Supporting Information

Probing a Sialyltransferase's Recognition Domain to Prepare α(2,8)-Linked Oligosialosides and Analogs

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General methods

Optical rotations were determined in a 5 cm cell at 25 ± 2°C. $[a]_D^{25}$ values are given in units of 10^{-1} deg cm^2 g^{-1}. Analytical TLC was performed on Silica Gel 60-F_{254} (Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with 5% sulfuric acid in water or with a ceric ammonium molybdate dip. All commercial reagents were used as supplied unless otherwise stated. Column chromatography was performed on Silica Gel 60 (Silicycle, Ontario). HPLC purification was conducted using a UV absorbance detector. Separations were performed on a reverse phase C18 semi-preparative silica gel column with combinations of water and methanol as eluent (flow rate 0.5–2.0 mL/min). Size exclusion chromatography was performed using either Sephadex G-15 resin and water as eluent or Sephadex LH-20 resin using methanol as eluent. Molecular sieves were stored in an oven at 100° C and flame-dried under vacuum before use. Organic solutions from extractions were dried with anhydrous Na_2SO_4 prior to concentration under vacuum at < 40 °C (bath). ^{1}H NMR spectra were recorded at 300, 400 and 600 MHz on Bruker spectrometers. The first order proton chemical shifts δ_H and δ_C are reported in δ (ppm) and referenced to either residual $CHCl_3$ (δ_H 7.24, δ_C 77.0, $CDCl_3$) or residual CD_2HOD (δ_H 3.30, δ_C 49.5, CD_3OD). ^{1}H and ^{13}C NMR spectra were assigned with the assistance of GCOSY, GHSQC spectra. Microanalyses and Electrospray Ionization (ESI) Mass Spectroscopy were performed by the analytical services of the Department of Chemistry, University of Calgary. For high resolution mass determination, spectra were obtained by voltage scan over a narrow range at a resolution of approximately 10000 and recorded by the analytical services of the Department of Chemistry, University of Alberta.

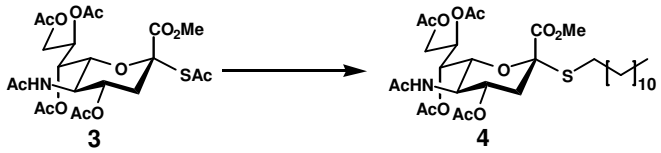
Experimental Procedures

CMP-NeuNAc synthetase from *Neisseria meningitides* (NYS-05). The enzyme was expressed in *Escherichia coli* AD202 and isolated according to published procedure.[1]

CSTII (α-2,8-sialyltransferase) from *Campylobacter jejuni* OH4384 was expressed in *Escherichia coli* AD202 according to published procedure.[1]

Cytidine 5'-monophosphate *N*-acetylneuraminic Acid (CMP-NeuNAc was prepared according to published procedure.[1]

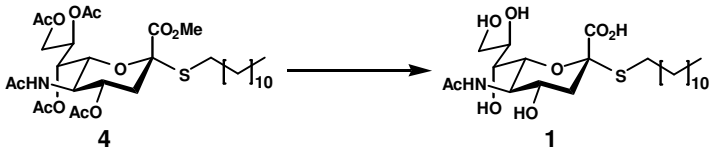
Methyl (Dodecyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (4):



Thioacetate **3**[2] (1.00 g, 1.82 mmol) was placed in a flame dried flask under an argon atmosphere. Iodododecane (0.9 mL, 3.64 mmol) was added dropwise followed by anhydrous DMF (7.5 mL). The reaction mixture was stirred for ten minutes at room temperature and diethylamine (0.9 mL) was added dropwise. After stirred for 24 hrs, ethyl acetate (~ 50 mL) was added and the organic solution was washed with water (4 × 50 mL), dried over anhydrous Na_2SO_4 and evaporated. The crude residue was purified by column chromatography on silica gel using a gradient of AcOEt – toluene (20% → 30%) as the eluent to afford the desired thioglycoside **4** (838 mg, 68% yield) as a foam. $[\alpha]^{D}_{25}$: -16.5° (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 5.32 (ddd, 1H, *J* = 2.6, 5.0, 10.8 Hz, H-8), 5.29 (dd, 1H, *J* = 1.8, 7.9 Hz, H-7), 5.22 (d, 1H, *J* = 10.2 Hz, NH), 4.82 (ddd, 1H, *J* = 4.7, 10.5, 11.7 Hz H-4), 4.27 (dd, 1H, *J* = 2.0, 12.3 Hz H-9a), 4.09 (dd, 1H, *J* = 4.7, 12.6 Hz, H-9b), 4.03 (ddd, 1H, 10.2, 10.2, 10.2 Hz, H-5), 3.79 (dd, 1H, *J* = 2.0, 10.8 Hz, H-6), 3.76 (s, 3H, OMe), 2.70 (overlapped, 1H, SC*H*aHb), 2.67 (dd, 1H, *J* = 4.4, 12.3 Hz, H-3eq), 2.49 (ddd, 1H, *J* = 6.7, 7.9, 12.0 Hz, SCHa*H*b), 2.13 (Ac), 2.10 (Ac), 2.00 (Ac), 2.00 (Ac), 1.95 (dd, 1H, *J* = 12.0, 12.0 Hz, H-3ax), 1.84 (Ac), 1.55-1.37 (m, 2H, SCHaHbCH_2), 1.34-1.13 (m, 18H, 9 × CH_2_dodecyl), 0.84 (t, 3H, *J* = 6.4 Hz, CH_3_dodecyl). ^{13}C NMR ($CDCl_3$, 100MHz): δ_C 170.92 (CO), 170.56 (CO), 170.11 (CO), 170.09 (CO), 170.01 (CO), 168.51 (CO), 83.18 (C-2), 74.18 (C-6), 69.72 (C-4), 68.89 (C-

8), 67.41 (C-7), 62.16 (C-9), 52.85 (OMe), 49.40 (C-5), 38.10 (C-3), 31.89, 29.64, 29.60, 29.57, 29.46, 29.32 (6 × CH_2_dodecyl), 29.20 (2 × CH_2_dodecyl), 28.91, 28.79 (2 × CH_2_dodecyl), 23.18 (Ac), 22.65 (CH_2_dodecyl), 21.13 (Ac), 20.83 (2 × Ac), 20.74 (Ac), 14.08 (CH_3_dodecyl).

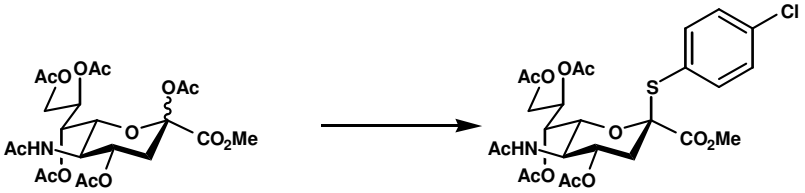
(Dodecyl 5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onic acid (1):



Thioglycoside **4** (200 mg, 0.30 mmol) was dissolved in anhydrous MeOH (5 mL) and a small amount of KOBu-*t* (70 mg, 0.60 mmol) was added. The reaction was stirred at room temperature for 45 min. The solvent was removed under reduced pressure and residue was re-dissolved in MeOH (5 mL) and H_2O (300 μL) was added. The reaction mixture was stirred at 50°C for 18 hours. The reaction mixture was cooled to room temperature and neutralized with one drop of acetic acid. After concentration under high vacuum, the residue was purified by reverse phase column chromatography on C18 silica gel using a gradient of MeOH – H_2O (33% → 75%) as the eluent. The product **1** was obtained as a white solid after lyophilization (111.0 mg, 76% yield). $[\alpha]^{D}_{25}$: +19.2° (*c* 0.24, MeOH). ^{1}H NMR (CD_3OD, 400 MHz): δ_H 3.84 (ddd, 1H, *J* = 2.8, 5.6, 8.8 Hz, H-8), 3.81 (dd, 1H, *J* = 2.8 Hz, 11.2 Hz, H-9a), 3.72 (ddd, 1H, *J* = 4.4, 9.6, 9.6 Hz, H-4), 3.67 (dd, 1H, *J* = 10.4, 10.4 Hz, H-5), 3.63 (dd, 1H, *J* = 12.4, 5.2 Hz, H-9b), 3.51 (dd, 1H, *J* = 2.0, 9.2 Hz, H-7), 3.44 (dd, 1H, *J* =2.0, 10.4 Hz, H-6), 2.87 (dd, 1H, *J* = 4.4, 12.0 Hz, H-3eq), 2.82 (ddd, 1H, *J* = 6.4, 8.4, 12.4 Hz, SC*Ha*Hb), 2.66 (ddd, 1H, *J* = 6.8, 8.0, 12.4 Hz, SCHa*Hb*), 2.00 (s, 1H, Ac), 1.63 (dd, 1H, *J* = 10.8, 12.4 Hz, H-3ax), 1.50-1.66 (m, 2H, 1 × CH_2_dodecyl), 1.20-1.40 (m, 18H, 9 × CH_2_dodecyl), 0.90 (t, 3H, *J* = 6.0 Hz, CH_3_dodecyl). ^{13}C NMR (CD_3OD, 100 MHz) δ 174.04 (NHAc), 173.71 (CO_2H), 85.71 (C-2), 75.18 (C-6), 71.63 (C-8), 68.85 (C-7), 68.27 (C-4), 63.04 (C-9), 52.65 (C-5), 41.86 (C-3), 31.66 (SCH_2), 29.51, 29.39 (2 × CH_2_dodecyl), 29.35 (2 × CH_2_dodecyl), 29.33,

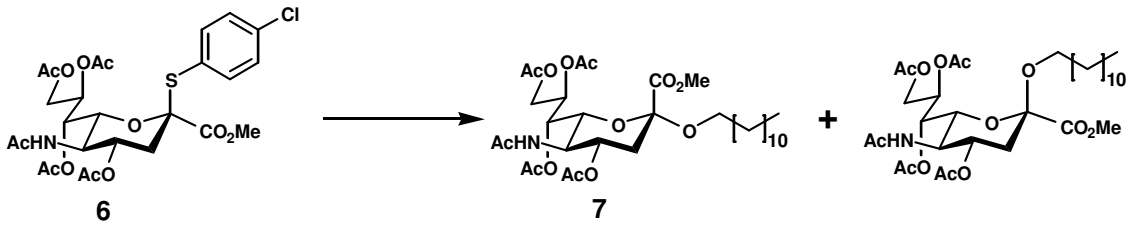
29.12, 29.06, 29.01, 28.89, 22.32 (6 × CH_2_dodecyl), 21.55 (NHAc), 13.03 (CH_3_dodecyl).

Methyl (p-chlorophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate (6)



To a solution of compound **5**[3] (533 mg, 1.0 mmol) and 4-chlorothiol phenol (159 mg, 1.1 mmol) in dry dichloromethane (10 mL) was added boron trifluoride etherate (300 μl, 2.5 mmol), The reaction mixture was kept overnight at room temperature. After diluted with dichloromethane (50 mL), the mixture was washed with saturated aqueous $NaHCO_3$, dried with $MgSO_4$, and concentrated. The residue was chromatographed using dichloromethane : acetone (6:1) as the eluent to give target compound (**6**) as a white powder (500 mg, 81%). $[\alpha]^{D}_{25}$: -140° (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 7.41 (d, 2H, *J* = 8.6 Hz, ClPh), 7.33 (d, 2H, *J* = 8.6 Hz, ClPh), 5.45 (dd, 1H, *J* = 2.4, 2.4 Hz, H-7), 5.38 (ddd, 1H, *J* = 4.7, 10.8, 11.5 Hz, H-4), 5.33 (br d, 1H, *J* = 10.0 Hz, NH), 4.93 (ddd, 1H, *J* = 2.0, 2.0, 8.6 Hz, H-8), 4.58 (dd, 1H, *J* = 2.5, 10.6 Hz, H-6), 4.47 (dd, 1H, *J* = 2.5, 12.3 Hz, H-9a), 4.14 (m, 1H, H-5), 4.02 (dd, 1H, *J* = 9.0, 12.3 Hz, H-9b), 3.65 (s, 3H, OMe), 2.67 (dd, 1H, *J* = 5.1, 13.9 Hz, H-3e), 2.10-2.17 (m, 4H, H-3ax + Ac), 2.10, (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.92 (s, 3H, Ac). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 171.24, 170.96, 170.39, 170.25, 170.16, 168.04 (CO), 137.40, 136.41, 129.35, 127.22 (Ar), 88.98 (C-2), 73.33 (C-6), 73.16 (C-8), 68.89 (C-7 + C-4), 62.64 (C-9), 52.73 (OMe), 49.37 (C-5), 37.43 (C-3), 23.17 (Ac), 21.07 (Ac), 20.88 (Ac), 20.72 (× 2, Ac × 2). ESI MS m/z calc'd for $C_{26}H_{32}ClNO_{12}SNa$ $(M+Na)^+$: 640.1; found: 640.2.

Methyl (dodecyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (7) and Methyl (dodecyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosid)onate



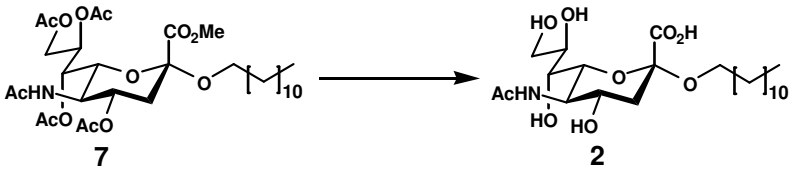
Thioglycoside **6** (798 mg, 1.29 mmol) and 1-dodecanol (481 mg, 2.401 mmol) were dissolved in a mixture of anhydrous dichloromethane (3.0 mL) and acetonitrile (6.0 mL) under an atomosphere of argon; 4 Å molecular sieves (300 mg) was added and the mixture was stirred at room temperature overnight. The temperature was cooled to -50 °C and *N*-iodosuccinimide (508 mg, 2.14 mmol) was added. After stirring for 10 minutes, a catalytic amount of trifluoromethanesulfonic acid (5 μL) was added dropwise and the reaction was slowly warmed to room temperature. TLC (CH_3CN : toluene 2 : 3) revealed that there were still starting material left. The temperature was cooled again to -50 °C and more *N*-iodosuccinimide (300 mg, 1.26 mmol) was added; subsequently another portion of trifluoromethanesulfonic acid (3 μL) was added and the reaction was slowly warmed to room temperature. TLC showed that all the staring material had been consumed. Et_3N (1.0 mL) was added and mixtire was diluted with ethyl acetate (20 mL). After filtration, the organic solution was washed with a 1 : 1 mixture of 10% $Na_2S_2O_3$ and sat. $NaHCO_3$, dried and evaporated. The residue was purified by column chromatography on silica gel using a mixture of acetone – CH_2Cl_2 (10 : 90) as eluent to afford sequentially the pure β-sialoside (**8**, 101.0 mg, yield: 11.9%), and the desired pure α-sialoside (**7**, 501.8 mg, yield 58.9%).

Data for the α-anomer (7): $[\alpha]^{D}_{25}$: -14.0° (*c* 0.47, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 5.38 (ddd, 1H, *J* = 8.1, 5.7, 2.7 Hz, H-8), 5.35 (d, 1H, *J* = 9.5 Hz, NHAc), 5.32 (dd, 1H, *J* = 8.1, 1.8 Hz, H-7), 4.83 (ddd, 1H, *J* = 12.4, 9.7, 4.6 Hz, H-4), 4.31 (dd, 1H, *J* = 12.4, 2.6 Hz, H-9a), 4.01-4.13 (m, 3H, H-9b + H-5 + H-6), 3.77 (s, 3H, OMe), 3.73 (ddd, 1H, *J* = 9.3, 6.6, 6.6 Hz, OC*Ha*CHb), 3.21 (ddd, 1H, *J* = 9.3, 6.6, 6.6 Hz, OCHa*CHb*),

2.57 (dd, 1H, *J* = 12.8, 4.8 Hz, H-3eq), 2.13 (s, 3H, Ac), 2.12 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.93 (dd, 1H, *J* = 12.6, 12.6 Hz, H-3ax), 1.86 (s, 3H, Ac), 1.46-1.55 (m, 2H, OCHaCHbCH_2), 1.19-1.34 (m, 18H, 9 × CH_2_dodecyl), 0.86 (t, 3H, *J* = 6.6 Hz, CH_3_dodecyl). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 171.00 (CO), 170.61 (CO), 170.22 (CO), 170.13 (CO), 170.05 (CO), 168.58 (CO), 98.75 (C-2), 72.47 (C-6), 69.26 (C-4), 68.86 (C-8), 67.45 (C-7), 65.09 (OCHaHb), 62.38 (C-9), 52.59 (OMe), 49.42 (C-5), 38.11 (C-3), 31.91, 29.68, 29.63, 29.62, 29.59 (× 2), 29.34, 29.33, 25.87 (9 × CH_2_dodecyl), 23.18 (Ac), 22.68 (1 × CH_2_dodecyl), 21.10 (Ac), 20.86 (Ac), 20.83 (Ac), 20.77 (Ac), 14.11 (CH_3_dodeyl). HRMS m/z calc'd for $C_{32}H_{53}NO_{13}Na$ ($M+Na^+$): 682.34091; found: 682.34115.

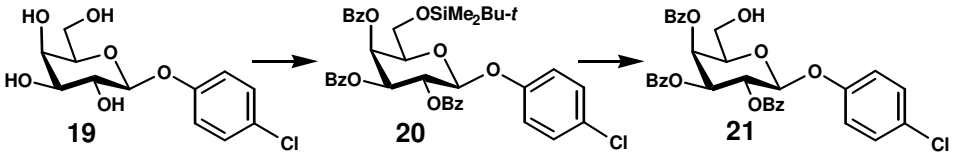
Data for the β-anomer: $[\alpha]^D_{25}$: -12.2° (*c* 0.51, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 5.39 (dd, 1H, *J* = 2.4, 3.5 Hz, H-7), 5.37 (d, 1H, *J* = 10.2 Hz, NHAc), 5.25 (ddd, 1H, *J* = 4.8, 11.0, 11.0 Hz, H-4), 5.18 (ddd, 1H, *J* = 2.6, 3.6, 7.5 Hz, H-8), 4.79 (dd, 1H, *J* = 12.4, 2.4 Hz, H-9a), 4.06-4.16 (m, 2H, H-9b + H-5), 3.92 (dd, 1H, *J* = 2.0, 10.6 Hz, H-6), 3.78 (s, 3H, OMe), 3.44 (ddd, 1H, *J* = 9.3, 6.4, 6.4 Hz, OC*Ha*CHb), 3.30 (ddd, 1H, *J* = 9.3, 6.8, 6.8 Hz, OCHa*CHb*), 2.45 (dd, 1H, *J* = 12.8, 4.9 Hz, H-3eq), 2.13 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.02 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.87 (s, 3H, Ac), 1.85 (dd, 1H, *J* = 12.6, 11.5 Hz, H-3ax), 1.49-1.60 (m, 2H, OCHaCHbCH_2), 1.20-1.37 (m, 18H, 9 × CH_2_dodecyl), 0.86 (t, 3H, *J* = 6.6 Hz, CH_3_dodecyl). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 171.08 (CO), 170.69 (CO), 170.51 (CO), 170.22 (CO), 170.17 (CO), 167.62 (CO), 98.48 (C-2), 72.23 (C-8), 71.72 (C-6), 69.04 (C-4), 68.49 (C-7), 64.26 (OCHaHb), 62.44 (C-9), 52.61 (OMe), 49.42 (C-5), 37.48 (C-3), 31.92, 29.67, 29.65 (× 2), 29.61, 29.56, 29.45, 29.35, 26.08 (9 × CH_2_dodecyl), 23.17 (Ac), 22.69 (1 × CH_2_dodecyl), 21.03 (Ac), 20.90 (Ac), 20.80 (2 × Ac), 14.12 (CH_3_dodecyl). HRMS m/z calc'd for $C_{32}H_{53}NO_{13}Na$ ($M+Na^+$): 682.34091; found: 682.34132.

Dodecyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid (2)



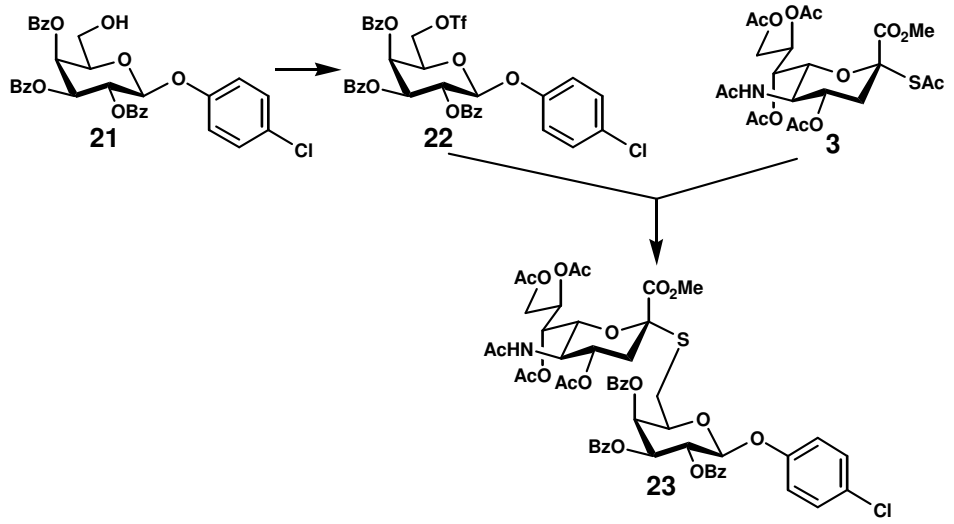
The fully protected α-sialoside (245.0 mg, 0.371 mmol) was dissolved in anhydrous methanol (20 mL), and a solution of 0.5 M NaOMe in MeOH (500 µL) was added. The mixture was stirred for 30 minutes and concentrated under vacuum. The residue was dissolved in an 1 : 1 H_2O – MeOH mixture (10 mL) and the saponification was continued at room temperature overnight. The pH of the solution was neutralized with acetic anhydride and the solvent was removed under reduced pressure. The residue was finally purified by HPLC on a reverse phase silica gel column using a gradient of H_2O – MeOH (0% → 100%) as eluent to afford the desired **2** as white solid after lyophilization (162 mg, 91% yield). $[\alpha]^{D}_{25}$: +3.5° (*c* 0.17, MeOH). ^{1}H NMR (CD_3OD, 400 MHz): δ_H 3.87 (ddd, 1H, *J* = 2.5, 5.4, 8.9 Hz, H-9), 3.83 (dd, 1H, *J* = 2.2, 11.4 Hz, H-9a), 3.76 (ddd, 1H, *J* = 8.9, 7.0, 7.0 Hz, -OC*Ha*Hb), 3.59-3.71 (m, 4H, H-4 + H-5 + H-9b + OC*H*aHb), 3.58 (dd, 1H, *J* = 1.6, 10.2 Hz, H-6), 3.51 (dd, 1H, *J* = 8.9, 1.6 Hz, H-7), 3.46 (ddd, 1H, *J* = 9.2, 6.7, 6.7 Hz, -OCHa*Hb*), 2.84 (high order dd, 1H, *J* = 4.1, 12.0 Hz, H-3eq), 2.02 (s, 3H, Ac), 1.57 (high order dd, 1H, *J* = 11.8, 11.8 Hz, H-3ax), 1.48-1.56 (m, 2H, OCHaHbCH_2), 1.17-1.38 (m, 18H, 9 × CH_2_dodecyl), 0.90 (t, 3H, *J* = 6.3 Hz, CH_3_dodecyl). ^{13}C NMR (CD_3OD, 100 MHz): δ_C 175.59 (CO), 174.71 (CO), 101.87 (C-2), 74.35 (C-6), 73.07 (C-8), 70.41 (C-7), 69.59 (C-4), 65.33 (OCH_2_dodecyl) 64.57 (C-9), 54.34 (C-5), 42.84 (C-3), 33.12, 31.10, 30.86, 30.81 (× 3), 30.72, 30.52, 27.71, 23.78 (10 × CH_2_dodecyl), 22.64 (NHAc), 14.49 (CH_3_dodecyl). HRMS m/z calc'd for $C_{23}H_{42}NO_9$ $(M-H)^-$: 476.28541; found: 476.28551.

4-Chlorophenyl 2,3,4-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (21)



To a solution of 4-chlorophenyl 1-thio-β-D-galactopyranoside (**19**)[4] (1.0 g, 3.25 mmol) in anhydrous pyridine (3.0 mL) was added tert-butylsimethylsilyl chloride (573 mg, 3.9 mmol) and 4-*N*,*N*-dimethylaminopyridine (cat. amount). The mixture was stirred at room temperature for 3 h. TLC shows a single product was formed (R_f = 0.6, 10:1 DCM/MeOH). The mixture was diluted with more anhydrous pyridine (10.0 mL) and benzoyl chloride (1.7 mL, 14.65 mmol) was added. The mixture was continued to stir at 40 ℃ overnight. The solvent was removed under reduced pressure. The residue was dissolved in ethyl acetate and the organic solution was washed with 2N HCl, saturated $NaHCO_3$, and brine and evaporated. The crude mixture was dissolved in the mixture of MeCN and water (9:1, 20 mL), and *p*-TsOH (1.37 g, 7.95 mmol) was added. The mixture was stirred at room temperature for 30 min. The reaction was quenched with $NaHCO_3$, and extracted with dichloromethane, The organic layer was washed with brine and concentrated. The residue was purified by column chromatography (4:1 toluene/ ethyl acetate) to give the desired product **21** as a white solid (1.69 g, 75% yield for three steps): R_f = 0.3 (3:1 toluene/ethyl acetate); $[\alpha]_D^{25}$:+53.0° (*c* 1.97, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 7.23–8.02 (m, 19H, ArH), 5.87 (dd, 1H, *J* = 3.2, ≈1.0 Hz, H-4), 5.75 (dd, 1H, *J* = 10.0, 10.0 Hz, H-2), 5.61 (dd, 1H, *J* = 10.0, 3.2 Hz, H-3), 4.99 (d, 1H, *J* = 10.0 Hz, H-1), 4.14 (dd, 1H, *J* = 6.8, <1.0 Hz, H-5), 3.87 (dd, 1H, *J* = 12.0, 6.8 Hz, H-6a), 3.64 (dd, 1H, *J* = 12.0, 6.8 Hz, H-6b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C166.3 (CO), 165.6 (CO), 165.2 (CO), 136.1, 135.1, 133.8, 133.4, 133.3, 130.1, 129.9, 129.8, 129.7, 129.2, 129.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 84.7 (C-1), 77.9 (C-5), 73.2 (C-3), 68.8 (C-4), 67.9 (C-2), 60.7 (C-6); ESI HRMS m/z calc'd for $C_{33}H_{27}O_8SClNa$ $(M+Na)^+$: 641.10074; found: 641.10089.

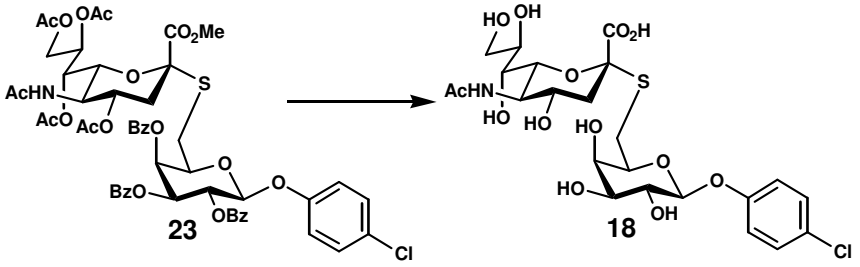
4-Chlorophenyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-*O*-benzoyl-6-deoxy-1,6-dithio-β-D-galactopyranoside (23)



Compound **21** (200 mg, 0.32 mmol) was dissolved in anhydrous dichloromethane (2.0 mL) and pyridine (0.1 mL, 1.24 mmol, 4.0 equiv.) with stirring under argon. After cooling the solution to -25 °C, trifluoromethanesulfonic anhydride (0.065 mL, 0.38 mmol, 1.2 equiv.) was added dropwise over approximately one minute. After stirring at constant temperature for 1 h, the reaction mixture was diluted with more dichloromethane (60 mL), and washed successively with cold 1M HCl, saturated $NaHCO_3$ solution, and ice-cold water; the organic layer was dried over Na_2SO_4, filtered, and concentrated at 18 °C under high vacuum. The crude triflate **22** was combined with **3** (260 mg, 0.47 mmol, 1.5 equiv.), and the mixture was dissolved in anhydrous DMF (2.0 mL) under argon. After cooling the solution to 0 °C, diethylamine (0.33 mL, 3.2 mmol, 10 equiv.) was added and the solution was stirred at room temperature for 4 h. The solvent was removed under reduce pressure and the residue was purified by column chromatography using a mixture of methanol - dichloromethane (1: 99) to afford **23** (100 mg, 30% yield) as amorphous solid. R_f = 0.4 (2:98 MeOH/DCM); $[\alpha]_D^{25}$: +89.6° (*c* 5.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 7.99 (m, 2H, Bz), 7.80 (m, 2H, Bz), 7.73 (m, 2H, Bz), 7.58-7.65 (m, 3H, 1H_Bz + 2H_PhCl), 7.52 (m, 1H, Bz), 7.32-7.50 (m, 7H, 5H_Bz + 2H_PhCl), 7.23 (m, 2H, Bz), 6.11 (dd, 1H, *J* = 3.2, ~1.0 Hz, H-4), 5.71 (dd, 1H, *J* = 10.0, 3.2 Hz, H-3), 5.63 (dd, 1H, *J* = 10.0, 10.0 Hz, H-2), 5.44 (ddd, 1H, *J* = 2.9, 5.1, 9.5 Hz, H-8'), 5.32 (dd, 1H, *J* = 8.8, ~1.0 Hz, Hz, H-7'), 5.30 (d, 1H, *J* = 10.0 Hz, H-1), 5.18 (high order d, 1H, *J* = 8.9 Hz, NHAc), 4.95 (high order m, 1H, H-4'), 4.40 (dd, 1H, *J* = 12.4, 2.4 Hz, H-9a'), 4.34 (ddd, 1H, *J* = 6.8, 6.8, ~1.0 Hz, H-5), 4.21 (dd, 1H, *J* = 12.4, 4.2 Hz, H-9b'),

3.90-4.00 (m, 2H, H-5' + H-6'), 3.82 (s, 3H, CH_3), 2.97 (dd, 1H, *J* =14.6, 6.7 Hz, H-6a), 2.79 (dd, 1H, *J* = 14.6, 7.9 Hz, H-6b), 2.75 (dd, 1H, *J* = 12.7, 4.7 Hz, H-3eq'), 2.25 (s, 3H, Ac), 2.19 (s, 3H, Ac), 2.03-2.07 (m, 4H, H-3ax' + Ac), 2.00 (s, 3H, Ac), 1.92 (s, 3H, Ac); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_H 170.87 (CO), 170.72 (CO), 170.61 (CO), 170.07 (CO), 169.94 (CO), 168.23 (CO), 165.25 (CO), 165.22 (CO), 165.05 (CO), 135.65, 134.48, 133.36, 133.23, 132.98, 129.81, 129.68, 129.45, 129.37, 129.25, 129.06, 128.77, 128.51, 128.37, 128.16, 84.13 (C-2'), 83.81 (C-1), 76.18 (C-5), 73.90 (C-6'), 73.19 (C-3), 69.12 (C-4'), 68.83 (C-3), 68.01 (C-8'), 67.80 (C-2), 66.93 (C-7'), 62.46 (C-9'), 53.18 (CO_2Me), 49.71 (C-5'), 38.32 (C-3'), 30.25 (C-6), 23.26 (Ac), 21.15 (Ac), 20.87 (Ac), 20.85 (Ac), 20.75 (Ac); ESI HRMS m/z calc'd for $C_{53}H_{54}NO_{19}S_2ClNa$ $(M+Na)^+$:1130.23122; found: 1130.23111.

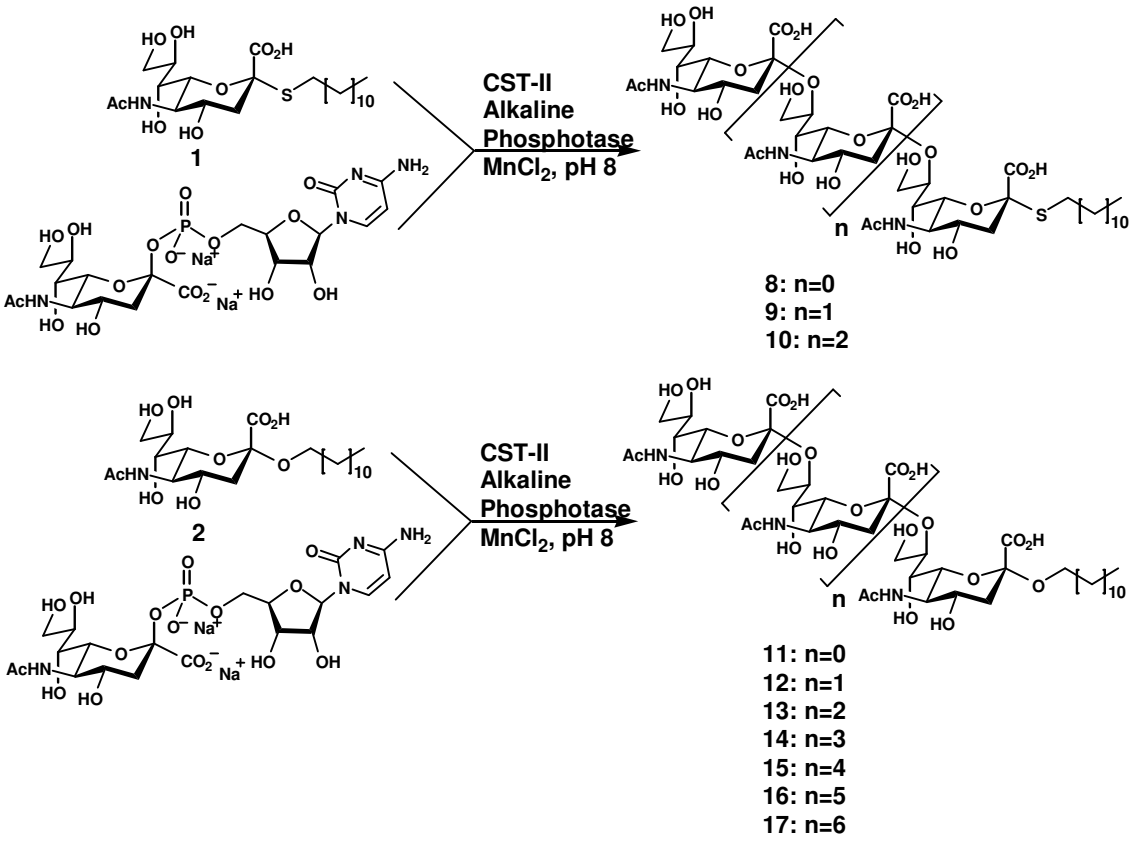
4-Chlorophenyl (5-acetamido-3,5-dideoxy-2-thio-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-6-deoxy-1,6-dithio-β-D-galactopyranoside (18)

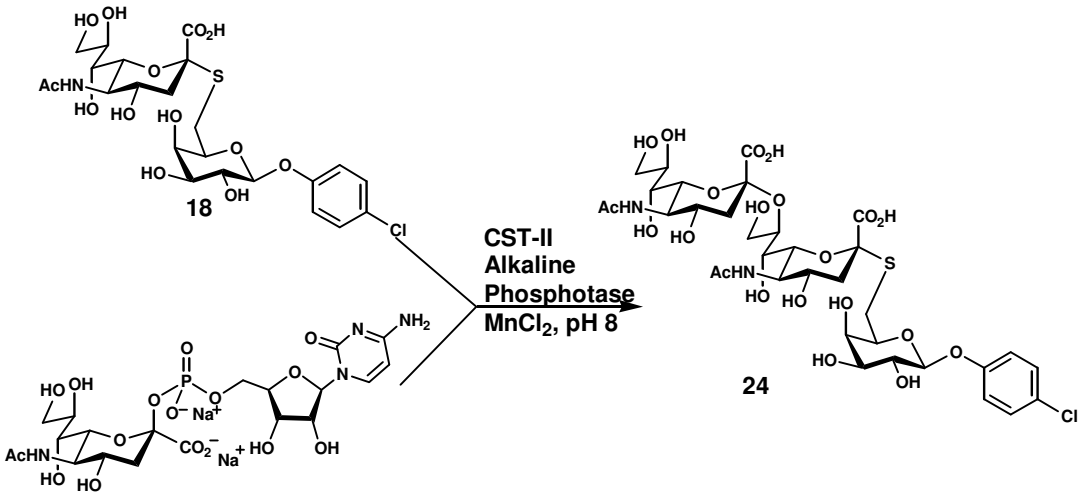


Compound **23** (160 mg, 1.36 mmol) was dissolved in anhydrous methanol (5.0 mL) and a solution of NaOMe in anhydrous methanol (2.0 M, 1.2 mL) was added under argon. The reaction mixture was stirred at room temperature for 8 h. The mixture was concentrated and the residue was redissolved in a 1:1 mixture of methanol- H_2O (4.0 mL) and the stirring was continued overnight. Several drops acetic anhydride was added to neutralize the solution and the mixture was concentrated. The residue was purified by reverse-phase C_{18} silica gel HPLC (elute 100% water) to afford compound **18** (65 mg, 75% yield) which was lyophilized as a white solid. R_f = 0.4 (65:35:5:0.1, DCM/MeOH/H_2O/HOAc); $[\alpha]_D^{25}$: +20.8° (*c* 2.4, MeOH). ^{1}H NMR (D_2O, 400 MHz): δ_H 7.86 (d, 2H, *J* = 8.4 Hz, PhCl), 7.76 (d, 2H, *J* = 8.4 Hz, PhCl), 5.02 (d, 1H, *J* = 9.6 Hz, H-1), 4.37 (dd, 1H, *J* =

2.8, ~1.0 Hz, H-4), 3.88– 4.09 (m, 10H, H-5 + H-3 + H-9a' + H-4' + H-8' + H-7' + H-6' + H-5' + H-9b' + H-2), 3.31 (dd, 1H, *J* = 14.0, 7.3 Hz, H-6a), 3.24 (dd, 1H, *J* = 14.0, 6.0 Hz, H-6b), 3.13 (dd, 1H, *J* = 12.8, 4.8 Hz, H-3_{eq}'), 2.36 (s, 3H, NHAc), 2.06 (dd, 1H, *J* = 12.7, 11.4 Hz, H-3_{ax}'); ^{13}C NMR (D_2O, 100 MHz): δ_H 174.90 (CO), 174.25 (CO), 173.18 (CO), 132.93, 132.13, 131.74, 129.20, 87.75 (C-1), 85.80 (C-2'), 78.03, 74.87, 73.94, 72.11, 69.05, 68.50, 68.00, 62.14 (C-9'), 52.14 (C-5'), 40.74 (C-3'), 29.56 (C-6), 22.13 (NHAc); ESI HRMS m/z calc'd for $C_{23}H_{31}NO_{12}S_2Cl$ $(M-H)^-$: 612.09817; found: 612.09841.

General procedure for enzymatic synthesis of oligosialosides 8-17 and 24 using α-thiosialoside 1, α-sialoside 2 and disaccharide 18 as substrates:





Procedure A: To a mixture of monosialoside (~0.061 mmol) and crude CMP-NeuNAc (2.8 equiv) was added a solution of $MnCl_2$ (0.5 M, 172 μL) and a solution of alkaline phosphatase (30 μL). A solution of crude CST-II (0.3 U/mL, 10.5 U) was added and the mixture was gently tumbled for 24 h. The reaction mixture was concentrated in vacuo. The residue was extracted with MeOH and concentrated under reduced vacuum. The residue was suspended in H_2O (5 mL), and the solution was recovered by centrifugation. The solid was extracted two more times (2 × 5 mL) as above, and the aqueous solutions were combined and concentrated (~ 3 mL). The mixture was purified on Sephadex G-25 using H_2O as eluent. The fractions containing oligomers were combined and concentrated and the residue was purified by HPLC on a reverse phase C18 column using a gradient of MeOH-H_2O (0→100%) as eluent to afford a(2,8)-linked oligosialosides up to tetramers.

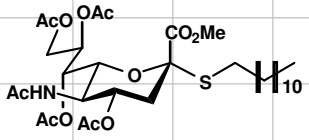
Procedure B: To a mixture of monosialosides (0.088 mmol) and crude CMP-NeuNAc (2.0 equiv) was added a solution of $MnCl_2$ (0.5 M, 200 μL) and a solution of alkaline phosphatase (20 μL). A solution of crude CST-II (0.3 U/mL, 10 mL, 3U) was added and the mixture was gently tumbled overnight at room temperature. Another batch of CST-II (0.3 U/mL, 10 mL, 3U), crude CMP-NeuNAc (2.0 equiv) and of alkaline phosphatase (20 μL) was added and the reaction was continued for another 24 hours. The reaction mixture was diluted with EtOH (50 mL) and concentrated under reduced vacuum. The residue was suspended in H_2O (5 mL), and the solution was recovered by centrifugation. The solid was extracted two more times (2 x 5 mL) as above, and the aqueous solutions were

combined and concentrated (~ 5 mL). The mixture was purified on Sephadex G-25 using H_2O as eluent. The fractions containing oligomers were combined and concentrated. The residue was purified by HPLC on a reverse phase C18 column using a gradient of MeOH-H_2O (0%→100%) as eluent to afford α(2,8)-disialoside – α(2,8)-octasialoside.

References:

1 Jacques, S.; Rich, J. R.; Ling, C.-C.; Bundle. D. R. *Org. Biomol. Chem.* **2006**, *4*, 142-154.
2 Warner, T. G.; Lee, L. A. **1988**, *176*, 211-218.
3 Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *187*, 35.
4 Sugiyama, S.; Diakur, J. M.; *Org. Lett.* **2000**, *2***,** 2713.

^{1}H NMR in $CDCl_3$, 400 MHz



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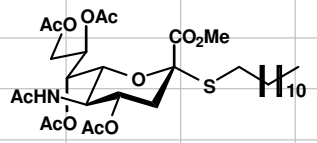

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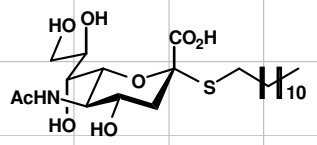
^{1}H NMR in CD_3OD, 400 MHz



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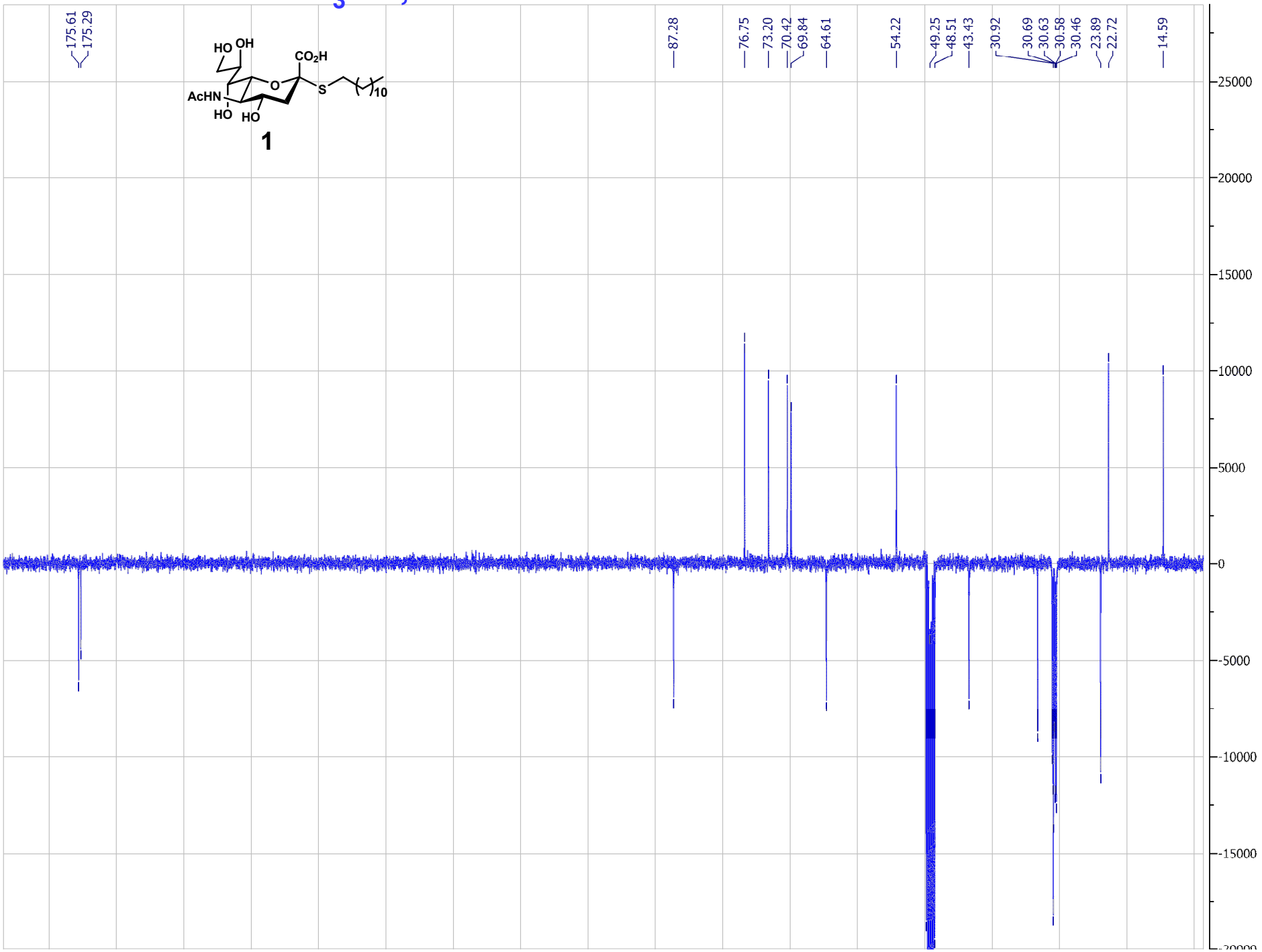

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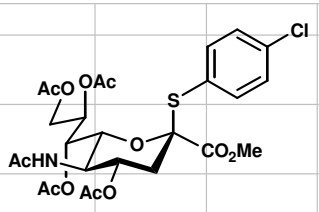
^{1}H NMR in $CDCl_3$, 400 MHz



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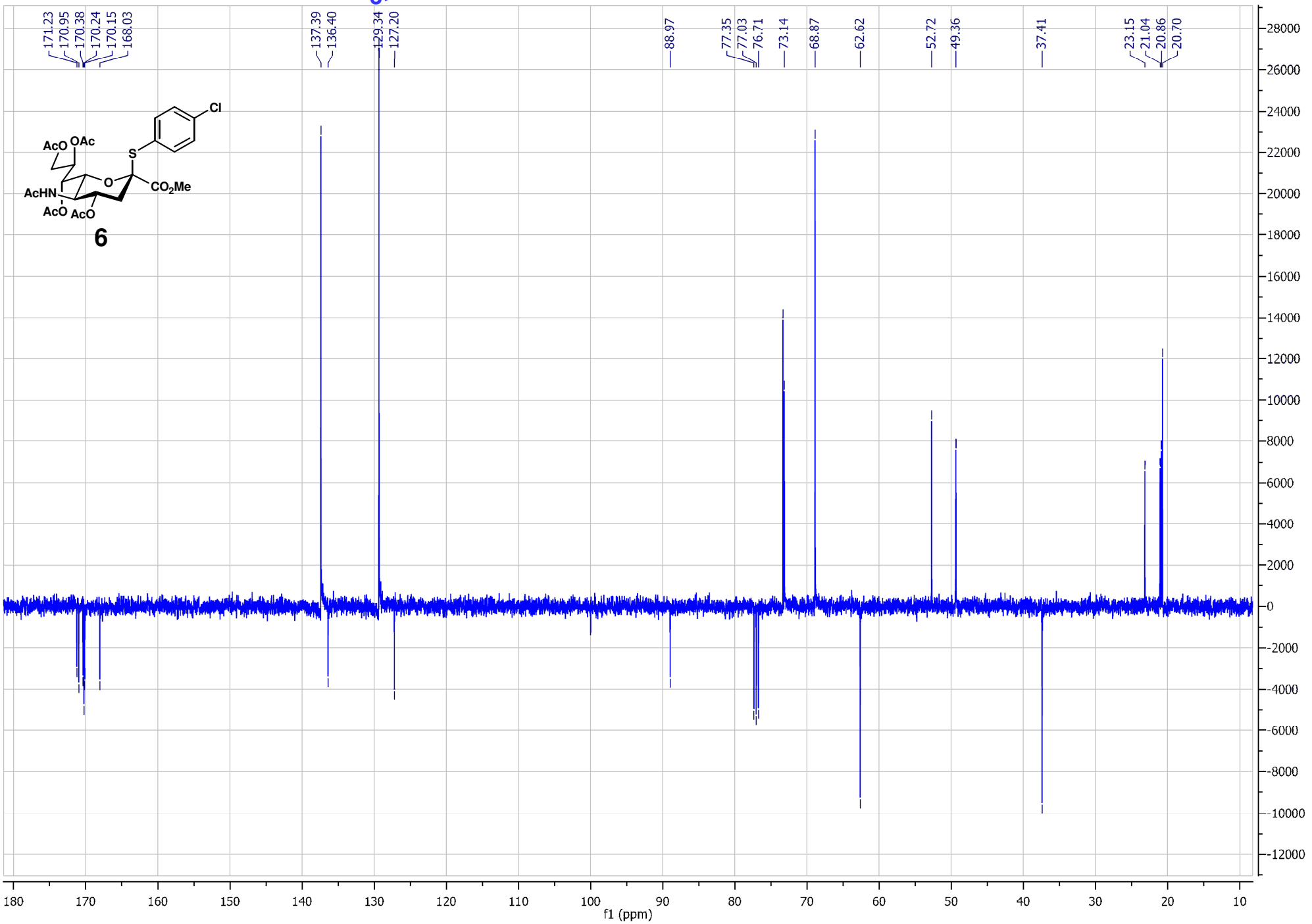

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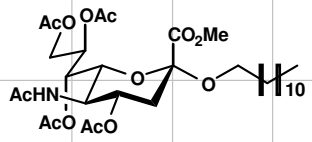

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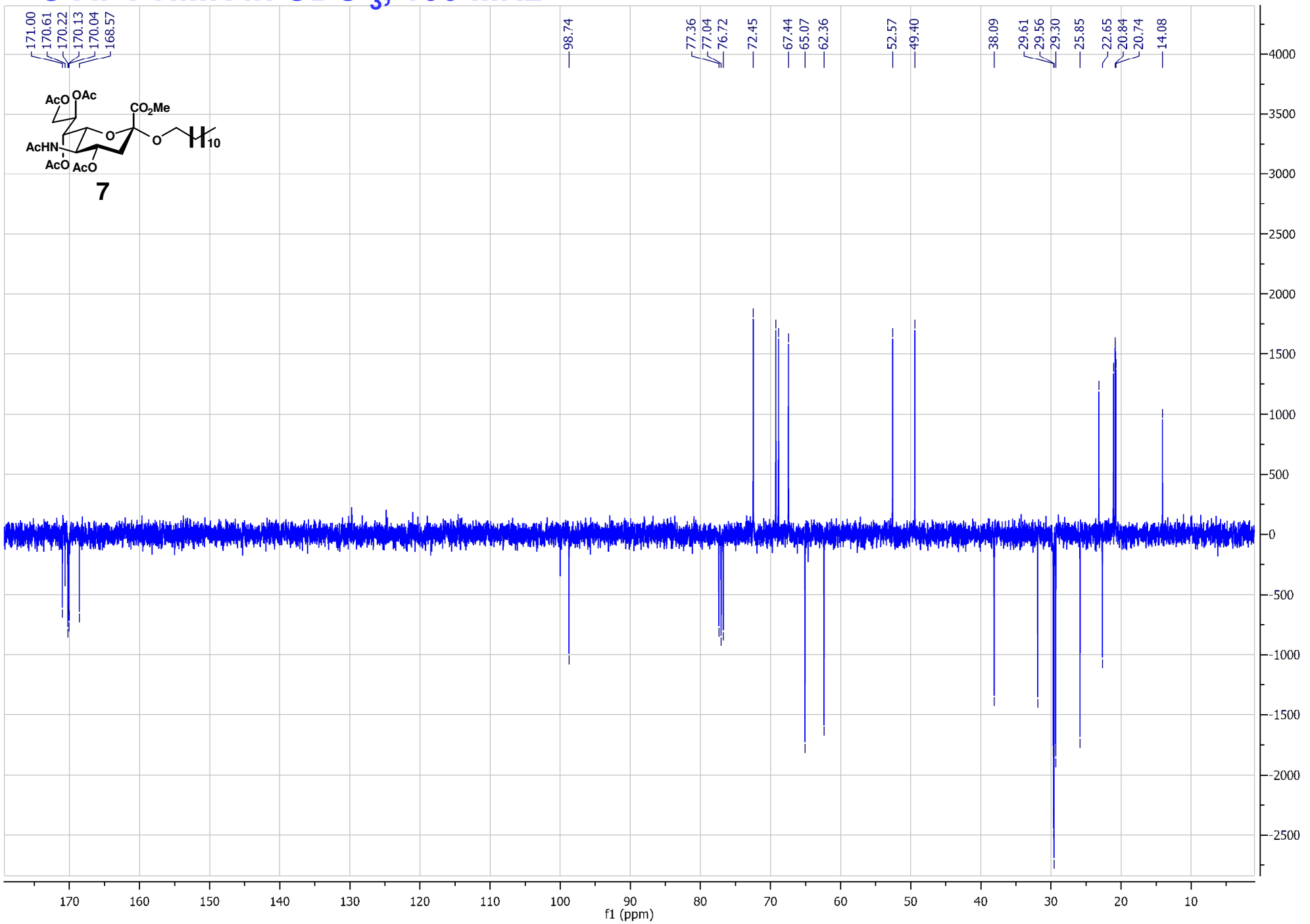

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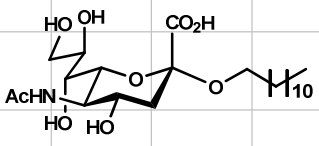

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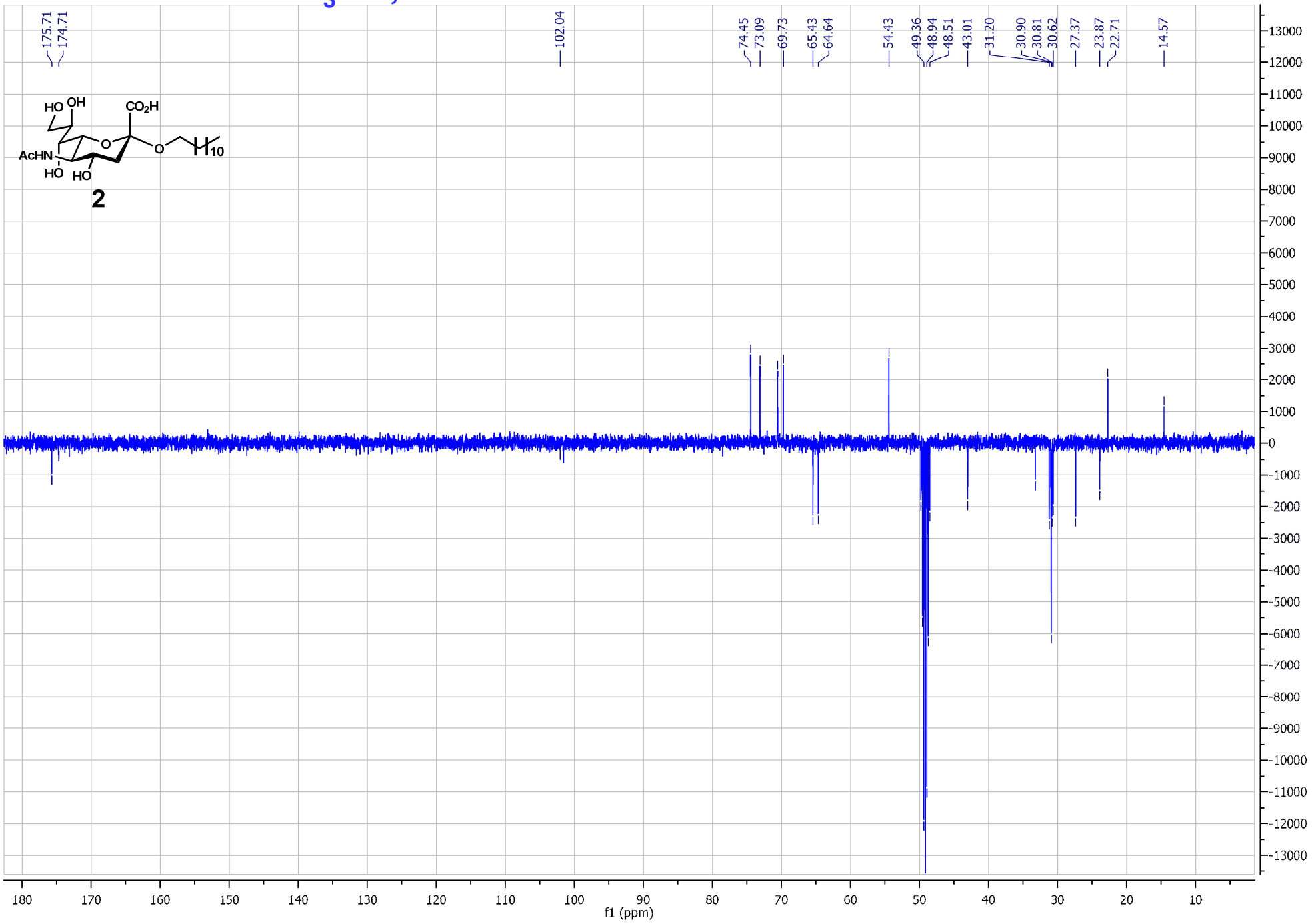
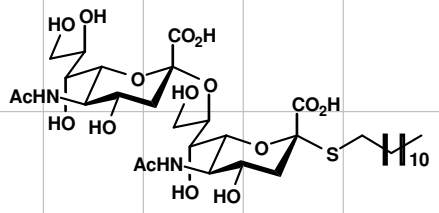


^{1}H NMR in CD_3OD, 600 MHz



8

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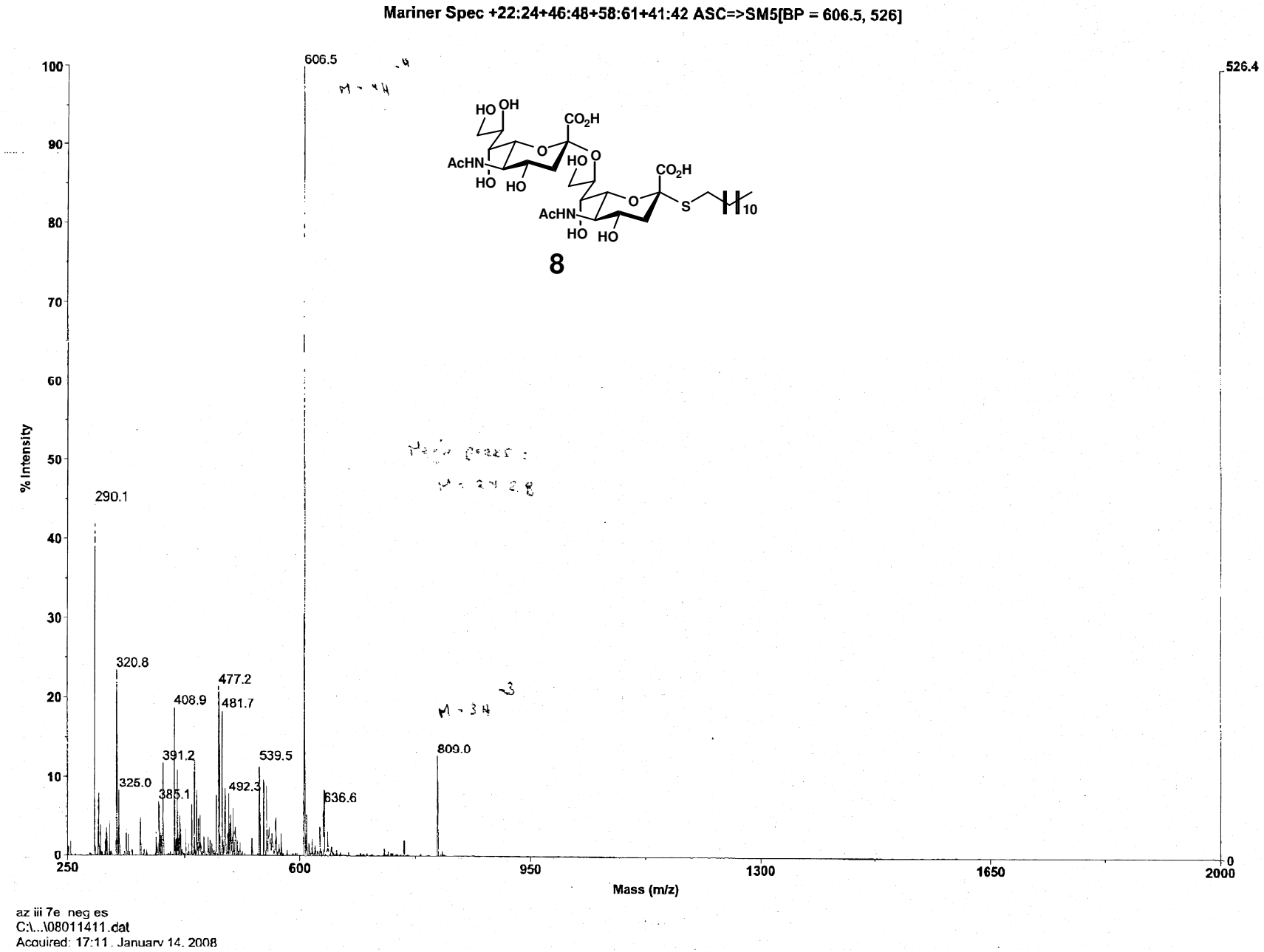

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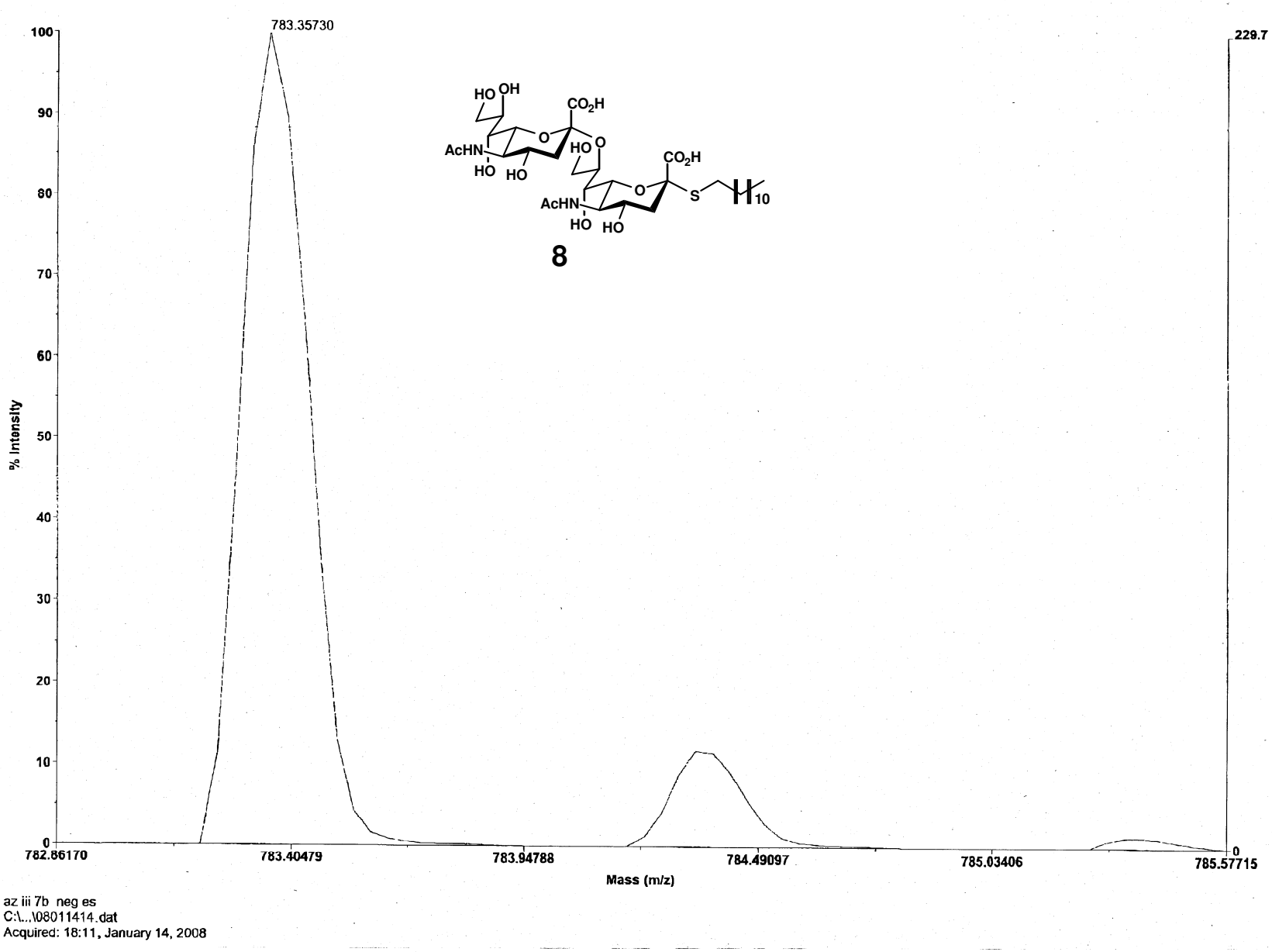

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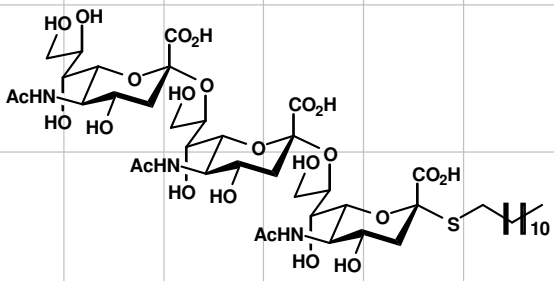

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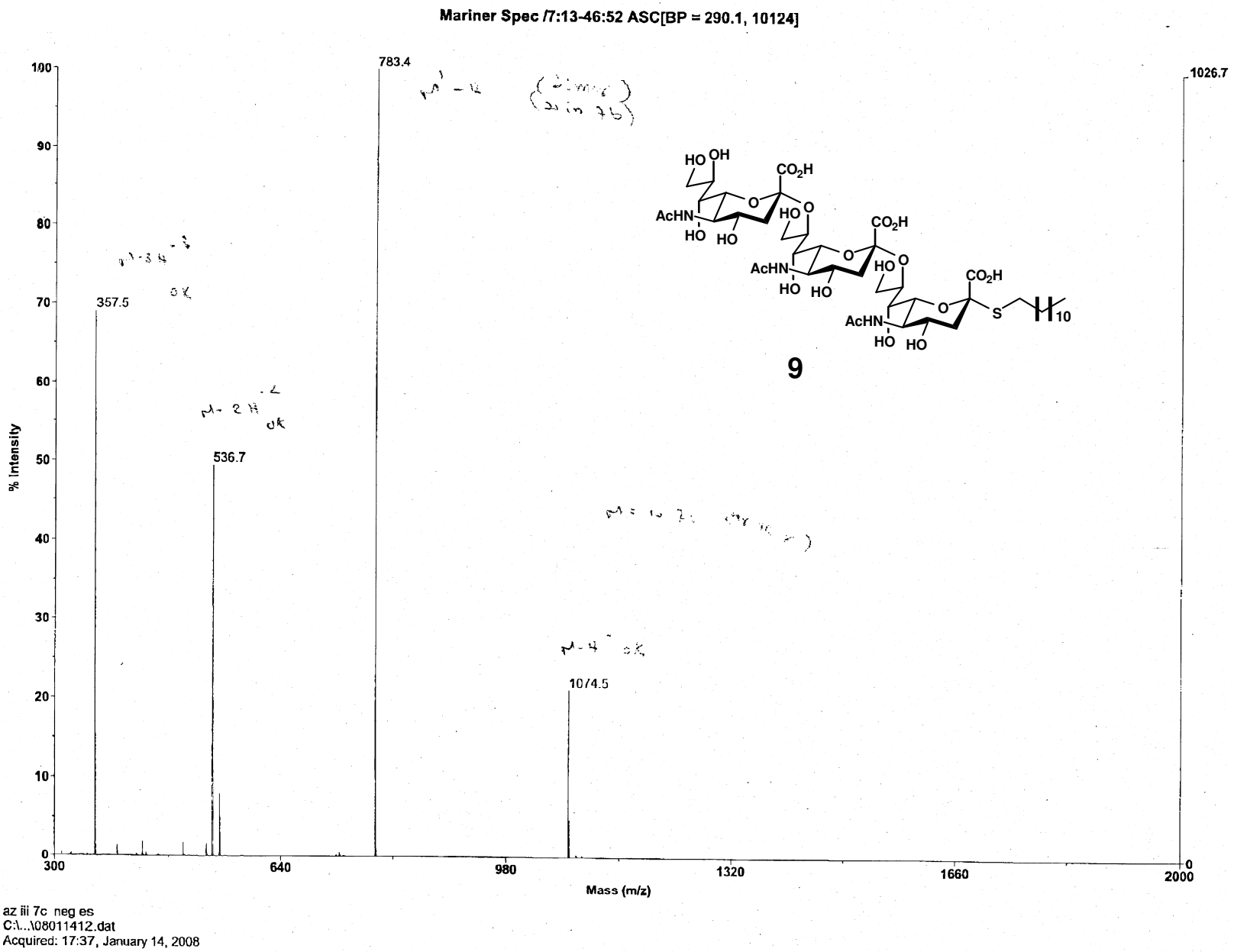

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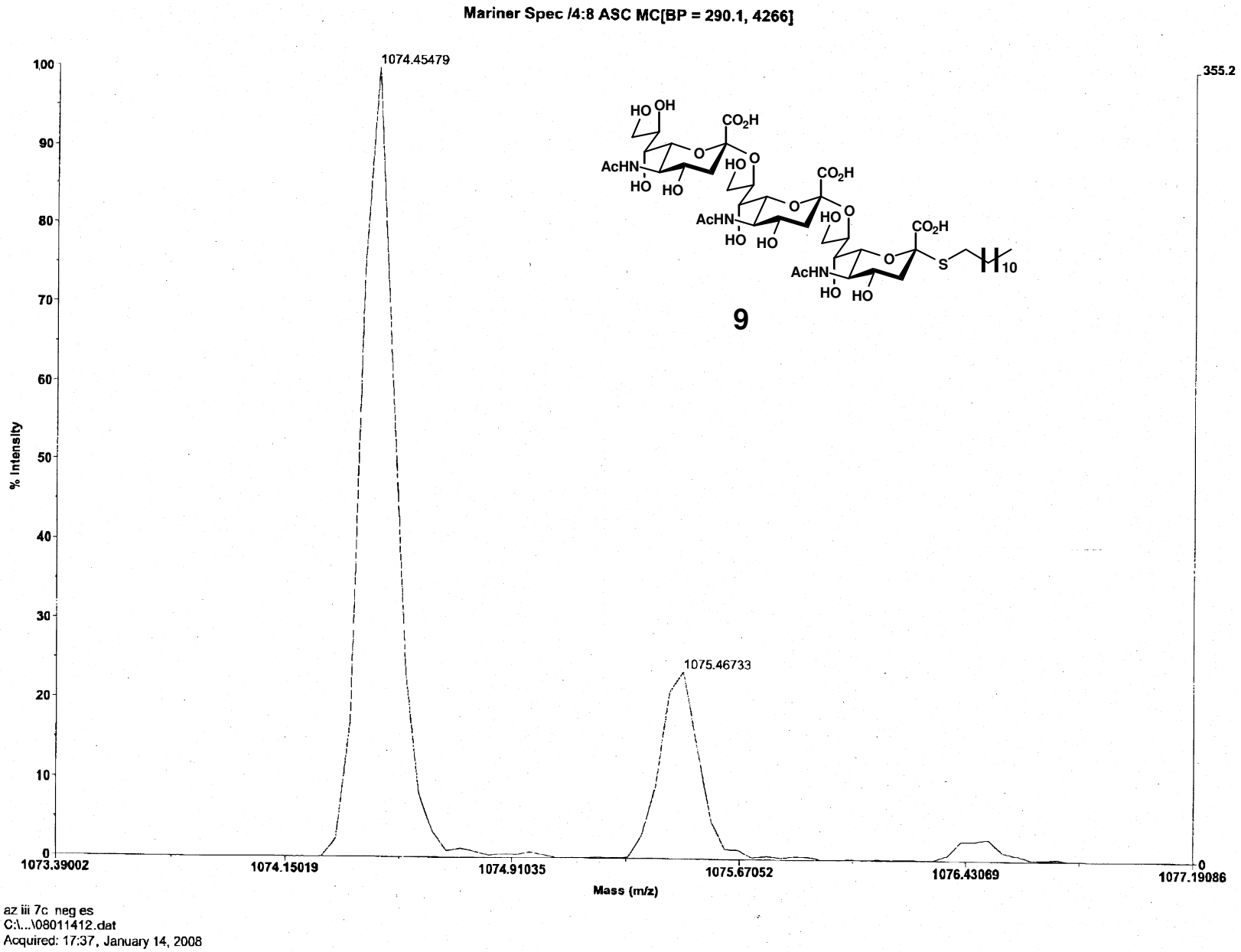

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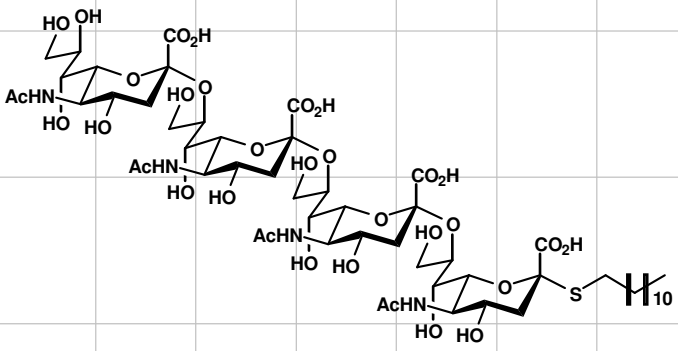

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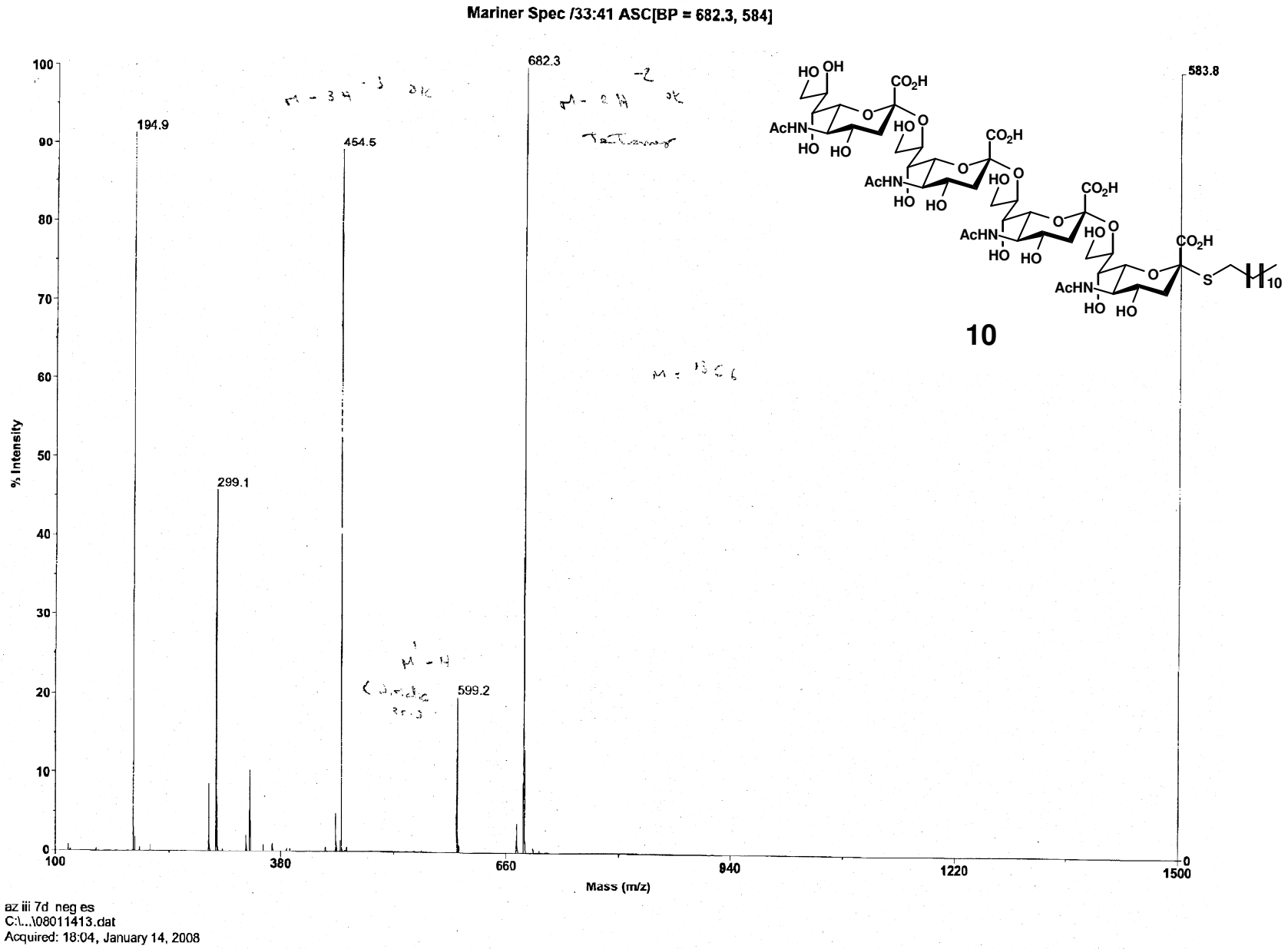

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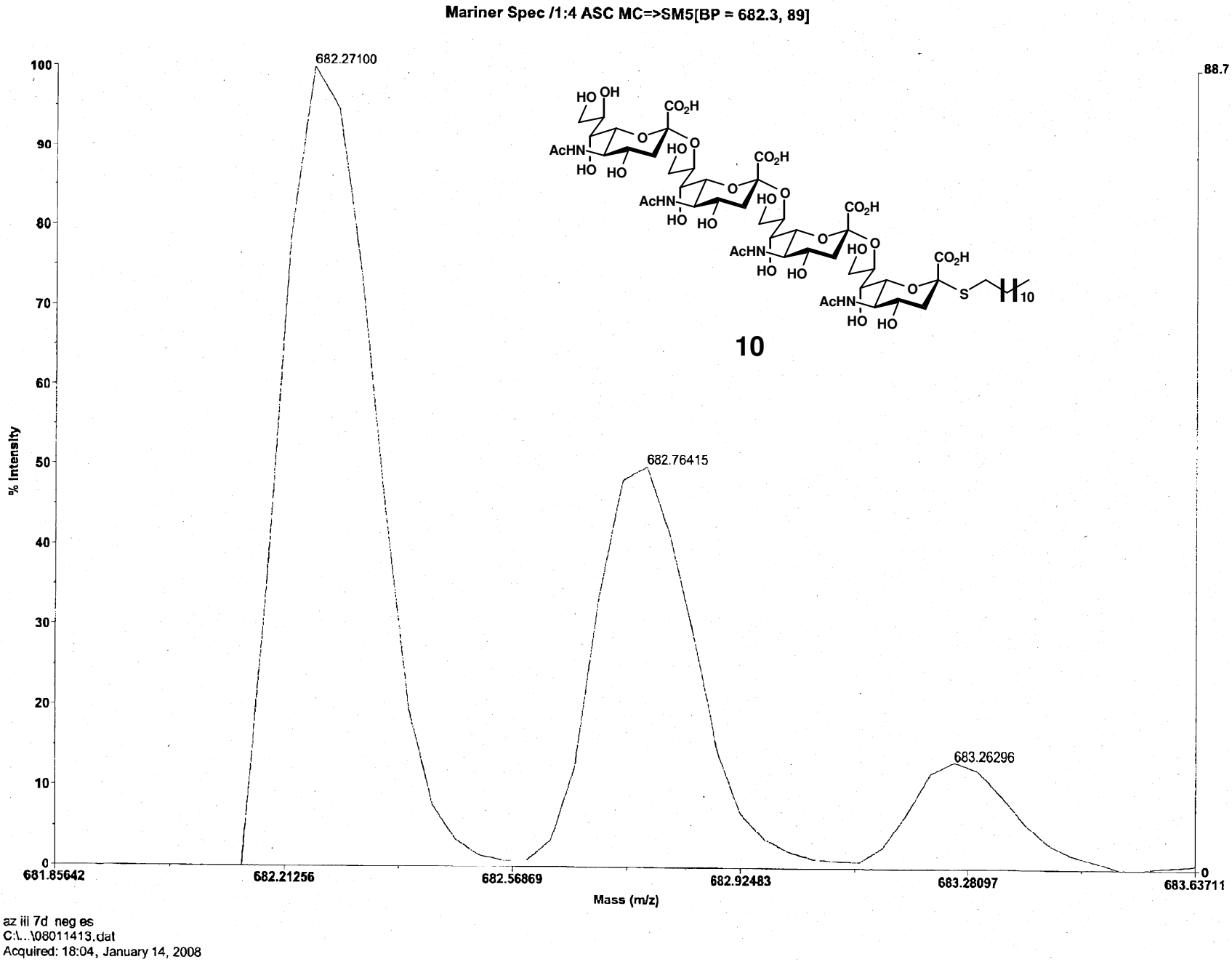

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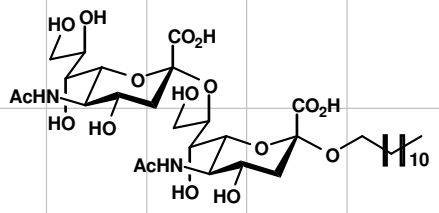

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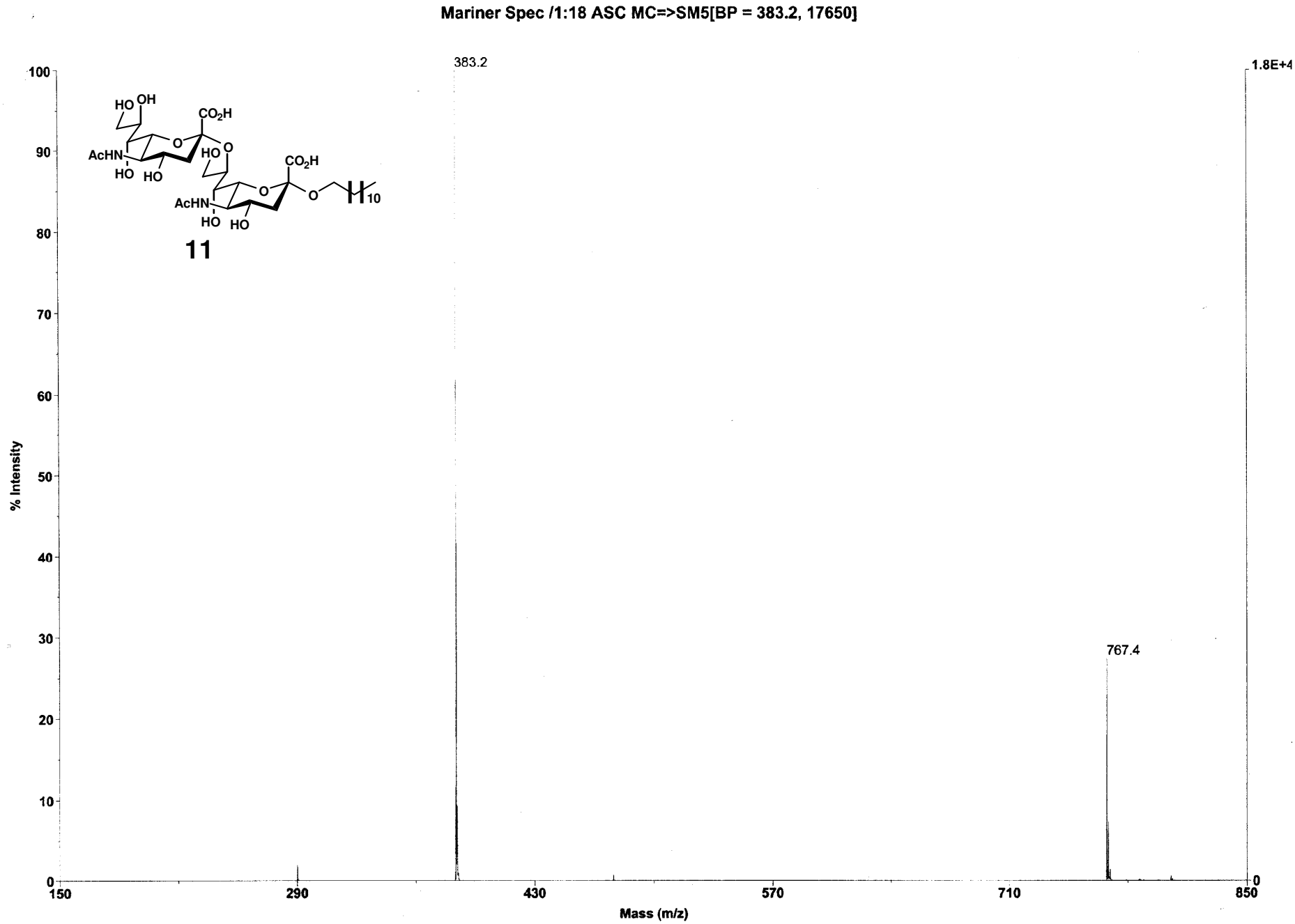

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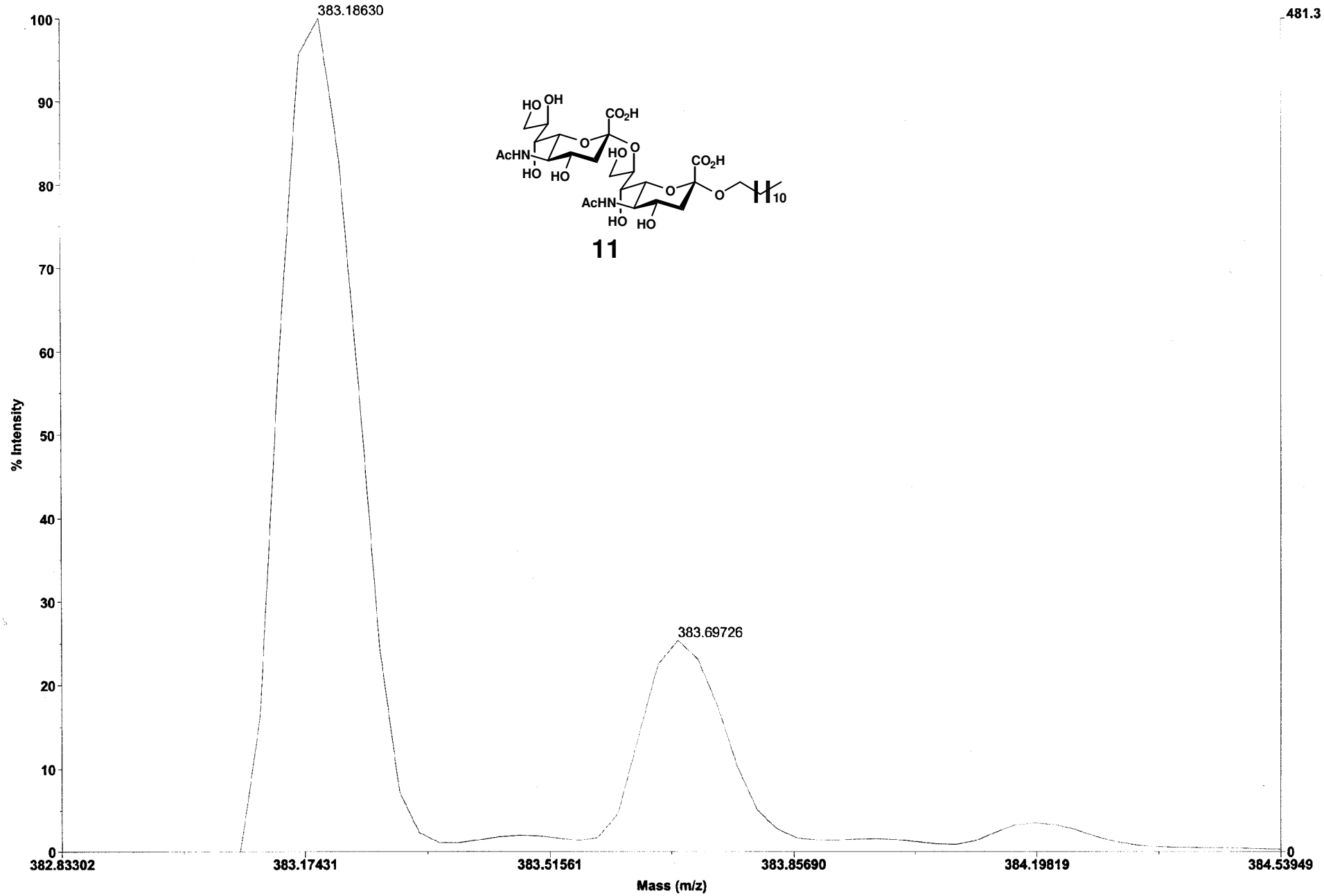

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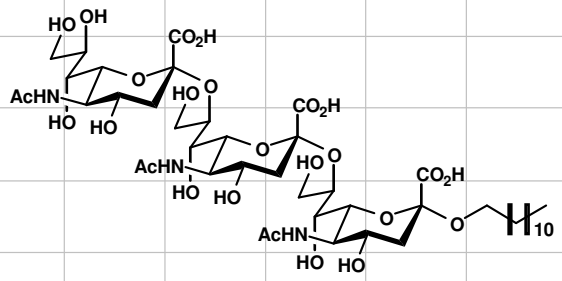

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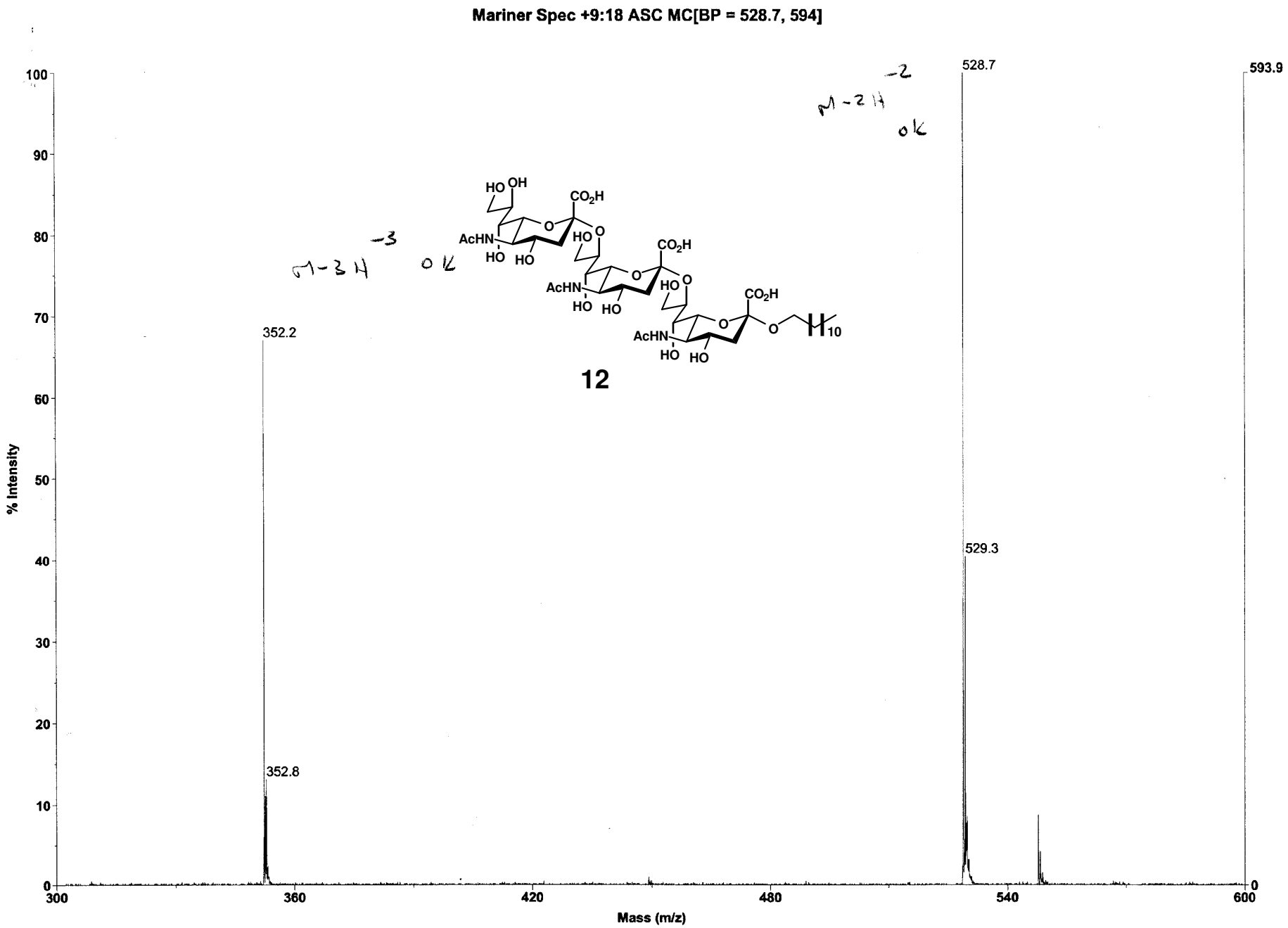

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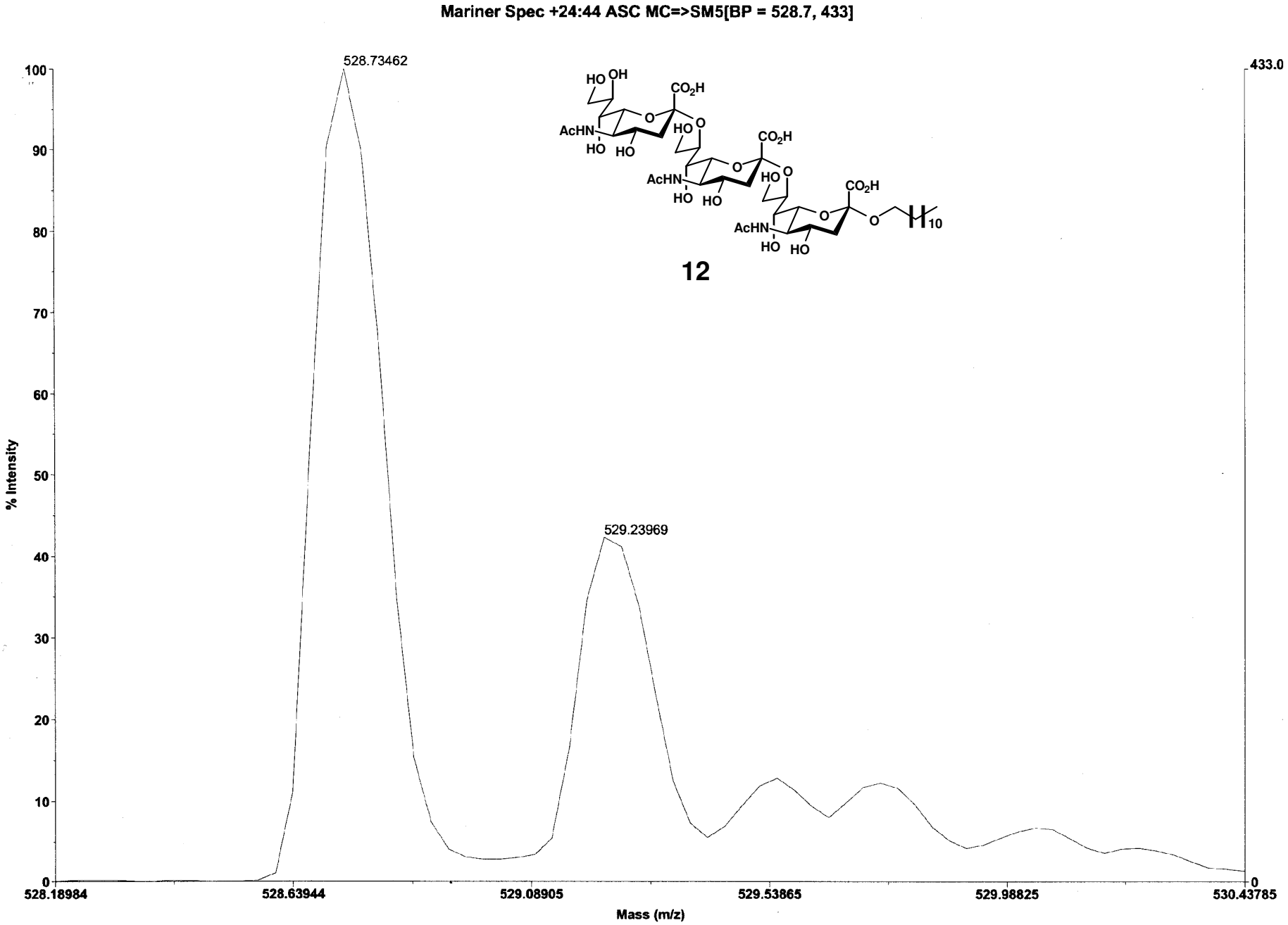

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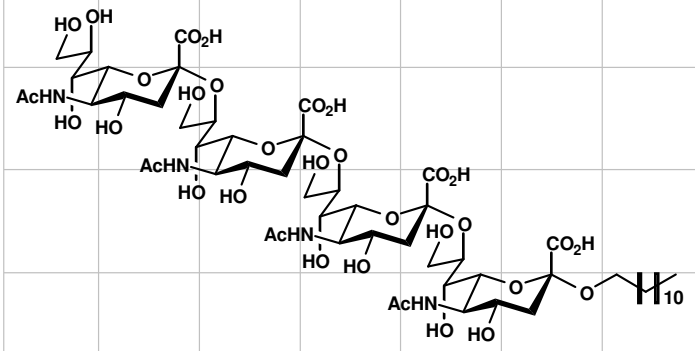

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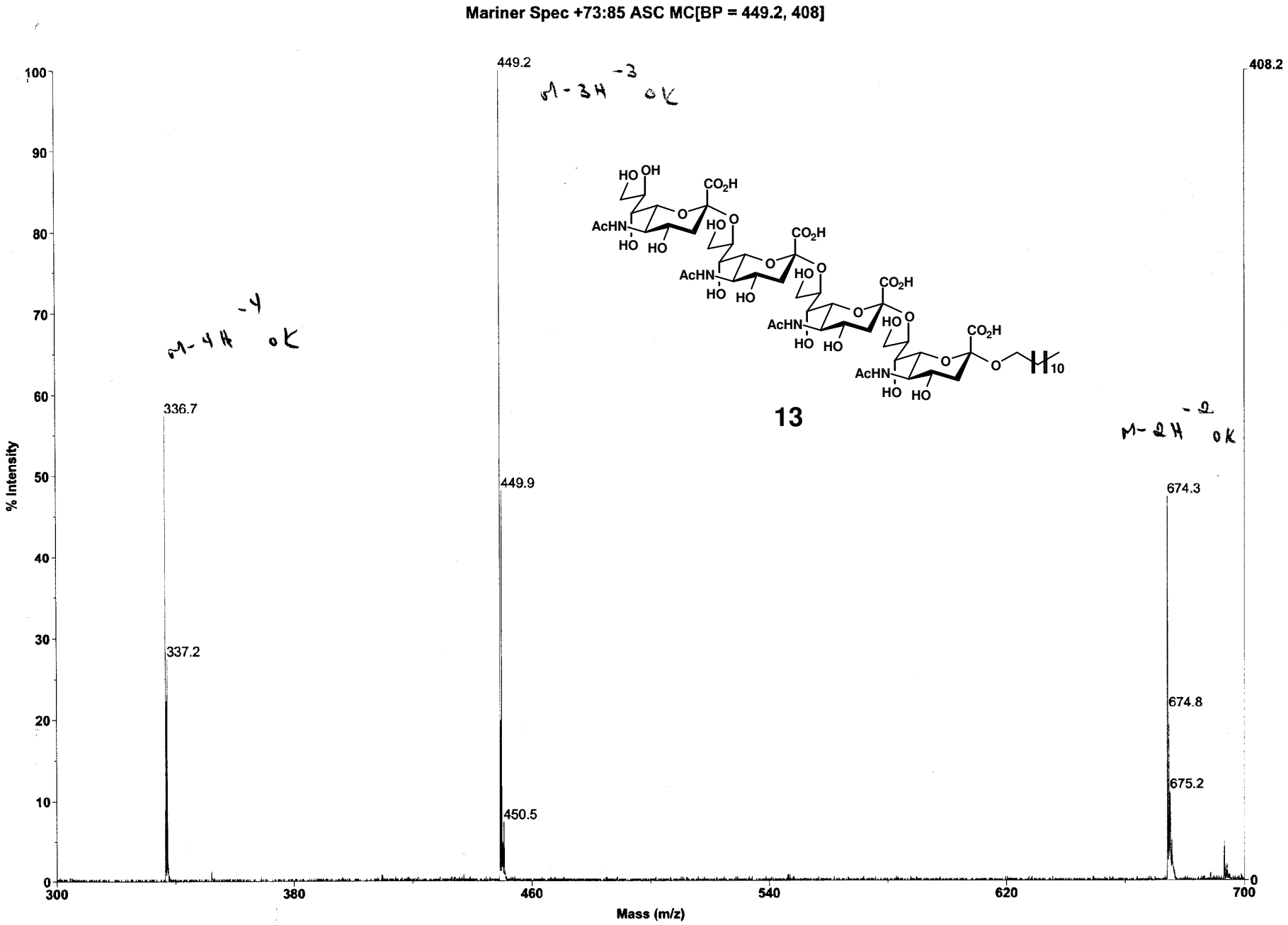

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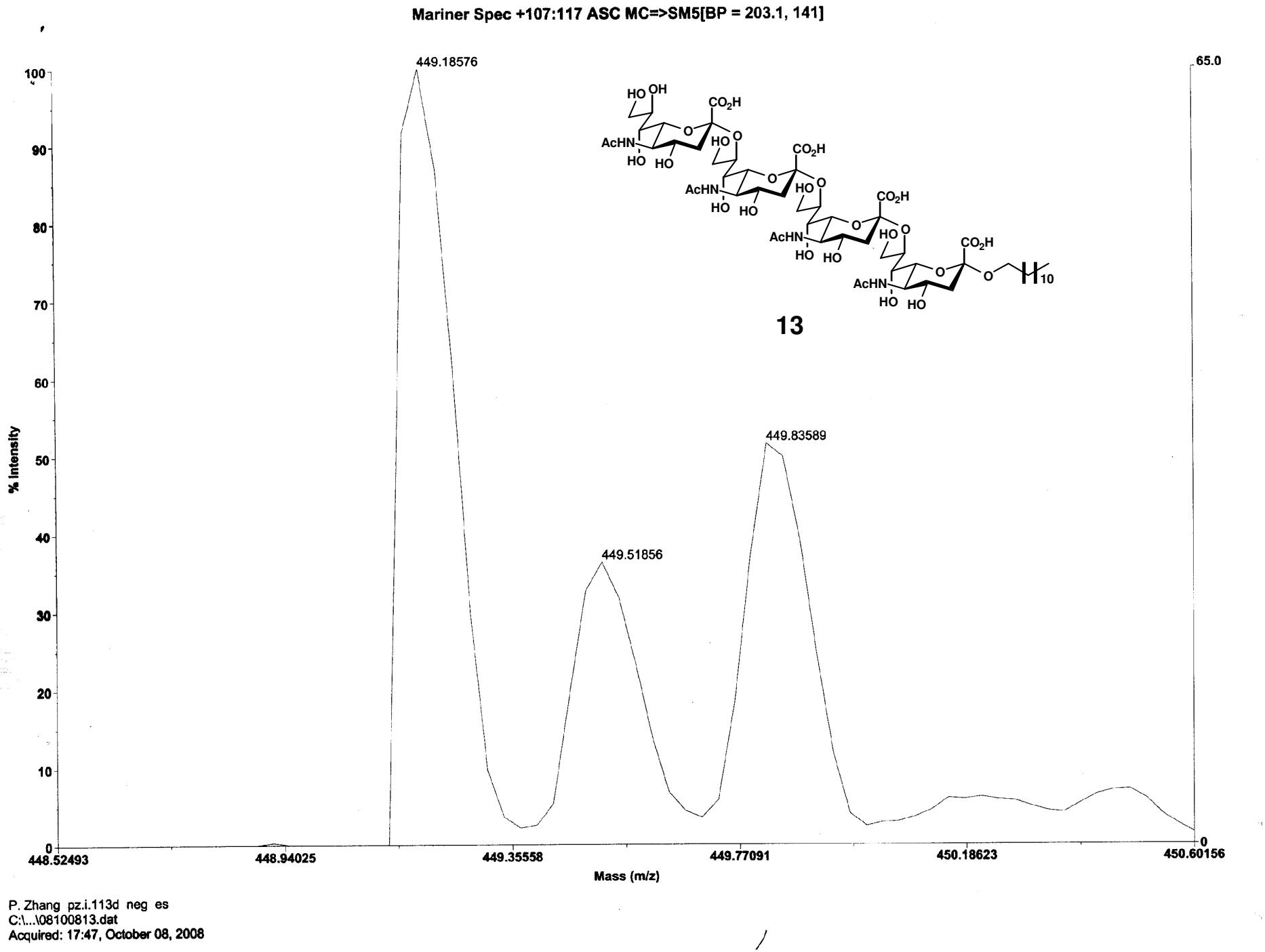

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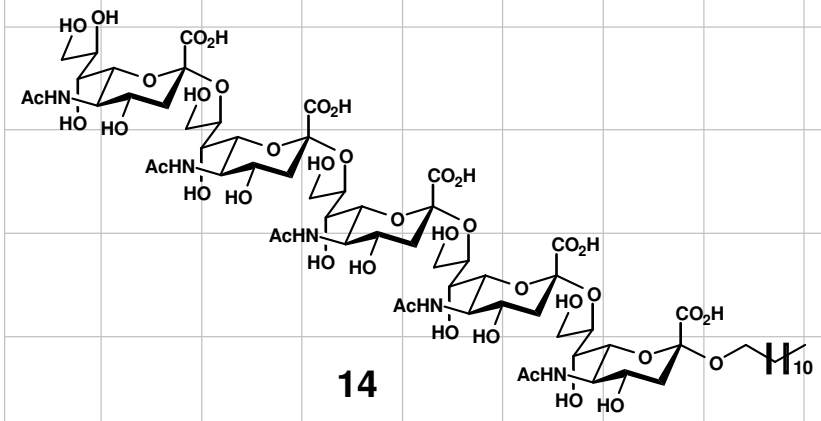

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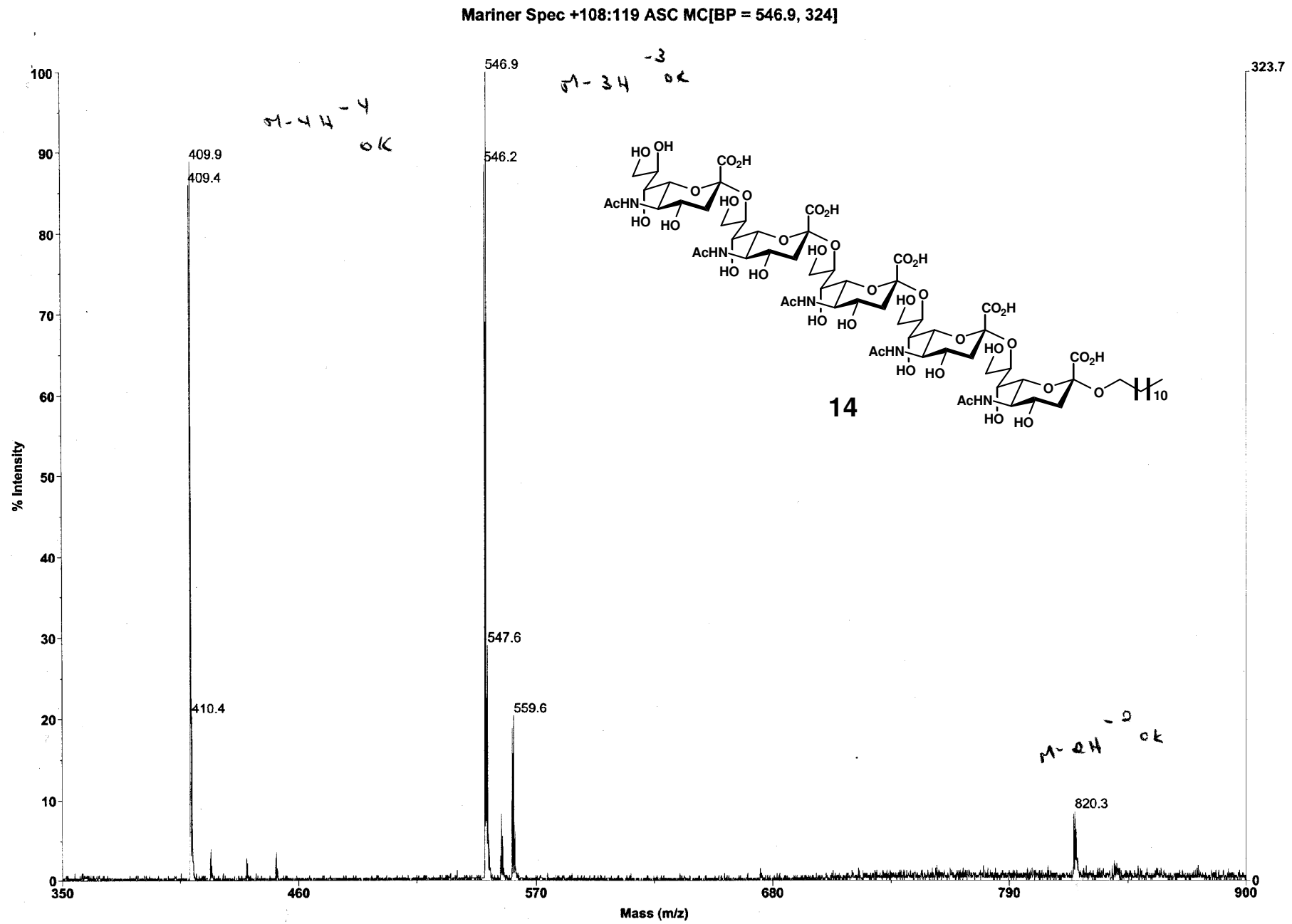
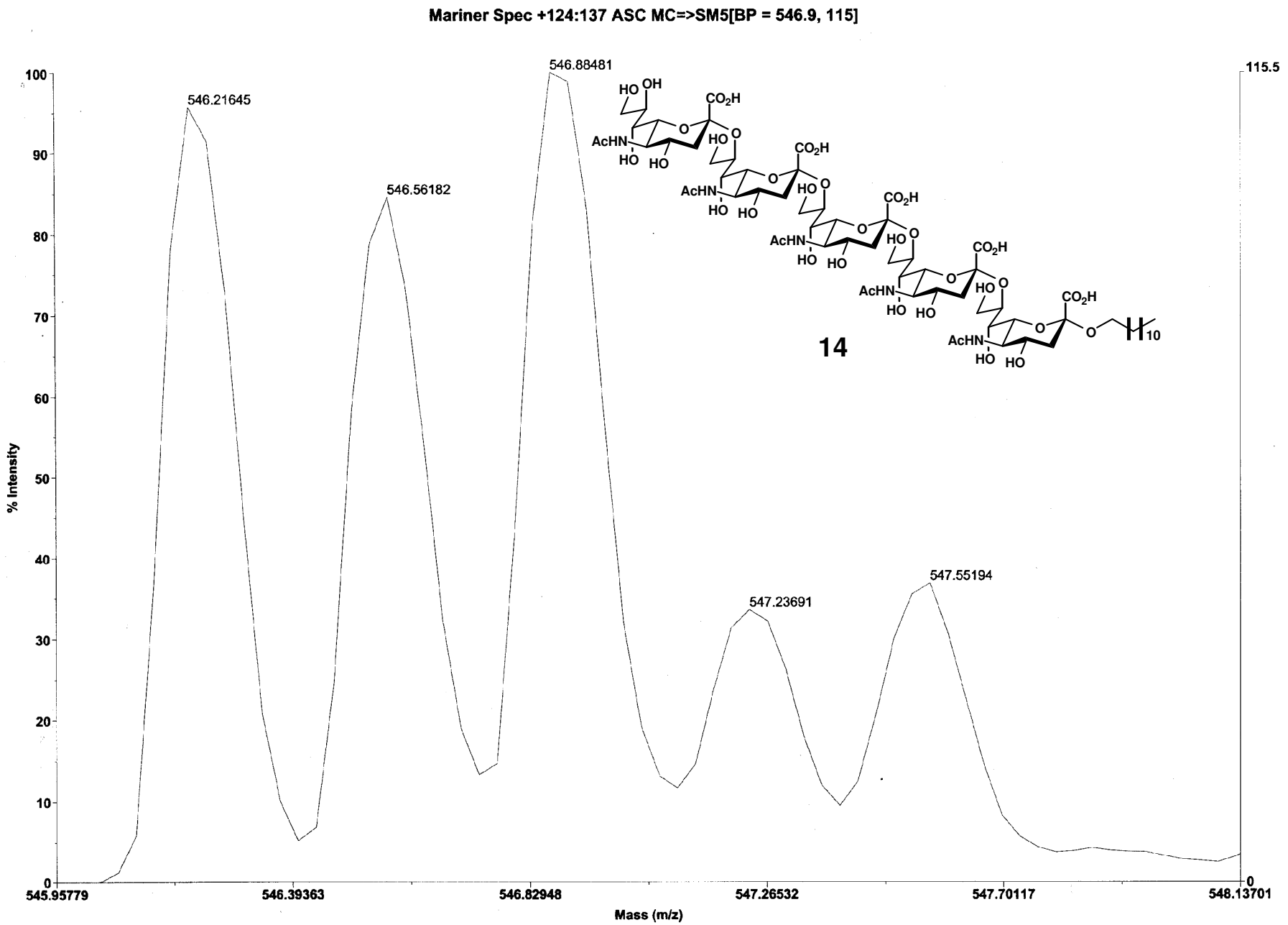


High Resolution Mass



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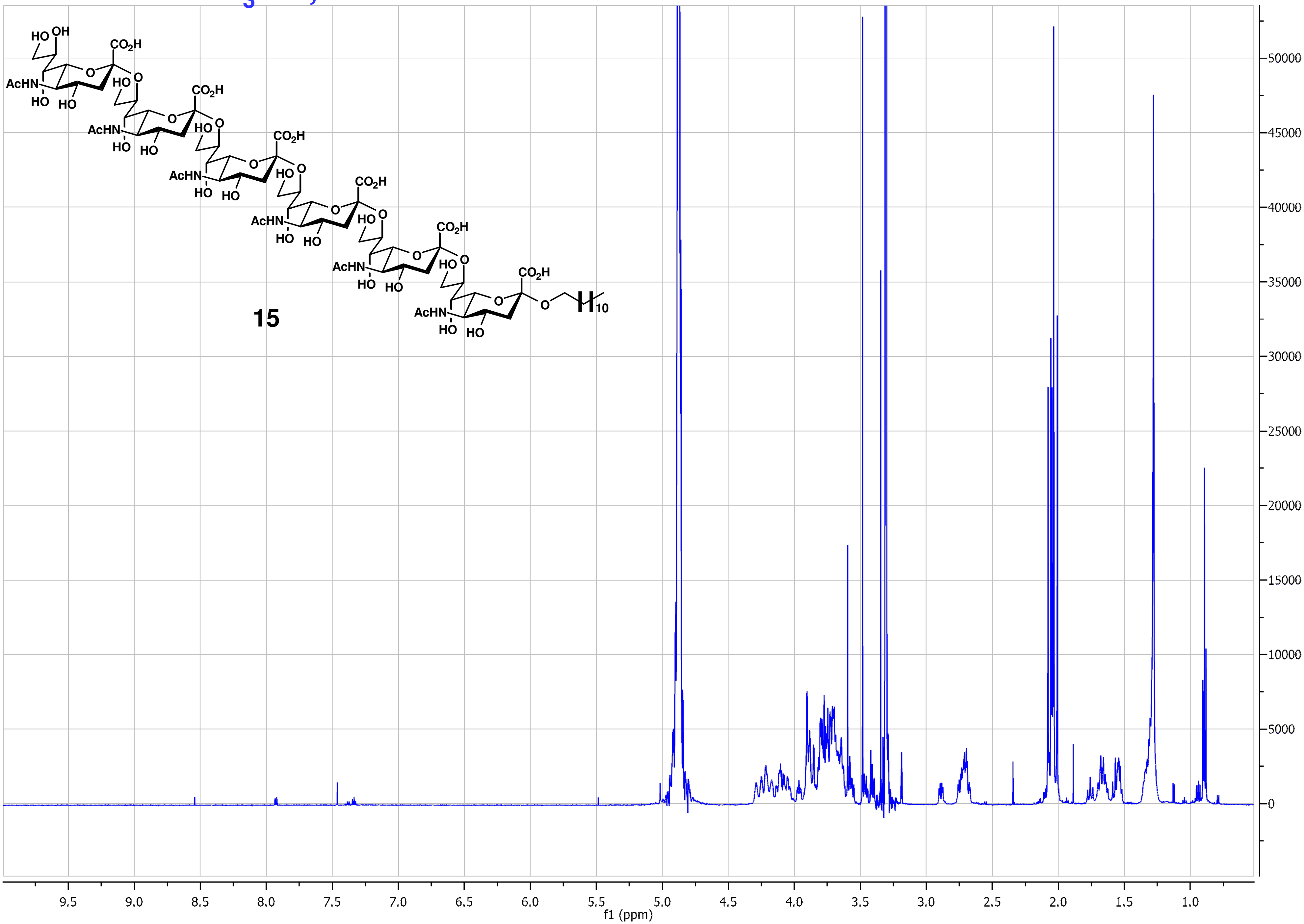
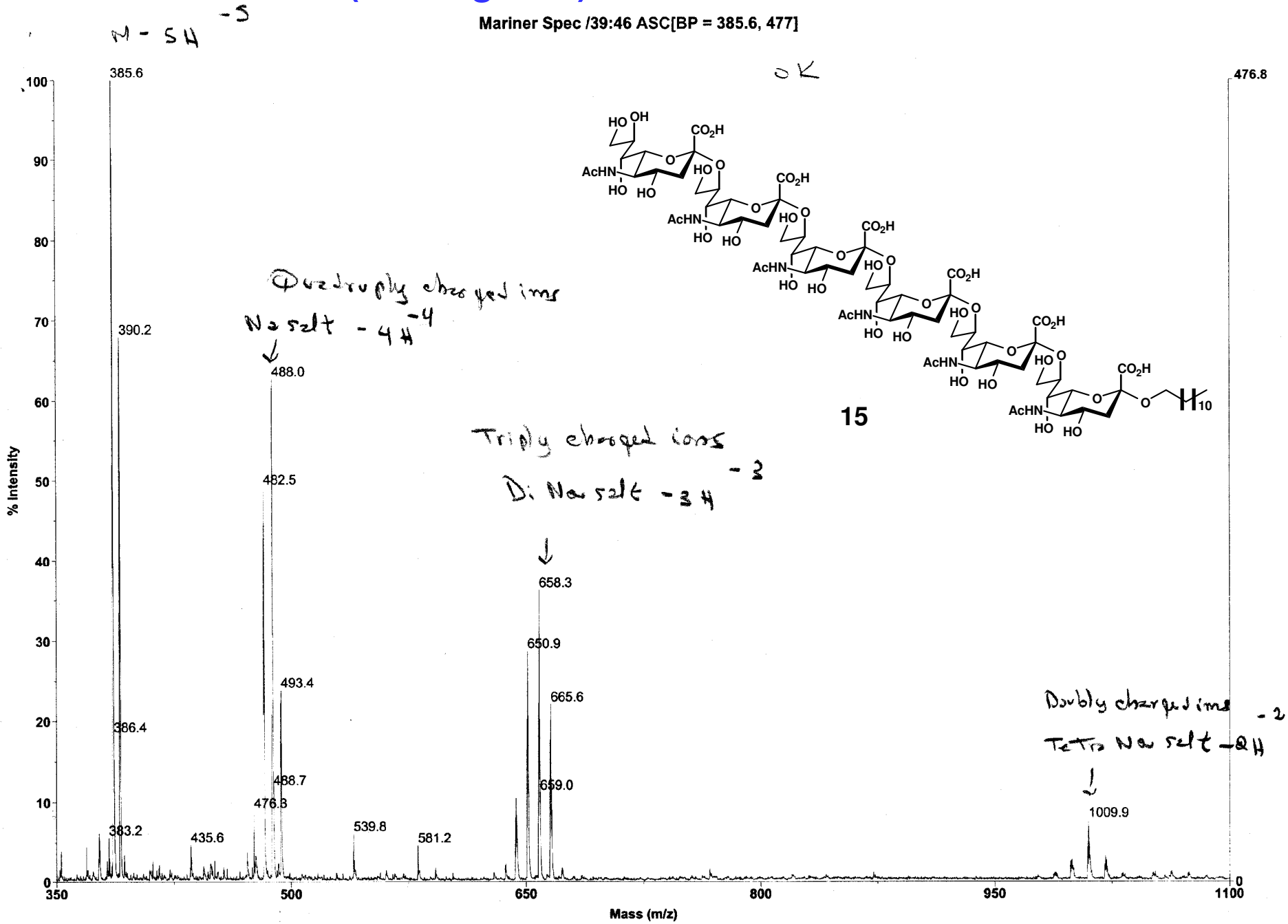
Acquired: 17:22, October 08, 2008

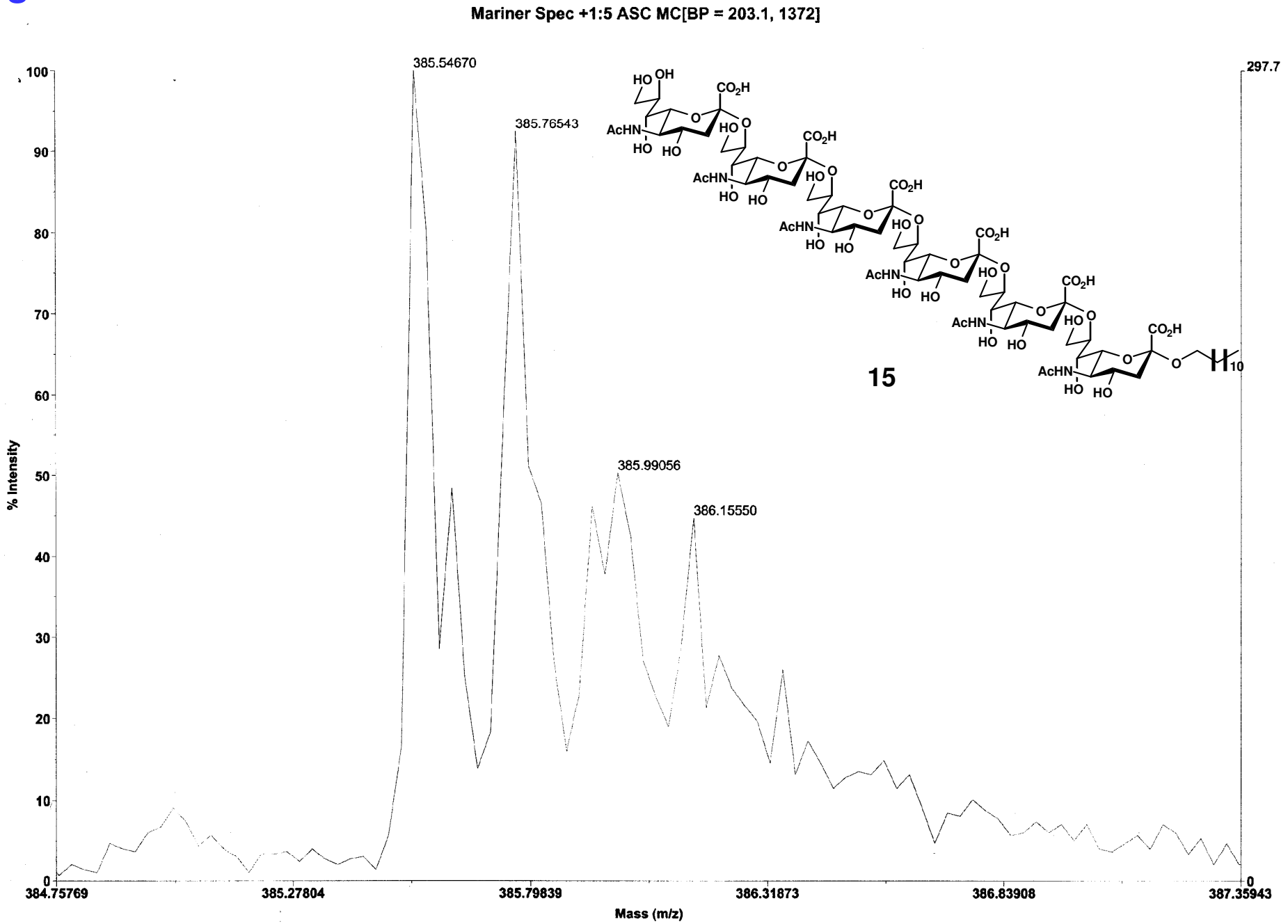
^{1}H NMR in CD_3OD, 600 MHz



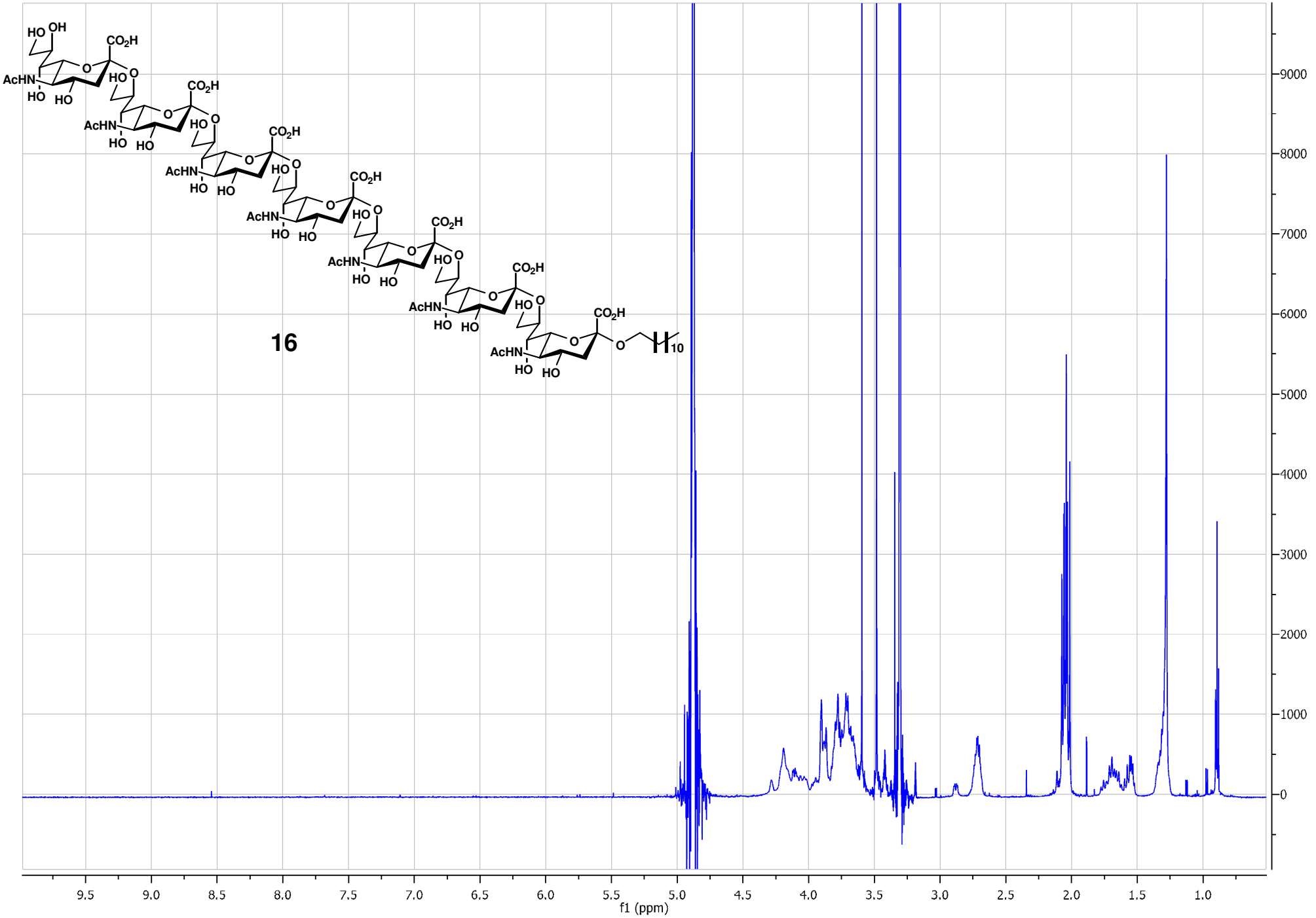
Low Resolution Mass (ESI negative)



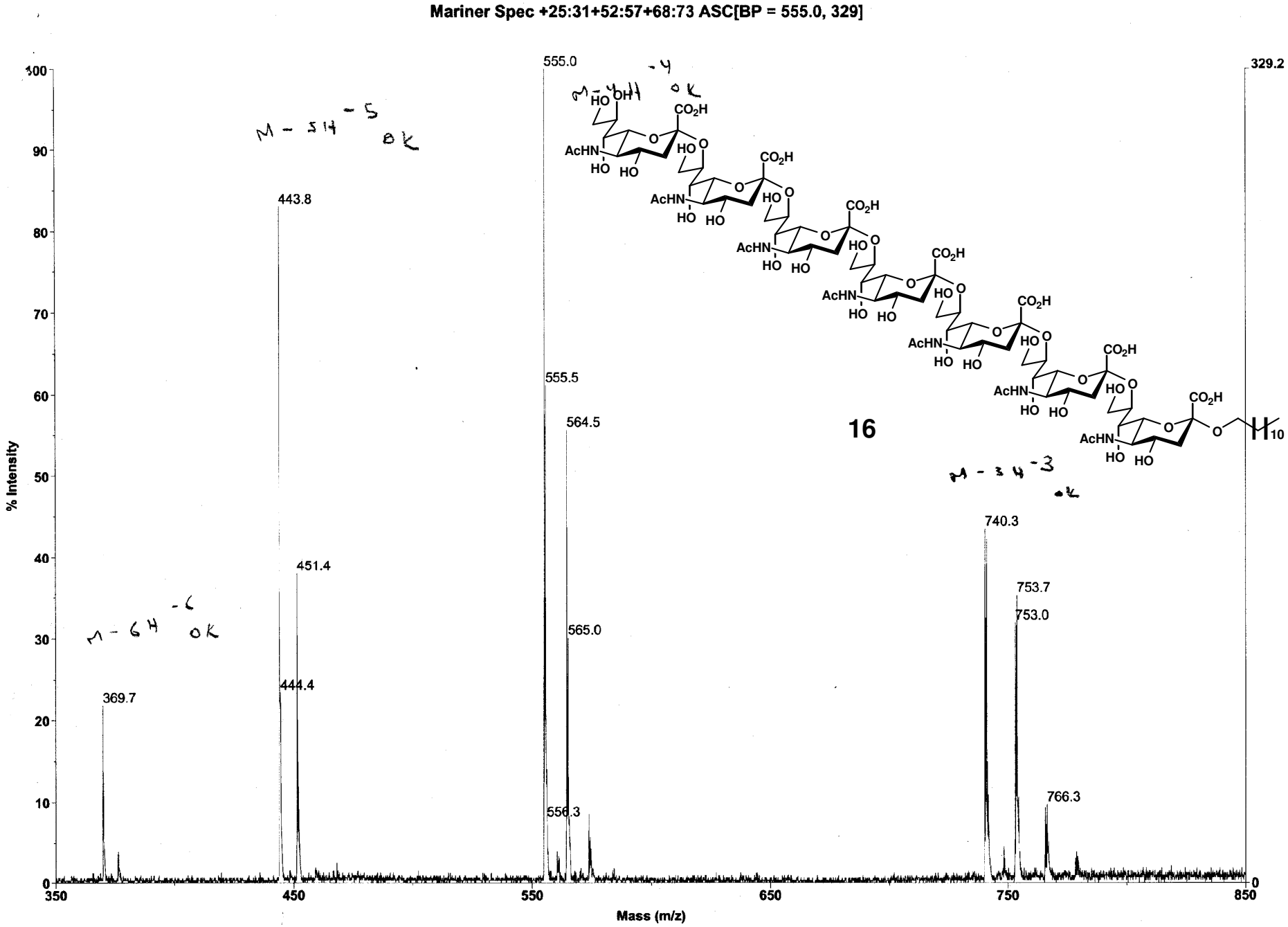
High Resolution Mass



^{1}H NMR in CD_3OD, 600 MHz



Low Resolution Mass (ESI negative)



P. Zhang pz.i.113a neg es
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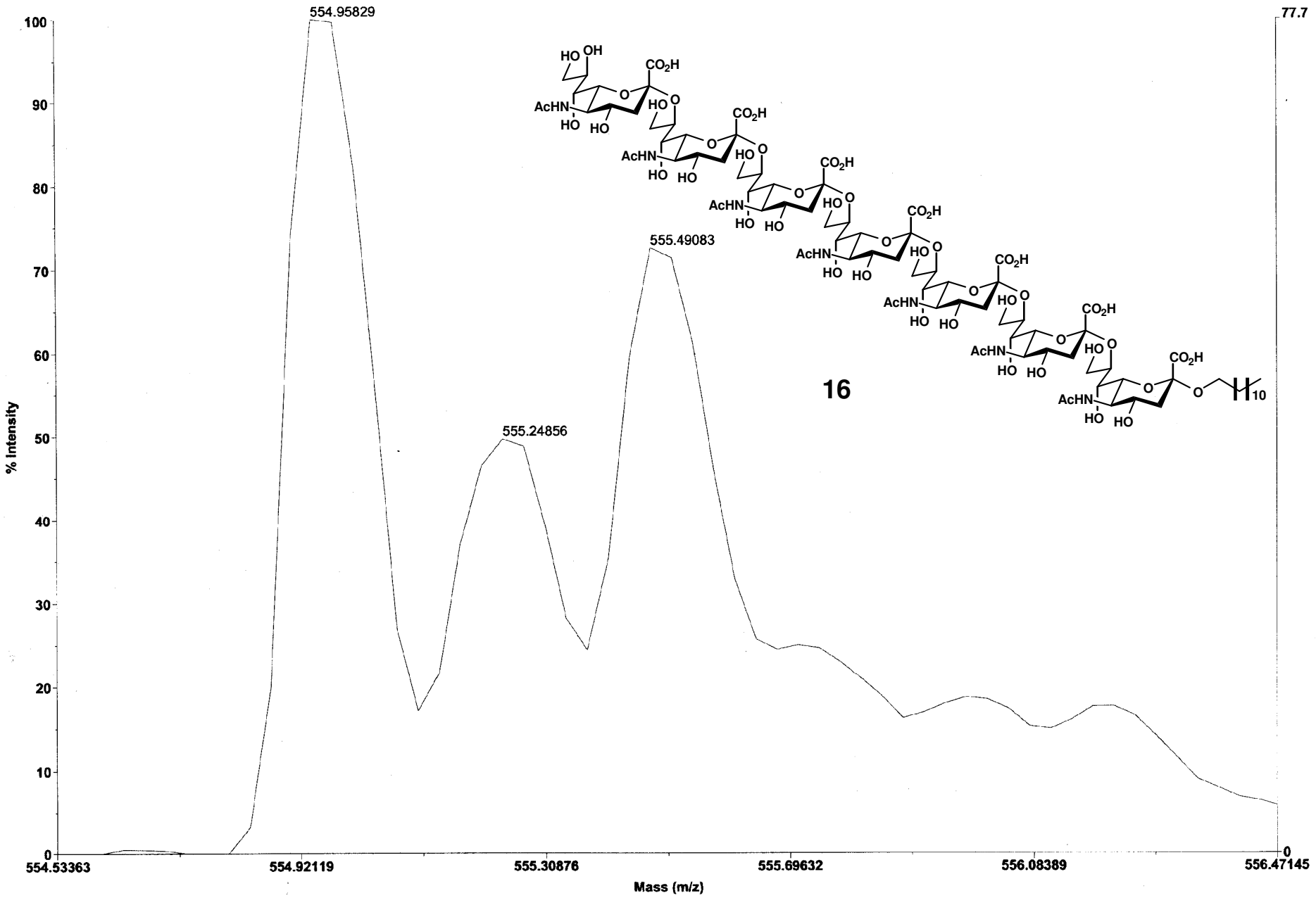
Acquired: 16:55, October 08, 2008

High Resolution Mass



P. Zhang pz.i.113a neg es
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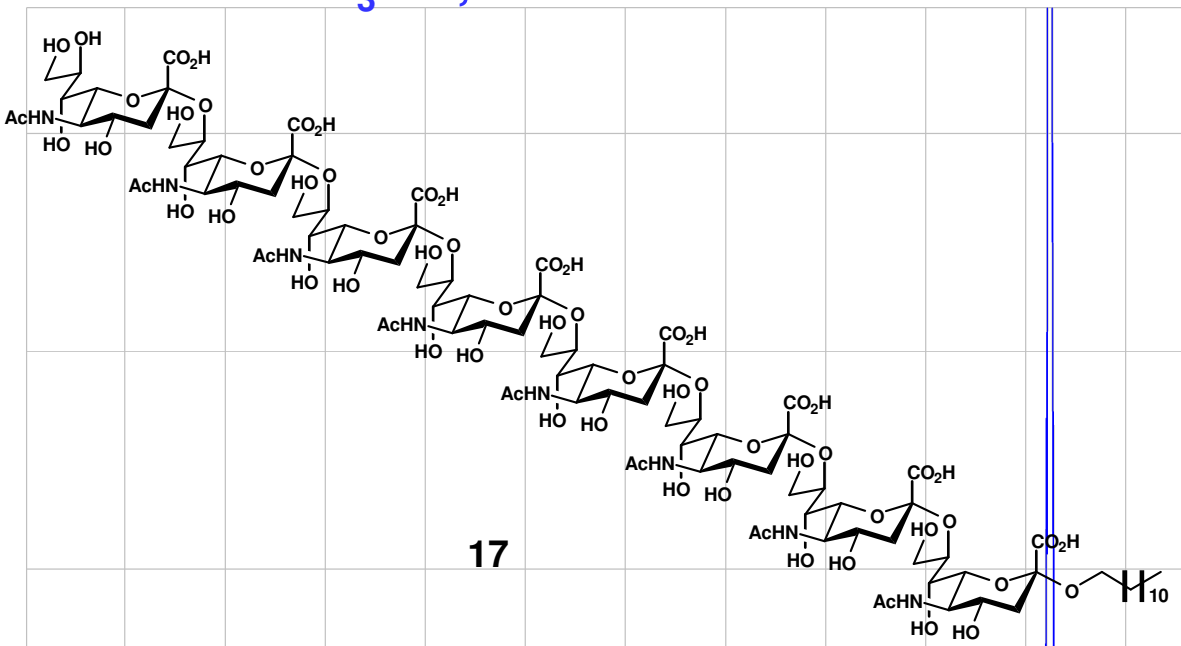
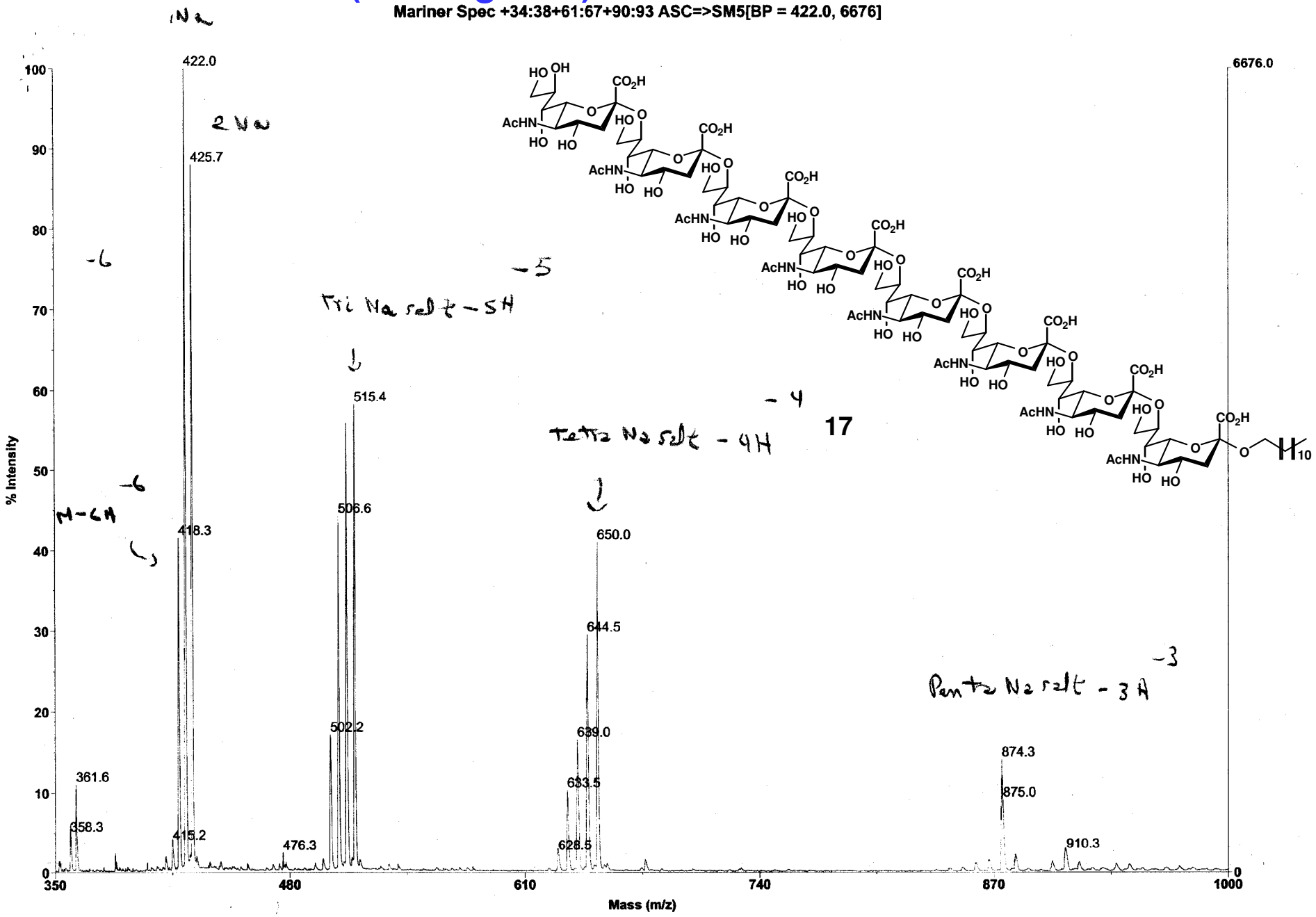
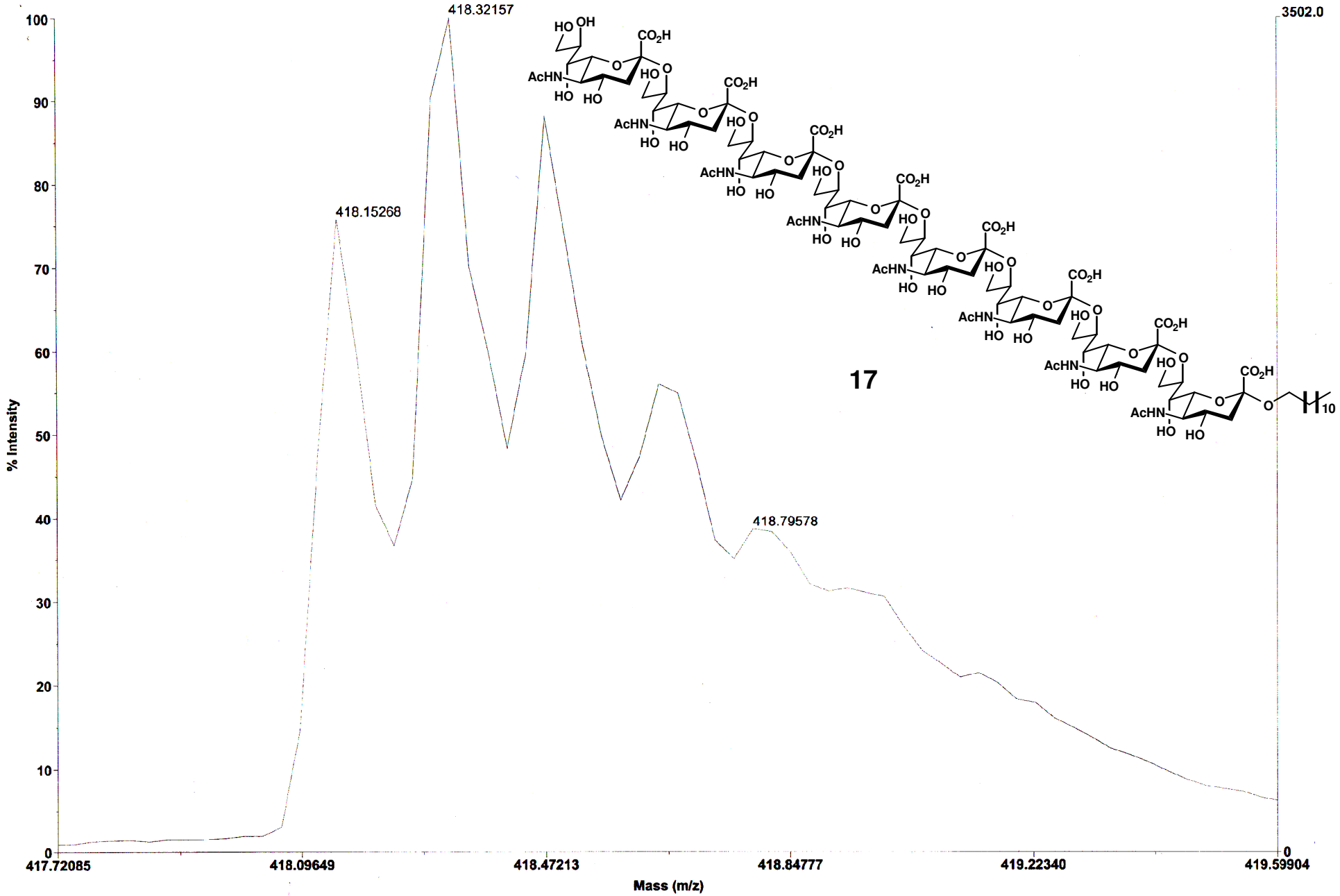
^{1}H NMR in CD_3OD, 600 MHz



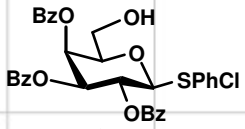
Low Resolution Mass (ESI negative)



High Resolution Mass

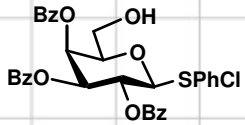


^{1}H NMR in $CDCl_3$, 400 MHz



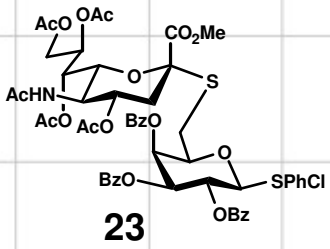
21

^{13}C APT NMR in $CDCl_3$, 100 MHz



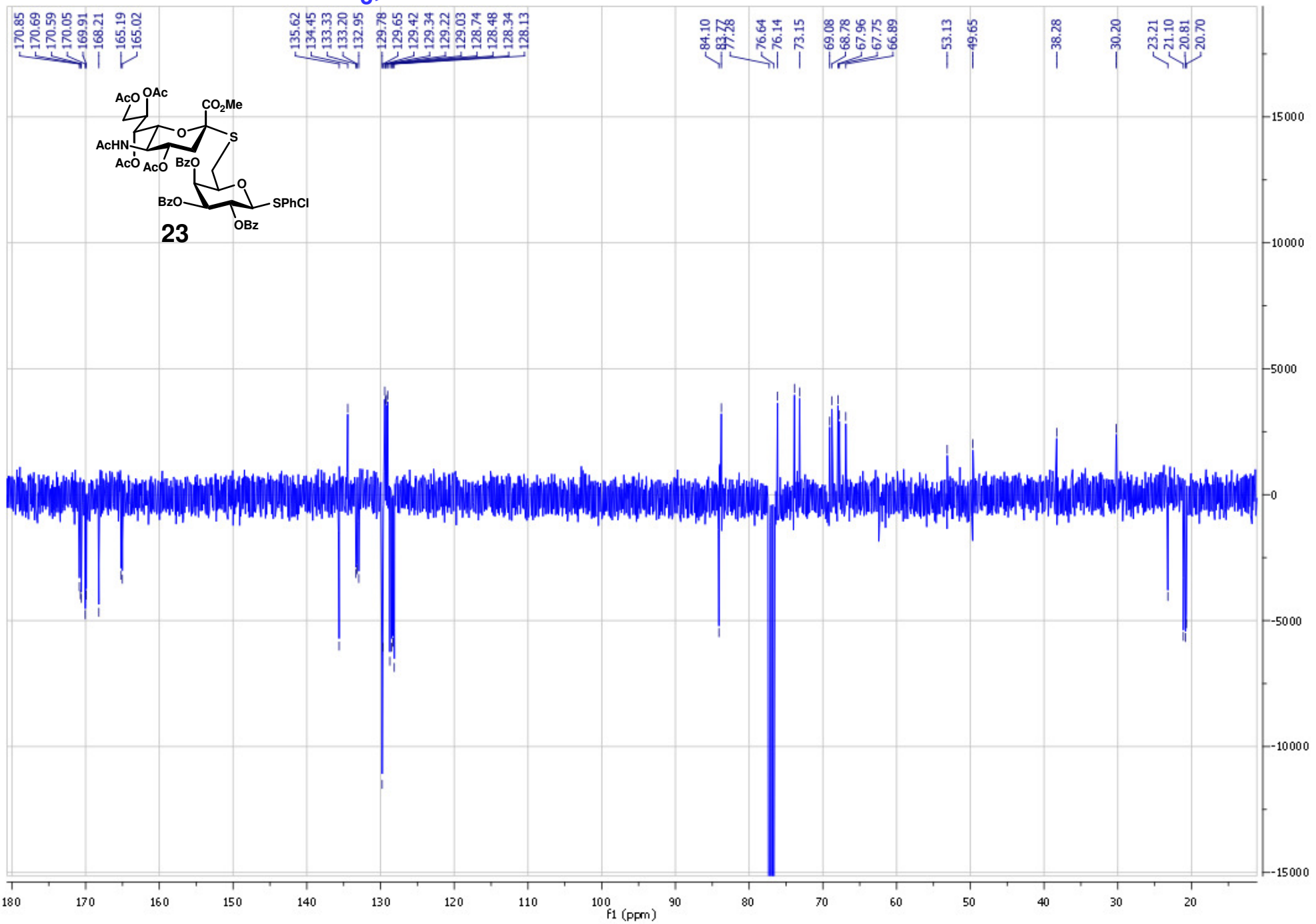
21

^{1}H NMR in $CDCl_3$, 400 MHz

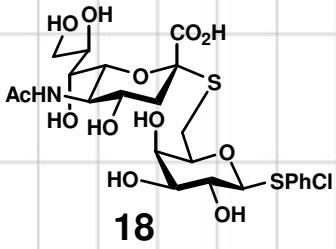


23

^{13}C APT NMR in $CDCl_3$, 100 MHz

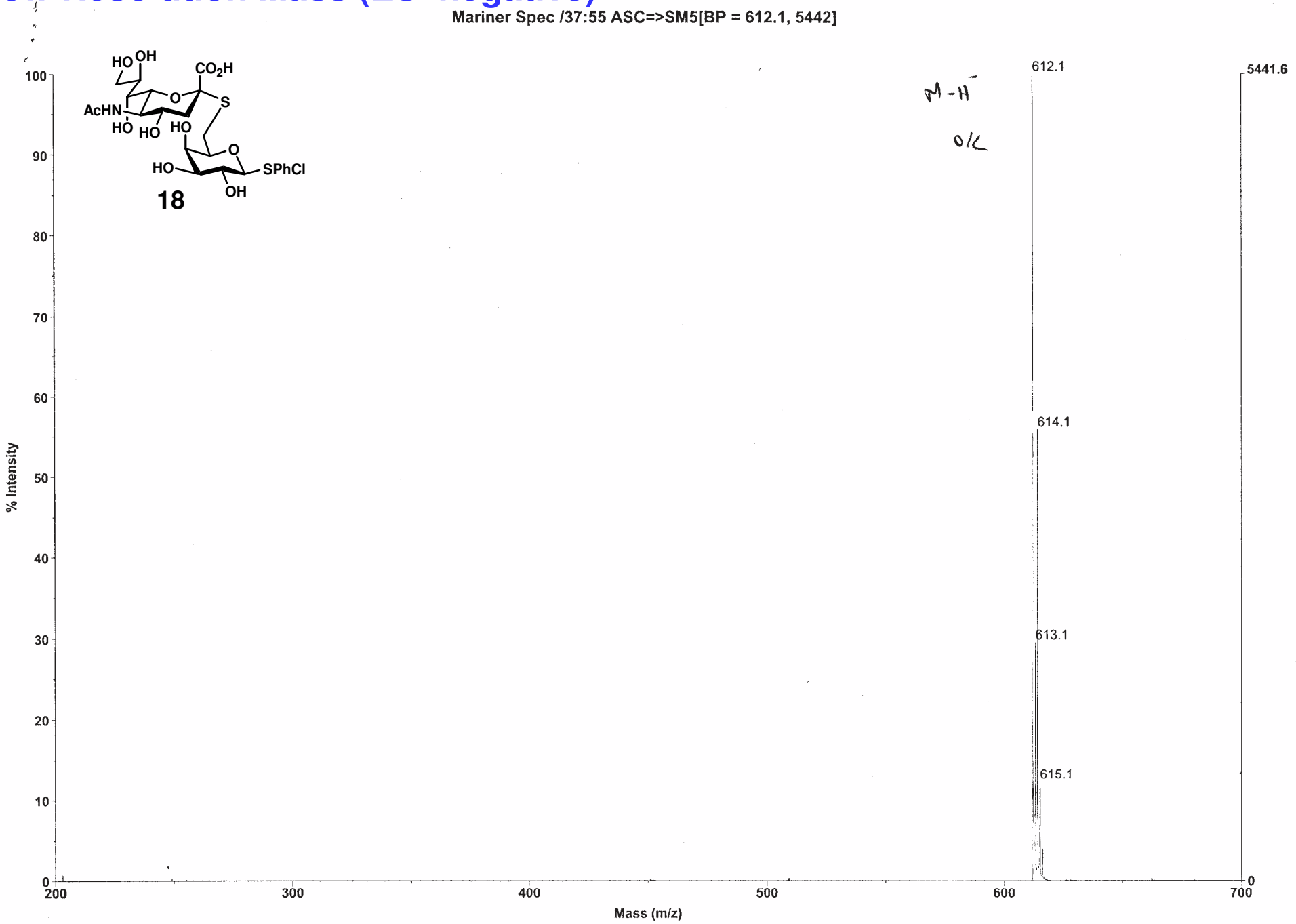


^{1}H NMR in CD_3OD, 400 MHz



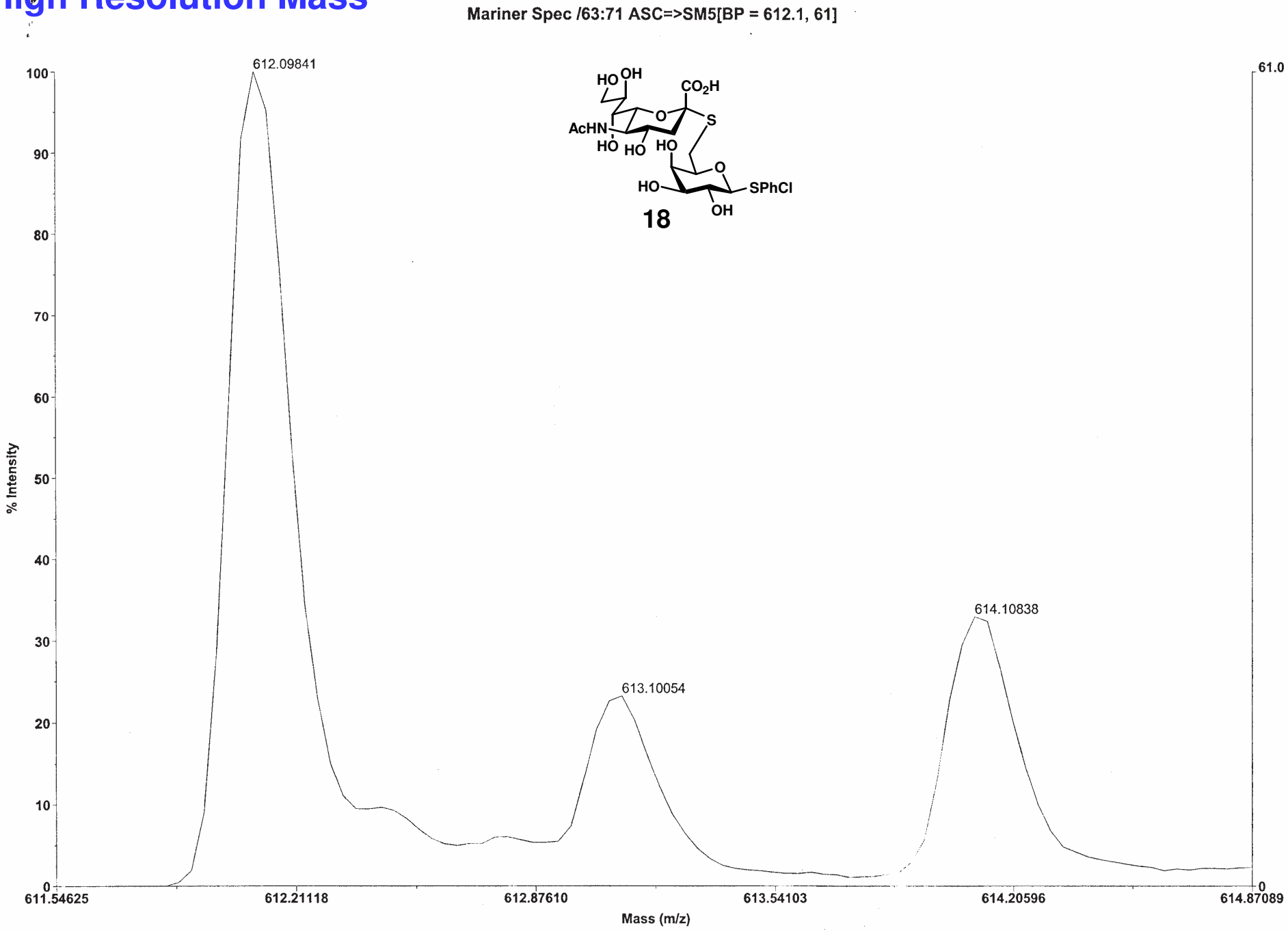
18

Low Resolution Mass (ESI negative)



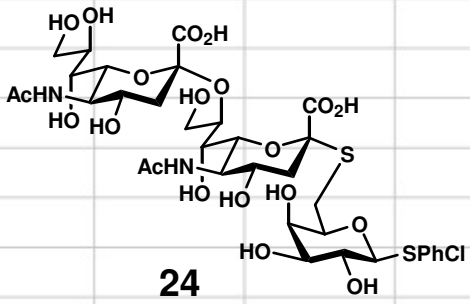
W. Li wl-i-37 neg es
C:\...\08052621.dat
Acquired: 20:33, May 26, 2008

High Resolution Mass



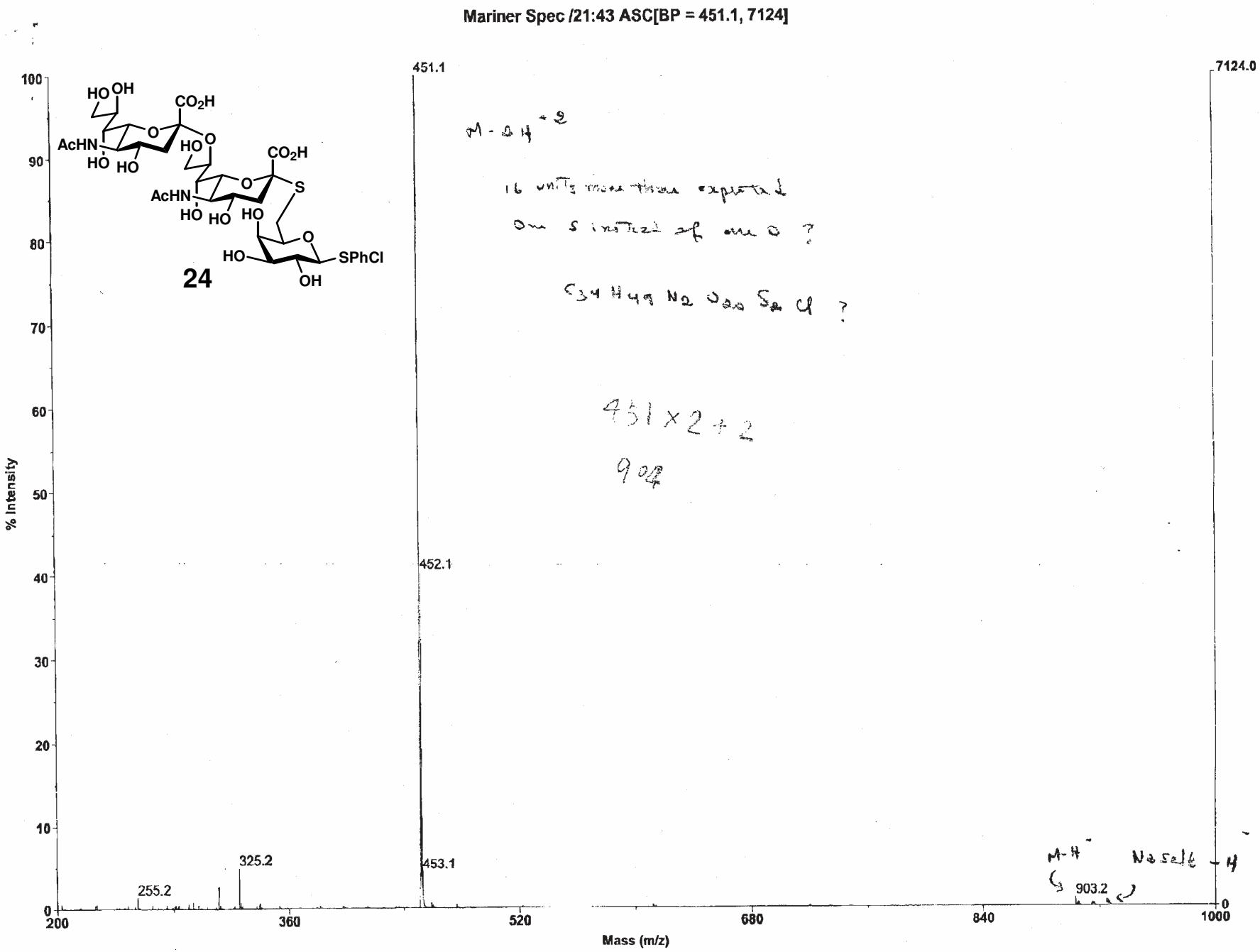
18

^{1}H NMR in CD_3OD, 400 MHz

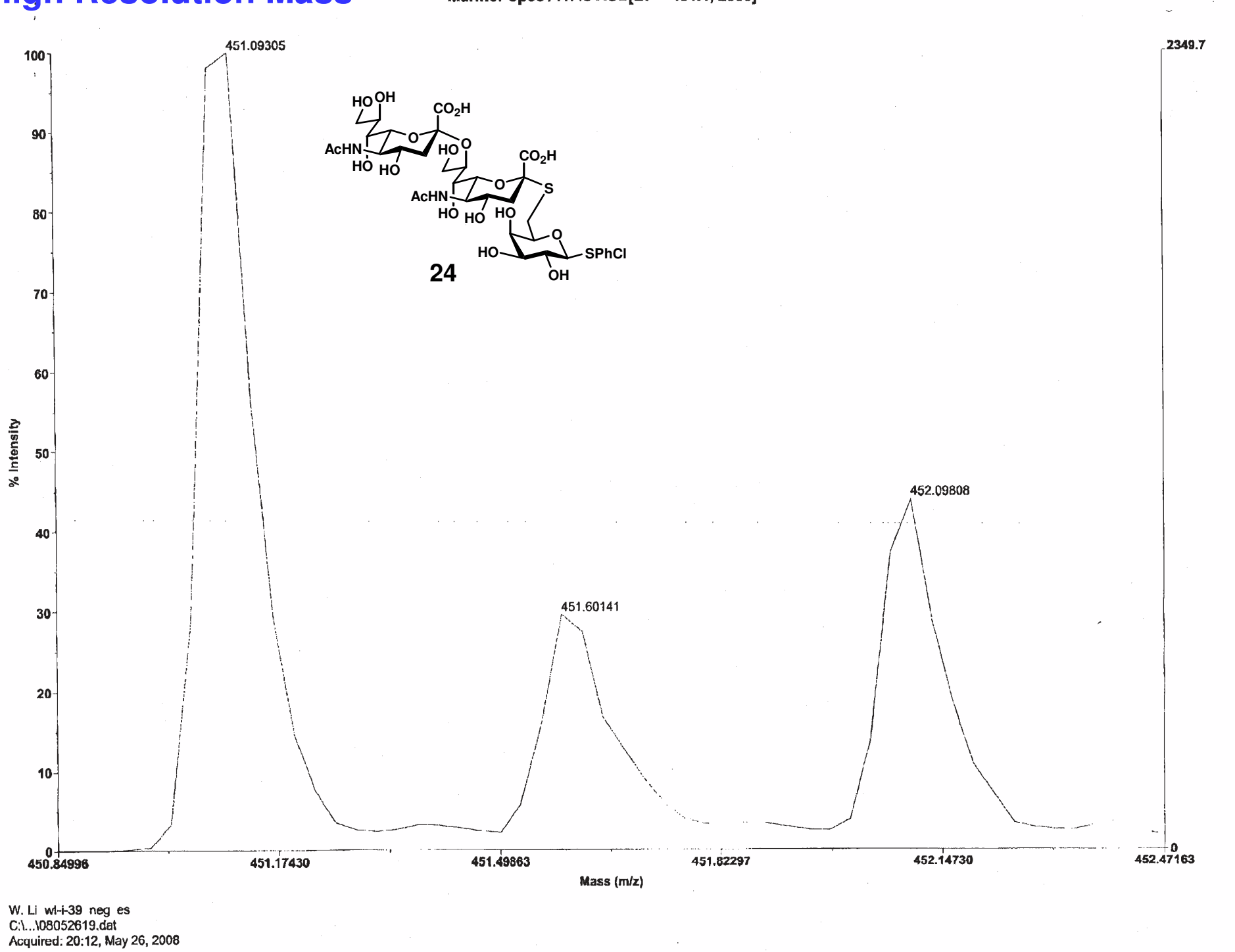


24

Low Resolution Mass (ESI negative)

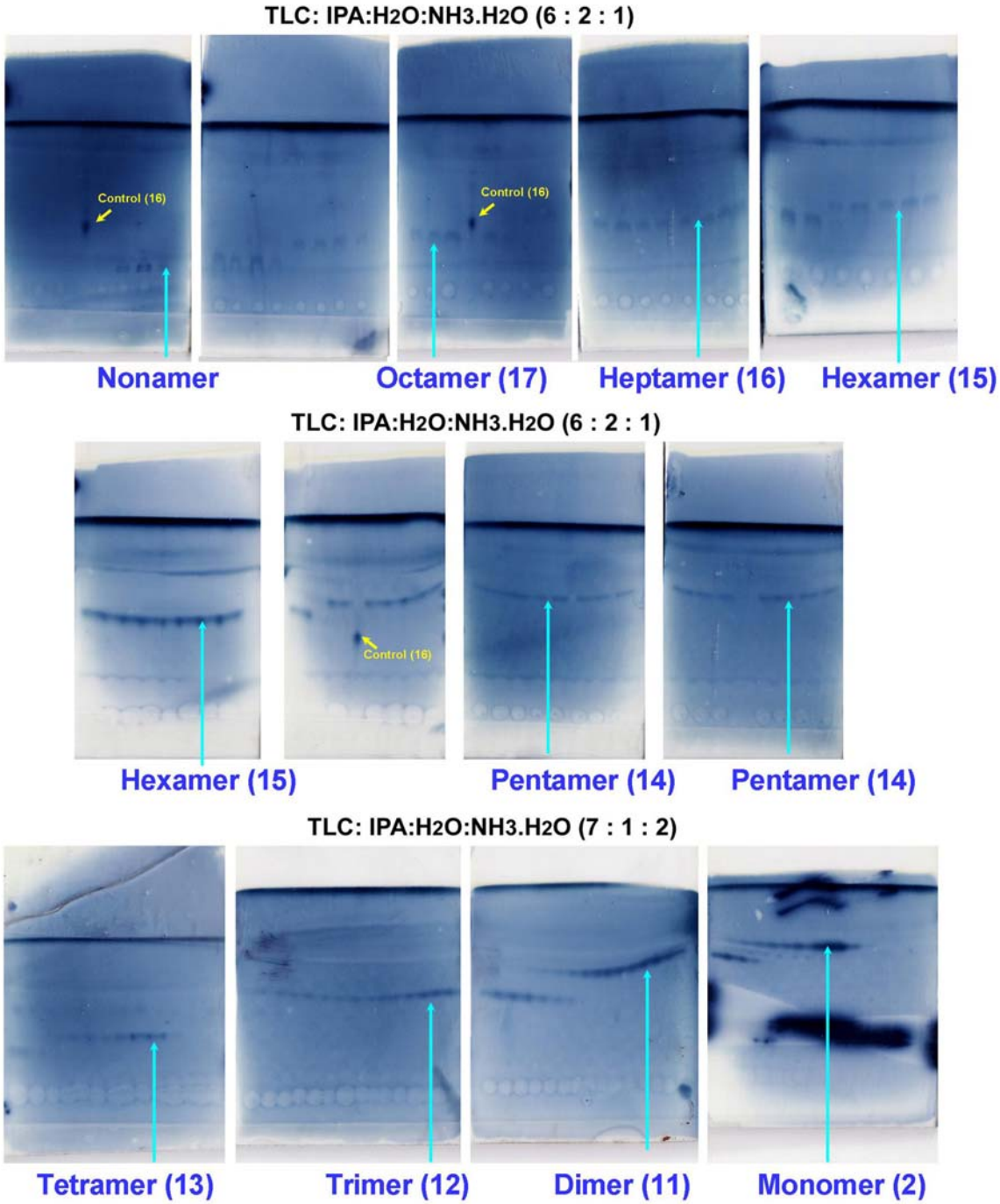


High Resolution Mass



TLC Profiles of Fractions from a Preparative Reverse Phase HPLC (C18)

Chemoenzymatic synthesis of α(2,8)-oligosialosides (11-17) using substrate 2.



All TLC's were visualized by immersion in a ceric ammonium molybdate dip and charred on a hot plate.