Supplemental Information

Sequential linkage of carbohydrate antigens to mimic capsular polysaccharides: Towards semisynthetic glycoconjugate vaccine candidates against *Streptococcus pneumoniae* serotype 14

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Part I. Molecular Dynamics Simulations

Two oligosaccharides (Figure S1 A) were modeled using the GLYCAM06$_{OSMO,14}$ force field [1, 2]. The system was solvated with TIP5P [3] water molecules to avoid excessive interactions between the monomers. Interaction parameters for the linker and terminal residues were obtained from the amber SB99 force field [4, 5] and partial charges were derived using the R.E.D. tools scripts [6]. Structure optimization for the charge derivation was performed with Gaussian at the HF/6-31G* level of theory.

Initial conformations were constructed with the Glycam Carbohydrate builder [7] and tleap. The topology was subsequently converted using the glycam2gmx.pl [8, 9] script. Molecular Dynamics simulations were performed at a constant temperature of 303 K and constant pressure of 1 bar using gromacs 5.1.2 [10]. For each molecule two independent simulations of 500 ns were performed, starting from the all gg and all gt conformations of the tetrasaccharides. Electrostatics were calculated using the particle mesh Ewald method [11], and water molecules were kept rigid with SETTLE [12].

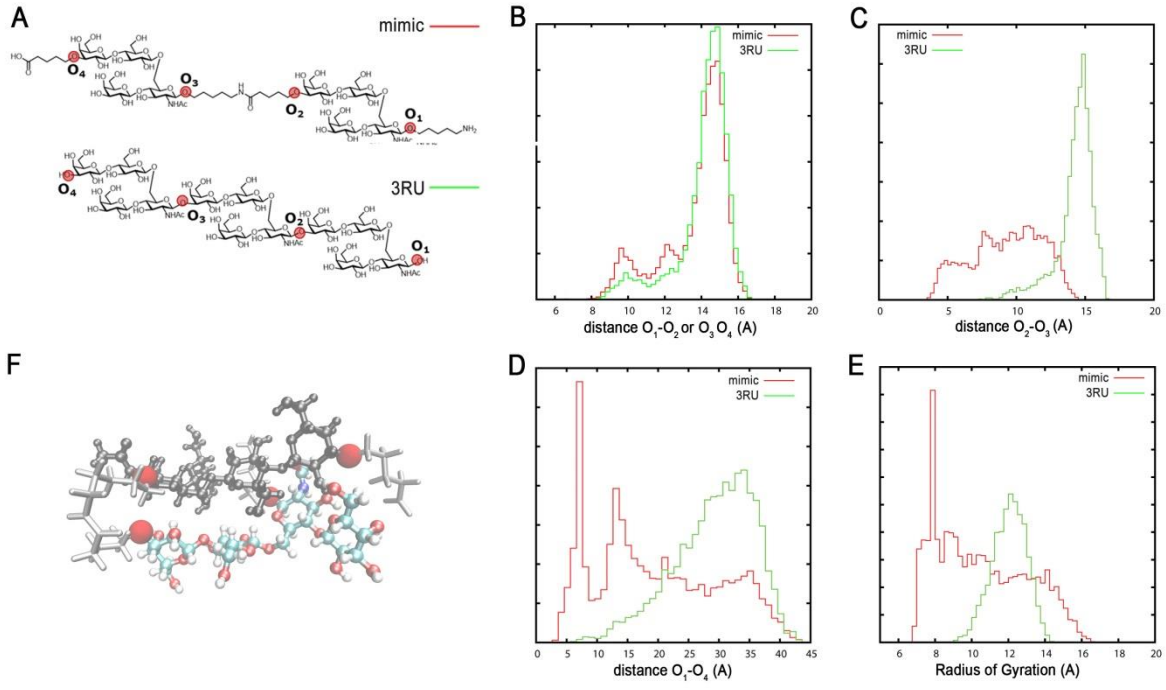
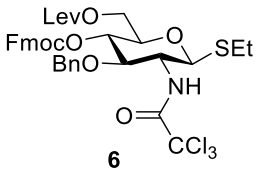


Figure S1: Molecular Dynamics Simulation Data. (A) The two simulated molecules, highlighting the oxygen atoms defining the distances plotted in panels (B-D). (B-E) probability distribution for the distances between atoms **O1** and **O2** or atoms **O3** and **O4** (B), atoms **O2** and **O3** (C) and atoms **O1** and **O4**.(D) as well as the radius of Gyration (E). The sharp peaks in the distributions for the mimic at short distances/ small radius of gyration in (C) and (D) correspond to folded conformations like the one shown in (F), where the tetrasaccharides stack together.

Part II. Syntheses

Chemicals were purchased as reagent grade and used without further purification unless stated otherwise. Anhydrous solvents were obtained from Waters Dry Solvent systems. Reactions were monitored by thin-layer chromatography (TLC) analysis, which was visualized by UV light (254 nm) and TLC sugar stain (1% (v/v) 3-methoxyphenol, 30% (v/v) sulfuric acid in ethanol). Flash column chromatography was performed on Kieselgel 60 with 230-400 mesh (Sigma-Aldrich, St. Louis, USA). ^{1}H-NMR, ^{13}C-NMR spectra were recorded on a 400 or 600 MHz Varian or Brucker spectrometer at room temperature. Chemical shifts (in ppm) were calibrated with the solvent residual peak. Coupling constants (*J*) are reported in Hertz (Hz). Optical rotations (OR) were measured with a Schmidt & Haensch UniPol L 1000 at 589 nm and concentration (c) expressed in g/100 mL. High resolution mass spectrometry (HRMS) was performed by Waters Xevo Q-Tof mass spectrometer.

Ethyl 3-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-6-*O*-levulinyl-2-deoxy-2-trichloroacetamino-1-thio-β-D-glucopyranoside (6)

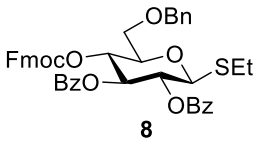


6

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (dd, *J* = 7.5, 3.7 Hz, 2H), 7.63 – 7.41 (m, 2H), 7.36 – 7.26 (m, 2H), 7.24 – 7.19 (m, 2H), 7.15 – 7.05 (m, 5H), 6.86 (d, *J* = 7.7 Hz, 1H), 4.98 (d, *J* = 10.3 Hz, 1H), 4.84 (dd, *J* = 9.9, 9.0 Hz, 1H), 4.54 (s, 2H), 4.42 (dd, *J* = 10.5, 6.8 Hz, 1H), 4.31 – 4.09 (m, 5H), 3.69 (ddd, *J* = 10.0, 5.2, 2.8 Hz, 1H), 3.56 (td, *J* = 10.1, 7.9 Hz, 1H), 2.73 – 2.58 (m, 4H), 2.56 – 2.49 (m, 2H), 2.09 (s, 3H), 1.28 – 1.12 (m, 3H).

NMR data was in accordance with previously reported values [13].

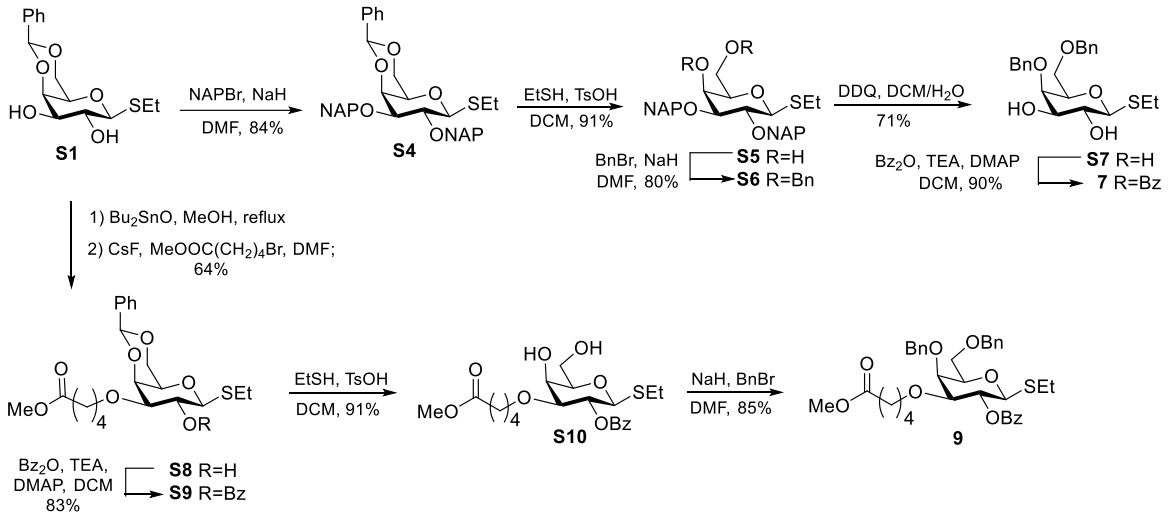
Ethyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-fluorenylmethoxycarbonyl-1-thio-β-D-glucopyranoside (8)



8

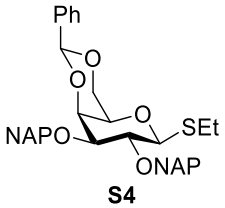
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.5 Hz, 2H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.71 (d, *J* = 7.4 Hz, 2H), 7.51 (t, *J* = 7.3 Hz, 1H), 7.48 – 7.21 (m, 15H), 7.17 (t, *J* = 7.5 Hz, 1H), 5.80 (t, *J* = 9.4 Hz, 1H), 5.49 (t, *J* = 9.6 Hz, 1H), 5.23 (t, *J* = 9.7 Hz, 1H), 4.76 (d, *J* = 10.1 Hz, 1H), 4.61 (d, *J* = 12.1 Hz, 1H), 4.56 (d, *J* = 12.0 Hz, 1H), 4.30 – 4.17 (m, 1H), 4.08 (t, *J* = 8.9 Hz, 1H), 4.01 – 3.90 (m, 2H), 3.74 (s, 2H), 2.87 – 2.68 (m, 2H), 1.28 (t, *J* = 7.5 Hz, 3H).

NMR data was in accordance with previously reported values [14].



Scheme S1: Synthesis of Building blocks **7** and **9**

Ethyl 4,6-*O*-benzylidene-2,3-di-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (S4)



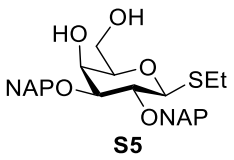
S4

Sodium hydride (768 mg, 19.2 mmol, 60% wt) was added to a solution of compound **S1** (2.0 g, 6.40 mmol) in DMF (20 mL) and THF (20 mL) at 0 °C. The reaction mixture was stirred for 15 min at room temperature followed by the addition of 2-bromomethylnaphthalene (4.3 g, 19.2

mmol). The reaction mixture was stirred for 3 h and quenched with saturated aqueous NH_4Cl solution and extracted with DCM. The aqueous layer was washed three times with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography using 30% ethyl acetate in hexanes to give compound **S4** (3.2 g, 5.4 mmol, 84%) as a pale yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 – 7.72 (m, 7H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.61 – 7.33 (m, 11H), 5.48 (s, 1H), 5.09 (d, *J* = 10.4 Hz, 1H), 5.02 (d, *J* = 10.4 Hz, 1H), 4.98 – 4.87 (m, 2H), 4.46 (d, *J* = 9.6 Hz, 1H), 4.29 (d, *J* = 12.3 Hz, 1H), 4.18 (d, *J* = 3.4 Hz, 1H), 4.02 – 3.89 (m, 2H), 3.66 (dd, *J* = 9.2, 3.4 Hz, 1H), 3.33 (s, 1H), 2.95 – 2.81 (m, 1H), 2.81 – 2.67 (m, 1H), 1.33 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.0, 136.1, 135.9, 133.4, 133.3, 133.2, 129.2, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.1, 126.7, 126.7, 126.6, 126.3, 126.1, 126.0, 125.9, 101.7, 84.6, 81.1, 75.9, 74.2, 72.0, 69.9, 69.5, 24.0, 15.2.

Ethyl 2,3-di-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (S5)

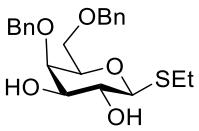


S5

Ethanethiol (11 mL, 151 mmol) and TsOH (191 mg, 1 mmol) were added to a solution of compound **S4** (2.98 g, 5.03 mmol) in DCM (30 mL). The reaction mixture was stirred for 1 h. After the starting material disappeared, the reaction was quenched with triethylamine and concentrated. The residue was purified by flash column chromatography with 70% ethyl acetate in hexanes to afford compound **S5** (152 mg, 0.34 mmol, 91%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 7.72 (m, 7H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 9.4 Hz, 1H), 7.50 – 7.39 (m, 5H), 5.07 (d, *J* = 10.5 Hz, 1H), 4.94 (d, *J* = 10.6 Hz, 1H), 4.88 (q, *J* = 11.9 Hz, 2H), 4.46 (d, *J* = 9.7 Hz, 1H), 4.09 (d, *J* = 3.0 Hz, 1H), 3.95 (dd, *J* = 11.8, 6.6 Hz, 1H), 3.83 – 3.72 (m, 2H), 3.62 (dd, *J* = 9.0, 3.2 Hz, 1H), 3.52 – 3.43 (m, 1H), 2.88 – 2.66 (m, 2H), 2.28 (s, 2H), 1.32 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 135.7, 135.1, 133.4, 133.3, 133.2, 128.6, 128.2, 128.1, 128.0, 127.9, 127.8, 127.1, 126.9, 126.5, 126.4, 126.3, 126.1, 126.0, 125.9, 85.4, 82.1, 78.0, 77.9, 76.0, 72.4, 67.7, 62.9, 25.1, 15.3; HRMS (ESI) calcd for $C_{30}H_{32}O_5SNa$ $[M+Na]^+$ 527.1862; found: 527.1866.

Ethyl 4,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside (S7)

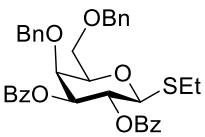


S7

Sodium hydride (1.07 g, 26.8 mmol, 60%) was added to a solution of compound **S5** (3.38 g, 6.70 mmol) in DMF (10 mL) and THF (10 mL) at 0 °C. The reaction was stirred for 15 min at room temperature followed by the addition of benzyl bromide (3.19 mL, 26.8 mmol). After 3 h, the reaction was quenched with aqueous saturated NH_4Cl solution and extracted three times with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography with 20% ethyl acetate in hexanes to give compound **S6** (3.65 g, 5.33 mmol, 80%) as white solid. Then compound **S6** was dissolved in DCM (30 mL) and H_2O (6 mL) followed by the addition of DDQ (6.08 g, 26.8 mmol) at 0 °C. The mixture was slowly warmed to room temperature and stirred for 1 h. The reaction mixture was quenched with water, extracted three times with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography using 30% ethyl acetate in hexanes to obtain compound **S7** (1.92 g, 4.76 mmol, 71%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 10H), 4.73 (d, *J* = 11.7 Hz, 1H), 4.68 (d, *J* = 11.7 Hz, 1H), 4.52 (d, *J* = 11.8 Hz, 1H), 4.47 (d, *J* = 11.8 Hz, 1H), 4.29 (d, *J* = 9.4 Hz, 1H), 3.92 (d, *J* = 3.1 Hz, 1H), 3.72 – 3.63 (m, 4H), 3.63 – 3.57 (m, 1H), 2.80 – 2.65 (m, 2H), 2.63 (s, 1H), 2.51 (s, 1H), 1.30 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.5, 137.8, 128.6, 128.6, 128.0, 128.0, 128.0, 127.9, 86.3, 77.6, 76.3, 75.5, 75.3, 73.7, 71.0, 68.6, 24.6, 15.4.

Ethyl 2,3-di-*O*-benzoyl-4,6-di-*O*-benzyl-1-thio-β-D-galactopyranoside (7)

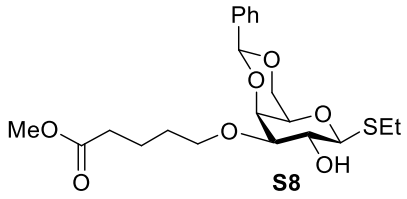


7

To a solution of compound **S7** (0.81 g, 1.99 mmol) in DCM (5 mL) were added Bz_2O (1.36 g, 5.99 mmol), triethylamine (2.2 mL, 15.98 mmol) and DMAP (48.8 mg, 0.40 mmol). The reaction was stirred for 3 h at room temperature and quenched by aqueous saturated $NaHCO_3$ solution. The aqueous layer was extracted three times with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography with 30% ethyl acetate in hexanes to give compound **7** (1.1 g, 1.79 mmol, 90%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 – 7.94 (m, 4H), 7.60 – 7.48 (m, 2H), 7.43 – 7.21 (m, 14H), 5.92 (t, *J* = 10.0 Hz, 1H), 5.43 (dd, *J* = 10.0, 3.0 Hz, 1H), 4.77 (d, *J* = 12.0 Hz, 1H), 4.73 (d, *J* = 12.0 Hz, 1H), 4.59 – 4.53 (m, 2H), 4.50 (d, *J* = 11.8 Hz, 1H), 4.30 (d, *J* = 2.6 Hz, 1H), 3.96 (t, *J* = 6.6 Hz, 1H), 3.79 – 3.65 (m, 2H), 2.90 – 2.70 (m, 2H), 1.30 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 165.6, 138.0, 137.9, 133.5, 133.2, 130.0, 129.9, 129.7, 129.2, 128.6, 128.6, 128.4, 128.4, 128.1, 128.0, 128.0, 127.8, 83.9, 77.5, 75.9, 75.1, 74.4, 73.7, 68.7, 68.3, 24.1, 15.0; HRMS (ESI) calcd for $C_{36}H_{36}O_7SNa$ $[M+Na]^+$ 635.2074; found: 635.2097.

Ethyl 4,6-*O*-benzylidene-3-*O*-(methyl 5-pentanoate)-1-thio-β-D-galactopyranoside (S8)



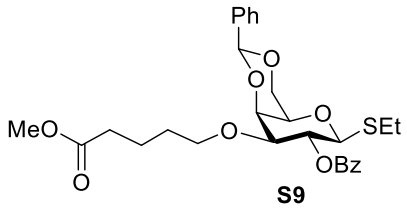
S8

Bu_2SnO (5.2 g, 20.8 mmol) was added to a solution of compound **S1** (5.0 g, 16.0 mmol) in MeOH (80 mL) and the mixture was heated to 80 °C overnight. The reaction mixture was concentrated under vacuum which was then dried azeotropically twice with toluene. The crude was dissolved in anhydrous DMF (80 mL) followed by the addition of methyl 5-bromopentanoate (2.8 mL, 19.2 mmol) and CsF (3.7 g, 24.0 mmol). The reaction was stirred at 60 °C overnight, quenched with water and extracted with DCM. The organic layer was washed with aqueous saturated $NaHCO_3$ solution, brine, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash column chromatography using 50% ethyl acetate in hexanes to afford compound **S8** (4.4 g, 10.2 mmol, 64%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.44 (m, 2H), 7.39 – 7.31 (m, 3H), 5.53 (s, 1H), 4.36 (t, *J* = 10.5 Hz, 2H), 4.32 (d, *J* = 3.4 Hz, 1H), 4.04 (d, *J* = 11.1 Hz, 1H), 3.98 (t, *J* = 9.3 Hz, 1H), 3.77 – 3.66 (m, 1H), 3.65 (s, 3H), 3.61 – 3.52 (m, 1H), 3.48 (s, 1H), 3.39 (dd, *J* = 9.2, 3.3 Hz,

1H), 2.89 – 2.80 (m, 1H), 2.79 – 2.69 (m, 1H), 2.62 (s, 1H), 2.33 (t, *J* = 7.0 Hz, 2H), 1.77 – 1.63 (m, 4H), 1.33 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.2, 137.9, 129.1, 128.3, 126.5, 101.3, 85.4, 81.4, 73.1, 70.3, 69.7, 68.9, 67.9, 51.7, 33.7, 29.2, 23.1, 21.7, 15.4; HRMS (ESI) calcd for $C_{21}H_{30}O_7SNa$ $[M+Na]^+$ 449.1604; found: 449.1604.

Ethyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-(methyl 5-pentanoate)-1-thio-β-D-galactopyranoside (S9)

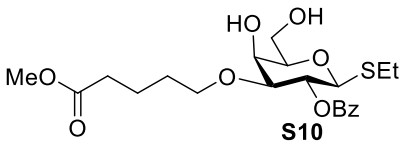


S9

To a solution of compound **S8** (1.44 g, 3.38 mmol) in DCM (15 mL) were added Bz_2O (1.15 g, 5.06 mmol), TEA (1.4 mL, 10.13 mmol) and DMAP (82.5 mg, 0.68 mmol). The reaction mixture was stirred for 3 h at room temperature. After the starting material disappeared, the reaction was quenched with saturated aqueous $NaHCO_3$ solution. The aqueous layer was washed three times with DCM. The combined organic layer was washed with brine solution, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography with 30% ethyl acetate in hexanes to get compound **S9** (1.48 g, 2.79 mmol, 83%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.3 Hz, 2H), 7.62 – 7.49 (m, 3H), 7.48 – 7.30 (m, 5H), 5.64 (t, *J* = 9.7 Hz, 1H), 5.57 (s, 1H), 4.59 (d, *J* = 9.8 Hz, 1H), 4.44 – 4.31 (m, 2H), 4.07 (d, *J* = 12.3 Hz, 1H), 3.72 – 3.61 (m, 2H), 3.55 (s, 4H), 3.44 (dt, *J* = 9.4, 5.8 Hz, 1H), 3.02 – 2.85 (m, 1H), 2.83 – 2.69 (m, 1H), 2.17 – 2.06 (m, 2H), 1.56 – 1.42 (m, 4H), 1.28 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 165.4, 137.8, 133.1, 130.2, 129.9, 129.2, 128.5, 128.3, 126.6, 101.5, 83.0, 79.9, 73.7, 70.4, 69.6, 69.4, 68.9, 51.5, 33.5, 29.1, 22.8, 21.5, 15.0; HRMS (ESI) calcd for $C_{28}H_{34}O_8SNa$ $[M+Na]^+$ 553.1866; found: 553.1874.

Ethyl 2-*O*-benzoyl-3-*O*-(methyl 5-pentanoate)-1-thio-β-D-galactopyranoside (S10)

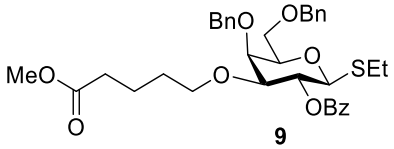


S10

Ethanethiol (0.8 mL, 11.3 mmol) and TsOH (14.3 mg, 76 μmol) were added to a solution of compound **S9** (200 mg, 0.38 mmol) in DCM (5 mL). The reaction mixture was stirred for 1 h. After the starting material disappeared, the reaction was quenched with triethylamine and concentrated. The residue was purified by flash column chromatography with 75% ethyl acetate in hexanes to give compound **S10** (152 mg, 0.34 mmol, 91%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 7.8 Hz, 2H), 7.58 (t, *J* = 7.2 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 2H), 5.45 (t, *J* = 9.7 Hz, 1H), 4.54 (d, *J* = 10.0 Hz, 1H), 4.18 (d, *J* = 2.9 Hz, 1H), 4.02 (dd, *J* = 11.7, 6.8 Hz, 1H), 3.85 (dd, *J* = 11.8, 4.5 Hz, 1H), 3.71 – 3.62 (m, 2H), 3.61 – 3.54 (m, 4H), 3.49 – 3.38 (m, 1H), 2.86 – 2.63 (m, 2H), 2.40 (brs, 2H), 2.14 (t, *J* = 6.6 Hz, 2H), 1.63 – 1.40 (m, 4H), 1.23 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.0, 165.5, 133.3, 130.0, 129.9, 128.5, 83.6, 81.1, 78.6, 70.2, 69.7, 67.3, 62.7, 51.6, 33.5, 29.2, 23.8, 21.5, 15.0.

Ethyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-(methyl 5-pentanoate)-1-thio-β-D-galactopyranoside (9)

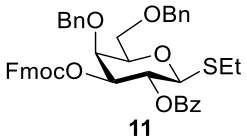


9

Benzyl bromide (160 µL, 1.36 mmol) and sodium hydride (54.2 mg, 1.36 mmol, 60% wt) were added to a solution of compound **S10** (150 mg, 0.34 mmol) in DMF (4 mL) at 0 °C. The reaction mixture was stirred for 30 min at room temperature and quenched by acetic acid. The mixture was diluted with DCM and then washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography with 30% ethyl acetate in hexanes to obtain compound **9** (181 mg, 0.29 mmol, 85%) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.5 Hz, 2H), 7.55 (t, *J* = 7.4 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.36 – 7.24 (m, 10H), 5.59 (t, *J* = 9.7 Hz, 1H), 4.95 (d, *J* = 11.7 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 4.51 (d, *J* = 9.9 Hz, 1H), 4.44 (q, *J* = 11.7 Hz, 2H), 4.00 (d, *J* = 2.0 Hz, 1H), 3.73 – 3.57 (m, 4H), 3.57 – 3.49 (m, 4H), 3.45 – 3.32 (m, 1H), 2.80 – 2.59 (m, 2H), 2.10 (t, *J* = 6.9 Hz, 2H), 1.57 – 1.36 (m, 4H), 1.20 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.9, 165.5, 138.8, 137.9, 133.1, 130.2, 129.8, 128.6, 128.5, 128.3, 128.1, 128.0, 127.6, 83.9, 82.9, 77.7, 74.6, 73.7, 73.3, 70.6, 70.5, 68.7, 51.5, 33.6, 29.4, 23.8, 21.5, 15.0; HRMS (ESI) calcd for $C_{35}H_{42}O_8SNa$ $[M+Na]^+$ 645.2492; found: 645.2521.

Ethyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-(9-fluorenylmethoxycarbonyl)-1-thio-β-D-galactopyranoside (11)

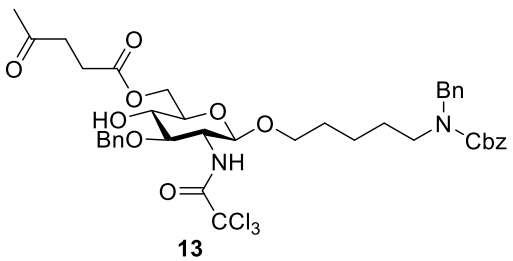


11

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 – 8.00 (m, 2H), 7.68 (m, 2H), 7.58 – 7.48 (m, 1H), 7.48 – 7.27 (m, 16H), 7.11 (m, 2H), 5.75 (t, *J* = 9.9 Hz, 1H), 5.07 (dd, *J* = 10.0, 3.0 Hz, 1H), 4.79 (d, *J* = 11.5 Hz, 1H), 4.60 (d, *J* = 9.9 Hz, 1H), 4.51 (d, *J* = 11.8 Hz, 2H), 4.46 (d, *J* = 11.7 Hz, 1H), 4.30 (dd, *J* = 10.4, 7.2 Hz, 1H), 4.21 (dd, *J* = 10.4, 7.8 Hz, 1H), 4.14 (d, *J* = 3.1 Hz, 1H), 4.06 (t, *J* = 7.4 Hz, 1H), 3.82 (t, *J* = 6.5 Hz, 1H), 3.72 – 3.62 (m, 2H), 2.74 (m, 2H), 1.23 (t, *J* = 7.5 Hz, 3H).

NMR data was in accordance with previously reported values [15].

***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (13)**

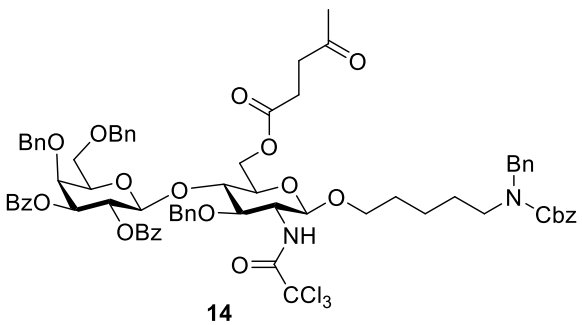


13

Monosaccharide **6** (500 mg, 0.64 mmol) and linker **5** (315 mg, 0.97 mmol) were mixed and co-evaporated three times with anhydrous toluene. Pre-activated molecular sieves AW-300 and anhydrous DCM (5 mL) were added and the mixture was stirred at room temperature for 20 min. The mixture was cooled down to -10 ^{o}C and NIS (215 mg, 0.97 mmol) followed by triflic acid (8.6 μL, 0.097 mmol) were added. The reaction mixture was stirred at -10 ^{o}C for 1.5 h. The reaction was quenched with saturated aqueous $NaHCO_3$ solution and 10% $Na_2S_2O_3$ solution. The aqueous layer was extracted three times with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated in *vacuo* to give crude compound **12**. The crude was then dissolved in DCM (5 mL) followed by the addition of TEA (1 mL), the reaction mixture was stirred for 2 hours at room temperature. The solvent was removed and the residue was purified by flash column chromatography with 15% acetone in toluene to yield compound **13** (468 mg, 0.57 mmol, 89%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.15 (m, 15H), 5.19 (d, *J* = 14.4 Hz, 2H), 4.92 – 4.75 (m, 3H), 4.59 (d, *J* = 12.1 Hz, 1H), 4.50 (s, 2H), 4.25 (d, *J* = 12.1 Hz, 1H), 4.08 (q, *J* = 10.2 Hz, 1H), 3.92 – 3.79 (m, 1H), 3.64 – 3.56 (m, 1H), 3.55 – 3.36 (m, 3H), 3.22 (m, 2H), 3.04 (d, *J* = 12.5 Hz, 1H), 2.80 (t, *J* = 6.5 Hz, 2H), 2.63 (t, *J* = 5.9 Hz, 2H), 2.21 (s, 3H), 1.53 (m, 4H), 1.28 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.1, 173.6, 162.1, 156.8, 156.3, 138.1, 137.9, 137.0, 136.8, 128.7, 128.7, 128.6, 128.3, 128.2, 128.1, 128.0, 127.4, 127.3, 99.5, 92.6, 79.3, 79.2, 75.0, 74.0, 71.1, 70.1, 67.3, 63.4, 58.7, 50.6, 50.4, 47.3, 46.2, 38.1, 30.0, 29.0, 28.1, 27.4, 23.5, 23.3; HRMS (ESI) calcd for $C_{40}H_{47}O_{10}N_2Cl_3Na$ $[M+Na]^+$ 845.2167; found: 845.2214.

***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 2,3-di-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-6-*O*-levulinyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (14)**

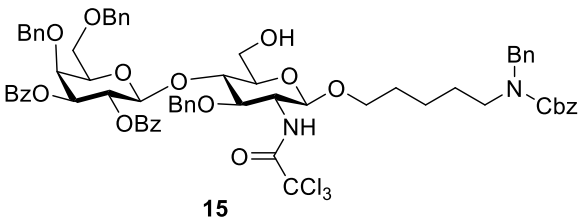


14

Both monosaccharides **13** (84 mg, 0.102 mmol) and **7** (75 mg, 0.123 mmol) were mixed and co-evaporated three times using anhydrous toluene. Pre-activated molecular sieves AW-300 and anhydrous DCM (3 mL) were added and the reaction mixture was stirred at room temperature for 20 min. Then the mixture was cooled down to -30 ^{o}C and NIS (34.5 mg, 0.153 mmol) followed by triflic acid (1.5 μL, 0.016 mmol) were added. The reaction mixture was stirred at -10 ^{o}C for 1.5 h. The reaction was quenched with triethylamine. The mixture was extracted with saturated aqueous $NaHCO_3$ solution. The combined organic layer was washed with brine solution, dried over Na_2SO_4, filtered and concentrated in *vacuo*. The residue was purified by flash column chromatography with 15% acetone in toluene to give compound **14** (126 mg, 0.092 mmol, 90%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.5 Hz, 2H), 7.86 (d, *J* = 7.4 Hz, 2H), 7.47 – 7.38 (m, 2H), 7.36 – 7.04 (m, 30H), 5.78 (dd, *J* = 10.3, 7.9 Hz, 1H), 5.44 (dd, *J* = 10.5, 2.9 Hz, 1H), 5.07 (d, *J* = 8.8 Hz, 2H), 4.94 (d, *J* = 10.8 Hz, 1H), 4.82 (d, *J* = 7.9 Hz, 1H), 4.64 (d, *J* = 11.3 Hz, 2H), 4.55 (d, *J* = 10.9 Hz, 1H), 4.38 (d, *J* = 11.0 Hz, 3H), 4.31 (d, *J* = 11.9 Hz, 1H), 4.27 – 4.14 (m, 3H), 4.11 – 4.03 (m, 1H), 4.01 – 3.91 (m, 2H), 3.87 (t, *J* = 7.8 Hz, 1H), 3.68 – 3.54 (m, 1H), 3.46 (t, *J* = 8.5 Hz, 2H), 3.42 – 3.31 (m, 2H), 3.24 – 3.02 (m, 3H), 2.84 – 2.71 (m, 1H), 2.67 (s, 1H), 2.62 – 2.44 (m, 2H), 2.41 – 2.30 (m, 1H), 2.14 (s, 3H), 1.48 – 1.30 (m, 4H), 1.18 – 1.05 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.7, 172.6, 165.9, 165.5, 138.2, 138.1, 138.0, 133.4, 133.3, 130.1, 130.0, 129.4, 129.3, 128.7, 128.6, 128.5 (2C), 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.3, 100.8, 99.5, 75.3, 74.8, 74.6, 74.5, 73.5, 70.9, 68.0, 67.3, 62.7, 60.5, 38.1, 30.0, 28.1, 21.2, 14.4; HRMS (ESI) calcd for $C_{74}H_{77}O_{17}N_2Cl_3Na$ $[M+Na]^+$ 1395.4175; found: 1395.4107.

_N_-Benzyl-_N_-benzyloxycarbonyl-5-pentyl 2,3-di-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (15)

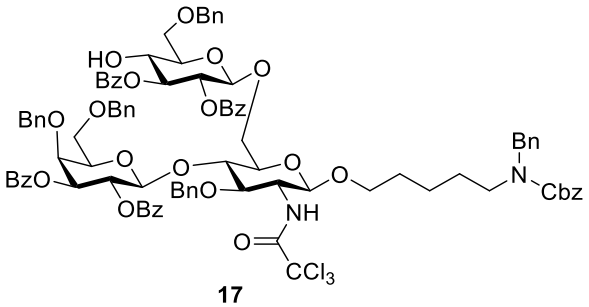


15

To a solution of compound **14** (30.6 mg, 0.022 mmol) in DCM (3 mL) was added a mixture of pyridine (0.06 mL) and acetic acid (0.04 mL) followed by the addition of hydrazine hydrate (2 μL, 0.045 mmol) at room temperature. The reaction was stirred for 4 h and diluted with ethyl acetate, quenched with acetone and poured into water. The aqueous layer was washed three times with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography with 30% acetone in hexanes to afford compound **15** (26.8 mg, 0.021 mmol, 94%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, *J* = 7.5 Hz, 2H), 7.92 (d, *J* = 7.5 Hz, 2H), 7.54 – 7.44 (m, 2H), 7.41 – 7.02 (m, 29H), 5.84 (dd, *J* = 10.3, 8.1 Hz, 1H), 5.36 (dd, *J* = 10.5, 2.9 Hz, 1H), 5.13 (d, *J* = 9.4 Hz, 2H), 5.03 (d, *J* = 10.5 Hz, 1H), 4.88 (d, *J* = 7.1 Hz, 1H), 4.81 – 4.67 (m, 2H), 4.59 (d, *J* = 10.6 Hz, 1H), 4.51 – 4.40 (m, 3H), 4.35 (d, *J* = 11.8 Hz, 1H), 4.29 – 4.17 (m, 2H), 4.02 (d, *J* = 8.4 Hz, 1H), 3.94 (t, *J* = 8.4 Hz, 1H), 3.88 – 3.78 (m, 1H), 3.77 – 3.60 (m, 3H), 3.54 (t, *J* = 8.5 Hz, 1H), 3.48 – 3.31 (m, 3H), 3.31 – 3.08 (m, 4H), 1.53 – 1.34 (m, 4H), 1.24 – 1.09 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 165.3, 138.1, 138.0, 133.5, 133.4, 130.0, 129.8, 129.5, 129.2, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3 (2C), 128.0, 127.9 (2C), 127.8, 127.7, 127.6, 127.4, 100.9, 99.5, 77.7, 76.5, 75.5, 75.3, 75.0, 74.6, 74.4, 73.6, 73.6, 71.0, 69.9, 67.8, 67.3, 60.9, 58.3, 50.4, 47.2, 29.1, 27.3, 23.3; HRMS (ESI) calcd for $C_{69}H_{71}O_{15}N_2Cl_3Na$ $[M+Na]^+$ 1297.3805; found: 1297.3794.

***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→6)-4-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (17)**



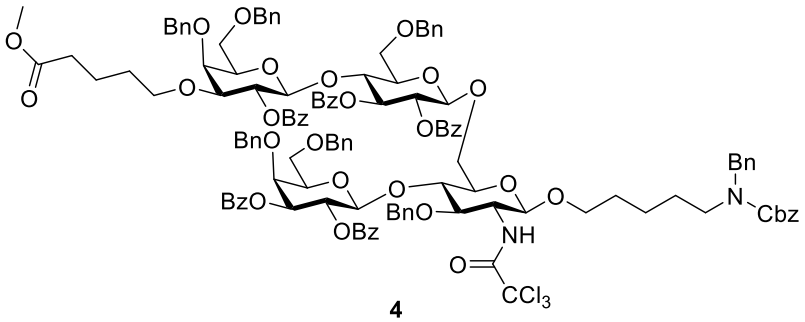
17

Disaccharide **15** (240 mg, 0.188 mmol) and thioglycoside **8** (168 mg, 0.226 mmol) were mixed and co-evaporated three times using anhydrous toluene. Pre-activated molecular sieves AW-

300 and anhydrous DCM (5 mL) were added and the mixture was stirred for 20 min at room temperature. The mixture was cooled down to -10 ^{o}C and NIS (83 mg, 0.367 mmol) followed by triflic acid (3.3 μL, 0.037 mmol) were added. The reaction was stirred at -10 ^{o}C for 1.5 h and quenched with saturated aqueous $NaHCO_3$ solution and 10% $Na_2S_2O_3$ solution. The aqueous layer was extracted three times with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to give crude compound **16**. The crude was dissolved in DCM (5 mL) followed by the addition of TEA (1 mL), the reaction mixture was stirred for 2 h at room temperature. The solvent was removed and the residue was purified by flash column chromatography with 15% acetone in toluene to afford compound **17** (300.1 mg, 0.173 mmol, 92%) as a white foam.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 – 7.99 (m, 2H), 7.97 – 7.92 (m, 4H), 7.79 (d, *J* = 7.3 Hz, 2H), 7.51 – 7.43 (m, 4H), 7.39 – 7.03 (m, 39H), 5.73 (t, *J* = 11.0 Hz, 1H), 5.47 – 5.33 (m, 3H), 5.14 (d, *J* = 24.6 Hz, 2H), 4.78 (t, *J* = 12.7 Hz, 1H), 4.74 – 4.65 (m, 2H), 4.63 – 4.51 (m, 4H), 4.48 – 4.35 (m, 5H), 4.30 (d, *J* = 11.1 Hz, 1H), 4.17 (d, *J* = 2.5 Hz, 1H), 4.00 – 3.90 (m, 3H), 3.85 – 3.75 (m, 4H), 3.70 (dd, *J* = 14.6, 7.3 Hz, 1H), 3.63 (s, 1H), 3.53 (t, *J* = 8.0 Hz, 2H), 3.47 – 3.38 (m, 3H), 3.33 – 3.13 (m, 3H), 2.98 – 2.86 (m, 1H), 1.47 – 1.22 (m, 4H), 1.18 – 1.03 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.1, 165.8, 165.6, 165.3, 138.1, 138.0, 133.5, 130.1, 130.0, 129.8, 129.4, 129.2, 128.7 (3C), 128.6 (3C), 128.57, 128.4, 128.3, 128.0, 127.9 (4C), 127.8, 127.7, 127.3, 101.4, 100.5, 99.7, 78.2, 76.4, 75.2, 74.3, 73.9, 73.5, 73.3, 71.3, 70.9, 70.3, 67.8, 67.3, 56.2, 50.4, 47.3, 46.3, 29.2, 27.5, 23.5; HRMS (ESI) calcd for $C_{96}H_{95}O_{22}N_2Cl_3Na$ $[M+Na]^+$ 1757.5341; found: 1757.5238.

_N_-Benzyl-_N_-benzyloxycarbonyl-5-pentyl 2-_O_-benzoyl-4,6-di-_O_-benzyl-3-_O_-(methyl 5-penanoate)-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranosyl-(1→6)-4-_O_-(2,3-di-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl)-3-_O_-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (4)

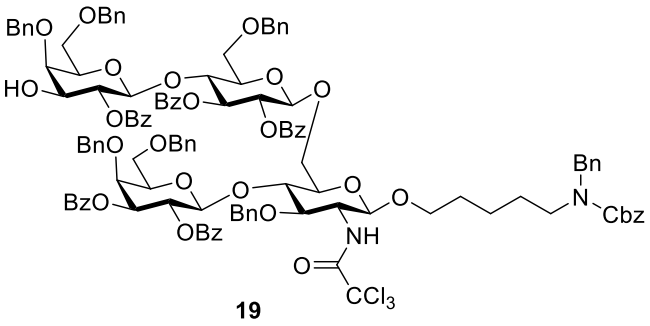


4

Both trisaccharides **17** (258 mg, 0.149 mmol) and monosaccharide **9** (130 mg, 0.208 mmol) were mixed and co-evaporated three times with anhydrous toluene. Pre-activated molecular sieves AW-300 and anhydrous DCM (6 mL) were added and the reaction mixture was stirred at room temperature for 20 min. Then the mixture was cooled down to -30 ^{o}C and NIS (65.2 mg, 0.290 mmol) followed by triflic acid (2.6 μL, 0.019 mmol) were added. The reaction mixture was stirred at -10 ^{o}C for 1.5 h and quenched with triethylamine. The mixture was extracted with aqueous saturated $NaHCO_3$ solution. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography with 15% acetone in toluene to afford compound **4** (301 mg, 0.131 mmol, 88%) as a white foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 – 7.94 (m, 6H), 7.91 – 7.86 (m, 2H), 7.82 – 7.77 (m, 2H), 7.60 – 7.15 (m, 56H), 5.74 (dd, *J* = 10.4, 7.9 Hz, 1H), 5.51 (t, *J* = 9.4 Hz, 1H), 5.45 (dd, *J* = 10.1, 8.0 Hz, 1H), 5.39 (dd, *J* = 10.5, 3.0 Hz, 1H), 5.33 (dd, *J* = 9.7, 7.9 Hz, 1H), 5.18 (d, *J* = 11.2 Hz, 2H), 4.87 (d, *J* = 11.7 Hz, 1H), 4.78 (d, *J* = 9.4 Hz, 1H), 4.70 (d, *J* = 11.4 Hz, 1H), 4.63 – 4.53 (m, 3H), 4.53 – 4.45 (m, 4H), 4.43 – 4.36 (m, 3H), 4.33 – 4.26 (m, 2H), 4.23 (d, *J* = 12.1 Hz, 1H), 4.18 (d, *J* = 2.8 Hz, 1H), 4.13 – 4.05 (m, 3H), 3.94 (dd, *J* = 10.4, 5.1 Hz, 1H), 3.86 – 3.80 (m, 2H), 3.78 – 3.72 (m, 2H), 3.72 – 3.61 (m, 2H), 3.60 – 3.44 (m, 9H), 3.41 – 3.35 (m, 1H), 3.35 – 3.26 (m, 3H), 3.23 – 3.09 (m, 3H), 2.98 – 2.82 (m, 3H), 2.09 (t, *J* = 7.0 Hz, 2H), 1.49 – 1.25 (m, 8H), 1.19 – 1.01 (m, 2H); ^{13}C NMR (152 MHz, $CDCl_3$) δ 173.8, 165.8, 165.5, 165.4, 165.3, 164.9, 139.0, 138.4, 138.0 (2C), 133.9, 133.4, 133.3, 133.2, 132.6, 130.6, 130.1, 130.0, 129.9 (2C), 129.7, 129.6, 129.5, 129.2, 128.9, 128.7, 128.6 (2C), 128.5, 128.3, 128.2 (3C), 128.0, 127.9 (3C), 127.8, 127.7, 127.4, 101.3, 101.1, 100.6, 99.6, 81.5, 75.3, 75.2, 74.5, 74.4, 74.2, 73.5, 73.4, 73.3, 73.2, 73.0, 72.7, 72.5, 72.0, 70.8, 70.2, 68.0, 67.6, 67.2, 67.2, 53.9, 51.5, 33.5, 29.4, 29.3, 21.5; HRMS (ESI) calcd for $C_{129}H_{131}O_{30}N_2Cl_3K$ $[M+K]^+$ 2334.7493; found: 2334.7359.

***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→6)-4-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (19)**



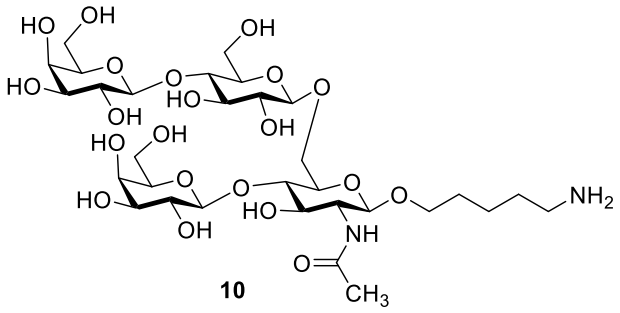
19

Trisaccharide **17** (17 mg, 9.8 μmol) and monosaccharide **11** (22 mg, 29 μmol) were mixed and co-evaporated three times using anhydrous toluene. Pre-activated molecular sieves AW-300 and anhydrous DCM (3 mL) were added and the mixture was stirred at room temperature for 20 min. The mixture was cooled down to -10 ^{o}C and NIS (10 mg, 44 μmol) followed by triflic acid (0.4 μL, 5 μmol) were added. The reaction was stirred at -10°C for 1 h and quenched with saturated aqueous $NaHCO_3$ solution and 10% $Na_2S_2O_3$ solution. The aqueous layer was washed three times with DCM. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated in *vacuo* to give crude compound **18**. Then the crude was dissolved in DCM (3 mL) followed by the addition of TEA (1 mL), the reaction mixture was stirred for 2 h at room temperature and the solvent was removed. The residue was purified by flash column chromatography with 15% acetone in toluene to yield compound **19** (17.5 mg, 8.0 μmol, 82%) as a white foam.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.6 Hz, 2H), 7.91 – 7.80 (m, 6H), 7.68 (d, *J* = 7.7 Hz, 2H), 7.53 – 6.80 (m, 55H), 5.64 (d, *J* = 8.0 Hz, 1H), 5.40 (t, *J* = 9.3 Hz, 1H), 5.32 – 5.22 (m, 2H), 5.11 – 5.01 (m, 3H), 4.70 (s, 1H), 4.60 (d, *J* = 11.4 Hz, 1H), 4.54 – 4.30 (m, 11H), 4.25 – 4.17 (m, 3H), 4.10 – 3.99 (m, 4H), 3.85 (dd, *J* = 10.3, 4.9 Hz, 1H), 3.79 – 3.55 (m, 7H), 3.52 – 3.25 (m, 9H), 3.12 – 3.00 (m, 3H), 2.93 – 2.80 (m, 2H), 2.72 (t, *J* = 9.2 Hz, 1H), 1.38 – 1.21 (m, 4H), 1.07 – 0.96 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.2, 165.8, 165.5, 165.4, 165.3, 138.3, 138.2, 138.0 (2C), 137.8, 133.8, 133.5, 133.4 (2C), 132.7, 130.5, 130.0 (3C), 129.9, 129.8, 129.7, 129.5, 129.3, 129.2, 128.8, 128.7, 128.6 (4C), 128.5, 128.4, 128.3, 128.2, 128.0 (2C), 127.9 (2C), 127.8, 127.7, 127.6, 127.5, 127.3, 101.3, 100.7, 100.5, 99.6, 78.2, 76.2, 75.2 (2C), 74.6, 74.3 (2C), 73.6, 73.4 (2C), 73.2 (2C), 73.0, 72.8, 72.0, 70.9, 69.4, 68.7, 67.9,

67.7, 67.3, 67.0, 56.4, 50.4, 47.3, 46.3, 29.8, 27.5, 23.4; HRMS (ESI) calcd for $C_{123}H_{121}Cl_3O_{28}N_2Na$ $[M+Na]^+$ 2203.7041; found: 2203.6987.

5-Aminopentyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-4-*O*-(β-D-galactopyranosyl)-2-deoxy-2-acetamino-β-D-glucopyranoside (10)

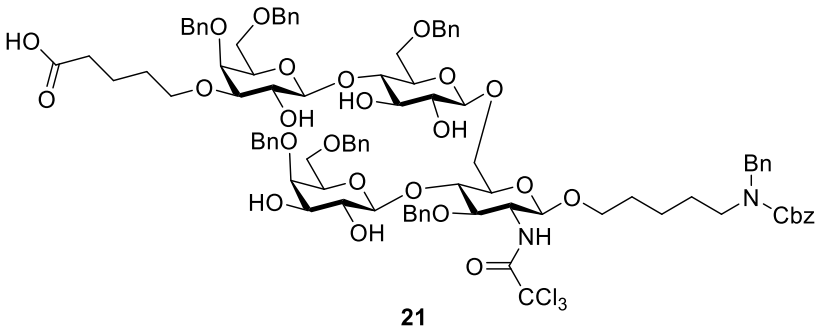


10

To a solution of tetrasaccharide **19** (13 mg, 5.96 μmol) in THF and MeOH (3 mL, v/v=1/1), excessive NaOMe (30 mg) was added. The reaction was stirred for 5 h, quenched with Amberlite H^+, filtered and concentrated under vacuum. The residue was purified by flash column chromatography with 5% MeOH in DCM to give compound **20** (9.7 mg, 5.84 μmol, 98%) as white solid. To a solution of compound **20** (9.7 mg, 5.84 μmol) in DCM/*t*-butanol/H_2O (2/4/1, 2.1mL), an excess amount of Pd-C was added. Then the reaction was stirred for 24 h at room temperature under hydrogen. After completion, the mixture was filtered to give compound **10** (4.0 mg, 5.1 μmol, 87%) as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.54 (d, *J* = 8.1 Hz, 1H), 4.51 (d, *J* = 7.8 Hz, 2H), 4.43 (d, *J* = 7.7 Hz, 1H), 4.27 (d, *J* = 10.0 Hz, 1H), 3.98 – 3.50 (m, 24H), 3.40 – 3.32 (m, 1H), 2.97 (t, *J* = 7.5 Hz, 2H), 2.01 (s, 3H), 1.70 – 1.55 (m, 4H), 1.43 – 1.33 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 174.4, 102.9, 102.7, 102.3, 101.2, 78.3, 77.7, 75.3, 75.2, 74.6, 74.2, 73.4, 72.6, 72.4, 72.2, 70.9, 70.2, 68.5, 67.3, 61.0, 60.0, 55.0, 39.3, 28.0, 26.3, 22.1, 22.0; HRMS (ESI) calcd for $C_{31}H_{57}O_{21}N_2$ $[M+H]^+$ 793.3454; found: 793.3425.

***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 4,6-di-*O*-benzyl-3-*O*-(5-penanoate acid)-β-D-galactopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (21)**

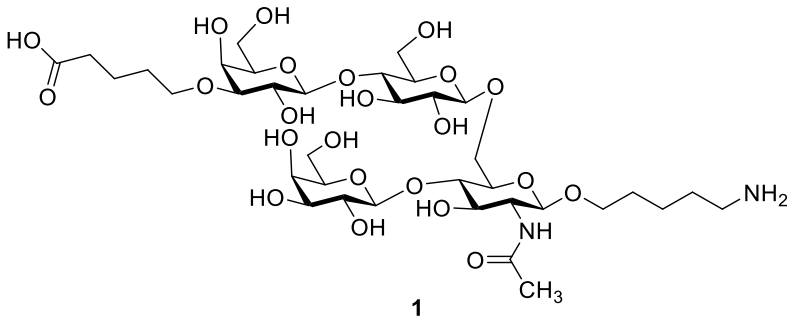


21

To a solution of tetrasaccharide **4** (27 mg, 0.012 mmol) in THF (2 mL) and MeOH (2 mL), 15% aqueous NaOH solution (100 μL) was added. The reaction mixture was stirred for 1 h until the starting material disappeared. Then excessive MeONa (50 mg) was added. The reaction mixture was stirred for another 5 h, quenched with Amberlite H^+, filtered and concentrated. The residue was purified by flash column chromatography with 5% MeOH in DCM (0.5% AcOH) to afford compound **21** (19.4 mg, 0.011 mmol, 92%) as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 – 6.85 (m, 41H), 5.20 – 5.09 (m, 2H), 4.95 (d, *J* = 10.7 Hz, 1H), 4.84 (brs, 1H), 4.78 (d, *J* = 11.7 Hz, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.60 – 4.49 (m, 6H), 4.48 – 4.42 (m, 3H), 4.39 – 4.34 (m, 2H), 4.31 (d, *J* = 11.8 Hz, 1H), 4.24 – 4.17 (m, 3H), 4.09 (brs, 1H), 3.96 (d, *J* = 8.3 Hz, 1H), 3.90 – 3.69 (m, 8H), 3.64 (s, 1H), 3.62 – 3.37 (m, 16H), 3.34 – 3.25 (s, 2H), 3.23 – 2.99 (m, 4H), 2.35 (s, 2H), 2.04 (s, 1H), 1.61 (brs, 2H), 1.52 – 1.39 (m, 4H), 1.27 – 1.17 (m, 4H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.8, 156.3, 138.8, 138.3, 138.0, 137.9, 137.6, 128.7, 128.6, 128.5 (2C), 128.4, 128.3, 128.2, 128.1, 128.0, 127.9 (3C), 127.8, 127.7, 127.3, 104.2, 103.8, 103.3, 103.2, 103.1, 99.4, 99.3, 82.9, 78.3, 76.1, 75.7, 75.2, 75.0, 74.6, 74.3, 74.1, 73.9, 73.8, 73.7, 73.5, 73.2, 73.1, 72.4, 70.8, 70.4, 70.1, 69.8, 69.1, 68.8, 68.6, 68.3, 68.0, 67.3, 58.2, 54.9, 50.7, 50.4, 49.8, 47.3, 47.0, 46.3, 46.1, 45.3, 43.6, 29.8, 29.4, 28.1, 27.5, 23.5, 23.3, 21.8; HRMS (ESI) calcd for $C_{93}H_{109}O_{25}N_2Cl_3Na$ $[M+Na]^+$ 1783.6255; found: 1783.6334.

5-Aminopentyl 3-*O*-(5-penanoate acid)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-4-*O*-(β-D-galactopyranosyl)-2-deoxy-2-acetamino-β-D-glucopyranoside (1)

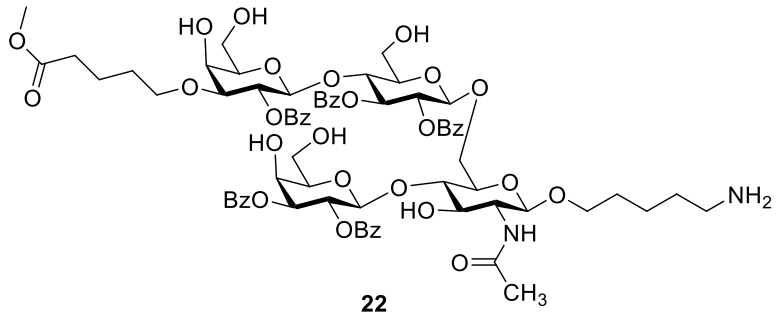


1

To a solution of compound **21** (5 mg, 2.84 μmol) in DCM/*t*-butanol/H_2O (2/4/1, 2.1 mL), an excess amount of Pd-C was added. The reaction was stirred at room temperature under hydrogen for 24 h. After completion, the mixture was filtered to give compound **1** (2.5 mg, 2.84 μmol, quant.) as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 4.59 (d, *J* = 8.0 Hz, 1H), 4.56 (d, *J* = 7.8 Hz, 2H), 4.49 (d, *J* = 7.7 Hz, 1H), 4.31 (d, *J* = 10.3 Hz, 1H), 4.18 (s, 1H), 4.04 – 3.96 (m, 2H), 3.96 – 3.89 (m, 2H), 3.87 – 3.68 (m, 15H), 3.65 – 3.54 (m, 5H), 3.47 (dd, *J* = 10.0, 3.1 Hz, 1H), 3.42 – 3.39 (m, 1H), 3.04 – 2.98 (m, 2H), 2.33 – 2.29 (m, 2H), 2.06 (s, 3H), 1.72 – 1.60 (m, 8H), 1.46 – 1.40 (m, 2H); ^{13}C NMR (152 MHz, D_2O) δ 174.5, 102.9, 102.8, 102.4, 101.2, 80.4, 78.3, 77.9, 75.3, 74.7, 74.3, 73.5, 72.7, 72.5, 72.3, 70.9, 70.2, 70.0, 69.2, 68.6, 67.4, 65.0, 61.1, 61.1, 60.0, 55.1, 39.3, 36.3, 28.5, 28.1, 26.3, 22.1, 22.1, 21.9; HRMS (ESI) calcd for $C_{36}H_{65}O_{23}N_2$ $[M+H]^+$ 893.3979; found: 893.4137.

5-Aminopentyl 2-*O*-benzoyl-3-*O*-(methyl 5-penanoate)-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-4-*O*-(2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-2-deoxy-2-acetamino-β-D-glucopyranoside (22)



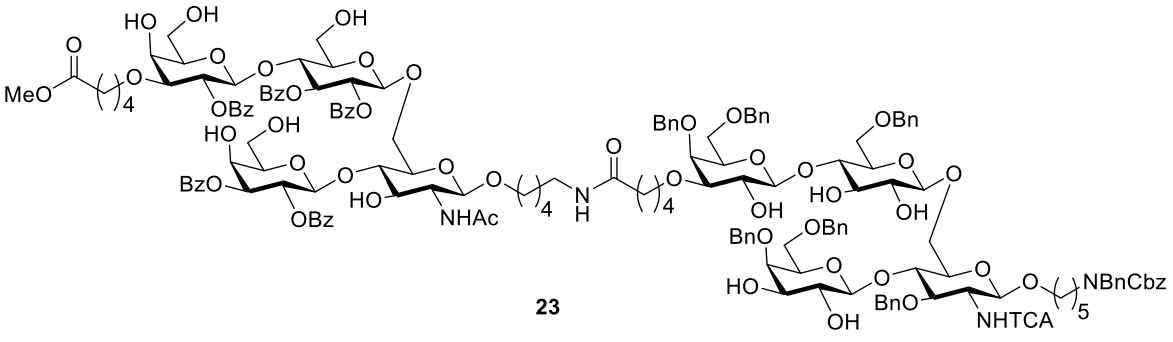
22

To a solution of compound **4** (26.8 mg, 11.7 μmol) in DCM/t-butanol/H_2O (2/4/1, 2.1mL), an excess amount of Pd-C was added. The reaction mixture was stirred for 24 h at room

temperature under hydrogen. After completion, the mixture was filtered to give compound **22** quantitatively.

^{1}H NMR (600 MHz, CD_3OD) δ 8.16 (d, *J* = 7.4 Hz, 2H), 8.11 (d, *J* = 7.3 Hz, 2H), 7.99 (d, *J* = 7.5 Hz, 2H), 7.93 (t, *J* = 7.2 Hz, 4H), 7.70 (t, *J* = 6.8 Hz, 2H), 7.63 (t, *J* = 7.6 Hz, 2H), 7.61 – 7.47 (m, 6H), 7.44 – 7.35 (m, 5H), 5.51 (dd, *J* = 10.2, 8.0 Hz, 1H), 5.38 – 5.28 (m, 2H), 5.09 (t, *J* = 8.0 Hz, 1H), 4.90 – 4.87 (m, 1H), 4.67 (d, *J* = 8.0 Hz, 1H), 4.29 (d, *J* = 8.1 Hz, 1H), 4.05 (t, *J* = 9.4 Hz, 1H), 4.02 (d, *J* = 2.8 Hz, 1H), 3.97 (d, *J* = 2.9 Hz, 1H), 3.95 (d, *J* = 7.9 Hz, 1H), 3.85 (d, *J* = 10.2 Hz, 1H), 3.78 – 3.74 (m, 1H), 3.69 – 3.54 (m, 8H), 3.52 (s, 3H), 3.46 – 3.40 (m, 3H), 3.38 – 3.32 (m, 3H), 3.30 – 3.24 (m, 2H), 3.19 (dd, *J* = 10.7, 5.9 Hz, 1H), 2.98 – 2.86 (m, 3H), 2.31 (d, *J* = 9.3 Hz, 1H), 2.12 – 2.05 (m, 2H), 1.95 (s, 3H), 1.69 – 1.62 (m, 2H), 1.58 – 1.53 (m, 2H), 1.50 – 1.40 (m, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 175.6, 173.5, 167.6, 167.1, 166.9, 166.8, 166.5, 135.6, 135.1, 134.6, 134.5, 134.3, 131.2, 131.1, 130.9 (3C), 130.8, 130.7, 130.6, 130.4, 130.3, 129.9, 129.5, 129.4, 102.9, 102.7, 102.5, 102.0, 82.4, 81.3, 76.9, 76.7, 76.6, 75.4, 74.5, 74.1, 74.0, 73.4, 73.1, 71.7, 70.2, 70.0, 68.5, 67.5, 66.3, 61.8, 61.1, 60.2, 56.2, 51.9, 40.7, 34.2, 30.0, 29.6, 27.9, 24.0, 23.0, 22.6; HRMS (ESI) calcd for $C_{72}H_{87}O_{28}N_2$ $[M+H]^+$ 1427.5440; found: 1427.5452.

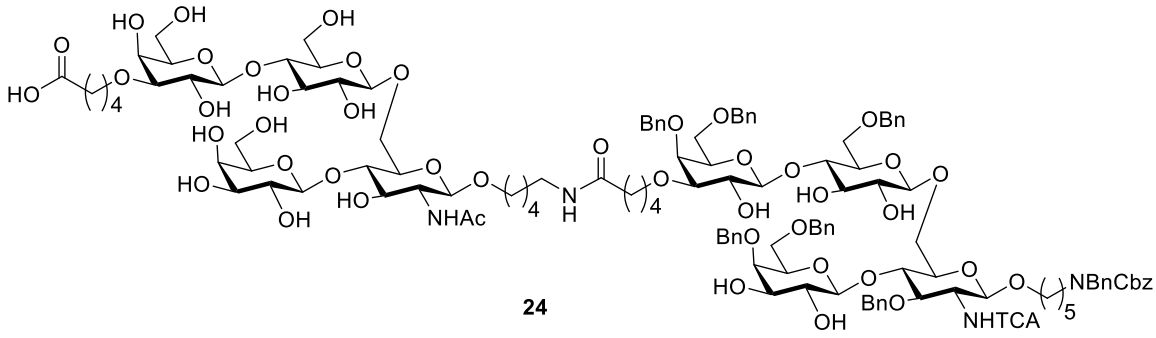
Protected divalent derivative (23)



Both compound **22** (16.7 mg, 11.7 μmol) and **21** (17.2 mg, 9.77 μmol) were dissolved in DMF (3 mL) followed by the addition of PyBOP (5.6 mg, 15.1 μmol) and DIPEA (0.2 mL). Then the reaction was stirred for 4 h at room temperature. The solvent was removed under vacuum and the residue was purified by flash column chromatography with 8% MeOH in dichloromethane to give **23** (24.7 mg, 7.8 μmol, 80%) as a white foam.

^{1}H NMR (700 MHz, CD_3OD) δ 8.23 – 7.87 (m, 11H), 7.74 – 7.47 (m, 12H), 7.45 – 7.21 (m, 41H), 7.10 (t, *J* = 7.5 Hz, 1H), 5.56 – 5.31 (m, 2H), 5.25 – 5.04 (m, 4H), 4.96 (d, *J* = 11.4 Hz, 1H), 4.71 – 4.41 (m, 14H), 4.34 – 4.19 (m, 4H), 4.14 – 3.99 (m, 6H), 3.95 – 3.50 (m, 39H), 3.49 – 3.41 (m, 5H), 3.37 (s, 3H), 3.31 – 3.11 (m, 9H), 2.24 (t, *J* = 7.6 Hz, 2H), 2.14 – 2.06 (m, 2H), 1.98 – 1.91 (m, 3H), 1.80 – 1.63 (m, 5H), 1.47 (m, 15H); HRMS (ESI) calcd for $C_{165}H_{193}Cl_3O_{52}N_4Na_2$ $[M+2Na]^{2+}$ 1606.5715; found: 1606.5713.

Partly protected divalent derivative (24)



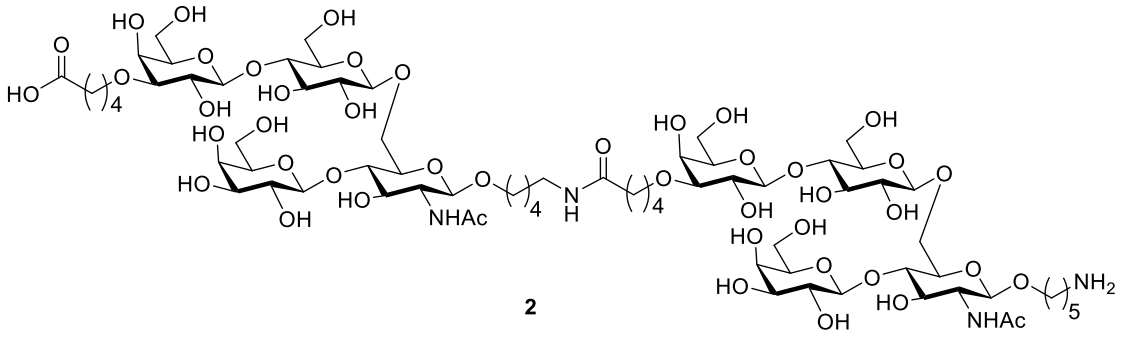
24

To a solution of **23** (8.7 mg, 2.74 μmol) in THF and MeOH (v/v=1/1, 2 mL), 15% NaOH solution (100 μL) was added. The reaction mixture was stirred for 1 h until starting material disappeared. Then excessive MeONa (30 mg) was added. The reaction was stirred for another 5 h, quenched with Amberlite H^+, filtered concentrated under vaccum. The residue was purified by size exclusion chromatography with 30% MeOH in chloroform to give compound **24** (7.1 mg, 2.69 μmol, 98%).

^{1}H NMR (400 MHz, CD_3OD) δ 7.49 – 6.99 (m, 40H), 5.14 (d, *J* = 11.8 Hz, 2H), 5.07 (d, *J* = 10.2 Hz, 1H), 4.99 – 4.92 (m, 1H), 4.82 (d, *J* = 11.2 Hz, 2H), 4.64 – 4.35 (m, 16H), 4.32 – 4.15 (m, 4H), 4.13 – 3.38 (m, 52H), 3.28 – 3.09 (m, 6H), 2.33 – 2.19 (m, 4H), 1.95 (s, 3H), 1.78 – 1.62 (m, 8H), 1.60 – 1.41 (m, 8H), 1.40 – 1.27 (m, 4H); ^{13}C NMR (101 MHz, CD_3OD) δ 176.0, 173.4, 140.5, 140.5, 140.3, 140.1, 139.8, 139.8, 139.3, 139.2, 138.1, 138.0, 129.6, 129.5, 129.4, 129.4, 129.4, 129.3, 129.2, 129.2, 129.2, 129.2, 129.0, 129.0, 128.9, 128.8, 128.7, 128.4, 105.1, 105.0, 104.8, 104.6, 104.5, 104.2, 102.9, 102.7, 102.3, 101.4, 86.0, 83.9, 83.6, 83.0, 82.7, 81.2, 80.8, 80.4, 79.6, 79.5, 78.8, 78.7, 77.7, 77.0, 76.8, 76.4, 76.2, 76.0, 75.7, 75.3, 74.8, 74.7, 74.6, 74.5, 74.4, 74.3, 74.1, 73.9, 73.4, 73.1, 72.7, 72.1, 71.7, 71.5, 70.6, 70.4, 70.1, 69.9, 68.6, 67.5,

66.9, 65.1, 62.6, 62.5, 62.0, 58.3, 56.7, 55.7, 40.3, 36.8, 36.7, 30.6, 30.2, 30.1, 24.4, 24.3, 24.2, 23.9, 23.4, 23.1, 14.6, 12.9; HRMS (ESI) calcd for $C_{129}H_{172}Cl_3O_{47}N_4Na$ $[M+H+Na]^{2+}$ 1328.5072; found: 1328.5741.

Divalent derivative (2)

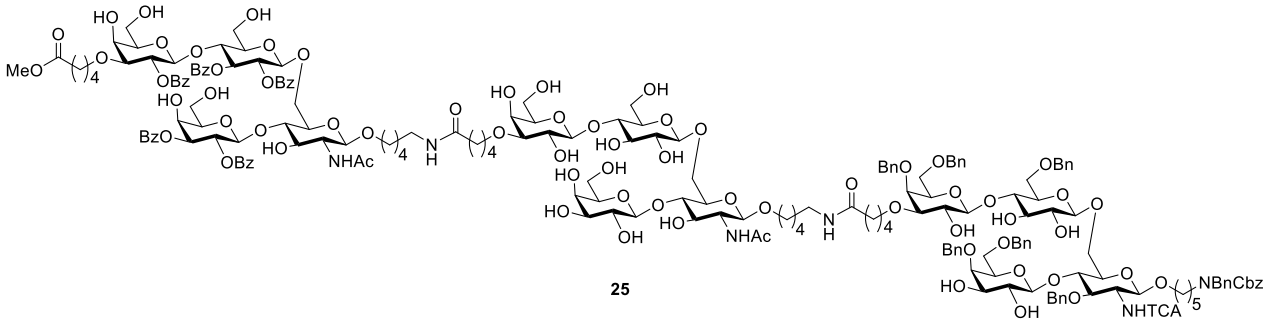


2

To a solution of compound **24** (7 mg, 2.66 μmol) in DCM/*t*-butanol/H_2O (2/4/1, 2.1mL), an excess amount of Pd-C was added. The reaction mixture was stirred for 24 h at room temperature under hydrogen. After completion, the mixture was filtered to give compound **2** (4.6 mg, 2.66 μmol) quantitatively as a white solid.

^{1}H NMR (700 MHz, D_2O) δ 4.53 – 4.45 (m, 6H), 4.40 (d, *J* = 7.8 Hz, 2H), 4.23 (d, *J* = 10.8 Hz, 2H), 4.09 (dd, *J* = 7.2, 2.8 Hz, 2H), 3.96 – 3.88 (m, 4H), 3.87 – 3.85 (m, 2H), 3.85 – 3.81 (m, 2H), 3.78 – 3.60 (m, 30H), 3.56 – 3.46 (m, 10H), 3.41 – 3.37 (m, 2H), 3.32 (t, *J* = 8.3 Hz, 2H), 3.14 – 3.08 (m, 2H), 2.94 – 2.89 (m, 2H), 2.21 (t, *J* = 7.2 Hz, 2H), 2.17 – 2.12 (m, 2H), 1.97 (s, 6H), 1.62 – 1.50 (m, 14H), 1.47 – 1.42 (m, 2H), 1.37 – 1.31 (m, 2H), 1.30 – 1.24 (m, 2H); ^{13}C NMR (175 MHz, D_2O) δ 183.6, 176.6, 174.4, 174.3, 102.9, 102.7, 102.4, 101.2, 101.1, 80.4, 78.3, 77.9, 75.3, 75.3, 74.7, 74.3, 73.5, 72.7, 72.5, 72.3, 70.9, 70.4, 70.2, 70.0, 69.3, 69.1, 68.6, 67.4, 65.0, 61.1, 60.0, 55.1, 39.3, 39.2, 37.2, 35.5, 28.7, 28.2, 28.1, 27.9, 26.4, 22.5, 22.3, 22.1, 22.0; HRMS (ESI) calcd for $C_{72}H_{126}O_{45}N_4Na$ $[M+Na]^+$ 1789.7592; found: 1789.7497.

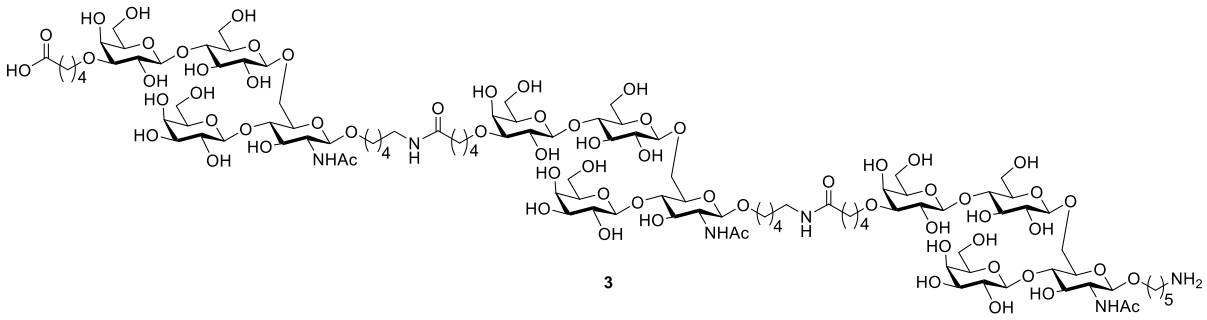
Protected trivalent derivative (25)



Both compound **22** (2.5 mg, 1.73 μmol) and **24** (3.8 mg, 1.44 μmol) were dissolved in DMF (1 mL) followed by the addition of PyBOP (2.2 mg, 4.3 μmol) and DIPEA (1.5 μL, 8.6 μmol). The reaction mixture was stirred for 2 h at room temperature. The solvent was removed under high vacuum and the residue was purified by size exclusion chromatography with 50% MeOH in chloroform to afford the compound **25** (5.6 mg, 1.4 μmol, 96%).

^{1}H NMR (700 MHz, CD_3OD) δ 8.24 – 7.89 (m, 9H), 7.75 – 7.08 (m, 56H), 5.54 (t, *J* = 9.1 Hz, 1H), 5.36 (t, *J* = 9.5 Hz, 2H), 5.18 – 5.08 (m, 7H), 4.71 – 4.40 (m, 26H), 4.27 – 4.20 (m, 4H), 4.12 – 3.53 (m, 77H), 3.19 – 3.14 (m, 4H), 2.27 – 2.19 (m, 4H), 2.13 – 2.06 (m, 2H), 1.96 (s, 6H), 1.81 – 1.69 (m, 6H), 1.60 – 1.43 (m, 18H), 1.38 – 1.32 (m, 6H); ^{13}C NMR (176 MHz, CD_3OD) δ 174.8, 174.6, 174.2, 172.0, 139.1, 138.7, 138.4, 138.0, 136.7, 129.9, 129.7, 129.5, 129.4, 129.3, 128.9, 128.6, 128.2, 128.0, 128.0, 127.9, 127.8, 127.8, 127.6, 127.6, 127.5, 127.4, 127.3, 127.0, 103.7, 103.6, 103.5, 103.1, 102.8, 101.5, 101.3, 101.2, 101.1, 100.9, 100.5, 82.5, 81.6, 79.9, 79.5, 79.1, 77.2, 75.6, 75.5, 75.1, 74.8, 74.6, 74.5, 74.3, 74.2, 74.0, 73.5, 73.3, 73.2, 73.1, 73.0, 72.7, 72.0, 71.3, 70.7, 70.3, 70.1, 69.1, 69.0, 68.7, 68.6, 67.2, 65.5, 64.9, 61.1, 60.7, 59.6, 55.3, 50.5, 38.9, 35.5, 35.4, 32.9, 29.2, 28.8, 28.6, 23.0, 22.5, 22.4, 21.7, 21.2; MALDI-TOF MS calcd for $C_{201}H_{255}Cl_3O_{74}N_6Na$ $[M+Na]^+$ 4064.533; found: 4064.444.

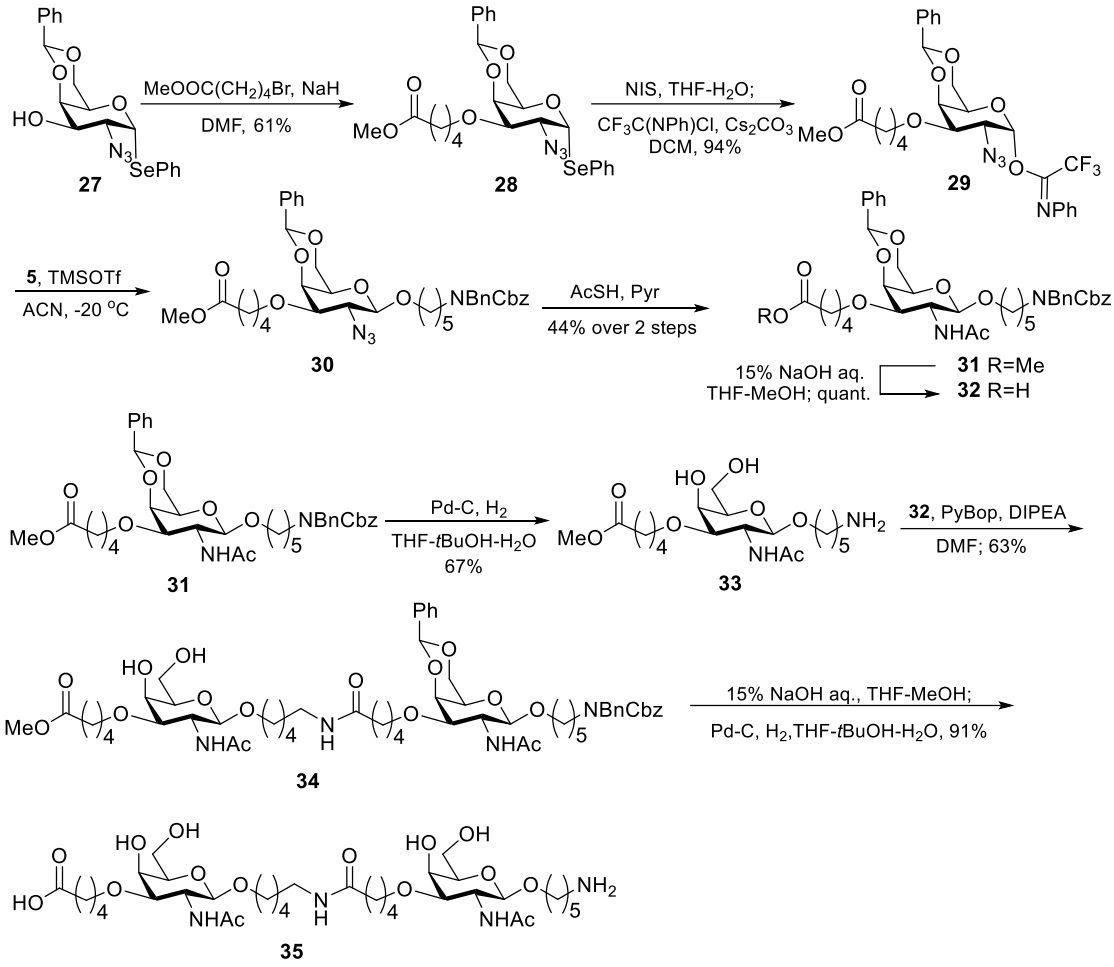
Trivalent derivative (3)



3

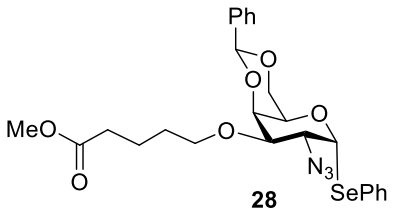
To a solution of tetrasaccharide **25** (3 mg, 0.74 μmol) in THF and MeOH (v/v=1/1, 2 mL), aqueous 15% NaOH solution (30 μL) was added. The reaction mixture was stirred for 1 h until starting material disappeared. Then excessive MeONa (30 mg) was added. The reaction mixture was stirred for another 5 h, quenched with Amberlite H^+, filtered and concentrated under vacuum to give crude product **26**. The residue **26** was dissolved in a mixture of *t*-butanol/H_2O (v/v=1/1, 1mL) followed by the addition of an excess amount of Pd-C. The reaction was stirred for 24 h at room temperature under hydrogen. The mixture was filtered to give compound **3** (1.3 mg, 0.49 μmol, 66%, over two steps) as a white solid.

^{1}H NMR (400 MHz, D_2O) δ 4.56 – 4.49 (m, 9H), 4.47 – 4.43 (m, 3H), 4.30 – 4.25 (m, 2H), 4.17 – 4.09 (m, 3H), 3.99 – 3.95 (m, 4H), 3.92 – 3.89 (m, 5H), 3.82 – 3.66 (m, 51H), 3.59 – 3.51 (m, 14H), 3.45 – 3.40 (m, 4H), 3.39 – 3.35 (m, 3H), 3.15 (t, *J* = 6.5 Hz, 4H), 2.25 (t, *J* = 7.3 Hz, 4H), 2.20 – 2.17 (m, 2H), 2.01 (s, 9H), 1.61 – 1.47 (m, 22H), 1.35 – 1.25 (m, 8H); ^{13}C NMR (176 MHz, D_2O) δ 183.7, 176.6, 174.4, 160.9, 102.9, 102.7, 102.4, 101.2, 80.4, 78.3, 77.8, 75.3, 74.7, 74.3, 73.5, 72.7, 72.5, 72.3, 70.9, 70.4, 70.0, 69.3, 69.1, 68.6, 67.4, 65.0, 61.1, 60.1, 55.1, 39.2, 37.2, 35.5, 28.7, 28.2, 27.9, 22.5, 22.3, 22.2, 22.1; HRMS (ESI) calcd for $C_{108}H_{190}O_{67}N_6$ $[M+2H]^{2+}$ 1321.5816; found: 1321.5756.



Scheme S2: Synthesis of compound **35**.

Phenyl 2-azido-2-deoxy-4,6-*O*-benzylidene-3-*O*-(methyl 5-pentanoate)-1-seleno-α-D-galactopyranoside (28)

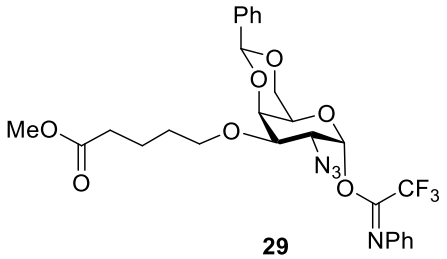


Sodium hydride (20 mg, 0.50 mmol, 60% wt) was added to a solution of phenyl 2-azido-2-deoxy-4,6-*O*-benzylidene-1-seleno-α-D-galactopyranoside **27** (144 mg, 0.33 mmol) in DMF (4 mL) at 0 °C, followed by the addition of methyl methyl 5-bromopentanoate (72 μL, 0.50 mmol). The reaction was stirred for 2 h at room temperature, quenched with saturated aqueous NH_4Cl and extracted three times with ethyl acetate. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column

chromatography with 20% ethyl acetate in hexanes to afford compound **28** (110 mg, 0.20 mmol, 61%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 – 7.53 (m, 2H), 7.54 – 7.47 (m, 2H), 7.41 – 7.32 (m, 3H), 7.28 – 7.26 (m, 3H), 6.03 (d, *J* = 5.2 Hz, 1H), 5.60 (s, 1H), 4.41 (dd, *J* = 3.4, 1.1 Hz, 1H), 4.33 (dd, *J* = 10.3, 5.2 Hz, 1H), 4.16 (dd, *J* = 12.8, 1.9 Hz, 1H), 4.13 – 4.05 (m, 2H), 3.75 (dt, *J* = 8.9, 6.1 Hz, 1H), 3.65 (s, 4H), 3.57 (dt, *J* = 8.8, 5.9 Hz, 1H), 2.36 (t, *J* = 7.2 Hz, 2H), 1.83 – 1.66 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 137.6, 133.9, 129.3, 129.2, 128.8, 128.3, 127.8, 126.3, 101.1, 85.7, 78.2, 72.3, 69.4, 68.6, 65.3, 59.8, 51.7, 33.7, 29.2, 21.7; HRMS (ESI) calcd for $C_{25}H_{29}O_6N_3SeNa$ $[M+Na]^+$ 570.1113; found: 570.1127.

_N_-Phenyltrifluoroacetimidate 2-azido-2-deoxy-4,6-_O_-benzylidene-3-_O_-(methyl 5-pentanoate)-α-D-galactopyranoside (29)



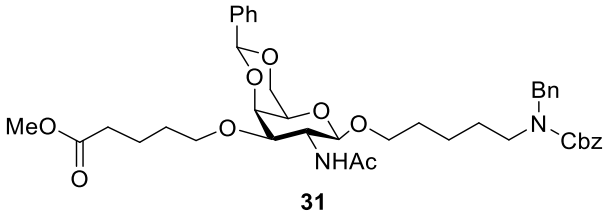
29

To a solution of monosaccharide **28** (98 mg, 0.18 mmol) in THF/H_2O (3.6 mL, v/v=5/1) was added NIS (121 mg, 0.54 mmol) at 0 °C. The reaction was stirred for 2 h at room temperature, then diluted with DCM and washed with 10% aq. $Na_2S_2O_3$ solution. The organic layer was dried over Na_2SO_4, filtered and concentrated to get crude hemi-acetal. The crude was dissolved in DCM (4 mL) followed by the addition of (*E*)-2,2,2-trifluoro-*N*-phenylacetimidoyl chloride (85 μL, 0.538 mmol) and Cs_2CO_3 (175 mg, 0.538 mmol) at 0 °C . After 2 h, the reaction mixture was filtered and concentrated. The residue was purified by flash column chromatography with 22% ethyl acetate in hexanes (1% triethylamine) to give imidate **29** (98.6 mg, 0.17 mmol, 94%) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 – 7.47 (m, 2H), 7.42 – 7.35 (m, 3H), 7.34 – 7.27 (m, 2H), 7.19 – 7.05 (m, 1H), 6.85 (d, *J* = 7.8 Hz, 2H), 5.58 (s, 1H), 4.40 – 4.24 (m, 2H), 4.06 (d, *J* = 13.0 Hz, 2H), 3.73 (dd, *J* = 7.1, 5.9 Hz, 1H), 3.64 (s, 3H), 3.56 (dd, *J* = 6.7, 5.7 Hz, 1H), 3.38 (s, 1H), 2.36 (t, *J* = 7.1 Hz, 2H), 1.82 – 1.64 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1,

143.5, 137.5, 129.3, 128.9, 128.4, 126.4, 124.5, 119.5, 101.2, 79.3, 71.7, 69.3, 69.0, 67.5, 61.0, 51.7, 33.7, 29.1, 21.7; HRMS (ESI) calcd for $C_{27}H_{29}F_3O_7N_4Na$ $[M+Na]^+$ 601.1880; found: 601.1876.

_N_-Benzyl-_N_-benzyloxycarbonyl-5-pentyl 2-acetamino-2-deoxy-4,6-_O_-benzylidene-3-_O_-(methyl 5-pentanoate)-β-D-galactopyranoside (31)

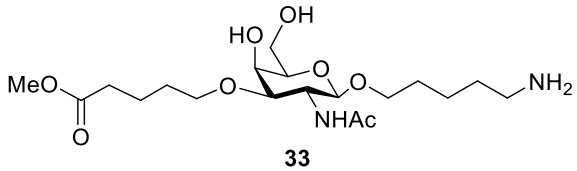


31

The imidate **29** (82.4 mg, 0.14 mmol) and *N*-(benzyl)-benzyloxycarbonyl-5-aminopentanol **5** (93 mg, 0.28 mmol) were co-evaporated twice with toluene and dried under vacuum. The residue was dissolved in acetonitrile (4 mL) and cooled to -20 ^oC. Trimethylsilyl trifluoromethanesulfonate (3 μL, 14.2 μmol) was added and the reaction was stirred for 1 h. The reaction was quenched with triethylamine and concentrated under vacuum to give crude **30**. The residue was dissolved in pyridine (4 mL), and thioacetic acid (1 mL) was added. The reaction was stirred for 72 h at room temperature. The reaction was concentrated under vacuum and purified by flash column chromatography with 50-80% ethyl acetate in hexanes to afford compound **31** (45 mg, 0.061 mmol, 44% over two steps) as a colorless oil.

^1H NMR (600 MHz, $CDCl_3$) δ 7.49 (d, *J* = 6.9 Hz, 2H), 7.41 – 7.20 (m, 12H), 7.15 (d, *J* = 7.4 Hz, 1H), 5.54 (s, 1H), 5.15 (d, *J* = 21.5 Hz, 2H), 5.09 (d, *J* = 8.3 Hz, 1H), 4.48 (d, *J* = 13.1 Hz, 2H), 4.30 (d, *J* = 12.3 Hz, 2H), 4.25 (d, *J* = 3.4 Hz, 1H), 4.06 (dd, *J* = 12.4, 1.8 Hz, 1H), 3.85 (d, *J* = 31.5 Hz, 1H), 3.66 (dt, *J* = 9.1, 5.8 Hz, 1H), 3.61 (s, 3H), 3.46 – 3.16 (m, 6H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.89 (s, 3H), 1.69 – 1.63 (m, 2H), 1.61 – 1.48 (m, 6H), 1.34 – 1.25 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 138.0, 128.9, 128.6, 128.6, 128.1, 128.0, 128.0, 127.3, 126.5, 101.1, 99.4, 74.9, 72.9, 69.7, 68.7, 67.3, 66.6, 55.0, 51.6, 50.5, 47.3, 46.2, 33.7, 29.2, 23.7, 21.7; HRMS (ESI) calcd for $C_{41}H_{52}O_{10}N_2Na$ $[M+Na]^+$ 755.3514; found: 755.3552.

5-Aminopentyl 2-acetamino-2-deoxy-3-*O*-(methyl 5-pentanoate)-β-D-galactopyranoside (33)

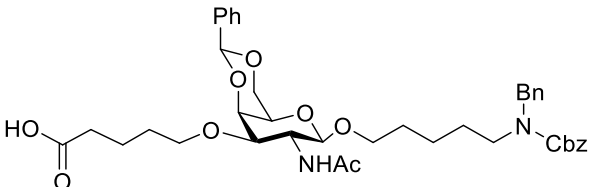


33

The compound **31** (10.3 mg, 0.014 mmol) was dissolved in a mixture of THF/*t*-butanol/H_2O (4/2/1, 2.1mL) followed by the addition of excess amount of Pd-C. The reaction was stirred for 20 h at room temperature under hydrogen. Then the reaction mixture was filtered to give compound **33** (4 mg, 9.5 μmol, 67%) as a colorless oil.

^{1}H NMR (400 MHz, D_2O) δ 4.31 (d, *J* = 8.6 Hz, 1H), 3.99 (d, *J* = 3.1 Hz, 1H), 3.81 – 3.58 (m, 5H), 3.55 (s, 3H), 3.51 – 3.44 (m, 2H), 3.38 – 3.23 (m, 2H), 2.84 (t, *J* = 7.7 Hz, 2H), 2.27 (t, *J* = 7.3 Hz, 2H), 1.87 (s, 3H), 1.55 – 1.44 (m, 8H), 1.32 – 1.21 (m, 2H); ^{13}C NMR (151 MHz, D_2O) δ 177.2, 174.2, 101.5, 78.7, 75.0, 69.9, 68.9, 64.4, 61.5, 61.0, 52.0, 51.2, 39.3, 33.2, 28.0, 28.0, 27.8, 26.3, 22.1, 20.9; HRMS (ESI) calcd for $C_{19}H_{37}O_8N_2$ $[M+H]^+$ 421.2544; found: 421.2529.

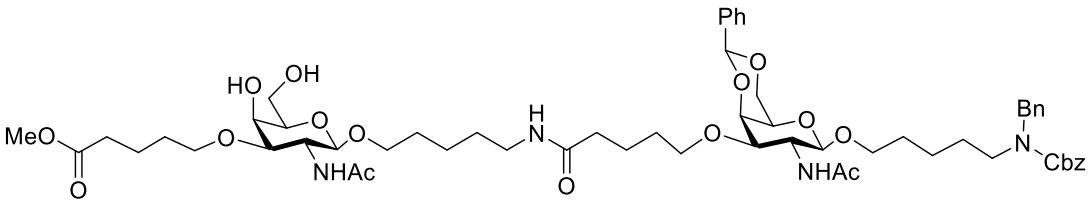
***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 2-acetamino-2-deoxy-4,6-*O*-benzylidene-3-*O*-(5-pentanoate acid)-β-D-galactopyranoside (32)**



32

To a solution of **31** (7.9 mg, 0.011 mmol) in THF and MeOH (2 mL, v/v=1/1), aqueous 15% NaOH solution (100 μL) was added. The reaction mixture was stirred for 4 h, quenched by Amberlite H^+, filtered and concentrated under vacuum. The residue **32** was used directly in the next step.

Protected divalent of *N*-acetyl-galactosamine (34)

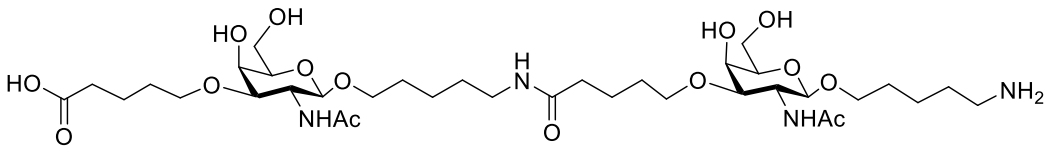


34

To a solution of crude **32** (11 μmol) and compound **33** (4 mg, 9.5 μmol) in DMF (2 mL), PyBOP (8 mg, 15.5 μmol) was added followed by the addition of DIPEA. The reaction was stirred for 2 h at room temperature. The solvent was removed *in vacuo*. The residue was purified by flash column chromatography using 7-9% methanol in dichloromethane to afford compound **34** (6.7 mg, 6 μmol, 63%) as a colorless oil.

^{1}H NMR (400 MHz, CD_3OD) δ 7.56 – 7.46 (m, 5H), 7.37 – 7.20 (m, 10H), 5.63 (s, 1H), 5.16 (d, *J* = 13.6 Hz, 2H), 4.51 (s, 3H), 4.45 – 4.34 (m, 2H), 4.18 (q, *J* = 12.3 Hz, 2H), 4.06 – 3.99 (m, 2H), 3.94 (t, *J* = 9.6 Hz, 1H), 3.86 (dt, *J* = 9.4, 6.1 Hz, 2H), 3.77 – 3.70 (m, 4H), 3.64 (s, 3H), 3.61 (dd, *J* = 11.2, 3.2 Hz, 1H), 3.51 – 3.42 (m, 5H), 3.38 (dd, *J* = 11.5, 4.0 Hz, 2H), 3.24 (d, *J* = 8.6 Hz, 2H), 3.08 (t, *J* = 6.6 Hz, 2H), 2.34 (t, *J* = 7.4 Hz, 2H), 2.17 (t, *J* = 7.4 Hz, 2H), 1.95 (s, 6H), 1.71 – 1.46 (m, 16H), 1.39 – 1.32 (m, 4H); ^{13}C NMR (176 MHz, CD_3OD) δ 176.0, 175.8, 173.5, 139.7, 129.9, 129.6, 129.1, 129.0, 128.7, 128.4, 128.1, 127.6, 126.9, 118.8, 111.6, 103.1, 102.7, 102.3, 80.9, 79.5, 78.8, 76.6, 73.5, 70.4, 70.2, 70.2, 70.1, 69.9, 68.1, 66.1, 62.6, 53.0, 52.8, 52.0, 40.3, 36.7, 34.5, 30.3, 30.2, 30.2, 30.0, 24.5, 24.2, 24.1, 23.2, 23.1, 22.8; HRMS (ESI) calcd for $C_{59}H_{84}O_{17}N_4Na$ $[M+Na]^+$ 1143.5724; found: 1143.5760.

Divalent of *N*-acetyl-galactosamine (35)



35

To a solution of compound **34** (6.5 mg, 5.8 μmol) in THF and MeOH (2 mL, v/v=1/1), an aqueous 15% NaOH solution (100 μL) was added. The reaction mixture was stirred overnight, quenched with Amberlite H^+, filtered and concentrated *in vacuo*. The residue was then

dissolved in a mixture of THF/*t*-butanol/H_2O (1/2/1, 2.1mL) followed by the addition of excess amount of Pd-C. The reaction mixture was stirred for 20 h at room temperature under hydrogen. The reaction mixture was filtered to give compound **35** (4.2 mg, 5.3 μmol, 91%) as a colorless oil.

^{1}H NMR (600 MHz, D_2O) δ 4.32 (d, *J* = 8.6 Hz, 2H), 3.99 (d, *J* = 3.1 Hz, 2H), 3.80 – 3.72 (m, 3H), 3.69 – 3.60 (m, 4H), 3.52 – 3.43 (m, 4H), 3.37 – 3.29 (m, 3H), 3.05 – 3.01 (m, 2H), 2.85 (t, *J* = 7.7 Hz, 2H), 2.57 (t, *J* = 6.4 Hz, 2H), 2.44 (t, *J* = 6.0 Hz, 2H), 2.24 (t, *J* = 7.3 Hz, 2H), 2.11 (t, *J* = 7.4 Hz, 2H), 1.88 (s, 3H), 1.87 (s, 3H), 1.75 – 1.66 (m, 4H), 1.59 – 1.34 (m, 12H), 1.30 – 1.24 (m, 2H), 1.20 (tt, *J* = 7.7, 4.6 Hz, 2H); ^{13}C NMR (151 MHz, D_2O) δ 179.2, 176.4, 174.1, 101.5, 101.5, 81.7, 78.7, 75.0, 75.0, 70.1, 69.9, 69.0, 68.9, 64.5, 64.4, 61.5, 61.0, 51.2, 39.3, 39.2, 35.4, 33.8, 28.2, 28.1, 28.0, 28.0, 27.9, 27.8, 26.3, 22.4, 22.2, 22.1, 22.1, 22.0, 21.1, 20.7, 20.7, 20.0, 19.6, 18.2; HRMS (ESI) calcd for $C_{36}H_{67}O_{15}N_4$ $[M+H]^+$ 795.4597; found: 795.4612.

Part III. Biochemistry experiments

Glycan array printing

Briefly, synthetic oligosaccharides and commercial pneumococcal polysaccharides were dissolved in 50 mM sodium phosphate buffer (PH 8.5) and spotted to *N*-hydroxysuccinimide activited hydrogel coated glass slides (CodeLink slides; Surmodics) using an S3 robotic non-contact microarray printer. Repetitive printing patterns were used as outlined in section 2.2.4. Slides were incubated in a humidified chamber overnight and quenched with 100 mM ethanolamine and 50 mM sodium phosphate (PH 9.0) for 1 h at room temperature. Slides were washed with water, dried by centrifugation and stored at 4 ^{o}C until use.

General procedure for glycan array analysis

Slides were blocked by incubation with 1% bovine serum albumin (BSA) in phosphate buffered saline (PBS) (1% BSA-PBS) for at least 30 min at room temperature. Slides were washed twice with PBS and dried by centrifugation. A 64-well incubation grid (Grace Biolabs) was applied to the slide. Dilutions of anti-ST14 mouse or human sera in 1% BSA-PBS were applied to the wells and incubated for 1 h at 37 ^{o}C in a humid and dark chamber. Wells were washed three times with PBS containing 0.1% Tween-20 (PBS-T). Secondary antibody dilutions in 1% BSA-PBS were applied to the wells and incubated for 1 h at 37 ^{o}C in a humid and dark chamber. The following secondary antibodies were used: goat anti-mouse IgG H+L AlexaFluor 635 (Thermo Fisher Scientific Invitrogen) and goat anti-human IgG H+L AlexaFluor 635 (Thermo Fisher Scientific Invitrogen). Wells were washed twice with PBS-T and once with PBS. The incubation grid was removed and the slides were rinsed with water and dried by centrifugation. Slides were scanned using a GenePix 4300A microarray scanner (Molecular Devices, Sunnyvale, CA, USA) and evaluated using GenePix Pro 7.2 (both Molecular Devices). The photomultiplier tube (PMT) voltage was adjusted such that scans were free of saturation signals and the wavelength was chosen based on the secondary antibody used.

Preparation of glycoconjugates

Bis(*p*-nitrophenyl adipate) (4 mg, 10 μmol) was added to a solution of oligosaccharide (1 μmol) in a mixture of dimethylsulfoxide/pyridine (8/1, 180 μL), followed by the addition of triethylamine (5 μL). The reaction was stirred for 6 h at room temperature. Then the solvent was removed by lyophilization overnight. The residue was triturated with chloroform (3×1 mL)

and dichloromethane (3×1 mL), transferred to a 1.8 mL type I class A glass vial using dimethylsulfoxide (150 μL) and lyophilized.

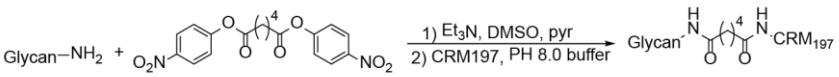
CRM197 (1 mg) or BSA was washed with autoclaved water (3×500 μL) and phosphate buffer 0.1 M pH 8.0 (3×500 μL) by dialysis using a centrifugal filter (10 KDa MWCO, Merck Milipore), concentrated 100 μL and transferred to the vial with activated oligosaccharide.

The reaction mixture was stirred for 18 h at room temperature, transferred to a centrifugal filter and washed with phosphate buffer 0.1 M pH 8.0 (3×400 μL) and autoclaved water (3×400 μL). A 10 μL sample was taken for analysis before washing the mixture with phosphate buffered saline (2×400 μL).

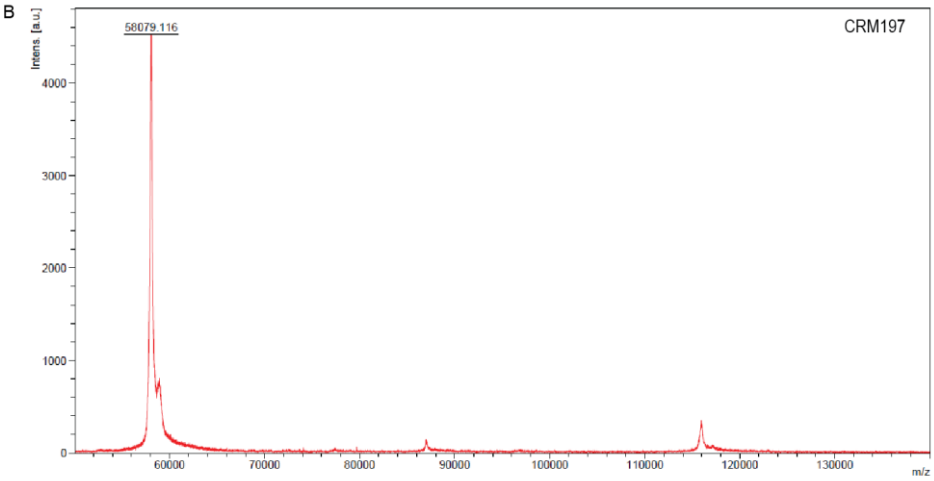
The average molecular size of the conjugates was characterized by Matrix-assisted laser desorption/ionization (MALDI) analysis using 2,4-dihydroxyacetophenone (DHAP) as matrix. The conjugate loading efficiency for CRM197-**1**, CRM197-**2**, CRM197-**3** and CRM197-**10** were 10, 11, 7, and 11 glycans per molecule of CRM respectively.

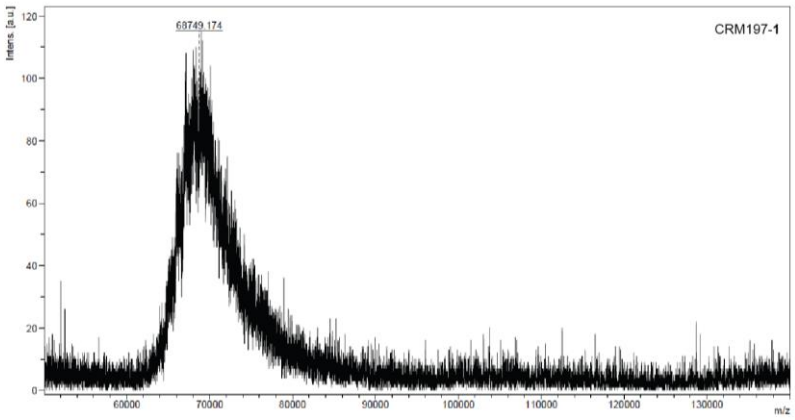
The conjugates were prepared in 6×SDS-PAGE sample loading dye and was resolved on 10% SDS-PAGE. The electrophoresis was carried out at 120 V and 25 mA for 90 min in electrode buffer and then the gel was soaked in PageBlue protein staining solution.

A



B

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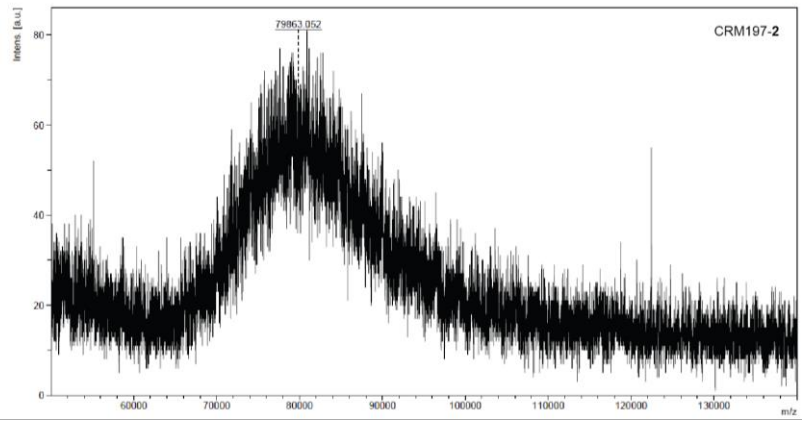
Intens. [a.u.]
m/z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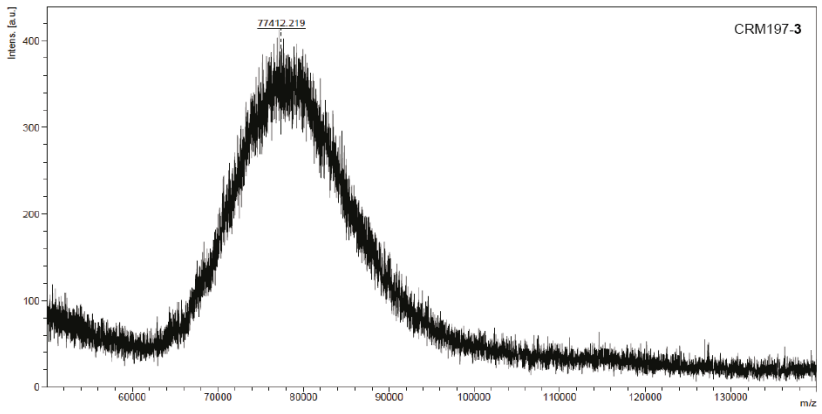
m/z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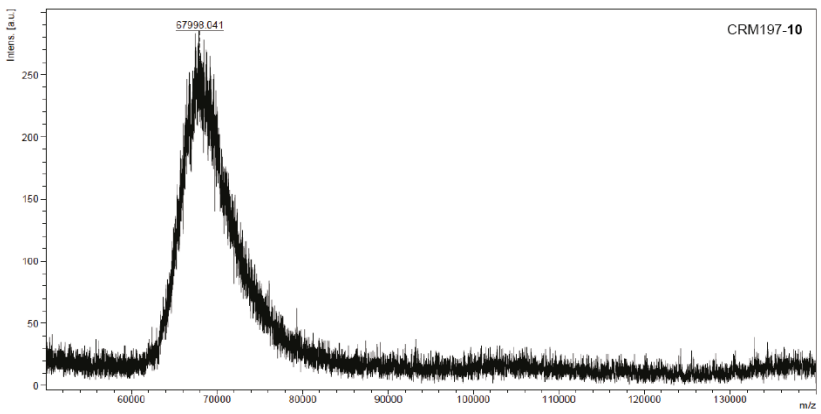
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Intens. [a.u.]
m/z

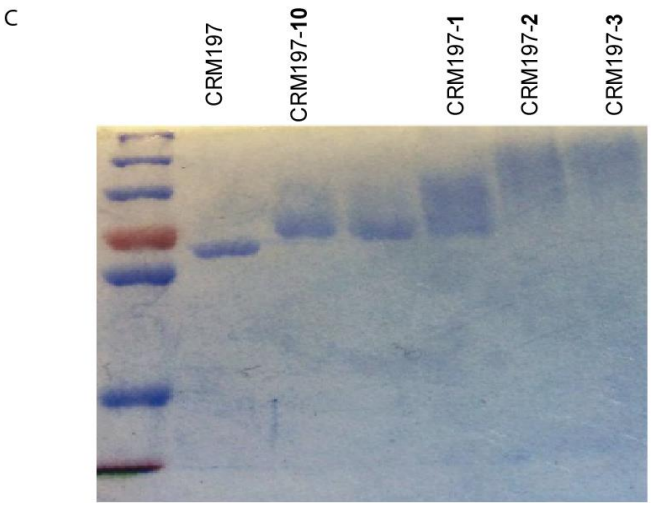


Figure S2: (A) Preparation of glycoconjugates. (B) Characterization of the conjugates with MALDI-TOF and (C) SDS-PAGE.

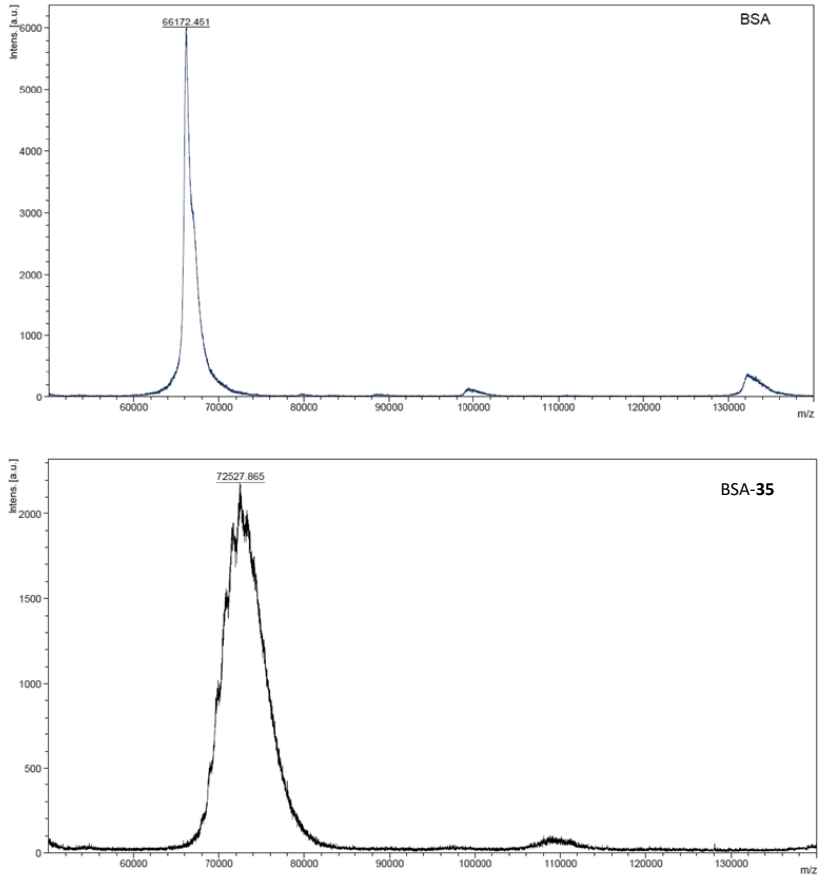


Figure S3: (A) Preparation of dummy glycoconjugates BSA-**35**. (B) Characterization of the conjugates with MALDI-TOF.

Immunization of mice

Four groups of five female 6-8 week-old C57BL/6 mice were immunized subcutaneously with each glycoconjugates (1 μg of oligosaccharide antigen per dose) adsorbed in 0.125mg of aluminum hydroxide (Brenntag, Mülheim, Germany). The control group was composed of three female 6-8 week-old C57BL/6 mice and received only PBS with aluminum hydroxide in the same dosage as the glycoconjugate group. On day 14 and 28, mice received a boost with the same formulation. Mice were bled via tail weekly and sera were collected after centrifugation. The antibody titers were measured using ELISA and glycan microarrays.

ELISA analysis

High binding 96-well polystyrene microtiter plates (Corning, USA) were coated overnight at 4 ^{o}C with 10 μg/mL of ST14 CPS in PBS, PH 7.2 (50 μL per well). The plates were washed three times with PBS-T and blocked with 1% BSA in PBS for 1 h at room temperature. After wash with PBS, the plate was incubated with each individual mouse serum at different dilutions (in order to find the optimal sera dilution without saturation) in triplicate for 1 h at room temperature. The plates were washed three times with PBS-T and incubated with horseradish peroxidase (HRP) conjugated goat anti-mouse IgG antibodies (Sigma-Aldrich, USA), then washed thoroughly with PBS-T. The absorbance was recorded at 450 nm using ELISA reader (Infinite® 200 NanoQuant, Tecan, Switzerland). The data analysis was performed with 1:100 sera dilution because no saturation in the absorbance measurement was detected. The same dilution was maintained in further analysis for comparison.

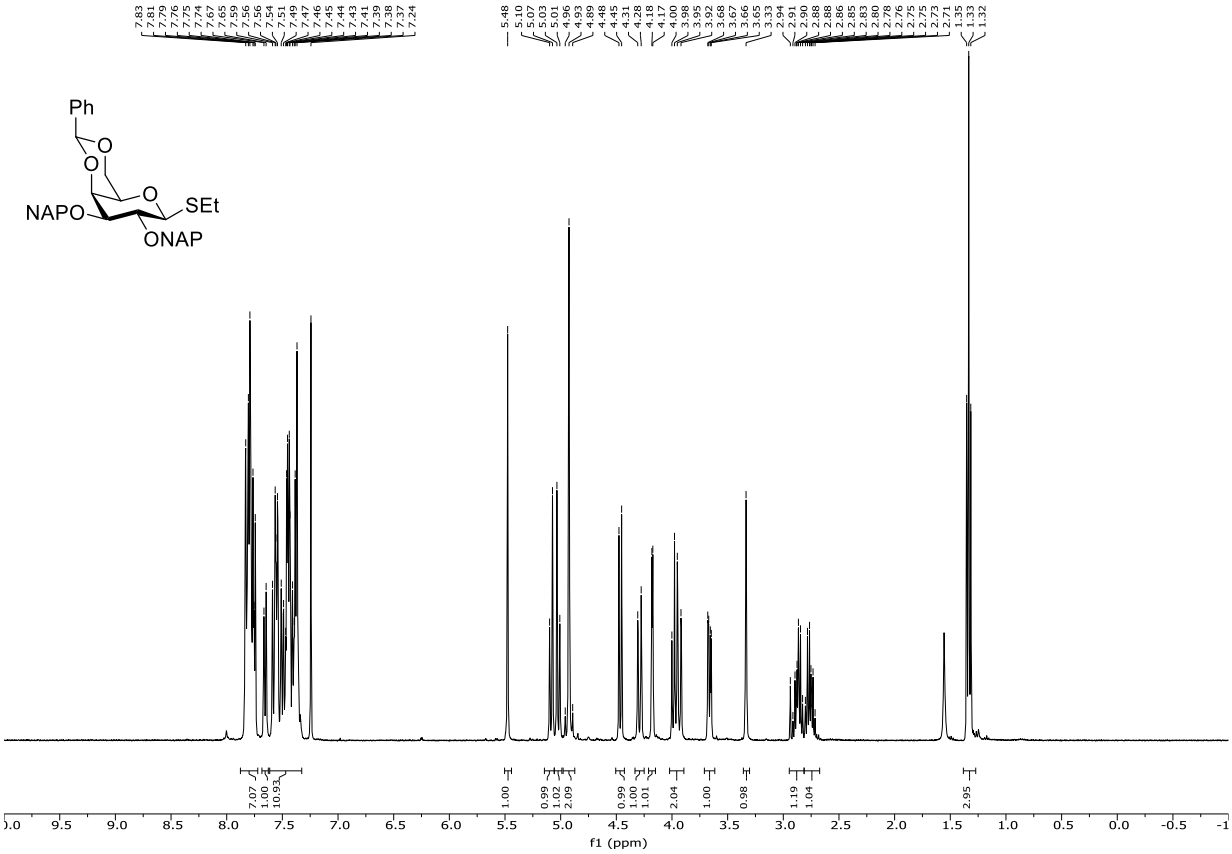
***In vitro* opsonophagocytic killing assay (OPKA)**

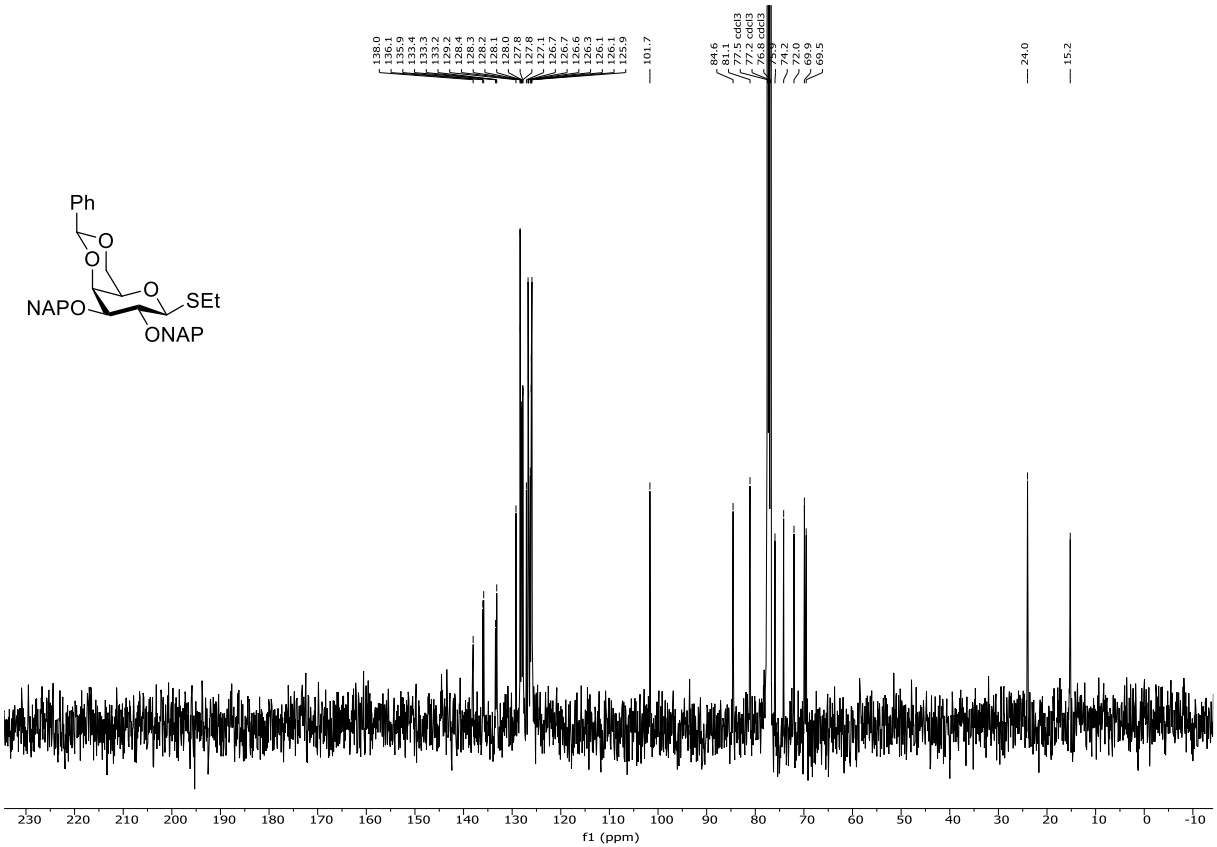
The assay was performed as described previously.[16] Concisely, HL-60 cell line was used as phagocytic cell. For granulocyte differentiation, approximately 4×10^5 cells/mL were seeded in tissue culture flasks (Corning, N.Y.) in complete medium (90% RPMI 1640, 10% FCS, 1 mM L-glutamine and penicillin-streptomycin solution; PAN Biotech, Germany) containing 0.8% N,N-dimethylformamide (DMF; 99.8% purity; Fisher Scientific, Fair Lawn, N.J.) for 5-6 days at 37°C in the presence of 5% CO_2. Cells were harvested by centrifugation (300 × g, 5 min) and counted by using 1% trypan blue exclusion and resuspended in opsonophagocytic buffer (HBSS with $Ca^{2+}$and Mg^{2+}, 0.1% gelatin, and 10% FBS; HyClone) at a final density of 1 x 10^7 cells/mL. A ratio of 400:1 effector to target cells was used in the assay. Glycerol stock of *S. pneumoniae* serotype 14 grown to mid-log phase (OD600= 0.25–0.30) were gently thawed

and diluted in opsonophagocytic buffer to a final density of 1000 CFU per 20 μL. Pooled (5 mice per group) heated inactivated (56°C, 30 min) mice sera (10 μL) from day 35 were aliquoted in round bottom non-treated 96-well plates in duplcicates at four-fold dilution intervals. A total of 20 μL of bacterial suspension was added to each well and incubated for 15 min at 37°C. Following, 10 μL of baby rabbit complement (10% (v/v), rabbit complement, Cedarlane) and 4×10^5 differentiated HL-60 cells (in 40 μL) were added to each well. The plates were incubated for 45 min at 37°C in 5% CO_2 with intermittent shaking. The phagocytic reaction was stopped by putting the plate on ice for 15 min. Viable extracellular pneumococci were determined by plating aliquots (5 μL) from each well on Columbia Agar plates with 5% (v/v) sheep blood and incubating at 37°C in 5% CO_2. CFUs were counted after overnight growth. Negative control containing only bacteria, complement, HL-60 cells, and buffer was used to calculate the percent killing of pneumococci. The assay was repeated two times independently. The percent killing was calculated as means ± SD of CFU. Opsonic index values where 50% bacterial killing occurs was obtained from four parameters logistic regression of individual opsonic curves.[17]

Part IV. NMR Spectra

Ethyl 4,6-*O*-benzylidene-2,3-di-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (S4)





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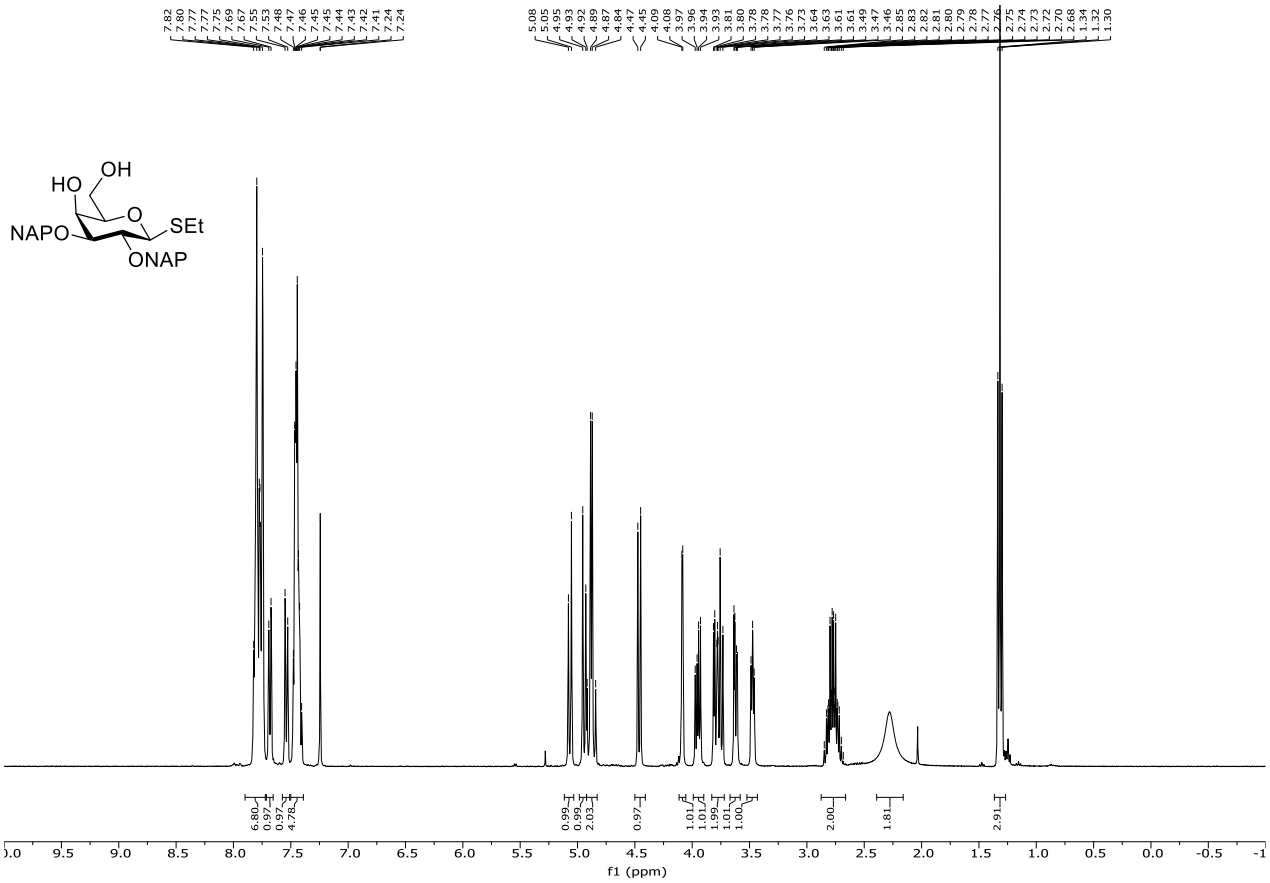

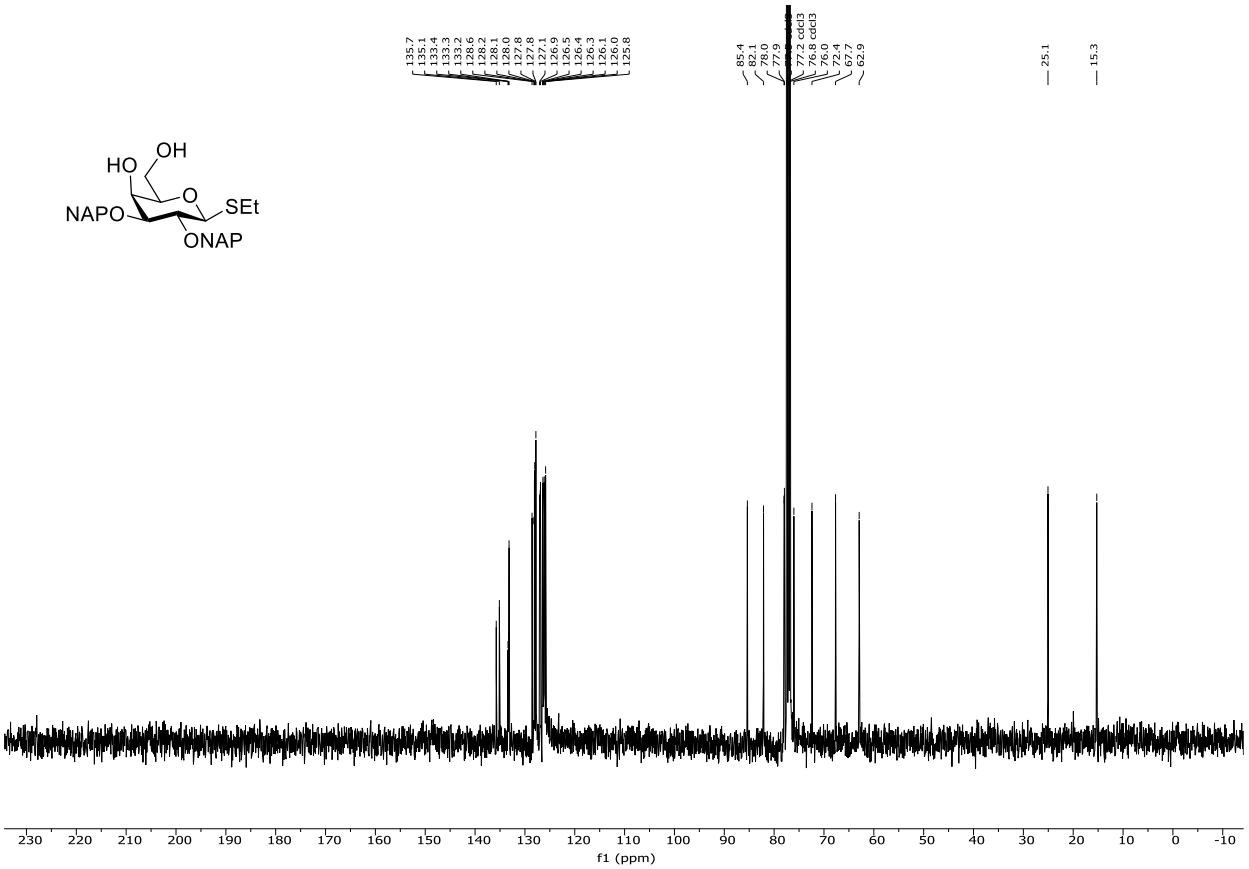

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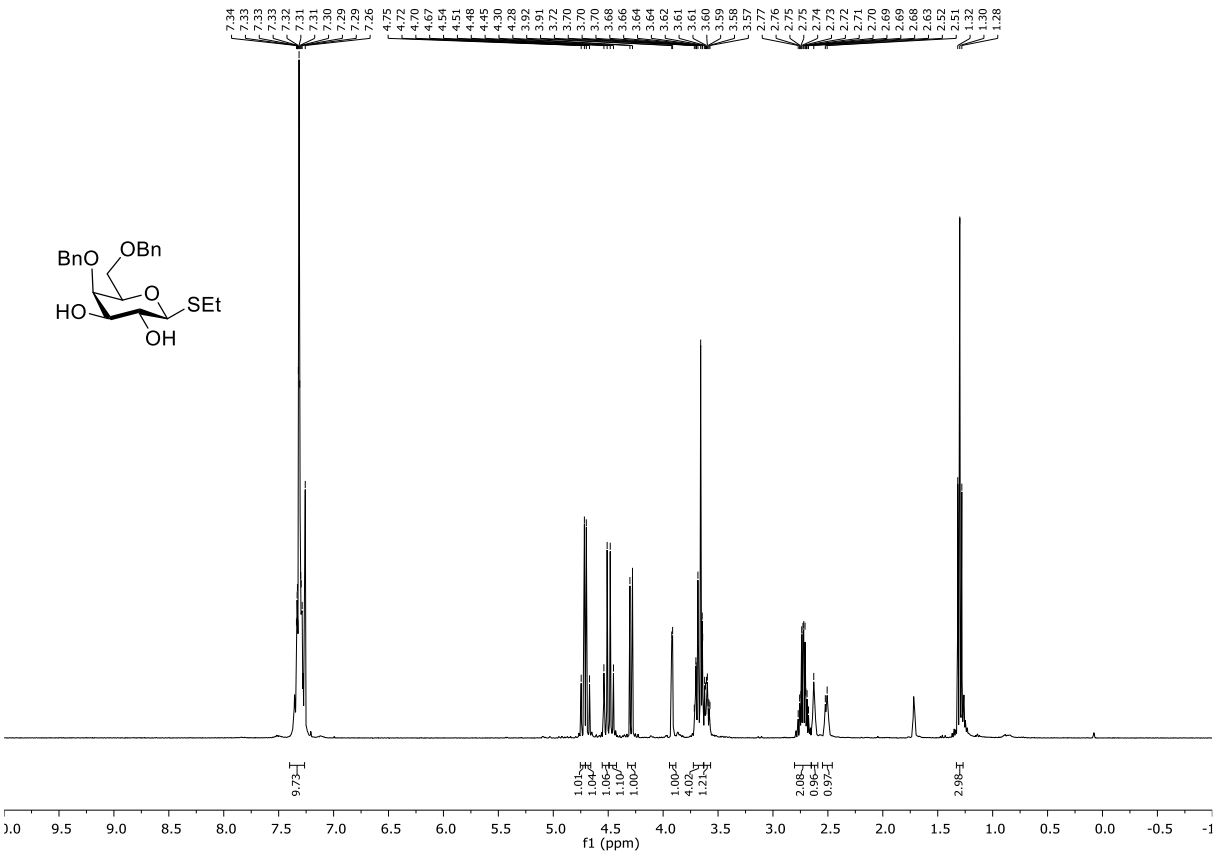

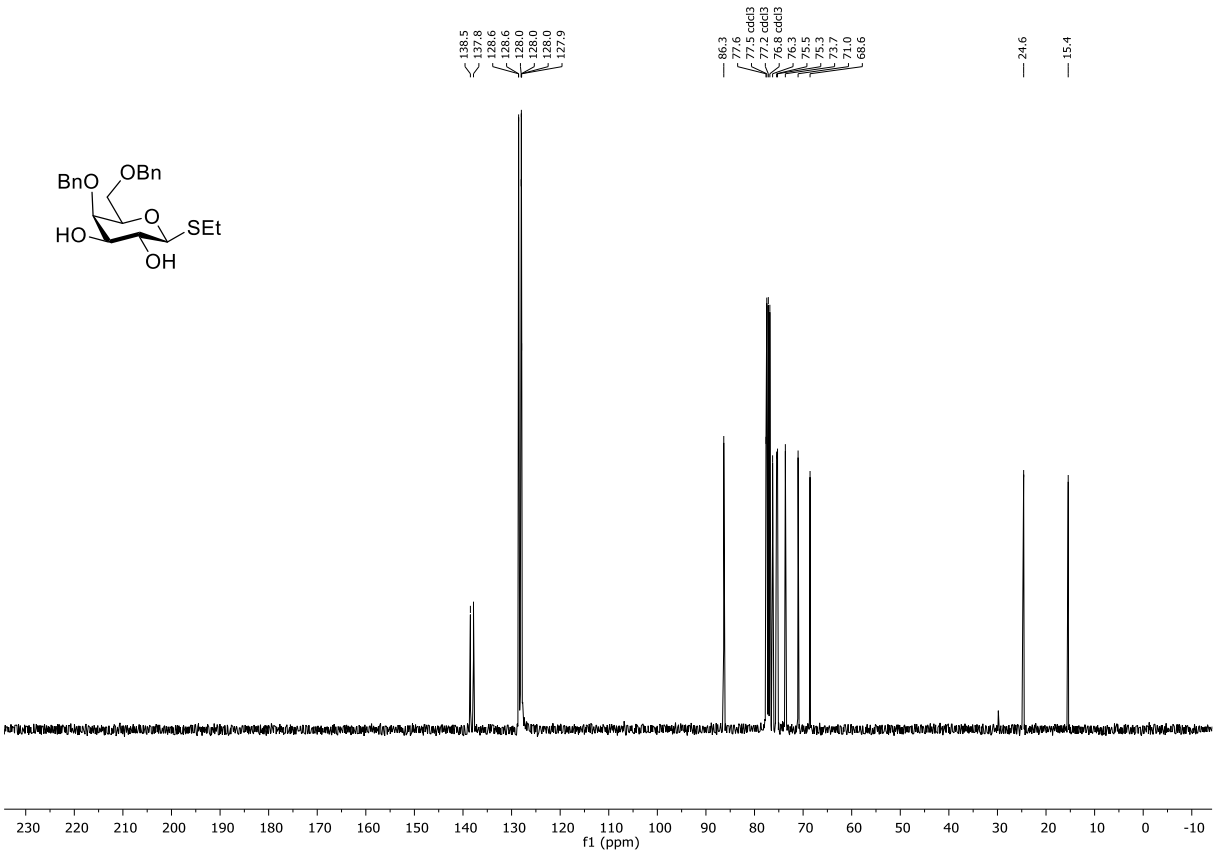

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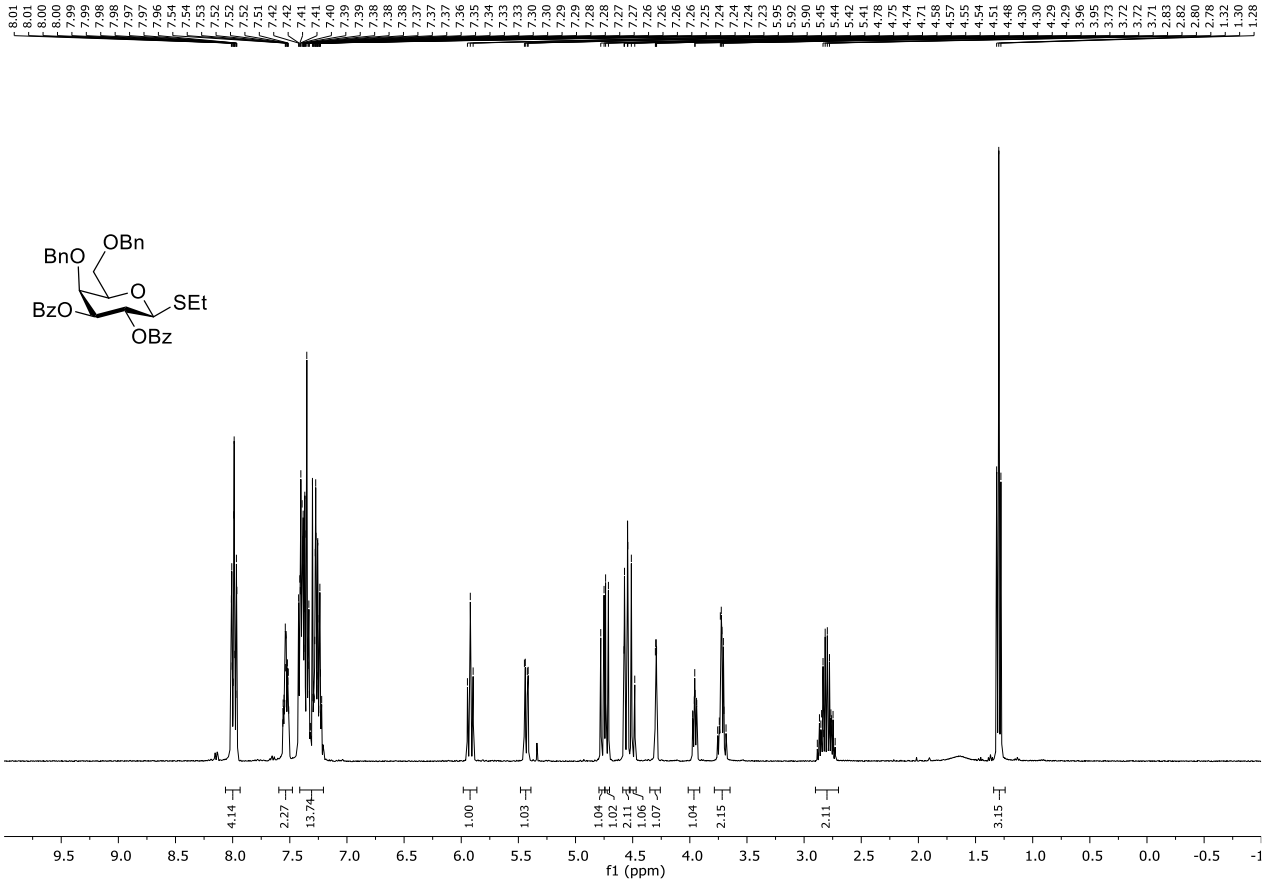

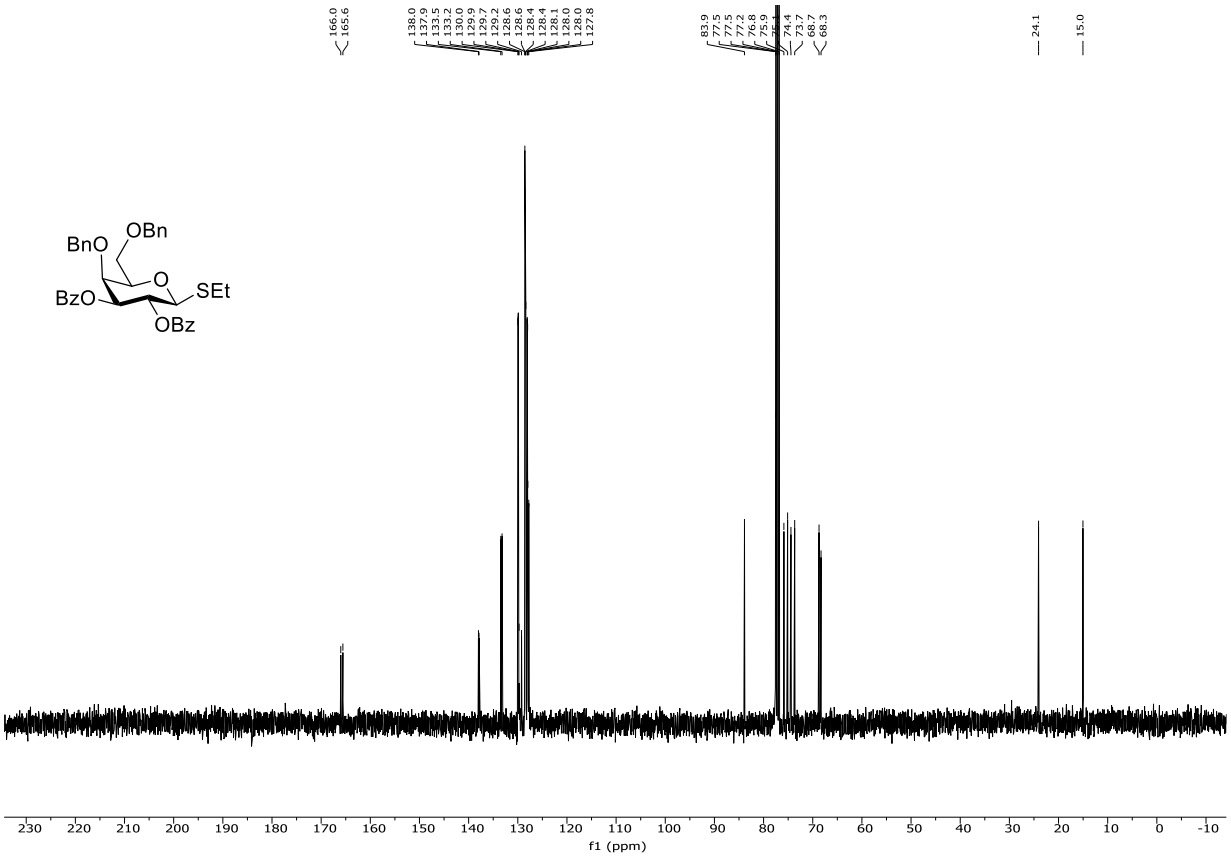

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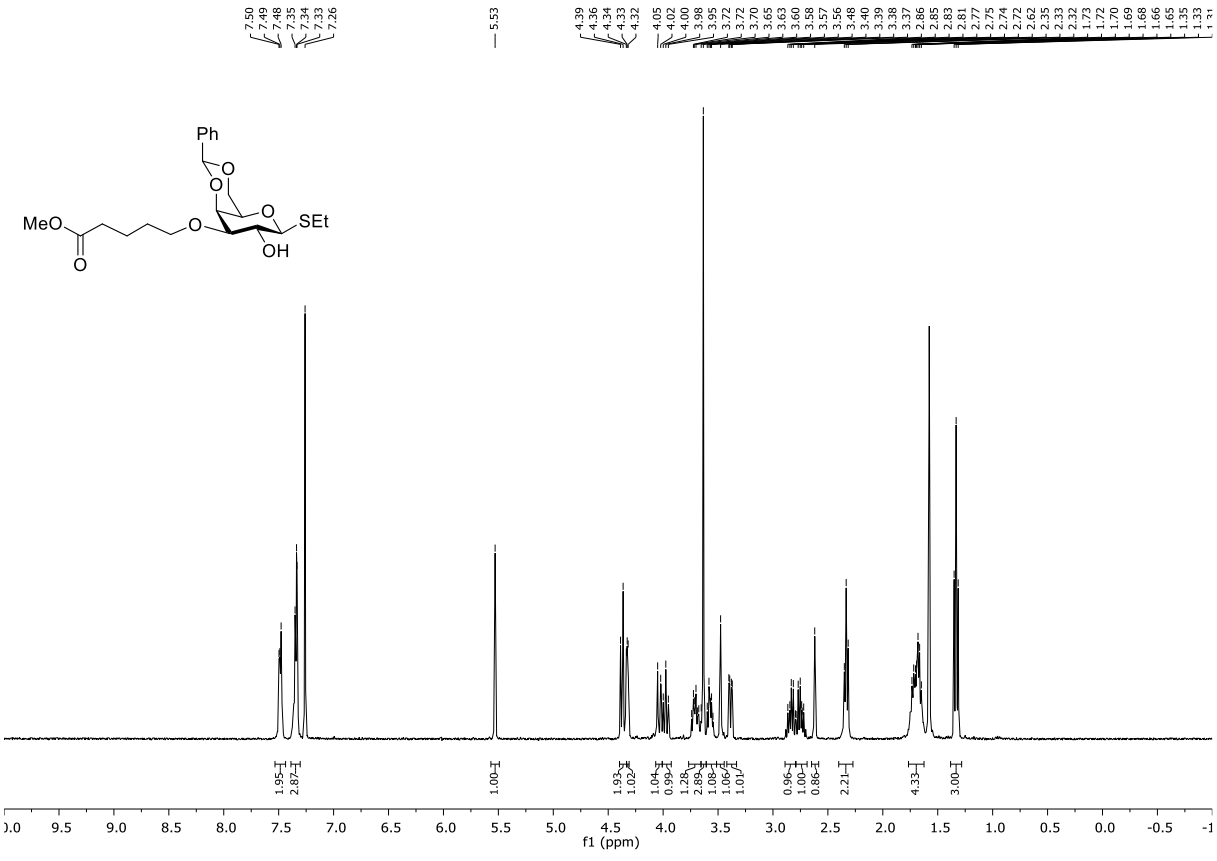

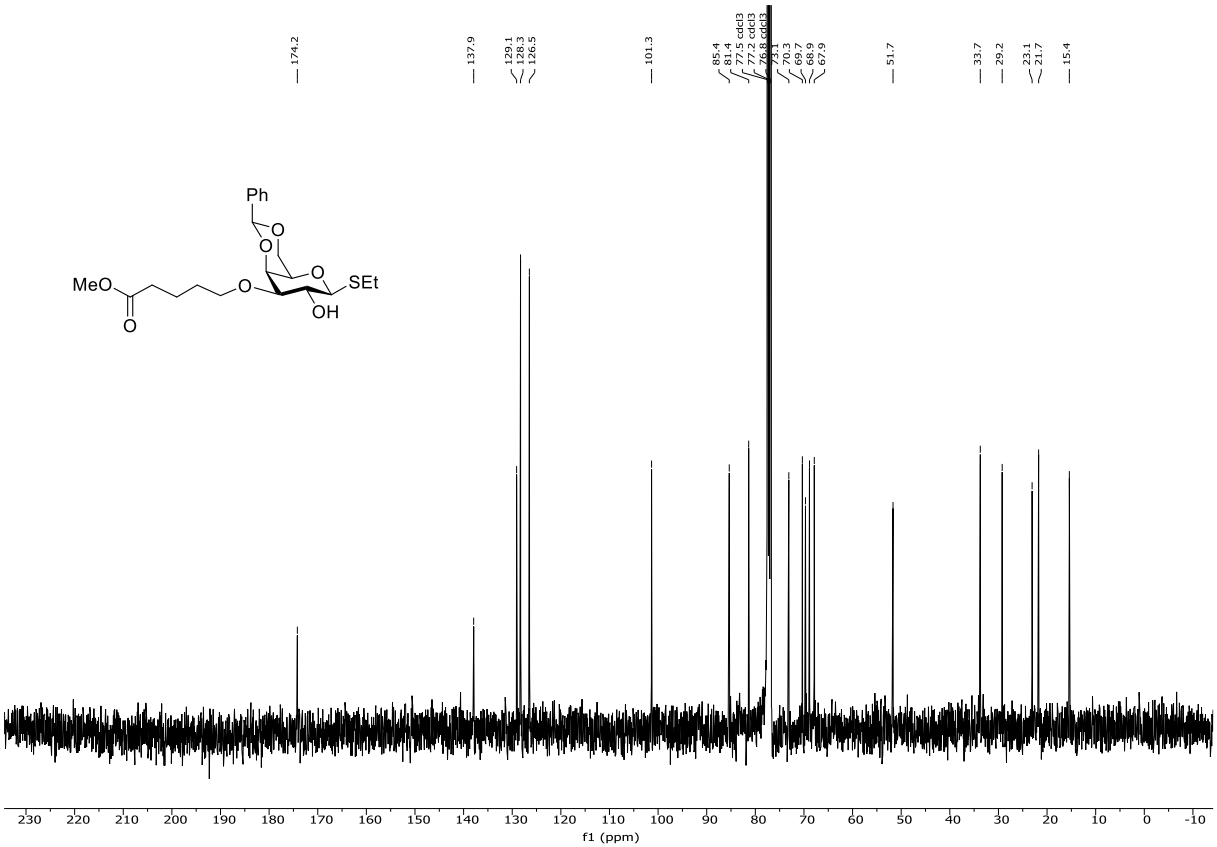

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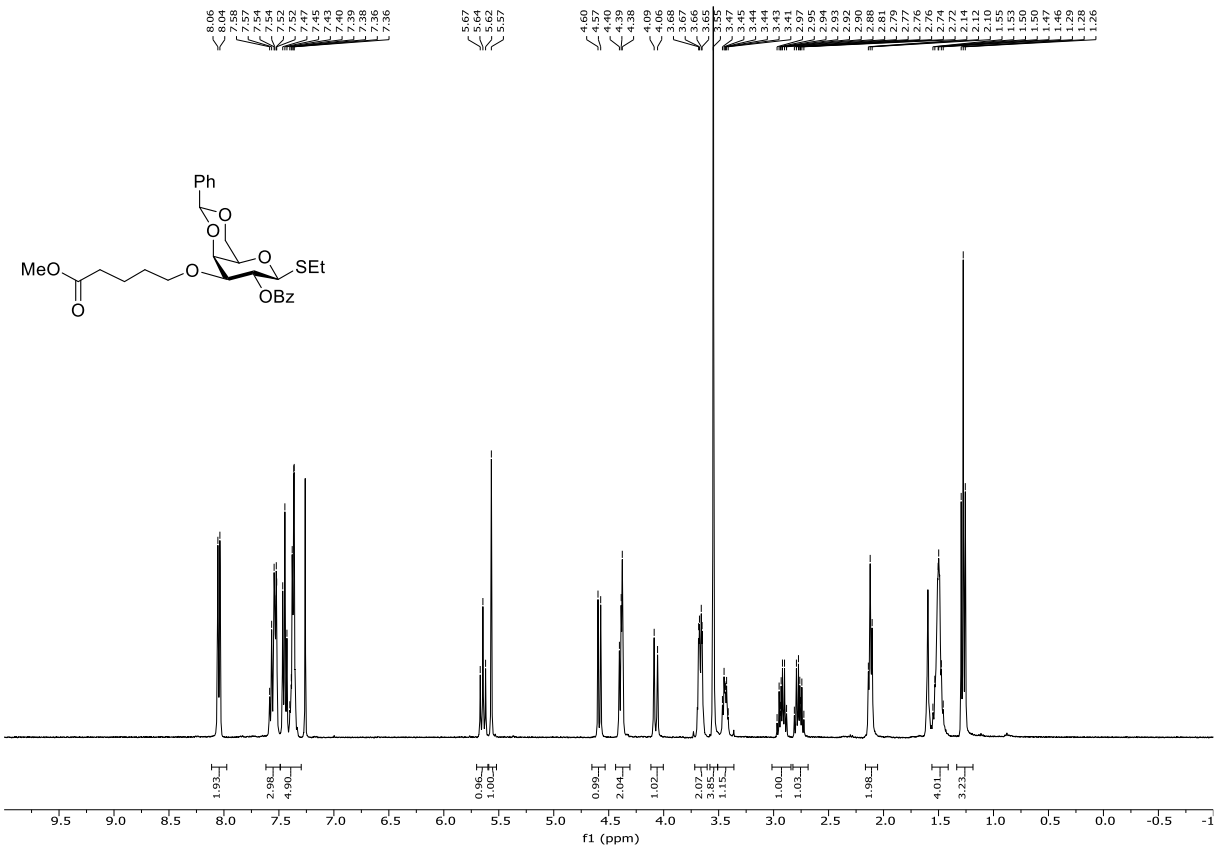

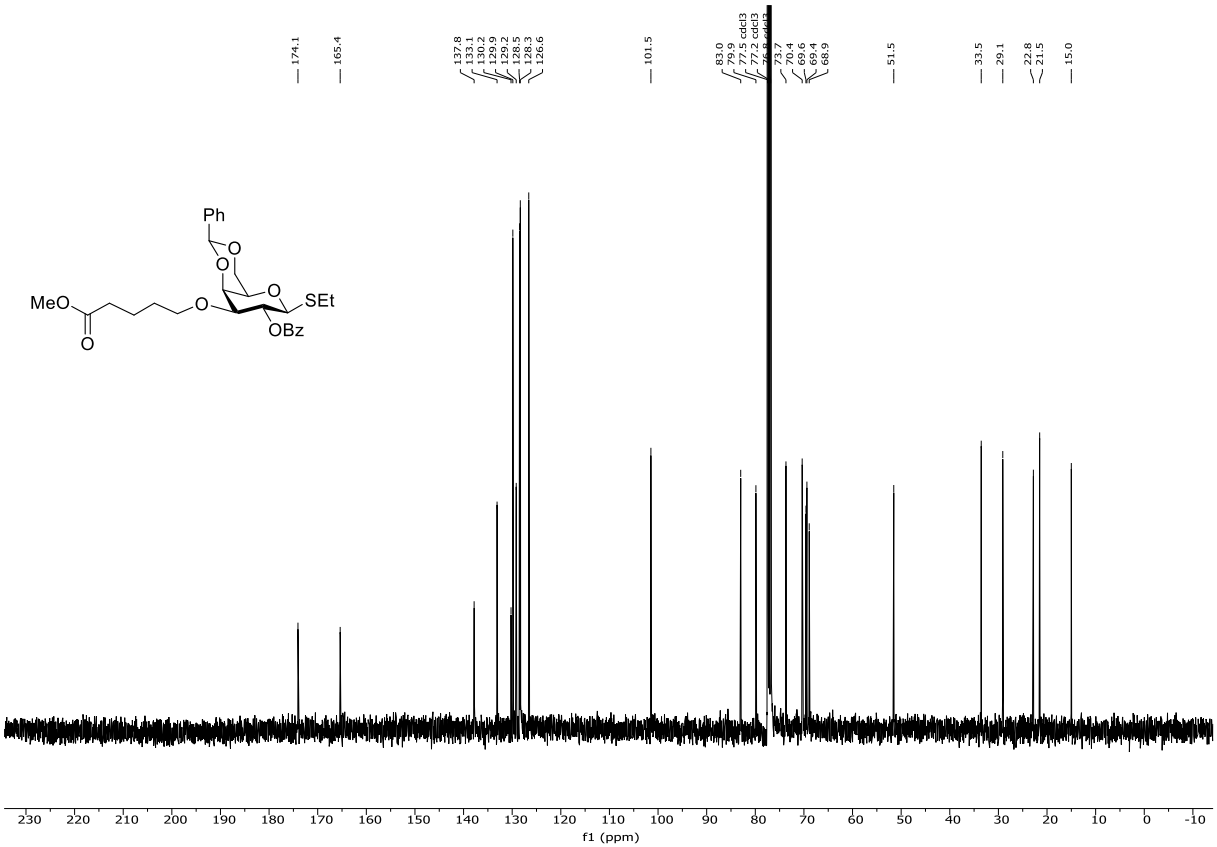

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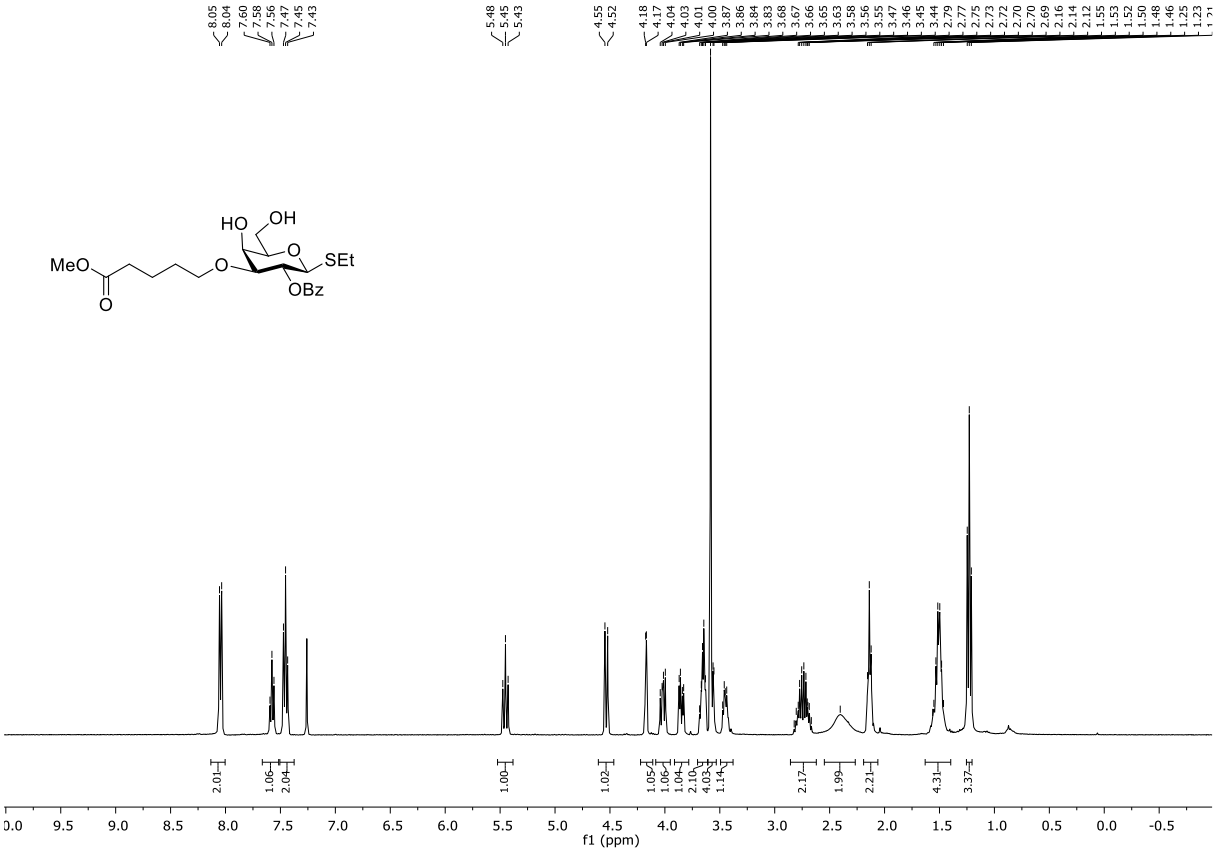
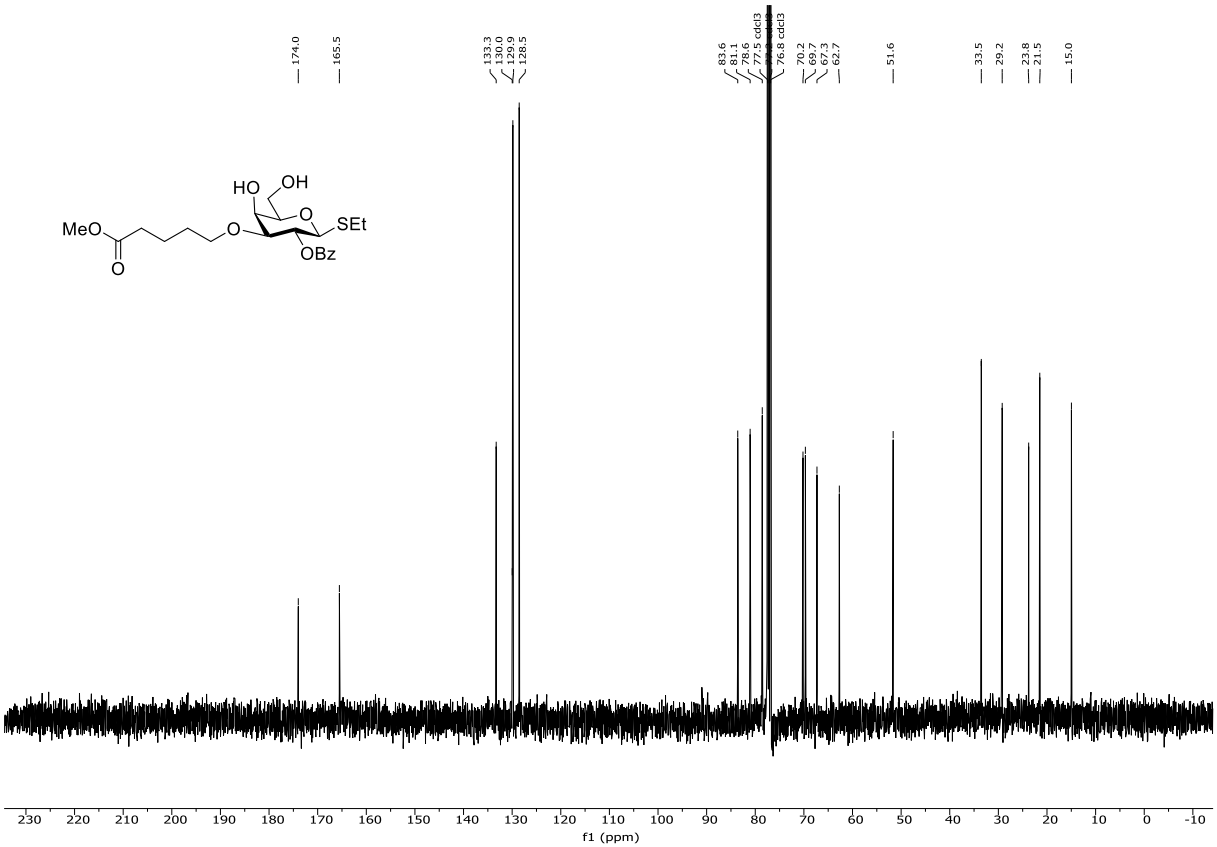
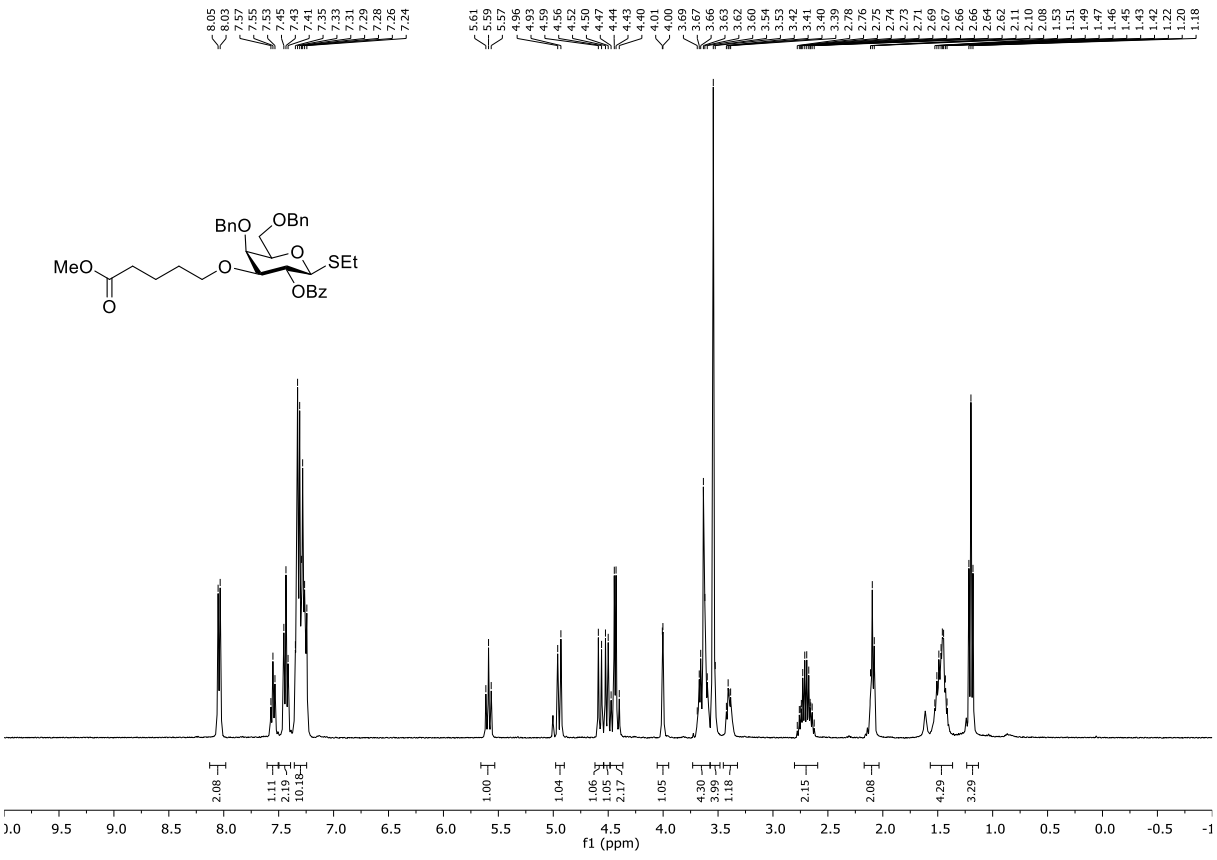
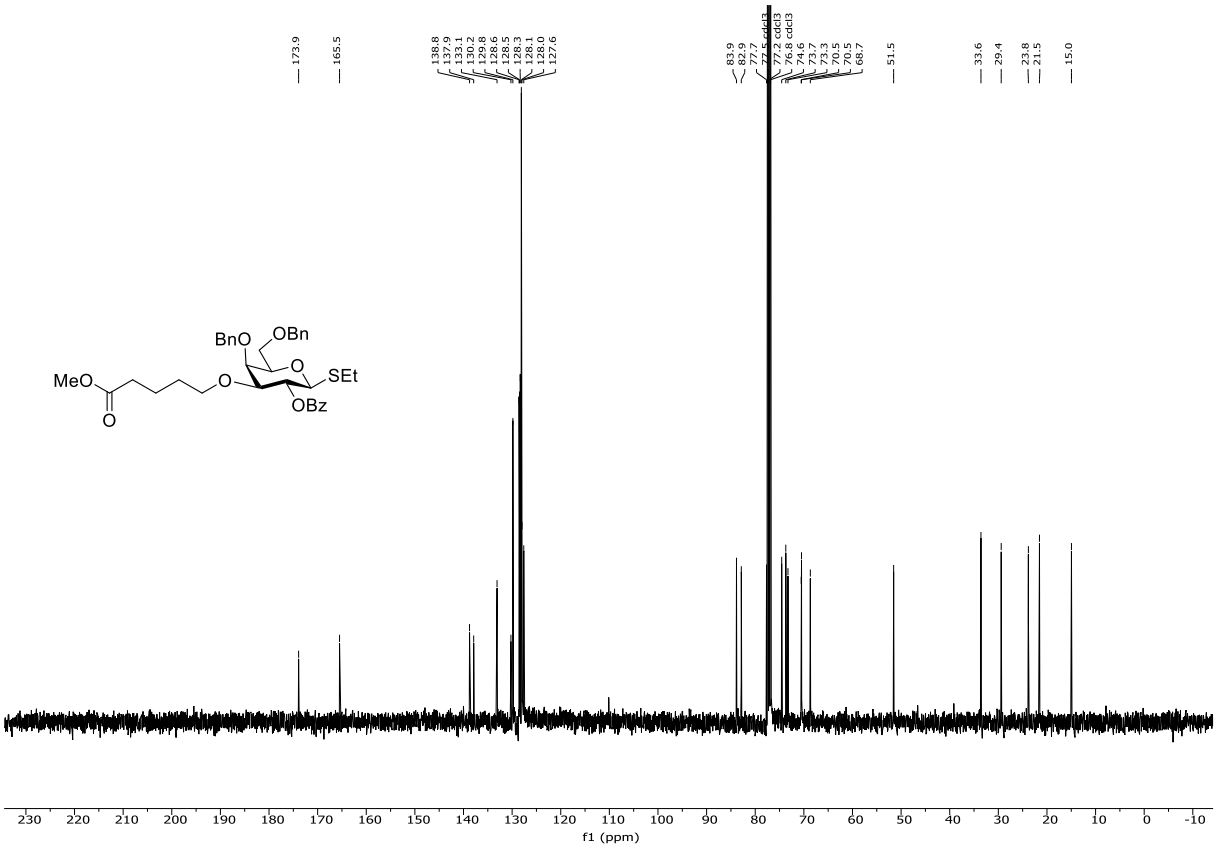


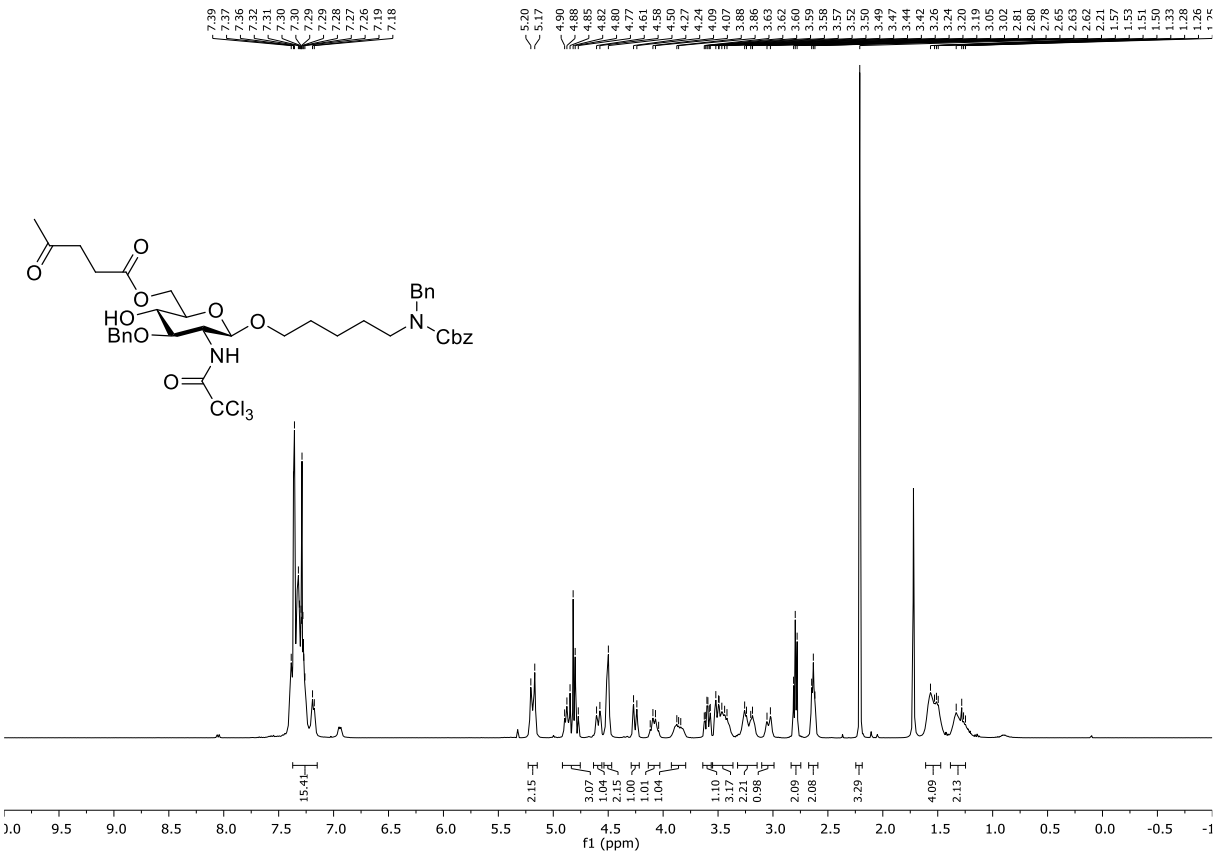


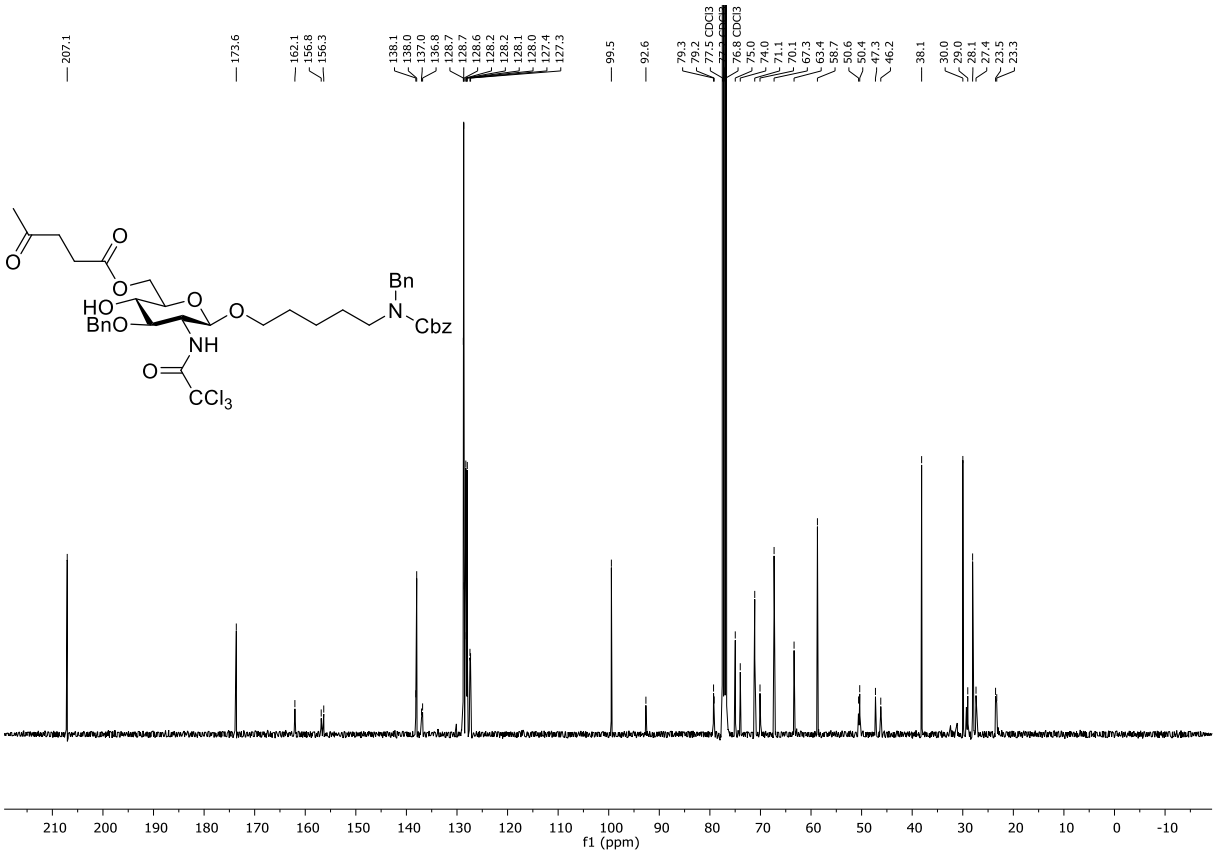
Ethyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-(methyl 5-pentanoate)-1-thio-β-D-galactopyranoside (9)



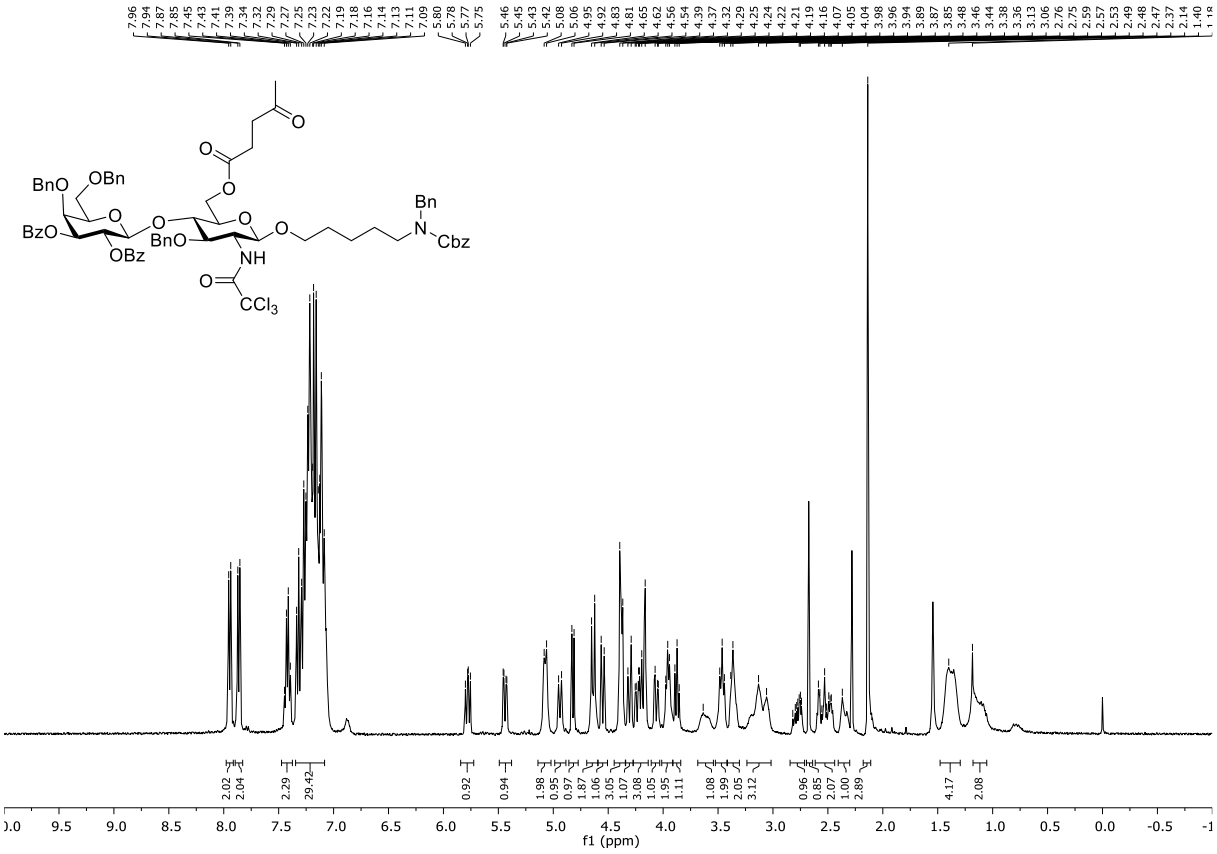


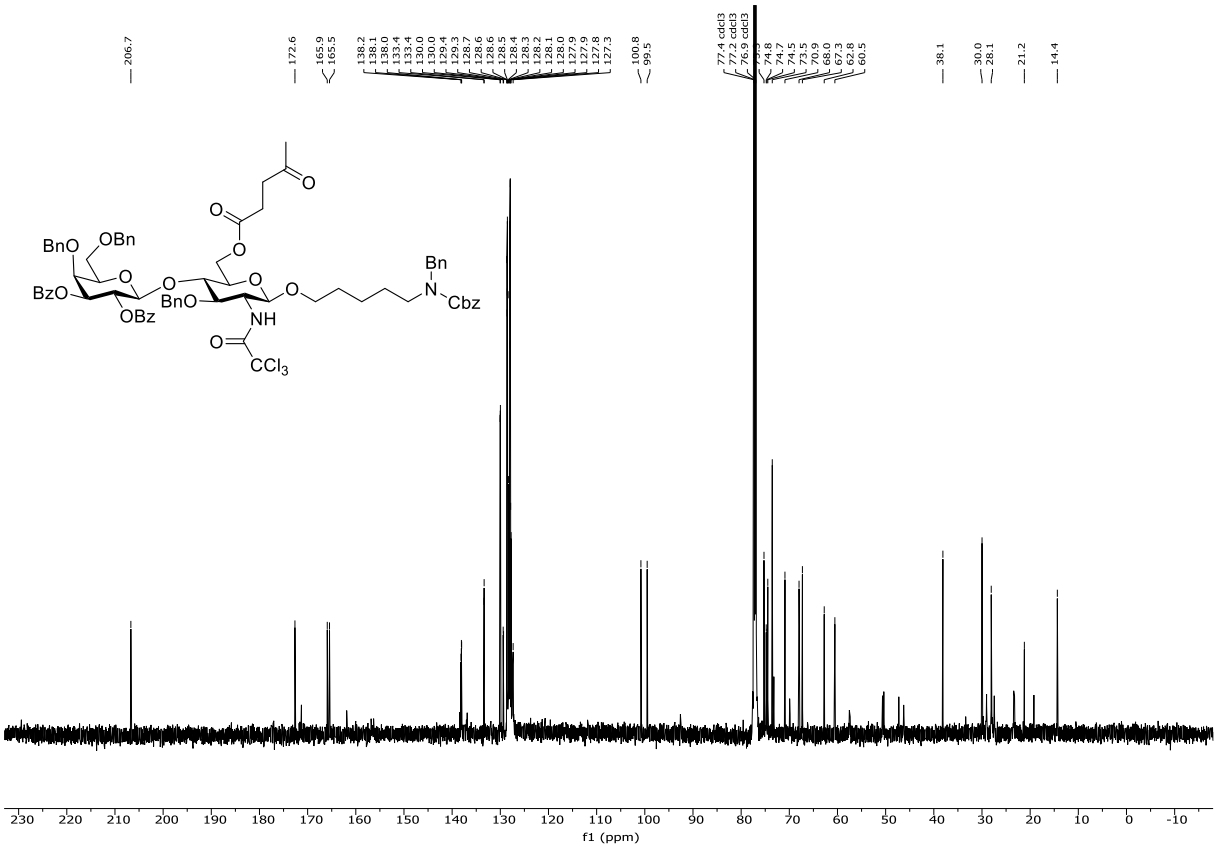
_N_-Benzyl-_N_-benzyloxycarbonyl-5-pentyl 3-_O_-benzyl-6-_O_-levulinyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (13)



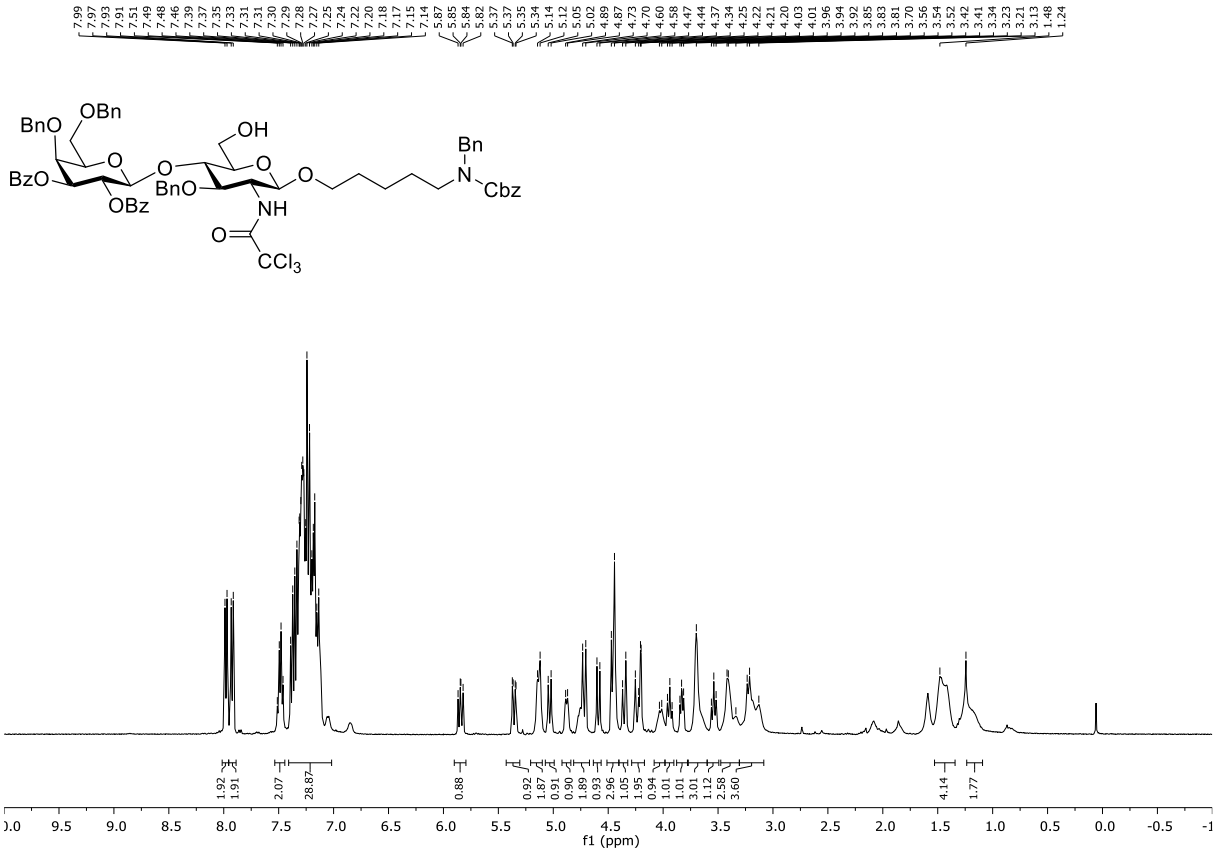


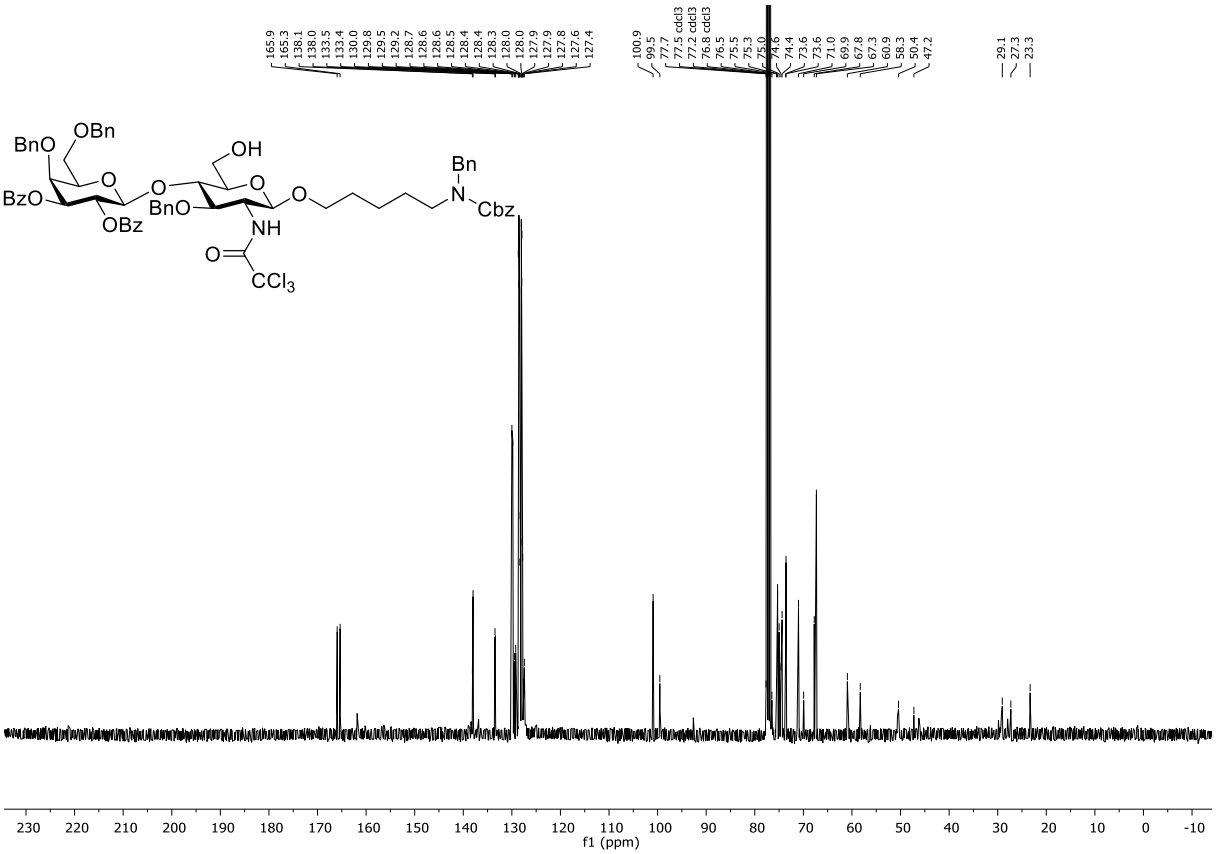
_N_-Benzyl-_N_-benzyloxycarbonyl-5-pentyl 2,3-di-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-6-_O_-levulinyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (14)





_N_-Benzyl-_N_-benzyloxycarbonyl-5-pentyl 2,3-di-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-3-_O_-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (15)

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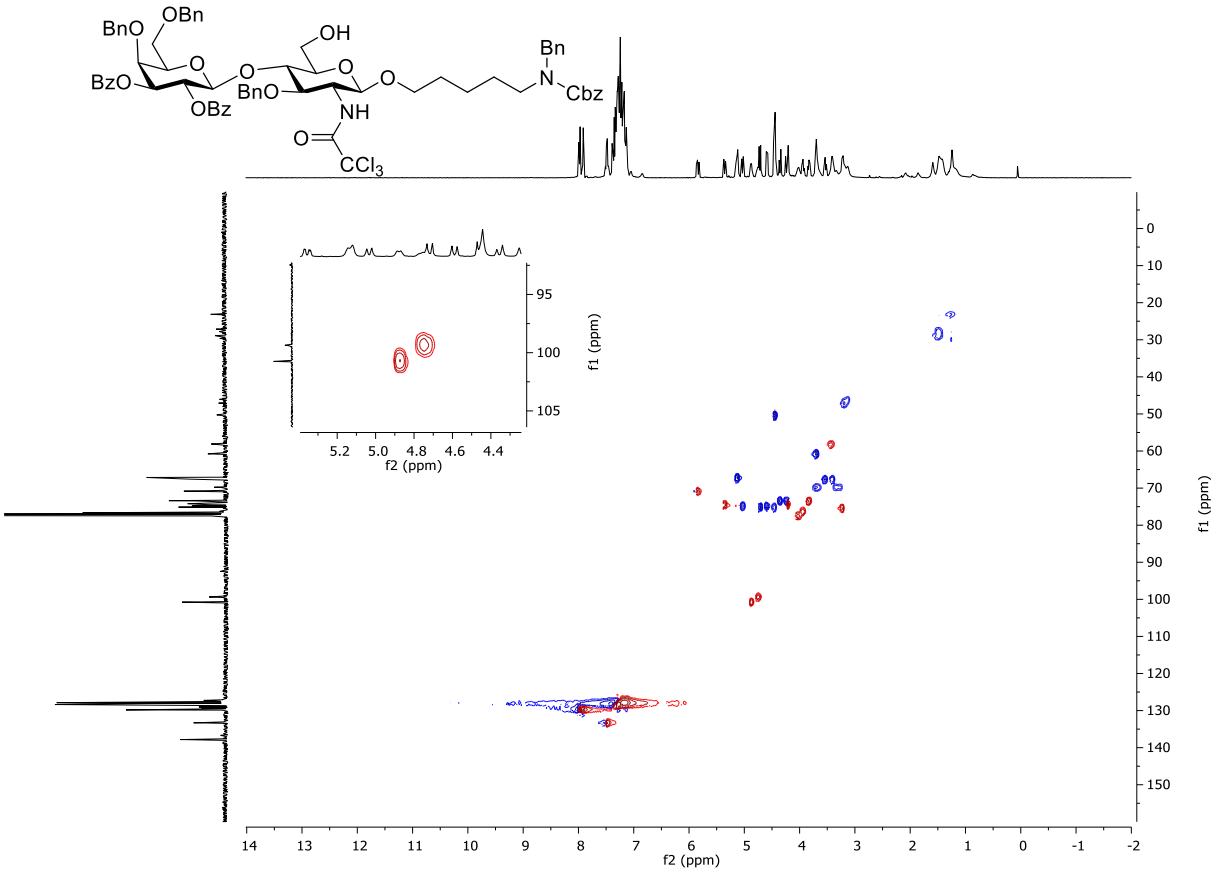
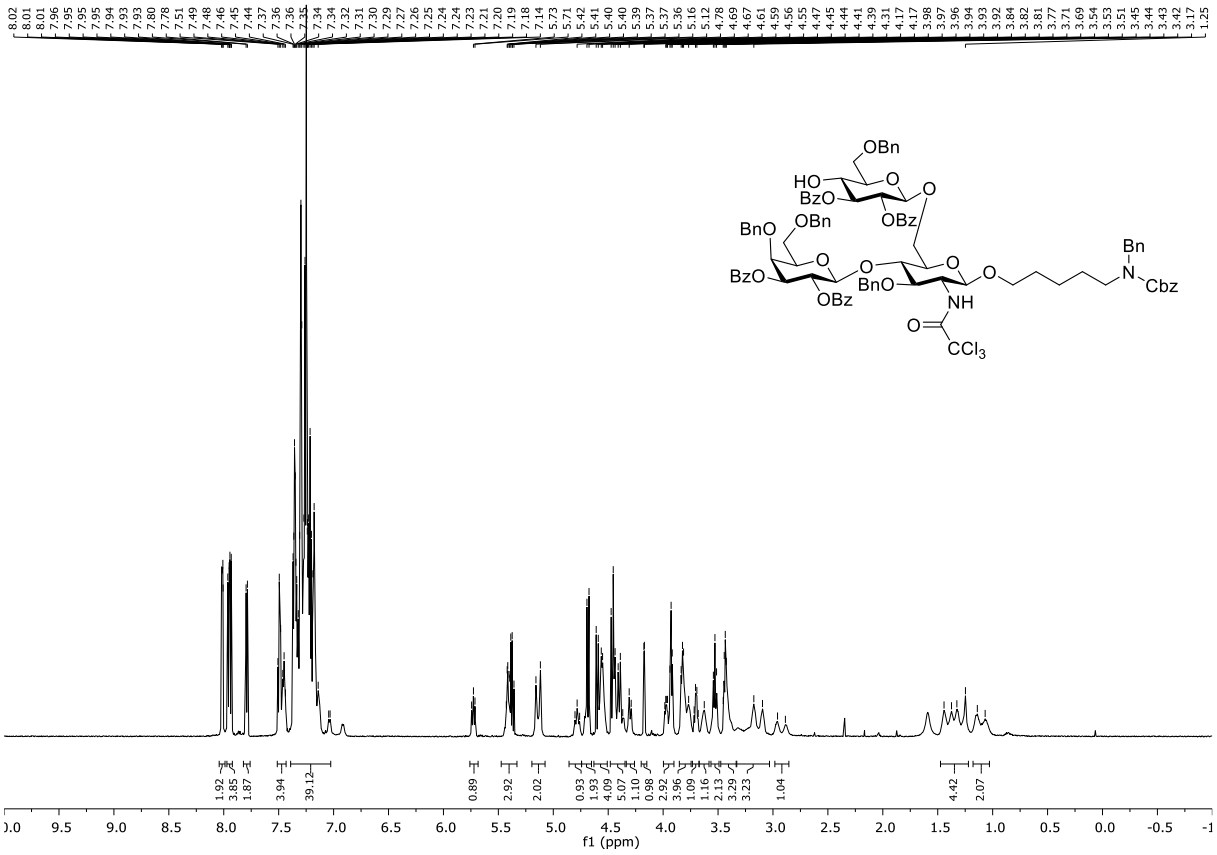
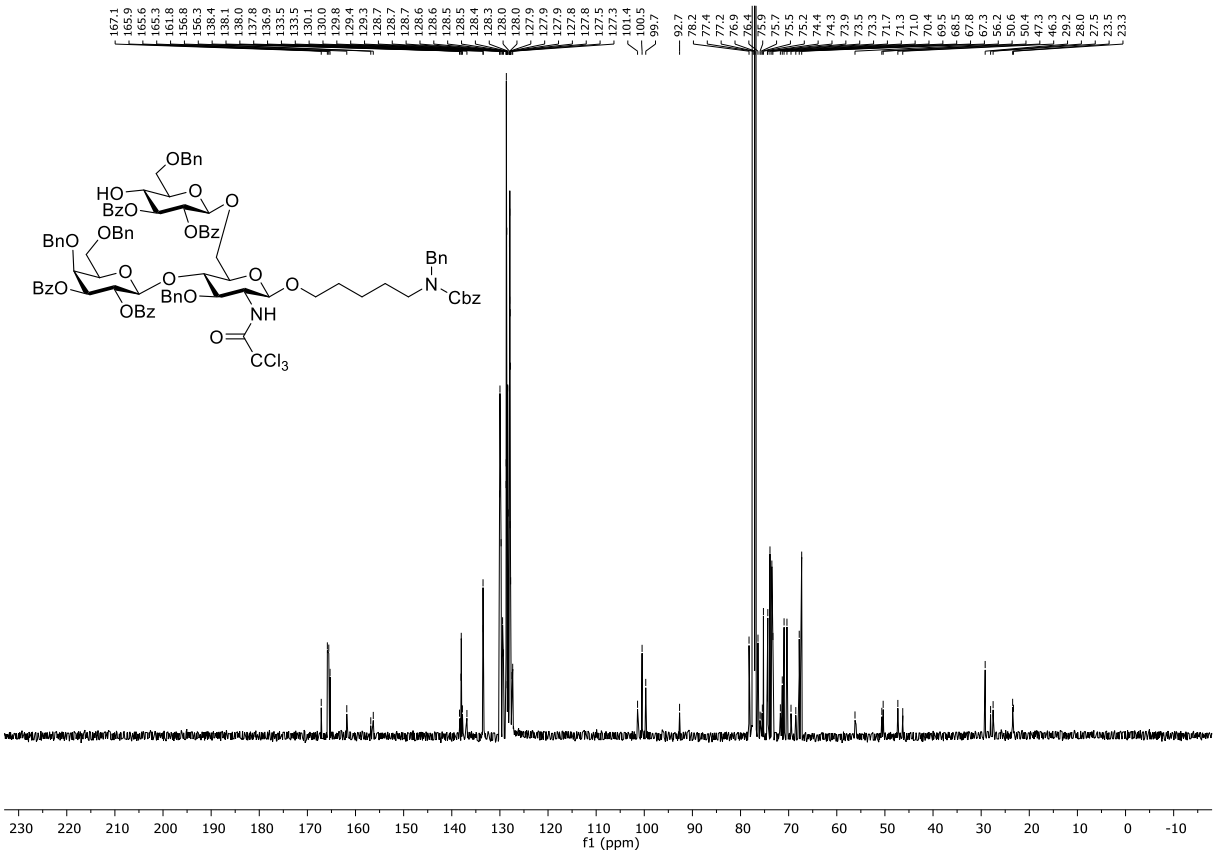
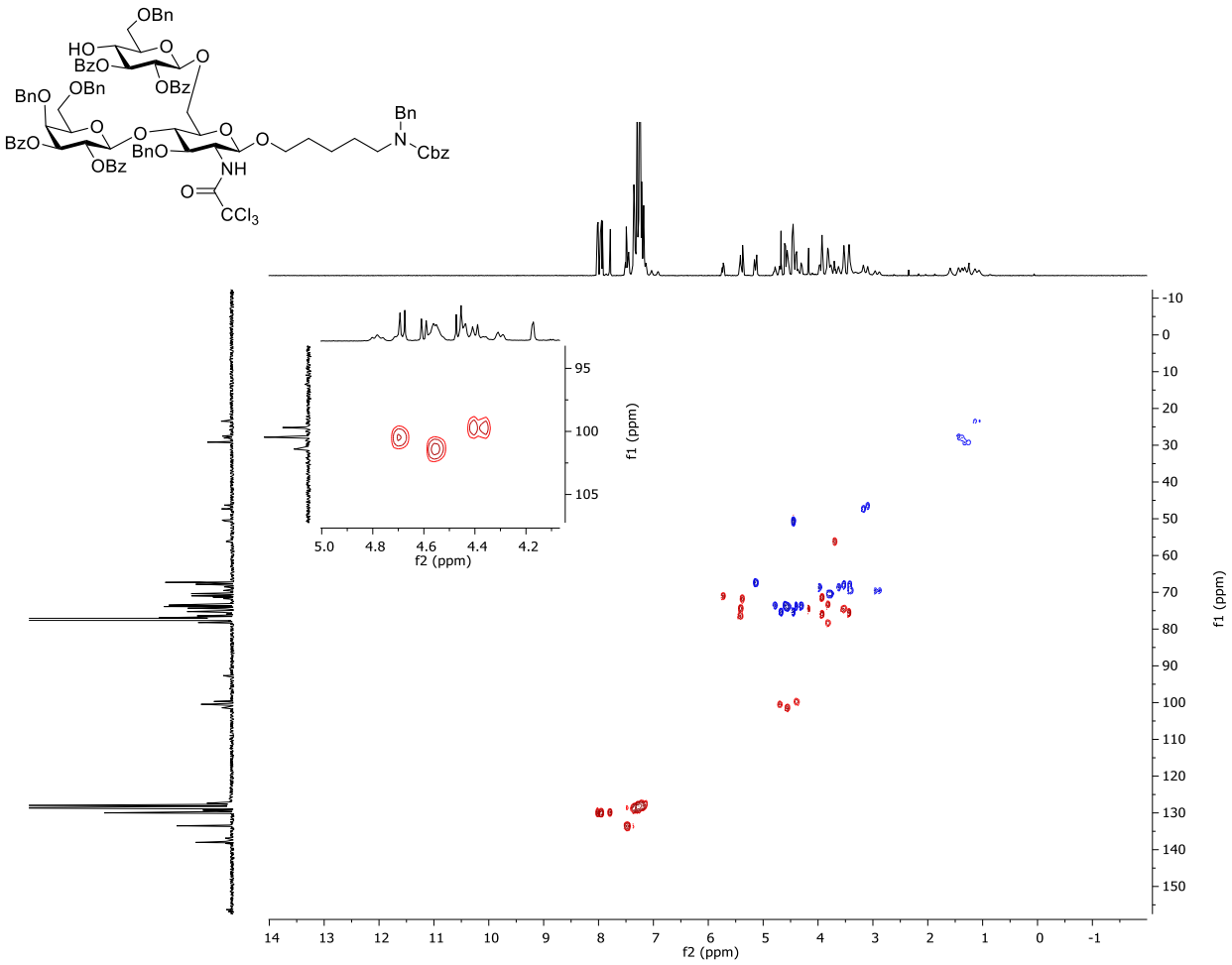
OBn
BzO
OBz
BnO
O
OH
NH
CCl3
Bn
N
Cbz
165.9 165.3 138.1 138.0 133.5 133.4 130.0 129.8 129.5 129.2 128.7 128.6 128.6 128.5 128.4 128.4 128.3 128.0 128.0 127.9 127.9 127.8 127.6 127.4
100.9 99.5 77.7 77.5 cdcl3 77.2 cdcl3 76.8 cdcl3 76.5 75.5 75.3 75.0 74.6 74.4 73.6 73.6 71.0 69.9 67.8 67.3 60.9 58.3 50.4 47.2
29.1 27.3 23.3
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)


BnO
OBn
BzO
OBz
BnO
O
OH
NH
CCl3
Bn
N
Cbz
95
100
105
5.2 5.0 4.8 4.6 4.4
f2 (ppm)
f1 (ppm)
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2
f2 (ppm)
f1 (ppm)

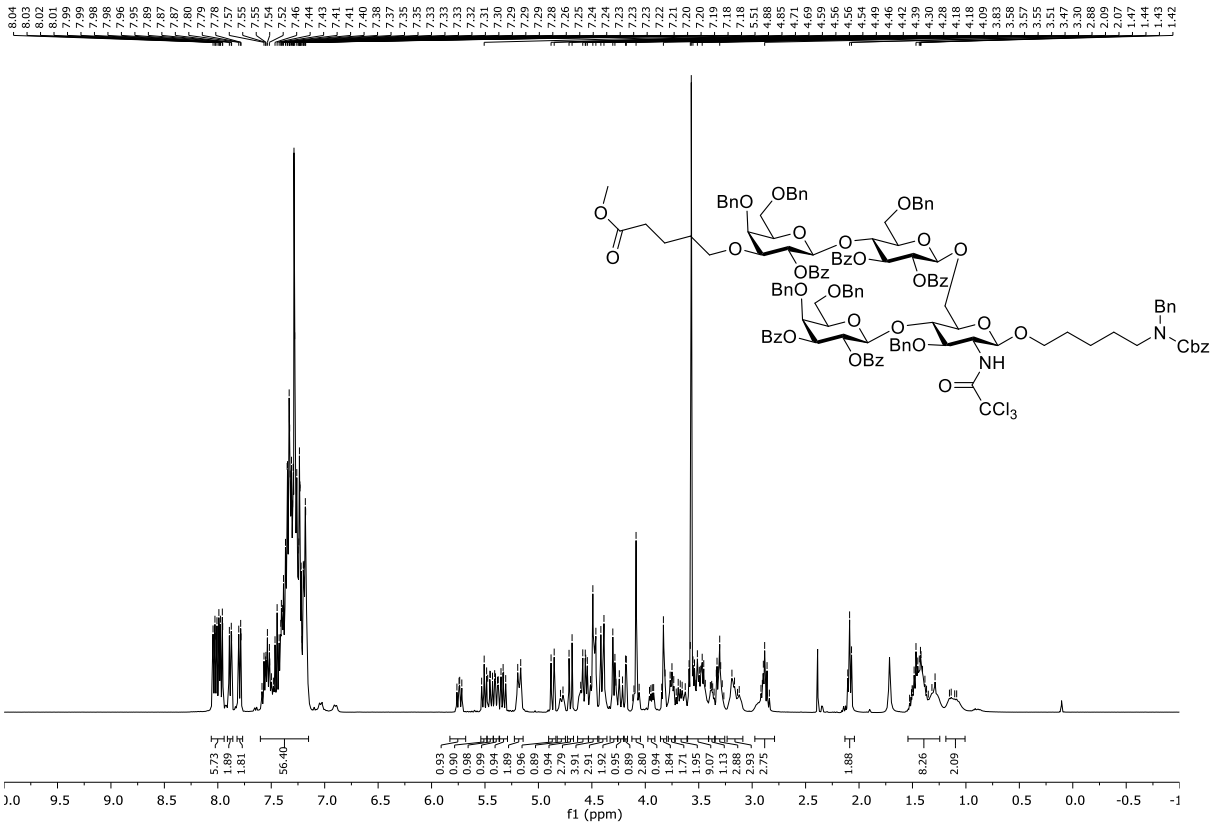
***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→6)-4-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (17)**

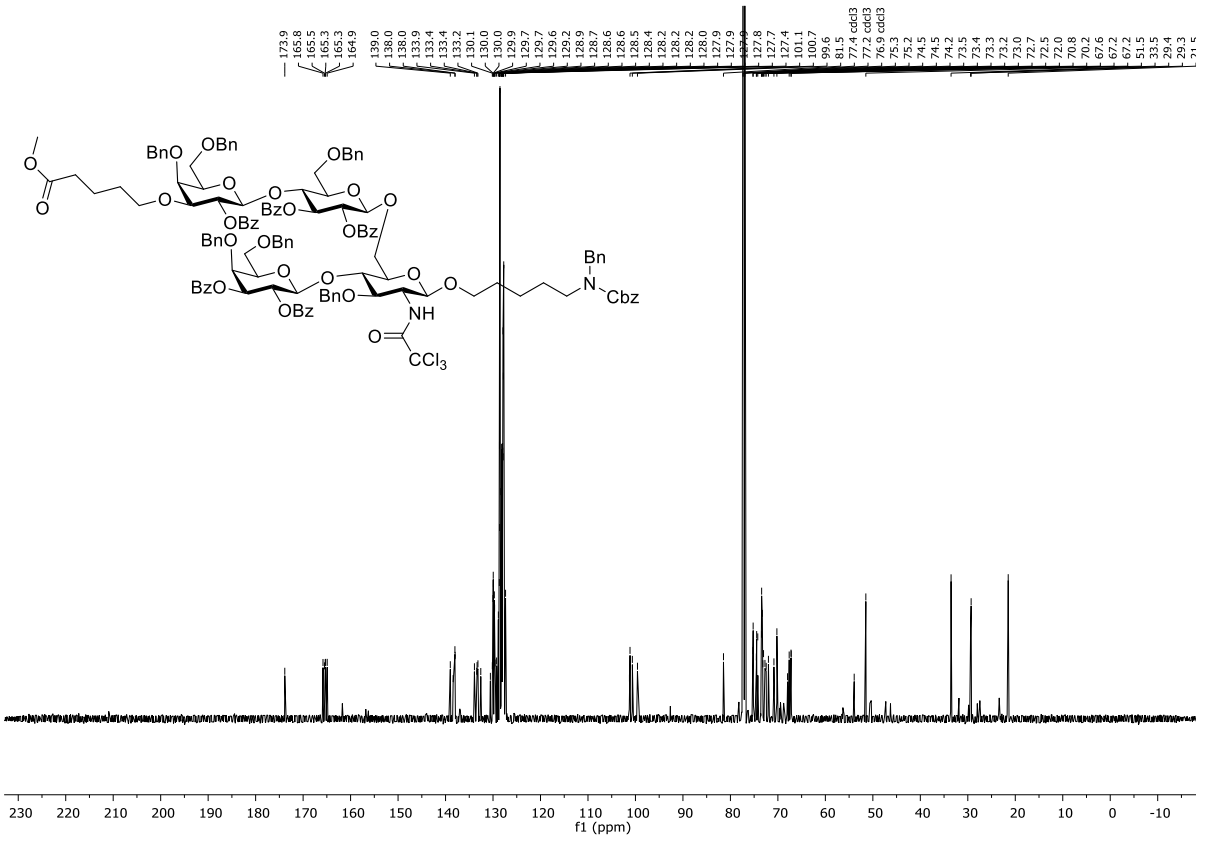


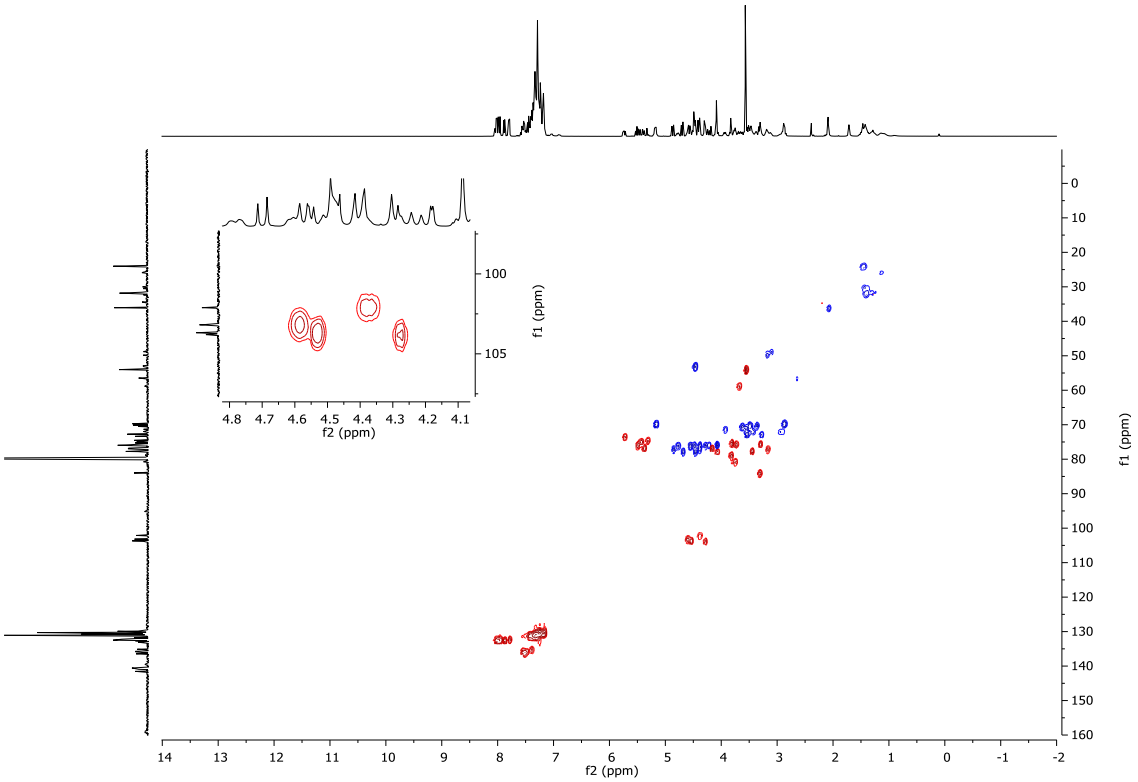



OBn
HO
BzO
OBz
BnO
OBn
BzO
OBz
BnO
NH
O
CCl3
Bn
N
Cbz
f1 (ppm)
f2 (ppm)
95
100
105
5.0
4.8
4.6
4.4
4.2
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150

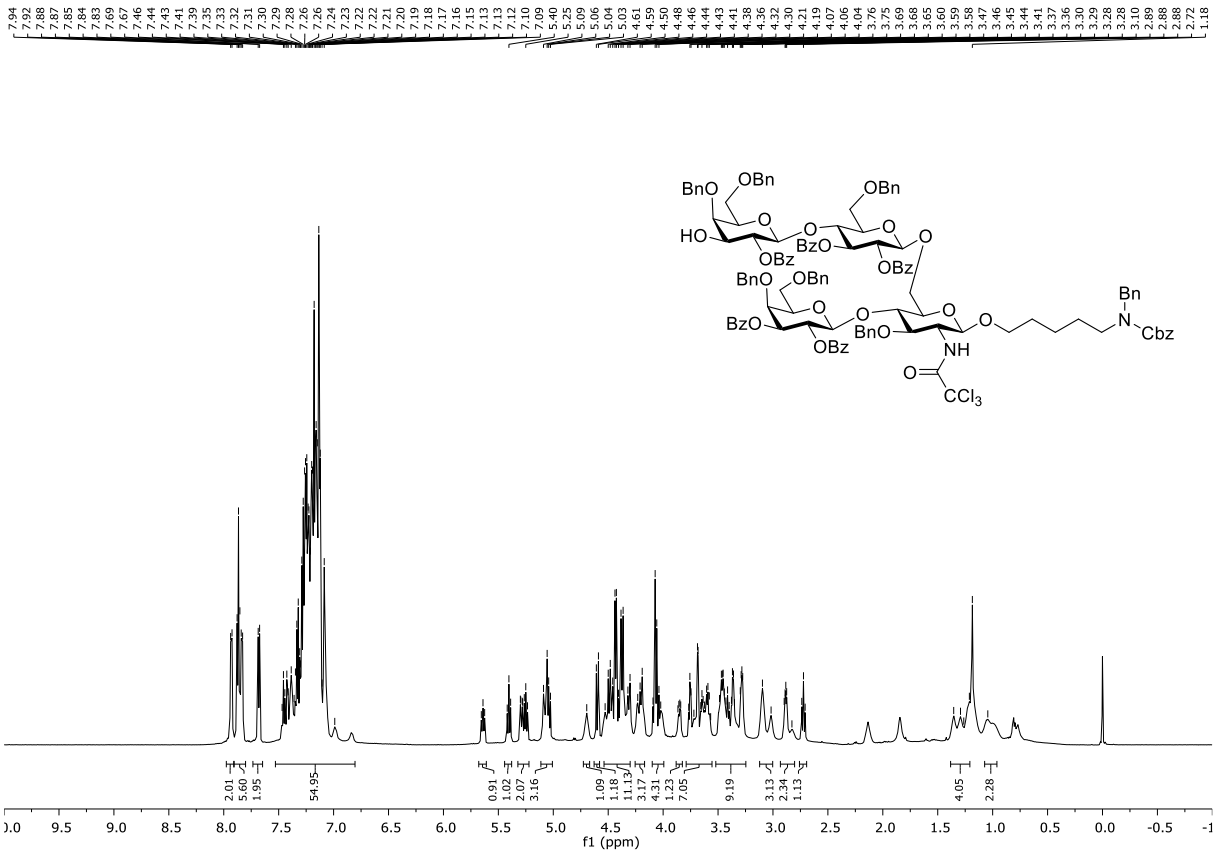
***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-3-*O*-(methyl 5-penanoate)-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-glucopyranosyl-(1→6)-4-*O*-(2,3-di-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-3-*O*-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (4)**

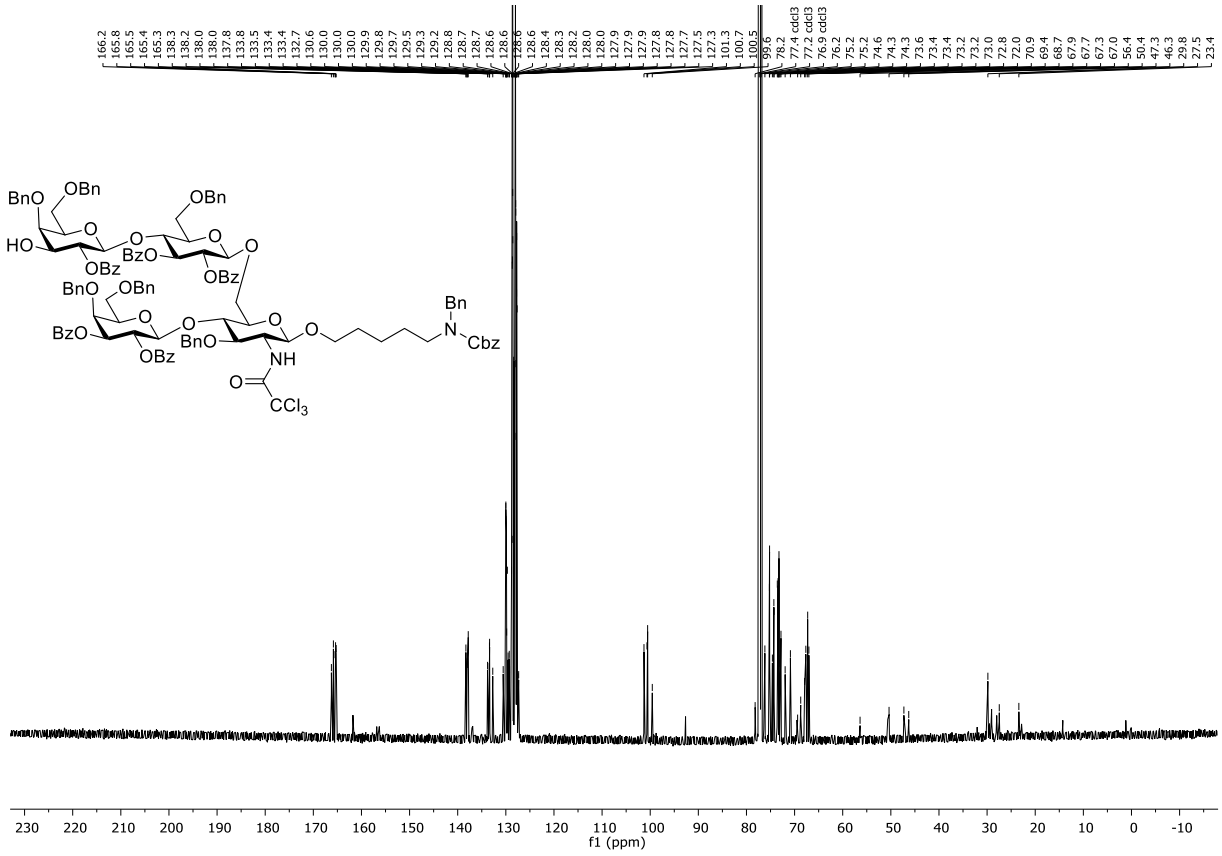






_N_-Benzyl-_N_-benzyloxycarbonyl-5-pentyl 2-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl-(1→4)-2,3-di-_O_-benzoyl-6-_O_-benzyl-β-D-glucopyranosyl-(1→6)-4-_O_-(2,3-di-_O_-benzoyl-4,6-di-_O_-benzyl-β-D-galactopyranosyl)-3-_O_-benzyl-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (19)




166.2
165.8
165.5
165.4
165.3
138.3
138.2
138.0
138.0
137.8
133.8
133.5
133.4
133.4
132.7
130.6
130.0
130.0
129.9
129.8
129.7
129.5
129.3
129.2
128.8
128.7
128.7
128.6
128.6
128.6
128.6
128.4
128.3
128.2
128.0
128.0
127.9
127.9
127.9
127.8
127.8
127.7
127.5
127.3
101.3
100.7
100.5
99.6
78.2
77.4 cdcl3
77.2 cdcl3
76.9 cdcl3
76.2
75.2
75.2
74.6
74.3
74.3
73.6
73.4
73.4
73.2
73.2
73.0
72.8
72.0
70.9
69.4
68.7
67.9
67.7
67.3
67.0
56.4
50.4
47.3
46.3
29.8
27.5
23.4
BnO
OBn
HO
OBz
BzO
OBn
O
OBz
BnO
OBn
BzO
OBz
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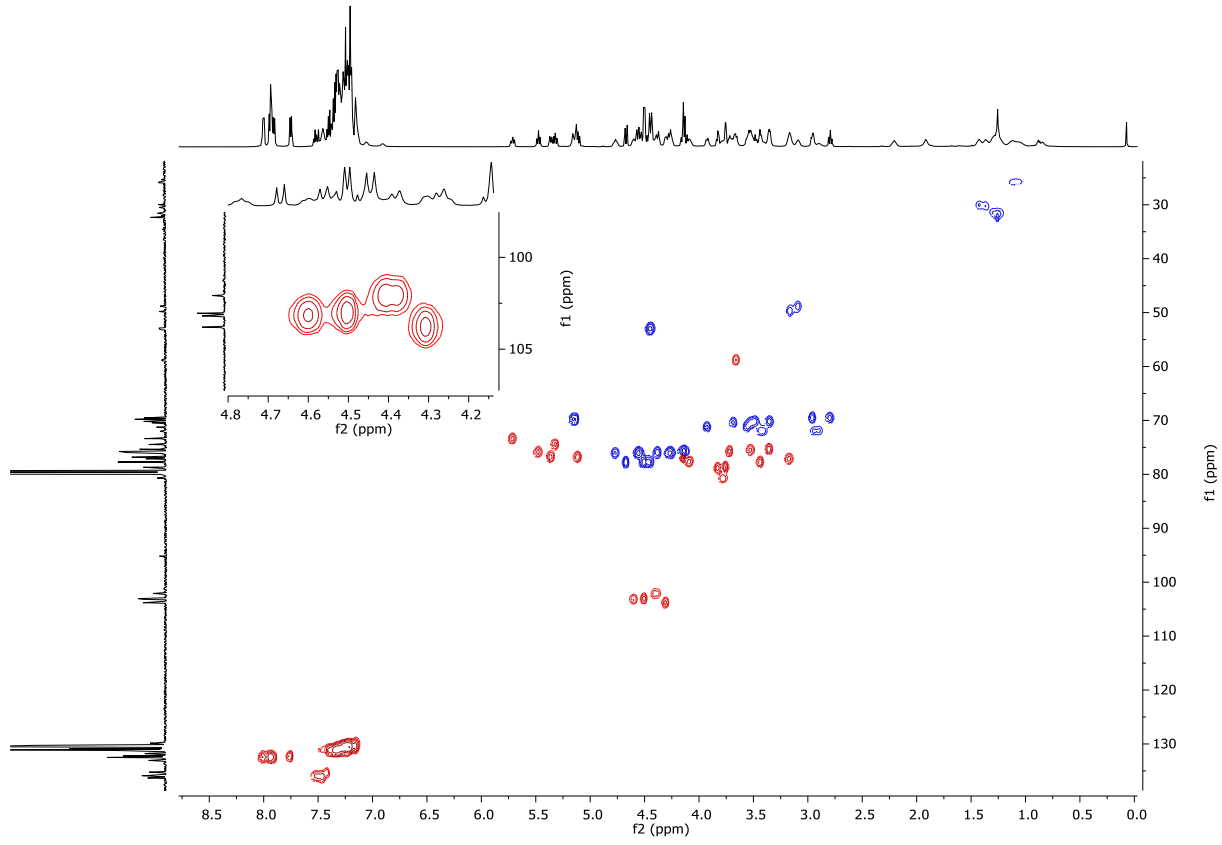
f1 (ppm)


100
105
f1 (ppm)
4.8 4.7 4.6 4.5 4.4 4.3 4.2
f2 (ppm)
30
40
50
60
70
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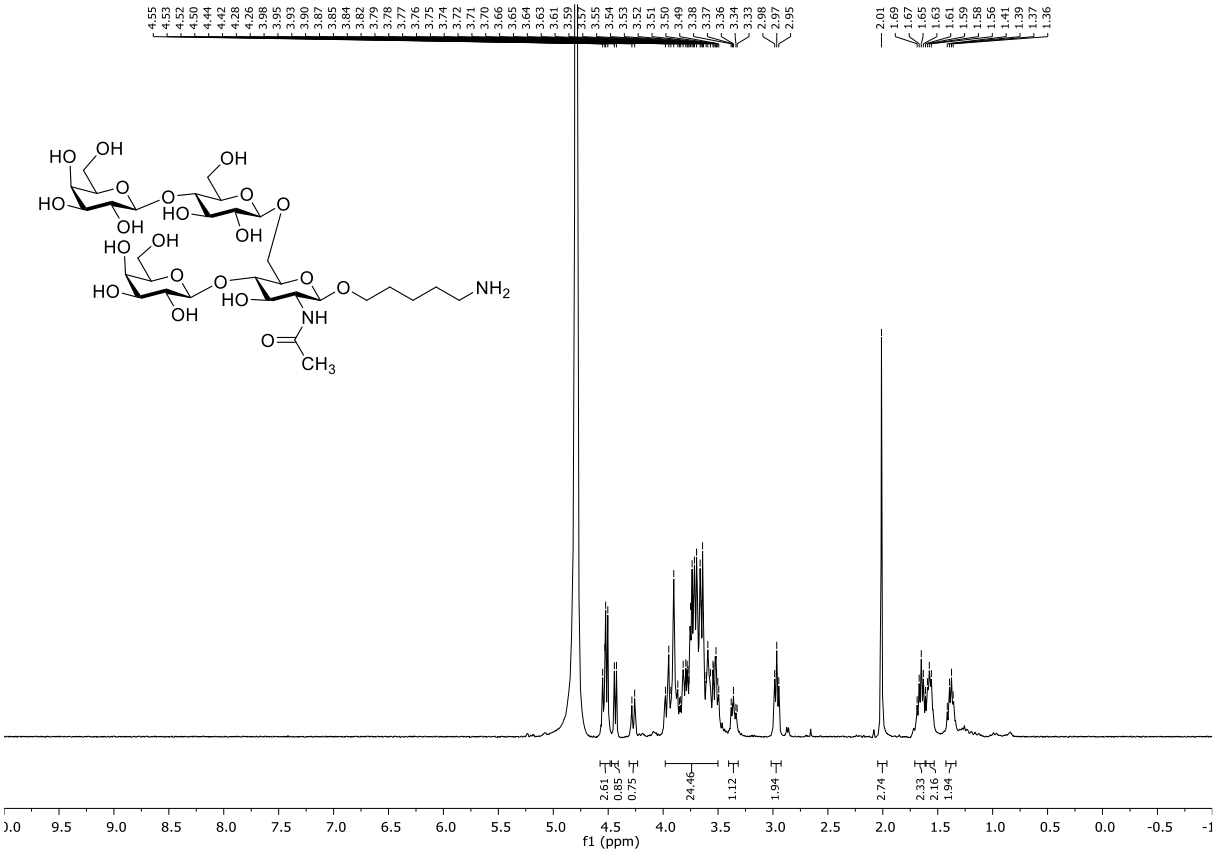
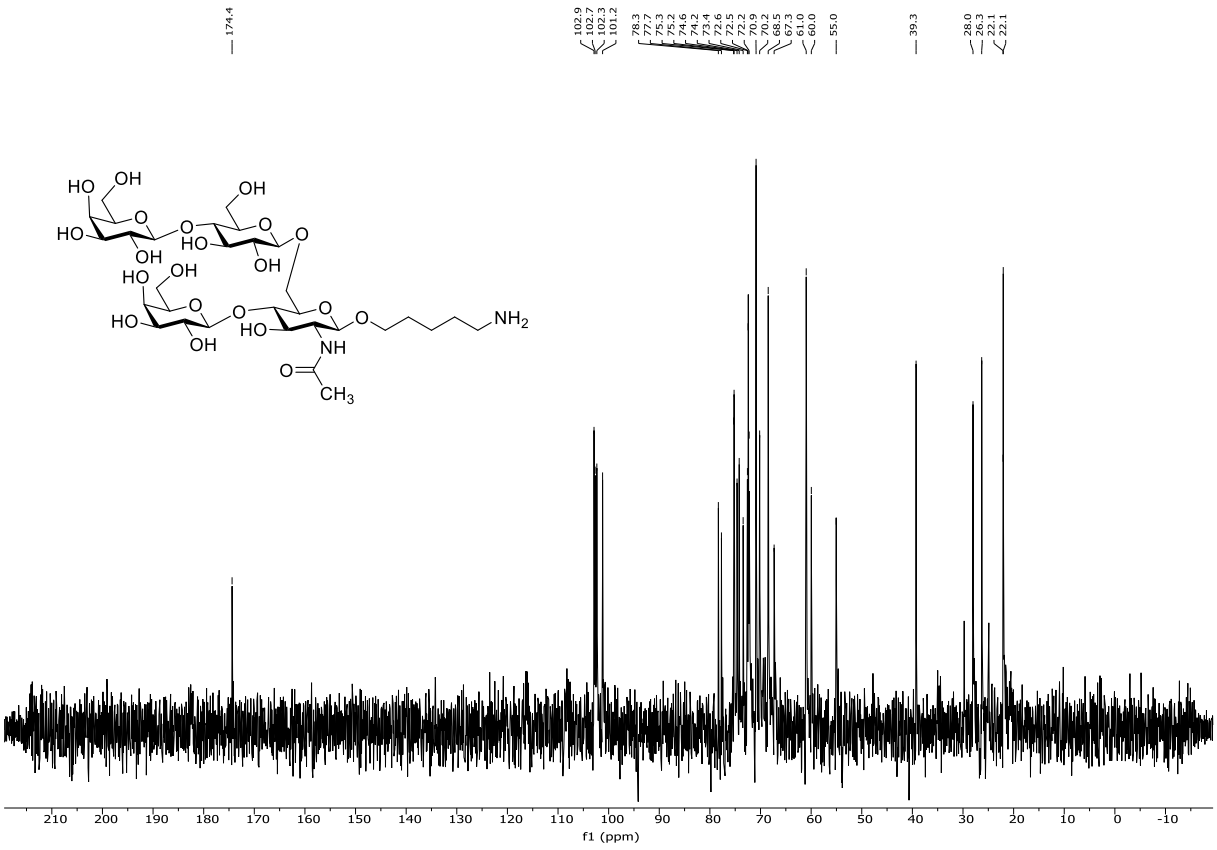
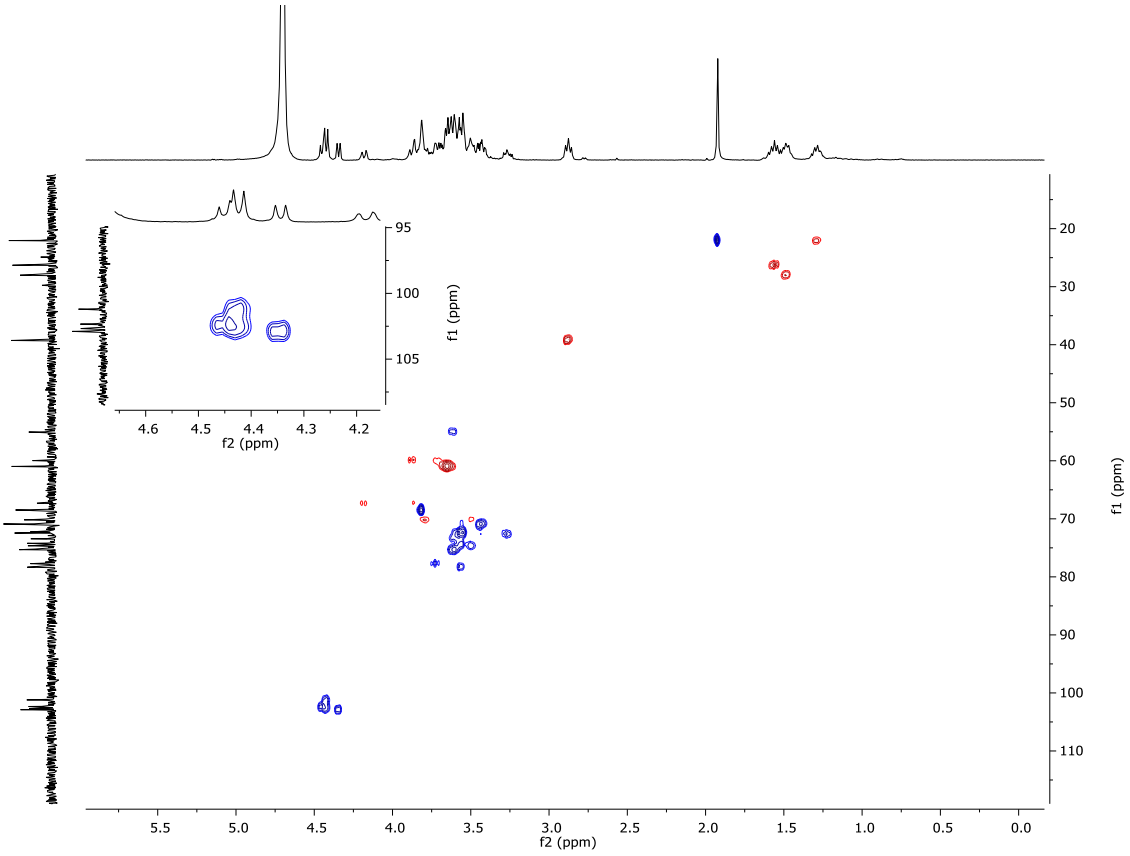
90
100
110
120
130
f1 (ppm)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f2 (ppm)

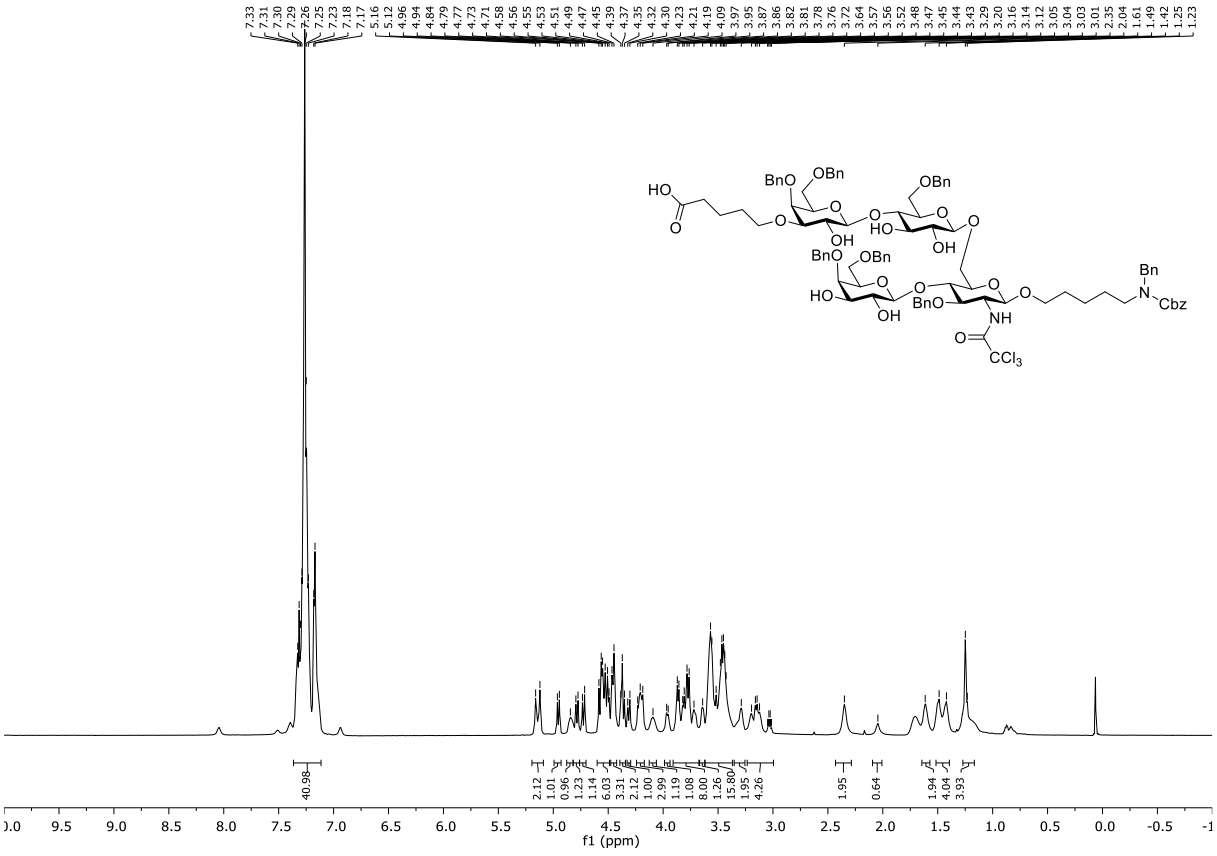
5-Aminopentyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-4-*O*-(β-D-galactopyranosyl)-2-deoxy-2-acetamino-β-D-glucopyranoside (10)

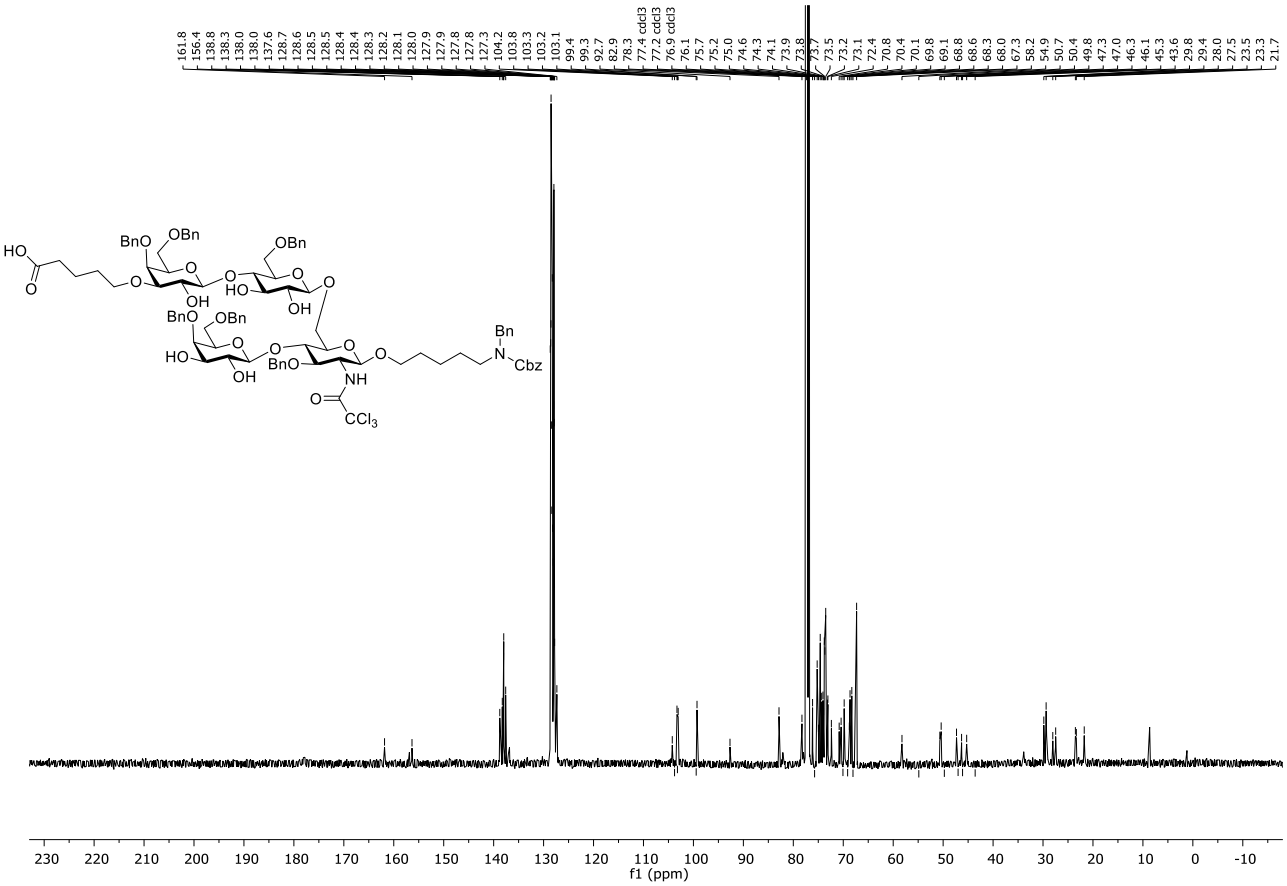






***N*-Benzyl-*N*-benzyloxycarbonyl-5-pentyl 4,6-di-*O*-benzyl-3-*O*-(5-penanoate acid)-β-D-galactopyranosyl-(1→4)-6-*O*-benzyl-β-D-glucopyranosyl-(1→6)-3-*O*-benzyl-4-*O*-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-2-deoxy-2-trichloroacetamino-β-D-glucopyranoside (21)**

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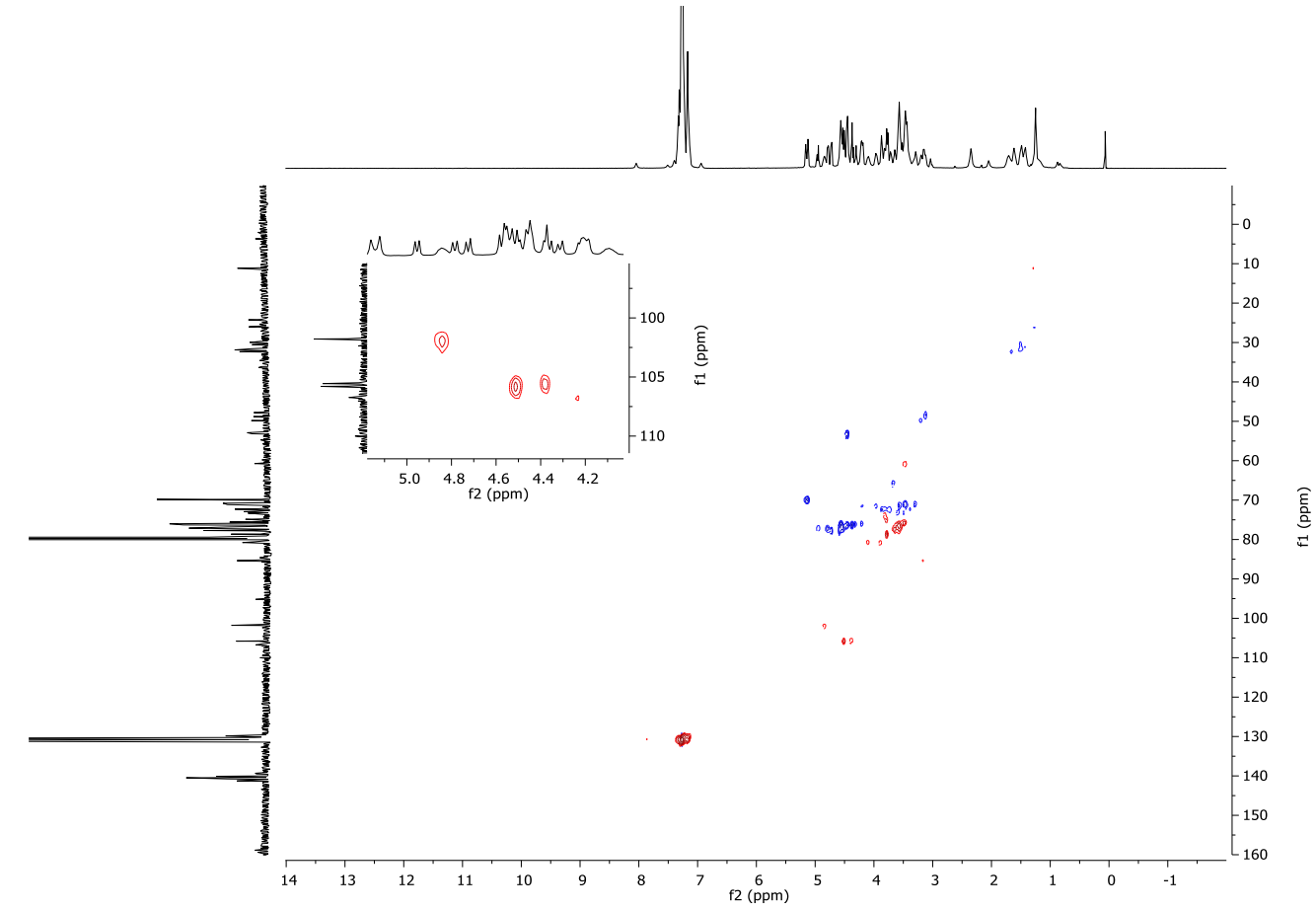
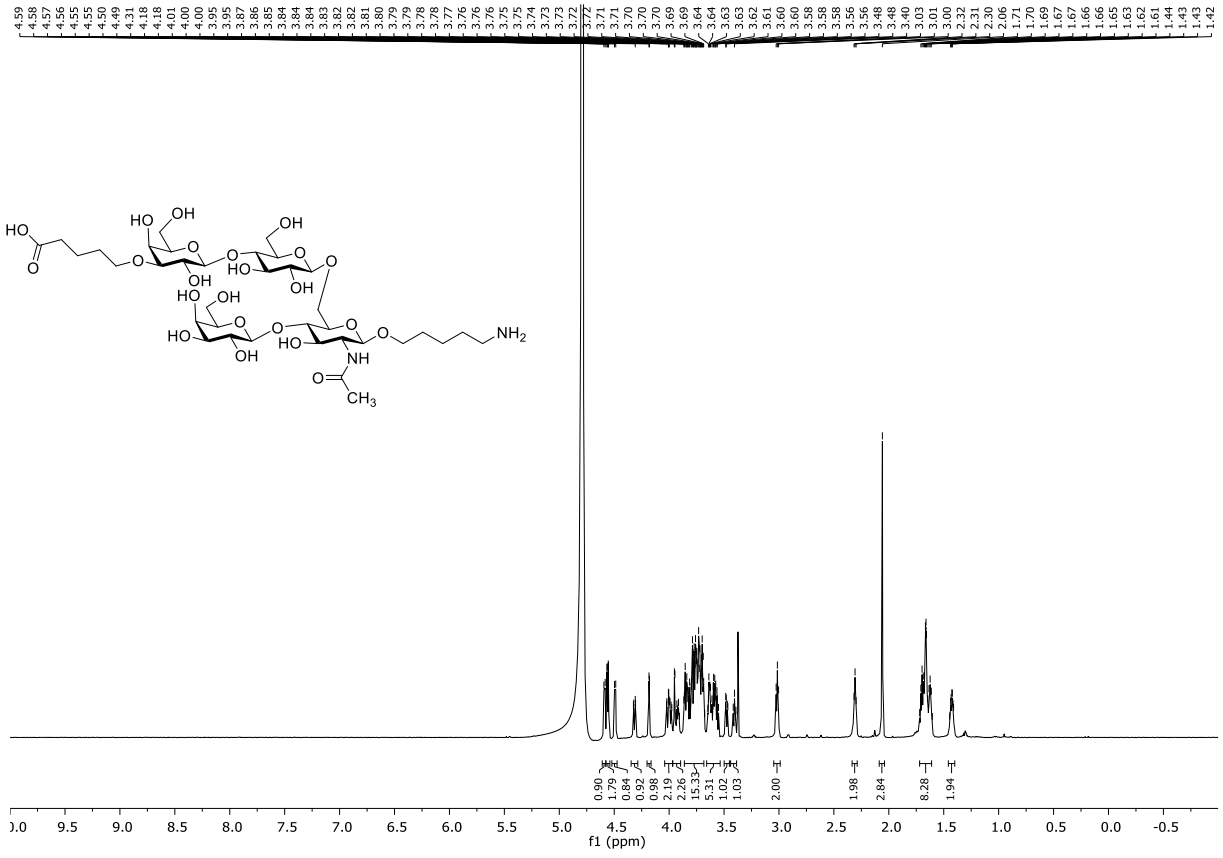
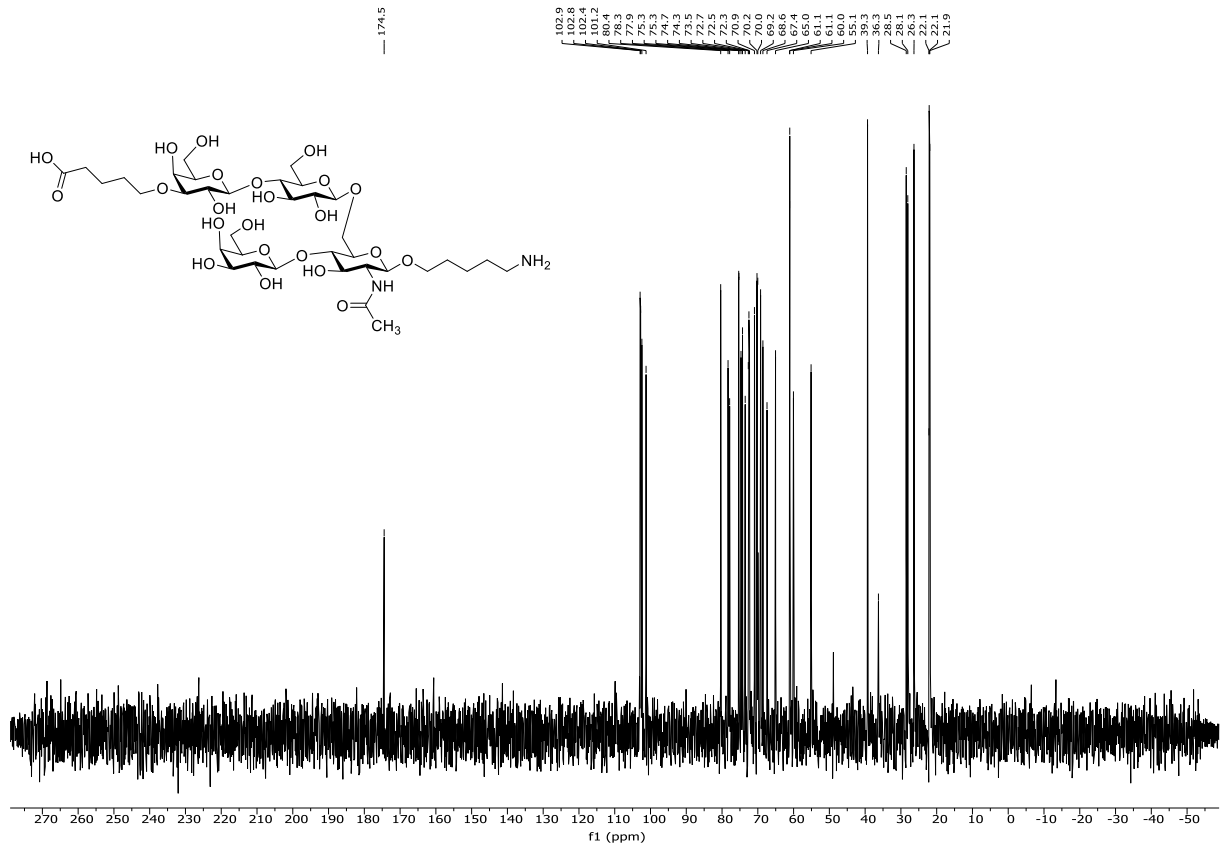
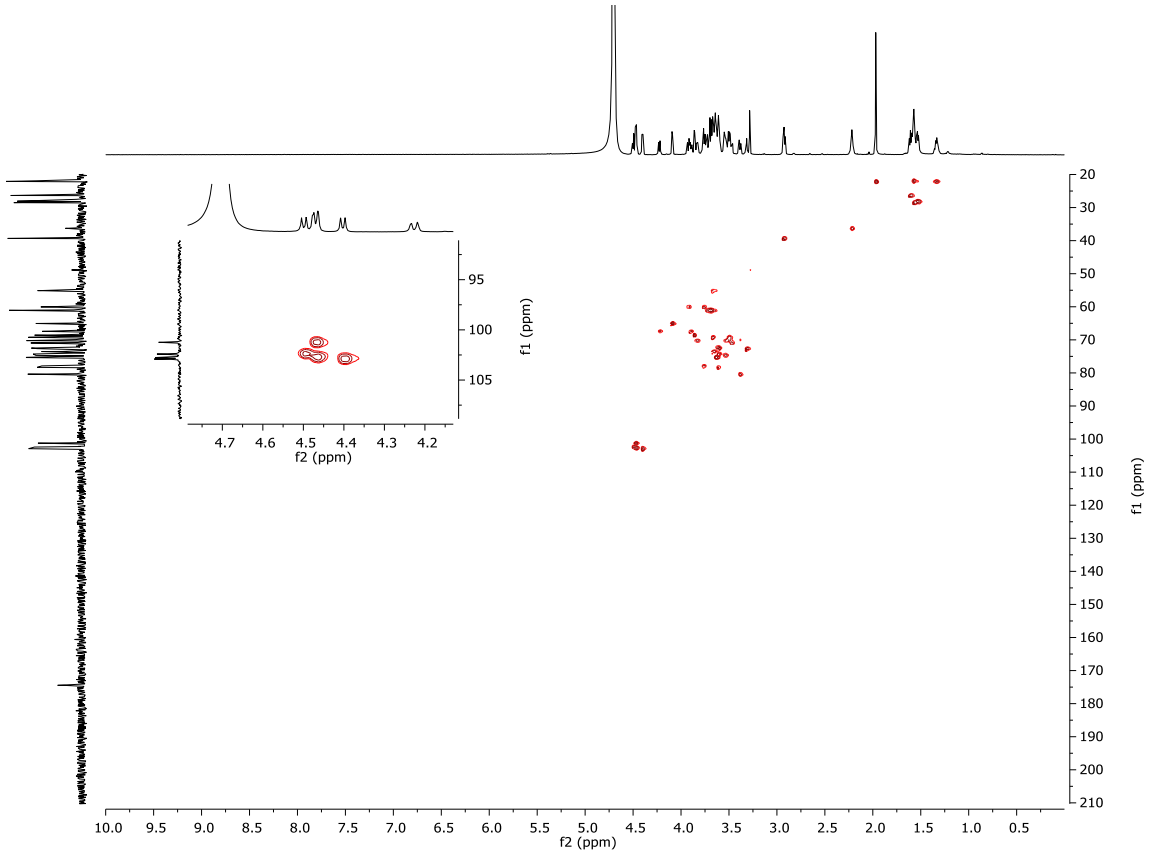
138.8
138.3
138.0
138.0
137.6
128.7
128.6
128.5
128.5
128.4
128.4
128.3
128.2
128.1
128.0
127.9
127.9
127.8
127.8
127.3
104.2
103.8
103.3
103.2
103.1
99.4
99.3
92.7
82.9
78.3
77.4 cdcl3
77.2 cdcl3
76.9 cdcl3
76.1
75.7
75.2
75.0
74.6
74.3
74.1
73.9
73.8
73.7
73.5
73.2
73.1
72.4
70.8
70.4
70.1
69.8
69.1
68.8
68.6
68.3
68.0
67.3
58.2
54.9
50.7
50.4
49.8
47.3
47.0
46.3
46.1
45.3
43.6
29.8
29.4
28.0
27.5
23.5
23.3
21.7
HO
O
BnO
OBn
O
OH
HO
Bn
N
Cbz
NH
O
CCl3
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)


5.0 4.8 4.6 4.4 4.2
f2 (ppm)
100
105
110
f1 (ppm)
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1
f2 (ppm)
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
f1 (ppm)

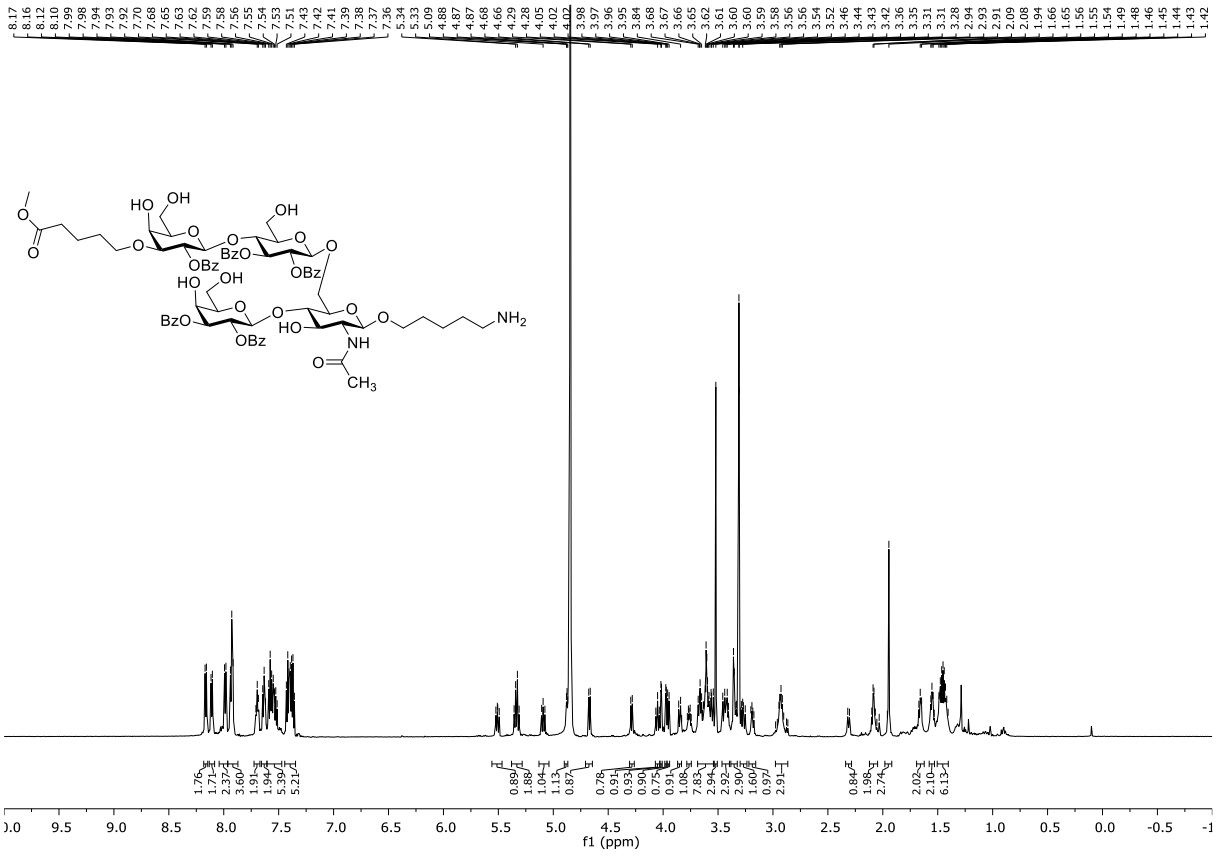
5-Aminopentyl 3-*O*-(5-penanoate acid)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-4-*O*-(β-D-galactopyranosyl)-2-deoxy-2-acetamino-β-D-glucopyranoside (1)

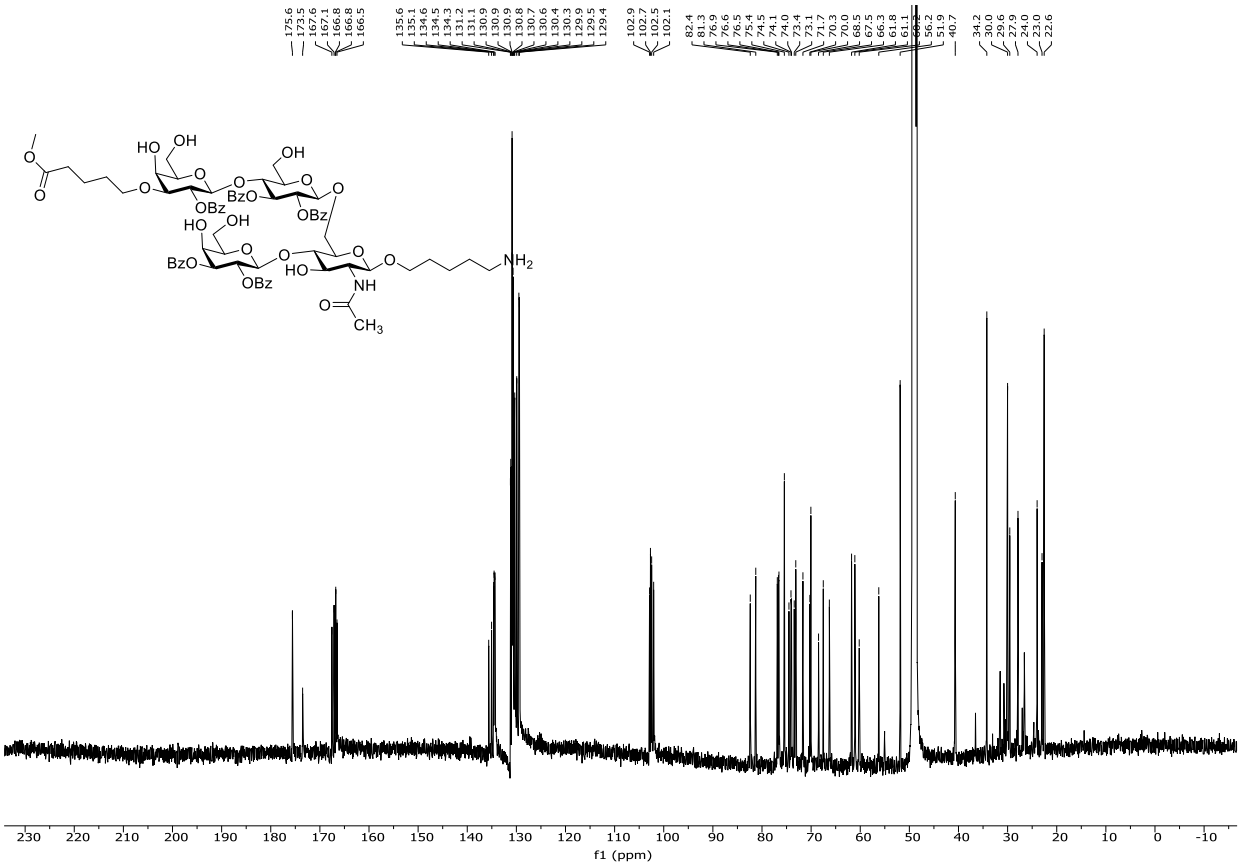


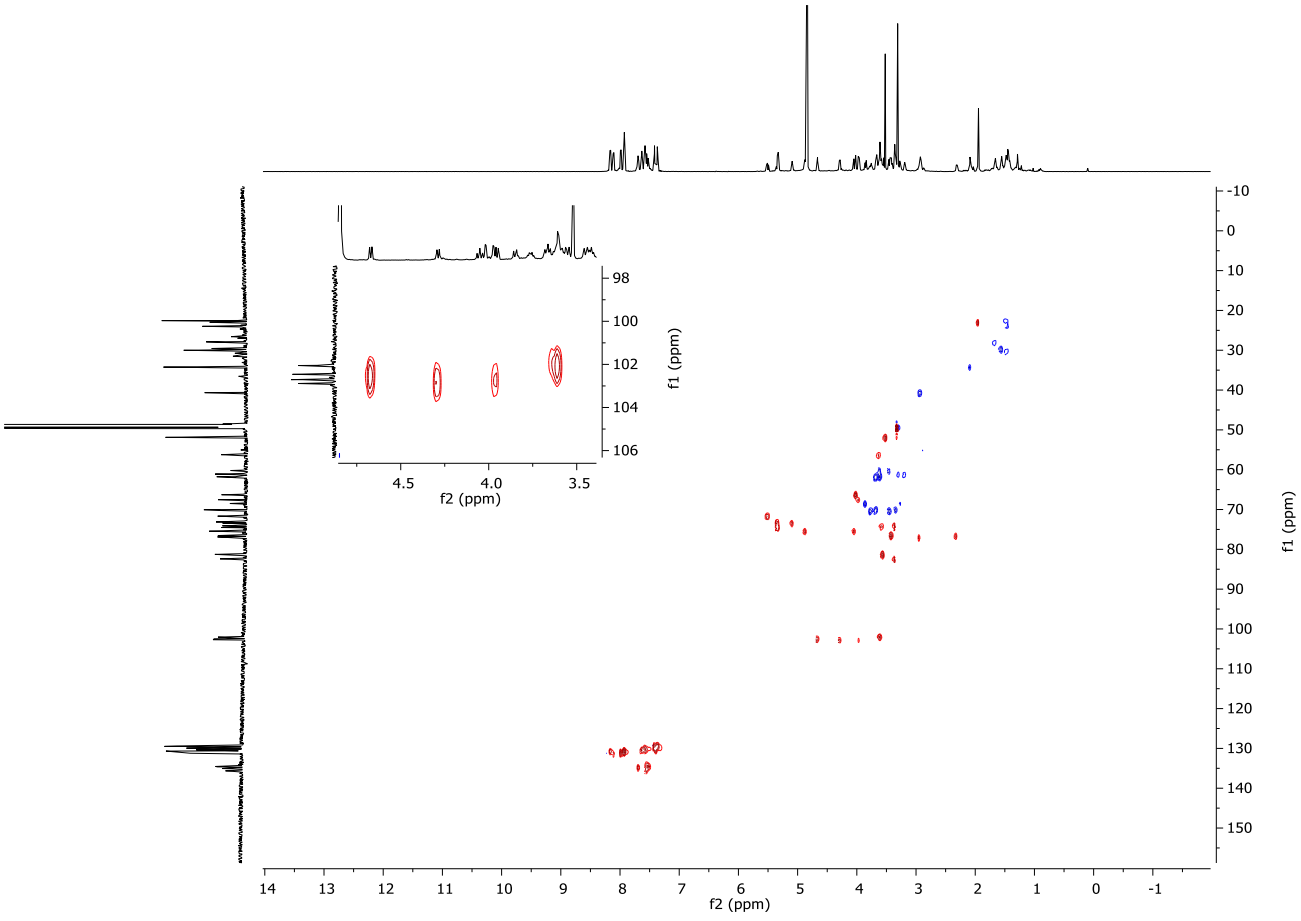




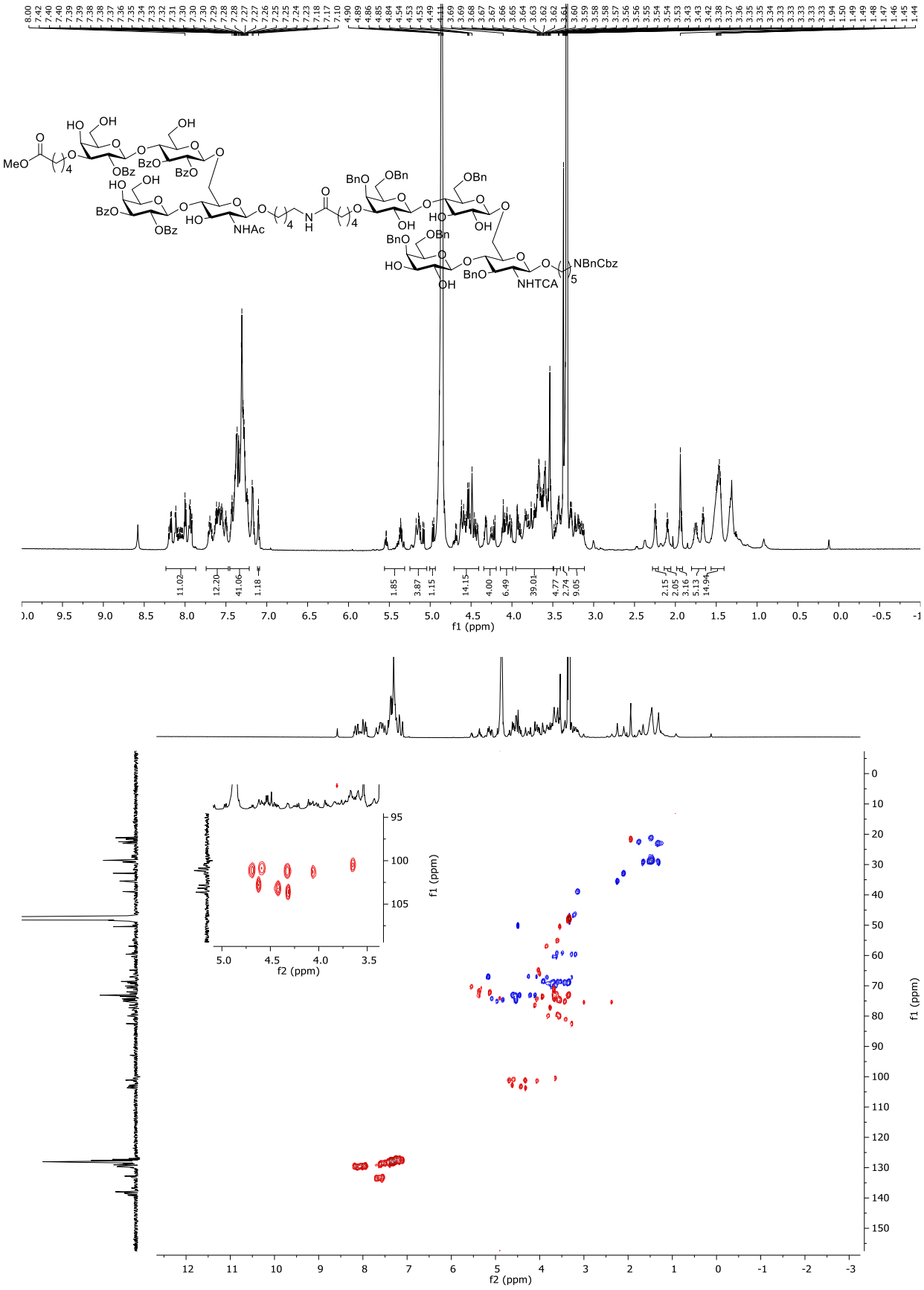
5-Aminopentyl 2-*O*-benzoyl-3-*O*-(methyl 5-penanoate)-β-D-galactopyranosyl-(1→4)-2,3-di-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-4-*O*-(2,3-di-*O*-benzoyl-β-D-galactopyranosyl)-2-deoxy-2-acetamino-β-D-glucopyranoside (22)



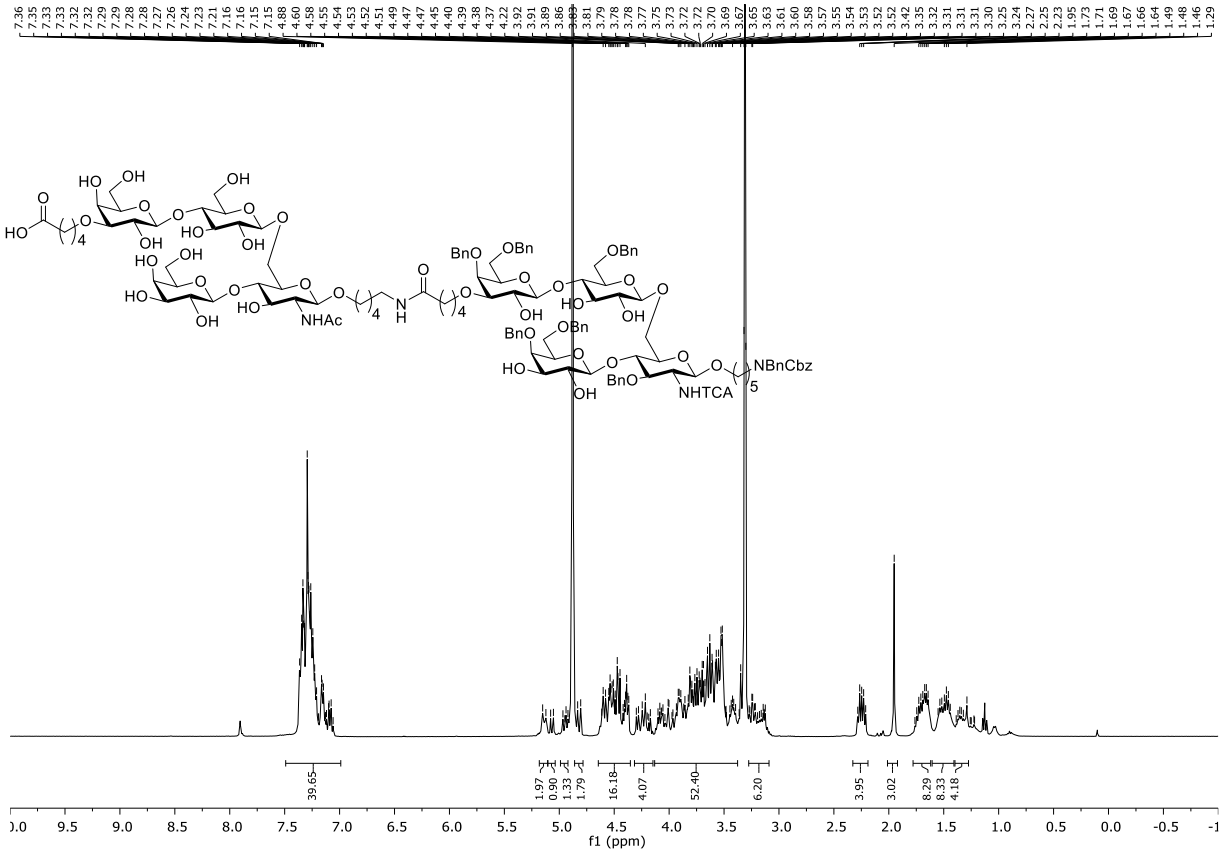


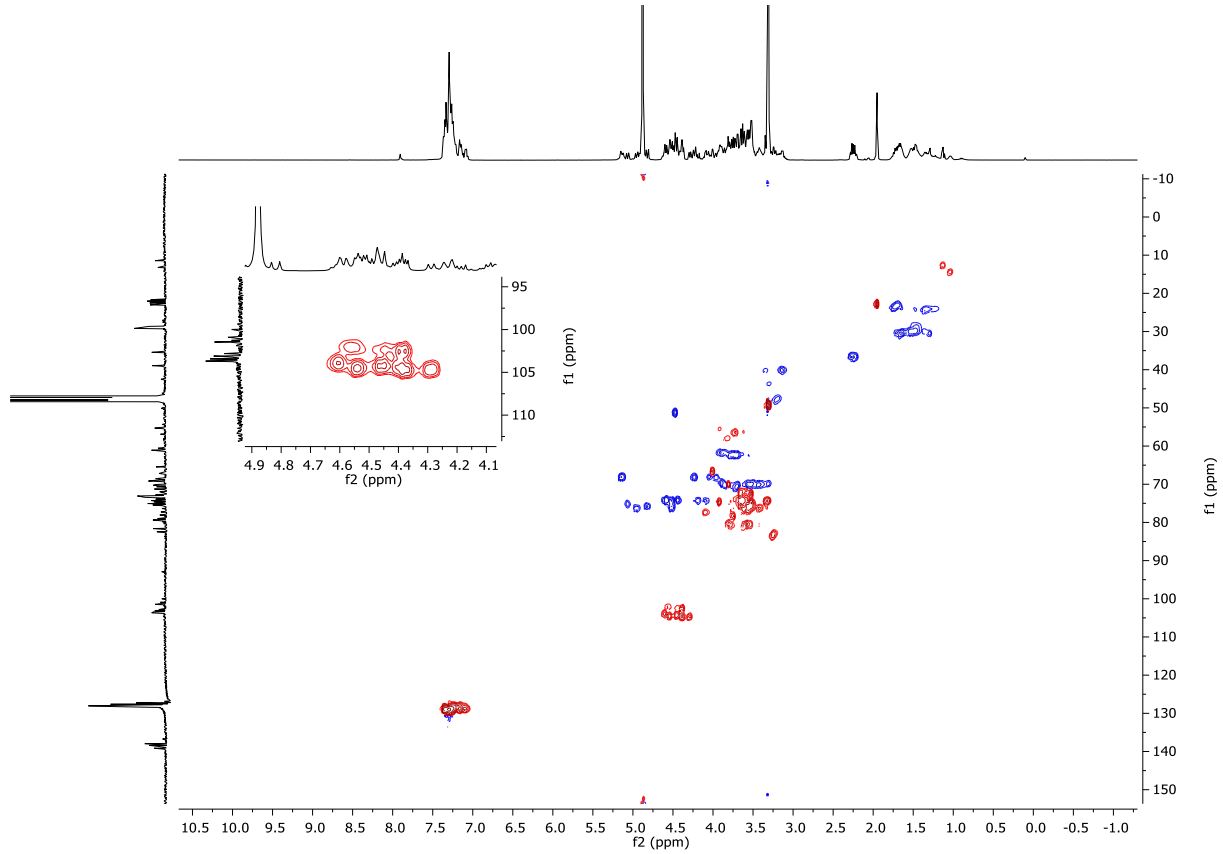


Protected divalent derivative (23)

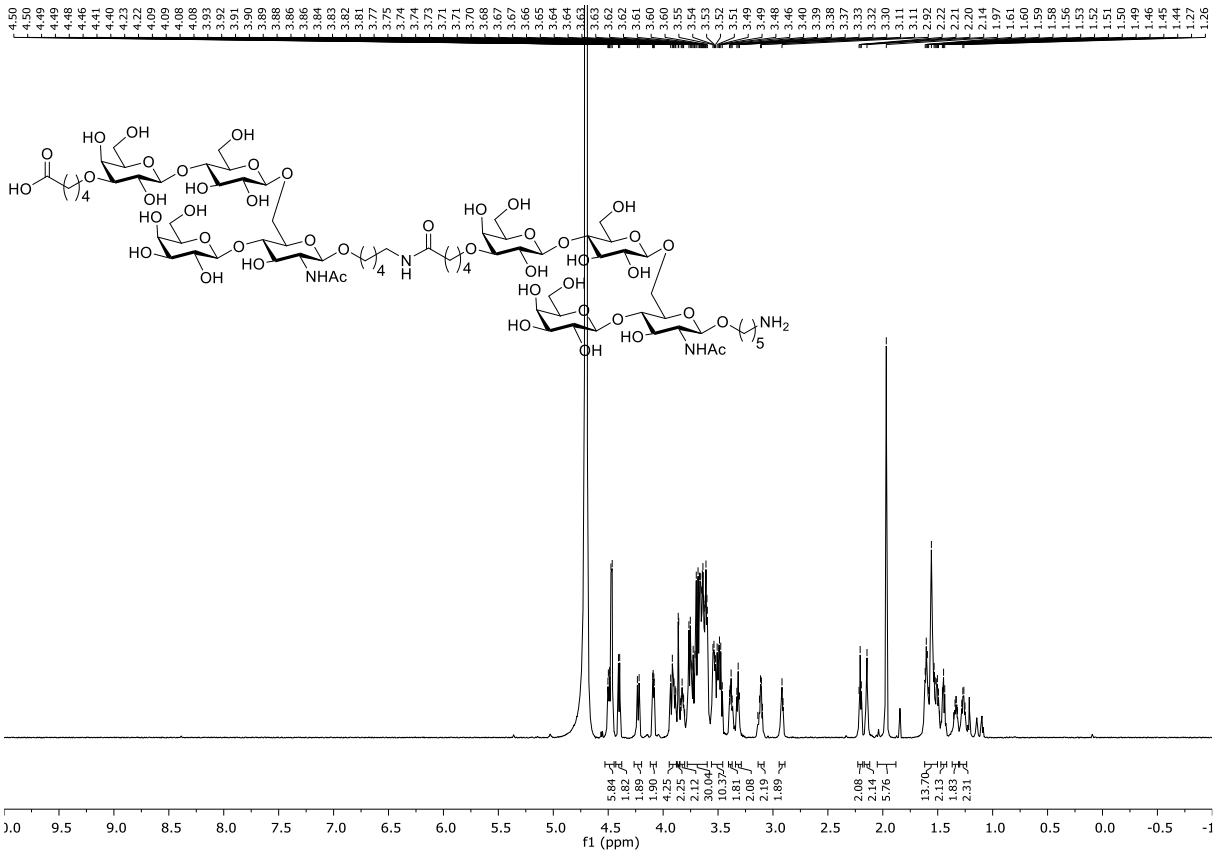


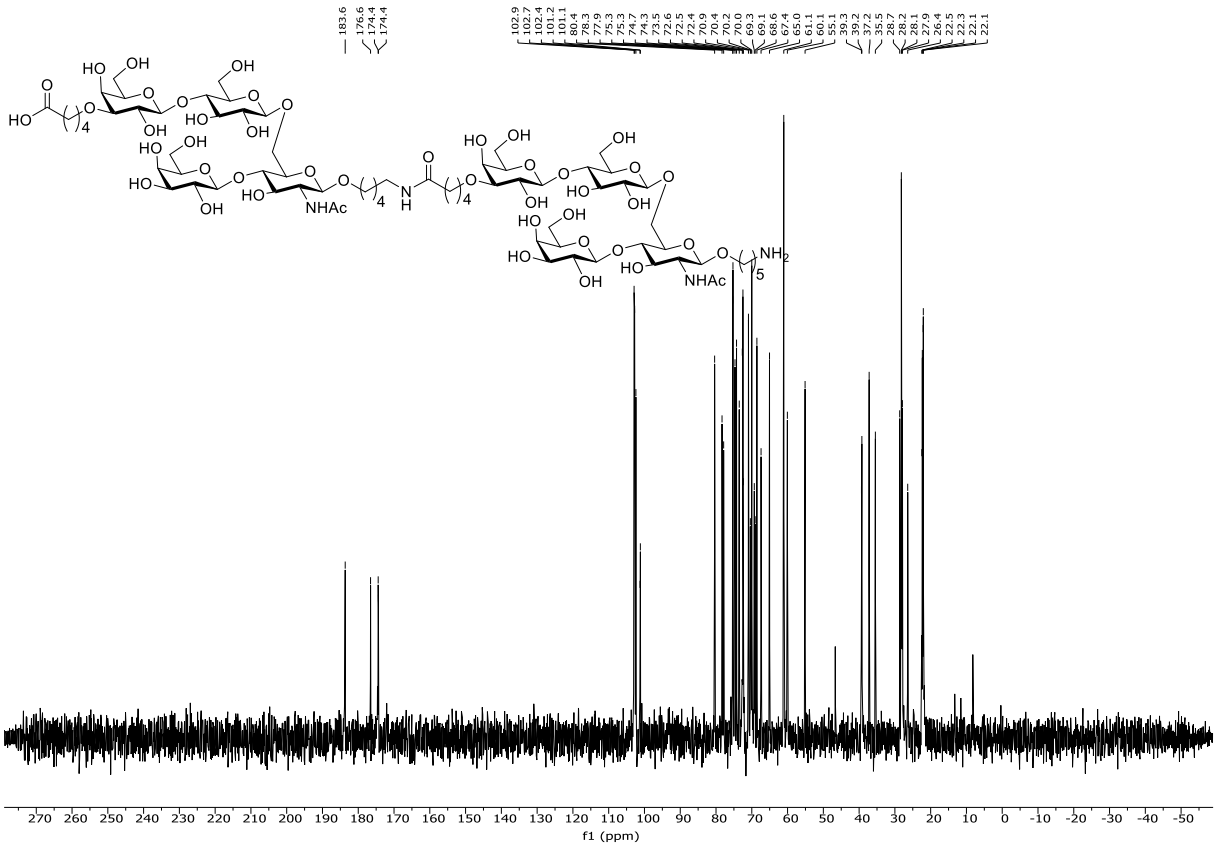
Partly protected divalent derivative (24)

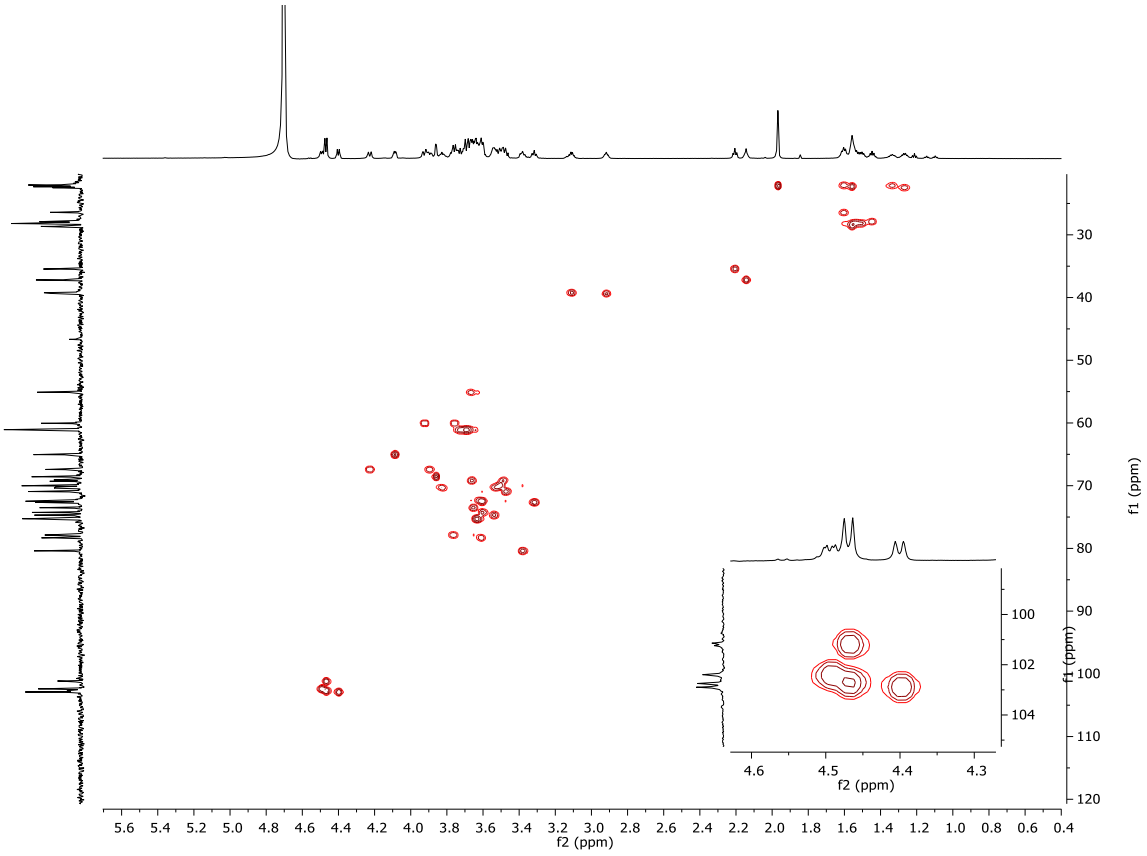




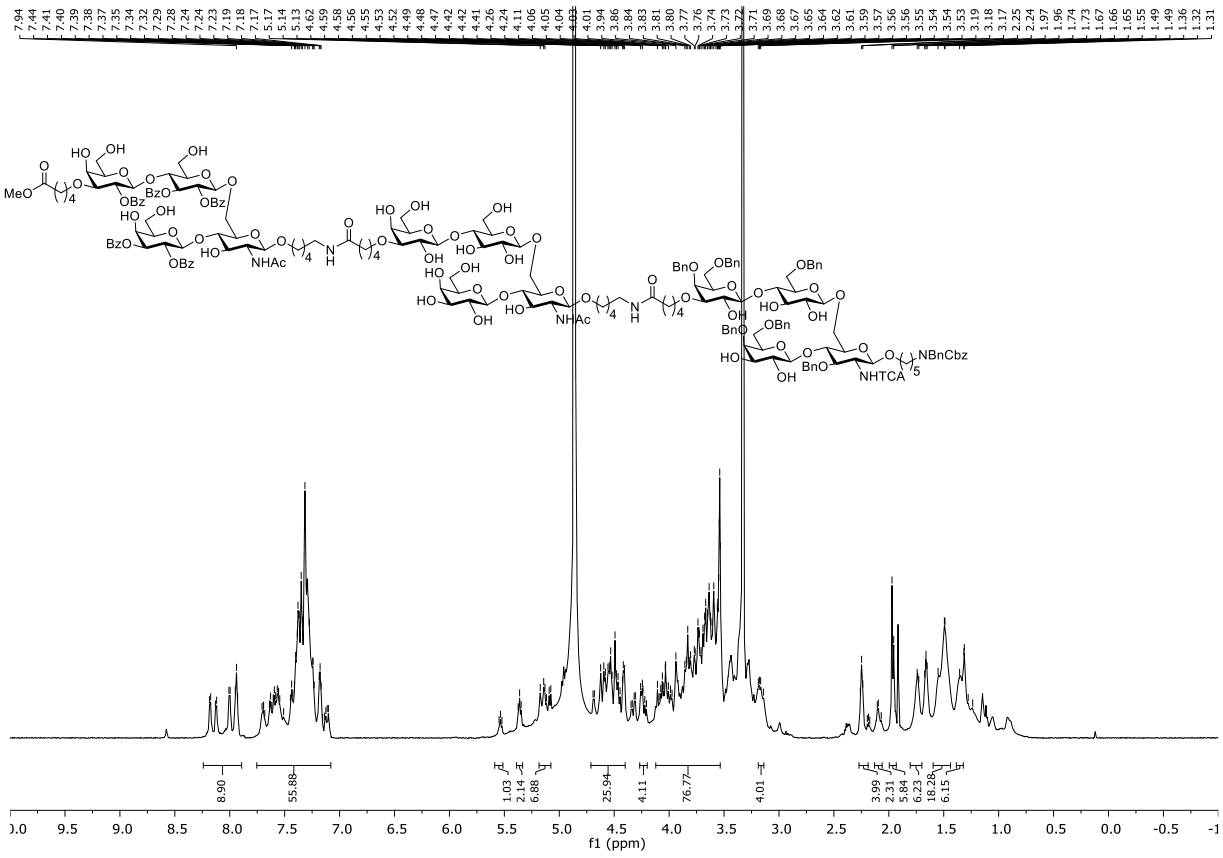
Divalent derivative (2)

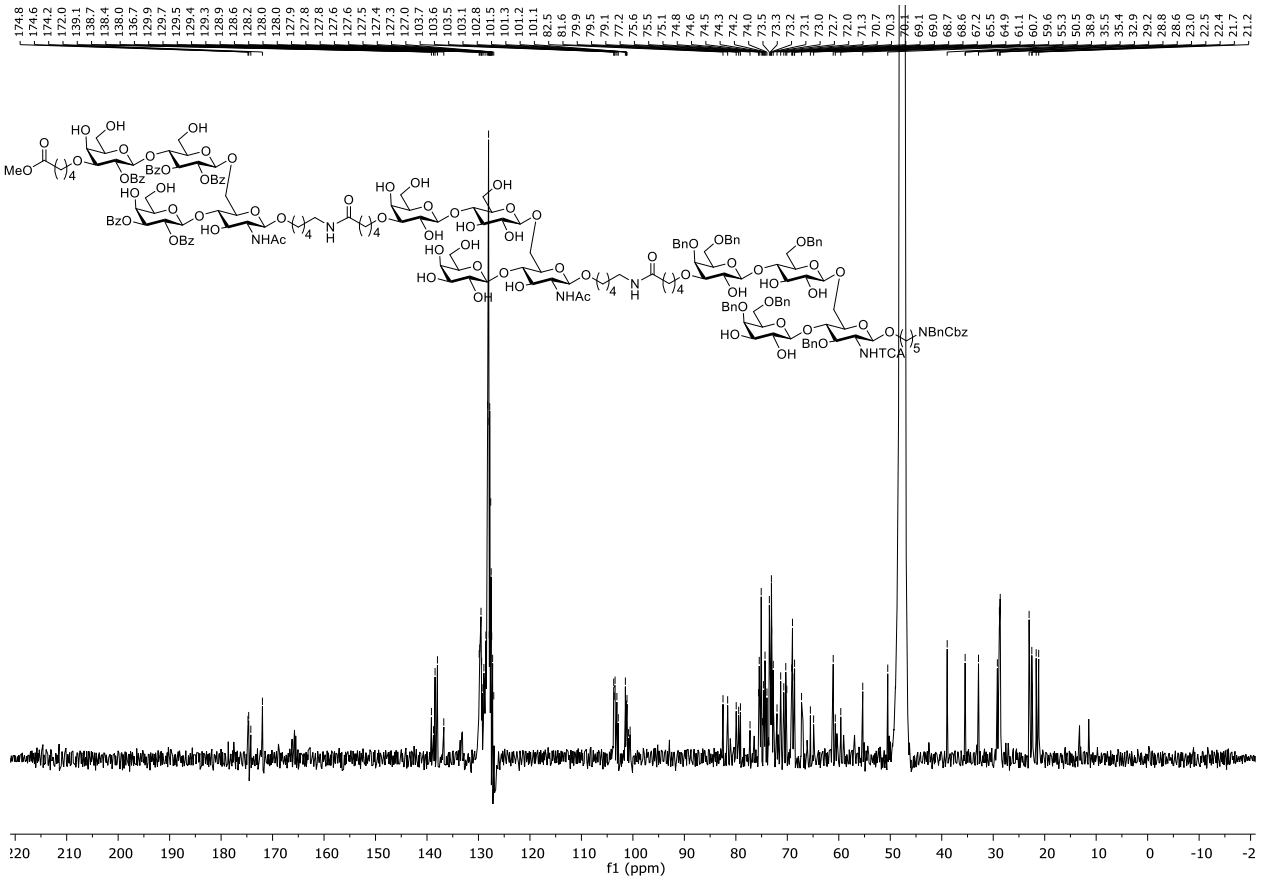

f1 (ppm)

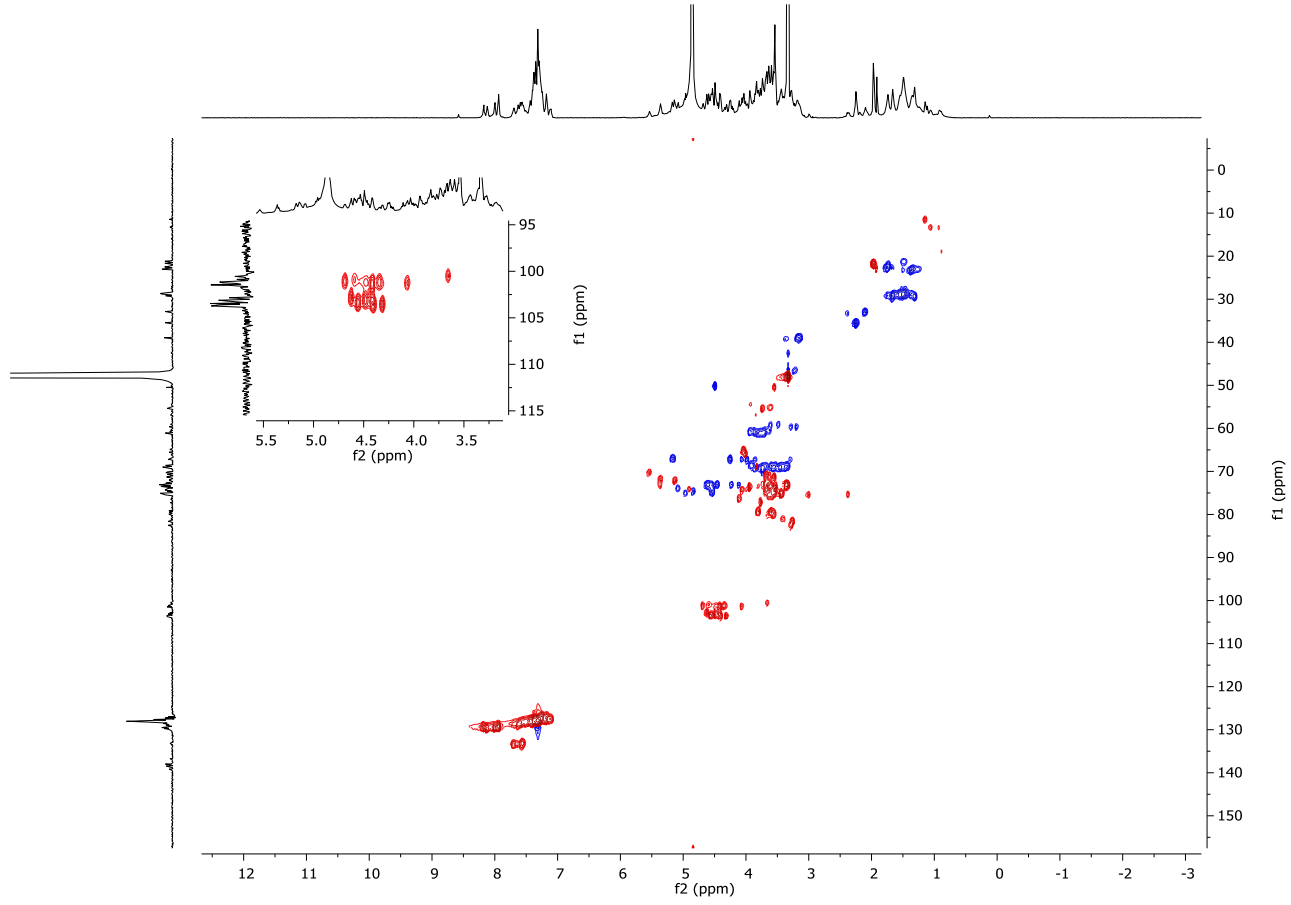

f1 (ppm)



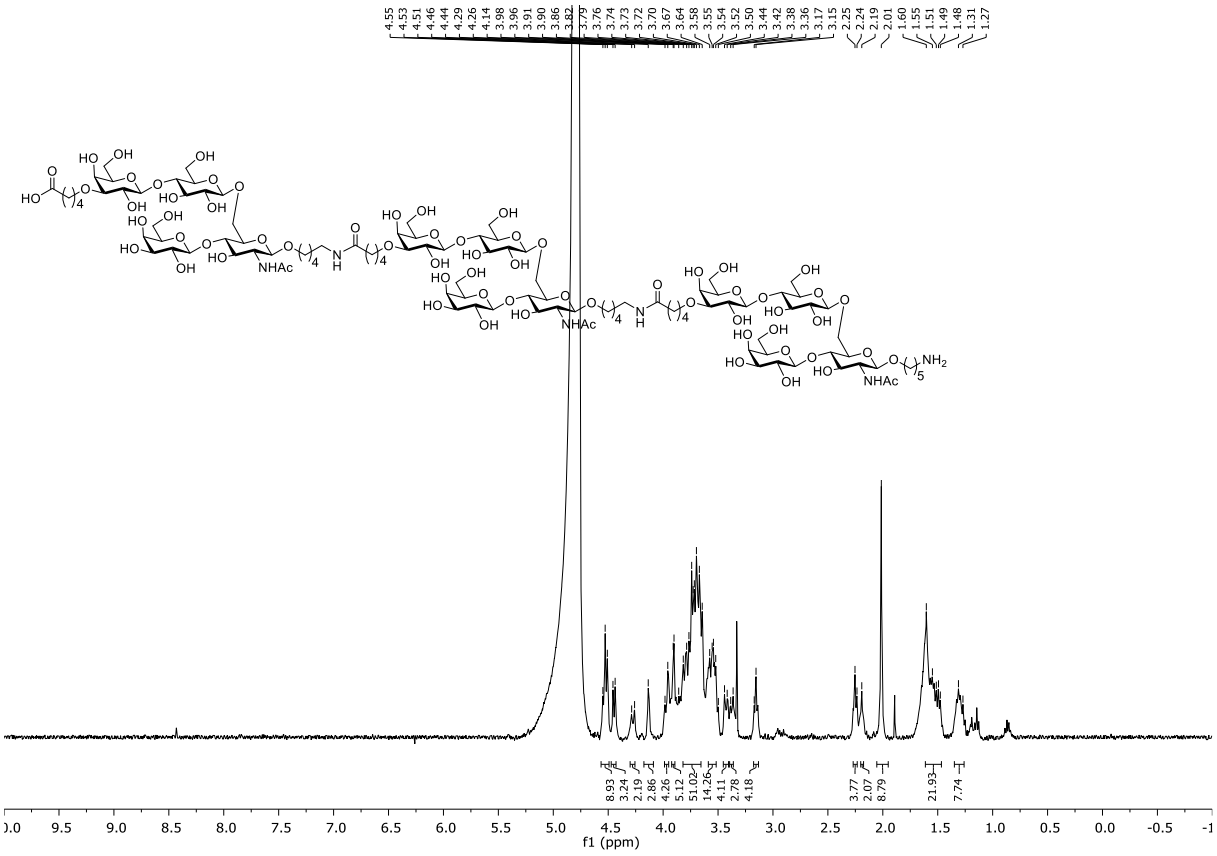
Protected trivalent derivative (25)

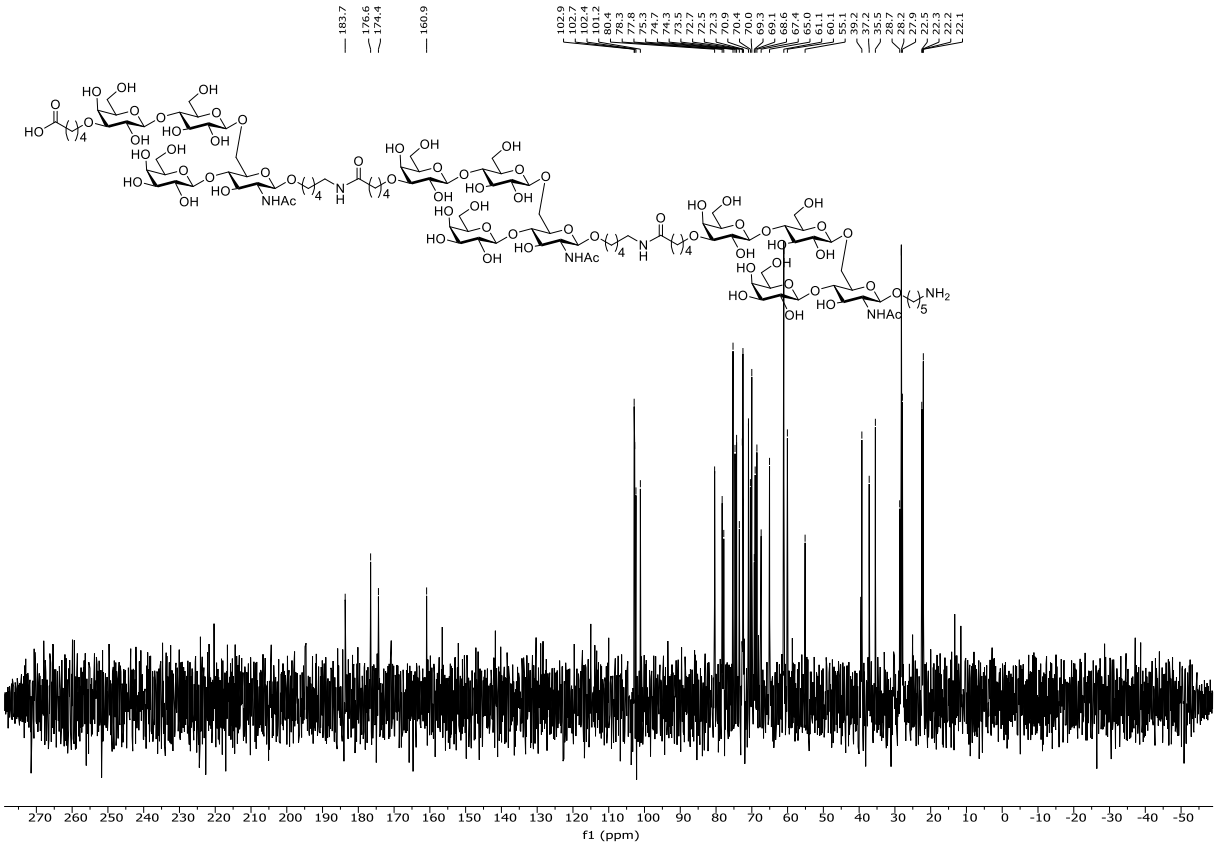


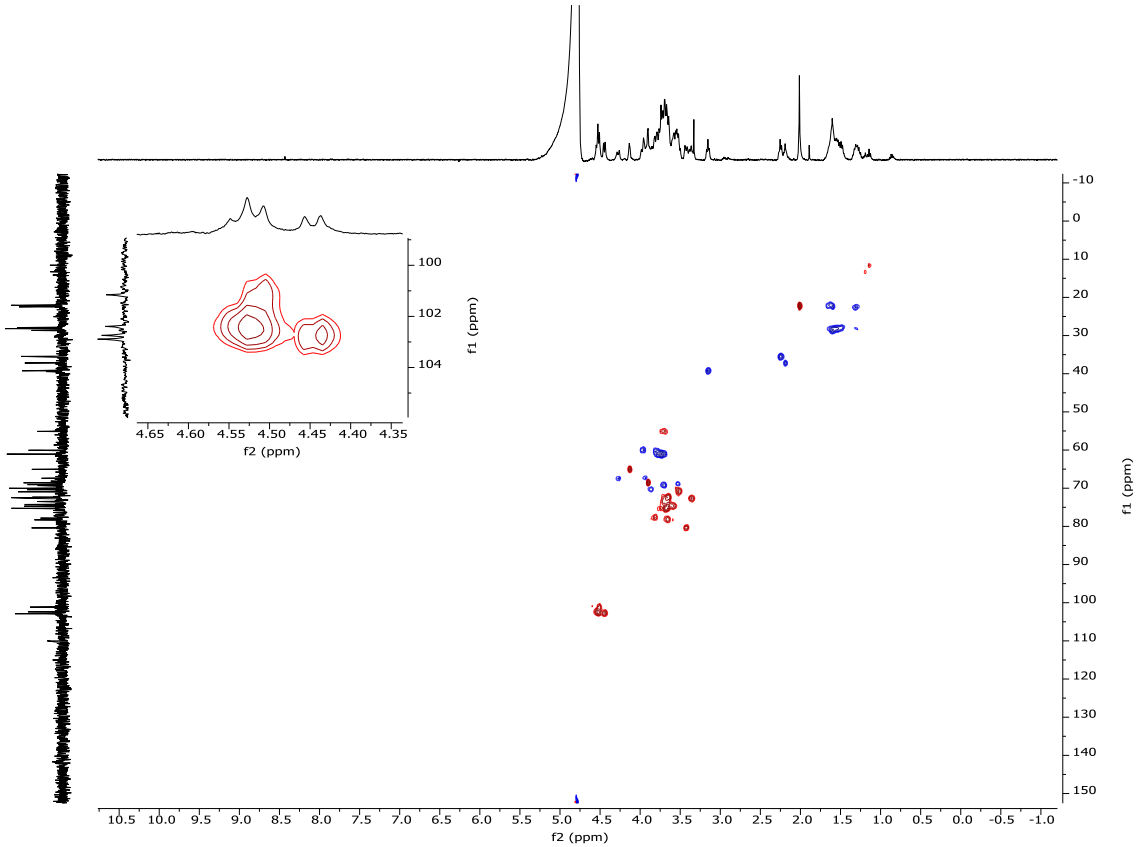




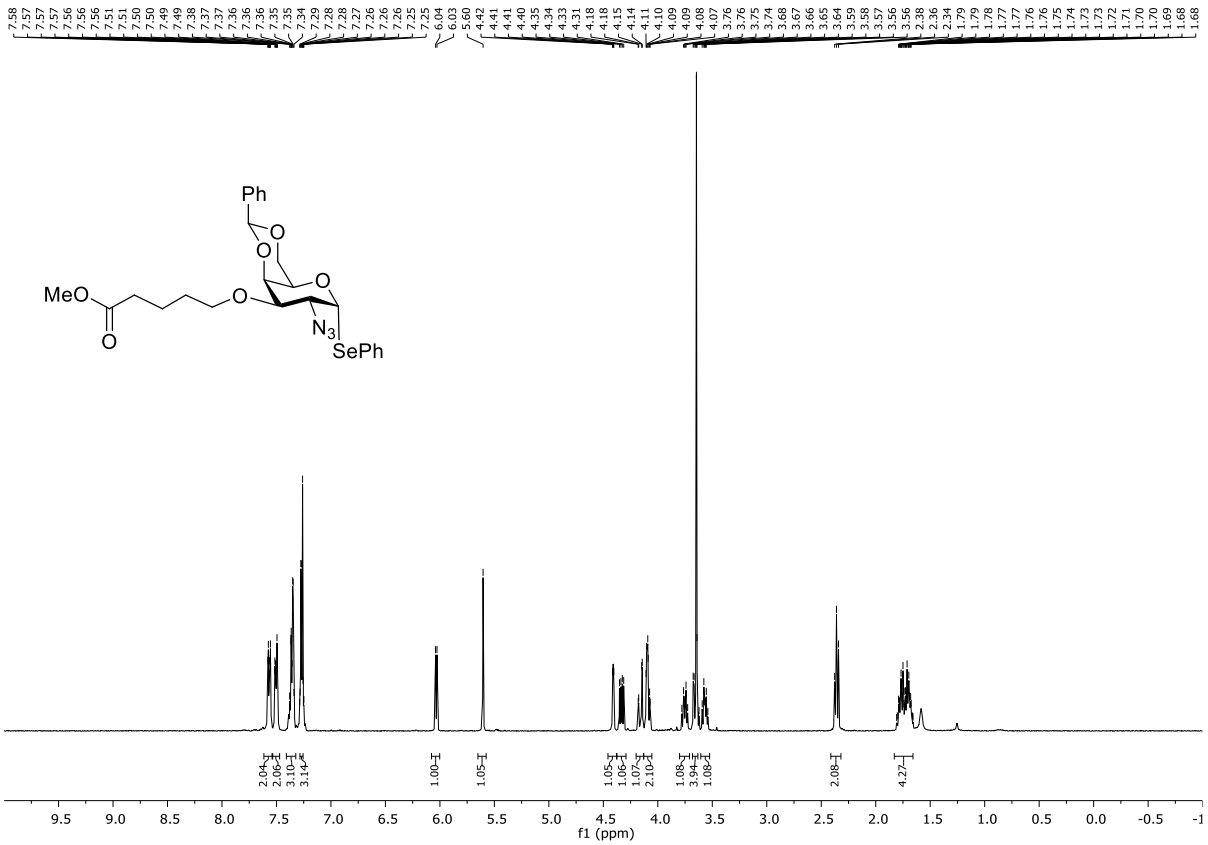
Trivalent derivative (3)

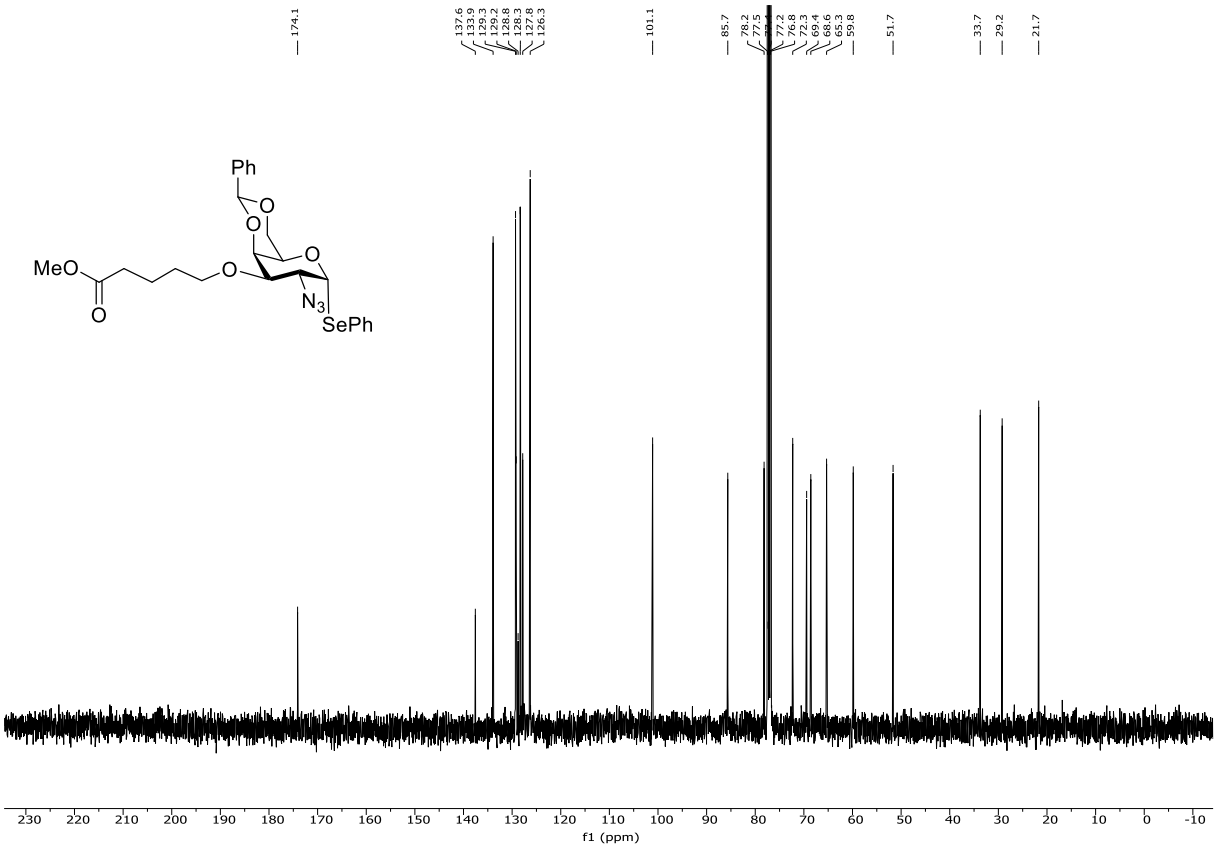

f1 (ppm)


f1 (ppm)

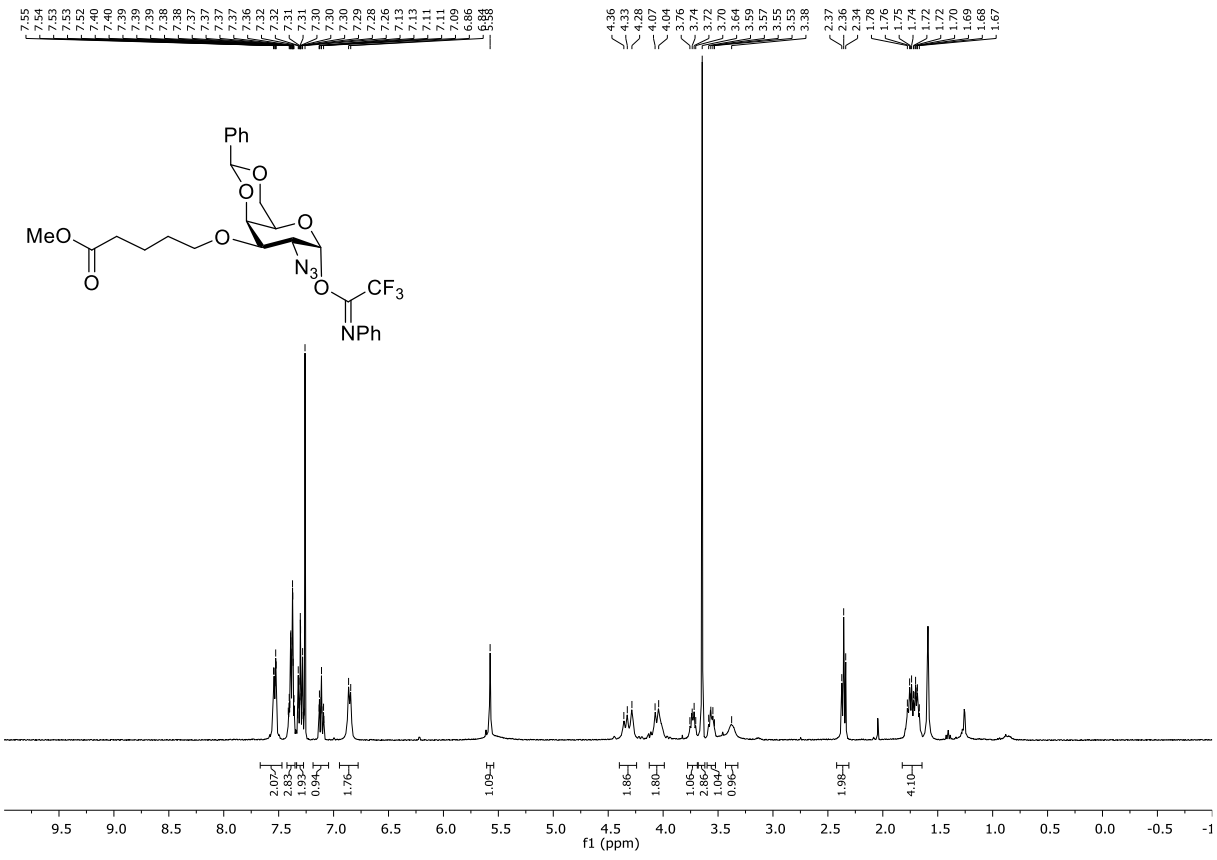


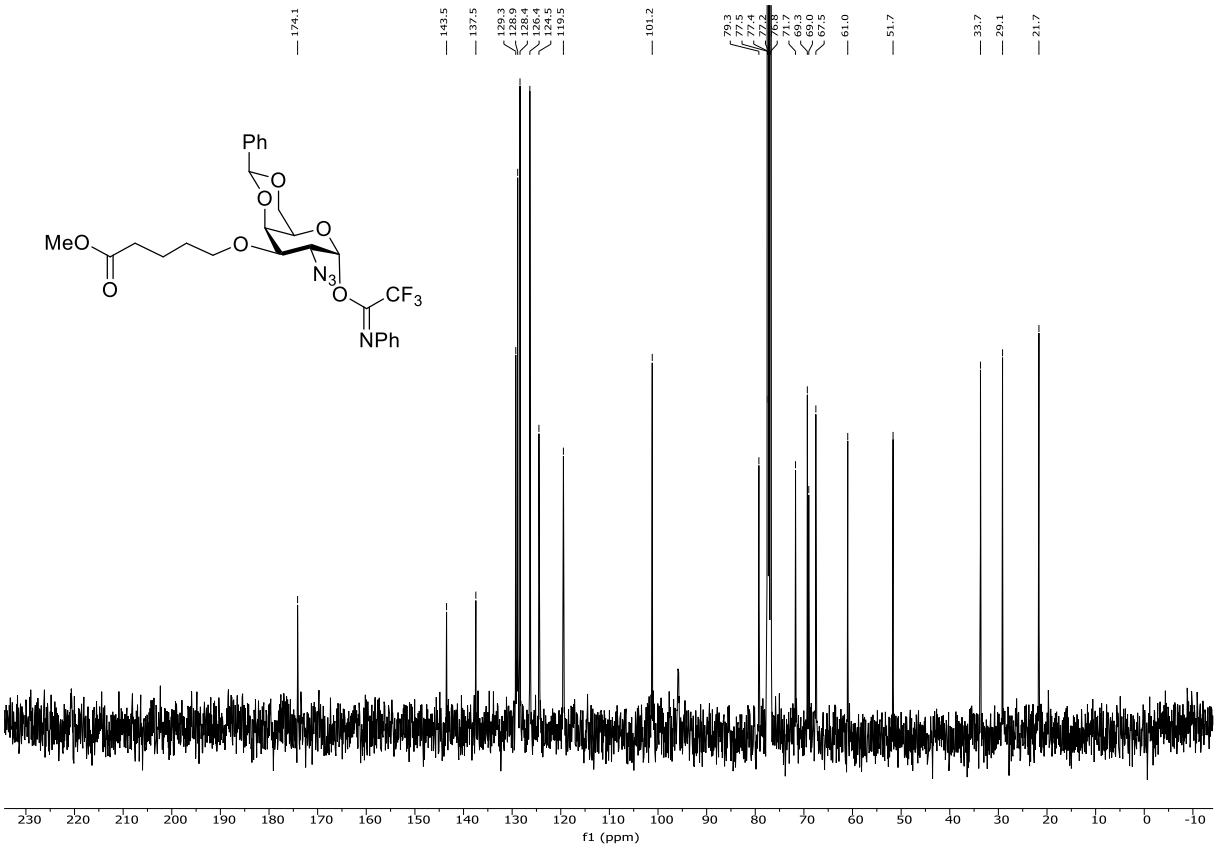
Phenyl 2-azido-2-deoxy-4,6-*O*-benzylidene-3-*O*-(methyl 5-pentanoate)-1-seleno-α-D-galactopyranoside (28)



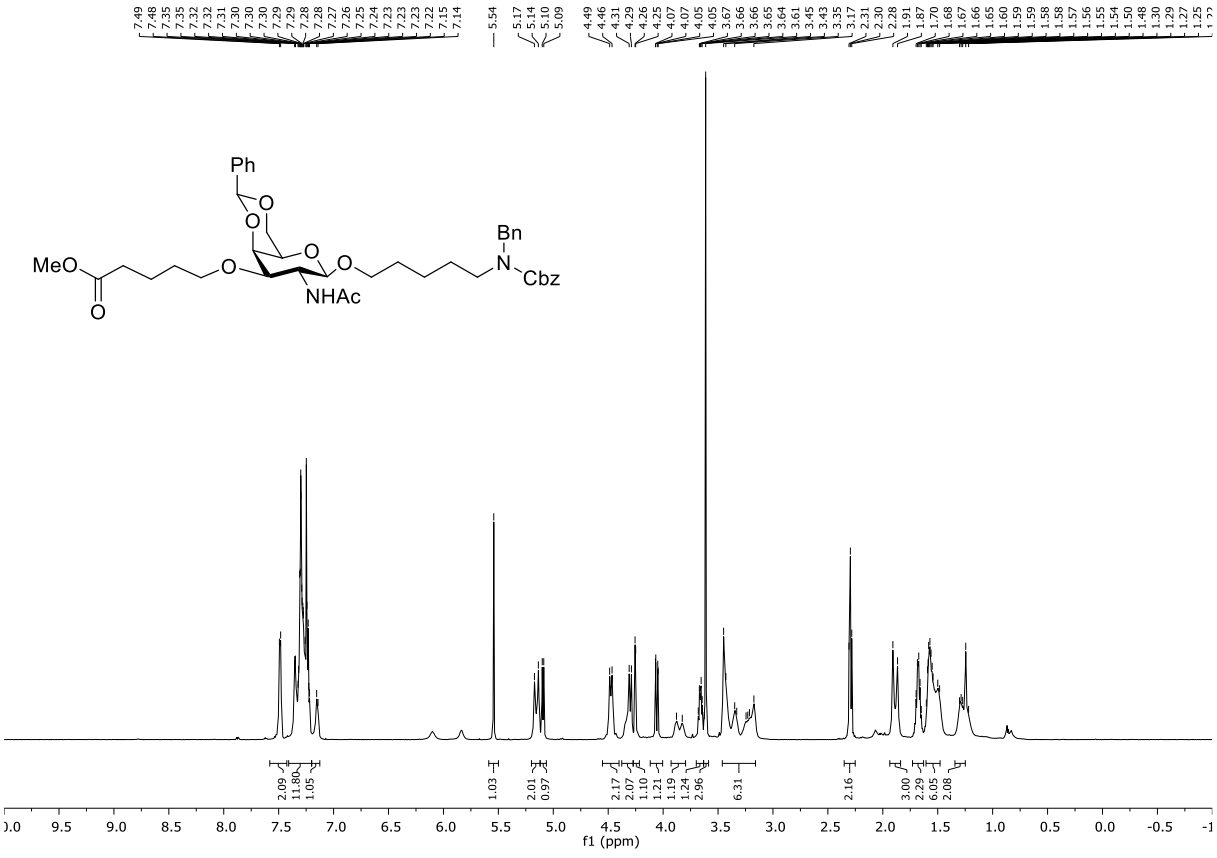


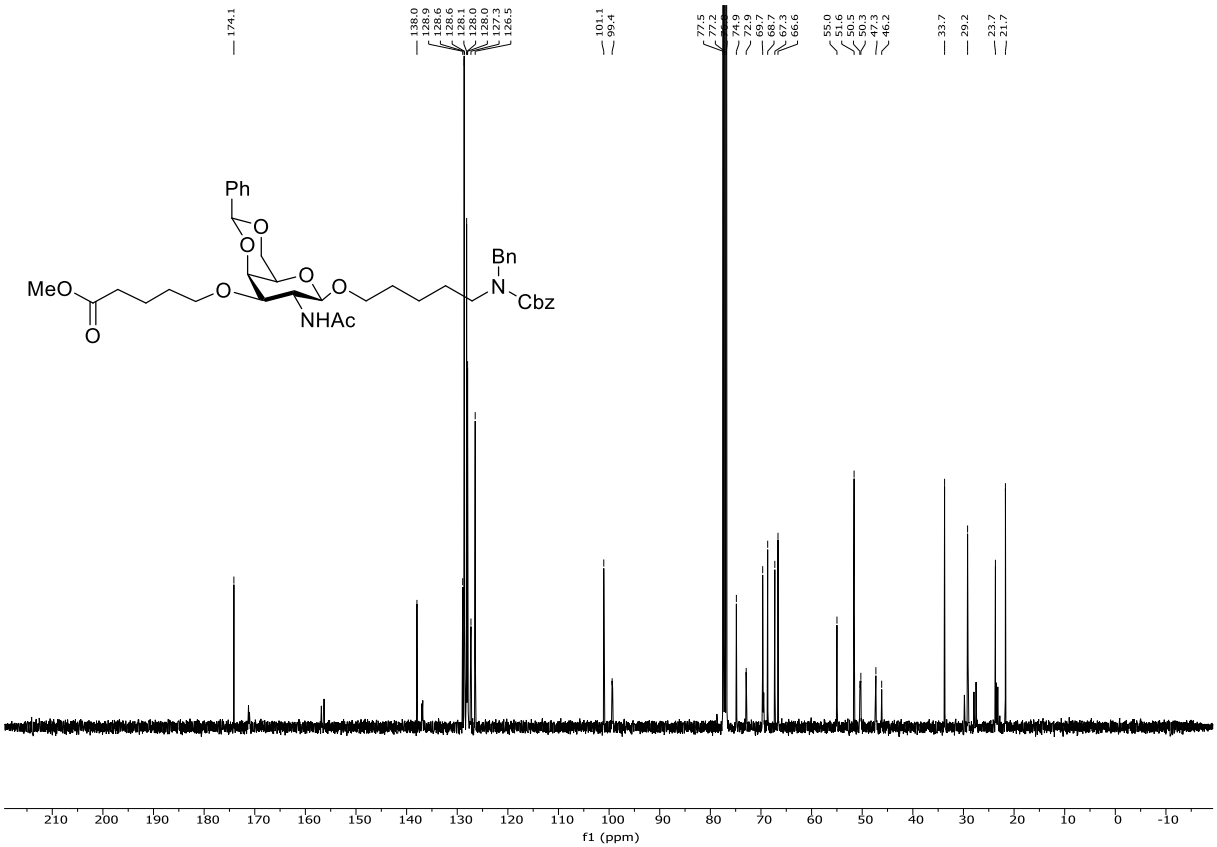
_N_-Phenyltrifluoroacetimidate 2-azido-2-deoxy-4,6-_O_-benzylidene-3-_O_-(methyl 5-pentanoate)-α-D-galactopyranoside (29)



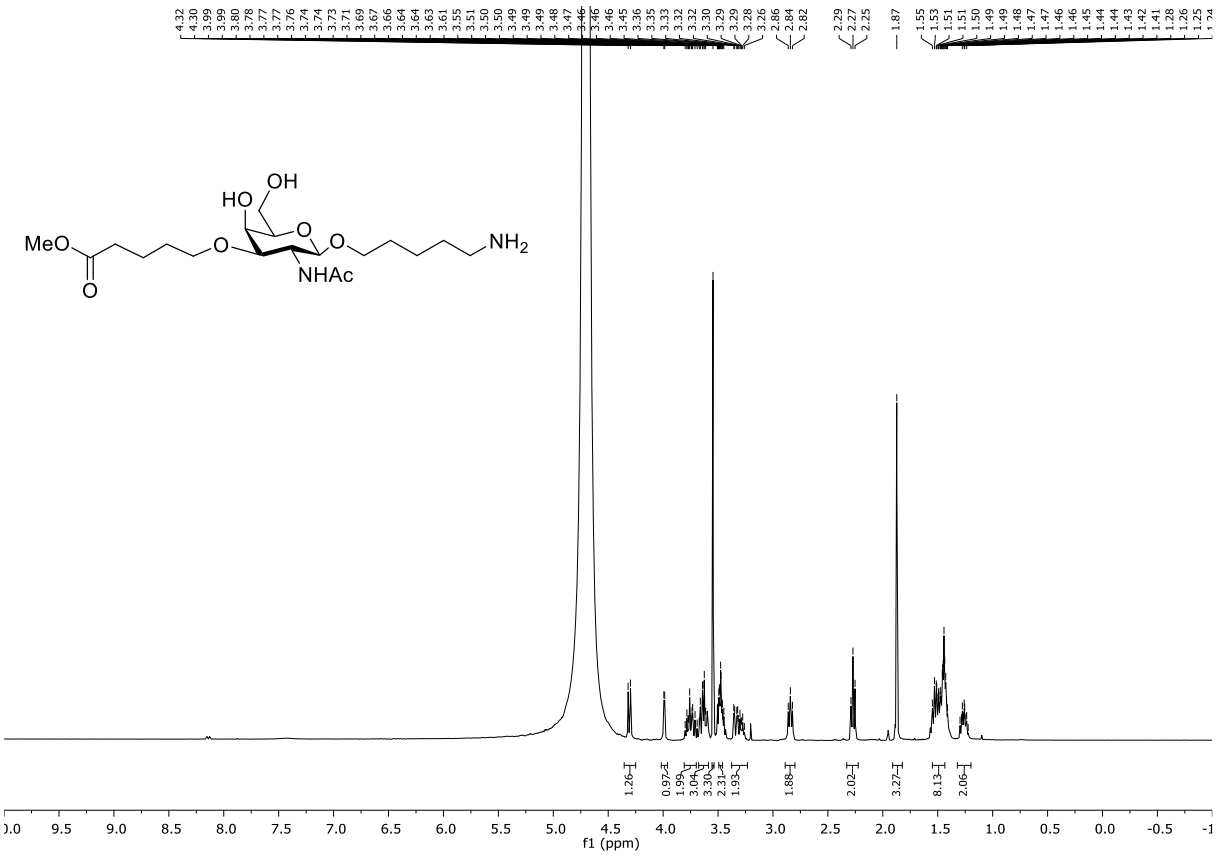


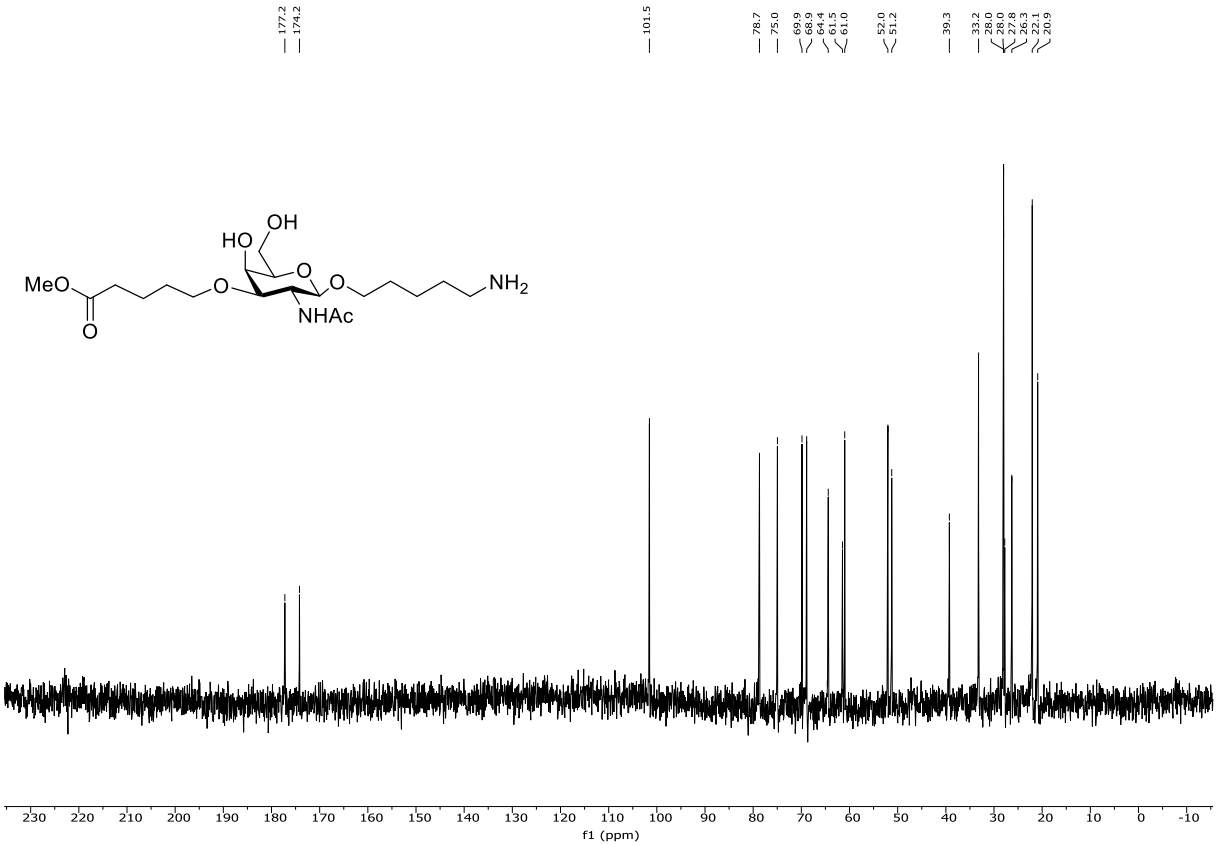
_N_-Benzyl-_N_-benzyloxycarbonyl-5-pentyl 2-acetamino-2-deoxy-4,6-_O_-benzylidene-3-_O_-(methyl 5-pentanoate)-β-D-galactopyranoside (31)



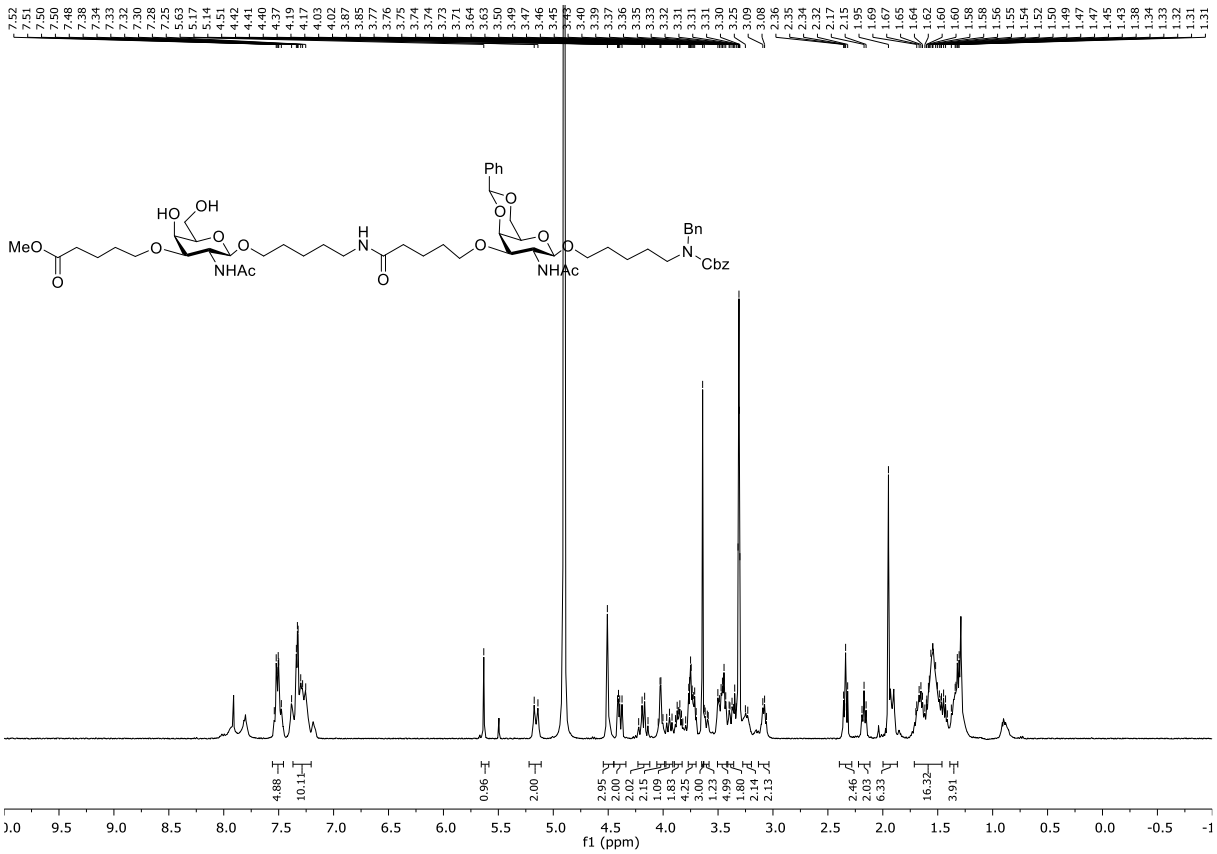


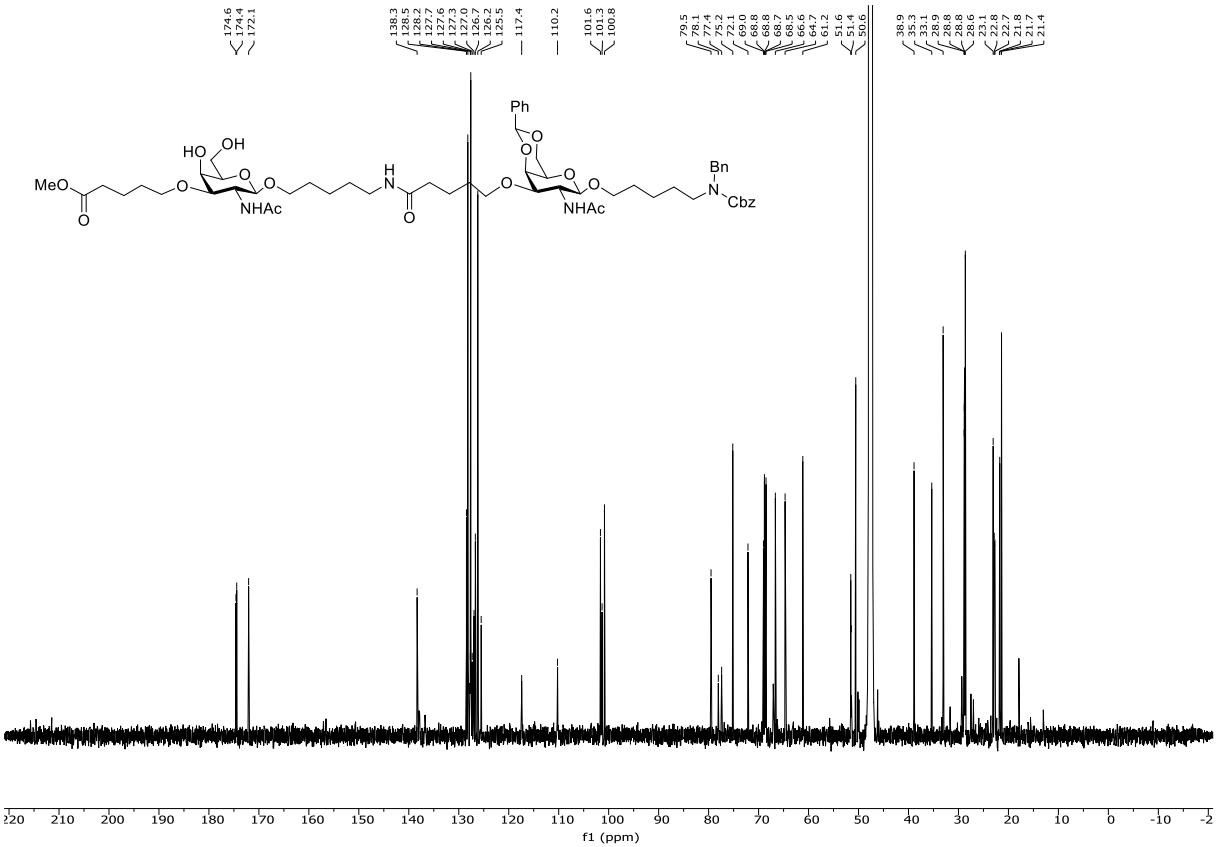
5-Aminopentyl 2-acetamino-2-deoxy-3-*O*-(methyl 5-pentanoate)-β-D-galactopyranoside (33)



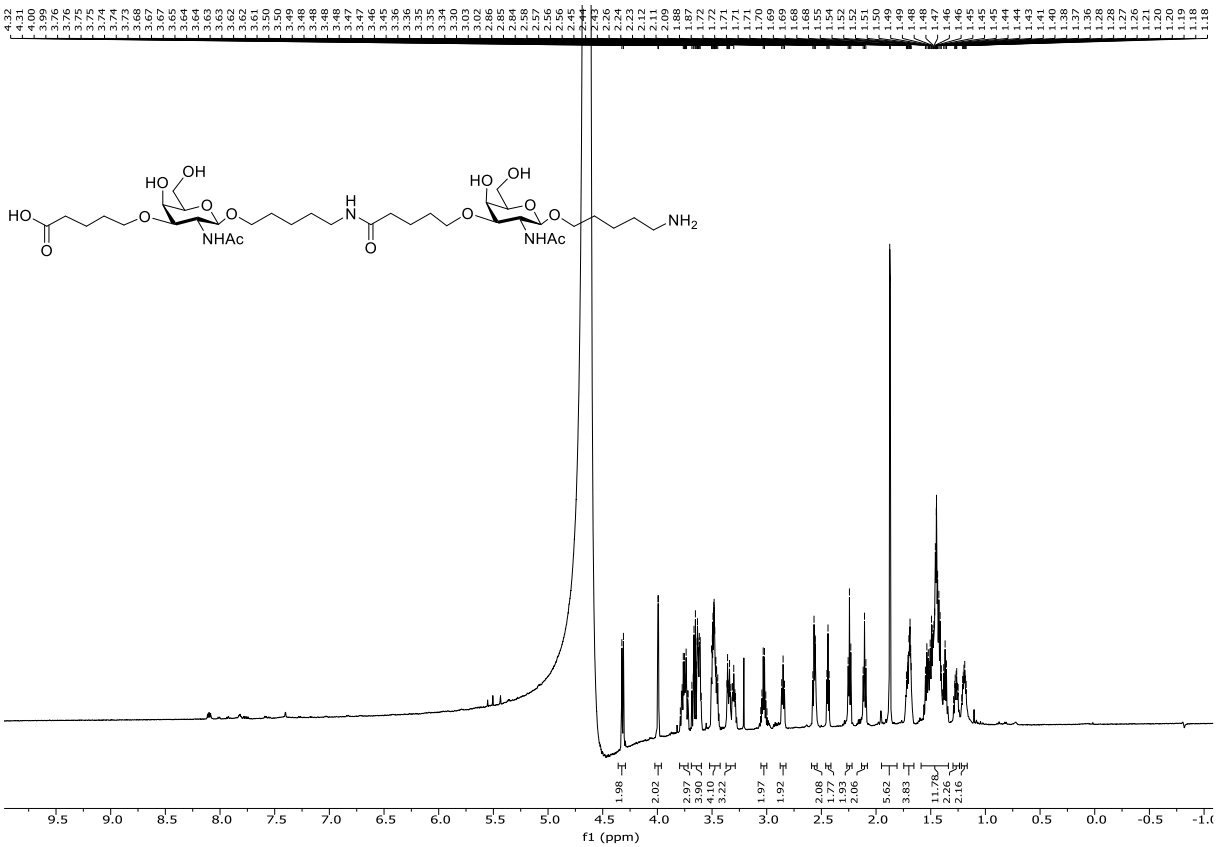


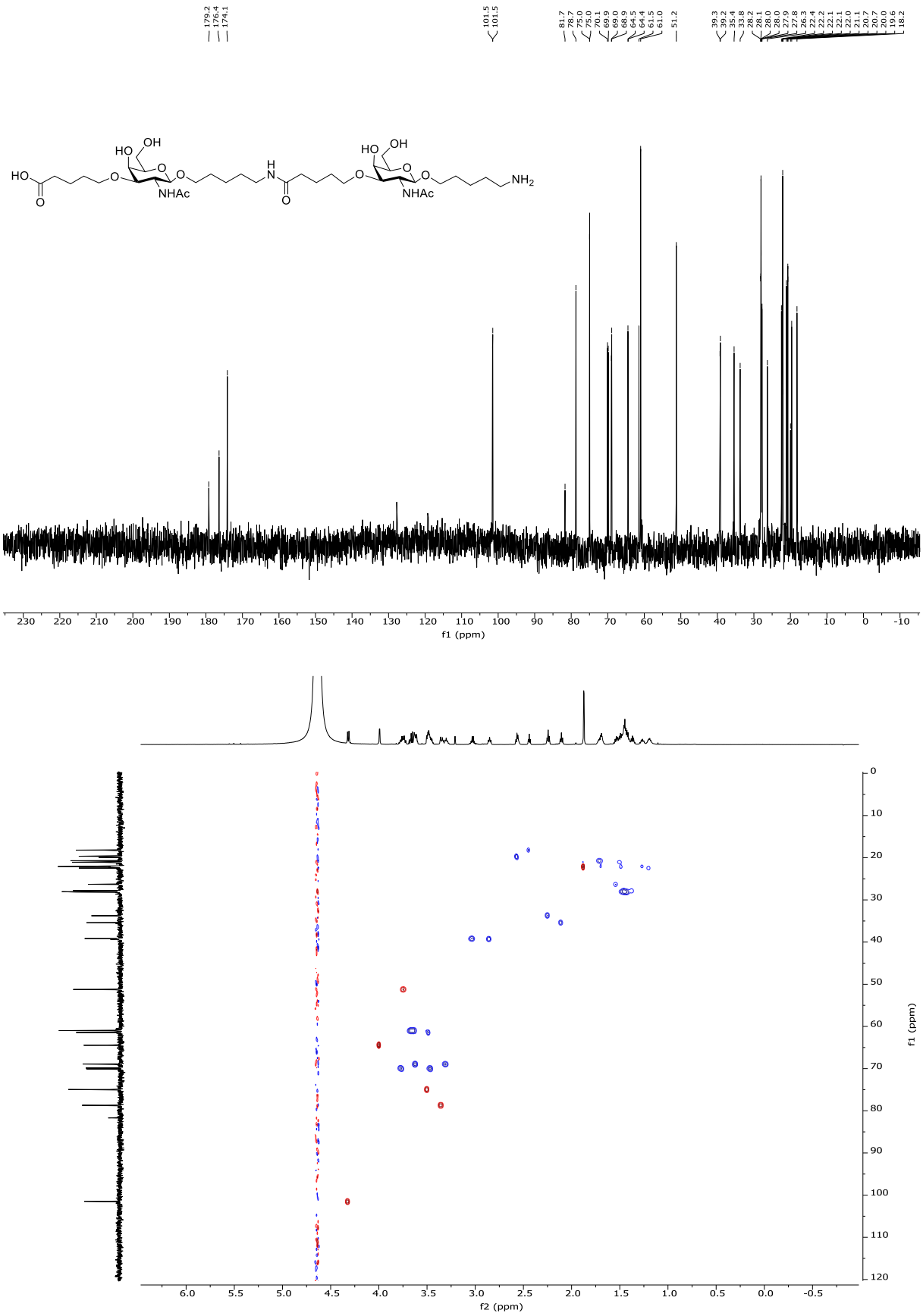
Protected divalent of *N*-acetyl-galactosamine (34)





Divalent of *N*-acetyl-galactosamine (35)




179.2
176.4
174.1
101.5
101.5
81.7
78.7
75.0
75.0
70.1
69.9
69.0
68.9
64.5
64.4
61.5
61.0
51.2
39.3
39.2
35.4
33.8
28.2
28.1
28.0
28.0
27.9
27.8
26.3
22.4
22.2
22.1
22.1
22.0
21.1
20.7
20.7
20.0
19.6
18.2
HO
OH
HO
O
O
O
NHAc
H
N
O
HO
OH
HO
O
O
O
NHAc
NH2
f1 (ppm)
f2 (ppm)
f1 (ppm)

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