Supporting Information for:

Disubstituted Sialic Acid Ligands Targeting Siglecs CD33 and CD22 Associated with Myeloid Leukaemias and B Cell Lymphomas

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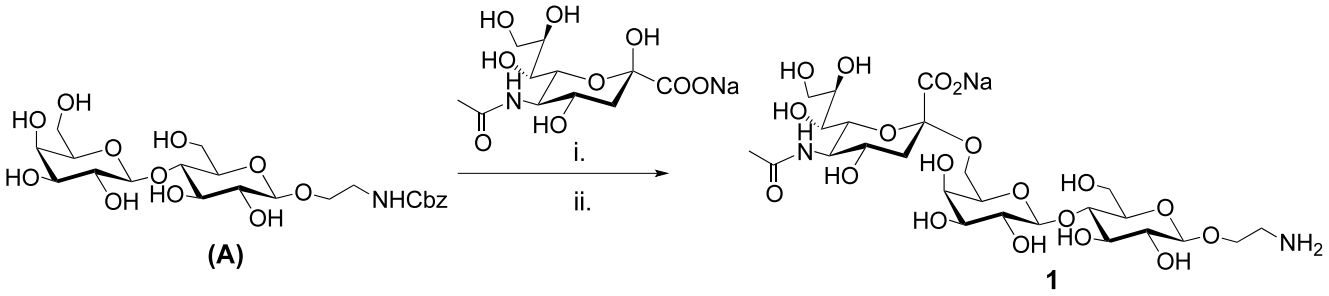
Supplementary Methods

General Methods for Synthesis. All chemicals were obtained from Sigma Aldrich or Acros Chemical Companies unless otherwise stated. NHS-activated-PEGylated-lipid (**Supplementary Scheme 8**) was purchased from NOF Corporation. C_{18} columns (2g) were obtained from Waters Corp. Reactions were monitored by thin-layer chromatography on 60F pre-coated TLC plates (EMD Chemicals, Inc.) using a 6:3:3:2 or 12:3:3:2 solvent system (EtOAc:AcOH:MeOH:H_2O, v:v:v:v). Compounds were visualized by UV light and/or dipping in 5% sulfuric acid in EtOH followed by charring on a hot plate. NMR spectra were obtained on a Bruker DRX-600 MHz instrument at 25°C. Spectra obtained in D_2O were referenced to external acetone (^{1}H δ 2.225 and ^{13}C δ 29.9). ESI-TOF high-accuracy mass spectrometry was recorded with an LC MSD TOF (Agilent Technologies). Silica gel column chromatography was performed with 60-200 mesh silica gel under medium pressure. Gel filtration was carried out with P-2 resin (Bio-Rad) as previously described with solvents degassed prior to use. Dialysis cassettes, used for the purification of the various Siglec-ligand lipids were obtained from Pierce. For enzymatic synthesis, bacteria expressing the CMP-NeuAc Synthetase from *N. Meningitidis* were obtained and grown as previously described[1]. The crude cell lysate was then used for synthesis. The *P. Damsella* α2,6 sialyltransferase (Pd2,6ST) was expressed and purified as reported by Yu et al[2]. The *C. Perfringens* NeuAc aldolase was purchased from the Toyobo Corporation. Compounds **B**[3], **D**[4], **I**[5], **5**[4], **6**[4], the Cu(I) stabilizing ligand, TTTA[6], Lac-HEG-Biotin[7] and BPC-NeuAc[8] were synthesized as previously reported.



Supplementary Scheme 1 - Conditions and Reagents: i) CTP, *N. Meningitidis* CMP-NeuAc Synthetase, *P. Damsella* α2,6 Sialyltransferase. ii) Pd/C, H_2, H_2O. Yield: 95% over 2 steps.

Synthesis of Compound 1 – Lactose-β-O-ethylazide[3] (Compound **B**, 520 mg, 1.26 mmol, 1 eq.) and Pd/C (100 mg) were dissolved in MeOH (30 mL) and left under a H_2 atmosphere overnight with stirring. The next day the catalyst was filtered over celite and the solvent evaporated. The residue was redissolved in MeOH (15 mL), *N*, *N*-diisopropylethylamine (DIEA) (52 μl, 0.315 mmol, 0.25 eq.) was added, followed by N-(benzyloxycarbonyloxy)succinimide (474 mg, 1.9 mmol, 1.5 eq). The reaction was left to proceed for two hours before evaporating and purifying on silica gel. The desired product was eluted with 15% MeOH in CH_2Cl_2. Further purification on Biogel P-2 (2.5 x 100 cm) running in 100 mM NH_4CO_3 yielded 437 mg (0.84 mmol, 67% yield) of compound **A**.

Compound **A**:

HRMS: $C_{22}H_{33}NO_{13,}$ $[M+H]^+$: Expected: 520.2025, Found: 520.2022

^{1}H NMR (600 MHz, MeOD) δ 7.43 – 7.21 (m, 5H), 5.06 (s, 2H), 4.34 (d, *J* = 7.7 Hz, 1H), 4.29 (d, *J* = 7.9 Hz, 1H), 3.93 – 3.85 (m, 2H), 3.85 – 3.79 (m, 2H), 3.76 (dd, *J* = 11.5, 7.5 Hz, 1H), 3.68 (dd, *J* = 11.4, 4.6 Hz, 1H), 3.63 (ddd, *J* = 10.8, 7.0, 4.2 Hz, 1H), 3.59 – 3.49 (m, 4H), 3.47 (dd, *J* = 9.7, 3.3 Hz, 1H), 3.39 (ddd, *J* = 14.5, 6.4, 4.1 Hz, 2H), 3.24 (dd, *J* = 9.1, 7.8 Hz, 1H). Note: 1H missing under MeOD peak.

^{13}C NMR (151 MHz, MeOD) δ 158.38, 137.77, 128.89 (2C's), 128.42, 128.27 (2C's), 104.51, 103.80, 79.84, 76.52, 75.89, 75.68, 74.17, 71.98, 69.72, 69.48, 66.92, 61.92, 61.19, 41.42.

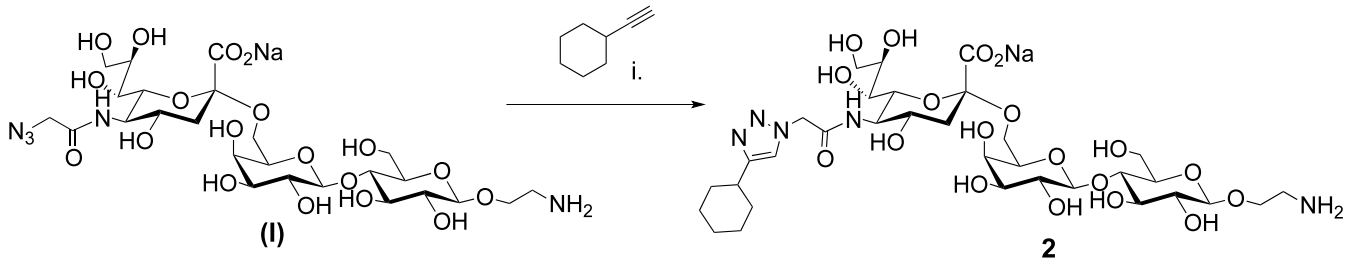
Compound **A** (7.5 mg, 14.4 μmol, 1 eq.), NeuAc (6.7 mg, 21.7 umol, 1.5 eq) and CTP (15.7 mg, 28.8 μmol, 2 eq.) were dissolved in Tris buffer solution (1.5 mL of a100 mM, 20 mM $MgCl_2$, pH 9.0) to which *N. Meningitidis* CMP-Synthetase (1.5 U) and *P. Damsella* α2-6 Sialyltransferase (0.15 U) were added. The reaction was left to proceed for 2.5 hrs at 37°C with end over end rotation after which time it was loaded onto a C18 column (2g, Waters), washed with H_2O (20 mL) and eluted with 30% MeOH in H_2O. After evaporation and lyophilization, Pd/C (3 mg) was added to the flask followed by H_2O (2 mL). The flask was then purged with H_2 and left under a H_2 atmosphere for 2 hrs after which time the reaction was complete. The reaction was then filtered through a 0.22 μm syringe filter and lyophilized to afford Compound **1** as a white solid (9.3 mg, 13.7 μmol, 95% yield).

Compound **1**:

HRMS: $C_{25}H_{44}N_2O_{19,}$ $[M+H]^+$: Expected: 677.2611, Found: 677.2611

^{1}H NMR (600 MHz, D_2O) δ 4.41 (d, *J* = 8.0 Hz, 1H), 4.29 (d, *J* = 7.9 Hz, 1H), 3.97 (ddd, *J* = 11.4, 4.9, 4.9 Hz, 1H), 3.83 (t, *J* = 9.6 Hz, 2H), 3.80-3.78 (m, 2H), 3.77 – 3.65 (m, 5H), 3.58 (dd, *J* = 10.5, 1.9 Hz, 1H), 3.56 – 3.48 (m, 6H), 3.46 (dd, *J* = 10.4, 3.7 Hz, 1H), 3.42 (d, *J* = 9.1 Hz, 1H), 3.39 (dd, *J* = 10.0, 7.8 Hz, 1H), 3.26 (t, *J* = 8.6 Hz, 1H), 3.08 (t, *J* = 5.1 Hz, 2H), 2.57 (dd, *J* = 12.5, 4.7 Hz, 1H), 1.89 (s, 3H), 1.60 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 175.68, 174.25, 103.93, 102.61, 101.04, 80.21, 75.42, 75.23, 74.51, 73.43, 73.29, 73.13, 72.58, 71.54, 69.28, 69.15, 69.14, 67.20, 64.42, 63.40, 60.89, 52.54, 40.86, 40.26, 22.83.



Supplementary Scheme 2 - Conditions and Reagents: i) CuI, TTTA, DMF, H_2O. Yield: 85%.

Synthesis of Compound 2- Compound **I** (15 mg, 20.3 μmol, 1 eq.) was dissolved in H_2O (200 μl) to which a solution of ethynylcyclohexane (5.5 mg, 50.7 μmol, 2.5 eq.) in DMF (100 μl) was added. In a separate vial, TTTA (6.8 mg, 16.2 μmol, 0.8 eq.), CuI (1.5 mg, 8.1 μmol, 0.4 eq.), and DMF (200 μl) were stirred until a homogenous solution was obtained (~15 minutes). The CuI/TTTA solution (100 μl) was added to azide/alkyne mixture, and the reaction was allowed to proceed overnight at room temperature.

The reaction was centrifuged to remove insoluble precipitate and then loaded onto a P-2 column (0.625 x 42.5 cm) running in 100 mM NH_4CO_3. Fractions containing the product were pooled, lyophilized, redissolved in H_2O and further purified by C18 column chromatography (2g, Waters) washing with H_2O (20 mL) and eluting with 35% MeOH (20 mL). After evaporation and lyophilization 14.6 mg of a white solid was obtained (14.7 mg, 17.3 μmol, 85% yield).

Compound **2**:

HRMS: $C_{33}H_{55}N_5O_{19,}$ $[M+H]^+$: Expected: 826.3564, Found: 826.3562

^{1}H NMR (600 MHz, D_2O) δ 7.66 (s, 1H), 5.10 (s, 2H), 4.40 (d, *J* = 7.8 Hz, 1H), 4.29 (d, *J* = 7.8 Hz, 1H), 3.98 (br s, 1H), 3.87 – 3.72 (m, 7H), 3.70 – 3.65 (m, 3H), 3.60 – 3.48 (m, 6H), 3.46 (dd, *J* = 10.4, 3.4 Hz, 1H), 3.43 (d, *J* = 9.1 Hz, 1H), 3.39 (dd, *J* = 10.0, 7.9 Hz, 1H), 3.26 (t, *J* = 8.3 Hz, 1H), 3.11 (br s, 2H), 2.62 (br s, 1H), 2.58 (dd, *J* = 12.4, 4.7 Hz, 1H), 1.89 – 1.80 (m, 2H), 1.67 – 1.53 (m, 4H), 1.32 – 1.22 (m, 4H), 1.16 – 1.08 (m, 1H).

^{13}C NMR (151 MHz, D_2O) δ 177.47, 174.21, 169.40, 124.08, 103.93, 102.60, 101.07, 80.25, 75.41, 75.22, 74.53, 73.42, 73.13, 73.02, 72.60, 71.53, 69.28, 69.13, 69.09, 64.47, 63.39, 60.88, 52.83, 52.77, 40.88, 35.24, 33.12 (2C's), 26.33 (2C's), 26.29.

Synthesis of Compounds 3-4, 7-8, 11-16 – Compound **A** (50 mg, 96.3 μmol, 1 eq.) along with CTP (105.1 mg, 192.6 μmol, 2 eq.) and 9-NH2-NeuAc (35.5 mg, 115.6 μmol,1.2 eq) were dissolved in Tris buffer solution (10 mL, 100 mM, 20 mM $MgCl_2$, pH 9.0). *N. Meningitidis* CMP-Synthetase (10 U) and *P. Damsella* α2-6 Sialyltransferase (1 U) were added and the reaction was left to proceed for 2.5 hrs at 37°C with end over end rotation, after which time TLC indicated the reaction was complete. The reaction was centrifuged and then loaded onto a 2g C18 Sep-Pak column (Waters Corp.). The column was washed with H_2O (20 mL) and the desired product was then eluted with 30% MeOH/H_2O. After evaporation of MeOH, the residual water was lyophilized to afford 76.4 mg of compound **C** as a white solid (94.4 μmol, 98% yield).

Compound **C**:

HRMS: $C_{33}H_{51}N_3O_{20,}$ $[M+H]^+$: Expected: 810.3139, Found: 810.3135

^{1}H NMR (600 MHz, D_2O) δ 7.34-7.26 (m, 5H), 4.99 (s, 2H), 4.33 (d, *J* = 8.0 Hz, 1H), 4.29 (d, *J* = 7.9 Hz, 1H), 3.94 (ddd, *J* = 9.1, 9.1, 3.0 Hz, 1H), 3.86 – 3.75 (m, 4H), 3.72 (t, *J* = 10.1 Hz, 1H), 3.69 (dd, *J* = 8.4, 3.8 Hz, 1H), 3.67 – 3.59 (m, 3H), 3.57 – 3.41 (m, 6H), 3.39 (dd, *J* = 9.9, 8.0 Hz, 2H), 3.32 – 3.15 (m, 4H), 2.86 (dd, *J* = 13.1, 9.5 Hz, 1H), 2.57 (dd, *J* = 12.4, 4.6 Hz, 1H), 1.89 (s, 3H), 1.60 (t, *J* = 12.2, 1H).

^{13}C NMR (151 MHz, D_2O) δ 175.74, 174.18, 159.25, 137.28, 129.57 (3C's), 129.16, 128.49, 104.00, 102.96, 101.16, 80.33, 75.34, 75.31, 74.45, 73.49 , 73.09, 73.06, 71.55, 71.11, 69.77, 69.32, 68.98, 68.95, 67.72 , 64.46 , 60.94 , 52.47 , 43.09 , 41.26 , 40.87 , 22.83 .

Compound **C** (5 mg, 6.0 μmol, 1 eq.) was dissolved in MeOH (1 mL) and NEt_3 was added (2 drops) and the solution was cooled to 0 °C. The appropriate acyl chloride (12.0 μmol, 2 eq.) dissolved in CH_2Cl_2 (0.5 mL) was then added dropwise and the reaction was stirred for 30 min at 0°C before being allowed to warm to room temperature. For the majority of reactions, this procedure resulted in the complete conversion. If a reaction was not complete, it was cooled back to 0 °C and additional acyl chloride was added until complete conversion was observed by TLC. In case of overacylation, a few drops of NaOMe (2M in MeOH) were added and the reaction was left to stir for 1 hr at room temperature. After this the solvents were evaporated to dryness, the residue resuspended in 100 mM NH_4CO_3 (0.5 mL), and loaded directly onto a P-2 column (0.625 x 42.5 cm) running in 100 mM NH_4CO_3 to yield the Cbz protected intermediates.

The intermediate was then dissolved in H_2O (2 mL) and Pd/C (2.5 mg) was added. The flask was purged with H_2 and the reaction allowed to proceed under a H_2 atmosphere until complete (~2 hrs). After this time the reaction was filtered through a 0.22 μm syringe filter and lyophilized. Yields of 90-99% over 2 steps were typically achieved with this method and with purity typically >95%. Selected NMR characterization and spectra are given below.

Compound **3**:

HRMS: $C_{32}H_{49}N_3O_{19,}$ $[M+H]^+$: Expected: 780.3033, Found: 780.3038.

^{1}H NMR (600 MHz, D_2O) δ 7.65 (d, *J* = 7.7 Hz, 2H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 2H), 4.37 (d, *J* = 8.0 Hz, 1H), 4.27 (d, *J* = 7.9 Hz, 1H), 3.98 – 3.91 (m, 2H), 3.83 (t, *J* = 9.8 Hz, 2H), 3.80 – 3.72 (m, 3H), 3.69 – 3.62 (m, 4H), 3.55 – 3.50 (m, 3H), 3.50 – 3.42 (m, 4H), 3.41 – 3.36 (m, 2H), 3.24 (t, *J* = 8.7 Hz, 1H), 3.07 (t, *J* = 5.0 Hz, 2H), 2.57 (dd, *J* = 12.4, 4.7 Hz, 1H), 1.86 (s, 3H), 1.60 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 175.62, 174.24, 172.10, 134.44, 132.88, 129.55 (2C's), 127.91 (2C's), 103.95, 102.61, 101.12, 80.30, 75.41, 75.26, 74.56, 73.41, 73.18, 73.12, 71.51, 70.89, 69.33, 69.10, 67.41, 64.58, 60.92, 52.55, 43.57, 40.88, 40.29, 22.80.

Compound **4**:

HRMS: $C_{38}H_{53}N_3O_{19,}$ $[M+H]^+$: Expected: 856.3346, Found: 856.3357.

^{1}H NMR (600 MHz, D_2O) δ 7.68 (d, *J* = 8.2 Hz, 2H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.56 (d, *J* = 7.8 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 1H), 4.27 (d, *J* = 8.0 Hz, 1H), 4.24 (d, *J* = 7.9 Hz, 1H), 3.93 (ddd, *J* = 8.8, 8.8, 3.4 Hz, 1H), 3.90 - 3.82 (m, 2H), 3.80-3.76 (m, 2H), 3.74 (d, *J* = 10.1 Hz, 1H), 3.72 - 3.58 (m, 5H), 3.56 - 3.35 (m, 9H), 3.19 (t, *J* = 8.6 Hz, 1H), 3.06 - 2.92 (m, 2H), 2.57 (dd, *J* = 12.2, 4.6 Hz, 1H), 1.87 (s, 3H), 1.61 (dd, *J* = 12.2, 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 175.64, 174.24, 171.26, 144.70, 140.10, 133.09, 129.96 (2C's), 129.16, 128.62 (2C's), 127.85 (4C's), 103.97, 102.58, 101.16, 80.31, 75.39, 75.28, 74.54, 73.34, 73.32, 73.13, 71.50, 71.14, 70.89, 69.30, 69.10, 67.45, 64.55, 60.93, 52.56, 43.76, 40.89, 40.25, 22.83.

Compound **7**:

HRMS: $C_{33}H_{51}N_3O_{19,}$ $[M+H]^+$: Expected: 794.3189, Found: 794.3187.

^{1}H NMR (600 MHz, D_2O) δ 7.48 (s, 1H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 7.5 Hz, 1H), 7.28 (t, *J* = 7.6 Hz, 1H), 4.35 (d, *J* = 8.0 Hz, 1H), 4.27 (d, *J* = 7.8 Hz, 1H), 3.95 – 3.90 (m, 2H), 3.85 – 3.80 (m, 2H), 3.78 (d, *J* = 3.3 Hz, 1H), 3.76 – 3.72 (m, 2H), 3.68 – 3.61 (m, 4H), 3.55 – 3.42 (m, 8H), 3.40 – 3.36 (m, 2H), 3.22 (dd, *J* = 9.1, 7.9 Hz, 1H) 3.03 – 2.98 (m, 2H), 2.56 (dd, *J* = 12.2, 4.7 Hz, 1H), 2.26 (s, 3H), 1.86 (s, 3H), 1.60 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 175.62, 174.25, 172.15, 139.81, 134.45, 133.52, 129.49, 128.40, 124.93, 103.96, 102.65, 101.14, 80.33, 75.41, 75.28, 74.55, 73.42, 73.19, 73.13, 71.52, 70.89, 70.88, 69.32

Compound **12**:

HRMS: $C_{38}H_{53}N_3O_{19,}$ $[M+H]^+$: Expected: 856.3346, Found: 856.3348.

^{1}H NMR (600 MHz, D_2O) δ 7.86 (dd, *J* = 3.0, 1.5 Hz, 1H), 7.72 (d, *J* = 7.7 Hz, 1H), 7.65 – 7.60 (m, 1H), 7.60 – 7.56 (m, 2H), 7.46 (ddd, *J* = 7.8, 7.8, 1.7 Hz, 1H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.35 – 7.30 (m, 1H), 4.27 (d, *J* = 8.0 Hz, 1H), 4.23 (d, *J* = 7.9 Hz, 1H), 3.97 – 3.95 (ddd, *J* = 3.5, 7.3, 9.0 Hz, 1H), 3.91 (ddd, *J* = 11.5, 5.0, 5.0 Hz, 1H), 3.84 (dd, *J* = 10.2, 8.6 Hz, 1H), 3.80 – 3.69 (m, 4H), 3.69 – 3.63 (m, 3H), 3.60 (dd, *J* = 12.3, 5.3 Hz, 1H), 3.55 – 3.43 (m, 5H), 3.43-3.39 (m, 2H), 3.39 – 3.33 (m, 2H), 3.19 (dd, *J* = 9.1, 8.1 Hz, 1H), 3.04 (t, *J* = 5.0 Hz, 2H), 2.57 (dd, *J* = 12.3, 4.5 Hz, 1H), 1.84 (s, 3H), 1.60 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 175.63, 174.24, 171.64, 141.68, 140.39, 135.08, 131.21, 130.22, 130.00 (2C's), 128.90, 127.81 (2C's), 126.99, 126.36, 103.94, 102.56, 101.14, 80.29, 75.37, 75.25, 74.56, 73.34, 73.21, 73.13, 71.49, 71.09, 70.88, 69.32, 69.10, 67.14, 64.60, 60.90, 52.56, 43.75, 40.87, 40.23, 22.80.

Compound **13**:

HRMS: $C_{34}H_{53}N_3O_{19,}$ $[M+H]^+$: Expected: 808.3346, Found: 808.3342

^{1}H NMR (600 MHz, D_2O) δ 7.28 (s, 2H), 7.17 (s, 1H), 4.35 (d, *J* = 8.0 Hz, 1H), 4.26 (d, *J* = 7.9 Hz, 1H), 3.98 – 3.89 (m, 2H), 3.85 – 3.80 (m, 2H), 3.79 (d, *J* = 3.4 Hz, 1H), 3.78 – 3.72 (m, 2H), 3.69 – 3.60 (m, 4H), 3.55 – 3.42 (m, 7H), 3.39 (dd, *J* = 9.8, 8.0 Hz, 2H), 3.23 (t, *J* = 8.7 Hz, 1H), 3.04 (t, *J* = 5.1 Hz, 2H), 2.57 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.22 (s, 6H), 1.85 (s, 3H), 1.60 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 175.61, 174.25, 172.17, 139.76 (2C's), 134.48, 134.22, 125.48 (2C's), 103.96, 102.62, 101.15, 80.33, 75.41, 75.27, 74.57, 73.39, 73.19, 73.14, 71.52, 70.88, 70.87, 69.33, 69.08, 67.70, 64.59, 60.94, 52.56, 43.52, 40.88, 40.33, 22.79 (2C's), 21.08.

Synthesis of Compounds 9-10 – Compound **D** (4.4 mg, 6.0 μmol, 1 eq.) was dissolved in MeOH (1 mL) and NEt_3 was added (2 drops) and the solution was cooled to 0 °C. The appropriate acyl chloride (12.0 μmol, 2 eq.) dissolved in CH_2Cl_2 (0.5 mL) was then added dropwise and the reaction was stirred for 30 min at 0 °C before being warmed to room temperature. Usually, this procedure resulted in the complete conversion. If a reaction was not complete, it was cooled back to 0 °C and additional acid chloride was added until complete conversion was observed by TLC. In the case of overacylation, a few drops of NaOMe (2M in MeOH) were added and the reaction was left to stir for 1 hr at room temperature. After this the reactions were evaporated to dryness, resuspended in 100 mM NH_4CO_3 (0.5 mL), and loaded directly onto a P-2 column (0.625 x 42.5 cm) running in 100 mM NH_4CO_3 to yield the azide protected intermediates.

The azide intermediate was then redissolved in THF:H_2O (1 mL, 1:1 v/v) to which a 1M solution of PMe_3 in THF was added (15 μL, 15 μmol, 2.5 eq.). After stirring for 2 hrs at room temperature the reaction was complete and the solution was evaporated. The desired products were then purified on a C18 column eluting in 30-40% MeOH in H_2O and obtained in ~75% yield over 2 steps.

Synthesis of Compounds 17-19, 21 – Compound **C** (5 mg, 6.0 μmol, 1 eq.) was dissolved in 100 mM $NaHCO_3$ (0.5 mL) and the solution was cooled to 0 °C. The appropriate NHS-Ester (12.0 μmol, 2 eq.), dissolved in DMF (0.5 mL), was added dropwise to the reaction which was then left to stir at 0°C and slowly warmed to room temperature. Once the reaction was complete, it was evaporated to dryness, redissolved in H_2O (0.5 mL), centrifuged to remove insoluble precipitate, and loaded onto a C18 column. After washing with H_2O, the desired compound was eluted with 30-40% MeOH/H_2O.

The Cbz protected intermediates were then subjected to hydrogenation as above, Pd/C removed by syringe filtration, and the desired products were obtained in good yield (typically >90%, with the exception of a 77% yield for the 3,5-dimethyl-4-hydroxy derivative) and excellent purity (>95%).

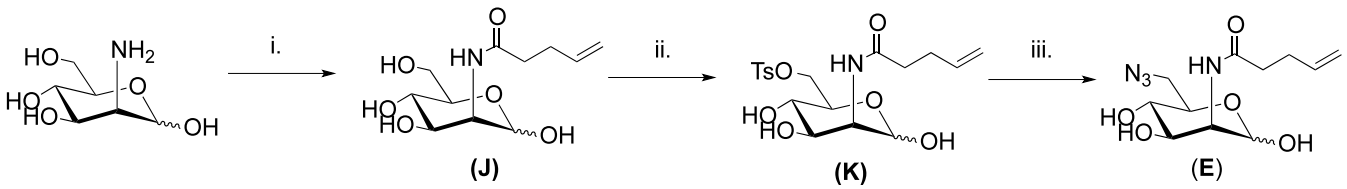
Compound **17**:

HRMS: $C_{34}H_{53}N_3O_{20,}$ $[M+H]^+$: Expected: 824.3895, Found: 824.3289

^{1}H NMR (600 MHz, D_2O) δ 7.33 (s, 2H), 4.28 (d, *J* = 8.0 Hz, 1H), 4.25 (d, *J* = 7.9 Hz, 1H), 3.93 (ddd, *J* = 10.7, 4.9, 4.9 Hz, 1H), 3.89 (ddd, *J* = 9.6, 7.1, 3.6 Hz, 1H), 3.85 – 3.77 (m, 3H), 3.74 (ddd, *J* = 10.2, 10.2, 4.6 Hz, 2H), 3.69-3.64 (m, 2H), 3.61 (dd, *J* = 12.2, 5.0 Hz, 1H), 3.58 (dd, *J* = 14.1, 3.5 Hz, 1H), 3.54 – 3.35 (m, 9H), 3.18 (t, *J* = 8.6 Hz, 1H), 3.04 (t, *J* = 5.1 Hz, 2H), 2.56 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.06 (s, 6H), 1.84 (s, 3H), 1.59 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 177.47, 175.60, 174.25, 171.72, 128.47 (2C's), 126.79 (2C's), 121.30, 103.97, 102.61, 101.19, 80.35, 75.38, 75.27, 74.52, 73.33, 73.32, 73.16, 71.54 , 71.02, 70.98, 69.28, 69.10, 67.69, 64.53, 60.98, 52.54, 43.51, 40.90, 40.32, 22.78, 17.40 (2C's).

Synthesis of Compound 20 - The acylation step was carried out as described above for compounds **17-19**, **21,** and the product was purified on a C18 column. The azide intermediate was then reduced with PMe_3 as above for compounds **9-10** (leaving the NO_2 group intact) and purified on Biogel P-2 (0.625 x 42.5 cm) running in 100 mM NH_4CO_3. The desired product was obtained in this way in good yield (72%).



Supplementary Scheme 3 - Conditions and Reagents: i) MeOH/H_2O, $NaHCO_3$, 4-Pentenoic Anhydride. Yield: 93%. ii) Pyridine, Tosyl Chloride, Yield: 76%. iii) NaN_3, DMF 65°C, Yield: 49%.

Synthesis of Compound E - Mannosamine-HCl (2 g, 9.27 mmol, 1 eq.) was dissolved in MeOH/H_2O (3:1, v/v, 65 mL) to which $NaHCO_3$ (2.95 g, 35.1 mmol, 3.8 eq) was added. The mixture was cooled to O°C and then pentenoic anhydride (1.86 g, 10.2 mmol, 1.1 eq) was added dropwise over 5 minutes. After 8 hrs at 0°C, another portion of pentenoic anhydride was added (169 mg, 0.93 mmol, 0.1 eq) and the reaction was left to proceed for another 2 hrs at which time TLC indicated the reaction was complete. The solution was warmed to room temperature, evaporated, and purified on silica gel eluting with MeOH:CH_2Cl_2 (1:7, v/v) yielding 2.25 g (8.60 mmol, 93% yield) of compound **J** a dark yellow oil.

Compound **J:**

HRMS: $C_{11}H_{19}NO_6$ $[M+H]^+$: Expected: 262.1285, Found: 262.1284.

Major Isolated Anomer (β)

^{1}H NMR (600 MHz, MeOD) δ 5.88 – 5.81 (m, 1H), 5.05 (dd, *J* = 17.0, 1.8 Hz, 1H), 4.97 (d, *J* = 1.8 Hz, 1H) 4.96 (dd, *J* = 10.5, 1.7 Hz, 1H), 4.27 (dd, *J* = 4.7, 1.6 Hz, 1H), 3.99 (dd, *J* = 9.7, 4.7 Hz, 1H), 3.78 – 3.73 (m, 2H), 3.57 (t, *J* = 9.7 Hz, 1H), 2.37 – 2.31 (m, 4H).

^{13}C NMR (151 MHz, MeOD) δ 175.59, 137.88, 115.13, 94.45, 72.87, 70.04, 67.90, 61.67, 54.48, 35.68, 30.39.

Compound **J** (2.2 g, 8.4 mmol, 1 eq) was dissolved in pyridine (40 mL) and cooled to 0 °C. Tosyl chloride (2.4 g, 12.6 mmol, 1.5 eq) dissolved in pyridine (10 mL) was then added to the flask dropwise over 5 minutes. The reaction was left to proceed for 2 hrs at 0 °C after which time another portion of tosyl chloride (1 g, 5.2 mmol, 0.6 eq) was added and the reaction was left to proceed another 1 hr at 0 °C. A final portion of tosyl chloride was added (0.2 g, 0.43 mmol, .05 eq) at 0 °C, the reaction was left to warm to room temperature and then methanol was added (20 mL). The reaction mixture was adsorbed on silica gel and eluted with 4% MeOH in EtOAc to yield the product (2.65 g, 6.4 mmol, 76% yield), compound **K**, as an oil.

Compound **K:**

HRMS: $C_{18}H_{25}NO_8S$ $[M+H]^+$: Expected: 416.1374, Found: 416.1373.

Major Isolated Anomer (β)

^{1}H NMR (600 MHz, MeOD) δ 7.79 (d, *J* = 8.3 Hz, 2H), 7.43 (d, *J* = 7.9 Hz, 2H), 5.83 (dddd, *J* = 16.5, 10.2, 6.8, 4.7 Hz, 1H), 5.04 (dd, *J* = 17.0, 1.4 Hz 1H), 4.96 (dd, *J* = 10.7, 1.4 Hz, 1H), 4.93 (d, *J* = 1.6 Hz, 1H), 4.29 (dd, *J* = 10.5, 1.9 Hz, 1H), 4.21 (dd, *J* = 4.7, 1.6 Hz, 1H), 4.18 (dd, *J* = 10.5, 6.6 Hz, 1H), 3.94 (dd, *J* = 9.7, 4.8 Hz, 1H), 3.91 (ddd, *J* = 10.1, 6.8, 2.2 Hz, 1H), 3.45 (t, *J* = 9.8 Hz, 1H), 2.44 (s, 3H), 2.38 – 2.29 (m, 4H).

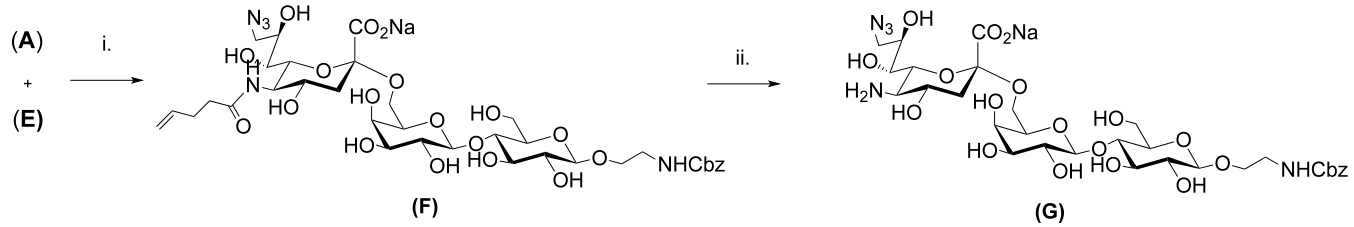
^{13}C NMR (151 MHz, MeOD) δ 175.68, 145.94, 137.82 , 133.69 , 130.46 (2C's), 128.55 (2C's), 115.21, 94.19, 70.72, 70.69, 69.55, 67.95, 54.51, 35.53, 30.40, 21.02.

Compound **K** (1.86 g, 4.5 mmol, 1 eq.) and NaN_3 (871 mg, 13.4 mmol, 3 eq.) were dissolved in DMF (20 mL) and the reaction was heated at 65 °C overnight. The mixture was then evaporated and purified on silica gel eluting with 1.5% MeOH in EtOAc. The desired product was inseparable from a by-product (believed to be the 1,6 anhydro elimination product). After evaporation 945 mg of a sticky off-white solid, compound **E**, was obtained. ^{1}H NMR the approximately 2:1 (mol:mol) ratio or product:byproduct, yielding 632 mg (2.21 mmol, 49% yield) of the desired product which was used without further purification.

Compound **E**:

HRMS: $C_{11}H_{18}N_4O_5$ $[M+H]^+$: Expected: 287.1350, Found: 287.1353.

1,6 Anhydro byproduct, HRMS: $C_{11}H_{17}NO_5$ $[M+H]^+$: Expected: 284.1185, Found: 284.1187



Supplementary Scheme 4 - Conditions and Reagents: i) (**A**), (**E**), Sodium Pyruvate, *C. Perfringens* NeuAc Aldolase, CTP, *N. Meningitidis* CMP-NeuAc Synthetase, *P. Damsella* ⟨2,6 Sialyltransferase. Yield: 96%. ii) H_2O, MeOH, I_2, pH 1.0. Yield: 75%.

Synthesis of Compound G – Compound **A** (60 mg, 115.5 μmol, 1 eq.), CTP (126 mg, 231 μmol, 2 eq.), pyruvate (124.5 mg, 808 μmol, 7 eq.), and the crude Compound **E** (60 mg crude = ~40 mg, 138.4 μmol, 1.2 eq.) were dissolved in Tris buffer solution (10 mL of a 100 mM, 20 mM $MgCl_2$, pH 9.0) and *C. Perfringens* NeuAc Aldolase (10 U), *N. Meningitidis* CMP-Synthetase (10 U) and *P. Damsella* α2-6 Sialyltransferase (1 U) were added. The reaction was allowed to proceed at room temperature overnight with end-over-end rotation (quantitative conversion), centrifuged, and loaded onto a C18 column (2g, Waters Corp.). After washing with H_2O (20 mL), the eluent was changed to 15% MeOH in H_2O (20 mL) then 50% MeOH in H_2O (20 mL). The desired product elutes at the end of the 15% MeOH elution and throughout the 50% MeOH elution. After evaporation of MeOH, the residual H_2O was lyophilized to obtain 97.2 mg of a white solid (111 μmol, 96% yield).

Compound **F**:

HRMS: $C_{36}H_{53}N_5O_{20,}$ $[M+H]^+$: Expected: 876.3356, Found: 876.3355

^{1}H NMR (600 MHz, D_2O) δ 7.38 – 7.21 (m, 5H), 5.73 (dddd, *J* = 16.6, 10.3, 6.3, 6.3 Hz, 1H), 5.04 – 4.95 (m, 3H), 4.93 (d, *J* = 10.3 Hz, 1H), 4.33 (d, *J* = 7.8 Hz, 1H), 4.27 (d, *J* = 7.9 Hz, 1H), 3.88 (ddd, *J* = 8.8, 6.1, 2.5 Hz, 1H), 3.85 – 3.76 (m, 4H), 3.70 (t, *J* = 10.2 Hz, 1H), 3.67 (dd, *J* = 8.3, 3.9 Hz, 1H), 3.66 – 3.58 (m, 4H), 3.57 – 3.42 (m, 8H), 3.39 (dd, *J* = 9.9, 7.9 Hz, 1H), 3.35 (dd, *J* = 13.2, 6.1 Hz, 1H), 3.29-3.17 (m, 3H), 2.57 (dd, *J* = *J* = 12.4, 4.6 Hz, 1H), 2.29-2.21 (m, 4H), 1.59 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 177.87, 174.23, 159.25, 137.82, 137.29, 129.57 (3C's), 129.16, 128.49, 116.55, 103.97, 102.95, 101.09, 80.31, 75.38, 75.30, 74.46, 73.49, 73.14, 73.13, 71.54, 71.10, 69.89, 69.74, 69.26, 68.87, 67.72, 64.31, 60.99, 60.08, 53.91, 52.50, 41.27, 41.00, 35.98, 30.15.

Compound **F** (109 mg, 124.5 μmol, 1 eq.) was dissolved in H_2O (5 mL, titrated to pH 1.0 with HCl). I_2 (94.8 mg, 373 μmol, 3 eq.) dissolved in 3 mL of MeOH was added and the reaction was left to proceed for 3 hrs at room temperature. The MeOH was then removed on a rotary evaporator (without heating) and the residual aqueous layer was then loaded directly onto a C18 column (2g, Waters Corp.). The column was washed with H_2O (15 mL), 30% MeOH (20 mL), 32.5% MeOH (20 mL), and 35% MeOH (20 mL). The desired product elutes cleanly throughout the 30% MeOH elution and the early fractions of 32.5% MeOH elution after which time it coelutes with a byproduct. From this initial purification 48 mg of pure product, compound **G**, and 57 mg of a mixture were obtained. The mixture is then repurified as above to afford another 26 mg of pure product (74 mg total, 93.4 μmol, 75% yield).

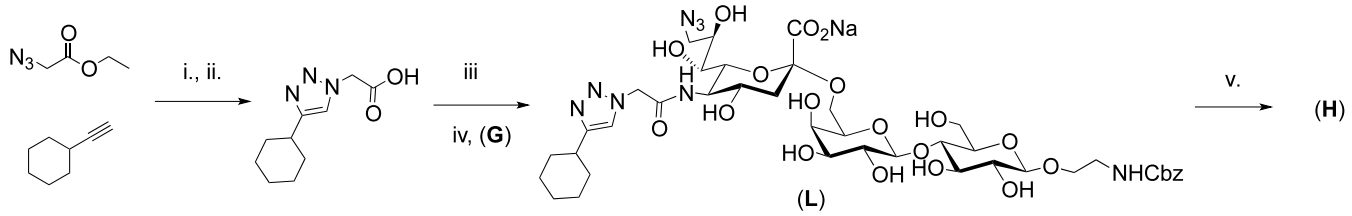
Note: The byproduct in this reaction forms by the competing opening of the iminolactone intermediate to a stable, 4-hydroxy-5-iodo-pentanamide (LC/MS $C_{36}H_{54}IN_5O_{21}$: $[M+H]^+$ Expected: 1020.2, Found: 1020.3). This side reaction was found to be reduced at lower pH.

Compound **G**:

HRMS: $C_{31}H_{47}N_5O_{19,}$ $[M+H]^+$: Expected: 794.2938, Found: 794.4939

^{1}H NMR (600 MHz, D_2O) δ 7.37 – 7.22 (m, 5H), 5.05-4.95 (m, 2H), 4.32 (d, *J* = 7.9 Hz, 1H), 4.27 (d, *J* = 7.9 Hz, 1H), 3.98 (ddd, *J* = 8.6, 5.8, 2.5 Hz, 1H), 3.90 – 3.83 (m, 2H), 3.83 – 3.76 (m, 3H), 3.68 (dd, *J* = 8.6, 3.1 Hz, 1H), 3.66 – 3.56 (m, 5H), 3.55-3.48 (m, 2H), 3.47 – 3.37 (m, 5H), 3.32-3.22 (m, 2H), 3.18 (t, *J* = 8.7 Hz, 1H), 3.06 (t, *J* = 10.1 Hz, 1H), 2.60 (dd, *J* = 12.5, 4.7 Hz, 1H), 1.63 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 173.86, 159.25, 137.28, 129.57 (3C's), 129.17, 128.50, 104.05, 102.95, 101.02, 80.55, 75.32, 75.26, 74.54, 73.50, 73.09, 72.25, 71.47, 70.98, 69.78, 69.56, 69.28, 67.93, 67.72, 64.64, 60.94, 53.72, 53.03, 41.27, 40.98.



Supplementary Scheme 5 - Conditions and Reagents: i) t-BuOH:H_2O, $CuSO_4$, Sodium Ascorbate. ii) NaOH, H_2O, Yield: 89% over 2-steps. iii) NHS, DCC, EtOAc. iv) (**G**), DMF, NEt_3. Yield: 86%. v) THF:H_2O (1:1), PMe_3, Yield: 97%.

Synthesis of Compound H - Ethynylcyclohexane (609mg, 5.6 mmol) and ethyl 2-azidoacetate (727 mg, 5.6 mmol) were suspended in tBuOH:H_2O (4mL, 1:1, v.v), $CuSO_4$ (560 µL of a 1M solution, 0.56 mmol) followed by sodium ascorbate (440 mg, 0.22 mmol) were added, and the mixture was stirred overnight. The reaction mixture was treated with EDTA solution (5 g/L, 5 mL) and the product was extracted with EtOAc (3 x 25 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated to dryness to give ethyl 2-(4-cyclohexyl-1H-1,2,3-triazol-1-yl)acetate (1.2 g, 90%) as a white powder.

Ethyl 2-(4-cyclohexyl-1H-1,2,3-triazol-1-yl)acetate**:**

LC/MS: $C_{12}H_{19}N_3O_2$, $[M+H]^+$: Expected: 238.3, Found: 238.3.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (s, 1H), 4.98 (s, 2H), 4.06 (q, J = 6.8 Hz, 2H), 2.60 (br s, 1H), 1.9- 1.2 (br m, 10 H), 1.11 (t, J = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.0, 153.5, 120.9, 61.8, 50.4, 34.9, 32.6, 25.8, 25.7, 13.7.

Ethyl 2-(4-cyclohexyl-1H-1,2,3-triazol-1-yl)acetate (400 mg, 1.69 mmol) was suspended in a 1M aqueous NaOH solution (3 mL, 1.8 eq.) and H_2O (8 mL) and stirred until a clear solution was obtained (~3 hrs). The mixture was neutralized with 2M HCl (3 mL) and the product was extracted with EtOAc (3 x 25 mL). The combined EtOAc extracts were dried (Na_2SO_4) and concentrated to afford 350 mg of 2-(4-cyclohexyl-1H-1,2,3-triazol-1-yl)acetic acid as an off white solid (99% yield).

Ethyl 2-(4-cyclohexyl-1H-1,2,3-triazol-1-yl)acetic acid**:**

HRMS: $C_{10}H_{15}N_3O_2$, $[M+H]^+$: Expected: 210.1237, Found: 210.1238.

^{1}H NMR (400 MHz, MeOD) δ 7.74 (s, 1H), 5.21 (s, 2H), 2.74 (ddddd, J = 14.5, 6.9, 3.5, 3.5, 3.4 Hz, 1H), 2.10 – 1.98 (m, 2H), 1.88 – 1.70 (m, 3H), 1.52-1.38 (m, 4H), 1.36-1.26 (m, 1H).

^{13}C NMR (101 MHz, MeOD) 168.53, 153.14, 121.98, 50.16 , 35.08, 32.66 (2C's), 25.83 (2C's), 25.70.

2-(4-cyclohexyl-1H-1,2,3-triazol-1-yl)acetic acid (50 mg, 238.9 μmol, 1 eq.) and N-hydroxysuccinimide (34.3 mg, 298.7 μmol, 1.25 eq.) were dissolved in EtOAc (2 mL) and cooled to 0 °C. DCC (61.7 mg, 298.7 μmol, 1.25 eq.) was then added and the reaction was left to proceed overnight at 0 °C. The next day the DCU was filtered off and the solvents were evaporated to dryness. The crude solid containing the NHS-Ester was used without further purification.

Compound **G** (16 mg, 20.2 μmol, 1 eq.) was dissolved in DMF (1 mL) and NEt_3 was added (3 drops). The reaction was cooled to 0 °C, and the above crude NHS-ester was then added in portions and stirred at 0 °C → RT checking by TLC every 2 hrs after each addition. If not complete, the reaction was cooled back to 0 °C and another portion was added, and the reaction was allowed to stir at 0°C → RT. This was continued until complete conversion was achieved. Due to some overacylation, a few drops of NaOMe were added at the end of the reaction, and the solution was left to stir for an hour before evaporation. The crude reaction was taken up in H_2O (1 mL), centrifuged, and loaded onto a C18 column. The column was washed with H_2O (20 mL), 45% MeOH (20 mL), and the product eluted with 60% MeOH. After evaporation of the MeOH, the residual water was lyophilized to afford 17 mg of the azide intermediate, Compound **L** (17.2 μmol, 86% yield).

The azide intermediate, Compound **L**, (17.0 mg, 17.2 μmol) was then dissolved in THF:H_2O (1.5 mL, 1:1 v/v) and 1M PMe_3 solution in THF was added (43 μL, 43 μmol, 2.5 eq.). The reaction was left to stir for 2 hrs and then the THF was evaporated. The remaining water layer was then loaded directly onto a P-2 column (0.625 x 42.5 cm) running in 100 mM NH_4CO_3. After purification, 16.0 mg of the desired product, Compound **H**, was obtained (16.6 μmol, 97% yield),

Compound **H:**

HRMS: $C_{41}H_{62}N_6O_{20,}$ $[M+H]^+$: Expected: 959.4091, Found: 959.4088.

^{1}H NMR (600 MHz, D_2O) δ 7.63 (s, 1H), 7.34-7.21 (m, 5H), 5.09 (s, 2H), 4.97 (s, 2H), 4.32 (d, *J* = 7.2 Hz, 1H), 4.28 (d, *J* = 7.8 Hz, 1H), 3.93 (t, *J* = 9.0 Hz, 1H), 3.87 – 3.76 (m, 5H), 3.73 (d, *J* = 10.4 Hz, 1H), 3.68 (d, *J* = 8.4 Hz, 1H), 3.65 – 3.36 (m, 11H), 3.28 – 3.16 (m, 4H), 2.86 (t, *J* = 11.4, 1H), 2.62 – 2.56 (m, 2H), 1.86 – 1.79 (m, 2H), 1.66-1.59 (m, 3H), 1.55 (d, *J* = 10.9 Hz, 1H), 1.29-1.20 (m, 4H), 1.15-1.06 (m, 1H).

^{13}C NMR (151 MHz, D_2O) δ 173.94, 169.03, 159.00, 154.57, 137.35, 129.39 (2C s), 129.01, 128.50 (2C's), 123.87, 104.18, 103.27, 101.14, 80.56, 75.53, 75.51, 74.75, 73.79, 73.46, 72.97, 71.76, 71.50, 69.73, 69.61, 69.37, 69.13, 67.50, 64.66, 61.11, 53.04, 52.69, 43.35, 41.37, 41.28, 35.56, 33.32 (2C's), 26.50 (2C's), 26.41

Synthesis of Compound 22 – Compound **H** (33.6 mg, 35.0 μmol, 1 eq.) was dissolved in 100 mM $NaHCO_3$ (1.5 mL) to which DMF (1.5 mL) was added. The reaction was cooled to 0 °C, the NHS ester of 4-hydroxy-3,5-dimethyl benzoic acid (13.8 mg, 52.6 μmol, 1.5 eq) was added, the reaction stirred for 2 hrs at 0 °C, and then warmed to room temperature. Due to some overacylation, a few drops of 2M NaOH were added and the reaction was left to stir at room temperature for 15 min before evaporation to dryness. The Cbz-protected intermediate was purified on a C18 column by washing with H_2O (20 mL), 35% MeOH (20 mL), and elution with 50% MeOH. In this way, 28.4 mg was obtained (25.7 μmol, 73% yield).

The Cbz-intermediate (25.0 mg, 22.9 μmol, 1 eq.) was deprotected by hydrogenation as described above, and after syringe filtration and subsequent P-2 column purification, 20.0 mg of the title compound, **22**, was obtained (20.5 μmol, 90% yield).

Compound **22**:

HRMS: $C_{42}H_{64}N_6O_{20,}$ $[M+H]^+$: Expected: 973.4248, Found: 973.4245.

^{1}H NMR (600 MHz, D_2O) δ 7.42 (s, 1H), 7.34 (s, 2H), 5.07 (d, *J* = 16.6 Hz, 1H), 4.97 (d, *J* = 16.6 Hz, 1H), 4.29 (d, *J* = 8.0 Hz, 1H), 4.26 (d, *J* = 7.9 Hz, 1H), 3.92 (dddd, *J* = 14.3, 9.3, 4.7, 4.7 Hz, 2H), 3.85-3.78 (m, 4H), 3.77 – 3.72 (m, 2H), 3.67 (dd, *J* = 8.1, 3.6 Hz, 1H), 3.62 (dd, *J* = 12.3, 5.1 Hz, 1H), 3.61 – 3.50 (m, 4H), 3.49 – 3.45 (m, 2H), 3.44 – 3.36 (m, 4H), 3.19 (t, *J* = 8.5 Hz, 1H), 3.08 – 3.02 (m, 2H), 2.57 (dd, *J* = 12.4, 4.6 Hz, 1H), 2.55-2.47 (m, 1H), 2.06 (s, 6H), 1.80-1.75 (m, 2H), 1.66 – 1.52 (m, 4H), 1.30 – 1.05 (m, 5H).

^{13}C NMR (151 MHz, D_2O) δ 174.22, 171.72, 169.31, 155.01, 128.5 (2C's), 126.62, 126.58, 123.72 (2C's), 103.97, 102.62, 101.20, 80.37, 75.38, 75.25, 74.53, 73.33, 73.15, 72.93, 71.53, 70.93, 70.67, 69.27, 68.90, 67.42, 64.53, 60.96, 52.92, 52.71, 43.31, 40.90, 40.28, 35.18, 33.11, 33.06, 26.34 (2C's), 26.25, 17.25 (2C's).

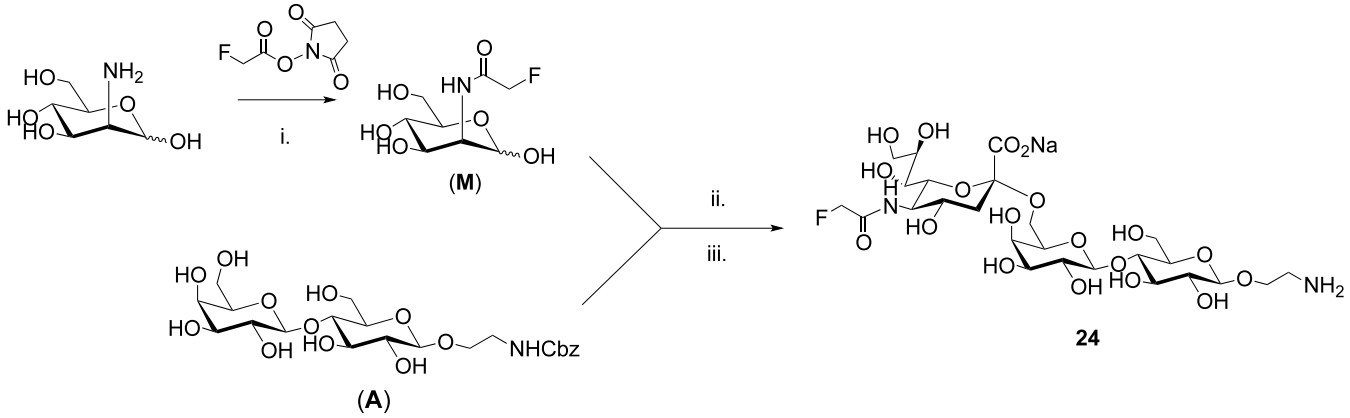
Synthesis of Compound 23 – This compound was made from compound **C**, in an analogous fashion as for compounds **3-4, 7-8, 11-16** described above, and obtained with a 94% yield over 2-steps.

Compound **23**:

HRMS: $C_{38}H_{53}N_3O_{20,}$ $[M+H]^+$: Expected: 872.3295, Found: 872.3304.

^{1}H NMR (600 MHz, D_2O) δ 7.43 – 7.40 (m, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.34 – 7.30 (m, 2H), 7.29 – 7.26 (m, 1H), 7.14 – 7.08 (m, 2H), 7.01 – 6.94 (m, 2H), 4.36 (d, *J* = 8.0 Hz, 1H), 4.27 (d, *J* = 7.9 Hz, 1H), 3.95 (ddd, *J* = 11.1, 4.8, 4.8 Hz, 1H), 3.91 (ddd, *J* = 10.4, 8.2, 3.1 Hz, 1H), 3.83 (dd, *J* = 7.2, 1.8 Hz, 1H), 3.81 (t, *J* = 4.5 Hz, 1H), 3.79 – 3.71 (m, 3H), 3.68 – 3.59 (m, 4H), 3.55-3.49 (m, 3H), 3.49 – 3.36 (m, 6H), 3.23 (dd, *J* = 9.2, 8.1 Hz, 1H), 3.05 (t, *J* = 5.1 Hz, 2H), 2.56 (dd, *J* = 12.4, 4.6 Hz, 1H), 1.85 (s, 3H), 1.60 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 175.62, 174.23, 171.12, 157.84, 157.11, 136.42, 131.23, 130.99 (2C's) , 125.00 , 123.04, 122.93, 119.90 (2C's), 118.13, 103.95, 102.62, 101.12, 80.31, 75.42, 75.27, 74.56, 73.41, 73.17, 73.13, 71.51, 70.88, 70.85, 69.33, 69.09, 67.54, 64.59, 60.94, 52.55, 43.60, 40.86, 40.30, 22.81.



Supplementary Scheme 6 - Conditions and Reagents: i) DMF, NEt_3, ii) Pyruvate, *C. Perfringens* NeuAc Aldolase, CTP, *N. Meningitidis* CMP-NeuAc Synthetase, *P. Damsella* α2,6 Sialyltransferase. Yield: 97%. Pd/C, H_2, H_2O. Yield: 89%.

Synthesis of Compound 24

Synthesis of Fluoroacetic Acid NHS-Ester: In an oven dried round bottom flask, *N*-hydroxysuccinimide (524 mg, 4.55 mmol, 1.1 eq.) was suspended in CH_2Cl_2 (15 mL) and NEt_3 was added (460.5 mg, 4.55 mmol, 1.1 eq). The flask was cooled to 0 °C and fluoroacetyl chloride (400 mg, 4.14 mmol, 1.0 eq.) was added dropwise over 5 minutes under a N_2 atmosphere. The reaction was left to proceed for 1 hr on ice and then warmed to room temperature after which time the precipitate was filtered and the reaction was evaporated to dryness, affording 1.43 g of a white solid (Theoretical Yield = 733.4 mg). Due to the instability of the compound, it was used

immediately in subsequent reactions without isolation assuming 100% conversion (i.e. 1.95 mg of solid/1 mg NHS Ester).

Mannosamine hydrochloride (25 mg, 116 μmol, 1 eq.) was suspended in DMF (0.5 mL), NEt_3 was added (23.4 mg, 232 μmol, 2 eq.), and the suspension was cooled to 0°C. Fluoroacetic acid NHS ester (49.8 mg crude = 25.4 mg NHS ester, 145 μmol, 1.25 eq.) was added as a solid and the reaction was left to proceed on ice for 1 hr before it was loaded directly onto a P-2 column (0.625 x 42.5 cm) running in 100 mM NH_4CO_3. Fractions containing the desired product, *N*-fluoroacetyl-mannosamine, were pooled and lyophilized to afford ~50 mg of a crude off white solid (~27.7 mg theoretical yield). This material was used in the next enzymatic step without further purification assuming 100% yield of the desired compound (i.e. 1.8 mg crude solid/mg of desired product).

Compound **A** (15 mg, 28.9 μmol, 1 eq.), CTP (31.5 mg, 57.8 μmol, 2 eq.), sodium pyruvate (31.2 mg, 202.3 μmol, 7 eq.), and the crude *N*-flouroacetyl-mannosamine (25 mg crude material = 13.8 mg compound, 57.8 μmol, 2 eq.) were dissolved in Tris buffer (1.5 mL of 100 mM Tris, 20 mM $MgCl_2$ pH 9.0 solution). *C. Perfringens* NeuAc Aldolase (4 U), *N. Meningitidis* CMP-Synthetase (4 U) and *P. Damsella* α2-6 Sialyltransferase (0.4 U) were added and the reaction was left to proceed with end over end rotation at 37°C for 3 hrs. The reaction was then centrifuged, and the supernatant was purified on a C18 column (2g, Waters) washing with H_2O (20 mL) and eluting the product with 15% MeOH (20 mL). After evaporation of MeOH and lyophilization of the residual water, 23.9 mg of a white solid (28.1 μmol, 97% yield) was obtained. To this solid (23.9 mg, 28.1 μmol), Pd/C was added (6.5 mgs) and the mixture was dissolved in H_2O (2 mLs), the flask was purged with H_2, and the reaction was left to proceed under a H_2 atmosphere for 3 hrs at which time TLC indicated complete the reaction was complete. After this time the reaction was filtered through a 0.22 μm syringe filter and lyophilized to afford 18.0 mg (25.1 μmol, 89% yield) of Compound **24** a white solid.

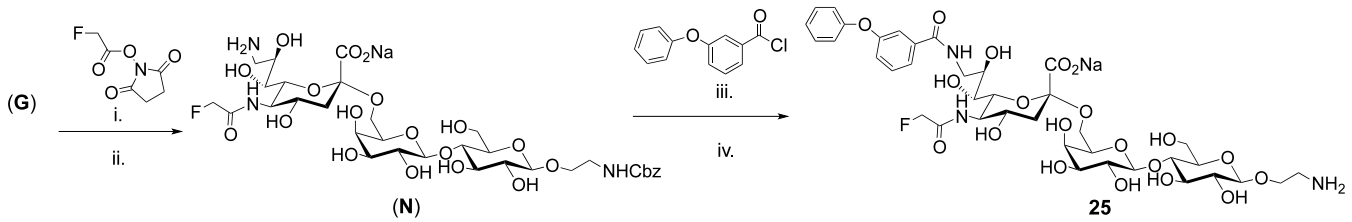
Compound **24**:

HRMS: $C_{25}H_{43}FN_2O_{19}$ $[M+H]^+$: Expected: 695.2517, Found: 695.2530.

^{1}H NMR (600 MHz, D_2O) δ 4.78 (d, J = 46.2 Hz, 2H), 4.41 (d, J = 8.0 Hz, 1H), 4.29 (d, J = 8.0 Hz, 1H), 4.01 - 3.93 (m, 1H), 3.88 - 3.82 (m, 3H), 3.80 - 3.66 (m, 7H), 3.60 (ddd, J = 12.0, 10.1, 4.7 Hz, 1H), 3.57 - 3.43 (m, 7H), 3.39 (dd, J = 9.8, 8.0 Hz, 1H), 3.26 (dd, J = 9.3, 8.0 Hz, 1H), 3.08 (t, J = 5.1 Hz, 2H), 2.59 (dd, J = 12.4, 4.7 Hz, 1H), 1.62 (t, J = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 174.24, 172.17 (d, *J* = 18.4 Hz), 103.94, 102.62 , 101.06, 80.59 (d, *J* = 181.2 Hz), 80.24, 79.99, 75.42, 75.24, 74.53, 73.44, 73.12, 72.88, 72.66, 71.53, 69.29, 69.07, 68.96, 67.33, 64.48, 63.39, 60.89, 52.17, 40.88, 40.27.

^{19}F NMR (375 MHz, D_2O) δ -226.45



Supplementary Scheme 7 - Conditions and Reagents: i) DMF, NEt_3. Yield: 74%. ii) PMe_3, THF:H_2O. Yield: 71%. iii) MeOH, CH_2Cl_2, NEt_3. Yield: 87%. iv) H_2, Pd/C, H_2O. Yield: 88%.

Synthesis of Compound 25

Compound **G** (66 mg, 83.2 μmol, 1.0 eq.) was dissolved in DMF (2.2 mL) and NEt_3 was added (4 drops). The solution was cooled to 0 °C and the above crude, fluoroacetic acid NHS-ester (35.5 mg crude = 18.2 mg NHS ester, 104 μmol, 1.25 eq.) was added in portions. The reaction was left to proceed on ice for 1.5 hrs, MeOH was added (1 mL), and the solution was brought to room temperature. After evaporation to dryness, the residue was taken up in H_2O (2-3 mL) and purified on a C18 column (2g, Waters) washing with H_2O (20 mL) and eluting with 30% MeOH. Upon concentration and lyophilization, 52.6 mg (61.6 μmol, 74% yield) of a white solid was obtained.

The 9-azido-Neu5FAc-α2-6-Lac-Et-NHCbz intermediate (26.1 mg, 30.6 μmol, 1.0 eq.) was dissolved in THF:H_2O (1.5 mL, 1:1 v/v) and PMe_3 (76.5 μL of a 1M solution in THF, 76.5 μmol, 2.5 eq.) was added. The reaction was left to proceed at room temperature for 3 hrs before the THF was removed by rotary evaporation. The reaction was then loaded onto a P-2 column (0.625 x 42.5 cm) running in 100 mM NH_4CO_3 and, after lyophilization, 17.9 mg of a white solid, Compound **N**, was obtained (21.6 μmol, 71% yield).

Compound **N:**

HRMS: $C_{33}H_{50}FN_3O_{20}$, $[M+H]^+$: Expected: 828.3044, Found: 828.3055.

^{1}H NMR (600 MHz, D_2O) δ 7.38-7.21 (m, 5H), 4.99 (s, 2H), 4.78 (d, *J* = 46.2 Hz, 2H), 4.33 (d, *J* = 7.8 Hz, 1H), 4.29 (d, *J* = 7.9 Hz, 1H), 3.94 (ddd, *J* = 9.1, 9.1, 2.9 Hz, 1H), 3.89 – 3.75 (m, 6H), 3.69 (dd, *J* = 8.4, 3.4 Hz, 1H), 3.66 – 3.59 (m, 3H), 3.55 – 3.37 (m, 7H), 3.29 – 3.19 (m, 4H), 2.86 (dd, *J* = 13.1, 9.6 Hz, 1H), 2.59 (dd, *J* = 12.4, 4.6 Hz, 1H), 1.63 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 174.16, 172.21 (d, *J* = 18.4 Hz), 159.25, 137.28, 129.57 (3C's), 129.16, 128.50, 104.00, 102.96, 101.16, 80.61 (d, *J* = 181.5 Hz), 80.35, 75.35, 75.31, 74.48, 73.50, 73.09, 72.65, 71.54, 70.99, 69.77, 69.33, 69.11, 68.79, 67.72, 64.54, 60.94, 52.09, 43.06, 41.26, 40.89.

^{19}F NMR (375 MHz, D_2O) δ -226.45 (s).

Compound **N** (27.0 mg, 31.8 μmol, 1 eq.) was dissolved in MeOH (1.2 mL), NEt_3 was added (2 drops), and the solution was cooled to 0 °C. 3-Phenoxylbenzoyl chloride (14.8 mg, 63.6 μmol, 2 eq.) dissolved in CH_2Cl_2 (0.8 mL) was then added dropwise over 2-3 minutes. After stirring for 30 mins at 0°C, the solution was warmed to room temperature and evaporated to dryness. The solid was resuspended in H_2O (0.6 mL) and loaded onto a P-2 column (0.625 x 42.5 cm) running in 100 mM NH_4CO_3. After purification, 28.8 mg (27.5 μmol, 87% yield) of a white solid was obtained. The Cbz proected intermediate was then deprotected in a similar way as described for **24** to afford 22.1 mg of **25** (24.2 μmol, 88% yield as a fluffy white solid.

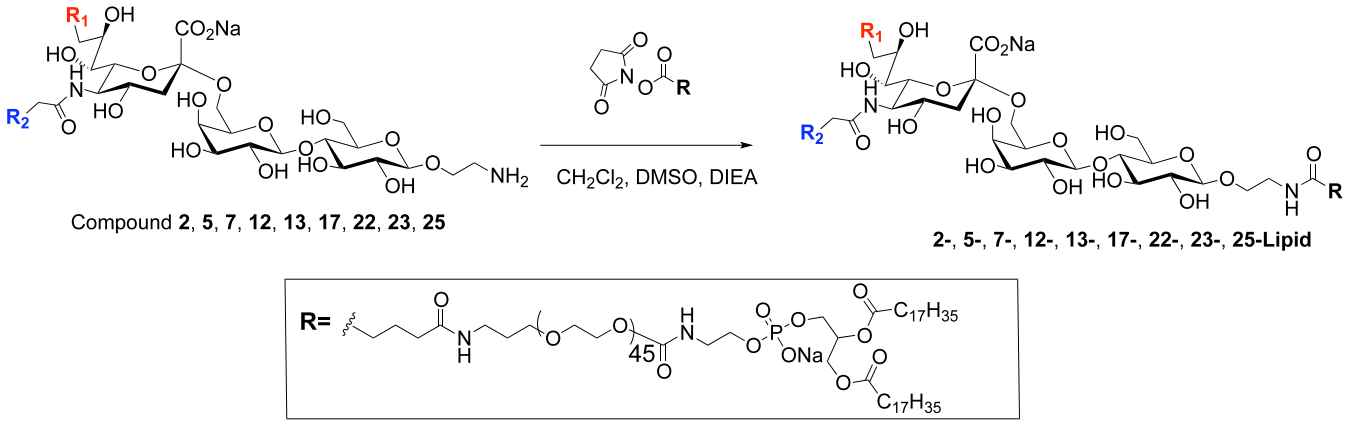
Compound **25**:

HRMS: $C_{38}H_{52}FN_3O_{20,}$ $[M+H]^+$: Expected: 890.3201, Found: 890.3196

^{1}H NMR (600 MHz, D_2O) δ 7.42 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.30 – 7.26 (m, 1H), 7.17 – 7.07 (m, 2H), 6.98 (d, *J* = 7.8 Hz, 2H), 4.74 (dd, *J* = 46.5, 9.9 Hz, 2H), 4.36 (d, *J* = 8.0 Hz, 1H), 4.27 (d, *J* = 7.9 Hz, 1H), 3.96-3.90 (m, 2H), 3.90 – 3.80 (m, 3H), 3.80 – 3.71 (m, 3H), 3.70-3.58 (m, 4H), 3.57 – 3.36 (m, 9H), 3.24 (dd, *J* = 9.0, 8.4 Hz, 1H), 3.04 – 2.99 (m, 2H), 2.59 (dd, *J* = 12.2, 4.4 Hz, 1H), 1.62 (t, *J* = 12.2 Hz, 1H).

^{13}C NMR (151 MHz, D_2O) δ 174.21, 172.13 (d, *J* = 18.5 Hz), 171.15, 157.85, 157.12, 136.42, 131.23, 130.99 (2C's), 125.00, 123.04, 122.93, 119.91 (2C's), 118.14, 103.96, 102.65, 101.13, 80.58 (d, *J* = 180 Hz), 80.35, 75.41, 75.29, 74.58, 73.43, 73.13, 72.77, 71.51, 70.91, 70.85, 69.34, 68.90, 68.10, 64.65, 60.95, 52.18, 43.64, 40.90, 40.38.

^{19}F NMR (375 MHz, D_2O) δ -226.35



Compound **2**, **5**, **7**, **12**, **13**, **17**, **22**, **23**, **25**

2-, **5-**, **7-**, **12-**, **13-**, **17-**, **22-**, **23-**, **25-Lipid**

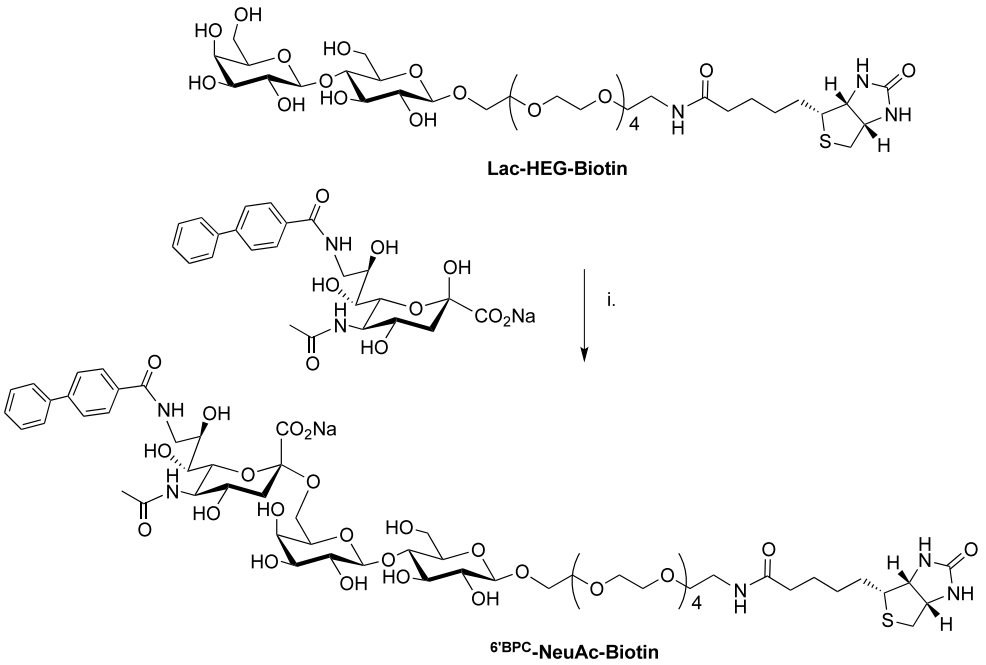
Supplementary Scheme 8 - Conditions and Reagents: CH_2Cl_2, DMSO, DIEA. Yields: 90-99%.

General Procedure for Synthesis of PEGylated Glycolipids for Liposomal Binding Studies.

The amine-terminated sialoside (4.0 μmol, 1.25 eq.) and NHS-PEG-Lipid (9.2 mg, 3.0 μmol, 1.0 eq.) were dissolved in CH_2Cl_2/DMSO (900 μl, 1:1, v/v) to which DIEA was added (50 μl). The reaction was left to proceed overnight at room temperature at which time the CH_2Cl_2 was evaporated under reduced pressure. The reaction mixture was diluted with H_2O (2.5 mL) and dialyzed against water (2 x 2L) using a 10,000 MWCO dialysis cassette (Pierce). After lyophilization, the glycolipid was afforded as a white solid in excellent yield (typically 90-99%). ^{1}H NMR analysis was done as previously described[4,5].

Synthesis of $^{6'BPC}$NeuAc-Biotin - Lac-HEG-Biotin (5 mg, 6 μmol, 1 eq.), BPC-NeuAc (4.4 mg, 9 μmol **,** 1.5 eq), and CTP (5.8 mg, 10.5 μmol, 1.75 eq.) were dissolved in Tris buffer (0.5 mL of 100 mM Tris, 20 mM $MgCl_2$, pH 9.0) to which *N. Meningitidis* CMP-Synthetase (1.5 U) and *P. Damsella* α2-6 Sialyltransferase (0.15 U) were added. After 2.5 hrs at 37° C the reaction was loaded onto a C18 column (2g) and washed with H_2O (20 mL), 25% MeOH (20 mL), 40% MeOH (20 mL), and 50% MeOH (20 mL). The desired product eluted at the end of the 40% MeOH washes and beginning of the 50% MeOH washes. After evaporation of the MeOH, the residual water was removed by lyophiization to afford 6.9 mgs of a white solid (5.2 μmol, 88% yield).

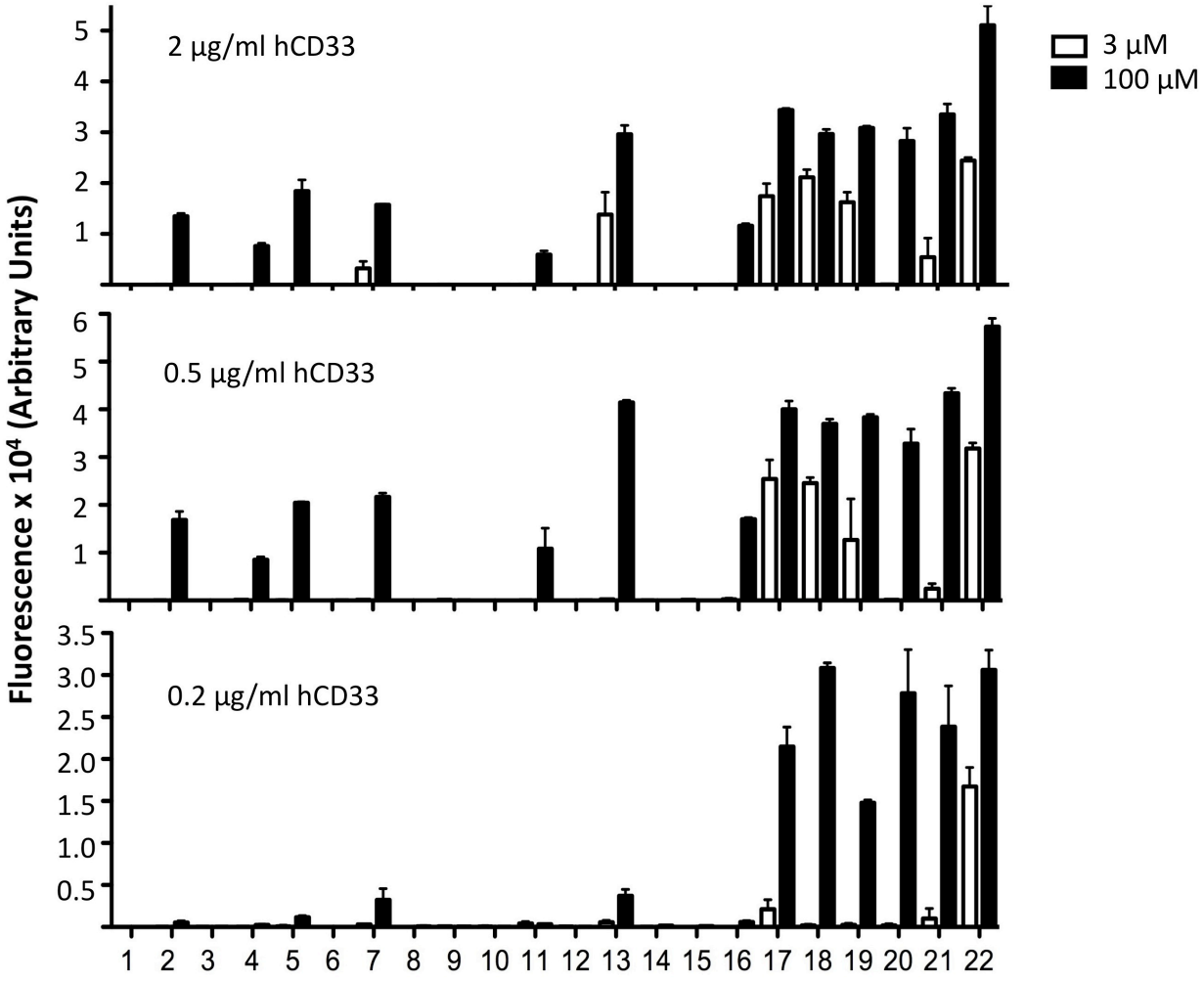
The ^{1}H-NMR Spectra is consistent with the structure and is given in the Appendix



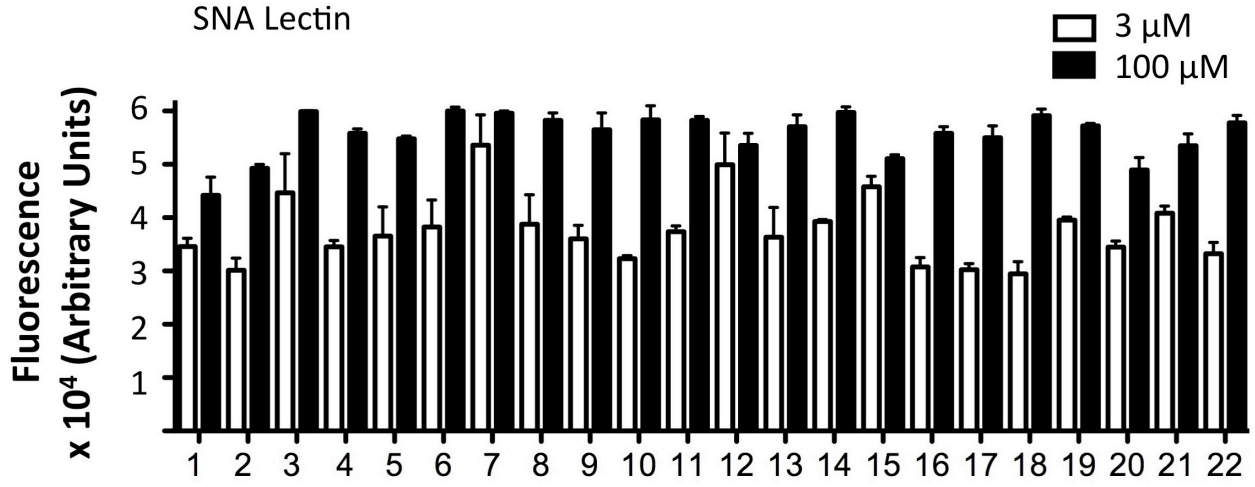
Supplementary Scheme 9 - Conditions and Reagents: *P. Damsella* α2-6 Sialyltransferase, *N. Meningitidis* CMP-NeuAc Synthetase Yields: 88%.

Supplementary Results

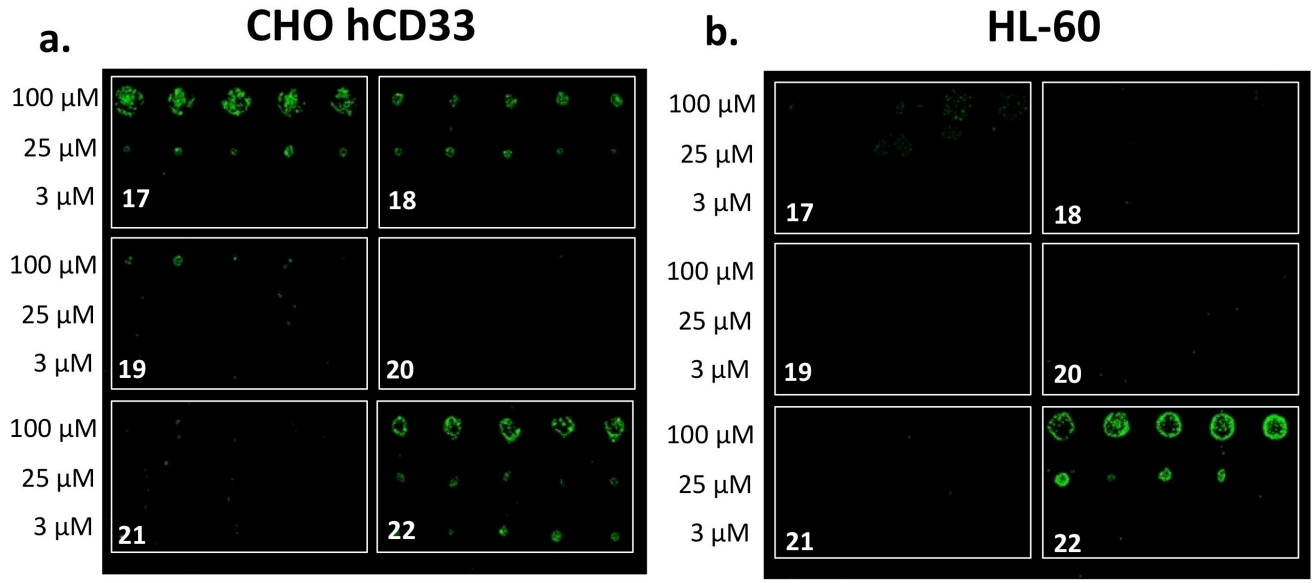
Supplementary Figure 1 – Full Titration Analysis for hCD33 on the Analog Array Described in Figure 1. This figure includes an intermediate concentration of hCD33-Fc applied to the array, 0.5 μg/ml, to further aid the reader in understanding our results shown in **Figure 1**.



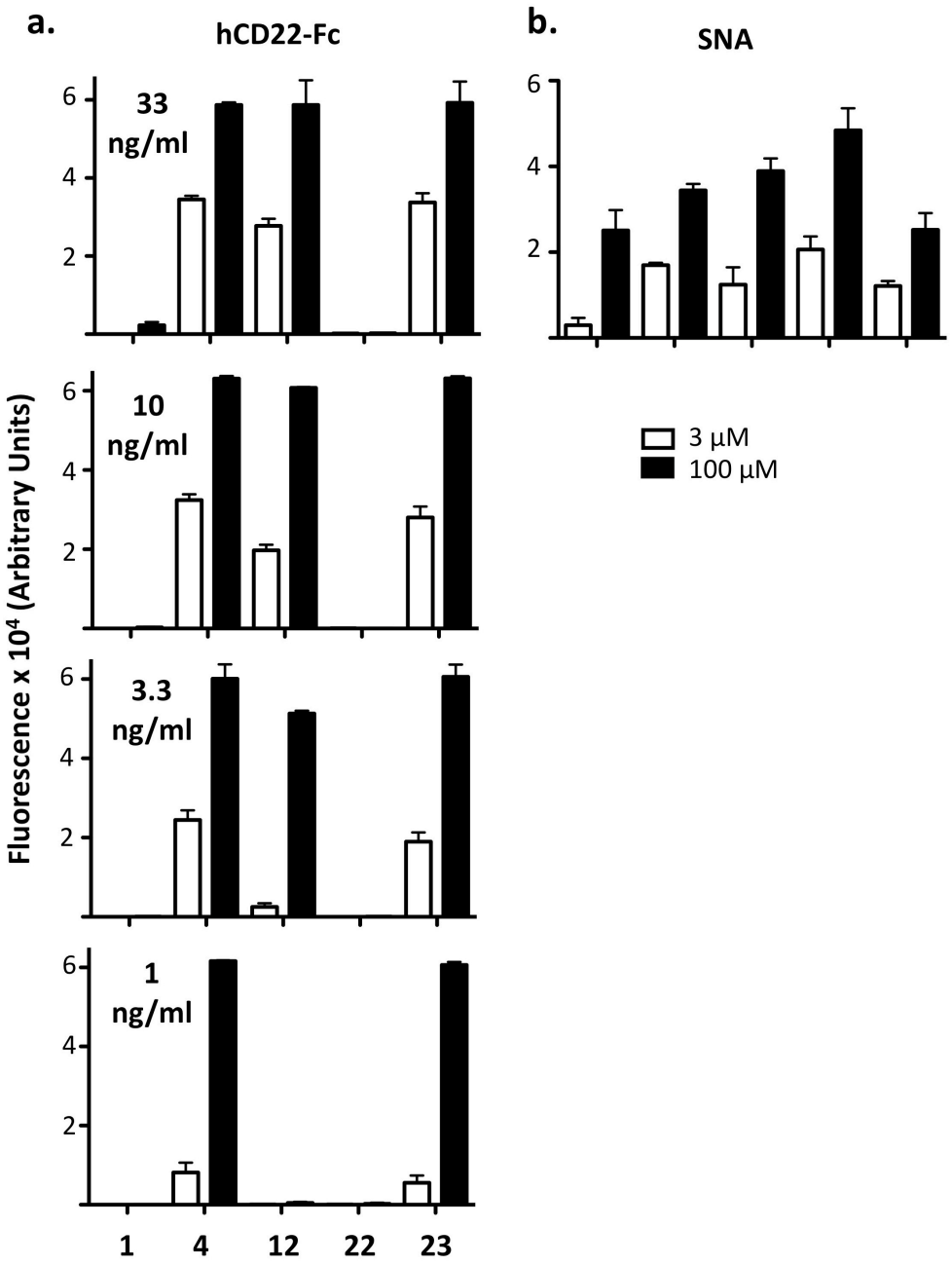
Supplementary Figure 2 – The α2-6 Linkage Specific Lectin SNA was used to show that all compounds were printed efficiently in order to rule out false positives. Biotinylated SNA (10 μg/ml, Vector Labs) was precomplexed with Alexa-Fluor-488 Streptavidin (2 μg/ml, Invitrogen) for 15 minutes and then serially diluted in PBS-Tween and applied to the analog array described in **Figure 1**. After binding for 45 minutes at room temperature in a humidified chamber, the slides were washed by dipping three times into PBS-Tween, PBS, H_2O, and centrifuged to dry. The results shown below are for a representative dilution where the signals are just below scanner saturation.



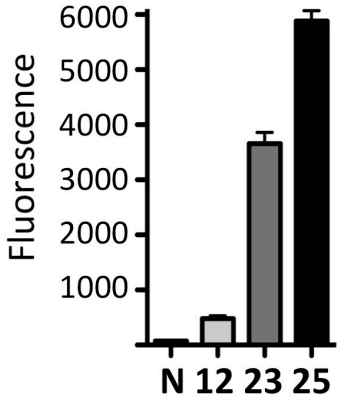
Supplementary Figure 3 – CD33-Expressing Cells Bind to High-Affinity Hits on the Analog Array Described in Figure 1. (**a**) CHO expressing CD33 cells (CHO-hCD33) or the (**b**) CD33-expressing AML cell line, HL-60, were fluorescently labeled with CMRA and applied to the analog array as previously described[5]. Briefly, cells were washed once in Opti-MEM and then resuspended in this media to 1 x 10^7 cells/ml. They were fluorescently labeled using 10 μM Cell-Tracker CMRA (Invitrogen) for 30 min at 37°C, pelleted, and washed 2x with HBSS containing 5 mg/ml BSA (HBSS/BSA), and resuspended to 1 x 10^7 cells/ml in this buffer. Cells were then applied to the glycan array for 1 h at room temperature, after which time the array was placed in a 50 ml conical tube containing HBSS/BSA (placed horizontally on a bench top) with the arrays facing down for 10 minutes. Unbound cells were removed by gravity in this way. The array was then further washed by dipping into a fresh solution of HBSS/BSA approximately 5–10 times, followed by water to remove residual salts. After centrifugation to dry the slide, the slide was scanned for fluorescence.



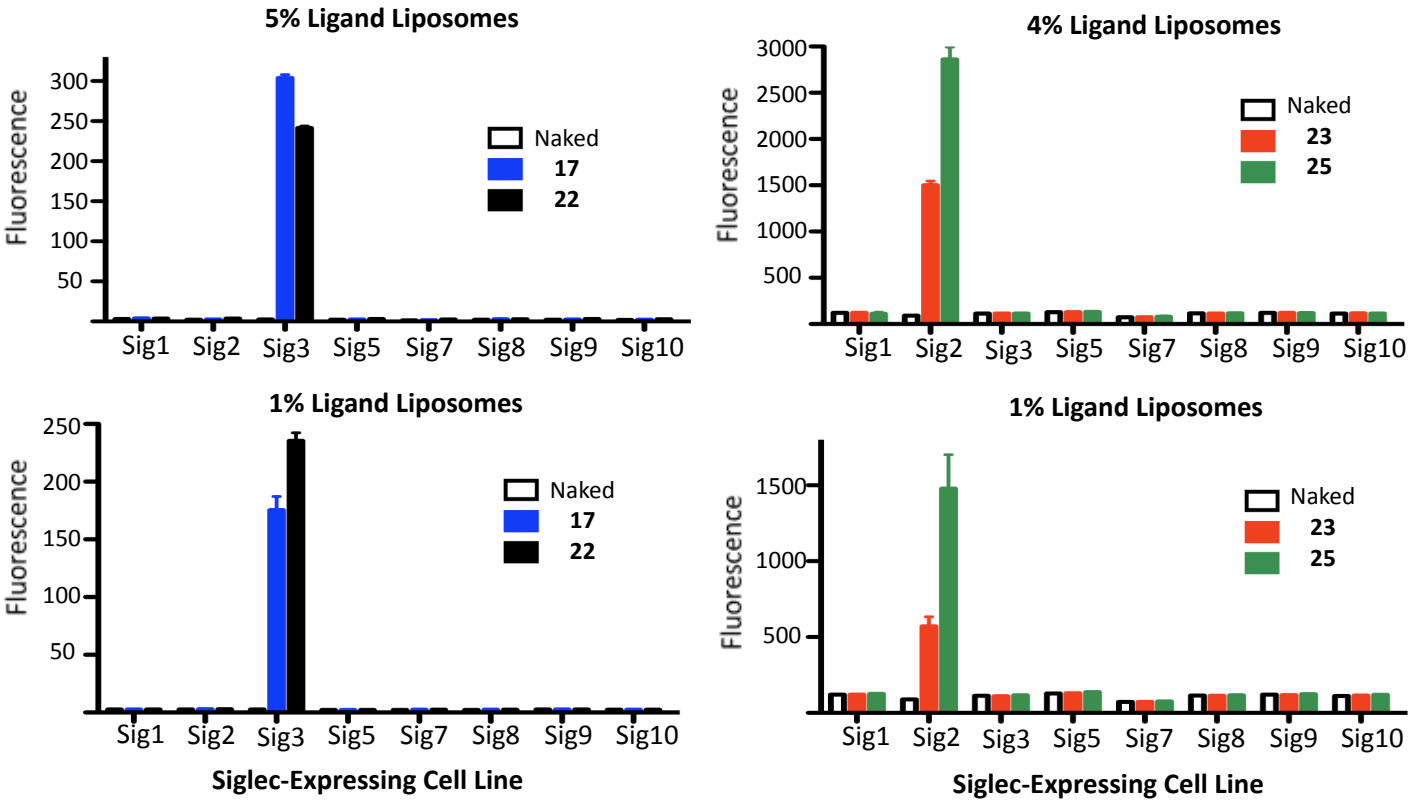
Supplementary Figure 4 – Full Titration Analysis for hCD22 and SNA binding to array in Figure 3. (**a**) This Figure includes higher (33 ng/ml) and intermediate (3.3 ng/ml) concentrations of hCD22-Fc applied to the array shown in **Figure 3** to further aid the reader in understanding our results. (**b**) SNA was applied to the array (as described above for **Supplementary Figure 3**) to show that all compounds were printed with similar efficiency.



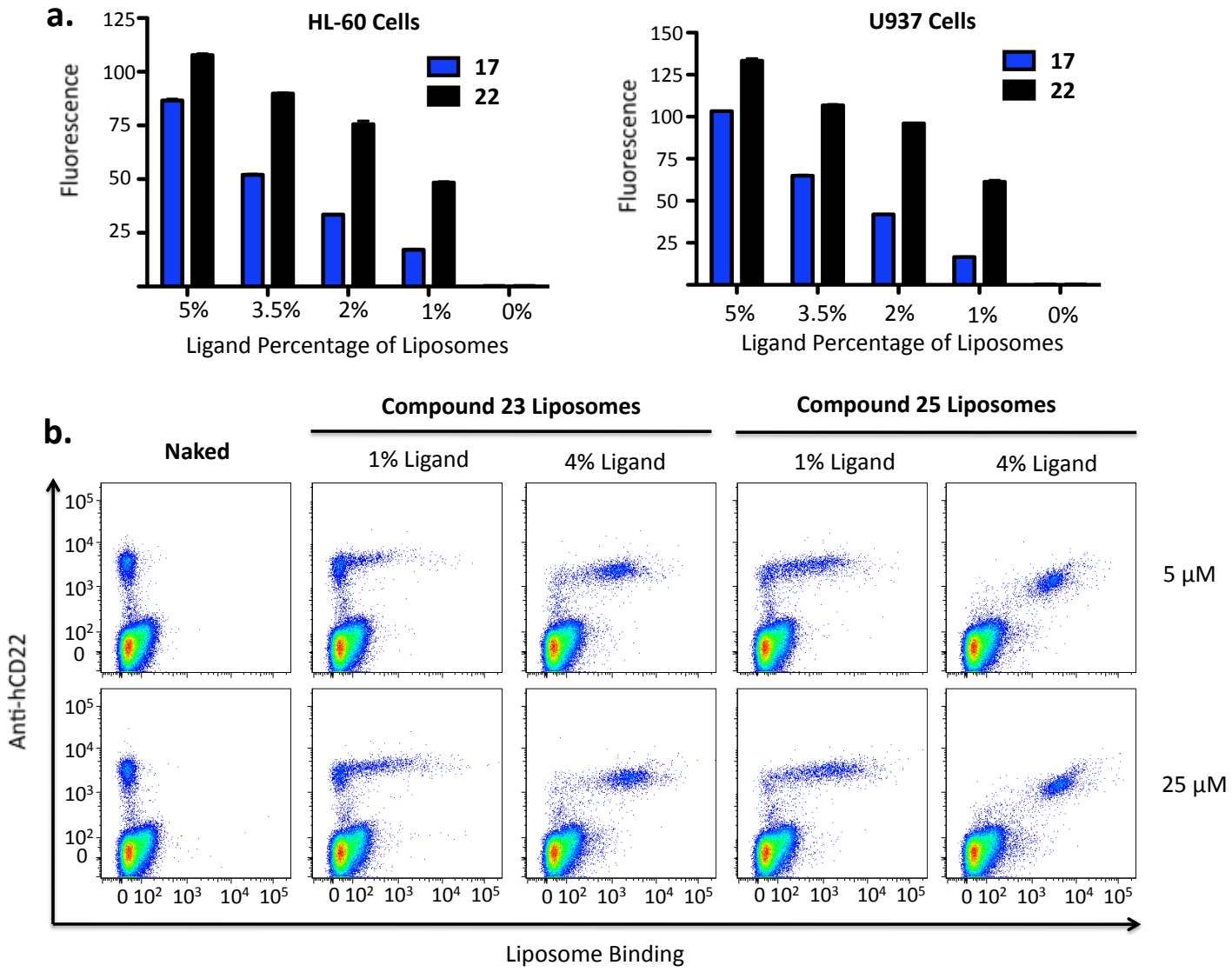
Supplementary Figure 5 – Comparison of Fluorescent, 12-, 23-, and 25-Displaying Liposomes to Ramos B-cells. 25 μM of Fluorescent, 4% Ligand-Displaying Liposomes were incubated with Ramos cells as described in the Experimental Section. The results of one of three independent experiments, carried out in triplicate, are shown below.



Supplementary Figure 6 – Ligand Percentage of Liposomes Does Not Affect Selectivity. 5 μM of fluorescent, hCD33 or hCD22 ligand-displaying liposomes were incubated with a panel of siglec-expressing cell lines and analyzed for binding by flow cytometry as described in the Experimental Section. Importantly, the results demonstrate that increasing the ligand % of CD33 targeted liposomes (**17** and **22**) does not affect the selectivity of these liposomes. Interestingly, compound **17** liposomes show slightly increased binding to CHO-CD33 (which have ~10-fold higher levels of CD33 as compared to AML cell lines or primary cells), than compound compound **22** liposomes at 5% ligand percentage, however, this is reversed at 1% ligand percentage.



Supplementary Figure 7 – Ligand Percentage of Liposomes Affects Binding to AML cells and Peripheral Blood B-cells. (a) 25 μM of fluorescent, hCD33 ligand-displaying liposomes, formulated with varying amounts of the indicated ligand-lipids, were incubated with HL60 or U937 cells for 1 hr at 37 °C and analyzed by flow cytometry. (b) Similarly, fluorescent hCD22 ligand-displaying liposomes, formulated with varying amount of the indicated ligand-lipids, were incubated with human peripheral blood cells as described in the Experimental Section. In both instances, the results clearly indicate that increasing ligand percentage improves binding to the desired cell population.



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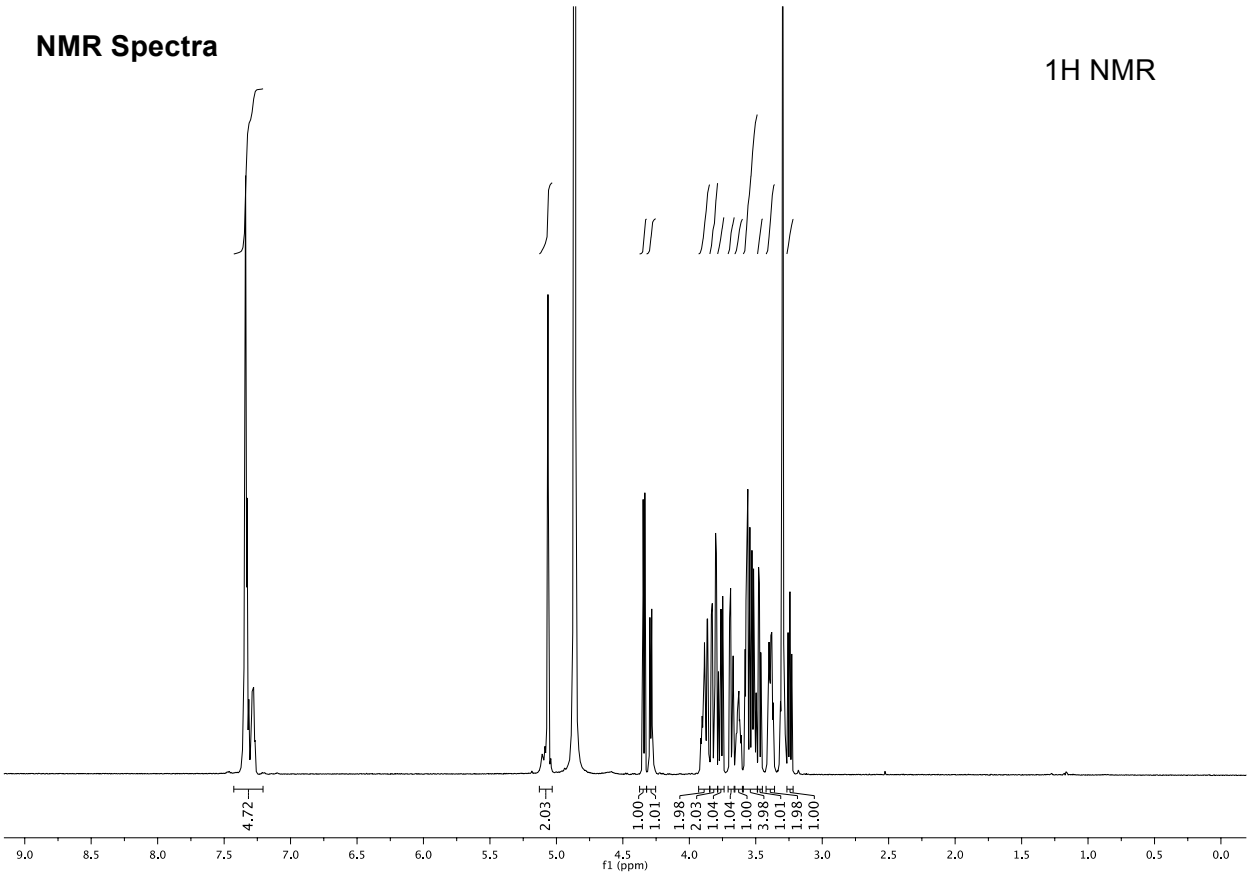
NMR Spectra

1H NMR



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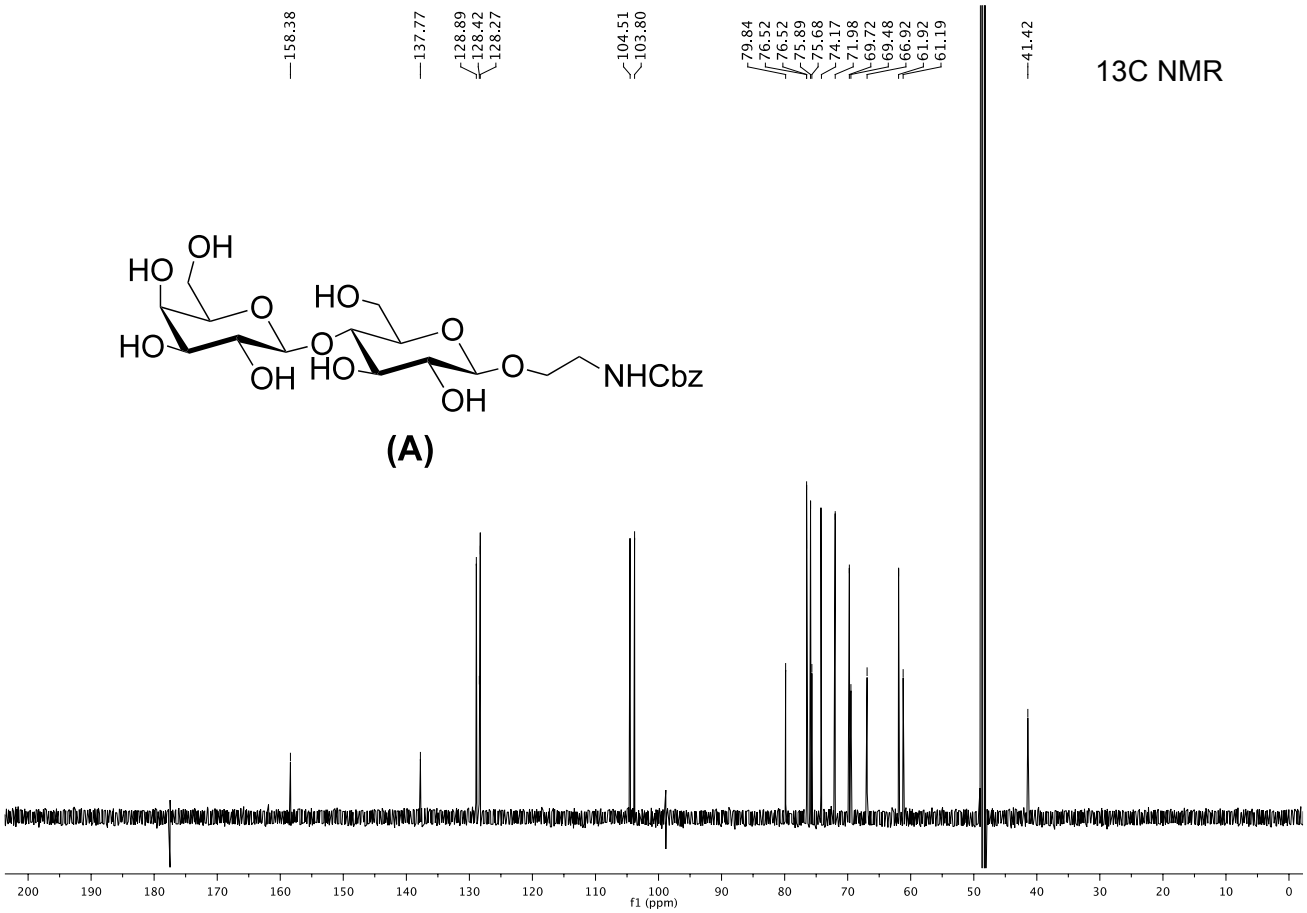

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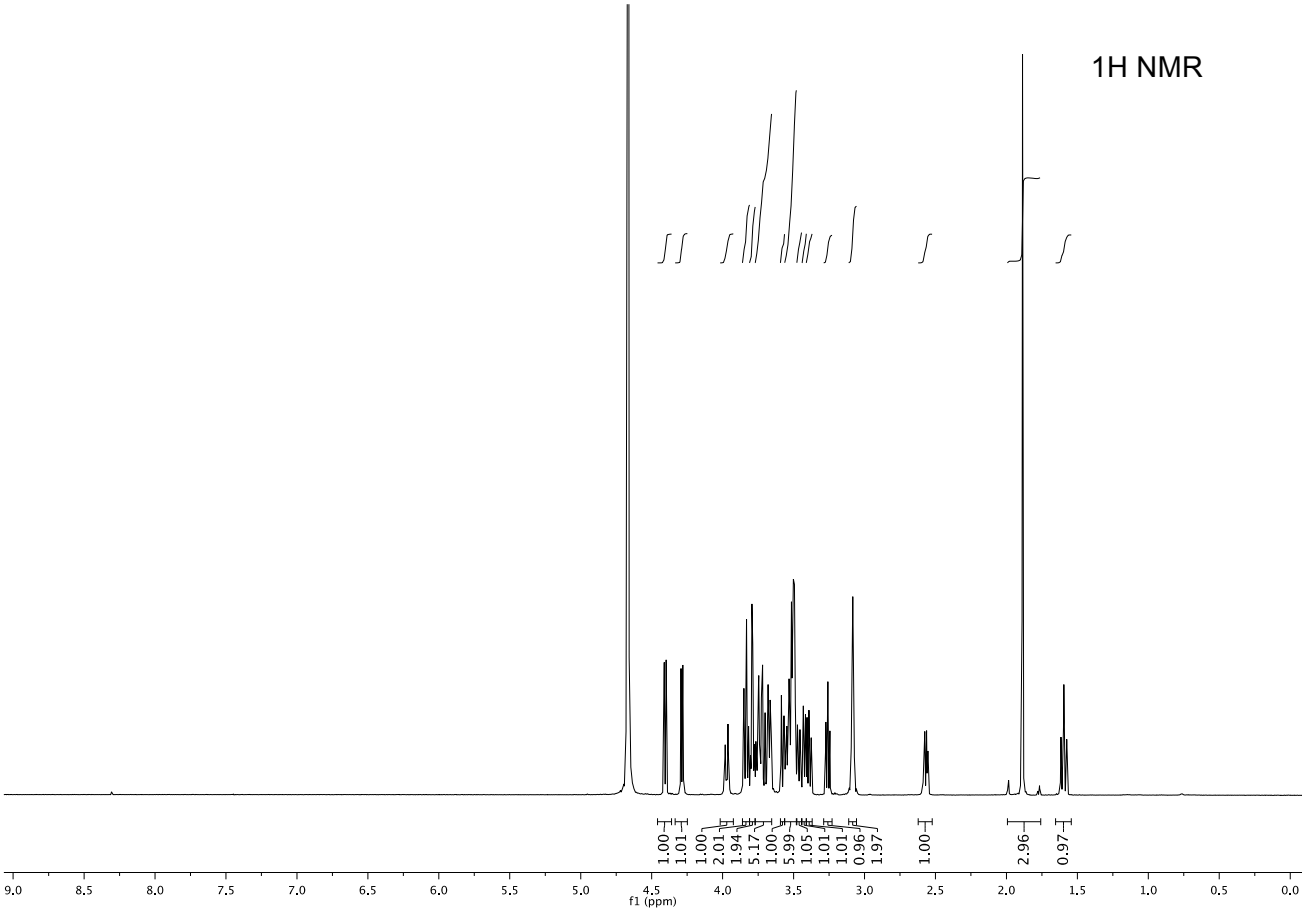
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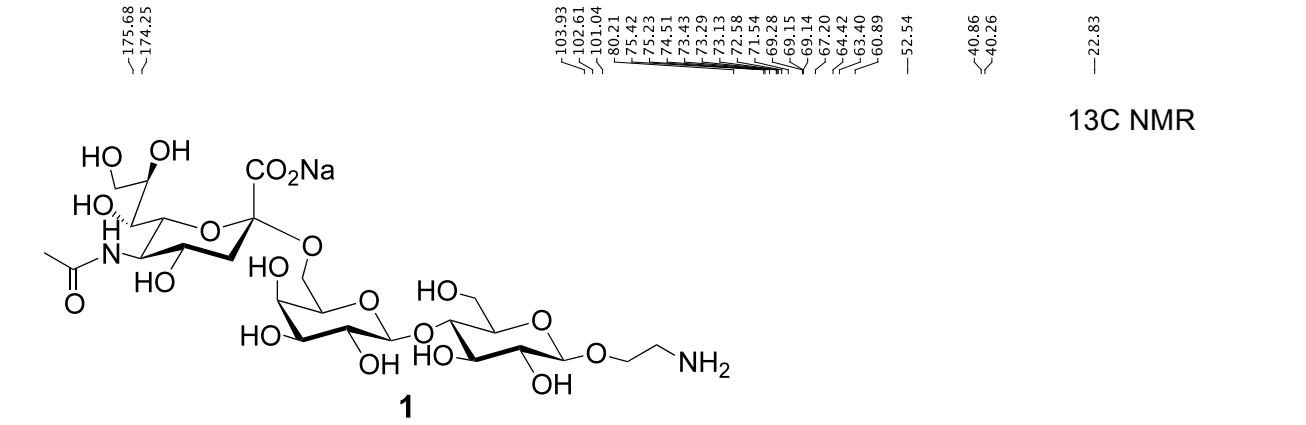
f1 (ppm)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HO
H
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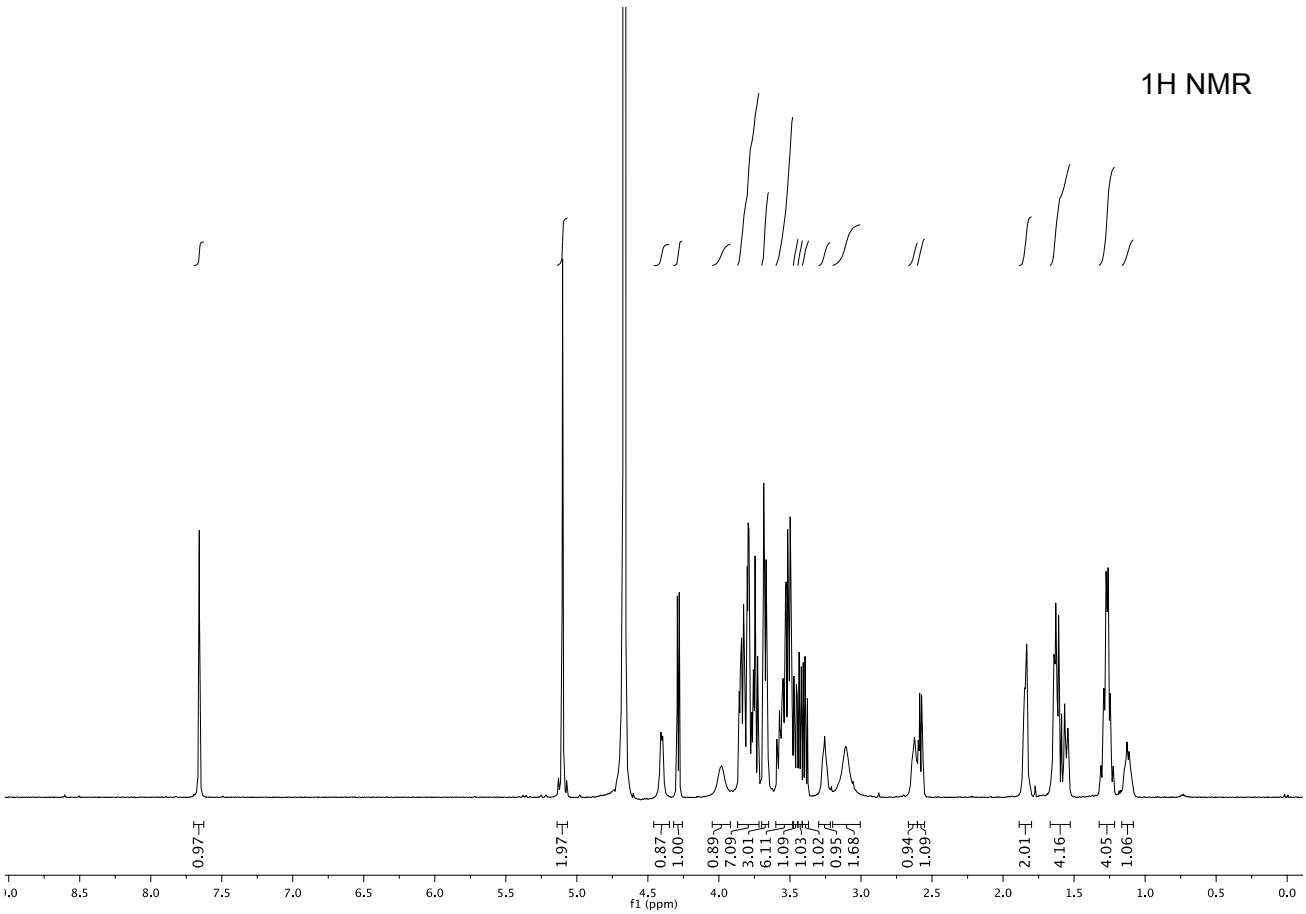
1H NMR



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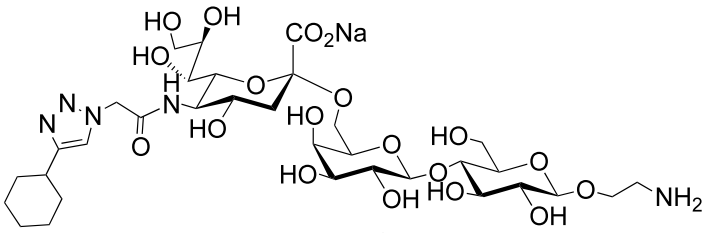
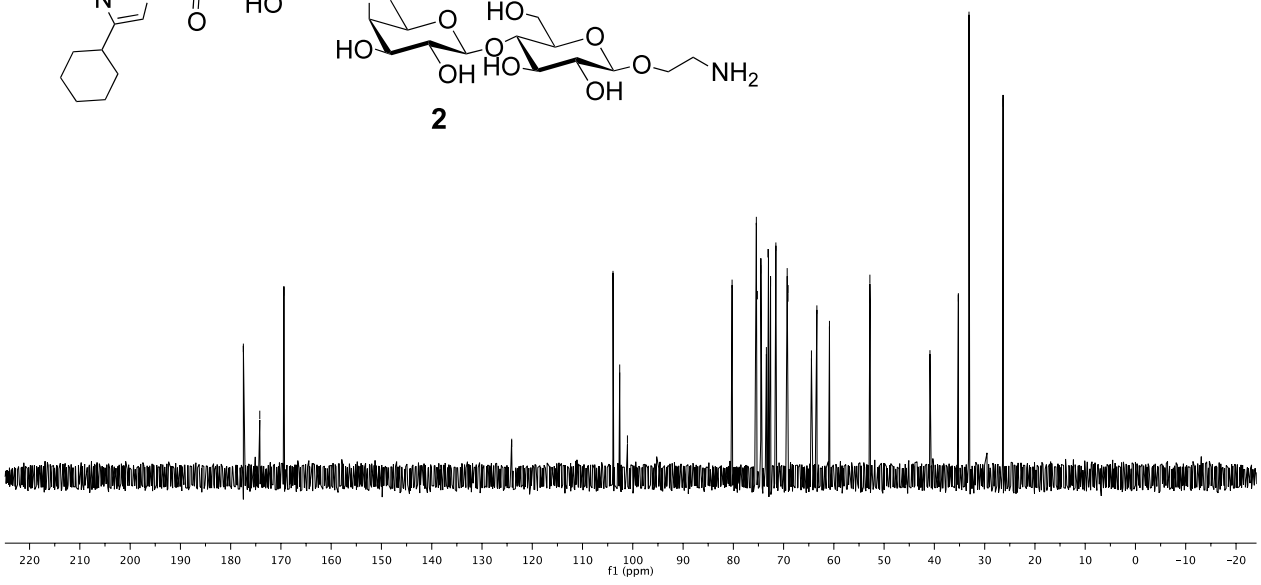
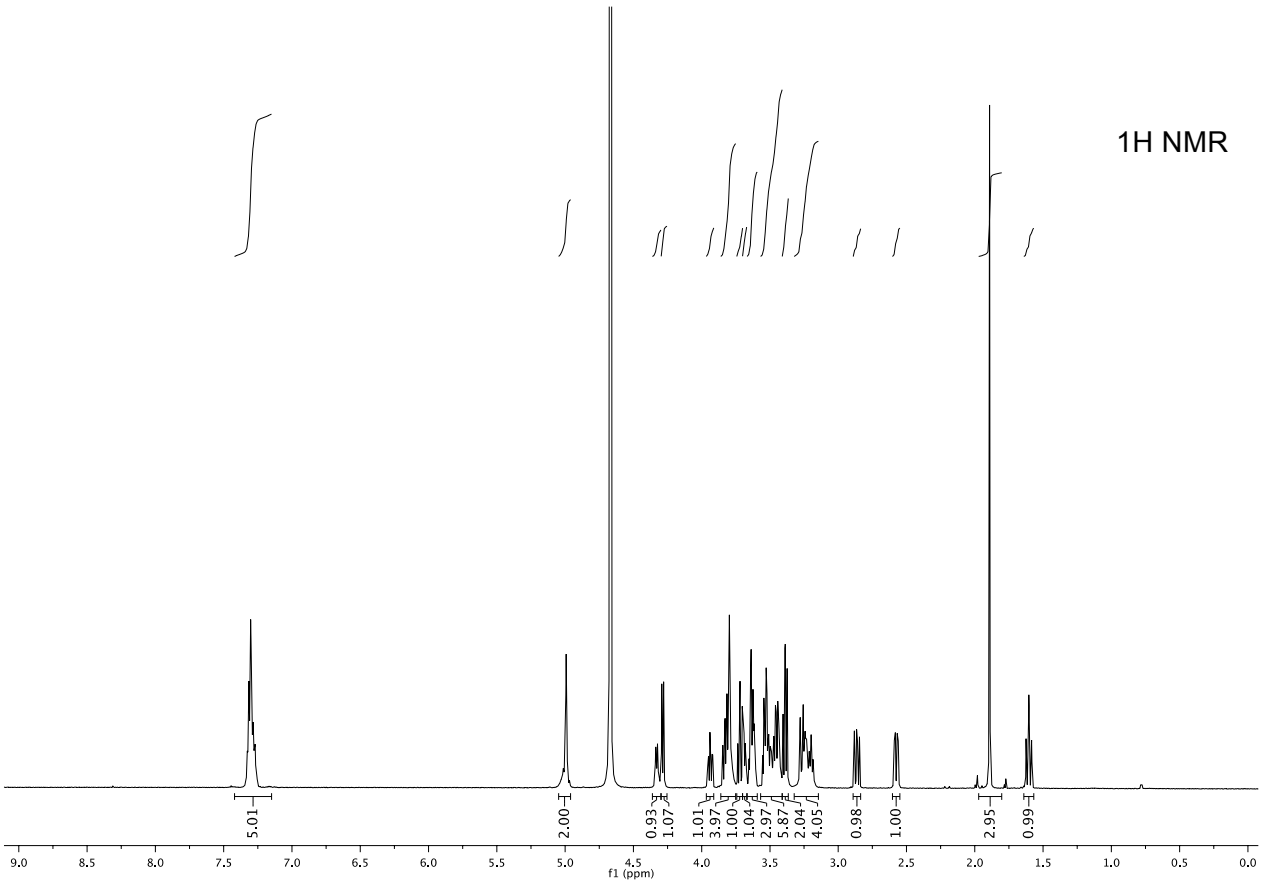
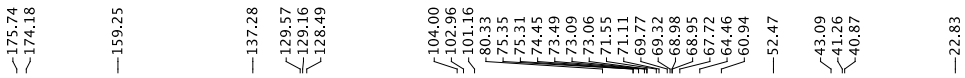


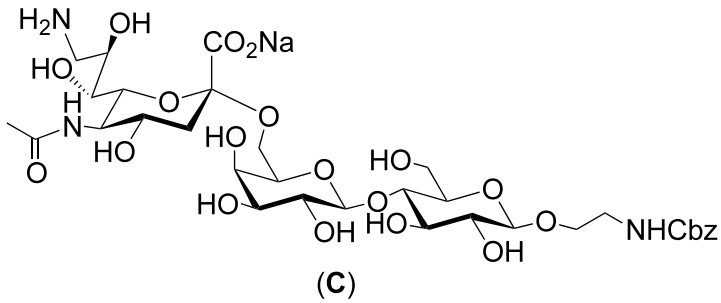
2



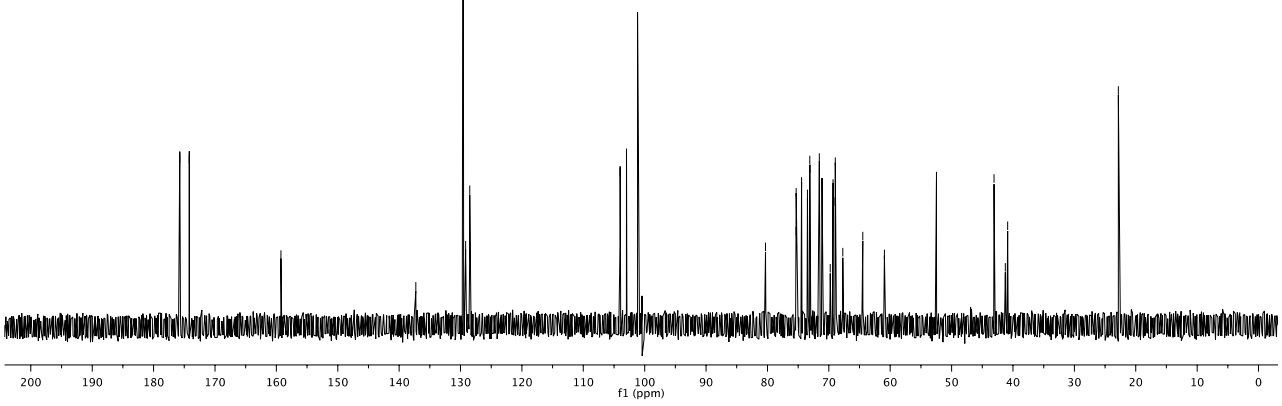

1H NMR
5.01
2.00
0.93
1.07
1.01
3.97
1.00
1.04
2.97
5.87
2.04
4.05
0.98
1.00
2.95
0.99
f1 (ppm)

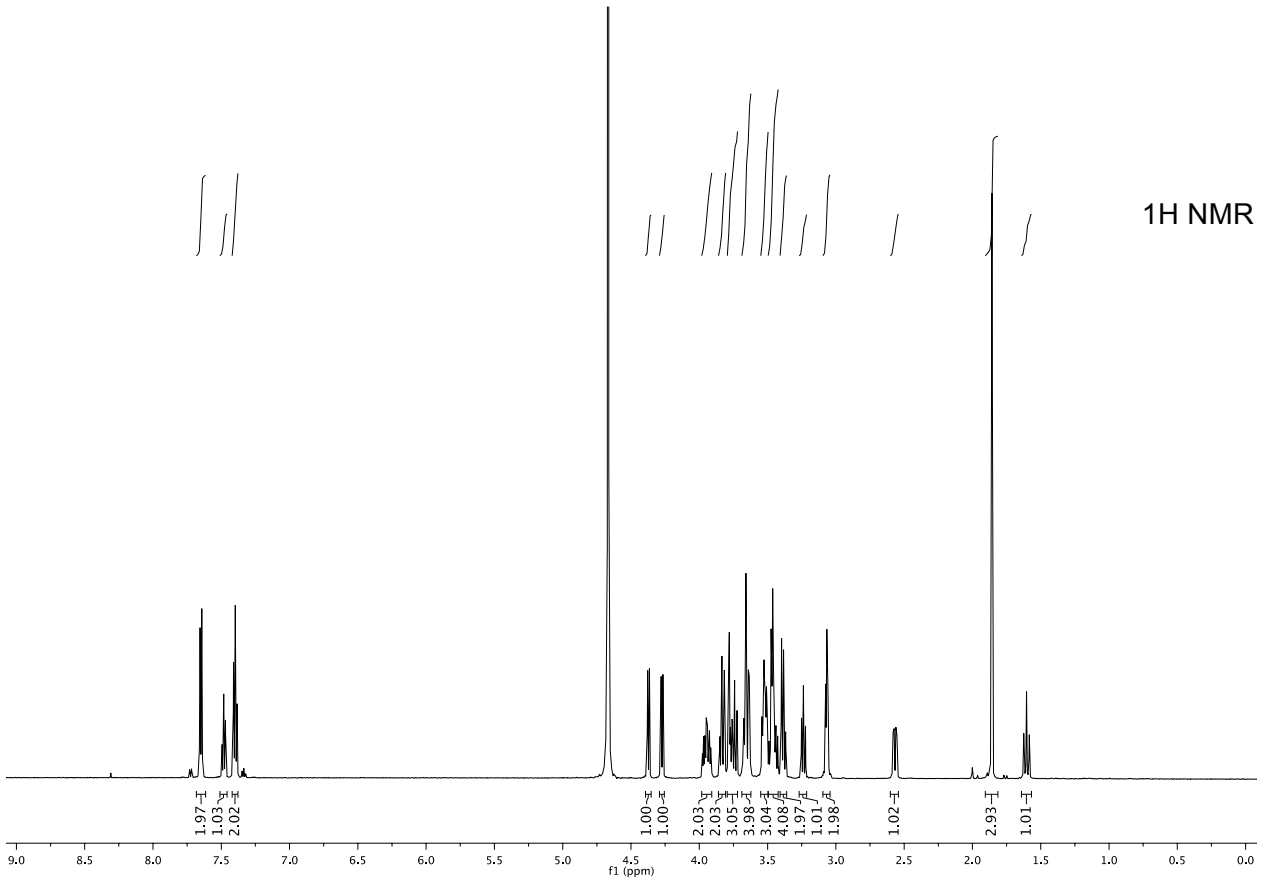

175.74
174.18
159.25
137.28
129.57
129.16
128.49
104.00
102.96
101.16
80.33
75.35
75.31
74.45
73.49
73.09
73.06
71.55
71.11
69.77
69.32
68.98
68.95
67.72
64.46
60.94
52.47
43.09
41.26
40.87
22.83

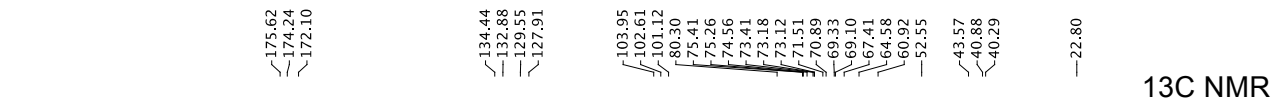
13C NMR


H2N
OH
HO
CO2Na
O
H
N
HO
O
HO
O
HO
HO
O
HO
O
OH
HO
O
NHCbz
OH

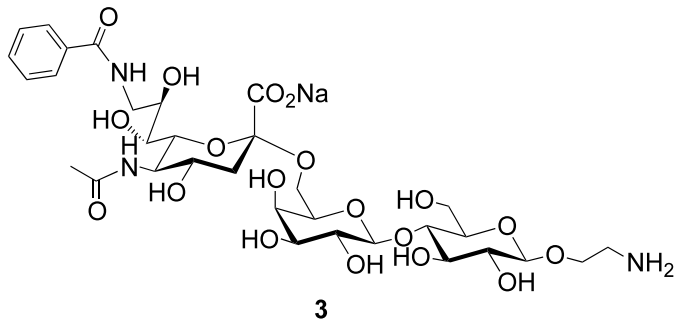
(**C**)


f1 (ppm)

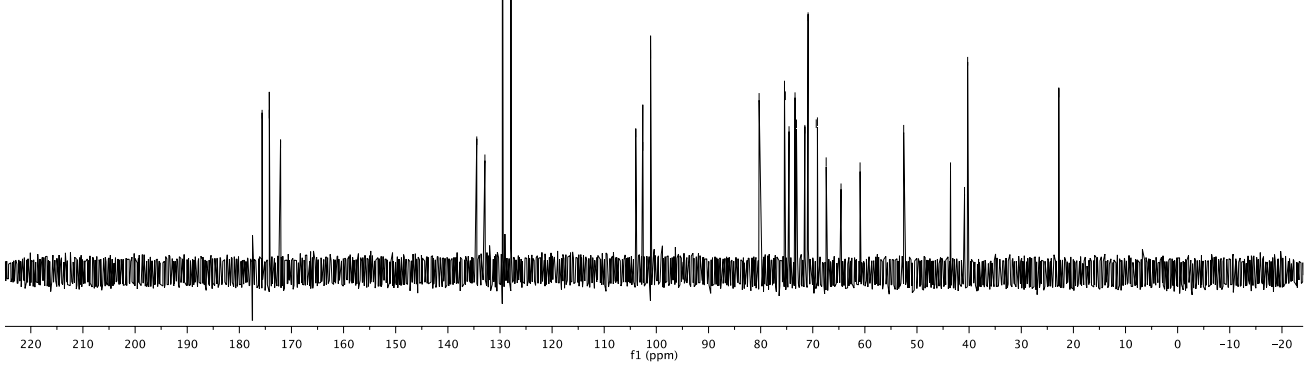

1H NMR
1.97
1.03
2.02
1.00
1.00
2.03
2.03
3.05
3.98
3.04
4.08
1.97
1.01
1.98
1.02
2.93
1.01
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

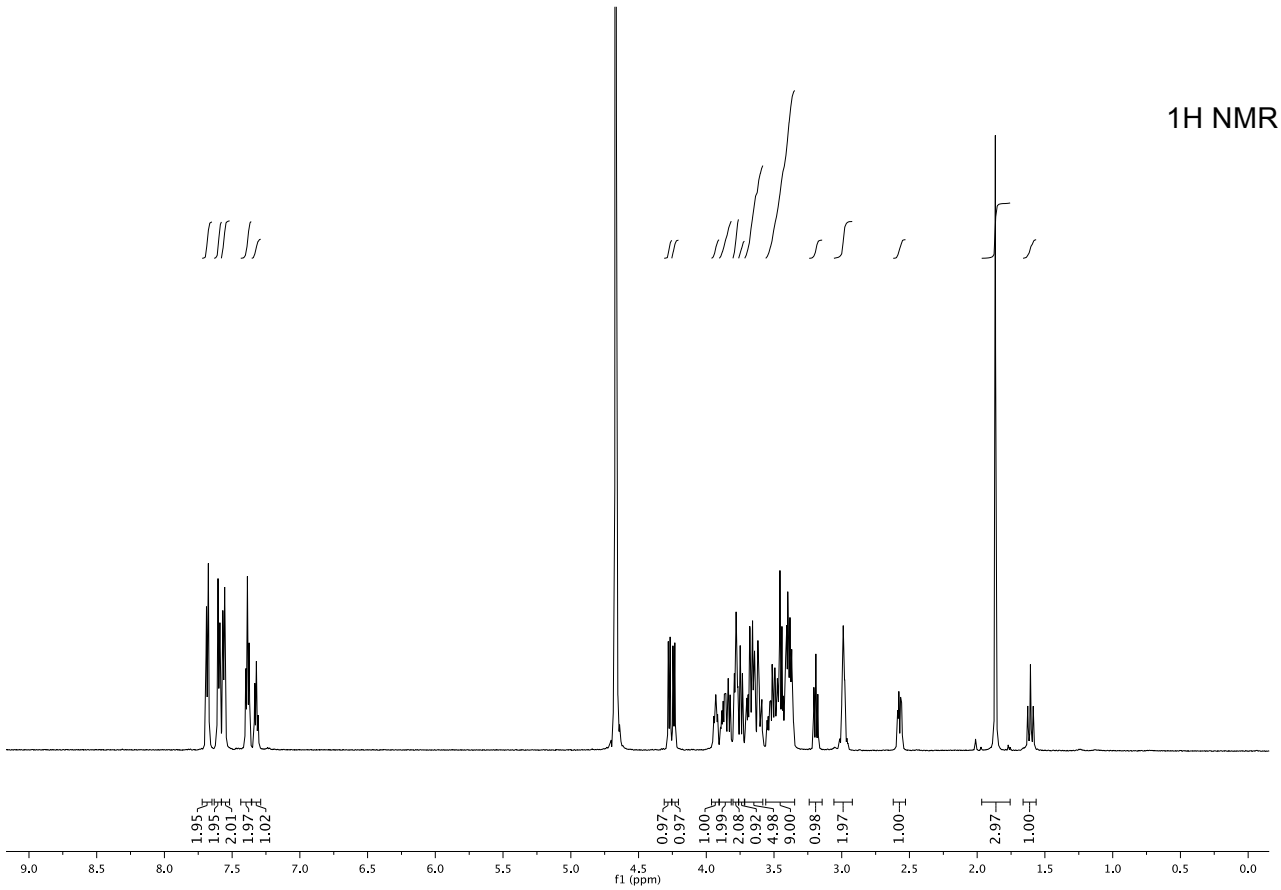

175.62
174.24
172.10
134.44
132.88
129.55
127.91
103.95
102.61
101.12
80.30
75.41
75.26
74.56
73.41
73.18
73.12
71.51
70.89
69.33
69.10
67.41
64.58
60.92
52.55
43.57
40.88
40.29
22.80

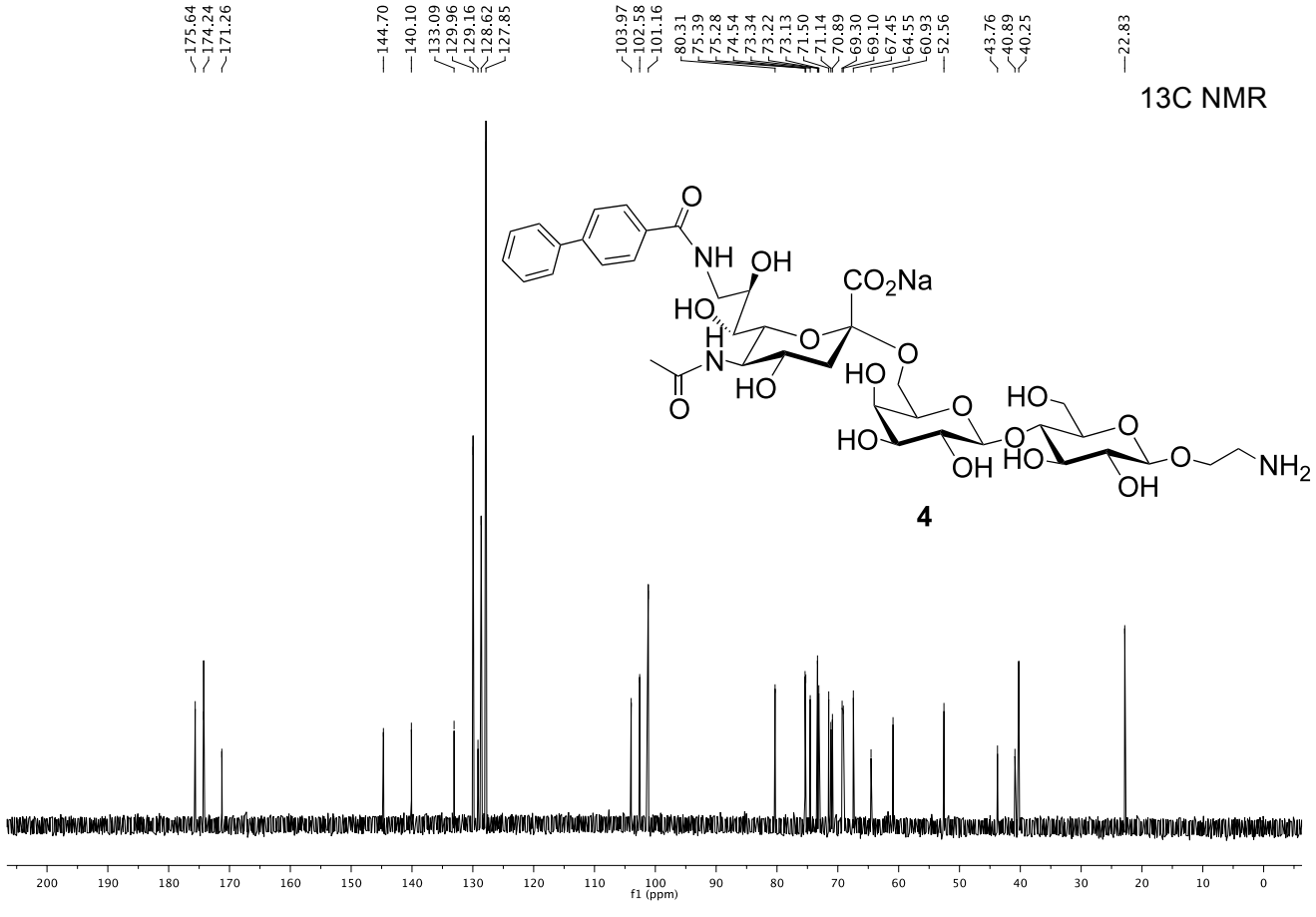
13C NMR


O
NH
OH
CO2Na
HO
H
N
O
HO
HO
O
O
HO
O
HO
HO
O
O
HO
OH
O
NH2
OH

3


220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


1H NMR
1.95
1.95
2.01
1.97
1.02
0.97
0.97
1.00
1.99
2.08
0.92
4.98
9.00
0.98
1.97
1.00
2.97
1.00
f1 (ppm)


13C NMR
175.64
174.24
171.26
144.70
140.10
133.09
129.96
129.16
128.62
127.85
103.97
102.58
101.16
80.31
75.39
75.28
74.54
73.34
73.22
73.13
71.50
71.14
70.89
69.30
69.10
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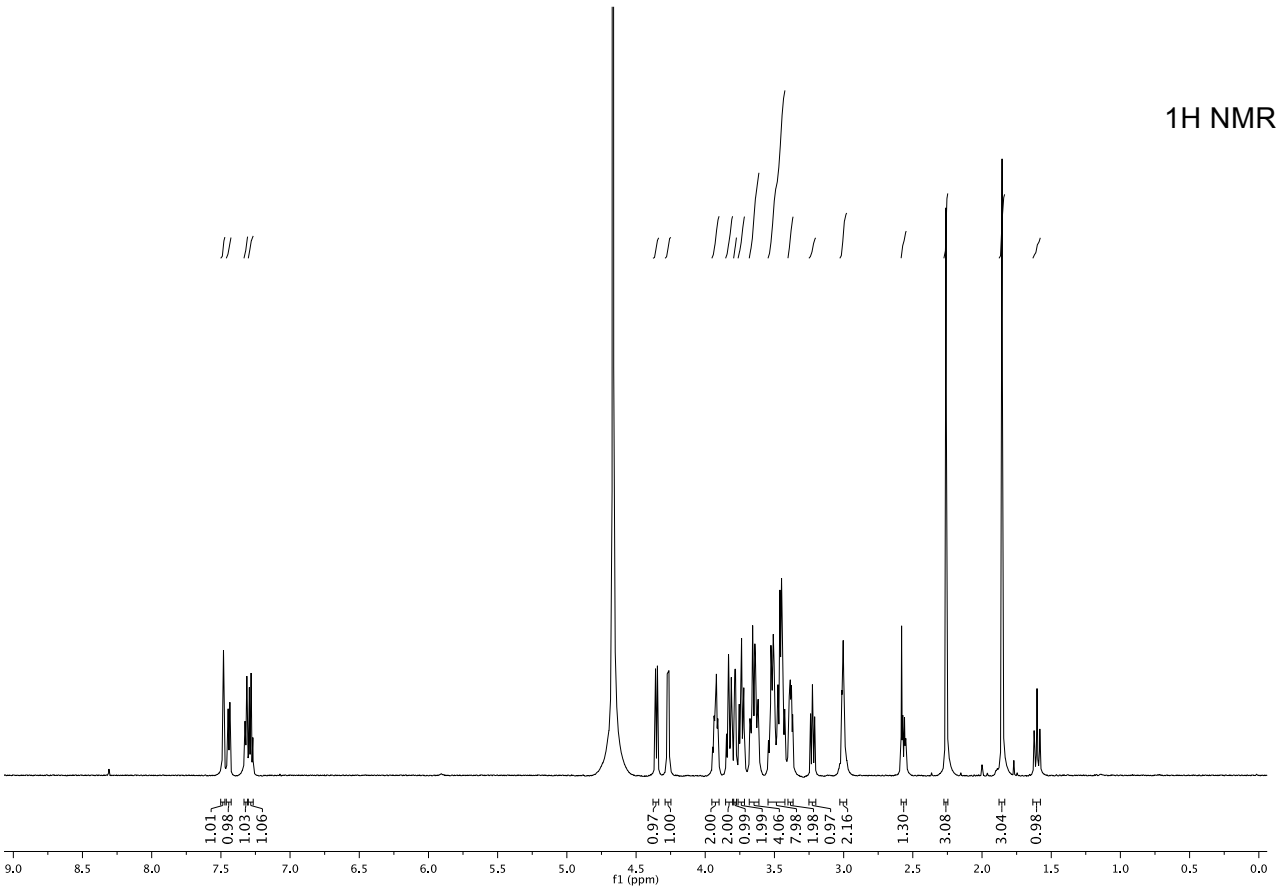
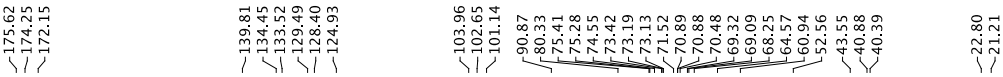
60.93
52.56
43.76
40.89
40.25
22.83
f1 (ppm)

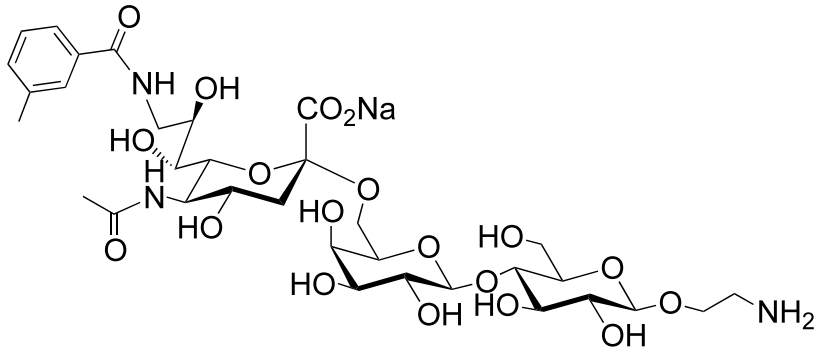
4

1H NMR

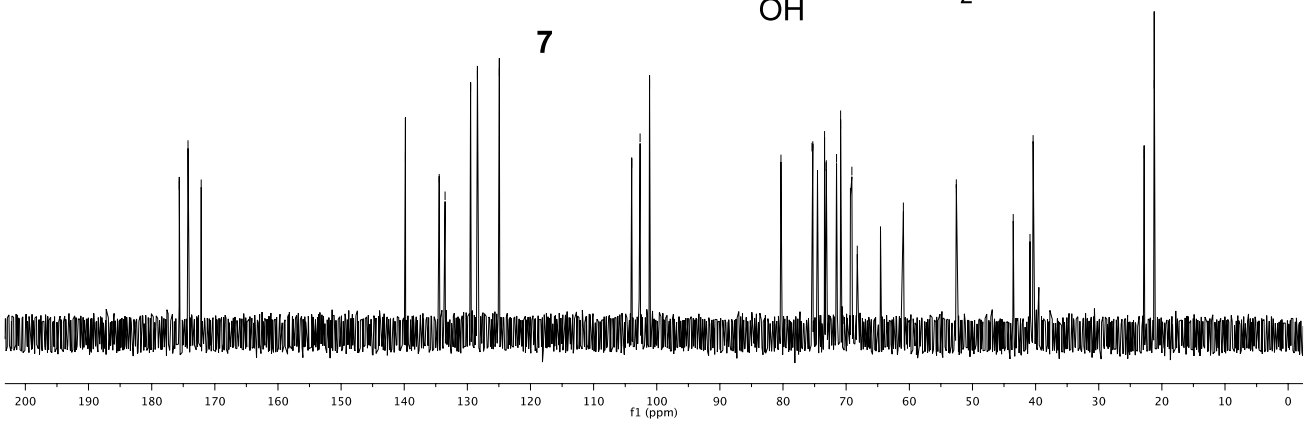


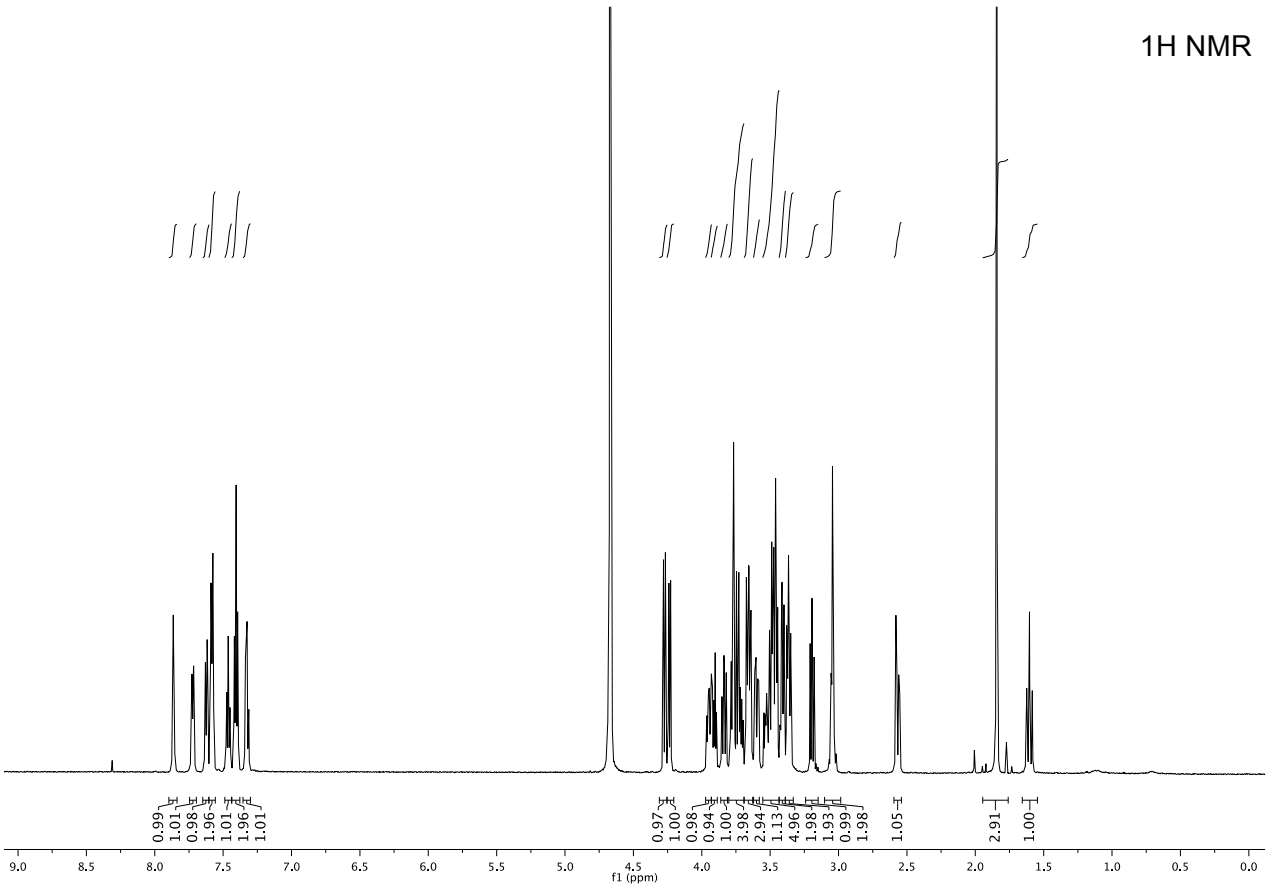


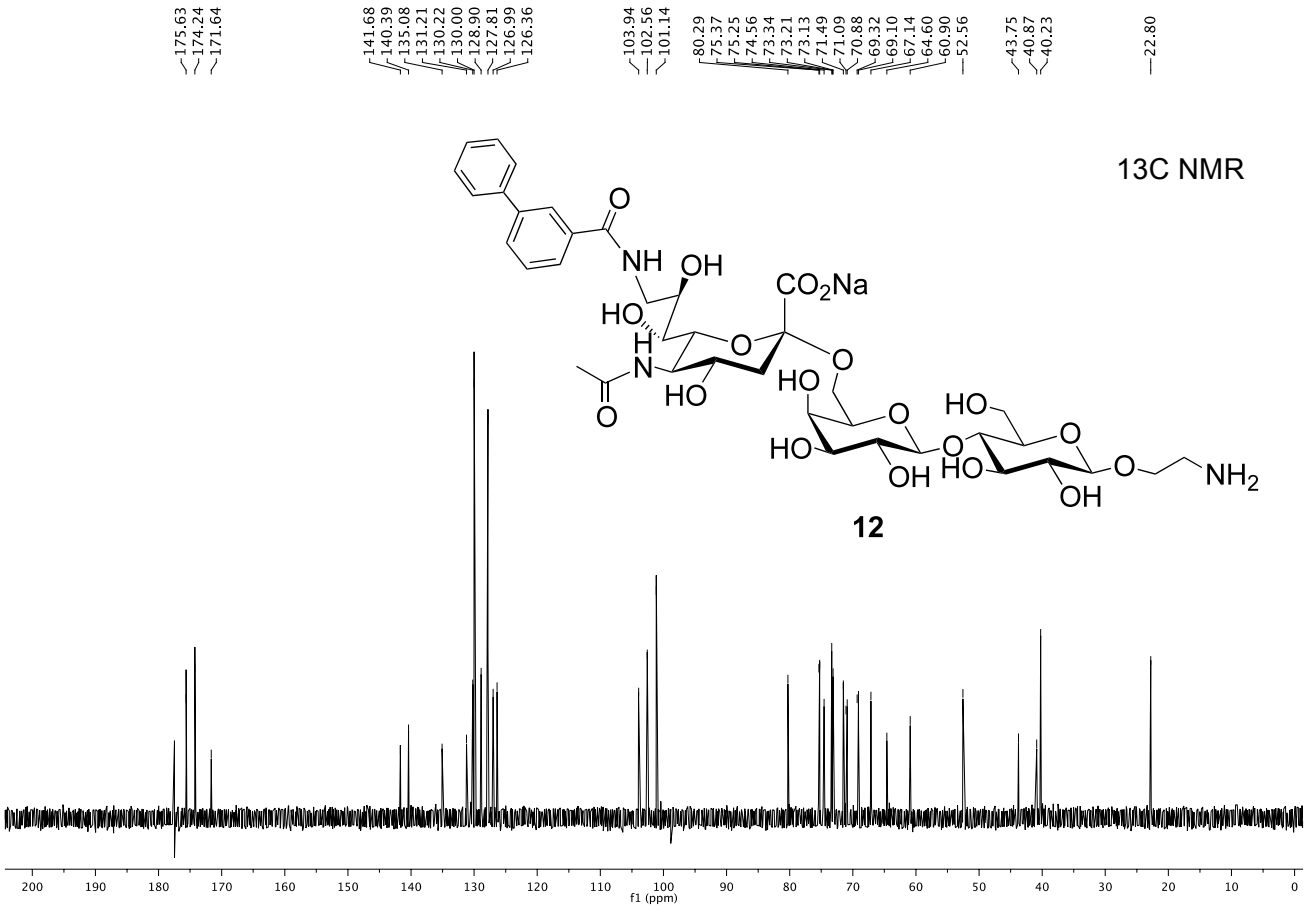
13C NMR



7



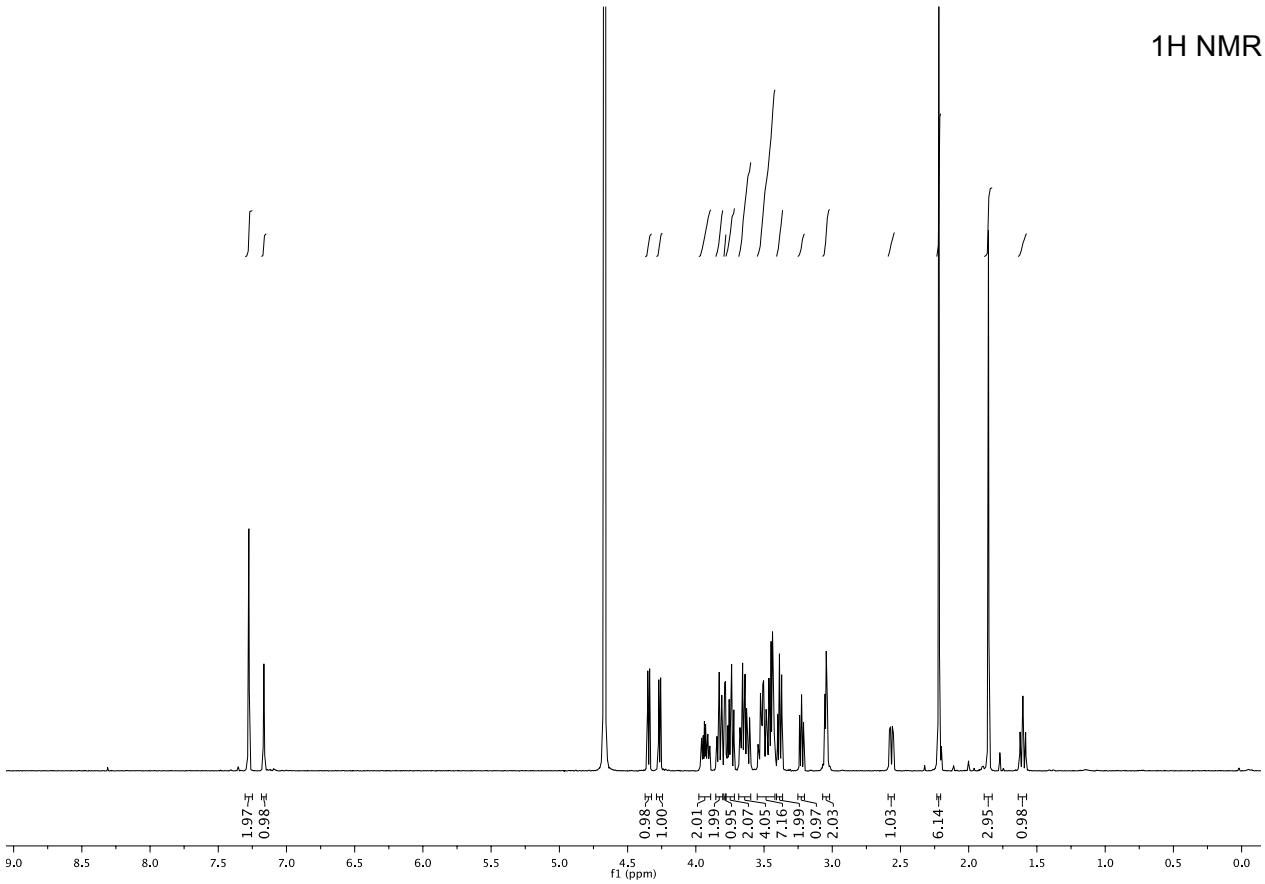

1H NMR
0.99
1.01
0.98
1.96
1.01
1.96
1.01
0.97
1.00
0.98
0.94
1.00
3.98
2.94
1.13
4.96
1.98
1.93
0.99
1.98
1.05
2.91
1.00
f1 (ppm)


13C NMR
175.63
174.24
171.64
141.68
140.39
135.08
131.21
130.22
130.00
128.90
127.81
126.99
126.36
103.94
102.56
101.14
80.29
75.37
75.25
74.56
73.34
73.21
73.13
71.49
71.09
70.88
69.32
69.10
67.14
64.60
60.90
52.56
43.75
40.87
40.23
22.80
f1 (ppm)

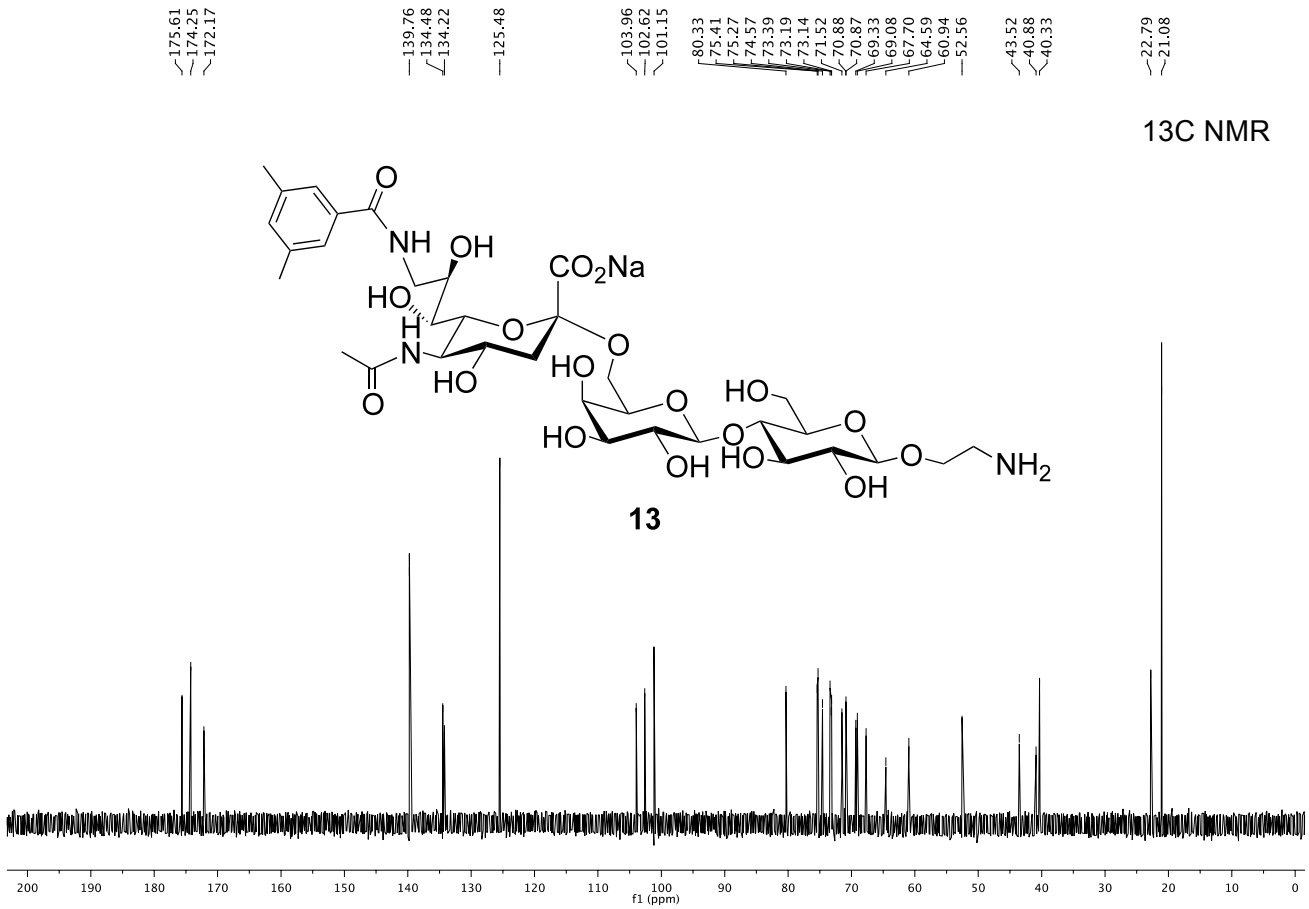
O
NH
OH
CO2Na
HO
H
N
O
HO
O
O
HO
HO
O
HO
O
O
HO
OH
HO
O
NH2
OH

12

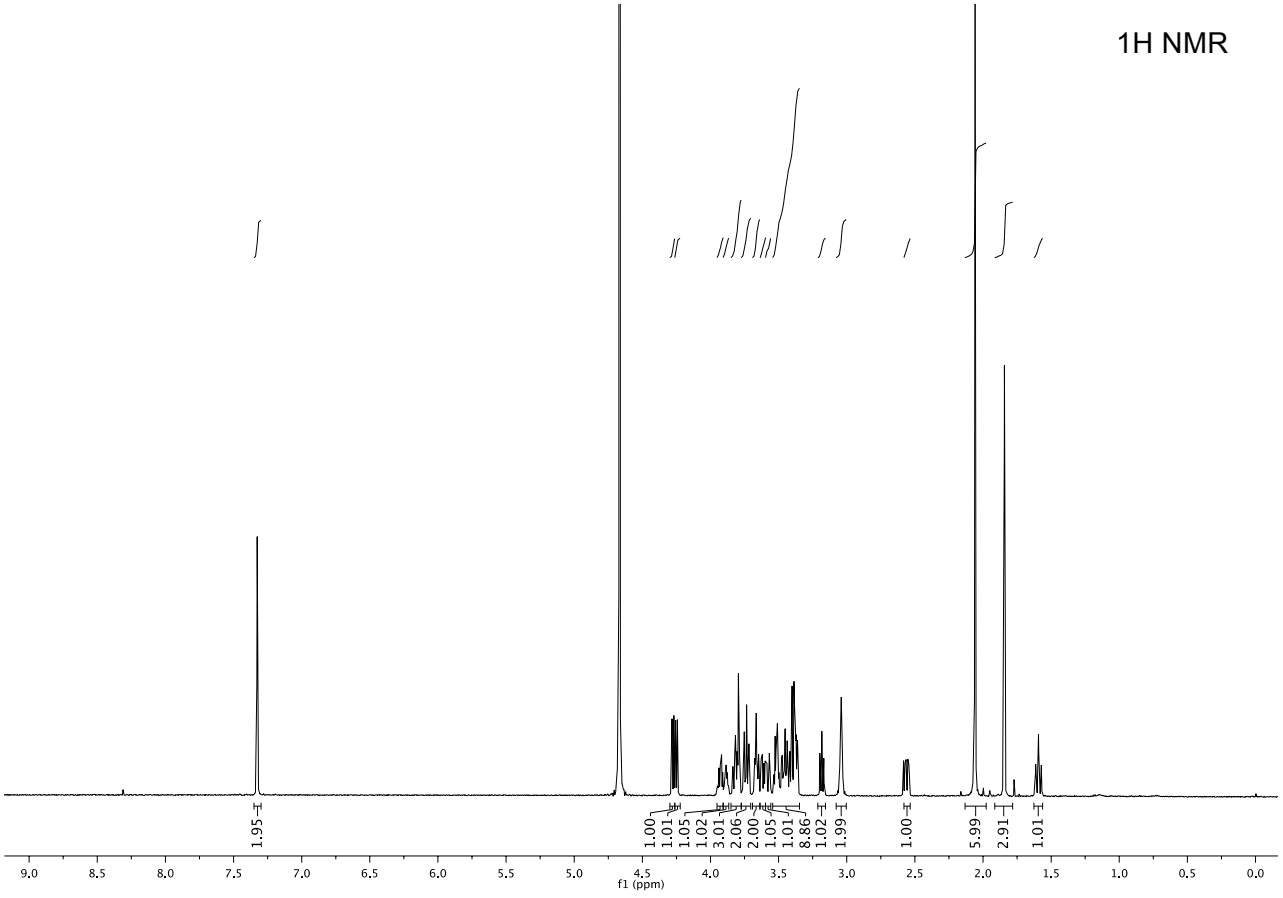
1H NMR

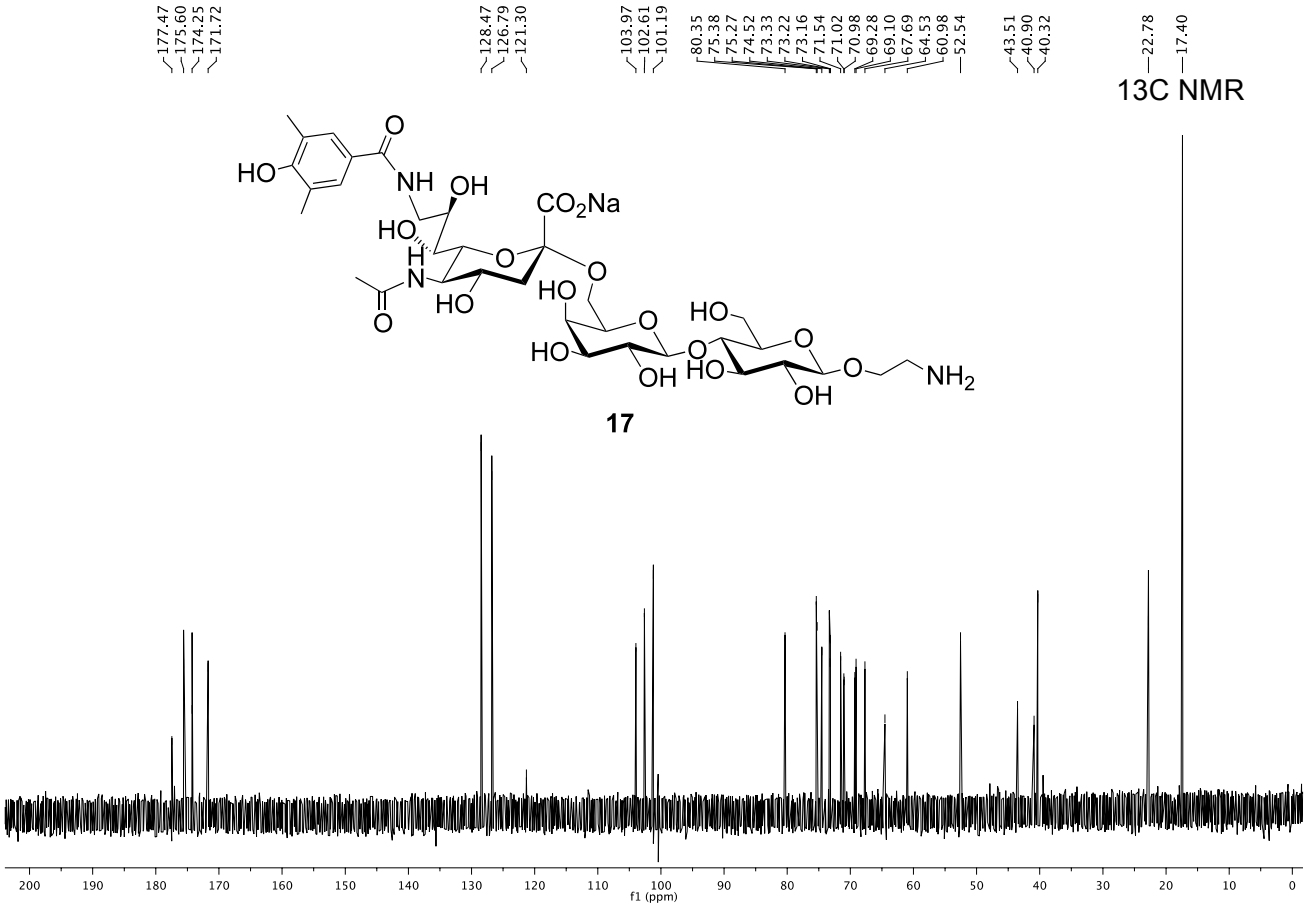


13C NMR

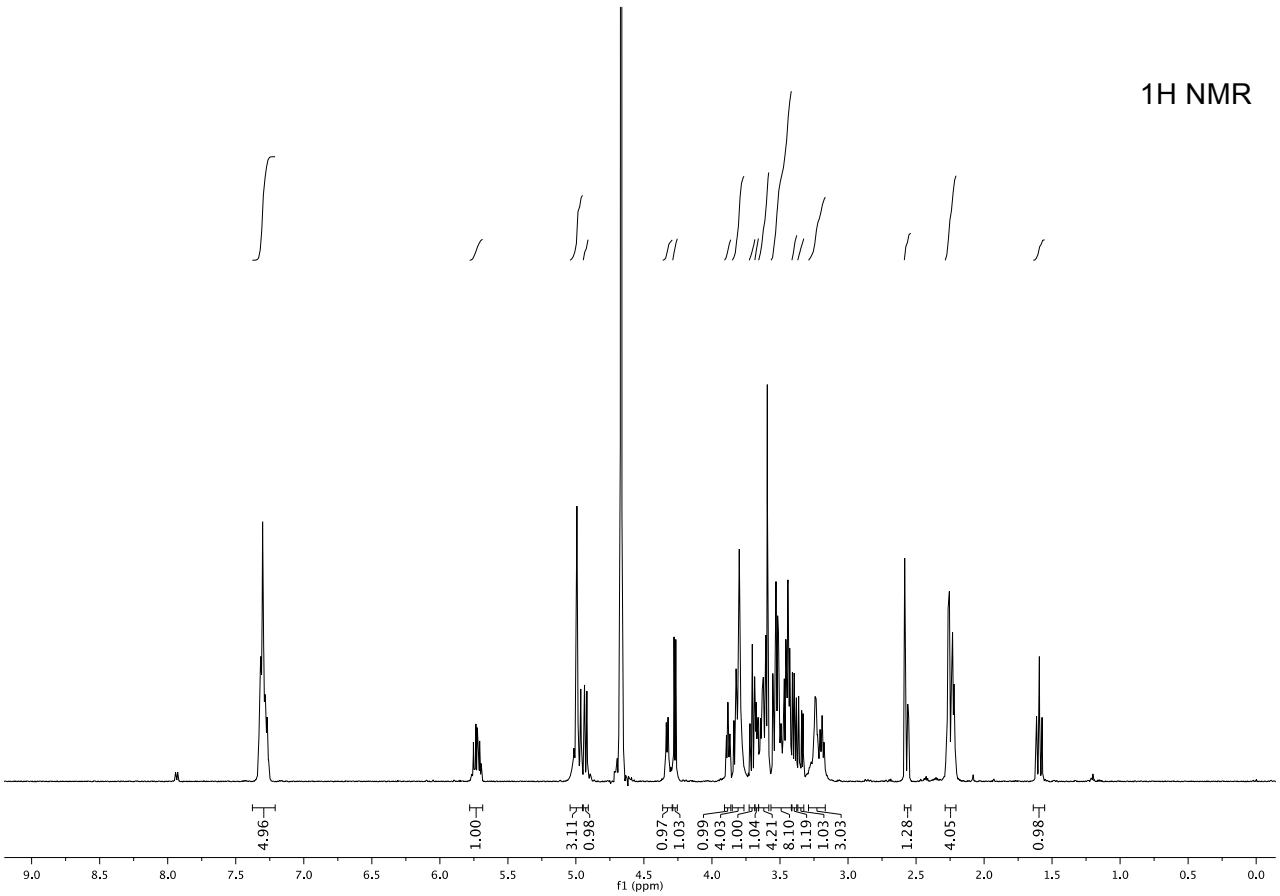


13

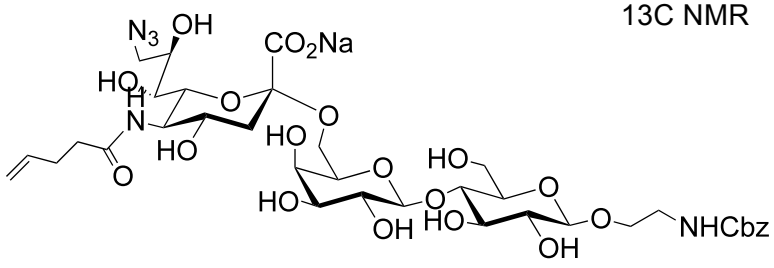

1H NMR
1.95
1.00
1.01
1.05
1.02
3.01
2.06
2.00
1.05
1.01
8.86
1.02
1.99
1.00
5.99
2.91
1.01
f1 (ppm)


13C NMR
177.47
175.60
174.25
171.72
128.47
126.79
121.30
103.97
102.61
101.19
80.35
75.38
75.27
74.52
73.33
73.22
73.16
71.54
71.02
70.98
69.28
69.10
67.69
64.53
60.98
52.54
43.51
40.90
40.32
22.78
17.40
HO
NH
OH
CO2Na
HO
H
N
O
HO
O
O
HO
O
HO
OH
HO
O
HO
O
NH2
OH
17
f1 (ppm)

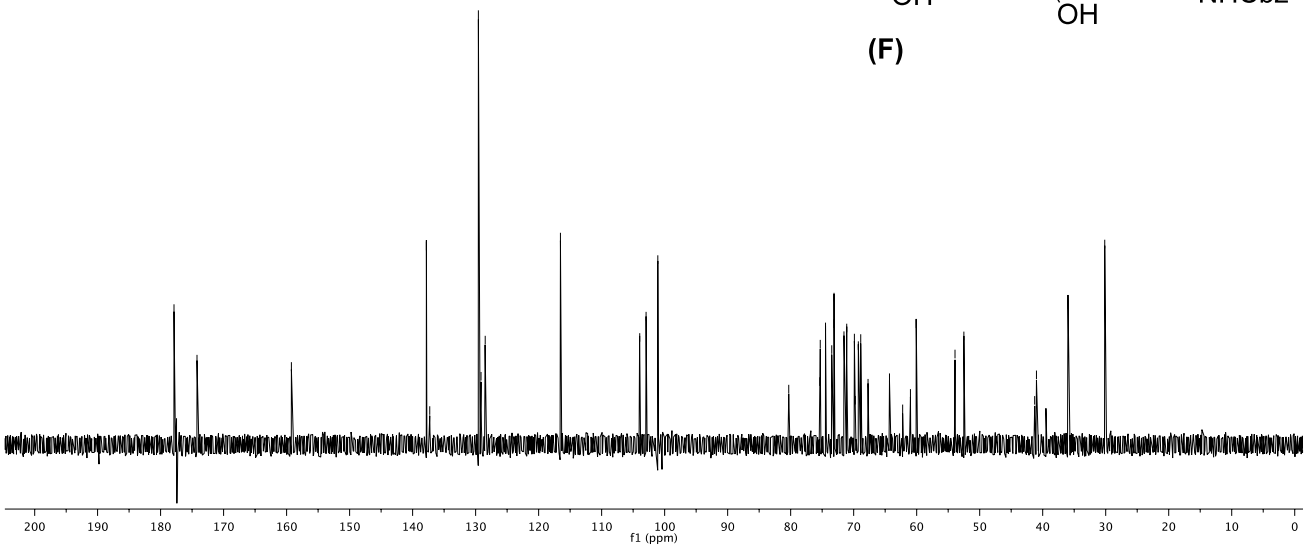
1H NMR



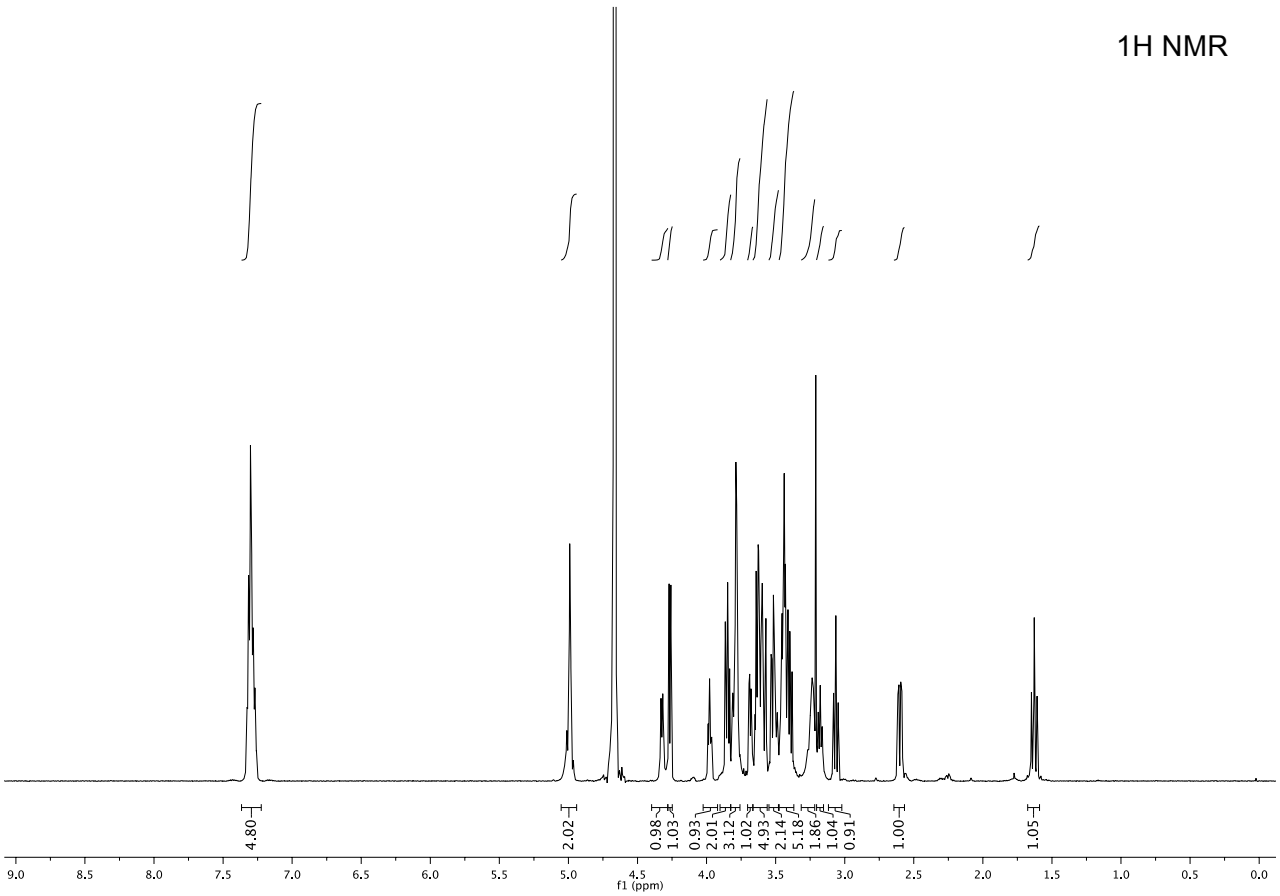
13C NMR



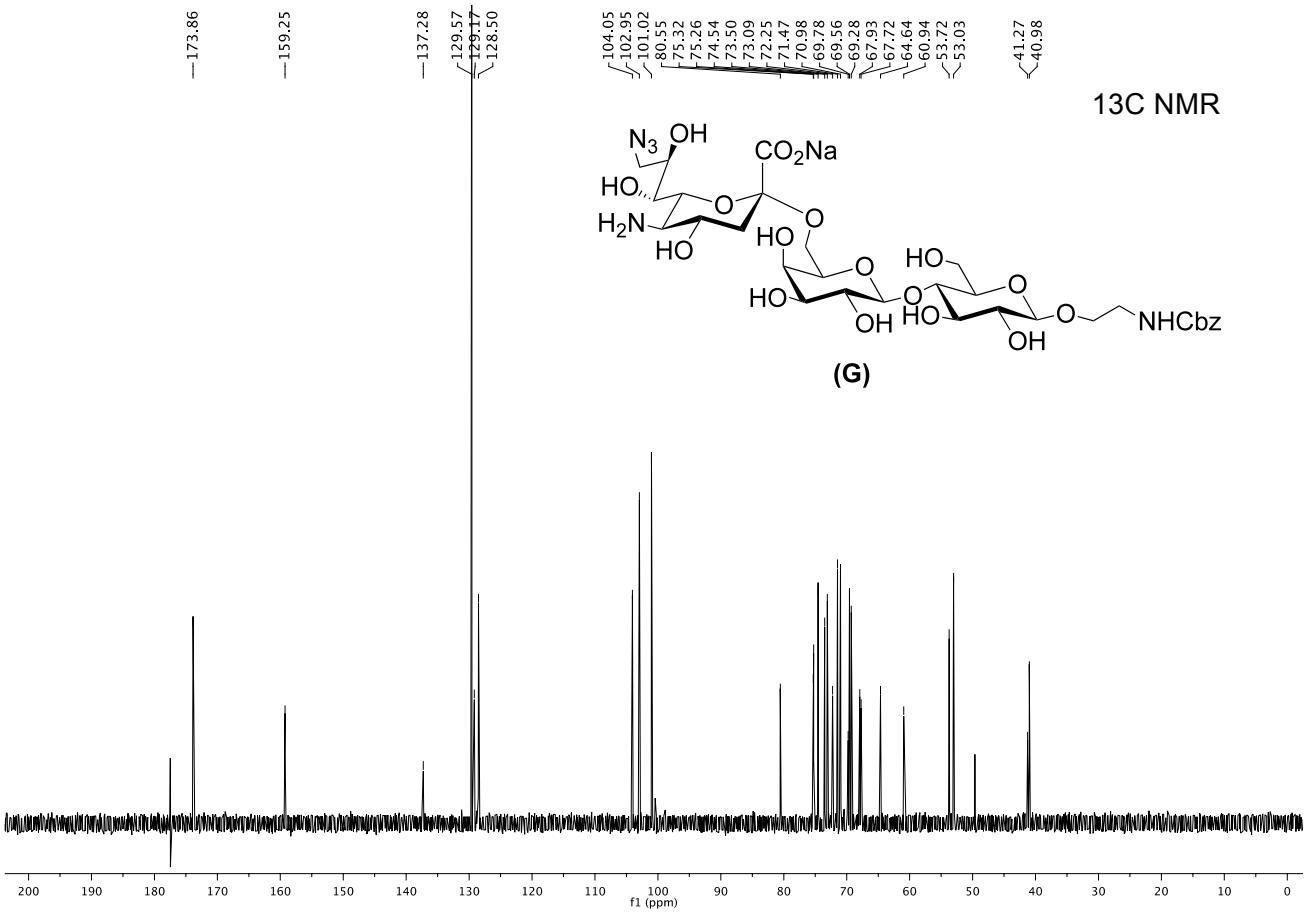
(F)



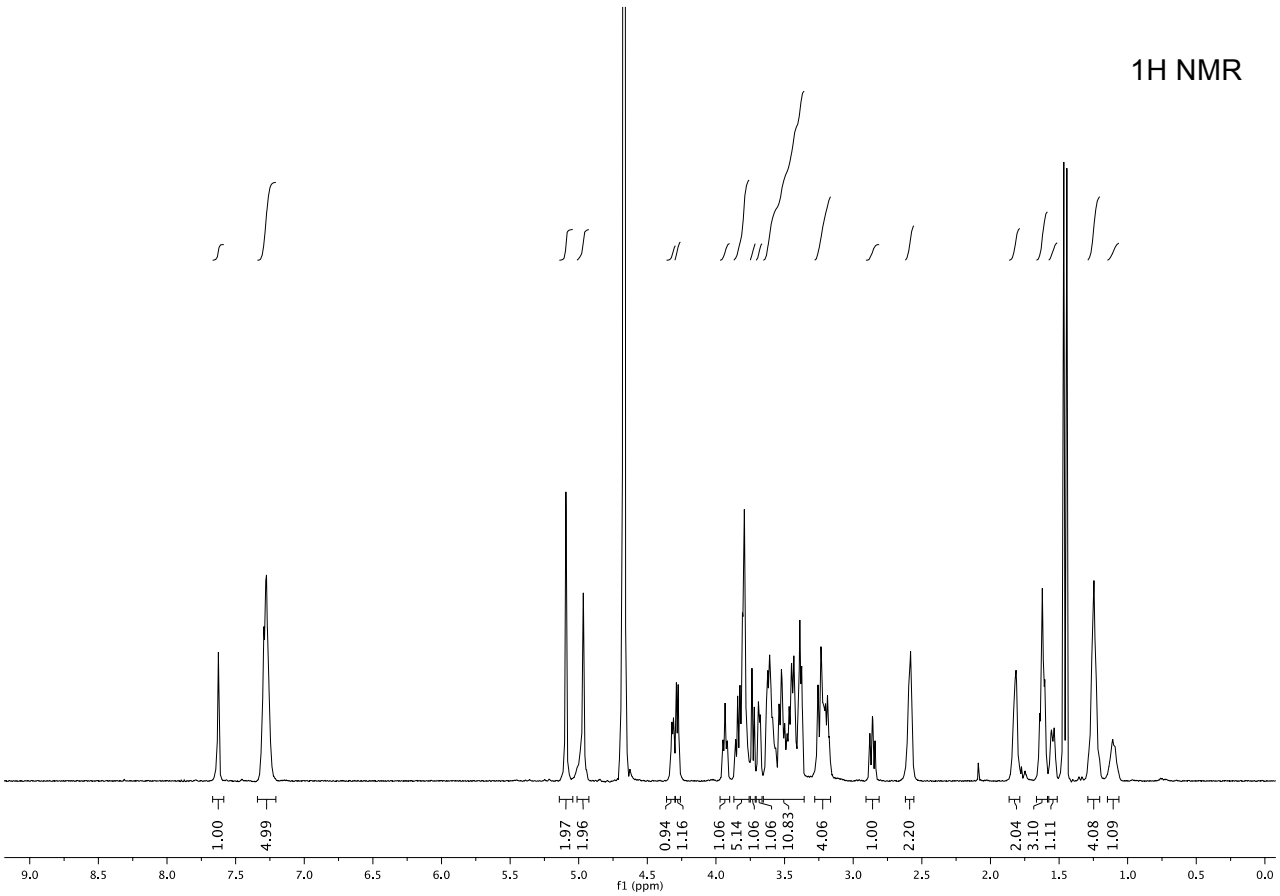
1H NMR

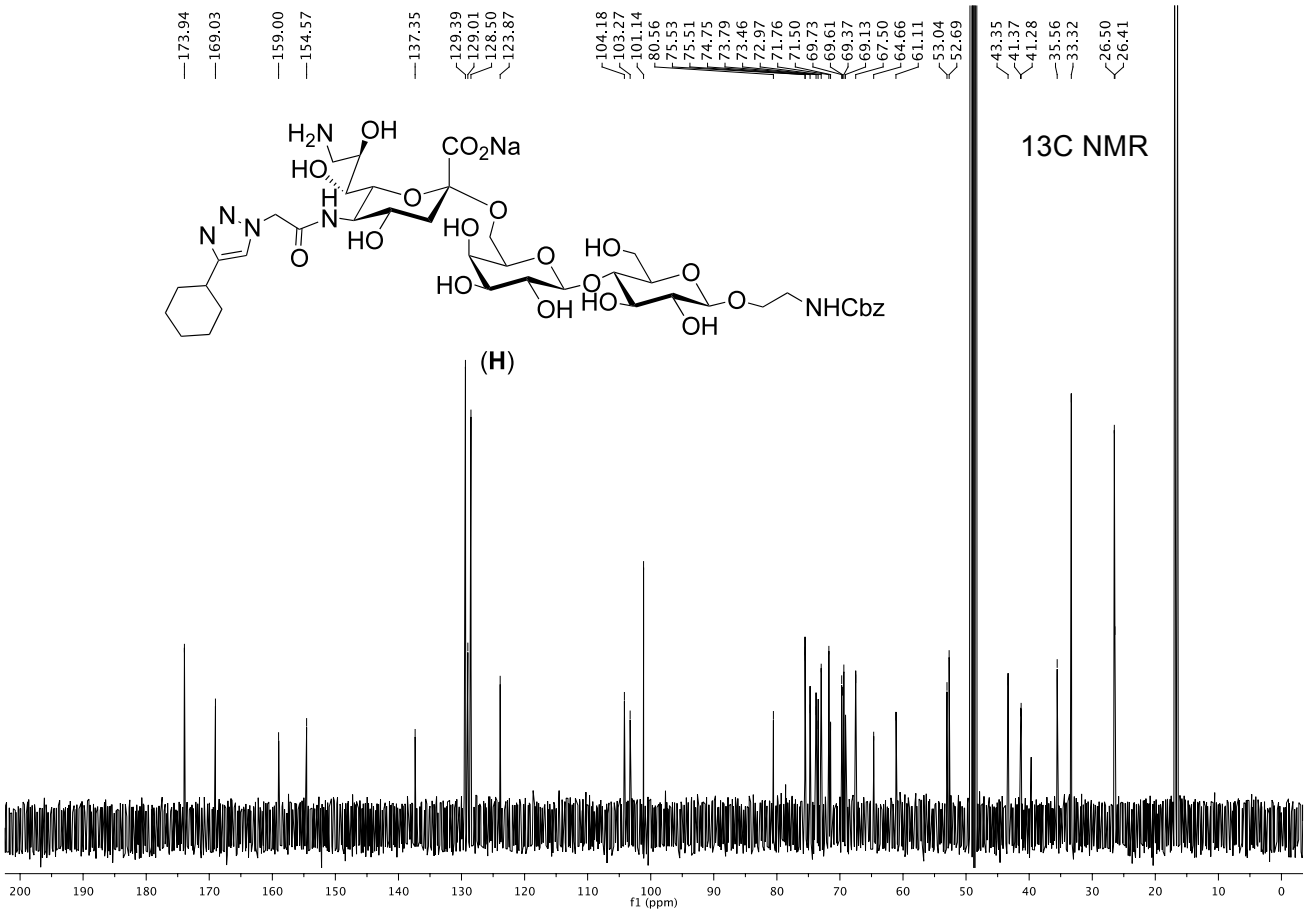


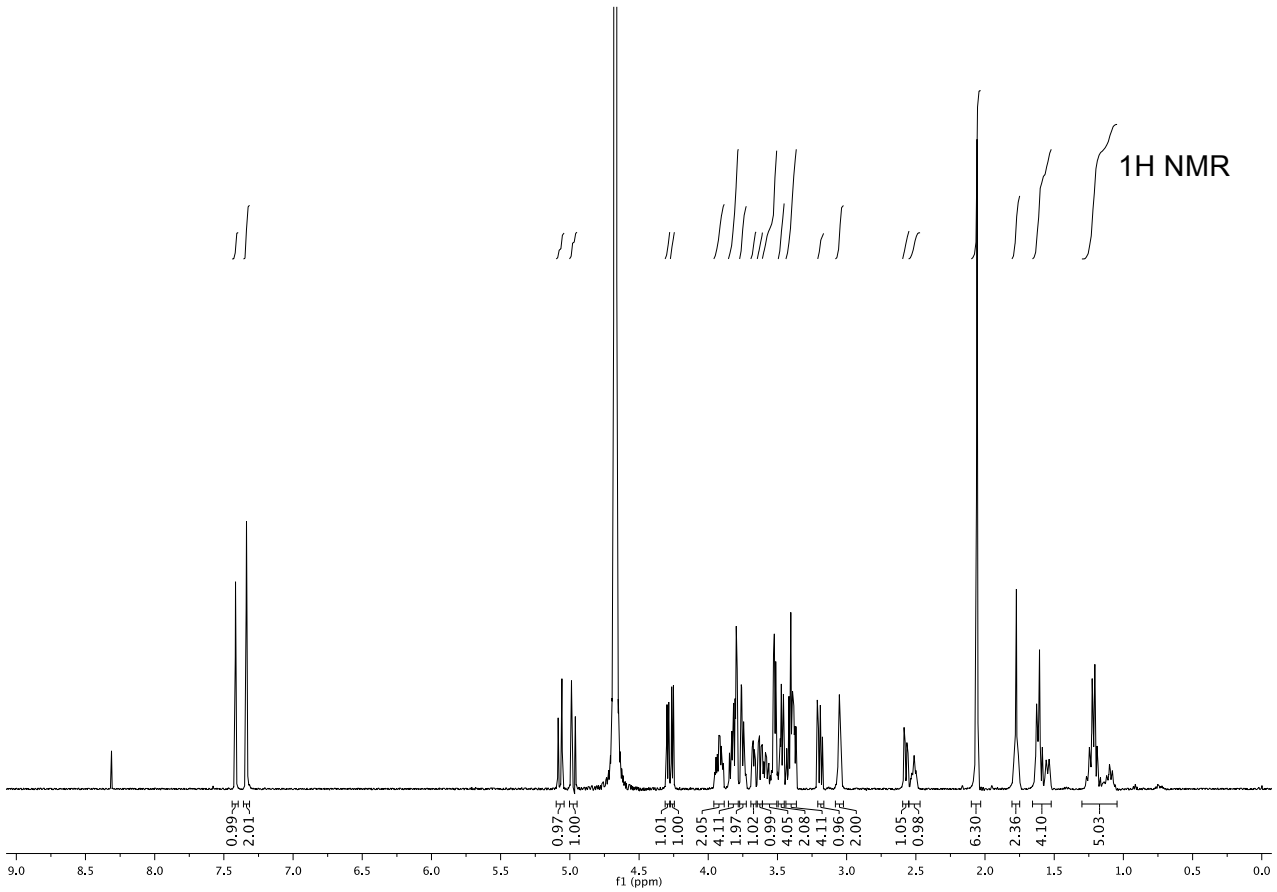
13C NMR

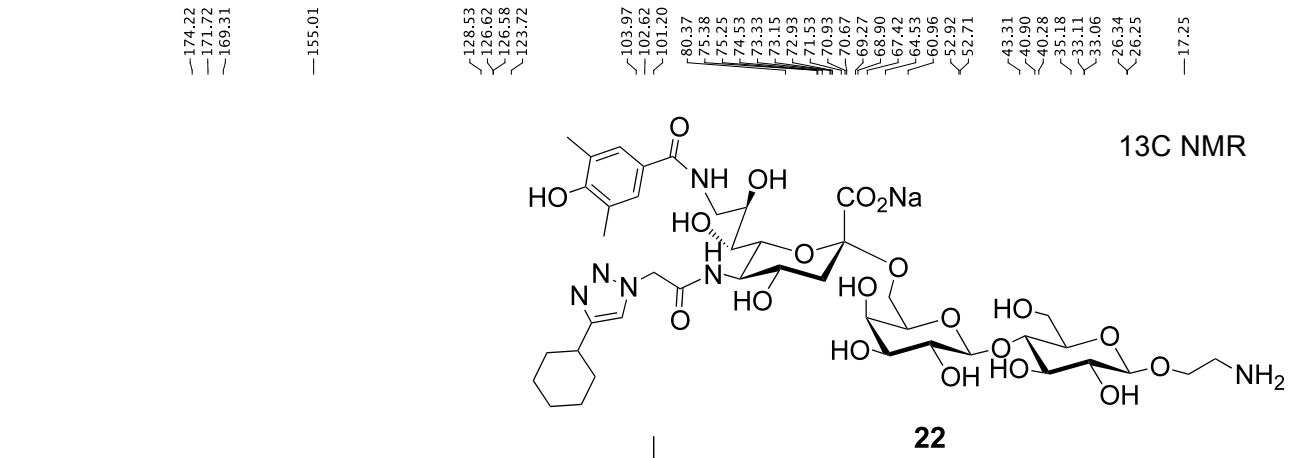


(G)


1H NMR
1.00
4.99
1.97
1.96
0.94
1.16
1.06
5.14
1.06
1.06
10.83
4.06
1.00
2.20
2.04
3.10
1.11
4.08
1.09
f1 (ppm)

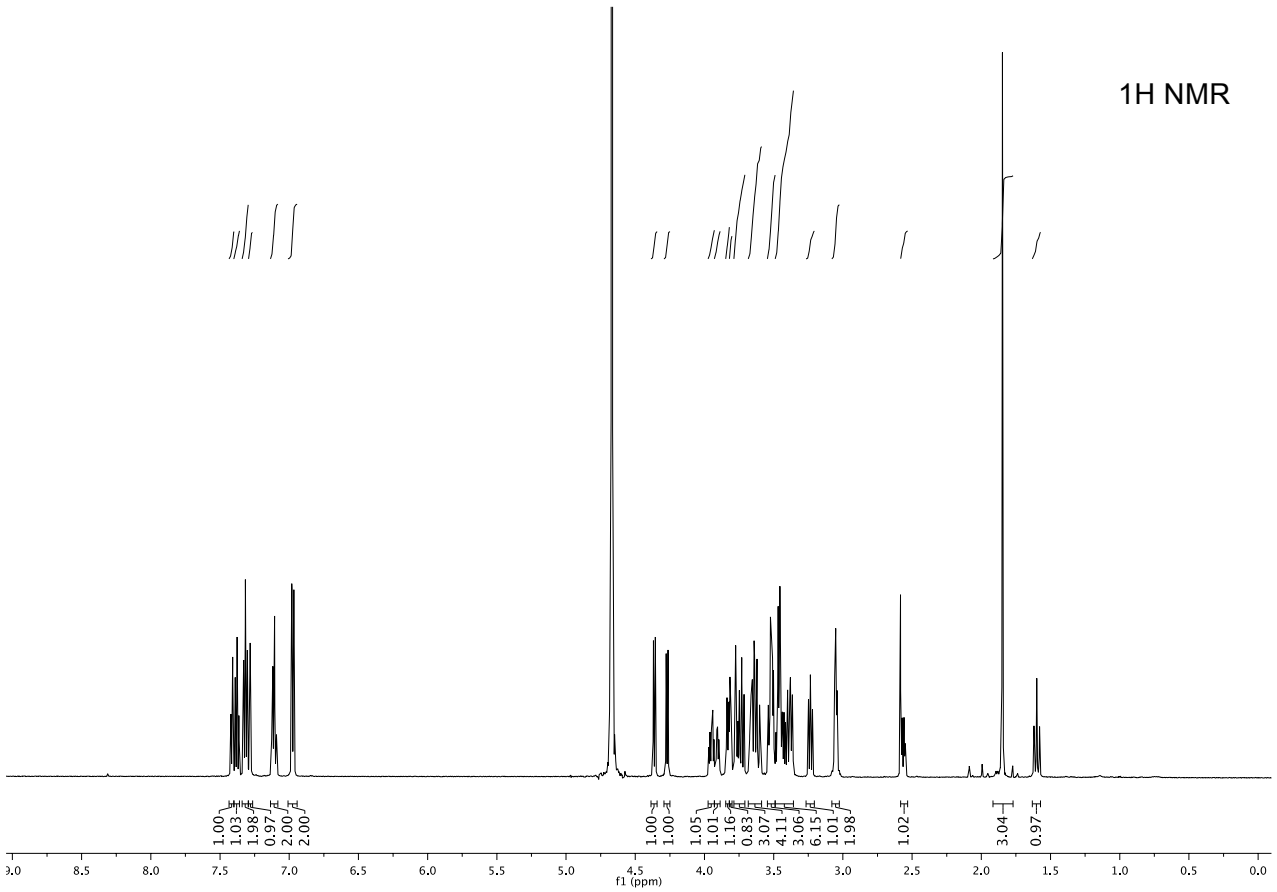

13C NMR
173.94
169.03
159.00
154.57
137.35
129.39
129.01
128.50
123.87
104.18
103.27
101.14
80.56
75.53
75.51
74.75
73.79
73.46
72.97
71.76
71.50
69.73
69.61
69.37
69.13
67.30
64.66
61.11
53.04
52.69
43.35
41.37
41.28
35.56
33.32
26.50
26.41
H2N
OH
HO
CO2Na
NHCbz
(H)
f1 (ppm)

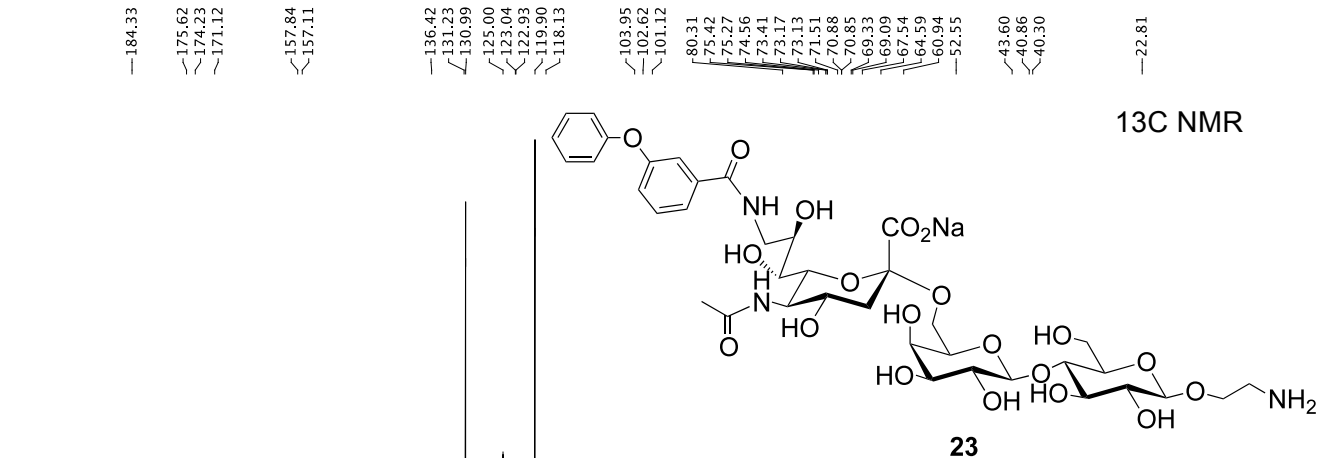

1H NMR
0.99
2.01
0.97
1.00
1.01
1.00
2.05
4.11
1.97
1.02
0.99
4.05
2.08
4.11
0.96
2.00
1.05
0.98
6.30
2.36
4.10
5.03
f1 (ppm)


13C NMR
174.22
171.72
169.31
155.01
128.53
126.62
126.58
123.72
103.97
102.62
101.20
80.37
75.38
75.25
74.53
73.33
73.15
72.93
71.53
70.93
70.67
69.27
68.90
67.42
64.53
60.96
52.92
52.71
43.31
40.90
40.28
35.18
33.11
33.06
26.34
26.25
17.25
f1 (ppm)

HO
NH
OH
HO
CO2Na
O
O
N
N
N
O
HO
HO
O
HO
O
HO
OH
HO
O
O
NH2
OH

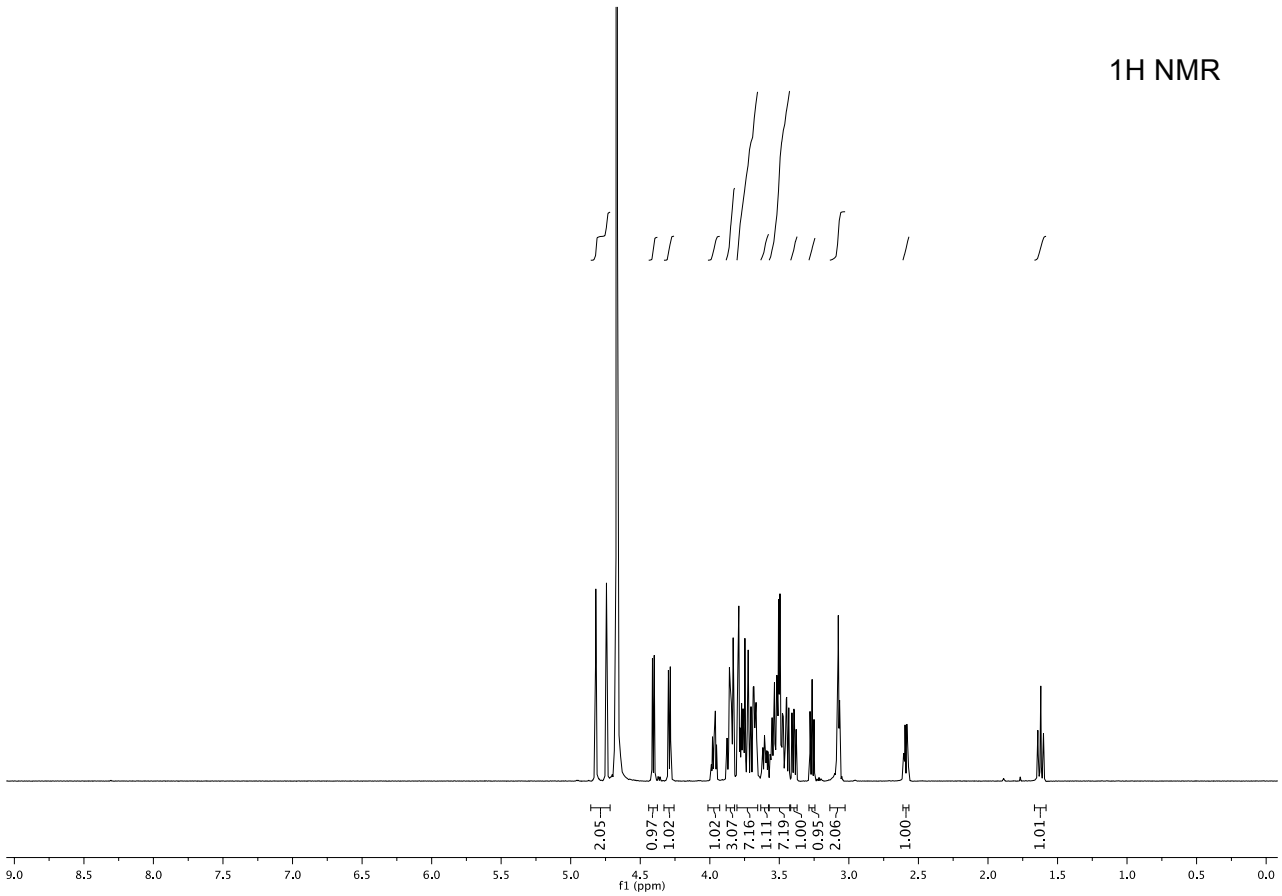
22

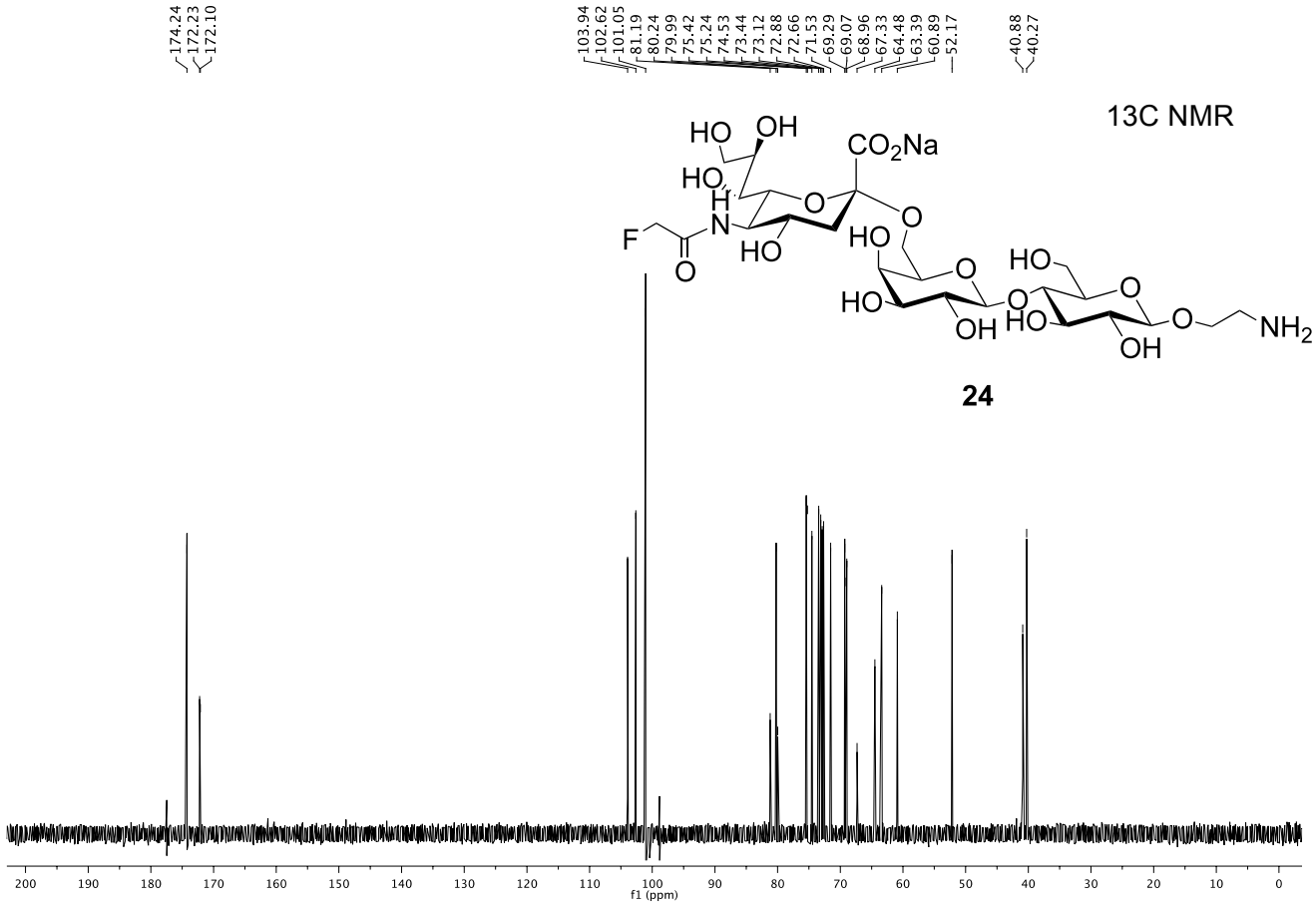

1H NMR
1.00
1.03
1.98
0.97
2.00
2.00
1.00
1.00
1.05
1.01
1.16
0.83
3.07
4.11
3.06
6.15
1.01
1.98
1.02
3.04
0.97
f1 (ppm)


13C NMR
184.33
175.62
174.23
171.12
157.84
157.11
136.42
131.23
130.99
125.00
123.04
122.93
119.90
118.13
103.95
102.62
101.12
80.31
75.42
75.27
74.56
73.41
73.17
73.13
71.51
70.88
70.85
69.33
69.09
67.54
64.59
60.94
52.55
43.60
40.86
40.30
22.81
f1 (ppm)

O
NH
OH
CO2Na
HO
H
N
O
HO
HO
O
HO
HO
OH
O
HO
HO
OH
O
NH2

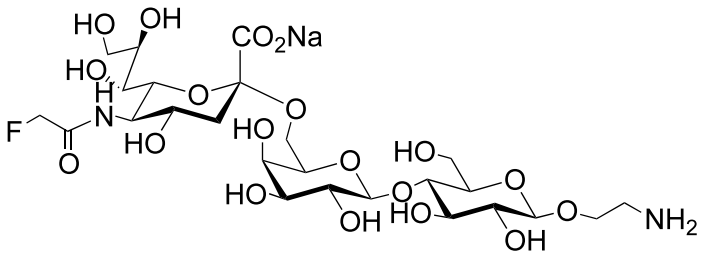
23


1H NMR
2.05
0.97
1.02
1.02
3.07
7.16
1.11
7.19
1.00
0.95
2.06
1.00
1.01
f1 (ppm)


174.24
172.23
172.10
103.94
102.62
101.05
81.19
80.24
79.99
75.42
75.24
74.53
73.44
73.12
72.88
72.66
71.53
69.29
69.07
68.96
67.33
64.48
63.39
60.89
52.17
40.88
40.27
13C NMR
HO
OH
CO2Na
HO
H
N
F
O
O
HO
O
O
HO
O
HO
O
HO
OH
HO
O
O
NH2
OH
f1 (ppm)

24

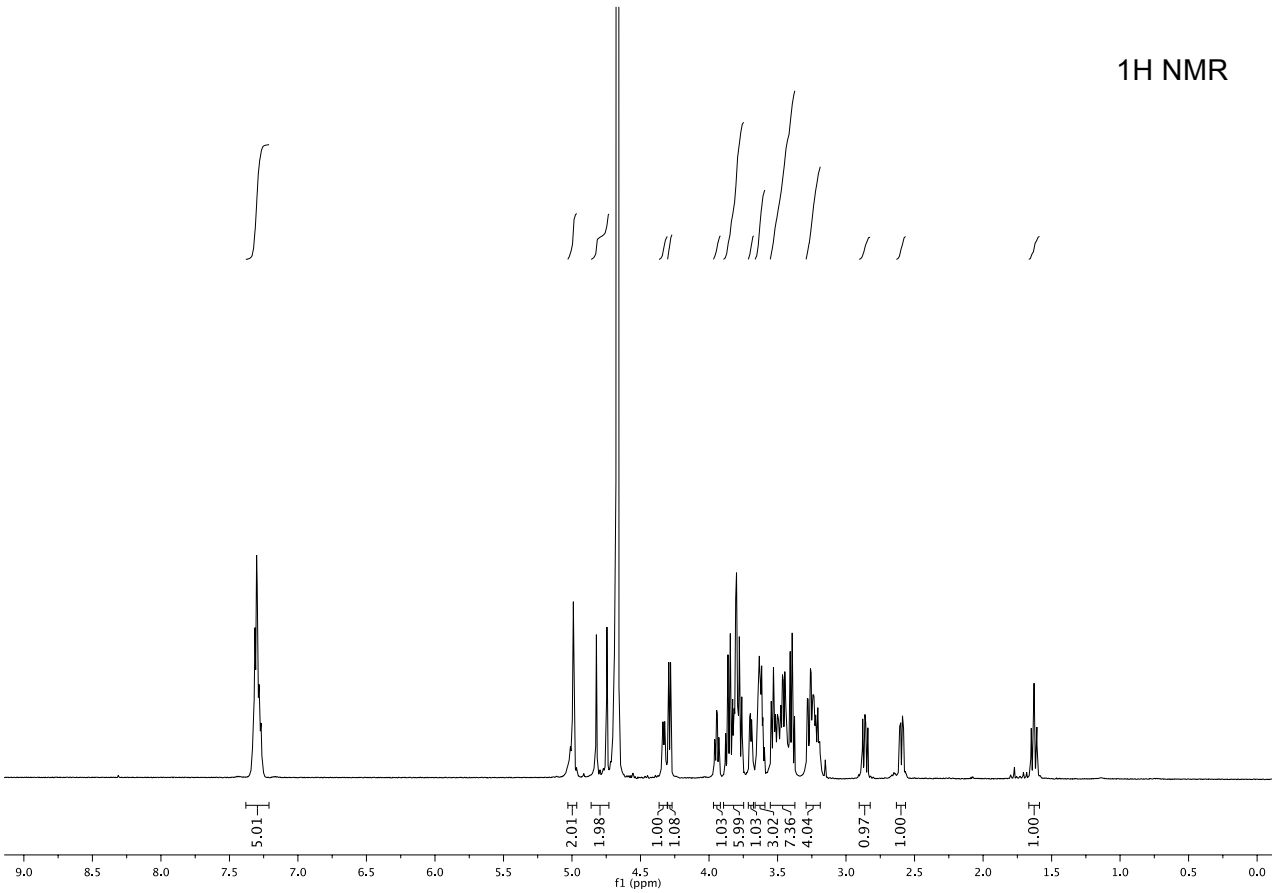
19F NMR


HO
OH
HO
H
N
F
O
HO
CO2Na
O
HO
O
HO
O
HO
OH
HO
O
HO
O
NH2
OH

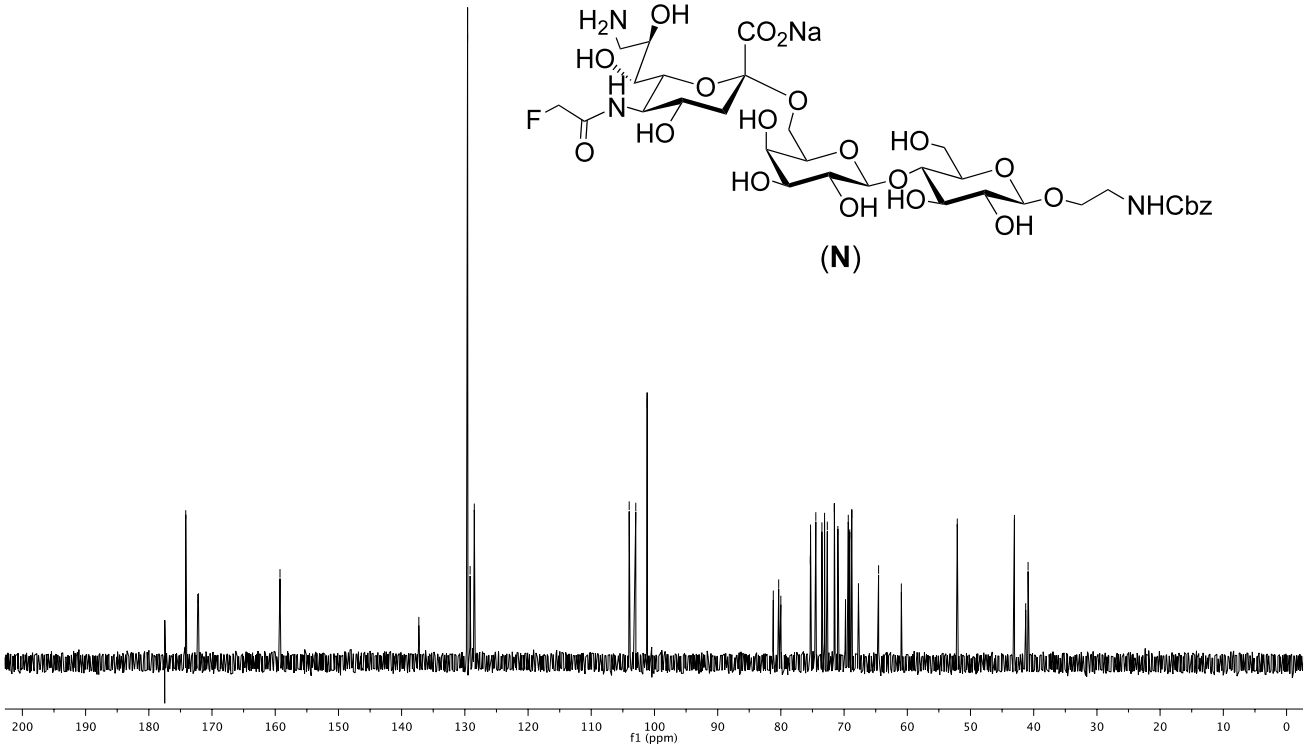
24

-226.45
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
-250
-260
-270
f1 (ppm)

1H NMR



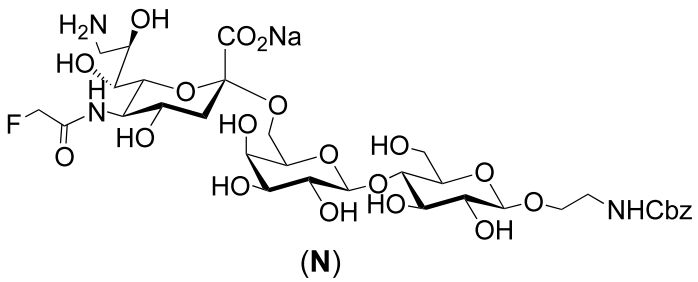
13C NMR



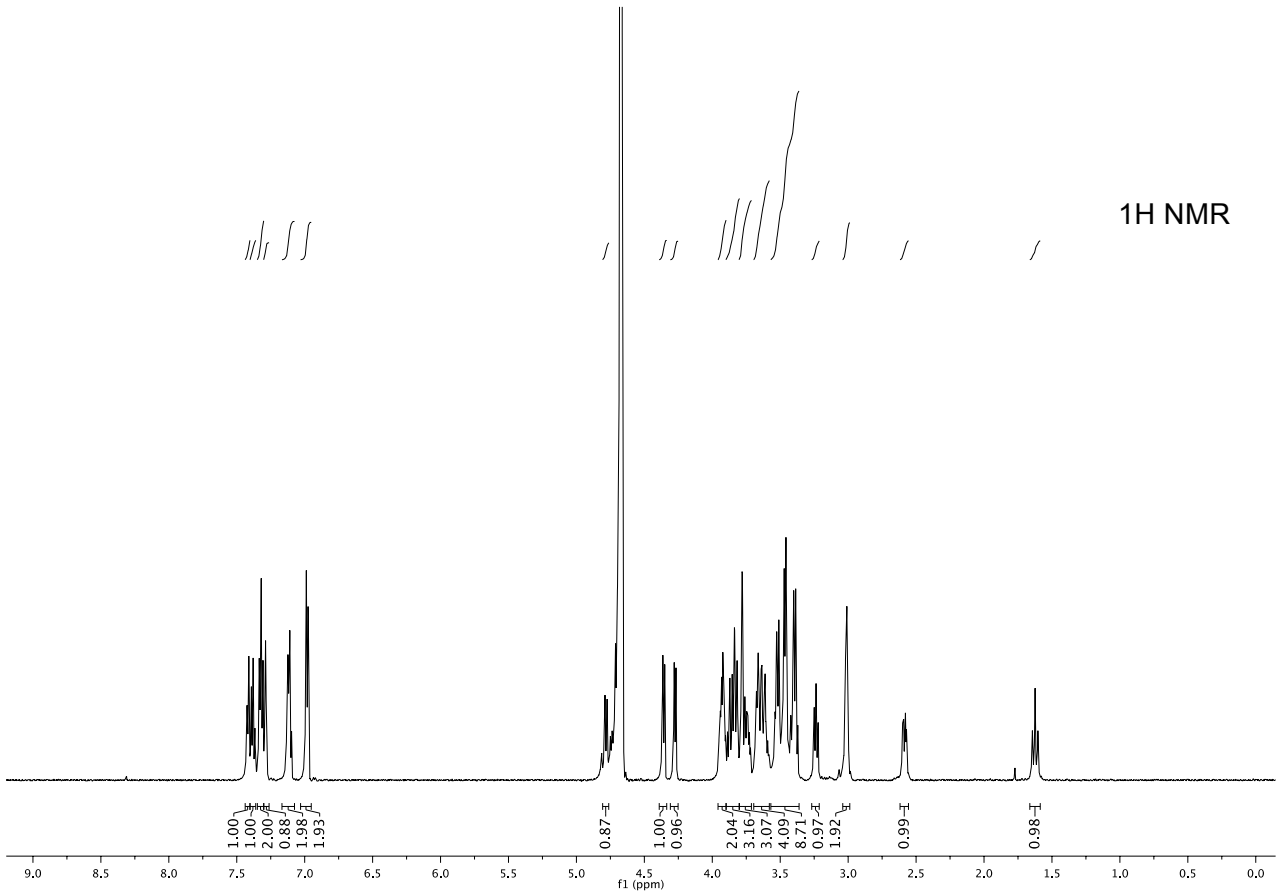
(**N**)

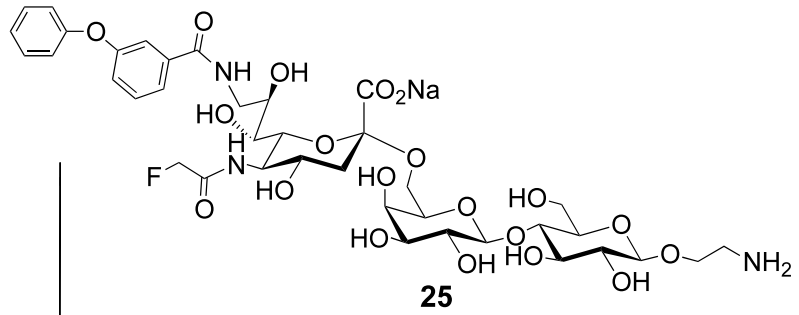
-226.45

19F NMR



(**N**)

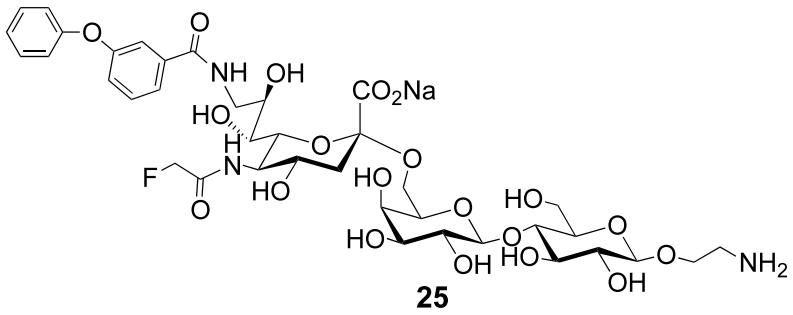

1H NMR
1.00
1.00
2.00
0.88
1.98
1.93
0.87
1.00
0.96
2.04
3.16
3.07
4.09
8.71
0.97
1.92
0.99
0.98
f1 (ppm)
174.21
172.19
172.07
171.15
157.85
157.12
136.42
131.23
130.99
125.00
123.04
122.93
119.91
118.14
103.96
102.65
101.13
81.18
80.35
79.98
75.41
75.29
74.58
73.43
73.13
72.77
71.51
70.91
70.85
69.34
68.90
68.10
64.65
60.95
52.18
43.64
40.90
40.38
13C NMR
f1 (ppm)


O
NH
OH
HO
CO2Na
H
N
F
O
HO
HO
O
HO
HO
OH
HO
O
HO
O
NH2
OH

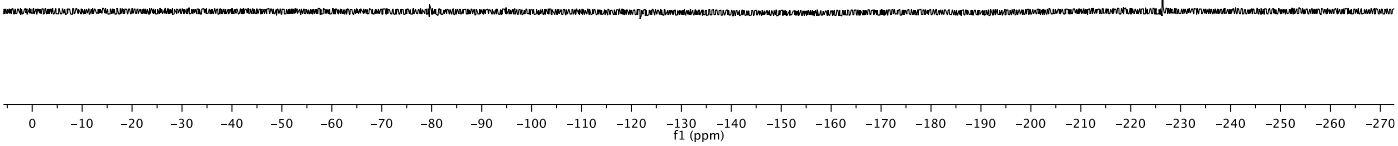
25

-226.35

19F NMR



25



1H NMR
6'BPC-NeuAc-Biotin
f1 (ppm)