Immunological Evaluation of Synthetic Glycosylphosphatidylinositol Glycoconjugates as Vaccine Candidates Against Malaria

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Supporting Information

General Methods

All purchased chemicals were of reagent grade and all anhydrous solvents were of high-purity grade and used as supplied except where noted otherwise. Reactions were performed in oven-dried glassware under an inert argon atmosphere unless noted otherwise. Reagent grade thiophene was dried over activated molecular sieves prior to use. Pyridine was distilled over CaH_2 prior to use. Sodium hydride suspension was washed with hexane and THF and stored in an anhydrous environment. Benzyl bromide was passed through activated basic aluminum oxide prior to use. Metal sodium was washed with hexane and stored in hexane. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plates (0.25mm). Compounds were visualized by UV irradiation or heating the plate after dipping in staining solution. The staining solutions were cerium sulfate-ammonium molybdate (CAM) solution, basic potassium permanganate solution, acidic ninhydrin-acetone solution, or a 3-methoxyphenol-sulfuric acid solution (Sugar Stain). Flash column chromatography was carried out using a forced flow of the indicated solvent on Sigma Aldrich silica gel high purity grade 60 Å (230-400 mesh particle size, for preparative column chromatography). Solvents were removed under reduced pressure using rotary evaporator and high vacuum (<1 mbar). Freeze drying of the aqueous solutions was performed using Alpha 2-4 LD Lyophilizer (Christ, Osterode am Harz, Germany)

^{1}H, ^{13}C and ^{31}P-NMR as well as all 2D-spectra (^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC) were recorded on a Varian 400 (400 MHz), a Varian 600 (600 MHz), a Bruker 400 (400 MHz) and a Bruker Ascend 400 (400 MHz) spectrometer in $CDCl_3$ (7.26 ppm ^{1}H, 77.1 ppm ^{13}C), D_2O (4.79 ppm ^{1}H), MeOD (4.87 ppm and 3.31 ppm ^{1}H, 49.00 ppm ^{13}C), Acetone-d_6 (2.05 ppm and 2.84 ppm ^{1}H, 206.26 ppm and 29.84 ppm ^{13}C) unless otherwise stated. The coupling constants (*J*) are reported in Hertz (Hz). Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; br, broad singlet; dd, doublet of doublets; m, multiplet; dt, doublet of triplets; h, hextet for ^{1}H NMR data. Signals were assigned by means of ^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC spectra and version thereof. ESI mass analyses were performed by Waters Xevo G2-XS Q-TOF with an Acquity H-class UPLC and a Bruker Autoflex-speed MALDI-TOF spectrometer.

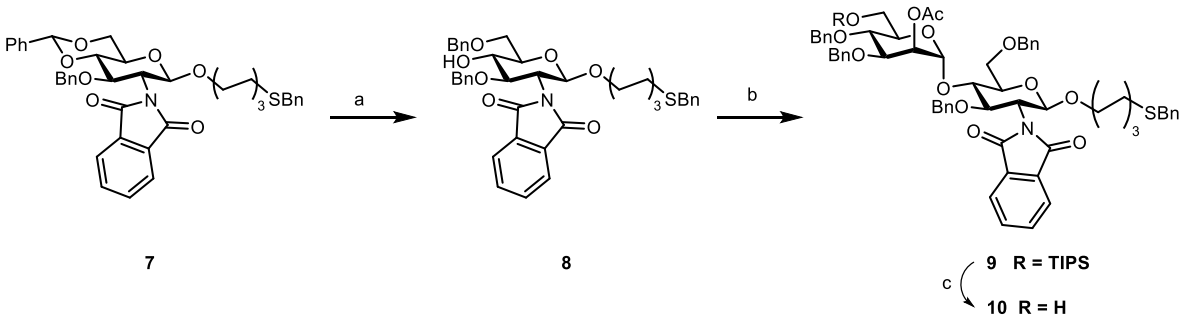
Synthesis of GPI fragments

Synthesis of fragment 6

The fragment **6** and all mannose building blocks were synthesized according to the previously published protocols.[1,2]

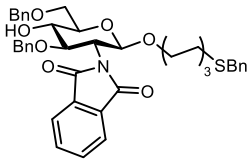
Synthesis of mannose α–(1→4) glucosamine disaccharide

The mannose α–(1→4) glucosamine disaccharide (10) is present in the fragments (1 to 4) and was used as a common building block to obtain the fragments *via* a convergent approach involving a [2+2] or a [2+3] glycosylation. α–(1→2)-dimannose and α–(1→2)-trimannose were synthesized using the established protocol.[3]



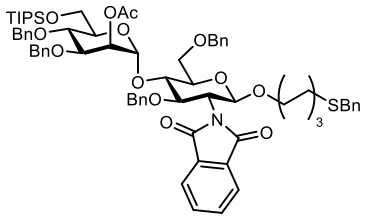
Scheme S1: Synthesis of pseudo-disaccharide **10**. a) TFAA, TFA, TES, CH_2Cl_2, 0 °C, 5 h, 80%; b) **2-6**, TMSOTf, CH_2Cl_2, -40 °C to -20 °C, 1 h, 88%; c) HF–py, THF, rt, 48 h, 83%.

1-O-(6-thiobenzyl)hexyl-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**8**)



To a stirred solution of **7**[4] (0.5 g, 0.72 mmol) in CH_2Cl_2 (8 mL) were added trifluoroacetic anhydride (0.31 mL, 2.16 mmol) and triethylsilane (0.58 mL, 3.6 mmol) were added at 0 °C followed by trifluoroacetic acid (0.28 mL, 3.6 mmol) drop wise. The reaction mixture was stirred at 0 °C. After 5 h, the reaction mixture was quenched with Et_3N and concentrated to give yellow oil that was purified by flash column chromatography to obtain glucoasamine acceptor **8** (0.4 g, 0.57 mmol, 80%) as a colorless oil. R_f = 0.33 (Hexanes/EtOAc = 3:1) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 – 7.60 (m, 4H), 7.39 – 7.18 (m, 8H), 7.04 (dt, *J* = 6.0, 2.2 Hz, 2H), 6.98 – 6.90 (m, 3H), 5.28 (s, 2H), 5.10 (dd, *J* = 8.3, 3.0 Hz, 1H), 4.73 (dd, *J* = 12.2, 1.6 Hz, 1H), 4.65 – 4.54 (m, 2H), 4.52 (dd, *J* = 12.2, 1.2 Hz, 1H), 4.21 (ddd, *J* = 10.8, 8.3, 1.3 Hz, 1H), 4.12 (ddd, *J* = 10.7, 8.3, 1.8 Hz, 1H), 3.85 – 3.69 (m, 4H), 3.63 (ddd, *J* = 9.5, 4.9, 1.6 Hz, 1H), 3.60 (s, 1H), 3.38 – 3.23 (m, 2H), 2.16 (t, *J* = 7.3 Hz, 1H), 1.44 – 1.23 (m, 3H), 1.21 – 0.93 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.1, 137.5, 133.8, 128.7, 128.5, 128.4, 128.1, 127.9, 127.8, 127.8, 127.3, 126.8, 98.2, 78.6, 74.6, 74.2, 73.7, 73.3, 70.8, 69.4, 55.3, 53.4, 44.7, 36.1, 32.3, 31.0, 29.0, 28.9, 28.3, 26.3, 25.3, 25.1. ESI-MS (*m*/*z*): $[M+Na]^+$ cald 718.86 obsd 718.6

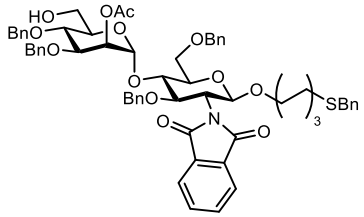
2-O-Acetyl-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→4)-1-O-(6-thiobenzyl)hexyl-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**9**)



The mannose donor (0.36 g, 0.52 mmol) and glucosamine acceptor **8** (0.3 g, 0.43 mmol) were co-evaporated with anhydrous toluene (3x7 mL) and dried under high vacuum for 2 h. The mixture was dissolved in anhydrous CH_2Cl_2 (8 mL) and activated molecular sieves were added. The solution was stirred for 10 min at room temperature and cooled to -40 °C. The mixture was treated with TMSOTf (19 µL, 0.086 mmol) and slowly warmed to -20 °C over a period of 1 h.

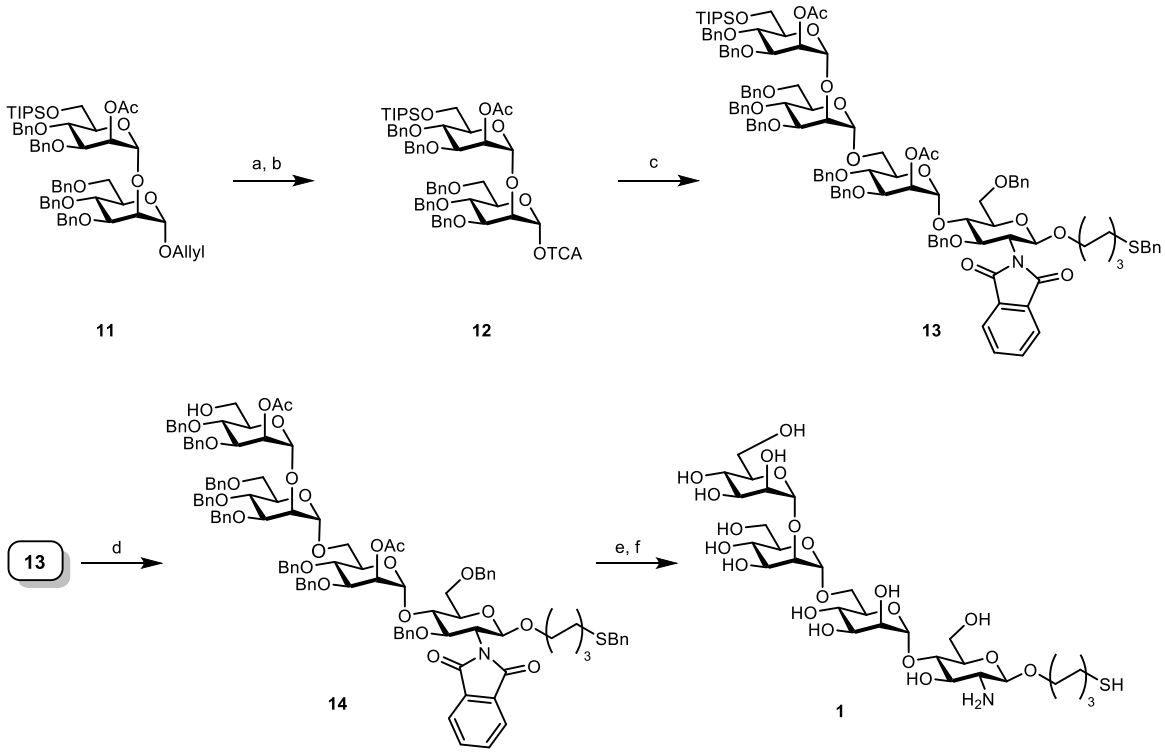
The reaction was diluted with CH_2Cl_2, quenched with Et_3N and concentrated. The crude product was purified by flash column chromatography to obtain disaccharide **9** (0.47 g, 0.38 mmol, 88%) as white solid. R_f= 0.5 (Hexanes/EtOAc = 3:1) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 16.0 Hz, 5H), 7.39 – 7.17 (m, 28H), 7.00 (d, *J* = 7.4 Hz, 2H), 6.88 (t, *J* = 7.6 Hz, 2H), 6.77 (t, *J* = 7.3 Hz, 1H), 5.42 (s, 1H), 5.27 (s, 1H), 5.05 (dd, *J* = 8.4, 2.7 Hz, 1H), 4.85 (dd, *J* = 15.8, 11.5 Hz, 3H), 4.76 – 4.47 (m, 7H), 4.37 (dd, *J* = 10.7, 8.7 Hz, 1H), 4.28 (d, *J* = 12.2 Hz, 1H), 4.20 – 3.98 (m, 3H), 3.95 – 3.85 (m, 4H), 3.79 – 3.70 (m, 5H), 3.63 (d, *J* = 25.1 Hz, 4H), 3.32 (dt, *J* = 9.7, 6.8 Hz, 1H), 2.15 (t, *J* = 7.2 Hz, 2H), 1.97 (s, 3H), 1.42 – 1.25 (m, 3H), 1.17 (dt, *J* = 15.0, 6.9 Hz, 3H), 1.13 – 1.03 (m, 7H), 1.04 (s, 22H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 138.7, 138.2, 138.0, 137.8, 133.7, 128.7, 128.4, 128.34, 128.3, 128.2, 128.0, 128.0, 127.9, 127.9, 127.9, 127.6, 127.6, 127.3, 127.29, 127.07, 126.83, 99.1, 98.0, 81.0, 77.9, 76.8, 75.2, 74.6, 74.5, 73.7, 73.5, 73.2, 71.8, 69.3, 69.2, 68.9, 62.1, 55.6, 36.1, 31.0, 29.0, 28.9, 28.3, 26.8, 25.3, 20.8, 18.0, 17.9, 12.0, 11.9. ESI-MS (*m*/*z*): $[M+Na]^+$ cald 1258.58 obsd 1528.4

2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-1-O-(6-thiobenzyl)hexyl-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**10**)



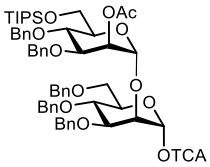
To a stirred solution of disaccharide **9** (0.16 g, 0.13 mmol) in THF (4 mL) was added 70% HF in pyridine (0.08 mL, 0.65 mmol). The reaction mixture was stirred for 48 h at room temperature. The reaction was quenched with aq. $NaHCO_3$, extracted with CH_2Cl_2, washed with brine and dried over Na_2SO_4. The solvents were removed under reduced pressure and the crude product was purified by flash column chromatography to obtain the disaccharide acceptor **10** (0.12 g, 0.11 mmol, 83%). R_f= 0.4 (Hexanes/EtOAc = 2:1) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 – 7.63 (m, 6H), 7.51 – 7.21 (m, 20H), 7.07 – 6.91 (m, 4H), 6.88 – 6.79 (m, 1H), 5.55 – 5.47 (m, 1H), 5.35 (d, *J* = 1.4 Hz, 1H), 5.07 (d, *J* = 8.5 Hz, 1H), 4.88 (dd, *J* = 24.8, 11.5 Hz, 1H), 4.78 – 4.60 (m, 3H), 4.63 – 4.51 (m, 1H), 4.44 (ddd, *J* = 11.4, 8.7, 2.9 Hz, 1H), 4.32 (d, *J* = 12.2 Hz, 1H), 4.24 – 4.11 (m, 1H), 4.10 – 3.89 (m, 2H), 3.90 – 3.58 (m, 10H), 3.36 (dt, *J* = 9.8, 6.6 Hz, 1H), 2.19 (t, *J* = 7.3 Hz, 1H), 2.05 (s, 3H), 1.38 (ddt, *J* = 30.1, 13.0, 6.8 Hz, 3H), 1.30 – 0.84 (m, 7H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.9, 138.5, 138.1, 138.0, 137.8, 137.7, 133.8, 130.0, 129.0, 128.8, 128.4, 128.4, 128.3, 128.1, 128.0, 128.0, 127.8, 127.8, 127.7, 127.6, 127.2, 127.1, 126.9, 99.2, 98.0, 81.2, 77.9, 76.9, 75.3, 74.9, 74.70, 74.0, 73.6, 73.1, 71.8, 69.4, 68.8, 68.6, 62.0, 55.8, 36.2, 31.0, 29.1, 28.9, 28.4, 25.4, 20.9. ESI-MS (*m*/*z*): $[M+Na]^+$ cald 1102.43 obsd 1102.4

Synthesis of fragment 1



Scheme S2: Synthesis of fragment GPI **1.** a) i. H_2, $[Ir(COD)(PMePh_2)_2]PF_6$, THF, rt, 12 h; ii. HgO, $HgCl_2$, acetone–H_2O (5:1), rt, 2 h, 81% ; b) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 2 h, 86%; c) TBSOTf, Et_2O, 0 °C, 2 h, 55% (α-isomer); d) $Sc(OTf)_3$, H_2O, CH_3CN, 50 °C, 12 h, 71%; e) $(CH_2NH_2)_2$, H_2O, *n*-BuOH, 90°C, 4 h; f) i. Na, liq. NH_3, THF, -78 °C, 1 h; ii. MeOH, rt, 1 h, 57% (over two steps).

2-O-Acetyl-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri- O-benzyl-α–D-mannopyranosyl trichloroacetimidate (**12**)

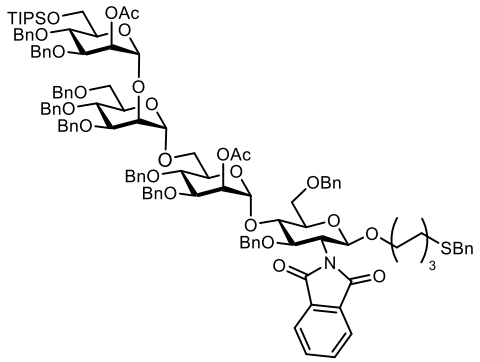


A solution of $[IrCOD(PPh_2Me)_2]PF_6$ (5.0 mg, 5.8 μmol) in THF (3 mL) was stirred under hydrogen atmosphere until the color turned from red to colorless to pale yellow. The hydrogen atmosphere was exchanged with Argon. The activated catalyst solution was added to a solution of disaccharide **11** (0.30 g, 0.29 mmol) in THF (10 mL) and stirred at room temperature. After 16 h, the solvent was removed and the residue was dissolved in a mixture of acetone (5.2 mL) and water (0.6 mL). Mercury (II) chloride (0.39 g, 1.45 mmol) and mercury (II) oxide (0.01 mg, 0.044 mmol) were added. After 1 h, saturated aq. $NaHCO_3$ was added to reaction mixture and was extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography to obtain disaccharide hemiacetal intermediate (0.24 g, 0.24 mmol, 81%) as colorless oil.

To a stirred solution of hemiacetal (0.285 g, 0.28 mmol) in CH_2Cl_2 (13 mL) at 0 °C were added CCl_3CN (0.29 mL, 2.88 mmol) and DBU (0.009 mL, 0.06 mmol). The reaction mixture was stirred for 1 h at 0 °C. The resulting mixture was concentrated and purified by flash column

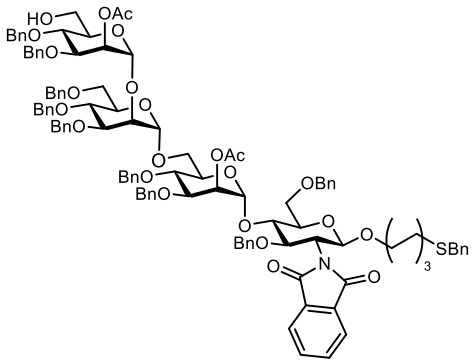
chromatography to obtain imidate donor **12** (0.28 g, 0.25 mmol, 86%) as clear oil. R_f = 0.4 (EtOAc/hexane = 2:3) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.39 – 7.31 (m, 5H), 7.31 – 7.13 (m, 17H), 6.29 (s, 1H), 5.51 (s, 1H), 5.15 (s, 1H), 4.93 – 4.85 (m, 2H), 4.79 – 4.68 (m, 3H), 4.67 – 4.56 (m, 2H), 4.47 (dd, *J* = 28.1, 11.5 Hz, 2H), 4.12 (s, 1H), 4.08 – 3.91 (m, 8H), 3.86 – 3.77 (m, 2H), 3.70 (d, *J* = 11.3 Hz, 1H), 2.11 (s, 3H), 1.08 (d, *J* = 4.7 Hz, 21H).

1-O-(6-thiobenzyl)hexyl-2-O-acetyl-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**13**)



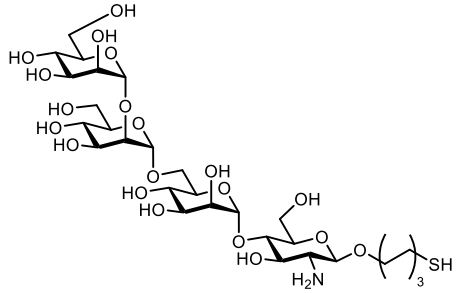
The disaccharide imidate **12** (0.18 g, 0.16 mmol) and disaccharide acceptor **9** (0.14 g, 0.13 mmol) were co-evaporated with toluene (3 x 5 mL) and dried under high vacuum for 2 h. The mixture was dissolved in a mixture of anhydrous diethylether and anhydrous CH_2Cl_2 (1:1, 6 mL) and activated molecular sieves were added. The solution was stirred for 10 min at room temperature and cooled to 0 °C. The mixture was treated with TBSOTf (9 µL, 0.04 mmol) and stirred at 0 °C for a period of 1 h. The reaction was diluted with CH_2Cl_2, quenched with Et_3N and concentrated. The crude product was purified by flash column chromatography to obtain tetrasaccharide **13** (0.15 g, 0.07 mmol, 55 % α–isomer). R_f = 0.3 (EtOAc/hexane = 2:3) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.59 (m, 6H), 7.31 (dd, *J* = 17.9, 11.8 Hz, 30H), 7.21 (dd, *J* = 14.7, 7.7 Hz, 36H), 7.17 – 7.08 (m, 7H), 7.01 (d, *J* = 7.4 Hz, 3H), 6.90 (t, *J* = 7.5 Hz, 3H), 6.79 (t, *J* = 7.2 Hz, 1H), 5.49 (d, *J* = 13.5 Hz, 3H), 5.34 (s, 1H), 5.15 (s, 1H), 5.08 – 5.01 (m, 2H), 4.93 – 4.79 (m, 8H), 4.72 – 4.27 (m, 23H), 4.23 – 3.99 (m, 7H), 4.02 – 3.79 (m, 16H), 3.77 (t, *J* = 10.2 Hz, 7H), 3.75 – 3.58 (m, 7H), 3.55 – 3.38 (m, 5H), 3.39 – 3.21 (m, 3H), 2.41 – 2.28 (m, 2H), 2.15 (t, *J* = 7.2 Hz, 1H), 2.07 (s, 3H), 1.98 (s, 3H), 1.08 (d, *J* = 4.1 Hz, 30H), 0.96 (d, *J* = 36.0 Hz, 2H), 0.92 – 0.81 (m, 23H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 169.8, 138.8, 138.5, 138.4, 138.1, 138.0, 138.0, 137.7, 137.6, 133.7, 129.9, 128.9, 128.7, 128.4, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.1, 127.9, 127.9, 127.9, 127.7, 127.5, 127.49, 127.4, 127.3, 127.2, 127.2, 127.1, 126.85, 99.3, 99.0, 98.8, 98.0, 80.9, 79.6, 78.4, 77.9, 75.2, 75.0, 74.7, 74.5, 74.2, 73.77, 73.3, 73.1, 71.88, 71.8, 71.7, 71.5, 69.3, 68.8, 68.5, 66.1, 62.5, 55.7, 36.2, 32.3, 31.9, 31.0, 29.7, 29.37, 29.0, 28.9, 28.3, 26.3, 25.3, 22.7, 21.0, 20.95, 18.1, 18.0, 14.1, 12.0. HRMS (*m*/*z*): $[M+H]^+$ cald 2053.0140 obsd 2053.0144

1-O-(6-thiobenzyl)hexyl-2-O-acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**14**)



To a stirred solution of tetrasaccharide **13** (0.13 g, 0.190 mmol) in CH_3CN (7 mL) were added water (50 μL) and $Sc(OTf)_3$ (0.093 g, 0.19 mmol). The reaction mixture was heated up to 50 °C ans stirred for 6 h. The reaction was quenched with Et_3N and concentrated. The crude product was purified by flash column chromatography to obtain **14** (0.09 g, 0.05 mmol, 71%). R_f = 0.35 (EtOAc/hexane = 1:1) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 16.4 Hz, 4H), 7.30 (dd, *J* = 9.4, 6.1 Hz, 17H), 7.23 (d, *J* = 7.3 Hz, 22H), 7.16 (dd, *J* = 14.8, 5.9 Hz, 9H), 7.00 (d, *J* = 7.5 Hz, 2H), 6.89 (t, *J* = 7.5 Hz, 2H), 6.77 (t, *J* = 7.3 Hz, 1H), 5.48 (s, 2H), 5.32 (s, 1H), 5.07 – 4.97 (m, 2H), 4.95 – 4.83 (m, 3H), 4.82 (d, *J* = 11.5 Hz, 3H), 4.69 – 4.54 (m, 8H), 4.50 (dd, *J* = 20.1, 9.3 Hz, 3H), 4.47 – 4.33 (m, 5H), 4.29 (d, *J* = 12.2 Hz, 1H), 4.22 – 4.13 (m, 1H), 4.07 (s, 1H), 4.01 – 3.68 (m, 24H), 3.65 – 3.53 (m, 5H), 3.53 – 3.44 (m, 3H), 3.37 – 3.21 (m, 2H), 2.14 (t, *J* = 6.7 Hz, 2H), 2.08 (s, 3H), 1.98 (s, 3H), 1.37 (dd, *J* = 15.8, 8.1 Hz, 3H), 1.35 – 1.22 (m, 3H), 1.15 (dd, *J* = 15.2, 7.9 Hz, 3H), 1.12 – 0.91 (m, 6H), 0.86 (d, *J* = 10.4 Hz, 3H). . ESI-MS (*m*/*z*): $[M+Na]^+$ cald 1918.80 obsd 1918.5

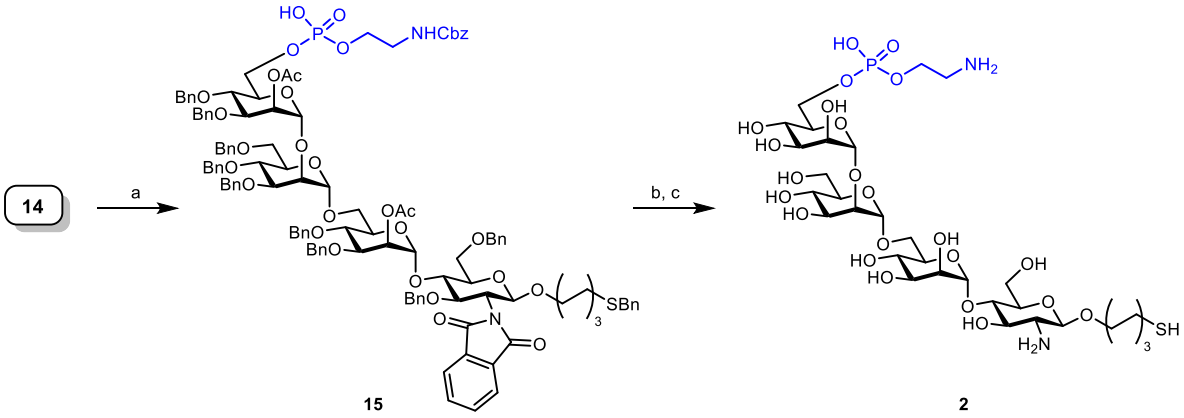
1-O-(6-thio)hexyl-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranoside (**1**)



Tetrasaccharide **14** (0.03 g, 0.02 mmol) was dissolved in a mixture of ethylenediamine (2 mL) and butanol (2 mL) and stirred at 90 °C for 3 h. After 3 h, the reaction mixture was concentrated to obtain partially deprotected crude intermediate. The crude intermediate was dissolved in anhydrous THF and MeOH. This solution was added dropwise to blue solution of 20 mL liquefied ammonia with sodium -78 °C. The reaction was stirred at -78 °C. After 1 h, the reaction was quenched with MeOH and stirred for additional 1h at rt. Sodium methoxide generated in the reaction was quenched by dropwise addition of glacial acetic acid. The reaction mixture was concentrated and the crude product was purified by size exclusion column chromatography using a Sephadex® super fine G-15 (GE Healthcare) column and 5% ethanol in water as eluent to obtain **1** (7 mg, 0.009 mmol, 57%) as white solid. ^{1}H NMR (600 MHz, Deuterium Oxide) δ 5.08 (s, 1H), 5.02 (s, 1H), 4.91 (s, 1H), 4.53 – 4.37 (m, 0H), 3.94 (ddd, *J* = 5.6, 3.1, 1.9 Hz, 3H), 3.91 – 3.88 (m, 2H), 3.85 – 3.74 (m, 9H), 3.75 – 3.52 (m, 22H), 3.53 – 3.42 (m, 4H), 3.23 – 3.08 (m, 1H), 2.97 – 2.91 (m, 1H), 2.86 – 2.72 (m, 1H), 2.64 (t, *J* = 7.2 Hz, 1H), 1.55 (ddd, *J* = 25.0, 14.1, 7.0 Hz, 6H), 1.30 – 1.25 (m, 6H). ^{13}C NMR (151 MHz, d_2o)

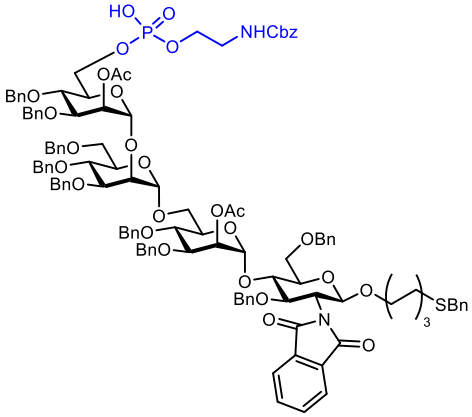
δ 104.9, 104.5, 100.9, 81.2, 79.7, 75.8, 75.3, 74.8, 72.9, 72.7, 72.5, 69.5, 69.4, 69.0, 63.7, 63.5, 30.7, 29.8, 27.2, 25.8. HRMS (*m*/*z*) of the oxidized compound: $[M+2H]^{2+}$ cald 781.3033 obsd 781.3027

Synthesis of fragment 2



Scheme S3: Synthesis of fragment GPI **2**. a) i. **2-3**, PivCl, py, rt, 16 h; ii. I_2, H_2O, py, rt, 1 h, 66% ; b) $(CH_2NH_2)_2$, H_2O, *n*-BuOH, 90 °C, 4 h; c) i. Na, liq. NH_3, THF, -78 °C, 1 h; ii. MeOH, rt, 1 h, 75% (over two steps).

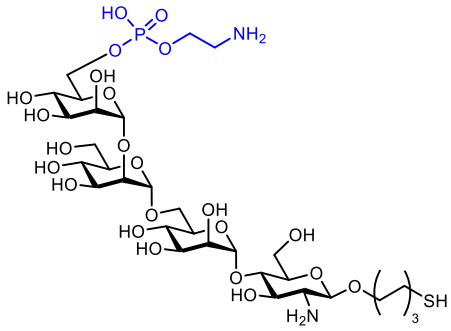
1-O-(6-thiobenzyl)hexyl-2-O-Acetyl-3,4-di-O-benzyl-6-O-(2-N-benzyloxycarbonyl) aminoethyl-phosphonato-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**15**)



Tetrasaccharide **14** (0.02 g, 10.5 µmol) and *H*–phosphonatethanolamine (0.01 g, 0.03 mmol) were co-evaporated with pyridine for three times and dried under high vacuum for 2 h. The mixture was dissolved in anhydrous pyridine (5 mL) and a solution of pivolyl chloride (4 µL, 0.03 mmol) in pyridine (1 mL) was added. The solution was stirred for 6 h at room temperature. After 6 h, iodine (8 mg, 0.03 mmol) and water (0.05 mL) were added and reaction was stirred for additional 2 h at room temperature. The reaction mixture was quenched with $Na_2S_2O_3$ and extracted with CH_2Cl_2. The organic layer were concentrated and purified by Et_3N deactivated silica gel flash column chromatography to obtain phosphorylated tetrasaccharide **15** (15 mg, 7 µmol, 66%). R_f = 0.5 (MeOH/ CH_2Cl_2 = 1:10) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 16.6 Hz, 4H), 7.21 (dd, *J* = 11.7, 8.2 Hz, 27H), 7.15 (dd, *J* = 10.7, 5.4 Hz, 15H), 7.14 – 7.01 (m,

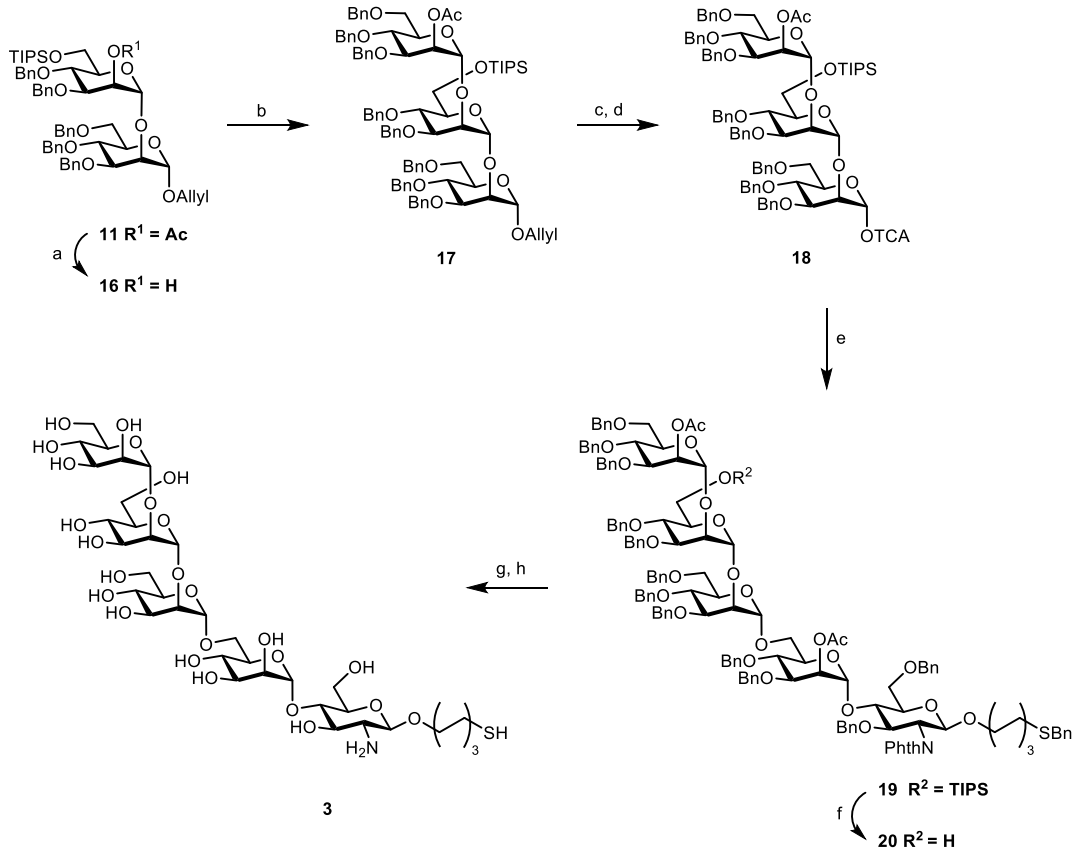
4H), 5.48 (d, J = 8.0 Hz, 2H), 5.37 (s, 1H), 5.25 (s, 0H), 5.09 (s, 1H), 5.03 – 4.93 (m, 1H), 4.87 – 4.67 (m, 5H), 4.64 – 4.34 (m, 9H), 3.96 – 3.85 (m, 5H), 3.76 (td, J = 23.3, 21.0, 9.8 Hz, 7H), 3.65 – 3.52 (m, 4H), 3.37 – 3.17 (m, 7H), 2.11 (t, J = 7.2 Hz, 1H), 2.00 – 1.90 (m, 6H), 1.53 (t, J = 7.3 Hz, 0H), 1.25 – 1.16 (m, 4H), 1.14 – 0.94 (m, 3H). ^{31}P NMR (162 MHz, $CDCl_3$) δ -1.95. ESI-MS (*m*/*z*): $[M+Na]^+$ cald 2176.83 obsd 2176.9

1-O-(6-thio)hexyl-6-O-aminoethyl-phosphonato-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-2-deoxy-2-amino-β-D-glucopyranoside (**2**)



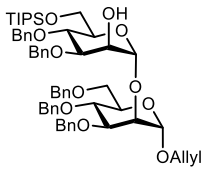
Tetrasaccharide **15** (16 mg, 7.5 µmol) was dissolved in a mixture of ethylenediamine (2 mL) and butanol (2 mL) and stirred at 90 °C for 3 h. After 3 h, the reaction mixture was concentrated to obtain partially deprotected crude intermediate. The crude intermediate was dissolved in anhydrous THF and MeOH. This solution was added dropwise to blue solution of 20 mL liquefied ammonia with sodium -78 °C. The reaction was stirred at -78 °C. After 1 h, the reaction was quenched with MeOH and stirred for additional 1h at rt. Sodium methoxide generated in the reaction was quenched by dropwise addition of glacial acetic acid. The reaction mixture was concentrated and the crude product was purified by size exclusion column chromatography using a Sephadex® super fine G-15 (GE Healthcare) column and 5% ethanol in water as eluent to obtain **2** (5 mg, 5.6 µmol, 75%) as white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.14 – 5.06 (m, 1H), 5.04 – 4.96 (m, 1H), 4.93 – 4.87 (m, 1H), 4.37 (d, J = 8.2 Hz, 1H), 4.06 – 3.39 (m, 26H), 3.21 – 3.13 (m, 2H), 2.83 – 2.74 (m, 1H), 2.67 (s, 1H), 1.62 (dt, J = 15.6, 7.5 Hz, 2H), 1.54 – 1.47 (m, 2H), 1.30 (ddd, J = 23.9, 13.9, 6.8 Hz, 4H). ^{13}C NMR (151 MHz, D_2O) δ 105.0, 104.4, 100.8, 81.5, 79.8, 77.4, 75.3, 74.8, 72.7, 72.4, 69.4, 68.8, 64.5, 63.4, 53.5, 42.6, 30.9, 29.9, 27.2, 26.4, 25.8. ^{31}P NMR (243 MHz, d_2o) δ -2.75. HRMS (*m*/*z*): $[M+H]^+$ cald 905.2961 obsd 905.2836

Synthesis of fragment 3



Scheme S4: Synthesis of fragment GPI **3**. a) NaOMe, MeOH/CH_2Cl_2, rt, 12 h, quant; b) TBSOTf, Et_2O, 0 °C, 2 h, 68%; c) i. H_2, $[Ir(COD)(PMePh_2)_2]PF_6$, THF, rt, 12 h; ii. HgO, $HgCl_2$, acetone-H_2O (5:1), rt, 2 h, 64% ; d) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 2 h, 80%; e) TBSOTf, Et_2O, 0 °C, 2 h, 65% (α–isomer); f) $Sc(OTf)_3$, H_2O, CH_3CN, 50 °C, 12h, 72%; g) $(CH_2NH_2)_2$, H_2O, *n*-BuOH, 90 °C, 4 h; h) i. Na, liq. NH_3, THF, -78 °C, 1 h; ii. MeOH, rt, 1 h, 74% (over two steps).

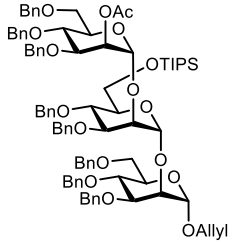
n-Allyl-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**16**)



To a stirred solution of dimannoside **11** (1.1 g, 1.07 mmol) in MeOH (12 mL) was added freshly prepared 1 M solution of NaOMe. After 1 h, the reaction was neutralized with Amberlite IR 120 H^+ resin, filtered and concentrated to obtain crude product **16** (0.96 g, 0.97 mmol, 91%). R_f = 0.35 (EtOAc/Hexanes 1:3) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 28H), 7.27 – 7.15 (m, 5H), 5.85 (ddt, *J* = 16.3, 10.9, 5.5 Hz, 1H), 5.25 (dd, *J* = 3.5, 1.5 Hz, 2H), 5.20 (d, *J* = 1.5 Hz, 1H), 5.17 – 5.12 (m, 2H), 4.91 – 4.80 (m, 4H), 4.76 – 4.58 (m, 5H), 4.60 – 4.49 (m, 4H), 4.14 (dd, *J* = 13.5, 4.5 Hz, 4H), 4.00 – 3.66 (m, 15H), 1.06 (d, *J* = 4.3 Hz, 21H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5, 138.5, 138.3, 138.1, 138.0, 133.7, 128.4, 128.4, 128.3, 128.3, 128.2, 127.9, 127.9, 127.8, 127.8, 127.7, 127.6, 127.6, 127.4, 127.4, 117.1, 100.1, 98.2, 80.2, 80.0,

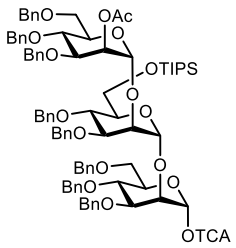
75.1, 75.0, 74.7, 74.1, 73.2, 72.5, 72.1, 72.1, 71.9, 69.2, 68.5, 67.8, 62.9, 18.0, 18.0, 11.9. ESI-MS (*m*/*z*): $[M+K]^+$ cald 1027.479 obsd 1027.291.

n-Allyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**17**)



The mannosyl imidate (0.19 g, 0.30 mmol) and disaccharide acceptor **16** (0.20 g, 0.20 mmol) were co-evaporated with toluene (5 mL x 3) and dried under high vacuum for 2 h. The mixture was dissolved in anhydrous diethylether (8 mL) and activated molecular sieves were added. The solution was stirred for 10 min at room temperature and cooled to 0 °C. The mixture was treated with TBSOTf (14 µL, 0.06 mmol) and stirred at 0 °C for a period of 1 h. The reaction was diluted with CH_2Cl_2, quenched with Et_3N and concentrated. The crude product was purified by flash column chromatography to obtain mannosyl trisaccharide **17** (0.2 g, 0.14 mmol, 68%) as colorless oil. R_f = 0.4 (EtOAc/Hexanes 1:4) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 36H), 7.30 – 7.16 (m, 39H), 7.15 (dd, *J* = 16.8, 5.4 Hz, 6H), 5.86 (ddt, *J* = 16.3, 10.8, 5.5 Hz, 1H), 5.55 (s, 1H), 5.30 (s, 1H), 5.27 – 5.11 (m, 3H), 5.03 (s, 1H), 4.88 (t, *J* = 8.2 Hz, 3H), 4.85 – 4.71 (m, 3H), 4.67 (dd, *J* = 11.5, 6.3 Hz, 3H), 4.67 – 4.50 (m, 9H), 4.53 – 4.46 (m, 3H), 4.50 – 4.35 (m, 3H), 4.27 (dd, *J* = 12.1, 5.4 Hz, 1H), 4.14 (dd, *J* = 12.9, 5.0 Hz, 2H), 4.06 (s, 3H), 4.02 – 3.86 (m, 13H), 3.89 – 3.83 (m, 3H), 3.77 (dt, *J* = 14.1, 10.9 Hz, 7H), 3.69 (d, *J* = 9.1 Hz, 3H), 3.64 – 3.54 (m, 2H), 3.46 (d, *J* = 10.0 Hz, 1H), 2.13 (s, 3H), 1.09 – 1.01 (m, 22H).^{13}C NMR (101 MHz, $CDCl_3$) δ 170.1, 138.6, 138.6, 138.5, 138.4, 138.3, 138.2, 138.1, 138.1, 133.7, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.9, 127.7, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 117.1, 100.0, 99.8, 98.1, 80.0, 78.4, 75.1, 75.0, 74.7, 74.2, 73.5, 73.3, 73.3, 72.1, 71.9, 71.8, 71.6, 69.2, 68.7, 67.7, 26.0, 21.1, 18.1, 11.9. ESI-MS (*m*/*z*): $[M+K]^+$ cald 1501.683 obsd 1501.592.

2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl trichloroacetimidate (**18**)

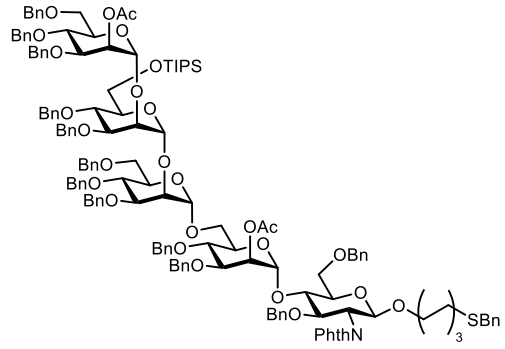


A solution of $[IrCOD(PPh_2Me)_2]PF_6$ (5.0 mg) in THF (3 mL) was stirred under hydrogen atmosphere until the color turned from red to colorless to pale yellow. The hydrogen atmosphere was exchanged with Argon. This solution was added to a solution of trisaccharide **17** (0.18 g, 0.13 mmol) in THF (10 mL). After 16 h, the solvent was removed and the residue was dissolved in a mixture of acetone (2.7 mL) and water (0.3 mL). Mercury (II) chloride (0.17

g, 0.63 mmol) and mercury (II) oxide (5.5 mg, 0.03 mmol) were added. After 1 h, saturated $NaHCO_3$ (aq) was added and the reaction mixture was extracted three times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography to give trisaccharide hemiacetal intermediate (0.12 g, 0.08 mmol, 64%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.21 (m, 25H), 7.23 – 7.12 (m, 33H), 7.16 – 7.02 (m, 13H), 5.47 (dd, *J* = 2.9, 1.7 Hz, 2H), 5.23 (d, *J* = 7.7 Hz, 2H), 4.93 (s, 1H), 4.86 – 4.66 (m, 7H), 4.68 – 4.32 (m, 20H), 4.29 – 4.14 (m, 2H), 4.06 (dd, *J* = 5.6, 2.3 Hz, 1H), 3.97 (s, 1H), 3.94 – 3.67 (m, 18H), 3.67 – 3.59 (m, 6H), 3.56 (ddd, *J* = 19.2, 9.9, 3.5 Hz, 7H), 3.42 (dd, *J* = 10.3, 6.3 Hz, 2H), 2.06 (s, 3H), 1.00 (d, *J* = 2.1 Hz, 22H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2, 138.6, 138.5, 138.4, 138.2, 138.1, 128.4, 128.4, 128.3, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.8, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 100.0, 99.8, 99.6, 79.7, 79.2, 78.4, 75.5, 75.1, 74.7, 74.2, 73.75, 73.4, 73.3, 72.1, 72.0, 71.7, 69.3, 69.1, 68.7, 68.6, 62.9, 62.8, 34.8, 31.9, 29.7, 26.1, 22.7, 21.2, 18.2, 14.2, 11.9.

To a stirred solution of hemiacetal (0.11 g, 0.08 mmol) in CH_2Cl_2 (10 mL) at 0 °C were added CCl_3CN (0.08 mL, 0.77 mmol) and DBU (4 µL, 0.02 mmol). The reaction mixture was stirred for 1 h at 0 °C. The resulting mixture was concentrated and purified by flash column chromatography to obtain imidate donor **18** (0.1 g, 0.06 mmol, 80%) as clear oil. R_f = 0.4 (EtOAc/hexane = 2:3)

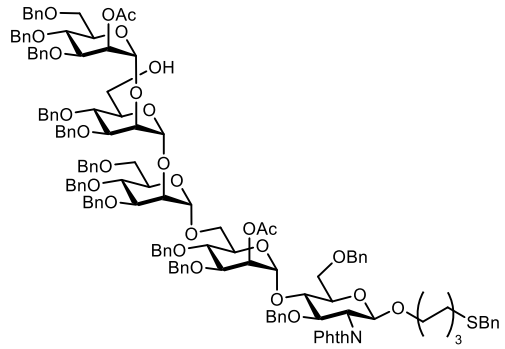
1-O-(6-thiobenzyl)hexyl-2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)- 3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**19**)



The trisaccharide imidate **18** (0.19 g, 0.12 mmol) and disaccharide acceptor **10** (0.11 g, 0.10 mmol) were co-evaporated with toluene (3 x 5 mL) and dried under high vacuum for 2 h. The mixture was dissolved in anhydrous diethylether (10 mL) and activated molecular sieves were added. The solution was stirred for 10 min at room temperature and cooled to 0 °C. The mixture was treated with TBSOTf (7 µL, 0.03 mmol) and stirred at 0 °C for a period of 1 h. The reaction was diluted with CH_2Cl_2, quenched with Et_3N and concentrated. The crude product was purified by flash column chromatography to obtain pentasaccharide **19** (0.17 g, 0.07 mmol, 65% α-isomer). R_f = 0.4 (EtOAc/hexane = 1:3) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 16.2 Hz, 3H), 7.34 – 7.17 (m, 57H), 7.20 – 7.07 (m, 12H), 7.10 – 7.04 (m, 4H), 7.03 – 6.84 (m, 6H), 6.77 (t, *J* = 7.4 Hz, 1H), 5.52 (d, *J* = 19.8 Hz, 1H), 5.30 (s, 2H), 5.06 – 4.99 (m, 2H), 4.92 – 4.20 (m, 32H), 4.15 (dd, *J* = 17.7, 7.1 Hz, 2H), 4.07 – 3.92 (m, 6H), 3.96 – 3.80 (m, 11H), 3.75 (ddd, *J* = 19.1, 15.0, 10.8 Hz, 11H), 3.60 (s, 2H), 3.50 – 3.22 (m, 6H), 2.13 (d, *J* = 14.8 Hz, 5H), 1.93 (d, *J* = 3.4 Hz, 3H), 1.44 – 1.17 (m, 5H), 1.21 – 1.00 (m, 27H), 1.01 – 0.80 (m, 5H). ^{13}C NMR

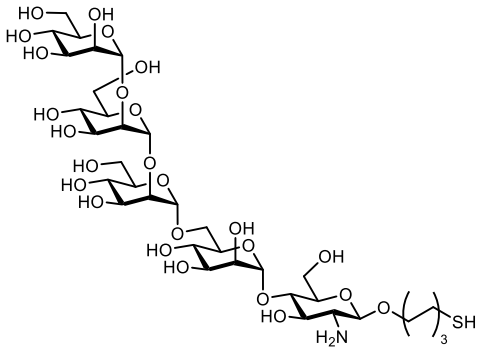
(101 MHz, $CDCl_3$) δ 170.1, 169.8, 138.8, 138.7, 138.5, 138.5, 138.4, 138.0, 137.7, 128.7, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0, 128.0, 127.9, 127.9, 127.7, 127.7, 127.6, 127.5, 127.4, 127.4, 127.3, 127.1, 127.0, 126.8, 100.0, 99.9, 99.3, 99.0, 98.0, 80.9, 79.4, 78.6, 78.5, 77.6, 75.0, 74.8, 74.6, 74.5, 74.4, 74.0, 73.3, 73.1, 72.7, 72.1, 72.0, 71.73, 71.4, 71.3, 69.3, 68.9, 68.7, 68.5, 55.7, 36.2, 31.0, 29.6, 29.0, 28.9, 28.3, 26.0, 25.3, 21.1, 20.9, 18.1, 11.9. HRMS (*m*/*z*): $[M+Na]^+$ cald 2486.3377 obsd 2486.3362

1-O-(6-thiobenzyl)hexyl-2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**20**)



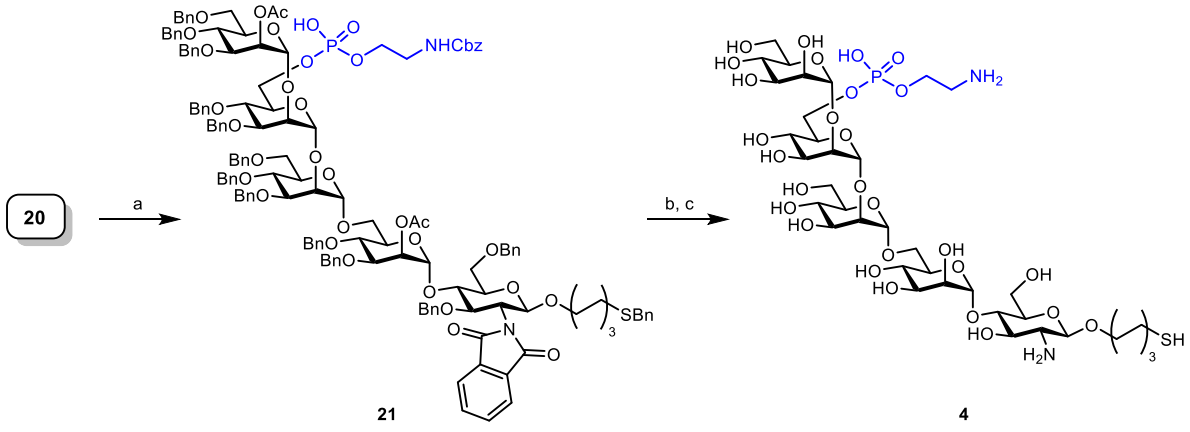
To a stirred solution of pentasaccharide **19** (0.07 g, 0.03 mmol) in CH_3CN (7 mL) were added water (50 μL) and $Sc(OTf)_3$ (0.04 g, 0.08 mmol). The reaction mixture was warmed to 50 °C and stirred for 6 h. The reaction was quenched with Et_3N and concentrated. The crude product was purified by flash column chromatography to obtain **20** (50 mg, 0.02 mmol, 72%). R_f = 0.25 (EtOAc/hexane = 2:3) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 15.8 Hz, 4H), 7.35 – 7.13 (m, 86H), 7.16 – 7.08 (m, 11H), 7.02 (dd, *J* = 16.1, 7.6 Hz, 4H), 6.88 (t, *J* = 7.5 Hz, 3H), 6.77 (t, *J* = 7.2 Hz, 1H), 5.50 (d, *J* = 18.3 Hz, 3H), 5.29 (s, 1H), 5.15 (s, 1H), 5.02 (d, *J* = 9.0 Hz, 3H), 4.92 – 4.75 (m, 8H), 4.76 – 4.60 (m, 4H), 4.62 – 4.49 (m, 11H), 4.51 – 4.39 (m, 7H), 4.42 – 4.24 (m, 7H), 4.22 – 4.13 (m, 1H), 4.07 (s, 2H), 3.99 – 3.57 (m, 33H), 3.58 – 3.39 (m, 4H), 3.35 – 3.18 (m, 1H), 2.16 (d, *J* = 7.1 Hz, 1H), 2.11 (s, 4H), 1.93 (s, 3H), 1.42 – 1.22 (m, 2H), 1.21 – 1.08 (m, 1H), 1.04 – 0.79 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 169.8, 138.4, 138.4, 138.3, 138.1, 137.9, 137.9, 137.8, 137.8, 137.6, 128.7, 128.4, 128.4, 128.3, 128.3, 128.2, 128.2, 128.2, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.4, 127.4, 127.3, 127.3, 127.1, 127.0, 126.8, 100.1, 99.3, 99.1, 98.9, 98.0, 81.0, 78.9, 78.3, 77.8, 75.1, 75.0, 74.8, 74.7, 74.5, 74.4, 74.2, 74.0, 73.6, 73.4, 73.3, 73.2, 72.6, 72.0, 71.9, 71.7, 71.6, 69.3, 69.2, 68.8, 68.6, 68.5, 68.4, 65.9, 62.0, 55.7, 36.1, 32.3, 31.0, 29.0, 28.9, 28.3, 26.3, 25.3, 21.1, 20.9. ESI-MS (*m*/*z*): $[M+Na]^+$ cald 2351.983 obsd 2351.651

1-O-(6-thio)hexyl-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-2-deoxy-2-amino-β-D-glucopyranoside (**3**)



Pentasaccharide **20** (0.02 g, 8.6 µmol) was dissolved in a mixture of ethylenediamine (2 mL) and butanol (2 mL) and stirred at 90 °C for 3 h. After 3 h, the reaction mixture was concentrated to obtain partially deprotected crude intermediate. The crude intermediate was dissolved in anhydrous THF and MeOH. This solution was added dropwise to blue solution of 20 mL liquefied ammonia with sodium -78 °C. The reaction was stirred at -78 °C. After 1 h, the reaction was quenched with MeOH and stirred for additional 1h at rt. Sodium methoxide generated in the reaction was quenched by dropwise addition of glacial acetic acid. The reaction mixture was concentrated and the crude product was purified by size exclusion column chromatography using a Sephadex® super fine G-15 (GE Healthcare) column and 5% ethanol in water as eluent to obtain **3** (6 mg, 6.36 µmol, 74%) as white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.19 – 5.15 (m, 1H), 5.10 – 5.06 (m, 1H), 5.00 (s, 1H), 4.92 (d, *J* = 1.5 Hz, 1H), 4.61 – 4.52 (m, 1H), 4.01 – 3.96 (m, 1H), 3.94 (s, 2H), 3.90 – 3.80 (m, 9H), 3.80 – 3.41 (m, 28H), 2.96 – 2.88 (m, 1H), 2.84 – 2.72 (m, 1H), 2.27 – 2.21 (m, 1H), 1.55 – 1.50 (m, 2H), 1.46 (p, *J* = 7.4 Hz, 1H), 1.30 (ddd, *J* = 28.5, 14.3, 7.2 Hz, 4H). ^{13}C NMR (151 MHz, d_2o) δ 104.8, 104.5, 103.2, 101.2, 100.9, 81.4, 81.1, 79.7, 77.5, 75.8, 75.3, 74.9, 73.2, 72.9, 72.7, 72.5, 69.6, 69.5, 69.4, 68.9, 63.7, 63.5, 63.2, 63.2, 58.4, 53.4, 30.9, 30.4, 29.8, 27.3, 26.4, 25.4, 24.1. HRMS (*m*/*z*): $[M+H]^+$ cald 944.3639 obsd 944.3621

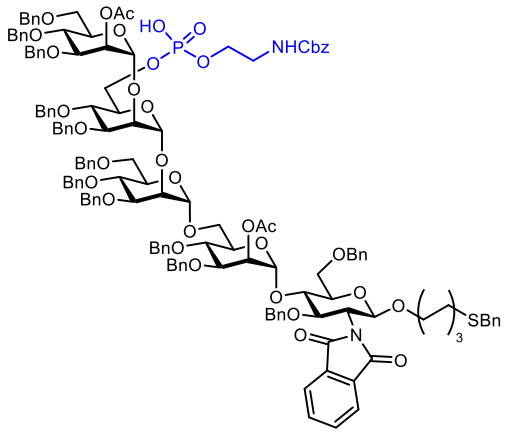
Synthesis of fragment 4



Scheme S5: Synthesis of fragment GPI **4**. a) i. **2-3**, PivCl, py, rt, 16 h; ii. I_2, H_2O, pyr, rt, 1 h, 86% b) $(CH_2NH_2)_2$, H_2O, *n*-BuOH, 90 °C, 4 h; c) i. Na, liq. NH_3, THF, -78 °C, 1 h; ii. MeOH, rt, 1 h, 60% (over two steps).

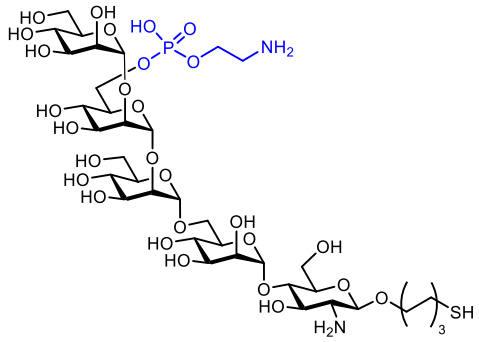
1-O-(6-thiobenzyl)hexyl-2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-6-O-(2-N-benzyloxycarbonyl)aminoethyl-phosphonato-α-D-mannopyranosyl-

(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**21**)



Pentasaccharide **20** (0.03 g, 0.01 mmol) and *H*-phosphonatethanolamine (0.02 g, 0.04 mmol) were co-evaporated with pyridine for three times and dried under high vacuum for 2 h. The mixture was dissolved in anhydrous pyridine (4 mL) and a solution of pivolyl chloride (5 µL, 0.04 mmol) in pyridine (1 mL) was added. The solution was stirred for 6 h at room temperature. After 6 h, iodine (0.01 g, 0.04 mmol) and water (0.05 mL) were added and reaction was stirred for 2 h. The reaction mixture was quenched with $Na_2S_2O_3$ and extracted with CH_2Cl_2. The organic layer were concentrated and purified by Et_3N deactivated silica gel flash column chromatography to obtain phosphorylated pentasaccharide **21** (0.03 g, 0.01 mmol, 86%). R_f = 0.5 (MeOH/CH_2Cl_2 = 1:10) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (d, *J* = 16.2 Hz, 6H), 7.33 (d, *J* = 7.7 Hz, 5H), 7.32 – 7.24 (m, 15H), 7.25 – 7.14 (m, 57H), 7.14 (d, *J* = 3.2 Hz, 5H), 7.11 (d, *J* = 7.1 Hz, 6H), 7.08 – 7.02 (m, 4H), 6.97 (d, *J* = 7.3 Hz, 4H), 6.87 (t, *J* = 7.6 Hz, 2H), 6.78 (d, *J* = 5.7 Hz, 2H), 5.53 (d, *J* = 25.7 Hz, 3H), 5.29 (s, 1H), 5.20 (s, 1H), 5.00 (td, *J* = 23.2, 22.0, 14.0 Hz, 4H), 4.79 (dt, *J* = 21.0, 11.9 Hz, 11H), 4.68 – 4.22 (m, 28H), 4.21 – 4.06 (m, 4H), 4.02 – 3.68 (m, 27H), 3.60 (d, *J* = 7.8 Hz, 4H), 3.51 – 3.36 (m, 5H), 3.29 (s, 3H), 2.14 (t, *J* = 7.3 Hz, 2H), 1.95 (s, 3H), 1.40 – 1.21 (m, 5H), 1.07 – 0.83 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0, 169.9, 156.4, 138.7, 138.4, 138.1, 138.0, 137.7, 137.6, 137.0, 133.8, 128.7, 128.4, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.8, 127.7, 127.5, 127.47, 127.4, 127.3, 127.2, 126.8, 100.2, 99.4, 99.3, 98.8, 98.0, 80.9, 78.4, 75.1, 74.9, 74.8, 74.6, 74.5, 74.4, 74.0, 73.5, 73.3, 73.1, 72.1, 72.0, 71.9, 71.7, 71.5, 71.3, 69.3, 68.5, 66.1, 55.8, 45.5, 36.1, 31.0, 29.6, 29.0, 28.8, 28.3, 25.3, 21.1, 20.9, 8.8. ^{31}P NMR (162 MHz, $CDCl_3$) δ 1.58. ESI-MS (*m*/*z*): $[M+Na]^+$ cald 2609.03 obsd 2609.6

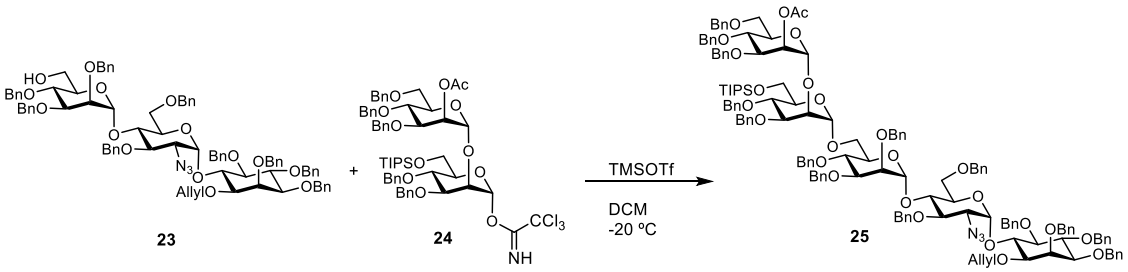
1-O-(6-thio)hexyl-α-D-mannopyranosyl-(1→2)-6-O-aminoethyl-phosphonato-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-2-deoxy-2-amino-β-D-glucopyranoside (**4**)



Pentasaccharide **21** (0.04 g, 0.02 mmol) was dissolved in a mixture of ethylenediamine (2 mL) and butanol (2 mL) and stirred at 90 °C for 3 h. After 3 h, the reaction mixture was concentrated to obtain partially deprotected crude intermediate. The crude intermediate was dissolved in anhydrous THF and MeOH. This solution was added dropwise to blue solution of 20 mL liquefied ammonia with sodium -78 °C. The reaction was stirred at -78 °C. After 1 h, the reaction was quenched with MeOH and stirred for additional 1h at rt. Sodium methoxide generated in the reaction was quenched by dropwise addition of glacial acetic acid. The reaction mixture was concentrated and the crude product was purified by size exclusion column chromatography using a Sephadex® super fine G-15 (GE Healthcare) column and 5% ethanol in water as eluent to obtain **4** (11 mg, 10.2 µmol, 60%) as white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.18 – 5.15 (m, 1H), 5.08 (s, 1H), 4.97 (s, 1H), 4.94 – 4.90 (m, 1H), 4.32 (ddd, *J* = 19.6, 7.9, 3.7 Hz, 1H), 4.03 – 3.95 (m, 5H), 3.94 (dt, *J* = 5.6, 2.7 Hz, 2H), 3.88 – 3.77 (m, 6H), 3.76 (d, *J* = 10.7 Hz, 2H), 3.74 – 3.51 (m, 14H), 3.48 (q, *J* = 8.8, 7.7 Hz, 2H), 3.47 – 3.41 (m, 2H), 3.21 – 3.14 (m, 2H), 3.01 (dt, *J* = 17.5, 6.4 Hz, 1H), 2.87 (dd, *J* = 10.2, 5.3 Hz, 0H), 2.65 (t, *J* = 7.2 Hz, 1H), 2.42 (t, *J* = 7.1 Hz, 0H), 1.62 – 1.45 (m, 3H), 1.32 – 1.23 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 184.0, 104.8, 104.4, 103.3, 100.9, 81.5, 81.0, 79.8, 77.4, 75.8, 75.3, 74.9, 74.7, 74.7, 73.2, 72.9, 72.7, 72.6, 72.4, 69.4, 69.1, 68.9, 67.2, 64.4, 63.6, 63.4, 58.9, 42.7, 42.6, 40.7, 31.1, 30.7, 29.8, 27.2, 27.1, 25.8. ^{31}P NMR (243 MHz, D_2O) δ -2.75. HRMS ESI-MS (*m*/*z*): $[M+H]^+$ cald 1066.3652 obsd 1066.3646

Synthesis of fragment 5

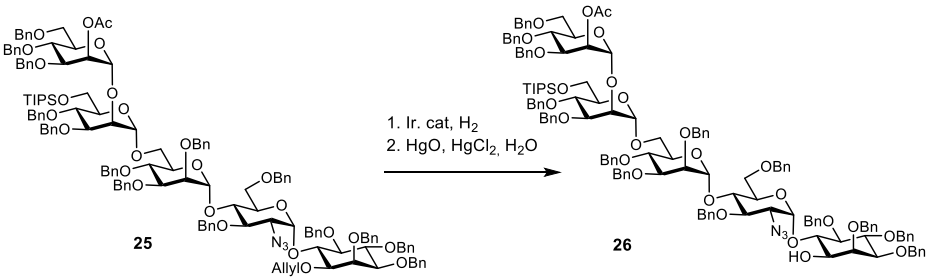
*2-O-Acetyl-3,4,6-O-tri-benzyl-α-D-mannopyranosyl-(1→2)-3,4-O-di-benzyl-6-O-(tri-isopropylsilyl)-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3,6-O-benzyl-α-D-glucopyranosyl-(1→6)-1-O-allyl-2,3,4,5-tetra-O-benzyl-D-myo-inositol (**25**)*



The dimannose imidate **24**[5] (74 mg, 0.065 mmol) and the trisaccharide acceptor **23**[5] (69 mg, 0.050 mmol) were co-evaporated with toluene 3 times and dry in high-vacuum overnight. The dry remaining was dissolved in DCM, activated 4 A molecular sieves were added (200 mg) and the mixture was stirred for 30 minutes at room temperature and then 15 min at -40 °C. TMSOTf was added slowly and the reaction mixture was allowed to gradually warm up to -20 °C. After 1h at -20 °C TLC indicated completion and the reaction was quenched with addition of Et_3N. The sieves were removed on celite and the solution was concentrated. The crude mixture was purified on a silica gel column with a gradient from 5% to 30% EtOAc in hexane to obtain 92 mg (0.039 mmol, 78 %) of the pentasaccharide **25**. R*f* = 0.52 (CH_2Cl_2/MeOH, 10:1), ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 7.48 – 7.41 (m, 2H), 7.40 – 7.06 (m, 80H), 6.02 – 5.89 (m, 1H), 5.79 (d, *J* = 3.7 Hz, 1H), 5.55 (t, *J* = 2.4 Hz, 1H), 5.34 (dd, *J* = 10.8, 2.1 Hz, 1H), 5.27 (dd, *J* = 4.1, 1.9 Hz, 2H), 5.21 (dd, *J* = 10.5, 1.6 Hz, 1H), 5.11 – 4.91 (m, 6H), 4.91 – 4.81 (m, 6H), 4.81 –

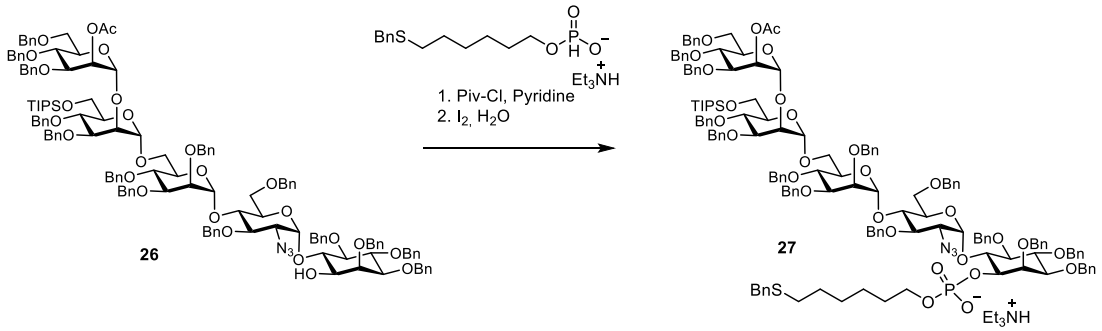
4.59 (m, 10H), 4.59 – 4.41 (m, 9H), 4.41 – 4.21 (m, 6H), 4.21 – 3.73 (m, 22H), 3.73 – 3.35 (m, 12H), 3.34 – 3.20 (m, 3H), 2.12 (s, 3H), 1.04 – 0.97 (m, 21H), ^{13}C NMR (101 MHz, $CDCl_3$) δ: 134.2, 128.1, 127.9, 127.8, 127.4, 125.3, 125.1, 97.5, 117.2, 117.1, 117.1, 100.5, 99.8, 99.3, 97.7, 81.9, 81.2, 79.8, 79.7, 78.6, 77.8, 77.2, 76.2, 75.8, 75.7, 75.5, 75.4, 75.2, 74.9, 74.9, 74.7, 74.3, 74.2, 74.1, 74.0, 73.8, 73.5, 73.3, 73.0, 72.7, 72.7, 72.2, 72.1, 72.0, 71.9, 71.8, 70.8, 69.7, 69.6, 68.8, 68.7, 68.6, 68.6, 68.5, 66.4, 63.2, 62.3, 21.2, 19.8, 18.1, 12,0, HRMS ESI-MS$^+$ M_{Calcd} ($C_{142}H_{161}N_3O_{26}Si$): 2352.1138; M_{found}: 2375.210 $(M + Na)^+$.

*2-O-Acetyl-3,4,6-O-tri-benzyl-α-D-mannopyranosyl-(1→2)-3,4-O-di-benzyl-6-O-(tri-isopropylsilyl)-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3,6-O-benzyl-α-D-glucopyranosyl-(1→6)-1-hydroxy-2,3,4,5-tetra-O-benzyl-D-myo-inositol (**26**)*



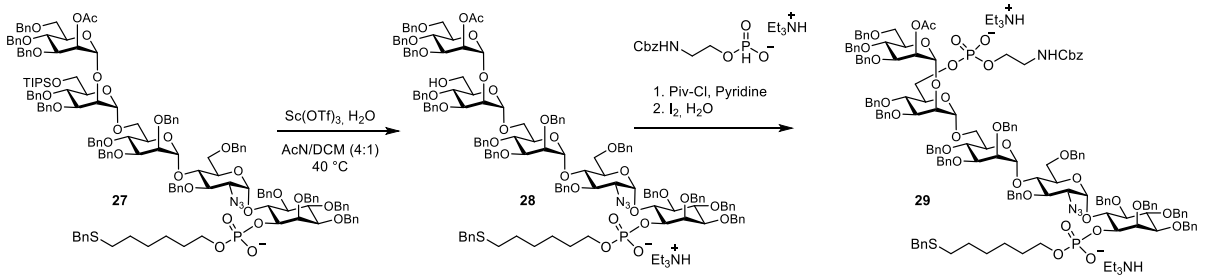
A solution of iridium catalyst (6.45 mg, 7.5 μmol) in THF (6 mL) was stirred for 15 min under hydrogen at 20 °C until the color turned from red to pale yellow. The H_2 atmosphere was exchanged with argon and this solution was added pseudo-pentasaccharide **25** (82 mg, 0.034 mmol). After 16 h, the solvent was removed and the residue was dissolved in 6 mL of acetone containing water (0.3 ml). Mercury (II) chloride (83 mg, 0.306 mmol) and mercury (II) oxide (2.76 mg, 0.013 mmol) were added. After 2 h, and not additional progress of the reaction, $NaHCO_3$ was added and the reaction mixture was extracted with DCM for three times. The combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography using n-hexane/EtOAc from 9:1 to 3:1 to give 58 mg (0.025 mmol) of the pseudo-pentasaccharide alcohol in 75% yield as a white powder. R*f* = 0.20 (n-hexan/AcOEt, 2:1), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 – 7.11 (m, 70H), 5.54 (d, *J* = 3.3 Hz, 2H), 5.27 (d, *J* = 2.1 Hz, 1H), 5.05 (d, *J* = 2.2 Hz, 1H), 5.01 (d, *J* = 11.6 Hz, 1H), 4.97 – 4.90 (m, 3H), 4.89 – 4.82 (m, 4H), 4.80 – 4.69 (m, 6H), 4.66 – 4.59 (m, 3H), 4.57 – 4.41 (m, 7H), 4.40 – 4.17 (m, 5H), 4.16 – 3.74 (m, 15H), 3.75 – 3.55 (m, 6H), 3.55 – 3.28 (m, 8H), 3.02 (d, *J* = 7.4 Hz, 1H), 2.12 (s, 3H), 1.00 (d, *J* = 6.4 Hz, 19H), 0.92 – 0.78 (m, 2H), ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.2 (C=O), 139.0, 138.9, 138.7, 138.5, 138.4, 138.4, 138.3, 138.3, 138.2, 138.2, 138.1, 137.7, 128.5, 128.5, 128.3, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 128.0, 127.8, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.3, 127.3, 127.2, 127.1, 126.8, 100.3, 99.8, 99.3, 97.6, 81.8, 81.3, 80.9, 80.5, 79.9, 79.6, 79.5, 78.6, 76.0, 75.8, 75.4, 75.1, 74.9, 74.6, 74.2, 73.9, 73.3, 73.2, 73.1, 72.8, 72.2, 72.1, 72.0, 72.0, 71.7, 71.5, 70.4, 68.8, 68.7, 66.4, 64.0, 62.3, 30.3, 29.7, 21.2, 18.1, 11.9. MALDI-MS for $C_{139}H_{157}N_3O_{26}Si$ M_{cald}: 2312.08, M_{found}: 2334.76 $(M+Na)^+$, 2350.74 $(M+K)^+$

*Triethylammonium (2-O-Acetyl-3,4,6-O-tri-benzyl-α-D-mannopyranosyl-(1→2)-3,4-O-di-benzyl-6-O-(tri-isopropylsilyl)-α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3,6-O-benzyl-α-D-glucopyranosyl-(1→6)-2,3,4,5-tetra-O-benzyl-1-O-(6-(benzylthio)hexyl-phosphonato)-D-myo-inositol (**27**)*



The pseudo-pentasaccharide alcohol **26** (58 mg, 0.025 mmol) and 6-(benzylthio)hexyl phosphonate (40 mg, 0.103 mmol) were dissolved in and co-evaporated three times with 5 mL of pyridine and dried in HV overnight. The remaining was dissolved in dry pyridine (3 ml) and pivaloyl chloride (0.013 ml, 0.103 mmol) was added under stirring and argon atmosphere. The reaction was stirred up to disappearance of the starting material by TLC (CH/AcOEt; 1.5:1) 14 h. I_2 (6.45 mg, 0.025 mmol) and water (100 µL) were added and the reaction was stirred for 1 h. The mixture was diluted with 30 mL of DCM, extracted with aqueous sat. $Na_2S_2O_3$ and water, dried with $MgSO_4$ and evaporated to dryness. The product was purified on a silica gel column deactivated with trimethylamine using DCM/MeOH from 50:1 to 20:1 as eluent. The fractions containing the product were collected and evaporated to deliver 62 mg (0.023 mmol, 92 %) of the phosphorylated pseudo-pentasaccharide **27**. R*f* = 0.24 (DCM/MeOH, 10:1), ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ 12.09 (s, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.74 – 7.58 (m, 4H), 7.22 (tdt, *J* = 30.5, 15.7, 6.7 Hz, 120H), 5.87 (s, 1H), 5.37 (s, 1H), 5.29 (s, 1H), 5.18 (s, 1H), 5.11 (s, 1H), 5.04 – 4.27 (m, 35H), 4.23 (d, *J* = 11.4 Hz, 2H), 4.18 – 3.98 (m, 5H), 3.97 – 3.40 (m, 25H), 3.35 (d, *J* = 10.8 Hz, 4H), 3.23 – 3.09 (m, 2H), 2.96 (dt, *J* = 9.4, 4.8 Hz, 6H), 2.57 – 2.22 (m, 6H), 1.91 (s, 3H), 1.61 – 1.36 (m, 5H), 1.25 (h, *J* = 7.1 Hz, 23H), 0.86 (dt, *J* = 13.5, 4.8 Hz, 2H), ^{31}P NMR (162 MHz, cdcl$_3$) δ -0.94, HRMS ESI-MS M_{calcd}: 2597.1544 $C_{152}H_{175}N_3O_{29}PSSi$, M_{found}: 2596.1443 $(M-H)^-$.

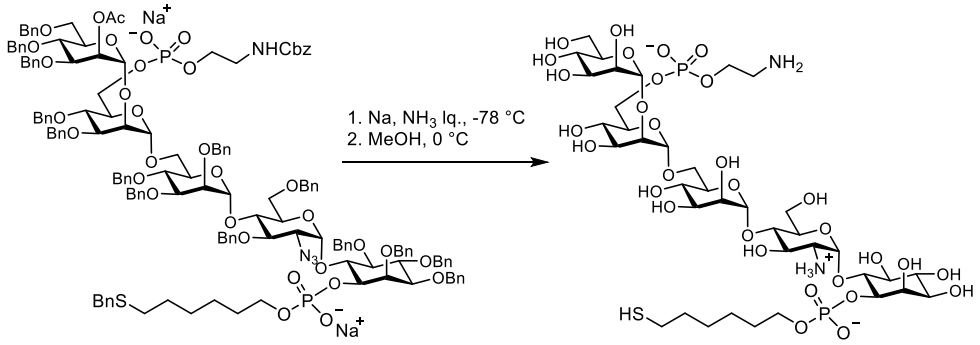
*Bistriethylammonium (2-O-Acetyl-3,4,6-O-tri-benzyl-α-D-mannopyranosyl-(1→2)-3,4-O-di-benzyl-6-O-(2-(N-benzyloxycarbonyl)aminoethylphosphonato)--α-D-mannopyranosyl-(1→6)-2,3,4-tri-O-benzyl-α-D-mannopyranosyl-(1→4)-2-azido-2-deoxy-3,6-O-benzyl-α-D-glucopyranosyl-(1→6)-2,3,4,5-tetra-O-benzyl-1-O-(6-(benzylthio)hexyl-phosphonato)-D-myo-inositol (**29**)*



The pseudo-pentasaccharide **27** (59 mg, 0.021 mmol) were dissolved in 4 mL of an acetonitrile/CH_2Cl_2 mixture (3:1). To this solution $Sc(OTf)_3$ (41.4 mg, 0.084 mmol) and water (10 µl, 0.555 mmol) were added. The mixture was stirred overnight at 40 °C. The reaction was quenched with 100 µL of triethylamine, the solvent was evaporated and the residue was dried *in vacuo*. The product was passed through a Sephadex LH 20 column (17 x 700 mm) and eluted with using a methanol/CH_2Cl_2 (1:2) mixture containing 0.05 % triethylamine. Collected fractions were evaporated and to obtain the crude product **28**, which was directly used in the next reaction. The monophosphorylated alcohol **28** was dissolved in 3 mL of pyridine and the H-phosphonate **2-3** (36 mg, 0,100 mmol) was added. The pyridine was removed and the residue

was co-evaporated three times with 5 mL of pyridine and dried in HV overnight. The reaction mixture was dissolved in anhydrous pyridine (3 mL) and PivCl (4.69 µl, 0.038 mmol) was added under stirring and argon atmosphere. The reaction was stirred up to disappearance of the starting material by TLC (CH/AcOEt; 1.5:1) 14 h. Iodine (5.42 mg, 0.021 mmol) and water (100 µL) were added to the reaction mixture and stirred for 1 h. The mixture was diluted with 30 mL of DCM and extracted with aq. saturated Na_2S_2O3 and brine, dried with MgSO4 and evaporated to dryness. The crude product was purified on a silica gel column deactivated with triethylamine using DCM/MeOH from 50:1 to 20:1 as eluent to give the bis-phosphorylated product **29** in 88% (46 mg,0.015 mmol). R*f* = 0.41 (CH_2Cl_2/MeOH, 10:1), ^{1}H NMR (400 MHz, Chloroform-*d*) δ 12.32 (s, 3H), 7.32 (d, *J* = 7.5 Hz, 2H), 7.26 (d, *J* = 7.7 Hz, 3H), 7.24 – 7.07 (m, 65H), 7.05 (dd, *J* = 13.3, 6.4 Hz, 14H), 6.64 (d, *J* = 4.8 Hz, 1H), 5.89 (d, *J* = 3.7 Hz, 1H), 5.45 (s, 1H), 5.11 (s, 1H), 4.97 (dd, *J* = 12.2, 8.1 Hz, 3H), 4.92 – 4.84 (m, 3H), 4.80 (ddd, *J* = 17.6, 9.1, 3.5 Hz, 6H), 4.74 (s, 3H), 4.73 – 4.63 (m, 3H), 4.60 – 4.43 (m, 6H), 4.41 – 4.29 (m, 9H), 4.25 (dd, *J* = 13.9, 7.6 Hz, 3H), 4.15 (d, *J* = 11.9 Hz, 2H), 4.08 – 3.62 (m, 23H), 3.61 – 3.38 (m, 10H), 3.17 (q, *J* = 4.9 Hz, 2H), 2.73 (s, 16H), 2.28 (t, *J* = 7.4 Hz, 2H), 2.01 (s, 3H), 1.51 (p, *J* = 7.1 Hz, 3H), 1.43 (p, *J* = 7.1 Hz, 2H), 1.32 – 1.10 (m, 11H), 1.08 – 1.00 (m, 26H), 0.84 – 0.74 (m, 3H). ^{31}P NMR (162 MHz, D_2O) δ 0.3, 0.2. MALDI-MS for $C_{153}H_{166}N_4O_{34}P_2S^-$ M_{calcd}: 2698.0663, M_{found}: 2697.240.

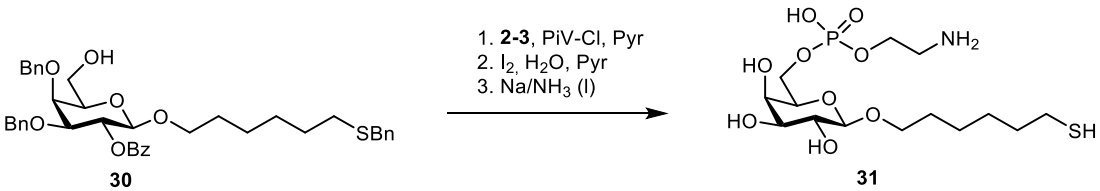
*α-D-mannopyranosyl- (1→2)- 6-O-Aminoethylphosphonato- α-D-mannopyranosyl- (1→6)- α-D-mannopyranosyl- (1→4)-2-amino-2-deoxy- α-D-glucopyranosyl- (1→6)-1-O-(thiohexyl-phosphonato)-D-myo-inositol (**5**)*



Ammonia (10 mL, 693 mmol)) was condensed in a dried two-necked flask at -78 °C. Sodium (50 mg, 2.175 mmol) was added until the solution was dark blue. The pseudo-pentasaccharide bisphosphate (20 mg, 6.36 µmol) was dissolved in 4 mL of THF) and added via cannula, followed by MeOH (0.2 mL). The resultant dark blue solution was stirred at -78°C for 60 min. Following disappearance of the blue color, methanol (5 ml, 124 mmol) was added to the mixture and ammonia was blown off with a stream of nitrogen. The reaction was warmed to room temperature and stirred for 1 h. The reaction was quenched by addition of acetic acid, diluted with ethanol and evaporated to dryness. Chromatography of the crude mixture on Sephadex G-25 (EtOH/H2O 1:20) gave the bis-phophorylated GPI-**5** (5 mg, 4.37 µmol, 77 % yield). R*f* = 0.30 (IPA/1M NH_4OAc, 2:1), ^{1}H NMR (600 MHz, Deuterium Oxide) δ 5.42 (d, *J* = 3.9 Hz, 1H), 5.10 (s, 1H), 5.02 (s, 1H), 4.91 (s, 1H), 4.11 – 4.03 (m, 4H), 4.00 (d, *J* = 5.5 Hz, 5H), 3.95 (s, 2H), 3.95 – 3.89 (m, 3H), 3.85 (dd, *J* = 13.9, 8.3 Hz, 4H), 3.80 (dd, *J* = 18.4, 8.9 Hz, 4H), 3.76 – 3.70 (m, 6H), 3.69 (s, 1H), 3.69 – 3.64 (m, 5H), 3.60 (dd, *J* = 19.4, 9.9 Hz, 6H), 3.49 (t, *J* = 10.0 Hz, 2H), 3.45 – 3.40 (m, 1H), 3.32 – 3.24 (m, 2H), 3.17 (t, *J* = 5.0 Hz, 3H), 2.79 (t, *J* = 8.0 Hz, 0H), 2.65 (t, *J* = 7.1 Hz, 2H), 2.25 (t, *J* = 7.7 Hz, 1H), 1.60 (dp, *J* = 14.3, 7.3 Hz, 3H), 1.56 – 1.50 (m, 3H), 1.45 (dd, *J* = 14.1, 6.7 Hz, 1H), 1.36 – 1.13 (m, 8H). ^{13}C NMR (151 MHz, d_2o) δ 97.9, 104.6, 101.1, 104.9, 78.8, 73.6, 74.1, 67.1, 64.4, 72.7, 81.2, 72.6, 68.9, 69.2, 80.0, 63.7, 72.9, 62.7, 74.8, 68.9, 74.3, 72.7, 73.0, 75.9, 79.0, 68.8, 63.7, 74.8, 69.5, 73.2, 75.4, 56.6,

42.7, 53.6, 40.8, 63.4, 30.9, 32.3, 29.8, 27.2, 29.2. MALDI-MS for $C_{38}H_{71}N_2O_{31}P_2S^-$ M_{calcd}: 1145.324 M_{found}: 1144.817

*6-O-Aminoethylphosphonato-1-O-(thiohexyl-phosphonato)-β-D-galactopyranose (**31**)*



30 **31**

The galactosyl alcohol **30**[6] (27 mg, 0.040 mmol) and the H-phosphonate **2-3** (50 mg, 0.140 mmol) were dissolved in co-evaporated three times with 5 mL of pyridine and dried *in vacuo*. The reaction mixture was dissolved in anhydrous pyridine (3 mL), pivaloyl chloride (17.2 µl, 0.4 mmol) was added and the reaction mixture was stirred for 4 h at room temperature. Iodine (10.3 mg, 0.040 mmol) and water (50 µL) were added to the reaction and the mixture was stirred for 1 h. The mixture was diluted with 10 mL of DCM and extracted with aq. saturated Na_2S_2O3 and brine, dried with MgSO4 and evaporated to dryness. The product was purified on a silica gel column using DCM/MeOH (10:1) as eluent. Ammonia (10 mL, 693 mmol)) was condensed in a dried two-necked flask at -78 °C. Sodium (50 mg, 2.175 mmol) was added until the solution was dark blue. The galactosyl phosphate was dissolved in 4 mL of THF and added via cannula, followed by MeOH (0.2 mL). The resultant dark blue solution was stirred at -78°C for 60 min. Following disappearance of the blue color, methanol (5 ml) was added to the mixture and ammonia was blown off with a stream of nitrogen. The reaction was warmed to room temperature and stirred for 1 h to remove the ester. The reaction was quenched by addition of acetic acid, diluted with ethanol and evaporated to dryness. Chromatography of the mixture on Sephadex G-25 using 5 % EtOH in water gave the galactose **31** as a mixture of the monomer and disulfide (5 mg, 0.012 mmol, 31 % yield). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.26 (dd, *J* = 8.1, 3.7 Hz, 1H), 3.96 (dd, *J* = 5.9, 4.3 Hz, 1H), 3.97 – 3.82 (m, 3H), 3.81 (d, *J* = 3.4 Hz, 1H), 3.72 (dq, *J* = 14.8, 6.7 Hz, 4H), 3.57 – 3.46 (m, 2H), 3.34 (dd, *J* = 9.9, 7.9 Hz, 1H), 3.12 (q, *J* = 5.3 Hz, 3H), 3.04 (q, *J* = 7.3 Hz, 1H), 2.80 – 2.71 (m, 1H), 2.65 – 2.47 (m, 3H), 1.65 – 1.53 (m, 1H), 1.49 (dt, *J* = 14.0, 6.5 Hz, 9H), 1.33 – 1.22 (m, 11H), 1.12 (td, *J* = 7.4, 2.3 Hz, 2H). ^{13}C NMR (101 MHz, D_2O) δ 102.7, 73.4, 73.3, 72.5, 70.6, 70.5, 68.1, 66.3, 64.2, 61.7, 61.6, 58.1, 50.9, 46.5, 39.9, 39.9, 38.0, 29.4, 29.4, 28.5, 28.4, 28.1, 27.4, 27.2, 24.5, 23.8, 20.9, 8.1. HRMS ESI-MS, M_{calcd} for the reduced form: 419.1379 ($C_{14}H_{30}NO_9PS$), M_{found}: 420.1422 $(M+H)^+$, M_{calcd} for the oxidized form: 836.2601($C_{28}H_{58}N_2O_{18}P_2S_2$), M_{found}: 837.2716 $(M+H)^+$.

Methods of Biochemistry

CRM_{197} was purchased from *Pf*ēnex Inc., 1M solution of resin bound TCEP was purchased from Thermo scientific and autoclaved sterile water was used for the conjugation.

Conjugation of GPI fragments 1-6 to CRM_{197}

250 µl of TCEP resin solution was centrifuged for 3 mins and excess of water was removed. TCEP resin was suspended in 0.1 M sodium phosphate buffer (pH 8) (150 µL) and centrifuged for 3 mins. The buffer excess was removed and autoclaved water was added. GPI fragment (in 120µL water) was transferred to the TCEP solution and incubated for 1h at rt. After 1 h, TCEP resin is filtered off using syringe and washed with autoclaved water (5x50 µL). All the water fractions are combined lyophilized. The reduction of disulfide to thiol is followed by LC-MS. To a stirred solution of CRM_{197} (3 mg) in 0.1 M sodium phosphate buffer (pH 7.4) was added a solution of SBAP (1.58 mg) in DMF (60 µL) at room temperature. The reaction mixture was stirred for 1 h. After 1 h, the solution was concentrated to 250 µL volume using an Amicon®

Ultra-4 Centrifugal Filter Unit (10 kDa cut off, Millipore) and washed with water (4x 1 mL) and once with 0.1 M sodium phosphate buffer (pH 8.0). Finally, the activated CRM_{197} is concentrated to obtain around 100 µL of solution. To the stirred solution of activated CRM_{197} solution was added the reduced glycan. The solution was stirred overnight at room temperature. The solution was concentrated to 250 µL of volume using an Amicon® Ultra-4 Centrifugal Filter Unit (10 kDa cut off, Millipore) and washed with water (4x 1 mL) and once with 0.1 M sodium phosphate buffer (pH 8.0) to obtain the CRM-GPI conjugate. cysteine (0.94 mg) was added directly to that solution in the Centricon and incubated for 1 h at r.t. After 1 h, the mixture was washed with water (4x 1 mL) re-buffered with PBS to obtain the desired CRM-GPI conjugate. MALDI-TOF mass spectroscopy and SDS-PAGE was done for all the intermediates and final samples.

Characterization of conjugates

CRM_{197}-GPI conjugates were characterized by MALDI-TOF mass spectrometry and SDS-PAGE gel electrophoresis using staining against carrier protein with Coomassie Brilliant Blue R250 (CBB) (Sigma-Aldrich, Munich, Germany, 6104-59-2) or used for western blotting. For gel electrophoresis, glycoconjugates were diluted 1:10 in sterile water; loading buffer was added, boiled for 5 minutes and loaded onto a 12% polyacrylamide gel. Gel electrophoresis was performed at 150 V, 150 mA for 60 minutes. Western blot transfer was accomplished at 100 V and 35 mA. The membrane was routinely tested for positive transfer with Ponceau S (Sigma-Aldrich, Munich, Germany, 6226-79-5), subsequently blocked for 2 h at RT with 5% BSA in PBS-T. Biotinylated Concavalin-A (Vector Laboratories, Burlingame, California, B-1005) was diluted 1:500 in 1x PBS 5% BSA 0.01 mM Mn^{2+} 0.1 mM Ca^{2+} and incubated 2 h shaking at RT. The membrane was subsequently washed and streptavidin HRP (BD Pharmingen, Heidelberg, Germany, 557630) was added 1:500 in 1x PBS 5% BSA 0.01 mM Mn^{2+} 0.1 mM Ca^{2+} and incubated 1 h shaking at RT. Finally, the membrane was washed again in 1x PBS 0.01mM Mn^{2+} 0.1mM Ca^{2+} and developed by enhanced luminol-based chemiluminescent according to manufacturer's instructions (Thermo Fisher Scientific, Darmstadt, Germany, 32109). Dual color precision protein standard (Bio-Rad Laboratories, Munich, Germany, 161-0374) was used as protein standard.

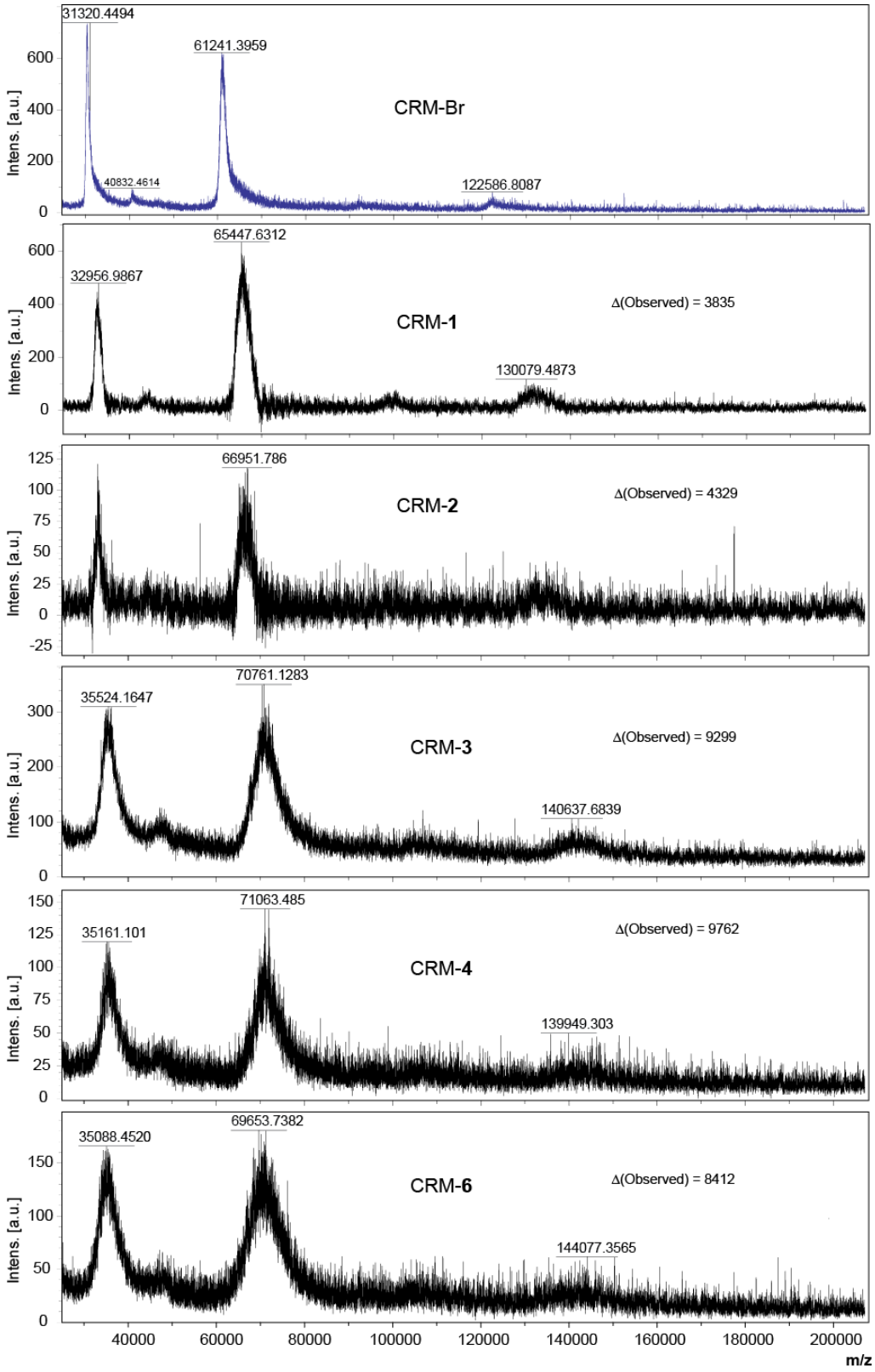


Figure S1: MALDI-TOF analysis of the activated CRM (CRM-AcBr) and CRM-GPI (CRM-Glycan) during glycoconjugate synthesis. Δ (observed) represents the difference in m/z for activated CRM_{197} and CRM-GPI and was used to calculate the glycan loading of the glycoconjugates. **(A-E)** represent the MALDI-TOF spectra for **CRM 1–4** and **6** respectively.

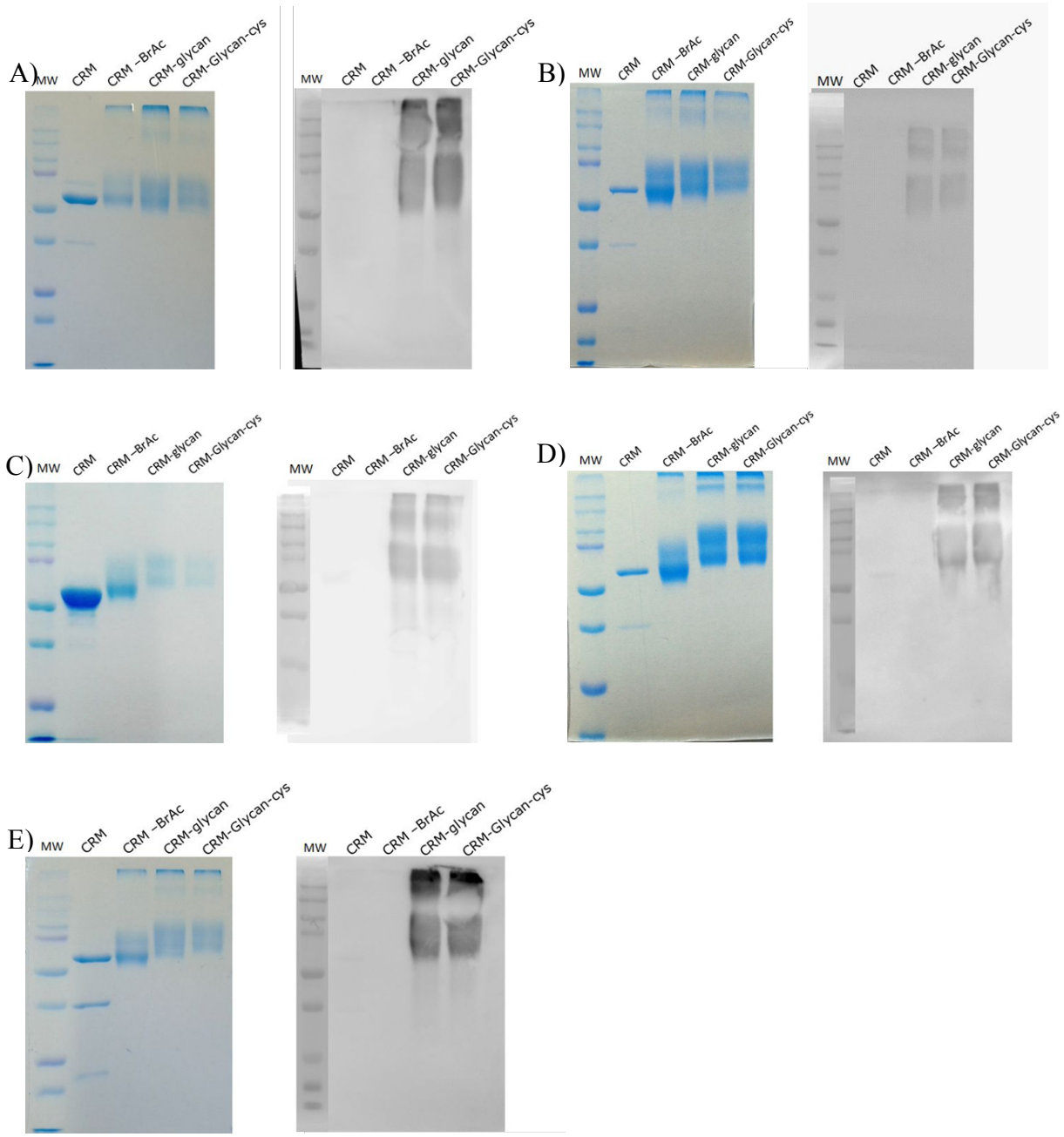


Figure S2: SDS PAGE and western blot analysis of the activated CRM (CRM-AcBr) and CRM-GPI (CRM-Glycan) and quenched glycoconjugate (CRM-glycan-cys) during glycoconjugate synthesis. **(A-E)** represent the SDS PAGE and western blot for **CRM 1–4** and **6** respectively.

GPI-CRM	Batch	Δ m/z	GPI molecular weight	Loading: GPI/CRM (% of mass)
GPI**1**	1	3835	781.82	4.92 (6.5% glycan)
	2	4778		6.12 (7.2% glycan)
GPI**2**	1	6972	904.87	7.4 (10.2% glycan)
	2	4329		5 (7% glycan)
GPI**3**	1	5668	943.96	5.7 (8.1% glycan)
	2	9299		9.8 (13% glycan)
GPI**4**	1	9865	1067.01	9.3 (14% glycan)
	2	9792		9.2 (13.7% glycan)
GPI**5**	1	5889	1146.99	5.2 (8.9% glycan)
	2	10240		8.9 (14.3% glycan)
GPI**6**	1	9322	1309.13	7.12 (13% glycan)
	2	8412		6.47 (12% glycan)

Figure S3: Glycan loading of the CRM glycoconjugates determined by MALDI-TOF mass spectrometry. Glycan loading was determined by MALDI and expressed as GPI molecules per CRM and as a percentage of weight for each **CRM–GPI** glycoconjugate, Δ m/z represents the difference in mass of the CRM-glycan and the activated CRM.

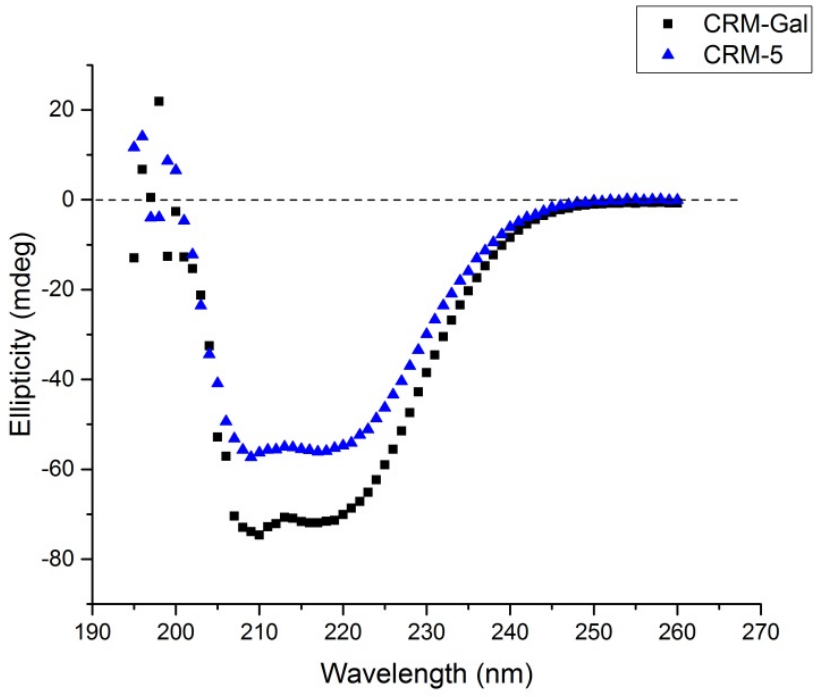


Figure S4. Circular dichroism analysis of CRM-**5** and CRM-**Gal.**

Animal Experiments

Animals were treated strictly according to German (Tierschutz-Versuchstierverordnung) and European Law (Directive 2010/63/EU). Recommendations of the Society for Laboratory Animal 43 Science (GV-SOLAS) and of the Federation of European Laboratory Animal Science Associations (FELASA) were followed. The Office for Health and Social Affairs Berlin (LAGeSo) approved the experiment conclusively (Permit Number: G0239/14). All efforts were made to minimize suffering. All C57BL/6JRj mice used in this study were obtained

from Janvier Labs (Saint-Berthevin, France). Mice were housed in individually-ventilated cages (IVCs) under specific pathogen free (SPF) conditions in the animal facility of the Federal Institute for Risk Assessment (BfR, Berlin, Germany). Mice were provided food and water *ad libitum*. Upon delivery (day -7), mice were allowed to rest for one week before experimental setting was started.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, US). Unpaired Student's *t* test was used to compare different sets of data, whereas two-way ANOVA was used to compare anti-GPI antibody levels over time between immunized and non-immunized groups. Log rank test was employed for analysis of survival between different groups. Statistical significance within figures is shown by asterisks: * represents $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

Immunization

On day 0, groups consisting of 15 C57BL/6JRj 5-week-old female mice were prime-immunized i.p. with CRM **1**-**6** or CRM_{197}-Gal as a control. Two boost-immunizations were performed in 14 day intervals (day 14 and day 28). Each mouse was injected a total of 100 µL i.p. of either GPI-CRM_{197} or CRM_{197}-Gal formulated with aluminum hydroxide (alum). Immunizations were performed with 5 µg GPI per vaccination. Due to diverging loadings of GPI on CRM_{197}, conjugates were diluted in sterile PBS accordingly. GPI-CRM_{197}-conjugates were formulated with aluminum hydroxide 1:2 (Alhydrogel®, Brenntag, Denmark) and rotated over night at 4 °C before immunization.

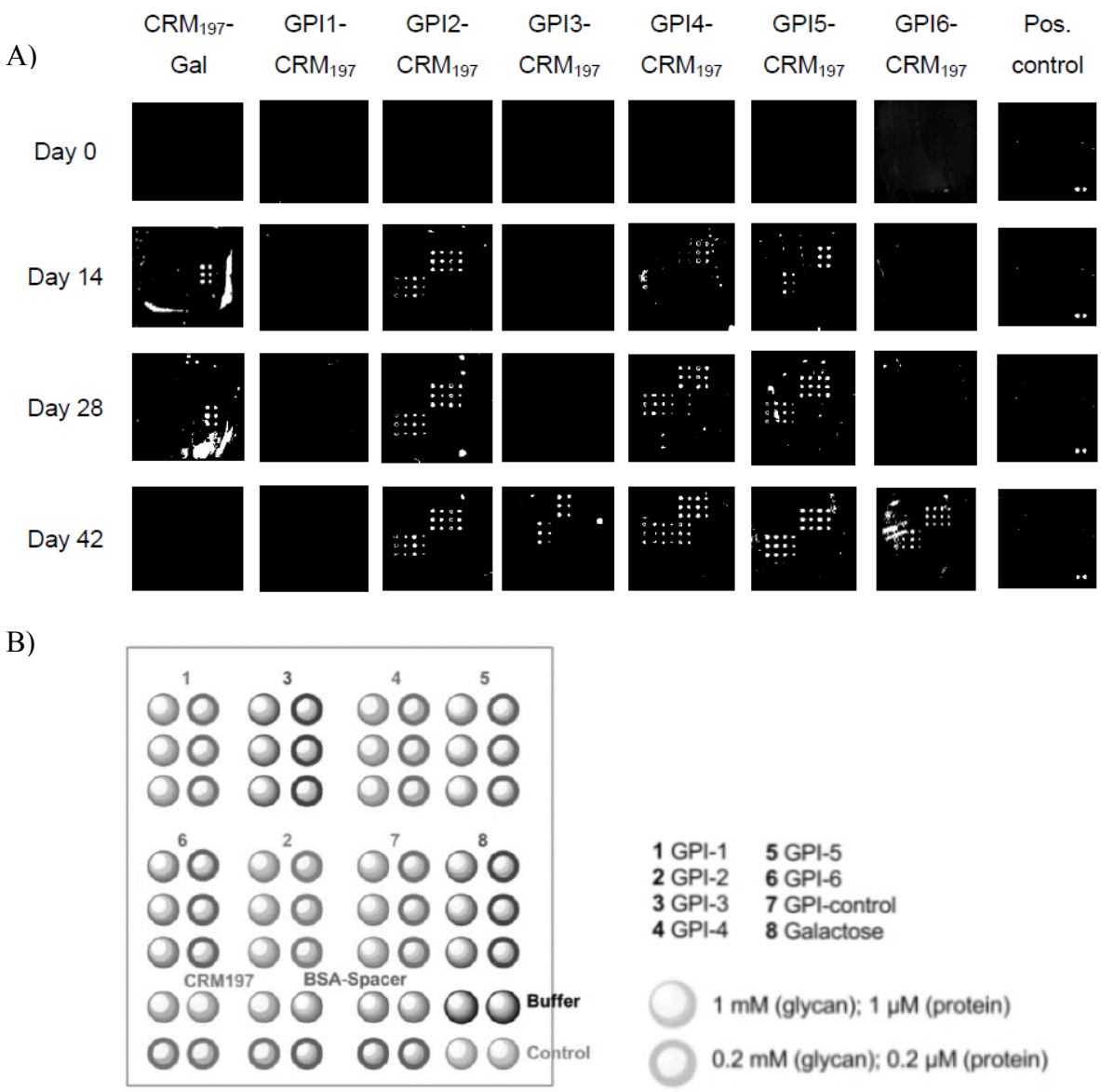


Figure S5: Glycan microarray for glycoconjugates at two concentrations for anti-GPI antibody level at days 0, 14, 28 and 42. (A) Representative microarray wells results from serum of mice immunized with CRM-**1**–**6** (day 0–42) and rabbit anti-*S. pneumoniae* antibodies as positive control. (B) Microarray printing pattern of maleimide treated glass slides (PolyAn, Berlin, Germany).

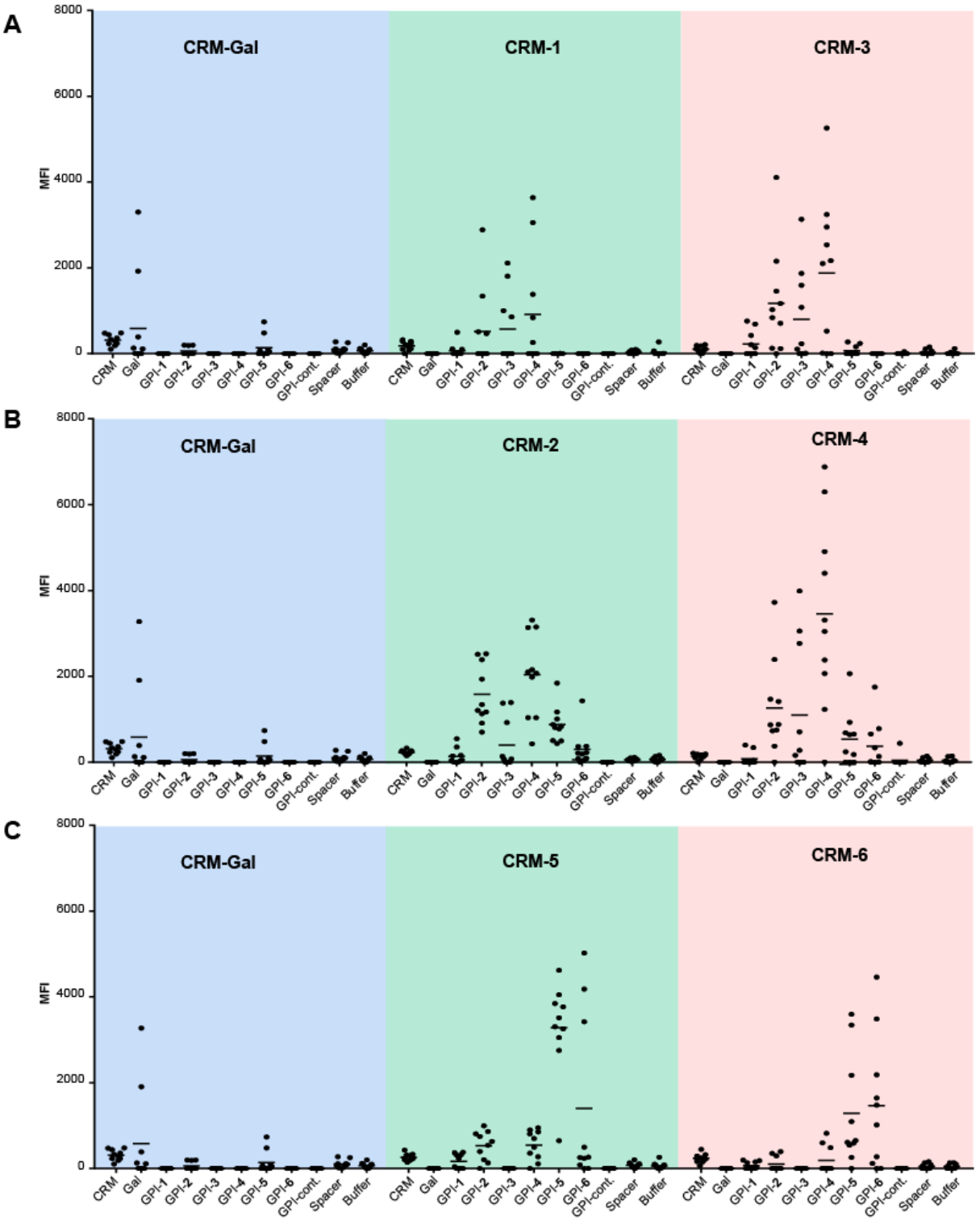


Figure S6: Cross-reactivity of anti-GPI antibodies after day 42. **(A)** Cross-reactivity of serum from **CRM-Gal, CRM-1** and **CRM-3** immunized mice against synthetic GPIs **(B)** Cross-reactivity of serum from **CRM-Gal, CRM-2** and **CRM-4** immunized mice against synthetic GPIs **(C)** Cross-reactivity of serum from **CRM-Gal, CRM-5** and **CRM-6** immunized mice against synthetic GPIs.

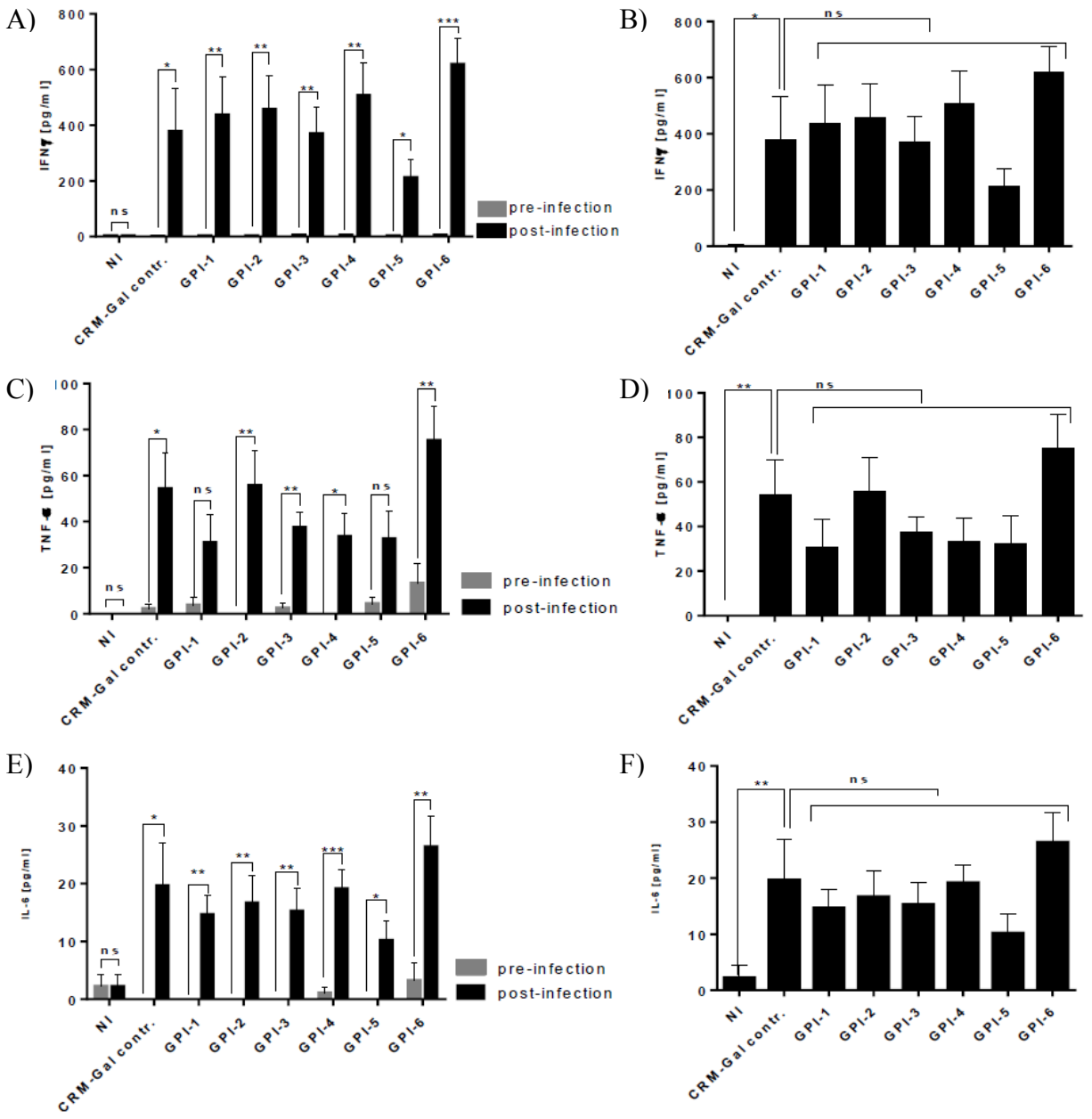


Figure S7: Pro-inflammatory cytokine levels of infected mice. (**A, C, E**) comparison in the level of IFN-γ, TNF-α and IL-6 for **CRM 1–6** immunized and control mice before and after infection. (**B, D, F**) Comparison in the serum levels of pro-inflammatory cytokines between non-infected mice and **CRM-GPI** vaccinated mice. Statistical significance was determined using the unpaired Student's t-test, significance shown by asterisks *($p < 0.05$), **($p < 0.01$) , ***($p < 0.001$); not significant results labeled ns ($p > 0.05$)

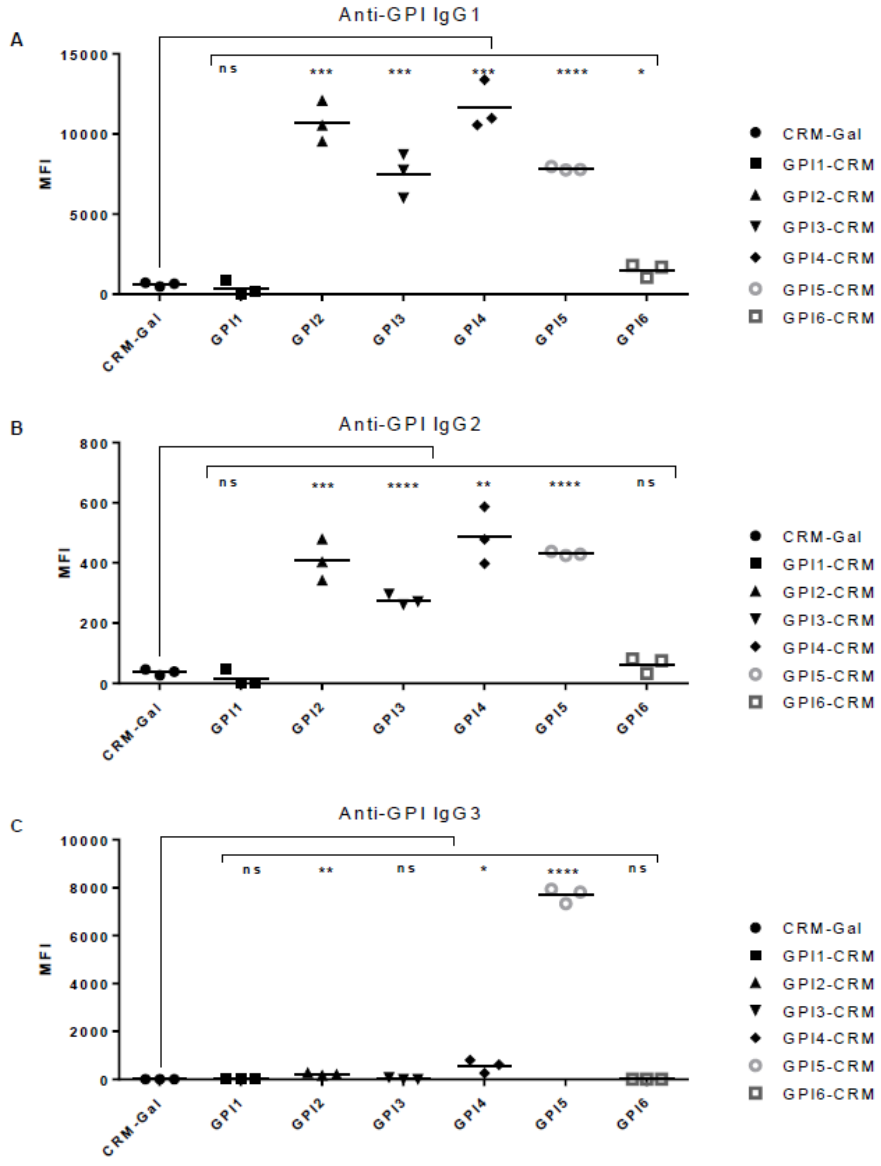


Figure S8. Endpoint anti-GPI antibody titer for IgG subclasses measured by glycan array for mice immunized with GPI-CRM-conjugates at day 42. Serum antibody levels at day 42 against CRM and synthetic GPI glycans 1-6 in mice immunized with CRM-Gal and CRM **1-6** for IgG subclasses IgG1 **(A)**, IgG2 **(B)** and IgG3 **(C).**

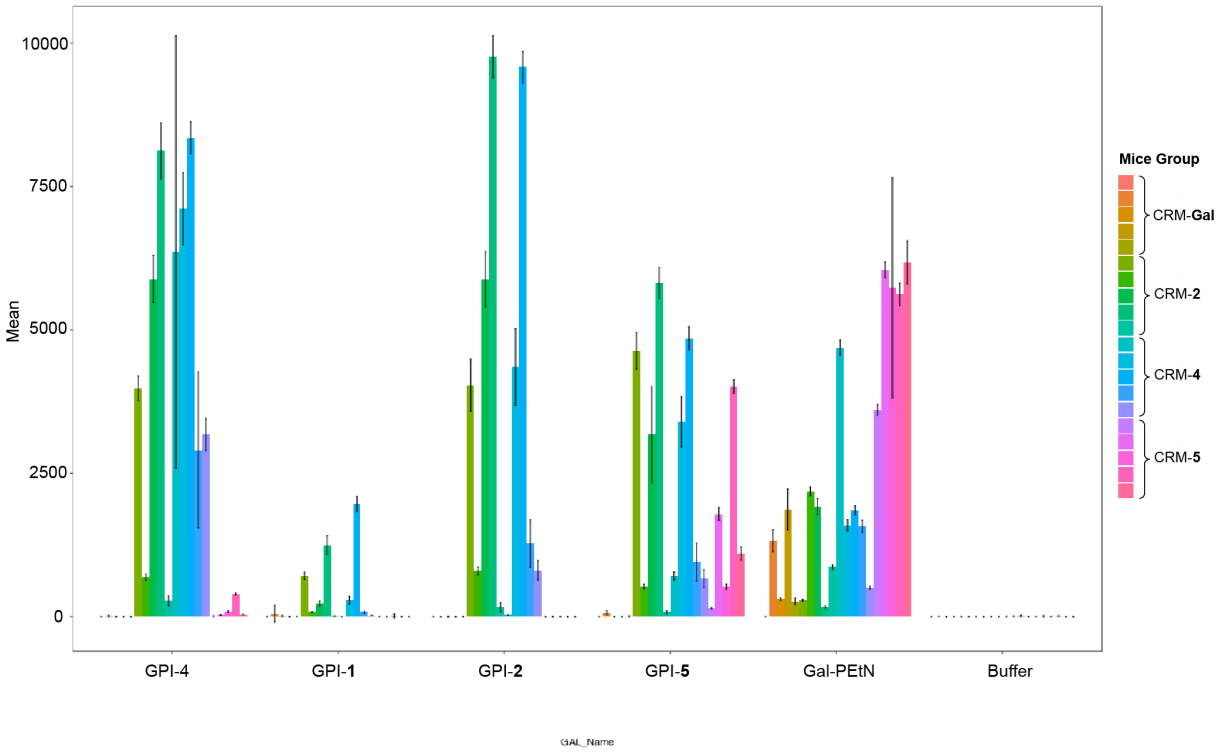


Figure **S9**. Binding of Antibodies to Gal-PEtN. Thea analysis was performed for the groups CRM-Gal, CRM-3, CRM-

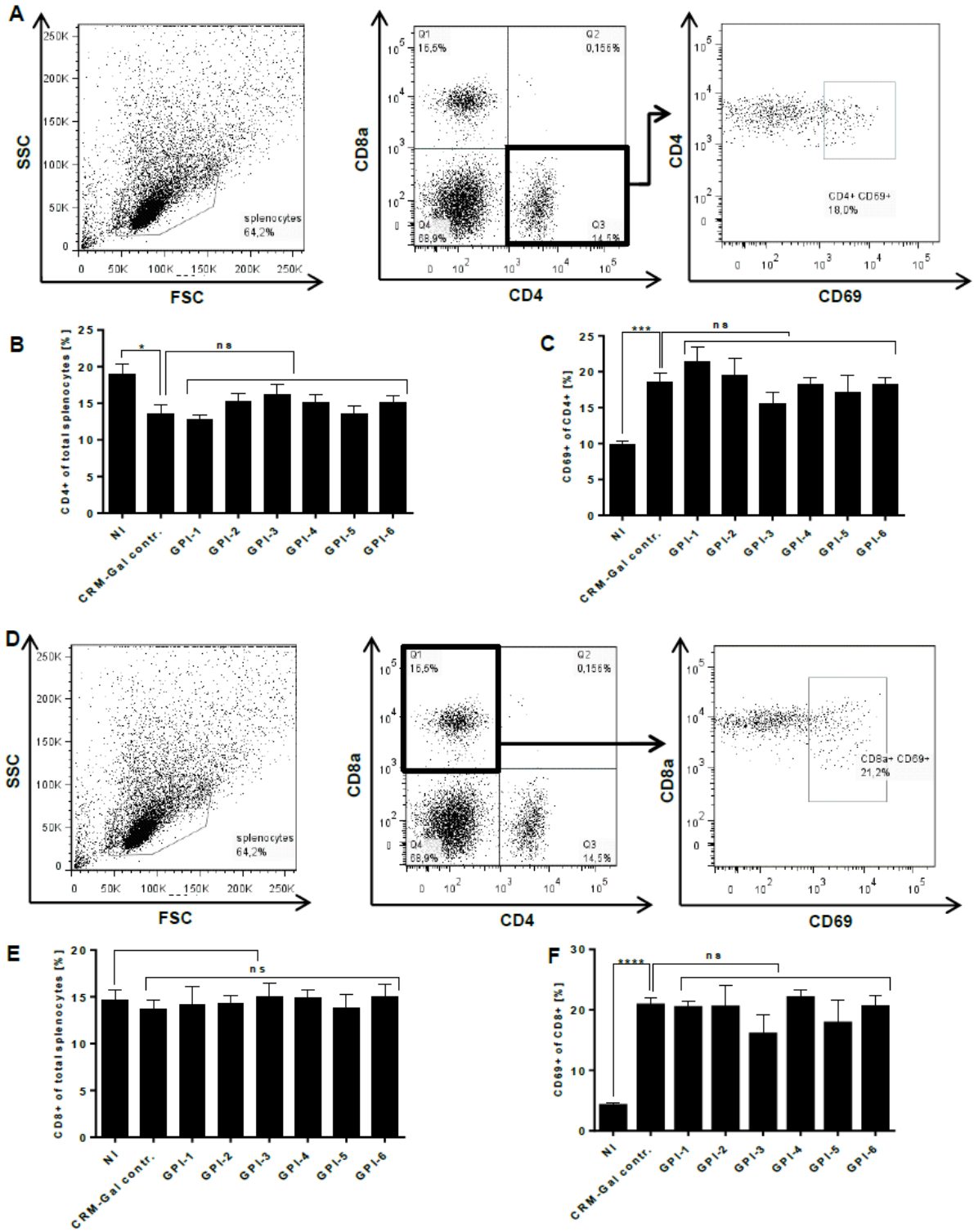
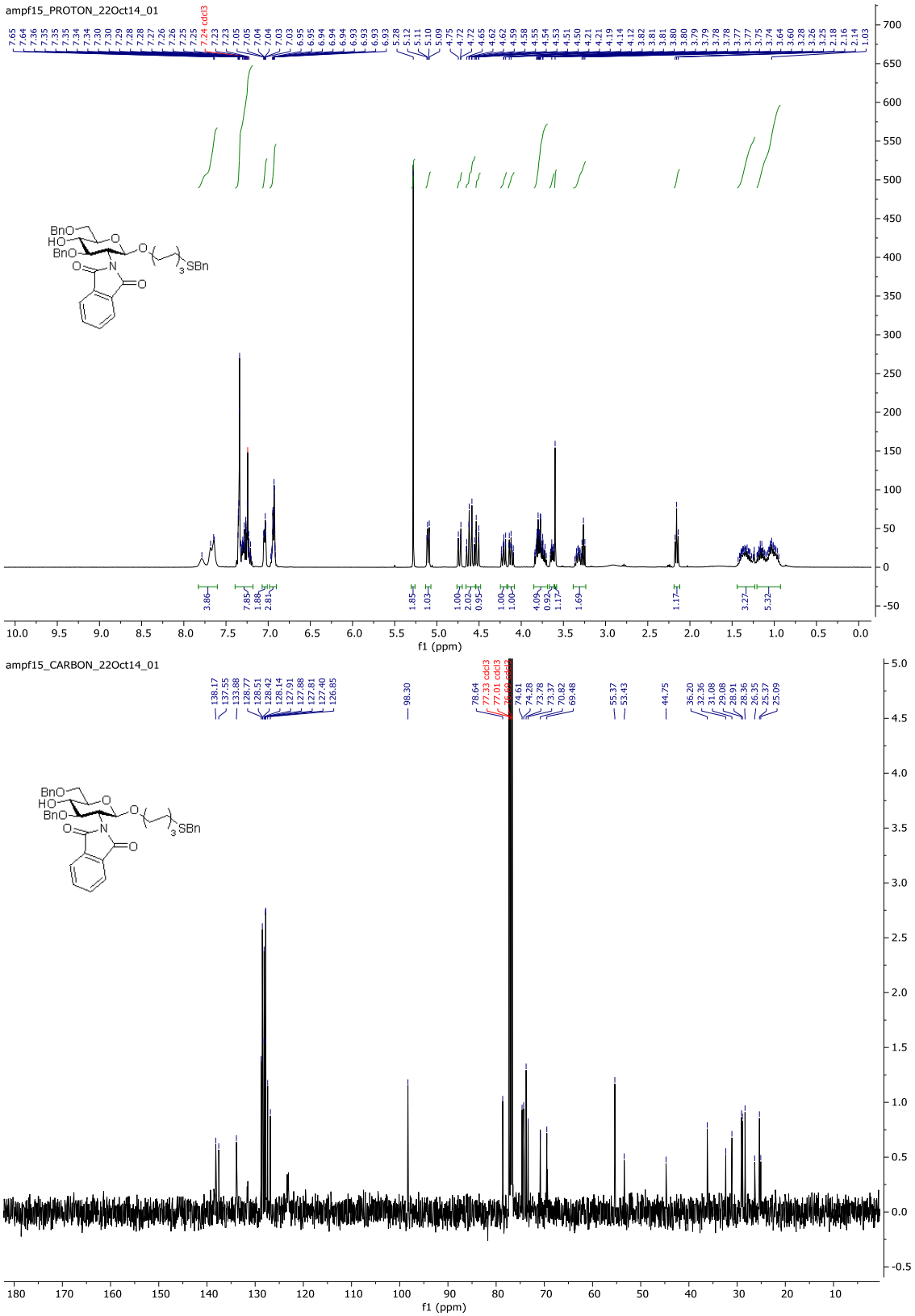


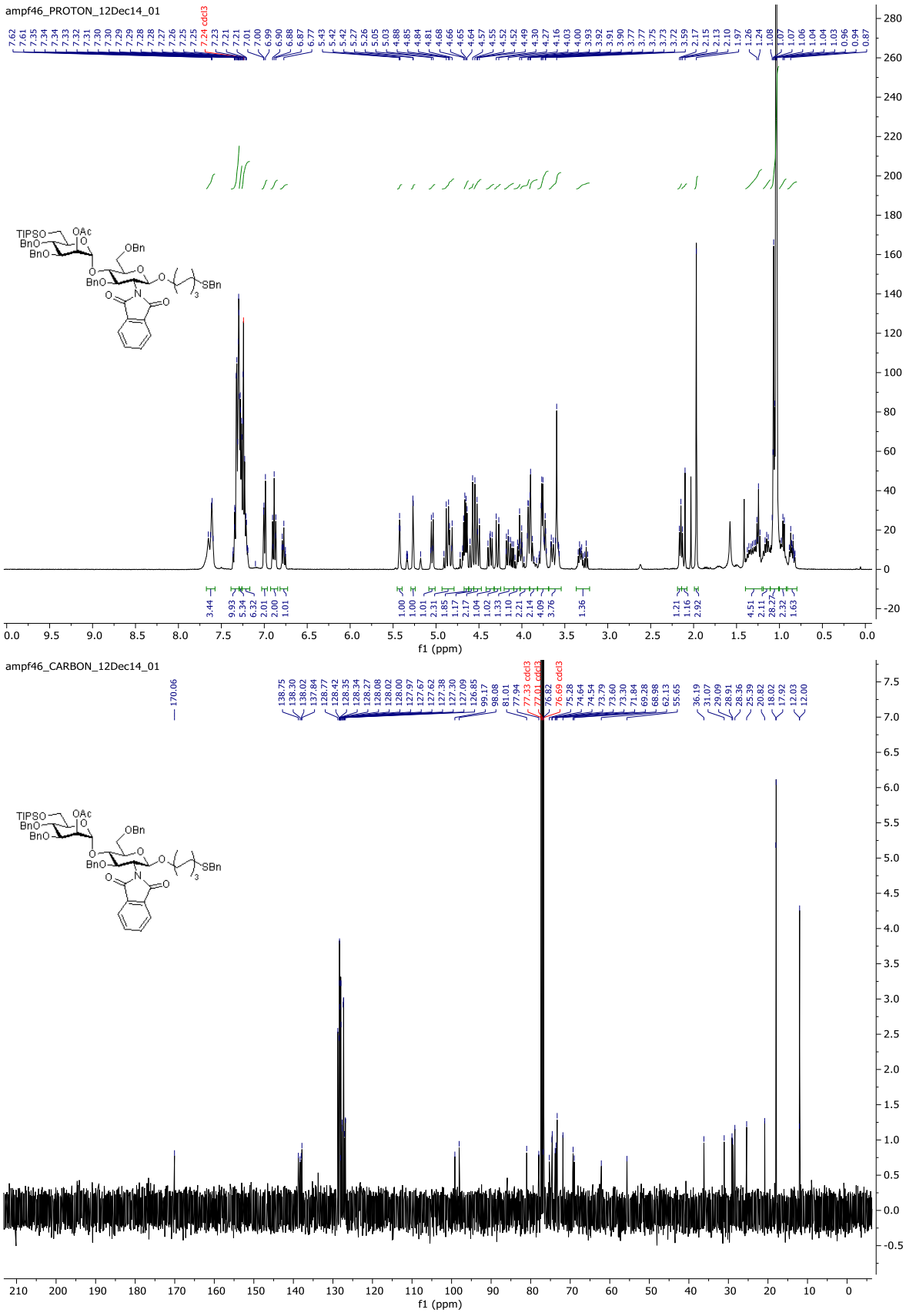
Figure S10. Spleen $CD8^+$ and $CD4^+$ T cell activation in immunized and *P. berghei* ANKA-challenged mice (**A**, **D**) Representative gatings of activated $CD4^+$ and $CD8^+$ spleen T cells. Spleen cells were isolated from immunized and *P. berghei* ANKA infected mice on day 6 *post* infection and quantified by flow cytometry. (**B**, **E**). (**C**, **F**) Cellular activation (measured by activation marker CD69) was marked in both $CD4^+$ and $CD8^+$ T cells compared to non-infected controls. Statistical significance was determined using Student's t-test, statistical significance shown by asterisks *($p<0.05$), ***($p<0.001$) and ****($p<0.0001$).

NMR Spectra

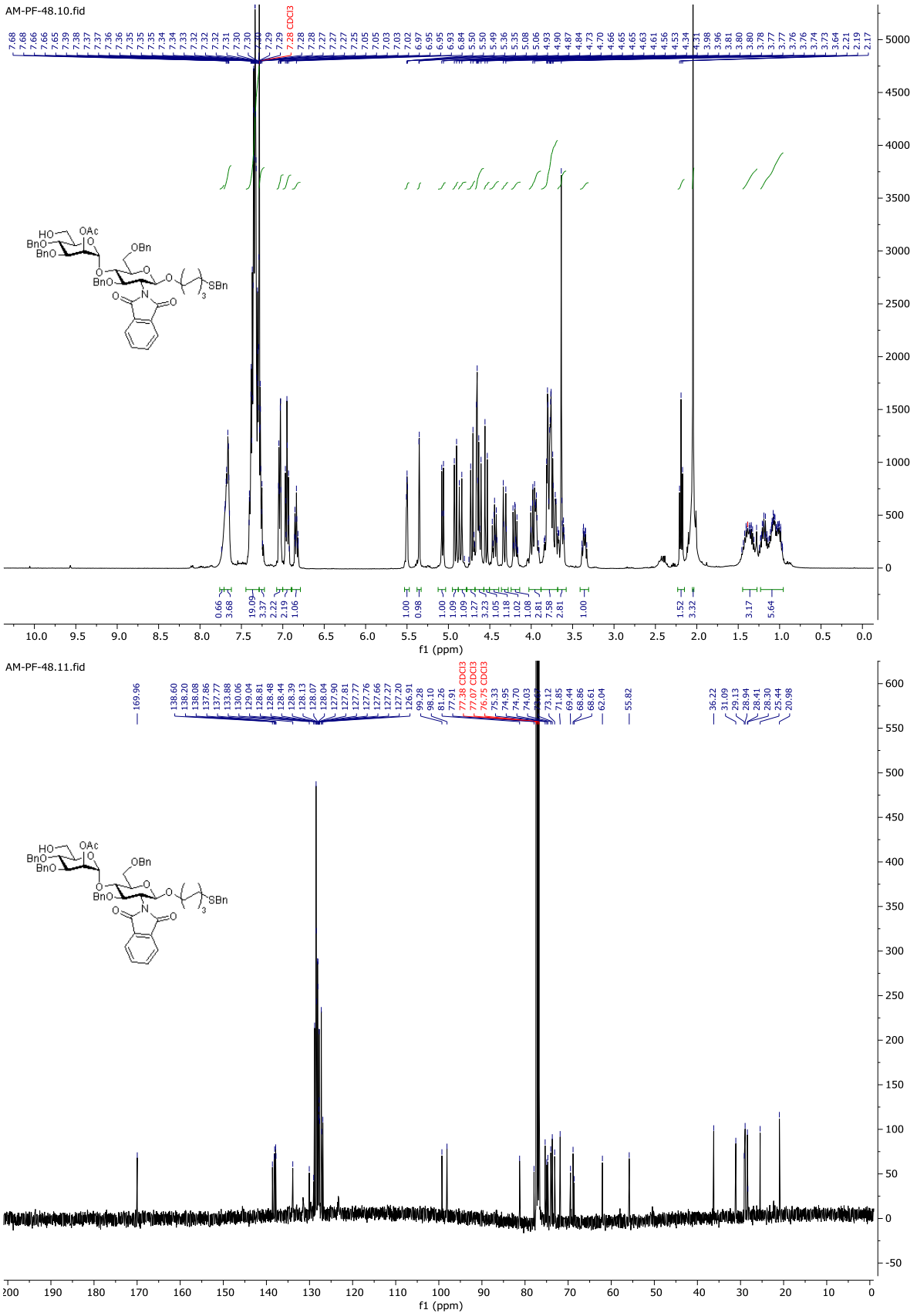
1-O-(6-thiobenzyl)hexyl-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**8**)



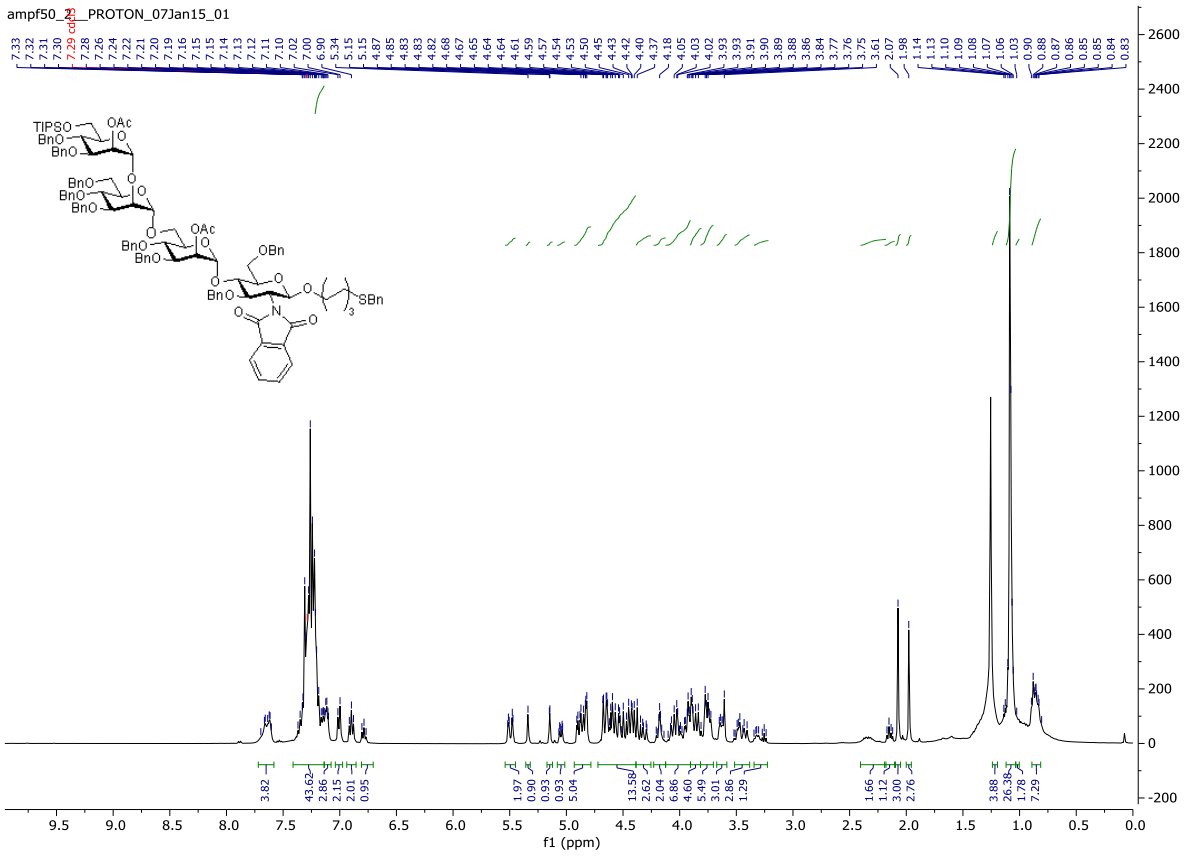
2-O-Acetyl-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→4)-1-O-(6-thiobenzyl)hexyl-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**9**)

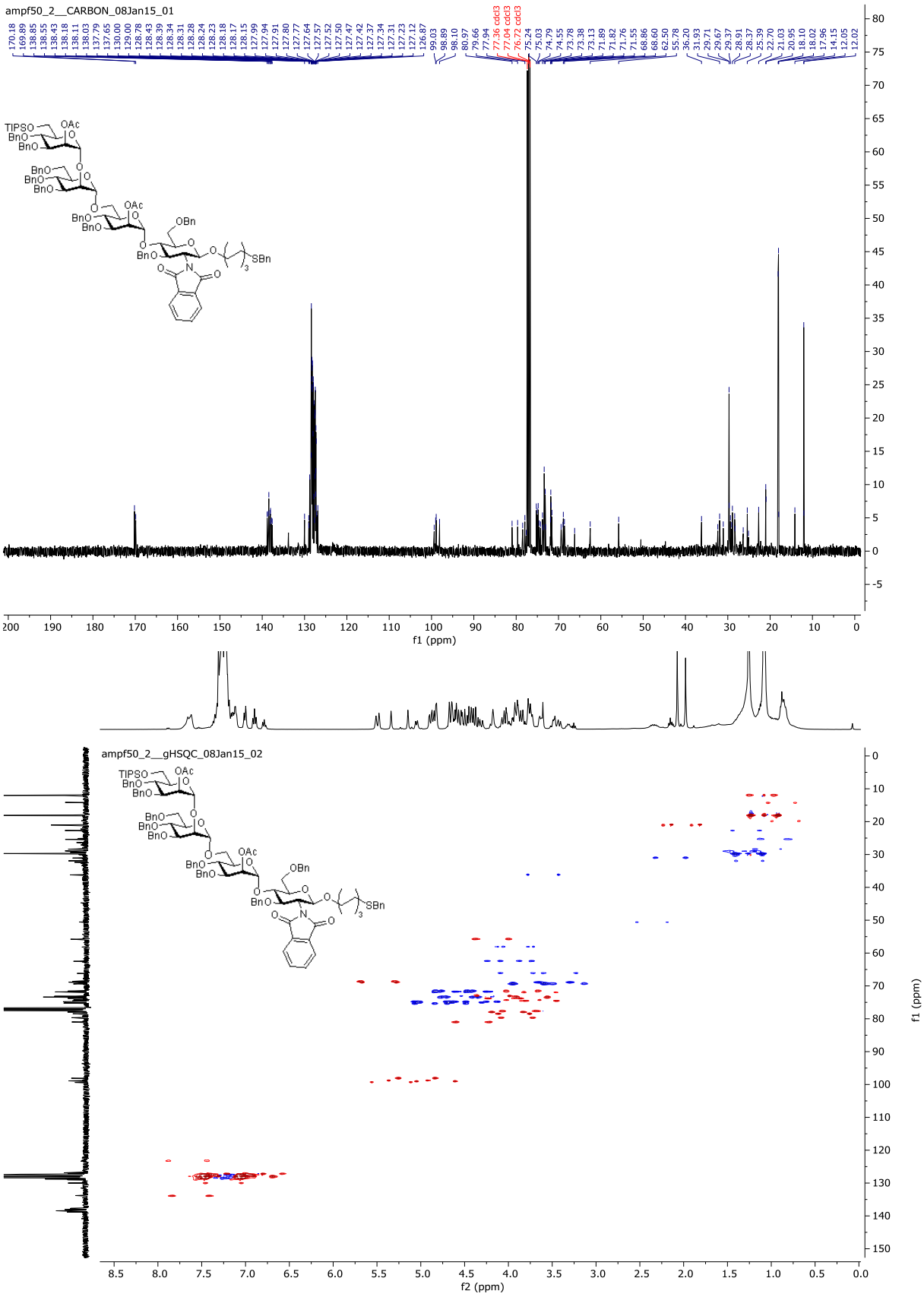


2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)- 1-O-(6-thiobenzyl)hexyl-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**10**)

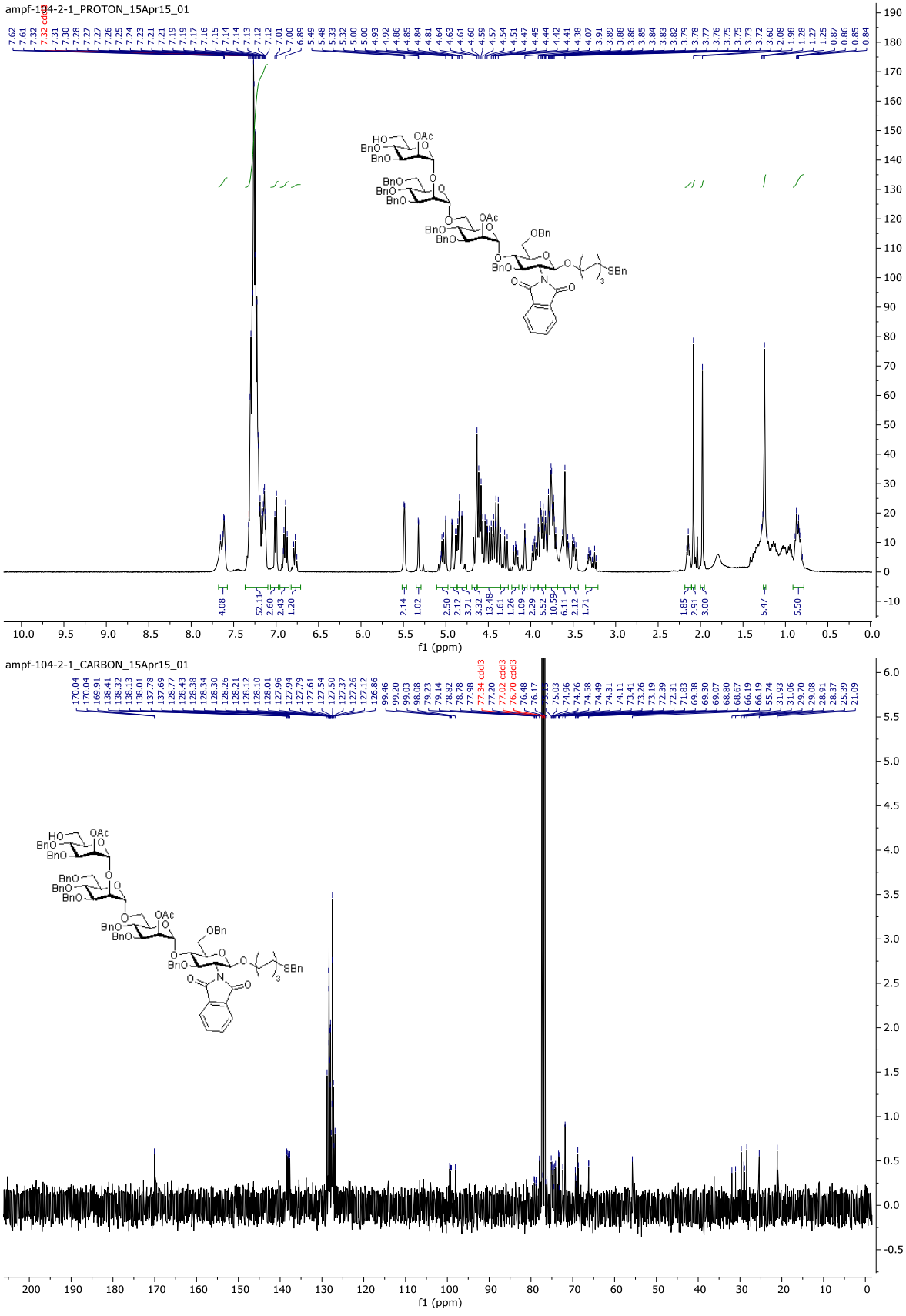


1-O-(6-thiobenzyl)hexyl-2-O-acetyl-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**13**)

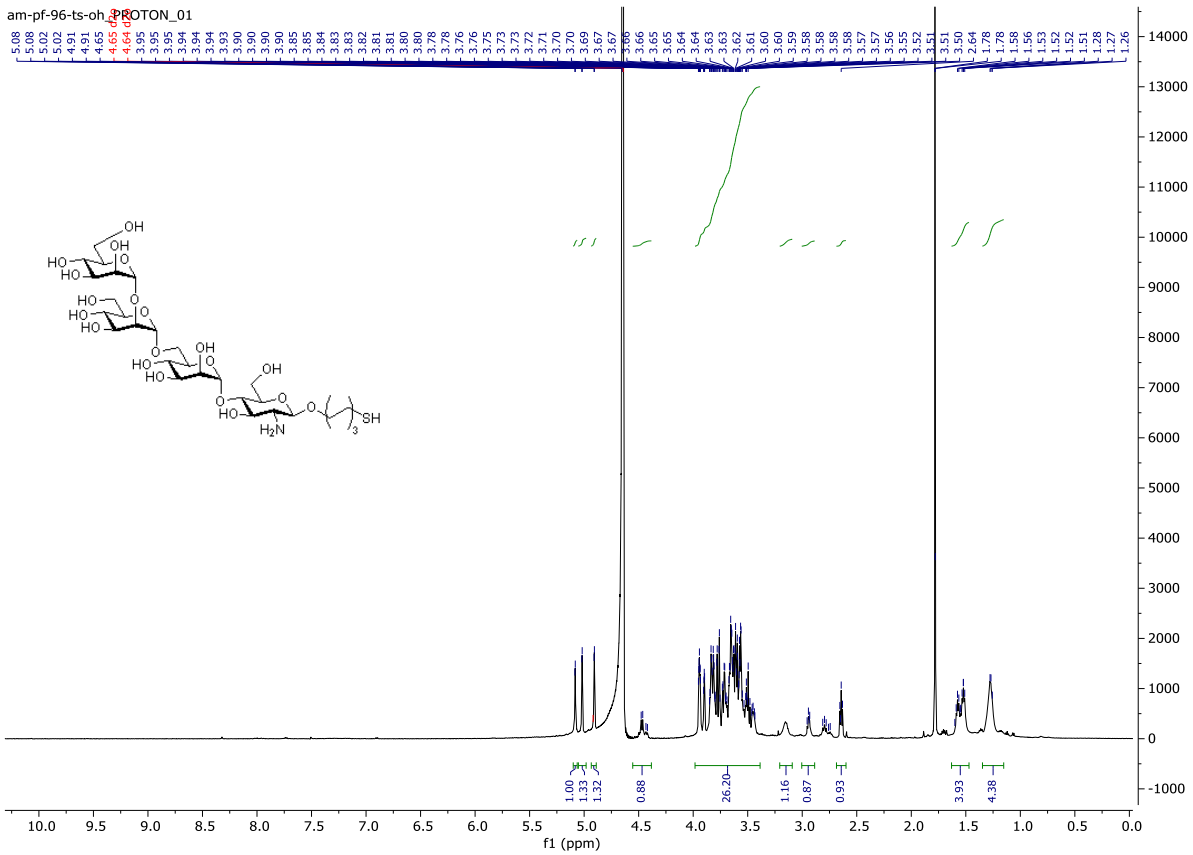

ampf50_2__PROTON_07Jan15_01
TIPSO
BnO
OAc
OBn
SBn
f1 (ppm)

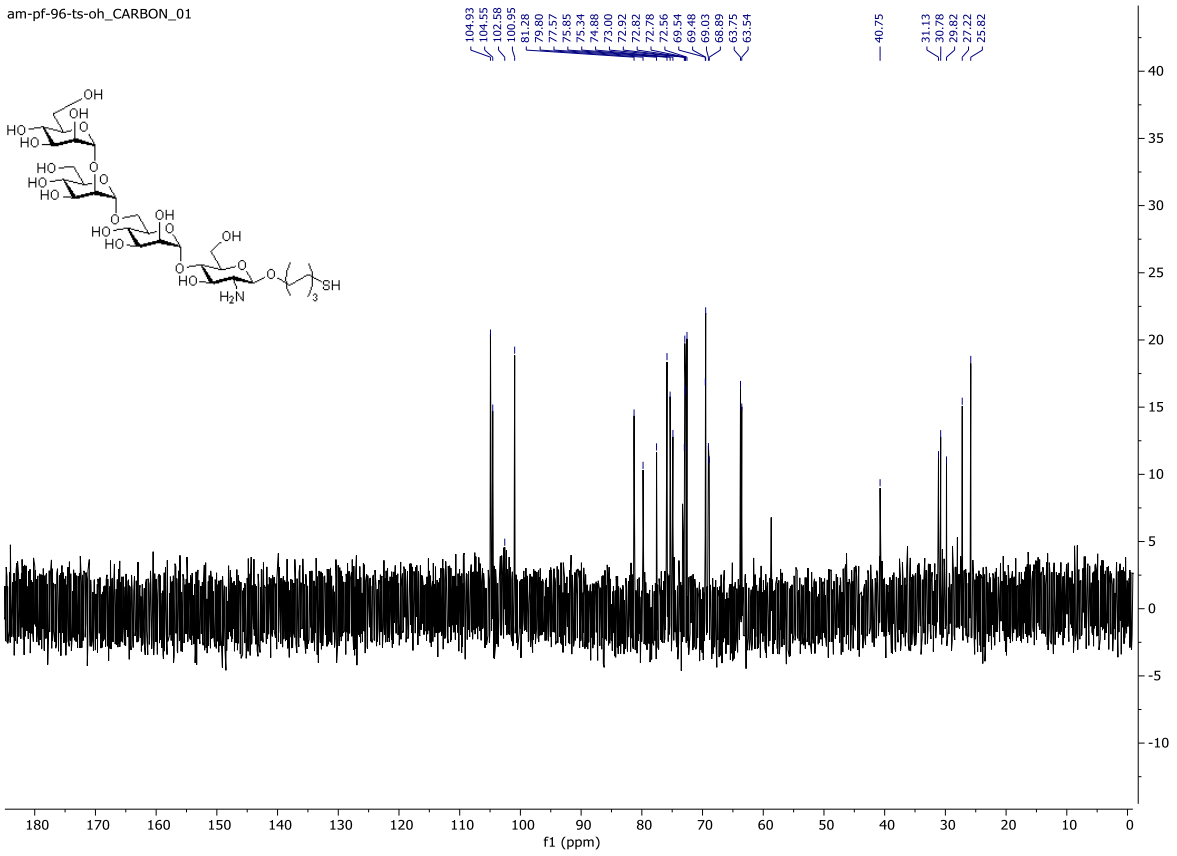

ampf50_2__CARBON_08Jan15_01
f1 (ppm)
ampf50_2__gHSQC_08Jan15_02
f2 (ppm)
f1 (ppm)

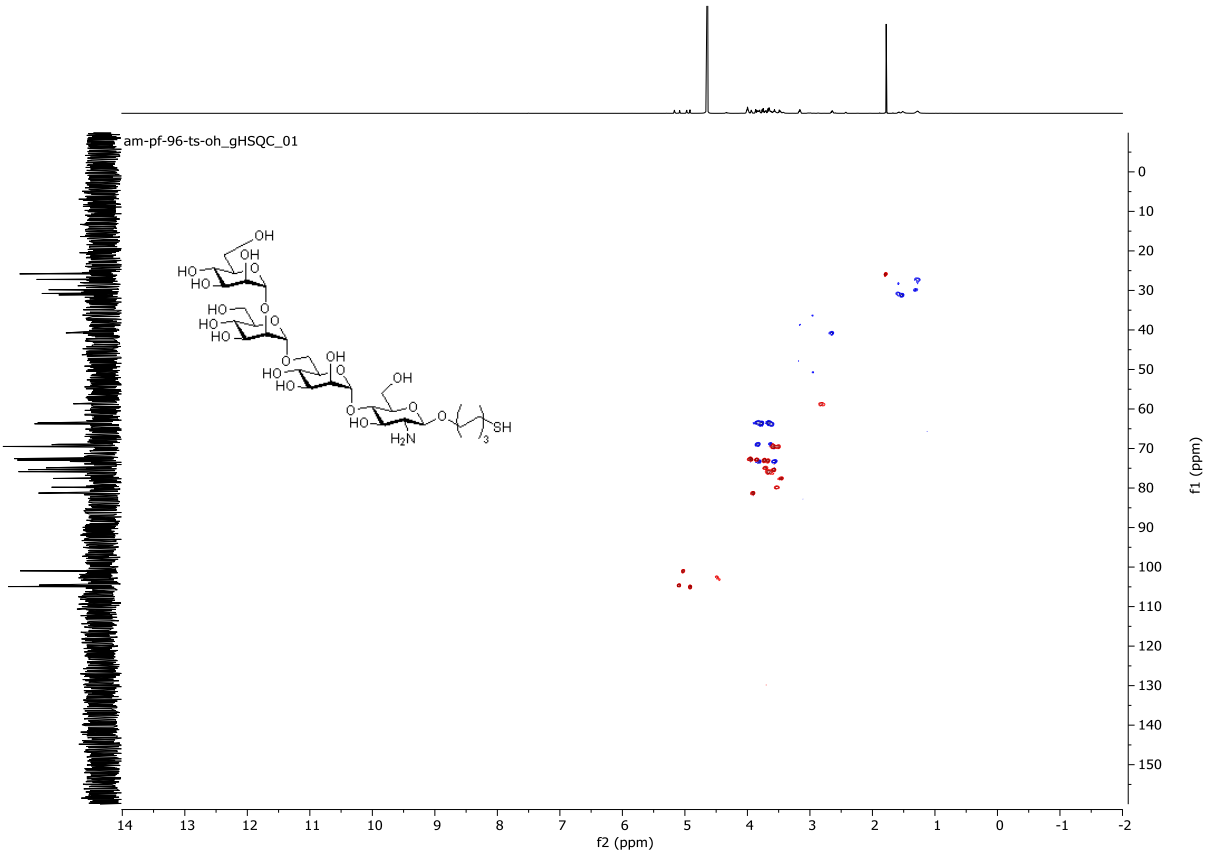
1-O-(6-thiobenzyl)hexyl-2-O-acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**14**)



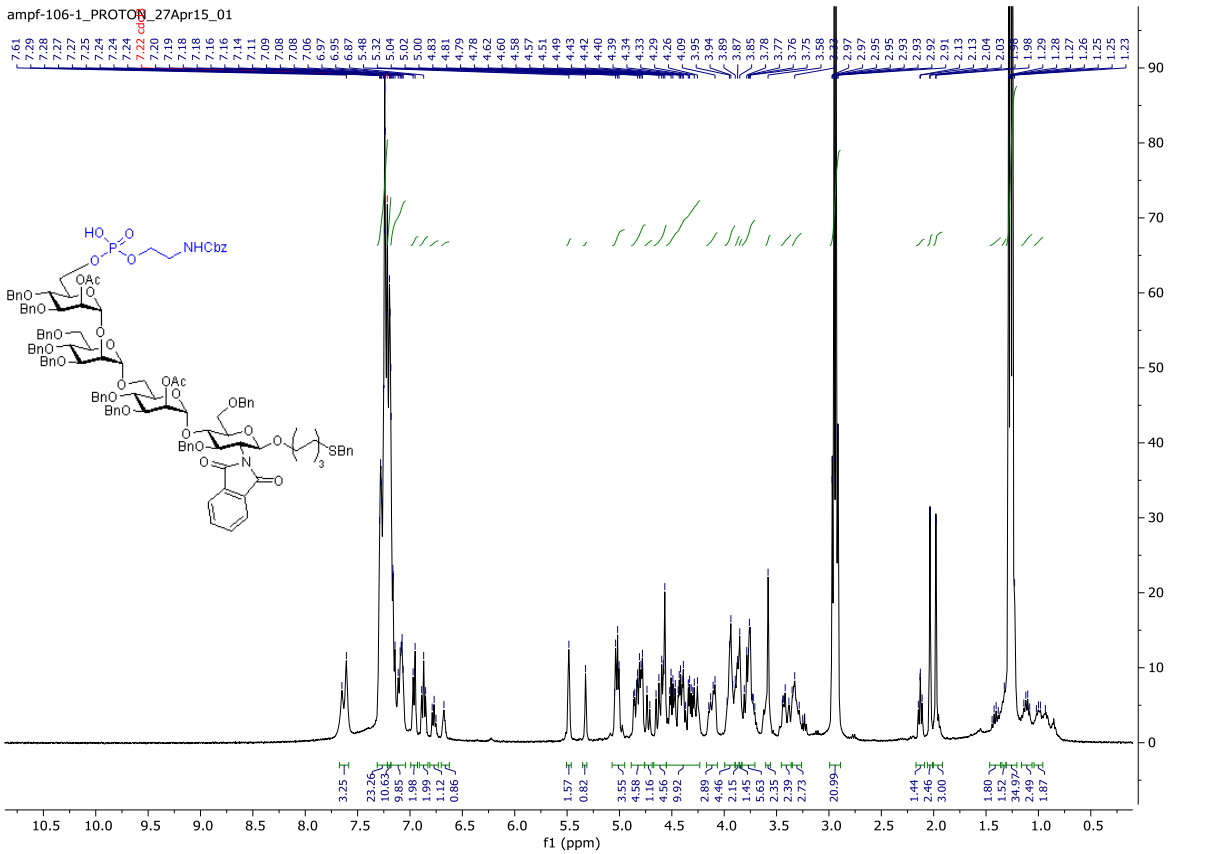
1-O-(6-thio)hexyl-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranoside (**1**)

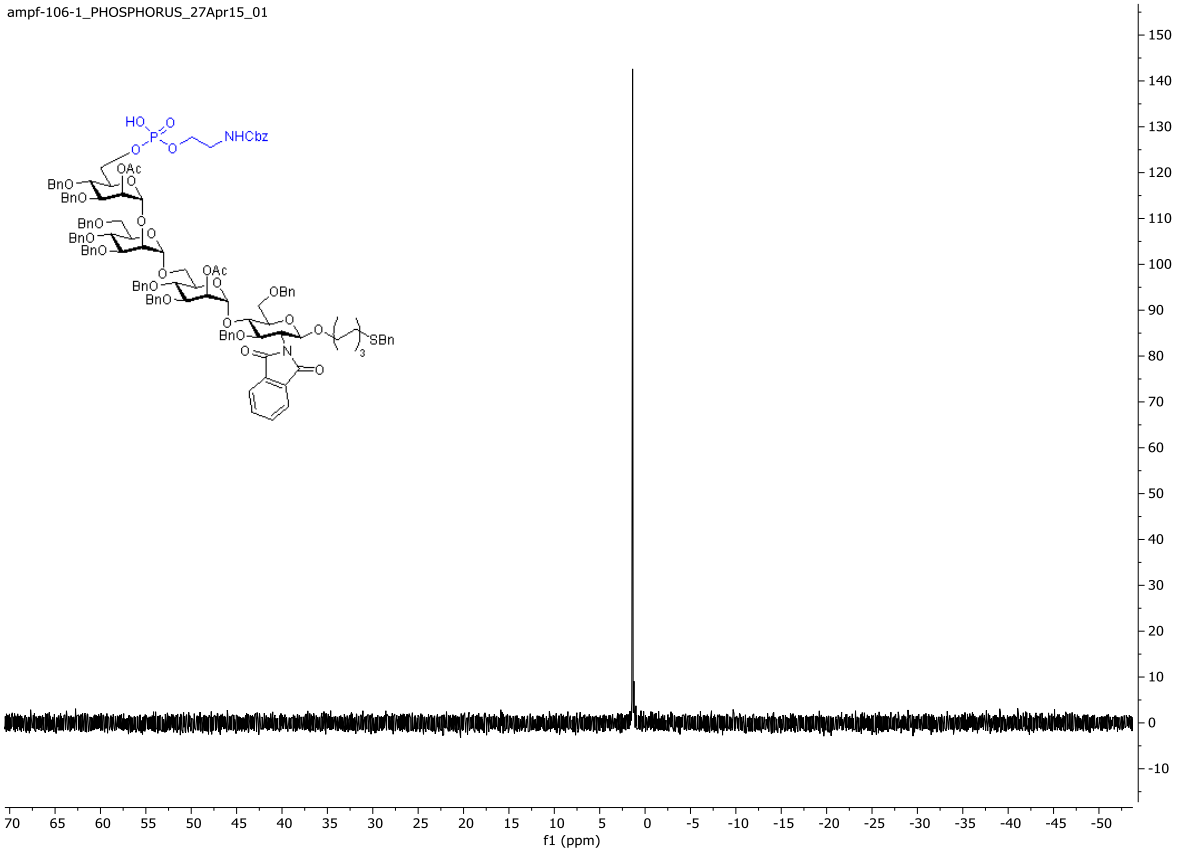




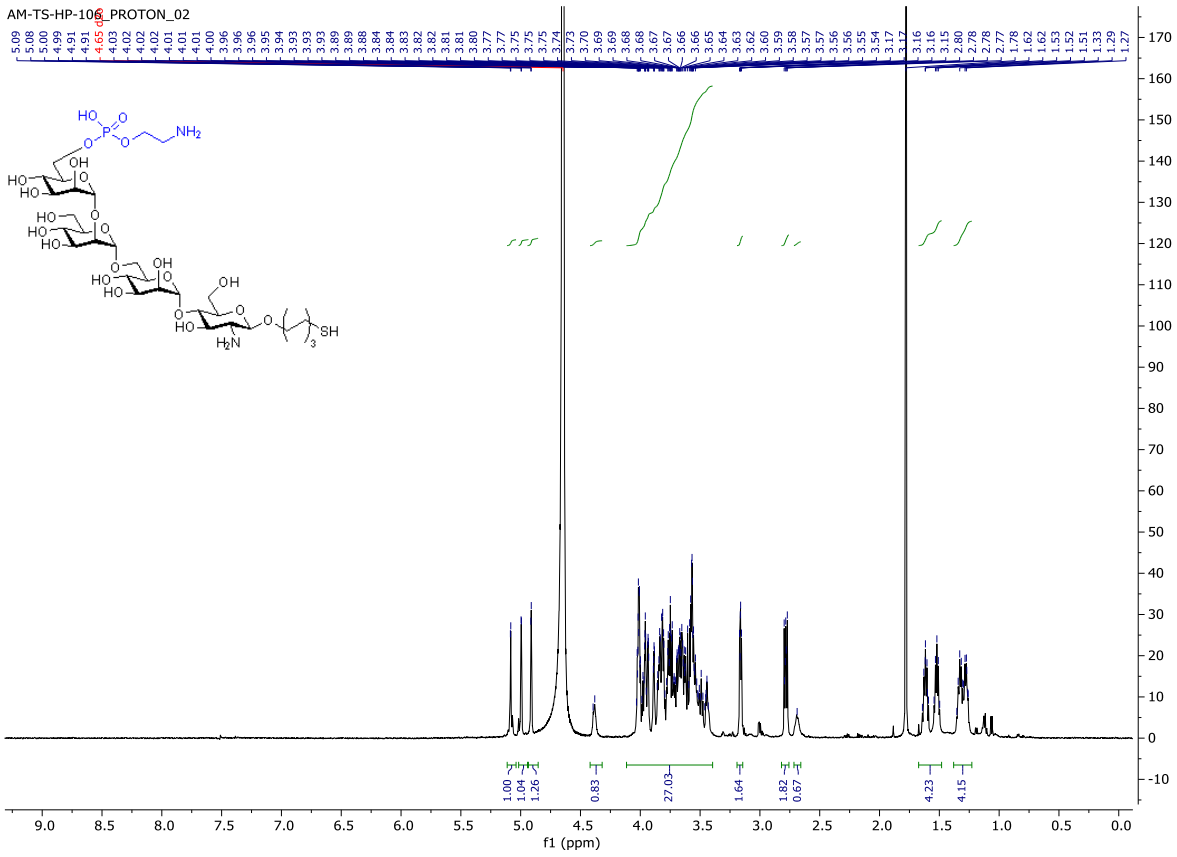


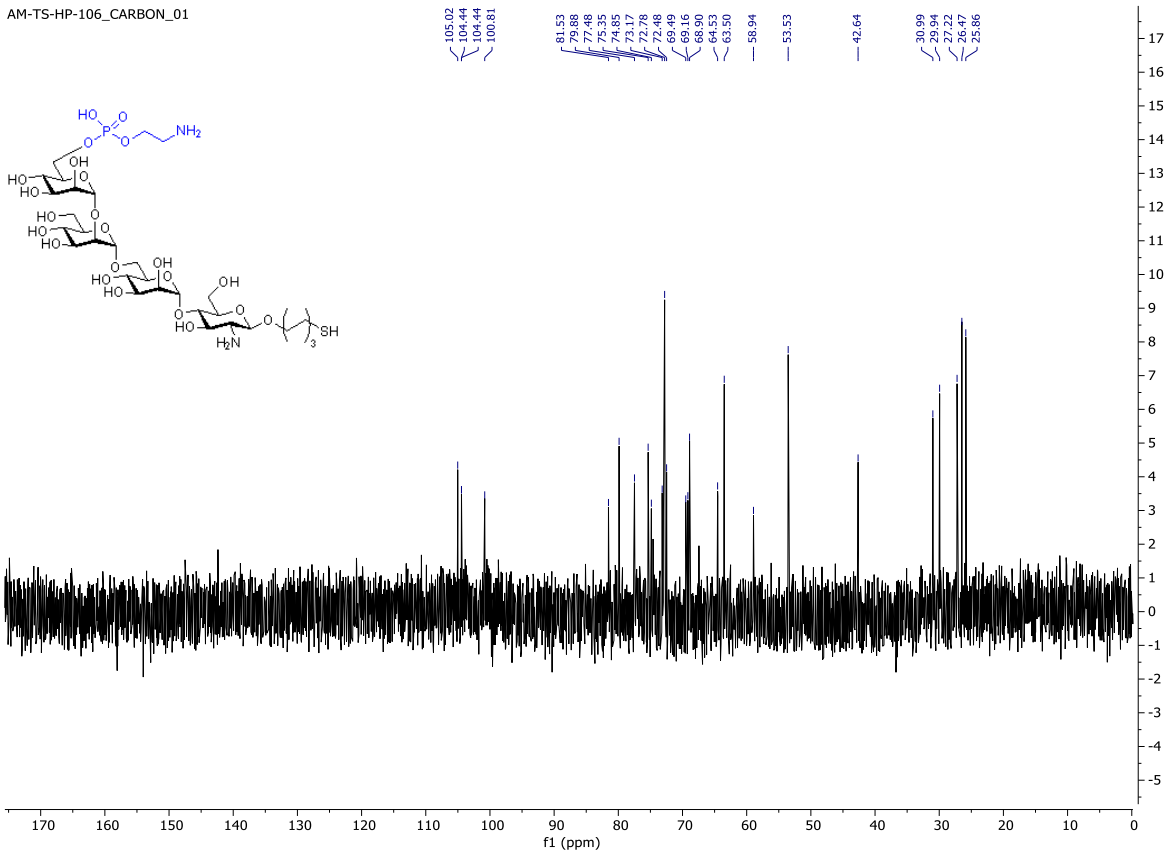
1-O-(6-thiobenzyl)hexyl-2-O-Acetyl-3,4-di-O-benzyl-6-O-(2-N-benzyloxycarbonyl) aminoethyl-phosphonato-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)- 3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**15**)

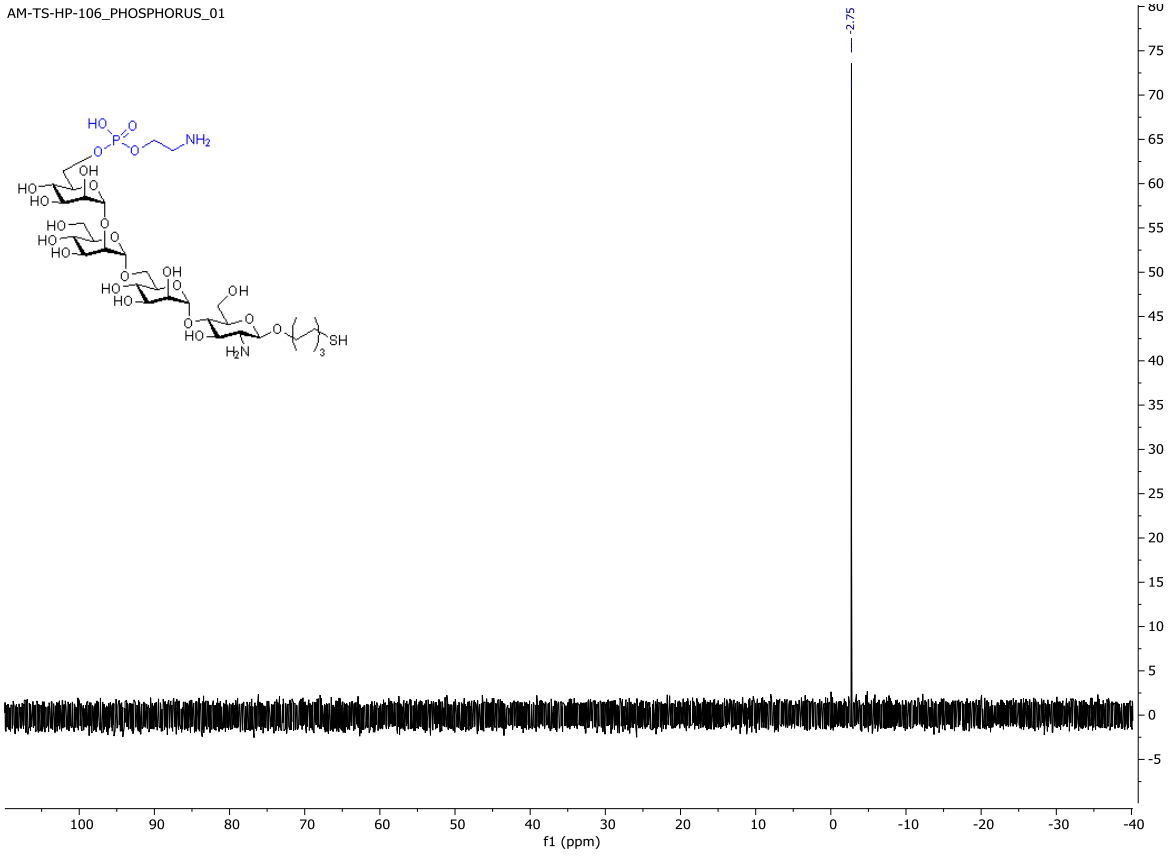


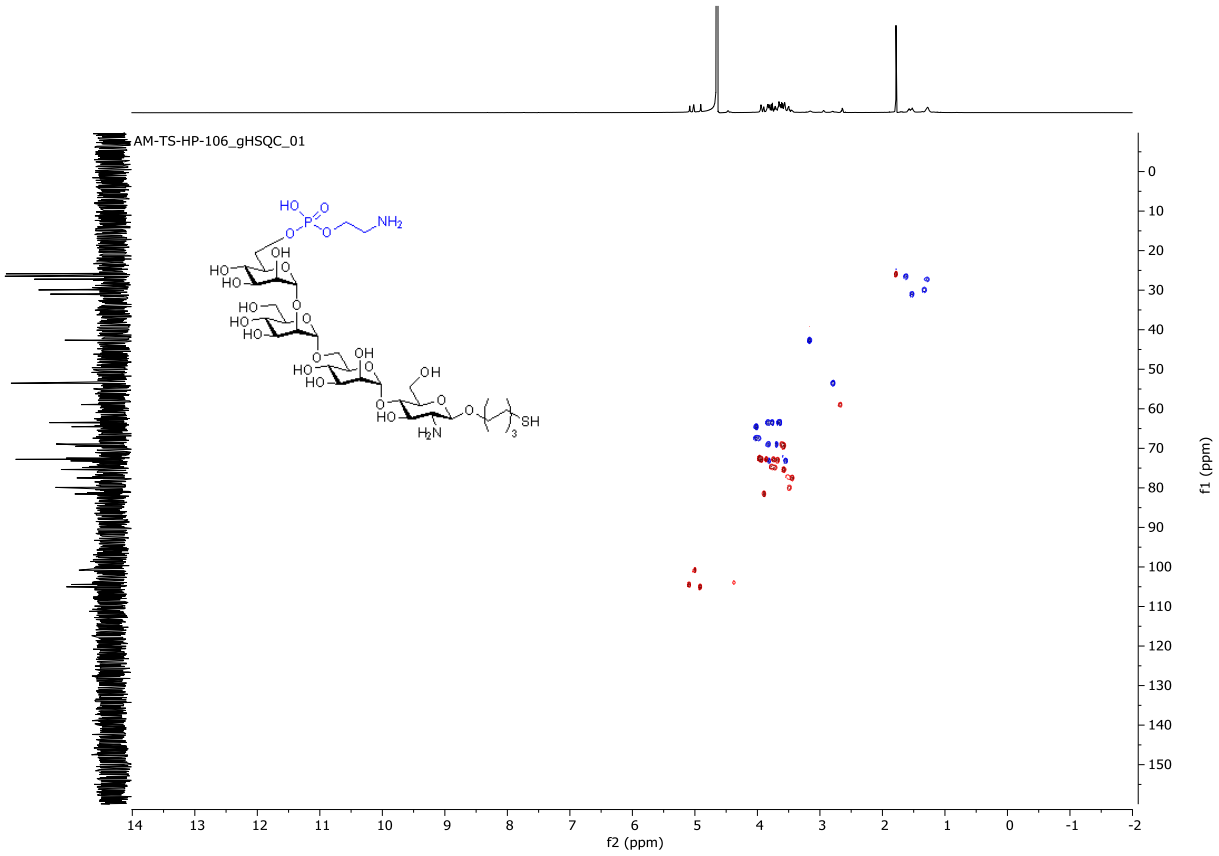


1-O-(6-thio)hexyl-6-O-aminoethyl-phosphonato-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-2-deoxy-2-amino-β-D-glucopyranoside (**2**)

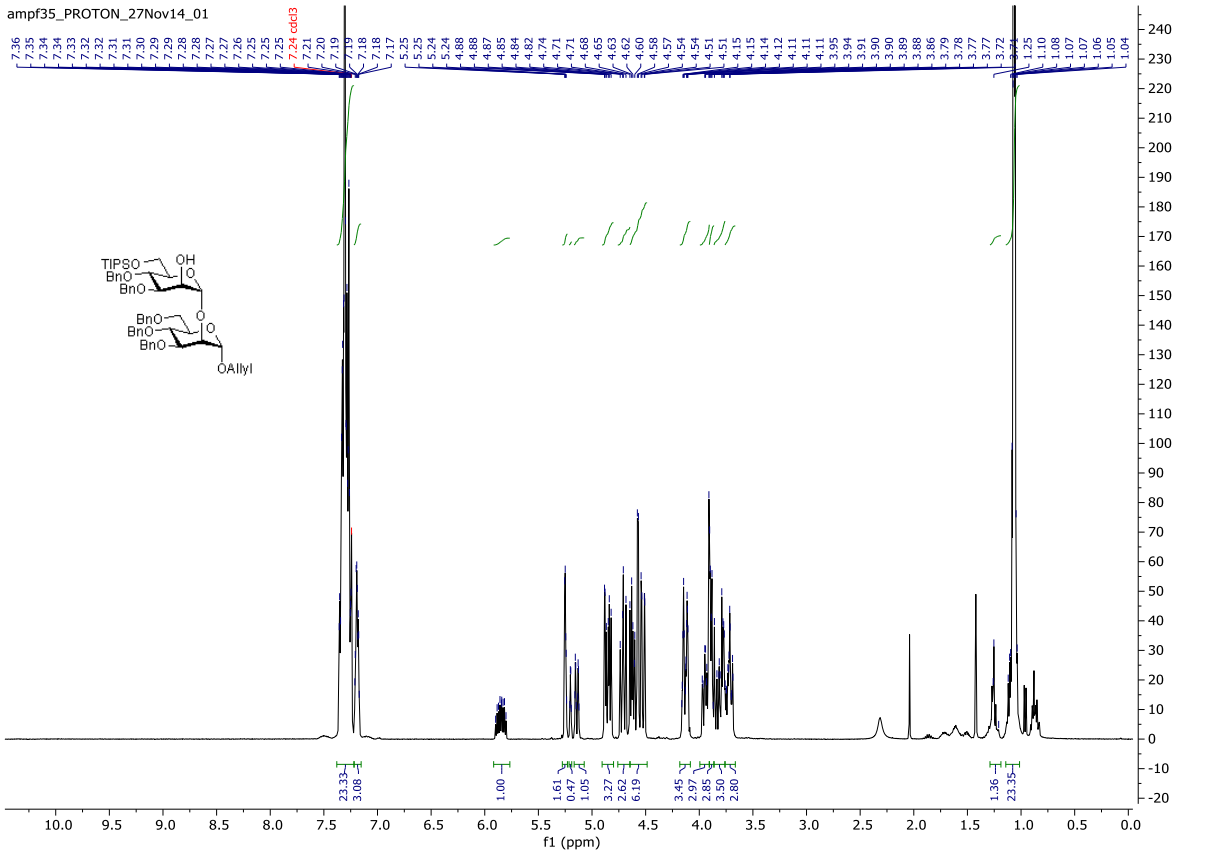


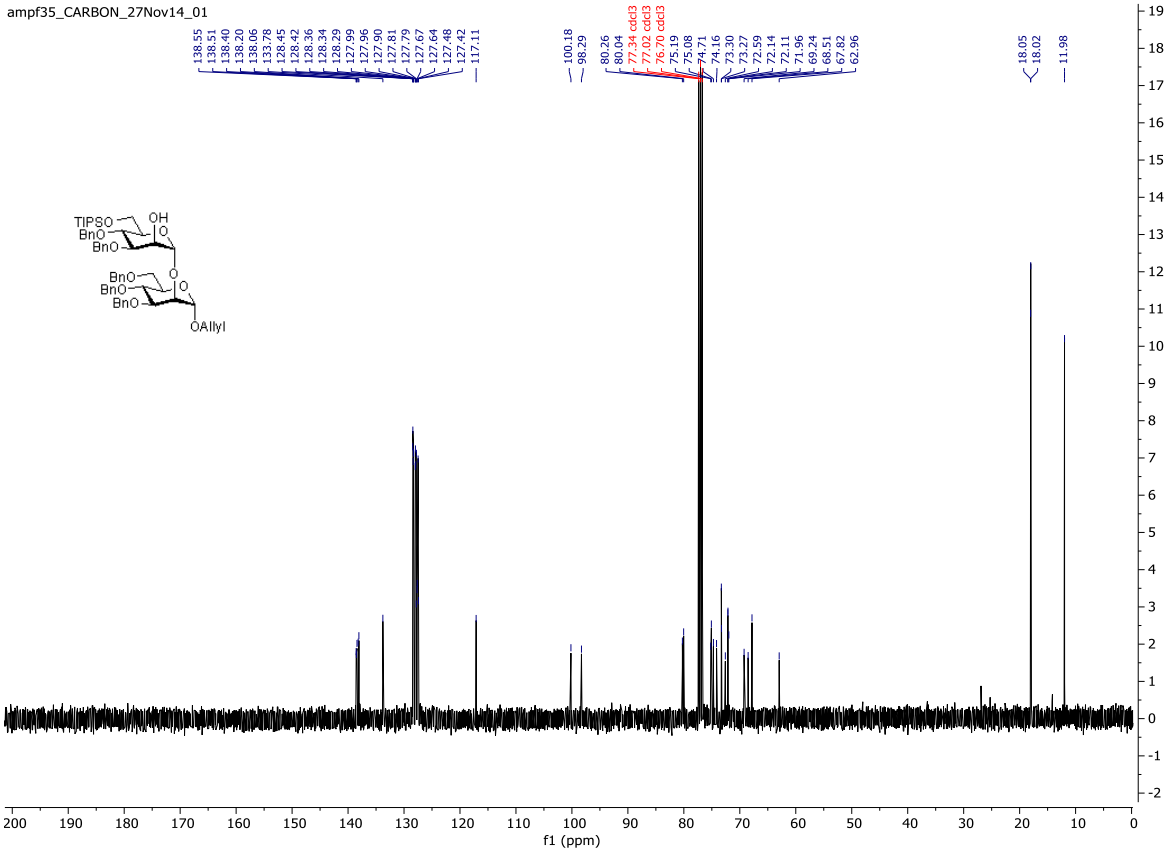

AM-TS-HP-106_CARBON_01
105.02
104.44
104.44
100.81
81.53
79.88
77.48
75.35
74.85
73.17
72.78
72.48
69.49
69.16
68.90
64.53
63.50
58.94
53.53
42.64
30.99
29.94
27.22
26.47
25.86
f1 (ppm)


AM-TS-HP-106_PHOSPHORUS_01
2.75
f1 (ppm)

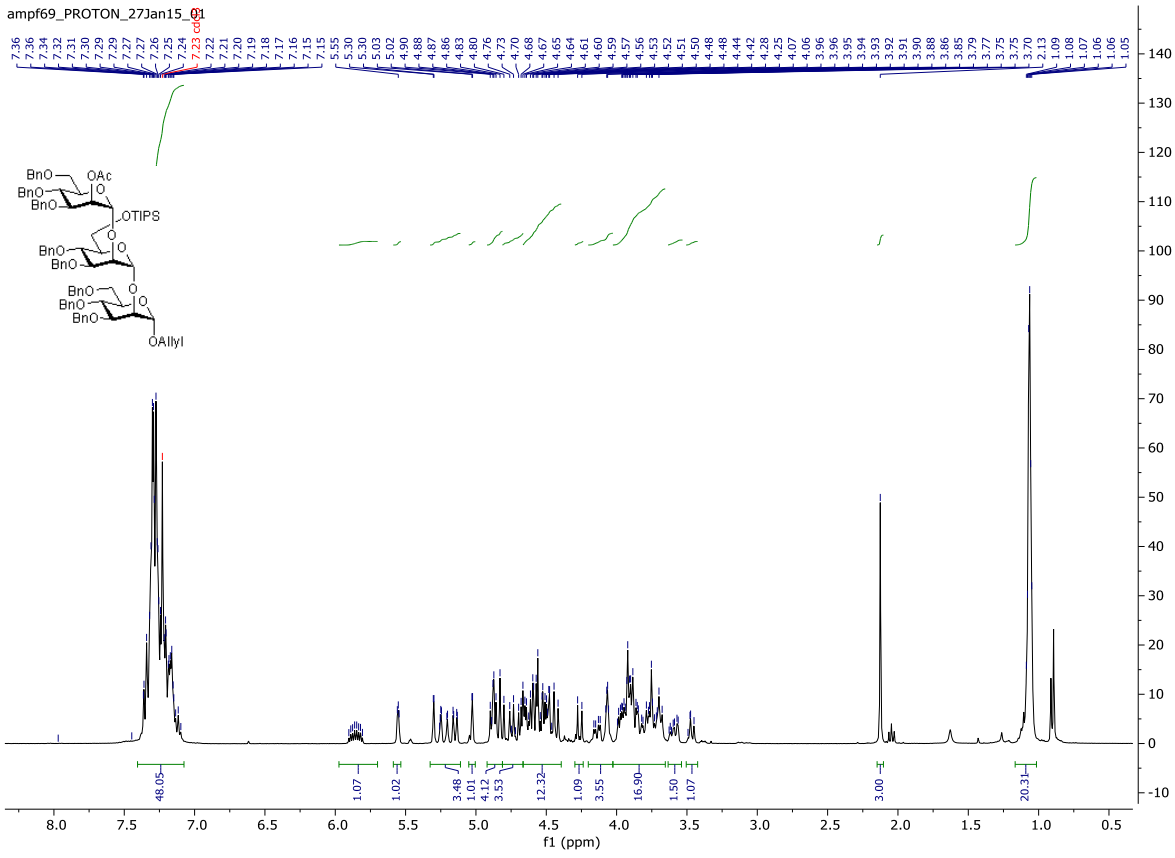


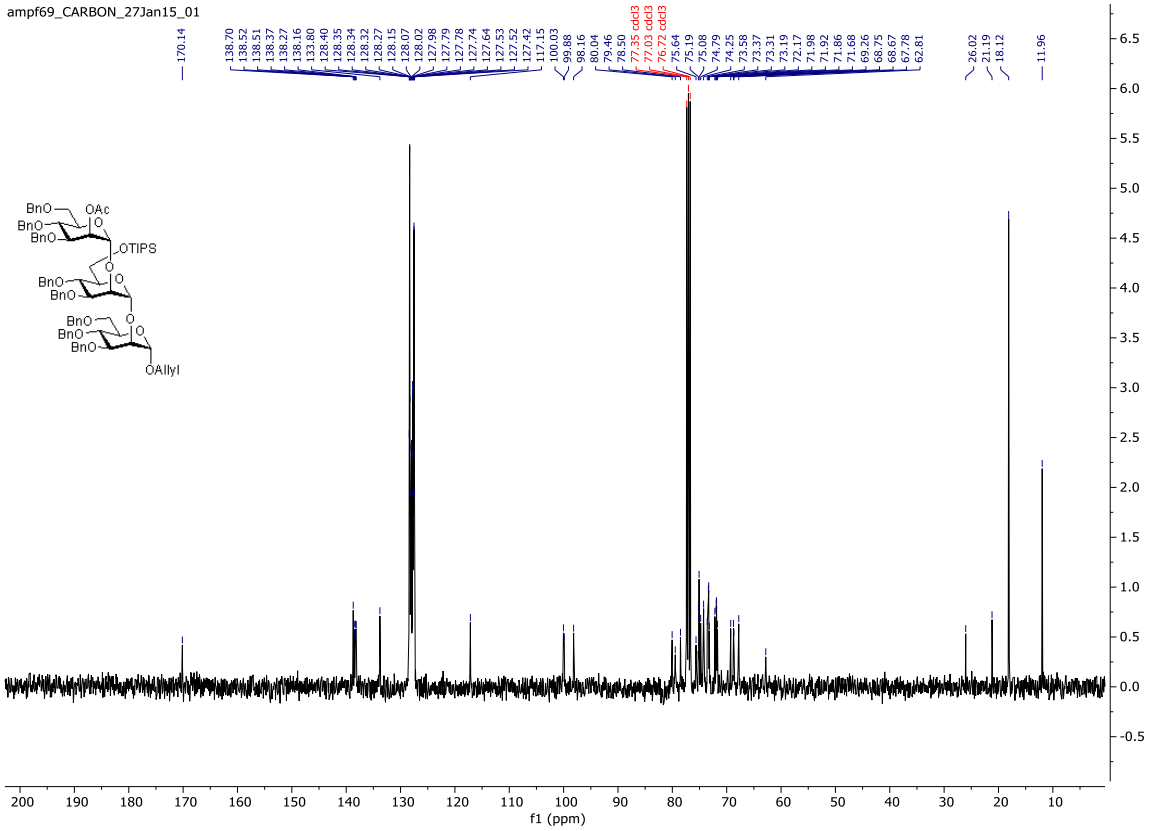
n-Allyl-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**16**)



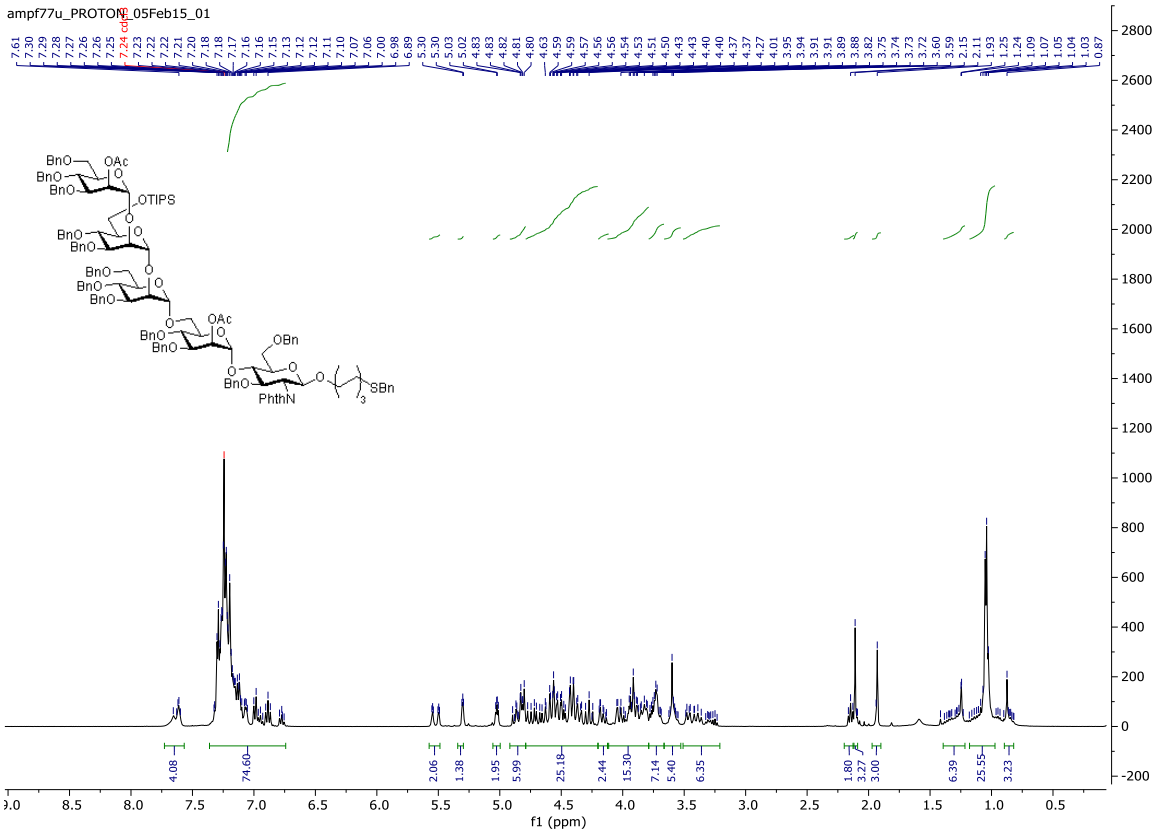


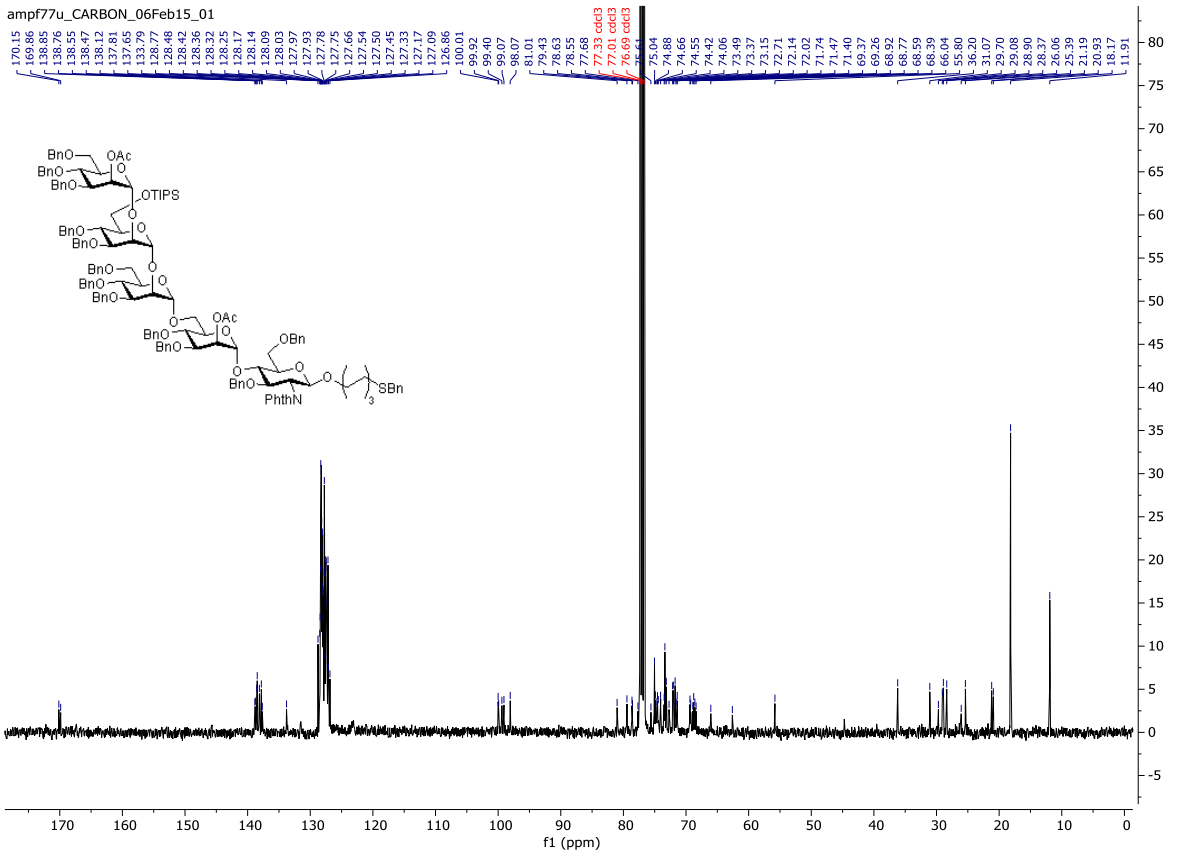
n-Allyl-2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**17**)



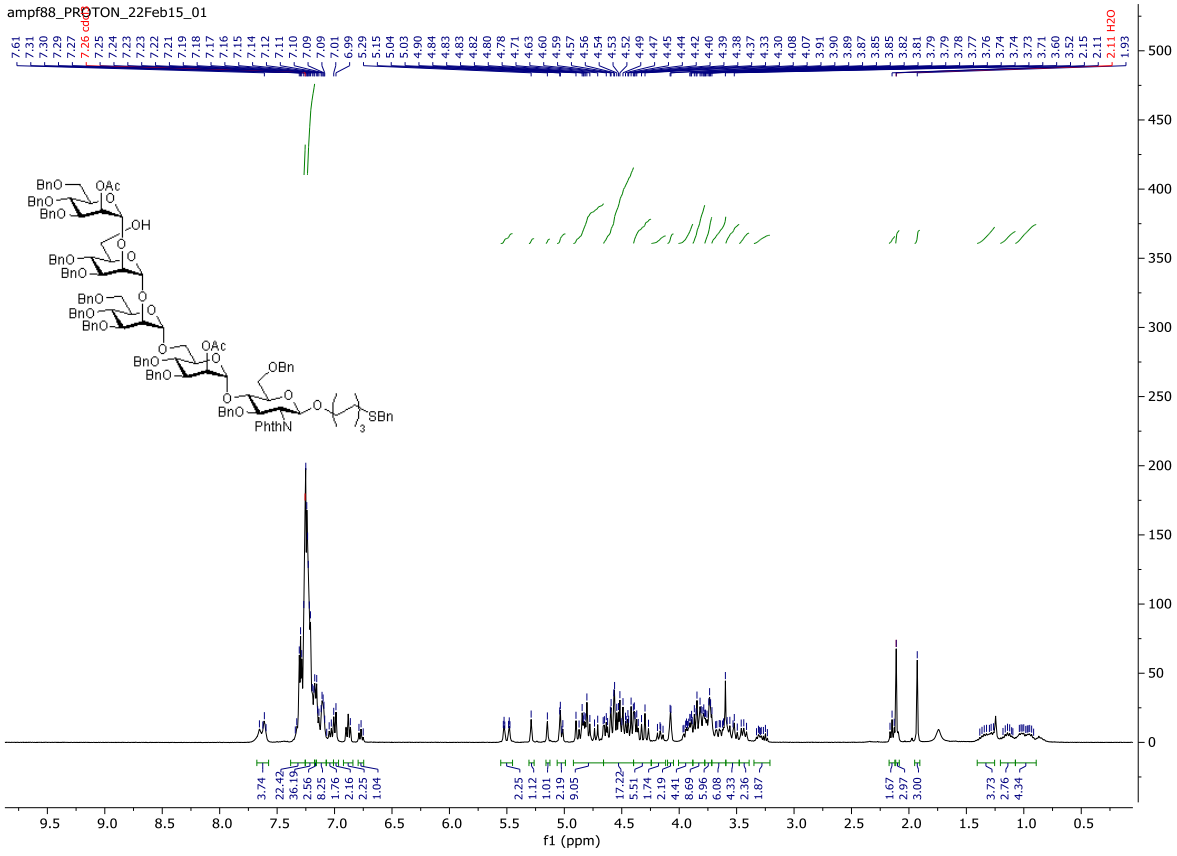


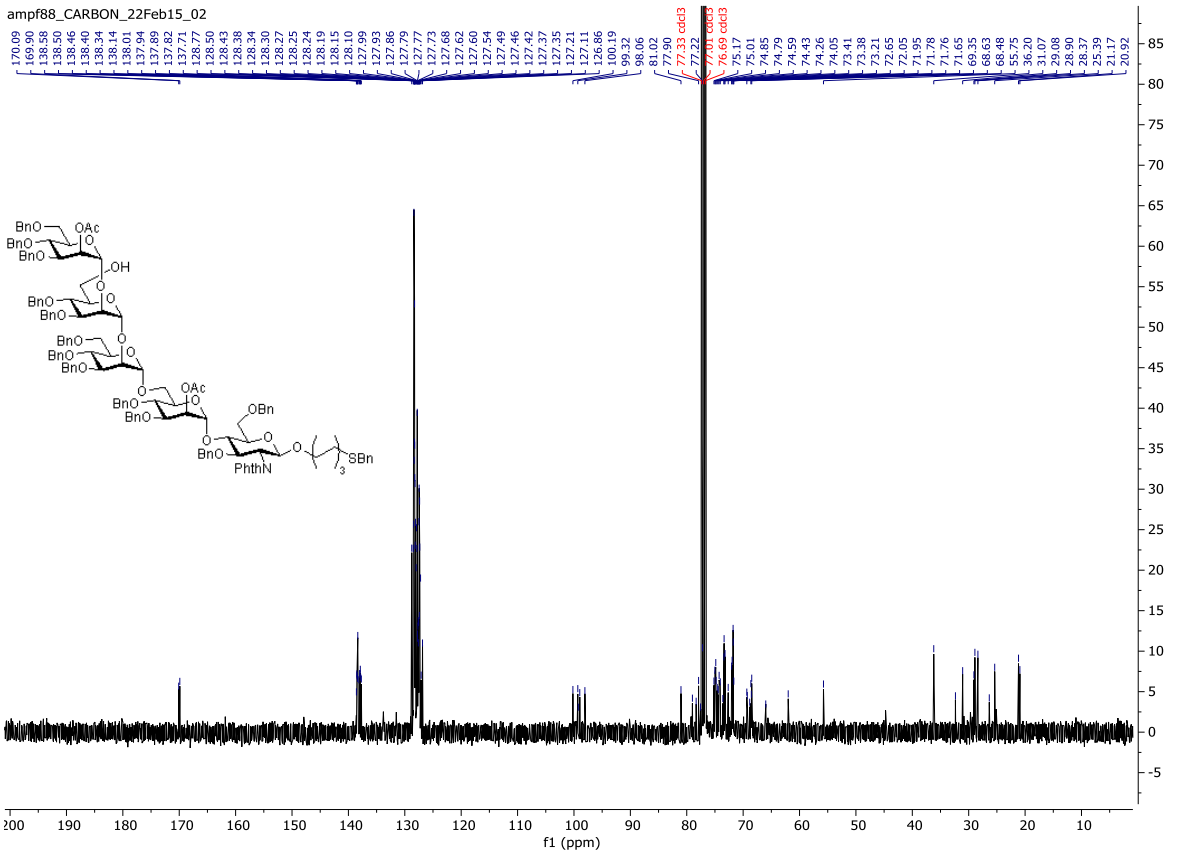
1-O-(6-thiobenzyl)hexyl-2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-6-O-triisopropylsilyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)- 3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**19**)



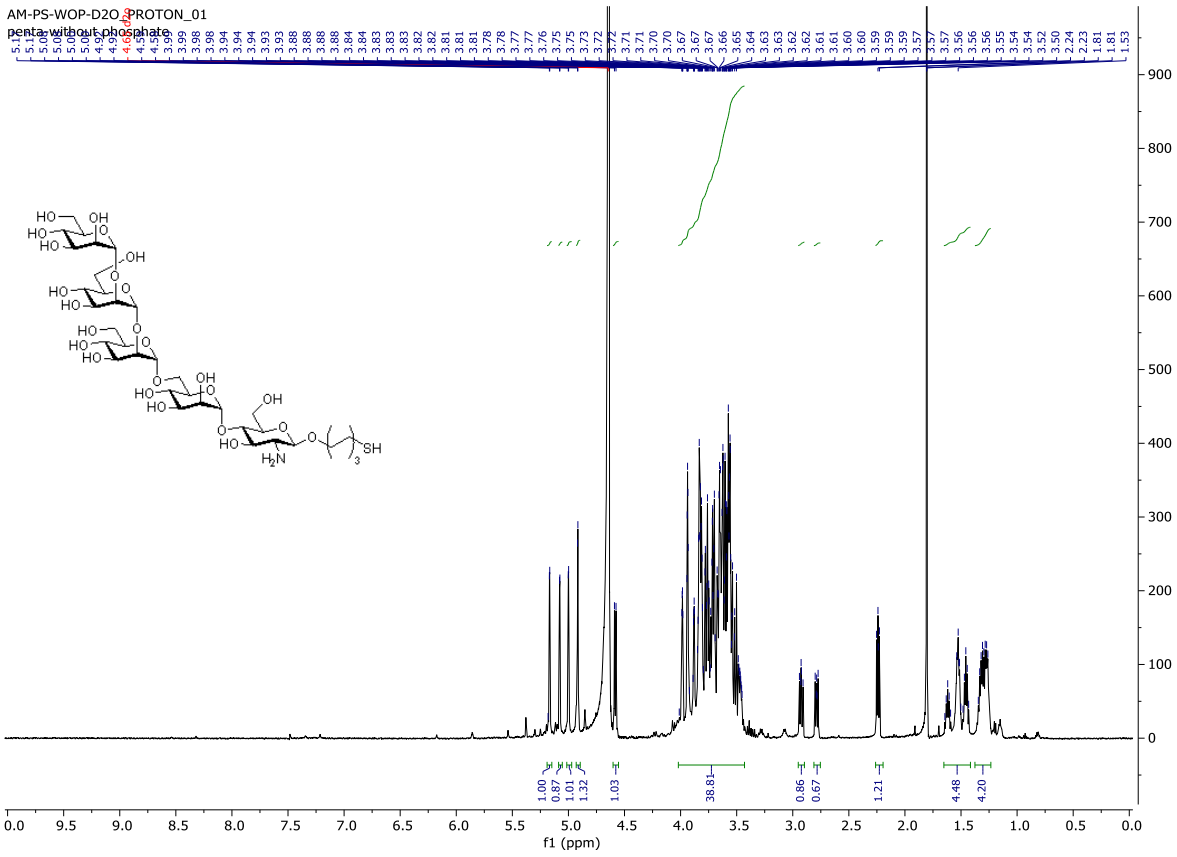


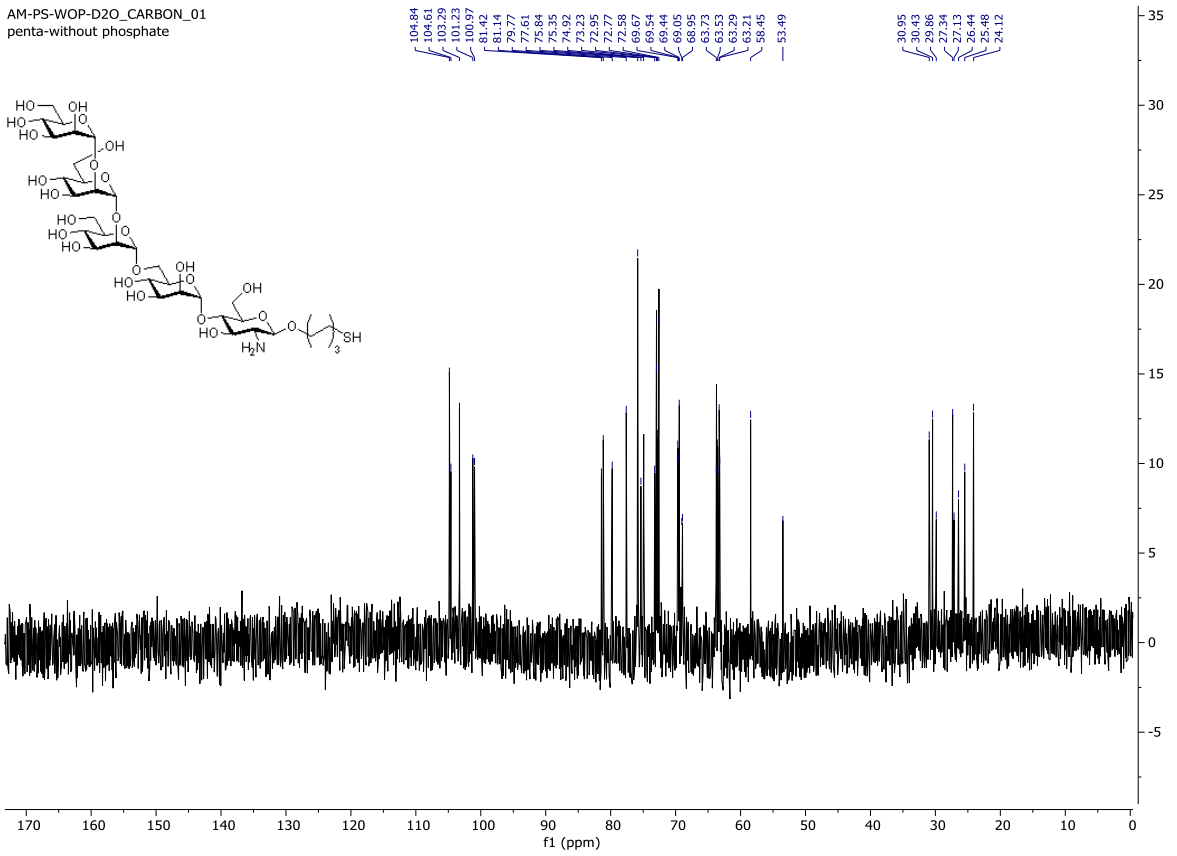
1-O-(6-thiobenzyl)hexyl-2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**20**)

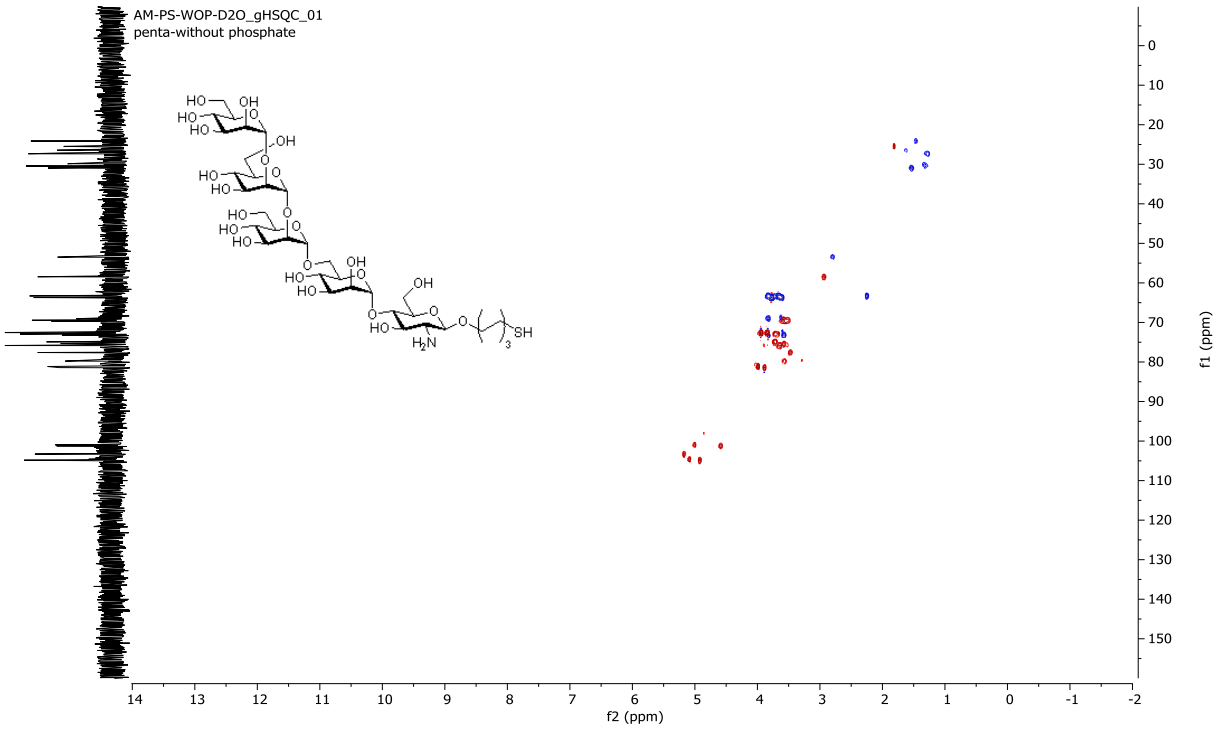




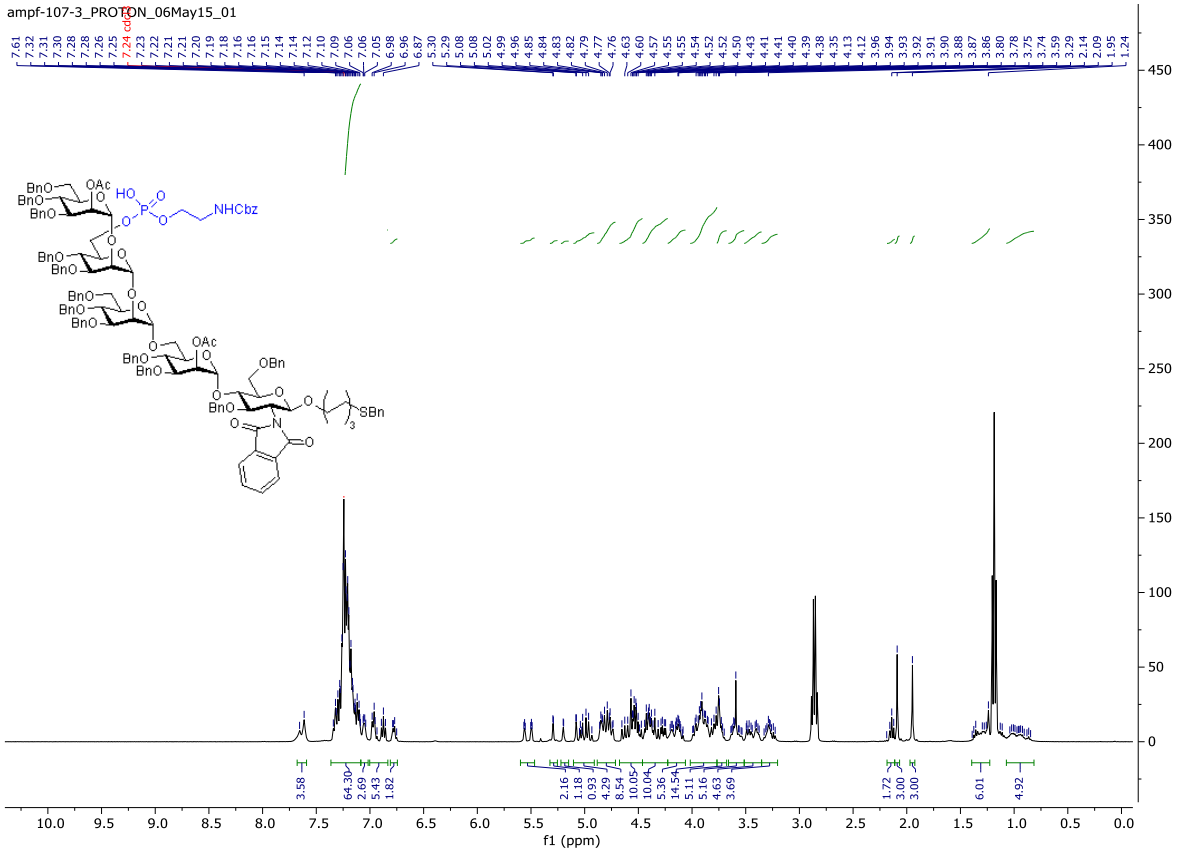
1-O-(6-thio)hexyl-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-2-deoxy-2-amino-β-D-glucopyranoside (**3**)

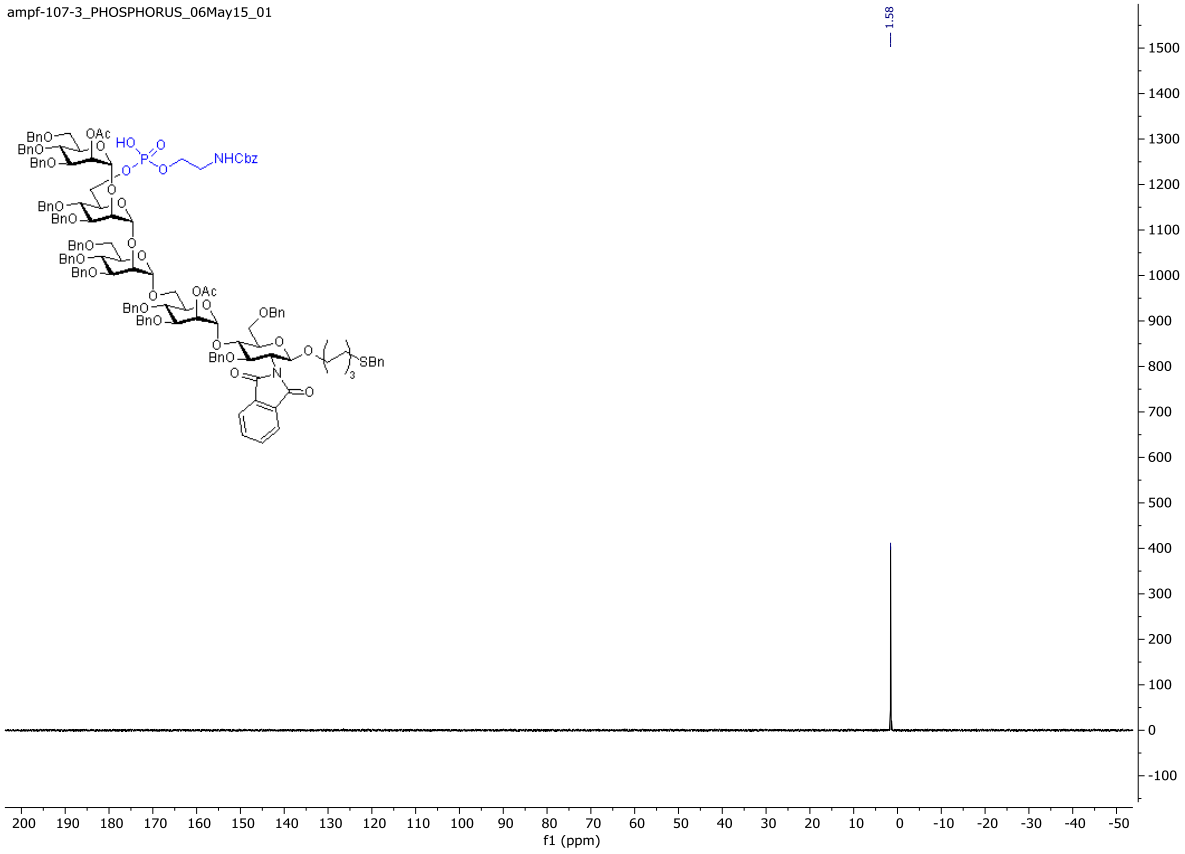


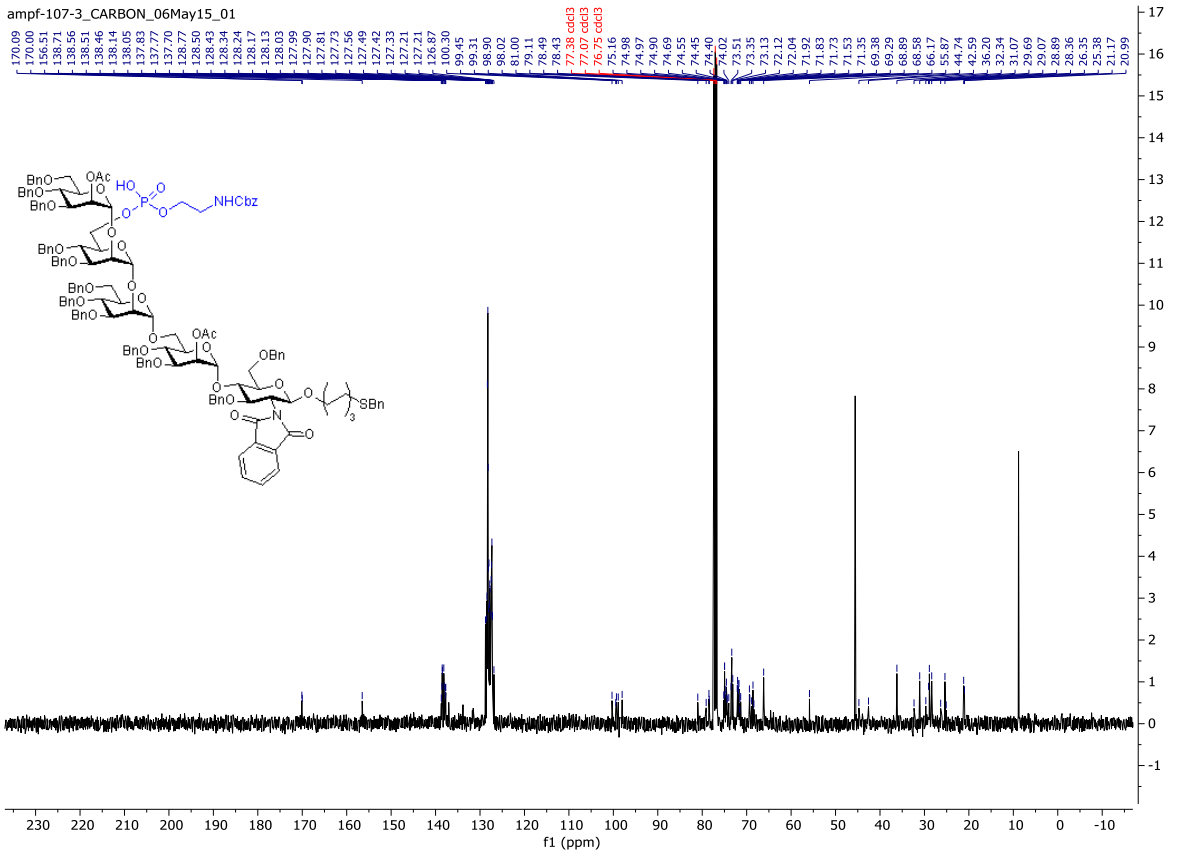

AM-PS-WOP-D2O_CARBON_01
penta-without phosphate
104.84
104.61
103.29
101.23
100.97
81.42
81.14
79.77
77.61
75.84
75.35
74.92
73.23
72.95
72.77
72.58
69.67
69.54
69.44
69.05
68.95
63.73
63.53
63.29
63.21
58.45
53.49
30.95
30.43
29.86
27.34
27.13
26.44
25.48
24.12
f1 (ppm)


AM-PS-WOP-D2O_gHSQC_01
penta-without phosphate
f2 (ppm)
f1 (ppm)

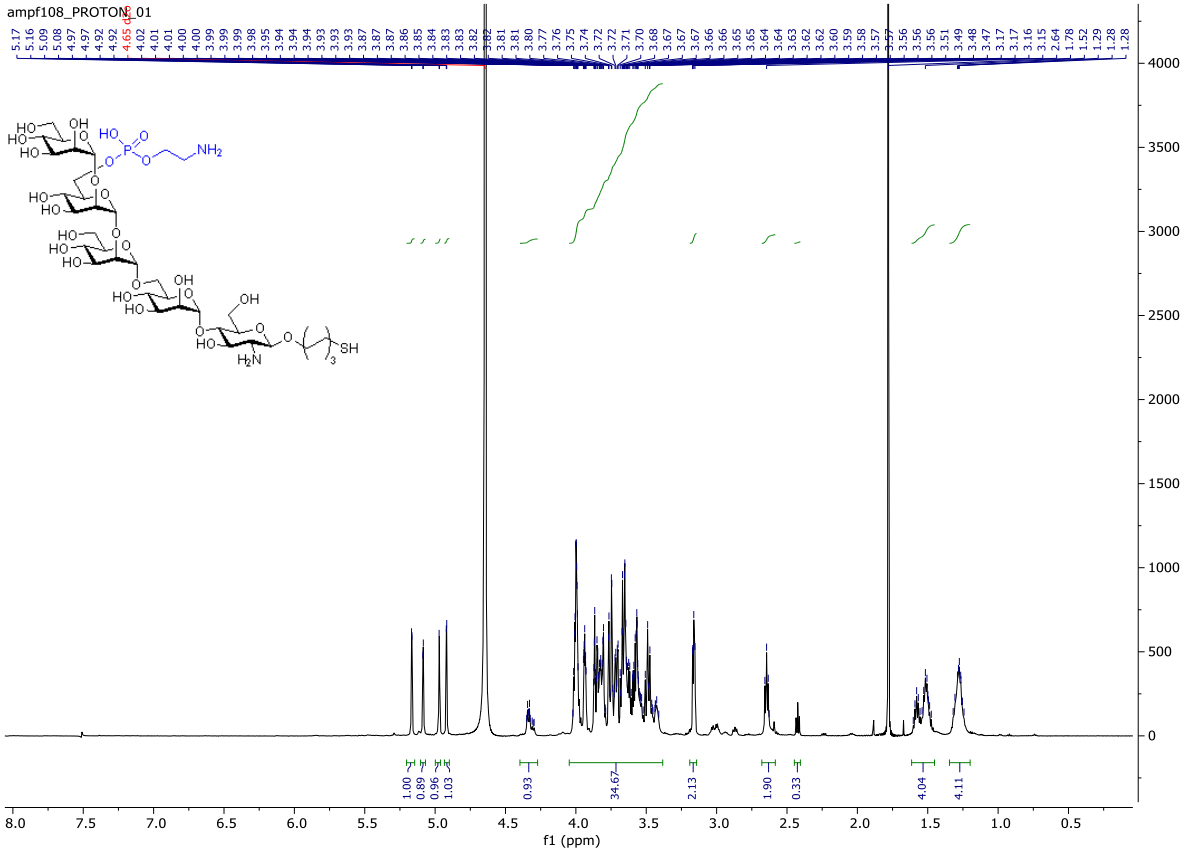
1-O-(6-thiobenzyl)hexyl-2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→2)-3,4-di-O-benzyl-6-O-(2-N-benzyloxycarbonyl)aminoethyl-phosphonato-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-2-O-Acetyl-3,4-di-O-benzyl-α-D-mannopyranosyl-(1→4)-3,6-O-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (**21**)

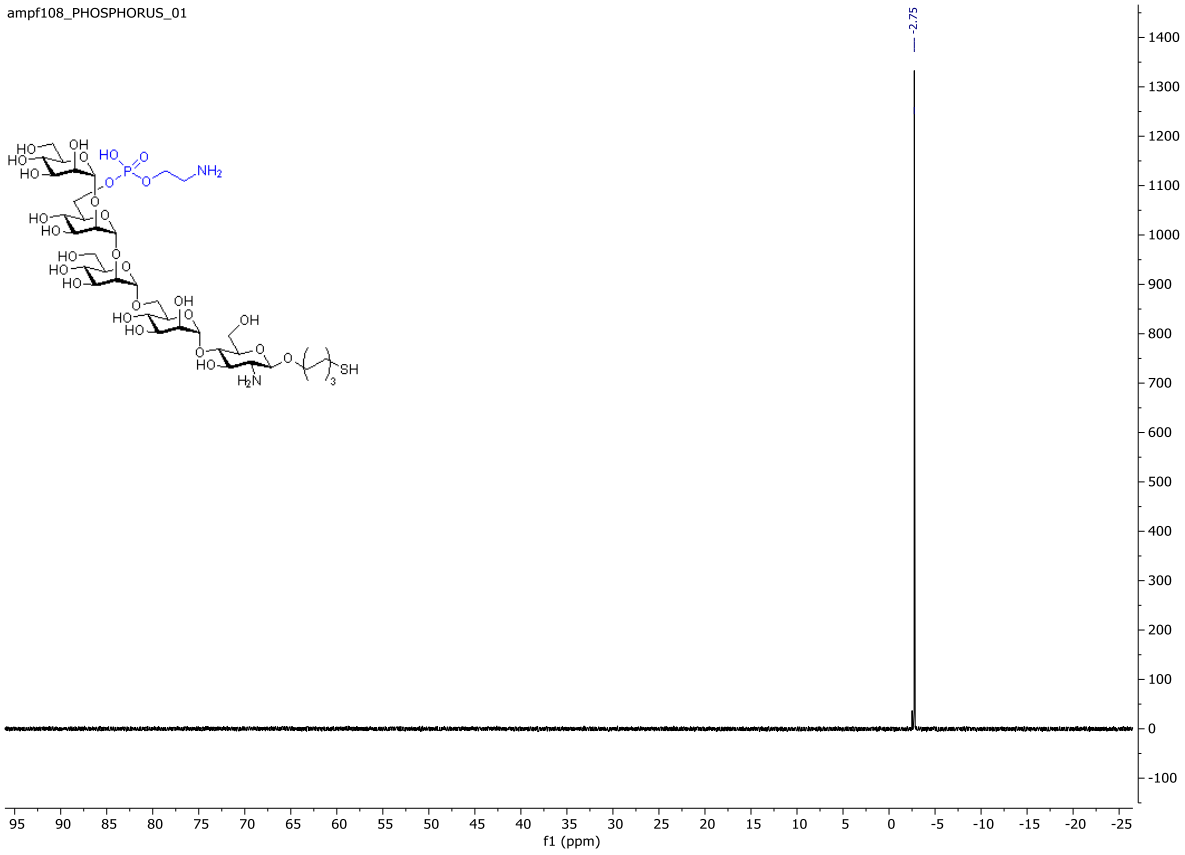


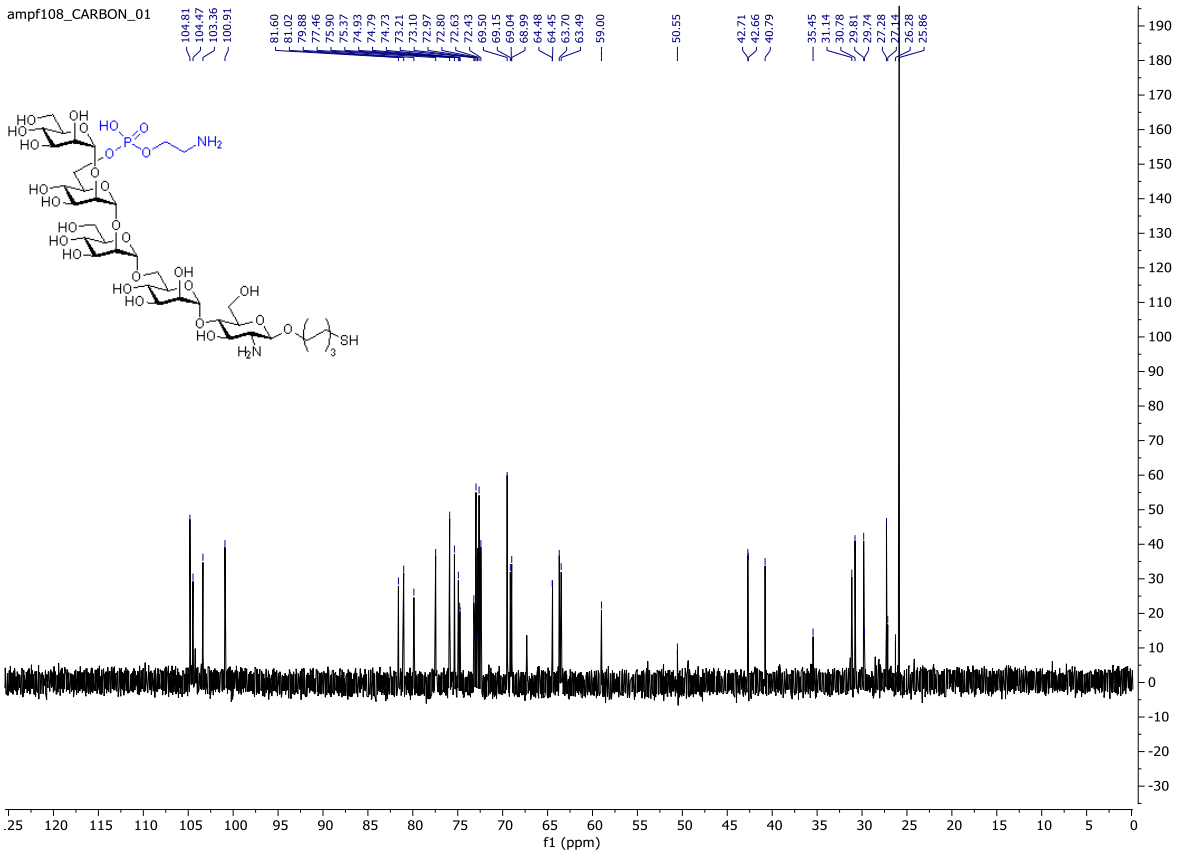

ampf-107-3_PHOSPHORUS_06May15_01
1.58
f1 (ppm)

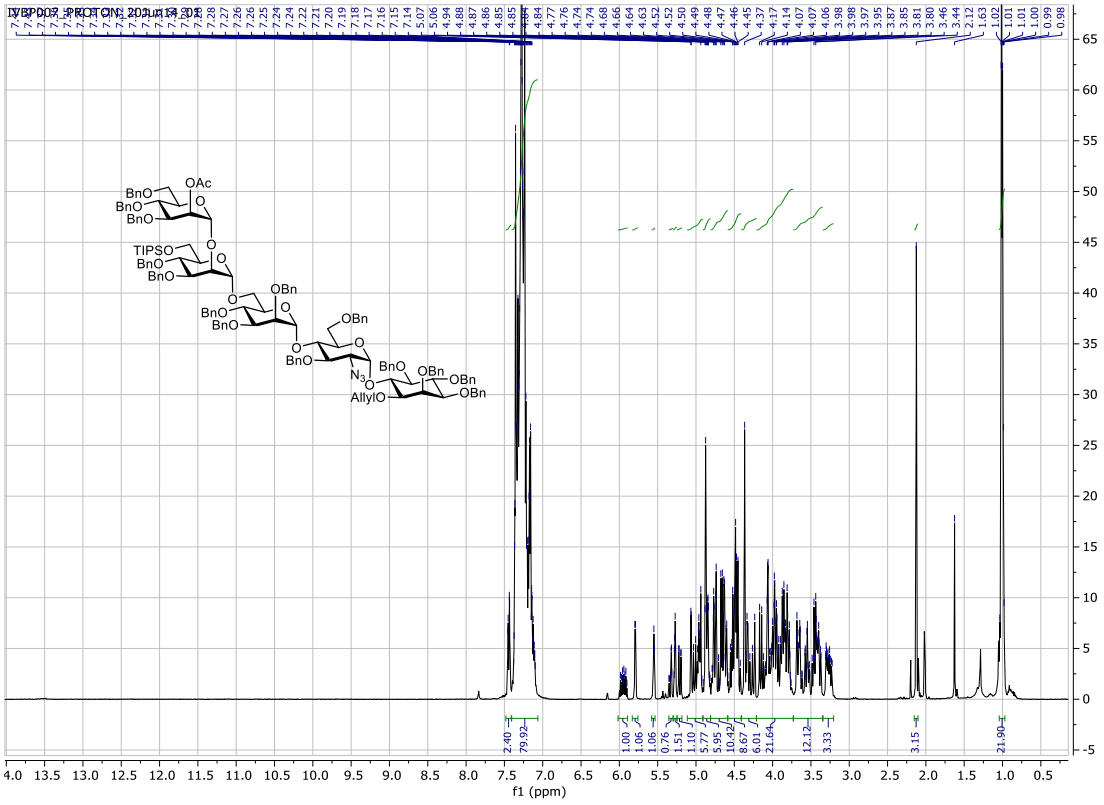

ampf-107-3_CARBON_06May15_01
f1 (ppm)

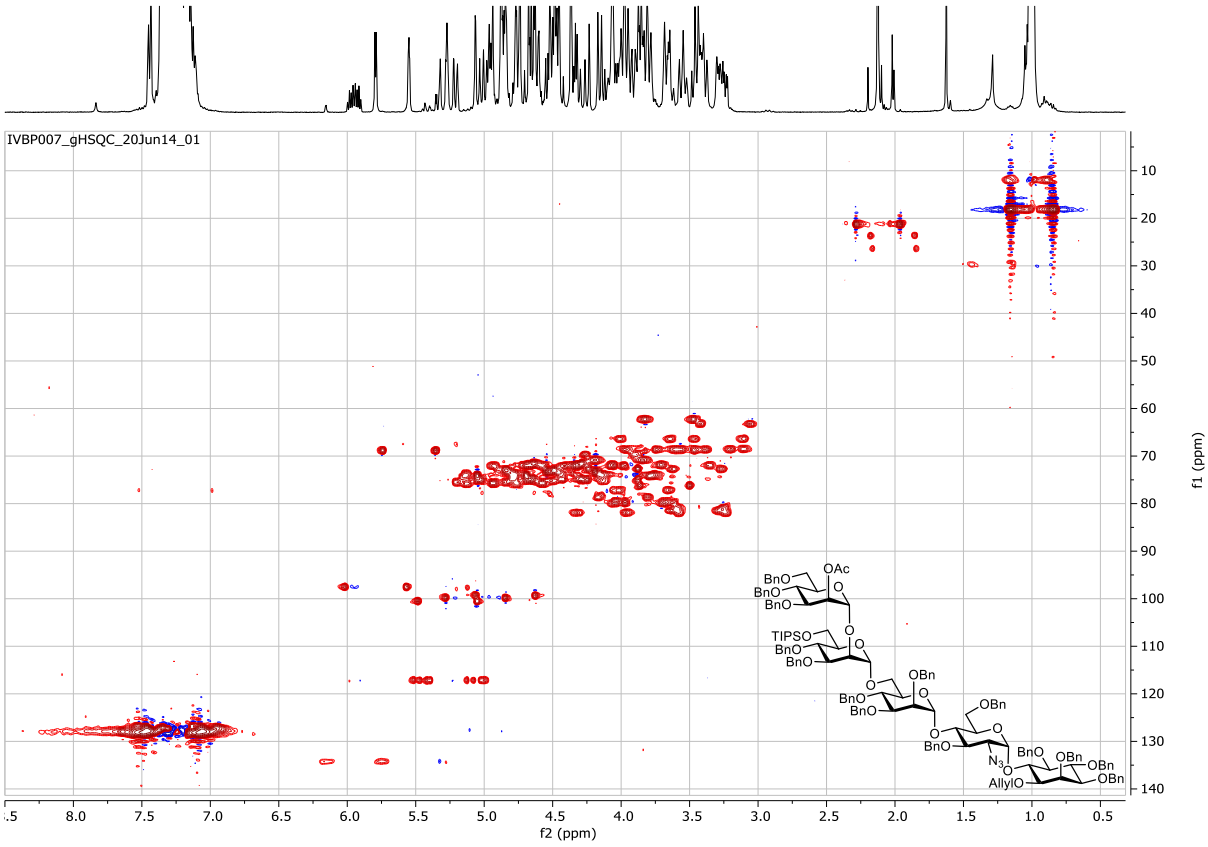
1-O-(6-thio)hexyl-α-D-mannopyranosyl-(1→2)-6-O-aminoethyl-phosphonato-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→4)-2-deoxy-2-amino-β-D-glucopyranoside (**4**)

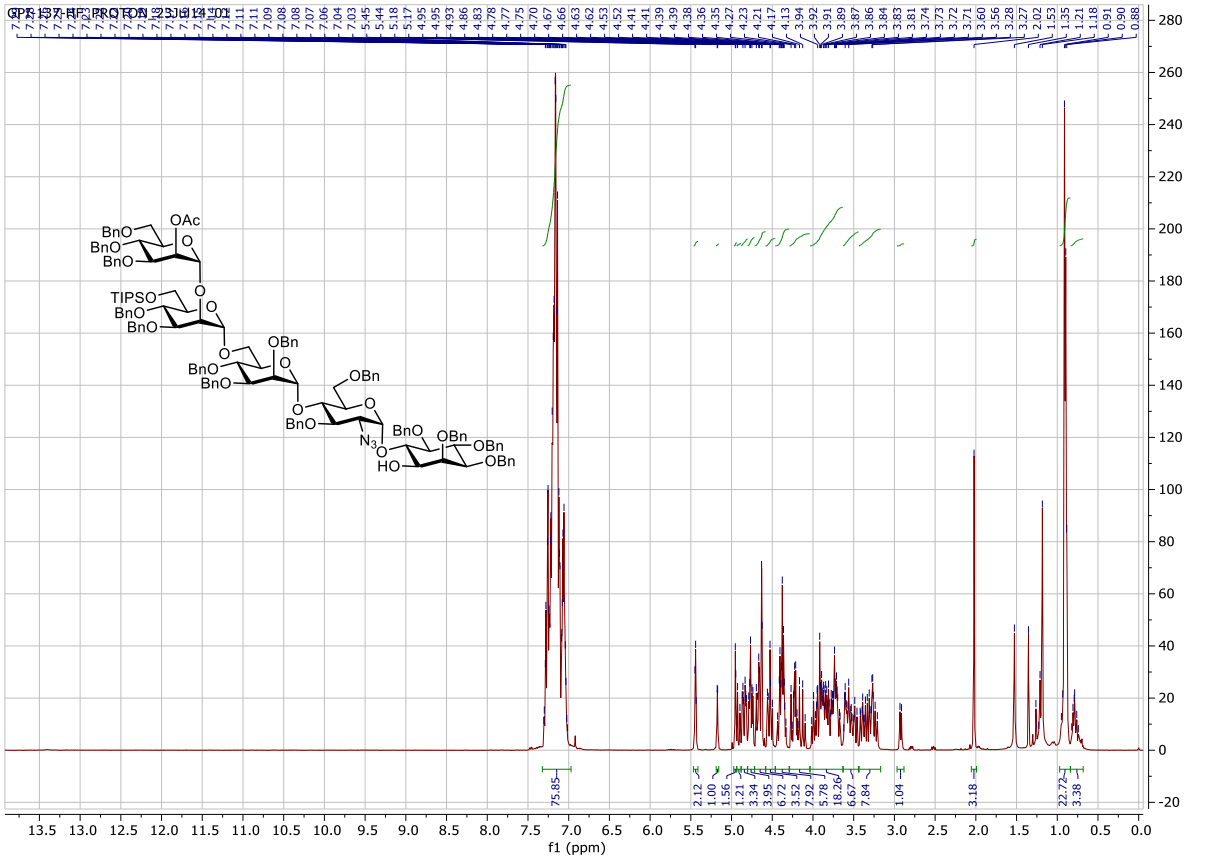


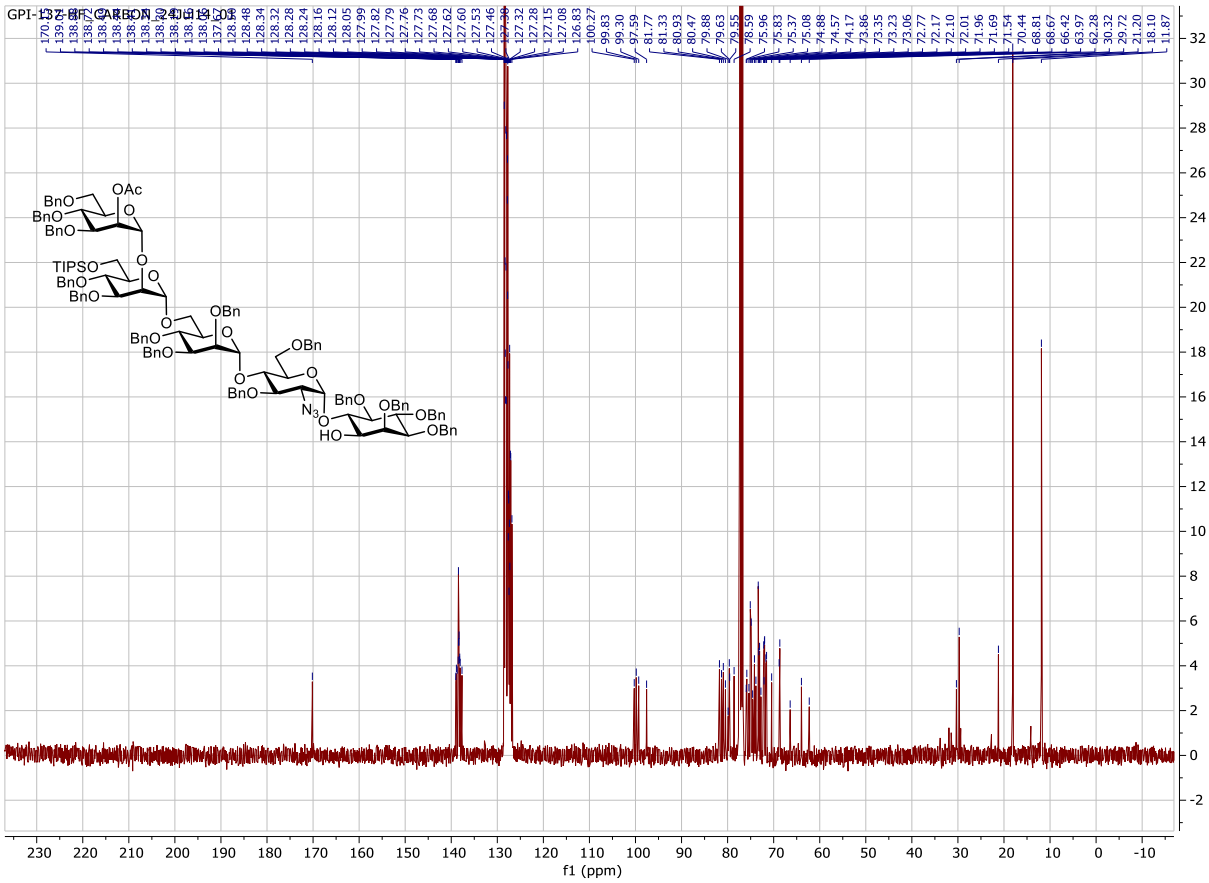


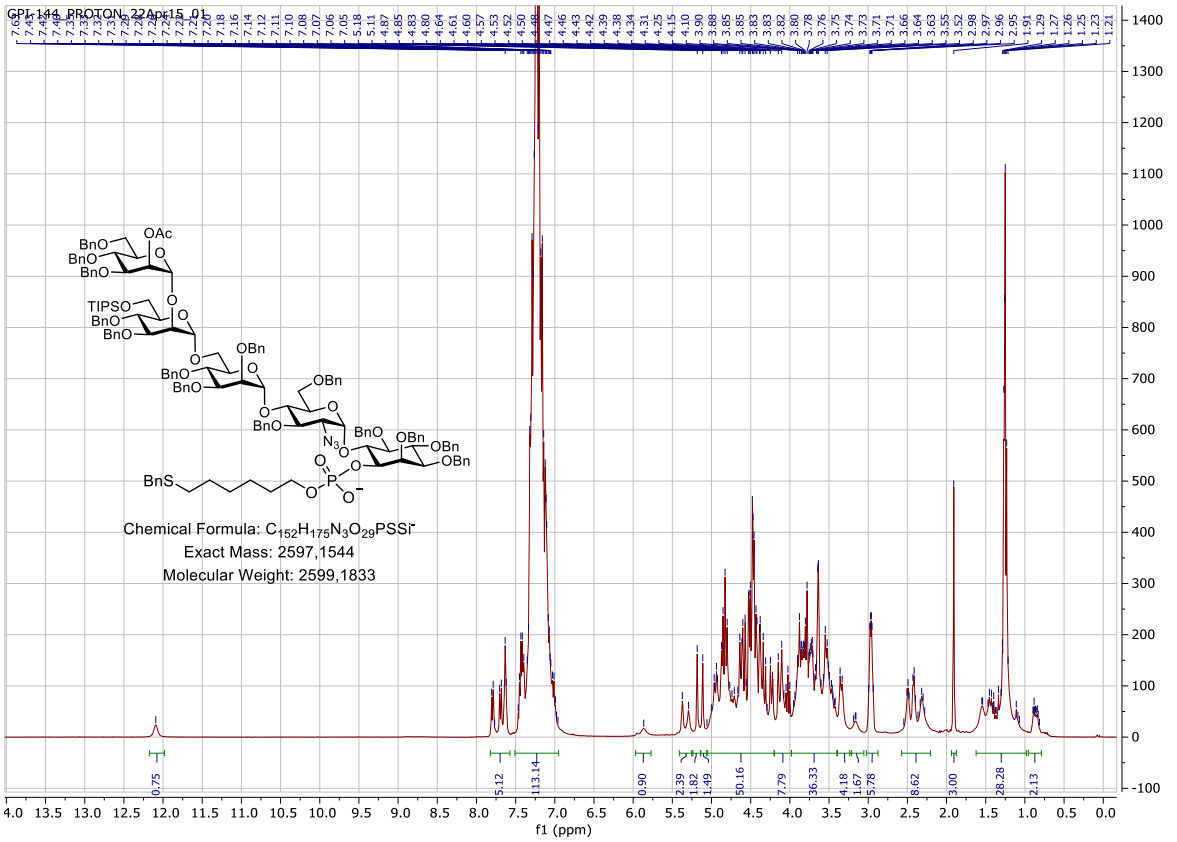

ampf108_CARBON_01
f1 (ppm)

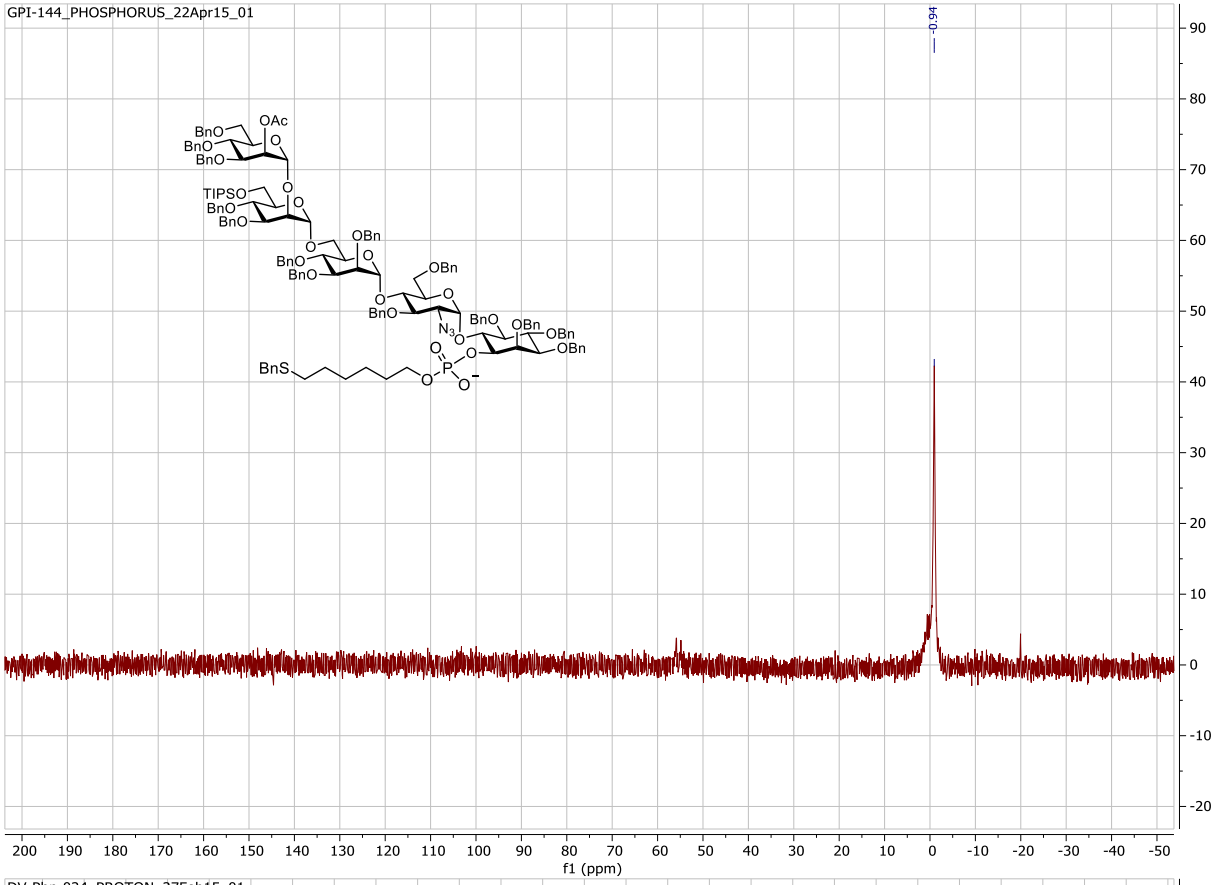

f1 (ppm)

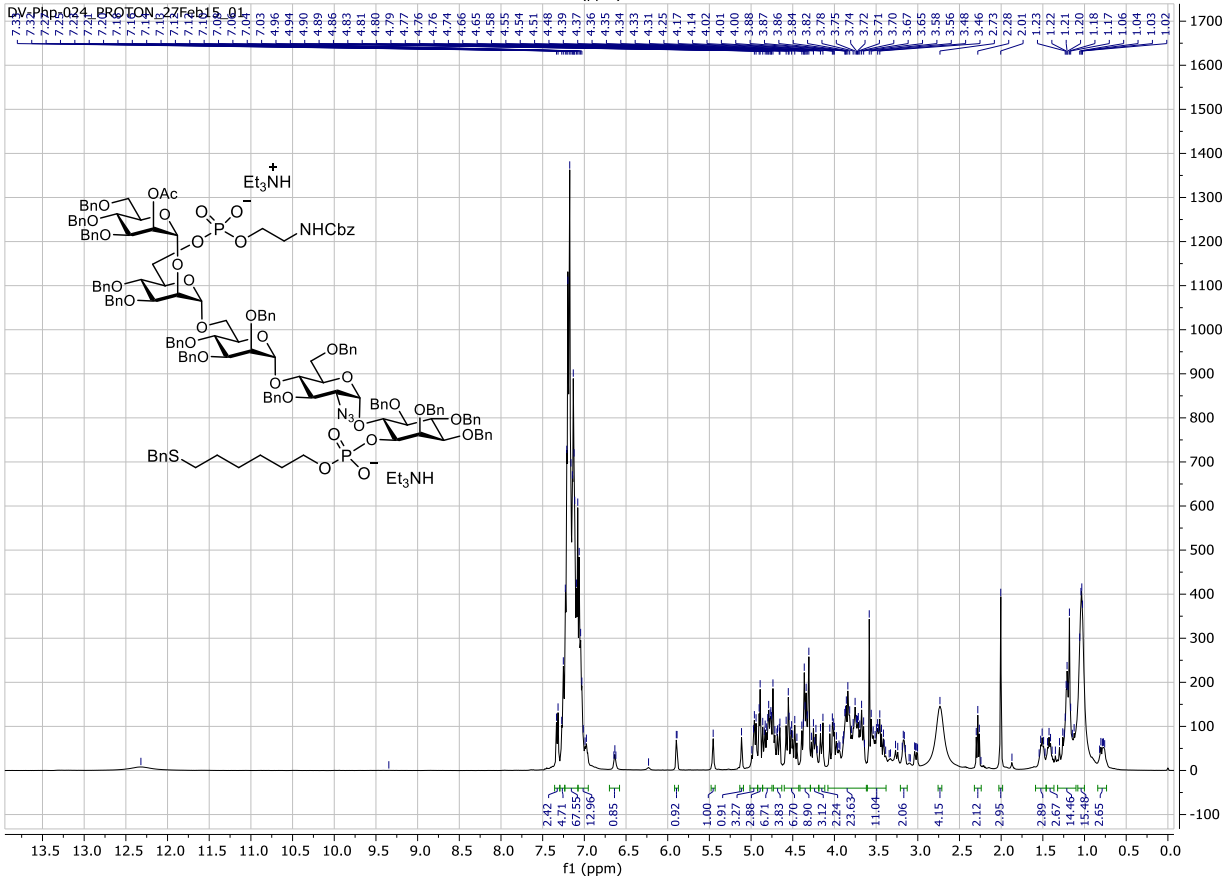

IVBP007_gHSQC_20Jun14_01
f2 (ppm)
f1 (ppm)

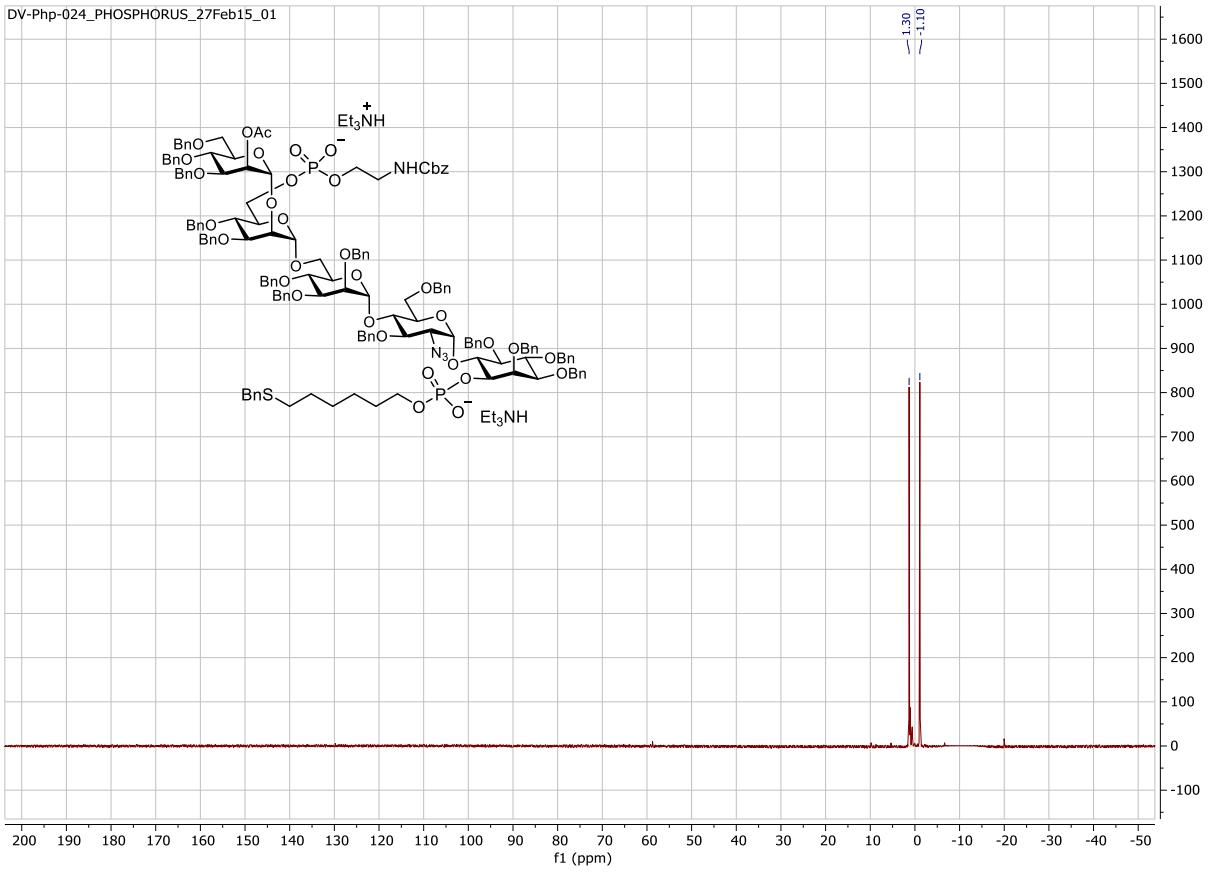

f1 (ppm)

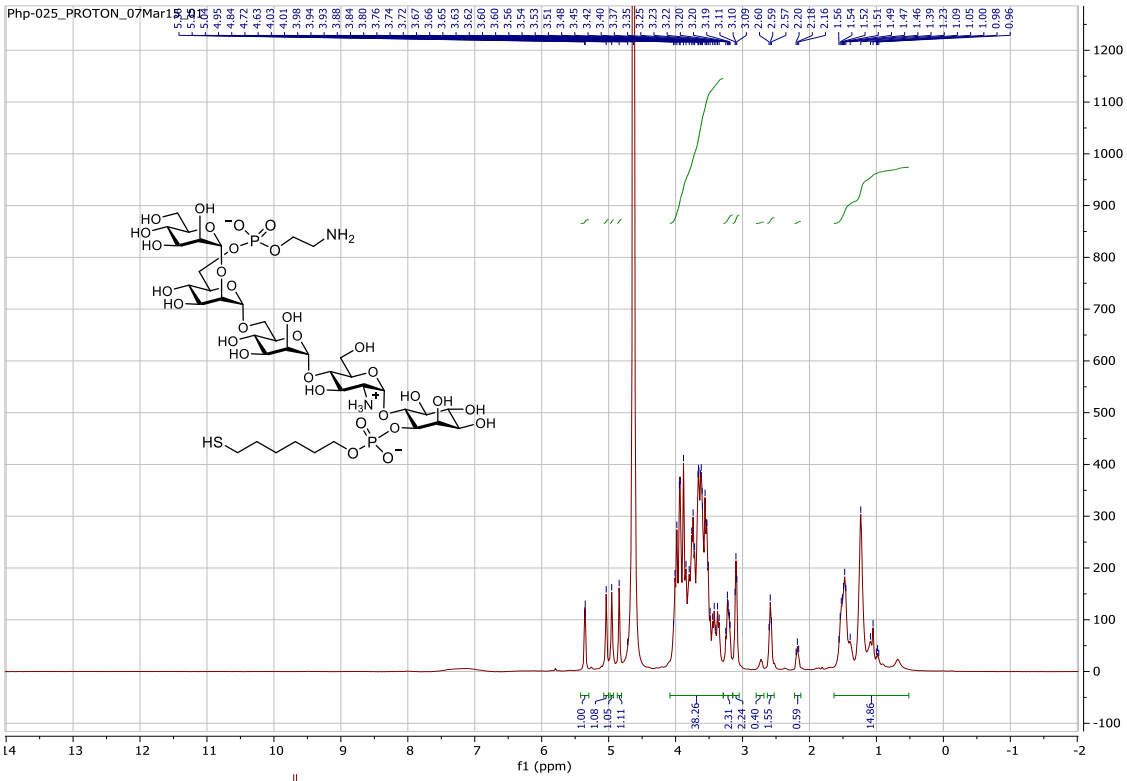

f1 (ppm)

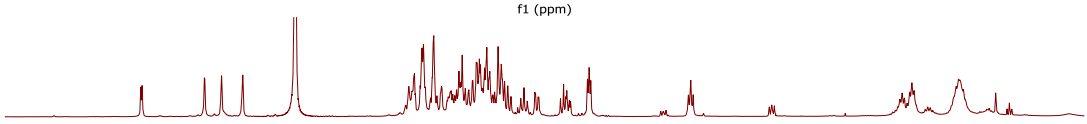

Chemical Formula: C152H175N3O29PSSi-
Exact Mass: 2597,1544
Molecular Weight: 2599,1833
f1 (ppm)

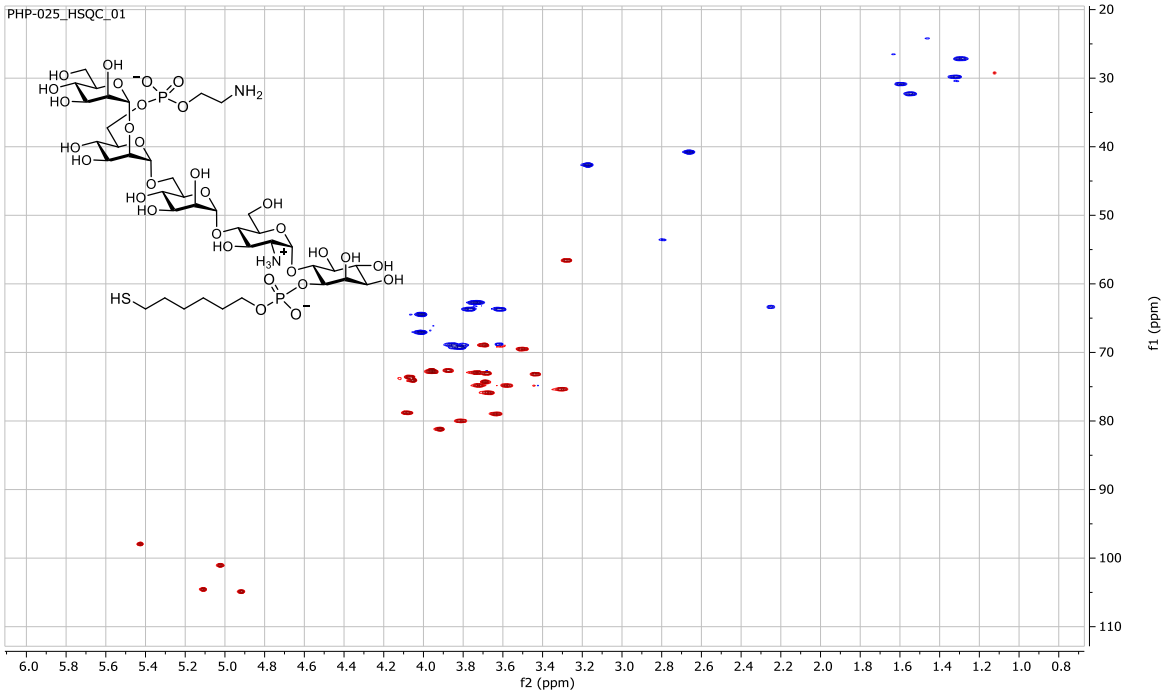

GPI-144_PHOSPHORUS_22Apr15_01
f1 (ppm)

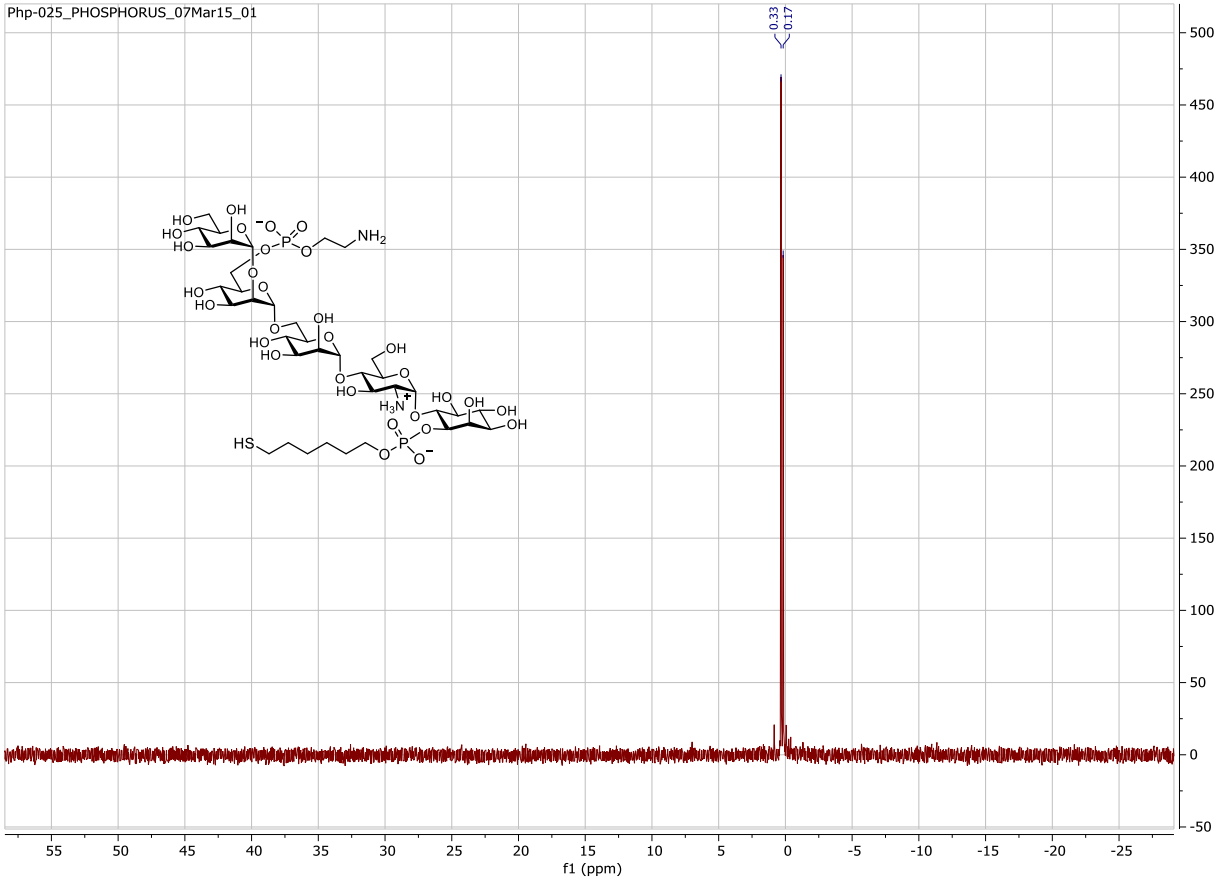

DV-Php-024_PROTON_27Feb15_01
f1 (ppm)

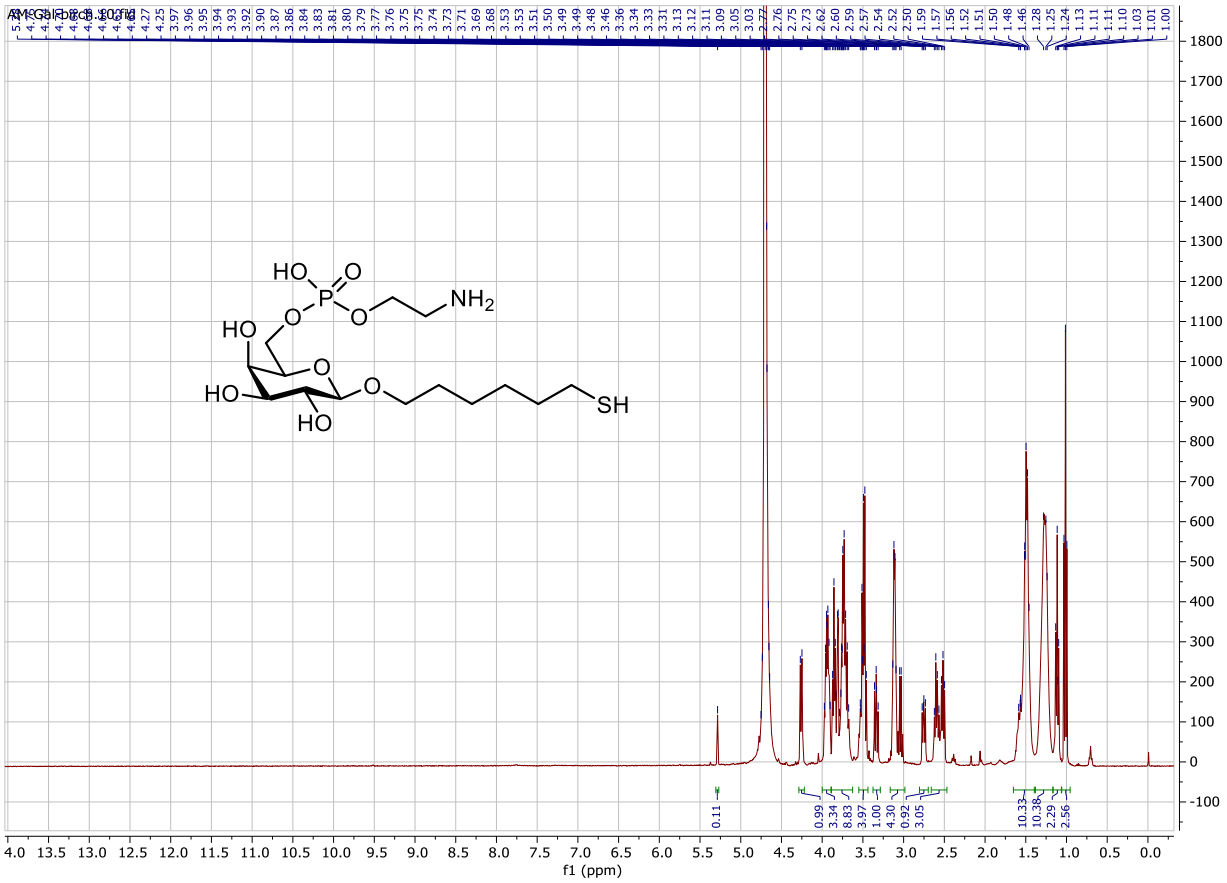

DV-Php-024_PHOSPHORUS_27Feb15_01
1.30
-1.10
f1 (ppm)

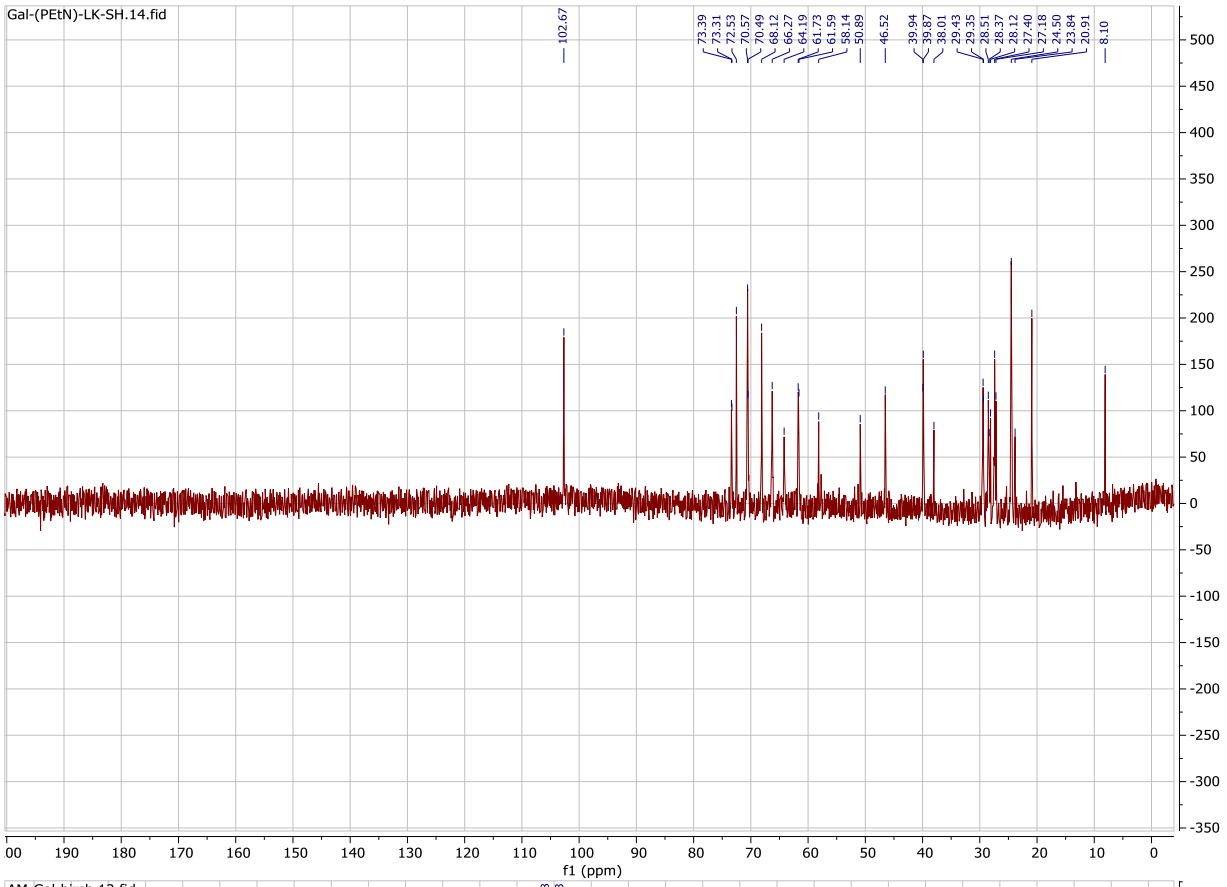

Php-025_PROTON_07Mar15_01
f1 (ppm)

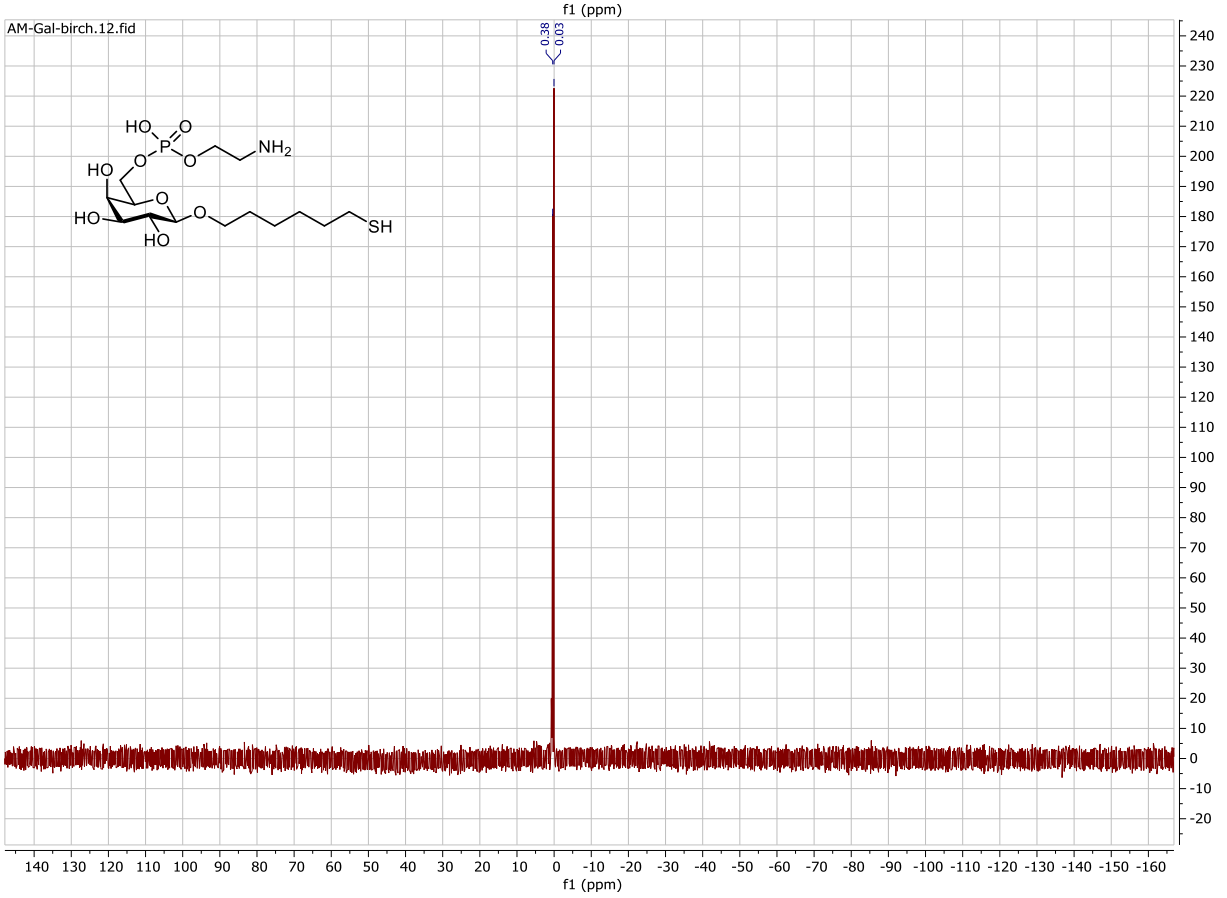



PHP-025_HSQC_01
f2 (ppm)
f1 (ppm)


Php-025_PHOSPHORUS_07Mar15_01
0.33
0.17
f1 (ppm)


f1 (ppm)


Gal-(PEtN)-LK-SH.14.fid
102.67
73.39
73.31
72.53
70.57
70.49
68.12
66.27
64.19
61.73
61.59
58.14
50.89
46.52
39.94
39.87
38.01
29.43
29.35
28.51
28.37
28.12
27.40
27.18
24.50
23.84
20.91
8.10
f1 (ppm)


AM-Gal-birch.12.fid
0.38
0.03
HO
O
P
O
O
NH2
HO
O
HO
O
SH
HO
f1 (ppm)

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