 130.06, 129.95, 129.48, 129.17, 128.62, 128.54, 128.50, 128.01, 127.99, 125.42 (Ar), 87.42 (C-1), 78.74 (C-5), 77.98 (C-3), 73.94 (*C*H_2Ph), 71.07 (C-4), 70.40 (C-2), 70.18 (C-6), 34.56 (Cq, *t*-Bu), 31.39 (Me, *t*-Bu), 20.45 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{40}O_7S$ 663,2387, found 663.2392.

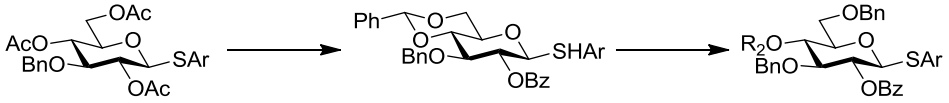
2-Methyl-5-tert-butylphenyl) 2,3-di-O-benzoyl-6-O-benzyl-4-O-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside: 11



11

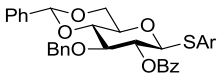
To a solution of compound **S4** (4.45 g, 6.94 mmol, 1.0 eq.) was added 9-fluorenylmethyl chloroformate (3.59 g, 13.88 mmol, 2.0 eq.) and pyridine (1.68 mL, 20.82 mmol, 3.0 eq.)

successively at 0°C, and stirred overnight at room temperature. After the mixture was quenched wtih 1M aqueous HCl, and diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **3** (5.74 g, 6.65 mmol, 96%). R_f : 0.18 (Hexane/EtOAc/DCM : 9/1/0.5). $[\alpha]_D^{25}$ = 3.09 (C= 3.05, $CHCl_3$). IR (thin film): υ = 2960, 1755, 1732, 1278, 1249 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.4 Hz, 2H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.71 (dd, *J* = 7.6, 3.3 Hz, 2H), 7.61 (d, *J* = 1.7 Hz, 1H), 7.56 – 7.50 (m, 1H), 7.46 – 7.20 (m, 16H), 7.17 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 5.80 (t, *J* = 9.5 Hz, 1H, H-3), 5.54 (t, *J* = 9.8 Hz, 1H, H-3), 5.25 (t, *J* = 9.8 Hz, 1H, H-4), 4.94 (d, *J* = 10.1 Hz, 1H, H-1), 4.58 (q, *J* = 12.2 Hz, 2H, C*H*$_2$Ph), 4.22 (dd, *J* = 10.4, 7.3 Hz, 1H, C*H*HPh of Fmoc), 4.08 (dd, *J* = 10.4, 7.8 Hz, 1H, H-6, C*H*HPh of Fmoc), 4.00 – 3.85 (m, 2H, H-5, C*H* of Fmoc), 3.74 (d, *J* = 3.9 Hz, 2H, H-6), 2.25 (s, 3H, Me), 1.26 (d, *J* = 0.8 Hz, 9H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 165.82 (OBz), 165.22 (OBz), 154.17, 149.86, 143.34, 143.04, 141.25, 141.20, 137.79, 137.34, 133.39, 132.13, 130.32, 130.09, 130.00, 129.98, 129.35, 128.93, 128.49, 128.46, 128.40, 127.90, 127.79, 127.23, 127.23, 125.54, 125.28, 125.11, 120.02 (Ar), 87.61 (C-1), 77.34 (C-5), 74.58 (C-3), 73.75 (*C*H_2Ph), 73.38 (C-4), 70.79 (C-2), 70.43 (*C*H_2 of Fmoc), 69.02 (C-6), 46.55 (*C*H of Fmoc), 34.54 (Cq, *t*-Bu), 31.37 (Me, *t*-Bu), 20.46 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{53}H_{50}O_9S$ 885.3068, found 885.3094.



S5 **S6: R_1 = H** **S7: R = H** **12: R = Fmoc**

(2-Methyl-5-tert-butylphenyl) 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside: S6

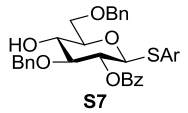


S6

To a solution of compound **S5**[31] (9.59 g, 17.17 mmol, 1.0 eq.) in MeOH (86 ml, 0.2 M) was added sodium methoxide (NaOMe) (0.93 g, 17.17 mmol, 1.0 eq.) at 0 °C, and stirred overnight at 40 °C. After completion, the mixture was neutralized with Amberlite IR-120 (H^+) ion exchange resin. The mixture was filtered off, concentrated, and coevaporated with toluene. To a solution of the crude in DMF (86 ml, 0.2 M) were added benzaldehyde dimethyl acetal (5.16 mL, 34.4 mmol, 2.0 eq.) and a catalytic amount of p-toluenesulfonic acid (pTsOH) (0.65 g, 3.44 mmol, 0.2 eq.) and the mixture was heated at 80°C. After the mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with ethyl acetate three times, after which the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and concentrated. To a solution of this crude in anhydrous DCM (86 ml, 0.2 M) was added benzoic anhydride (Bz_2O) (7.77 g, 34.3 mmol, 2.0 eq.), triethylamine (7.2 mL, 51.5 mmol, 3.0 eq.), and a catalytic amount of DMAP (0.42 g, 3.43 mmol, 0.2 eq.) at 0°C, and stirred overnight at room temperature. After the mixture was quenched by saturated aqueous $NaHCO_3$, the organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **S6** (9.5 g, 15.21 mmol, 89% over three steps). R_f : 0.23 (hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = + 69.86 (C= 3.18, $CHCl_3$); IR (thin film): υ = 2962, 2870, 1729, 1452, 1265 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ*: 8.06 – 8.00 (m, 2H),

7.65 – 7.57 (m, 1H), 7.56 – 7.37 (m, 8H), 7.21 (dd, *J* = 8.0, 2.1 Hz, 1H), 7.17 – 7.05 (m, 6H), 5.64 (s, 1H, CHPh), 5.38 (ddd, *J* = 10.2, 5.9, 2.8 Hz, 1H, H-2), 4.83 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 4.82 (d, *J* = 10.2 Hz, 1H, H-2), 4.70 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 4.40 (dd, *J* = 10.5, 5.0 Hz, 1H, H-6), 3.96 – 3.83 (m, 3H, H-3, H-4, H-6), 3.56 (dt, *J* = 14.4, 4.9 Hz, 1H, H-5), 2.19 (s, 3H), 1.28 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 165.07 (Bz), 149.50, 137.68, 137.11, 136.87, 133.17, 132.21, 129.91, 129.88, 129.76, 129.73, 129.03, 128.34, 128.27, 128.13, 128.06, 127.55, 125.96, 125.21 (Ar), 101.27 (CHPh), 87.86 (C-1), 81.48 (C-3 or C-4), 79.27 (C-3 or C-4), 74.20 (Bn), 72.08 (C-2), 70.50 (C-5), 68.65 (C-6), 34.39 (Cq, *t*-Bu), 31.23 (Me, *t*-Bu), 20.19 (Me). MS ESI-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{38}H_{40}O_6SNa$ 647.2443, found 647.2462.

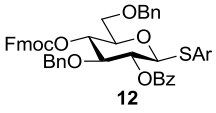
2-Methyl-5-*turt*-butylphenyl-2-*O*-benzoyl -3, 6-di-*O*-benzyl-1-thio-β-D-glucopyranose S7



S7

Compound **S6** (4.7 g, 7.36 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (41 mL, 0.18 M). To a solution of compound **S6** (10.0 g, 15.7 mmol, 1.0 eq.) was added triethylsilane (7.05 mL, 44.1 mmol, 6.0 eq.) and trifluoroacetic anhydride (0.520 mL, 3.68 mmol) at 0°C. After 30 min, trifluoroacetic acid (2.83 mL, 36.8 mmol, 5.0 eq.) was added to the mixture that was stirred and allowed to warm to room temperature for 8 h. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **S7** (4.44 g, 6.94 mmol, 94%). R_f : 0.25 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = 38.25 (C= 1.00, $CHCl_3$). IR (thin film): υ = 3458, 2961, 1729, 1274 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 – 8.05 (m, 2H), 7.63 – 7.56 (m, 2H), 7.50 – 7.43 (m, 2H), 7.39 – 7.27 (m, 5H), 7.22 – 7.16 (m, 6H), 7.07 (d, *J* = 8.0 Hz, 1H), 5.35 (dd, *J* = 10.1, 9.1 Hz, 1H, H-2), 4.76 (d, *J* = 10.1 Hz, 1H, H-1), 4.71 (dd, *J* = 20.0, 9.6 Hz, 2H, CH_2Ph), 4.60 (q, *J* = 12.0 Hz, 2H, CH_2Ph), 3.85 (t, *J* = 9.2 Hz, 1H, H-4), 3.80 (d, *J* = 4.7 Hz, 2H, H-6), 3.71 (t, *J* = 9.0 Hz, 1H, H-3), 3.57 (dt, *J* = 9.5, 4.7 Hz, 1H, H-5), 2.78 (br, 1H, OH), 2.20 (s, 3H), 1.25 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.38 (Bz), 149.69, 138.00, 137.76, 136.89, 133.34, 132.95, 129.98, 129.92, 129.70, 128.60, 128.55, 128.53, 128.17, 127.99, 127.97, 127.94, 125.08 (Ar), 87.60 (C-1), 83.70 (C-3), 78.23 (C-5), 74.73 (Bn), 73.89 (Bn), 72.29 (C-2), 72.22 (C-4), 70.49 (C-6), 34.53 (Cq, *t*-Bu), 31.38 (Me, *t*-Bu), 20.38 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{38}H_{42}O_6SNa$ 649,2600, found 649.2585.

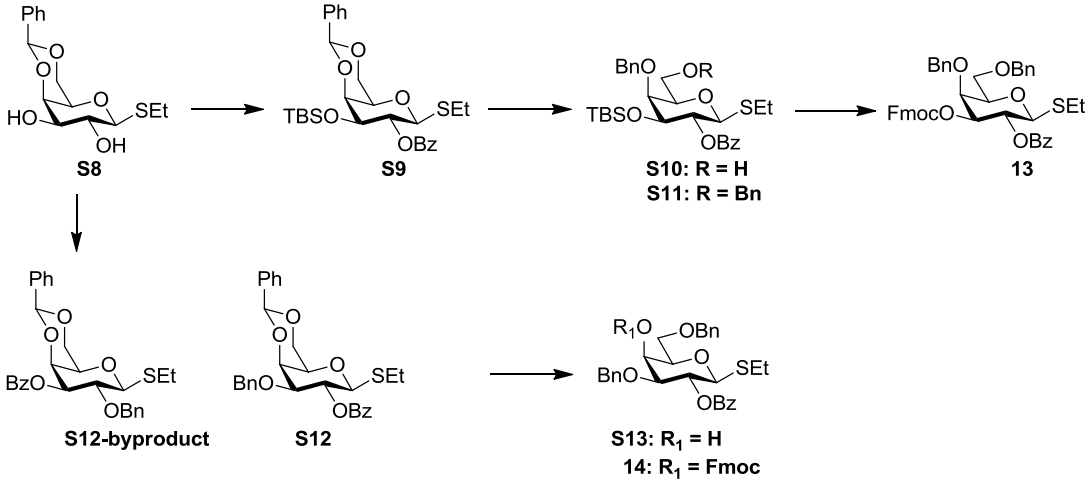
2-Methyl-5-turt-butylphenyl-2-O-benzoyl -3, 6-di-O-benzyl-1-thio-β-D-glucopyranose: 12



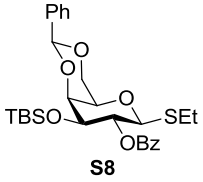
12

To a solution of compound **S7** (4.45 g, 6.94 mmol, 1.0 eq.) was added 9-fluorenylmethyl chloroformate (3.59 g, 13.88 mmol, 2.0 eq.) and pyridine (1.68 mL, 20.82 mmol, 3.0 eq.) successively at 0°C, and stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl and diluted with DCM, the organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate/DCM = 9:0.5:0.5 to 9:1:0.5) to afford **3**

(5.74 g, 6.65 mmol, 96%). R_f : 0.17 (Hexane/EtOAc/DCM : 9/1/0.5). $[\alpha]_D^{25}$ = 35.70 (C= 2.95, $CHCl_3$). IR (thin film): υ = 2960, 1754, 1732, 1451, 1248 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (dd, *J* = 7.5, 0.9 Hz, 2H), 7.75 (dd, *J* = 7.5, 3.8 Hz, 2H), 7.63 – 7.51 (m, 4H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.39 (td, *J* = 7.5, 2.3 Hz, 2H), 7.33 – 7.17 (m, 8H), 7.10 – 7.02 (m, 6H), 5.39 (t, *J* = 9.6 Hz, 1H, H-2), 5.03 (t, *J* = 9.5 Hz, 1H, H-4), 4.76 (d, *J* = 10.1 Hz, 1H, H-1), 4.57 (dd, *J* = 20.3, 12.6 Hz, 2H, CH_2Ph), 4.53 (s, 2H, CH_2Ph), 4.32 (d, *J* = 7.1 Hz, 2H, CH_2, Fmoc), 4.12 (t, *J* = 7.1 Hz, 1H, CH, Fmoc), 3.92 (t, *J* = 9.2 Hz, 1H, H-3), 3.80 – 3.71 (m, 1H, H-5), 3.71 – 3.62 (m, 2H, H-6), 2.20 (s, 3H), 1.23 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 165.15 (Bz), 154.33 (Fmoc), 149.82, 143.43, 143.24, 141.44, 141.40, 137.89, 137.44, 137.03, 133.41, 132.74, 130.02, 129.98, 129.83, 128.56, 128.46, 128.29, 128.04, 127.88, 127.76, 127.31, 125.23, 125.14, 120.21 (Ar), 87.69 (C-1), 81.19 (C-3), 77.40 (C-5), 75.55 (C-4), 74.40 (Bn), 73.73 (Bn), 72.18 (C-2), 70.19 (CH_2, Fmoc), 69.76 (C-6), 46.84 (CH, Fmoc), 34.55 (Cq, *t*-Bu), 31.37 (Me, *t*-Bu), 20.43 (Me). MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{53}H_{52}O_8SNa$ 871.3281, found 871.3311.



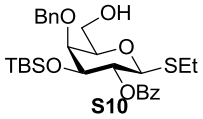
Ethyl 2-O-benzoyl-4,6-O-benzylidene-3-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside: S9



To a solution of compound **S8**[31] (13 g, 41.6 mmol, 1.0 eq.) in anhydrous DCM (104 mL, 0.4 M) was added TBDMSCl (7.53 g, 49.9 mmol, 1.2 eq.) and imidazole (3.97 g, 58.3 mmol, 1.4 eq.) successively at 0°C, stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, and diluted with DCM, the organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. To a solution of this crude in anhydrous DCM (100 mL, 0.4 M) was added benzoic anhydride (18.0 g, 80 mmol, 2.0 eq.), triethylamine (16.7 mL, 120 mmol, 3.0 eq.), and a catalytic amount of DMAP (0.974 g, 7.97 mmol, 0.2 eq.) at 0°C. The mixture was stirred overnight at room temperature. Afterwards, the mixture was quenched with saturated aqueous $NaHCO_3$ and diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude was

purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **S8** (19.2 g, 36.2 mmol, 91%) over two steps. R_f = 0.57 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +35.44 (c = 1.00, chloroform); IR (thin film): υ = 3090, 2957, 2857, 1721, 1602, 1451, cm^{-1}; ^{1}H NMR (CDCl3) δ ^{1}H NMR (400 MHz, $cdcl_3$) δ 8.07 – 8.01 (m, 2H), 7.60 – 7.52 (m, 3H), 7.46 – 7.33 (m, 5H), 5.60 (t, *J* = 9.6 Hz, 1H, H-2), 5.53 (s, 1H, C*H*Ph), 4.56 (d, *J* = 9.9 Hz, 1H, H-1), 4.39 (dd, *J* = 12.4, 1.5 Hz, 1H, H-6a), 4.14 (dd, *J* = 3.6, 0.7 Hz, 1H, H-4), 4.05 (dd, *J* = 12.4, 1.8 Hz, 1H. H-6b), 4.03 – 3.99 (m, 1H, H-3), 3.55 (d, *J* = 1.1 Hz, 1H, H-5), 2.99 – 2.65 (m, 2H, CH_2), 1.26 (t, *J* = 7.5 Hz, 3H, CH_3), 0.75 (s, 9H, *tert*-Bu), 0.04 (s, 3H, Me), -0.12 (s, 3H. Me). ^{13}C NMR (100 MHz, $cdcl_3$) δ 165.41(OBz), 138.01, 133.02, 130.45, 129.88, 128.99, 128.41, 128.29, 126.39(Ar), 101.24(*C*HPh), 82.90(C-1), 76.97(C-4), 73.65(C-3), 70.36(C-5), 70.20(C-2), 69.53(C-6), 25.57(*t*-Bu), 22.85(CH_2), 18.05(Cq), 15.00(CH_3), -4.44(Me), -4.58(Me); MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{38}O_6SSiNa$ 553.2056, found 553.1976.

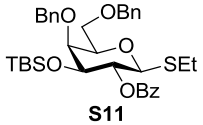
Ethyl 4-O-benzyl-3-O-tert-butyldimethylsilyl-2-O-benzoyl-1-thio-β-D-galactopyranoside: S10



S10

Compound **S9** (20 g, 37.7 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (220 mL, 0.17 M). To a solution of compound **S9** was added 1 M solution of BH_3 in THF (151 mL, 151 mmol, 4.0 eq.), and TMSOTf (3.40 mL, 18.84 mmol, 0.5 eq.) successively at 0°C. The mixture was stirred for 5 h at 0°C. After the mixture was quenched with saturated aqueous $NaHCO_3$ and diluted with DCM, the organic layer was dried over $MgSO_4$ and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:0.5) to afford **S10** (19.5 g, 36.6 mmol, 95%). R_f = 0.28 (hexane/ethyl acetate, 1:1); $[\alpha]_D^{20}$ = +48.68 (c = 2.00, $CHCl_3$); IR (thin film): υ = 3030, 2930, 1719, 1602, 1452, 1352^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 – 8.08 (m, 2H), 7.69 – 7.61 (m, 1H), 7.55 – 7.49 (m, 2H), 7.48 – 7.34 (m, 6H), 5.72 (t, *J* = 9.5 Hz, 1H, H-2), 5.18 (d, *J* = 11.7 Hz, 1H, CH*H*Ph), 4.67 (d, *J* = 11.7 Hz, 1H, CH*H*Ph), 4.58 (d, *J* = 9.8 Hz, 1H, H-1), 4.09 – 4.00 (m, 1H, H-3), 3.92 (dd, *J* = 10.9, 6.4 Hz, 1H, H-6a), 3.85 (d, *J* = 2.2 Hz, 1H, H-4), 3.71 – 3.66 (m, 1H, H-5), 3.63 (dd, *J* = 10.9, 5.2 Hz, 1H, H-6b), 2.92 – 2.66 (m, 2H) 1.28 (t, *J* = 7.5 Hz, 3H, Me), 0.86 (d, *J* = 2.8 Hz, 9H, *t*-Bu), 0.20 (s, 3H, Me), -0.00 (s, 3H, Me). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.50 (Bz), 138.62, 133.10, 130.41, 129.95, 128.57, 128.44, 128.15, 127.94 (Ar), 83.91(C-1), 79.13(C-5), 77.00(C-4), 75.90(C-3), 74.97(*C*H_2Ph), 71.07(C-2), 62.40(C-6), 25.67(*t*-Bu), 23.76(CH_2), 17.94(Cq), 14.97(Me), -3.85(Me), -4.90(Me); MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{28}H_{40}O_6SSiNa$ 555.2213, found 555.2126.

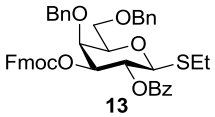
Ethyl 2-O-benzoyl-4,6-di-O-benzyl-3-O-tert-butyldimethylsilyl-1-thio-β-D-galactopyranoside: S11



S11

To a solution of compound **S10** (4.01 g, 7.51 mmol, 1.0 eq.) in anhydrous DMF and THF (58.7 mL, 1:9), were added BnBr (2.68 mL, 22.52 mmol, 3.0 eq.) and sodium hydride (0.721 mg, 18.02 mmol, 2.4 eq) portionwise at 0°C. After the reaction mixture was stirred for 2 h at 0°C the mixture was quenched with saturated aqueous NH_4C, diluted with DCM, over Mg_2SO_4. Following evaporation of the solvent, the crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:0.5:0.5 to 9:1:1) to afford **S11** (4.58g, 7.36 mmol, 98%). R_f (hexane:ethylacetate:DCM = 8:2:1) = 0.75; $[\alpha]_D^{20}$= +19.04 (c = 1.0, $CHCl_3$). IR (thin film): υ = 3032, 1726, 1603, 1497, 1453 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.03 (m, 2H), 7.59 – 7.53 (m, 1H), 7.48 – 7.41 (m, 2H), 7.39 – 7.26 (m, 10H), 5.64 (t, *J* = 9.5 Hz, 1H, H-2), 5.10 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.54 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.50 (d, *J* = 9.7 Hz, 1H, H-1), 4.46 (dd, *J* = 16.0, 11.8 Hz, 1H, CH_2Ph), 3.97 (d, *J* = 9.3 Hz, 1H, H-3), 3.86 (d, *J* = 2.2 Hz, 1H, H-4), 3.74 (dd, *J* = 9.7, 3.6 Hz, 1H, H-5), 3.64 (d, *J* = 6.3 Hz, 2H, H-6), 2.81 – 2.60 (m, 1H, CH_2), 1.20 (t, *J* = 7.5 Hz, 2H, Me), 0.78 (s, 9H), 0.12 (s, 3H), -0.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.49(Bz), 139.02, 138.06, 133.02, 130.50, 129.94, 128.56, 128.40, 128.33, 128.04, 127.92, 127.79, 127.51(Ar), 83.78(C-1), 77.66(C-5), 77.44(C-4), 75.80(C-3), 75.30(*C*H_2Ph), 73.69(*C*H_2Ph), 71.19(C-2), 68.81(C-6), 25.67(*t*-Bu), 23.67(CH_2 thio), 17.94(Cq), 14.95(Me), -3.89(Me), -4.90(Me). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{35}H_{46}O_6SSiNa$ 645.2682, found 645.2607.

Ethyl 2-O-benzoyl-4,6-di-O-benzyl-3-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside: 13

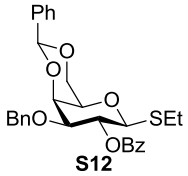


13

To a solution of compound **S11** (3.77 g, 6.05 mmol, 1.0 eq.) in anhydrous acetonitrile was added boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$) (0.920 ml, 7.26 mmol, 1.2 eq.) at 0°C. After the reaction mixture was stirred for 20 min at 0°C the mixture was quenched with saturated aqueous $NaHCO_3$ (30 mL), diluted with DCM, and dried over $MgSO_4$. The solvent was evaporated *in vacuo*. To a solution of this crude in anhydrous DCM (30 mL) was added 9-fluorenylmethylchloroformate (3.91 g, 15.12 mmol, 2.5 eq.) and pyridine (1.47 mL, 18.14 mmol, 3.0 eq.) successively at 0°C, and stirred overnight at room temperature. After the mixture was quenched with 1 M aqueous HCl, and diluted with DCM. The organic layer was dried over $MgSO_4$ and the solvent evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **13** (4.21 g, 5.76 mmol, 95%). **NMR after first step S11:** ^1H NMR (400 MHz, $cdcl_3$) δ 8.09 – 8.00 (m, 2H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 7.40 – 7.25 (m, 10H), 5.30 (t, *J* = 9.7 Hz, 1H, H-2), 4.73 (q, *J* = 11.8 Hz, 2H, Bn), 4.62 – 4.47 (m, 3H, H-1, Bn), 4.01 (d, *J* = 3.5 Hz, 1H, H-4), 3.84 – 3.68 (m, 3H, H-3,5,6), 2.87 – 2.57 (m, 3H, CH_2, thio), 2.37 (d, *J* = 9.7 Hz, 1H, *H*O-), 1.24 (t, *J* = 7.4 Hz, 3H, , Me, thio).^{13}C NMR (101 MHz, $cdcl_3$) δ 166.70(C=O, Bz), 138.22, 137.77, 133.34, 130.08, 129.92, 128.73, 128.64, 128.49, 128.10, 128.08, 128.05(Ar), 83.47(C-1), 77.56(C-5), 76.84(C-4), 75.58(Bn), 74.20(C-3), 73.74(Bn), 72.47(C-2), 68.30(C-6), 24.01(CH_2, thio), 15.10(Me, thio). **Product 13:** R_f (hexane:ethylacetate:DCM = 8:2:1) = 0.49; $[\alpha]_D^{20}$= +34.08 (c = 2.69, $CHCl_3$). IR (thin film): υ = 2870, 1730, 1602, 1496, 1451 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.06 (dt, *J* = 8.5, 1.6 Hz, 2H), 7.74 – 7.66 (m, 2H), 7.62 – 7.27 (m, 9H), 7.13 (dtd, *J* = 8.6, 7.5, 1.1 Hz, 17H), 5.76 (t, *J* = 9.9 Hz, 1H, H-2), 5.09 (dd, *J* = 10.0, 3.0 Hz, 1H, H-3), 4.80 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.62 (d, *J* = 9.9 Hz, 1H, H-1), 4.58 – 4.42 (m, 3H, Bn, , CH*H*Ph), 4.31 (dd, *J* = 10.4, 7.1 Hz, 1H, H-6a), 4.23 (dd, *J* = 10.4, 7.8 Hz, 1H, H-6b), 4.18 – 4.14 (m, 1H, H-4), 4.07 (t, *J* = 7.4 Hz, 1H, H-5), 3.84 (t, *J*

= 6.9 Hz, 1H, CH Fmoc), 3.68 (d, *J* = 6.7 Hz, 2H, CH_2 Fmoc), 2.76 (qq, *J* = 12.4, 7.5 Hz, 2H, CH_2), 1.25 (t, *J* = 7.5 Hz, 3H, Me). ^{13}C NMR (101 MHz, cdcl$_3$) δ 165.35(Bz), 154.64(Fmoc), 143.38, 142.95, 141.32, 141.21, 138.04, 137.84, 133.31, 130.05, 129.70, 128.57, 128.49, 128.42, 128.26, 128.00, 127.98, 127.92, 127.83, 127.22, 127.19, 125.28, 125.07, 120.07, 120.06(Ar), 83.84(C-1), 79.14(C-3), 77.36(CH, Fmoc), 75.20(Bn), 74.13(C-4), 73.67(Bn), 70.21(C-6), 68.70(C-2), 68.18(CH_2, Fmoc), 46.59(C-5), 23.99(CH_2), 14.93(CH_3). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{44}H_{42}O_8SNa$ 753.2498, found 753.2442.

Ethyl 2-O-benzoyl-4,6-di-O-benzyl-4-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside: S12



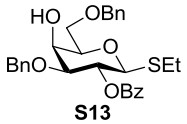
S12

To a solution of **S8** (14.7 g, 47.1 mmol, 1.0 eq.) in DCM (118 mL, 0.4 M) were added TBSCl (7.80 g, 51.8 mmol, 1.1 eq.) and imidazole (4.48 g, 65.9 mmol, 1.4 eq.). After the mixture was stirred for 12 h at room temperature, the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$, the organic layer was evaporated *in vacuo*. To a solution of the crude in anhydrous DMF (234 mL, 0.2 M) were added BnBr (16.7 mL, 141 mmol, 3.0 eq.) and sodium hydride (4.50 g, 113 mmol, 2.4 eq) portionwise at 0°C. The reaction mixture was stirred at room temperature for 1 h and quenched with saturated aqueous NH_4Cl, extracted with Et_2O, and dried with Mg_2SO_4. To a solution of the crude mixture in THF (117 mL, 0.4 M) was added tetra-*N*-butylammonium fluoride (TBAF) (94 mL, 94 mmol, 2.0 eq.) at room temperature. After 4 h, the reaction mixture was concentrated *in vacuo*, re-dissolved in DCM, and extracted with 0.1 M of $CuSO_4$ aqueous solution. After the organic layer was dried over $MgSO_4$, and evaporated *in vacuo*, to a solution of this crude mixture in anhydrous DCM (157 mL, 0.4 M) were added benzoic anhydride (21.36 g, 94 mmol, 2.0 eq.), triethylamine (26.3 mL, 189 mmol, 4.0 eq.), and a catalytic amount of DMAP (1.15 g, 9.44 mmol, 0.2 eq.) at 0°C, and the mixture was stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **S12-byproduct** (4.01g, 7.92 mmol, 16.8%) and **S12** (19.0 g, 37.5 mmol, 79%) over four steps. **S12-byproduct** R_f: 0.19 (Hexane:EtOAc:DCM = 9:1:1); $[\alpha]_D^{20}$= +12.85 (c = 2.01, $CHCl_3$); IR (thin film): υ = 2871, 1719, 1453, 1295, 1261 cm-1; δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 – 8.03 (m, 2H), 7.60 – 7.55 (m, 1H), 7.52 – 7.41 (m, 4H), 7.39 – 7.32 (m, 3H), 7.25 – 7.15 (m, 5H), 5.51 (s, 1H), 5.23 (dd, *J* = 9.7, 3.6 Hz, 1H, H-3), 4.91 (d, *J* = 10.5 Hz, 1H, C*H*HPh), 4.70 (d, *J* = 10.5 Hz, 1H, C*H*HPh), 4.60 (d, *J* = 9.6 Hz, 1H, H-1), 4.54 (dd, *J* = 3.6, 0.9 Hz, 1H, H-4), 4.37 (dd, *J* = 12.4, 1.6 Hz, 1H, H-6), 4.10 (d, *J* = 9.6 Hz, 1H, H-2), 4.07 – 4.00 (m, 1H, H-6), 3.60 (d, *J* = 1.1 Hz, 1H, H-5), 2.97 – 2.72 (m, 2H), 1.36 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.23 (Bz), 138.03, 137.93, 133.39, 129.97, 129.03, 128.58, 128.37, 128.24, 128.23, 127.85, 126.42 (Ar), 101.00 (CHPh), 84.86 (C-1), 76.01 (C-3), 75.74 (CH_2Ph), 75.57 (C-2), 74.34 (C-4), 69.76 (C-6), 69.38 (C-5), 24.42, 15.16. MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{29}H_{30}O_6SNa$ 529.1661, found 529.1655.

S12, Rf = 0.18 (hexane/ethyl acetate/DCM, 9:1:1); $[\alpha]_D^{20}$ = +28.01 (c = 2.75, chloroform); IR (thin film): υ = 2871, 1719, 1261 cm-1; δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.02 (m, 2H), 7.62 – 7.53 (m, 3H), 7.50 – 7.43 (m, 2H), 7.43 – 7.34 (m, 3H), 7.26 – 7.16 (m, 5H), 5.74 (t, *J*

= 9.7 Hz, 1H, H-2), 5.52 (s, 1H, CHPh), 4.66 (q, *J* = 12.8 Hz, 2H, CH_2Ph), 4.55 (d, *J* = 9.9 Hz, 1H, H-1), 4.36 (dd, *J* = 12.3, 1.5 Hz, 1H, H-6), 4.28 (dd, *J* = 3.4, 0.8 Hz, 1H, H-4), 4.02 (dd, *J* = 12.4, 1.7 Hz, 1H, H-6), 3.76 (dd, *J* = 9.6, 3.4 Hz, 1H, H-3), 3.47 (d, *J* = 1.1 Hz, 1H, H-5), 2.93 (dq, *J* = 12.3, 7.5 Hz, 1H, C*H*HCH_3), 2.78 (dq, *J* = 12.3, 7.5 Hz, 1H, C*H*HCH_3), 1.28 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.37 (Bz), 137.95, 137.89, 133.11, 130.27, 129.98, 129.15, 128.44, 128.42, 128.32, 127.83, 127.78, 126.59 (Ar), 101.47 (C*H*Ph), 83.04 (C-1), 78.29 (C-3), 73.57 (C-4), 71.13 (CH_2Ph), 70.28 (C-5), 69.50 (C-6), 68.91 (C-2), 22.88, 14.99.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{29}H_{30}O_6SNa$ 529.1661, found 529.1656.

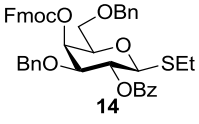
Ethyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside S13



S13

Compound **S12** (8.25 g, 16.28 mmol, 1.0 eq.) was co-evaporated with toluene and dissolved under an Ar atmosphere in DCM (96 mL, 0.17 M). To a solution of **S12** were added triethylsilane (15.6 mL, 98 mmol, 6.0 eq.) and trifluoroacetic anhydride (1.15 mL, 8.14 mmol, 0.5 eq.) at 0°C. After 30 min, trifluoroacetic acid (6.27 mL, 81 mmol, 5.0 eq.) was added dropwise, and the reaction mixture was stirred and allowed to warm to room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9:1 to 7:3) to afford **S13** (8.01 g, 15.73 mmol, 97 %). R_f : 0.21 (Hexane/EtOAc/DCM : 8/2/0.5). $[\alpha]_D^{25}$ = 32.14 (C= 3.05, $CHCl_3$). IR (thin film): υ = 2871, 1723, 1453, 1268 cm-1 δ ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 – 8.00 (m, 2H), 7.62 – 7.56 (m, 1H), 7.49 – 7.43 (m, 2H), 7.39 – 7.28 (m, 5H), 7.23 – 7.14 (m, 5H), 5.53 (t, *J* = 9.7 Hz, 1H, H-2), 4.68 (d, *J* = 12.3 Hz, 1H, CH_2Ph) 4.60 (s, 2H, CH_2Ph), 4.54 (d, *J* = 12.3 Hz, 1H, CH_2Ph), 4.49 (d, *J* = 10.0 Hz, 1H, H-1), 4.19 (d, *J* = 2.8 Hz, 1H, H-4), 3.84 (dd, *J* = 9.8, 6.4 Hz, 1H, H-6), 3.77 (dd, *J* = 9.8, 5.6 Hz, 1H, H-6), 3.69 (d, *J* = 5.9 Hz, 1H, H-5), 3.66 (dd, *J* = 9.3, 3.2 Hz, 1H, H-3), 2.82 – 2.64 (m, 2H), 2.61 (br, 1H, OH), 1.22 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cdcl3) δ 165.56 (Bz), 138.07, 137.27, 133.22, 130.09, 130.01, 128.58, 128.55, 128.48, 128.08, 127.97, 127.97, 127.93 (Ar), 83.59 (C-1), 79.46 (C-3), 77.43 (C-5), 73.88 (CH_2Ph), 71.46 (CH_2Ph), 69.67 (C-2), 69.12 (C-6), 66.49 (C-4), 23.76, 15.01. MS ESI-HRMS *m/z* $[M+Na]^+$ calcd for $C_{29}H_{32}O_6SNa$ 531.1817, found 531.1811.

Ethyl 2-O-benzoyl-4,6-di-O-benzyl-4-O-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside: 14

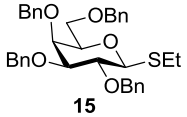


14

To a solution of compound **S13** (7.7 g, 15.14 mmol, 1.0 eq) in anhydrous DCM (76 mL) were added 9-fluorenylmethylchloroformate (7.83 g, 30.3 mmol, 2.0 eq.) and pyridine (3.67 mL, 45.4 mmol, 3.0 eq.) successively at 0°C, and stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, and diluted with DCM he organic layer was dried over MgSO4 and the solvent was evaporated *in vacuo*. The crude was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **14** (10.2 g, 13.96 mmol, 92%). R_f = 0.15 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ =

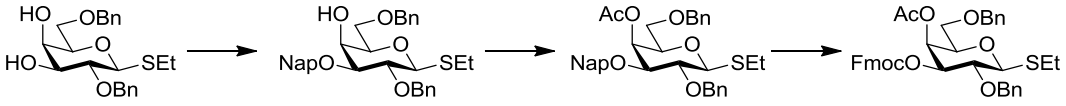
+34.81 (c = 3.30, $CHCl_3$); IR (thin film): υ = 2871, 1748, 1727, 1451 cm-1; ^{1}H NMR (CDCl3) 8.01 (dd, *J* = 7.3, 1.1 Hz, 2H), 7.77 (dd, *J* = 7.2, 2.5 Hz, 2H), 7.70 (d, *J* = 7.4 Hz, 1H), 7.60 (ddd, *J* = 7.4, 6.6, 4.1 Hz, 2H), 7.50 – 7.28 (m, 10H), 7.26 – 7.20 (m, 1H), 7.16 – 6.99 (m, 5H), 5.60 (t, *J* = 9.8 Hz, 1H, H-2), 5.56 (d, *J* = 3.2 Hz, 1H, H-4), 4.70 (d, *J* = 12.6 Hz, 1H, C*H*HPh), 4.57 – 4.46 (m, 5H, H-1, C*H*HPh, CH_2Ph, C*H*H of Fmoc), 4.29 – 4.22 (m, 2H, C*H*H of Fmoc, CH of Fmoc), 3.85 (t, *J* = 6.5 Hz, 1H, H-5), 3.76 – 3.70 (m, 2H, H-3, H-6), 3.65 (dd, *J* = 9.3, 7.5 Hz, 1H, H-6)., 2.87 – 2.68 (m, 2H), 1.26 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (100 MHz, cdcl3) δ 165.33 (Bz), 155.17 (Fmoc), 143.80, 143.29, 141.45, 141.32, 137.67, 137.38, 133.21, 130.09, 130.07, 128.60, 128.46, 128.32, 128.11, 128.05, 127.96, 127.92, 127.77, 127.44, 127.40, 125.87, 125.42, 120.08, 120.04 (Ar), 84.02 (C-1), 77.61 (C-3), 76.11 (C-4), 73.95 (CH_2Ph), 71.15 (CH_2Ph), 70.72 (C-4), 70.38 (CH_2, Fmoc), 69.45 (C-2), 68.02 (C-6), 46.69 (CH, Fmoc), 24.08, 15.02.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{44}H_{42}O_8SNa$ 753.2498, found 753.2474.

Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside 15

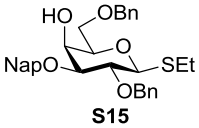


15

The known compound **15** was prepared according to literature[27].



Ethyl 2,6-di-*O*-benzyl-3-*O*-(2-naphthylmathyl)-1-thio-β-D-galactopyranoside S15

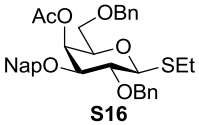


S15

Dibutyltin oxide (2.2 g, 8.78 mmol, 1.2 eq.) was added to a solution of **S14** (2.96 g, 7.32 mmol, 1.0 eq.) in methanol (36.6 ml, 0.2 M) and the reaction mixture was heated to reflux for 4 h. Removal of solvent from the reaction mixture gave stannylene acetal that kept under vacuum for 4 h. To a solution of the crude acetal in anhydrous DMF (37 mL, 0.2 M) were added 2-naphtylmethyl bromide (2.43 g, 11.0 mmol, 1.5 eq.) and tetrabutylammonium iodide (NBu_4I) (3.24 g, 8.78 mmol, 1.2 eq.). The reaction mixture was stirred at 60 °C for 3 h. Afterwards, the mixture was diluted with EtOAc (75 mL) and quenched with saturated aqueous NH_4Cl. The combined organic layer was washed with saturated aqueous NH_4Cl and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **S15** (3.4 g, 6.24 mmol, 85%). R_f = 0.31 (hexane/ethyl acetate/DCM, 7:3); $[\alpha]_D^{20}$ = 3.19 (c = 3.00, chloroform); IR (thin film): υ = 3479, 3030, 2869, 1453, 1366, 1093 cm-1; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 – 7.74 (m, 4H), 7.48 (tt, *J* = 3.9, 3.1 Hz, 4H), 7.44 – 7.40 (m, 2H), 7.37 – 7.27 (m, 7H), 4.91 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.91 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.86 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.81 (d, *J* = 10.2 Hz, 1H, C*H*HPh), 4.58 (s, 2H, CHPh), 4.44 (d, *J* = 9.7 Hz, 1H, H-1), 4.13 (d, *J* = 2.6 Hz, 1H, H-4), 3.79 (dd, *J* = 9.9, 5.9 Hz, 1H, H-6), 3.75 – 3.70 (m, 2H, H-2, H-6), 3.60 (dd, *J* = 9.0, 3.2 Hz, 1H, H-3), 3.57 (t, *J* = 5.8 Hz, 1H, H-

5), 2.84 – 2.70 (m, 2H), 2.57 (d, *J* = 2.4 Hz, 1H, OH), 1.32 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 138.33, 138.05, 135.33, 133.35, 133.20, 128.56, 128.49, 128.47, 128.05, 127.92, 127.90, 127.84, 126.83, 126.34, 126.19, 125.91 (Ar), 85.24 (C-1), 82.38 (C-3), 78.08 (C-2), 77.04 (C-5), 75.97 (CH_2Ph), 73.87 (CHPh), 72.28 (CH_2Ph), 69.49 (C-6), 67.15 (C-4), 24.90, 15.27. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{33}H_{38}O_5SNa$ 567.2176, found 567.2201.

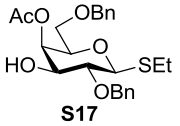
Ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-(2-naphthylmathyl)-1-thio-β-D-galactopyranoside S16



S16

To a solution of **S15** (3.4 g, 6.24 mmol, 1.0 eq.) in DCM (31 mL, 0.2 M) were added acetic anhydride (1.77 mL, 18.7 mmol, 3.0 eq.), triethylamine (5.22 mL, 37.5 mmol, 6.0 eq.), and DMAP (0.076 g, 0.624 mmol, 0.1 eq.) in the ice bath. The reaction mixture was stirred overnight at room temperature. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **S16** (3.44 g, 6.24 mmol, 94%). R_f = 0.19 (hexane/ethyl acetate/DCM, 7:3); $[\alpha]_D^{20}$ = 27.30 (c = 3.07, chloroform); IR (thin film): υ = 2867, 1741, 1454, 1372, 1231, 1097, 940 cm-1; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.84 – 7.69 (m, 4H), 7.49 – 7.43 (m, 3H), 7.42 – 7.29 (m, 10H), 5.70 (dd, *J* = 3.3, 0.8 Hz, 1H, H-4), 4.95 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.83 (dd, *J* = 25.2, 10.2 Hz, 2H, CH_2Nap), 4.69 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.58 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.50 (d, *J* = 9.7 Hz, 1H, H-1), 4.48 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 3.76 – 3.73 (m, 1H, H-5), 3.69 (dd, *J* = 9.2, 3.3 Hz, 1H, H-3), 3.64 – 3.59 (m, 2H, H-2, H-6), 3.52 (dd, *J* = 9.5, 6.9 Hz, 1H, H-6), 2.83 – 2.72 (m, 2H), 2.13 (s, 3H, Ac), 1.33 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.53 (Ac), 138.31, 137.75, 135.34, 133.38, 133.15, 128.58, 128.44, 128.41, 128.25, 128.17, 128.08, 127.99, 127.88, 127.76, 127.06, 126.29, 126.11, 126.00, 85.55, 81.04, 78.02, 76.00, 75.96, 73.84, 72.05, 68.33, 67.14, 25.22, 21.11 (Ac), 15.20. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{35}H_{38}O_6SNa$ 609.2281, found 609.2245.

Ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside S17

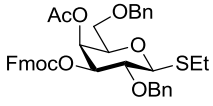


S17

To a solution of **S16** (1.4 g, 2.39 mmol, 1.0 eq.) in DCM and phosphate buffer (DCM:buffer, 5:1, 14 ml, 0.17 M) was added DDQ (1.4 g, 5.97 mmol, 2.5 eq.) at room temperature. After stirring for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM. The combined organic layer was washed with saturated aqueous $NaHCO_3$, and brine, and dried over $MgSO_4$. After the solvent was evaporated *in vacuo* the crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8/1/0.5 to 8/2/0.5) to give **S17** (0.78 g, 1.75 mmol, 73%). R_f = 0.11 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ = -11.53 (c = 4.00, chloroform); IR (thin film): υ = 3472, 2869, 1741, 1454, 1374, 1233, 1099, 1047 cm-1; ^{1}H NMR ($CDCl_3$) δ 7.53 – 7.09 (m, 10H), 5.39 (d, *J* = 3.3 Hz, 1H, H-4), 4.94 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 4.68 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 4.55 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.48 (d, *J* = 9.7 Hz, 1H, H-1), 4.44 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 3.80 (dd, *J* = 9.1, 3.4 Hz, 1H, H-3), 3.75 (t, *J* = 6.3 Hz, 1H, H-5), 3.56 (dd, *J* = 9.6, 6.1 Hz, 1H, H-6), 3.53 – 3.46 (m, 2H, H-2, H-6), 2.87 – 2.69 (m, 2H), 2.07 (s, 3H, Ac), 1.34 (t, *J* = 7.4 Hz,

3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.30 (Ac), 137.99, 137.76, 128.64, 128.54, 128.41, 128.17, 128.01, 127.93, 85.31, 79.01, 76.07, 75.68, 73.88, 73.71, 70.26, 68.33, 25.41, 21.00 (Ac), 15.17. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{24}H_{30}O_6SNa$ 469.1655, found 469.1754.

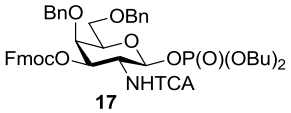
Ethyl 4-*O*-acetyl-2,6-di-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-galactopyranoside 16



16

To a solution of **S17** (0.78 g, 1.75 mmol, 1.0 eq) in anhydrous DCM (8.7 mL) were added 9-fluorenylmethylchloroformate (0.90 g, 3.49 mmol, 2.0 eq.) and pyridine (0.40 mL, 5.24 mmol, 3.0 eq.) successively at 0 °C, and stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, diluted with DCM, and was dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **16** (1.08 g, 1.62 mmol, 92%). R_f = 0.32 (hexane/ethyl acetate/DCM, 9:1:0.5); $[\alpha]_D^{20}$ = -1.01 (c = 3.45, chloroform); IR (thin film): υ = 1269, 1748, 1451, 1261, 1223, 1102 cm-1; ^{1}H NMR ($CDCl_3$) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (dd, *J* = 17.2, 7.6 Hz, 2H), 7.44 – 7.38 (m, 3H), 7.37 – 7.23 (m, 11H), 5.63 (d, *J* = 2.5 Hz, 1H), 4.87 (d, *J* = 9.7 Hz, 2H), 4.71 (d, *J* = 10.6 Hz, 1H), 4.56 (d, *J* = 10.1 Hz, 2H), 4.54 – 4.48 (m, 1H), 4.43 (d, *J* = 12.0 Hz, 1H), 4.33 – 4.25 (m, 2H), 3.84 (t, *J* = 6.2 Hz, 1H), 3.71 (t, *J* = 9.7 Hz, 1H), 3.57 (dd, *J* = 9.3, 6.3 Hz, 1H), 3.49 (t, *J* = 8.1 Hz, 1H), 2.86 – 2.71 (m, 2H), 2.09 (d, *J* = 2.3 Hz, 3H), 1.34 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.33 (Ac), 154.27 (Fmoc), 143.88, 143.24, 141.46, 141.39, 137.79, 137.75, 128.59, 128.47, 128.26, 128.08, 128.02, 128.00, 127.98, 127.95, 127.31, 127.27, 125.44, 125.26, 120.14 (Ar), 85.53 (C-1), 78.70 (C-3), 76.34 (C-2), 75.85 (CH_2Ph), 75.78 (C-5), 73.72 (CH_2Ph), 70.42 (CH_2, Fmoc), 68.05 (C-6), 68.02 (C-4), 46.82 (CH, Fmoc), 25.47, 20.90 (Ac), 15.20. MS ESI+-HRMS m/z $[M+Na]^+$ calcd for $C_{39}H_{40}O_8SNa$ 691.2342, found 691.2245.

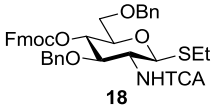
Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside 17



17

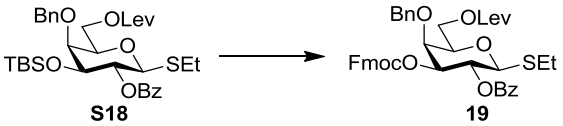
The known compound **17** was prepared according to literature[33].

Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside 18

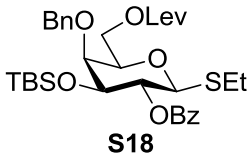


18

The known compound **18** was prepared according to literature[27].

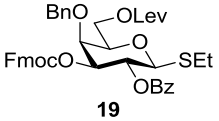


Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-6-*O*-levulinyl-1-thio-β-D-galactopyranoside S18



To a solution of compound **S9** (4.280 g, 8.03 mmol, 1.0 eq.) and DMAP (0.294 g, 2.41 mmol, 0.3 eq.) in anhydrous DCM (40 mL, 0.2 M) were added levulinic acid (2.80 g, 24.10 mmol, 3.0 eq.), and DIC (3.75 mL, 24.10 mmol) dropwise in an ice bath. After the mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and was dried over $MgSO_4$ the solvent was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane/ethyl acetate = 9/1/1 to 7/3/1) to afford **S18** (4.7 g, 7.45 mmol, 93 %). R_f (hexane:ethylacetate:DCM = 7:3:1) = 0.51; $[\alpha]_D^{20}$= +48.68 (c = 2.0, $CHCl_3$). IR (thin film): □ = 2886, 1719, 1452, 1352, 1259, 1069cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.07 – 7.99 (m, 2H), 7.62 – 7.50 (m, 1H), 7.48 – 7.41 (m, 2H), 7.41 – 7.31 (m, 4H), 7.31 – 7.25 (m, 1H), 5.63 (t, *J* = 9.5 Hz, 1H, H-2), 5.12 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.59 (d, *J* = 11.5 Hz, 1H, CH*H*Ph), 4.51 (d, *J* = 9.7 Hz, 1H, H-1), 4.27 (dd, *J* = 11.2, 6.6 Hz, 1H, H-6a), 4.19 (dd, *J* = 11.2, 5.8 Hz, 1H, H-6b), 3.98 (d, *J* = 9.2 Hz, 1H, H-3), 3.82 – 3.70 (m, 2H, H-4,5), 2.82 – 2.61 (m, 4H, CH_2 thio, CH_2 Lev), 2.57 – 2.46 (m, 2H, CH_2 of Lev), 2.18 (s, 3H, CH_3 of Lev), 1.21 (t, *J* = 7.5 Hz, 3H, CH_3 thio), 0.78 (s, 9H *tert*-Bu of Si), 0.13 (s, 3H, Me of Si), -0.07 (s, 3H, Me of Si). ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.60(C=O, Lev), 172.61(C=O, Lev), 165.46(C=O, Bz), 138.66, 133.07, 130.41, 129.93, 128.42, 128.41, 127.94, 127.67(Ar), 83.85(C-1), 77.20(C-4), 76.23(C-15, 75.71(C-3), 75.16(CH_2Ph), 70.99(C-2), 63.62(C-6), 38.07(CH_2 Lev), 29.95(Me, Lev), 28.02(CH_2, Lev), 25.66(*tert*-Bu, Si), 23.86(CH_2, thio), 17.92(Cq, Si), 14.96(Me, thio), -3.89(Me, Si), -4.94(Me, Si). MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{33}H_{46}O8SSiNa$ 653.2580, found 653.2530.

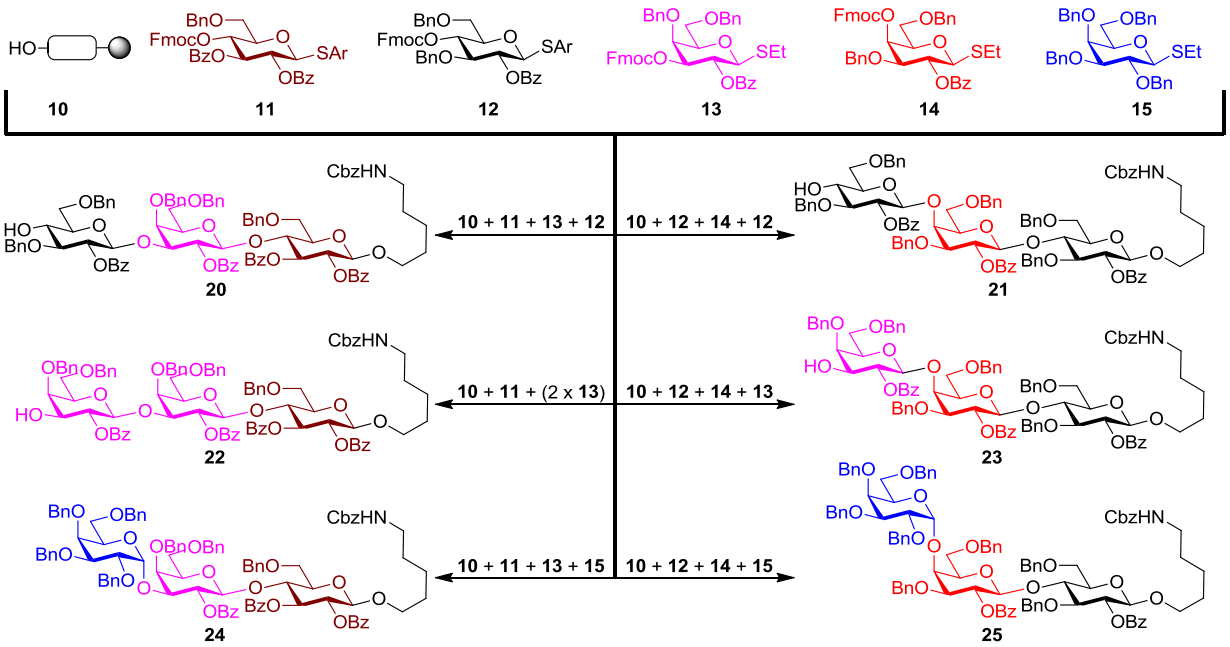
Ethyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-1-thio-β-D-galactopyranoside 19



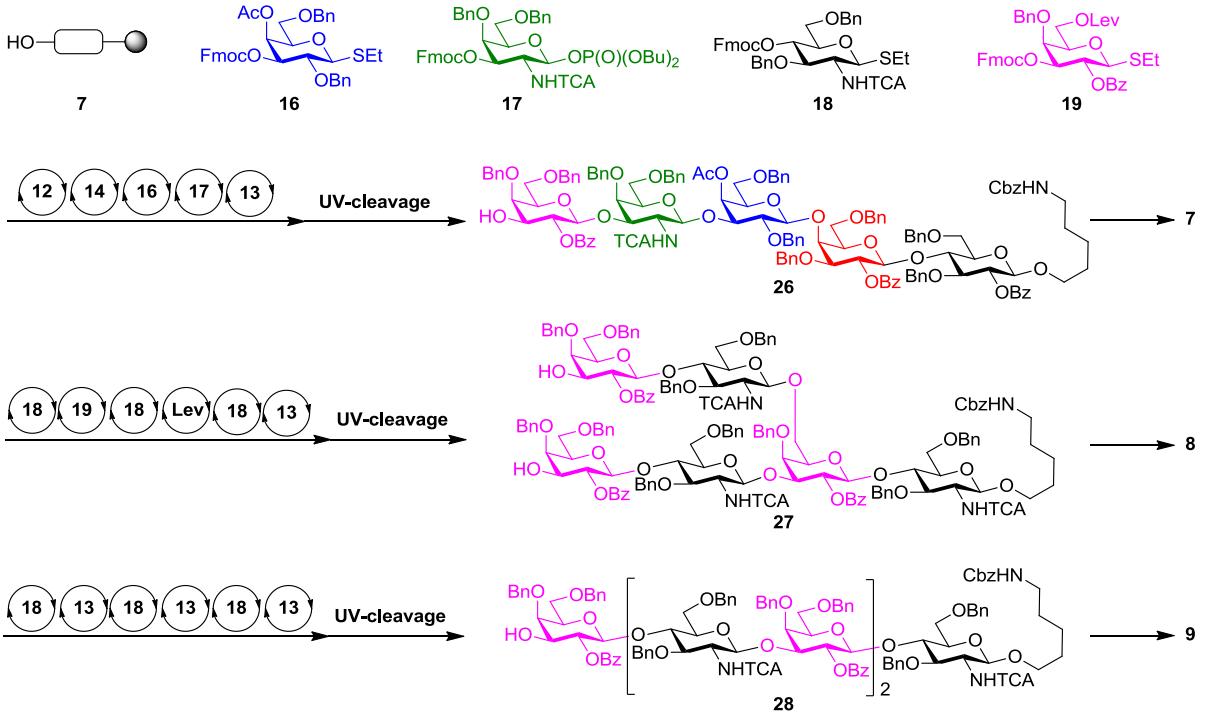
To a solution of compound **S18** (4.7 g, 7.45 mmol, 1.0 eq.) in dry acetonitrile (93 mL, 0.08 M) was added $BF_3{\cdot}OEt_2$ (1.13 mL, 8.94 mmol, 1.2eq.). After 30 min, the reaction mixture was quenched with saturated aqueous $NaHCO_3$, diluted with DCM, and dried over $MgSO_4$; the combined organic layer was evaporated *in vacuo*. To a solution of this crude product in anhydrous DCM (36 mL) were added 9-fluorenylmethylchloroformate (4.63 g, 17.91 mmol, 2.5 eq.) and pyridine (1.74 mL, 21.49 mmol, 3.0 eq.) successively at 0 °C, and the reaction mixture was stirred overnight at room temperature. After the mixture was quenched with 1M aqueous HCl, diluted with DCM, and dried over $MgSO_4$ the organic layer was evaporated *in vacuo*. The crude product was purified by column chromatography on silica gel (hexane:ethylacetate:DCM = 8:1:1 to 8:2:1) to afford **21** (5.0 g, 6.77 mmol, 94%). R_f (hexane:ethylacetate:DCM = 7:3:1) = 0.29; $[\alpha]_D^{20}$= +33.58 (c = 2.0, $CHCl_3$). IR (thin film):

υ = 3060, 1732, 1602, 1451, 1359, 1272, 1156 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.14 – 7.92 (m, 2H), 7.71 – 7.65 (m, 2H), 7.56 – 7.50 (m, 1H), 7.49 – 7.27 (m, 11H), 7.18 – 7.06 (m, 2H), 5.75 (t, *J* = 10.0 Hz, 1H, H-2), 5.08 (dd, *J* = 10.0, 2.9 Hz, 1H, H-3), 4.85 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.62 (d, *J* = 9.9 Hz, 1H, H-1), 4.55 (d, *J* = 11.4 Hz, 1H, CH*H*Ph), 4.37 – 4.20 (m, 3H, CH_2 Fmoc, H-6a), 4.16 (dd, *J* = 11.1, 6.4 Hz, 1H, H-6b), 4.07 (dd, *J* = 13.9, 5.2 Hz, 2H, C*H* Fmoc, H-4), 3.82 (dd, *J* = 6.8, 6.0 Hz, 1H, H-5), 2.84 – 2.66 (m, 4H, CH_2 Lev, CH_2 thio), 2.53 (t, *J* = 6.4 Hz, 2H, CH_2 Lev), 2.19 (s, 3H, Me Lev), 1.24 (t, *J* = 7.5 Hz, 3H, Me Lev). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.41(C=O, Lev), 172.30(C=O, Lev), 165.20(C=O, Bz), 154.49(C=O, Fmoc), 143.21, 142.75, 141.20, 141.08, 137.42, 133.25, 129.93, 129.47, 128.41, 128.39, 128.36, 127.94, 127.83, 127.11, 127.07, 125.15, 124.91, 119.98, 119.97(Ar), 83.82(C-1), 78.96(C-2), 75.90(C-5), 75.01(CH_2Ph), 73.52(C-4), 70.21(C-6), 68.36(C-2), 62.66(CH_2 Fmoc), 46.43(CH Fmoc), 37.86(CH_2 Lev), 29.85(Me Lev), 27.75(CH_2 Lev), 24.06(CH_2 thio), 14.82(Me thio), MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{42}H_{42}O_{10}SNa$ 761.2396, found 761.2333.

3 Automated Glycan Assembly



Scheme S1. Automated glycan assembly of oligosaccharides 20-25.



Scheme S2. Automated glycan assembly of oligosaccharides 26-28.

Preparation of the resin and the synthesizer for automated synthesis: The functionalized resin was loaded into the reaction vessel of the synthesizer and swollen in 2 mL DCM. To start the synthesis sequence, the resin was washed using Module 1. The building blocks were co-evaporated with toluene for three times, dissolved in DCM under an argon atmosphere and transferred into the vials that were placed on the corresponding port in the synthesizer. Reagents were dissolved in the corresponding solvents under an Ar atmosphere in bottles that were placed on the corresponding port in the synthesizer.

Module 1 – Acidic TMSOTf Wash: The resin is washed with DMF, THF, DCM (three times each, with 2 mL for 25 s), and 0.350 mL of solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in DCM once at -20 °C. The resin is swollen in 2 mL DCM and the temperature of the reaction vessel is adjusted to $\mathbf{T_a}$.

Module 2 – Glycosylation using thioglycoside: For glycosylation the DCM is drained and a solution of thioglycoside building block (5 eq. in 1.0 mL DCM) is delivered to the reaction vessel. After the set temperature is reached ($\mathbf{T_a}$), the reaction starts with the addition of 1 mL of NIS (5 eq. in 1.0 mL DCM), and TfOH (0.1 eq. in 1.0 mL DCM) solution. The glycosylation is performed for $\mathbf{t_1}$ at $\mathbf{T_a}$ and for $\mathbf{t_2}$ at $\mathbf{T_i}$. After the reaction the solution is drained and the resin is washed with DCM (six times with 2 mL for 15 s). This procedure is repeated twice.

Module 3 - Fmoc Deprotection: The resin is washed with DMF (six times with 2 mL for 15 s), swollen in 2 mL DMF and the temperature of the reaction vessel is adjusted to 25°C. For Fmoc deprotection the DMF is drained and 2 mL of a solution of 20% Et_3N in DMF is delivered to the reaction vessel. After 5 min the reaction solution is collected in the fraction collector of the oligosaccharide synthesizer and 2 mL of a solution of 20% Et_3N in DMF is delivered to the resin. This procedure is repeated three times.

Module 4 - Glycosylation using phosphate: For glycosylation the DCM is drained and a solution of phosphate building block (5 eq. in 1.0 mL DCM) is delivered to the reaction vessel. After the set temperature is reached ($\mathbf{T_a}$), the reaction starts with the addition of 1 mL of solution of TMSOTf. The glycosylation is performed for $\mathbf{t_1}$ at $\mathbf{T_a}$ and for $\mathbf{t_2}$ at $\mathbf{T_i}$. After the reaction the solution is drained and the resin is washed with DCM (six times with 2 mL for 15 s). This procedure is repeated twice.

Module 5 – Levulinoyl (Lev) deprotection: The resin is washed with DCM (six times with 2 mL for 25 s), swollen in 1.3 mL DCM and the temperature of the reaction vessel is adjusted to 25 °C. For Lev deprotection 0.8 mL of the hydrazine hydrate solution is delivered into the reaction vessel. After 30 min the reaction solution is drained and the resin is washed with 0.2 M acetic acid in DCM and DCM (six times each with 2 mL for 25 s). The entire procedure is performed three times.

Automated Glycan Assembly: The functionalized resin **10** (65 mg; loading 0.39 mmol/g; 0.0250 mmol) (Scheme S1) was loaded into the reaction vessel of the synthesizer and swollen in 2 mL CH_2Cl_2. To start the synthesis sequence, following sequences are carried out to achieve the target molecule.

The target oligosaccharide was synthesized with the corresponding sequences (Table S1). This crude molecule was confirmed with MALDI, and crude NMR (^{1}H, and HSQC), and analytical HPLC. This crude was purified using preparative HPLC. All data were collected for fully protected trisaccharide.

Table S1. Sequences for automated trisaccharide synthesis.

Sequence	Module	Details	Condition
I	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	2	5 eq. building block for **11, 12** and **18**, 5 eq. of NIS Solution	T_a = -30 °C, t_1 = 5 min T_i = -30 °C, t_2 = 25 min
	3	Fmoc Removal	r.t for 5 min
II	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	2	5 eq. building block for **13, 14,** and **16**, 5 eq. of NIS Solution	T_a = -40 °C, t_1 = 5 min T_i = -20 °C, t_2 = 25 min
	3	Fmoc Removal	r.t for 5 min
III	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	2	5 eq. building block for **15**, 5 eq. of NIS Solution	T_a = -40 °C, t_1 = 5 min T_i = -20 °C, t_2 = 25 min
IV	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	4	5 eq. building block **17**, 5 eq. of TMSOTf Solution	T_a = -40 °C, t_1 = 5 min T_i = -20 °C, t_2 = 25 min
	3	Fmoc Removal	r.t for 5 min
V	1	2.5 eq. of TMSOTf solution	-20 °C, for 1 min
	5	5 eq. building block for **19**, 5 eq. of NIS Solution	T_a = -40 °C, t_1 = 5 min T_i = -20 °C, t_2 = 25 min
	5	Lev Removal	r.t for 5 min

4 Purification of the Fully Protected Oligosaccharides

Analytical HPLC: The crude material was analyzed by HPLC (column: Luna 5μm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: 280 nm, and ELSD).

Preparative HPLC: The crude mixture was carefully dissolved in a minimum volume of DCM and 0.9 mL of 20% hexane in ethyl acetate. The crude solution was injected for purification using preparative HPLC (column: Luna 5μm Silica (260 x 10 mm); flow rate: 5 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: 280 nm, and ELSD) to afford the fully protected target oligosaccharide.

Table S2. Structures of oligosaccharides **20-28**.

Entry	Sequence	Isolated Yield
20	Glcβ(1→3)-Galβ(1→4)-Glcβ-O-$(CH_2)_5$-NHCbz	38%
21	Galβ(1→3)-Galβ(1→4)-Glcβ-O-$(CH_2)_5$-NHCbz	37%
22	Galα(1→3)-Galβ(1→4)-Glcβ-O-$(CH_2)_5$-NHCbz	35%
23	Glcβ(1→4)-Galβ(1→4)-Glcβ-O-$(CH_2)_5$-NHCbz	40%
24	Galβ(1→4)-Galβ(1→4)-Glcβ-O-$(CH_2)_5$-NHCbz	39%
25	Galα(1→4)-Galβ(1→4)-Glcβ-O-$(CH_2)_5$-NHCbz	35%
26	Galβ(1→3)-GalNAcβ(1→3)-Galα(1→4)-Galβ(1→4)-Glcβ-O-$(CH2)_5$-NHCbz	24%
27	Galβ(1→4)-GlcNAcβ(1→3)-[Galβ(1→4)-GlcNAcβ(1→6)]-Galβ(1→4)-GlcNAcβ-O-$(CH2)_5$-NHCbz	crude
28	Galβ(1→4)-GlcNAcβ(1→3)-Galβ(1→4)-GlcNAcβ(1→3)-Galβ(1→4)-GlcNAcβ-O-$(CH2)_5$-NHCbz	crude

***N*-benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside 20**

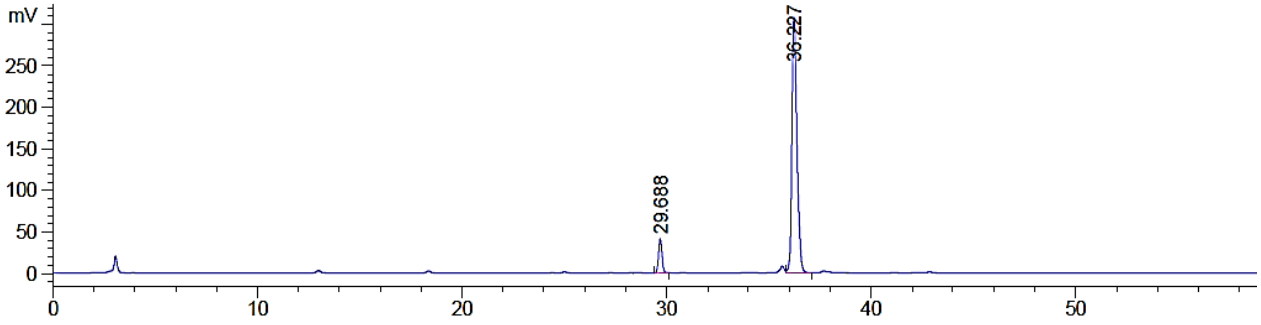


Figure S1. Purification of trisaccharide **20**. Conditions: column: Luna 5µm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.90 (d, *J* = 7.5 Hz, 2H), 7.82 (d, *J* = 7.4 Hz, 2H), 7.65 (d, *J* = 7.9 Hz, 4H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.48 (t, *J* = 7.4 Hz, 1H), 7.45 (t, *J* = 7.4 Hz, 1H), 7.40 – 7.20 (m, 30H), 7.18 – 7.10 (m, 7H), 7.08 – 7.03 (m, 2H), 5.50 (t, *J* = 9.4 Hz, 1H, H-3), 5.34

(dd, *J* = 9.9, 8.2 Hz, 1H, H'-2), 5.29 (dd, *J* = 9.5, 8.2 Hz, 1H, H-2), 5.20 – 5.15 (m, 1H, H''-2), 5.06 (s, 2H, CH_2, Cbz), 4.97 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.62 (d, *J* = 7.9 Hz, 1H, H''-1), 4.57 – 4.47 (m, 5H, 5 x C*H*HPh), 4.46 (d, *J* = 7.9 Hz, 1H, H-1), 4.42 (d, *J* = 4.2 Hz, 1H, C*H*HPh), 4.41 (d, *J* = 8.1 Hz, 1H, H'-1), 4.23 (d, *J* = 12.3 Hz, 1H, C*H*HPh), 4.06 (q, *J* = 11.9 Hz, 2H, CH_2Ph), 4.01 (t, *J* = 9.4 Hz, 1H, H-4), 3.88 (d, *J* = 1.9 Hz, 1H, H'-4), 3.82 – 3.74 (m, 3H, H'-3, H-6, OC*H*H$(CH_2)_4$NHCbz), 3.73 – 3.65 (m, 2H, H''-4, H-6), 3.55 – 3.49 (m, 1H, H-5), 3.49 – 3.30 (m, 6H, H''-3, 2 x H-5, H-6, OC*H*H$(CH_2)_4$NHCbz), 2.87 (ddd, *J* = 27.1, 13.7, 7.1 Hz, 4H, H-6, CH_2NHCbz), 2.67 (br, 1H, OH), 1.52 – 1.35 (m, 2H, CH_2, pentane), 1.31 – 1.24 (m, 2H, CH_2, pentane), 1.18 – 1.09 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.33 (Bz), 165.31 (Bz), 164.92 (Bz), 163.92 (Bz), 156.36 (Cbz), 139.23, 138.34, 138.18, 137.90, 137.74, 136.83, 133.14, 132.95, 132.72, 132.47, 130.58, 129.93, 129.80, 129.70, 129.67, 129.09, 128.62, 128.60, 128.53, 128.49, 128.46, 128.43, 128.41, 128.30, 128.20, 128.15, 128.08, 128.05, 127.98, 127.93, 127.87, 127.86, 127.82, 127.75, 127.72, 127.70, 127.19 (Ar), 101.65 (C''-1), 101.04 (C-1), 100.85 (C'-1), 82.33, 79.12, 77.37, 77.16, 76.95, 75.73, 75.15, 74.73, 74.68, 74.53, 74.48, 73.91, 73.44, 73.41, 73.17, 73.15, 72.47, 72.09, 71.95, 70.46, 69.71, 67.70, 67.41, 66.59, 40.90, 29.45, 28.96, 23.14.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{94}H_{95}NO_{22}Na$ 1612.6243, found 1612.6133.

_N_-benzyloxycarbonyl-5-amino-pentyl 2-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→3)-2-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside 21

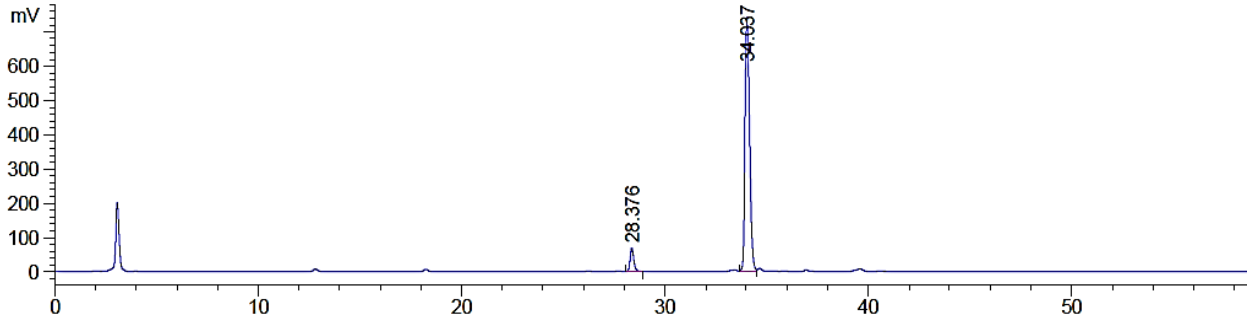


Figure S2. Purification of trisaccharide 21. Conditions: column: Luna 5 µm Silica 100 Å, (260 x4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz,$CDCl_3$) δ 7.91 (d, *J* = 8.3 Hz, 2H), 7.86 – 7.82 (m, 2H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.72 – 7.67 (m, 2H), 7.57 – 7.51 (m, 1H), 7.50 – 7.42 (m, 2H), 7.42 – 7.12 (m, 39H), 5.54 (t, *J* = 9.4 Hz, 1H, H-3), 5.40 (t, *J* = 8.8 Hz, 1H, H'-2), 5.30 (dd, *J* = 9.6, 8.1 Hz, 1H, H-2), 5.20 (dd, *J* = 9.9, 8.0 Hz, 1H, H''-2), 5.06 (s, 2H, CH_2, Cbz), 4.92 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.71 (dd, *J* = 36.2, 11.8 Hz, 2H, CH_2Ph), 4.62 (d, *J* = 7.8 Hz, 1H, H''-1), 4.57 (s, 1H, NHCbz), 4.52 – 4.42 (m, 6H, H-1, H'-1, CH_2Ph, 2 x C*H*HPh), 4.25 (d, *J* = 12.3 Hz, 1H, C*H*HPh), 4.08 – 3.99 (m, 3H, H-4, CH_2Ph), 3.87 (dd, *J* = 8.6, 5.9 Hz, 3H, H'-3, H''-3, H''-4), 3.82 – 3.75 (m, 1H, -OC*H*H$(CH_2)_4$-NHCbz), 3.72 – 3.62 (m, 3H, H-5, H-6), 3.56 (br, 1H, H'-4), 3.50 (dd, *J* = 11.0, 3.7 Hz, 1H, H-6), 3.41 (dd, *J* = 21.3, 12.5 Hz, 2H, H-5, H-6), 3.36 (dd, *J* = 15.2, 6.7 Hz, 1H, -OC*H*H$(CH_2)_4$-NHCbz), 3.30 (dd, *J* = 8.0, 4.9 Hz, 1H, H-5), 2.94 – 2.86 (m, 3H, H-6, CH_2NHCbz), 2.83 (t, *J* = 8.5 Hz, 1H, H-6), 2.38 (br, 1H, OH), 1.51 – 1.40 (m, 2H, CH_2, pentane), 1.32 – 1.25 (m, 2H, CH_2, pentane), 1.21 – 1.10 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.73 (Bz), 165.34 (Bz), 165.33 (Bz), 164.14

(Bz), 156.35 (Cbz), 139.12, 138.35, 138.21, 138.10, 137.65, 136.82, 133.14, 133.05, 132.86, 132.48, 130.57, 129.94, 129.89, 129.79, 129.67, 129.53, 128.65, 128.59, 128.50, 128.47, 128.43, 128.41, 128.29, 128.16, 128.13, 128.09, 128.08, 128.02, 128.00, 127.96, 127.90, 127.79, 127.71, 127.68, 127.16 (Ar), 101.35 (C''-1), 101.04 (C'-1), 100.90 (C-1), 78.71, 76.70, 75.59, 75.41, 75.18, 74.69, 74.59, 73.94, 73.84, 73.79, 73.47, 73.40, 73.18, 73.13, 73.09, 72.66, 72.11, 69.71, 68.52, 67.76, 67.51, 66.57, 40.89, 29.44, 28.96, 23.13.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{94}H_{95}NO_{22}Na$ 1612.6243, found 1612.6151.

_N_-Benzyloxycarbonyl-5-amino-pentyl 2,3,4,6-tetra-_O_-benzyl-α-D-galactopyranosyl-(1→3)-2-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranoside 22

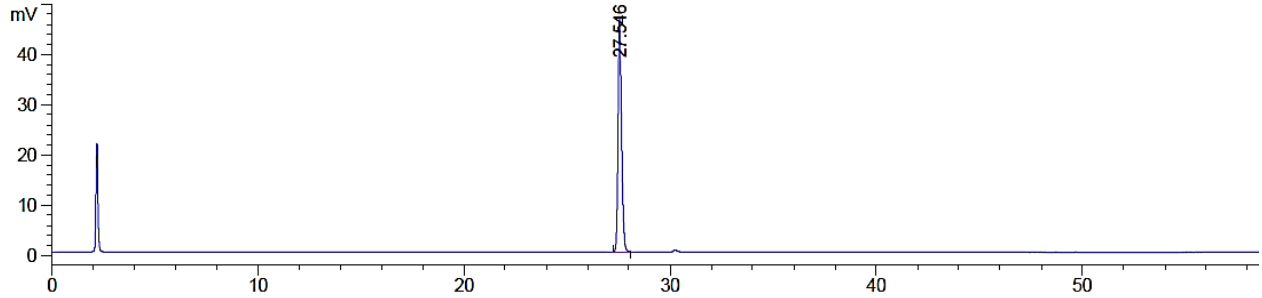


Figure S3. Purification of trisaccharide 22. Conditions: column: Luna 5µm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz,$CDCl_3$) δ 7.97 – 7.88 (m, 4H), 7.83 (d, *J* = 7.3 Hz, 2H), 7.47 (dd, *J* = 15.3, 7.5 Hz, 2H), 7.40 – 7.06 (m, 47H), 5.55 (m, 2H, H'-2, H-3), 5.31 (br, 1H, H-2), 5.07 (s, 2H, CH_2, Cbz), 4.96 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.90 (d, *J* = 3.3 Hz, 1H, H''-1), 4.78 (dd, *J* = 11.4, 6.0 Hz, 2H, 2 x C*H*HPh), 4.62 – 4.42 (m, 6H, H-1, H'-1), 4.37 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.31 – 4.14 (m, 4H), 4.10-4.00 (m, 3H, H-4, CH_2Ph), 3.97 (dd, *J* = 10.1, 3.3 Hz, 1H, H''-2), 3.90 – 3.83 (m, 2H, H'-4, H''-5), 3.81 (br, 1H, -OC*H*H$(CH_2)_4$), 3.73 (dd, *J* = 10.1, 2.5 Hz, 1H, H''-3), 3.67 – 3.46 (m, 5H, H'-3, H''-4, H-5, H-6), 3.39 (s, 1H, -OC*H*H$(CH_2)_4$-NHCbz), 3.21 (dd, *J* = 9.9, 7.1 Hz, 2H, H'-5, H''-6), 2.99 (dd, *J* = 8.7, 5.5 Hz, 1H, H''-6), 2.91 (s, 2H, C*H*$_2$NHCbz), 2.86 (d, *J* = 6.6 Hz, 2H, H'-6), 1.54 – 1.37 (m, 2H, CH_2, pentane), 1.34 – 1.24 (m, 2H, CH_2, pentane), 1.18 (s, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.36 (Bz), 165.29 (Bz), 164.84 (Bz), 156.39 (Cbz), 139.39, 138.85, 138.74, 138.55, 138.41, 138.26, 136.88, 133.15, 133.05, 132.58, 130.50, 130.19, 130.03, 129.92, 129.87, 129.76, 128.62, 128.48, 128.41, 128.23, 128.15, 128.12, 128.07, 127.85, 127.80, 127.75, 127.66, 127.61, 127.54, 127.49, 127.43, 127.05, 101.27 (C-1 or C'-1), 101.14 (C-1 or C'-1), 99.11 (C''-1), 80.51, 79.04, 77.36, 76.53, 75.63, 74.95, 74.92, 74.85, 74.63, 74.25, 73.80, 73.60, 73.37, 73.28, 73.12, 72.72, 72.26, 69.82, 69.75, 68.11, 68.06, 67.30, 66.62, 40.95, 29.52, 29.03, 23.19.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{101}H_{103}NO_{21}Na$ 1688.6920, found 1688.6920.

***N*-Benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 23**

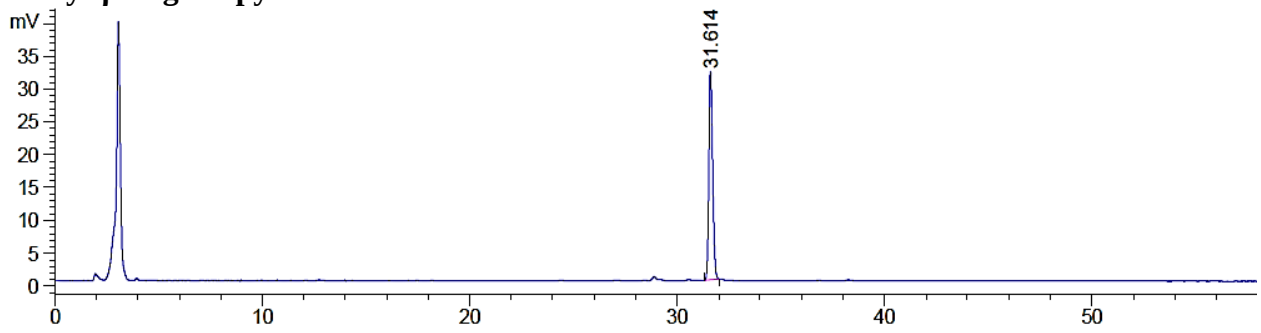


Figure S4. Purification of trisaccharide 23. Conditions: column: Luna 5μm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.09 (d, *J* = 7.7 Hz, 2H), 7.96 (d, *J* = 7.6 Hz, 2H), 7.84 (d, *J* = 7.7 Hz, 2H), 7.58 (t, *J* = 7.4 Hz, 1H), 7.53 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.4 Hz, 1H), 7.46 – 6.97 (m, 41H), 5.32 (d, *J* = 18.0 Hz, 1H, H-2), 5.27 (t, *J* = 8.7 Hz, 1H, H-2), 5.18 (d, *J* = 7.9 Hz, 1H, H-1), 5.14 – 5.09 (m, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 4.73 - 4.71 (m, 2H, 2 x C*H*HPh), 4.60 (d, *J* = 11.7 Hz, 1H, C*H*HPh), 4.58 (d, *J* = 12.4 Hz, 1H, C*H*HPh), 4.56 – 4.49 (m, 4H, H-1, CH_2Ph, C*H*HPh), 4.40 – 4.33 (m, 4H, H'-4, 3 x C*H*HPh), 4.31 – 4.26 (m, 2H, H-1, C*H*HPh), 4.23 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 3.88 (t, *J* = 9.2 Hz, 1H, H-4), 3.82 – 3.64 (m, 6H, H-3, H'-4, 3 x H-6, -OC*H*H$(CH_2)_4$-NHCbz), 3.53 – 3.40 (m, 6H, 2 x H-3, 2 x H-5, 2x H-6), 3.35 (dd, *J* = 9.6, 5.3 Hz, 1H, H-6), 3.29 (d, *J* = 6.6 Hz, 1H, -OC*H*H$(CH_2)_4$-NHCbz), 3.16 (d, *J* = 9.5 Hz, 1H, H-5), 2.88 (d, *J* = 6.1 Hz, 2H, CH_2NHCbz), 2.67 (br, 1H, OH), 1.50 – 1.33 (m, 2H, CH_2, pentane), 1.28 (t, *J* = 12.6 Hz, 2H, CH_2, pentane), 1.20 – 1.05 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.56 (Bz), 165.15 (Bz), 164.41 (Bz), 156.37 (Cbz), 138.77, 138.72, 138.41, 138.16, 137.90, 137.67, 136.86, 133.10, 133.04, 132.54, 130.49, 130.36, 130.30, 130.04, 129.80, 129.77, 128.61, 128.52, 128.48, 128.44, 128.43, 128.31, 128.19, 128.17, 128.15, 127.96, 127.94, 127.89, 127.88, 127.86, 127.78, 127.75, 127.69, 127.66, 127.55, 127.01 (Ar), 101.26 (C-1), 100.83 (C-1), 100.34 (C-1), 82.62, 80.56, 79.84, 74.97, 74.94, 74.55, 74.29, 74.00, 73.87, 73.80, 73.50, 73.40, 72.29, 72.12, 71.74, 70.66, 70.31, 69.40, 68.92, 67.99, 66.58, 40.92, 29.46, 28.97, 23.18.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{94}H_{97}NO_{21}Na$ 1598.6451, found 1599.6566.

***N*-Benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-4,6-di-O-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 24**

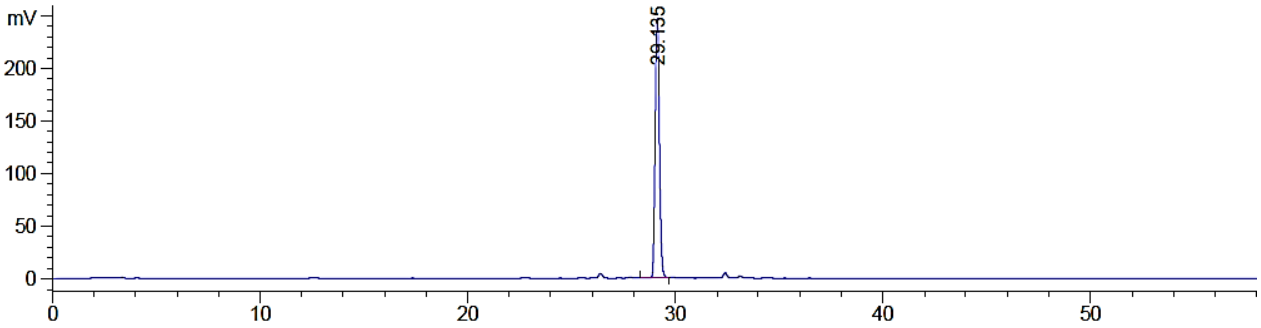


Figure S5: Purification of trisaccharide 17. Conditions: column: Luna 5µm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (d, *J* = 8.0 Hz, 2H), 7.97 (d, *J* = 7.7 Hz, 2H), 7.92 (d, *J* = 7.9 Hz, 2H), 7.61 (t, *J* = 7.0 Hz, 1H), 7.53 (dd, *J* = 13.1, 6.2 Hz, 2H), 7.47 (t, *J* = 7.4 Hz, 2H), 7.44 – 7.20 (m, 31H), 7.16 (t, *J* = 7.2 Hz, 2H), 7.12 (d, *J* = 7.2 Hz, 2H), 7.04 – 6.98 (m, 3H), 6.95 (t, *J* = 6.8 Hz, 1H), 5.48 (t, *J* = 8.8 Hz, 1H, H-2), 5.28 – 5.23 (m, 1H, H-2), 5.22 (d, *J* = 7.6 Hz, 1H, H''-1), 5.11 (t, *J* = 8.5 Hz, 1H, H-2), 5.06 (s, 2H, CH_2, Cbz), 4.92 (d, *J* = 11.5 Hz, 1H, C*H*HPh), 4.72 (d, *J* = 11.6 Hz, 1H, C*H*HPh), 4.66 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.59 – 4.54 (m, 3H, H-1, 2 x C*H*HPh), 4.50 – 4.43 (m, 4H, H'-4, CH_2Ph, C*H*HPh), 4.40 (d, *J* = 12.0 Hz, 1H, C*H*HPh), 4.30 (d, *J* = 7.9 Hz, 1H, H-1), 4.26 (d, *J* = 12.0 Hz, 2H, 2 x C*H*HPh), 4.16 (d, *J* = 10.7 Hz, 1H, C*H*HPh), 3.93 (d, *J* = 2.4 Hz, 1H, H''-4), 3.90 (t, *J* = 9.0 Hz, 1H, H-4), 3.82 – 3.72 (m, 2H, H''-3, OC*H*H$(CH_2)_4$), 3.68 (dd, *J* = 12.4, 6.4 Hz, 1H, H-6), 3.65 – 3.57 (m, 3H, H-5, 2 x H-6), 3.57 – 3.50 (m, 2H, H-3, H-6), 3.49 – 3.42 (m, 3H, H-3, H-5, H-6), 3.34 – 3.27 (m, 2H, H-6, OC*H*H$(CH_2)_4$), 3.19 (d, *J* = 8.1 Hz, 1H, H-5), 3.00 (br, 1H, OH), 2.89 (d, *J* = 6.7 Hz, 2H, C*H*$_2$NHCbz), 1.51 – 1.35 (m, 2H, CH_2, pentane), 1.34 – 1.22 (m, 2H, CH_2, pentane), 1.20 – 1.06 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.92 (Bz), 165.09 (Bz), 164.57 (Bz), 156.35 (Cbz), 138.67, 138.47, 138.42, 138.35, 137.91, 137.66, 136.84, 133.16, 133.04, 132.87, 130.66, 130.25, 130.03, 129.81, 129.71, 128.59, 128.59, 128.52, 128.47, 128.44, 128.42, 128.36, 128.16, 128.12, 127.96, 127.92, 127.87, 127.77, 127.58, 127.46, 127.05 (Ar), 101.26 (C-1), 100.76 (C-1), 99.73 (C-1), 80.67, 80.13, 77.01, 76.66, 75.87, 75.25, 74.99, 74.97, 74.20, 73.79, 73.63, 73.60, 73.50, 73.44, 72.30, 71.79, 69.39, 69.11, 68.66, 68.53, 67.96, 66.55, 40.90, 29.81, 29.43, 28.94, 23.15, 22.81, 14.24.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{94}H_{97}NO_{21}Na$ 1598.6451, found 1599.6567.

***N*-Benzyloxycarbonyl-5-amino-pentyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 25**

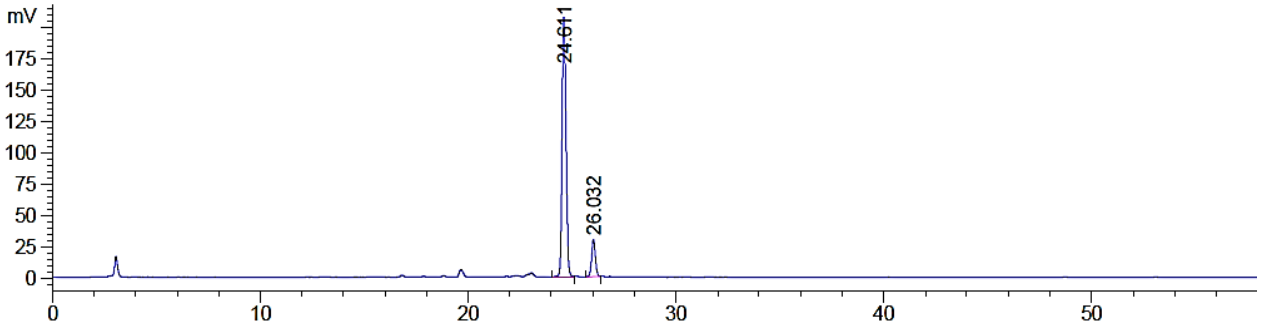


Figure S6. Purification of trisaccharide 25. Conditions: column: Luna 5μm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.7 Hz, 2H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.3 Hz, 1H), 7.46 (t, *J* = 7.6 Hz, 2H), 7.41 – 7.13 (m, 38H), 7.12 – 7.04 (m, 6H), 7.01 – 6.94 (m, 3H), 5.63 – 5.54 (m, 1H, H-2), 5.13 (t, *J* = 8.5 Hz, 1H, H'-2), 5.07 – 5.02 (m, 3H, H''-1, CH_2 of Cbz), 4.87 (dd, *J* = 11.6, 7.2 Hz, 2H, 2 x C*H*HPh), 4.80 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.74 (d, *J* = 11.8 Hz, 1H, C*H*HPh), 4.71 – 4.62 (m, 3H, H-1, 2 x C*H*HPh), 4.60 (d, *J* = 12.1 Hz, 1H, C*H*HPh), 4.56 – 4.48 (m, 3H, H-,2 x C*H*HPh), 4.41 (d, *J* = 11.3 Hz, 1H, C*H*HPh), 4.35 – 4.04 (m, 13H), 3.78 – 3.73 (m, 1H, H-6), 3.69 (t, *J* = 8.9 Hz, 1H, H'-3), 3.65 – 3.59 (m, 1H, H-6), 3.56 (t, *J* = 9.1 Hz, 3H, 2 x H-6, $OCH_2(CH_2)_4NHCbz$), 3.44 (br, 1H, H-5), 3.40 (d, *J* = 10.2 Hz, 1H, H-3), 3.29 (d, *J* = 7.4 Hz, 2H, H-5, H-6), 3.19 (dd, *J* = 8.6, 4.6 Hz, 1H, H-6), 2.85 (d, *J* = 6.0 Hz, 2H, C*H*$_2$NHCbz), 1.50 – 1.32 (m, 2H, CH_2, pentane), 1.31 – 1.19 (m, 2H, CH_2, pentane), 1.18 – 1.02 (m, 2H, CH_2, pentane). ^{13}C NMR (150 MHz, $CDCl_3$) δ 165.16 (Bz), 165.10 (Bz), 156.37 (Cbz), 139.19, 138.81, 138.76, 138.65, 138.60, 138.35, 138.31, 138.03, 136.87, 133.25, 133.01, 130.22, 130.15, 129.95, 129.89, 128.75, 128.62, 128.56, 128.48, 128.37, 128.35, 128.32, 128.26, 128.22, 128.20, 128.19, 128.16, 127.95, 127.84, 127.80, 127.58, 127.41, 127.39, 127.28, 127.18, 101.31 (C'-1), 101.15 (C''-1), 100.71 (C-1), 79.87, 79.52, 78.90, 76.79, 76.56, 75.13, 75.08, 74.92, 74.29, 74.16, 73.92, 73.62, 73.52, 73.31, 73.22, 73.00, 72.68, 72.24, 71.20, 69.48, 69.23, 68.10, 67.83, 67.46, 66.59, 40.92, 29.45, 28.96, 23.18.; MS ESI$^+$-HRMS *m/z* $[M+Na]^+$ calcd for $C_{101}H_{105}NO_{20}Na$ 1674.7128, found 1674.7037.

***N*-Benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→3)- 4,6-di-*O*-benzyl-2-deoxy-2-trichloracetamido-β-D-galactopyranosyl-(1→3)-4-*O*-acetyl-2-*O*-benzoyl-6-*O*-benzyl-α-D-galactopyranosyl-(1→3)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-glucopyranoside 26**

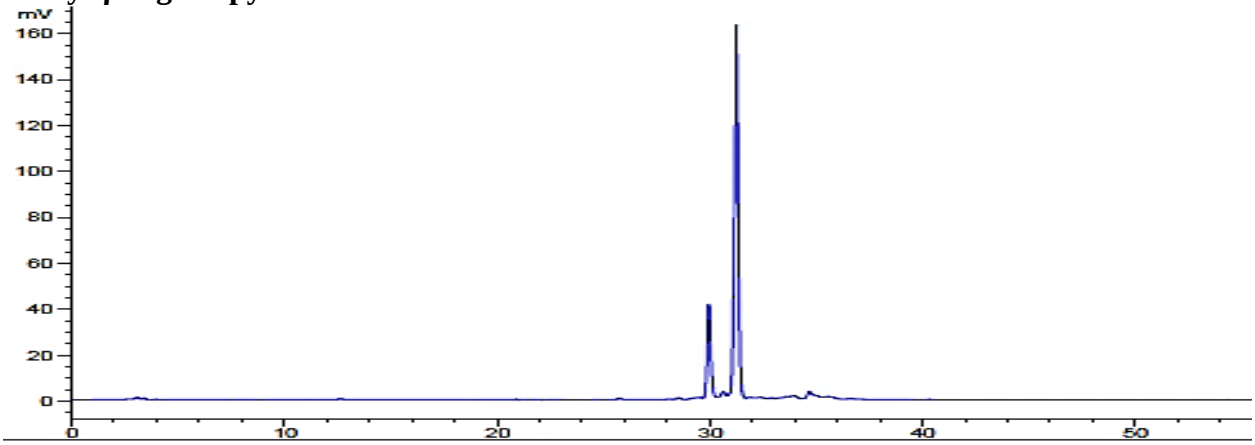


Figure S7. Purification of trisaccharide 26. Conditions: column: Luna 5μm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.5 Hz, 2H), 7.94 (d, *J* = 7.2 Hz, 4H), 7.59 (t, *J* = 7.3 Hz, 1H), 7.54 – 7.48 (m, 2H), 7.46 – 7.03 (m, 59H), 6.99 (d, *J* = 7.4 Hz, 2H), 6.61 (d, *J* = 7.7 Hz, 1H), 5.50 (s, 1H), 5.42 (dd, *J* = 10.1, 8.1 Hz, 1H), 5.31 (dd, *J* = 9.9, 8.0 Hz, 1H), 5.18 (t, *J* = 8.4 Hz, 1H), 5.06 (s, 2H), 4.96 (d, *J* = 3.0 Hz, 1H), 4.92 (d, *J* = 11.4 Hz, 1H), 4.87 (d, *J* = 11.8 Hz, 1H), 4.79 – 4.64 (m, 7H), 4.57 – 4.42 (m, 9H), 4.36 (dd, *J* = 19.2, 11.2 Hz, 3H), 4.29 – 4.13 (m, 7H), 4.08 (s, 1H), 4.04 (t, *J* = 8.8 Hz, 1H), 4.01 (s, 1H), 3.98 – 3.93 (m, 2H), 3.86 (ddd, *J* = 18.9, 10.5, 5.7 Hz, 2H), 3.81 – 3.59 (m, 8H), 3.54 (td, *J* = 12.8, 5.0 Hz, 3H), 3.43 – 3.26 (m, 7H), 2.88 (d, *J* = 6.0 Hz, 2H), 2.38 (d, *J* = 9.8 Hz, 1H), 1.93 (d, *J* = 9.6 Hz, 3H), 1.42 (dd, *J* = 37.1, 6.0 Hz, 2H), 1.31 – 1.21 (m, 2H), 1.14 (dd, *J* = 16.8, 7.8 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.21 (Ac), 166.94(Bz), 165.11(Bz), 165.03(Bz), 161.74(TCA), 156.35 (Cbz), 139.10, 138.85, 138.83, 138.64, 138.43, 138.18, 138.14, 137.73, 137.67, 136.84, 133.23, 133.11, 130.27, 130.19, 130.05, 129.96, 129.84, 128.70, 128.60, 128.54, 128.51, 128.40, 128.36, 128.28, 128.16, 128.14, 128.07, 128.05, 127.99, 127.94, 127.83, 127.79, 127.70, 127.64, 127.61, 127.59, 127.56, 127.37, 127.24 (Ar), 101.30, 101.06, 100.57, 100.32, 99.29 (**C''-1**), 92.61 (TCA), 80.86, 78.34, 77.50, 76.61, 75.71, 75.54, 75.47, 75.25, 74.82, 74.76, 74.16, 74.11, 73.99, 73.77, 73.74, 73.66, 73.52, 73.40, 73.26, 73.16, 73.00, 72.18, 71.21, 69.44, 69.29, 68.44, 68.21, 68.15, 68.01, 67.56, 66.58, 56.10, 40.91, 29.46, 28.98, 23.18, 21.24.; MS ESI^{+}-HRMS *m/z* $[M+NH_4]^+$ calcd for $C_{138}H_{147}N_3O_{32}Cl_3$ 2462.9028, found 2462.8802.

5 Fully Deprotected Trisaccharides and Final Purification

Deprotection: To the solution of the fully protected oligosaccharide in MeOH (0.2 mL/μmol of oligosaccharide) was added 58 μL of 0.5 M NaOMe solution (0.25 eq. per acetyl of benzoyl group) in MeOH at 40°C. The reaction was monitored by MS until it was completed, then neutralized by 200 mg of Amberite (400 mg per 100 μL of NaOMe solution). After filtering off the suspension, the crude mixture was dissolved in MeOH, EtOAc, and AcOH (v/v/v =5:0.5:0.2), followed adding 5% palladium on carbon (Pd/C) (50% w/w = Pd/oligosaccharide), purged first with argon and then with hydrogen, left to stir overnight at

room temperature under balloon pressure. The reaction mixture was filtered through modified cellulose filter, washed with 20 mL of water/MeOH, 9:1 and the combined solution was evaporated *in vacuo* to provide the crude.

5.1 Purification of Oligosaccharide Products

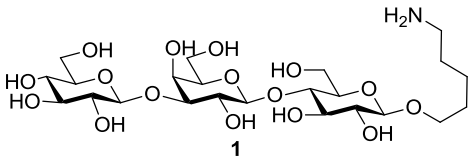
Analytical HPLC: The crude material was analyzed by HPLC (column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in acetonitrile/0.1% FA in triple distilled water (TDW); gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD).

Preparative HPLC: The crude solution is purified by preparative HPLC (column: Hypercarb®, (150 x 10.00 mm); flow rate: 3.6 mL/min; eluents: gradient: 0.1% FA in acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD) to afford the unprotected oligosaccharide.

Table S3. Structures of the trisaccharides **1-6**.

Entry	Sequence	Isolated Yield
1	Glcβ(1→3)-Galβ(1→4)-Glcβ(1→4)-O-$(CH_2)_5$-NH_2	55%
2	Galβ(1→3)-Galβ(1→4)-Glcβ(1→4)-O-$(CH_2)_5$-NH_2	52%
3	Galα(1→3)-Galβ(1→4)-Glcβ(1→4)-O-$(CH_2)_5$-NH_2	48%
4	Glcβ(1→4)-Galβ(1→4)-Glcβ(1→4)-O-$(CH_2)_5$-NH_2	50%
5	Galβ(1→4)-Galβ(1→4)-Glcβ(1→4)-O-$(CH_2)_5$-NH_2	53%
6	Galα(1→4)-Galβ(1→4)-Glcβ(1→4)-O-$(CH_2)_5$-NH_2	51%
7	Galβ(1→3)-GalNAcβ(1→3)-Galβ(1→4)-Galβ(1→4)-Glcβ-O-(CH2)5-NHCbz	24%
8	Galβ(1→4)-GlcNAcβ(1→3)-[Galβ(1→4)-GlcNAcβ(1→6)]-Galβ(1→4)-GlcNAcβ-O-(CH2)5-NHCbz	
9	Galβ(1→4)-GlcNAcβ(1→3)-Galβ(1→4)-GlcNAcβ(1→3)-Galβ(1→4)-GlcNAcβ-O-(CH2)5-NHCbz	

5-Amino-pentyl β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 1



1

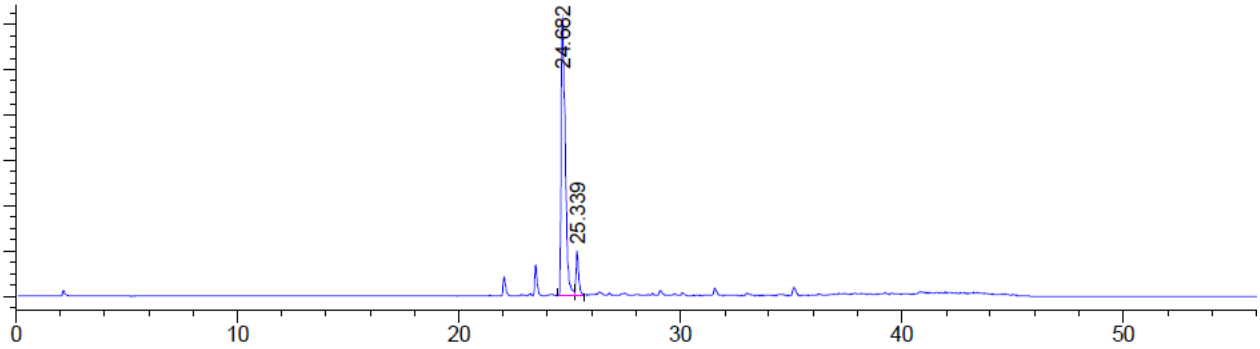
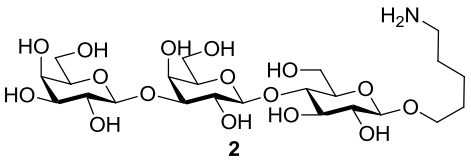


Figure S8. Purification of trisaccharide 1. Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 8.48 (s, 1H, *H*CO_2H), 5.17 (d, *J* = 4.0 Hz, 1H, H, H''-1), 4.55 (d, *J* = 8.0 Hz, 1H, H-1), 4.52 (d, *J* = 8.1 Hz, 1H, H-1), 4.25 – 4.19 (m, 2H), 4.05 (d, *J* = 3.1 Hz, 1H), 4.02 (dd, *J* = 12.3, 1.8 Hz, 1H), 3.99 – 3.94 (m, 2H), 3.89 (dd, *J* = 10.4, 3.9 Hz, 1H), 3.86 – 3.65 (m, 12H), 3.64 – 3.61 (m, 1H), 3.36 – 3.32 (m, 1H), 2.98 (t, *J* = 7.3 Hz, 2H, $OCH_2(CH_2)_4NH_2$), 1.73 – 1.66 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.52 – 1.44 (m, 2H, -$CH_2CH_2CH_2CH_2$-).). ^{13}C NMR (150 MHz, D_2O) δ 172.06 (CO_2H), 106.93 (C-1), 105.16 (C-1), 104.62 (C-1), 84.51, 80.88, 77.67, 77.60, 77.37, 77.06, 75.44, 75.12, 73.63, 72.79, 72.69, 71.18, 71.00, 63.59, 63.57, 62.71, 41.96 (CH_2NH_2), 30.76, 29.00, 24.68.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{43}NO_{16}Na$ 612.2474, found 612.2479.

5-Amino-pentyl β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 2



2

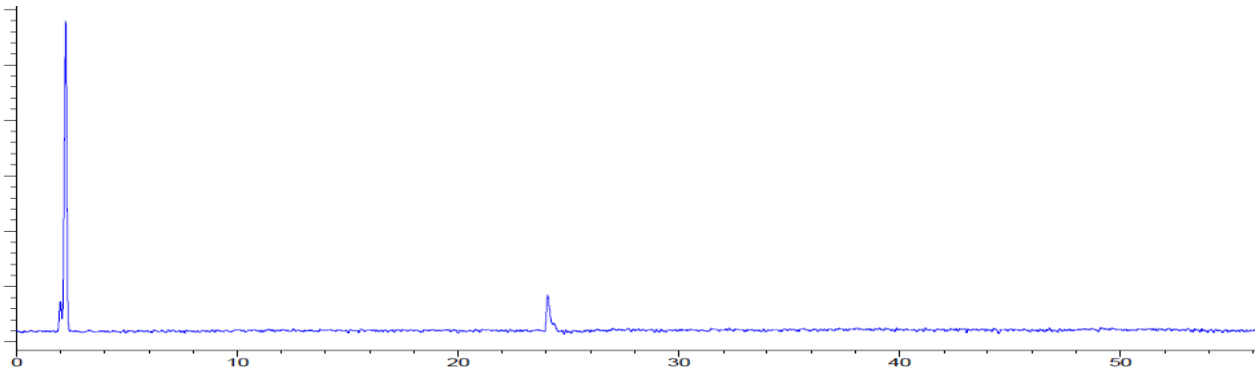
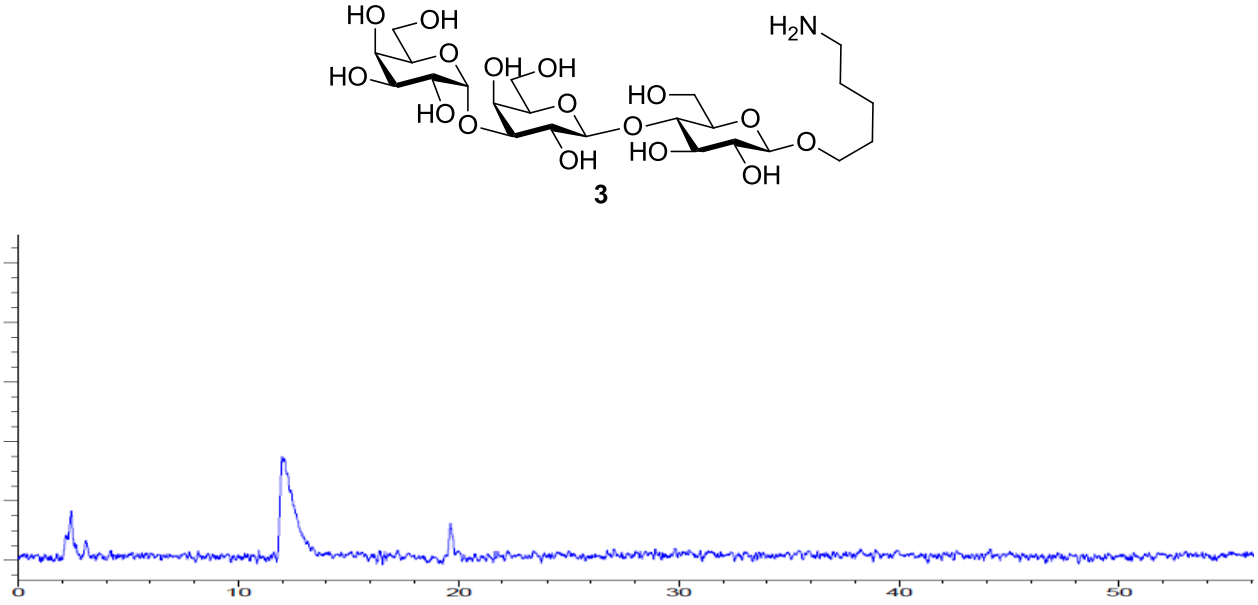


Figure S9: Purification of trisaccharide **2.** Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 8.42 (s, 1H, *H*CO_2H), 4.68 (d, *J* = 7.9 Hz, 1H, H-1), 4.51 (d, *J* = 10.3 Hz, 1H, H-1), 4.50 (d, *J* = 10.5 Hz, 1H, H-1), 4.20 (d, *J* = 3.2 Hz, 1H), 4.01 – 3.87 (m, 3H), 3.86 – 3.63 (m, 10H), 3.62 – 3.57 (m, 1H), 3.51 (t, *J* = 8.9 Hz, 1H), 3.48 – 3.41 (m, 2H), 3.38 (dd, *J* = 9.3, 8.0 Hz, 1H), 3.32 (t, *J* = 8.5 Hz, 1H, H-2), 3.02 (t, *J* = 7.5 Hz, 2H, O*C*H_2(CH_2)$_4$$NH_2$), 1.74 – 1.64 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.51 – 1.43 (m, 2H, -$CH_2CH_2CH_2CH_2$-). ^{13}C NMR (150 MHz, D_2O) δ 171.84 (CO_2H), 106.36 (C-1), 105.15 (C-1), 104.62 (C-1), 84.61, 80.86, 78.37, 78.12, 77.61, 77.36, 77.06, 75.88, 75.45, 72.74, 72.69, 72.00, 70.88, 63.59, 63.09, 62.70, 41.96 (*C*H_2NH_2), 30.76, 29.00, 24.68.; MS ESI$^+$-HRMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{43}NO_{16}Na$ 612.2474, found 612.2483.

5-Amino-pentyl α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 3

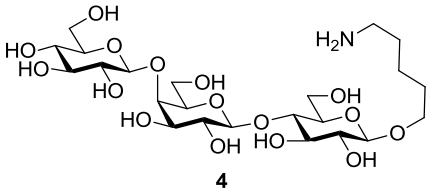


3

Figure S10. Purification of trisaccharide 3. Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 8.48 (s, 1H, *H*CO_2H), 5.17 (d, *J* = 4.0 Hz, 1H, H, H’’-1), 4.55 (d, *J* = 8.0 Hz, 1H, H-1), 4.52 (d, *J* = 8.1 Hz, 1H, H-1), 4.25 – 4.19 (m, 2H), 4.05 (d, *J* = 3.1 Hz, 1H), 4.02 (dd, *J* = 12.3, 1.8 Hz, 1H), 3.99 – 3.94 (m, 2H), 3.89 (dd, *J* = 10.4, 3.9 Hz, 1H), 3.86 – 3.65 (m, 12H), 3.64 – 3.61 (m, 1H), 3.36 – 3.32 (m, 1H), 2.98 (t, *J* = 7.3 Hz, 2H, O*C*H_2(CH_2)$_4$$NH_2$), 1.73 – 1.66 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.52 – 1.44 (m, 2H $OCH_2CH_2CH_2CH_2CH_2NH_2$). ^{13}C NMR (150 MHz, D_2O) δ 173.63 (CO_2H), 105.45 (C-1), 104.62(C-1), 98.03(C’’-1), 81.28, 79.81, 77.66, 77.35, 77.13, 75.41, 73.44, 72.75, 72.18, 71.89, 71.73, 70.79, 67.41, 63.59, 63.53, 62.77, 42.09 (*C*H_2NH_2), 30.80, 29.63, 24.73.; MS ESI^+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{44}NO_{16}Na$ 590.2655, found 590.2644.

5-Amino-pentyl β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 4



4

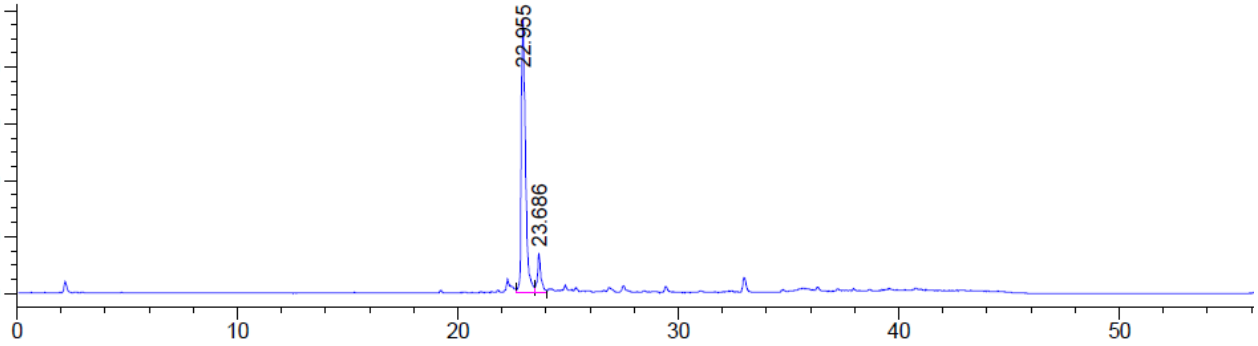
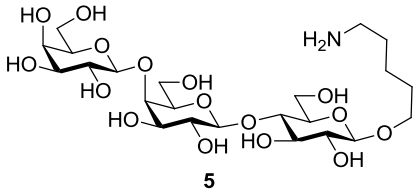


Figure S11. Purification of trisaccharide 4. Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 8.42 (s, 1H, *H*CO_2H), 4.67 (d, *J* = 7.9 Hz, 1H, H-1), 4.48 (d, *J* = 8.0 Hz, 1H, H-1), 4.48 (d, *J* = 7.8 Hz, 1H, H-1), 4.19 (d, *J* = 3.0 Hz, 1H), 4.01 – 3.89 (m, 3H), 3.85 – 3.56 (m, 11H), 3.51 (t, *J* = 9.0 Hz, 1H), 3.46 – 3.38 (m, 2H), 3.37 – 3.27 (m, 2H), 3.01 (t, *J* = 7.5 Hz, 2H, O*C*H_2(CH_2)$_4$$NH_2$), 1.74 – 1.64 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.50 – 1.42 (m, 2H, -$CH_2CH_2CH_2$-). ^{13}C NMR (150 MHz, D_2O) δ 172.18 (CO_2H), 106.17 (C-1), 105.47 (C-1), 104.61 (C-1), 80.97, 79.83, 78.44, 78.39, 77.38, 77.16, 77.05, 76.29, 75.52, 75.42, 73.96, 72.68, 72.13, 63.49, 63.28, 62.64, 41.96 (*C*H_2NH_2), 30.76, 29.00, 24.68.; MS ESI$^+$-HRMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{43}NO_{16}Na$ 612.2474, found 612.2565.

5-Amino-pentyl β-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 5



5

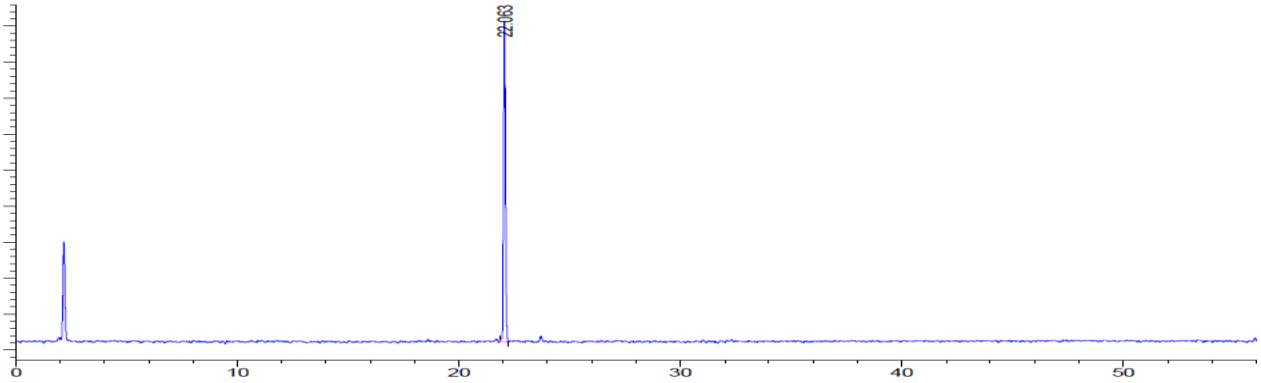
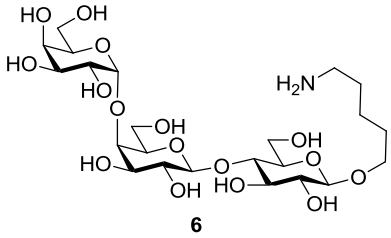


Figure S12. Purification of trisaccharide 5. Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 8.34 (s, 1H, HCO_2H), 4.48 (d, J = 7.9 Hz, 1H, H-1), 4.36 (d, J = 8.3 Hz, 1H, H-1), 4.36 (d, J = 7.8 Hz, 1H, H-1), 4.07 (d, J = 2.9 Hz, 1H), 3.88 – 3.77 (m, 3H), 3.74 – 3.61 (m, 7H), 3.60 – 3.42 (m, 8H), 3.20 – 3.15 (m, 1H), 2.88 (t, J = 7.5 Hz, 2H, $OCH_2(CH_2)_4NH_2$), 1.60 – 1.51 (m, 4H, $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.37 – 1.30 (m, 2H, -$CH_2CH_2CH_2$-). 13C NMR (150 MHz, D_2O) δ 173.02 (CO_2H), 106.80 (C-1), 105.51 (C-1), 104.60 (C-1), 81.07, 79.75, 77.74, 77.38, 77.11, 77.06, 75.56, 75.41, 74.05, 73.96, 72.68, 71.21, 63.59, 63.34, 62.65, 41.96 (CH_2NH_2), 30.76, 29.00, 24.68.; MS ESI+-HRMS *m/z* $[M+Na]^+$ calcd for $C_{23}H_{43}NO_{16}Na$ 612.2474, found 612.2484.

5-Amino-pentyl α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 6



6

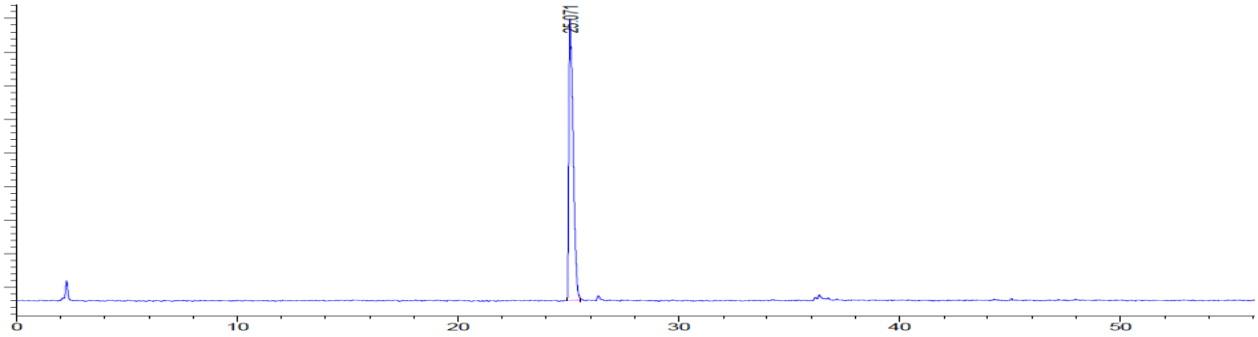
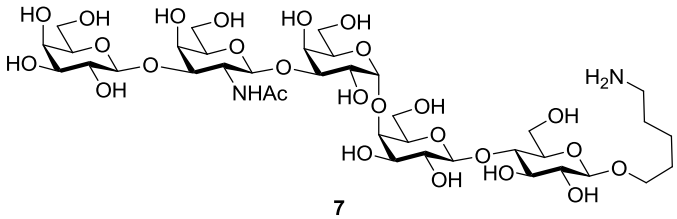


Figure S13. Purification of trisaccharide 6. Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 4.96 (d, *J* = 3.9 Hz, 1H, H''-1), 4.52 (d, *J* = 7.8 Hz, 1H, H-1), 4.50 (d, *J* = 8.0 Hz, 1H, H-1), 4.37 (t, *J* = 6.4 Hz, 1H), 4.07 – 3.56 (m, 18H), 3.34 – 3.29 (m, 1H), 3.04 – 3.00 (m, 2H, $OCH_2(CH_2)_4NH_2$), 1.75 – 1.64 (m, 4H, 2H $OCH_2CH_2CH_2CH_2CH_2NH_2$), 1.47 (dt, *J* = 15.3, 7.6 Hz, 2H, $-CH_2CH_2CH_2-$). ^{13}C NMR (150 MHz, D_2O) δ 108.44 (C-1), 107.14 (C-1), 105.48 (C''-1), 83.90, 82.54, 80.62, 79.99, 79.70, 78.09, 77.35, 76.10, 76.01, 75.25, 74.31, 74.12, 73.73, 65.69, 65.56, 65.24, 44.54 (CH_2NH_2), 33.33, 31.58, 28.43, 27.26.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{23}H_{43}NO_{16}Na$ 612.2474, found 612.2484.

5-Amino-pentyl β-D-galactopyranosyl-(1→3)-2-deoxy-2-*N*-amidoacetyl-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 7



7

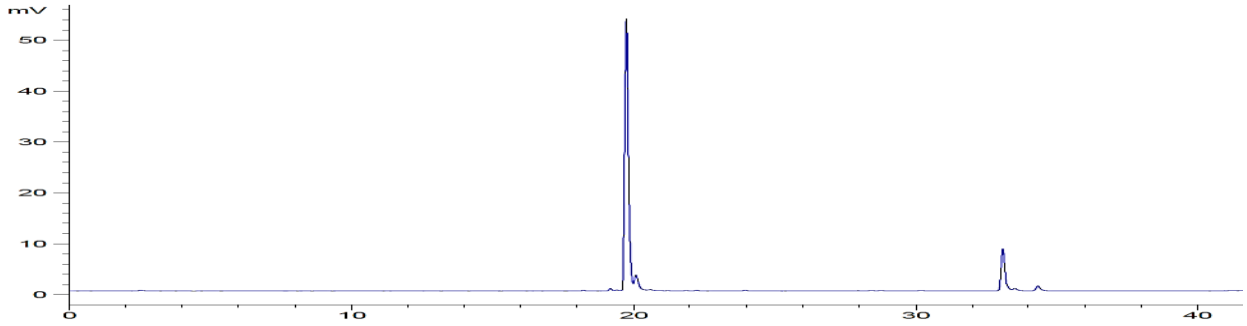
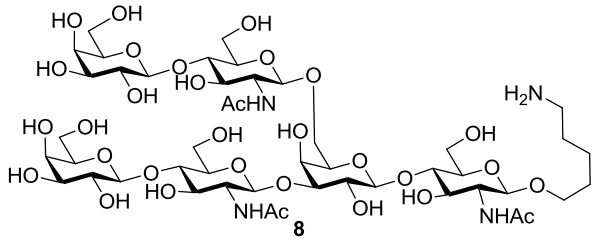


Figure S14. Purification of trisaccharide 7. Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (500 MHz, D_2O) δ 4.93 (d, *J* = 3.8 Hz, 1H, **H''-1**), 4.71 (d, *J* = 8.5 Hz, 1H), 4.53 (d, *J* = 7.7 Hz, 1H), 4.50 (d, *J* = 8.0 Hz, 1H), 4.47 (d, *J* = 7.6 Hz, 1H), 4.40 (s, 1H), 4.26 (s, 1H), 4.20 (d, *J* = 3.1 Hz, 1H), 4.10 – 3.90 (m, 10H), 3.88 – 3.51 (m, 21H), 3.32 (t, *J* = 8.5 Hz, 1H), 3.02 (t, *J* = 7.4 Hz, 2H), 1.70 (dd, *J* = 18.4, 8.1 Hz, 4H), 1.48 (d, *J* = 7.3 Hz, 2H). ^{13}C NMR (176 MHz, D_2O) δ 175.12 (Ac), 169.24 (Formic acid), 104.80, 103.31, 102.90, 101.97, 100.40 (C''-2), 79.58, 78.81, 78.67, 77.21, 75.46, 75.00, 74.81, 74.61, 74.55, 72.94, 72.46, 72.13, 70.89, 70.60, 70.29, 70.08, 68.92, 68.57, 67.99, 67.60, 61.00, 60.96, 60.38, 60.33, 60.07, 51.50, 39.35.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{23}H_{43}NO_{16}Na$ 612.2474, found 956.4018.

5-Amino-pentyl α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 8



8

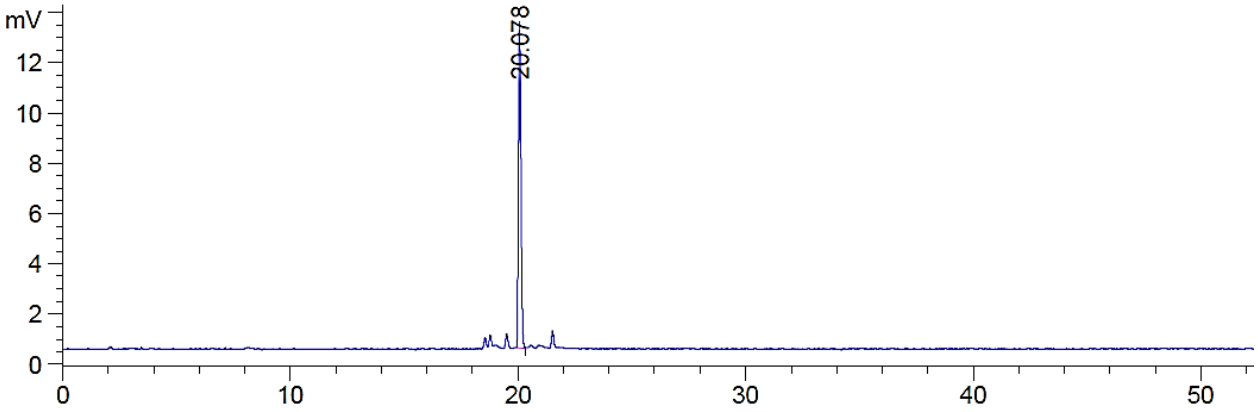
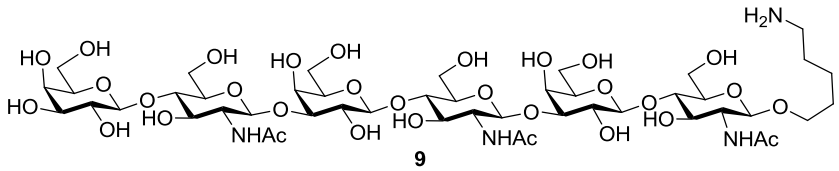


Figure S15. Purification of trisaccharide 8. Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 4.72 (d, *J* = 8.4 Hz, 1H, **H-1**), 4.63 (d, *J* = 7.8 Hz, 1H, **H-1**), 4.54 (d, *J* = 8.2 Hz, 1H, **H-1**), 4.51 – 4.47 (m, 3H, 3 x **H-1**), 4.17 (d, *J* = 3.0 Hz, 1H), 4.02 – 3.90 (m, 7H), 3.89 – 3.54 (m, 28H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.08 (s, 3H), 2.05 (d, *J* = 4.0 Hz, 6H), 1.69 (dt, *J* = 15.4, 7.7 Hz, 2H), 1.65 – 1.59 (m, 2H), 1.46 – 1.38 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.47 (NHAc), 177.07 (NHAc), 176.90 (NHAc), 171.67 (formic acid), 105.53 (**C-1)**, 105.47 (2 x **C-1**), 105.32 (**C-1)**, 103.71 (**C-1)**, 103.50 (**C-1)**, 84.42, 81.60, 80.97, 80.77, 77.95, 77.28, 77.16, 76.32, 75.07, 74.95, 74.78, 73.57, 72.71, 72.41, 71.26, 71.15, 63.62, 62.59, 57.81, 57.64, 41.94, 30.68, 28.98, 25.03, 24.78, 24.72.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{47}H_{83}N_4O_{31}Na$ 1221.4855, found 1221.4836.

5-Amino-pentyl α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside 9



9

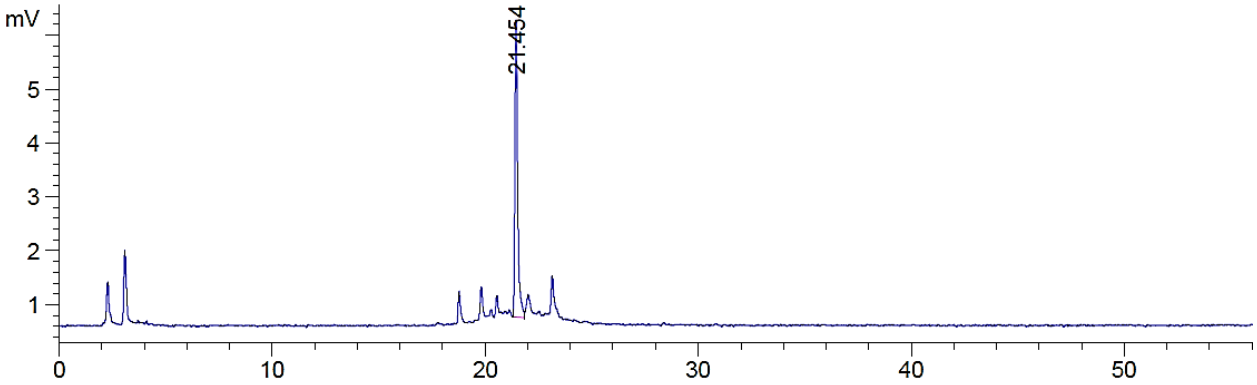
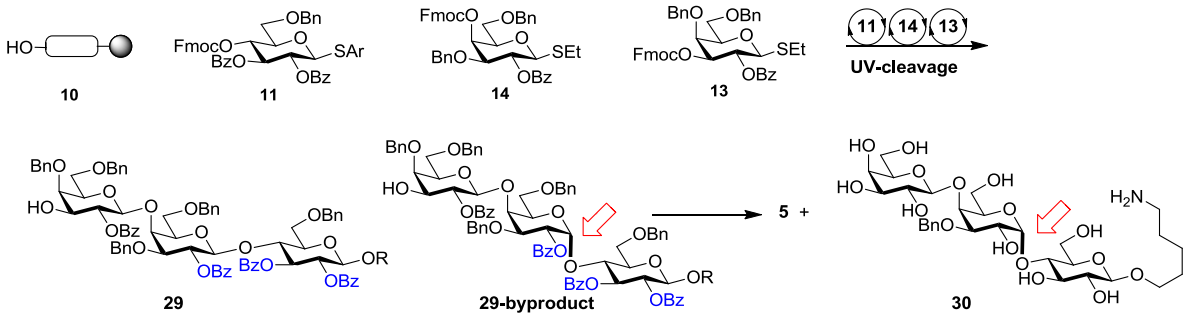


Figure S16. Purification of trisaccharide 28. Condition: column: Hypercarb®, (150 x 4.60 mm); flow rate: 0.8 mL/min; eluents: 0.1% FA in Acetonitrile/0.1% FA in TDW; gradient: 0% (10 min) 30% (in 30 min) 100% (in 5 min); detection: ELSD.

^{1}H NMR (600 MHz, D_2O) δ 4.57 (d, *J* = 8.1 Hz, 2H, 2 x **H-1**), 4.39 (d, *J* = 7.3 Hz, 1H, **H-1**), 4.34 (dd, *J* = 13.3, 6.8 Hz, 3H, 3 x **H-1**), 4.03 (s, 2H), 3.89 – 3.38 (m, 36H), 2.86 (t, *J* = 7.4 Hz, 2H), 1.91 (s, 9H), 1.58 – 1.51 (m, 2H), 1.50 – 1.42 (m, 2H), 1.31 – 1.22 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 177.50 (2 x NHAc), 177.01 (NHAc), 173.63 (Formic acid), 105.50 (3 x **C-1**), 105.47 (**C-1**), 105.35 (**C-1**), 103.71 (**C-1**), 84.68, 81.12, 80.80, 77.96, 77.48, 77.36, 77.16, 75.12, 75.01, 74.79, 73.57, 72.71, 72.56, 71.15, 70.91, 63.63, 63.55, 62.67, 62.47, 57.80, 57.67, 41.94, 30.68, 28.99, 24.78.; MS ESI+-HRMS *m/z* [M+H]+ calcd for $C_{47}H_{84}N_4O_{31}$ 1199.5036, found 1199.4991

5.2 Alternative synthesis of trisaccharide 5



Scheme S3. Automated glycan assembly of 29. The alternative synthesis of **5** using building block **11** instead of **12** leads to the formation of a by-product **30**.

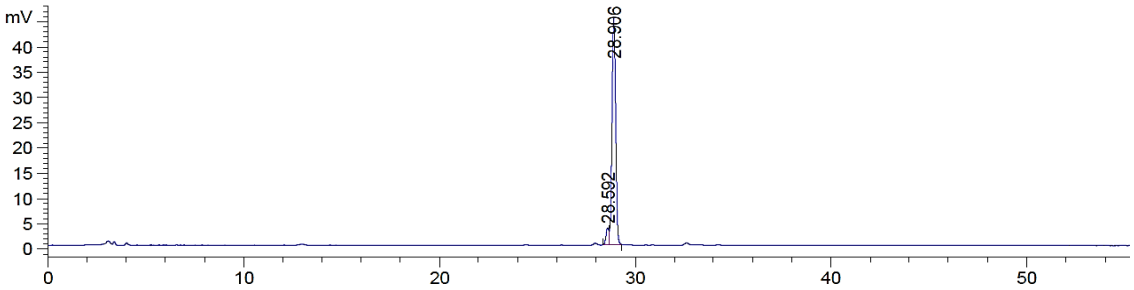


Figure S17. HPLC analysis of trisaccharide 29. Conditions: column: Luna 5µm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

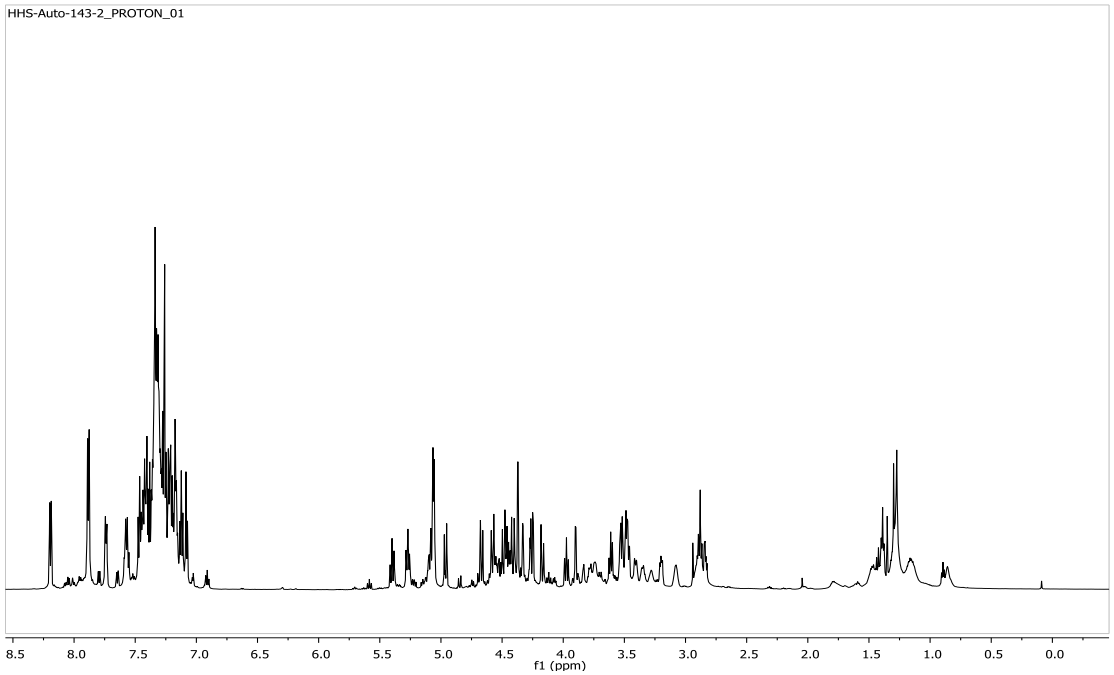


Figure S18. 1H NMR spectra of the crude product **29**.

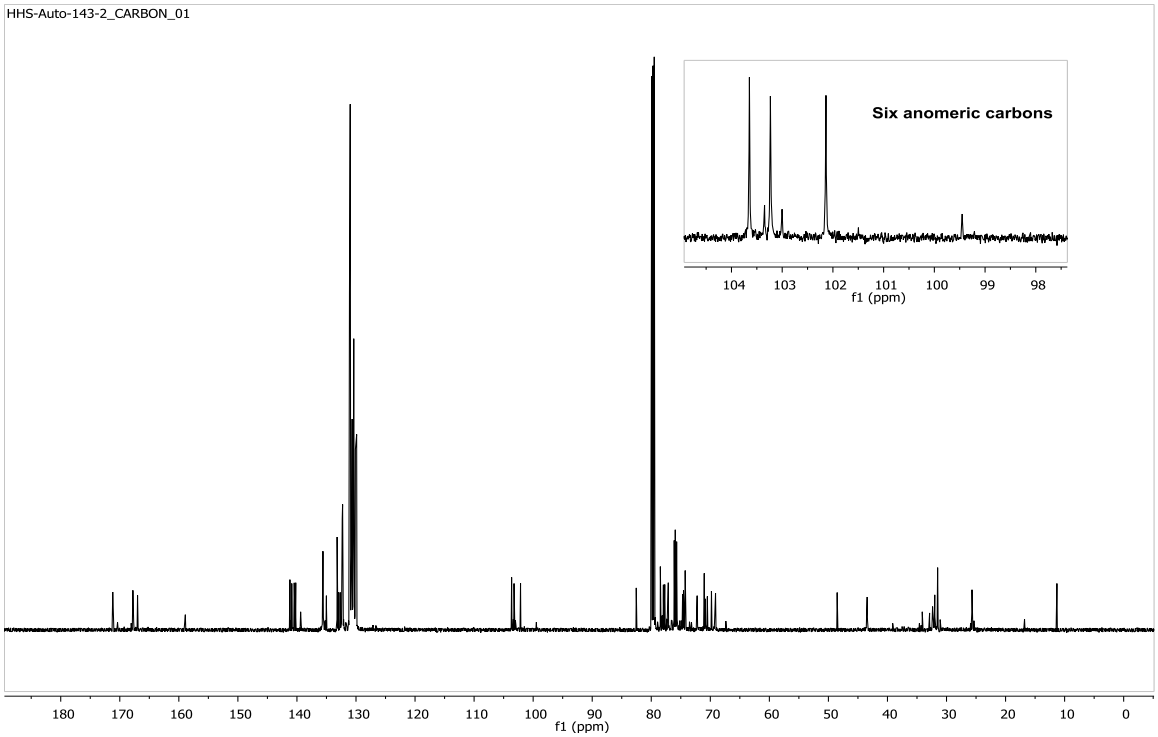


Figure S19. ^{13}C NMR spectra of the crude product **29**.

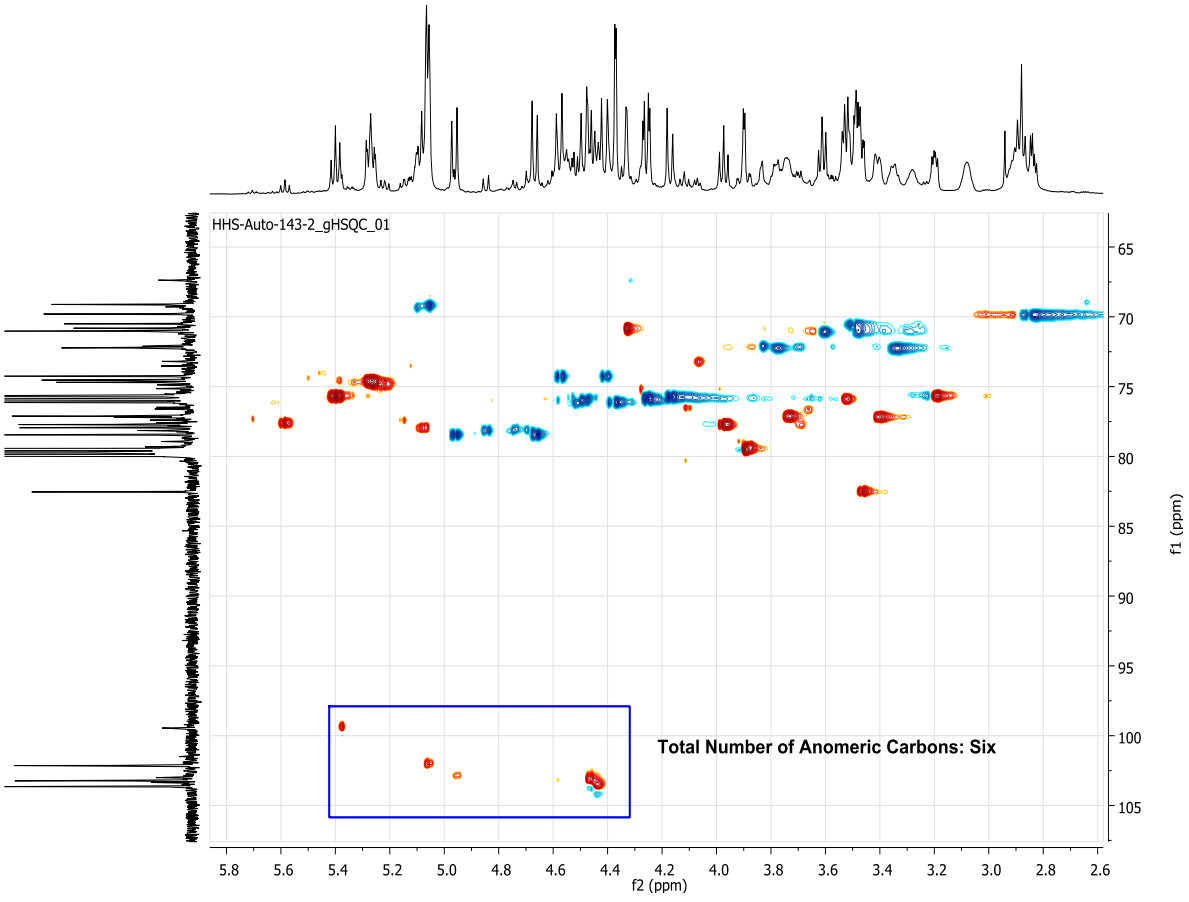


Figure S20. ^{1}H-^{13}C HSQC NMR spectra of the crude product **29**.

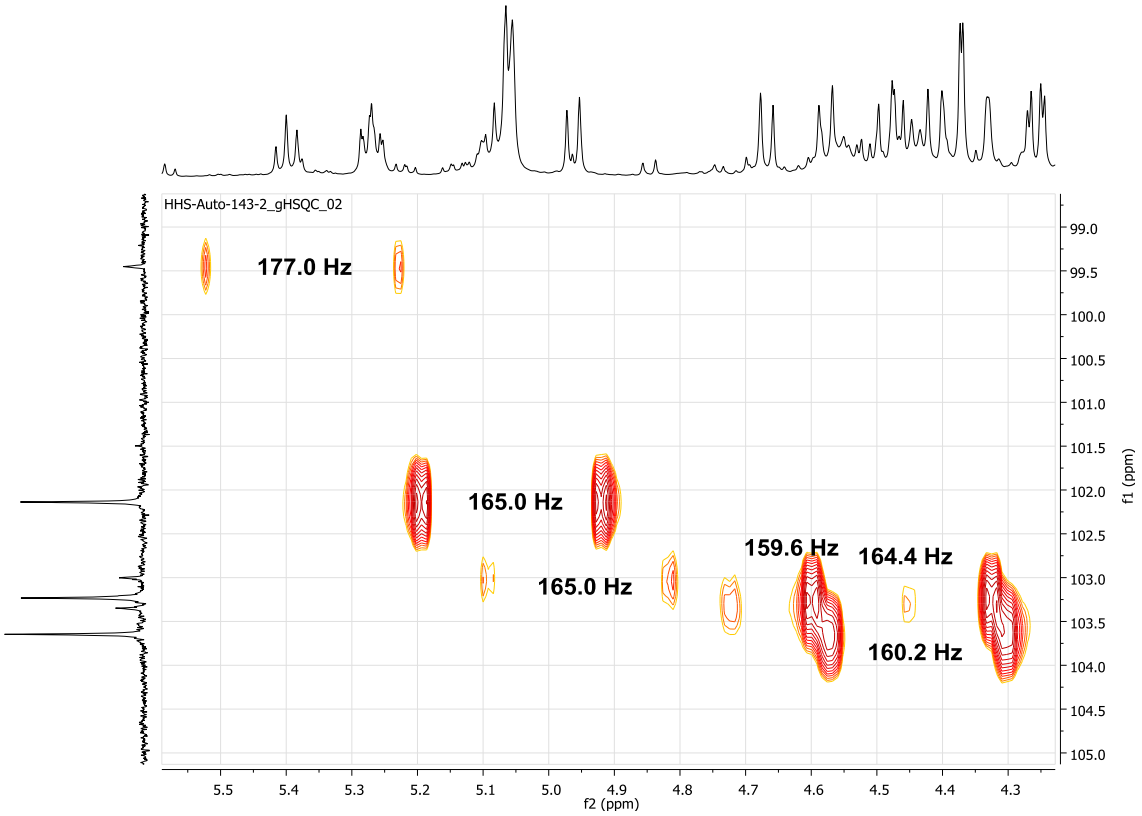
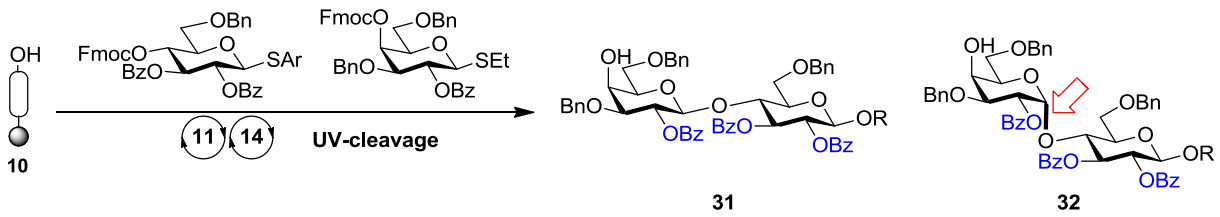


Figure S21. ^{1}H-^{13}C coupled-HSQC NMR spectra of the crude product **29**.

To obtain the trisaccharide **5**, which exhibits only *β*-glycosidic bonds, the synthesis following Scheme 3 was performed. The NMR analysis of the fully protected crude product **29**, however, indicated the unexpected result of one *α*-anomeric carbon and two *β*-anomeric carbons. Because the installation of 1,2-*cis* glycosidic linkage has not been reported on the automated synthesis, it was assumed that the glycosylation reaction between the linker (**10**) and the first donor (**11**) bearing neighboring participation groups as a benzoyl ester at C2 provided the exclusive 1,2-*trans* linkage. Therefore, we hypothesized that the second glycosylation resulted in the unexpected glycosylation outcome upon assembly of the trisaccharide. To confirm this hypothesis, the disaccharide **31** was synthesized (Scheme S4) using building block **11** and **14**. Following automated glycan assembly, two products were purified by the preparative NP-HPLC. The characterization by NMR experiments shows that the byproduct **32** is a stereoisomer of the desired product **31** (Scheme S4 and Figure S20). In conclusion, the trisaccharide **29-byproduct**, as a stereoisomer of **29** was rationally elucidated based on this disaccharide synthesis.



Scheme S4. Automated glycan assembly of 31.

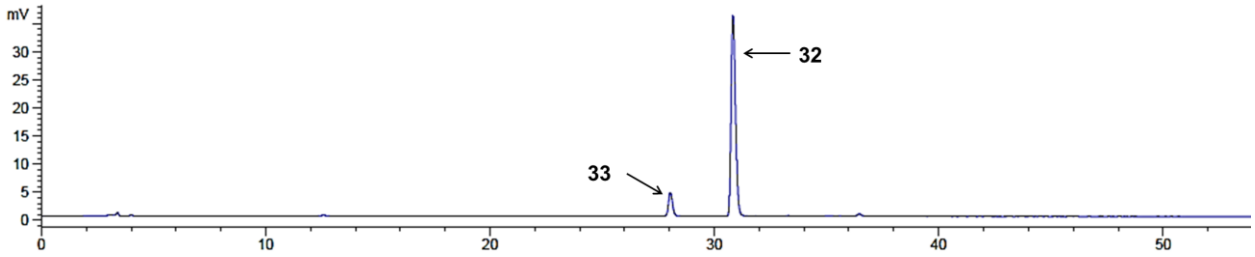


Figure S22. Analysis of disaccharide **31** and **32**. Conditions: column: Luna 5µm Silica 100 Å, (260 x 4.60 mm); flow rate: 1 mL/min; eluents: 5% DCM in hexane/5% DCM in ethyl acetate; gradient: 20% (5 min) 60% (in 40 min) 100% (in 5 min); detection: ELSD.

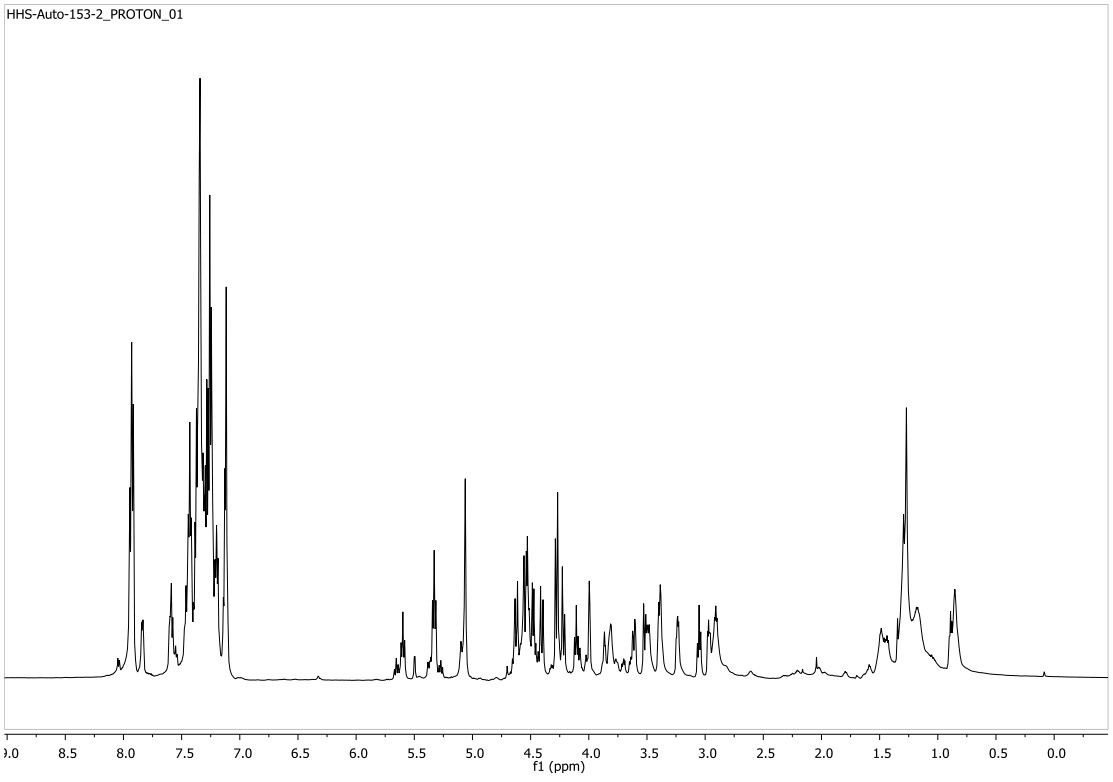


Figure S23. ^{1}H NMR experiments of the crude product **31**.

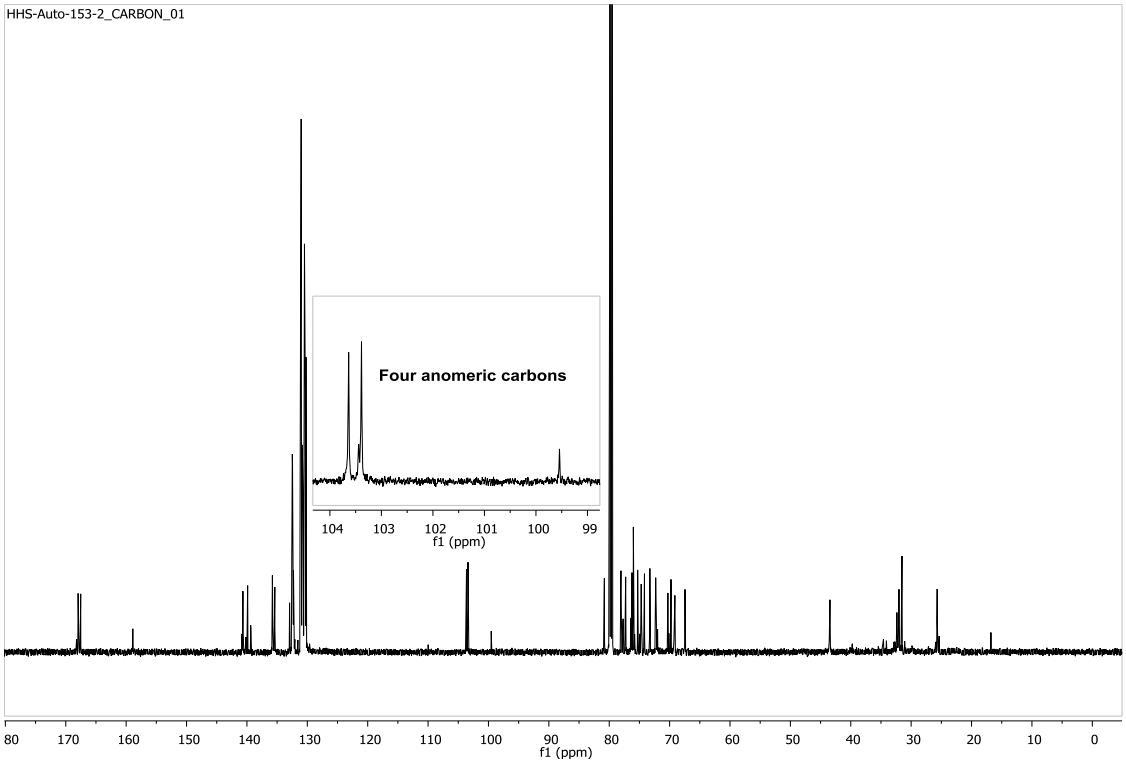


Figure S24. ^{13}C NMR experiments of the crude product 31.

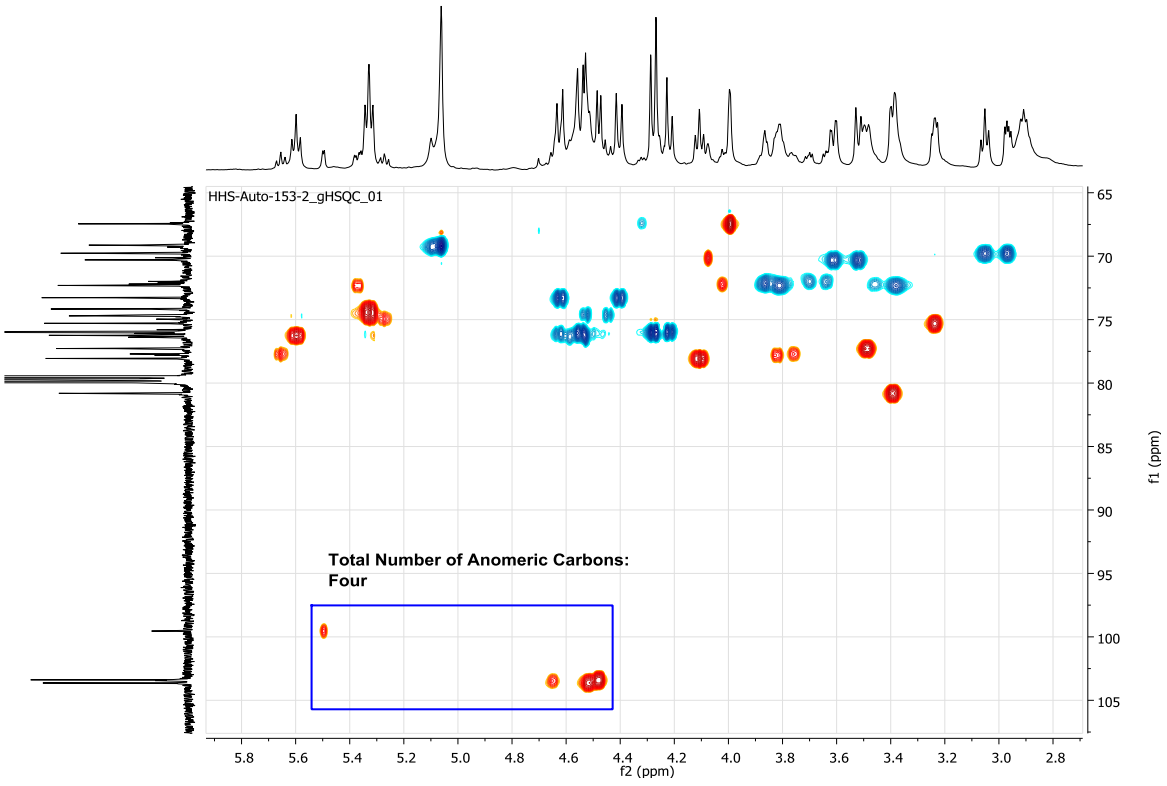


Figure S25. ^{11}H-^{13}C HSQC NMR experiments of the crude product 31.

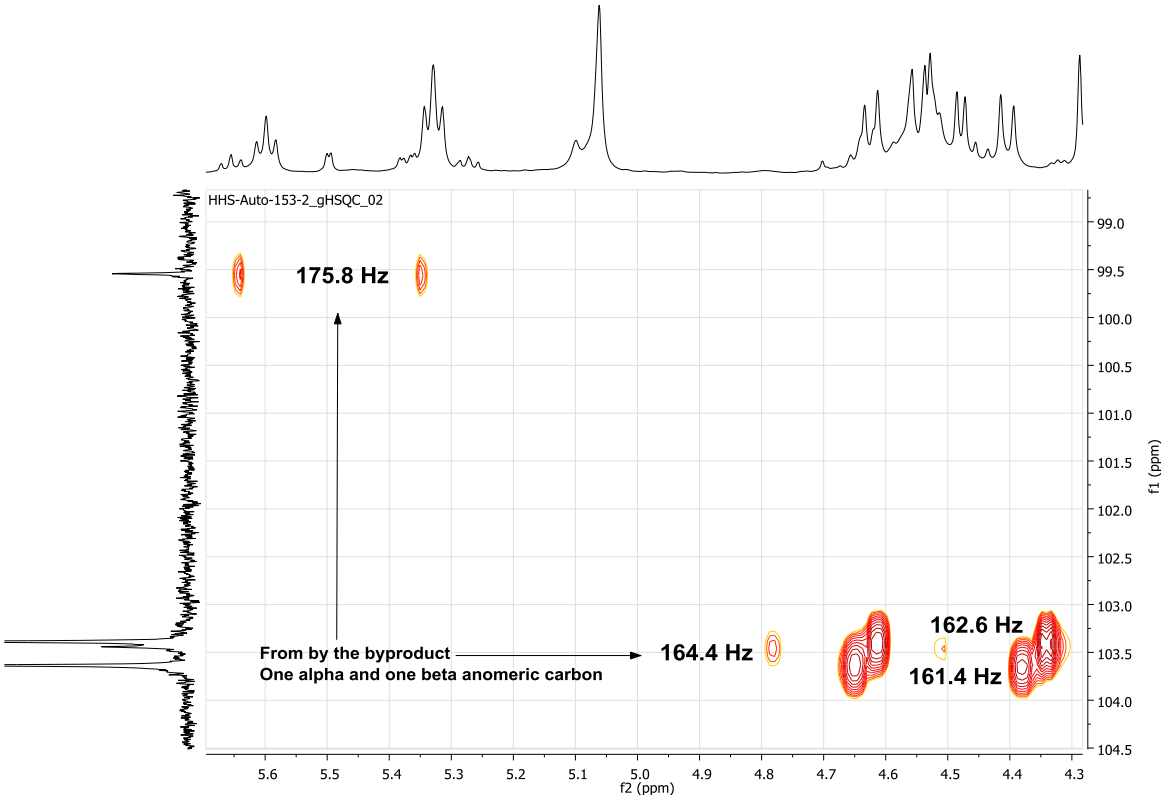


Figure S26. ^{1}H-^{13}C coupled-HSQC NMR experiments of the crude product 31.

N-benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside 31

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (t, *J* = 7.9 Hz, 6H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.51 – 7.16 (m, 24H), 7.15 – 7.08 (m, 4H), 5.59 (t, *J* = 9.4 Hz, 1H, H-3), 5.32 (dd, *J* = 9.7, 8.0 Hz, 2H, H-2, H'-2), 5.06 (s, 2H, CH_2, Cbz), 4.62 (d, *J* = 12.5 Hz, 1H, C*H*HPh), 4.56 – 4.44 (m, 4H, **H-1**, **H'-1**, C*H*HPh, NH), 4.40 (d, *J* = 12.5 Hz, 1H, C*H*HPh), 4.26 (d, *J* = 12.2 Hz, 1H, C*H*HPh), 4.24 (dd, *J* = 27.3, 11.9 Hz, 2H, CH_2Ph), 4.10 (t, *J* = 9.4 Hz, 1H, H-4), 4.01 – 3.96 (m, 1H, H'-4), 3.81 (dt, *J* = 11.6, 5.9 Hz, 1H, OC*H*H, linker), 3.60 (dd, *J* = 11.1, 3.8 Hz, 1H, H-6), 3.50 (t, *J* = 9.9 Hz, 2H, H-5, H-6), 3.37 (dt, *J* = 9.4, 4.7 Hz, 2H, H'-3, OC*H*H, linker), 3.22 (dd, *J* = 8.1, 5.1 Hz, 1H, H'-5), 3.03 (t, *J* = 8.7 Hz, 1H, H'-6), 2.95 (dd, *J* = 9.1, 5.0 Hz, 1H, H'-6), 2.93 – 2.83 (m, 2H, C*H*$_2$NHCbz), 2.21 (br, 1H, OH), 1.54 – 1.37 (m, 2H), 1.34 – 1.22 (m, 2H), 1.21 – 1.07 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.41 (Bz), 165.33 (Bz), 164.99 (Bz), 156.36 (Cbz), 138.23, 138.09, 137.32, 136.80, 133.26, 133.22, 132.86, 130.37, 129.96, 129.92, 129.82, 129.62, 128.62, 128.56, 128.52, 128.49, 128.30, 128.22, 128.17, 127.95, 127.89, 127.87, 127.81, 127.71 (Ar), 101.10 (**C-1** or **C'-1**), 100.84 (**C'-1** or **C-1**), 78.19, 77.36, 75.52, 74.70, 73.67, 73.47, 73.41, 72.70, 72.12, 71.58, 70.67, 69.80, 67.70, 67.16, 66.60, 64.85, 40.90, 29.48, 28.97, 23.15.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{67}H_{69}NO_{16}Na$ 1166.4509, found 1166.4521.

N-benzyloxycarbonyl-5-amino-pentyl 2-*O*-benzoyl-3,6-di-*O*-benzyl-α-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranoside 32

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 – 7.79 (m, 4H), 7.56 (dd, *J* = 17.9, 7.3 Hz, 4H), 7.46 – 7.14 (m, 25H), 7.11 (d, *J* = 7.3 Hz, 2H), 5.64 (t, *J* = 9.5 Hz, 1H, H-3), 5.48 (d, *J* = 3.8 Hz, 1H,

H'-1), 5.35 (dd, *J* = 10.4, 3.9 Hz, 1H, H'-2), 5.26 (dd, *J* = 9.7, 7.9 Hz, 1H, H-2), 5.06 (s, 2H, Cbz), 4.64 (d, *J* = 7.6 Hz, 1H, **H-1**), 4.62 (d, *J* = 12.1 Hz, 1H, C*H*HPh), 4.53 (dd, *J* = 13.2, 4.6 Hz, 5H, 2 x C*H*HPh, CH_2Ph, NH), 4.44 (d, *J* = 11.9 Hz, 1H, C*H*HPh), 4.24 (t, *J* = 9.3 Hz, 1H, H-4), 4.06 (br, 1H, H'-4), 4.00 (t, *J* = 5.7 Hz, 1H, H'-5), 3.87 (d, *J* = 10.0 Hz, 3H, H-6, OC*H*H, linker), 3.80 (dd, *J* = 10.4, 2.9 Hz, 1H, H'-3), 3.77 – 3.72 (m, 1H, H-5), 3.69 (dd, *J* = 9.5, 5.9 Hz, 1H, H'-6), 3.62 (dd, *J* = 9.5, 5.9 Hz, 1H, H'-6), 3.45 (d, *J* = 8.5 Hz, 1H, OC*H*H, linker), 2.96 – 2.86 (m, 2H, CH_2NH), 2.56 (br, 1H, OH), 1.55 – 1.41 (m, 2H), 1.37 – 1.12 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.68 (Bz), 165.39 (Bz), 165.31 (Bz), 156.38 (Cbz), 138.31, 138.07, 137.63, 133.21, 133.16, 133.06, 130.07, 129.77, 129.68, 129.51, 128.98, 128.64, 128.58, 128.49, 128.38, 128.31, 128.21, 127.96, 127.87, 127.76, 127.70 (Ar), 100.92 (**C-1**), 97.02 (**C'-1**), 77.36, 75.21, 75.15, 73.87, 73.56, 73.22, 72.40, 72.10, 69.72, 69.61, 69.53, 69.43, 67.60, 66.65, 40.93, 29.54, 29.04, 23.20.; MS ESI+-HRMS *m/z* [M+Na]+ calcd for $C_{67}H_{69}NO_{16}Na$ 1166.4509, found 1166.4517.

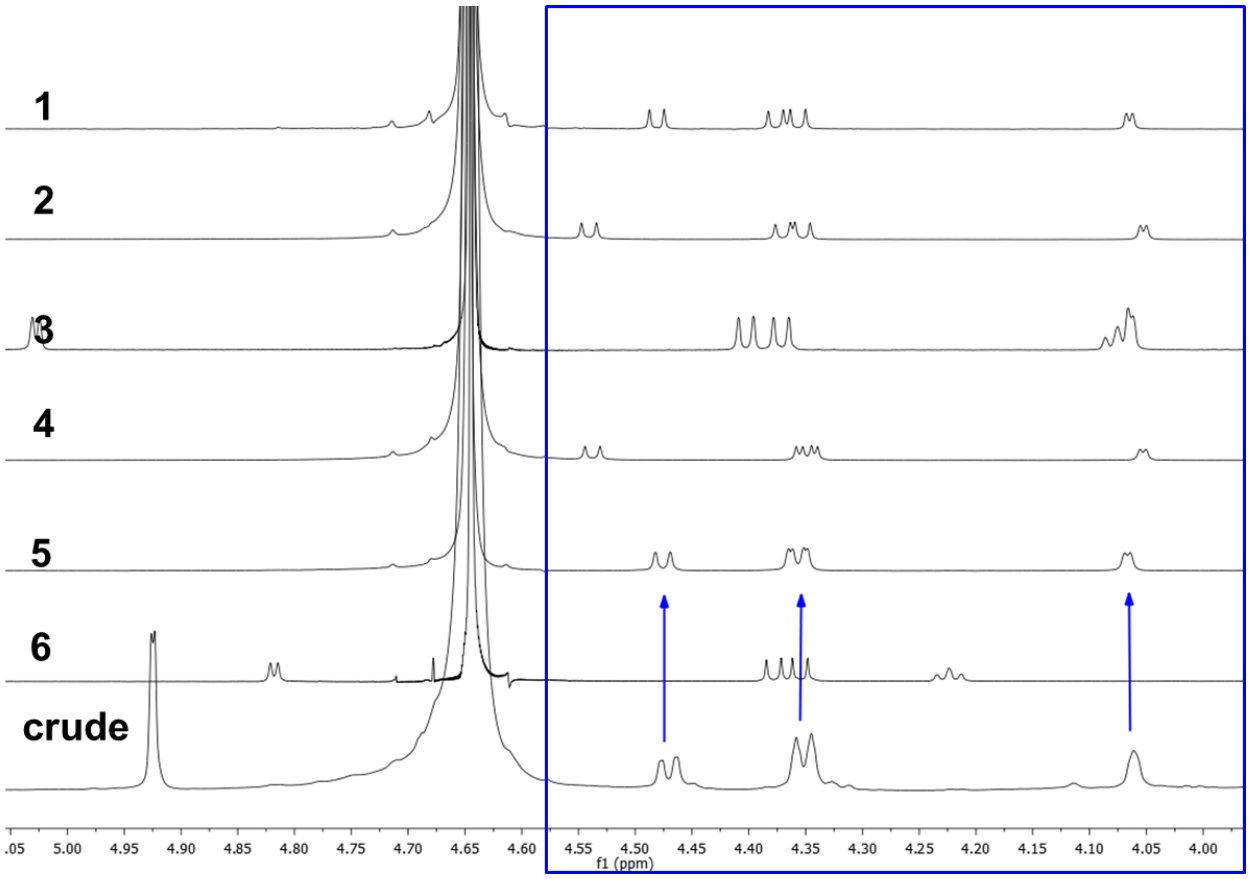


Figure S27. Comparison of ^{1}H NMR of the trisaccharides 1-5 and the final crude mixture **5/30** (Scheme S3).

6 Ion Mobility-Mass Spectrometry

Ion mobility-mass spectrometry (IM-MS) experiments were performed on a travelling wave quadrupole/IMS/oa-ToF MS instrument, Synapt G2-S HDMS (Waters, Manchester, U.K.)[22], which was mass calibrated prior to measurements using a solution of caesium iodide (100 $mg \cdot mL^{-1}$). IM-MS data analysis was performed using MassLynx 4.1, DriftScope 2.4 (Waters, Manchester, UK), and OriginPro 8.5 (OriginLab Corporation, Northampton) software.

6.1 Offline nano-ESI Measurements

For IM-MS analysis compounds **1-9** and the crude mixture of **5**/**30** were each dissolved in water/methanol (1:1, *v*:*v*) at a concentration of 1-10 µmol/L. A nano-electrospray source (nESI) was used to ionize 3-5 µL of sample from platinum-palladium-coated borosilicate capillaries prepared in-house. Typical settings were: source temperature, 20 °C; needle voltage, 0.8 kV; sample cone voltage, 25 V; cone gas, 0 L/h. The ion mobility parameters were optimized to achieve maximum resolution without excessive heating of the ions upon injection into the IM cell. Values were: trap gas flow, 2 mL/min; helium cell gas flow, 180 mL/min; IMS gas flow, 90 mL/min; trap DC bias, 35 V; IMS wave velocity, 800 m/s; IMS wave height, 40 V. For MS/MS experiments the trap collision energy was increased to 40-60 V.

IM-MS Spectra of each individual carbohydrate, three trisaccharide mixtures (**6/3**, **3/2** and **5/6**) and the crude mixture **5**/**30** were recorded separately in positive and negative ion mode. Arrival time distributions (ATD) were extracted from raw data using MassLynx and drift times were determined manually via Gaussian fitting using Origin 8.5. For the measurement of the individual carbohydrates, the *m/z* signal intensity was kept at approximately 10^3 counts per second to avoid saturation and subsequent broadening of the corresponding drift peak (for an example see Extended Data Figure 7). In order to avoid discrimination of a minor component, an average signal intensity of 10^4 counts per second was used for the semiquantitative assessment of mixtures (Extended Data Figure 7b). Under these conditions, minor components with relative concentrations below 1% can still be detected qualitatively, but a semiquantitative assessment is no longer possible. For unknown mixtures, we therefore suggest to acquire data at both high and low intensity settings when possible. In the former case, minor components with relative concentrations below 1% can be qualitatively detected, while the latter case typically yields a better IM resolution and enables a semiquantitative assessment (Extended Data Figure 7). In addition, an acquisition at different intensity settings can help to evaluate mixtures in which the isomers cannot be fully resolved. For broad and inconclusive ATDs, a comparison with neighbouring peaks of similar mass and charge can furthermore be used to distinguish between overlapping and saturated peaks.[29]

CCS estimations were performed using an established protocol and dextran as the calibrant (Dextran MW=1000 and Dextran MW=5000, Sigma Aldrich)[29,30]. The calibration solution consisted of 0.1 mg/mL dextran1000, 0.5 mg/mL dextran5000, and 1 mM NaH_2PO_4 in water:methanol (1:1, *v*:*v*). The calibrant and each sample were measured on a travelling wave Synapt instrument at five wave velocities in both positive and negative ion mode. Drift times where extracted from raw data by fitting a Gaussian distribution to the arrival time distribution (ATD) of each ion and corrected for their *m/z* dependent flight time. CCS reference values[30] of dextran were corrected for charge and mass and a logarithmic plot of corrected CCSs against corrected drift times was used as a calibration curve to estimate CCSs. One calibration curve was generated for every wave velocity and each ion polarity. The resulting five estimated CCSs for each sample ion were averaged. These measurements

where repeated three times and the averaged values for different ions are presented in Extended Data Table 2.

6.2 Semiquantitative Analysis of Trisaccharide Mixtures

For the semiquantitative analysis of anomeric trisaccharide mixtures a quantification experiment was performed using isomers **2** and **3**. Stock solutions of **2** and **3** with identical concentration were prepared in water/methanol (1:1, *v*:*v*). Each stock solution was diluted individually to yield relative concentrations (rel. conc.) of 80, 56, 43, 25, 11, 5, 1, 0.1, and 0.01%. The serial dilutions were used to obtain isomer mixtures with concentration ratios $x(\mathbf{3}) = \left(\frac{c[\mathbf{3}]}{c[\mathbf{2}]+c[\mathbf{3}]}\right)$ between 0 and 1 (see Table S4). A value of 0.5 represents equal amounts of **2** and **3**, while 0 and 1 indicate the presence of only **2** or **3**, respectively.

Table S4. Relative concentrations of **2** and **3** in the investigated mixtures and their corresponding concentration ratios x(**3**). Measured relative intensities Int_{rel}(**3**) were calculated from the drift peak areas (A) of the deprotonated species $[M-H]^-$=588.4.

rel. conc. **3**	rel. conc. **2**	theoretical x(**3**)	measured Int_{rel}(**3**)	STD
1	100	0.01	0.04	0.011
5	100	0.05	0.07	0.004
11	100	0.10	0.10	0.007
25	100	0.20	0.18	0.005
43	100	0.30	0.27	0.005
56	100	0.36	0.35	0.005
80	100	0.44	0.42	0.010
100	100	0.50	0.49	0.007
100	80	0.56	0.55	0.016
100	56	0.64	0.60	0.005
100	43	0.70	0.69	0.008
100	25	0.80	0.78	0.005
100	11	0.90	0.89	0.010
100	5	0.95	0.93	0.012
100	1	0.99	0.97	0.007

To achieve constant experimental conditions, the semiquantitative analysis was performed on a Synapt instrument equipped with an online nano-ESI source that was coupled to an ACQUITY UPLC System (Waters, Manchester, U.K.). Settings were: eluents, 0.1% FA in methanol/0.1% FA in water at a constant rate of 50%, flow rate 8 µL/min, sample injection: 10 µL. Data were acquired in negative ion mode with following settings: source temperature, 80 °C; needle voltage, 2.7 kV; sample cone voltage, 25 V; desolvation temperature 150°C, cone gas, 0 L/h, nanoflow gas 1.3 bar, purge gas flow 500.0 mL/h. Ion mobility parameter were: trap gas flow, 0.4 mL/min; helium cell gas flow, 180 mL/min; IMS gas flow, 90 mL/min; trap DC bias, 45 V; IMS wave velocity, 800 m/s; IMS wave height, 40 V.

Extraction of the ATD of the 588.4 *m/z* ion showed two separate arrival times, each of which corresponded to one of the two isomers. The area under the ATD is related to the

concentration of the sample. Therefore, the theoretical concentration ratio x(**3)** was compared to the ratio of the drift time peak areas $Int_{rel}(\mathbf{3})=\frac{A[\mathbf{3}]}{A[\mathbf{2}]+A[\mathbf{3}]}$ (Figure 3C). A linear correlation was observed, demonstrating the semiquantification of one isomer in the presence of another, down to contents of 1% of the minor component. Relative concentrations between 1 and 0.1% were still qualitatively detectable, but a determination of the relative content was not possible anymore due to detector saturation caused by the major component.

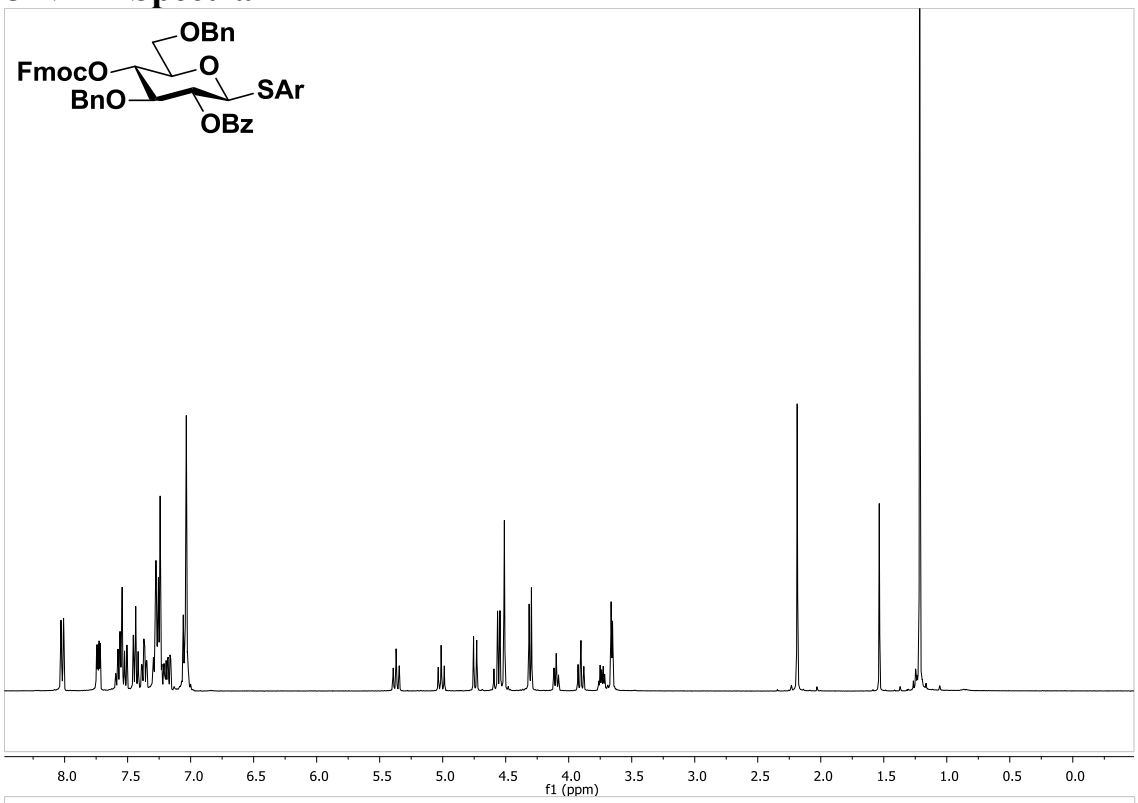
7 Additional References

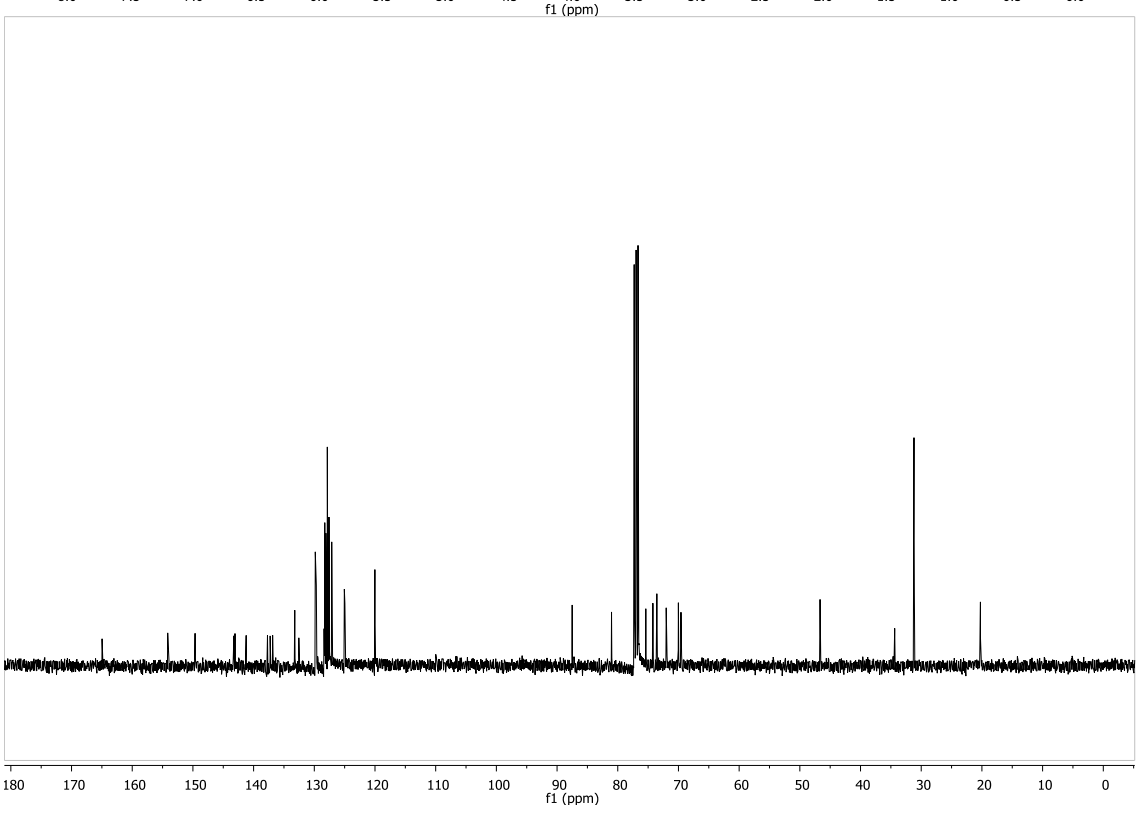
31. Eller, S., Collot, M., Yin, J., Hahm, H. S. & Seeberger, P. H. Automated solid-phase synthesis of chondroitin sulfate glycosaminoglycans. *Angew. Chem. Int. Ed.* **52**, 5858-5861 (2013).

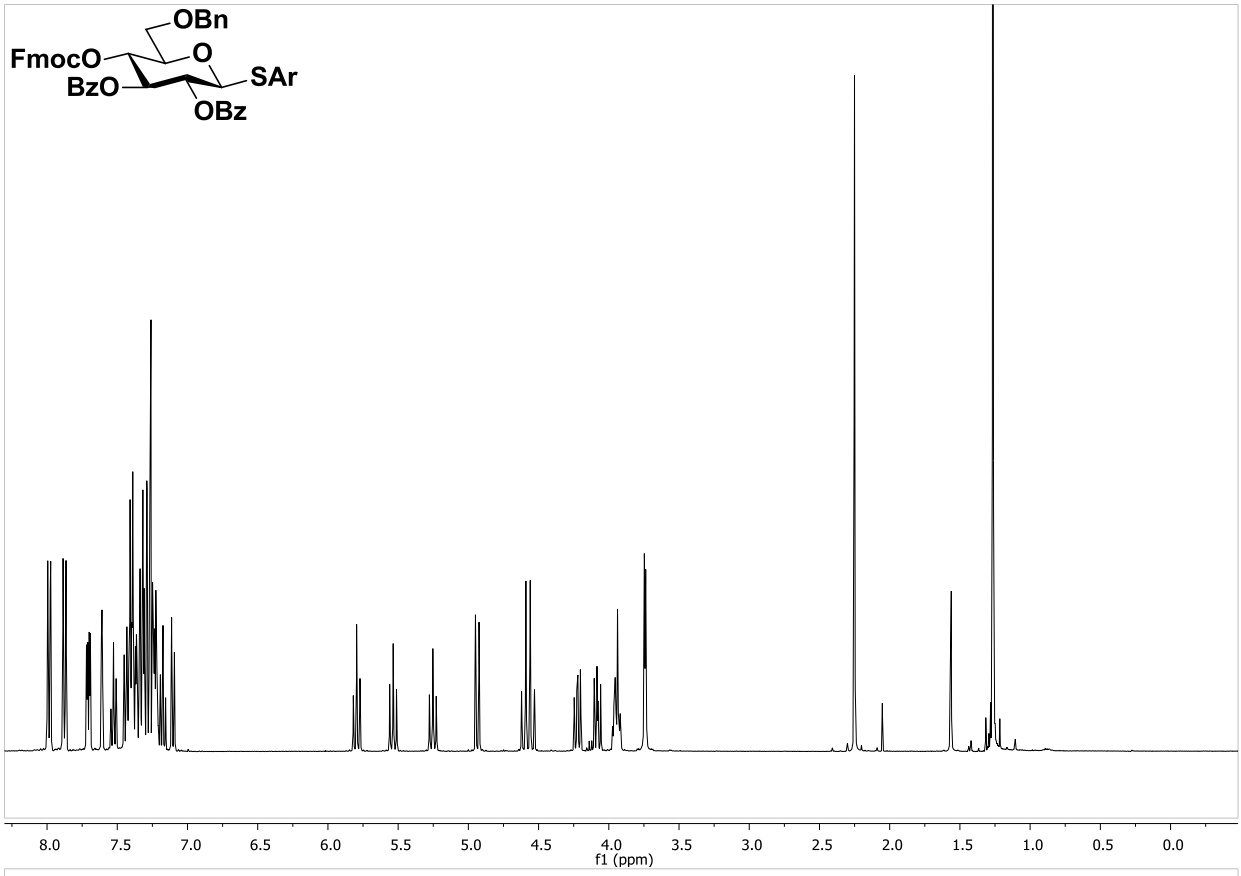
32. Martin, C. E., Weishaupt, M. W. & Seeberger, P. H. Progress toward developing a carbohydrate-conjugate vaccine against Clostridium difficile ribotype 027: synthesis of the cell-surface polysaccharide PS-I repeating unit. *Chem. Commun.* **47**, 10260-10262 (2011).

33. Werz, D. B., Carstagner, B. & Seeberger, P. H. Automated synthesis of the tumor-associated carbohydrate antigens Gb-3 and Globo-H: Incorporation of α-galactosidic linkages. *J. Am. Chem. Soc.* **129**, 2770-2771 (2007).

8 NMR Spectra



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FmocO
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BzO
SAr
OBz
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7.0
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6.0
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3.5
3.0
2.5
2.0
1.5
1.0
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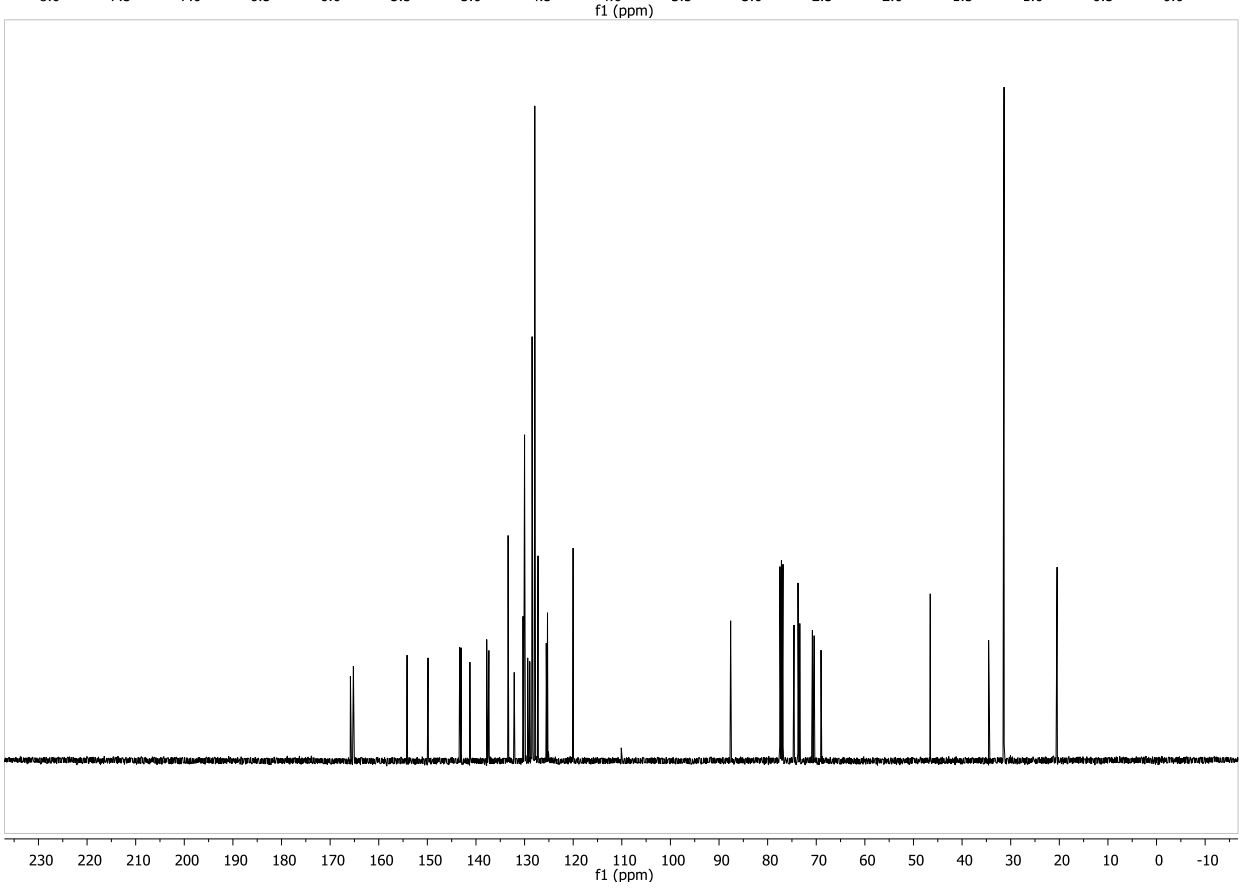
0.0
f1 (ppm)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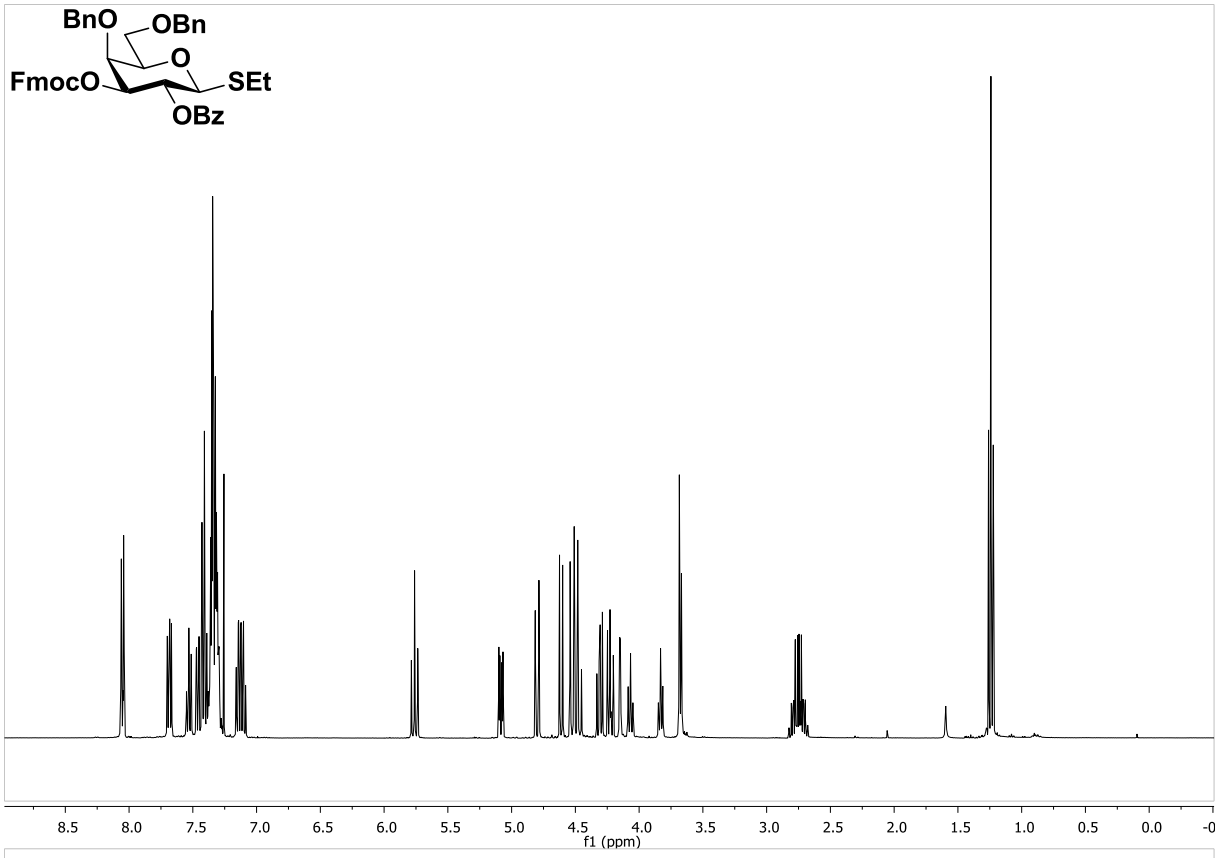
-10
f1 (ppm)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O
FmocO
SEt
OBz
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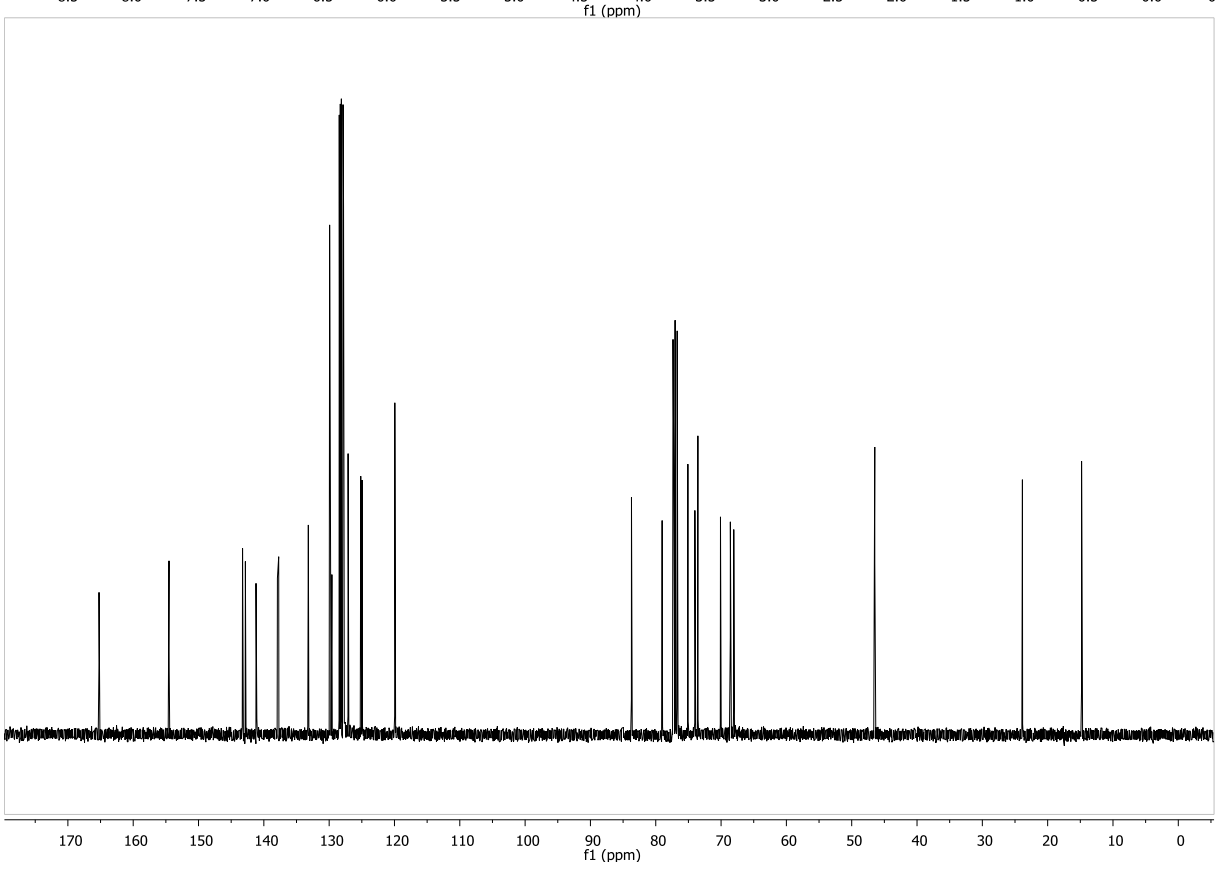
f1 (ppm)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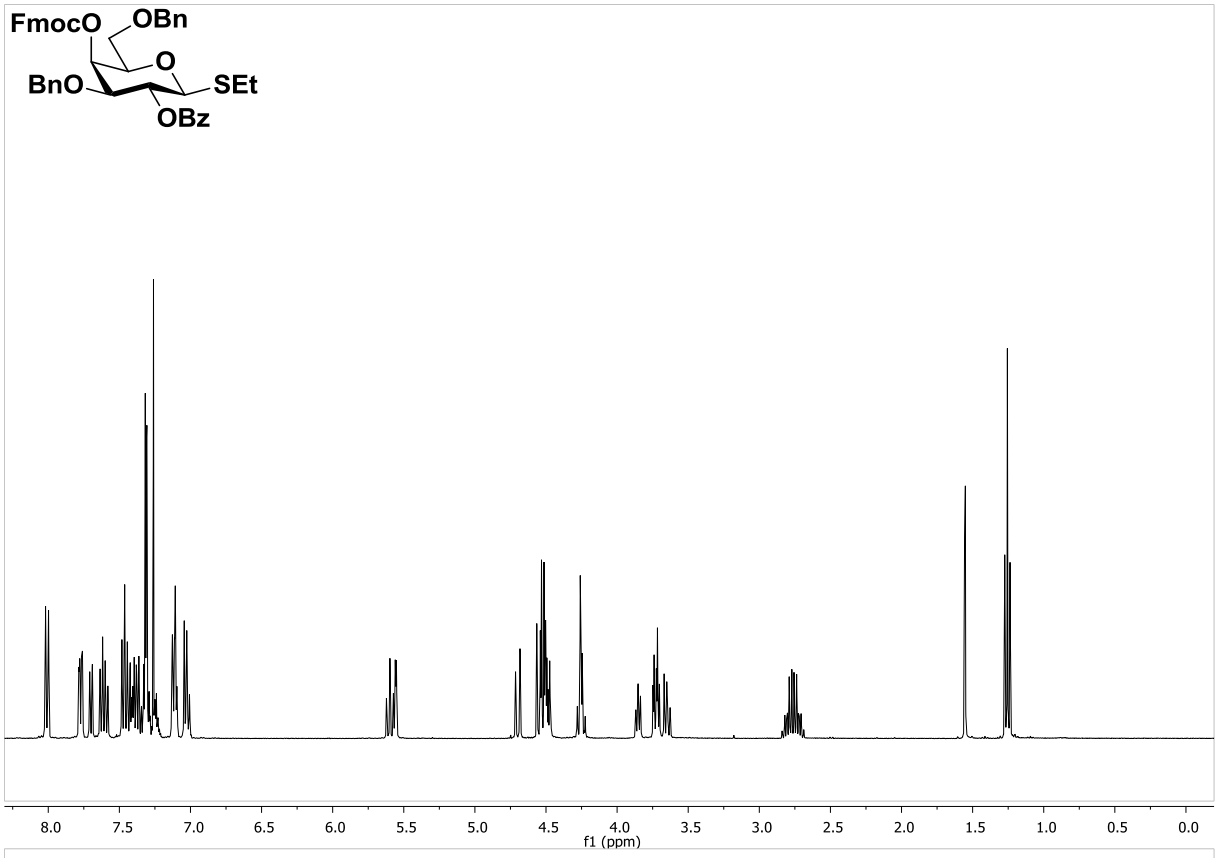
f1 (ppm)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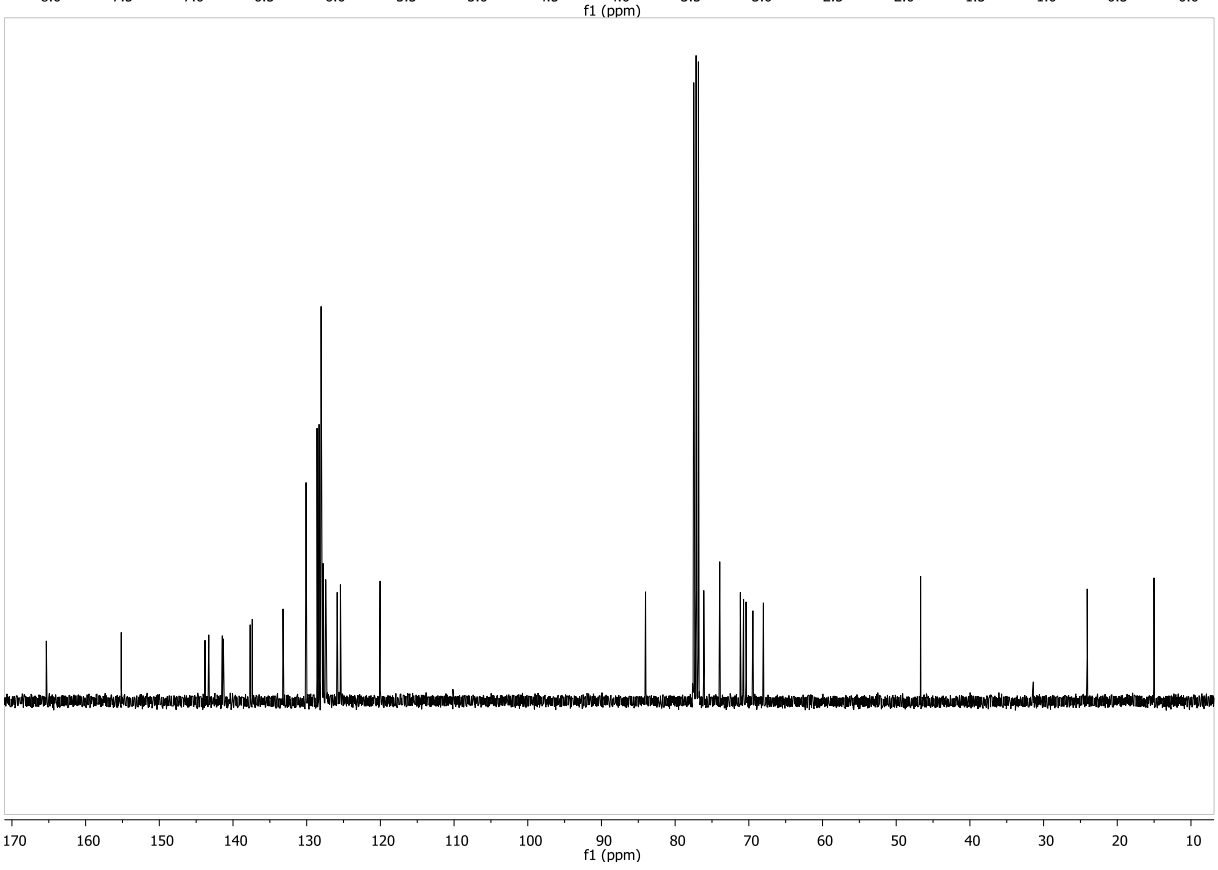
f1 (ppm)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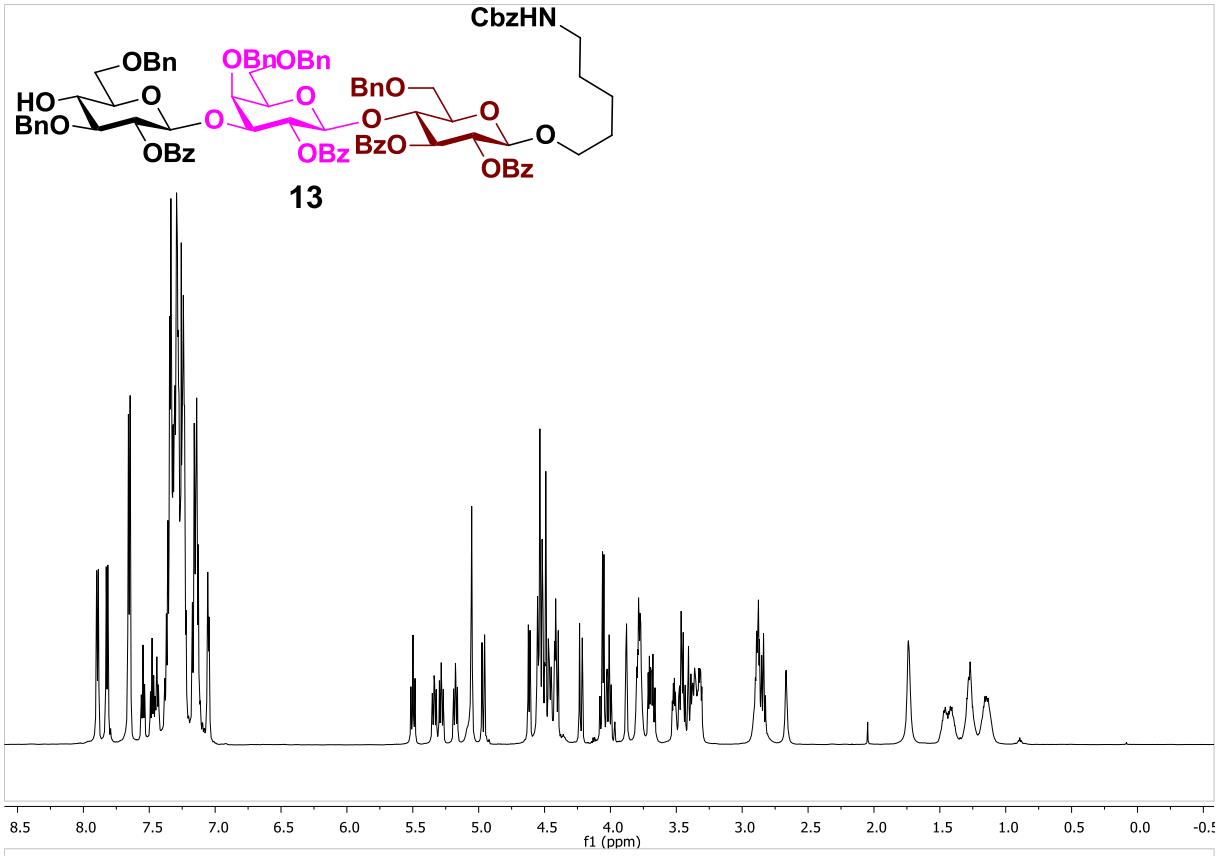
f1 (ppm)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BnO
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OBz
OBnOBn
O
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OBz
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BzO
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13
8.5
8.0
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7.0
6.5
6.0
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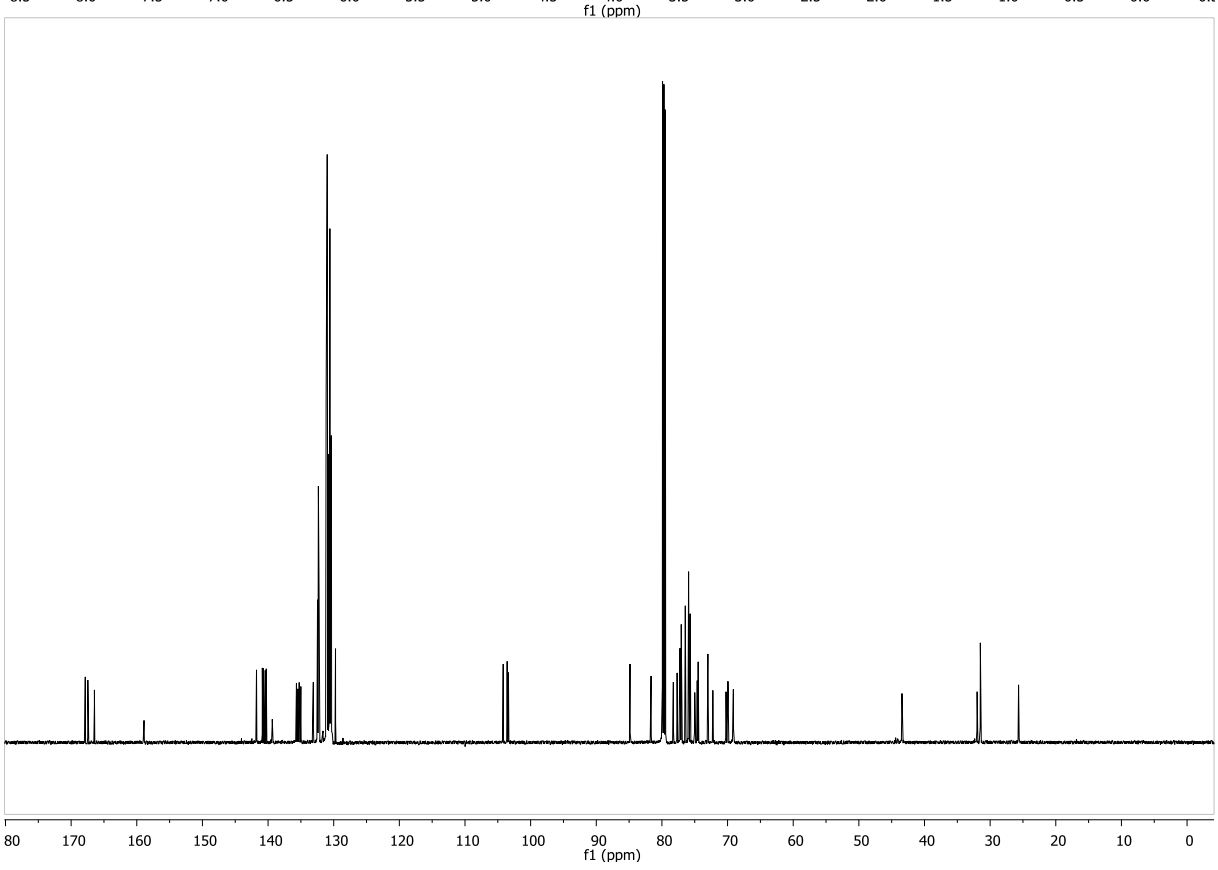
0.5
0.0
f1 (ppm)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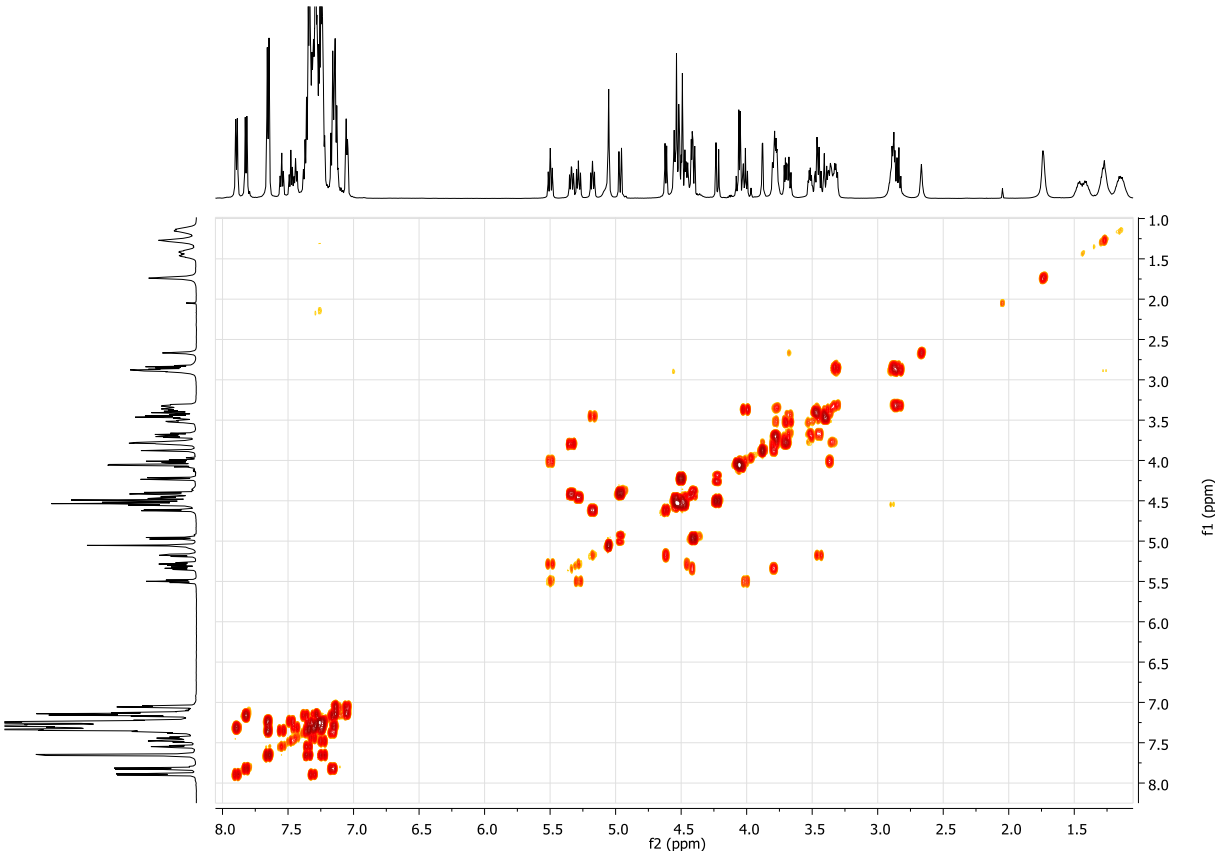
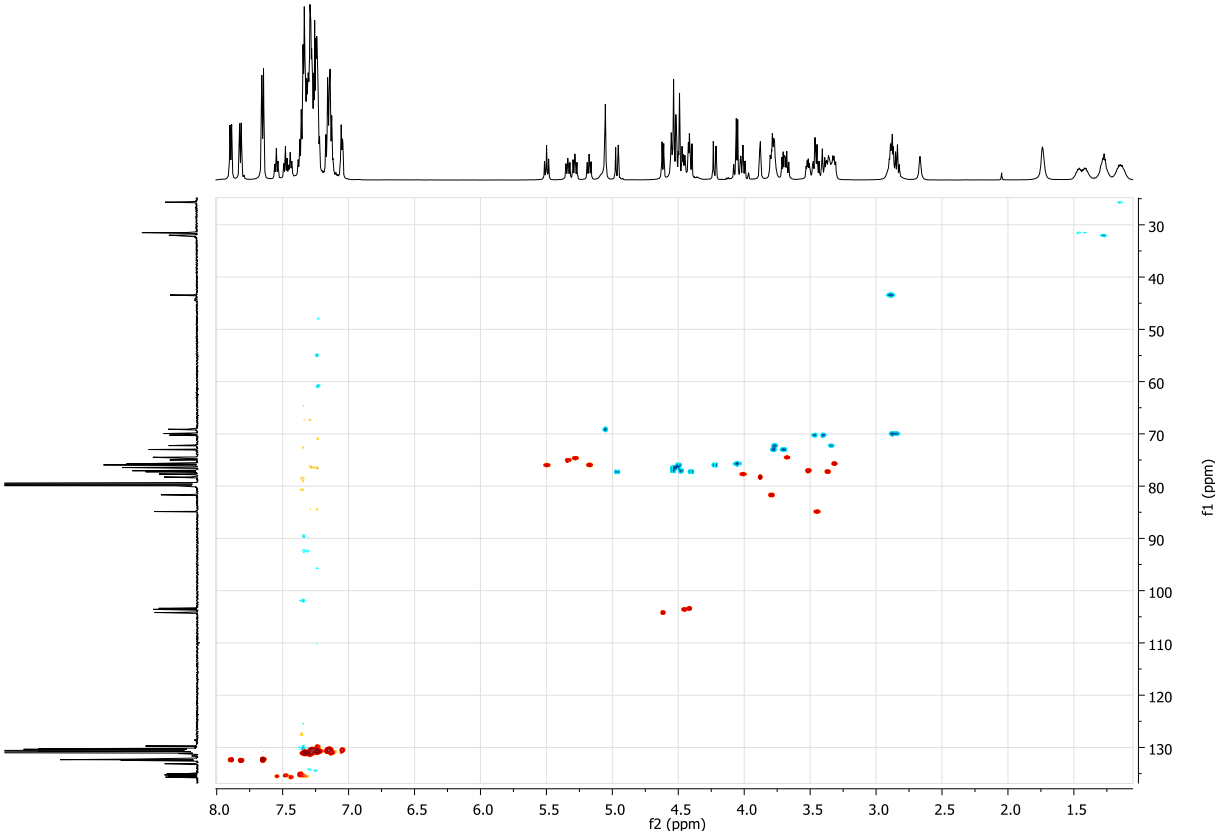
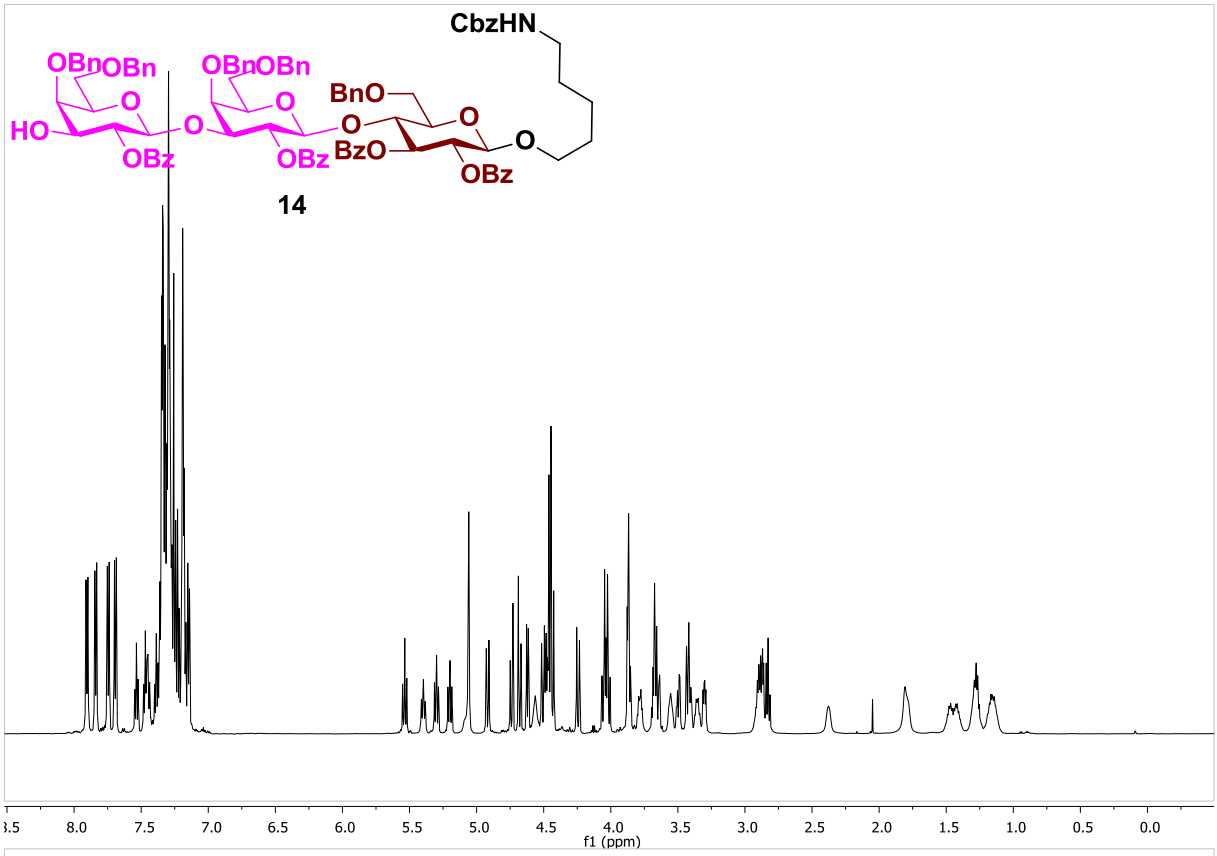
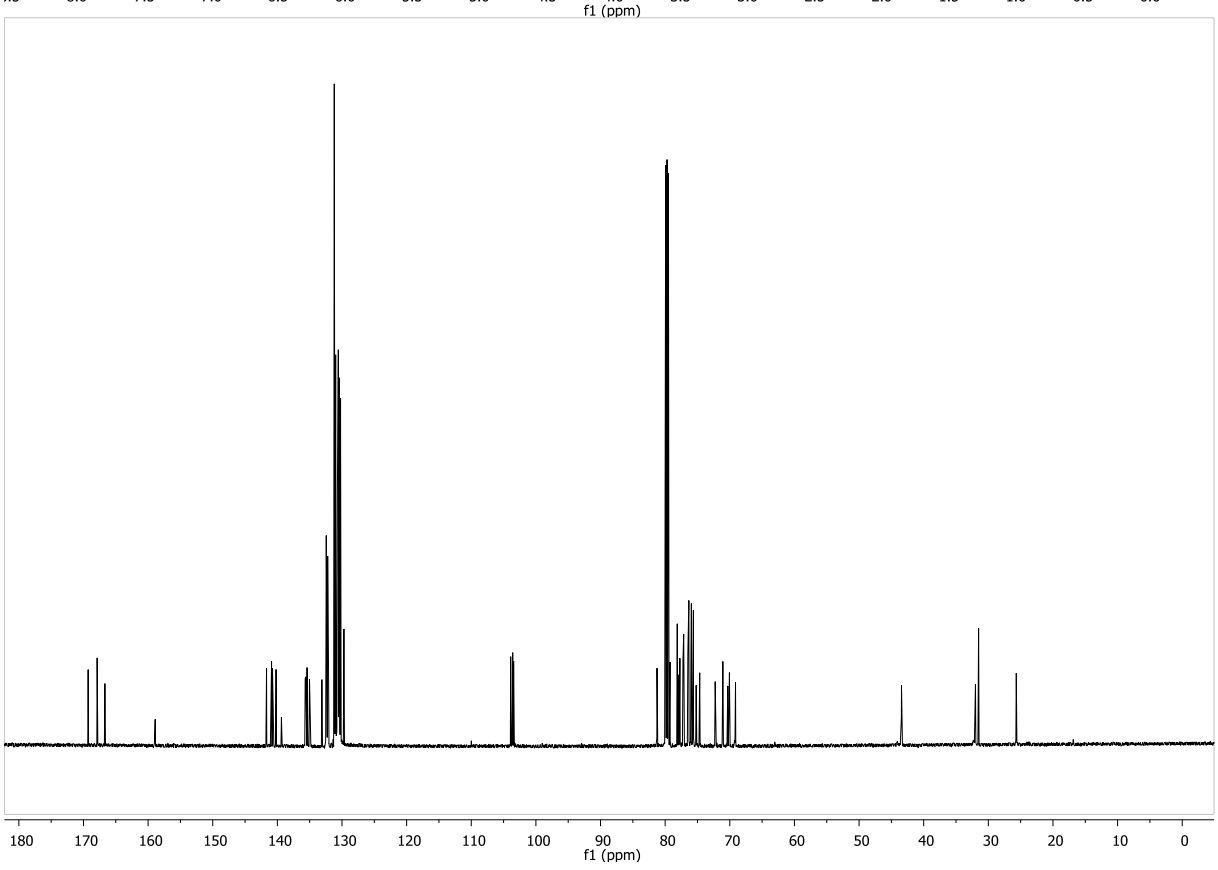
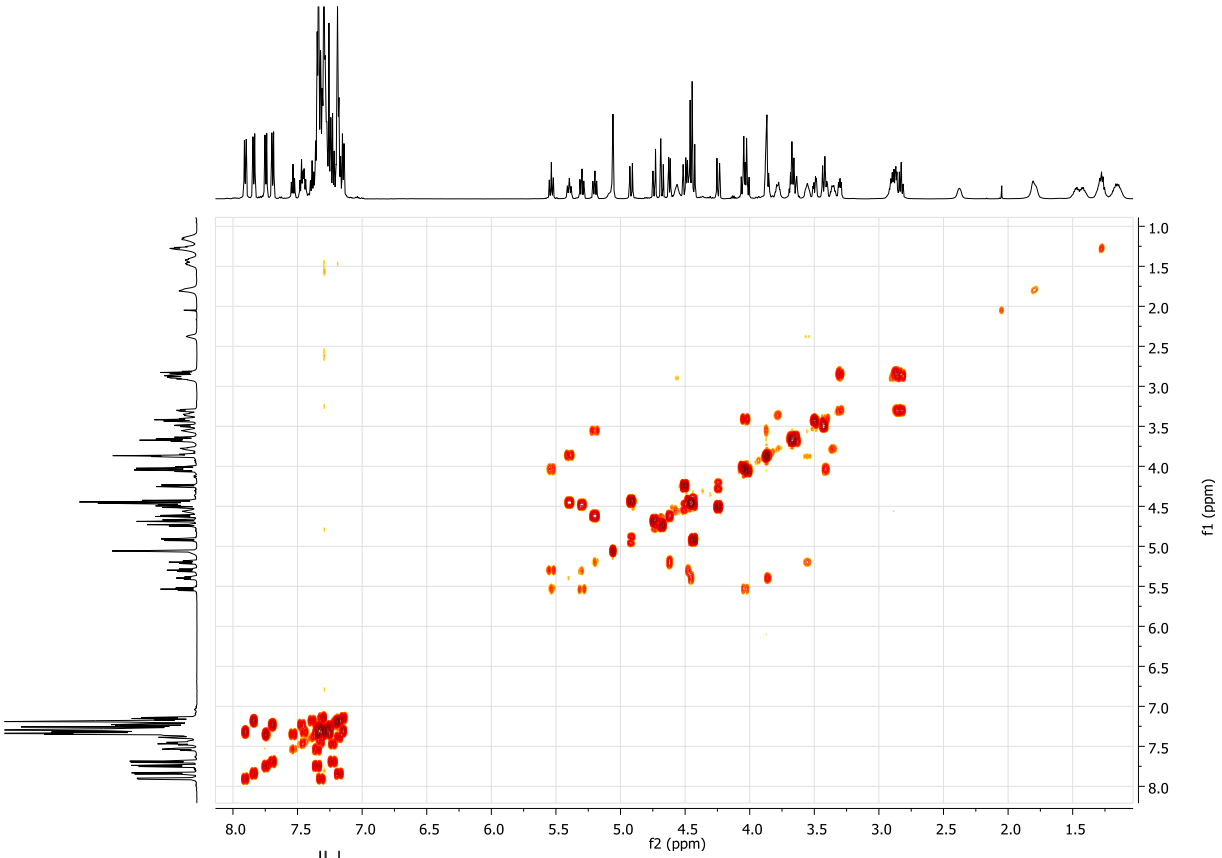
0
f1 (ppm)

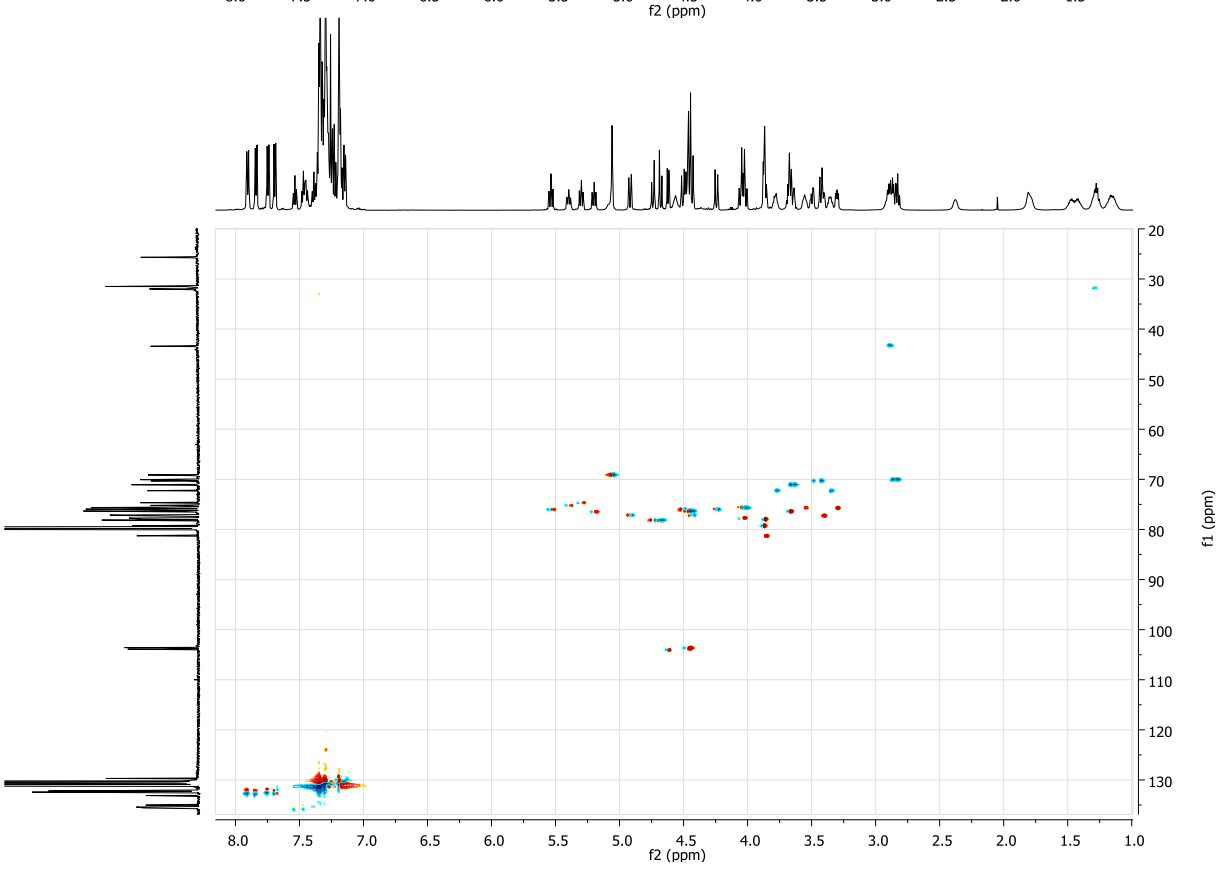

f1 (ppm)
f2 (ppm)

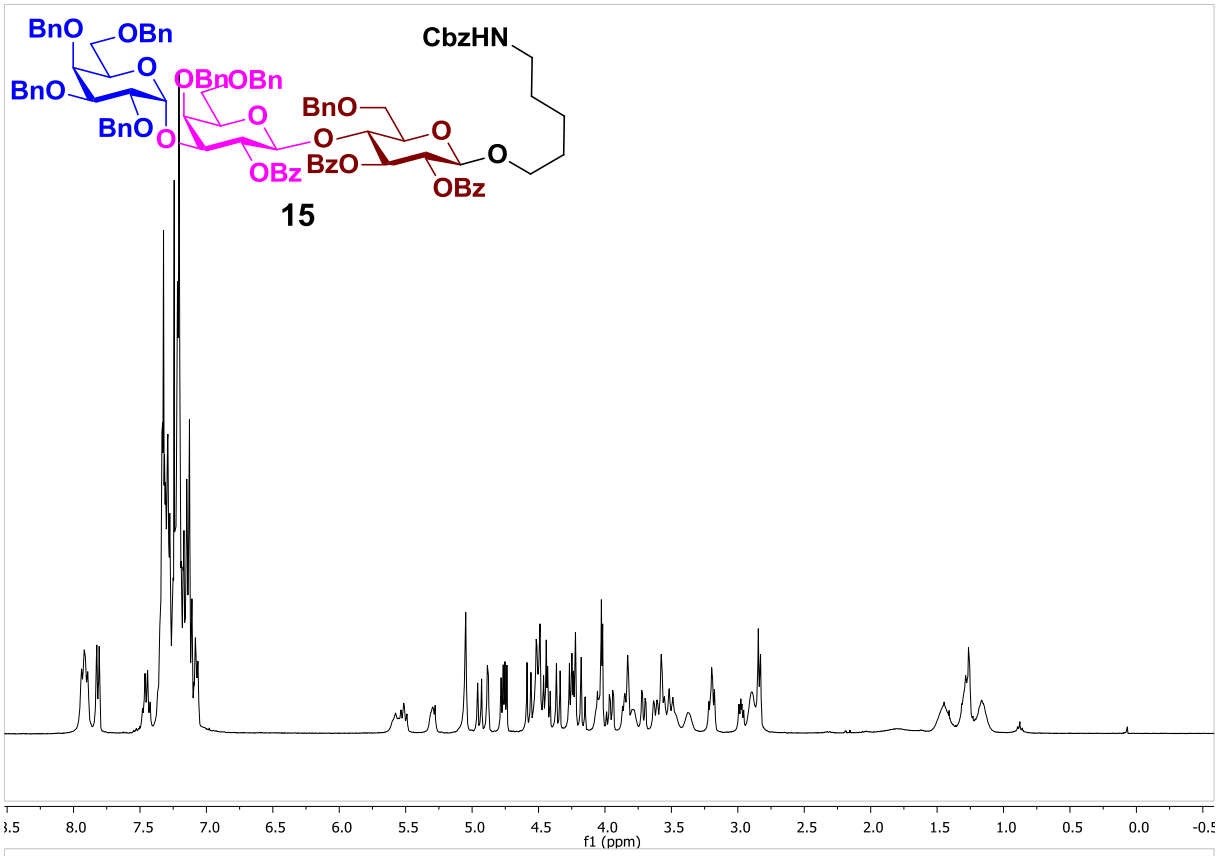

f1 (ppm)
f2 (ppm)

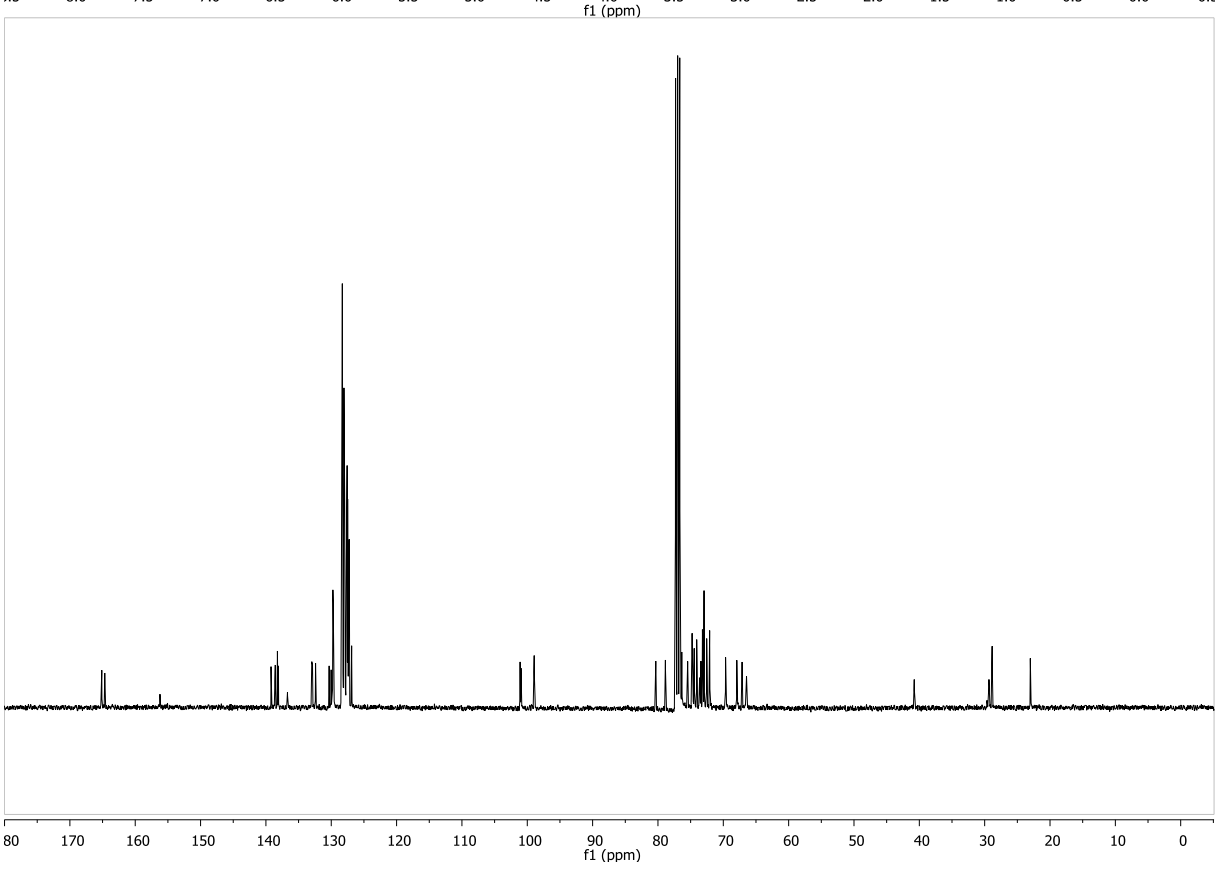

CbzHN
OBn
OBn
HO
O
OBz
OBn
OBn
O
O
OBz
BnO
O
O
BzO
O
OBz
14
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

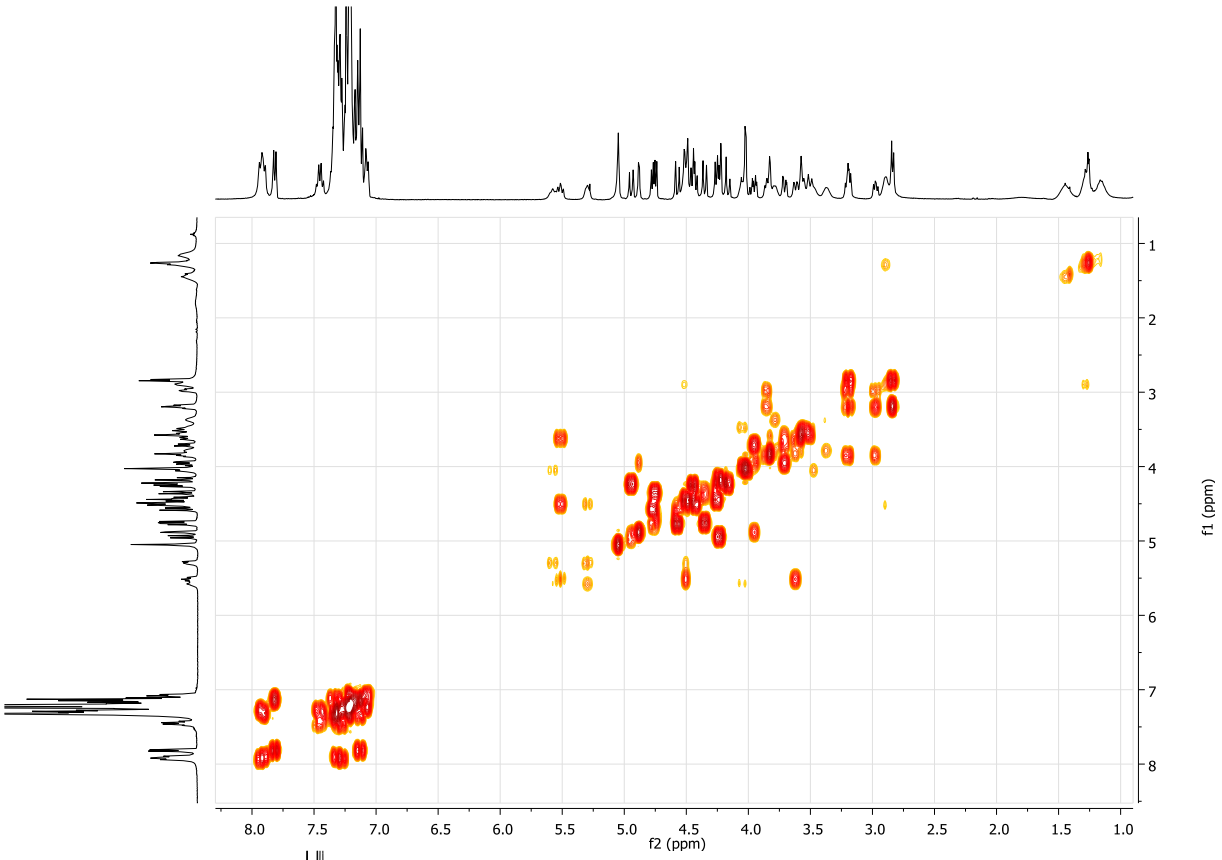

180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

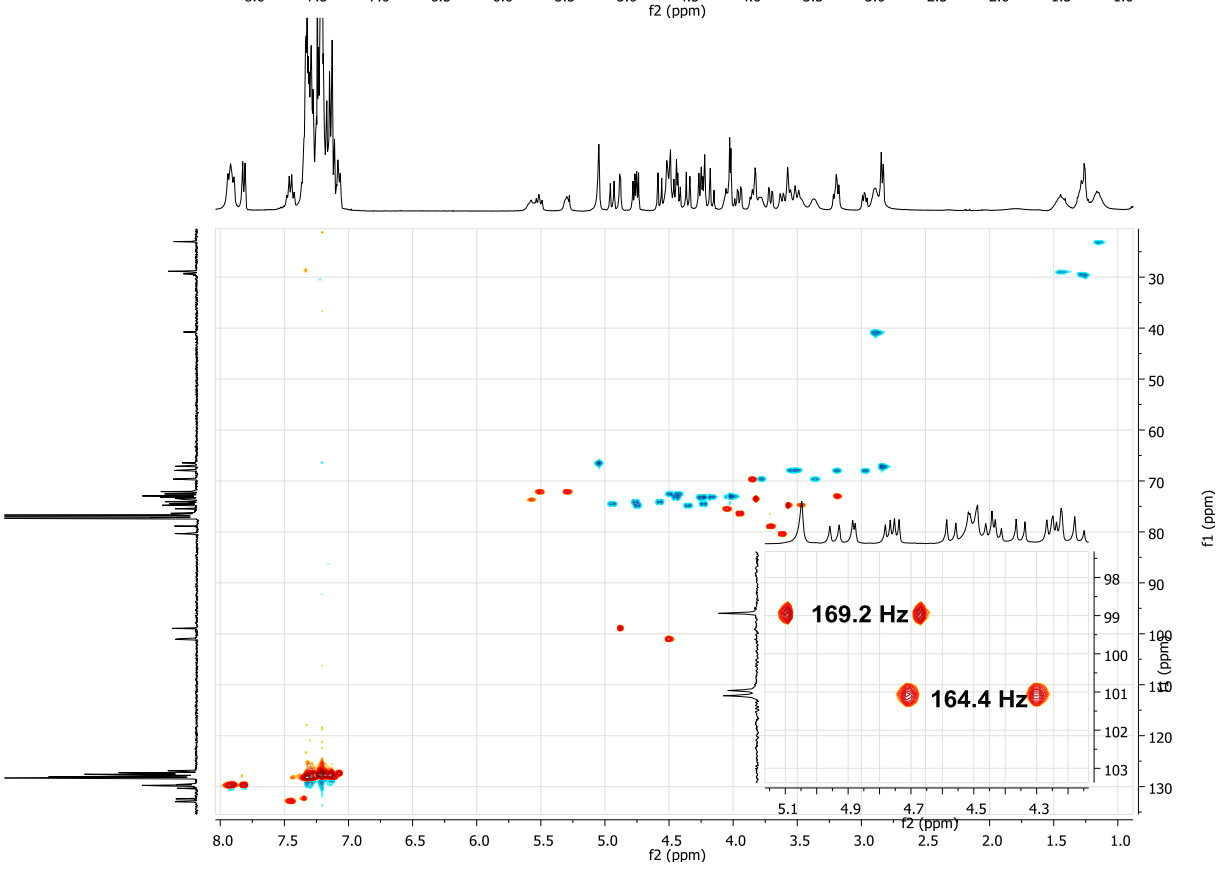

f2 (ppm)
f1 (ppm)

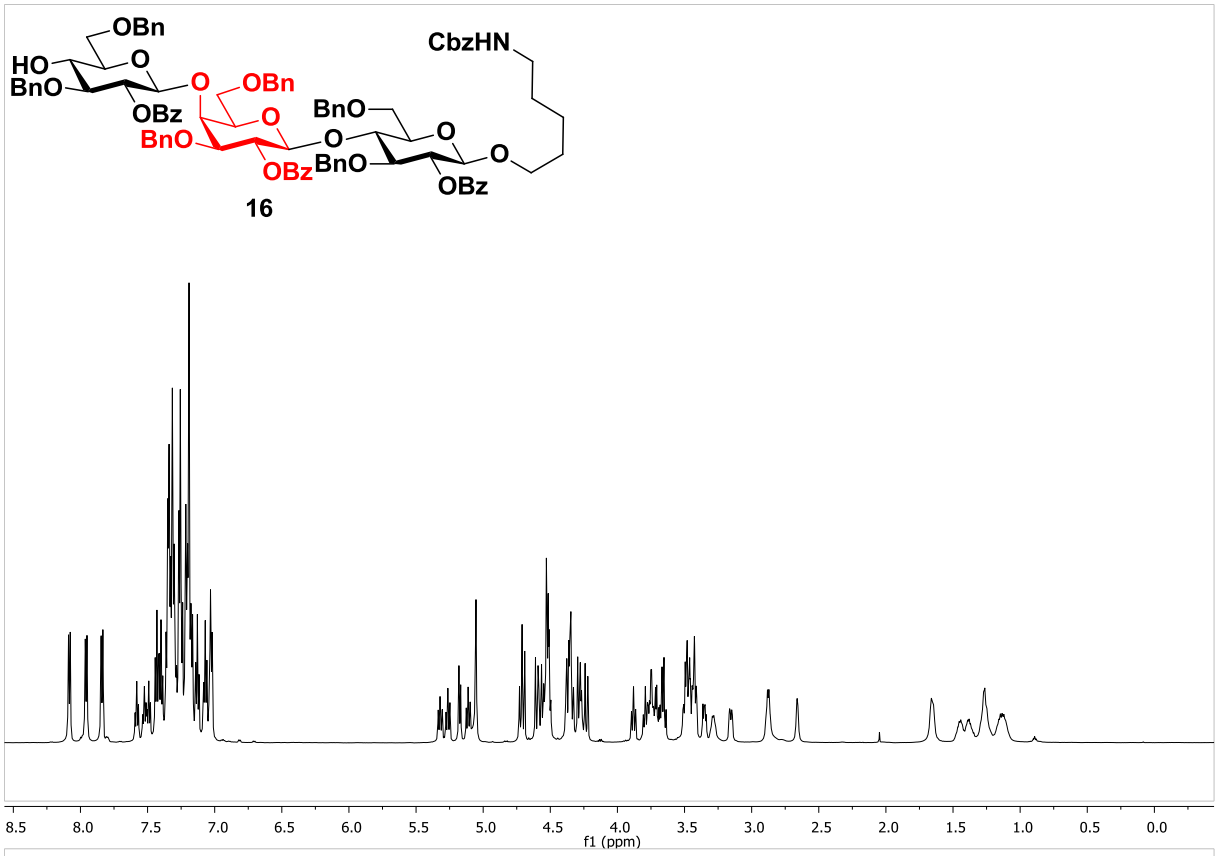

f2 (ppm)
f1 (ppm)

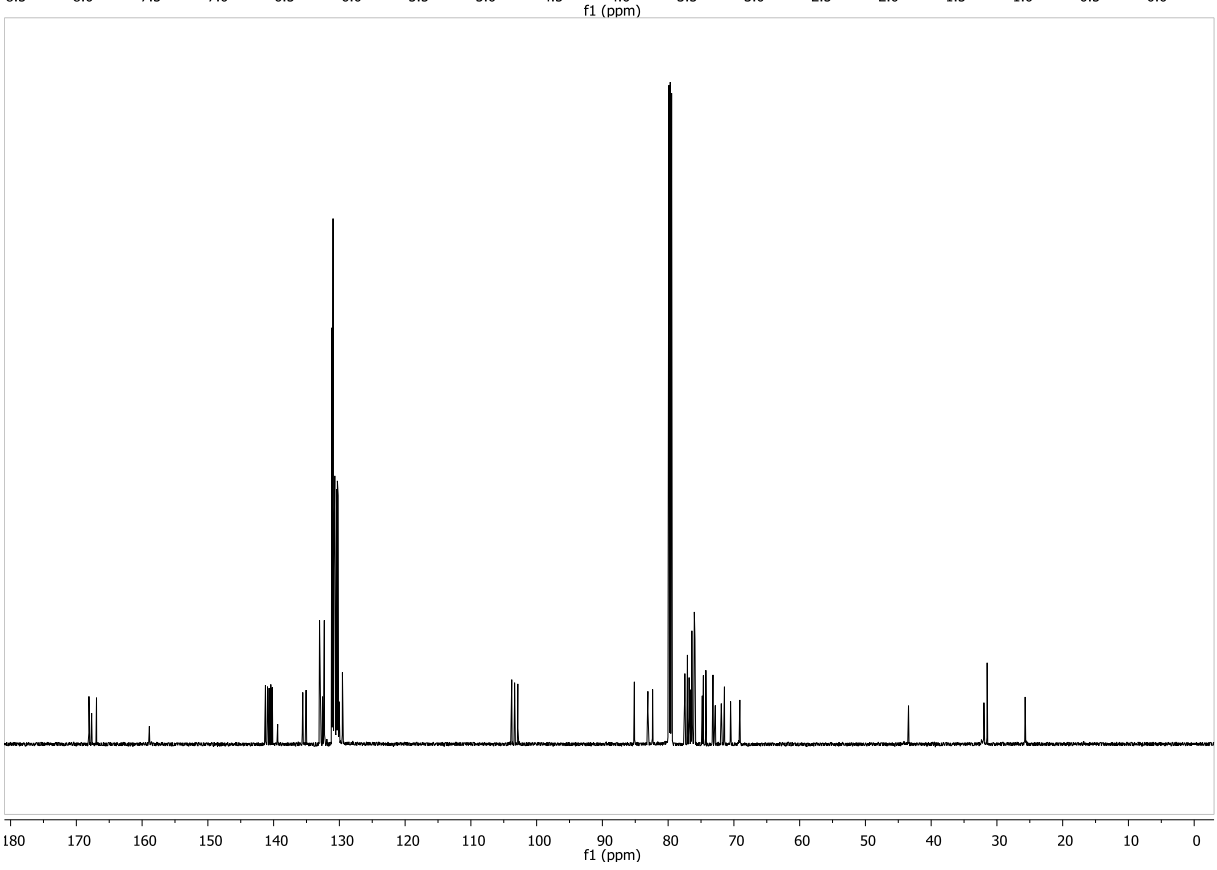

BnO
OBn
O
BnO
BnO
O
OBnOBn
O
OBz
BnO
O
BzO
O
O
OBz
CbzHN
15
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

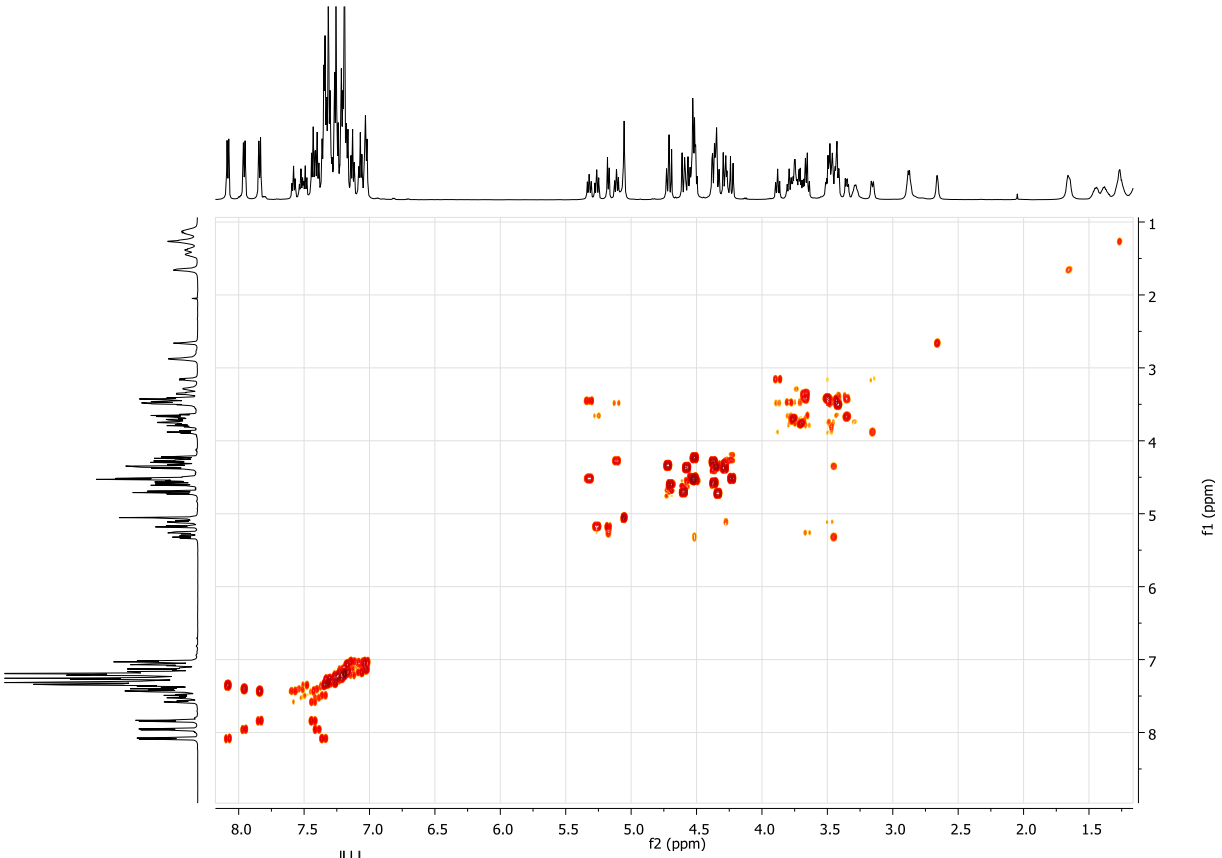

170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

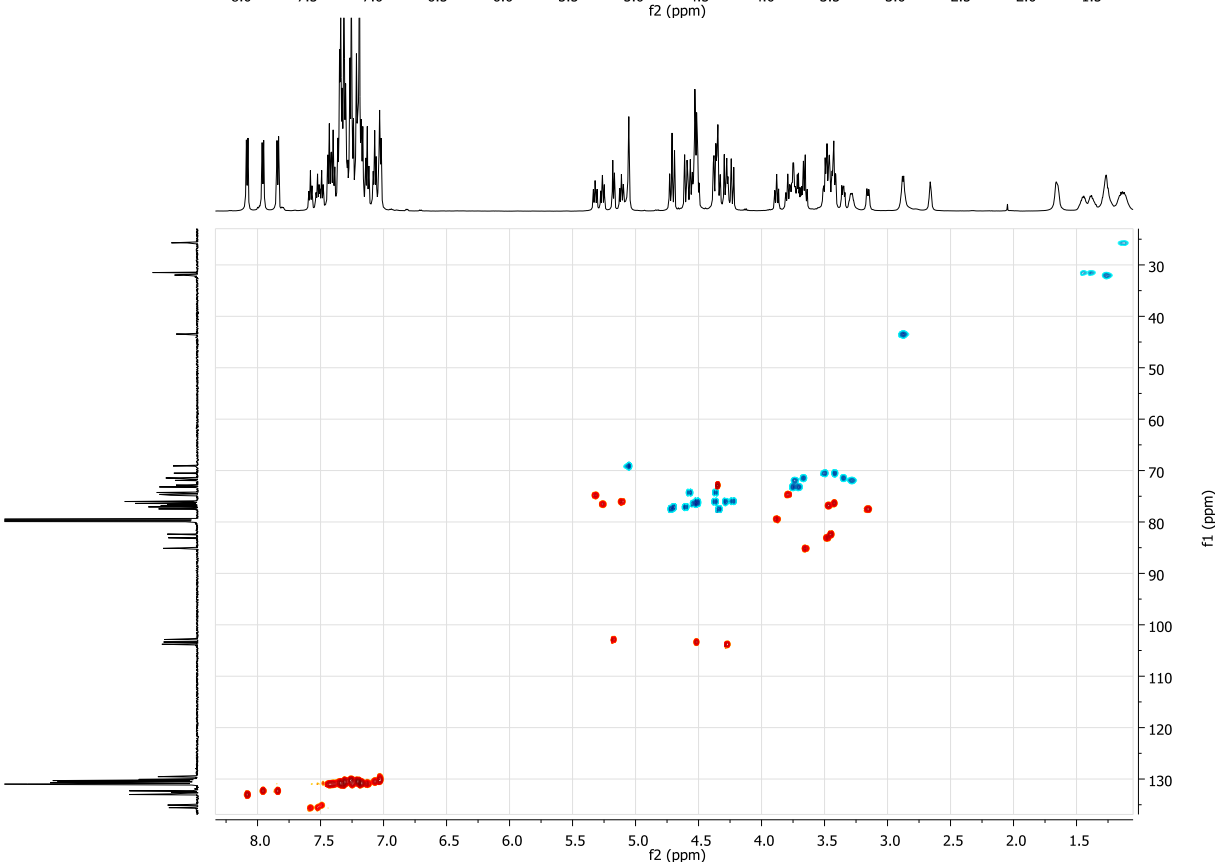

f2 (ppm)
f1 (ppm)

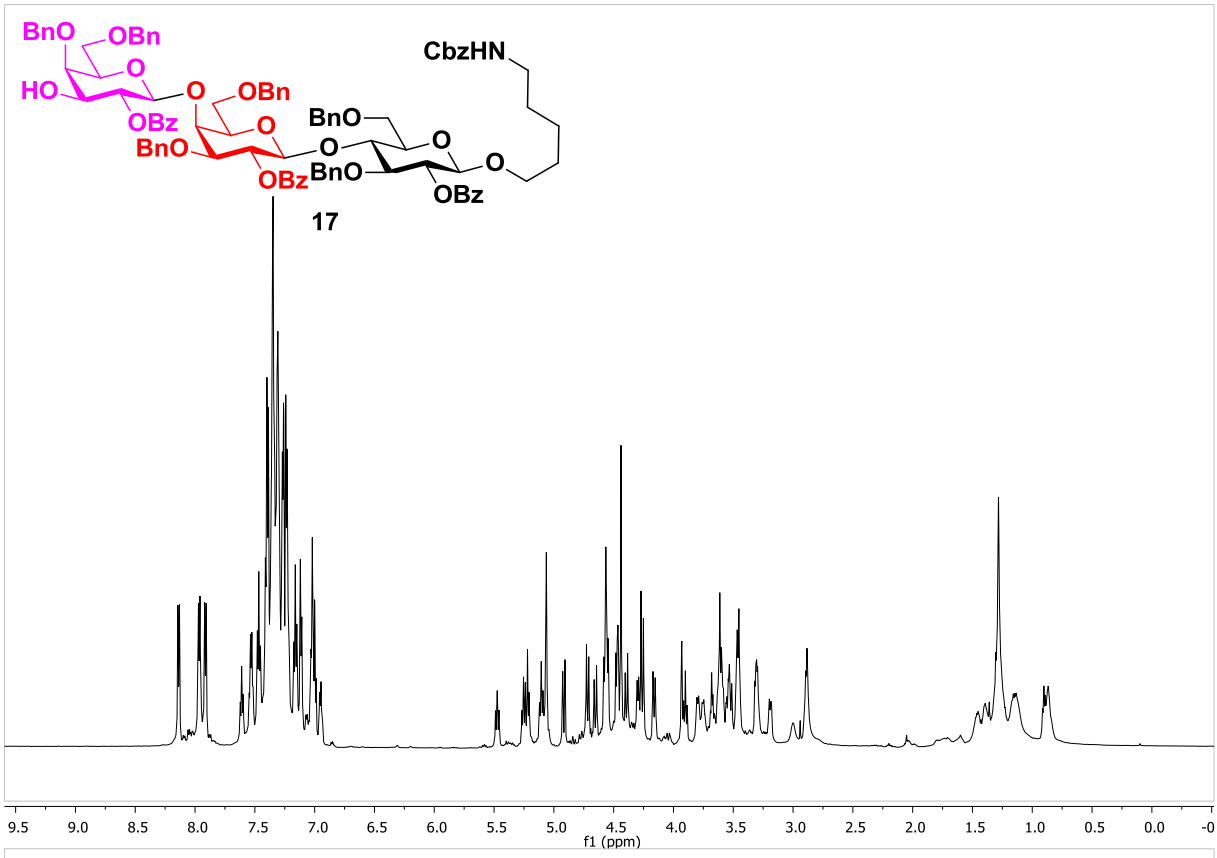

169.2 Hz
164.4 Hz
f2 (ppm)
f1 (ppm)

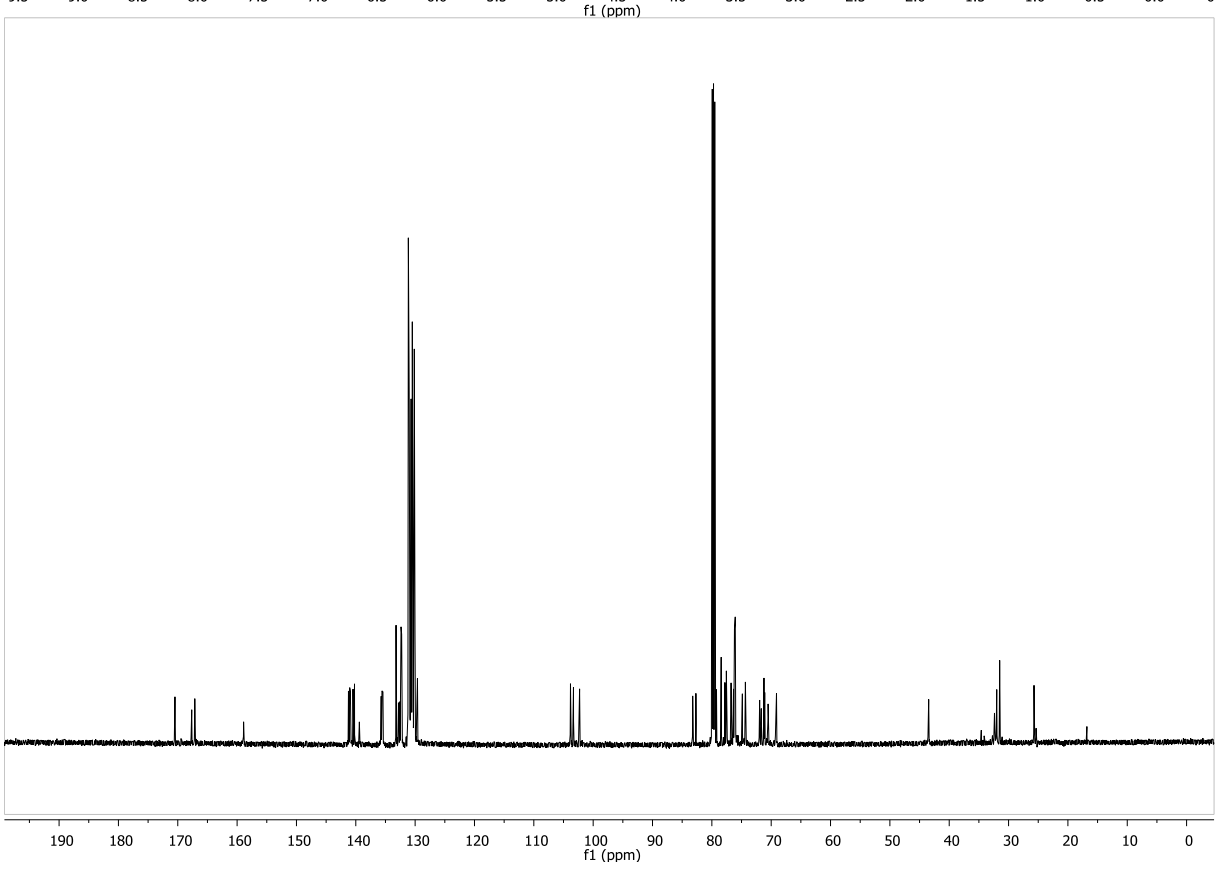

OBn
HO
BnO
O
OBz
O
OBn
O
BnO
OBz
BnO
O
BnO
O
O
OBz
CbzHN
16
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

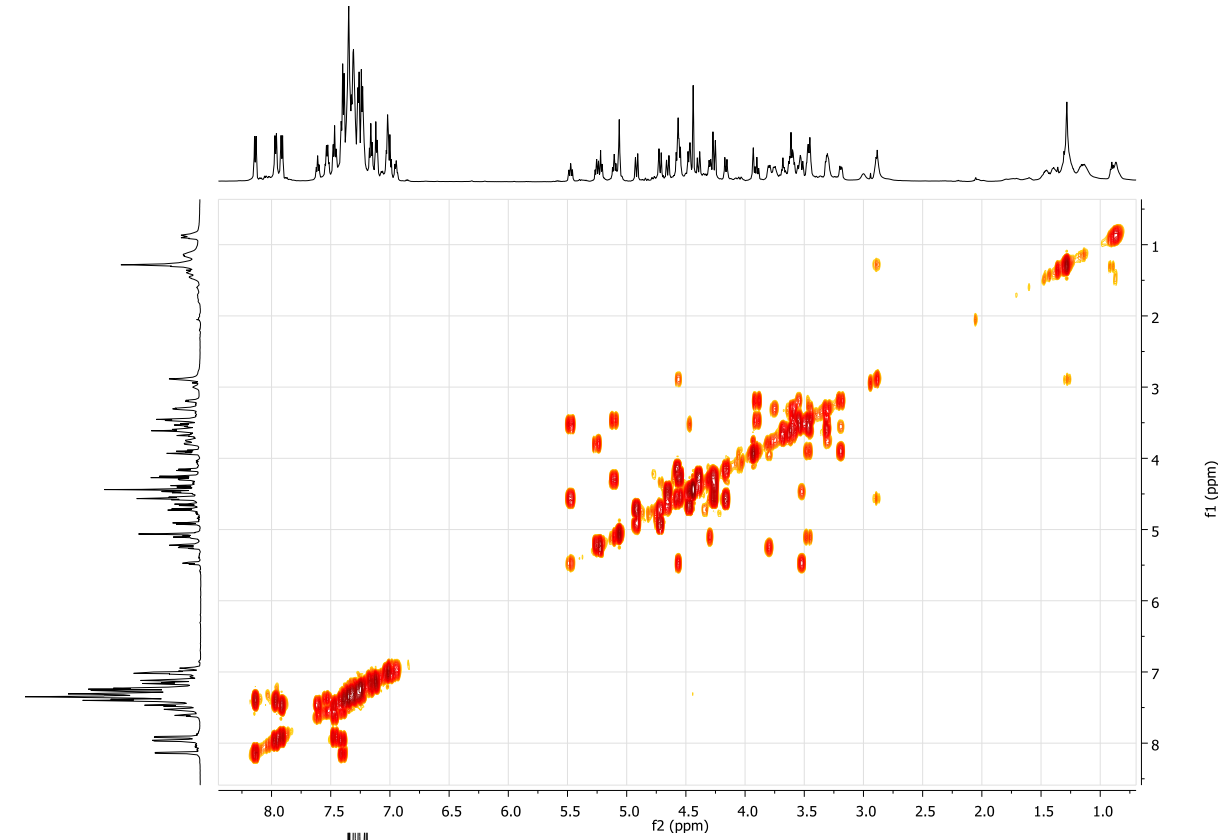

180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

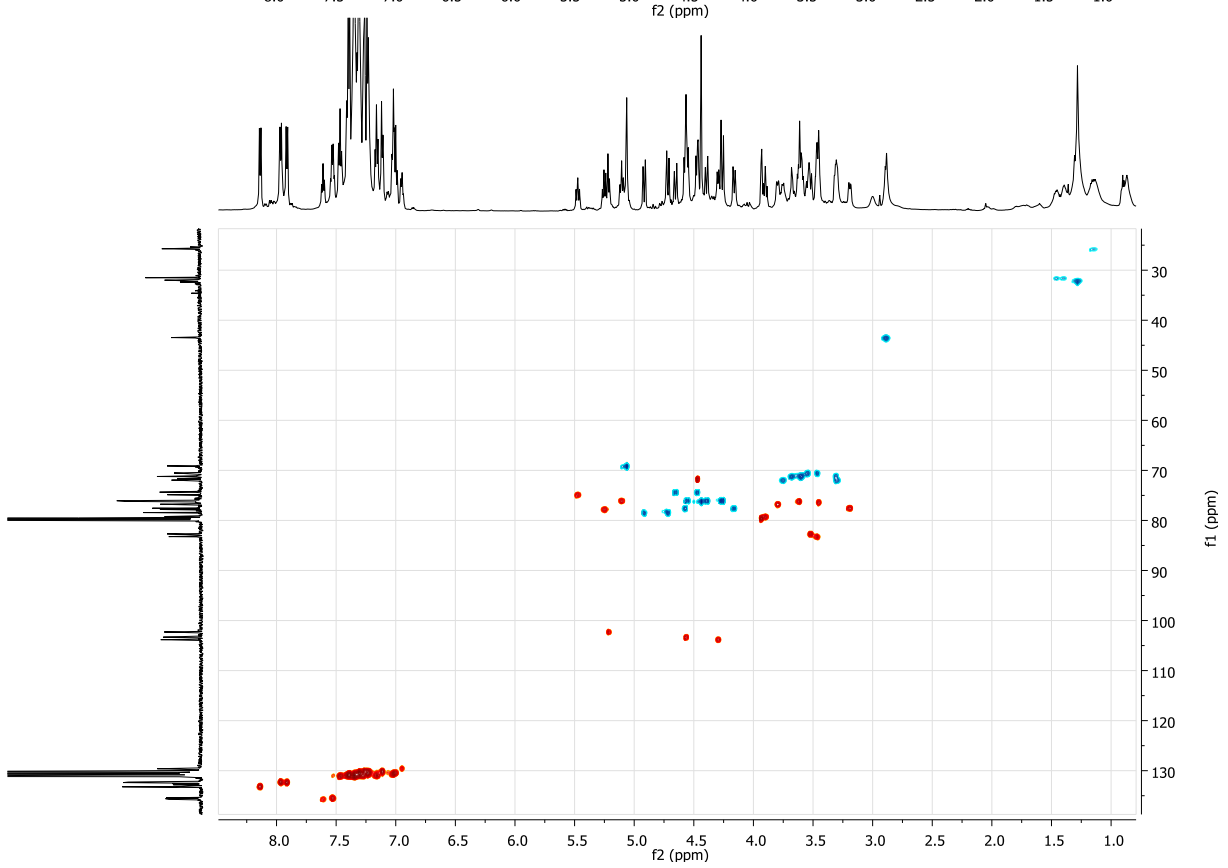

f1 (ppm)
f2 (ppm)

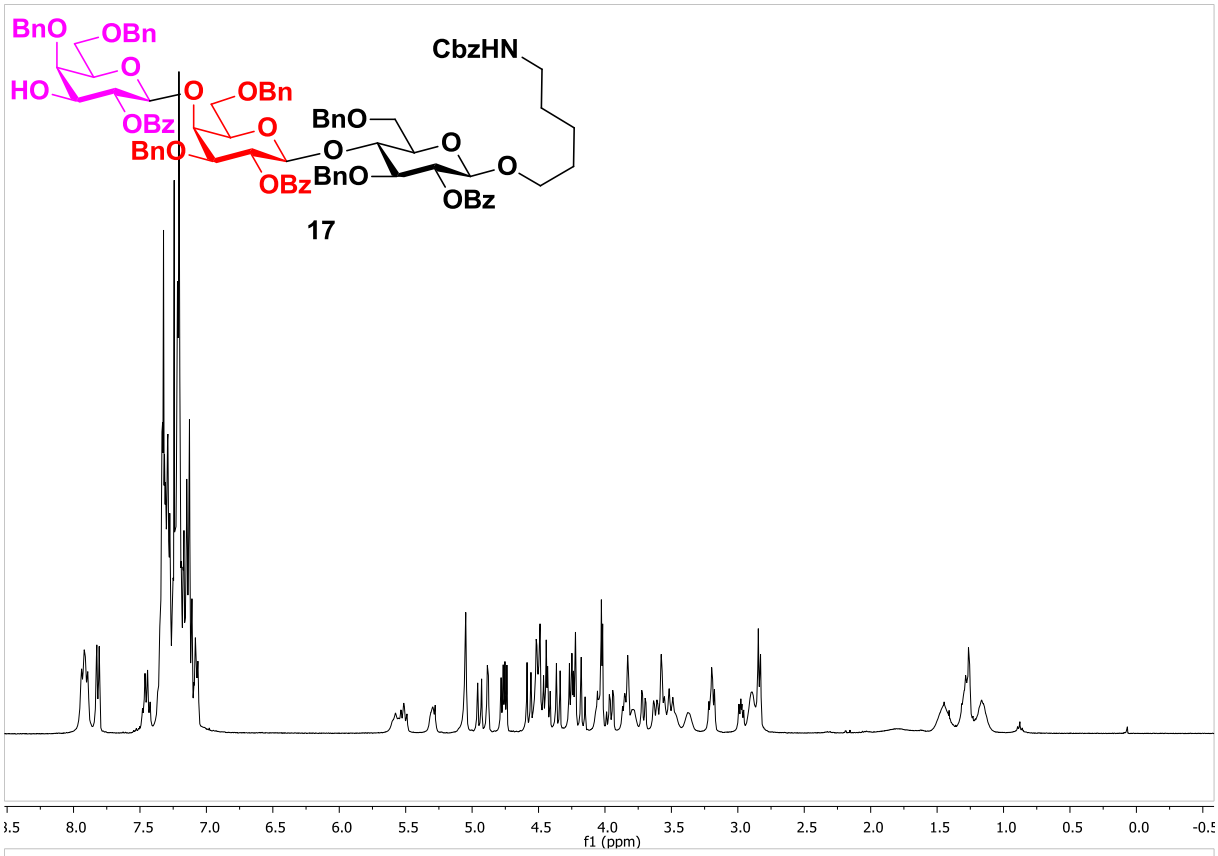

f1 (ppm)
f2 (ppm)

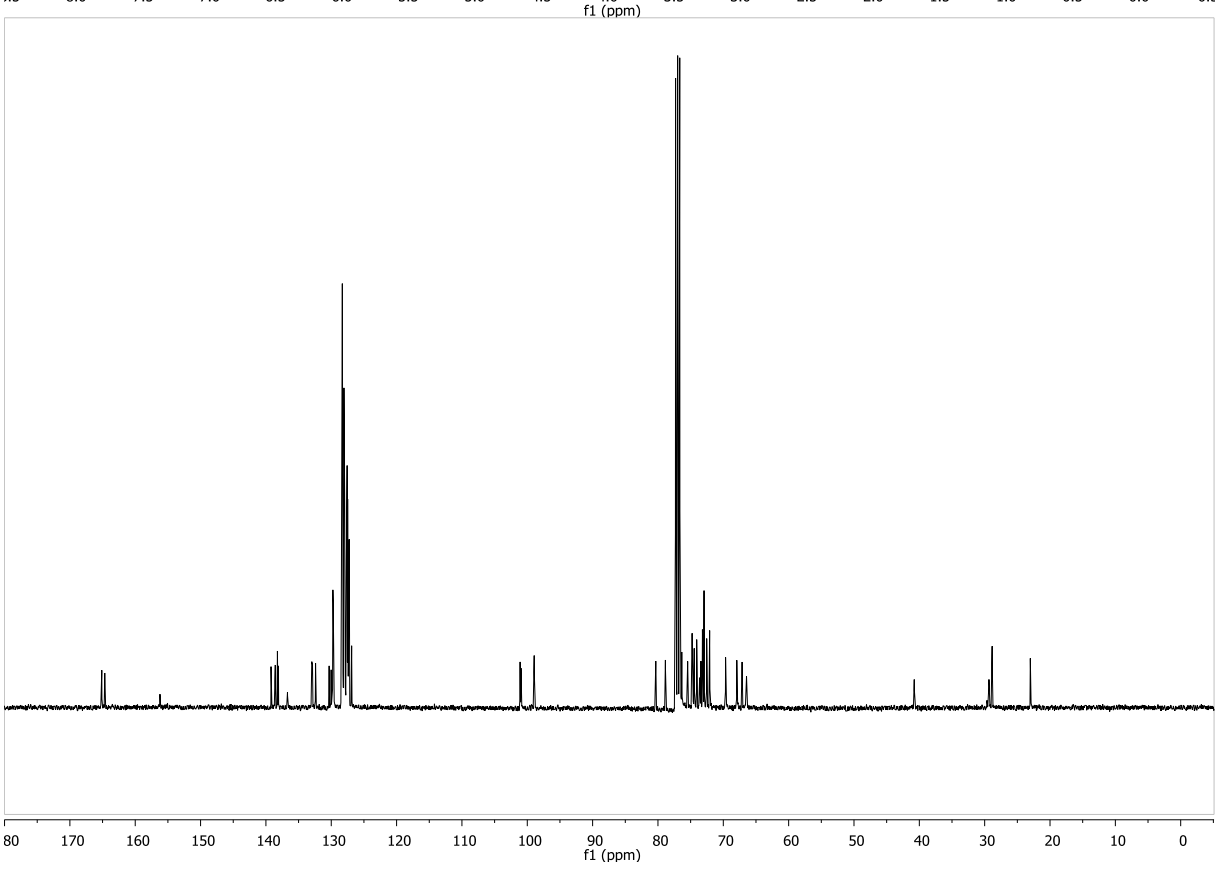

BnO
OBn
HO
O
OBz
BnO
O
OBn
O
OBz
BnO
O
BnO
O
O
OBz
CbzHN
17
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0
f1 (ppm)

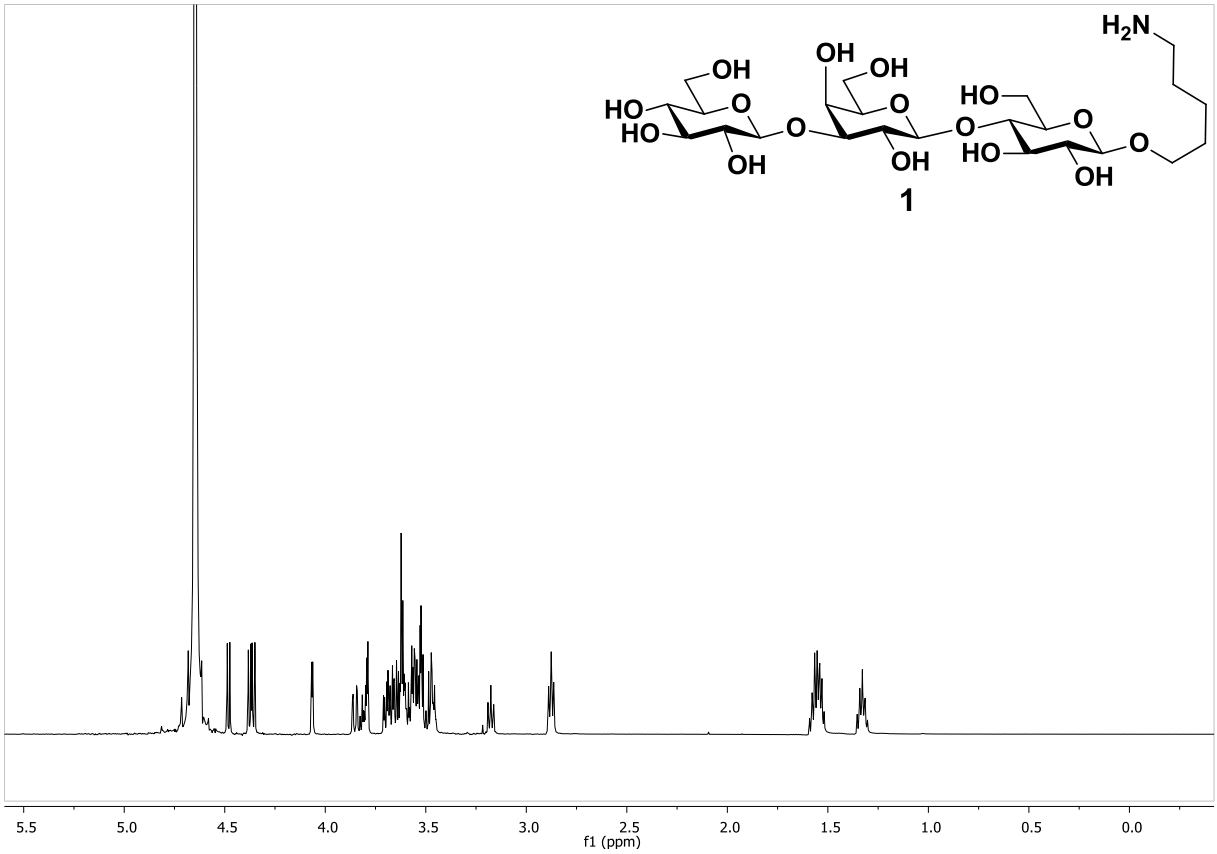

190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

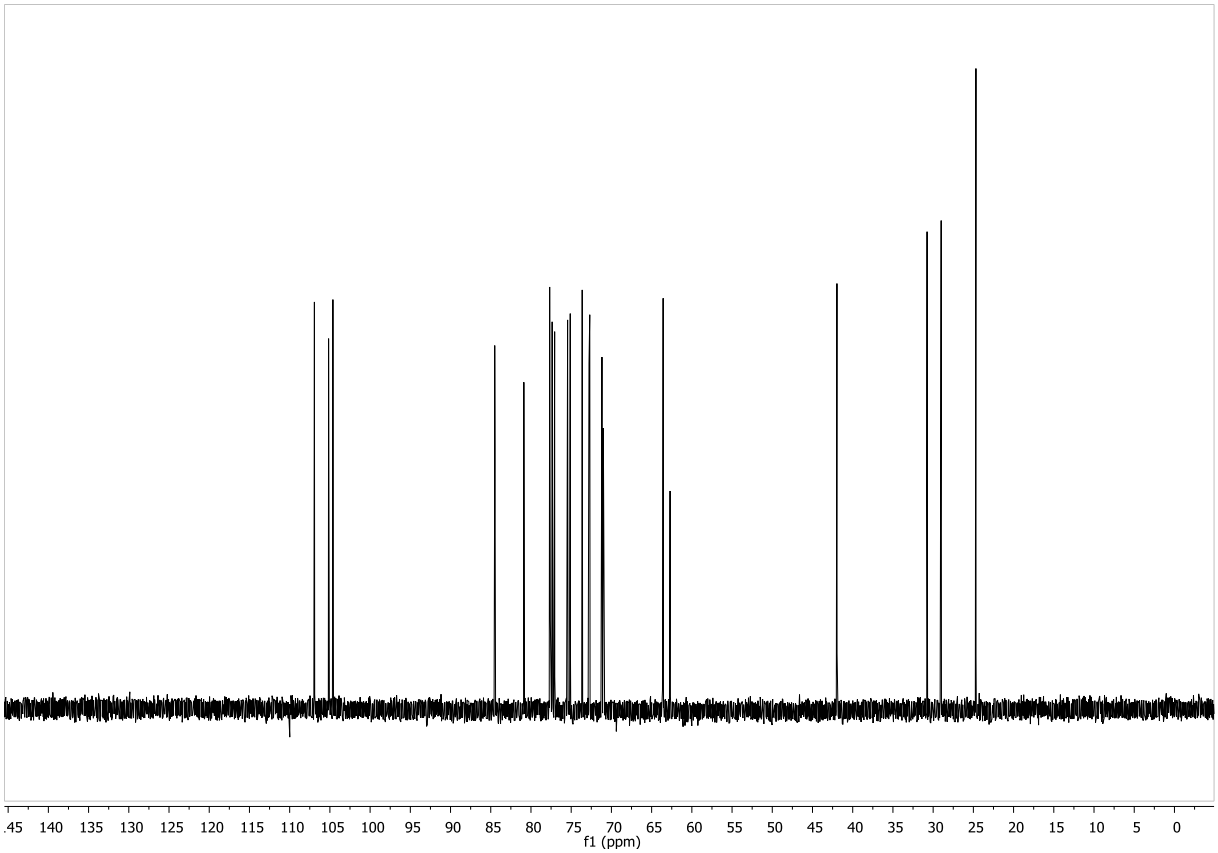

f1 (ppm)
f2 (ppm)

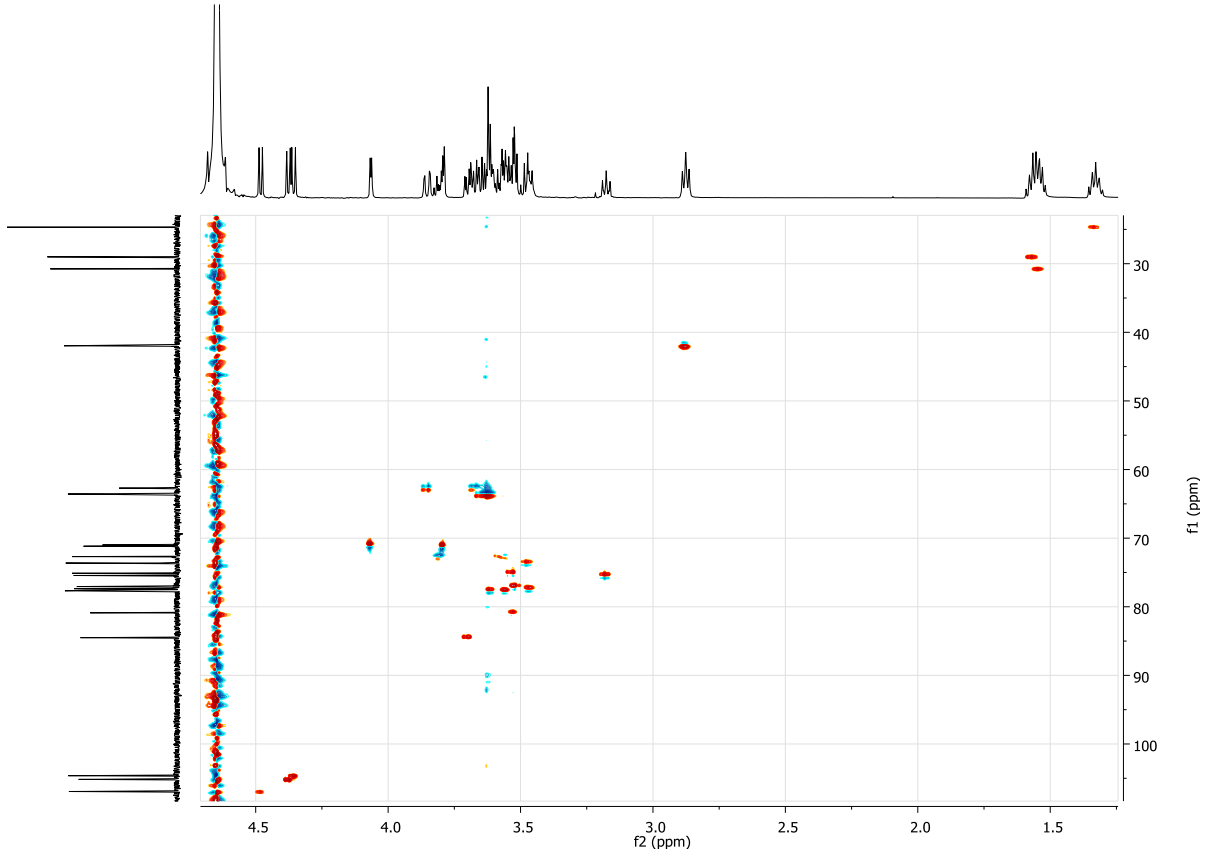

f1 (ppm)
f2 (ppm)

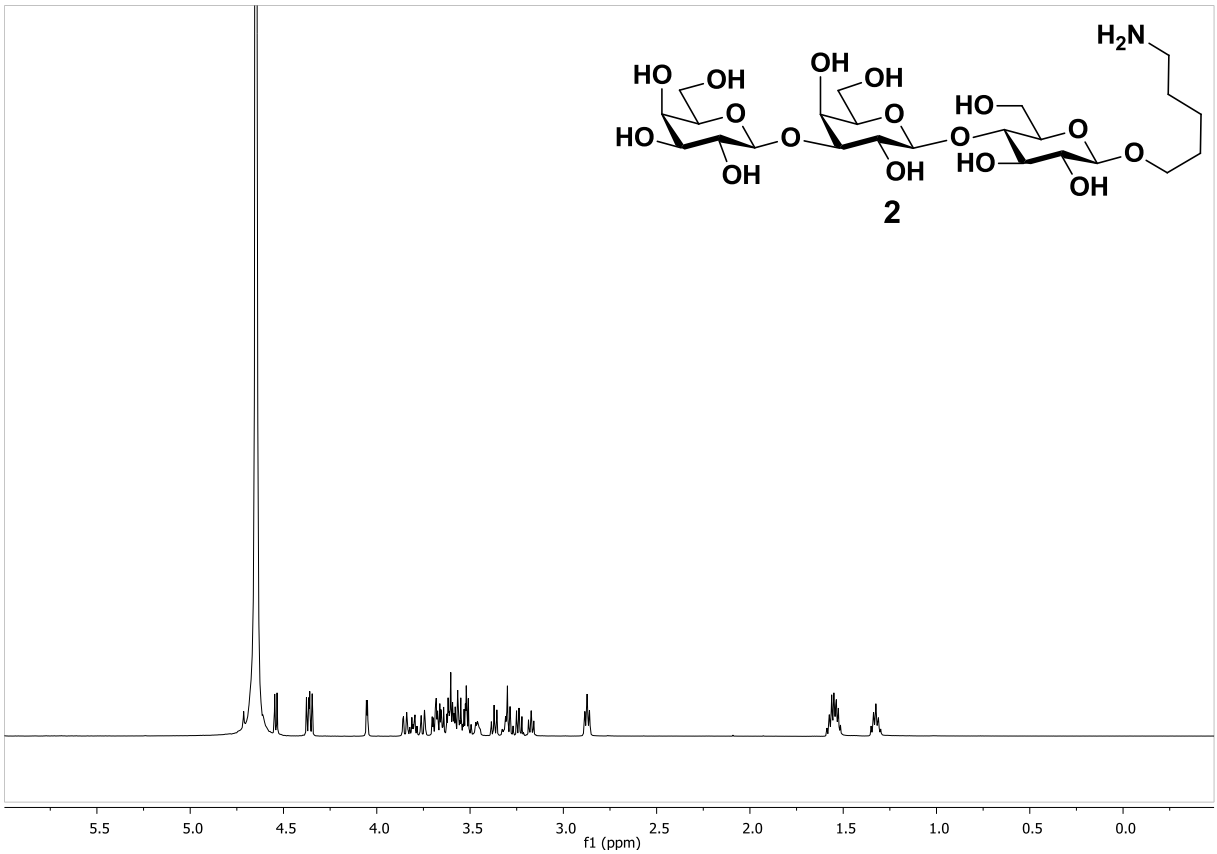

BnO
OBn
O
HO
OBz
O
OBn
O
BnO
OBz
BnO
O
BnO
O
O
OBz
CbzHN
17
f1 (ppm)

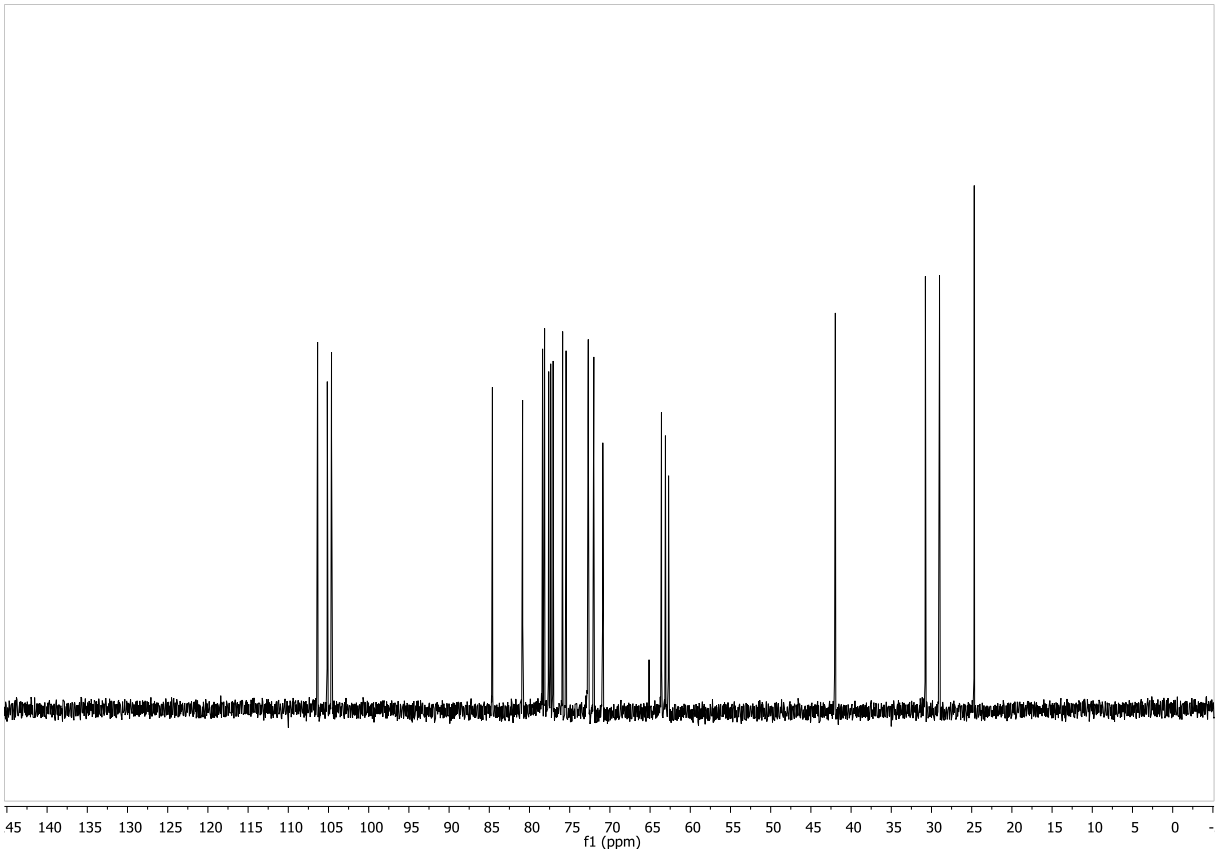

f1 (ppm)

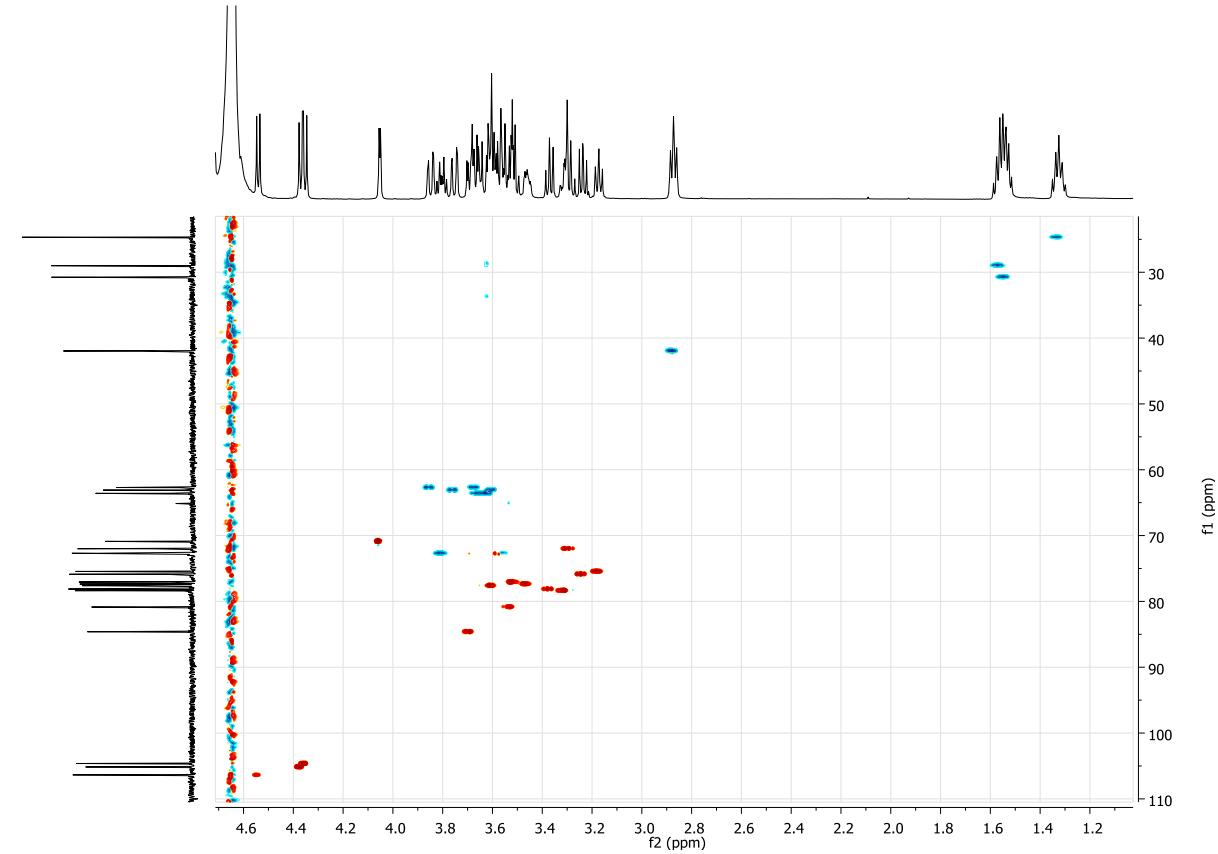

H2N
OH
OH
OH
HO
HO
O
O
O
O
HO
HO
O
O
OH
OH
OH
1
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

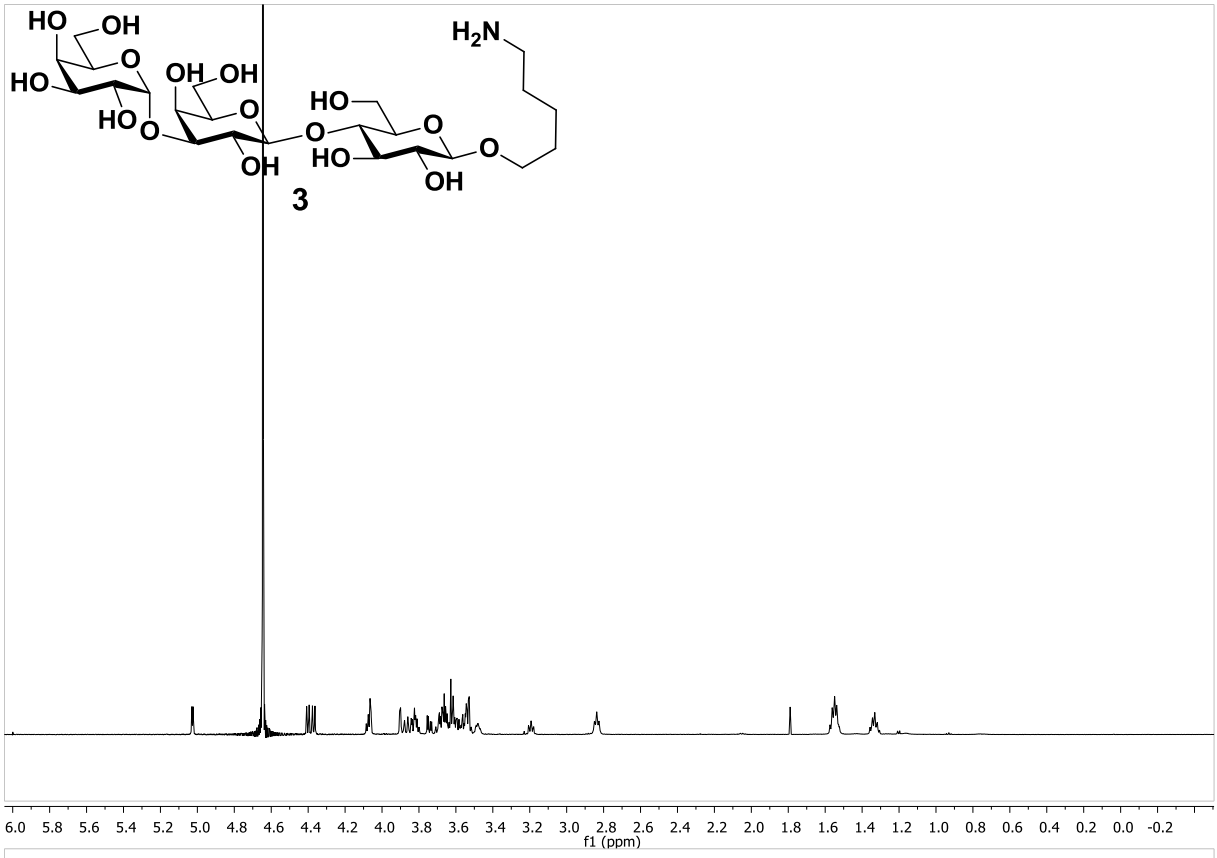

.45
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
f1 (ppm)

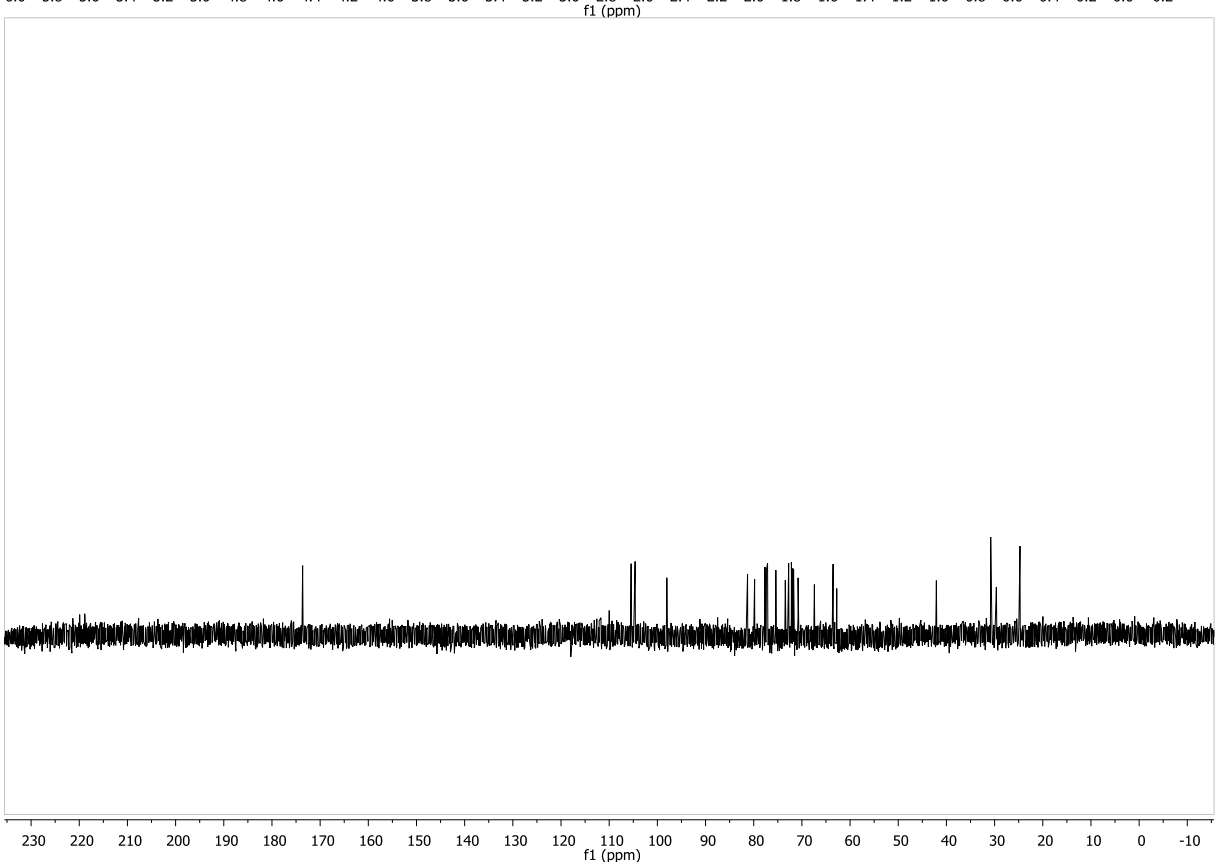

30
40
50
60
70
80
90
100
f1 (ppm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
f2 (ppm)

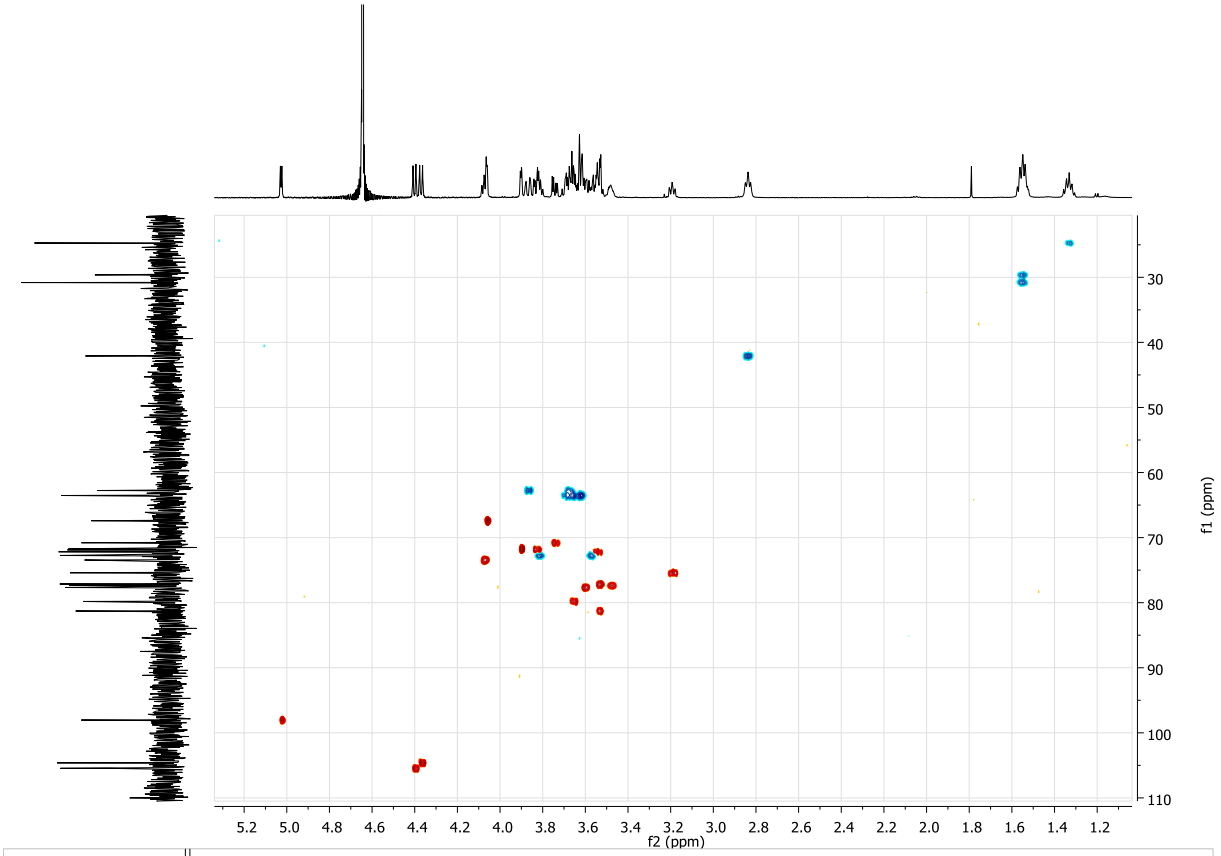

H2N
HO
OH
O
HO
OH
O
OH
OH
O
HO
O
O
HO
OH
O
2
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

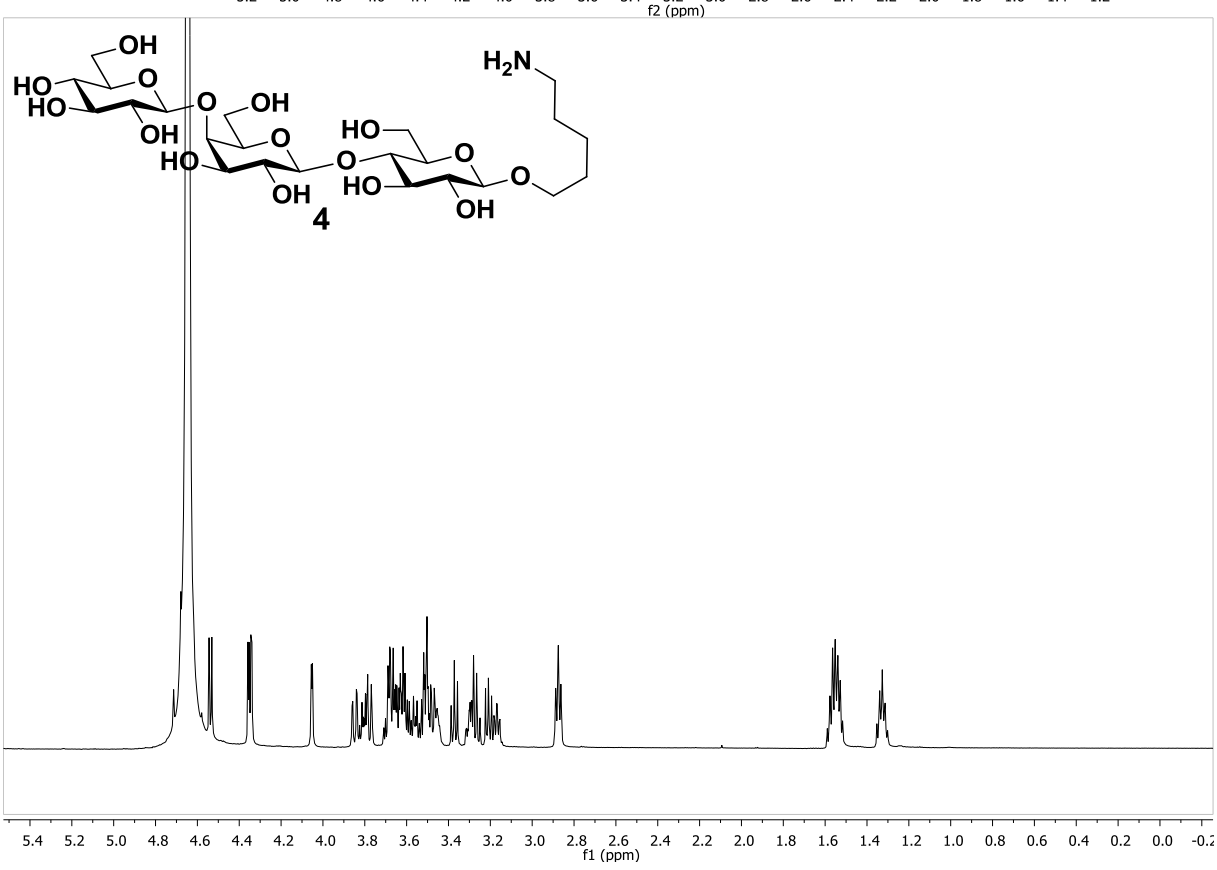

45 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0 -
f1 (ppm)

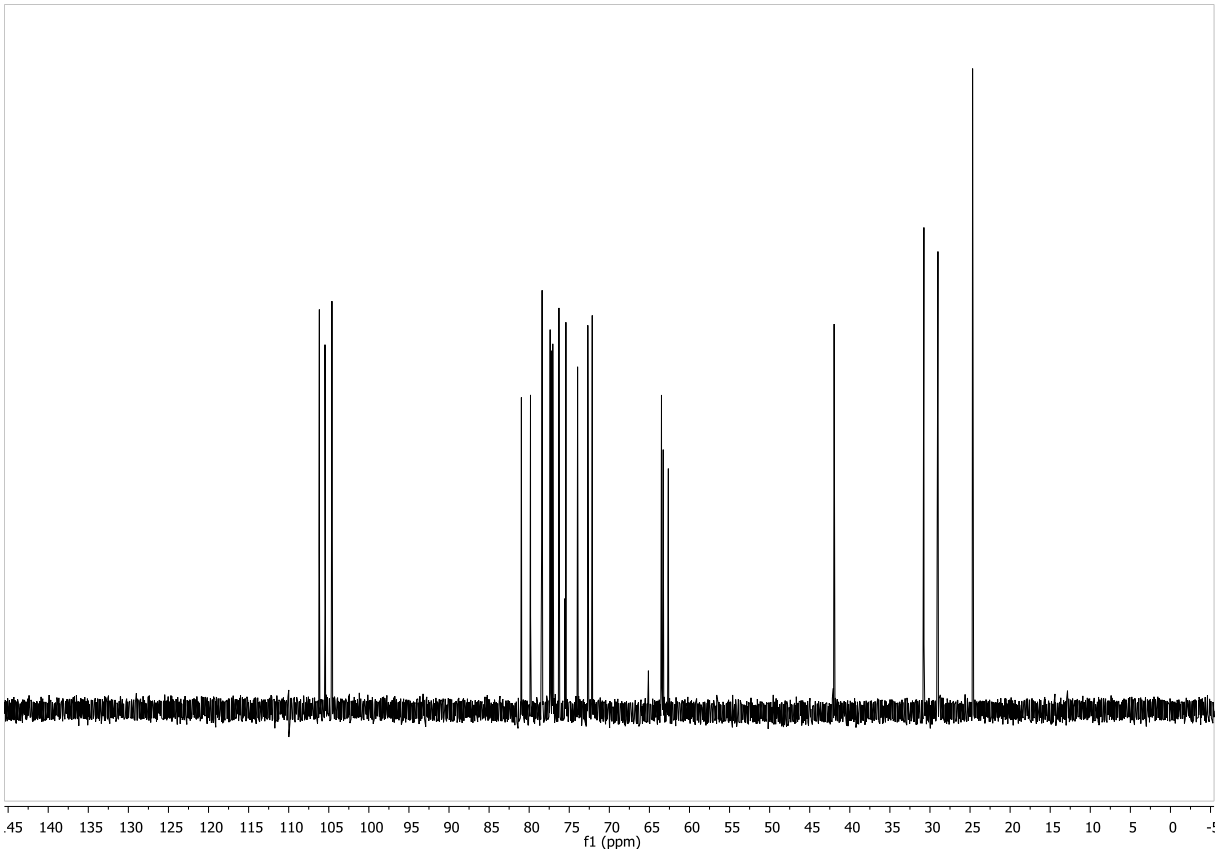

4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
f2 (ppm)
30 40 50 60 70 80 90 100 110
f1 (ppm)

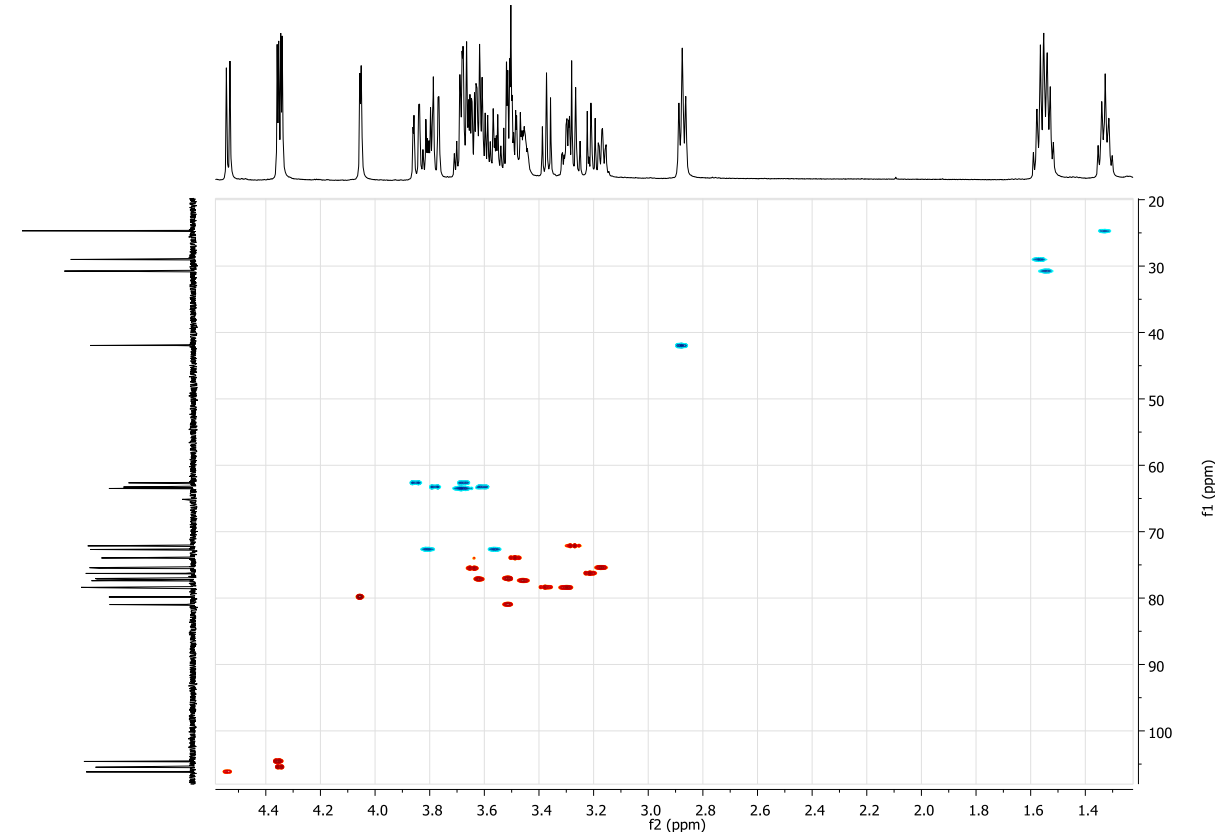

HO
OH
O
HO
HO
O
OH
OH
O
O
OH
HO
O
HO
OH
O
H_2N
3
6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0 -0.2
f1 (ppm)

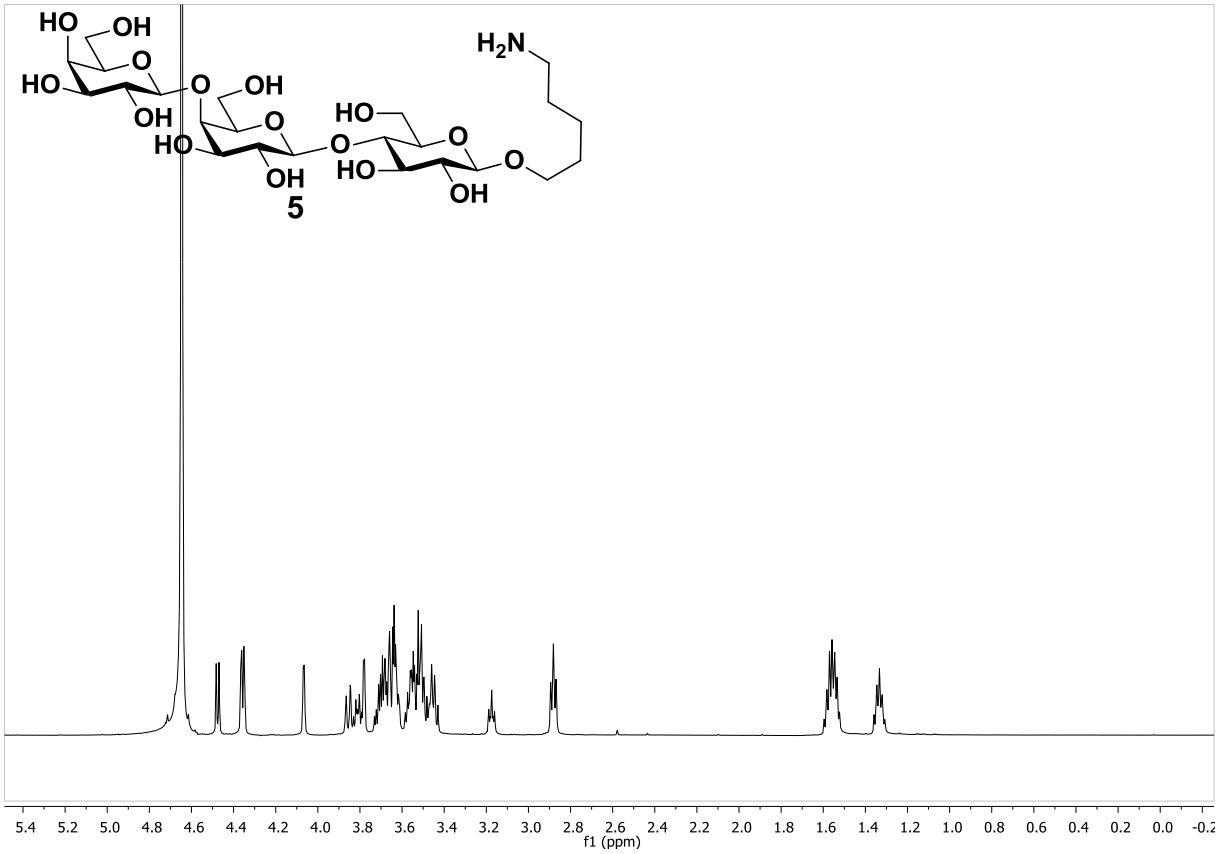

230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

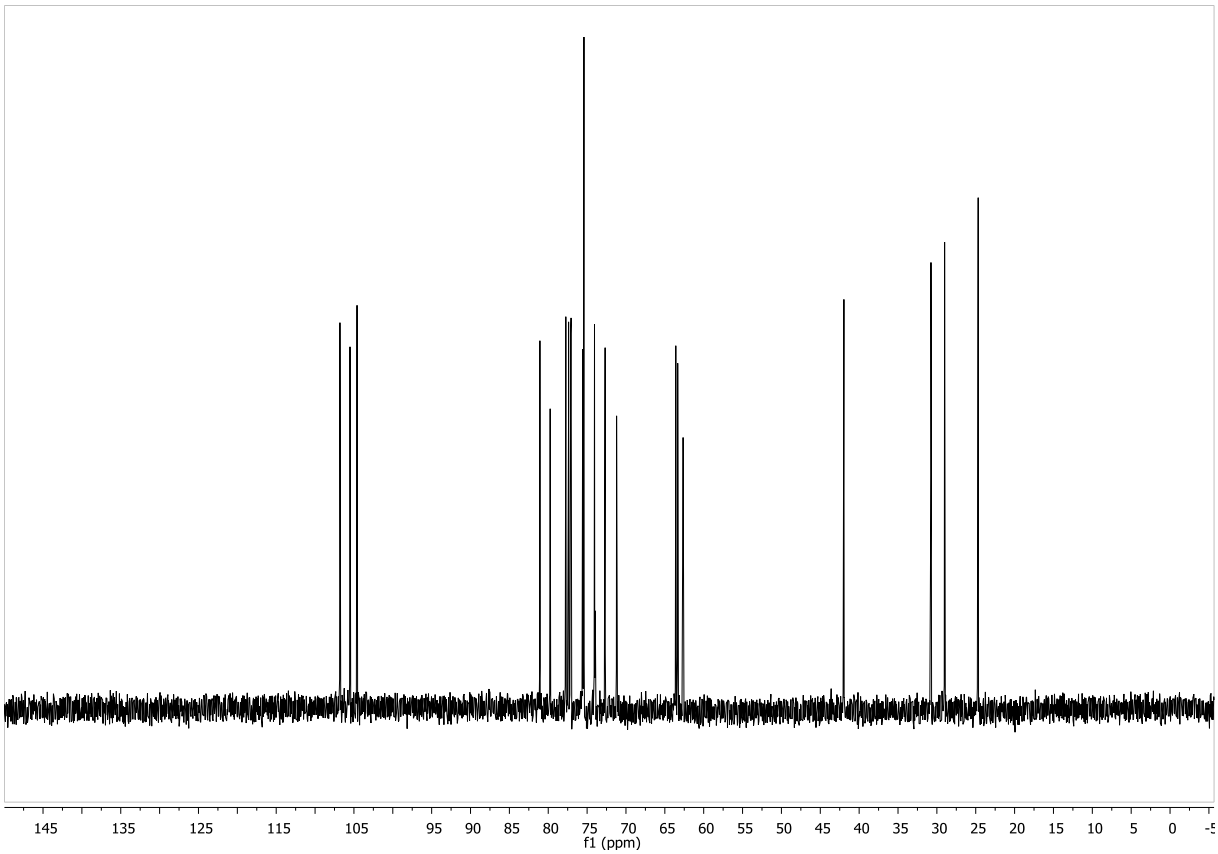

f1 (ppm)
f2 (ppm)

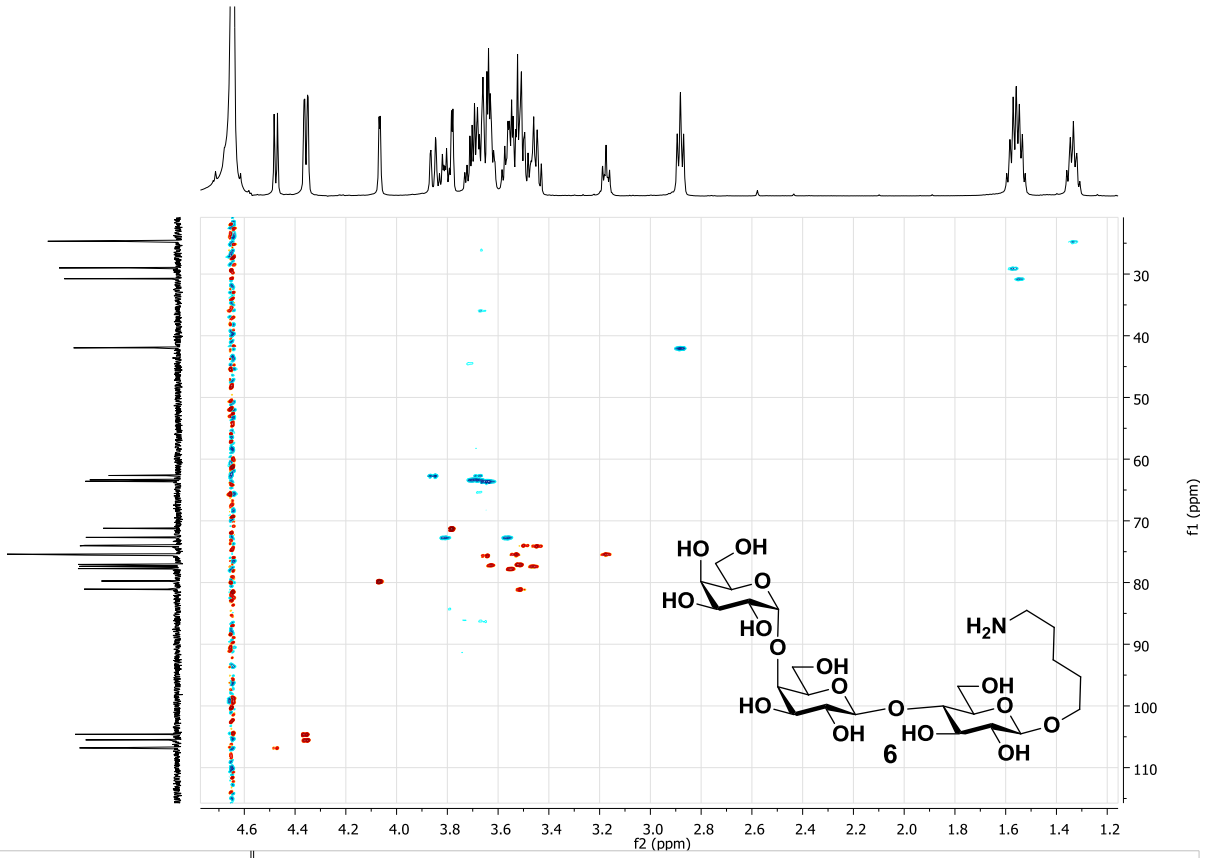

OH
HO
HO
O
OH
HO
O
OH
O
HO
O
HO
OH
4
H2N
f1 (ppm)

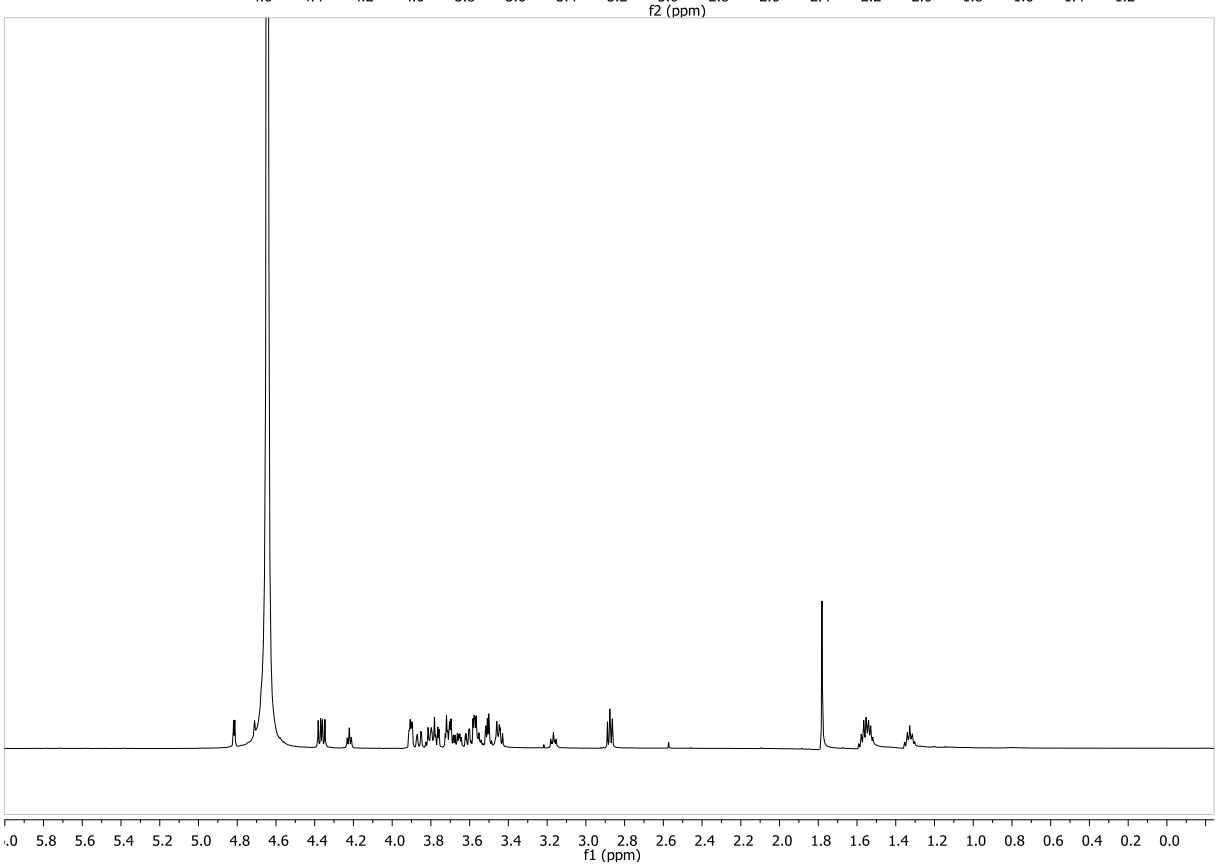

f1 (ppm)

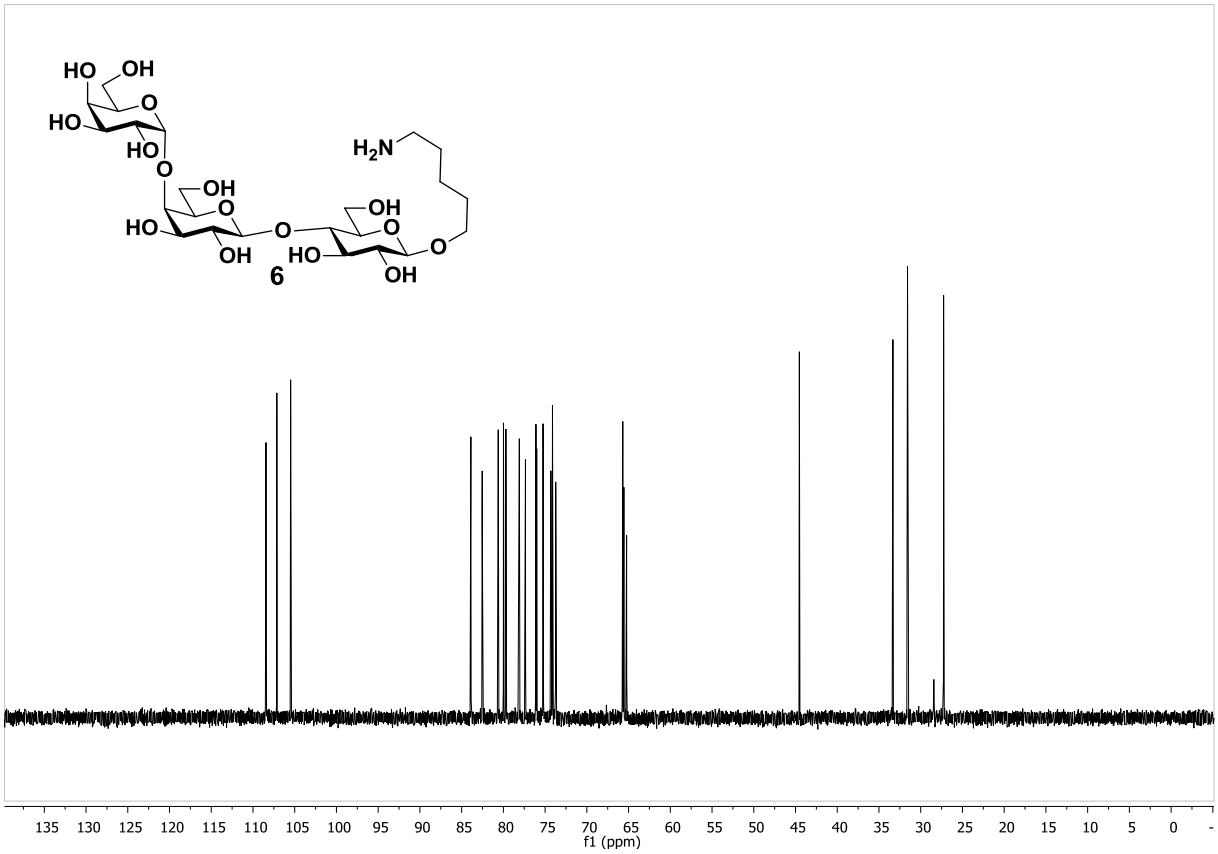

f2 (ppm)
f1 (ppm)

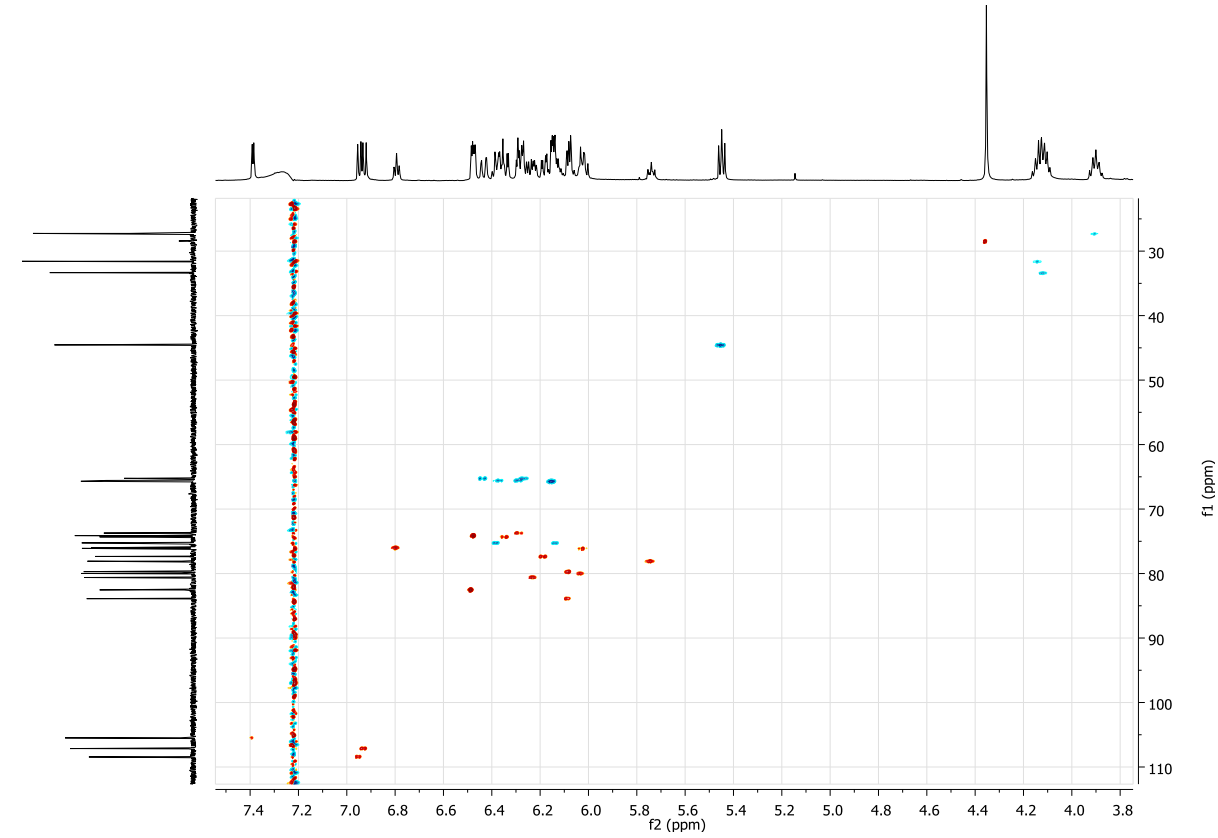

HO
OH
O
HO
O
OH
OH
HO
O
HO
O
OH
5
H_2N
HO
O
O
HO
OH
5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0
f1 (ppm)

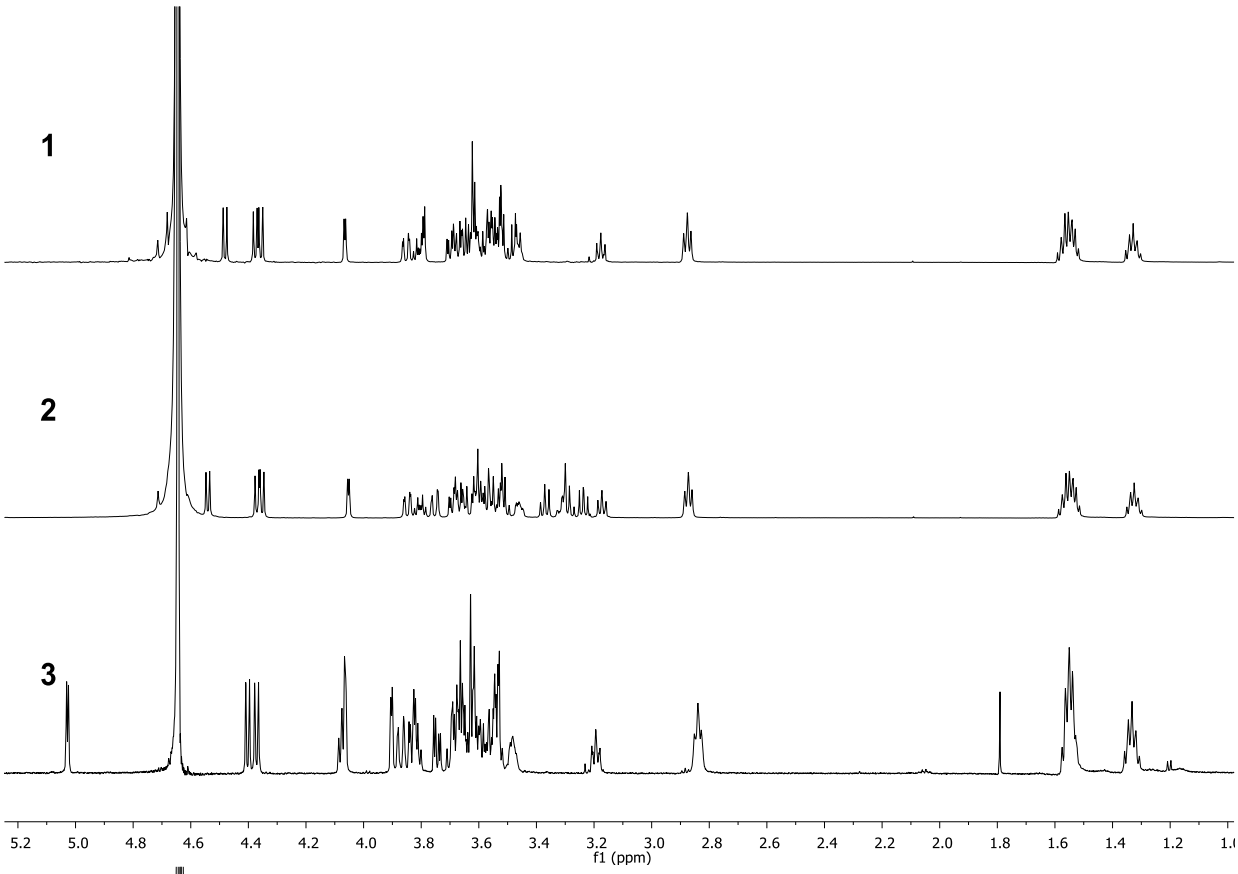

145 135 125 115 105 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
f1 (ppm)

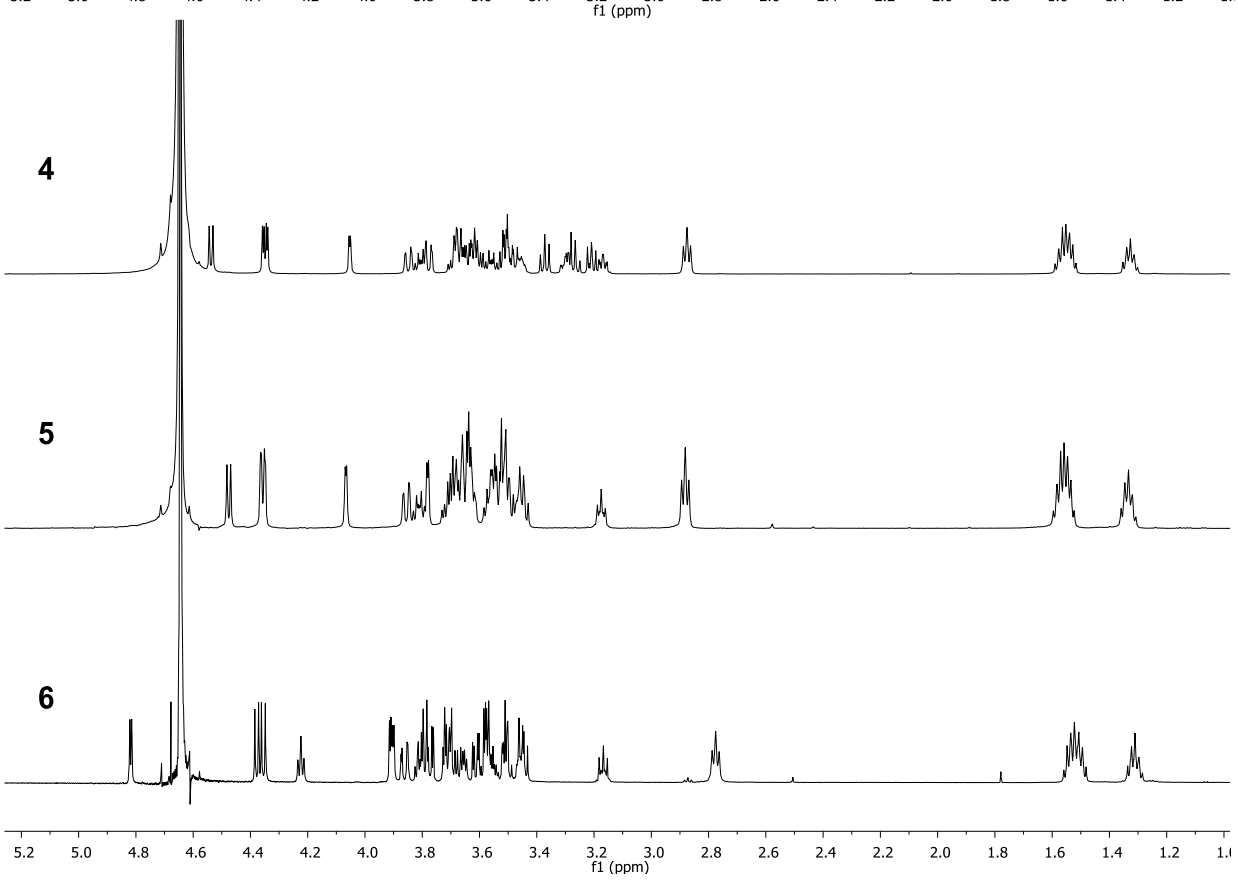

HO
OH
O
HO
HO
O
OH
O
HO
OH
O
H2N
OH
O
HO
O
OH
6
f1 (ppm)
f2 (ppm)

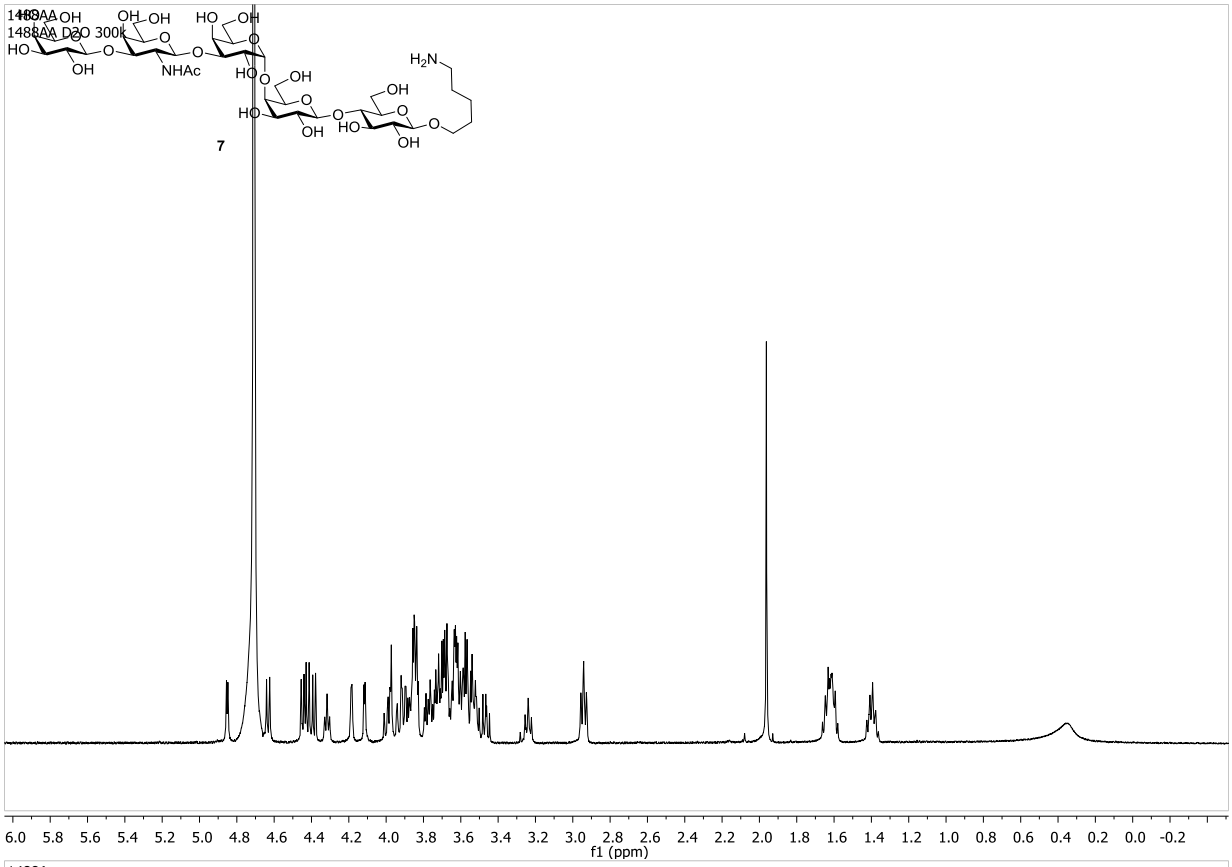

f1 (ppm)

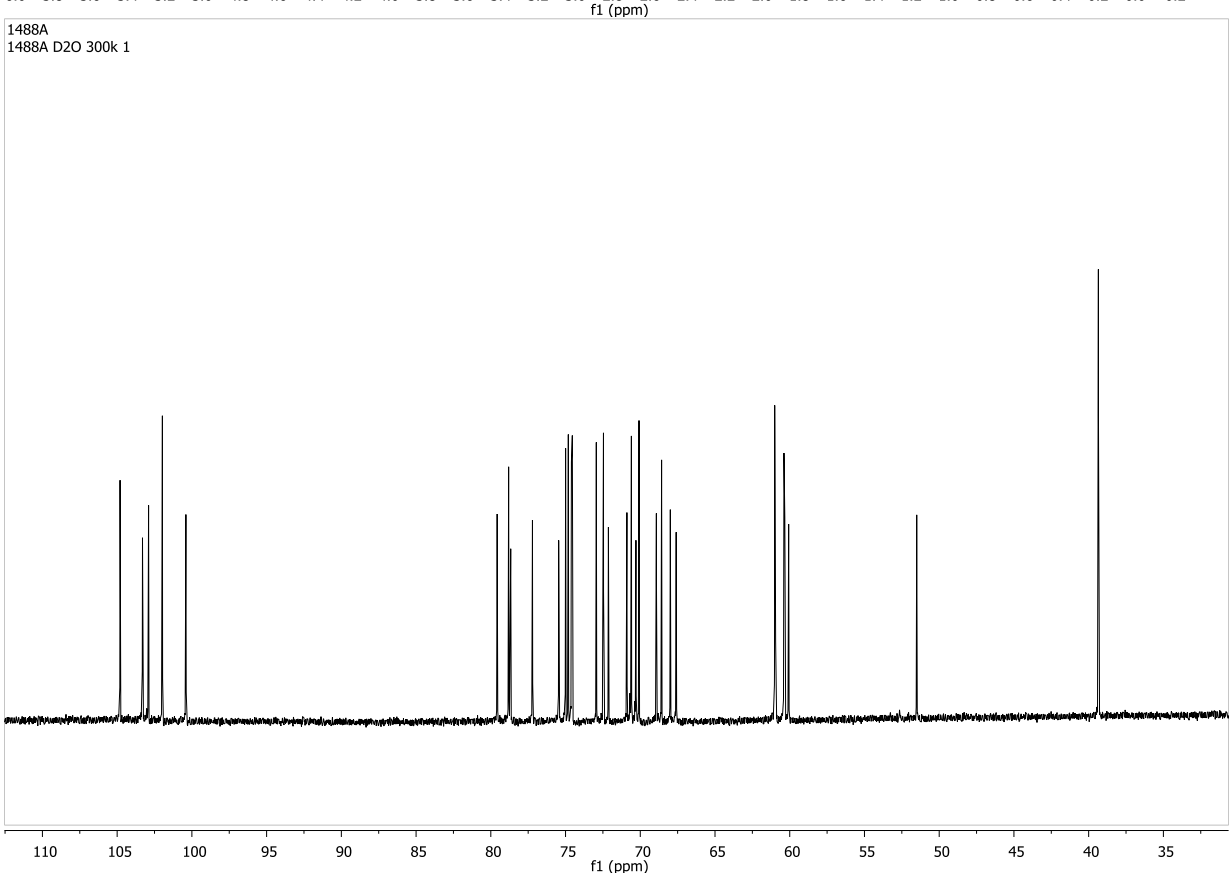

HO
OH
O
HO
HO
O
OH
O
HO
O
OH
6
H2N
OH
O
HO
O
OH
135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5 0
f1 (ppm)

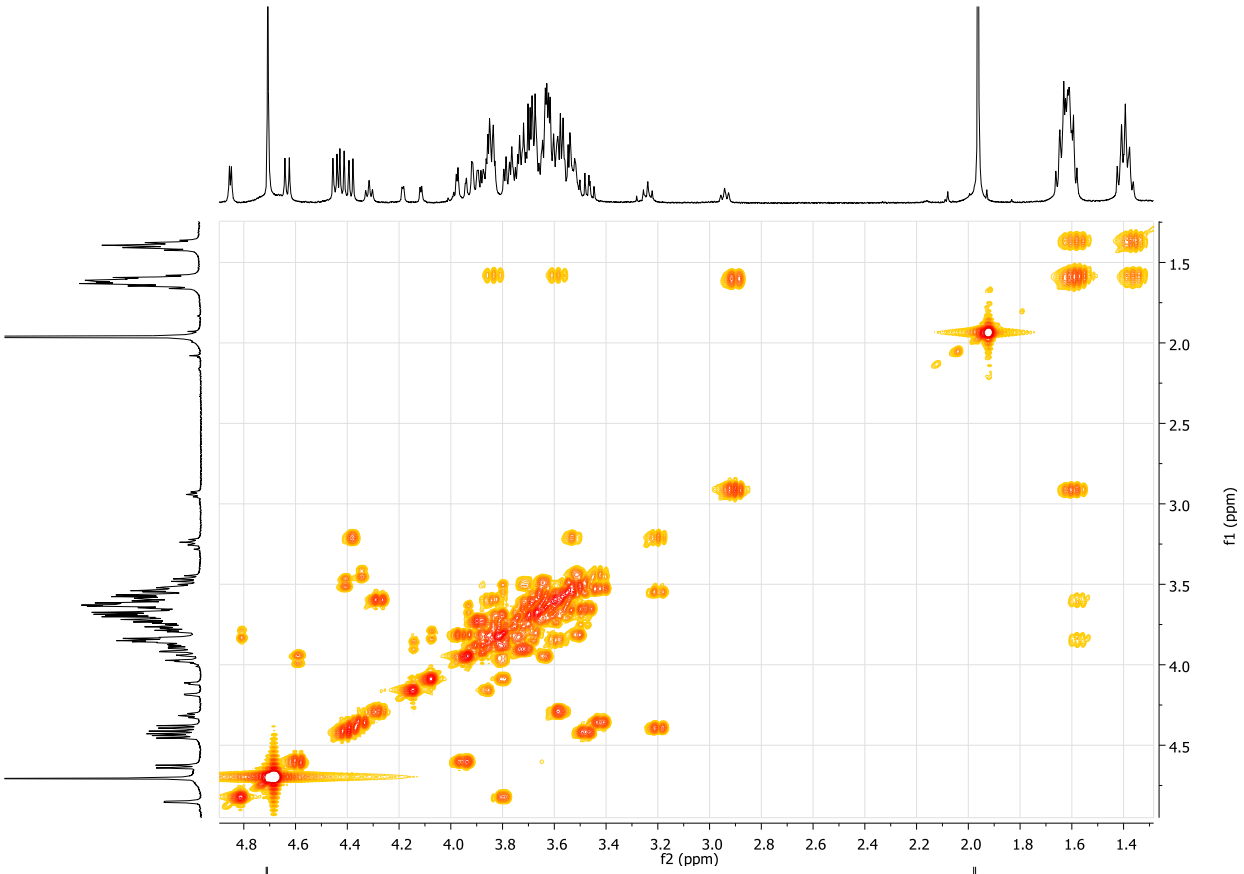

30
40
50
60
70
80
90
100
110
f1 (ppm)
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8
f2 (ppm)

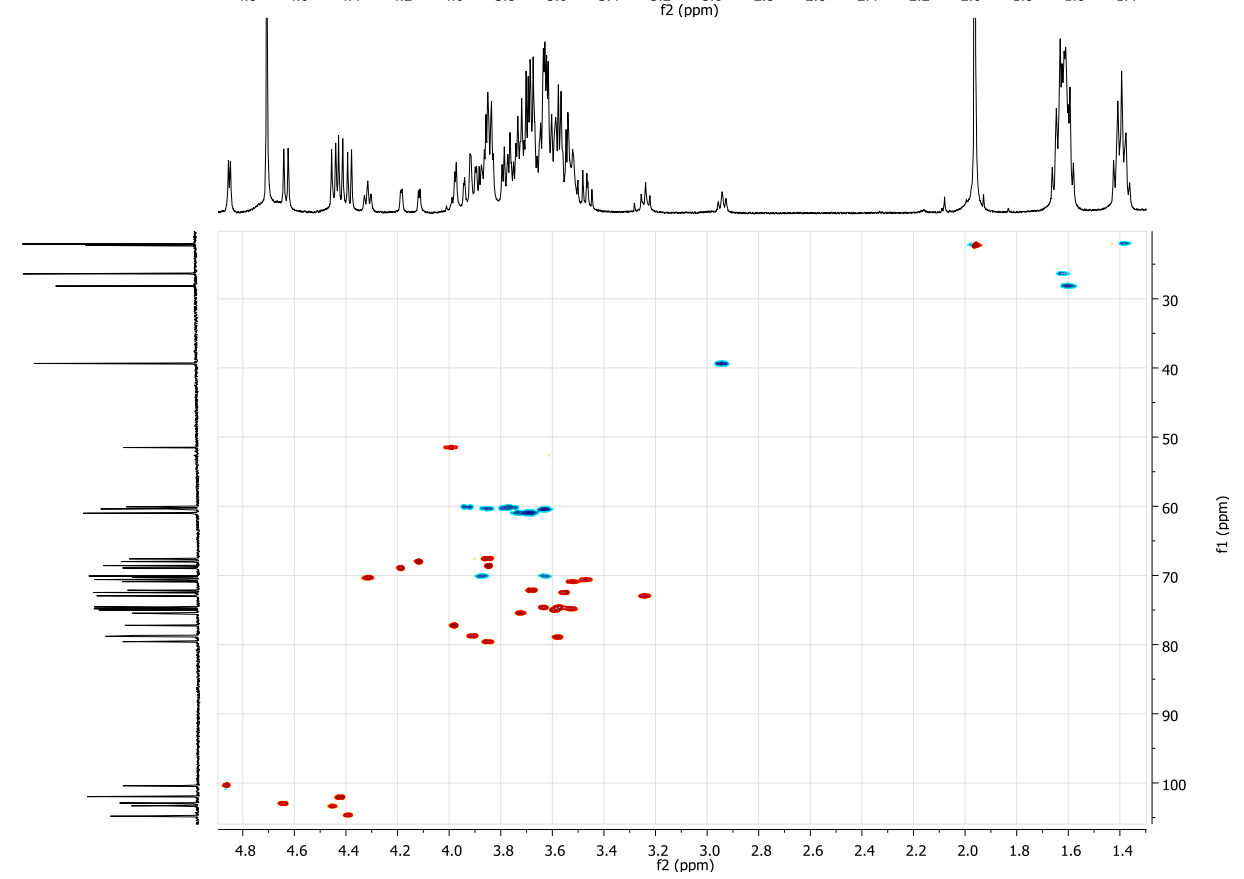

1
2
3
5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
f1 (ppm)

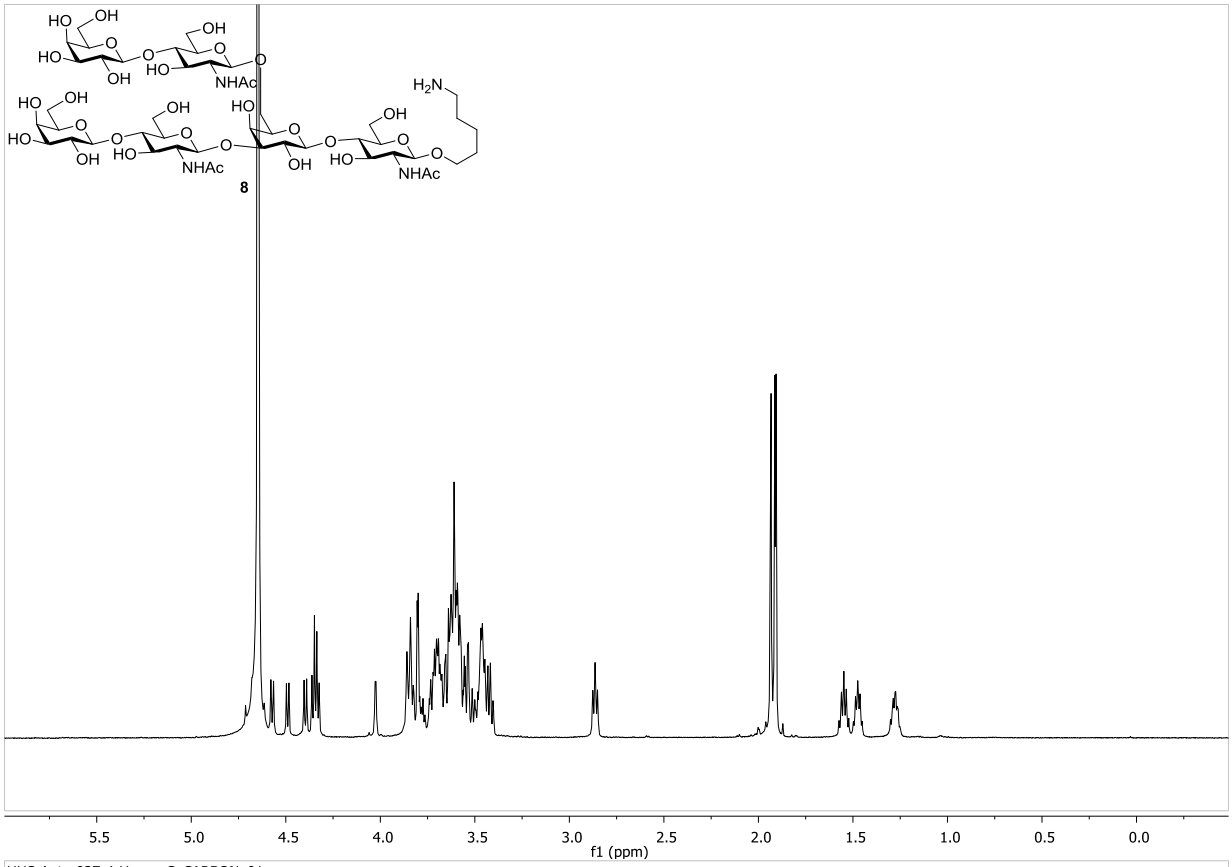

4
5
6
5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
f1 (ppm)

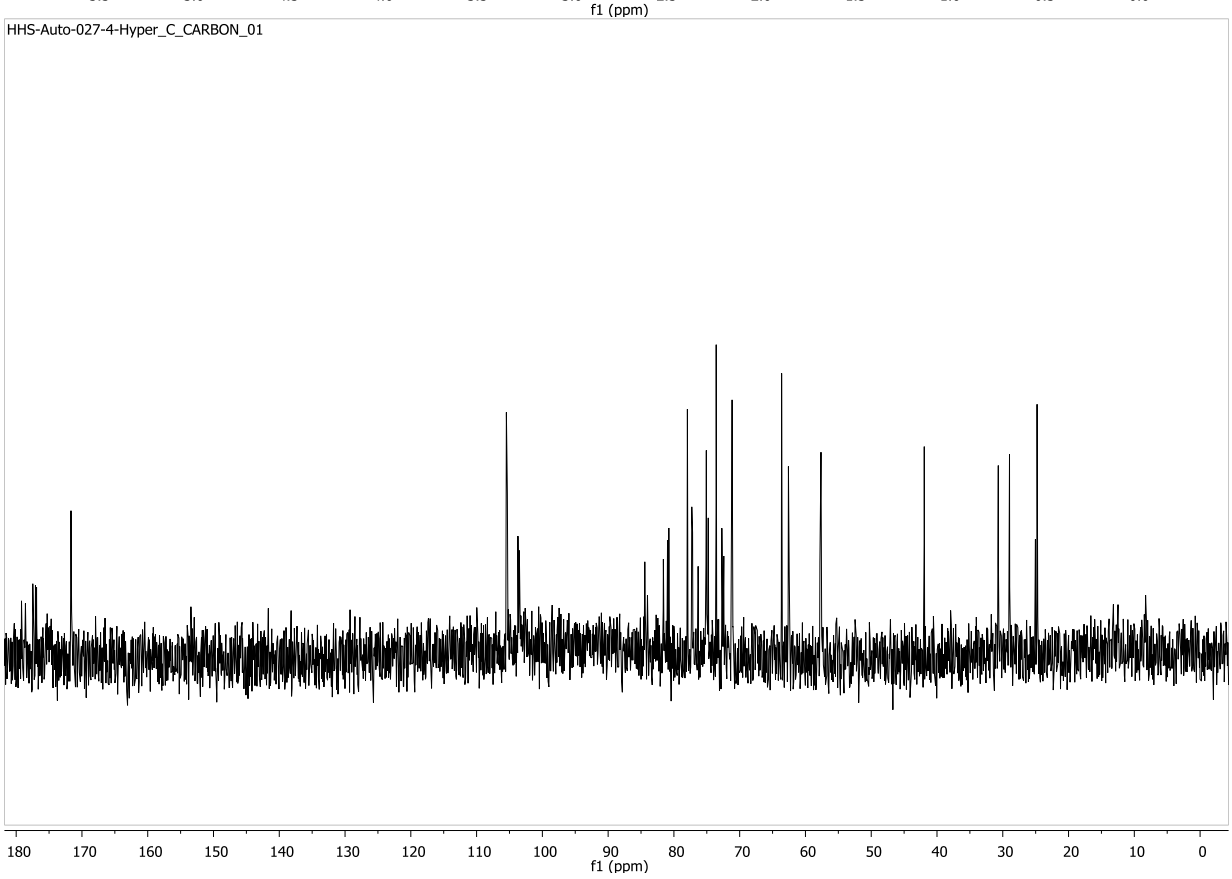

1488AA
1488AA D2O 300k
7
6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0 -0.2
f1 (ppm)

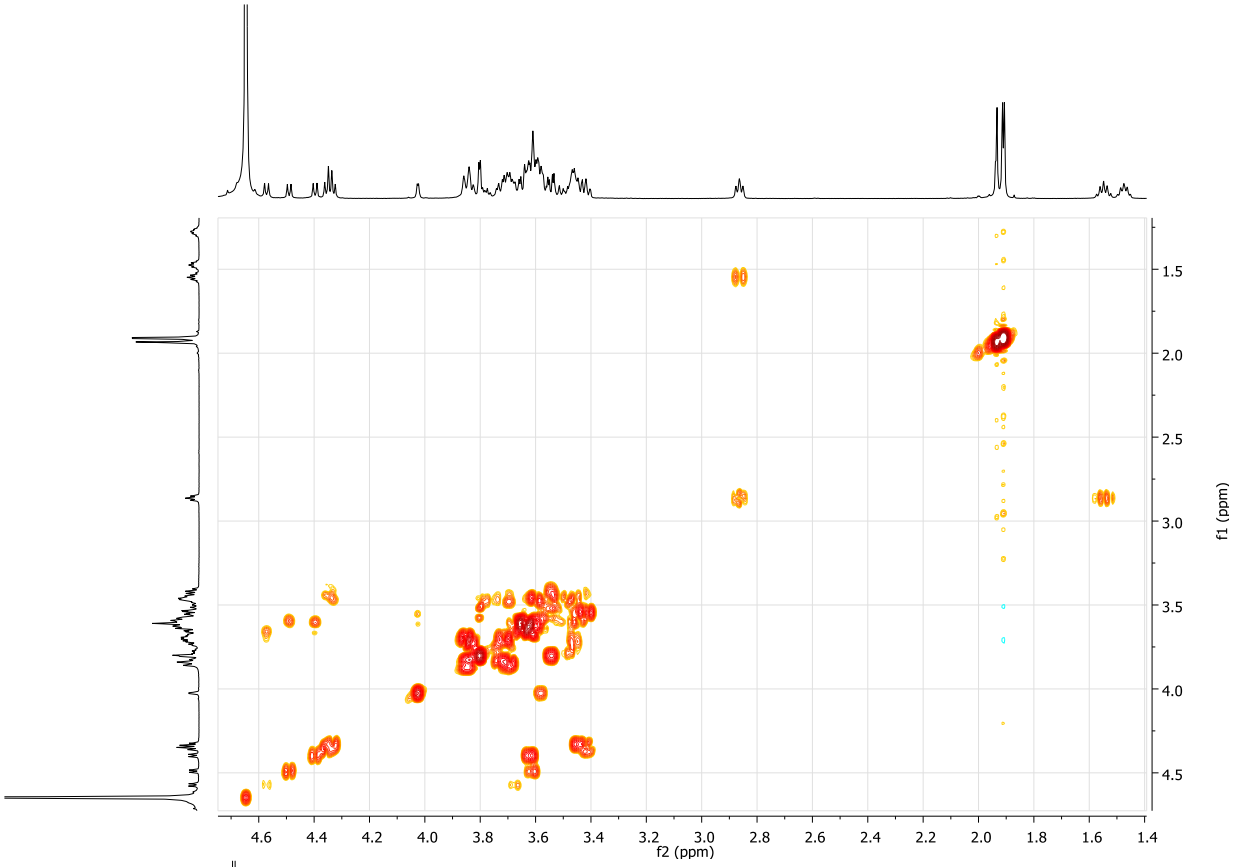

1488A
1488A D2O 300k 1
110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35
f1 (ppm)

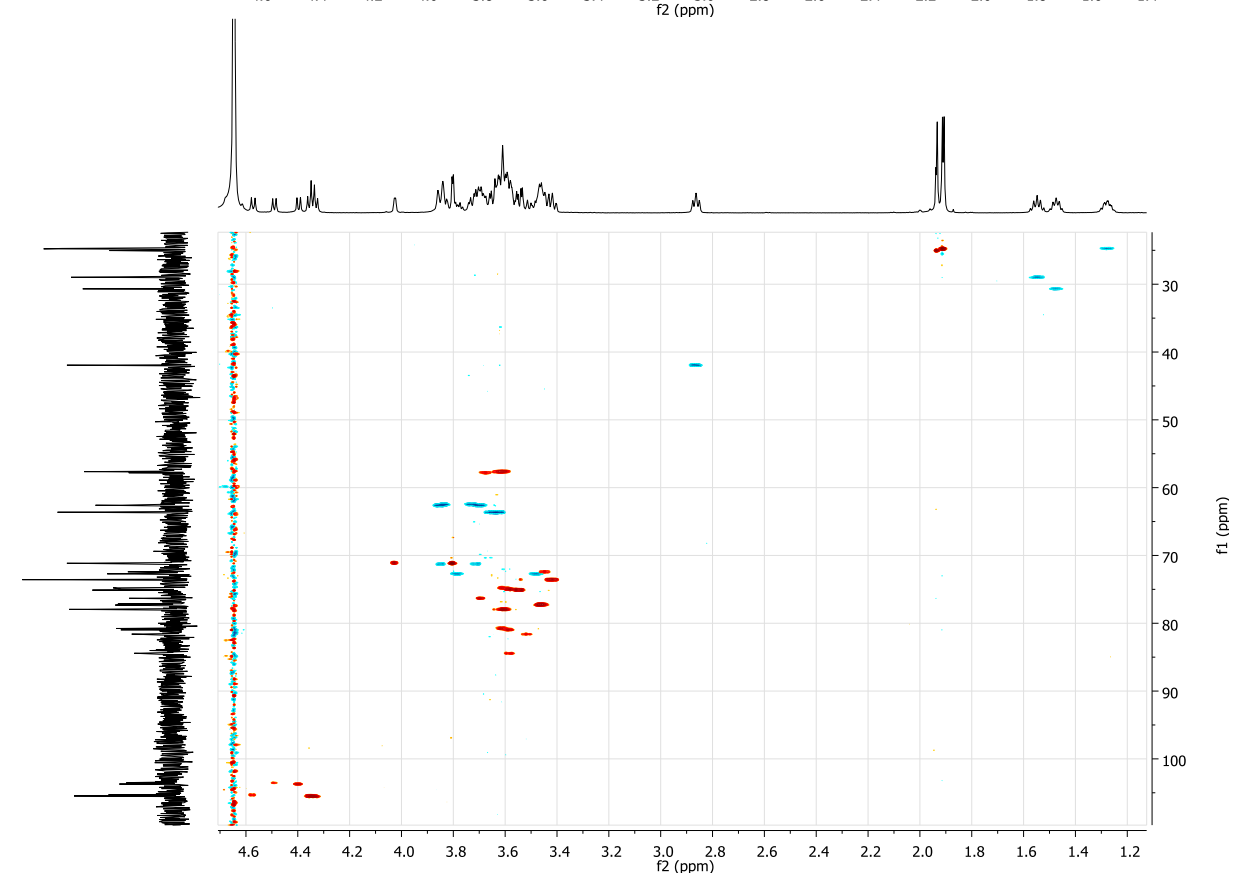

1.5
2.0
2.5
3.0
3.5
4.0
4.5
f1 (ppm)
4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4
f2 (ppm)

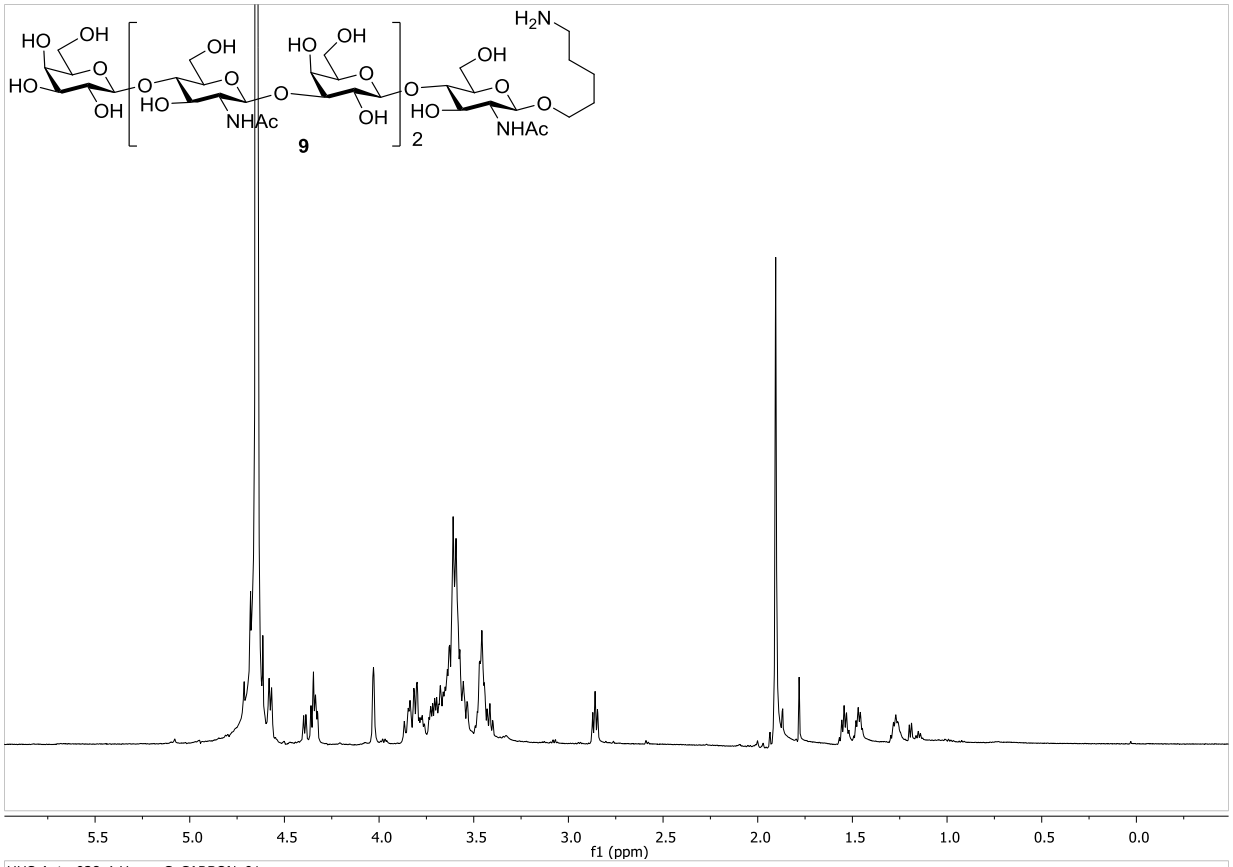

30
40
50
60
70
80
90
100
f1 (ppm)
4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4
f2 (ppm)

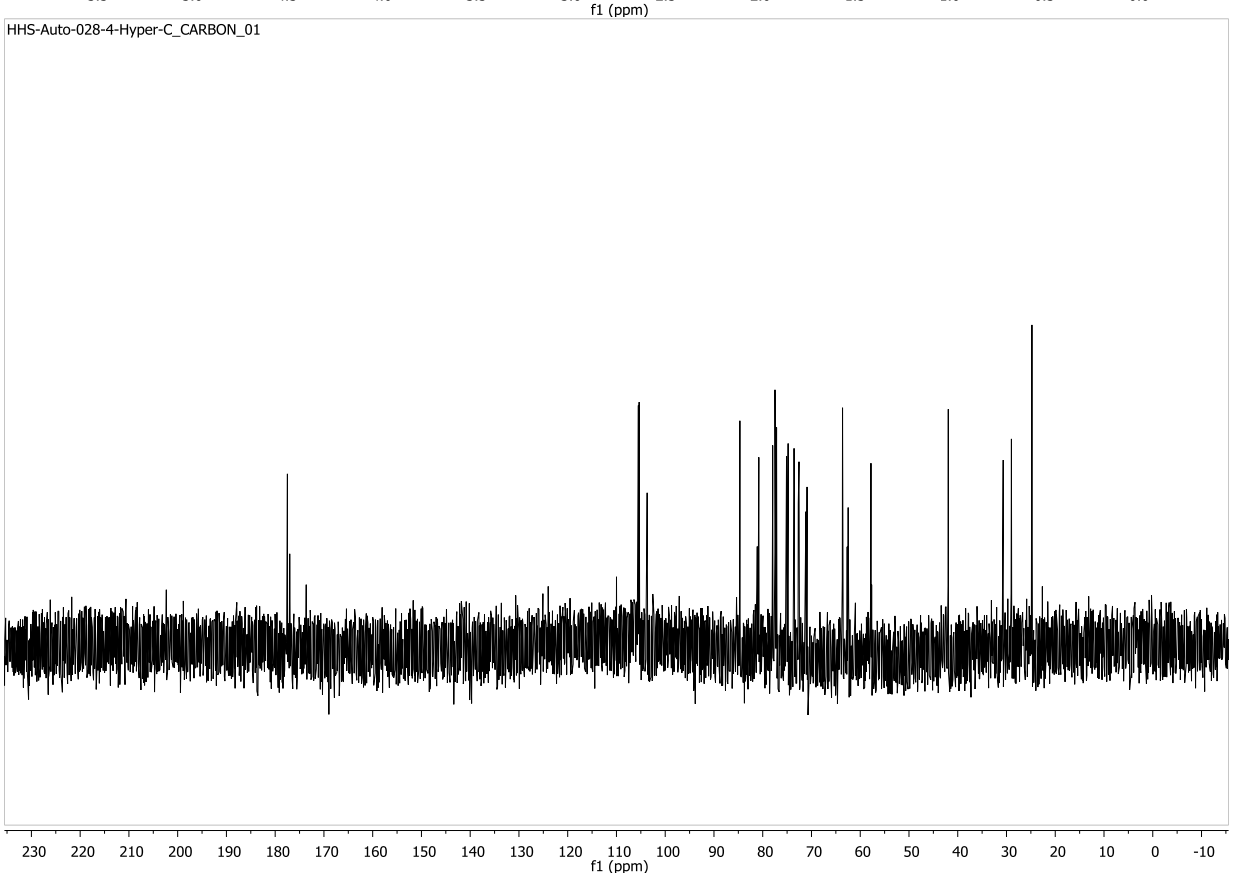

8
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

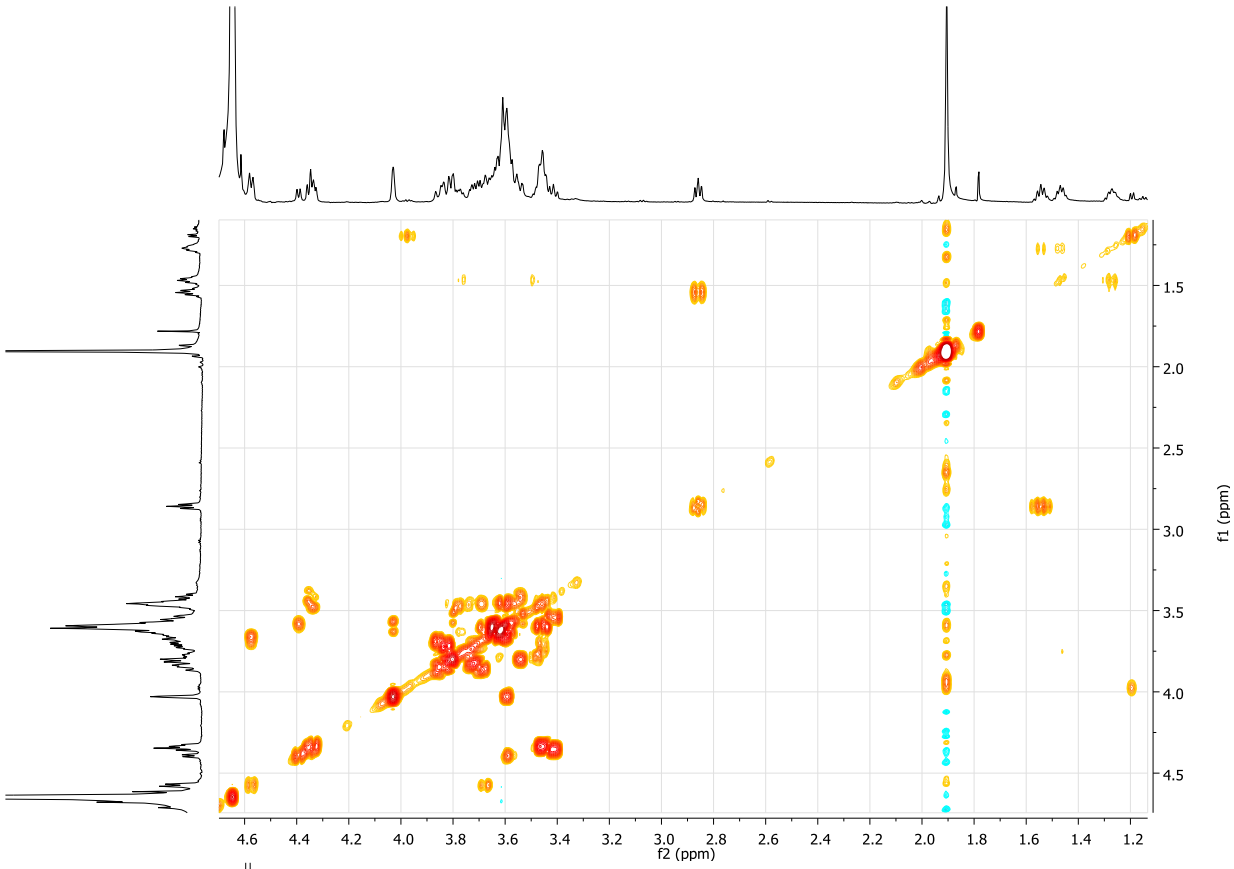

HHS-Auto-027-4-Hyper_C_CARBON_01
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

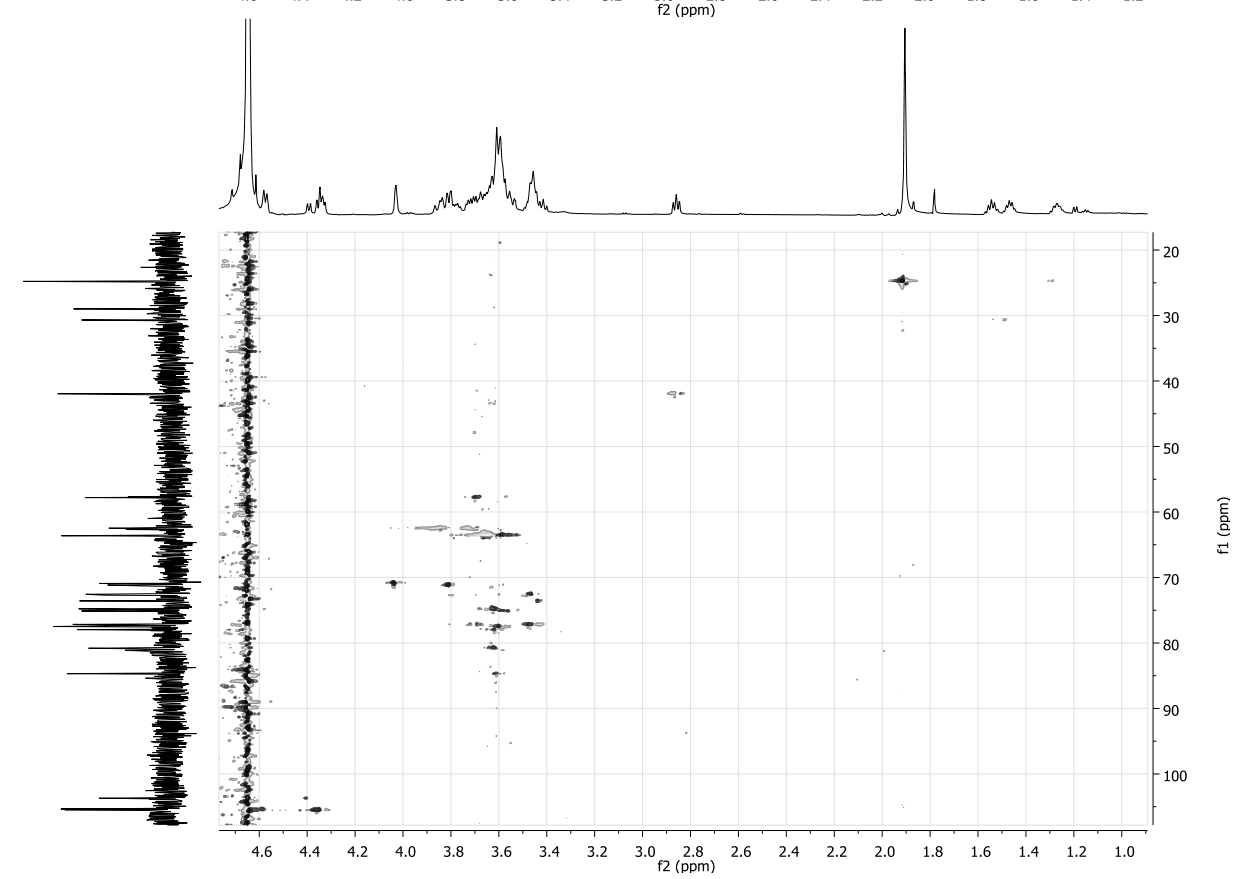

1.5
2.0
2.5
3.0
3.5
4.0
4.5
f1 (ppm)
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
f2 (ppm)

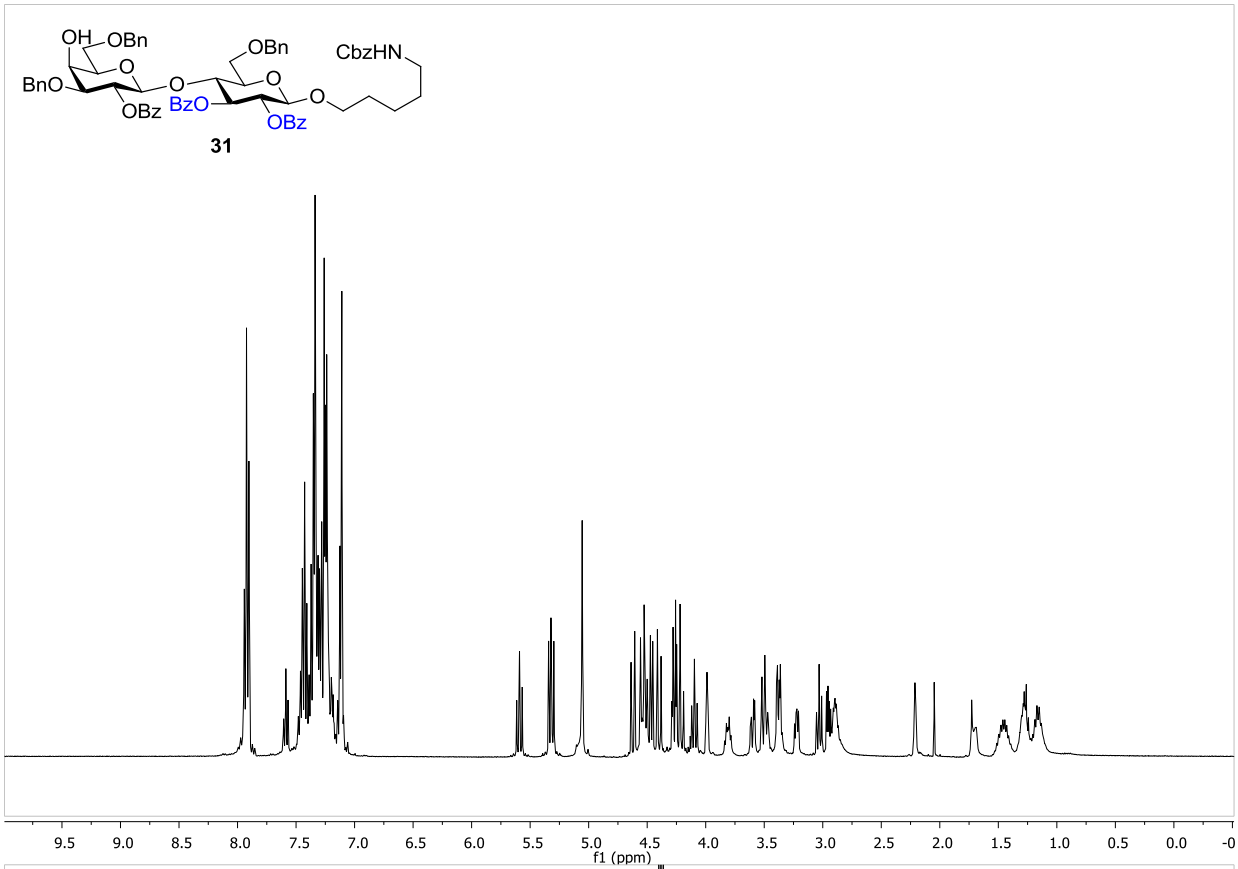

30
40
50
60
70
80
90
100
f1 (ppm)
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
f2 (ppm)

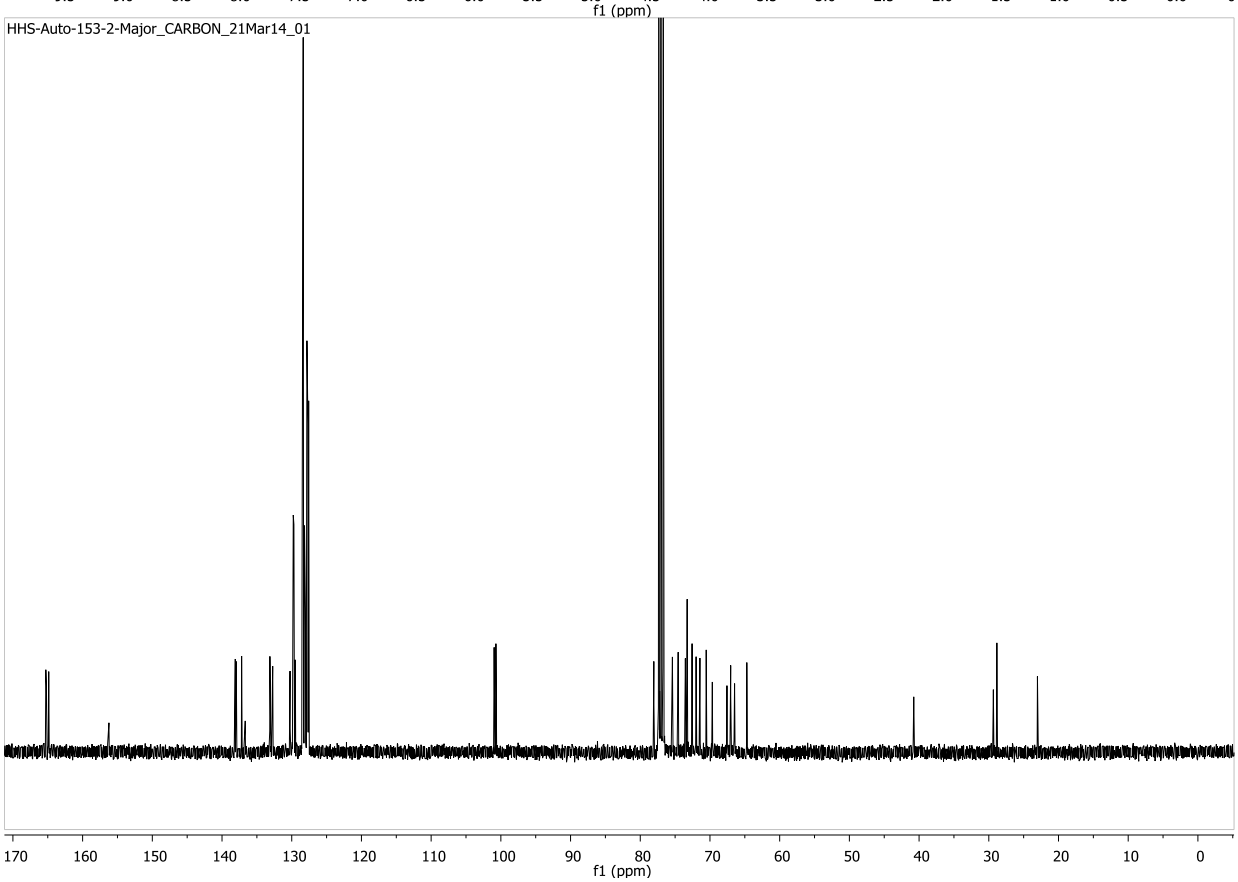

HO
OH
O
HO
OH
O
HO
NHAc
OH
O
HO
OH
O
HO
OH
O
HO
NHAc
H_2N
9
2
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

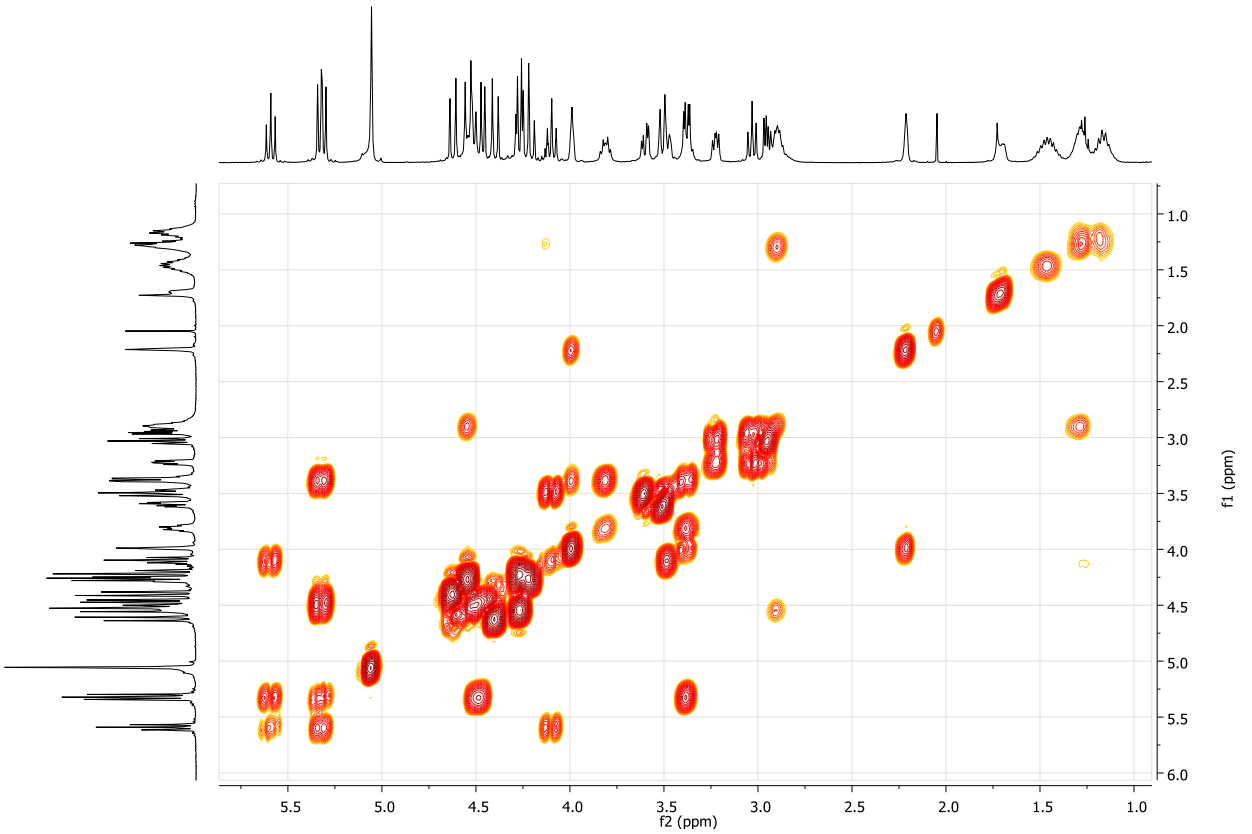

HHS-Auto-028-4-Hyper-C_CARBON_01
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

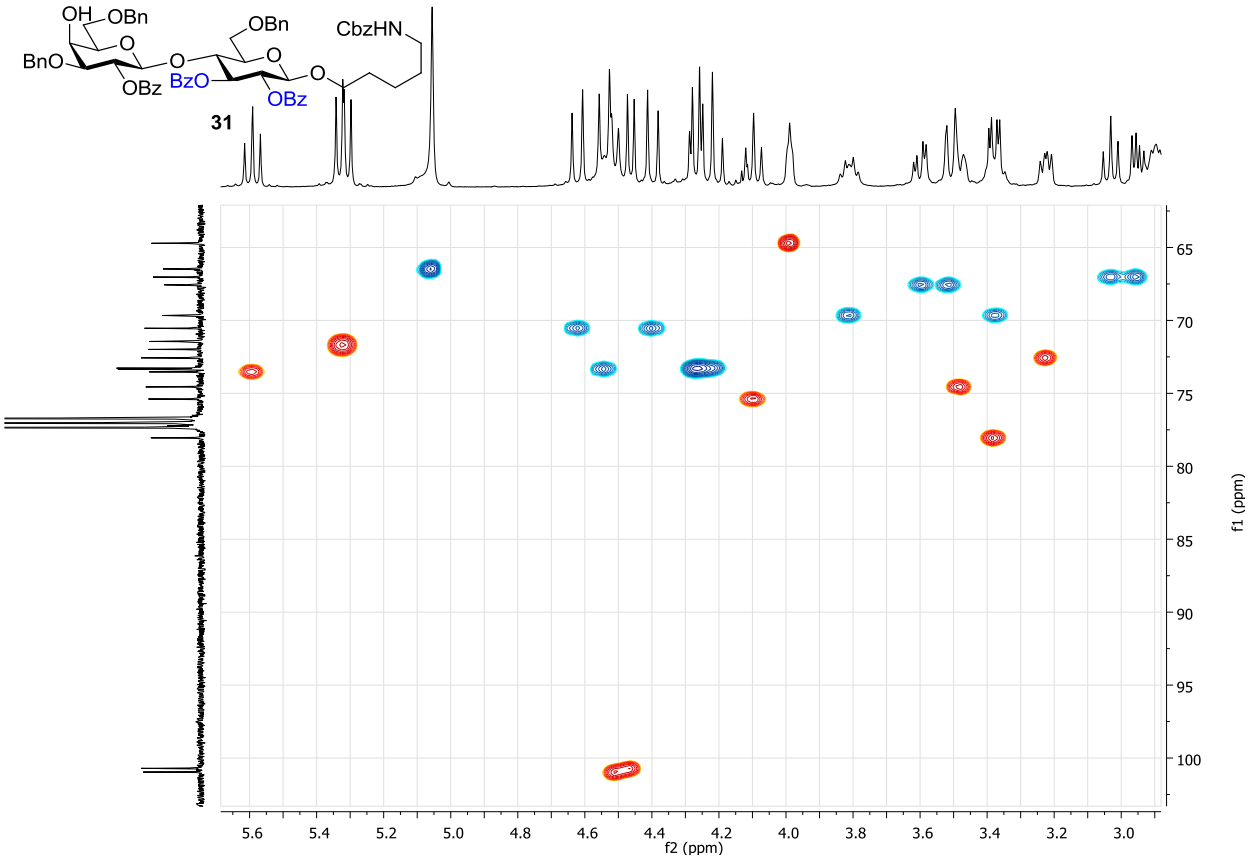

4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2
f2 (ppm)
1.5 2.0 2.5 3.0 3.5 4.0 4.5
f1 (ppm)


4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
f2 (ppm)
20 30 40 50 60 70 80 90 100
f1 (ppm)

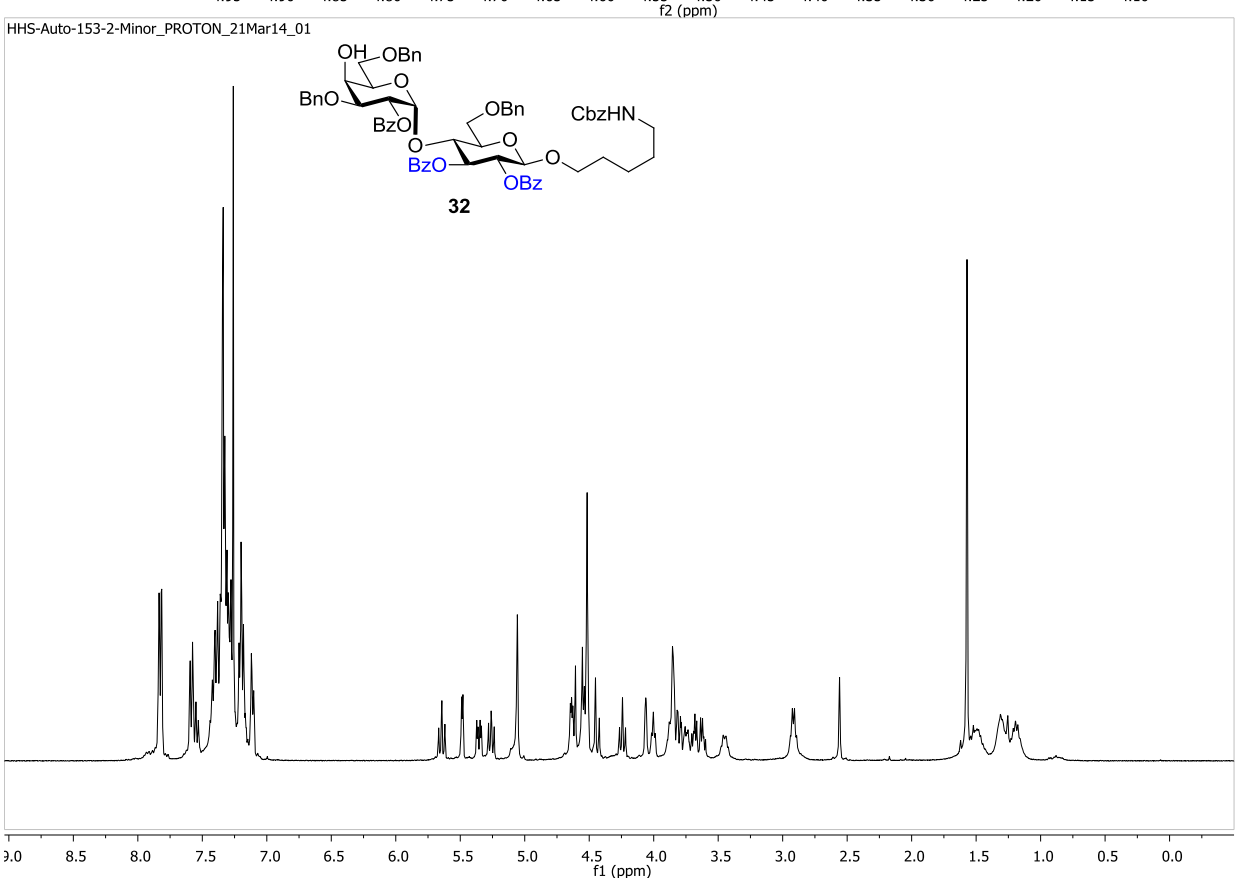

OH
OBn
BnO
O
OBz
O
BzO
OBn
O
OBz
O
CbzHN
31
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0
f1 (ppm)

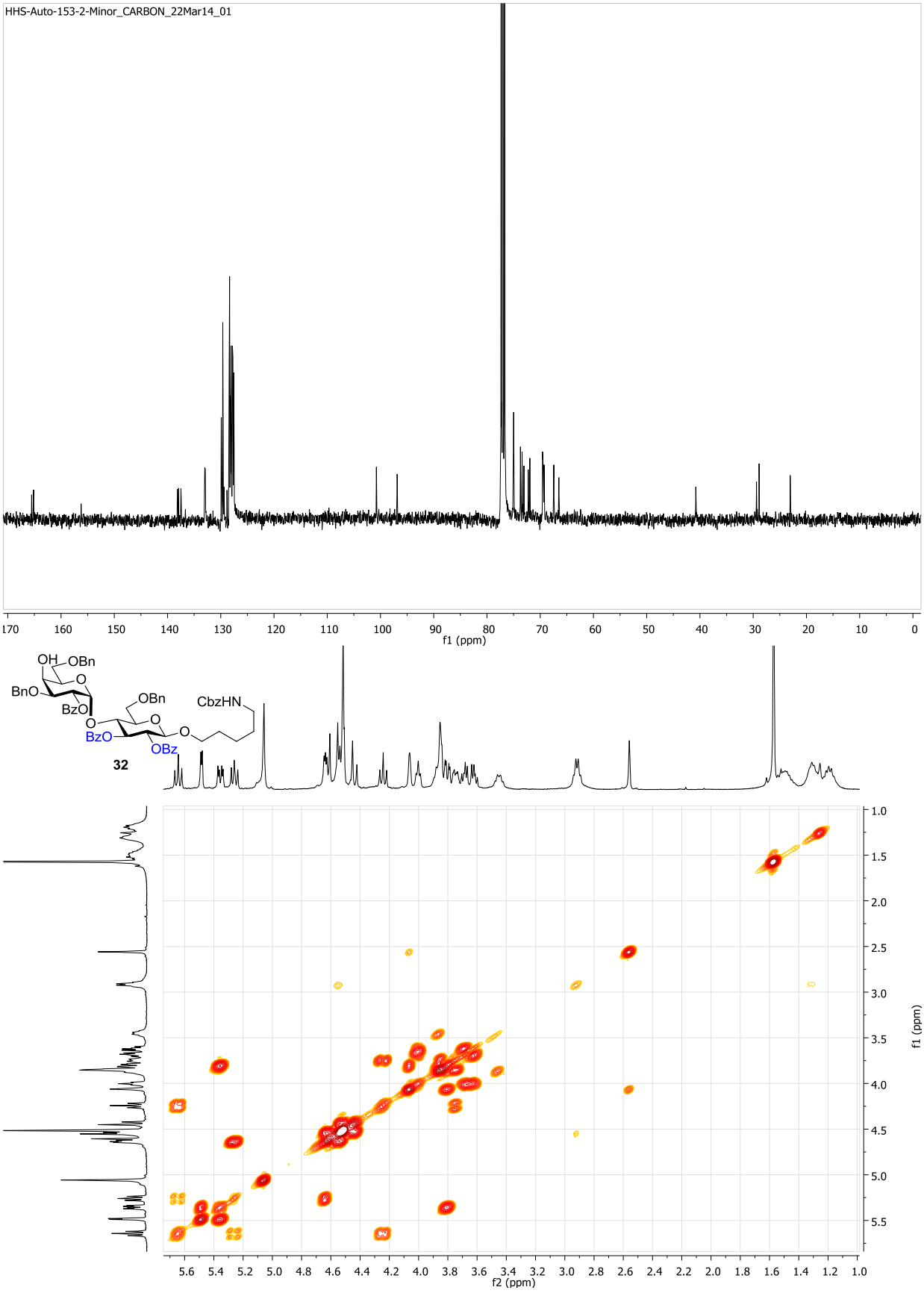

HHS-Auto-153-2-Major_CARBON_21Mar14_01
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

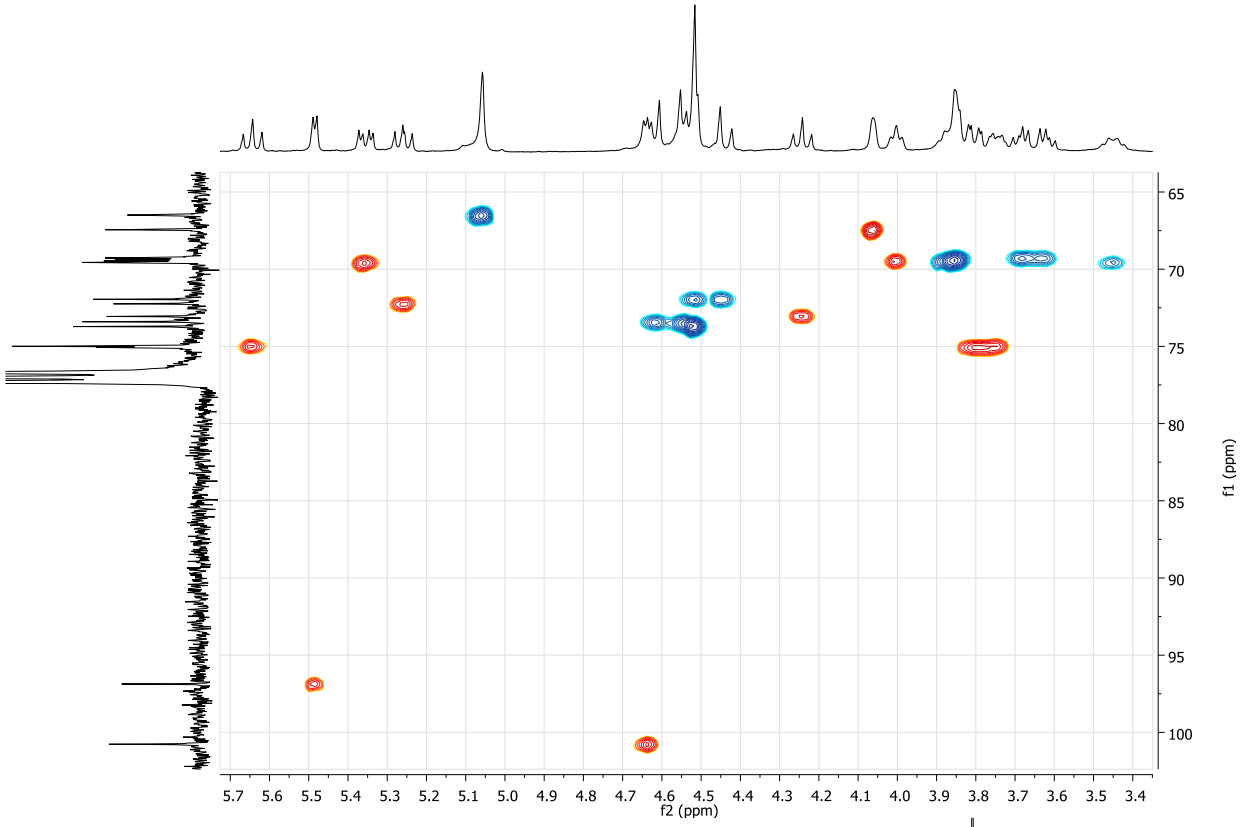

f2 (ppm)
f1 (ppm)

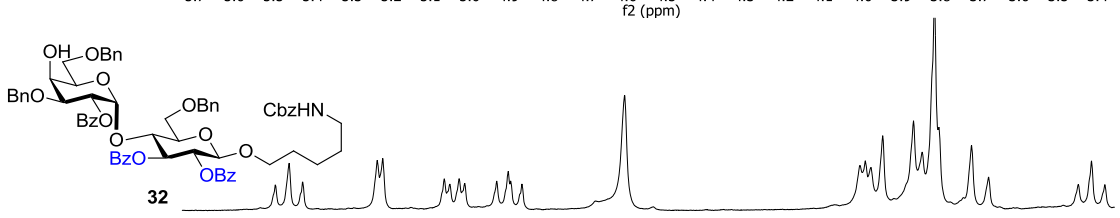

OH
OBn
O
BnO
OBz
O
BzO
OBz
OBn
O
O
CbzHN
31
f2 (ppm)
f1 (ppm)


162.0 Hz
161.2 Hz
f2 (ppm)
f1 (ppm)

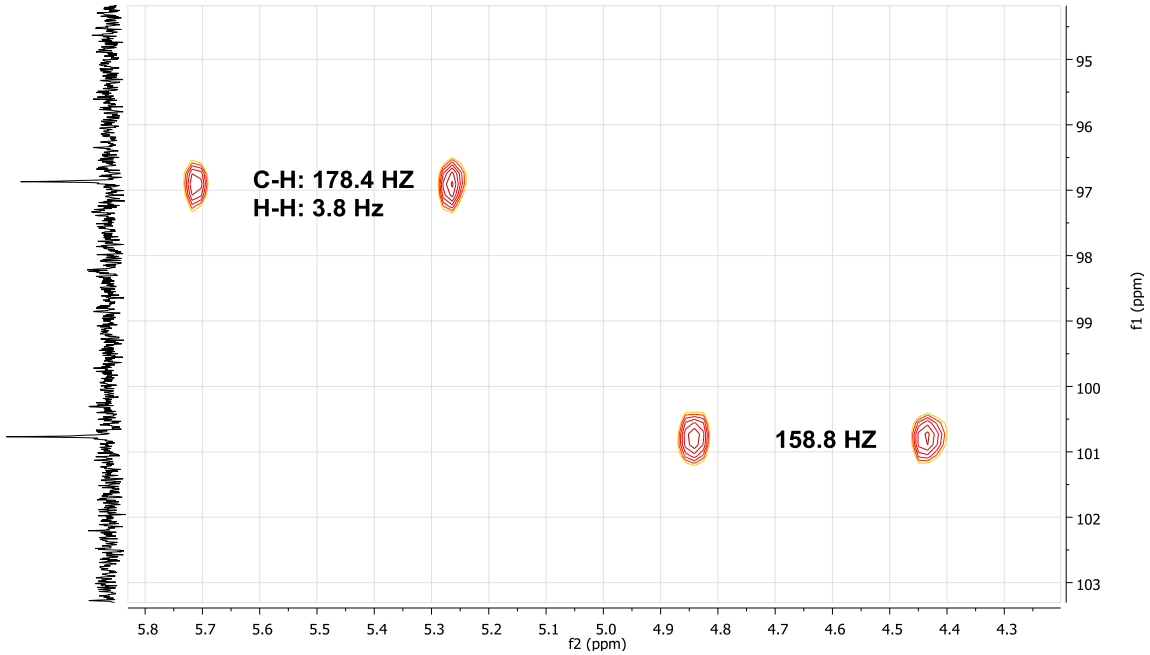

HHS-Auto-153-2-Minor_PROTON_21Mar14_01
OH
OBn
BnO
BzO
OBn
CbzHN
BzO
OBz
32
f1 (ppm)


HHS-Auto-153-2-Minor_CARBON_22Mar14_01
f1 (ppm)
OH
OBn
BnO
O
BzO
OBn
CbzHN
O
BzO
OBz
32
f2 (ppm)
f1 (ppm)


65
70
75
80
85
90
95
100
f1 (ppm)
5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4
f2 (ppm)


OH
OBn
O
BnO
BzO
O
OBn
O
CbzHN
O
BzO
OBz

32


C-H: 178.4 HZ
H-H: 3.8 Hz
158.8 HZ
95
96
97
98
99
100
101
102
103
f1 (ppm)
5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3
f2 (ppm)