Chemical synthesis of highly congested gp120 V1V2 *N*-glycopeptide antigens for potential HIV-1–directed vaccines

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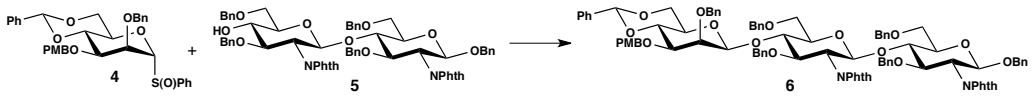
SUPPORTING INFORMATION

General Information. All non-aqueous reactions were carried out under an atmosphere of argon or nitrogen in flame- or oven-dried glassware with magnetic stirring unless otherwise indicated. Benzene, dichloromethane, diethyl ether, tetrahydrofuran, and toluene were purified by passage through an activated alumina column. Dichloromethane for glycosylation reactions was distilled from calcium hydride. All other commercially obtained reagents were used as received, except where specified otherwise. Flash chromatography was performed on Silicycle SiliaFlash P60 silica gel (60 Å pore size, 230-400 mesh). Analytical thin layer chromatography was performed on Silicycle SiliaPlate glass-backed plates coated with silica gel (250 μm thickness, 60 Å pore size, F-254 indicator) and visualized by exposure to ultraviolet light and/or staining with aqueous ceric ammonium molybdate solution or 5% sulfuric acid in methanol. ^{1}H NMR spectra were recorded on a Bruker AVANCE DRX-500 (500 MHz) or DRX-600 (600 MHz) spectrometer at 24 °C, unless otherwise stated. Chemical shifts are reported in parts per million from $CDCl_3$, C_6D_6, D_2O, or DMSO-d_6 internal standard (7.26, 7.15, 4.79, and 2.50 ppm, respectively). Data are reported as follows: (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, dd = doublet of doublets, ddd = doublet of doublet of doublets, br = broad; coupling constant(s) in Hz; integration). Proton-decoupled ^{13}C NMR spectra were recorded on a Bruker AVANCE DRX-500 (125 MHz) or DRX-600 (150 MHz) spectrometer at 24 °C, unless otherwise stated. Chemical shifts are reported in ppm from $CDCl_3$, C_6D_6, or DMSO-d_6 internal standard (77.0, 128.0, 39.52 ppm, respectively). Peaks that are split due to coupling to ^{19}F are reported as individual resonances. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were recorded on a JASCO FT/IR-6100 spectrometer. Optical rotations were recorded on a JASCO P-2000 digital polarimeter. Low resolution electrospray ionization (ESI) mass spectra were obtained on a JEOL JMS-DX303 HF mass spectrometer or Waters Micromass ZQ mass spectrometer in the NMR Analytical Core Facility at MSKCC.

Experimental Procedures: Carbohydrates.



Benzyl 2-*O*-benzyl-3-*O*-*p*-methoxybenzyl-4,6-*O*-(*R*)-benzylidene-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (6). Freshly activated AW-300 MS (8 g) were added to a solution of mannose sulfoxide **4**[1] (3.97 g, 6.78 mmol) in anhydrous CH_2Cl_2 (50 mL). After 1 h at r.t., the mixture was cooled to –78 °C, and di-*tert*-butyl pyridine (3.5 mL, 15.8 mmol) and Tf_2O (1.2 mL, 7.23 mmol) were added. The mixture was allowed to warm up to –50 °C over 30 min, cooled to –78 °C and a solution of acceptor **5**[2] (4.75 g, 4.52 mmol) in CH_2Cl_2 (50 mL) (cooled separately at –78 °C) was added dropwise via cannula. The mixture was stirred at –78 °C for 8 h, filtered through a pad of Celite, washed with saturated aqueous $NaHCO_3$, water, brine, dried over $MgSO_4$ and concentrated. Purification by flash chromatography (hexanes:CH_2Cl_2:EtOAc, 4:4:1) afforded **6** (5.87 g, 86%) as an amorphous white solid in single diastereomeric form.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87–6.58 (m, 47H), 5.41 (s, 1H), 5.20 (d, *J* = 8.3 Hz, 1H), 4.87 (d, *J* = 8.4 Hz, 1H), 4.84–4.70 (m, 4H), 4.61 (d, *J* = 12.3 Hz, 1H), 4.58 (d, *J* = 11.9 Hz, 1H), 4.52–4.36 (m, 6H), 4.36–4.24 (m, 3H), 4.22–4.08 (m, 4H), 4.07–4.01 (m, 2H), 4.01–3.94 (m, 2H), 3.69 (s, 3H), 3.65 (d, *J* = 3.1 Hz, 1H), 3.53 (dd, *J* = 11.3, 2.0 Hz, 1H), 3.49 (dd, *J* = 11.2, 1.6 Hz, 1H), 3.44 (t, *J* = 10.4 Hz, 1H), 3.39–3.27 (m, 3H), 3.23 (ddd, *J* = 9.9, 3.8, 1.7 Hz, 1H), 3.11 (dt, *J* = 10.1, 2.6 Hz, 1H), 3.05 (td, *J* = 9.6, 4.8 Hz, 1H).

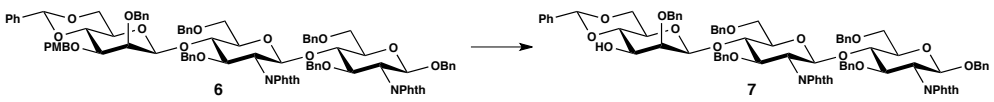
^{13}C NMR (150 MHz, $CDCl_3$) δ 167.63, 167.56, 159.20, 138.91, 138.72, 138.67, 138.55, 137.89, 137.70, 137.22, 134.01, 133.81, 133.47, 131.72, 130.62, 129.17, 129.13, 128.81, 128.57, 128.55, 128.38, 128.34, 128.31, 128.29, 128.25, 128.23, 128.20, 128.17, 128.15, 128.11, 128.08, 127.98, 127.81, 127.80, 127.79, 127.75, 127.69, 127.65, 127.60, 127.59, 127.57, 127.56, 127.51, 127.49, 127.47, 127.44, 127.41, 127.36, 127.13, 126.92, 126.85, 126.20, 126.11, 123.67, 123.12, 113.77, 113.73, 101.90, 101.33, 97.17, 97.07, 79.37, 78.68, 78.04, 77.31, 77.24, 77.09, 76.88, 76.57, 75.78, 75.13, 74.71, 74.67, 74.55, 74.34, 73.31, 72.73, 72.32, 70.54, 68.57, 68.24, 67.97, 67.38, 56.61, 55.78, 55.31, 55.30.

IR (ATR-FTIR, thin film) 3030, 2867, 1712, 1386, 1046 cm^{-1}.

$[\alpha]^{22}_D$ (*c* 1.0, CH_2Cl_2) –32.8.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{91}H_{86}N_2NaO_{19}$) requires 1533.6, found 1533.9.

(1) Crich, D.; Li, H.; Yao, Q.; Wink, D. J.; Sommer, R. D.; Rheingold, A. L. *J. Am. Chem. Soc.* **2001**, *123*, 5826–5828.
(2) Walczak, M. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2012**, *134*, 16430–16433.



Benzyl 2-*O*-benzyl-4,6-*O*-(*R*)-benzylidene-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (7). Trisaccharide **6** (5.35 g, 3.54 mmol) was dissolved in CH_2Cl_2 (100 mL), followed by addition of H_2O (100 mL), and the mixture treated with DDQ (2.68 g, 11.8 mmol). The mixture was stirred vigorously at r.t., in the dark for 2 h. The reaction was quenched with a buffer solution (0.7% ascorbic acid + 1.3% citric acid + 1.9 % NaOH in H_2O, w/v) (20 mL), diluted with CH_2Cl_2 (200 mL), washed with water (2×), brine, dried over $MgSO_4$ and concentrated. Purification by flash chromatography (hexanes:CH_2Cl_2:EtOAc, 4:4:1) afforded **7** (4.1 g, 83%) as an amorphous white solid.

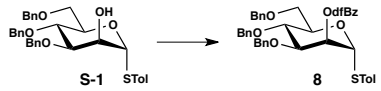
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.98–6.74 (m, 43H), 5.46 (s, 1H), 5.33 (d, *J* = 7.9 Hz, 1H), 5.05 (d, *J* = 11.6 Hz, 1H), 5.00 (d, *J* = 8.4 Hz, 1H), 4.94 (d, *J* = 12.2 Hz, 1H), 4.89 (d, *J* = 12.8 Hz, 1H), 4.78–4.69 (m, 3H), 4.65 (d, *J* = 12.0 Hz, 1H), 4.61–4.53 (m, 3H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.43 (t, *J* = 12.8 Hz, 2H), 4.34–4.21 (m, 4H), 4.21–4.11 (m, 3H), 3.76 (d, *J* = 3.7 Hz, 1H), 3.73 (t, *J* = 9.5 Hz, 1H), 3.69 (dd, *J* = 11.3, 2.0 Hz, 1H), 3.65–3.59 (m, 2H), 3.57–3.45 (m, 3H), 3.36 (ddd, *J* = 9.8, 3.7, 1.5 Hz, 1H), 3.24 (dt, *J* = 9.9, 2.4 Hz, 1H), 3.16 (td, *J* = 9.6, 4.9 Hz, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 167.64, 138.81, 138.67, 138.55, 138.22, 137.75, 137.30, 137.20, 134.47, 133.48, 131.70, 129.76, 129.07, 129.02, 128.62, 128.60, 128.55, 128.42, 128.39, 128.32, 128.29, 128.24, 128.22, 128.16, 128.10, 128.08, 128.06, 128.02, 127.99, 127.98, 127.97, 127.95, 127.90, 127.84, 127.82, 127.80, 127.78, 127.57, 127.56, 127.48, 127.46, 127.44, 127.35, 127.33, 127.32, 127.18, 127.14, 126.98, 126.86, 126.84, 126.38, 126.30, 123.12, 101.95, 97.17, 97.03, 79.42, 79.20, 79.01, 77.29, 77.14, 77.08, 76.86, 76.58, 75.83, 75.74, 74.68, 74.66, 74.54, 74.31, 73.45, 73.43, 72.69, 70.96, 70.55, 68.50, 68.25, 67.82, 66.88, 56.55, 55.78.

IR (ATR-FTIR, thin film) 3477, 3030, 2871, 1775, 1712, 1386, 1075 cm^{-1}.

$[\alpha]^{22}_D$ (*c* 1.0, CH_2Cl_2) –35.7.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{83}H_{78}N_2NaO_{18}$) requires 1413.5, found 1413.9.



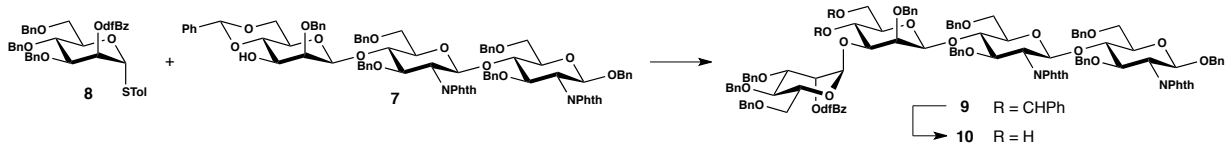
***p*-Tolyl 3,4,6-tri-*O*-benzyl-(2,5-difluorobenzoyl)-1-thio-α-D-mannopyranoside.** Thioglycoside **S-1**[3] (10.0 g, 18.0 mmol) and 4-dimethylaminopyridine (0.22 g, 1.8 mmol) were dissolved in pyridine (50 mL), and then 2,5-difluorobenzoyl chloride (6.7 mL, 54.0 mmol) was added. The mixture was stirred at room temperature overnight and then diluted with CH_2Cl_2 (300 mL). The mixture was washed with saturated aqueous $NaHCO_3$ (150 mL), water (150 mL) and 1 N HCl (150 mL), and then dried over Na_2SO_4, filtered and concentrated. Purification by flash chromatography (9:1 to 85:15 hexanes/ethyl acetate) afforded difluorobenzoyl ester **8** (11.0 g, 88% yield) as a clear oil.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72–7.65 (m, 1H), 7.42–7.20 (m, 18H), 7.13–7.05 (m, 3H), 5.85 (dd, *J* = 2.9, 1.7 Hz, 1H), 5.59 (d, *J* = 1.7 Hz, 1H), 4.90 (d, *J* = 10.8 Hz, 1H), 4.81 (d, *J* = 11.4 Hz, 1H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 11.4 Hz, 1H), 4.54 (d, *J* = 10.7 Hz, 1H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.41 (ddd, *J* = 9.6, 4.4, 1.9 Hz, 1H), 4.14–4.04 (m, 2H), 3.89 (dd, *J* = 10.8, 4.5 Hz, 1H), 3.79 (dd, *J* = 10.7, 2.0 Hz, 1H), 2.32 (s, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 162.21, 162.20, 162.19, 162.17, 159.14, 159.12, 158.8, 158.7, 157.43, 157.41, 157.13, 157.12, 138.3, 138.2, 138.0, 137.5, 132.4, 129.8, 129.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 121.64, 121.58, 121.5, 121.4, 118.52, 118.47, 118.45, 118.36, 118.30, 118.28, 86.3, 78.4, 75.3, 74.6, 73.3, 72.5, 71.9, 71.5, 68.9, 21.1.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{41}H_{38}F_2O_6SNa$) requires 719.2, found 719.3.

(3) Chayajarus, K.; Chambers, D. J.; Chughtai, M. J.; Fairbanks, A. J. *Org. Lett.* **2004**, *6*, 3797–3800.



Benzyl [3,4,6-tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→3)]-2-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (10). A mixture of trisaccharide acceptor **7** (4.0 g, 2.87 mmol) and mannose thioglycoside donor **8** (2.6 g, 3.74 mmol) was dissolved in anhydrous CH_2Cl_2 (100 mL). Freshly activated AW-300 MS (6 g) was added and stirred at r.t. for 1 h. The mixture was cooled to 0 °C, NIS (0.9 g, 4.0 mmol) and TMSOTf (100 µl, 0.57 mmol) were added sequentially, and the mixture was allowed to warm up to r.t. over 5 h. The mixture was filtered through a pad of Celite and the organic layer was washed with sat aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, water, brine, dried over $MgSO_4$ and concentrated.

The crude tetrasaccharide **9** was dissolved in acetic acid (30 mL). H_2O (4.5 mL) was added dropwise with stirring and the reaction mixture was heated at 70 °C for 3 h. The mixture was co-evaporated with toluene and the crude mass was purified by flash chromatography (hexanes:EtOAc, 1:1) to give **10** (3.39 g, 63% over two steps) as an amorphous white solid. Measurement of $^1J_{CH}$ coupling constants[4] confirmed the anomeric configuration at each inter-residue glycosidic bond (data listed below).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.88–6.57 (m, 56H), 5.52 (d, *J* = 3.0 Hz, 2H), 5.21–5.14 (m, 1H), 4.93–4.85 (m, 2H), 4.84–4.74 (m, 3H), 4.69 (d, *J* = 11.2 Hz, 1H), 4.62 (m, *J* = 12.0, 9.3 Hz, 2H), 4.52–4.40 (m, 8H), 4.42–4.34 (m, 2H), 4.29 (d, *J* = 12.3 Hz, 1H), 4.22 (d, *J* = 12.2 Hz, 1H), 4.17–4.08 (m, 4H), 4.05 (dd, *J* = 10.7, 8.4 Hz, 1H), 3.99 (m, 2H), 3.96–3.91 (m, 1H), 3.88 (t, *J* = 9.6 Hz, 1H), 3.74 (d, *J* = 3.1 Hz, 1H), 3.69 (t, *J* = 9.6 Hz, 1H), 3.65 (dd, *J* = 10.2, 2.0 Hz, 1H), 3.55 (m, 2H), 3.51–3.45 (m, 2H), 3.43–3.34 (m, 3H), 3.31 (dd, *J* = 11.7, 5.7 Hz, 1H), 3.23 (ddd, *J* = 9.8, 3.9, 1.6 Hz, 1H), 3.13 (dt, *J* = 10.1, 2.5 Hz, 1H), 2.97 (ddd, *J* = 9.2, 5.8, 3.3 Hz, 1H).

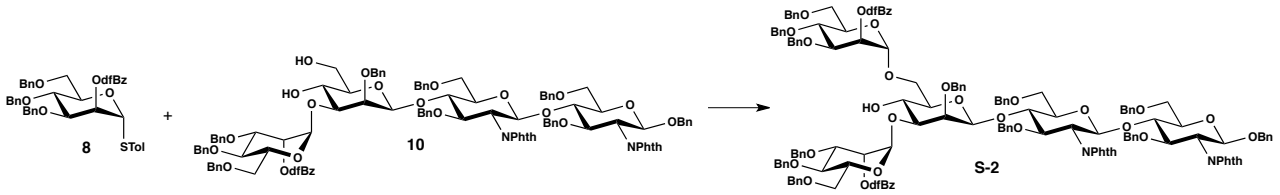
^{13}C NMR (150 MHz, $CDCl_3$) δ 168.52, 167.65, 162.18, 159.08, 158.75, 157.38, 157.13, 138.63, 138.56, 138.50, 138.36, 138.22, 137.79, 137.64, 137.19, 134.49, 134.07, 133.85, 133.47, 133.42, 131.69, 131.43, 130.91, 130.13, 129.77, 129.58, 129.29, 129.02, 128.82, 128.71, 128.61, 128.55, 128.51, 128.39, 128.31, 128.28, 128.22, 128.13, 128.08, 128.01, 127.96, 127.90, 127.86, 127.82, 127.79, 127.72, 127.66, 127.57, 127.48, 127.38, 127.35, 127.29, 127.20, 127.09, 127.04, 127.02, 126.92, 126.85, 125.96, 123.71, 123.12, 121.61, 121.55, 121.45, 121.39, 118.52, 118.46, 118.36, 118.29, 109.63, 101.12 ($^1J_{CH}$ = 160.3 Hz, *β*-Man), 97.20 ($^1J_{CH}$ = 165.3 Hz, *β*-GlcN), 97.14, 97.09 ($^1J_{CH}$ = 174.3 Hz, *α*-Man), 80.85, 79.12, 78.71, 77.88, 76.61, 76.55, 75.89, 75.86, 75.81, 75.73, 75.02, 74.75, 74.70, 74.54, 74.50, 74.49, 74.46, 73.56, 73.33, 72.74, 71.89, 71.80, 70.54, 70.14, 69.49, 68.21, 67.63, 66.39, 62.57, 56.59, 56.51, 55.77.

IR (ATR-FTIR, thin film) 3472, 2925, 1775, 1712, 1387, 1073, 698 cm^{-1}.

$[\alpha]^{24}_D$ (*c* 1.0, CH_2Cl_2) –15.8.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{110}H_{104}F_2N_2NaO_{24}$) requires 1897.7, found 1897.6.

(4) (a) Bock, K.; Lundt, I.; Pedersen, C. *Tetrahedron Lett*. **1973**, *14*, 1037–1040. (b) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.



Benzyl [3,4,6-tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→3)]-[3,4,6-tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→6)]-2-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (S-2). A mixture of tetrasaccharide acceptor **10** (1.05 g, 0.56 mmol) and mannose thioglycoside donor **8** (390 mg, 0.56 mmol) was dissolved in anhydrous CH_2Cl_2 (100 mL). Freshly activated AW-300 MS (2.0 g) was added and stirred at r.t. for 1 h. The mixture was cooled to –40 °C, NIS (189 mg, 0.84 mmol) and TMSOTf (20 μl, 0.11 mmol) were added sequentially, and the mixture was allowed to warm up to 0 °C over 5 h. The mixture was filtered through a pad of Celite and the organic layer was washed with saturated aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, water, brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (hexanes:EtOAc, 2:1) to give the pentasaccharide **S-2** (1.29 g, 94%) as an amorphous white solid.

The regioselectivity of glycosylation was confirmed by a range of 2D-NMR experiments. The HMBC spectrum of pentasaccharide **S-2** showed a cross peak between H-1 of the newly installed α-Man (5.16 ppm) and C-6 of the central, branched β-Man (67.2 ppm) confirming that the glycosylation had occurred at the primary alcohol at the C-6 position. This assignment was also supported by the change in chemical shift of the C-6 carbon from 62.6 ppm to 67.0 ppm while C-4 remained relatively unchanged from 66.4 (in the case of diol) to 66.7 ppm (after the glycosylation). Further evidence was obtained from the NOESY spectrum, which revealed cross peaks between the H-1 of α-Man (5.16 ppm) and H-6a and H-6b of β-Man (4.12 and 3.86 ppm respectively).

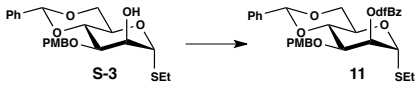
^{1}H NMR (600 MHz, C_6D_6) δ 7.99–7.07 (m, 62H), 7.06–6.90 (m, 5H), 6.88–6.75 (m, 3H), 6.75–6.66 (m, 1H), 6.58–6.36 (m, 3H), 6.22–6.15 (m, 1H), 6.01 (d, J = 1.9 Hz, 1H), 5.94 (t, J = 2.4 Hz, 1H), 5.76 (d, J = 8.3 Hz, 1H), 5.49 (d, J = 12.8 Hz, 1H), 5.34 (d, J = 11.8 Hz, 1H), 5.31 (d, J = 8.5 Hz, 1H), 5.20 (d, J = 13.0 Hz, 1H), 5.16–5.10 (m, 2H), 5.08 (d, J = 11.3 Hz, 1H), 5.04–4.95 (m, 3H), 4.90 (d, J = 12.8 Hz, 1H), 4.88–4.82 (m, 2H), 4.81–4.38 (m, 22H), 4.31–4.24 (m, 3H), 4.25–4.16 (m, 3H), 4.13 (dd, J = 11.0, 3.9 Hz, 1H), 3.98 (dd, J = 10.6, 1.8 Hz, 1H), 3.91–3.78 (m, 5H), 3.77–3.69 (m, 1H), 3.68–3.59 (m, 2H), 3.55 (dd, J = 10.9, 1.7 Hz, 1H), 3.44 (dt, J = 10.2, 2.5 Hz, 1H), 3.41 (dt, J = 9.4, 3.3 Hz, 1H), 2.97 (ddd, J = 10.1, 3.4, 1.6 Hz, 1H).

^{13}C NMR (150 MHz, C_6D_6) δ 167.67, 162.50, 159.19, 158.98, 157.49, 157.38, 157.37, 157.29, 139.78, 139.50, 139.41, 139.33, 139.15, 139.13, 138.81, 138.75, 138.66, 138.50, 138.09, 133.14, 132.36, 129.05, 128.99, 128.95, 128.85, 128.75, 128.72, 128.67, 128.65, 128.62, 128.58, 128.56, 128.55, 128.51, 128.47, 128.44, 128.42, 128.37, 128.35, 128.31, 128.26, 128.25, 128.18, 128.13, 128.09, 128.02, 127.97, 127.95, 127.94, 127.91, 127.86, 127.73, 127.70, 127.68, 127.65, 127.58, 127.55, 127.53, 127.50, 127.49, 127.45, 127.30, 127.28, 127.03, 127.02, 123.03, 120.16, 118.62, 118.54, 118.50, 118.46, 118.41, 118.37, 118.33, 118.25, 118.19, 102.26, 98.69, 98.11, 97.76, 81.01, 80.13, 79.41, 79.28, 78.79, 77.51, 77.10, 76.47, 75.99, 75.58, 75.26, 75.21, 75.10, 75.03, 74.93, 74.85, 74.82, 73.69, 73.50, 73.40, 73.10, 72.87, 72.84, 72.02, 71.91, 70.61, 70.51, 70.33, 70.31, 69.58, 68.51, 67.88, 67.10, 67.01, 57.32, 56.55.

IR (ATR-FTIR, thin film) 2926, 1776, 1714, 1495, 1387, 1077, 698 cm^{-1}.

$[\alpha]^{24}_D$ (*c* 1.0, CH_2Cl_2) –13.4.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{144}H_{134}F_4N_2NaO_{30}$) requires 2469.9, found 2470.0.



Ethyl 4,6-*O*-benzylidene-2-*O*-(2,5-difluorobenzoyl)-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (11). To a solution of alcohol **S-3**[5] (768 mg, 1.77 mmol) and 4-dimethylaminopyridine (43.4 mg, 0.355 mmol) in pyridine (5.0 mL) was added 2,5-difluorobenzoyl chloride (0.44 mL, 3.55 mmol) via syringe pump over 10 min. The reaction mixture was stirred at room temperature; gradual formation of a white precipitate was observed over time. An additional portion of 2,5-difluorobenzoyl chloride (0.11 mL, 0.887 mmol) was added at 19.5 h. After a total reaction time of 44 h, MeOH (0.80 mL) was added. The resulting mixture was stirred for 1 h, then diluted with CH_2Cl_2 (80 mL) and washed with water (120 mL). The aqueous phase was back-extracted with CH_2Cl_2 (60 mL), then the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. Purification by flash chromatography (10% EtOAc/hexanes) afforded an oily white solid that was taken up in EtOAc (80 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) (to remove residual 2,5-difluorobenzoic acid). The combined aqueous phases were back-extracted with EtOAc (40 mL). The organic layers were combined, washed with water (20 mL) and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated to provide difluorobenzoyl ester **11** as a yellowish foam in 94% yield (960 mg, 1.68 mmol).

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.67 (ddd, *J* = 8.5, 5.4, 3.2 Hz, 1H), 7.53–7.48 (m, 2H), 7.41–7.34 (m, 3H), 7.26–7.22 (m, 3H), 7.14 (apparent td, *J* = 9.4, 4.1 Hz, 1H), 6.81 (d, 2H), 5.65 (s, 2H), 5.65 (dd, *J* = 3.3, 1.5 Hz, 1H), 5.38 (d, *J* = 1.4 Hz, 1H), 4.66 (d, *J* = 11.8 Hz, 1H), 4.62 (d, *J* = 11.7 Hz, 1H), 4.31–4.23 (m, 2H), 4.19–4.14 (m, 1H), 4.04 (dd, *J* = 9.8, 3.3 Hz, 1H), 3.92–3.86 (m, 1H), 3.78 (s, 3H), 2.72–2.59 (m, 2H), 1.30 (t, *J* = 7.4 Hz, 3H).

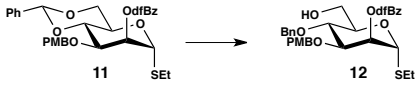
^{13}C NMR (150 MHz, $CDCl_3$) δ 162.30, 162.28, 162.27, 162.26, 159.24, 159.07, 159.05, 158.79, 158.77, 157.36, 157.35, 157.17, 157.15, 137.38, 129.69, 129.44, 128.94, 128.16, 126.13, 121.74, 121.68, 121.58, 121.52, 119.31, 119.26, 119.23, 119.18, 118.60, 118.55, 118.47, 118.43, 118.38, 118.30, 113.74, 101.64, 83.29, 78.78, 73.70, 72.85, 71.92, 68.61, 64.65, 55.23, 25.66, 14.94.

IR (ATR-FTIR, thin film) 3419, 3067, 3033, 2953, 2928, 2871, 1721, 1612, 1595, 1587, 1514, 1496, 1454, 1428, 1371, 1308, 1270, 1244, 1186, 1098, 1081, 1063, 1029, 969, 945, 908, 890, 826 cm^{-1}.

$[\alpha]^{22}_D$ (*c* 0.15, CH_2Cl_2) +19.2.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{30}H_{30}F_2NaO_7S$) requires 595.2, found 595.1.

(5) Cherif, S.; Clavel, J.-M.; Monneret, C. *J. Carbohydr. Chem.* **2002**, *21*, 123–130.



Ethyl 4-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-3-*O*-*p*-methoxybenzyl-1-thio-α-D-mannopyranoside (12). To a cooled (0 °C) round-bottom flask containing benzylidene acetal **11** (960 mg, 1.68 mmol) was added borane-THF complex (1.0 M in THF, 8.4 mL, 8.40 mmol). The resulting clear, colorless solution was stirred for 10 min at 0 °C. Copper(II) trifluoromethanesulfonate (60.7 mg, 0.168 mmol) was then added in one portion, giving a light brown suspension that was maintained at 0 °C for 25.5 h with vigorous stirring. The reaction was carefully quenched while cold by successive addition of triethylamine (0.24 mL) and MeOH (3.0 mL) (**CAUTION:** H_2 evolution!). Volatiles were removed on a rotary evaporator, and the residue was co-evaporated with MeOH a few times, resulting in a cloudy, dark brown oil. Purification by flash chromatography (20% EtOAc/hexanes) afforded alcohol **12** as a clear, very pale yellow oil in 96% yield (929 mg, 1.62 mmol). The regioselectivity of the ring opening was assigned on the basis of the multiplicity of the OH proton, and the observation of a ^{1}H-^{1}H correlation between the OH and C-6 protons in the 2D COSY.

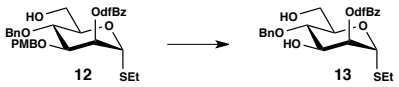
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.65 (ddd, *J* = 8.5, 5.4, 3.3 Hz, 1H), 7.37–7.32 (m, 2H), 7.32–7.28 (m, 3H), 7.29–7.21 (m, 1H), 7.24 (d, *J* = 8.6 Hz, 2H), 7.14 (apparent td, *J* = 9.4, 4.2 Hz, 1H), 6.82 (d, *J* = 8.6 Hz, 2H), 5.66 (apparent t, *J* = 2.3 Hz, 1H), 5.36 (d, *J* = 1.7 Hz, 1H), 4.90 (d, *J* = 10.9 Hz, 1H), 4.68 (d, *J* = 11.1 Hz, 1H), 4.64 (d, *J* = 10.9 Hz, 1H), 4.51 (d, *J* = 11.1 Hz, 1H), 4.07 (dt, *J* = 9.3, 3.3 Hz, 1H), 4.02–3.94 (m, 2H), 3.85–3.79 (m, 2H), 3.78 (s, 3H), 2.70–2.58 (m, 2H), 1.78 (dd, *J* = 7.6, 5.5 Hz, 1H), 1.29 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 162.39, 162.38, 162.37, 162.35, 159.29, 159.03, 159.01, 158.80, 158.79, 157.32, 157.31, 157.19, 157.17, 138.17, 129.76, 129.64, 128.38, 128.05, 127.77, 121.68, 121.62, 121.52, 121.46, 119.38, 119.33, 119.30, 119.25, 118.63, 118.58, 118.46, 118.44, 118.41, 118.28, 113.77, 82.25, 77.98, 75.18, 74.03, 72.40, 71.79, 71.40, 62.04, 55.18, 25.61, 14.85.

IR (ATR-FTIR, thin film) 3500, 3073, 3032, 2962, 2930, 2874, 2837, 1721, 1612, 1587, 1514, 1496, 1454, 1427, 1368, 1345, 1308, 1268, 1247, 1185, 1094, 1075, 1031, 968, 942, 892, 825 cm^{-1}.

$[\alpha]^{22}_D$ (*c* 1.0, CH_2Cl_2) +34.3.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{30}H_{32}F_2NaO_7S$) requires 597.2, found 597.2.



Ethyl 4-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-1-thio-α-D-mannopyranoside (13). To a solution of PMB ether **12** in CH_2Cl_2 (7.2 mL) was added water (0.40 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (263 mg, 1.16 mmol), resulting in a dark greenish-black color that became reddish-orange over time. After stirring for 4 h at room temperature, the reaction was quenched with a solution of ascorbic acid/citric acid/NaOH (0.7%/1.3%/0.9% in water, 50 mL) and diluted with EtOAc (100 mL). The layers were separated, and the aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic layers were filtered through a pad of Celite, and the filtrate was washed with saturated aqueous $NaHCO_3$ (100 mL), brine (100 mL), dried (Na_2SO_4), filtered, and concentrated. Purification by flash chromatography (20% EtOAc/hexanes) afforded diol **13** as a clear, colorless oil in 89% yield (312 mg, 0.685 mmol).

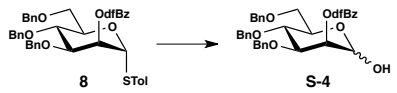
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.63 (ddd, *J* = 8.5, 5.4, 3.2 Hz, 1H), 7.41–7.34 (m, 4H), 7.34–7.29 (m, 1H), 7.29–7.24 (m, 1H), 7.16 (apparent td, *J* = 9.4, 4.1 Hz, 1H), 5.42 (dd, *J* = 3.3, 1.6 Hz, 1H), 5.41 (d, *J* = 1.6 Hz, 1H), 4.83 (d, *J* = 11.3 Hz, 1H), 4.78 (d, *J* = 11.3 Hz, 1H), 4.17 (ddd, *J* = 9.1, 5.7, 3.2 Hz, 1H), 4.08 (dt, *J* = 9.7, 3.1 Hz, 1H), 3.93 (t, *J* = 9.5 Hz, 1H), 3.91–3.83 (m, 2H), 2.72–2.57 (m, 2H), 2.08 (d, *J* = 5.7 Hz, 1H), 1.81 (dd, *J* = 7.7, 5.4 Hz, 1H), 1.30 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 162.90, 162.89, 162.87, 162.86, 158.96, 158.95, 158.92, 158.90, 157.30, 157.28, 157.27, 157.25, 137.96, 128.65, 128.17, 128.14, 121.89, 121.83, 121.73, 121.67, 119.28, 119.23, 119.20, 119.15, 118.66, 118.61, 118.57, 118.56, 118.49, 118.44, 118.40, 118.39, 82.07, 75.70, 75.53, 75.03, 72.20, 70.84, 61.90, 25.72, 14.89.

IR (ATR-FTIR, thin film) 3454, 3126, 3076, 3032, 2967, 2929, 2879, 1722, 1627, 1595, 1496, 1454, 1429, 1310, 1270, 1242, 1187, 1092, 1074, 965, 943, 891, 827 cm^{-1}.

$[\alpha]^{22}_D$ (*c* 1.1, CH_2Cl_2) +63.9.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{22}H_{24}F_2NaO_6S$) requires 477.1, found 477.1.



3,4,6-Tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-D-mannopyranoside (S-4).[6] To a cooled (0 °C) solution of thioglycoside **8** (1.88 g, 2.70 mmol) in acetone/water (9:1, 40 mL) was added *N*-bromosuccinimide (1.44 g, 8.09 mmol). The resulting clear, orange solution was stirred at 0 °C, with additional portions of *N*-bromosuccinimide (480 mg, 2.70 mmol) added at 1 h and 4 h. After a total reaction time of 6 h, the reaction mixture was concentrated until turbidity was evident. The residue was then taken up in EtOAc (500 mL), washed with saturated aqueous $NaHCO_3$ (3 × 120 mL), water (3 × 120 mL), dried (Na_2SO_4), filtered, and concentrated. Purification by flash chromatography (25% EtOAc/hexanes) afforded anomeric alcohol **S-4** as a clear, colorless oil in 86% yield (1.37 g, 2.33 mmol, α:β mixture).

^{1}H NMR (600 MHz, $CDCl_3$, major anomer) δ 7.66 (ddd, *J* = 8.5, 5.4, 3.3 Hz, 1H), 7.39–7.31 (m, 6H), 7.31–7.25 (m, 7H), 7.25–7.21 (m, 1H), 7.19–7.14 (m, 2H), 7.11 (apparent td, *J* = 9.3, 4.2 Hz, 1H), 5.59 (dd, *J* = 3.1, 2.0 Hz, 1H), 5.33 (dd, *J* = 3.9, 2.0 Hz, 1H), 4.86 (d, *J* = 10.8 Hz, 1H), 4.77 (d, *J* = 11.4 Hz, 1H), 4.62 (d, *J* = 12.2 Hz, 1H), 4.59 (d, *J* = 11.4 Hz, 1H), 4.54 (d, *J* = 12.1 Hz, 1H), 4.48 (d, *J* = 10.9 Hz, 1H), 4.14 (dd, *J* = 9.4, 3.0 Hz, 1H), 4.13–4.09 (m, 1H), 3.87 (apparent t, *J* = 9.6 Hz, 1H), 3.74 (dd, *J* = 10.4, 2.2 Hz, 1H), 3.69 (dd, *J* = 10.5, 5.8 Hz, 1H), 3.65 (d, *J* = 3.7 Hz, 1H).

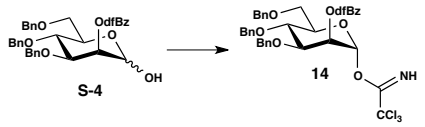
^{13}C NMR (150 MHz, $CDCl_3$, major anomer) δ 162.34, 162.33, 162.32, 162.30, 159.08, 159.07, 158.73, 158.71, 157.37, 157.36, 157.11, 157.09, 138.10, 137.83, 137.82, 128.32, 128.29, 127.99, 127.91, 127.67, 127.66, 127.65, 121.57, 121.51, 121.41, 121.35, 119.43, 119.38, 119.35, 119.30, 118.53, 118.47, 118.44, 118.36, 118.31, 118.27, 92.13, 77.53, 75.13, 74.51, 73.31, 71.69, 71.19, 70.11, 69.28.

IR (ATR-FTIR, thin film) 3406, 3087, 3064, 3032, 2924, 2868, 1738, 1720, 1627, 1596, 1496, 1454, 1428, 1363, 1342, 1309, 1270, 1254, 1242, 1188, 1119, 1075, 1063, 1038, 978, 943, 910, 892, 825 cm^{-1}.

$[\alpha]^{21}_D$ (*c* 1.9, CH_2Cl_2) –25.5.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{34}H_{32}F_2NaO_7$) requires 613.2, found 613.3.

(6) The procedure for conversion to the hemiacetal is based on the following report: Motawia, M. S.; Marcussen, J.; Møller, B. L. *J. Carbohydr. Chem.* **1995**, *14*, 1279–1294.



3,4,6-Tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl trichloroacetimidate (14). To a cooled (0 °C) solution of anomeric alcohol **S-4** (1.35 g, 2.29 mmol) and trichloroacetonitrile (2.3 mL, 22.9 mmol) in CH_2Cl_2 (9.0 mL) was added and 1,8-diazabicyclo[5.4.0]undec-7-ene (40 μL, 0.267 mmol) dropwise via syringe. The resulting clear, yellow solution was stirred at 0 °C for 4 h. The reaction mixture was loaded directly on a short silica gel column and purified by flash chromatography (20% EtOAc/hexanes) to afford trichloroacetimidate **14** as a clear, yellow oil in 96% yield (1.61 g, 2.19 mmol, ~95% α-anomer).

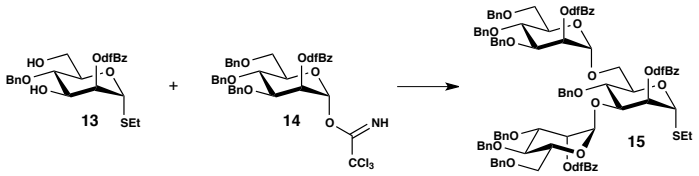
^{1}H NMR (600 MHz, $CDCl_3$, α-anomer) δ 8.71 (s, 1H), 7.70 (ddd, J = 8.5, 5.4, 3.2 Hz, 1H), 7.37–7.23 (m, 14H), 7.22–7.18 (m, 2H), 7.11 (apparent td, J = 9.4, 4.2 Hz, 1H), 6.42 (d, J = 2.1 Hz, 1H), 5.71 (apparent t, J = 2.6 Hz, 1H), 4.87 (d, J = 10.7 Hz, 1H), 4.79 (d, J = 11.4 Hz, 1H), 4.70 (d, J = 12.1 Hz, 1H), 4.64 (d, J = 11.4 Hz, 1H), 4.55 (d, J = 10.6 Hz, 1H), 4.52 (d, J = 12.1 Hz, 1H), 4.18 (apparent t, J = 9.6 Hz, 1H), 4.13 (dd, J = 9.5, 3.0 Hz, 1H), 4.03 (ddd, J = 9.8, 3.9, 1.8 Hz, 1H), 3.86 (dd, J = 11.1, 3.8 Hz, 1H), 3.75 (dd, J = 11.2, 1.9 Hz, 1H).

^{13}C NMR (150 MHz, $CDCl_3$, α-anomer) δ 162.12, 162.10, 162.09, 162.08, 159.91, 159.18, 159.17, 158.77, 158.75, 157.47, 157.45, 157.15, 157.14, 138.22, 137.98, 137.38, 128.42, 128.37, 128.28, 128.25, 128.18, 127.91, 127.82, 127.61, 127.48, 121.86, 121.80, 121.70, 121.64, 119.06, 119.01, 118.98, 118.93, 118.62, 118.57, 118.55, 118.46, 118.40, 118.38, 95.07 ($^1J_{CH}$ = 180.5 Hz), 90.71, 77.14, 75.52, 74.48, 73.66, 73.34, 72.06, 68.43, 68.35.

IR (ATR-FTIR, thin film) 3337, 3087, 3064, 3032, 2904, 2869, 1742, 1726, 1675, 1627, 1596, 1496, 1454, 1428, 1362, 1320, 1307, 1267, 1239, 1187, 1164, 1101, 1076, 1067, 1046, 1028, 972, 946, 929, 828 cm^{-1}.

$[\alpha]^{22}_D$ (*c* 1.1, CH_2Cl_2) +14.9.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{36}H_{32}{}^{35}Cl_3F_2NNaO_7$) requires 756.1, found 756.1.



Ethyl 3,4,6-tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→3)-[3,4,6-tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→6)]-4-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-1-thio-α-D-mannopyranoside (15). Diol acceptor **14** (294 mg, 0.647 mmol) and trichloroacetimidate donor **13** (1.17 g, 1.60 mmol) were azeotroped three times with benzene then dried for 2 h *in vacuo*. The residue was dissolved in anhydrous CH_2Cl_2 (6.5 mL), and the clear, yellow solution was stirred in the presence of acid-washed molecular sieves (AW-300, 1.6 mm pellets, 900 mg) for 15 min at room temperature. The mixture was cooled to 0 °C, then trimethylsilyl trifluoromethanesulfonate (5% in CH_2Cl_2, 0.24 mL, 66.4 μmol) was added dropwise via syringe. After stirring for 2 h at 0 °C, the reaction medium was neutralized with a few drops of triethylamine, then filtered and concentrated. Purification by flash chromatography (0–1% EtOAc/CH_2Cl_2) afforded trisaccharide **15** as a white foam in 75% yield (771 mg, 0.482 mmol). Measurement of $^1J_{CH}$ coupling constants[4] confirmed the α-anomeric configuration at the newly formed glycosidic bonds (data listed below).

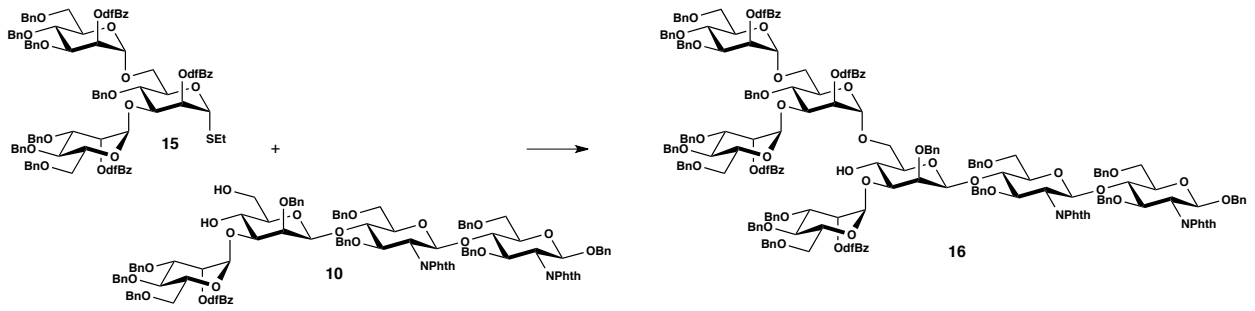
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68–7.58 (m, 3H), 7.39–7.19 (m, 28H), 7.19–7.11 (m, 8H), 7.11–7.04 (m, 5H), 5.72 (apparent t, *J* = 2.5 Hz, 1H), 5.59 (apparent t, *J* = 2.3 Hz, 1H), 5.54 (dd, *J* = 2.7, 1.5 Hz, 1H), 5.38 (d, *J* = 1.1 Hz, 1H), 5.30 (d, *J* = 1.9 Hz, 1H), 5.11 (d, *J* = 2.1 Hz, 1H), 4.83 (d, *J* = 10.8 Hz, 1H), 4.81–4.77 (m, 2H), 4.76 (d, *J* = 11.1 Hz, 1H), 4.71 (d, *J* = 12.1 Hz, 1H), 4.66 (d, *J* = 12.2 Hz, 1H), 4.61–4.53 (m, 3H), 4.49–4.38 (m, 5H), 4.27 (dd, *J* = 9.4, 3.1 Hz, 1H), 4.21–4.15 (m, 1H), 4.08 (dd, *J* = 9.3, 3.1 Hz, 1H), 4.06–3.98 (m, 3H), 3.97–3.91 (m, 2H), 3.85–3.79 (m, 2H), 3.77–3.72 (m, 2H), 3.68 (dd, *J* = 10.8, 3.6 Hz, 1H), 3.66–3.61 (m, 2H), 2.67–2.49 (m, 2H), 1.23 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 162.85, 162.84, 162.82, 162.81, 162.08, 162.06, 162.05, 162.03, 162.00, 161.99, 161.97, 161.96, 159.12, 159.10, 158.90, 158.89, 158.86, 158.85, 158.71, 157.41, 157.39, 157.25, 157.23, 157.20, 157.19, 157.10, 138.37, 138.37, 138.25, 138.20, 137.78, 137.69, 137.55, 128.47, 128.35, 128.23, 128.21, 128.17, 128.13, 128.06, 127.89, 127.85, 127.72, 127.63, 127.60, 127.59, 127.46, 127.36, 121.86, 121.80, 121.70, 121.64, 121.61, 121.55, 121.53, 121.47, 121.46, 121.39, 121.37, 121.31, 119.45, 119.40, 119.37, 119.32, 119.28, 119.23, 119.20, 119.18, 119.15, 119.10, 118.90, 118.85, 118.73, 118.68, 118.50, 118.46, 118.45, 118.33, 118.29, 99.62 ($^1J_{CH}$ = 173.4 Hz), 97.64 ($^1J_{CH}$ = 174.0 Hz), 81.72, 78.30, 78.13, 77.64, 75.32, 75.21, 75.12, 74.75, 74.68, 74.16, 73.90, 73.32, 73.26, 72.60, 71.82, 71.69, 71.56, 71.44, 69.93, 69.31, 68.59, 68.23, 65.62, 25.63, 14.95.

IR (ATR-FTIR, thin film) 3087, 3066, 3031, 2928, 2869, 1739, 1722, 1627, 1595, 1496, 1454, 1428, 1362, 1308, 1269, 1239, 1187, 1145, 1079, 1028, 981, 943, 910, 892, 826 cm^{-1}.

$[\alpha]^{22}_D$ (*c* 1.0, CH_2Cl_2) +14.3.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{90}H_{84}F_6NaO_{18}S$) requires 1621.5, found 1621.3.



Benzyl [3,4,6-tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→3)]-[[3,4,6-tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→3)]-[3,4,6-tri-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→6)]]-4-*O*-benzyl-2-*O*-(2,5-difluorobenzoyl)-α-D-mannopyranosyl-(1→6)]-2-*O*-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (16). A mixture of tetrasaccharide acceptor **10** (1.0 g, 0.53 mmol) and trimannose thioglycoside donor **15** (0.86 g, 0.54 mmol) was dissolved in anhydrous CH_2Cl_2 (40 mL). Freshly activated AW-300 MS (1.8 g) was added and stirred at r.t. for 1 h. The mixture was cooled to 0 °C, NIS (180 mg, 0.8 mmol) and TMSOTf (20 µl, 0.11 mmol) were added sequentially, and the mixture was allowed to warm up to r.t. over 4 h. The mixture was filtered through a pad of Celite and the organic layer was washed with saturated aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, water, brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash chromatography (hexanes:EtOAc, 2:1) to give the heptasaccharide **16** (1.35 g, 75%) as an amorphous white solid. The regioselectivity of glycosylation was confirmed by analogy to the case of the pentasaccharide, noting a shift in the C-6 carbon of the central, branched β-Man from 62.6 ppm to 65.6 ppm. Measurement of $^1J_{CH}$ coupling constants[4] confirmed the anomeric configuration at each inter-residue glycosidic bond (data listed below).

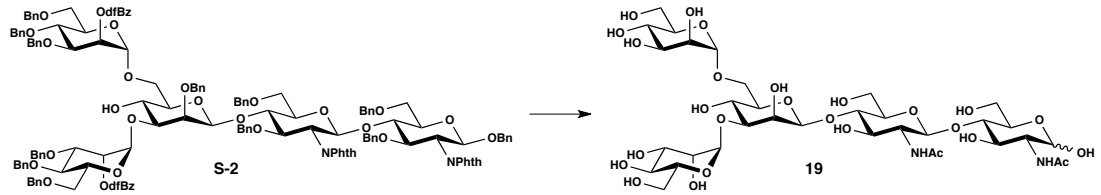
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81–6.56 (m, 100H), 5.69 (t, *J* = 2.5 Hz, 1H), 5.67 (t, *J* = 2.5 Hz, 1H), 5.56 (t, *J* = 2.6 Hz, 1H), 5.45–5.41 (m, 1H), 5.39 (d, *J* = 1.9 Hz, 1H), 5.20 (d, *J* = 7.7 Hz, 1H), 5.18 (d, *J* = 1.9 Hz, 1H), 5.09 (d, *J* = 1.9 Hz, 1H), 4.93 (d, *J* = 12.3 Hz, 1H), 4.91 (d, *J* = 8.6 Hz, 1H), 4.87 (d, *J* = 1.8 Hz, 1H), 4.84–4.69 (m, 8H), 4.69–4.61 (m, 3H), 4.60–4.37 (m, 17H), 4.37–4.30 (m, 3H), 4.23 (dd, *J* = 9.5, 3.2 Hz, 1H), 4.20 (d, *J* = 12.1 Hz, 1H), 4.18–4.03 (m, 7H), 4.03–3.91 (m, 6H), 3.91–3.83 (m, 3H), 3.80 (m, 2H), 3.78–3.67 (m, 5H), 3.65 (m, 2H), 3.60 (dd, *J* = 10.7, 1.9 Hz, 1H), 3.58–3.53 (m, 1H), 3.53–3.39 (m, 5H), 3.35 (m, 2H), 3.24 (ddd, *J* = 9.7, 4.0, 1.8 Hz, 1H), 3.14 (dt, *J* = 9.8, 2.5 Hz, 1H), 3.05 (dt, *J* = 9.4, 3.8 Hz, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 168.21, 167.59, 167.40, 162.59, 162.13, 161.91, 159.06, 158.78, 158.71, 157.35, 157.16, 157.09, 138.66, 138.62, 138.50, 138.46, 138.38, 138.07, 137.94, 137.89, 137.83, 137.63, 137.21, 133.77, 133.49, 133.40, 131.82, 131.71, 131.48, 128.52, 128.46, 128.39, 128.35, 128.32, 128.27, 128.22, 128.17, 128.12, 128.08, 128.05, 128.01, 127.98, 127.88, 127.84, 127.81, 127.77, 127.74, 127.68, 127.63, 127.54, 127.53, 127.49, 127.44, 127.41, 127.38, 127.32, 127.29, 127.24, 127.17, 127.12, 126.84, 126.75, 123.56, 123.06, 121.37, 121.20, 119.62, 119.58, 119.55, 119.53, 119.49, 119.48, 119.45, 119.43, 119.40, 119.35, 119.18, 119.13, 119.10, 119.04, 118.79, 118.74, 118.62, 118.54, 118.47, 118.40, 118.37, 118.30, 118.23, 118.19, 101.99 ($^1J_{CH}$ = 158.8 Hz, β-Man), 99.59 ($^1J_{CH}$ = 174.5 Hz, α-Man), 98.62 ($^1J_{CH}$ = 175.2 Hz, α-Man), 97.83 ($^1J_{CH}$ = 175.0 Hz, α-Man), 97.08 (×2) ($^1J_{CH}$ = 168.3 Hz, β-GlcN), 97.06 ($^1J_{CH}$ = 174.9 Hz, α-Man), 80.75, 79.69, 78.22, 78.19, 77.99, 77.91, 77.80, 76.70, 75.95, 75.37, 75.21, 74.97, 74.74, 74.58, 74.55, 74.51, 74.40, 74.38, 74.35, 74.31, 74.19, 73.90, 73.42, 73.29, 73.13, 73.01, 72.65, 72.61, 72.60, 72.18, 71.84, 71.50, 70.98, 70.44, 69.90, 69.83, 69.36, 69.25, 68.65, 68.32, 68.14, 67.68, 67.62, 67.49, 65.54.

IR (ATR-FTIR, thin film) 3031, 2929, 2869, 1715, 1495, 1387, 1269, 1078, 738, 698 cm^{-1}.

$[\alpha]^{24}_D$ (*c* 1.0, CH_2Cl_2) –11.5.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{198}H_{182}F_8N_2NaO_{42}$) requires 3434.2, found 3434.0.



[α-D-mannopyranosyl-(1→3)]-[α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→4)-2-deoxy-2-*N*-acetyl-D-glucopyranoside (19). To a solution of oligosaccharide **S-2** (846 mg, 0.35 mmol) in CH_2Cl_2/MeOH (1:9, 20 mL), was added Na-metal (16 mg, 0.69 mmol). The mixture was stirred at r.t. for 4 h, quenched with Dowex 50W X8 resin, filtered, and evaporated to dryness. The residue was dissolved in toluene (16 mL), *n*-butanol (32 mL), ethylenediamine (9.6 mL), and heated at 90 °C for 24 h. The mixture was co-evaporated with toluene.

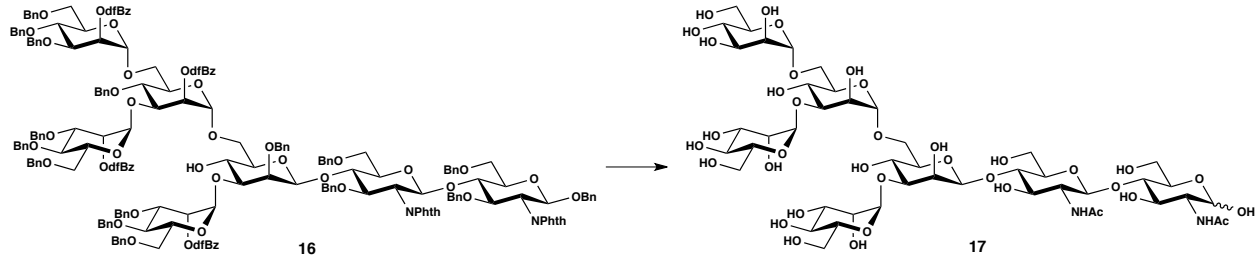
The residue was dissolved in MeOH (40 mL). Acetic anhydride (2.6 mL) and triethylamine (4.0 mL) were sequentially added to the mixture and stirred at r.t. for 12 h. The reaction was monitored by LCMS at each stage. The residue was purified by flash chromatography (hexanes:CH_2Cl_2:acetone, 1:1:1) to give the partially deprotected oligosaccharide (620 mg) as an amorphous white solid.

To a solution of partially deprotected pentasaccharide (620 mg) in MeOH (60 mL) was added H_2O (6.0 mL) dropwise, at r.t. under an atmosphere of argon. $Pd(OH)_2$/C (20% by wt., 620 mg) was added to the mixture under argon atmosphere. Argon was replaced by hydrogen and the mixture was stirred at r.t. for 12 h under 1 atm pressure. The mixture was filtered by PTFE GL 0.45 μm cartridge, evaporated, and purified using C18 SepPak column. The product eluted in neat H_2O. The pure fractions were combined and lyophilized to give compound **19** (240 mg, 0.26 mmol) as a mixture of anomers in 74% overall yield over 4 steps.

^{1}H NMR data were consistent with previously published values.[7]

LRMS (ESI+) m/z calc'd for $[M+H]^+$ ($C_{34}H_{59}N_2O_{26}$) requires 911.3, found 911.5.

(7) Paulsen, H.; Lebuhn, R. *Carbohydr. Res.* **1984**, *130*, 85–101.



[α-D-mannopyranosyl-(1→3)]-[[α-D-mannopyranosyl-(1→3)]-[α-D-mannopyranosyl-(1→6)]]-α-D-mannopyranosyl-(1→6)]-β-D-mannopyranosyl-(1→4)-2-deoxy-2-*N*-acetyl-β-D-glucopyranosyl-(1→4)-2-deoxy-2-*N*-acetyl-D-glucopyranoside (17). To a solution of oligosaccharide **16** (1.2 g, 0.35 mmol) in CH_2Cl_2/MeOH (1:10, 22 mL), was added Na-metal (33 mg, 1.4 mmol). The mixture was stirred at r.t. for 8 h, quenched with Dowex 50W X8 resin, filtered, and evaporated to dryness. The residue was dissolved in toluene (16 mL), *n*-butanol (32 mL), ethylenediamine (9.6 mL), and heated at 90 °C for 24 h. The mixture was co-evaporated with toluene.

The residue was dissolved in MeOH (40 mL). Acetic anhydride (2.6 mL) and triethylamine (4.0 mL) were sequentially added to the mixture and stirred at r.t. for 12 h. The reaction was monitored by LCMS at each stage. The residue was purified by flash chromatography (hexanes:CH_2Cl_2:acetone, 1:1:1) to give the partially deprotected oligosaccharide (940 mg) as an amorphous white solid.

To a solution of partially deprotected heptasaccharide (800 mg) in MeOH (60 mL) was added H_2O (6.0 mL) dropwise, at r.t. under an atmosphere of argon. $Pd(OH)_2/C$ (20% by wt., 800 mg) was added to the mixture under argon atmosphere. Argon was replaced by hydrogen and the mixture was stirred at r.t. for 12 h under 1 atm pressure. The mixture was filtered by PTFE GL 0.45 μm cartridge, evaporated, and purified using C18 SepPak column. The product eluted in neat H_2O. The pure fractions were combined and lyophilized to give compound **17** (288 mg, 0.23 mmol) as a mixture of anomers in 77% overall yield over 4 steps.

^{1}H NMR (600 MHz, D_2O, α-anomer) δ 5.19 (d, J = 2.6 Hz, 1H), 5.12–5.07 (m, 2H), 4.91 (d, J = 1.9 Hz, 1H), 4.87 (br d, 1H), 4.63–4.57 (m, 1H), 4.26 (d, J = 2.6 Hz, 1H), 4.15 (dd, J = 3.4, 1.7 Hz, 1H), 4.08 (dd, J = 3.4, 1.7 Hz, 1H), 4.07 (dd, J = 3.6, 1.7 Hz, 1H), 4.03–3.58 (m, 38H), 2.07 (s, 3H), 2.04 (s, 3H).

LRMS (ESI+) m/z calc'd for $[M+H]^+$ ($C_{46}H_{79}N_2O_{36}$) requires 1235.4, found 1235.6.

General procedures for peptide and glycopeptide synthesis.

Solid-phase peptide synthesis by Fmoc-strategy. Automated peptide synthesis was performed on an Applied Biosystems Pioneer continuous S3 flow peptide synthesizer. Peptides were synthesized under standard automated Fmoc protocols on Fmoc-Arg(Pbf)-TGT resin or TG Sieber resin. The deblock mixture was a mixture of 100:2:2 of DMF/piperidine/DBU. The following Fmoc amino acids from Novabiochem were employed: Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Dmcp)-OH, Fmoc-Asp(OAll)-OH, Fmoc-Asp(O*t*Bu)-OH, Fmoc-Asp(OMpe)-OH, Boc-Cys(Trt)-OH, Fmoc-Gln(Dmcp)-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-His(Trt)-OH, Fmoc-Ile-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Val-OH. The following pseudoproline dipeptides were used: Fmoc-Ile-Thr($\Psi^{Me,Me}$pro)-OH (Novabiochem) and Fmoc-Met-Thr($\Psi^{Me,Me}$pro)-OH (**S-8**, synthesized in the laboratory).

Acid-labile protecting group removal.

Cocktail B. Peptides were subjected to Cocktail B (1 mL / 10 mg of peptide) consisting of trifluoroacetic acid (88% by volume), water (5% by volume), phenol (5% by weight), and *i*-Pr_3SiH (2% by volume). The resulting solution was triturated in ice-cold diethyl ether (3 x 15 mL) to give a white precipitate, which was centrifuged. The supernatant was discarded and the precipitate was solubilized in water/acetonitrile (1:1, 0.05% trifluoroacetic acid), lyophilized and the resulting solid was purified by HPLC.

Cocktail R. Peptides were subjected to Cocktail R (3 mL / 100 mg of peptide) consisting of trifluoroacetic acid (90% by volume), thioanisole (5% by volume), 1,2-ethanedithiol (3% by weight), and anisole (2% by volume). The resulting solution was triturated in ice-cold diethyl ether (3 x 15 mL) to give a white precipitate, which was centrifuged. The supernatant was discarded and the precipitate was solubilized in water/acetonitrile (1:1, 0.05% trifluoroacetic acid), lyophilized and the resulting solid was purified by HPLC.

HPLC. All separations involved a mobile phase of 0.05% TFA (v/v) in water (solvent A) / 0.04% TFA in acetonitrile (solvent B).

HPLC LC-MS analytical separations were performed using a Waters 2695 Separations Module and a Waters 2996 Photodiode Array Detector equipped with Varian Microsorb C18 column (150 x 2 mm) or Waters C8 X-Bridge column (150 x 2.1 mm) or Varian 300-5 C4 column (250 x 2 mm) at a flow rate of 0.2 mL/min.

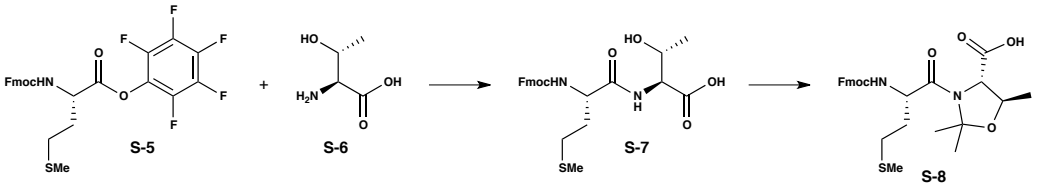
UPLC LC-MS analytical separations were performed using a Waters Acquity system equipped with an Acquity UPLC BEH C4 column (100 x 2.1 mm).

Preparatory HPLC separations were performed using a WATERS 2545 Binary Gradient Module equipped with a WATERS 2996 Photodiode Array Detector using either Microsorb 100-5 C18 column (250 x 21.4 mm), Microsorb 100-5 C8 column (250 x 21.4 mm) or Waters C8 X-Bridge column (150 x 19 mm) at a flow rate of 16 mL/min.

Native chemical ligation (NCL) buffer. The buffer required for native chemical ligation (NCL) was freshly prepared prior to the reaction. Na_2HPO_4 (56.6 mg, 0.4 mmol) was solubilized in water (1 mL), Guanidine·HCl (1.146 g, 12 mmol), and TCEP·HCl (10.8 mg, 0.04 mmol) were then added and solubilized. The pH was brought to 7 with a solution of NaOH (5 M, 20 μL). After 15 min degassing with argon, 4-mercaptophenylacetic acid (MPAA) (67 mg, 0.4 mmol) was added and the pH was brought to 7.2 with a solution of NaOH (5 M, 120 μL). After 15 min degassing the solution was ready for use.

Glycan anomeric amine installation (Kochetkov reaction). Oligosaccharide was dissolved in water (5 mL) and added to $(NH_4)HCO_3$ (6 g, BioUltra, 99.5% (T), Cat. No. 09830 Fluka). The resultant slurry was warmed to 40 °C and stirred very slowly at this temperature for three days. After three days, the clear supernatant was filtered through a plug of cotton. The remaining material was rinsed with the same amount of cold water (2 x 5 mL), filtered, pooled with the clear supernatant, immediately frozen and lyophilized. The lyophilization was deemed complete when the mass of the product remained constant. This provided the glycosyl amine as a white solid (quantitative). Oligosaccharides were stored at room temperature on the lyophilizer.

Experimental Procedures: Peptides and Glycopeptides.



Fmoc-Met-Thr($\Psi^{Me,Me}$pro)-OH (S-8).[8] L-Threonine (**S-6**) (1.03 g, 8.7 mmol) was dissolved in a minimal volume of aqueous sodium carbonate (10% w/v) at pH 9 (9 mL), and the solution was added to a suspension of Fmoc-Met-OPfp (**S-5**) (1.55 g, 2.9 mmol) in acetone (23 mL). After vigorous stirring for 3 h, the reaction mixture was cooled to 0 ºC and acidified with 1 N HCl to pH ~1. The solution was then concentrated *in vacuo* to less than half of the initial volume and ethyl acetate (100 mL) and water (60 mL) were added. The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 60 mL). The combined organic extracts were washed with water (30 mL) and brine (2 × 30 mL), dried over $MgSO_4$, filtered and evaporated to dryness. The residue was crystallized from ethyl acetate/hexane to give Fmoc-Met-Thr-OH (**S-7**) as a white solid.

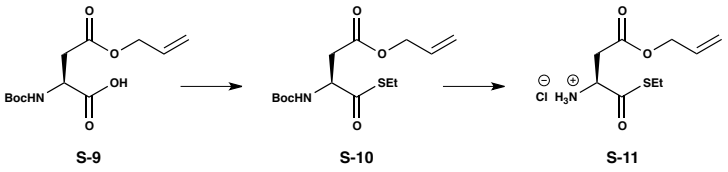
Dipeptide **S-7** (2.88 mmol) was then suspended in dry THF (55 mL), and pyridyl toluene-4-sulfonate (145 mg, 0.58 mmol) and 2,2-dimethoxypropane (1.8 mL, 14.4 mmol) were added. The suspension was then heated to reflux overnight under Ar, the condensate being bypassed over molecular sieves (4 Å). After cooling, triethylamine was added (120 µL, 0.86 mmol) and the mixture was evaporated to dryness. The residue was taken up in ethyl acetate (100 mL), and washed with water (2 × 50 mL). The aqueous layer was extracted with ethyl acetate (2 × 60 mL) and the combined organics were dried over $MgSO_4$, filtered and concentrated. The residue was purified by flash chromatography (20:1 to 10:1 CH_2Cl_2/MeOH) to give the desired pseudoproline dipeptide Fmoc-Met-Thr($\Psi^{Me,Me}$pro)-OH (**S-8**) (1.3 g, 88% yield) as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79–7.69 (m, 3H), 7.62–7.46 (m, 3H), 7.44–7.33 (m, 3H), 7.33–7.23 (m, 4H), 5.86 (d, *J* = 8.8 Hz, 1H), 4.48–4.33 (m, 3H), 4.33–4.23 (m, 3H), 4.21–4.10 (m, 2H), 2.60–2.41 (m, 3H), 2.09 (s, 3H), 2.01–1.84 (m, 3H), 1.67 (s, 3H), 1.59 (s, 3H), 1.49 (d, *J* = 6.0 Hz, 3H), 1.42 (d, *J* = 5.9 Hz, 1H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 172.2, 170.0, 156.2, 143.7, 143.2, 141.1, 141.0, 127.6, 127.0, 125.1, 125.0, 119.81, 119.79, 97.2, 74.9, 72.7, 67.5, 65.5, 52.8, 46.8, 33.6, 29.8, 26.2, 23.4, 19.9, 15.4.

LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{27}H_{32}N_2O_6SNa$) requires 535.6, found 535.3; m/z calc'd for $[M+K]^+$ ($C_{27}H_{32}N_2O_6SK$) requires 551.7, found 551.2.

(8) General procedure for pseudoproline synthesis: Wöhr, T.; Wahl, F.; Nefzi, A.; Rohwedder, B.; Sato, T.; Sun, X.; Mutter, M. *J. Am. Chem. Soc.* **1996**, *118*, 9218–9227.



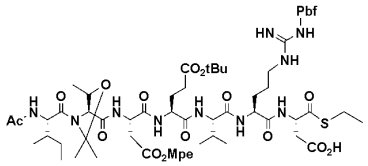
S-9 S-10 S-11

H-Asp(OAll)-SEt·HCl (S-11). Boc-Asp(OAll)-OH (**S-9**) (2.73 g, 10 mmol) was solubilized in dichloromethane (50 mL). To this solution EDC (1.77 mL, 10 mmol), HOBt (4.05 g, 30 mmol) and ethanethiol (3.6 mL, 50 mmol) were added. The mixture was stirred for 3 h 30 min, concentrated *in vacuo* and purified by flash chromatography (10–15% EtOAc/hexanes) to afford after concentration and lyophilization Boc-Asp(OAll)-SEt (**S-10**) (1.11 g, 3.5 mmol, 35% yield) as a white solid.

Boc-Asp(OAll)-SEt (454 mg, 1.4 mmol) was directly solubilized in a solution of HCl in dioxane (4 M, 24 mL). After 1 h 30 min at room temperature, the solution was concentrated *in vacuo*, resuspended in water and lyophilized twice to afford H-Asp(OAll)-SEt·HCl (**S-11**) as white solid (373 mg, 1.4 mmol, quantitative yield).

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.83 (br s, 3H), 5.91 (ddt, J = 17.3, 10.7, 5.5 Hz, 1H), 5.33 (apparent dq, J = 17.3, 1.6 Hz, 1H), 5.24 (apparent dq, J = 10.5, 1.4 Hz, 1H), 4.60 (apparent dq, J = 5.5, 1.3 Hz, 2H), 4.45 (t, J = 5.7 Hz, 1H), 3.13 (dd, J = 17.5, 5.4 Hz, 2H), 3.08 (dd, J = 17.5, 6.1 Hz, 1H), 3.00–2.90 (m, 2H), 1.19 (t, J = 7.3 Hz, 3H).

^{13}C NMR (150 MHz, DMSO-d_6) δ 195.2, 168.4, 132.1, 118.3, 65.4, 54.9, 35.1, 23.3, 14.4.



Protected *N*-terminal fragment (S-12). Upon completion of automated synthesis on 0.2 mmol of Fmoc-Arg(Pbf)-NovaSynTGT resin, the peptide-resin was subjected to acetylation. The peptide-resin was washed with DMF into a peptide synthesis vessel and treated with acetic anhydride (366 µL, 4 mmol), DIEA (768 µL, 4.4 mmol) in DMF (4 mL) for 25 min. The peptide-resin was then washed with DMF, dichloromethane and methanol. After drying, the resin was subjected to a cleavage cocktail (1:1:8 of acetic acid/trifluoroethanol/methylene chloride) 3 times for 30 min. The resulting portions of cleavage solution were pooled and concentrated at room temperature. The oily residue was resuspended in a minimum amount of trifluoroethanol and precipitated with water. The resulting mixture was immediately lyophilized to afford the peptide as white solid (175 mg, 73% yield).

To a solution of this peptide (157 mg, 130 µmol) in chloroform (10 mL) was added EDC (57.6 µL, 325.4 µmol), HOOBt (51.5 mg, 315.7 µmol) and finally H-Asp(OAll)-SEt·HCl (**S-11**) (96 mg, 378.3 µmol). The mixture was stirred for 1 h 30 min at room temperature. After concentration, the oily residue was resuspended in a minimum amount of trifluoroethanol and precipitated with water containing 0.05% trifluoroacetic acid. The resulting mixture was immediately lyophilized. The peptide was solubilized in chloroform (10 mL), then $Pd(PPh_3)_4$ (93.5 mg, 80.9 µmol) was added, followed by phenylsilane (75.7 µL, 614.2 µmol). The reaction was stirred in the dark for 20 min. After concentration, the oily residue was resuspended in a minimum amount of trifluoroethanol and diluted in water/acetonitrile (1:1, 0.05% trifluoroacetic acid). The resulting mixture was immediately lyophilized. The lyophilized mixture was resuspended in water/acetonitrile (1:1, 0.05% trifluoroacetic acid) and pre-purified on Sephadex LH-20 equilibrated with water/acetonitrile (1:1, 0.05% trifluoroacetic acid). The peptide-containing fractions were pooled and immediately lyophilized. The pre-purified peptide was solubilized in water/acetonitrile (1:1, 0.05% trifluoroacetic acid) and purified to homogeneity by RP-HPLC (C4 semiprep, 40% to 85% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 18 min. Lyophilization of the collected fractions provided peptide **S-12** (77 mg, 43% yield) as a white solid.

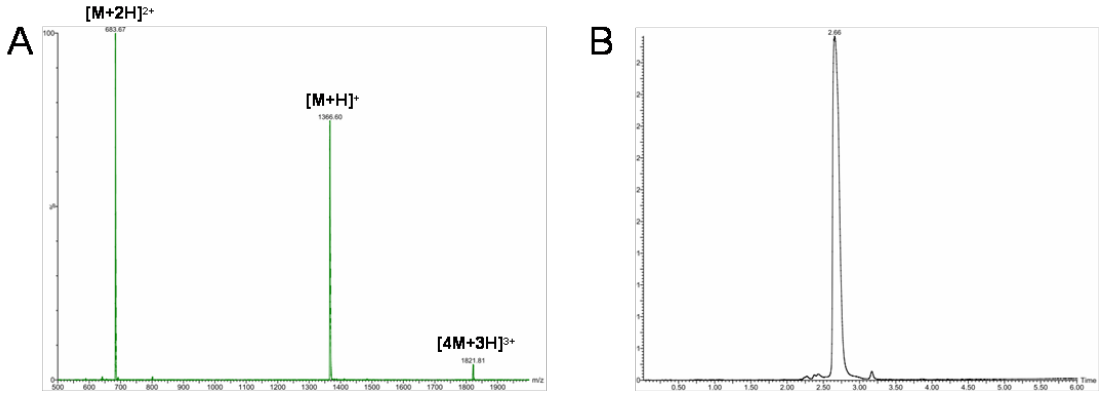
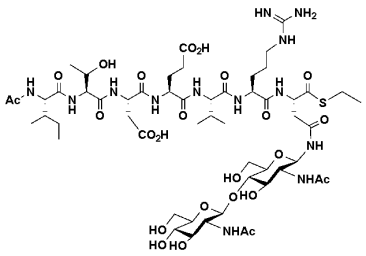


Figure S1: A - ESI-MS of compound **S-12**. ESI calculated for $C_{64}H_{104}N_{10}O_{18}S_2$ $[M+H]^+$ *m/z*: 1366.7, found: 1366.6; $[M+2H]^{2+}$ *m/z*: 683.85, found: 683.67; $[4M+3H]^{3+}$ *m/z*: 1821.93, found: 1821.81. **B -** UV trace from UPLC analysis of purified compound **S-12**; gradient: 50% to 95% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column.



GlcNAc$_2$ *N*-terminal fragment (S-13). Peptide **S-12** (15 mg, 11 μmol) and chitobiose anomeric amine (13 mg, 30.7 μmol) were combined and solubilized in anhydrous DMSO (343 μL). To this mixture, a freshly prepared solution of PyAOP in anhydrous DMSO (0.5 mg/μL, 15.6 μL, 15 μmol) was added, followed by DIEA (4 μL, 23 μmol). The solution turned a deep, golden-yellow color and this was stirred for 30 min. The reaction mixture was then frozen and lyophilized.

The protected glycopeptide was then subjected to Cocktail B for 1 h 15 min, precipitated, centrifuged, resuspended and lyophilized as described in the general procedure. The crude peptide was purified to homogeneity by RP-HPLC (C18 semiprep, 10% to 35% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 18.4 min. Lyophilization of the collected fractions provided peptide **S-13** (8 mg, 54% yield) as a white solid.

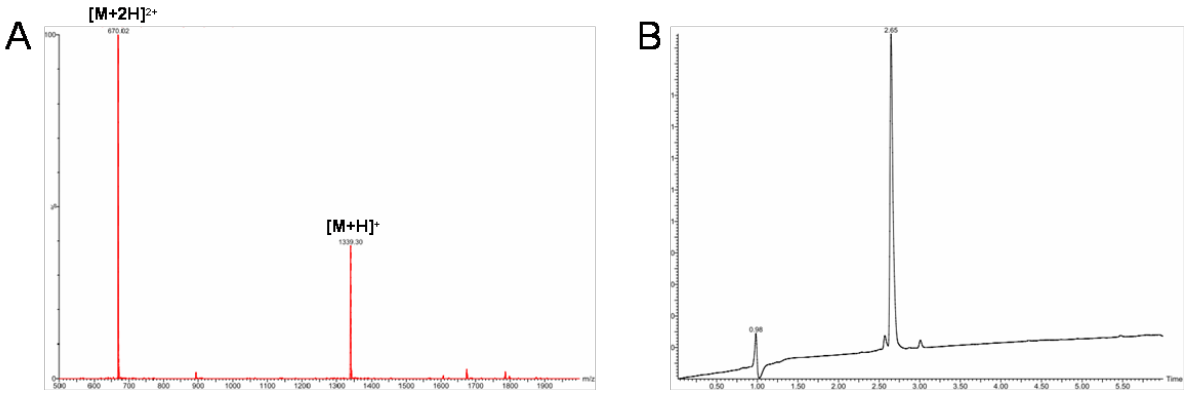
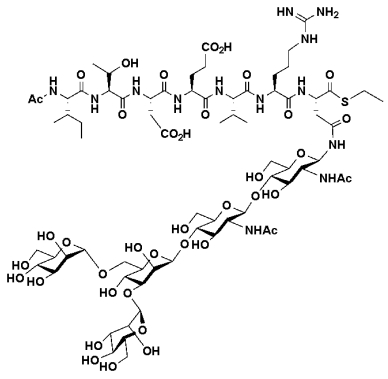


Figure S2: A - ESI-MS of compound **S-13**. ESI calculated for $C_{54}H_{91}N_{13}O_{24}S$ $[M+H]^+$ *m/z*: 1339.44, found: 1339.30; $[M+2H]^{2+}$ *m/z*: 670.02, found: 670.22. **B -** UV trace from UPLC analysis of purified compound **S-13**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C18 column.



$Man_3GlcNAc_2$ *N*-terminal fragment (S-14). Peptide **S-12** (50.4 mg, 36.9 μmol, 1.2 equiv) and glycosyl amine **20** (28 mg, 30.8 μmol, 1 equiv) were combined and solubilized in anhydrous DMSO (288 μL). To this mixture, a freshly prepared solution of PyAOP in anhydrous DMSO (288 μL, 0.25 mg/μL, 138.6 μmol, 4.5 equiv) was added, followed by DIEA (22.2 μL, 127.7 μmol, 4.1 equiv). The solution turned a deep, golden-yellow color and this was stirred for 30 min. The reaction mixture was then frozen and lyophilized.

The glycopeptide was then subjected to Cocktail B (1.5 mL) for 1 h 15 min. The peptide was precipitated, centrifuged, resuspended and lyophilized as described in the general procedure. The resulting solid was purified to homogeneity by RP-HPLC (C18 semiprep, 10% to 35% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 16.27 min. Lyophilization of the collected fractions provided peptide **S-14** (20.6 mg, 37% yield) as a white solid.

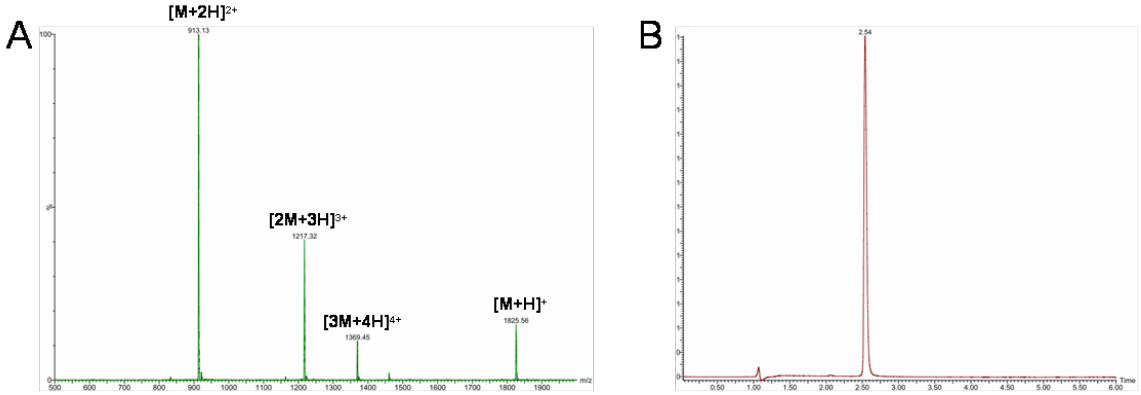
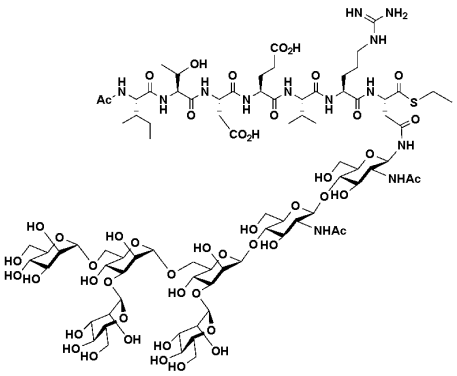


Figure S3: A - ESI-MS of compound **S-14**. ESI calculated for $C_{72}H_{121}N_{13}O_{39}S$ $[M+2H]^{2+}$ *m/z*: 913.43, found: 913.13; $[2M+3H]^{3+}$ *m/z*: 1217.57, found: 1217.32; $[3M+4H]^{4+}$ *m/z*: 1369.64, found: 1369.45. **B -** UV trace from UPLC analysis of purified compound **S-14**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C18 column.



$Man_5GlcNAc_2$ *N*-terminal fragment (S-15). Peptide **S-12** (37.8 mg, 27.7 μmol, 1.2 equiv) and glycosyl amine **18** (28.4 mg, 22.2 μmol, 1 equiv) were combined and solubilized in anhydrous DMSO (216 μL). To this mixture, a freshly prepared solution of PyAOP in anhydrous DMSO (216 μL, 0.25 mg/μL, 104 μmol, 4.5 equiv) was added, followed by DIEA (16.6 μL, 95.5 μmol, 4.1 equiv). The solution turned a deep, golden-yellow color and this was stirred for 30 min. The reaction mixture was then frozen and lyophilized.

The glycopeptide was then subjected to Cocktail B (1.5 mL) for 1 h 15 min. The peptide was precipitated, centrifuged, resuspended and lyophilized as described in the general procedure. The resulting solid was purified to homogeneity by RP-HPLC (C18 semiprep, 10% to 35% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 15.95 min. Lyophilization of the collected fractions provided peptide **S-15** (21.1 mg, 44% yield) as a white solid.

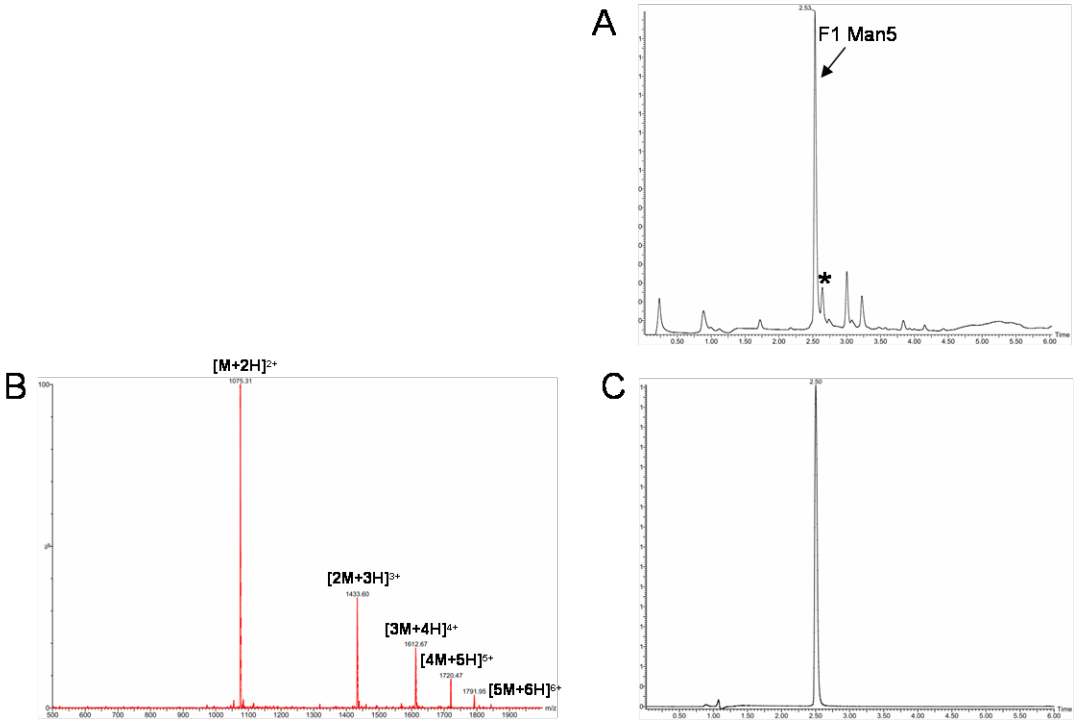
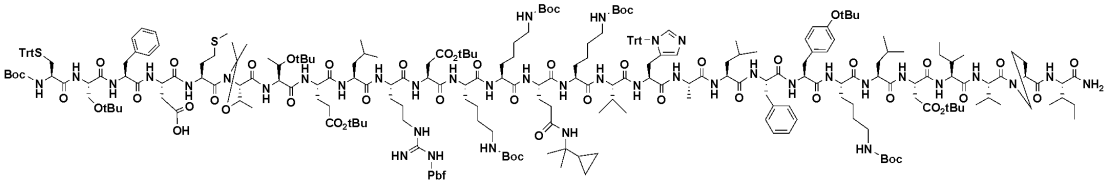


Figure S4: A - UV trace from UPLC analysis of the crude mixture obtained after one-flask aspartylation/deprotection. The star (*) indicates a side product of identical mass, presumably due to epimerization of the thioester; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C18 column. **B** - ESI-MS of compound **S-15**. ESI calculated for $C_{84}H_{141}N_{13}O_{49}S$ $[M+2H]^{2+}$ *m/z*: 1075.57, found: 1075.31; $[2M+3H]^{3+}$ *m/z*: 1433.76, found: 1433.60; $[3M+4H]^{4+}$ *m/z*: 1612.85, found: 1612.67; $[4M+5H]^{5+}$ *m/z*: 1720.31, found: 1720.47; $[5M+6H]^{6+}$ *m/z*: 1791.91, found: 1791.95. **C -** UV trace from UPLC analysis of purified compound **S-15**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C18 column.



Protected *C*-terminal fragment (S-16). Upon completion of automated synthesis on 0.05 mmol of TG Sieber resin, the peptide-resin was subjected to deallylation. The peptide-resin was washed with a mixture of dichloromethane/DMF (1:1) into a peptide synthesis vessel and treated with $Pd(PPh_3)_4$ (5 mg, 4.3 μmol, 0.086 equiv) and phenylsilane (50 μL, 0.4 mmol, 8.6 equiv) in dichloromethane/DMF (1:1, 2.5 mL). After 20 min, the $Pd(PPh_3)_4$/phenylsilane treatment was repeated once. The peptide-resin was then washed with DMF, dichloromethane and methanol. After drying, the peptide-resin was subjected to a cleavage cocktail (1:99 of trifluoracetic acid/methylene chloride, 2 mL) 5 times for 5 min, (2:98 of trifluoracetic acid/methylene chloride, 2 mL) 5 times for 5 min, and (3:97 of trifluoracetic acid/methylene chloride, 2 mL) 5 times for 5 min. The resulting portions of cleavage solution were systematically pooled in cold diethyl ether and concentrated. The oily residue was resuspended in a minimum amount of trifluoroethanol and precipitated with water. The resulting mixture was immediately lyophilized to give peptide **S-16** as a white solid (150 mg). The peptide was used without further purification.

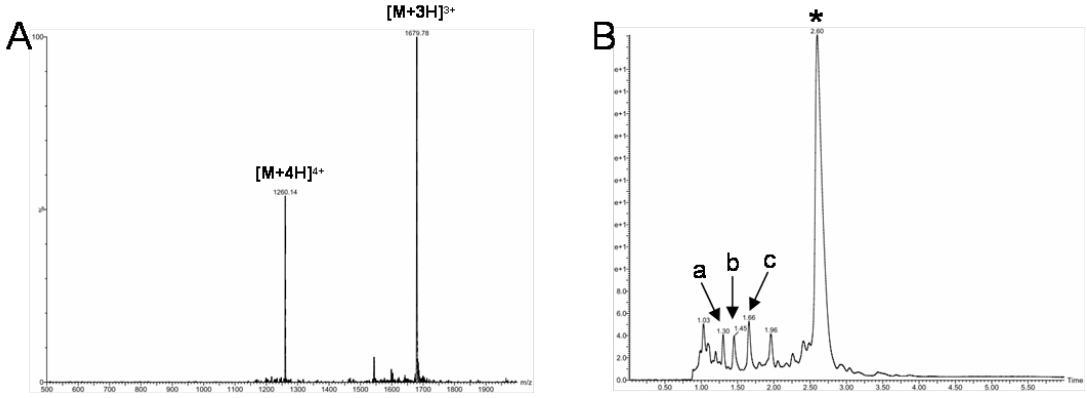
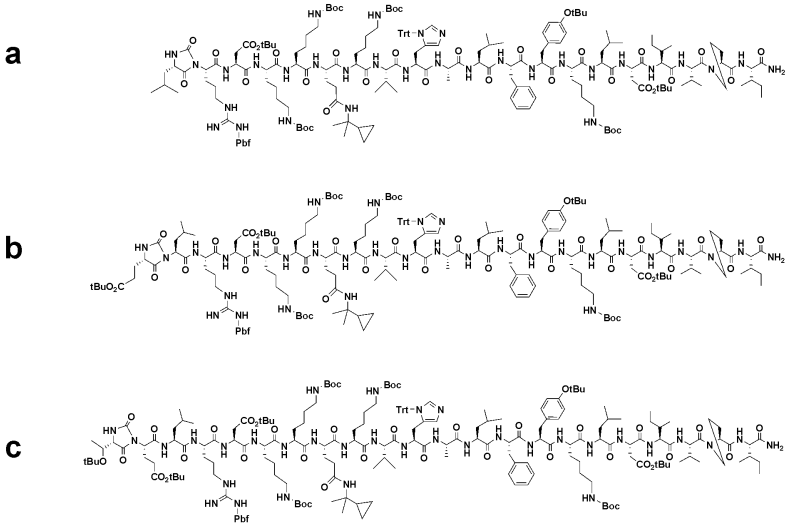
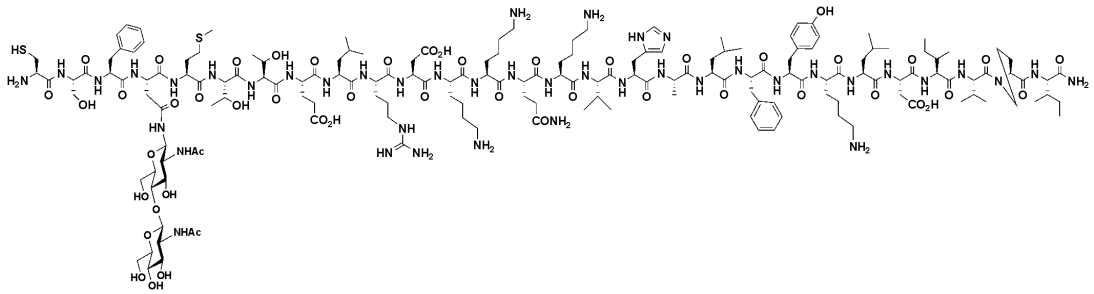


Figure S5: A - ESI-MS of compound **S-16**. ESI calculated for $C_{547}H_{858}N_{104}O_{146}S_8$ $[M+3H]^{3+}$ *m/z*: 1259.8 , found: 1260.1; $[M+4H]^{4+}$ *m/z*: 1679.4, found: 1679.8. **B -** UV trace from UPLC analysis of compound **S-16**; The star (*) indicates product **S-16**, **a-c** correspond to capped truncation products with the presumed structures shown below; gradient: 85% to 99% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column.





GlcNAc$_2$ *C*-terminal fragment (S-17). Peptide **S-16** (40 mg, 7.95 μmol, 1 equiv) and chitobiose anomeric amine (10.4 mg, 24.6 μmol, 3 equiv) were combined and solubilized in anhydrous DMSO (643 μL). To this mixture, a freshly prepared solution of PyAOP in anhydrous DMSO (0.5 mg/μL) was added (23.2 μL, 22.2 μmol, 2.8 equiv), followed by DIEA (3.2 μL, 18.5 μmol, 2.3 equiv). The solution turned a deep, golden-yellow color and this was stirred for 30 min. The reaction was then quenched by the addition of 1.5 mL of ice-cold water + 0.05% trifluoracetic acid. The precipitate formed was isolated by centrifugation, resuspended in water/acetonitrile (1:1, 0.05% trifluoroacetic acid) and immediately lyophilized.

The dry solid was then subjected to Cocktail R for 1 h 30 min. The peptide was precipitated, centrifuged, and lyophilized. The crude peptide was purified to homogeneity by RP-HPLC (C8 semiprep, 25% to 55% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 12.2 min. Lyophilization of the collected fractions provided peptide **S-17** (7.1 mg, 25% yield) as a white solid.

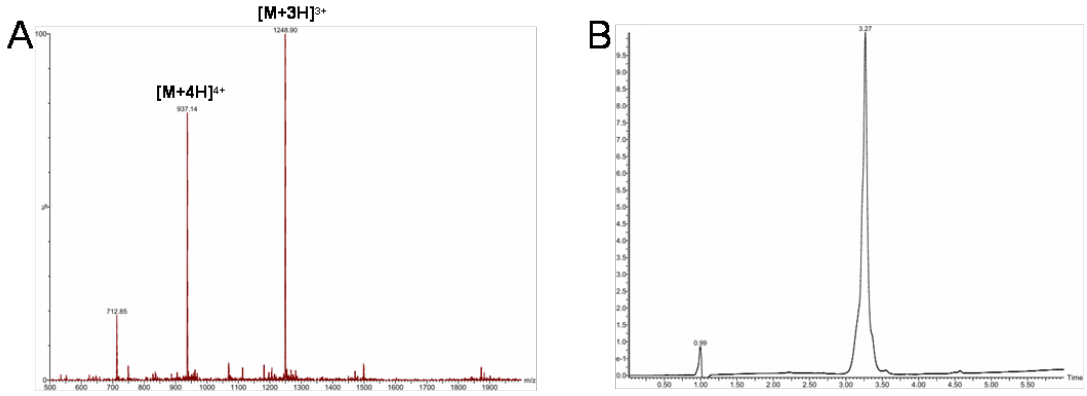
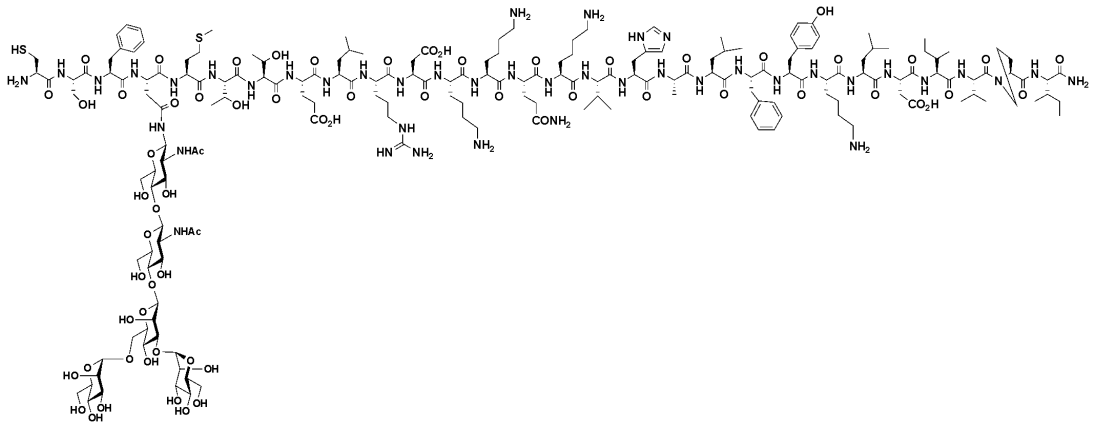


Figure S6: A - ESI-MS of compound **S-17**. ESI calculated for $C_{168}H_{272}N_{42}O_{50}S_2$ $[M+4H]^{4+}$ *m/z*: 937.1, found: 937.1; $[M+3H]^{3+}$ *m/z*: 1249.1, found: 1248.9. **B -** UV trace from UPLC analysis of purified compound **S-17**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column.



$Man_3GlcNAc_2$ *C*-terminal fragment (S-18). Peptide **S-16** (45.4 mg, 9 μmol, 1 equiv) and glycosyl amine **20** (10.8 mg, 11.86 μmol, 1.3 equiv) were combined and solubilized in anhydrous DMSO (300 μL). To this mixture, a freshly prepared solution of PyAOP in anhydrous DMSO (2.7 mg/μL) was added (50 μL, 25.6 μmol, 2.8 equiv), followed by DIEA (3.9 μL, 22.6 μmol, 2.5 equiv). The solution turned a deep, golden-yellow color and this was stirred for 30 min. The reaction was quenched by addition of 1.5 mL of ice-cold water + 0.05% trifluoracetic acid. The precipitate formed was isolated by centrifugation, resuspended in water/acetonitrile (1:1, 0.05% trifluoroacetic acid) and immediately lyophilized.

The glycopeptide was then subjected to Cocktail R (3 mL) for 1 h 30 min. The peptide was precipitated, centrifuged, resuspended and desalted by size exclusion chromatography (Bio-Gel P-6, fine, acetonitrile/water (2:8, 0.05% trifluoroacetic acid)). The crude peptide was purified to homogeneity by RP-HPLC (C8 X-bridge semiprep, 20% to 40% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 12.9 min. Lyophilization of the collected fractions provided peptide **S-18** (11.9 mg, 31% yield) as a white solid.

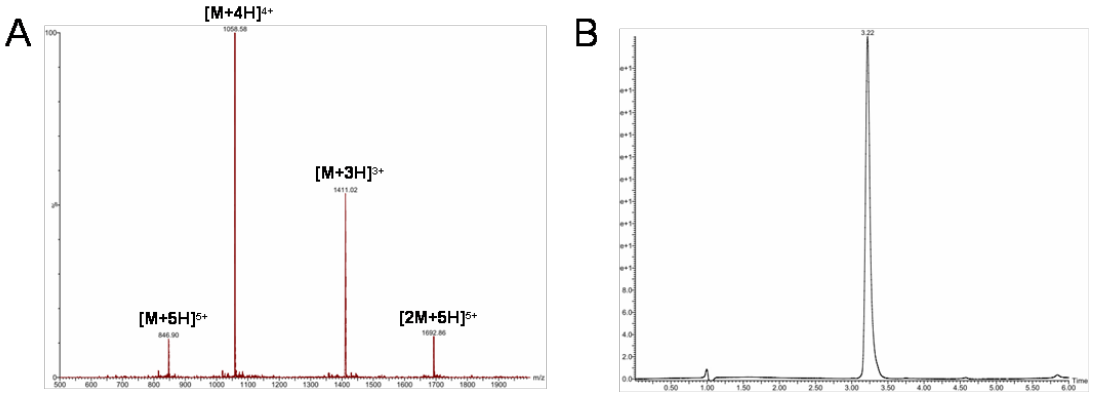
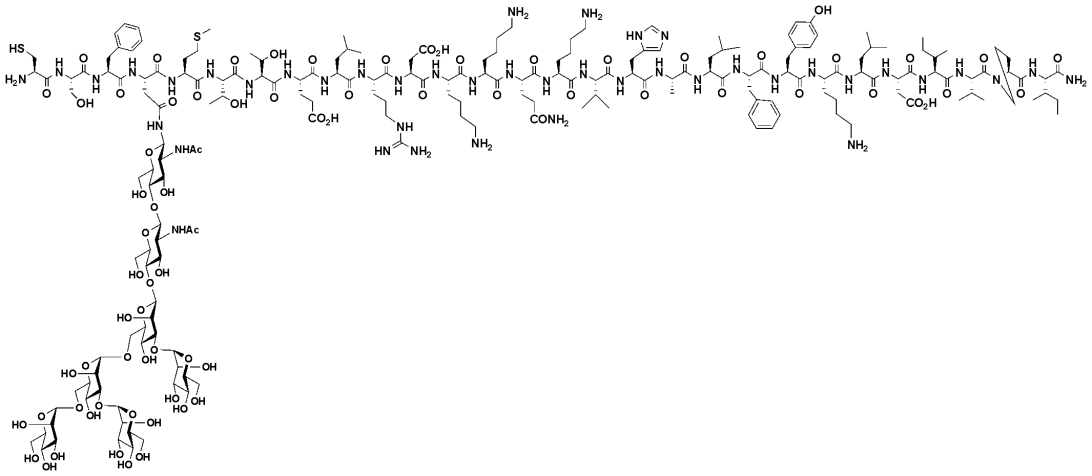


Figure S7: A - ESI-MS of compound **S-18**. ESI calculated for $C_{186}H_{302}N_{42}O_{65}S_2$ $[M+5H]^{5+}$ *m/z*: 847.15, found: 846.9; $[M+4H]^{4+}$ *m/z*: 1058.69, found: 1058.58; $[M+3H]^{3+}$ *m/z*: 1411.25, found: 1411.02; $[2M+5H]^{5+}$ *m/z*: 1693.3, found: 1692.86. **B -** UV trace from UPLC analysis of purified compound **S-18**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column.



$Man_5GlcNAc_2$ *C*-terminal fragment (24). Peptide **S-16** (45.4 mg, 9 μmol, 1 equiv) and glycosyl amine **18** (14.6 mg, 11.9 μmol, 1.3 equiv) were combined and solubilized in anhydrous DMSO (300 μL). To this mixture, a freshly prepared solution of PyAOP in anhydrous DMSO (2.7 mg/μL) was added (50 μL, 25.6 μmol, 2.8 equiv), followed by DIEA (3.9 μL, 22.6 μmol, 2.5 equiv). The solution turned a deep, golden-yellow color and this was stirred for 30 min. The reaction was quenched by addition of 1.5 mL of ice-cold water + 0.05% trifluoracetic acid. The precipitate formed was isolated by centrifugation, resuspended in water/acetonitrile (1:1, 0.05% trifluoroacetic acid) and immediately lyophilized.

The glycopeptide was then subjected to Cocktail R (3 mL) for 1 h 30 min. The peptide was precipitated, centrifuged, resuspended and desalted by size exclusion chromatography (Bio-Gel P-6, fine, acetonitrile/water (2:8, 0.05% trifluoroacetic acid)). The crude peptide was purified to homogeneity by RP-HPLC (C8 X-bridge semiprep, 20% to 40% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 13.25 min. Lyophilization of the collected fractions provided peptide **24** (9 mg, 22% yield) as a white solid.

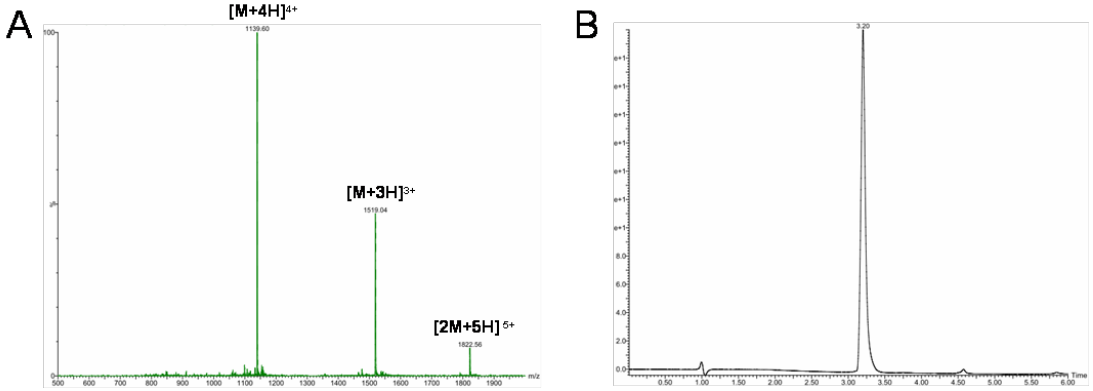
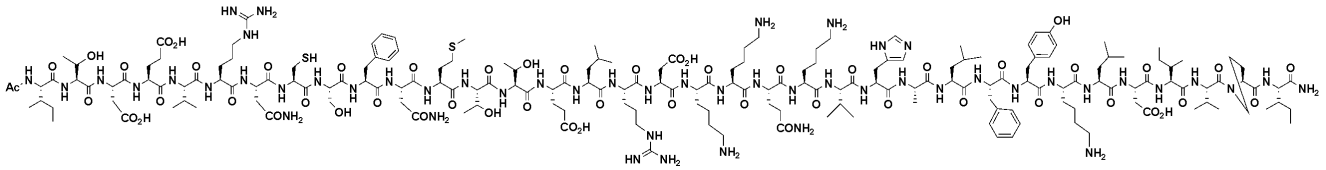


Figure S8: A - ESI-MS of compound **24**. ESI calculated for $C_{198}H_{322}N_{42}O_{75}S_2$ $[M+4H]^{4+}$ *m/z*: 1139.76, found:1139.60; $[M+3H]^{3+}$ *m/z*: 1519.34, found:1519.04; $[2M+5H]^{5+}$ *m/z*: 1823.01, found: 1822.56. **B -** UV trace from UPLC analysis of purified compound **24**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column.



Aglycone V1V2 (S-19).

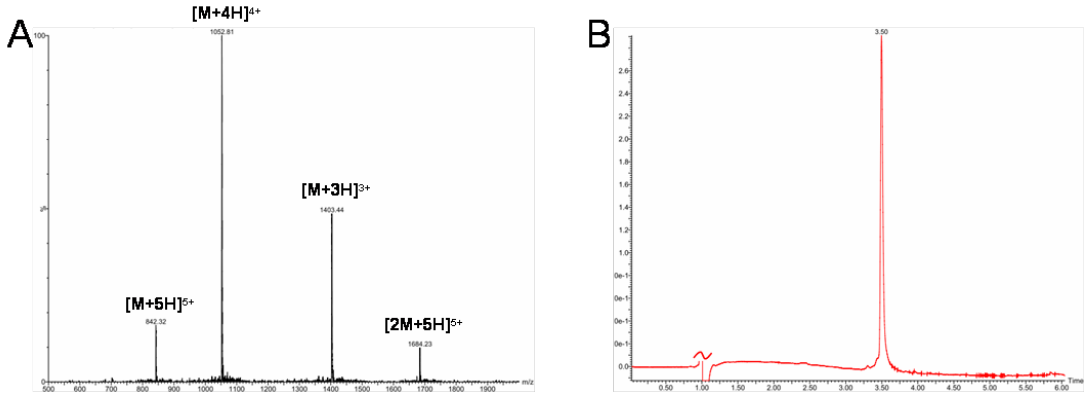
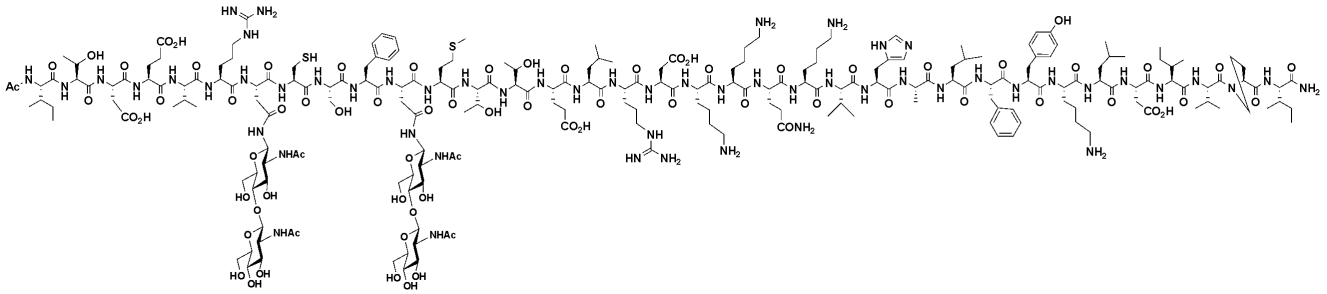


Figure S9: A - ESI-MS of compound **S-19**. ESI calculated for $C_{188}H_{305}N_{51}O_{54}S_2$ $[M+5H]^{5+}$ *m/z*: 842.6, found:842.3; $[M+4H]^{4+}$ *m/z*: 1053.0, found: 1052.8; $[M+3H]^{3+}$ *m/z*: 1403.6, found: 1403.4; $[2M+5H]^{5+}$ *m/z*: 1684.1, found: 1684.2. **B -** UV trace from UPLC analysis of purified compound **S-19**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column.



GlcNAc$_2$ V1V2 (3). Freshly purified *N*-terminal fragment **S-13** (8 mg, 5.98 μmol) and *C*-terminal fragment **S-17** (10 mg, 2.67 μmol) were combined and solubilized in NCL buffer (324 μL, 7 mM, prepared as described in general procedure). To this mixture was added neutral TCEP solution (0.5 M, 36 μL). After 2 h another portion of neutral TCEP solution (0.5 M, 36 μL) was added and the reaction was stirred for 3 h 30 min. After completion of the ligation, the mixture was diluted dropwise with water/acetonitrile (1:1, 0.05% trifluoroacetic acid) and desalted by size exclusion chromatography (Bio-Gel P-6, medium, acetonitrile/water (2:8, 0.05% trifluoroacetic acid)). The crude peptide was purified to homogeneity by RP-HPLC (C8 semiprep, 20% to 45% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 20.25 min. Lyophilization of the collected fractions provided **3** (4.2 mg, 34% yield) as a white solid.

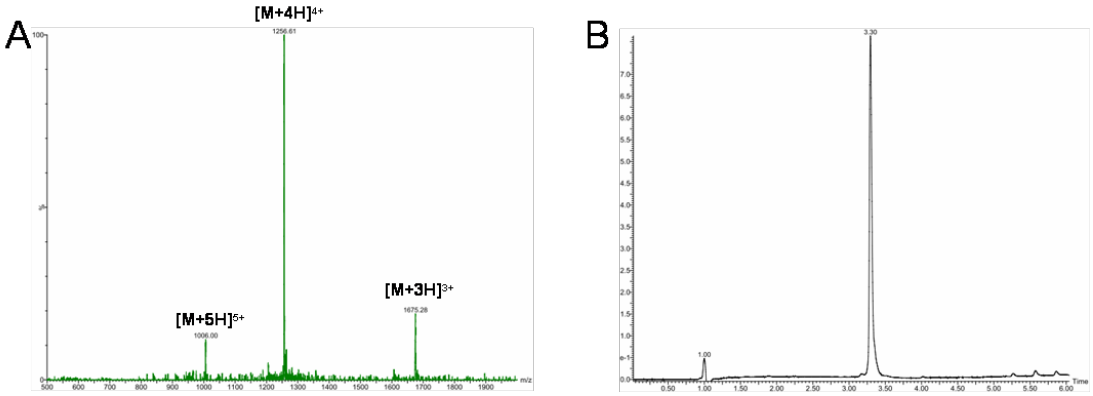
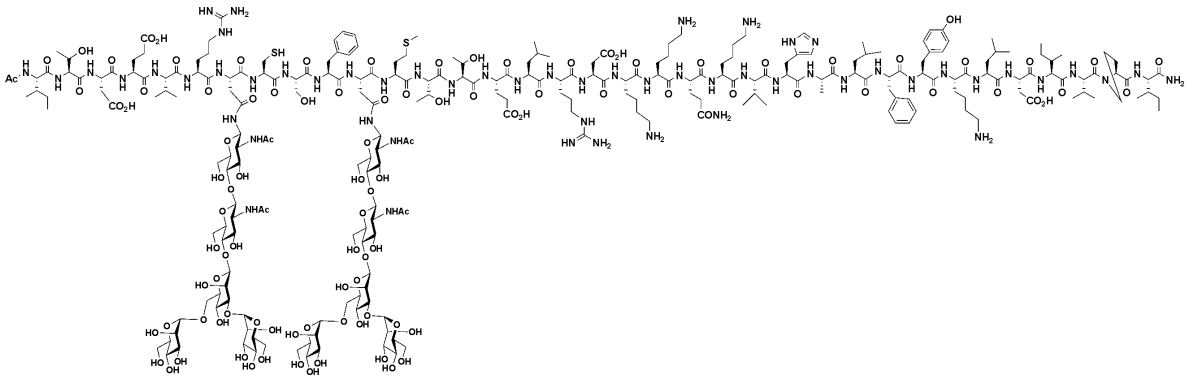


Figure S10: A - ESI-MS of compound **3**. ESI calculated for $C_{220}H_{357}N_{55}O_{74}S_2$ $[M+5H]^{5+}$ *m/z*: 1005.1, found: 1006.0; $[M+4H]^{4+}$ *m/z*: 1256.2, found: 1256.6; $[M+3H]^{3+}$ *m/z*: 1674.5, found: 1675.3. **B -** UV trace from UPLC analysis of purified compound **3**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column.



$Man_3GlcNAc_2$ V1V2 (2). Freshly purified *N*-terminal fragment **S-14** (9.7 mg, 5.3 μmol) and *C*-terminal fragment **S-18** (7.5 mg, 1.77 μmol) were combined and solubilized in NCL buffer (224 μL, 7 mM, prepared as described in general procedure). To this mixture was added neutral TCEP solution (0.5 M, 24 μL). After 2 h another portion of neutral TCEP solution (0.5 M, 24 μL) was added and the reaction was stirred for 6 h. After completion of the ligation, the mixture was diluted dropwise with water/acetonitrile (1:1, 0.05% trifluoroacetic acid) and desalted by size exclusion chromatography (Bio-Gel P-6, fine, acetonitrile/water (2:8, 0.05% trifluoroacetic acid)). The crude peptide was purified to homogeneity by RP-HPLC (C8 X-bridge semiprep, 20% to 40% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 14.78 min. Lyophilization of the collected fractions provided **2** (5 mg, 47% yield) as a white solid.

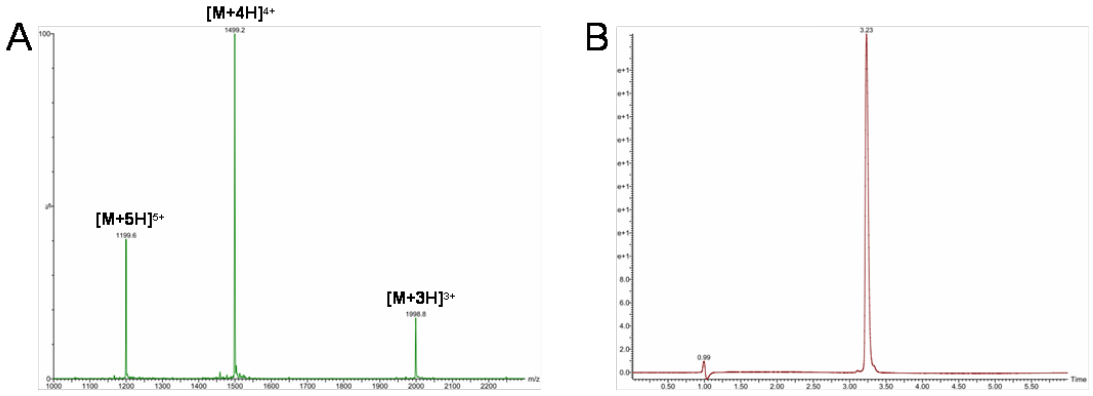
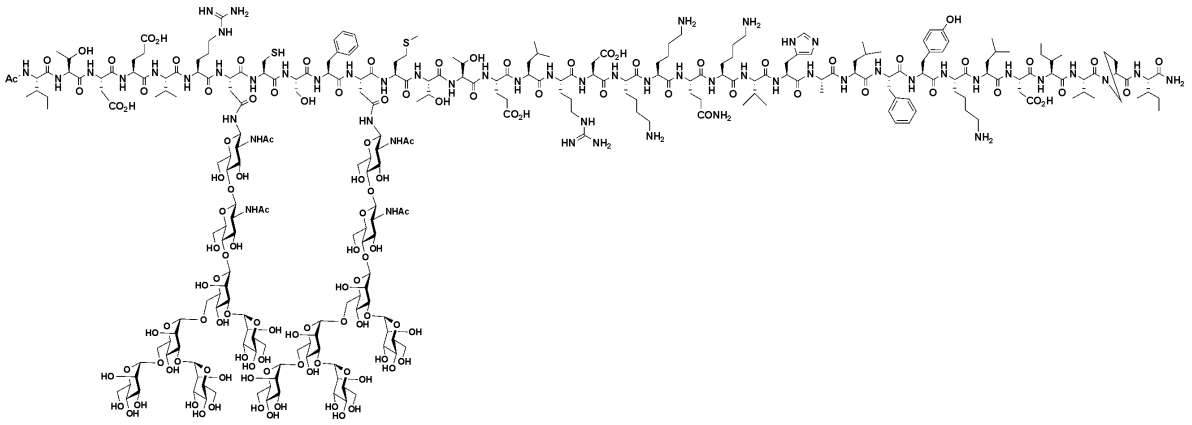


Figure S11: A - ESI-MS of compound **2**. ESI calculated for $C_{256}H_{417}N_{55}O_{104}S_2$ $[M+5H]^{5+}$ *m/z*: 1198.8, found: 1199.6; $[M+4H]^{4+}$ *m/z*: 1499.4, found: 1499.2; $[M+5H]^{5+}$ *m/z*: 1998.8, found: 1998.8. **B -** UV trace from UPLC analysis of purified compound **2**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column.



$Man_5GlcNAc_2$ V1V2 (1). Freshly purified *N*-terminal fragment **22** (11.4 mg, 5.3 μmol) and *C*-terminal fragment **24** (8.1 mg, 1.77 μmol) were combined and solubilized in NCL buffer (224 μL, 7 mM, prepared as described in general procedure). To this mixture was added neutral TCEP solution (0.5 M, 24 μL). After 2 h another portion of neutral TCEP solution (0.5 M, 24 μL) was added and the reaction was stirred for 6 h. After completion of the ligation, the mixture was diluted dropwise with water/acetonitrile (1:1, 0.05% trifluoroacetic acid) and desalted by size exclusion chromatography (Bio-Gel P-6, fine, acetonitrile/water (2:8, 0.05% trifluoroacetic acid)). The crude peptide was purified to homogeneity by RP-HPLC (C8 X-bridge semiprep, 20% to 40% acetonitrile/water over 30 min, 16 mL/min). Product eluted at 14.25 min. Lyophilization of the collected fractions provided **1** (6.5 mg, 55% yield) as a white solid.

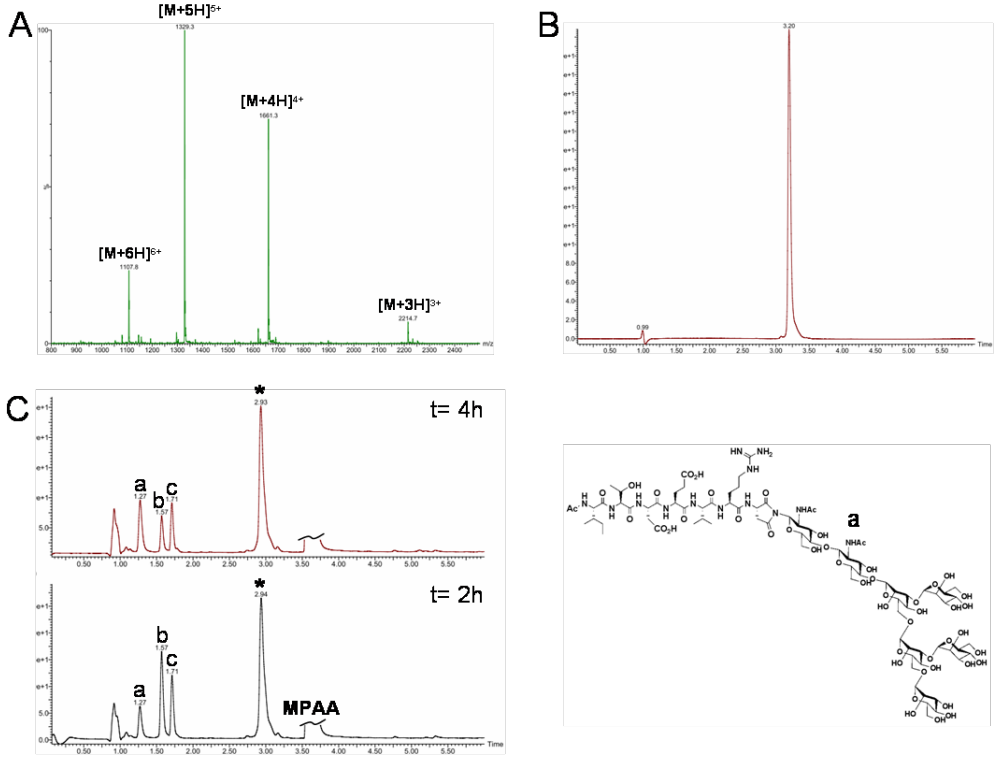


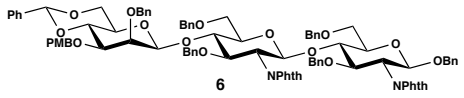
Figure S12: A - ESI-MS of compound **1**. ESI calculated for $C_{280}H_{457}N_{55}O_{124}S_2$ $[M+6H]^{6+}$ *m/z*: 1108.0, found: 1107.8; $[M+5H]^{5+}$ *m/z*: 1329.4, found: 1329.3; $[M+4H]^{4+}$ *m/z*: 1661.5, found: 1661.3; $[M+3H]^{3+}$ *m/z*: 2215.0, found: 2214.7. **B -** UV trace from UPLC analysis of purified compound **1**; gradient: 10% to 60% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C4 column. **C -** UV trace from UPLC analysis of the native chemical ligation performed to access **1** (*); peak **a** corresponds to the cyclized product shown above, peak **b** corresponds to **22**, and peak **c** corresponds to the transthioesterification product of **22** with MPAA; gradient: 20% to 55% acetonitrile/water over 6 min at a flow rate of 0.3 mL/min, BEH C8 column.

Surface Plasmon Resonance. V1V2 glycopeptide binding K_d and rate constant measurements were carried out on a BIAcore 3000 instrument using an anti-human Ig Fc capture assay as described earlier.[9] Anti-human IgG Fc antibody (Sigma Chemicals) was immobilized on a CM5 sensor chip to about 10000 response units (RU), and each antibody was captured to about 300 RU. Anti-RSV Synagis mAb was captured on the same sensor chip as a control surface. Non-specific binding and drift in signal was double referenced by subtracting binding to the control surface and blank buffer flow for each of the peptide binding interactions. V1V2 glycopeptides were injected at concentrations ranging from 1 to 40 μg/mL as indicated in Figure 2 of the manuscript. All curve-fitting analyses were performed using global fit of multiple titrations to the 1:1 Langmuir model. All data analysis was performed using the BIAevaluation 4.1 analysis software (GE Healthcare).

(9) Alam, S. M.; McAdams, M.; Boren, D.; Rak, M.; Scearce, R. M.; Gao, F.; Camacho, Z. T.; Gewirth, D.; Kelsoe, G.; Chen, P.; Haynes, B. F. *J. Immunol.* **2007**, *178*, 4424–4435.

Selected NMR Spectra

^{1}H NMR (600 MHz, $CDCl_3$)



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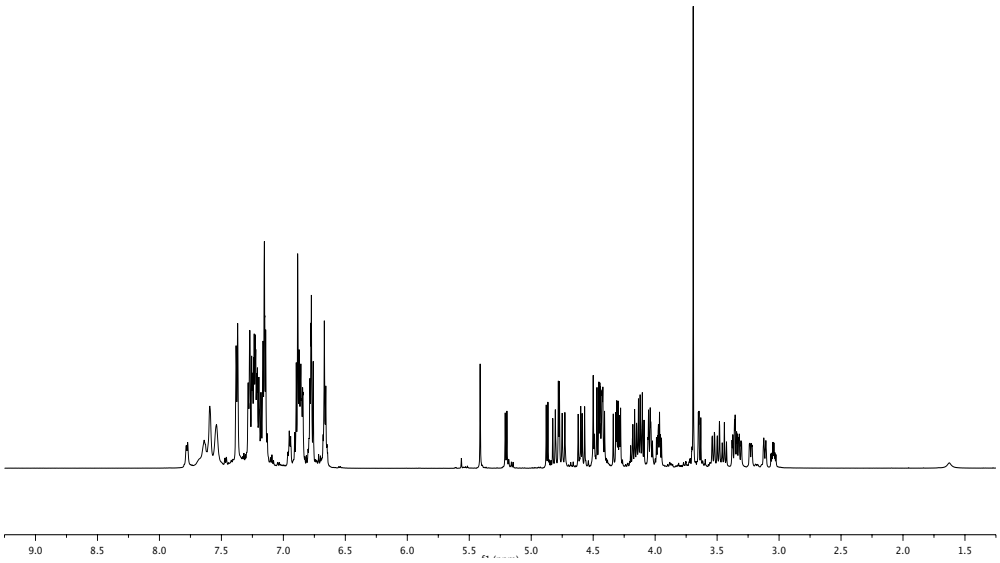
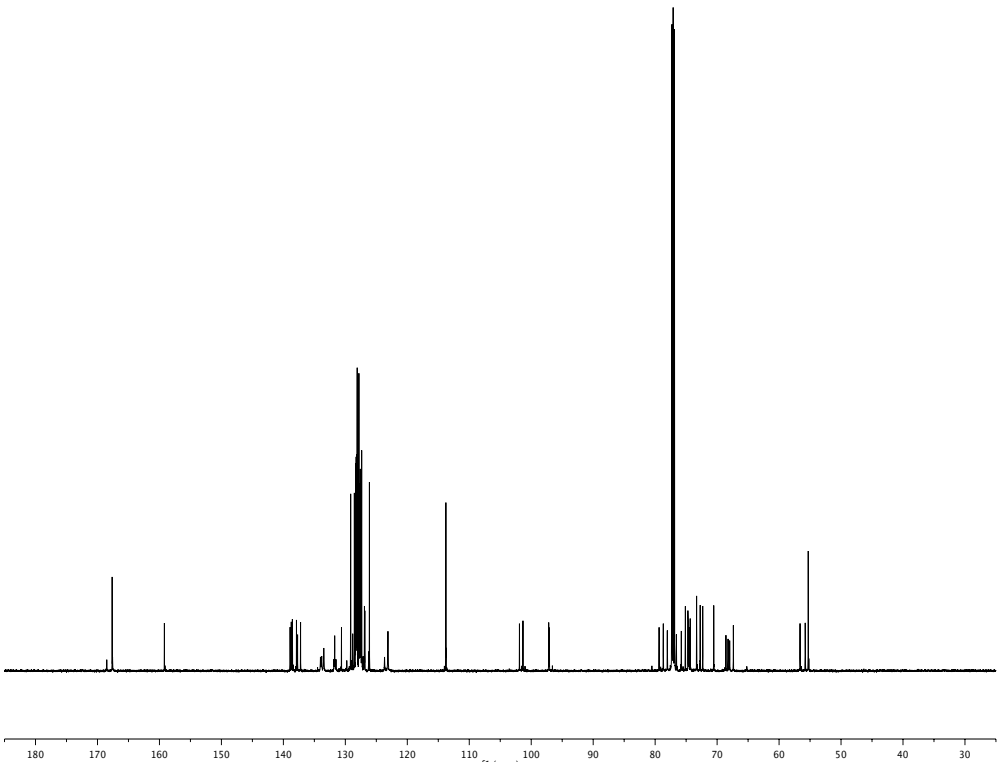


^{13}C NMR (150 MHz, $CDCl_3$)



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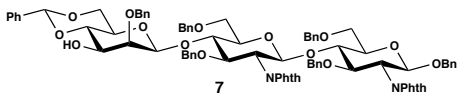

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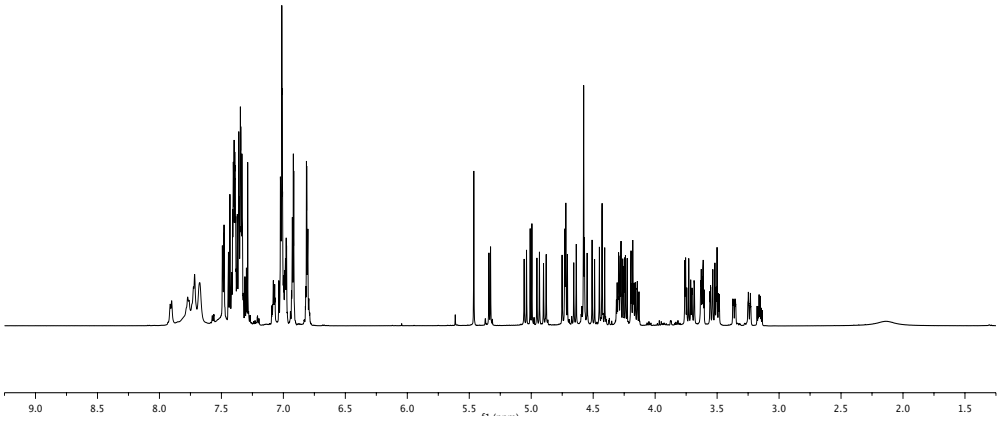

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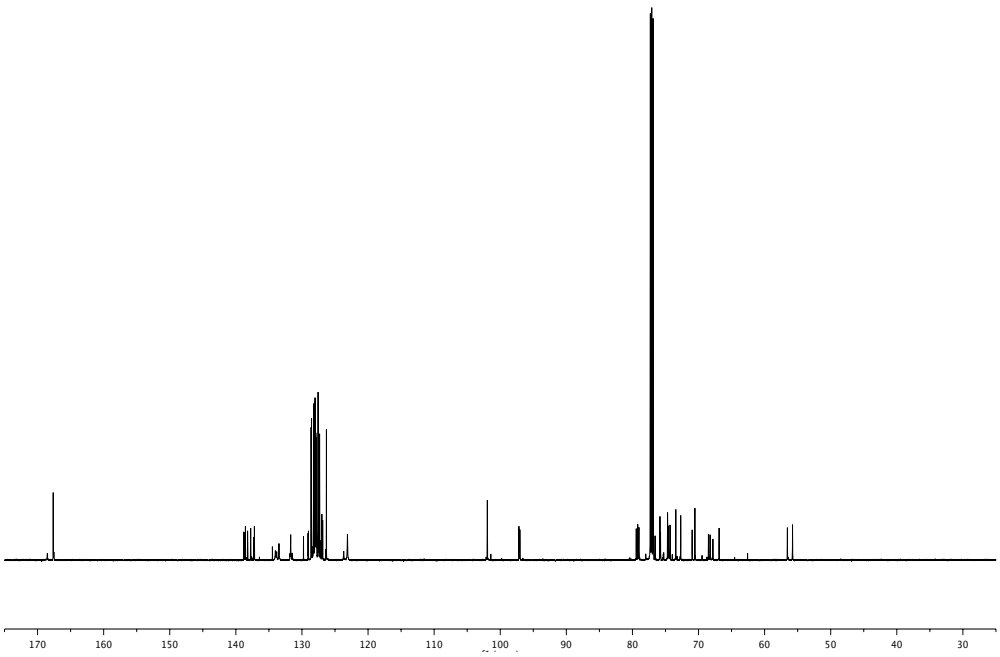
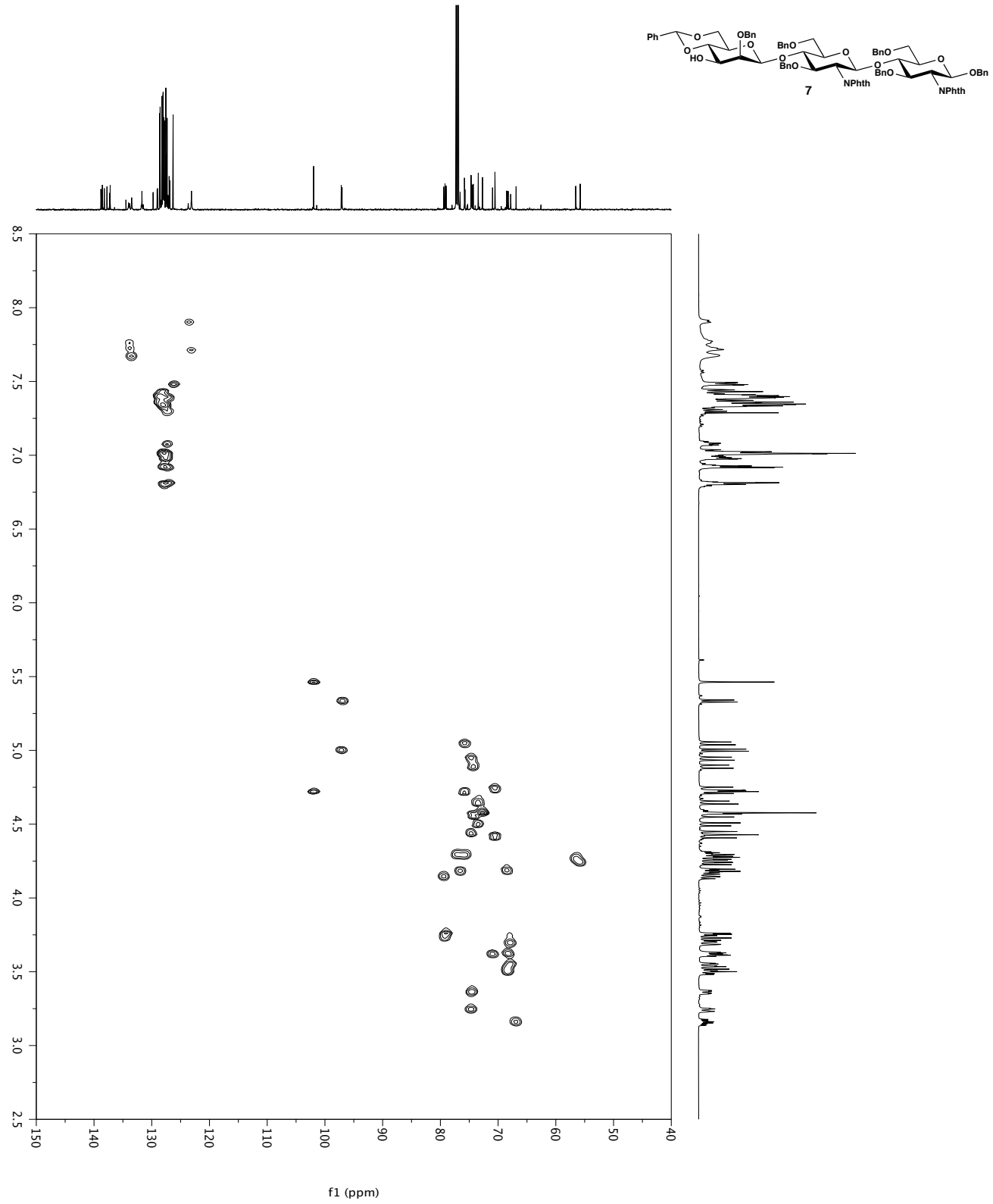
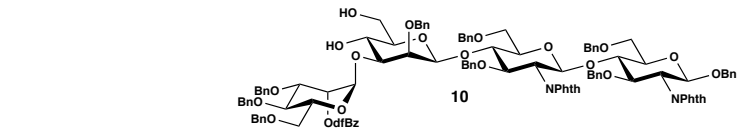


HSQC (600 MHz in F2, $CDCl_3$)



^{1}H NMR (600 MHz, $CDCl_3$)



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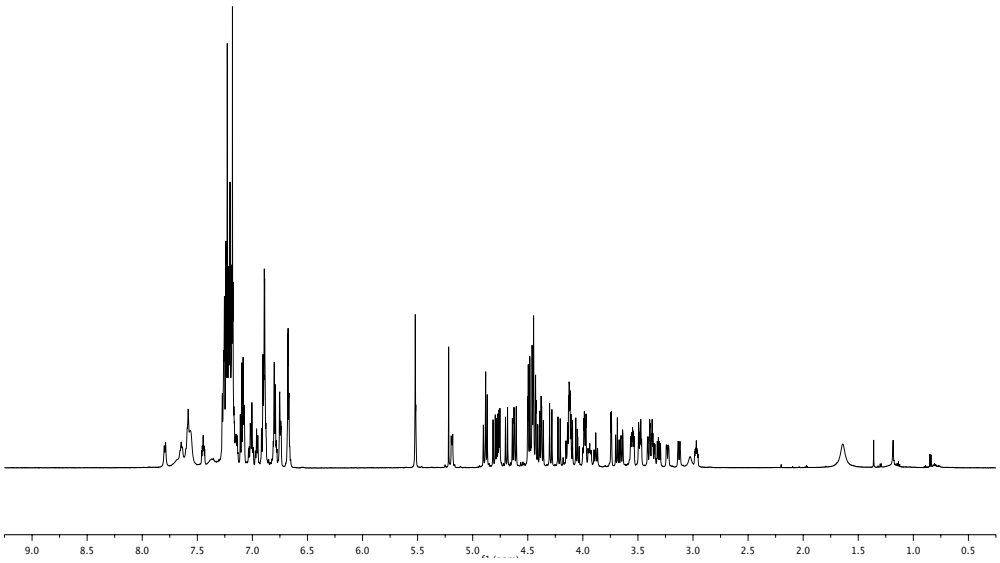

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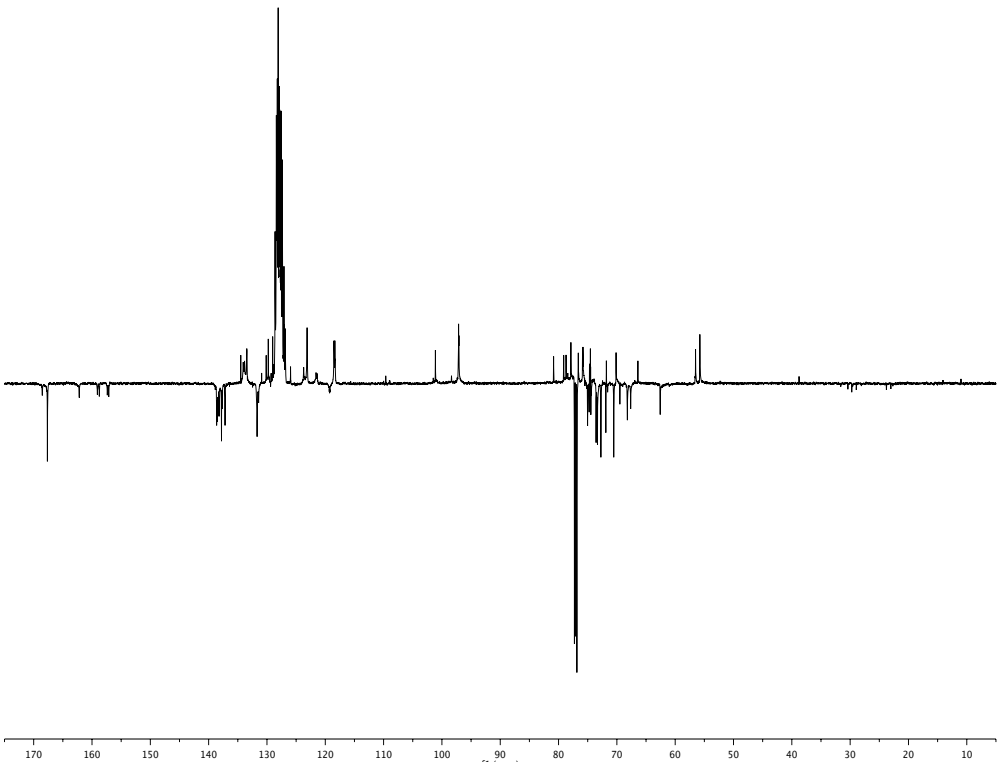
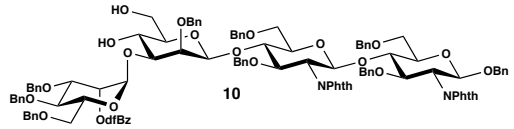


HSQC (600 MHz in F2, $CDCl_3$)



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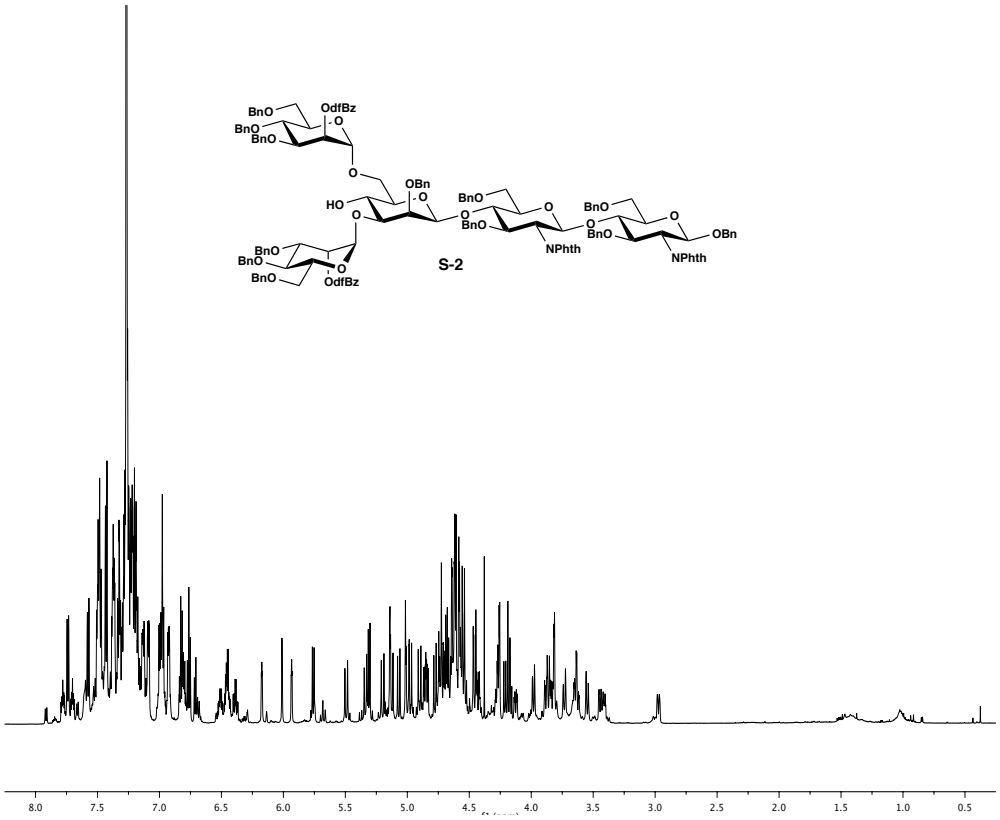
^{1}H NMR (600 MHz, C_6D_6)



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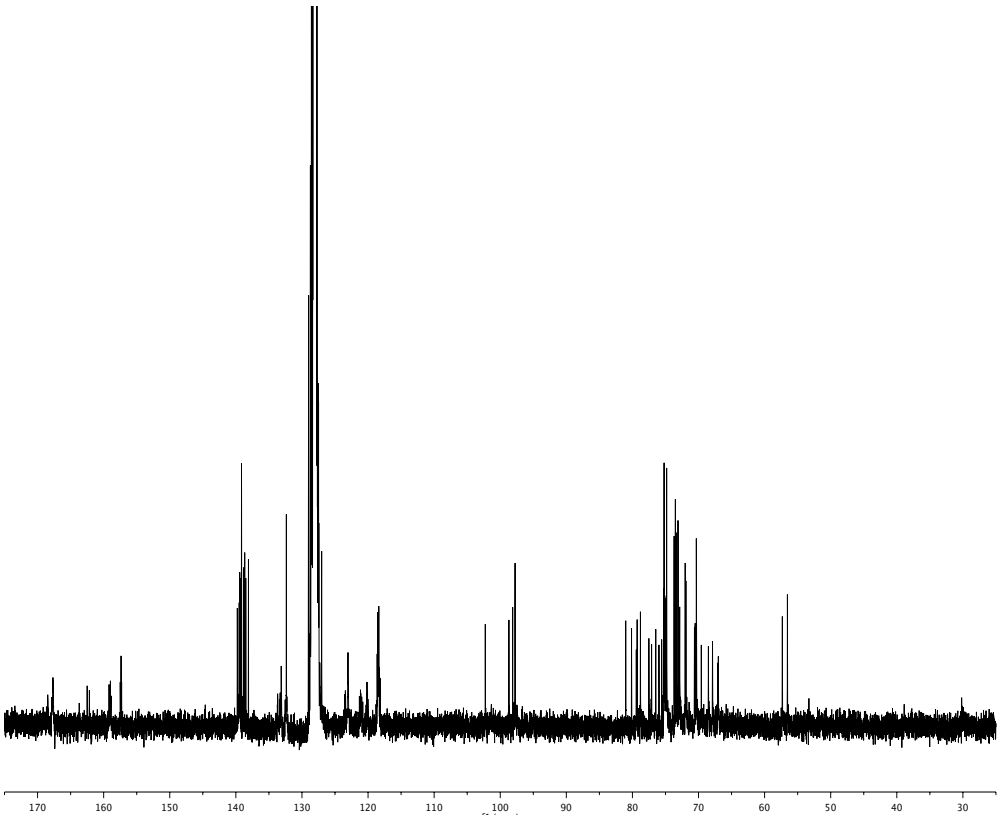
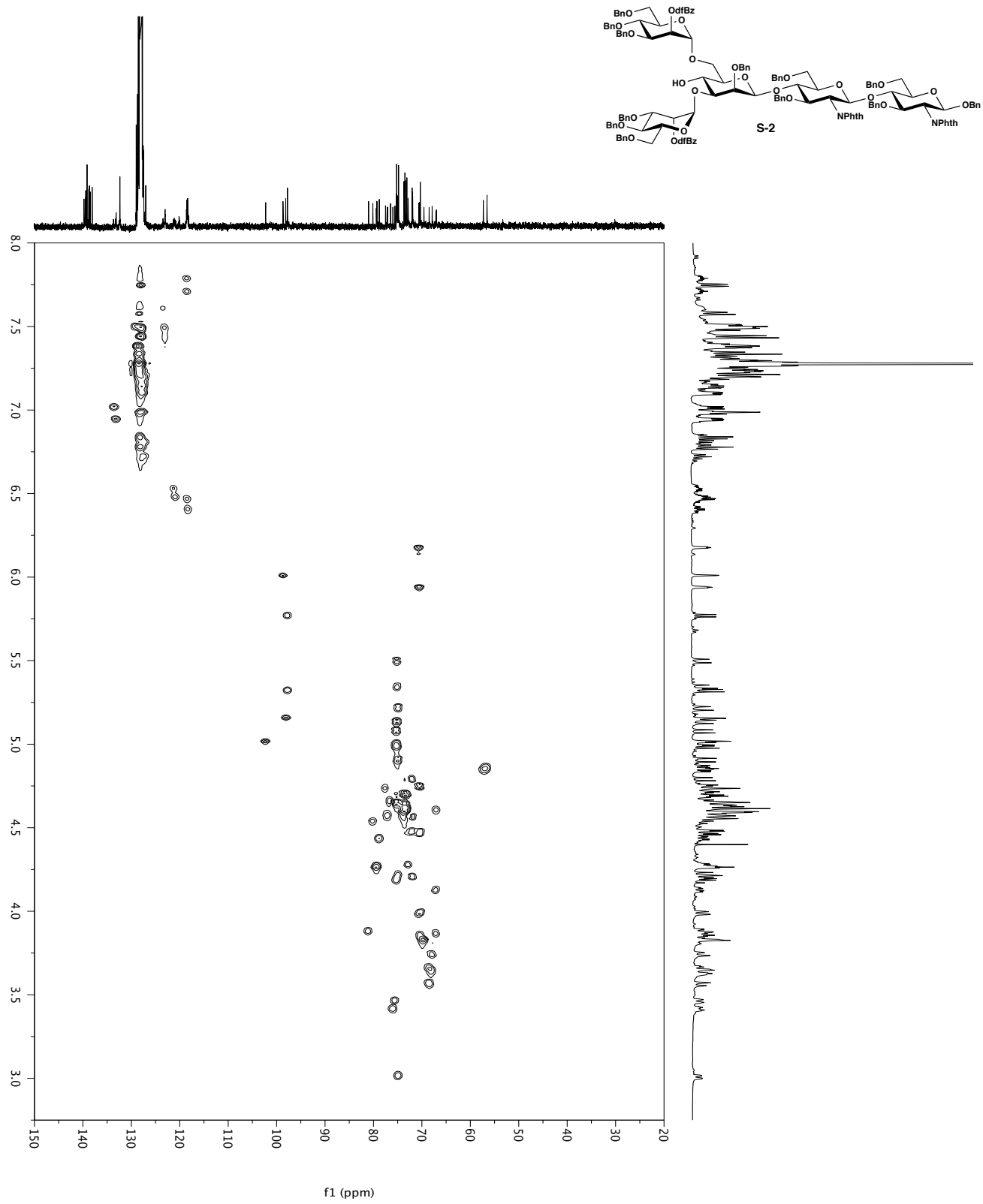


HSQC (600 MHz in F2, C_6D_6)



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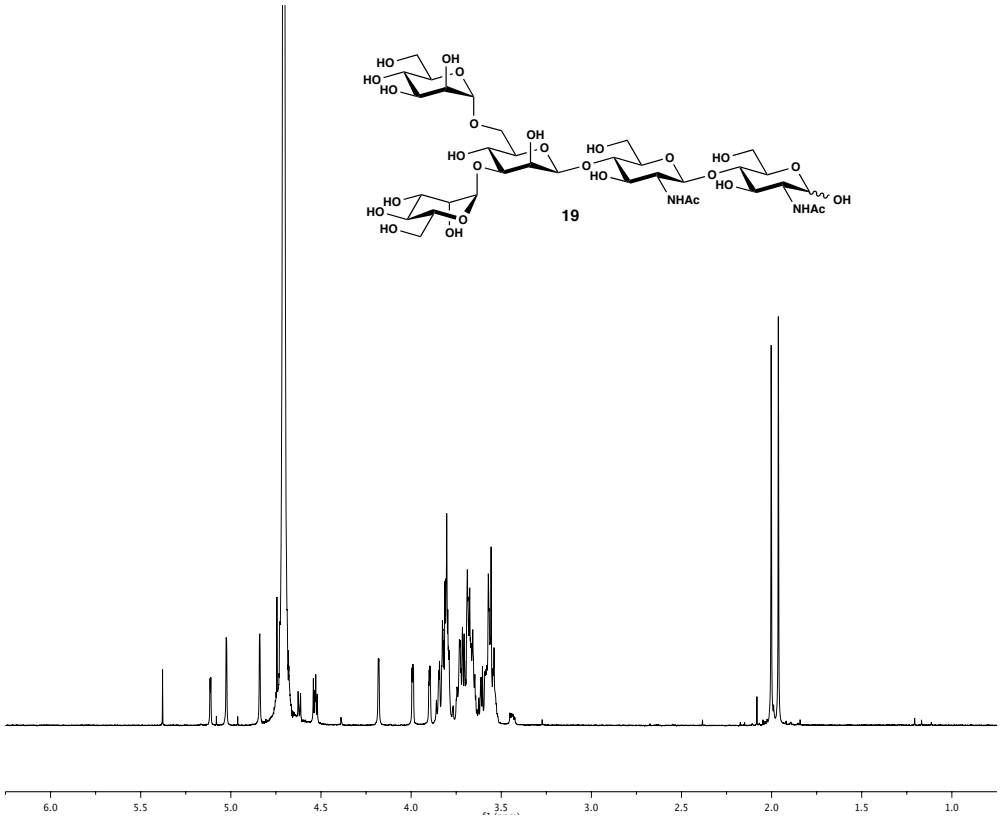

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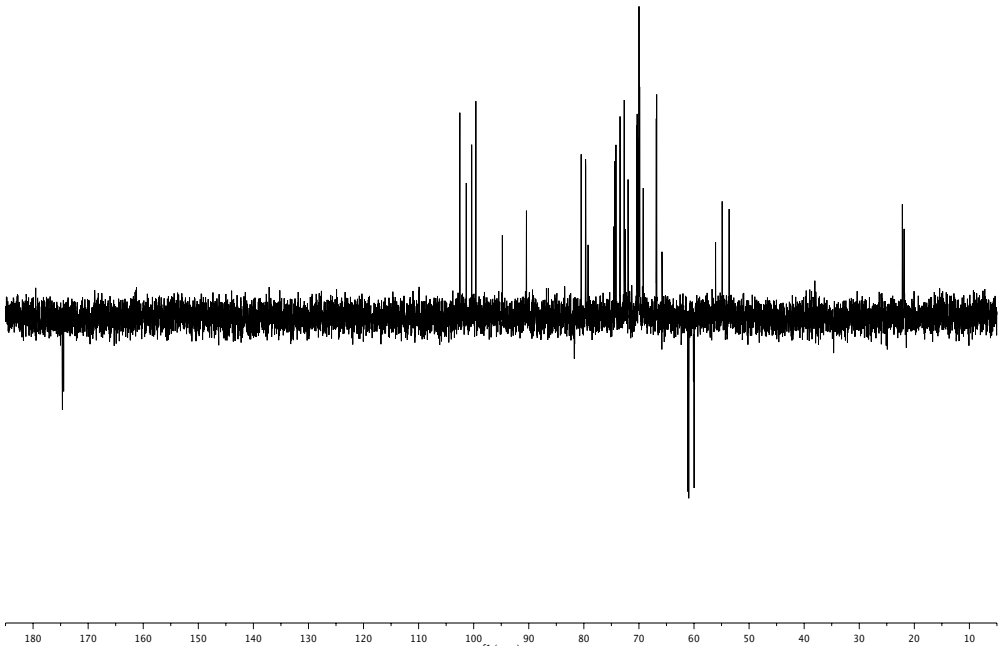

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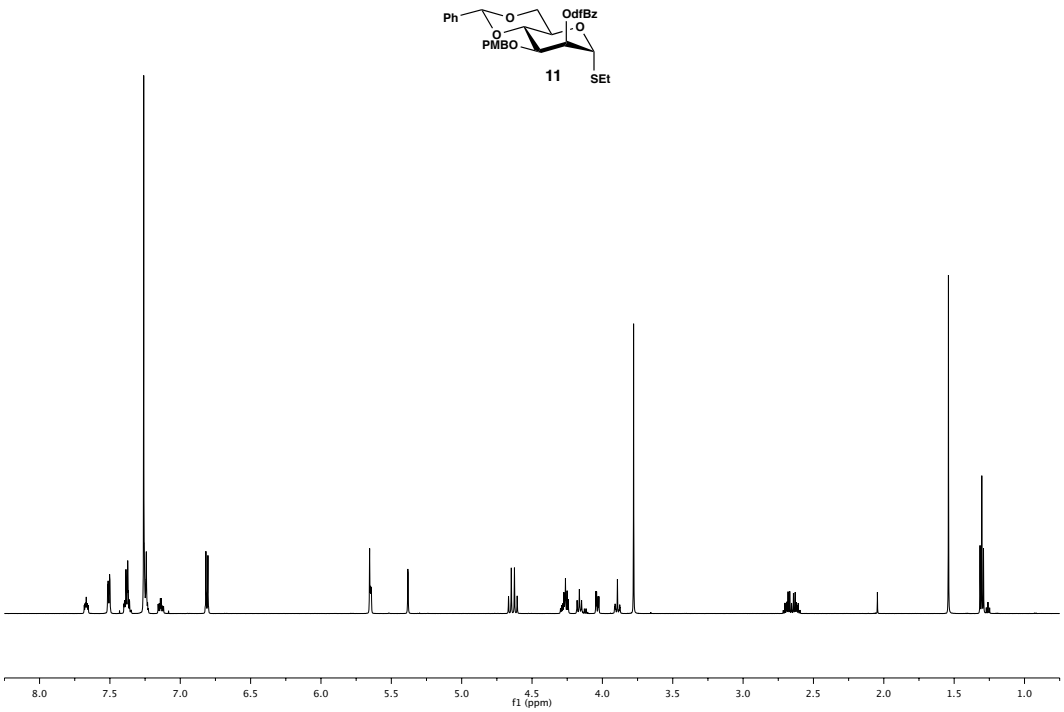

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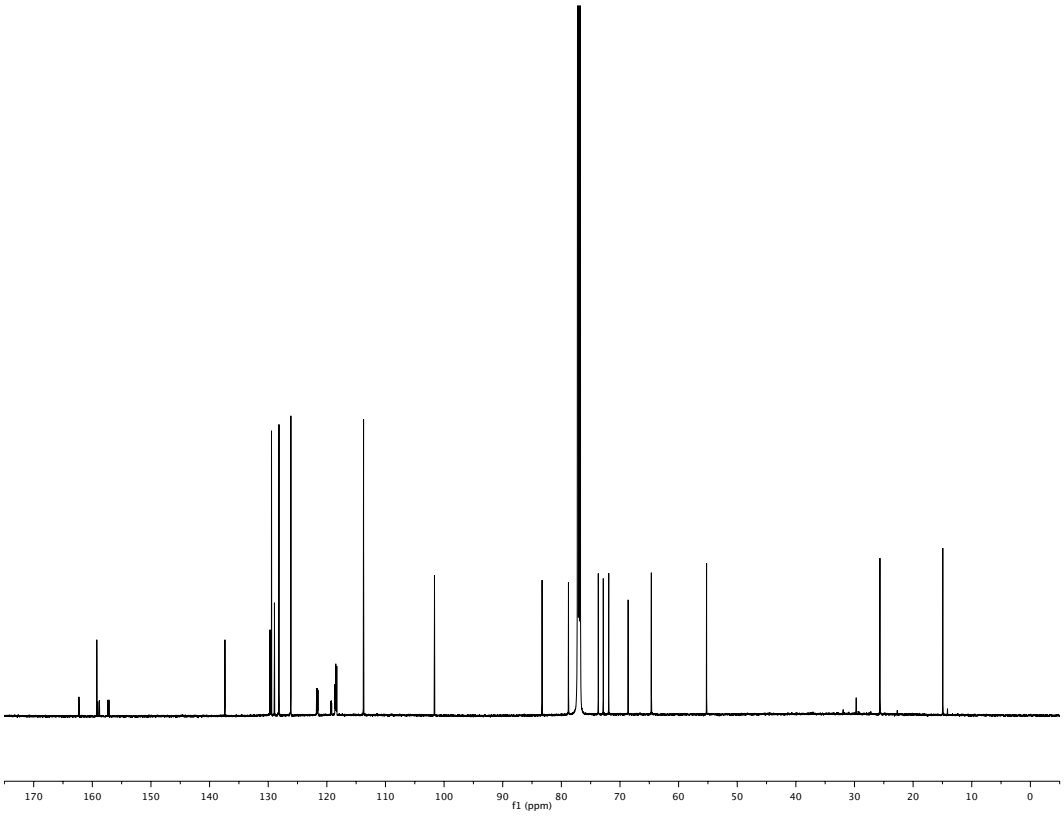
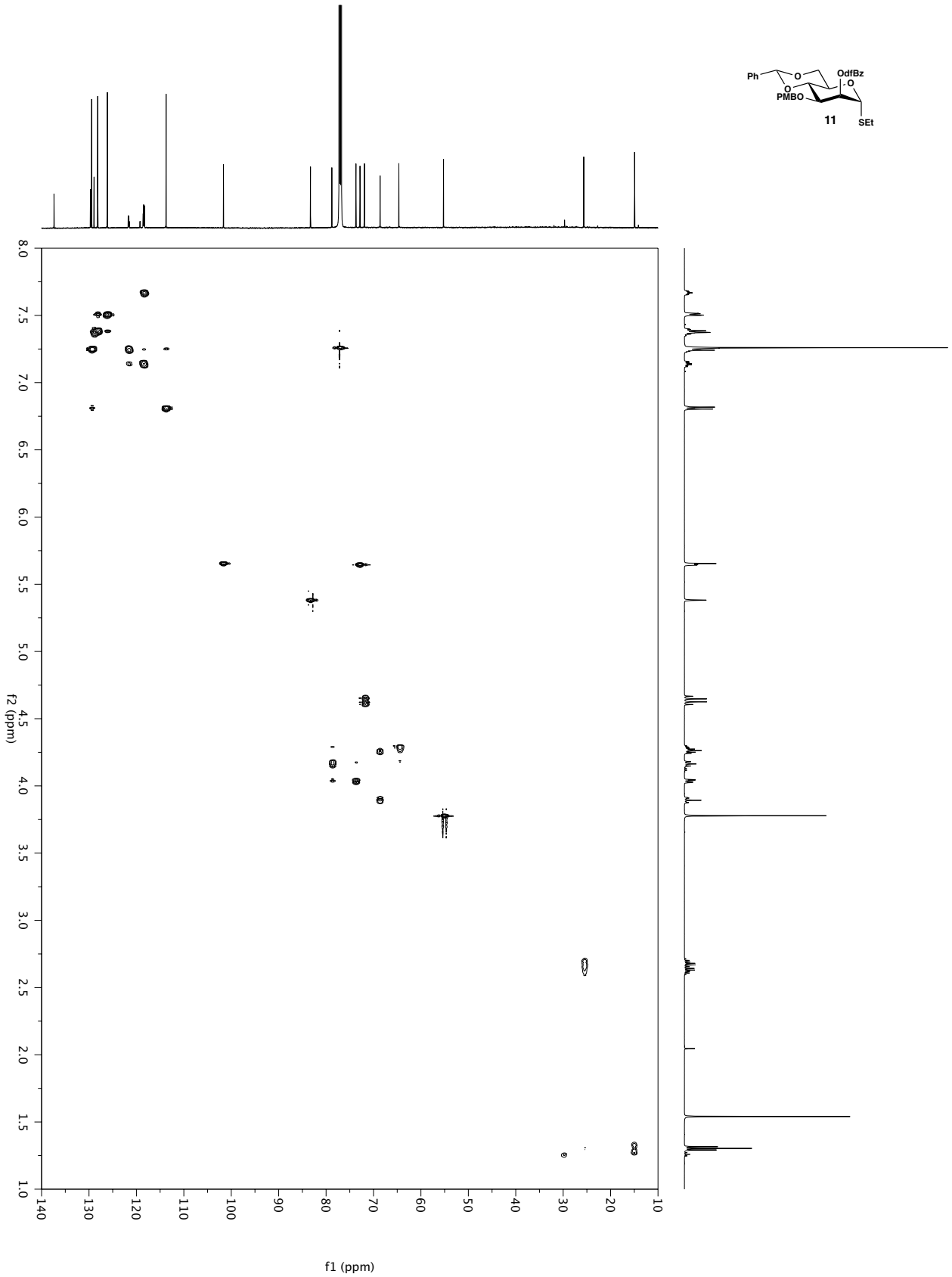


HSQC (600 MHz in F2, $CDCl_3$)



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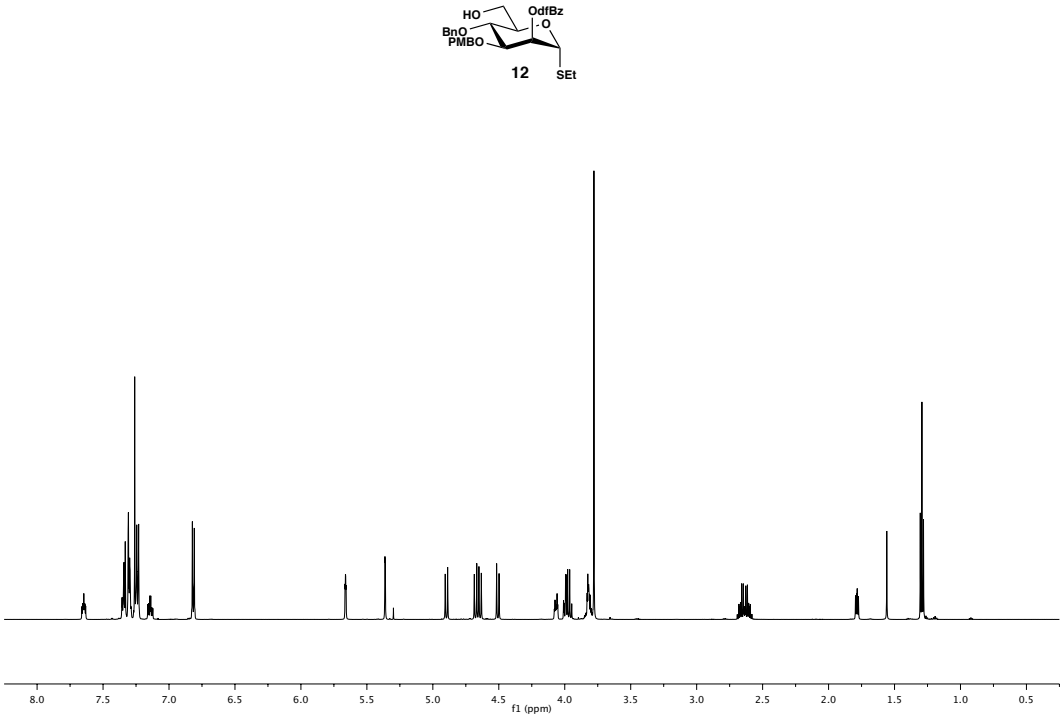

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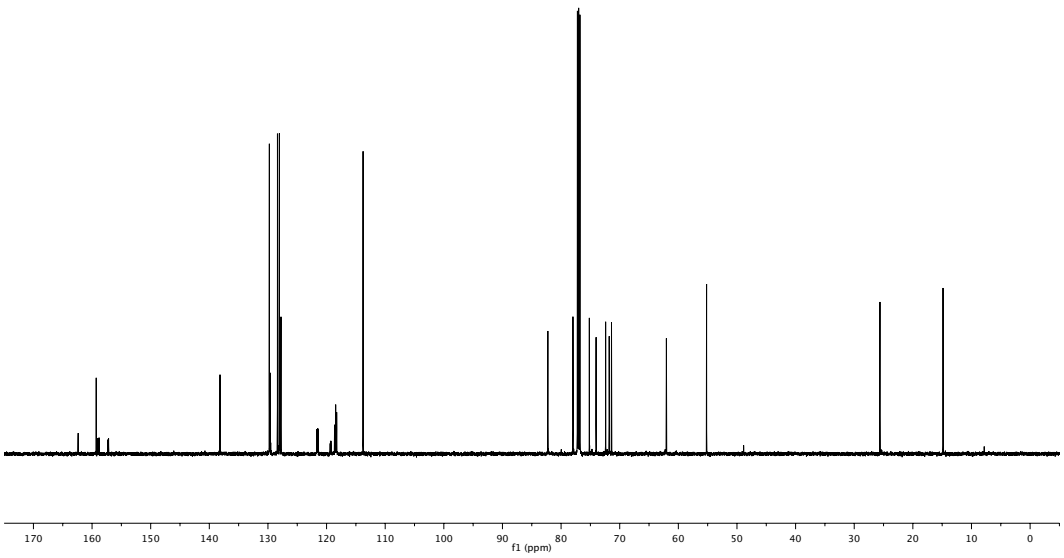

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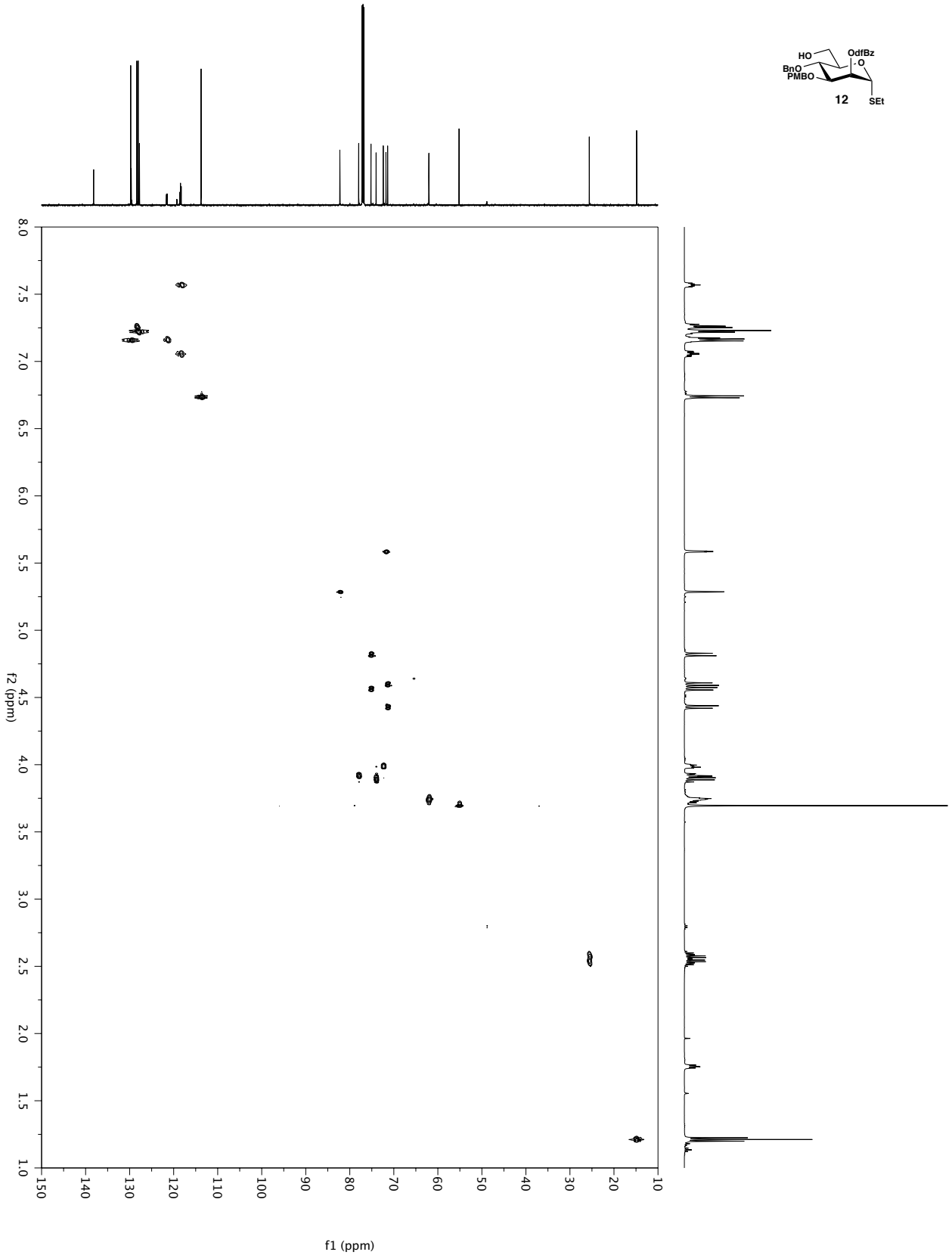

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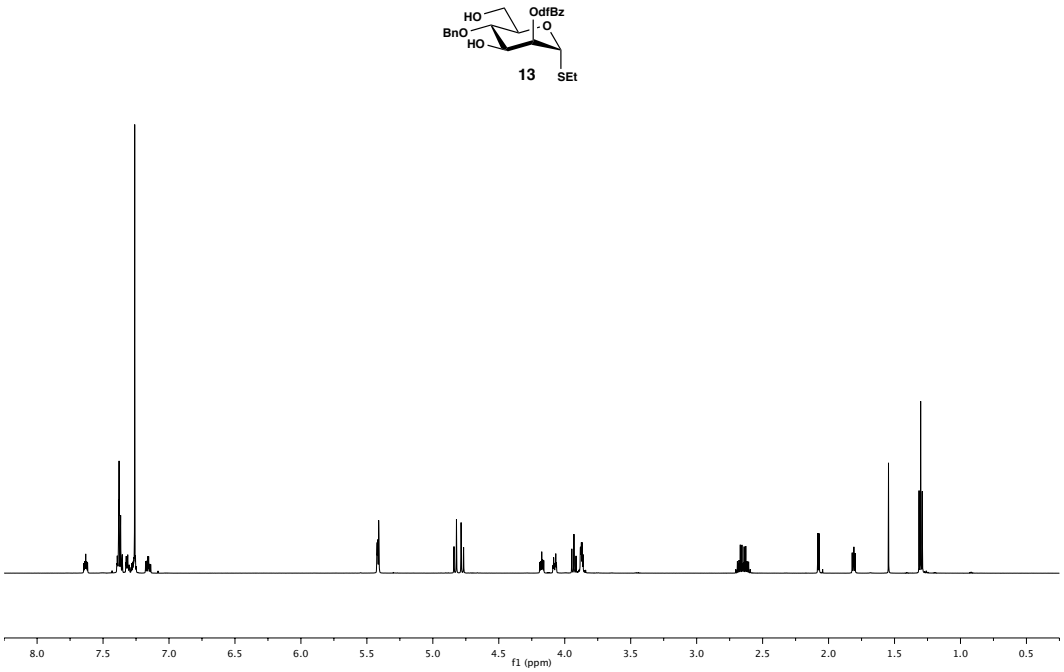

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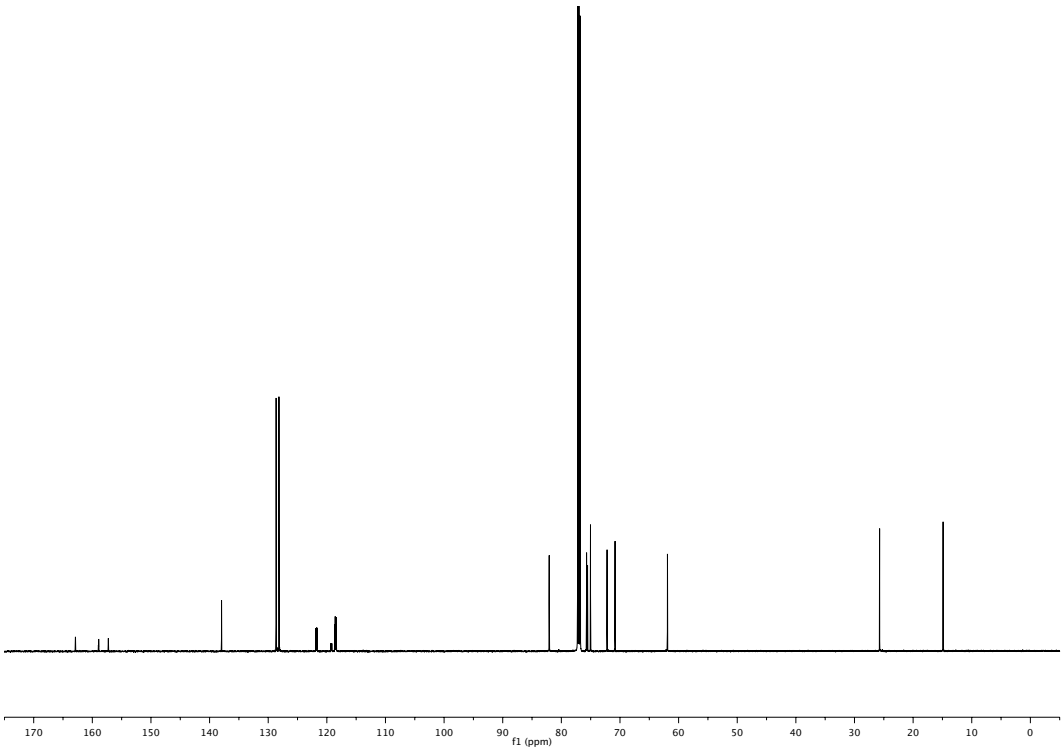

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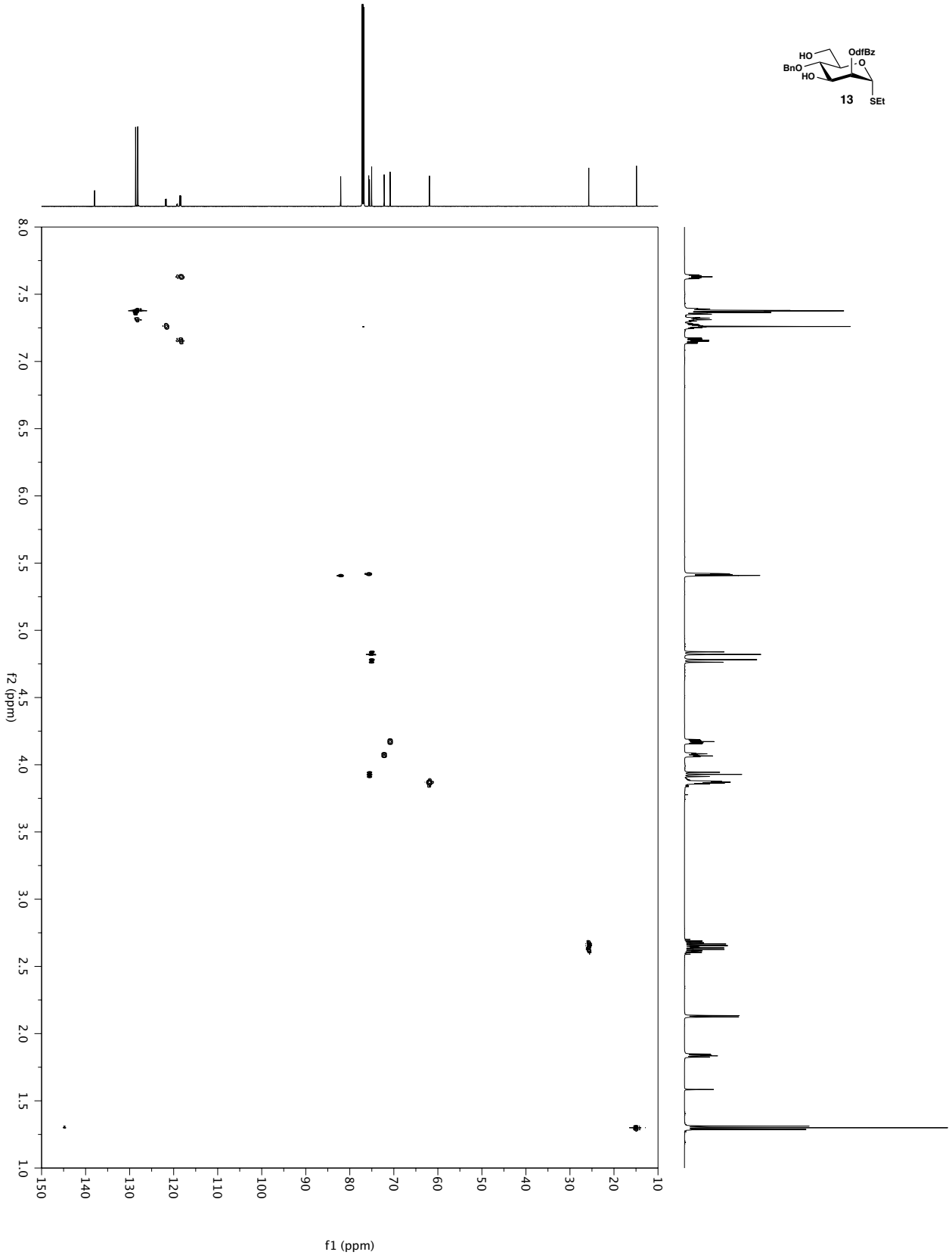

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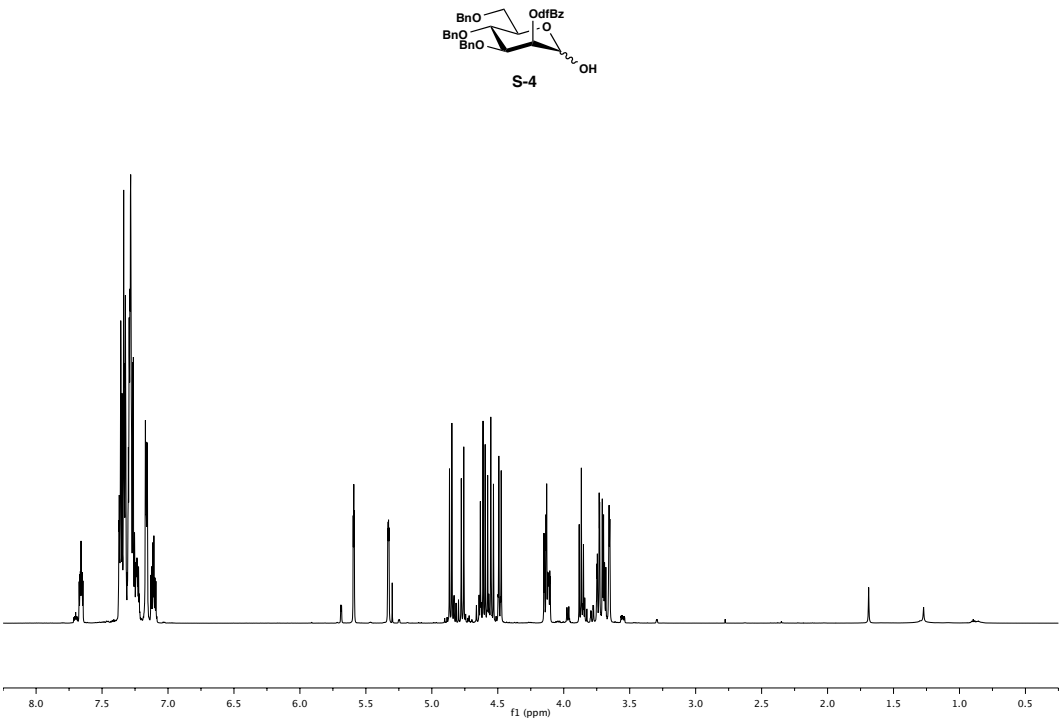

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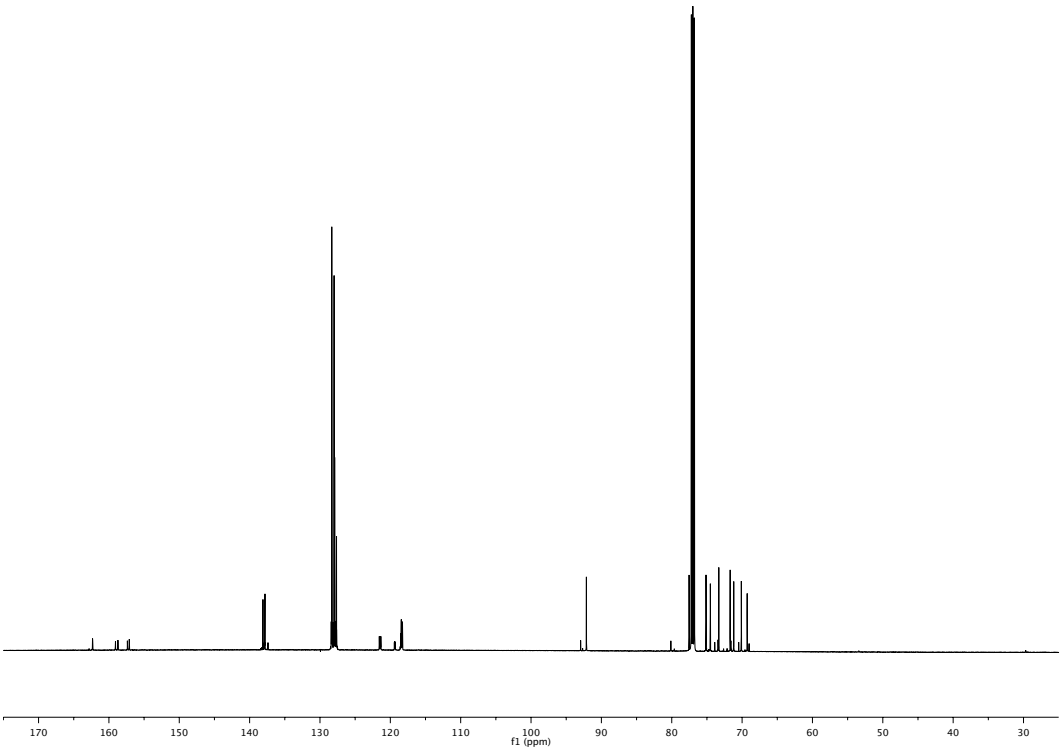

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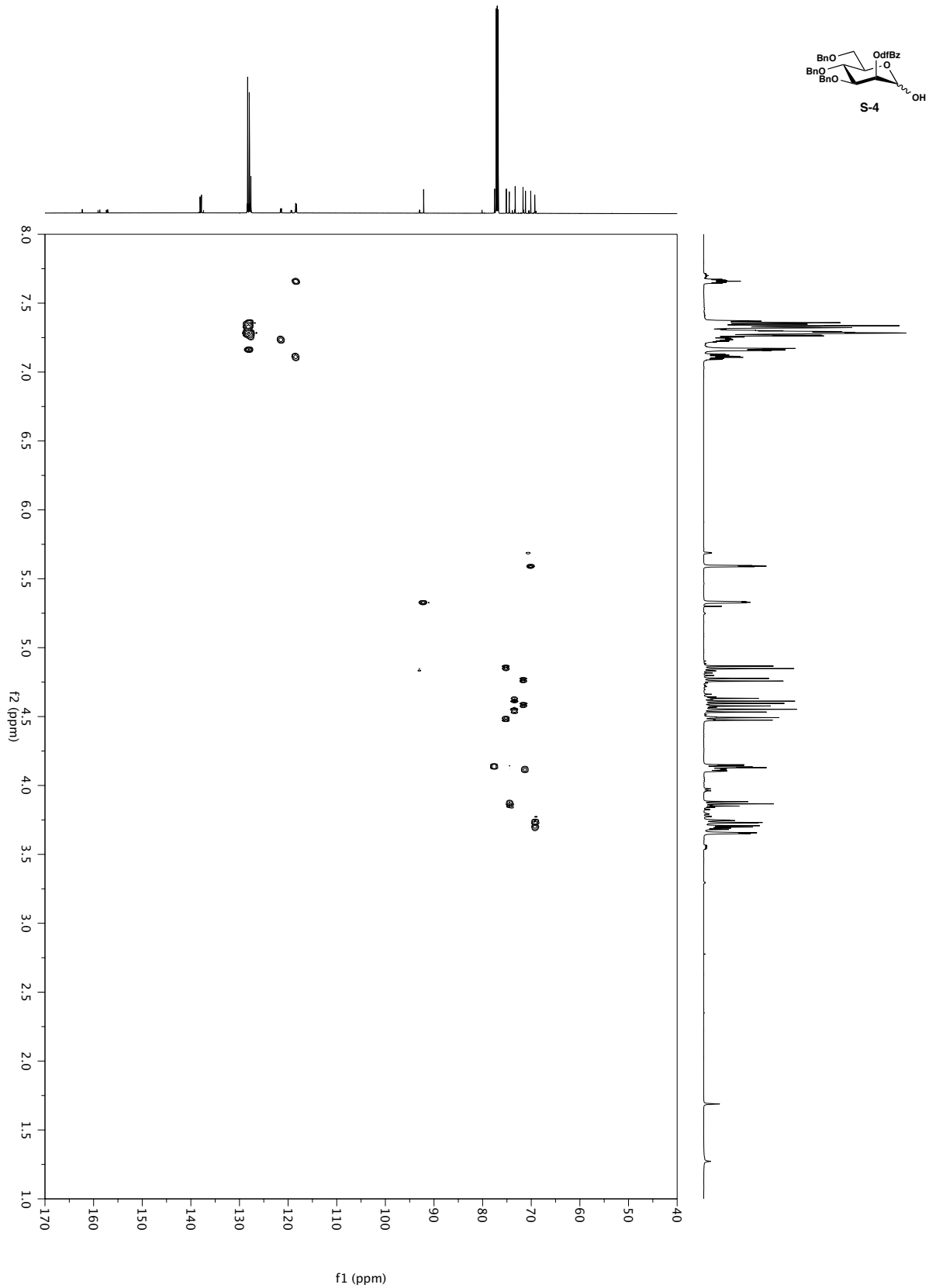

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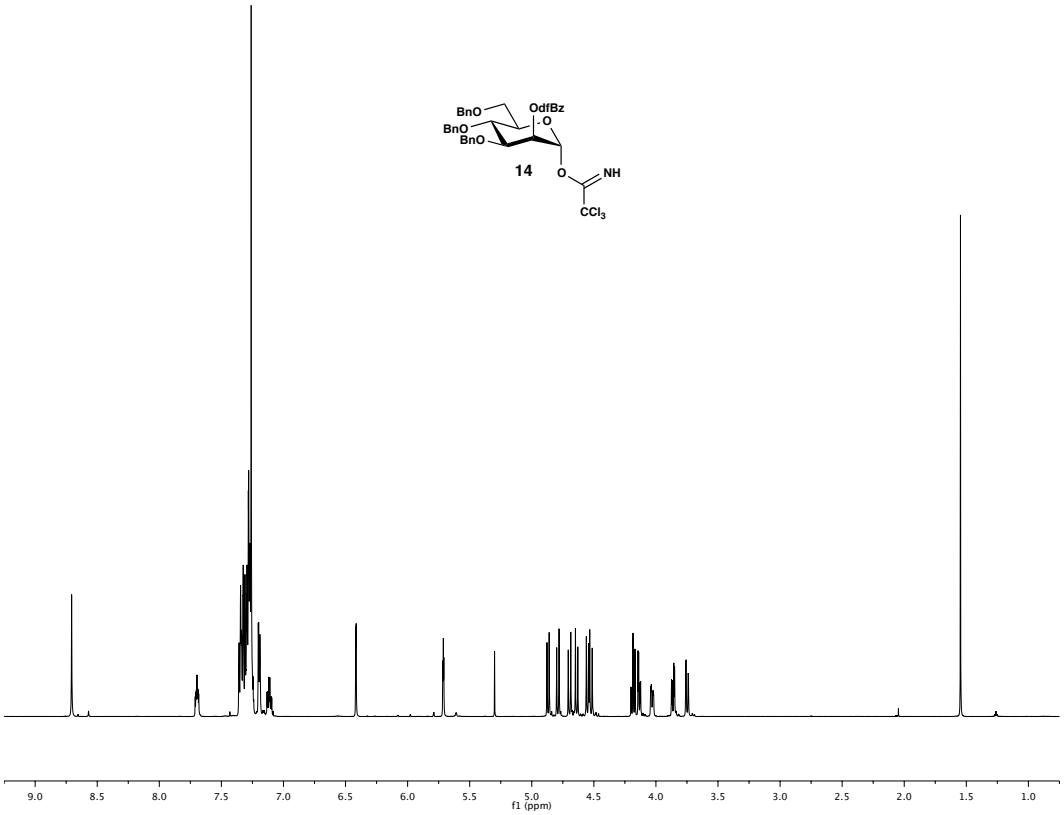

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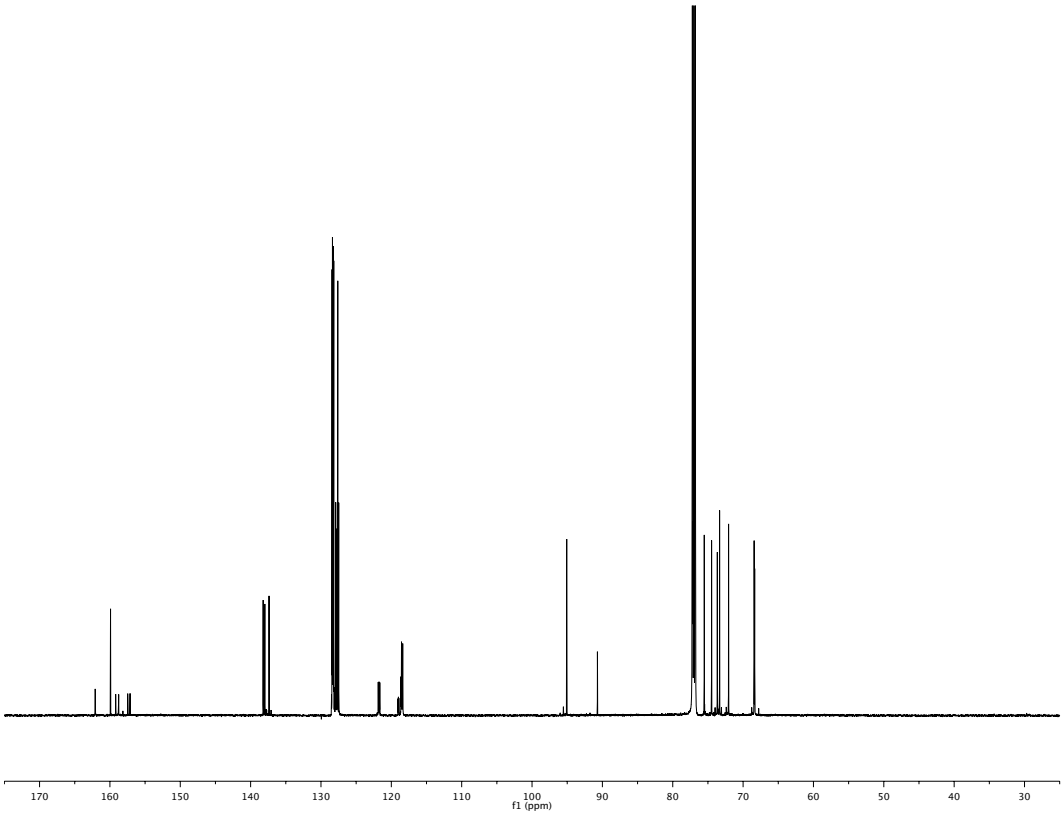
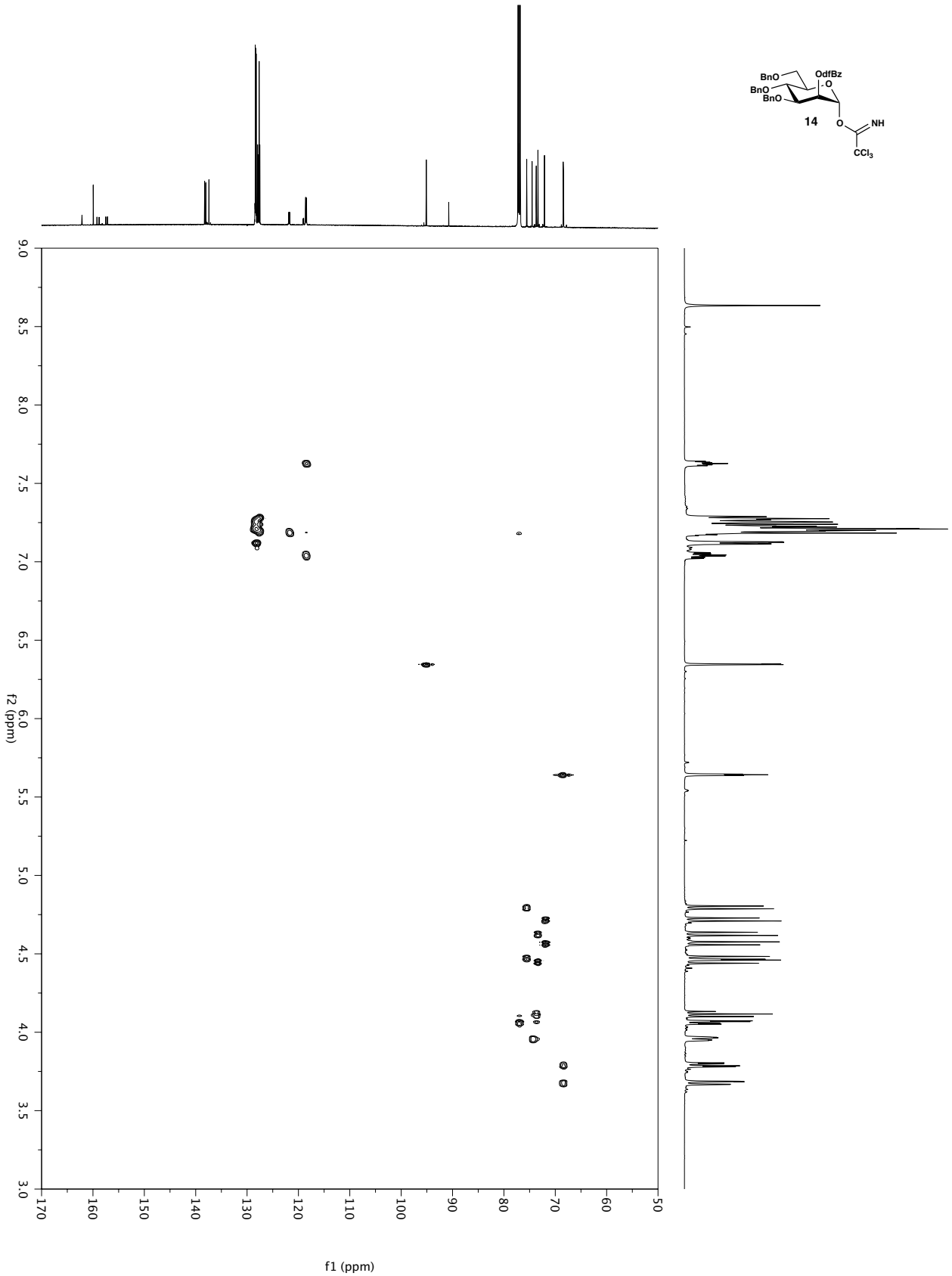


HSQC (600 MHz in F2, $CDCl_3$)



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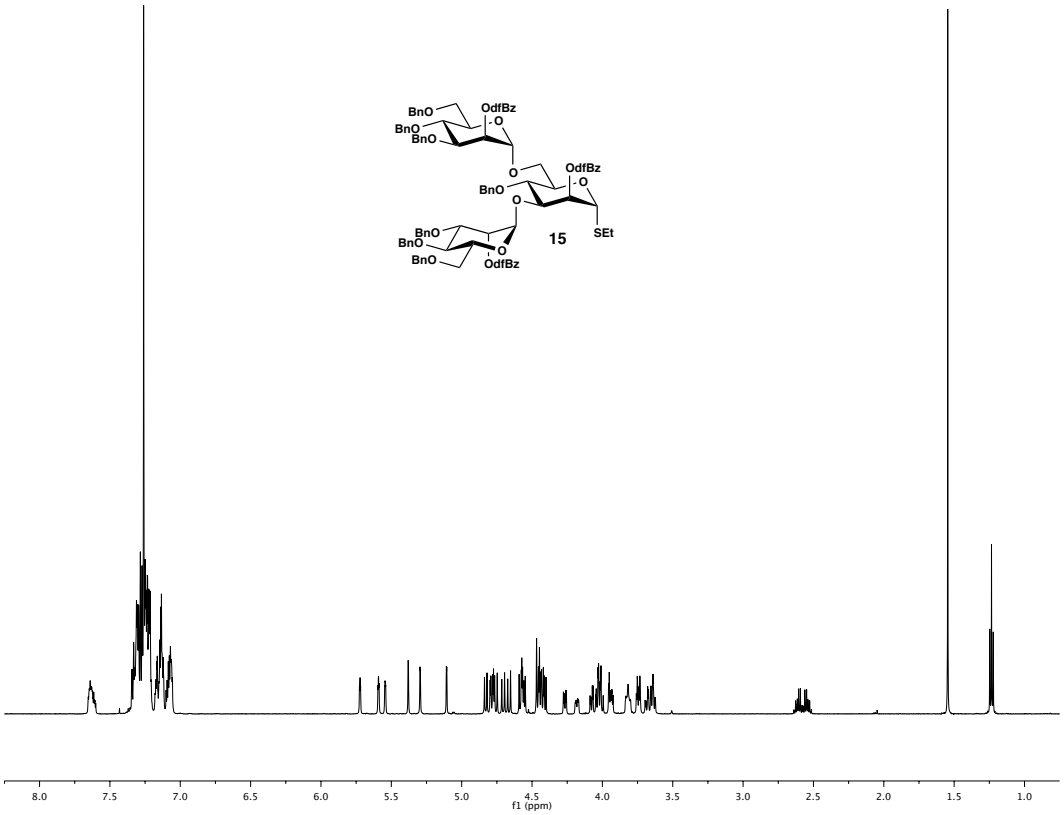

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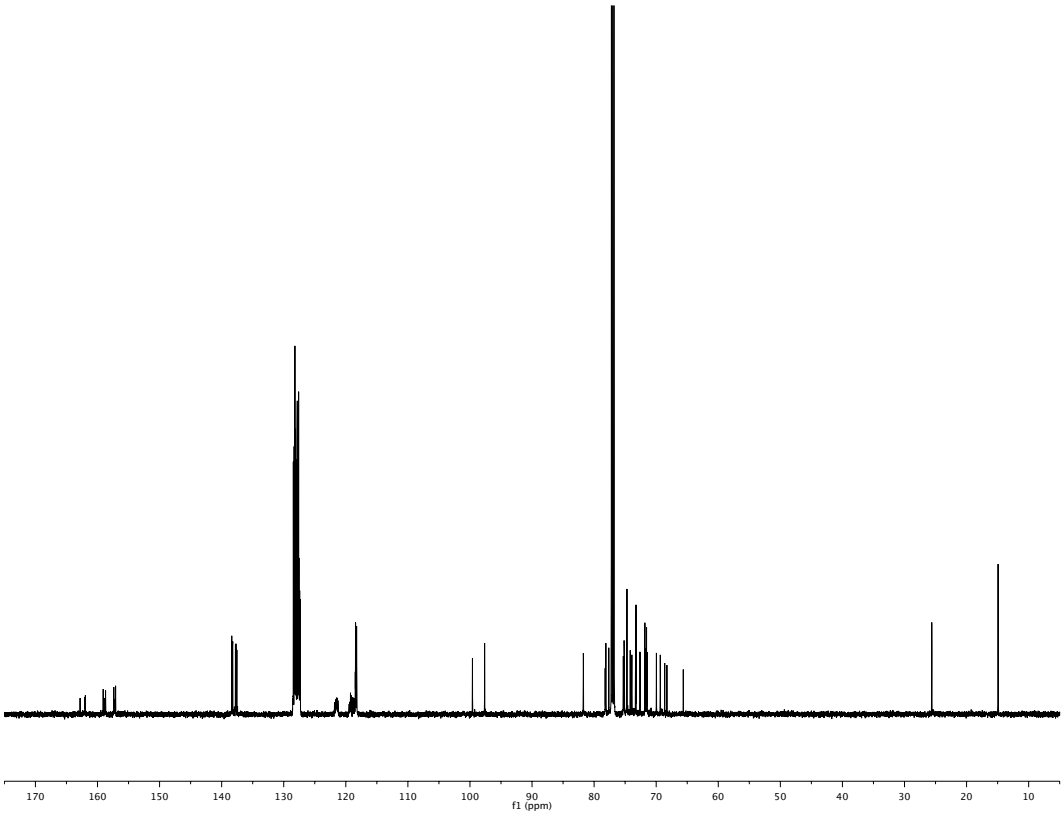
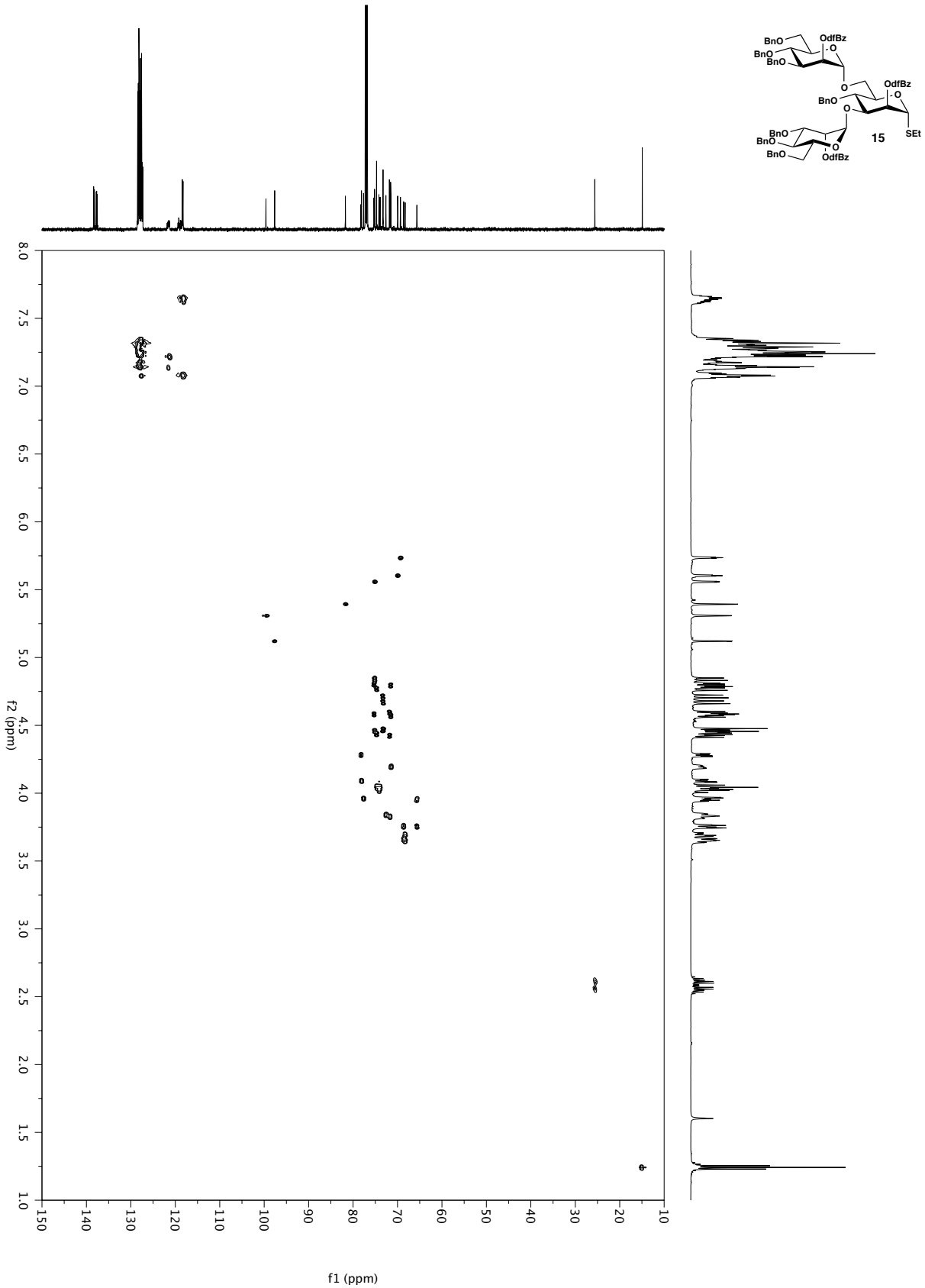


HSQC (600 MHz in F2, $CDCl_3$)



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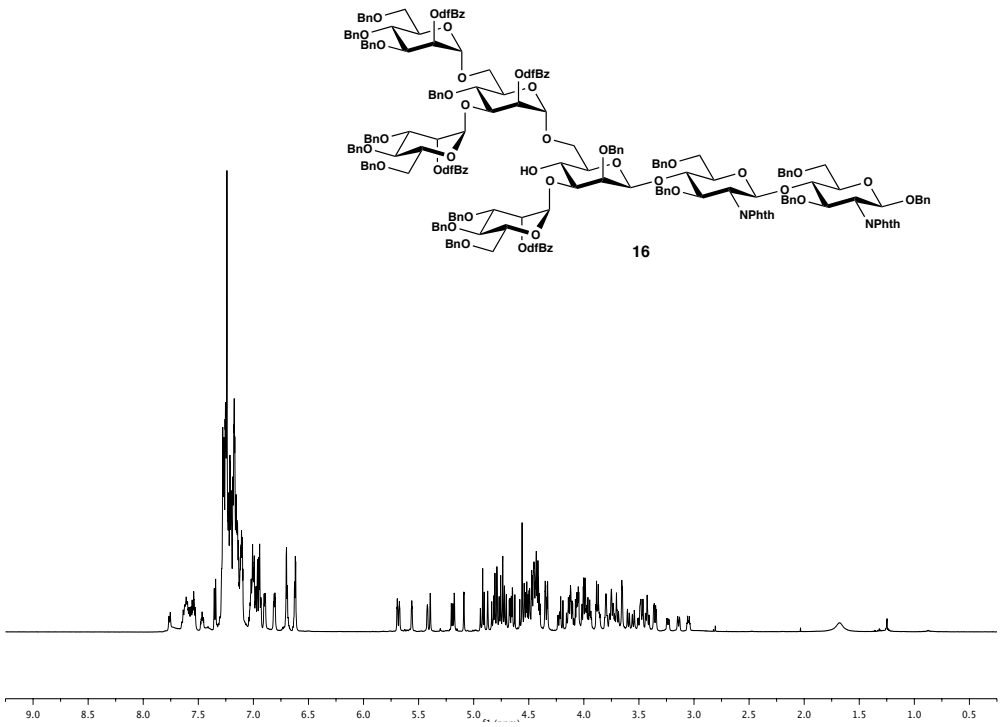

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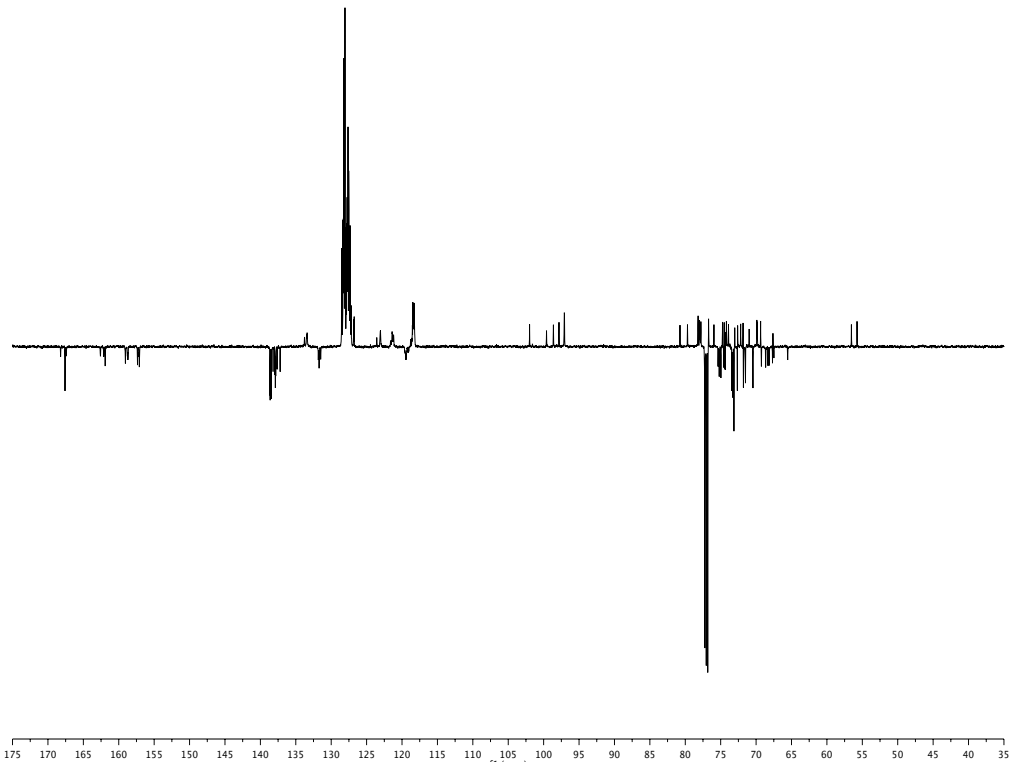
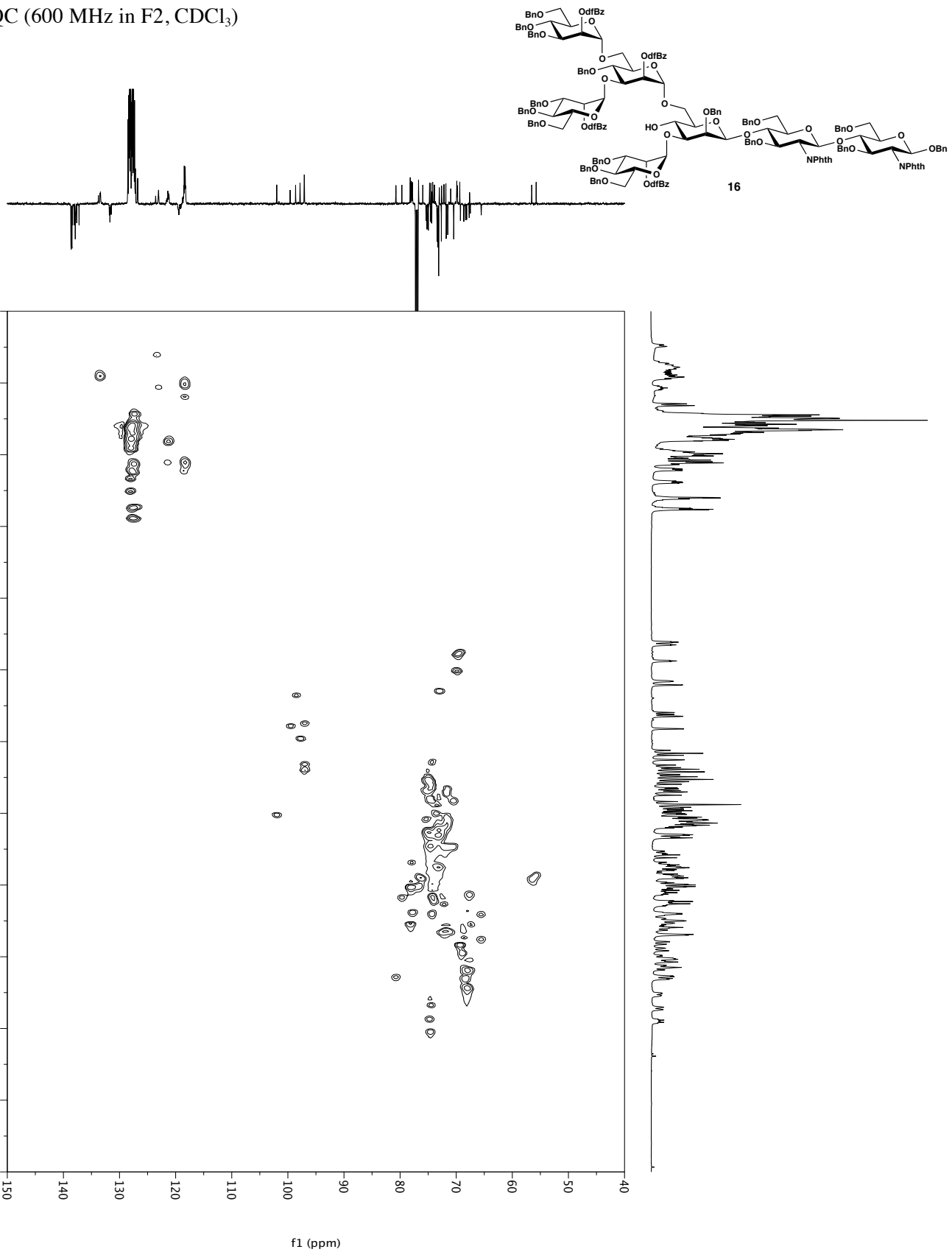


HSQC (600 MHz in F2, $CDCl_3$)



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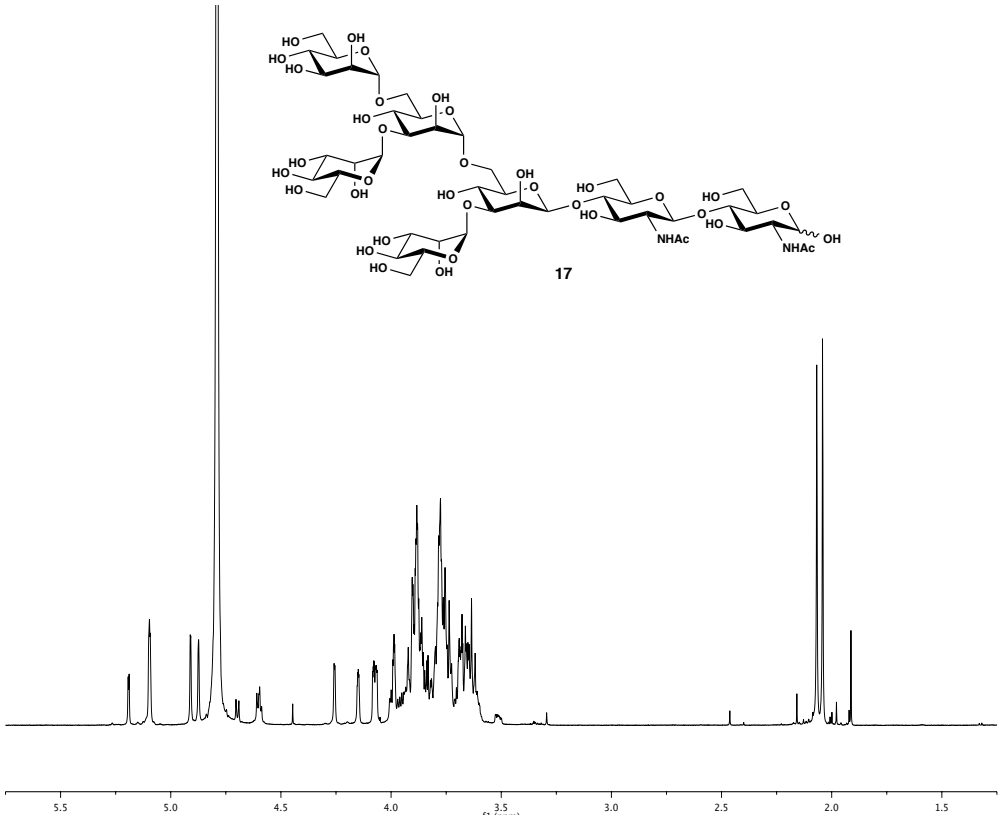
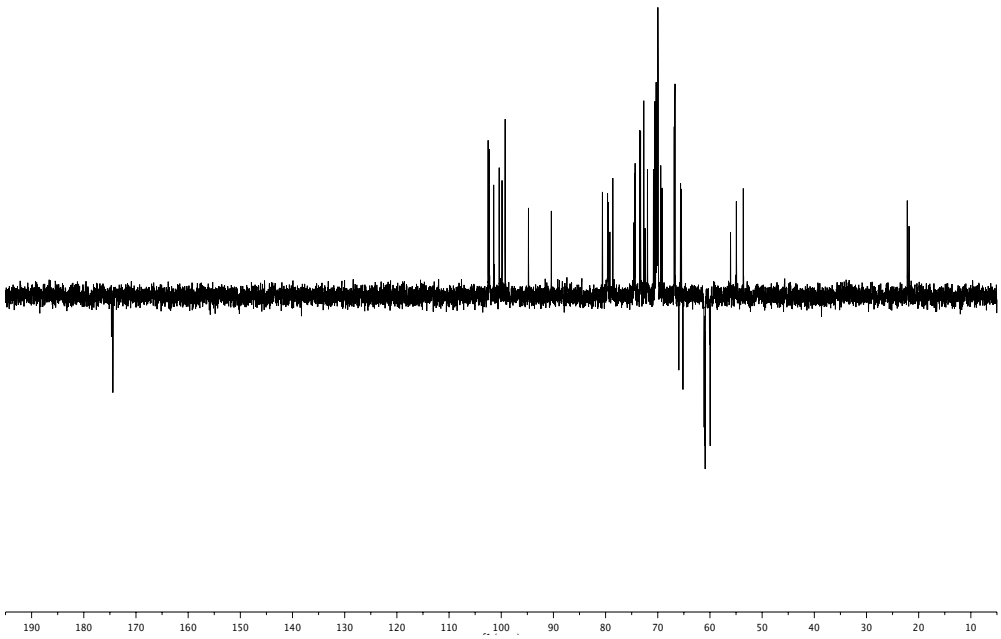


^{13}C APT NMR (150 MHz, D_2O)



HSQC (600 MHz in F2, D_2O)