Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Facile Chemoenzymatic Synthesis of O-Mannosyl Glycans

Shuaishuai Wang+, Qing Zhang+, CongCong Chen, Yuxi Guo, Madhusudhan Reddy Gadi, Jin Yu, Ulrika Westerlind, Yunpeng Liu, Xuefeng Cao, Peng G. Wang, and Lei Li**

anie_201803536_sm_miscellaneous_information.pdf

Supporting Information

Table of Contents

I. Chemical Synthesis 2

II. Enzymatic Extensions 14

III. Glycan Microarray preparation, assay and data 18

IV. HPLC, MS and NMR analysis of purified O-mannosyl glycans 21

V. NMR spectra 66

VI. Reference 103

VII. Microarray Data 104

I. Chemical Synthesis

Materials:

All chemicals were purchased as reagent grade and used without further purification. Anhydrous dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN), tetrahydrofuran (THF), *N,N*-dimethyl formamide (DMF), diethyl ether (Et_2O), toluene, and methanol (MeOH) were purchased from a commercial source without further distillation. Pulverized Molecular Sieves MS-4 Å (Aldrich) for glycosylation was activated by heating at 350 ºC for 3 h. Reactions were monitored by analytical thin-layer chromatography (TLC) in EM silica gel 60 F254 plates and visualized under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate or *p*-anisadehyde. Flash chromatography was performed on silica gel (Merck) of 40-63μm particle size and P2 gel (Biorad). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 (400 MHz), and Bruker AVANCE 600 (600 MHz) spectrometer at 25 ºC. All ^{1}H Chemical shifts (in ppm) were assigned according to $CDCl_3$ (δ= 7.24 ppm) and D_2O (δ = 4.79 ppm) and all ^{13}C NMR was calibrated with $CDCl_3$ (δ = 77.00 ppm). Coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are described using the following abbreviations: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. 1H NMR spectra are reported in the following order: chemical shift, multiplicity, coupling constant(s), and number(s) of protons. All NMR signals were assigned on the basis of ^{1}H NMR, COSY, HSQC, HMQC, and ^{13}C NMR experiments. HPLC-MS experiments were performed on an LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher) equipped with EASY-spray source and nano-LC UltiMate 3000 high-performance liquid chromatography system (Thermo Fisher). Samples were transmitted into MS with a silica column. LTQ-Orbitrap Elite mass spectrometer was operated in the data-dependent mode. A full-scan survey MS experiment (m/z range was set according to the molecular weight of O-mannose glycan; automatic gain control target, 1,000,000 ions; resolution at 400 m/z, 240,000; maximum ion accumulation time, 200 ms) was acquired by the Orbitrap mass spectrometer. MALDI-TOF MS analyses were performed on UltrafleXtreme MALDI TOF/TOF Mass Spectrometer (Bruker). Scan range of MS was set according to the molecular weight of O-mannose glycans, and reflector mode was used for O-mannose glycan analysis. Mass spectra were obtained in both positive and negative extraction mode with the following voltage settings: ion source 1 (19.0 kV), ion source 2 (15.9 kV), and lens (9.3 kV). The reflector voltage was set to 20 kV. The laser was pulsed at 7 Hz and the pulsed ion extraction time was set at 400 ns. The laser power was kept in the range of 40–90%. Fmoc-Thr(OH)-O*t*Bu (**4**) was purchased from Sigma-Aldrich.

General Procedures

A) Glycosylation of *N*-phenyltrifluoroacetimidate donor

A mixture of donor (1.2 mmol), acceptor (1 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 was stirred at room temperature under argon for 1 h. TMSOTf (0.2 mmol) was added at -60 °C. The reaction mixture was stirred at -60 °C for 1 h before it was quenched with a few drops of triethylamine. The resulting mixture was filtered. The filtrate was concentrated *in vacuo* and purified on a silica gel column to produce the product.

B) Glycosylation of thioether donor procedure

A mixture of thioether donor (1 mmol), amino acid acceptor **4** (1.5-2 mmol) and 4 Å molecular sieves in dry Et_2O/CH_2Cl_2 (1:1, v/v) were stirred at room temperature under argon for 1 h. NIS (1.5 mmol) and AgOTf (0.2 mmol) were added at 0 °C. The reaction mixture was stirred for 10 h before it was quenched with a few drops of triethylamine. The resulting mixture was filtered. The filtrate was diluted with CH_2Cl_2 and washed with 5% aqueous $Na_2S_2O_3$, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified on a silica gel column to produce the product.

C) Global deprotection of Ac and *t*Bu and reintroducing of Fmoc

Oligosaccharide glycosyl amino acid derivative (1 mmol) was dissolved in TFA/CH_2Cl_2 (1:1, v/v) and stirred at room temperature under argon for 4 h. The mixture was concentrated *in vacuo* and the residue was dissolved in MeOH, and NaOMe in MeOH was added until pH was 10. After stirring at room temperature for 2 h, the solution was neutralized with ion-exchange resin (H+), then filtered and concentrated *in vacuo*. The crude product, NaHCO3 (4 mmol) and 9-fluorenylmethyl-Nsuccimidylcarbonate (3 mmol) were dissolved in H_2O/acetone (1:1, v/v) and this mixture was stirred at room temperature. After 2 h, the mixture was concentrated *in vacuo* and purified on a silica gel column to afford the product.

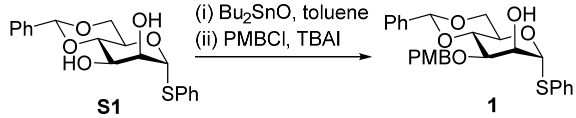
D) Transformation of PMB to Ac and cleavage of benzylidene

A solution of oligosaccharide (1 mmol) in a mixture of CH_2Cl_2/H_2O (30:1, v/v) was treated with DDQ (1.2 mmol) in ice bath and stirred at 25 °C for 3 h. Triethylamine was added and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2 was cooled down to 0 °C, followed by slow addition of acetic anhydride (3 mmol) and TEA (5 mmol). The reaction mixture was stirred at room temperature overnight and concentrated *in vacuo*. The crude product was dissolved in anhydrous MeOH, followed by addition of TsOH (0.1 mmol) and EtSH (6 mmol). The reaction mixture was stirred at rt. for 6 h and then quenched with triethylamine and concentrated *in vacuo*. The mixture was purified with silica column to get the product.

E) Transformation of NHTroc to NHAc

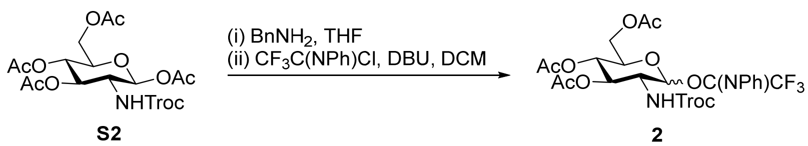
N-Troc protected oligosaccharide (1 mmol) was dissolved in AcOH at room temperature, followed by addition of Zn dust (<10 micron, 10 mmol). After being stirred at 40 °C for 24 h, the mixture was concentrated *in vacuo* to give a residue for the next step without further purification. To a solution of the residue in pyridine was added Ac_2O. After being stirred at room temperature for 12 h, the solution was diluted with ethyl acetate and washed with aqueous HCl (1 M), saturated aqueous $NaHCO_3$, and brine solution. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give a residue, which was purified by silica gel column chromatography to generate NHAc containing compound.

Preparation of the versatile precursor 1.



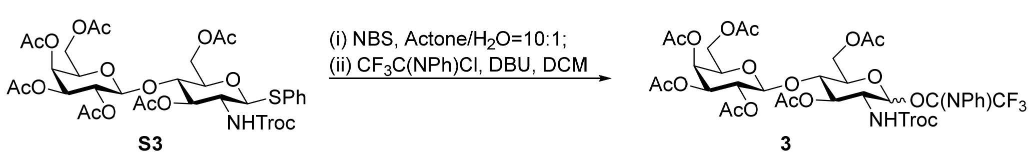
Compound **S1**[1] (13.40 g, 37.2 mmol), dibutyltin oxide (10.23 g, 40.9 mmol) and toluene (250 mL) were refluxed for 3 h, followed by concentration *in vacuo*. The residue, tetrabutylammonium iodide (15.09 g, 40.9 mmol), 4-Methoxybenzyl chloride (6.39 mL, 40.9 mmol), and toluene (250 mL) were stirred at 70 °C overnight. Toluene was removed by concentration *in vacuo* and the residue was diluted with ethyl acetate (300 mL). The resulting solution was washed with water and saturated aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate, 4:1, v/v) to afford precursor **1** (16.26 g, 91%) as white foams. R_f = 0.28 (hexane/ethyl acetate, 4:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (dd, *J* = 7.6, 1.8 Hz, 2H), 7.46 – 7.34 (m, 5H), 7.35 – 7.25 (m, 5H), 6.88 (d, *J* = 8.7 Hz, 2H), 5.61 (s, 1H), 5.57 (s, 1H), 4.81 (d, *J* = 11.4 Hz, 1H), 4.66 (d, *J* = 11.4 Hz, 1H), 4.33 (td, *J* = 9.8, 4.9 Hz, 1H), 4.25 – 4.11 (m, 3H), 3.93 (dd, *J* = 9.5, 3.4 Hz, 1H), 3.84 (t, *J* = 10.3 Hz, 1H), 3.80 (s, 3H), 2.95 (d, *J* = 1.2 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.47, 137.42, 133.27, 131.65, 129.74, 129.61, 129.10, 128.94, 128.20, 127.63, 126.05, 113.90, 101.57, 87.76, 78.92, 75.35, 72.87, 71.32, 68.48, 64.57, 55.23; MALDI-MS: $[M+Na]^+$ $C_{27}H_{28}O_6SNa$ calculated for 503.1504, found 503.1515.

Preparation of N-phenyltrifluoroacetimidate donor 2.



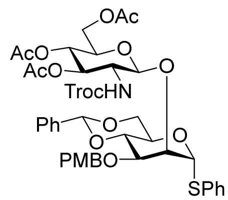
Compound **S2** (5.70 g, 10.9 mmol) was dissolved in THF (100 mL) followed by the addition of benzylamine (2.10 mL, 19.6 mmol). After the reaction mixture was stirred at room temperature for 8 h under dry atmosphere, it was evaporated and the residue was diluted with ethyl acetate and washed successively with 1 M aqueous HCl, saturated aqueous NaHCO3, and brine. The organic layer was dried over MgSO4, filtrated and concentrated under high vacuum. The crude product was dissolved in CH_2Cl_2 (200 mL), then 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride (3.42 mL, 21.8 mmol) and 1,8-diazabicyclo-[5,4,0]-7-undecene (2.44 mL, 16.4 mmol) were added at 0 °C under dry atmosphere. After the reaction mixture was stirred at room temperature for 2 h, it was evaporated followed by purification by silica gel column chromatography (hexane/ethyl acetate 4/1) to afford imidate donor **2** (5.50 g, 77% over two steps) as a white foams, which were found unstable and used immediately in the subsequent glycosylation without further characterization.

Preparation of N-phenyltrifluoroacetimidate donor 3.



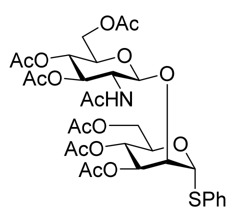
Compound **S3**[2] (5.10 g, 5.9 mmol) was dissolved in Actone/H_2O (10:1, v/v, 60 mL) at -30 °C and N-Bromosuccinimide (5.25 g, 29.5 mmol) was added to the solution. After stirred at -30 °C for 2 h, the reaction was quenched with 5% aqueous $Na_2S_2O_3$ (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The mixture was diluted with ethyl acetate and washed with brine. The organic layer was dried over $NaSO_4$, filtered, and concentrated. The residue was dissolved in

CH_2Cl_2 (100 mL), then 2,2,2-Trifluoro-N-phenylacetimidoyl Chloride (1.85 mL, 11.8 mmol) and 1,8-diazabicyclo-[5,4,0]-7-undecene (1.32 mL, 8.9 mmol) were added at 0 °C under dry atmosphere. After the reaction mixture was stirred at room temperature for 2 h, it was evaporated followed by purification by silica gel column chromatography (hexane/acetone 4/1) to afford imidate donor **3** (4.29 g, 75% over two steps) as a white foams, which were found instable and used immediately in the subsequent glycosylation without further characterization.



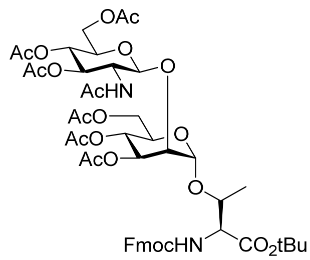
Phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-4,6-O-benzylidene-3-(4-Methoxybenzyl)-1-thio-α-D-Mannopyranoside (5)

Compound **1** (3.37 g, 7.0 mmol) was glycosylated with fresh-made donor **2** (5.50 g, 8.43 mmol) by following general procedure **A** to get the desired compound **5 (**5.63 g, 85%) as white foam. R_f = 0.30 (hexane/ethyl acetate, 3:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 7.49 (m, 2H), 7.49 – 7.37 (m, 5H), 7.37 – 7.30 (m, 5H), 6.94 – 6.84 (m, 2H), 5.64 (s, 1H), 5.54 (dd, *J* = 10.7, 9.3 Hz, 1H), 5.48 (d, *J* = 1.4 Hz, 1H), 5.37 – 5.30 (m, 1H), 5.14 – 5.01 (m, 2H), 4.78 (d, *J* = 11.2 Hz, 1H), 4.74 – 4.63 (m, 2H), 4.52 (d, *J* = 12.5 Hz, 1H), 4.40 (dd, *J* = 3.3, 1.6 Hz, 1H), 4.35 – 4.11 (m, 5H), 3.96 (dd, *J* = 9.8, 3.2 Hz, 1H), 3.88 – 3.78 (m, 4H), 3.77 – 3.70 (m, 1H), 3.44 (dt, *J* = 10.9, 8.1 Hz, 1H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.63, 170.27, 169.58, 159.40, 137.50, 133.42, 131.72, 129.87, 129.66, 129.23, 128.94, 128.25, 127.91, 126.06, 113.87, 101.55, 87.40, 78.75, 76.40, 74.40, 72.49, 72.09, 68.80, 68.49, 65.32, 62.16, 60.42, 55.26, 21.06, 20.71, 20.65, 20.58; MALDI-MS: $[M+Na]^+$ $C_{42}H_{46}Cl_3NO_{15}SNa$ calculated for 964.1552, found 964.1538.



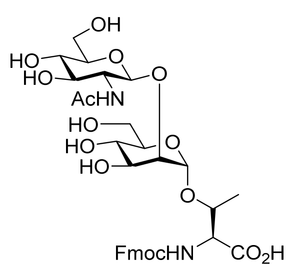
Phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-acetyl-1-thio-α-D-Mannopyranoside (6)

Compound **5** (2.83 g, 3.0 mmol) was dissolved in TFA/CH_2Cl_2 (10:1, v/v, 50 mL) and stirred at room temperature under argon for 1 h. CH_2Cl_2 (100 mL) was added to dilute the reaction and then neutralized with saturated aqueous $NaHCO_3$. The organic layer was separated and washed with brine. After dried over anhydrous Na_2SO_4 and concentrated, the residue was treated by following general procedure **E** to afford compound **6** (1.74 g, 80%) as white foam. R_f = 0.30 (hexane/acetone, 3:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.42 (m, 2H), 7.41 – 7.24 (m, 3H), 5.73 (d, *J* = 7.9 Hz, 1H), 5.51 (t, *J* = 9.9 Hz, 1H), 5.45 (s, 1H), 5.30 (t, *J* = 10.0 Hz, 1H), 5.12 – 4.95 (m, 2H), 4.53 – 4.41 (m, 2H), 4.33 – 4.20 (m, 2H), 4.11 – 3.97 (m, 2H), 3.72 (dd, *J* = 10.2, 5.1 Hz, 1H), 3.59 (dt, *J* = 11.5, 8.1 Hz, 1H), 2.11 – 2.07 (m, 6H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 – 1.99 (m, 6H), 1.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.78, 170.67, 170.62, 170.50, 170.35, 169.56, 169.51, 133.14, 131.55, 129.18, 127.87, 98.40, 85.07, 75.47, 71.84, 71.47, 70.46, 69.53, 68.83, 66.20, 62.71, 62.05, 55.63, 23.19, 20.75, 20.72, 20.70, 20.67, 20.63; $[M+Na]^+$ $C_{32}H_{41}NO_{16}SNa$ calculated for 750.2044, found 750.2032.



N$^{\alpha}$-9-Fluorenylmethyloxycarbonyl-O-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-3,4,6-tri-O-acetyl-α-D-mannopyranosyl]-L-threonine tertbutyl ester (7)

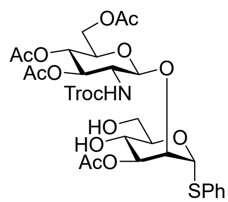
Compound **6** (1.74 g, 2.4 mmol) was glycosylated with compound **4** (1.43 g, 3.6 mmol) by following general procedure **B** to get the desired compound **7** (2.27 g, 93%) as white foam. R_f = 0.29 (hexane/acetone, 3:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.4 Hz, 2H), 7.72 – 7.54 (m, 2H), 7.47 – 7.29 (m, 4H), 5.76 (d, *J* = 7.9 Hz, 1H), 5.56 (d, *J* = 9.0 Hz, 1H), 5.43 (t, *J* = 9.8 Hz, 1H), 5.23 (t, *J* = 9.7 Hz, 1H), 5.10 – 4.94 (m, 2H), 4.91 – 4.80 (m, 2H), 4.58 – 4.14 (m, 8H), 4.14 – 4.05 (m, 2H), 4.05 – 3.88 (m, 2H), 3.72 – 3.51 (m, 2H), 2.08 (s, 6H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.95 (s, 3H), 1.53 (s, 9H), 1.32 (d, *J* = 5.6 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.65, 170.60, 170.56, 169.47, 169.32, 156.52, 143.84, 143.74, 141.31, 127.78, 127.11, 125.18, 120.04, 99.00, 98.89, 82.67, 74.15, 71.86, 71.65, 69.95, 69.21, 68.79, 67.36, 66.09, 62.89, 62.10, 58.92, 55.31, 47.17, 28.03, 23.24, 20.75, 20.71, 20.64, 17.88; $[M+Na]^+$ $C_{49}H_{62}N_2O_{21}Na$ calculated for 1037.3743, found 1037.3752.



N$^{\alpha}$-9-Fluorenylmethyloxycarbonyl-O-[2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-L-threonine (8, M100)

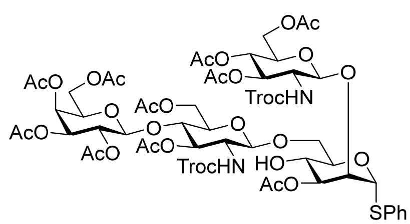
Compound **7** (2.13 g, 2.1 mmol) was treated by following general procedure **C** to afford compound **8** (1.34 g, 90%) as white foam. R_f = 0.40 (ethyl acetate/methanol/H_2O, 4:2:0.5, v/v); ^{1}H NMR (400 MHz, D_2O) δ 7.74 – 7.60 (m, 2H), 7.60 – 7.40 (m, 2H), 7.40 – 7.18 (m, 4H), 4.60

(dd, J = 10.7, 4.9 Hz, 1H), 4.42 – 4.27 (m, 2H), 4.23 (d, J = 6.3 Hz, 1H), 4.12 – 3.96 (m, 1H), 3.83 – 3.71 (m, 4H), 3.71 – 3.42 (m, 7H), 3.42 – 3.30 (m, 3H), 3.27 (s, 1H), 1.96 (s, 3H), 0.94 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 174.87, 158.05, 143.91, 143.49, 140.96, 140.91, 127.96, 127.41, 124.90, 124.78, 120.09, 100.01, 98.04, 77.19, 75.59, 73.28, 73.05, 69.84, 69.26, 67.22, 65.92, 61.60, 60.52, 55.26, 47.10, 22.35, 18.33; $[M-H]^-$ $C_{33}H_{41}N_2O_{15}$ calculated for 705.2585, found 705.2470.



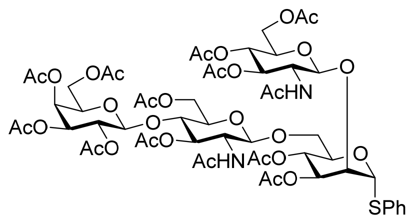
Phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-3-O-acetyl-1-thio-α-D-Mannopyranoside (9)

Compound **5** (3.58 g, 3.8 mmol) was treated by following general procedure **C** to afford compound **9** (2.27 g, 77%) as white foam. R_f = 0.33 (hexane/acetone, 4:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, J = 6.5 Hz, 2H), 7.36 – 7.19 (m, 3H), 6.00 (d, J = 8.2 Hz, 1H), 5.35 (s, 1H), 5.25 (t, J = 9.9 Hz, 1H), 4.99 (t, J = 9.5 Hz, 1H), 4.88 (d, J = 9.6 Hz, 1H), 4.71 – 4.56 (m, 2H), 4.52 – 4.37 (m, 2H), 4.33 – 4.15 (m, 2H), 4.15 – 3.97 (m, 2H), 3.87 (t, J = 10.5 Hz, 1H), 3.80 – 3.45 (m, 4H), 2.93 (d, J = 8.7 Hz, 1H), 2.10 (s, 3H), 2.08 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.99, 170.89, 170.77, 169.52, 154.57, 133.42, 131.69, 129.27, 127.94, 100.41, 95.00, 85.81, 76.94, 74.56, 73.42, 73.20, 71.77, 71.35, 68.58, 64.12, 62.12, 61.50, 56.05, 20.91, 20.68, 20.59; $[M+Na]^+$ $C_{29}H_{36}Cl_3NO_{15}SNa$ calculated for 798.0769, found 798.0778.



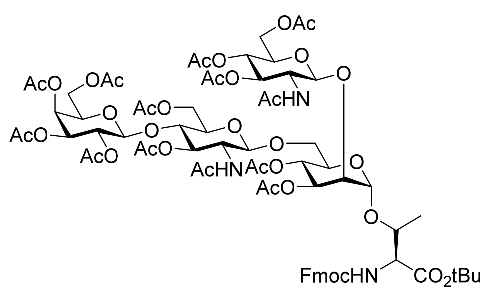
Phenyl 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-glucopyranosyl-(1→2)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-(1→6)-3-O-acetyl-1-thio-α-D-mannopyranoside (10)

Compound **9** (2.10 g, 2.7 mmol) was glycosylated with fresh-made donor **3** (3.05 g, 3.2 mmol) by following general procedure **A** to get the desired compound **10** (3.26 g, 79%) as white foam. R_f = 0.35 (hexane/acetone, 2:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 6.7 Hz, 2H), 7.39 – 7.24 (m, 3H), 6.32 (d, J = 8.4 Hz, 1H), 6.05 (d, J = 8.4 Hz, 1H), 5.45 (s, 1H), 5.43 – 5.31 (m, 3H), 5.09 (dd, J = 10.3, 7.9 Hz, 1H), 5.05 – 4.91 (m, 3H), 4.86 (dd, J = 9.8, 2.6 Hz, 1H), 4.78 (d, J = 8.2 Hz, 1H), 4.76 – 4.62 (m, 3H), 4.59 – 4.44 (m, 3H), 4.41 (s, 1H), 4.26 (dd, J = 12.3, 5.4 Hz, 1H), 4.20 – 4.00 (m, 6H), 3.96 (d, *J* = 10.9 Hz, 1H), 3.92 – 3.81 (m, 2H), 3.79 – 3.65 (m, 2H), 3.61 (dd, *J* = 8.7, 3.8 Hz, 1H), 3.49 (dq, *J* = 17.4, 8.7 Hz, 2H), 3.00 (s, 1H), 2.15 (s, 3H), 2.13 (s, 6H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.77, 170.68, 170.50, 170.40, 170.19, 170.12, 169.52, 169.11, 155.08, 154.61, 133.76, 130.97, 129.19, 127.63, 101.24, 100.97, 99.16, 95.48, 95.35, 85.16, 76.47, 74.73, 74.52, 73.30, 72.72, 72.47, 71.97, 71.14, 70.99, 70.74, 69.15, 68.88, 67.77, 66.67, 63.87, 62.07, 60.93, 56.75, 56.14, 21.03, 20.94, 20.88, 20.72, 20.69, 20.63, 20.60, 20.50; $[M+Na]^+$ $C_{56}H_{70}Cl_6N_2O_{32}SNa$ calculated for 1547.1661, found 1547.1603.



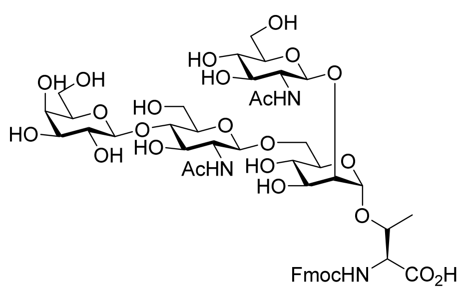
Phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-glucopyranosyl-(1→2)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→6)]-3,4-di-O-acetyl-1-thio-α-D-mannopyranoside (11)

Compound **10** (3.10 g, 2.0 mmol) was treated by following general procedure **C** to afford compound **11** (2.30 g, 87%) as white foam. R_f = 0.22 (hexane/acetone, 3:2, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.36 (m, 2H), 7.35 – 7.26 (m, 3H), 7.26 – 7.20 (m, 1H), 6.46 (d, *J* = 10.0 Hz, 1H), 5.89 (dd, *J* = 10.7, 9.2 Hz, 1H), 5.68 (d, *J* = 1.1 Hz, 1H), 5.50 – 5.37 (m, 2H), 5.34 (d, *J* = 2.8 Hz, 1H), 5.14 – 4.99 (m, 3H), 4.99 – 4.82 (m, 2H), 4.54 – 4.36 (m, 3H), 4.26 (dd, *J* = 12.2, 5.9 Hz, 1H), 4.22 – 4.11 (m, 3H), 4.11 – 4.00 (m, 4H), 3.95 (dd, *J* = 12.1, 2.2 Hz, 1H), 3.85 (t, *J* = 7.1 Hz, 1H), 3.80 – 3.66 (m, 2H), 3.53 (ddd, *J* = 9.7, 5.0, 1.8 Hz, 1H), 3.15 (d, *J* = 10.8 Hz, 1H), 3.02 – 2.89 (m, 1H), 2.14 (s, 3H), 2.11 (s, 3H), 2.08 (s, 6H), 2.07 (s, 3H), 2.05 (s, 3H), 2.04 (d, *J* = 3.8 Hz, 3H), 2.02 (d, *J* = 4.8 Hz, 6H), 1.99 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.69, 172.18, 170.86, 170.60, 170.31, 170.29, 170.08, 170.00, 169.93, 169.78, 169.08, 133.09, 130.04, 129.24, 127.24, 102.96, 101.07, 96.29, 83.45, 77.41, 77.09, 76.78, 76.15, 74.31, 72.83, 72.75, 71.88, 70.88, 70.62, 70.55, 70.43, 69.98, 69.53, 69.15, 68.44, 66.51, 65.77, 62.35, 62.07, 60.57, 56.92, 53.49, 23.28, 23.25, 21.02, 20.89, 20.80, 20.67, 20.64, 20.61, 20.58, 20.52, 20.48; $[M+Na]^+$ $C_{56}H_{74}N_2O_{31}SNa$ calculated for 1325.3894, found 1325.3935.



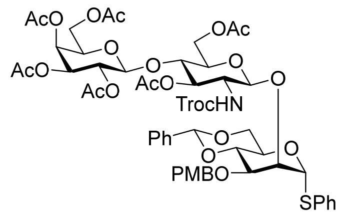
N$^{\alpha}$-9-Fluorenylmethyloxycarbonyl-O-{2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-glucopyranosyl-(1→2)-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl]-(1→6)-3,4-di-O-acetyl-α-D-mannopyranosyl}-L-threonine tertbutyl ester (12)

Compound **11** (2.00 g, 1.5 mmol) was glycosylated with compound **4** (1.19 g, 3.0 mmol) by following general procedure **B** to get the desired compound **12** (2.12 g, 89%) as white foam. R_f = 0.24 (hexane/acetone, 3:2, v/v); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 – 7.74 (m, 2H), 7.69 – 7.52 (m, 2H), 7.44 – 7.37 (m, 2H), 7.37 – 7.30 (m, 3H), 6.44 (d, J = 10.0 Hz, 1H), 5.95 – 5.85 (m, 1H), 5.60 (d, J = 9.3 Hz, 1H), 5.43 – 5.31 (m, 3H), 5.17 – 5.01 (m, 3H), 4.96 (dd, J = 10.4, 3.4 Hz, 1H), 4.94 – 4.83 (m, 2H), 4.54 – 4.42 (m, 3H), 4.42 – 4.23 (m, 5H), 4.23 – 4.02 (m, 7H), 3.97 – 3.83 (m, 2H), 3.83 – 3.70 (m, 2H), 3.70 – 3.60 (m, 1H), 3.60 – 3.52 (m, 1H), 3.14 (d, J = 11.3 Hz, 1H), 2.91 (dd, J = 17.8, 7.9 Hz, 1H), 2.16 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.09 (s, 3H), 2.07 (t, J = 2.7 Hz, 6H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.52 (s, 9H), 1.31 – 1.24 (m, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 172.57, 172.20, 170.87, 170.63, 170.32, 170.11, 170.04, 169.97, 169.93, 169.89, 169.10, 169.03, 156.59, 143.95, 143.79, 141.29, 127.71, 127.11, 127.08, 125.25, 119.98, 119.95, 102.90, 101.11, 98.35, 96.47, 82.46, 78.01, 76.15, 72.90, 72.80, 72.71, 71.80, 70.91, 70.64, 70.62, 70.07, 69.85, 69.17, 69.09, 68.32, 67.25, 66.52, 65.79, 62.39, 61.97, 60.57, 59.03, 56.99, 53.51, 47.20, 28.11, 23.30, 23.28, 21.04, 20.93, 20.85, 20.81, 20.69, 20.63, 20.61, 20.58, 20.50, 18.06; $[M+Na]^+$ $C_{73}H_{95}N_3O_{36}Na$ calculated for 1612.5593, found 1612.5678.



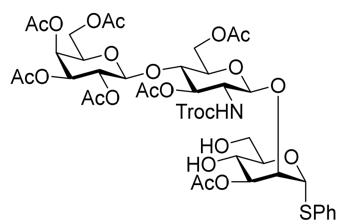
N$^{\alpha}$-9-Fluorenylmethyloxycarbonyl-O-{2-acetamido-2-deoxy-β-glucopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→6)-α-D-mannopyranosyl}-L-threonine (13, M301)

Compound **12** (1.90 g, 1.2 mmol) was treated by following general procedure **C** to afford compound **13** (1.11 g, 85%) as white foam. R_f = 0.30 (ethyl acetate/methanol/H_2O, 4:2:0.5, v/v); ^{1}H NMR (400 MHz, MeOD) δ 7.79 (d, J = 7.5 Hz, 2H), 7.74 – 7.58 (m, 2H), 7.44 – 7.27 (m, 4H), 4.55 (d, J = 7.7 Hz, 1H), 4.50 – 4.31 (m, 5H), 4.23 (t, J = 6.7 Hz, 1H), 4.18 – 4.02 (m, 2H), 4.01 – 3.84 (m, 5H), 3.84 – 3.51 (m, 14H), 3.51 – 3.30 (m, 6H), 2.08 (s, 3H), 2.06 (s, 3H), 1.22 (d, J = 5.9 Hz, 2H); ^{13}C NMR (100 MHz, MeOD) δ 173.40, 172.73, 157.41, 144.06, 143.80, 141.18, 127.42, 126.85, 124.88, 119.55, 103.81, 101.78, 98.66, 79.69, 79.33, 76.38, 75.74, 75.04, 73.89, 73.41, 72.46, 71.20, 70.54, 69.85, 68.93, 68.64, 67.77, 66.59, 61.16, 60.88, 60.39, 55.28, 47.08, 22.35, 22.12, 18.44; $[M-H]^-$ $C_{47}H_{64}N_3O_{25}$ calculated for 1071.3907, found 1071.3741.



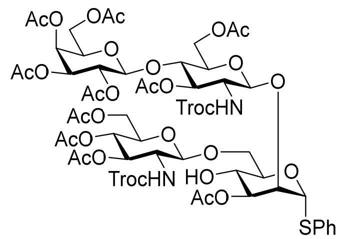
Phenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-4,6-O-benzylidene-3-(4-Methoxybenzyl)-1-thio-α-D-Mannopyranoside (14)

Compound **1** (1.8 g, 3.8 mmol) was glycosylated with fresh-made donor **3** (4.23 g, 4.5 mmol) by following general procedure **A** to get the desired compound **14 (**3.46 g, 75%) as white foam. R_f = 0.33 (hexane/acetone, 3:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (dd, *J* = 7.6, 1.8 Hz, 2H), 7.46 – 7.25 (m, 10H), 6.87 (d, *J* = 8.7 Hz, 2H), 5.62 (s, 1H), 5.48 (s, 1H), 5.43 – 5.23 (m, 3H), 5.12 (dd, *J* = 10.4, 7.9 Hz, 1H), 4.97 (dd, *J* = 10.4, 3.4 Hz, 1H), 4.81 (d, *J* = 8.2 Hz, 1H), 4.75 – 4.62 (m, 3H), 4.59 – 4.45 (m, 3H), 4.34 (d, *J* = 1.7 Hz, 1H), 4.30 – 4.03 (m, 6H), 3.94 – 3.86 (m, 2H), 3.85 – 3.72 (m, 5H), 3.65 – 3.51 (m, 2H), 2.15 (s, 3H), 2.06 (s, 3H), 2.06 (s, 6H), 2.04 (s, 3H), 1.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.40, 170.33, 170.15, 170.08, 169.09, 159.30, 153.99, 137.55, 133.40, 131.49, 130.00, 129.44, 129.24, 128.90, 128.23, 127.84, 126.10, 113.80, 101.53, 100.98, 99.17, 95.32, 86.79, 78.47, 76.24, 74.48, 74.23, 73.00, 71.90, 71.38, 70.97, 70.75, 69.13, 68.46, 66.69, 65.30, 62.24, 60.94, 55.98, 55.26, 20.82, 20.79, 20.66, 20.63, 20.61, 20.52; $[M+Na]^+$ $C_{54}H_{62}Cl_3NO_{23}SNa$ calculated for 1252.2397, found 1252.2444.



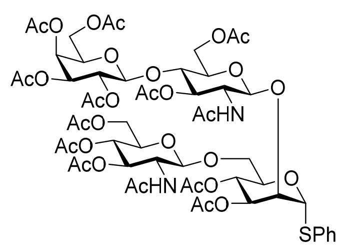
Phenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-3-O-acetyl-1-thio-α-D-Mannopyranoside (15)

Compound **14** (3.3 g, 2.7 mmol) was treated by following general procedure **C** to afford compound **15** (2.31 g, 81%) as white foam. R_f = 0.22 (hexane/acetone, 2:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 6.6 Hz, 2H), 7.34 – 7.21 (m, 3H), 6.07 (d, *J* = 9.1 Hz, 1H), 5.35 (s, 1H), 5.32 (d, *J* = 3.1 Hz, 1H), 5.10 – 4.99 (m, 2H), 4.94 (dd, *J* = 10.4, 3.3 Hz, 1H), 4.87 (dd, *J* = 9.9, 2.6 Hz, 1H), 4.72 (d, *J* = 11.9 Hz, 1H), 4.52 – 4.42 (m, 2H), 4.38 (d, *J* = 11.7 Hz, 3H), 4.31 – 4.19 (m, 1H), 4.15 – 3.98 (m, 4H), 3.94 – 3.78 (m, 2H), 3.78 – 3.63 (m, 3H), 3.63 – 3.53 (m, 1H), 3.43 (d, *J* = 3.9 Hz, 1H), 2.97 (d, *J* = 8.7 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.12, 170.46, 170.42, 170.15, 170.13, 169.44, 154.88, 133.34, 131.56, 129.27, 127.88, 101.17, 100.94, 95.06, 85.69, 76.09, 74.58, 73.61, 73.28, 72.73, 71.63, 70.83, 70.69, 69.19, 66.66, 63.99, 62.23, 61.48, 60.91, 55.97, 20.87, 20.78, 20.75, 20.64, 20.58, 20.47; $[M+Na]^+$ $C_{41}H_{52}Cl_3NO_{23}SNa$ calculated for 1086.1614, found 1086.1658.



Phenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl-(1→2)-[3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-glucopyranosyl-(1→6)]-3-O-acetyl-1-thio-α-D-Mannopyranosyl (16)

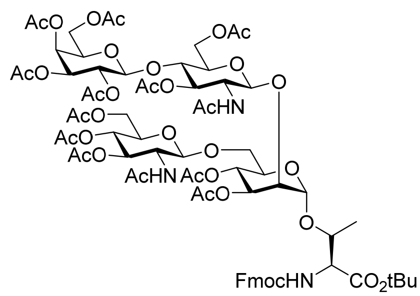
Compound **15** (2.10 g, 2.0 mmol) was glycosylated with fresh-made donor **2** (1.54 g, 2.4 mmol) by following general procedure **A** to get the desired compound **16** (2.75 g, 82%) as white foam. R_f = 0.25 (hexane/acetone, 2:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 7.5, 1.7 Hz, 2H), 7.34 – 7.21 (m, 3H), 6.96 (d, *J* = 7.0 Hz, 1H), 5.93 (d, *J* = 9.7 Hz, 1H), 5.73 (t, *J* = 10.0 Hz, 1H), 5.43 (s, 1H), 5.33 (d, *J* = 3.2 Hz, 1H), 5.18 – 4.89 (m, 6H), 4.81 (dd, *J* = 10.0, 2.7 Hz, 1H), 4.72 – 4.53 (m, 3H), 4.50 – 4.38 (m, 3H), 4.36 (s, 1H), 4.24 (dd, *J* = 12.3, 4.4 Hz, 1H), 4.16 – 4.03 (m, 4H), 4.03 – 3.89 (m, 4H), 3.85 (t, *J* = 6.6 Hz, 1H), 3.83 – 3.65 (m, 3H), 3.65 – 3.56 (m, 1H), 3.17 (dd, *J* = 18.1, 8.0 Hz, 1H), 2.12 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 (s, 6H), 2.01 (s, 3H), 2.01 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.80, 170.59, 170.51, 170.42, 170.14, 170.10, 169.76, 169.29, 154.95, 133.57, 130.98, 129.18, 127.65, 101.23, 100.94, 100.49, 96.05, 94.96, 85.58, 77.60, 76.27, 75.19, 74.19, 73.55, 72.93, 72.63, 71.62, 71.44, 70.96, 70.87, 70.45, 69.34, 69.09, 67.65, 66.80, 63.26, 62.51, 62.13, 61.26, 57.10, 55.69, 21.02, 20.88, 20.81, 20.75, 20.64, 20.60, 20.57, 20.55, 20.48; $[M+Na]^+$ $C_{56}H_{70}Cl_6N_2O_{32}SNa$ calculated for 1547.1661, found 1547.1738.



Phenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-glucopyranosyl-(1→6)]-3,4-di-O-acetyl-1-thio-α-D-Mannopyranoside (17)

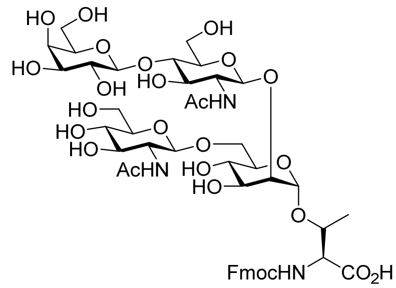
Compound **16** (2.50 g, 1.6 mmol) was treated by following general procedure **C** to afford compound **17** (1.75 g, 82%) as white foam. R_f = 0.22 (hexane/acetone, 3:2, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 – 7.36 (m, 2H), 7.36 – 7.26 (m, 2H), 7.26 – 7.19 (m, 1H), 7.10 (d, *J* = 6.9 Hz, 1H), 6.55 (d, *J* = 9.9 Hz, 1H), 5.84 (dd, *J* = 10.6, 8.9 Hz, 1H), 5.74 (s, 1H), 5.50 – 5.38 (m, 2H), 5.33 (d, *J* = 2.8 Hz, 1H), 5.15 – 4.98 (m, 4H), 4.94 (dd, *J* = 10.4, 3.4 Hz, 1H), 4.48 (d, *J* = 7.9 Hz, 1H), 4.37 – 3.97 (m, 11H), 3.85 (t, *J* = 7.0 Hz, 1H), 3.77 – 3.55 (m, 3H), 3.19 (d, *J* = 10.9 Hz, 1H), 2.90 (dt, *J* = 10.5, 8.1 Hz, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.03 (s, 6H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.91

(s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.73, 172.09, 171.03, 170.70, 170.63, 170.53, 170.35, 170.26, 170.10, 169.92, 169.37, 169.32, 168.87, 133.13, 129.74, 129.24, 127.13, 103.01, 100.62, 96.29, 83.11, 77.85, 74.47, 72.98, 72.86, 71.86, 70.99, 70.57, 70.47, 70.36, 69.49, 69.07, 68.55, 68.49, 66.62, 65.93, 62.44, 61.96, 60.78, 57.07, 53.41, 23.32, 23.16, 21.08, 21.05, 20.70, 20.69, 20.67, 20.61, 20.57, 20.55, 20.52, 20.48; $[M+Na]^+$ $C_{56}H_{74}N_2O_{31}SNa$ calculated for 1325.3894, found 1325.3946.



N$^{\alpha}$-9-Fluorenylmethyloxycarbonyl-O-{2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-glucopyranosyl-(1→6)]-3,4-di-O-acetyl-α-D-Mannopyranosyl}-L-threonine tertbutyl ester (18)

Compound **17** (1.57 g, 1.2 mmol) was glycosylated with compound **4** (0.98 g, 2.4 mmol) by following general procedure **B** to get the desired compound **18** (1.56 g, 83%) as white foam. R_f = 0.23 (hexane/acetone, 3:2, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.4 Hz, 2H), 7.71 – 7.51 (m, 2H), 7.49 – 7.25 (m, 5H), 7.05 (d, J = 7.0 Hz, 1H), 6.51 (d, J = 9.6 Hz, 1H), 5.87 – 5.72 (m, 1H), 5.57 (d, J = 9.3 Hz, 1H), 5.41 – 5.28 (m, 3H), 5.19 – 5.01 (m, 4H), 5.01 – 4.87 (m, 2H), 4.59 – 4.41 (m, 3H), 4.41 – 3.96 (m, 16H), 3.90 – 3.81 (m, 1H), 3.81 – 3.70 (m, 1H), 3.70 – 3.55 (m, 3H), 3.17 (d, J = 11.2 Hz, 1H), 2.88 (dd, J = 17.5, 8.1 Hz, 1H), 2.18 (s, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 2.04 (s, 6H), 2.02 (s, 3H), 1.97 (s, 3H), 1.94 (s, 3H), 1.52 (s, 9H), 1.27 (d, J = 8.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.45, 172.04, 171.00, 170.70, 170.62, 170.57, 170.35, 170.22, 170.12, 169.82, 169.38, 169.01, 156.57, 143.95, 143.78, 141.28, 127.70, 127.10, 125.25, 119.98, 102.88, 101.06, 98.03, 96.56, 82.47, 78.21, 77.93, 72.88, 72.79, 71.91, 71.03, 70.88, 70.52, 69.85, 69.50, 69.22, 69.11, 68.61, 68.38, 67.25, 66.53, 65.93, 62.51, 62.00, 60.61, 58.98, 57.02, 53.84, 53.48, 47.19, 29.28, 28.13, 23.28, 23.18, 21.09, 20.72, 20.68, 20.63, 20.59, 20.53, 17.95; $[M+Na]^+$ $C_{73}H_{95}N_3O_{36}Na$ calculated for 1612.5593, found 1612.5663.



N$^{\alpha}$-9-Fluorenylmethyloxycarbonyl-O-{β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-[2-acetamido-2-deoxy-β-glucopyranosyl-(1→6)]-α-D-Mannopyranosyl}-L-threonine (19, M201)

Compound **18** (1.90 g, 1.2 mmol) was treated by following general procedure **C** to afford compound **19** (1.09 g, 87%) as white foam. R_f = 0.30 (ethyl acetate/methanol/H_2O, 4:2:0.5, v/v); ^{1}H NMR (400 MHz, D_2O) δ 7.68 – 7.56 (m, 2H), 7.56 – 7.36 (m, 2H), 7.36 – 7.16 (m, 5H), 4.59 – 4.51 (m, 1H), 4.47 (d, *J* = 8.4 Hz, 1H), 4.43 – 4.23 (m, 4H), 4.18 (d, *J* = 6.4 Hz, 1H), 4.09 (d, *J* = 10.3 Hz, 1H), 3.98 (d, *J* = 4.8 Hz, 1H), 3.90 – 3.80 (m, 4H), 3.80 – 3.55 (m, 16H), 3.55 – 3.42 (m, 5H), 3.41 – 3.24 (m, 5H), 1.97 (s, 3H), 1.95 (s, 4H), 0.95 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 174.59, 174.15, 158.00, 143.88, 143.45, 140.96, 140.90, 127.97, 127.43, 124.80, 120.11, 102.90, 101.16, 99.84, 98.22, 78.31, 77.18, 76.85, 75.90, 75.31, 74.56, 73.93, 72.48, 71.95, 70.94, 70.06, 69.21, 68.56, 67.59, 65.98, 61.02, 60.87, 59.88, 55.57, 54.82, 48.89, 47.09, 22.50, 22.33, 18.49; $[M+Na]^+$ $C_{47}H_{65}N_3O_{25}Na$ calculated for 1094.3805, found 1094.3876.

II. Enzymatic Extensions

Materials and enzymes

N-Acetylneuraminic acid(Neu5Ac), *N*-Glycolylneuraminic acid (Neu5Gc) and cytidine 5'-triphosphate (CTP) were purchased from Carbosynth Limited. Sugar nucleotides uridine 5'-diphospho-galactose (UDP-Gal)[3], guanosine 5'-diphospho-L-fucose (GDP-Fuc)[4], uridine 5′-diphosphoglucuronic acid(UDP-GlcA)[5] were prepared as described previously reported. Enzymes including CMP-sialic acid synthetase from *Neisseria meningitidis* (NmCSS),[6] mutant M144D of α2,3-sialyltransferase 1 from *Pasteurella multocida* (PmST1-M144D),[7] mutant E271F/R313Y of PmST1 (PmST1m),[8] α2,6-sialyltransferase from *Photobacterium damselae* (Pd2,6ST),[9] β1,4-galactosyltransferase from *N. meningitidis* (NmLgtB),[10] and C-terminal 66 amino acid truncated α1,3-fucosyltransferase from *Helicobacter pylori* (Hp3FT)[11] were expressed and purified as previously described. All enzymes were desalted against 50 mM Tris-HCl, and 20% glycerol, and stored at -20 °C for long-term use. β-galactosidase (βGalD) from *Streptococcus pneumoniae* was purchased from Prozyme, CA.

Cloning and expression of human GlcAT-P

The human β1,3-glucuronyltransferase gene (GlcAT-P) gene (GenBank: AB029396.1) was codon optimized, synthesized and cloned into vector pET15b vector (Genescript, NJ). The recombinant plasmid harboring GlcAT-P was transformed into *E. coli* BL21 (DE3) for hetergenous expression. The recombinant strain was cultured in LB medium at 37 °C with brief shaking (180 rpm) untill OD_{600nm} reached 0.6 to 0.8, followed by addition of isopropyl-b-D-thiogalactopyranoside (IPTG) to a final concentration of 0.2 mM. After 20 h induction at 16 °C, the cells were harvested by centrifuging at 4000 rpm for 30 min. The target protein was expressed as inclusion body. *E. coli* cell lysate was used to in GlcAT-P-catalyzed reactions.

Optimized GlcAT gene sequence

CATCAATCCACCCTGGCACCGCTGCTGGCAGTCCATAAGGACGAAGGTTCCGATCCG
CGTCGTGAGACCCCGCCGGGCGCAGACCCGCGTGAGTACTGCACCAGCGATCGTGA
CATCGTGGAAGTGGTGCGTACCGAATACGTTTACACCCGTCCGCCGCCGTGGAGCG
ATACCCTGCCGACCATCCACGTGGTTACCCCGACCTACAGCCGTCCGGTGCAGAAGG
CTGAGCTGACCCGTATGGCTAACACCCTGCTGCACGTTCCGAACCTGCACTGGCTGG
TTGTGGAAGATGCGCCGCGTCGTACCCCGCTGACCGCTCGTCTGCTGCGTGACACCG
GTCTGAACTACACCCACCTGCACGTGGAAACCCCGCGTAACTACAAACTGCGTGGT
GACGCTCGTGACCCGCGTATCCCGCGTGGTACCATGCAGCGTAACCTGGCTCTGCGT
TGGCTGCGTGAAACCTTCCCGCGTAACAGCAGCCAGCCGGGCGTGGTTTACTTCGCG
GACGATGACAACACCTACAGCCTGGAACTGTTCGAGGAAATGCGTAGCACCCGTCG
TGTGAGCGTTTGGCCGGTGGCTTTCGTTGGTGGCCTGCGTTACGAGGCGCCGCGTGT
GAACGGTGCTGGCAAGGTGGTTCGTTGGAAAACCGTTTTCGATCCGCACCGTCCGTT
CGCGATCGACATGGCGGGTTTCGCTGTGAACCTGCGTCTGATCCTGCAGCGTAGCCA
GGCTTACTTCAAGCTGCGTGGCGTGAAAGGTGGCTACCAGGAGAGCAGCCTGCTGC
GTGAACTGGTTACCCTGAACGACCTGGAACCGAAGGCGGCTAACTGCACCAAAATC
CTGGTGTGGCACACCCGTACCGAGAAGCCGGTGCTGGTGAATGAAGGCAAGAAAGG
TTTTACCGACCCGTCCGTTGAGATT

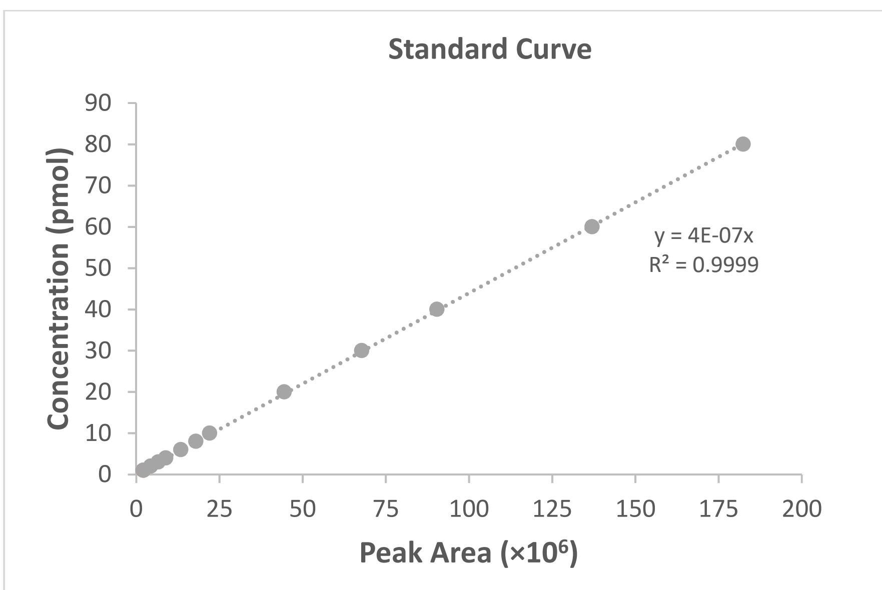
General HPLC methods

<u>HPLC method for monitoring reactions and purity analysis of final products</u>: An analytical GL Science Inertsil ODS-4 column (100 Å, 5 µm, 4.6 mm × 250 mm) was used for separation, monitored by a UV detector (260 nm) or fluorescent detector (Ex 260nm, Em 310 nm).[12] The running solvents are solvent A(H_2O with 0.1% TFA) and solvent B (acetonitrile with 0.1% TFA). The running condition is gradient elution with solvent B% linear increased from 20% to 40% within 25 mins, with a total flow rate of 1 mL/min.

HPLC method for purifying final products: An analytical GL Science Inertsil ODS-4 column (100 Å, 5 µm, 4.6 mm × 250 mm) was used for separating small reactions with 3 mg or less products, and a semipreparative Inertsil ODS-4 column (100 Å, 5 µm, 20 mm × 250 mm) was used for separating products with over 5 mgs. The method for using the analytical column is same as above and monitored by a UV detector (260 nm). The method for using the semipreparative column is similar as that for the analytical column, with the only difference of flow rate at 18.9 mL/min instead. Commonly, the analytical column enabled up to 1 mg product separation per run, while the semipreparative one enabled up to 15 mg product separation per run.

HPLC quantification of purified glycans

Firstly, 10 mg M201 was weighted out accurately and diluted to 1 µM as standard solution. Then different volumes of standard solution, 1 µL, 2 µL, 3 µL, 4 µL, 6 µL, 8 µL, 10 µL, 20 µL, 30 µL, 40 µL, 60 µL, 80 µL, were injected into the HPLC by the method mentioned above using fluorescent (Ex. 260 nm, Em. 310 nm) in three replicates. The peak area of each injection was recorded and calculated to make the standard curve. All the purified glycans was quantified by the same condition as built the standard curve.



General methods for O-mannosyl glycan preparation and purification

A) β1,4-galactosylation by NmLgtB

Reaction mixtures contain Tris-HCl (100 mM, pH 7.5), an acceptor O-mannosyl glycan (10 mM), UDP-Gal (15 mM), $MgCl_2$ (10 mM), and an appropriate amount of NmLgtB. Reactions were incubated at 37 °C overnight and monitored by HPLC and/or MALDI-MS. After over 90% acceptor was converted, the reaction was quenched by freezing at -80 °C for 30 min, thaw and brief centrifugation to remove protein precipitants. The sample was then concentrated and subject for HPLC separation, product-containing fractions were pooled and lyophilized for characterization and next step use. The purity and quantification of each glycan was confirmed by HPLC as described above. NmLgtB-catalyzed reactions give 90-96% yields (HPLC purified glycan product/starting glycan substrate × 100%)

B) One-pot two-enzyme α2,3-sialylation catalyzed by PmST1-M144D or PmST1m

Reaction mixtures contain Tris-HCl (100 mM, pH 7.5), an acceptor glycan (10 mM), CTP (15 mM), Neu5Ac or Neu5Gc (15 mM), $MgCl_2$ (10 mM), and appropriate amount of NmCSS and PmST1-M144D (or PmST1m). PmST1-M144D-catalyzed reactions were incubated at 37 °C overnight and monitored by HPLC and/or MALDI-TOF. After over 90% acceptor was converted, the reaction was quenched, concentrated and subject for HPLC separation. PmST1m-catalyzed reactions were incubated at 37 °C for 30 min, quenched, and concentrated for HPLC separation. Product-containing fractions were pooled and lyophilized for characterization and next step use. PmST1-catalyzed reactions give 85-92% yields (HPLC purified glycan product/starting glycan substrate × 100%).

C) One-pot two-enzyme α2,6-sialylation catalyzed by Pd26ST

Reaction mixtures contain Tris-HCl (100 mM, pH 7.5), an acceptor glycan (10 mM), CTP (15 mM), Neu5Ac or Neu5Gc (15 mM), $MgCl_2$ (10 mM), and appropriate amount of NmCSS and Pd26ST. Reactions were incubated at 37 °C overnight and monitored by HPLC and/or MALDI-TOF. After over 90% acceptor was converted, reactions were quenched, concentrated before HPLC separation. Product-containing fractions were pooled and lyophilized for characterization and next step use. Pd26ST-catalyzed reactions give 89-94% yields (HPLC purified glycan product/starting glycan substrate × 100%).

D) α1,3-fucosylation catalyzed by Hp3FT

Reaction mixtures contain Tris-HCl (100 mM, pH 7.5), an acceptor glycan (10 mM), GDP-Fuc (15 mM), $MgCl_2$ (10 mM), and appropriate amount of Hp3FT. Reactions were incubated at 37 °C overnight and were monitored by HPLC and/or MALDI-TOF. After over 90% acceptor was converted, reactions were quenched, concentrated before HPLC separation. Product-containing fractions were pooled and lyophilized for characterization and next step use. Hp3FT-catalyzed reactions give 85-86% yields (HPLC purified glycan product/starting glycan substrate × 100%).

E) GlcAT-P-catalyzed addition of GlcA

The reaction mixture contains Tris-HCl (100 mM, pH 7.5), **M101** (10 mM), UDP-GlcA (15 mM), $MgCl_2$ (10 mM), and appropriate amount of GlcAT-P cell lysis. Reaction was incubated at 37 °C overnight. The reaction was monitored by HPLC until 90% M101 was converted. The reaction was then quenched andconcentrated for HPLC separation. Product-containing fractions were pooled and lyophilized for characterization. The reaction gives a yield of 90% (HPLC purified glycan product/starting glycan substrate × 100%).

F) β-galactosidase-catalyzed reaction

The reaction was performed according to the instruction using commercial β-galactosidase in the presence of **M201**. The reaction was monitored and product was purified by HPLC. The reaction gives a yield of 96% (HPLC purified glycan product/starting glycan substrate × 100%)

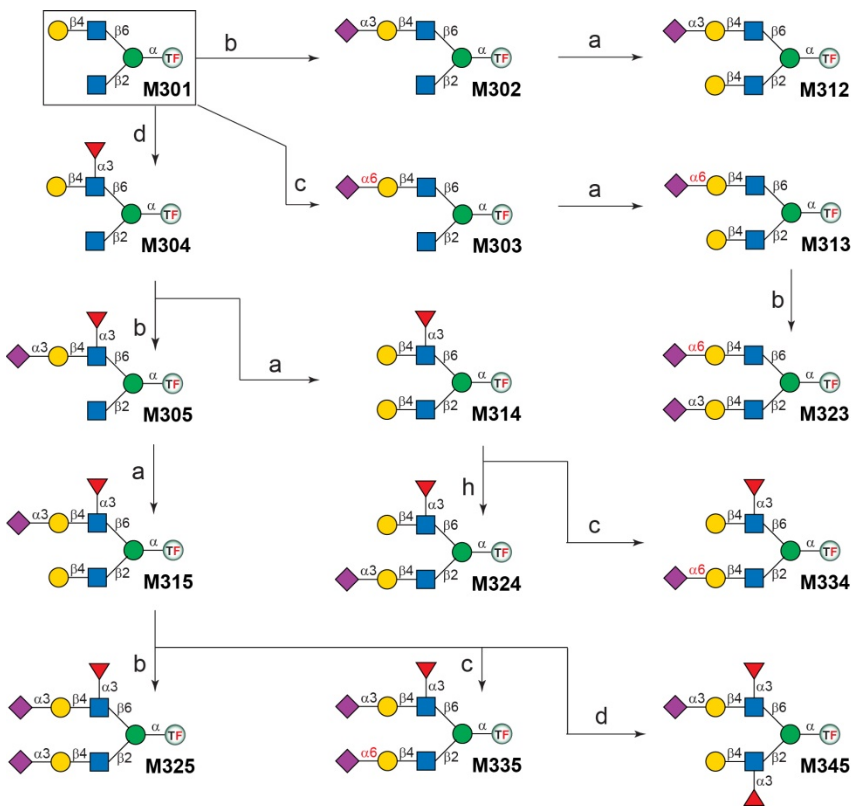


Figure S1. Enzymatic extension of M301 to generate core M2 O-mannosyl glycans. (a) NmLgtB, UDP-Gal, Mg^{2+}; (b) PmST1-M144D, NmCSS, Neu5Ac, CTP, Mg^{2+}; (c) Pd26ST, NmCSS, Neu5Ac, CTP, Mg^{2+}; (d) Hp3FT, GDP-Fuc, Mg^{2+}; (h) PmST1m, NmCSS, Neu5Ac, CTP, Mg^{2+}.

III. Glycan Microarray preparation, assay and data

A) Method for removing Fmoc

O-mannosyl glycans (50 ug) were dissolved in 200 uL H_2O, and 30 uL triethylamine was added to remove the Fmoc group at room temperature for 4 h. The reactions were then lyophilized and hexane extraction was used to remove free Fmoc.

B) Method for microarray preparation

O-mannosyl glycans microarray was prepared as previously reported,[13] briefly, 8 subarrays was printed on N-hydroxysuccinimide (NHS)-derivatized slides by Z Biotech (Aurora, CO, USA). Within each subarray, each glycan was printed in six replicates with print buffer, and print buffer was also printed as a negative control. In addition, Biotin-PEG2-Amine (0.01 mg/mL) (positive control 1), Rabbit IgG (0.1 mg/mL) (positive control 2) were printed in six replicates with print buffer to serve as a positive control. A marker containing human IgG conjugate with Cy3 (0.01 mg/mL) and human IgG conjugate with Alexa 647 (0.01 mg/mL) was also printed in the replicates of six.

C) Method for microarray assay

Materials: All biotinylated lectins were purchased from EYLabs (San Mateo, CA). Cy5-streptavidin, Cy3- streptavidin, goat anti-rabbit IgG-Alexa Fluor 647 conjugate, goat anti-mouse IgG-Alexa Fluor 647 conjugate were purchased from ThermoFisher Scientific (Waltham, MA). Mouse anti-human CD15s (sialyl-lewis X) antibody was purchased from BD Biosciences (Franklin Lakers, NJ). Mab(IIH6) was a kind gift from Dr. Kevin Campbell (HHMI, University of Iowa).[15]

Procedures: Microarray slides were rehydrated for 30 min in blocking buffer (50 mM ethanolamine in 50 mM sodium borate, pH 9.2) and wash with H_2O before assay. All assay were performed as previously reported.[13] Plant lectins, including Concanavalin A from *Canavalia ensiformis* (Con A, 10 μg/mL), *Aleuria aurantia* lectin (AAL, 10 μg/mL), *Ricinus Communis* lectin I (RCA-I, 10 μg/mL), *Erythrina cristagalli* Lectin (ECA, 10 μg/mL) were applied with appropriate concentrations, and detected by Cy3-streptavidin or Cy5-streptavidin (1 μg/mL). Anti-CD15s antibody (10 μg/mL), and IIH6 antibody (1:200 dilution) antibody were also tested. The primary antibodies were bound by goat anti-mouse IgG-Alexa Fluor 647 conjugate (5 μg/mL). Twelve subarrays were assayed with rabbit serums from Dr. Ulrika Westerlind. Goat anti-rabbit IgG-Alexa Fluor 647 conjugate (5 μg/mL) was used to bound with the primary antibodies. After binding, the slides were scanned with a microarray scanner (GenePix 4000B).

D) Results and analysis

Firstly, binding specificities *Aleuria aurantia* lectin (AAL, specific to α-linked Fuc) was profiled. As shown in Figure S2, AAL bound to all O-mannosyl glycans with an α1,3-linked Fuc residue (**Fig 6A**). Meanwhile, weaker bindings were observed towards **M3X4** and **M3X5** (contain one Fuc on the β1,6-branch) than **M2X4** and **M2X5** (contain one Fuc on the β1,2-branch) with the exception of **M34**5 and **M24**5 (contain Fuc residues on both branches), indicating AAL possesses a branch preference towards the β1,2-branch. In addition, AAL showed higher bindings to glycans that contain the Le^X epitope (e.g., **M104**, **M040**, **M204**,

M214, **M304**, **M314**) than those contain the sLe^X epitope (e.g., **M105**, **M050**, **M205**, **M215**, **M305**, **M315**), suggesting a same-chain glycosylation (α2,3-sialylation) influence. Besides ECA and RCA-I's branch preferences, When comparing binding specificities of RCA-I towards **M212**, **M213**, **M214** and **M215**, a series of glycans with the same β1,6-branch (LN disaccharide) but differs in the β1,2-branch, an apparent preference of **M213**>**M214**>**M215**>**M212** was observed, suggesting a side-chain influence on RCA-I binding. Such influences were also found for ECA (**Fig 6A**) and AAL (**Fig S2**, varied bindings to **M104**, **M204**, **M214**, **M224**, **M234**), but in different orders.

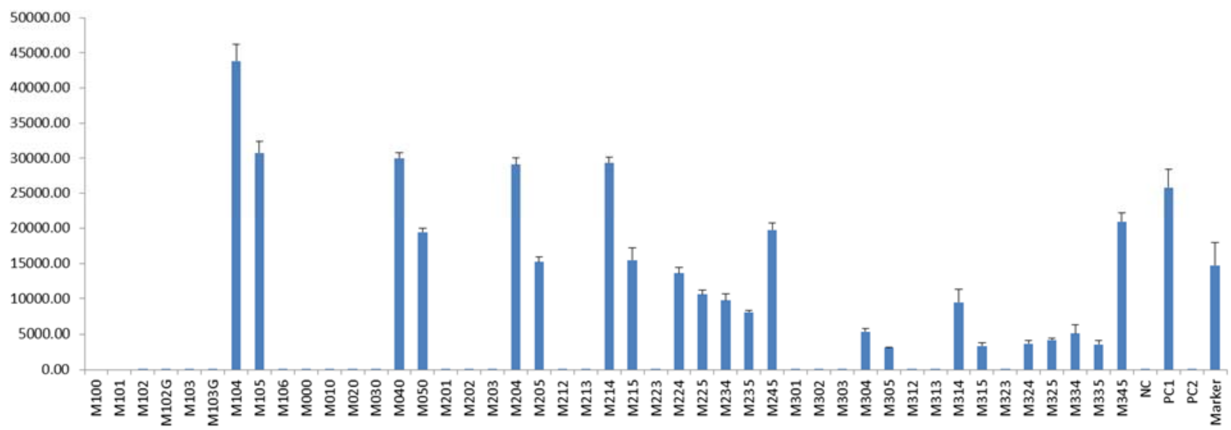


Figure S2. Microarray analysis and binding profile of O-mannosyl glycans towards Aleuria aurantia lectin (AAL, 10 μg/mL), Cy5- streptavidin was used to detect binding.

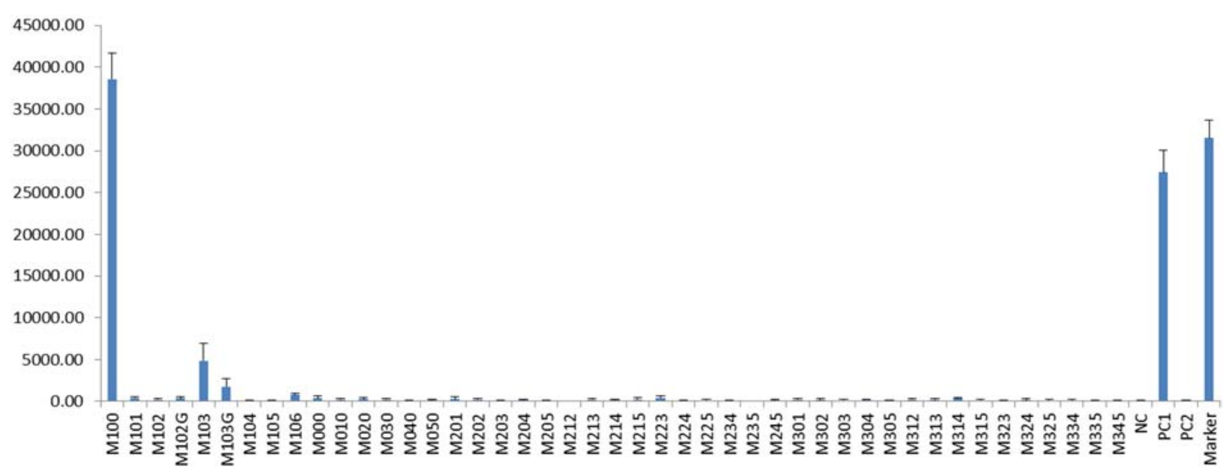


Figure S3. Microarray analysis and binding profile of O-mannosyl glycans towards Concanavalin A from Canavalia ensiformis (Con A, 10 μg/mL), Cy5- streptavidin was used to detect binding.

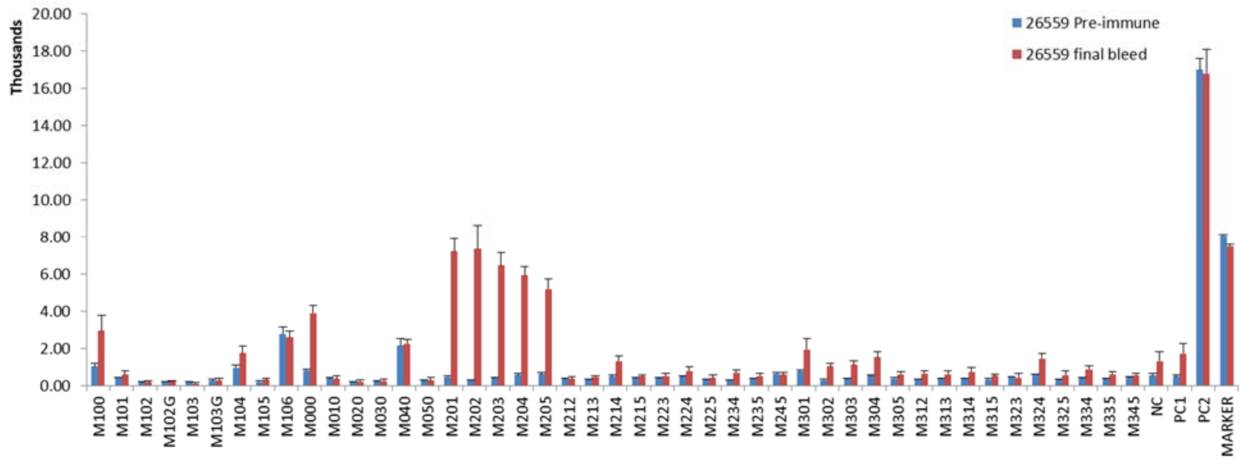


Figure S4. Microarray analysis and binding profile of O-mannosyl glycans towards rabbit 26559 anti-core M2 trisaccharide containing glycoconjugate antiserum (1:25), goat anti-rabbit IgG-Alexa Fluor 647 conjugate was used to detect binding.

As expected, a α-DG antibody (IIH6) failed to bind any core M1 and branched core M2 structures (**Fig S5**), as it is specific for core M3 glycans.[15]

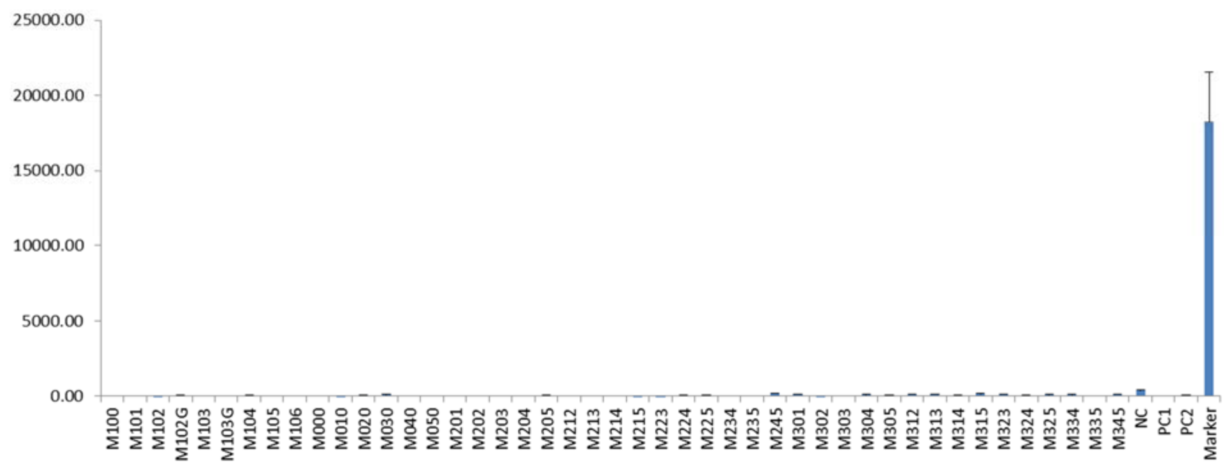
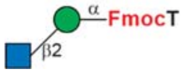
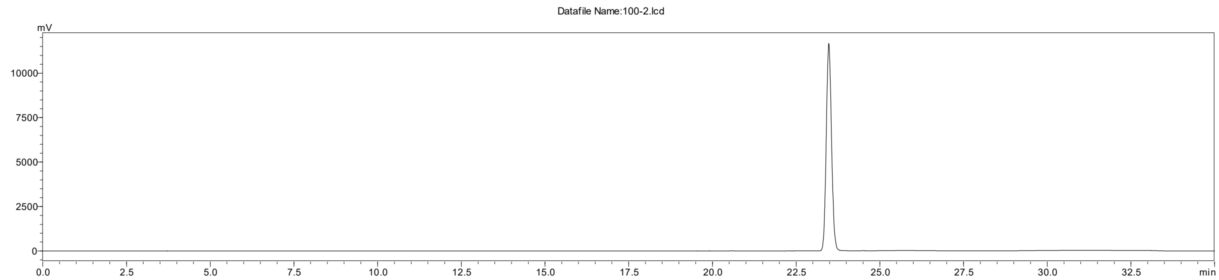


Figure S5. Microarray analysis and binding profile of O-mannosyl glycans towards mouse IIH6 antibody (1:200 dilution), goat anti-mouse IgG-Alexa Fluor 647 conjugate was used to detect binding.

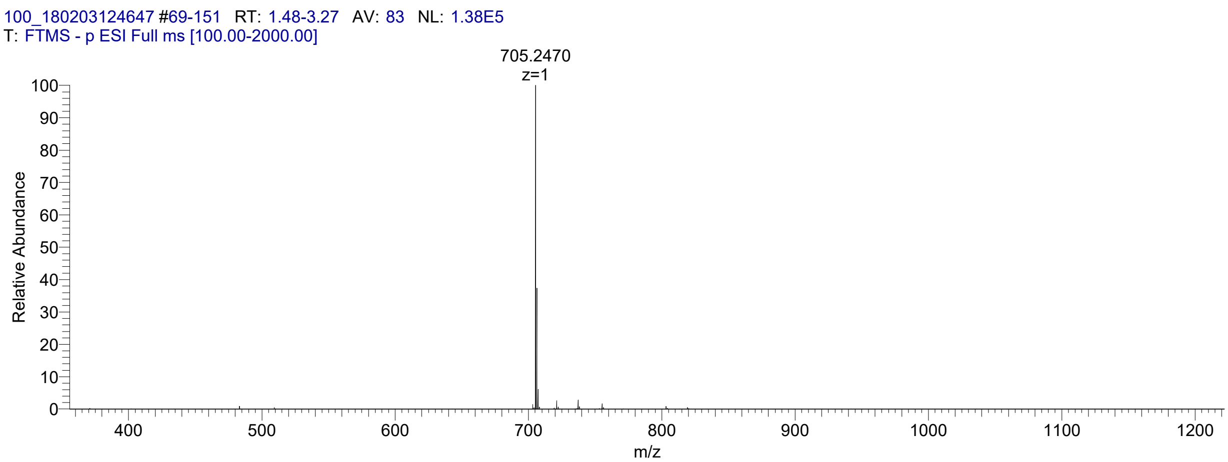
IV. HPLC, MS and NMR analysis of purified O-mannosyl glycans

M100 

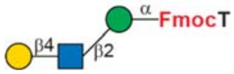
HPLC-FL, T_R = 23.47 min



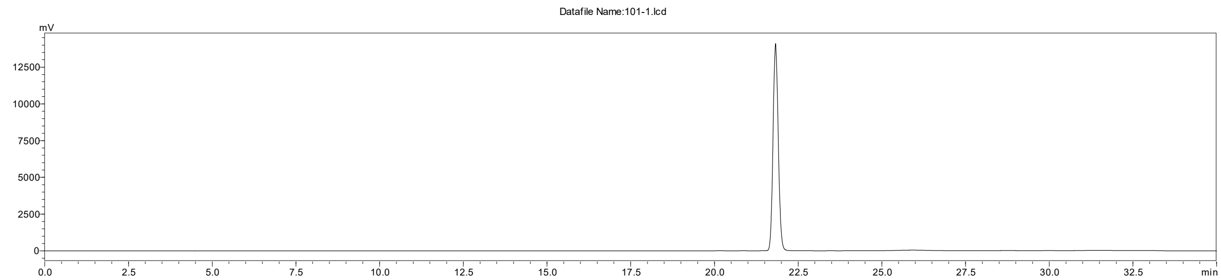
ESI-MS, calculated: 706.2585; found $[M-H]^-$ 705.2470



^{1}H NMR (400 MHz, D_2O) δ 7.74 – 7.60 (m, 2H), 7.60 – 7.40 (m, 2H), 7.40 – 7.18 (m, 4H), 4.60 (dd, *J* = 10.7, 4.9 Hz, 1H), 4.42 – 4.27 (m, 2H), 4.23 (d, *J* = 6.3 Hz, 1H), 4.12 – 3.96 (m, 1H), 3.83 – 3.71 (m, 4H), 3.71 – 3.42 (m, 7H), 3.42 – 3.30 (m, 3H), 3.27 (s, 1H), 1.96 (s, 3H), 0.94 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 174.87, 158.05, 143.91, 143.49, 140.96, 140.91, 127.96, 127.41, 124.90, 124.78, 120.09, 100.01, 98.04, 77.19, 75.59, 73.28, 73.05, 69.84, 69.26, 67.22, 65.92, 61.60, 60.52, 55.26, 47.10, 22.35, 18.33; MALDI-MS: [$C_{33}H_{42}N_2O_{15}Na$ calculated for 729.2483, found 729.2478 M+Na]$^+$

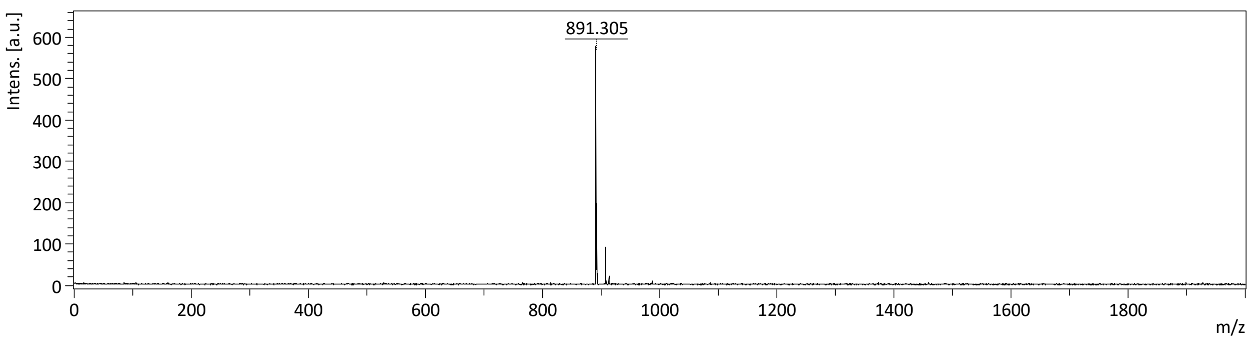
M101 

HPLC-FL, T_R = 21.82 min

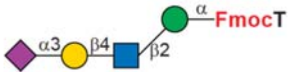


ESI-MS, calculated: 868.3113; found $[M-H]^-$ 867.2972

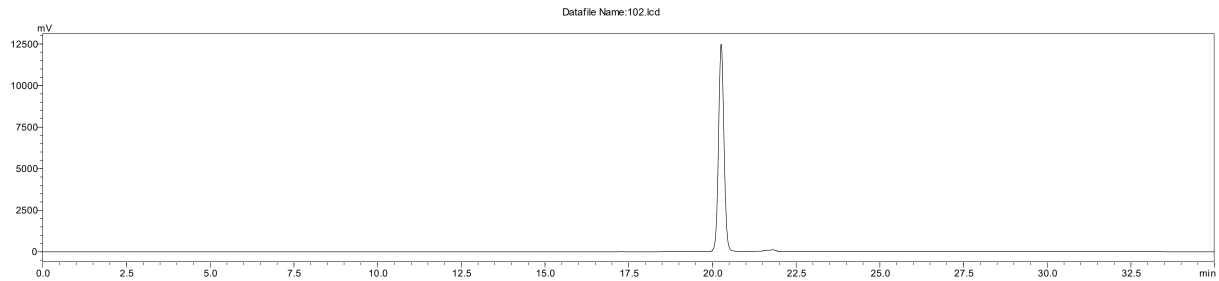
MALDI-MS, found $[M+Na]^+$ 891.305, $[M+K]^+$ 907.279



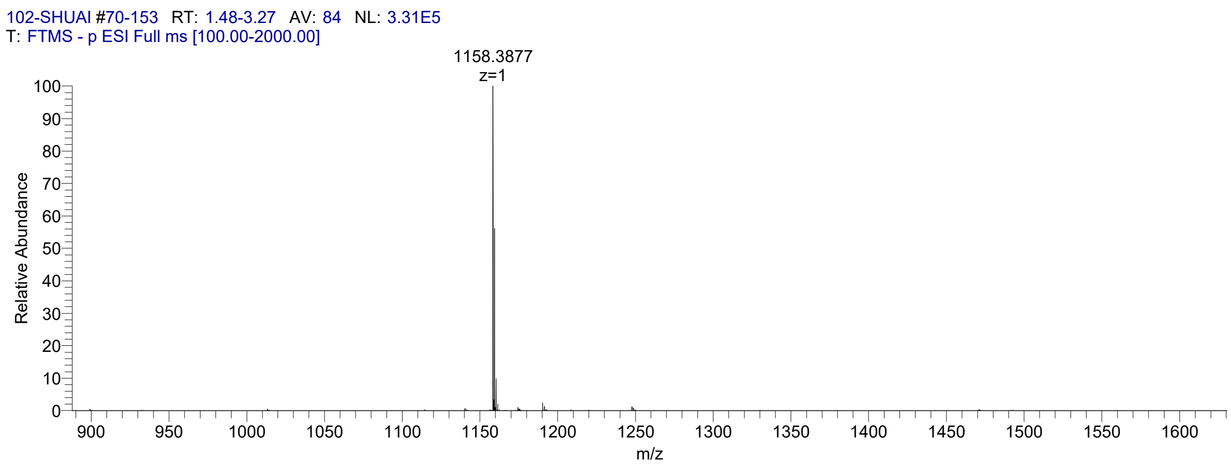
^{1}H NMR (600 MHz, D_2O) δ 7.71 – 7.55 (m, 2H), 7.71 – 7.55 (m, 2H), 7.51 – 7.29 (m, 4H), 4.77 (d, *J* = 4.9 Hz, 1H), 4.75 (d, *J* = 4.8 Hz, 1H), 4.65 (m, 1H), 4.63 (m, 1H), 4.57 (dd, *J* = 11.0, 4.6 Hz, 1H), 4.38 (d, *J* = 7.8 Hz, 1H), 4.35 (d, *J* = 5.5 Hz, 1H), 4.29 – 4.20 (m, 2H), 3.95 (d, *J* = 2.1 Hz, 1H), 3.86 (m, 2H), 3.81 – 3.57 (m, 10H), 3.57 – 3.42 (m, 4H), 3.38 (t, *J* = 9.7 Hz, 1H), 1.96 (s, 3H), 0.96 (d, *J* = 6.4 Hz, 3H).

M102 

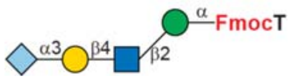
HPLC-FL, T_R = 20.26 min



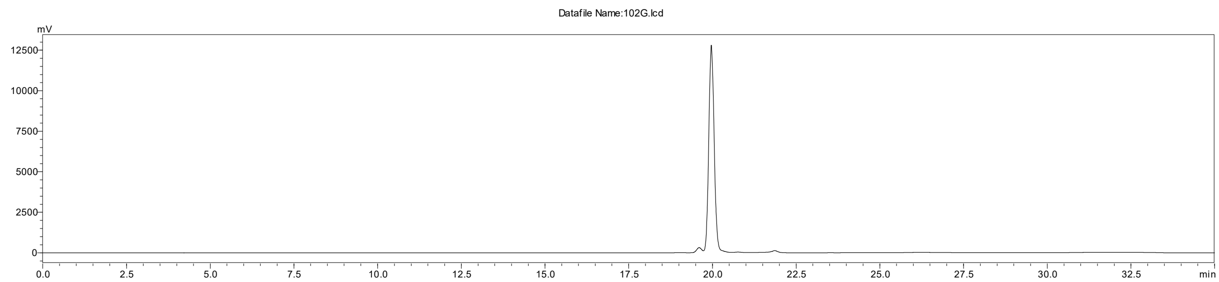
ESI-MS, calculated: 1159.4068; found $[M-H]^-$ 1158.3877



^{1}H NMR (600 MHz, D_2O) δ 7.90 – 7.73 (m, 2H), 7.71 – 7.50 (m, 2H), 7.45 – 7.25 (m, 4H), 4.65 (s, 1H), 4.59 (s, 1H), 4.54 (dd, *J* = 11.0, 4.6 Hz, 1H), 4.41 (d, *J* = 7.8 Hz, 1H), 4.30 (d, *J* = 7.1 Hz, 1H), 4.23 (s, 1H), 4.17 (d, *J* = 5.6 Hz, 1H), 4.00 (d, *J* = 12.5 Hz, 1H), 3.89 – 3.66 (m, 8H), 3.65 – 3.36 (m, 16H), 3.32 (t, *J* = 9.6 Hz, 1H), 2.64 (dd, *J* = 12.4, 4.4 Hz, 1H), 1.91 (s, 3H), 1.90 (s, 3H), 1.69 (t, *J* = 12.1 Hz, 1H), 0.91 (d, *J* = 6.2 Hz, 3H).

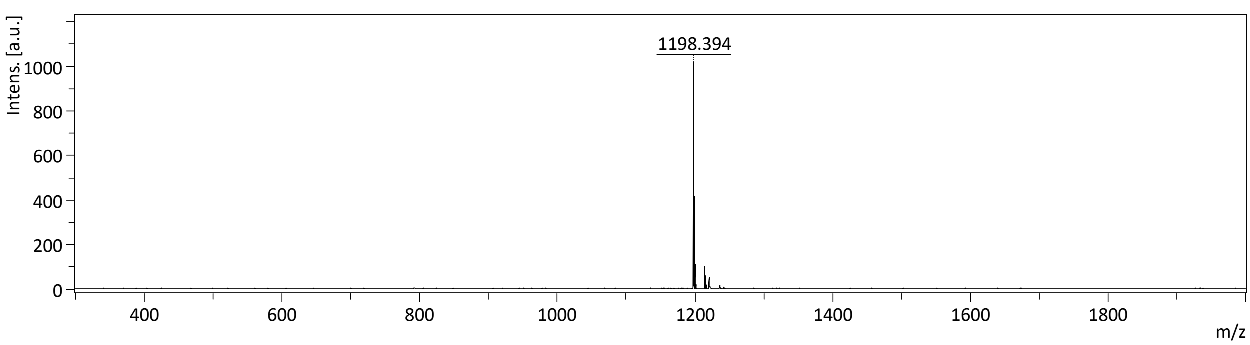
M102G 

HPLC-FL, T_R = 19.97 min

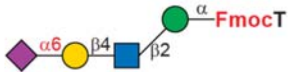


ESI-MS, calculated: 1175.4017; found $[M-H]^-$ 1174.3850

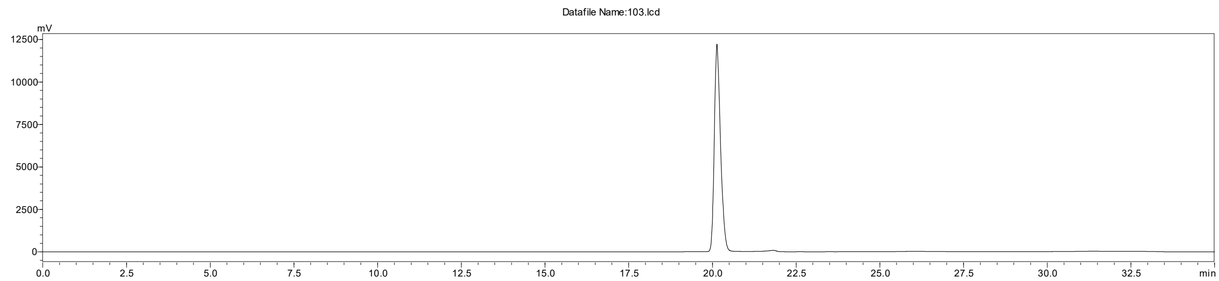
MALDI-MS, found $[M+Na]^+$ 1198.394, $[M+K]^+$ 1214.375



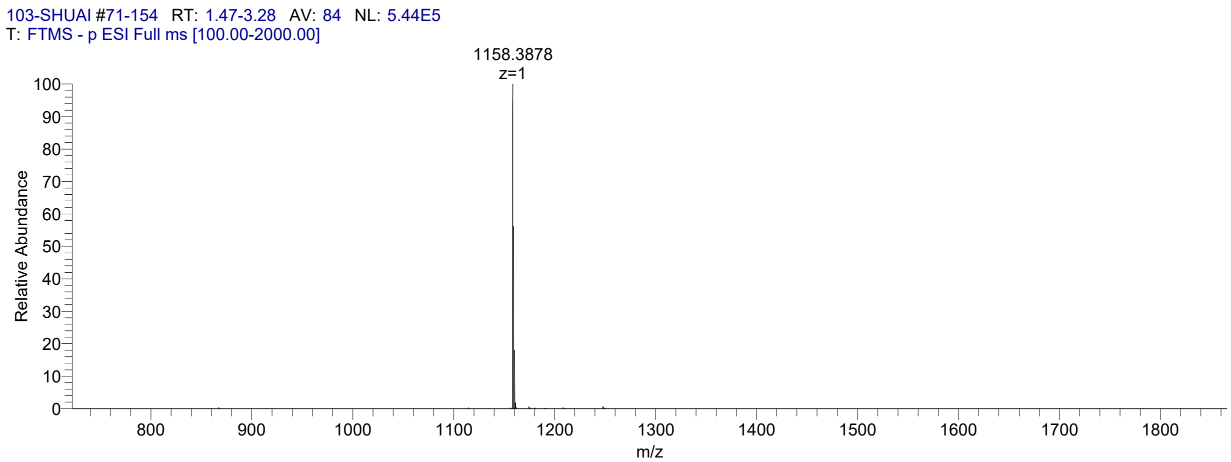
^{1}H NMR (600 MHz, D_2O) δ 7.86 – 7.73 (m, 2H), 7.67 – 7.49 (m, 2H), 7.41 – 7.25 (m, 4H), 4.64 – 4.60 (m, 1H), 4.57 – 4.50 (m, 1H), 4.40 (d, *J* = 8.0 Hz, 1H), 4.28 (d, *J* = 6.7 Hz, 1H), 4.23 (s, 2H), 4.17 (d, *J* = 6.7 Hz, 1H), 4.03 – 3.97 (m, 3H), 3.94 (s, 1H), 3.86 – 3.35 (m, 24H), 3.31 (t, *J* = 9.7 Hz, 1H), 2.64 (d, *J* = 8.4 Hz, 1H), 1.88 (s, 3H), 1.69 (t, *J* = 12.1 Hz, 1H), 0.90 (d, *J* = 6.2 Hz, 3H).

M103 

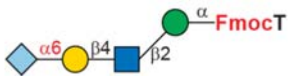
HPLC-FL, T_R = 20.14 min



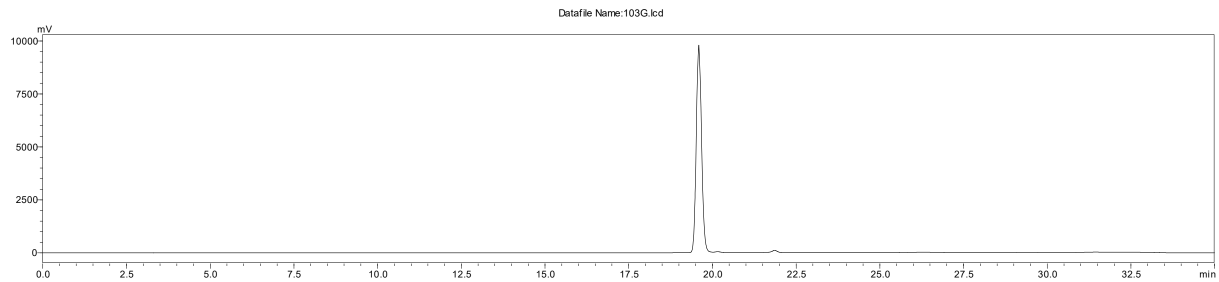
ESI-MS, calculated: 1159.4068; found $[M-H]^-$ 1158.3878



^{1}H NMR (600 MHz, D_2O) δ 7.83 – 7.72 (m, 2H), 7.63 – 7.49 (m, 2H), 7.42 – 7.25 (m, 4H), 4.69 (s,1H), 4.68 (s,1H), 4.53 (dd, *J* = 11.0, 3.4 Hz, 1H), 4.35 – 4.25 (m, 2H), 4.20 (s, 2H), 4.02 (s, 1H), 3.89 (t, *J* = 9.4 Hz, 1H), 3.86 – 3.67 (m, 7H), 3.67 – 3.37 (m, 15H), 3.34 (t, *J* = 9.4 Hz, 1H), 2.53 (d, *J* = 8.7 Hz, 1H), 1.92 (s, 3H), 1.91 (s, 3H), 1.64 (t, *J* = 12.2 Hz, 1H), 0.92 (d, *J* = 6.0 Hz, 3H).

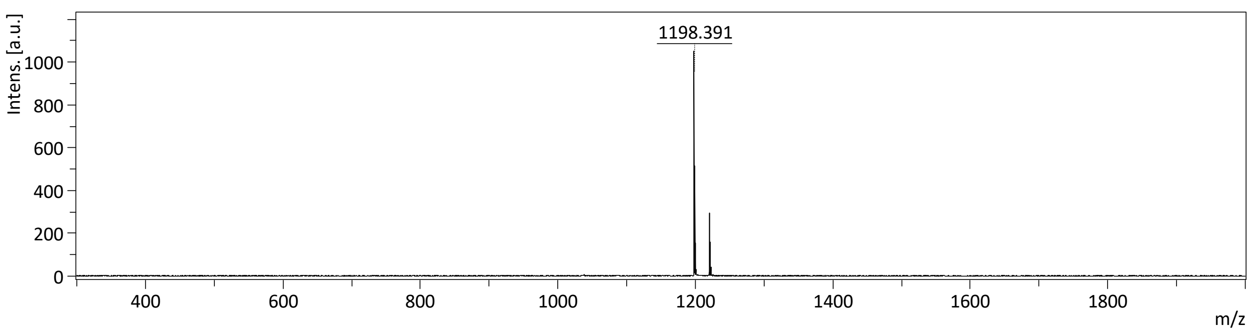
M103G 

HPLC-FL, T_R = 19.59 min

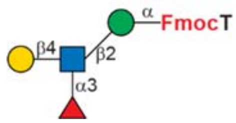


ESI-MS, calculated: 1175.4017; found $[M-H]^-$ 1174.3792

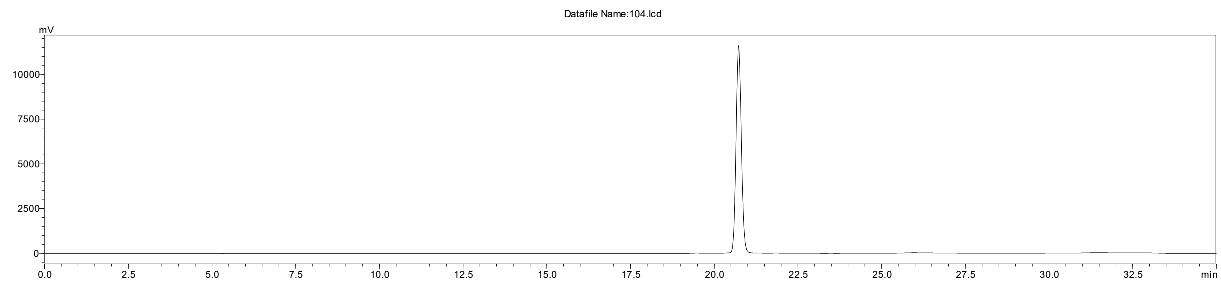
MALDI-MS, found $[M+Na]^+$ 1198.391, $[M+K]^+$ 1214.372



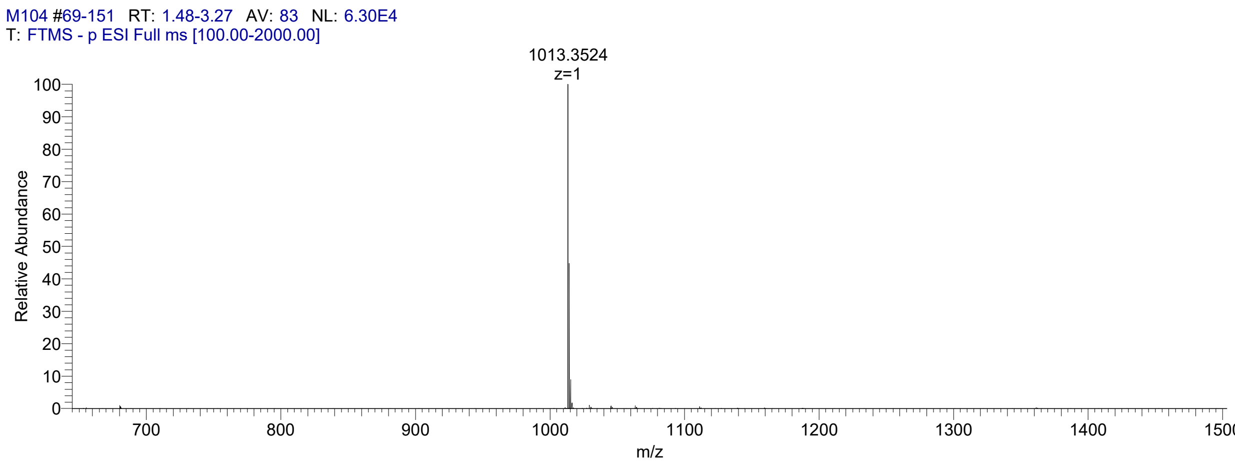
^{1}H NMR (600 MHz, D_2O) δ 7.89 – 7.74 (m, 2H), 7.71 – 7.53 (m, 2H), 7.49 – 7.29 (m, 4H), 4.74 (s, 1H), 4.67 – 4.60 (m, 1H), 4.62 – 4.54 (m, 1H), 4.40 – 4.30 (m, 2H), 4.24 (d, *J* = 5.1 Hz, 2H), 4.08 (s, 1H), 4.04 (s, 2H), 3.95 (t, *J* = 9.5 Hz, 1H), 3.89 – 3.71 (m, 10H), 3.71 – 3.34 (m, 14H), 2.59 (dd, *J* = 12.5, 4.4 Hz, 1H), 2.03 – 1.93 (m, 3H), 1.70 (t, *J* = 12.2 Hz, 1H), 0.97 (d, *J* = 6.4 Hz, 3H).

M104 

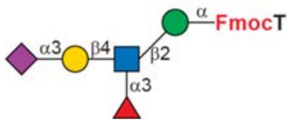
HPLC-FL, T_R = 20.73 min



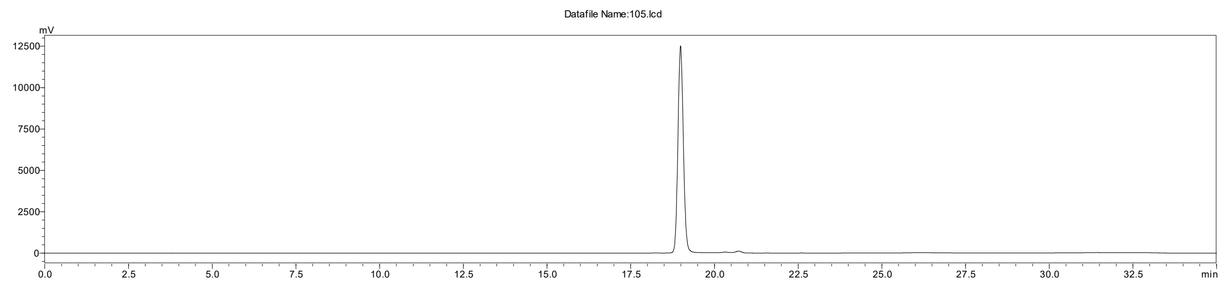
ESI-MS, calculated: 1014.3693; found $[M-H]^-$ 1013.3524



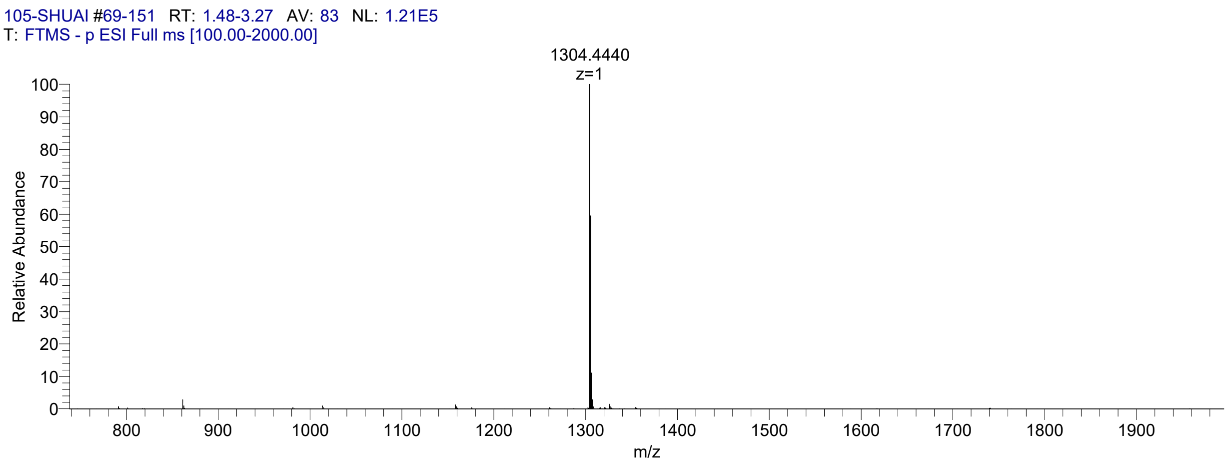
^{1}H NMR (600 MHz, D_2O) δ 7.83 – 7.60 (m, 2H), 7.60 – 7.40 (m, 2H), 7.38 – 7.18 (m, 4H), 5.04 (d, *J* = 3.8 Hz, 1H), 4.76 (d, *J* = 6.8 Hz, 1H), 4.64 – 4.54 (m, 1H), 4.43 (s, 1H), 4.36 (d, *J* = 7.6 Hz, 2H), 4.25 (s, 1H), 4.12 (s, 1H), 4.05 (s, 1H), 3.90 – 3.70 (m, 9H), 3.70 – 3.32 (m, 11H), 1.94 (s, 3H), 1.11 (d, *J* = 6.2 Hz, 3H), 0.98 (d, *J* = 5.8 Hz, 3H).

M105 

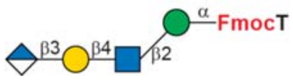
HPLC-FL, T_R = 18.99 min



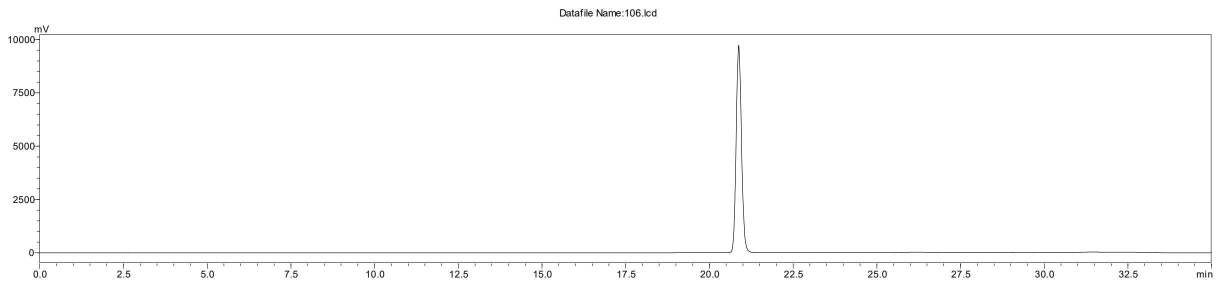
ESI-MS, calculated: 1305.4647; found $[M-H]^-$ 1304.4440



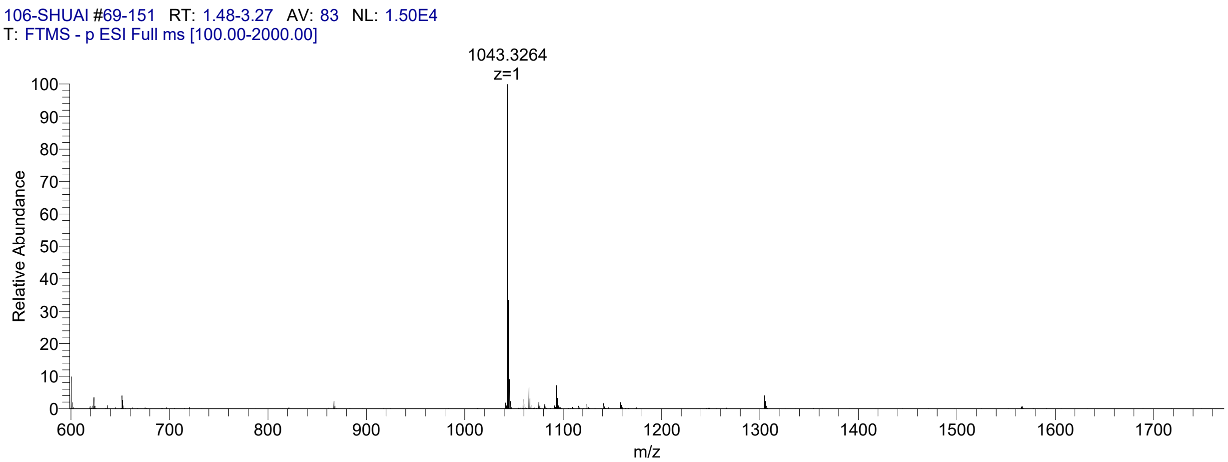
^{1}H NMR (600 MHz, D_2O) δ 7.85 – 7.73 (m, 2H), 7.68 – 7.48 (m, 2H), 7.42 – 7.23 (m, 4H), 4.96 (d, J = 3.2 Hz, 1H), 4.65 (s, 1H), 4.64 (s, 1H), 4.53 (dd, J = 10.9, 4.5 Hz, 2H), 4.36 (d, J = 7.9 Hz, 1H), 4.28 (d, J = 8.0 Hz, 1H), 4.25 – 4.14 (m, 2H), 4.00 – 3.88 (m, 2H), 3.87 – 3.60 (m, 13H), 3.60 – 3.34 (m, 14H), 3.28 (t, J = 9.6 Hz, 1H), 2.62 (d, J = 8.0 Hz, 1H), 1.89 (s, 3H), 1.88 (s, 3H), 1.67 (t, J = 12.1 Hz, 1H), 1.03 (d, J = 6.2 Hz, 3H), 0.90 (d, J = 6.1 Hz, 3H).

M106 

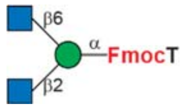
HPLC-FL, T_R = 20.87 min



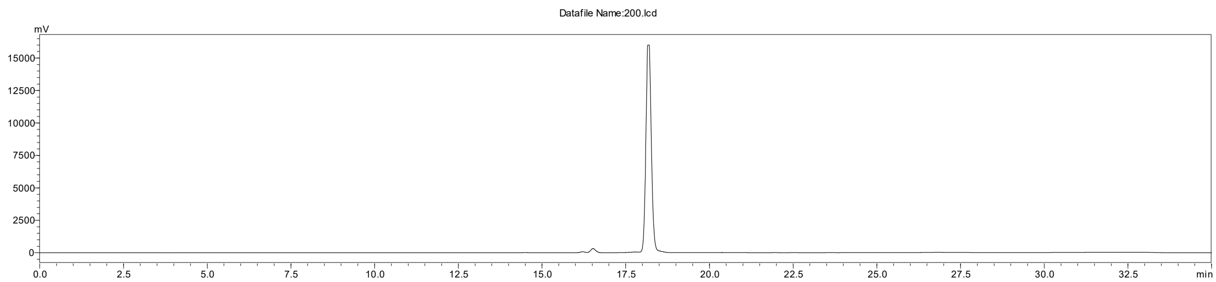
ESI-MS, calculated: 1044.3434; found $[M-H]^-$ 1043.3264



^{1}H NMR (600 MHz, D_2O) δ 7.84 – 7.64 (m, 2H), 7.64 – 7.44 (m, 2H), 7.41 – 7.19 (m, 4H), 4.65 (s, 1H), 4.58 (d, J = 7.9 Hz, 1H), 4.50 (dd, J = 11.0, 4.7 Hz, 1H), 4.36 (d, J = 7.8 Hz, 1H), 4.32 – 4.23 (m, 1H), 4.22 – 4.12 (m, 2H), 4.02 (d, J = 2.9 Hz, 1H), 3.92 (d, J = 2.1 Hz, 1H), 3.83 – 3.51 (m, 15H), 3.51 – 3.36 (m, 5H), 3.36 – 3.24 (m, 2H), 1.88 (s, 3H), 0.90 (d, J = 6.3 Hz, 3H).

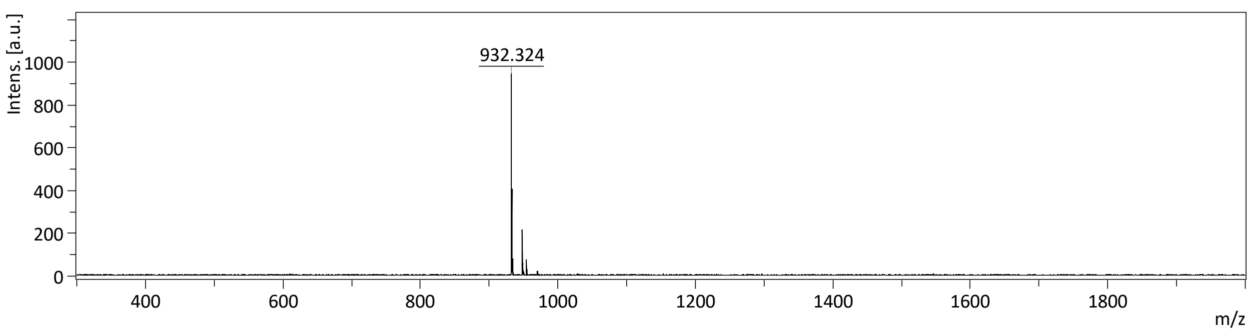
M000 

HPLC-FL, T_R = 18.20 min

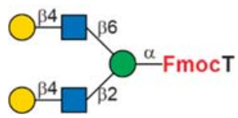


ESI-MS, calculated: 909.3379; found $[M-H]^-$ 908.3226

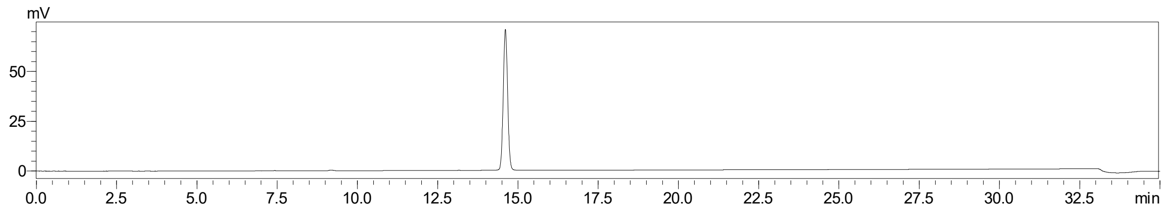
MALDI-MS, found $[M+Na]^+$ 932.324, $[M+K]^+$ 948.318



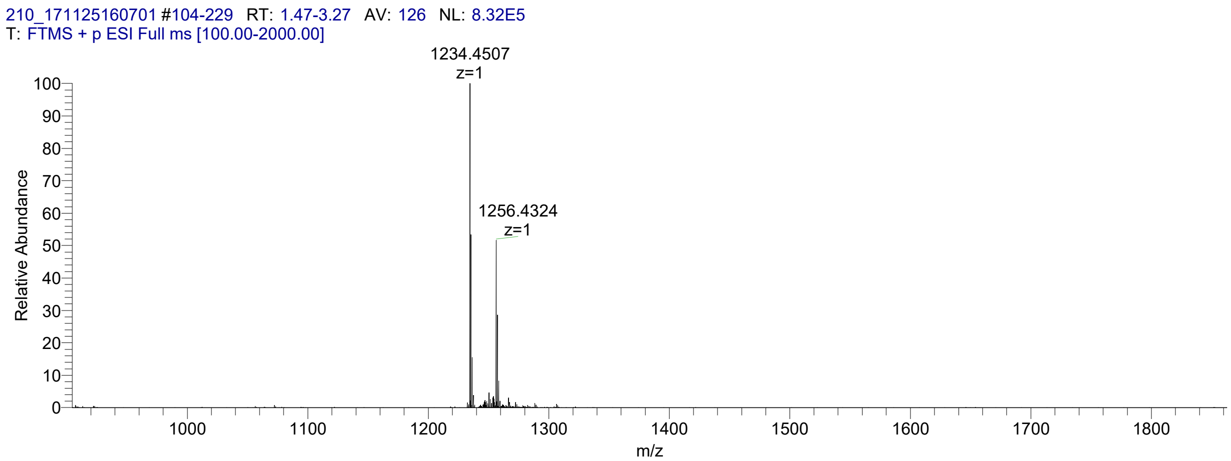
^{1}H NMR (600 MHz, D_2O) δ 7.94 – 7.79 (m, 2H), 7.71 – 7.54 (m, 2H), 7.50 – 7.29 (m, 4H), 4.76 (dd, *J* = 10.8, 4.9 Hz, 1H), 4.67 (s, 1H), 4.56 (dd, *J* = 11.0, 4.7 Hz, 1H), 4.47 (d, *J* = 8.5 Hz, 1H), 4.34 (d, *J* = 8.3 Hz, 1H), 4.27 (s, 1H), 4.18 (d, *J* = 6.1 Hz, 1H), 4.12 (d, *J* = 10.4 Hz, 1H), 4.01 (s, 1H), 3.86 (d, *J* = 12.3 Hz, 1H), 3.80 (d, *J* = 10.9 Hz, 1H), 3.75 – 3.43 (m, 10H), 3.43 – 3.24 (m, 5H), 1.96 (s, 3H), 1.94 (s, 3H), 0.96 (d, *J* = 6.4 Hz, 3H).

M010 

HPLC-UV260nm, TR = 14.61 min

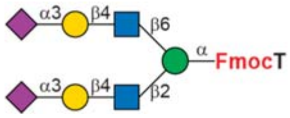


ESI-MS, calculated: 1233.4435; found $[M+H]^+$ 1234.4497, $[M+Na]^+$ 1256.4307

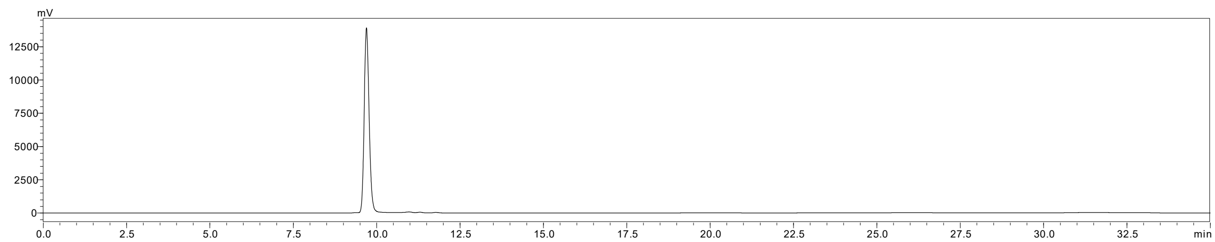


^{1}H NMR (600 MHz, D_2O) δ 7.91 – 7.72 (m, 2H), 7.72 – 7.49 (m, 2H), 7.49 – 7.27 (m, 4H), 4.74 (d, *J* = 3.3 Hz, 1H), 4.66 (d, *J* = 4.5 Hz, 1H), 4.60 – 4.51 (m, 1H), 4.51 – 4.43 (m, 2H), 4.41 – 4.35 (m, 2H), 4.34 – 4.24 (m, 2H), 4.20 (d, *J* = 5.8 Hz, 2H), 4.11 (d, *J* = 10.8 Hz, 1H), 4.03 (d, *J* = 14.8 Hz, 1H), 4.00 – 3.79 (m, 4H), 3.79 – 3.39 (m, 21H), 3.31 (t, *J* = 10.0 Hz, 1H), 1.96 (s, 3H), 1.94 (s, 3H), 0.99 (d, *J* = 4.8 Hz, 3H).

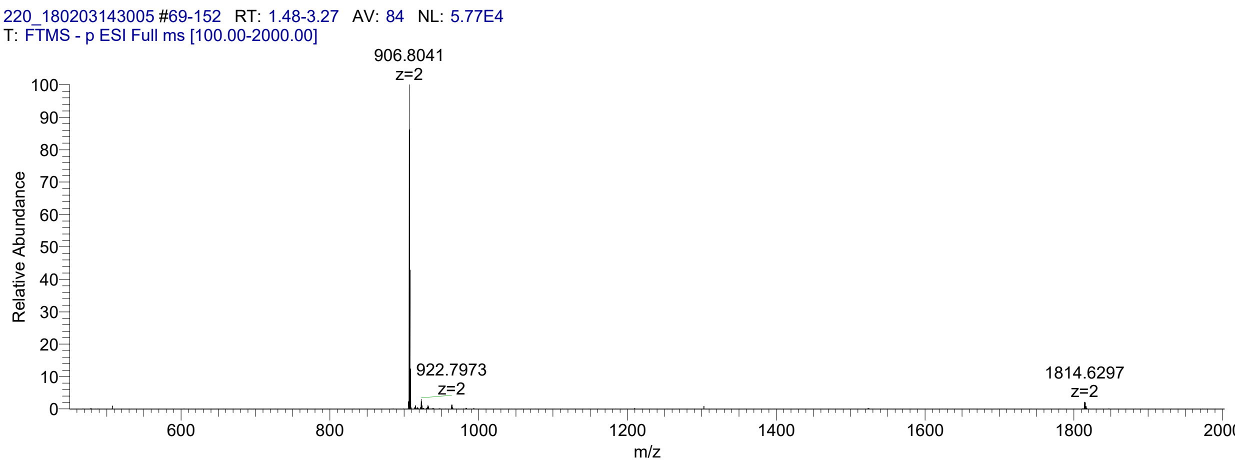
M020



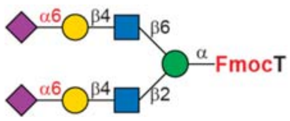
HPLC-FL, T_R = 9.69 min



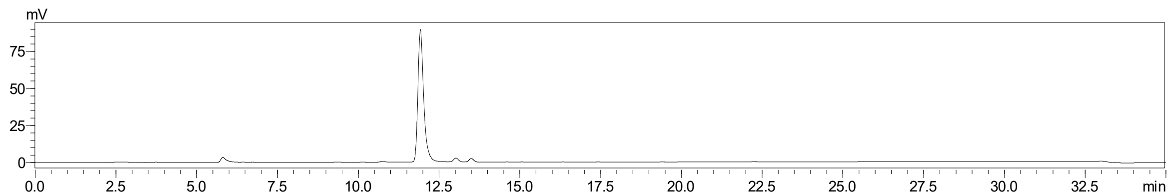
ESI-MS, calculated: 1815.6344; found $[M-H]^-$ 1814.6297, $[M-2H]^{2-}$ 906.8041



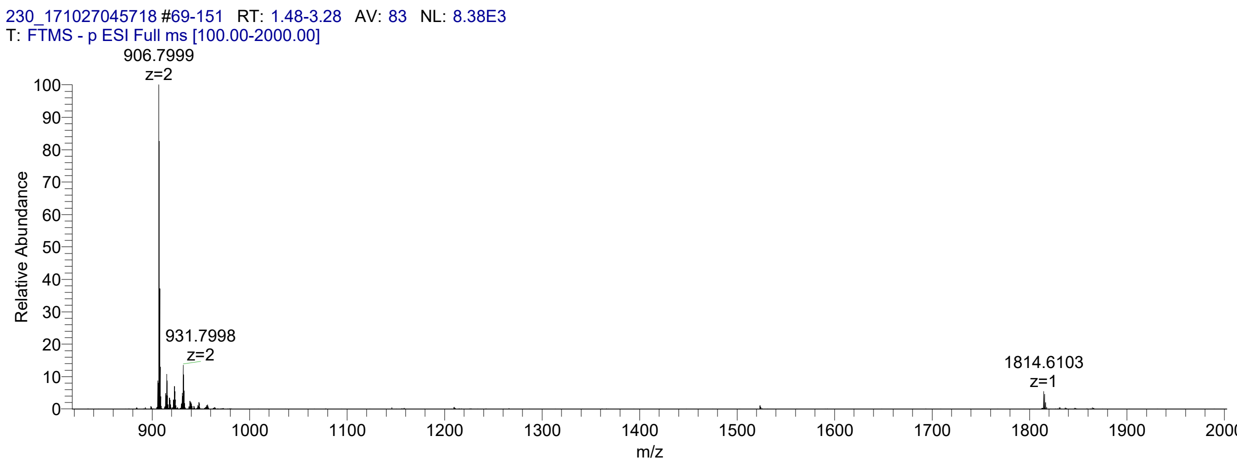
^{1}H NMR (600 MHz, D_2O) δ 7.92 – 7.78 (m, 2H), 7.72 – 7.54 (m, 2H), 7.52 – 7.29 (m, 4H), 4.76 – 4.74 (m, 1H), 4.68 – 4.66 (m, 1H), 4.59 – 4.50 (m, 2H), 4.52 – 4.44 (m, 2H), 4.40 (d, *J* = 7.7 Hz, 1H), 4.35 (d, *J* = 7.5 Hz, 1H), 4.28 (s, 2H), 4.20 (d, *J* = 6.4 Hz, 1H), 4.17 – 4.00 (m, 4H), 3.98 – 3.39 (m, 38H), 3.30 (t, *J* = 10.3 Hz, 1H), 2.68 (dd, *J* = 12.6, 4.0 Hz, 2H), 1.96 (s, 9xH), 1.92 (s, 3H), 1.78 (t, *J* = 12.5 Hz, 2H), 0.99 (d, *J* = 6.1 Hz, 3H).

M030 

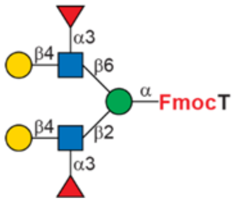
HPLC-UV$_{260nm}$, T_R = 11.90 min



ESI-MS, calculated: 1815.6344; found $[M-H]^-$ 1814.6103, $[M-2H]^{2-}$ 906.7999

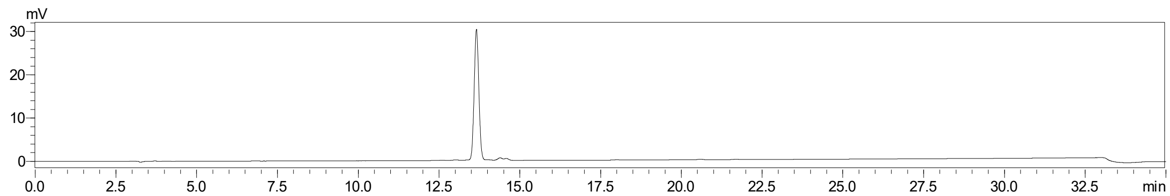


^{1}H NMR (600 MHz, D_2O) δ 7.90 – 7.78 (m, 2H), 7.71 – 7.54 (m, 2H), 7.50 – 7.29 (m, 4H), 4.62 – 4.53 (m, 1H), 4.50 (d, *J* = 8.4 Hz, 2H), 4.42 – 4.33 (m, 2H), 4.33 – 4.25 (m, 2H), 4.22 (d, *J* = 4.7 Hz, 1H), 4.17 – 4.05 (m, 2H), 4.00 – 3.42 (m, 40H), 3.31 (t, *J* = 9.8 Hz, 1H), 2.62 – 2.56 (m, 2H), 1.98 (s, 3H), 1.96 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.68 (t, *J* = 12.2 Hz, 2H), 1.00 (d, *J* = 6.4 Hz, 3H).



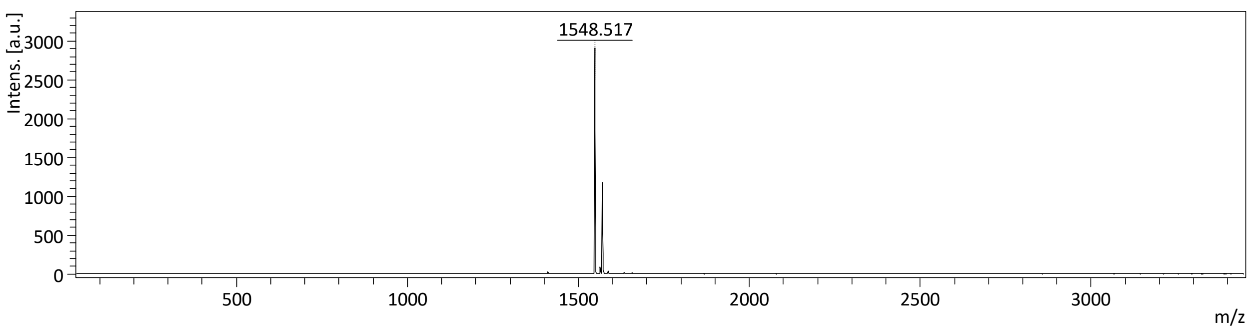
M040

HPLC-UV$_{260nm}$, T_R = 13.71 min

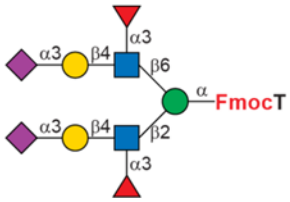


ESI-MS, calculated: 1525.5594; found $[M-H]^-$ 1524.5373

MALDI-MS, found $[M+Na]^+$ 1548.517, $[M+K]^+$ 1564.491

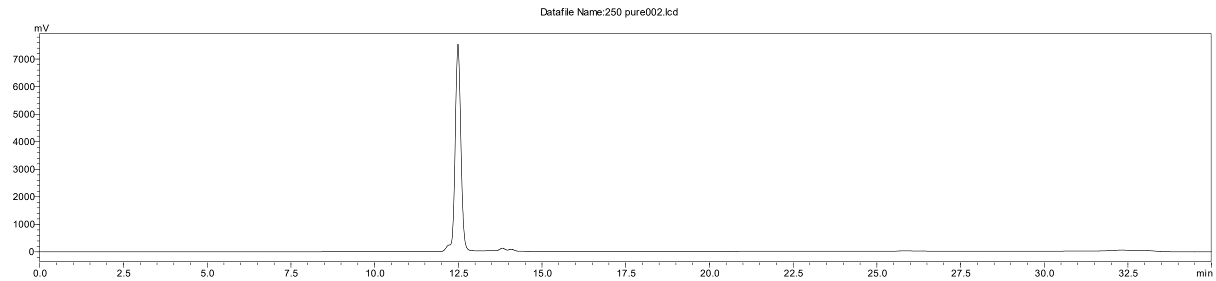


^{1}H NMR (600 MHz, D_2O) δ 7.71 – 7.54 (m, 2H), 7.54 – 7.32 (m, 2H), 7.23 (m, *J* = 31.1 Hz, 4H), 4.99 – 4.92 (m, 2H), 4.61 (d, *J* = 7.0 Hz, 1H), 4.48 (d, *J* = 9.1 Hz, 1H), 4.44 – 4.35 (m, 1H), 4.35 – 4.21 (m, 3H), 4.18 (d, *J* = 7.1 Hz, 1H), 4.14 (d, *J* = 5.5 Hz, 1H), 4.03 (d, *J* = 34.7 Hz, 3H), 3.90 – 3.16 (m, 35H), 1.88 (s, 3H), 1.85 (s, 3H), 1.08 – 0.98 (m, 6H), 0.93 (d, *J* = 5.0 Hz, 3H).

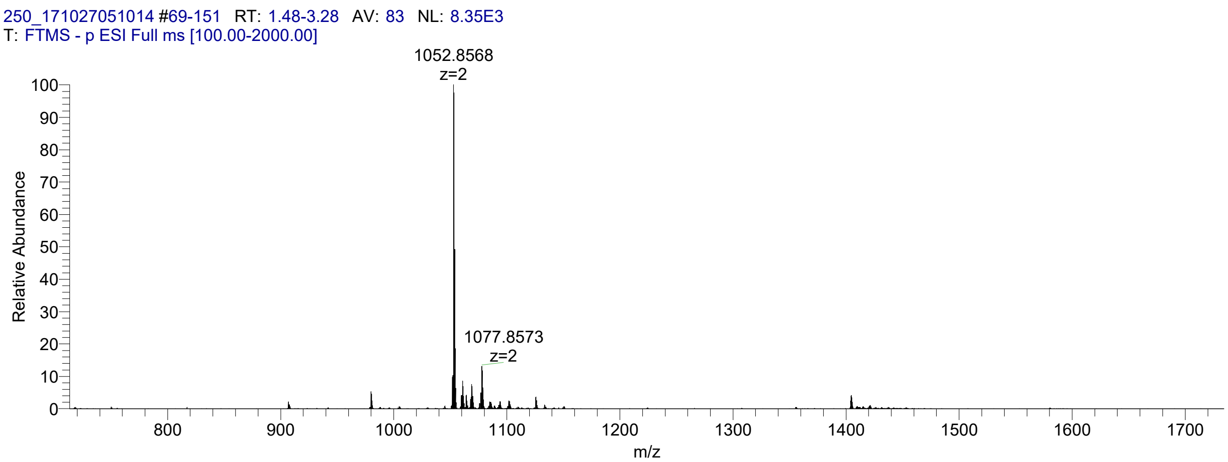


M050

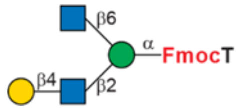
HPLC-UV_{260nm}, T_R = 11.78 min



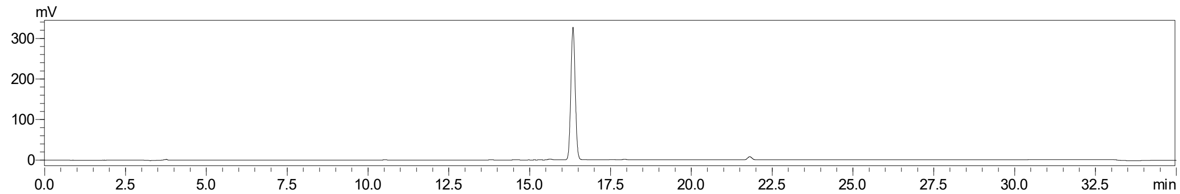
ESI-MS, calculated: 2107.7502; found $[M-2H]^{2-}$ 1052.8568



^{1}H NMR (600 MHz, D_2O) δ 7.87 – 7.72 (m, 2H), 7.67 – 7.48 (m, 2H), 7.44 – 7.26 (m, 4H), 5.00 – 4.91 (m, 2H), 4.64 – 4.63 (m, 1H), 4.62 – 4.58 (m, 2H), 4.54 – 4.46 (m, 2H), 4.46 – 4.35 (m, 3H), 4.35 – 4.27 (m, 2H), 4.23 (s, 2H), 4.14 (d, J = 5.5 Hz, 1H), 4.10 – 3.91 (m, 5H), 3.91 – 3.61 (m, 21H), 3.61 – 3.31 (m, 22H), 3.23 (t, J = 9.9 Hz, 1H), 2.66 – 2.56 (m, 2H), 1.89 (s, 6H), 1.88 (s, 3H), 1.85 (s, 3H), 1.68 (t, J = 12.0 Hz, 2H), 1.08 – 0.99 (m, 6H), 0.91 (d, J = 5.7 Hz, 3H).

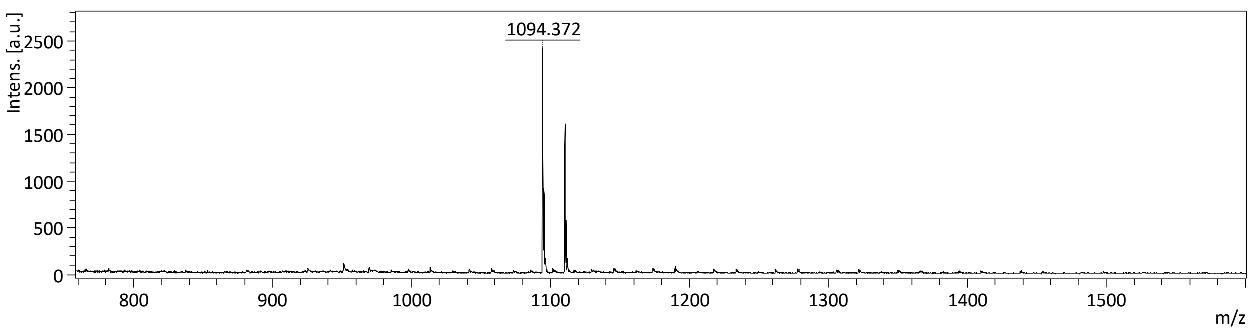
M201 

HPLC-UV_{260nm}, T_R = 16.26 min

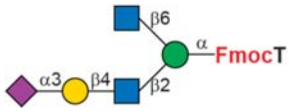


ESI-MS, calculated: 1071.3907; found $[M-H]^-$ 1070.3732

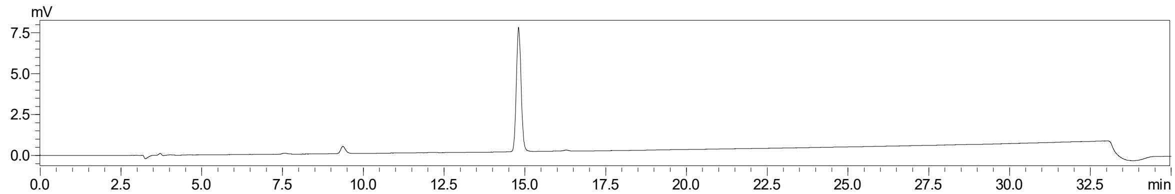
MALDI-MS, found $[M+Na]^+$ 1094.372, $[M+K]^+$ 1110.358



^{1}H NMR (400 MHz, D_2O) δ 7.68 – 7.56 (m, 2H), 7.56 – 7.36 (m, 2H), 7.36 – 7.16 (m, 5H), 4.59 – 4.51 (m, 1H), 4.47 (d, J = 8.4 Hz, 1H), 4.43 – 4.23 (m, 4H), 4.18 (d, J = 6.4 Hz, 1H), 4.09 (d, J = 10.3 Hz, 1H), 3.98 (d, J = 4.8 Hz, 1H), 3.90 – 3.80 (m, 4H), 3.80 – 3.55 (m, 16H), 3.55 – 3.42 (m, 5H), 3.41 – 3.24 (m, 5H), 1.97 (s, 3H), 1.95 (s, 4H), 0.95 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 174.59, 174.15, 158.00, 143.88, 143.45, 140.96, 140.90, 127.97, 127.43, 124.80, 120.11, 102.90, 101.16, 99.84, 98.22, 78.31, 77.18, 76.85, 75.90, 75.31, 74.56, 73.93, 72.48, 71.95, 70.94, 70.06, 69.21, 68.56, 67.59, 65.98, 61.02, 60.87, 59.88, 55.57, 54.82, 48.89, 47.09, 22.50, 22.33, 18.49

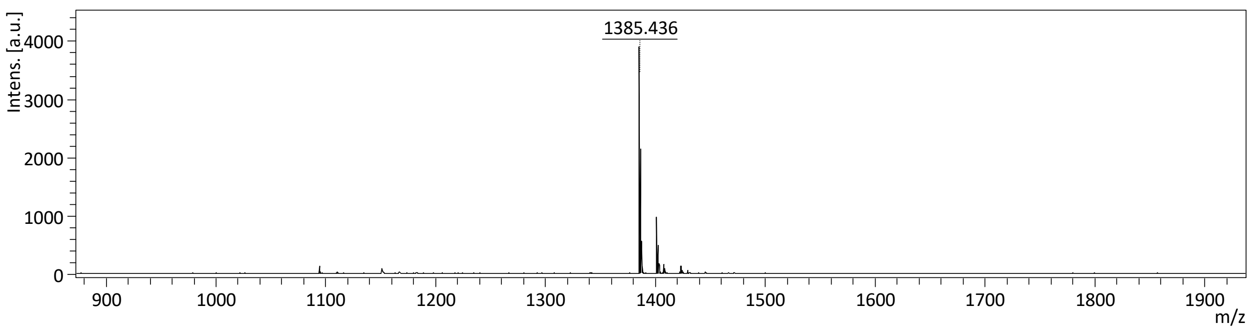
M202 

HPLC-UV_{260nm}, T_R = 14.86 min

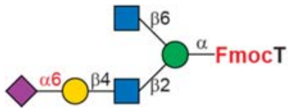


ESI-MS, calculated: 1362.4861; found $[M-H]^-$ 1361.4659, $[M-2H]^{2-}$ 680.2284

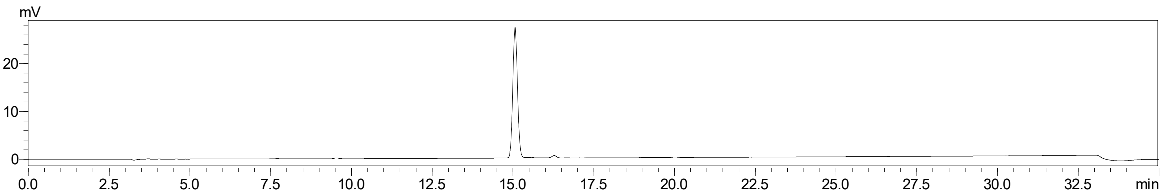
MALDI-MS; found $[M+Na]^+$ 1385.436, $[M+K]^+$ 1401.414



^{1}H NMR (600 MHz, D_2O) δ 7.63 – 7.47 (m, 2H), 7.47 – 7.26 (m, 2H), 7.26 – 7.07 (m, 4H), 4.63 (s, 1H), 4.50 – 4.34 (m, 3H), 4.31 – 4.19 (m, 2H), 4.15 (s, 1H), 4.08 – 3.97 (m, 3H), 3.93 (d, J = 11.3 Hz, 1H), 3.90 – 3.50 (m, 21H), 3.50 – 3.35 (m, 5H), 3.35 – 3.20 (m, 3H), 2.63 (d, J = 8.5 Hz, 1H), 1.90 (s, 6H), 1.87 (s, 3H), 1.75 (t, J = 12.2 Hz, 1H), 0.93 (d, J = 5.5 Hz, 3H).

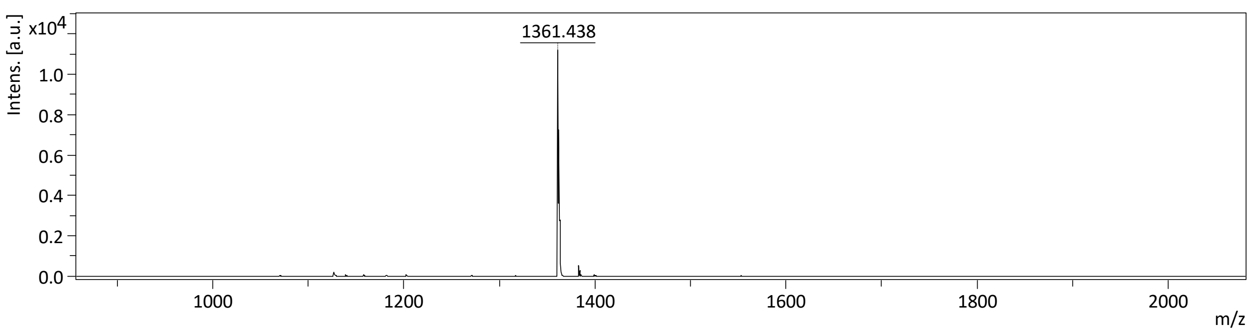
M203 

HPLC-UV_{260nm}, T_R = 15.12 min

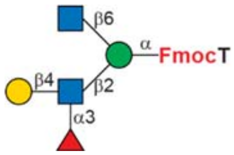


ESI-MS, calculated: 1362.4861; found $[M-H]^-$ 1361.4653, $[M-2H]^{2-}$ 680.2283

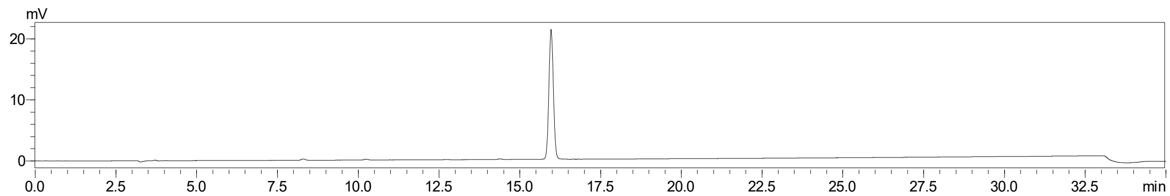
MALDI-MS; found $[M-H]^-$ 1361.438



^{1}H NMR (600 MHz, D_2O) δ 7.92 – 7.78 (m, 2H), 7.72 – 7.54 (m, 2H), 7.49 – 7.30 (m, 4H), 4.63 – 4.55 (m, 2H), 4.47 (d, *J* = 8.6 Hz, 1H), 4.37 (m, 2H), 4.29 (m, 1H), 4.20 (m, 1H), 4.14 (d, *J* = 10.0 Hz, 1H), 4.07 (s, 1H), 3.94 (m, 2H), 3.91 – 3.43 (m, 33H), 3.43 – 3.27 (m, 4H), 2.59 (dd, *J* = 12.5, 4.5 Hz, 1H), 1.98 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.68 (t, *J* = 12.2 Hz, 1H), 0.97 (d, *J* = 6.4 Hz, 3H).

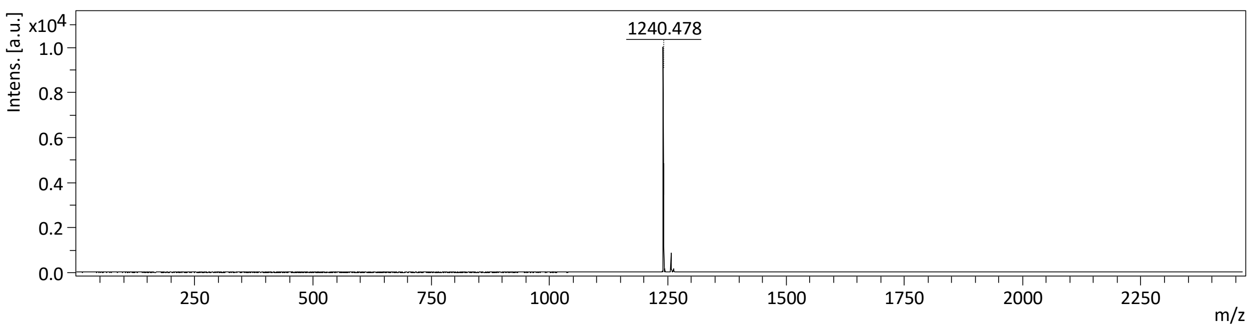
M204 

HPLC-UV$_{260nm}$, T_R = 16.01 min

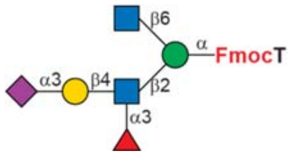


ESI-MS, calculated: 1217.4486; found $[M-H]^-$ 1216.4292

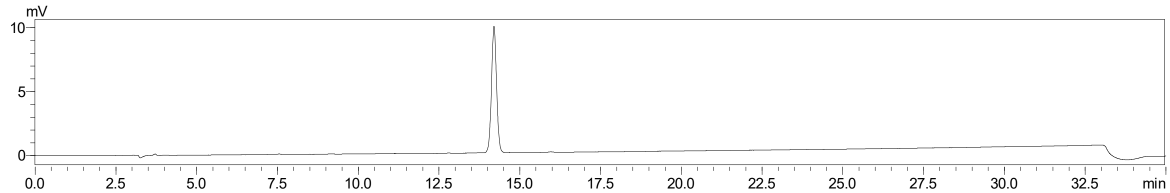
MALDI-MS; found $[M+Na]^+$ 1240.478,, $[M+K]^+$ 1256.458



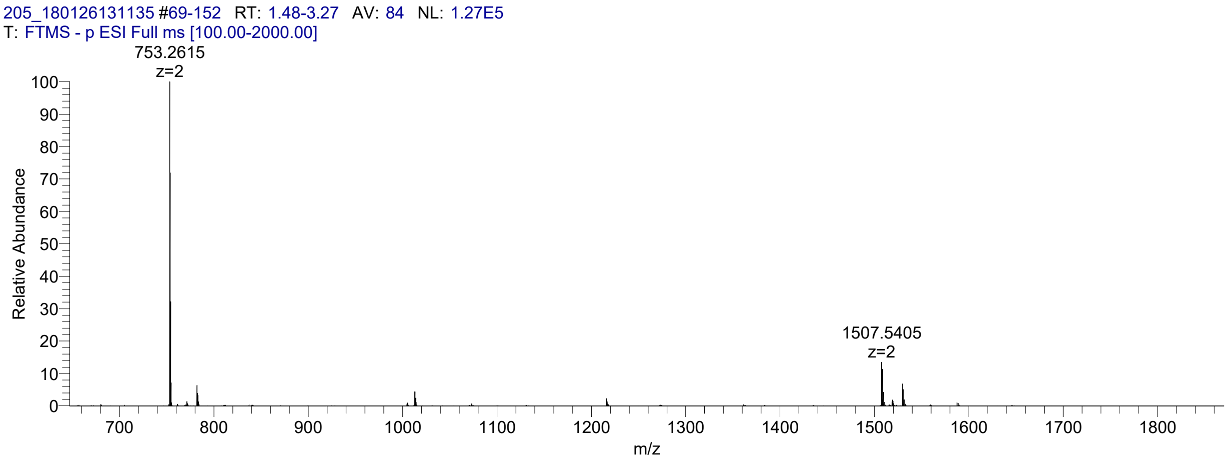
^{1}H NMR (600 MHz, D_2O) δ 7.90 – 7.68 (m, 2H), 7.67 – 7.50 (m, 2H), 7.46 – 7.26 (m, 4H), 5.03 (d, *J* = 3.5 Hz, 1H), 4.66 (s, 1H), 4.59 (d, *J* = 11.8 Hz, 1H), 4.54 – 4.42 (m, 2H), 4.36 (d, *J* = 7.8 Hz, 2H), 4.27 – 4.16 (m, 2H), 4.11 (d, *J* = 10.2 Hz, 2H), 3.99 (s, 1H), 3.94 – 3.55 (m, 18H), 3.55 – 3.26 (m, 8H), 1.96 (s, 3H), 1.94 (s, 3H), 1.10 (d, *J* = 6.4 Hz, 3H), 0.96 (d, *J* = 6.1 Hz, 3H).

M205 

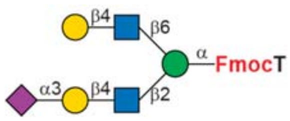
HPLC-UV260nm, T_R = 14.24 min



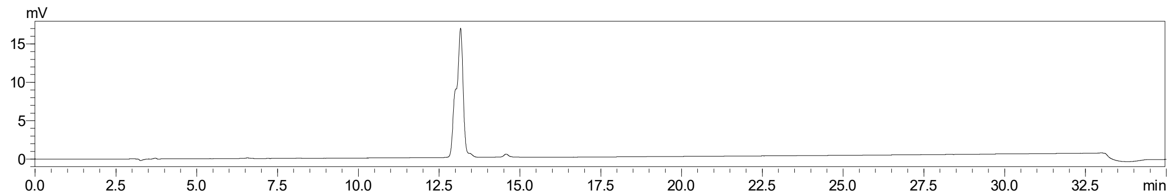
ESI-MS, calculated: 1508.5440; found $[M-H]^-$ 1507.5277



^{1}H NMR (600 MHz, D_2O) δ 7.84 – 7.72 (m, 2H), 7.66 – 7.48 (m, 2H), 7.43 – 7.26 (m, 4H), 4.98 (d, *J* = 3.8 Hz, 1H), 4.61 (s, 1H), 4.56 – 4.50 (m, 1H), 4.45 – 4.35 (m, 2H), 4.31 (d, *J* = 8.0 Hz, 1H), 4.24 – 4.17 (m, 1H), 4.14 (dd, *J* = 6.4, 2.1 Hz, 1H), 4.07 (d, *J* = 10.1 Hz, 1H), 4.02 (s, 1H), 3.98 (dd, *J* = 9.8, 2.8 Hz, 1H), 3.88 – 3.68 (m, 11H), 3.68 – 3.64 (m, 2H), 3.64 – 3.50 (m, 10H), 3.50 – 3.37 (m, 7H), 3.37 – 3.22 (m, 4H), 2.65 (dd, *J* = 12.4, 4.4 Hz, 1H), 1.91 (s, 3H), 1.90 (s, 3H), 1.88 (s, 3H), 1.71 (t, *J* = 12.2 Hz, 1H), 1.05 (d, *J* = 6.5 Hz, 3H), 0.92 (d, *J* = 6.4 Hz, 3H).

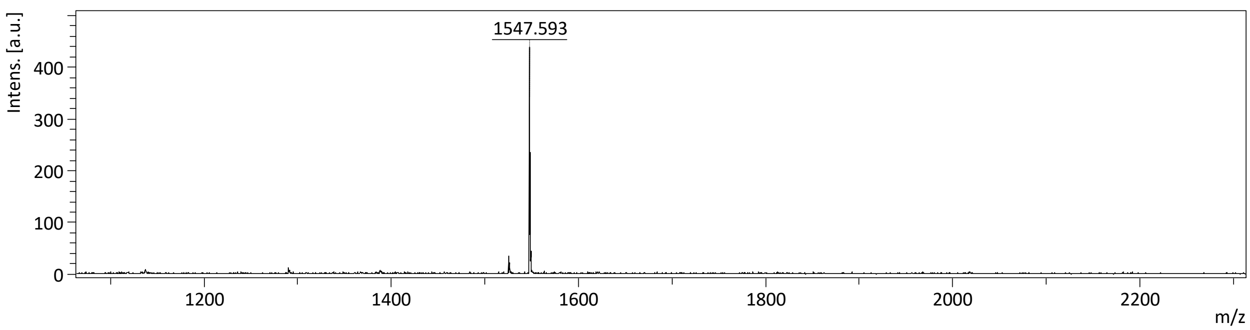
M212 

HPLC-UV$_{260nm}$, T_R = 13.15 min

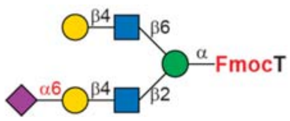


ESI-MS, calculated: 1524.5390; found $[M-H]^-$ 1523.5161, $[M-2H]^{2-}$ 761.2533

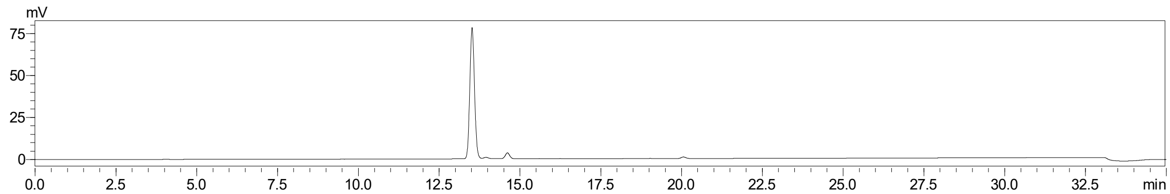
MALDI-MS; found $[M+Na]^+$ 1547.593



^{1}H NMR (600 MHz, D_2O) δ 7.93 – 7.79 (m, 2H), 7.72 – 7.55 (m, 2H), 7.50 – 7.30 (m, 4H), 4.78 (d, *J* = 5.7 Hz, 1H), 4.64 (d, *J* = 3.2 Hz, 1H), 4.55 (d, *J* = 10.7 Hz, 2H), 4.52 – 4.43 (m, 2H), 4.43 – 4.33 (m, 2H), 4.33 – 4.24 (m, 2H), 4.20 (d, *J* = 4.9 Hz, 1H), 4.16 – 3.98 (m, 3H), 3.98 – 3.38 (m, 32H), 3.30 (t, *J* = 9.8 Hz, 1H), 2.68 (dd, *J* = 12.5, 4.4 Hz, 1H), 1.96 (s, 6H), 1.93 (s, 3H), 1.75 (t, *J* = 12.2 Hz, 1H), 1.00 (d, *J* = 6.0 Hz, 3H).

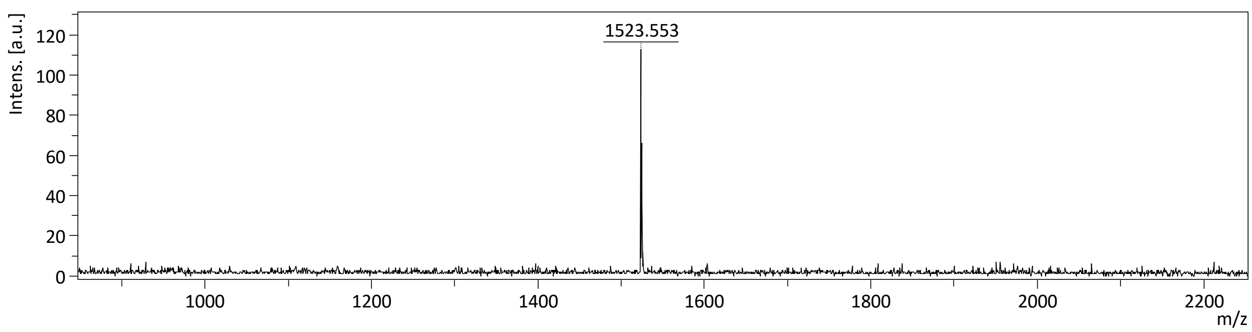
M213 

HPLC-UV$_{260nm}$, T_R = 13.52 min

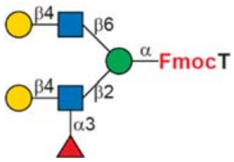


ESI-MS, calculated: 1524.5390; found $[M-H]^-$ 1523.5166, $[M-2H]^{2-}$ 761.2534

MALDI-MS; found $[M-H]^-$ 1523.553

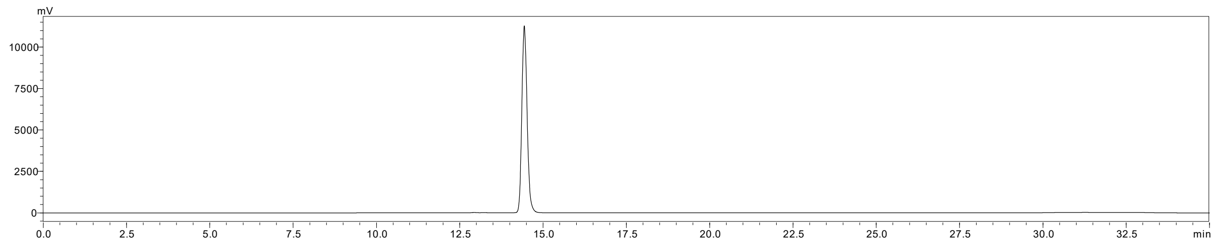


^{1}H NMR (600 MHz, D_2O) δ 7.88 – 7.75 (m, 2H), 7.68 – 7.51 (m, 2H), 7.49 – 7.29 (m, 4H), 4.78 – 4.72 (m, 1H), 4.70 – 4.65 (m, 1H), 4.61 – 4.51 (m, 1H), 4.47 (d, *J* = 10.6 Hz, 1H), 4.42 – 4.33 (m, 2H), 4.30 (d, *J* = 7.7 Hz, 1H), 4.28 – 4.18 (m, 2H), 4.12 (s, 2H), 3.99 – 3.38 (m, 35H), 3.31 (t, *J* = 9.9 Hz, 1H), 2.57 (dd, *J* = 12.7, 4.3 Hz, 1H), 1.98 (s, 3H), 1.96 (s, 3H), 1.93 (s, 3H), 1.73 (t, *J* = 12.2 Hz, 1H), 1.00 (d, *J* = 6.2 Hz, 3H).

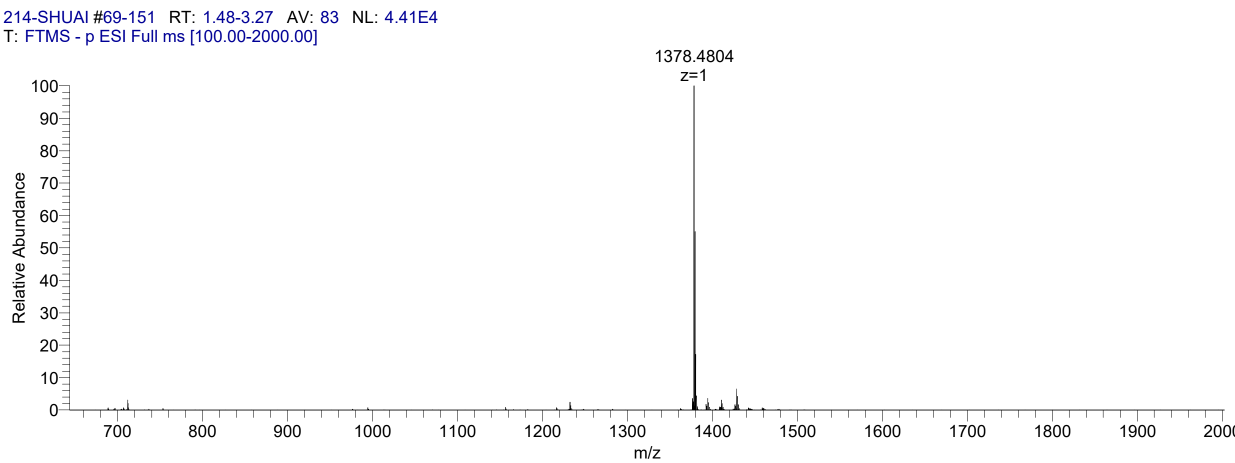


M214

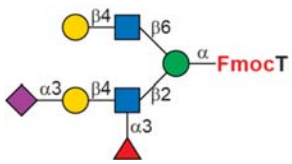
HPLC-UV$_{260nm}$, T_R = 14.41 min



ESI-MS, calculated: 1379.5014; found $[M-H]^-$ 1378.4804

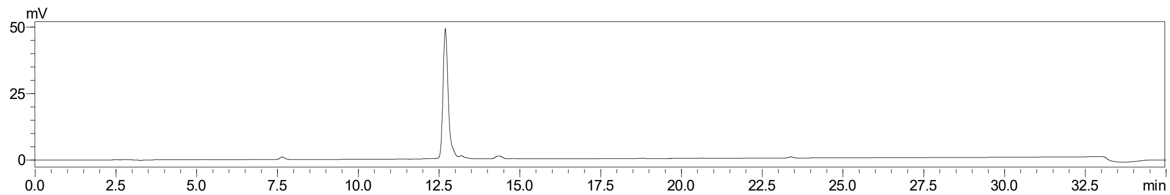


[1]H NMR (600 MHz, D_2O) δ 7.94 – 7.78 (m, 2H), 7.72 – 7.54 (m, 2H), 7.54 – 7.30 (m, 4H), 5.03 (d, *J* = 3.8 Hz, 1H), 4.77 – 4.75 (m, 1H), 4.67 (s, 1H), 4.49 (d, *J* = 7.9 Hz, 1H), 4.36 (d, *J* = 7.8 Hz, 2H), 4.31 (d, *J* = 7.7 Hz, 1H), 4.27 (s, 1H), 4.21 (d, *J* = 5.5 Hz, 1H), 4.11 (d, *J* = 11.3 Hz, 1H), 4.03 (s, 1H), 3.97 – 3.38 (m, 33H), 3.30 (t, *J* = 10.0 Hz, 1H), 1.96 (s, 3H), 1.94 (s, 3H), 1.11 (d, *J* = 6.2 Hz, 3H), 0.99 (d, *J* = 6.3 Hz, 3H).

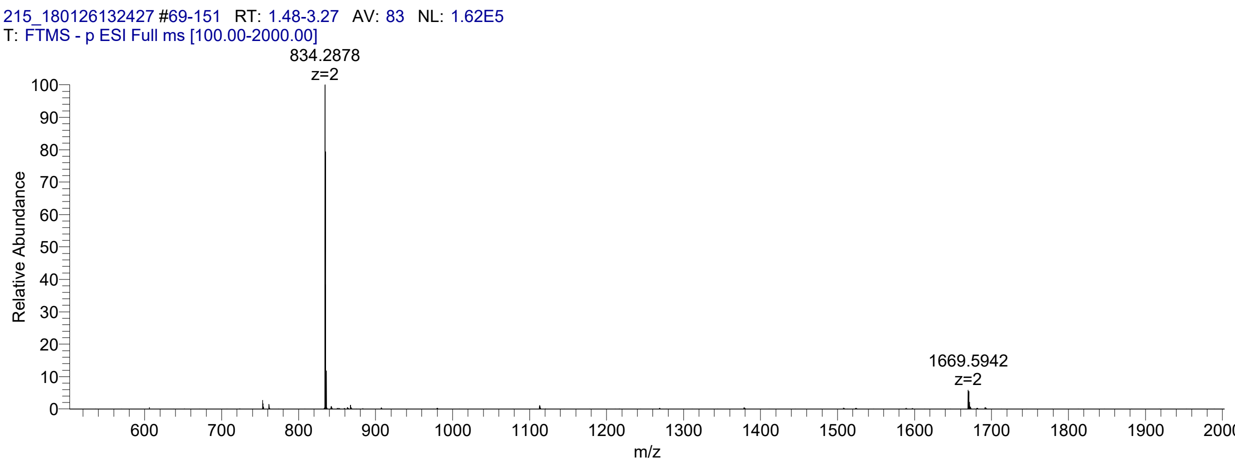


M215

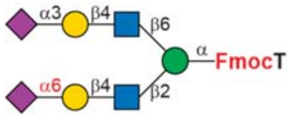
HPLC-UV$_{260nm}$, T_R = 12.69 min



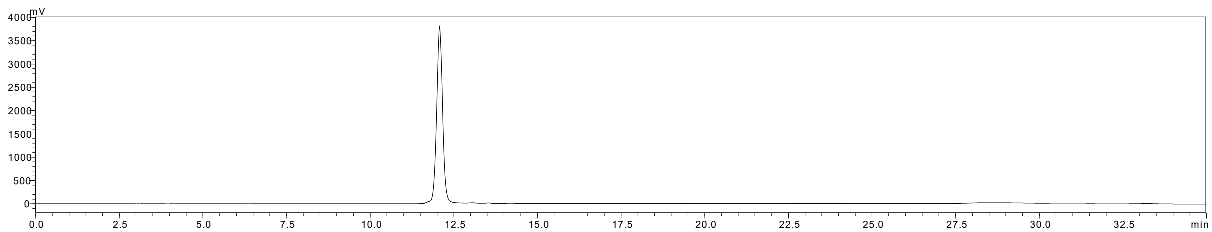
ESI-MS, calculated: 1670.5969; found $[M-H]^-$ 1669.5942, $[M-2H]^{2-}$ 834.2878



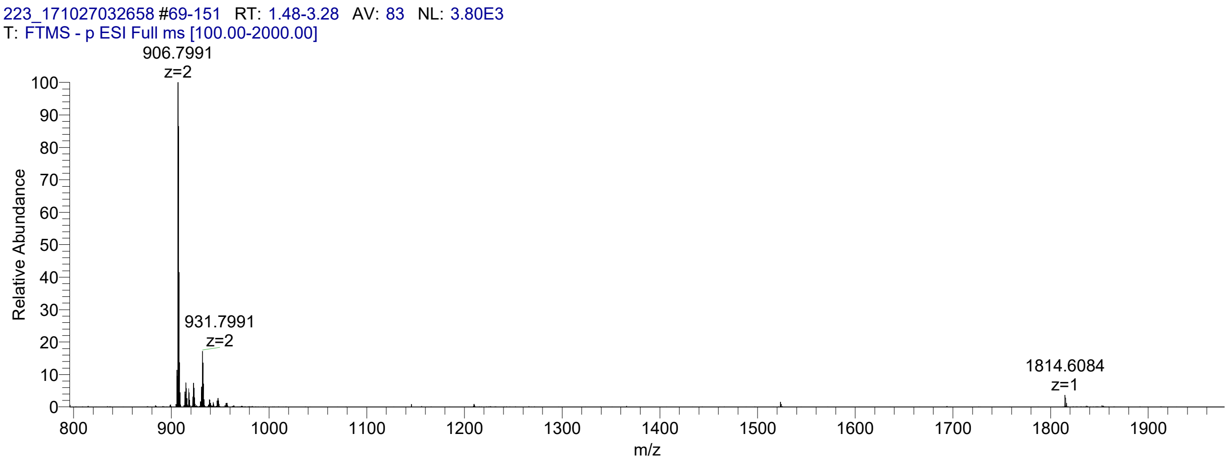
^{1}H NMR (600 MHz, D_2O) δ 7.91 – 7.80 (m, 2H), 7.71 – 7.55 (m, 2H), 7.49 – 7.31 (m, 4H), 5.03 (d, *J* = 3.9 Hz, 1H), 4.77 – 4.75 (m, 1H), 4.68 – 4.65 (m, 1H), 4.56 (dd, *J* = 10.9, 5.0 Hz, 1H), 4.51 – 4.41 (m, 2H), 4.38 (d, *J* = 8.1 Hz, 1H), 4.34 – 4.25 (m, 2H), 4.21 (dd, *J* = 6.3, 2.2 Hz, 1H), 4.15 – 4.06 (m, 2H), 4.03 (dd, *J* = 9.8, 3.0 Hz, 1H), 3.96 – 3.76 (m, 12H), 3.76 – 3.42 (m, 26H), 3.30 (t, *J* = 9.8 Hz, 1H), 2.70 (dd, *J* = 12.4, 4.6 Hz, 1H), 1.96 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.75 (t, *J* = 12.1 Hz, 1H), 1.10 (d, *J* = 6.6 Hz, 3H), 1.00 (d, *J* = 6.3 Hz, 3H).

M223 

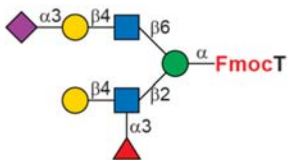
HPLC-FL, T_R = 12.07 min



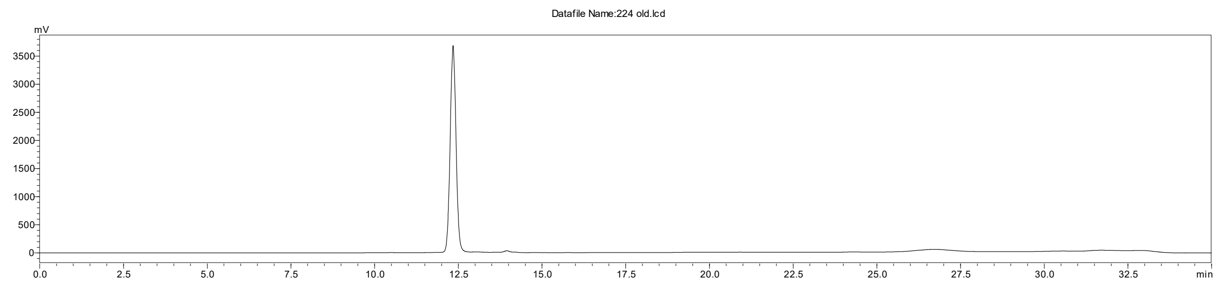
ESI-MS, calculated: 1815.6344; found $[M-H]^-$ 1814.6084, $[M-2H]^{2-}$ 906.7991



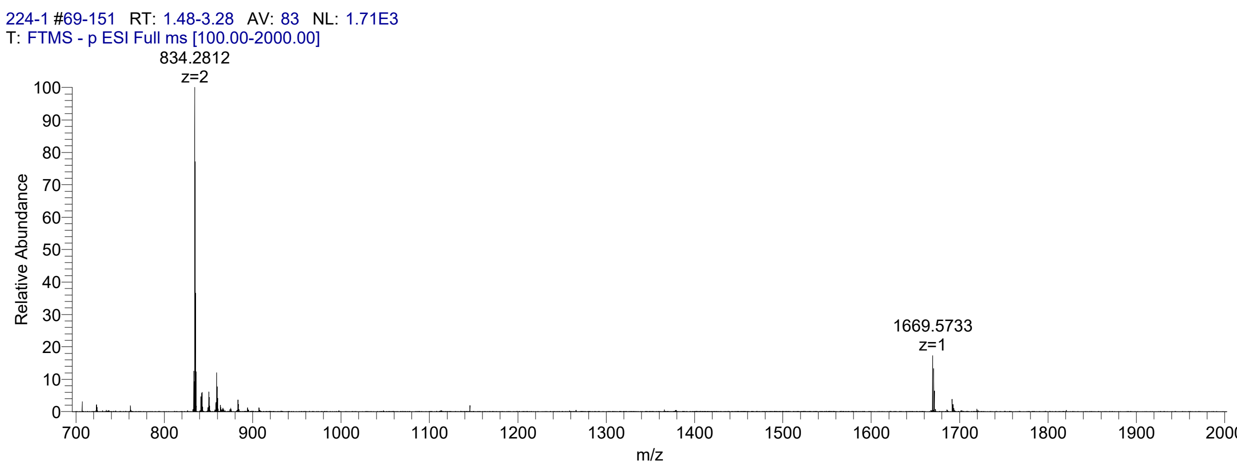
^{1}H NMR (600 MHz, D_2O) δ 7.91 – 7.77 (m, 2H), 7.63 (ddd, *J* = 28.7, 16.5, 7.2 Hz, 2H), 7.54 – 7.27 (m, 4H), 4.57 (dd, *J* = 11.0, 5.1 Hz, 1H), 4.48 (d, *J* = 8.4 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 4.40 – 4.34 (m, 2H), 4.34 – 4.25 (m, 1H), 4.25 – 4.18 (m, 1H), 4.17 – 4.00 (m, 3H), 4.00 – 3.41 (m, 40H), 3.31 (t, *J* = 9.8 Hz, 1H), 2.69 (dd, *J* = 12.5, 4.5 Hz, 1H), 2.59 (dd, *J* = 12.5, 4.5 Hz, 1H), 1.98 (s, 3H), 1.96 (s, 6H), 1.93 (s, 3H), 1.76 (t, *J* = 12.2 Hz, 1H), 1.69 (t, *J* = 12.2 Hz, 1H), 1.00 (d, *J* = 6.3 Hz, 3H).

M224 

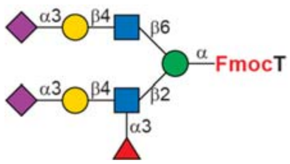
HPLC-UV_{260nm}, T_R = 12.09 min



ESI-MS, calculated: 1670.5969; found $[M-H]^-$ 1669.5733, $[M-2H]^{2-}$ 834.2812

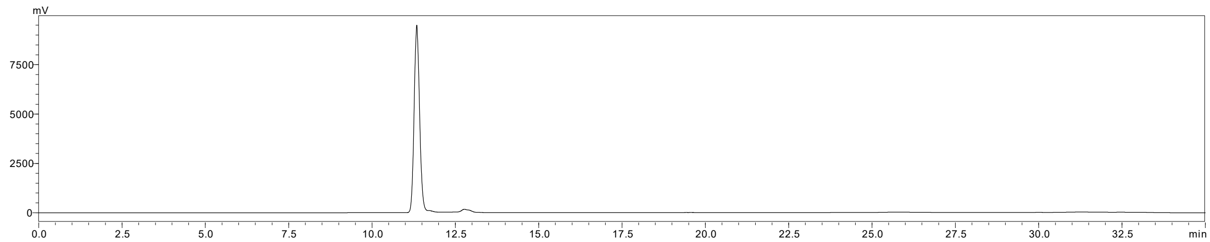


^{1}H NMR (600 MHz, D_2O) δ 7.94 – 7.81 (m, 2H), 7.78 – 7.56 (m, 2H), 7.50 – 7.30 (m, 4H), 5.07 – 4.99 (m, 2H), 4.54 – 4.46 (m, 2H), 4.46 – 4.33 (m, 2H), 4.29 (s, 1H), 4.26 – 4.14 (m, 1H), 4.08 (d, J = 10.8 Hz, 1H), 4.05 – 3.98 (m, 1H), 3.92 (t, J = 12.1 Hz, 1H), 3.89 – 3.55 (m, 28H), 3.55 – 3.38 (m, 9H), 3.28 (t, J = 9.8 Hz, 1H), 2.69 (dd, J = 12.3, 4.4 Hz, 1H), 2.00 – 1.86 (m, 9H), 1.73 (t, J = 12.1 Hz, 1H), 1.15 – 1.02 (m, 6H), 0.94 (d, J = 6.3 Hz, 3H).

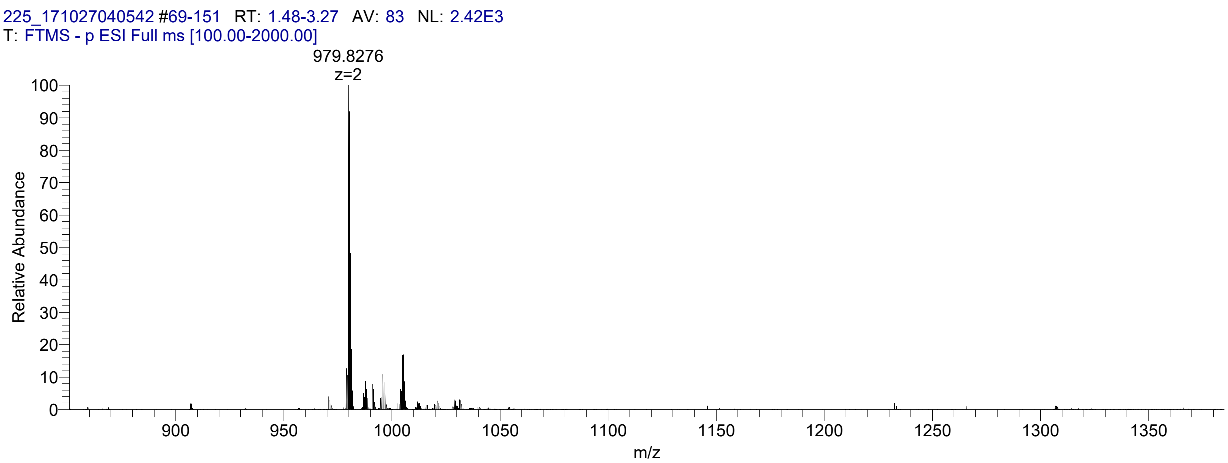


M225

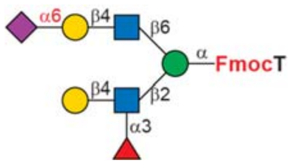
HPLC-FL, T_R = 11.34 min



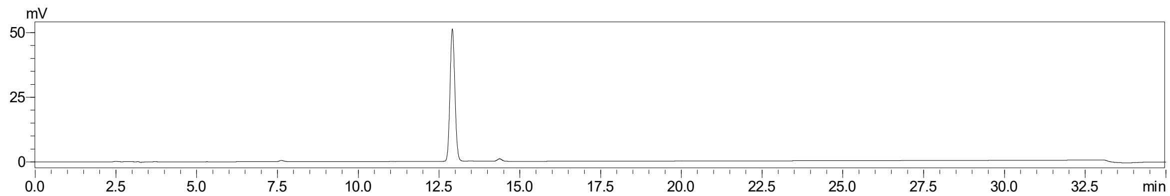
ESI-MS, calculated: 1961.6923; found $[M-2H]^{2-}$ 979.8275



^{1}H NMR (600 MHz, D_2O) δ 7.93 – 7.80 (m, 2H), 7.72 – 7.55 (m, 2H), 7.53 – 7.33 (m, 4H), 5.03 (d, *J* = 4.0 Hz, 1H), 4.61 – 4.53 (m, 2H), 4.52 – 4.35 (m, 5H), 4.30 (s, 2H), 4.24 – 4.15 (m, 1H), 4.05 (m, 4H), 3.98 – 3.38 (m, 39H), 3.37 – 3.25 (m, 2H), 3.23 – 3.15 (m, 1H), 2.74 – 2.65 (m, 2H), 1.96 (s, 6H), 1.95 (s, 3H), 1.93 (s, 3H), 1.92 (s, 3H), 1.74 (t, *J* = 11.9 Hz, 2H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 6.4 Hz, 3H).

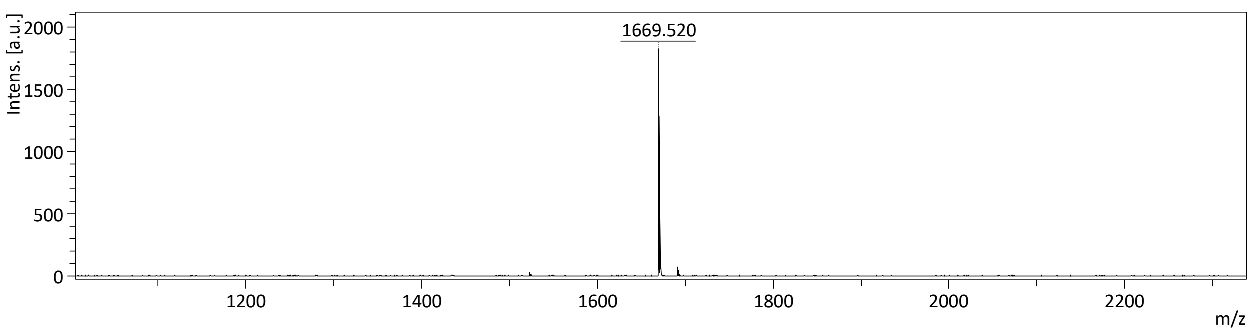
M234 

HPLC-UV$_{260nm}$, T_R = 12.96 min

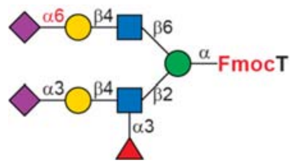


ESI-MS, calculated: 1670.5969; found $[M-H]^-$ 1669.5733, $[M-2H]^{2-}$ 834.2816

MALDI-MS; found $[M-H]^-$ 1669.520

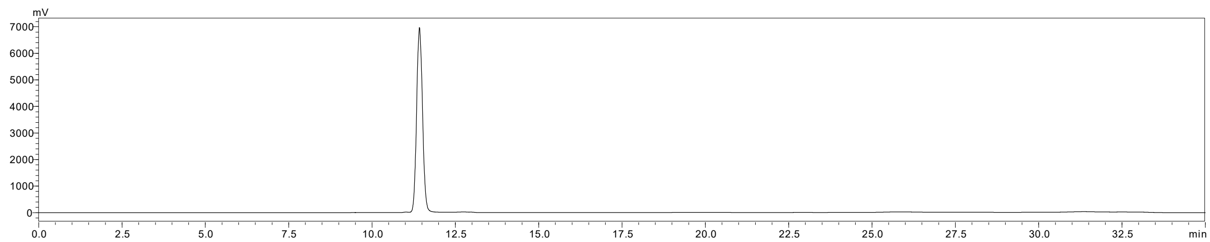


^{1}H NMR (600 MHz, D_2O) δ 7.92 – 7.78 (m, 2H), 7.72 – 7.55 (m, 2H), 7.53 – 7.32 (m, 4H), 5.03 (d, *J* = 3.8 Hz, 1H), 4.56 (d, *J* = 12.5 Hz, 1H), 4.51 (d, *J* = 8.3 Hz, 1H), 4.45 – 4.33 (m, 3H), 4.29 (s, 2H), 4.21 (d, *J* = 6.3 Hz, 1H), 4.15 – 4.02 (m, 2H), 4.02 – 3.35 (m, 38H), 3.35 – 3.26 (m, 1H), 2.60 (dd, *J* = 12.2, 4.6 Hz, 1H), 1.97 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.70 – 1.63 (m, 1H), 1.10 (d, *J* = 6.3 Hz, 3H), 0.99 (d, *J* = 5.4 Hz, 3H).

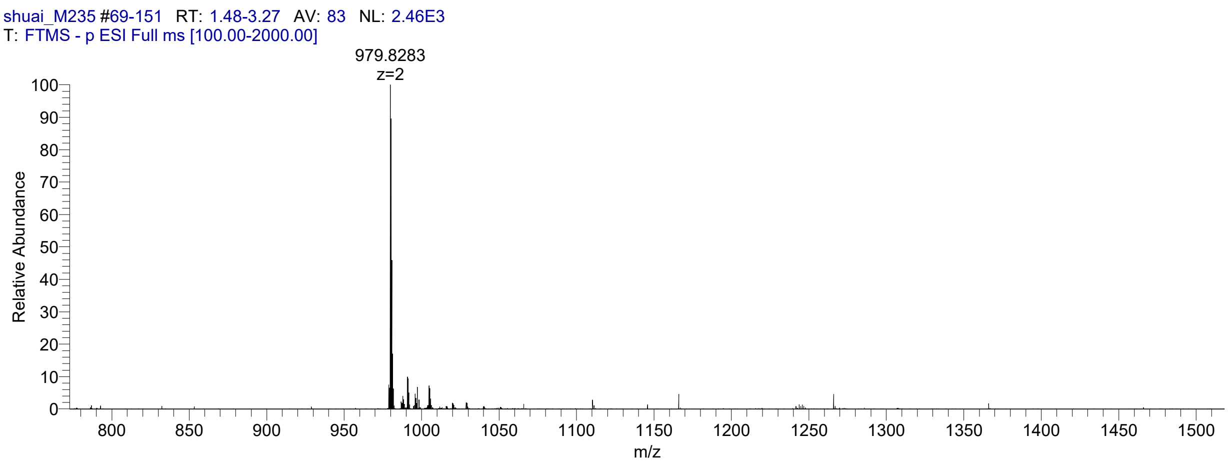


M235

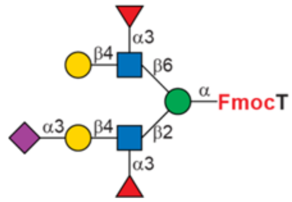
HPLC-FL, T_R = 11.42 min



ESI-MS, calculated: 1961.6923; found $[M-2H]^{2-}$ 979.8283

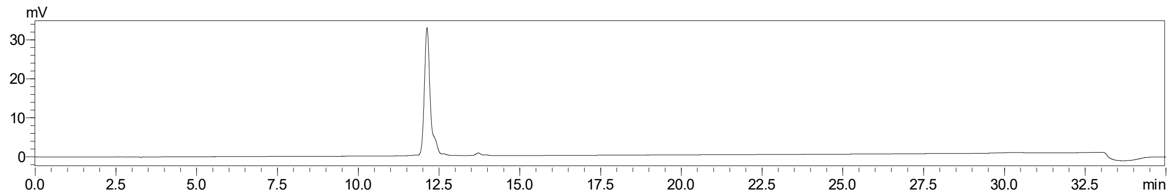


[1]H NMR (600 MHz, D_2O) δ 7.92 – 7.79 (m, 2H), 7.78 – 7.54 (m, 2H), 7.51 – 7.32 (m, 4H), 5.03 (d, *J* = 4.0 Hz, 1H), 4.62 – 4.56 (m, 2H), 4.50 (d, *J* = 8.1 Hz, 1H), 4.49 – 4.44 (m, 1H), 4.43 (d, *J* = 8.0 Hz, 1H), 4.41 – 4.34 (m, 1H), 4.32 – 4.27 (m, 2H), 4.21 (d, *J* = 5.2 Hz, 1H), 4.15 – 4.05 (m, 2H), 4.02 (dd, *J* = 10.1, 2.7 Hz, 1H), 3.99 – 3.39 (m, 46H), 3.35 – 3.27 (m, 1H), 2.69 (dd, *J* = 12.4, 4.5 Hz, 1H), 2.60 (dd, *J* = 12.1, 4.3 Hz, 1H), 1.96 (s, 3H), 1.96 (s, 3H), 1.92 (s, 3H), 1.75 (t, *J* = 12.3 Hz, 1H), 1.71 – 1.64 (m, 1H), 1.10 (d, *J* = 6.5 Hz, 3H), 1.00 (d, *J* = 6.0 Hz, 3H).

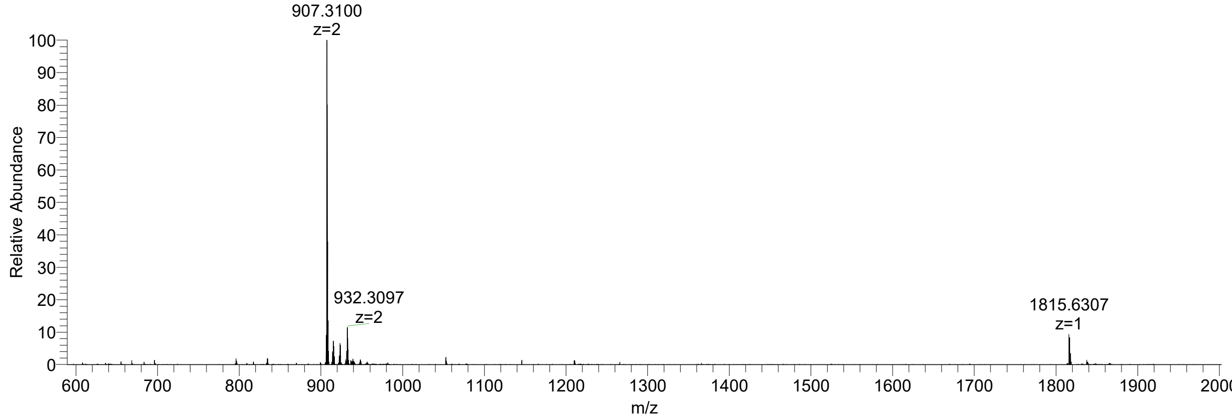


M245

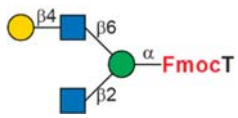
HPLC-UV$_{260nm}$, T_R = 12.13 min



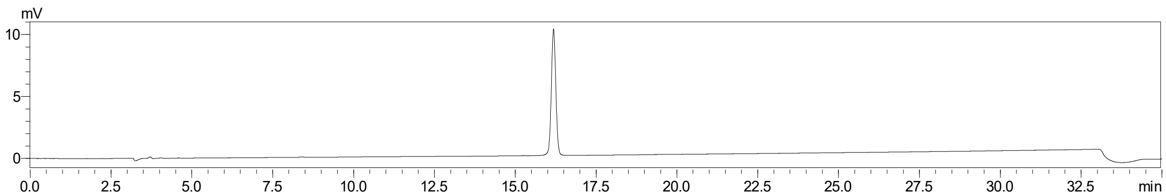
ESI-MS, calculated: 1816.6548; found $[M-H]^-$ 1815.6307, $[M-2H]^{2-}$ 907.3100



^{1}H NMR (600 MHz, D_2O) δ 7.87 – 7.75 (m, 2H), 7.68 – 7.48 (m, 2H), 7.46 – 7.26 (m, 4H), 4.96 (d, *J* = 3.2 Hz, 2H), 4.60 – 4.58 (m, 2H), 4.48 – 4.39 (m, 3H), 4.36 (d, *J* = 7.6 Hz, 2H), 4.31 (d, *J* = 9.5 Hz, 2H), 4.22 (d, *J* = 5.9 Hz, 2H), 4.16 – 4.15 (m, 1H), 4.13 (d, *J* = 5.7 Hz, 1H), 4.02 (d, *J* = 10.4 Hz, 1H), 3.95 (d, *J* = 9.9 Hz, 2H), 3.88 – 3.61 (m, 18H), 3.61 – 3.49 (m, 9H), 3.49 – 3.29 (m, 9H), 3.27 – 3.17 (m, 2H), 2.62 (dd, *J* = 12.2, 3.7 Hz, 1H), 1.89 (s, 6H), 1.87 (s, 3H), 1.66 (t, *J* = 12.3 Hz, 1H), 1.03 (d, *J* = 6.0 Hz, 6H), 0.91 (d, *J* = 6.2 Hz, 3H).

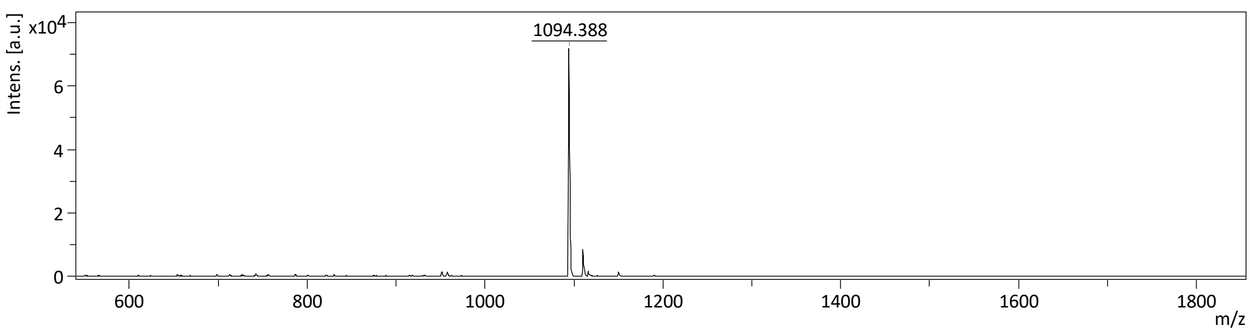
M301 

HPLC-UV$_{260nm}$, T$_R$ = 16.19 min

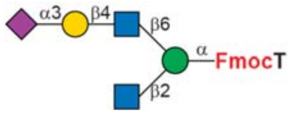


ESI-MS, calculated: 1071.3907; found $[M-H]^-$ 1070.3777

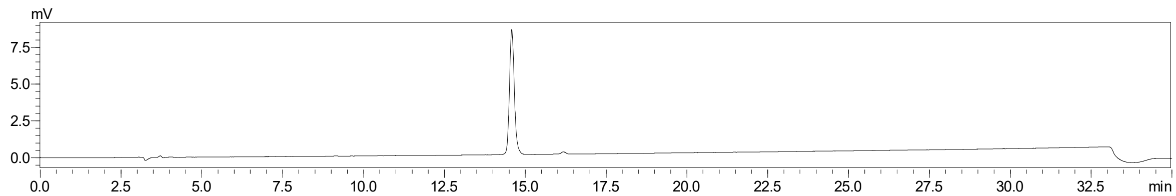
MALDI-MS; found $[M+Na]^+$ 1094.388



^{1}H NMR (400 MHz, MeOD) δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.74 – 7.58 (m, 2H), 7.44 – 7.27 (m, 4H), 4.55 (d, *J* = 7.7 Hz, 1H), 4.50 – 4.31 (m, 5H), 4.23 (t, *J* = 6.7 Hz, 1H), 4.18 – 4.02 (m, 2H), 4.01 – 3.84 (m, 5H), 3.84 – 3.51 (m, 14H), 3.51 – 3.30 (m, 6H), 2.08 (s, 3H), 2.06 (s, 3H), 1.22 (d, *J* = 5.9 Hz, 2H); ^{13}C NMR (100 MHz, MeOD) δ 173.40, 172.73, 157.41, 144.06, 143.80, 141.18, 127.42, 126.85, 124.88, 119.55, 103.81, 101.78, 98.66, 79.69, 79.33, 76.38, 75.74, 75.04, 73.89, 73.41, 72.46, 71.20, 70.54, 69.85, 68.93, 68.64, 67.77, 66.59, 61.16, 60.88, 60.39, 55.28, 47.08, 22.35, 22.12, 18.44.

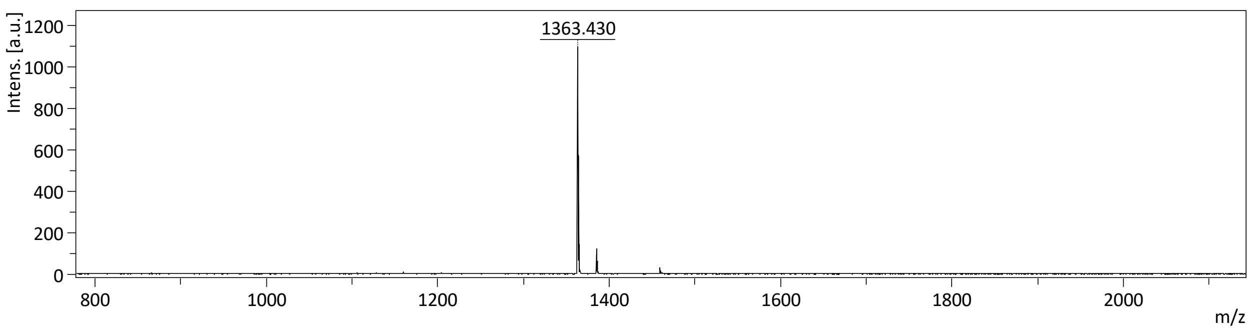
M302 

HPLC-UV$_{260nm}$, T_R = 14.58 min



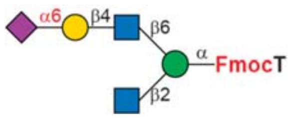
ESI-MS, calculated: 1362.4861; found $[M-H]^-$ 1361.4634, $[M-2H]^{2-}$ 680.2272

MALDI-MS; found $[M+H]^+$ 1363.4380, $[M+Na]^+$ 1385.418

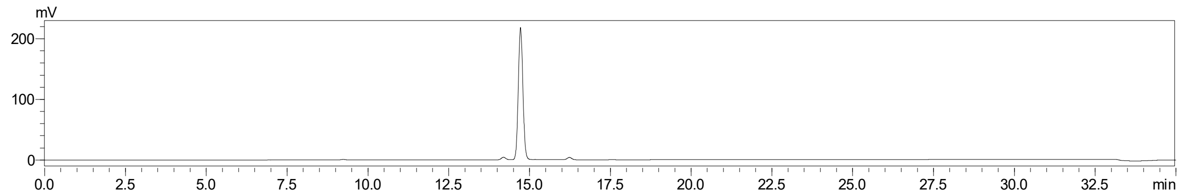


^{1}H NMR (600 MHz, D_2O) δ 7.79 – 7.66 (m, 2H), 7.61 – 7.42 (m, 2H), 7.38 – 7.22 (m, 4H), 4.68 – 4.58 (m, 1H), 4.58 – 4.47 (m, 1H), 4.47 – 4.36 (m, 1H), 4.33 (d, J = 7.8 Hz, 1H), 4.29 (t, J = 7.7 Hz, 1H), 4.22 (d, J = 7.6 Hz, 1H), 4.20 – 3.96 (m, 3H), 3.93 – 3.80 (m, 1H), 3.80 – 3.34 (m, 30H), 3.34 – 3.20 (m, 3H), 2.63 (dd, J = 12.4, 4.1 Hz, 1H), 1.91 (s, 6H), 1.87 (s, 3H), 1.74 (t, J = 12.2 Hz, 1H), 0.94 (d, J = 6.2 Hz, 3H).

M303

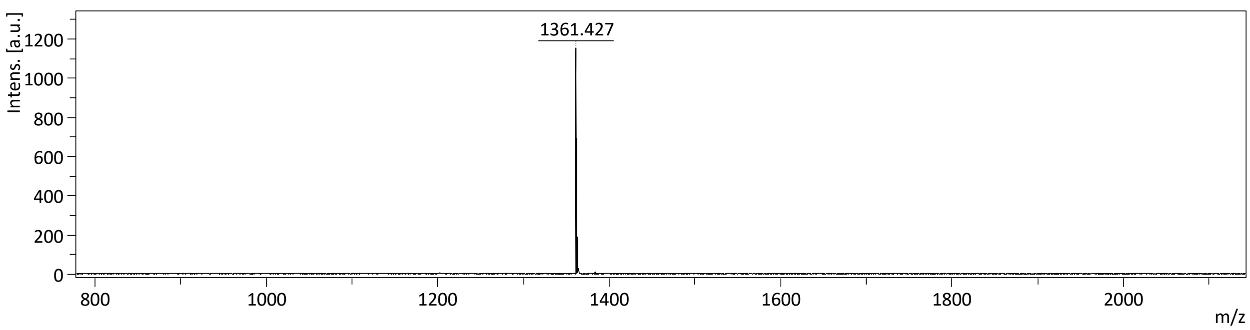


HPLC-FL, T_R = 14.57 min

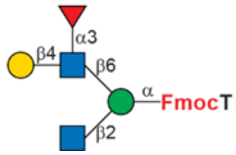


ESI-MS, calculated: 1362.4861; found $[M-H]^-$ 1361.4637, $[M-2H]^{2-}$ 680.2274

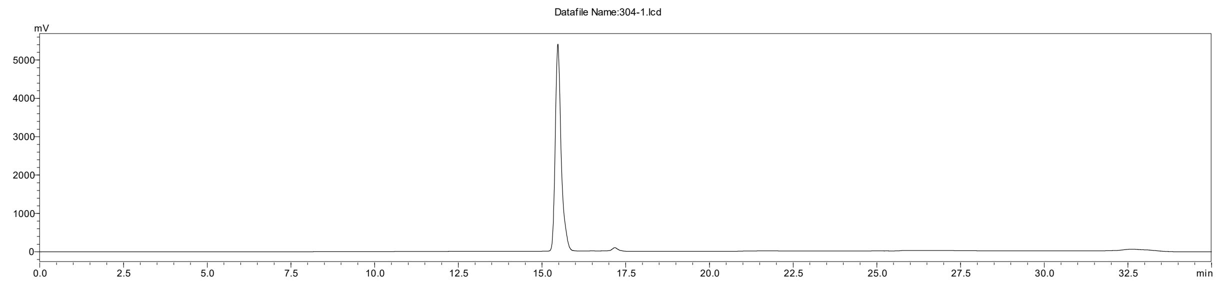
MALDI-MS; found $[M-H]^-$ 1361.427



^{1}H NMR (600 MHz, D_2O) δ 7.92 – 7.73 (m, 2H), 7.73 – 7.49 (m, 2H), 7.49 – 7.29 (m, 4H), 4.68 (s, 1H), 4.60 – 4.43 (m, 2H), 4.36 (d, *J* = 7.9 Hz, 1H), 4.29 (d, *J* = 6.5 Hz, 1H), 4.28 – 4.18 (m, 3H), 4.18 – 4.05 (m, 3H), 4.04 – 3.88 (m, 4H), 3.88 – 3.41 (m, 21H), 3.41 – 3.26 (m, 4H), 2.59 (dd, *J* = 12.4, 3.8 Hz, 1H), 1.99 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.75 (t, *J* = 12.1 Hz, 1H), 1.02 (d, *J* = 6.3 Hz, 3H).

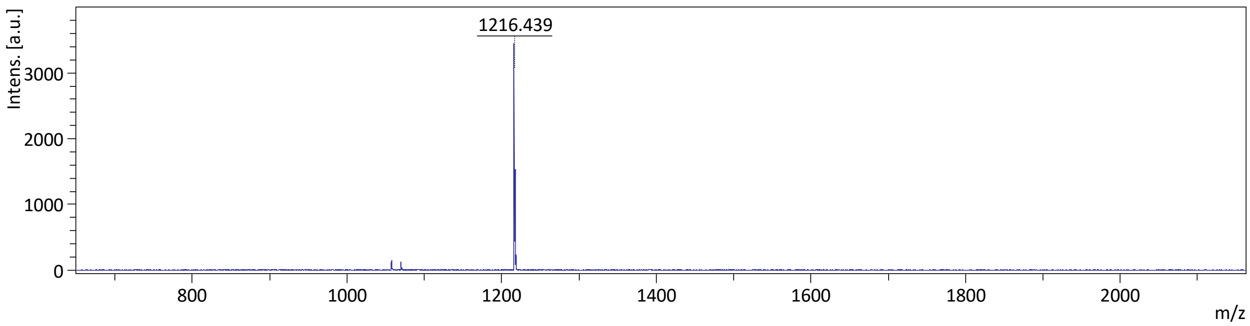
M304 

HPLC-FL, T_R = 15.19 min

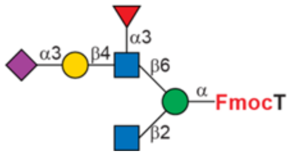


ESI-MS, calculated: 1217.4486; found $[M+H]^+$ 1218.4589, $[M+Na]^+$ 1240.4386

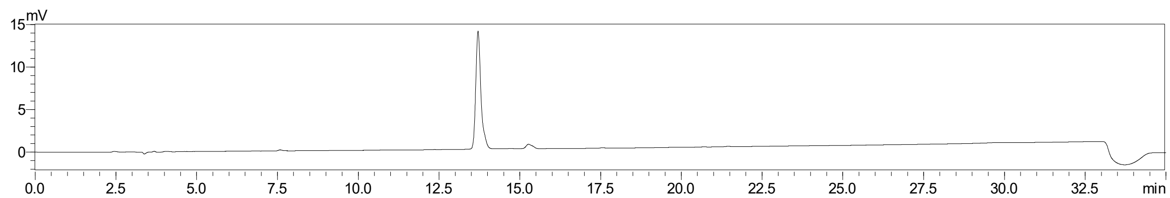
MALDI-MS, found $[M-H]^-$ 1216.436



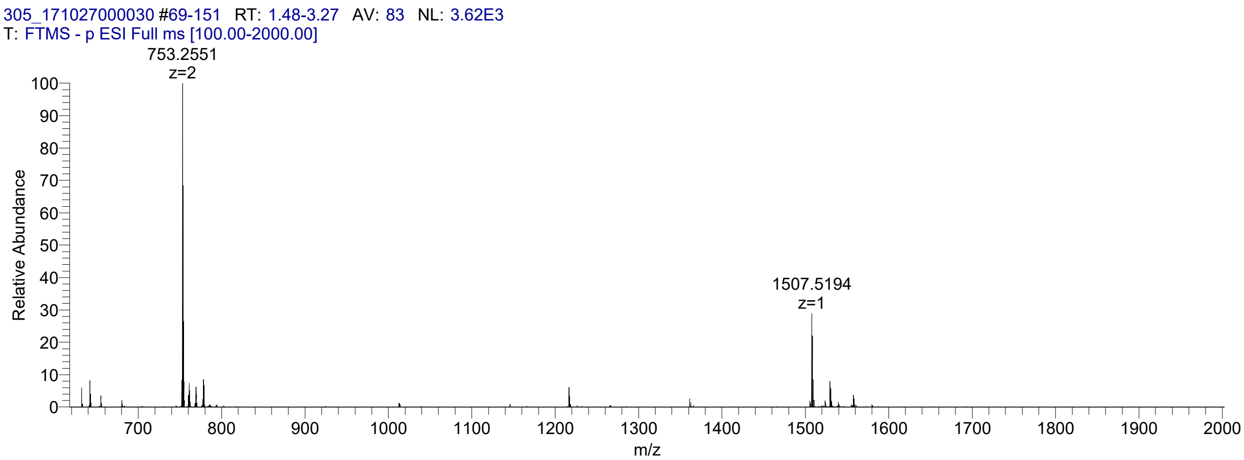
^{1}H NMR (600 MHz, D_2O) δ 7.87 – 7.73 (m, 2H), 7.74 – 7.51 (m, 2H), 7.51 – 7.29 (m, 4H), 5.02 (d, *J* = 3.9 Hz, 1H), 4.75 (d, *J* = 7.1 Hz, 1H), 4.65 – 4.54 (m, 1H), 4.54 – 4.44 (m, 2H), 4.41 – 4.32 (m, 1H), 4.27 (d, *J* = 7.8 Hz, 1H), 4.21 (d, *J* = 4.7 Hz, 2H), 4.16 – 4.02 (m, 2H), 3.90 (d, *J* = 10.4 Hz, 1H), 3.89 – 3.29 (m, 26H), 1.96 (s, 3H), 1.93 (s, 3H), 1.10 (d, *J* = 6.5 Hz, 3H), 1.00 (d, *J* = 6.3 Hz, 3H).

M305 

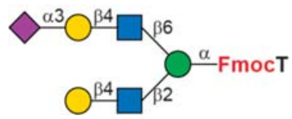
HPLC-UV$_{260nm}$, T_R = 13.55 min



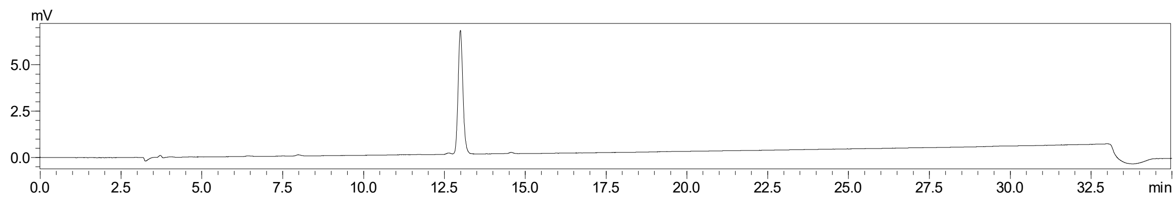
ESI-MS, calculated: 1508.544; found $[M-H]^-$ 1507.5194, $[M-2H]^{2-}$ 753.2551



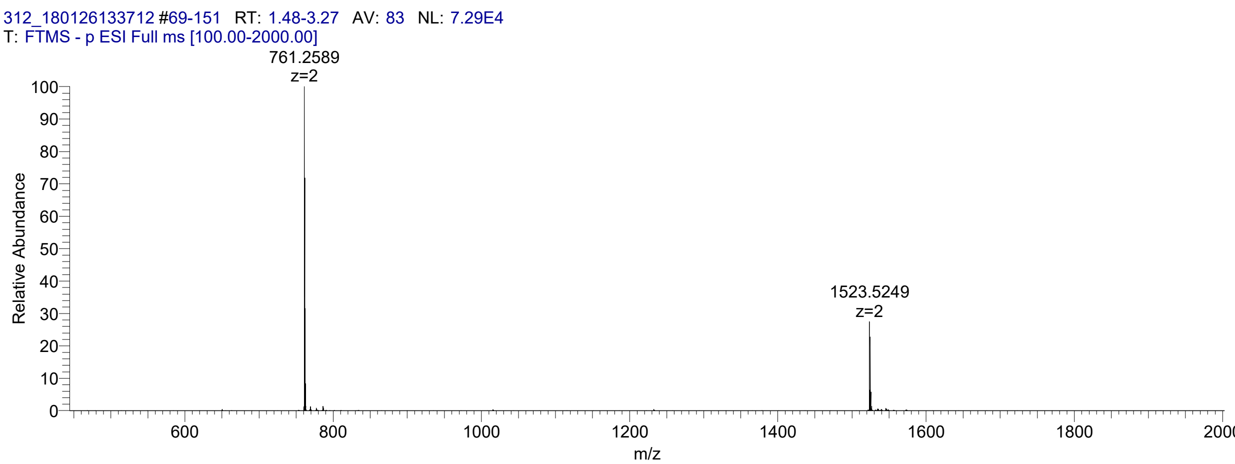
^{1}H NMR (600 MHz, D_2O) δ 7.77 – 7.59 (m, 2H), 7.59 – 7.37 (m, 2H), 7.37 – 7.15 (m, 4H), 4.98 (d, *J* = 3.4 Hz, 1H), 4.66 (s, 1H), 4.54 (s, 1H), 4.51 – 4.39 (m, 2H), 4.39 – 4.26 (m, 3H), 4.26 – 3.95 (m, 5H), 3.95 – 3.20 (m, 32H), 2.66 (dd, *J* = 12.6, 4.4 Hz, 1H), 1.93 (s, 6H), 1.89 (s, 3H), 1.80 (t, *J* = 12.2 Hz, 1H), 1.06 (d, *J* = 6.2 Hz, 3H), 0.97 (d, *J* = 6.2 Hz, 3H).

M312 

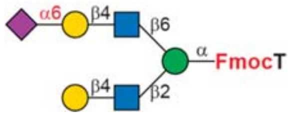
HPLC-UV_{260nm}, T_R = 12.99 min



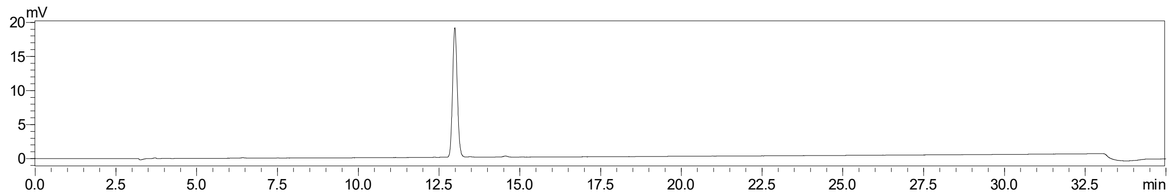
ESI-MS, calculated: 1524.5390; found $[M-H]^-$ 1523.5249, $[M-2H]^{2-}$ 761.2589



^{1}H NMR (600 MHz, D_2O) δ 7.92 – 7.73 (m, 2H), 7.73 – 7.56 (m, 2H), 7.54 – 7.33 (m, 4H), 4.80 – 4.74 (m, 1H), 4.65 – 4.58 (m, 1H), 4.59 – 4.46 (m, 4H), 4.38 (d, *J* = 7.8 Hz, 2H), 4.34 – 4.23 (m, 2H), 4.19 (d, *J* = 5.0 Hz, 1H), 4.11 (d, *J* = 10.3 Hz, 1H), 3.99 (s, 1H), 3.95 – 3.78 (m, 7H), 3.78 – 3.42 (m, 26H), 3.39 – 3.26 (m, 1H), 2.61 (dd, *J* = 12.4, 4.4 Hz, 1H), 1.97 (s, 6H), 1.96 (s, 3H), 1.66 (t, *J* = 11.9 Hz, 1H), 0.99 (d, *J* = 6.2 Hz, 3H).

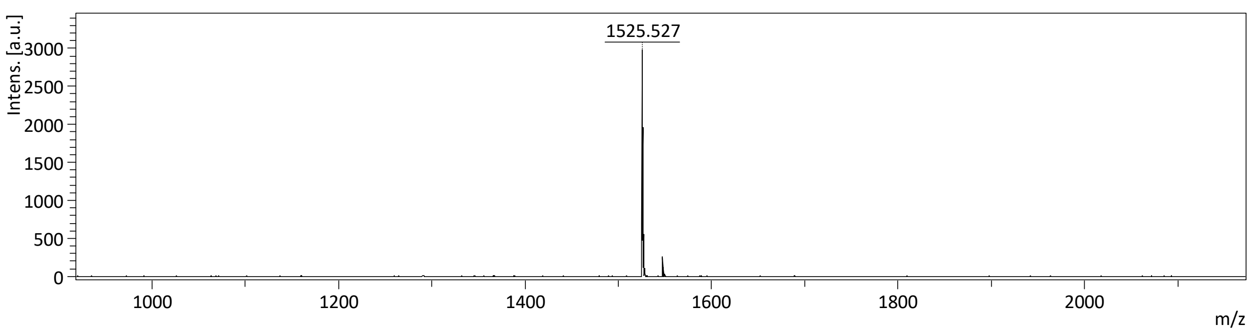
M313 

HPLC-UV$_{260nm}$, T_R = 12.98 min

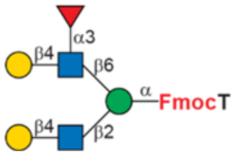


ESI-MS, calculated: 1524.5390; found $[M-H]^-$ 1523.5148, $[M-2H]^{2-}$ 761.2527

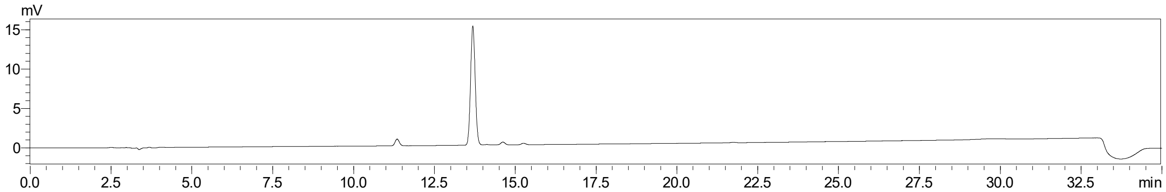
MALDI-MS; found $[M+H]^+$ 1525.527, $[M+Na]^+$ 1547.527



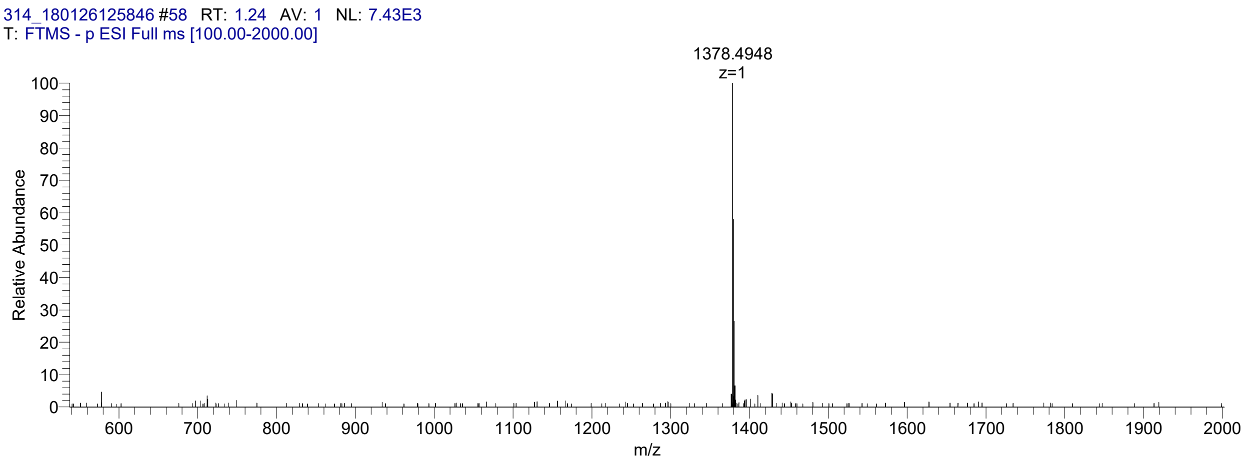
^{1}H NMR (600 MHz, D_2O) δ 7.94 – 7.82 (m, 2H), 7.75 – 7.57 (m, 2H), 7.52 – 7.31 (m, 4H), 4.75 – 4.73 (m, 1H), 4.69 – 4.67 (m, 1H), 4.52 (d, *J* = 7.7 Hz, 2H), 4.44 – 4.35 (m, 2H), 4.35 – 4.25 (m, 2H), 4.18 (d, *J* = 6.4 Hz, 1H), 4.11 (d, *J* = 10.4 Hz, 1H), 3.98 – 3.89 (m, 2H), 3.89 – 3.78 (m, 6H), 3.78 – 3.42 (m, 28H), 3.33 – 3.26 (m, 1H), 2.61 (dd, *J* = 12.8, 4.3 Hz, 1H), 1.98 (s, 6H), 1.96 (s, 3H), 1.65 (t, *J* = 11.9 Hz, 1H), 0.96 (d, *J* = 5.7 Hz, 3H).

M314 

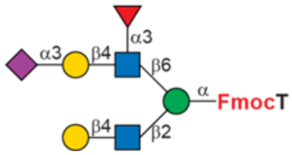
HPLC-UV_{260nm}, T_R = 13.65 min



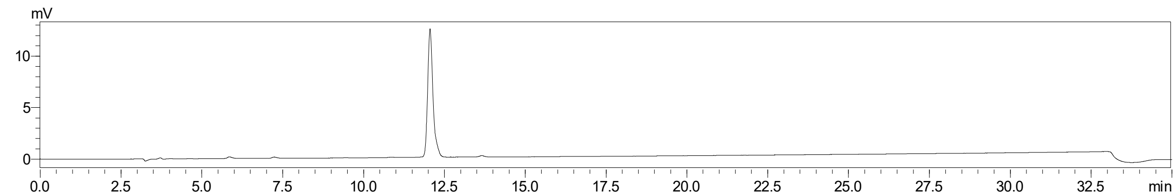
ESI-MS, calculated: 1379.5014; found $[M-H]^-$ 1378.4948



^{1}H NMR (600 MHz, D_2O) δ 7.89 – 7.78 (m, 2H), 7.70 – 7.53 (m, 2H), 7.49 – 7.28 (m, 4H), 5.02 (d, *J* = 3.8 Hz, 1H), 4.76 – 4.74 (m, 1H), 4.69 – 4.67 (m, 1H), 4.54 – 4.46 (m, 2H), 4.35 (d, *J* = 8.2 Hz, 2H), 4.32 – 4.17 (m, 4H), 4.09 (d, *J* = 10.0 Hz, 1H), 4.04 (s, 1H), 3.91 (d, *J* = 11.2 Hz, 2H), 3.88 – 3.69 (m, 10H), 3.69 – 3.46 (m, 14H), 3.46 – 3.26 (m, 6H), 1.96 (s, 3H), 1.93 (s, 3H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 6.3 Hz, 3H).

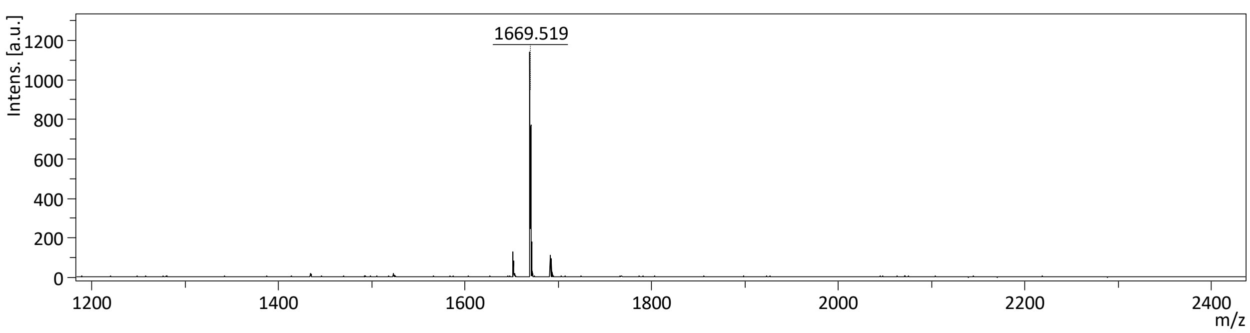
M315 

HPLC-UV$_{260nm}$, T_R = 12.05 min

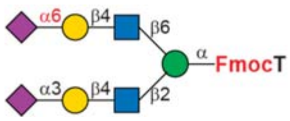


ESI-MS, calculated: 1670.5969; found [M-H]$^-$ 1669.5717, [M-2H]$^{2-}$ 834.2809

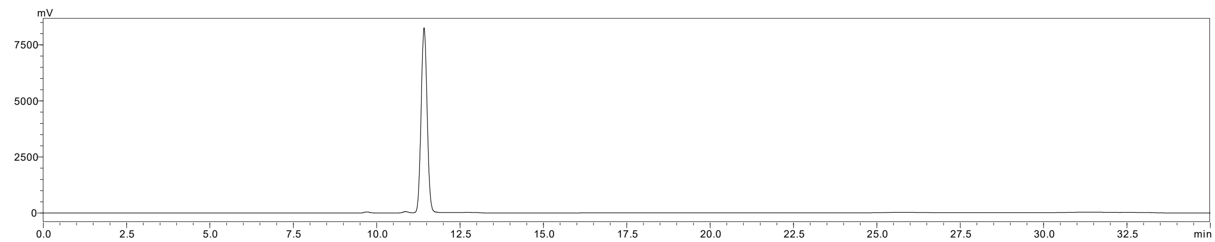
MALDI-MS; found [M+H]$^+$ 1669.519



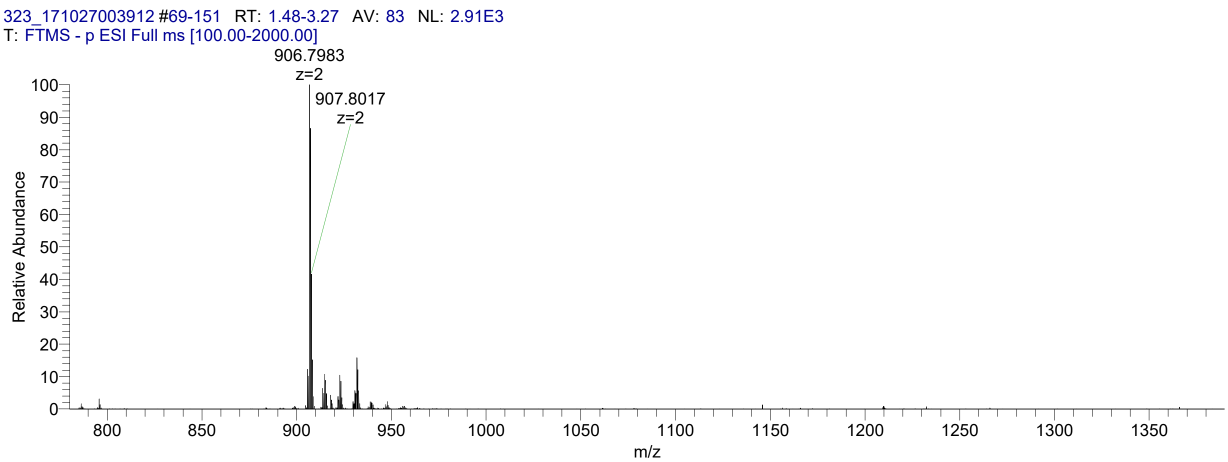
^{1}H NMR (600 MHz, D_2O) δ 7.88 – 7.74 (m, 2H), 7.69 – 7.50 (m, 2H), 7.47 – 7.30 (m, 4H), 5.01 (d, *J* = 3.4 Hz, 1H), 4.83 – 4.79 (m, 1H), 4.65 – 4.63 (m, 1H), 4.53 – 4.31 (m, 5H), 4.29 – 4.17 (m, 3H), 4.16 – 4.00 (m, 3H), 3.98 – 3.37 (m, 37H), 3.31 (t, *J* = 10.4 Hz, 1H), 2.69 (dd, *J* = 12.7, 4.4 Hz, 1H), 1.96 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.81 (t, *J* = 12.3 Hz, 1H), 1.09 (d, *J* = 6.0 Hz, 3H), 0.99 (d, *J* = 6.3 Hz, 3H).

M323 

HPLC-FL, T_R = 11.67 min

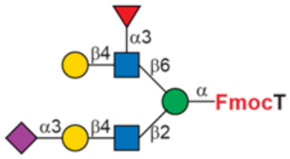


ESI-MS, calculated: 1815.6344; found $[M-H]^-$ 1814.6059, $[M-2H]^{2-}$ 906.7983

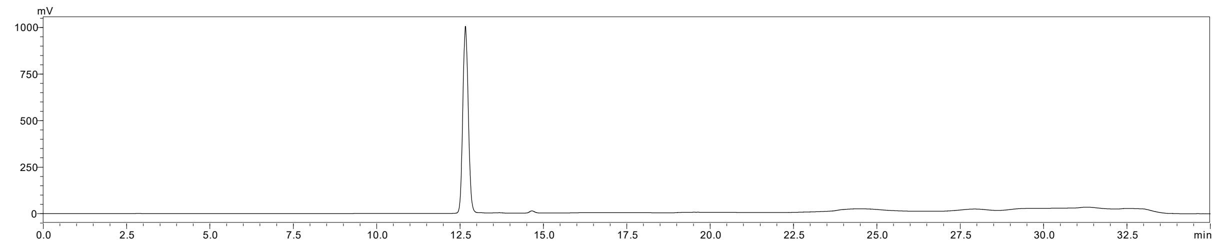


^{1}H NMR (600 MHz, D_2O) δ 7.90 – 7.76 (m, 2H), 7.67 – 7.52 (m, 2H), 7.48 – 7.29 (m, 4H), 4.74 – 4.72 (m, 1H),4.69 – 4.67 (m, 1H), 4.54 (d, *J* = 14.9 Hz, 2H), 4.50 (d, *J* = 8.1 Hz, 1H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.36 (d, *J* = 7.8 Hz, 1H), 4.32 – 4.18 (m, 3H), 4.16 – 4.03 (m, 3H), 4.05 – 3.36 (m, 40H), 3.31 (t, *J* = 9.8 Hz, 1H), 2.80 – 2.63 (m, 1H), 2.62 – 2.53 (m, 1H), 1.96 (s, 9H), 1.95 (s, 3H), 1.80 (t, *J* = 12.1 Hz, 1H), 1.77 – 1.68 (m, 1H), 1.01 (d, *J* = 6.2 Hz, 3H).

M324

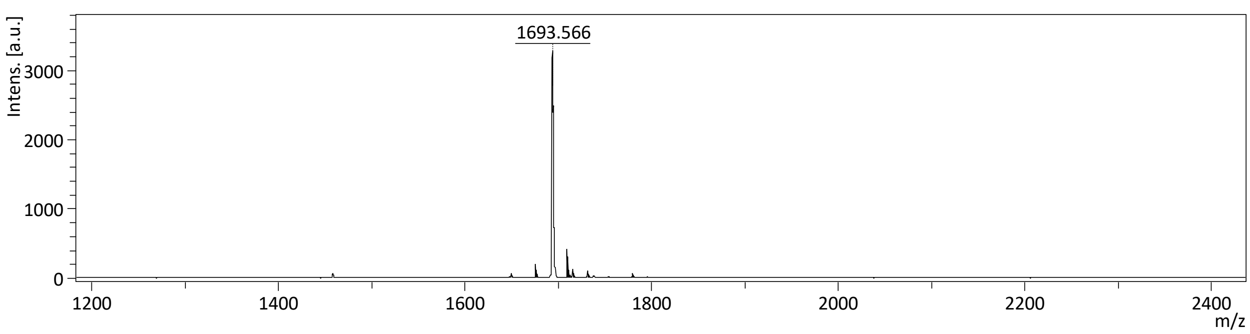


HPLC-FL, T_R = 12.52 min

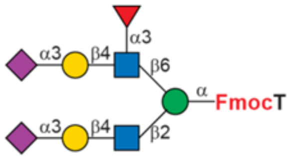


ESI-MS, calculated: 1670.5969; found $[M-H]^-$ 1669.5715, $[M-2H]^{2-}$ 834.2804

MALDI-MS, found $[M+Na]^+$ 1693.566, $[M+K]^+$ 1709.538

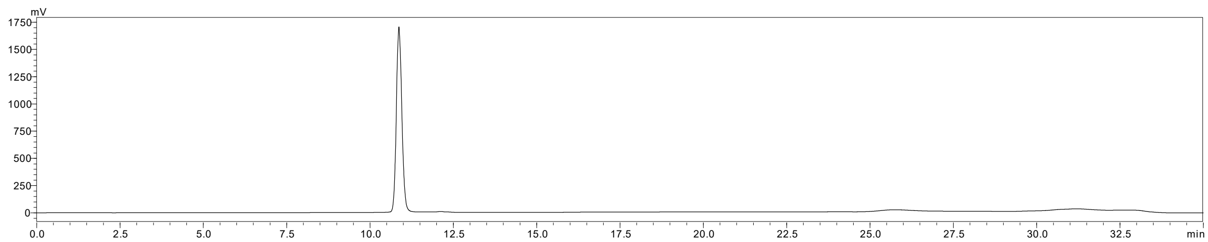


^{1}H NMR (600 MHz, D_2O) δ 7.90 – 7.78 (m, 2H), 7.71 – 7.54 (m, 2H), 7.52 – 7.30 (m, 4H), 5.01 (d, *J* = 3.8 Hz, 1H), 4.58 – 4.52 (m, 2H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 7.7 Hz, 2H), 4.35 (d, *J* = 8.1 Hz, 2H), 4.27 (d, *J* = 6.8 Hz, 2H), 4.20 (d, *J* = 5.5 Hz, 1H), 4.14 – 4.02 (m, 3H), 3.99 – 3.55 (m, 28H), 3.54 – 3.44 (m, 5H), 3.44 – 3.36 (m, 2H), 3.31 (t, *J* = 9.7 Hz, 1H), 2.68 (dd, *J* = 11.9, 3.4 Hz, 1H), 1.96 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H), 1.80 (t, *J* = 12.5 Hz, 1H), 1.09 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 6.2 Hz, 3H).

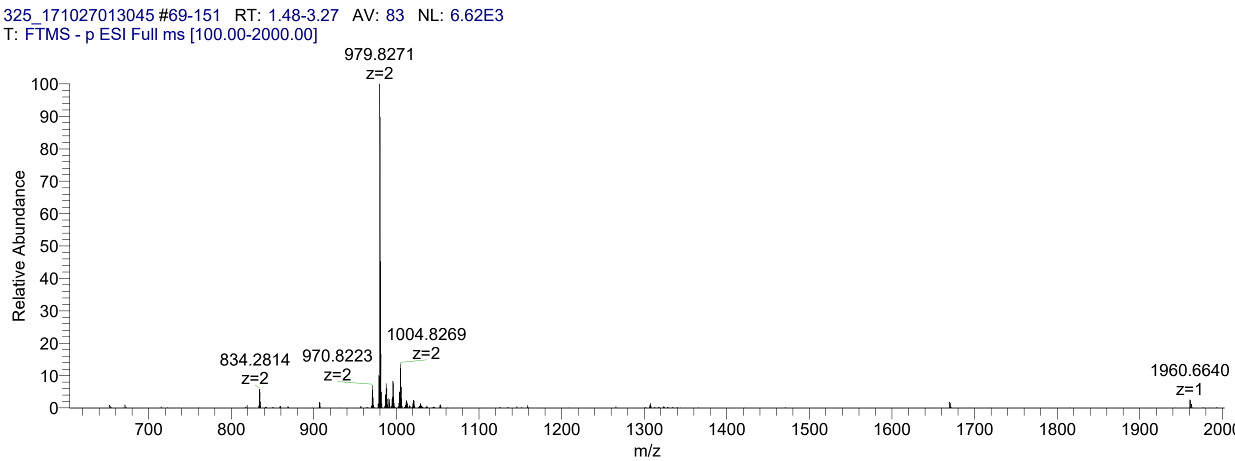


M325

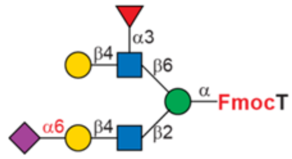
HPLC-FL, T_R = 11.02 min



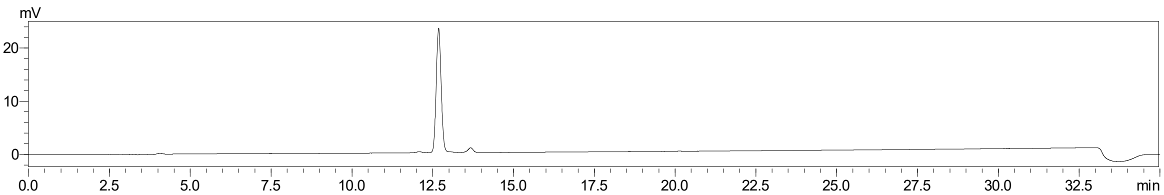
ESI-MS, calculated: 1961.6923; found $[M-H]^-$ 1960.6640, $[M-2H]^{2-}$ 979.8271



^{1}H NMR (600 MHz, D_2O) δ 7.92 – 7.78 (m, 2H), 7.74 – 7.53 (m, 2H), 7.48 – 7.30 (m, 4H), 5.02 (d, J = 4.0 Hz, 1H), 4.55 (dd, J = 8.8, 4.4 Hz, 1H), 4.53 – 4.42 (m, 3H), 4.37 (dd, J = 17.1, 7.9 Hz, 2H), 4.34 – 4.24 (m, 2H), 4.24 – 4.15 (m, 1H), 4.15 – 3.99 (m, 4H), 3.99 – 3.39 (m, 42H), 3.31 (t, J = 9.7 Hz, 1H), 2.75 – 2.62 (m, 2H), 1.96 (s, 3H), 1.95 (s, 3H), 1.92 (s, 6H), 1.77 (t, J = 12.4 Hz, 2H), 1.09 (d, J = 6.3 Hz, 3H), 0.99 (d, J = 6.2 Hz, 3H).

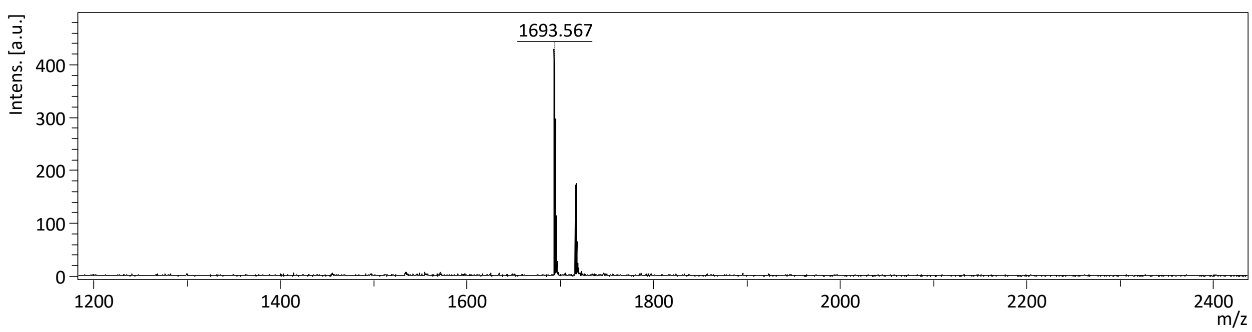
M334 

HPLC-UV$_{260nm}$, T_R = 12.58 min



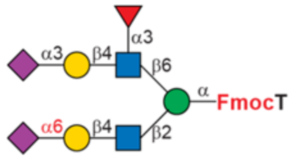
ESI-MS, calculated: 1670.5969; found $[M-H]^-$ 1669.5713, $[M-2H]^{2-}$ 834.2806

MALDI-MS, found $[M+Na]^+$ 1693.567, $[M+K]^+$ 1709.894

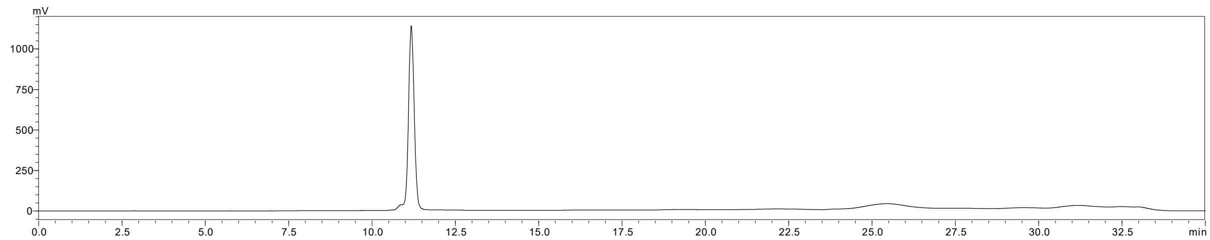


^{1}H NMR (600 MHz, D_2O) δ 7.91 – 7.78 (m, 2H), 7.72 – 7.53 (m, 2H), 7.52 – 7.29 (m, 4H), 5.02 (d, J = 3.7 Hz, 1H), 4.58 – 4.51 (m, 2H), 4.49 (d, J = 7.2 Hz, 1H), 4.41 – 4.33 (m, 2H), 4.31 – 4.17 (m, 3H), 4.16 – 4.05 (m, 2H), 4.01 – 3.44 (m, 36H), 3.44 – 3.37 (m, 2H), 3.31 (t, J = 9.9 Hz, 1H), 2.57 (dd, J = 12.7, 2.6 Hz, 1H), 1.97 (s, 3H), 1.96 (s, 3H), 1.92 (s, 3H), 1.73 (t, J = 12.3 Hz, 1H), 1.09 (d, J = 6.4 Hz, 3H), 1.00 (d, J = 6.3 Hz, 3H).

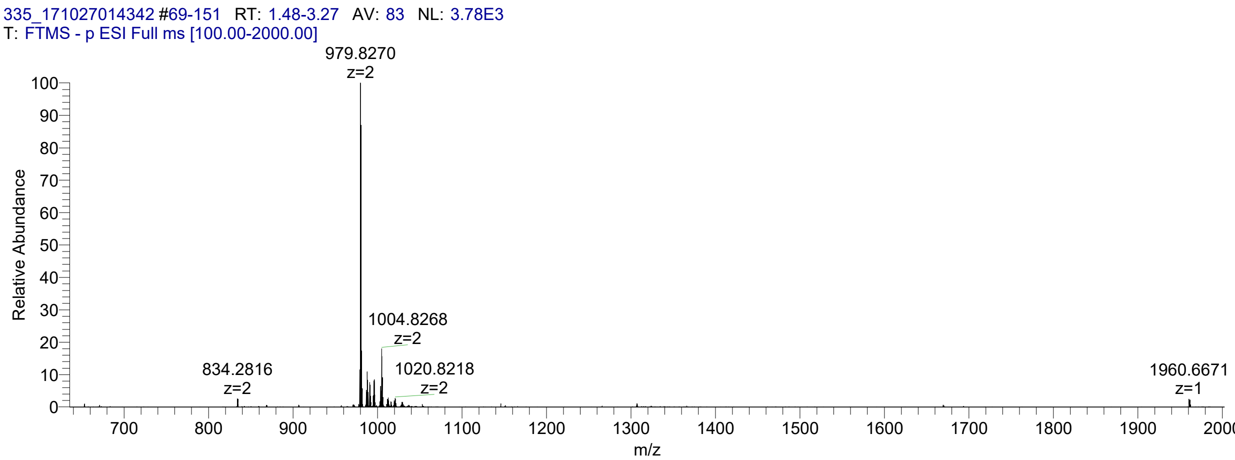
M335



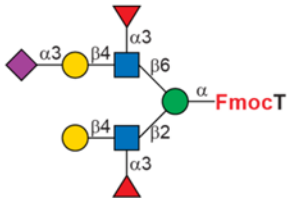
HPLC-FL, T_R = 11.33 min



ESI-MS, calculated: 1961.6923; found $[M-H]^-$ 1960.6671, $[M-2H]^{2-}$ 979.8270

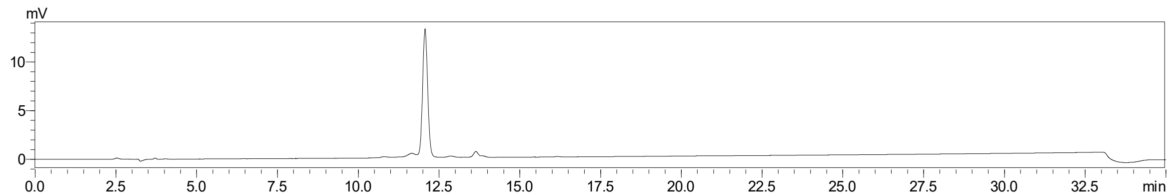


^{1}H NMR (600 MHz, D_2O) δ 7.93 – 7.79 (m, 2H), 7.74 – 7.56 (m, 2H), 7.51 – 7.31 (m, 4H), 5.02 (d, *J* = 3.7 Hz, 1H), 4.60 – 4.53 (m, 1H), 4.53 – 4.43 (m, 2H), 4.43 – 4.34 (m, 2H), 4.34 – 4.25 (m, 1H), 4.21 (d, *J* = 2.7 Hz, 1H), 4.17 – 4.06 (m, 2H), 4.01 (d, *J* = 9.6 Hz, 1H), 3.93 (d, *J* = 10.0 Hz, 2H), 3.90 – 3.38 (m, 46H), 3.31 (t, *J* = 9.8 Hz, 1H), 2.70 (dd, *J* = 12.3, 4.5 Hz, 1H), 2.59 (dd, *J* = 12.4, 4.4 Hz, 1H), 1.98 (s, 3H), 1.96 (s, 6H), 1.92 (s, 3H), 1.76 (t, *J* = 11.9 Hz, 1H), 1.72 – 1.64 (m, 1H), 1.09 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 6.2 Hz, 3H).

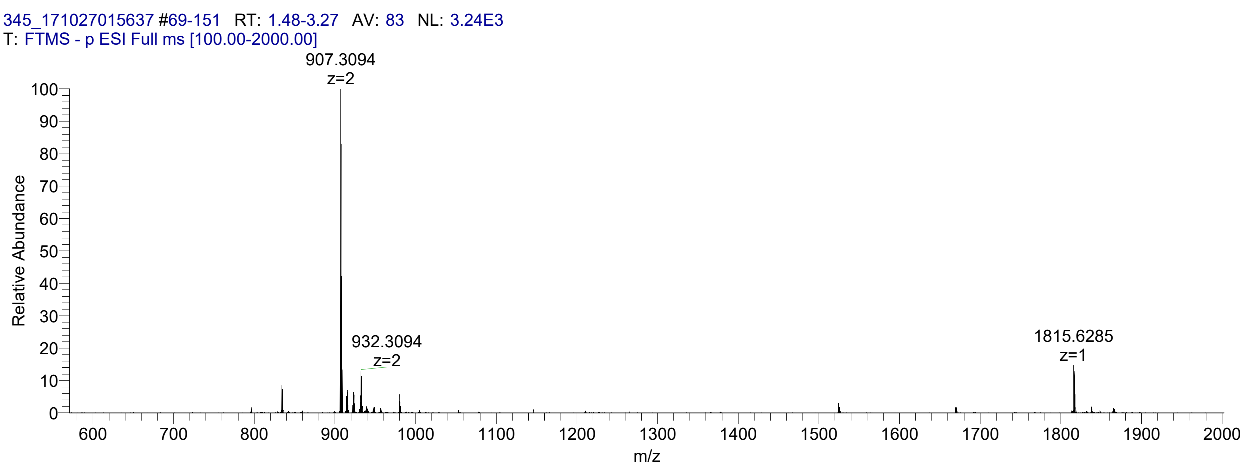


M345

HPLC-UV$_{260nm}$, T_R = 12.06 min



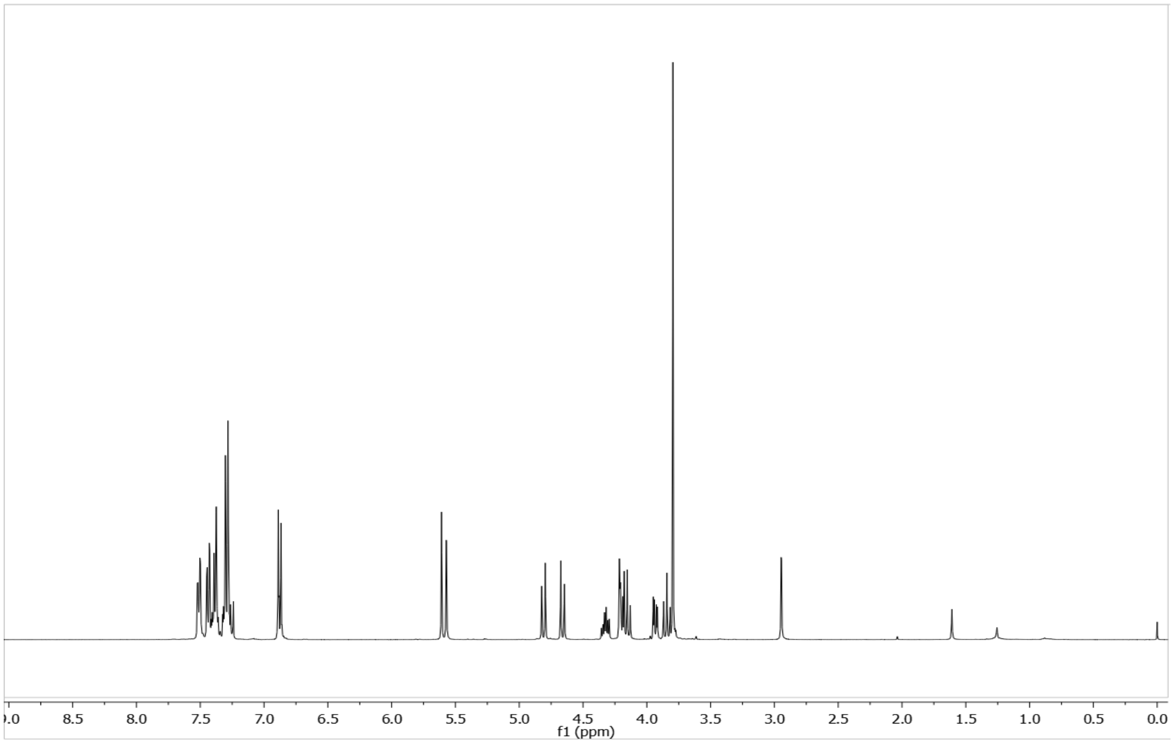
ESI-MS, calculated: 1816.6548; found $[M-H]^-$ 1815.6285, $[M-2H]^{2-}$ 907.3094



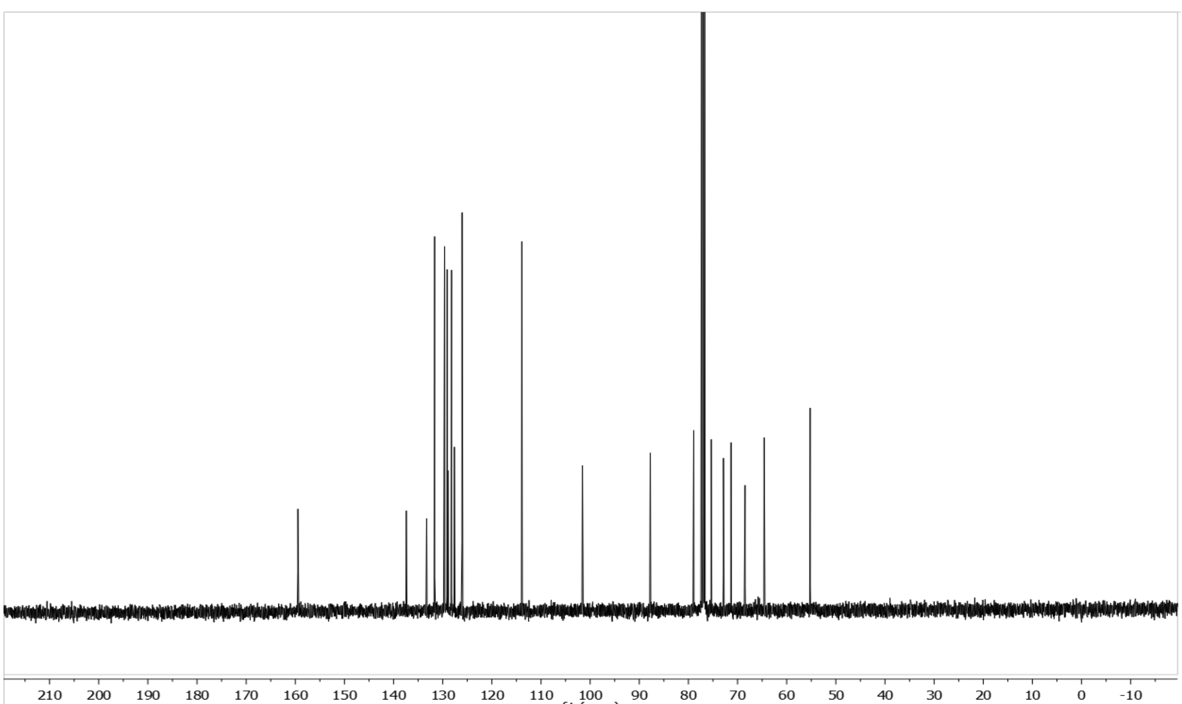
^{1}H NMR (600 MHz, D_2O) δ 7.94 – 7.79 (m, 2H), 7.77 – 7.54 (m, 2H), 7.51 – 7.30 (m, 4H), 5.03 (d, *J* = 4.1 Hz, 2H), 4.57 – 4.52 (m, 1H), 4.52 – 4.43 (m, 1H), 4.37 (dd, *J* = 14.6, 7.7 Hz, 2H), 4.33 – 4.25 (m, 1H), 4.21 (dd, *J* = 6.3, 2.3 Hz, 1H), 4.13 – 4.07 (m, 1H), 4.07 – 3.98 (m, 1H), 3.93 (d, *J* = 10.7 Hz, 1H), 3.90 – 3.54 (m, 32H), 3.54 – 3.38 (m, 10H), 3.31 (t, *J* = 9.7 Hz, 1H), 2.70 (dd, *J* = 12.5, 4.5 Hz, 1H), 1.96 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H), 1.77 (t, *J* = 12.1 Hz, 1H), 1.15 – 1.05 (m, 6H), 0.99 (d, *J* = 6.3 Hz, 3H).

V. NMR spectra

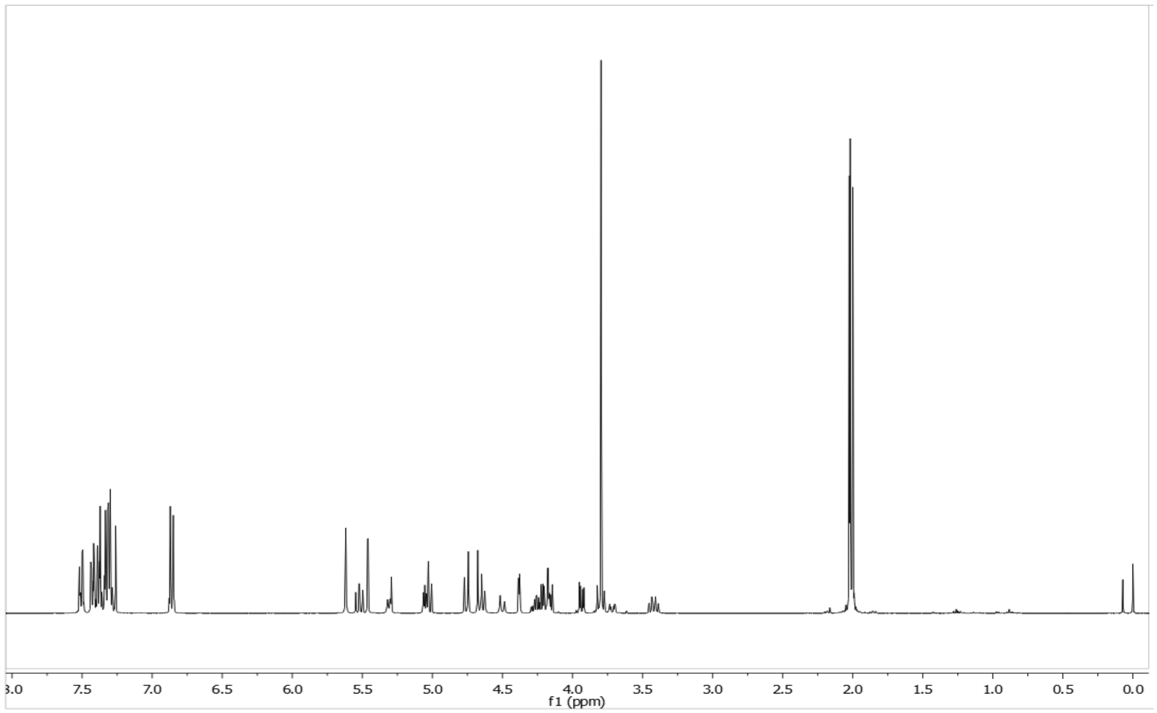
[1]H NMR of Compound 1



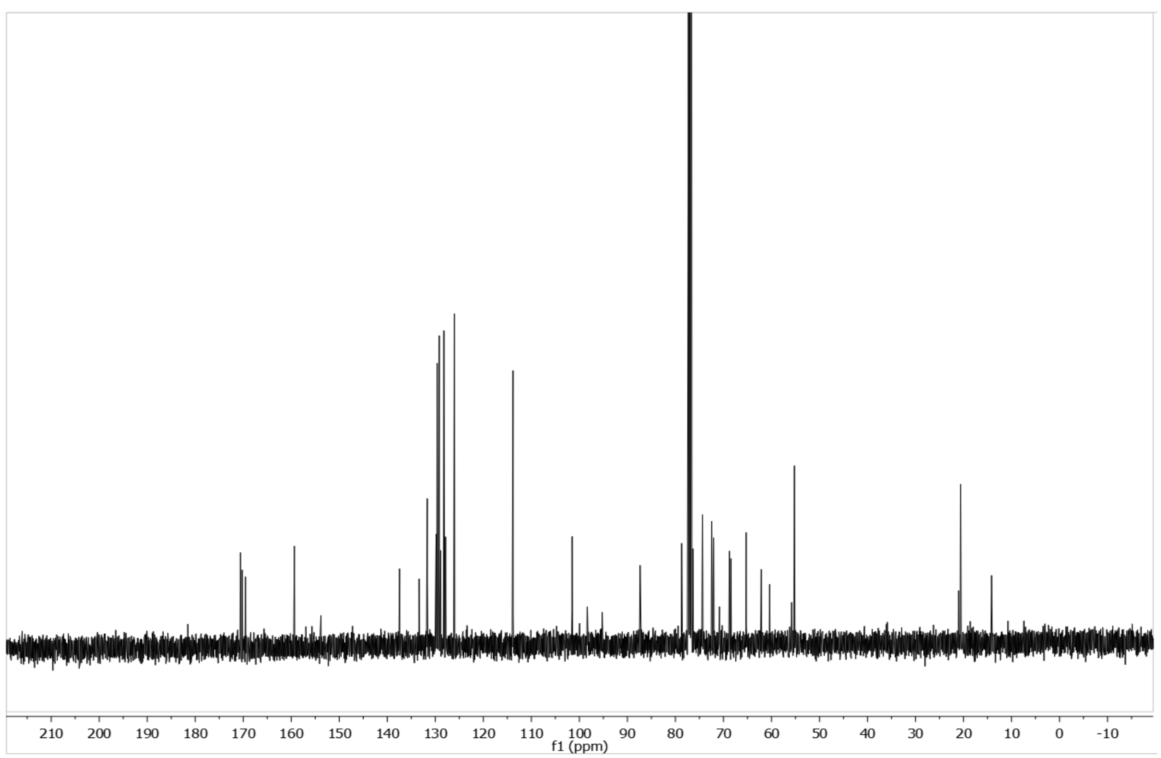
[13]C NMR of Compound 1



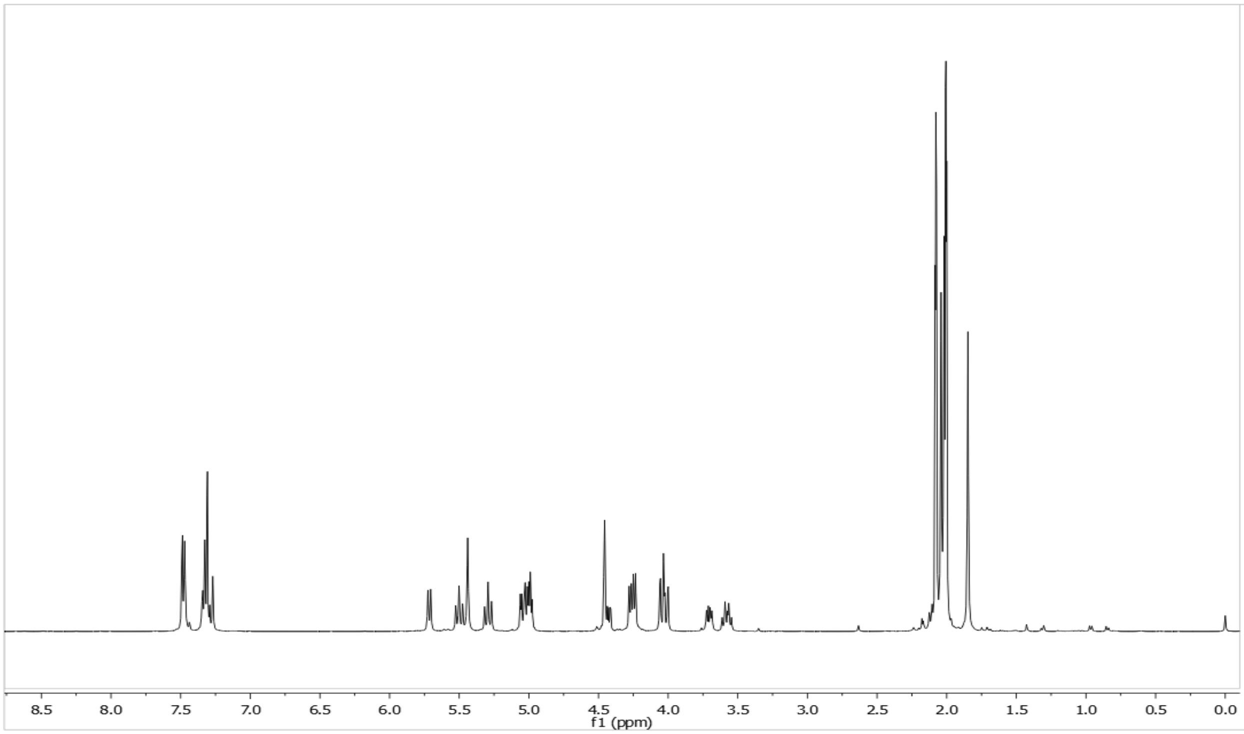
[1]H NMR of Compound 5



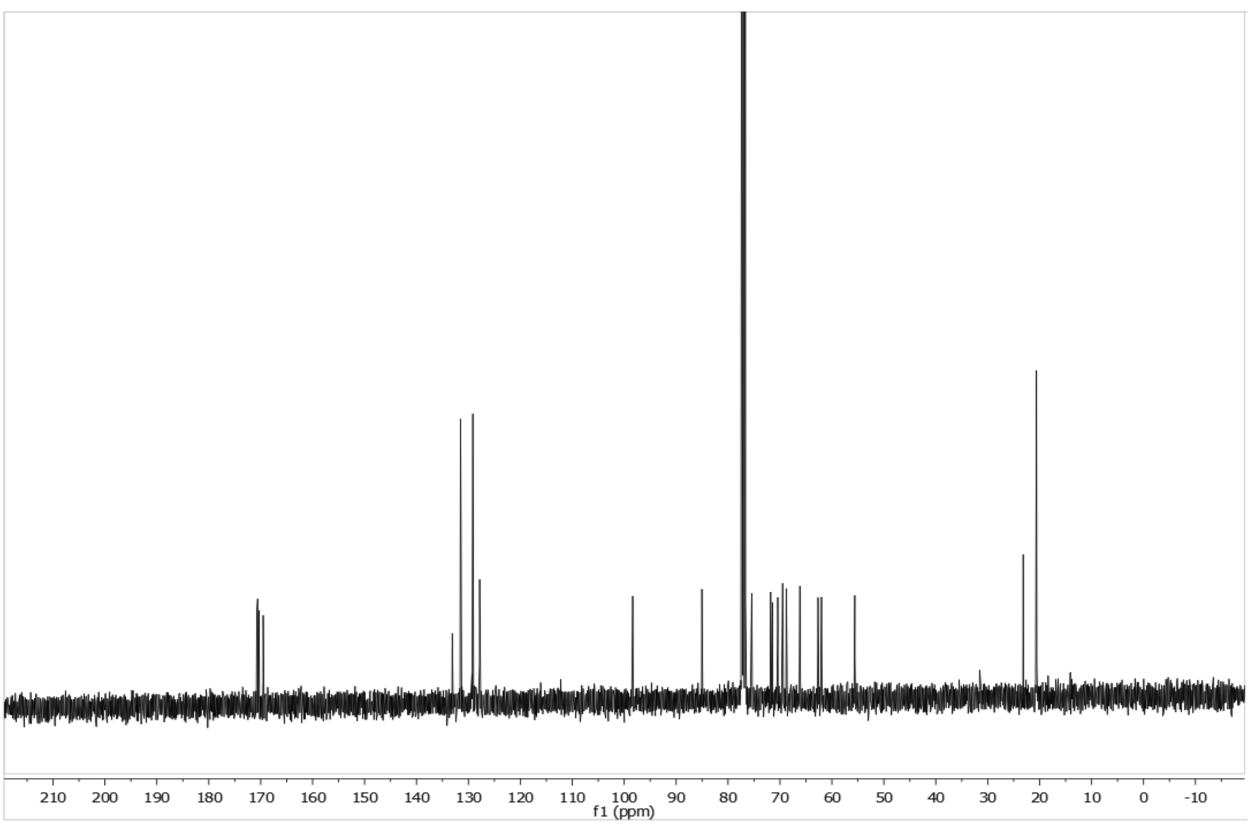
[13]C NMR of Compound 5



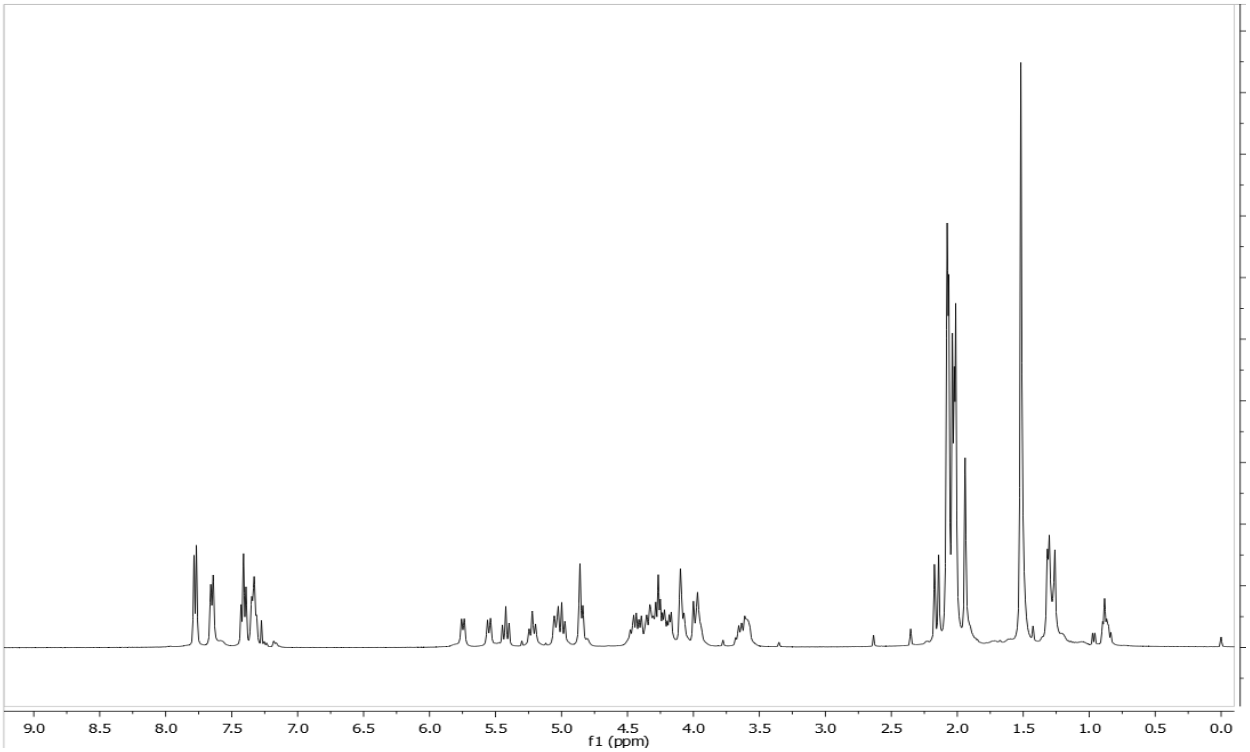
[1]H NMR of Compound 6



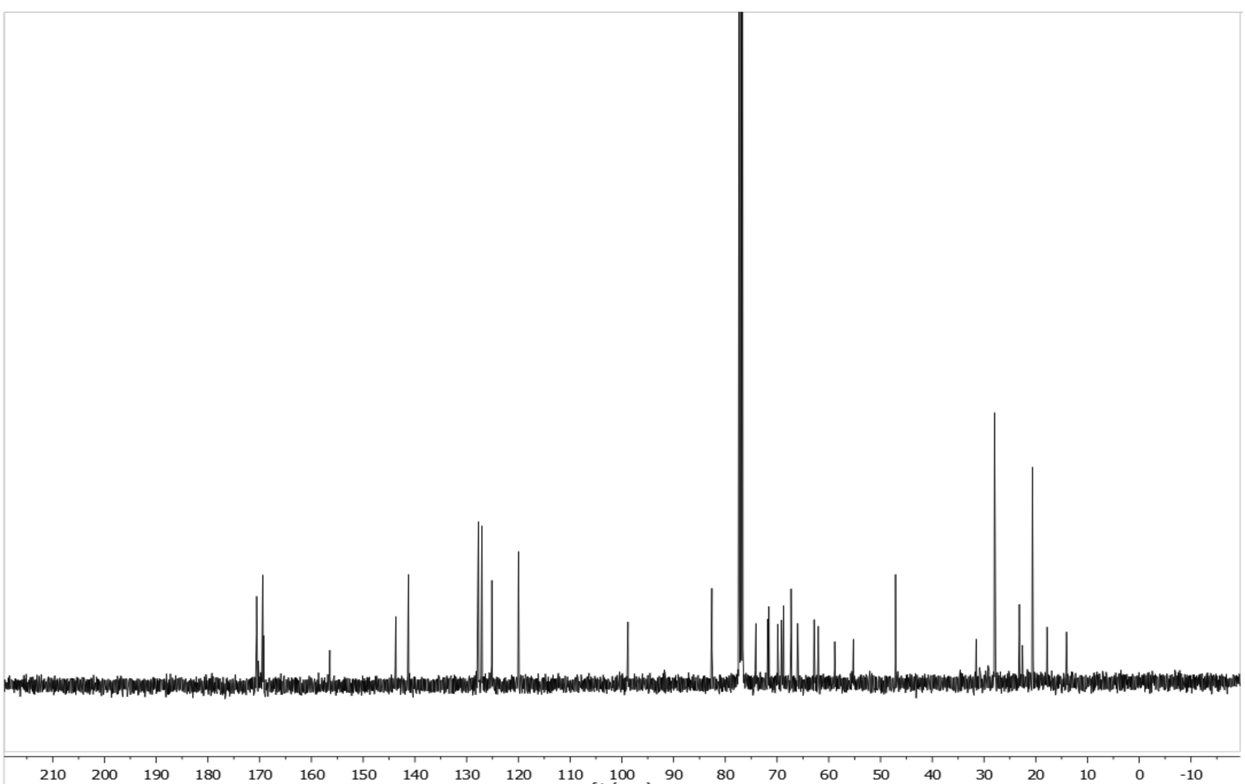
[13]C NMR of Compound 6



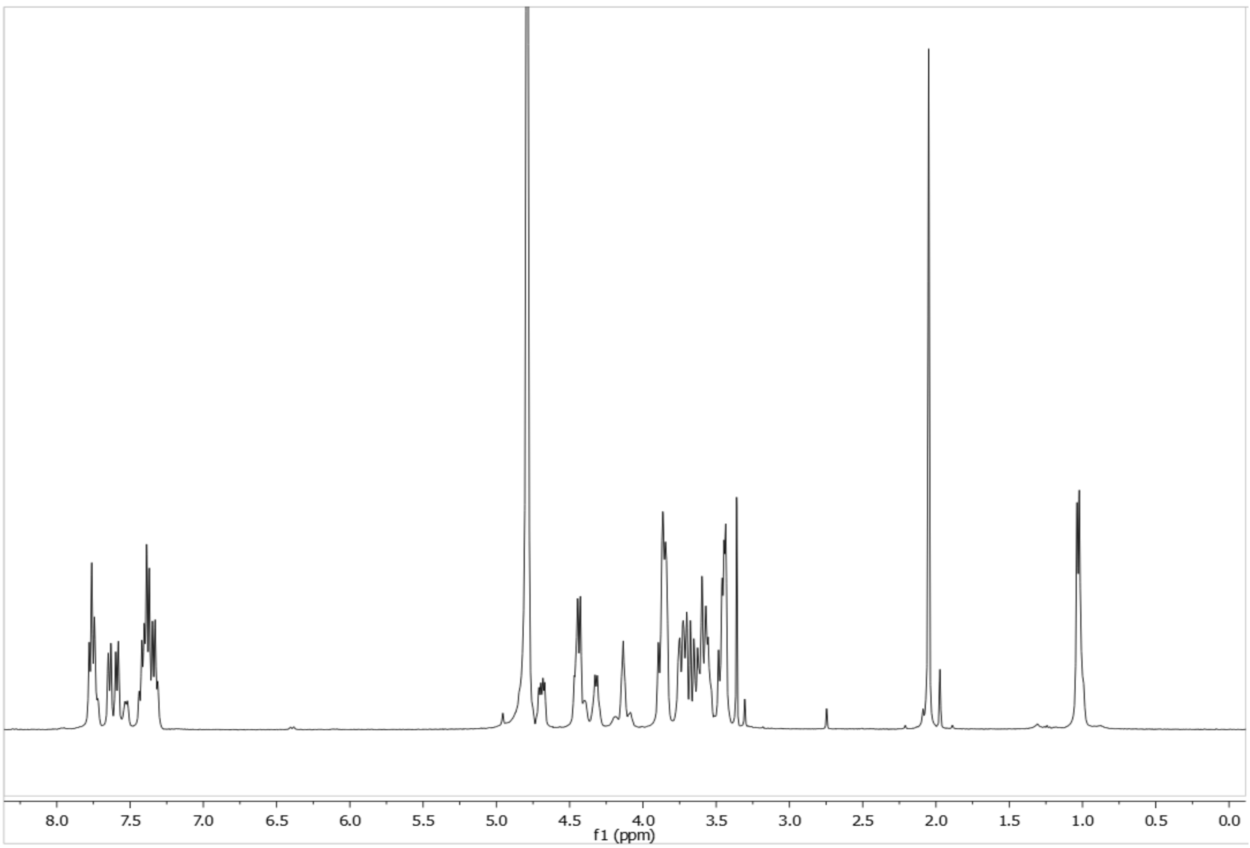
[1]H NMR of Compound 7



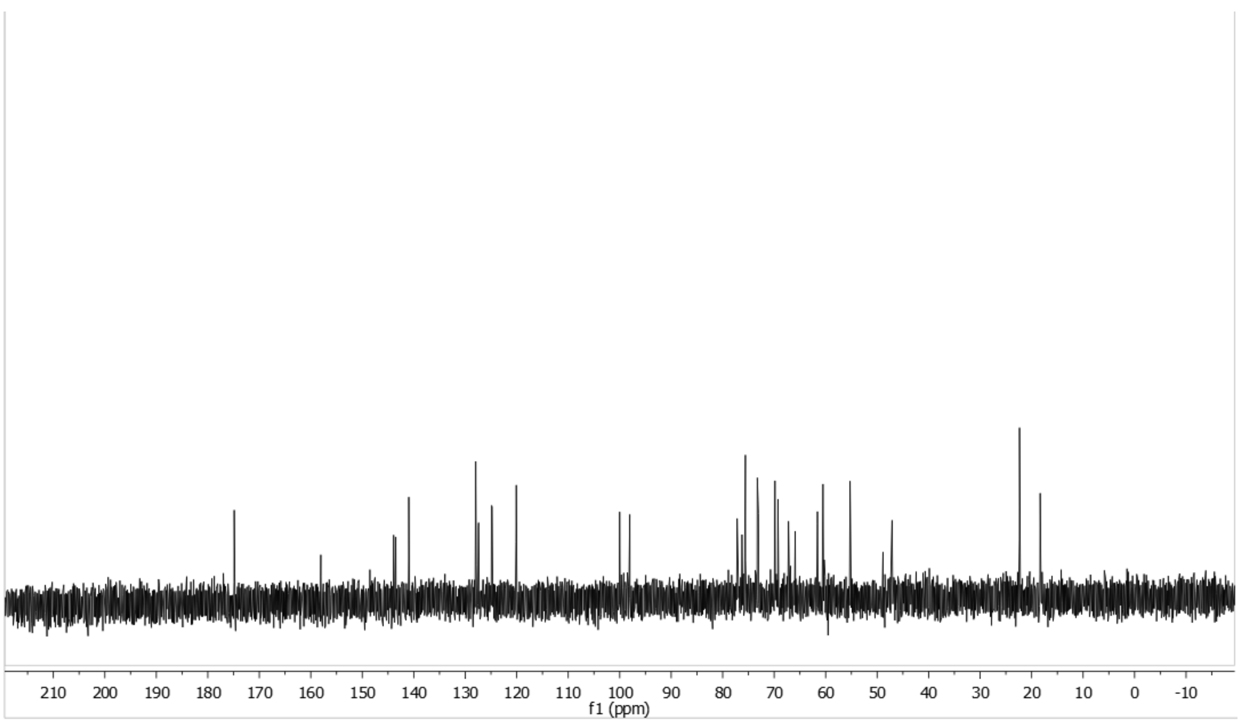
[13]C NMR of Compound 7



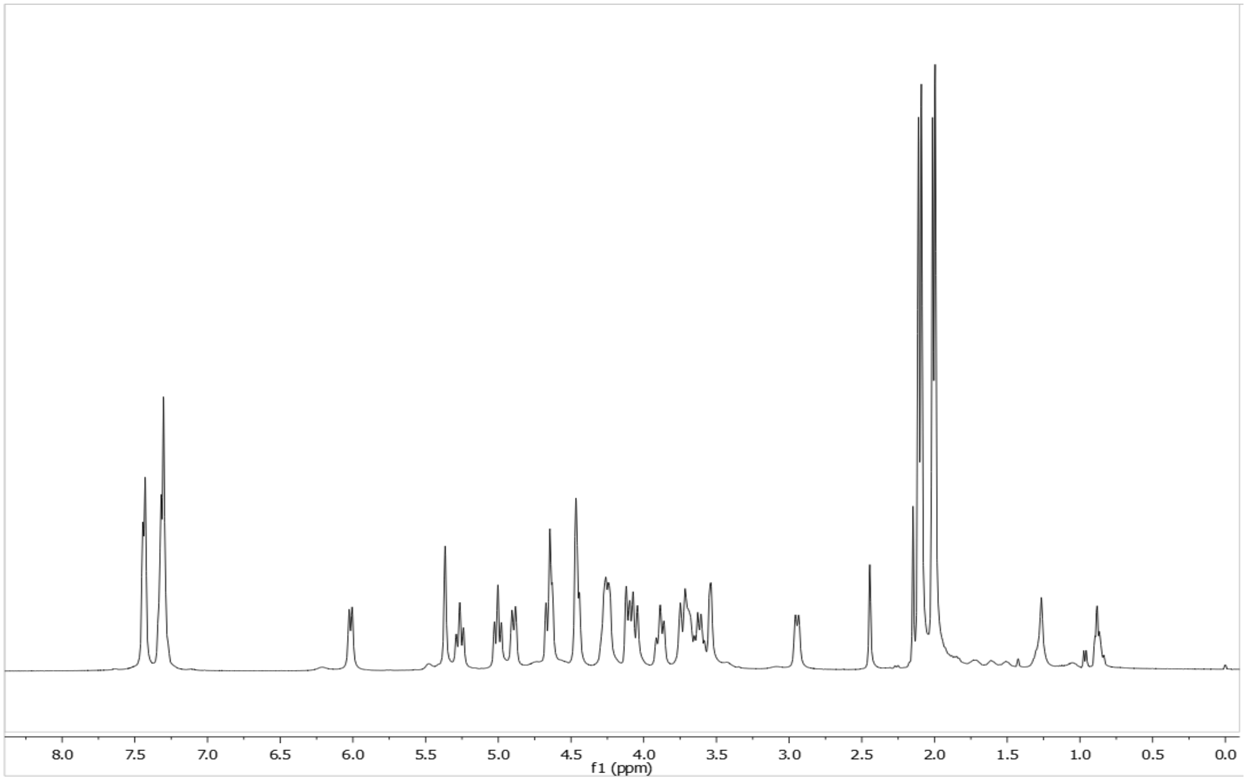
^{1}H NMR of Compound 8 (M100)



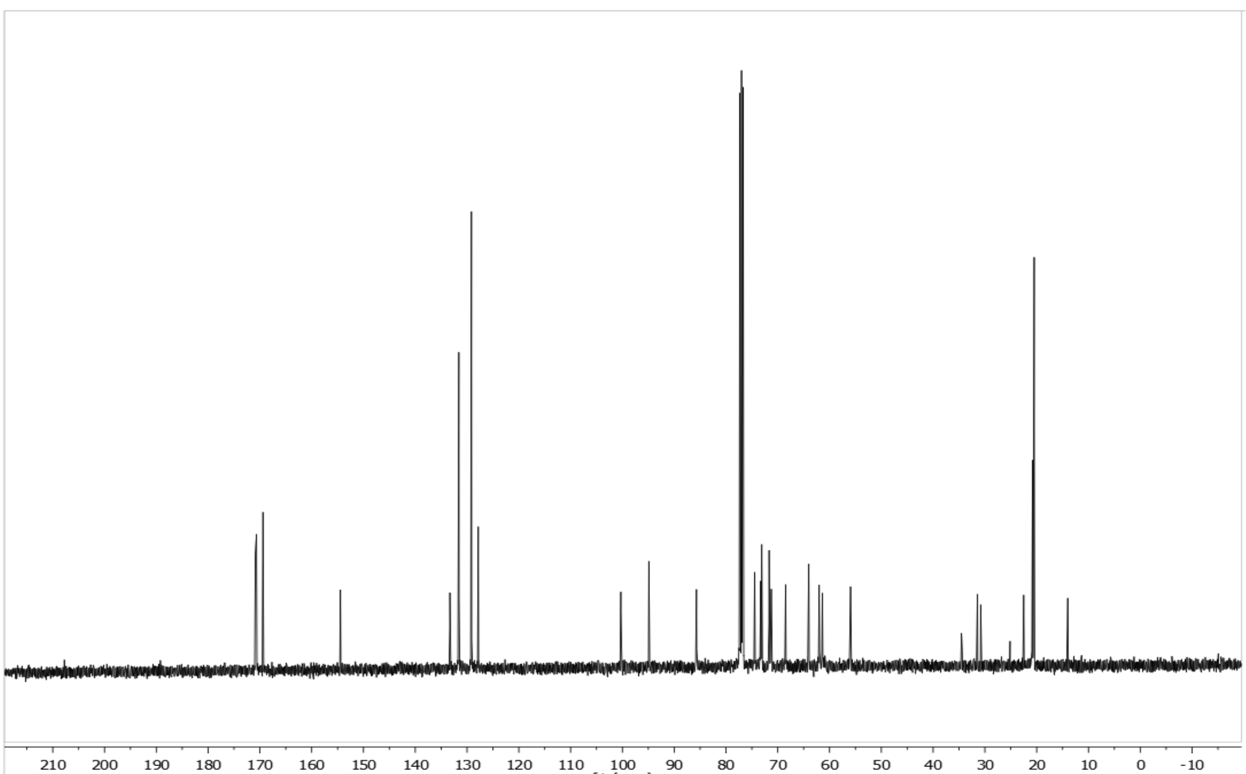
^{13}C NMR of Compound 8 (M100)



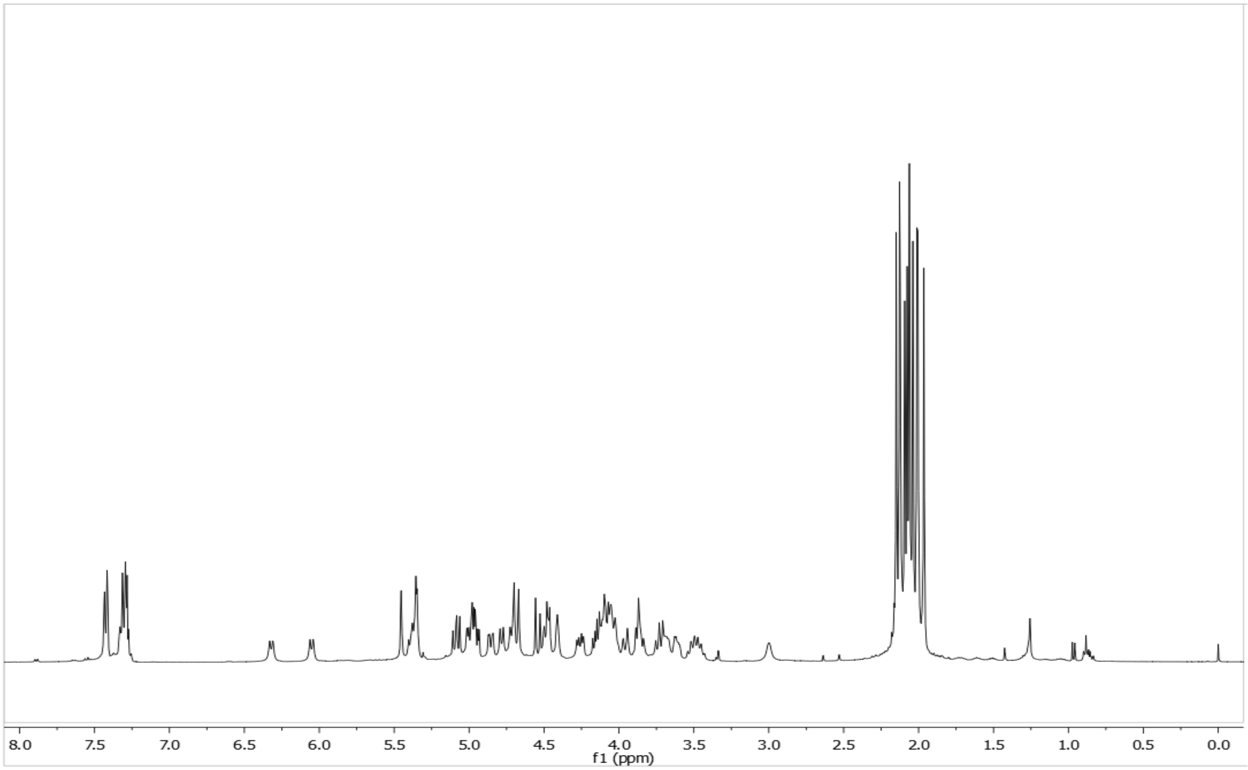
^{1}H NMR of Compound 9



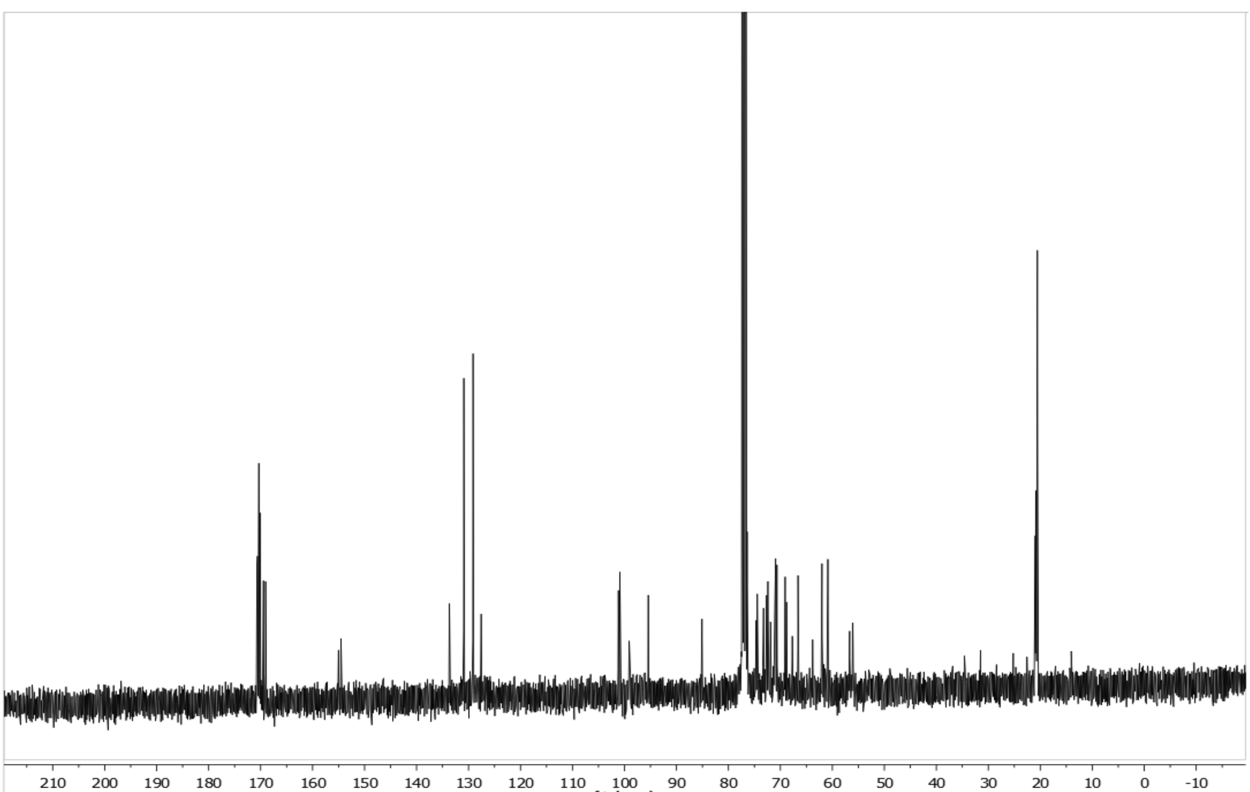
^{13}C NMR of Compound 9



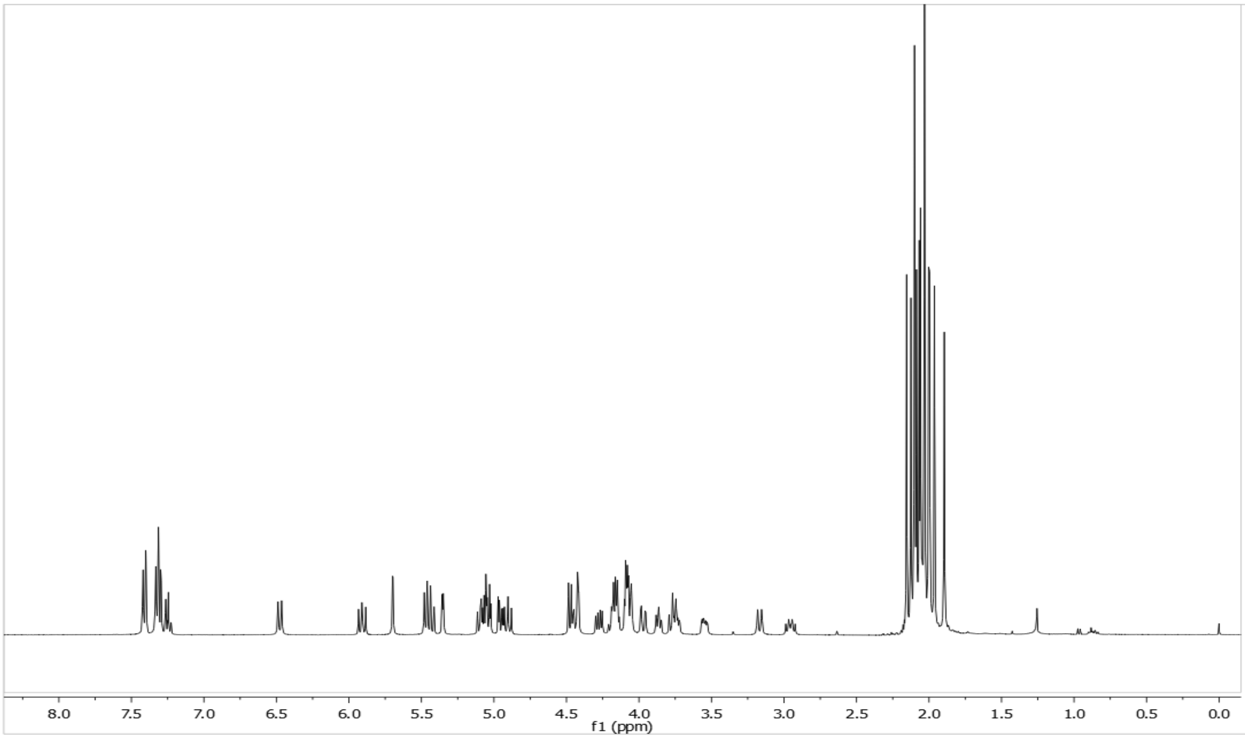
^{1}H NMR of Compound 10



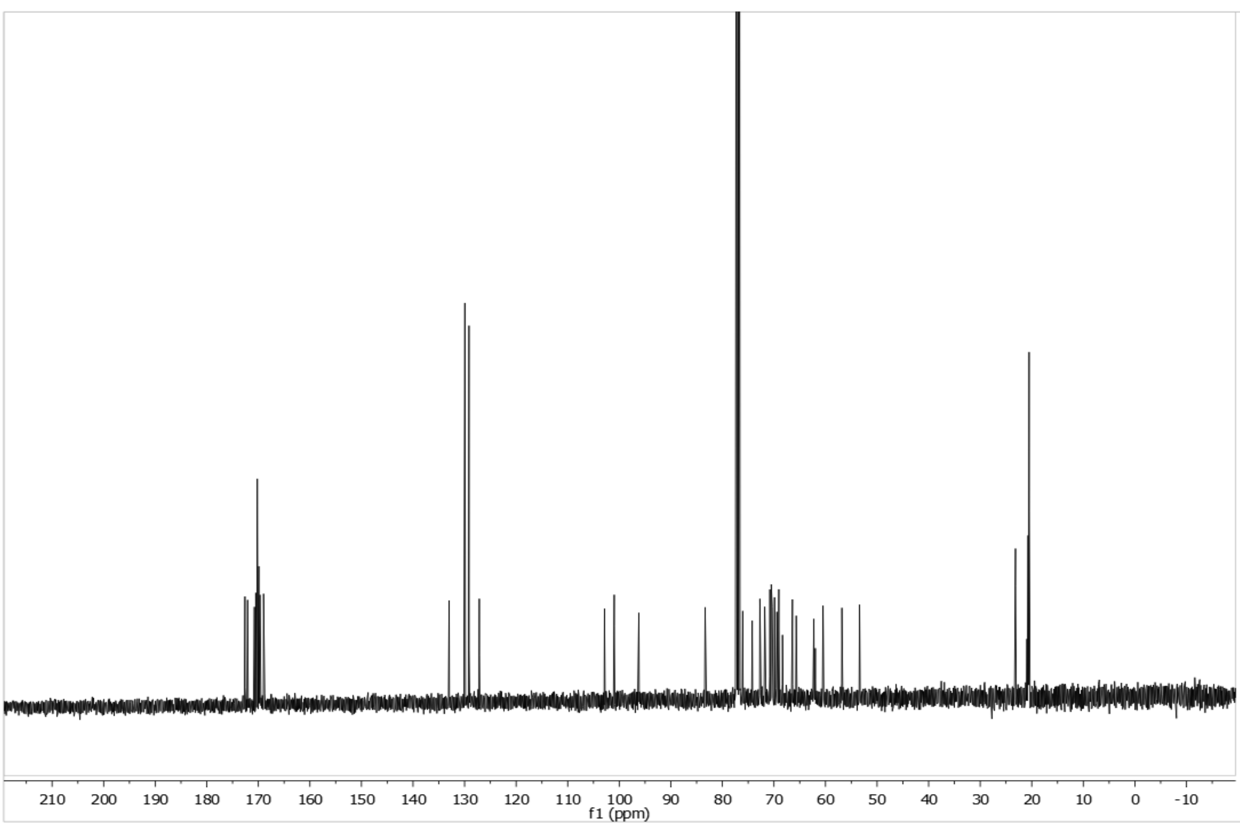
^{13}C NMR of Compound 10



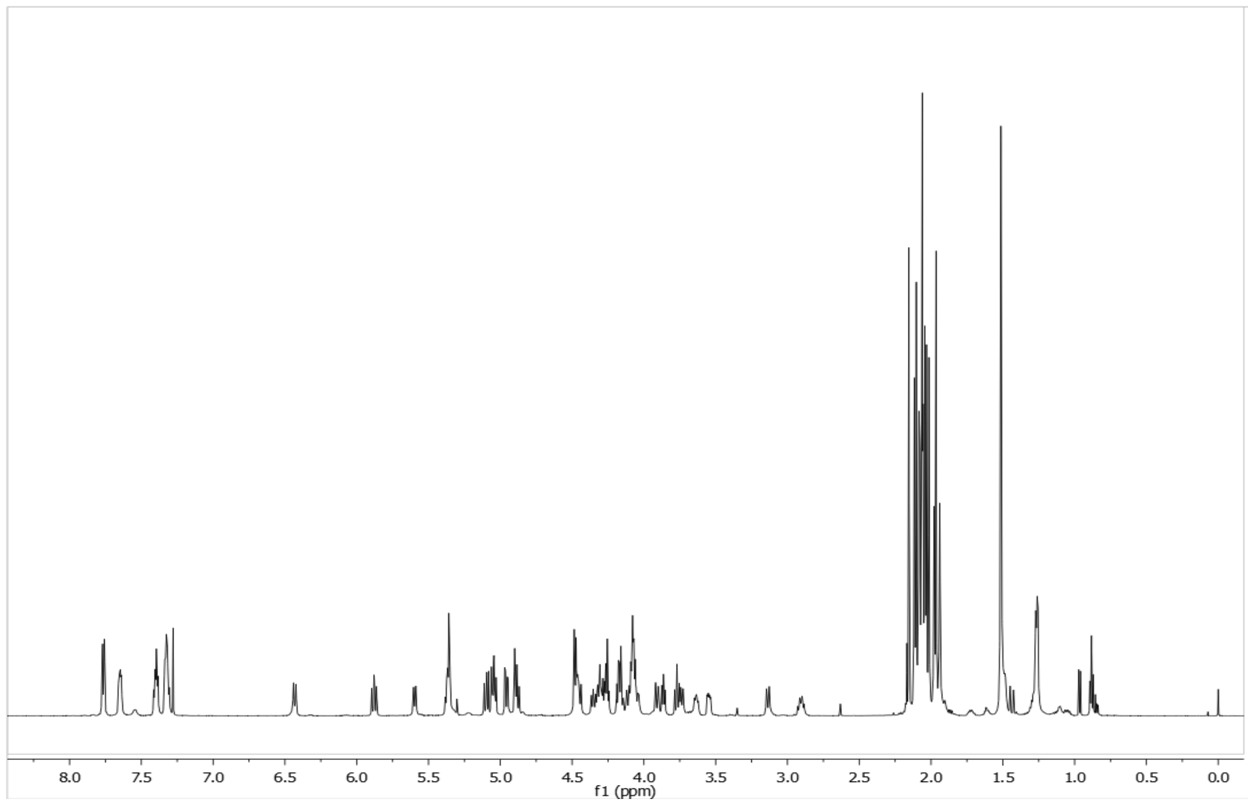
[1]H NMR of Compound 11



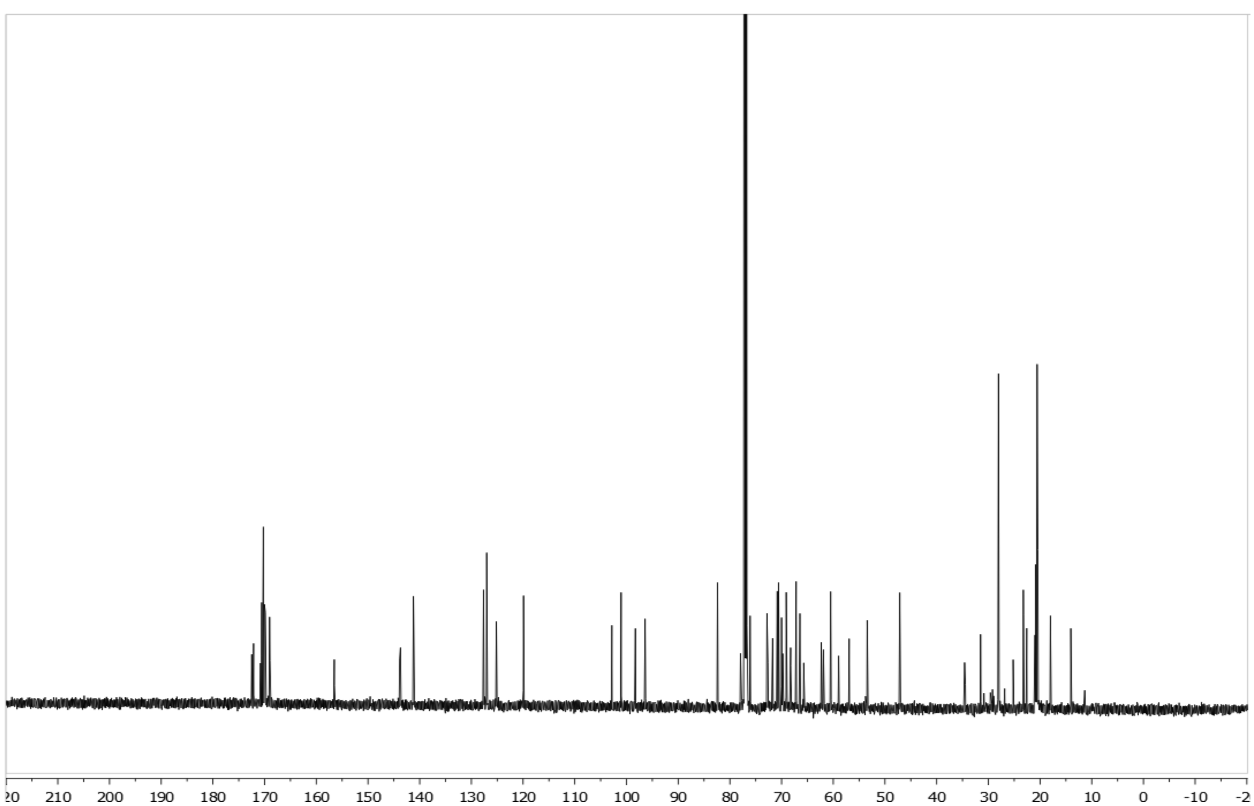
[13]C NMR of Compound 11



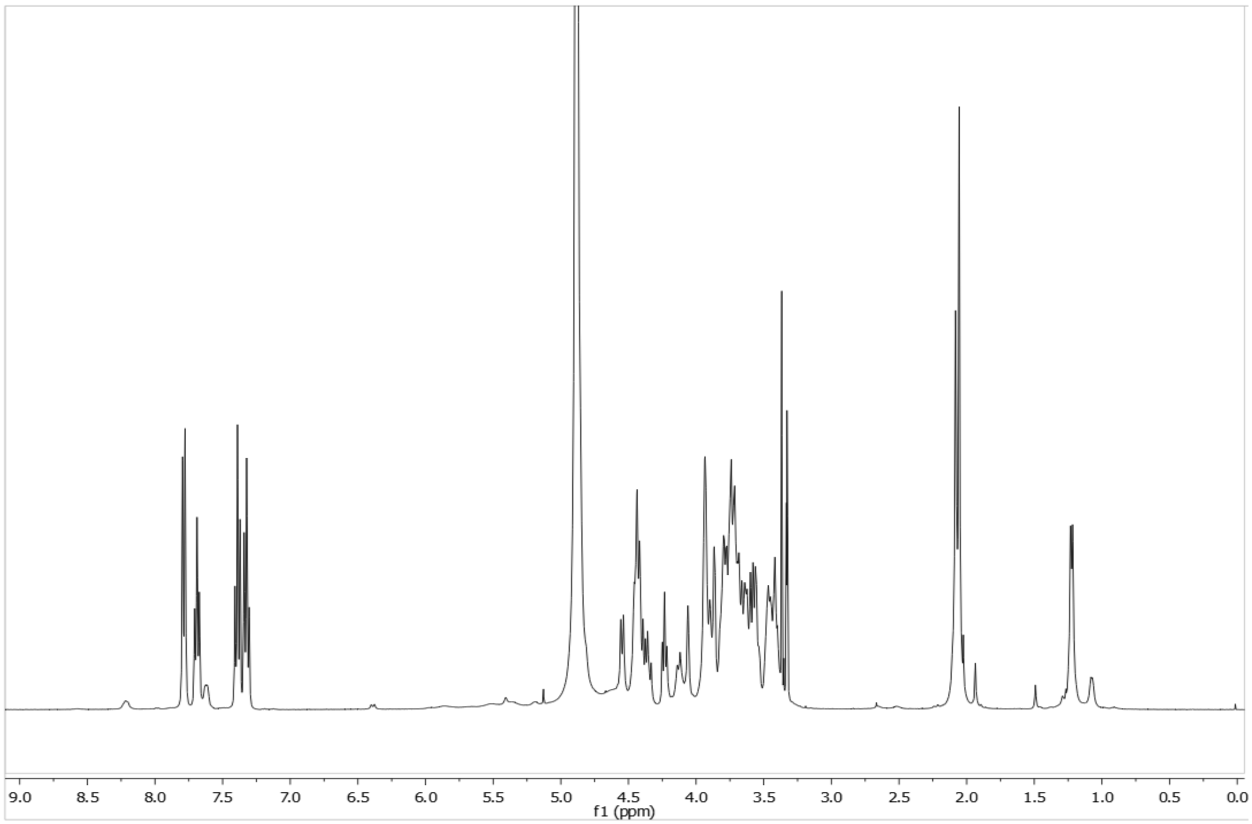
[1]H NMR of Compound 12



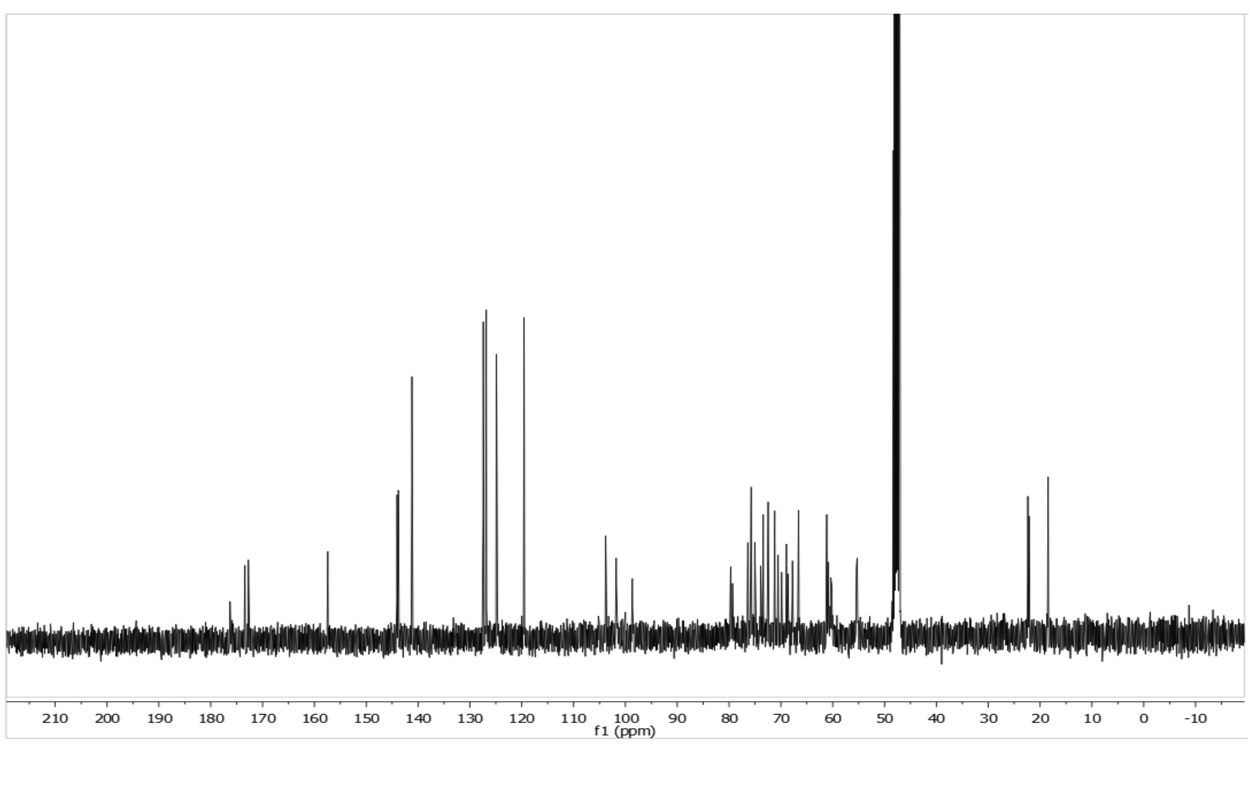
[13]C NMR of Compound 12



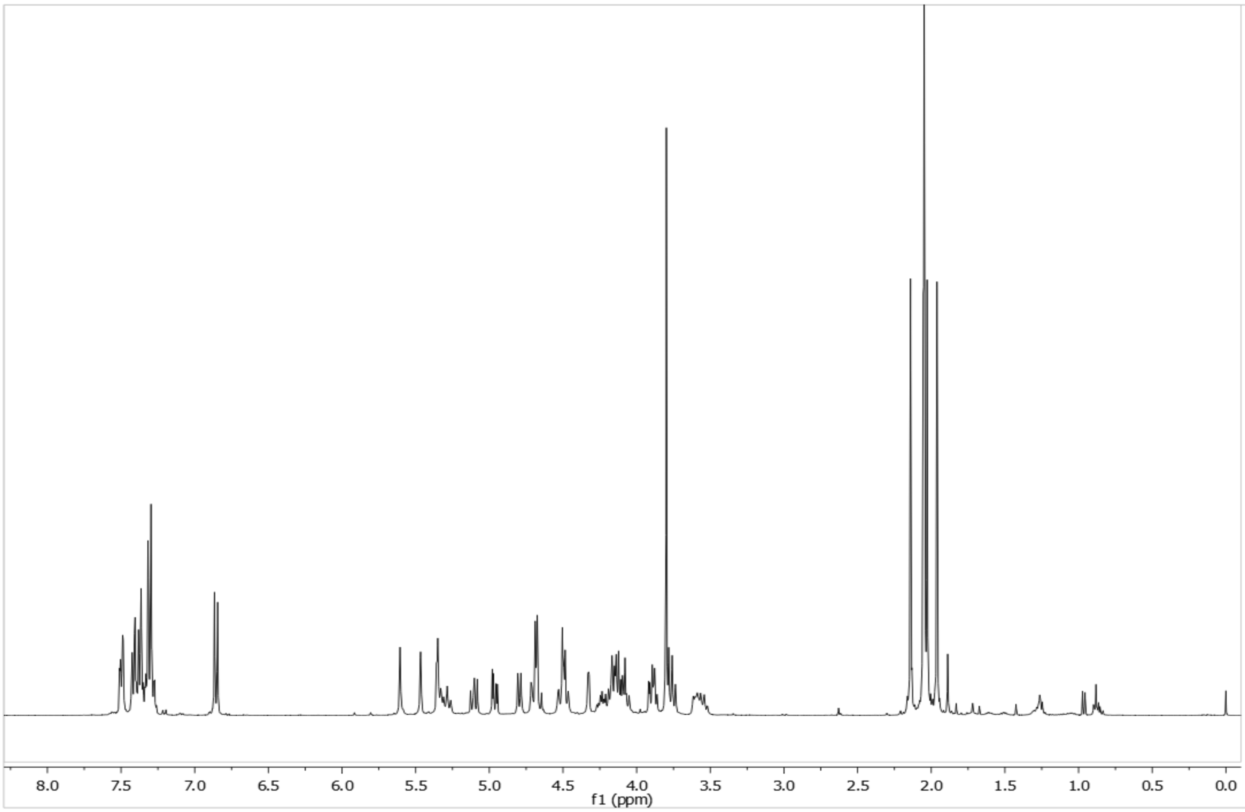
^{1}H NMR of Compound 13 (M301)



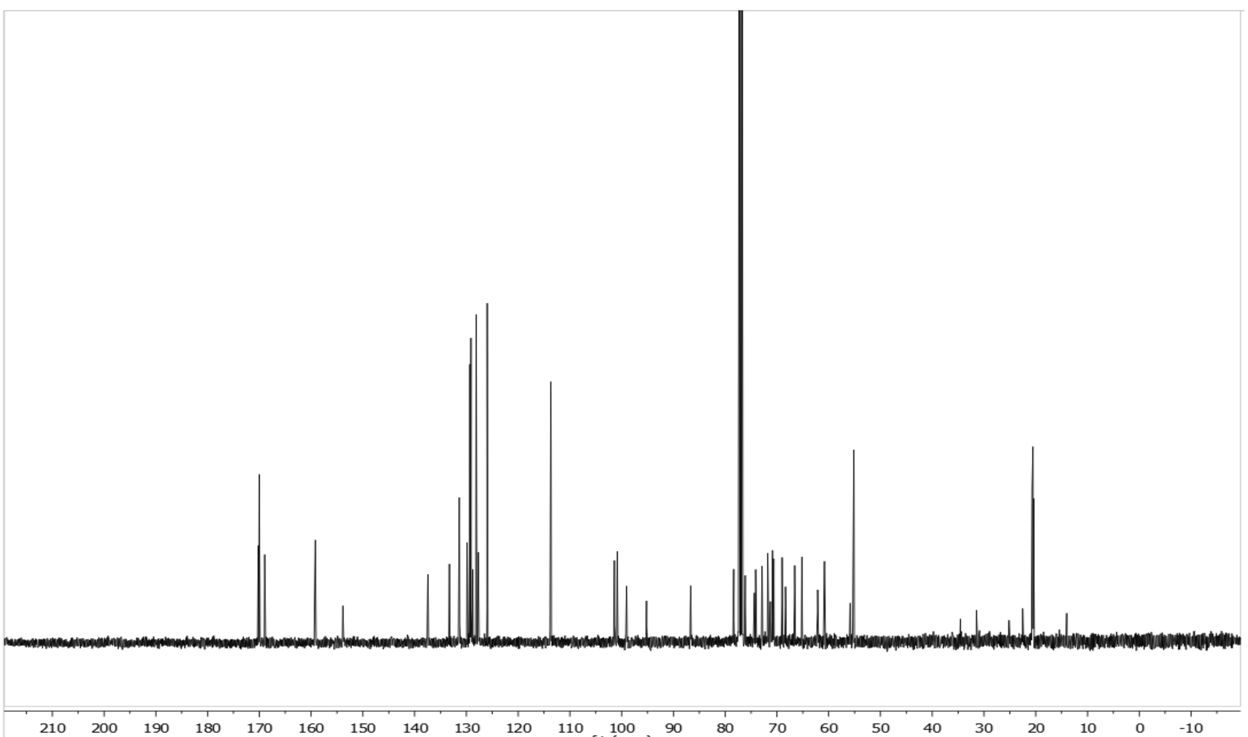
^{13}C NMR of Compound 13 (M301)



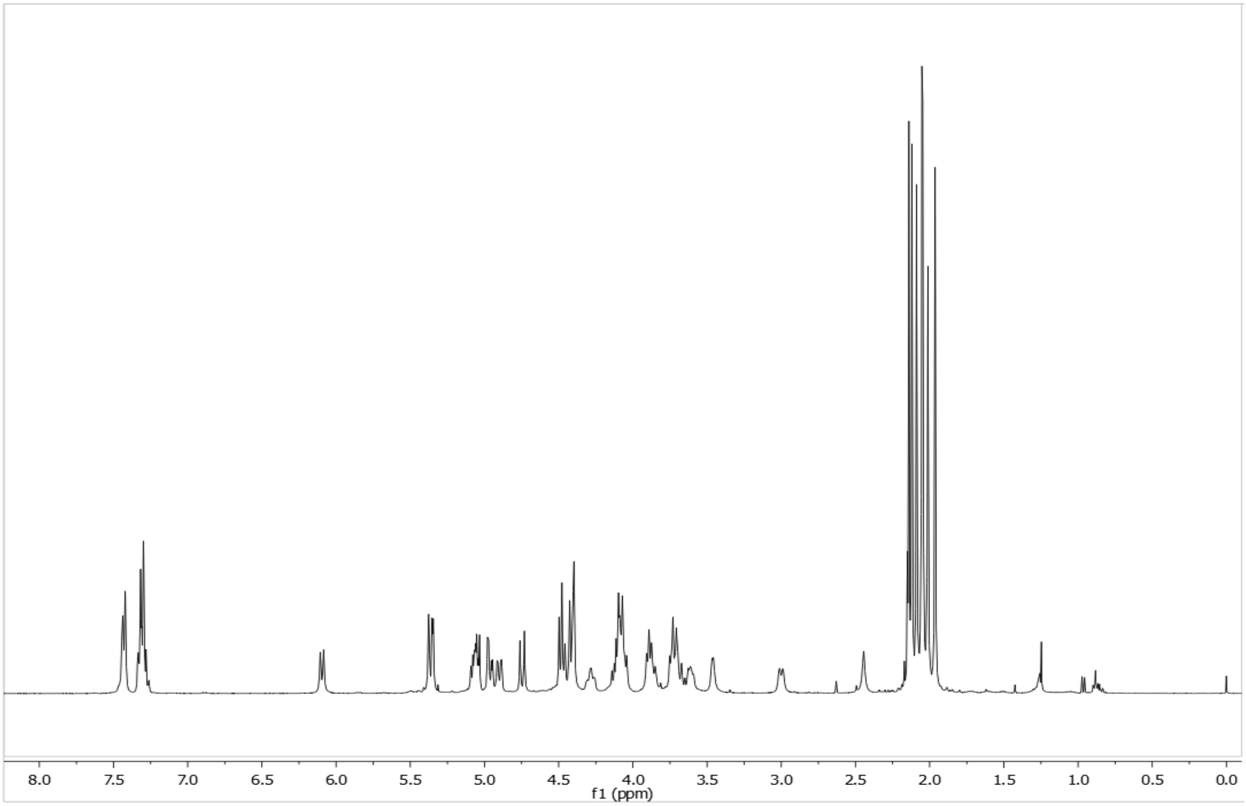
^{1}H NMR of Compound 14



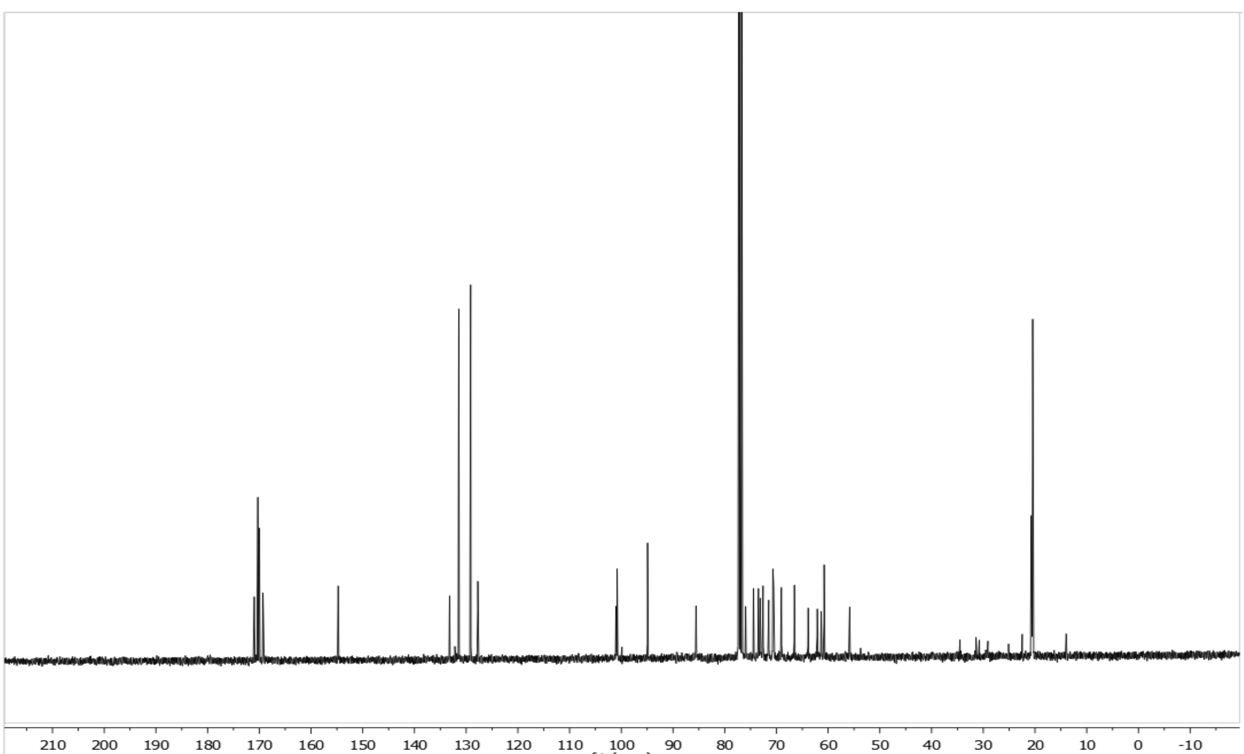
^{13}C NMR of Compound 14



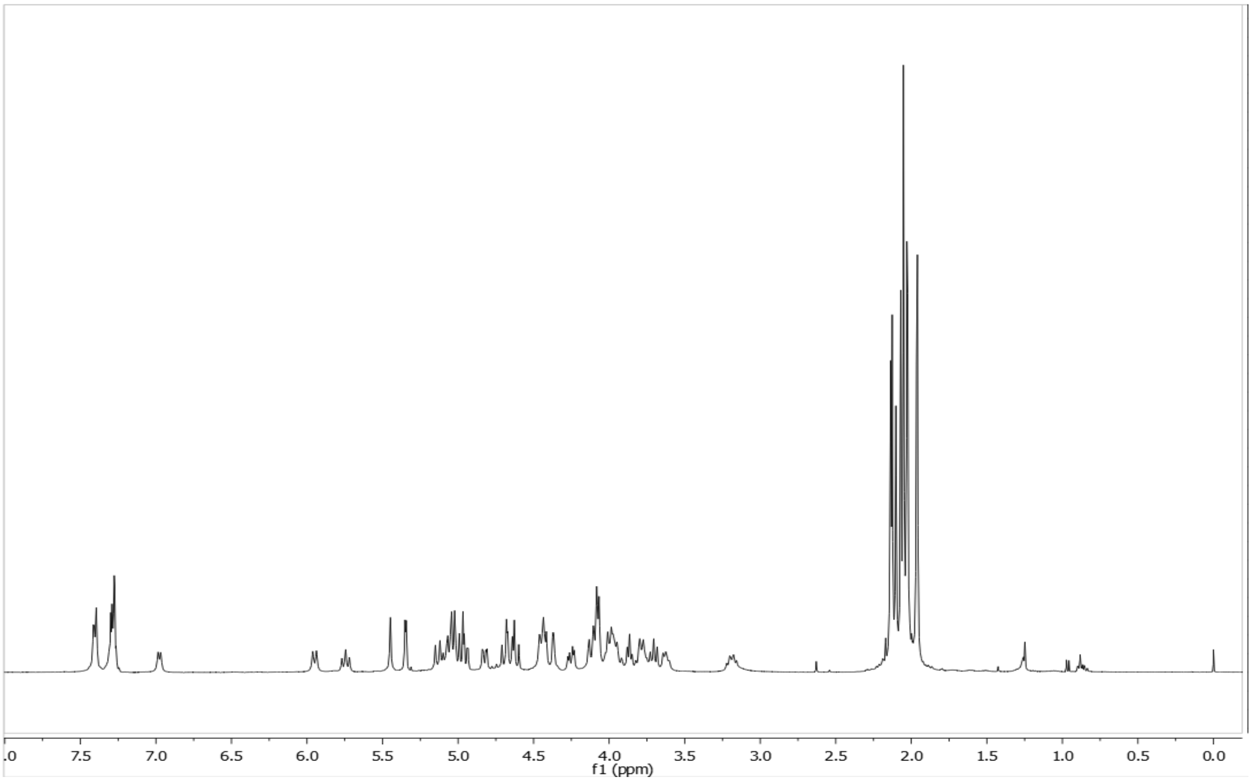
^{1}H NMR of Compound 15



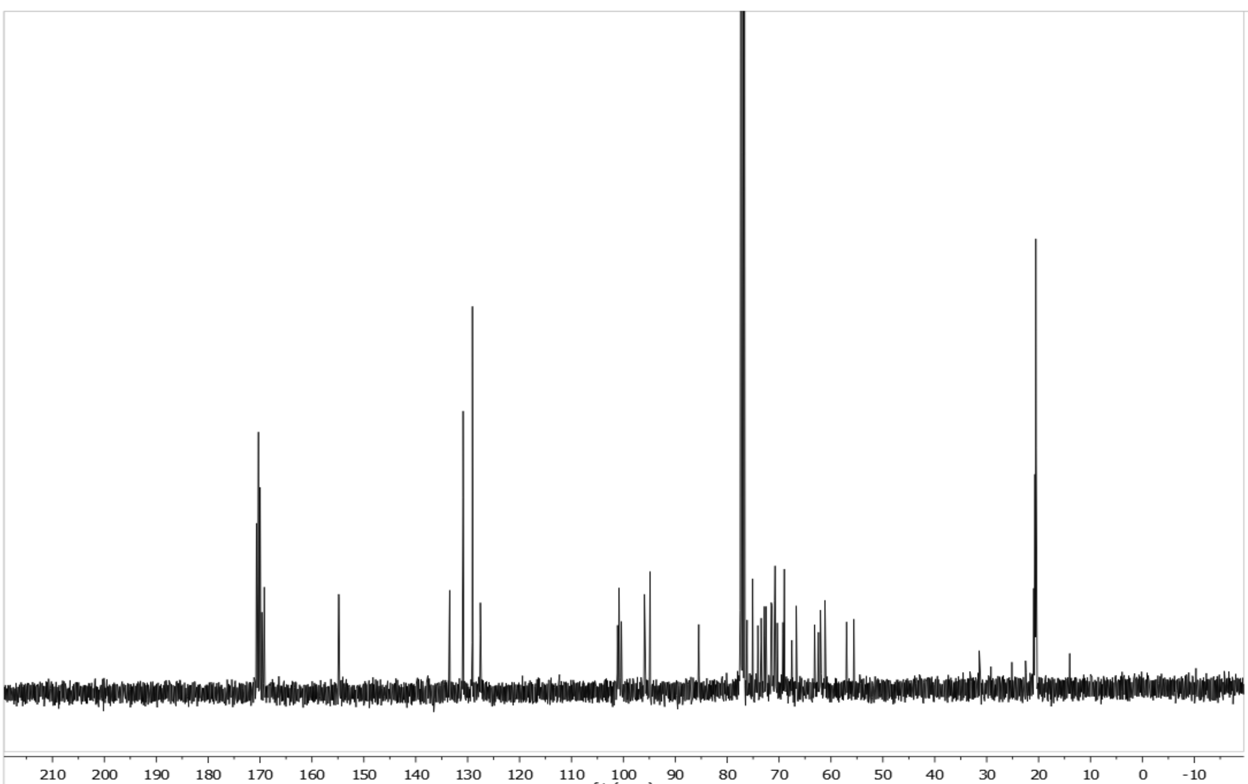
^{13}C NMR of Compound 15



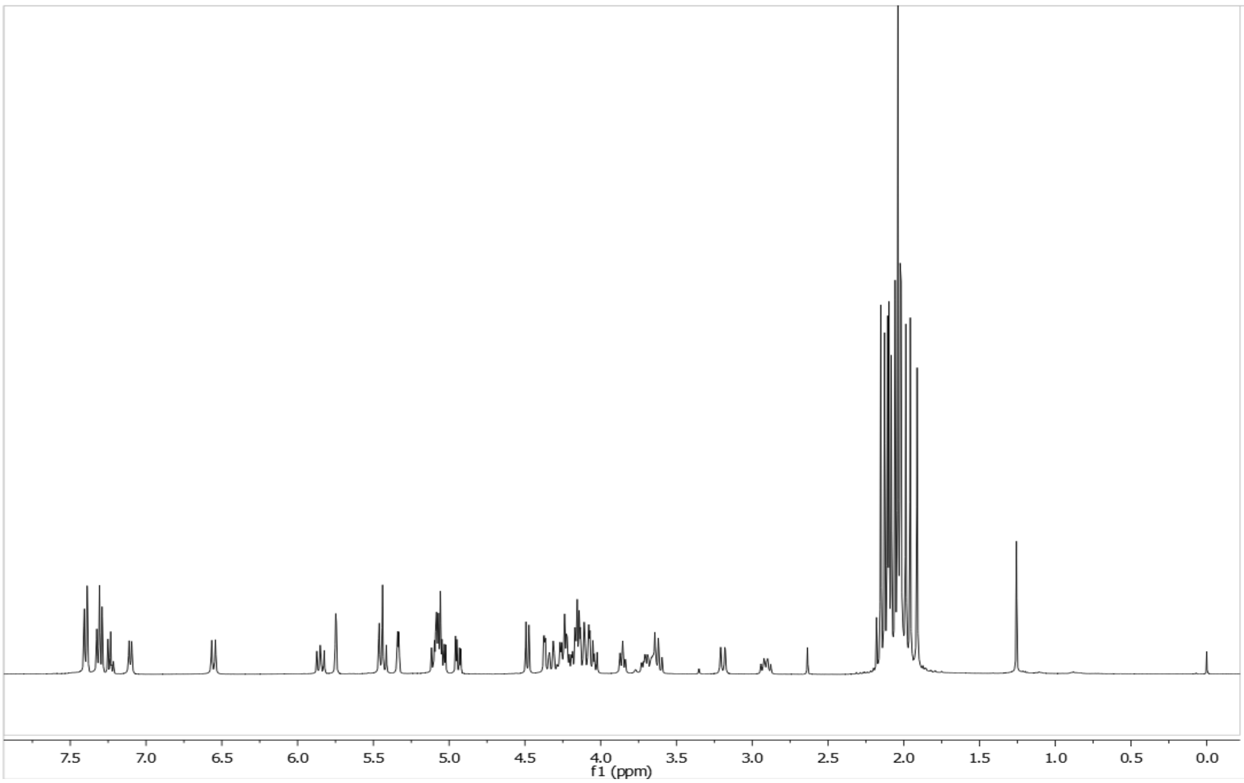
^{1}H NMR of Compound 16



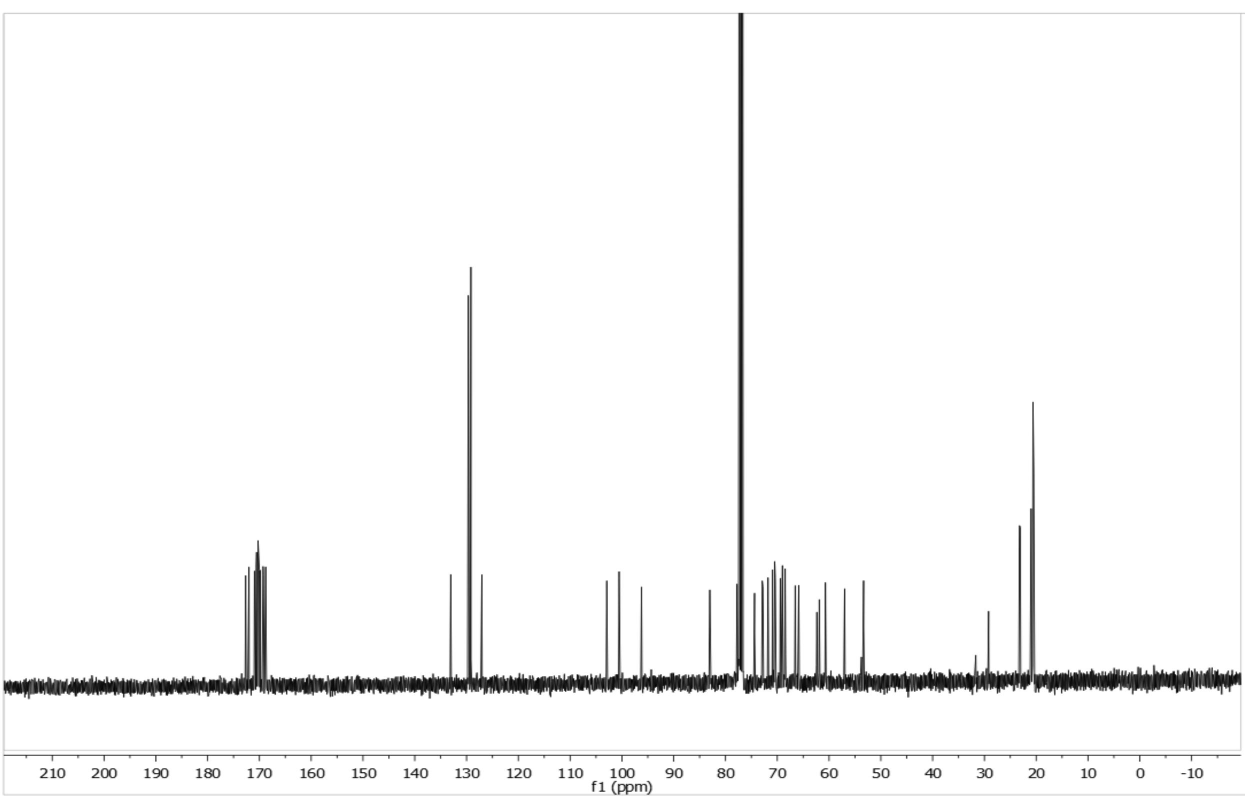
^{13}C NMR of Compound 16



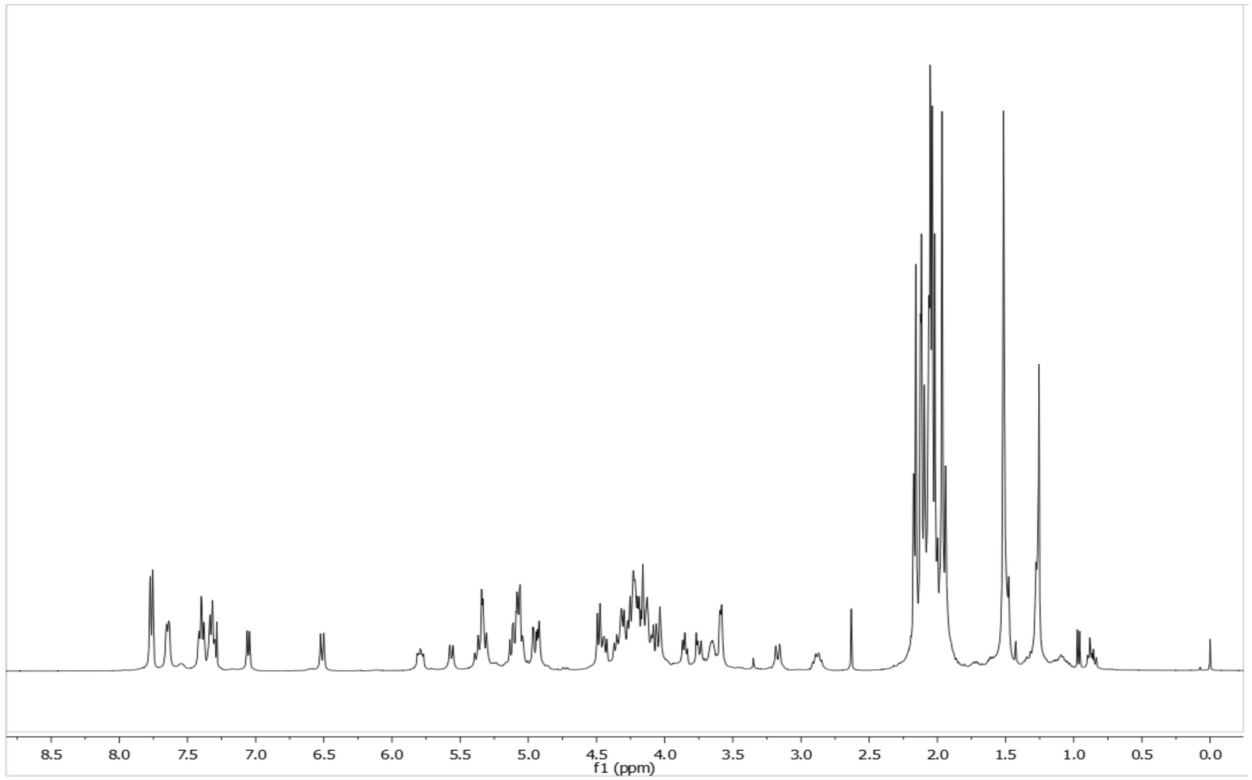
[1]H NMR of Compound 17



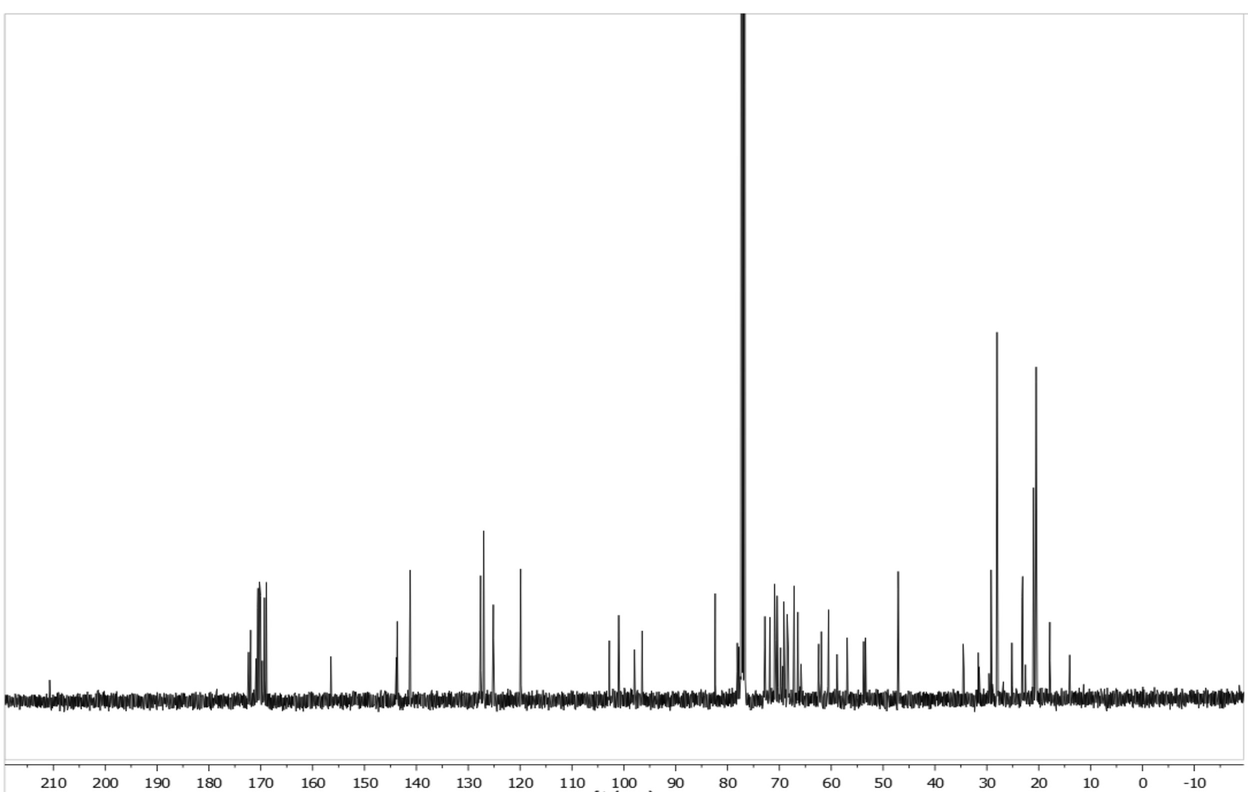
[13]C NMR of Compound 17



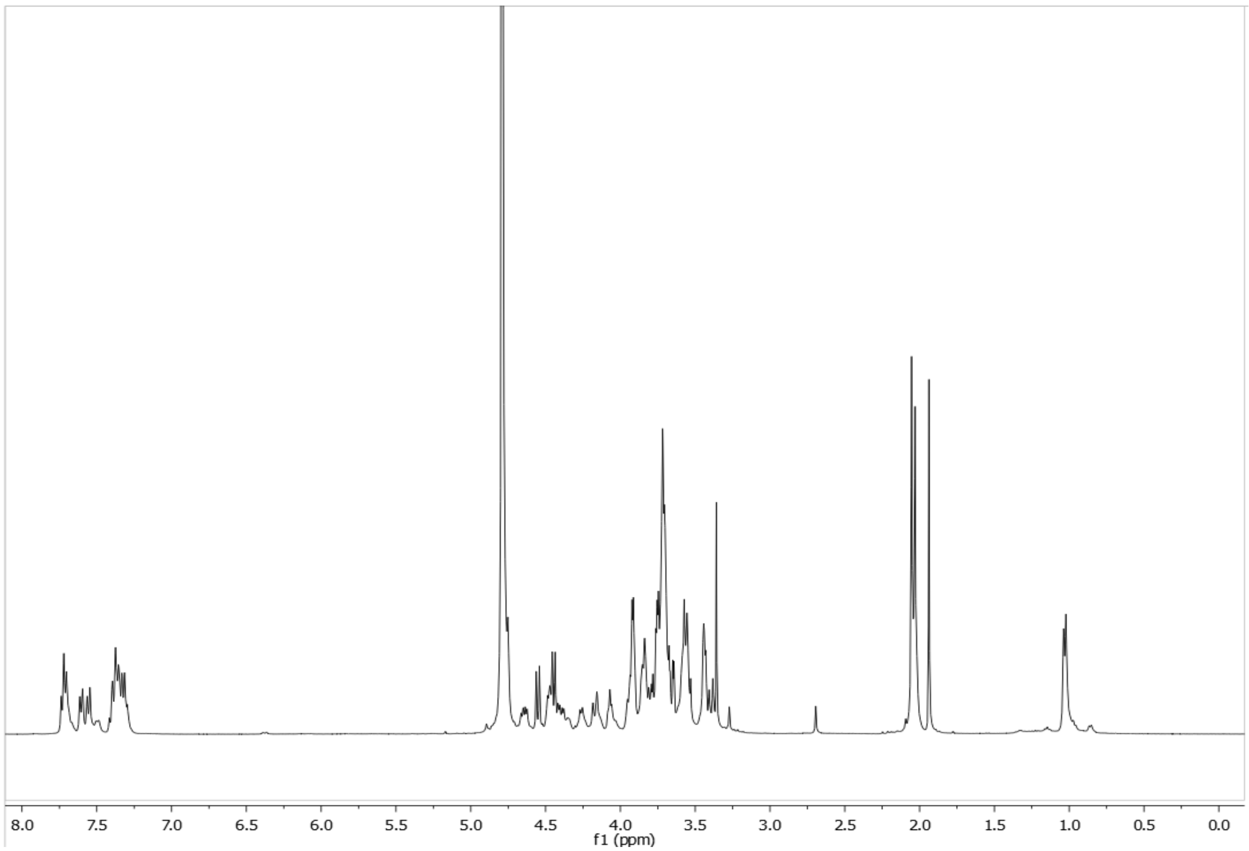
^{1}H NMR of Compound 18



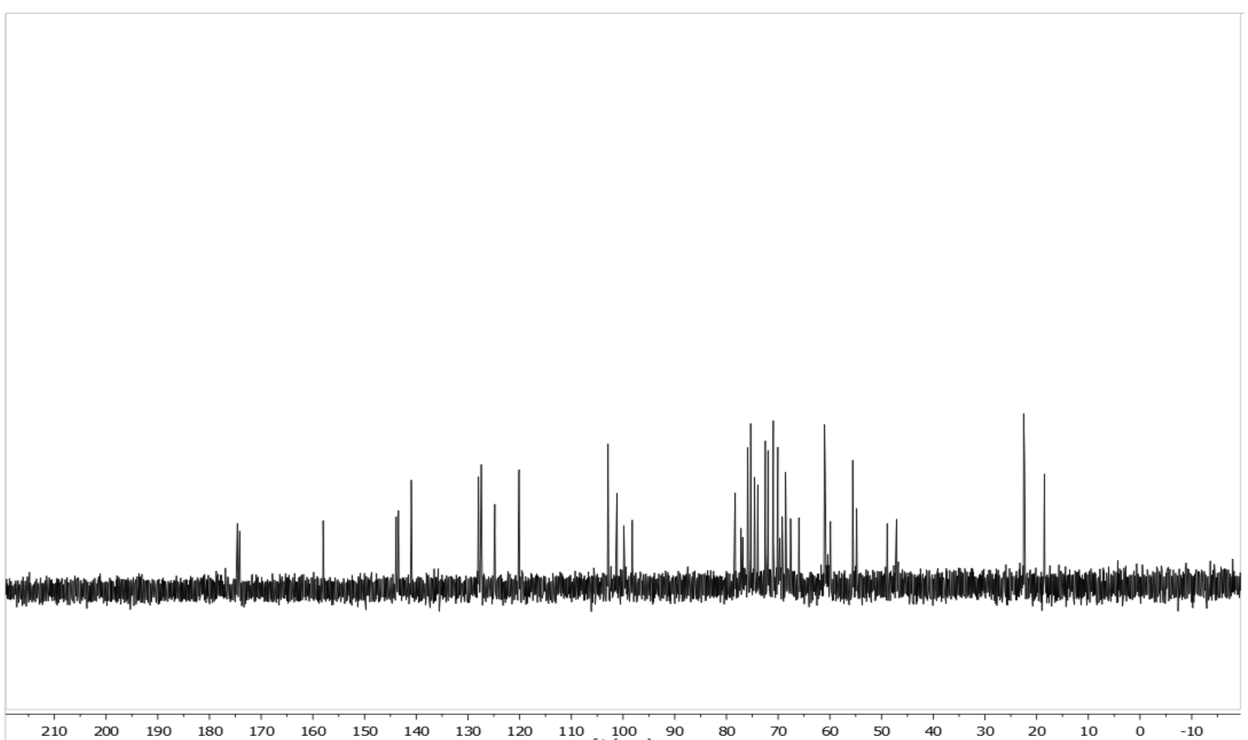
^{13}C NMR of Compound 18



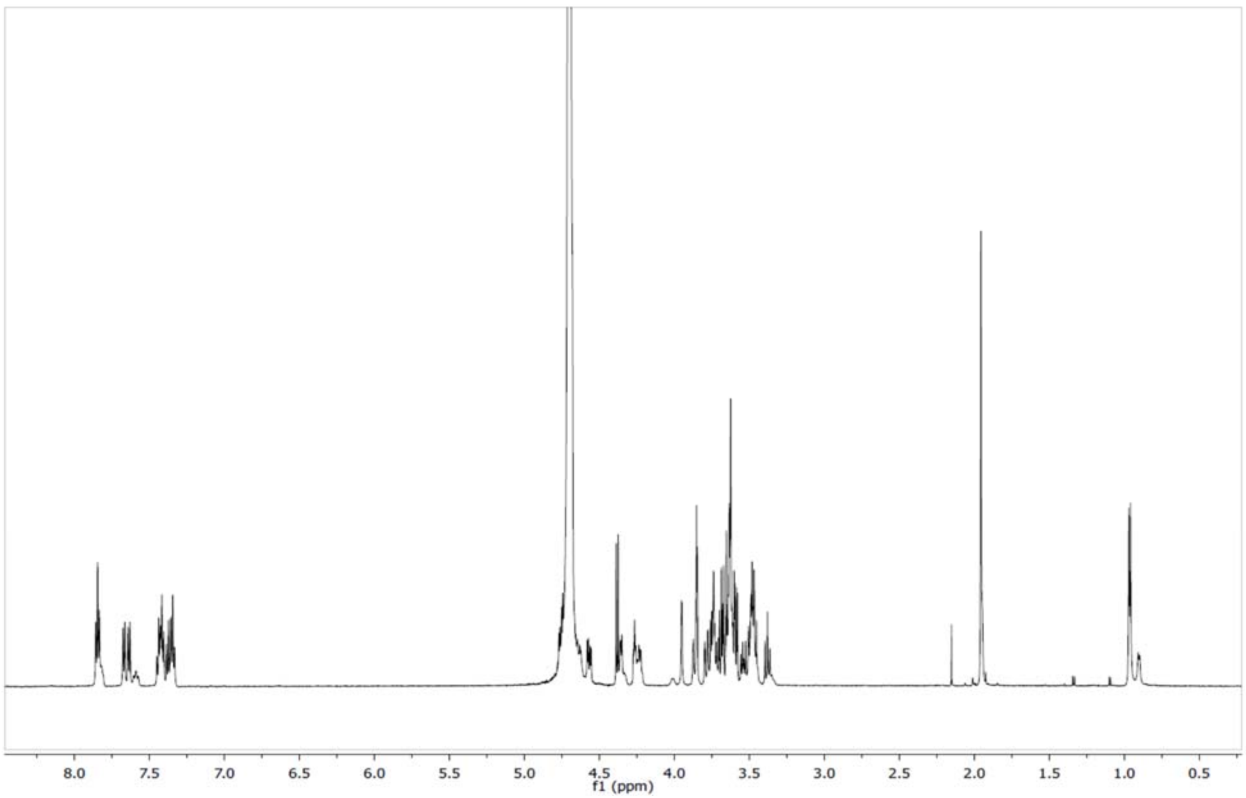
[1]H NMR of Compound 19 (M201)



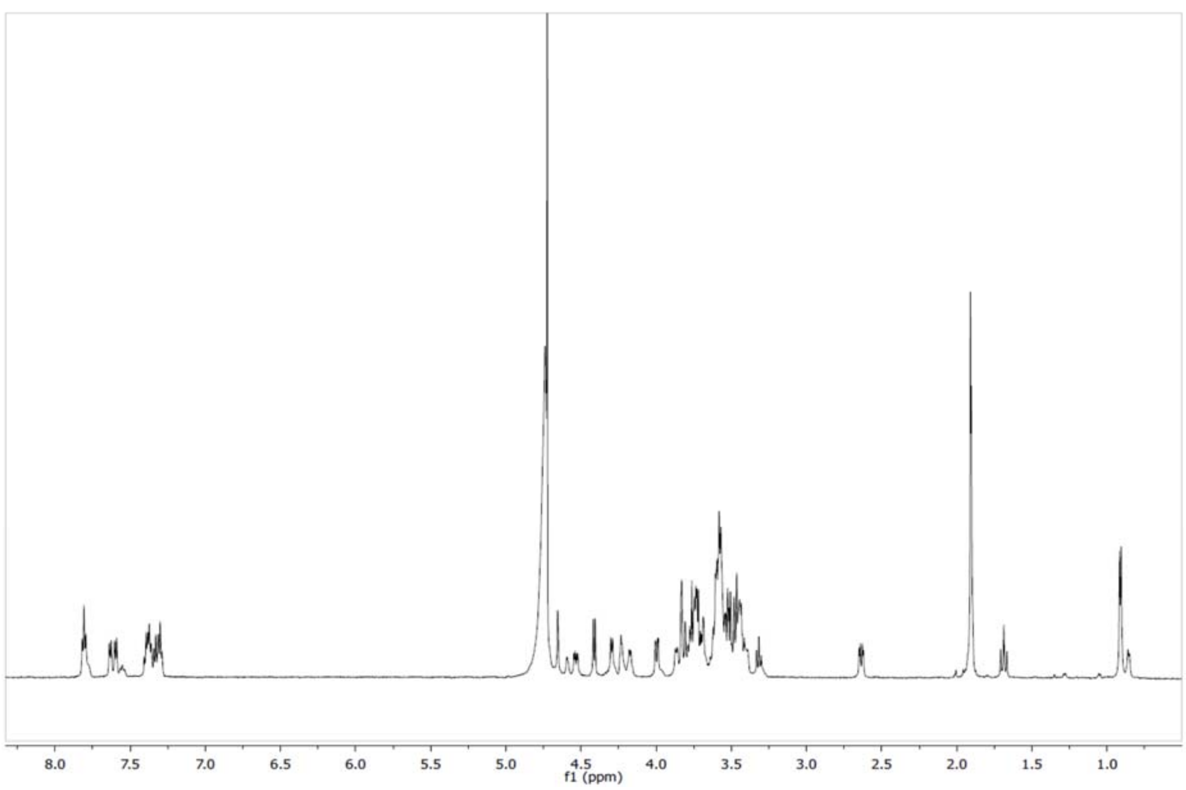
[13]C NMR of Compound 19 (M201)



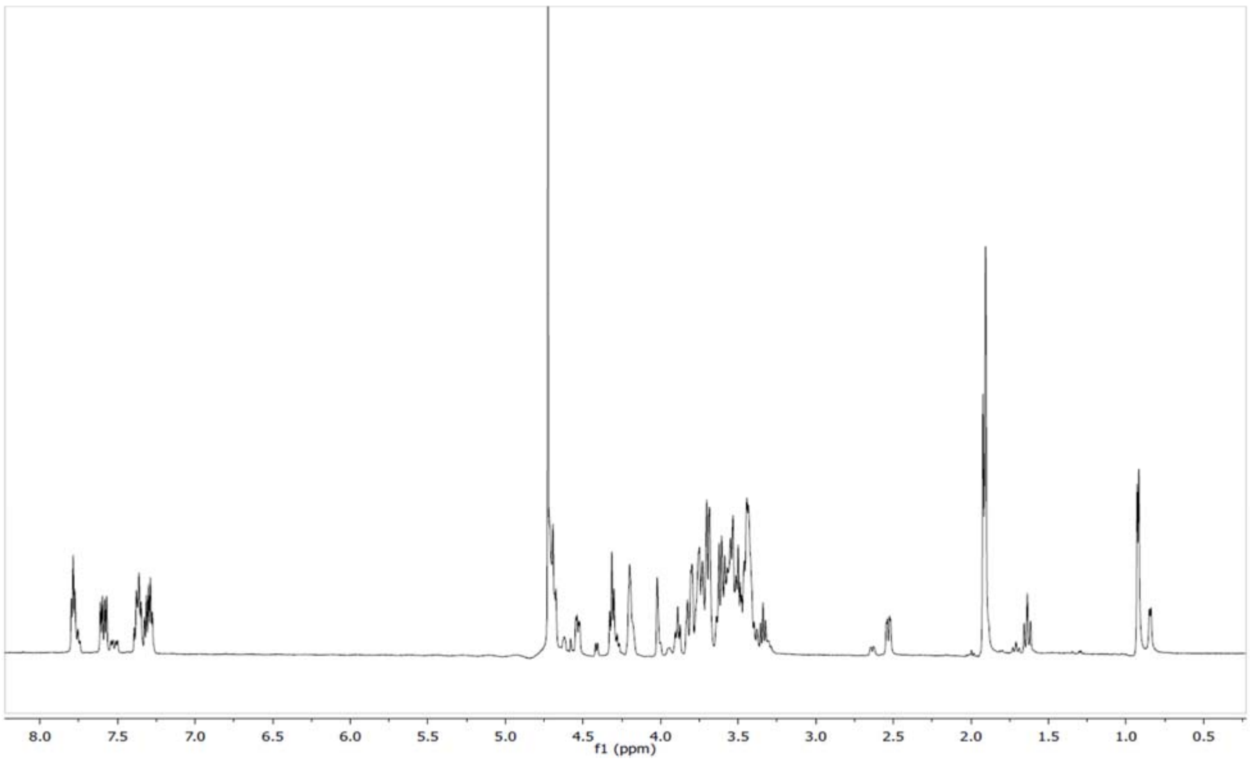
^{1}H NMR of M101



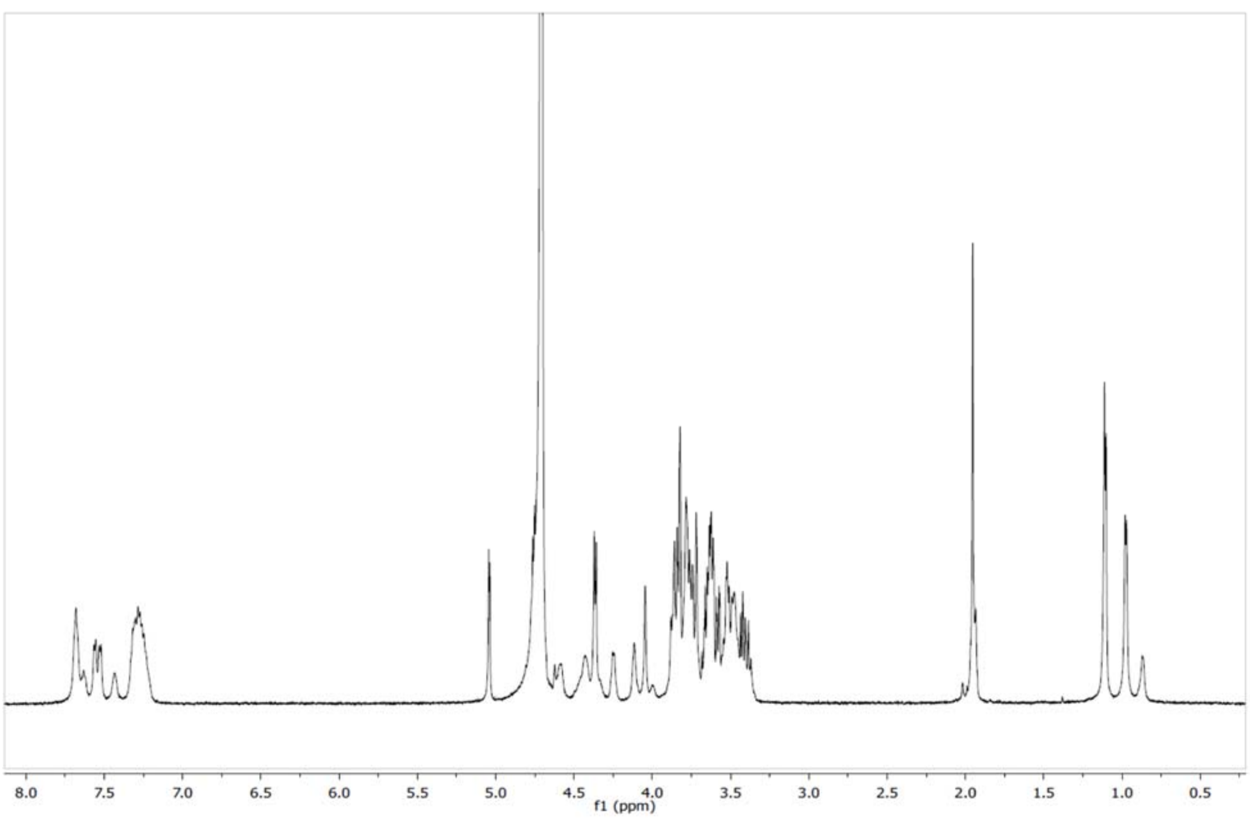
^{1}H NMR of M102



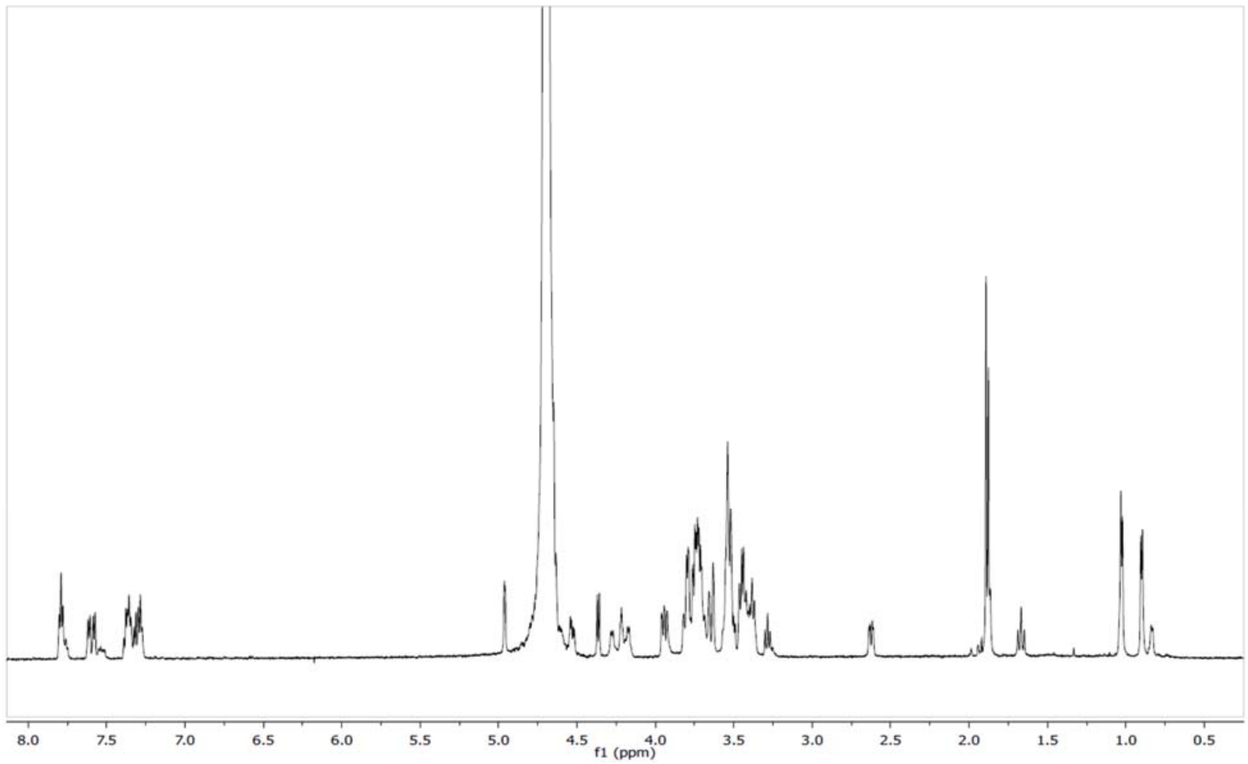
^{1}H NMR of M103



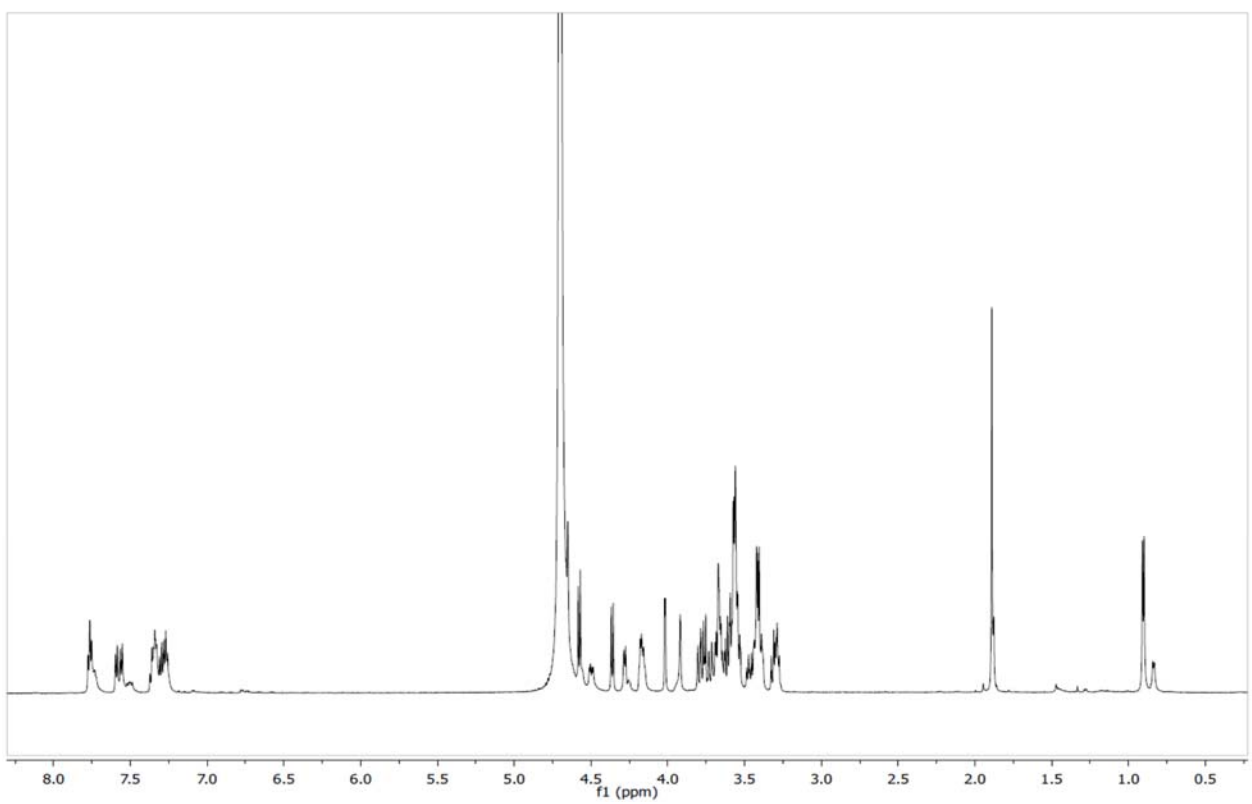
^{1}H NMR of M104



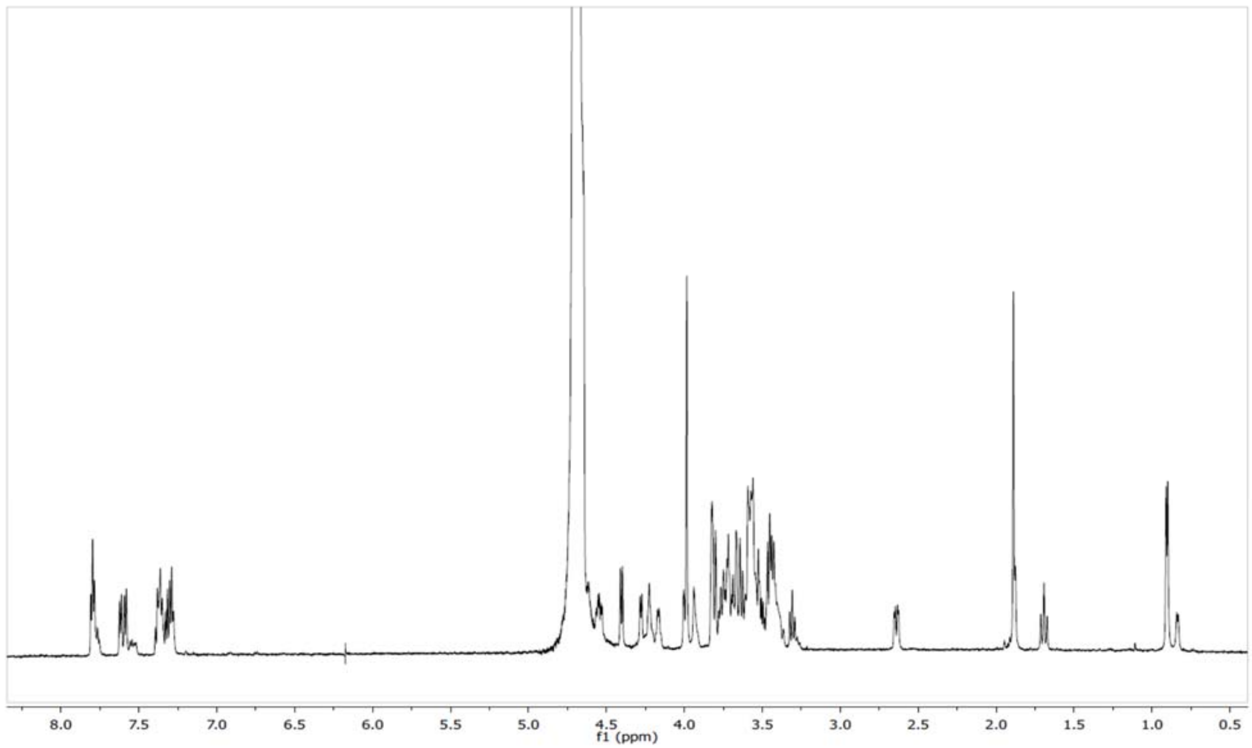
[1]H NMR of M105



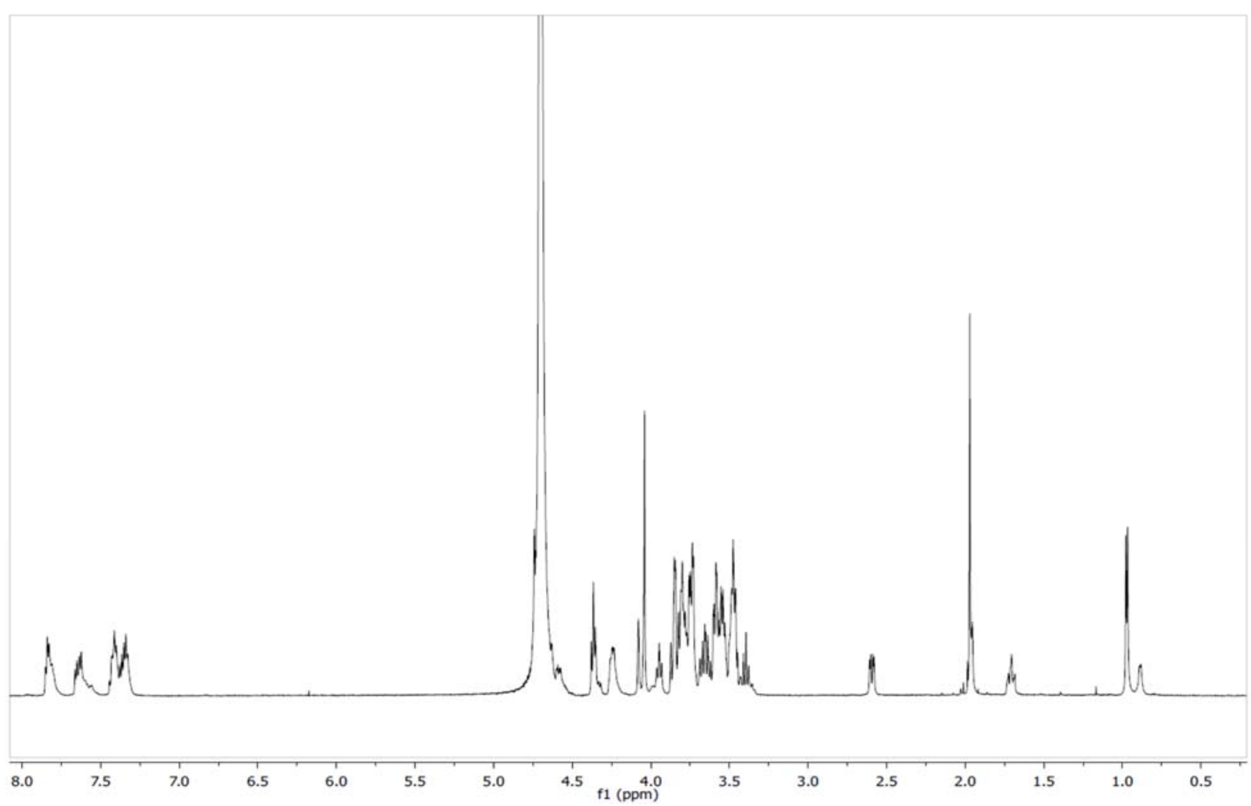
[1]H NMR of M106



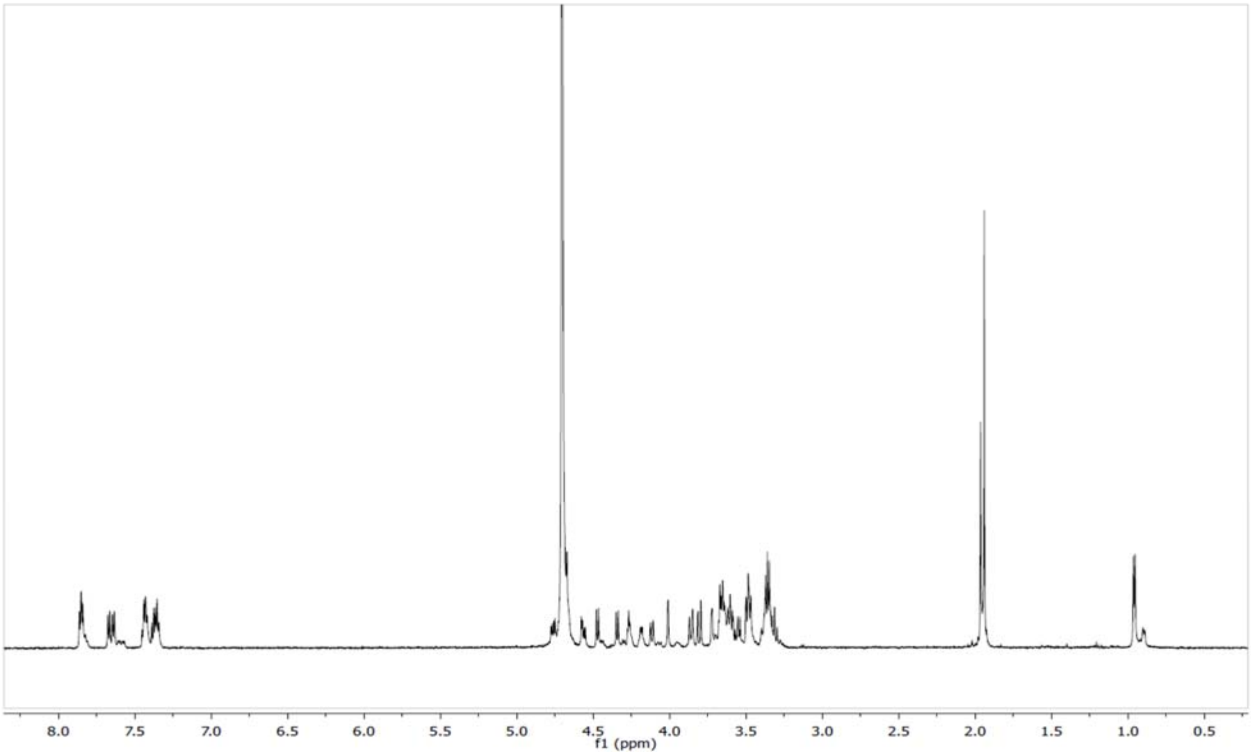
^{1}H NMR of M102G



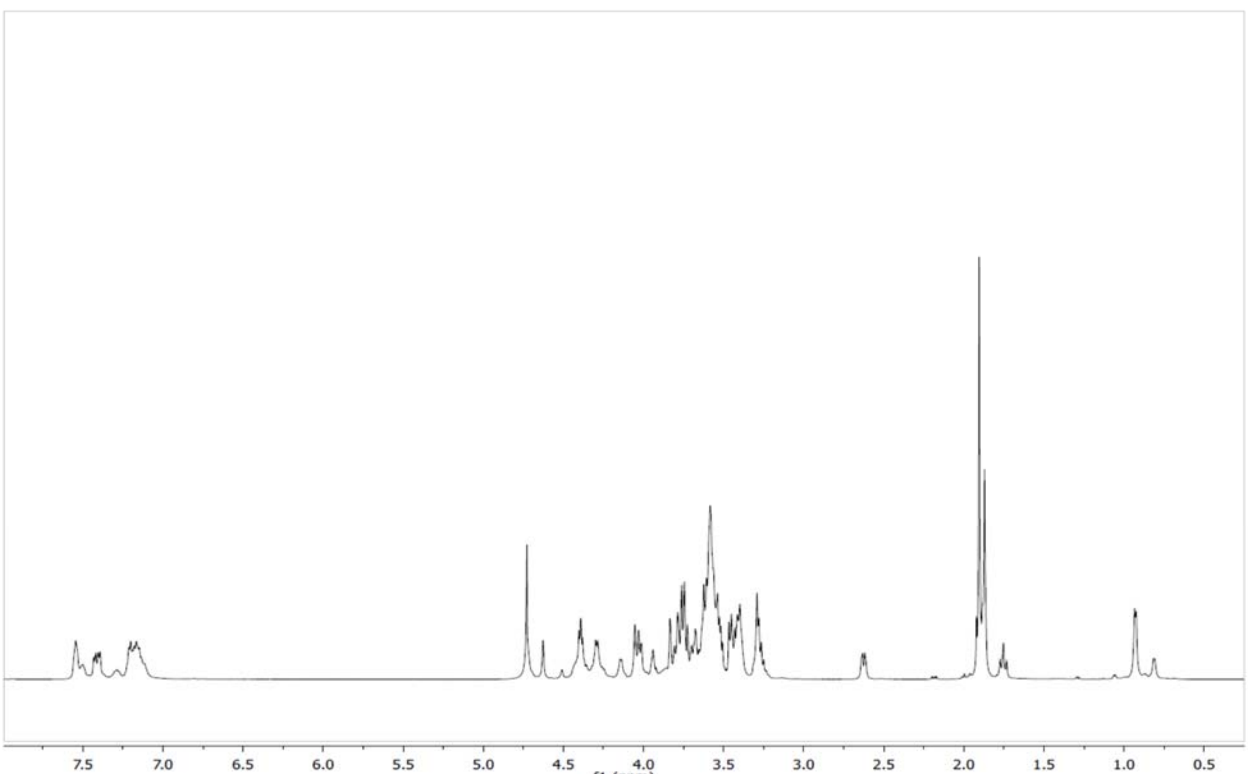
^{1}H NMR of M103G



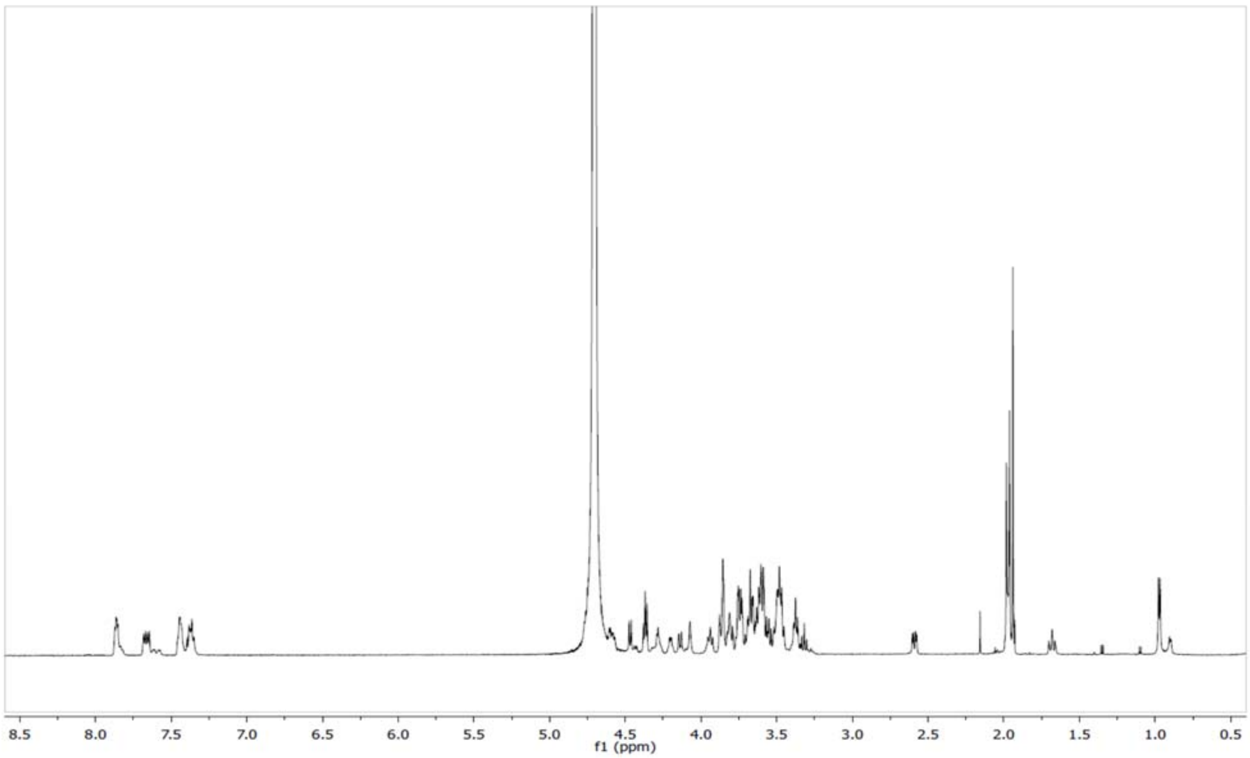
^{1}H NMR of M000



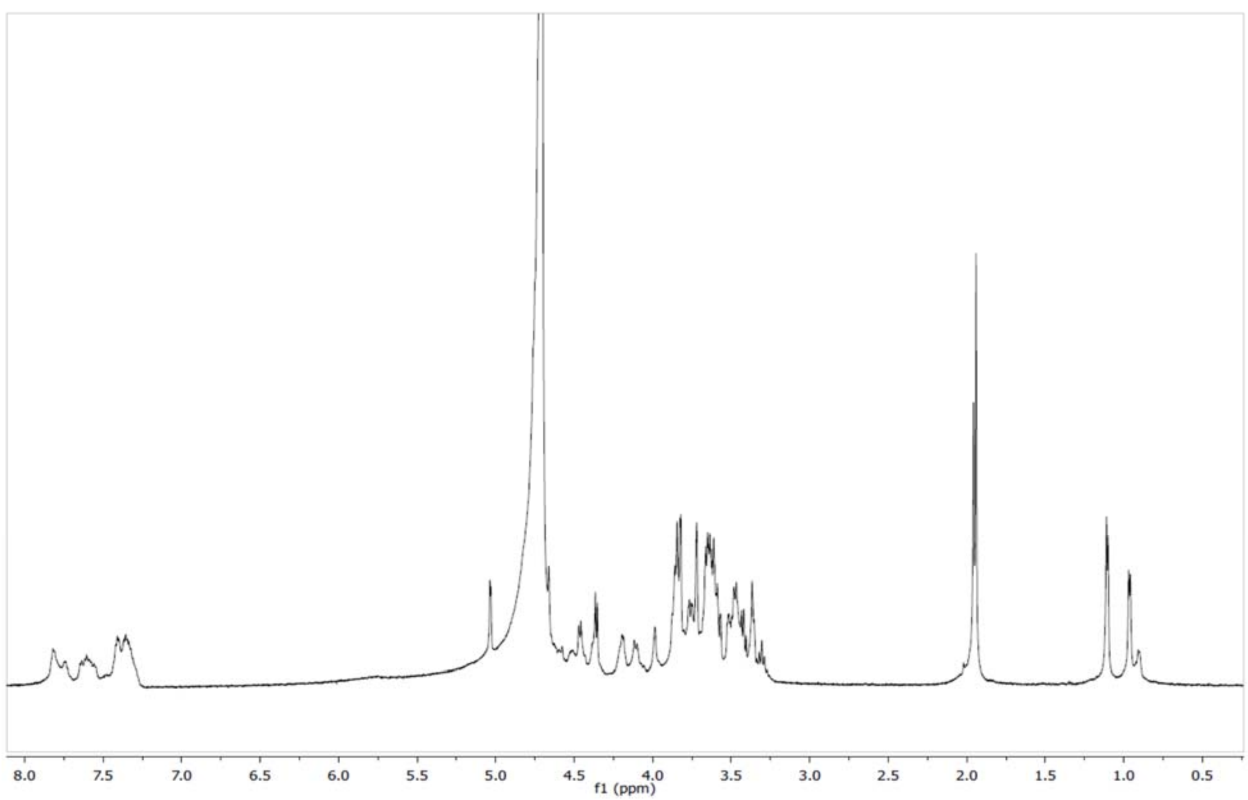
^{1}H NMR of M202



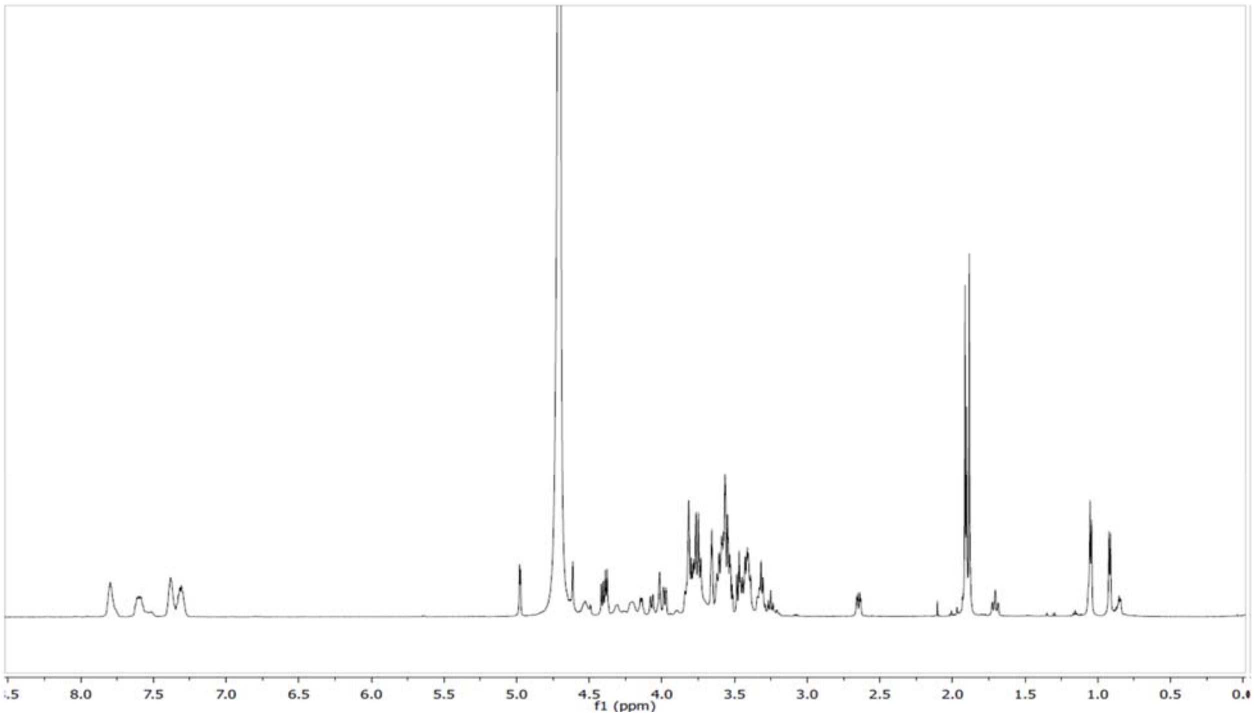
^{1}H NMR of M203



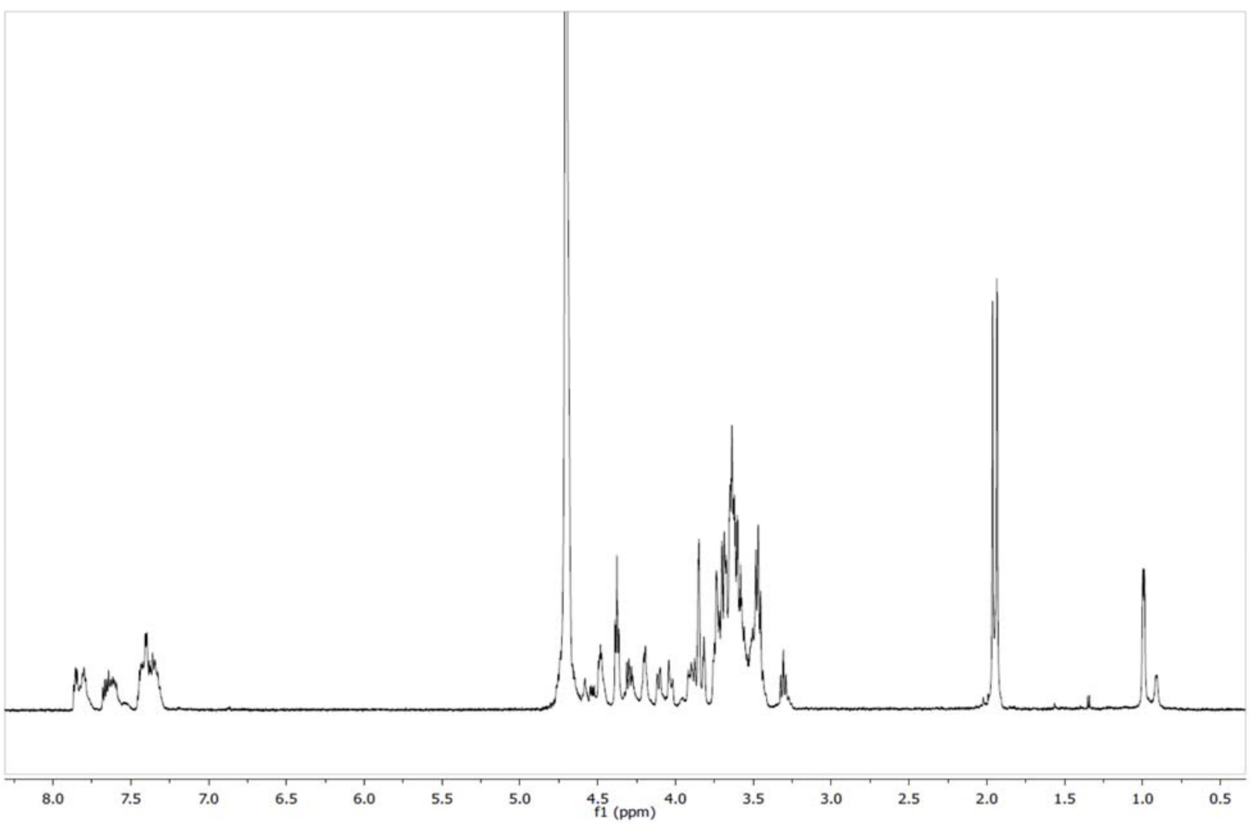
^{1}H NMR of M204



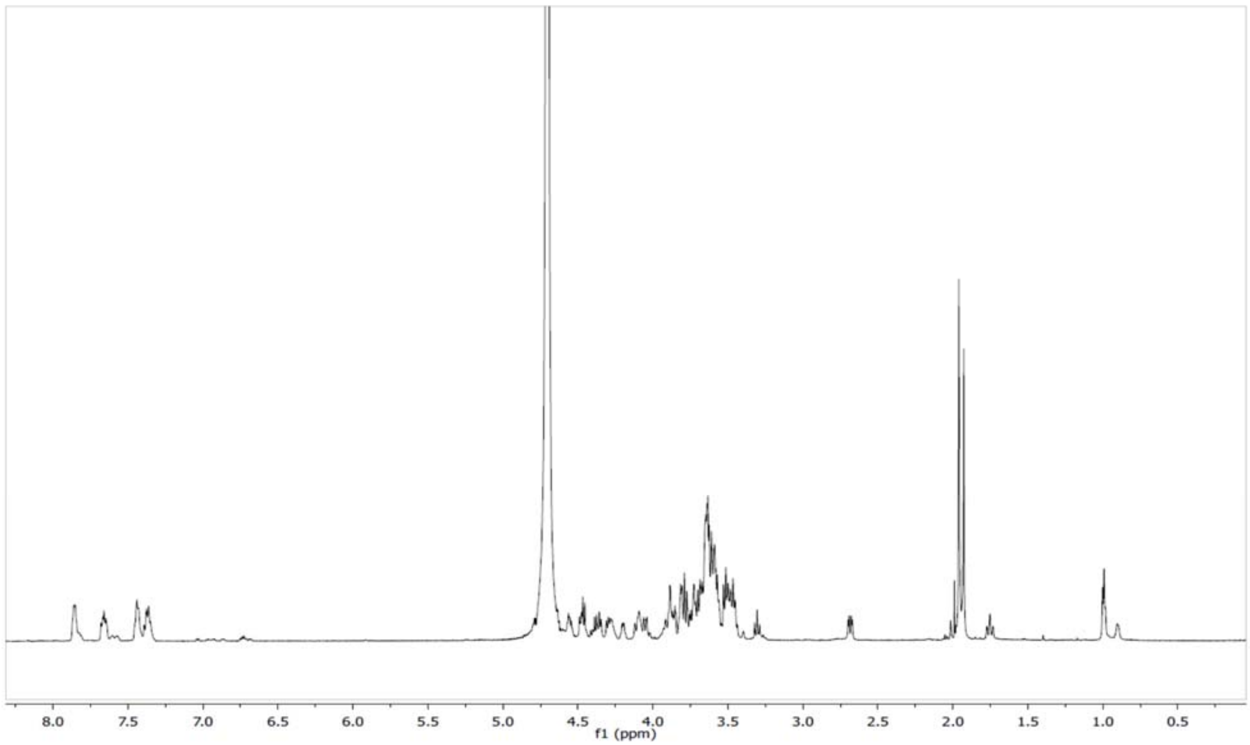
^{1}H NMR of M205



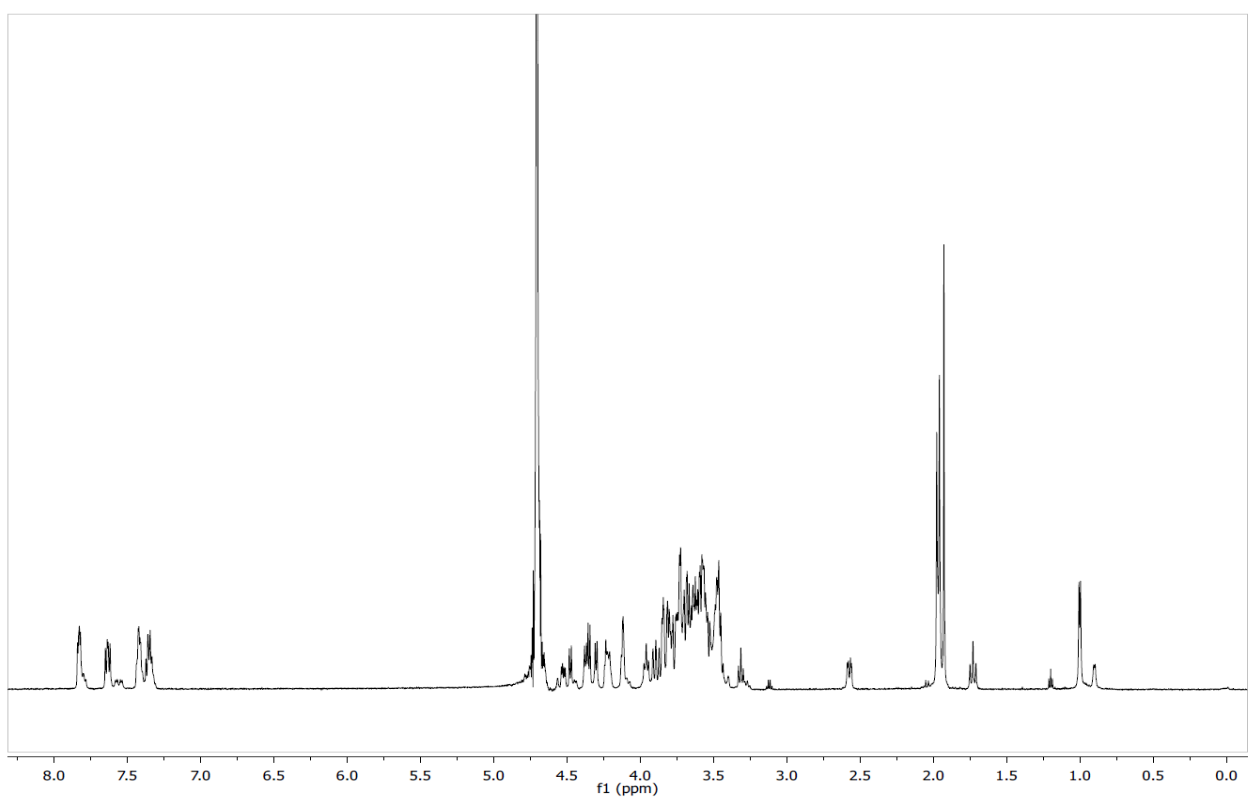
^{1}H NMR of M010



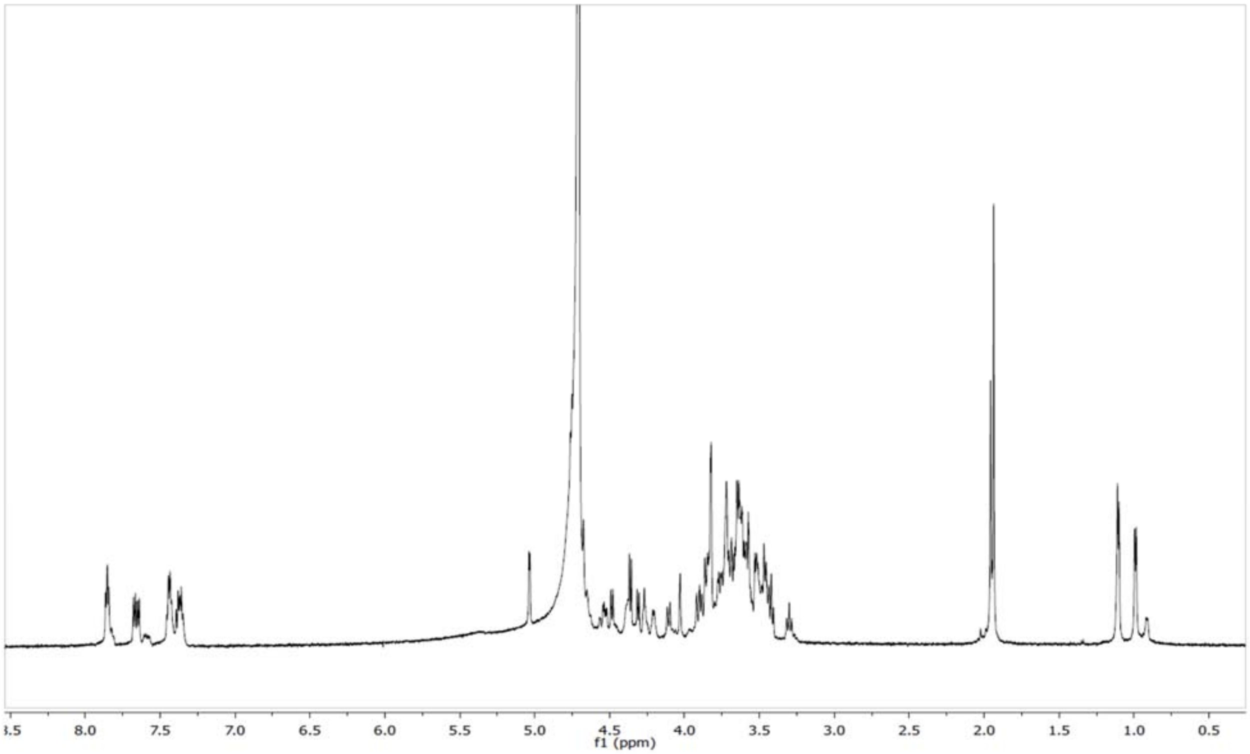
^{1}H NMR of M212



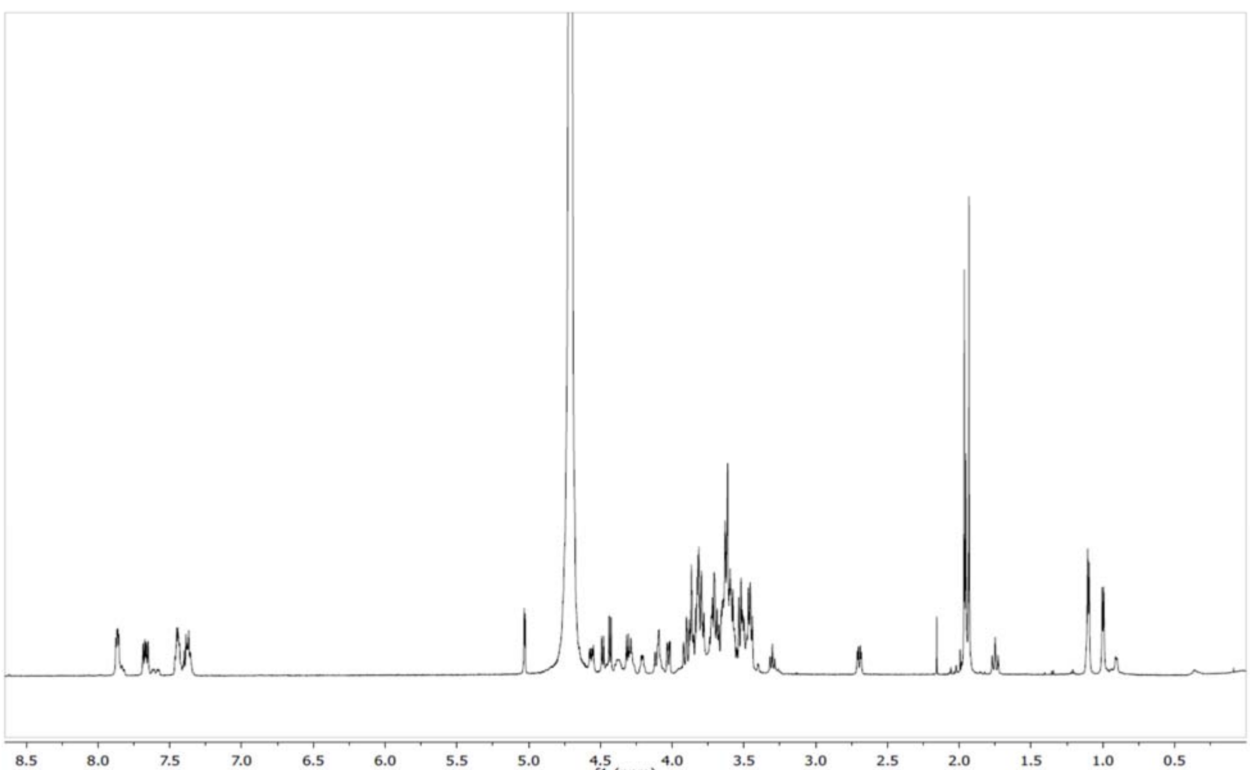
^{1}H NMR of M213



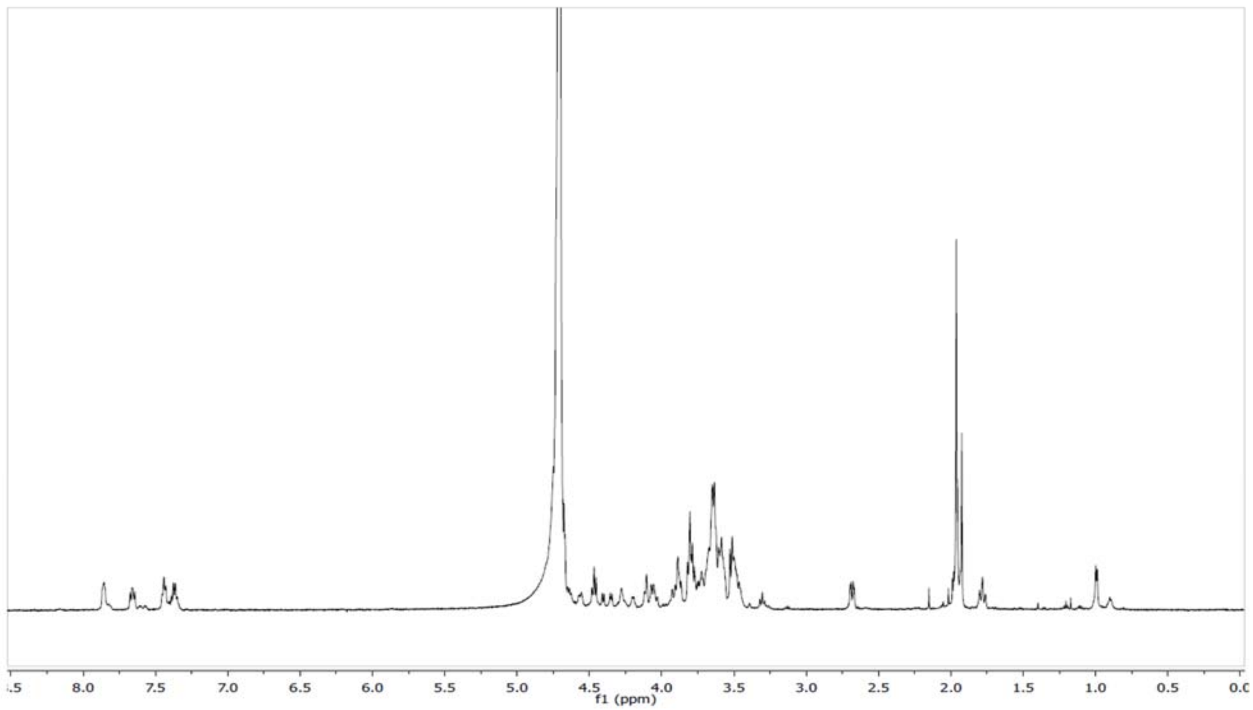
[1]H NMR of M214



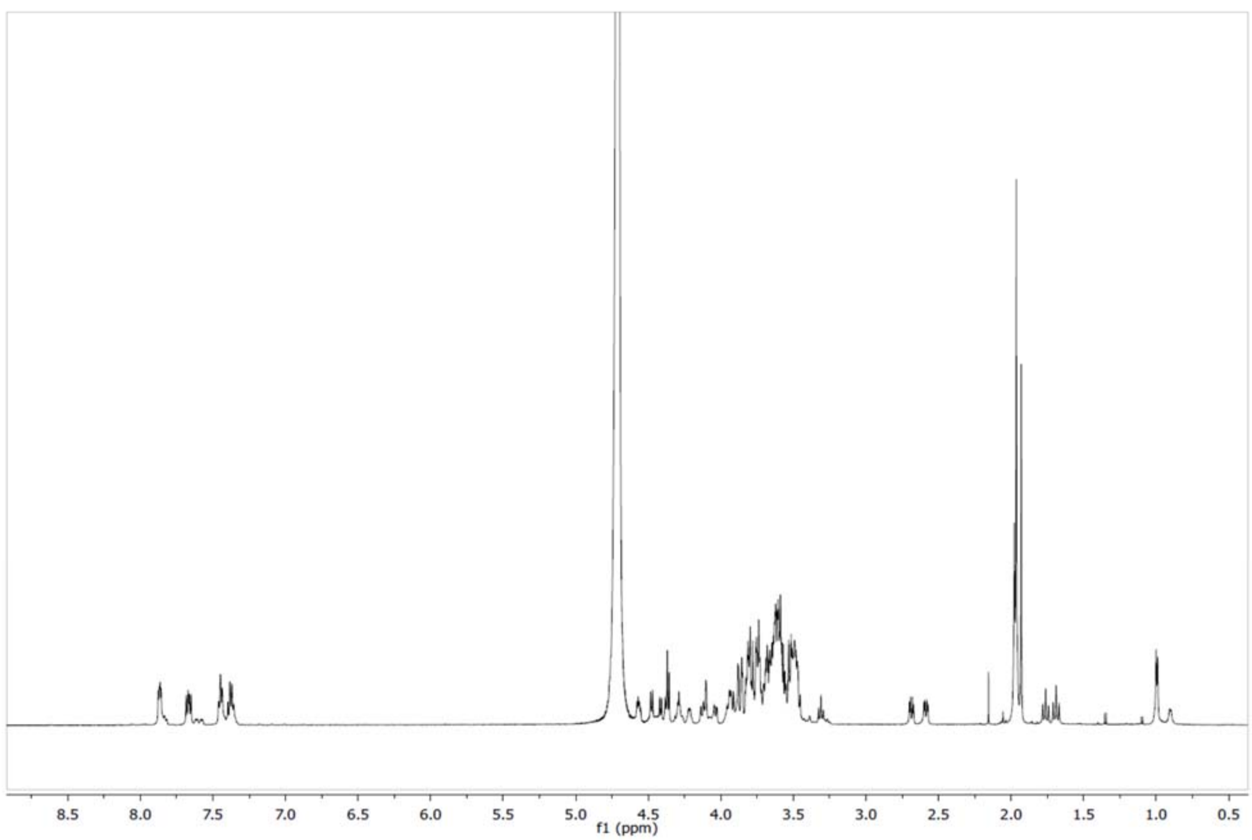
[1]H NMR of M215



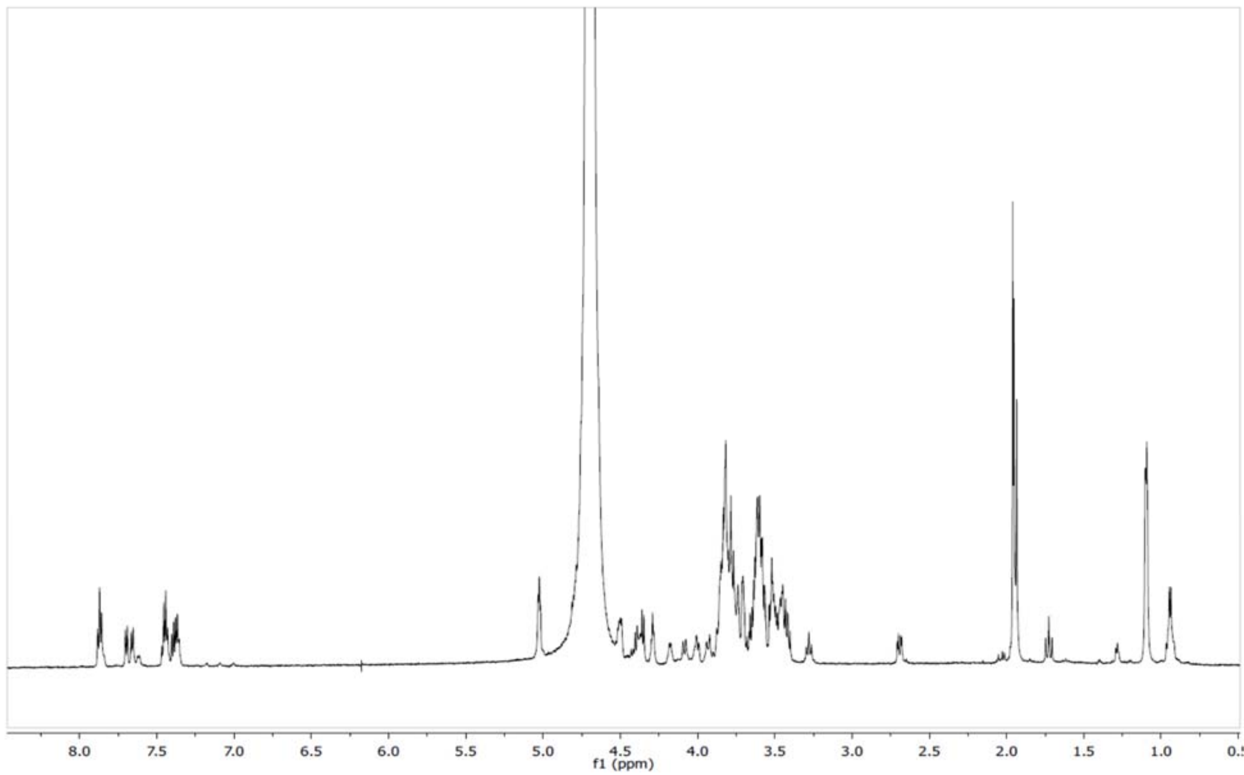
^{1}H NMR of M020



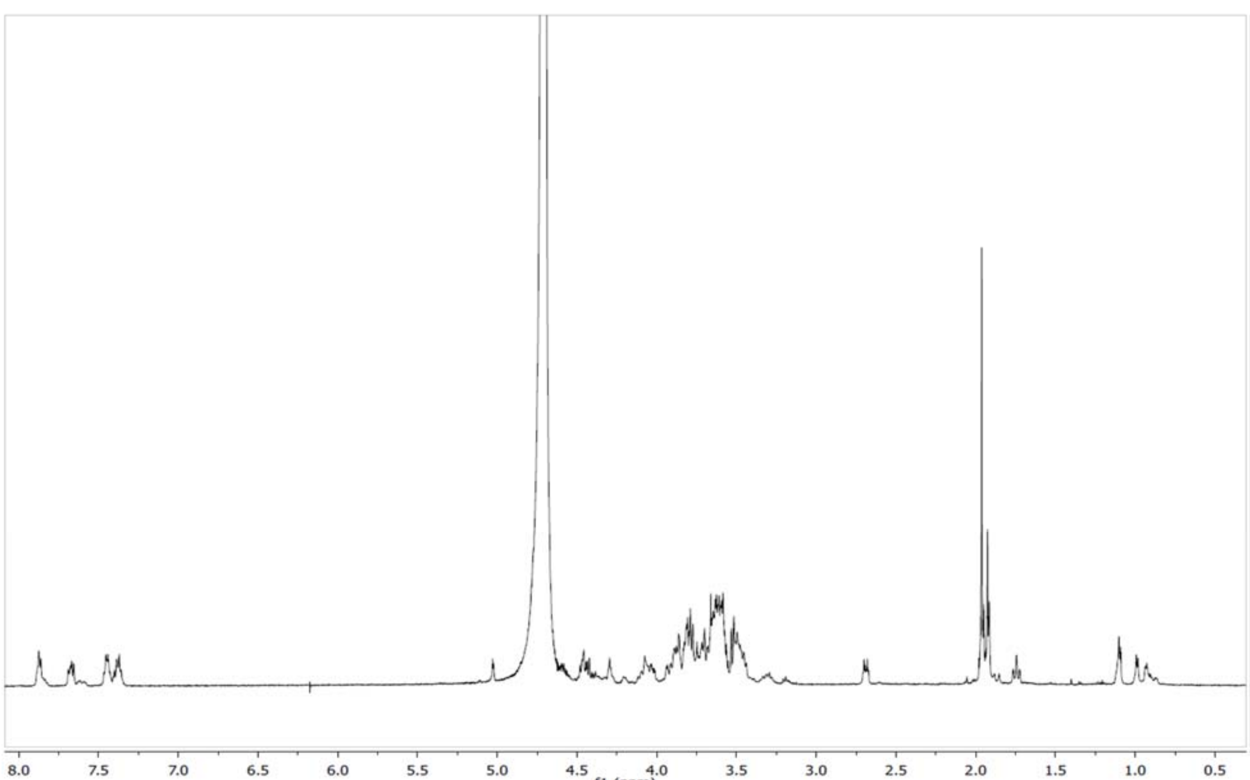
^{1}H NMR of M223



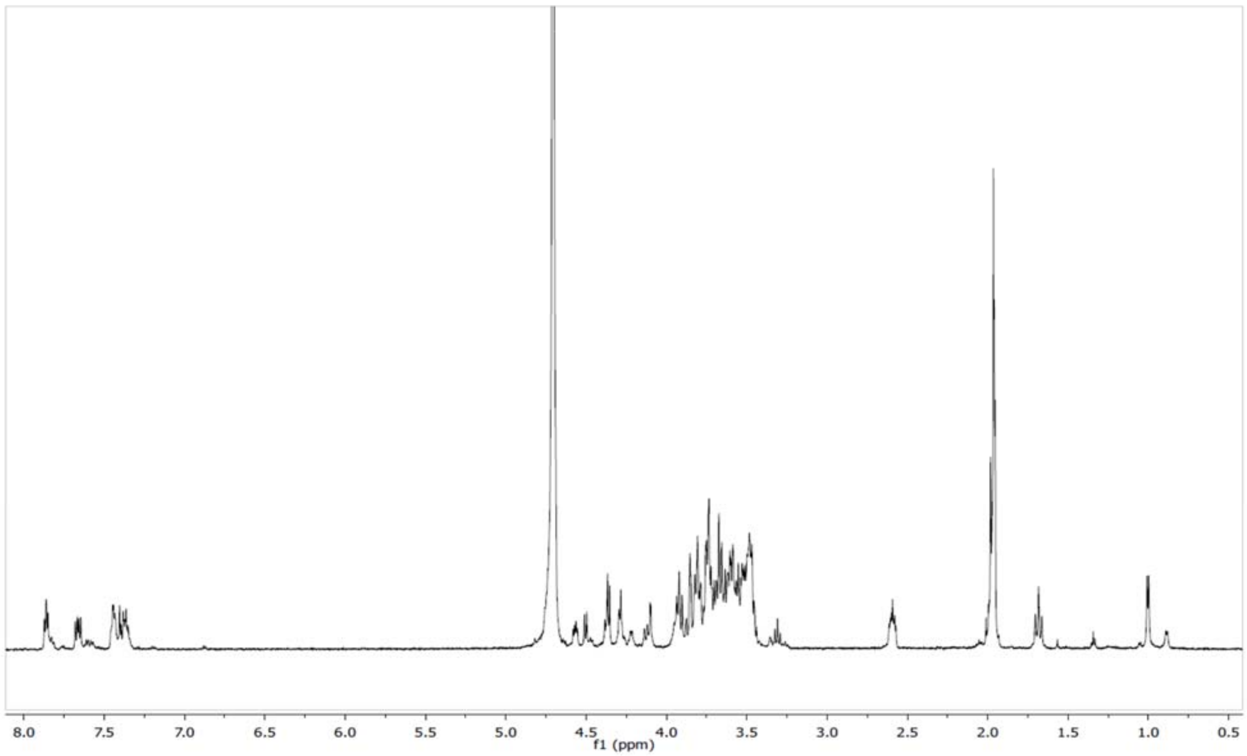
^{1}H NMR of M224



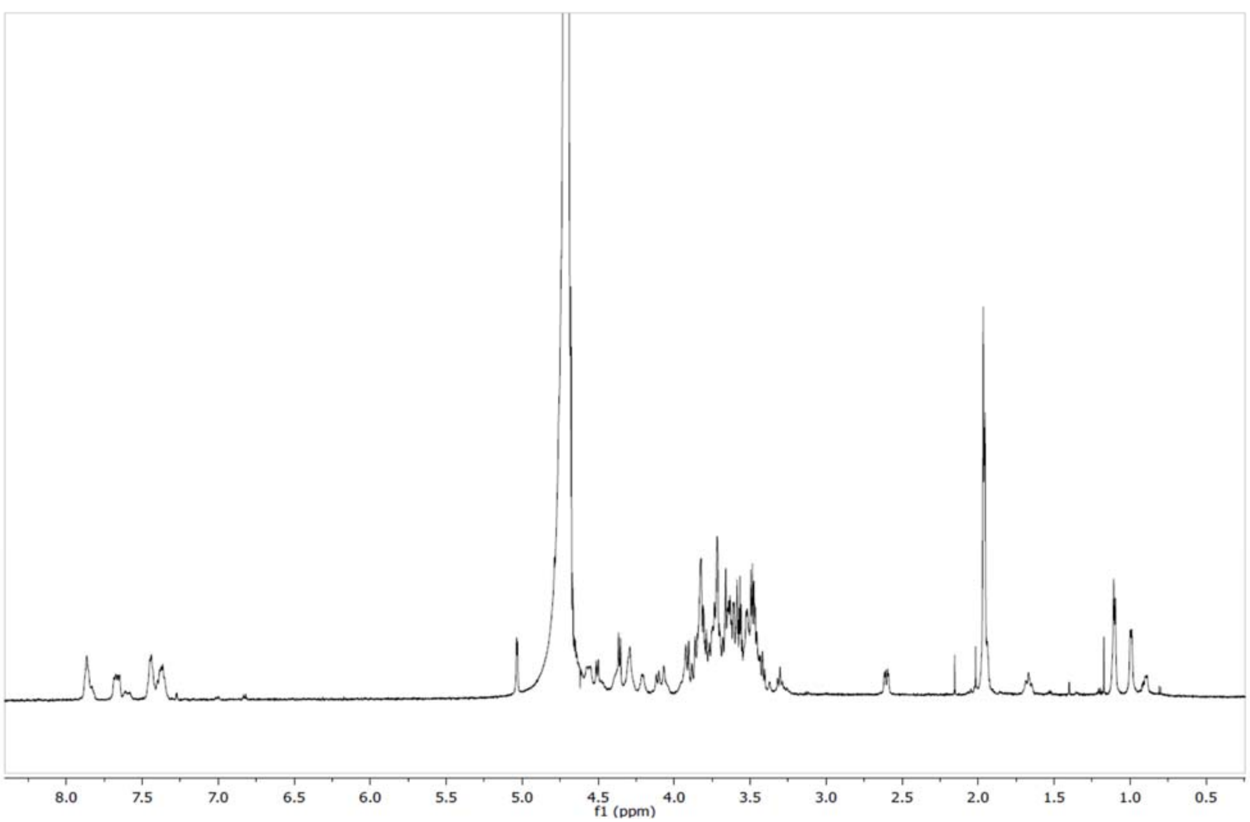
^{1}H NMR of M225



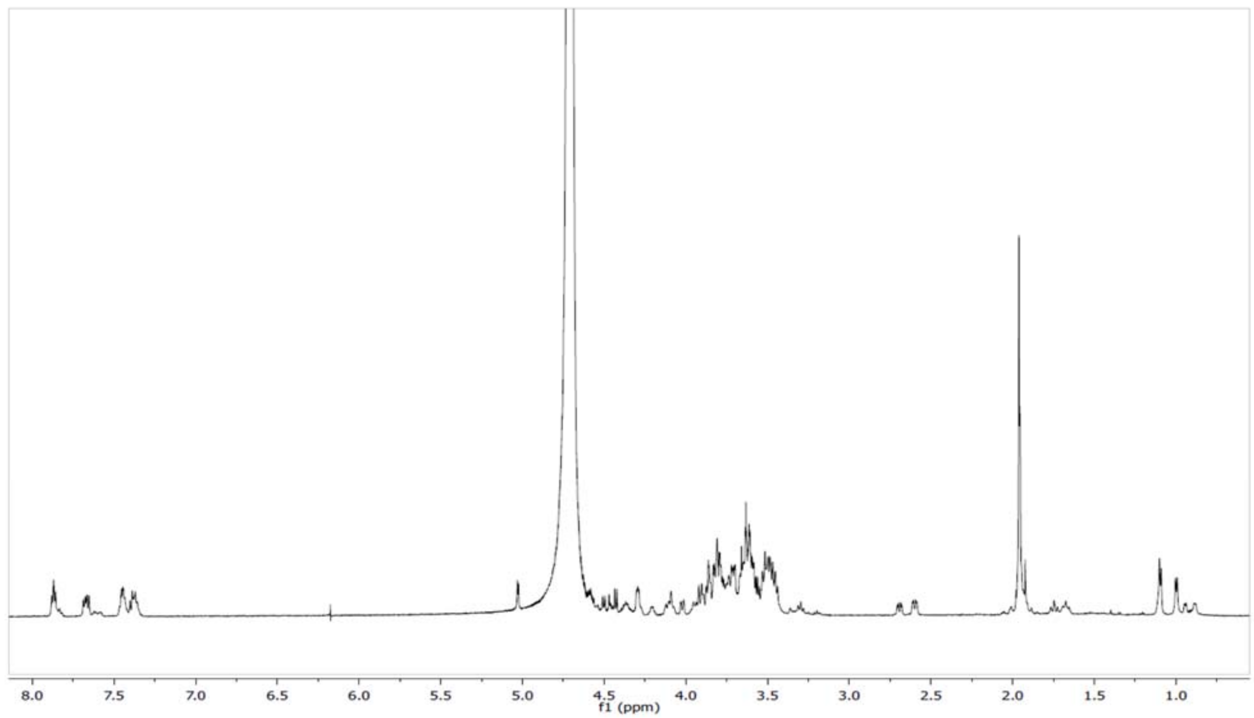
^{1}H NMR of M030



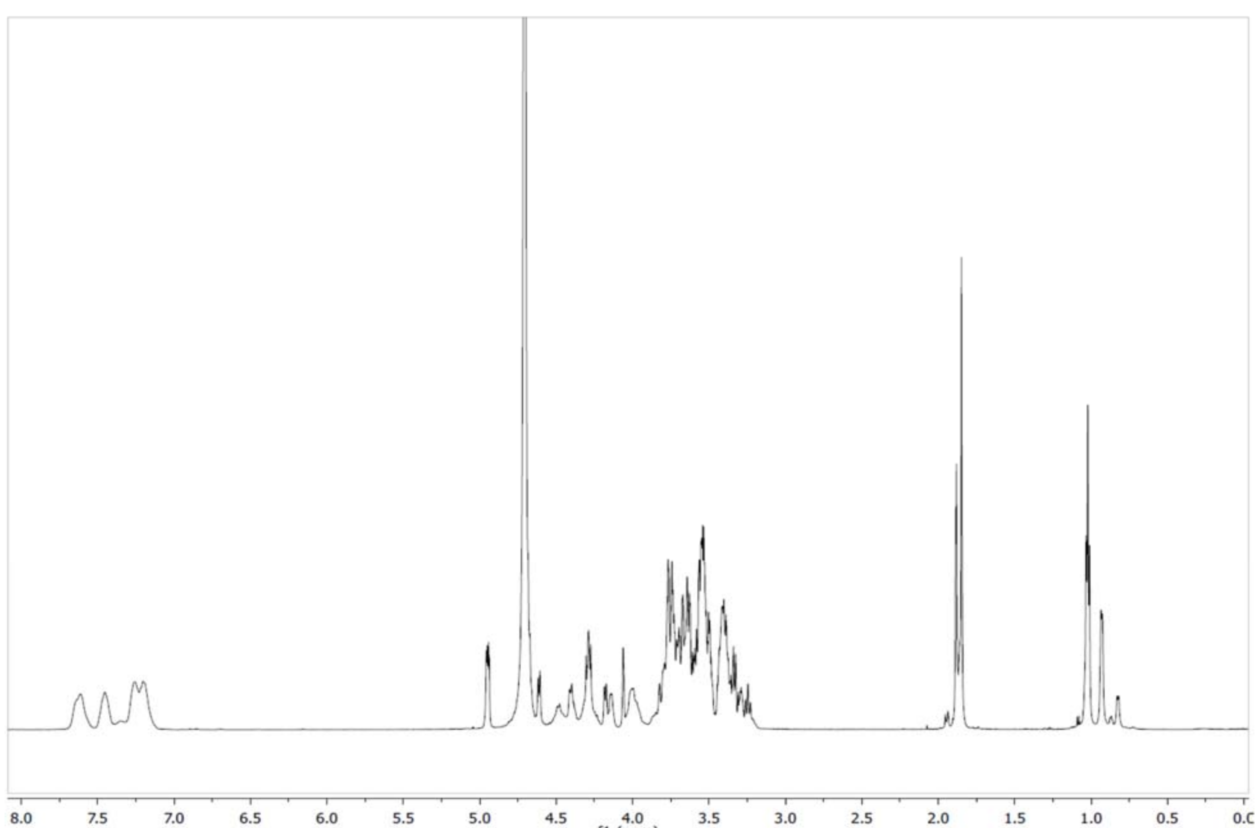
^{1}H NMR of M234



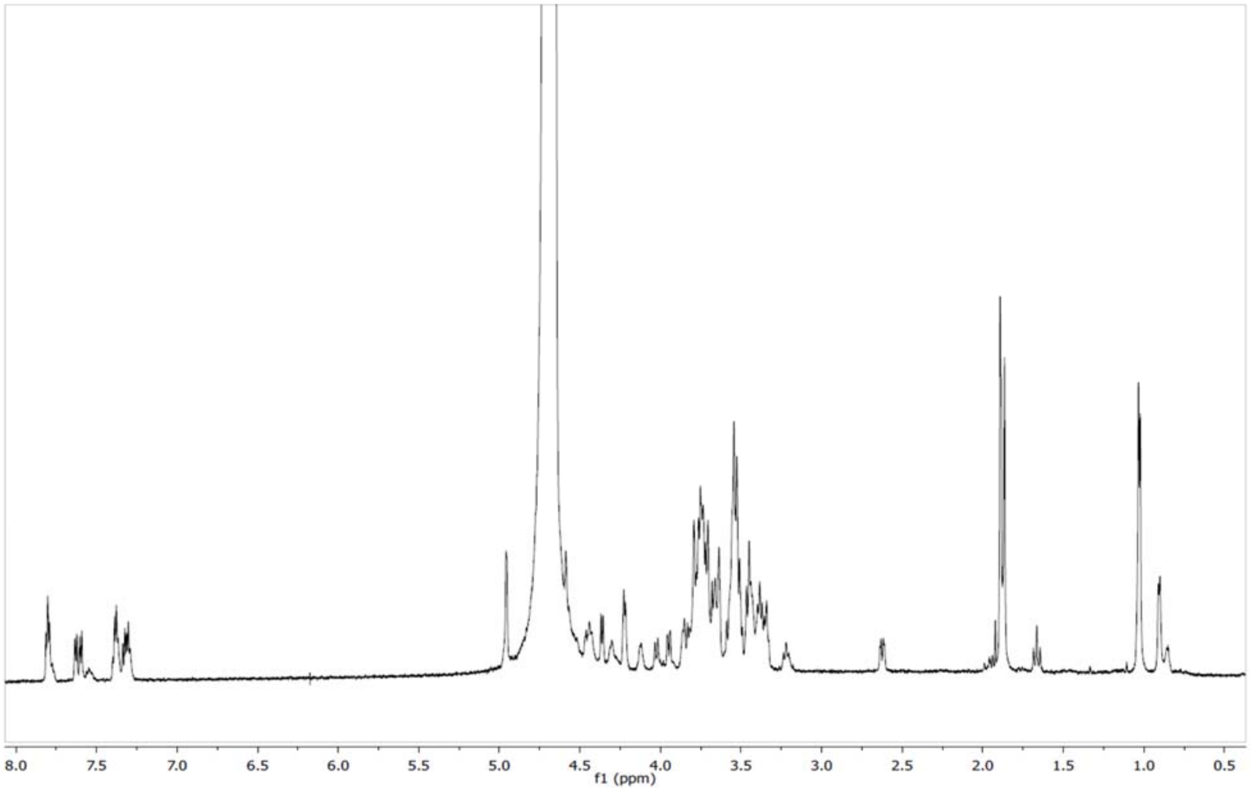
^1H NMR of M235



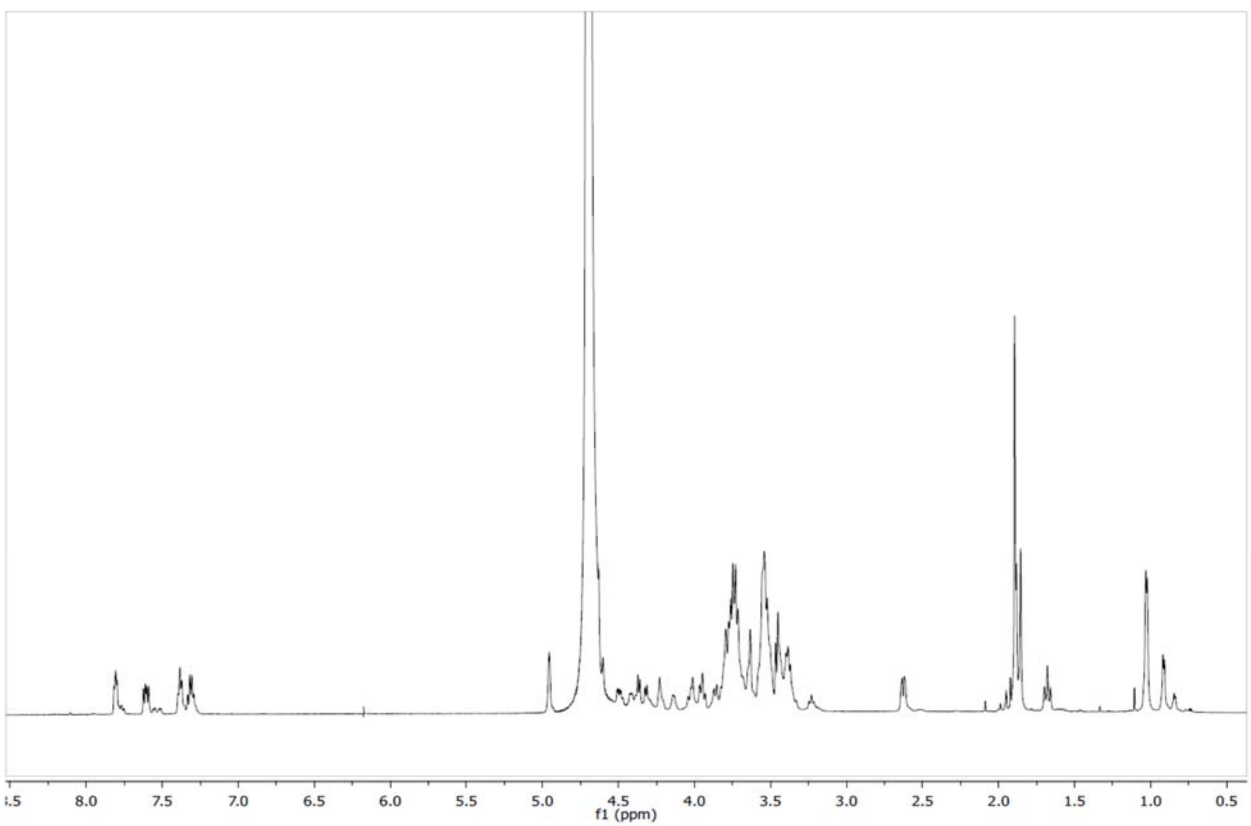
^1H NMR of M040



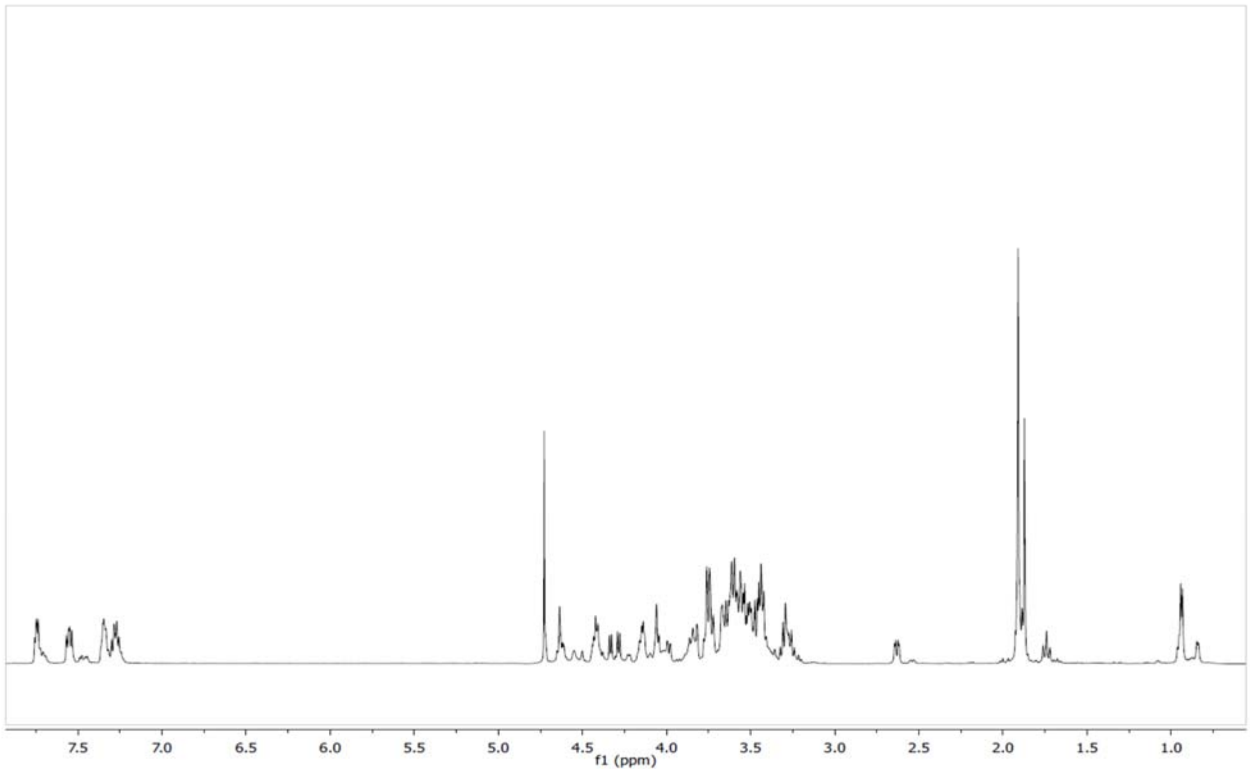
^{1}H NMR of M245



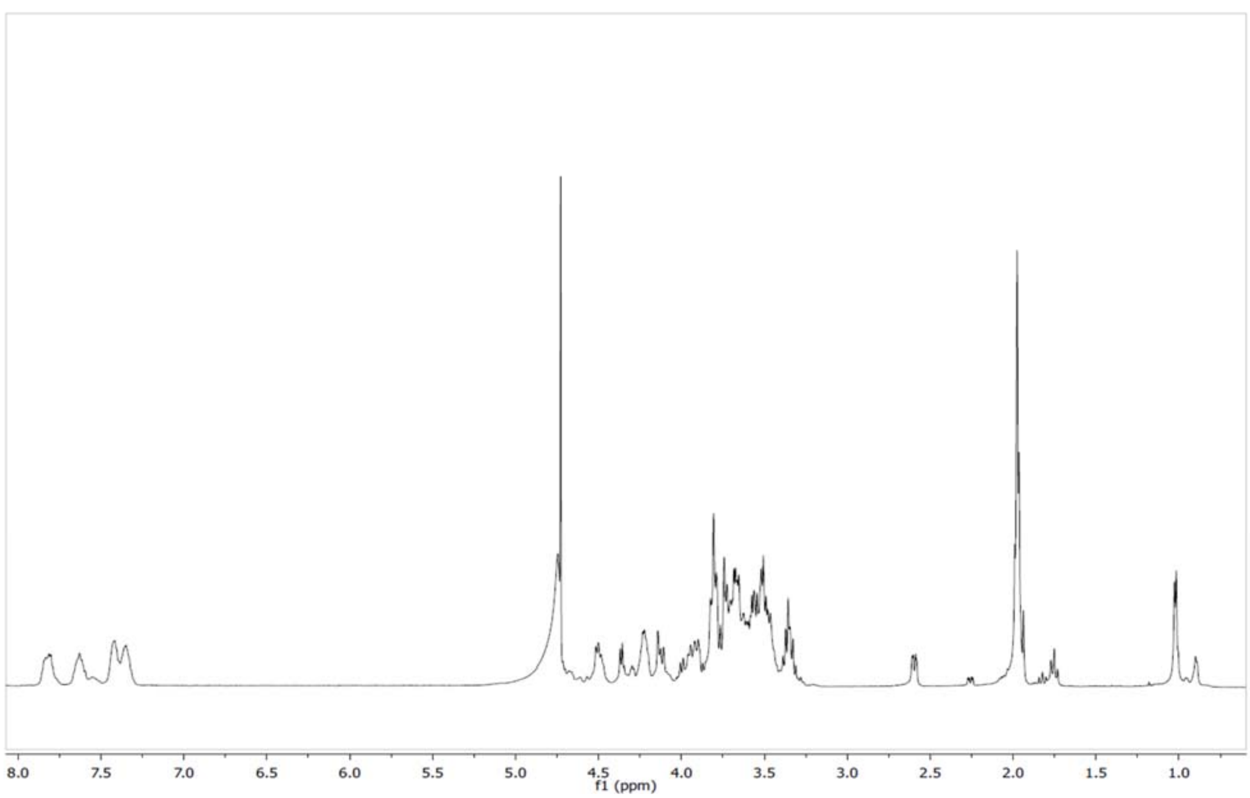
^{1}H NMR of M050



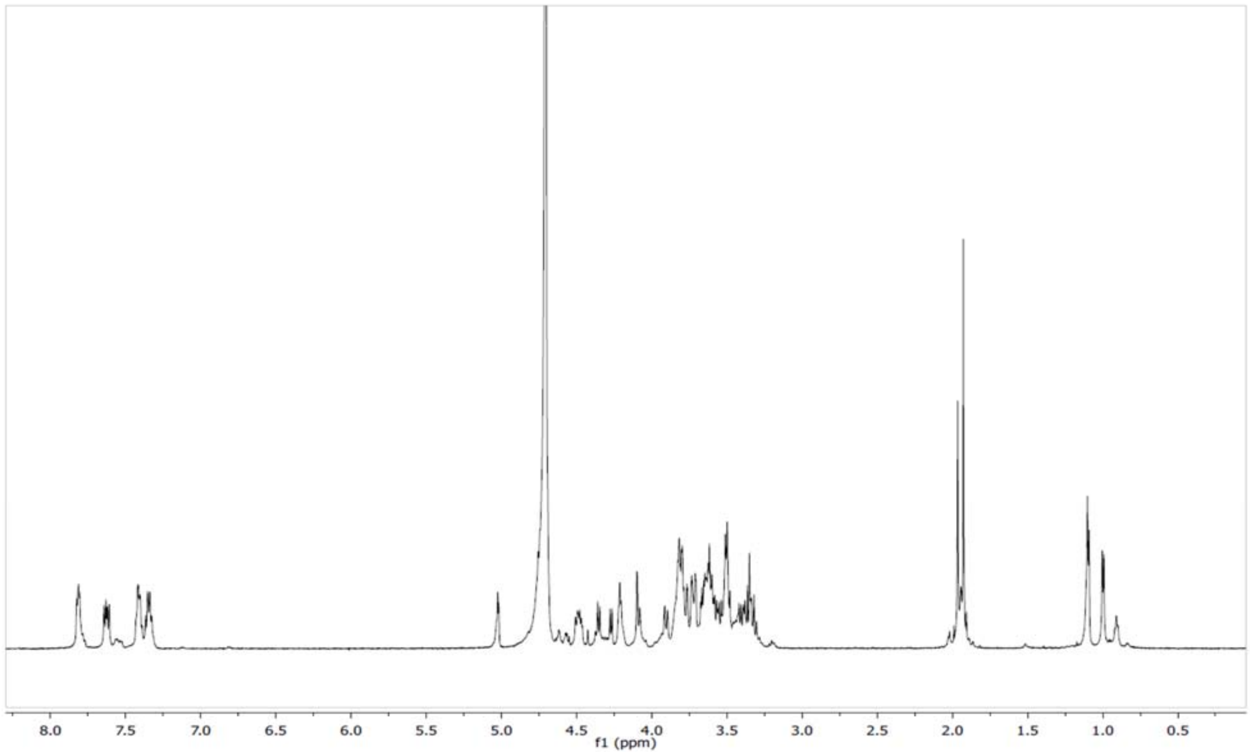
^{1}H NMR of M302



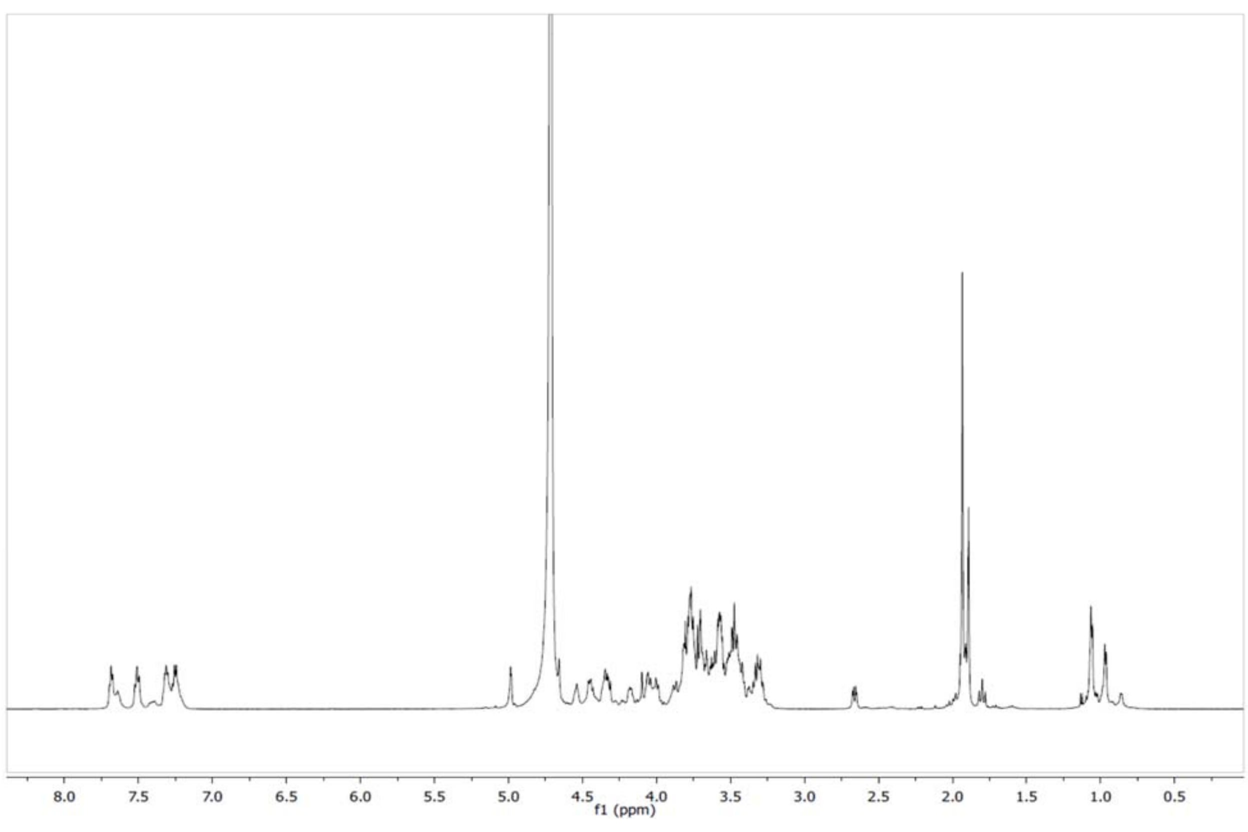
^{1}H NMR of M303



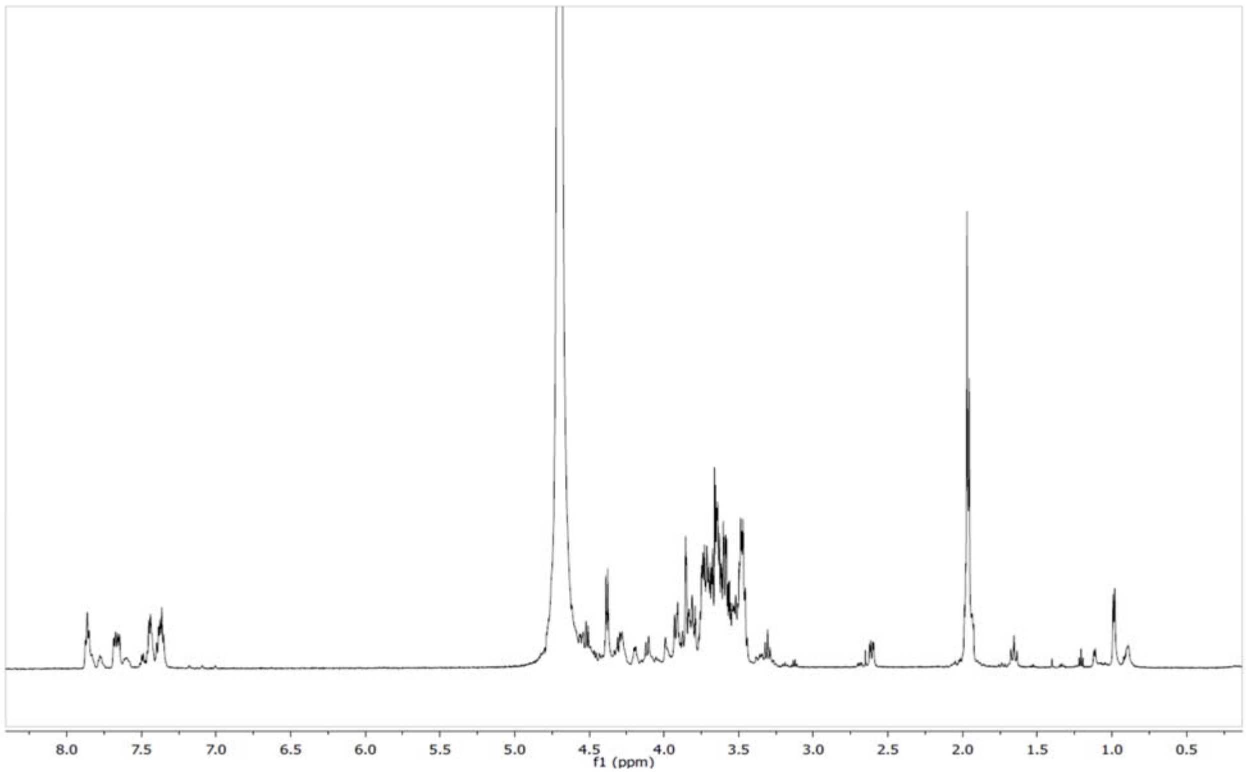
^{1}H NMR of M304



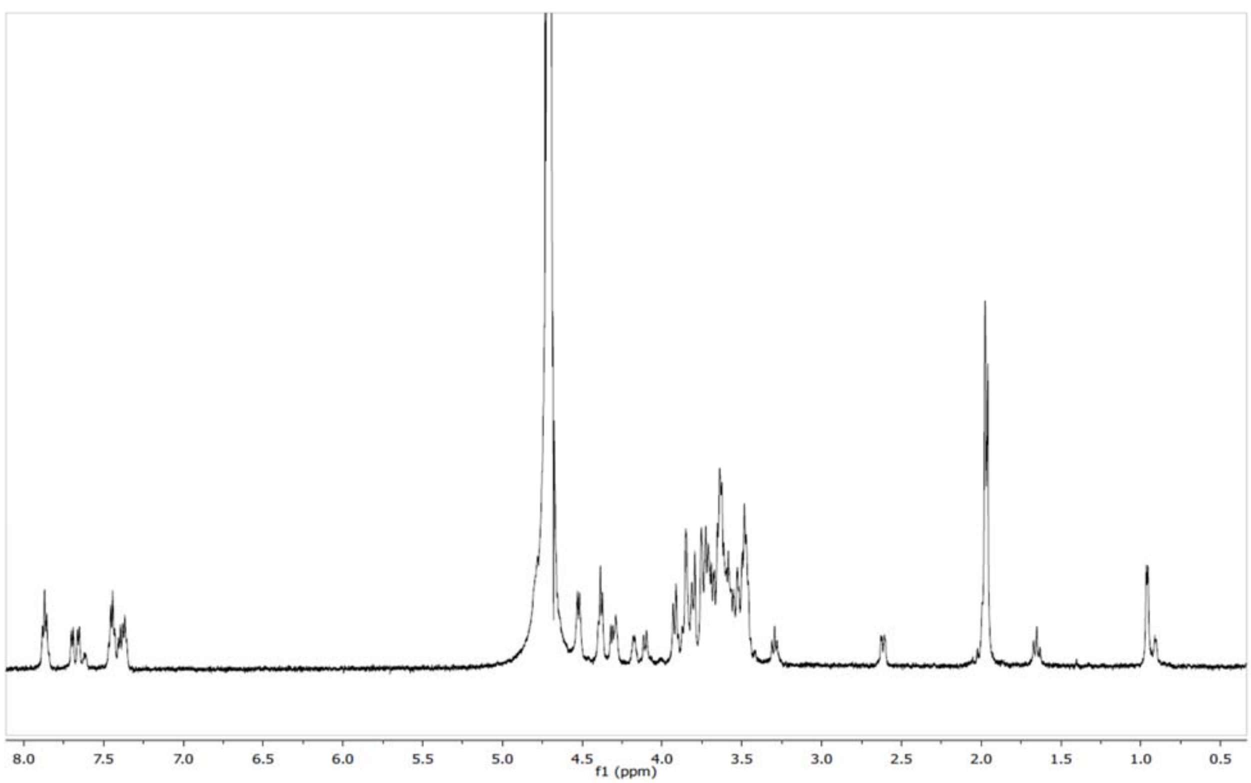
^{1}H NMR of M305



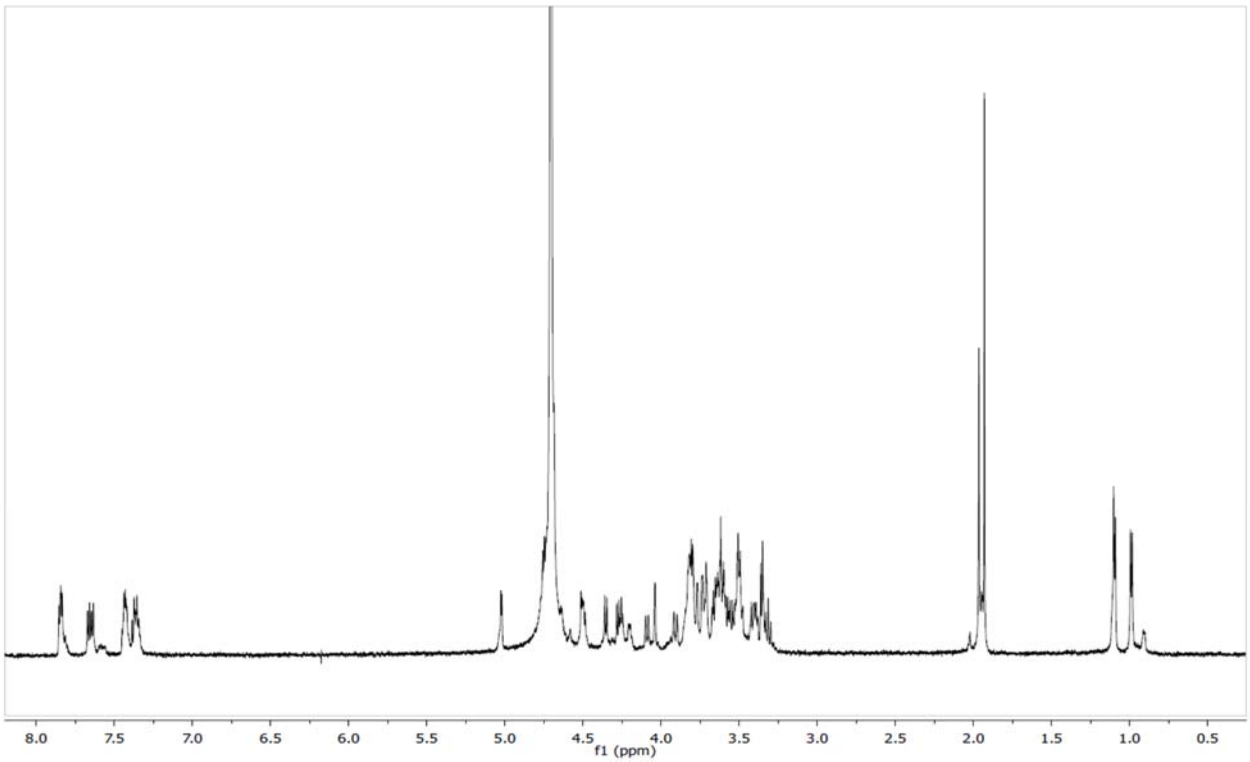
[1]H NMR of M312



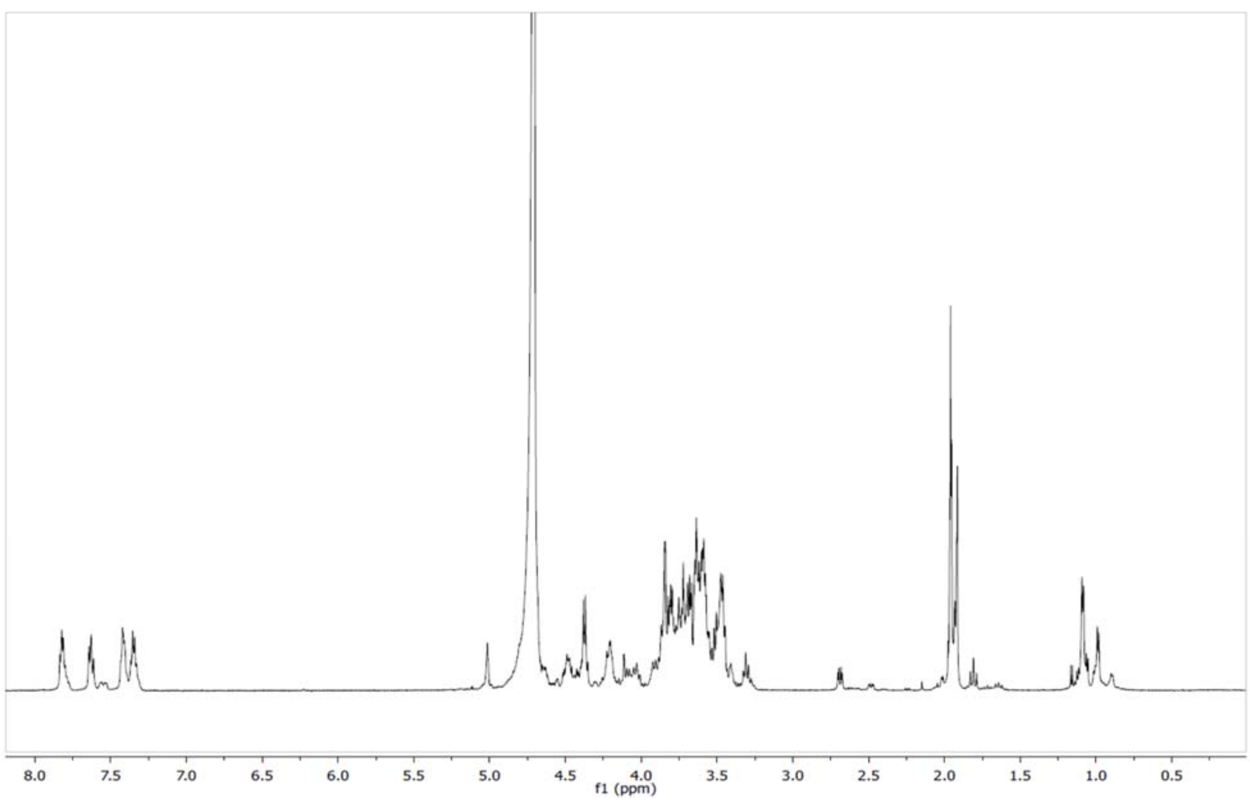
[1]H NMR of M313



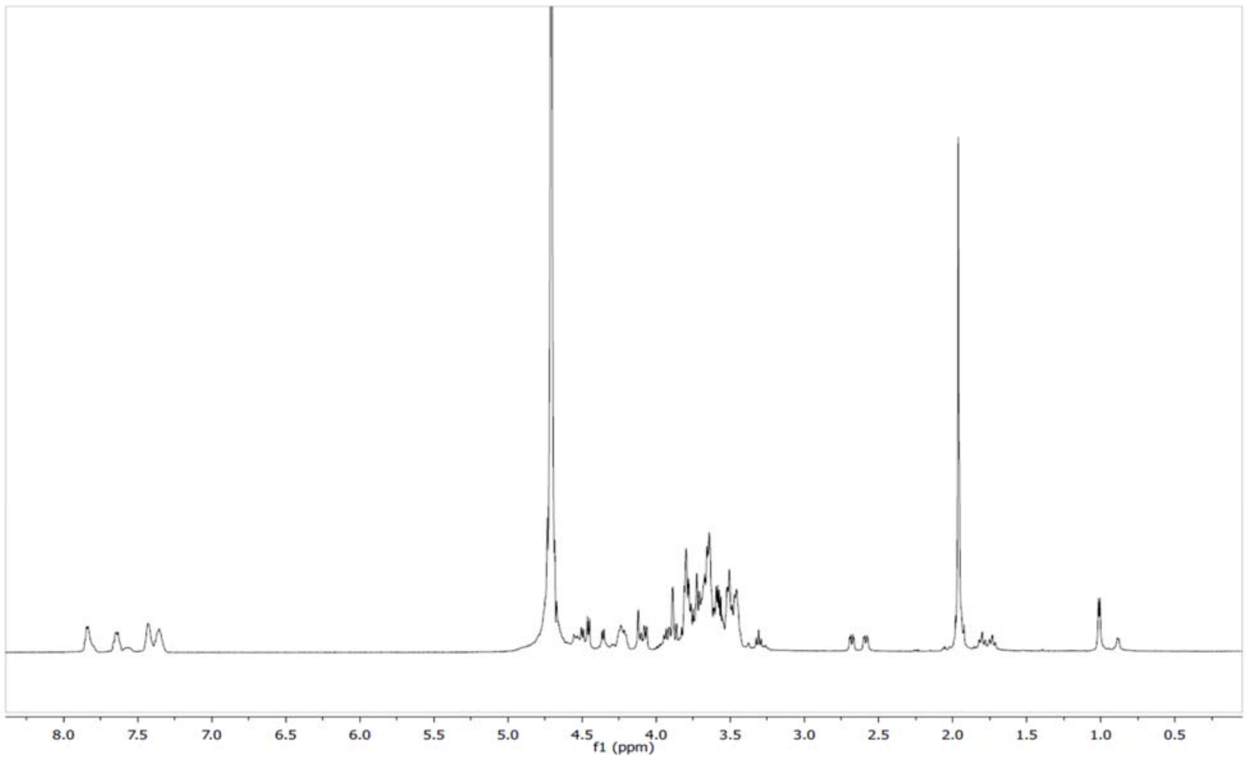
[1]H NMR of M314



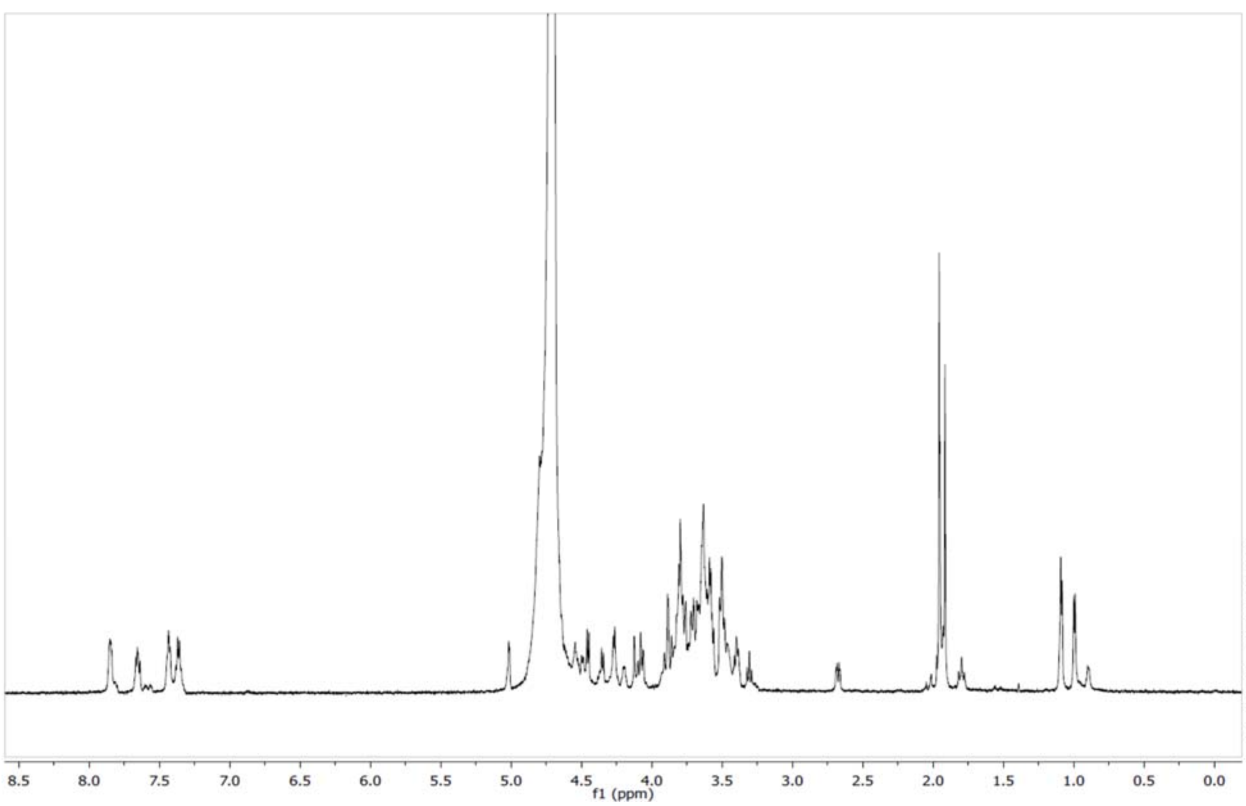
[1]H NMR of M315



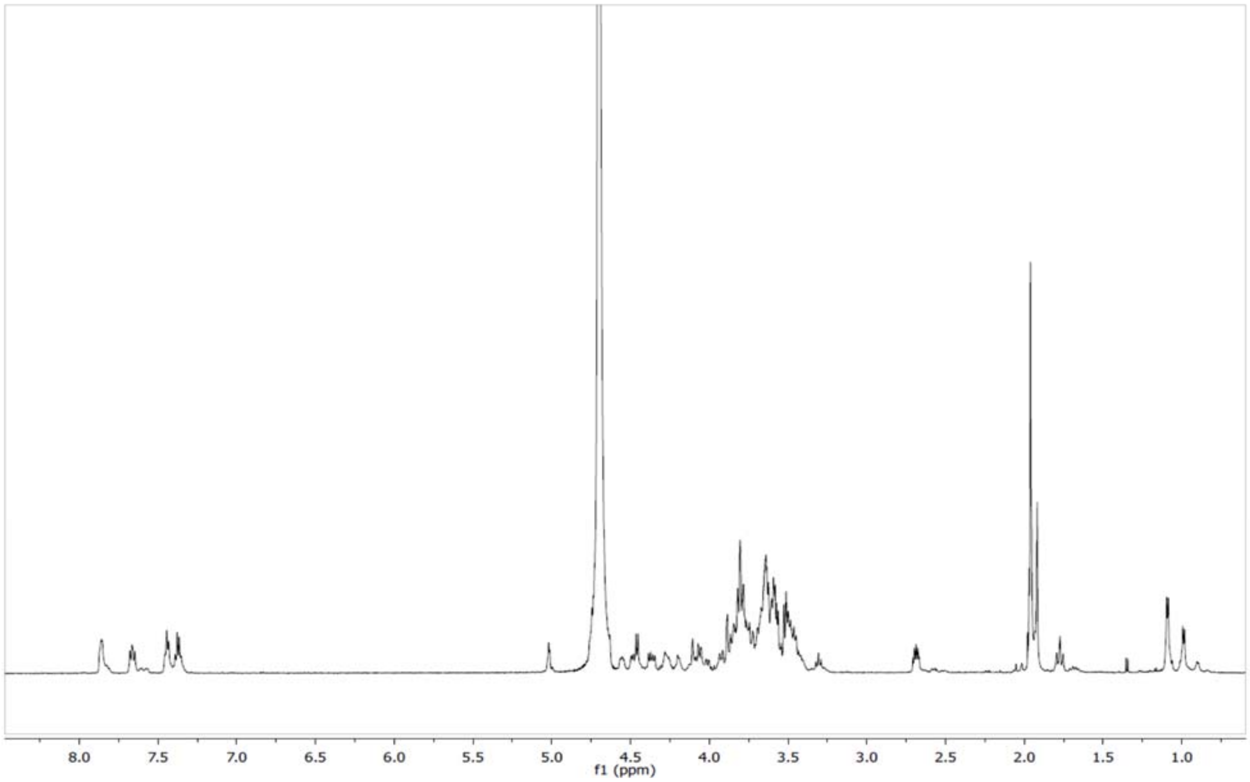
[1]H NMR of M323



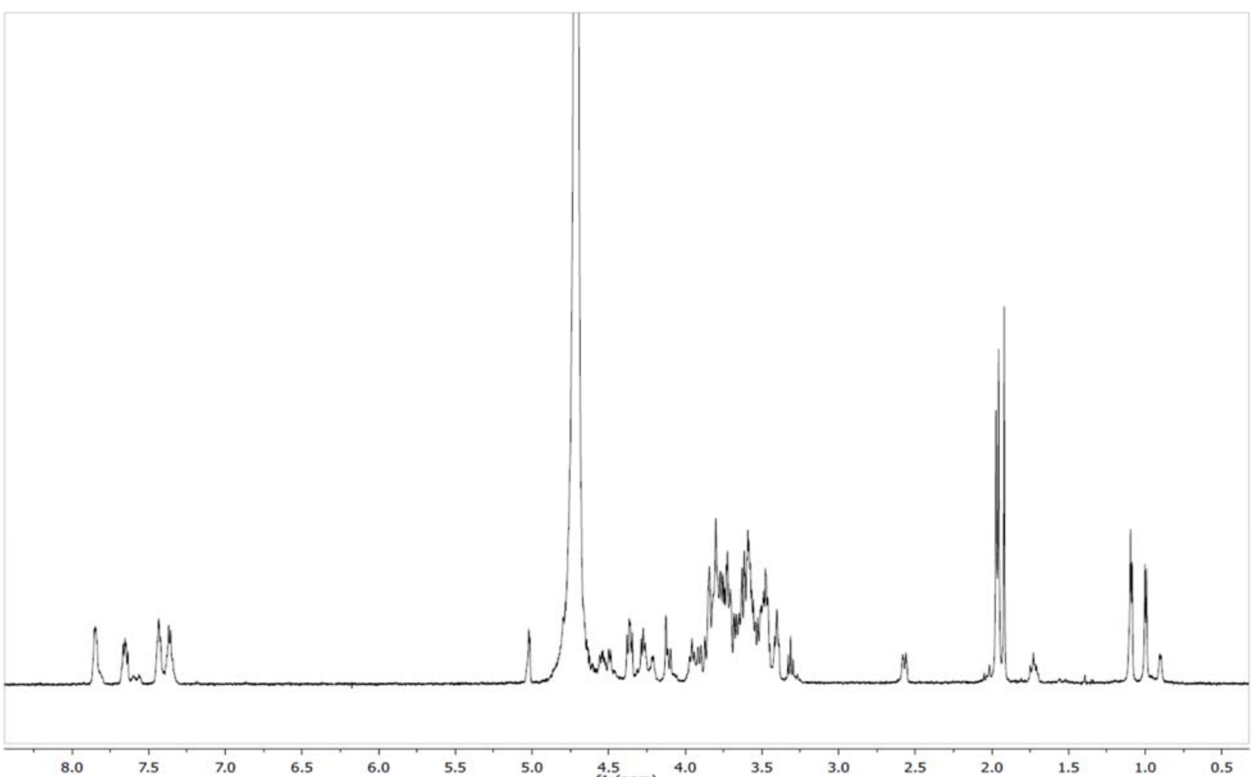
[1]H NMR of M324



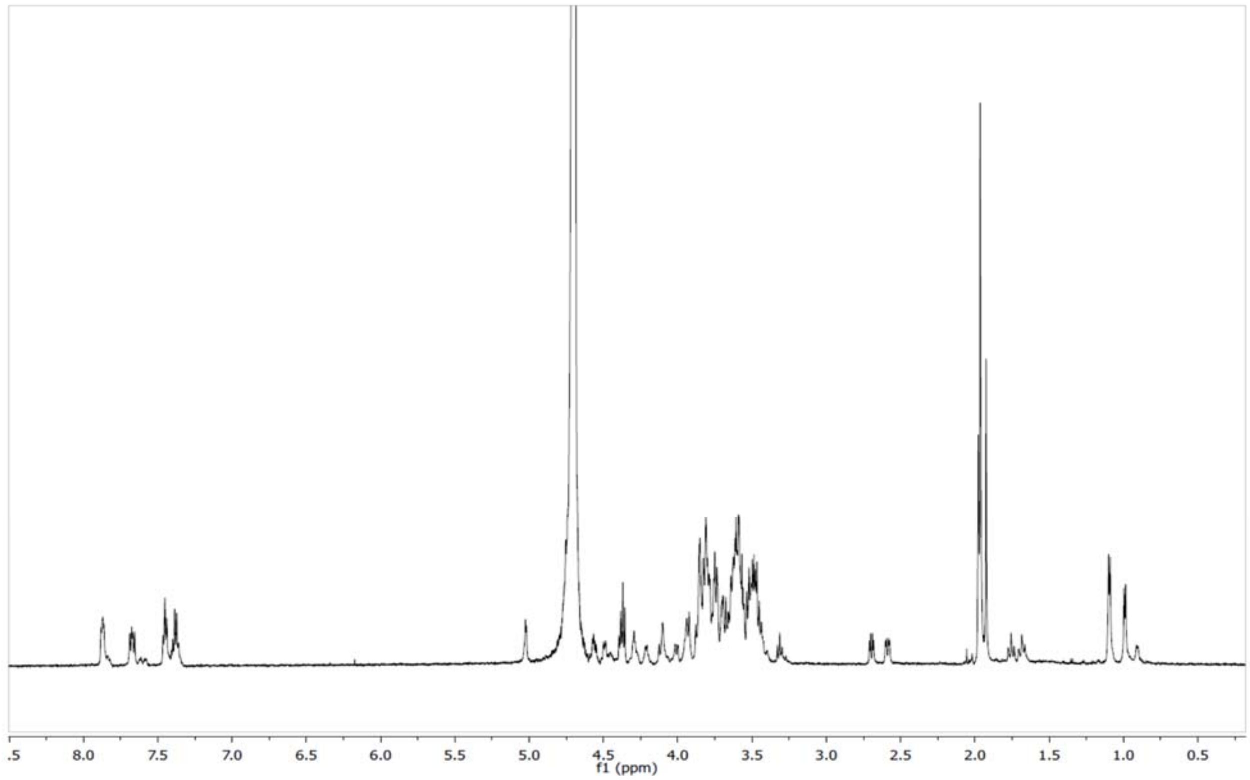
^{1}H NMR of M325



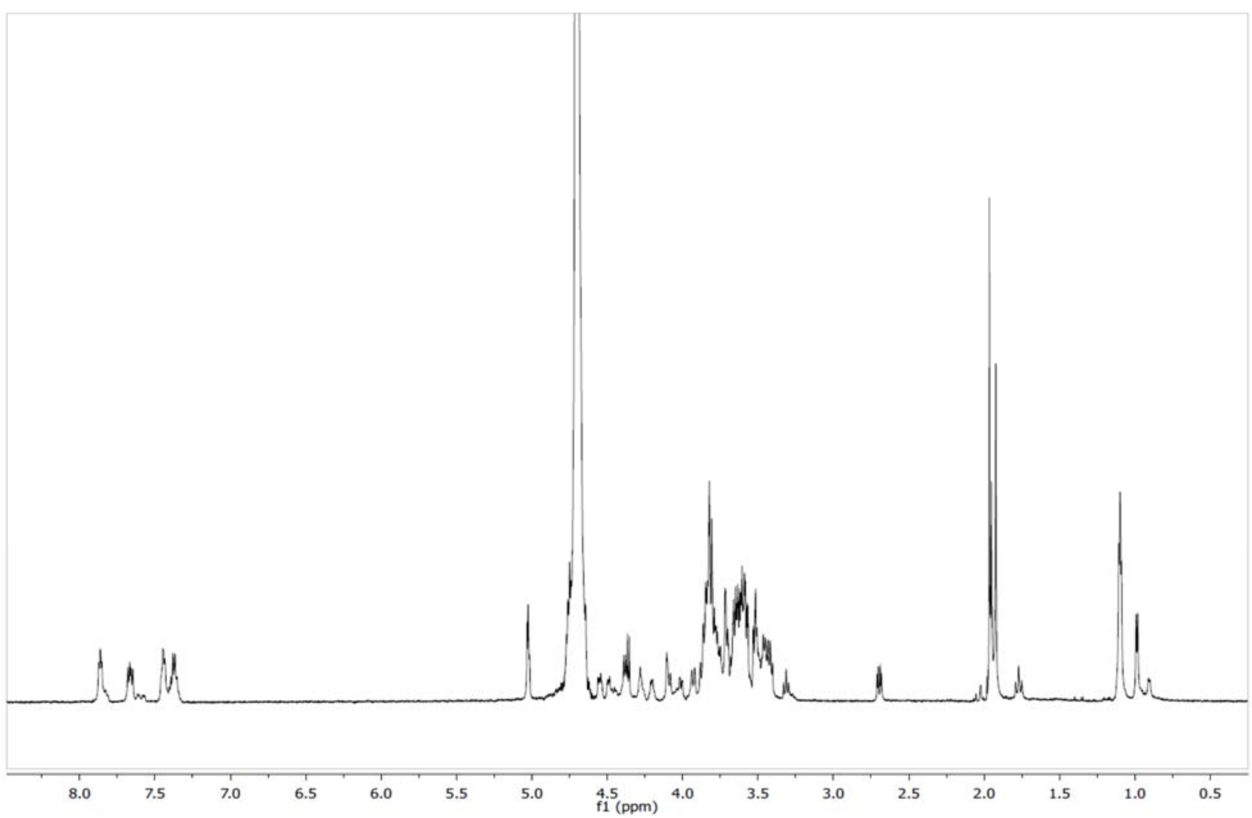
^{1}H NMR of M334



^{1}H NMR of M335



^{1}H NMR of M345



VI. Reference

[1] Z. Wang, Z. S. Chinoy, S. G. Ambre, W. Peng, R. McBride, R. P. de Vries, J. Glushka, J. C. Paulson, G. J. Boons, *Science* **2013**, *341*, 379-383.

[2] F. Yan, S. Mehta, E. Eichler, W. W. Wakarchuk, M. Gilbert, M. J. Schur, D. M. Whitfield, *J Org Chem* **2003**, *68*, 2426-2431.

[3] M. M. Muthana, J. Qu, Y. Li, L. Zhang, H. Yu, L. Ding, H. Malekan, X. Chen, *Chem Commun (Camb)* **2012**, *48*, 2728-2730.

[4] G. Zhao, W. Guan, L. Cai, P. G. Wang, *Nat Protoc* **2010**, *5*, 636-646.

[5] Y. Guo, J. Fang, T. Li, X. Li, C. Ma, X. Wang, P. G. Wang, L. Li, *Carbohydr Res* **2015**, *411*, 1-5.

[6] H. Yu, H. Yu, R. Karpel, X. Chen, *Bioorg Med Chem* **2004**, *12*, 6427-6435.

[7] G. Sugiarto, K. Lau, J. Qu, Y. Li, S. Lim, S. Mu, J. B. Ames, A. J. Fisher, X. Chen, *ACS Chem Biol* **2012**, *7*, 1232-1240.

[8] G. Sugiarto, K. Lau, Y. Li, Z. Khedri, H. Yu, D. T. Le, X. Chen, *Mol Biosyst* **2011**, *7*, 3021-3027.

[9] H. Yu, S. Huang, H. Chokhawala, M. Sun, H. Zheng, X. Chen, *Angew Chem Int Ed Engl* **2006**, *45*, 3938-3944.

[10] K. Lau, V. Thon, H. Yu, L. Ding, Y. Chen, M. M. Muthana, D. Wong, R. Huang, X. Chen, *Chem Commun (Camb)* **2010**, *46*, 6066-6068.

[11] H. Yu, Y. Li, Z. Wu, L. Li, J. Zeng, C. Zhao, Y. Wu, N. Tasnima, J. Wang, H. Liu, M. R. Gadi, W. Guan, P. G. Wang, X. Chen, *Chem Commun (Camb)* **2017**, *53*, 11012-11015.

[12] S. Einarsson, B. Josefsson, S. Lagerkvist, *J Chromatogr A* **1983**, *282*, 609-618.

[13] Z. Wu, Y. Liu, L. Li, X. F. Wan, H. Zhu, Y. Guo, M. Wei, W. Guan, P. G. Wang, *Org Biomol Chem* **2017**, *15*, 8946-8951.

[14] J. Yu, O. C. Grant, C. Pett, S. Stahl, R. J. Woods, U. Westerlind, *Chemistry* **2017**, *23*, 3466-3473.

[15] J. M. Ervasti, K. Ohlendieck, S. D. Kahl, M. G. Gaver, K. P. Campbell, *Nature* **1990**, *345*, 315-319.

VII. Microarray Data

Microarray assay using Lectins

Glycan	AAL	STDEV		RCA-I	STDEV		ECA	STDEV		Con A	STDEV
M100	-0.60	2.15		66.50	3.87		-4.33	5.96		38589.33	3089.79
M101	-3.80	2.76		206.80	57.07		3377.50	358.19		445.83	87.57
M102	6.20	2.80		38.00	7.34		-2.50	6.43		245.67	82.94
M102G	11.80	3.03		27.60	7.54		-5.17	5.45		392.33	162.75
M103	7.00	3.40		26.80	6.02		-4.50	6.49		4856.33	2058.03
M103G	6.80	1.74		39.80	9.10		5.50	5.54		1794.17	892.14
M104	43846.50	2352.23		12.60	3.96		11.17	10.88		123.67	59.88
M105	30774.40	1658.07		46.60	8.50		5.33	9.07		73.50	37.68
M106	5.80	1.66		30.60	8.42		0.33	9.37		779.00	242.76
M000	6.20	2.04		15.40	2.19		129.67	43.33		385.17	302.59
M010	4.80	2.69		26129.70	1604.66		21723.50	2869.31		232.50	98.02
M020	9.20	2.31		22.20	4.44		243.00	32.51		300.00	119.21
M030	12.20	2.20		380.10	78.78		20.67	1.94		214.50	105.40
M040	30008.80	838.23		20.40	6.31		939.33	169.00		76.33	28.05
M050	19419.80	534.20		23.00	7.42		-6.50	8.86		171.50	67.10
M201	16.40	4.10		81.40	27.33		5408.67	1016.71		288.33	230.32
M202	6.60	1.33		15.00	5.76		15.33	8.57		208.50	115.46
M203	20.40	2.46		30.90	7.07		-15.83	6.04		75.00	36.63
M204	29119.90	911.55		19.60	5.73		99.33	52.27		172.33	107.19
M205	15216.20	706.74		25.80	5.14		165.50	15.61		83.17	62.60
M212	15.20	2.72		1280.10	97.46		3453.33	527.96		39.33	26.41
M213	10.40	0.99		12480.70	1347.74		5220.17	594.38		215.67	97.29
M214	29372.40	757.98		5783.70	486.87		6128.17	534.20		201.17	52.39
M215	15444.30	1731.53		4325.50	579.71		10088.50	1616.05		246.17	180.51
M223	28.20	2.56		39.40	4.23		270.67	47.39		416.17	237.03
M224	13595.80	823.96		36.10	7.80		384.00	45.12		44.33	64.16
M225	10597.00	602.63		18.20	5.78		148.17	53.37		146.17	87.03
M234	9742.20	885.93		98.90	18.89		38.50	7.89		60.33	73.73
M235	8098.10	266.10		417.40	61.60		371.17	93.35		35.50	51.96
M245	19742.50	985.93		27.60	8.51		14.50	8.88		168.17	70.27
M301	66.20	6.79		1965.10	571.47		4328.50	684.17		225.50	149.91
M302	18.00	1.83		89.60	37.82		4.67	11.27		167.83	143.12
M303	15.80	1.51		36.60	6.38		7.00	9.83		104.50	111.79
M304	5411.40	381.44		30.80	8.95		105.00	36.20		227.50	56.02
M305	3084.00	95.30		24.10	8.91		19.50	12.63		108.00	44.20
M312	5.40	2.02		829.00	108.92		2460.67	494.91		233.17	146.94
M313	15.60	2.59		2883.90	590.92		3682.17	491.71		230.33	80.99

M314	9448.80	1881.91		75.20	31.44		2151.50	182.50		359.50	115.25
M315	3379.10	419.13		8.40	5.41		1848.00	240.68		147.33	126.87
M323	18.60	3.77		110.00	31.31		86.33	27.50		111.33	78.14
M324	3700.40	473.38		17.00	2.26		137.67	29.21		153.83	172.10
M325	4253.60	223.48		26.70	8.86		15.67	6.23		119.67	88.29
M334	5117.50	1205.63		22.20	3.24		85.67	34.88		113.83	107.47
M335	3592.40	563.46		43.50	18.77		13.67	6.57		124.67	69.86
M345	20909.40	1360.96		4.20	1.82		23.33	14.68		104.50	56.80
NC	27.20	3.37		24.70	3.33		23.17	25.37		74.00	90.41
PC1	25869.00	2541.00		29592.90	384.17		35474.67	1490.64		27411.67	2617.91
PC2	32.05	20.13		2.00	10.23		1523.67	157.12		125.37	23.05
Marker	14695.26	3210.15		15246.26	2104.16		29284.83	1215.30		31535.33	2122.57

Microarray assay using antibodies

Anti-CD15S	STDEV		**IIH6**	STDEV
75.50	14.45		13.40	16.22
30.80	8.14		28.40	16.12
20.60	3.20		-28.40	11.97
28.80	9.45		36.40	22.06
51.60	8.60		11.00	18.56
28.20	10.05		28.80	15.49
18.40	2.33		42.20	29.33
60.80	7.61		8.00	14.53
45.40	10.00		39.00	10.03
28.20	5.18		24.00	10.40
2.20	2.77		-5.60	18.28
20.80	5.86		97.20	5.09
62.70	11.89		95.20	20.95
77.30	13.50		41.00	12.36
22442.00	2410.34		31.80	3.40
31.60	8.10		10.60	9.21
12.60	2.16		19.80	6.88
60.60	8.62		40.00	6.88
45.40	12.05		49.60	3.86
20.20	4.16		70.80	2.18
12.00	3.32		21.40	15.27
6.80	0.92		22.40	13.07
51.80	9.20		36.00	10.31
61.20	11.69		-36.80	21.22

55.40	10.01		-11.80	9.26
10.40	0.90		68.20	12.92
38.40	8.51		70.40	12.16
31.50	9.17		5.40	1.43
29.90	7.01		16.60	4.45
54.60	12.17		212.80	9.37
29.60	8.92		134.30	11.22
19.80	2.77		-4.60	9.01
81.10	7.77		40.20	13.49
52.20	11.04		107.20	11.60
18647.50	1142.41		52.00	15.78
43.20	8.10		112.40	14.80
19.40	3.05		121.20	14.98
96.36	15.37		72.80	15.74
12649.70	2040.37		159.00	14.23
29.80	9.02		141.80	10.11
38.80	9.77		72.40	15.96
13917.20	2398.72		93.20	30.69
8.40	0.39		107.40	16.96
2716.30	140.60		42.60	9.66
6877.80	670.14		133.60	28.27
34.70	5.28		374.80	26.81
16.60	3.49		8.60	14.52
36.89	23.00		56.32	12.46
17562.24	2332.56		18253.27	3326.24

Microarray assay using antisera

Glycan	26559 Pre-immune	SDTEV		26559 final bleed	SDTEV		26560 Pre-Immune	SDTEV		26560 final bleed	SDTEV
M100	1010.50	160.84		2927.50	839.73		686.00	278.29		8663.67	1212.61
M101	412.72	34.71		576.17	186.61		368.33	102.96		398.67	40.85
M102	175.89	22.15		177.50	48.46		218.00	65.53		364.25	53.21
M102G	185.56	20.92		206.50	56.07		129.83	62.26		256.17	48.73
M103	182.11	16.11		98.17	44.03		98.50	53.77		192.83	76.81
M103G	256.94	61.60		274.50	105.82		216.33	93.97		227.33	56.52
M104	949.33	163.94		1716.83	381.91		1063.33	293.60		712.08	127.72
M105	200.50	40.33		297.83	91.91		150.83	95.89		319.92	89.50
M106	2774.89	383.53		2563.33	374.84		3150.50	511.36		4902.17	870.92
M000	809.17	65.64		3874.50	400.15		999.67	231.17		9278.08	993.38
M010	397.00	33.98		348.17	176.41		312.50	138.60		614.75	71.65

M020	182.56	25.11		189.17	107.16		98.33	72.88		272.83	71.57
M030	209.72	33.86		239.00	101.84		187.50	76.98		277.67	101.31
M040	2128.39	391.24		2246.33	229.15		1583.00	464.69		698.50	224.01
M050	254.83	41.17		257.17	160.91		157.50	19.88		312.50	51.11
M201	452.72	52.69		7193.67	680.67		678.33	207.29		9913.17	821.91
M202	275.44	27.39		7333.17	1293.18		299.00	83.62		9767.75	1128.59
M203	391.11	17.36		6436.33	686.92		329.00	75.67		8361.50	674.47
M204	560.56	68.62		5915.67	466.22		836.00	232.76		9023.50	1452.68
M205	617.67	72.40		5176.50	546.60		333.50	70.74		7084.75	515.67
M212	352.39	17.49		346.50	141.75		211.50	73.31		433.83	34.25
M213	296.28	26.03		457.67	67.96		225.83	107.85		310.75	42.10
M214	475.11	79.59		1302.00	277.23		723.83	204.32		495.92	138.73
M215	391.78	54.13		482.50	87.85		309.50	55.14		232.58	62.31
M223	403.22	27.58		509.17	129.57		210.33	46.53		196.00	102.77
M224	480.61	25.22		760.83	224.88		347.83	77.22		432.00	79.59
M225	304.33	33.02		390.67	160.33		271.00	64.43		423.33	100.46
M234	279.56	21.45		690.00	147.30		503.50	109.39		446.17	65.53
M235	361.94	19.19		510.83	129.64		256.67	89.91		458.42	97.35
M245	626.67	86.75		569.67	115.16		569.33	153.76		461.75	136.74
M301	742.33	104.16		1923.83	580.28		598.33	277.21		13042.42	2218.76
M302	292.28	40.43		1006.00	167.66		391.17	79.99		5847.50	573.02
M303	341.00	45.43		1112.00	218.63		414.17	159.72		4121.50	834.74
M304	520.67	30.33		1504.67	277.75		572.17	183.05		7448.75	467.74
M305	375.83	65.87		594.50	125.38		237.00	58.21		5323.92	1035.37
M312	328.50	25.74		634.17	132.92		217.00	83.70		288.33	75.52
M313	345.33	27.77		596.17	206.69		315.83	78.52		414.08	93.22
M314	345.89	36.03		736.33	236.41		269.67	98.58		546.33	54.92
M315	331.72	28.70		522.83	87.44		300.50	160.57		410.42	105.46
M323	456.89	34.34		422.83	209.41		241.50	27.25		501.50	81.72
M324	591.11	22.55		1444.50	290.68		869.00	213.42		647.17	91.15
M325	294.06	36.48		548.50	254.14		286.67	47.79		364.50	91.68
M334	390.33	33.13		854.67	189.55		1240.50	353.16		428.50	98.59
M335	353.44	43.41		583.00	152.61		268.00	88.15		433.00	34.53
M345	436.78	50.18		546.83	108.41		335.33	61.25		477.25	90.52
NC	533.61	97.57		1312.67	485.88		488.33	91.15		731.17	55.22
PC1	489.39	90.10		1697.50	554.79		297.33	149.36		763.58	54.34
PC2	16988.11	615.76		16789.17	1300.35		29767.83	2581.69		28637.75	434.17
MARKER	8066.89	36.81		7485.17	110.24		12823.83	121.19		13982.33	71.52