Sequential one-pot enzymatic synthesis of oligo-*N*-acetyllactosamine and its multi-sialylated extensions

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Supporting Information

Materials and Methods

The enzymes and reagents used in the molecular biology experiments, the DNA ladders and the deoxynucleotide triphosphates (dNTPs) were purchased from Violet BioScience (Taipei, Taiwan) or New England Biolabs (NEB, Ipswich, MA, USA). DNA primers for the PCR reaction were purchased from Mission Biotech (Taipei, Taiwan). The synthetic oligonucleotides encoding the β-1,3-*N*-acetyl-glucosaminyl transferase gene (*jhp1032*) from *Helicobacter pylori* were purchased from GeneArt (now Life Technologies, Carlsbad, CA, USA). The genomic DNA of *Bifidobacterium longum* (BCRC 11847) and *Photobacterium damselae* subsp. Damselae (ATCC 33539) were purchased from the Bioresource Collection and Research Centre (Hsinchu, Taiwan). Protein molecular weight standards and P-2 gel were obtained from BioRad (Hercules, CA, USA). QlAprep® Spin Miniprep Kit and QlAquick® Gel Extraction Kit were purchased from Qiagen (Hilden, Germany). The pGEM-T Easy Vector System I was purchased from Promega (Fitchburg, WI, USA). All other chemicals and enzymes were obtained from the Sigma-Aldrich Company unless otherwise stated. The procedures for standard DNA manipulation, including plasmid DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, DNA ligation, and the transformation of *E. coli*, were performed according to the instructions provided by the manufacturer. PCR was carried out in a T3000 Thermocycler (Biometra, Göttingen, Germany). The SDS-PAGE for protein analysis was stained with InstantBlue (Expedeon, Cambridgeshire, UK). The protein concentrations were determined with the BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA) using bovine serum albumin as the standard.

Cloning and overexpression of enzymes

Gene resource: MtGalK,[1] RmlA,[2] NmGalT[2] and PmST[3] were cloned and overexpressed as previously reported by our group. NahK and Pd2,6ST were cloned and overexpressed using the IMPACT™ system, while HpGnT was cloned and overexpressed using the pMAL™ protein fusion and purification system according to the instruction manual provided by the manufacturer (NEB).

Gene cloning: DNA amplification was performed in a solution (50 μL) that contained 50 ng of template DNA, 0.4 μM of each primer, 0.2 mM dNTP, 1.5 mM

$MgCl_2$, and 2.5 U *Taq* DNA polymerase. PCR was performed with denaturation at 95 °C for 30 sec, annealing at 55-65 °C for 30 sec and extension at 72 °C for 1 min. The amplified fragment was sub-cloned into the pGEM-T Easy Vector. The insert was further confirmed by a blue/white screen and sequence analysis. In the case of NahK, the target gene was digested from the constructed T vector and inserted into the *Nde*I/*Sap*I-treated vector pTXB1; the gene encoding Pd2,6ST was inserted into *Nde*I/*Xho*I-treated pTXB1, while the HpGnT gene was digested from the constructed T vector and inserted into the *Sac*I/*Xho*I-treated vector pMYB5. The ligation mixture was then transformed into the DH5α and BL21 (DE3) strain of *E. coli*. DNA sequencing was used to confirm the in-frame cloning of the full length target gene. The genes for the enzymes were isolated by PCR amplification using the following primers: *nahK*-F: 5'-CAT ATG ACC GAA AGC AAT GAA GTT TTA TTC GGC ATC GCC T-3' and *nahK*-R: 5'-GCT CTT CAG CA TCC TCC TCC CTT GGC AGC CTC CAT G-3'; *pd2,6ST*-F: 5'-CAT ATG TGT AAT AGT GAC AAT ACC AGC TTG AAA GAA ACG GT-3'; *pd2,6ST*-R: 5'-CTC GAG AGC CCA GAA CAG AAC ATC TTT TTC-3'; *hpGnT*-F: 5'-TCG AGC TCG ATC GAG GGA AGG TGT GGG GGG GGG ATG ACC AGC GCA AGC AGC-3'; *hpGnT*-R: 5'-CTC GAG GCC TTT TTT CAG CAG TTT CAG TTT AAT CAG AAT TTT TTT CAC-3'.

Protein overexpression: *E. coli* BL21 (DE3) carrying the above recombinant plasmids was cultured in Luria-Bertani (LB) medium (10 g L^{-1} peptone, 5 g L^{-1} yeast extract, 10 g L^{-1} NaCl; 5 mL) with ampicillin (100 μg mL^{-1}) overnight at 37 °C in an orbital shaking incubator S300R (Firstek, New Taipei City, Taiwan) with vigorous shaking at 200 rpm, then transferred to fresh LB medium (500 mL) with ampicillin for another 2 h at 37 °C. When the optical density OD_{600} value reached 0.6–0.8, the culture was induced with 0.5 mM IPTG for 16–24 h at 16 °C. The bacterial cells were harvested by centrifugation at 4 ^{o}C and 5000 x *g* for 15 min. The cell pellet was resuspended at 25 mL per liter of cell culture in column buffer (pH 8.0, 20 mM HEPES containing 0.1% Triton X-100, 500 mM NaCl, and 0.1 mM EDTA). The cells were disrupted by sonication on ice for 20 min in 3-s intervals, and then the debris was removed by centrifugation for 60 min at 24000 x *g* and 4 °C.

Protein purification: For the IMPACT system purification, the cell lysate was

collected and applied to a 6-mL chitin bead column that was pre-equilibrated with column buffer. After the sample was loaded onto the chitin column and incubated with chitin affinity beads for 30 min at 4 °C, the column was washed with 20 column volumes of the same buffer. The resin was then quickly washed with 1 column volume of the same buffer containing 80 mM DTT, and the effluent was reloaded. The column was clamped at both ends, and on-column cleavage of the intein tag from the fusion protein was performed by incubating the column at 4 °C for 16 h. The purified protein was eluted using the same buffer without DTT. The effluent was concentrated by centrifugation with a centrifugal filter device (Amicon Ultra, Millipore), divided into aliquots, and stored at -30 °C.

For the pMAL system, the supernatant was applied to amylose resin equilibrated in 20 mM Tris-HCl (pH 7.5 with 500 mM NaCl). MBP-HPGnT was eluted with 10 mM maltose in the same buffer, and the fractions were analyzed by SDS-PAGE. Fractions with significant amounts of MBP-HPGnT were pooled and then concentrated by centrifugation, divided into aliquots, and stored at -30 °C.

Synthesis of UDP-Gal and UDP-GlcNAc

The synthesis of UDP-gal was performed in a 10 mL reaction buffer containing 100 mM Tris-HCl (pH 7.5), 20 mM $MgCl_2$, 20 mM ATP, 20 mM UTP, 20 mM Gal, 10 enzyme units (EU) of inorganic pyrophosphatase (IP), 200 μg MtGalK and 20 mg RmlA. The reaction was carried out at 55 °C for 4 h and monitored by RP-HPLC coupled with a UV detector, according to our previous literature reports.[2] For the synthesis of UDP-GlcNAc, the reaction was performed in a 10 mL reaction buffer containing 100 mM Tris-HCl (pH 7.5), 20 mM $MgCl_2$, 20 mM ATP, 20 mM UTP, 20 mM GlcNAc, 10 EU IP, 5 mg NahK, and 20 mg RmlA. The reaction was first carried out at 37 °C for 2 h and then was increased to 55 °C. The reaction mixture was incubated for another 2 h. The formation of UDP-GlcNAc was monitored by RP-HPLC.

To isolate the UDP-Gal/UDP-GlcNAc, the reaction mixture was centrifuged for 5 min at 16,200 x *g* at room temperature, and the clear supernatant was collected. The diluted solution was first desalted by P-2 Gel followed by Q FF anion-exchange column purification (GE Healthcare, Little Chalfont, UK) eluted with a gradient of NH_4HCO_3 solution (0→500 mM). The fraction containing UDP-Gal/UDP-GlcNAc

was collected and lyophilized.

One-Pot synthesis of oligo-LacNAcs

The one-pot synthesis of oligo-LacNAc was performed in two steps. In one vessel, the UDP-Gal synthesis was performed in 10 mL of reaction buffer containing 200 mM Tris-HCl (pH 7.5), 200 mM $MgCl_2$, 200 mM ATP, 200 mM UTP, 200 mM Gal, 320 μg MtGalK, 20 mg RmlA, and 10 EU IP. The reaction proceeded as according to the aforementioned procedures. In another vessel, the UDP-GlcNAc reaction was performed in 10 mL of reaction buffer containing 200 mM Tris-HCl pH 7.5, 200 mM $MgCl_2$, 200 mM ATP, 200 mM UTP, 200 mM GlcNAc, 320 μg NahK, 20 mg RmlA, and 10 EU IP. The reaction proceeded according to the aforementioned procedures. When the yield of UDP-Gal and UDP-GlcNAc reached 50%, the two vessels were combined as one. The solution temperature was cooled to 25 °C, followed by the addition of azido-GlcNAc **1** (10 mM), 200 μg of NmGalT, 400 μg of HpGnT, and dithiothreitol (DTT) to a final concentration of 0.2 mM. The reaction was monitored by thin-layer chromatography (TLC: methanol (MeOH)/dichloromethane (DCM) = 1/1). The product was desalted by the P-2 gel and then analyzed by MALDI-TOF mass spectrometry.

Synthesis of LacNAc (2)

A buffered (100 mM Tris-HCl, pH of 7.5) solution of Gal (20 mM) containing $MgCl_2$ (40 mM) was incubated at 55 °C with ATP (20 mM) and UTP (20 mM) in the presence of IP (10 EU), MtGalK (10 μg mL^{-1}) and RmlA (1 mg mL^{-1}) for 4 h. The solution was cooled to 25 °C, followed by the addition of azido-GlcNAc **1** (35 mg, 0.1 mmol), DTT (0.2 mM), and NmGalT (20 μg mL^{-1}). The resulting mixture was incubated at 25 °C for 4 h. When TLC analysis (MeOH/DCM = 1/1) indicated the completion of the reaction, the reaction solution was centrifuged (10,000 x *g*, 10 min) to remove the proteins and insoluble precipitates. The supernatant was filtered (0.45-μm filter; Millipore), concentrated, and passed through a P-2 gel to obtain the desired product. The product-containing fractions were identified by TLC analysis and were pooled together then lyophilized to give an isolation yield of 90% (46 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.21-1.26 (m, 4H), 1.43 (q, *J* = 6.9 Hz, 2H), 1.47 (q, *J* = 7.0 Hz, 2H), 1.91 (s, 3H), 3.20 (t, *J* = 6.9 Hz, 2H), 3.40 (dd, *J* = 8.1, 10.2 Hz, 1H),

3.43-3.48 (m, 2H), 3.53 (dd, *J* = 3.3, 10.1 Hz, 1H), 3.56-3.66 (m, 6H), 3.70 (dd, *J* = 5.2, 12.3 Hz, 1H), 3.75-3.79 (m, 2H), 3.85 (dd, *J* = 1.9, 12.3 Hz, 1H), 4.34 (d, *J* = 7.9 Hz, 1H), 4.39 (d, *J* = 7.9 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 22.02, 24.47, 25.40, 27.81, 28.25, 50.97, 54.96, 59.92, 60.85, 68.38, 70.22, 70.80, 72.29, 72.34, 74.58, 75.18, 78.31, 100.88, 102.71, 174.22; HRMS (ESI-TOF) calcd for $C_{20}H_{37}N_4O_{11}$ $[M+H]^+$ = 509.2459; found 509.2451.

Synthesis of GlcNAc-LacNAc (3)

A buffered (100 mM Tris-HCl, pH of 7.5) solution of GlcNAc (20 mM) containing $MgCl_2$ (40 mM) was incubated at 37 °C with ATP (20 mM) and UTP (20 mM) in the presence of NahK (1 mg mL^{-1}) and RmlA (1 mg mL^{-1}) for 2 h. The reaction temperature was increased to 55 °C and reacted for another 4 h. The reaction was cooled to 25 °C, followed by the addition of **2** (51 mg, 0.1 mmol), DTT (308 μg, 2 μmol), and HpGnT (40 μg mL^{-1}). The resulting mixture was incubated at 25 °C for 4 h. When TLC analysis (MeOH/DCM = 1/1) indicated the completion of the reaction, the reaction solution was centrifuged (10,000 x *g*, 10 min) to remove the proteins and insoluble precipitates. The supernatant was filtered (0.45-μm filter; Millipore), concentrated, and passed through a P-2 gel to obtain the desired product. The product-containing fractions were identified by TLC analysis and pooled together then lyophilized to give an isolation yield of 99% (70 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.12-1.27 (m, 4H), 1.39-1.49 (m, 4H), 1.89 (s, 6H), 3.18 (t, *J* = 6.6 Hz, 2H), 3.30-3.36 (m, 2H), 3.41-3.47 (m, 4H), 3.55-3.64 (m, 9H), 3.68 (dd, *J* = 4.4, 12.3 Hz, 1H), 3.74-3.79 (m, 2H), 3.84 (d, *J* = 11.9 Hz, 2H), 4.00-4.02 (m, 1H), 4.31 (d, *J* = 7.8 Hz, 1H), 4.38 (d, *J* = 7.6 Hz, 1H), 4.53 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.68 (x2), 24.12, 25.06, 27.46, 27.91, 50.64, 54.59, 55.17, 59.60, 59.99, 60.44, 67.92, 69.20, 69.51, 69.88, 71.94, 73.08, 74.24, 74.38, 75.16, 78.04, 81.48, 100.54, 102.33, 102.41, 173.87, 174.45; HRMS (ESI-TOF) calcd for $C_{28}H_{48}N_5O_{16}$ $[M-H]^-$: 710.3096; found 710.3101.

Synthesis of $(LacNAc)_2$ (4)

Compound **4** was prepared by following the procedures described for the synthesis of LacNAc (**2**), with an isolated yield of 96% (84 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.19-1.29 (m, 4H), 1.42-1.51 (m, 4H), 1.91 (s, 6H), 3.20 (t, *J* = 6.9 Hz, 2H), 3.41 (dd,

J = 7.8, 9.9 Hz, 1H), 3.45-3.49 (m, 4H), 3.54 (d, *J* = 3.2 Hz, 1H), 3.56 (dd, *J* = 3.5, 5.4 Hz, 1H), 3.57-3.67 (m, 12H), 3.68-3.74 (m, 3H), 3.76-3.79 (m, 1H), 3.80 (d, *J* = 3.4 Hz, 1H), 3.83 (dd, *J* = 2.0, 12.1 Hz, 1H), 3.86 (dd, *J* = 2.0, 12.3 Hz, 1H), 4.03 (d, *J* = 3.24 Hz, 1H), 4.33 (d, *J* = 7.9 Hz, 1H), 4.35 (d, *J* = 7.9 Hz, 1H), 4.40 (d, *J* = 7.9 Hz, 1H), 4.58 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.93 (x2), 24.38, 25.31, 27.72, 28.16, 50.89, 54.83, 54.94, 59.61, 59.83, 60.69, 60.78, 68.06, 68.30, 69.71, 70.14, 70.72, 71.94, 72.19, 72.26, 74.30, 74.49, 74.62, 75.10, 77.92, 78.25, 81.82, 100.80, 102.50, 102.62, 102.66, 174.14, 174.66; HRMS (ESI-TOF) calcd for $C_{34}H_{58}N_5O_{21}$ $[M-H]^-$: 872.3624; found 872.3635.

Synthesis of GlcNAc-(LacNAc)$_2$ (5)

Compound **5** was prepared by following the procedures described for the synthesis of GlcNAc-LacNAc (**3**), with an isolated yield of 89% (49 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.19-1.26 (m, 4H), 1.40-1.49 (m, 4H), 1.89 (s, 9H), 3.18 (t, *J* = 6.9 Hz, 2H), 3.29-3.35 (m, 2H), 3.41-3.48 (m, 6H), 3.54-3.64 (m, 16H), 3.65-3.66 (m, 1H), 3.70 (dd, *J* = 4.8, 12.2 Hz, 2H), 3.74-3.78 (m, 2H), 3.81 (dd, *J* = 2.2, 12.6 Hz, 1H), 3.84 (dd, *J* = 1.8, 12.2 Hz, 1H), 4.01 (d, *J* = 3.2 Hz, 2H), 4.31 (d, *J* =7.8 Hz, 1H), 4.33 (d, *J* = 7.8 Hz, 1H), 4.38 (d, *J* = 7.8 Hz, 1H), 4.54 (d, *J* = 8.5 Hz, 1H), 4.56 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.98 (x3), 24.42, 25.36, 27.76, 28.21, 50.94, 54.89, 54.96, 55.47, 59.68, 59.74, 59.89, 60.29, 60.74 (x2), 68.12, 69.50, 69.77, 69.80, 70.19, 71.98, 72.24, 73.37, 74.35, 74.54, 74.68 (x2), 75.47, 78.02, 78.32, 81.78, 81.86, 100.85, 102.53, 102.64, 102.70 (x2), 174.18, 174.70, 174.76; HRMS (ESI-TOF) calcd for $C_{42}H_{72}N_6O_{26}Na$ $[M+Na]^+$: 1099.4394; found 1099.4402.

Synthesis of (LacNAc)$_3$ (6)

Compound **6** was prepared by following the procedures described for the synthesis of LacNAc (**2**), with an isolated yield of 99% (62 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.16-1.23 (m, 4H), 1.37-1.45 (m, 4H), 1.87 (s, 9H), 3.15 (t, *J* = 6.9 Hz, 2H), 3.36-3.45 (m, 6H), 3,50 (dd, *J*= 3.3, 9.9 Hz, 2H), 3.51-3.57 (m, 14H), 3.58-3.70 (m, 10H), 3.72-3.83 (m, 5H), 3.98-4.00 (m, 2H), 4.28-4.32 (m, 3H), 4.36 (d, *J* = 7.4 Hz, 1H), 4.52-4.54 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 22.09 (x3), 24.47, 25.42, 27.78, 28.30, 51.05, 55.03, 55.10, 55.14, 59.91 (x2), 60.12, 60.78 (x2), 60.86, 68.19 (x2), 68.47, 69.88 (x2), 70.24, 70.88, 72.10 (x2), 72.32, 72.48, 74.47 (x2), 74.63, 74.75

(x2), 75.23, 78.37 (x2), 78.69, 81.93 (x2), 100.90, 102.47 (x2), 102.79 (x2), 174.20, 174.72 (x2); HRMS (ESI-TOF) calcd for $C_{48}H_{82}N_6O_{31}Na$ $[M+Na]^+$: 1261.4922; found 1261.4926.

Synthesis of GlcNAc-(LacNAc)$_3$ (7)

Compound **7** was prepared by following the procedures described for the synthesis of GlcNAc-LacNAc (**3**), with an isolated yield of 93% (34 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.18-1.28 (m, 4H), 1.40-1.49 (m, 4H), 1.90 (s, 12H), 3.19 (t, *J* = 6.9 Hz, 2H), 3.30-3.36 (m, 2H), 3.41-3.38 (m, 8H), 3.55-3.61 (m, 22H), 3.62-3.72 (m, 10H), 3.75-3.77 (m, 2H), 3.81-3.85 (m, 3H), 4.02 (d, *J* = 2.5 Hz, 3H), 4.32-3.34 (m, 3H), 4.38 (d, *J* = 7.7 Hz, 1H), 4.54-4.57 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 23.25 (x4), 25.71, 26.64, 29.06, 29.49, 52.22, 56.17, 56.23 (x2), 56.74, 60.40 (x3), 60.93 (x2), 61.15, 61.56, 62.04 (x2), 62.53, 69.41 (x2), 70.77, 71.05 (x2), 71.48, 73.26 (x2), 73.53, 74.65, 75.63 (x2), 75.83, 75.96 (x2), 76.74, 79.24 (x2), 79.56, 83.07, 83.14 (x2), 102.13, 103.84 (x2), 103.97 (x4), 175.48, 176.00 (x3); HRMS (ESI-TOF) calcd for $C_{56}H_{93}N_7O_{36}Na_2$ $[M+2Na]^{2+}$: 743.7807; found 743.7816.

Synthesis of (LacNAc)$_4$ (8)

Compound **8** was prepared by following the procedures described for the synthesis of LacNAc (**2**), with an isolated yield of 43% (15 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.13-1.22 (m, 4H), 1.40-1.49 (m, 4H), 1.85 (s, 12H), 3.14 (t, *J* = 7.0 Hz, 2H), 3.34-3.43 (m, 18H), 3.49-3.59 (m, 24H), 3.60-3.68 (m, 8H), 3.69-3.81 (m, 6H), 3.94-3.99 (m, 3H), 4.25-4.30 (m, 4H), 4.33 (d, *J* = 7.5 Hz, 1H), 4.49-4.53 (m, 3H); ^{13}C NMR (150 MHz, D_2O) δ 23.26 (x4), 25.72, 26.65, 29.06, 29.50, 52.22, 56.17, 56.23 (x2), 56.27, 60.93 (x3), 61.15, 62.04 (x2), 62.12, 62.93 (x2), 69.41 (x2), 69.64, 71.05 (x2), 71.48, 72.05, 73.26 (x2), 73.53, 73.59, 75.64 (x2), 75.83, 75.96 (x2), 76.44, 79.23 (x2), 79.55, 83.15 (x2), 102.14, 103.85 (x3), 103.98 (x4), 175.48, 176.00 (x3); HRMS (ESI-TOF) calcd for $C_{62}H_{106}N_7O_{41}$ $[M+H]^+$: 1604.6425; found 1604.6416.

Synthesis of α-2,3-Sialyl LacNAc (9)

A buffered (50 mM Tris-HCl, pH of 8.5) solution of **2** (10 mM) containing $MgCl_2$ (10 mM) was incubated at 37 °C with CMP-Neu5Ac (15 mM) in the presence of PmST

(50 μg mL^{-1}) for 8 h. The reaction was quenched by the addition of an equal volume of EtOH when TLC analysis (EtOAc/MeOH/H_2O/HOAc=3/2/1/0.5) indicated the completion of the reaction. The reaction solution was centrifuged (10,000 x *g*, 10 min) to remove any proteins and insoluble precipitates. The supernatant was filtered (0.45-μm filter; Millipore), concentrated, and passed through a P-2 gel to obtain the desired product. The product-containing fractions identified by TLC analysis were pooled together and then lyophilized to give **9** with an isolation yield of 82% (12.9 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.21-1.29 (m, 4H), 1.42-1.51 (m, 4H), 1.68 (t, *J* = 12.2 Hz, 1H), 1.91 (s, 6H), 2.63 (dd, *J* = 4.6, 12.4 Hz, 1H), 3.20 (t, *J* = 6.9 Hz, 2H), 3.43-3.53 (m, 6H), 3.55-3.65 (m, 8H), 3.71-3.75 (m, 2H), 3.76-3.80 (m, 2H), 3.83 (d, *J* = 3.1 Hz, 1H), 3.88 (dd, *J* = 2.2, 12.3 Hz, 1H), 4.00 (dd, *J* = 3.1, 9.9 Hz, 1H), 4.40 (d, *J* = 7.9 Hz, 1H), 4.43 (d, *J* = 7.9 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 22.01, 22.15, 24.59, 25.53, 27.94, 28.38, 39.60, 51.11, 51.66, 55.08, 60.05, 60.18, 60.99, 62.56, 67.45, 68.08, 68.31, 69.36, 70.34, 71.73, 72.39, 72.86, 74.72, 75.14, 75.46, 78.32, 99.79, 101.06, 102.54, 173.83, 174.34, 174.99; HRMS (ESI-TOF) calcd for $C_{31}H_{52}N_5O_{19}$ $[M-H]^-$: 798.3257; found 798.3248.

Synthesis of α-2,3-Sialyl $(LacNAc)_2$ (10)

Compound **10** was prepared by following the procedures described for the synthesis of α-2,3-Sialyl LacNAc (**9**), with an isolated yield of 54% (6.4 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.19-1.27 (m, 4H), 1.41-1.50 (m, 4H), 1.67 (t, *J* = 12.2 Hz, 1H), 1.90 (s, 9H), 2.63 (dd, *J* = 4.6, 12.4 Hz, 1H), 3.19 (t, *J* = 6.9 Hz, 2H), 3.43-3.48 (m, 6H), 3.50 (d, *J* = 1.9 Hz, 1H), 3.52 (dd, *J* = 1.9, 3.6 Hz, 1H), 3.53-3.62 (m, 12H), 3.62-3.65 (m, 2H), 3.66-3.71 (m, 3H), 3.72-3.78 (m, 5H), 3.83-3.87 (m, 3H), 3.99 (dd, *J* = 3.2, 9.9 Hz, 1H), 4.03 (d, *J* = 3.3 Hz, 1H), 4.33 (d, *J* = 7.9 Hz, 1H), 4.39 (d, *J* = 7.9 Hz, 1H), 4.43 (d, *J* = 7.9 Hz, 1H), 4.57 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 22.00, 22.14 (x2), 24.59, 25.52, 27.93, 28.37, 39.61, 51.10, 51.65, 55.04, 55.15, 59.82, 60.05, 60.93, 61.00, 62.55, 67.45, 68.06, 68.26, 68.31, 69.35, 69.94, 70.35, 71.73, 72.12, 72.41, 72.86, 74.53, 74.72, 74.86, 75.14, 75.47, 77.99, 78.49, 82.04, 99.78, 101.02, 102.52, 102.76, 102.87, 173.82, 174.35, 174.86, 174.99; HRMS (ESI-TOF) calcd for $C_{45}H_{75}N_6O_{29}$ $[M-H]^-$: 1163.4578; found: 1163.4567.

Synthesis of α-2,3-Sialyl $(LacNAc)_3$ (11)

Compound **11** was prepared by following the procedures described for the synthesis of α-2,3-Sialyl LacNAc (**9**), with an isolated yield of 73% (9 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.29-1.36 (m, 4H), 1.51-1.59 (m, 4H), 1.76 (t, *J* = 12.2 Hz, 1H), 1.99 (s, 12H), 2.72 (dd, *J* = 4.6, 12.3 Hz, 1H), 3.28 (t, *J* = 6.9 Hz, 2H), 3.52-3.57 (m, 8H), 3.59-3.61 (m, 2H), 3.62-3.64 (m, 1H), 3.65-3.74 (m, 21H), 3.75-3.80 (m, 4H), 3.81-3.83 (m, 2H), 3.84-3.87 (m, 3H), 3.90-3.95 (m, 4H), 4.08 (dd, *J* = 3.0, 9.9 Hz, 1H), 4.12-4.13 (m, 2H), 4.42 (d, *J* = 4.8 Hz, 1H), 4.43 (d, *J* = 4.8 Hz, 1H), 4.48 (d, *J* = 7.8 Hz, 1H), 4.52 (d, *J* = 7.8 Hz, 1H), 4.65-4.67 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 22.02, 22.16 (x2), 24.51, 25.54, 27.95, 28.39, 39.63, 51.12 (x2), 51.67, 55.07, 55.13, 55.17, 59.53, 59.84 (x2), 60.06, 60.94 (x2), 61.02, 62.57, 67.46, 68.08, 68.30 (x2), 69.37, 69.95 (x2), 70.37, 71.75, 72.16 (x2), 72.43, 72.88, 74.54 (x2), 74.73, 74.86 (x2), 75.16, 75.48, 78.00, 78.17, 78.48, 82.05 (x2), 99.80, 101.03, 102.54, 102.73, 102.78, 102.88 (x2), 173.84, 174.37, 174.88 (x2), 175.01; HRMS (ESI-TOF) calcd for $C_{59}H_{98}N_7O_{39}$ $[M-H]^-$: 1528.5900; found 1528.5914.

Synthesis of α-2,6-Sialyl LacNAc (12)

Compound **12** was prepared by following the procedures described for the synthesis of α-2,3-Sialyl LacNAc (**9**), except using Pd2,6ST (50 μg mL^{-1}), with an isolated yield of **12** of 99% (16.4 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.21-1.29 (m, 4H), 1.41-1.50 (m, 4H), 1.59 (t, *J* = 12.2 Hz, 1H), 1.91 (s, 3H), 1.94 (s, 3H), 2.55 (dd, *J* = 4.6, 12.4 Hz, 1H), 3.20 (t, *J* = 6.9 Hz, 2H), 3.40-3.45 (m, 3H), 3.47-3.57 (m, 7H), 3.58-3.62 (m, 4H), 3.66-3.72 (m, 3H), 3.74-3.79 (m, 3H), 3.85-3.88 (m, 2H), 4.33 (d, *J* = 8.0 Hz, 1H), 4.43 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.96, 22.23, 24.56, 25.49, 27.90, 28.35, 40.02, 51.07, 51.83, 54.84, 59.33, 60.33, 62.59, 63.27, 68.13, 68.33, 70.28, 70.67, 71.63, 72.36, 72.41, 72.48, 73.61, 74.40, 80.69, 100.09, 100.81, 103.40, 173.44, 174.32, 174.87; HRMS (ESI-TOF) calcd for $C_{31}H_{52}N_5O_{19}$ $[M-H]^-$: 798.3257; found 798.3265.

Synthesis of α-2,6-Sialyl GlcNAc-LacNAc (13)

Compound **13** was prepared by following the procedures described for the synthesis of **12**, with an isolated yield of 57% (15.1 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.18-1.26 (m, 4H), 1.40-1.48 (m, 4H), 1.56 (t, *J* = 12.2 Hz, 1H), 1.88 (s, 3H), 1.89 (s, 3H), 1.91 (s, 3H), 2.52 (dd, *J* = 4.6, 12.3 Hz, 1H), 3.18 (t, *J* = 6.9 Hz, 2H), 3.37-3.42 (m,

3H), 3.43-3.52 (m, 6H), 3.54-3.59 (m, 6H), 3.61-3.63 (m, 2H), 3.65-3.68 (m, 3H), 3.71-3.78 (m, 5H), 3.82-3.85 (m, 2H), 4.02 (d, *J* = 3.2 Hz, 1H), 4.29 (d, *J* = 8.0 Hz, 1H), 4.40 (d, *J* = 8.1 Hz, 1H), 4.51 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.85, 21.99, 22.12, 24.45, 25.39, 27.79, 28.24, 39.94, 50.97, 51.72, 54.70, 55.49, 60.23, 60.31, 62.48, 63.05, 67.87, 68.02, 68.22, 69.45, 69.53, 70.18, 71.49, 72.33, 72.38, 73.08, 73.47, 74.30, 75.48, 80.61, 82.05, 99.99, 100.72, 102.71, 103.31, 173.33, 174.22, 174.74, 174.77; HRMS (ESI-TOF) calcd for $C_{39}H_{65}N_6O_{24}$ $[M-H]^-$: 1001.4050; found 1001.4043.

Synthesis of mono-α-2,6-Sialyl (LacNAc)$_2$ (14)

Compound **14** was prepared by following the procedures described for the synthesis of **12**, with an isolated yield of 72% (8.6 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.20- 1.28 (m, 4H), 1.43-1.51 (m, 4H), 1.60 (t, *J* = 12.1 Hz, 1H), 1.91 (s, 6H), 1.94 (s, 3H), 2.56 (dd, *J* = 4.6, 12.1 Hz, 1H), 3.20 (t, *J* = 6.9 Hz, 2H), 3.40-3.54 (m, 11H), 3.58-3.62 (m, 12H), 3.66-3.71 (m, 5H), 3.74-3.90 (m, 8H), 4.03-4.06 (m, 1H), 4.34 (t, *J* = 7.2 Hz, 2H), 4.40 (d, *J* = 7.2 Hz, 1H), 4.62 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.99, 22.14, 22.25, 24.58, 25.52, 27.93, 28.37, 40.04, 51.10, 51.85, 54.91, 55.02, 59.83, 60.03, 60.11, 60.65, 60.92, 62.62, 63.31, 68.17, 68.26, 68.37, 69.94, 70.34, 70.70, 71.68, 72.20, 72.40, 72.50, 73.66, 74.23, 74.71, 74.84, 78.48, 80.43, 81.97, 100.11, 101.02, 102.54, 102.87, 103.42, 173.50, 174.34, 174.89 (x2); HRMS (ESI-TOF) calcd for $C_{45}H_{75}N_6O_{29}$ $[M-H]^-$: 1163.4578; found 1163.4557.

Synthesis of di-α-2,6-Sialyl (LacNAc)$_2$ (15)

Compound **15** was prepared by following the procedures described for the synthesis of **12** but using 2.5 equivalents of CMP-NeuAc, with an isolated yield of 99% (16.6 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.20-1.28 (m, 4H), 1.43-1.52 (m, 4H), 1.58 (t, *J* = 12.1 Hz, 1H), 1.60 (t, *J* = 12.2 Hz, 1H), 1.91 (s, 6H), 1.93 (s, 3H), 1.94 (s, 3H), 2.54 (dd, *J* = 4.3, 12.3 Hz, 1H), 2.55 (dd, *J* = 4.0, 12.4 Hz, 1H), 3.21 (t, *J* = 6.9 Hz, 2H), 3.40-3.56 (m, 16H), 3.57-3.62 (m, 7H), 3.66-3.72 (m, 7H), 3.74-3.89 (m, 10H), 4.05 (d, *J* = 3.1 Hz, 1H), 4.32 (d, *J* = 7.9 Hz, 1H), 4.33 (d, *J* = 7.8 Hz, 1H), 4.43 (d, *J* = 7.7 Hz, 1H), 4.59 (d, *J* = 7.9 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.90 (x2), 22.17 (x2), 24.49, 25.43, 27.83, 28.28, 39,98, 51.01, 51.76 (x2), 54.74, 54.82, 59.59 (x2), 60.02, 60.26, 62.54 (x2), 63.12, 63.20, 67.91, 68.08 (x2), 68.28 (x2), 69.49, 70.22, 70.61,

71.52, 71.58, 72.20, 72.29, 72.42 (x2), 73.13, 73.54, 74.14, 74.36, 80.40, 80.66, 82.12, 100.03 (x2), 100.77, 102.49, 103.35, 103.39, 173.40 (x2), 174.26, 174.74, 174.79 (x2); HRMS (ESI-TOF) calcd for $C_{56}H_{93}N_7O_{37}$ $[M-2H]^{2-}$: 726.7727; found 726.7730.

Synthesis of mono-α-2,6-Sialyl (LacNAc)$_3$ (16) and di-α-2,6-Sialyl (LacNAc)$_3$ (17)

Compounds **16** and **17** were prepared by following the procedures described for the synthesis of **12** but using 2 equivalents of CMP-NeuAc, with isolated yields of 32% (5.1 mg) and 56% (10.6 mg), respectively; for mono-α-2,6-Sialyl (LacNAc)$_3$ (**16**): ^{1}H NMR (600 MHz, D_2O) δ 1.22-1.31 (m, 4H), 1.45-1.54 (m, 4H), 1.63 (t, *J* = 12.2 Hz, 1H), 1.94 (s, 3H), 1.95 (s, 6H), 1.96 (s, 3H), 2.58 (dd, *J* = 4.6, 12.3 Hz, 1H), 3.23 (t, *J* = 6.9 Hz, 2H), 3.43-3.48 (m, 3H), 3.49-3.58 (m, 10H), 3.59-3.61 (m, 6H), 3.62-3.67 (m, 11H), 3.68-3.75 (m, 9H), 3.76-3.81 (m, 5H), 3.83-3.85 (m, 2H), 3.87-3.92 (m, 3H), 4.06-4.08 (m, 2H), 4.35-4.39 (m, 3H), 4.43 (d, *J* = 7.8 Hz, 1H), 4.60-4.62 (m, 2H); ^{13}C NMR (150 MHz, D_2O) δ 21.87, 22.02 (x2), 22.14, 24.45, 25.39, 27.79, 28.25, 39.93, 50.98, 51.74, 54.81, 54.93, 54.98, 59.71, 59.93 (x2), 60.01, 60.79 (x2), 62.52, 63.19, 68.05, 68.14, 68.27 (x2), 69.82 (x2), 70.22, 70.59, 71.55, 72.03, 72.07, 72.28 (x2), 72.39, 73.54, 74.13, 74.40, 74.58, 74.72 (x2), 78.09, 78.39, 80.32, 81.85, 81.90, 100.00, 100.88, 102.40, 102.57, 102.74 (x2), 103.29, 173.36, 174.21, 174.76 (x3); HRMS (ESI-TOF) calcd for $C_{59}H_{98}N_7O_{39}$ $[M-H]^-$: 1528.5900; found 1528.5914.

For di-α-2,6-Sialyl (LacNAc)$_3$ (**17**): ^{1}H NMR (600 MHz, D_2O) δ 1.23-1.30 (m, 4H), 1.45-1.52 (m, 4H), 1.59 (t, *J* = 12.1 Hz, 1H), 1.61 (t, *J* = 12.1 Hz, 1H), 1.92 (s, 6H), 1.93 (s, 3H), 1.95 (s, 3H), 1.96 (s, 3H), 2.55 (dd, *J* = 4.1, 12.1 Hz, 1H), 2.57 (dd, *J* = 4.2, 12.1 Hz, 1H), 3.22 (t, *J* = 6.9 Hz, 2H), 3.42-3.46 (m, 5H), 3.47-3.51 (m, 6H), 3.53-3.57 (m, 7H), 3.57-3.64 (m, 10H), 3.67-3.74 (m, 11H), 3.75-3.84 (m, 8H), 3.84-3.91 (m, 3H), 4.05-4.07 (m, 2H), 4.33-4.37 (m, 3H), 4.41 (d, *J* = 7.7 Hz, 1H), 4.61 (d, *J* = 7.9 Hz, 1H), 4.62 (d, *J* = 8.5 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.89 (x2), 22.04, 22.15 (x2), 24.48, 25.42, 27.82, 28.27, 39.96, 51.01, 51.76 (x2), 54.82 (x2), 54.93, 60.02 (x2), 60.81, 62.53, 63.13, 63.20, 67.90, 68.08 x2, 68.18, 68.28 (x2), 69.49, 69.86 (x2), 70.24, 70.61, 71.53, 71.57, 72.13, 72.20, 72.30 (x2), 72.41 (x2), 73.14, 73.54, 74.15 (x2), 74.61, 74.75 (x2), 78.40, 80.36, 80.42, 80.67, 81.87, 82.12 (x2), 100.03 (x2), 100.91, 102.48 (x2), 102.76, 103.33, 103.40, 173.38 (x2), 174.24, 174.78 (x4); HRMS (ESI-TOF) calcd for $C_{70}H_{114}N_8O_{47}H_2$ $[M-2H]^{2-}$: 909.3388; found 909.3391.

Synthesis of tri-α-2,6-Sialyl (LacNAc)$_3$ (18)

Compound **18** was prepared by following the procedures described for the synthesis of **12** but using 5 equivalents of CMP-NeuAc, with an isolated yield of 85% (18.8 mg); ^{1}H NMR (600 MHz, D_2O) δ 1.22-1.29 (m, 4H), 1.43-1.52 (m, 4H), 1.57-1.63 (m, 3H), 1.91 (s, 9H), 1.93 (s, 6H), 1.94 (s, 3H), 2.53-2.57 (m, 3H), 3.21 (t, *J* = 6.9 Hz, 2H), 3.41-3.54 (m, 24H), 3.58-3.62 (m, 9H), 3.66-3.73 (m, 11H), 3.74-3.89 (m, 16H), 4.05 (d, *J* = 3.1 Hz, 2H), 4.32-4.34 (m, 3H), 4.43 (d, *J* = 8.1 Hz, 1H), 4.58 (d, *J* = 8.1 Hz, 1H), 4.59 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (150 MHz, D_2O) δ 21.86 (x3), 22.12 (x3), 24.45, 25.39, 27.79, 28.24, 39.93 (x2), 50.97, 51.72 (x3), 54.71 (x2), 54.76 (x2), 59.26 (x2), 59.96 (x2), 60.22, 62.50 (x2), 63.08 (x2), 63.16, 67.89, 68.04 (x3), 68.24 (x3), 69.46 (x2), 69.81, 70.18 (x2), 70.57, 71.49 (x2), 71.54, 72.16 (x2), 72.25, 72.37 x3, 73.08 (x2), 73.50, 74.10 (x2), 74.32, 80.36 (x2), 80.61, 82.03 (x2), 99.99 x3, 100.73, 102.46 (x2), 103.30, 103.35 (x2), 173.35 (x3), 174.22, 174.75 (x5); HRMS (ESI-TOF) calcd for $C_{81}H_{133}N_9O_{55}$ $[M-3H]^{3-}$: 702.9218; found 702.9227; Calcd for $C_{81}H_{133}N_9O_{55}$ $[M-2H]^{2-}$: 1054.8865; found 1054.8875.

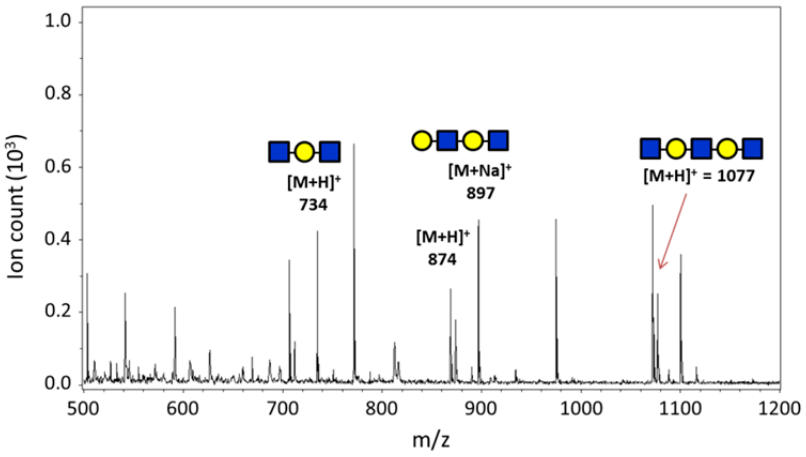

Figure S1. MALDI-TOF mass spectrometry analysis of the one-pot synthesis of oligo-LacNAc.

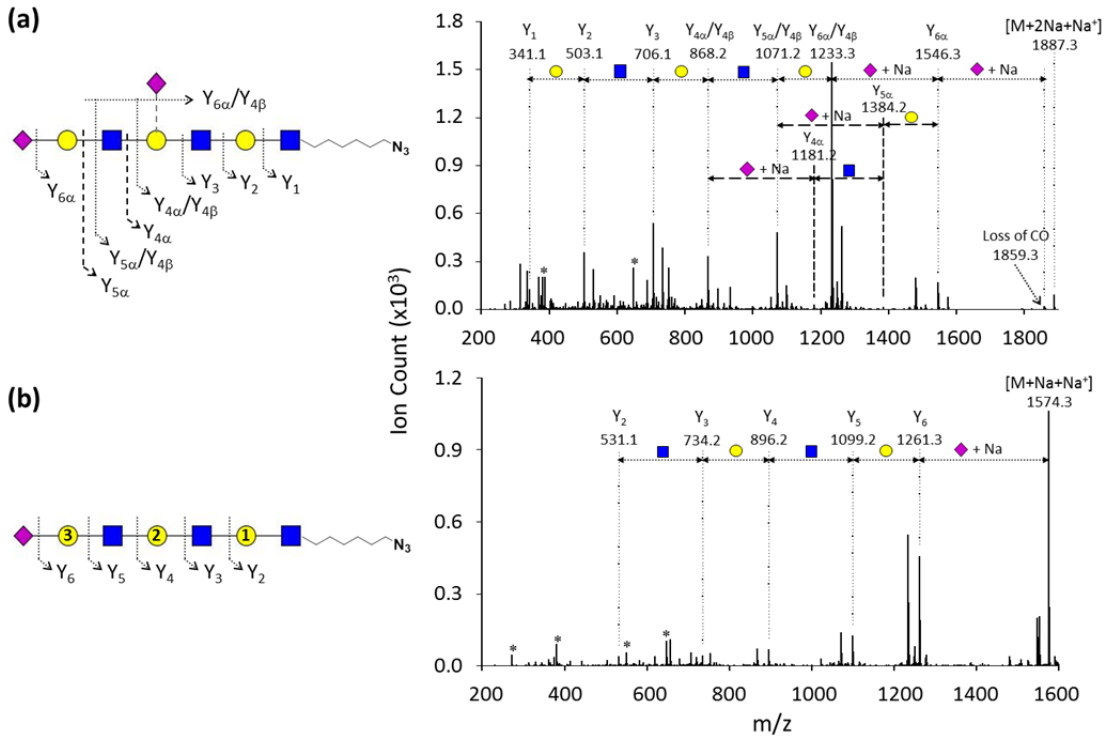

Figure S2. Site Determination Analysis: DHB@Fe_3O_4 nanoparticle-assisted MALDI mass spectra of (a) disialylated $(LacNAc)_3$ and (b) monosialylated $(LacNAc)_3$ at a ratio of 1:0.1 (DHB:DHB@Fe_3O_4) matrix. The matrix-derived ("*") peaks are shown.

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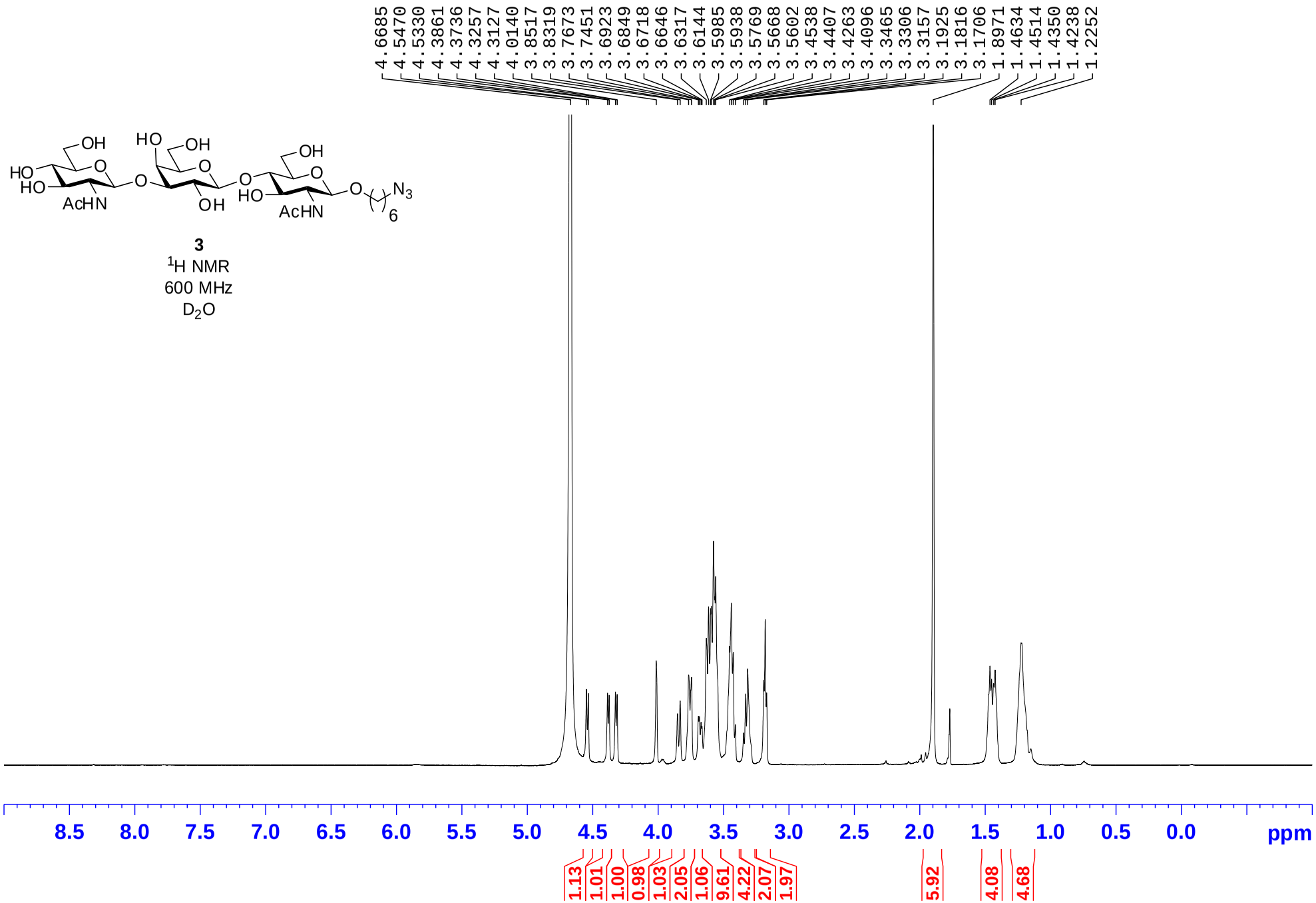
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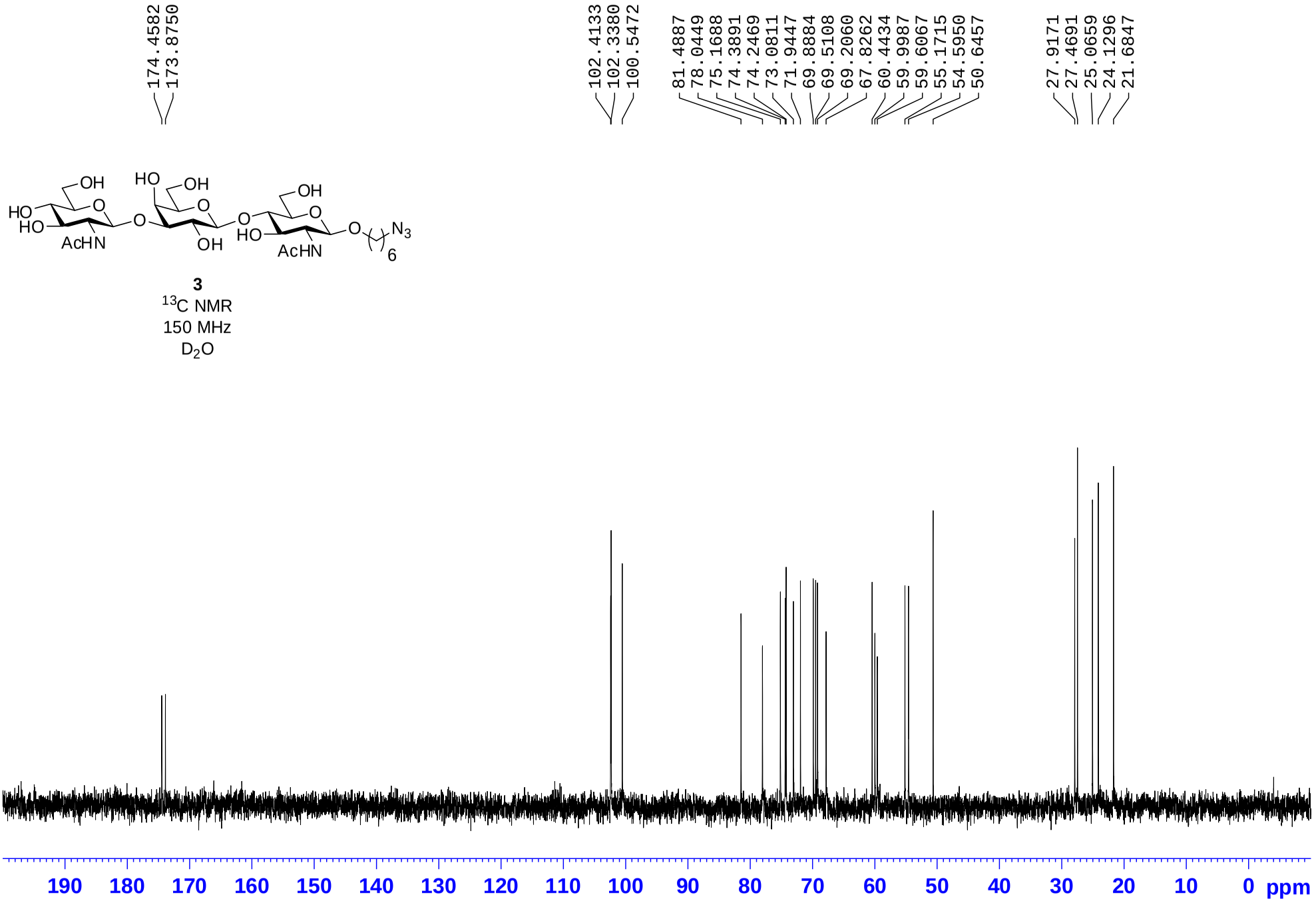
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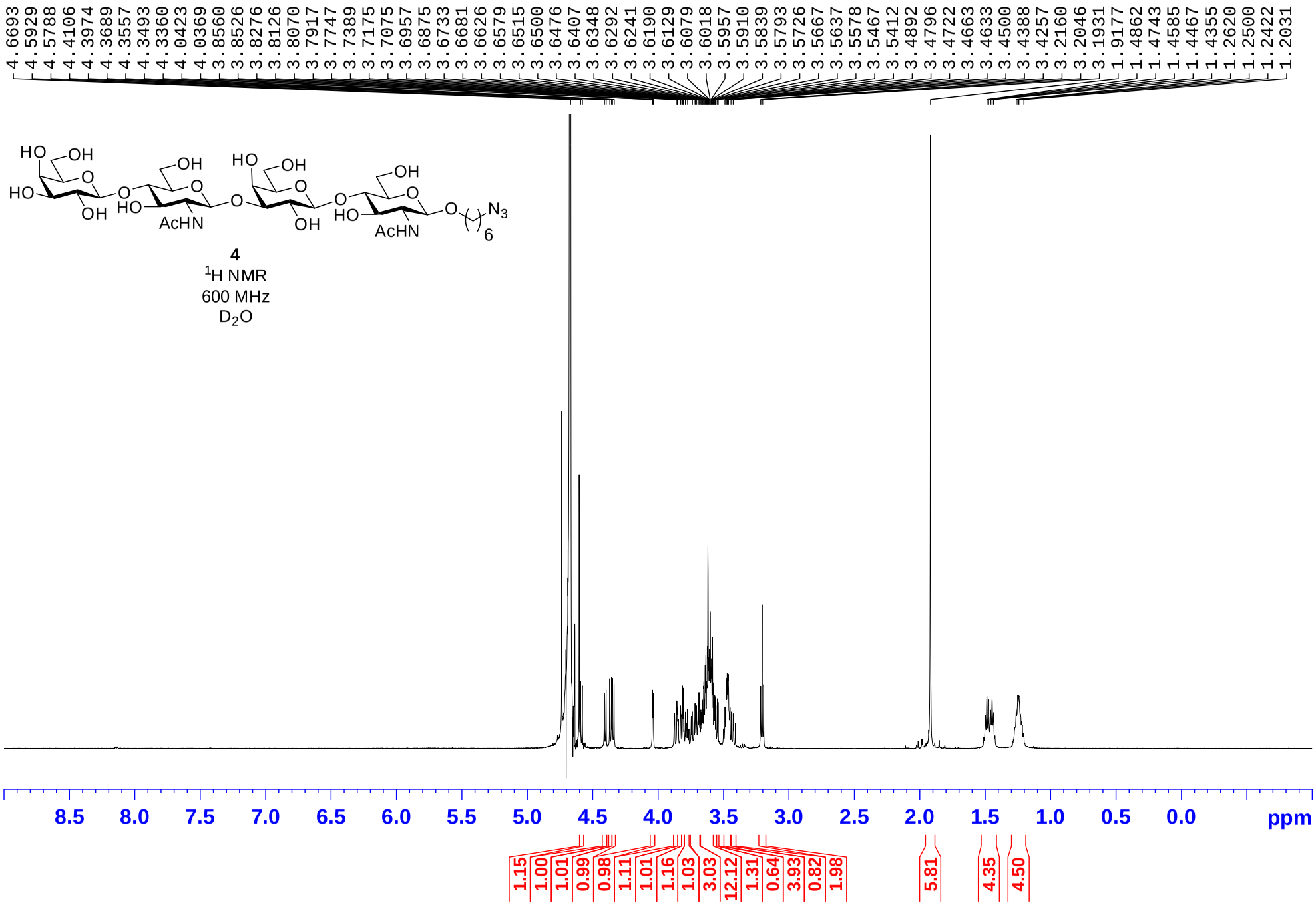
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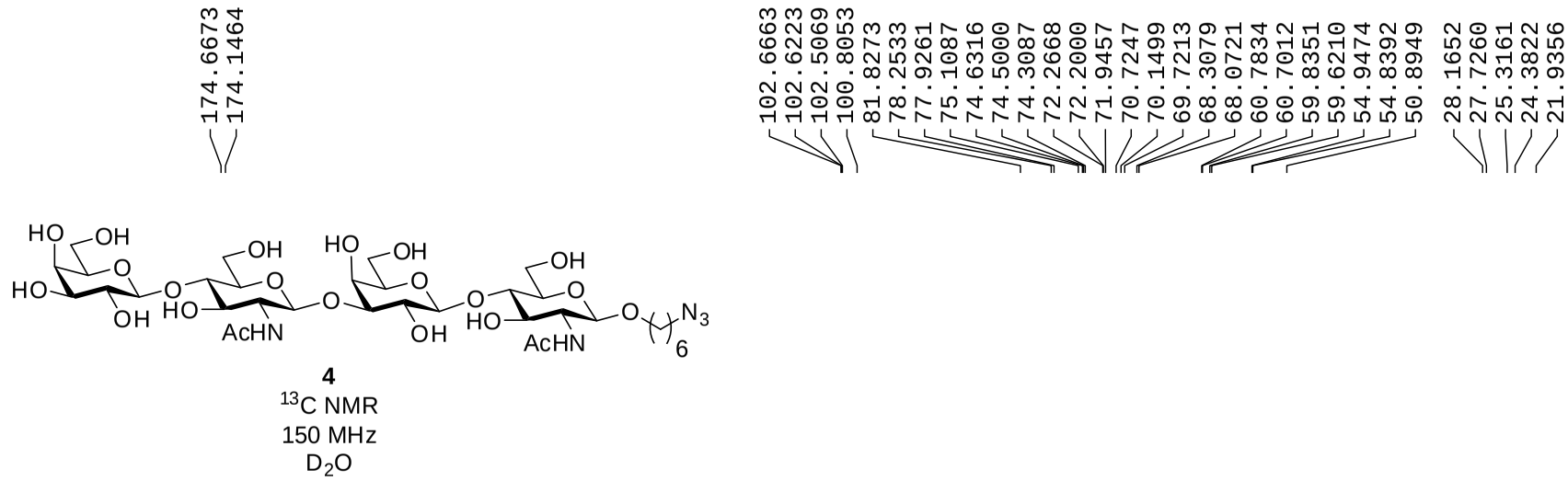
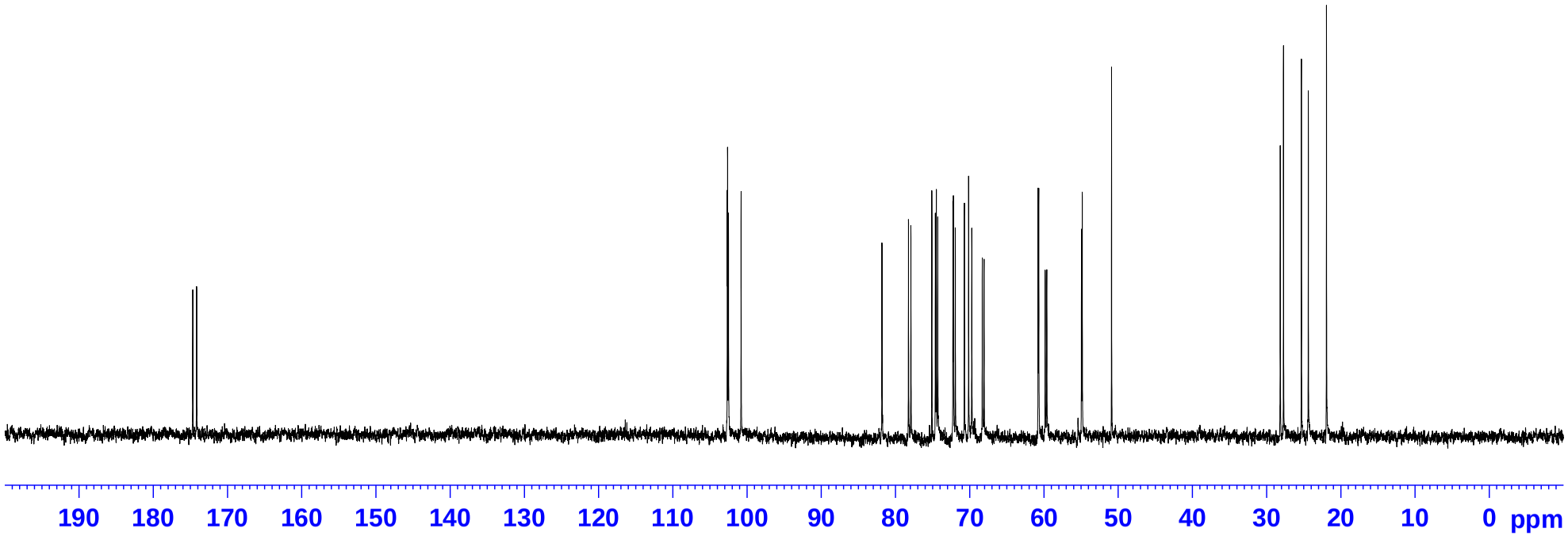


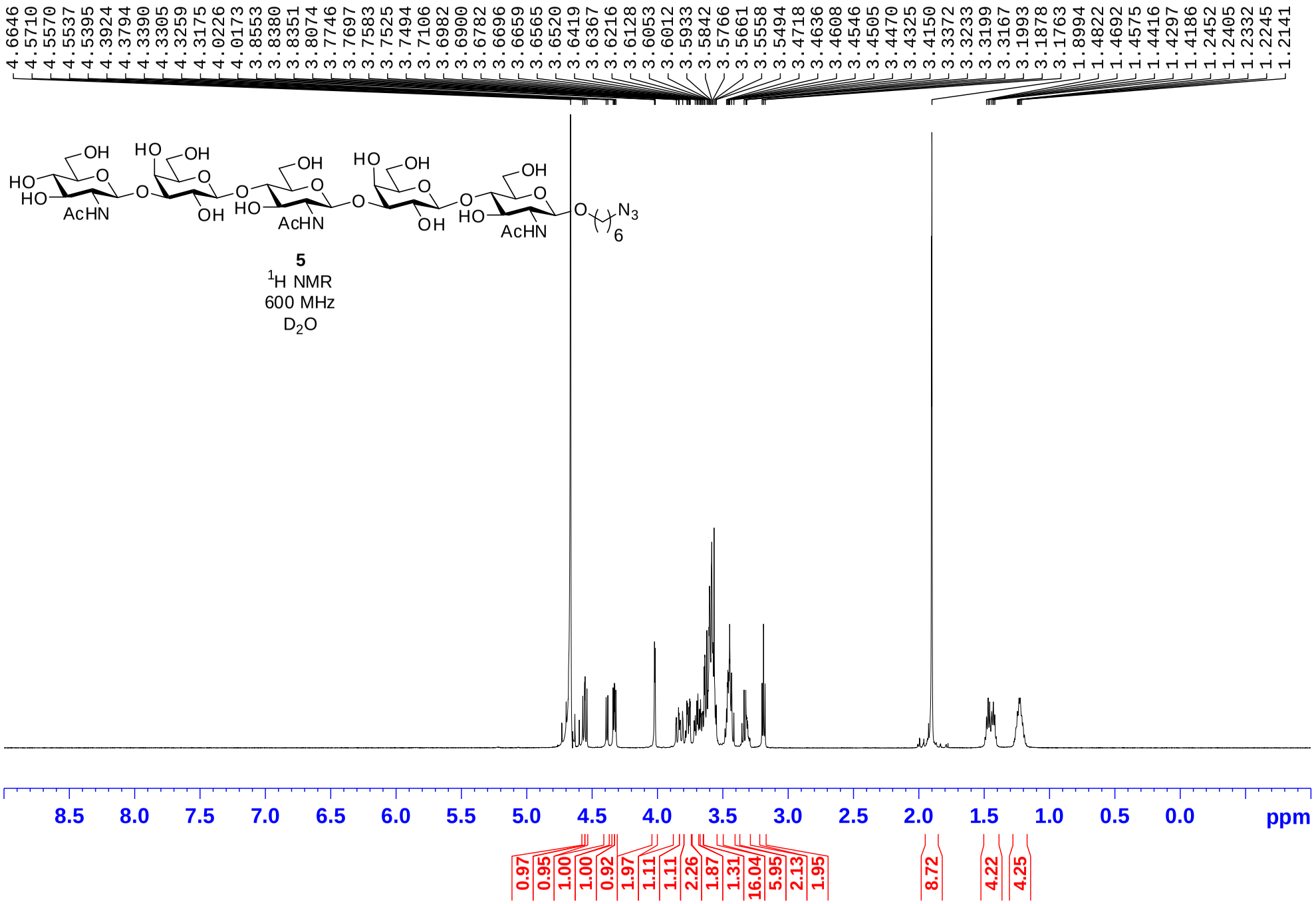
4

^{13}C NMR

150 MHz

D_2O

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4.5570
4.5537
4.5395
4.3924
4.3794
4.3390
4.3305
4.3259
4.3175
4.0226
4.0173
3.8553
3.8380
3.8351
3.8074
3.7746
3.7697
3.7583
3.7525
3.7494
3.7106
3.6982
3.6900
3.6782
3.6696
3.6659
3.6565
3.6520
3.6419
3.6367
3.6216
3.6128
3.6053
3.6012
3.5933
3.5842
3.5766
3.5661
3.5558
3.5494
3.4718
3.4636
3.4608
3.4546
3.4505
3.4470
3.4325
3.4150
3.3372
3.3233
3.3199
3.3167
3.1993
3.1878
3.1763
1.8994
1.4822
1.4692
1.4575
1.4416
1.4297
1.4186
1.2452
1.2405
1.2332
1.2245
1.2141
5
1H NMR
600 MHz
D2O
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
ppm
0.97
0.95
1.00
1.00
0.92
1.97
1.11
1.11
2.26
1.87
1.31
16.04
5.95
2.13
1.95
8.72
4.22
4.25

174.7648
174.7122
174.1900

102.7141
102.6513
102.5415
100.8557
81.8674
81.7921
78.3332
78.0250
75.4755
74.6870
74.5540
74.3622
73.3823
72.2526
71.9918
70.1995
69.8142
69.7802
69.5069
68.1298
60.7507
60.3032
59.8981
59.7513
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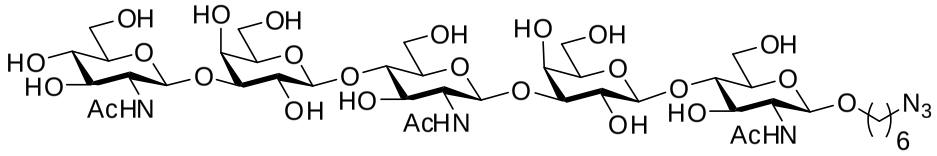
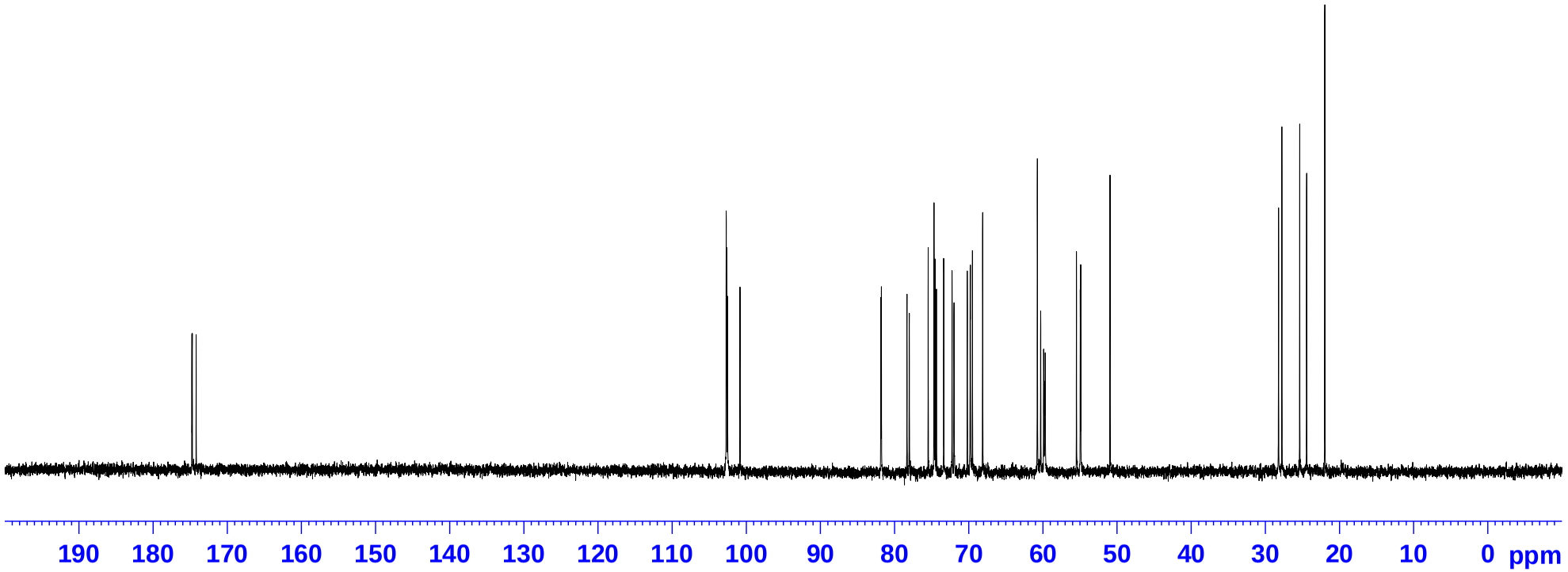
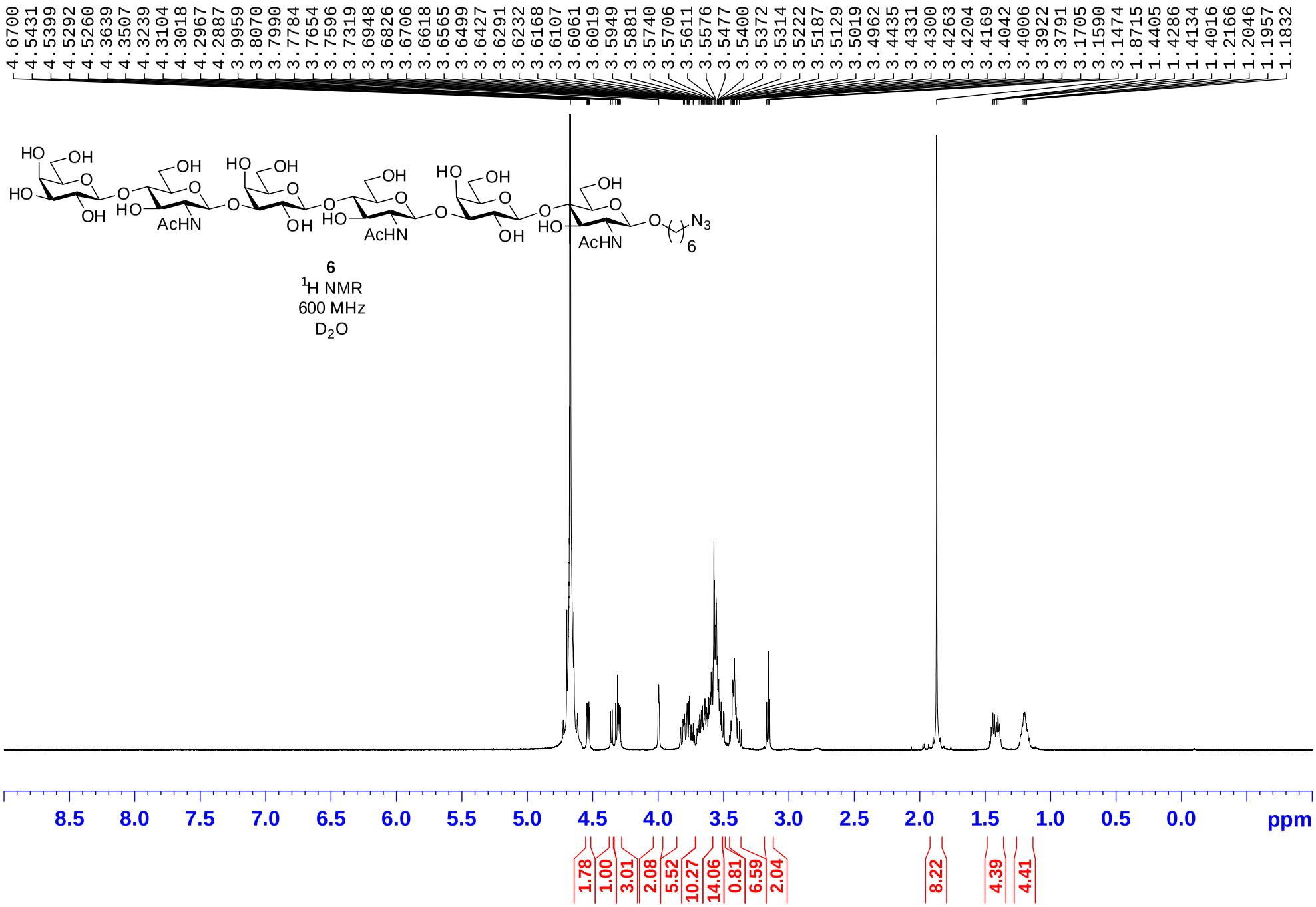
21.9879



5
^{13}C NMR
150 MHz
D_2O

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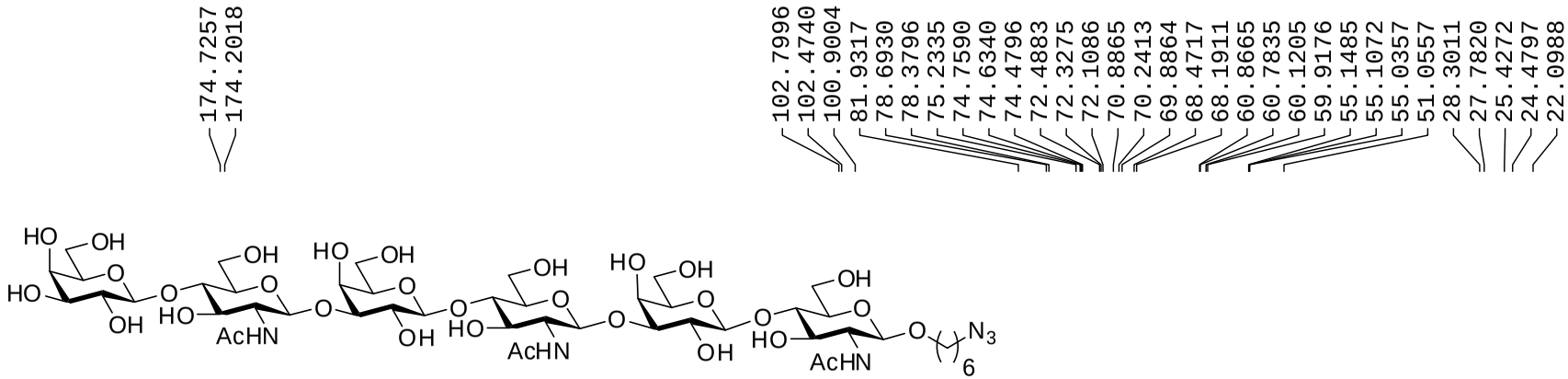
4.5399
4.5292
4.5260
4.3639
4.3507
4.3239
4.3104
4.3018
4.2967
4.2887
3.9959
3.8070
3.7990
3.7784
3.7654
3.7596
3.7319
3.6948
3.6826
3.6706
3.6618
3.6565
3.6499
3.6427
3.6291
3.6232
3.6168
3.6107
3.6061
3.6019
3.5949
3.5881
3.5740
3.5706
3.5611
3.5576
3.5477
3.5400
3.5372
3.5314
3.5222
3.5187
3.5129
3.5019
3.4962
3.4435
3.4331
3.4300
3.4263
3.4204
3.4169
3.4042
3.4006
3.3922
3.3791
3.1705
3.1590
3.1474
1.8715
1.4405
1.4286
1.4134
1.4016
1.2166
1.2046
1.1957
1.1832
HO
OH
O
HO
OH
AcHN
N3
6
6
1H NMR
600 MHz
D2O
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
ppm
1.78
1.00
3.01
2.08
5.52
10.27
14.06
0.81
6.59
2.04
8.22
4.39
4.41

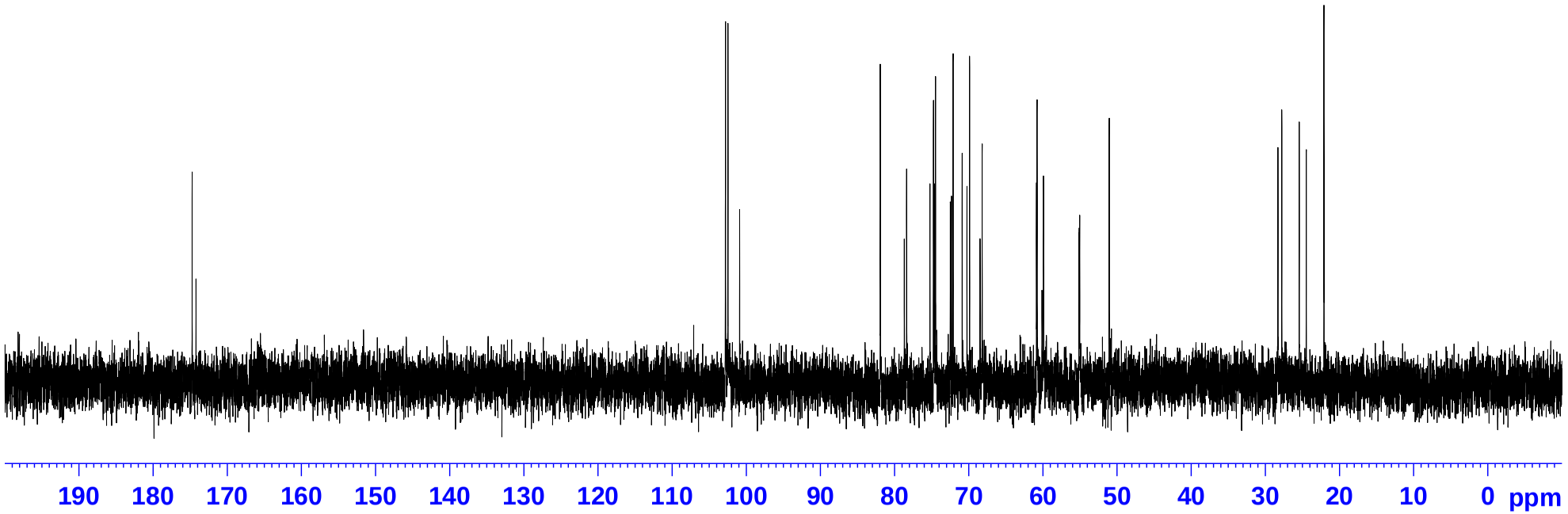


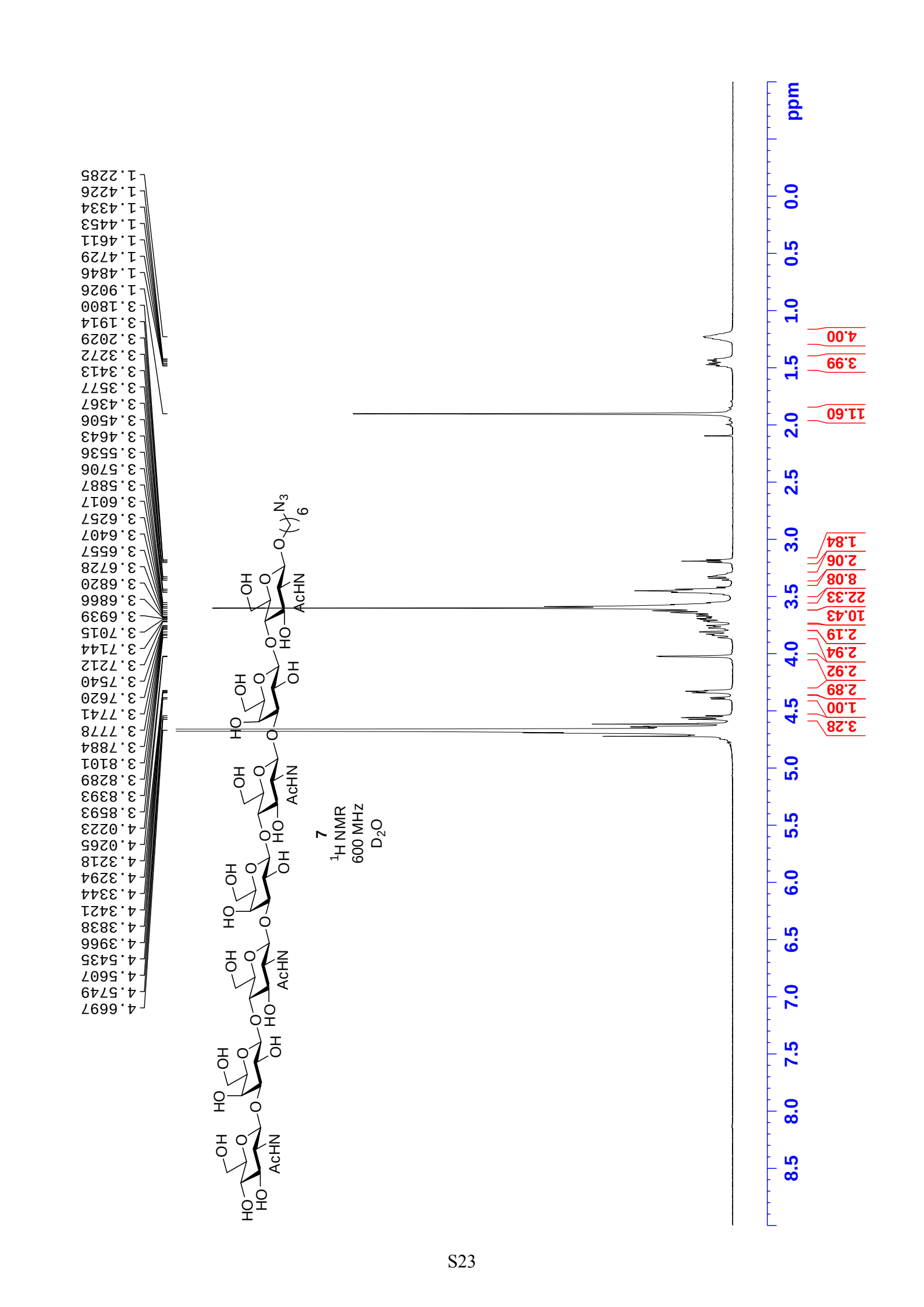
6

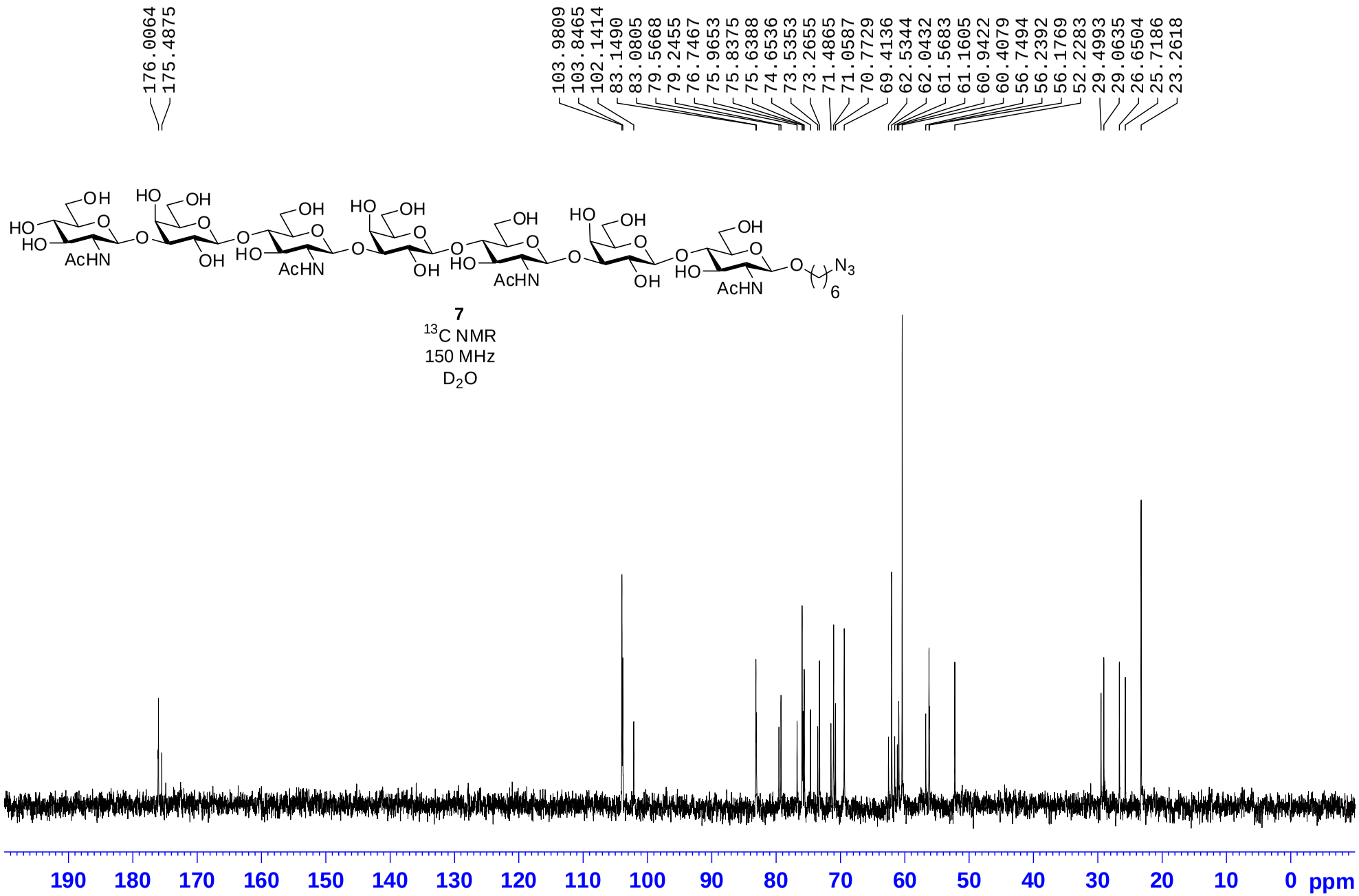
^{13}C NMR

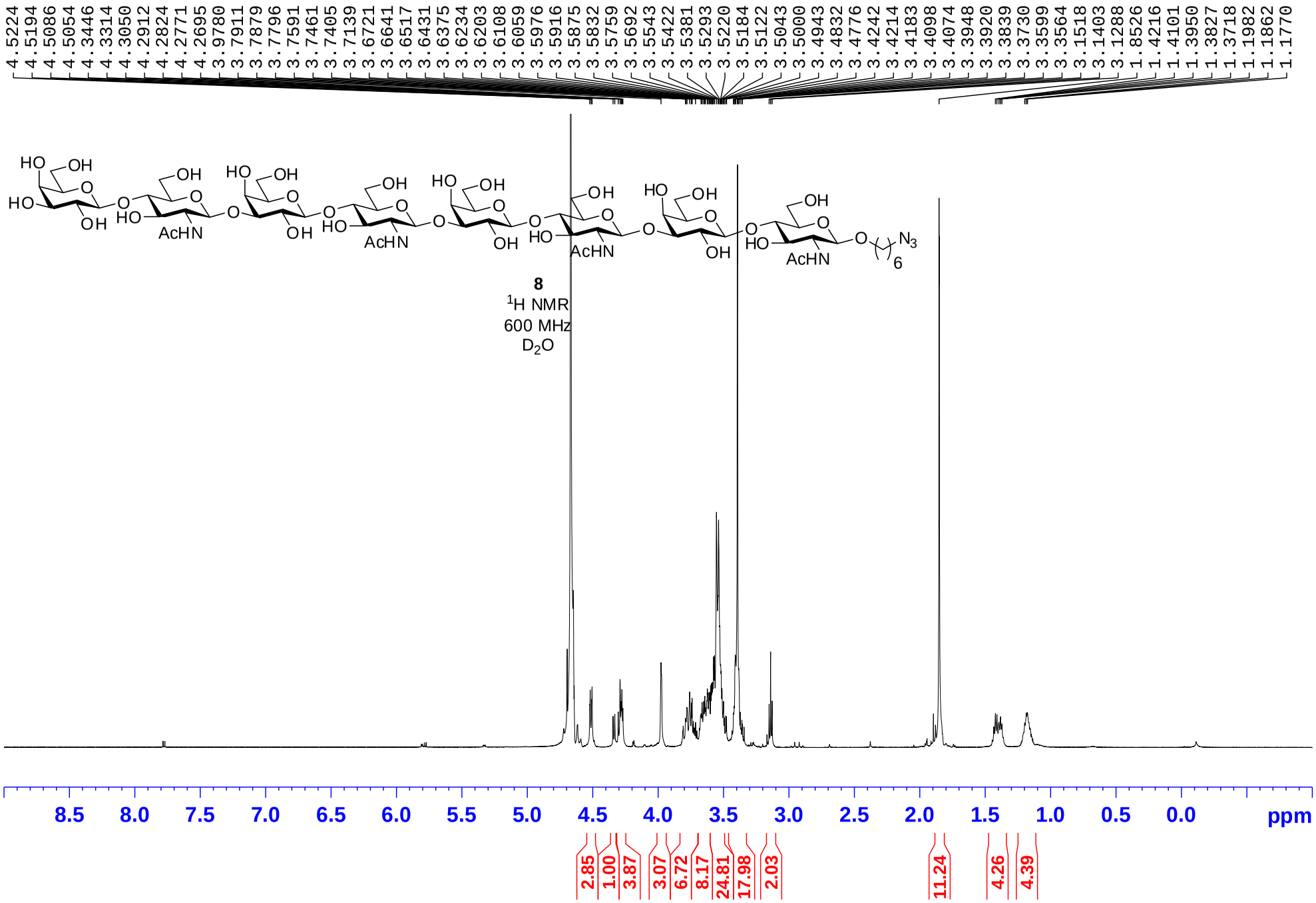
150 MHz

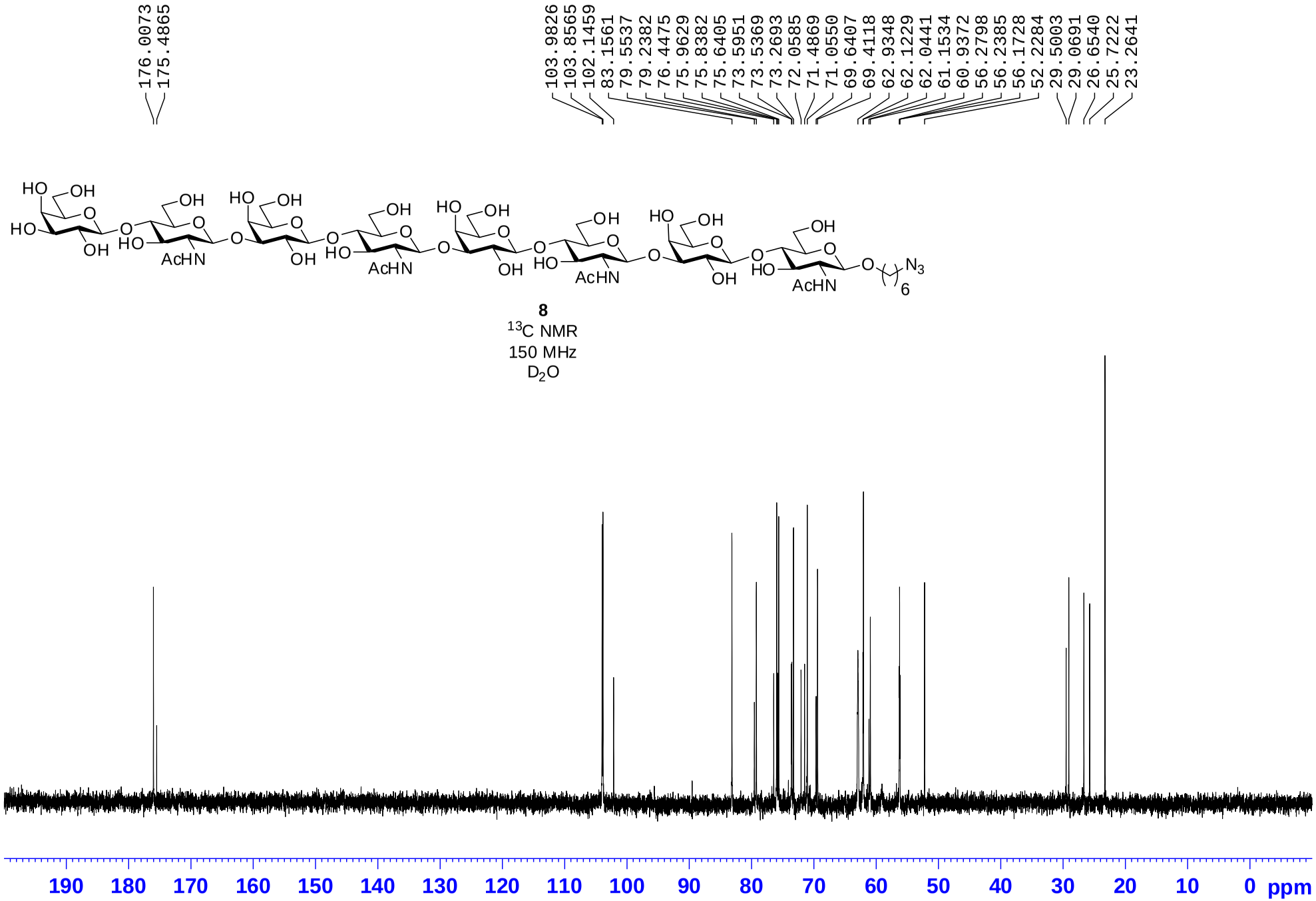
D_2O




7
^{1}H NMR
600 MHz
D_2O
ppm
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
3.28
1.00
2.89
2.92
2.94
2.19
10.43
22.33
8.08
2.06
1.84
11.60
3.99
4.00
AcHN
OH
HO
O
N_3
6


176.0064
175.4875
103.9809
103.8465
102.1414
83.1490
83.0805
79.5668
79.2455
76.7467
75.9653
75.8375
75.6388
74.6536
73.5353
73.2655
71.4865
71.0587
70.7729
69.4136
62.5344
62.0432
61.5683
61.1605
60.9422
60.4079
56.7494
56.2392
56.1769
52.2283
29.4993
29.0635
26.6504
25.7186
23.2618
AcHN
N3
6
7
13C NMR
150 MHz
D2O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


4.5224 4.5194 4.5086 4.5054 4.3446 4.3314 4.3050 4.2912 4.2824 4.2771 4.2695 3.9780 3.7911 3.7879 3.7796 3.7591 3.7461 3.7405 3.7139 3.6721 3.6641 3.6517 3.6431 3.6375 3.6234 3.6203 3.6108 3.6059 3.5976 3.5916 3.5875 3.5832 3.5759 3.5692 3.5543 3.5422 3.5381 3.5293 3.5220 3.5184 3.5122 3.5043 3.5000 3.4943 3.4832 3.4776 3.4242 3.4214 3.4183 3.4098 3.4074 3.3948 3.3920 3.3839 3.3730 3.3599 3.3564 3.1518 3.1403 3.1288 1.8526 1.4216 1.4101 1.3950 1.3827 1.3718 1.1982 1.1862 1.1770
8
1H NMR
600 MHz
D2O
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 ppm
2.85 1.00 3.87 3.07 6.72 8.17 24.81 17.98 2.03 11.24 4.26 4.39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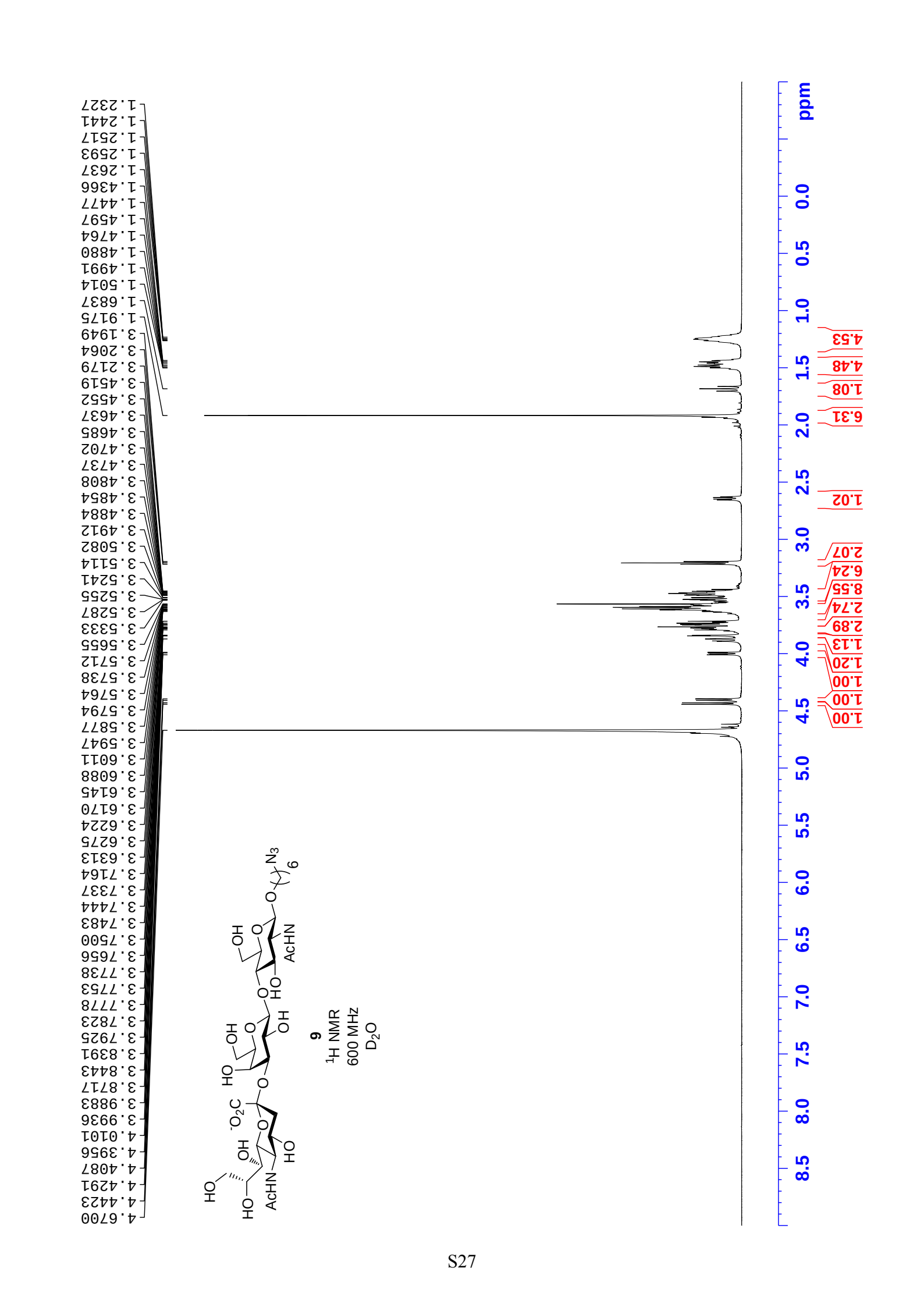
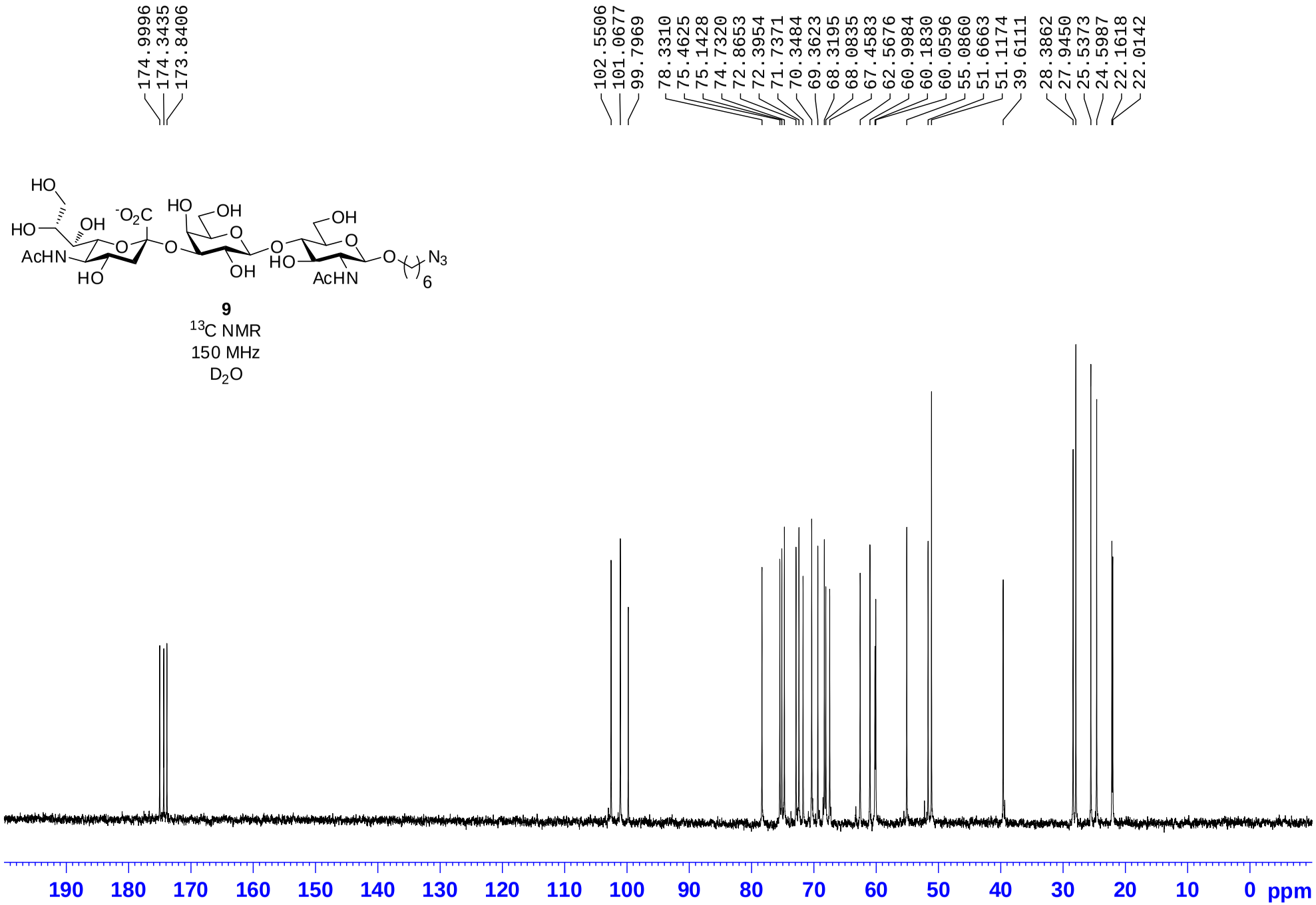
103.9826
103.8565
102.1459
83.1561
79.5537
79.2382
76.4475
75.9629
75.8382
75.6405
73.5951
73.5369
73.2693
72.0585
71.4869
71.0550
69.6407
69.4118
62.9348
62.1229
62.0441
61.1534
60.9372
56.2798
56.2385
56.1728
52.2284
29.5003
29.0691
26.6540
25.7222
23.2641
HO
OH
O
AcHN
N3
6
8
13C NMR
150 MHz
D2O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm


9
^{1}H NMR
600 MHz
D_2O
ppm
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
1.00
1.00
1.00
1.20
1.13
2.89
2.74
8.55
6.24
2.07
1.02
6.31
1.08
4.48
4.53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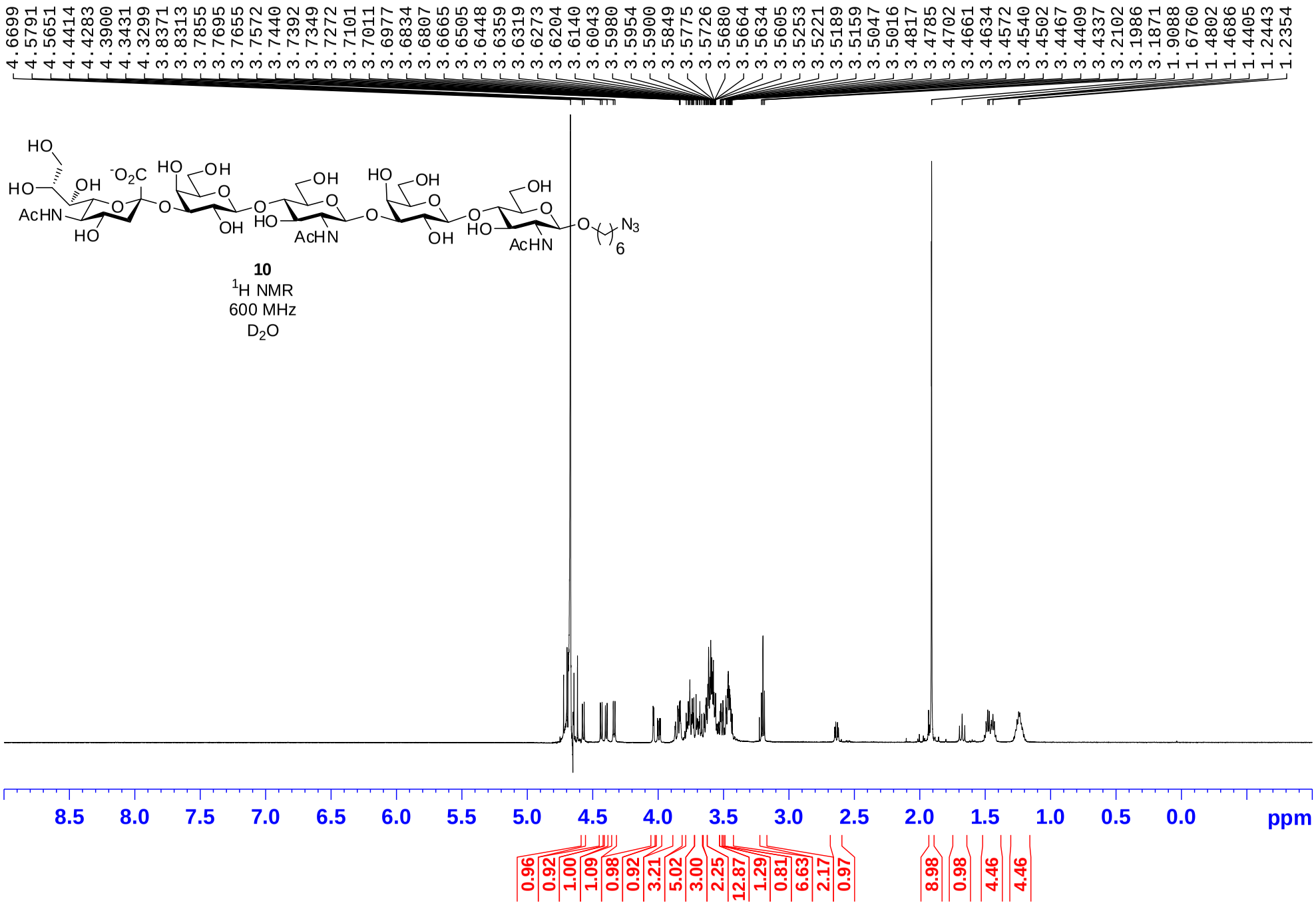
174.3435
173.8406
102.5506
101.0677
99.7969
78.3310
75.4625
75.1428
74.7320
72.8653
72.3954
71.7371
70.3484
69.3623
68.3195
68.0835
67.4583
62.5676
60.9984
60.1830
60.0596
55.0860
51.6663
51.1174
39.6111
28.3862
27.9450
25.5373
24.5987
22.1618
22.0142
HO
HO
OH
$^{-}O_2C$
HO
OH
OH
AcHN
HO
HO
AcHN
N_3
6
9
^{13}C NMR
150 MHz
D_2O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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4.4283
4.3900
4.3431
4.3299
3.8371
3.8313
3.7855
3.7695
3.7655
3.7572
3.7440
3.7392
3.7349
3.7272
3.7101
3.7011
3.6977
3.6834
3.6807
3.6665
3.6505
3.6448
3.6359
3.6319
3.6273
3.6204
3.6140
3.6043
3.5980
3.5954
3.5900
3.5849
3.5775
3.5726
3.5680
3.5664
3.5634
3.5605
3.5253
3.5221
3.5189
3.5159
3.5047
3.5016
3.4817
3.4785
3.4702
3.4661
3.4634
3.4572
3.4540
3.4502
3.4467
3.4409
3.4337
3.2102
3.1986
3.1871
1.9088
1.6760
1.4802
1.4686
1.4405
1.2443
1.2354
10
1H NMR
600 MHz
D2O
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
ppm
0.96
0.92
1.00
1.09
0.98
0.92
3.21
5.02
3.00
2.25
12.87
1.29
0.81
6.63
2.17
0.97
8.98
0.98
4.46
4.46

174.9979
174.8636
174.3532
173.8237

102.8723
102.7624
102.5266
101.0207
99.7875
82.0496
78.4939
77.9963
75.4701
75.1497
74.8622
74.7211
74.5300
72.8641
72.4119
72.1243
71.7364
70.3535
69.9441
69.3541
68.3093
68.0694
67.4500
62.5592
61.0011
60.9300
60.0565
59.8211
55.1564
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51.1099
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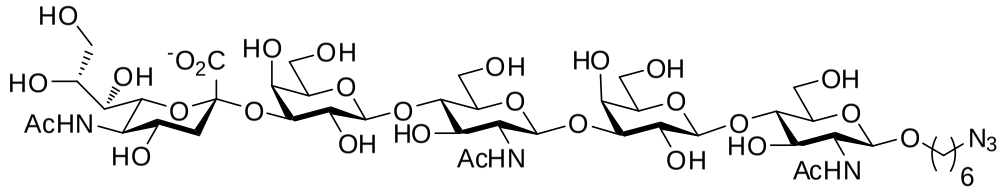
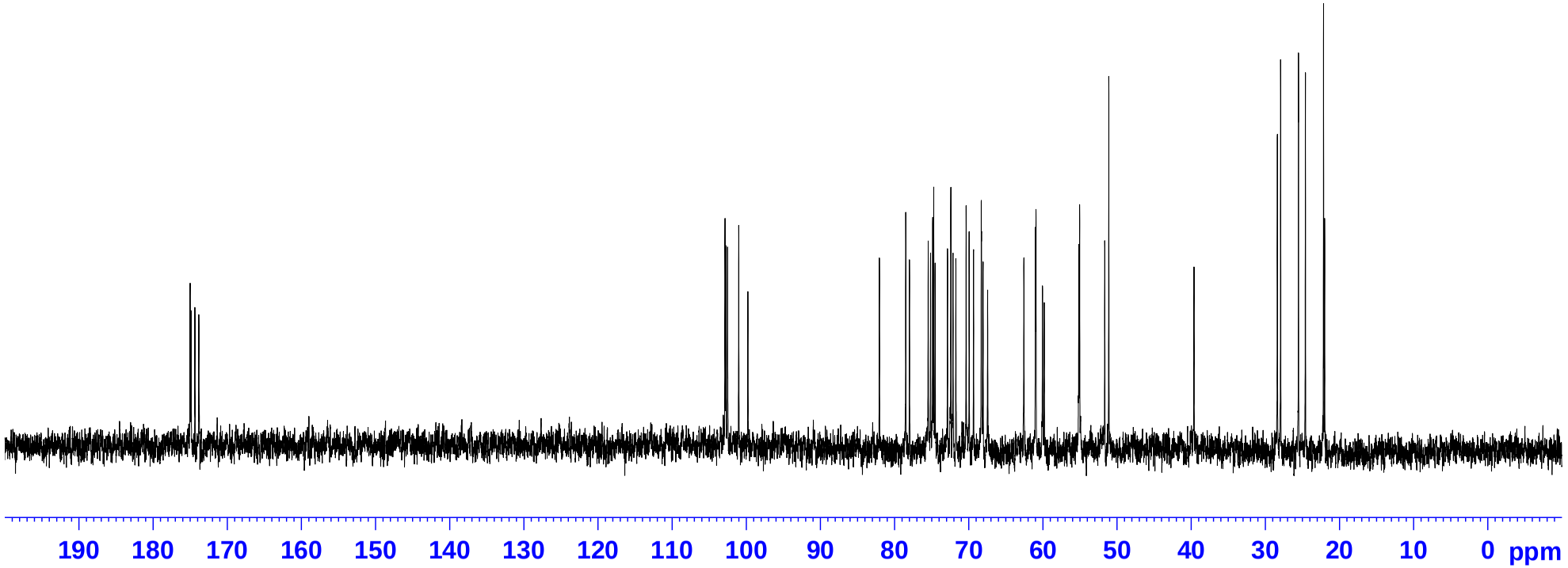
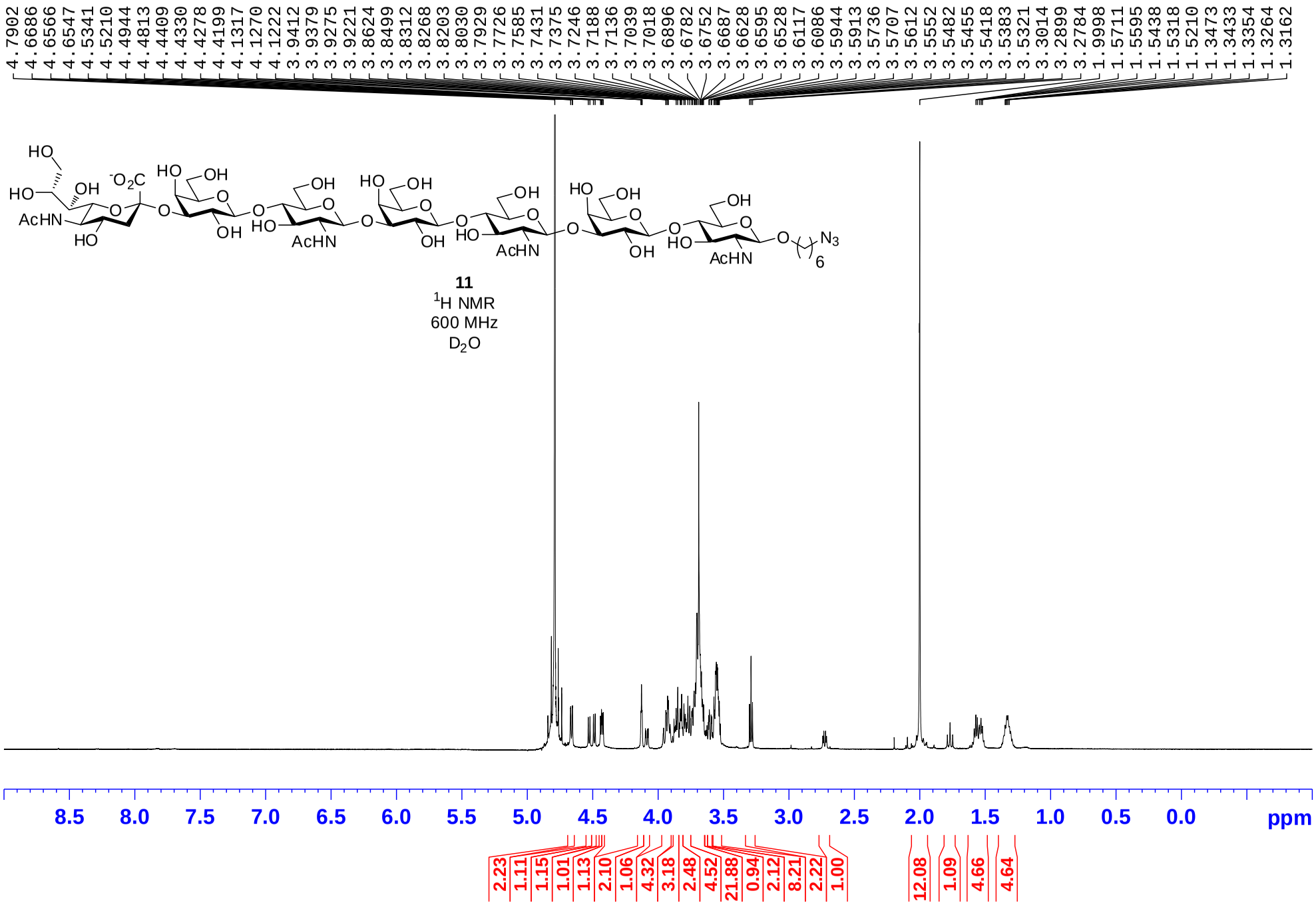
28.3774
27.9354
25.5277
24.5926
22.1482
22.0021



10
^{13}C NMR
150 MHz
D_2O

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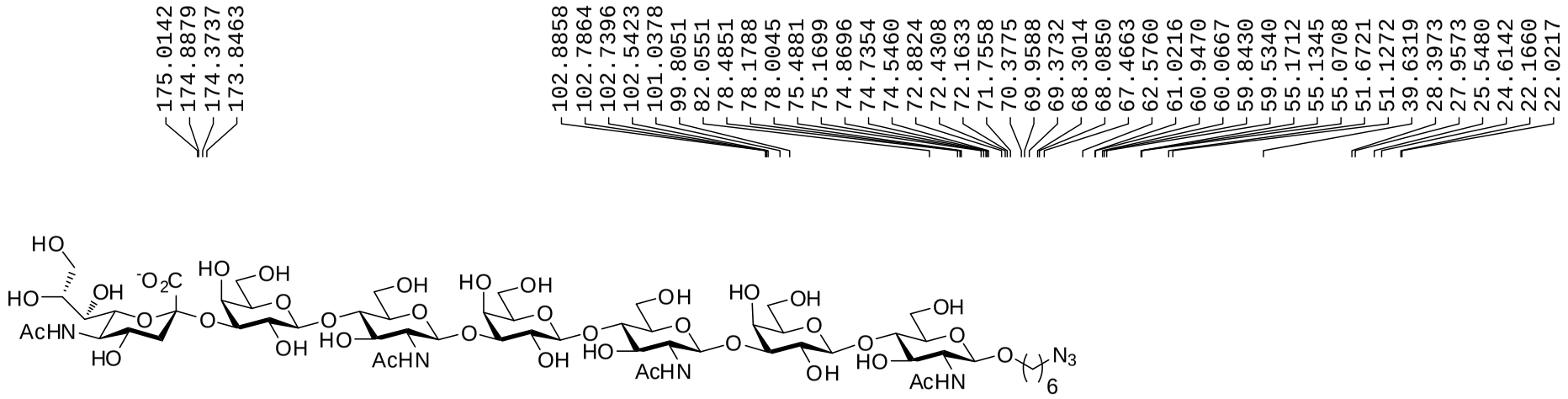
4.6566
4.6547
4.5341
4.5210
4.4944
4.4813
4.4409
4.4330
4.4278
4.4199
4.1317
4.1270
4.1222
3.9412
3.9379
3.9275
3.9221
3.8624
3.8499
3.8312
3.8268
3.8203
3.8030
3.7929
3.7726
3.7585
3.7431
3.7375
3.7246
3.7188
3.7136
3.7039
3.7018
3.6896
3.6782
3.6752
3.6687
3.6628
3.6595
3.6528
3.6117
3.6086
3.5944
3.5913
3.5736
3.5707
3.5612
3.5552
3.5482
3.5455
3.5418
3.5383
3.5321
3.3014
3.2899
3.2784
1.9998
1.5711
1.5595
1.5438
1.5318
1.5210
1.3473
1.3433
1.3354
1.3264
1.3162
11
1H NMR
600 MHz
D2O
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
ppm
2.23
1.11
1.15
1.01
1.13
2.10
1.06
4.32
3.18
2.48
4.52
21.88
0.94
2.12
8.21
2.22
1.00
12.08
1.09
4.66
4.64

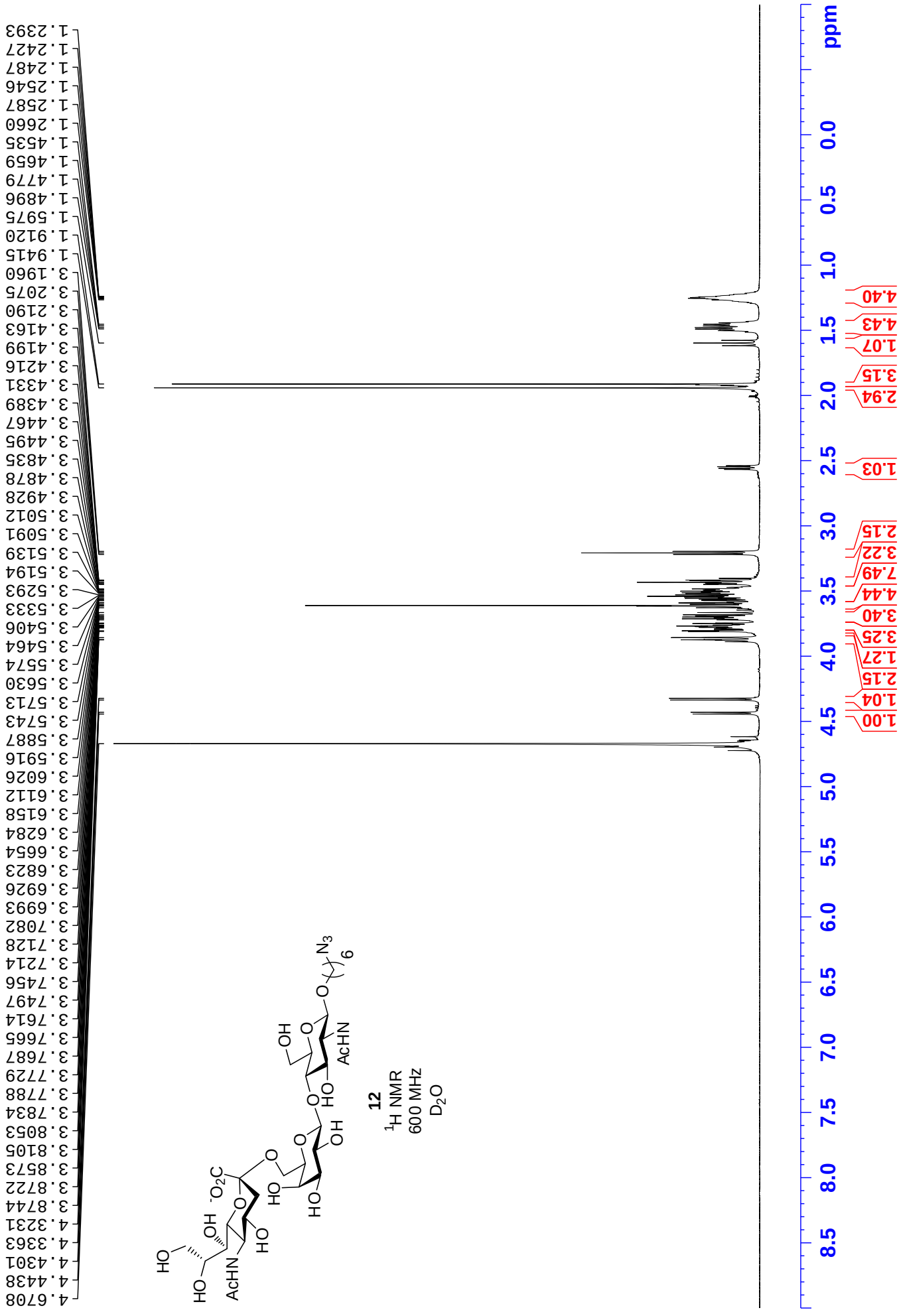


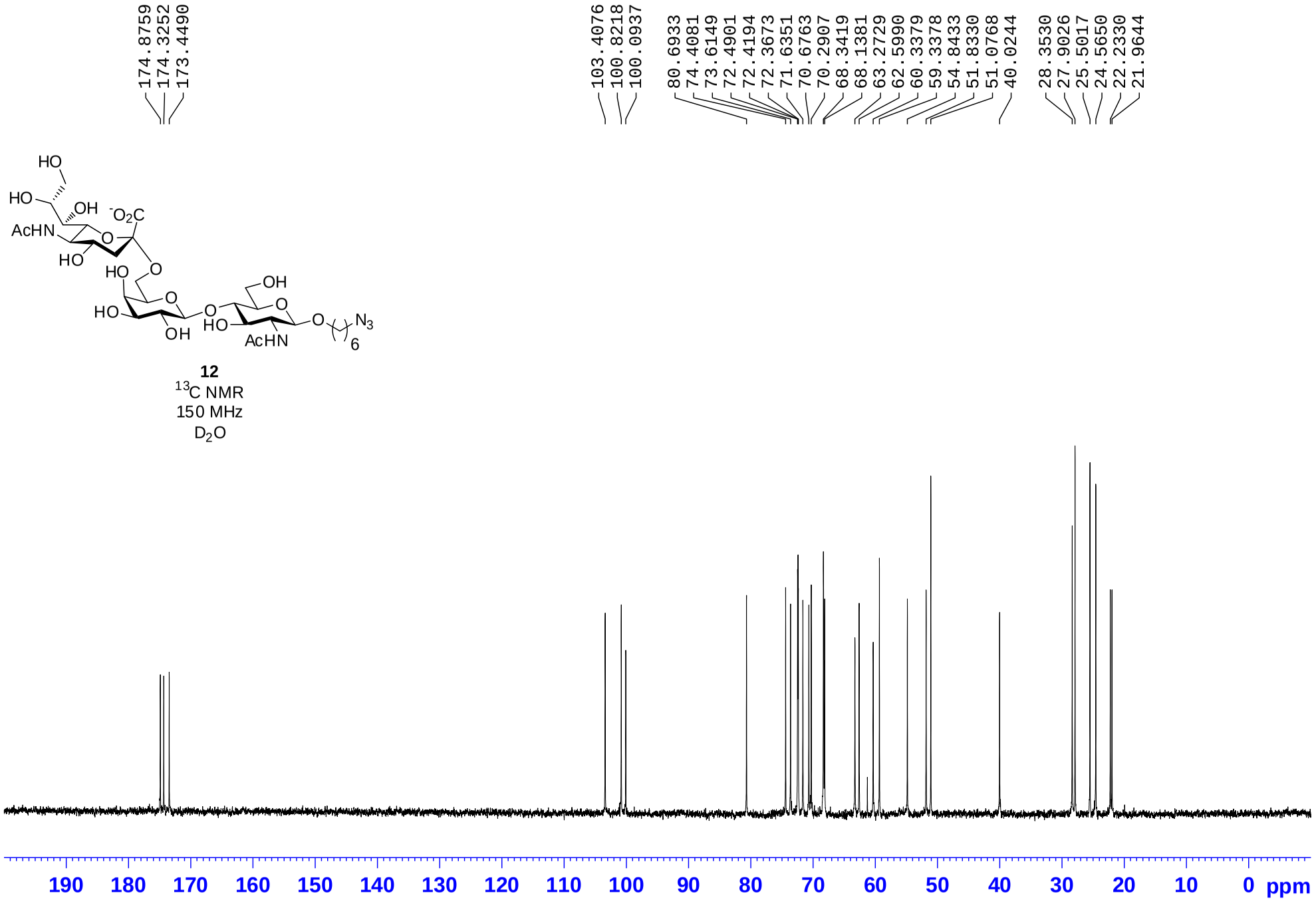
11

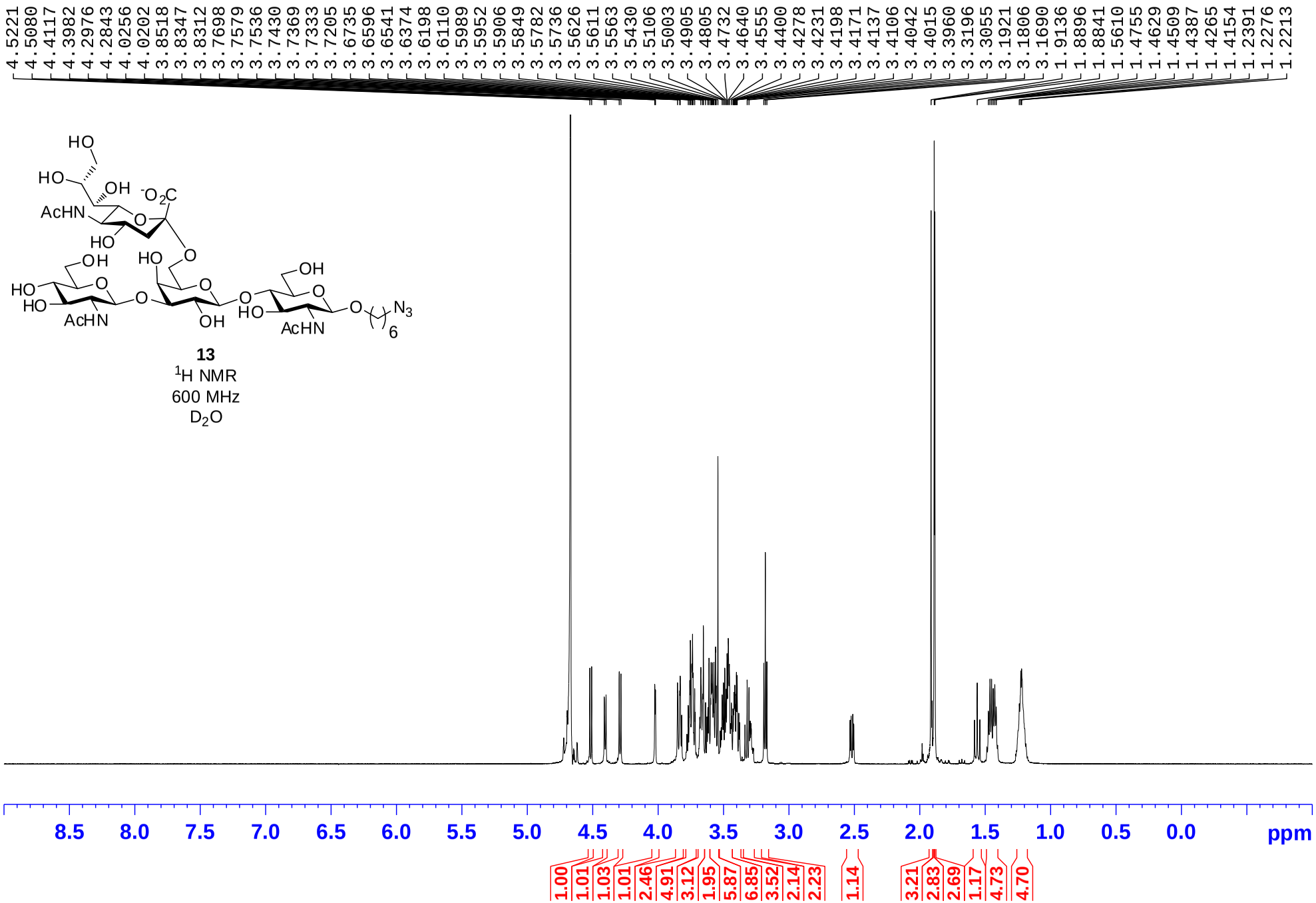
^{13}C NMR

150 MHz

D_2O


12
1H NMR
600 MHz
D2O
ppm
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
1.00
1.04
2.15
1.27
3.25
4.40
3.40
7.49
4.44
3.22
2.15
1.03
2.94
3.15
1.07
4.43
4.40


174.8759
174.3252
173.4490
103.4076
100.8218
100.0937
80.6933
74.4081
73.6149
72.4901
72.4194
72.3673
71.6351
70.6763
70.2907
68.3419
68.1381
63.2729
62.5990
60.3379
59.3378
54.8433
51.8330
51.0768
40.0244
28.3530
27.9026
25.5017
24.5650
22.2330
21.9644
HO
HO
OH
$^{-}O_2C$
AcHN
O
HO
HO
O
HO
O
OH
HO
OH
O
HO
AcHN
O
N_3
6
12
^{13}C NMR
150 MHz
D_2O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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3.7698
3.7579
3.7536
3.7430
3.7369
3.7333
3.7205
3.6735
3.6596
3.6541
3.6374
3.6198
3.6110
3.5989
3.5952
3.5906
3.5849
3.5782
3.5736
3.5626
3.5611
3.5563
3.5430
3.5106
3.5003
3.4905
3.4805
3.4732
3.4640
3.4555
3.4400
3.4278
3.4231
3.4198
3.4171
3.4137
3.4106
3.4042
3.4015
3.3960
3.3196
3.3055
3.1921
3.1806
3.1690
1.9136
1.8896
1.8841
1.5610
1.4755
1.4629
1.4509
1.4387
1.4265
1.4154
1.2391
1.2276
1.2213
13
1H NMR
600 MHz
D2O
8.5
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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
ppm
1.00
1.01
1.03
1.01
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4.91
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1.95
5.87
6.85
3.52
2.14
2.23
1.14
3.21
2.83
2.69
1.17
4.73
4.70

174.7712
174.7406
174.2233
173.3332

103.3100
102.7153
100.7216
99.9913
82.0573
80.6207
75.4823
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72.3882
72.3325
71.4931
70.1866
69.5380
69.4575
68.2313
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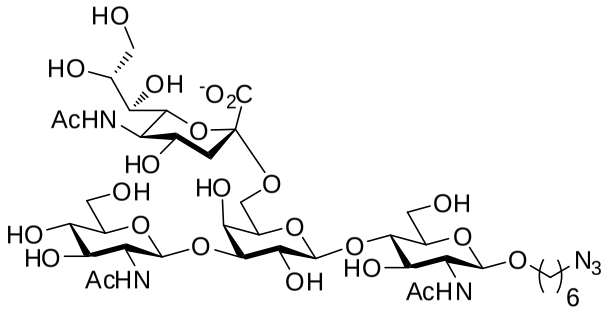
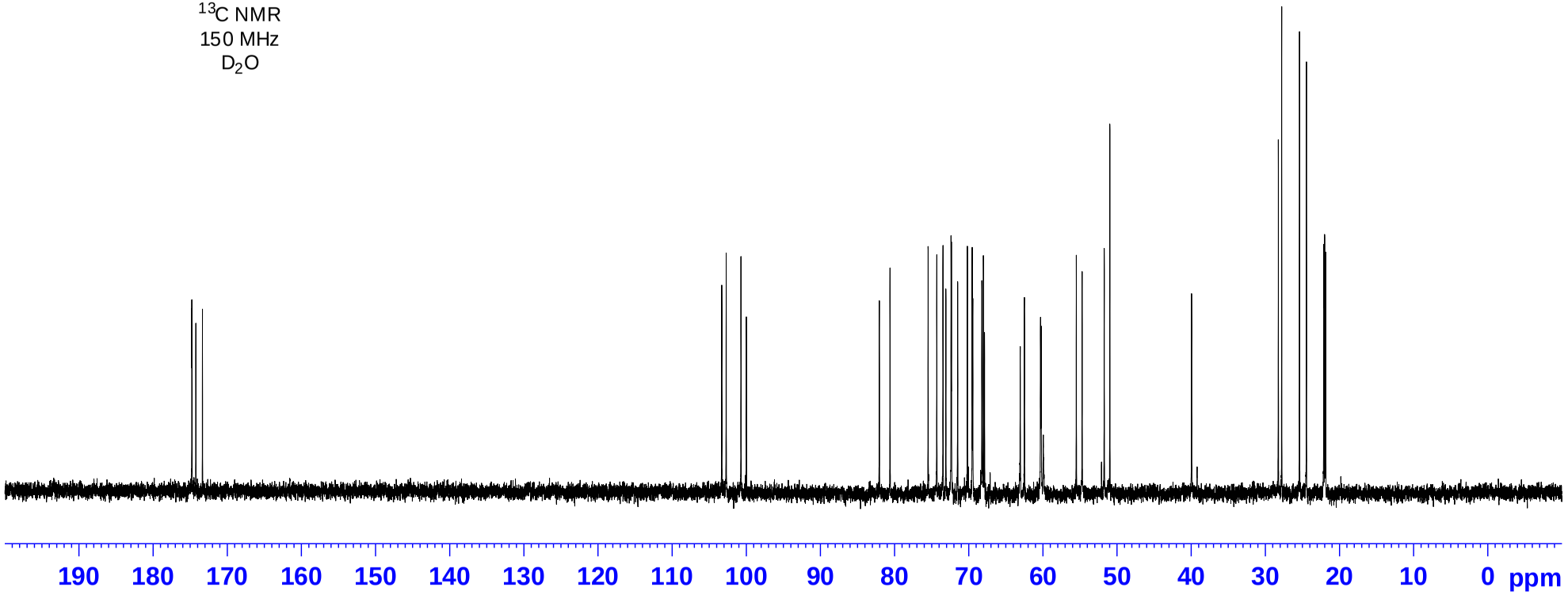
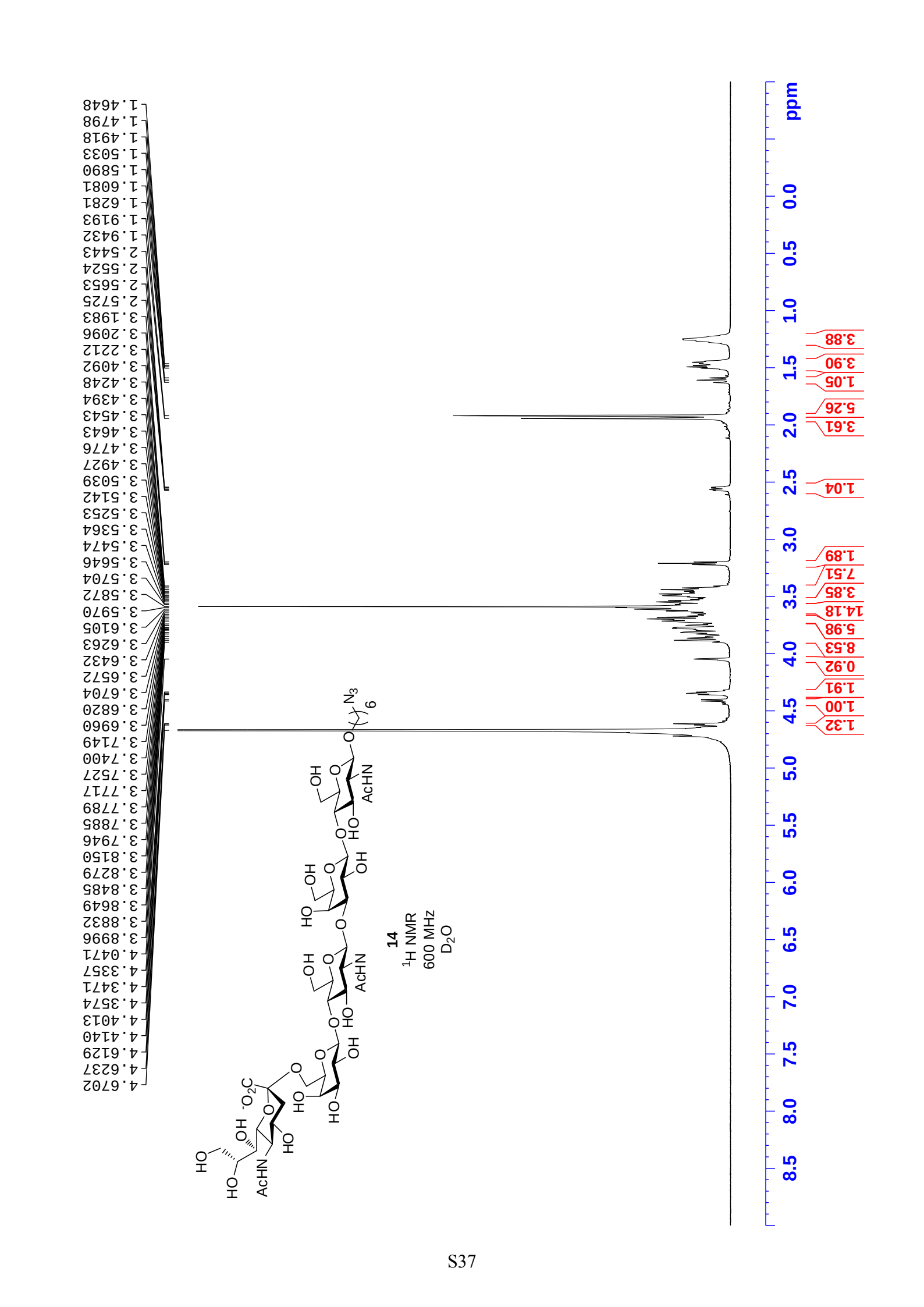
21.8583



13
^{13}C NMR
150 MHz
D_2O

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1H NMR
600 MHz
D2O
ppm
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0.5
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1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
1.32
1.00
1.91
0.92
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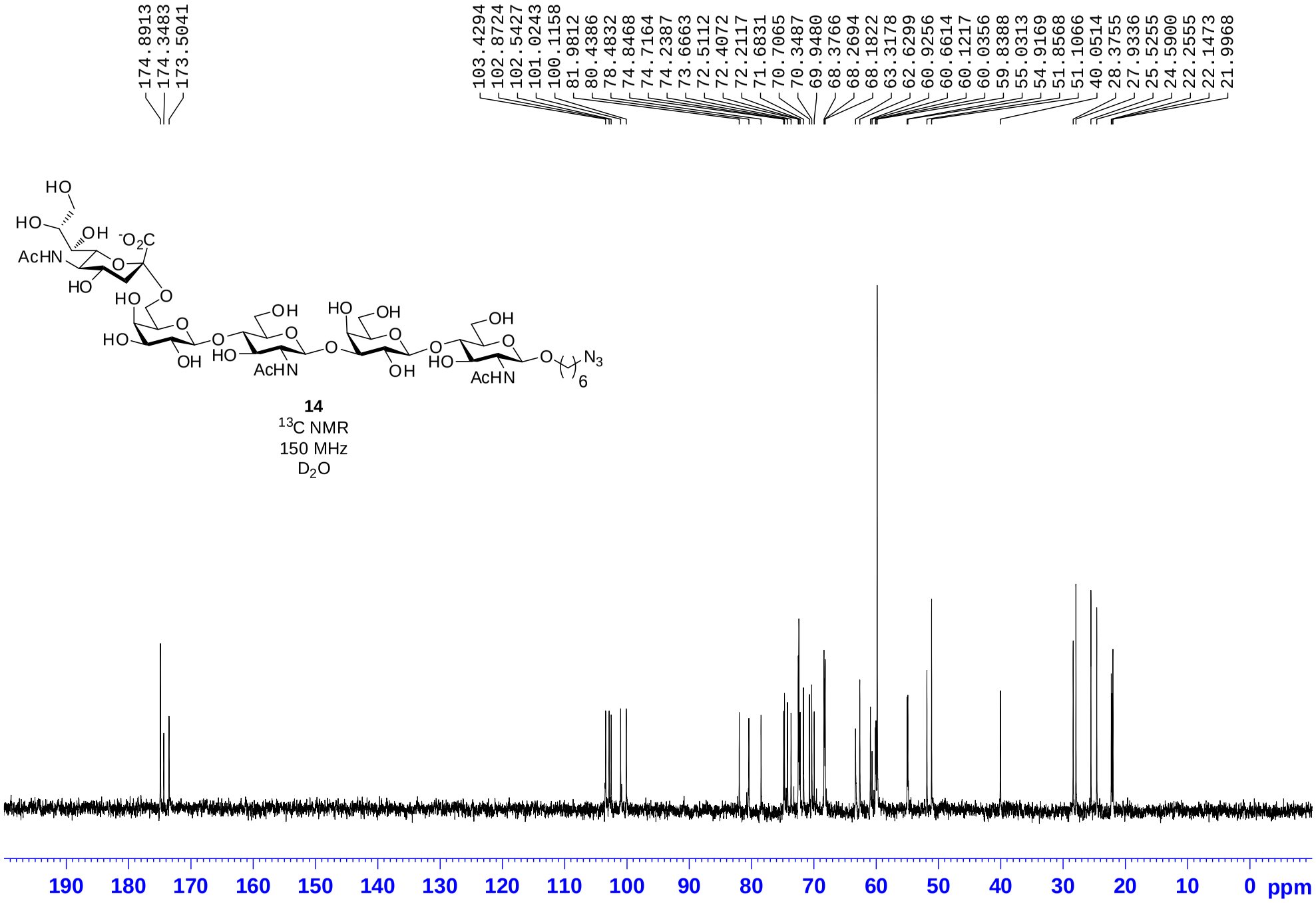
14.18
3.85
7.51
1.89
1.04
3.61
5.26
1.05
3.90
3.88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101.0243
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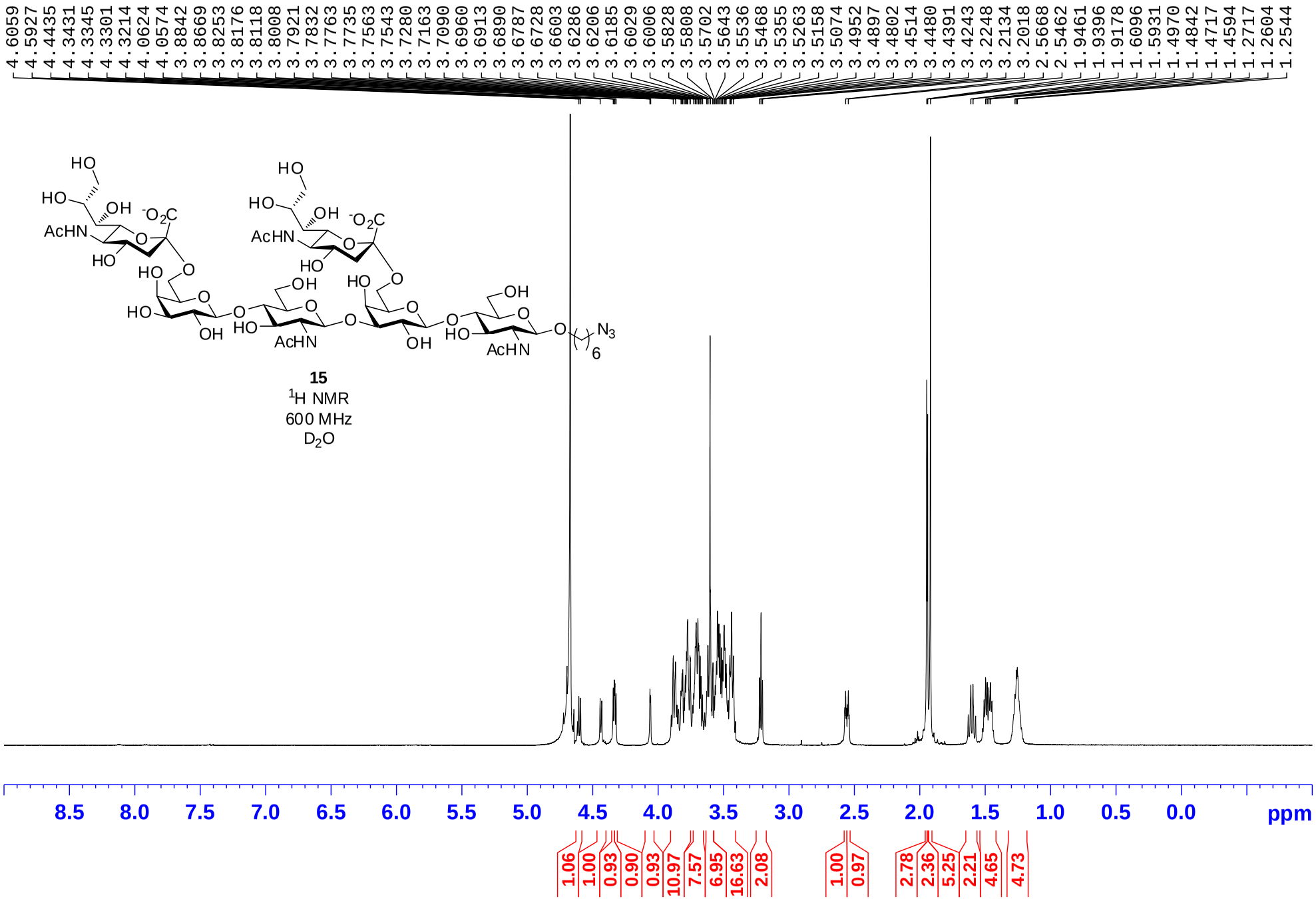
78.4832
74.8468
74.7164
74.2387
73.6663
72.5112
72.4072
72.2117
71.6831
70.7065
70.3487
69.9480
68.3766
68.2694
68.1822
63.3178
62.6299
60.9256
60.6614
60.1217
60.0356
59.8388
55.0313
54.9169
51.8568
51.1066
40.0514
28.3755
27.9336
25.5255
24.5900
22.2555
22.1473
21.9968
HO
HO
OH
$^{-}O_2C$
AcHN
O
HO
HO
O
HO
O
OH
HO
AcHN
O
OH
HO
OH
O
HO
AcHN
O
OH
HO
OH
O
HO
AcHN
O
N_3
6
14
^{13}C NMR
150 MHz
D_2O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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4.3345
4.3301
4.3214
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4.0574
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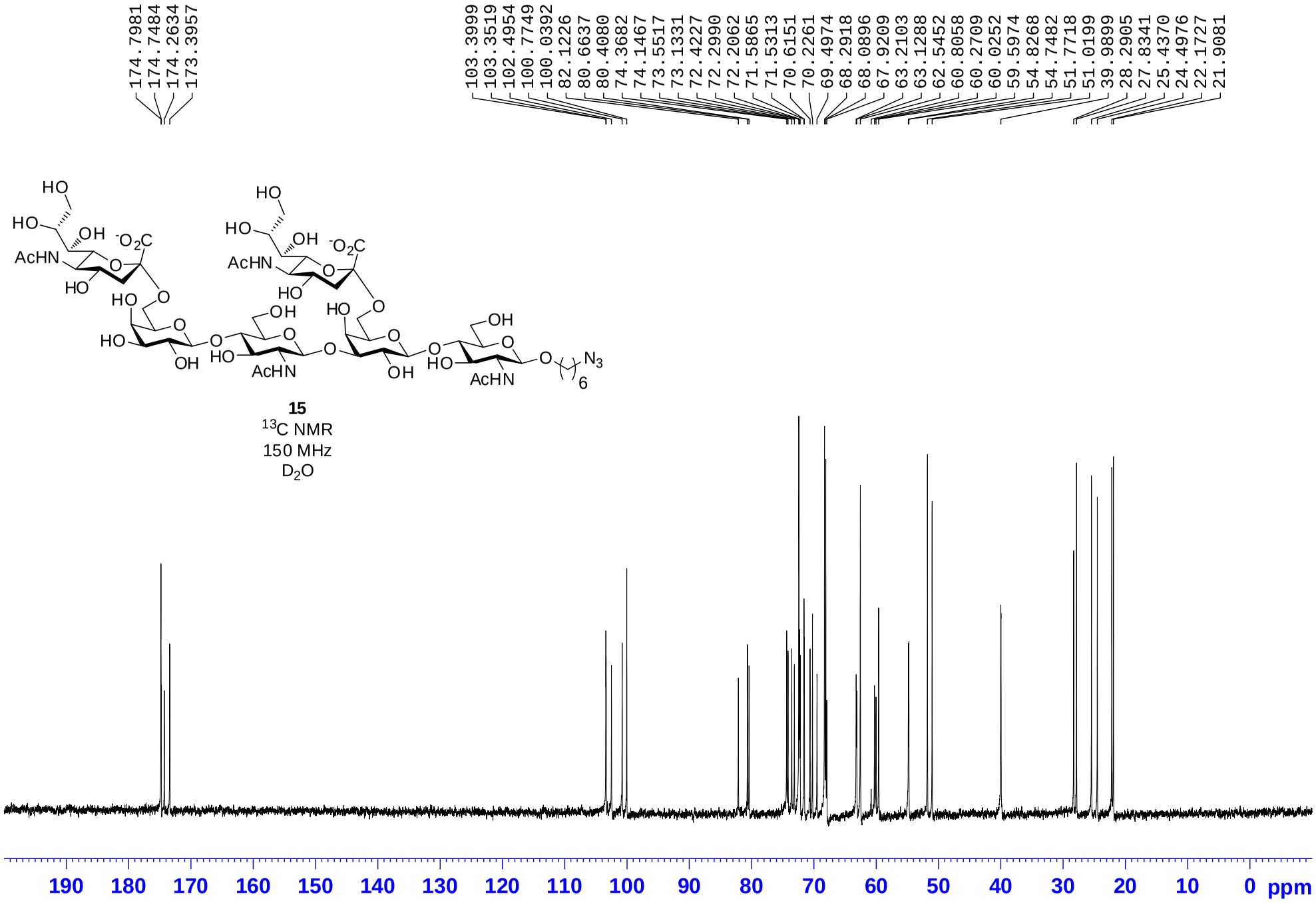
3.8669
3.8253
3.8176
3.8118
3.8008
3.7921
3.7832
3.7763
3.7735
3.7563
3.7543
3.7280
3.7163
3.7090
3.6960
3.6913
3.6890
3.6787
3.6728
3.6603
3.6286
3.6206
3.6185
3.6029
3.6006
3.5828
3.5808
3.5702
3.5643
3.5536
3.5468
3.5355
3.5263
3.5158
3.5074
3.4952
3.4897
3.4802
3.4514
3.4480
3.4391
3.4243
3.2248
3.2134
3.2018
2.5668
2.5462
1.9461
1.9396
1.9178
1.6096
1.5931
1.4970
1.4842
1.4717
1.4594
1.2717
1.2604
1.2544
15
1H NMR
600 MHz
D2O
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
ppm
1.06
1.00
0.93
0.90
0.93
10.97
7.57
6.95
16.63
2.08
1.00
0.97
2.78
2.36
5.25
2.21
4.65
4.73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100.0392
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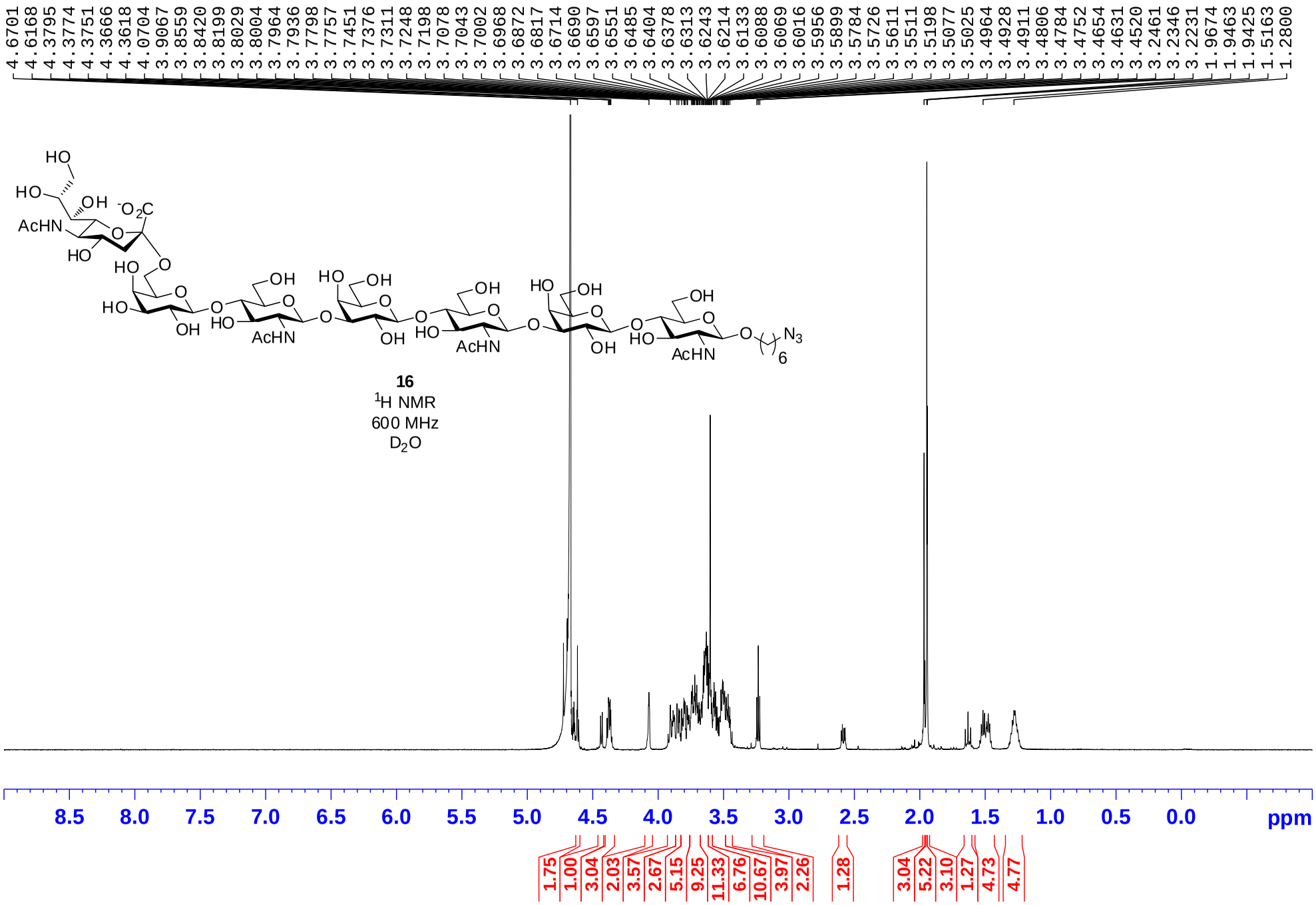
80.6637
80.4080
74.3682
74.1467
73.5517
73.1331
72.4227
72.2990
72.2062
71.5865
71.5313
70.6151
70.2261
69.4974
68.2918
68.0896
67.9209
63.2103
63.1288
62.5452
60.8058
60.2709
60.0252
59.5974
54.8268
54.7482
51.7718
51.0199
39.9899
28.2905
27.8341
25.4370
24.4976
22.1727
21.9081
15
13C NMR
150 MHz
D2O
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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3.8420
3.8199
3.8029
3.8004
3.7964
3.7936
3.7798
3.7757
3.7451
3.7376
3.7311
3.7248
3.7198
3.7078
3.7043
3.7002
3.6968
3.6872
3.6817
3.6714
3.6690
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3.6551
3.6485
3.6404
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3.6313
3.6243
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3.5726
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3.5511
3.5198
3.5077
3.5025
3.4964
3.4928
3.4911
3.4806
3.4784
3.4752
3.4654
3.4631
3.4520
3.2461
3.2346
3.2231
1.9674
1.9463
1.9425
1.5163
1.2800
16
1H NMR
600 MHz
D2O
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
ppm
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1.00
3.04
2.03
3.57
2.67
5.15
9.25
11.33
6.76
10.67
3.97
2.26
1.28
3.04
5.22
3.10
1.27
4.73
4.77

174.7668
174.2183
173.3603

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102.4019
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100.0085
81.9065
81.8568
80.3244
78.4008
78.1008
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74.4067
74.1341
73.5521
72.3953
72.2895
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70.5962
70.2275
69.8302
68.2722
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68.0611
63.1967
62.5275
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60.0214
59.9414
59.7154
54.9921
54.9378
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25.3981
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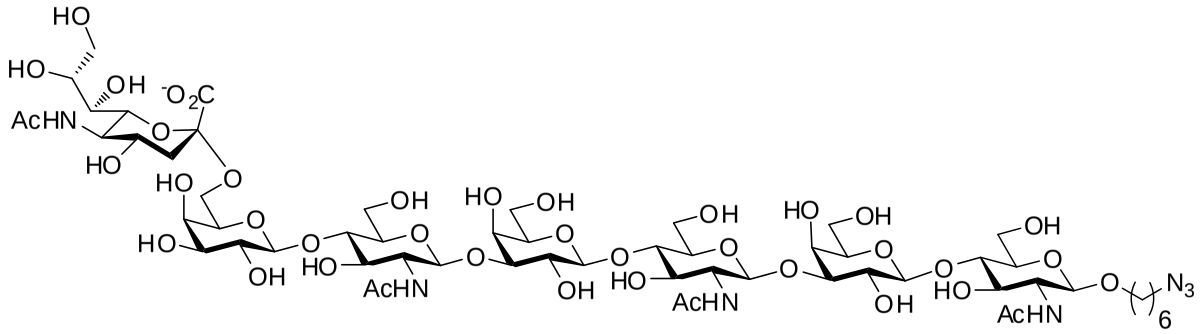
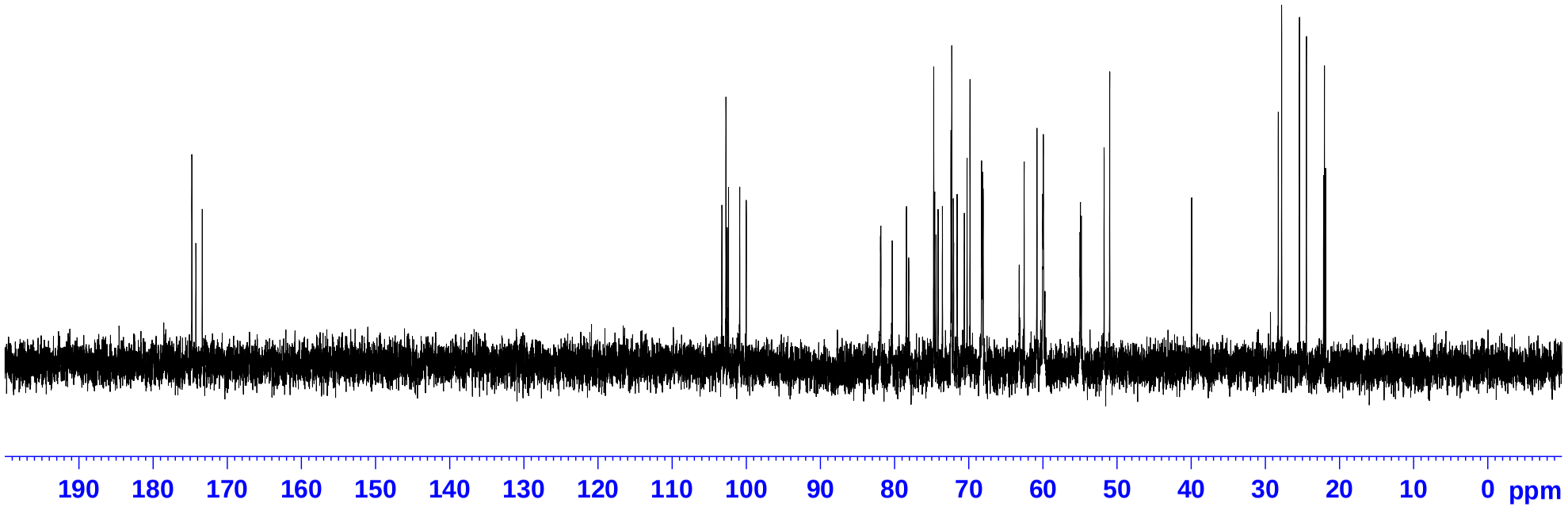
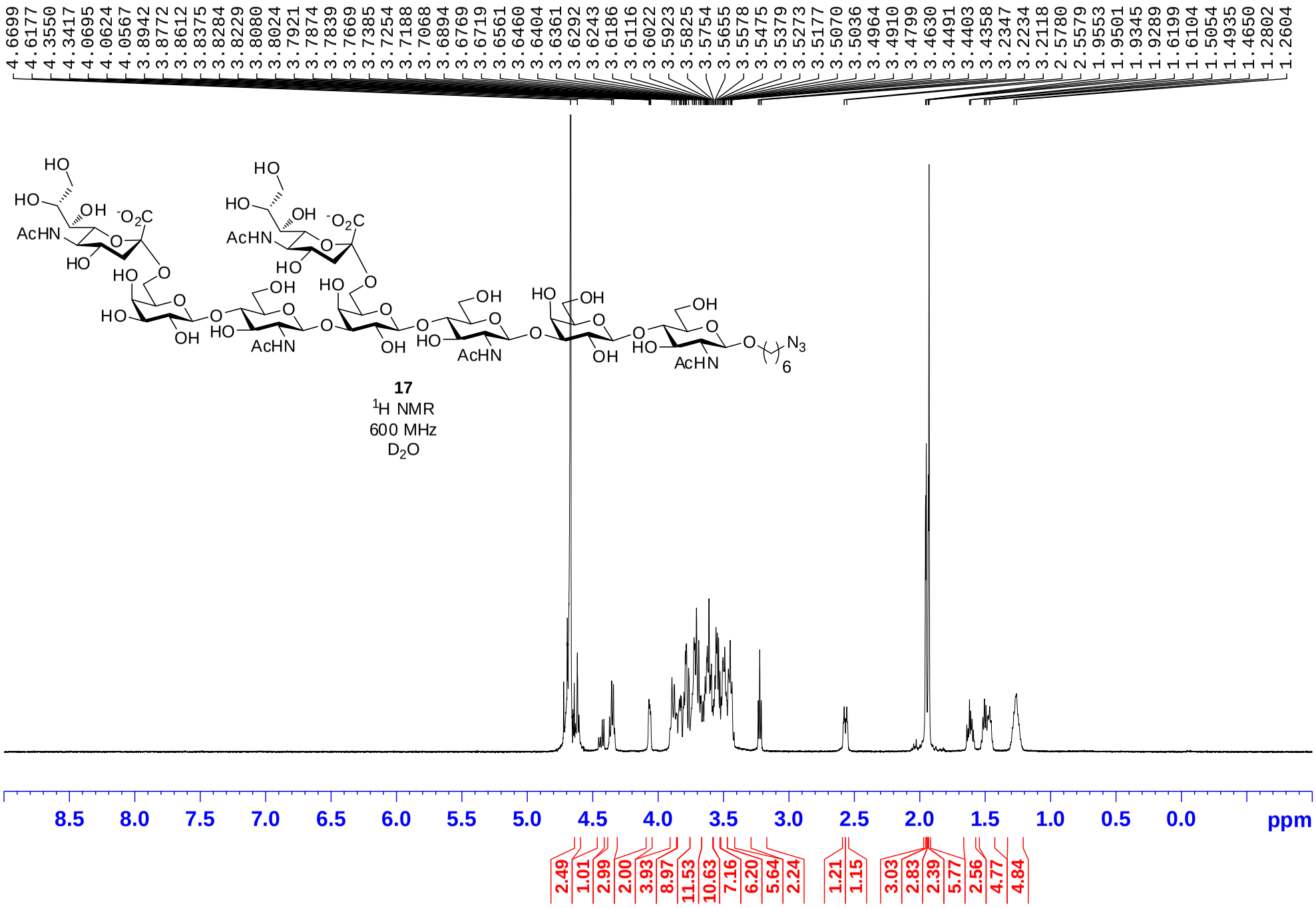
22.0277
21.8778



16
^{13}C NMR
150 MHz
D_2O

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3.8284
3.8229
3.8080
3.8024
3.7921
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3.7839
3.7669
3.7385
3.7254
3.7188
3.7068
3.6894
3.6769
3.6719
3.6561
3.6460
3.6404
3.6361
3.6292
3.6243
3.6186
3.6116
3.6022
3.5923
3.5825
3.5754
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3.5578
3.5475
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3.5273
3.5177
3.5070
3.5036
3.4964
3.4910
3.4799
3.4630
3.4491
3.4403
3.4358
3.2347
3.2234
3.2118
2.5780
2.5579
1.9553
1.9501
1.9345
1.9289
1.6199
1.6104
1.5054
1.4935
1.4650
1.2802
1.2604
17
1H NMR
600 MHz
D2O
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
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2.99
2.00
3.93
8.97
11.53
10.63
7.16
6.20
5.64
2.24
1.21
1.15
3.03
2.83
2.39
5.77
2.56
4.77
4.84

174.7857
174.2490
173.3889

103.4004
103.3345
102.7672
102.4871
100.9198
100.0315
82.1205
81.8726
80.6714
80.4241
80.3677
78.4096
74.7503
74.6195
74.1530
73.5484
73.1460
72.4146
72.3027
72.2066
72.1302
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71.5300
70.6125
70.2410
69.8620
69.4946
68.2882
68.1661
68.0876
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63.1342
62.5395
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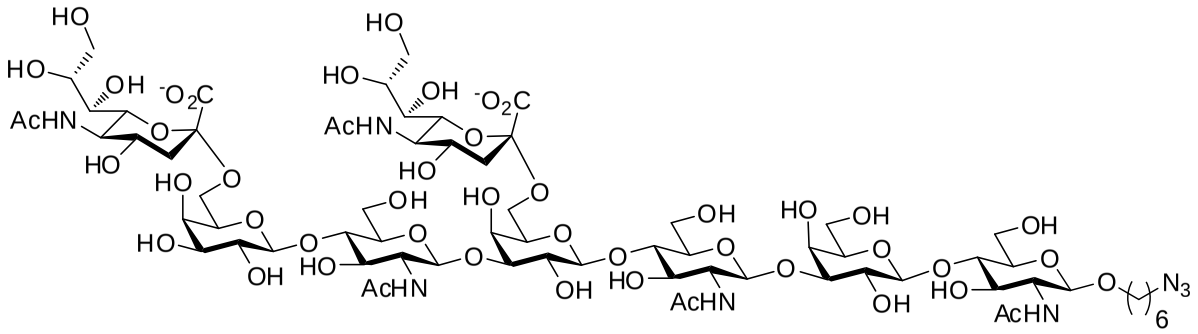
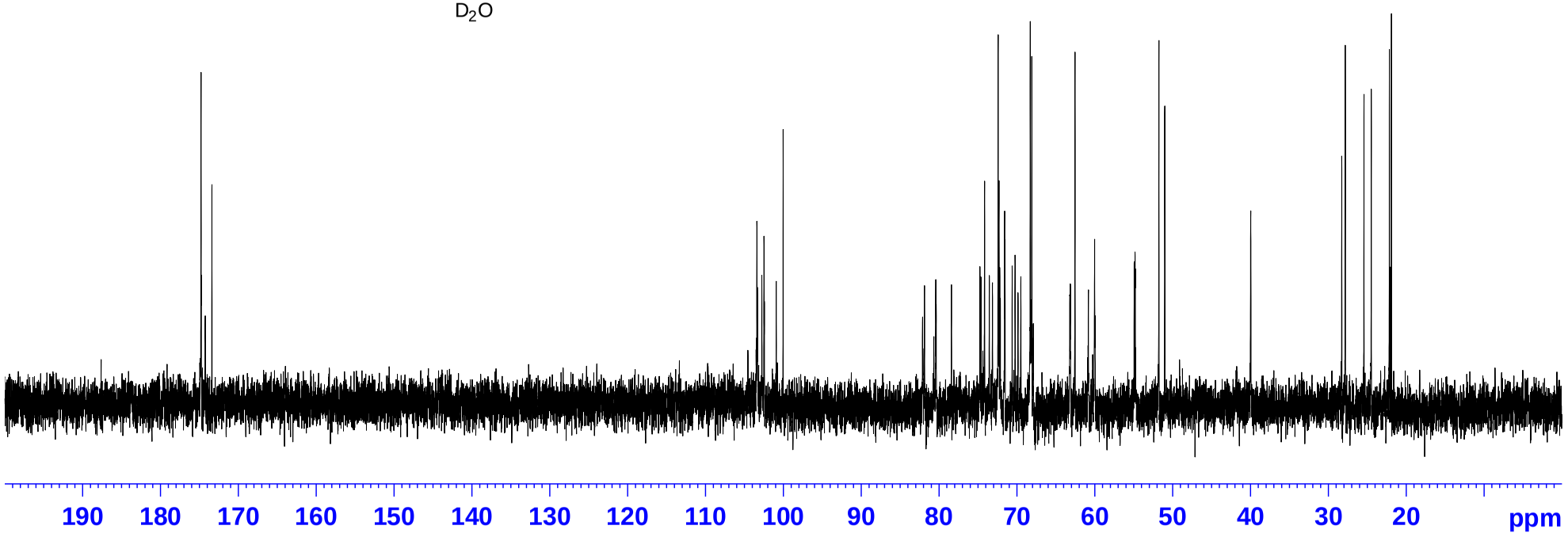
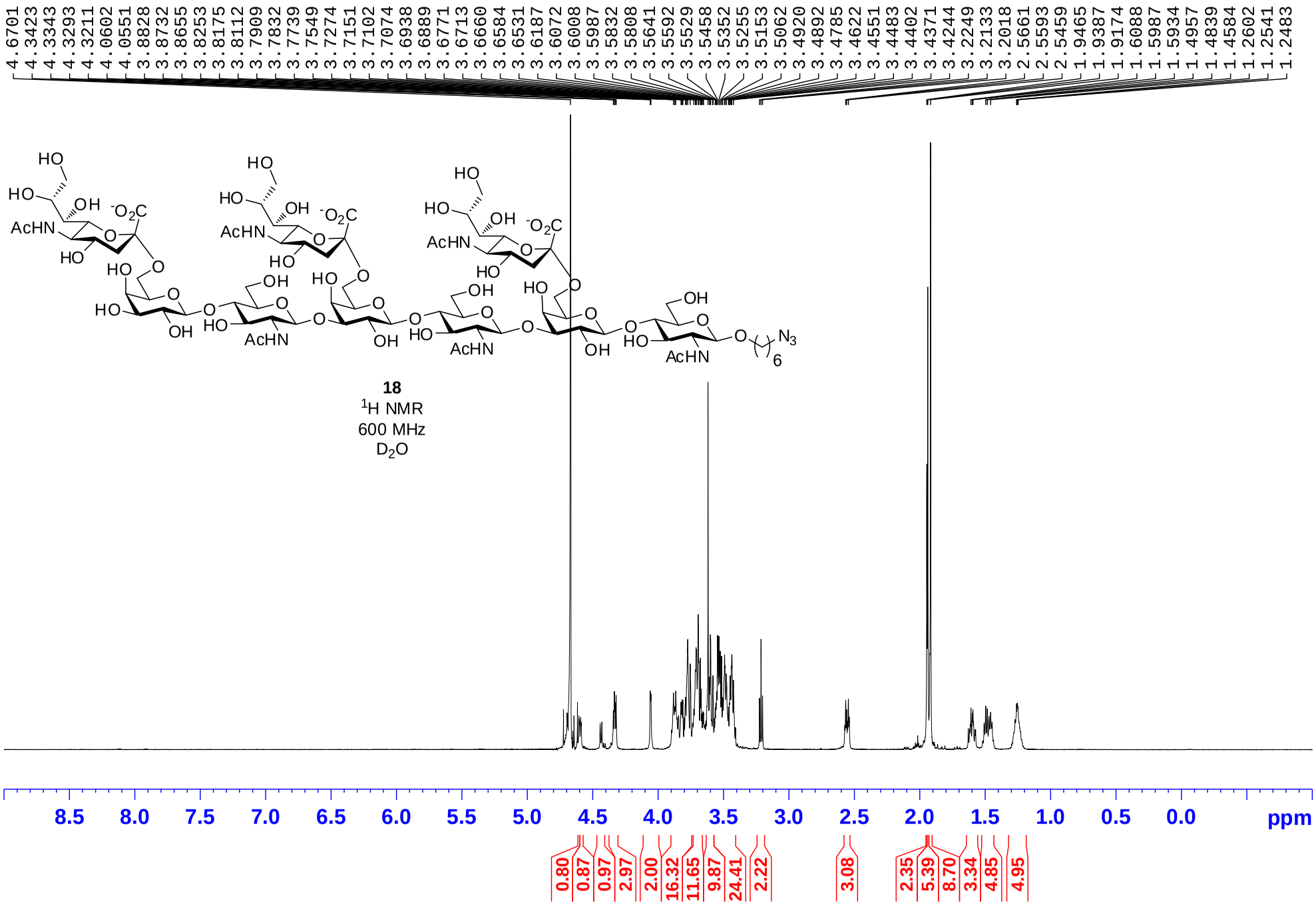
27.8217
25.4225
24.4845
22.1596



17
^{13}C NMR
150 MHz
D_2O

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4.3293
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4.0602
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3.8253
3.8175
3.8112
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3.7832
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3.7274
3.7151
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3.7074
3.6938
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3.6771
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3.2249
3.2133
3.2018
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1.9465
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1.6088
1.5987
1.5934
1.4957
1.4839
1.4584
1.2602
1.2541
1.2483
18
1H NMR
600 MHz
D2O
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
ppm
0.80
0.87
0.97
2.97
2.00
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5.39
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3.34
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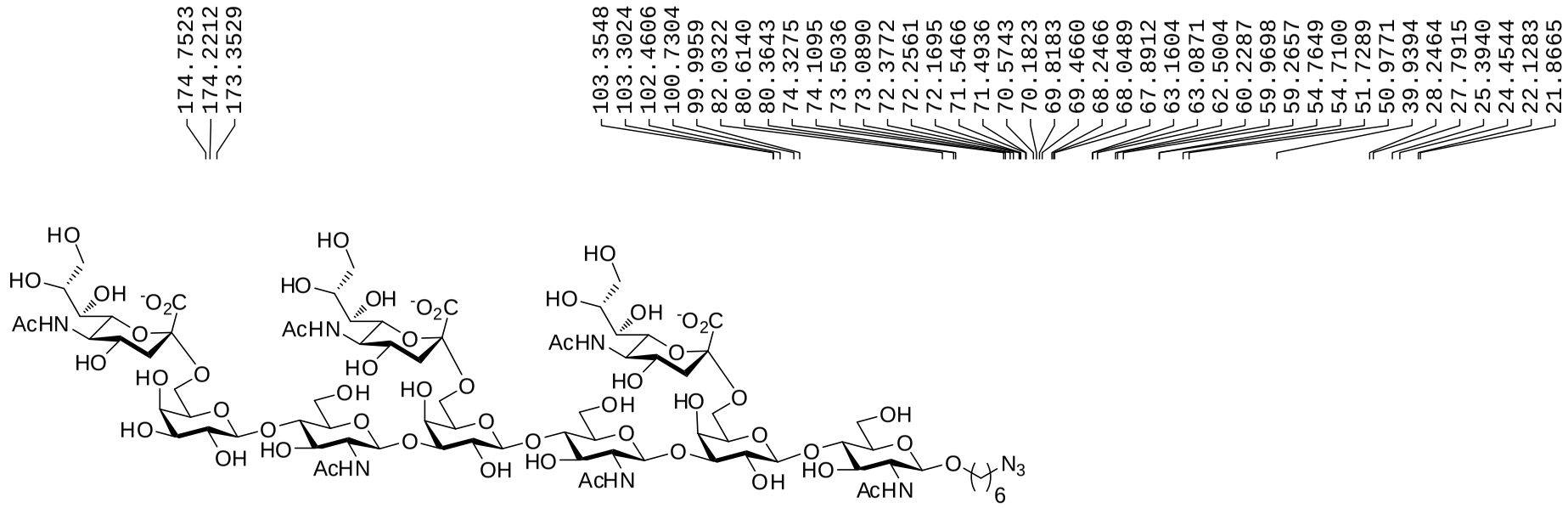
4.95



18

^{13}C NMR

150 MHz

D_2O