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Materials and Methods

All solvents were of reagent grade and used as received. Chemicals and reagents were used as commercially supplied without any further purification unless otherwise stated. Mannosamine hydrochloride and *N*-acetylneuraminic acid (Neu5Ac) were purchased from Carbosynth Ltd. Cytidine 5′-triphosphate disodium salt (CTP), Inorganic Pyrophosphatase (IPPase) from baker's yeast (*S. cerevisiae*), Sialic Acid Aldolase (SA aldolase) from *Escherichia coli* K12, CMP-Sialic Acid Synthetase (CMP-SA synthetase) from *Neisseria meningitidis* group B, fetuin and asialofetuin were purchased from Sigma-Aldrich. Alkaline Phosphatase from calf intestine (CIAP) was purchased from Calbiochem EMD Millipore. All enzymatic reactions were performed in aqueous buffered system. MeO-DIBO,[1] MeO-DIBAC,[2] BCN[3] and BCN-biotin[3] conjugate were synthesized as previously described.

Column chromatography was performed on silica gel G60 (Silicycle, 60-200 μm, 60 Å). TLC-analysis was conducted on Silicagel 60 F_{254} (EMD Chemicals inc.) with detection by UV-absorption (254 nm) were applicable, and by spraying with 20% sulfuric acid in ethanol followed by charring at ~150 °C or by spraying with a solution of Hanessian's stain followed by charring at ~150 °C. Molecular sieves (4 Å) were flame activated under vacuum prior to use.

All nuclear magnetic resonance (NMR) spectra were acquired on a 300 MHz or 400 MHz Bruker spectrometer operating at 25 °C. Chemical shifts are reported in parts per million (ppm) relative to the NMR solvent as the internal standard. NMR data is presented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances), coupling constant in Hertz (Hz), integration. All NMR signals were assigned on the basis of ^{1}H NMR, COSY and HSQC experiments.

High-resolution mass spectra were recorded on a Thermo Exactive orbitrap (ESI) mass spectrometer.

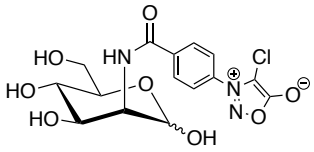
Reverse-Phase HPLC was performed on an Agilent 1260 Infinity II system equipped with an automated injector, UV-detector, fraction-collector and Agilent Poroshell 120 EC-C18 column (4 μm, 4.6 × 150 mm).

Enzyme activity measurements were obtained through the use of a phosphatase-coupled assay utilizing colorimetric detection of inorganic phosphate with malachite green-based reagents – Malachite green phosphate detection kit from R&D Systems, recombinant human 5'-nucleotidase (CD73) from Bon Opus Biosciences, the absorbance was read using a SpectraMax M2E Multi-Mode Cuvette/Microplate Reader (Molecular Devices).

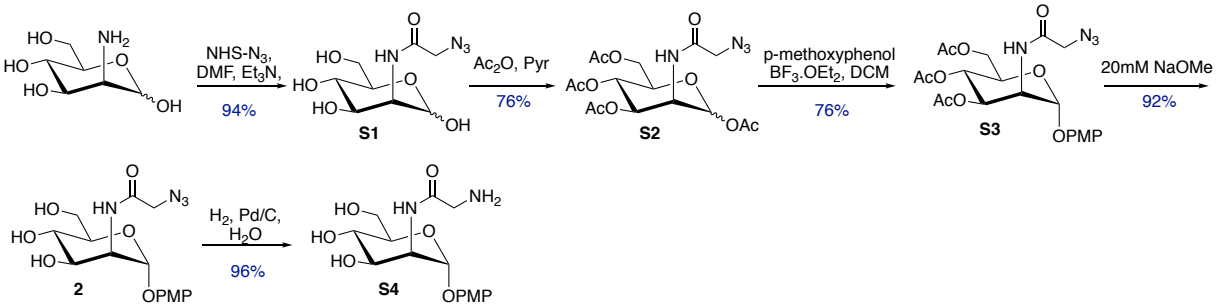
In-gel fluorescence scanning was performed on a ChemiDoc MP imager (BioRad)

Experimental Procedures

2-Deoxy-2-*N*-(1,2,3-oxadiazol-3-ium-5-olate-4-chloro-3-benzamido)-D-mannopyranose (1).

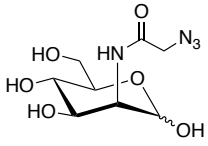


Et_3N (212 µL, 1.52 mmol) was added to a solution of **mannosamine hydrochloride** (82 mg, 0.38 mmol) in dry DMF (9 mL) and the mixture was left to stir for 10 min, followed by the addition of NHS-activated chlorosydnone[4] (154 mg, 0.46 mmol). The reaction mixture was left to stir for 18 h at room temperature under an atmosphere of argon. The reaction mixture was concentrated *in vacuo* and the residue was azeotropically dried with toluene (3 × 25 mL). The resulting residue was purified by flash chromatography on silica gel (gradient of 0% → 10% MeOH:DCM) to afford **1** (147 mg, 96%) as an orange solid. ^{1}H NMR (300 MHz, D_2O): δ 3.46-3.53 (m, 0.5H, H-5α), 3.67 (t, *J* = 9.9 Hz, 0.5H, H-4α), 3.77 (t, *J* = 9.8 Hz, 0.5H, H-4β), 3.83-4.01 (m, 3H, H-6a, H-6b, H-5β, H-3α), 4.20 (dd, *J* = 9.7, 4.7 Hz, 0.6H, H-3β), 4.63 (dd, *J* = 4.6, 1.3 Hz, 0.8H, H-2β), 4.77 (H-2α, under the D_2O peak), 5.19 (d, *J* = 1.6 Hz, 0.5H, H-1α), 5.32 (d, *J* = 1.2 Hz, 0.5H, H-1β), 7.91 (dd, *J* = 8.7, 2.0 Hz, 2H, 2 × aromatic CH), 8.07-8.15 (m, 2H, 2 × aromatic CH). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 53.79 (C-2β), 54.65 (C-2α), 59.80, 65.82 (C-4α), 66.25 (C-4β), 68.83, 71.84, 76.14, 92.90, 124.70 (2×), 129.43 (2×). HRMS ESI: *m/z* for $[M+H]^+$ $C_{15}H_{17}ClN_3O_8^+$, calcd 402.0699; obsd 402.0699.



Scheme S1. Chemical synthesis of ManNAz-PMP **2** and ManNNH$_2$-PMP **S4** from mannosamine.

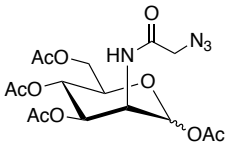
2-(Azidoacetamido)-2-deoxy-D-mannopyranose (S1).



Et_3N (2.6 µL, 18.56 mmol) was added to a solution of **mannosamine hydrochloride** (1 g, 4.64 mmol) in dry DMF (55 mL) and the mixture was left to stir for 10 min, followed by the addition of NHS-activated azido acetic acid[5] (1.1 mg, 5.57 mmol). The reaction mixture was left to stir for 18 h at room temperature under an atmosphere of argon. The reaction mixture was concentrated *in vacuo* and the residue was azeotropically dried with toluene (3 × 50 mL). The resulting residue was purified by flash chromatography on silica gel (gradient of 0% → 20% MeOH:DCM) to afford **4** (1.14 g, 94%) as a white solid. ^{1}H NMR (300 MHz, D_2O): δ 3.43-3.67

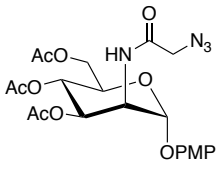
(m, 1.3H, H-5α, H-4α, H-4β), 3.80-3.95 (m, 3.5H, H-6a, H-6b, H-5β, H-3α), 4.08-4.14 (m, 2.8H, H-3β, CH_2N_3), 4.40 (dd, *J* = 4.6, 1.6 Hz, 0.5H, H-2β), 4.53 (dd, *J* = 4.4, 1.6 Hz, 0.5H, H-2α), 5.09 (d, *J* = 1.6 Hz, 0.5H, H-1α), 5.18 (d, *J* = 1.6 Hz, 0.5H, H-1β). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 51.64, 52.93 (C-2β), 53.79 (C-2α), 60.23, 66.25, 68.40 (C-3α), 71.41, 76.14 (C-5α), 92.47. In agreement with the literature data.[6]

2-(Azidoacetamido)-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-mannopyranose (S2).



Acetic anhydride (8 mL) was added to a solution of **S1** (1.14 g, 4.36 mmol) in pyridine (10 mL) and the resulting reaction mixture was left to stir for 18 h. The solution was diluted with ethyl acetate, washed with a saturated solution of $NaHCO_3$ and a solution of $CuSO_4$, dried over $MgSO_4$, filtered and the filtrate was concentrated *in vacuo*. The resulting residue was purified by flash chromatography on silica gel (petroleum ether:EtOAc, 2:8, v:v) to afford **3** (1.42 g, 76%) as an amorphous white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.01-2.19 (m, 12H, 4 × OAc), 3.79-3.85 (m, 0.4H, H-5α), 4.01-4.09 (m, 2.6H, CH_2N_3, H-5β), 4.15 (dd, *J* = 12.0, 2.3 Hz, 1H, H-6a), 4.25 (dt, *J* = 12.4, 4.4 Hz, 1H, H-6b), 4.62 (ddd, *J* = 9.3, 4.1, 1.8 Hz, 0.6H, H-2β), 4.74 (ddd, *J* = 9.0, 3.6, 1.5 Hz, 0.4H, H-2α), 5.06 (dd, *J* = 9.9, 3.8 Hz, 0.4H, H-3α), 5.19 (dt, *J* = 16.4, 9.8 Hz, 1H, H-4), 5.35 (dd, *J* = 10.1, 4.2 Hz, 0.6H, H-3β), 5.89 (d, *J* = 1.6 Hz, 0.4H, H-1α), 6.05 (d, *J* = 1.6 Hz, 0.6H, H-1β), 6.55 (d, *J* = 9.3 Hz, 0.6H, N-Hβ), 6.63 (d, *J* = 8.7 Hz, 0.4H, N-Hα). ^{13}C NMR assigned from HSQC (75 MHz, $CDCl_3$): δ 20.48 (4×), 49.70, 52.28, 61.74, 64.32, 68.62 (C-3α), 69.47 (C-5β), 71.19 (C-3β), 72.91 (C-5α), 89.25 (C-1α), 90.10 (C-1β). In agreement with the literature data.[6]

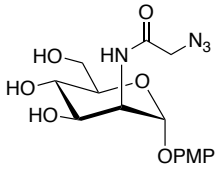
***para*-Methoxyphenyl-2-(azidoacetamido)-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-mannopyranoside (S3)**.



4-Methoxyphenol (74 mg, 0.599 mmol) was added to a solution of **S2** (224 mg, 0.52 mmol) in dry DCM (30 mL) and the mixture was cooled to 0 °C, followed by the slow addition of $BF_3.OEt_2$ (193 μL, 1.562 mmol) and the reaction left to stir, while warming to room temperature, for 18 h. followed by the addition of NHS-activated azido acetic acid[7] (1.1 mg, 5.57 mmol). The solution was diluted with DCM, washed with a saturated solution of $NaHCO_3$, extracted with DCM (2 × 25 mL), dried over Na_2SO_4, filtered and the filtrate was concentrated *in vacuo*. The resulting residue was purified by flash chromatography on silica gel (petroleum ether:acetone, 15:7, v:v) to afford **S3** (195 mg, 76%). ^{1}H NMR (300 MHz; $CDCl_3$): δ 2.03 (s, 3H, OAc), 2.07 (d, *J* = 4.6 Hz, 6H, 2 × OAc), 3.78 (s, 3H, OMe), 4.07-4.16 (m, 4H, CH_2N_3, H-6a, H-5),

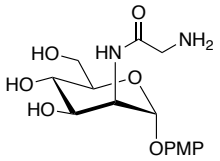
4.23 (dd, J = 12.4, 4.9 Hz, 1H, H-6b), 4.80 (dd, J = 9.3, 4.3 Hz, 1H, H-2), 5.21 (t, J = 10.1 Hz, 1H, H-4), 5.36 (d, J = 1.7 Hz, 1H, H-1), 5.56 (dd, J = 10.1, 4.4 Hz, 1H, H-3), 6.59 (d, J = 9.3 Hz, 1H, NH), 6.83 (d, J = 9.2 Hz, 2H, 2 × aromatic CH), 7.02 (d, J = 9.2 Hz, 2H, 2 × aromatic CH). ^{13}C NMR assigned from HSQC (75 MHz, $CDCl_3$): δ 20.26 (3×), 50.35, 52.07, 55.51, 61.52, 65.39, 68.40 (2×), 97.19, 114.39 (2×), 117.39 (2×). HRMS ESI: *m/z* for $[M+H]^+$ $C_{21}H_{27}N_4O_{10}^+$, calcd 495.1722; obsd 495.1727.

***para*-Methoxyphenyl-2-(azidoacetamido)-2-deoxy-α-D-mannopyranoside (2)**.



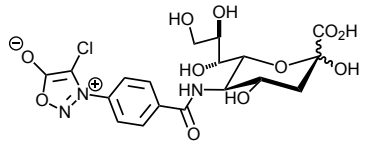
Mannopyranoside **S3** (193 mg) was dissolved in 20 mM NaOMe (7 mL), and the reaction left to stir at room temperature for 2.5 h. The reaction was neutralized with Dowex 50WX8 H^+ resin, filtered, concentration and azeotropically dried with toluene (4 × 5 mL). The resulting residue was purified by gel filtration over Biogel P-2 (eluent 5% aqueous *n*-butanol) to afford **2** (132 mg, 92%) as an amorphous white solid. ^{1}H NMR (300 MHz, D_2O): δ 3.67-3.86 (m, 7H, H-4, H-6a, H-6b, OMe, H-5), 4.10 (s, 2H, CH_2N_3), 4.27 (dd, J = 9.6, 4.8 Hz, 1H, H-3), 4.64 (dd, J = 4.6, 1.7 Hz, 1H, H-2), 5.48 (d, J = 1.6 Hz, 1H, H-1), 6.99 (d, J = 9.3 Hz, 2H, 2 × aromatic CH), 7.13 (d, J = 9.2 Hz, 2H, 2 × aromatic CH). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 51.21, 52.49, 55.51, 60.23, 66.25, 68.83, 72.69, 97.63, 114.82 (2×), 118.26 (2×). HRMS ESI: *m/z* for $[M-H]^-$ $C_{15}H_{19}N_4O_7^-$, calcd 367.1259; obsd 367.1267.

***para*-Methoxyphenyl-2-(aminoacetamido)-2-deoxy-α-D-mannopyranoside (S4)**.

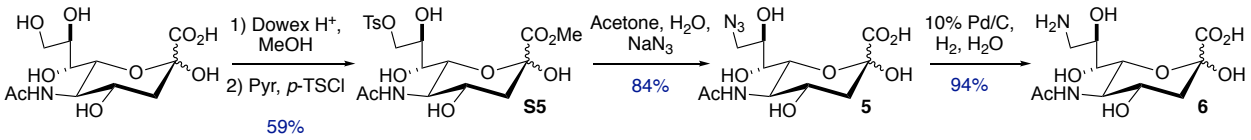


10% Pd on activated carbon (2.2 mg) was added to **2** (21.6 mg, 58.6 μmol) in a mixture of water (1 mL) and methanol (1 mL), and left to stir for 3 h under an atmosphere of hydrogen. The reaction mixture was filtered through a syringe filter (cellulose acetate, 0.45 μm) and lyophilized to give **S4** (19.3 mg, 96%) as an amorphous white solid. ^{1}H NMR (300 MHz, D_2O): δ 3.45 (s, 2H, CH_2NH_2), 3.68-3.84 (m, 7H, H-4, H-6a, H-6b, OMe, H-5), 4.25 (dd, J = 9.6, 4.8 Hz, 1H, H-3), 4.61 (dd, J = 4.7, 1.6 Hz, 1H, H-2), 5.46 (d, J = 1.6 Hz, 1H, H-1), 6.98 (d, J = 9.2 Hz, 2H, 2 × aromatic CH), 7.13 (d, J = 9.3 Hz, 2H, 2 × aromatic CH). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): 43.04, 52.07, 60.23, 66.25, 68.83, 72.69, 98.06, 114.82 (2×), 118.26 (2×). HRMS ESI: *m/z* for $[M+H]^+$ $C_{15}H_{23}N_2O_7^+$, calcd 343.1500; obsd 343.1516.

5-(3-[4-Chloro-1,2,3-oxadiazol-3-ium-5-olate]benzamido)-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (4).

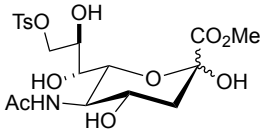


Sialic acid aldolase (13 µL, 8.86 mU/µmol mannosamine) was added to a solution of compound **1** (11.2 mg, 27.9 µmol) and sodium pyruvate (92.0 mg, 836.3 µmol) in phosphate buffer (656 µL, 100 mM, pH 7.7) containing $MgCl_2$ (27.9 µL, 20 mM) to achieve a 40 mM final concentration of compound **1**. The resulting reaction mixture was incubated at 37 °C for 18 h. Another 13 µL of sialic acid aldolase was added and the reaction mixture was further incubated at 37 °C for another 18 h. The reaction mixture was lyophilized, dissolved in water (100 µL), centrifuged and the supernatant subjected to gel filtration over Biogel P-2 (eluent 5% aqueous *n*-butanol). All the fractions containing the desired product were combined and lyophilized to give the C-5 modified sialic acid **4** (12.1 mg, 88%) as amorphous off-white solid. ^{1}H NMR (300 MHz, D_2O): δ 1.88-1.96 (m, 1H, H-3a), 2.30 (dd, *J* = 13.2, 3.4 Hz, 1H, H-3e), 3.60-3.69 (m, 2H, H-7, H-9b), 3.79-3.90 (m, 2H, H-8, H-9a), 4.15-4.27 (m, 3H, H-6, H-5, H-4), 7.93 (d, *J* = 8.8 Hz, 2H, 2 × aromatic CH), 8.12 (d, *J* = 8.9 Hz, 2H, 2 × aromatic CH). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 39.17, 52.93, 63.24, 67.11, 68.40, 69.69, 70.12, 124.70 (2×), 129.00 (2×). HRMS ESI: *m/z* for $[M-H]^-$ $C_{18}H_{19}ClN_3O_{11}^-$, calcd 488.0714, obsd 488.0713.



Scheme S2. Chemical synthesis of Neu9Az **5** and Neu9NH_2 **6** from Neu5Ac.

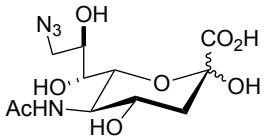
Methyl-5-(acetamido)-9-*O*-tosyl-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonate (S5).



Dowex 50WX8 H^+ resin (1.08 g) was added to a solution of ***N*-acetylneuraminic acid** (**Neu5Ac**) (4 g, 12.93 mmol) in anhydrous methanol (80 mL) and the reaction was left to stir at room temperature under an atmosphere of argon for 48 h. The solution turned clear. The resin was filtered and washed with methanol. The filtrate was concentrated *in vacuo* and azeotropically dried with toluene (4 × 40 mL). *p*-Toluenesulfonyl chloride (2.61 g, 13.71 mmol) was added to a solution of the resulting residue in anhydrous pyridine (40 mL) at 0 °C and the reaction was left to stir, while warming to room temperature, for 48 h. The reaction mixture was concentrated *in vacuo* and azeotropically dried with toluene (5 × 40 mL). The resulting residue was purified by flash chromatography on silica gel (MeOH:EtOAc, 1:16, v:v) to afford **S5** (3.65 g, 59%) as an amorphous white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 1.89 (dd, *J* = 12.7, 11.5 Hz, 1H, H-3a), 2.04

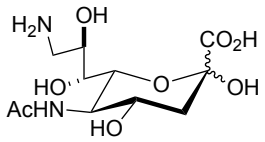
(s, 3H, NAc), 2.23 (dd, *J* = 13.0, 5.0 Hz, 1H, H-3e), 2.49 (s, 3H, Ts-CH_3), 3.46 (dd, *J* = 9.1, 1.2 Hz, 1H, H-7), 3.75 (d, *J* = 10.3 Hz, 1H, H-5), 3.80 (s, 3H, CO_2CH_3), 3.88 (ddd, *J* = 12.4, 6.7, 3.7 Hz, 1H, H-8), 3.96 (dd, *J* = 10.5, 1.3 Hz, 1H, H-6), 4.00-4.10 (m, 2H, H-4, H-9a), 4.31 (dd, *J* = 10.0, 2.2 Hz, 1H, H-9b), 7.47 (d, *J* = 8.1 Hz, 2H, 2 × Ts-aromatic CH), 7.82 (d, *J* = 8.3 Hz, 2H, 2 × Ts-aromatic CH). ^{13}C NMR assigned from HSQC (75 MHz, CD_3OD): δ 19.62, 20.48, 39.39, 51.42, 52.28, 66.04, 67.76 (2×), 69.47, 72.05, 127.07 (2×), 128.79 (2×). In agreement with the literature data.[5]

5-(Acetamido)-9-azido-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (5).



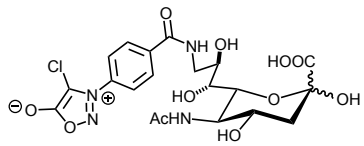
Compound **S5** (723 mg, 1.51 mmol) and sodium azide (404 mg, 6.21 mmol) were dissolved in acetone (8 mL) and water (2 mL). The reaction mixture was refluxed at 70 °C for 18 h. The reaction mixture was cooled to room temperature, concentrated *in vacuo* and the residue was azeotropically dried with toluene (3 × 25 mL). The resulting residue was purified by flash chromatography on silica gel (gradient of 5% → 50% MeOH:DCM) to afford **5** (428 mg, 84%) as an amorphous brownish-green solid. ^{1}H NMR (300 MHz, D_2O): δ 1.85 (dd, *J* = 12.9, 11.2 Hz, 1H, H-3a), 2.08 (s, 3H, NAc), 2.23 (dd, *J* = 12.9, 4.7 Hz, 1H, H-3e), 3.46-3.56 (m, 2H, H-9b, H-7), 3.63 (dd, *J* = 13.1, 2.8 Hz, 1H, H-9a), 3.89-4.07 (m, 4H, H-5, H-8, H-6, H-4). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 21.98, 39.17, 52.07, 53.79 67.11, 68.83 (2×), 69.69. In agreement with the literature data.[5]

5-(Acetamido)-9-amino-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (6).



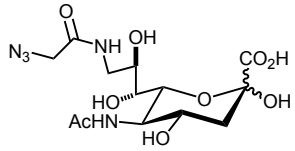
A mixture of **5** (95 mg, 0.28 mmol) and 10% Pd on activated carbon (9.5 mg) in water (3 mL) was stirred for 4 h under an atmosphere of H_2 (1 atm). The reaction mixture was filtered through a syringe filter (cellulose acetate, 0.45 μm) and lyophilized to give **6** (82.2 mg, 94%) as an amorphous yellow solid. ^{1}H NMR (300 MHz, D_2O): δ 1.83 (t, *J* = 12.0 Hz, 1H, H-3a), 2.05 (s, 3H, NAc), 2.22 (dd, *J* = 12.9, 4.5 Hz, 1H, H-3e), 2.69 (dd, *J* = 13.4, 8.5 Hz, 1H, H-9b), 3.08 (dd, *J* = 13.4, 3.0 Hz, 1H, H-9a), 3.43 (d, *J* = 8.9 Hz, 1H, H-7), 3.74 (td, *J* = 8.7, 3.2 Hz, 1H, H-8), 3.87-4.05 (m, 3H, H-5, H-6, H-4). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 21.34, 38.53, 42.83, 52.28, 67.76, 69.47 (2×), 70.33. In agreement with the literature data.[5]

5-(Acetamido)-9-(3-[4-chloro-1,2,3-oxadiazol-3-ium-5-olate]benzamido)-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (7).



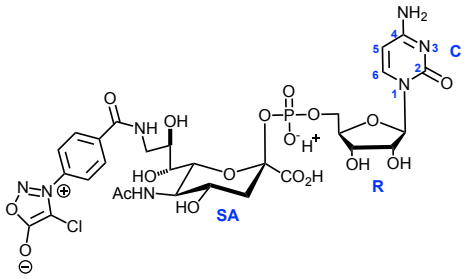
Triethylamine (183 μL, 1.31 mmol) was added to a solution of amine **6** (101 mg, 0.33 mmol) in dry DMF (3.9 mL) and the mixture was left to stir for 10 min, followed by portion-wise addition of NHS-activated chlorosydnone[4] (155 mg, 0.46 mmol). The reaction mixture was left to stir for 18 h at room temperature under an atmosphere of argon. The reaction mixture was concentrated *in vacuo* and the residue was azeotropically dried with toluene (3 × 25 mL). The resulting residue was purified by flash chromatography on silica gel (gradient of 30% → 70% MeOH:DCM) to afford **7** (106 mg, 61%) as an amorphous yellowish off-white solid. ^{1}H NMR (300 MHz, D_2O): δ 1.86 (dd, *J* = 12.7, 11.6 Hz, H-3a), 2.03 (s, 3H, NAc), 2.25 (dd, *J* = 12.9, 4.6 Hz, 1H, H-3e), 3.52-3.59 (m, 2H, H-7, H-9b), 3.82 (dd, *J* = 14.1, 3.1 Hz, 1H, H-9a), 3.95-4.08 (m, 4H, H-5, H-8, H-4, H-6), 7.92 (d, *J* = 8.6 Hz, 2H, 2 × aromatic CH), 8.08 (d, *J* = 8.6 Hz, 2H, 2 × aromatic CH). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 21.98, 39.17, 43.04, 52.07, 66.68, 68.83, 69.69, 70.12, 125.13 (2×), 128.57 (2×). HRMS ESI: *m/z* for $[M+H]^+$ $C_{20}H_{24}ClN_4O_{11}^+$, calcd 531.1125, obsd 531.1127.

5-(Acetamido)-9-azidoacetamido-3,5,9-trideoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (8).



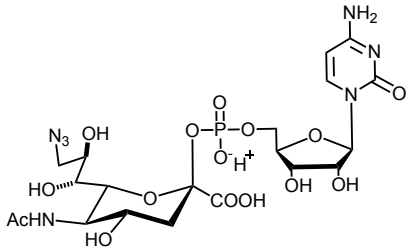
Triethylamine (181 μL, 1.30 mmol) was added to a solution of **6** (100 mg, 0.32 mmol) in dry DMF (4 mL) and the mixture was left to stir for 10 min, followed by the addition of NHS-activated azidoacetic acid (84 mg, 0.42 mmol). The reaction mixture was left to stir for 48 h at room temperature under an atmosphere of argon. The reaction mixture was concentrated *in vacuo* and the residue was azeotropically dried with toluene (3 × 25 mL). The resulting residue was purified by flash chromatography on silica gel (gradient of 20% → 80% MeOH:DCM) to afford **8** (61 mg, 48%) as an amorphous yellowish off-white solid. ^{1}H NMR (300 MHz, D_2O): δ 1.83 (dd, *J* = 12.7, 11.3 Hz, 1H, H-3a), 2.05 (s, 3H, NAc), 2.22 (dd, *J* = 12.9, 4.6 Hz, 1H, H-3e), 3.33 (dd, *J* = 14.1, 7.7 Hz, 1H, H-9b), 3.45 (d, *J* = 9.0 Hz, 1H, H-7), 3.64 (dd, *J* = 14.1, 3.0 Hz, 1H, H-9a), 3.82 (td, *J* = 8.3, 3.2 Hz, 1H, H-8), 3.88-4.06 (m, 5H, H-5, H-6, H-4, CH_2N_3). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 21.98, 39.17, 42.61, 51.64, 52.07, 67.11, 68.83, 69.26, 69.69. In agreement with the literature data.[8]

Cytidine-5'-yl-5-(acetamido)-9-(3-[4-chloro-1,2,3-oxadiazol-3-ium-5-olate]benzamido)-3,5,9-trideoxy-β-D-*glycero*-D-*galacto*-non-2-ulopyranosid-2"-yl phosphate (9).



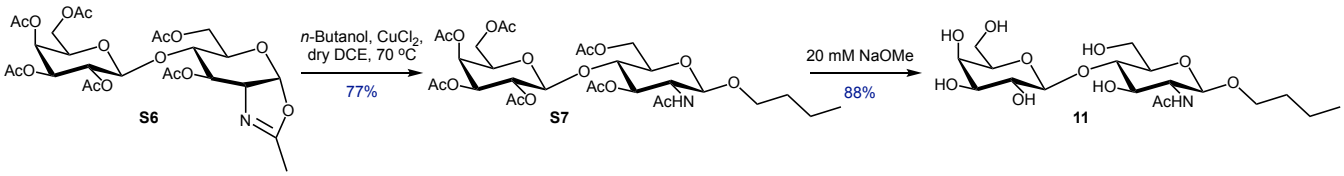
Sialic acid derivative **7** (4.1 mg, 7.72 μmol) and CTP (4.3 mg, 8.11 μmol) were dissolved in Tris-HCl buffer (910 μL, 100 mM, pH 8.3) containing $MgCl_2$ (38.6 μL, 20 mM). To this, inorganic pyrophosphatase from S. *cerevisiae* (IPPase) (3.1 μL, 310 mU) and CMP-sialic acid synthetase from *N. meningitis* (13.3 μL, 133 mU) were added to achieve an 8 mM final concentration of the monosaccharide **7**. The resulting reaction mixture was incubated at 37 °C for 18 h. CIAP (15 μL, 15 U) was added to the reaction and left to incubate further at 37 °C for 3 h. The reaction was mixture was lyophilized, dissolved in 5% aq. *n*-butanol (70 μL), centrifuged and the supernatant subjected to gel filtration over Biogel P-2 (eluent 5% aq. *n*-butanol). Fractions containing the product were combined and lyophilized to give the CMP-Neu9NSydCl **9** (3.1 mg, 51%) as amorphous white solid. ^{1}H NMR (300 MHz; D2O): δ 1.67 (ddd, *J* = 13.1, 11.3, 6.0 Hz, 1H, SA H-3a), 2.04 (s, 3H, NAc), 2.50 (dd, *J* = 13.2, 4.6 Hz, 1H, SA H-3e), 3.37-3.47 (m, 2H, SA H-9b, SA H-7), 4.02-3.93 (m, 2H, SA H-9a, SA H-5), 4.13-4.05 (m, 2H, SA H-8, SA H-4), 4.24-4.18 (m, 5H, SA H-6, R H-5a, R H-5b, R H-4), 4.33-4.29 (m, 2H, R H-2, R H-3), 5.93 (d, *J* = 4.1 Hz, 1H, R H-1), 6.21 (d, *J* = 7.8 Hz, 1H, C H-6), 7.89 (d, *J* = 8.8 Hz, 2H, 2 × aromatic CH), 8.07-8.02 (m, 3H, 2 × aromatic CH, C H-5). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 21.98, 40.89, 42.61, 51.64, 64.96, 67.97 (2×), 70.12, 71.41, 73.99, 83.44, 88.60, 95.48, 124.70 (2×), 128.57 (2×), 143.18. HRMS ESI: *m/z* for $[M-H]^-$ $C_{29}H_{34}ClN_7O_{18}P^-$, calcd 834.1392, obsd 834.1392.

Cytidine-5'-yl-5-(acetamido)-9-azido-3,5,9-trideoxy-β-D-*glycero*-D-*galacto*-non-2-ulopyranosid-2"-yl phosphate (10).



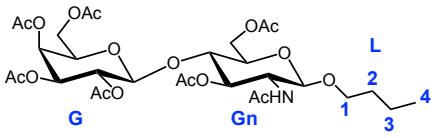
Sialic acid derivative **8** (5.9 mg, 17.65 μmol) and CTP (9.8 mg, 18.53 μmol) were dissolved in Tris-HCl buffer (829.4 μL, 100 mM, pH 8.3) containing $MgCl_2$ (35.3 μL, 20 mM). To this, inorganic pyrophosphatase from S. *cerevisiae* (IPPase) (3.1 μL, 310 mU) and CMP-sialic acid synthetase from *N. meningitis* (14.7 μL, 147 mU) were added to achieve a 20 mM final concentration of the monosaccharide **8**. The resulting reaction mixture was incubated at 37 °C for 18 h. CIAP (27 μL, 27 U) was added to the reaction and left to incubate further at 37 °C for 2.5 h. The reaction was mixture was lyophilized, dissolved in 5% aq. *n*-

butanol (70 µL), centrifuged and the supernatant subjected to gel filtration over Biogel P-2 (eluent 5% aq. *n*-butanol). Fractions containing the product were combined and lyophilized to give the CMP-Neu9Az **10** (7.2 mg, 64%) as amorphous white solid. ^{1}H NMR (300 MHz; D_2O): δ 1.65 (ddd, *J* = 13.2, 11.2, 5.8 Hz, 1H, SA H-3a), 2.06 (s, 3H, NAc), 2.50 (dd, *J* = 13.3, 4.7 Hz, 1H, SA H-3e), 3.54-3.46 (m, 2H, SA H-7, SA H-9b), 3.65 (dd, *J* = 13.1, 2.5 Hz, 1H, SA H-9a), 4.09-3.92 (m, 3H, SA H-5, SA H-4, SA H-8), 4.14 (d, *J* = 10.4 Hz, 1H, SA H-6), 4.36-4.18 (m, 6H, R H-5, R H-4, R H-2, R H-3), 5.97 (d, *J* = 3.5 Hz, 1H, R H-1), 6.27 (d, *J* = 7.8 Hz, 1H, C H-6), 8.16 (d, *J* = 7.9 Hz, 1H, C H-5). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): δ 21.98, 40.89, 51.64, 52.93, 64.53, 67.54 (2×), 68.83, 69.69, 70.98, 73.99, 82.58, 88.60, 96.34, 141.46. In agreement with the literature data.[9]



Scheme S3. Chemical synthesis of LacNAc-butyl **11**.

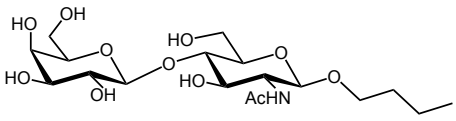
Butyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→4)-O-2-deoxy-2-acetamido-3,6-di-O-acetyl-β-D-glucopyranoside (S7).



n-Butanol (550 µL, 6.02 mmol) was added to a solution of oxazoline **S6**[10] (372 mg, 0.602 mmol) in dry DCE (2.6 mL), followed by the addition of $CuCl_2$ (81 mg, 0.602) and the reaction stirred at 70 °C for 1 h and then at room temperature for 18 h. The reaction mixture was diluted with acetone and aq. $NaHCO_3$, filtered through celite, and the filtrate concentrated *in vacuo*. The crude product was extracted from the remaining aqueous solution with DCM (4 × 50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting residue was purified by flash chromatography on silica gel (petroleum ether:acetone, 11:9, v:v), to give the Ac_6LacNAc-butyl **S7** (321 mg, 77%). ^{1}H NMR (300 MHz; $CDCl_3$): δ 0.87 (t, *J* = 7.3 Hz, 3H, L H-4), 1.37-1.27 (m, 2H, L H-3), 1.55-1.46 (m, 2H, L H-2), 1.95 (d, *J* = 3.3 Hz, 6H, OAc, NAc), 2.04 (d, *J* = 4.8 Hz, 9H, 3 × OAc), 2.09 (s, 3H, OAc), 2.13 (s, 3H, OAc), 3.45-3.37 (m, 1H, L H-1a), 3.61 (ddd, *J* = 8.5, 5.3, 3.0 Hz, 1H, Gn H-5), 3.89-3.74 (m, 3H, Gn H-4, L H-1b, G H-5), 4.14-3.96 (m, 4H, Gn H-2, G H-6a, G H-6b, Gn H-6a), 4.50-4.41 (m, 3H, Gn H-1, Gn H-6b, G H-1), 4.96 (dd, *J* = 10.5, 3.4 Hz, 1H, G H-3), 5.08 (m, 2H, Gn H-2, G H-2), 5.34 (d, *J* = 2.4 Hz, 1H, G H-4), 5.71 (d, *J* = 9.4 Hz, 1H, Gn NH). ^{13}C NMR assigned from HSQC (75 MHz, $CDCl_3$):

13.39, 18.54, 20.26 (5×), 23.27, 31.01, 52.93, 60.66, 62.38, 66.25, 68.83, 69.26, 70.55 (2×), 72.27 (2×), 75.28, 100.63. HRMS ESI: *m/z* for $[M+H]^+$ $C_{30}H_{46}NO_{17}^+$, calcd 692.2760, obsd 692.2750.

Butyl (β-D-galactopyranosyl)-(1→4)-O-2-deoxy-2-acetamido-β-D-glucopyranoside (11).



Peracetylated butyl-linkered LacNAc **S7** (320 mg, 0.463 mmol) was dissolved in 14.6 mL of a 20 mM sodium methoxide solution and left to stir at RT for 3 h. The reaction mixture was neutralized by the addition of Dowex 50WX8 H^+ resin, filtered and concentrated under reduced pressure to yield the desired butyl-linkered LacNAc **11** (180 mg, 88%) as an amorphous white solid. ^{1}H NMR (300 MHz; D_2O): δ 0.86 (t, *J* = 7.4 Hz, 3H, L H-4), 1.30 (dq, *J* = 14.5, 7.3 Hz, 2H, L H-3), 1.55-1.46 (m, 2H, L H-2), 2.00 (s, 3H, NAc), 3.91-3.48 (m, 13H, G H-2, Gn H-5, L H-1a, G H-3, Gn H-4, Gn H-3, G H-5, Gn H-2, G H-6a, G H-6b, Gn H-6a, L H-1b, G H-4), 3.96 (dd, *J* = 12.3, 2.2 Hz, 1H, Gn H-6b), 4.45 (d, *J* = 7.7 Hz, 1H, G H-1), 4.50 (d, *J* = 7.9 Hz, 1H, Gn H-1). ^{13}C NMR assigned from HSQC (75 MHz, D_2O): 12.85, 18.22, 21.88, 30.47, 54.97, 59.91, 60.77, 68.51, 70.23, 70.87, 72.38 (2×), 74.31, 75.17, 78.18, 100.96, 102.68. HRMS ESI: *m/z* for $[M-H]^-$ $C_{18}H_{32}NO_{11}^-$, calcd 438.1981, obsd 438.1989.

ManNSydCl and ManNAz-PMP stability study

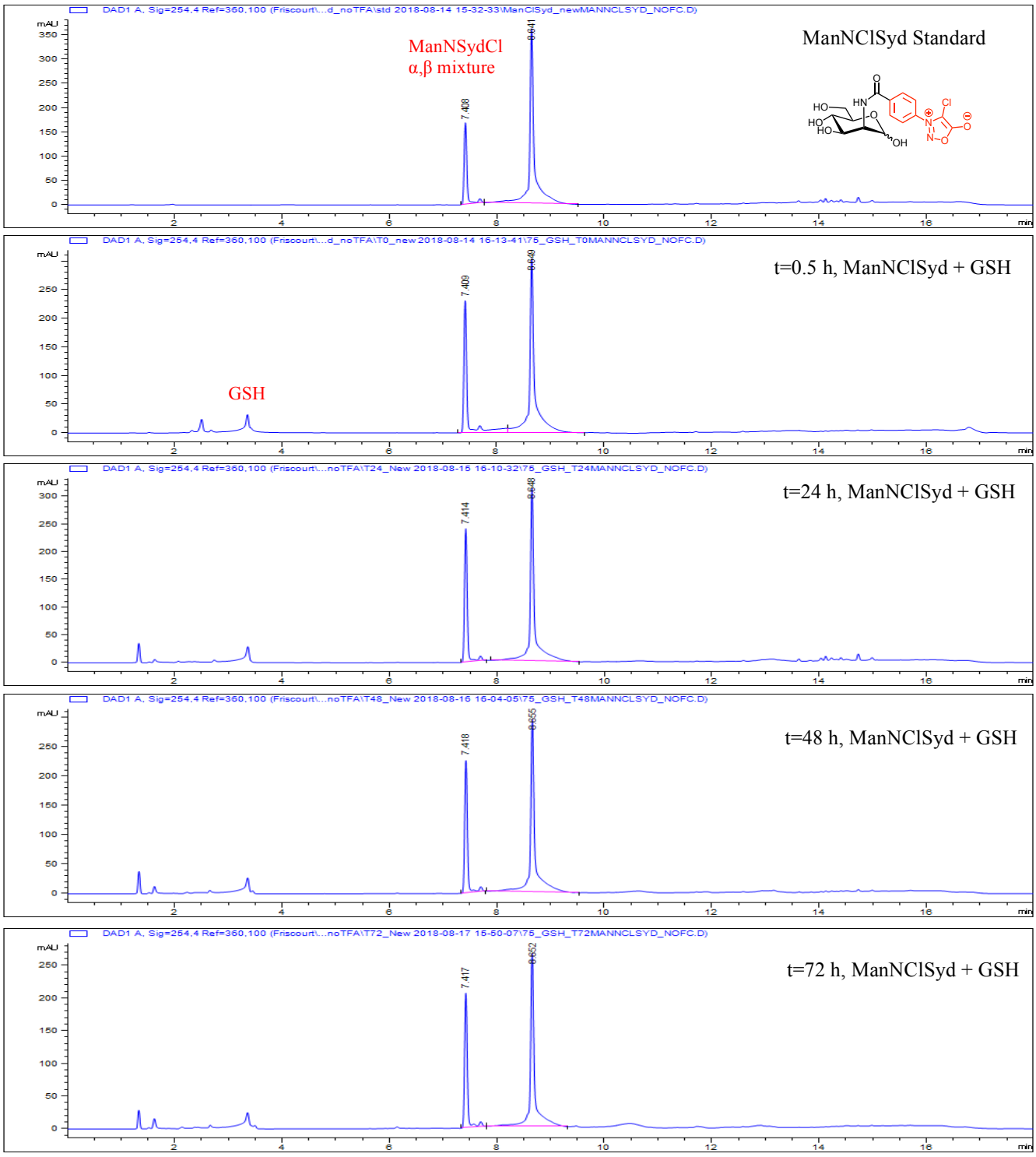


Figure S1. HPLC traces visualized at λ=254 nm of a 1:100 mM solution of ManNSydCl **1** : glutathione in degassed DI H_2O incubated at 37 °C. At various time points (0, 0.5, 24, 48 and 72 h), 10 µL aliquots were analyzed by RP-HPLC using a gradient of MeOH in water (0→50% over 10 min).

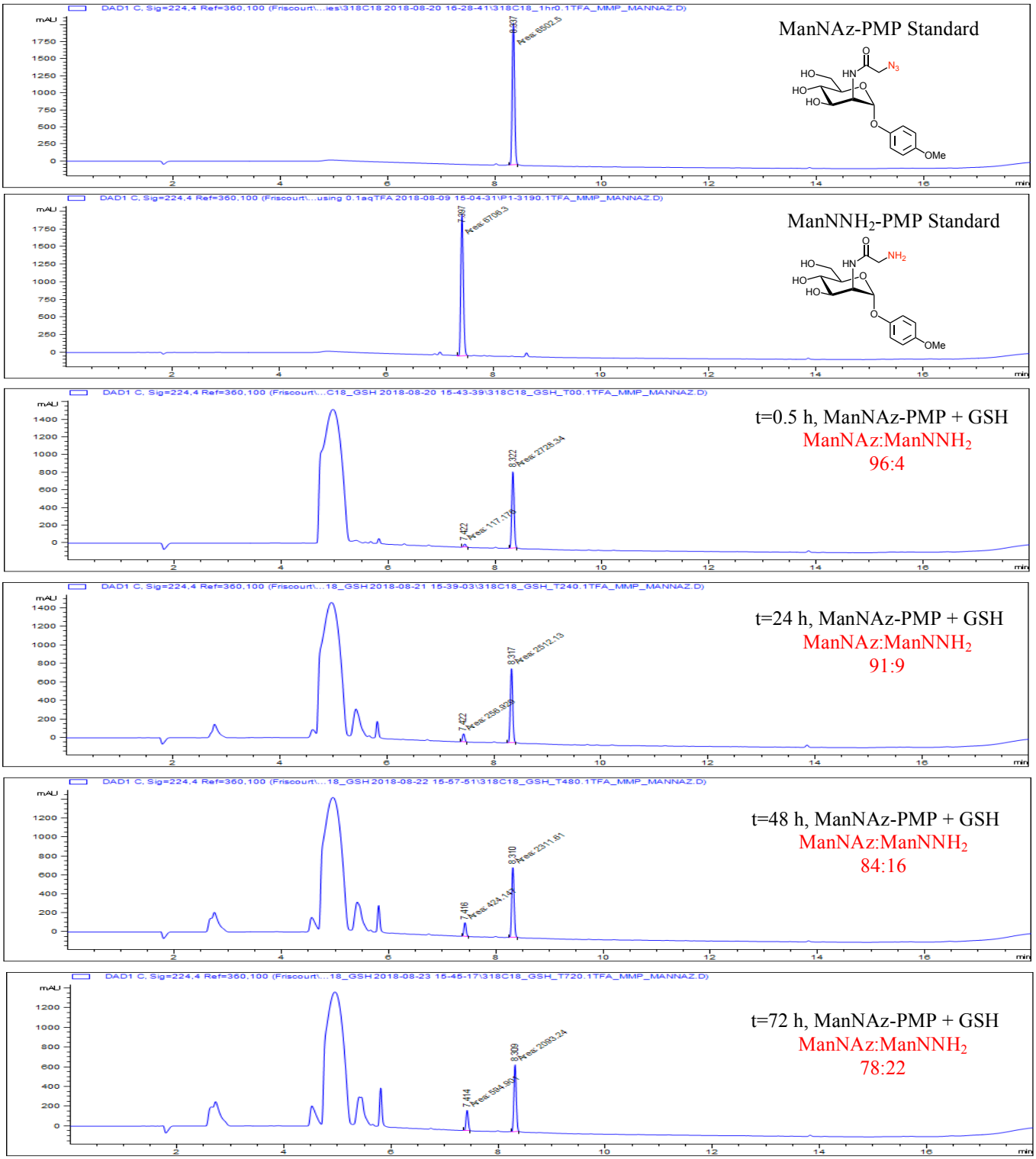


Figure S2. HPLC traces visualized at λ=224 nm of a 1:100 mM solution of ManNAz-PMP **2** : gluthathione in degassed DI H_2O incubated at 37 °C. At various time points (0, 0.5, 24, 48 and 72 h), 10 µL aliquots were analyzed by RP-HPLC using a gradient of MeOH in 0.1% TFA/water (0→85% over 10 min).

Kinetics measurements

The rate measurements of cycloaddition various cyclooctynes (MeO-DIBO, MeO-DIBAC, BCN) with 4-chloro-3-phenylsydnone were conducted by using ^{1}H NMR spectroscopy (Brüker 400 MHz) at 25 °C. A 20 mM solution of the sydnone (0.2 mL) in CD_3OD was added to a thermally equilibrated solution of cyclooctynes (20 mM, 0.2 mL) in CD_3OD, leading to a mixture of both reactants in 1:1 ratio with a respective concentration of 6.66 mM. Reactions were monitored by following the decay of characteristic peaks of the cyclooctynes and the sydnones as well as the formation of characteristic pyrazole peaks. Consumption of starting materials followed a second-order equation and the second-order rate constants were obtained by least squares fitting of the data to a linear equation.

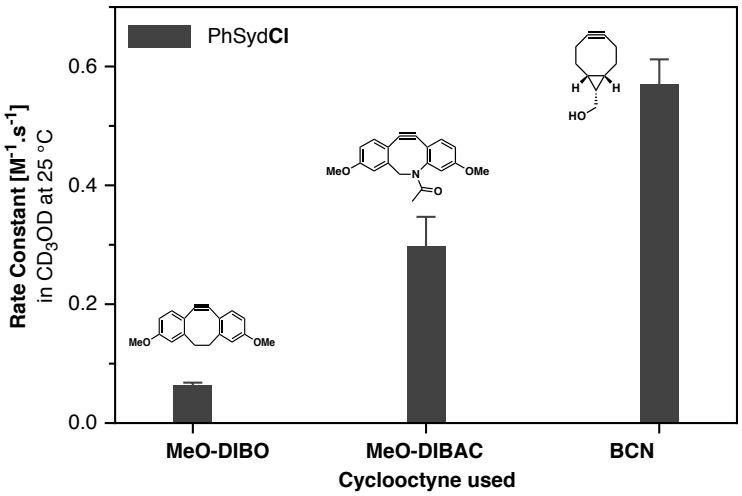


Figure S3. General representation of rate constants of cycloaddition reactions between cyclooctynes and 4-chloro-3-phenylsydnone (PhSydCl).

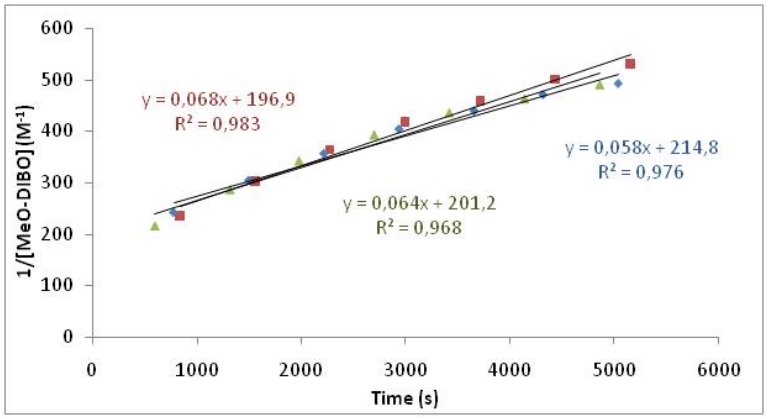


Figure S4. Kinetics measurement plots of MeO-DIBO and 4-chloro-3-phenylsydnone. Decay of MeO-DIBO: 2.22-2.39 (m, CH_2), 6.92 (d, 2 × CHar) and formation of the pyrazole product: 3.75 (s, 2×OCH_3), 6.87 (d, CHar).

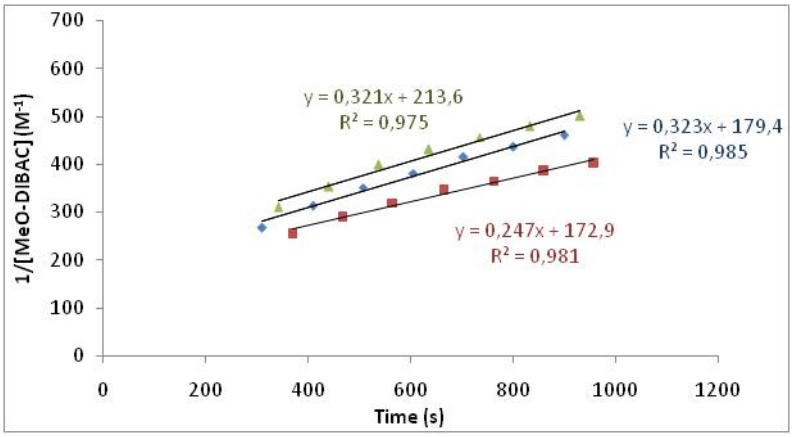


Figure S5. Kinetics measurement plots of MeO-DIBAC and 4-chloro-3-phenylsydnone. Decay of MeO-DIBAC: 1.765-1.825 (m, CH_3) and formation of the pyrazole product: 1.58-1.65 (2×s, CH_3), 4.36-4.52 (2×d, CH_2), 5.86-6.08 (2×d, CH_2).

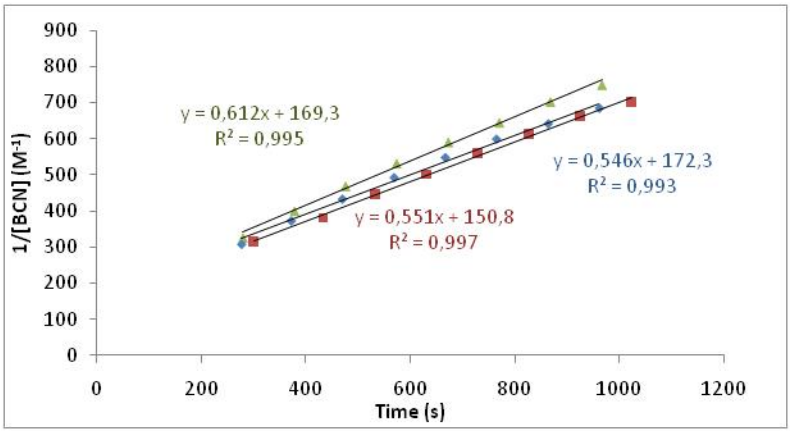


Figure S6. Kinetics measurement plots of BCN and 4-chloro-3-phenylsydnone. Decay of BCN: 0.82-0.96 (m, CH_2) and sydnone: 7.65-7.82 (m, 5×CHar) and formation of the pyrazole product: 2.47-2.59 (m, CH), 7.39-7.56 (m, 5×CHar).

Cell culture

U2OS and A549 cells were obtained from ATTC. Both cells lines were maintained in DMEM high glucose (4.5 g/L) (GIBCO) supplemented with 10% of foetal bovine serum (FBS, Dominique Dutscher), glutamine (2 mM), penicillin (Sigma, 100 µg/L) and streptomycin (Sigma, 100 µg/L), at 37 °C, 5% CO_2, in a humidified atmosphere. Mycoplasma contamination check was carried out using the VenorGeM Kit (Minerva Biolabs GmbH, Germany). When indicated, sugars were added to media at the indicated concentration (2 mM) for 2 days. Cell number was determined using the TC20 Automated Cell Counter (Bio-Rad) according to the manufacturer's instructions.

Cell-surface glycan labeling and imaging by confocal microscopy

1.5×10^6 Cells (U2OS or A549) were grown in 12-well plates on glass coverslips with the respective sugars for 48 h. Thereafter, cells were washed three times with PBS and incubated with BCN-biotin conjugate (50 µM) in DMEM (without phenol red, glucose 4.5 g/L, FBS 10%) during 1 h at room temperature. Then, cells were washed four times with PBS and stained with Streptavidin-Alexa 568 (5 µg/mL) in DMEM (without phenol red, glucose 4.5 g/L, FBS 10%) during 1 h at room temperature. After three washes with cold PBS at 4 °C, cells were fixed using paraformaldehyde 4% in PBS during 30 min at room temperature. Finally, the slides were mounted using Prolong Diamond with DAPI (Thermo Fisher) and samples were imaged using a Leica TCS SP8 confocal microscope (Figure 2 and Figure S7).

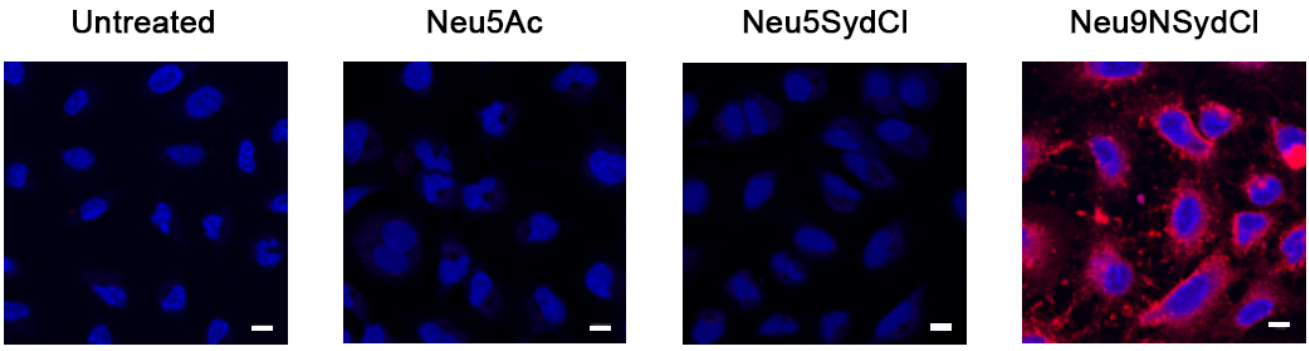


Figure S7. Metabolic cell-surface glycan labeling of living A549 cells. Fluorescence microscope images of A549 cells cultured with Neu5Ac (2 mM), Neu5SydCl (**4**) (2 mM), Neu9NSydCl (**7**) (2 mM) or no sugar (untreated) for 48 h, treated with BCN-biotin conjugate (50 µM) for 1 h and stained with Streptavidin-AlexaFluor568 (Red), fixed and mounted in the presence of DAPI (Blue), Scale bars: 10 µm.

Flow cytometry

2.5×10^5 cells per well (U2OS) were seeded in 6-well plates. After the treatment, cells were washed four times with PBS and incubated with BCN-biotin conjugate (50 µM) in DMEM (without phenol red, glucose 4.5 g/L, FBS 10%) during 1 h at room temperature. Then, cells were washed four times with PBS and stained with Streptavidin-Alexa 568 (5 µg/mL) in DMEM (without phenol red, glucose 4.5 g/L, FBS 10%) during 1 h at room temperature. After three washes with PBS, cells were detached using trypsin/EDTA and collected with fresh medium. After centrifugation at 300g during 5 minutes, cells were resuspended in 300 µL of resuspension buffer (BSA 0.1%, EDTA 1 mM in PBS). Then, cells were analyzed using a BDFACS Canto BD LSRFortessa (BD Biosciences) flow cytometer. The analysis of the data was performed using the free software FACSDiva (Figure 2B and Figure S8).

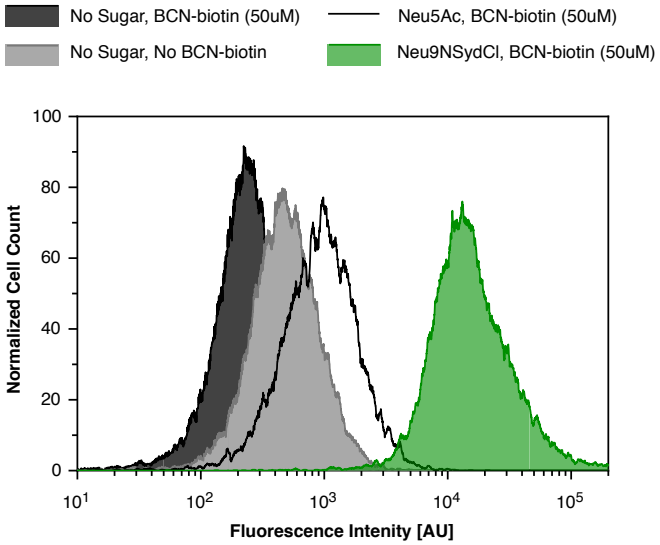


Figure S8. Flow cytometry analysis of U2OS cells cultured respectively with No Sugar, Neu5Ac and Neu9NSydCl (**7**) and treated or not with BCN-biotin. All samples were however stained with Streptavidin-AlexaFluor568. This data highlights that potential unspecific fluorescence due to BCN treatment is within autofluorescence background.

Cell viability

Cell viability was assessed using the Cell Titer-Blue Cell Viability Assay (Promega). Briefly, 1×10^4 cells per well (U2OS or A549) were seeded in triplicate in 96-well plates. After feeding the cells with the respective sugars (2 mM), 20 µL of the reagent was added to each well and the plate was incubated for 2 h at 37 °C, 5% CO_2 in a humidified atmosphere. Fluorescence was recorded at 560/590nm with a

Tristar2 LB942 device (Berthold) to determine the cell viability. Etoposide (10 μM) was used as a positive control for cell death induction (Figure S9-S10).

Statistics

The results are expressed as a mean ± s.e.m. of at least three independent experiments. One-way ANOVA followed by Bonferroni's comparison as a post hoc test was used to evaluate the statistical difference of the results. Statistical significance was estimated when $P < 0.01$.

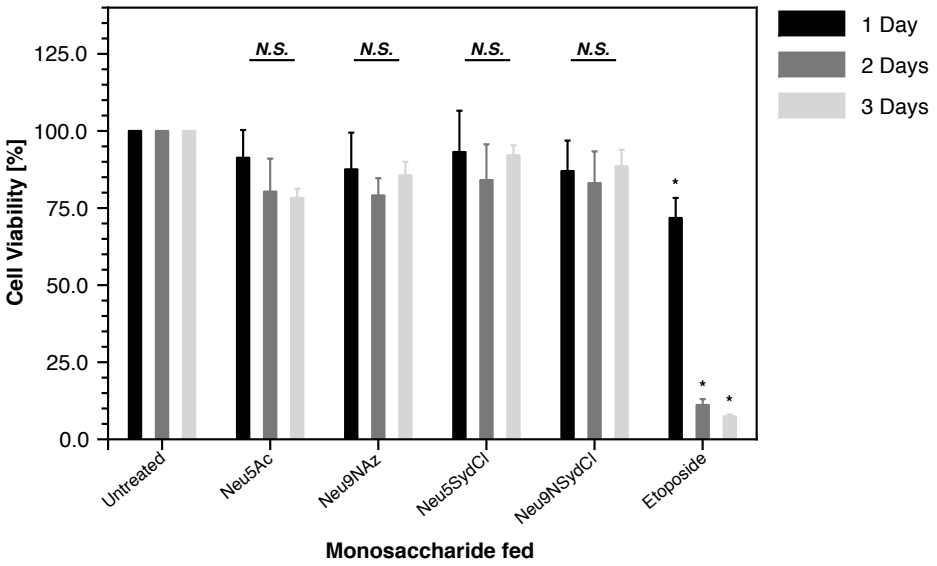


Figure S9. U2OS Cell viability assay with various sialic acid monosaccharides (2 mM). * $P < 0.01$, *N.S.* not significant.

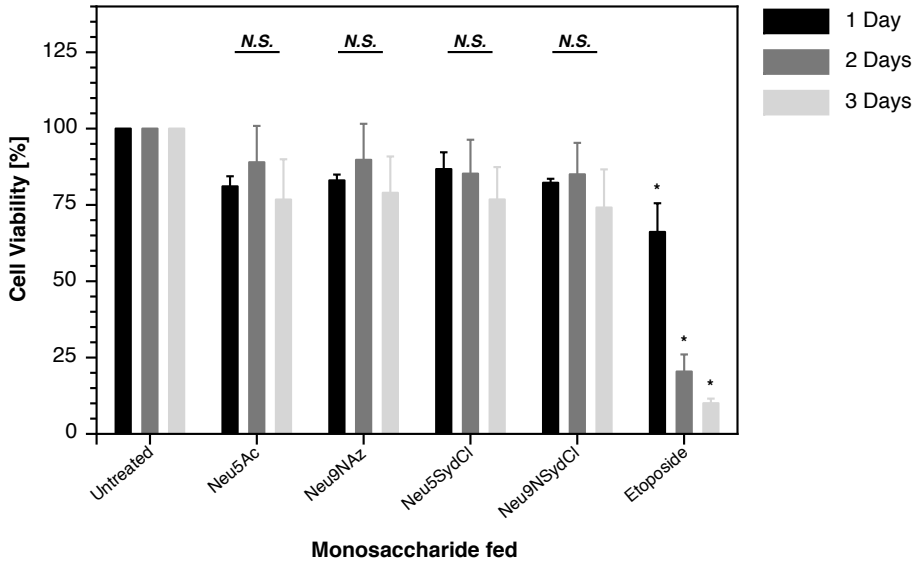


Figure S10. A549 Cell viability assay with various sialic acid monosaccharides (2 mM). * $P < 0.01$, *N.S.* not significant.

Enzymatic-^{1}H-NMR coupled assay for the detection of CMP-sialic acid formation

Inorganic Pyrophosphatase (16.74 mU/μmol CTP) and CMP-sialic acid synthetase (8.36 mU/μmol sialic acid derivative) were added to a solution of sialic acid derivatives **Neu5Ac**, **Neu5SydCl** (**4**), **Neu9NSydCl** (**7**) and **Neu9NAz** (**8**) (6.78 μmol – 14.23 μmol) and CTP (1.05 eq) in Tris buffer (100 mM, pH 8.93) containing $MgCl_2$ (20 mM) to achieve a 20 mM final concentration of the sialic acid derivative. The resulting reaction mixture was incubated at 37 °C for 5 h. The mixture was centrifuged and the supernatant was lyophilized. The lyophilized reaction mixture was dissolved in 400 μL of D_2O and immediately analyzed by ^{1}H NMR (Figure 3 and Figure S11-S14).

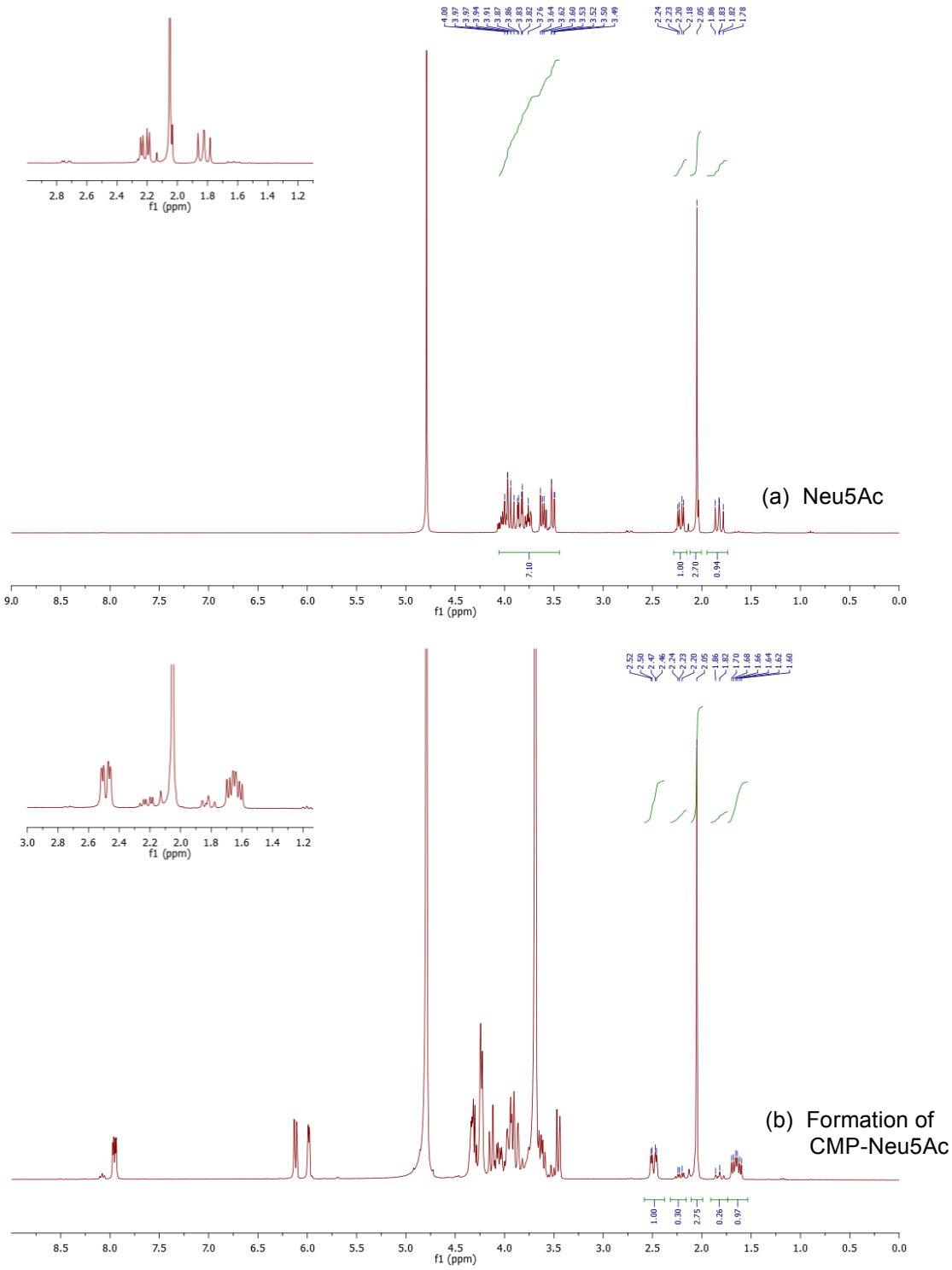


Figure S11. [1]H-NMR spectrum of (a) starting Neu5Ac and (b) crude mixture of CMP-Neu5Ac formation after 5 h of reaction. C-3 proton shifts for Neu5Ac (1.89 ppm (H-3a) and 2.23 ppm (H-3e)). C-3 proton shifts for CMP-Neu5Ac (1.65 ppm (H-3a) and 2.49 ppm (H-3e)).

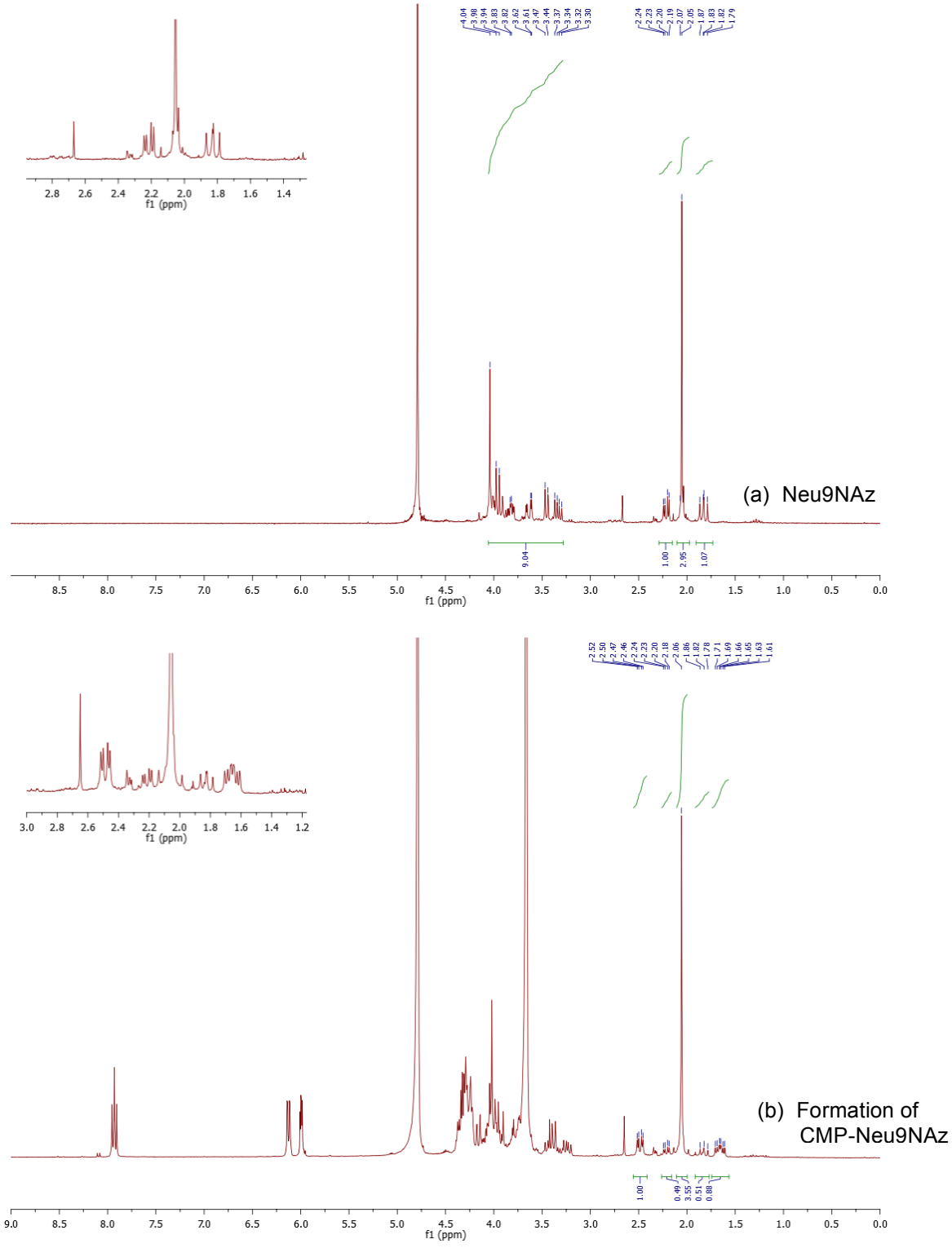
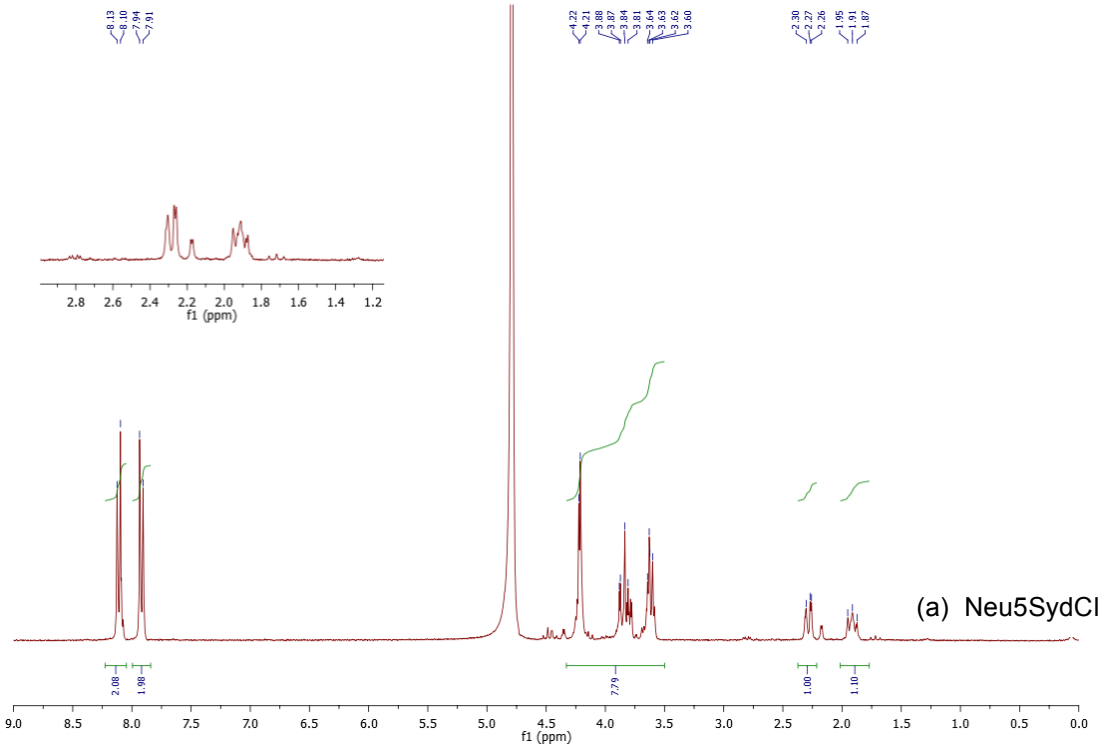
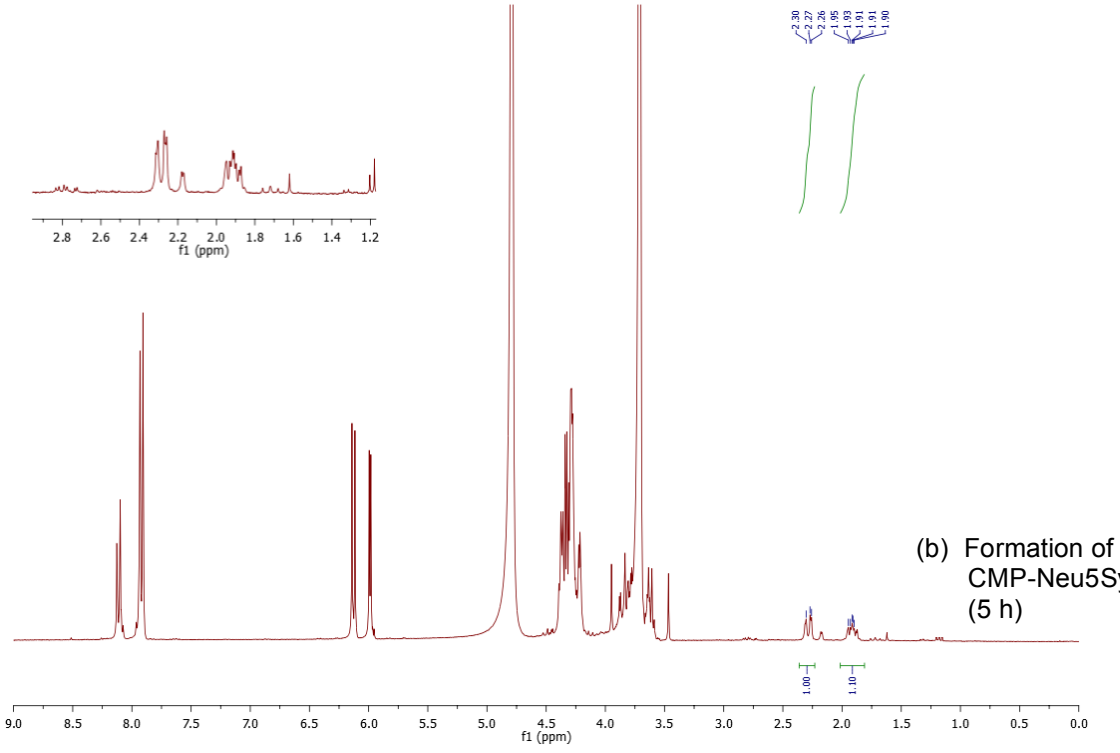


Figure S12. ^{1}H-NMR spectrum of (a) starting neuraminic acid Neu9NAz (**8**) and (b) crude mixture of CMP-Neu9NAz formation after 5 h of reaction. C-3 proton shifts for Neu9NAz (1.83 ppm (H-3a) and 2.23 ppm (H-3e)). C-3 proton shifts for CMP-Neu9NAz (1.66 ppm (H-3a) and 2.48 ppm (H-3e)).



(a) Neu5SydCl



(b) Formation of CMP-Neu5SydCl (5 h)

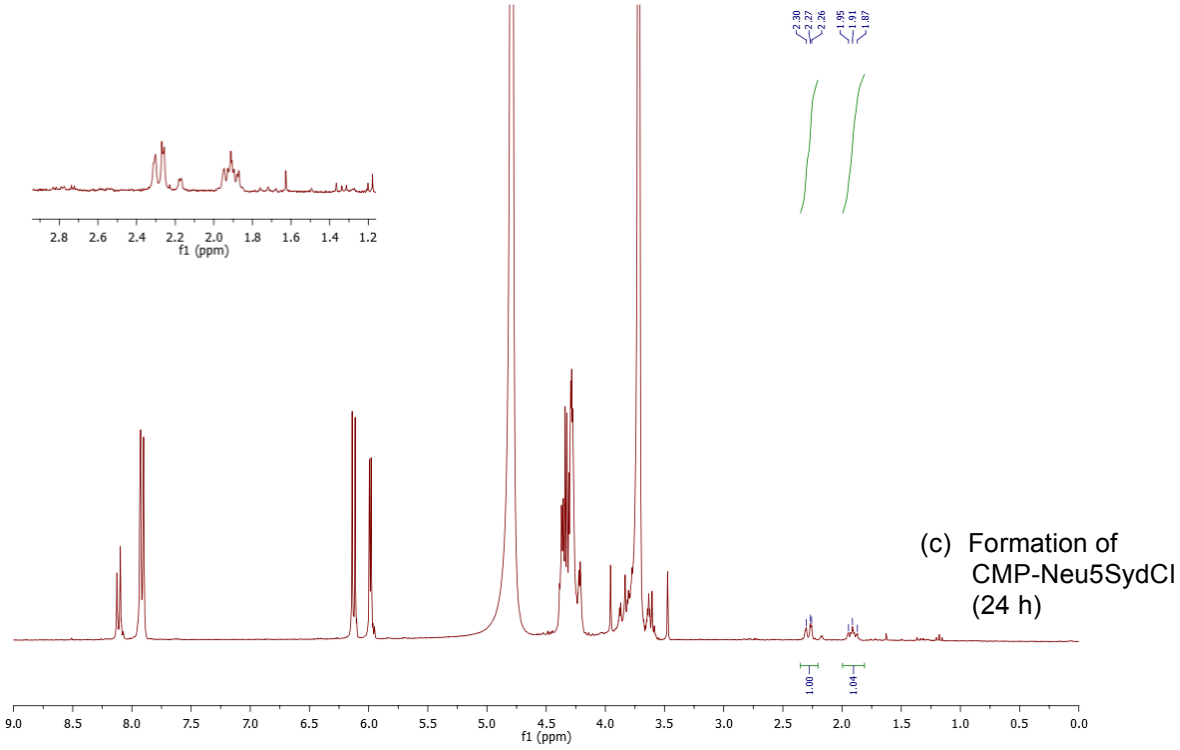


Figure S13. ^{1}H-NMR spectrum of (a) starting neuraminic acid Neu5SydCl (**4**), (b) crude mixture of CMP-Neu5SydCl formation after 5 h of reaction and (c) after 24 h of reaction. C-3 proton shifts for Neu5SydCl (1.92 ppm (H-3a) and 2.2 ppm (H-3e)). C-3 proton shifts for CMP-Neu5SydCl (Same as Neu5SydCl (No product formed)).

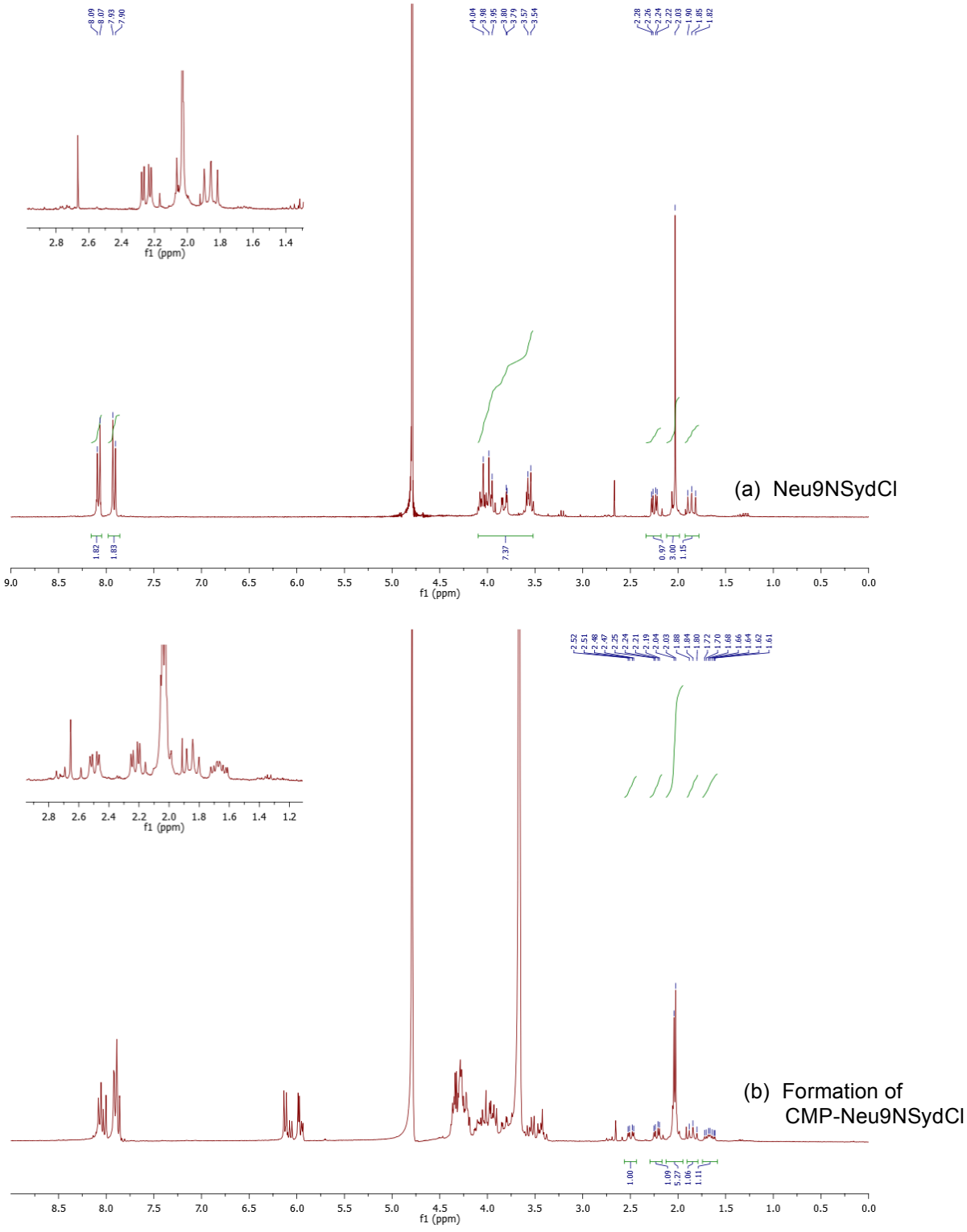


Figure S14. [1]H-NMR spectrum of (a) starting neuraminic acid Neu9NSydCl (**7**), (b) crude mixture of CMP-Neu9NSydCl formation after 5 h of reaction. C-3 proton shifts for Neu9NSydCl (1.86 ppm (H-3a) and 2.25 ppm (H-3e)). C-3 proton shifts for CMP-Neu9NSydCl (1.67 ppm (H-3a) and 2.50 ppm (H-3e)).

Expression of ST6Gal-I and ST3Gal-IV

Human ST6Gal-I and ST3Gal-IV were generated as secreted fusion proteins in HEK293 cells and purified as previously described.[11]

Kinetic studies of ST6Gal-I and ST3Gal-IV

Kinetic analysis of **ST6Gal-I** with CMP-Neu5Ac derivatives were performed in a 50 μL reaction volume of MES buffer (100 mM, pH 6.5) containing 0.32 μg of recombinant human ST6Gal-I, LacNAc-butyl **11** (2.4 mM), CD73 (0.5 ng/μL) and CMP-Neu5Ac (0.025-1 mM), CMP-Neu9Az **10** (0.025-1 mM) or CMP-Neu9NSydCl **9** (0.025-1 mM). Enzyme assay mixtures were incubated for 30 min at 37 °C and the reaction was stopped by adding 10 μL of malachite green phosphate detection reagent A, incubation for 10 min at 25 °C, followed by addition of 10 μL reagent B. After 20 min at 25 °C, the absorbance was read using a microplate reader at 620 nm and compared to equivalent analyses for a phosphate standard curve. Control reactions containing all assay components except ST6Gal-I were performed in parallel for all enzyme assays as a reaction blank and absorbance values were subtracted from all enzyme reactions. ST6Gal-I enzyme activity values (nmol/min/μg) were determined at varied donor concentrations, and kinetic data were fit using KaleidaGraph (Version 4.5) to determine $K_{M(app)}$ and $k_{cat(app)}$ values (Table 1 and Figure S15). For each graph, all data points were collected in triplicates. In addition, two individual experiments were performed (each graph pair).

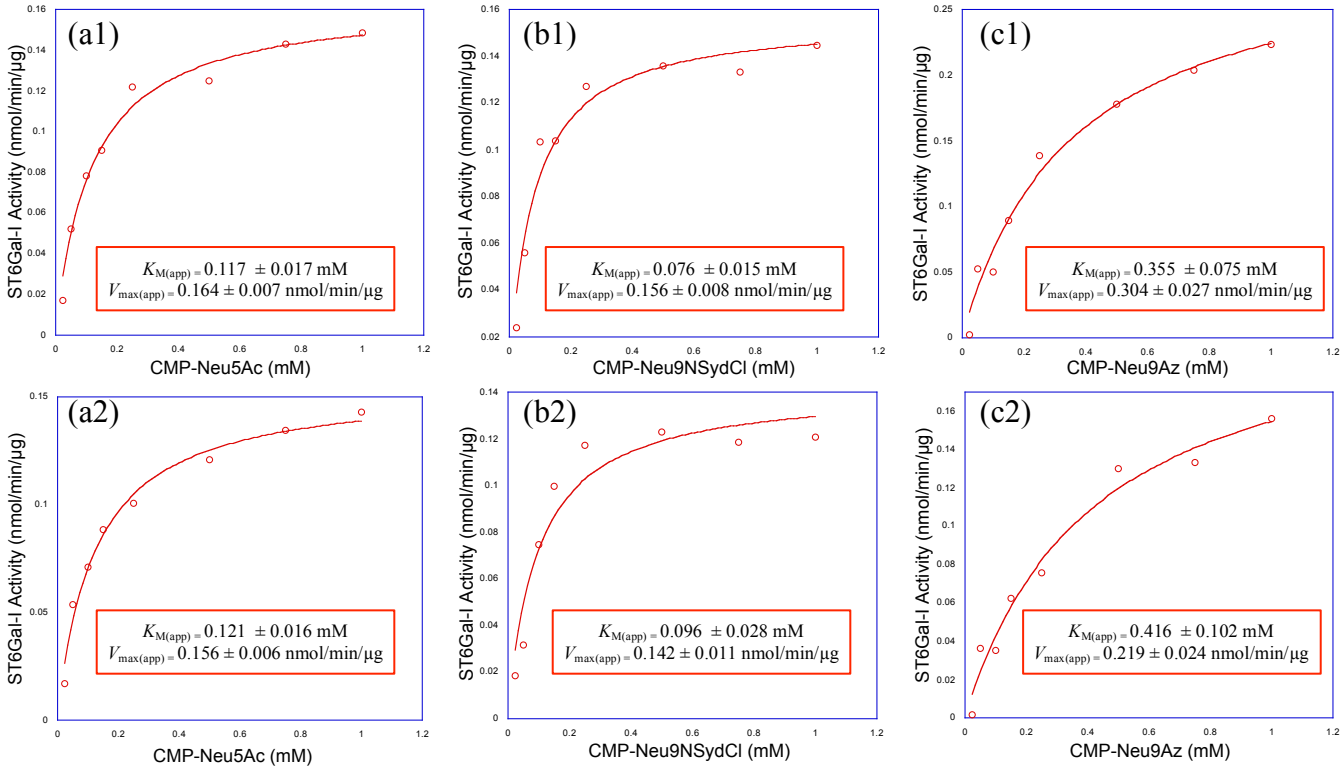


Figure S15. Kinetic analysis of ST6Gal-I with (a1-2) CMP-Neu5Ac, (b1-2) CMP-Neu9NSydCl (**9**) and (c1-2) CMP-Neu9Az (**10**) as the sugar donor and LacNAc-butyl as acceptor. $V_{max(app)}$ and $K_{M(app)}$ were determined by fitting the data to the Michaelis–Menten equation. For each graph, all data points were collected in triplicates. Each graph pair is representative of two individual experiments.

Kinetic analysis of **ST3Gal-IV** with CMP-NeuAc derivatives were performed in a 50 µL reaction volume of MES buffer (100 mM, pH 6.5) containing 0.96 µg of recombinant ST3Gal-IV, LacNAc-butyl **11** (2.4 mM), CD73 (0.5 ng/µL) and CMP-Neu5Ac (0.025-1 mM), CMP-Neu9Az **10** (0.025-1 mM) or CMP-Neu9NSydCl **9** (0.025-1 mM). Enzyme assay mixtures were incubated for 60 min at 37 °C and the reaction was stopped by adding 10 µL of malachite green phosphate detection reagent A, incubation for 10 min at 25 °C, followed by addition of 10 µL reagent B. After 20 min at 25 °C, the absorbance was read using a microplate reader at 620 nm and compared to equivalent analyses for a phosphate standard curve. Control reactions containing all assay components except ST3Gal-IV were performed in parallel for all enzyme assays as a reaction blank and absorbance values were subtracted from all enzyme reactions. ST3Gal-IV enzyme activity values (nmol/min/µg) were determined at varied donor concentrations, and kinetic data were fit using KaleidaGraph (Version 4.5) to determine $K_{M(app)}$ and $k_{cat(app)}$ values (Table 1 and Figure S16). For each graph, all data points were collected in triplicates. In addition, two individual experiments were performed (each graph pair).

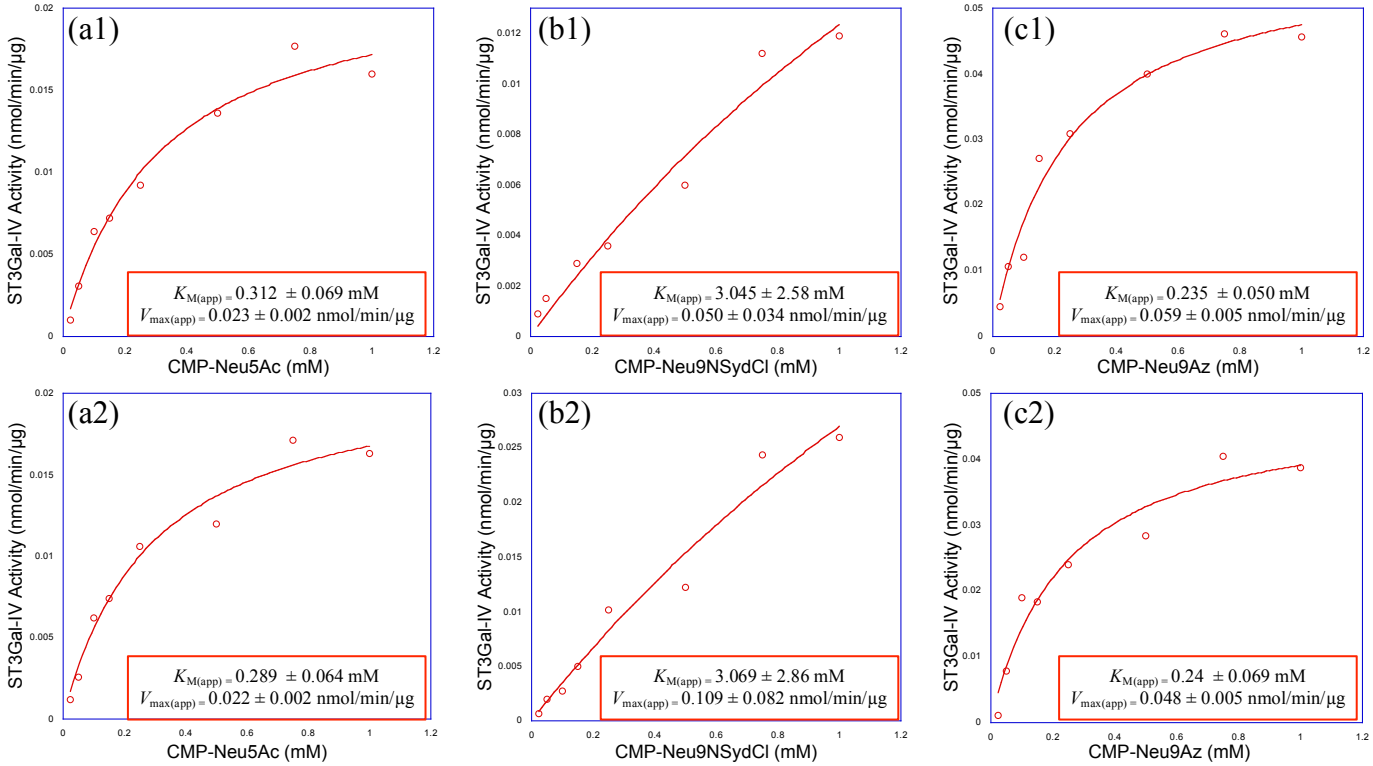


Figure S16. Kinetic analysis of ST3Gal-IV with a) CMP-Neu5Ac, b) CMP-Neu9NSydCl (**9**) and c) CMP-Neu9Az (**10**) as the sugar donor and LacNAc-butyl as the acceptor. V_{max} and K_M were determined by fitting the data to the Michaelis–Menten equation. For each graph, all data points were collected in triplicates. Each graph pair is representative of two individual experiments.

Enzymatic sialylation of asialofetuin.

To a solution of asialofetuin (0.5 mg) and CMP-sialic acid derivatives (0.25 mg of Neu5Ac, 0.35 mg of Neu9NSydCl **9**, 0.25 mg of Neu9Az **10**) in sodium cacodylate buffer (196.2 µL, 50 mM, pH 5.4) containing BSA (0.4%), CIAP (6 µL, 6 U) and ST6Gal-I (2 µL, 4 µg) or ST3Gal-IV (4 µL, 4 µg) was added and the reaction incubated at 37 °C for 18 h.

BCN-FITC conjugate (1 µL, 45 mM in DMF) was incubated individually with each previously described enzymatic reaction (49 µL aliquot) at 37 °C for 6 h. Some protein samples were analyzed via SDS-PAGE (4-15% gradient) and visualized by in-gel fluorescence imaging (Figure 4).

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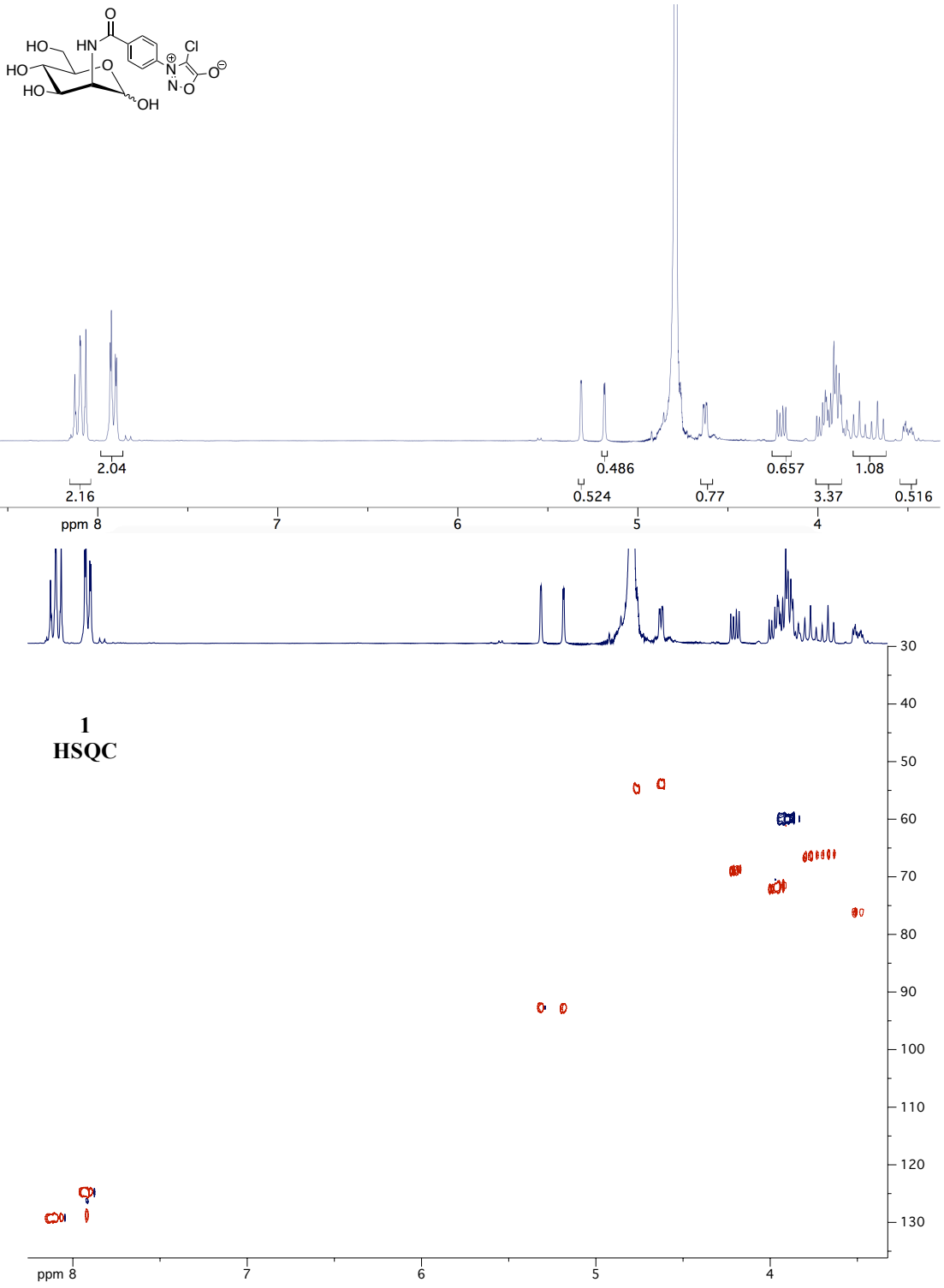
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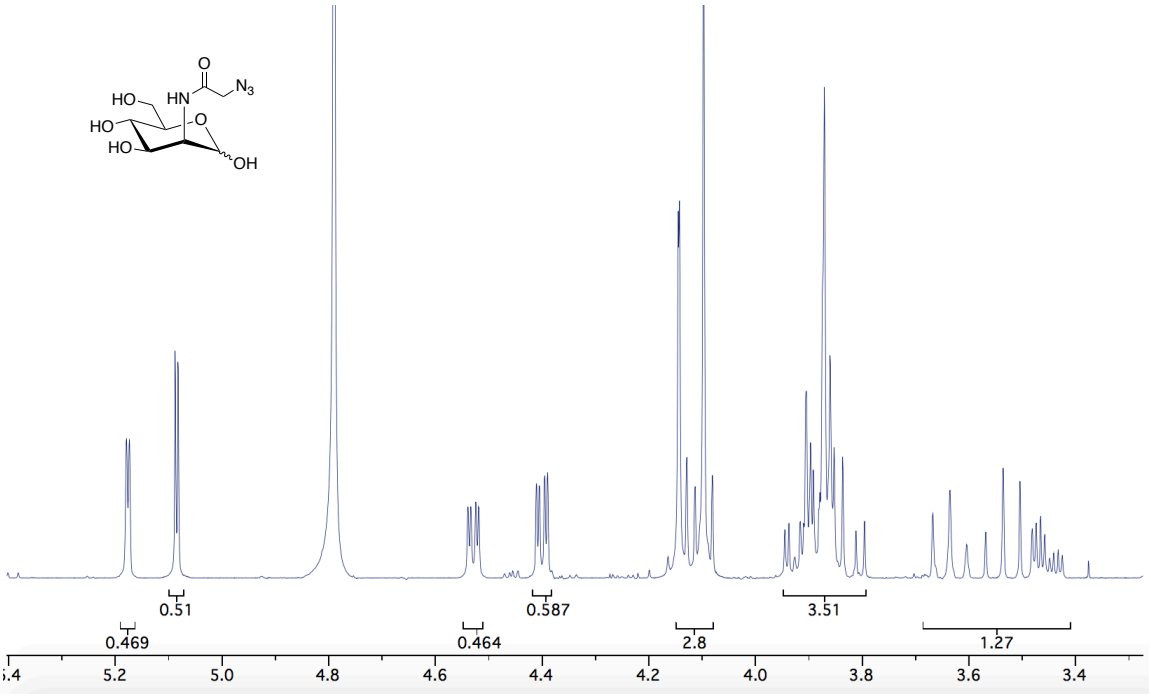
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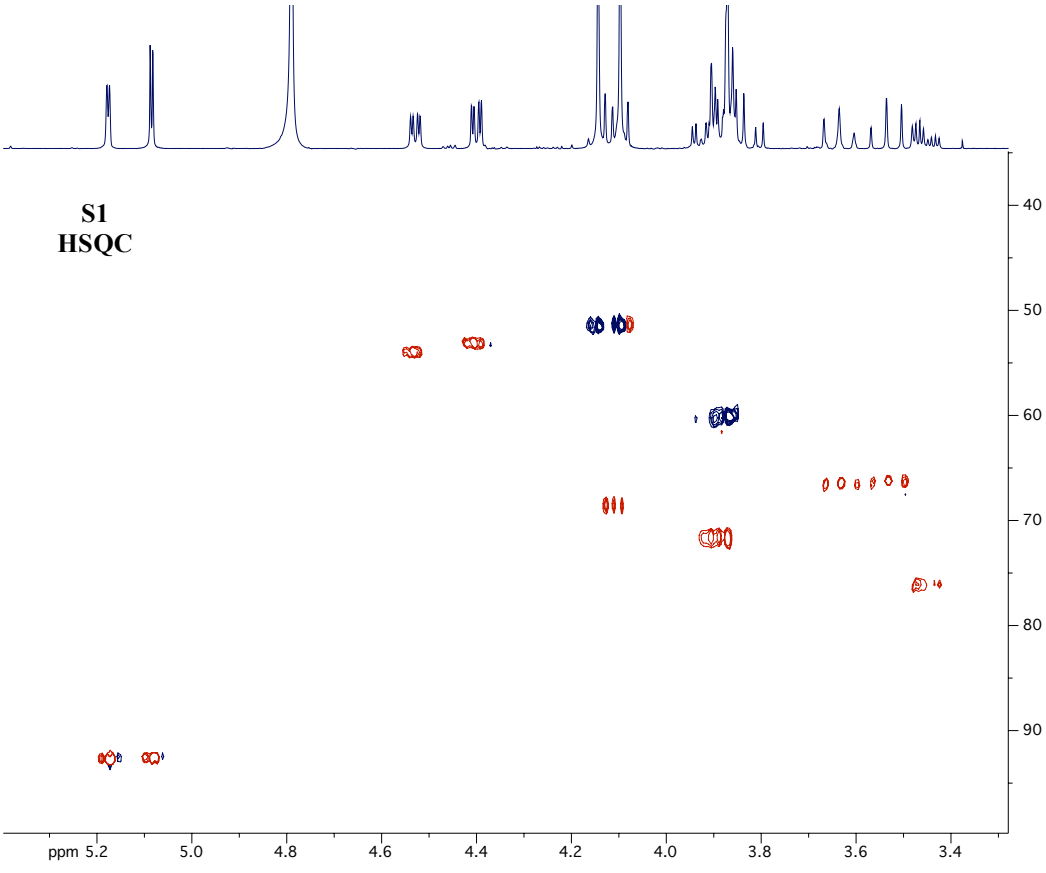
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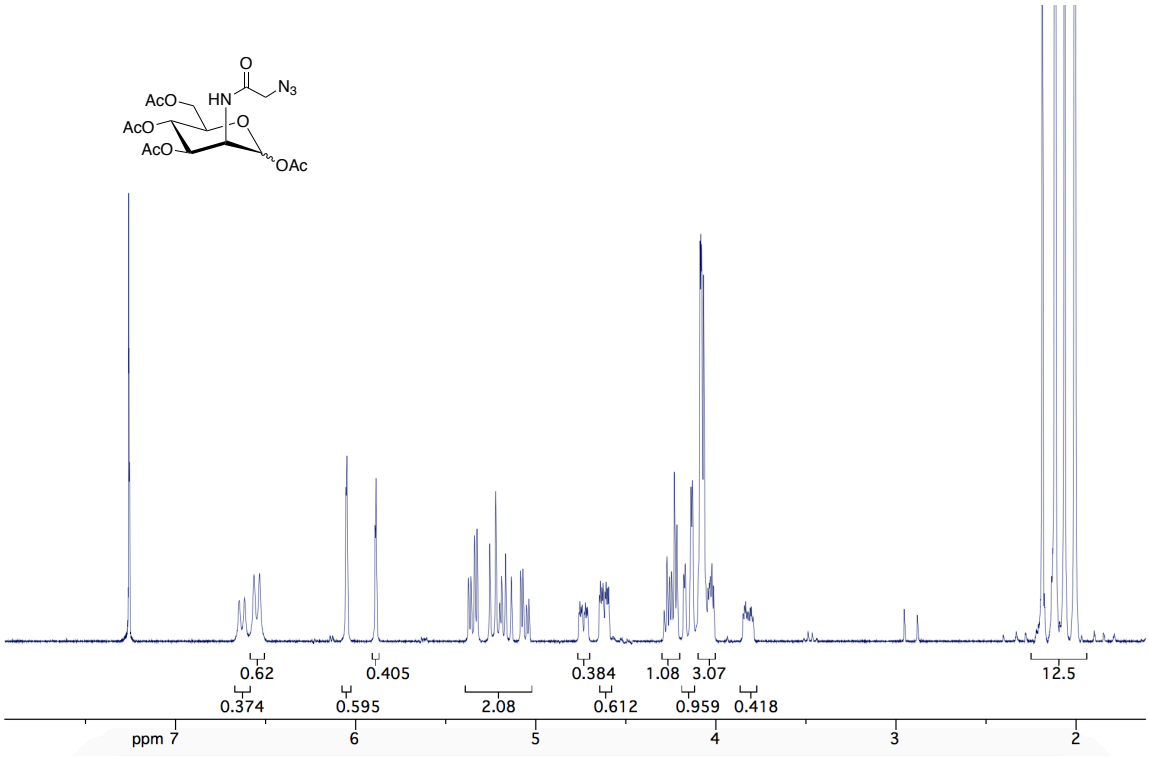
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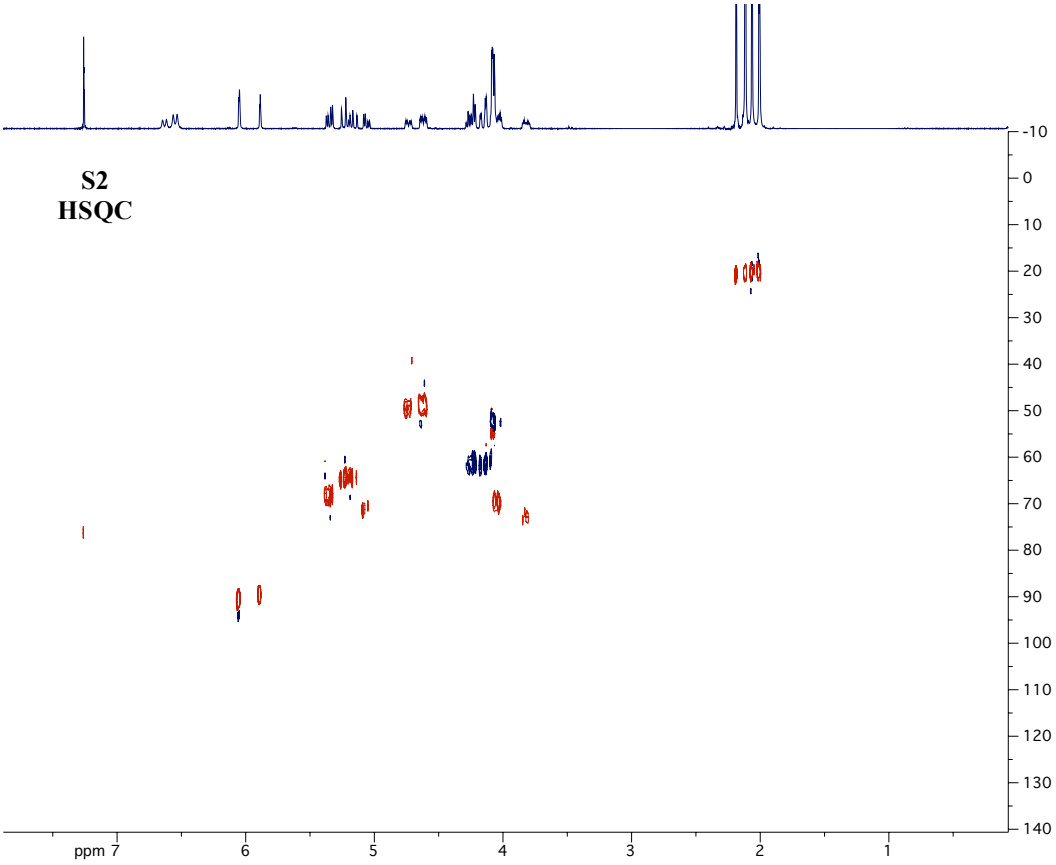
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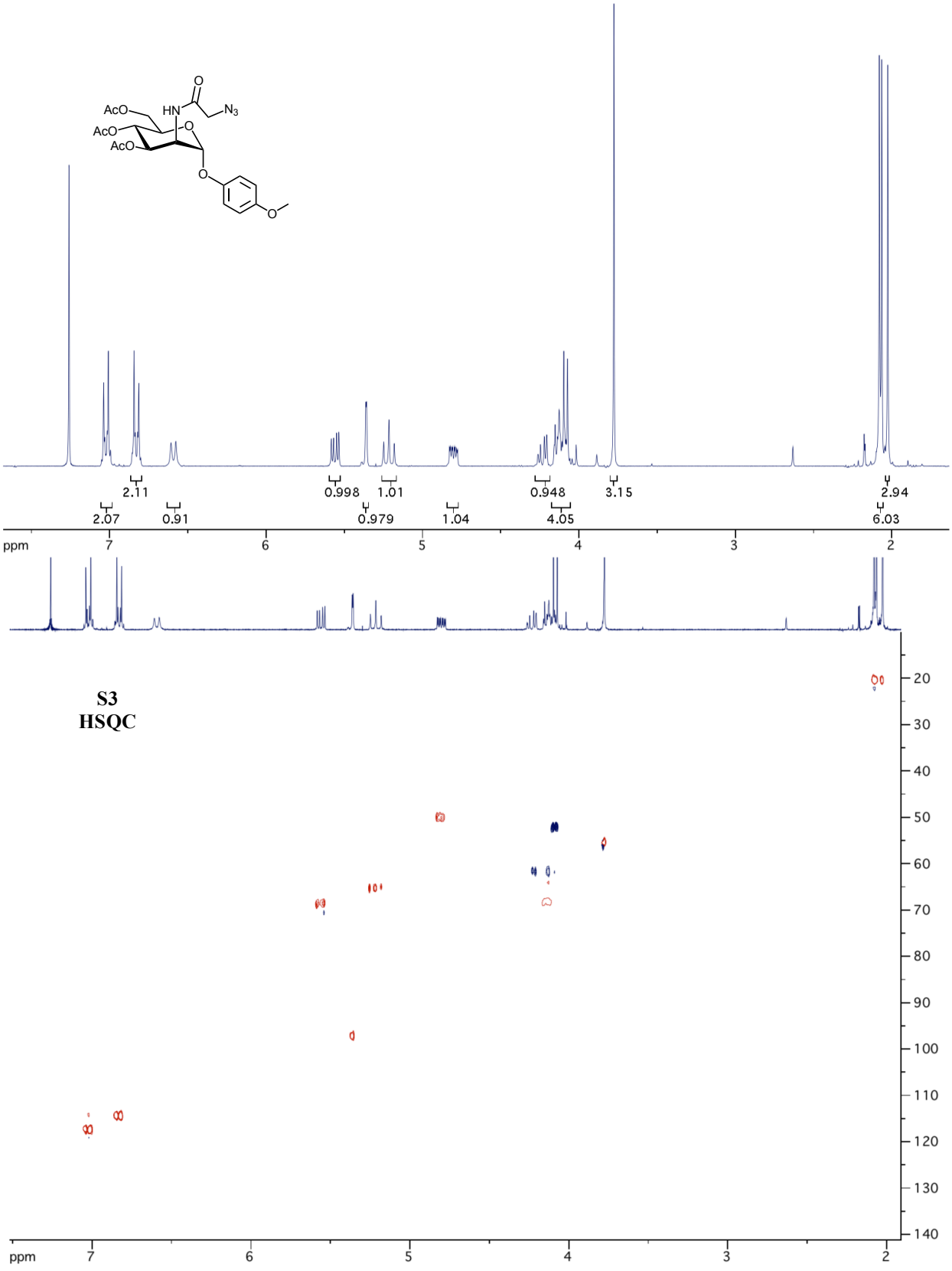
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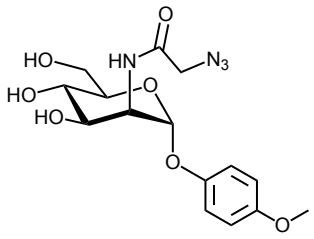
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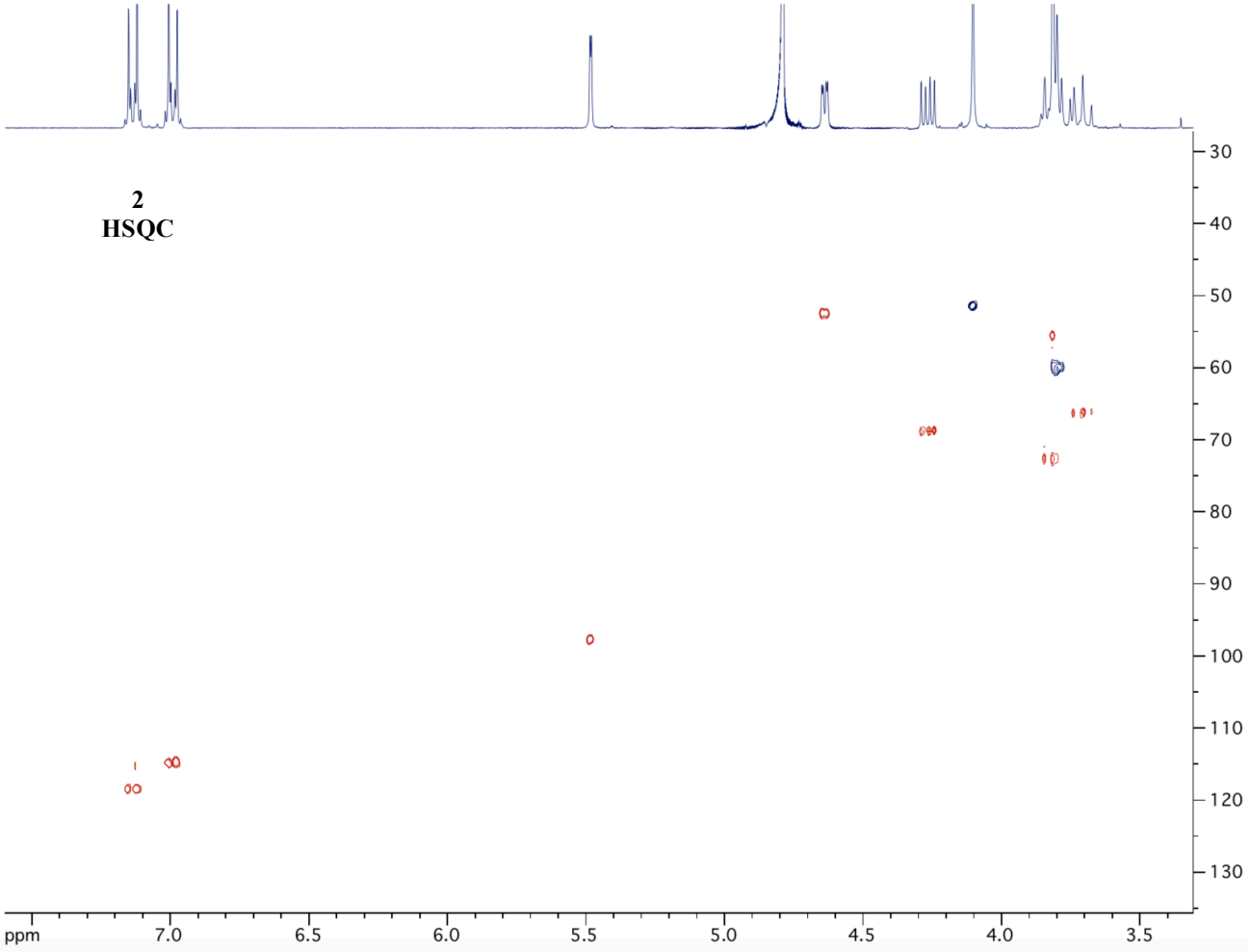
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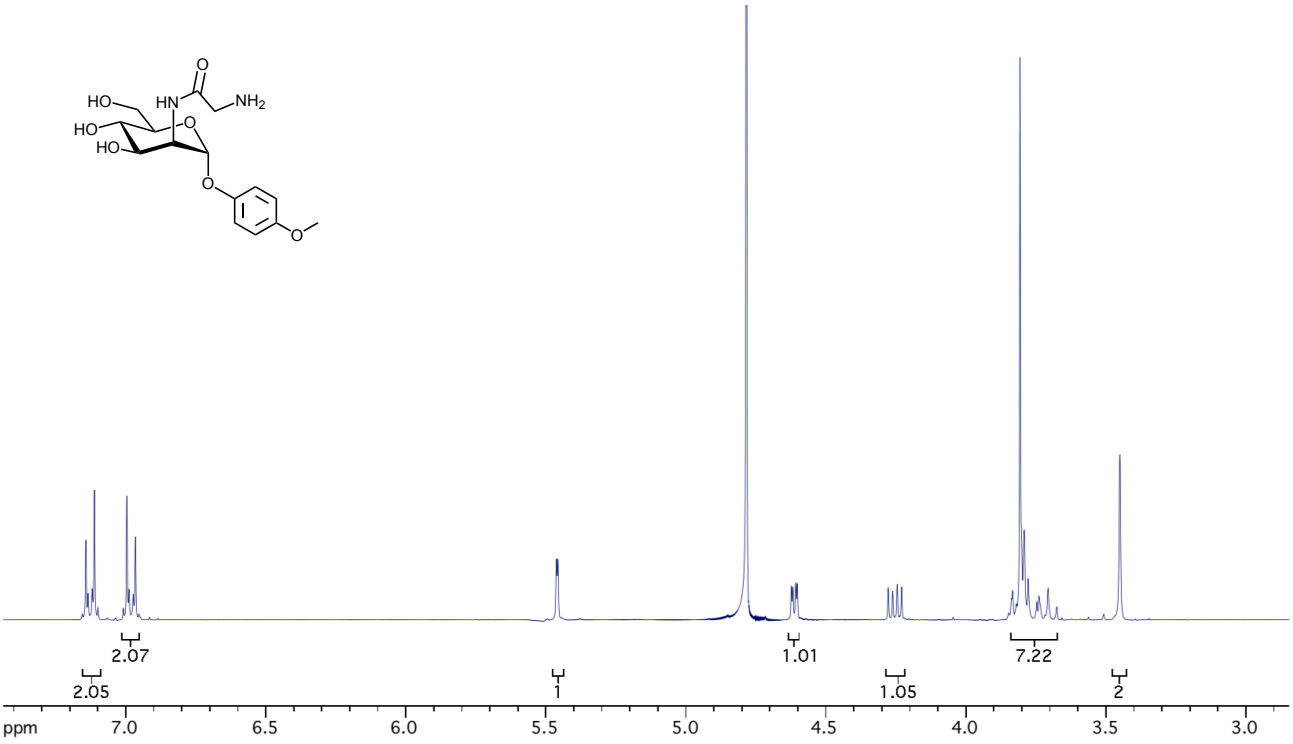
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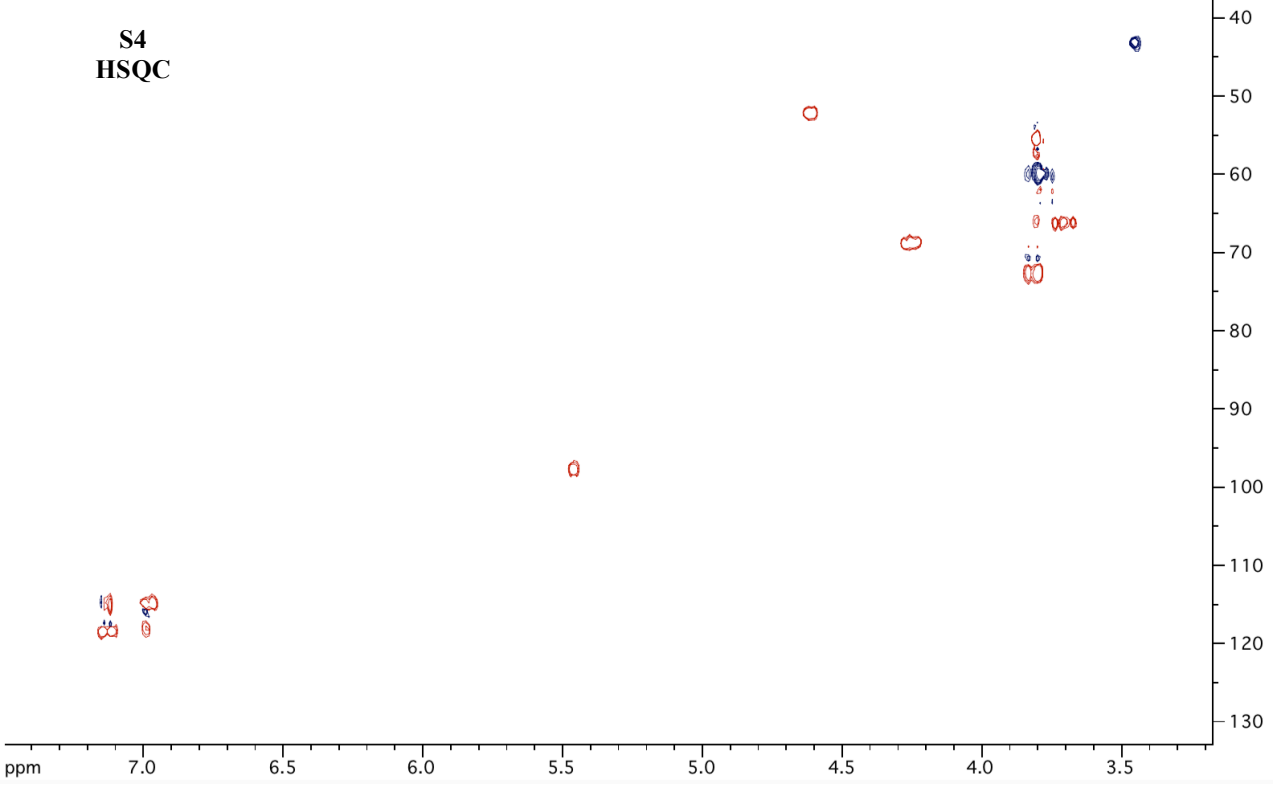
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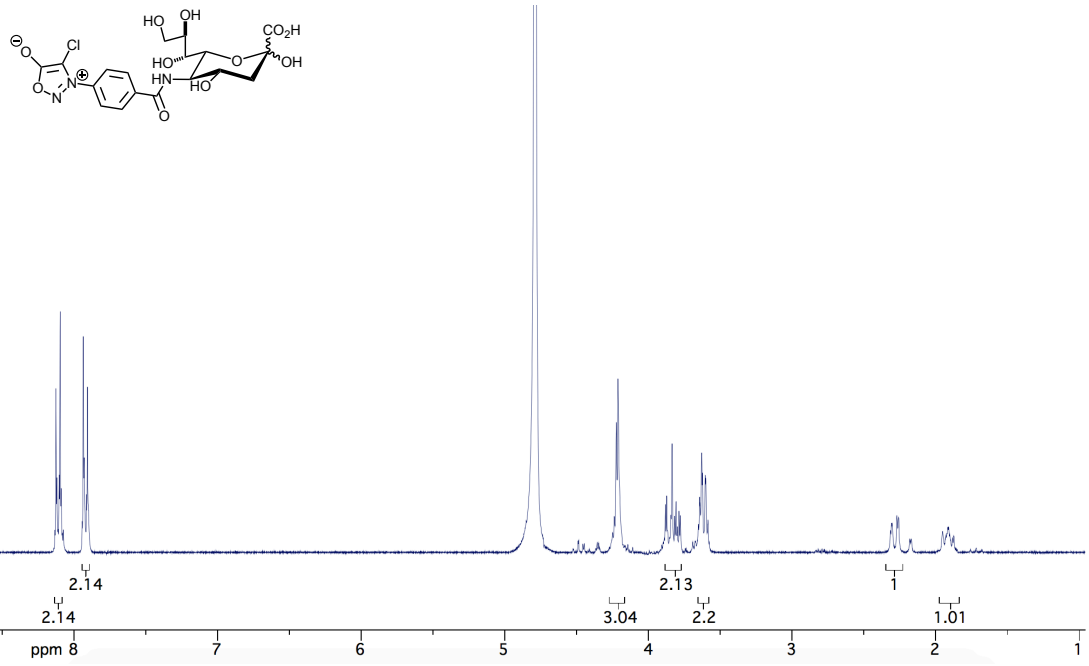
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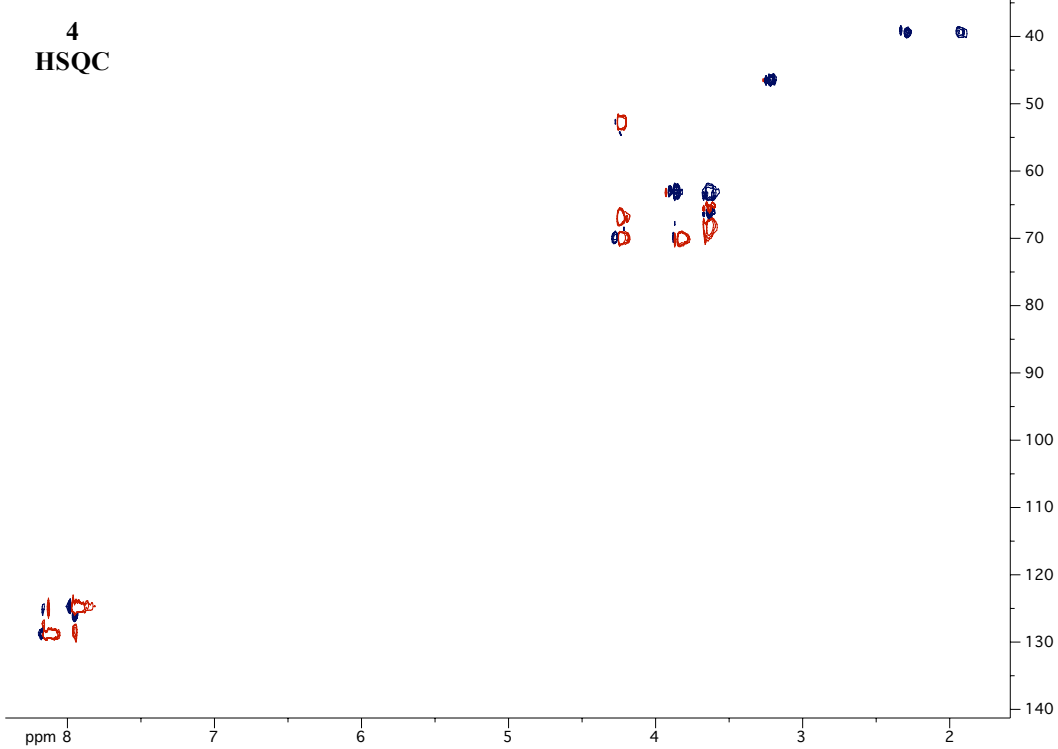
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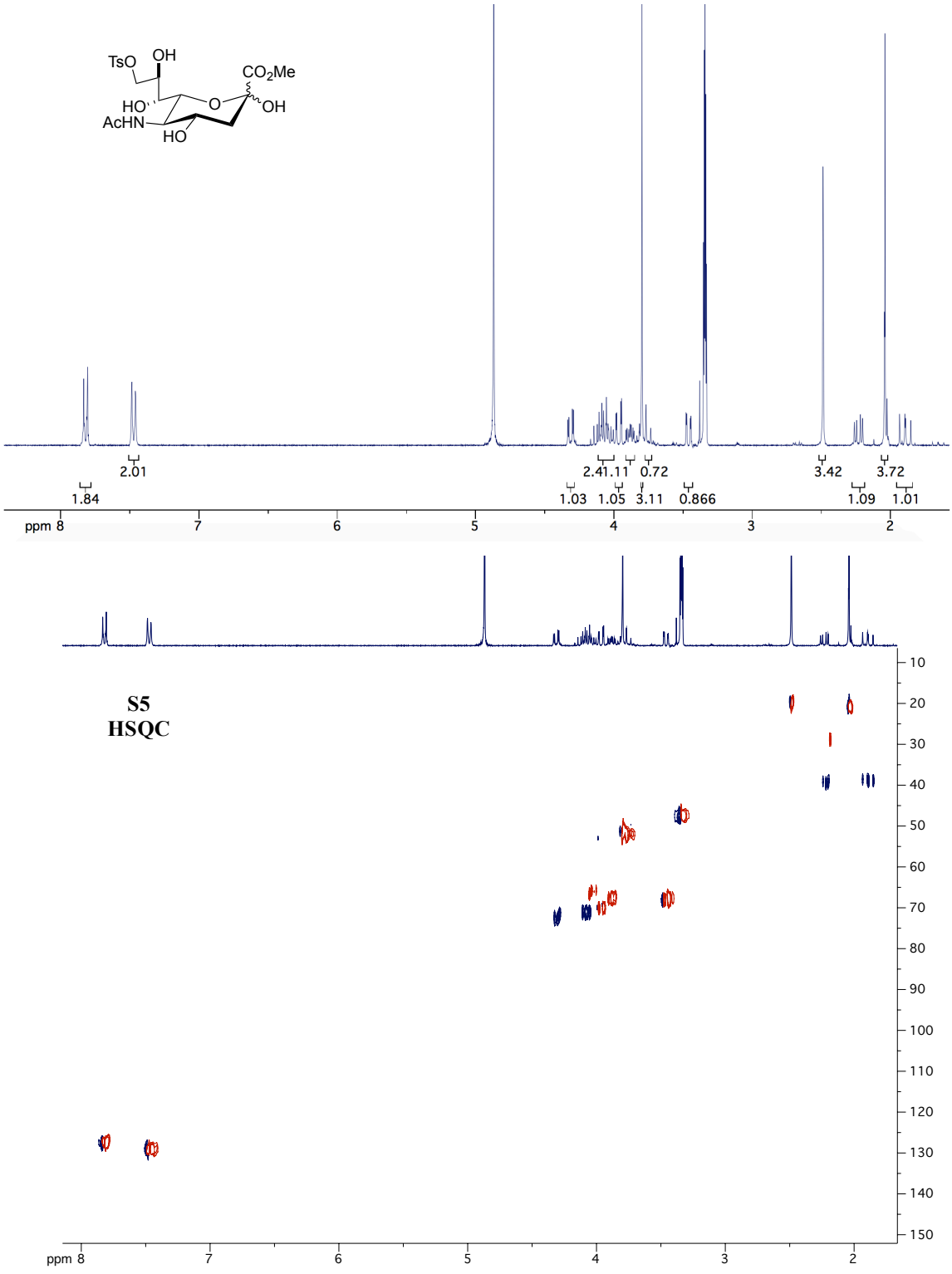
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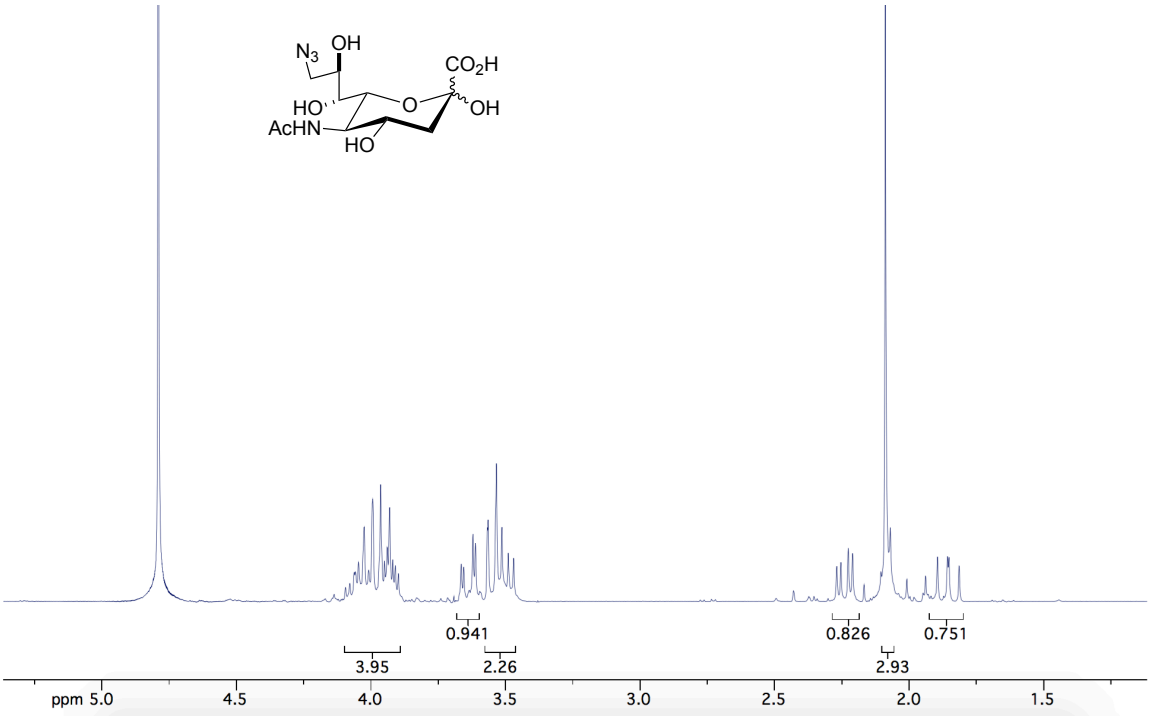
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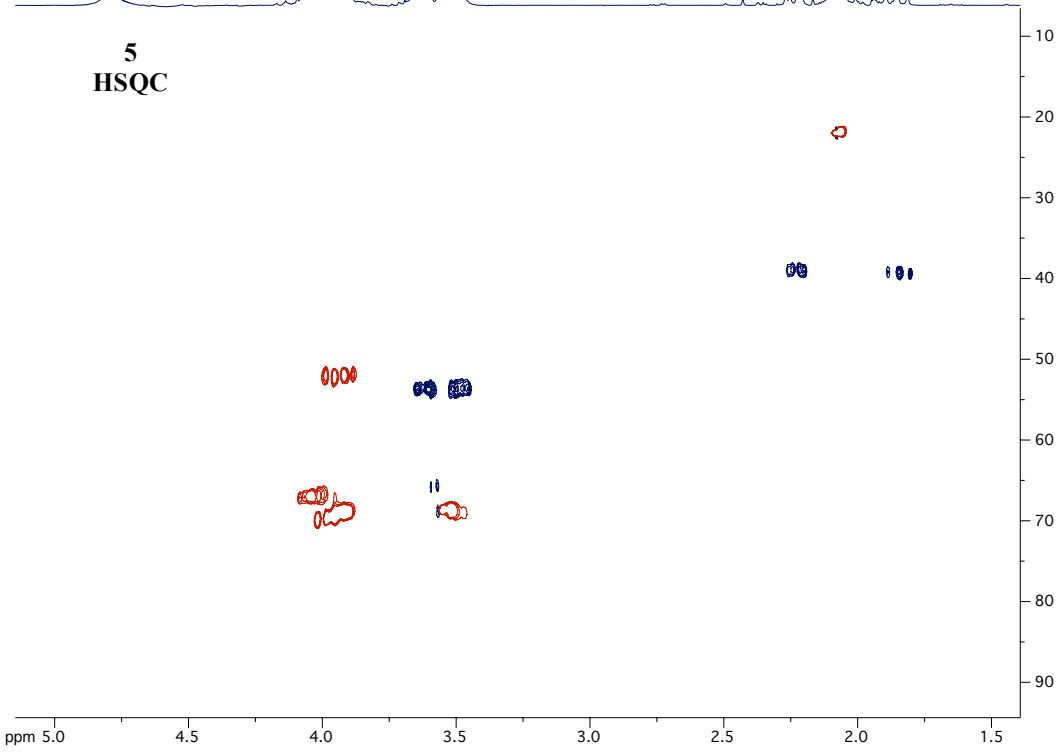
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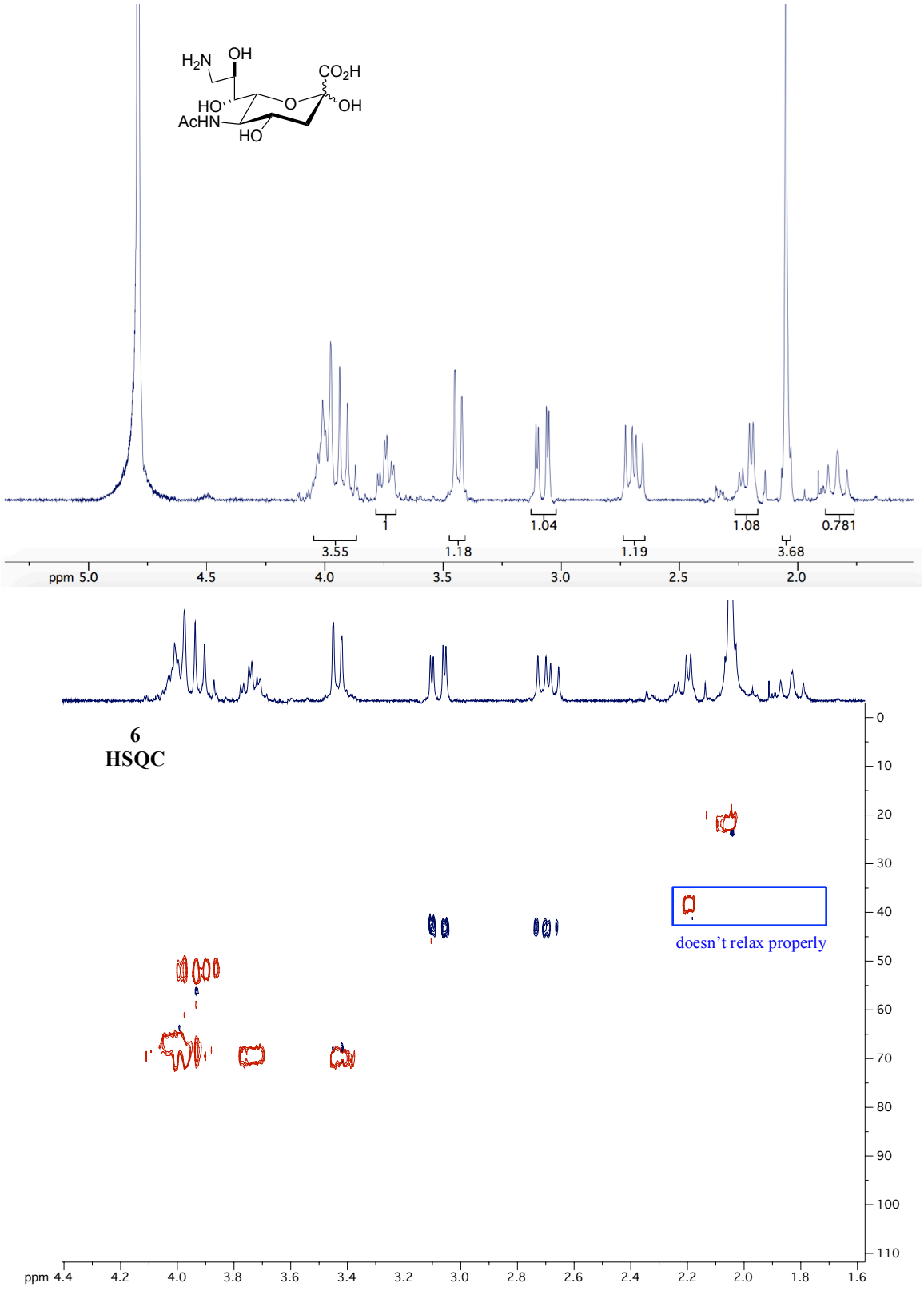
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HSQC
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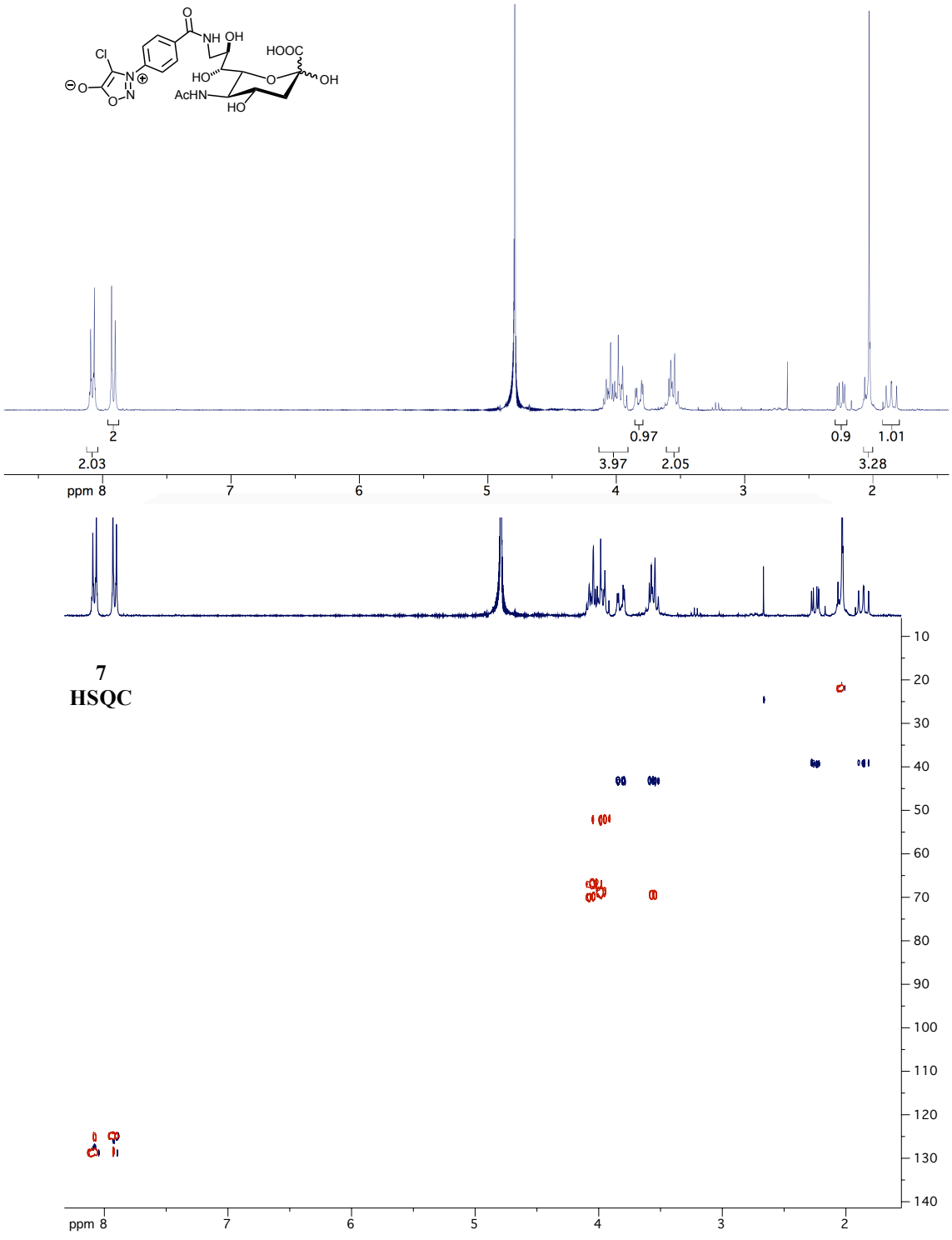
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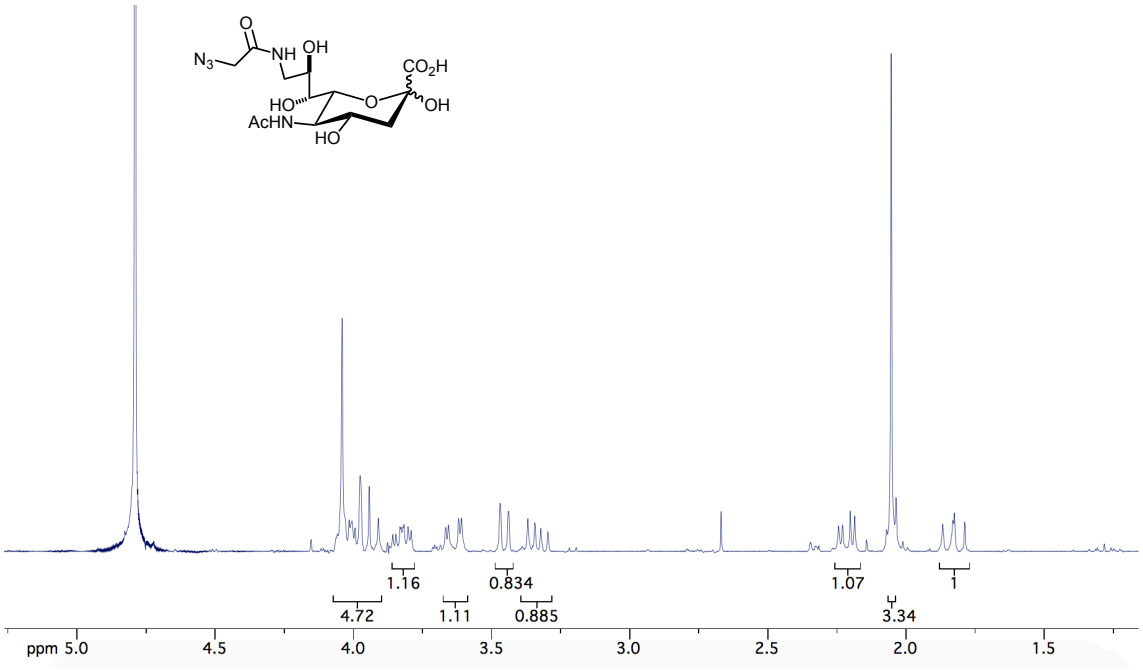
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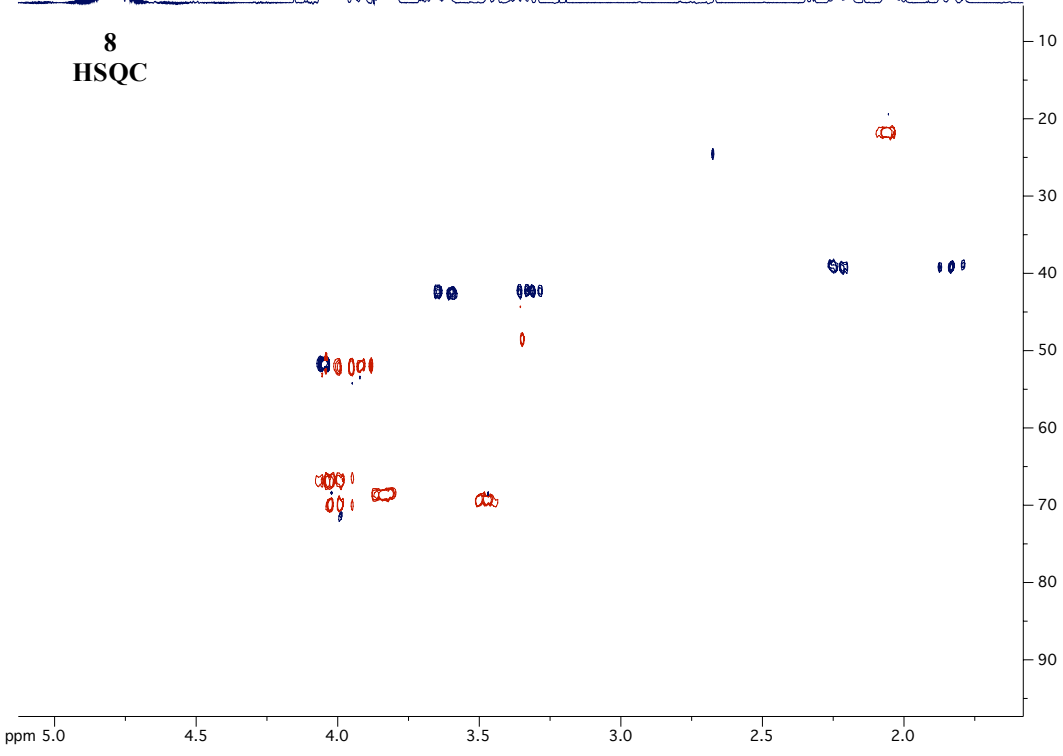
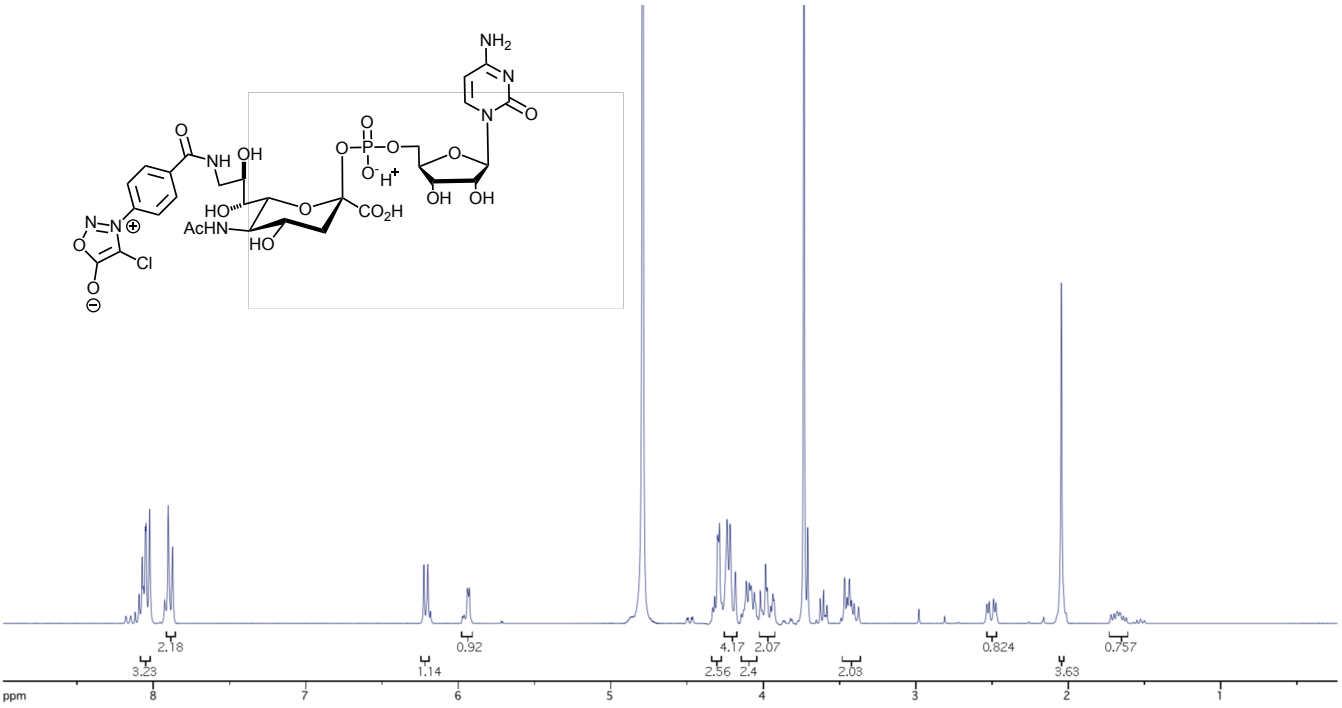
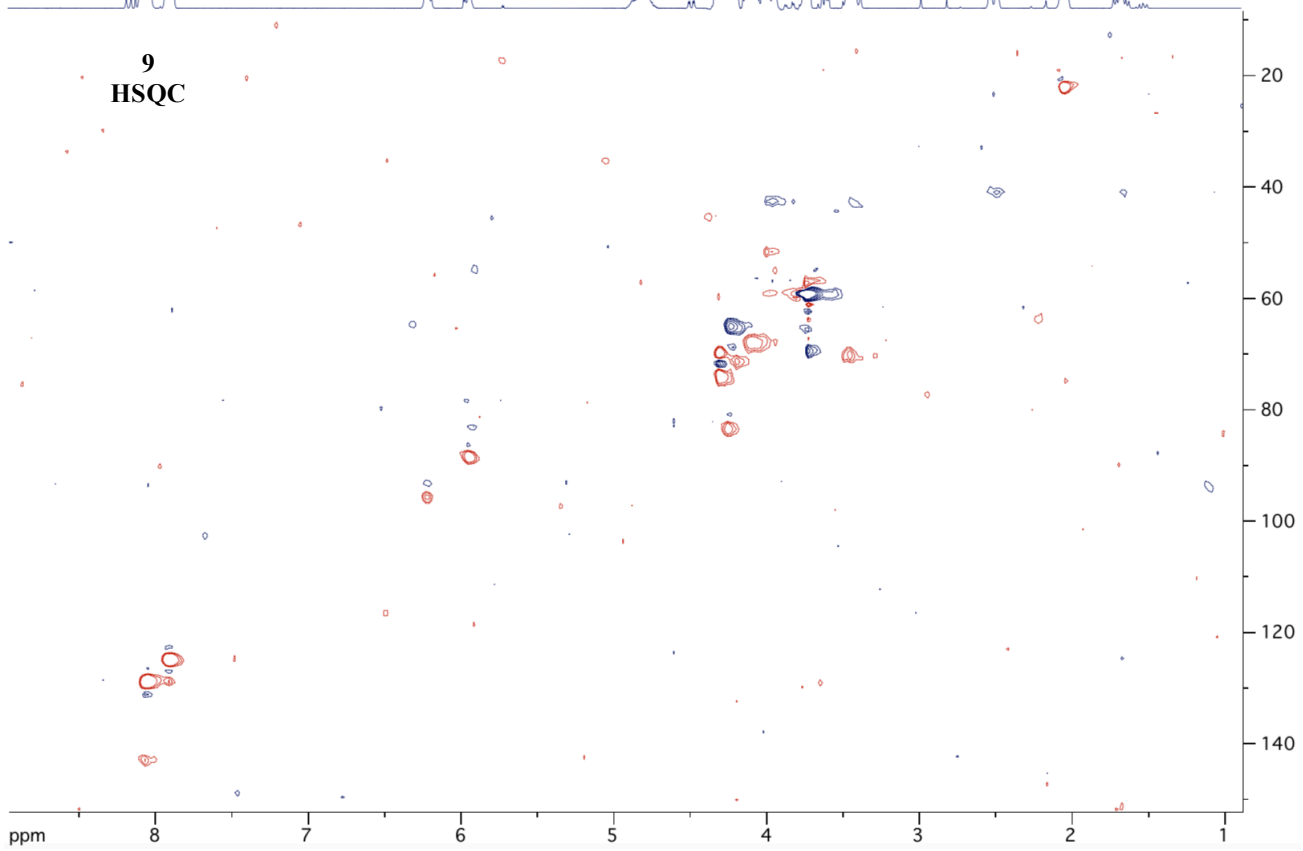
1.5

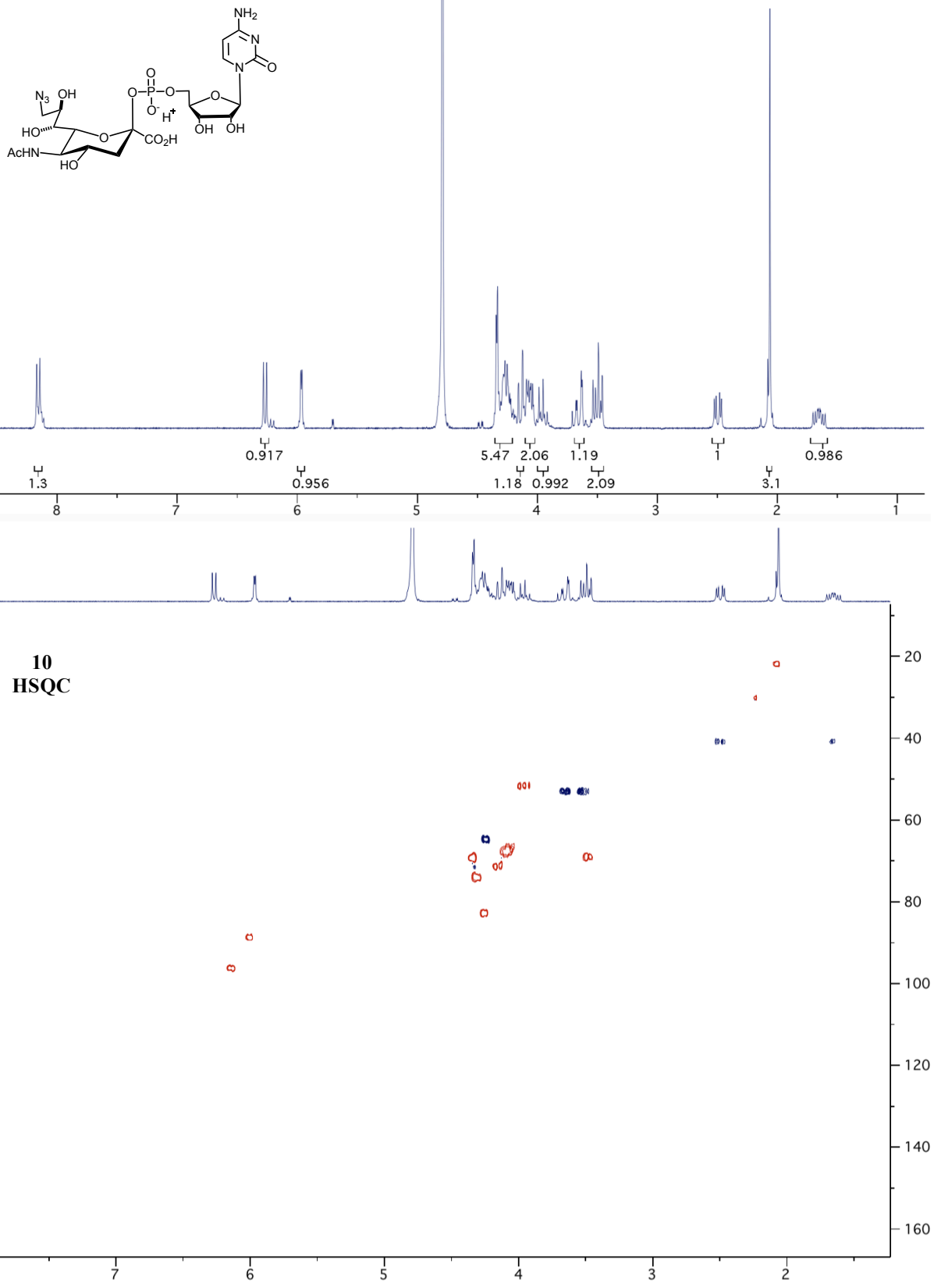
8
HSQC

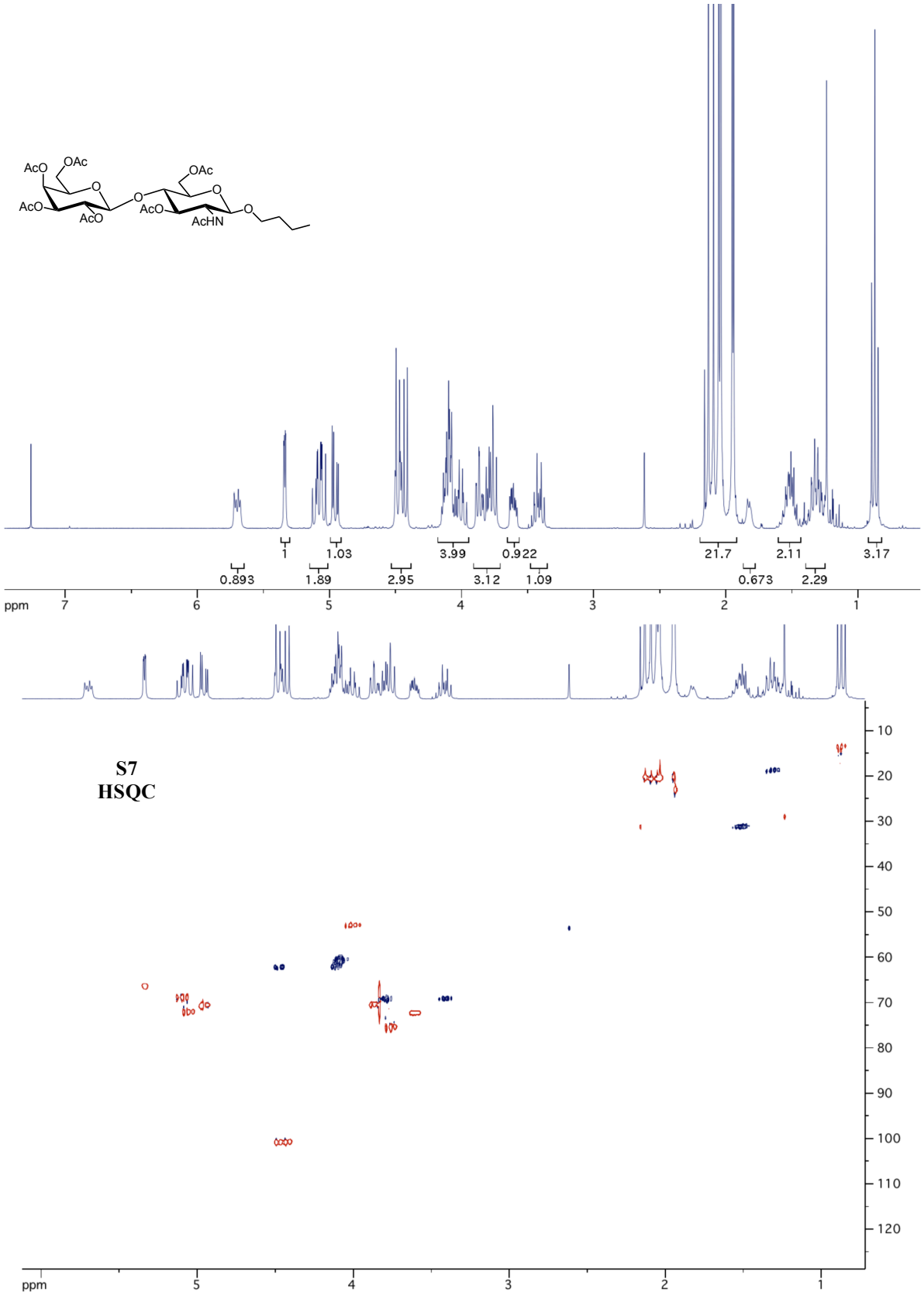

10
20
30
40
50
60
70
80
90
ppm 5.0
4.5
4.0
3.5
3.0
2.5
2.0

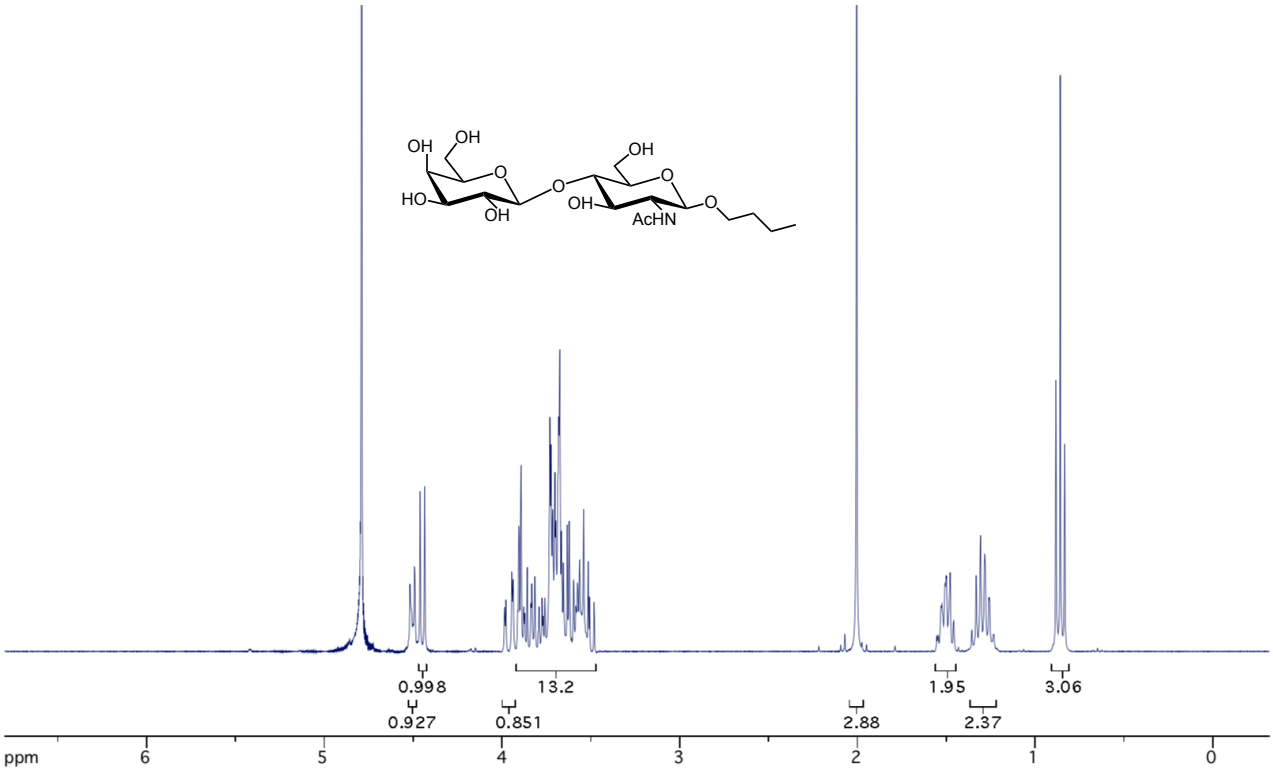

NH2
N
N
O
O
NH
OH
O
P
O
O
H+
O
HO
AcHN
HO
O
CO2H
OH
OH
N
N
O
Cl
O
2.18
3.23
0.92
1.14
4.17
2.07
2.56
2.4
2.03
0.824
3.63
0.757
ppm
8
7
6
5
4
3
2
1

9
HSQC

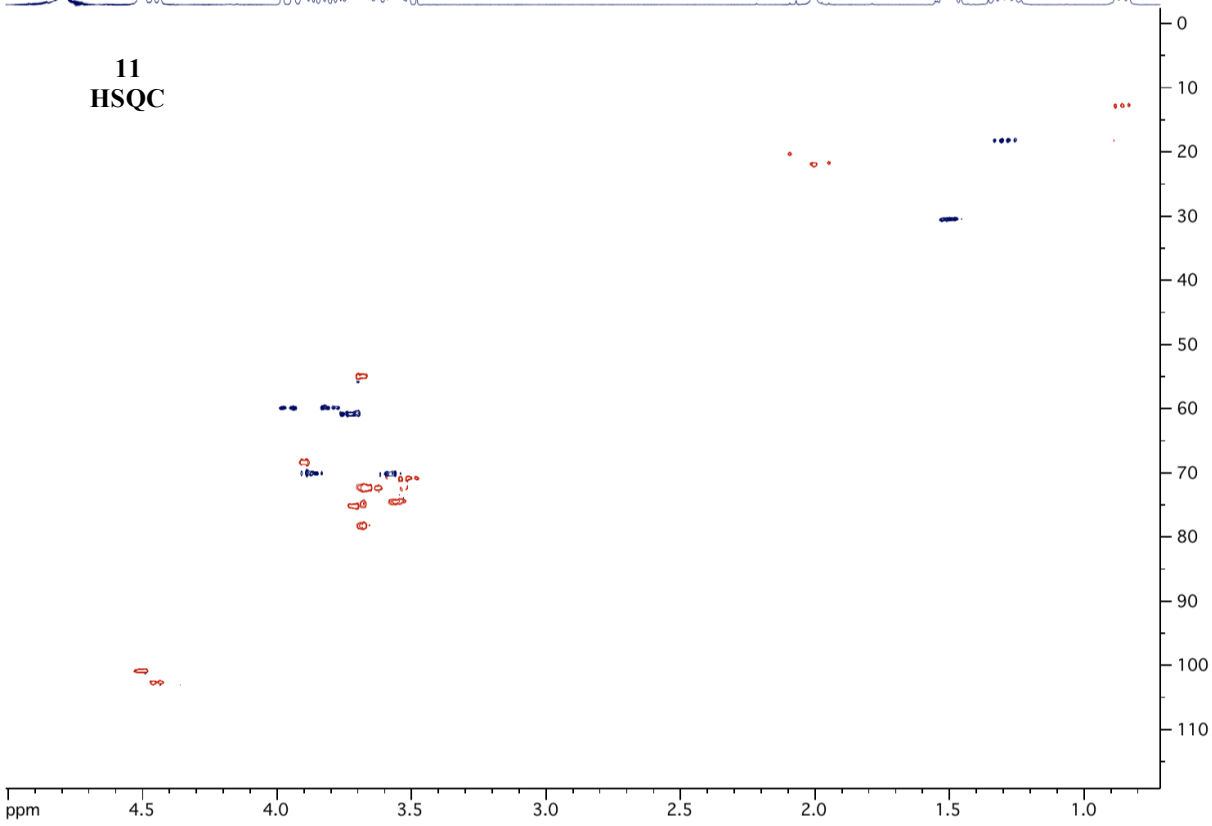

20
40
60
80
100
120
140
ppm
8
7
6
5
4
3
2
1


10
HSQC
0.917
5.47
2.06
1.19
1
0.986
1.3
0.956
1.18
0.992
2.09
3.1
ppm


AcO
OAc
AcO
AcO
AcO
OAc
AcO
AcHN
1
1.03
3.99
0.922
21.7
2.11
3.17
0.893
1.89
2.95
3.12
1.09
0.673
2.29
ppm
7
6
5
4
3
2
1
S7
HSQC
10
20
30
40
50
60
70
80
90
100
110
120
ppm
5
4
3
2
1


OH
OH
HO
OH
O
O
OH
O
OH
AcHN
O
0.998
13.2
1.95
3.06
0.927
0.851
2.88
2.37
ppm
6
5
4
3
2
1
0

11
HSQC


0
10
20
30
40
50
60
70
80
90
100
110
ppm
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0