C-mannosyl lysine for solid phase assembly of mannosylated peptide conjugate cancer vaccines

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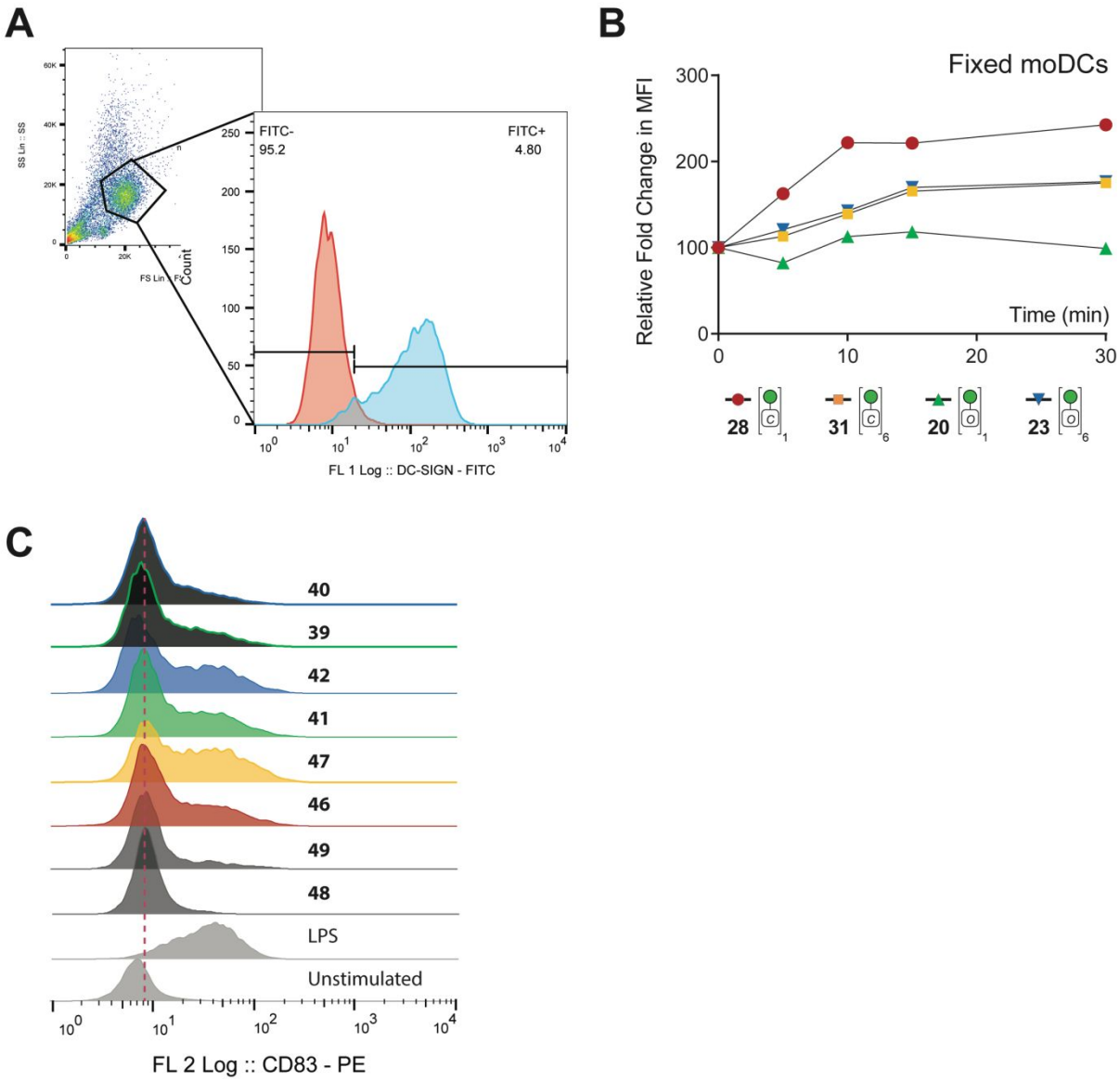
Supplementary Material

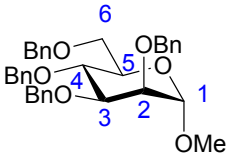
Supplementary figures and schemes

SM Figure 1 | Binding profiles upon blocking of various CLRs (A) Flow cytometric analysis of DC-SIGN expression on moDCs, one donor is depicted as representative of eight. (B) Binding of the biotinylated clusters to moDCs was quantified using flow cytometry after blocking the DC-SIGN receptor with an antibody. The binding was reduced, although minimal cluster-dependent residual binding remained. (C)) The expression of co-stimulatory marker CD83 was measured using flow cytometry. One of four donors are depicted. LPS (10 ng/mL) and **49** were included as positive control, as well as a naked **48** negative control. Overnight moDC stimulation with the trivalent conjugates induced CD83 expression, independent of mannoside variation, mannoside valency, and conjugate sequence.



Synthetic procedures

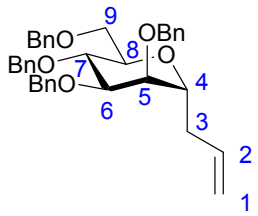
Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside (3).



Methyl α-D-mannopyranoside (29.13 g, 150 mmol) was co-evaporated with toluene (2x), dissolved in DMF (900 mL, 0.17 M) and cooled to 0 °C. NaH (60%

dispersion in mineral oil) (36.0 g, 900 mmol, 6 eq) was added in small portions under a continuous flow of argon. BnBr (80.28 mL, 675 mmol, 4.5 eq) and TBAI (5.54 g, 15 mmol, 0.1 eq) were added and the reaction mixture was stirred at room temperature. After three days the mixture was with MeOH at 0°C, diluted in Et_2O and washed with brine. The aqueous layer was back extracted with Et_2O, the organic fractions combined, washed with brine (2x), dried over $MgSO_4$ (s), filtered and concentrated *in vacuo*. Purification using silica gel column chromatography (1/8, → 1/4, PE/Et_2O/PE, v/v) yielded compound **3** as a colorless oil (64.5 g, 116.3 mmol, 77.5%). TLC R_f: 0.67 (1/4, EtOAc/PE, v/v); IR (neat, cm^{-1}): 3020, 2905, 1495, 1453, 1362, 1261, 1098, 1058, 1026, 967, 910, 846, 801, 733, 695; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 - 7.22 (m, 18H, H_{arom}), 7.18 - 7.14 (m, 2H, H_{arom}), 4.88 (d, *J* = 10.8 Hz, 1H, C*H*H Bn), 4.79 - 4.64 (m, 4H, C*H*H-Bn, CH_2-Bn, H-1), 4.61 (s, 2H, CH_2-Bn), 4.53 (dd, *J* = 20.7, 11.5 Hz, 2H, CH*H*-Bn (2x)), 3.97 (t, *J* = 9.1 Hz, 1H, H-4), 3.88 (dd, *J* = 9.3, 3.1 Hz, 1H, H-3), 3.81 - 3.70 (m, 4H, H-2, H-5, H-6), 3.32 (s, 3H, OMe); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.6, 138.6, 138.5, 138.5 (C_q), 128.4, 128.4, 128.4, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5 (CH_{arom}), 99.0 (C-1), 80.3 (C-3), 75.1 (CH_2-Bn), 75.0(C-4), 74.6 (C-2), 73.4, 72.6, 72.2 (CH_2-Bn), 71.8 (C-5), 69.4 (C-6), 54.8 (OMe); HRMS $[C_{35}H_{38}O_6 + H]^+$: 555.2751 found, 555.2741 calculated.

3-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranoside)-1-propene (4).[1,2]



Compound **3** (53.61 g, 96.64 mmol) was co-evaporated with toluene (3x) under argon, dissolved in ACN (133 mL, 0.73 M) and cooled to 0 °C. Allyltrimethylsilane (41.5 mL, 260.93 mmol, 2.7 eq) and TMSOTf (21.0 mL, 116.0 mmol, 1.2 eq) were added and the reaction mixture was irradiated with ultrasound for 50 min after which the reaction mixture was quenched by addition of Et_3N. The reaction mixture was diluted in Et_2O washed with brine (3x), dried over $MgSO_4$ (s), filtered and concentrated *in vacuo*. Purification using silica gel column chromatography (1/4, → 1/1, Et_2O/PE, v/v) yielded compound **4** as a colorless oil (56.1 g, 70.48 mmol, 73%). TLC R_f: 0.61 (EtOAc/PE, 1/4, v/v); IR (neat, cm^{-1}): 3021, 2905, 2855, 1495, 1453, 1363, 1262, 1090, 1072, 1027, 1001, 912, 733, 695; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 - 7.24 (m, 18H, H_{arom}), 7.22 - 7.18 (m, 2H, H_{arom}), 5.83 - 5.68 (m, 1H, H-2), 5.08 - 4.93 (m, 2H, H-1), 4.70 (d, *J* = 11.3 Hz, 1H, C*H*H-Bn), 4.62 - 4.49 (m, 7H, CH_2-Bn (3x), CH*H*-Bn), 4.04 (q, *J* = 6.5 Hz, 1H, H-4), 3.92 - 3.66 (m, 5H, H-6, H-7, H-8, H-9), 3.62 (dd, *J* = 4.6, 3.1 Hz, 1H, H-5), 2.41 – 2.25 (m, 2H, H-3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 138.5, 138.4, 138.3 (C_q), 134.4 (C-2), 128.5, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.6 (CH_{arom}), 117.4 (C-1), 77.0 (C-6/7/8), 75.2 (C-5), 75.0 (C-6/7/8), 74.0 (CH_2-Bn), 73.8 (C-6/7/8), 73.4 (CH_2-Bn), 72.4 (C-4), 72.2, 71.6 (CH_2-Bn), 69.2 (C-9), 34.8 (C-3); HRMS $[C_{37}H_{40}O_5 + H]^+$: 565.2957 found, 565.2949 calculated.

3-(2,3,4,6-Tetra-O-Acetyl-α-D-mannopyranoside)-1-propene (5).

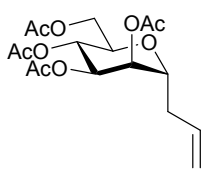
Method a) BCl_3

Compound **4** (6.44 g, 11.4 mmol) was co-evaporated with toluene (3x) under argon, dissolved in DCM (10 mL) and cooled to -78°C. To this solution BCl_3 (100 mL, 1.0 M in DCM, 100 mmol, >8 eq) was added dropwise. After overnight stirring at -78°C the reaction mixture was quenched by the addition of MeOH (100 mL) at -78°C after which the mixture was concentrated *in vacuo* and co-evaporated with toluene (5x). The crude pink oil was dissolved in pyridine (20 mL, 0.6 M) and Ac_2O (6.5 mL, 69.0 mmol, 6 eq) was added dropwise at 0°C. After overnight stirring at rt the reaction was quenched with MeOH, diluted in Et_2O and washed with HCl (1 M, aq., 4x). The organic layer was collected, dried over $MgSO_4$

(s), filtered, and concentrated *in vacuo*. Purification using silica gel column chromatography (1/9, → 1/1, Et_2O/PE, v/v) yielded title compound **5** as a colorless oil (4.003 g, 10.7 mmol, 95%).

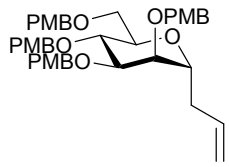
Method b) Li-napthalenide

Compound **4** (45.8 g, 81 mmol) was co-evaporated with toluene (3x) under argon and dissolved in distilled anhydrous THF (180 mL). This solution was added dropwise to a lithium napthalenide solution at -78°C and stirred for five days at -20°C with a glass stirring rod. Li-napthalenide solution was prepared from Li (9.2 g, 1.3 mol, 14 eq) and naphthalene (15.5 g, 121 mmol, 1.5 eq) in distilled anhydrous THF (400 mL). The reaction was quenched with MeOH, neutralized with Amberlite H^+ resin, filtered and concentrated *in vacuo*. After co-evaporation with toluene (3x) the mixture was dissolved in pyridine (135 mL, 0.6 M), cooled to 0°C and Ac_2O (46 mL, 486 mmol, 6 eq) was added dropwise. After overnight stirring at rt, the reaction was quenched with MeOH, diluted with Et_2O and washed with HCl (1 M, aq.). The aqueous layer was back-extracted with Et_2O (2x) and the organic layers were combined, washed with HCl (1.0 M, aq., 2x), dried over $MgSO_4$ (s), filtered and concentrated *in vacuo.* Purification using silica gel column chromatography (1/9, → 1/1, Et_2O/PE, v/v) yielded title compound **5** as a colorless oil (16.38 g, 44 mmol, 54%).



TLC R_f: 0.34 (Et_2O/PE, 6/4, v/v); IR (neat, cm^{-1}): 2905, 1739, 1369, 1213, 1144, 1114, 1045, 986, 918, 802; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (ddt, *J* = 17.1, 10.2, 6.9 Hz, 1H, H-2), 5.27 (dd, *J* = 8.9, 3.3 Hz, 1H, H-6), 5.23 - 5.13 (m, 4H, H-7, H-5, H-1), 4.33 (dd, *J* = 12.1, 6.3 Hz, 1H, H-9a), 4.11 (dd, *J* = 12.1, 2.9 Hz, 1H, H-9b), 4.08 - 4.02 (m, 1H, H-4), 3.90 (ddd, *J* = 8.9, 6.5, 2.9 Hz, 1H, H-8), 2.59 - 2.48 (m, 1H, H-3a), 2.48 - 2.36 (m, 1H, H-3b), 2.13 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.03 (s, 3H, Ac); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.3, 170.0, 169.8 (C=O), 132.6 (C-2), 118.4 (C-1), 74.2 (C-4), 70.7 (C-8), 70.1 (C-5), 68.9 (C-6), 67.1 (C-7), 62.5 (C-9), 33.7 (C-3), 21.0, 20.8, 20.8 (Ac (4x)); HRMS $[C_{17}H_{24}O_9 + H]^+$: 373.1493 found, 373.1493 calculated.

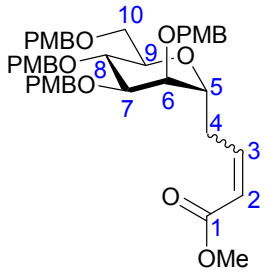
3-(2,3,4,6-Tetra-*O*-parамethoxybenzyl-α-D-mannopyranoside)-1-propene (6).



Compound **5** (18.84 g, 50.6 mmol) was dissolved in MeOH (83 mL, 0.6 M) and NaOMe (4.5 mL, ~5.4 M, 20.25 mmol, 0.4 eq) was added dropwise. After overnight stirring, the mixture was neutralized with Amberlite H^+ resin, filtrated and concentrated *in vacuo*. After co-evaporation with toluene (3x) the crude was dissolved in DMF (253 mL, 0.2 M) cooled to 0°C and NaH (12.14 g, 60% wt dispersion in mineral oil, 303.6 mmol, 6 eq), PMBCl (41.2 mL, 303.6 mmol, 6 eq) and TBAI (3.7 g, 10.1 mmol, 0.2 eq) were added in portions. After overnight stirring at rt, the mixture was quenched with H_2O at 0°C and extracted with Et_2O (2x). The organic layers were combined, washed with brine (3x), dried over $MgSO_4$ (s), filtrated and concentrated *in vacuo*. Purification using silica gel column chromatography (1/9, → 1/4, EtOAc/PE, v/v) yielded title compound **6** as a colorless oil (23.8 g, 34.7 mmol, 69%). TLC R_f: 0.38 (EtOAc/PE, 1/4, v/v); IR (neat, cm^{-1}): 3054, 2988, 1612, 1514, 1422, 1264, 1174, 1083, 896, 824, 732, 704; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (t, *J* = 8.3 Hz, 4H, H_{arom}), 7.18 (d, *J* = 8.6 Hz, 2H, H_{arom}), 7.09 (d, *J* = 8.6 Hz, 2H, H_{arom}), 6.87 - 6.79 (m, 8H, H_{arom}), 5.74 (ddt, *J* = 18.2, 9.1, 6.9 Hz, 1H, H-2), 5.06 - 4.96 (m, 2H, H-1), 4.61 (d, *J* = 10.9 Hz, 1H, C*H*H-PMB), 4.56 - 4.37 (m, 7H, CH_2-PMB (3x), CH*H*-PMB), 4.04 - 3.94 (m, 1H, H-4), 3.84 - 3.75 (m, 14H, OMe (4x), H-6, H-8), 3.71 (dt, *J* = 7.4, 4.0 Hz, 2H, H-9a, H-7), 3.65 (dd, *J* = 10.1, 2.9 Hz, 1H, H-9b), 3.57 (dd, *J* = 4.6, 3.1 Hz, 1H, H-5), 2.32 (tt, *J* = 12.2, 6.6 Hz, 2H, H-3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.3, 159.3, 159.2 (C_q), 134.6 (C-2), 130.7, 130.6, 130.5, 130.4 (C_q), 129.7, 129.7, 129.6, 129.5 (CH_{arom}), 117.2 (C-2), 113.8, 113.8, 113.8 (CH_{arom}), 76.5 (C-

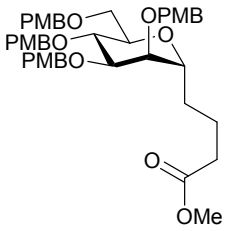
7), 74.7 (C-5), 74.6, 73.8 (C-6, C-8), 73.5, 73.0 (CH_2-PMB), 72.4 (C-4)), 71.7, 71.1 (CH_2-PMB), 68.9 (C-9), 55.4, 55.3 (OMe (4x)), 34.8 (C-3); HRMS $[C_{41}H_{48}O_9 + Na]^+$: 707.3191 found, 707.3191 calculated.

Methyl-but-4-(2,3,4,6-tetra-O-paramethoxybenzyl-α-D-mannopyranoside)-cis/trans-2-enoate (7).



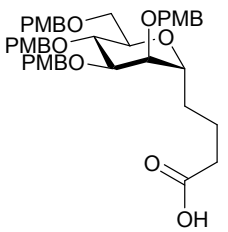
Compound **6** (24.77 g, 36.2 mmol) was co-evaporated with $CHCl_3$ (1x), dissolved in DCM (40 mL, 1 M) and purged by bubbling with N_2 gas for twenty minutes. To this mixture methyl acrylate (16.3 mL, 181 mmol, 5 eq) and Grubbs second generation catalyst (0.68 g, 0.80 mmol, 0.02 eq) were added, the mixture was purged with N_2 for twenty minutes more, after which it was refluxed protected from light. After two days the mixture was concentrated on Celite and purified via silica gel column chromatography (1/9 → 1/3, EtOAc/PE, v/v) to yield unreacted starting material **6** (6.27 g, 9.16 mmol, 25%) and product **7** as a brown oil (19.60 g, 26.4 mmol, 73%). TLC R_f: 0.22 (EtOAc/PE, 1/4, v/v); IR (neat, cm^{-1}): 2936, 2837, 1720, 1659, 1611, 1586, 1512, 1463, 1440, 1362, 1302, 1245, 1172, 1082, 1033, 819, 733, 702; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 - 7.09 (m, 8H, H_{arom}), 6.96 - 6.87 (m, 1H, H-3), 6.87 - 6.80 (m, 8H, H_{arom}), 5.83 (d, *J* = 15.7 Hz, 1H, H-2), 4.51 (d, *J* = 11.4 Hz, 1H, C*H*H-PMB), 4.47 - 4.37 (m, 7H, CH_2-PMB (3x), CH*H*-PMB), 4.04 - 3.96 (m, 1H, H-5), 3.89 - 3.83 (m, 1H, H-9), 3.83 - 3.75 (m, 12H, OMe (3x)), 3.75 - 3.65 (m, 6H, H-7, H-8, CO_2Me , H-10a), 3.65 - 3.59 (m, 1H, H-10b), 3.50 (dd, *J* = 6.4, 2.5 Hz, 1H, H-6), 2.55 - 2.37 (m, 2H, H-4); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7 (C-1), 159.3, 159.1 (C_q), 145.3 (C-3), 130.4, 130.2, 130.1, 130.0 (C_q), 129.7, 129.6, 129.4 (CH_{arom}), 123.0 (C-2), 113.8, 113.7 (CH_{arom}), 74.9 (C-6), 74.7, 74.2 (C-7, C-8), 73.9 (C-9), 72.9, 72.6, 71.8, 71.0 (CH_2-PMB), 70.3 (C-5), 68.2 (C-10), 55.3 (OMe (4x)), 51.4 (COO*Me*), 33.7 (C-4); HRMS $[C_{43}H_{50}O_{11} + Na]^+$: 765.3246 found, 765.3245 calculated.

Methyl-4-(2,3,4,6-tetra-*O*-paramethoxybenzyl-α-D-mannopyranoside)-butanoate (8).



Compound **7** (19.60 g, 26.4 mmol) and $RuCl_3$ (1.1 g, 5.3 mmol, 0.2 eq) were dissolved in 1,2-dichloroethane (100 mL, 0.26 M) and purged with N_2 for twenty minutes. To this solution $NaBH_4$ (3.2 g, 84.5 mmol, 3.2 eq) was added, the flask was sealed with a septum and three empty balloons were fitted. The mixture was cooled to 0°C and MeOH (34 mL) was added dropwise over thirty minutes time after which the reaction was allowed to warm up and stirred for four hours. The septum was removed and the reaction was quenched by addition of a small amount of H_2O at 0°C, after which it was concentrated and purified via silica gel column chromatography (1/9 → 3/7, EtOAc/PE, v/v) to yield **8** as a yellow oil (18.35 g, 24.6 mmol, 93%). TLC R_f: 0.34 (EtOAc/PE, 3/7, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 - 7.22 (m, 4H, H_{arom}), 7.19 (d, *J* = 8.6 Hz, 2H, H_{arom}), 7.08 (d, *J* = 8.6 Hz, 2H, H_{arom}), 6.88 - 6.80 (m, 8H, H_{arom}), 4.63 - 4.38 (m, 8H, CH_2-PMB), 3.90 (dd, *J* = 8.6, 4.2 Hz, 1H, H-5), 3.84 - 3.60 (m, 20H, OMe (5x), H-7, H-8, H-9, H-10), 3.50 (dd, *J* = 4.7, 3.1 Hz, 1H, H-6), 2.30 (t, *J* = 7.1 Hz, 2H, H-2), 1.83 - 1.41 (m, 4H, H-3, H-4); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.6 (C-1), 159.1, 159.1, 159.0, 130.4, 130.3, 130.2 (C_q), 129.9, 129.4, 129.3, 129.2, 129.0, 113.6, 113.5, 113.5 (CH_{arom}), 76.5 (C-7, C-8, C-9), 75.4 (C-6), 74.3, 73.4 (C-7, C-8, C-9), 73.1, 72.7 (CH_2-PMB), 72.1 (C-5), 71.5, 71.0 (CH_2-PMB), 68.5 (C-10), 55.0, 55.0 (OMe), 51.2 (COO*Me*), 33.5 (C-2), 29.0, 21.1 (C-3, C-4); HRMS $[C_{43}H_{52}O_{11} + H]^+$: 745.3573 found, 745.3582 calculated.

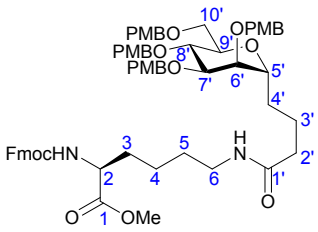
4-(2,3,4,6-tetra-*O*-paramethoxybenzyl-α-D-mannopyranoside)-butanoic acid (9).



Methyl ester **8** (18.34 g, 24.6 mmol) was dissolved in THF (123 mL, 0.2 M) and cooled to 0°C, followed by the dropwise addition of KOH (31 mL, 4.0 M, aq., 124 mmol, 5 eq). The mixture was heated to 50°C and stirred overnight. After acidification of the reaction mixture with HCl (1 M aq., pH = ± 2), the product was

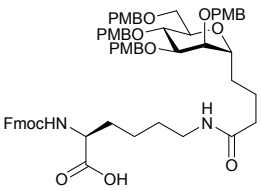
extracted with Et_2O (3x), the organic layers were combined, dried over $MgSO_4$ (s), filtered and concentrated *in vacuo*. Purification using silica gel column chromatography (3/7 → 1/0, EtOAc/PE, v/v) yielded title compound **9** as a clear oil (18.01 g, 24.6 mmol, qnt.). TLC R_f: 0.27 (EtOAc/PE, 7/3, v/v); IR (neat, cm^{-1}): 3055, 2920, 1513, 1422, 1264, 1034, 896, 733, 704; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 - 7.21 (m, 4H, H_{arom}), 7.18 (d, *J* = 8.6 Hz, 2H, H_{arom}), 7.08 (d, *J* = 8.6 Hz, 2H, H_{arom}), 6.83 (ddd, *J* = 8.5, 5.4, 2.5 Hz, 8H, H_{arom}), 4.60 - 4.36 (m, 8H, CH_2-PMB), 3.90 (dt, *J* = 8.5, 4.6 Hz, 1H, H-5), 3.83 - 3.66 (m, 18H, OMe (4x) H-7, H-8, H-9, H-10a), 3.66 - 3.60 (m, 1H, H-10b), 3.50 (dd, *J* = 5.0, 2.9 Hz, 1H, H-6), 2.34 (t, *J* = 7.0 Hz, 2H, H-2), 1.81 - 1.49 (m, 4H, H-3, H-4); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.5 (C-1), 159.3, 159.2, 130.5, 130.5, 130.4 (C_q), 129.7, 129.7, 129.6, 129.6, 113.9 (CH_{arom}), 76.6 (C-7, C-8, C-9), 75.6 (C-6), 74.5, 73.6 (C-7, C-8, C-9), 73.3, 73.0 (CH_2-PMB), 72.2 (C-5), 71.9, 71.3 (CH_2-PMB), 68.8 (C-10), 55.4, 55.4 (OMe), 33.6 (C-2), 29.1 (C-4), 21.2 (C-3); HRMS $[C_{42}H_{50}O_{11} + Na]^+$: 753.3247 found, 753.3245 calculated.

N_α-Fmoc-N_ε-[butan-4-(2,3,4,6-tetra-*O*-parmethoxybenzyl-α-D-mannopyranoside)-amide]-L-lysine-methyl ester (10).



carboxylic acid **8** (6.25 g, 8.55 mmol) was combined with amine **11** (3.58 g, 8.55 mmol, 1 eq) and HCTU (3.54 g, 8.55 mmol, 1 eq), dissolved in DMF (42.8 mL, 0.2 M) and DIPEA (4.47 mL, 25.65 mmol, 3 eq) was added dropwise. After two hours the mixture diluted with EtOAc, washed with a mixture of HCl (1 M, aq.) and brine (1/1, v/v), the aqueous layer was back extracted with EtOAc (1x), the organic layers were combined, washed with brine (1x), dried over $MgSO_4$ (s), filtered and concentrated *in vacuo*. Purification using silica gel column chromatography (1/1 → 9/1, EtOAc/PE, v/v) yielded title compound **10** as a white solid (9.30 g, 8.49 mmol, 99%). TLC R_f: 0.34 (EtOAc/PE, 4/1, v/v); IR (neat, cm^{-1}): 3331, 2934, 1720, 1648, 1611, 1585, 1512, 1451, 1301, 1246, 1173, 1082, 1033, 820, 760, 740; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.5 Hz, 2H, H_{arom}), 7.60 (d, *J* = 7.1 Hz, 2H, H_{arom}), 7.39 (t, *J* = 7.4 Hz, 2H, H_{arom}), 7.30 (t, *J* = 7.4 Hz, 2H, H_{arom}), 7.27 - 7.14 (m, 6H, H_{arom}), 7.09 (d, *J* = 8.4 Hz, 2H, H_{arom}), 6.83 (dd, *J* = 7.5, 5.0 Hz, 8H, H_{arom}), 5.94 (s, 1H, $N_\varepsilon H$), 5.48 (d, *J* = 8.1 Hz, 1H, $N_\alpha H$), 4.59 (d, *J* = 11.1 Hz, 1H, C*H*H-PMB), 4.53 - 4.36 (m, 9H, CH*H*-PMB, CH_2-PMB (3x), CH_2-Fmoc), 4.36 - 4.27 (m, 1H, H-2), 4.22 (t, *J* = 6.9 Hz, 1H, CH-Fmoc), 3.94 - 3.86 (m, 1H, H-5'), 3.84 - 3.55 (m, 21H, OMe (5x), H-7', H-8', H-9', H-10'), 3.51 - 3.45 (m, 1H, H-6'), 3.18 - 3.03 (m, 2H, H-6), 2.29 - 2.09 (m, 2H, H-2'), 1.86 - 1.58 (m, 4H, H-3, H-3'), 1.58 - 1.44 (m, 2H, H-4'), 1.44 - 1.25 (m, 4H, H-4, H-5); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.4, 173.1 (C-1, C-1'), 159.4, 159.4, 159.4, 159.3, 143.9, 141.4, 130.5, 130.5, 130.3 (C_q), 129.7, 129.6, 129.6, 127.8, 127.2, 125.2, 120.1, 113.9, 113.9, 113.9 (CH_{arom}),76.8 (C-7',C-8', C-9'), 75.9 (C-6'), 74.8, 73.6 (C-7',C-8', C-9'), 73.4 (CH_2-PMB), 73.2 (C-5'), 73.2, 71.9, 71.4(CH_2-PMB), 69.4 (C-10'), 67.1 (CH_2-Fmoc), 53.8 (C-2), 52.5 (COO*Me*), 47.3 (CH-Fmoc), 39.0 (C-6), 35.7 (C-2'), 32.1 (C-3), 29.2 (C-5), 28.3 (C-4'), 22.9 (C-3'), 22.6 (C-4); HRMS $[C_{64}H_{74}N_2O_{14} + Na]^+$: 1117.5038 found, 1117.5032 calculated.

N_α-Fmoc-N_ε-[butan-4-(2,3,4,6-tetra-*O*-parmethoxybenzyl-α-D-mannopyranoside)-amide]-L-lysine (1).



Methyl ester **10** (2.19 g, 2.0 mmol) was dissolved in THF (20 mL, 0.1 M) and *t*-BuOH (2 mL) and cooled to 0°C. A solution of LiOH (240 mg, 10.0 mmol, 5 eq) in H_2O_2 (50% wt. aq., 4 mL, 2 M) was added dropwise (pH >10) and stirred at 0°C for four hours. The mixture was diluted with EtOAc, washed with HCl (1 M, aq., 1x), the organic layer was dried over $MgSO_4$ (s), filtered, concentrated *in vacuo* and purified using silica gel column chromatography (2/8 → 8/2,

Acetone/DCM, v/v) to yield title compound **1** as a fluffy white powder after lyophilization from 1,4-dioxane (1.70 g, 1.57 mmol, 79%). TLC R_f: 0.23 (AcOH/EtOAc/PE, 1/80/20, v/v/v); IR (neat, cm^{-1}): 3333, 2933, 1718, 1612, 1586, 1512, 1451, 1301, 1246, 1174, 1080, 1032, 819, 760, 740; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H, H_{arom}), 7.61 (d, *J* = 5.9 Hz, 2H, H_{arom}), 7.39 (t, *J* = 7.4 Hz, 2H, H_{arom}), 7.30 (t, *J* = 7.1 Hz, 2H, H_{arom}), 7.27 - 7.08 (m, 8H, H_{arom}), 6.89 - 6.78 (m, 8H, H_{arom}), 5.93 (t, *J* = 5.9 Hz, 1H, $N_\varepsilon H$), 5.61 (d, *J* = 7.1 Hz, 1H, $N_\alpha H$), 4.58 (d, *J* = 11.2 Hz, 1H, C*H*H-PMB), 4.52 - 4.30 (m, 10H, CH*H*-PMB, CH_2-PMB (3x), CH_2-Fmoc, H-2), 4.22 (t, *J* = 7.1 Hz, 1H, CH-Fmoc), 3.93 - 3.87 (m, 1H, H-5'), 3.83 - 3.76 (m, 13H, OMe (4x), H-7'/ H-8'/ H-9'), 3.72 - 3.55 (m, 4H, H-10', H-7'/ H-8'/ H-9'), 3.49 (dd, *J* = 5.4, 2.5 Hz, 1H, H-6'), 3.20 (dt, *J* = 11.6, 5.3 Hz, 1H, H-6a), 3.09 - 2.96 (m, 1H, H-6b), 2.26 (dt, *J* = 14.3, 6.5 Hz, 1H, H-2a'), 2.11 (dt, *J* = 13.8, 6.3 Hz, 1H, H-2b'), 1.90 - 1.58 (m, 4H, H-3, H-3'), 1.53 - 1.27 (m, 6H, H-4', H-4, H-5); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.1, 173.8 (C-1, C-1'), 159.5, 159.5, 159.4, 156.1, 143.9, 141.4, 130.2 (C_q), 129.9, 129.8, 129.8, 129.6, 127.8, 127.2, 125.3, 120.1, 113.9, 113.9, 113.9 (CH_{arom}), 76.2 (C-7',C-8', C-9'), 75.9 (C-6'), 74.7, 73.5 (C-7',C-8', C-9'), 73.3 (CH_2-PMB), 73.2 (C-5'), 73.2, 72.0, 71.5 (CH_2-PMB), 69.1 (C-10'), 67.1 (CH_2-Fmoc), 55.4 (OMe), 53.6 (C-2), 47.3 (CH-Fmoc), 39.0 (C-6), 35.5 (C-2'), 31.8 (C-3), 29.8 (C-5), 28.8 (C-4'), 23.2 (C-3'), 21.9 (C-4); HRMS $[C_{63}H_{72}N_2O_{14} + H]^+$: 1081.5081 found, 1081.5059 calculated.

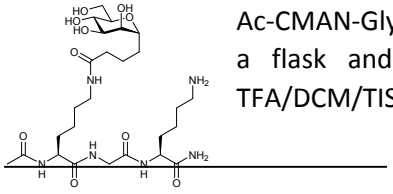
General procedure for manual solid phase synthesis:

The solid-phase peptide synthesis was performed starting with Tentagel S-RAM resin (~0.22 mmol/g) on a 45-50 µmol scale using established Fmoc protocols.[3] The consecutive steps performed in each cycle were:

1) DMF wash (1x) followed by nitrogen purge; *2)* Deprotection of the Fmoc-group with 20% piperidine in DMF (4 mL, 3 x 5 min); *3)* DMF wash (3x) followed by nitrogen purge; *4)* Coupling of the appropriate amino acid[1] in five-fold excess (unless stated otherwise)[2,3]; *5)* DMF wash (3x) followed by nitrogen purge; *6)* capping with a Ac_2O/DMF/DIPEA solution (4mL, 20/88/2, v/v/v) for 2 min; *7)* DMF wash (2x).

After the complete sequence capping was achieved by utilization of steps 1,2 & 3 followed by 6 and washing with DMF (3x), DCM (3x) and Et_2O (2x) followed by nitrogen purge.

Ac-CMAN-Gly-Lys-NH_2 (16).



Ac-CMAN-Gly-Lys(Boc)-Tentagel-S-RAM (**12**) (loading: 50 µmol) was transferred to a flask and treated for 120 minutes with a cleavage cocktail (20 mL, TFA/DCM/TIS/H_2O/phenol[4]/octanethiol, 3600/60/100/72/72/100, v/v/v/v/w/v).

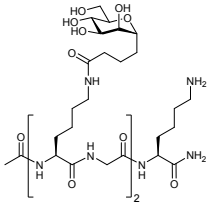
[1] The Fmoc amino acids applied in this synthesis were: Fmoc-Lys(Boc)-OH, Fmoc-Gly-OH, **1**[c].

[2] For couplings on 50 µmol scale: generally the Fmoc amino acid*** was dissolved in a HCTU solution in DMF (1.25 mL ,0.20 M, 0.25 mmol, 5 eq) This solution was transferred to the reaction vessel followed by a DIPEA solution in DMF (1.00 mL, 0.50 M, 0.50 mmol, 10 eq) to initiate the coupling. Next, the reaction vessel was shaken for 60 min at room temperature.

[3] For C-mannoside couplings less equivalents with prolonged reaction times were used. Generally **1** (2eq) was dissolved in a solution of HCTU in DMF (0,5 mL ,0.20 M, 100 µmol, 2 eq), followed by a DIPEA solution in DMF (0.40 mL, 0.5 M, 200 µmol, 4 eq) and shaken overnight.

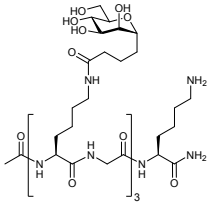
The resin was filtered off and washed with neat TFA (3 x 1 mL). The filtrate was concentrated and purified via gel filtration (Toyopearl HW40S, 150 mM NH_4HCO_3, 1.6x60 cm, 1 mL/min, 51.5 - 60 mL) followed by purification via RP-HPLC (linear-gradient 0 - 30 % B in A, 10 min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm, 5 mL/min) yielded title compound **16** as a white powder after lyophilization (2.11 mg, 3.49 µmol, 7.0% over 3 couplings). LC-MS: R_t = 3.42 min (0 - 50% ACN; 13 min); ^{1}H NMR (500 MHz, D_2O) δ 4.27 (dd, *J* = 9.5, 4.9 Hz, 1H), 4.19 (dd, *J* = 8.6, 5.8 Hz, 1H), 3.96 – 3.86 (m, 3H), 3.86 – 3.79 (m, 2H), 3.76 (dd, *J* = 9.4, 3.3 Hz, 1H), 3.69 (dd, *J* = 12.2, 6.2 Hz, 1H), 3.59 (t, *J* = 9.5 Hz, 1H), 3.50 – 3.44 (m, 1H), 3.15 (t, *J* = 7.0 Hz, 2H), 2.90 (t, *J* = 7.7 Hz, 2H), 2.26 (hept, *J* = 7.2 Hz, 2H), 2.01 (s, 3H), 1.91 – 1.27 (m, 16H); ^{13}C NMR (126 MHz, D_2O) δ 176.7, 176.3, 175.2, 174.5, 171.4, 77.9, 73.5, 71.4, 70.8, 67.3, 61.2, 54.1, 53.2, 42.5, 39.3, 38.9, 35.2, 30.3, 27.8, 26.9, 26.7, 22.3, 22.0, 21.8, 21.6; HRMS $[C_{26}H_{48}N_6O_{10} + H]^+$: 605.3503 found, 605.3505 calculated.

Ac-CMAN-Gly-CMAN-Gly-Lys-NH_2 (17).



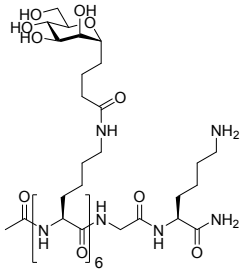
Ac-CMAN-Gly-CMAN-Gly-Lys(Boc)-Tentagel-S-RAM (**13**) (loading: 45 µmol) was transferred to a flask and treated for 120 minutes with a cleavage cocktail (10 mL, TFA/TIS/H_2O, 190/5/5, v/v/v). The mixture was concentrated to approximately one mL after which the resin was filtered off into cold Et_2O (45 mL) and the resin was washed off with neat TFA (3 x 1 mL) into the ether solution. This solution was centrifuged (10 min, 5000 rpm) after which the supernatant was removed and the precipitate was dried under nitrogen flow. Purification via RP-HPLC (linear gradient 0 - 30 % B in A, 10 min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm, 5 mL/min) yielded title compound **17** as a white powder after lyophilization (6.43 mg, 6.29 µmol, 14% over 5 couplings). LC-MS: R_t = 0.91 min (10 - 90% ACN; 13 min); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.28 (td, *J* = 8.6, 7.9, 5.3 Hz, 2H), 4.21 (dd, *J* = 8.7, 5.7 Hz, 1H), 3.96 – 3.91 (m, 4H), 3.91 – 3.86 (m, 2H), 3.86 – 3.80 (m, 4H), 3.77 (dd, *J* = 9.3, 3.3 Hz, 2H), 3.70 (dd, *J* = 12.1, 6.2 Hz, 2H), 3.61 (t, *J* = 9.5 Hz, 2H), 3.48 (ddd, *J* = 9.1, 6.2, 2.2 Hz, 2H), 3.16 (t, *J* = 6.9 Hz, 4H), 2.98 (t, *J* = 7.5 Hz, 2H), 2.34 – 2.19 (m, 4H), 2.02 (s, 3H), 1.93 – 1.23 (m, 26H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.6, 176.3, 176.3, 175.2, 174.8, 174.5, 171.6, 171.4, 77.9, 73.5, 71.4, 70.8, 67.3, 61.3, 54.2, 53.9, 53.1, 42.5, 42.4, 39.2, 38.9, 35.2, 30.3, 27.8, 26.7, 26.1, 22.4, 22.0, 21.9, 21.7; ESI-MS $[C_{44}H_{79}N_9O_{18} + H]^+$: 1022.400 found, 1022.562 calculated.

Ac-CMAN-Gly-CMAN-Gly-CMAN-Gly-Lys-NH_2 (18).



Ac-CMAN-Gly-CMAN-Gly-CMAN-Gly-Lys(Boc)-Tentagel-S-RAM (**14**) (loading: 45 µmol) was transferred to a flask and treated for 120 minutes with a cleavage cocktail (10 mL, TFA/TIS/H_2O, 190/5/5, v/v/v). The resin was filtered off into cold Et_2O (45 mL) and the resin was washed off with neat TFA (3 x 1 mL) into the ether solution. This solution was centrifuged (10 min, 5000 rpm) after which the supernatant was removed and the precipitate was dried under nitrogen flow. Purification via RP-HPLC (linear gradient 0 - 30 % B in A, 10 min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm, 5 mL/min) yielded title compound **18** as a white powder after lyophilization (3.91 mg, 2.71 µmol, 6.0% over 7 couplings). LC-MS: R_t = 4.54 min (20 - 50% ACN; 13 min); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.32 – 4.24 (m, 3H), 4.21 (dd, *J* = 8.6, 5.6 Hz, 1H), 3.96 – 3.86 (m, 9H), 3.86 – 3.80 (m, 6H), 3.77 (dd, *J* = 9.3, 3.3 Hz, 3H), 3.70 (dd, *J* = 12.1, 6.2 Hz, 3H), 3.61 (t, *J* = 9.5 Hz, 3H), 3.48 (ddd, *J* = 9.3, 6.2, 2.2 Hz, 3H), 3.15 (d, *J* = 6.5 Hz, 6H), 2.98 (t, *J* = 7.4 Hz, 2H), 2.34 – 2.18 (m, 6H), 2.02 (s, 3H), 1.92 – 1.22 (m, 36H); ESI-MS $[C_{62}H_{110}N_{12}O_{26} + H]^+$: 1439.533 found, 1439.773 calculated.

Ac-CMAN-CMAN-CMAN-CMAN-CMAN-CMAN-Gly-Lys-NH_2 (19).

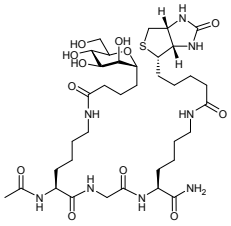


Ac-CMAN-CMAN-CMAN-CMAN-CMAN-CMAN-Gly-Lys(Boc)-Tentagel-S-RAM (**15**) (loading: 50 µmol) was transferred to a flask and treated for 120 minutes with a cleavage cocktail (20 mL, TFA/DCM/TIS/H_2O/phenol[4]/octanethiol, 3600/60/100/72/72/100, v/v/v/v/w/v). The resin was filtered off and washed with neat TFA (3 x 1 mL). The filtrate was concentrated and purified via gel filtration (Toyopearl HW40S, 150 mM NH_4HCO_3, 1.6x60 cm, 1 mL/min, 34 - 49 mL) followed by purification via RP-HPLC (linear gradient 0 - 30 % B in A, 10 min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm, 5 mL/min) to yield title compound **19** as a white powder after lyophilization (2.56 mg, 1.06 µmol, 2.1% over 8 couplings). LC-MS: R_t = 4.22 min (0 - 50% ACN; 13 min); 1H NMR (500 MHz, D2O) δ 4.30 – 4.18 (m, 6H), 4.18 – 4.14 (m, 1H), 3.93 (s, 2H), 3.88 (dd, *J* = 10.0, 3.4 Hz, 6H), 3.86 – 3.79 (m, 12H), 3.76 (dd, *J* = 9.4, 3.3 Hz, 6H), 3.69 (dd, *J* = 12.1, 6.2 Hz, 6H), 3.60 (t, *J* = 9.5 Hz, 6H), 3.47 (ddd, *J* = 9.0, 6.2, 2.1 Hz, 6H), 3.17 – 3.09 (m, 12H), 2.97 (t, *J* = 7.2 Hz, 2H), 2.24 (hept, *J* = 7.6 Hz, 12H), 2.01 (s, 3H), 1.89 – 1.25 (m, 66H); HRMS $[C_{106}H_{188}N_{16}O_{45} + 3H]^{3+}$: 803.1054 found, 803.1055 calculated.

General procedure for biotinylation:

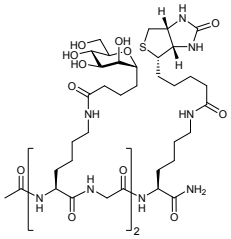
The "general procedure" to introduce the biotin handle: Glycoclusters described above with a free amine were dissolved in DMSO (0.02 M). To this, a stock solution of Biotin-NHS (0.15 M, 3-4 eq) and DIPEA (0.015M, 0.3-0.4 eq) in DMSO was added and shaken overnight after which compounds were purified via RP-HPLC (linear-gradient 10 - 16 % B in A, 12 min, 5 mL/min, Develosil RPAQUEOUS 10.0 x 250 mm) followed by lyophilization.

Ac-CMAN-Gly-Lys(biotin)-NH_2 (20).



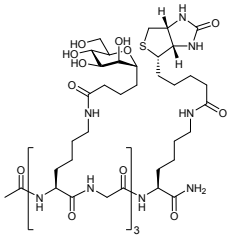
Compound **16** (1.54 mg, 1.23 µmol) was coupled with biotin-NHS using the general procedure. Compound **20** was obtained after purification by RP-HPLC as a white powder (1.98 mg, 2.38 µmol, 94%). LC-MS: R_t = 5.23 min (0 - 50% ACN; 13 min); HRMS $[C_{36}H_{62}N_8O_{12}S + H]^+$: 831.4281 found, 831.4281 calculated.

Ac-CMAN-Gly-CMAN-Gly-Lys(biotin)-NH_2 (21).



Compound **17** (6.43 mg, 6.30 µmol) was coupled with biotin-NHS using the general procedure. Compound **21** was obtained after purification by RP-HPLC as a white powder (5.64 mg, 4.51 µmol, 72%). LC-MS: R_t = 5.21 min (0 - 50% ACN; 13 min); ^{1}H NMR (500 MHz, D_2O) δ 4.62 (dd, *J* = 7.9, 4.4 Hz, 1H), 4.44 (dd, *J* = 7.9, 4.5 Hz, 1H), 4.33 – 4.27 (m, 2H), 4.25 (dd, *J* = 8.7, 5.7 Hz, 1H), 3.98 – 3.95 (m, 4H), 3.95 – 3.91 (m, 2H), 3.90 – 3.84 (m, 4H), 3.81 (dd, *J* = 9.4, 3.4 Hz, 2H), 3.74 (dd, *J* = 12.1, 6.2 Hz, 2H), 3.65 (t, *J* = 9.5 Hz, 2H), 3.52 (ddd, *J* = 9.2, 6.2, 2.3 Hz, 2H), 3.35 (dt, *J* = 9.7, 5.3 Hz, 1H), 3.20 (dt, *J* = 6.9, 3.6 Hz, 6H), 3.01 (dd, *J* = 13.1, 5.0 Hz, 1H), 2.79 (d, *J* = 13.0 Hz, 1H), 2.36 – 2.23 (m, 6H), 2.06 (s, 3H), 1.92 – 1.30 (m, 32H); HRMS $[C_{54}H_{93}N_{11}O_{20}S + 2H]^{2+}$: 624.8236 found, 624.8232 calculated.

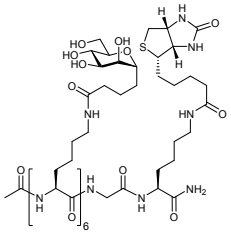
Ac-CMAN-Gly-CMAN-Gly-CMAN-Gly-Lys(biotin)-NH_2 (22).



Compound **18** (3.91 mg, 2.72 µmol) was coupled with biotin-NHS using the general procedure. Compound **22** was obtained after purification by RP-HPLC as

a white powder (9.29 mg, 5.58 µmol, 99%). LC-MS: R_t = 5.16 min (0 - 50% ACN; 13 min); HRMS $[C_{72}H_{124}N_{14}O_{28}S + 2H]^{2+}$: 833.4289 found, 833.4288 calculated.

Ac-CMAN-CMAN-CMAN-CMAN-CMAN-CMAN-Gly-Lys(biotin)-NH_2 (23).



Compound **19** (2.09 mg, 0.87 µmol) was coupled with biotin-NHS using the general procedure. Compound **23** was obtained after purification by RP-HPLC as a white powder (1.84 mg, 0.70 µmol, 80%). LC-MS: R_t = 5.06 min (0 - 50% ACN; 13 min); HRMS $[C_{116}H_{202}N_{18}O_{47}S + 3H]^{3+}$: 1317.1939 found, 1317.1935 calculated.

General procedure for automated solid phase synthesis of gp100 peptides:

The solid-phase peptide synthesis was performed on a TRIBUTE® Peptide Synthesizer (Gyros Protein Technologies AB, Arizona, USA) applying Fmoc based protocol starting with Tentagel S-RAM resin (~0.22 mmol/g) on a 100-250 µmol scale using established synthetic protocols.[3] The consecutive steps for synthesis on 250 µmol scale[4] performed in each cycle were:

1) DMF wash (1x) followed by nitrogen purge; *2)* Deprotection of the Fmoc-group with 20% piperidine in DMF (8 mL)(3 x 3 min at 50 °C); *3)* DMF wash (3x) followed by nitrogen purge; *4.1)* Coupling of the appropriate amino acid[5] in four-fold excess (unless stated otherwise);[6,7,8] *4.2)* Step 4.1 was repeated *5)* DMF wash (3x) followed by nitrogen purge; *6)* capping with a solution of Ac_2O/DMF/DIPEA (8mL, 10/88/2, v/v/v) for 2 min; *7)* DMF wash (2x).

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[4] All amounts were scaled-down in equimolar proportions for smaller scale.

[5] The amino acids applied in this synthesis were: Fmoc-Lys(Mmt)-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Trp(Boc)-OH, Fmoc-L-α-aminobutyric acid, Fmoc-Asp(O*t*Bu)-OH[d], Fmoc-Leu-OH[d], Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Glu(O*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, Fmoc-Pro-OH, Fmoc-Tyr(*t*Bu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Val-OH, Fmoc-His(Trt)-OH, Fmoc-AEEA-OH *(Fmoc-8-amino-3,6-dioxaoctanoic acid)*, **32** and **1**.[e]

[6] Generally, the Fmoc amino acid is dissolved in a HCTU solution in DMF (5.00 mL ,0.20 M, 1.0 mmol, 4 eq) The resulting solution was transferred to the reaction vessel followed by a DIPEA solution in DMF (4.00 mL, 0.50 M, 2.0 mmol, 8 eq) to initiate the coupling. The reaction vessel was shaken for 30 min at 50 °C (unless stated otherwise).

[7] Aspartic acid and the adjacent Leucine and Arginine were introduced at with one hour reaction time at room temperature. Fmoc removal was achieved with piperide/DMF in 3 x 5 min at room temperature.[8]

[8] For C-mannoside couplings less equivalents with prolonged reaction times were used. Generally for elongation on 100 µmol scale, **1** (2eq) was dissolved in a solution of HCTU in DMF (1,0 mL ,0.20 M, 200 µmol, 2 eq), followed by a DIPEA solution in DMF (0.80 mL, 0.5 M, 400 µmol, 4 eq) and shaken overnight.

After the complete sequence the resin was washed with DMF (3x), DCM (3x), Et_2O (3x), followed by nitrogen purge before treatment with the cleavage cocktail.

Val-Thr(*t*Bu)-His(Trt)-Thr(*t*Bu)-Tyr(*t*Bu)-Leu-Glu(O*t*Bu)-Pro-Gly-Pro-Val-Thr(*t*Bu)-Ala-Asn(Trt)-Arg(Pbf)-Gln(Trt)-Leu-Tyr(*t*Bu)-Pro-Glu(O*t*Bu)-Trp(Boc)-Thr(*t*Bu)-Glu(O*t*Bu)-Ala-Gln(Trt)-Arg(Pbf)-Leu-Asp(O*t*Bu)-$_\alpha$Abu-Trp(Boc)-Arg(Pbf)-Gly-Lys(Mmt)-Tentagel-S-Rink amide (50).
Peptide synthesis was performed on a 250 µmol scale using the general procedure. Resulting in functionalized resin used for the assembly of resins **33** and **34**.

Resin 33.

Immobilized gp100 peptide previously described[5] (theoretical loading: 25 µmol) was elongated with **1** (54 mg, 50 µmol, 2 eq) by shaking overnight at room temperature with HCTU (20.7 mg, 50 µmol, 2 eq) and DIPEA (17.4 µL, 100 µmol, 4 eq) in DMF (450 µL, 0.11 M of **1**). After washing with DMF (3x), the Fmoc was removed with piperidine/DMF (1/4, v/v, 3 x 5 min), and the resin was washed with DMF (3x) to obtain resin **33**.

Resin 34.

Immobilized gp100 peptide previously described[5] (theoretical loading: 100 µmol) was elongated with **1** (216 mg, 200 µmol, 2 eq) by shaking overnight at room temperature with HCTU (82.6 mg, 200 µmol, 2 eq) and DIPEA (69.5 µL, 400 µmol, 4 eq) in DMF (1.8 mL, 0.11 M of **1**). After washing with DMF (3x), the Fmoc was removed with piperidine/DMF (1/4, v/v, 3 x 5 min), was washed with DMF (3x), and the sequence was repeated (6x in total) to obtain resin **34**.

Conjugate 39.

Resin **33** (theoretical loading: 25 µmol) was elongated using the general procedure (see SI) to introduce Fmoc-AEEA-OH (4eq). Further elongation with **32** (46 mg, 100 µmol, 4 eq) was achieved by shaking for two hours with HCTU (41 mg, 100 µmol, 4 eq) and DIPEA (34.8 µL, 200 µmol, 8 eq) in DMF (0.90 mL, 0.11 M of **32**) after which the resin was washed with DMF (3x), DCM (3x), Et_2O (3x) and dried by nitrogen purge. This resin was transferred to a flask and treated for 120 minutes with a cleavage cocktail (20 mL, TFA/DCM/TIS/H_2O/phenol[4]/octanethiol, 3600/60/100/72/72/100, v/v/v/v/w/v). The resin was filtered off and washed with neat TFA (3 x 1 mL). The filtrate was concentrated and transferred dropwise into a cold mixture of Et_2O/pentane (45 mL, 5/4, v/v). This solution was centrifuged (10 min, 5000 rpm) after which the supernatant was removed, and the precipitate was dried under nitrogen flow, re-dissolved in magic (5 mL, *t*-BuOH/ACN/H_2O, 1/1/1, v/v/v) and lyophilized. Purification via RP-HPLC (linear-gradient 25 - 35 % B in A, 10 min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm, 5 mL/min) yielded conjugate **39** as a white powder after lyophilization (3.09 mg, 651 nmol, 2.60 % over 36 couplings). LC-MS: R_t = 4.46 min (10 - 90% ACN; 13 min); R_t = 7.29 min (0 - 50% ACN; 13 min); HRMS $[C_{215}H_{323}N_{59}O_{63} + 6H]^{6+}$: 791.23965 found, 791.23964 calculated.

Conjugate 40.

Resin **34** (theoretical loading: 50 µmol) was elongated using the general procedure (see SI) to introduce Fmoc-AEEA-OH (4eq). Further elongation with **32** (92 mg, 200 µmol, 4eq) was achieved by shaking for two hour with HCTU (82 mg, 200 µmol, 4eq) and DIPEA (70 µL, 400 µmol, 8 eq) in DMF (1.80 mL, 0.11 M of **32**) after which the resin was washed with DMF (3x), DCM (3x), Et_2O (3x) and dried by nitrogen purge. This resin was transferred to a flask and treated for 120 minutes with a cleavage cocktail (20 mL, TFA/DCM/TIS/H_2O/phenol[4]/octanethiol, 3600/60/100/72/72/100, v/v/v/v/w/v). The resin was filtered off and washed with neat TFA (3 x 1 mL). The filtrate was concentrated and transferred

dropwise into a cold mixture of Et_2O/pentane (45 mL, 5/4, v/v). This solution was centrifuged (10 min, 5000 rpm) after which the supernatant was removed, and the precipitate was dried under nitrogen flow, re-dissolved in magic (5 mL, *t*-BuOH/ACN/H_2O, 1/1/1, v/v/v) and lyophilized. Purification via RP-HPLC (linear-gradient 16 - 28 % B in A, 10 min, Gemini-NX 5µm C18, 110 Å, 250 x 12.0 mm, 5 mL/min) yielded conjugate **40** as a white powder after lyophilization. (4.125 mg, 650 nmol, 1.30% over 41 couplings). LC-MS: R_t = 7.73 min (15 - 40% ACN; 15 min); R_t = 5.82 min (10 - 50% ACN; 13 min); HRMS $[C_{295}H_{463}N_{69}O_{98} + 6H]^{6+}$: 1091.56505 found, 1091.56485 calculated.

Conjugate 41.

Resin **33** (theoretical loading: 25 µmol) was N-terminally capped with Ac_2O/DMF/DIPEA (4mL, 10/88/2, v/v/v, 3 x 5 min) and the resin was washed with DMF (3x), DCM (3x), Et_2O (3x). This resin was loaded in a syringe with frit and treated with a mixture of AcOH in TFE and DCM (1/2/7, v/v/v) shaken for 15 minutes followed by filtration[6]. This was repeated until the filtrate lost the yellow color (~ 8x). After which the resin was washed with DCM (5x), DMF (5x) Piperidine/DMF (1/4, v/v, 2x), and DMF (5x). It was C-terminally elongated using the general procedure (see SI) to introduce Fmoc-AEEA-OH (4eq). Further elongation with **32** (46 mg, 100 µmol, 4 eq) was achieved by shaking for two hour with HCTU (41 mg, 100 µmol, 4 eq) and DIPEA (34.8 µL, 200 µmol, 8 eq) in DMF (0.90 mL, 0.11 M of **32**) after which the resin was washed with DMF (3x), DCM (3x), Et_2O (3x) and dried by nitrogen purge, transferred to a flask and treated for 120 minutes with a cleavage cocktail (20 mL, TFA/DCM/TIS/H_2O /phenol[4]/octanethiol, 3600/60/100/72/72/100, v/v/v/v/w/v). The resin was filtered off and washed with neat TFA (3 x 1 mL). The filtrate was concentrated and transferred dropwise into a cold mixture of Et_2O/pentane (45 mL, 5/4, v/v) This solution was centrifuged (10 min, 5000 rpm) after which the supernatant was removed, and the precipitate was dried under nitrogen flow, re-dissolved in magic (5 mL, *t*-BuOH/ACN/H_2O, 1/1/1, v/v/v) and lyophilized. Purification via RP-HPLC (linear gradient 21 - 36 % B in A, 15 min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm, 5 mL/min) yielded conjugate **41** as a white powder after lyophilization (2.51 mg, 525 nmol, 2.10% over 36 couplings) LC-MS: R_t = 4.53 min (10 - 90% ACN; 13 min); HRMS $[C_{217}H_{325}N_{59}O_{64} + 5H]^{5+}$: 957.68821 found, 957.68822 calculated.

Conjugate 42.

Resin **34** (theoretical loading: 62.5 µmol) was N-terminaly capped with Ac_2O/DMF/DIPEA (8mL, 10/88/2, v/v/v, 3 x 5 min) and the resin was washed with DMF (3x), DCM (3x), Et_2O (3x). This resin was loaded in a syringe with frit and treated with a mixture of AcOH in TFE and DCM (1/2/7, v/v/v) shaken for 15 minutes followed by filtration[6]. This was repeated until the filtrate lost the yellow color (~ 8x). After which the resin was washed with DCM (5x), DMF (5x) Piperidine/DMF (1/4, v/v, 2x) and DMF (5x). It was C-terminaly elongated using the general procedure (see SI) to introduce Fmoc-AEEA-OH (4eq). Further elongation with **32** (115 mg, 250 µmol, 4 eq) was achieved by shaking for two hour with HCTU (103 mg, 250 µmol, 4 eq) and DIPEA (87 µL, 500 µmol, 8 eq) in DMF (2.25 mL, 0.11 M of **32**) after which the resin was washed with DMF (3x), DCM (3x) and Et_2O (3x) and dried by nitrogen purge, transferred to a flask and treated for 120 minutes with a cleavage cocktail (40 mL, TFA/DCM/TIS/H_2O /phenol[4]/octanethiol, 3600/60/100/72/72/100, v/v/v/v/w/v). The resin was filtered off and washed with neat TFA (3 x 1 mL). The filtrate was concentrated and transferred dropwise into a cold mixture of Et_2O/pentane (45 mL, 5/4, v/v) This solution was centrifuged (10 min, 5000 rpm) after which the supernatant was removed and the precipitate was dried under nitrogen flow, re-dissolved in magic (5 mL, *t*-BuOH/ACN/H_2O, 1/1/1, v/v/v) and lyophilized. Purification via RP-HPLC (linear gradient 22.5 - 30 % B in A, 15 min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm, 5 mL/min) yielded conjugate **42** as a white powder after lyophilization (2.48 mg, 376 nmol, 0.60% over 41 couplings). LC-MS: R_t = 7.11 min (0 - 50% ACN; 13 min); HRMS $[C_{297}H_{465}N_{69}O_{99} + 4H]^{4+}$: 1647.34697 found, 1647.34628 calculated.

General alkyne introduction procedure:

The "general procedure" to introduce the alkyne handle: A solution of glycoclusters **24** or **27**[5] (0.2 M, aq., 1 eq) was mixed with a stock solution of pent-4-ynoic (0.15 M, 3 eq) and DIPEA (0.05 M, 1 eq) in DMSO and shaken for one hour. Reaction progress was followed via LC-MS and when completed, the 4-pentynoic amides were purified via gel filtration (Toyopearl HW-40 S, 150 mM NH_4HCO_3, 1.6x60 cm, 1 mL/min) or RP-HPLC (linear-gradient, 5 mL/min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm) followed by lyophilization.

Ac-Lys(Man_1)-Gly-Lys(pent-4-ynoic amide)-NH_2 (43).

Compound **24** (5.72 mg, 9.28 µmol) was coupled with pent-4-ynoic using the general procedure. Compound **43** was obtained after RP-HPLC (linear-gradient 5 - 45% B, 10min) as a white powder (4.01 mg, 5.75 µmol, 62%). LC-MS: R_t = 4.48 min (0 - 50% ACN; 13 min); ^{1}H NMR (400 MHz, D_2O) δ 8.03 (s, 1H), 4.93 (d, *J* = 1.7 Hz, 1H), 4.82 – 4.80 (m, 1H), 4.68 (d, *J* = 12.4 Hz, 1H), 4.42 (t, *J* = 6.9 Hz, 2H), 4.26 – 4.14 (m, 2H), 3.92 – 3.57 (m, 8H), 3.16 (t, *J* = 6.9 Hz, 2H), 2.49 – 2.42 (m, 2H), 2.42 – 2.36 (m, 2H), 2.32 (t, *J* = 2.5 Hz, 1H), 1.97 (s, 3H), 1.89 (q, *J* = 7.3 Hz, 2H), 1.85 – 1.62 (m, 4H), 1.48 (q, *J* = 8.2, 7.5 Hz, 2H), 1.34 (ddd, *J* = 23.0, 15.9, 7.4 Hz, 4H); ^{13}C NMR (101 MHz, D_2O) δ 174.9, 171.3, 99.4, 72.9, 70.4, 70.1, 69.9, 66.6, 60.8, 59.7, 53.8, 50.1, 42.4, 39.0, 34.5, 34.5, 30.5, 22.3, 21.8, 21.6, 14.6;HRMS [$C_{30}H_{48}N_8O_{11}$ + H]$^+$: 697.3515 found, 697.3515 calculated.

Ac-Lys(Man_1)-Lys(Man_1)-Lys(Man_1)-Lys(Man_1)-Lys(Man_1)-Lys(Man_1)-Gly-Lys(pent-4-ynoic amide)-NH_2 (44).

Compound **27** (8.0 mg, 2.1 µmol) was coupled with pent-4-ynoic using the general procedure. Compound **44** was obtained after purification by gel filtration (eluted at 41 - 52 mL) or RP-HPLC (linear-gradient 5 - 45% B, 10min) as a white powder (3.52 mg, 1.38 µmol, 66%). LC-MS: R_t = 5.09 min (0 - 50% ACN; 13 min); ^{1}H NMR (500 MHz, D_2O) δ 7.95 (d, *J* = 5.6 Hz, 6H), 4.87 (d, *J* = 1.7 Hz, 6H), 4.71 (d, *J* = 12.5, 3.9 Hz, 6H), 4.58 (d, *J* = 12.5 Hz, 6H), 4.31 (t, *J* = 6.5 Hz, 12H), 4.20 – 3.99 (m, 7H), 3.91 – 3.49 (m, 38H), 3.08 (t, *J* = 6.8 Hz, 2H), 2.39 – 2.22 (m, 5H), 1.92 (s, 3H), 1.86 – 1.08 (m, 42H); ^{13}C NMR (126 MHz, D_2O) δ 160.5, 99.4, 72.9, 70.5, 69.9, 66.6, 60.8, 59.8; HRMS [$C_{105}H_{168}N_{28}O_{46}$ + 2H]$^{2+}$: 1280.0931 found, 1280.0921 calculated.

General procedure for the CuAAC of alkyne mannoside clusters and azido-gp100 peptides:

The "general click protocol" used for the conjugation of alkynes and azido-gp100 peptides: All solvents used in these reactions were degassed by sonicating while bubbling argon through the solutions. A solution of azido-peptide **45**[5] in DMSO was mixed with a solution of alkyne functionalized glycoclusters in water **43** or **44** followed by addition of an aliquot of a stock solution of CuI (0.1 eq), THPTA (0.3 eq) and DIPEA (0.2 eq) in water ([Cu^+] = 0.5 M). The reaction was stirred at 45°C and the process was followed via LC-MS. When reactions did not progress and turned blue, a stock solution of sodium ascorbate (0.25 M) and arginine[7] (0.5 M, 0.2 - 1 eq ascorbate) in water was added. After completion a small amount of Quadrasil® AP (washed with water) was added, stirred for 1 h, filtered and purified by either gel filtration (Toyopearl HW-40S, 150 mM NH_4HCO_3, buffer contained 20% ACN, 1.6x60 cm, 1 mL/min) and/or RP-HPLC (linear-gradient, 5 mL/min, Gemini-NX 5µm C18, 110 Å, 250 x 10.0 mm) followed by lyophilization.

Ac-Lys(*[Ac-Lys(Man$_1$)-Gly-Lys(*triazolylpentyl-5-amide)-NH$_2$])-Val-Thr-His-Thr-Tyr-Leu-Glu-Pro-Gly-Pro-Val-Thr-Ala-Asn-Arg-Gln-Leu-Tyr-Pro-Glu-Trp-Thr-Glu-Ala-Gln-Arg-Leu-Asp-$_\alpha$Abu-Trp-Arg-Gly-Lys(Peg-TLR7L)-NH$_2$ (46).
A solution of mannosides cluster **43** (75 µL, 0.02 M, 1.50 µmol, 1.5 eq, aq.) and azido-peptide **45**[5] in DMSO (100 µL, 0.01 M, 1.0 µmol, 1 eq) were conjugated using the general click protocol. After purification by RP-HPLC (linear-gradient 7 - 42% B, 11 min) compound **46** was obtained as a white powder (1.800 mg, 341 nmol, 34%). LC-MS: R_t = 4.51 min (10 - 90% ACN; 13 min); HRMS [$C_{237}H_{355}N_{69}O_{69}$ + 5H]$^{5+}$: 1055.9369 found, 1055.9368 calculated.

Ac-Lys(*[Ac-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Lys(Man$_1$)-Gly-Lys(*triazolylpentyl-5-amide)-NH$_2$])-Val-Thr-His-Thr-Tyr-Leu-Glu-Pro-Gly-Pro-Val-Thr-Ala-Asn-Arg-Gln-Leu-Tyr-Pro-Glu-Trp-Thr-Glu-Ala-Gln-Arg-Leu-Asp-$_\alpha$Abu-Trp-Arg-Gly-Lys(Peg-TLR7L)-NH$_2$ (47).
A solution of mannosides cluster **44** (120 µL, 0.01 M, 1.20 µmol, 1.2 eq, aq.) and azido-peptide **45**[5] in DMSO (100 µL, 0.01 M, 1.0 µmol, 1 eq) were conjugated using the general click protocol. After purification by RP-HPLC (linear-gradient 8 - 42% B, 10 min) compound **47** was obtained as a white powder (2.085 mg, 292 nmol, 29%). LC-MS: R_t = 7.19 min (0 - 50% ACN; 13 min); R_t = 4.38 min (10 - 90% ACN; 13 min); HRMS [$C_{312}H_{475}N_{89}O_{104}$ + 6H]$^{6+}$: 1190.4189 found, 1190.4194 calculated.

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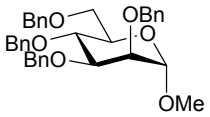
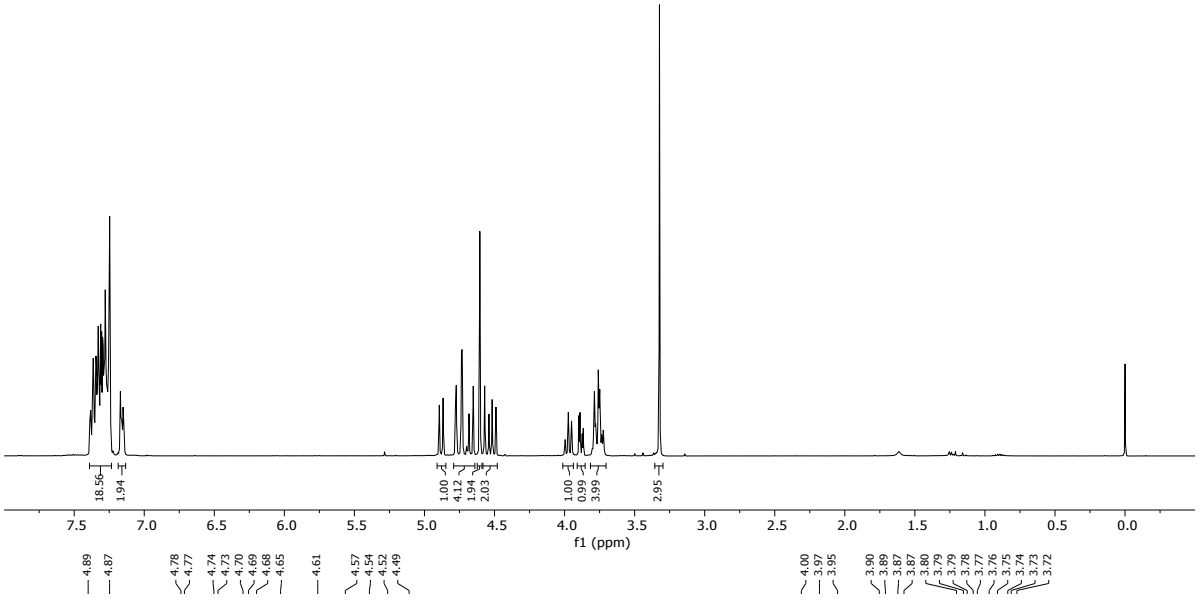
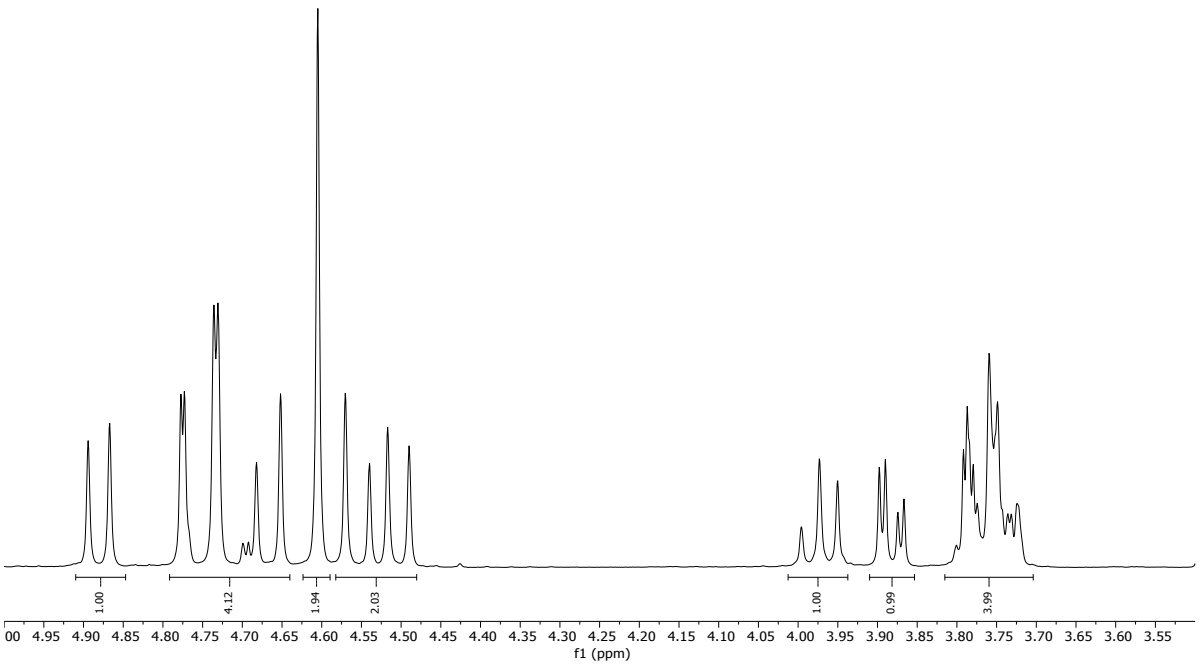
Spectral data

Compound 3

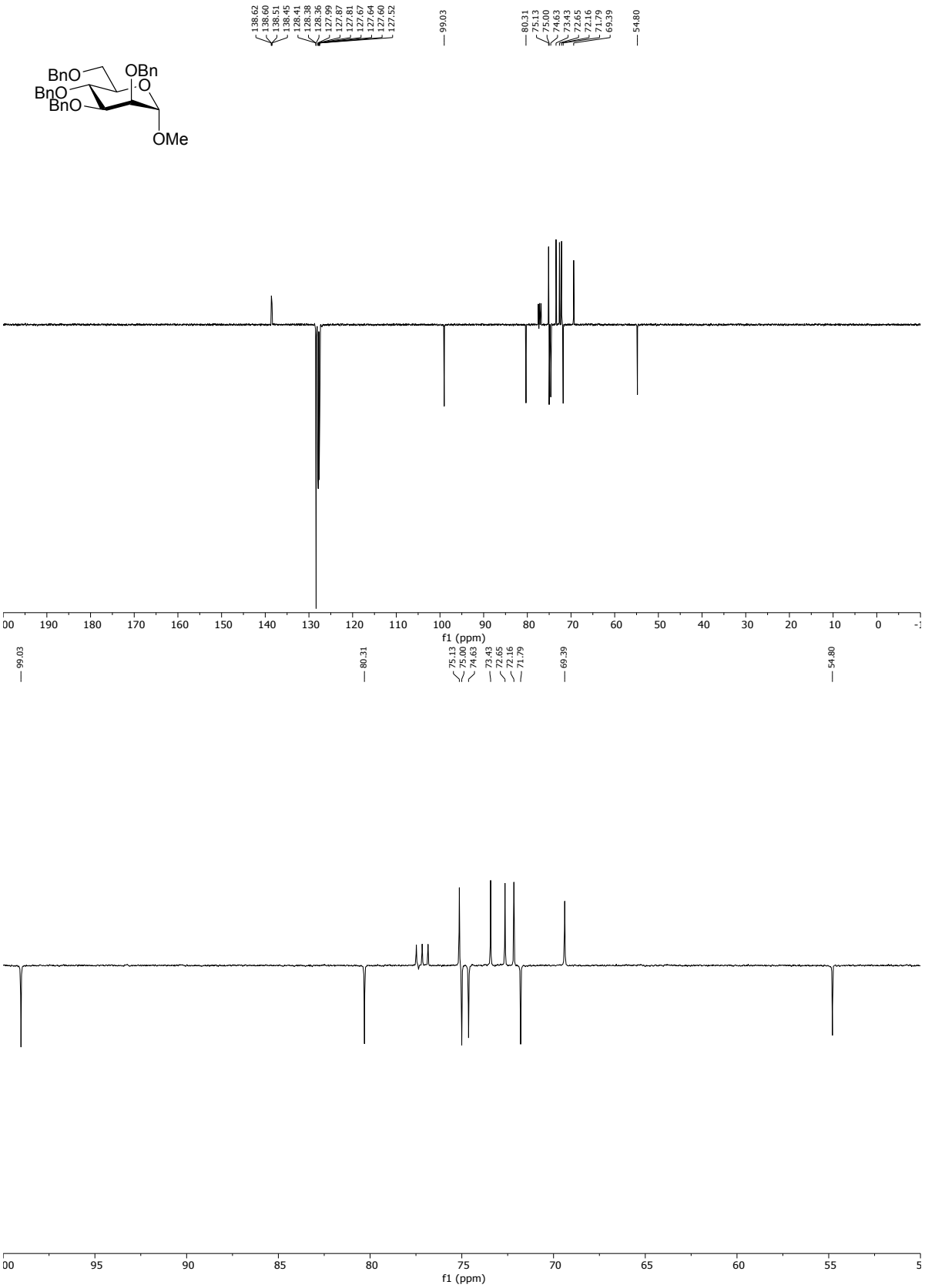
^{1}H- NMR







^{13}C APT NMR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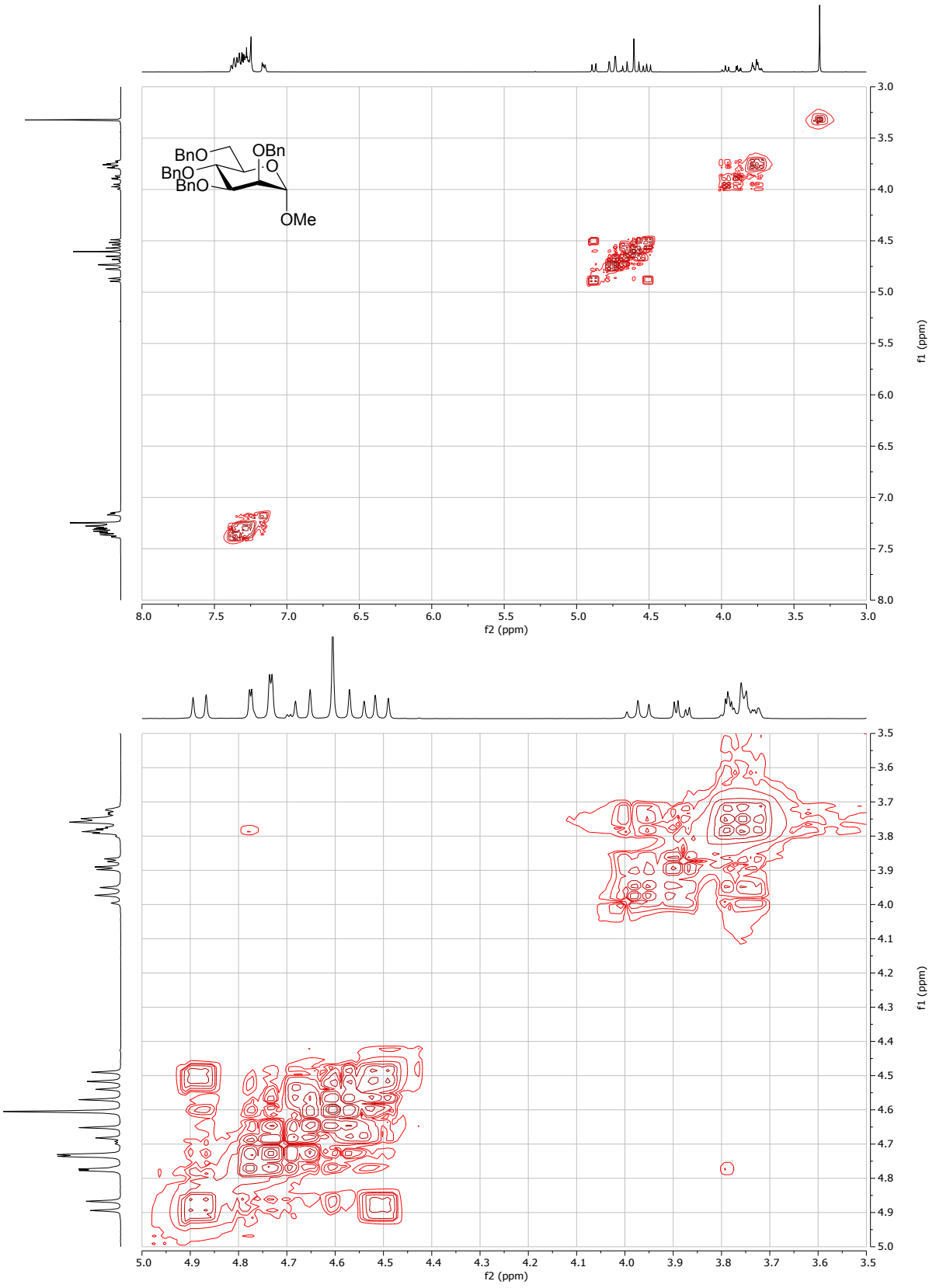

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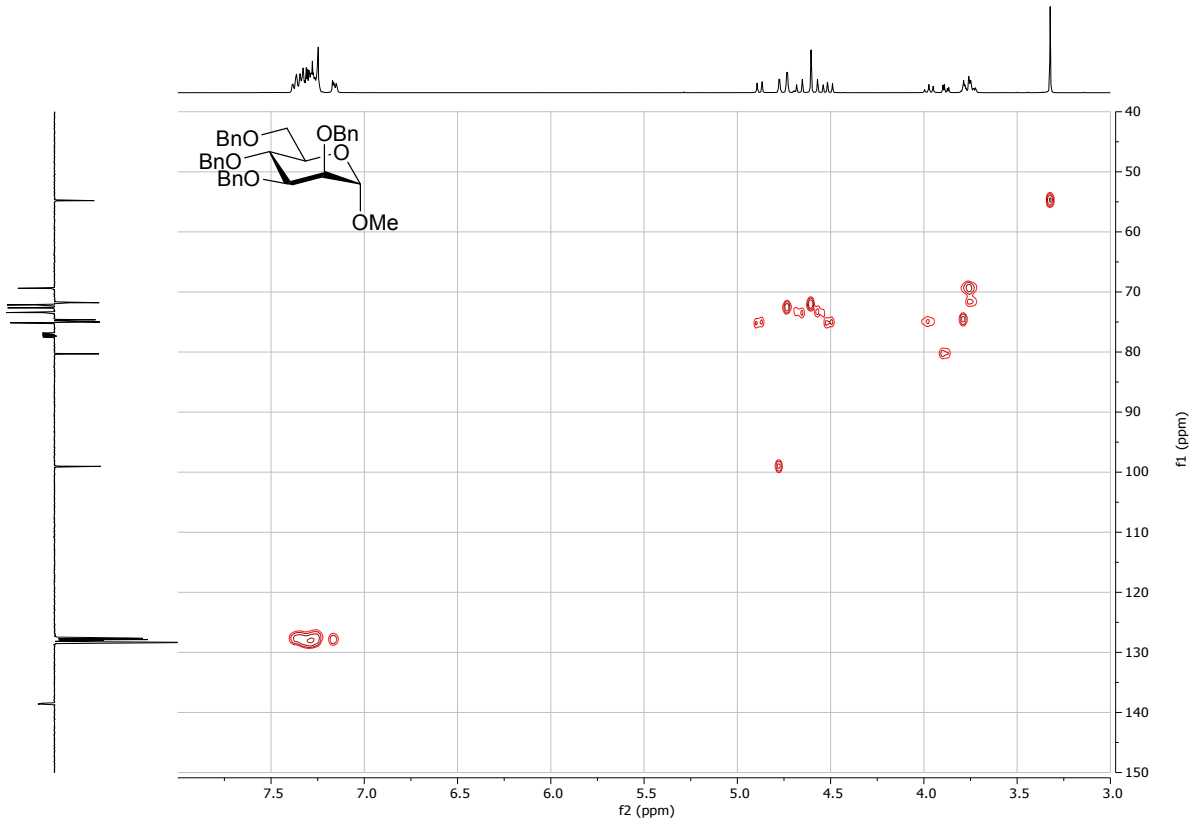
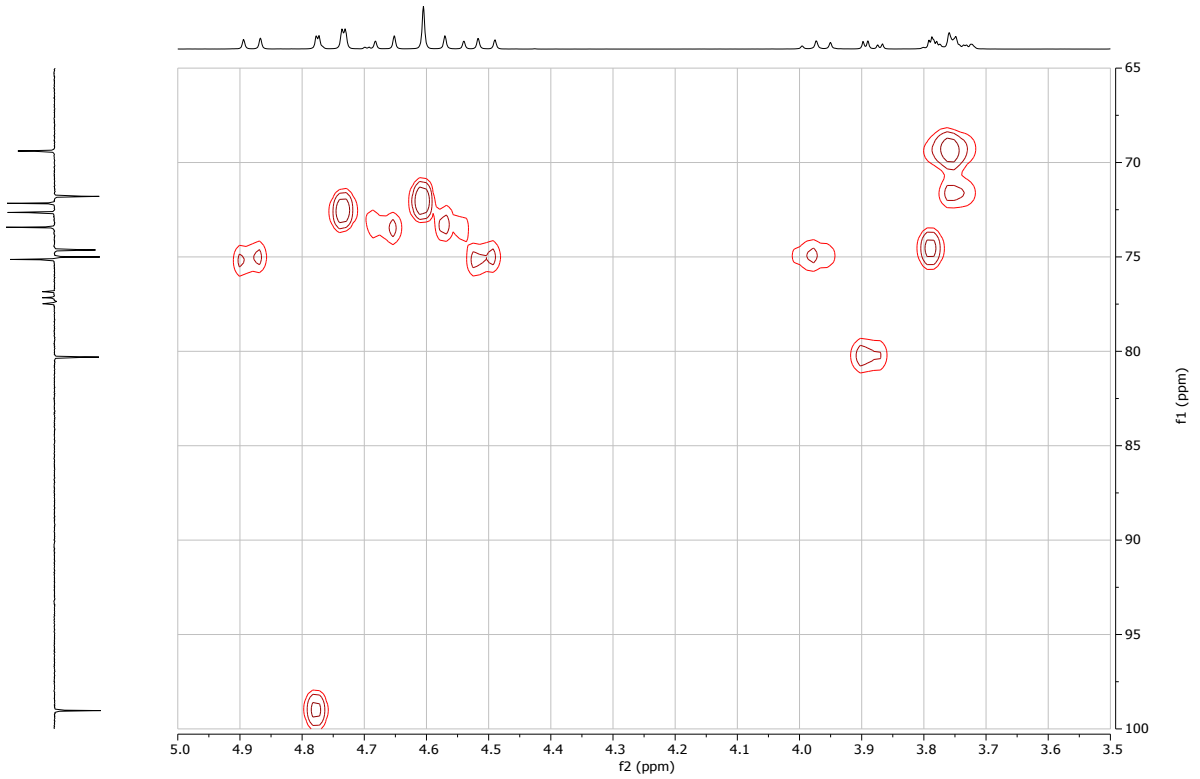
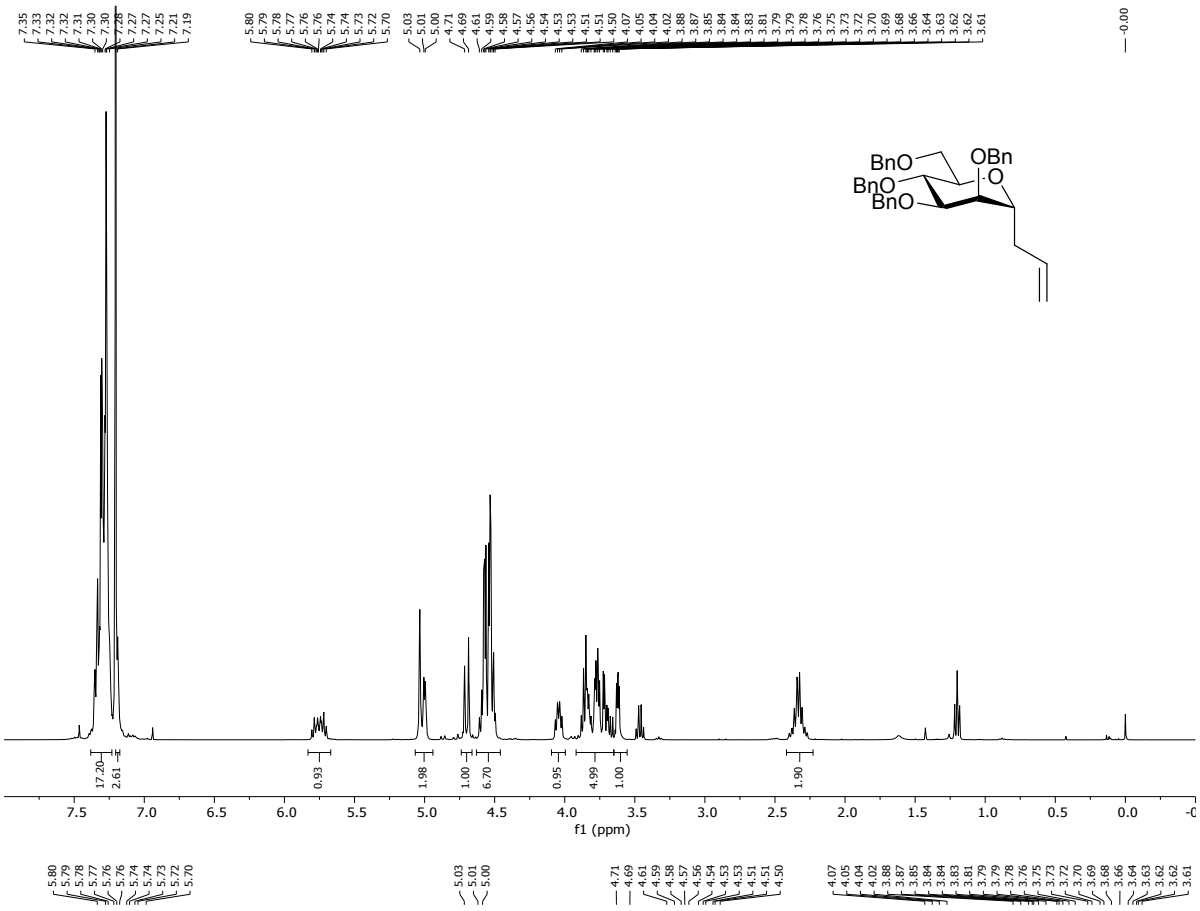
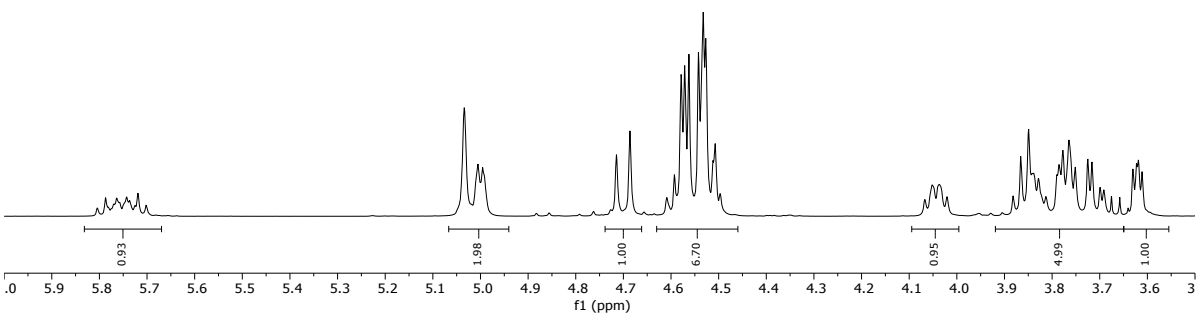




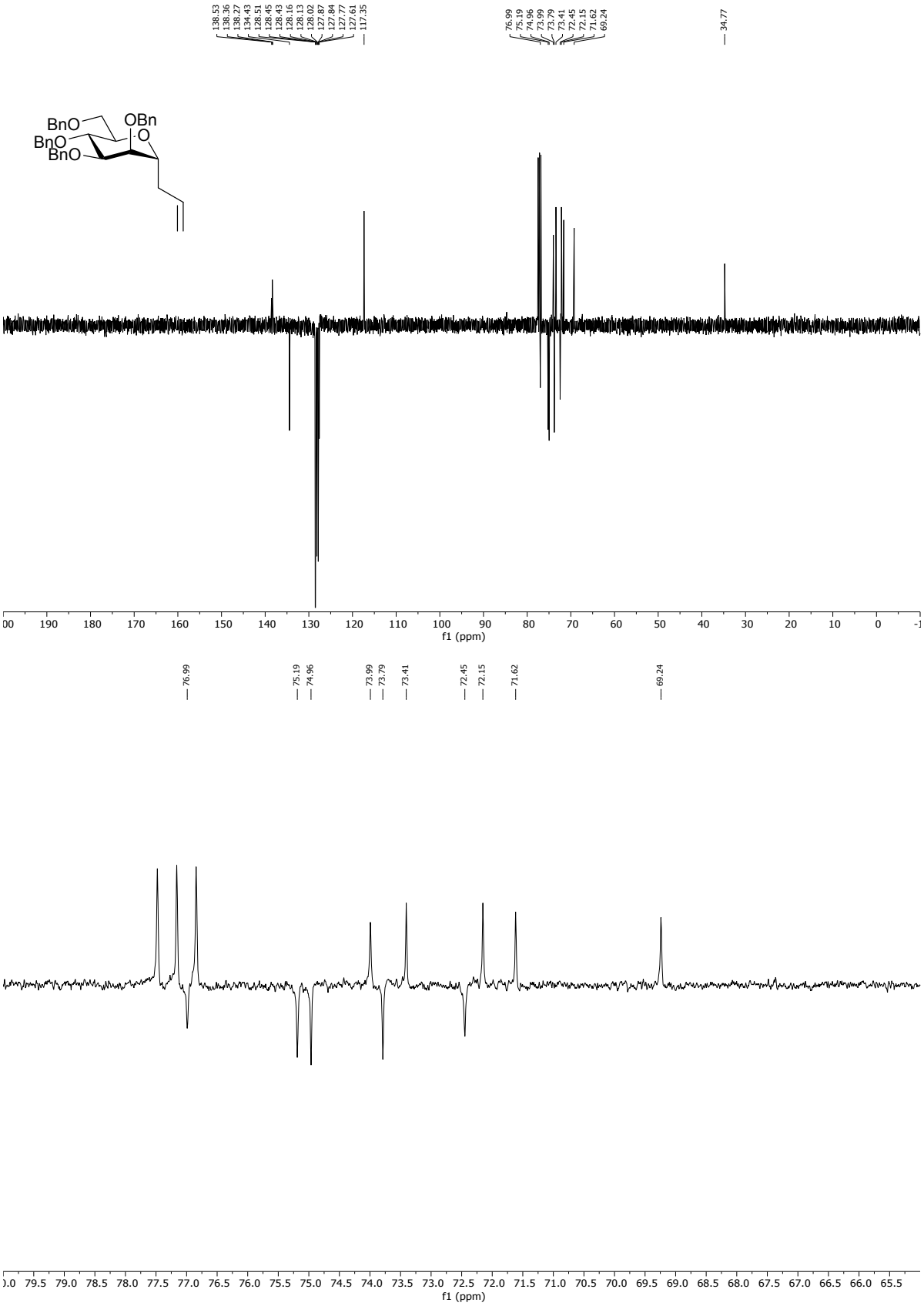
Compound 4

^{1}H NMR





^{13}C APT NMR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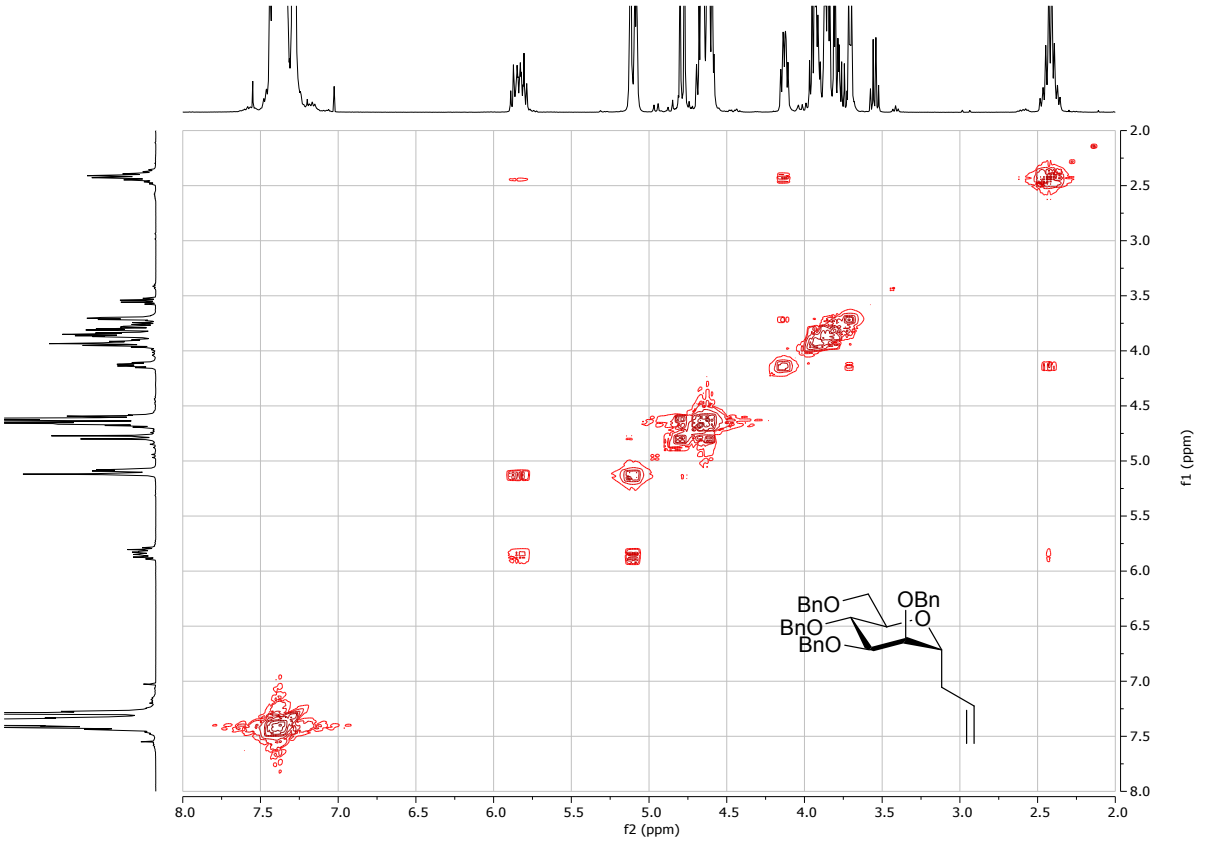
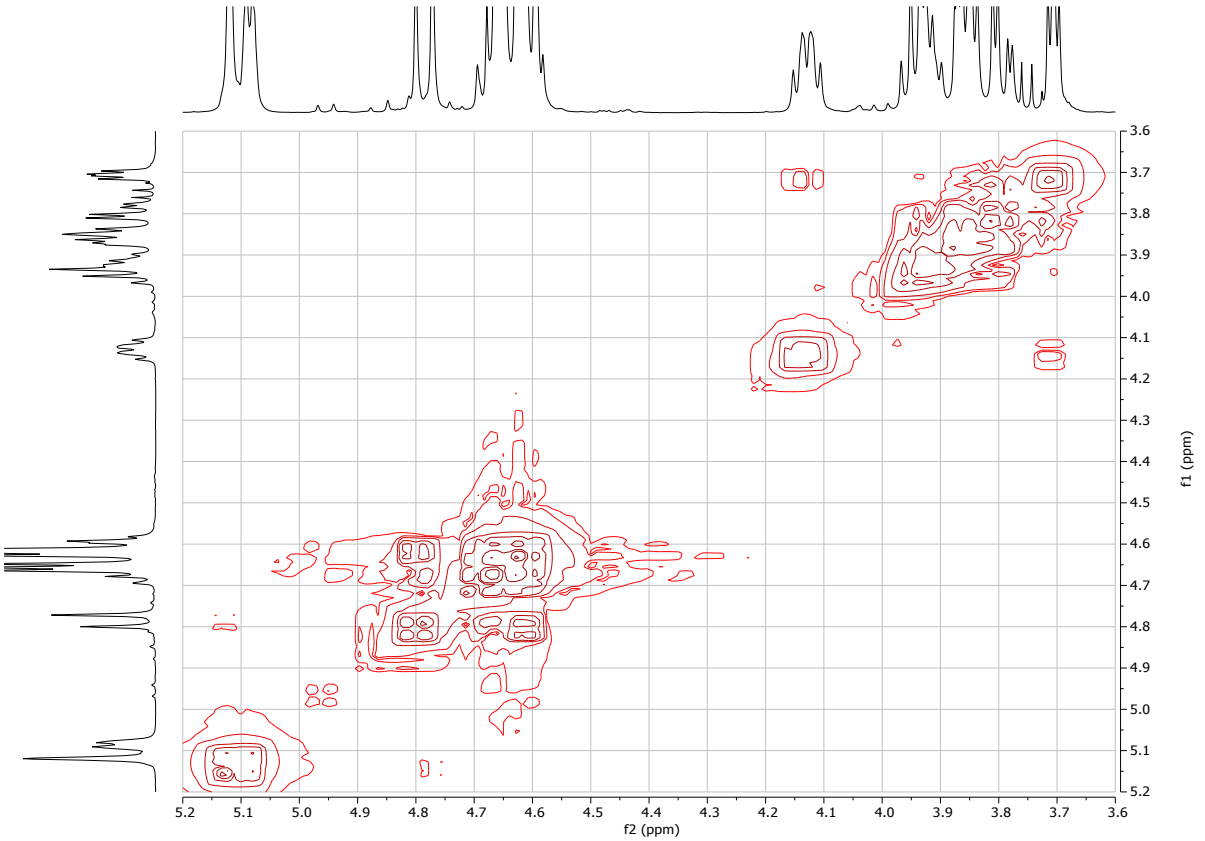
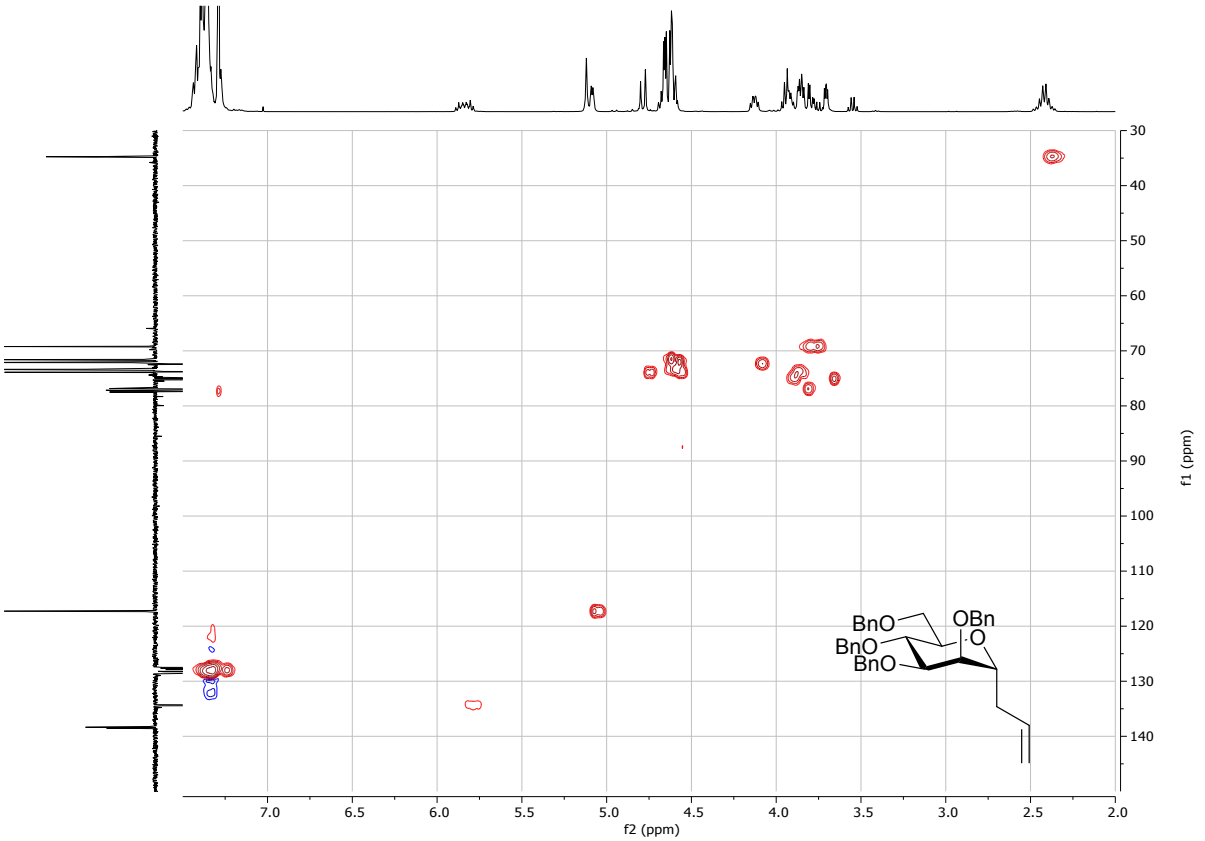
72.45
72.15
71.62
69.24
f1 (ppm)

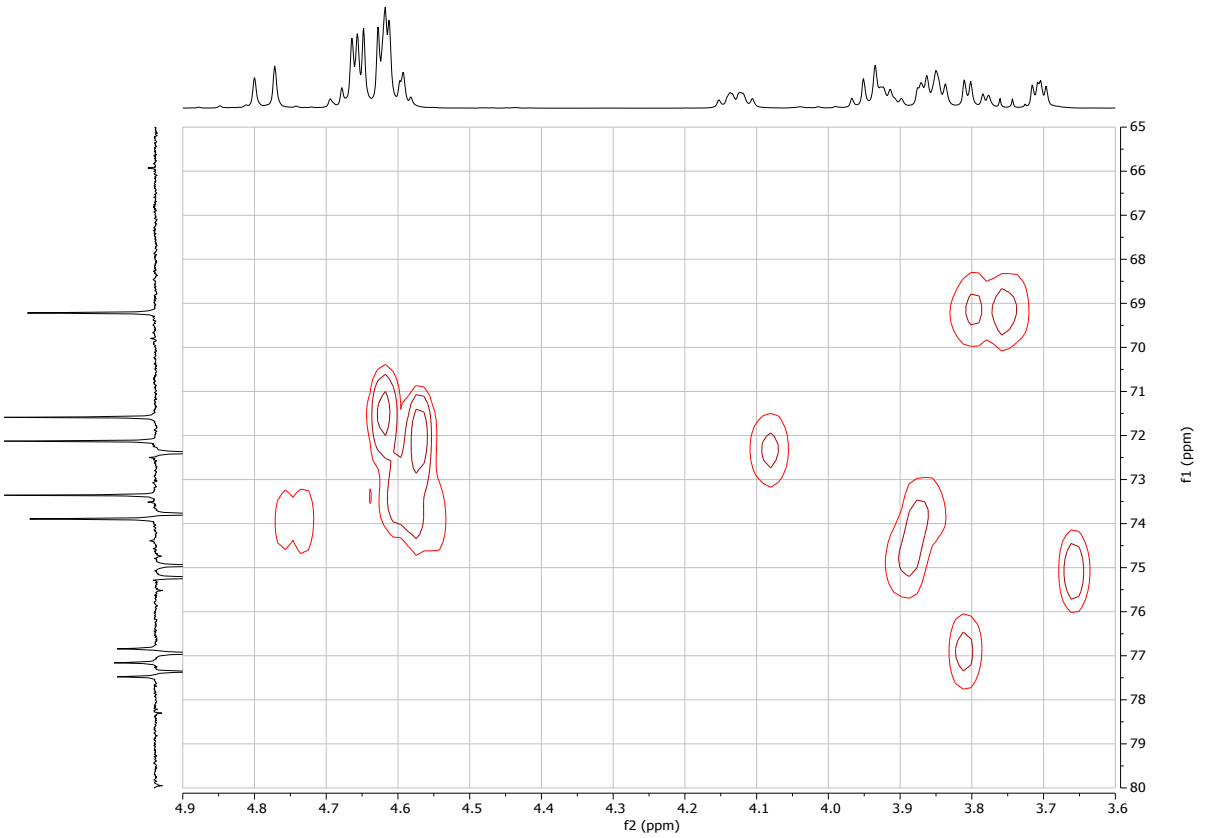
HH-COSY NMR





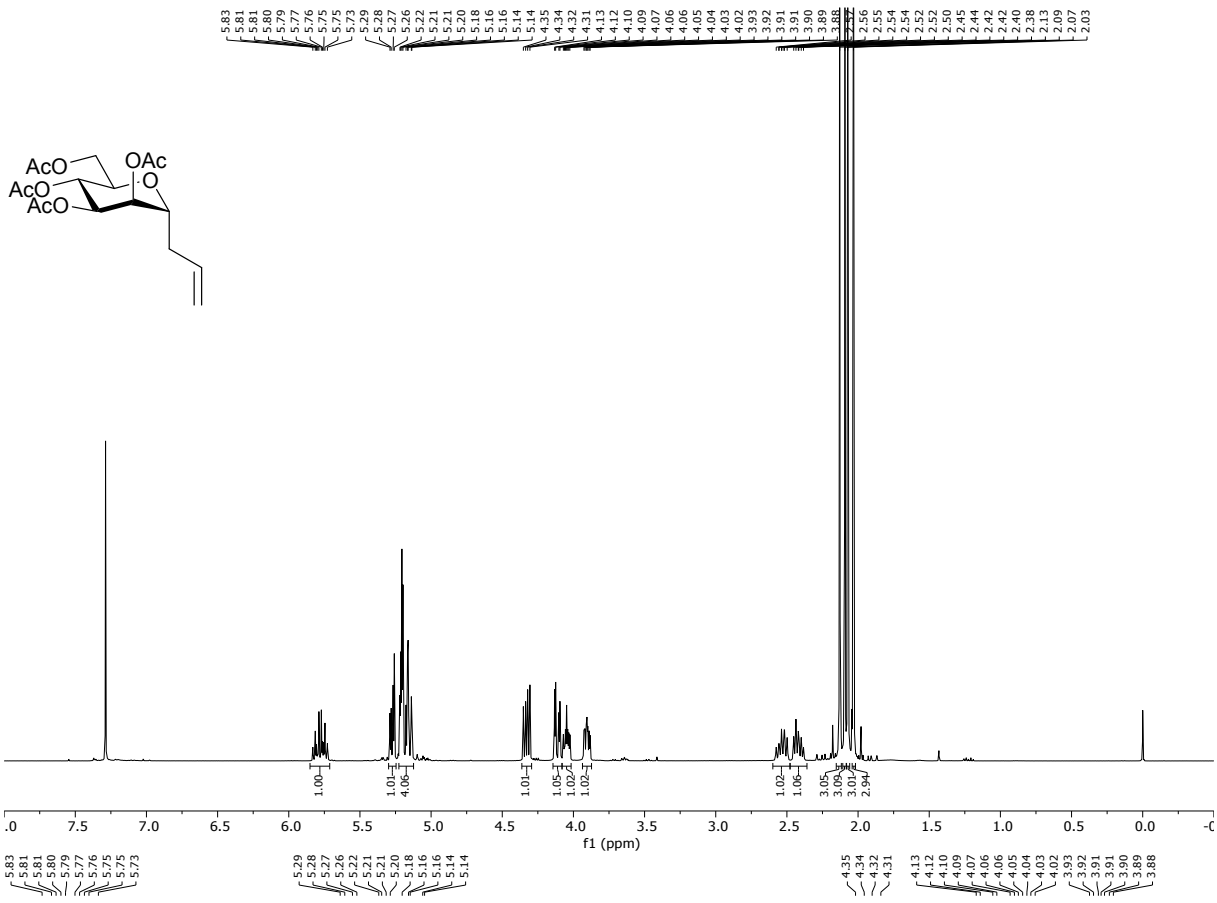
HSQC NMR

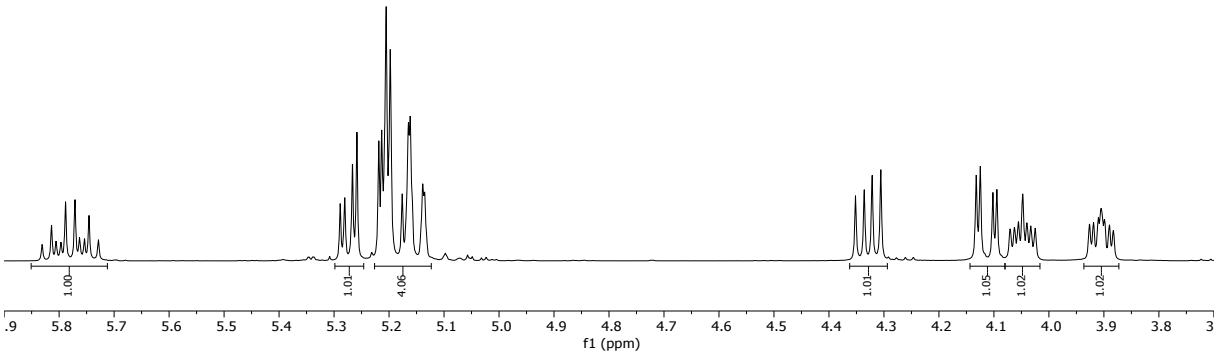




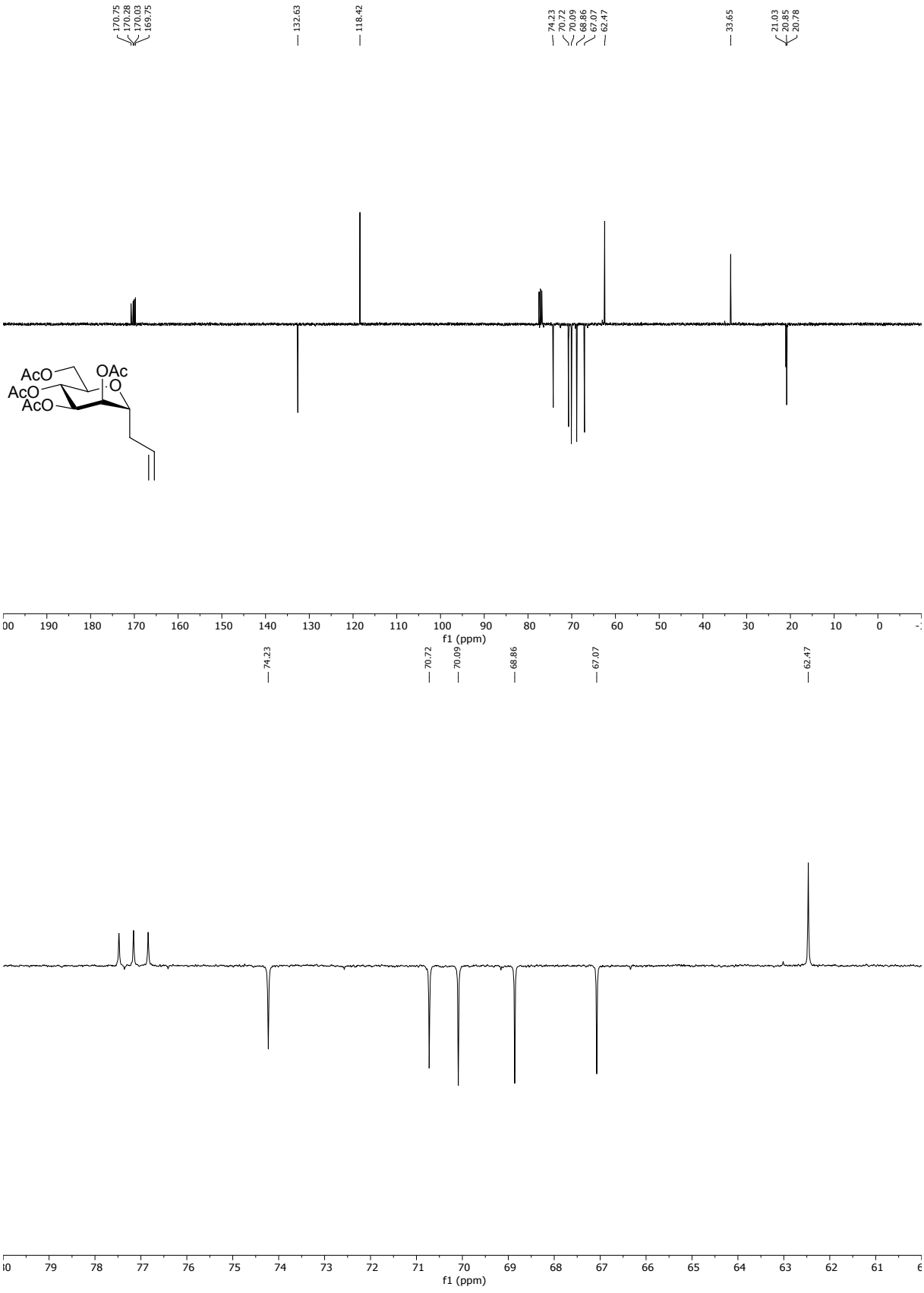
Compound 5

^{1}H NMR



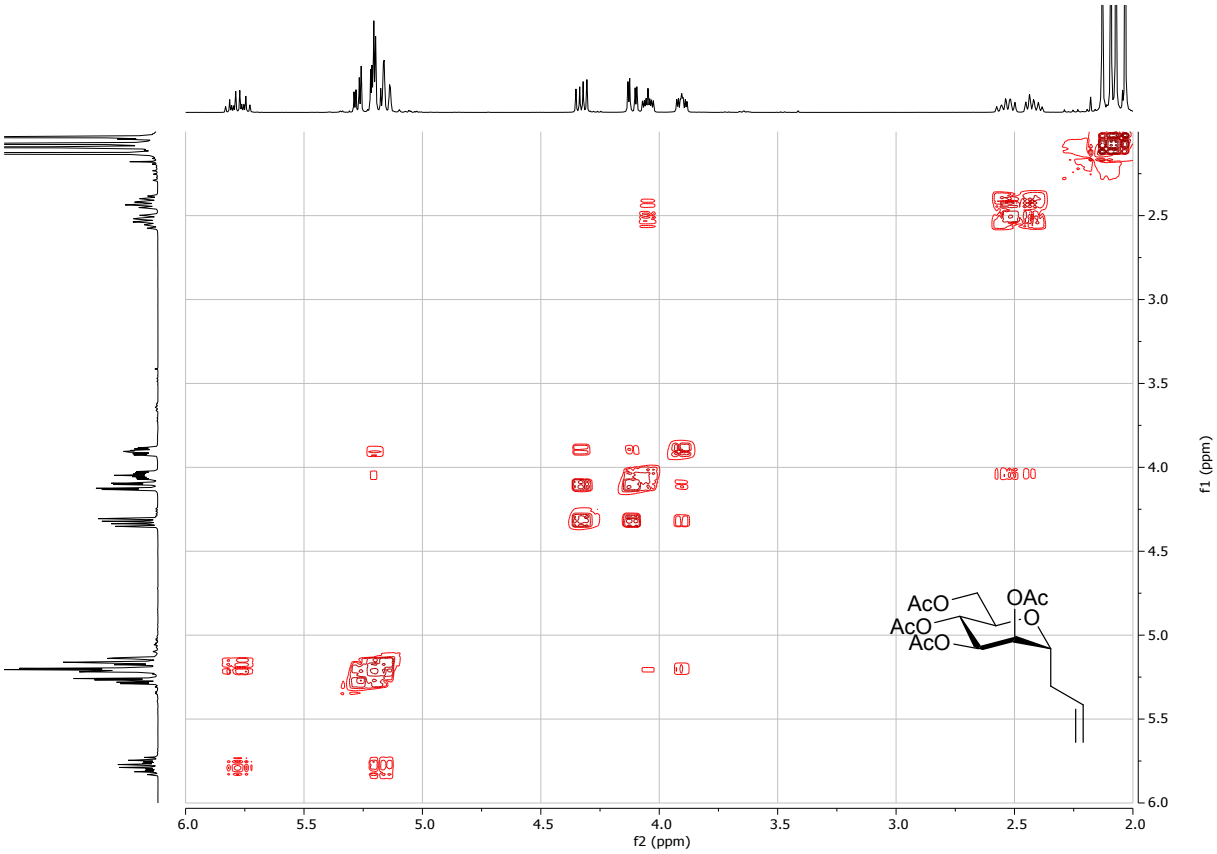


^{13}C APT NMR

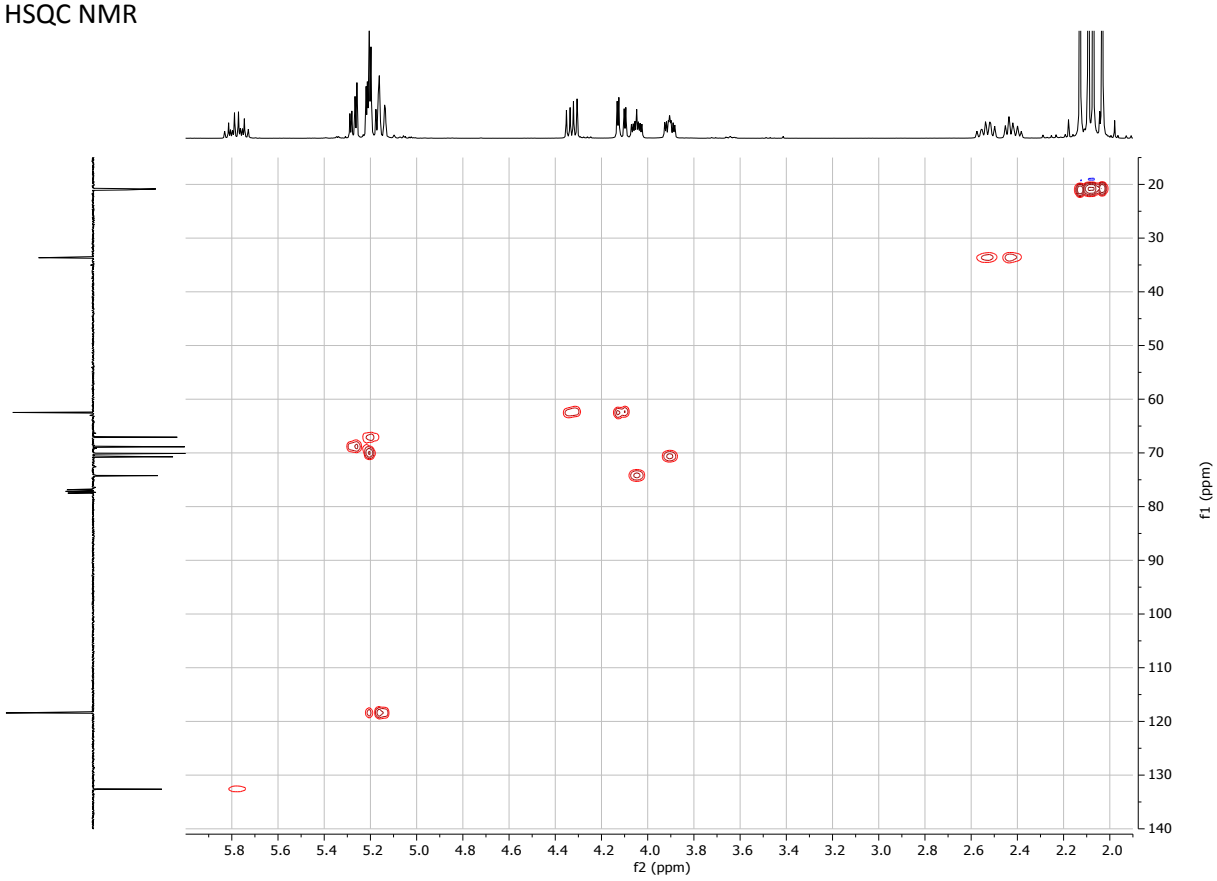

170.75
170.28
170.03
169.75
132.63
118.42
74.23
70.72
70.09
68.86
67.07
62.47
33.65
21.03
20.85
20.78
AcO
OAc
AcO
O
AcO
f1 (ppm)

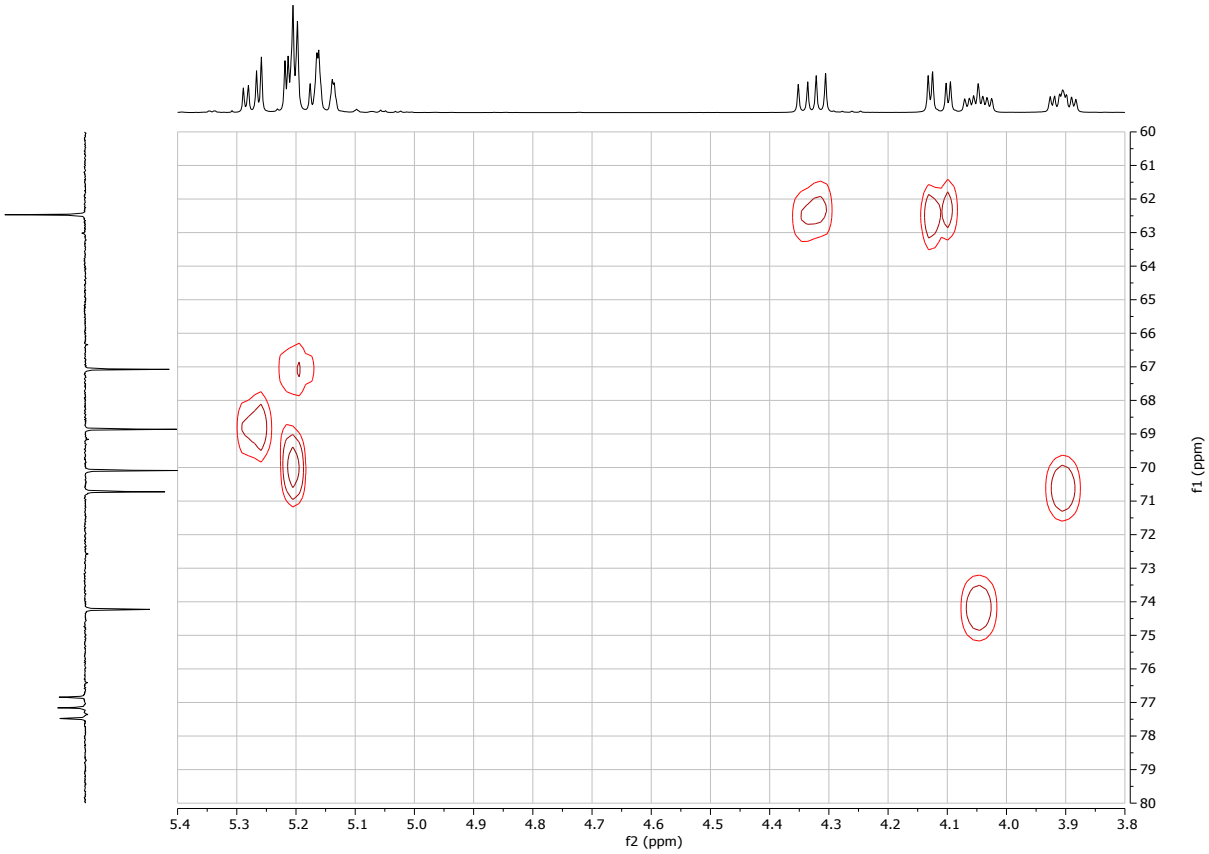
74.23
70.72
70.09
68.86
67.07
62.47
f1 (ppm)

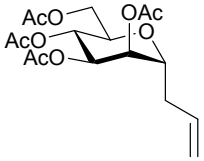
HH-COSY NMR



HSQC NMR

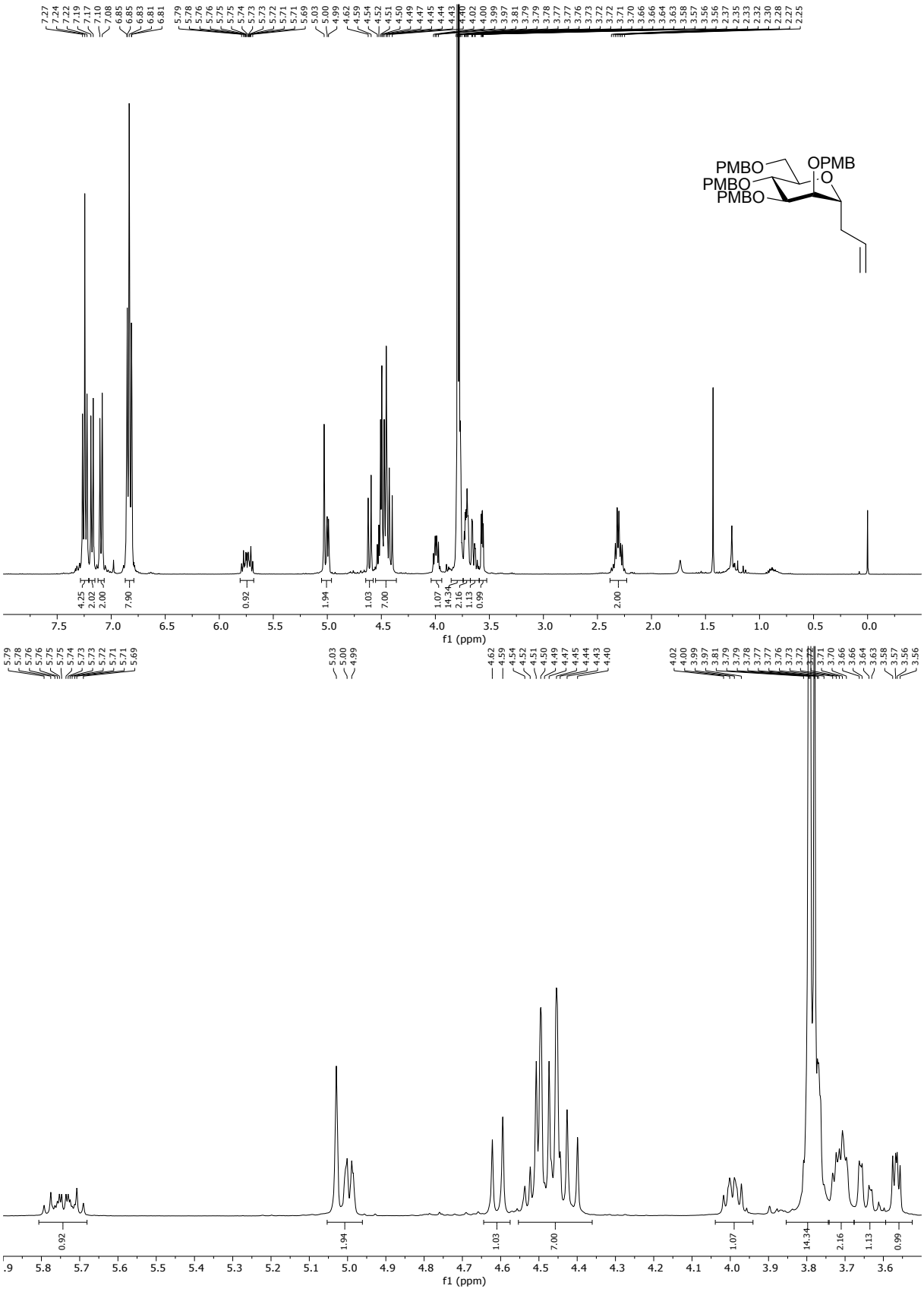



60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
f1 (ppm)
5.4
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
f2 (ppm)

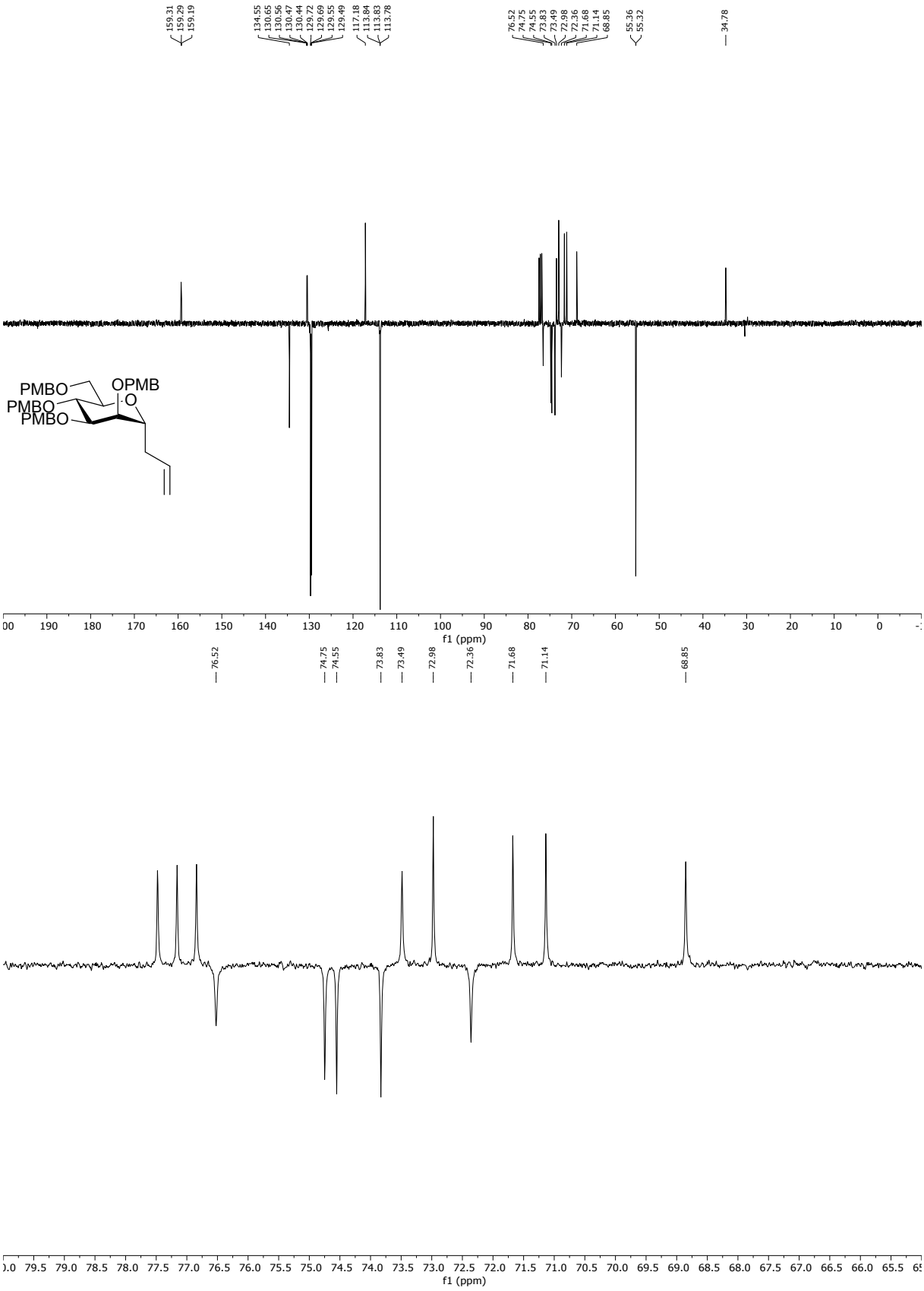

AcO
AcO
AcO
OAc
O

Compound 6

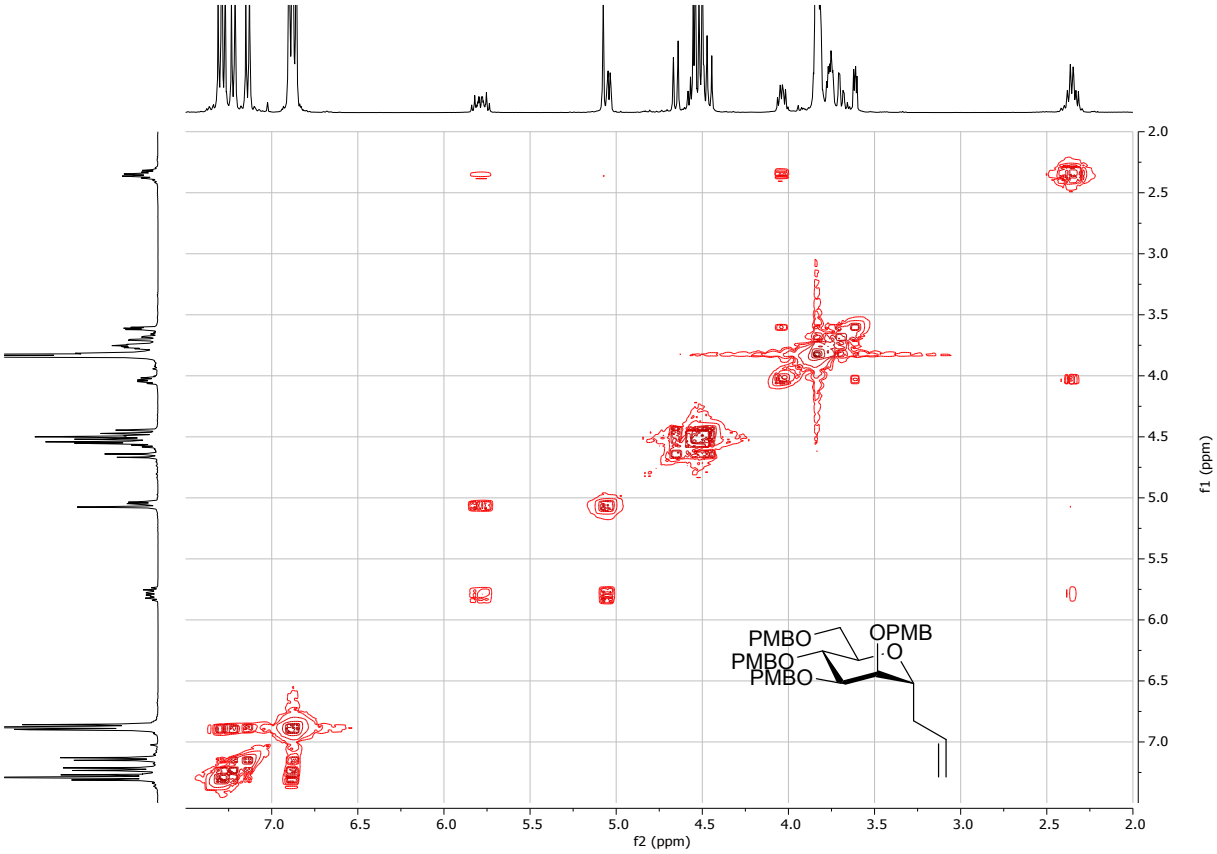
^{1}H NMR



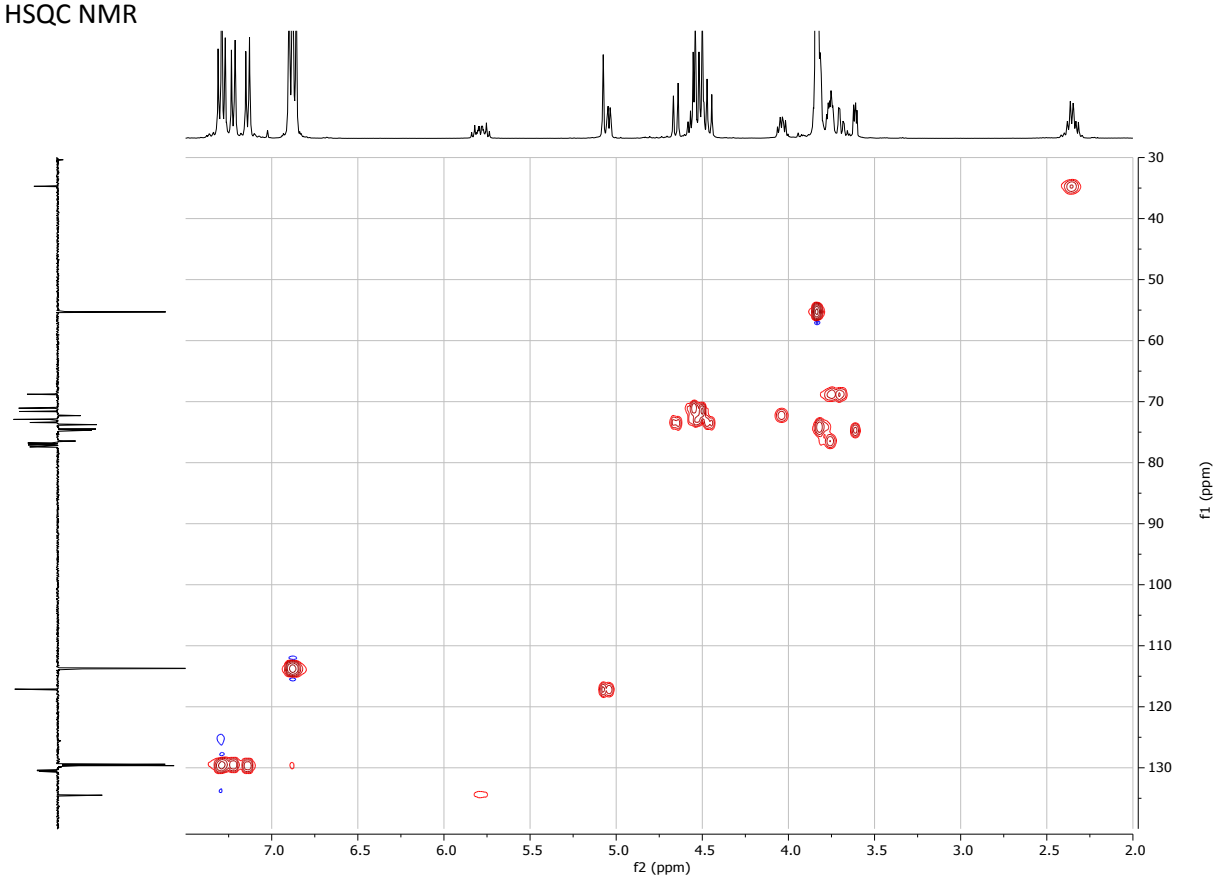
^{13}C APT NMR


159.31
159.29
159.19
134.55
130.65
130.56
130.47
130.44
129.72
129.69
129.55
129.49
117.18
113.84
113.83
113.78
76.52
74.75
74.55
73.83
73.49
72.98
72.36
71.68
71.14
68.85
55.36
55.32
34.78
PMBO
PMBO
PMBO
OPMB
O
f1 (ppm)

HH-COSY NMR

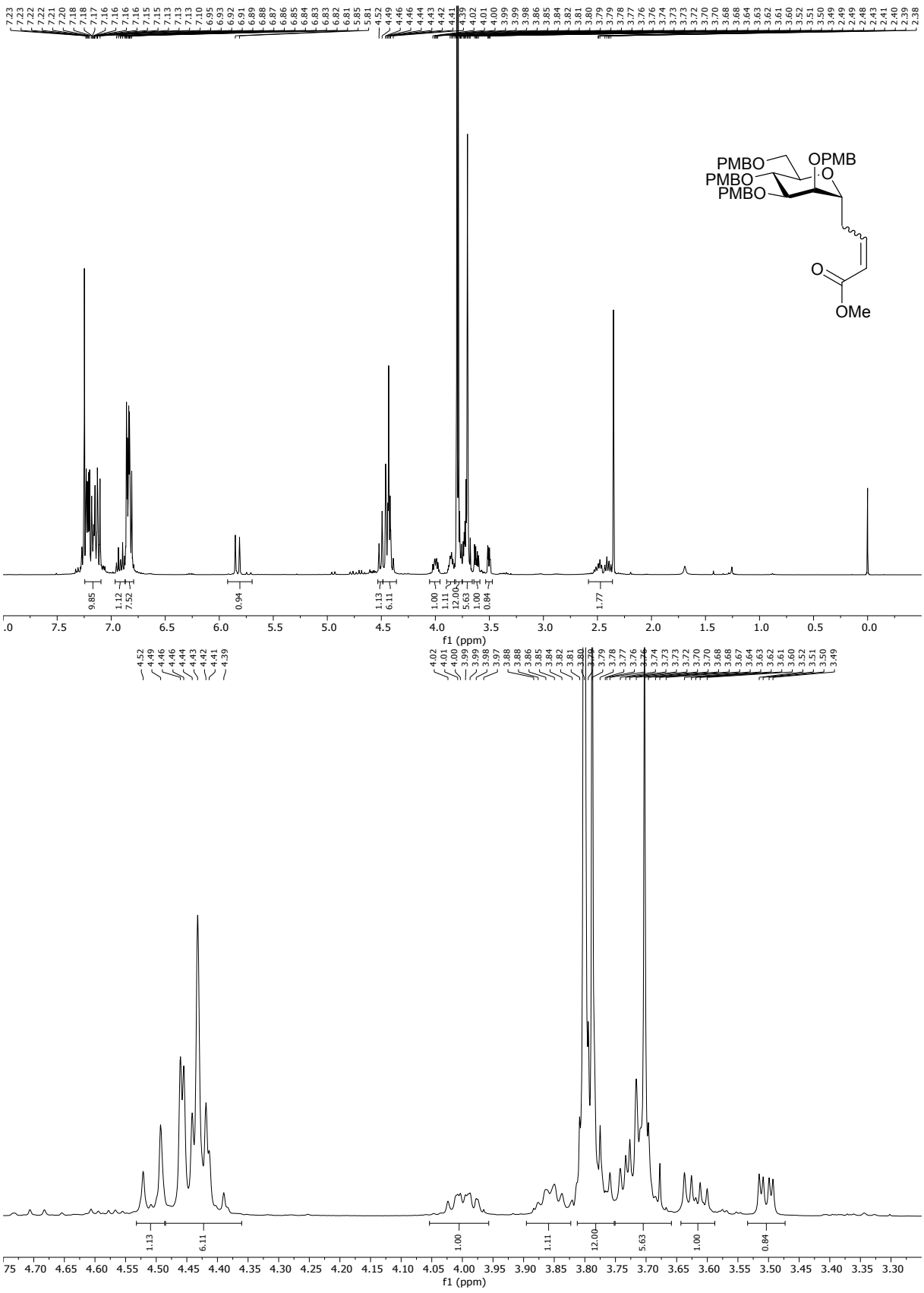


HSQC NMR

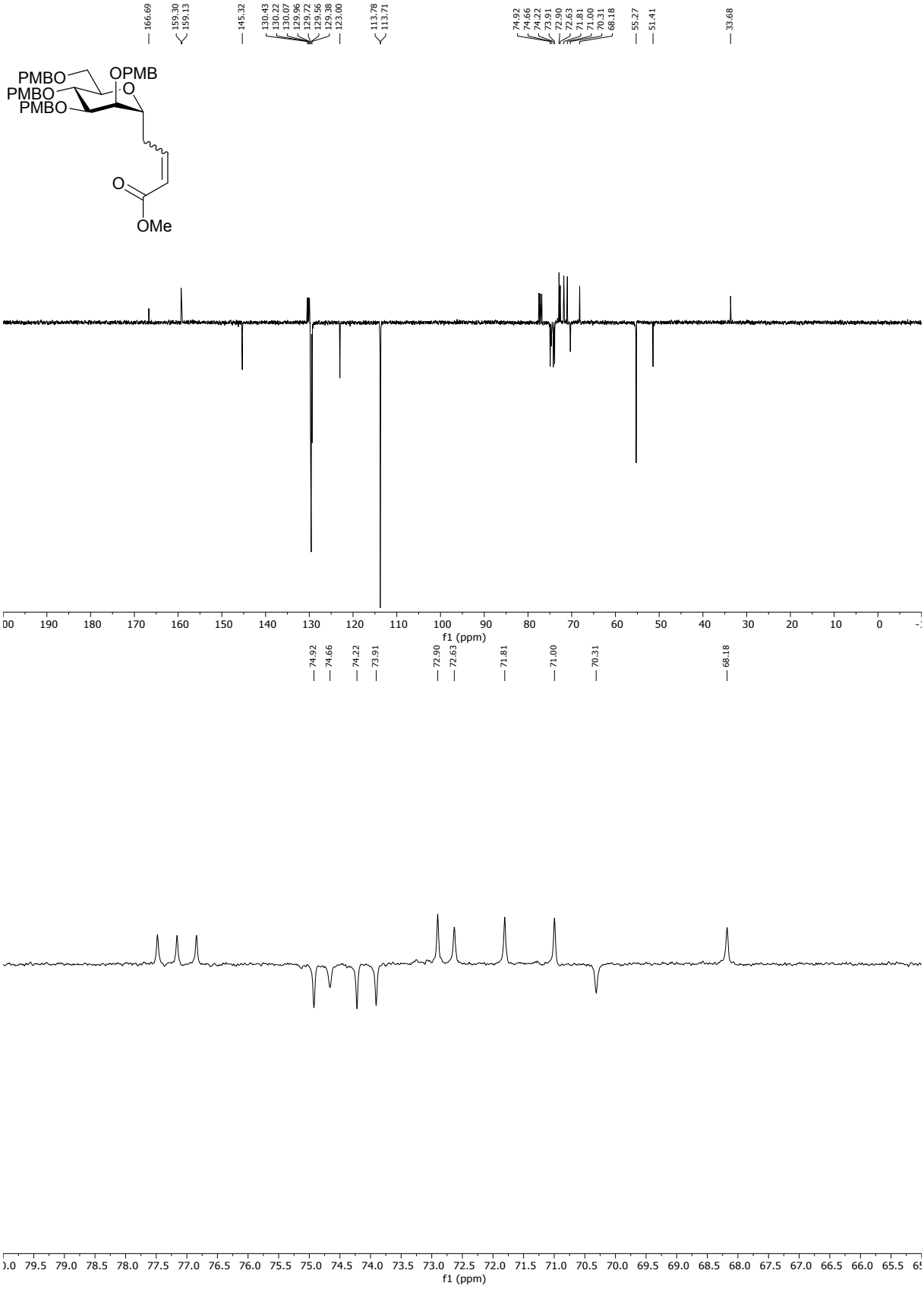


Compound 7

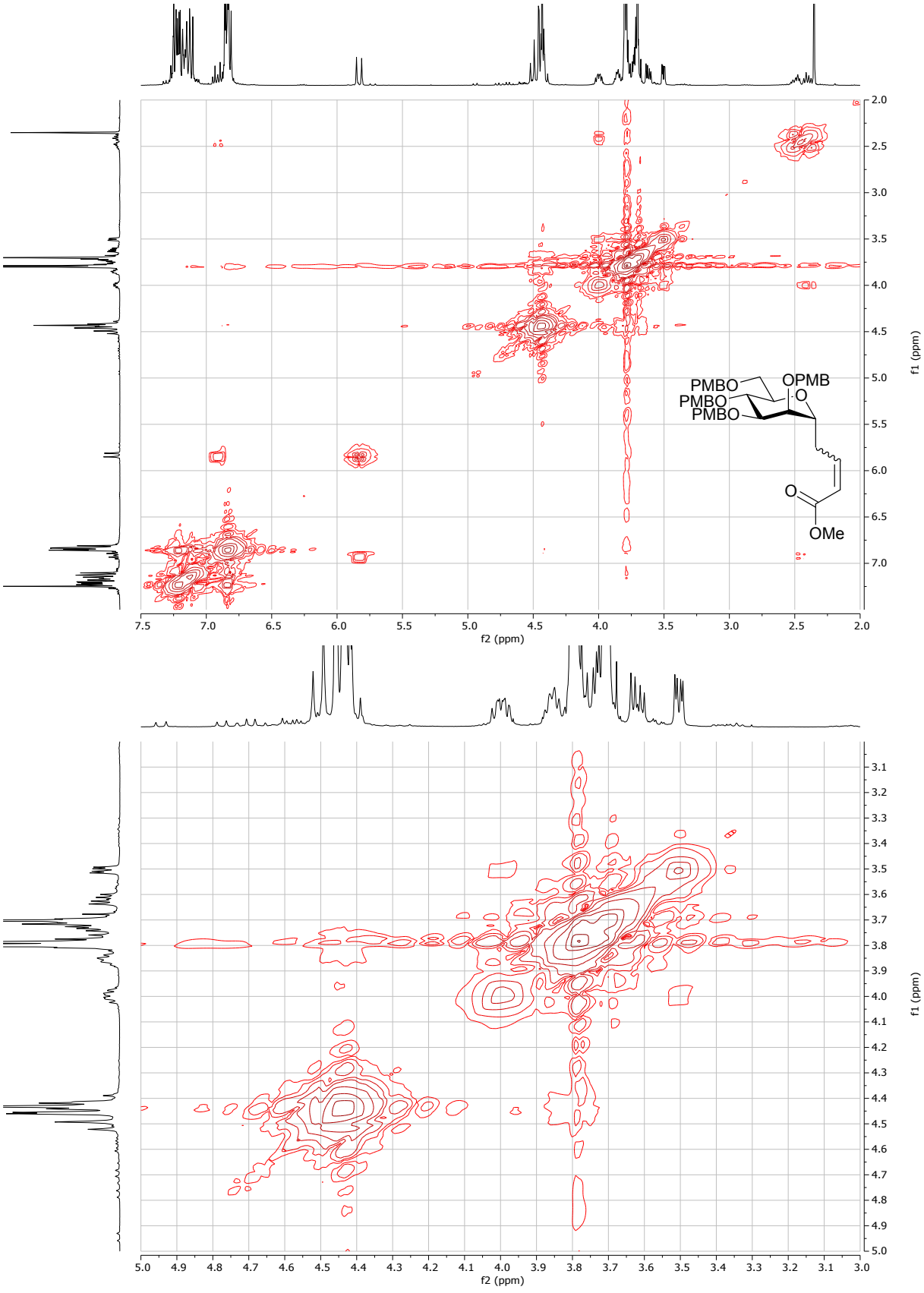
^{1}H NMR



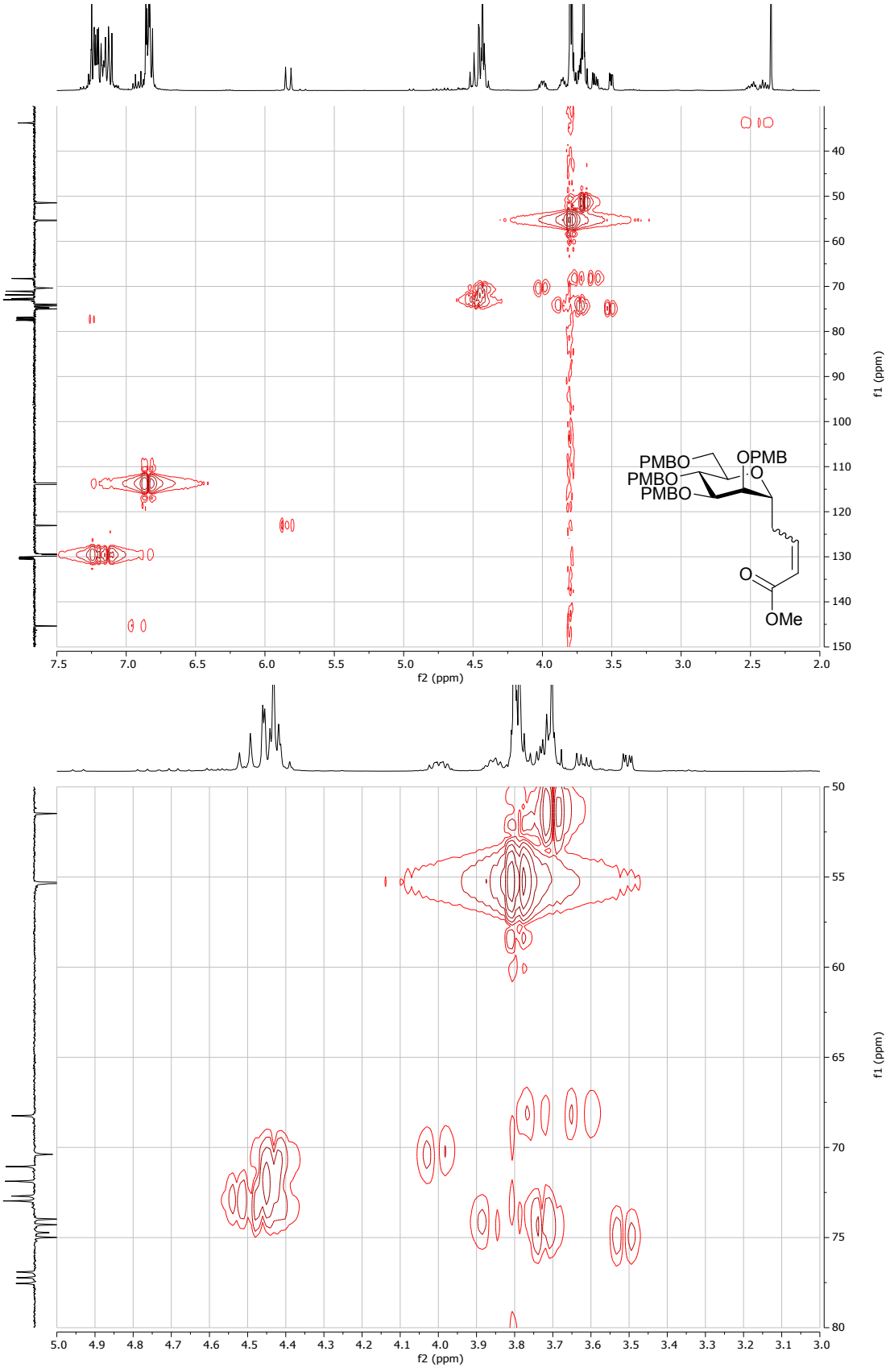
^{13}C APT NMR


166.69
159.30
159.13
145.32
130.43
130.22
130.07
129.96
129.72
129.56
129.38
123.00
113.78
113.71
74.92
74.66
74.22
73.91
72.90
72.63
71.81
71.00
70.31
68.18
55.27
51.41
33.68
PMBO
PMBO
PMBO
OPMB
O
O
OMe
f1 (ppm)

HH COSY NMR

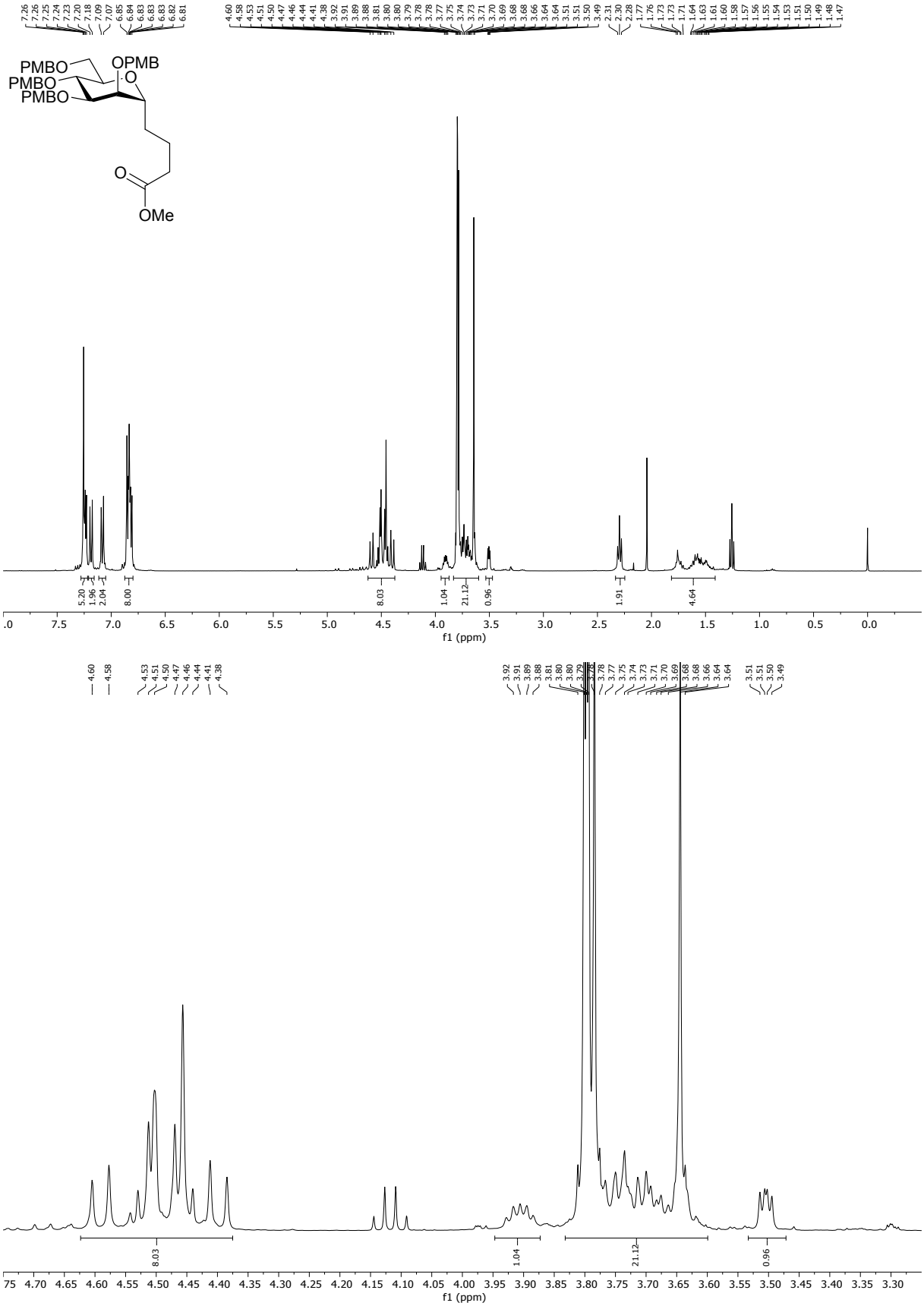


HSQC NMR

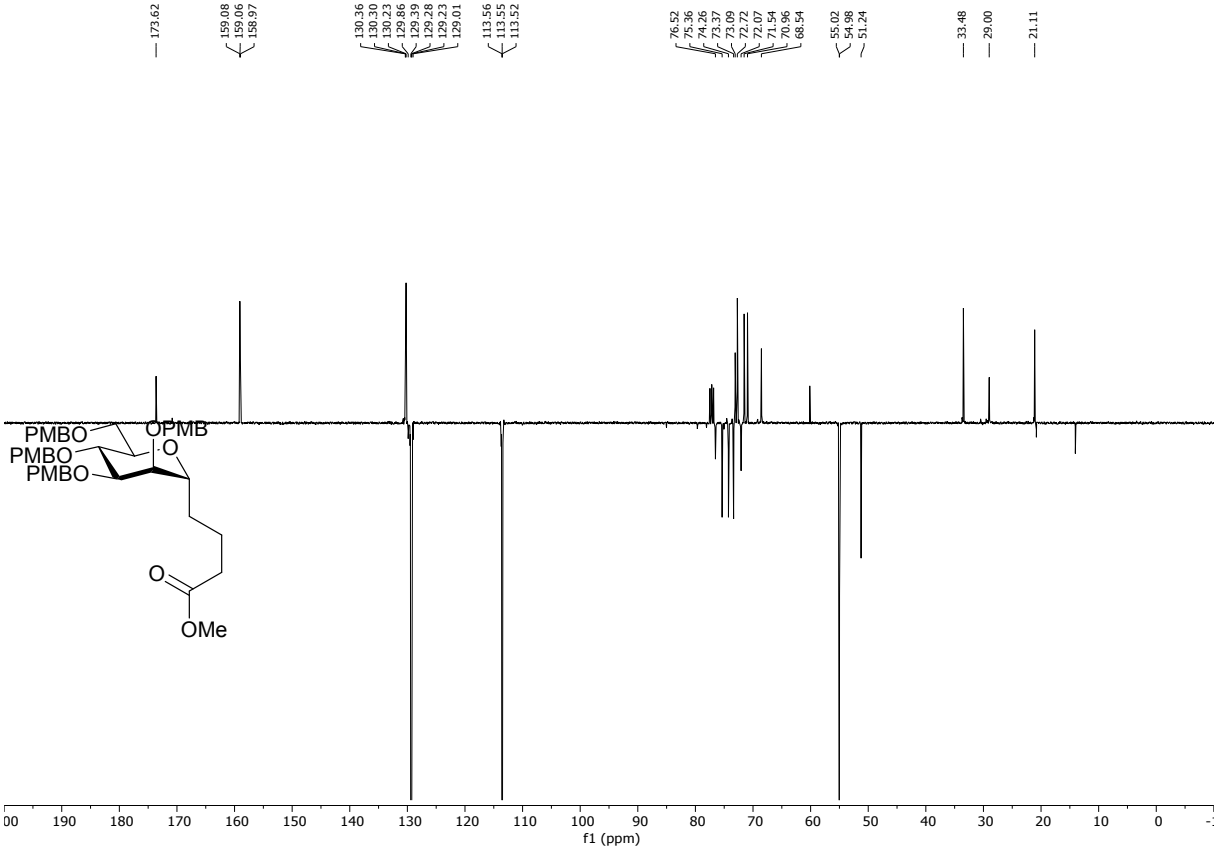


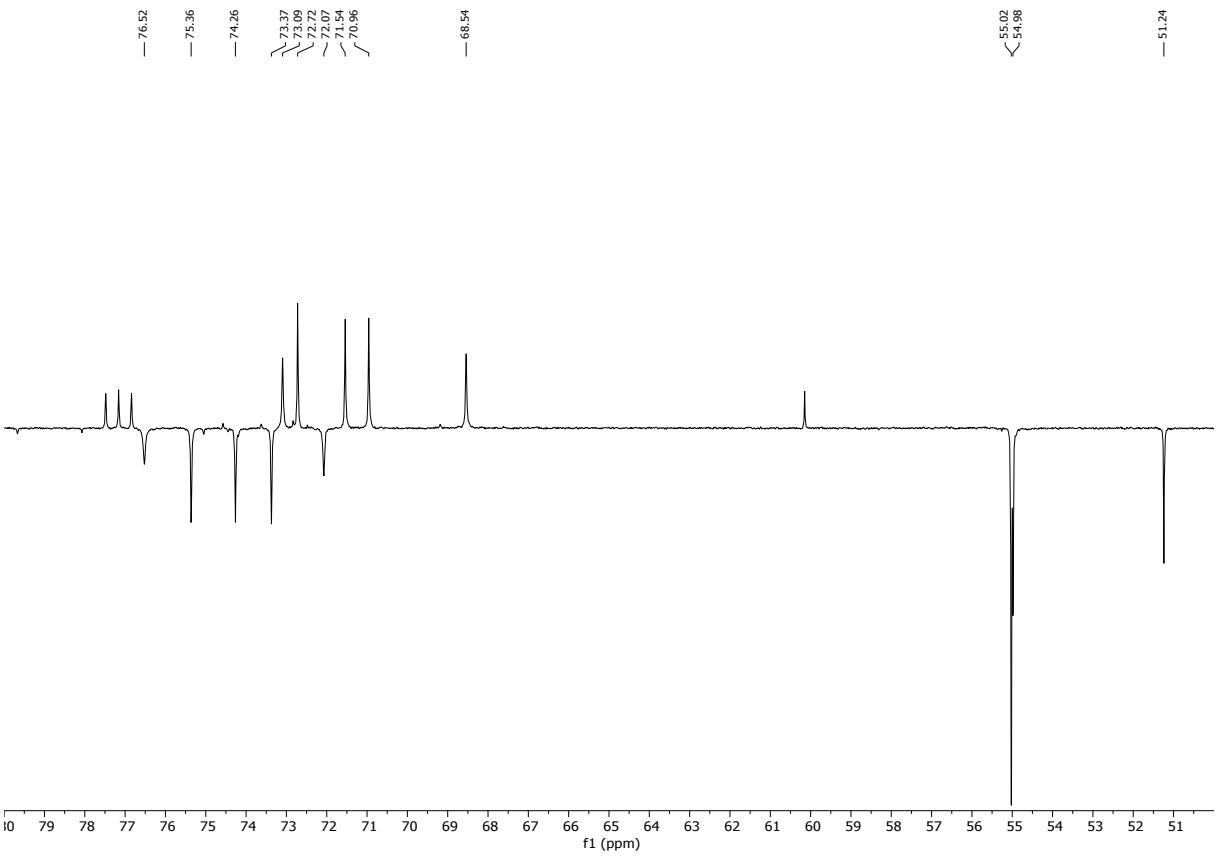
Compound 8

^{1}H NMR

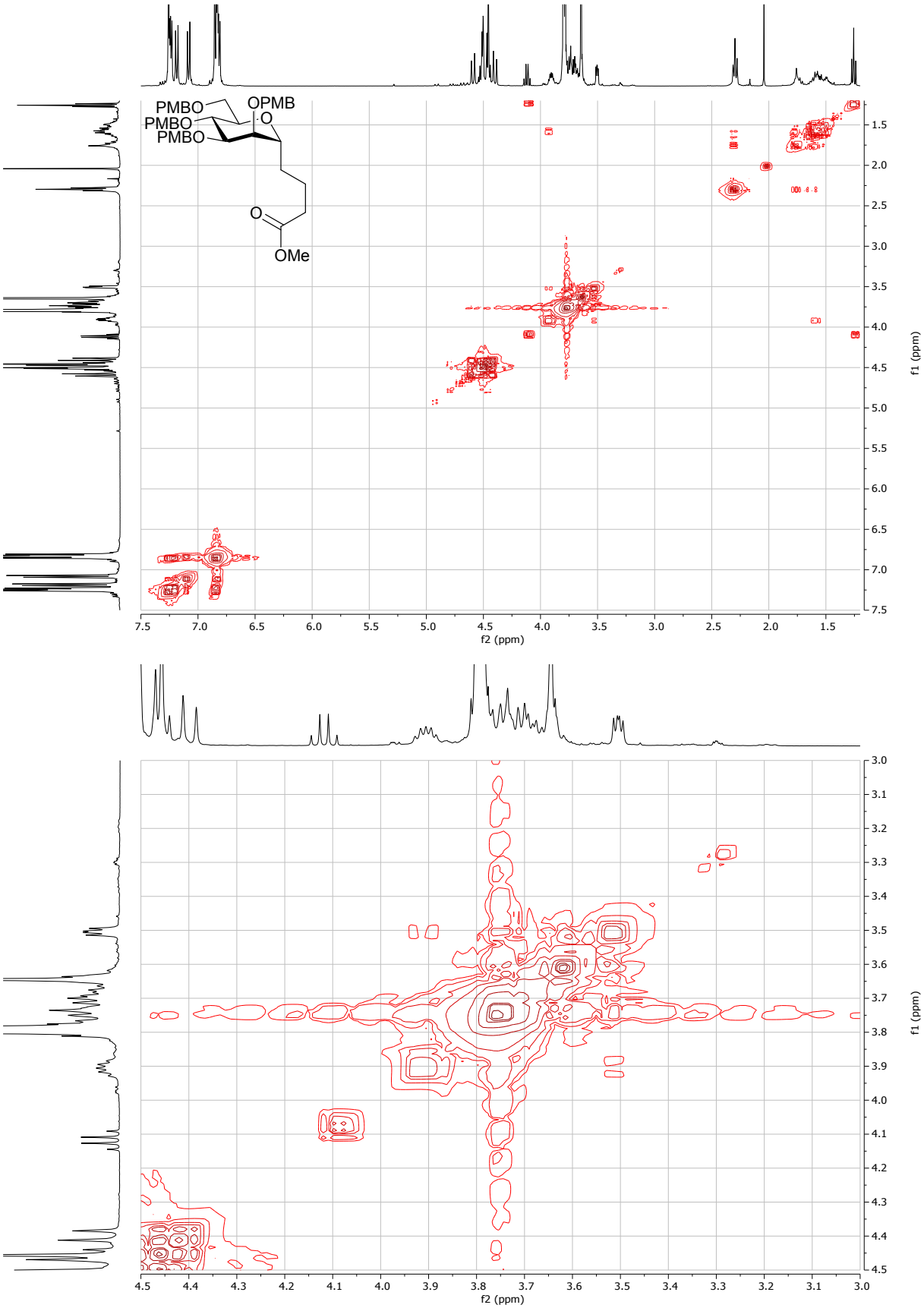


^{13}C APT NMR

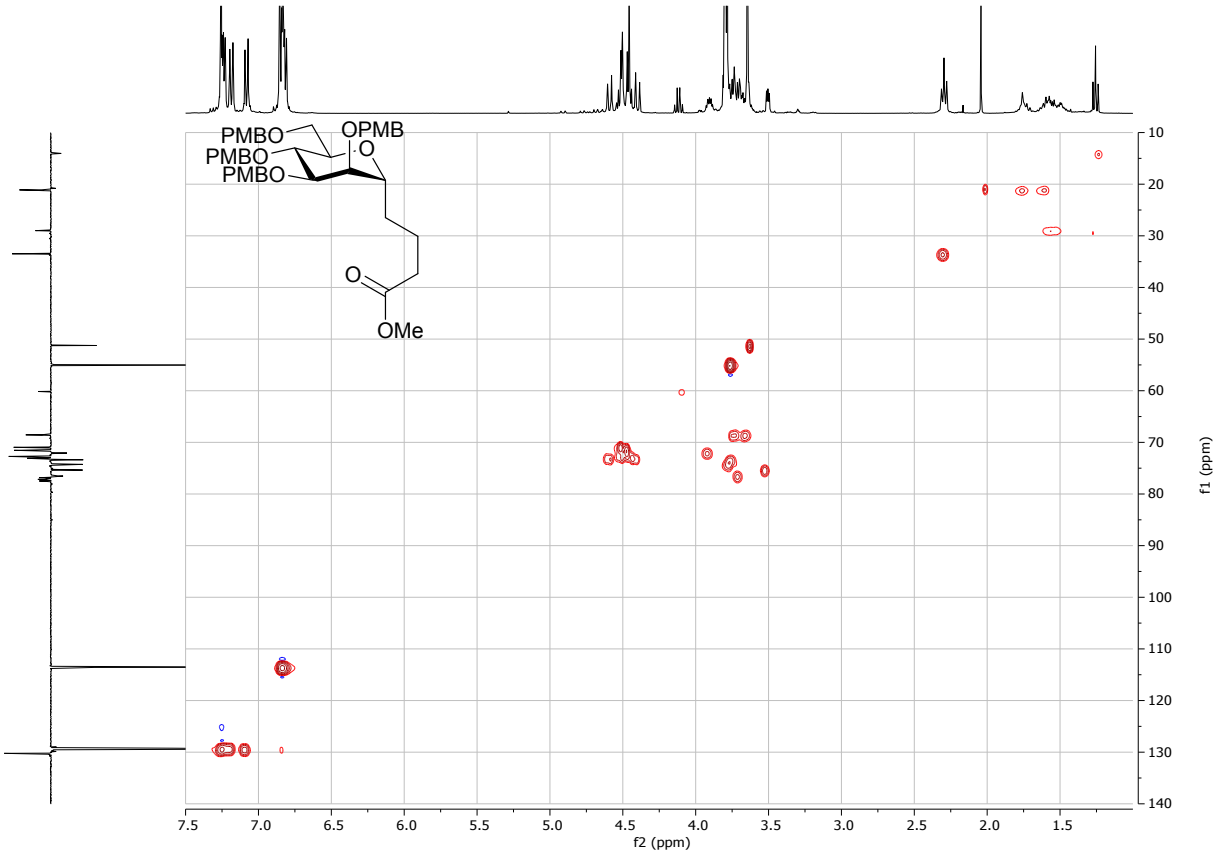

173.62
159.08
159.06
158.97
130.36
130.30
130.23
129.86
129.39
129.28
129.23
129.01
113.56
113.55
113.52
76.52
75.36
74.26
73.37
73.09
72.72
72.07
71.54
70.96
68.54
55.02
54.98
51.24
33.48
29.00
21.11
PMBO
PMBO
PMBO
OPMB
O
O
OMe
f1 (ppm)

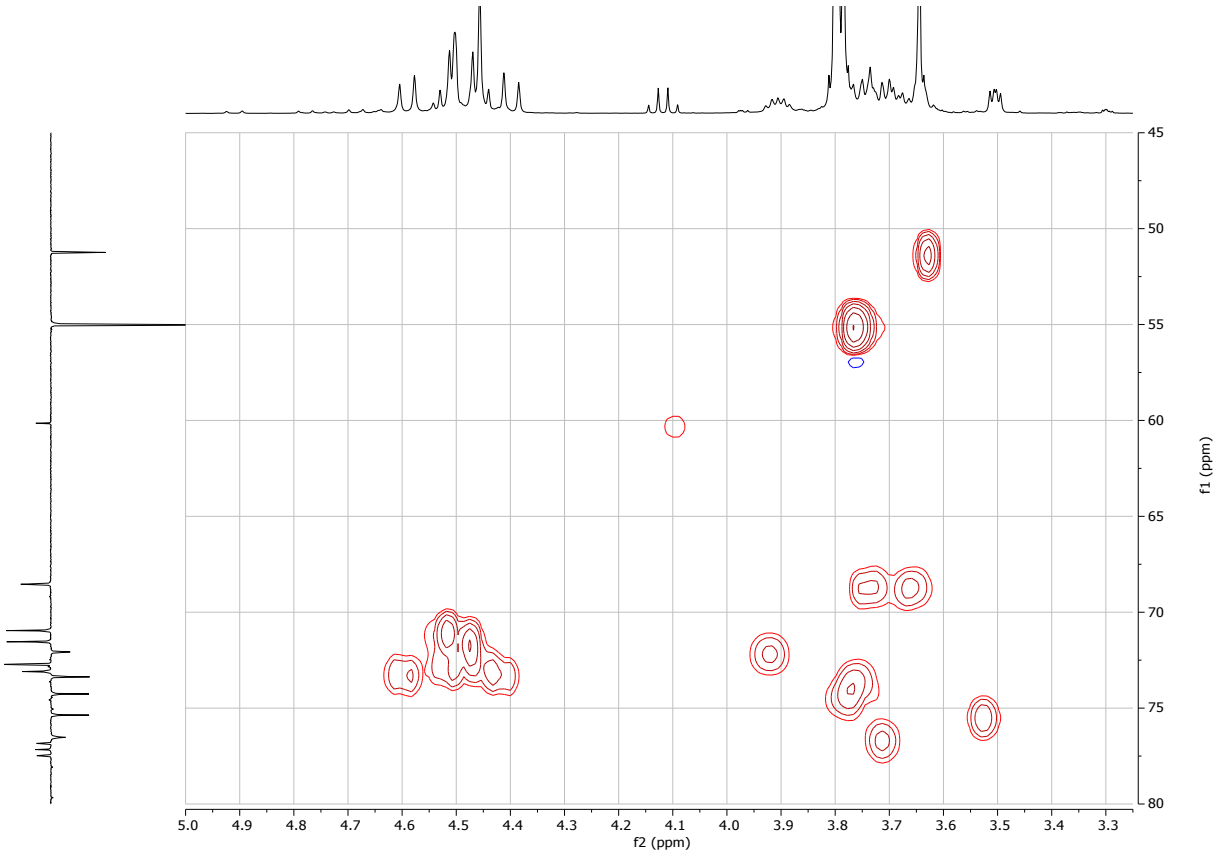

76.52
75.36
74.26
73.37
73.09
72.72
72.07
71.54
70.96
68.54
55.02
54.98
51.24
f1 (ppm)

HH-COSY NMR



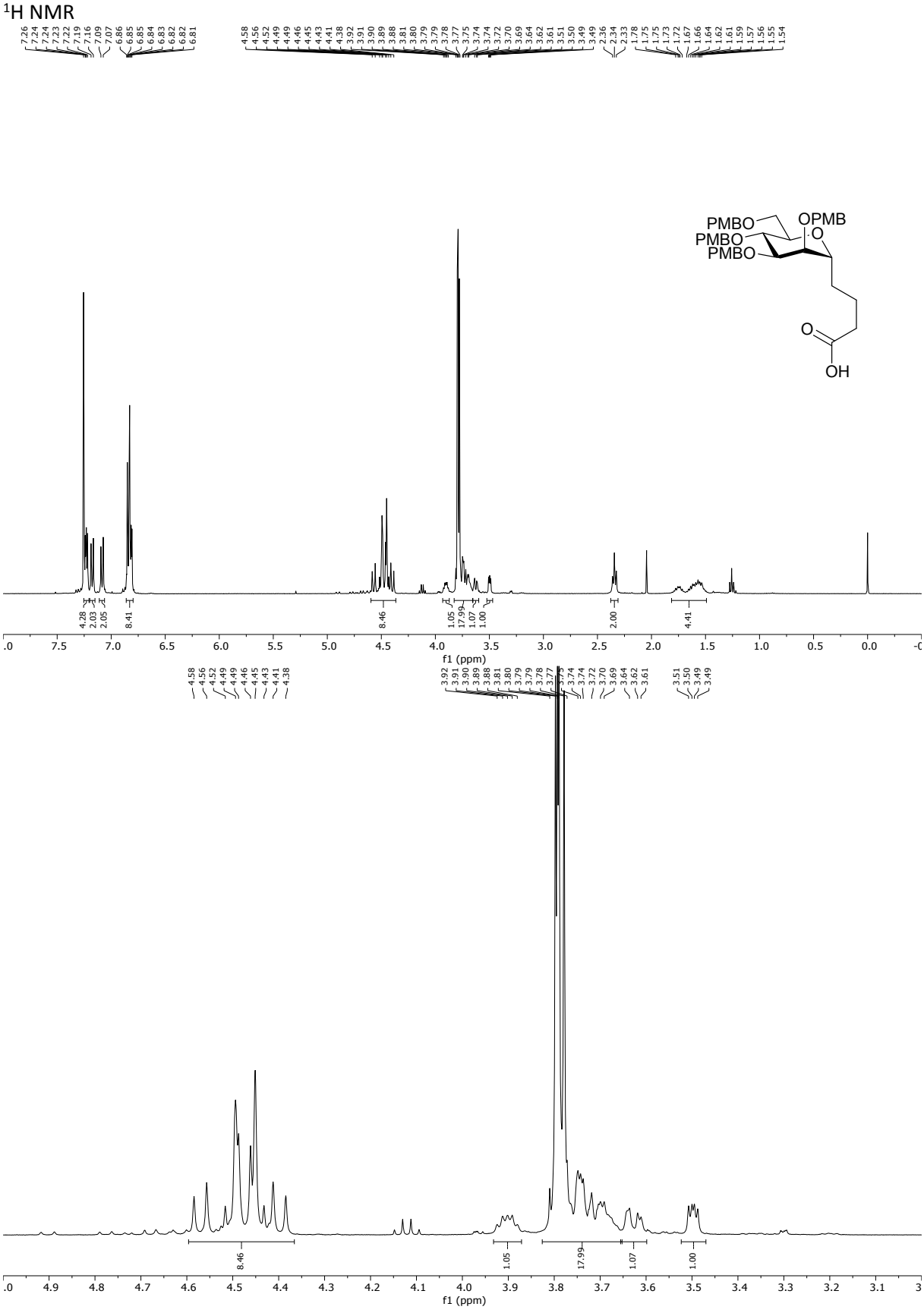
HSQC NMR



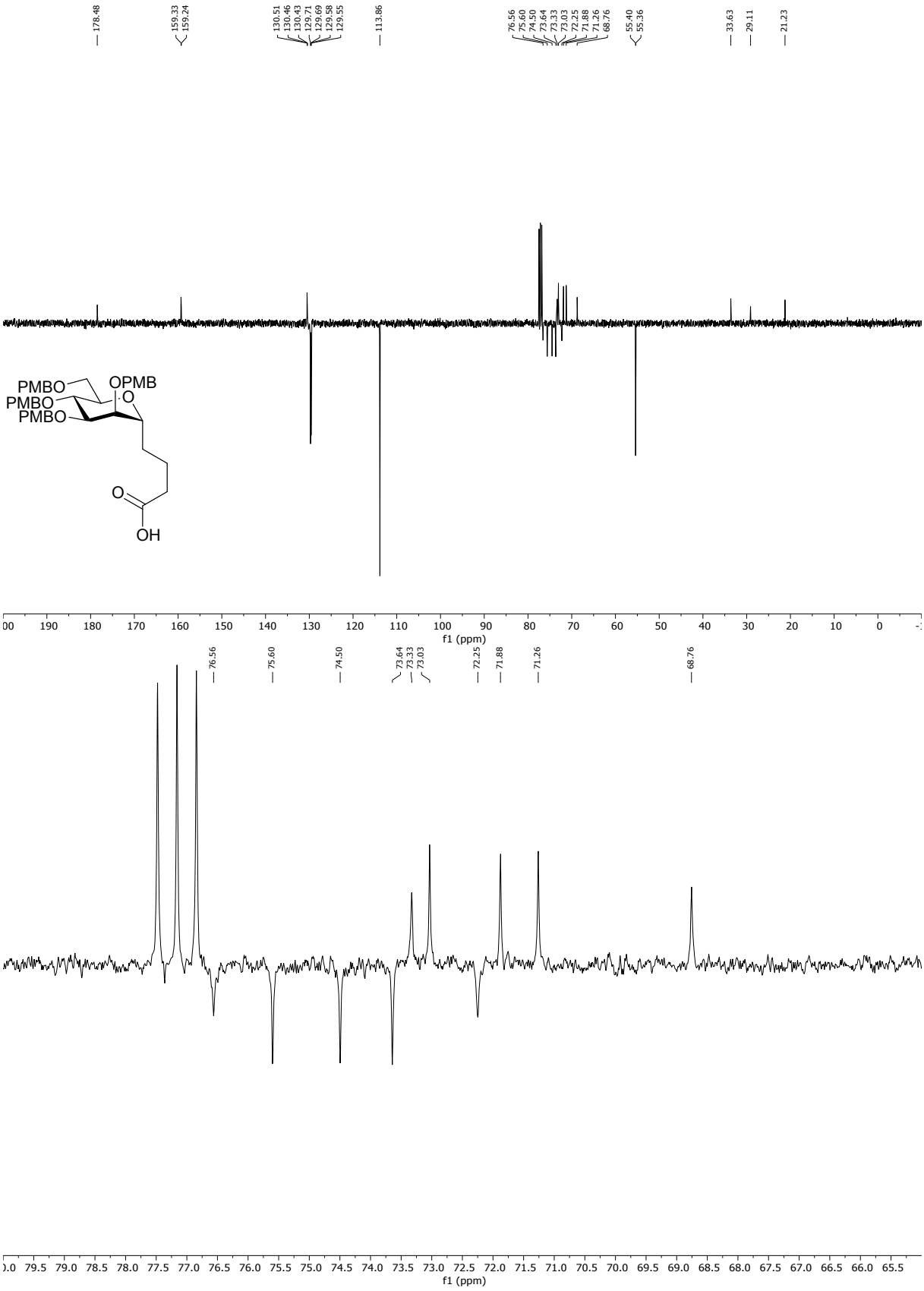


Compound 9

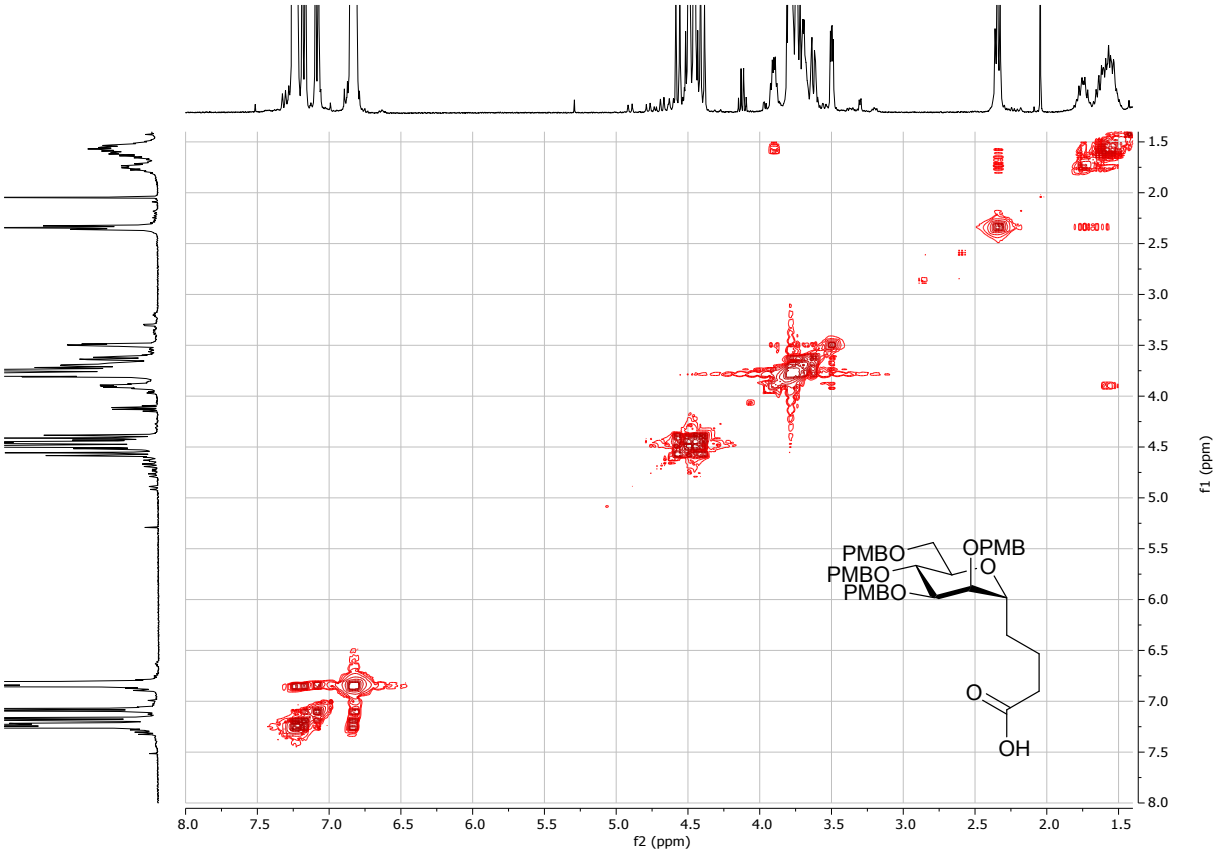
^{1}H NMR



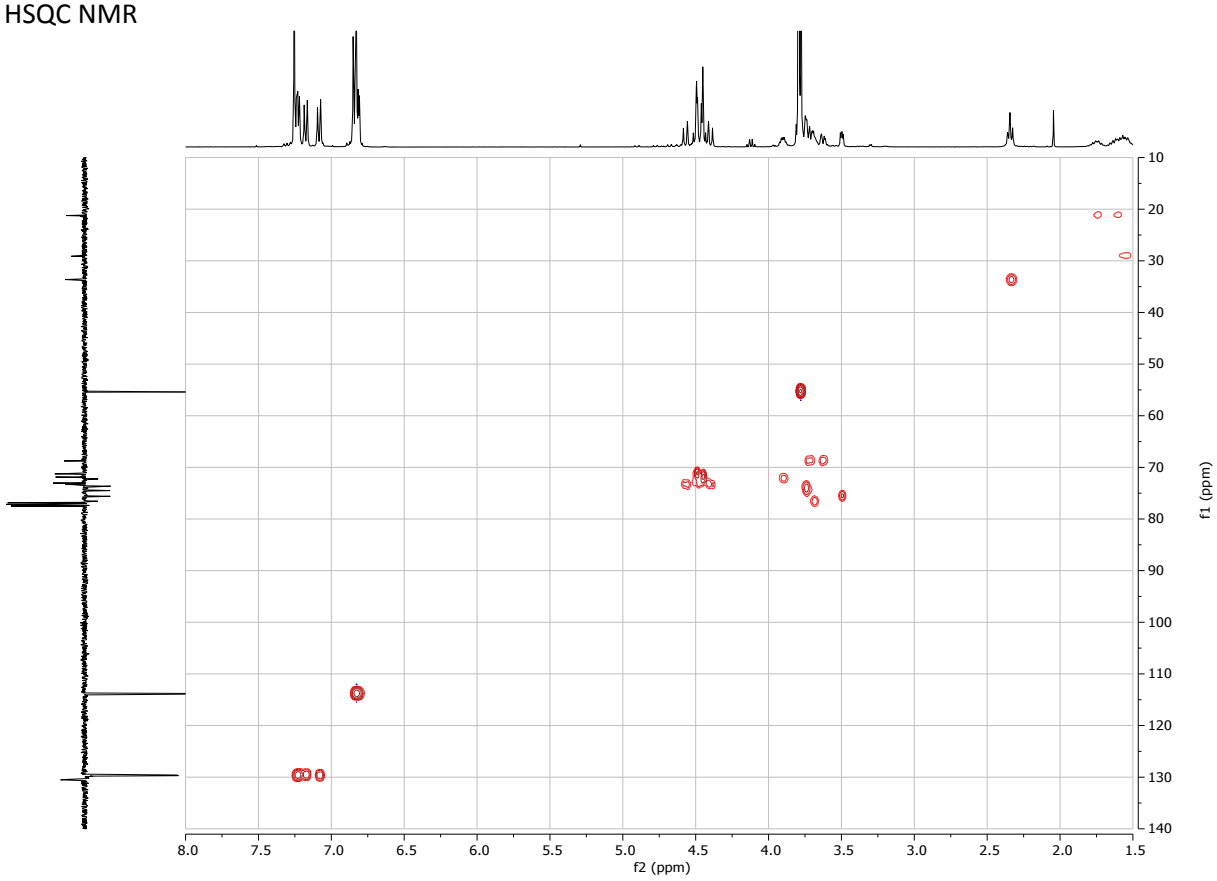
^{13}C APT NMR


178.48
159.33
159.24
130.51
130.46
130.43
129.71
129.69
129.58
129.55
113.86
76.56
75.60
74.50
73.64
73.33
73.03
72.25
71.88
71.26
68.76
55.40
55.36
33.63
29.11
21.23
PMBO
PMBO
PMBO
OPMB
O
O
OH
f1 (ppm)
f1 (ppm)

HH-COSY NMR

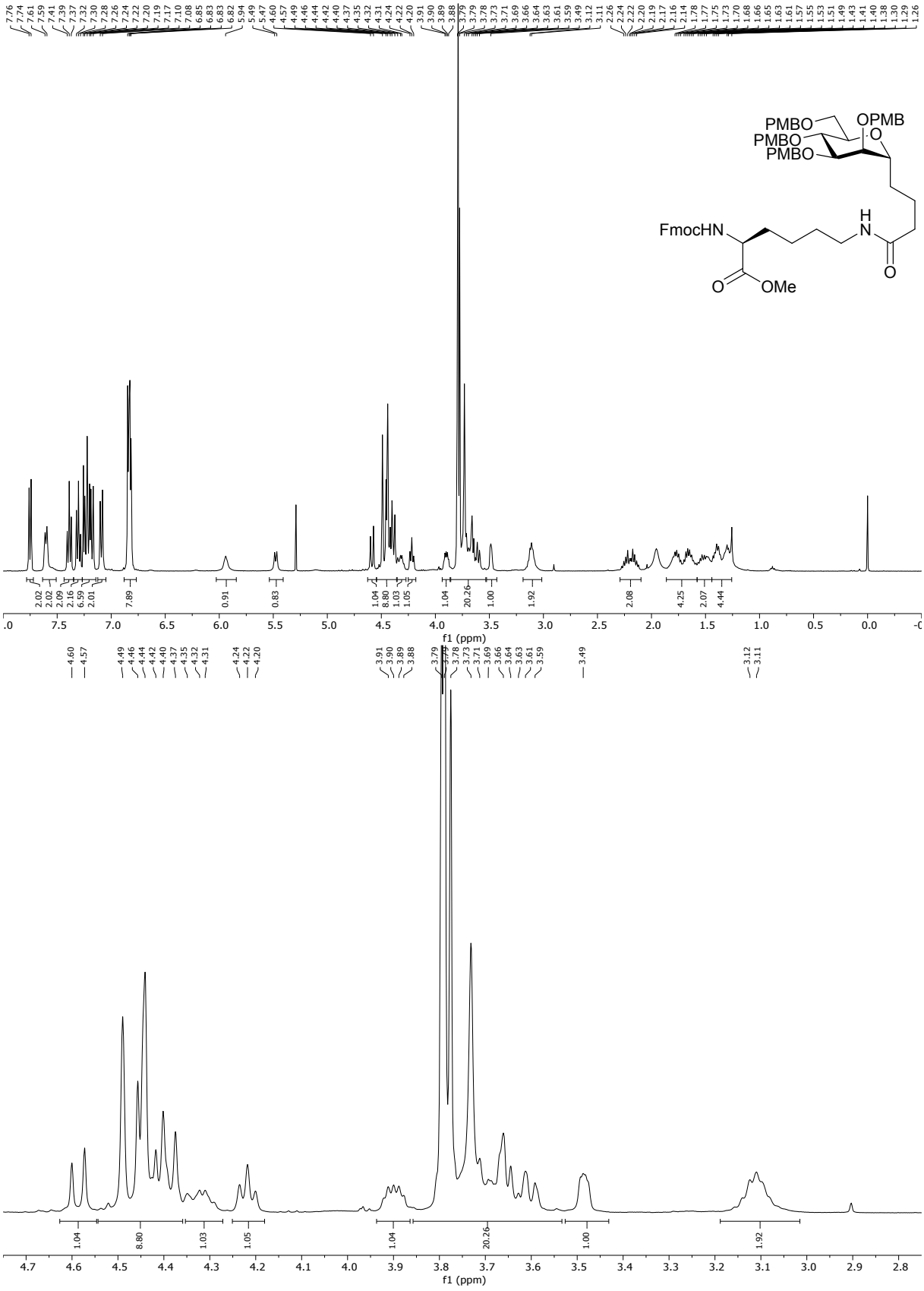


HSQC NMR

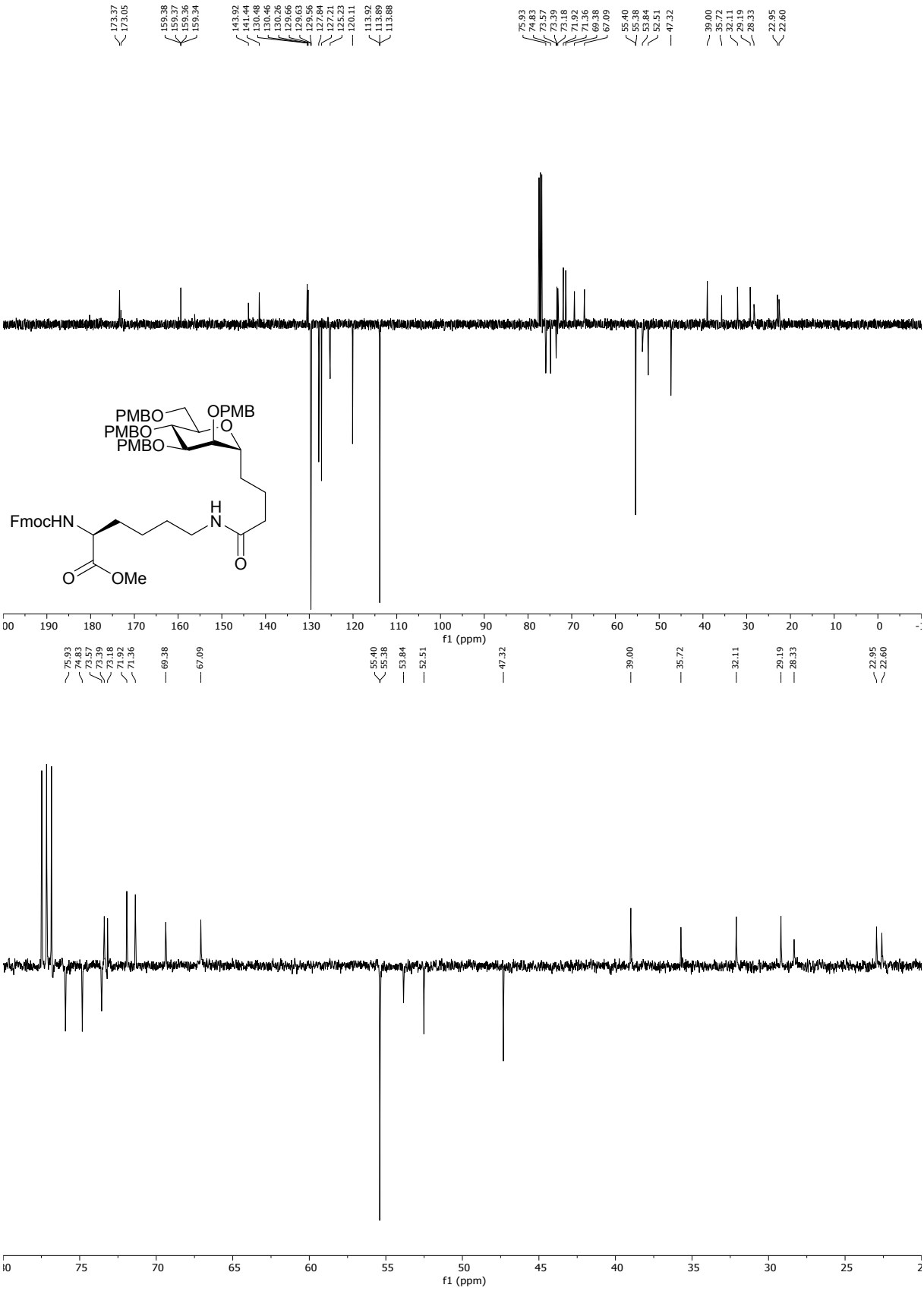


Compound 10

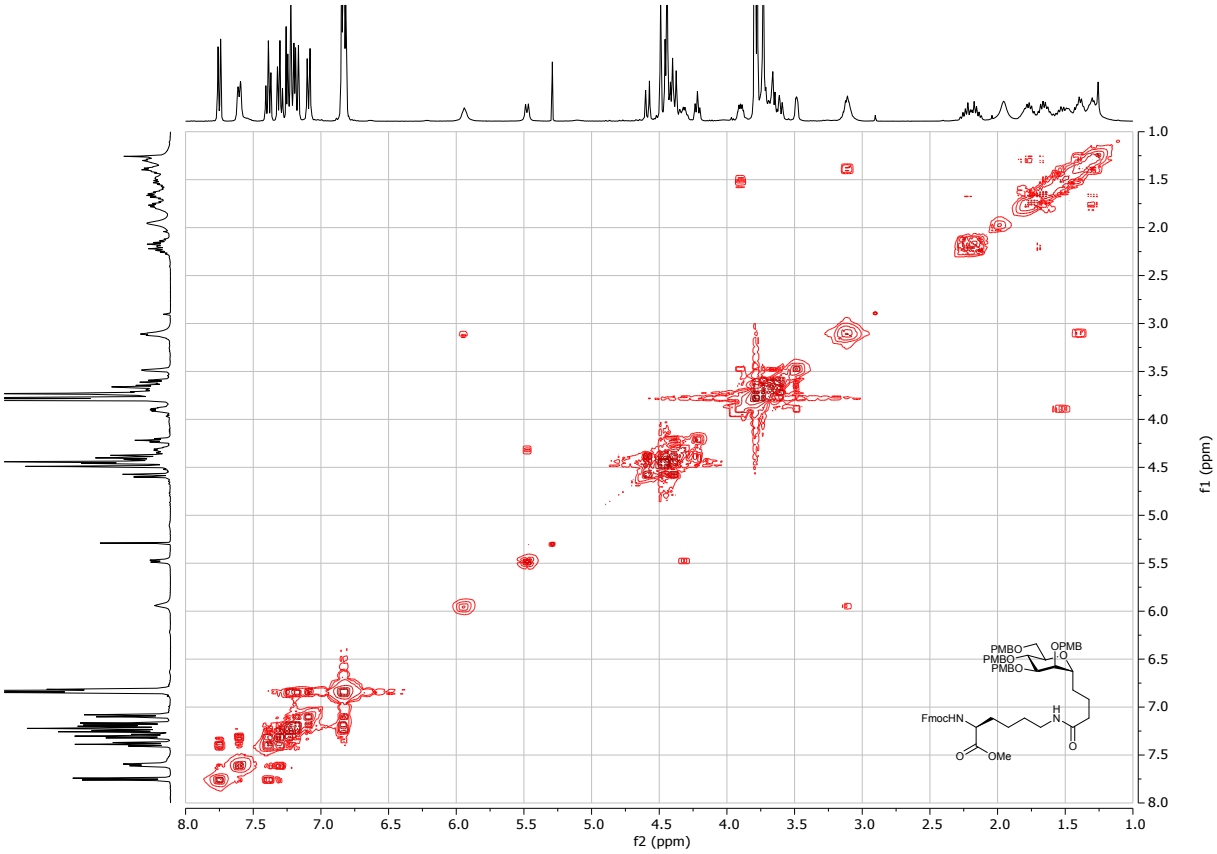
^{1}H NMR



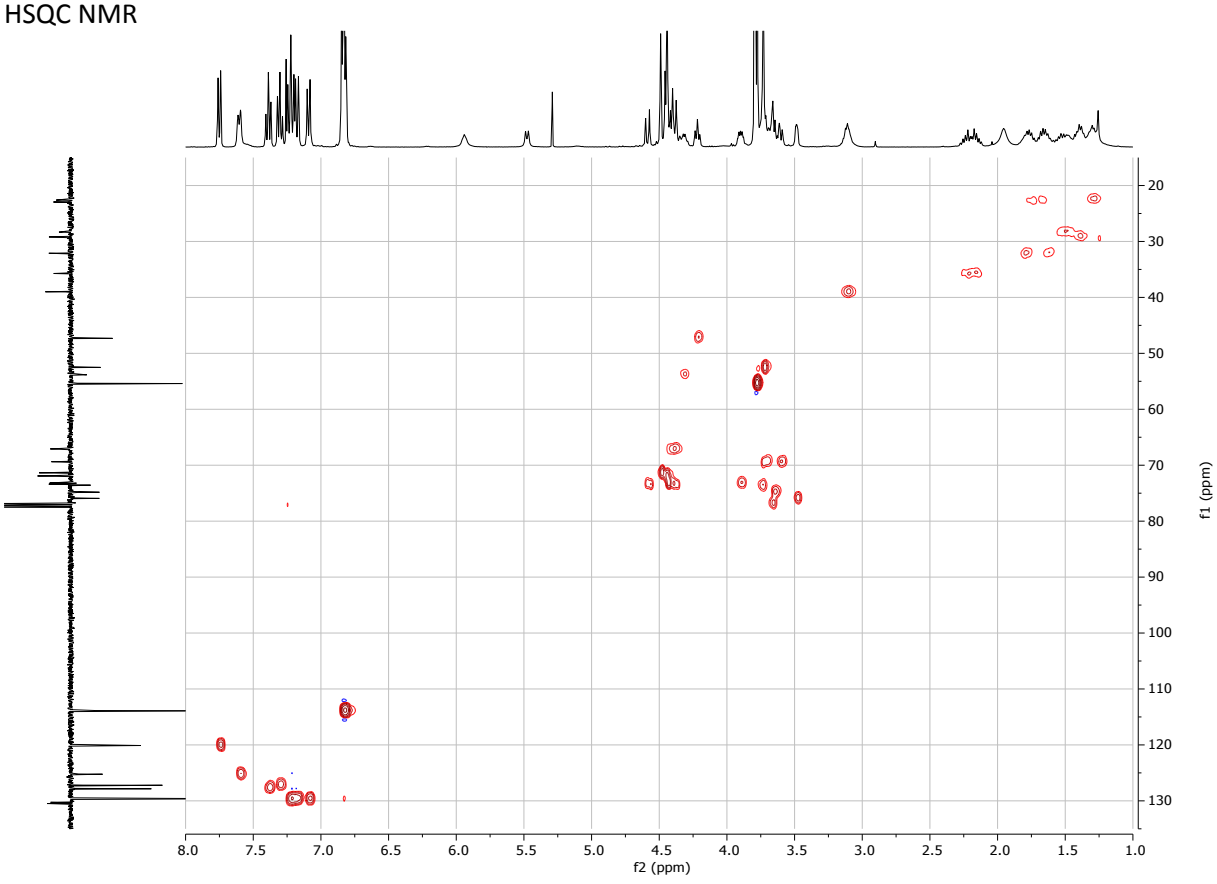
^{13}C APT NMR



HH- COSY NMR



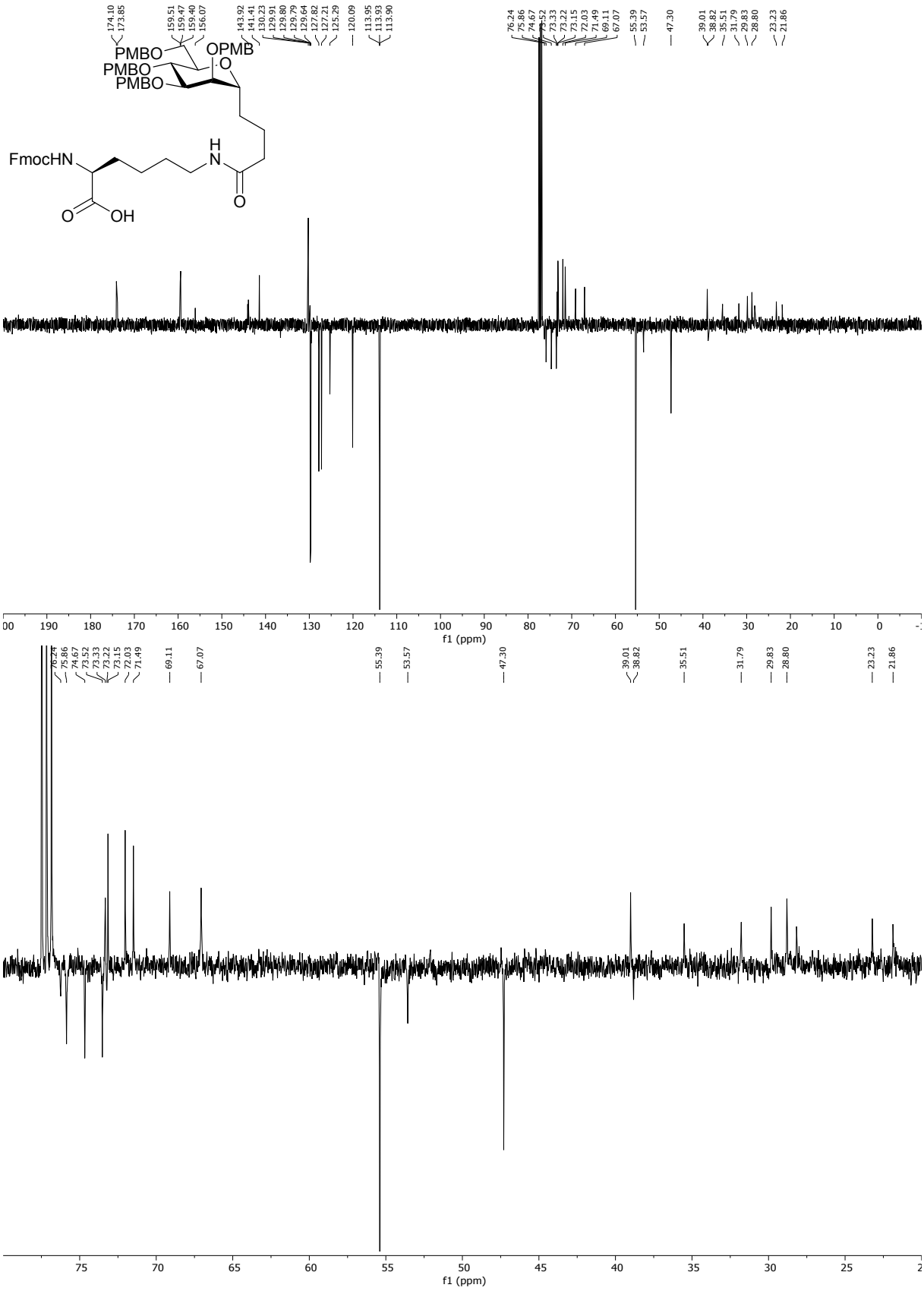
HSQC NMR



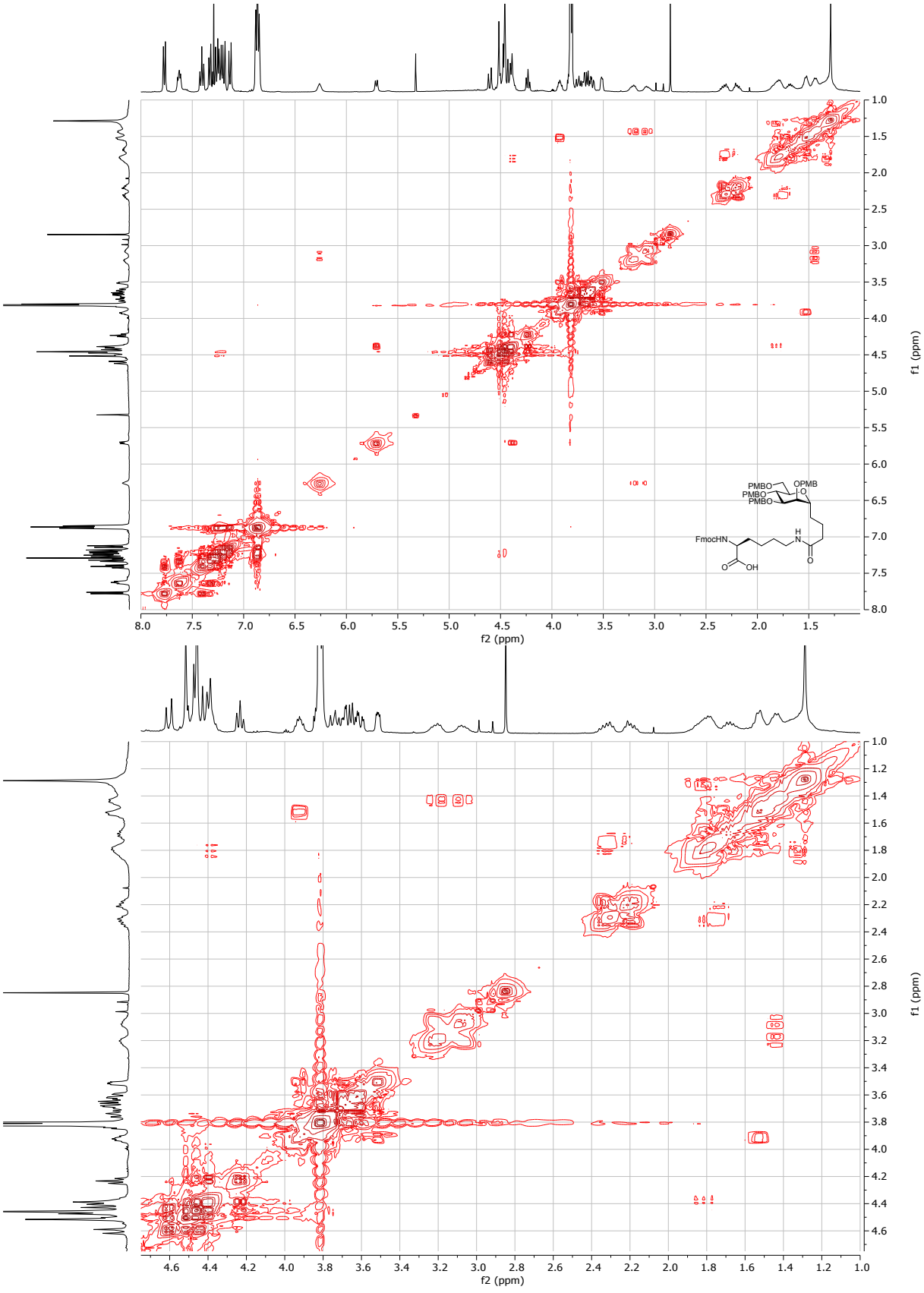
Compound 1

^{1}H NMR

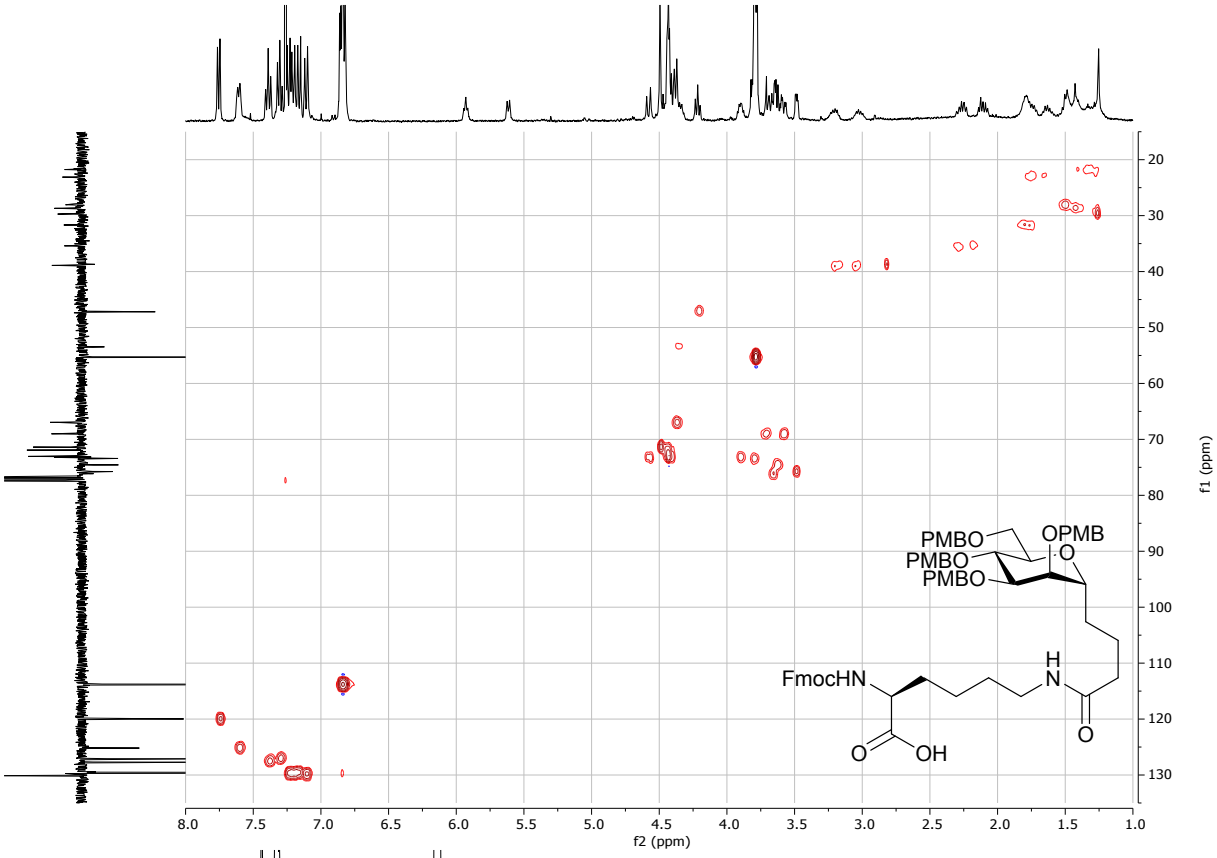
^{13}C APT NMR

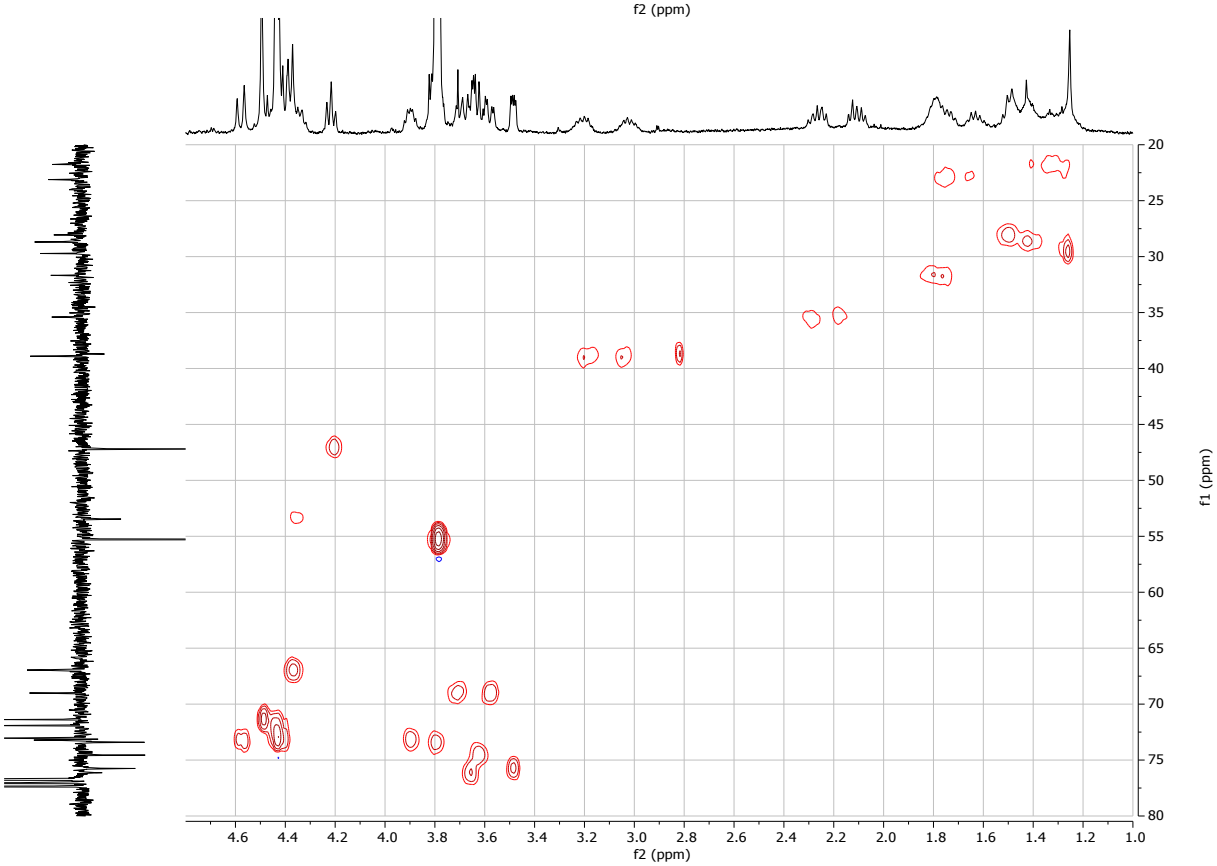

174.10
173.85
159.51
159.47
159.40
156.07
143.92
141.41
130.23
129.91
129.80
129.79
129.64
127.82
127.21
125.29
120.09
113.95
113.93
113.90
76.24
75.86
74.67
73.52
73.33
73.22
73.15
72.03
71.49
69.11
67.07
55.39
53.57
47.30
39.01
38.82
35.51
31.79
29.83
28.80
23.23
21.86
PMBO
PMBO
PMBO
OPMB
O
H
N
FmocHN
O
O
OH
f1 (ppm)

HH-COSY NMR



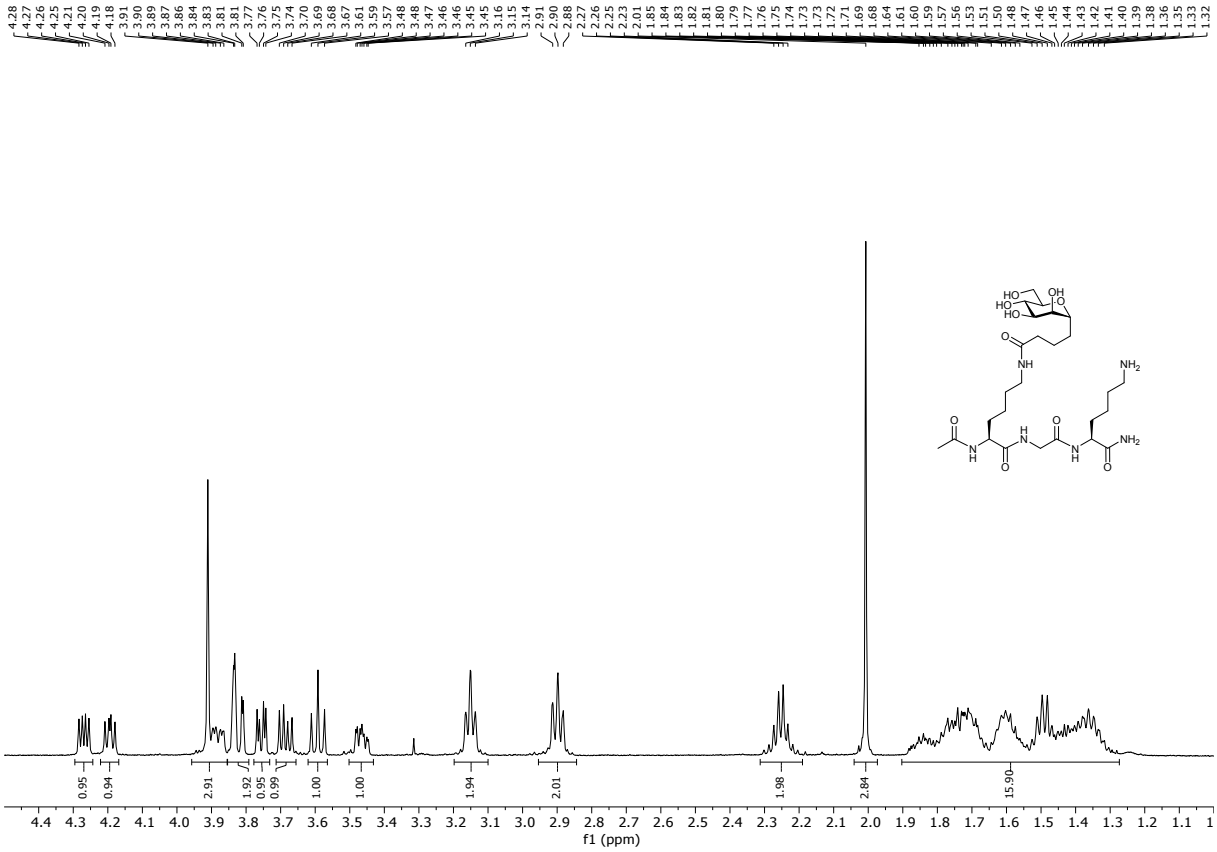
HSQC NMR



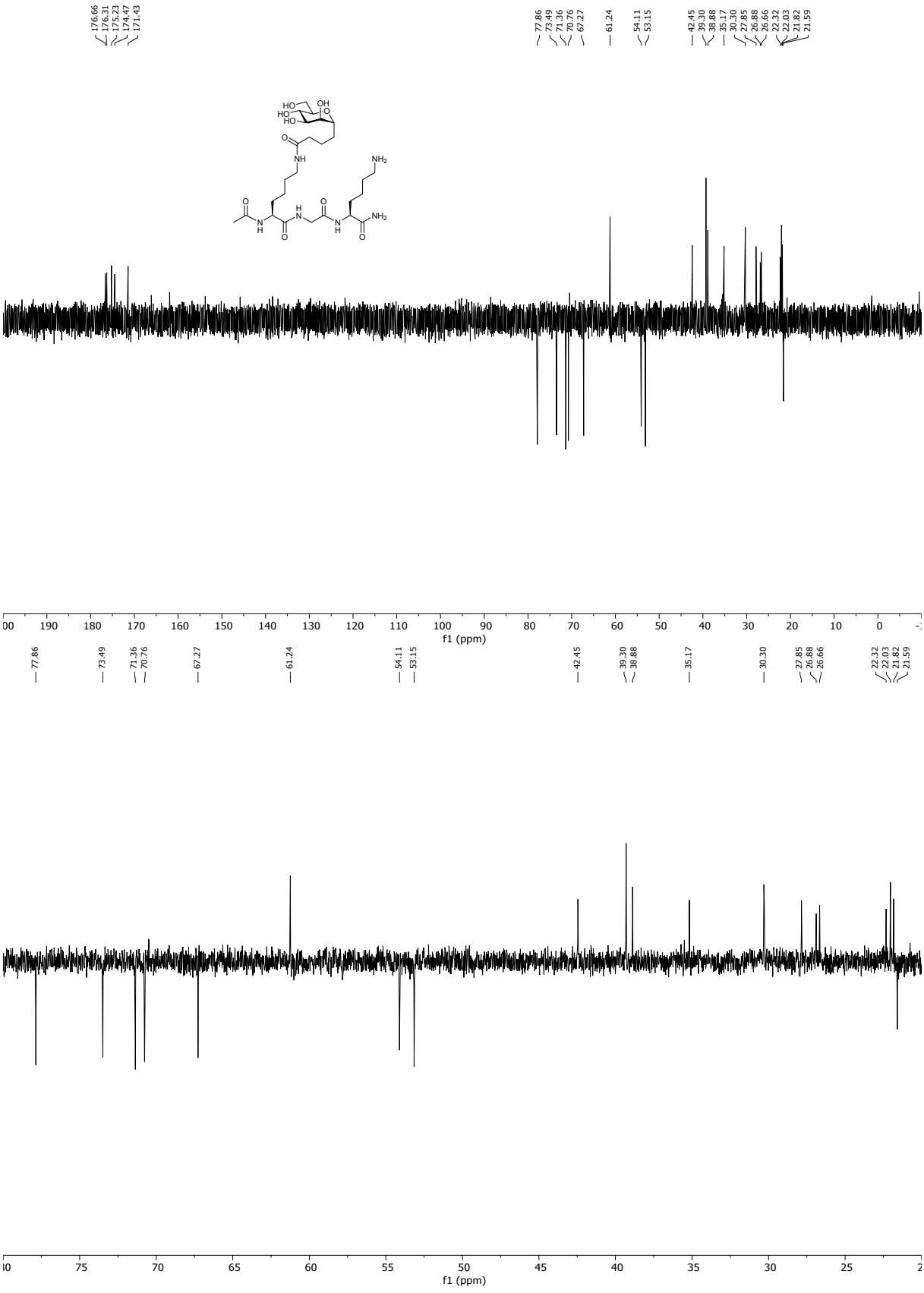


Compound 16

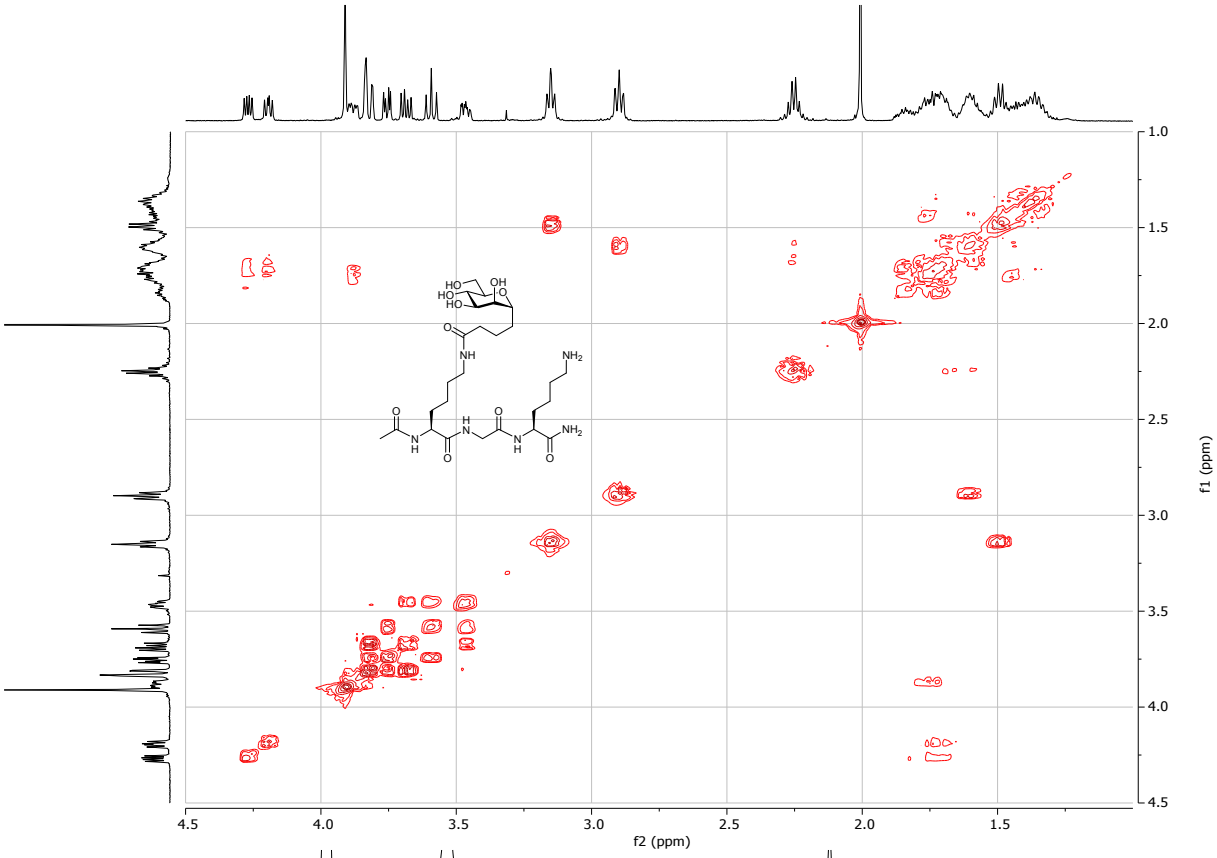
^{1}H NMR

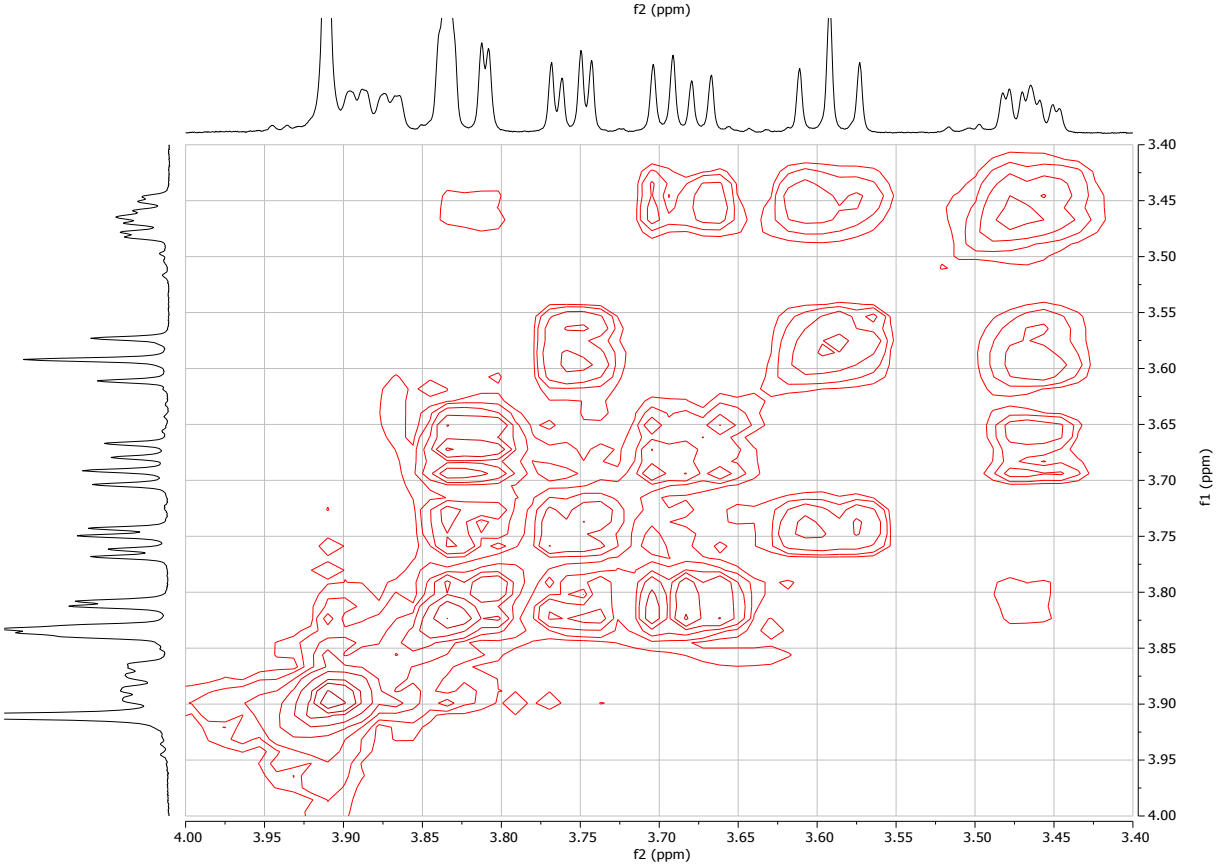


^{13}C APT NMR

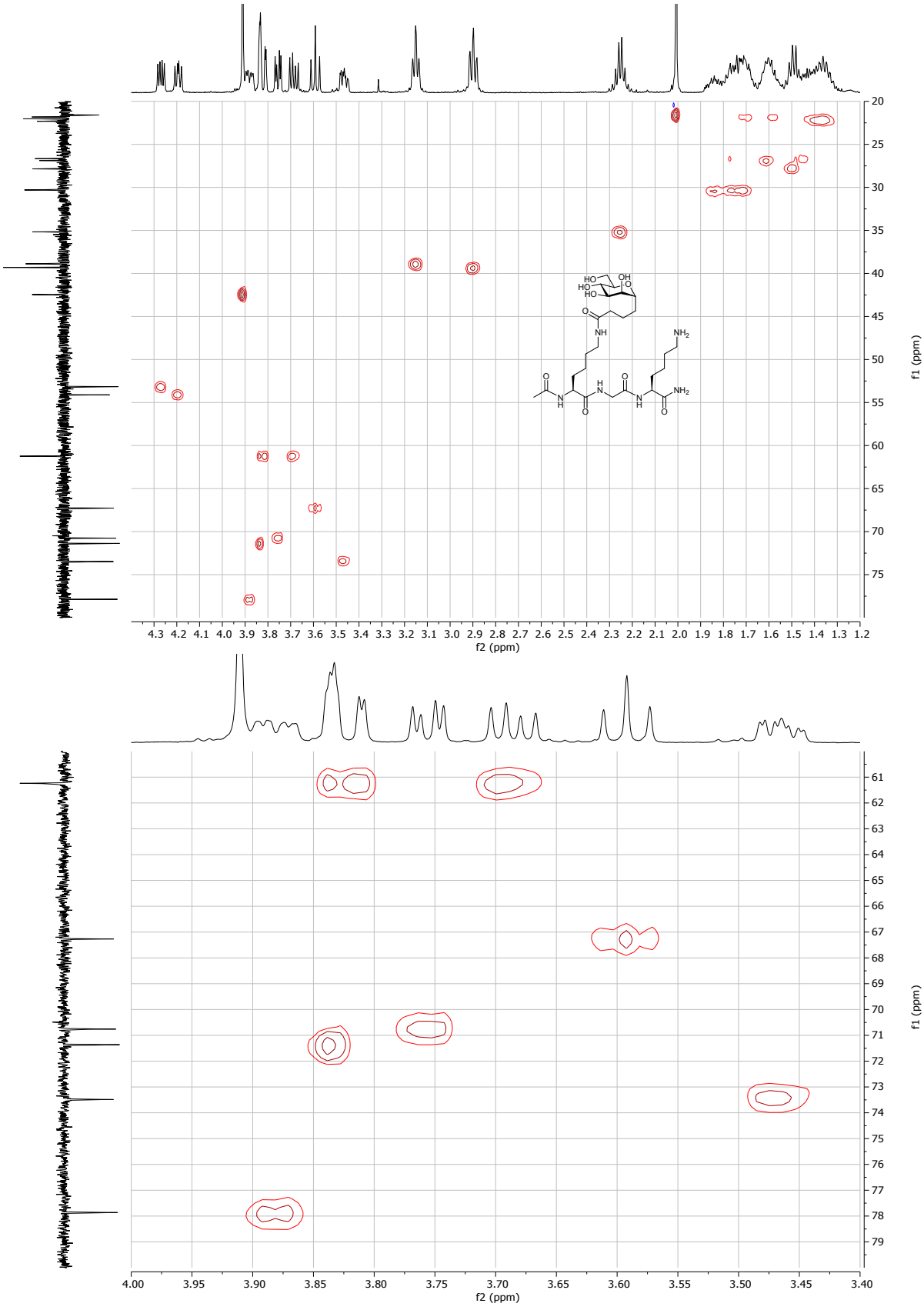

176.66
176.31
175.23
174.47
171.43
77.86
73.49
71.36
70.76
67.27
61.24
54.11
53.15
42.45
39.30
38.88
35.17
30.30
27.85
26.88
26.66
22.32
22.03
21.82
21.59
f1 (ppm)

HH- COSY NMR

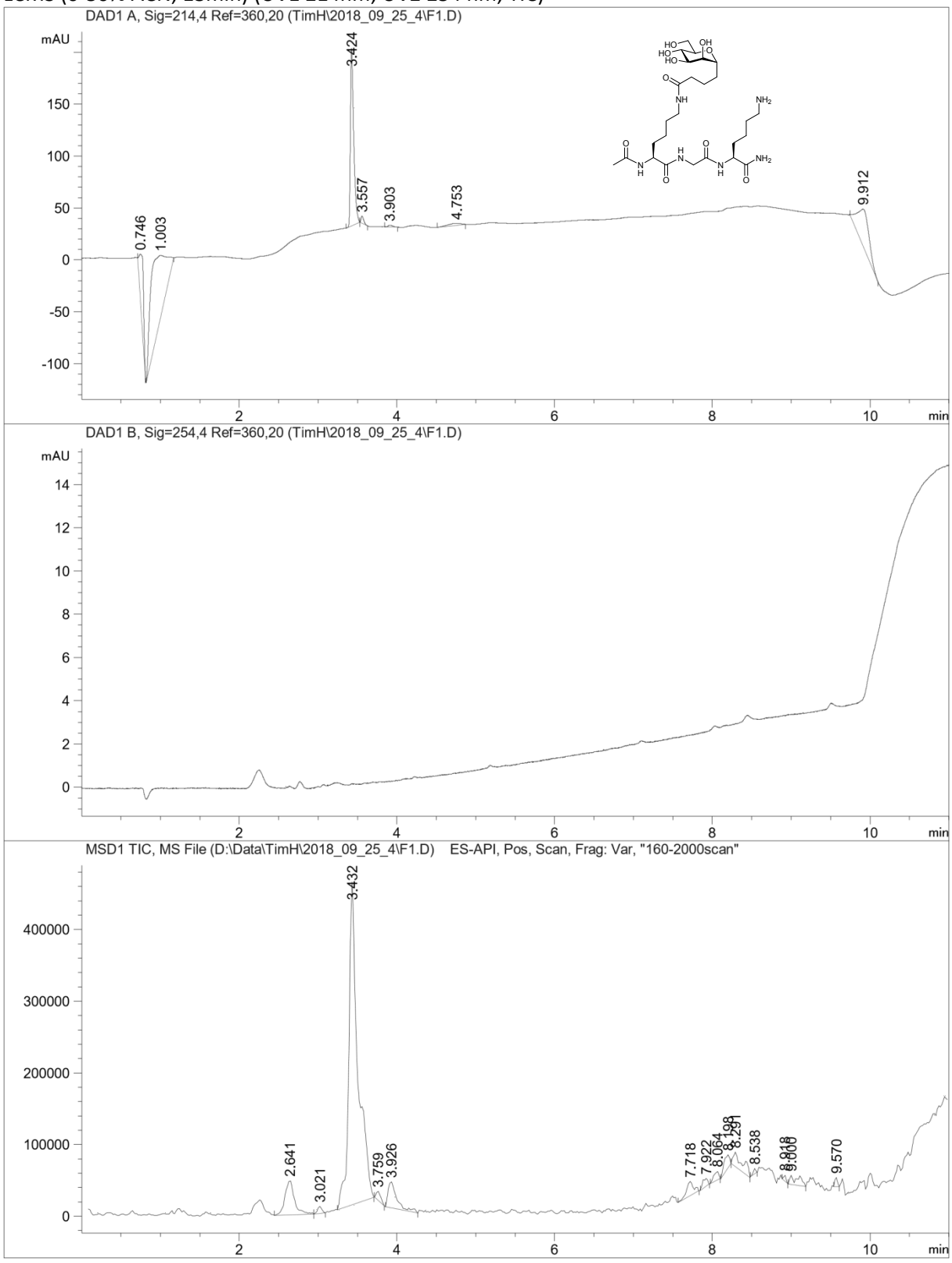

f2 (ppm)
f1 (ppm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


f2 (ppm)
f1 (ppm)
4.00
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
3.55
3.50
3.45
3.40

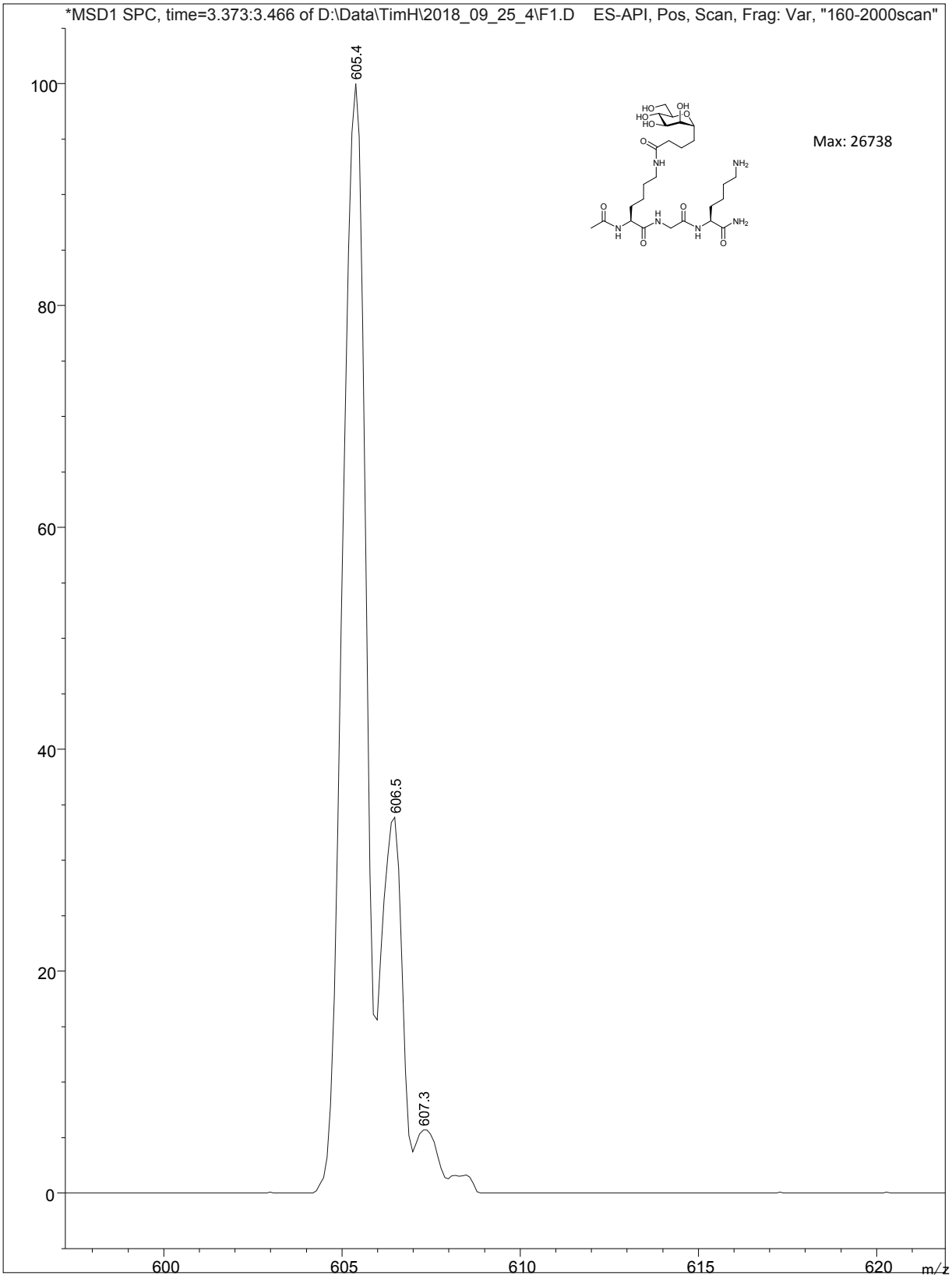
HSQC NMR



LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)

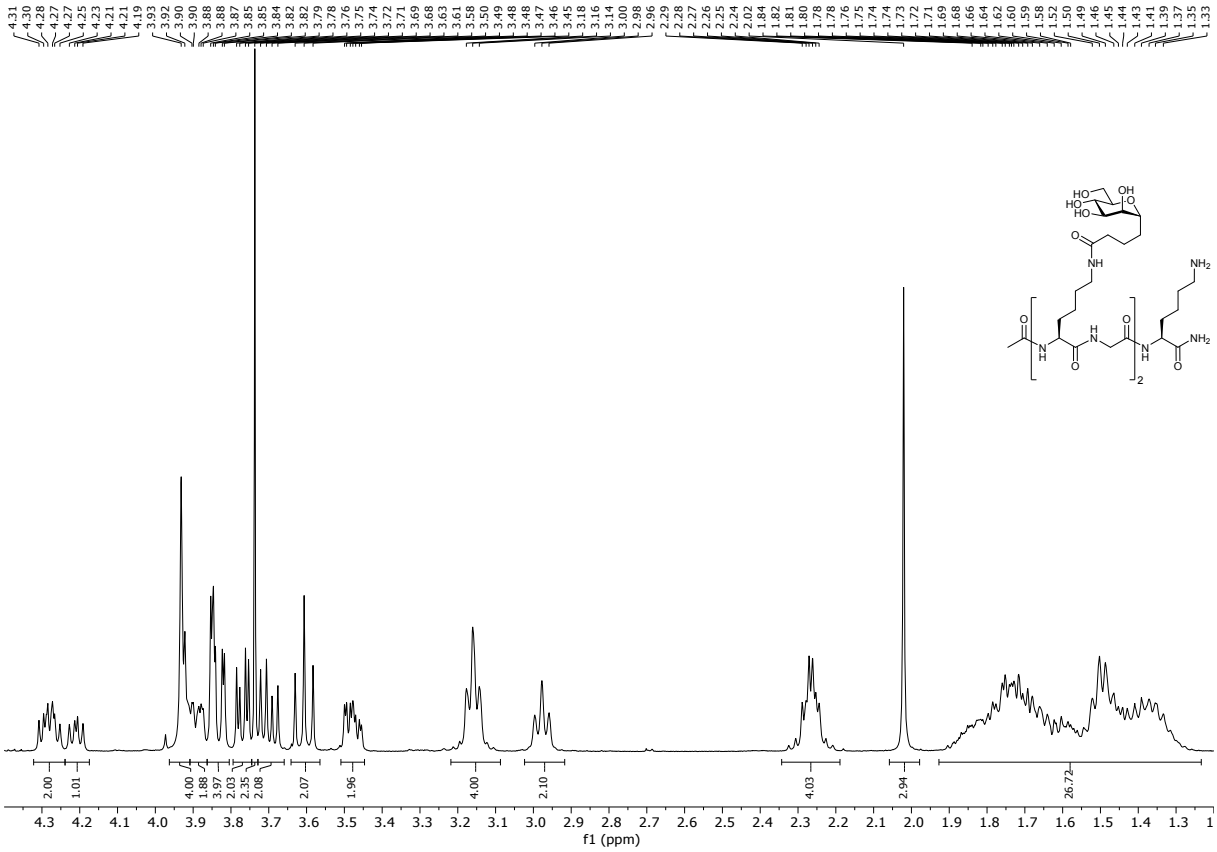


MS

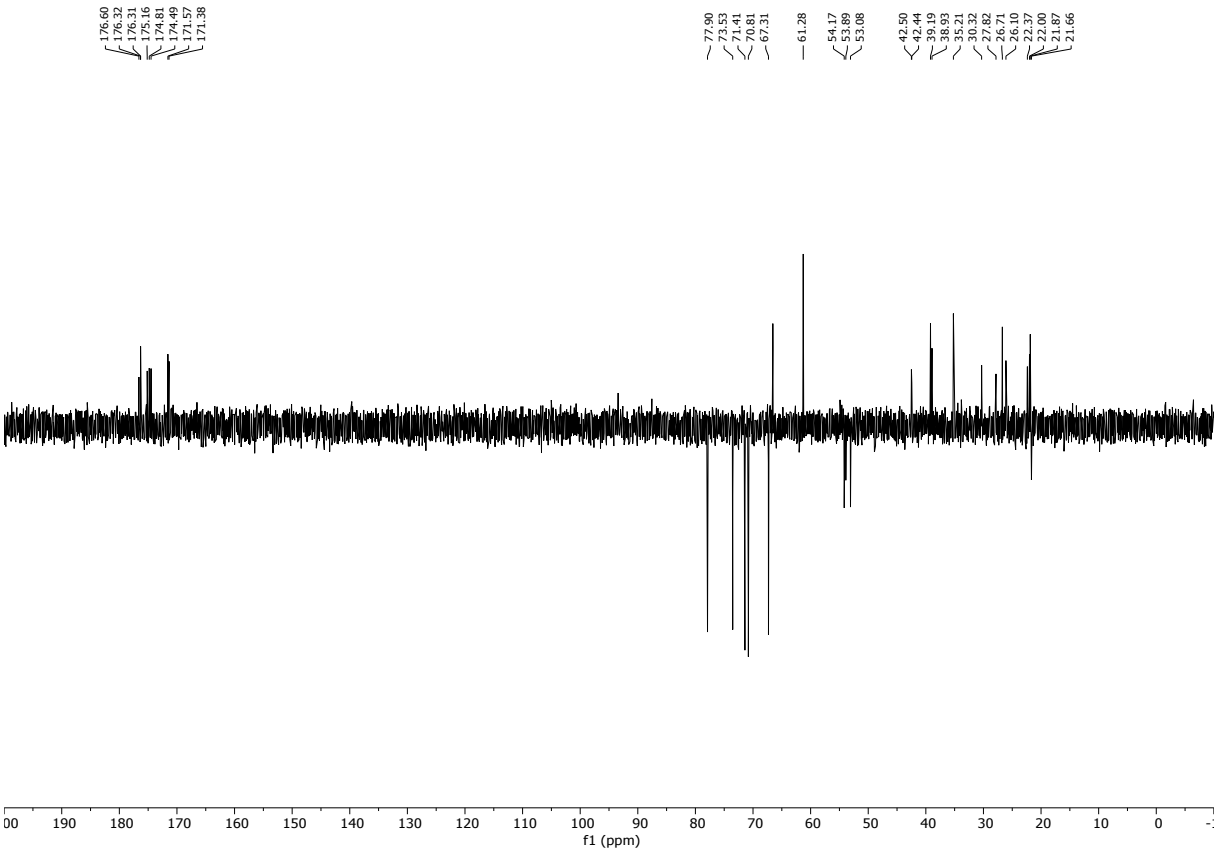

*MSD1 SPC, time=3.373:3.466 of D:\Data\TimH\2018_09_25_4\F1.D ES-API, Pos, Scan, Frag: Var, "160-2000scan"
605.4
606.5
607.3
Max: 26738
100
80
60
40
20
0
600
605
610
615
620
m/z

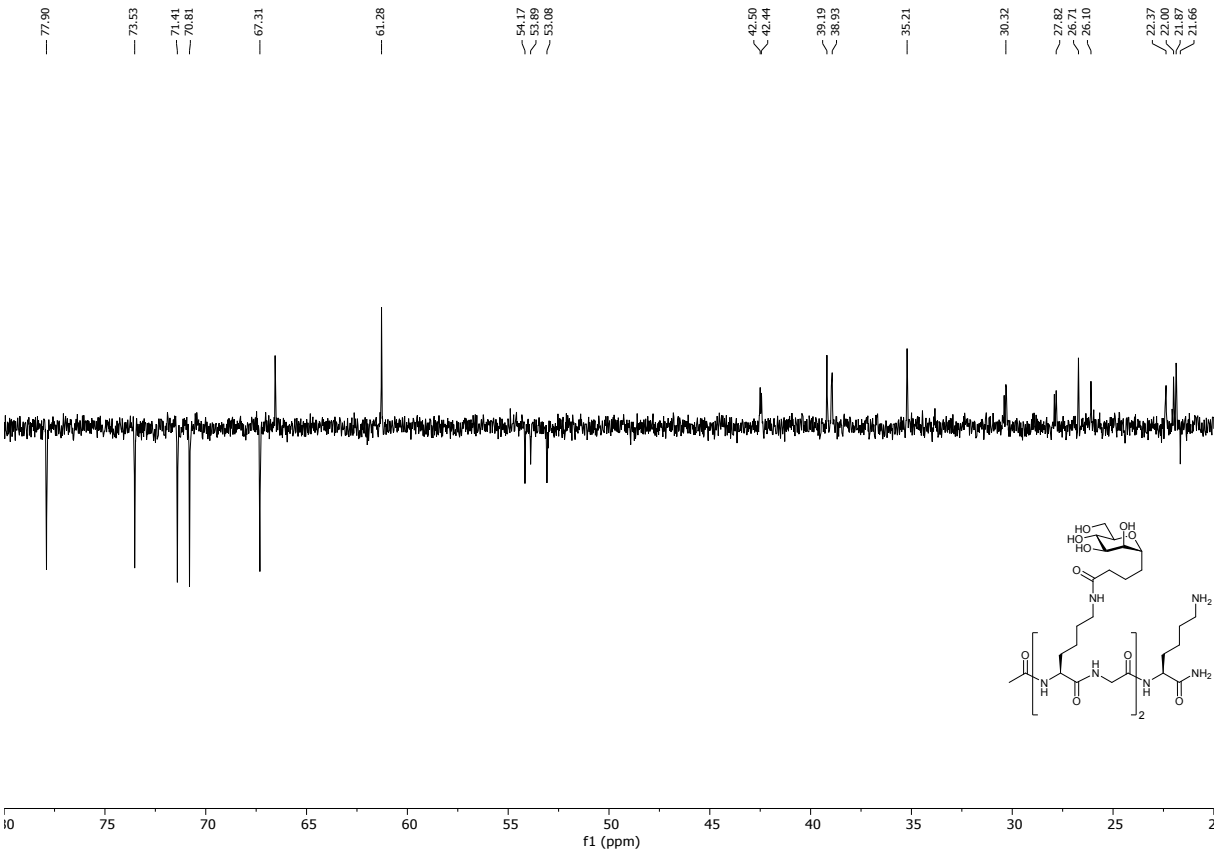
Compound 17

^{1}H NMR

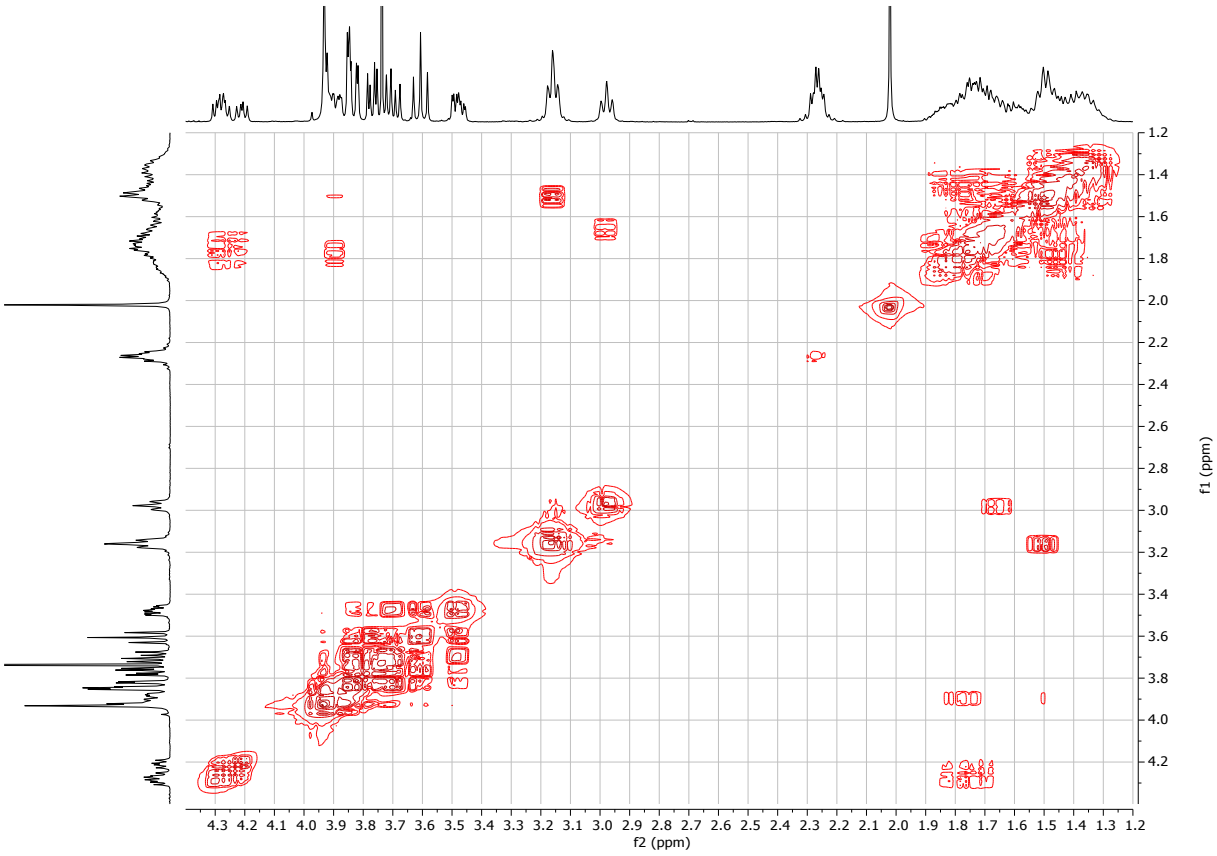


^{13}C APT NMR



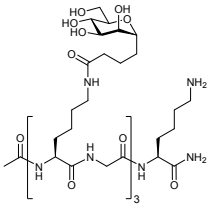

77.90
73.53
71.41
70.81
67.31
61.28
54.17
53.89
53.08
42.50
42.44
39.19
38.93
35.21
30.32
27.82
26.71
26.10
22.37
22.00
21.87
21.66
f1 (ppm)

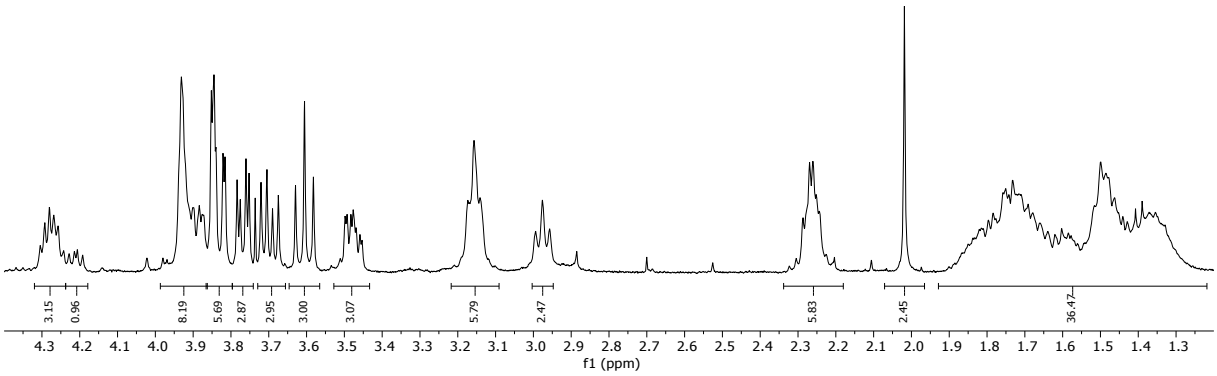
HH-COSY NMR


f2 (ppm)
f1 (ppm)

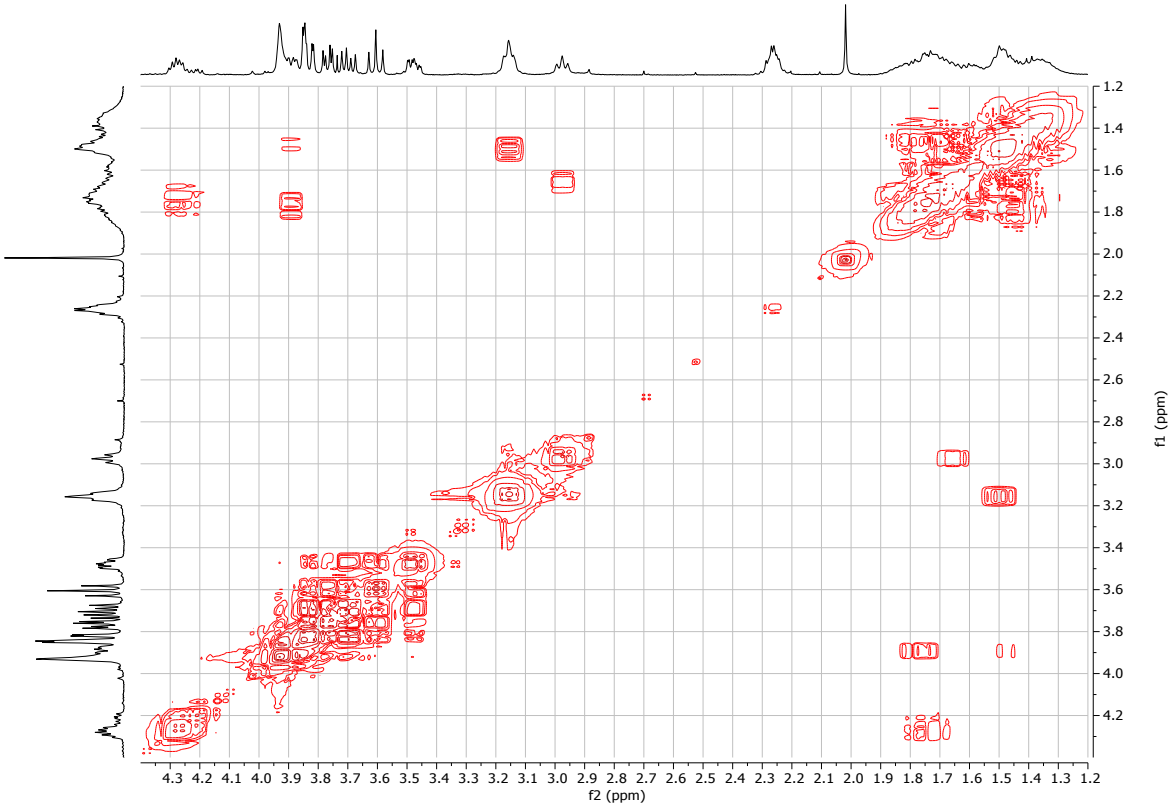
Compound 18

^{1}H NMR



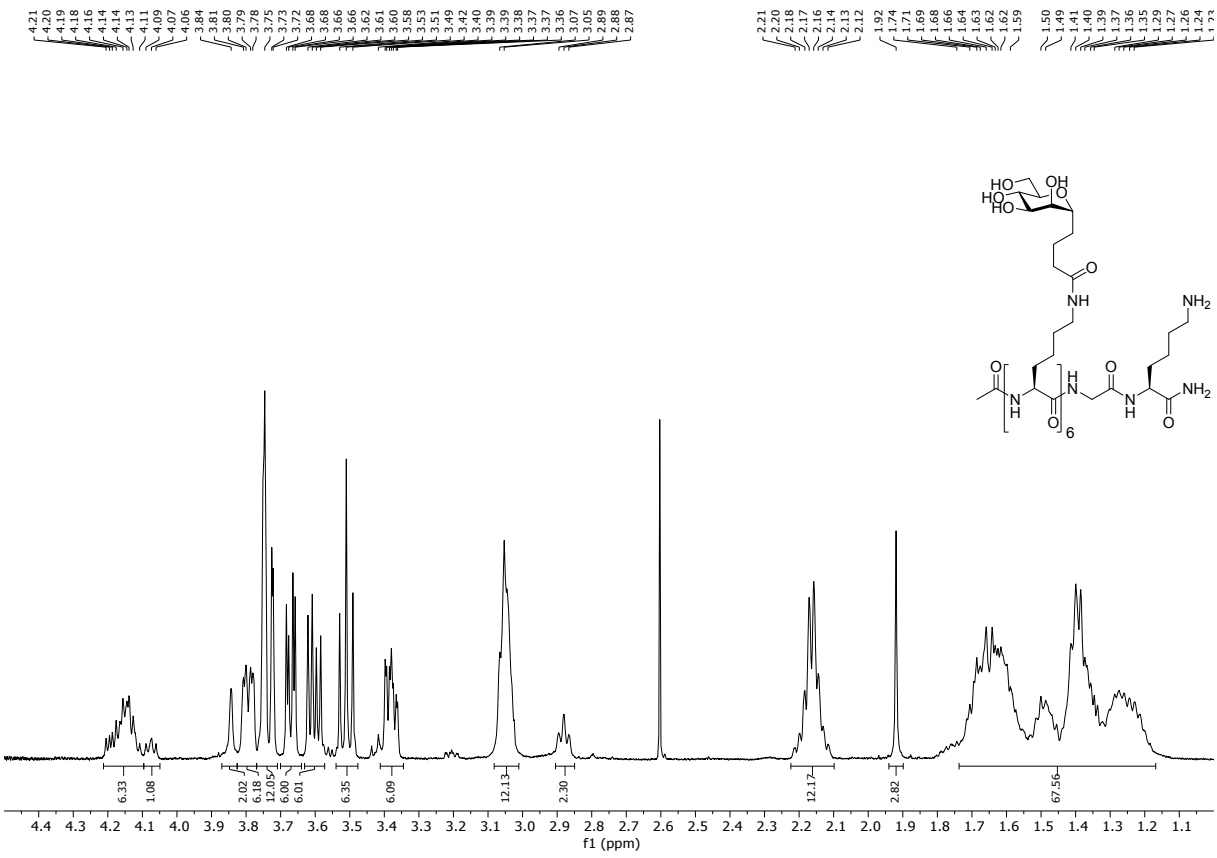


HH-COSY NMR

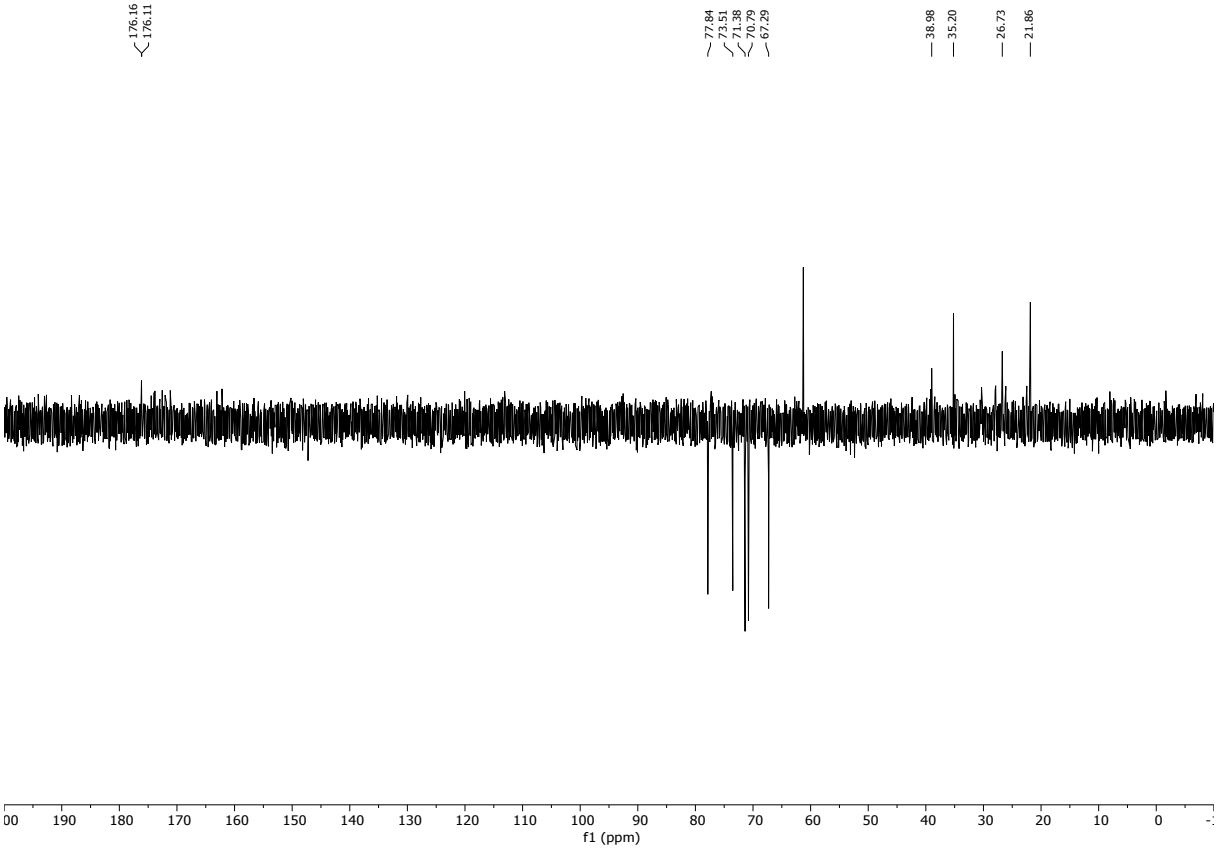


Compound 19

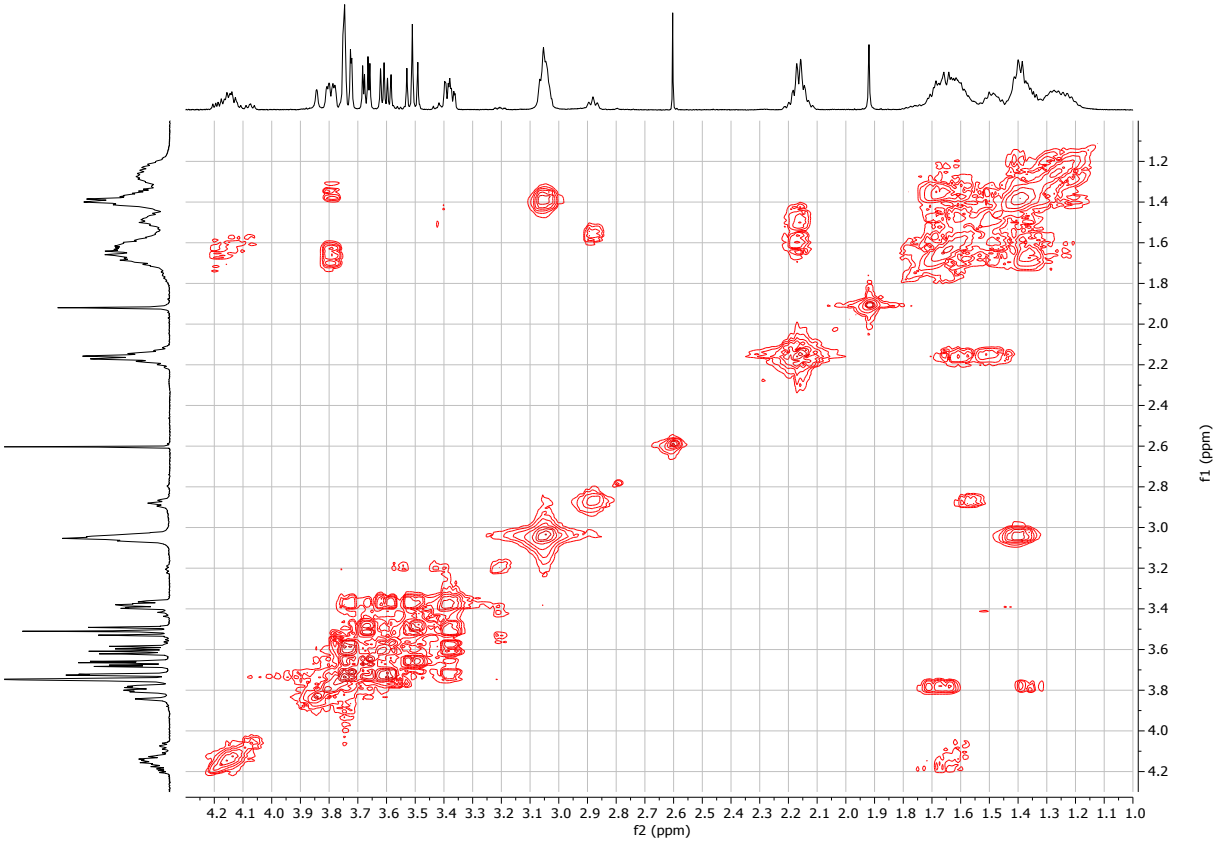
^{1}H NMR



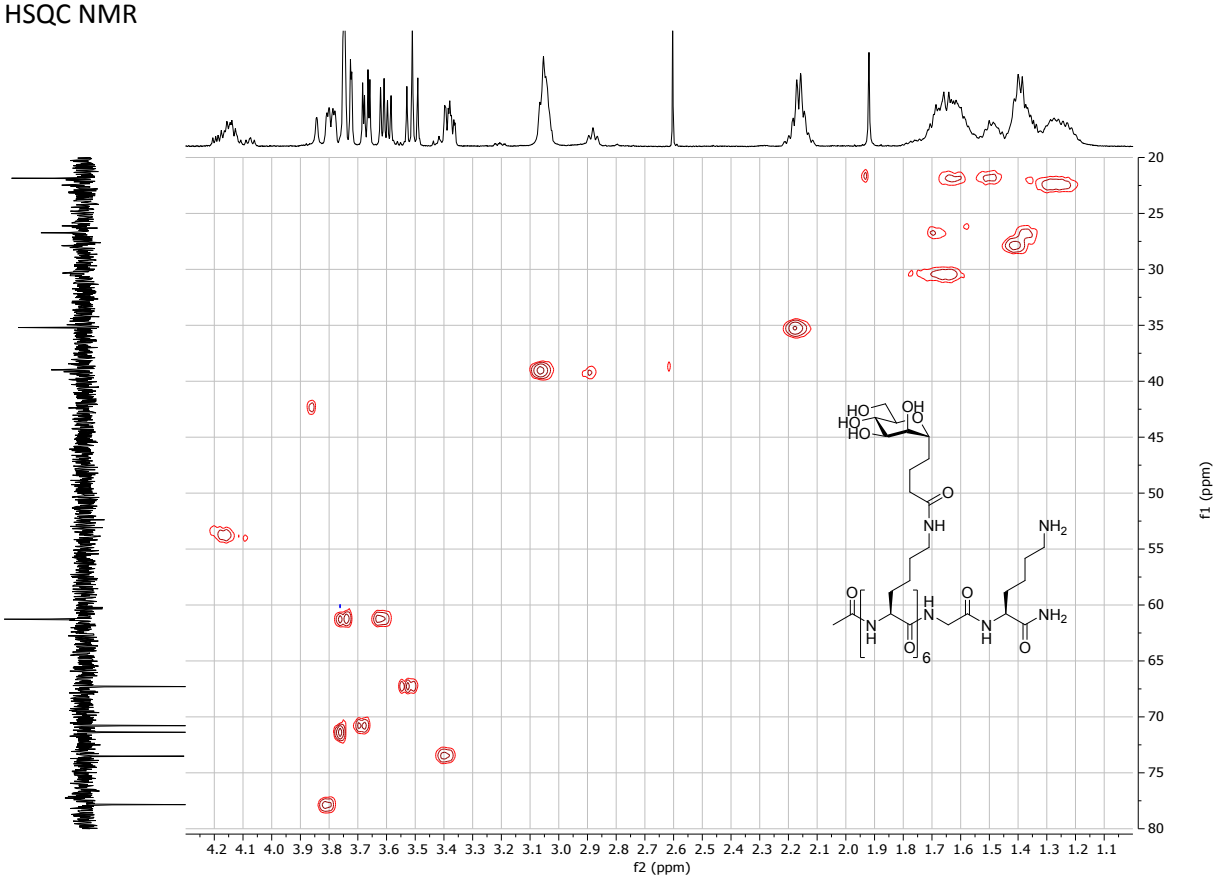
^{13}C APT NMR



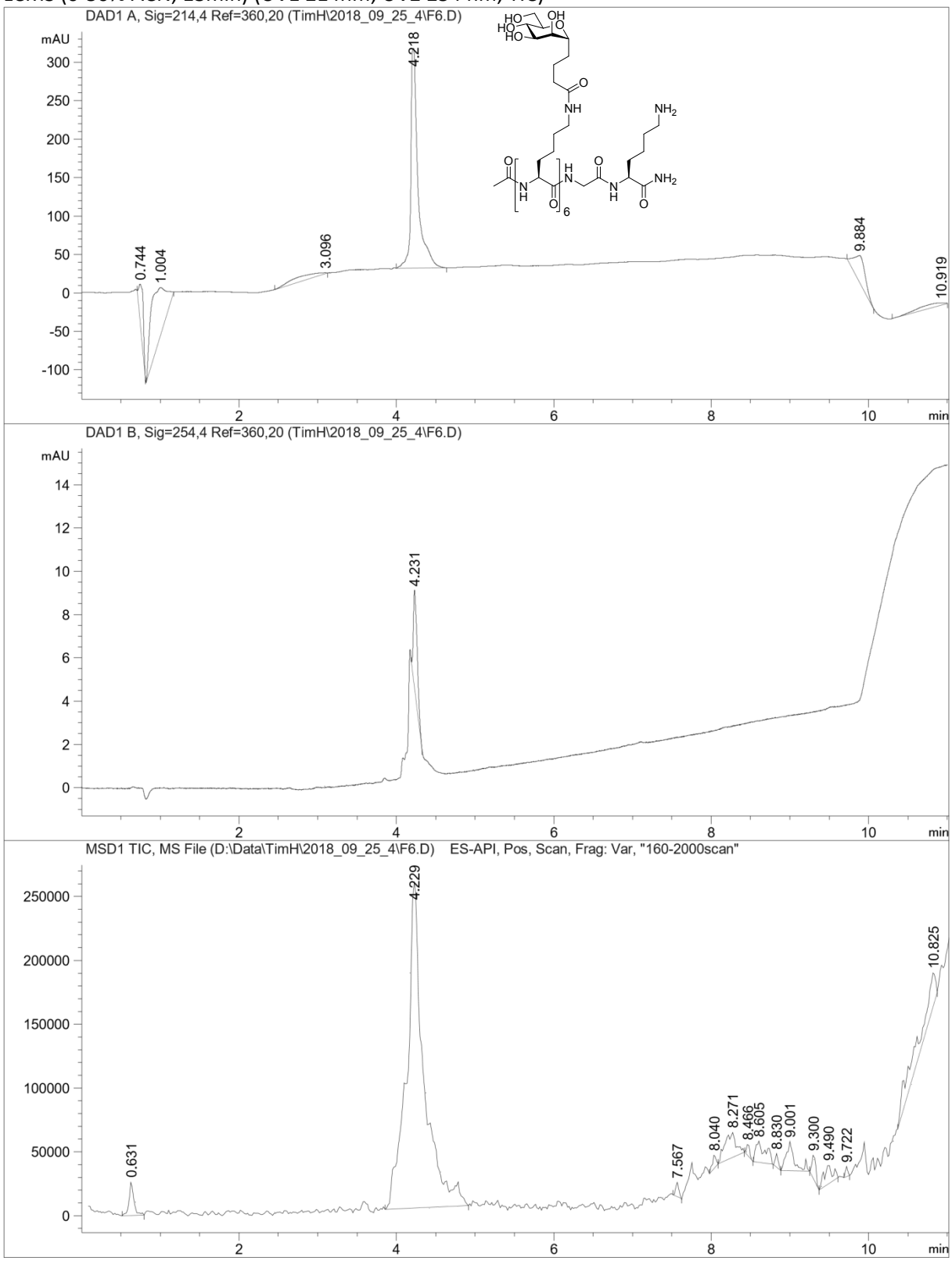
HH-COSY NMR



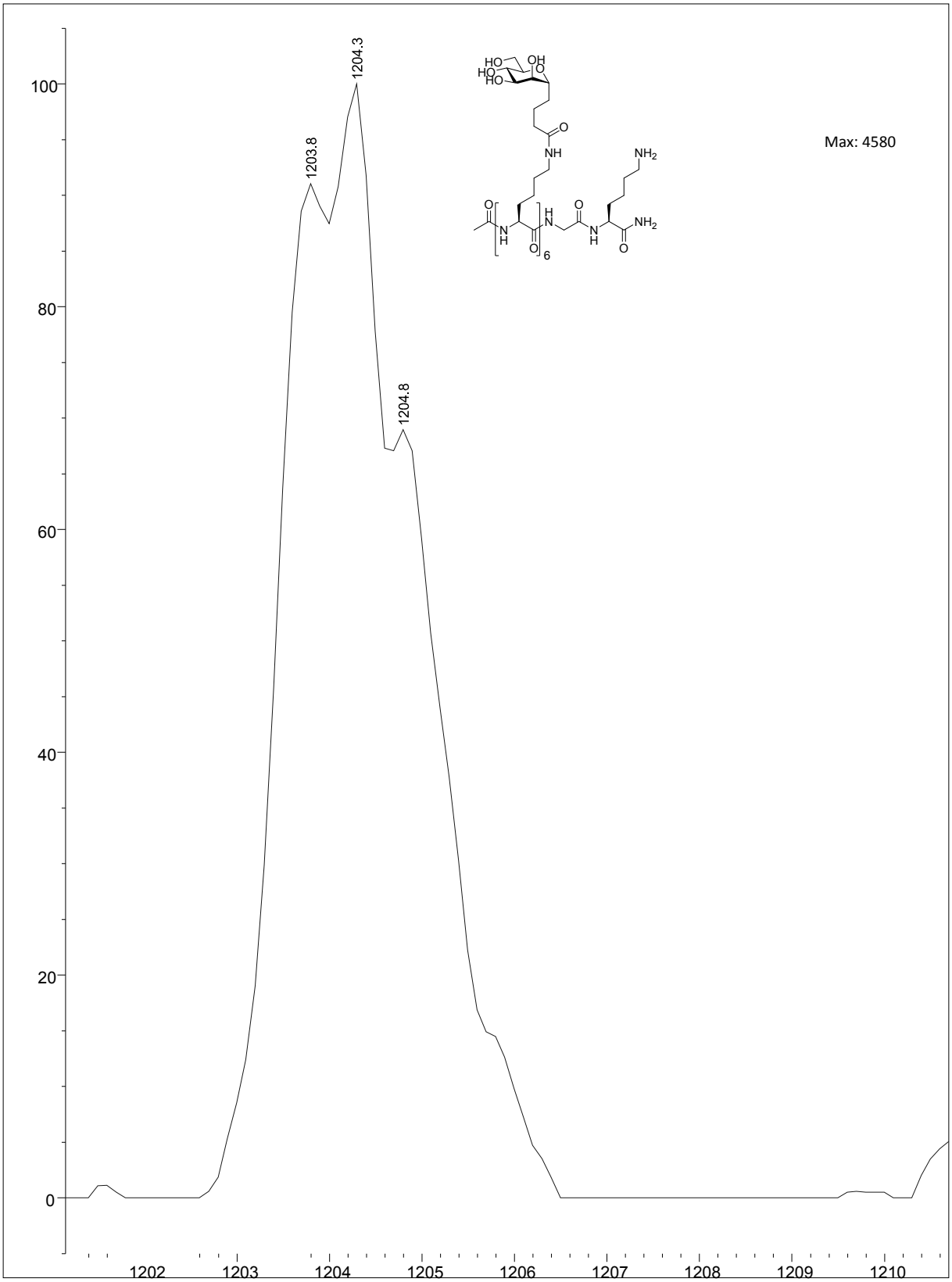
HSQC NMR



LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)

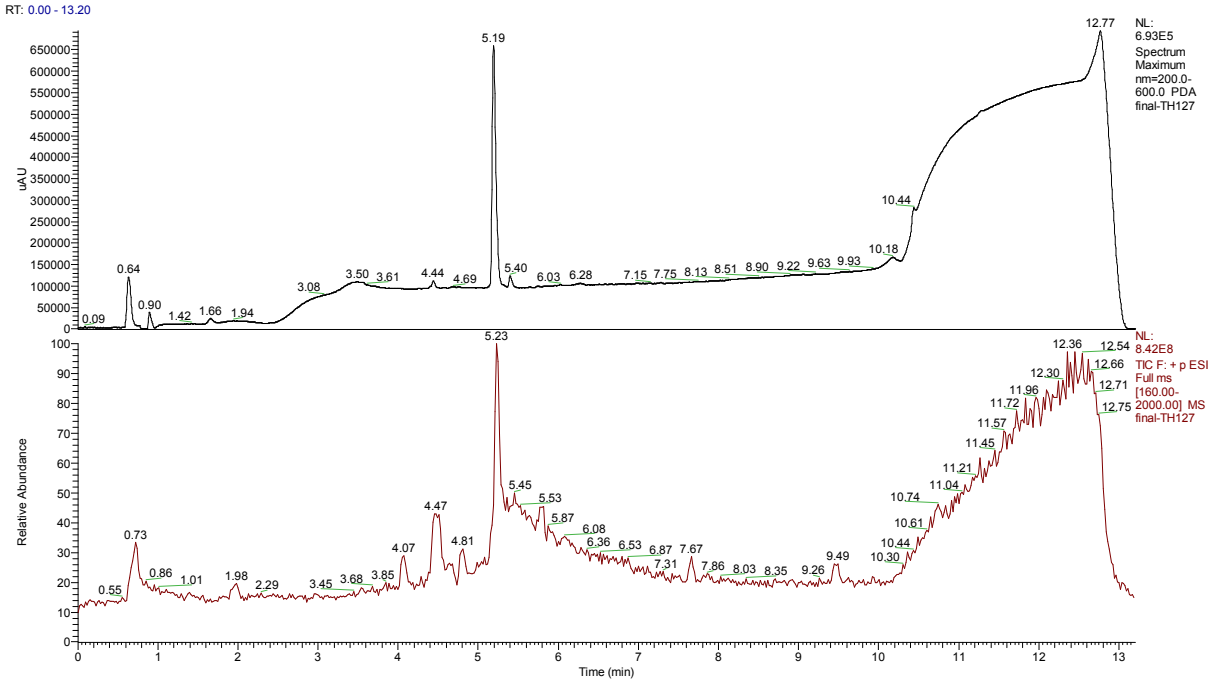


MS

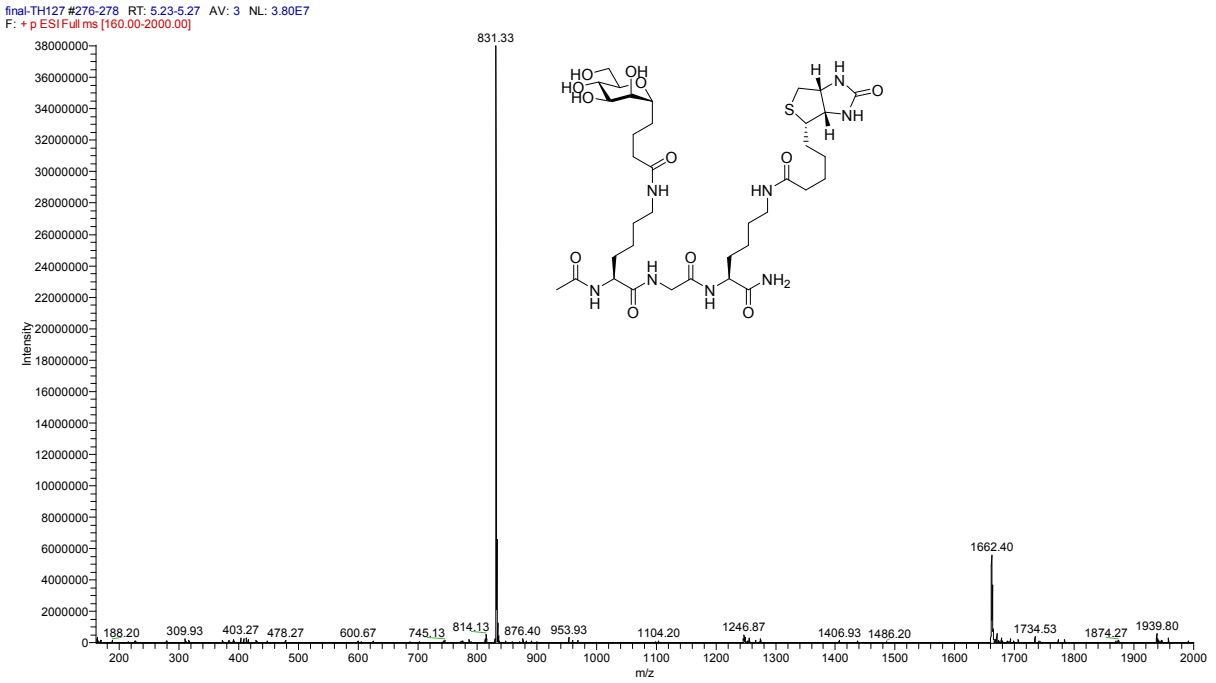

1204.3
1203.8
1204.8
Max: 4580
100
80
60
40
20
0
1202
1203
1204
1205
1206
1207
1208
1209
1210

Compound 20

LCMS (0-50% ACN; 13min) (UV 200-600nm; TIC)

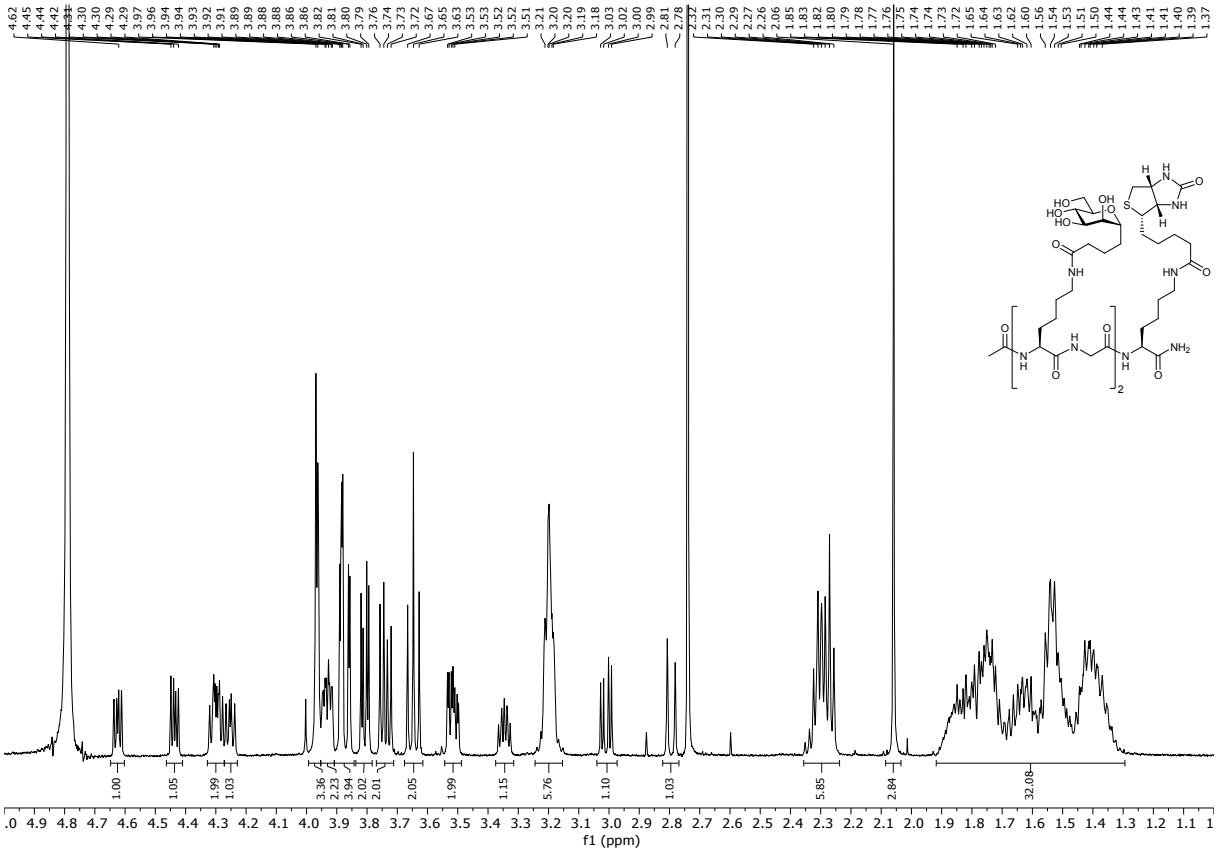


MS

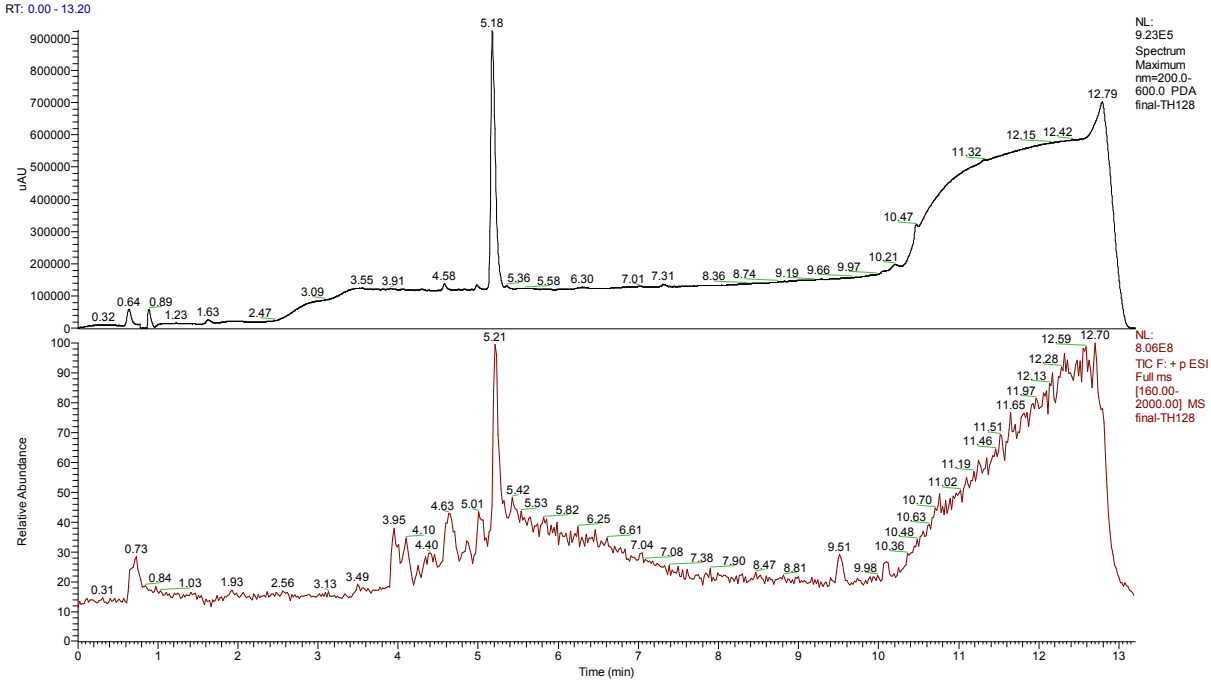


Compound 21

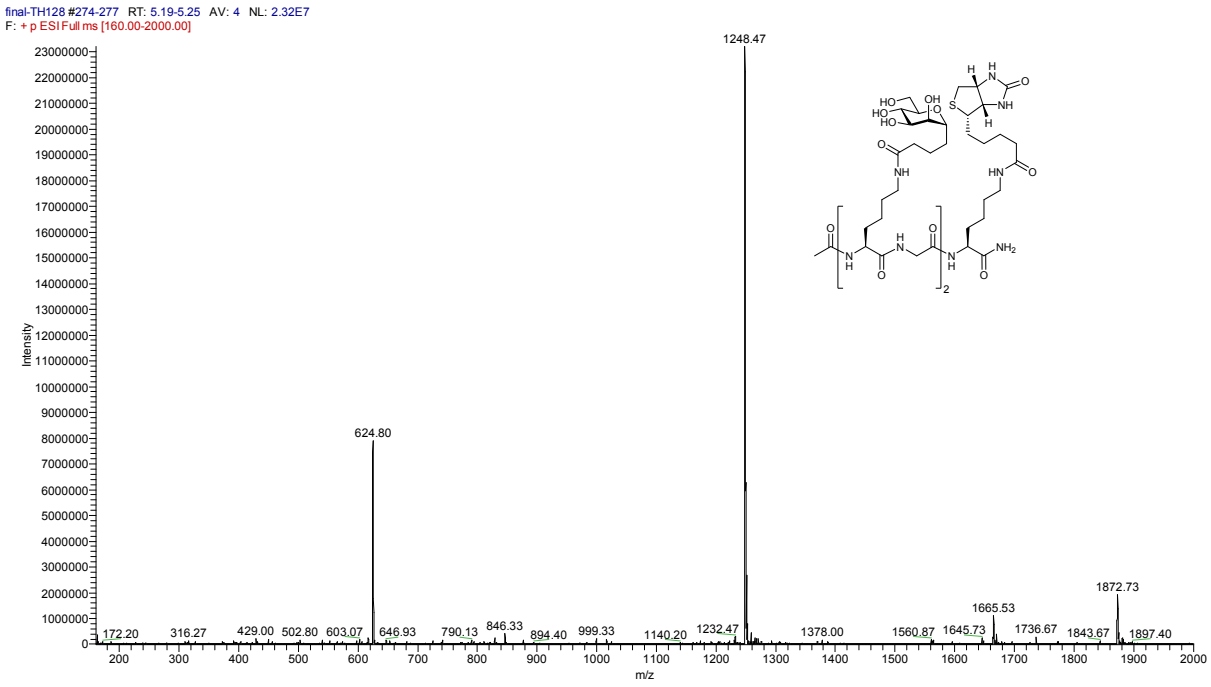
^{1}H NMR



LCMS (0-50% ACN; 13min) (UV 200-600nm; TIC)

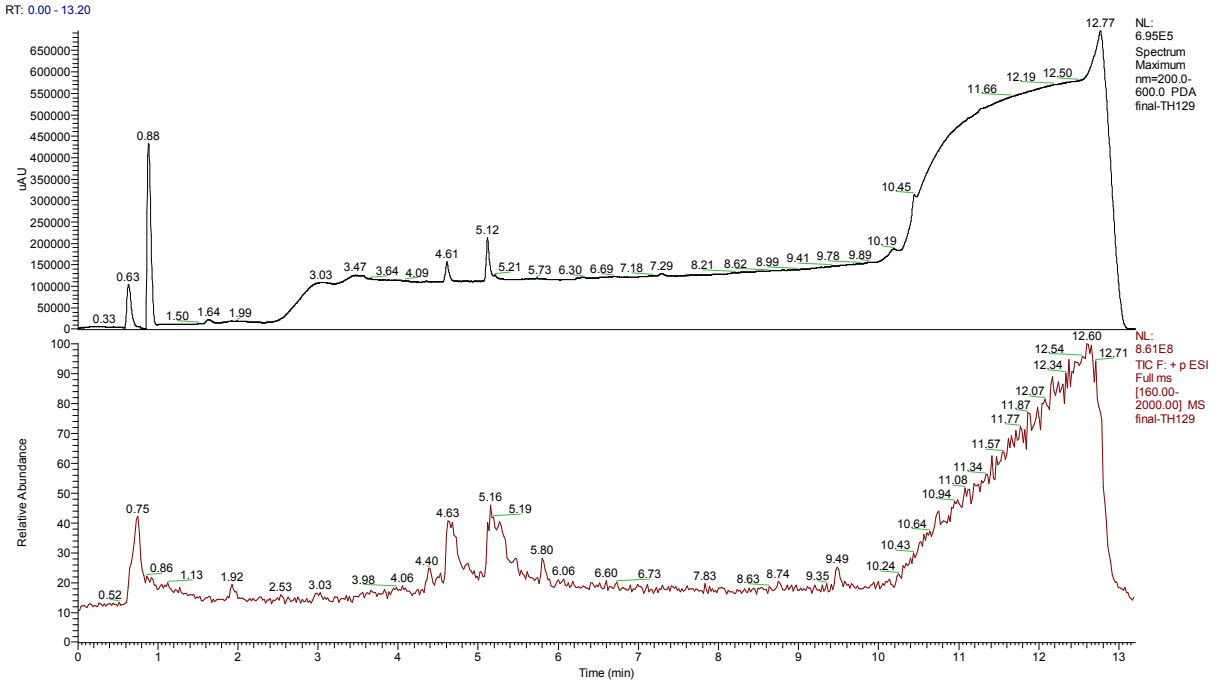


MS

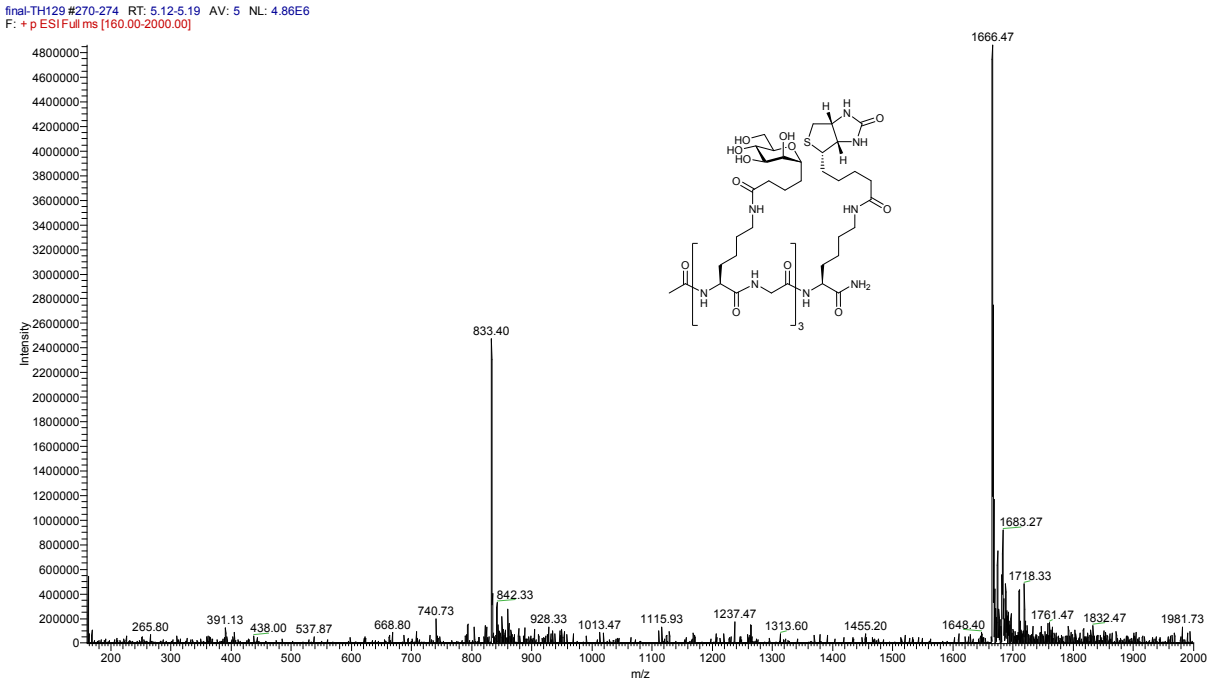


Compound 22

LCMS (0-50% ACN; 13min) (UV 200-600nm; TIC)

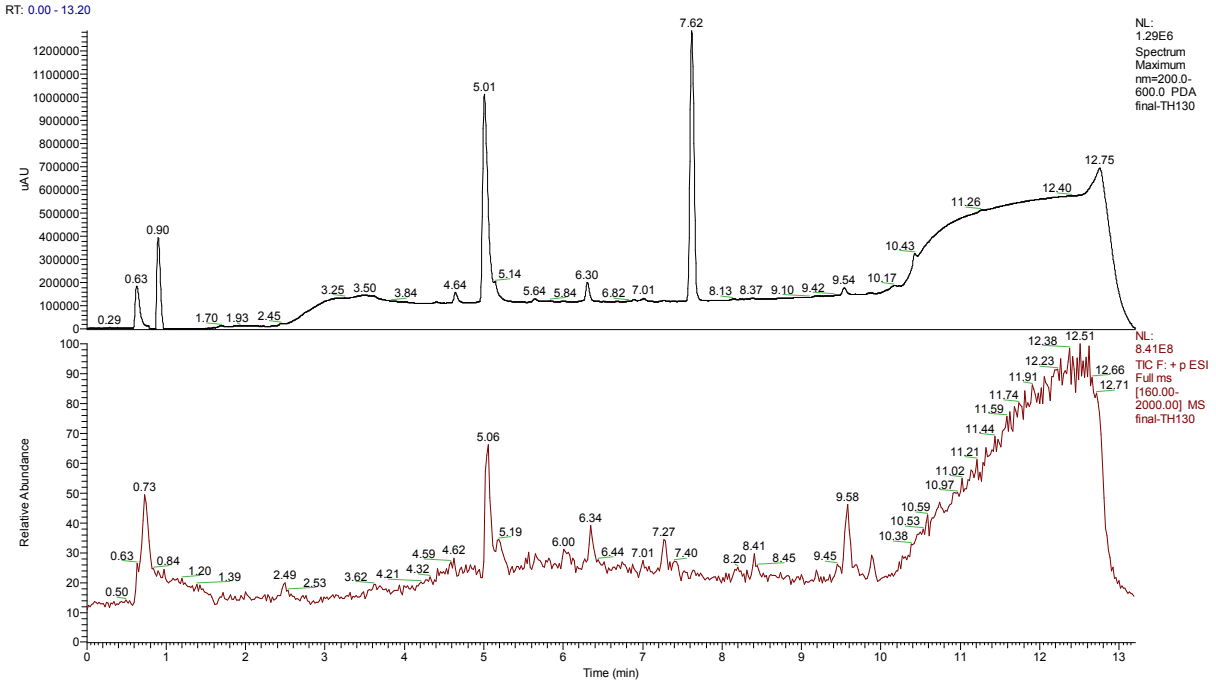


MS

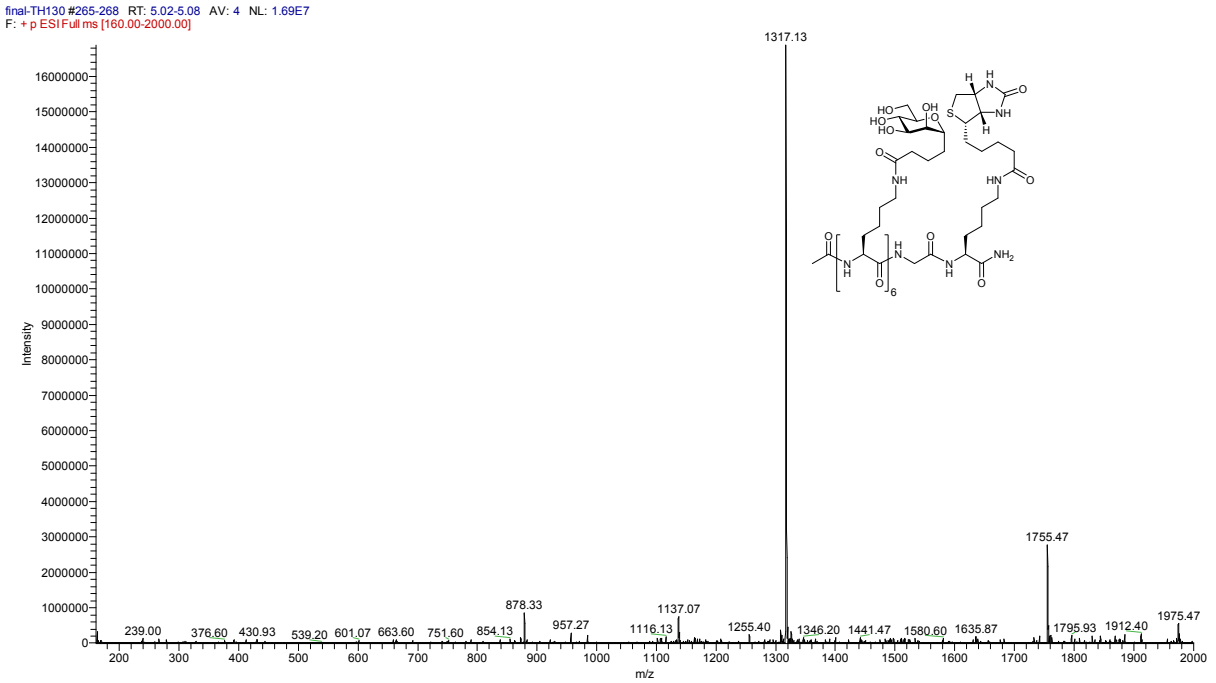


Compound 23

LCMS (0-50% ACN; 13min) (UV 200-600nm; TIC)

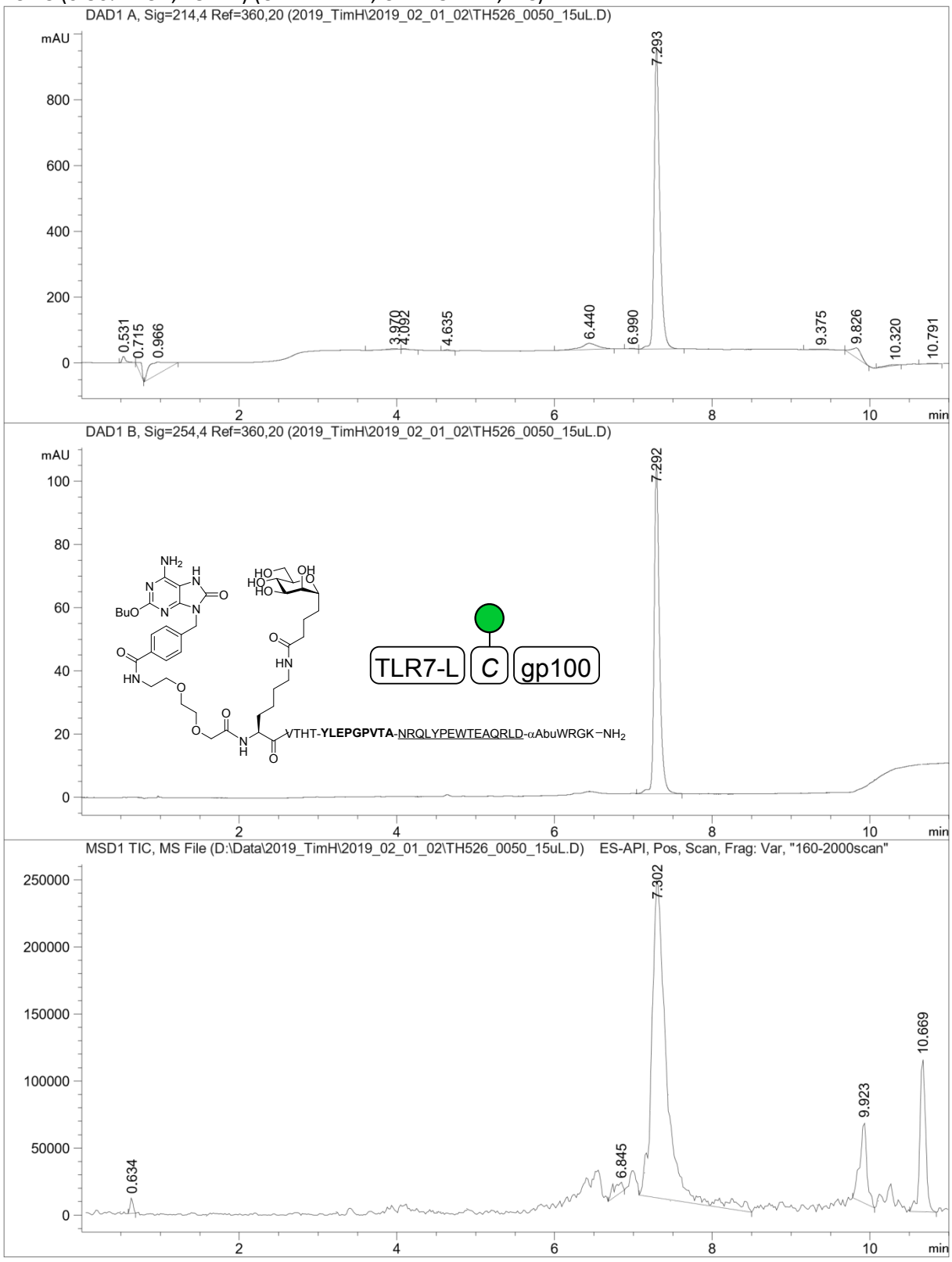


MS

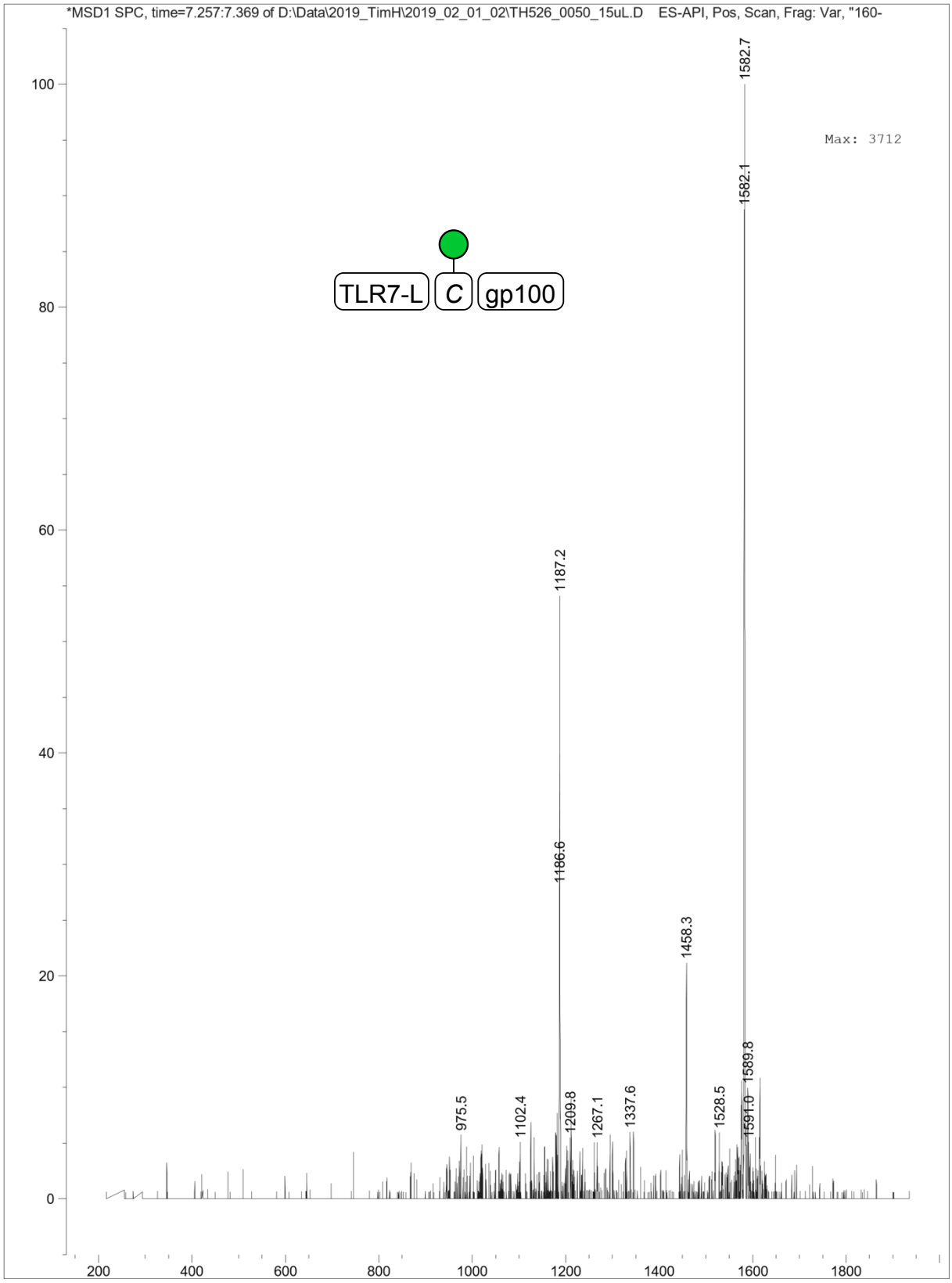


Compound 39

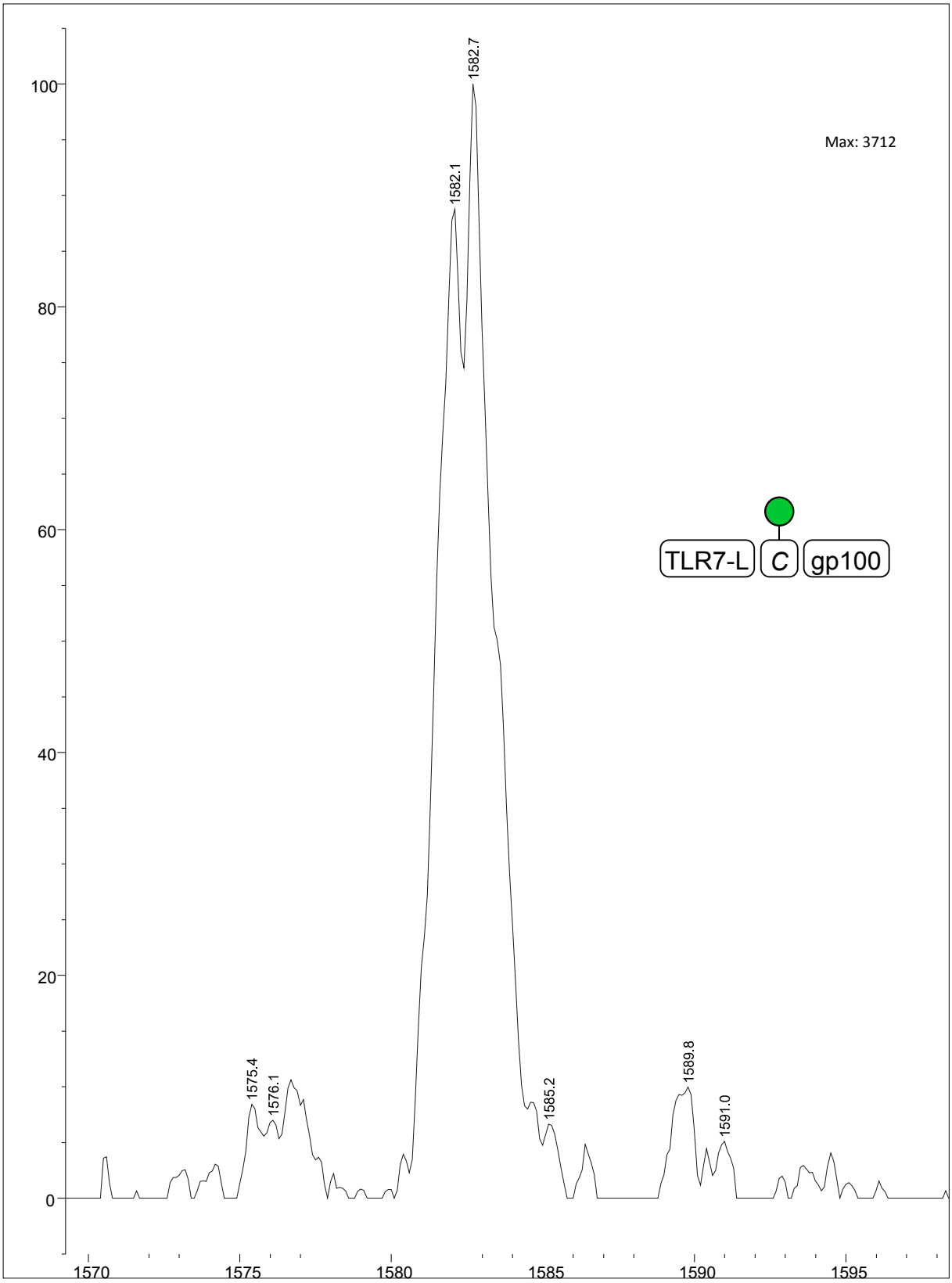
LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)



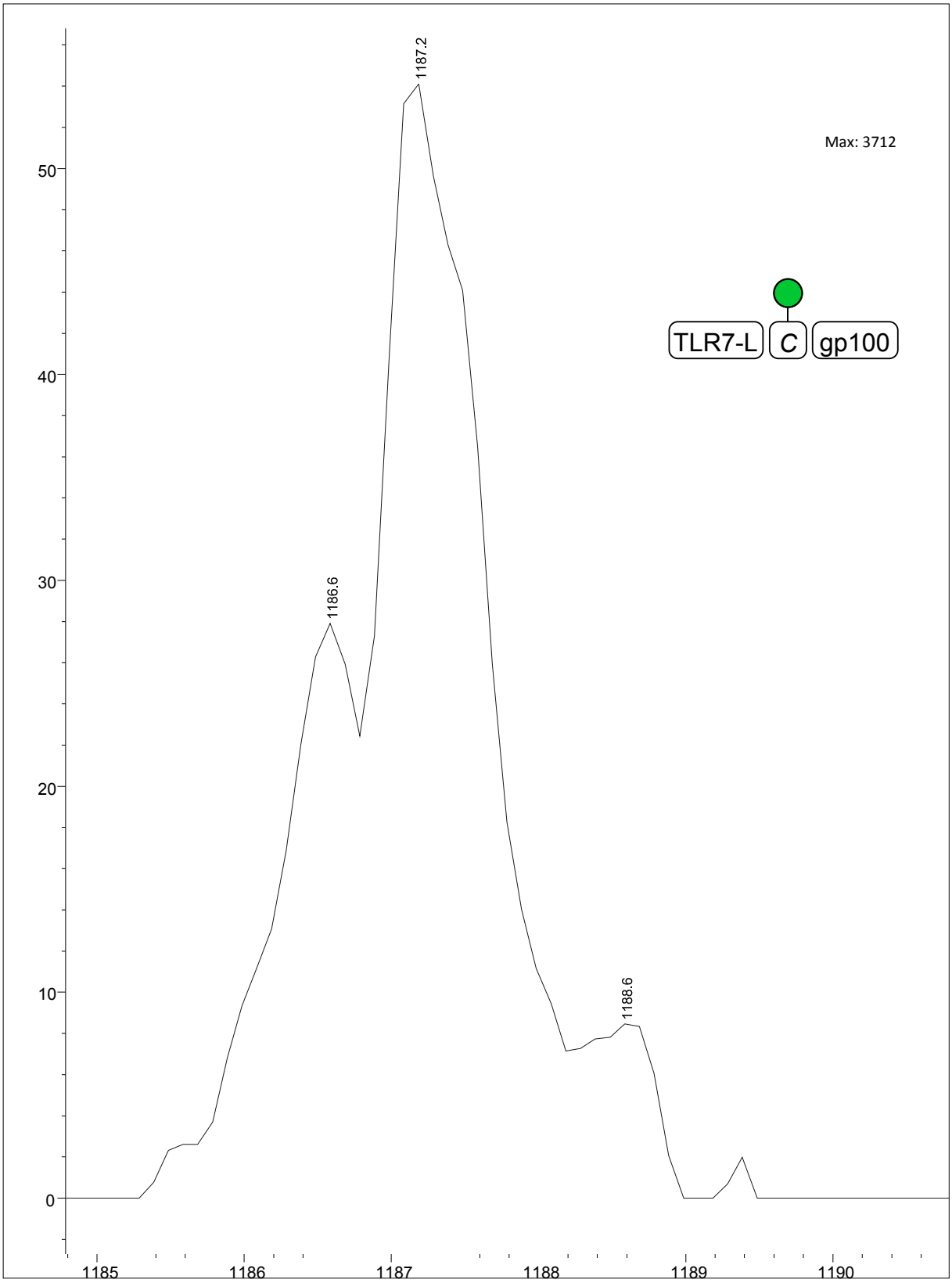
MS


*MSD1 SPC, time=7.257:7.369 of D:\Data\2019_TimH\2019_02_01_02\TH526_0050_15uL.D ES-API, Pos, Scan, Frag: Var, "160-
Max: 3712
TLR7-L
C
gp100
1582.7
1582.1
1187.2
1186.6
1458.3
1589.8
975.5
1102.4
1209.8
1267.1
1337.6
1528.5
1591.0
100
80
60
40
20
0
200
400
600
800
1000
1200
1400
1600
1800

MS

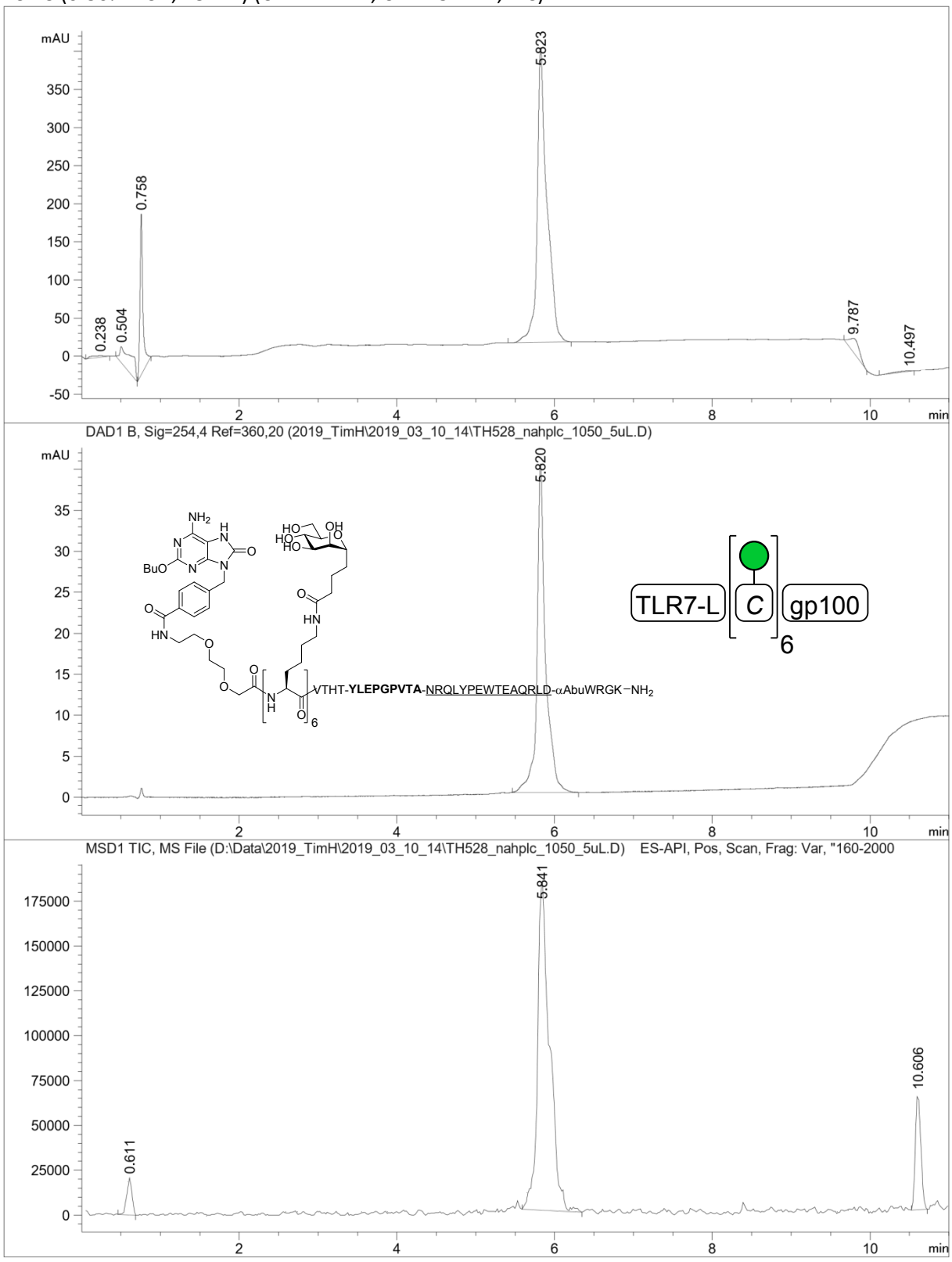

100
80
60
40
20
0
1570
1575
1580
1585
1590
1595
Max: 3712
1582.7
1582.1
1575.4
1576.1
1585.2
1589.8
1591.0
TLR7-L
C
gp100

MS

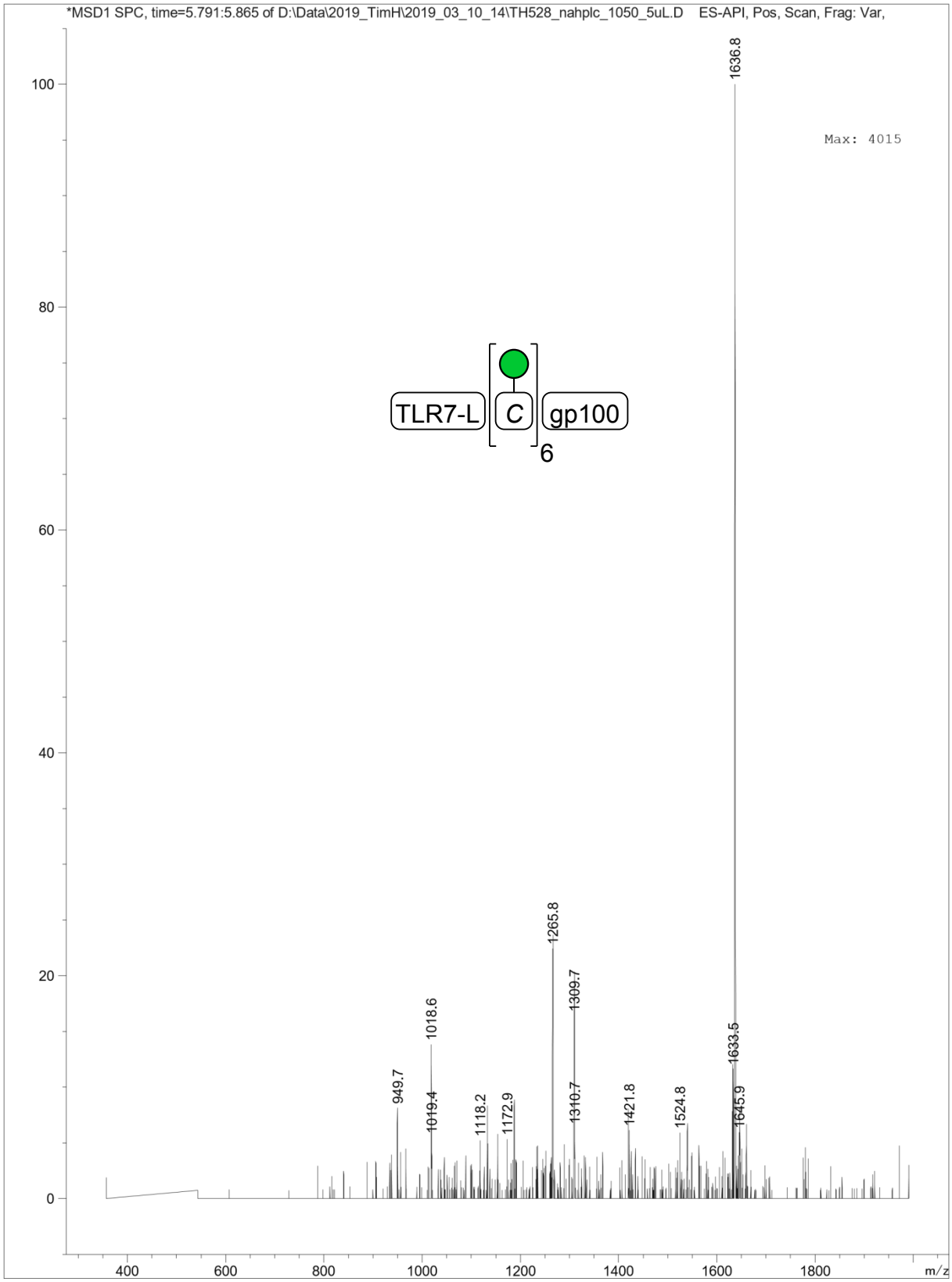

1187.2
Max: 3712
50
40
30
20
10
0
1186.6
1188.6
TLR7-L
C
gp100
1185
1186
1187
1188
1189
1190

Compound 40

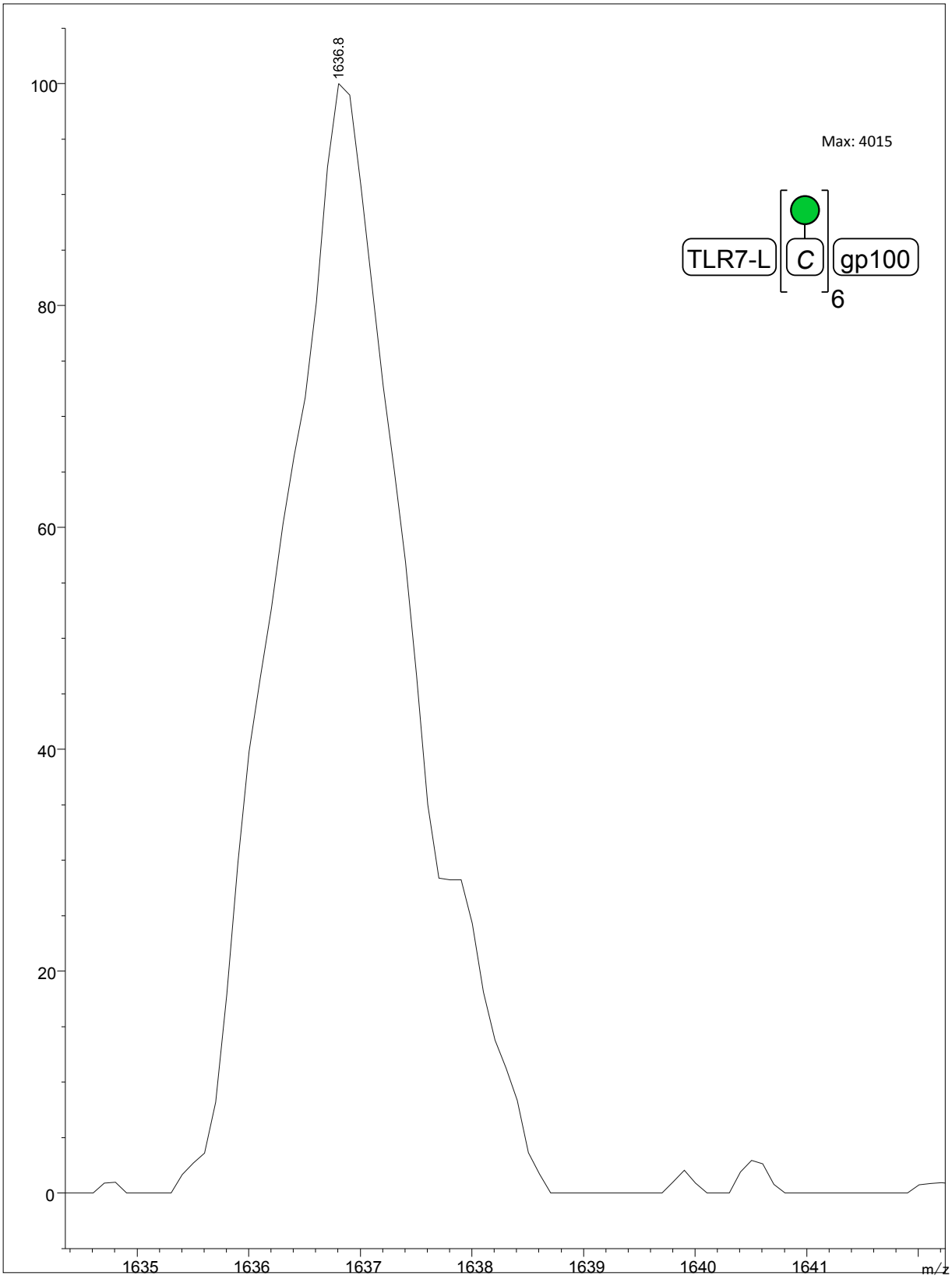
LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)



MS

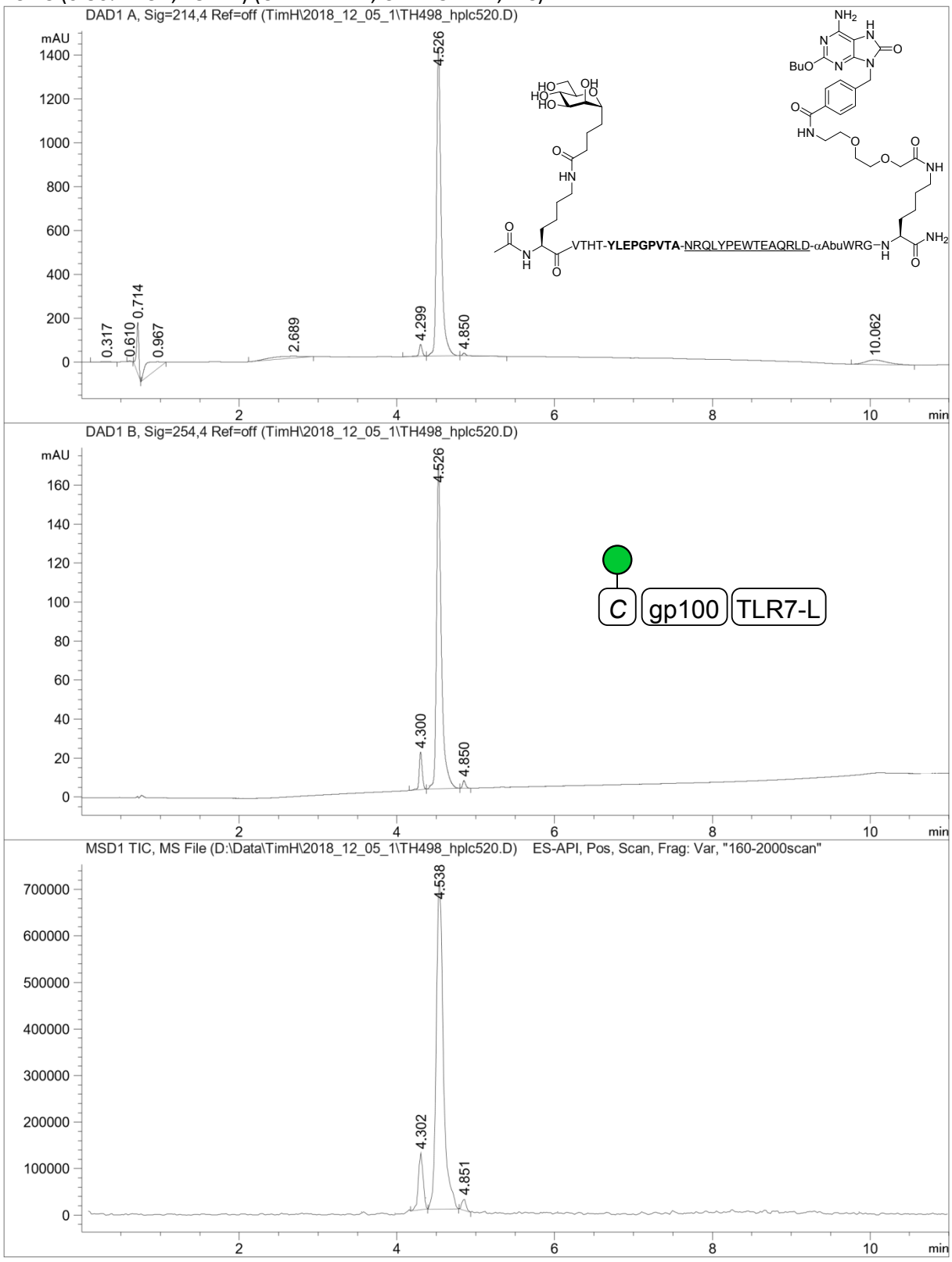

*MSD1 SPC, time=5.791:5.865 of D:\Data\2019_TimH\2019_03_10_14\TH528_nahplc_1050_5uL.D ES-API, Pos, Scan, Frag: Var,
Max: 4015
1636.8
1265.8
1309.7
1018.6
1633.5
949.7
1019.4
1118.2
1172.9
1310.7
1421.8
1524.8
1645.9
TLR7-L
C
gp100
6
100
80
60
40
20
0
400
600
800
1000
1200
1400
1600
1800
m/z

MS

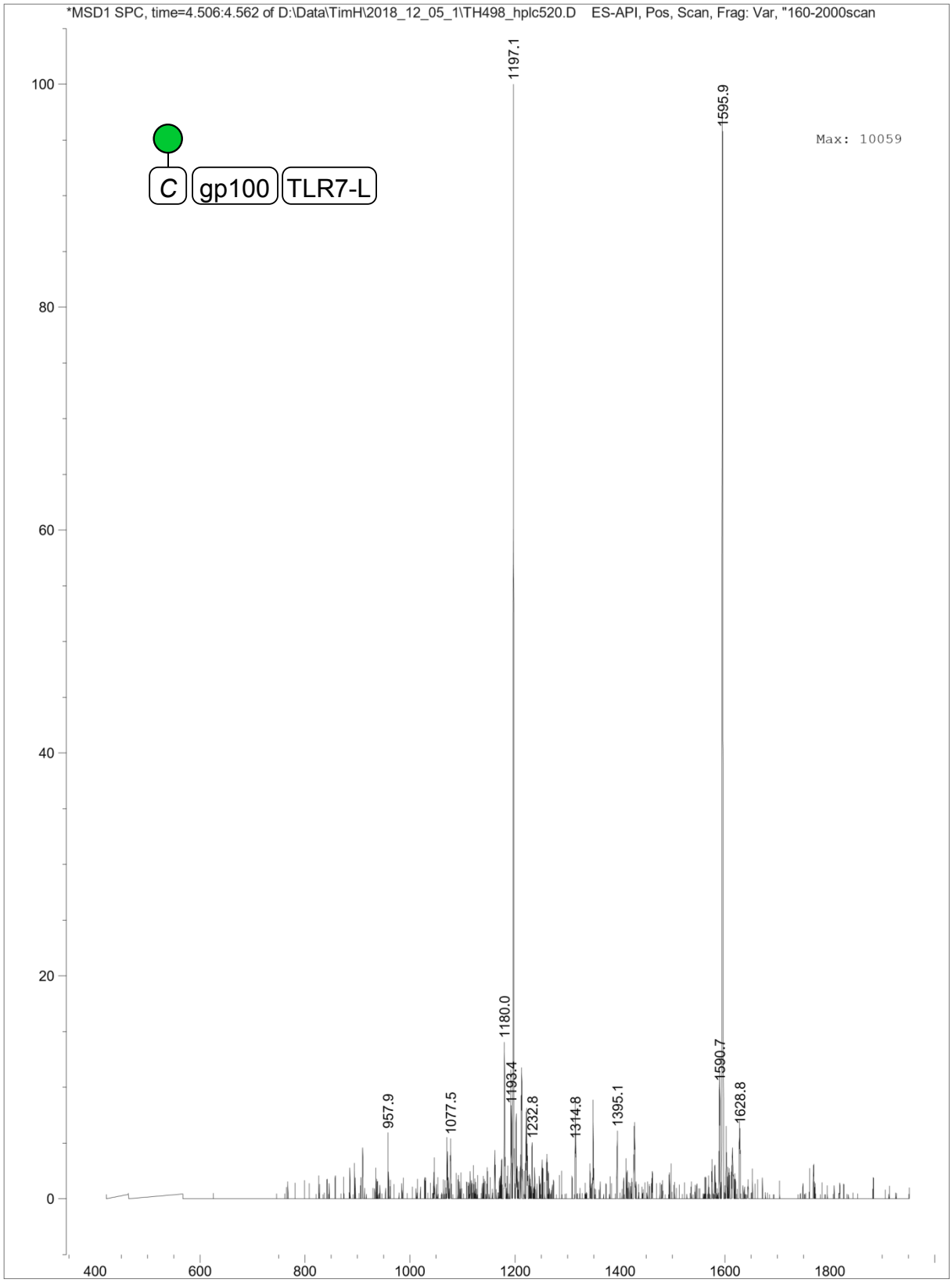

1636.8
100
80
60
40
20
0
Max: 4015
TLR7-L
C
gp100
6
1635
1636
1637
1638
1639
1640
1641
m/z

Compound 41

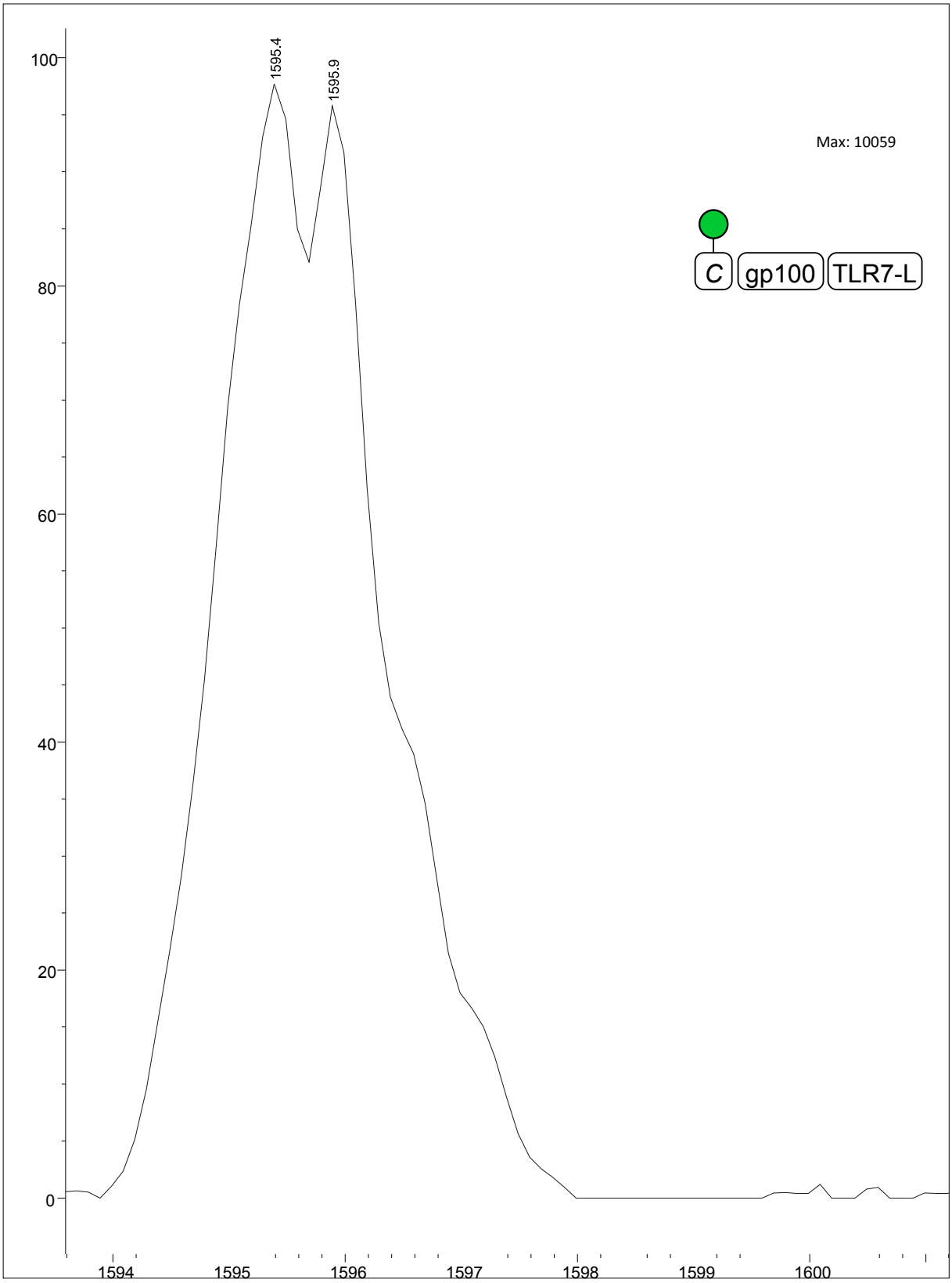
LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)



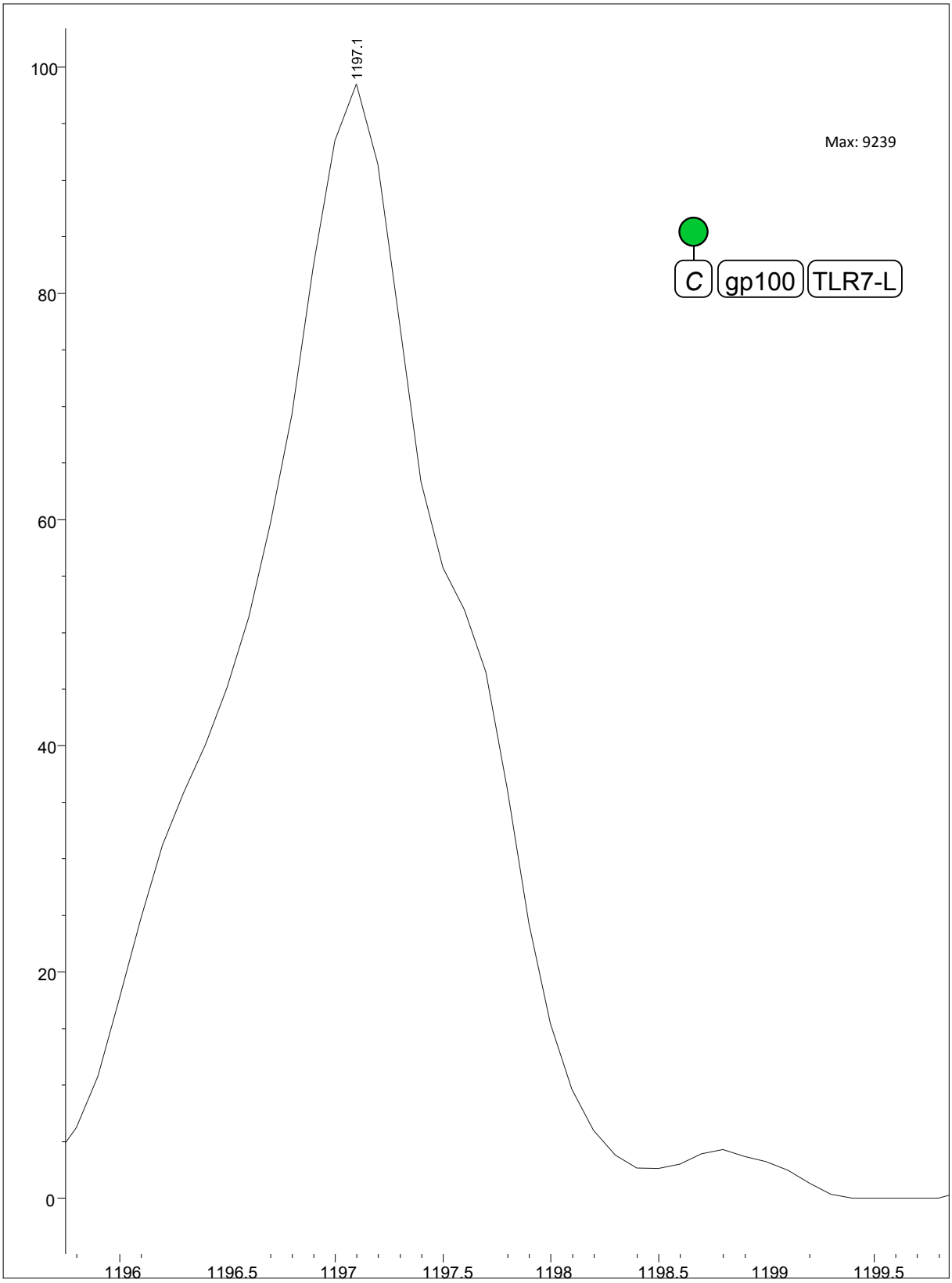
MS


*MSD1 SPC, time=4.506:4.562 of D:\Data\TimH\2018_12_05_1\TH498_hplc520.D ES-API, Pos, Scan, Frag: Var, "160-2000scan
Max: 10059
C
gp100
TLR7-L
1197.1
1595.9
1180.0
1590.7
957.9
1077.5
1193.4
1232.8
1314.8
1395.1
1628.8
100
80
60
40
20
0
400
600
800
1000
1200
1400
1600
1800

MS

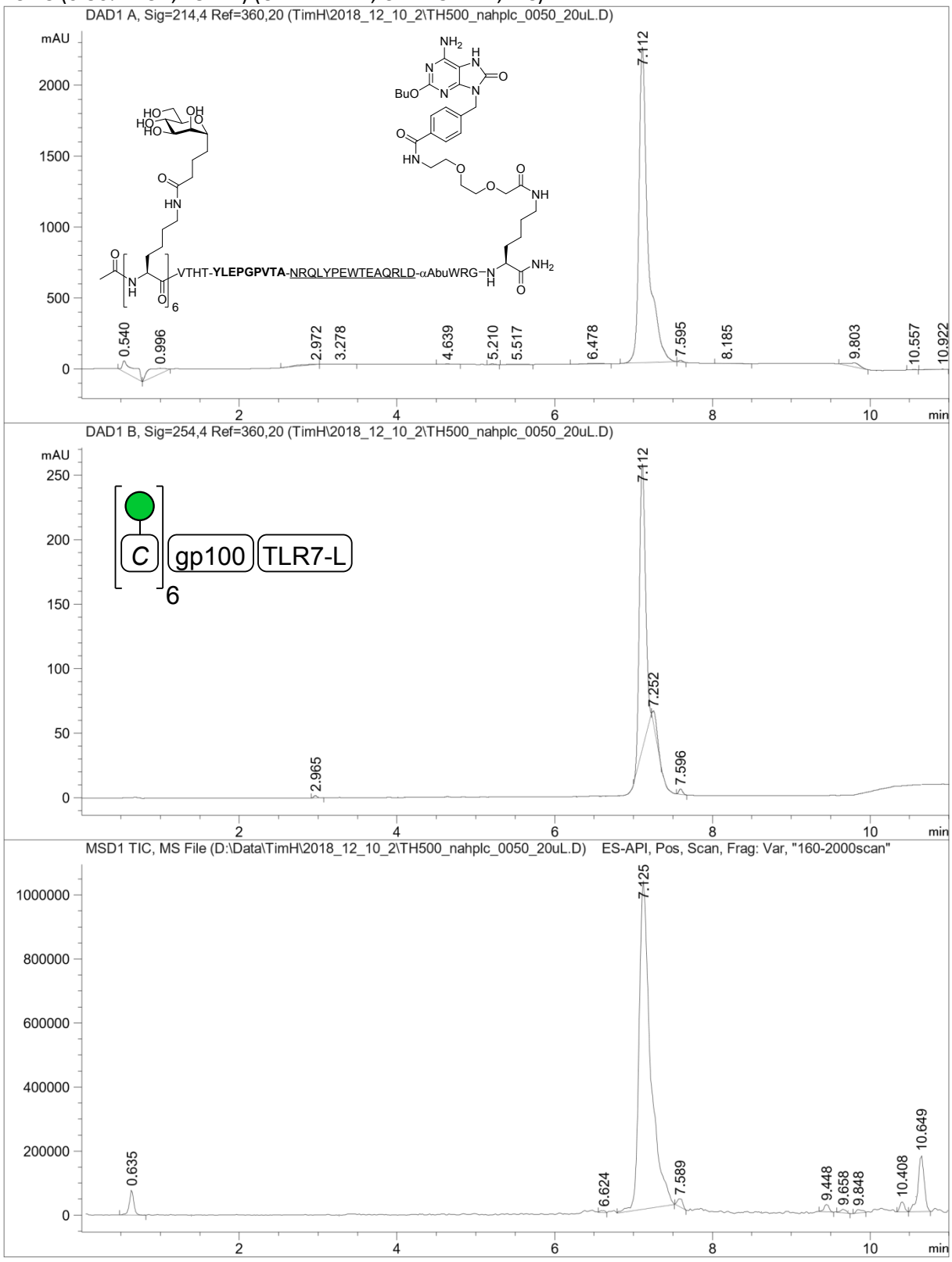

1595.4
1595.9
Max: 10059
C
gp100
TLR7-L
100
80
60
40
20
0
1594
1595
1596
1597
1598
1599
1600

MS

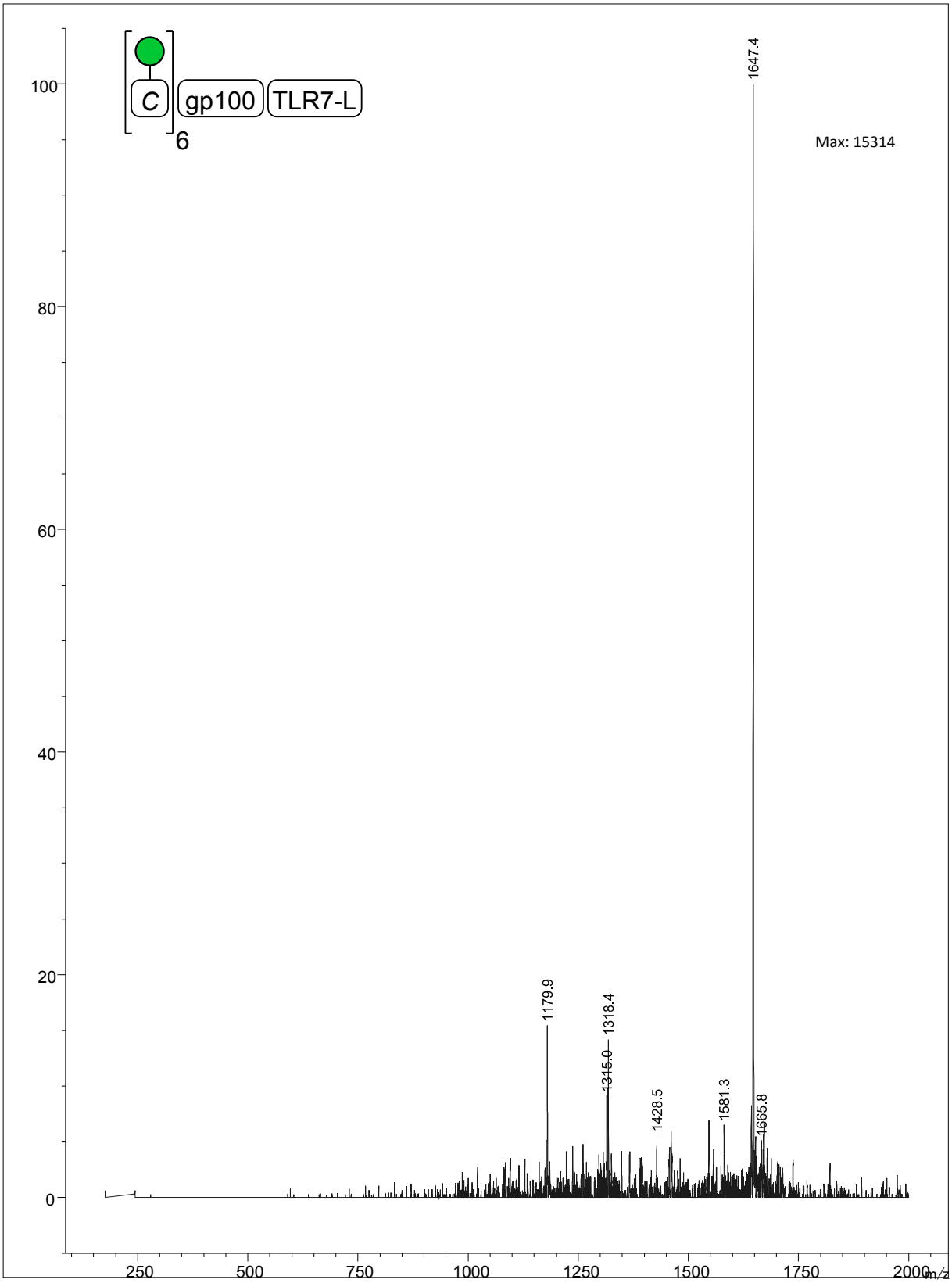

1197.1
Max: 9239
C
gp100
TLR7-L
100
80
60
40
20
0
1196
1196.5
1197
1197.5
1198
1198.5
1199
1199.5

Compound 42

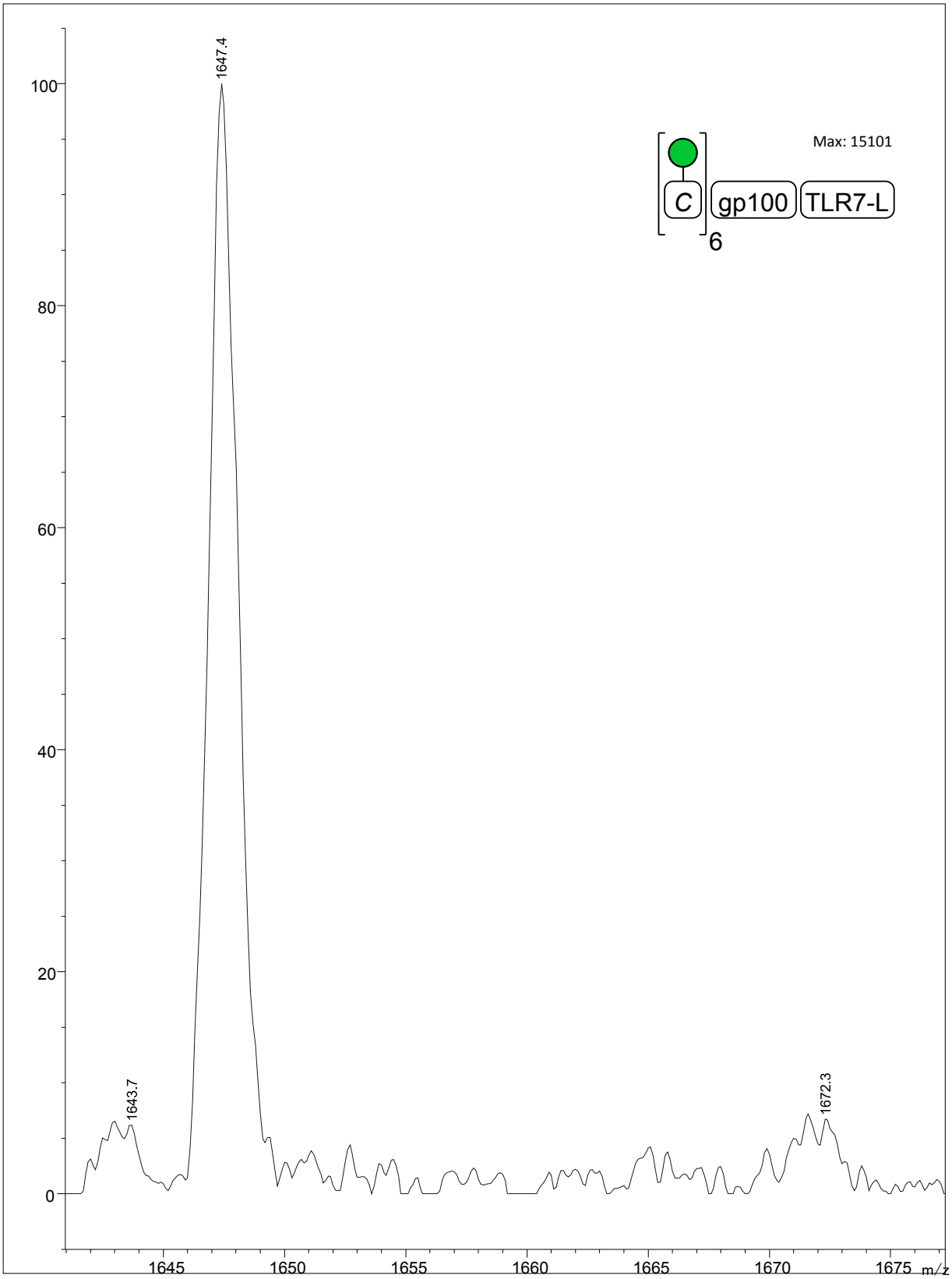
LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)



MS

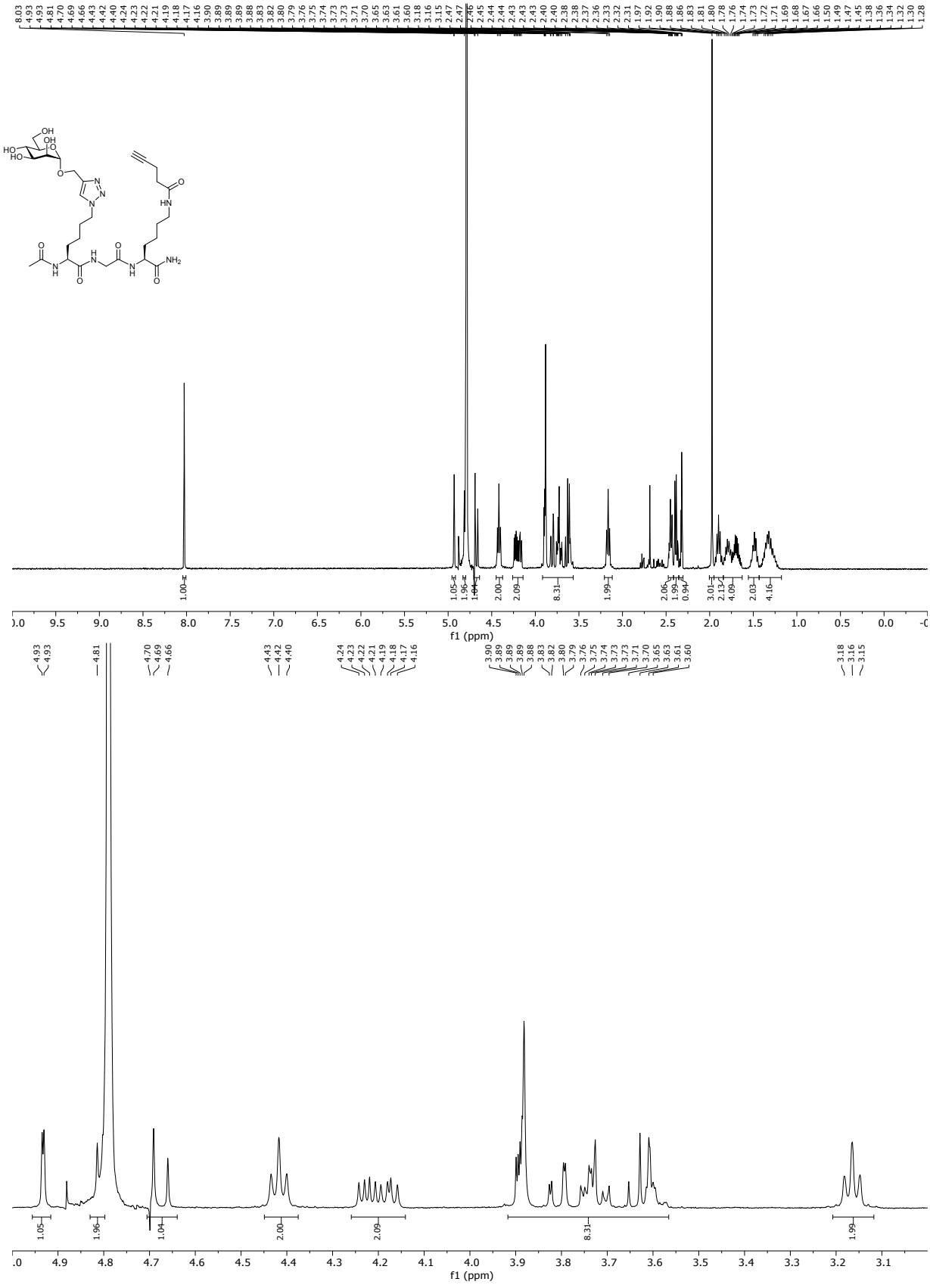

C
gp100
TLR7-L
6
Max: 15314
1647.4
1179.9
1318.4
1315.0
1428.5
1581.3
1665.8
100
80
60
40
20
0
250
500
750
1000
1250
1500
1750
2000
m/z

MS

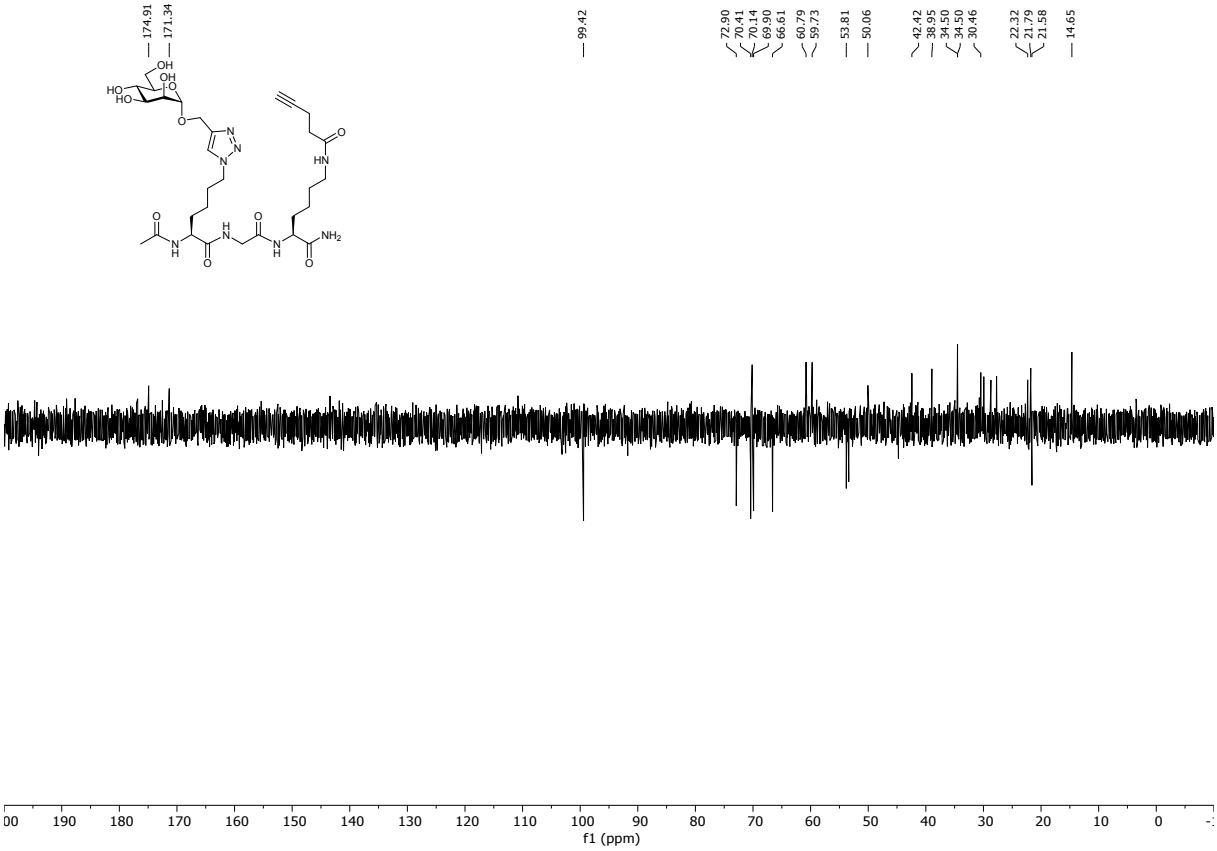

1647.4
100
80
60
40
20
0
1643.7
1672.3
Max: 15101
C
gp100
TLR7-L
6
1645
1650
1655
1660
1665
1670
1675
m/z

Compound 43

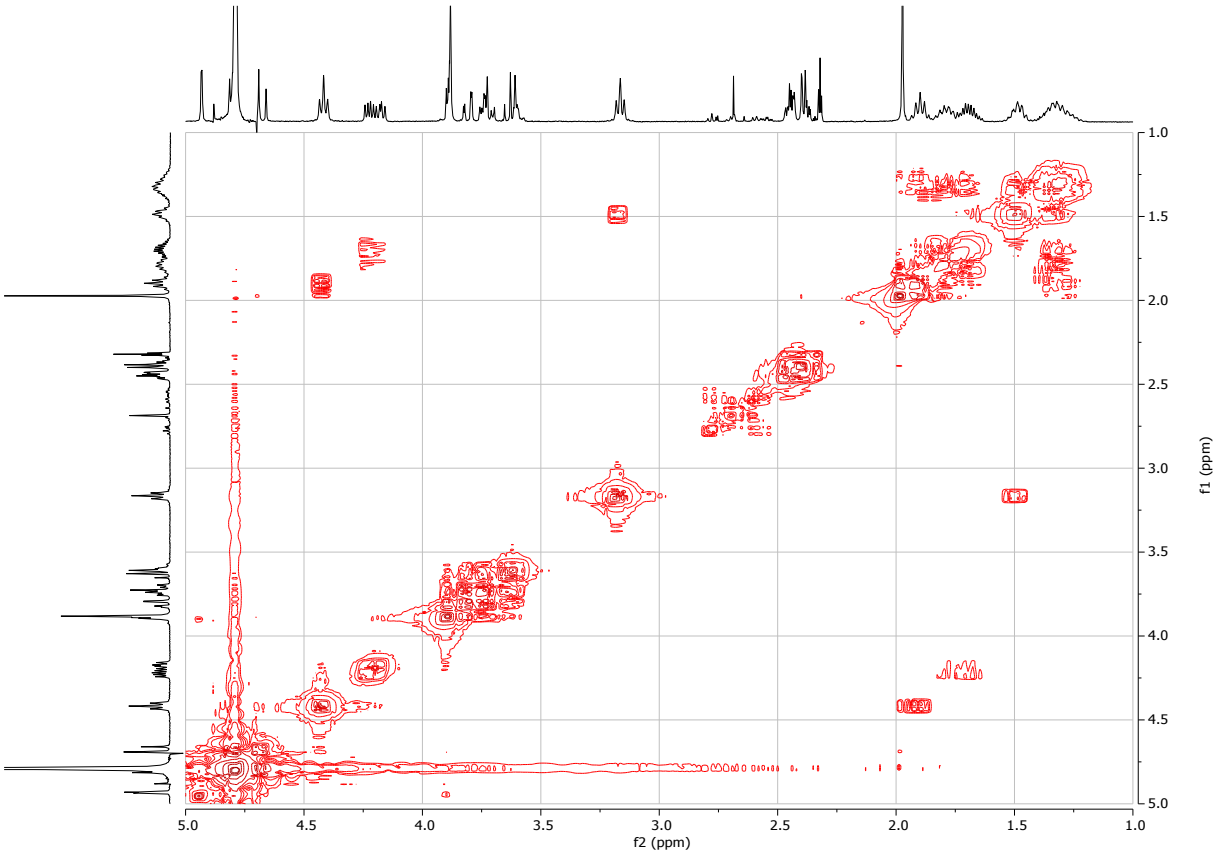
^{1}H NMR



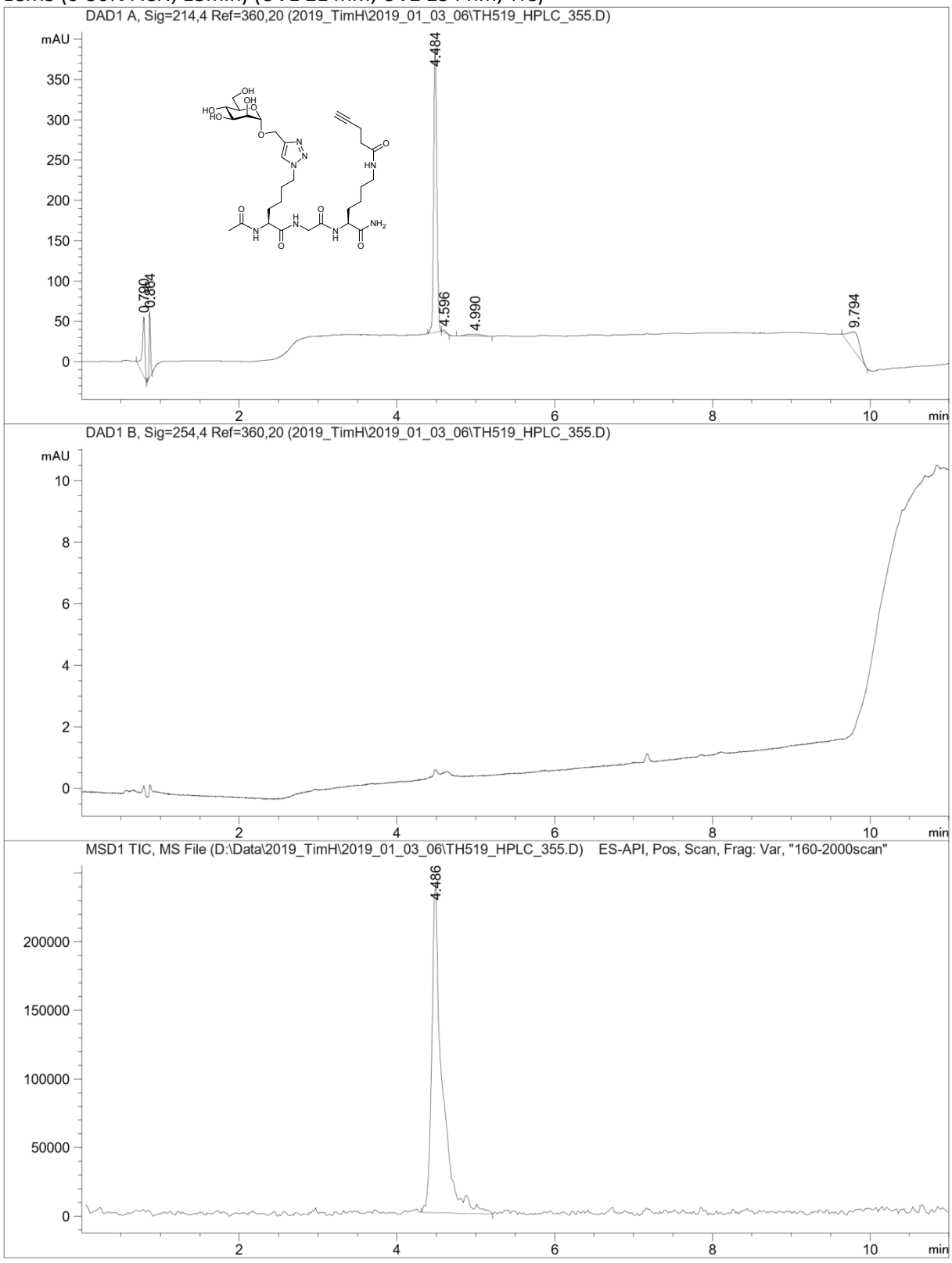
^{13}C APT NMR



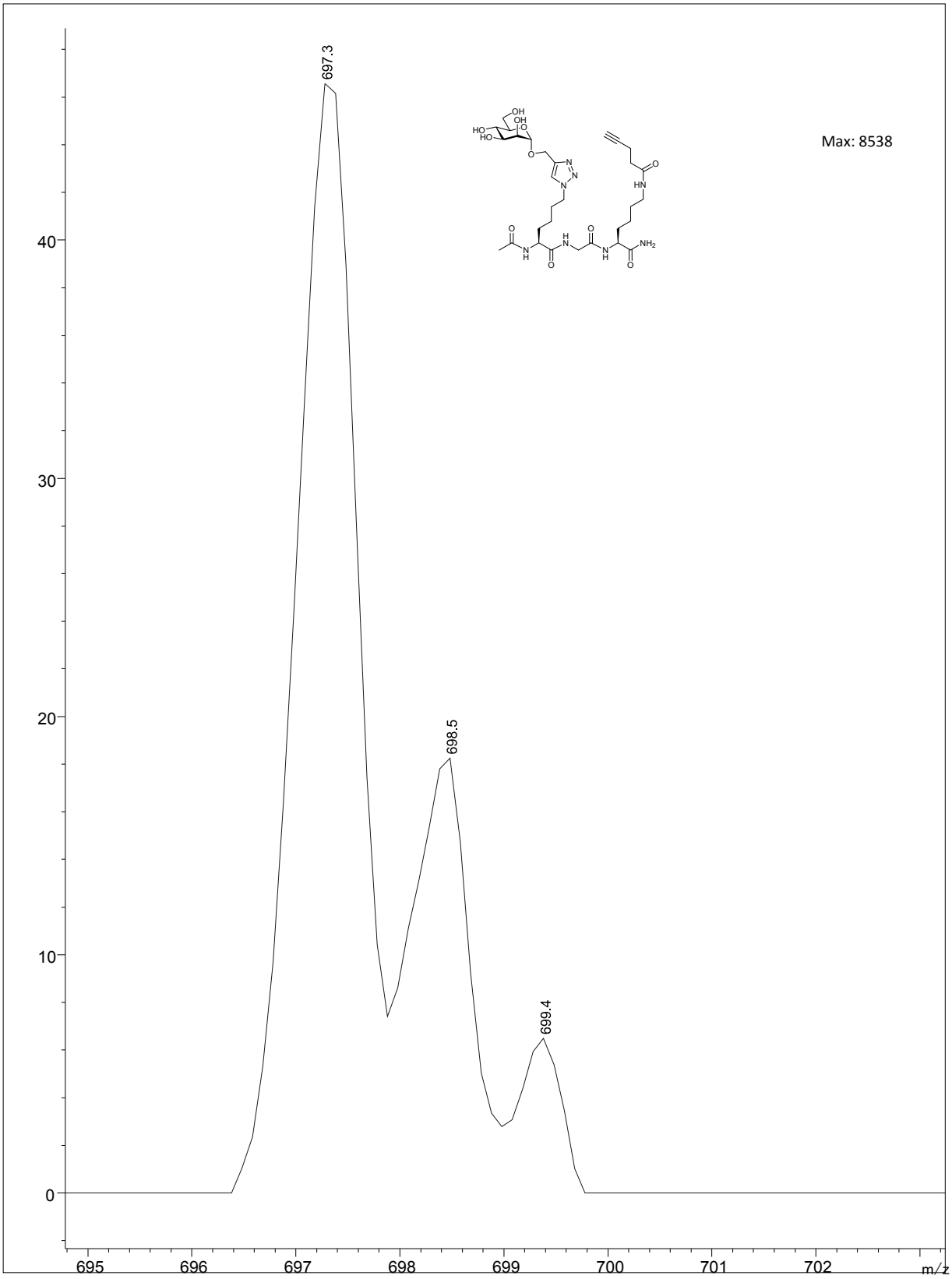
HH-COSY NMR



LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)

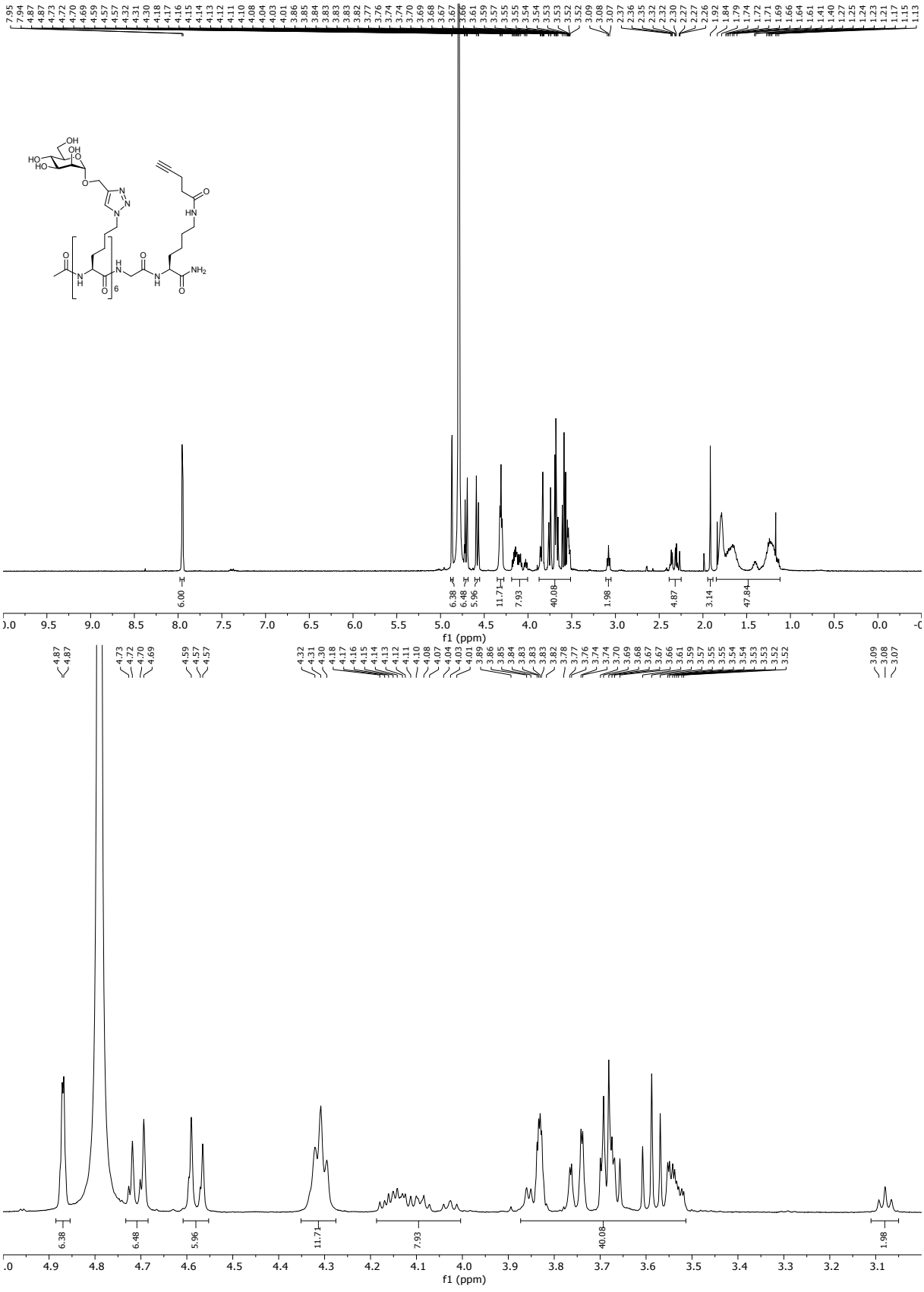


MS

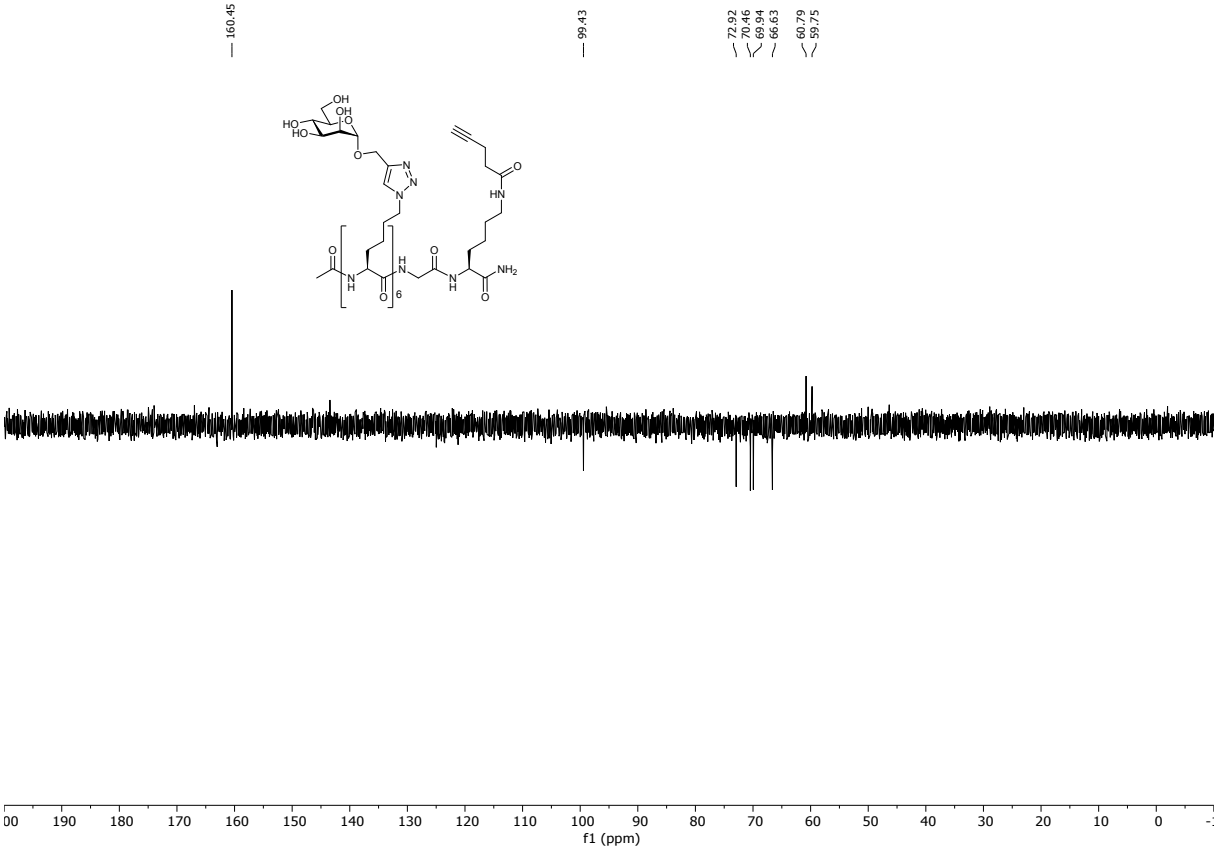

697.3
698.5
699.4
Max: 8538
40
30
20
10
0
695
696
697
698
699
700
701
702
m/z

Compound 44

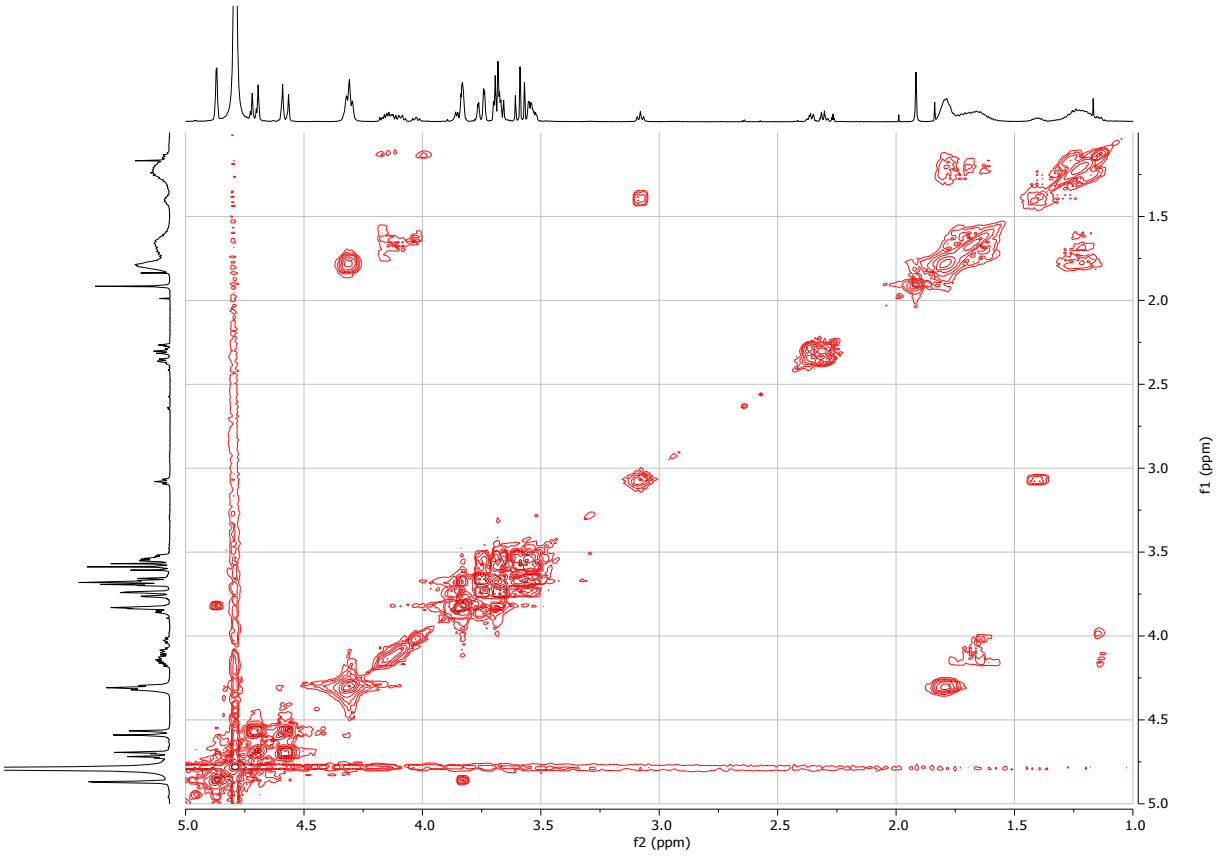
^{1}H NMR



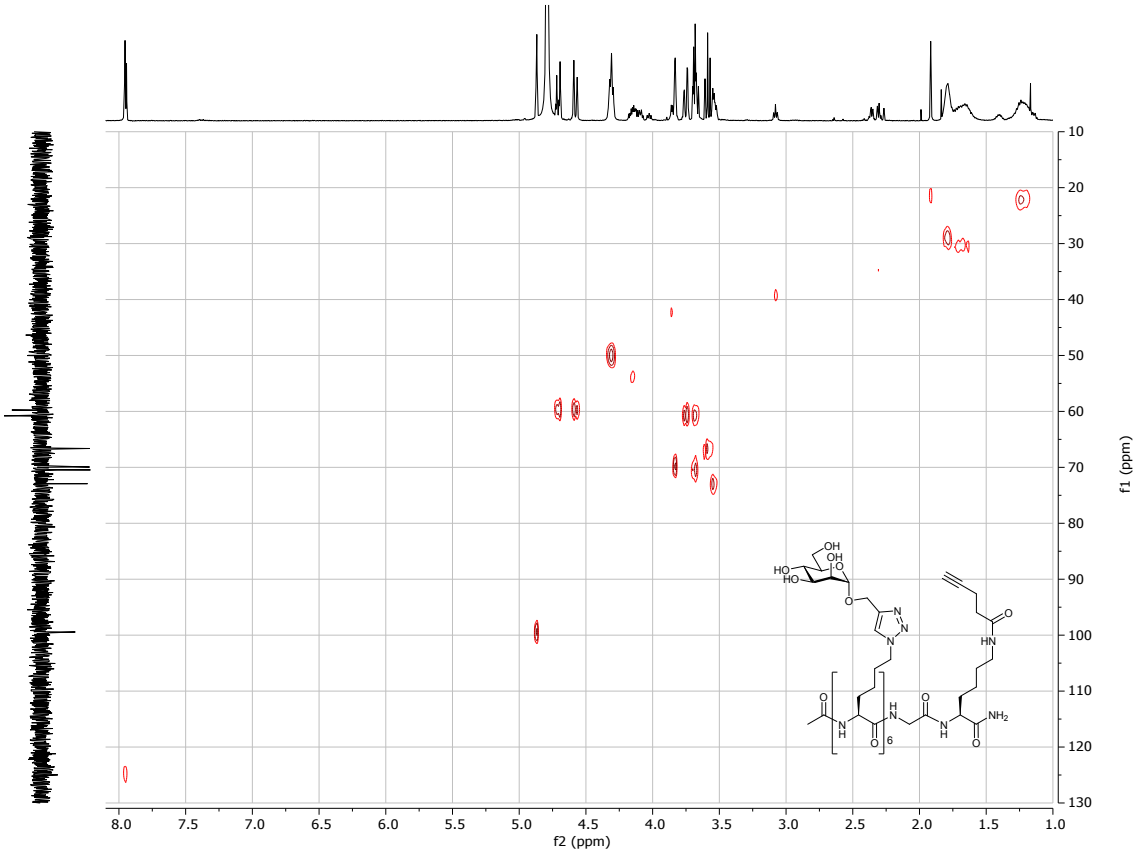
^{13}C APT NMR



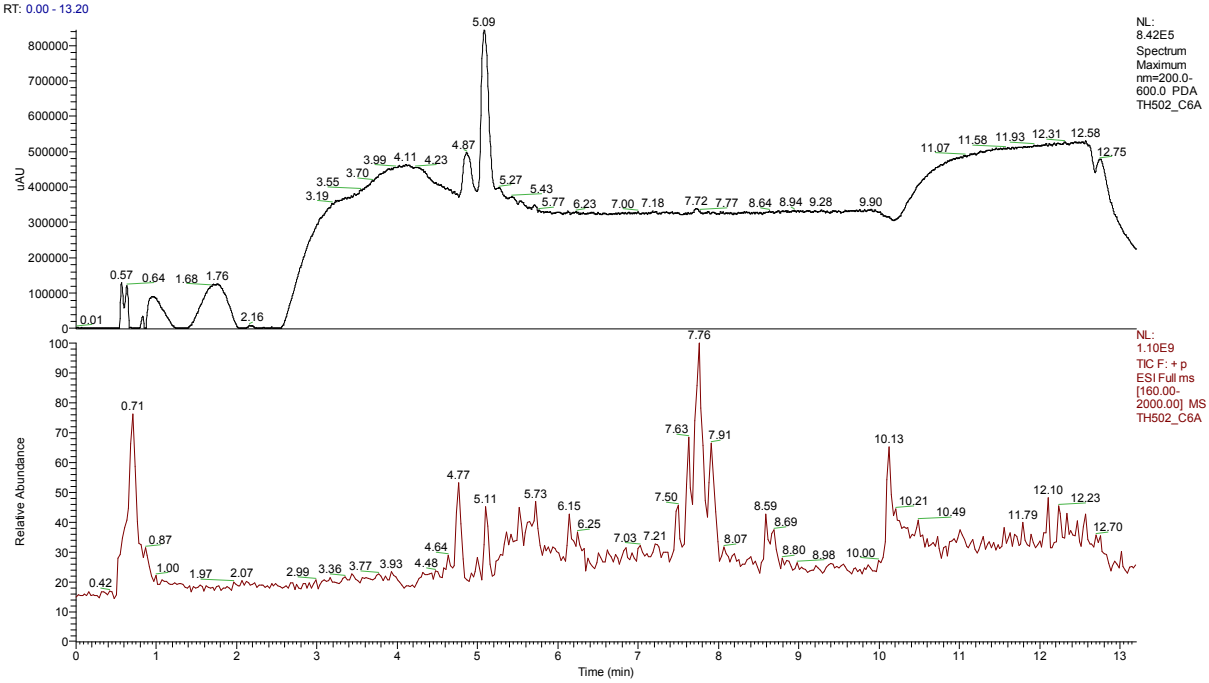
HH-COSY NMR



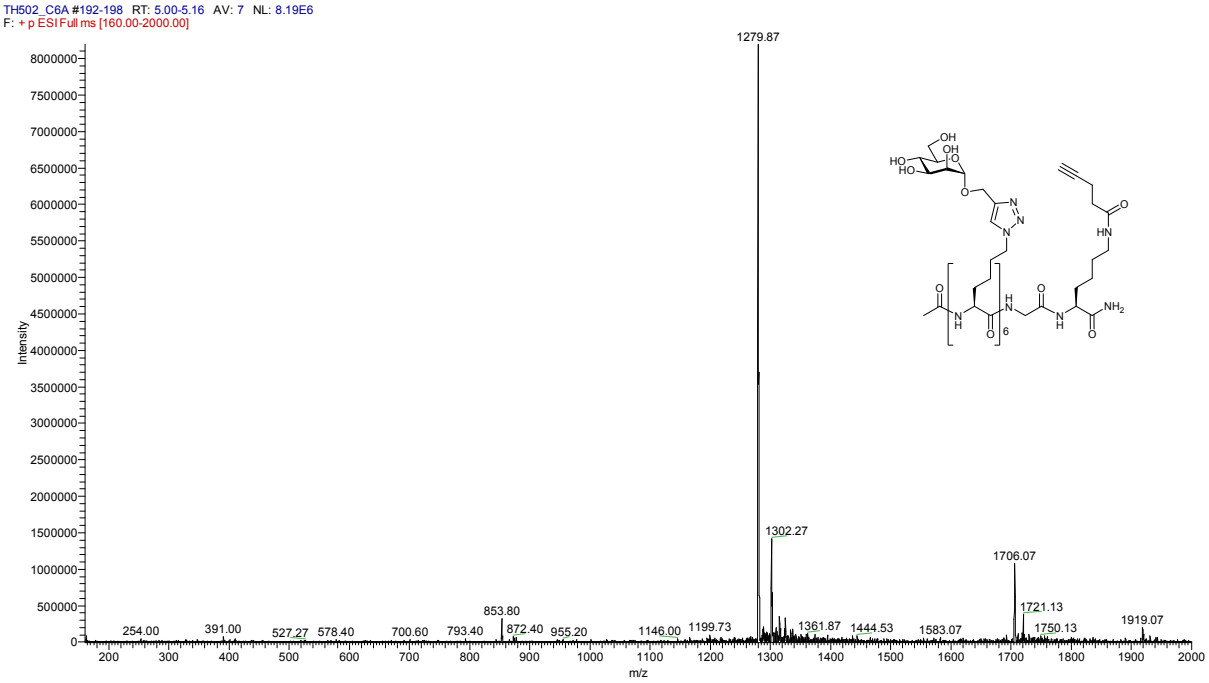
HSQC NMR


f2 (ppm)
f1 (ppm)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
10
20
30
40
50
60
70
80
90
100
110
120
130
OH
OH
HO
HO
O
N
N
N
O
HN
H
N
O
6
O
H
N
O
N
H
O
NH2

LCMS (0-50% ACN; 13min) (UV 200-600nm; TIC)

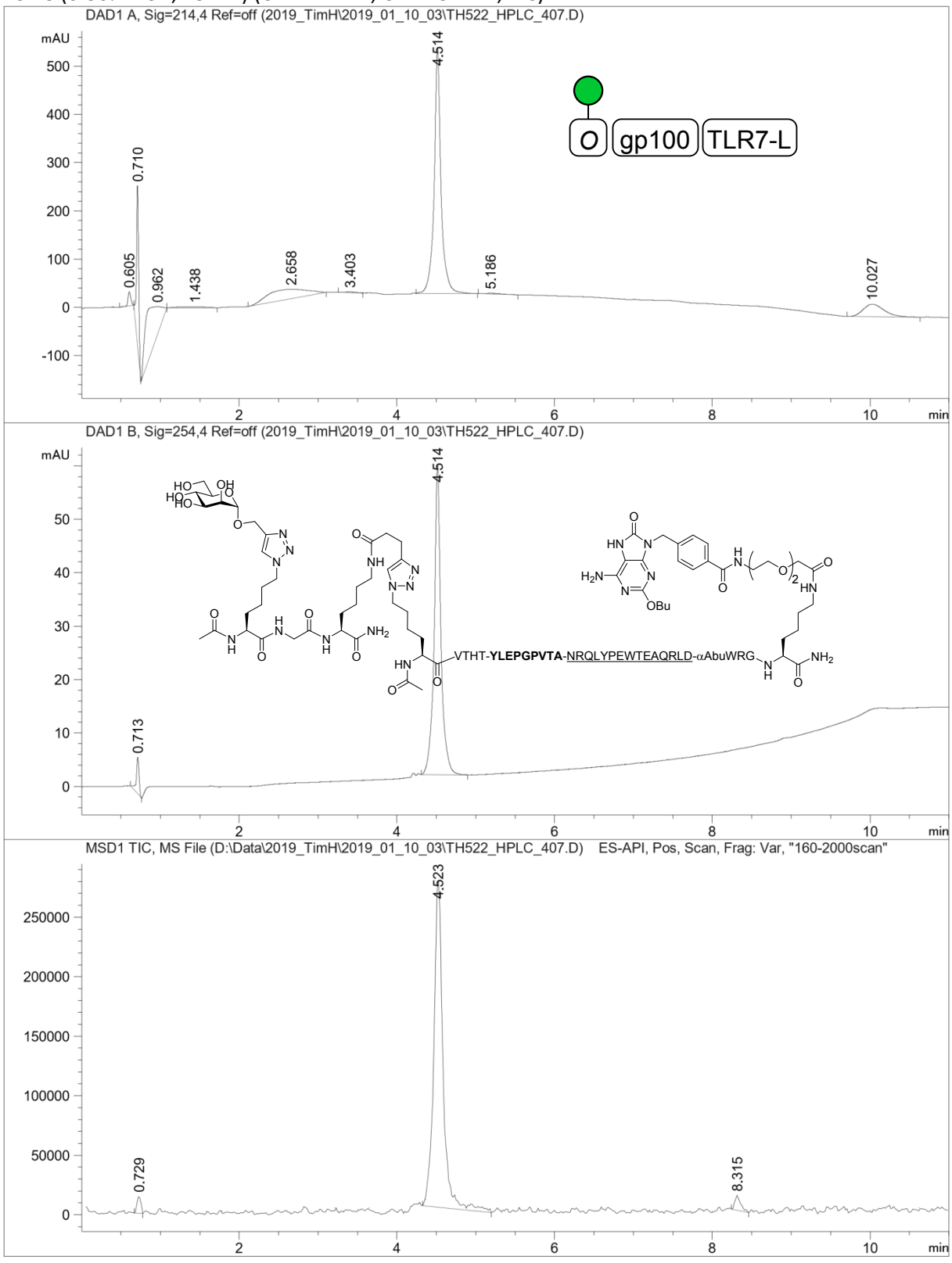


MS

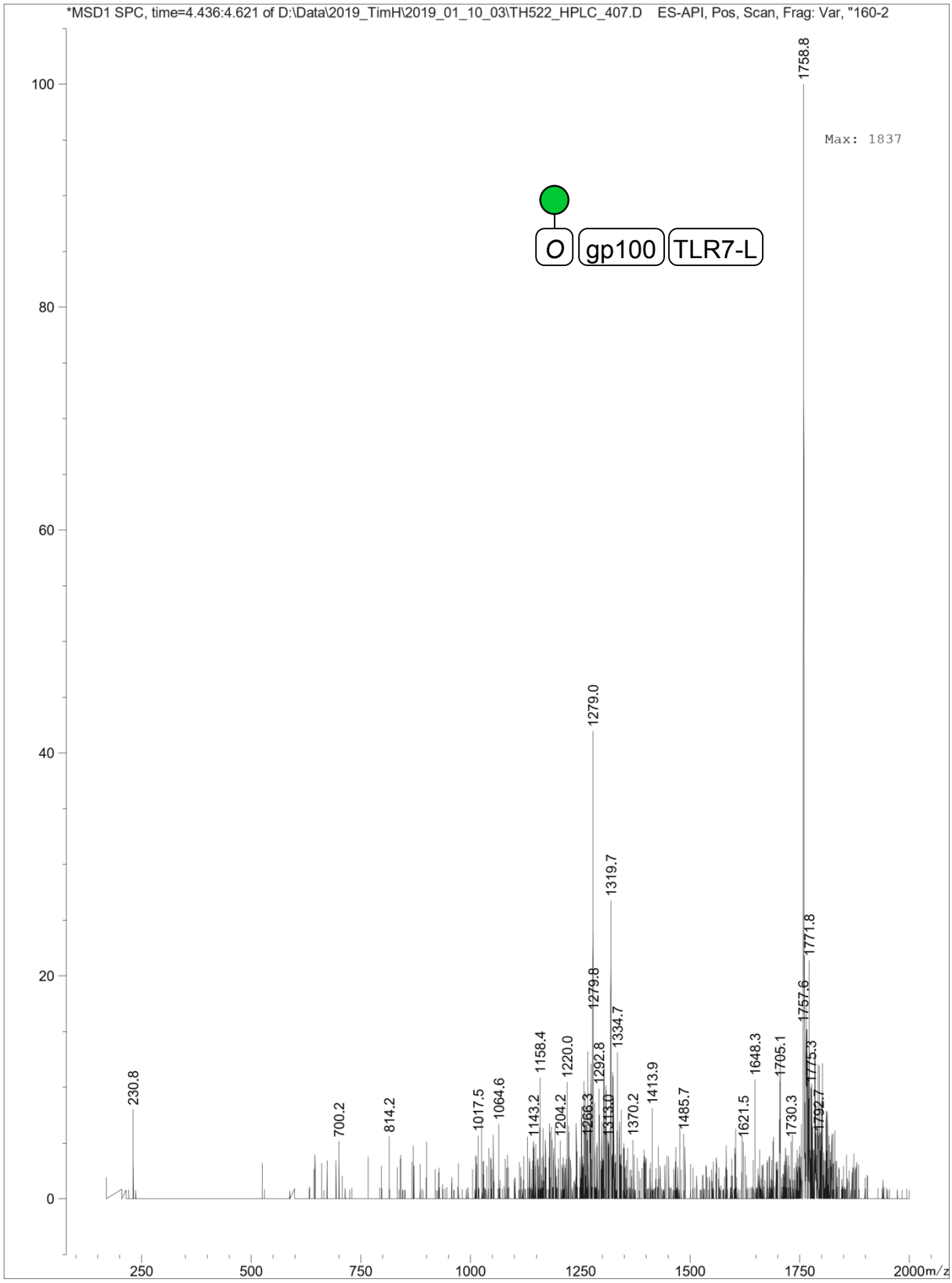


Compound 46

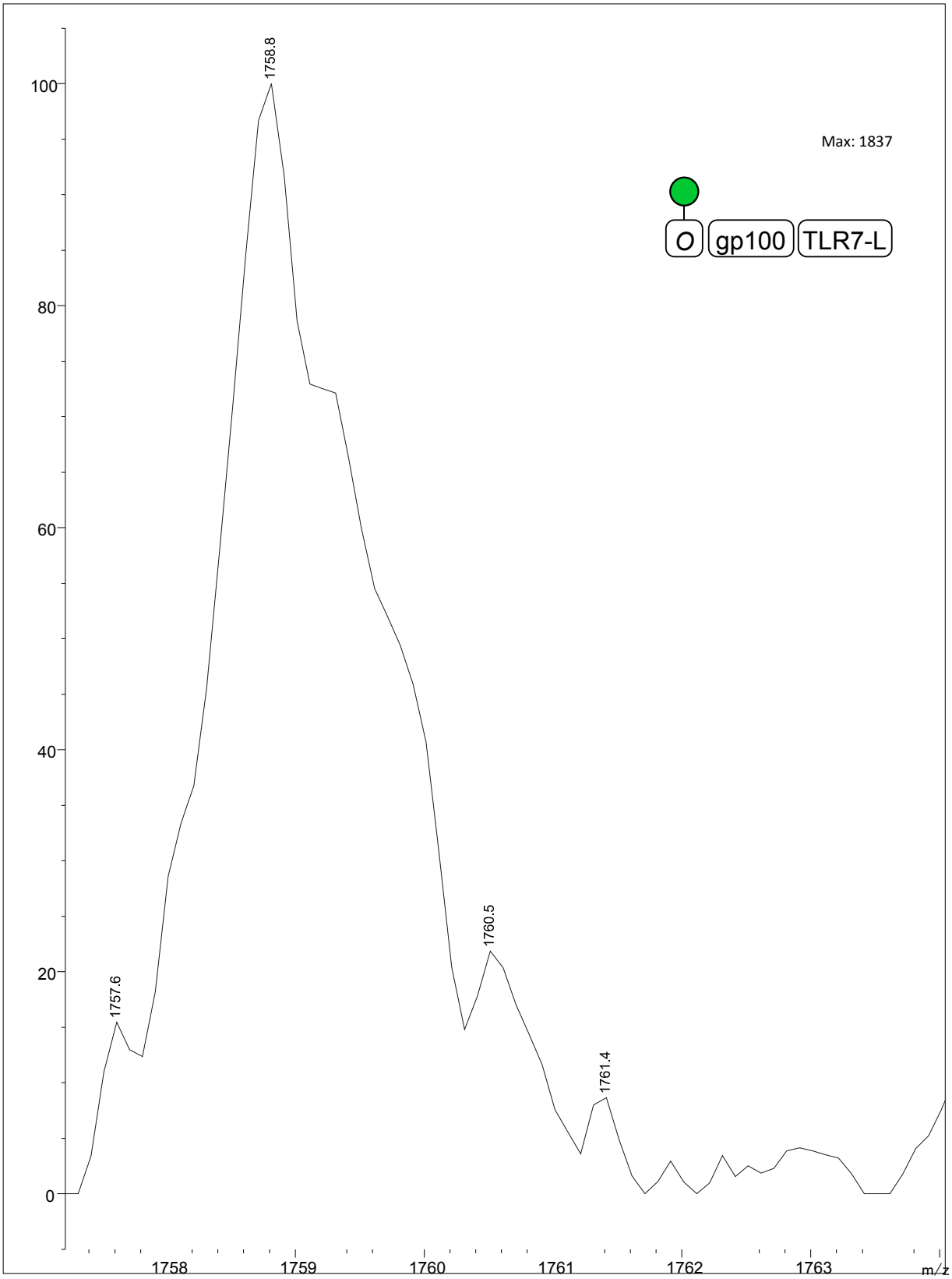
LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)



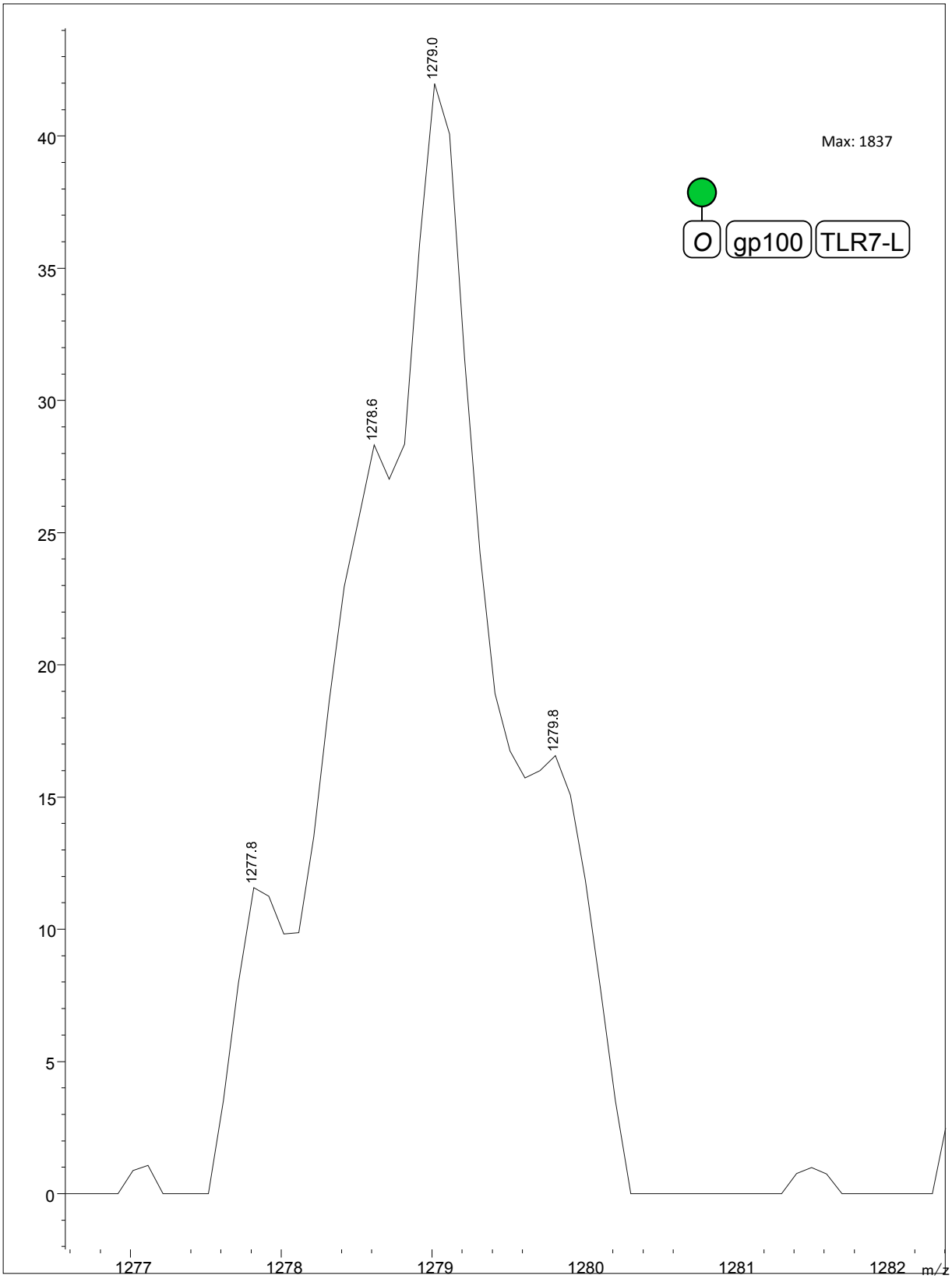
MS


*MSD1 SPC, time=4.436:4.621 of D:\Data\2019_TimH\2019_01_10_03\TH522_HPLC_407.D ES-API, Pos, Scan, Frag: Var, "160-2
Max: 1837
O
gp100
TLR7-L
1758.8
1279.0
1319.7
1771.8
1279.8
1334.7
1158.4
1220.0
1292.8
1757.6
1648.3
1705.1
1775.3
1792.7
1730.3
1621.5
1485.7
1413.9
1370.2
1313.0
1266.3
1204.2
1143.2
1017.5
1064.6
814.2
700.2
230.8
100
80
60
40
20
0
250
500
750
1000
1250
1500
1750
2000m/z

MS

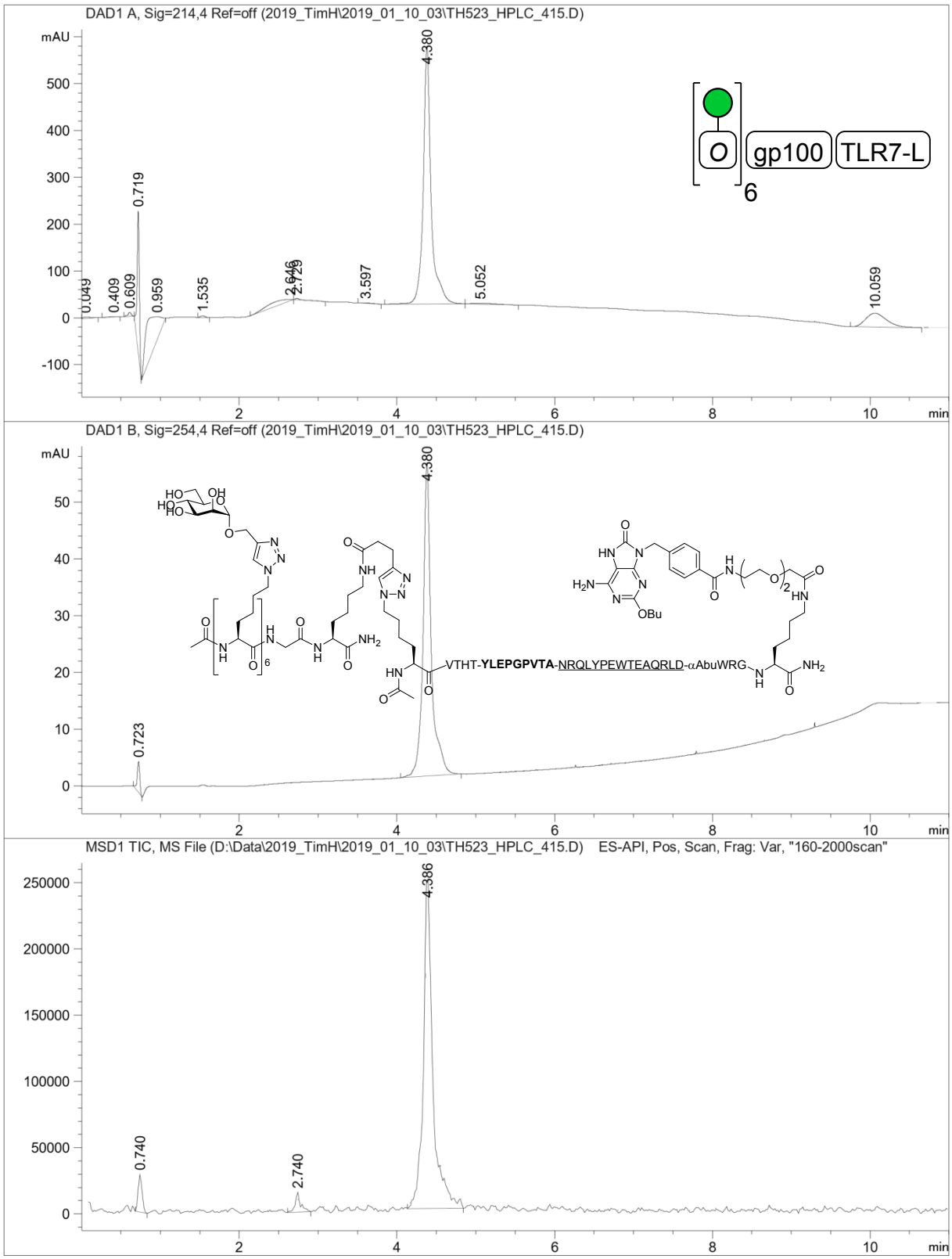

1758.8
100
Max: 1837
O
gp100
TLR7-L
80
60
40
20
1757.6
1760.5
1761.4
0
1758
1759
1760
1761
1762
1763
m/z

MS

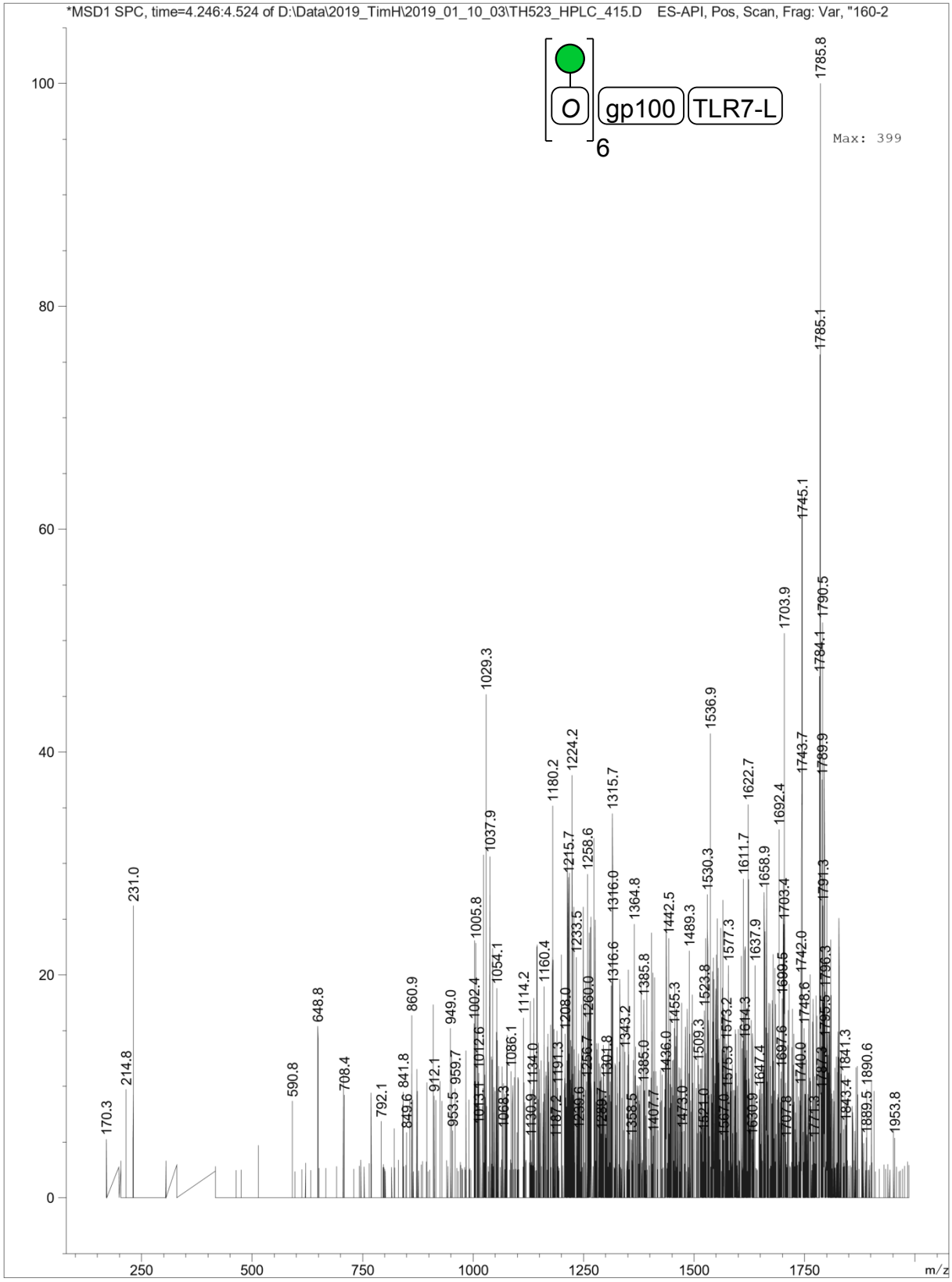

1279.0
1278.6
1277.8
1279.8
Max: 1837
O
gp100
TLR7-L
40
35
30
25
20
15
10
5
0
1277
1278
1279
1280
1281
1282
m/z

Compound 47

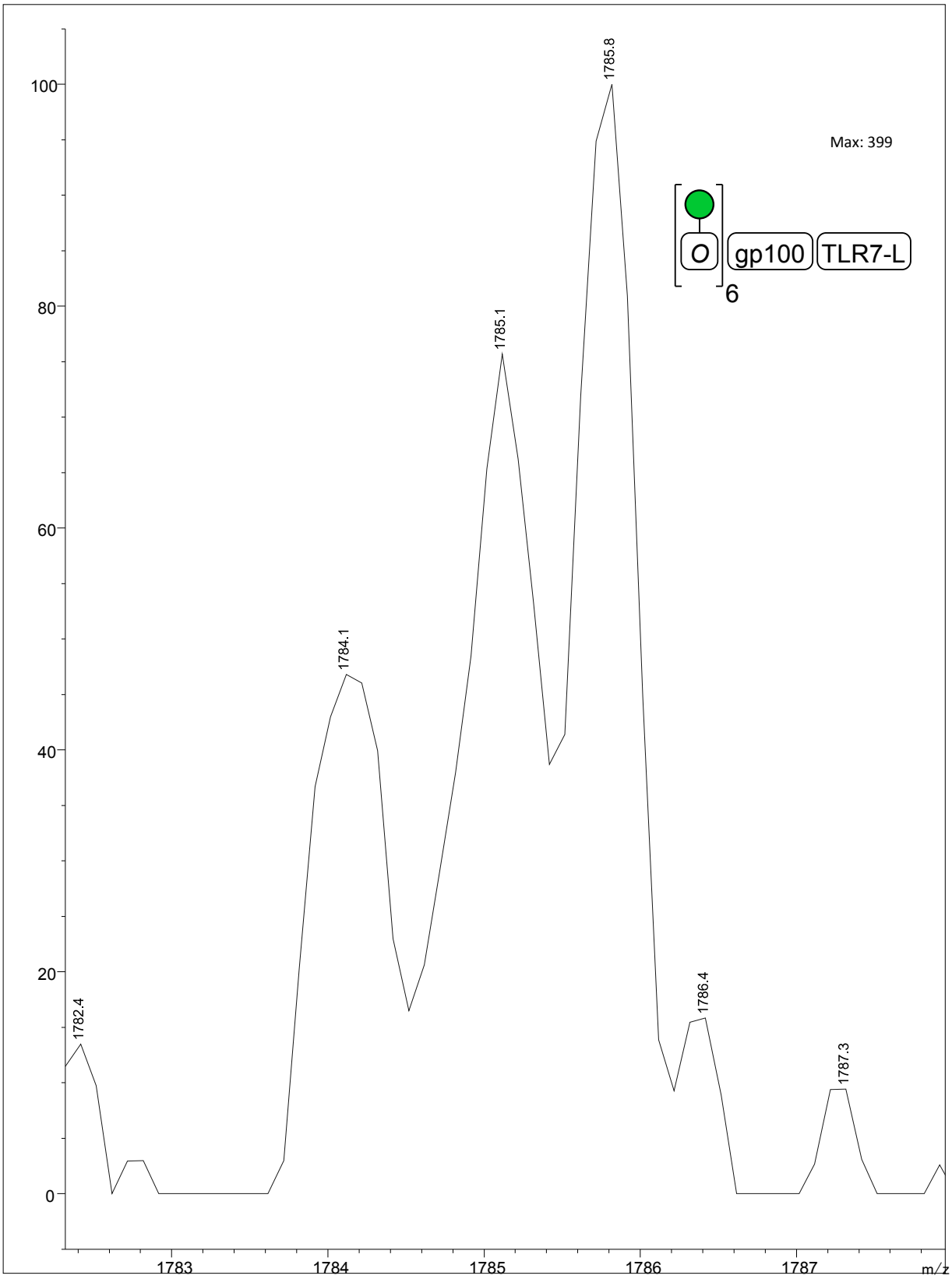
LCMS (0-50% ACN; 13min) (UV1 214nm; UV2 254 nm; TIC)



MS


*MSD1 SPC, time=4.246:4.524 of D:\Data\2019_TimH\2019_01_10_03\TH523_HPLC_415.D ES-API, Pos, Scan, Frag: Var, "160-2
O
gp100
TLR7-L
6
Max: 399
m/z

MS


100
80
60
40
20
0
1782.4
1784.1
1785.1
1785.8
1786.4
1787.3
Max: 399
O
gp100
TLR7-L
6
1783
1784
1785
1786
1787
m/z