Supporting Information

for

Chemoenzymatic synthesis of C8-modified sialic acids and related α2–3- and α2–6-linked sialosides

Hai Yu[a], Hongzhi Cao[b], Vinod Kumar Tiwari[c], Yanhong Li[a], Xi Chen[a,*]

[a]*Department of Chemistry, University of California, One Shields Avenue, Davis, California 95616*
[b]*Current Address: National Glycoengineering Research Center, Shandong University, Jinan, Shandong 250012, China*
[c]*Current Address: Banaras Hindu University, Chemistry, Faculty of Science, Varanasi, UP, India 221005.*

*Corresponding author: Tel: +1 530 754 6037; fax: +1 530 752 8995; e-mail address: chen@chem.ucdavis.edu

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General Methods

^{1}H NMR (300, 400, or 600 MHz) and ^{13}C NMR (75 or 100 MHz) spectra were recorded on a Varian Mercury-300, a Varian Inova-400, or a Varian Inova-600 spectrometer. High resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility in the University of California, Davis. Silica gel 60 Å (40–63 μm, Sorbent technologies) was used for flash column chromatography. Analytical thin-layer chromatography was performed on silica gel plates 60 GF_{254} (Sorbent technologies) using anisaldehyde stain for detection. Gel filtration chromatography was performed using a column (100 cm × 2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad, Hercules, CA).

Synthesis of 5-*O*-methyl ManNAc, 5-*O*-methyl ManNGc, 5-*O*-methyl mannose, 5-deoxyl mannose

Benzyl 3-*O*-benzyl-5,6-carbonate-D-glucofuranoside (8). A solution of 3-*O*-benzyl-5,6-carbonate 1,2-*O*-isopropylidene α-D-glucofuranose **7**[1] (2.89 g, 8.59 mmol) in benzyl alcohol (35.6 mL) was stirred for 4 h at 80 °C in the presence of Dowex-50 H^+ ion exchange resin (3.0 mL). The filtered solution was purified on silica gel column and washed first with hexane to remove most of the benzyl alcohol, then washed with hexane:ethyl acetate (10:1 to 1:1, v/v) to give benzyl 3-*O*-benzyl-5,6-carbonate-α-D-glucofuranoside (1.58 g, 48%) as syrup and benzyl 3-*O*-benzyl-5,6-carbonate-β-D-glucofuranoside (1.32 g, 40%) as syrup. For benzyl 3-*O*-benzyl-5,6-carbonate-α-D-glucofuranoside: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.20–7.39 (m, 10H), 5.26 (t, 1H, *J* = 13.2 Hz), 4.83–4.88 (m, 2H), 4.72 (d, 1H, *J* = 11.4 Hz), 4.60–4.63 (m, 2H), 4.49 (d, 1H, *J* = 11.4 Hz), 4.44 (dd, 1H, *J* = 6.6, 9.0 Hz), 4.38 (d, 1H, *J* = 9.0 Hz), 4.22 (s, 1H), 4.08 (dd, 1H, *J* = 3.0 and 6.0 Hz), 2.93 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 155.20, 137.25, 136.82, 128.88, 128.79, 128.55, 128.53, 128.43, 128.03, 100.55, 83.70, 78.12, 77.19, 75.43, 72.01, 70.52, 66.07. For 3-*O*-benzyl-5,6-carbonate-β-D-glucofuranoside: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.22–7.35 (m, 10H), 5.18 (s, 1H), 4.85 (dt, 1H, *J* = 1.8, 10.2 Hz), 4.79–4.82 (m, 2H), 4.77 (dd, 1H, *J* = 6.0 and 9.0 Hz), 4.64 (d, 1H, *J* = 11.4 Hz), 4.50 (d, 1H, *J* = 12.0 Hz), 4.40 (d, 1H, *J* = 6.0 Hz), 4.39 (s, 1H), 4.28 (t, 1H, *J* = 9.0 Hz), 4.04 (d, 1H, *J* = 4.8 Hz), 3.51 (d, 1H, *J* = 4.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 156.24, 137.53, 137.43, 128.78, 128.75, 128.27, 128.12, 127.90, 108.92, 83.48, 82.22, 77.68, 76.77, 72.14, 70.33, 67.18.

Benzyl 2-azido-3-*O*-benzyl-5,6-carbonate-2-deoxy-α-D-mannofuranoside (9). To a solution of benzyl 3-*O*-benzyl-5,6-carbonate-β-D-glucofuranoside **8** (4.82 g, 12.47 mmol) in CH_2Cl_2 (100 mL) in presence of pyridine (2 mL, 24.73 mmol) was added trifluoromethanesulfonic anhydride (2.53 mL, 14.98 mmol) at -30 °C. After 30 minute, the mixture was diluted with dichloromethane (100 mL) and washed successively with cold 1 M HCl, saturated $NaHCO_3$ and brine, dried over $MgSO_4$, filtrated and concentrated to give crude triflate ester. The crude triflate ester was used directly in the next step without further purification. To the solution of crude triflate ester in dry DMF (30 mL), sodium azide (2.75 g, 42.31 mmol) was added at 0 °C. After stirred for 2 h, the mixture was allowed warm to room temperature and stirred over night. The solution was diluted with dichloromethane (200 mL), washed with brine, dried over $MgSO_4$ and purified by flash chromatography (hexane:ethyl acetate = 5:1) to give Benzyl 2-azido-3-*O*-benzyl-5,6-carbonate-2-deoxy-α-D-manofuranoside (4.65 g, 91%) as syrup. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.29–7.39 (m, 10 H), 5.16 (d, 1H, *J* = 1.8 Hz), 4.84–4.87 (m, 1H), 4.71 (d, 1H, *J* = 11.4 Hz), 4.69 (d, 1H, *J* = 12.0 Hz), 4.66 (dd, 1H, *J* = 6.6 and 9.0 Hz), 4.48–4.53 (m, 4H), 4.39 (t, 1H, *J* = 8.4 Hz), 4.01 (dd, 1H, *J* = 1.8, 5.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 155.09, 136.72, 136.67, 129.01, 128.84, 128.78, 128.45, 128.42, 128.22, 104.53, 78.58, 77.69, 75.07, 74.67, 70.20, 66.08, 65.96.

Benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-mannofuranoside (10). To a solution of Benzyl 2-azido-3-*O*-benzyl-5,6-carbonate-2-deoxy-α-D-manofuranoside **9** (4.60 g, 11.18 mmol) in dry methanol (100 mL) was added sodium methoxide (100 mg, 1.85 mmol). After stirring over night at room temperature the mixture was neutralized with Dowex H^+ resin, filtrated, concentrated and purified flash chromatography (hexane:ethyl acetate 2:1 to 1:1, v/v) to give Benzyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-manofuranoside (4.31 g, 100%) as white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.30–7.41 (m, 10H), 5.14 (d, 1H, *J* = 1.8 Hz), 4.81 (d, 1H, *J* = 10.8 Hz), 4.69 (d, 1H, *J* = 12.0 Hz), 4.60 (t, 1H, *J* = 6.0 Hz), 4.56 (d, 1H, *J* = 11.4 Hz), 4.50 (d, 1H, *J* = 12.0 Hz), 4.13 (dd, 1H, *J* = 6.6 and 9.0 Hz), 4.02–4.06 (m, 1H), 3.95 (dd, 1H, *J* = 1.2 and 5.4 Hz), 3.82 (d, 1H, *J* = 10.2 Hz), 3.69 (d, 1H, *J* = 4.8 Hz), 3.01 (s br, 1H), 2.11 (s br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.19, 136.79, 129.10, 128.79, 128.34, 128.32, 104.38, 80.12, 77.33, 74.40, 70.36, 70.03, 65.44, 64.11.

The suspended mixture of benzyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-manofuranoside (2.96 g, 7.68 mmol) and dibutyltin oxide (2.86 g, 11.49 mmol) in dry toluene (70 mL) were heated under reflux for 2 h. Approximately 50 mL solvent was azeotropicly removed using a Dean-stark apparatus under Ar, then Bu_4NI (200 mg) and benzyl bromide (0.9 mL, 7.68 mmol) were introduced. The reaction was heated under reflux for 24 h, solvent evaporated, the residue diluted with ethyl acetate and washed successively with 10% aqueous KF solution and brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography with hexane:EtOAc, (10:1 to 5:1, v/v) to give benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-mannofuranoside (3.16 g, 86%) as a syrup: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37–7.27 (m, 15H), 5.18 (d, 1H, *J* = 2.4 Hz), 4.79 (d, 1H, *J* = 11.4 Hz), 4.70 (d, 1H, *J* = 12.0 Hz), 4.61 (dd, 2H, *J* = 4.8, 12.0 Hz), 4.56 (d, 1H, *J* = 12.0 Hz), 4.49 (dd, 2H, *J* = 6.0, 11.4 Hz), 4.19 (dd, 1H, *J* = 4.8 and 9.0 Hz), 4.17–4.14 (m, 1H), 3.85 (dd, 1H, *J* = 2.4, 5.4 Hz), 3.72 (dd, 1H, *J* = 3.0 and 10.2 Hz), 3.61 (dd, 1H, *J* = 4.8 and 9.6 Hz), 2.87 (d, 1H, *J* = 4.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 138.38, 137.30, 137.17, 128.90, 128.75, 128.64, 128.50, 128.37, 128.28, 128.22, 128.00, 127.93,104.54, 80.06, 78.23, 74.56, 73.74, 71.46, 70.18, 69.11, 66.42.

Benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-5-*O*-methyl-α-D-mannofuranoside (11). To a solution of benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-α-D-mannofuranoside **10** (1.39 g, 2.93 mmol) in dry DMF (20 mL), sodium hydride (60% in mineral oil, 352 mg, 8.80 mmol) was added slowly. After stirring for 20 minute, MeI (0.36 mL, 5.78 mmol) was added and the reaction mixture was stirred over night at room temperature. The reaction mixture was diluted with dichloromethane (80 mL) and washed successively with 0.5 M HCl, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and purified flash chromatography (hexane:ethyl acetate = 5:1) to give Benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-5-*O*-methyl-α-D-mannofuranoside (1.43 g, 100%) as syrup. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.39–7.27 (m, 15H), 5.20 (d, 1H, *J* = 3.0 Hz), 4.80 (d, 1H, *J* = 10.8 Hz), 4.71 (d, 1H, *J* = 11.4 Hz), 4.62 (d, 1H, *J* = 11.4 Hz), 4.60 (d, 1H, *J* = 12.0), 4.57 (d, 1H, *J* = 12.0 Hz), 4.49 (d, 1H, *J* = 11.4 Hz), 4.33 (t, 1H, *J* = 4.8 Hz), 4.27 (dd, 1H, *J* = 4.2 and 8.4 Hz), 3.85 (dd, 1H, *J* = 1.8 and 10.8 Hz), 3.81 (dd, 1H, *J* = 3.6 and 4.8 Hz), 3.73–3.70 (m, 1H), 3.59 (dd, 1H, *J* = 4.2 and 10.8 Hz), 3.73 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 138.67, 137.93, 137.42, 128.71, 128.64, 128.55, 128.26, 128.14, 128.07, 128.06, 127.83, 127.74, 104.67, 79.65, 78.26, 77.44, 74.54, 73.74, 70.35, 68.62, 67.52, 57.58.

Benzyl 2-*N*-acetyl-3,6-di-*O*-benzyl-2-deoxy-5-*O*-methyl-α-D-mannofuranoside (12). To a solution of benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-5-*O*-methyl-α-D-mannofuranoside **11** (500 mg, 1.02 mmol) in dry pyridine (0.5 mL), the AcSH (11.0 mL) was added at 0 °C. The mixture was allowed warm to room temperature and stirred overnight, then it was concentrated and purified flash chromatography (hexane:ethyl actate = 3:1, v/v) to give Benzyl 2-*N*-acetyl-3,6-di-*O*-benzyl-2-deoxy-5-*O*-methyl-α-D-mannofuranoside (450 mg, 87%) as white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.36–

7.25 (m, 15H), 6.57 (d, 1H, *J* = 7.8 Hz), 4.95 (d, 1H, *J* = 1.8 Hz), 4.66 (d, 1H, *J* = 12.0 Hz), 4.58–4.44 (m, 7H), 4.31 (t, 1 H, *J* = 6.0Hz), 3.78 (dd, 1H, *J* = 3.6 and 10.2 Hz), 3.76–3.73 (m, 1H), 3.68 (dd, 1H, *J* = 4.8 and 10.2 Hz), 3.53 (s, 3H), 1.87 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.02, 138.43, 137.86, 137.81, 128.83, 128.63, 128.58, 128.25, 128.01, 127.96, 127.89, 127.85, 105.97, 79.78, 78.53, 78.45, 73.97, 73.67, 69.58, 69.55, 58.89, 55.32, 23.52.

2-*N*-Acetyl-2-deoxy-5-*O*-methyl-D-mannofuranoside (13). The benzyl 2-*N*-acetyl-3,6-di-*O*-benzyl-2-deoxy-5-*O*-methyl-α-D-mannofuranoside **12** (450 mg, 0.89 mmol) was dissolved in ethanol (30 mL), then 10% Pd/C (200 mg) was added. The mixture was hydrogenated at 55 Psi for 4 hours. The catalyst was filtered off and evaporation gave pure 2-*N*-acetyl-2-deoxy-5-*O*-methyl-D-mannose (188 mg, 90%). ^{1}H NMR (300 MHz, D_2O): δ 5.17 (d, 0.43H, *J* = 5.1 Hz), 5.12 (d, 0.57H, *J* = 6.0 Hz), 4.18–4.14 (m, 1H), 4.12 (4.05 (m, 2H), 3.86–3.79 (m, 1 H), 3.76 (d, 0.44H, *J* = 2.1 Hz), 3.51 (d, 0.57H, *J* = 3.0 Hz), 3.47 (d, 1H, *J* = 4.8 Hz), 3.43–3.36 (m, 1H), 3.27 (s, 3H), 1.90 (s, 1.24H), 1.88 (s, 1.88H); ^{13}C NMR (75 MHz, D_2O): δ 174.53, 174.30, 99.81, 94.95, 79.02, 78.86, 78.67, 78.32, 70.72, 69.24, 60.14, 59.41, 59.16, 57.49, 57.10, 54.74, 21.93, 21.88.

Benzyl 3,6-di-*O*-benzyl-2-deoxy-2-glycolamido-5-*O*-methyl-α-D-mannofuranoside (15). To a solution of benzyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-5-*O*-methyl-α-D-mannofuranoside **11** (491 mg, 1.00 mmol) in pyridine-H_2O (60 mL, 5:1, v/v), the 1,3-propanedithiol (1.81 mL) and Et_3N (1.87 mL) were added. The mixture was stirred at 50 °C for 24 hours, then it was concentrated and purified by flash chromatography (dichloromethane:MeOH = 20:1, v/v) to give benzyl 3,6-di-*O*-benzyl-2-deoxy-5-*O*-methyl-α-D-mannofuranosamine **14** (469 mg, 100%) as syrup. The syrup (469 mg) was dissolved in DMF (5.0 mL) and *N*-hydroxysuccinimidyl glycolate (260 mg, 1.50 mmol) was added. After stirring overnight, the reaction mixture was concentrated and purified by flash chromatography (hexane:ethyl acetate = 2:1, v/v) to give benzyl 3,6-di-*O*-benzyl-2-deoxy-2-*N*-glycolyl-5-*O*-methyl-α-D-mannofuranoside **15** (450 mg, 86%) as white solid. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.53 (d, 1H, *J* = 8.4 Hz), 7.34–7.26 (m, 15H), 4.99 (d, 1H, *J* = 2.4 Hz), 4.67 (d, 1H, *J* = 12.0 Hz), 4.63 (dt, 1H, *J* = 1.8, 7.8 Hz), 4.56 (d, 2H, *J* = 3.6 Hz), 4.50–4.47 (m, 3H), 4.45 (t, 1H, *J* = 5.4 Hz), 4.33 (t, 1H, *J* = 6.6 Hz), 3.96 (d, 2H, *J* = 6.6 Hz), 3.80 (dd, 1H, *J* = 3.0 and 10.2 Hz), 3.76–3.74 (m, 1H), 3.67 (dd, 1H, *J* = 4.8 and 10.2 Hz), 3.50 (s, 3H), 3.08 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.07, 138.46, 137.83, 137.76, 128.85, 128.77, 128.64, 128.61, 128.26, 128.18, 128.04, 127.91, 127.90, 105.93, 79.37, 78.60, 78.54, 74.18, 73.68, 69.63, 69.47, 62.37, 58.61, 55.41.

2-Deoxy-2-glycolamido-5-*O*-methyl-D-mannofuranoside (16). The benzyl 3,6-di-*O*-benzyl-2-deoxy-2-glycolamido-5-*O*-methyl-α-D-mannofuranoside **15** (430 mg, 0.89 mmol) was dissolved in ethanol (30 mL) , then 10% Pd/C (200 mg) was added. The mixture was hydrogenated at 55 Psi for 4 hours. The catalyst was filtered off and the filtration was concentrated and purified by flash chromatography (dichloromethane: methanol = 2:1, v/v) to give 2-deoxy-2-glycolamido-5-*O*-methyl-D-mannose **16** (157 mg, 76%). ^{1}H NMR (600 MHz, D_2O): δ 5.24 (d, 0.5H, *J* = 4.8 Hz), 5.18 (d, 0.5H, *J* = 5.4 Hz), 4.24–4.18 (m, 2H), 4.13 (dd, 0.5H, *J* = 3.6 and 9.0 Hz), 4.00 (s, 1H), 3.99 (d, 1H, *J* = 0.6 Hz), 3.90 (dd, 0.5H, *J* = 3.6 and 8.4 Hz), 3.86 (dd, 0.5 H, *J* = 3.6 and 13.8 Hz), 3.81 (dd, 0.5H, *J* = 3.0 and 12.6 Hz), 3.55–3.52 (m, 1H), 3.49 (dd, 0.5H, *J* = 3.6 and 12.6 Hz), 3.44–3.41 (m, 0.5H), 3.30 (s, 1.5H), 3.29 (s, 1.5H); ^{13}C NMR (150 MHz, D_2O): δ 175.12, 174.73, 100.13, 94.91, 79.08, 78.91, 78.72, 78.50, 70.86, 69.19, 61.06, 61.02, 59.39, 59.33, 59.22, 57.56, 57.15, 53.93.

Benzyl 2,3: 5,6-di-*O*-isopropylidene-D-mannofuranoside (18). 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose **17** (3.50 g, 13.46 mmol) was dissolved in DMF (20 mL), NaH (0.45 g, 18.75 mmol) was added at 0^{o}C and stirred the reaction for 10 min. Benzyl bromide (1.8 mL, 15.15 mmol) was added

slowly through syringe to the reaction mixture and then the reaction was stirred at room temperature for 10 h and filtered. The filtrate was extracted with ethyl acetate, washed with brine, dried over anhydrous $MgSO_4$. The solvent was concentrated under reduced pressure. The crude mass on column chromatography (Hexane:EtOAc, 10:1) gave **18** as α/β isomers (4.6 g, 99%). **18**α isomers: Solid, Yield 57% ; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25 (m, 5H, Ar-H), 4.99 (d, 1H, *J* = 2.7 Hz, H-1), 4.59–4.54 (m, 2H), 4.43–4.39 (m, 2H), 4.03 (m, 1H), 3.91–3.88 (m, 2H), 1.38 (s, 3H), 1.31 (s, 3H), 1.30 (s, 3H), 1.24 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 134.93, 126.17, 125.72, 125.57, 110.27, 106.93, 103.27, 82.77, 78.09, 77.21, 70.78, 66.80, 64.62, 24.59, 23.55, 22.89, 22.17; **18**β isomers: Oil, Yield 42%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32, 4.91 (d, 1H, *J* = 12.3 Hz, H-1), 4.76–4.65 (m, 2H), 4.58 (dd, 1H, *J* = 3.9 Hz and 6.3 Hz), 4.75 (dt, 1H), 4.09 (d, 2H, *J* =5.1 Hz), 3.56 (dd, 1H, *J* = 3.9 Hz and 7.8 Hz), 1.56 (s, 3H), 1.42(s, 3H), 1.33 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.37, 128.64, 128.31, 128.11, 113.96, 109.47, 101.45, 79.99, 79.33, 76.86, 73.54, 71.73, 67.08, 27.25, 25.96, 25.51,25.28 .

Benzyl 2,3-*O*-isopropylidene-α-D-mannofuranoside (19). The substrate **18** (1.9 g, 5.43 mmol) was dissolved in 70% AcOH (15 mL) and stirred at 30 °C for 16 h. and concentrated under reduced pressure. The residue was dissolved in dicholomethane, washed sequentially with $NaHCO_3$, water, and brine, dried over anhydrous $MgSO_4$. After filtered the solvent was concentrated under reduced pressure to a colorless oil which was subjected to column chromatography to yield the pure product **19** as Oil (1.59 g, 95%); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.35 (m, 5H, Ar-H), 5.12 (s, 1H, H-1), 4.85 (dd, 1H,*J* = 3.6 Hz and 6.0 Hz, H-3), 4.67 (d, 1H, *J* = 6.0 Hz, H-2), 4.62 (d, 1H, *J* = 12.0 Hz), 4.50 (d, 1H, *J* = 12.0 Hz), 3.99 (m, 2H, H-4 and H-5), 3.82 (dd, 1H, *J* = 3.3 Hz and 8.1 Hz, H-6a), 3.67 (dd, 1H, *J* = 5.1 Hz and Hz, H-6b), 1.47 (s, 3H), 1.33(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.68, 128.67, 128.24, 128.06, 112.96, 105.83, 85.15, 80.42, 79.68, 70.66, 69.47, 64.70, 26.16, 24.87.

Benzyl 6-*O*-benzyl-2,3-*O*-isopropylidene-mannofuranoside (20). A solution of benzyl 2,3-*O*-isopropylide-mannofuranoside **19** (1.4 g, 4.51 mmol) and dibutyltin oxide (1.7 g, 6.82 mmol) in anhydrous toluene (100 mL) was heated at refluxed for 5 h under dean stark condition. Then n-Bn_4NBr (200 mg) and benzyl bromide (1.3 mL, 10.9 mmol) were added. The reaction mixture was stirred at reflux for overnight. The solvent was then evaporated under vacuum and the residue was purified by chromatography on silica gel and eluted with ethyl acetate/hexane gave 1.56 g (86%) of desired compound **20**. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.37–7.28 (m, 5H, Ar-H), 5.09 (s, 1H), 4.86 (dd, 1H, *J* = 3.6 Hz and 6.0 Hz), 4.64 (d, 1H, *J* = 3.0 Hz), 4.62 (t, 1H, *J* = 5.4 Hz), 4.58 (d, 1H, *J* = 12.0 Hz), 4.45 (d, 1H, *J* = 12.0 Hz), 4.04 (m, 1H), 4.04 (dd, 1H, *J* = 3.6 Hz and 8.4 Hz), 3.73 (dd, 1H, *J* = 3.0 Hz and 9.6 Hz), 3.67 (dd, 1H, *J* = 5.4 Hz and 9.6 Hz), 1.45 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 138.28, 137.56, 128.76, 128.73, 128.71, 128.68, 128.33, 128.29, 128.10, 127.98, 127.19, 112.81, 105.60, 85.11, 80.28, 79.25, 73.76, 72.08, 69.19, 69.12, 26.22, 24.87.

Benzyl 6-*O*-benzyl-5-*O*-methyl-2,3-*O*-isopropylidene-mannofuranoside (21). The compound **20** (1.5 g, 3.75 mmol) was dissolved in DMF (20 mL) and then NaH (135 mg, 1.5 eq) was added at 0 °C. The reaction mixture was stirred for 10 min before the addition of CH_3I (0.7 mL, 11.24 mmol) and 20 mg of TBAI. The reaction mixture was stirred for overnight and quenched by the addition of methanol. The mixture was diluted with EtOAc, washed with water and dried with $MgSO_4$. After filtered through celite, the solvent was evaporated and the residue was purified by chromatography using 20% EtOAc/Hexane to give product **21** (1.55 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46–7.30 (m, 5H, Ar-H), 5.11 (s, 1H), 4.89 (m, 1H), 4.85–4.46 (m, 5H), 4.14 (dd, 1H, *J* = 3.0 Hz and 9.0 Hz), 4.00 (m, 1H), 3.76 (dd, 1H, *J* = 3.6 Hz and 9.0 Hz), 3.58 (dd, 1H, *J* = 5.4 Hz and 10.8 Hz), 3.46 (s, 3H), 1.49 (s,

3H), 1.37 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 139.10, 137.72, 128.74, 128.48, 128.47, 128.37, 128.10, 127.778, 112.44, 105.66, 85.11, 80.10, 78.67, 76.24, 73.74, 73.40, 69.05, 59.68, 26.42, 25.22.

5-*O*-Methyl-mannofuranoside (22). The compound **21** (1.2 g, 2.90 mmol) was dissolved in 15 mL of TFA (75% in water). Reaction mixture was stirred for 2 h at 0 ^{o}C. Solvent evaporated under reduced pressure. Compound dried under vacuum. The obtained residue was dissolved in methanol (15 mL) with Pd-C (100 mg). Reaction was stirred under H_2 atmosphere for overnight. After filtered, the solvent was evaporated under reduced pressure and crude mass was purified using EtOAc/methanol that afforded 506 mg of desired compound (90%). ^{1}H NMR (600 MHz, D_2O): δ 5.17 (d, 1H, *J* = 6.0 Hz), 4.22–4.11 (m, 2 H), 4.03 (t, 1 H, *J* = 9.6 Hz), 3.91–3.49 (m, 3 H), 3.39 (s, 3 H); ^{13}CNMR (D_2O, 75MHz): δ 101.01, 79.11, 78.51, 77.88, 71.52, 59.40, 57.47.

Benzoyl 6-*O*-benzoyl-2,3-*O*-isopropylidene-D-mannofuranoside (24). To a solution of benzoyl 2,3-*O*-isopropylidene-D-mannofuranoside **23**[2] (2.0 g, 6.16 mmol) in dry pyridine (20 mL) was added dropwise a solution of benzoyl chloride (0.8 mL, 6.8 mmol) in dry CH_2Cl_2 (5 mL) at 0 °C and the mixture was stirred at 0^{o}C and stirred until TLC indicated that the reaction was complete. The mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with 1N HCl and satd. aq. $NaHCO_3$. After concentrated, the residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **24** as a syrup (1.9 g, 90 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06–7.95 (m, 10H), 6.51 (s, 1H), 6.00 (m, 1H), 5.75–5.45 (m, 3H), 4.49 (m, 2H), 1.54 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.22, 165.10, 133.78, 133.41, 130.02, 129.95, 129.66, 128.75, 128.60, 113.77, 101.53, 85.21, 81.42, 80.01, 68.93, 67.17, 26.29, 24.99.

Benzoyl 6-*O*-benzoyl-2,3-*O*-isopropylidene-5-*O*-phenoxythiocarbonyl-D-mannofuranoside (25). The compound **24** (0.75 g, 1.75 mmol) was dissolved in anhydrous dichloromethane (20 mL) and phenyl chlorothionoformate (0.356 mL, 2.62 mmol) was added slowly at 0 °C. After added pyridine (1.0 mL) the reaction mixture was stirred at for overnight and concentrated. The crude product was purified using silica gel column chromatography (10% EtOAc in Hexane) to give of desired **25** (0.94 g, 95%). ^{13}C NMR (150MHz, $CDCl_3$): δ 166.19, 153.17, 133.79, 133.25, 130.04, 129.94, 129.75, 128.75, 128.53, 126.85, 122.08, 114.07, 101.34, 85.06, 80.07, 79.46, 78.88, 62.93, 26.29, 25.16.

Benzoyl 6-*O*-benzoyl-5-deoxyl-2,3-*O*-isopropylidene-D-mannofuranoside (26). 0.94 g of phenoxythiocarbonyl derivative **25** (1.75 mmol) was dissolved in anhydrous toluene (70 mL). Bu_3SnH (0.5 mL) followed by AIBN (20 mg) was added. The reaction mixture was refluxed for overnight at 90 °C. The solvent was evaporated under reduced pressure and the product was purified by silica gel column chromatography (10% EtOAc in Hexane) to yield **26** in 71% yield as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.04–7.93 (m, 10H), 6.42 (s, 1H), 4.90–4.83 (m, 2H), 4.54–4.34 (m, 3H), 2.37–2.24 (m, 2H), 1.52 (s, 3H), 1.36 (s, 3H); ^{13}C NMR (150MHz) δ 166.75, 165.34, 133.64, 133.19, 130.46, 129.99, 129.89, 129.79, 128.74, 128.68, 128.57, 115.61, 113.35, 101.49, 85.66, 80.29, 80.09, 62.25, 28.13, 26.38, 25.18.

5-Deoxyl-D-mannofuranoside (27). Compound **26** (480 mg, 1.16 mmol) was dissolved in 60% TFA (15 mL) and reaction was stirred for 3h. The solvent removed under reduced pressure and co-evaporated with toluene. The residue was then dissolved in MeOH (15 mL), and NaOMe (50mg) was added at 0 °C and stirred for overnight. The reaction mixture was neutralized with Amberlite IR-120 H resin, filtered and concentrated. The residue was purified by flash chromatography (2:1 EtOAc-MeOH) to give 5-deoxy mannose (174 mg, 91%); ^{13}C NMR (150MHz, D_2O): δ 101.83, 99.09, 82.99, 77.65, 76.15, 75.39, 72.86, 69.02, 62.50, 37.55, 37.37.

Enzymatic synthesis of C8-modified sialic acids and sialosides

General procedure for the synthesis of sialic acids: A sialic acid precursor (e.g. C5-modified ManNAc, ManNGc, or mannose derivatives, 20 mM, 1.0 equiv.) and sodium pyruvate (100 mM, 5.0 equiv.) were incubated at 37 °C with *Pasteurella multocida* sialic acid aldolase (4–6 mg) in 100 mM Tris-HCl buffer (pH 7.5) and with gentle stirring. The reaction was monitored by thin layer chromatography (TLC) developed with *i*-PrOH:H_2O:HOAc = 5:1:1 (by volume) and stained with *p*-anisaldehyde sugar stain. After 48 hours, the reaction was stopped by adding the same volume of ice-cold EtOH and incubating at 4 °C for 30 min. The mixture was centrifuged to remove insoluble precipitates. The supernatant was concentrated and passed through an anion exchange chromatography on Dowex 1-X8 (formate, 100–200 mesh) using a gradient of formic acid as eluent. The fractions containing sialic acids were collected and further purified by a BioGel P-2 gel filtration column to give pure sialic acid products.

General procedure for one-pot three enzyme preparative synthesis of α2–3/6-linked sialosides. LacβProN_3 (30–50 mg, 20 mM) and a sialic acid precursor (e.g. C5-modified ManNAc or mannose derivatives, 1.5 equiv.) were incubated at 37 °C in 100 mM of Tris-HCl buffer (pH 8.8) containing sodium pyruvate (7.5 equiv.), CTP (1.5 equiv.), 20 mM of $MgCl_2$ (0.5 M, 0.4 mL), appropriate amount of an *P. multocida* sialic acid aldolase, an *N. meningitidis* CMP-sialic acid synthetase, and *Photobacterium damselae* α2–6-sialyltransferase (Pd2,6ST, for producing α2–6-linked sialosides) or *Pasteurella multocida* α2–3-sialyltransferase (PmST1, for producing α2–3-linked sialosides). The reaction was monitored by TLC developed with EtOAc:MeOH:H_2O:HOAc = 4:2:1:0.5 (by volume) and stained with *p*-anisaldehyde sugar stain. After 1 to 24 h, the reaction was quenched by adding the same volume of EtOH and centrifuged to remove insoluble precipitates. The supernatant was concentrated and passed through a BioGel P-2 gel filtration column with water to obtain desired products.

5-Acetamido-3,5-dideoxy-8-*O*-methyl-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (Neu5Ac8Me, 1). Yield 89%; 73 mg, white powder. ^{1}H NMR (600 MHz, D_2O): δ 4.00 (m, 1 H), 3.93–3.90 (m, 3H), 3.64 (dd, 1 H, *J* = 4.8 and 12.6 Hz), 3.61 (d, 1 H, *J* = 9.0 Hz), 3.42 (m, 1H), 3.41 (s, 3H), 2.21 (dd, 1 H, *J* = 4.8 and 12.6 Hz), 2.03 (s, 3 H), 1.83 (t, 1 H, *J* = 12.0 Hz); ^{13}C NMR (150 MHz, D_2O): δ 176.66, 174.93, 96.73, 80.12, 70.58, 67.35, 67.24, 59.93, 57.98, 52.41, 39.72, 22.25; HRMS (ESI) *m/z* calcd for $C_{12}H_{20}NO_9Na$ (M+Na) 345.1036, found 345.1042.

5-(2-Hydroxy)-acetamido-3,5-dideoxy-8-*O*-methyl-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid (Neu5Gc8Me, 2). Yield 86%; 59 mg, white powder. ^{1}H NMR (600 MHz, D_2O): δ 4.12 (s, 2H), 4.01 (m, 1 H), 3.99 (t, 1 H, *J* = 10.2 Hz), 3.97 (t, 1 H, *J* = 10.8 Hz), 3.92 (t, 1 H, *J* = 6.0 Hz), 3.64 (dd, 1 H, *J* = 4.8 and 12.6 Hz), 3.55 (d, 1 H, *J* = 9.6 Hz), 3.41 (s, 3H), 2.21 (dd, 1 H, *J* = 4.8 and 12.6 Hz), 1.84 (t, 1 H, *J* = 12.0 Hz); ^{13}C NMR (150 MHz, D_2O): δ 176.65, 175.75, 96.53, 80.21, 70.30, 67.35, 67.19, 61.22, 60.10, 58.09, 52.25, 39.86; HRMS (ESI) *m/z* calcd for $C_{12}H_{20}NO_{10}Na$ (M+Na) 361.0985, found 361.0994.

3-Deoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (Kdn8Me, 3). Yield 85%; 54 mg, white powder. ^{1}H NMR (600 MHz, D_2O): δ 3.97–3.91 (m, 3 H), 3.83 (d, 1 H, *J* = 9.6 Hz), 3.67 (dd, 1 H, *J* = 5.4 and 12.6 Hz), 3.56 (t, 1 H, *J* = 9.6 Hz), 3.44 (m, 1H), 3.43 (s, 3H), 2.15 (dd, 1 H, *J* = 4.8 and 12.6 Hz), 1.78 (t, 1 H, *J* = 12.6 Hz); ^{13}C NMR (150 MHz, D_2O): δ 176.78, 96.59, 80.56, 71.92, 70.56, 69.29, 66.99, 60.36, 58.02, 39.53; HRMS (ESI) *m/z* calcd for $C_{10}H_{17}O_9Na$ (M+Na) 304.0770, found 304.0768.

3,8-Dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (Kdn8Deoxy, 4). Yield 90%; 84 mg, white powder. ^{1}H NMR (400 MHz, D_2O): δ 4.15 (dd, 1 H, *J* = 4.4 and 8.8 Hz), 3.93 (m, 1 H), 3.72–3.69 (m, 2 H), 3.57 (t, 1 H, *J* = 10.4 Hz), 3.55 (t, 1 H, *J* = 10.0 Hz), 2.17 (dd, 1 H, *J* = 4.8 and 12.8 Hz), 1.89 (m, 1H), 1.77 (t, 1 H, *J* = 12.4 Hz), 1.72 (m, 1H); ^{13}C NMR (100 MHz, D_2O): δ 177.21, 96.62, 75.12, 71.07, 69.38, 65.31, 58.94, 52.25, 39.48, 35.74; HRMS (ESI) *m/z* calcd for $C_9H_{15}O_8Na$ (M+Na) 274.0665, found 274.0671.

3-Azidopropyl *O*-(5-acetamido-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5Ac8Meα2–3LacProN_3, 29). Yield 93%; 78 mg, white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.69 (d, 1H, *J* = 7.8 Hz), 4.64 (d, 1H, *J* = 7.8 Hz), 4.05 (dd, 1H, *J* = 3.0 and 10.2 Hz), 4.00–3.95 (m, 4H), 3.87–3.53 (m, 16H), 3.48 (m, 1H), 3.46 (s, 3H), 3.44 (t, 2H, *J* = 6.6 Hz,), 3.28 (t, 1H, *J* = 9.0 Hz), 2.65 (dd, 1H, *J* = 4.8 and 12.6 Hz, H-3$_{eq}$''), 2.00 (s, 3H), 1.89 (m, 2H), 1.73 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (150 MHz, D_2O) δ 175.07, 173.75, 102.90, 102.26, 100.31, 80.41, 78.32, 75.85, 75.34, 74.93, 74.41, 72.94 (2C), 69.50, 68.13, 67.81, 67.49, 67.08, 61.14, 60.15, 59.44, 57.63, 52.11, 47.98, 39.91, 28.36, 22.20; HRMS (ESI) *m/z* calcd for $C_{27}H_{45}N_4O_{19}Na_2$ (M+Na) 775.2473, found 775.2480.

3-Azidopropyl *O*-(3,8-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Kdn8Deoxyα2–3LacβProN_3, 30). Yield 91%; 54 mg, white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.51 (d, 1H, *J* = 7.8 Hz), 4.48 (d, 1H, *J* = 7.8 Hz), 4.00–3.92 (m, 5H), 3.82–3.48 (m, 16H), 3.45 (t, 2H, *J* = 6.6 Hz), 3.30 (t, 1H, *J* = 7.8 Hz), 2.62 (dd, 1H, *J* = 4.2 and 12.0 Hz, H-3$_{eq}$''), 1.99–1.85 (m, 4H), 1.71 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (150 MHz, D_2O) δ 174.31, 102.84, 102.29, 100.74, 78.40, 76.89, 75.64, 75.34, 74.95, 74.49, 72.97, 70.56, 70.05, 69.59, 67.77, 67.52, 65.45, 61.19, 60.21, 58.65, 48.03, 39.40, 35.75, 28.39; HRMS (ESI) *m/z* calcd for $C_{24}H_{40}N_3O_{19}Na_2$ (M+Na) 720.2051, found 720.2059.

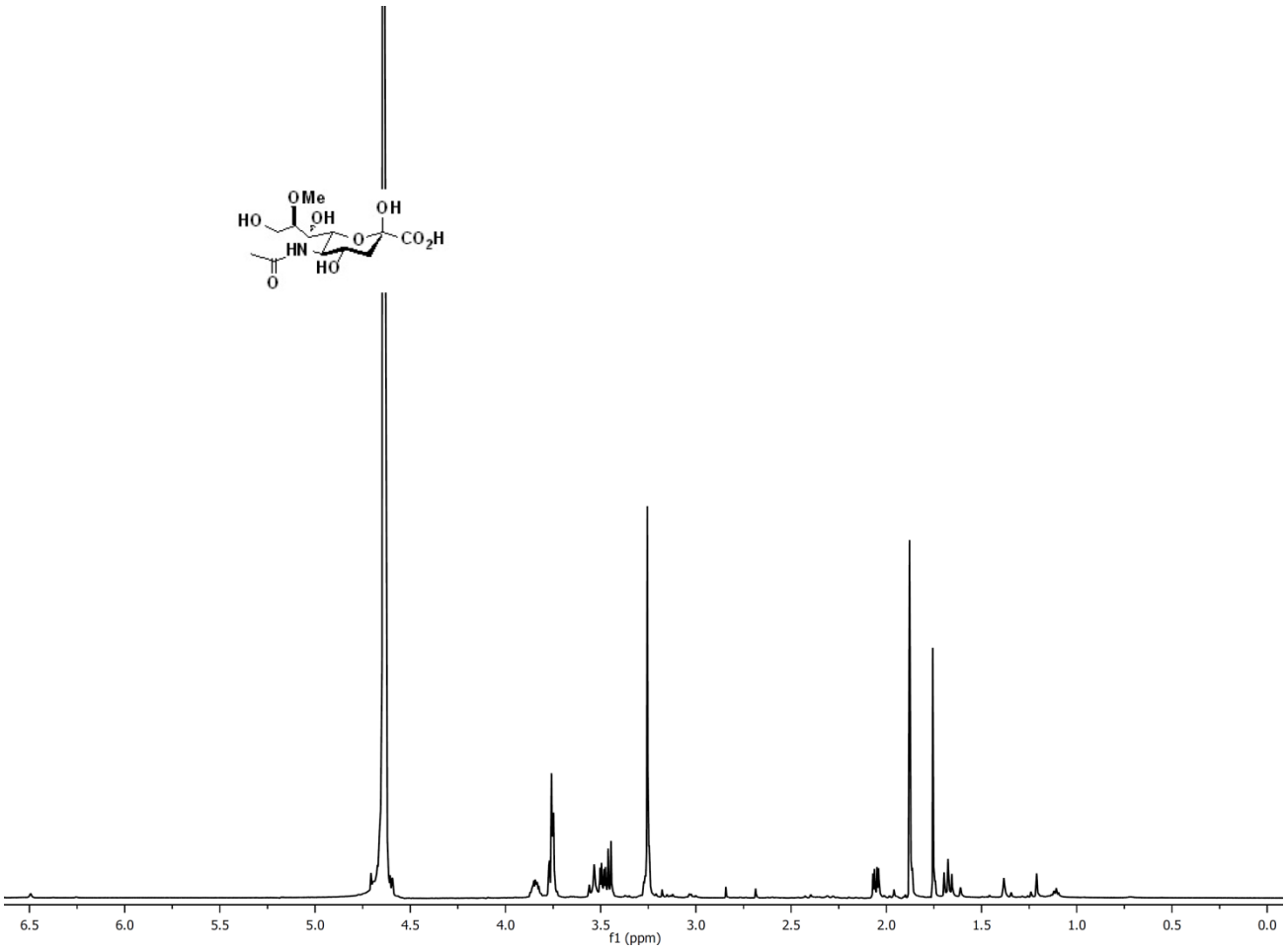
3-Azidopropyl *O*-(5-acetamido-3,5-dideoxy-8-*O*-methyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5Ac8Meα2–6LacβProN_3, 31). Yield 95%; 82 mg, white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.64 (d, 1H, *J* = 7.8 Hz), 4.40 (d, 1H, *J* = 8.4 Hz), 4.00–3.92 (m, 4H), 3.85 (t, 1H, *J* = 10.2 Hz), 3.81–3.49 (m, 15H), 3.47 (s, 3H), 3.46 (m, 1H), 3.44 (t, 2H, *J* = 6.6 Hz), 3.30 (t, 1H, *J* = 8.4 Hz), 2.56 (dd, 1H, *J* = 4.2 and 12.0 Hz, H-3$_{eq}$''), 2.00 (s, 3H), 1.88 (m, 2H), 1.67 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (150 MHz, D_2O) δ 174.98, 173.84, 103.33, 102.14, 100.74, 80.60, 79.69, 73.88, 72.89, 72.66, 72.50, 70.92, 68.63, 68.16, 67.55, 67.46, 63.62, 61.57, 60.35, 59.92, 59.46, 57.82, 52.23, 48.01, 40.27, 28.37, 22.26; HRMS (ESI) *m/z* calcd for $C_{27}H_{45}N_4O_{19}Na_2$ (M+Na) 775.2473, found 775.2486.

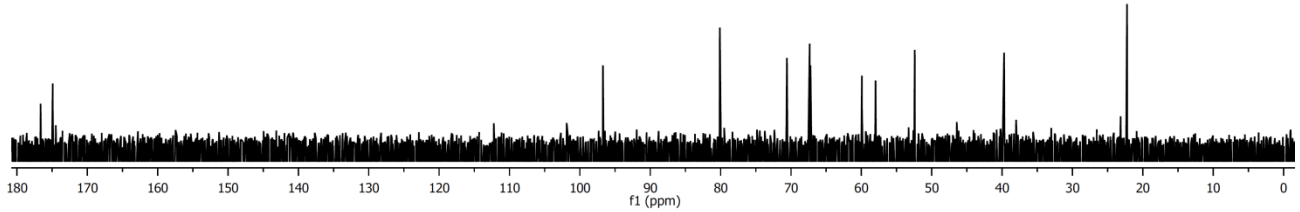
3-Azidopropyl *O*-(3,8-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Kdn8Deoxyα2–6LacβProN_3, 32). Yield, 92%; 61 mg, white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.49 (d, 1H, *J* = 7.8 Hz), 4.42 (d, 1H, *J* = 7.8 Hz), 4.06–3.93 (m, 5H), 3.80–3.49 (m, 16H), 3.45 (t, 2H, *J* = 6.6 Hz), 3.32 (t, 1H, *J* = 8.4 Hz), 2.57 (dd, 1H, *J* = 3.6 and 12.0 Hz, H-3$_{eq}$''), 2.00–1.87 (m, 4H), 1.65 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (150 MHz, D_2O) δ 174.06, 103.35, 102.18, 100.71, 79.75, 76.59, 74.80, 74.76, 73.95, 72.88, 72.50, 70.96, 70.74, 69.90, 68.66, 67.50, 65.86, 63.58, 60.40, 58.73, 48.03, 39.49, 35.82, 28.39; HRMS (ESI) *m/z* calcd for $C_{24}H_{40}N_3O_{19}Na_2$ (M+Na) 720.2051, found 720.2050.

Reference

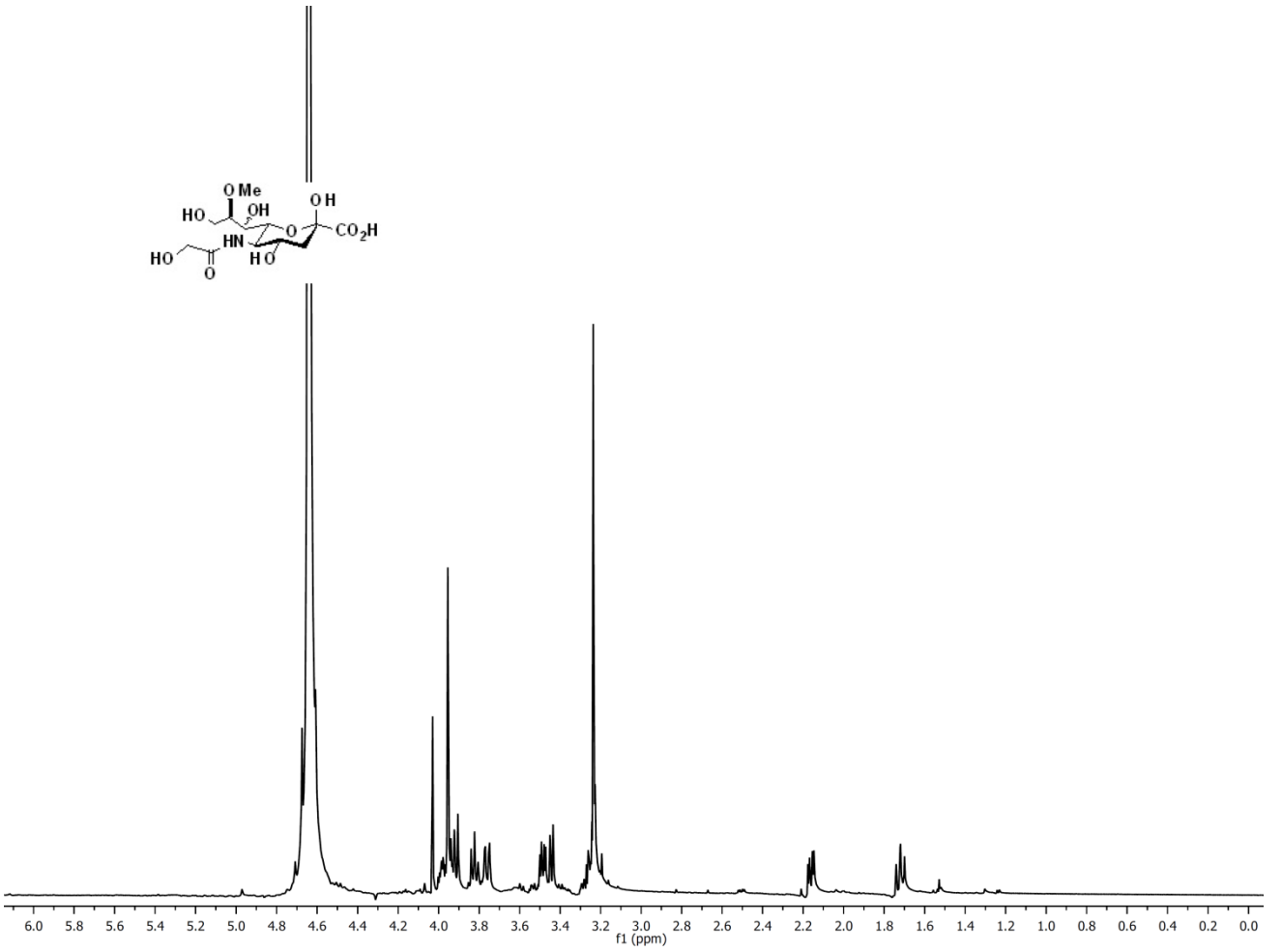
(1) Shing, T. K. M.; Zhong, Y. L. *Tetrahedron* **2001**, *57*, 1573.
(2) Suhara, Y.; Ono, K.; Yoshida, A.; Fujishima, T.; Saito, N.; Honzawa, S.; Kishimoto, S.; Sugiura, T.; Waku, K.; Takayama, H.; Kittaka, A. *Heterocycles* **2004**, *62*, 423.

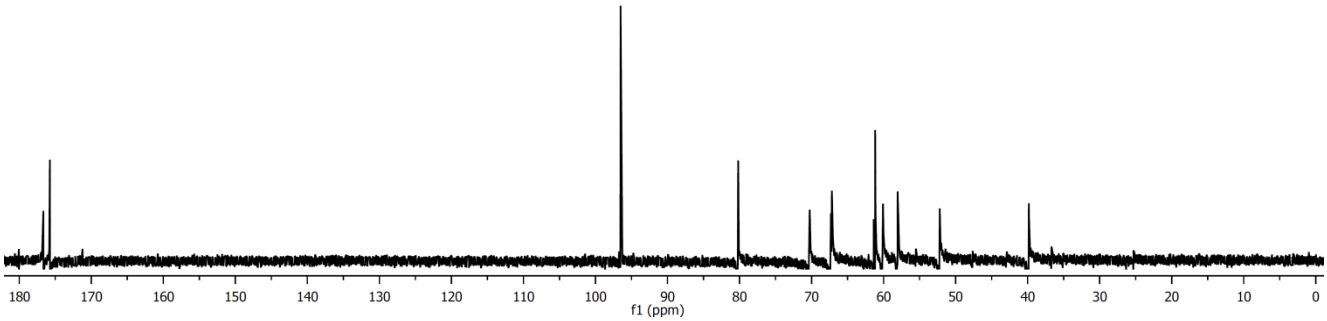
^{1}H and ^{13}C NMR for Neu5Ac8Me (**1**)



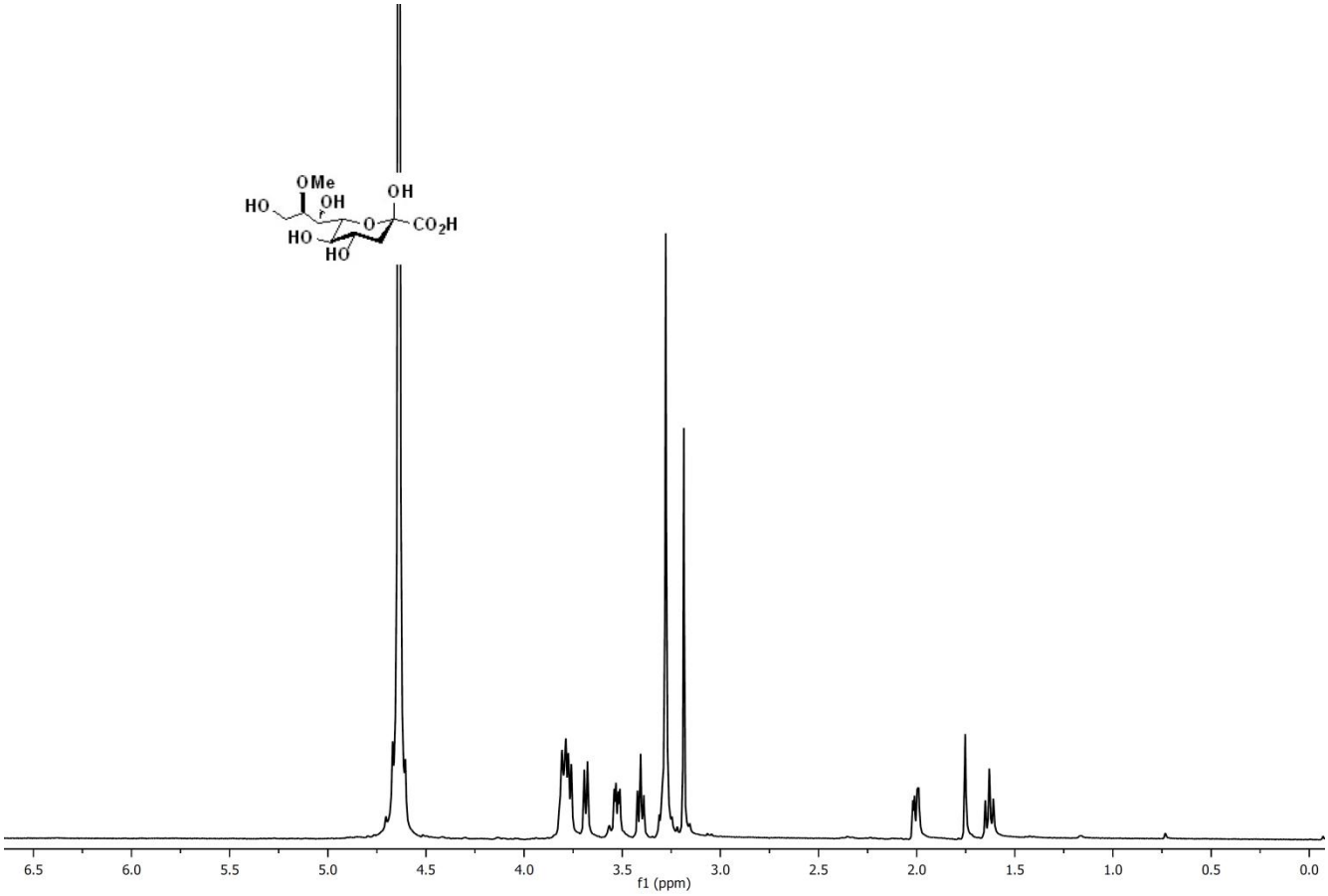


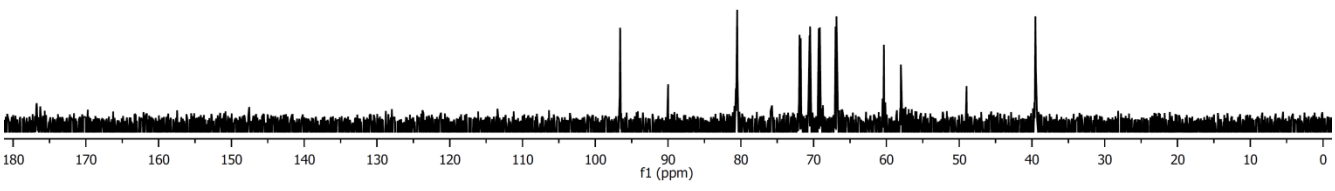
^{1}H and ^{13}C NMR for Neu5Gc8Me (**2**)



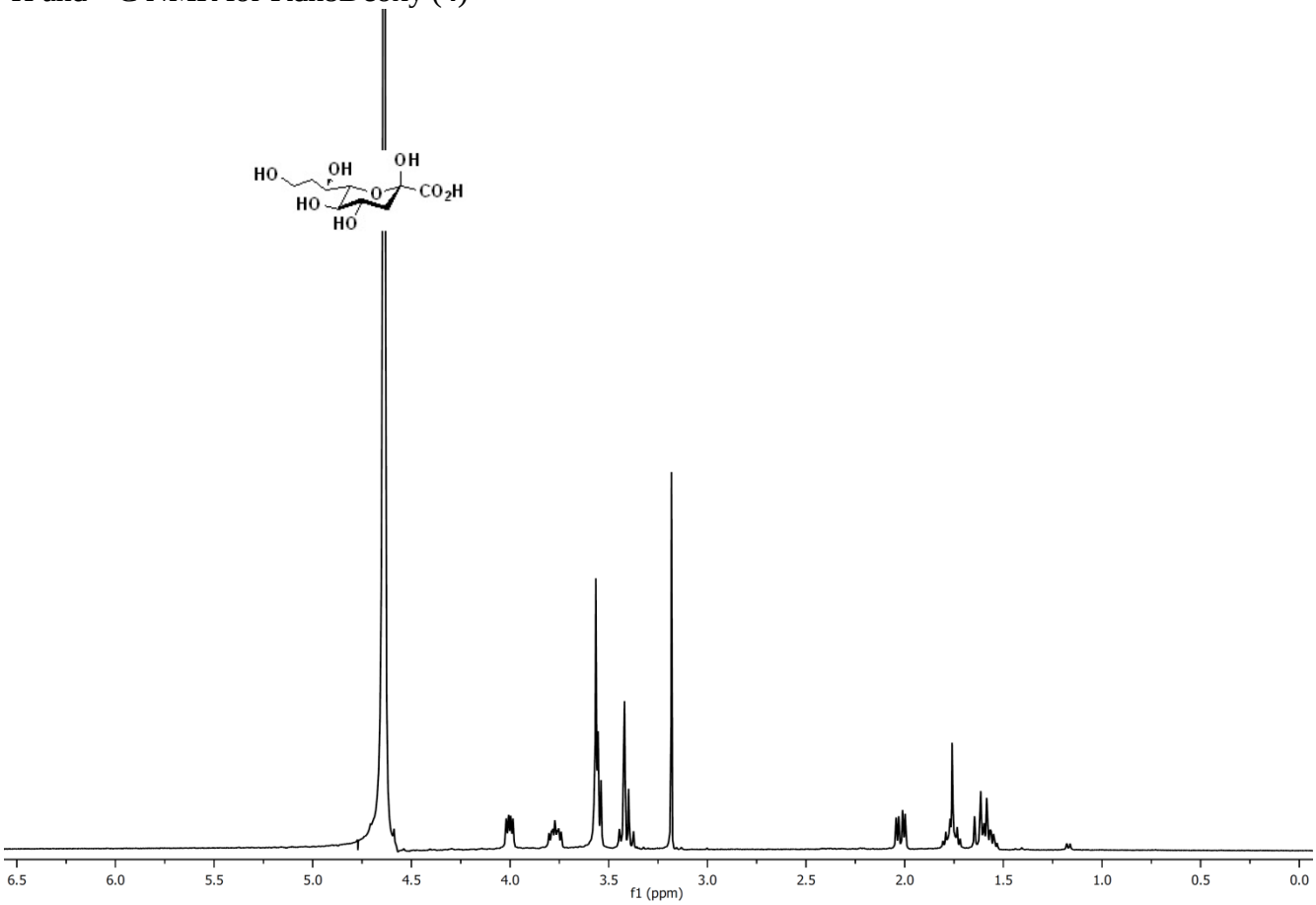


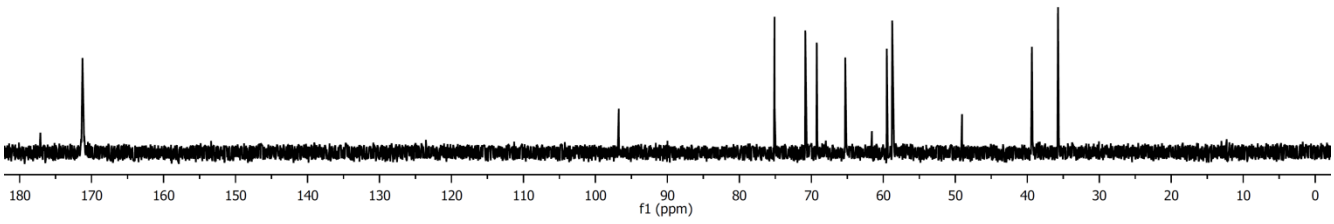
^{1}H and ^{13}C NMR for Kdn8Me (**3**)



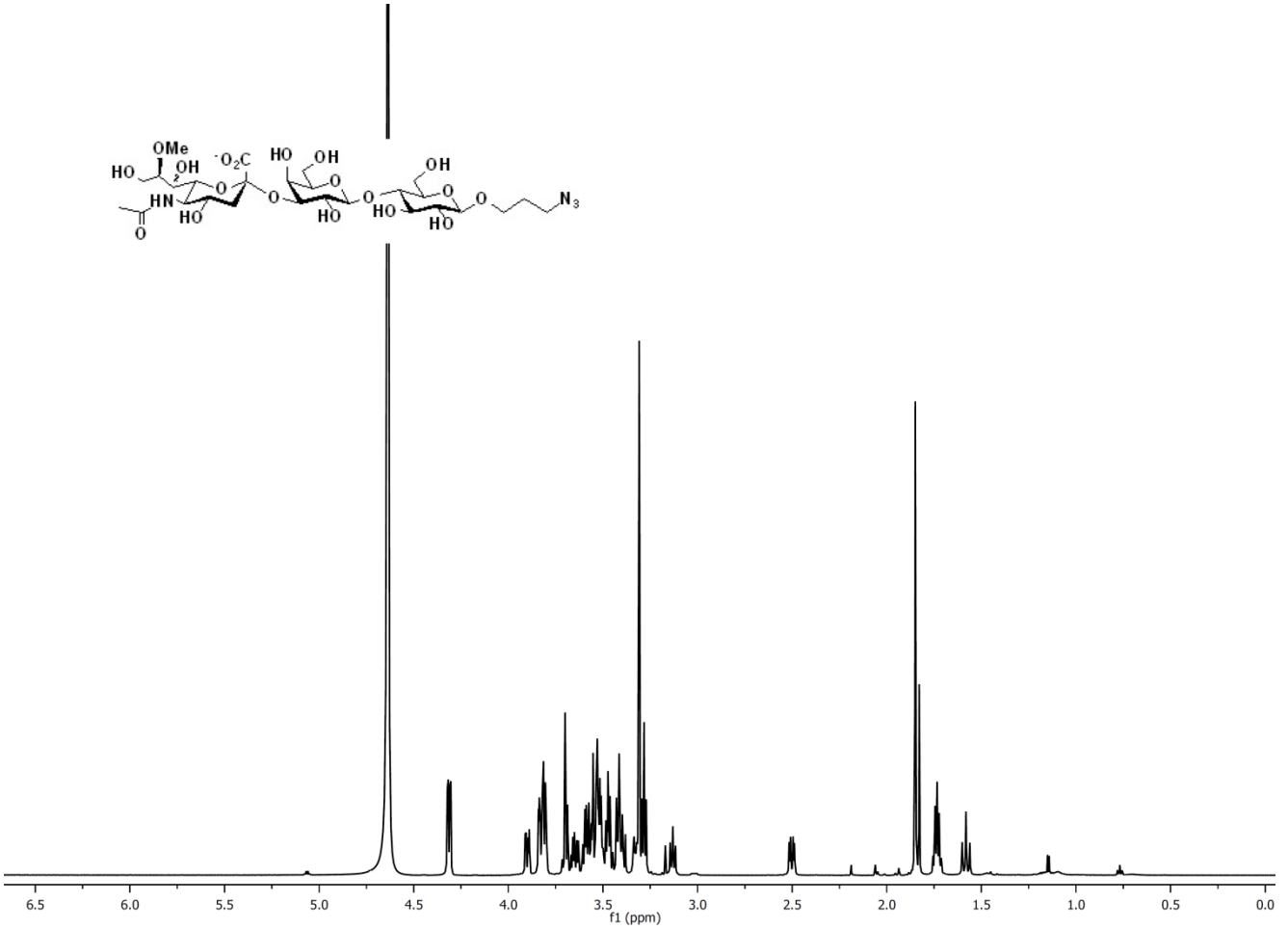


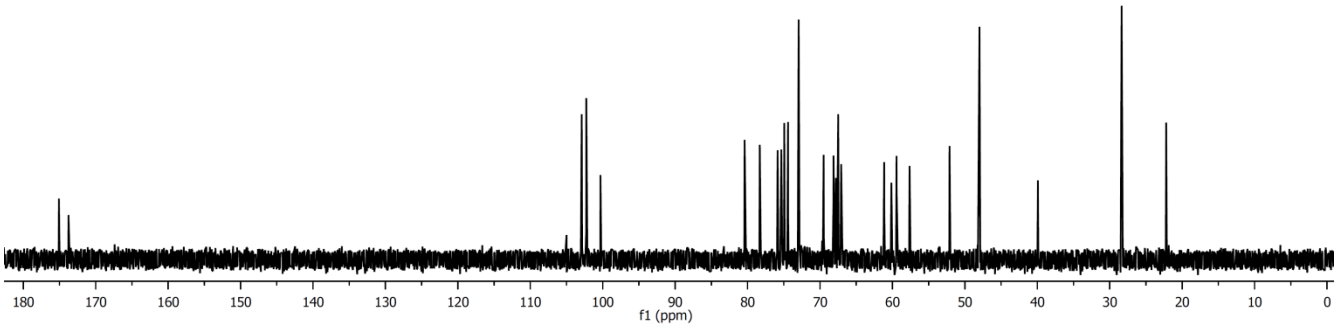
^{1}H and ^{13}C NMR for Kdn8Deoxy (**4**)



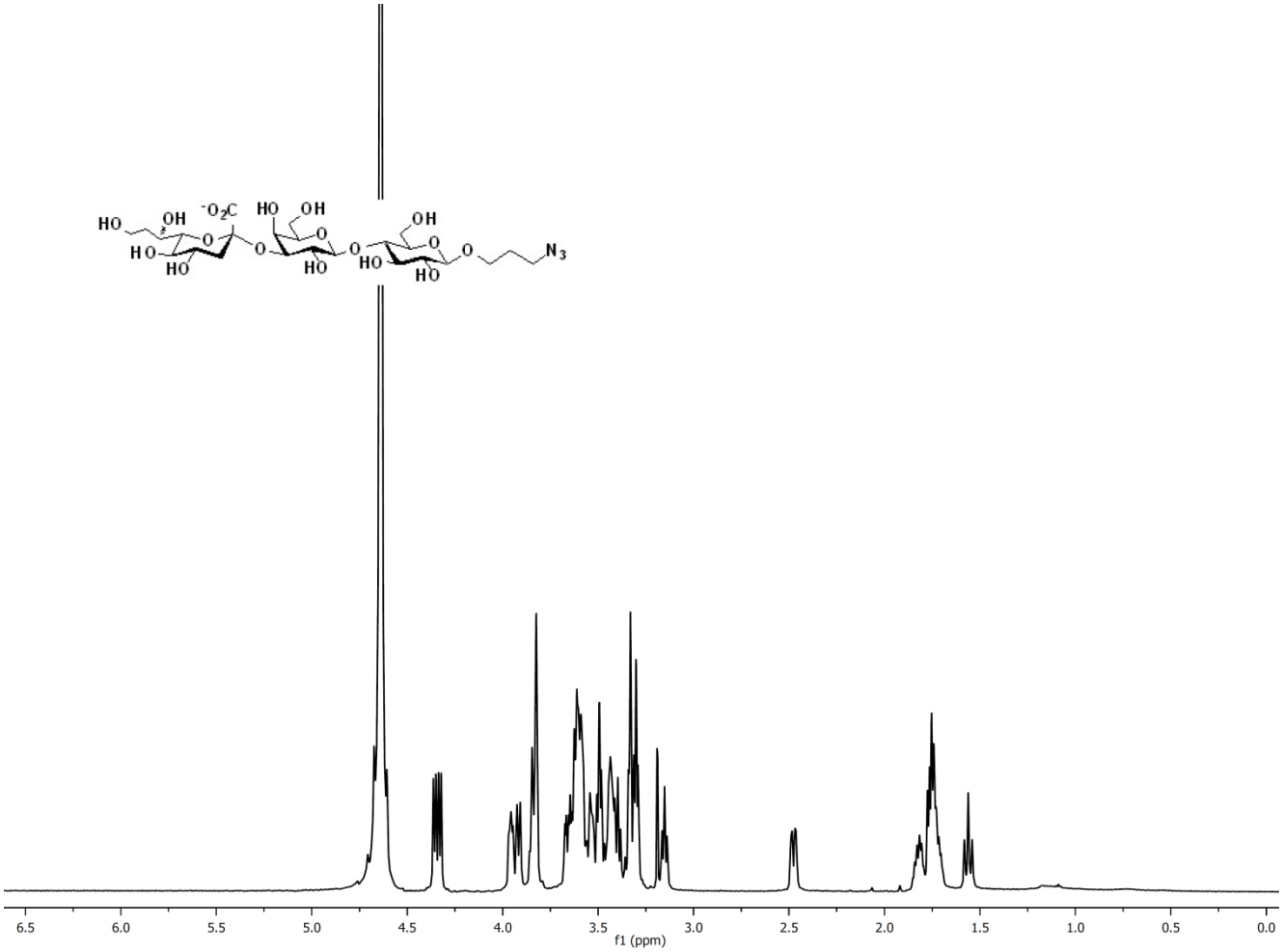


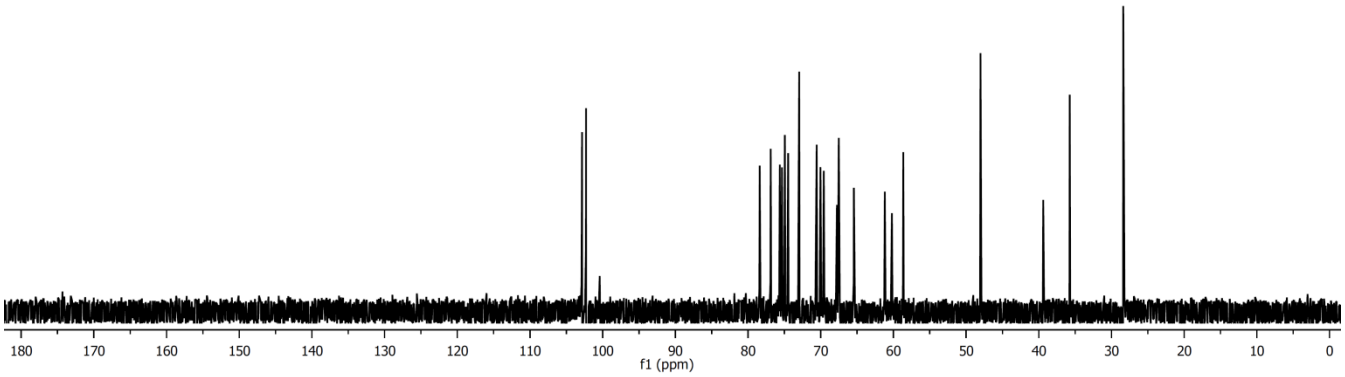
^{1}H and ^{13}C NMR for Neu5Ac8Meα2–3LacProN_3 (**29**)



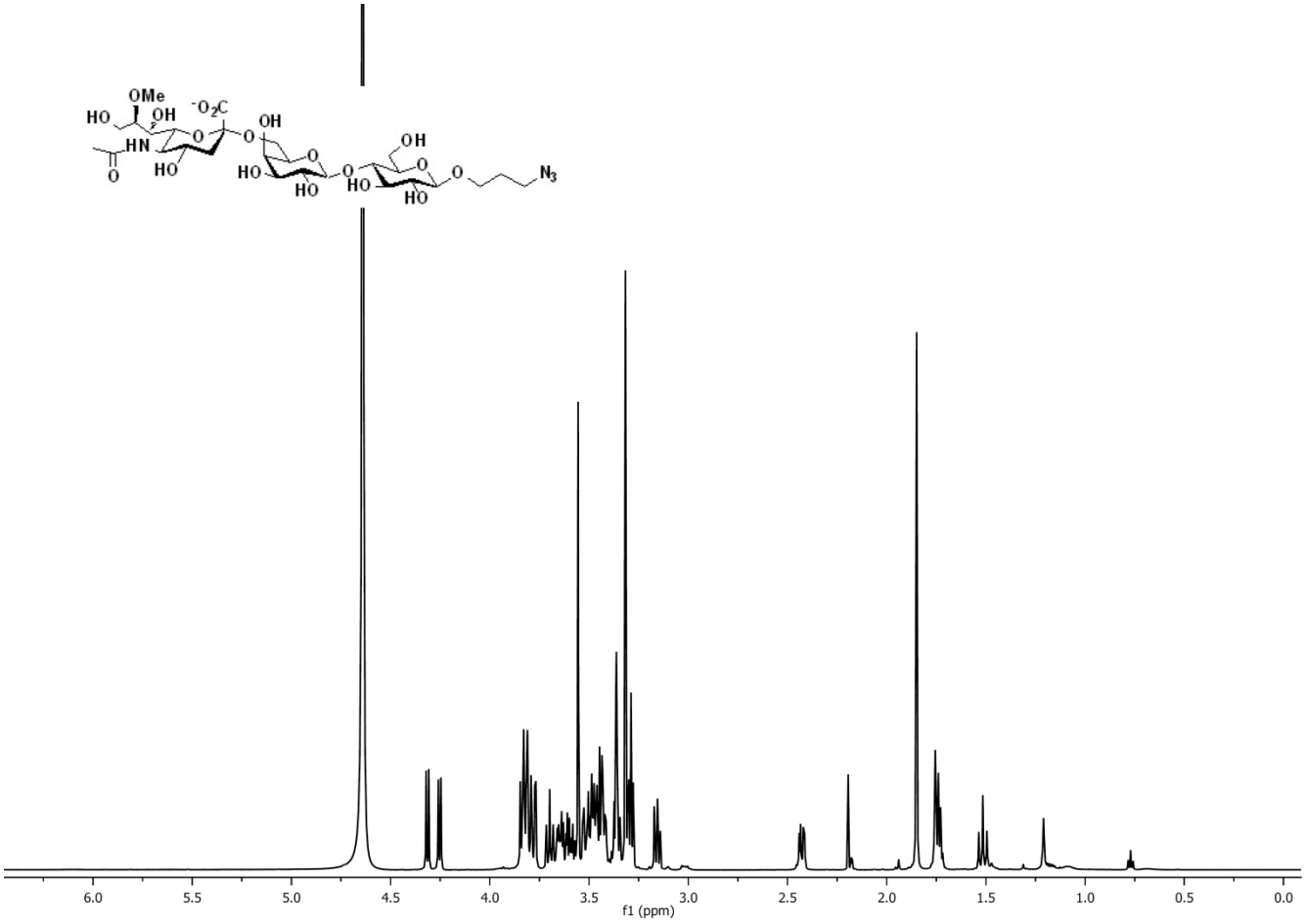


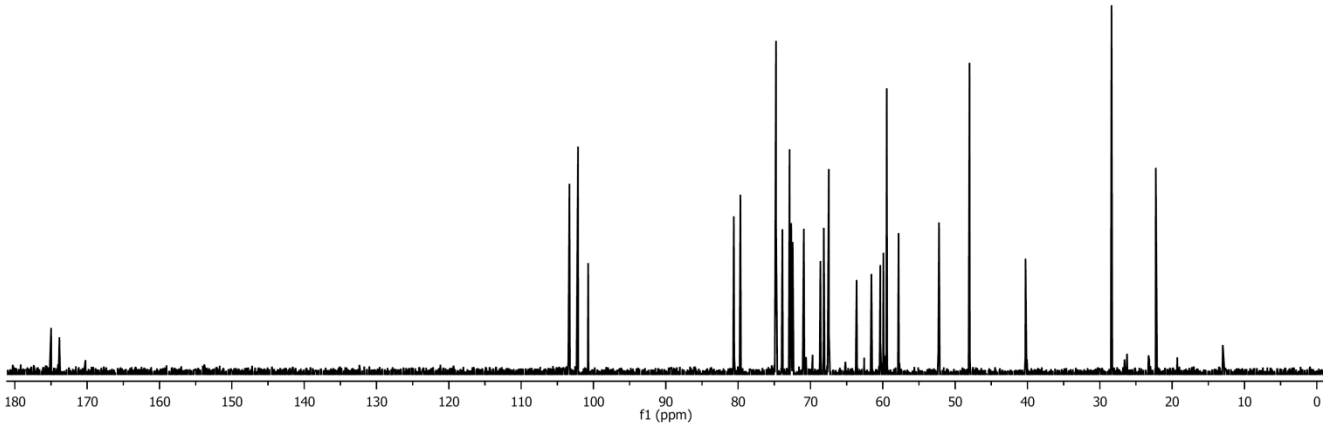
^{1}H and ^{13}C NMR for Kdn8Deoxyα2–3LacProN_3 (**30**)





^{1}H and ^{13}C NMR for Neu5Ac8Meα2–6LacProN_3 (**31**)

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90
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60
50
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30
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f1 (ppm)

^{1}H and ^{13}C NMR for Kdn8Deoxyα2–6LacProN_3 (**32**)