Supplementary Data

Biofunctional thiosialosides inhibit influenza virus

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Contents

I. Materials and Methods:

1. Chemical synthesis:

All solvents were dried prior to use. Thin-layer chromatography (TLC) was purchased from EMD Co. Ltd. (German). All compounds were stained with 5% H_2SO_4 in ethanol followed by heating. Detection with UV light was employed when possible. Flash column chromatography was performed on silica gel 200-300 mesh. NMR spectra were recorded on Bruker-AMX (400 MHz) instruments and analyzed by MestReNova. Chemical shifts (δ) were reported in parts per million downfield from TMS, the internal standard; *J* values were given in Hertz. Mass spectra were recorded on a ABI API 3200 instrument. Neuraminidase was purchased from Sino Biological Inc. (Beijing, China). Viruses were obtained from BEIresources, NIAID. **SA** and **SG**, compounds with free thiols are present as free thiols and disulfides as observed by NMR spectroscopy. These compounds were passed through Pierce Immobilized Reductant Column as per the manufacturers protocol to ensure that any disulfides are converted to the free thiols before performing the assays.

2. Cell and Viruses:

MDCK (NBL2) (ATCC® CCL34™) cells were cultured in DMEM medium (Life Technologies, USA) with 10% fetal bovine serum and 1% antibiotic-antimycotic. The viruses were obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID including Influenza A Virus, A/Hong Kong/8/1968 (H3N2) egg isolate (produced in eggs), NR-346 and A/California/07/2009 (H1N1) egg isolate (produced in eggs), NR-13663.

3. Virus Propagation.

The viruses used in the plaque reduction assay and the MUNANA based enzyme inhibition assay were propagated from the initial virus stocks. MDCK cells were grown to 100% confluent and then infected with a 1:10,000 dilution of the initial stock of virus isolates. The cells were subsequently grown in DMEM with 0.3% BSA and 1% antibiotic-antimycotic for three days. Supernatants of the cultures were collected and followed by ultracentrifugation at 70,000g for 3 hours to purify and concentrate the viruses. Viruses had their plaque forming units (pfu) determined by plaque assay in MDCK cells as previously described and were stored in Phosphate Buffered Saline (PBS) at -80 degrees until using.

4. Inhibition assay.

Assays were conducted in 0.1 M sodium acetate buffer pH 5.5 containing 10 mM CaCl2. MUNANA (Sigma) was used as the fluorescent substrate. Serial dilutions of inhibitors were premixed with the protein or virus for 30 min. The protein concentrations used were 5×10^{-4} and 4×10^{-5} Units for N1 and N2, respectively. The virus concentration used was 7.5×10^{4} and 1.8×10^{3} for A/Hong Kong/8/1968 (H3N2) and A/California/07/2009 (H1N1), respectively. The fluorescence was monitored at 3 min intervals for 2 h after addition of the substrate. The IC50 was calculated as the concentration of the inhibitor resulting in a 50% decrease of the rate compared to the control.

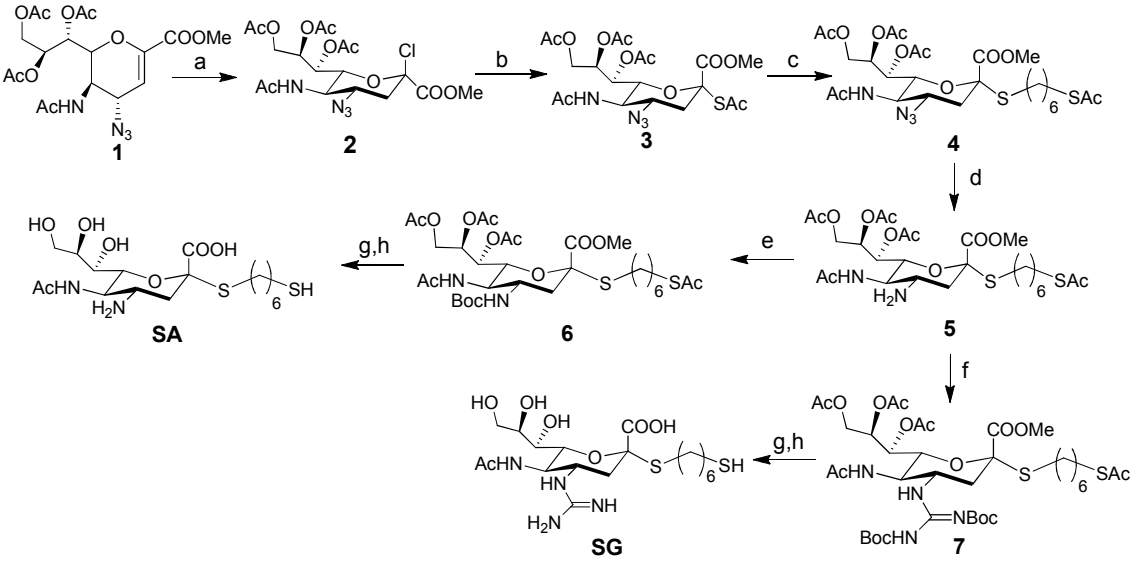
5. Plaque Reduction Assays.

Plaque reduction assays (PRA) in MDCK cells were performed by the addition of serial ten-fold dilutions of inhibitors in the overlay of serum-free DMEM with 0.6% molecular biology grade agarose (Fisher, USA) containing 1 µg/mL of L-1-tosylamido-2-phenylethyl chloromethyl ketone ('TPCK')-treated trypsin (Sigma, USA). The cells were seeded at a concentration of 1 x 10^6 per well and inoculated with virus when they reached confluence. The agarose overlays were removed after 5 days; cells were fixed with 70% ethanol and then stained with 0.2% crystal violet. The IC50 is the concentration of inhibitor causing a 50% decrease in plaque sizes. A range was used when the 50% reduction in plaque size fell between two inhibitor concentrations.

Abbreviations: N,N Dimethyl formamide, DMF; Ethyl acetate, EtOAc; Trifluroacetic acid, TFA; Acetonitrile, CH_3CN; Azidotrimethylsilane, $TMSN_3$; Diethylamine DEA; Trimethylsilyltrifluoromethanesulfonate, TMSOTf; Tetrabutylammonium bisulfate, TBAB; p-Toluene sulfonyl, Tos; Di-tert-butyl dicarbonate, $(Boc)_2O$; tert-Butyl alcohol, t-BuOH; Methanesulfonyl chloride, MsCl.

I. Chemical Synthesis.

a. Synthesis of S-sialomonomers.



Scheme 1. Synthesis of **SA** and **SG**. *Reagents and Conditions:* a) HCl (g), LiCl, CH_3CN, 6 days; b) KSAc, TBAB (Tetrabutylammonium bisulfate), CH_2Cl_2/H_2O, overnight, 50% over 2 steps; c) $TosOC_6H_{12}SAc$, DEA, DMF, 65%; d) PPh_3, THF/H_2O, 12h; e) $(Boc)_2O$, TEA, THF, 60% over 2 steps f) MeS-(C=NBoc)NHBoc, $HgCl_2$, TEA, CH_2Cl_2, 80% over 2 steps; g) MeOH/NaOH (aq), 1 h; h) TFA/DCM (1:1). 90% over two steps for **SA** and **SG**, respectively.

Methyl (5-acetamido-7, 8, 9-tri-O-acetyl-4-azido-2-chloro-3, 4, 5-trideoxy -β-D- glycerol -D-galacto-2-nonulopyranosid)onate 2. Anhydrous HCl was bubbled through a solution of

glycal **1** (1g, 2.1 mmol) in CH_3CN (20 ml) containing LiCl (500 mg, 11.7 mmol) for 30 min. The reaction mixture was stirring at room temperature for 4 days. HCl gas was bubbled again for 30 min through the solution again and the reaction was stirred for 2 more days. The solvent was evaporated to dryness, suspended in CH_2Cl_2, washed with ice-cold saturated $NaHCO_3$, dried over Na_2SO_4, concentrated to dryness. The crude material was directly used in next step.

Methyl (5-acetamido- 7, 8, 9-tri-O-acetyl-4-azido-2-thioacetyl-3, 4, 5-trideoxy -α-D-glycero-D-galacto-2-nonulopyranosid) onate 3. KSAc (1.25 g, 0.01 mmol, 5eq based on **1**) and TBAB (740mg, 2.2 mmol, 1eq based on **1**) were dissolved in water; a solution of crude chloride in CH_2Cl_2 was added dropwise. The two phase reaction system was stirred for 12 h. The organic phase was extracted by CH_2Cl_2 and washed with water, dried over Na_2SO_4. The solvent was removed in vacuum to give a residue, which was purified by flash chromatography to afford the title compound (580 mg, 50%) and 1 as a mixture (molar ratio 1:1 determinated by NMR).

6-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-4-azido-3, 4, 5-trideoxy -D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-1-thiolacete -hexane 4. Thioacetate **3** (200 mg, 0.37 mmol) and $TosOC_6H_{12}SAc$ (88.6 mg, 0.3 mmol) were dissolved in dry DMF (8 ml). DEA (312 μL, 3 mmol) was added dropwise and the reaction mixture was stirred for 2 h at room temperature. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography to give the product as a colorless oil (215 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 (d, *J* = 8.3 Hz, 1H, -N*H*Ac), 5.35-5.29 (m, 2H, H-7, H-8), 4.31 (d, *J* = 11.4 Hz, 1H), 4.20 (dd, *J* = 12.5, 4.3 Hz, 1H), 4.09 (d, *J* = 10.7 Hz), 3.99 (br, 1H), 3.83 (d, *J* = 10.5 Hz, 3H), 3.32-3.31 (m, 1H), 2.87 (t, *J* = 7.3 Hz, 2H), 2.82 – 2.63 (m, 2H, -SC*H*a, H-3eq), 2.62 – 2.45 (m, 1H, -SC*H*a), 2.33, 2.17, 2.05, 2.00 (4s, 12H), 1.86 – 1.32 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.9, 170.9, 170.5, 170.1, 169.8, 168.3, 162.5, 82.9, 77.5, 77.2, 76.8, 73.5, 68.5, 67.4, 62.0, 58.7, 52.7, 50.3, 38.0, 36.5, 31.4, 30.5, 29.2, 29.0, 28.9, 28.6, 28.1, 28.0, 23.1, 21.0, 20.8, 20.6. ESI-HRMS calcd for $C_{26}H_{41}N_4O_{11}S_2$: 649.2213, found: *m/z* 649.2230 $[M + H]^+$; $C_{26}H_{40}N_4O_{11}S_2Na$: 671.2038, found: *m/z* 671.2033 $[M + Na]^+$.

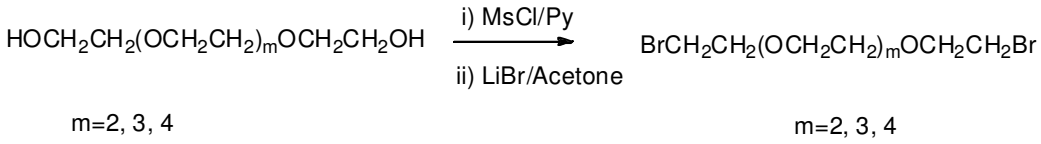
6-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-3, 4, 5-trideoxy-4-(bis-N, N'-tert-butyloxycarbonyl)-guanidino-*D*-glycero-α-D-galacto-non-2-ulopyranosyl) onate] -1-thioacetyl-hexane 7. A solution of azide **4** (200 mg, 0.33 mmol) and PPh_3 (0.4 mmol) in THF/H_2O (1:1, 10 ml) was stirred overnight. The solvent was removed in vacuo. The crude product **5** was dissolved in anhydrous CH_2Cl_2, and TEA was added dropwise to obtain a clear solution. Then 1, 3-bis (tert-butoxycarbonyl)-2-methylthiopseudourea (115 mg, 0.4 mmol) and $HgCl_2$ (109 mg, 0.4 mmol) was added under 0 ℃. The solution was warmed to room temperature and stirred for 12 h to obtain a suspension. The reaction was filtered. The filtrate was concentrated and purified by silica gel chromatography to obtain title compound **7** as a colorless amorphous solid (135mg, 80% over two steps). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 11.29 (s, 1H, N*H*Boc), 8.39 (1H, d, *J*=6.8 Hz, -NHBoc), 6.01 (1H, d, *J*=8.8 Hz, -NHAc), 5.39-5.30 (m, 2H, H-7, H-8), 4.34 (dd, 1H, *J*=2.0, 12.0 Hz, H-9a), 4.10-4.00 (m, 3H, H-9b, H-6, H-4), 3.83 (s, 3H, -OMe), 3.81-3.76 (m, 1H, H-5), 3.54 (t, 2H, *J*=6.8 Hz, -CH_2SAc), 2.82-2.72 (m, 2H, -SCH_a-, H_{3eq}), 2.58-2.53 (m, 1H, -SCH_b-), 2.17, 2.14, 2.04, 1.86 (4s, 4×3H, 4×-CH_3CO), 1.89-1.21 (m, 26H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.9, 170.7, 170.6, 170.1, 170.1, 168.7, 162.9, 156.7, 152.6, 83.8, 83.3, 79.4, 77.3, 77.0, 76.7, 75.4, 69.0, 67.7, 62.3, 52.8, 50.4, 49.7, 38.8, 30.6, 29.3, 29.2, 29.1, 29.0, 28.7, 28.3, 28.2, 28.2, 28.0, 23.0, 21.2, 20.9, 20.7. ESI-HRMS calcd for $C_{37}H_{61}N_4O_{15}S_2$: 865.3575, found: *m/z* 865.3590 $[M + H]^+$.

6-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-3, 4, 5-trideoxy -4-(N-tert-butyloxycarbonyl)-amino-D-glycero-α-*D*-galacto-non-2-ulopyranosyl)onate] -1-thioacetyl-hexane 6. The crude product **5** (1 mmol) was dissolved in THF, $(Boc)_2O$ (218 mg, 1 mmol) was added and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was concentrated under reduced pressure. The crude product was purified by silica gel chromatography to obtain title product as a colorless amorphous solid (134 mg, 60%).^{1}H NMR (400 MHz, $CDCl_3$) δ 5.60 – 5.21 (m, 3H), 4.70 (d, *J* = 8.4 Hz, 1H), 4.31 (d, *J* = 12.0 Hz, 1H), 4.11 (dd, *J* = 12.4, 4.2 Hz, 1H), 4.03 – 3.70 (m, 5H), 3.53-3.52 (m, 1H), 2.86 (t, *J* = 7.3 Hz, 1H), 2.79 – 2.70 (m, 2H), 2.55-2.48 (m,1H), 2.32, 2.16, 2.12, 2.04, 1.90 (5s, 5OAc), 1.75-1.69 (m, 1H), 1.56 – 1.26 (m, 17H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 195.9, 170.9, 170.6, 169.9, 168.6, 155.9, 83.4, 79.9, 77.3, 77.0, 76.7, 74.8, 68.5, 67.4, 62.1, 52.9, 50.2, 50.1, 39.2, 30.6, 29.3, 29.1, 29.0, 28.7, 28.3, 28.2, 28.2, 23.2, 21.1, 20.7. ESI-HRMS calcd for $C_{31}H_{50}N_2O_{13}S_2Na$: 745.2652, found: *m/z* 745.2657 $[M + Na]^+$.

6-S-[5-acetamido-3, 4, 5-trideoxy-4- amino-*D*-glycero-α-*D*-galacto-enonic acid] -1- thiol -hexane SA. 6 was treated with MeOH and 1 M NaOH. After stirred for 1 h, the solution was neutralized to pH 7 with Dowex 50W X 8 (H^+) resin, filtered and evaporated to dryness. Then TFA/DCM (1:1) was added, and the reaction mixture was stirred for 2 h at room temperature. The solution was evaporated to dryness. The residue was lyophilized to get a colorless amorphous solid (90%). ^{1}H NMR (400 MHz, MeOD) δ 4.19-4.14 (m, 1H); 3.85-3.61 (m, 5H), 3.44-3.32 (m, 1H); 2.90-2.67 (m, 5H, HSCH_2-, -SCH_2-, H_{3eq}), 2.02 (s, 3H, -NHAc), 1.57-1.28 (m, 8H); ^{13}C NMR (100 MHz, MeOD) δ 174.8, 172.5, 75.5, 71.5, 68.2, 62.9, 50.6, 38.1, 36.2, 28.9, 28.8, 28.6, 28.1, 27.5, 21.5. ESI-HRMS calcd for $C_{17}H_{33}N_2O_7S_2$: 441.1729, found: *m/z* 440.1639 $[M + H]^+$.

6-S-[5-acetamido-4-guanidino-3, 4, 5-trideoxy -D-glycero-α-D -galacto-non-2 -ulopyranosyl) onate]-1-thiol-hexane SG.
The guanidine compound **7** was deprotected with the same procedure described above to get a colorless amorphous solid (90%). ^{1}H NMR (400 MHz, D_2O) δ 3.72-3.30 (m, 7H), 2.64-2.51 (m, 5H, HSCH_2-, -SCH_2-, H_{3eq}), 1.81 (s, 3H, -NHAc), 1.57-1.28 (m, (m, 8H); ^{13}C NMR (125 MHz, D_2O) δ 174.8, 174.5, 162.7, 158.4, 117.8, 114.9, 86.3, 75.5, 72.0, 68.2, 62.5, 52.1, 50.8, 39.7, 38.2, 29.5, 29.2, 28.2, 27.6, 27.1, 22.0. ESI-HRMS calcd for $C_{18}H_{35}N_4O_7S_2$: 483.1946, found: *m/z* 483.1947 $[M + H]^+$.

General Protocol for the synthesis of oligoethylene glycol dibromide



Scheme 2. Synthesis of oligoethylene glycol dibromides. *Reagent and conditions* i) MsCl/Py; ii) LiBr/Acetone.

To a solution of the diol (1.0 eq) in pyridine, methanesulfonyl chloride (3.0 eq) was added dropwise at ^{o}C. The reaction mixture was stirred at room temperature for 3 h. The reaction was washed with HCl (1 M) and $NaHCO_3$ (aq), extracted with CH_2Cl_2 and dried with Na_2SO_4. The solvent was removed in vacuo and the residue was dissolved in acetone. Lithium bromide (4 eq) was added. The reaction

mixture was stirred and heated up to reflux overnight. The reaction was concentrated in vacuo and the crude product was purified by chromatography to obtain the product.

Tetraethylene glycol dibromide (m=2) was prepared by the procedure described above as a yellow oil (87%).^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (t, *J* = 6.0 Hz, 4H, $BrCH_2$ C*H*$_2$O), 3.67 (s, 8H, OC*H*$_2$C*H*$_2$O), 3.48 (t, *J* = 6.0 Hz, 4H, BrC*H*$_2$CH$_2$O); ^{13}C NMR (101 MHz, $CDCl_3$) δ 71.2, 70.7, 70.5, 30.4.

Pentaethylene glycol dibromide (m=3) was prepared by the procedure described above as a yellow oil (85%).^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81 (t, *J* = 6.0 Hz, 4H, $BrCH_2$ C*H*$_2$O), 3.67 (s, 12H, OC*H*$_2$C*H*$_2$O), 3.48 (t, *J* = 6.0 Hz, 4H, BrC*H*$_2$CH$_2$O); ^{13}C NMR (101 MHz, $CDCl_3$) δ 71.2, 70.7, 70.6, 70.5, 30.4.

Hexaethylene glycol dibromide (m=4) was prepared by the procedure described above as a yellow oil (85%).^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (t, *J* = 6.0 Hz, 4H, $BrCH_2$ C*H*$_2$O), 3.67 (s, 16H, OC*H*$_2$C*H*$_2$O), 3.45 (t, *J* = 6.4 Hz, 4H, BrC*H*$_2$CH$_2$O); ^{13}C NMR (101 MHz, $CDCl_3$) δ 71.2, 70.7, 70.6, 70.5, 30.4.

b. Synthesis of S-sialodimers.

General Protocol for the synthesis of S-sialodimers.

Thioacetate **3** (2.2 eq) and dibromide (1eq) were dissolved in dry DMF. DEA was added dropwise and the reaction was stirred for 3 h at room temperature. Then the reaction mixture was washed with 1 M HCl (aq), extracted with CH_2Cl_2, dried with Na_2SO_4. The solution was concentrated and purified by column chromatography.

1, 6-Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-4-azido-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-hexane 8. Thioacetate **3** and 1, 6- dibromohexane were used as described above. The crude product was purified by column chromatography to give the title compound as colorless oil (86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (d, *J* = 8.3 Hz, 2H, -N*H*Ac), 5.32-5.28 (m, 4H, H-7, H-8), 4.30 (d, *J* = 12.4 Hz, 2H, H-9a), 4.17 (dd, *J* = 12.4, 4.0 Hz, 2H, H-9b), 4.06 (d, *J* = 10.5 Hz, 2H, H-6), 3.91-3.90 (m, 2H, H-4), 3.80 (s, 6H, -OMe), 3.35-3.34 (m, 2H, H-5), 2.76-2.73 (m, 4H, -SC*H*$_a$-, H_{3eq}), 2.62 – 2.42 (m, 2H, -SC*H*$_b$-), 2.34 – 1.90 (m, 24H, -OAc), 1.75 (t, *J* = 12.6 Hz, 2H, H-3ax), 1.37 (dd, *J* = 53.7, 48.4 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.80, 170.73, 170.60, 170.00, 168.31, 82.97, 77.37, 77.05, 76.74, 72.69, 68.42, 67.77, 62.04, 57.77, 52.93, 51.68, 38.22, 29.10, 28.78, 28.35, 23.47, 21.12, 20.92, 20.73.

1, 12-Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-4-azido-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-dodecane 9. Thioacetate **3** and 1, 12- dibromo dodecane were used as described above. The crude product was purified by column chromatography to give the title compound as colorless oil (76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (br, 2H, -N*H*Ac), 5.31-5.28 (m, 4H, H-7, H-8), 4.30 (dd, *J* = 12.4, 2.0 Hz, 2H, H-9a), 4.17 (d, *J* = 12.4 Hz, 2H, H-9b), 4.02 (d, *J* = 6.4 Hz, 2H, H-6), 3.92-3.79 (m, 2H, H-4), 3.79 (s, 6H, -OMe), 3.36-3.34 (m, 2H, H-5), 2.78-2.71 (m, 4H, -SC*H*$_a$-, H_{3eq}), 2.57-2.52 (m, 2H, -SC*H*$_a$-), 2.29 – 1.90 (m, 24H), 1.75 (t, *J* = 12.5 Hz, 1H, H_{3ax}), 1.58 – 1.18 (m, 20H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.82, 170.62, 170.03,

168.35, 83.00, 77.38, 77.07, 76.75, 72.87, 68.55, 67.76, 62.05, 57.94, 53.45, 52.91, 51.46, 38.20, 29.54, 29.45, 29.26, 29.19, 28.90, 28.76, 23.44, 21.12, 20.91, 20.74.

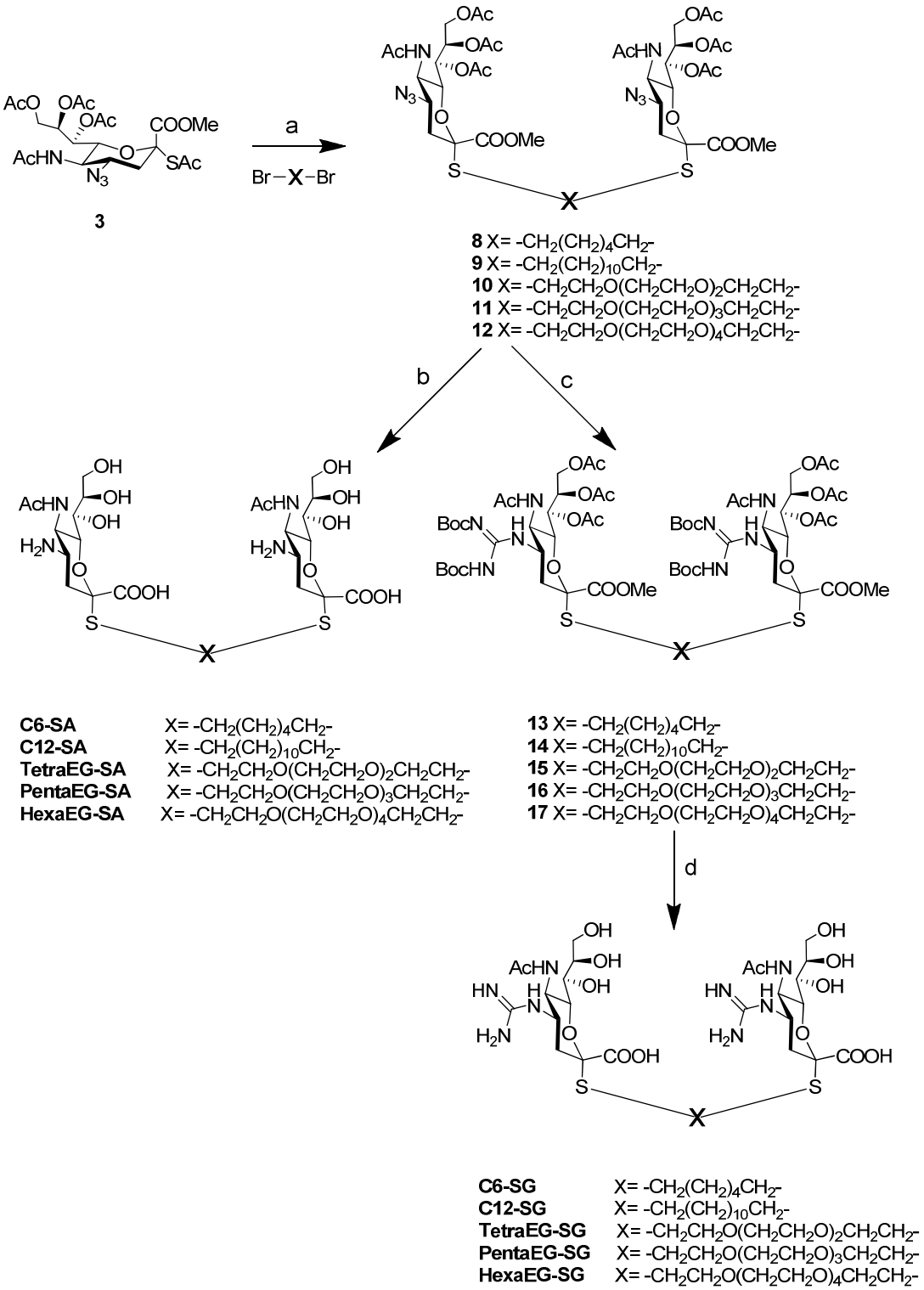
Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-4-azido-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-tetraethylene glycol 10. Thioacetate **3** and tetraethylene glycol dibromide were used as described above. The crude product was purified by column chromatography to give the title compound as colorless oil (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (br, 2H, -N*H*Ac), 5.31-5.28 (m, 4H, H-7, H-8), 4.29 (d, *J* = 12.4 Hz, 2H, H-9a), 4.23 – 4.09 (m, 2H, H-9b), 4.03 (d, *J* = 10.4 Hz, 2H, H-6), 3.89-3.85 (m, 2H, H-4), 3.80 (s, 6H, OMe), 3.61-3.50 (m, 12H), 3.37-3.36 (m, 2H, H-5), 3.05 – 2.87 (m, 2H, -SCH_a-), 2.86 – 2.70 (m, 4H, m, 4H, -SCH_b-, H_{3eq}), 2.39 – 1.89 (m, 24H), 1.77 (t, *J* = 12.6 Hz, 2H, H_{3ax}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.87, 170.65, 170.12, 168.36, 82.78, 77.39, 77.07, 76.75, 72.82, 70.44, 70.24, 68.35, 67.69, 62.09, 57.80, 53.05, 51.54, 38.09, 28.93, 23.43, 21.12, 20.90, 20.72.

Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-4-azido-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-pentaethylene glycol 11. Thioacetate **3** and pentaethylene glycol dibromide were used as described above. The crude product was purified by column chromatography to give the title compound as colorless oil (87%).^{1}H NMR (400 MHz, $CDCl_3$) δ 5.81 (d, *J* = 8.3 Hz, 2H, -N*H*Ac), 5.29-5.27 (m, 4H, H-7, H-8), 4.30 (d, *J* = 12.3 Hz, 2H, H-9a), 4.14 (dd, *J* = 12.4, 4.2 Hz, 2H, H-9b), 4.05 (d, *J* = 10.5 Hz, 2H, H-6), 3.98 – 3.87 (m, 2H, H-4), 3.86 – 3.77 (m, 8H, H-6, OMe), 3.61-3.60 (m, 16H), 3.32 (d, *J* = 9.3 Hz, 2H, H-5), 3.05 – 2.87 (m, 2H, -SCH_a-), 2.86 – 2.70 (m, 4H, m, 4H, -SCH_b-, H_{3eq}), 2.39 – 1.89 (m, 24H), 1.77 (t, *J* = 12.6 Hz, 2H, H_{3ax}); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.74, 170.62, 170.48, 169.98, 168.41, 82.84, 77.35, 77.03, 76.71, 72.82, 70.57, 70.43, 70.24, 68.50, 67.87, 62.11, 57.69, 52.92, 51.78, 38.20, 28.99, 23.35, 21.01, 20.80, 20.60.

Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-4-azido-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-hexaethylene glycol 12. Thioacetate **3** and hexaethylene glycol dibromide were used as described above. The crude product was purified by column chromatography to give the title compound as colorless oil (85%).^{1}H NMR (400 MHz, $CDCl_3$) δ 5.88 (s, br, 2H, -N*H*Ac), 5.29-5.27 (m, 4H, H-7, H-8), 4.27 (d, *J* = 12.0 Hz, 2H, H-9a), 4.13 (d, *J* = 9.6 Hz, 2H, H-9b), 4.00 (d, *J* = 9.3 Hz, 2H, H-4), 3.83-3.78 (M, 8H, H-6, OMe), 3.61-3.60 (m, 20H), 3.38-3.37 (m, 2H, H-5), 3.03 – 2.85 (m, 2H, -SCH_a-), 2.85 – 2.65 (m, 4H, -SCH_b-, H_{3eq}), 2.36 – 1.88 (m, 24H), 1.75 (t, *J* = 12.6 Hz, 1H, H-3ax); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.83, 170.62, 170.05, 168.33, 82.73, 77.41, 77.09, 76.77, 72.82, 70.55, 70.52, 70.39, 70.21, 68.24, 67.63, 62.07, 57.82, 53.47, 53.02, 51.42, 38.09, 28.93, 23.42, 21.12, 20.90, 20.72.

General protocol for the deprotection of the azide.

The azide compound was treated with MeOH and 1 M NaOH (1:1 v/v). After stirred for 1 h, the solution was neutralized to pH 7 with Dowex 50W X 8 (H^+) resin, filtered and evaporated to dryness. Then the residue in EtOH/EtOAc (1:1) with 2 drop of acetic acid was hydrogenated with $Pd(OH)_2$/C under atmosphere of hydrogen (3 psi) for 12 h. Then the mixture was filtered and washed with MeOH. The filtrate was concentrated to dryness, purified by Bio-Gel® (P-2 Polyacrylamide Gel column) and lyophilized to get a colorless amorphous solid.



Scheme 2. Synthesis of bivalent analogs of S-Sialosides**.** *Reagents and Conditions:* a) DEA, DMF; 76-85%; b) i) NaOH/CH_3OH (aq), ii) H_2/$Pd(OH)_2$; 90-92% % over 2 steps; c) i) H_2/$Pd(OH)_2$, ii)MeS-(C=NBoc)NHBoc, $HgCl_2$, TEA, CH_2Cl_2. 85-89% % over 2 steps; d) NaOH/CH_3OH (aq), 1 h; i) TFA/DCM (1:1), 80-85% over 2 steps.

1, 6-Di-S-[5-acetamido- -4-amino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-enonic acid]-hexane C6-SA. ^{1}H NMR (500 MHz, D_2O) δ 3.89-3.47 (m, 14H), 2.78-2.63 (m, 4H, -SCH_a-, H_{3eq}), 2.58 – 2.53 (m, 2H, -SCH_b-), 1.94 (s, 6H, -NHAc), 1.46-1.24 (m, 8H); ^{13}C NMR (125 MHz, D_2O) δ 174.5, 174.3, 170.4, 83.0, 74.3, 70.3, 67.5, 67.3, 62.3, 58.3, 53.1, 49.3, 36.8, 28.5, 28.1, 26.9, 21.6, 21.5. ESI-HRMS calcd for $C_{28}H_{51}N_4O_{14}S_2$: 730.2843, found: *m/z* 730. 2868 $[M + H]^+$.

1, 12-Di-S-[5-acetamido- -4-amino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-enonic acid]-dodecane C12SA. ^{1}H NMR (500 MHz, D_2O) δ 3.90-3.74 (m, 14H), 2.71-2.61 (m, 4H, -SCH_a-, H_{3eq}), 2.58 – 2.53 (m, 2H, -SCH_b-), 1.95 (s, 6H, -NHAc), 1.73-1.71 (m, 1H, H_{3ax}) 1.49-1.07 (m, 20H); ^{13}C NMR (125 MHz, D_2O) δ 174.2, 172.4, 84.3, 74.4, 71.0, 67.4, 67.2, 62.3, 61.9, 58.9, 49.4, 37.5, 28.9, 28.7, 28.4, 28.1, 21.6. ESI-HRMS calcd for $C_{34}H_{51}N_4O_{14}S_2$: 815.3782, found: *m/z* 815.3787 $[M + H]^+$.

Di-S-[5-acetamido--4-amino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-enonic acid]-tetraethylene glycol TetraEG-SA. ^{1}H NMR (500 MHz, D_2O) δ 3.76-3.48 (m, 26H), 2.85-2.78 (m, 6H, -SCH_2-, H_{3eq}), 1.93 (s, 6H, -NHAc), 1.87-1.85 (m, 1H, H_{3ax}); ^{13}C NMR (125 MHz, D_2O) δ 174.5, 172.4, 84.9, 74.1, 71.4, 69.2, 68.9, 68.8, 67.2, 62.1, 61.9, 50.3, 46.9, 36.1, 28.5, 21.6, 21.5. ESI-HRMS calcd for $C_{30}H_{55}N_4O_{17}S_2$: 807.3004, found: *m/z* 807.3022 $[M + H]^+$.

Di-S-[5-acetamido-4-amino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-enonic acid]-pentaethylene glycol PentaEG-SA. 1H NMR (500 MHz, D2O) δ 3.76-3.48 (m, 30H), 2.85-2.78 (m, 6H, -SCH_2-, H_{3eq}), 1.93 (s, 6H, -NHAc), 1.87-1.85 (m, 1H, H_{3ax}); 13C NMR (125 MHz, D2O) δ 174.5, 174.2, 173.2, 172.5, 85.4, 85.0, 74.4, 74.1, 71.5, 71.4, 69.3, 69.2, 69.1, 68.9, 68.8, 68.4, 67.4, 67.3, 67.2, 66.1, 62.0, 61.9, 59.3, 50.3, 49.2, 47.0, 37.4, 36.1, 28.5, 21.6, 21.5. ESI-HRMS calcd for $C_{32}H_{59}N_4O_{18}S_2$: 851.3266, found: *m/z* 851.3258 $[M + H]^+$.

Di-S-[5-acetamido-4-amino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-enonic acid]-hexaethylene glycol HexaEG-SA. ^{1}H NMR (500 MHz, D_2O) δ 3.76-3.41 (m, 34 H), 2.85-2.71 (m, 6H, -SCH_2-, H_{3eq}), 1.93 (s, 6H, -NHAc), 1.71-1.69 (m, H_{3ax}); ^{13}C NMR (125 MHz, D_2O) δ 174.5, 174.2, 173.2, 85.4, 74.1, 71.4, 69.4, 69.1, 68.9, 68.7, 68.4, 67.4, 66.1, 62.0, 59.4, 50.3, 49.2, 37.5, 28.5, 22.7, 21.5, 21.5. ESI-HRMS calcd for $C_{34}H_{63}N_4O_{19}S_2$: 895.3528, found: *m/z* 895.3521 $[M + H]^+$.

General protocol for the changing of the azide to guanidine.

The azide in EtOH/EtOAc (1:1) was hydrogenated with $Pd(OH)_2/C$ under atmosphere of hydrogen (3 psi) for 12 h. Then the mixture was filtered and washed with MeOH. The filtrate was concentrated to dryness. The guanidine compound was synthesized according to the procedure described above for the synthesis of the monoer guanidino product as colorless syrup (85-89%).

1, 6-Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-3, 4, 5-trideoxy-4-(bis-N, N'-tert-butyloxycarbonyl)-guanidino-*D*-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-hexane 13. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.30 (s, 2H, -N*H*Boc), 8.39 (d, *J* = 7.1 Hz, 2H, -N*H*Boc), 6.03 (d, *J* = 8.7 Hz, 2H, -N*H*Ac), 5.36-5.29 (m, 4H, H-7, H-8), 4.35 (d, *J* = 12.3 Hz, 1H, H-9a), 4.12-3.99 (m, 6H, H-9b, H-4, H-5), 3.95 – 3.67 (m, 8H, OMe, H-6), 2.81 – 2.70 (m, 4H, -SCH_a-, H_{3eq}), 2.67 – 2.47 (m, 2H, -SCH_b-), 2.16, 2.14, 2.04, 1.83 (4s, 24H, -Ac), 1.69 – 1.14 (m, 34H).^{13}C NMR

(101 MHz, $CDCl_3$) δ 170.81, 170.65, 170.13, 168.79, 162.99, 156.79, 152.70, 83.81, 83.36, 79.50, 77.35, 77.03, 76.71, 75.46, 69.19, 67.83, 62.35, 52.89, 50.46, 49.69, 38.82, 29.69, 29.09, 28.83, 28.43, 28.26, 28.02, 23.02, 21.21, 20.98, 20.81.

1, 12-Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-3, 4, 5-trideoxy-4-(bis-N, N'-tert-butyloxycarbonyl)-guanidino-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-dodecane 14. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.30 ((s, 2H, -N*H*Boc), 8.39 (d, *J* = 7.2 Hz, 2H, -N*H*Boc), 6.05 (d, *J* = 9.1 Hz, 2H, -N*H*Ac), 5.63 – 5.16 (m, 4H, H-7, H-8), 4.35 (dd, *J* = 12.4, 2.6 Hz, 1H, H-9a), 4.12-3.99 (m, 6H, H-9b, H-4, H-5), 3.95 – 3.70 (m, 8H, OMe, H-6), 2.81 – 2.70 (m, 4H, -SCH_a-, H_{3eq}), 2.67 – 2.47 (m, 2H, -SCH_b-), 2.18, 2.16, 2.03, 1.82 (4s, 24H, -Ac), 1.68 – 1.19 (m, 56H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.81, 170.65, 170.17, 168.81, 162.98, 156.79, 152.70, 83.80, 83.38, 79.49, 77.36, 77.04, 76.73, 75.51, 69.32, 67.88, 62.35, 52.85, 50.47, 49.69, 38.84, 29.61, 29.51, 29.27, 28.95, 28.85, 28.25, 28.02, 23.02, 21.20, 20.96, 20.80.

Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-3, 4, 5-trideoxy-4-(bis-N, N'-tert-butyloxycarbonyl)-guanidino-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-tetraethylene glycol 15. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.31 (s, 2H, -N*H*Boc), 8.40 (d, *J* = 6.8 Hz, 2H, -N*H*Boc), 6.06 (d, *J* = 8.6 Hz, 2H, -N*H*Ac), 5.35-5.27 (m, 4H, H-7, H-8), 4.33 (d, *J* = 12.3 Hz, 2H, H-6), 4.21 – 3.95 (m, 6H, H-9a, H-9b, H-4), 3.85 (s, 6H, -OMe), 3.76 (d, *J* = 9.9 Hz, 1H, H-5), 3.67-3.63 (m, 12H), 3.03-2.82 (m, 6H, -SCH_a-, H_{3eq}-SCH_b-), 2.18, 2.17, 2.04, 1.89 (4s, 12H, -Ac), 1.90-1.87 (m, 1H, H-3ax), 1.49 (s, 36H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.82, 170.19, 168.78, 162.97, 156.81, 152.70, 83.84, 83.15, 79.53, 77.35, 77.03, 76.71, 75.47, 70.45, 70.30, 70.22, 68.98, 67.76, 62.41, 52.97, 50.42, 49.63, 38.73, 30.92, 28.93, 28.26, 28.03, 23.02, 21.22, 20.98, 20.80.

Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-3, 4, 5-trideoxy-4-(bis-N, N'-tert-butyloxycarbonyl)-guanidino-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-pentaethylene glycol 16. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.28 (s, 2H, -N*H*Boc), 8.38 (d, *J* = 6.8 Hz, 2H, -N*H*Boc), 6.06 (d, *J* = 8.6 Hz, 2H, -N*H*Ac), 5.34-5.26 (m, 4H, H-7, H-8), 4.33 (d, *J* = 12.3 Hz, 2H, H-6), 4.07 – 3.97 (m, 6H, H-9a, H-9b, H-4), 3.81 (s, 6H, -OMe), 3.76-3.73 (d, *J* = 9.9 Hz, 1H, H-5), 3.63-3.61 (m, 16H), 2.94-2.82 (m, 6H, -SCH_a-, H_{3eq}, -SCH_b-), 2.18, 2.17, 2.04, 1.89 (4s, 12H, -Ac), 1.90-1.87 (m, 1H, H-3ax), 1.49 (s, 36H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3, 170.2, 169.7, 169.6, 168.3, 162.4, 156.3, 152.1, 83.3, 82.6, 79.1, 74.9, 70.0, 69.9, 69.8, 69.7, 68.4, 67.2, 61.9, 52.3, 49.8, 49.1, 38.1, 28.4, 27.7, 27.5, 22.5, 20.7, 20.4, 20.3,

Di-S-[Methyl 5-acetamido-7, 8, 9-tri-O- acetyl-3, 4, 5-trideoxy-4-(bis-N, N'-tert-butyloxycarbonyl)-guanidino-D-glycero-α-D-galacto-non-2-ulopyranosyl) onate]-hexaethylene glycol 17.^{1}H NMR (400 MHz, $CDCl_3$) δ 11.29 (s, 2H, -N*H*Boc), 8.39 (d, *J* = 6.8 Hz, 2H, -N*H*Boc), 6.05 (d, *J* = 8.8 Hz, 2H, -N*H*Ac), 5.35-5.26 (m, 4H, H-7, H-8), 4.33 (d, *J* = 12.3 Hz, 2H, H-6), 4.09 – 3.98 (m, 6H, H-9a, H-9b, H-4), 3.82 (s, 6H, -OMe), 3.77-3.74 (d, *J* = 9.9 Hz, 1H, H-5), 3.64-3.62 (m, 20H), 2.95-2.78 (m, 6H, -SCH_a-, H_{3eq}, -SCH_b-), 2.15, 2.13, 2.03, 1.83 (4s, 12H, -Ac), 1.90-1.87 (m, 1H, H-3ax), 1.48 (s, 36H);^{13}C NMR (101 MHz, $CDCl_3$) δ 170.8, 170.7, 170.2, 168.8, 162.9, 156.8, 152.6, 83.8, 83.1, 79.5, 77.3, 77.0, 76.7, 75.4, 70.5, 70.4, 70.2, 70.2, 68.9, 67.7, 62.4, 52.9, 50.3, 49.6, 38.7, 28.9, 28.2, 28.0, 23.0, 21.2, 20.9, 20.7.

General protocol for the deprotection of the guanidine.

The guanidine compound was treated with MeOH and 1 M NaOH. After stirred for 1 h, the solution was neutralized to pH 7 with Dowex 50W X 8 (H^+) resin, filtered and evaporated to dryness. Then TFA/DCM (1:1) was added, and the reaction mixture was stirred for 2 h at room temperature. The solution was evaporated to dryness. The residue was purified by Bio-Gel® (P-2 Polyacrylamide Gel column) and lyophilized to get a colorless amorphous solid.

1, 6-Di-S-[5-acetamido- -4-guanidino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-enonic acid]-hexane C6-SG. ^{1}H NMR (500 MHz, D_2O) δ 3.90-3.49 (m, 14H), 2.68-2.62 (m, 4H, -SCH_a-, H_{3eq}), 2.58 – 2.54 (m, 2H, -SCH_b-), 1.88 (s, 6H, -NHAc), 1.79 (t, *J*= 10, 15 Hz, H_{3ax}), 1.46-1.24 (m, 8H); ^{13}C NMR (125 MHz, D_2O) δ 174.5, 172.8, 162.3, 156.1, 84.7, 74.3, 71.2, 67.4, 51.4, 49.3, 37.8, 29.0, 28.5, 28.9, 28.4, 27.0, 21.3. ESI-HRMS calcd for $C_{30}H_{55}N_8O_{14}S_2$: 815.3279, found: *m/z* 815.3282 $[M + H]^+$.

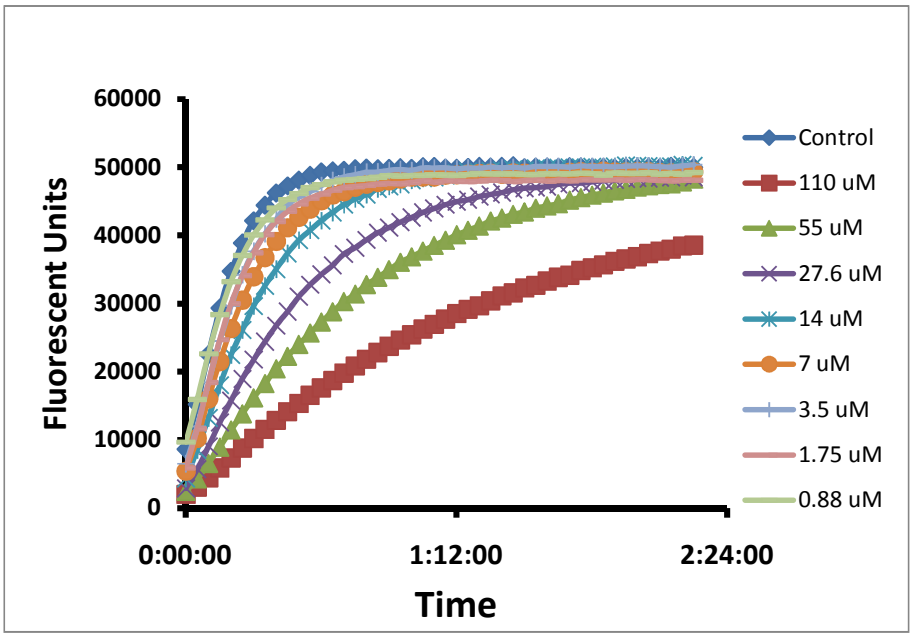
1, 12-Di-S-[5-acetamido- -4- guanidino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2-enonic acid]-dodecane C12-SG. ^{1}H NMR (500 MHz, D_2O) δ 3.95-3.55 (m, 14H), 2.69-2.57 (m, 6H, -SCH_a-, H_{3eq}, SCH_b-), 1.90 (s, 6H, -NHAc), 1.49-1.07 (m, 20H); ^{13}C NMR (125 MHz, D_2O) δ 174.1, 170.0, 162.5, 162.2, 156.2, 117.0, 114.7, 85.4, 82.7, 74.5, 70.4, 67.6, 62.2, 53.2, 50.4, 49.5, 37.3, 29.0, 28.9, 28.6, 28.4, 28.1, 21.4. ESI-HRMS calcd for $C_{36}H_{67}N_8O_{14}S_2$: 899.4218, found: *m/z* 899.4221 $[M + H]^+$.

Di-S-[5-acetamido--4-guanidino-3,4, 5-trideoxy-D-glycero-α-D-galacto-non-2- enonic acid]-tetraethylene glycol TetraEG-SG). ^{1}H NMR (500 MHz, D_2O) δ 3.87-3.48 (m, 26H), 2.85-2.78 (m, 6H, -SCH_2-, H_{3eq}), 1.93 (s, 6H, -NHAc), 1.77 (t, *J*= 10, 15 Hz, H_{3ax}), ^{13}C NMR (125 MHz, D_2O) δ 173.9, 173.0, 156.2, 85.0, 74.4, 71.4, 69.3, 69.0, 68.9, 68.8, 67.4, 61.9, 51.5, 49.4, 37.8, 28.3, 21.3. ESI-HRMS calcd for $C_{32}H_{59}N_8O_{17}S_2$: 891.9843, found: *m/z* 815.9832 $[M + H]^+$.

Di-S-[5-acetamido-4-guanidino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2- enonic acid]-pentaethylene glycol PentaEG-SG. 1H NMR (500 MHz, D2O) δ 3.90-3.51 (m, 30H), 2.85-2.68 (m, 6H, -SCH_2-, H_{3eq}), 1.93 (s, 6H, -NHAc), 1.79 (1.79 (t, *J*= 10, 15 Hz, H3ax),); 13C NMR (125 MHz, D2O) δ 173.9, 173.1, 156.1, 85.0, 74.4, 71.4, 69.3, 69.0, 68.9, 68.8, 67.4, 61.9, 51.5, 49.4, 37.8, 28.4, 21.3. ESI-HRMS calcd for $C_{34}H_{63}N_8O_{18}S_2$: 925.3702, found: *m/z* 815.3701 $[M + H]^+$.

Di-S-[5-acetamido-4-guanidino-3, 4, 5-trideoxy-D-glycero-α-D-galacto-non-2- enonic acid]-hexaethylene glycol HexaEG-SG. ^{1}H NMR (500 MHz, D_2O) δ 3.89-3.48 (m, 34 H), 2.84-2.68 (m, 6H, -SCH_2-, H_{3eq}), 1.88 (s, 6H, -NHAc), 1.77 1.79 (t, *J*= 10, 15 Hz, H_{3ax}); ^{13}C NMR (125 MHz, D_2O) δ 173.9, 173.3, 156.1, 85.4, 74.1, 71.5, 69.4, 69.1, 68.9, 68.7, 67.4, 61.9, 51.7, 49.4, 37.9, 28.4, 21.3. ESI-HRMS calcd for $C_{36}H_{66}N_8O_{19}S_2$: 979.3964, found: *m/z* 979.3976 $[M + H]^+$.

II. Neuraminidase inhibition assay.



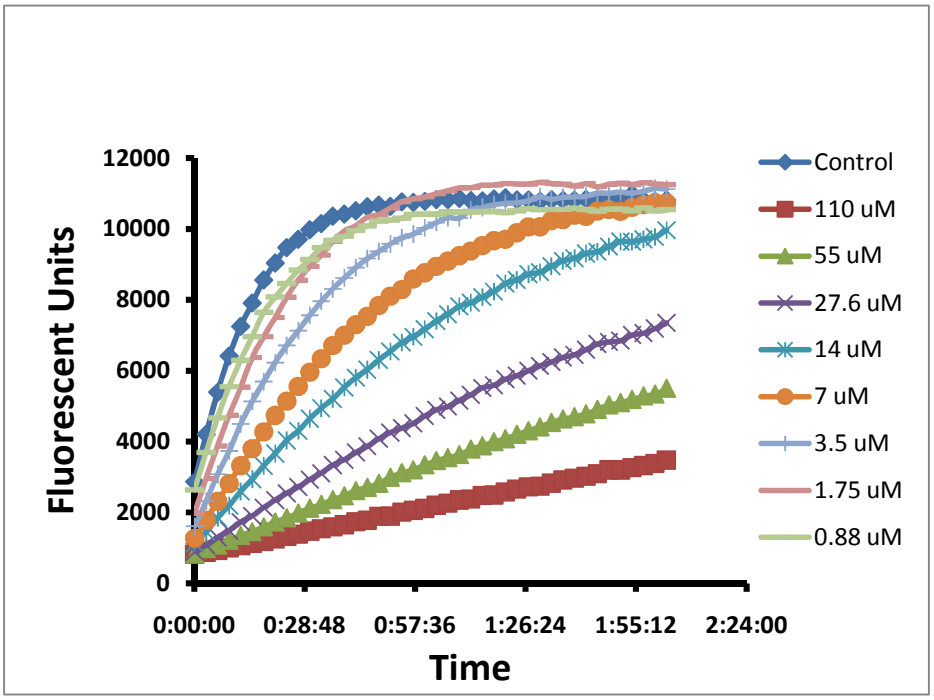
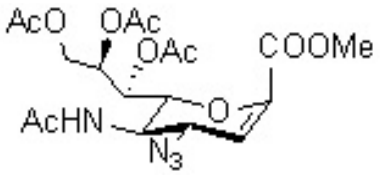
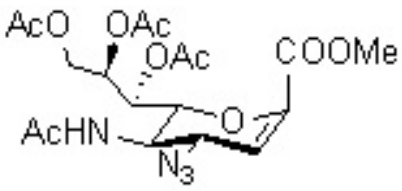


Figure S1. Selected raw data of the NA inhibition assays. **Top:** H3N2 inhibition assays for **C6-SA**. **Bottom:** b) Influenza H1N1 (A/California/07/2009) inhibition assays for **C6-SA**. NA or intact viral and inhibitor in serial dilutions were preincubated for 30 mins followed by the addition of MUNANA. The protein concentrations used were 5 x 10^{-4} and 4 x 10^{-5} Units for N1 and N2, respectively. The virus concentration used was 7.5 x 10^{4} and 1.8 x 10^{3} for A/Hong Kong/8/1968 (H3N2) and A/California/07/2009 (H1N1), respectively. Fluorescence was monitored at 3 min intervals for 2 h after the addition of the substrate.

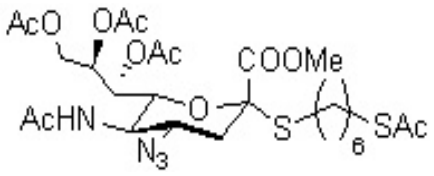
^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100 MHz, $CDCl_3$)

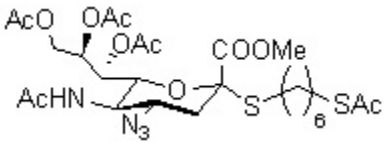


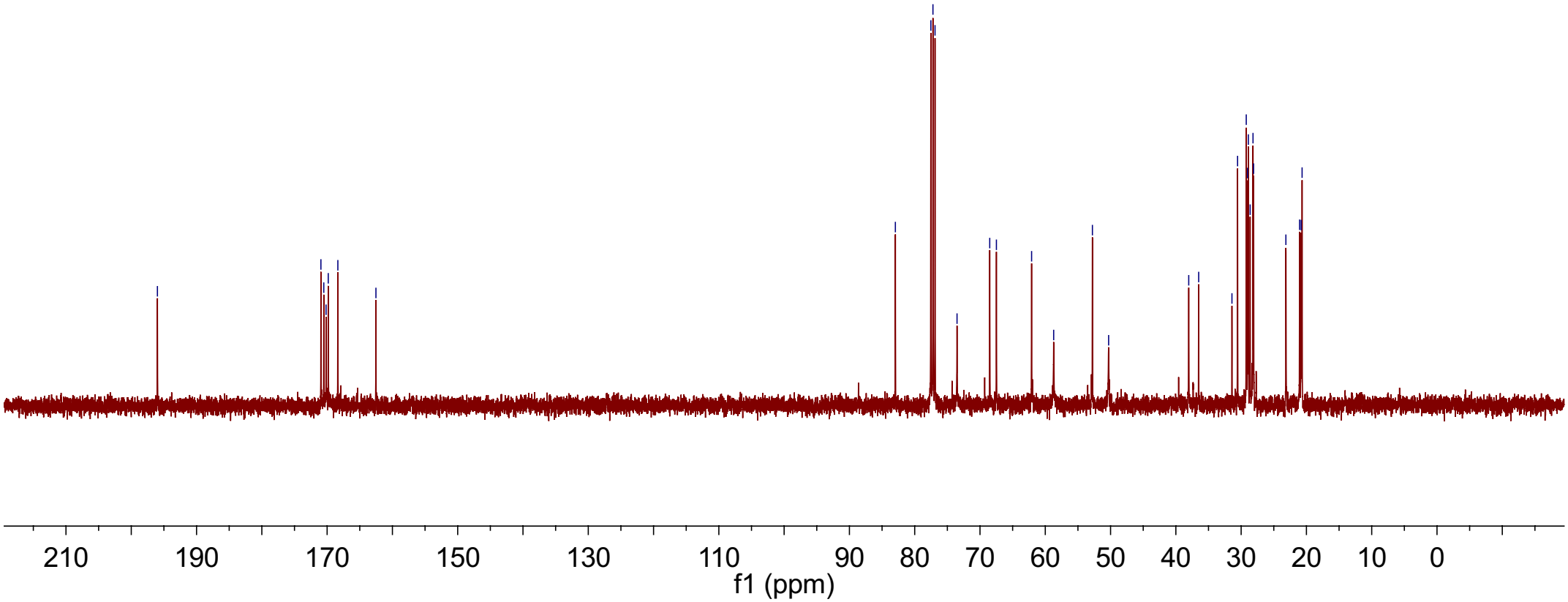
^{1}H NMR (400 MHz, $CDCl_3$)



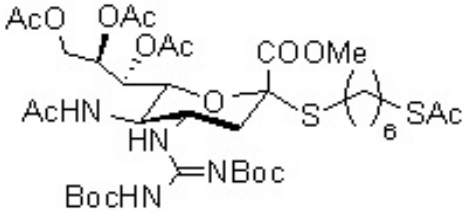
196.0
171.0
170.2
169.8
168.4
162.5
83.0
73.5
67.5
58.7
50.3
38.0
36.5
28.2
23.1
21.0
20.8
20.7

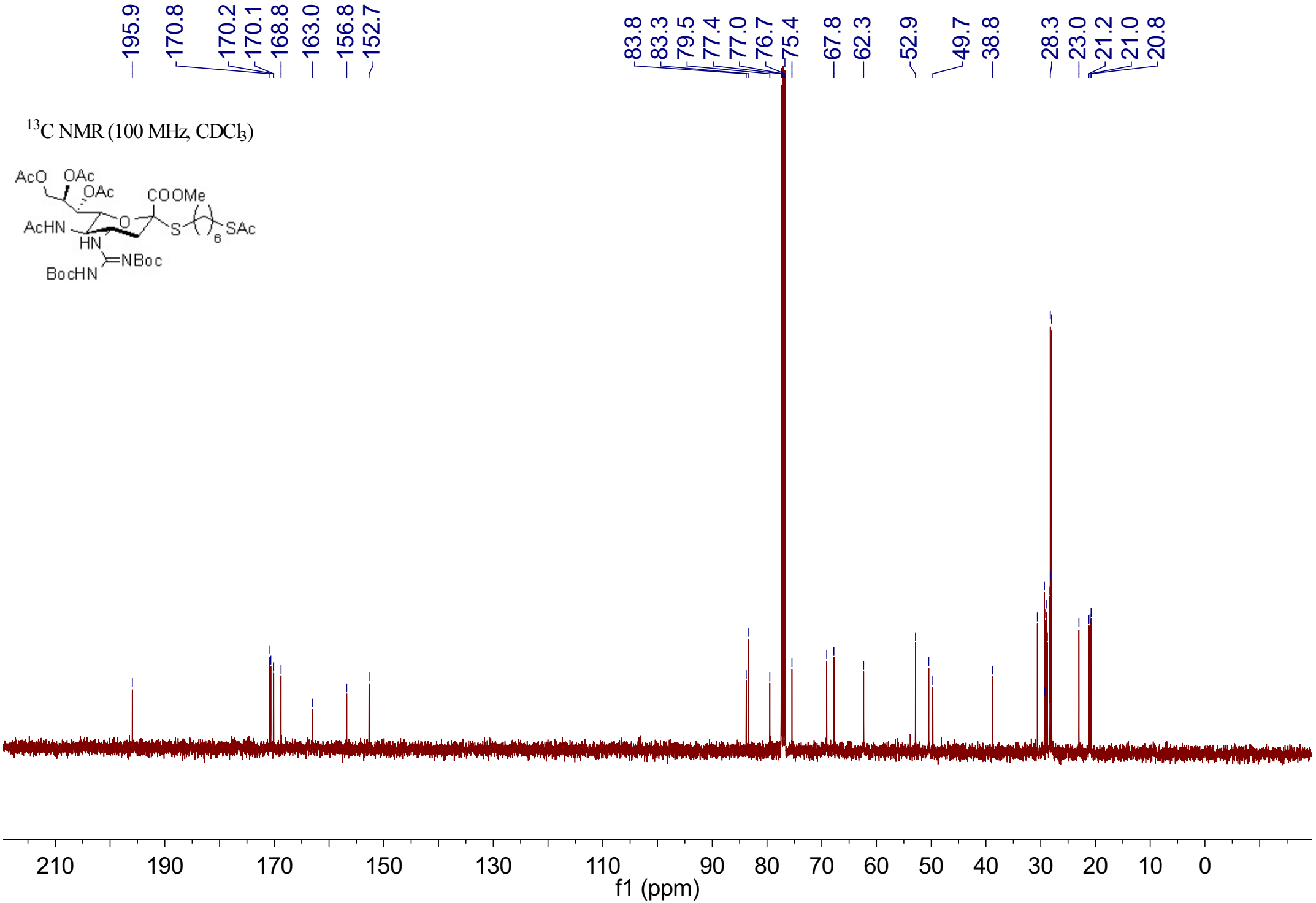
^{13}C NMR (100 MHz, $CDCl_3$)



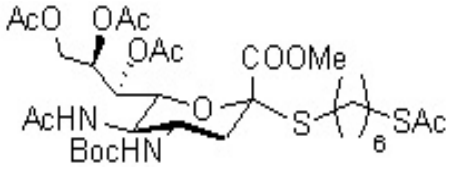


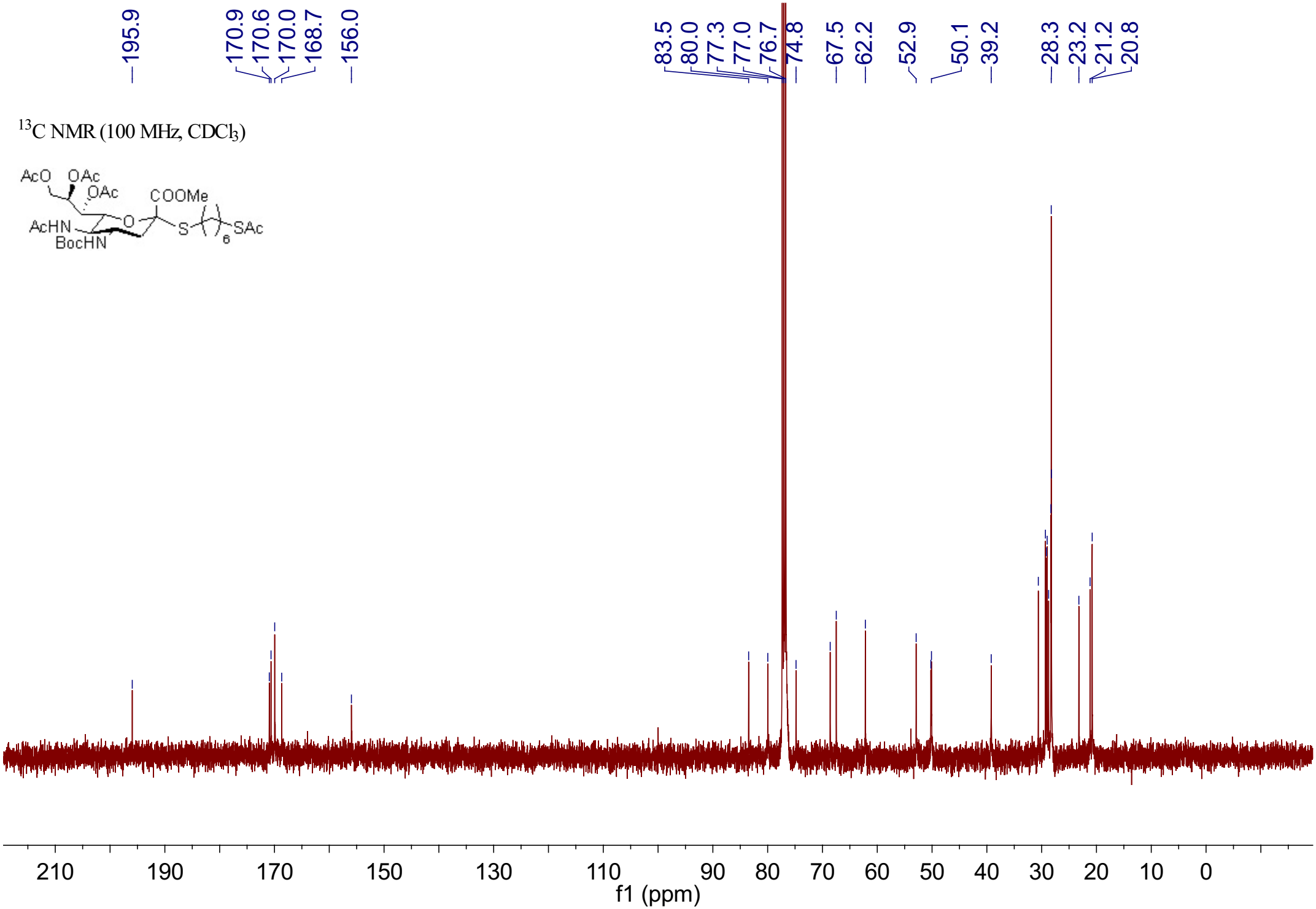
^{1}H NMR (400 MHz, $CDCl_3$)

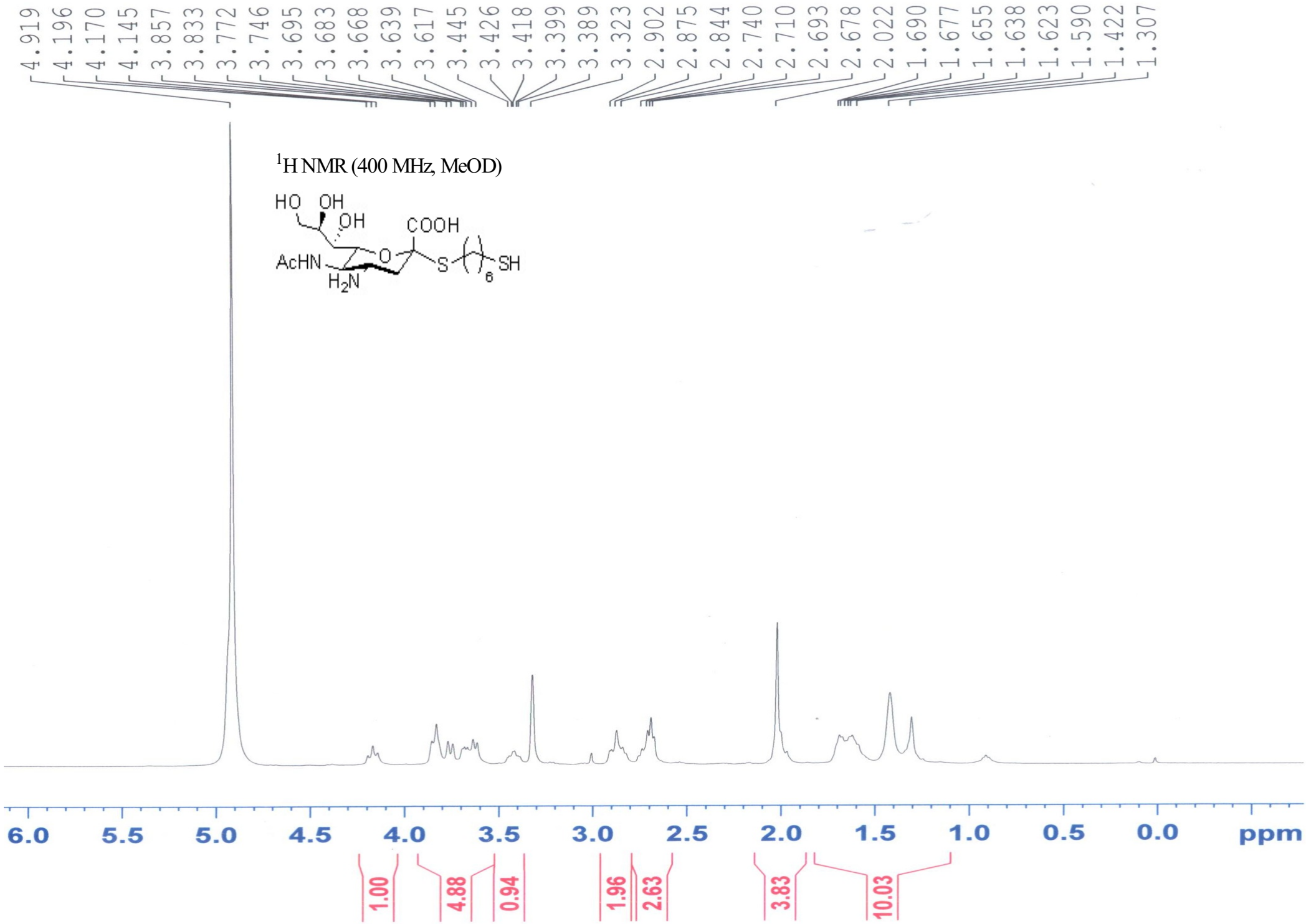



195.9
170.8
170.2
170.1
168.8
163.0
156.8
152.7
83.8
83.3
79.5
77.4
77.0
76.7
75.4
67.8
62.3
52.9
49.7
38.8
28.3
23.0
21.2
21.0
20.8
^{13}C NMR (100 MHz, $CDCl_3$)
AcO
OAc
OAc
COOMe
AcHN
O
S
6
SAc
HN
NBoc
BocHN
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

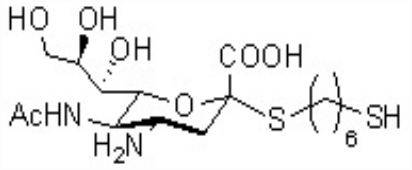
^{1}H NMR (400 MHz, $CDCl_3$)



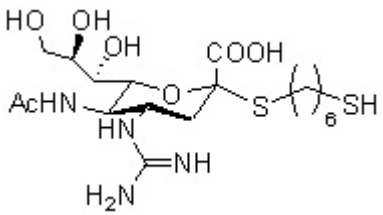

195.9
170.9
170.6
170.0
168.7
156.0
83.5
80.0
77.3
77.0
76.7
74.8
67.5
62.2
52.9
50.1
39.2
28.3
23.2
21.2
20.8
13C NMR (100 MHz, CDCl3)
AcO
OAc
OAc
COOMe
AcHN
BocHN
O
S
6
SAc
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


4.919
4.196
4.170
4.145
3.857
3.833
3.772
3.746
3.695
3.683
3.668
3.639
3.617
3.445
3.426
3.418
3.399
3.389
3.323
2.902
2.875
2.844
2.740
2.710
2.693
2.678
2.022
1.690
1.677
1.655
1.638
1.623
1.590
1.422
1.307
1H NMR (400 MHz, MeOD)
HO
OH
OH
COOH
AcHN
O
S
SH
6
H2N
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
1.00
4.88
0.94
1.96
2.63
3.83
10.03

^{13}C NMR (100 MHz, MeOD)

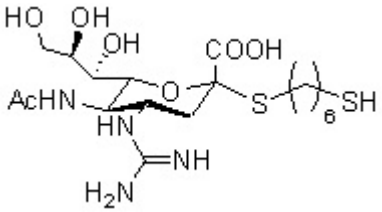


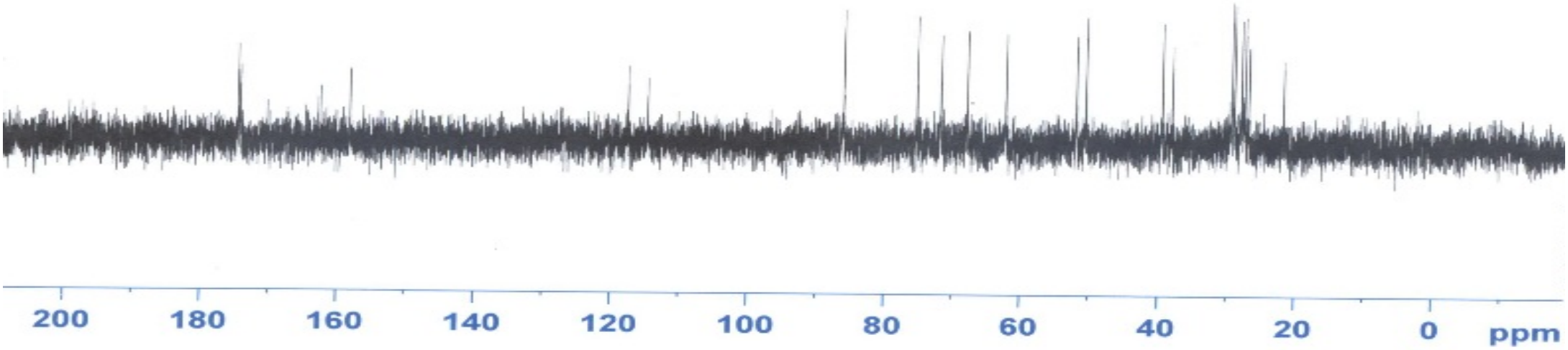
^{1}H NMR (400 MHz, D_2O)



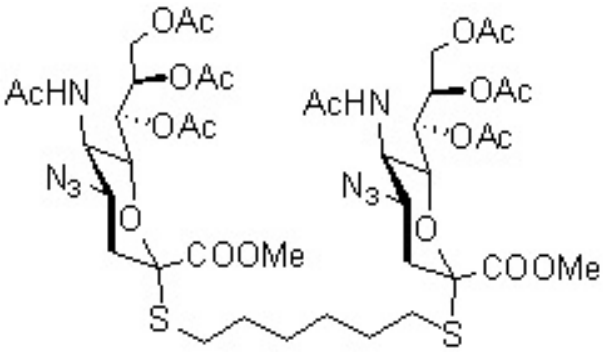
174.83
174.49
162.73
158.44
117.80
114.90
86.35
75.56
72.05
68.21
62.51
52.19
50.81
39.74
38.28
29.59
29.20
28.20
27.66
27.09
22.02

^{13}C NMR (100 MHz, D_2O)





^{1}H NMR (400 MHz, $CDCl_3$)

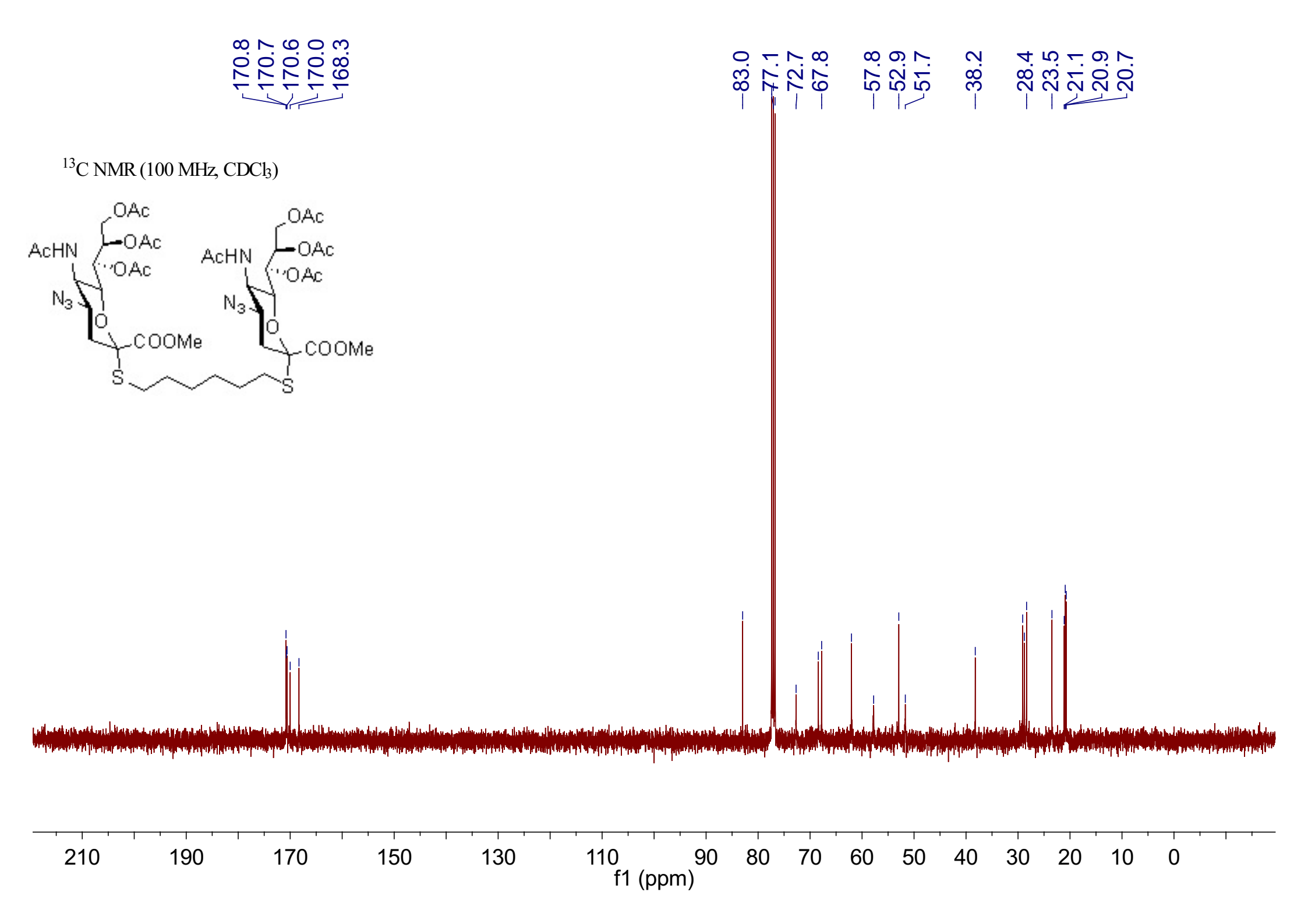


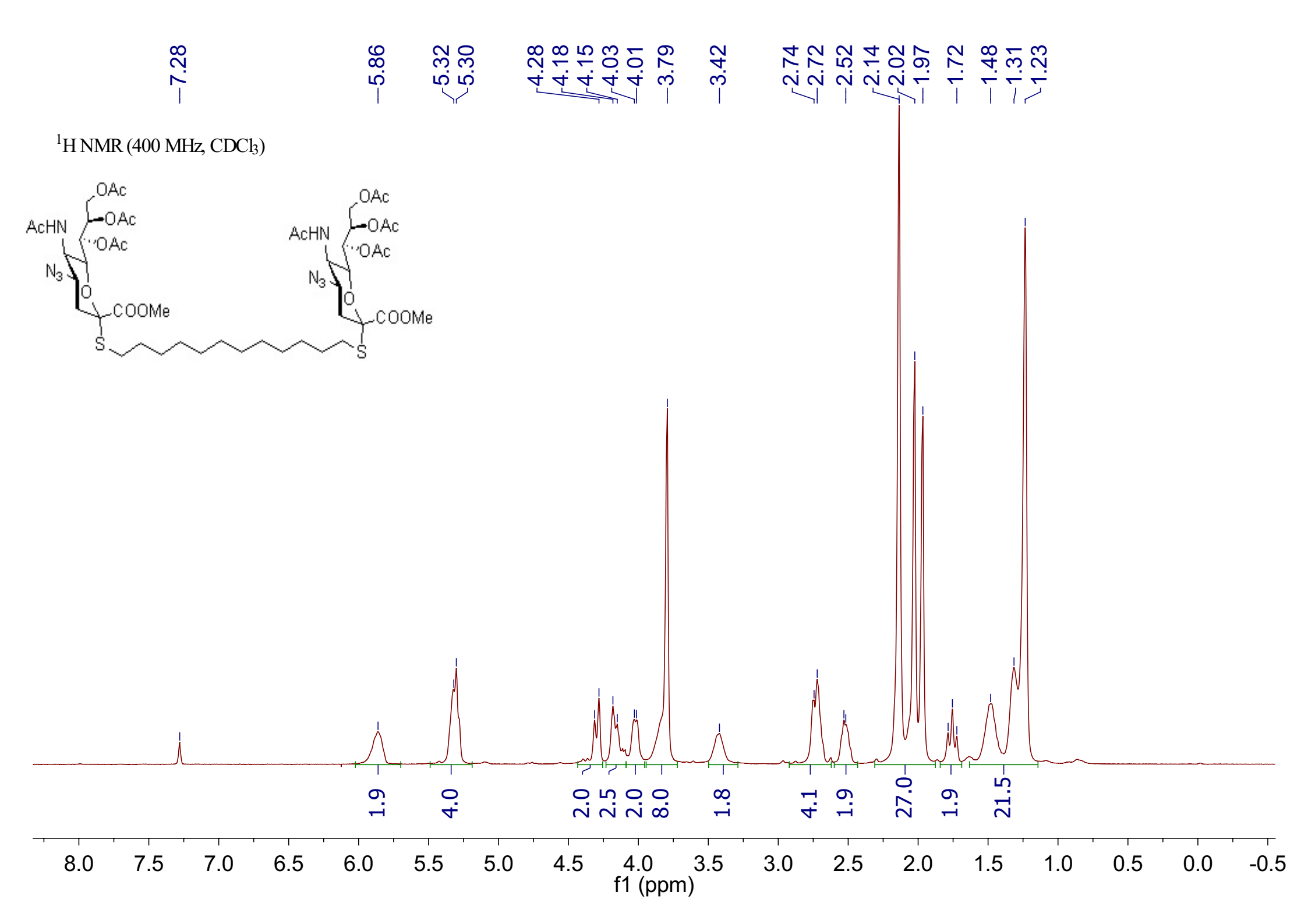
7.28
5.75
5.73
5.33
5.30
5.28
4.29
4.18
4.15
4.04
3.80
3.36
3.34
2.73
2.54
2.49
1.98
1.71
1.51
1.36
1.34
1.25

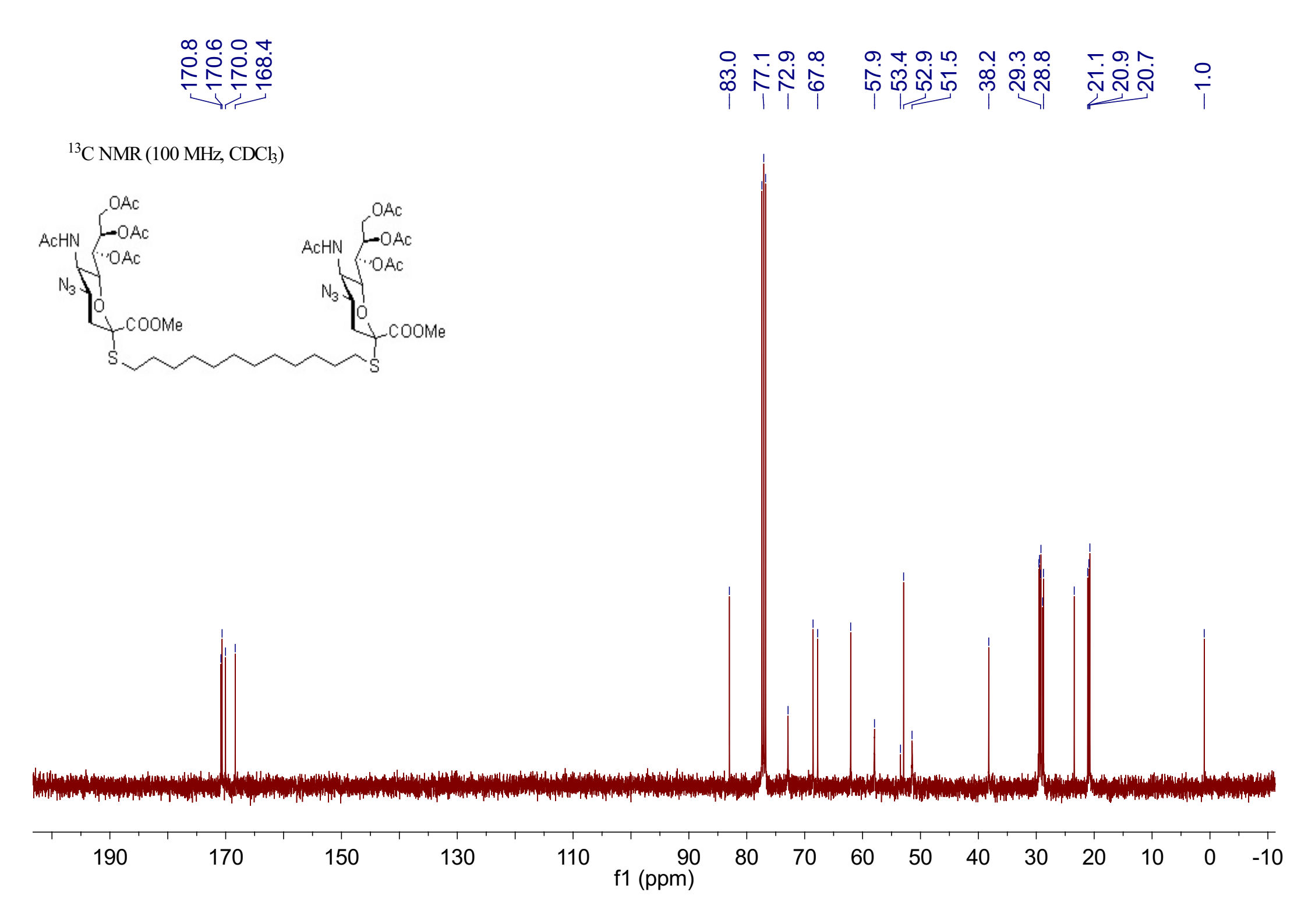
1.6 3.7 2.1 2.2 1.8 2.1 5.7 2.0 4.7 2.4 26.5 2.2 8.9

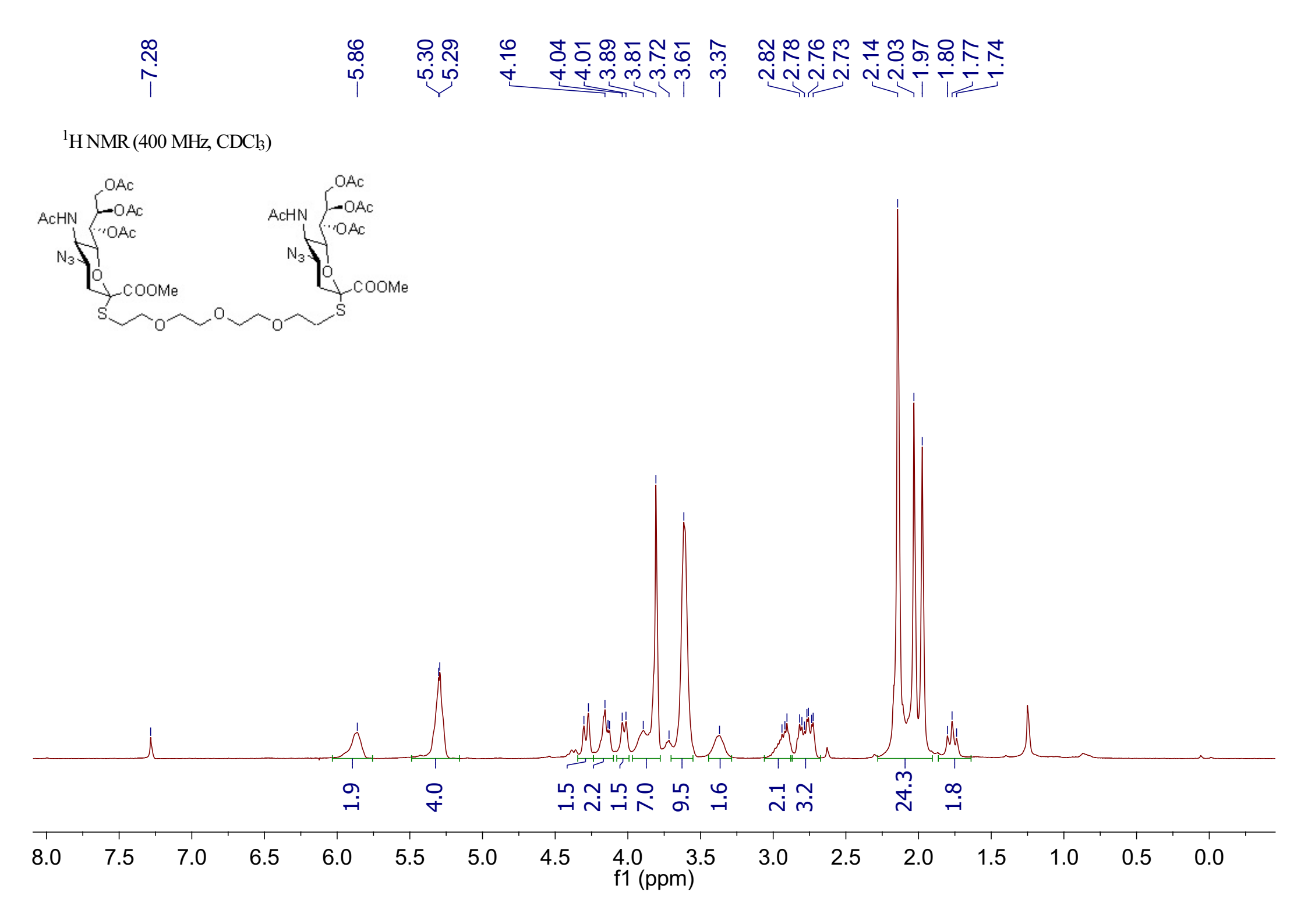
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5

f1 (ppm)

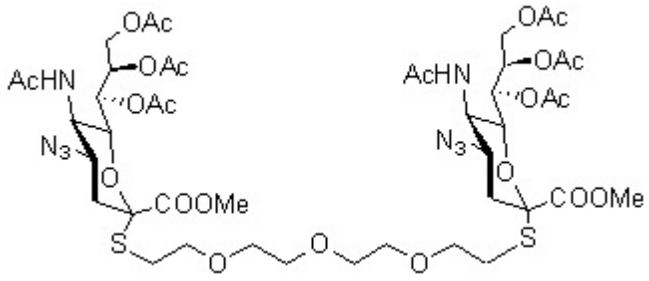

170.8
170.7
170.6
170.0
168.3
83.0
77.1
72.7
67.8
57.8
52.9
51.7
38.2
28.4
23.5
21.1
20.9
20.7
13C NMR (100 MHz, CDCl3)
OAc
OAc
AcHN
OAc
N3
O
COOMe
S
OAc
OAc
AcHN
OAc
N3
O
COOMe
S
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.28
5.86
5.32
5.30
4.28
4.18
4.15
4.03
4.01
3.79
3.42
2.74
2.72
2.52
2.14
2.02
1.97
1.72
1.48
1.31
1.23
^{1}H NMR (400 MHz, $CDCl_3$)
OAc
OAc
AcHN
OAc
N_3
O
COOMe
S
1.9
4.0
2.0
2.5
2.0
8.0
1.8
4.1
1.9
27.0
1.9
21.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

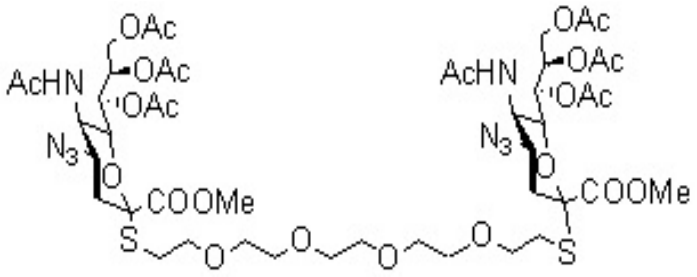

170.8
170.6
170.0
168.4
83.0
77.1
72.9
67.8
57.9
53.4
52.9
51.5
38.2
29.3
28.8
21.1
20.9
20.7
1.0
13C NMR (100 MHz, CDCl3)
OAc
OAc
AcHN
OAc
N3
O
COOMe
S
OAc
OAc
AcHN
OAc
N3
O
COOMe
S
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

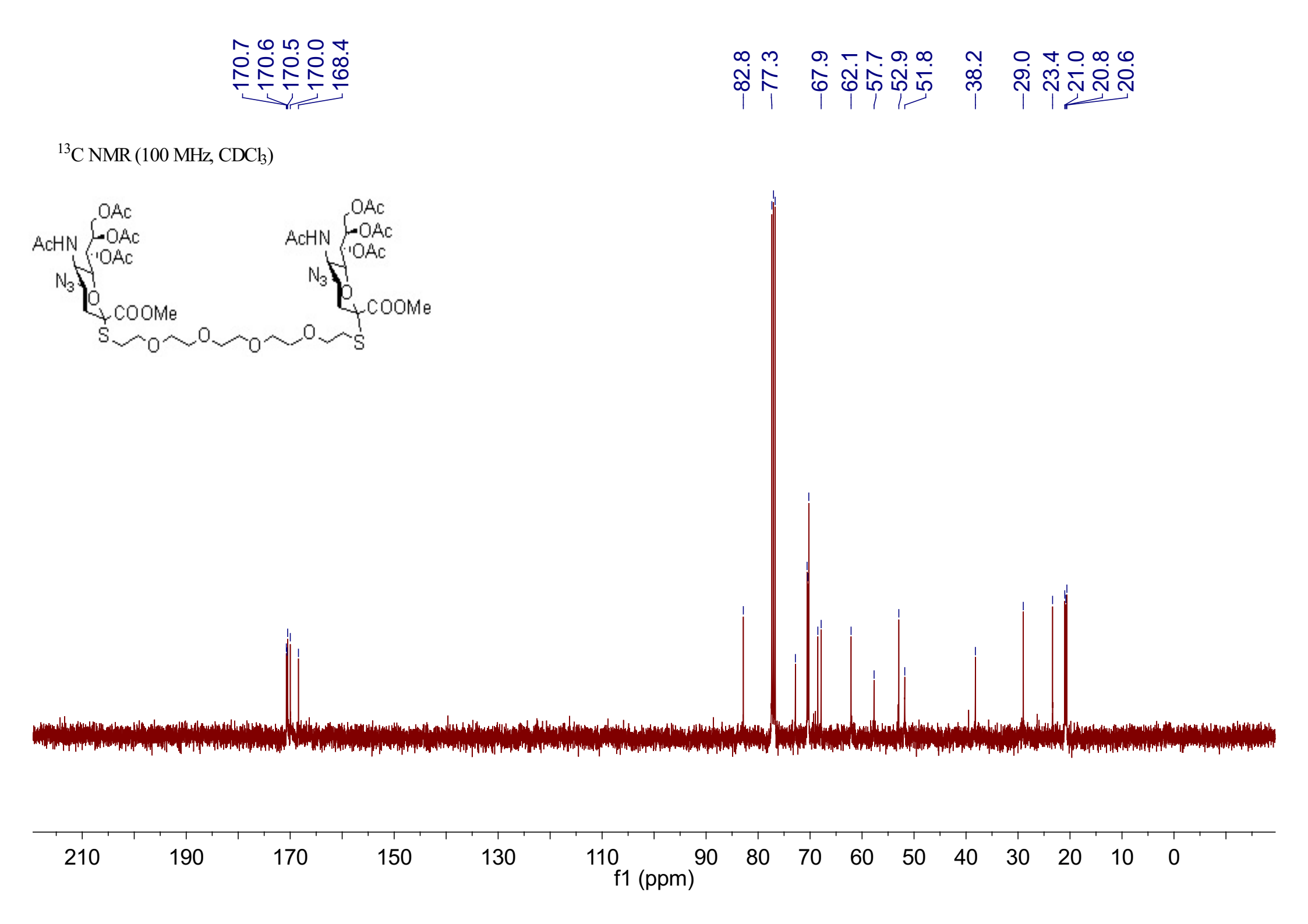

7.28
5.86
5.30
5.29
4.16
4.04
4.01
3.89
3.81
3.72
3.61
3.37
2.82
2.78
2.76
2.73
2.14
2.03
1.97
1.80
1.77
1.74
1H NMR (400 MHz, CDCl3)
OAc
OAc
AcHN
OAc
N3
O
COOMe
S
O
O
O
1.9
4.0
1.5
2.2
1.5
7.0
9.5
1.6
2.1
3.2
24.3
1.8
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

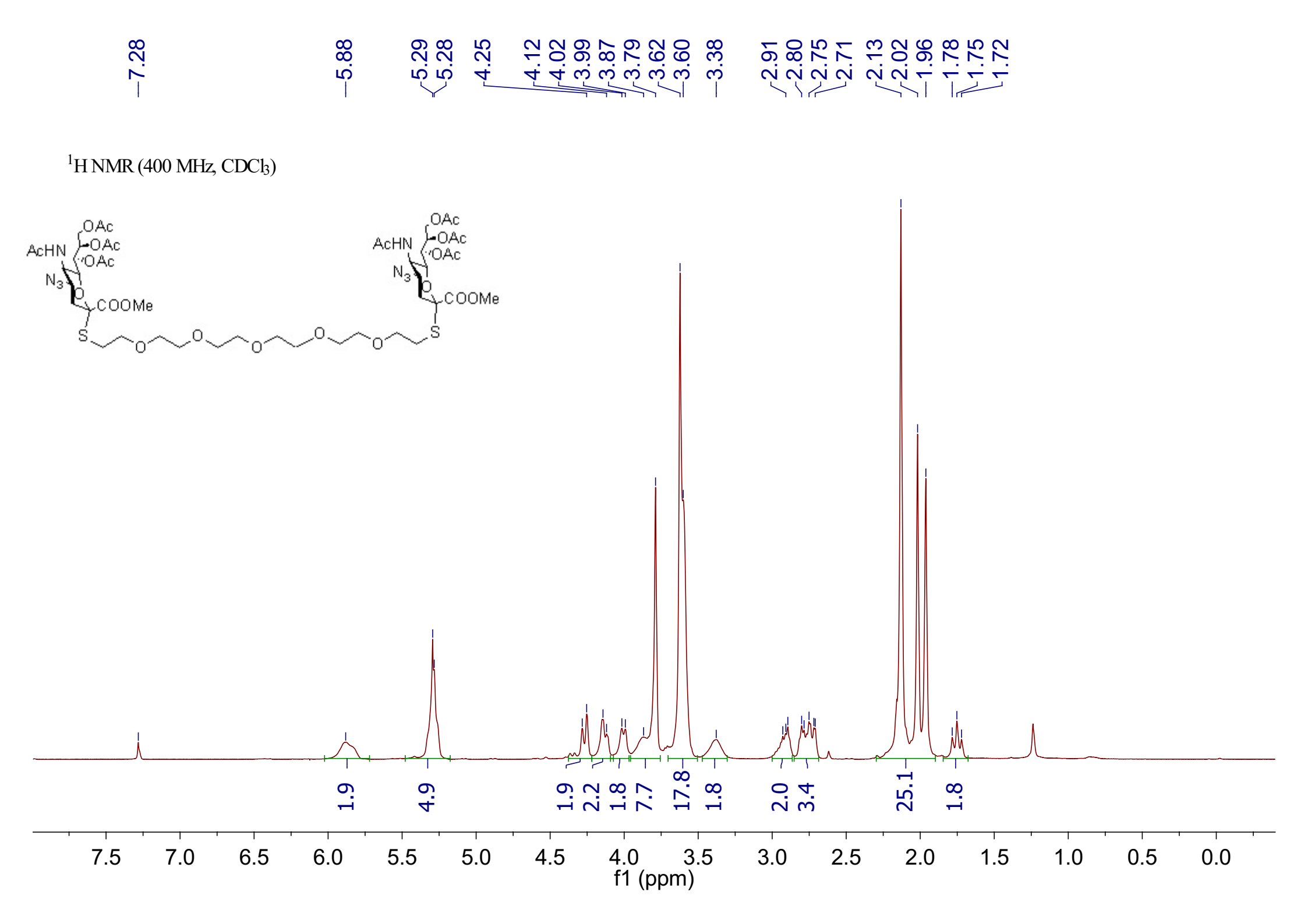
^{13}C NMR (100 MHz, $CDCl_3$)

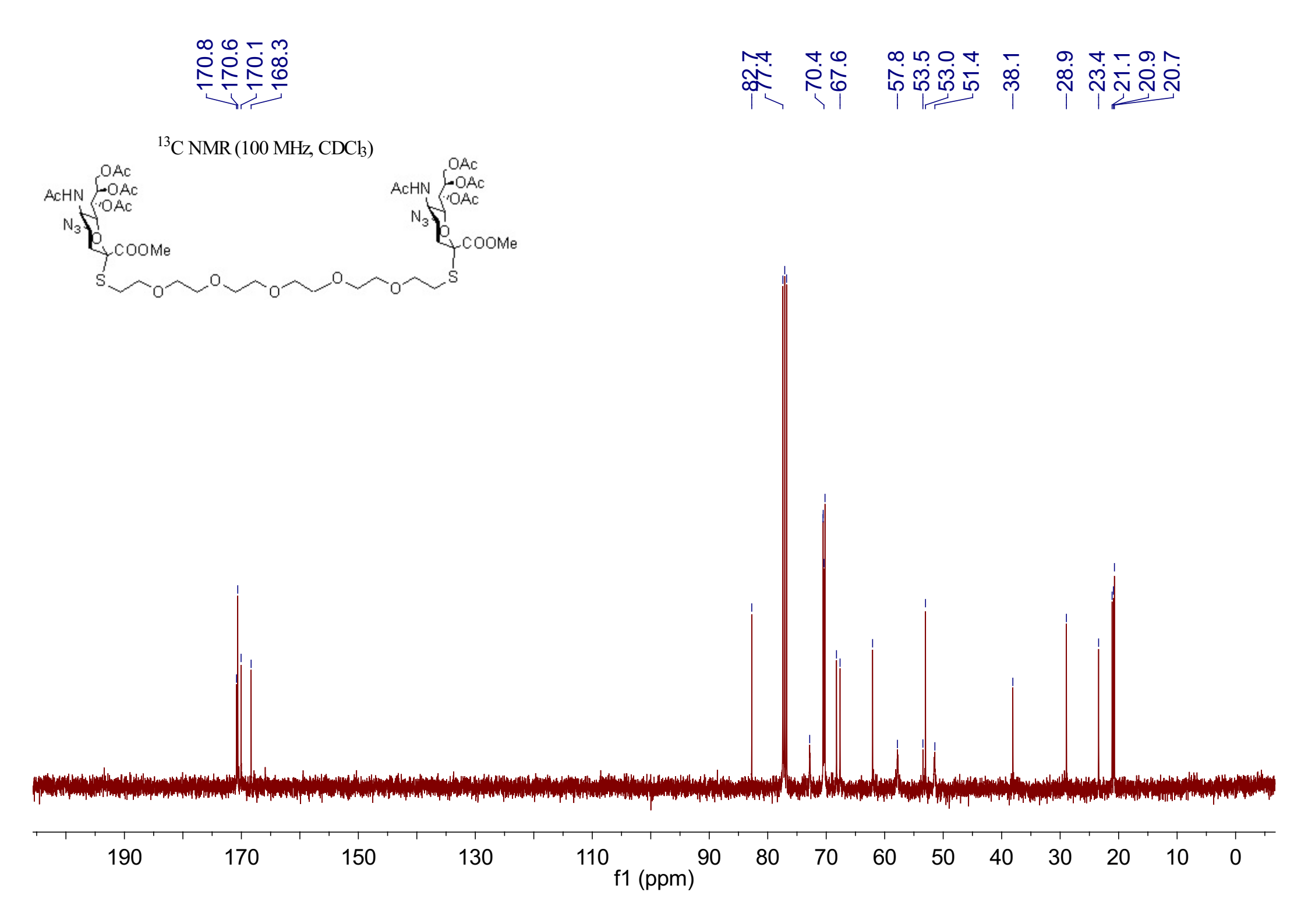


^{1}H NMR (400 MHz, $CDCl_3$)

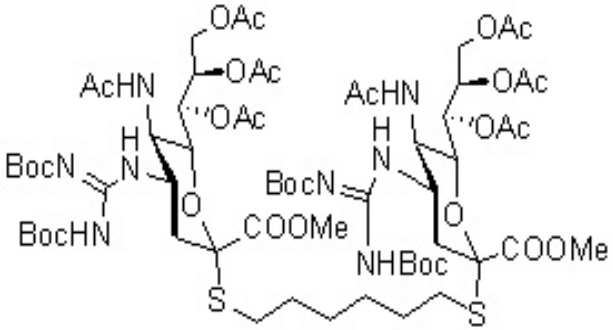


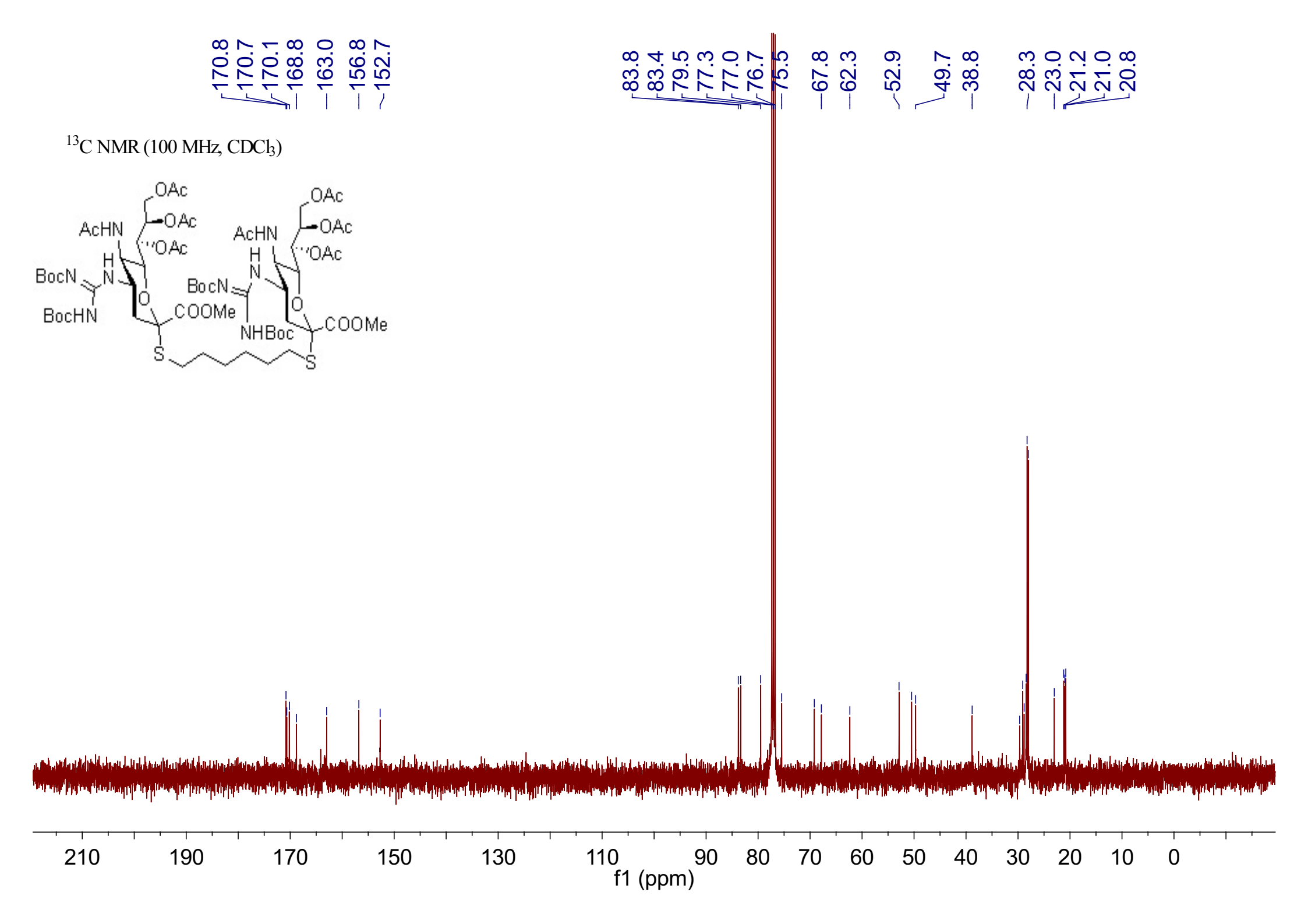

170.7
170.6
170.5
170.0
168.4
82.8
77.3
67.9
62.1
57.7
52.9
51.8
38.2
29.0
23.4
21.0
20.8
20.6
13C NMR (100 MHz, CDCl3)
OAc
OAc
OAc
AcHN
N3
O
COOMe
S
O
O
O
O
S
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.28
5.88
5.29
5.28
4.25
4.12
4.02
3.99
3.87
3.79
3.62
3.60
3.38
2.91
2.80
2.75
2.71
2.13
2.02
1.96
1.78
1.75
1.72
1H NMR (400 MHz, CDCl3)
OAc
OAc
AcHN
OAc
N3
O
COOMe
S
O
O
O
O
O
O
S
1.9
4.9
1.9
2.2
1.8
7.7
17.8
1.8
2.0
3.4
25.1
1.8
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

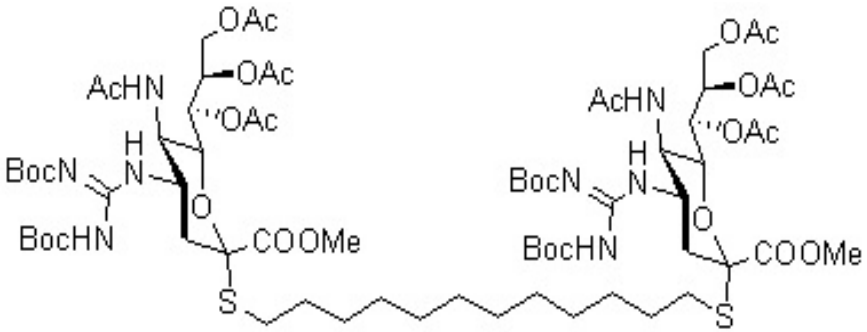

170.8
170.6
170.1
168.3
82.7
77.4
70.4
67.6
57.8
53.5
53.0
51.4
38.1
28.9
23.4
21.1
20.9
20.7
^{13}C NMR (100 MHz, CDCl$_3$)
OAc
OAc
OAc
AcHN
N$_3$
O
COOMe
S
O
O
O
O
O
O
S
OAc
OAc
OAc
AcHN
N$_3$
O
COOMe
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

^{1}H NMR (400 MHz, $CDCl_3$)




170.8
170.7
170.1
168.8
163.0
156.8
152.7
83.8
83.4
79.5
77.3
77.0
76.7
75.5
67.8
62.3
52.9
49.7
38.8
28.3
23.0
21.2
21.0
20.8
^{13}C NMR (100 MHz, $CDCl_3$)
OAc
OAc
AcHN
OAc
H
N
BocN
BocHN
O
COOMe
S
OAc
OAc
AcHN
OAc
H
N
BocN
NHBoc
O
COOMe
S
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

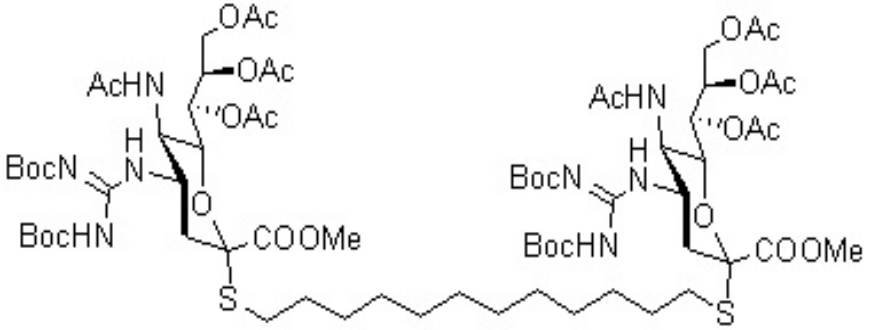
^{1}H NMR (400 MHz, $CDCl_3$)



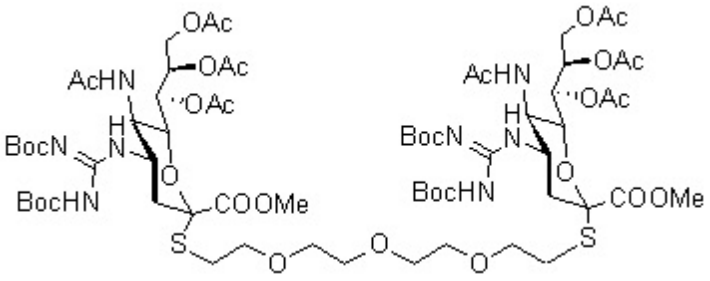
170.8
170.6
170.2
168.8
163.0
156.8
152.7

83.8
83.4
79.5
77.4
77.0
76.7
75.5
67.9
62.4
52.9
49.7
38.8
28.0
23.0
21.2
21.0
20.8

^{13}C NMR (100 MHz, $CDCl_3$)



^{1}H NMR (400 MHz, $CDCl_3$)

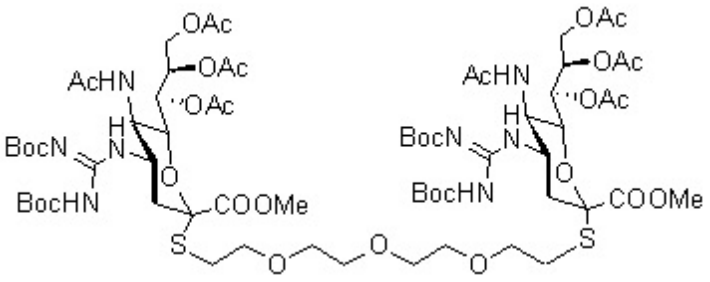


11.31
8.41
8.39
7.28
7.28
6.07
6.05
5.30
3.84
3.63
2.80
2.18
2.18
2.17
2.15
2.15
2.04
1.88
1.84
1.49

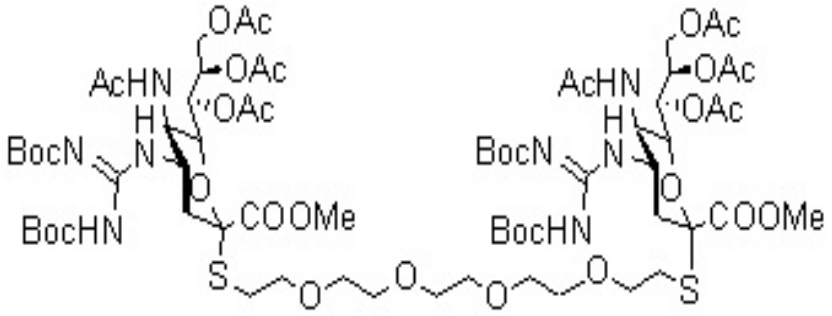
2.0
2.1
2.0
4.0
2.2
5.6
17.4
5.6
33.1
34.6

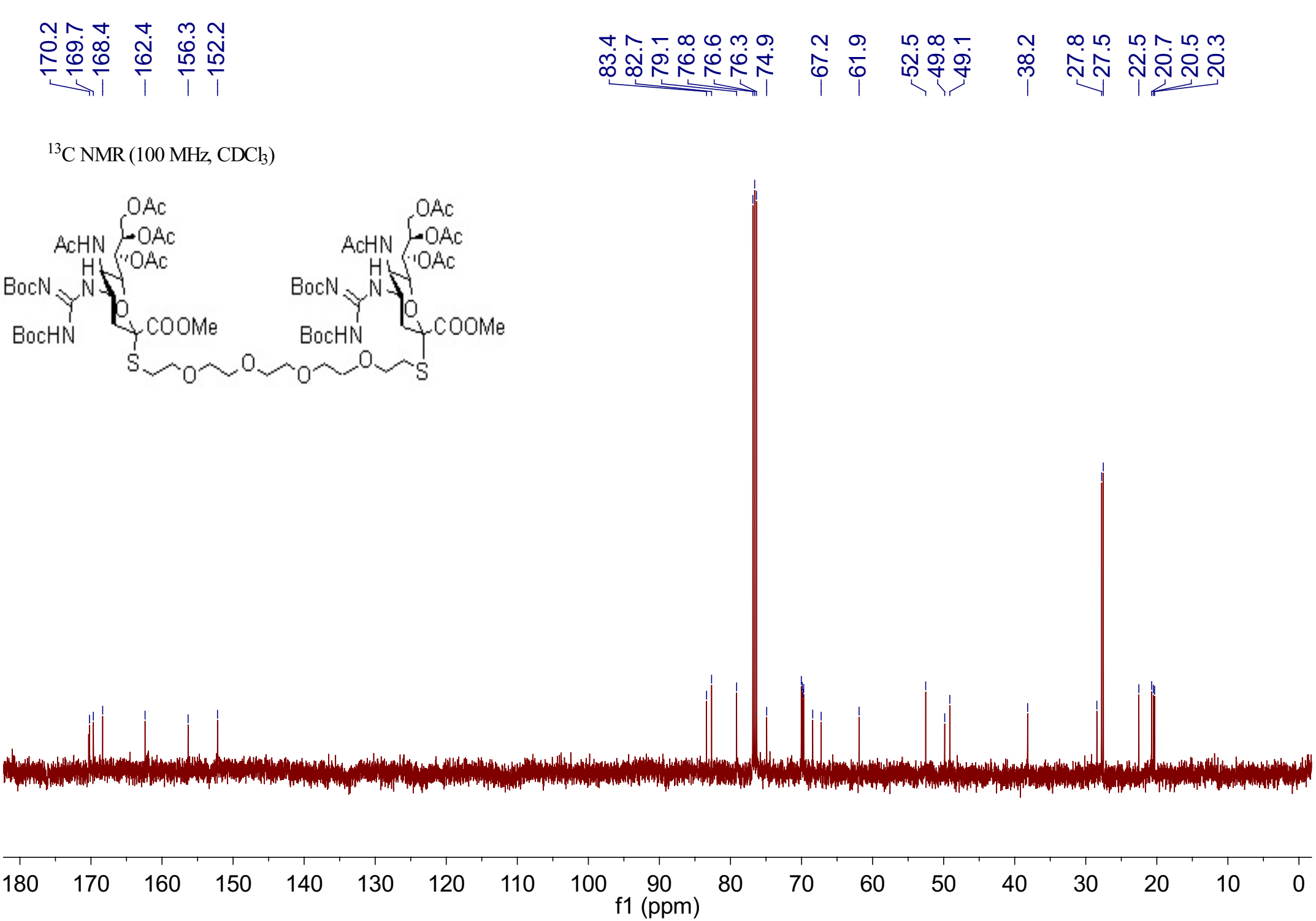
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3
f1 (ppm)

^{13}C NMR (100 MHz, $CDCl_3$)

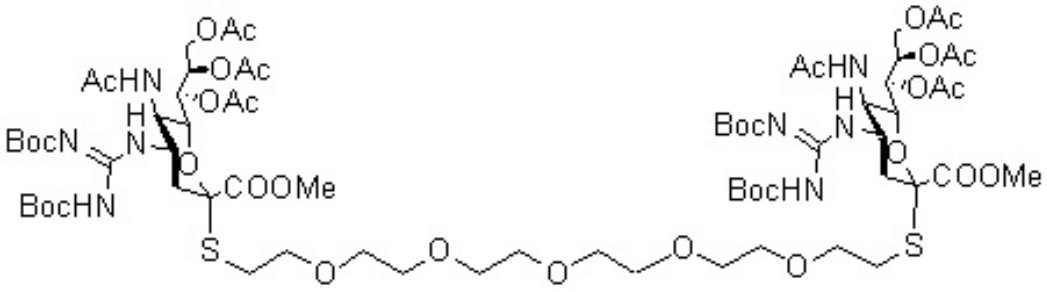


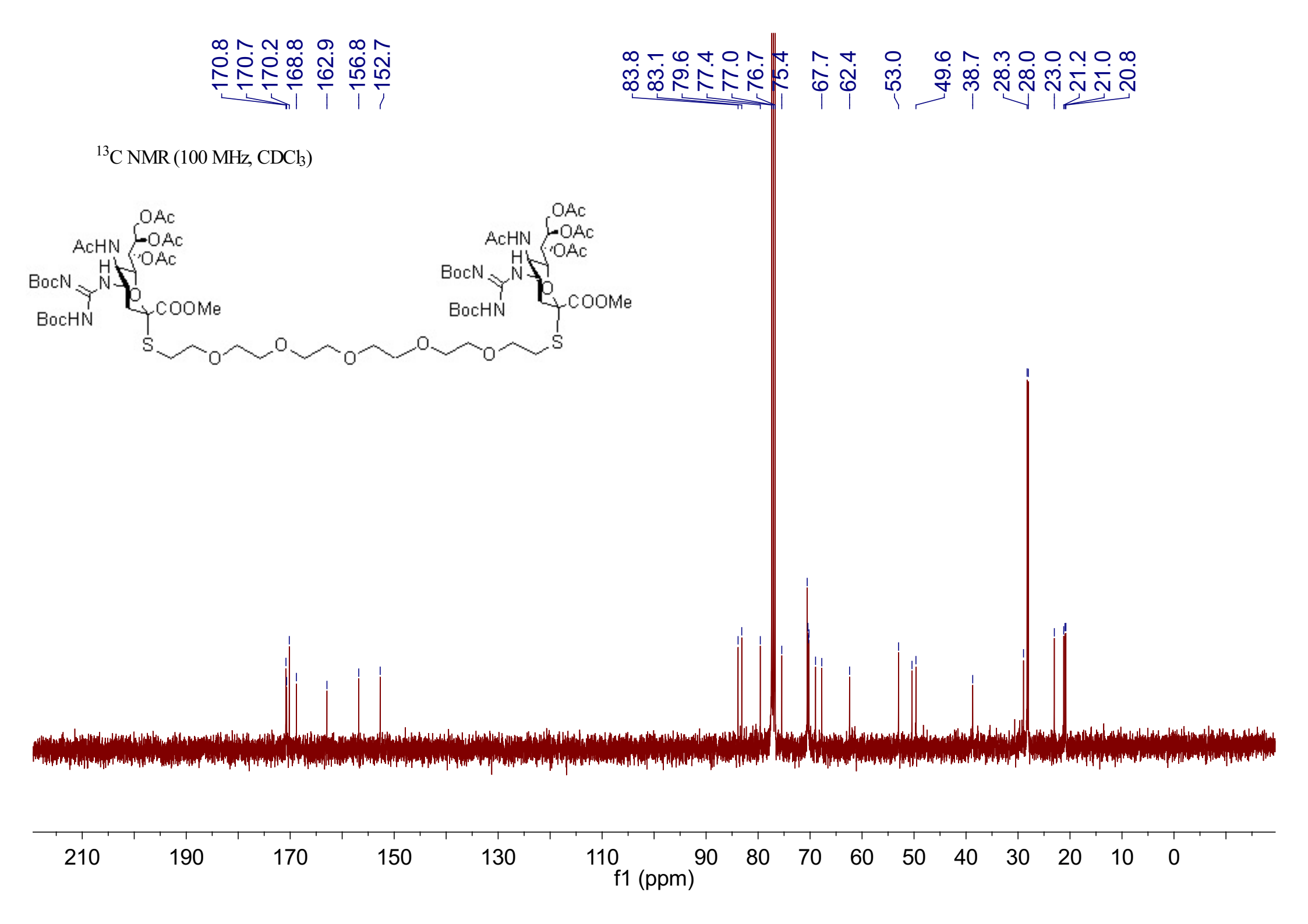
^{1}H NMR (400 MHz, $CDCl_3$)

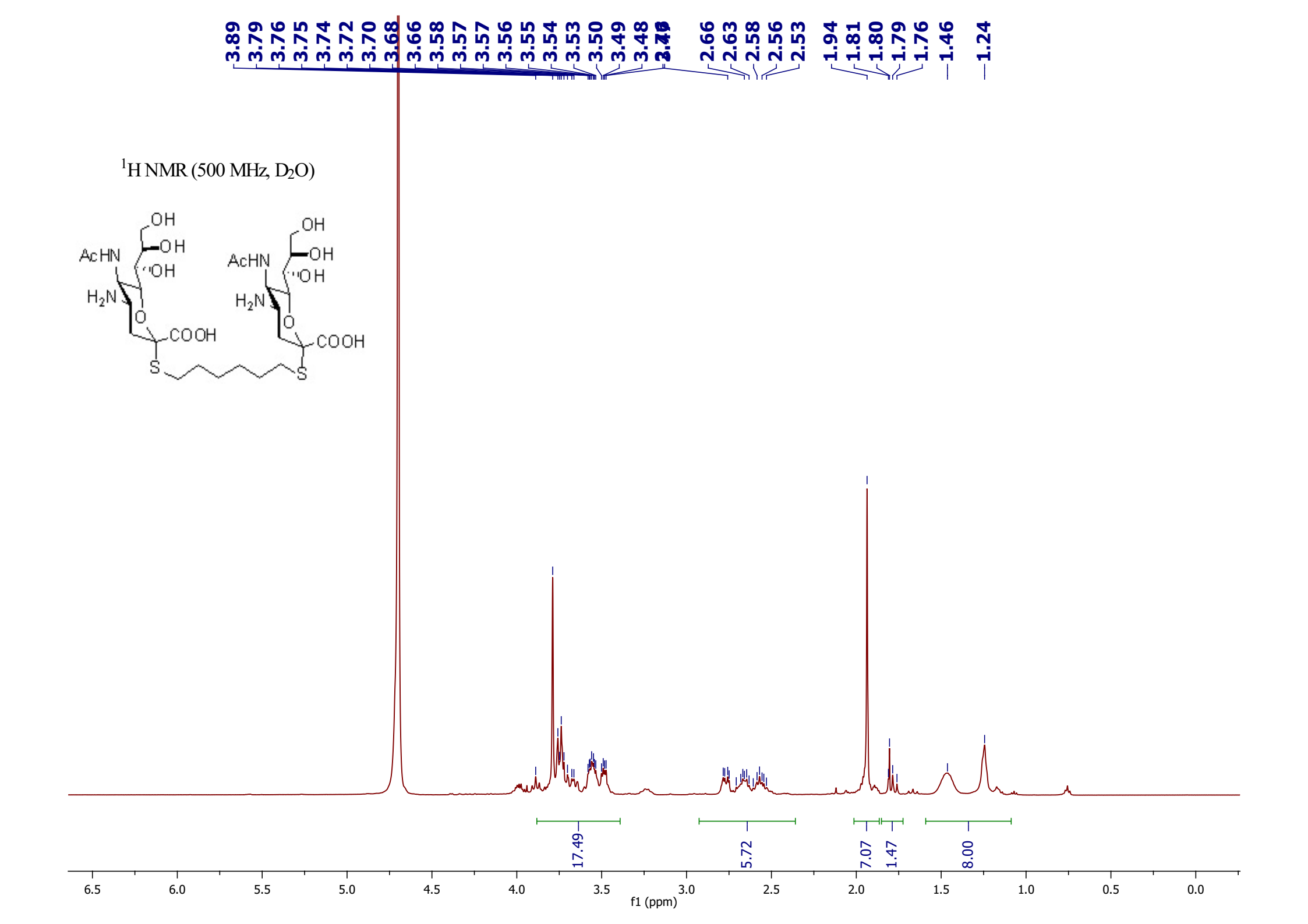



170.2
169.7
168.4
162.4
156.3
152.2
83.4
82.7
79.1
76.8
76.6
76.3
74.9
67.2
61.9
52.5
49.8
49.1
38.2
27.8
27.5
22.5
20.7
20.5
20.3
^{13}C NMR (100 MHz, $CDCl_3$)
OAc
AcHN
OAc
OAc
H
BocN
N
O
COOMe
BocHN
S
O
O
O
O
S
f1 (ppm)
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

^{1}H NMR (400 MHz, $CDCl_3$)



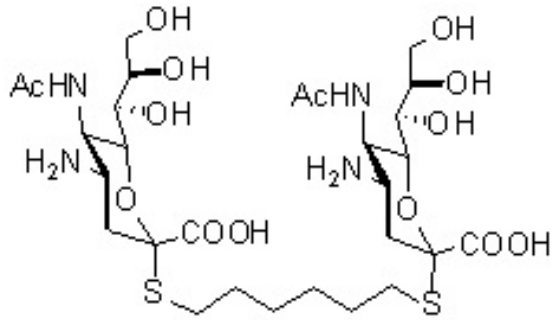

170.8
170.7
170.2
168.8
162.9
156.8
152.7
83.8
83.1
79.6
77.4
77.0
76.7
75.4
67.7
62.4
53.0
49.6
38.7
28.3
28.0
23.0
21.2
21.0
20.8
^{13}C NMR (100 MHz, CDCl$_3$)
OAc
AcHN
OAc
OAc
H
BocN
N
O
COOMe
BocHN
S
O
O
O
O
O
S
f1 (ppm)
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0

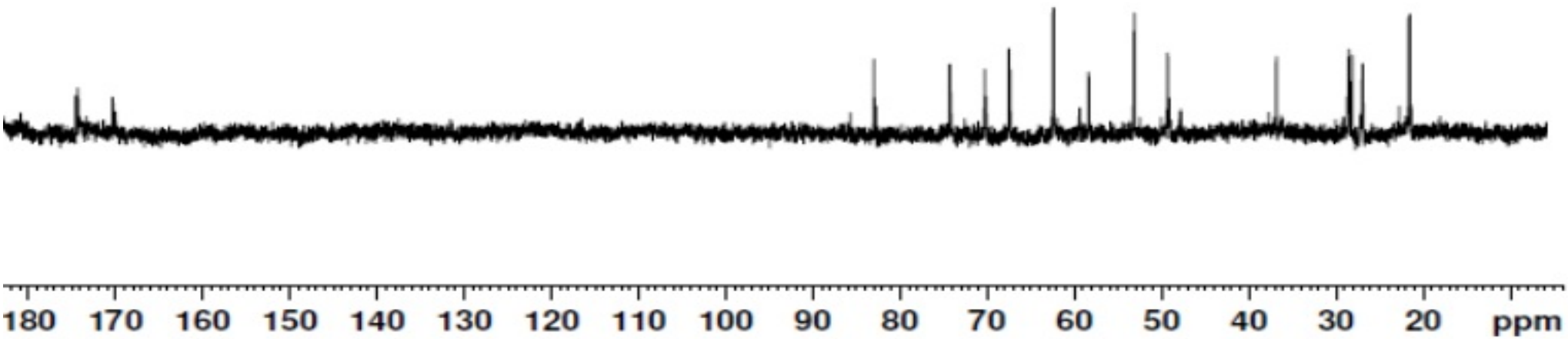

3.89
3.79
3.76
3.75
3.74
3.72
3.70
3.68
3.66
3.58
3.57
3.57
3.56
3.55
3.54
3.53
3.50
3.49
3.48
2.66
2.63
2.58
2.56
2.53
1.94
1.81
1.80
1.79
1.76
1.46
1.24
1H NMR (500 MHz, D2O)
OH
OH
AcHN
OH
H2N
O
COOH
S
OH
OH
AcHN
OH
H2N
O
COOH
S
17.49
5.72
7.07
1.47
8.00
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

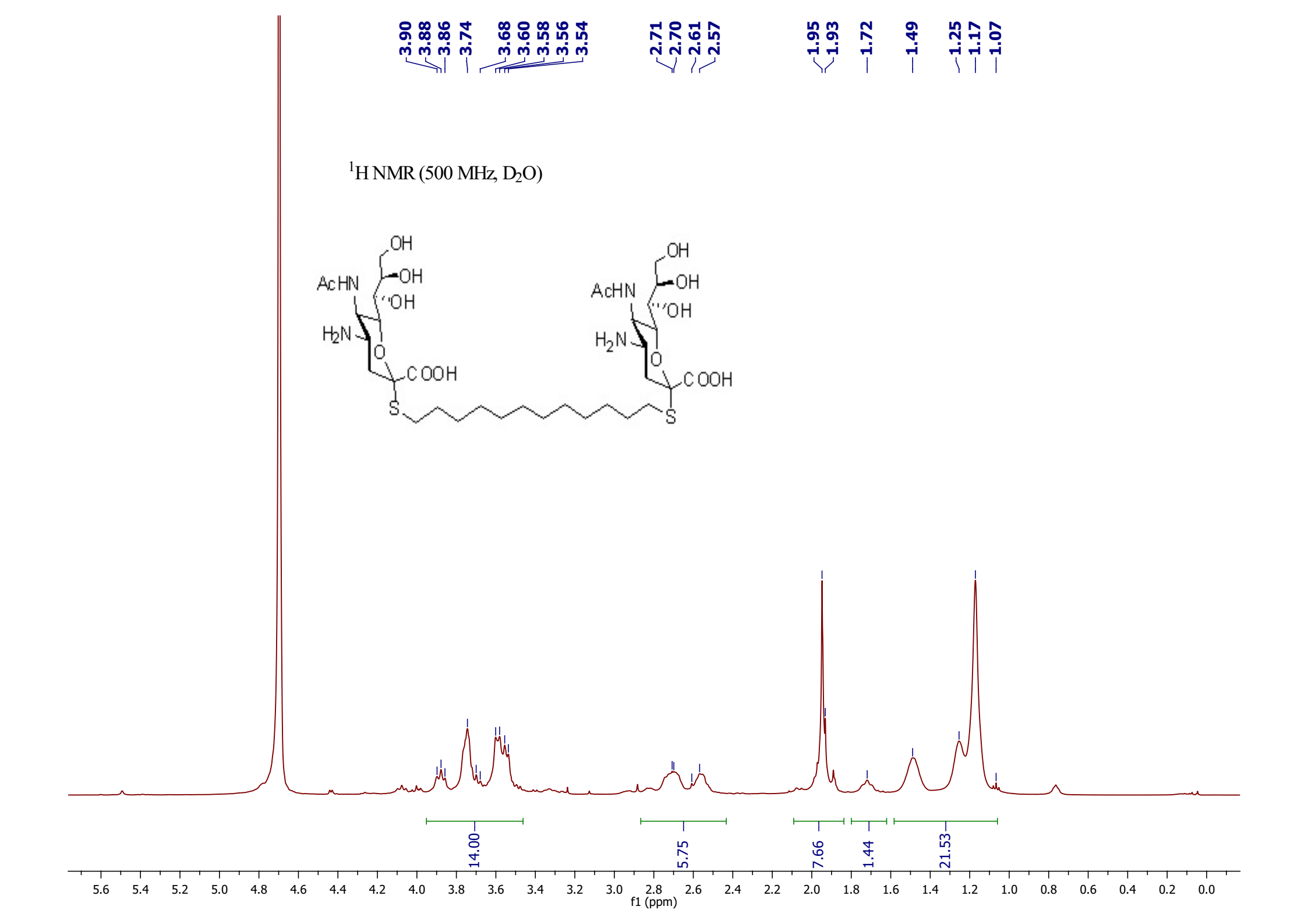
174.57
174.33
170.32

^{13}C NMR (125 MHz, D_2O)

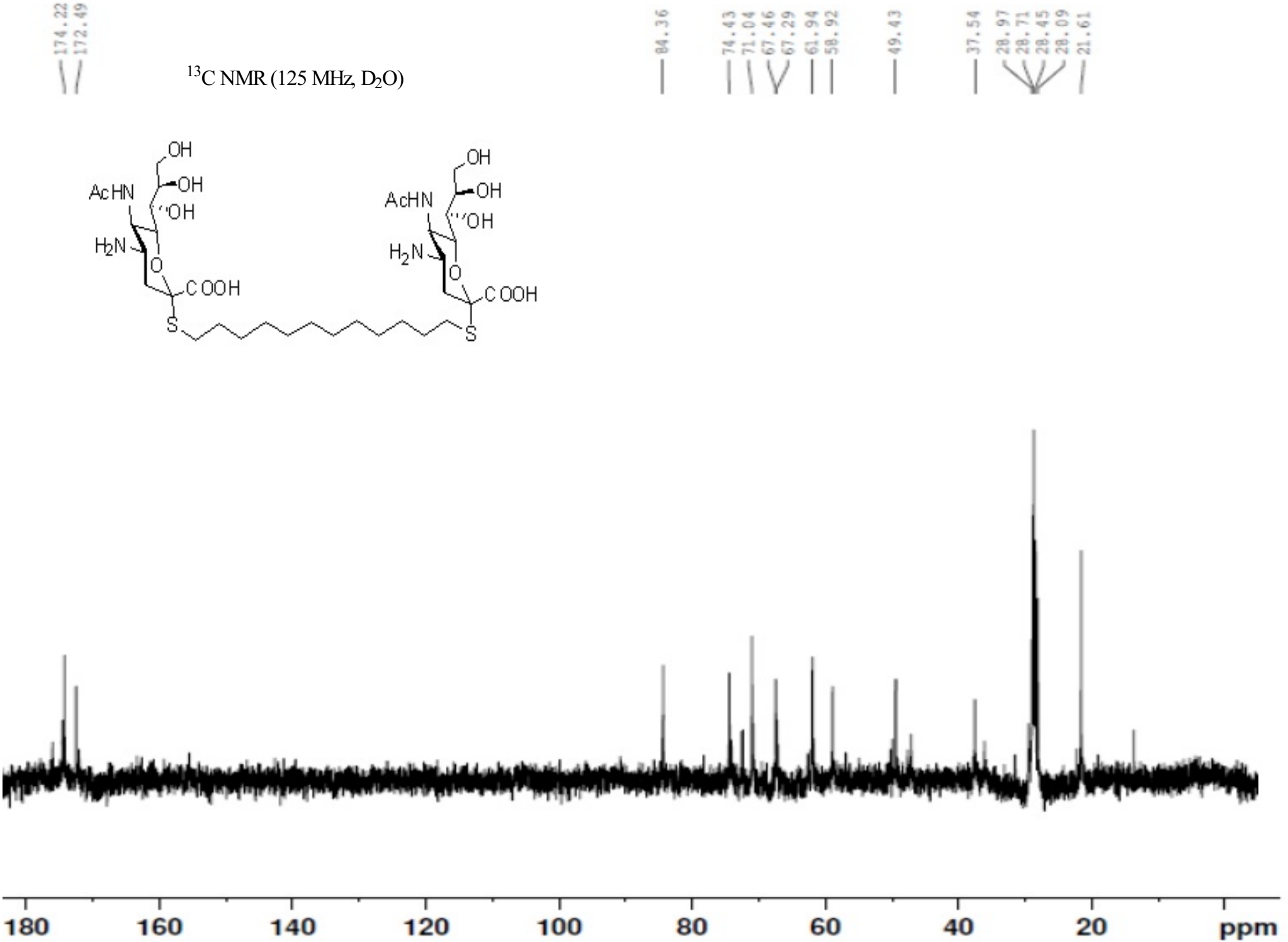
83.02
74.33
70.32
67.50
67.39
62.41
58.37
53.14
49.29
36.83
28.50
28.16
26.92
21.62
21.54

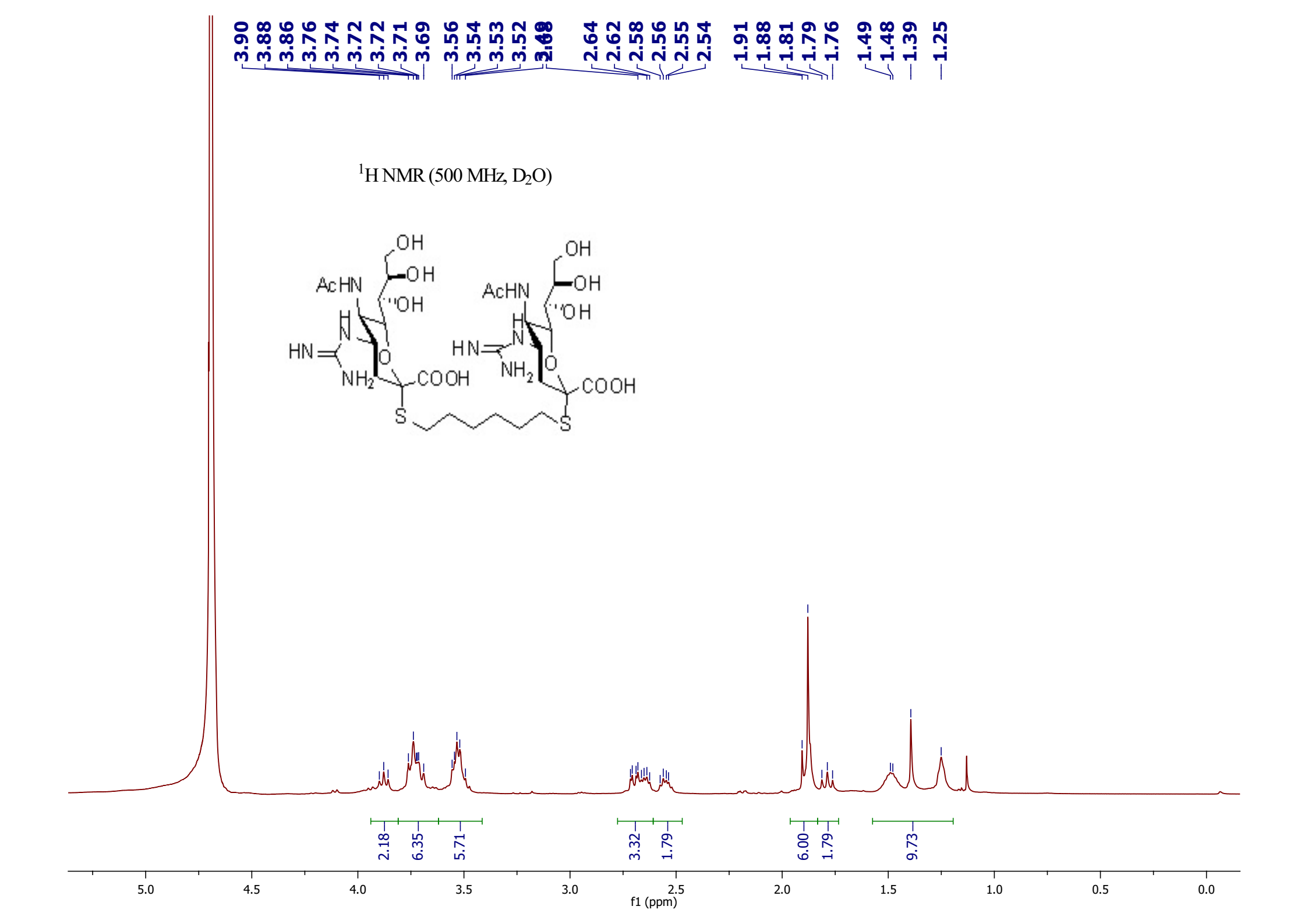





3.90
3.88
3.86
3.74
3.68
3.60
3.58
3.56
3.54
2.71
2.70
2.61
2.57
1.95
1.93
1.72
1.49
1.25
1.17
1.07
1H NMR (500 MHz, D2O)
OH
AcHN
OH
OH
H2N
O
COOH
S
14.00
5.75
7.66
1.44
21.53
f1 (ppm)

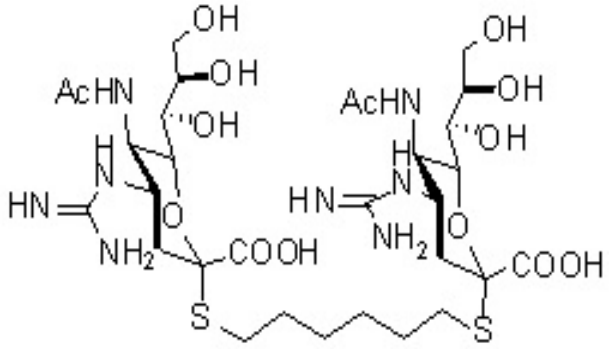
^{13}C NMR (125 MHz, D_2O)

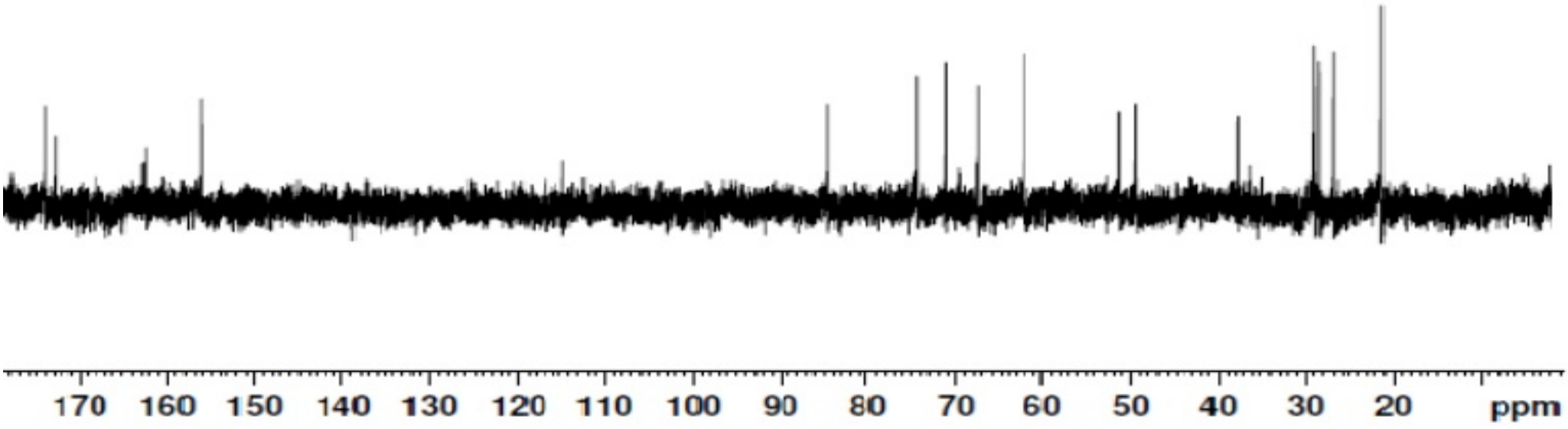


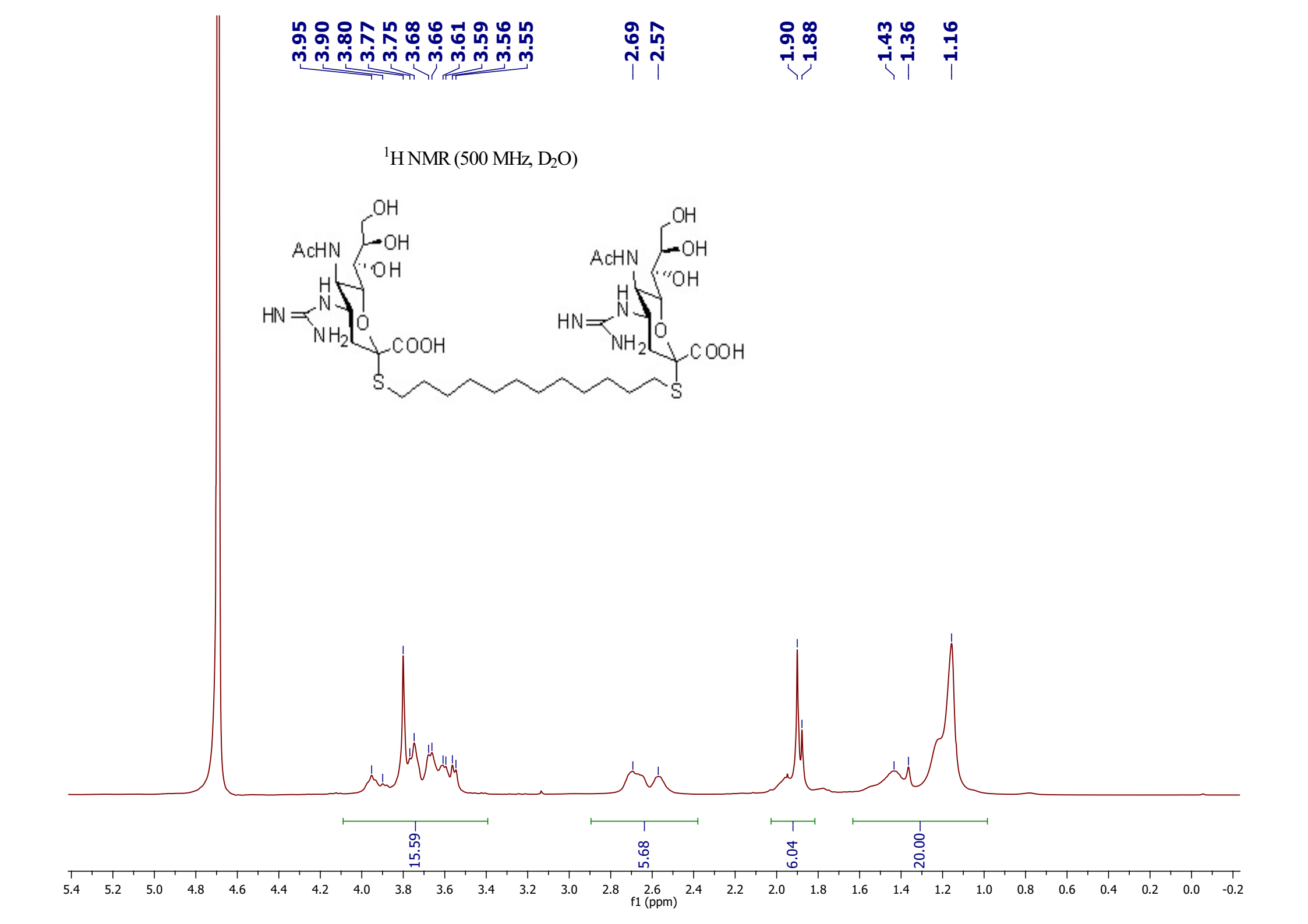

3.90
3.88
3.86
3.76
3.74
3.72
3.72
3.71
3.69
3.56
3.54
3.53
3.52
2.64
2.62
2.58
2.56
2.55
2.54
1.91
1.88
1.81
1.79
1.76
1.49
1.48
1.39
1.25
^{1}H NMR (500 MHz, D_2O)
OH
OH
AcHN
OH
HN
NH$_2$
COOH
S
2.18
6.35
5.71
3.32
1.79
6.00
1.79
9.73
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

174.04
172.81
162.36
156.17
84.70
74.33
71.19
67.46
62.03
51.40
49.46
37.84
29.08
28.98
28.49
27.05
21.35

^{13}C NMR (125 MHz, D_2O)

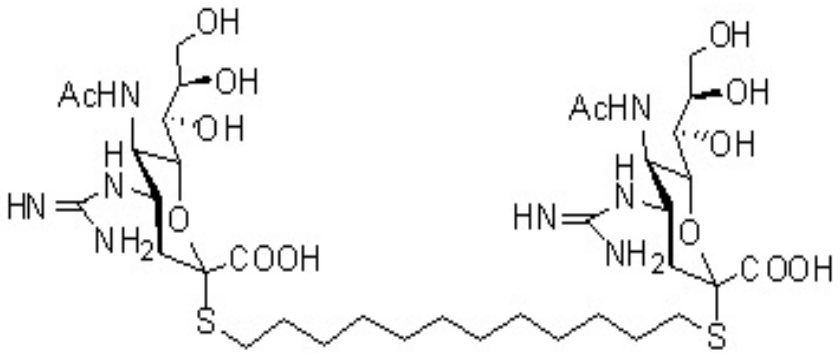


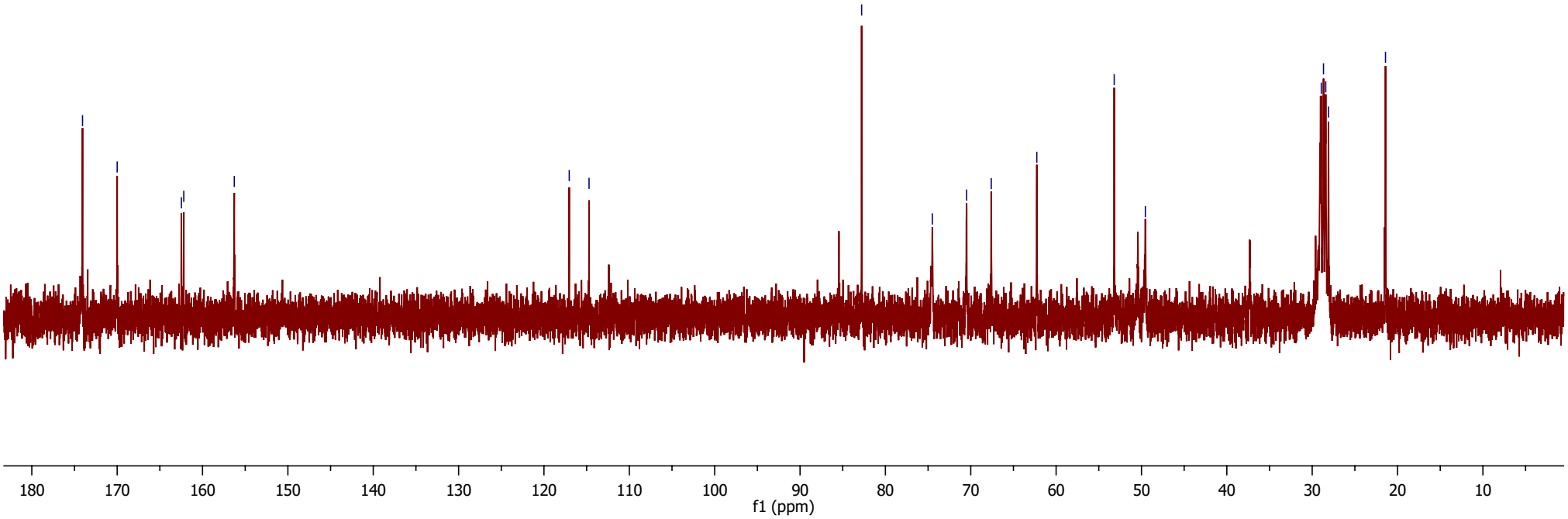


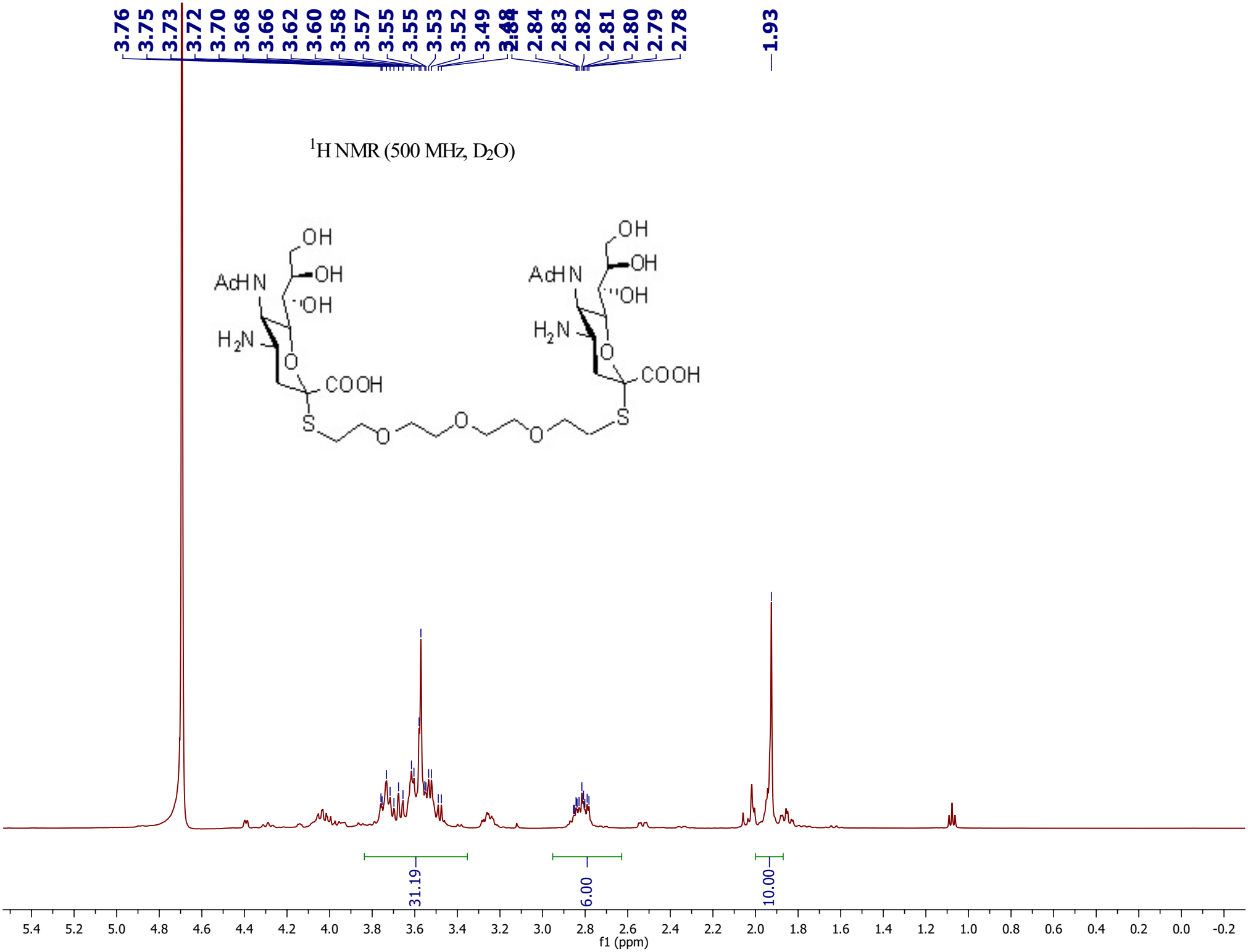

3.95
3.90
3.80
3.77
3.75
3.68
3.66
3.61
3.59
3.56
3.55
2.69
2.57
1.90
1.88
1.43
1.36
1.16
^{1}H NMR (500 MHz, D_2O)
OH
AcHN
OH
OH
H
N
HN
O
NH_2
COOH
S
15.59
5.68
6.04
20.00
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
f1 (ppm)

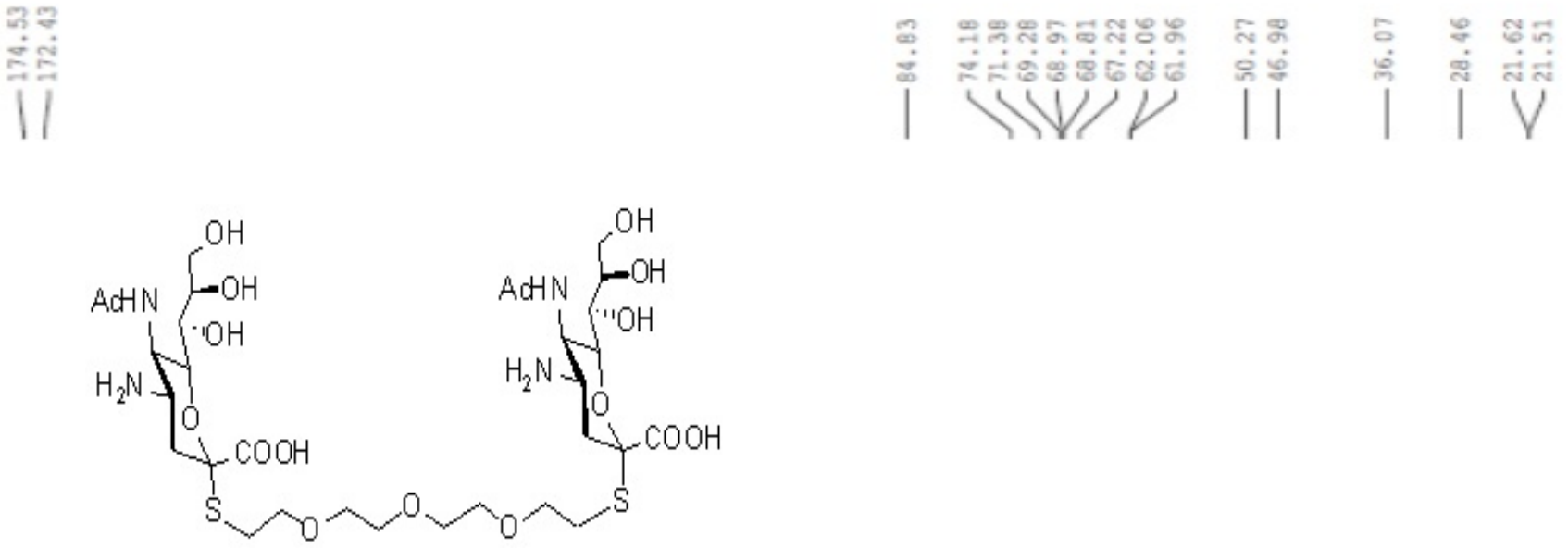
174.06
170.00
162.48
162.20
156.28
117.04
114.72
82.78
74.50
67.60
62.26
53.19
49.54
28.93
28.68
28.41
28.09
21.41

^{13}C NMR (125 MHz, D_2O)

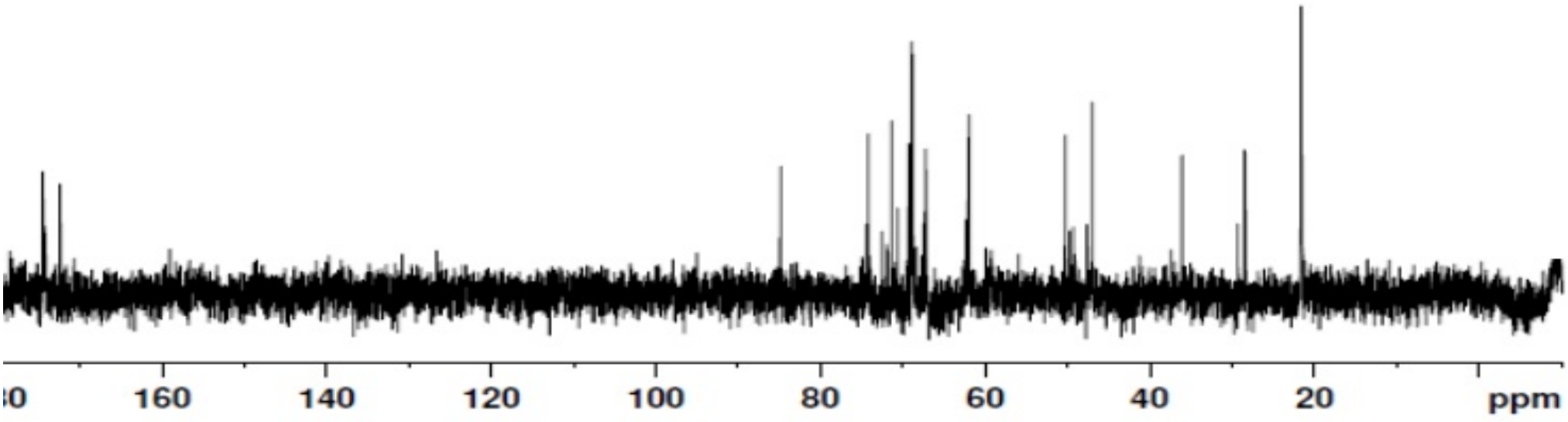


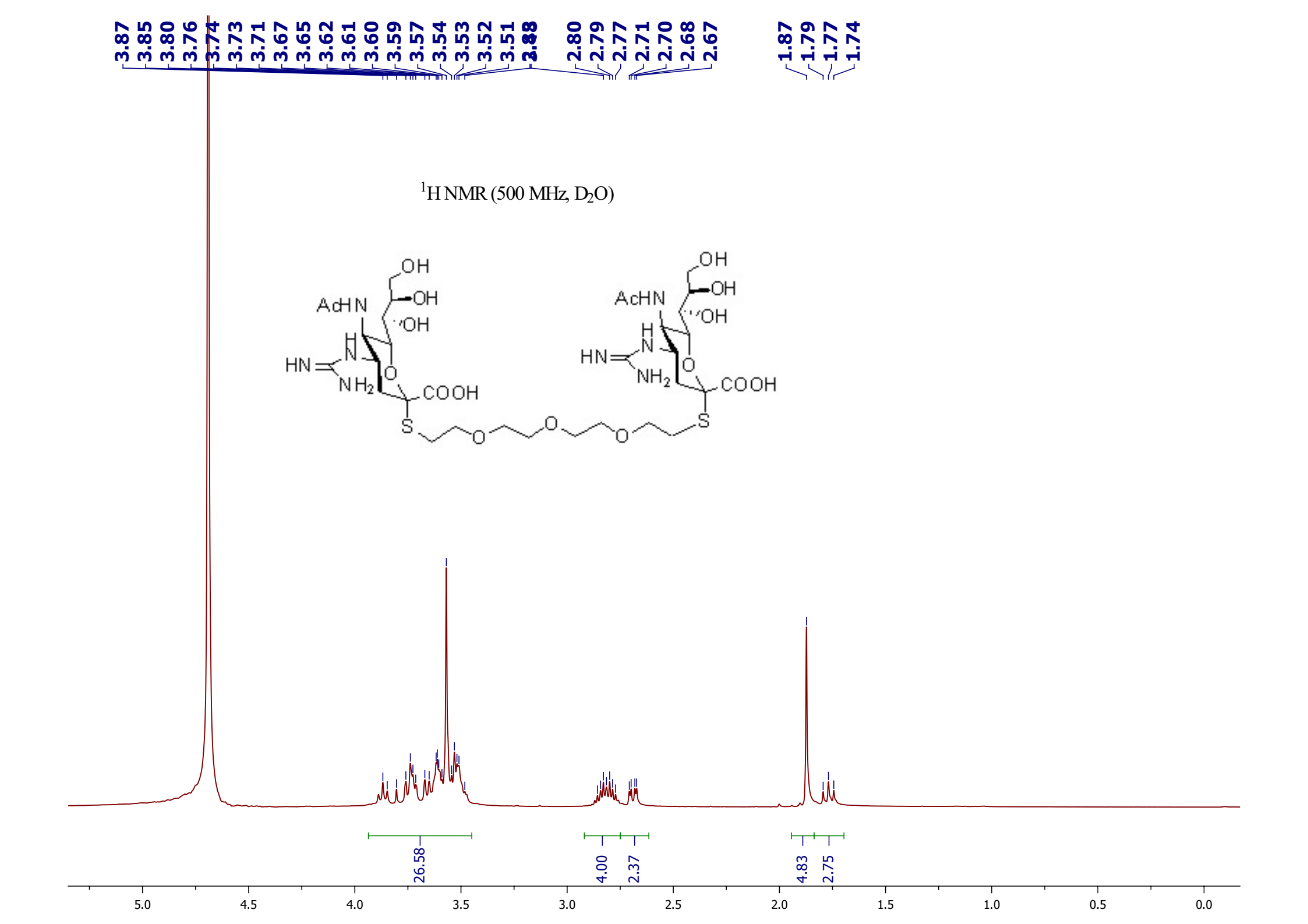



3.76
3.75
3.73
3.72
3.70
3.68
3.66
3.62
3.60
3.58
3.57
3.55
3.55
3.53
3.52
3.49
2.84
2.83
2.82
2.81
2.80
2.79
2.78
1.93
^{1}H NMR (500 MHz, D_2O)
OH
OH
AcHN
OH
H_2N
O
COOH
S
O
O
O
S
31.19
6.00
10.00
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
f1 (ppm)

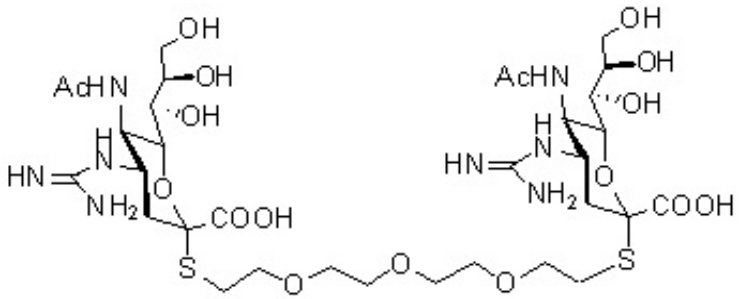


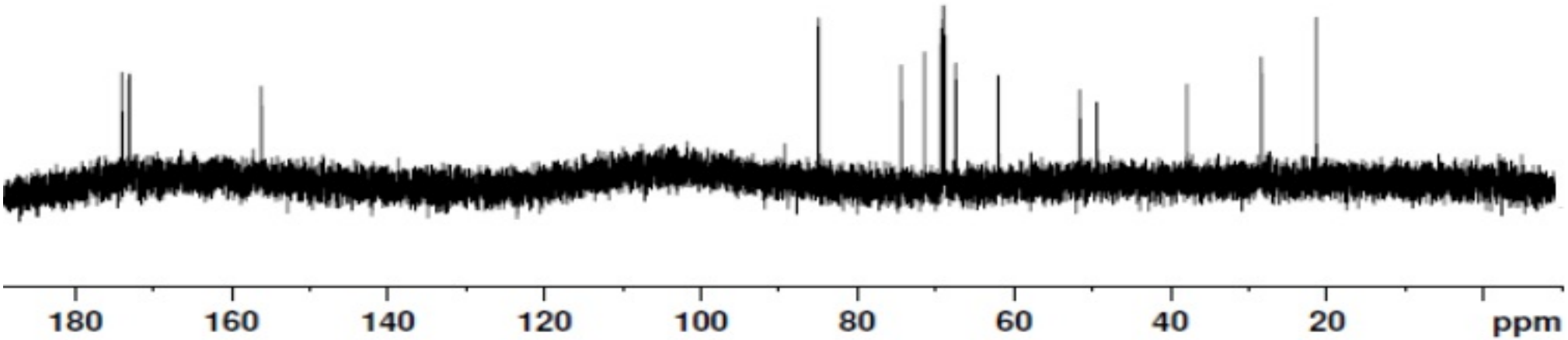
^{13}C NMR (125 MHz, D_2O)

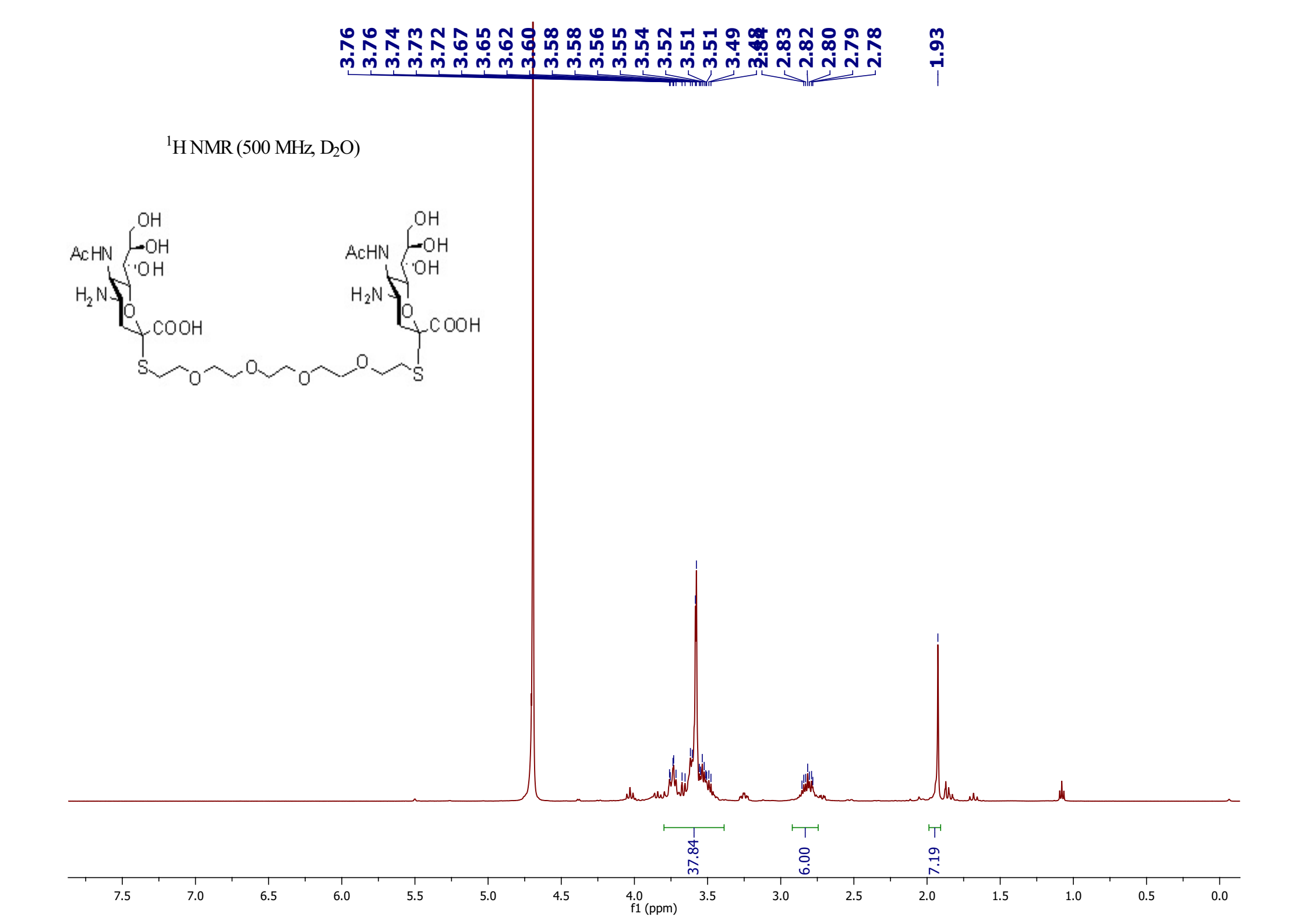



3.87
3.85
3.80
3.76
3.74
3.73
3.71
3.67
3.65
3.62
3.61
3.60
3.59
3.57
3.54
3.53
3.52
3.51
2.80
2.79
2.77
2.71
2.70
2.68
2.67
1.87
1.79
1.77
1.74
1H NMR (500 MHz, D2O)
OH
AcHN
OH
OH
HN
NH2
COOH
S
O
O
O
26.58
4.00
2.37
4.83
2.75
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

^{13}C NMR (125 MHz, D_2O)



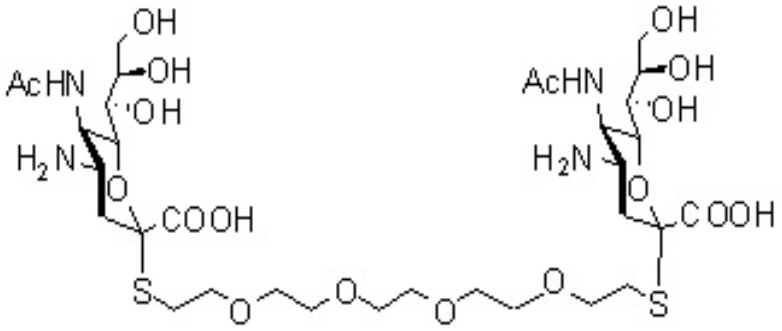


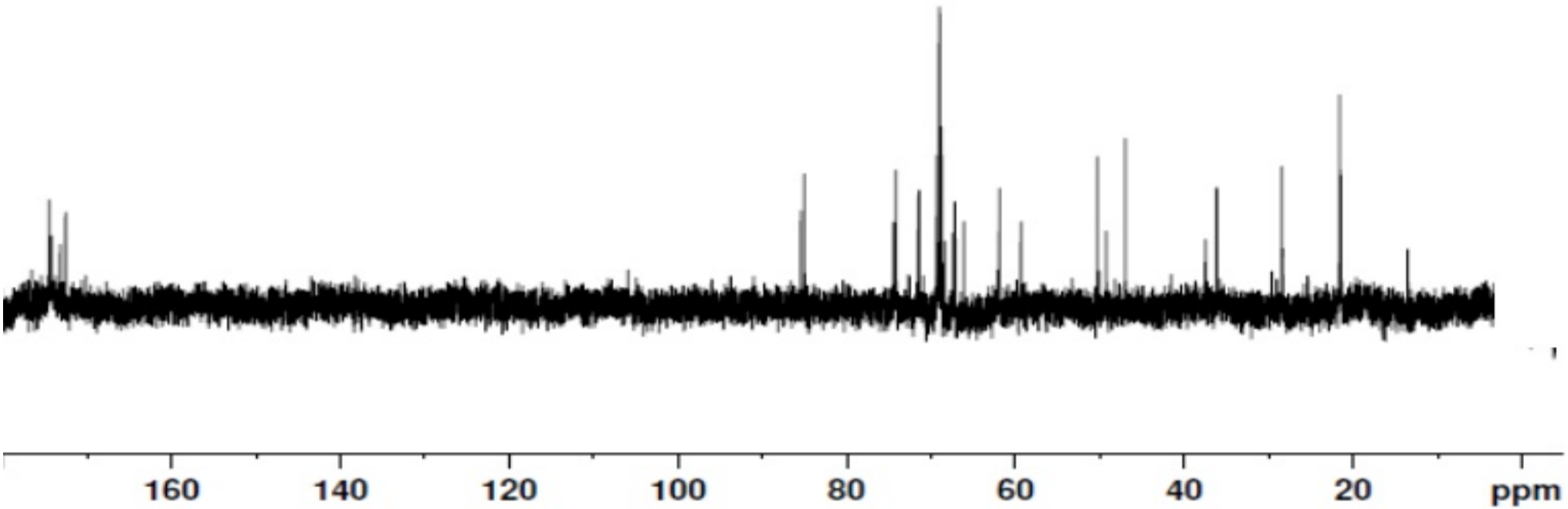

3.76
3.76
3.74
3.73
3.72
3.67
3.65
3.62
3.60
3.58
3.58
3.56
3.55
3.54
3.52
3.51
3.51
3.49
2.83
2.82
2.80
2.79
2.78
1.93
1H NMR (500 MHz, D2O)
OH
OH
OH
AcHN
H2N
O
COOH
S
O
O
O
O
S
37.84
6.00
7.19
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

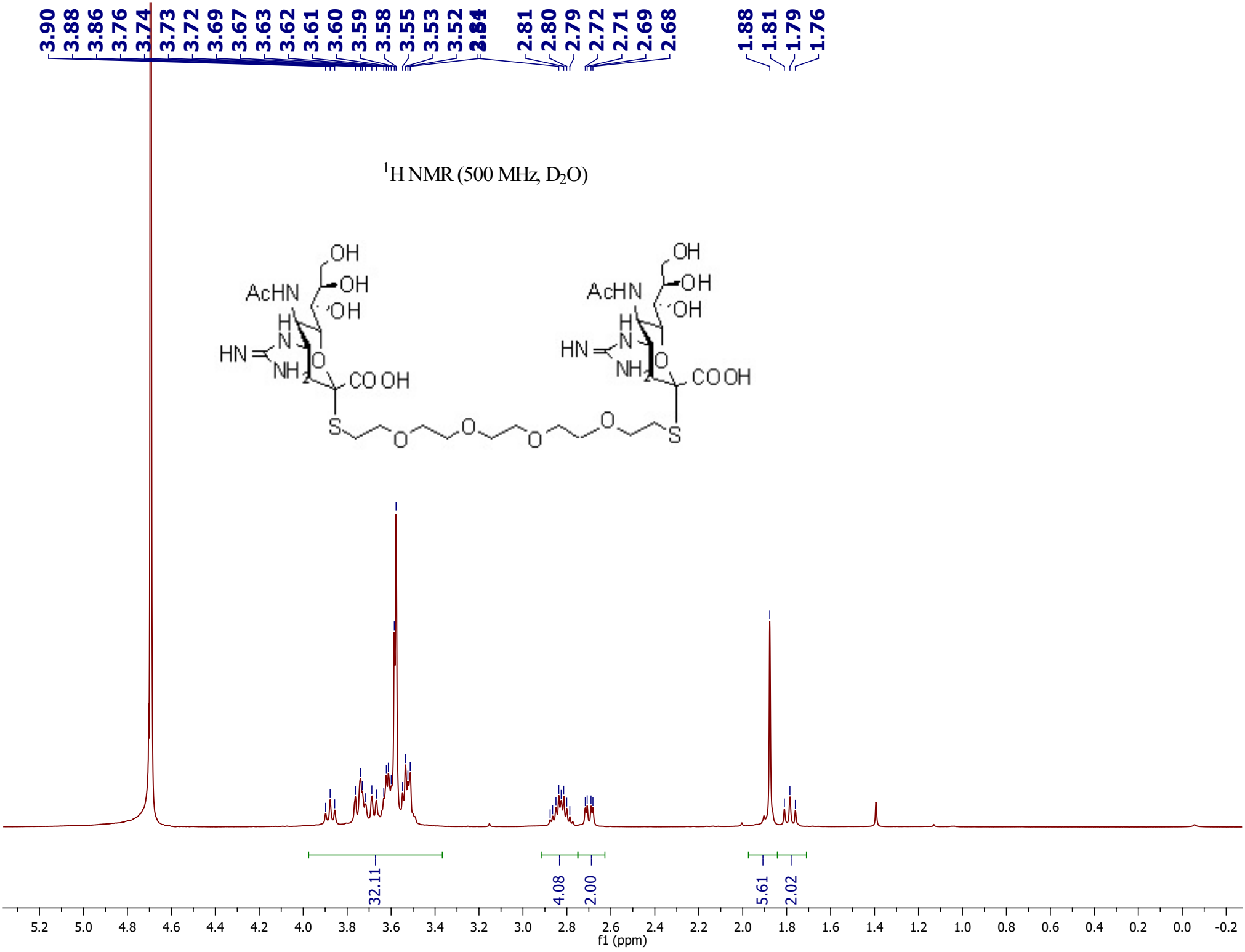
174.51
174.27
173.20
172.57

85.43
85.02
74.43
74.19
71.56
71.45
69.39
69.31
69.06
68.98
68.79
68.40
67.39
67.27
67.18
66.11
61.97
61.90
59.37
50.33
49.25
47.01
37.49
36.16
28.43
21.59
21.48
13.56

^{13}C NMR (125 MHz, D_2O)

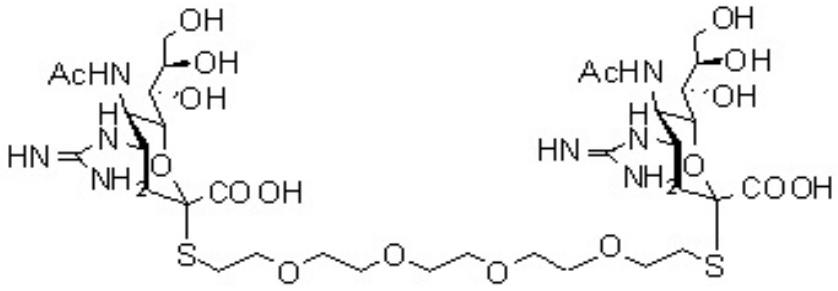


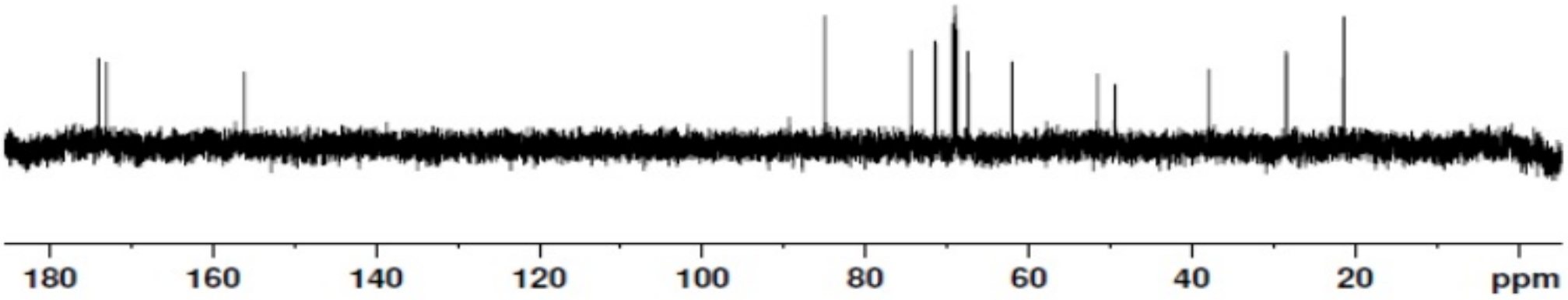


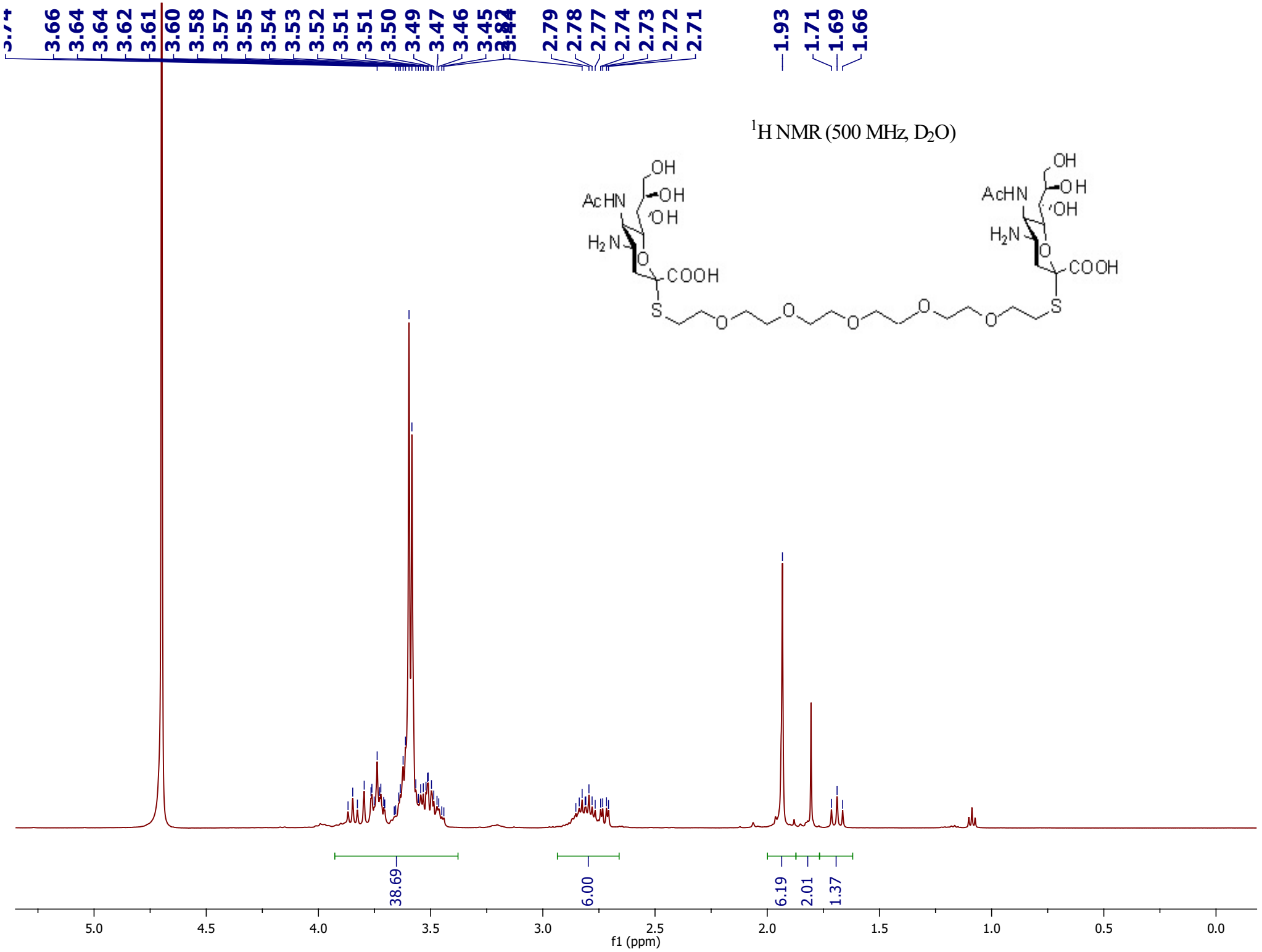


^{1}H NMR (500 MHz, D_2O)

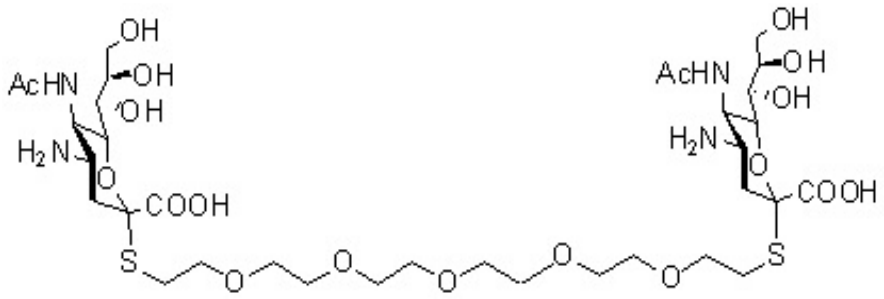
^{13}C NMR (125 MHz, D_2O)

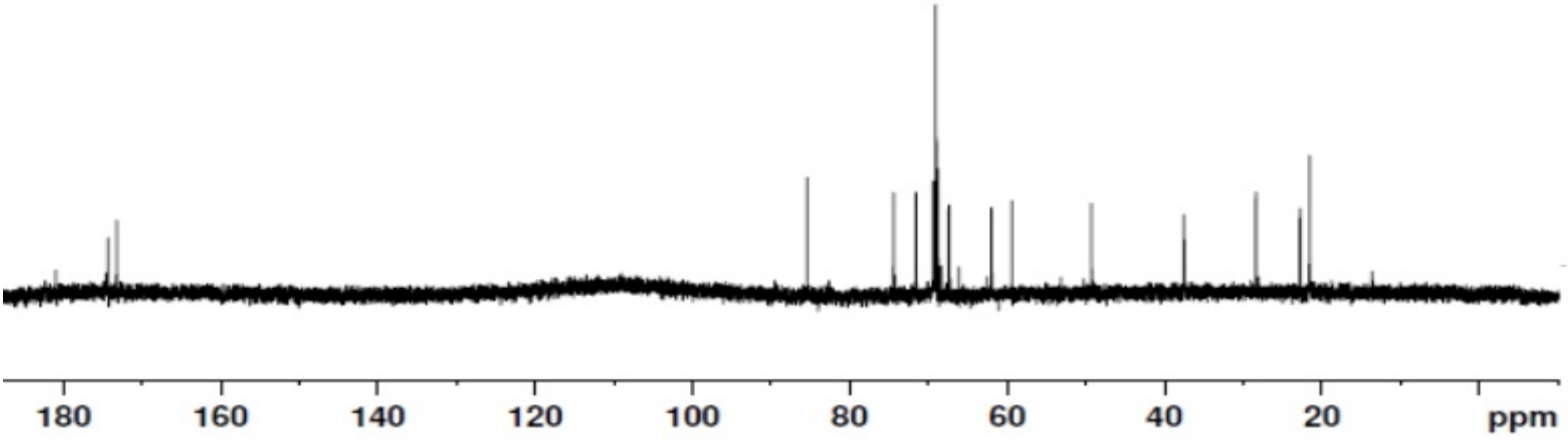


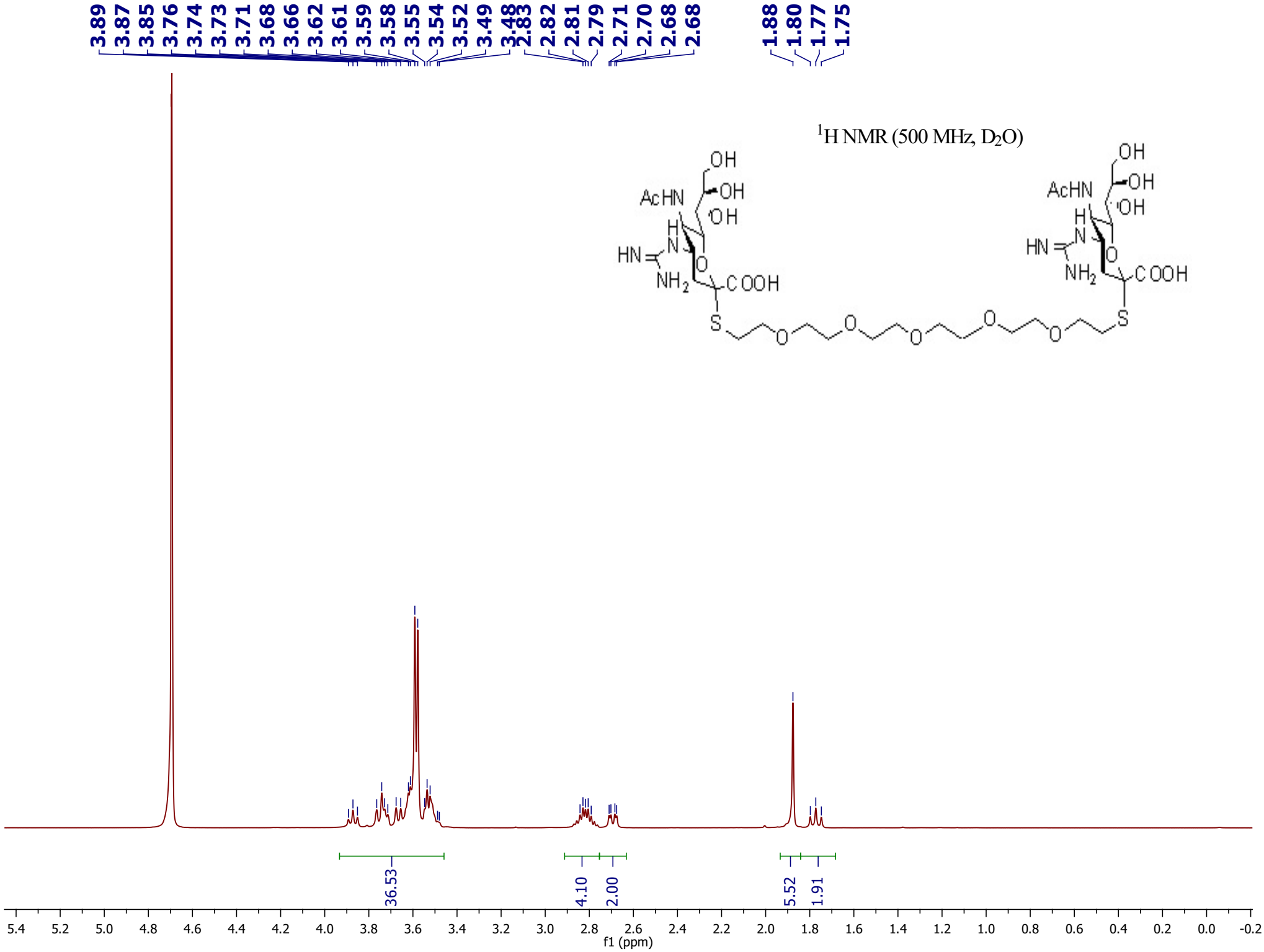



3.66
3.64
3.64
3.62
3.61
3.60
3.58
3.57
3.55
3.54
3.53
3.52
3.51
3.51
3.50
3.49
3.47
3.46
3.45
2.79
2.78
2.77
2.74
2.73
2.72
2.71
1.93
1.71
1.69
1.66
1H NMR (500 MHz, D2O)
OH
AcHN
OH
OH
H2N
O
COOH
S
O
O
O
O
O
S
38.69
6.00
6.19
2.01
1.37
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

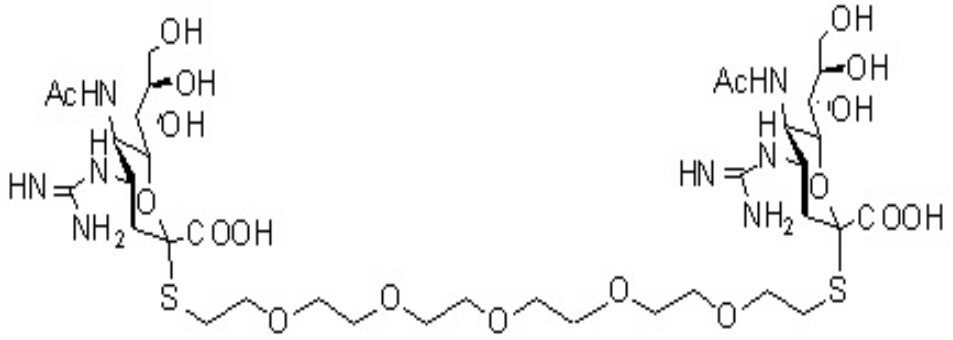
^{13}C NMR (125 MHz, D_2O)

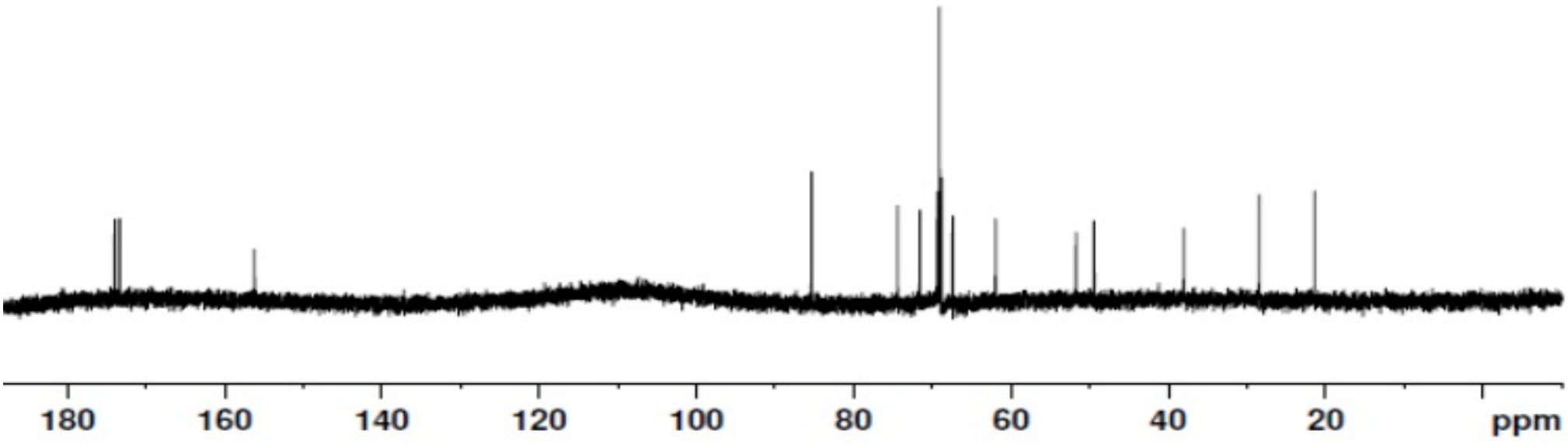




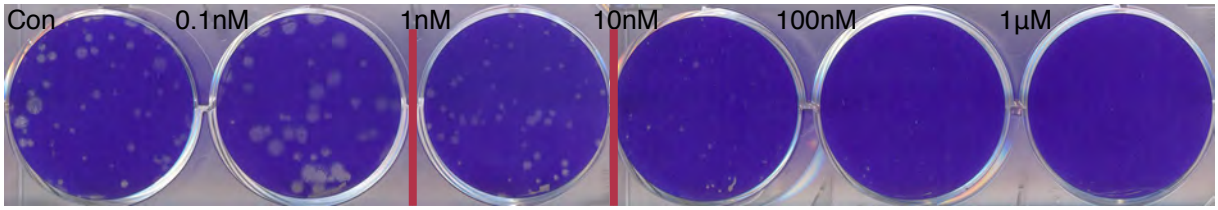

3.89
3.87
3.85
3.76
3.74
3.73
3.71
3.68
3.66
3.62
3.61
3.59
3.58
3.55
3.54
3.52
3.49
3.48
2.83
2.82
2.81
2.79
2.71
2.70
2.68
2.68
1.88
1.80
1.77
1.75
1H NMR (500 MHz, D2O)
OH
AcHN
OH
OH
H
N
HN
NH2
O
COOH
S
O
O
O
O
O
S
36.53
4.10
2.00
5.52
1.91
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
f1 (ppm)

^{13}C NMR (125 MHz, D_2O)

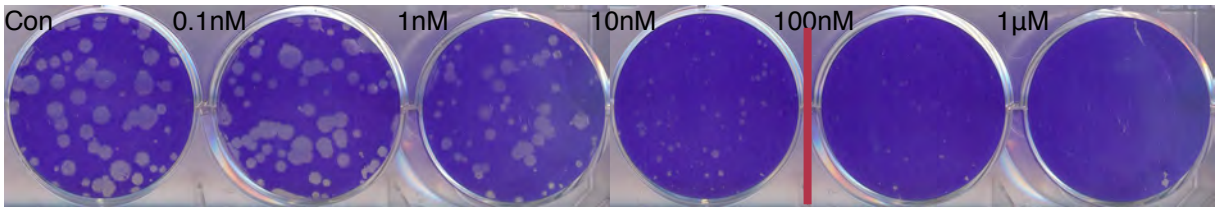




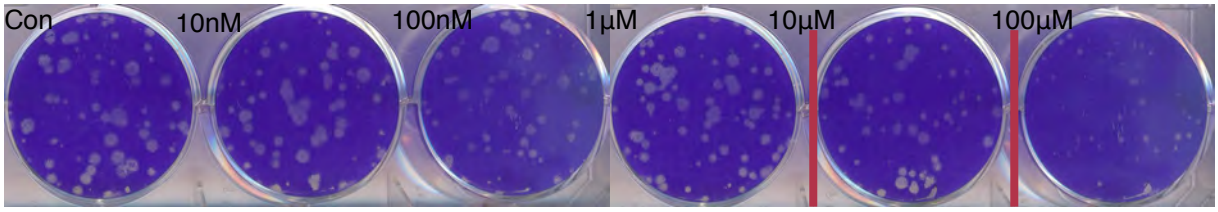
Zanamivir



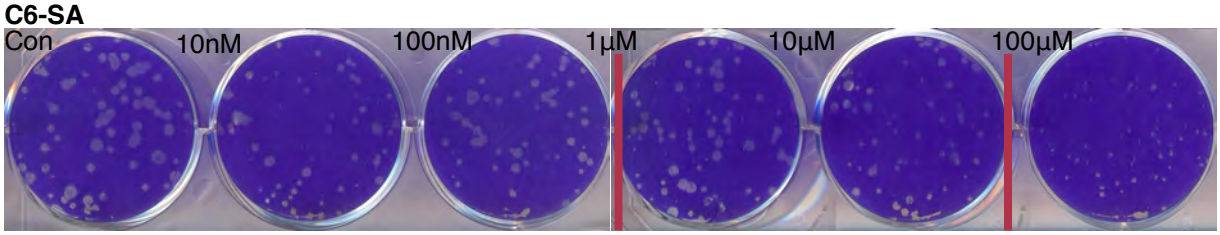
Oseltamivir



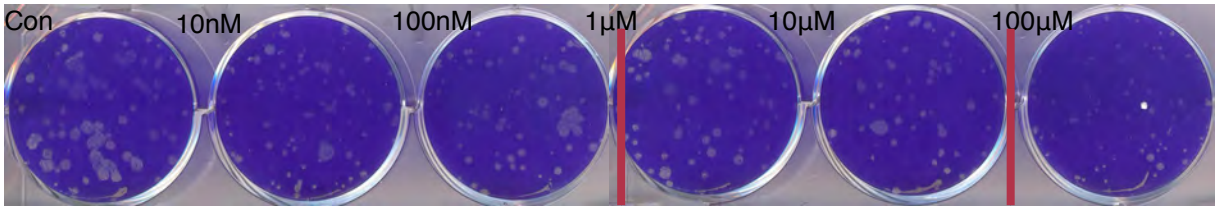
SA



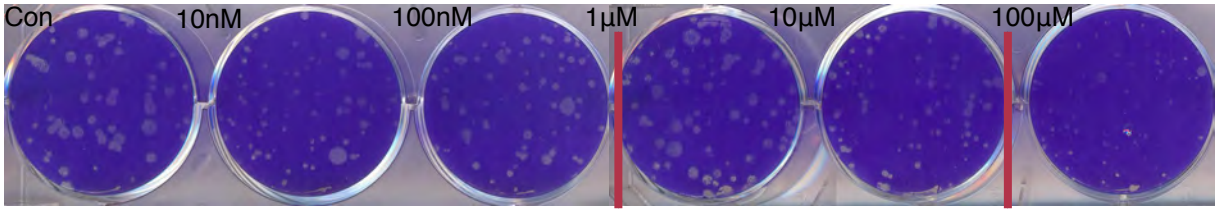
C6-SA



C12-SA



TetraEG-SA



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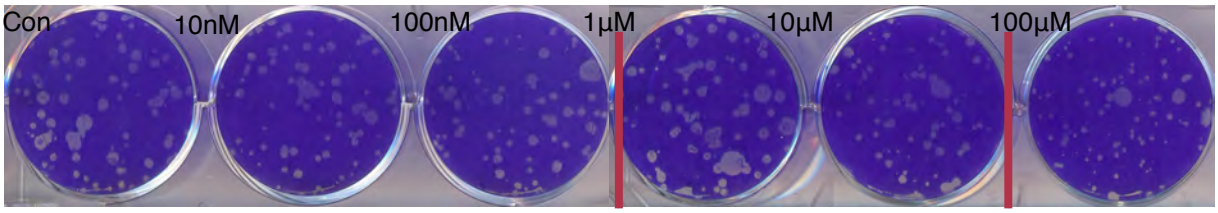
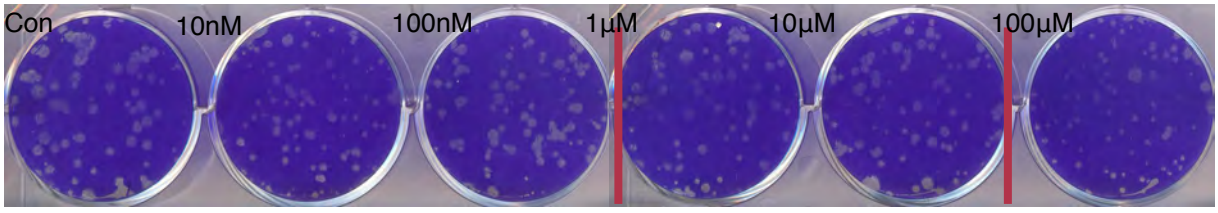


HexaEG-SA



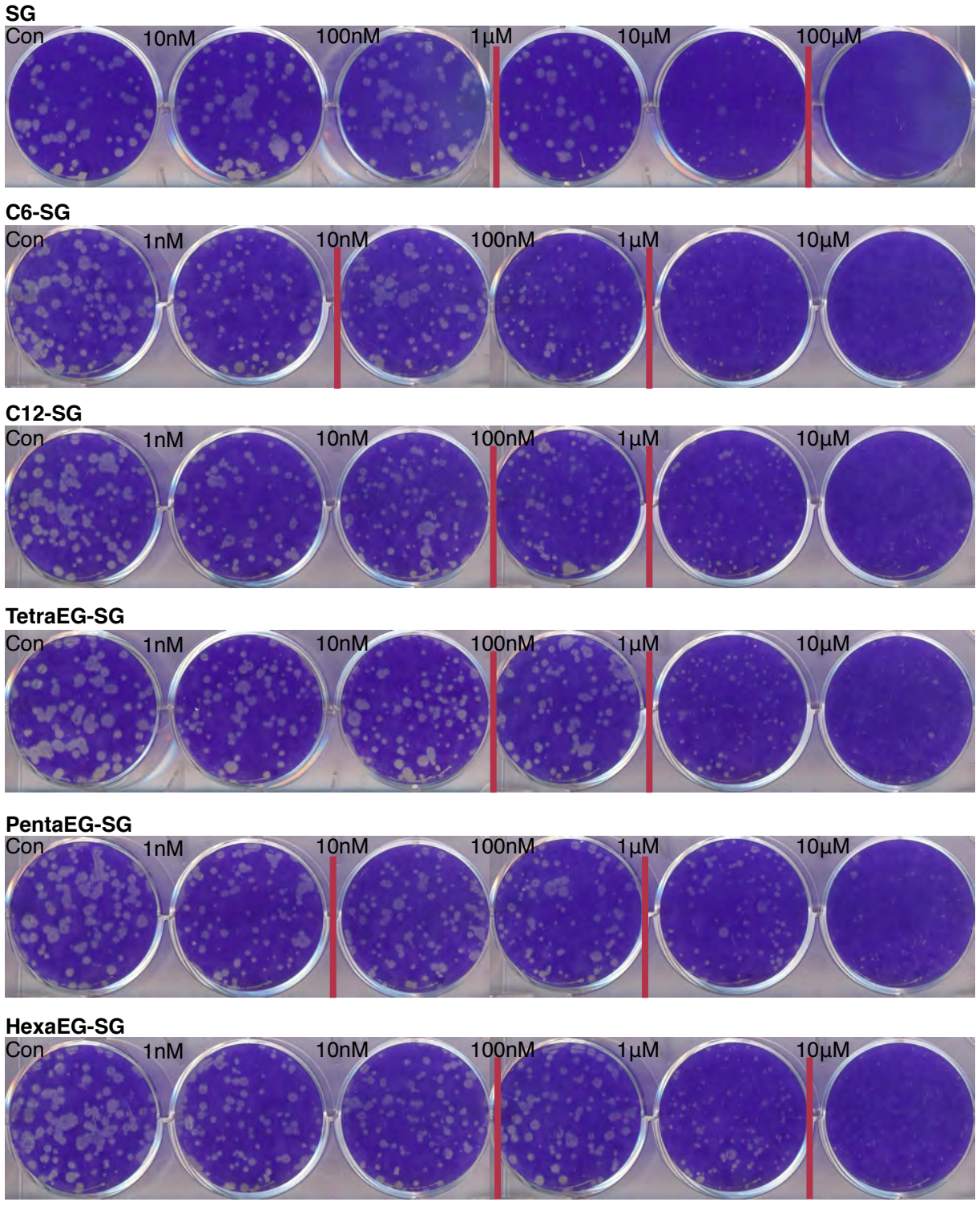
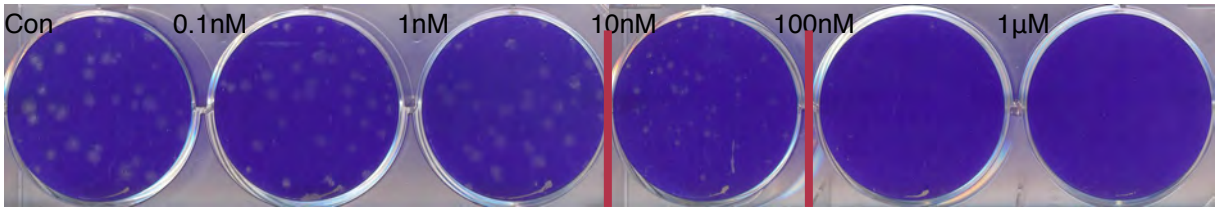
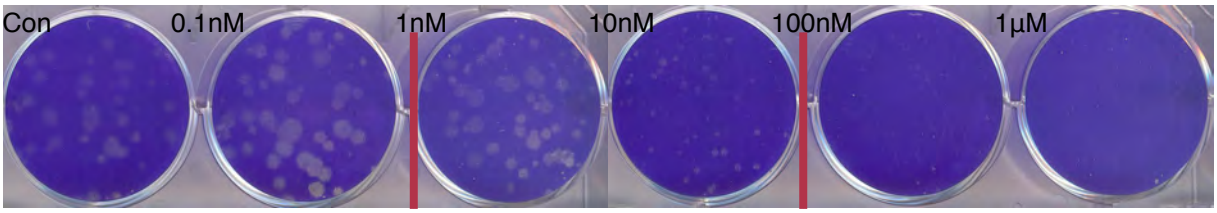


Figure S2 Images of the plaque size reduction assay by the sialosides synthesized in this report and current antivirals using A/Hongkong/8/1968 (H3N2) virus

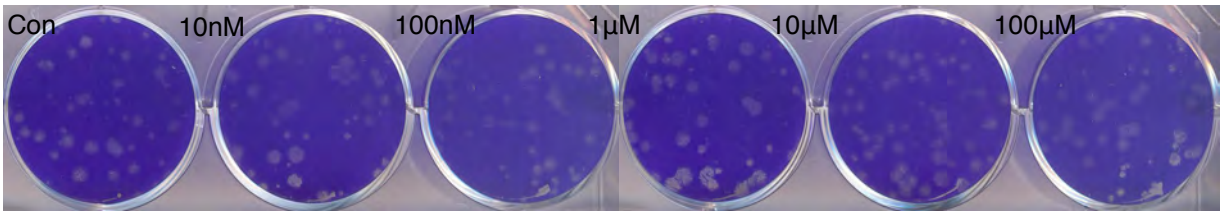
Zanamivir



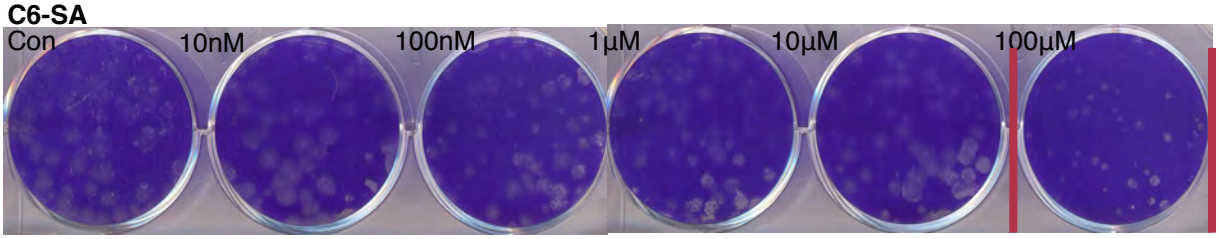
Oseltamivir



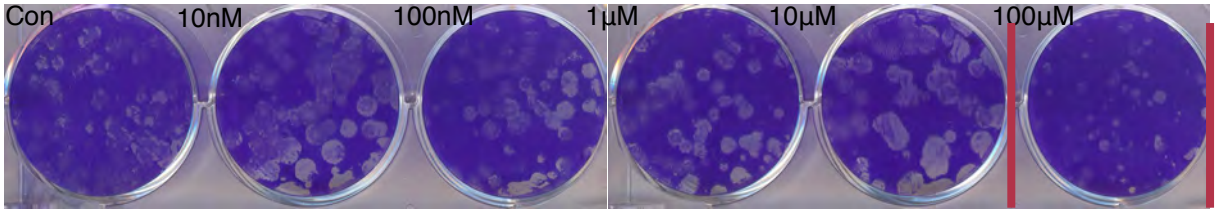
SA



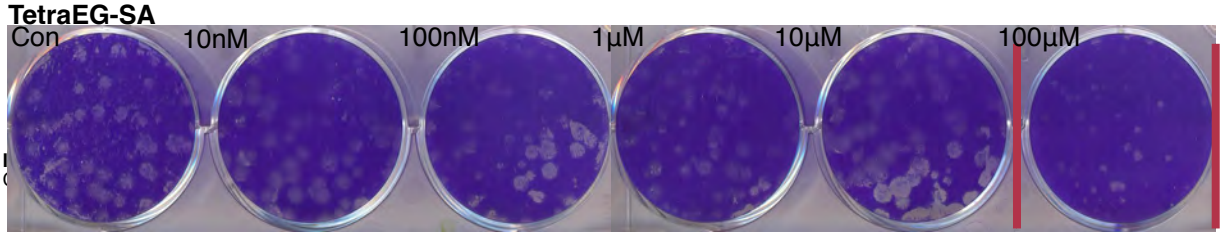
C6-SA



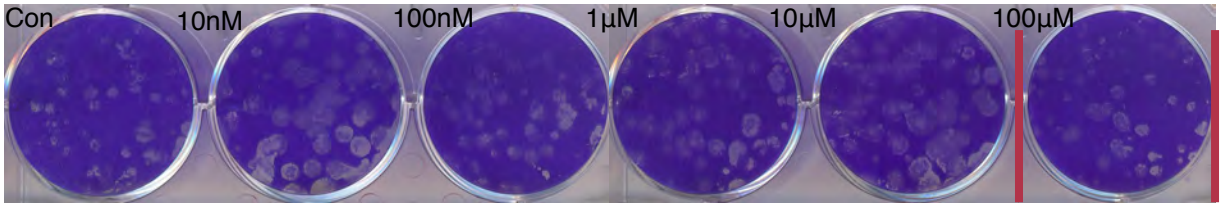
C12-SA



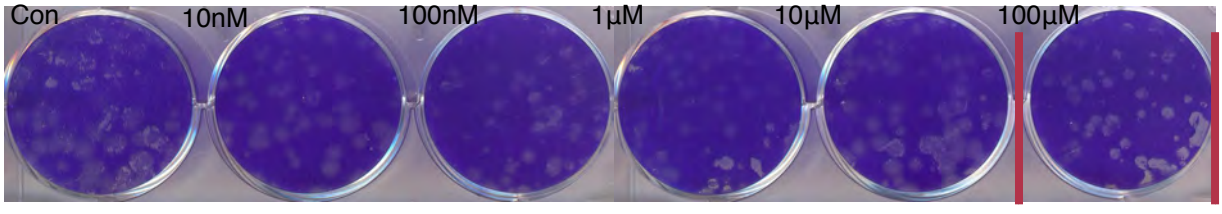
TetraEG-SA



PentaEG-SA



HexaEG-SA



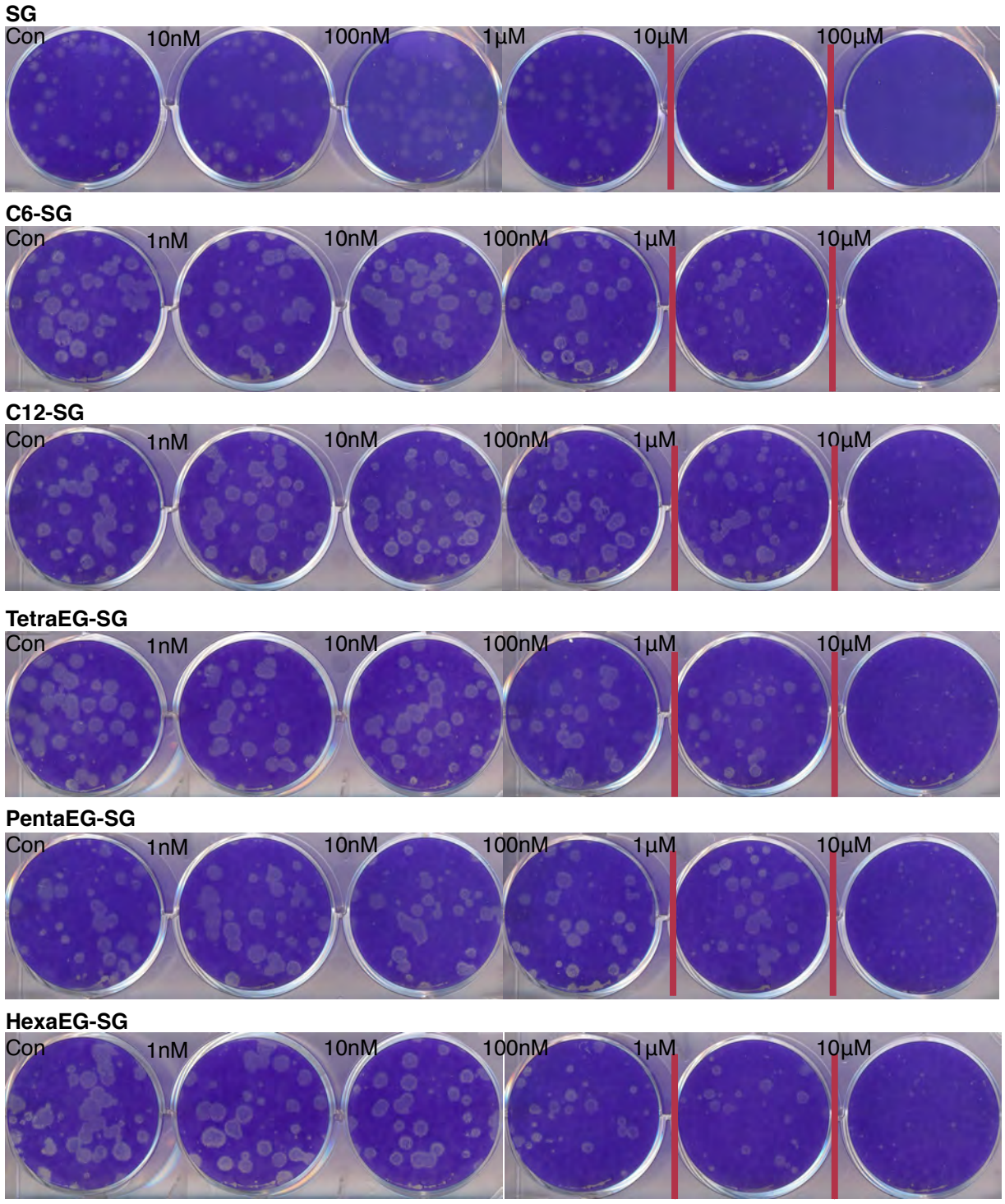


Figure S3 Images of the plaque size reduction assay by the sialosides synthesized in this report and current antivirals using A/California/07/2009 (H1N1) virus