Supporting Information

for

Probe sialidase substrate specificity using chemoenzymatically synthesized sialosides containing C9-modified sialic acids

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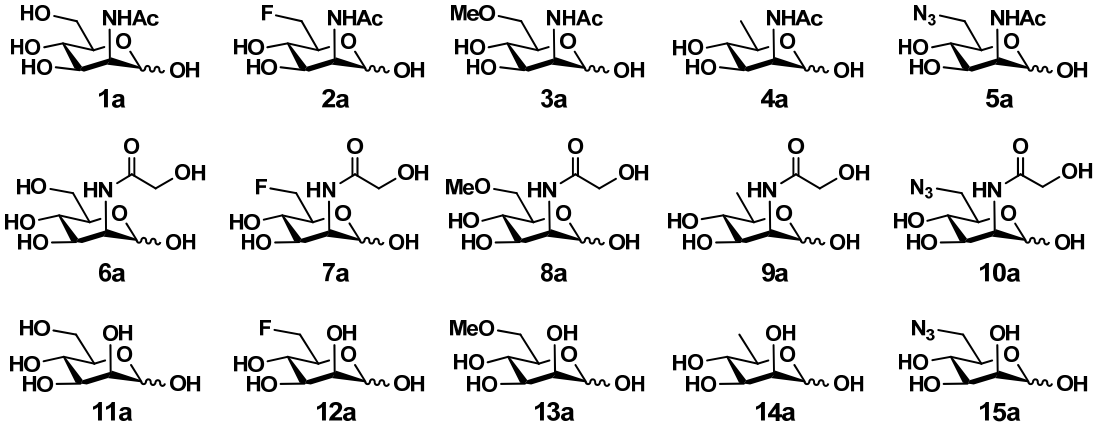
General methods

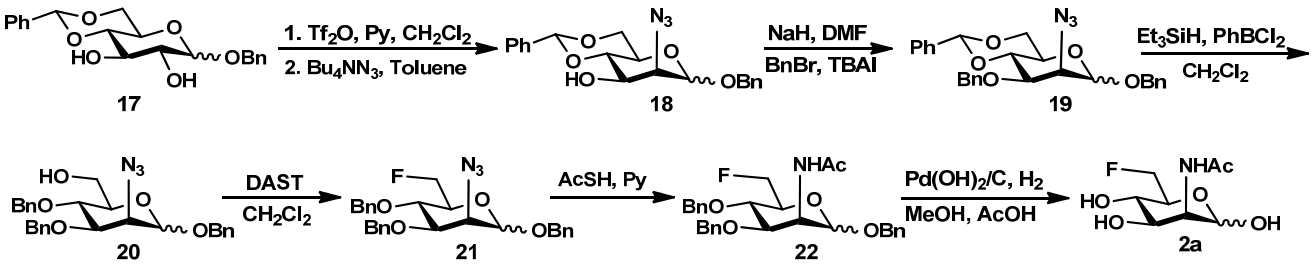
All chemicals were obtained from commercial suppliers and used without further purification unless otherwise noted. Anhydrous solvents were used to carry out organic reactions under inert argon or nitrogen environment. ^{1}H NMR (600 MHz) and ^{13}C NMR (151 MHz) spectra were recorded on a Varian Inova-600 spectrometer and ^{19}F NMR (282 MHz) spectra were recorded on a Mercury-300 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 (200–425 mesh, Fisher Chemical) was used for flash column chromatography. Thin-layer chromatography (TLC) was performed on silica gel plates 60 GF_{254} (Sorbent technologies) using anisaldehyde sugar stain or 5% sulfuric acid in ethanol stain for detection. Gel filtration chromatography was performed using a column (100 cm × 2.5 cm) packed with Bio-gel P-2 Fine resins (Bio-Rad, Hercules, CA).

Synthesis of ManNAc, ManNGc, and mannose derivatives as precursors for sialosides



N-Acetylmannosamine (ManNAc) **1a** and mannose **11a** were purchased from Sigma. *N*-Glycolylmannosamine (ManNGc) **6a** and mannose derivatives 6-N_3-mannose **5a** and 6-N_3-ManNAc **15a** were synthesized as described previously.[1, 2] Compounds **2a–4a, 7a–10a, and 12a–14a** were synthesized as described in the following:

2-Acetamido-2,6-dideoxy-6-fluoro-D-mannopyranoside (6-F-ManNAc, 2a)



To a solution of compound **17**[3] (9.04 g, 25.22 mmol) in CH_2Cl_2 (100 mL) and pyridine (20 mL), trifluoromethanesulfonic acid anhydride (Tf_2O) (4.67 mL, 27.74 mmol) was added drop wisely at -20 °C. The reaction mixture was stirred at between -20 °C to 0 °C for 3 h. The solvent was removed by rotovap, co-evaporated with toluene, and dried under vacuum for overnight. The crude product was then dissolved in anhydrous toluene (150 mL) and tetra-*n*-butylammonium azide (10.76 g, 37.82 mmol) was added. The mixture was stirred at 65 °C for 1 h and then 100 °C for 1.5 h. After cooled, the reaction mixture was diluted with toluene, washed with water, and concentrated. The residue was subjected to silica gel column chromatography (Hexanes:EtOAc = 9:1) to give **18** (8.02 g, 83%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51–7.50 (m, 2H), 7.42–7.35 (m, 8H), 5.57 (s, 1H), 4.87 (s, 1H), 4.72 (d, *J* = 12.0 Hz, 1H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.29–4.27 (m, 1H), 4.23 (dd, *J* = 9.0, 3.6 Hz, 1H), 3.94–3.90 (m, 2H), 3.84–3.79 (m, 2H), 2.89 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 137.10, 136.59, 129.43, 128.70, 128.50, 128.32, 128.17, 126.37, 102.35, 98.32, 79.10, 69.64, 68.96, 68.70, 63.83, 63.71.

A solution of **18** (5.50 g, 14.34 mmol) in dry DMF (40 mL) was stirred with NaH (0.52 g, 21.51 mmol) for 30 min at 0 °C. Tetra-*n*-butylammonium iodide (0.53 g, 1.43 mmol) and benzyl bromide (3.5 mL, 28.70 mmol) were added and the reaction was stirred at 0 °C to room temperature for

overnight. The reaction was stopped by adding MeOH (2 mL), CH_2Cl_2 was then added and the mixture was washed with water, dried over $MgSO_4$, and concentrated. Silica gel column chromatography of the residue (Hexanes:EtOAc = 9.5:0.5) gave **19** (6.45 g, 95%).

Compound **19** (1.85 g, 3.90 mmol) was dissolved in anhydrous CH_2Cl_2 (7 mL) with 4 Å molecular sieves (1.5 g) and stirred for 1 h at room temperature. The resulting mixture was cooled to -78 °C and Et_3SiH (0.33 mL, 2.08 mmol) and $PhBCl_2$ (0.24 mL, 1.88 mmol) were added successively to the stirred solution. After being stirred at -78 °C for 1.5 h, Et_3N (1 mL) and MeOH (1 mL) were added successively and the mixture was filtered over Celite and concentrated. The residue was diluted with CH_2Cl_2, washed with an aqueous solution of $NaHCO_3$, dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel column (Hexanes:EtOAc = 8:2) to give **20** (1.76 g, 95%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.47 (d, *J* = 6.6 Hz, 2H), 7.44–7.36 (m, 13H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.93 (d, *J* = 1.8 Hz, 1H), 4.80 (dd, *J* = 19.2, 11.4 Hz, 2H), 4.74 (d, *J* = 3.0 Hz, 1H), 4.73 (d, *J* = 1.8 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.22 (dd, *J* = 6.0, 3.6 Hz, 1H), 4.03 (dd, *J* = 3.6, 1.8 Hz, 1H), 4.00 (t, *J* = 9.6 Hz, 1H), 3.90–3.84 (m, 2H), 3.79–3.76 (m, 1H), 2.44 (t, *J* = 5.4 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.10, 137.77, 136.68, 128.55, 128.51, 128.14, 128.09, 128.07, 128.05, 127.92, 127.90, 97.45, 79.77, 75.41, 74.24, 72.64, 72.40, 69.35, 61.81, 61.43.

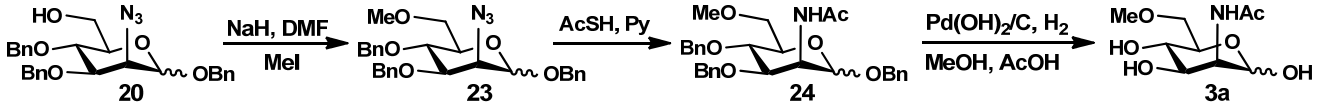
To a solution of **20** (1.48 g, 3.11 mmol) in anhydrous CH_2Cl_2 (15 mL) in a Teflon flask, (diethylamino)sulfur trifluoride (DAST) (2.05 mL, 15.55 mmol) was slowly added at -40 °C. The reaction was stirred for 3 days at room temperature. After cooled to -20 °C, MeOH (5 mL) was added and the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2, washed with water for 3 times, dried over $MgSO_4$, and concentrated. The crude product was purified using flash column chromatography (Hexanes:EtOAc = 10:1) to yield **21** (0.77 g, 52%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42–7.30 (m, 15 H), 4.94 (d, *J* = 10.8 Hz, 1H), 4.90 (s, 1H), 4.76 (dd, *J* = 24.0, 11.4 Hz, 2H), 4.70–4.59 (m, 4H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.16 (dd, *J* = 9.0, 3.6 Hz, 1H), 3.98 (dd, *J* = 3.6, 1.8 Hz, 1H), 3.92 (t, *J* = 9.6 Hz, 1H), 3.83–3.76 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 137.99, 137.74, 136.70, 128.65, 128.23, 128.06, 97.58, 82.03 (d, *J* = 175.9 Hz), 79.87, 75.60, 73.58, 72.77, 71.37 (d, *J* = 18.6 Hz), 69.61, 61.39; ^{19}F NMR (282 MHz, $CDCl_3$) δ -233.30 (ddd, *J* = 48.2, 48.2, 27.3 Hz, 1F).

Compound **21** (0.78 g, 1.63 mmol) was dissolved in pyridine (5 mL) and thioacetic acid (10 mL) and stirred for overnight at room temperature. The solvent was removed under diminished pressure and the crude product was purified using silica gel column chromatography (Hexanes:EtOAc = 8:2) to afford compound **22** (0.68 g, 84%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.28 (m, 15H), 5.70 (d, *J* = 8.4 Hz, 1H), 4.98 (d, *J*= 1.8 Hz, 1H), 4.95 (d, *J* = 10.2 Hz, 1H), 4.76–4.45 (m, 8H), 4.14 (dd, *J* = 9.6, 4.8 Hz, 1H), 3.77 (dd, *J* = 10.2, 3.0 Hz, 1H), 3.65 (t, *J* = 9.9 Hz, 1H), 2.05 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.44, 138.22, 137.97, 136.94, 128.71, 128.69, 128.67, 128.50, 128.23, 128.18, 128.07, 99.10, 82.75 (d, *J* = 172.1 Hz), 77.75, 75.64, 73.64, 73.38 (d, *J* = 6.0 Hz), 71.52, 70.55 (d, *J* = 16.6 Hz), 70.11, 49.59, 23.73; ^{19}F NMR (282 MHz, $CDCl_3$) δ -234.29 (ddd, *J* = 47.4, 47.4, 26.5 Hz, 1F).

Compound **22** (0.68 g, 1.38 mmol) was dissolved in AcOH (5 mL) and MeOH (25 mL), and $Pd(OH)_2/C$ (80 mg) was added. The resulted mixture was hydrogenated for 2 days with vigorous shaking under H_2 atmosphere (80 mbar), and then the catalyst was removed by filtration over Celite and washed with MeOH. The concentrated product was purified by flash column chromatography

(EtOAc:MeOH:H_2O = 10:1:0.1) to afford **2a** (0.26 g, 85%). ^{1}H NMR (600 MHz, D_2O) δ 5.13 (s, 0.7H), 5.05 (d, *J* = 1.2 Hz, 0.3H), 4.75–4.60 (m, 2H), 4.46 (dd, *J* = 4.8, 1.8 Hz, 0.3H), 4.31 (d, *J* =1.2 Hz, 0.7H), 4.08–3.54 (m, 3H), 2.08 (s, 1H), 2.04 (s, 2H); ^{13}C NMR (151 MHz, D_2O) δ 175.90, 174.99, 93.31, 93.28, 82.99 (d, *J* = 21.6 Hz), 81.85 (d, *J* = 21.6 Hz), 75.09 (d, *J* = 18.1 Hz), 71.92, 70.96 (d, *J* = 17.3 Hz), 68.65, 65.96 (d, *J* = 7.2 Hz), 65.74 (d, *J* = 4.1 Hz), 54.07, 53.37, 22.19, 22.03; ^{19}F NMR (282 MHz, D_2O) δ -234.17 (ddd, *J* = 47.9, 47.9, 25.4 Hz, 0.4F), -235.04 (ddd, *J* = 50.7, 50.7, 19.7 Hz, 0.6F).

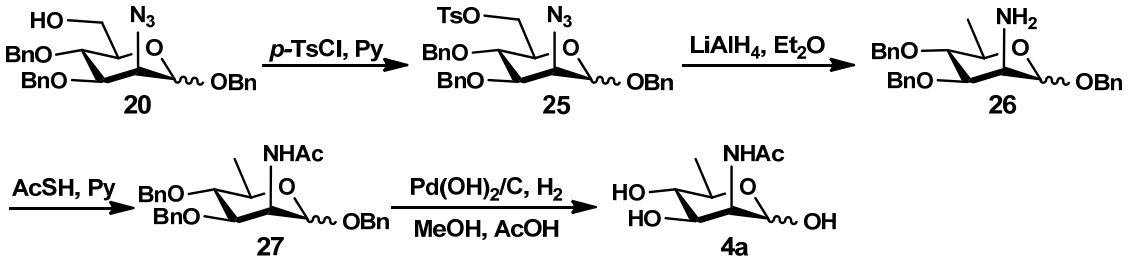
2-Acetamido-2-deoxy-6-*O*-methyl-D-mannopyranoside (6-*O*-Me-ManNAc, 3a)



A solution of **20** (1.70 g, 3.57 mmol) in anhydrous DMF (15 mL) was stirred with NaH (0.35 g, 14.30 mmol) at room temperature for 30 min. Methyl iodide (0.9 mL, 14.30 mmol) was added slowly at 0 °C and the mixture was stirred for overnight at room temperature. The mixture was diluted with CH_2Cl_2, washed with water, dried over $MgSO_4$, and concentrated. Purification of the residue by a silica gel column chromatography (Hexanes:EtOAc = 9.5:0.5) gave **23** (1.69 g, 97%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40–7.29 (m, 15H), 4.91-4.89 (m, 2H), 4.74 (dd, *J* = 19.8, 11.4 Hz, 2H), 4.62 (d, *J* = 10.8 Hz, 1H), 4.47 (d, *J* = 12.0 Hz, 1H), 4.12 (dd, *J* = 9.6, 3.6 Hz, 1H), 3.97 (dd, *J* = 3.6, 1.2 Hz, 1H), 3.93 (t, *J* = 9.9 Hz, 1H), 3.78–3.76 (m, 1H), 3.64 (dd, *J* = 10.8, 4.2 Hz, 1H), 3.58 (dd, *J* = 10.8, 1.8 Hz, 1H), 3.41 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.49, 138.09, 137.05, 128.71, 128.67, 128.27, 128.06, 97.70, 80.05, 75.59, 74.67, 72.84, 71.83, 71.43, 69.45, 61.70, 59.56.

Compound **23** (1.26 g, 2.57 mmol) was then dissolved in pyridine (5 mL) and thioacetic acid (10 mL) and stirred for overnight at room temperature. The solvent was removed under diminished pressure and the crude product was purified using silica gel column (Hexanes:EtOAc = 7:3) to afford compound **24** (1.09 g, 84%), which was dissolved in AcOH (5 mL) and MeOH (25 mL), and $Pd(OH)_2/C$ (80 mg) was added. The resulted mixture was hydrogenated for 2 days with vigorous shaking under H_2 atmosphere (80 mbar), the catalyst was removed by filtration over Celite and washed with MeOH. The concentrated product was purified by silica gel column chromatography (EtOAc:MeOH:H_2O = 10:1:0.2) to afford **3a** (0.42 g, 83%). ^{1}H NMR (600 MHz, D_2O) δ 5.11 (s, 0.6H), 5.02 (d, *J* = 1.8 Hz, 0.4H), 4.45 (dd, *J* = 1.8, 4.2 Hz, 0.4H), 4.32 (dd, *J* = 4.8, 1.2 Hz, 0.6H), 4.04 (dd, *J* = 9.6, 4.2 Hz, 0.4H), 3.96–3.94 (m, 0.6H), 3.83–3.81 (m, 0.4H), 3.76–3.69 (m, 2H), 3.62 (t, *J* = 10.2 Hz, 0.6H), 3.54–3.51 (m, 1H), 3.43 (s, 1.2H), 3.42 (s, 1.8H), 2.09 (s, 1.2H), 2.05 (s, 1.8H); ^{13}C NMR (151 MHz, D_2O) δ 175.87, 174.94, 93.33, 93.24, 75.10, 72.14, 71.01, 70.90, 70.76 68.90, 67.06, 66.73, 58.69, 58.61, 54.22, 53.36, 22.22, 22.08.

2-Acetamido-2,6-dideoxy-D-mannopyranoside (6-Deoxy-ManNAc, 4a)



A stirred solution of **20** (0.72 g, 1.51 mmol) in anhydrous pyridine (10 mL) was treated with a solution of *p*-toluenesulfonyl chloride (0.87 g, 4.53 mmol) in pyridine (2 mL) at 0 °C and the mixture was stirred for overnight at room temperature. The reaction mixture was stopped by adding MeOH (2 mL) and concentrated. Purification of the residue by column chromatography on silica gel (Hexanes:EtOAc = 8.5:1.5) afforded **25** (0.89 g, 93%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (dd, *J* = 8.4, 1.8 Hz, 2H), 7.37–7.20 (m, 17H), 4.85 (dd, *J* = 10.8, 1.8 Hz, 1H), 4.77 (s, 1H), 4.74–4.67 (m, 2H), 4.59 (dd, *J* = 12.0, 1.2 Hz, 1H), 4.50 (dd, *J* = 10.8, 1.2 Hz, 1H), 4.41 (dd, *J* = 11.4, 1.2 Hz), 4.21 (s, 2H), 4.09 (m, 1H), 3.92 (d, *J* = 1.2 Hz, 1H), 3.81–3.79 (m, 1H), 3.76–3.73 (m, 1H), 2.42 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 144.88, 137.75, 137.60, 136.55, 133.03, 129.85, 128.61, 128.21, 97.22, 79.83, 75.36, 73.76, 72.67, 70.15, 69.49, 68.81, 61.15, 21.74.

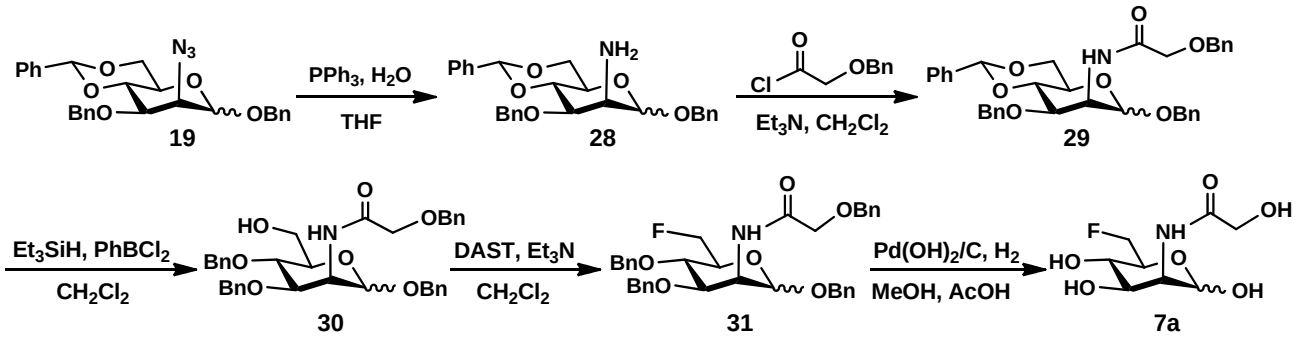
To a solution of **25** (0.89 g, 1.41 mmol) in Et_2O (50 mL), $LiAlH_4$ (0.23 g, 6.06 mmol) was slowly added and the mixture was refluxed for 50 min at 50 °C. The resulting mixture was cooled in an ice bath, diluted with toluene (35 mL) and followed by gradual addition of EtOAc (2 mL). After removing the solvents under reduced pressure, column chromatography on silica gel (Hexanes:EtOAc = 3:7) afforded **26** (0.57 g, 96%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38–7.29 (m, 15H), 4.91 (d, *J* = 10.8 Hz, 1H), 4.81 (s, 1H), 4.70–4.63 (m, 4H), 4.47 (d, *J* = 12.0 Hz, 1H), 3.92 (dd, *J* = 6.0, 1.8 Hz, 1H), 3.84–3.80 (m, 1H), 3.42 (t, *J* = 9.6 Hz, 1H), 3.40 (d, *J* = 3.6 Hz, 1H), 1.52 (s, 2H), 1.32 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.59, 138.53, 137.55, 128.57, 128.54, 128.53, 128.16, 128.08, 127.91, 127.85, 100.11, 80.37, 80.11, 75.44, 71.82, 69.16, 67.81, 52.33, 18.25.

Compound **26** (0.57 g, 1.31 mmol) was dissolved in pyridine (4 mL) and thioacetic acid (8 mL) and stirred for overnight at room temperature. The solvent was removed under diminished pressure and the crude product was purified using silica gel column (Hexanes:EtOAc = 7:3) to afford compound **27** (0.52 g, 84%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38–7.34 (m, 15H), 5.86 (d, *J* = 12.0 Hz, 1H), 4.98 (d, *J* = 12.0 Hz, 1H), 4.93 (s, 1H), 4.78–4.75 (m, 2H), 4.66 (dd, *J* = 24.0, 12.0 Hz, 2H), 4.51 (dd, *J* = 12.0, 6.0 Hz, 2H), 4.13 (dd, *J* = 4.0, 1.8 Hz, 1H), 3.89–3.84 (m, 1H), 3.24 (t, *J* = 6.0 Hz, 1H), 2.07 (s, 3H), 1.35 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.49, 138.32, 137.94, 137.03, 128.44, 128.30, 127.96, 127.92, 127.81, 98.39, 80.39, 77.59, 75.45, 71.26, 69.41, 67.11, 49.69, 23.54, 18.16.

Compound **27** (0.52 g, 1.09 mmol) was dissolved in AcOH (4 mL) and MeOH (20 mL), and $Pd(OH)_2$/C (50 mg) was added. The resulted mixture was hydrogenated for 2 days with vigorous shaking under H_2 atmosphere (80 mbar), and then the catalyst was removed by filtration over Celite and washed with MeOH. The concentrated product was purified by flash column chromatography (EtOAc:MeOH:H_2O = 10:1:0.1) to afford **4a** (0.18 g, 82%). ^{1}H NMR (600 MHz, D_2O) δ 5.07 (s,

0.6H), 5.00 (s, 0.4H), 4.45 (d, *J* = 3.6 Hz, 0.4H), 4.31 (d, *J* = 4.8 Hz, 0.6H), 4.01 (dd, *J* = 9.6, 4.8 Hz, 0.5H), 3.93–3.91 (m, 0.5H), 3.78 (dd, *J* = 10.2, 4.8 Hz, 0.5H), 3.46–3.38 (m, 1H), 3.29 (t, *J* = 9.6 Hz, 0.5H), 2.11 (s, 1.4H), 2.07 (s, 1.6H), 1.31 (d, *J* = 6.6 Hz, 1.4H), 1.29 (d, *J* = 6.0 Hz, 1.6H); ^{13}C NMR (151 MHz, D_2O) δ 178.45, 177.53, 95.63, 95.52, 75.24, 75.20, 74.86, 74.43, 71.12, 70.81, 56.94, 56.22, 24.92, 24.73, 19.52, 19.50.

2,6-Dideoxy-2-glycolamido-6-fluoro-D-mannopyranoside (6-F-ManNGc, 7a)



Triphenylphosphine (2.05 g, 7.80 mmol) and water (1.23 mL, 0.07 mmol) were added to a stirred solution of **19** (1.23 g, 2.60 mmol) in THF (40 mL) and the mixture was heated at 45 °C for 3 h. The solvent was evaporated. Purification of the residue by a silica gel column (Hexanes:EtOAc = 9:1) afforded product **28** (1.12 g, 97%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 (d, *J* = 7.8 Hz, 2H), 7.36–7.24 (m, 13H), 5.59 (s, 1H), 4.82 (s, 1H), 4.79 (d, *J* = 11.4 Hz, 1H), 4.66 (t, *J* = 12.0 Hz, 2H), 4.46 (d, *J* = 12.0 Hz, 1H), 4.22 (dd, *J* = 10.2, 4.8 Hz, 1H), 4.05 (t, *J* = 9.0 Hz, 1H), 3.97 (dd, *J* = 10.2, 4.8 Hz, 1H), 3.94–3.88 (m, 1H), 3.82 (t, *J* = 10.2 Hz, 1H), 3.43 (d, *J* = 3.6 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.51, 137.59, 137.03, 128.84, 128.47, 128.33, 128.17, 128.02, 127.94, 127.58, 126.05, 101.56, 100.99, 78.90, 75.75, 72.74, 69.23, 68.90, 63.86, 53.72.

Compound **28** (1.12 g, 2.50 mmol) was dissolved in CH_2Cl_2 (20 mL) and Et_3N (0.72 mL, 5.20 mmol) was added drop wisely at 0 °C. Benzyloxyacetyl chloride (0.49 mL, 3.12 mmol) was added to the reaction mixture and stirred at 0 °C for 2 h and then room temperature for 2 h. The residue was concentrated and purified by silica gel flash column chromatography (Hexanes:EtOAc = 7:3) to give **29** (1.43 g, 96%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53–7.52 (d, *J* = 7.8 Hz, 2H), 7.42–7.26 (m, 18H), 6.99 (d, *J* = 7.8 Hz, 1H), 5.54 (s, 1H), 5.04 (s, 1H), 4.76–4.65 (m, 4H), 4.63–4.54 (m, 4H), 4.24–3.93 (m, 4H), 3.76–3.69 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.25, 138.13, 137.43, 137.00, 136.83, 129.09, 128.75, 128.65, 128.43, 128.34, 128.31, 128.19, 128.14, 127.89, 127.81, 127.73, 126.20, 101.79, 99.28, 79.01, 73.58, 73.44, 71.82, 69.86, 69.63, 68.91, 63.48, 50.46.

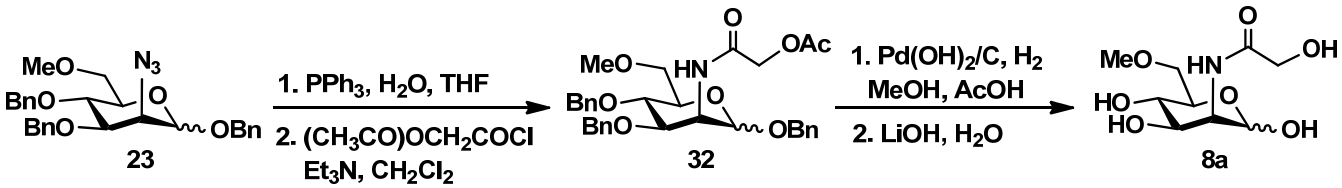
Compound **29** (0.37 g, 0.62 mmol) was dissolved in anhydrous CH_2Cl_2 (7 mL) containing 4 Å molecular sieves (1.5 g). After the mixture was stirred for 1 h at room temperature, it was cooled to -78 °C and Et_3SiH (0.30 mL, 1.85 mmol) and $PhBCl_2$ (0.27 mL, 2.10 mmol) were added successively to the stirred solution. After being stirred at -78 °C for 1.5 h, Et_3N (1 mL) and MeOH (1 mL) were added successively and the mixture was filtered over Celite and concentrated. The residue was diluted with CH_2Cl_2, washed with an aqueous solution of $NaHCO_3$, dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel flash column chromatography (Hexanes:EtOAc = 1:1) to give **30**

(0.31 g, 85%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39–7.30 (m, 20H), 6.95 (d, *J* = 9.0 Hz, 1H), 5.01 (s, 1H), 4.92 (d, *J* = 10.8 Hz, 1H), 4.80 (d, *J* = 10.8 Hz, 1H), 4.76 (dd, *J* = 9.0, 4.2 Hz, 1H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.62–4.60 (m, 2H), 4.56–4.53 (m, 3H), 4.20 (dd, *J* = 9.0, 4.0 Hz, 1H), 4.05 (dd, *J* = 24.6, 15.6 Hz, 2H), 3.81–3.74 (m, 3H), 3.62 (t, *J* = 9.6 Hz, 1H), 1.89 (s, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.27, 138.37, 138.20, 137.13, 137.05, 128.88, 128.75, 128.74, 128.66, 128.64, 128.41, 128.39, 128.28, 128.12, 127.99, 127.88, 98.59, 77.89, 75.47, 74.14, 73.63, 71.66, 71.46, 69.85, 69.73, 62.13, 49.21.

To a solution of **30** (0.31 g, 0.52 mmol) and Et_3N (0.14 mL, 1.04 mmol) in anhydrous CH_2Cl_2 (12 mL) in a Teflon flask, DAST (0.27 mL, 2.08 mmol) was slowly added at -40 °C. The reaction was stirred for 3 days at room temperature. After cooled to -20 °C, MeOH (1 mL) was added and the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2, washed with water for 3 times, dried over $MgSO_4$, and concentrated. The crude product was purified using silica gel flash column chromatography (Hexanes:EtOAc = 7:3) to yield **31** (0.14 g, 45%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.26 (m, 20 H), 6.91 (d, *J* = 8.4 Hz, 1H), 4.99 (s, 1H), 4.89 (d, *J* = 10.8 Hz, 1H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.76–4.74 (m, 1H), 4.67–4.50 (m, 8H), 4.16 (dd, *J* = 9.6, 4.2 Hz, 1H), 4.03 (d, *J* = 4.8 Hz, 2H), 3.80 (dd, *J* = 28.2, 10.2 Hz, 1H), 3.60 (t, *J* = 9.6 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.12, 138.11, 138.02, 137.00, 136.87, 128.75, 128.64, 128.59, 128.53, 128.33, 128.18, 128.16, 128.03, 127.89, 127.73, 98.68, 82.03, (d, *J* = 173.5 Hz), 77.76, 75.52, 73.51, 73.28 (d, *J* = 6.8 Hz), 71.34, 70.59 (d, *J* = 18.6 Hz), 69.93, 69.59, 48.90; ^{19}F NMR (282 MHz, $CDCl_3$) δ -234.76 (ddd, *J* = 48.8, 48.8, 30.4 Hz, 1F).

Compound **31** (0.14 g, 0.23 mmol) was dissolved in AcOH (4 mL) and MeOH (20 mL), and $Pd(OH)_2/C$ (25 mg) was added. The resulted mixture was hydrogenated for 2 days with vigorous shaking under H_2 atmosphere (80 mbar), and then the catalyst was removed by filtration over Celite and washed with MeOH. The concentrated product was purified by silica gel column chromatography (EtOAc:MeOH:H_2O = 10:1:0.1) to afford **7a** (47 mg, 85%). ^{1}H NMR (600 MHz, D_2O) δ 5.16 (s, 0.6H), 5.09 (s, 0.4H), 4.73–4.59 (m, 2H), 4.48 (dd, *J* = 4.2, 1.8 Hz, 0.3H), 4.34 (dd, *J* = 4.8, 1.8 Hz, 0.7H), 4.16 (s, 1H), 4.12 (s, 2H), 3.99 (dd, *J* = 28.8, 15.6 Hz, 0.6H), 3.87 (dd, *J* = 7.2, 4.2 Hz, 0.4H), 3.68 (t, *J* = 10.2 Hz, 0.5H), 3.57 (t, *J* = 9.6 Hz, 0.5H); ^{13}C NMR (151 MHz, D_2O) δ 176.17, 175.25, 93.14 (2C), 82.87 (d, *J* = 28.0 Hz), 81.75 (d, *J* = 29.7 Hz), 75.10 (d, *J* = 8.3 Hz), 71.88, 70.99 (d, *J* = 18.6 Hz), 68.65, 65.93, 65.70, 61.04 (d, *J* = 16.9 Hz), 53.85, 53.00; ^{19}F NMR (282 MHz, D_2O) δ -234.78 (ddd, *J* = 48.2, 48.2, 30.2 Hz, 0.4F), -235.50 (ddd, *J* = 46.8, 46.8, 28.2 Hz, 0.6F).

2-Deoxy-2-glycolamido-6-*O*-methyl-D-mannopyranoside (6-*O*-Me-ManNGc, 8a)

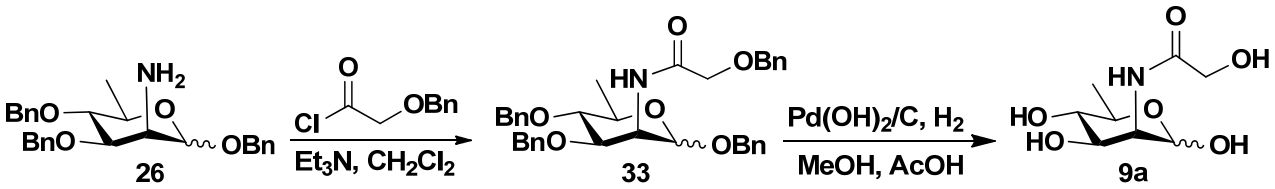


Triphenylphosphine (0.42 g, 1.59 mmol) and water (0.25 mL, 0.01 mmol) were added to a stirred solution of **23** (0.26 g, 0.53 mmol) in THF (10 mL) and the mixture was heated at 45 °C for 3 h. The solvent was removed and the crude product was dried under vacuum. The dried compound was

dissolved in CH_2Cl_2 (10 mL) and Et_3N (0.15 mL, 1.06 mmol) was added dropwise at 0 °C. Then, acetoxyacetyl chloride (0.07 mL, 0.64 mmol) was added to the reaction mixture and stirred at 0 °C for 2 h and then room temperature for 2 h. The residue was concentrated and purified by flash silica gel column chromatography (Hexanes:EtOAc = 1:1) to give **32** (0.28 g, 96%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.29 (m, 15H), 6.43 (d, *J* = 9.0 Hz, 1H), 4.97 (s, 1H), 4.91 (d, *J* = 10.8 Hz, 1H), 4.77 (d, *J* = 10.8 Hz, 1H), 4.74 (dd, *J* = 9.0, 6.0 Hz, 1H), 4.68 (d, *J* = 12.0 Hz, 1H), 4.60 (dd, *J* = 24.6, 15.6 Hz, 3H), 4.51 (dd, *J* = 10.8, 6.6 Hz, 2H), 4.15 (dd, *J* = 9.6, 4.8 Hz, 1H), 3.79 (d, *J* = 9.6 Hz, 1H), 3.70 (t, *J* = 9.6 Hz, 1H), 3.66 (dd, *J* = 10.2 Hz, 1H), 3.55 (dd, *J* = 10.8, 1.8 Hz, 1H), 3.37 (s, 3H), 2.14 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.37, 167.26, 138.32, 137.99, 136.86, 128.57, 128.48, 128.34, 128.18, 128.09, 127.89, 127.84, 98.42, 77.69, 75.27, 73.62, 71.34, 70.97, 70.77, 69.69, 63.09, 59.38, 49.04, 20.67.

Compound **32** (0.28 g, 0.49 mmol) was dissolved in AcOH (5 mL) and MeOH (25 mL), and $Pd(OH)_2/C$ (30 mg) was added. The resulted mixture was hydrogenated for 2 days with vigorous shaking under H_2 atmosphere (80 mbar), and then the catalyst was removed by filtration over Celite, washed with MeOH, dried under reduced pressure. The concentrated product was dissolved in aqueous solution of LiOH (0.5 M, 20 mL) and stirred at room temperature for overnight. The solvent was removed and the crude product was purified by silica gel column chromatography (EtOAc:MeOH:H_2O = 10:1:0.2) to afford **8a** (103 mg, 83%). ^{1}H NMR (600 MHz, D_2O) δ 5.12 (s, 0.7H), 5.05 (s, 0.3H), 4.47 (d, *J* = 4.2 Hz, 0.3H), 4.35 (d, *J* = 4.8 Hz, 0.7H), 4.16 (s, 0.6H), 4.13 (s, 1.4H), 4.07–3.46 (m, 5H), 3.40 (s, 1H), 3.39 (s, 2H); ^{13}C NMR (151 MHz, D_2O) δ 175.32, 175.27, 93.35, 93.24, 75.45, 72.30, 71.38, 71.05, 69.12, 67.43, 67.18, 61.37, 58.99, 54.13, 53.25.

2,6-Dideoxy-2-glycolamido-D-mannopyranoside (6-Deoxy-ManNGc, 9a)

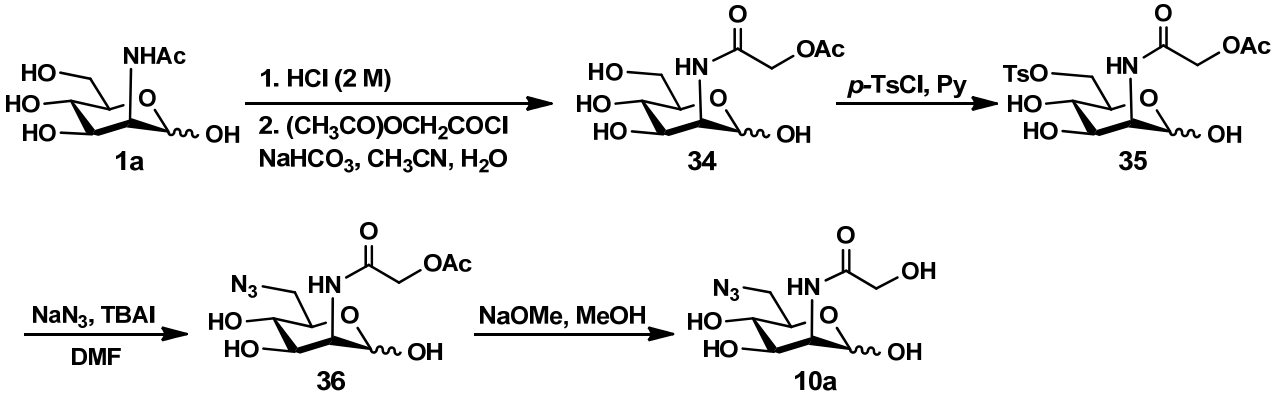


To a solution of **26** (0.25 g, 0.57 mmol) in CH_2Cl_2 (15 mL) was added Et_3N (0.16 mL, 1.15 mmol) at 0 °C. Benzyloxyacetyl chloride (0.11 mL, 0.68 mmol) was added to the reaction mixture and stirred at 0 °C for 2 h and then room temperature for 2 h. The residue was concentrated and purified by silica gel flash column chromatography (Hexanes:EtOAc = 1:1) to give **33** (0.24 g, 74%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38–7.27 (m, 20H), 6.90 (d, *J* = 9.0 Hz, 1H), 4.90 (d, *J* = 7.8 Hz, 0.7H), 4.88 (s, 0.3H), 4.77 (d, *J* = 10.8 Hz, 1H), 4.73 (dd, *J* = 9.0, 4.2 Hz, 1H), 4.67 (d, *J* = 12.0 Hz, 1H), 4.58 (dd, *J* = 25.8, 13.8 Hz, 3H), 4.53–4.49 (m, 3H), 4.11 (dd, *J* = 9.0, 4.2 Hz, 1H), 4.04 (dd, *J* = 27.6, 15.0 Hz, 2H), 3.85–3.81 (m, 1H), 3.13 (t, *J* = 9.6 Hz, 1H), 1.28 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.00, 138.38, 138.19, 137.20, 137.06, 128.72, 128.56, 128.48, 128.30, 128.23, 128.16, 128.07, 128.02, 127.87, 127.79, 127.72, 98.27, 80.42, 77.75, 75.52, 73.47, 71.28, 69.66, 69.49, 67.31, 49.17, 18.23.

Compound **33** (0.24 g, 0.41 mmol) was dissolved in AcOH (5 mL) and MeOH (25 mL), and $Pd(OH)_2/C$ (30 mg) was added. The resulted mixture was hydrogenated for 2 days with vigorous

shaking under H_2 atmosphere (80 mbar), and then the catalyst was removed by filtration over Celite and washed with MeOH. The concentrated product was purified by silica gel column chromatography (EtOAc:MeOH:H_2O = 10:1:0.1) to afford **9a** (75 mg, 83%). ^{1}H NMR (600 MHz, D_2O) δ 5.08 (s, 0.5H), 5.03 (s, 0.5H), 4.46 (d, *J* = 4.8 Hz, 0.5H), 4.32 (d, *J* = 4.8 Hz, 0.5H), 4.16 (s, 1H), 4.13 (s, 1H), 4.02 (dd, *J* = 10.2, 4.8 Hz, 0.5H), 3.95–3.90 (m, 0.5H), 3.79 (dd, *J* = 10.2, 4.2 Hz, 0.5H), 3.47–3.44 (m, 0.5H), 3.35 (t, *J* = 9.6 Hz, 0.5H), 3.25 (t, *J* = 9.6 Hz, 0.5H), 1.30 (d, *J* = 6.0 Hz, 1.5H), 1.27 (d, *J* = 6.0 Hz, 1.5H); ^{13}C NMR (151 MHz, D_2O) δ 176.17, 175.28, 92.92, 92.72, 72.60, 72.32, 71.80, 71.74, 68.47, 68.14, 61.09, 61.02, 54.05, 53.21, 53.17, 16.85, 16.80.

6-Azido-2,6-dideoxy-2-glycolamido-D-mannopyranoside (6-N_3-ManNGc, 10a)



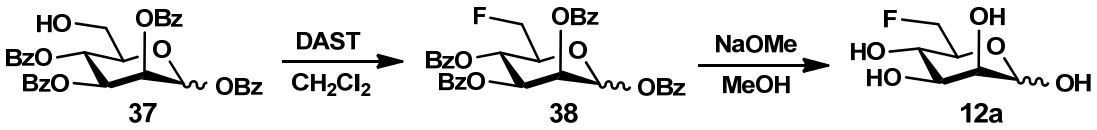
ManNAc **1a** (1.0 g, 4.52 mmol) was dissolved in an aqueous solution of HCl (2 M, 40 mL) and stirred at 60 °C for overnight. The solvent was removed and the residue was dried under vacuum. The resulting compound was dissolved in a mixture of CH_3CN (70 mL) and water (70 mL) and followed by addition of $NaHCO_3$ (3.80 g, 45.20 mmol) at 0 °C. Acetoxyacetyl chloride (0.97 mL, 9.04 mmol) was added to the reaction mixture and stirred at 0 °C for 2 h and then room temperature for 2 h. MeOH was added to the mixture and the solvents were removed. The residue was purified by silica gel flash column chromatography (EtOAc:MeOH:H_2O = 8:1:0.2) to give **34** (1.20 g, 95%). ^{1}H NMR (600 MHz, D_2O) δ 5.11 (d, *J* = 1.2 Hz, 0.6H), 5.02 (d, *J* = 1.2 Hz, 0.4H), 4.71 (d, *J* = 1.2 Hz, 0.7H), 4.66 (m, 2.3H), 4.47 (dd, *J* = 4.8, 1.8 Hz, 0.4H), 4.33 (dd, *J* = 4.2, 1.2 Hz, 0.6H), 4.04 (dd, *J* = 9.6, 4.8 Hz, 0.6H), 3.85–3.80 (m, 2.4H), 3.59 (t, *J* = 9.6 Hz, 0.6H), 3.48 (t, *J* = 10.2 Hz, 0.4H), 2.16 (s, 1H), 2.15 (s, 2H); ^{13}C NMR (151 MHz, D_2O) δ 173.62, 173.51, 172.46, 171.47, 170.69, 93.02, 92.97, 76.49, 72.12, 68.93, 66.83, 66.58, 62.73, 61.40, 60.46, 54.26, 53.31, 20.08.

A stirred solution of **34** (1.20 g, 4.30 mmol) in anhydrous pyridine (8 mL) was treated with a solution of *p*-toluenesulfonyl chloride (0.90 g, 4.73 mmol) in pyridine (3 mL) at 0 °C and the mixture was stirred for 3 h. The reaction mixture was stopped by adding MeOH (2 mL) and concentrated. Purification of the residue by column chromatography (EtOAc:MeOH = 9:1) afforded **35** (1.21 g, 65%). A mixture containing compound **35** (1.21 g, 2.79 mmol), NaN_3 (1.09 g, 16.70 mmol), and tetra-*n*-butylammonium iodide (0.20 g, 0.55 mmol) in anhydrous DMF (15 mL) was stirred at 60 °C for overnight. The mixture was concentrated and purified by silica gel flash column chromatography (EtOAc:MeOH = 9.5:0.5) to afford product **36** (0.63 g, 75%). ^{1}H NMR (600 MHz, D_2O) δ 5.21 (d, *J* = 2.4 Hz, 0.5H), 5.16 (d, *J* = 6.6 Hz, 0.5H), 4.72 (d, *J* = 1.8 Hz, 1.5H), 4.69 (s, 1.5H), 4.49–3.51 (m, 5H),

2.22 (s, 1.5H), 2.20 (s, 1.5H); ^{13}C NMR (151 MHz, D_2O) δ 173.56, 170.81, 170.52, 170.17, 93.07, 91.02, 70.62, 70.13, 69.98, 69.42, 68.67, 67.84, 67.11, 64.38, 62.83, 61.48, 53.97, 53.40, 51.10, 20.15, 20.07.

Compound **36** (0.63 g, 2.07 mmol) was dissolved in dry MeOH (15 mL) containing a catalytic amount of NaOMe. The mixture was stirred at room temperature for overnight and neutralized using Dowex (H^+) resin. The resulted suspension was filtrated and concentrated. Silica gel column chromatography (EtOAc:MeOH:H_2O = 8:1:0.3) of the crude product afforded product **10a** (0.49 g, 90%). ^{1}H NMR (600 MHz, D_2O) δ 5.20 (d, *J* = 3.6 Hz, 0.7H), 5.14 (s, 0.3H), 4.47 (d, *J* = 4.2 Hz, 0.5H), 4.35 (d, *J* = 4.8 Hz, 0.5H), 4.17 (s, 0.4H), 4.13–4.12 (m, 2.6H), 4.09–3.45 (m, 4H); ^{13}C NMR (151 MHz, D_2O) δ 176.00, 175.28, 94.90, 93.08, 76.65, 76.15, 73.87, 72.27, 71.80, 70.96, 70.19, 69.01, 67.03, 61.10, 60.78, 56.58, 53.81, 53.07.

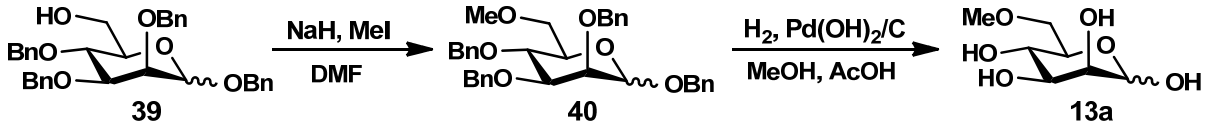
6-Deoxy-6-fluoro-D-mannopyranoside (6-F-Man, 12a)



To a solution of **37**[4] (0.36 g, 0.60 mmol) in anhydrous CH_2Cl_2 (10 mL) in a Teflon flask, DAST (0.60 mL, 4.54 mmol) was slowly added at -40 °C. The reaction was stirred for 3 days at room temperature. After cooled down to -20 °C, MeOH (2 mL) was added and the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2, washed with water for 3 times, dried over $MgSO_4$, and concentrated. The crude product was purified using silica gel flash column chromatography (Hexanes:EtOAc = 4:1) to yield **38** (0.21 g, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23–7.27 (m, 20 H), 6.63 (d, *J* = 2.1 Hz, 1H), 6.14–6.02 (m, 2H), 5.88 (dd, *J* = 2.7, 2.1 Hz, 1H), 4.72 (t, *J* = 2.1 Hz, 1H), 4.56 (t, *J* = 2.4 Hz, 1H), 4.49–4.34 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 165.87, 165.50, 165.48, 164.01, 134.32, 133.99, 133.89, 133.67, 130.39, 130.27, 130.14, 130.05, 129.99, 129.83, 128.93, 128.76, 128.64, 91.49, 73.12, 72.12, 70.04, 69.53, 65.81; ^{19}F NMR (282 MHz, $CDCl_3$) δ -233.40 (ddd, *J* = 49.5, 49.5, 25.5 Hz, 1F).

To a suspension of **38** (0.21 g, 0.35 mmol) in dry MeOH (10 mL) was added NaOMe (40 mg) and the mixture was stirred overnight at room temperature. The reaction was neutralized with Dowex (H^+) resin, filtrated, concentrated and purified by silica gel flash column chromatography (EtOAc:MeOH:H_2O = 10:1:0.3) to afford **12a** (62 mg, 98%). ^{1}H NMR (600 MHz, D_2O) δ 5.18 (d, *J* = 1.8 Hz, 0.6H), 4.92 (s, 0.4H), 4.76–4.59 (m, 2H), 3.94–3.93 (m, 1H), 3.85 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.76 (t, *J* = 10.2 Hz, 1H), 3.67–3.66 (m, 1H); ^{13}C NMR (151 MHz, D_2O) δ 94.37, 93.9, 83.05 (d, *J* = 18.6 Hz), 81.92 (d, *J* = 18.6 Hz), 74.74 (d, *J* = 17.7 Hz), 72.99, 71.27 (d, *J* = 17.7 Hz), 70.67, 70.21, 65.72 (d, *J* = 7.2 Hz), 65.47 (d, *J* = 6.8 Hz); ^{19}F NMR (282 MHz, D_2O) δ -234.16 (ddd, *J* = 49.5, 49.5, 27.3 Hz, 0.6F), -235.03 (ddd, *J* = 49.5, 49.5, 29.3 Hz, 0.4F).

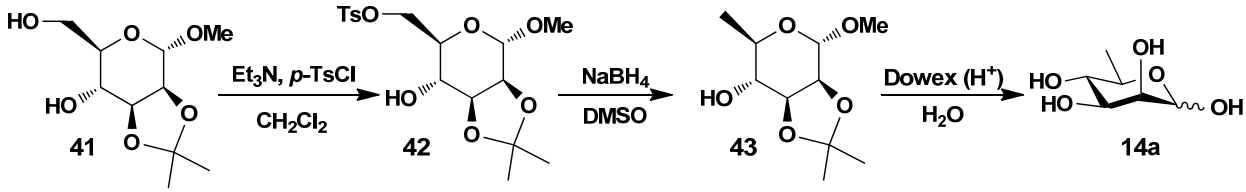
6-*O*-Methyl-D-mannopyranoside (6-*O*-Me-Man, 13a)



A solution of **39**[2] (2.23 g, 4.12 mmol) in anhydrous DMF (20 mL) was stirred with NaH (0.39 g, 16.48 mmol) at room temperature for 30 min. Methyl iodide (1.02 mL, 16.48 mmol) was added slowly at 0 °C and the mixture was stirred for 6 h. The mixture was diluted with CH_2Cl_2, washed with water, dried over $MgSO_4$, and concentrated. Purification of the residue by silica gel column chromatography (Hexanes:EtOAc = 10:0.5) gave **40** (2.26 g, 99%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39–7.30 (m, 20H), 5.00 (s, 1H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.75–4.73 (m, 3H), 4.66–4.64 (m, 3H), 4.48 (d, *J* = 12.0 Hz, 1H), 4.05–3.98 (m, 2H), 3.85–3.84 (m, 2H), 3.71 (dd, *J* = 10.2, 4.8 Hz, 1H), 3.65 (dd, *J* = 10.8, 1.8 Hz, 1H), 3.44 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.82, 138.51, 137.60, 128.65, 128.59, 128.57, 128.30, 128.18, 128.06, 128.00, 127.93, 127.88, 127.77, 97.60, 80.44, 75.50, 75.22, 74.78, 72.83, 72.41, 71.97 (2C), 69.23, 59.54.

Compound **40** (2.26 g, 4.07 mmol) was dissolved in AcOH (8 mL) and MeOH (40 mL), and $Pd(OH)_2/C$ (400 mg) was added. The resulted mixture was hydrogenated for 2 days with vigorous shaking under H_2 atmosphere (80 mbar), and then the catalyst was removed by filtration over Celite and washed with MeOH. The concentrated product was purified by silica gel column chromatography (EtOAc:MeOH:H_2O = 10:1:0.1) to afford **13a** (0.63 g, 80%). ^{1}H NMR (600 MHz, D_2O) δ 5.13 (s, 0.7H), 4.86 (d, *J* = 1.8 Hz, 0.3H), 3.91–3.71 (m, 3H), 3.66–3.45 (m, 3H), 3.39 (s, 1H) 3.38 (s, 2H); ^{13}C NMR (151 MHz, D_2O) δ 94.12, 93.78, 74.70, 73.10, 71.60, 71.29, 70.90, 70.75, 70.27, 67.11, 66.80, 58.62, 58.53.

6-Deoxy-D-mannopyranoside (6-Deoxy-Man, 14a)



To a solution of **41**[5] (1.44 g, 6.15 mmol) in CH_2Cl_2 (15 mL) was added Et_3N (1.04 mL, 7.40 mmol) and *p*-toluenesulfonyl chloride (1.46 g, 7.40 mmol). The reaction was stirred at room temperature for overnight. The mixture was concentrated under reduced pressure and the crude product was purified using silica gel flash column chromatography (Hexanes:EtOAc = 10:1) to give **42** (2.10 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 4.83 (s, 1H), 4.29 (d, *J* = 3.2 Hz, 2H), 4.09 (d, *J* = 3.2 Hz, 2H), 3.75–3.70 (m, 1H), 3.62–3.59 (m, 1H), 3.34 (s, 3H), 2.75 (d, *J* = 5.2 Hz, 1H), 2.44 (s, 3H), 1.47 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.14, 133.08, 130.06, 128.19, 109.99, 98.56, 78.13, 75.56, 69.35, 68.77, 68.49, 55.41, 28.02, 26.18, 21.86.

Sodium borohydride (0.96 g, 25.46 mmol) was added slowly to a stirred solution of **42** (1.97 g, 5.07 mmol) in DMSO (20 mL). The mixture was stirred at 80 °C for 2 h. The reaction solution was

poured into cold water (70 mL), extracted with Et_2O, filtrated, and dried over $MgSO_4$. Solvent was removed under reduced pressure and the crude product was purified using column chromatography on silica gel (Hexanes:EtOAc = 3:7) to afford **43** (1.03 g, 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.84 (s, 1H), 4.13–4.03 (m, 2H), 3.66–3.61 (m, 1H), 3.38 (s, 3H), 2.60 (d, *J* = 5.6 Hz, 1H), 1.52 (s, 3H), 1.35 (m, 3H), 1.30 (d, *J* = 8.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.74, 98.40, 78.80, 75.85, 74.66, 65.97, 55.18, 28.21, 26.35, 17.71.

To a solution of compound **43** (0.96 g, 4.40 mmol) in water (50 mL) was added Dowex (H^+) resin (5.2 g). The mixture was stirred vigorously and refluxed for 16 h. The suspension was filtered and the solvent was removed under diminished pressure. Silica gel column chromatography purification (EtOAc:MeOH:H_2O = 10:0.8:0.1) gave compound **14a** (0.68 g, 94%). ^{1}H NMR (600 MHz, D_2O) δ 5.06 (s, 0.6H), 4.82 (s, 0.4H), 3.88–3.79 (m, 1.6H), 3.74 (dd, *J* = 9.6, 3.0 Hz, 0.4H), 3.54 (dd, *J* = 9.0, 3.0 Hz, 0.4H), 3.40–3.29 (m, 1.6H), 1.24 (d, *J* = 5.4 Hz, 1H), 1.22 (d, *J* = 6.0 Hz, 2H); ^{13}C NMR (151 MHz, D_2O) δ 94.11, 93.62, 72.86, 72.30, 72.14, 71.94, 71.45, 70.93, 70.06, 68.39, 16.93, 16.88.

Enzymatic synthesis of sialosides 1b–15b and 1c–15c

General procedure for one-pot three-enzyme preparative synthesis of α2–3- (1b–15b) and α2–6-linked (1c–15c) sialosides using Galβ*p*NP as the sialyltransferase acceptor. Galβ*p*NP (1.0 eq, 10 mM, 0.1 mmol), a sialic acid precursor (1.2–1.5 eq, 12 mM, 0.12 mmol), sodium pyruvate (6.0 eq, 60 mM, 0.6 mmol), and CTP (2.0 eq, 20 mM, 0.2 mmol) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.5) and $MgCl_2$ (20 mM). After addition of appropriate amount of an Pm aldolase, an *N. meningitides* CMP-sialic acid synthetase, and a sialyltransferase (PmST1 or Pd2,6ST), water was added to bring the volume of the reaction mixture to 10 mL. The reaction was incubated in an isotherm incubator with agitating in 120 rpm for 1–16 h at 37 °C or for 12–48 h at room temperature. The product formation was monitored by TLC using EtOAc:MeOH:H_2O:AcOH = 7:2:0.5:0.1 (by volume) as the developing solvent and stained with *p*-anisaldehyde sugar stain solution. The reaction was terminated by adding the same volume of ice-cold EtOH and incubating at 4 °C for 30 min. The mixture was centrifuged to remove precipitates. The supernatant was concentrated and passed through a Bio-gel P-2 gel filtration column with water to obtain the desired product. Silica gel column purification (EtOAc:MeOH:H_2O = 7:2:0.5) was used for further purification.

4-Nitrophenyl *O*-(5-acetamido-9-fluoro-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Neu5Ac9Fα2–3Galβ*p*NP, 2b). Yield, 98%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.26 (d, *J* = 9.0 Hz, 2H), 7.24 (d, *J* = 9.6 Hz, 2H), 5.28 (d, *J* = 7.8 Hz, 1H), 4.71–4.56 (m, 2H), 4.22 (dd, *J* = 10.2, 3.0 Hz, 1H), 4.05–3.97 (m, 2H), 3.91–3.85 (m, 3H), 3.74 (d, *J* = 6.0 Hz, 2H), 3.71–3.64 (m, 3H), 2.77 (dd, *J* = 12.6, 4.8 Hz, 1H), 2.01 (s, 3H), 1.82 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.10, 173.97, 161.84, 142.66, 126.24, 116.56, 100.05, 99.80, 84.47 (d, *J* = 165.6 Hz), 75.61, 72.84 (d, *J* = 11.0 Hz), 70.58, 70.45, 68.93, 68.55, 67.39, 67.00, 60.81, 51.79, 39.85, 22.22; ^{19}F NMR (282 MHz, D_2O) δ -234.76 (ddd, *J* =

46.5, 46.5, 27.9 Hz, 1F); HRMS (ESI) calculated for $C_{23}H_{31}FN_2O_{15}Na$ (M+H) 617.1606, found 617.1579.

4-Nitrophenyl *O*-(5-acetamido-3,5-dideoxy-9-*O*-methyl-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Neu5Ac9OMeα2–3Galβ*p*NP, 3b). Yield, 94%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.25 (d, *J* = 9.0 Hz, 2H), 7.24 (d, *J* = 8.4 Hz, 2H), 5.28 (d, *J* = 8.4 Hz, 1H), 4.22 (dd, *J* = 9.6, 2.4 Hz, 1H), 4.03 (d, *J* = 3.0 Hz, 1H), 3.98–3.96 (m, 1H), 3.92–3.83 (m, 3H), 3.75 (d, *J* = 6.0 Hz, 2H), 3.70–3.62 (m, 3H), 3.57 (dd, *J* = 9.0, 1.8 Hz, 1H), 3.52 (dd, *J* = 10.2, 6.6 Hz, 1H), 3.34 (s, 3H), 2.78 (dd, *J* = 12.0, 4.8 Hz, 1H), 2.01 (s, 3H), 1.81 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.16, 173.98, 161.92, 142.68, 126.30, 116.62, 99.92, 99.88, 75.66 (2C), 73.40, 72.97, 70.42, 68.97, 68.58, 68.32, 67.47, 60.87, 58.61, 51.83, 39.88, 22.25; HRMS (ESI) calculated for $C_{24}H_{34}N_2O_{16}Na$ (M+H) 629.1806, found 629.1781.

4-Nitrophenyl *O*-(5-acetamido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Neu5Ac9Deoxyα2–3Galβ*p*NP, 4b). Yield, 90%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.22 (d, *J* = 7.8 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 5.24 (d, *J* = 7.8 Hz, 1H), 4.18 (dd, *J* = 9.6, 1.8 Hz, 1H), 3.99 (d, *J* = 2.4 Hz, 1H), 3.92–3.79 (m, 4H), 3.71 (d, *J* = 6.0 Hz, 2H), 3.65–3.61 (m, 2H), 3.33–3.29 (m, 1H), 2.73 (dd, *J* = 12.0, 4.2 Hz, 1H), 1.98 (s, 3H), 1.77 (t, *J* = 12.0 Hz, 1H), 1.19 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 177.63, 176.61, 164.42, 145.20, 128.82, 119.14, 102.61, 102.41, 78.18, 78.16, 75.77, 75.48, 71.48, 71.16, 70.27, 69.99, 63.40, 54.49, 42.34, 24.78, 21.14; HRMS (ESI) calculated for $C_{23}H_{32}N_2O_{15}Na$ (M+H) 599.1700, found 599.1691**.**

4-Nitrophenyl *O*-(5-acetamido-9-azido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Neu5Ac9N$_3$α2–3Galβ*p*NP, 5b). Yield, 87%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.21 (d, *J* = 9.0 Hz, 2H), 7.21 (d, *J* = 9.0 Hz, 2H), 5.26 (d, *J* = 7.8 Hz, 1H), 4.22 (dd, *J* = 9.6, 3.0 Hz, 1H), 4.03–3.99 (m, 2H), 3.91–3.83 (m, 2H), 3.75 (d, *J* = 6.0 Hz, 2H), 3.71 (s, 1H), 3.69–3.42 (m, 5H), 2.77 (dd, *J* = 12.0, 4.2 Hz, 1H), 2.02 (s, 3H), 1.81 (t, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.17, 173.98, 161.88, 142.64, 126.25, 116.59, 100.01, 99.88, 75.61, 72.88, 70.54, 68.92, 68.50, 67.40, 62.64, 60.83, 59.48, 53.18, 51.84, 39.89, 22.21; HRMS (ESI) calculated for $C_{23}H_{31}N_5O_{15}Na$ (M+H) 640.1714, found 640.1684.

4-Nitrophenyl *O*-(9-fluoro-5-glycolylamido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Neu5Gc9Fα2–3Galβ*p*NP, 7b). Yield, 98%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.26 (d, *J* = 9.6 Hz, 2H), 7.24 (d, *J* = 9.0 Hz, 2H), 5.28 (d, *J* = 7.8 Hz, 1H), 4.72–4.56 (m, 2H), 4.23 (dd, *J* = 10.2, 3.0 Hz, 1H), 4.10 (s, 2H), 4.03 (d, *J* = 3.0 Hz, 1H), 3.96 (t, *J* = 10.2 Hz, 1H), 3.92–3.88 (m, 2H), 3.81–3.70 (m, 6H), 2.80 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.84 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.89, 174.01, 161.86, 142.64, 126.24, 116.57, 100.06, 99.81, 84.45 (d, *J* = 170.8 Hz), 75.66, 75.59, 72.57, 70.58 (d, *J* = 14.6 Hz), 68.94, 68.28, 67.37, 66.93, 61.14, 60.80, 51.48, 39.91; ^{19}F NMR (282 MHz, D_2O) δ -234.83 (ddd, *J* = 45.7, 45.7, 27.6 Hz, 1F); HRMS (ESI) calculated for $C_{23}H_{31}FN_2O_{16}Na$ (M+H) 633.1555, found 633.1525.

4-Nitrophenyl *O*-(5-glycolylamido-3,5-dideoxy-9-*O*-methyl-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Neu5Gc9OMeα2–3Galβ*p*NP, 8b).

Yield, 40%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.26 (d, *J* = 9.6 Hz, 2H), 7.24 (d, *J* = 9.6 Hz, 2H), 5.28 (d, *J* = 7.8 Hz, 1H), 4.23 (dd, *J* = 10.2, 3.0 Hz, 1H), 4.10 (s, 2H), 4.03 (d, *J* = 3.6 Hz, 1H), 3.99–3.88 (m, 4H), 3.80–3.74 (m, 4H), 3.67 (dd, *J* = 10.8, 1.8 Hz, 1H), 3.57 (dd, *J* = 9.6, 1.8 Hz, 1H), 3.52 (dd, *J* = 10.8, 6.6 Hz, 1H), 3.34 (s, 3H), 2.79 (dd, *J* = 12.6, 4.2 Hz, 1H), 1.83 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.89, 174.00, 161.87, 142.67, 126.24, 116.57, 100.00, 99.85, 75.67, 75.63, 73.34, 72.66, 70.43, 68.93, 68.28, 68.22, 67.41, 61.13, 60.83, 58.53, 51.48, 39.90; HRMS (ESI) calculated for $C_{24}H_{34}N_2O_{17}Na$ (M+H) 645.1755, found 645.1727.

4-Nitrophenyl *O*-(5-glycolylamido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Neu5Gc9Deoxyα2–3Galβ*p*NP, 9b). Yield, 90%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.29 (d, *J* = 9.0 Hz, 2H), 7.28 (d, *J* = 9.0 Hz, 2H), 5.31 (d, *J* = 7.8 Hz, 1H), 4.26 (dd, *J* = 9.6, 3.0 Hz, 1H), 4.14 (s, 2H), 4.07 (d, *J* = 3.0 Hz, 1H), 3.98–3.91 (m, 3H), 3.82–3.78 (m, 4H), 3.75 (s, 1H), 3.39 (dd, *J* = 9.0, 1.8 Hz, 1H), 2.82 (dd, *J* = 12.0, 4.8 Hz, 1H), 1.86 (t, *J* = 12.6 Hz, 1H), 1.26 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 175.85, 174.10, 161.88, 142.67, 126.27, 116.59, 100.08, 99.83, 75.60 (2C), 73.15, 72.66, 68.90, 68.33, 67.76, 67.42, 61.10, 60.84, 51.63, 39.84, 18.53; HRMS (ESI) calculated for $C_{23}H_{32}N_2O_{16}Na$ (M+H) 615.1650, found 615.1624.

4-Nitrophenyl *O*-(9-azido-5-glycolylamido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Neu5Gc9N$_3$α2–3Galβ*p*NP, 10b). Yield, 85%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.22 (d, *J* = 9.0 Hz, 2H), 7.22 (d, *J* = 9.6 Hz, 2H), 5.28 (d, *J* = 7.8 Hz, 1H), 4.25 (dd, *J* = 10.2, 3.0 Hz, 1H), 4.11 (s, 2H), 4.04 (d, *J* = 3.0 Hz, 1H), 3.96–3.75 (m, 9H), 3.62–3.57 (m, 2H), 2.80 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.84 (t, *J* = 12.0 Hz, 1H); ^{13}CNMR (151 MHz, D_2O) δ 176.04, 174.07, 162.02, 142.78, 126.37, 116.75, 100.18, 100.02, 75.73, 72.91, 72.10, 69.08, 68.34 (2C), 67.58, 62.86, 61.30, 60.98, 59.65, 51.69, 40.05; HRMS (ESI) calculated for $C_{23}H_{29}N_5O_{16}Na$ (M-H) 631.1609, found 631.1574.

4-Nitrophenyl *O*-(9-fluoro-3,9-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Kdn9Fα2–3Galβ*p*NP, 12b). Yield, 94%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.22 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 5.24 (d, *J* = 7.8 Hz, 1H), 4.73–4.57 (m, 2H), 4.17 (dd, *J* = 9.6, 3.0 Hz, 1H), 4.02–3.94 (m, 3H), 3.89–3.84 (m, 2H), 3.72 (d, *J* = 6.0 Hz, 2H), 3.62–3.51 (m, 3H), 2.70 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.75 (t, *J* = 12.0, 1H); ^{13}C NMR (151 MHz, D_2O) δ 176.90, 164.80, 145.61, 129.13, 119.64, 103.05, 102.95, 87.81 (d, *J* = 164.6 Hz), 78.57, 76.84, 73.65 (d, *J* = 16.6 Hz), 73.31, 72.84, 71.87, 70.37, 69.80, 69.77 (d, *J* = 15.1 Hz), 63.80, 42.43; ^{19}F NMR (282 MHz, D_2O) δ -234.43 (ddd, *J* = 47.9, 47.9, 25.4 Hz, 1F); (ESI) calculated for $C_{21}H_{28}NO_{15}FNa$ (M+H) 576.1341, found 576.1372.

4-Nitrophenyl *O*-(9-*O*-methyl-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Kdn9OMeα2–3Galβ*p*NP, 13b). Yield, 78%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.22 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.4 Hz, 2H), 5.24 (d, *J* = 7.2 Hz, 1H), 4.16 (dd, *J* = 9.0, 3.0 Hz, 1H), 3.98 (d, *J* = 3.0 Hz, 1H), 4.00 (t, *J* = 7.2, 1H), 3.88–3.48 (m, 10H), 3.32 (s, 3H), 2.68 (dd, *J* = 12.0, 4.2 Hz, 1H), 1.72 (t, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 174.16, 161.90, 142.71, 126.29, 116.63, 100.02, 99.93, 75.67 (2C), 74.01, 73.57, 70.60, 70.45, 69.90, 68.96,

68.03, 67.40, 60.86, 58.60, 39.54; HRMS (ESI) calculated for $C_{22}H_{31}NO_{16}Na$ (M+H) 588.1541, found 588.1533.

4-Nitrophenyl *O*-(3,9-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Kdn9Deoxyα2–3Galβ*p*NP, 14b). Yield, 94%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.22 (d, *J* = 9.0 Hz, 2H), 7.20 (d, *J* = 9.6 Hz, 2H), 5.23 (d, *J* = 7.8 Hz, 1H), 4.15 (dd, *J* = 9.6, 2.4 Hz, 1H), 3.98 (d, *J* = 3.0 Hz, 1H), 3.91–3.82 (m, 3H), 3.70 (d, *J* = 6.0 Hz, 2H), 3.59–3.47 (m, 4H), 2.68 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.72 (t, *J* = 12.6 Hz, 1H), 1.22 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.19, 161.83, 142.65, 126.23, 116.52, 99.99, 99.77, 75.54 (2C), 73.91, 72.69, 70.40, 69.93, 68.87, 67.94, 67.29, 60.76, 39.37, 18.46; HRMS (ESI) calculated for $C_{21}H_{29}NO_{15}Na$ (M+H) 558.1435, found 558.1429.

4-Nitrophenyl *O*-(9-azido-3,9-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-*O*-β-D-galactopyranoside (Kdn9N$_3$α2–3Galβ*p*NP, 15b). Yield, 91%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.28 (d, *J* = 9.0 Hz, 2H), 7.28 (d, *J* = 9.6 Hz, 2H), 5.32 (d, *J* = 7.8 Hz, 1H), 4.27 (dd, *J* = 10.2, 3.0 Hz, 1H), 4.10–4.05 (m, 2H), 3.98–3.59 (m, 9H), 3.54 (dd, *J* = 13.2, 6.6 Hz, 1H), 2.78 (dd, *J* = 12.0, 4.2 Hz, 1H), 1.84 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 174.31, 161.92, 142.74, 126.33, 116.69, 100.22, 100.13, 75.74, 74.02, 70.92, 70.44, 69.98, 69.01, 68.64, 67.44, 62.73, 60.89, 53.36, 39.58. HRMS (ESI) calculated for $C_{21}H_{28}N_4O_{15}Na$ (M+H) 599.1449, found 599.1476.

4-Nitrophenyl *O*-(5-acetamido-9-fluoro-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Ac9Fα2–6Galβ*p*NP, 2c). Yield, 95%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.27 (d, *J* = 9.0 Hz, 2H), 7.25 (d, *J* = 9.0 Hz, 2H), 5.17 (d, *J* = 7.2 Hz, 1H), 4.71–4.56 (m, 2H), 4.00–3.92 (m, 4H), 3.83 (t, *J* = 7.8 Hz, 1H), 3.78–3.64 (m, 6H), 2.74 (dd, *J* = 12.6, 3.6 Hz, 1H), 1.99 (s, 3H), 1.64 (t, *J* = 12.0 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 175.11, 173.67, 162.04, 142.69, 126.29, 116.57, 100.40, 100.00, 84.52 (d, *J* = 166.8 Hz), 74.18, 72.48 (2C), 70.42 (2C), 68.57, 68.38, 67.10, 63.20, 51.94, 40.42, 22.17; ^{19}F NMR (282 MHz, D_2O) δ -234.70 (ddd, *J* = 46.5, 46.5, 27.9 Hz, 1F); HRMS (ESI) calculated for $C_{23}H_{31}FN_2O_{15}Na$ (M+H) 617.1606 , found 617.1571.

4-Nitrophenyl *O*-(5-acetamido-3,5-dideoxy-9-*O*-methyl-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Ac9OMeα2–6Galβ*p*NP, 3c). Yield, 92%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.27 (d, *J* = 9.0 Hz, 2H), 7.25 (d, *J* = 9.6 Hz, 2H), 5.17 (d, *J* = 7.2 Hz, 1H), 4.01–3.99 (m, 2H), 3.95–3.91 (m, 2H), 3.84 (dd, *J* = 9.6, 7.8 Hz, 1H), 3.77 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.72 (dd, *J* = 15.6, 5.4 Hz, 1H), 3.69–3.63 (m, 4H), 3.52–3.49 (m, 2H), 3.35 (s, 3H), 2.74 (dd, *J* = 12.0, 4.2 Hz, 1H), 1.99 (s, 3H), 1.63 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.13, 173.70, 162.04, 142.67, 126.28, 116.56, 100.39, 100.00, 74.16, 73.37, 72.62, 72.49, 70.42, 70.31, 68.53 (2C), 68.36, 63.14, 58.60, 51.95, 40.40, 22.15; HRMS (ESI) calculated for $C_{24}H_{34}N_2O_{16}$ (M+H) 629.1806, found 629.1773.

4-Nitrophenyl *O*-(5-acetamido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Ac9Deoxyα2–6Galβ*p*NP, 4c). Yield, 97%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.25 (d, *J* = 9.0 Hz, 2H), 7.22 (d, *J* = 9.6 Hz, 2H), 5.14 (d, *J* = 7.8 Hz, 1H), 3.98–3.96 (m, 2H), 3.89 (dd, *J* = 15.0, 6.6 Hz, 2H), 3.81 (dd, *J* = 10.2, 7.8 Hz, 1H), 3.74 (dd,

J = 10.2, 3.6 Hz, 1H), 3.72–3.68 (m, 1H), 3.66–3.59 (m, 3H), 3.27 (dd, *J* = 9.0, 1.8 Hz, 1H), 2.71 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.96 (s, 3H), 1.60 (t, *J* = 12.6 Hz, 1H), 1.19 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 174.96, 173.67, 161.95, 142.67, 126.25, 116.51, 100.34, 99.92, 74.14, 73.27, 72.60, 72.45, 70.39, 68.51, 68.41, 67.61, 63.13, 52.03, 40.36, 22.11, 18.52; HRMS (ESI) calculated for $C_{23}H_{32}N_2O_{15}Na$ (M+H) 599.1700, found 599.1692.

4-Nitrophenyl *O*-(5-acetamido-9-azido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Ac9N$_3$α2–6Galβ*p*NP, 5c). Yield, 81%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.28 (d, *J* = 9.6 Hz, 2H), 7.26 (d, *J* = 9.6 Hz, 2H), 5.17 (d, *J* = 7.8 Hz, 1H), 4.01–3.91 (m, 4H), 3.84 (t, *J* = 7.8 Hz, 1H), 3.79–3.62 (m, 6H), 3.52 (d, *J* = 9.0 Hz, 1H), 3.43 (dd, *J* = 13.2, 6.6 Hz, 1H), 2.73 (dd, *J* = 12.6, 4.2 Hz, 1H), 2.00 (s, 3H), 1.63 (t, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.12, 173.67, 162.01, 142.67, 126.27, 116.56, 100.45, 100.01, 74.17, 72.54, 72.48, 70.47, 70.41, 68.97, 68.54, 68.31, 63.18, 53.21, 51.98, 40.38, 22.14; HRMS (ESI) calculated for $C_{23}H_{31}N_5O_{15}Na$ (M+H) 640.1714, found 640.1682.

4-Nitrophenyl *O*-(9-fluoro-5-glycolylamido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Gc9Fα2–6Galβ*p*NP, 7c). Yield, 95%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.28 (d, *J* = 9.6 Hz, 2H), 7.25 (d, *J* = 9.0 Hz, 2H), 5.18 (d, *J* = 7.2 Hz, 1H), 4.71–4.55 (m, 2H), 4.08 (s, 2H), 4.01–3.93 (m, 4H), 3.86–3.75 (m, 5H), 3.68–3.64 (m, 2H), 2.76 (dd, *J* = 12.6, 4.2 Hz, 1H), 1.66 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.89, 173.73, 162.05, 142.71, 126.30, 116.58, 100.46, 100.04, 84.52 (d, *J* = 167.3 Hz), 74.21, 72.51, 72.28, 70.57, 70.45, 68.59, 68.12, 67.06, 63.24, 61.12, 51.67, 40.47; ^{19}F NMR (282 MHz, D_2O) δ -234.80 (ddd, *J* = 48.8, 48.8, 27.3 Hz, 1F); HRMS (ESI) calculated for $C_{23}H_{31}FN_2O_{16}Na$ (M+H) 633.1555, found 633.1522.

4-Nitrophenyl *O*-(5-glycolylamido-3,5-dideoxy-9-*O*-methyl-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Gc9OMeα2–6Galβ*p*NP, 8c). Yield, 30%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.27 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 9.0 Hz, 2H), 5.17 (d, *J* = 7.2 Hz, 1H), 4.08 (s, 2H), 4.01 (d, *J* = 2.4 Hz, 1H), 4.00 (d, *J* = 4.2 Hz, 1H), 3.93 (dd, *J* = 15.6, 7.2 Hz, 2H), 3.86–3.76 (m, 5H), 3.53–3.50 (m, 2H), 3.53–3.50 (m, 2H), 3.35 (s, 3H), 2.76 (dd, *J* = 12.0, 3.6 Hz, 1H), 1.65 (t, *J* = 12.0 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 175.89, 173.69, 162.05, 142.71, 126.31, 116.59, 100.42, 100.04, 74.20, 73.39, 72.52, 72.37, 70.46, 70.40, 68.57, 68.33, 68.12, 63.19, 61.12, 58.61, 51.67, 40.47; HRMS (ESI) calculated for $C_{24}H_{34}N_2O_{17}Na$ (M+H) 645.1755, found 645.1720.

4-Nitrophenyl *O*-(5-glycolylamido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Gc9Deoxyα2–6Galβ*p*NP, 9c). Yield, 84%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.28 (d, *J* = 9.6 Hz, 2H), 7.26 (d, *J* = 9.0 Hz, 2H), 5.18 (d, *J* = 7.8 Hz, 1H), 4.08 (s, 2H), 4.01–3.99 (m, 2H), 3.95–3.91 (m, 2H), 3.85–3.74 (m, 5H), 3.66 (dd, *J* = 10.8, 4.2 Hz, 1H), 3.30 (dd, *J* = 8.4, 1.2 Hz, 1H), 2.75 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.64 (t, *J* = 12.0 Hz, 1H), 1.22 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 175.85, 173.75, 162.05, 142.71, 126.31, 116.60, 100.44, 100.06, 74.20, 73.26, 72.52, 72.38, 70.46, 68.56, 68.19, 67.71, 63.18, 61.12, 51.80, 40.47, 18.56; HRMS (ESI) calculated for $C_{23}H_{32}N_2O_{16}Na$ (M+H) 615.1650, found 615.1616.

4-Nitrophenyl *O*-(9-azido-5-glycolylamido-3,5,9-trideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Gc9N$_3$α2–6Galβ*p*NP, 10c). Yield, 82%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.30 (d, *J* = 9.0 Hz, 2H), 7.28 (d, *J* = 9.0 Hz, 2H), 5.21 (d, *J* = 7.8 Hz, 1H), 4.12 (s, 2H), 4.06–4.03 (m, 2H), 3.99 (t, *J* = 8.4 Hz, 1H), 3.83-3.72 (m, 7H), 3.63 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.56 (dd, *J* = 12.0, 6.6 Hz, 1H), 3.49 (d, *J* = 9.0 Hz, 1H), 2.80 (dd, *J* = 12.0, 3.6 Hz, 1H), 1.69 (t, *J* = 12.0 Hz, 1H); ^{13}CNMR (151 MHz, D_2O) δ 175.96, 173.66, 161.98, 142.62, 126.25, 116.53, 100.37, 100.01, 74.14, 72.46, 71.94, 70.42, 68.53, 68.24, 68.03, 63.16, 62.71, 61.08, 59.57, 51.68, 40.42; HRMS (ESI) calculated for $C_{23}H_{29}N_5O_{16}$ (M-Na-H) 631.1609, found 631.1565.

4-Nitrophenyl *O*-(3,9-dideoxy-9-fluoro-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Kdn9Fα2–6Galβ*p*NP, 12c). Yield, 98%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.25 (d, *J* = 9.0 Hz, 2H), 7.23 (d, *J* = 9.6 Hz, 2H), 5.15 (d, *J* = 7.8 Hz, 1H), 4.73–4.56 (m, 2H), 4.02–3.88 (m, 5H), 3.81 (t, *J* = 8.4 Hz, 1H), 3.75 (dd, *J* = 9.6, 3.0 Hz, 1H), 3.61–3.55 (m, 3H), 3.41 (t, *J* = 9.6 Hz, 1H), 2.67 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.57 (t, *J* = 12.6 Hz, 1H); ^{13}C NMR (151 MHz, D_2O) δ 173.85, 161.99, 142.63, 126.25, 116.50, 100.35, 99.98, 84.55 (d, *J* = 166.8 Hz), 74.17, 73.50, 72.45, 70.62, 70.38, 70.24, 69.97, 68.55, 66.75, 66.70, 63.22, 40.02; ^{19}F NMR (282 MHz, D_2O) δ -234.42 (ddd, *J* = 47.9, 47.9, 28.2 Hz, 1F); HRMS (ESI) calculated for $C_{21}H_{28}NO_{15}FNa$ (M+H) 576.1341, found 576.1330.

4-Nitrophenyl *O*-(9-*O*-methyl-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Kdn9OMeα2–6Galβ*p*NP, 13c). Yield, 62%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.24 (d, *J* = 7.2 Hz, 2H), 7.21 (d, *J* = 7.8 Hz, 2H), 5.13 (d, *J* = 7.2 Hz, 1H), 3.96–3.95 (m, 2H), 3.89 (t, *J* = 8.4 Hz, 2H), 3.80 (t, *J* = 8.4 Hz, 1H), 3.74–3.67 (m, 3H), 3.60–3.50 (m, 4H), 3.83 (t, *J* = 9.0 Hz, 1H), 3.34 (s, 3H), 2.65 (dd, *J* = 12.0, 3.6 Hz, 1H), 1.55 (t, *J* = 12.0 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 174.01, 162.36, 143.06, 126.56, 117.05, 100.82, 100.46, 74.55, 73.97, 73.93, 72.91, 70.98, 70.82, 70.66, 70.40, 68.92, 68.60, 63.48, 58.90, 40.28; HRMS (ESI) calculated for $C_{22}H_{31}NO_{16}Na$ (M+H) 588.1541, found 588.1531.

4-Nitrophenyl *O*-(3,9-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Kdn9Deoxyα2–6Galβ*p*NP, 14c). Yield, 70%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.25 (d, *J* = 9.6 Hz, 2H), 7.22 (d, *J* = 9.6 Hz, 2H), 5.14 (d, *J* = 7.2 Hz, 1H), 3.97–3.95 (m, 2H), 3.89 (t, *J* = 8.4 Hz, 2H), 3.82–3.79 (m, 1H), 3.74 (dd, *J* = 10.2, 3.6 Hz, 1H), 3.60–3.51 (m, 4H), 3.39 (t, *J* = 9.6 Hz, 1H), 2.66 (dd, *J* = 12.6, 4.8 Hz, 1H), 1.55 (t, *J* = 12.6 Hz, 1H), 1.22 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) δ 173.90, 161.99, 142.69, 126.26, 116.52, 100.35, 99.99, 74.17, 73.59, 72.80, 72.46, 70.40, 70.31, 70.11, 68.50, 67.92, 63.18, 40.05, 18.54; HRMS (ESI) calculated for $C_{21}H_{29}NO_{15}Na$ (M+H) 558.1435, found 558.1424.

4-Nitrophenyl *O*-(9-azido-3,9-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Kdn9N$_3$α2–6Galβ*p*NP, 15c). Yield, 59%; white solid. ^{1}H NMR (600 MHz, D_2O) δ 8.28 (d, *J* = 9.0 Hz, 2H), 7.25 (d, *J* = 9.0 Hz, 2H), 5.17 (d, *J* = 8.4 Hz, 1H) 4.00–3.90 (m, 4H), 3.85–3.76 (m, 3H), 3.68–3.61 (m, 4H), 3.46 (dd, *J* = 13.2, 6.6 Hz, 1H), 3.41 (t, *J* = 9.6 Hz, 1H), 2.69 (dd, *J* = 12.0, 4.2 Hz, 1H), 1.59 (t, *J* = 12.0, 1H); ^{13}C NMR (151 MHz, D_2O) δ 171.69,

159.89, 140.57, 124.14, 114.44, 98.34, 97.69, 72.07, 71.42, 70.37, 68.70, 68.29, 68.11, 67.91, 66.53, 66.43, 61.10, 51.19, 37.87; HRMS (ESI) calculated for $C_{21}H_{28}N_4O_{15}Na$ (M+H) 599.1449, found 599.1442.

Sialidase substrate specificity assays

Materials: *Clostridium perfringens* sialidase (type VI), *Vibrio cholerae* sialidase (type III), and β-galactosidase from *Aspergillus oryzae* were purchased from Sigma. *Salmonella typhimurium* sialidase and *Streptococcus pneumoniae* sialidase were bought from Prozyme. All of these enzymes were used without further purification. PmST1 was expressed in *E. coli* and purified as described previously.[6] Sodium pyruvate (Fisher Biotech), *N*-acetylmannosamine (ManNAc, Sigma), mannose (Acros Organics), *para*-nitrophenyl β-D-galactopyranoside (Galβ*p*NP, Sigma), and cytidine 5'-triphosphate disodium salt (CTP, Sigma) were from commercially available sources. 384-Well plates for sialidase assays were from Fisher Biotech.

All sialidase assays were carried out at 37 °C in duplicate for 30 min in 384-well plates in a final volume of 20 μL containing a sialoside substrate (0.3 mM) and the β-galactosidase (12 μg, 126 mU). The amount of the β-galactosidase required to completely hydrolyze Galβ*p*NP (0.3 mM) within the time frame of the assay was predetermined. The assay conditions for various sialidases were as follows: *S. typhimurium* sialidase (1.5 mU) in sodium acetate buffer (100 mM, pH 5.5) containing NaCl (100 mM); *C. perfringens* sialidase (2 mU) in MES buffer (100 mM, pH 5.0); *V. cholerae* sialidase (1 mU) in sodium acetate buffer (100 mM, pH 5.5) containing NaCl (150 mM) and $CaCl_2$ (10 mM); *S. pneumoniae* sialidase (0.2 mU) in sodium acetate buffer (100 mM, pH 6.0); PmST1 (7.5 μg) in sodium acetate buffer (100 mM, pH 5.5); NEU2 (6 μg) in MES buffer (100 mM, pH 5.0). The reactions were stopped by adding 40 μL of CAPS buffer (*N*-cyclohexyl-3-aminopropane sulfonic acid, 0.5 M, pH 10.5). The amount of *para*-nitrophenolate formed was determined by measuring the A_{405nm} of the reaction mixtures using a microplate reader. Reactions of Galβ*p*NP and an excess amount of β-galactosidase were used as controls.

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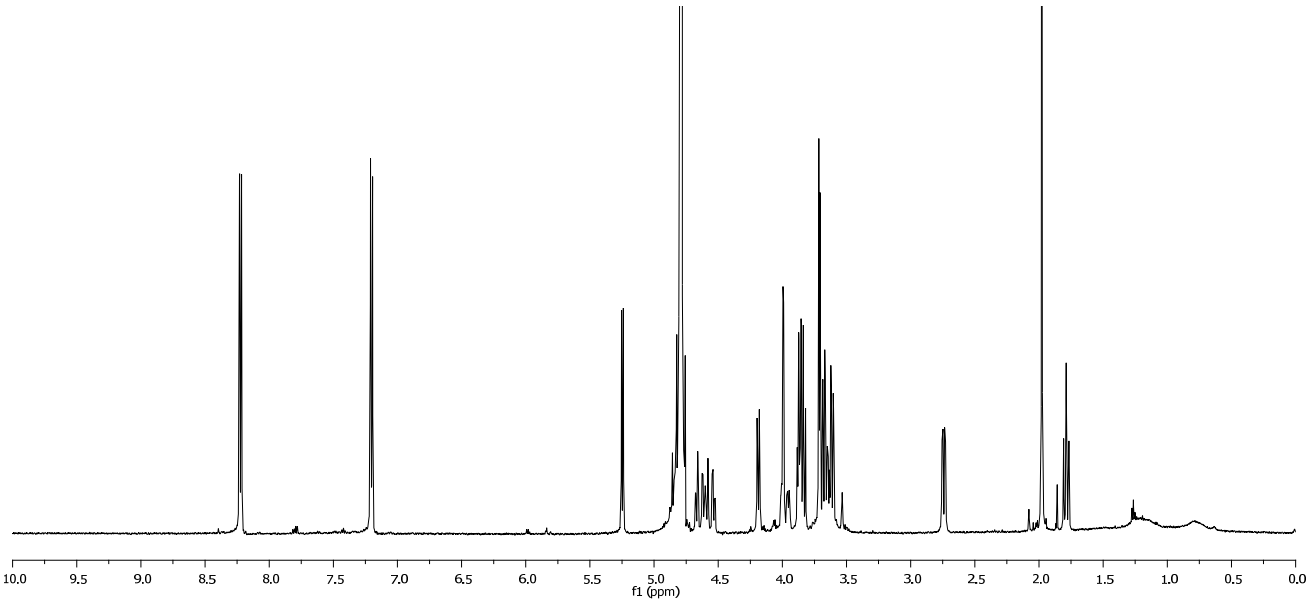
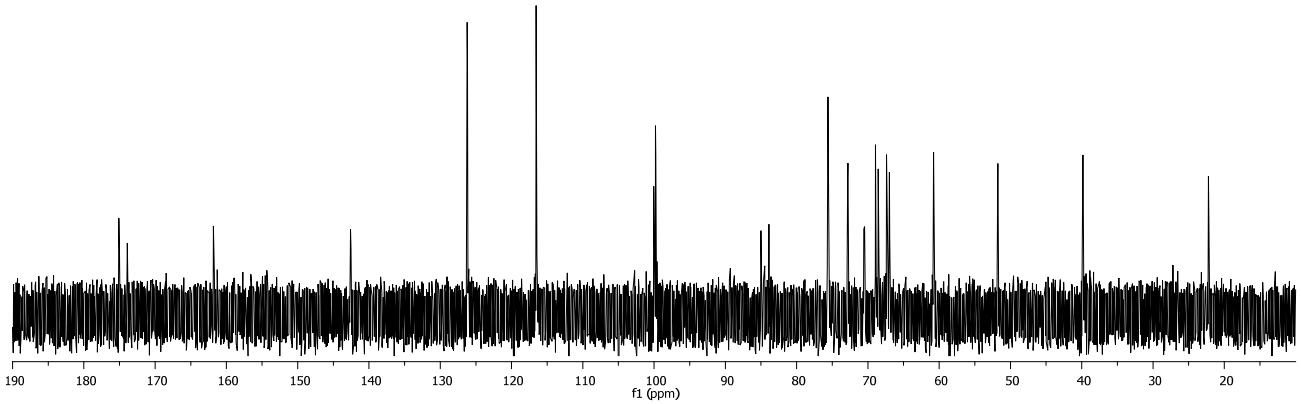
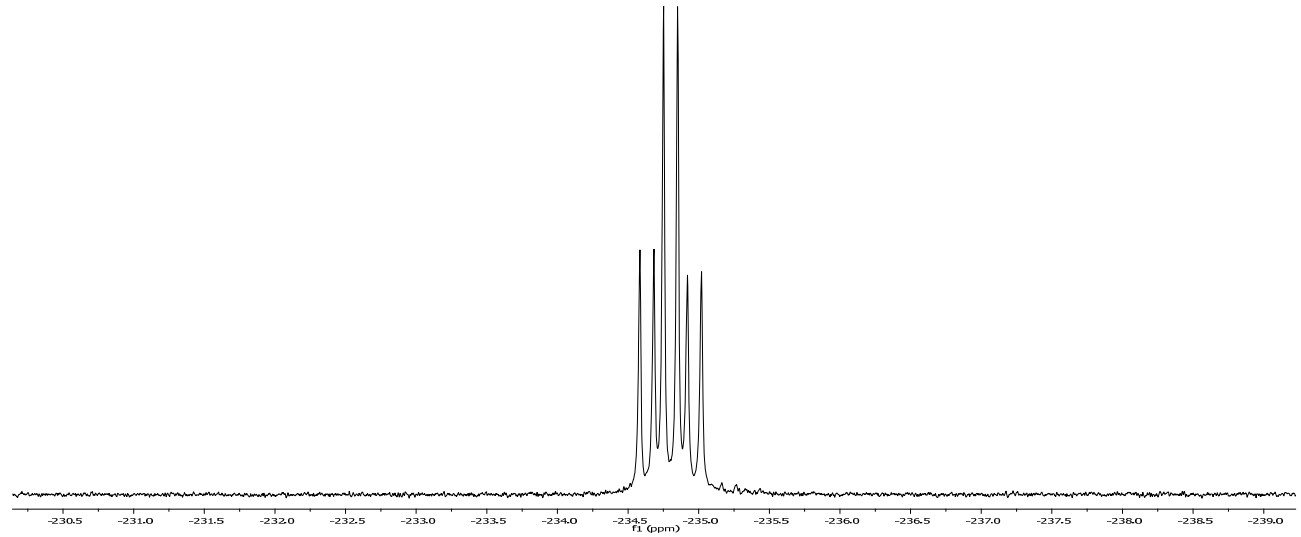
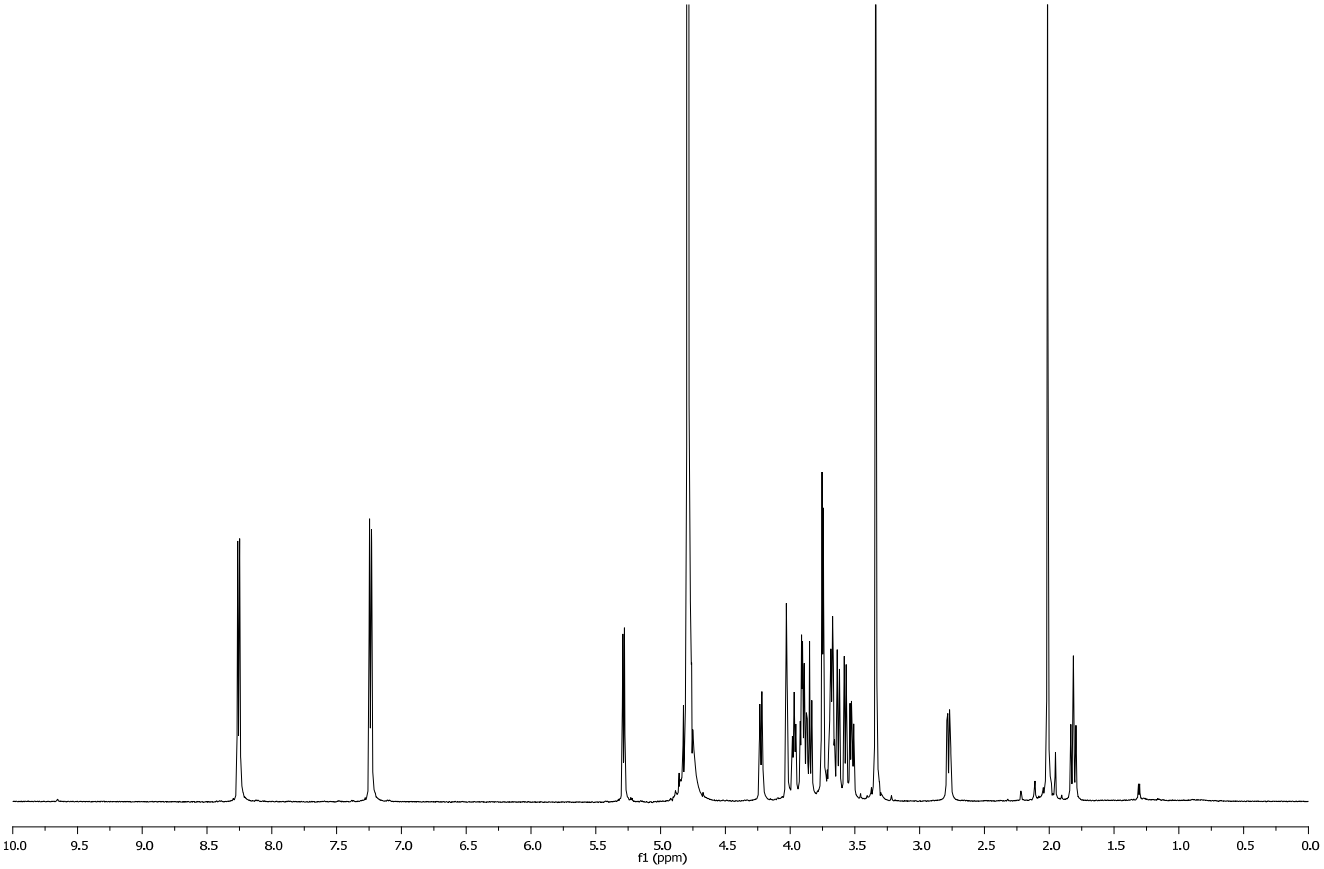
Compound **2b**, Neu5Ac9Fα2–3Galβ*p*NP

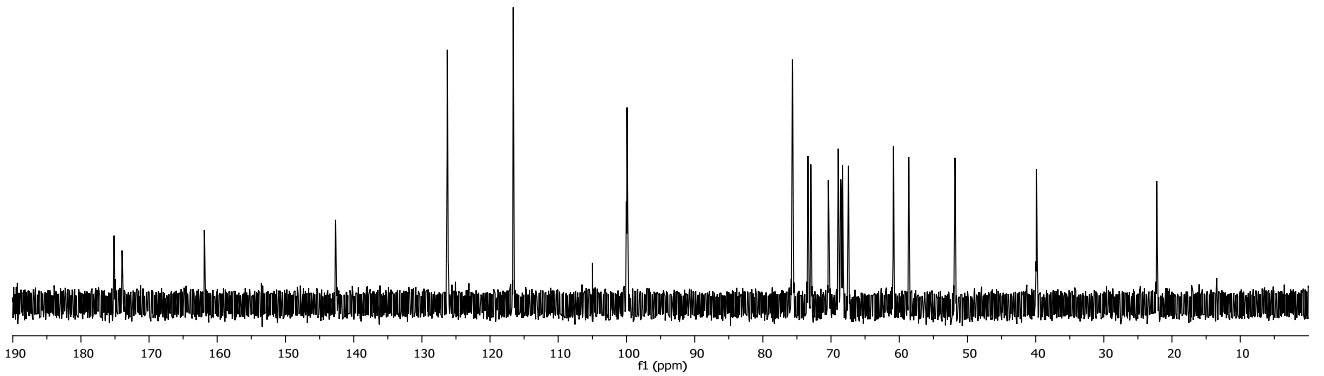




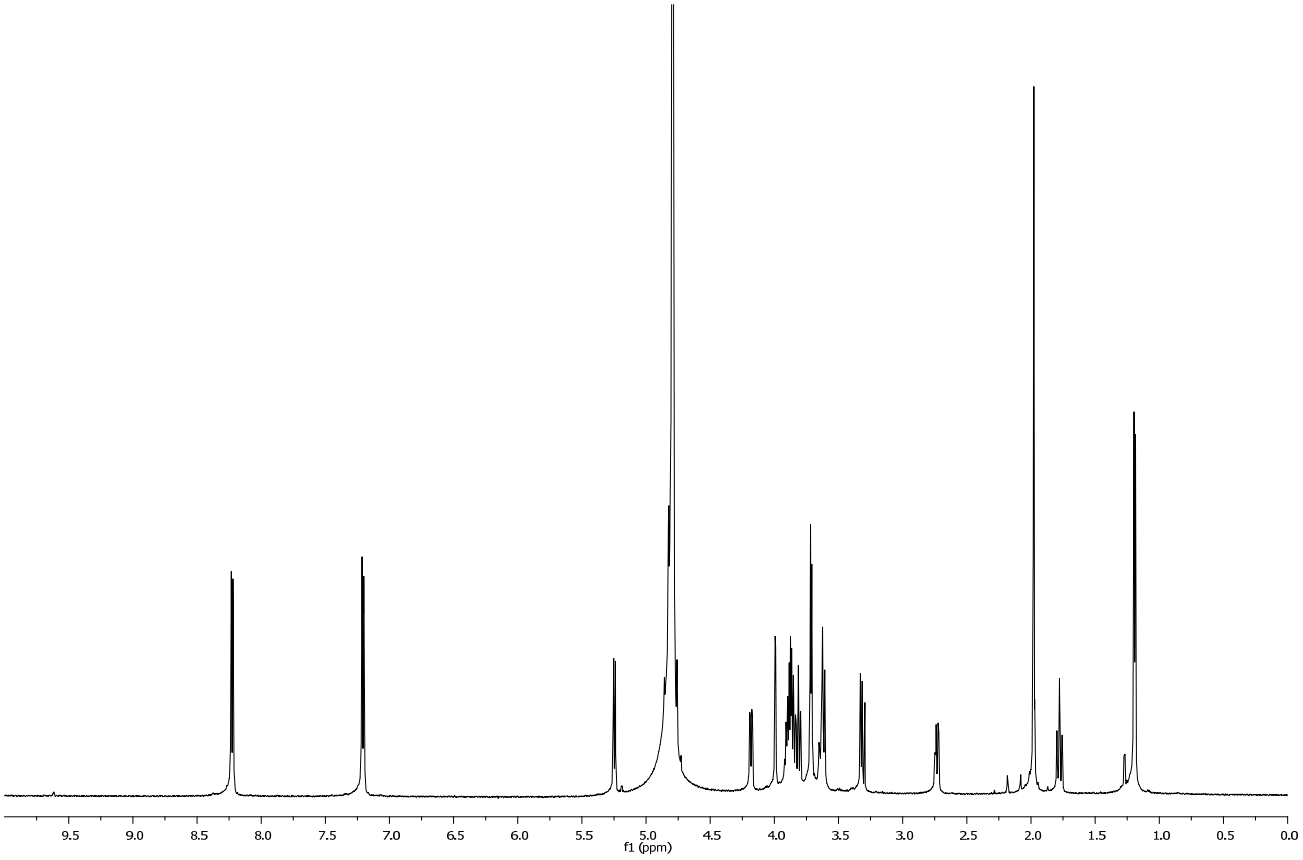


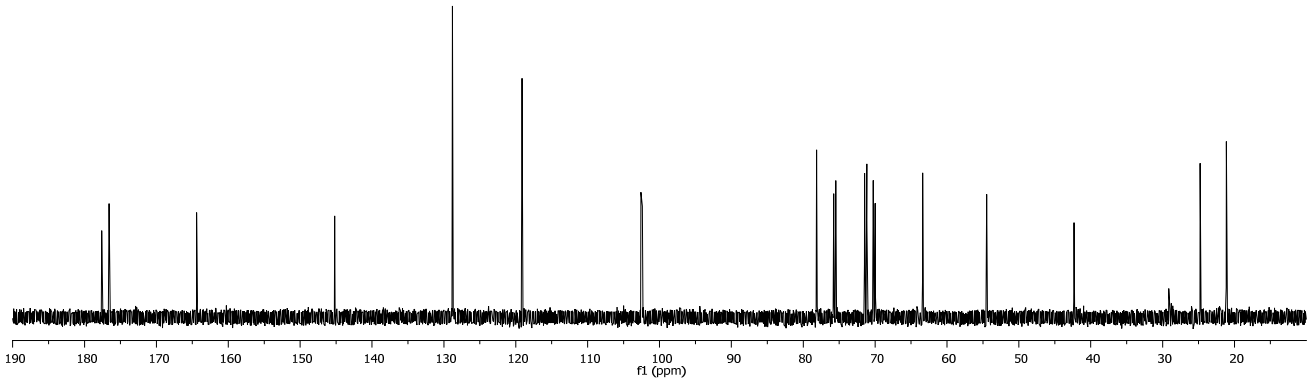
Compound **3b**, Neu5Ac9OMeα2–3Galβ*p*NP



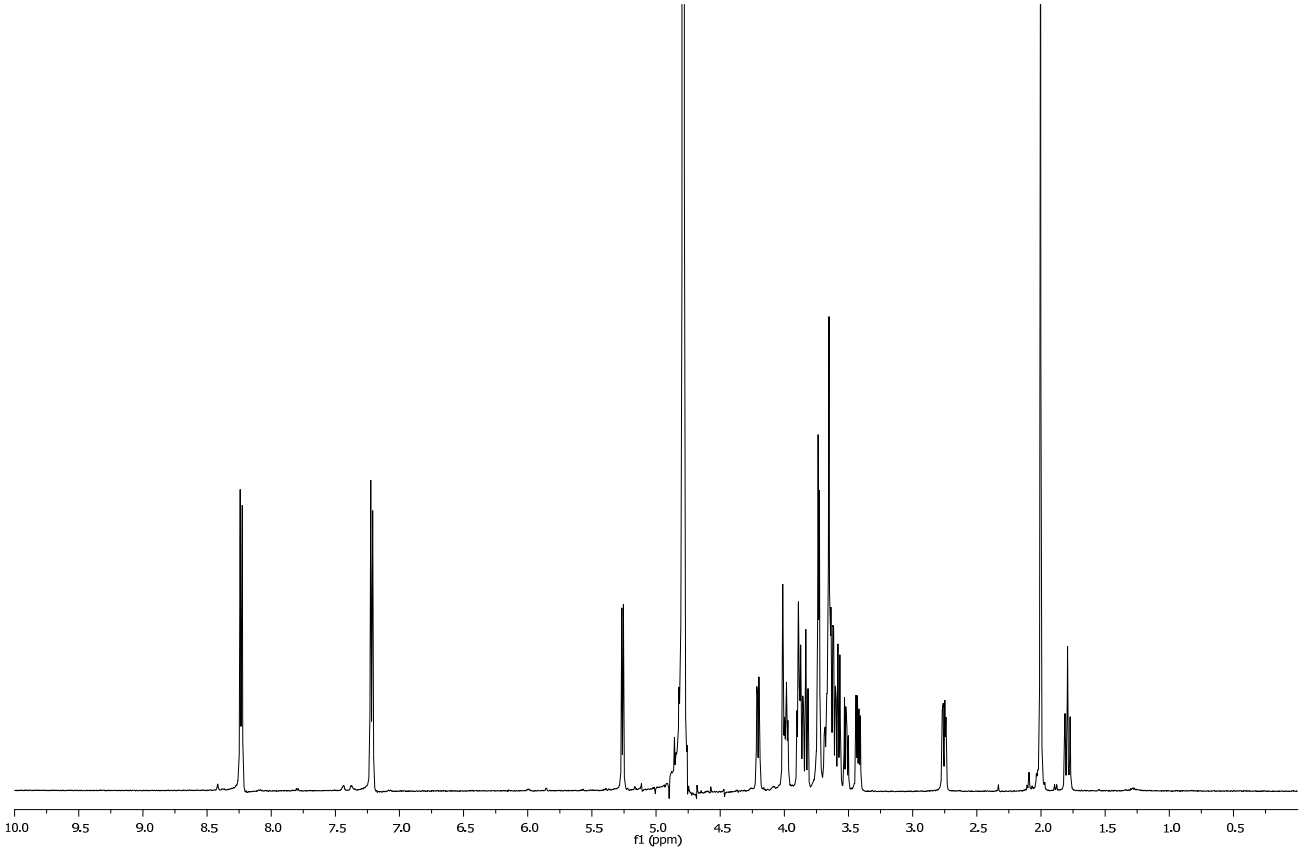


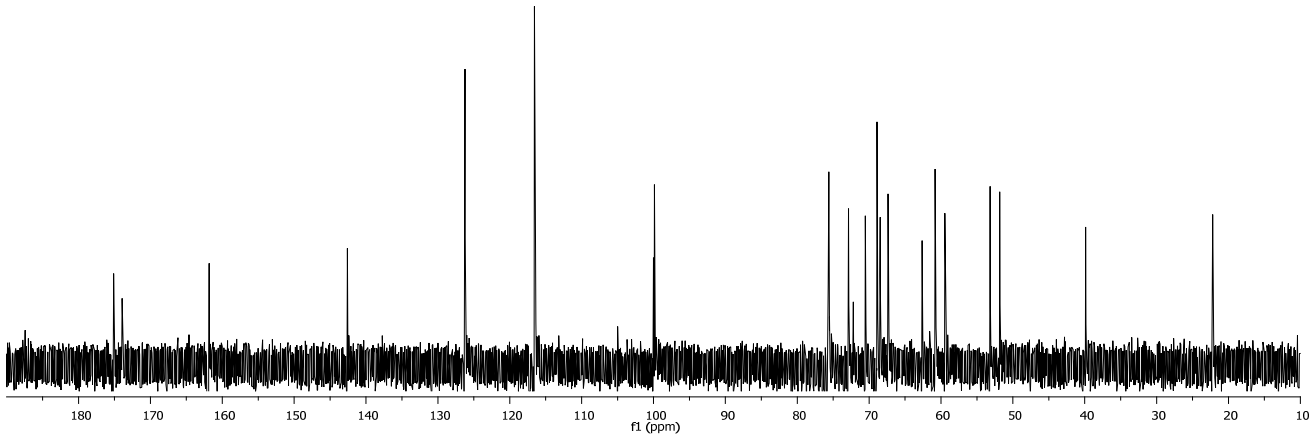
Compound **4b**, Neu5Ac9Deoxyα2–3Galβ*p*NP



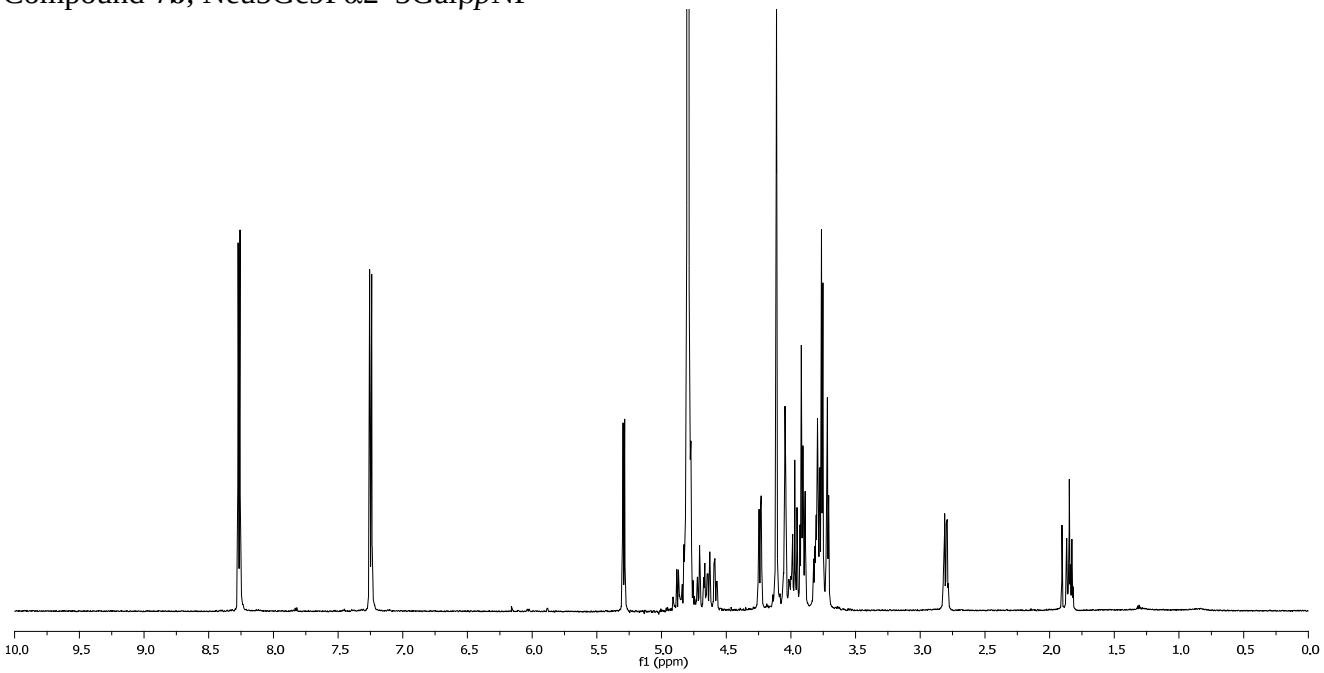


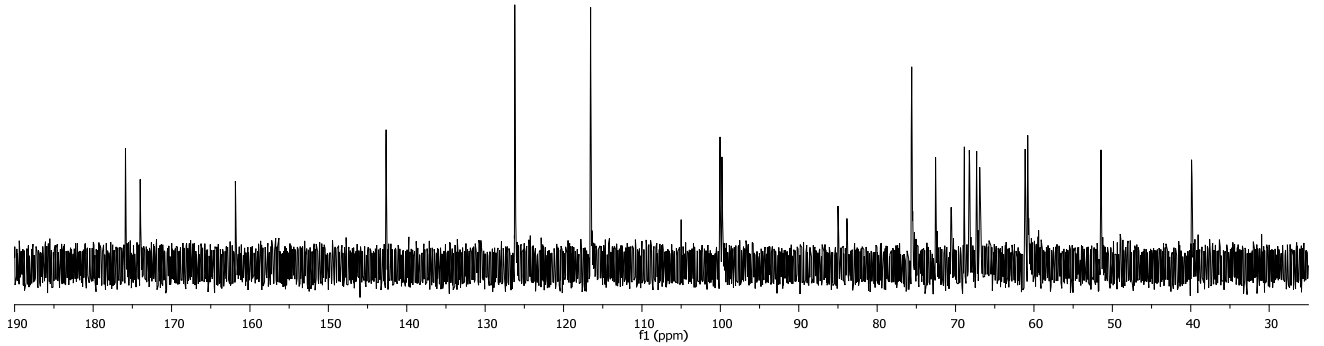
Compound **5b**, Neu5Ac9N$_3$α2–3Galβ*p*NP

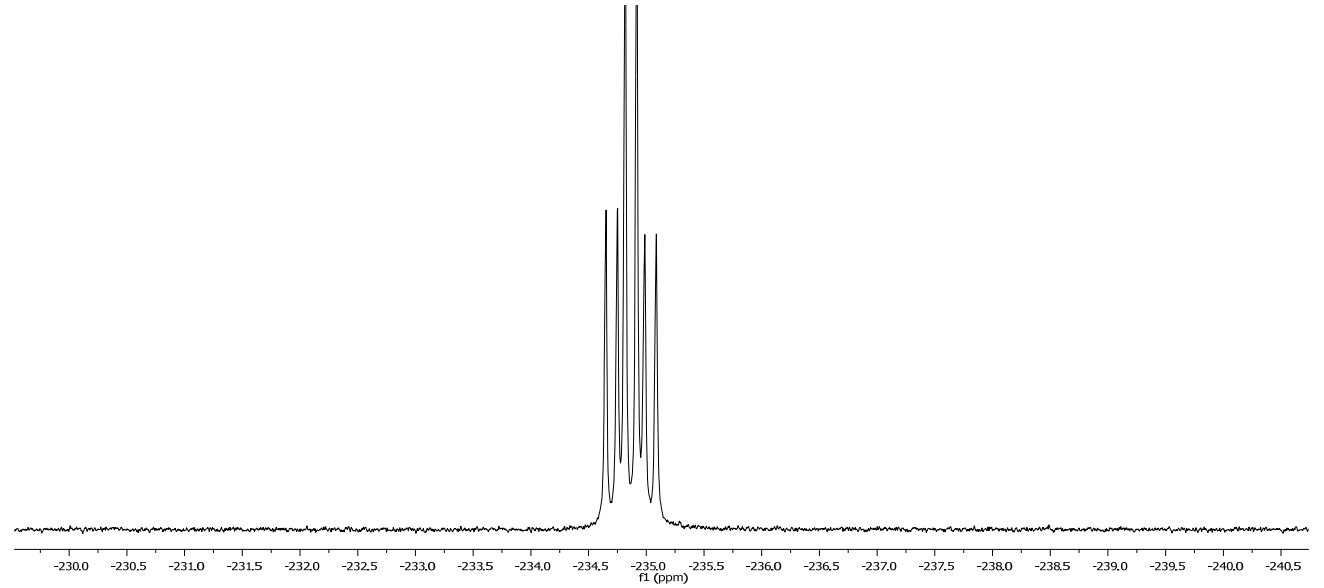




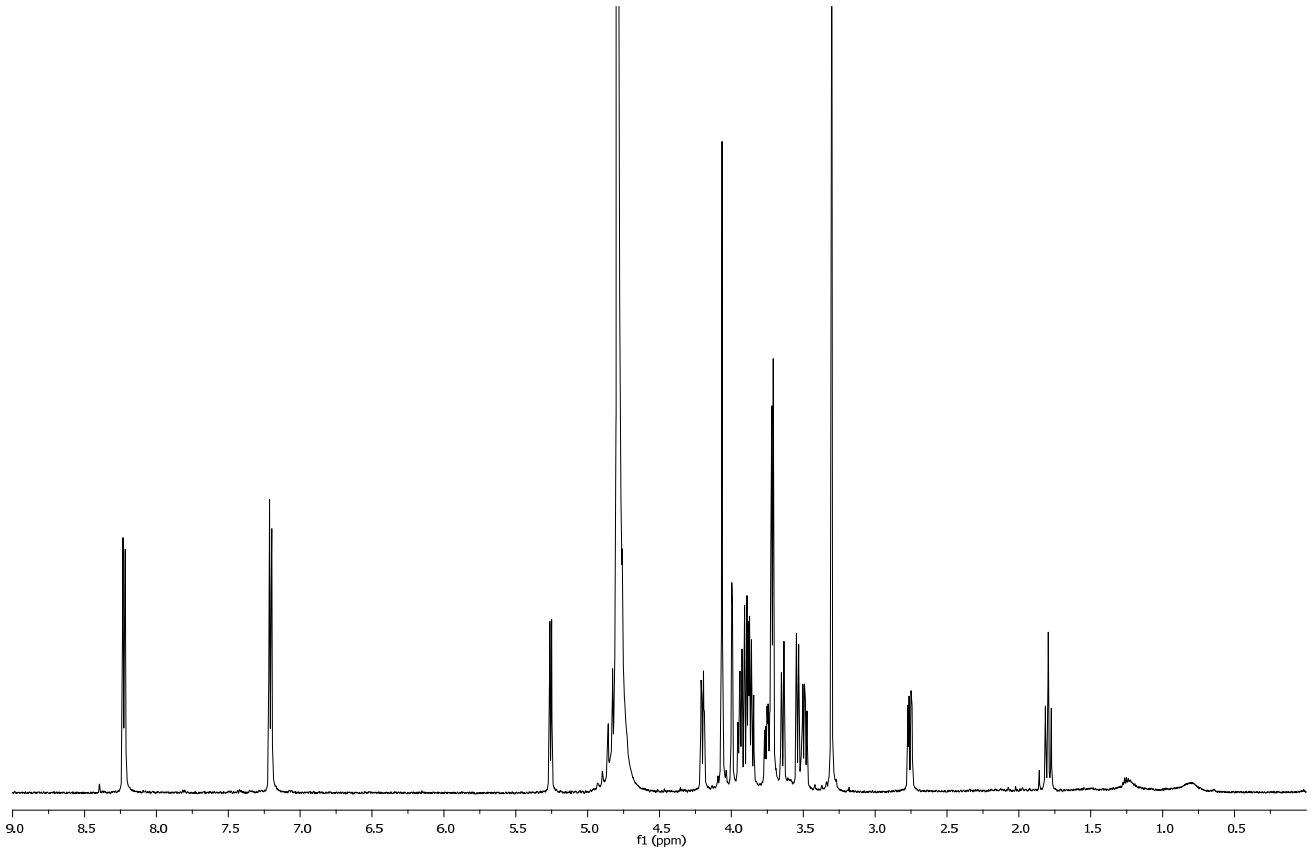
Compound **7b**, Neu5Gc9Fα2–3Galβ*p*NP

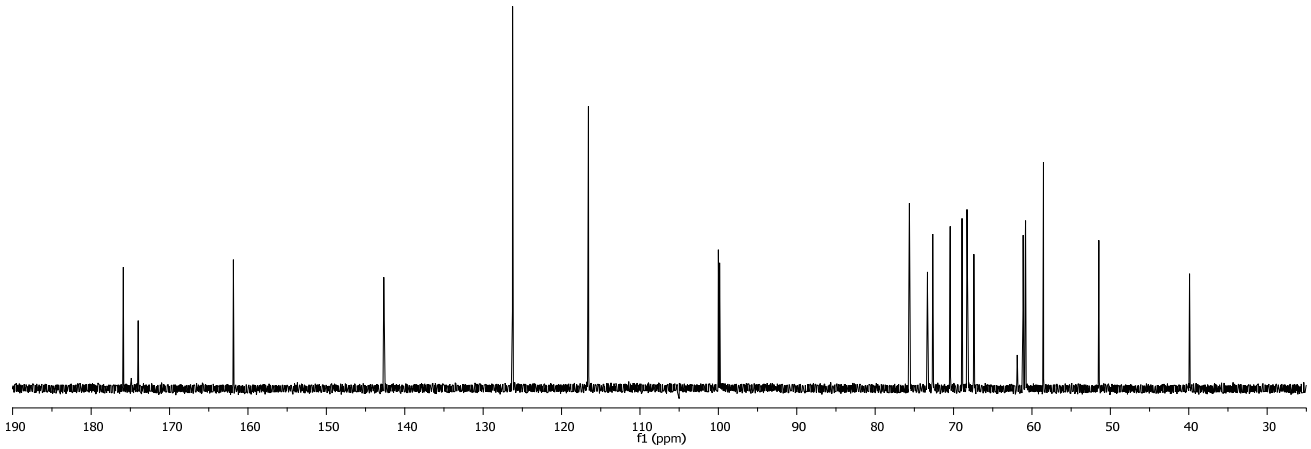






Compound **8b**, Neu5Gc9OMeα2–3Galβ*p*NP





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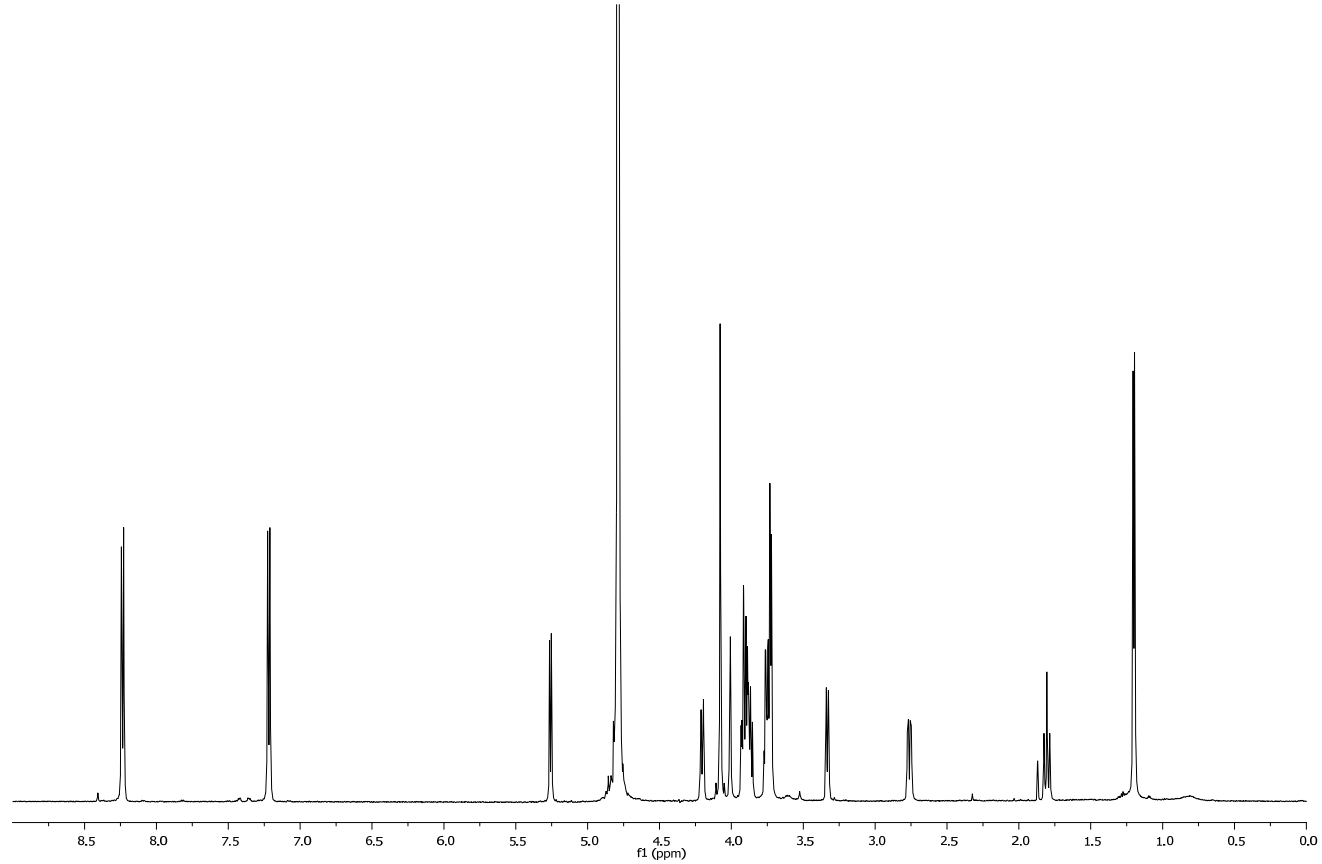
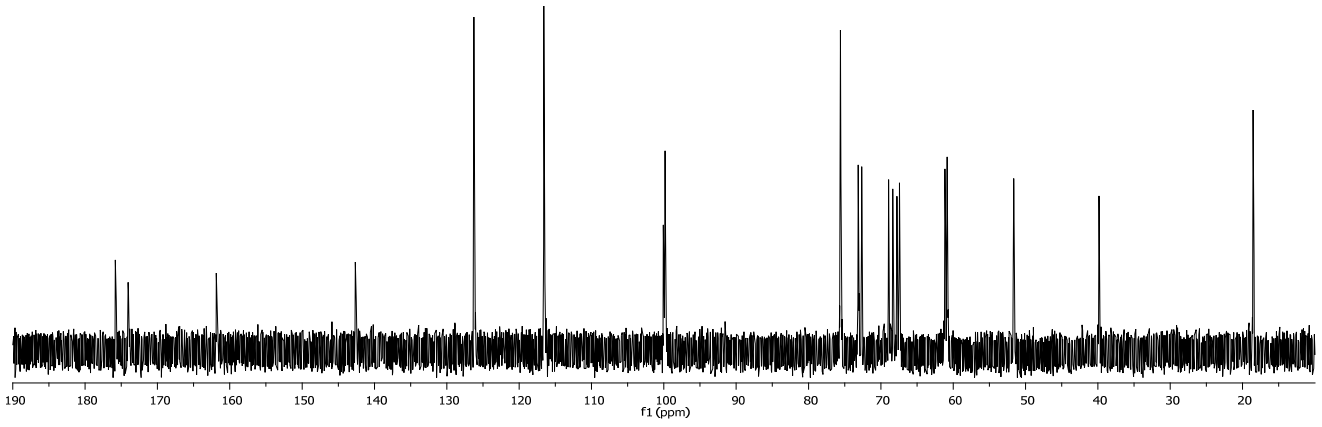
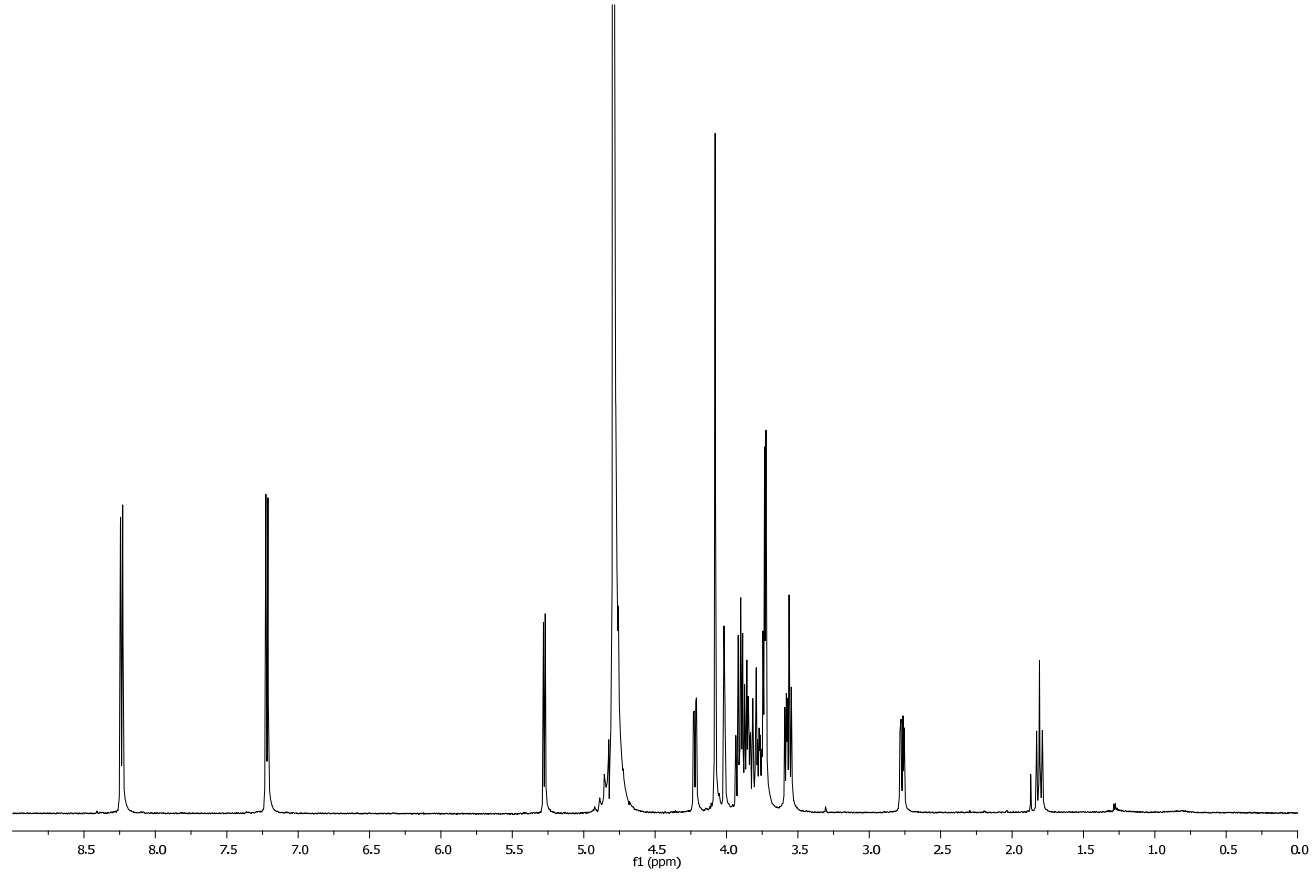
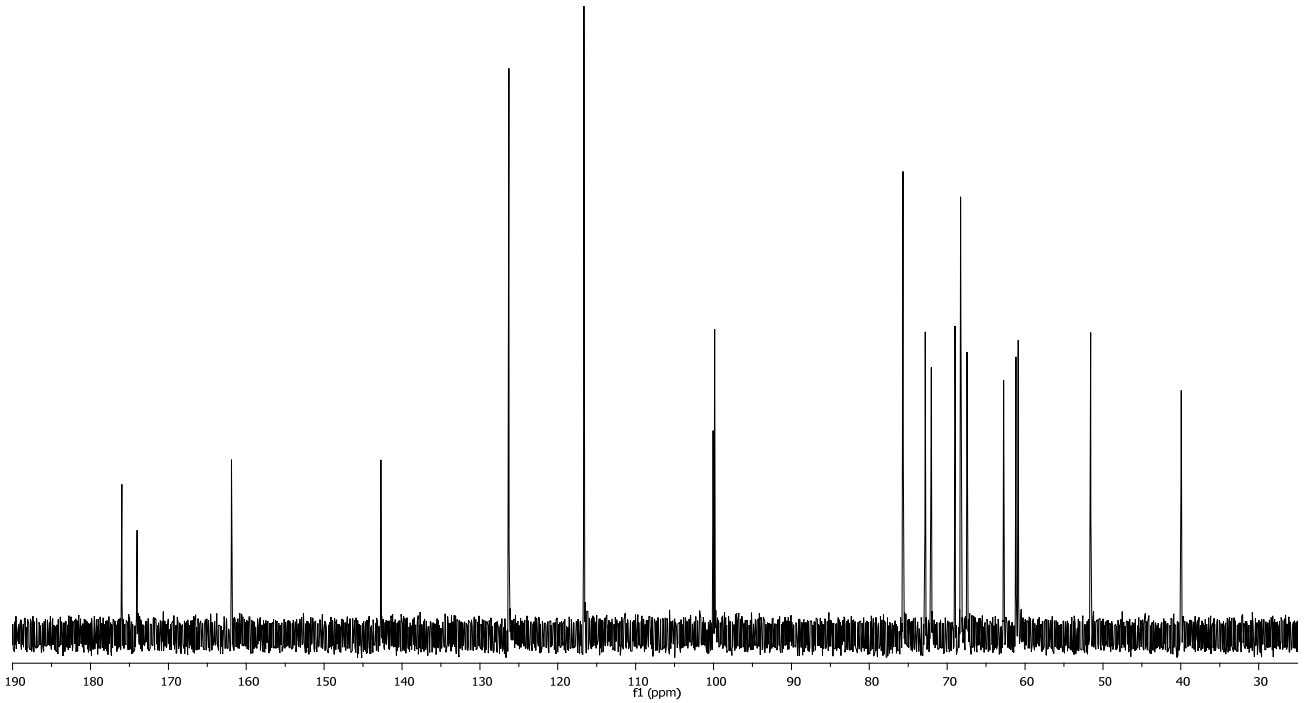


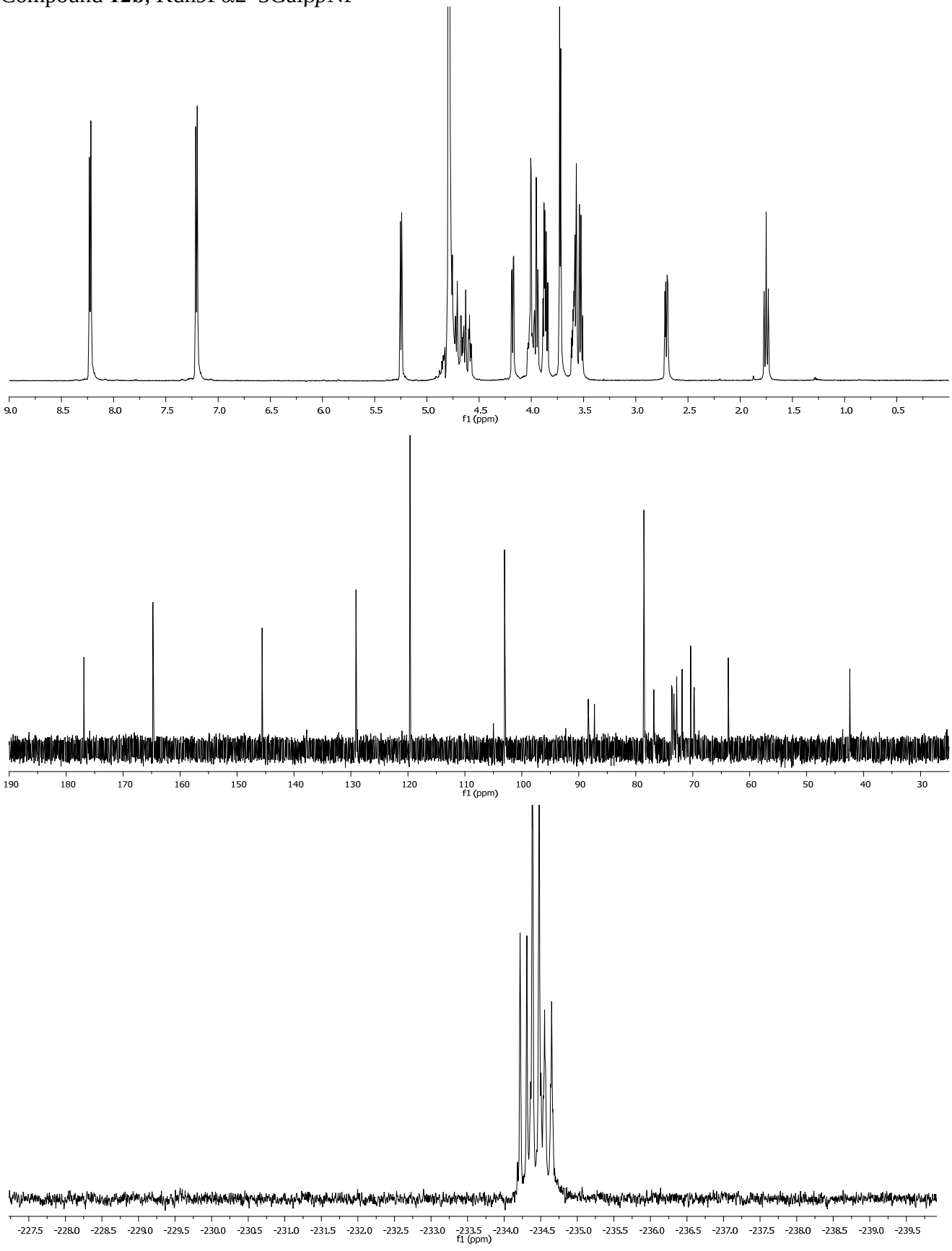


Compound **10b**, Neu5Gc9N$_3$α2–3Galβ*p*NP

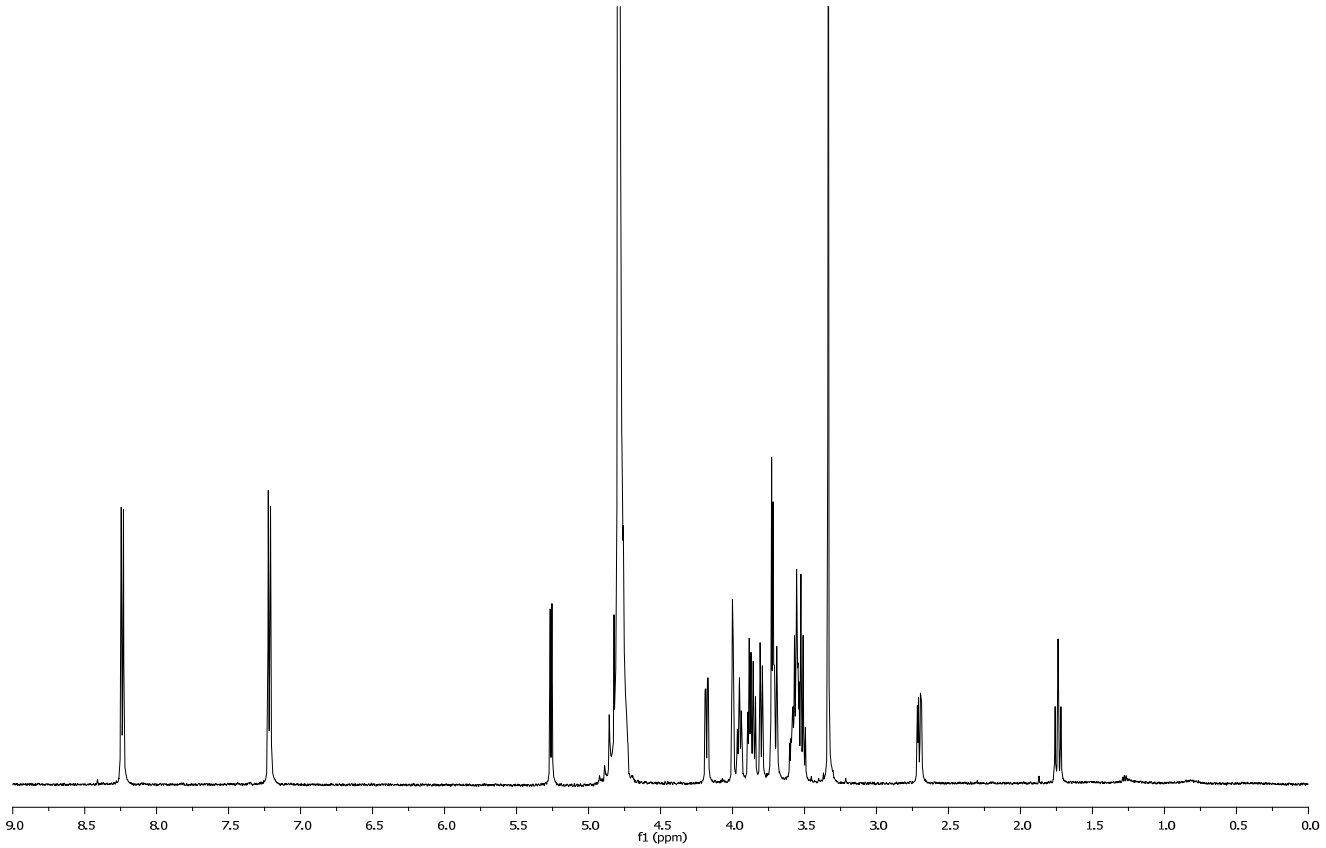


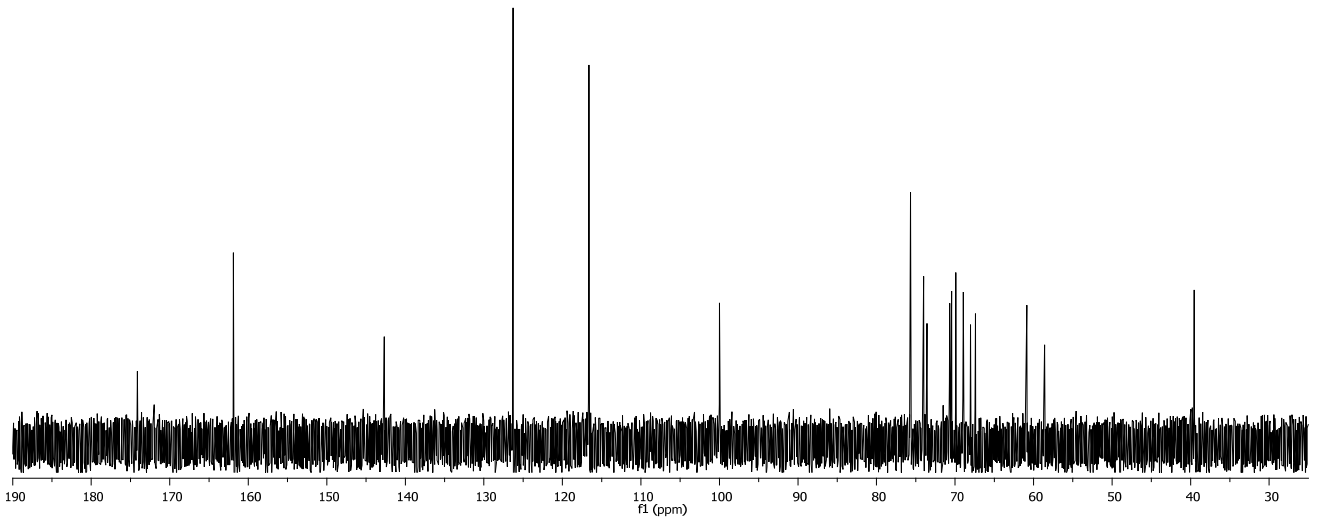


Compound **12b**, Kdn9Fα2–3Galβ*p*NP

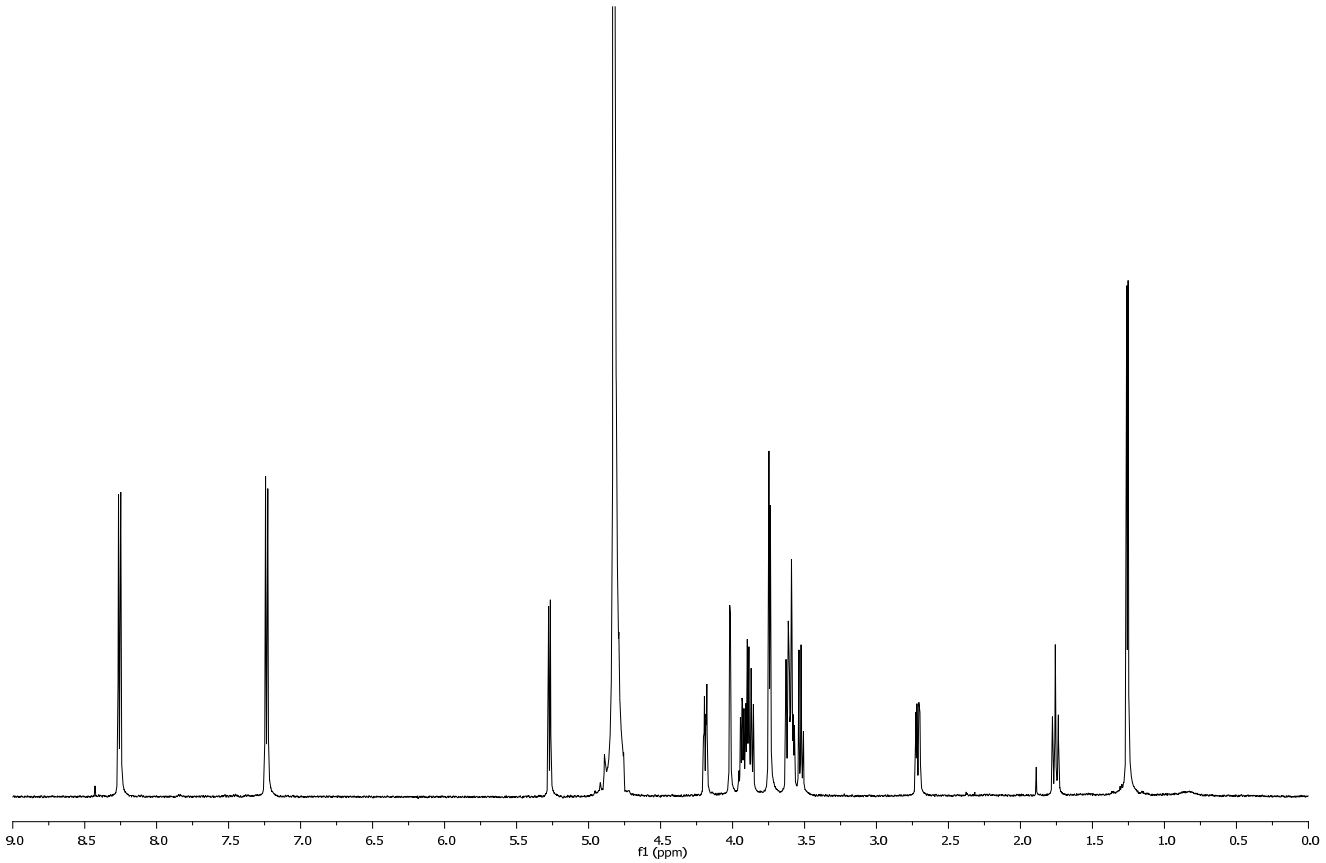


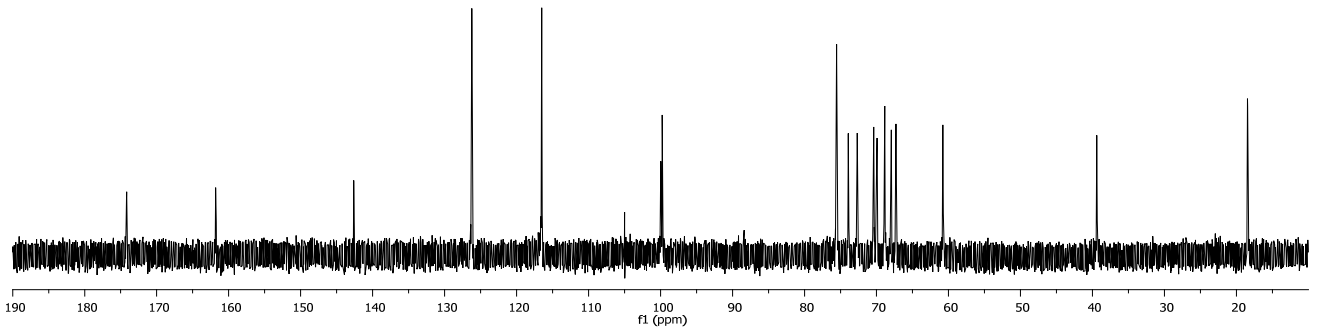
Compound **13b**, Kdn9OMeα2–3Galβ*p*NP



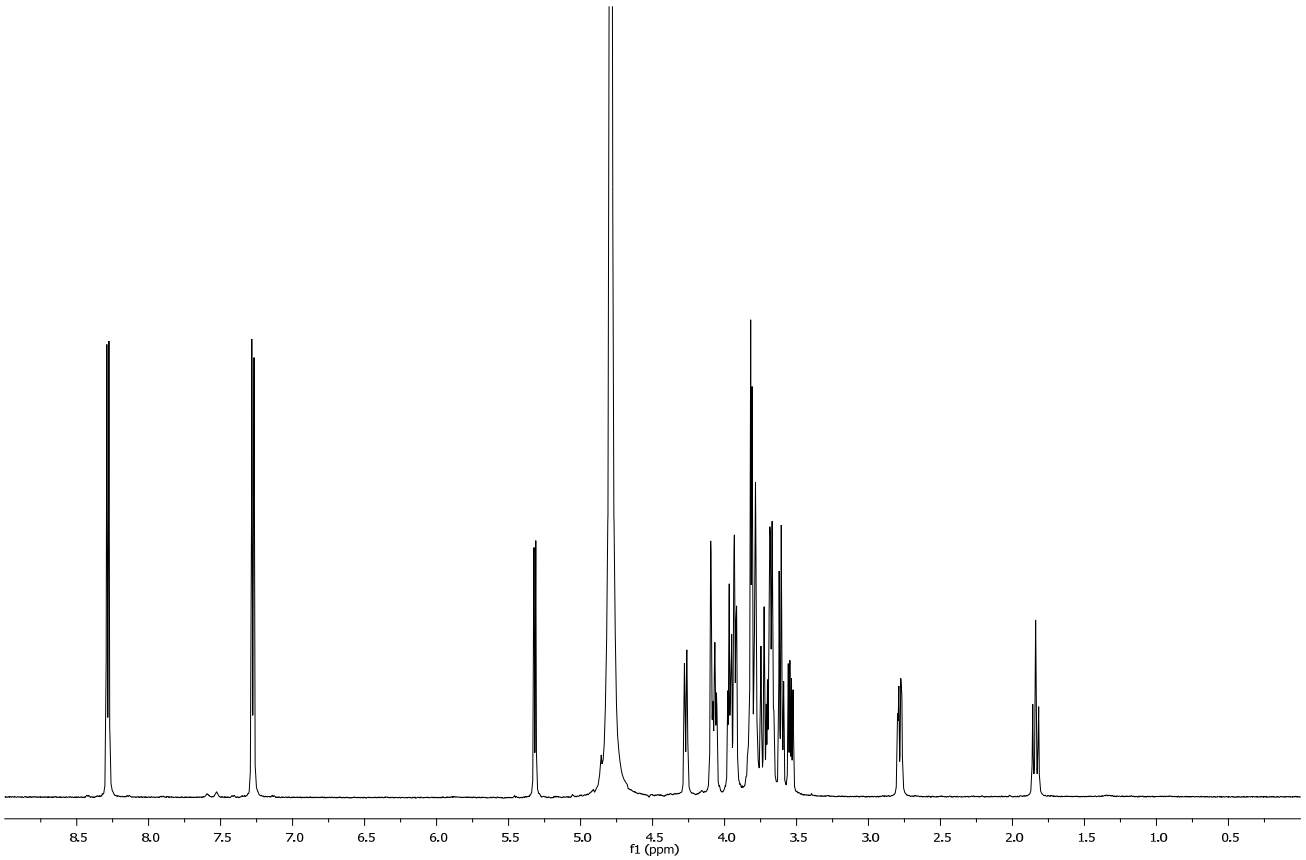


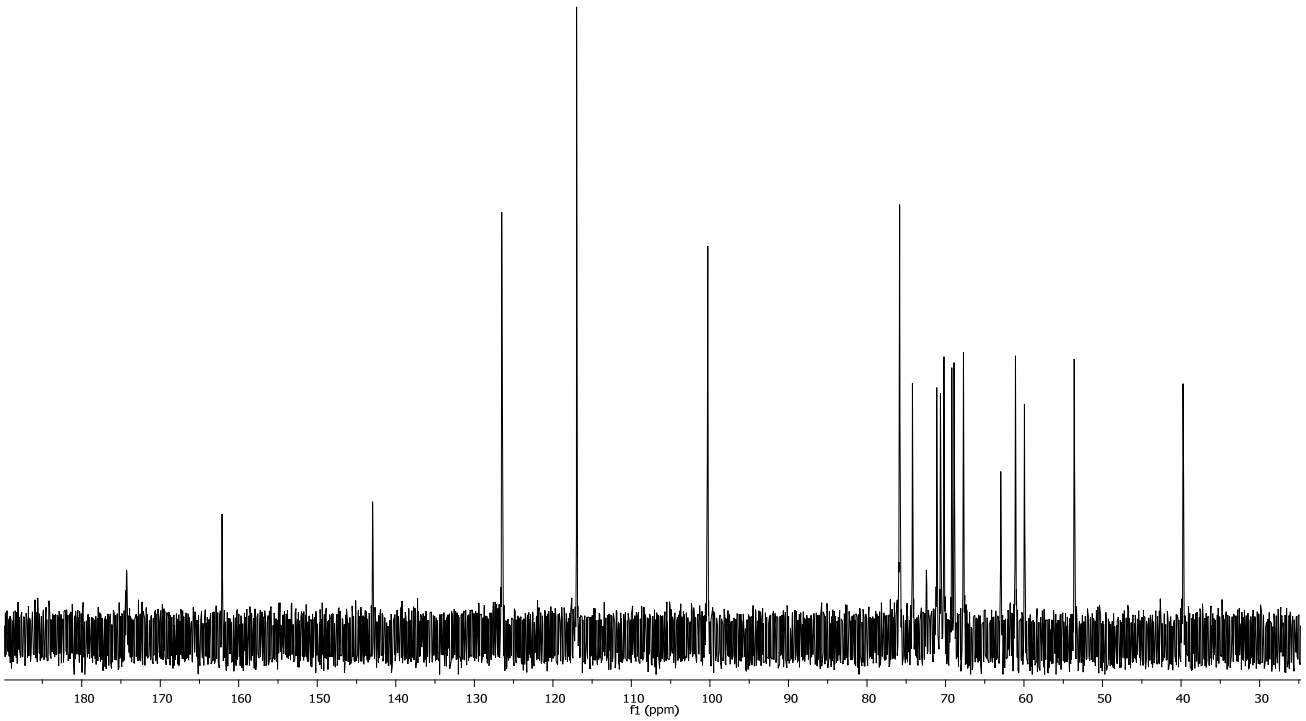
Compound **14b**, Kdn9Deoxyα2–3Galβ*p*NP



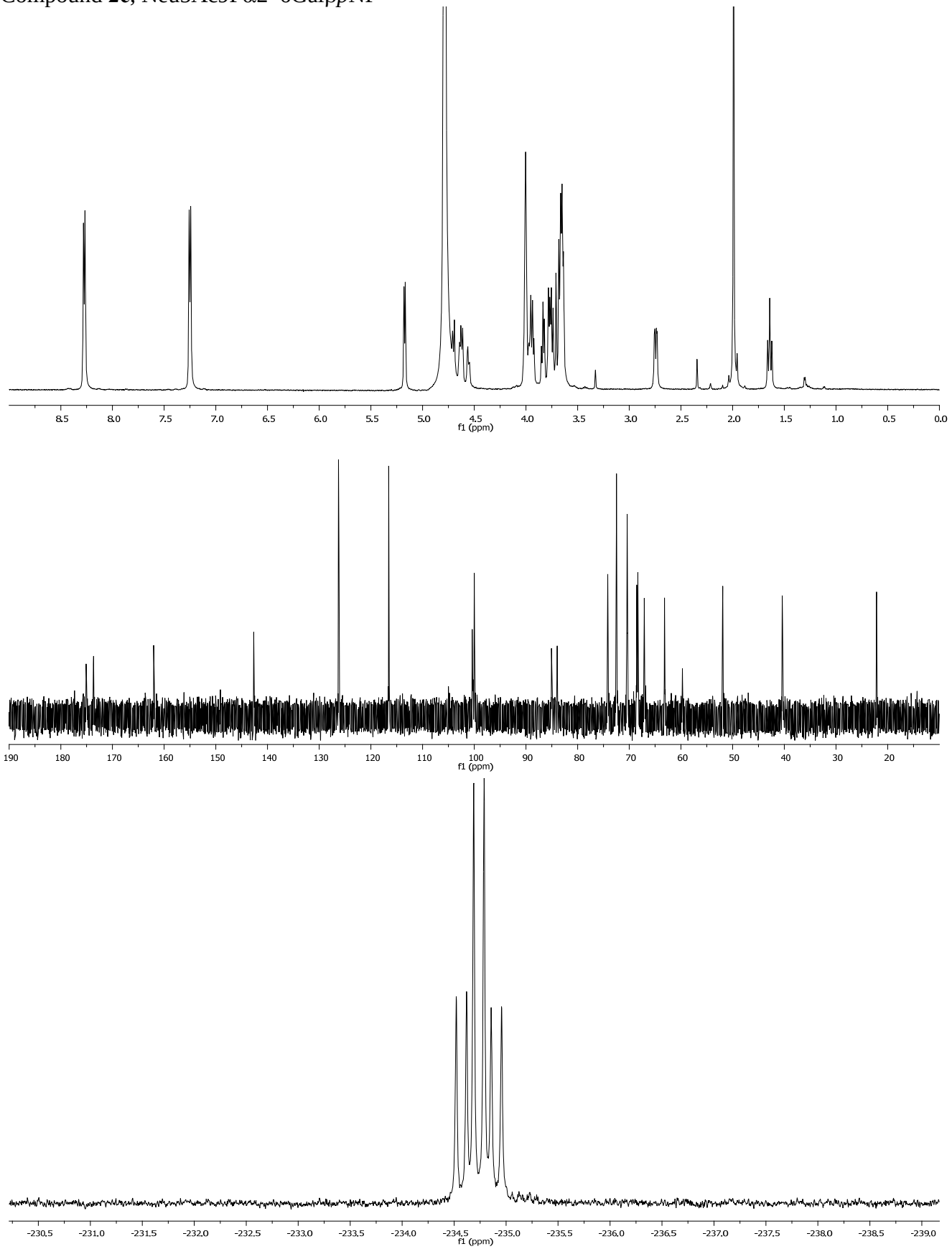


Compound **15b**, Kdn9N$_3$α2–3Galβ*p*NP

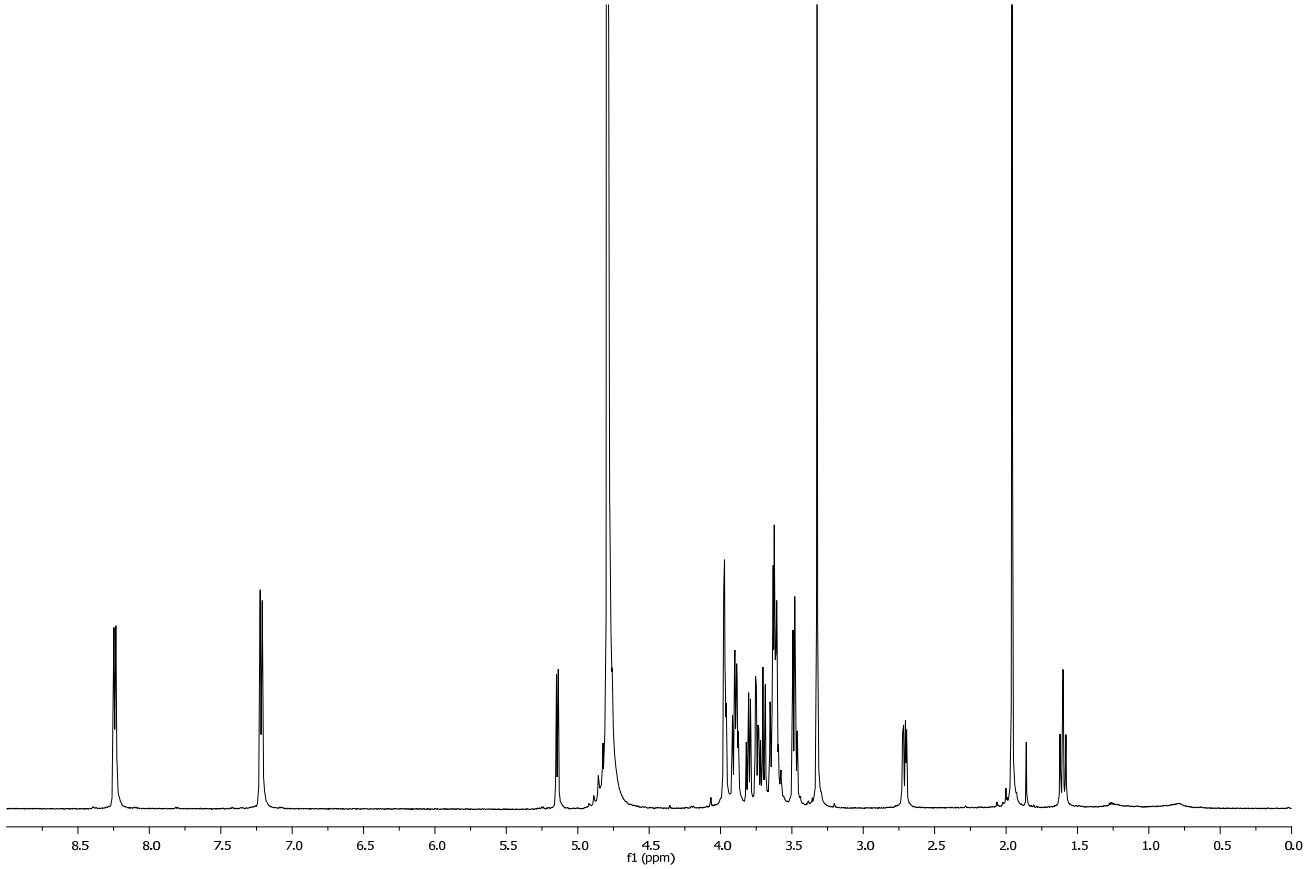


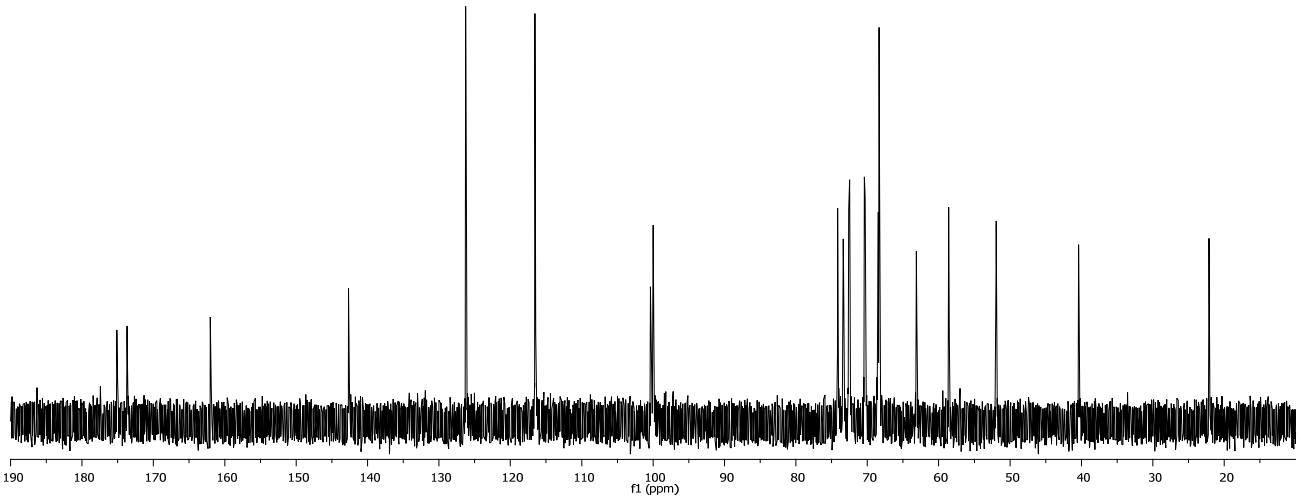


Compound **2c**, Neu5Ac9Fα2–6Galβ*p*NP

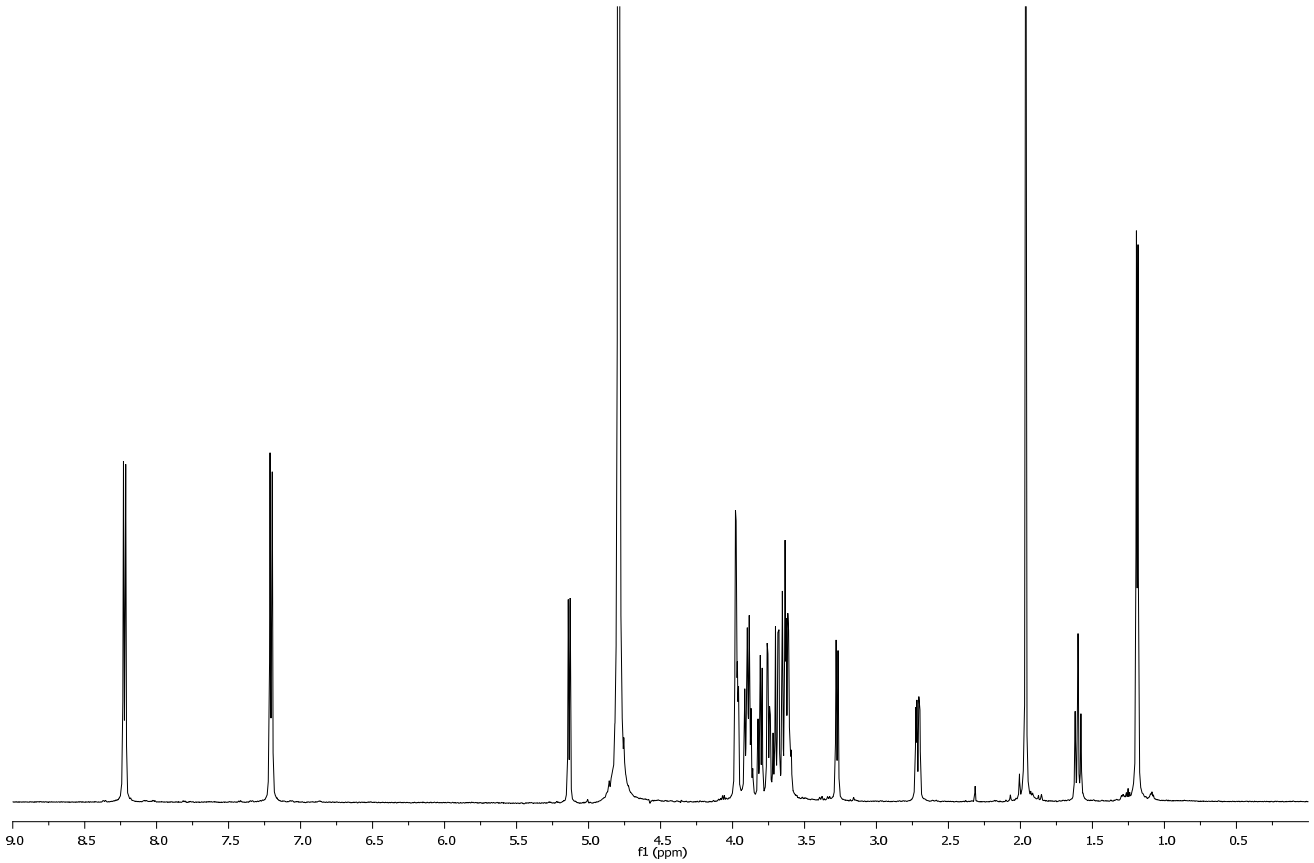


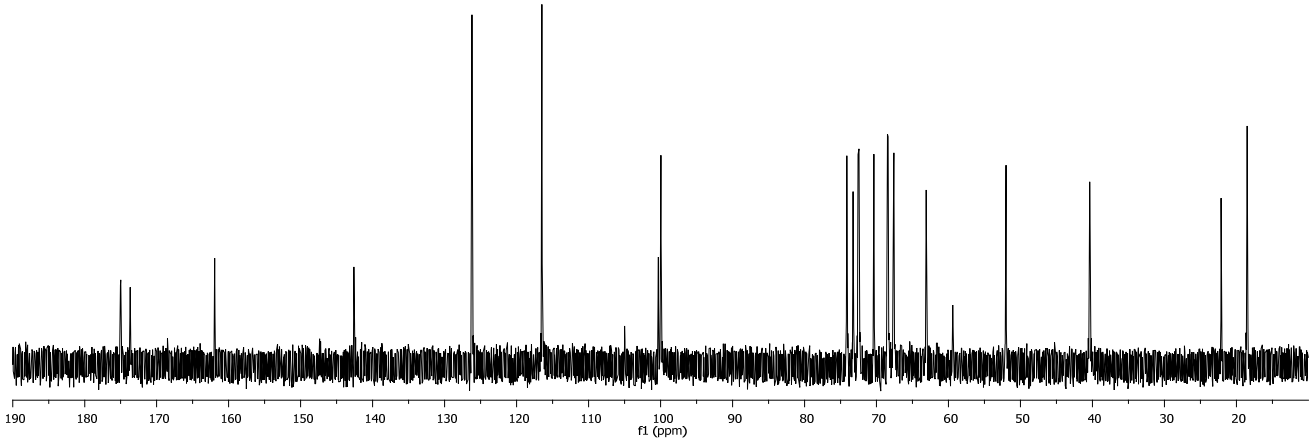
Compound **3c**, Neu5Ac9OMeα2–6Galβ*p*NP



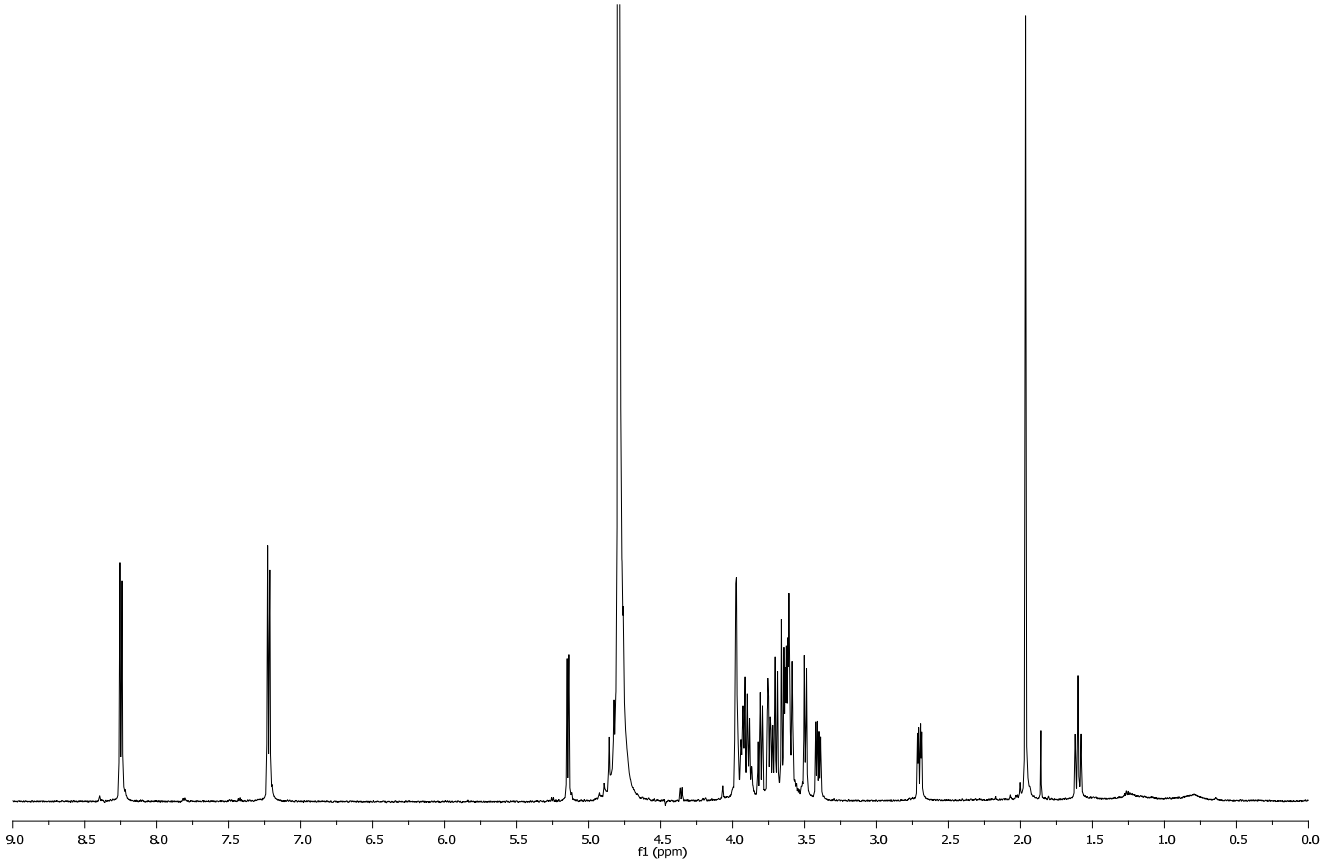


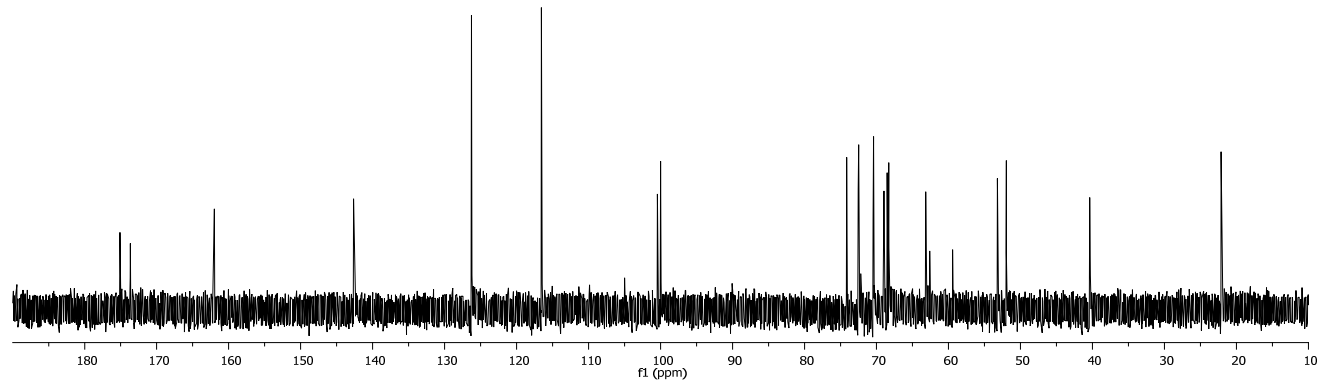
Compound **4c**, Neu5Ac9Deoxyα2–6Galβ*p*NP



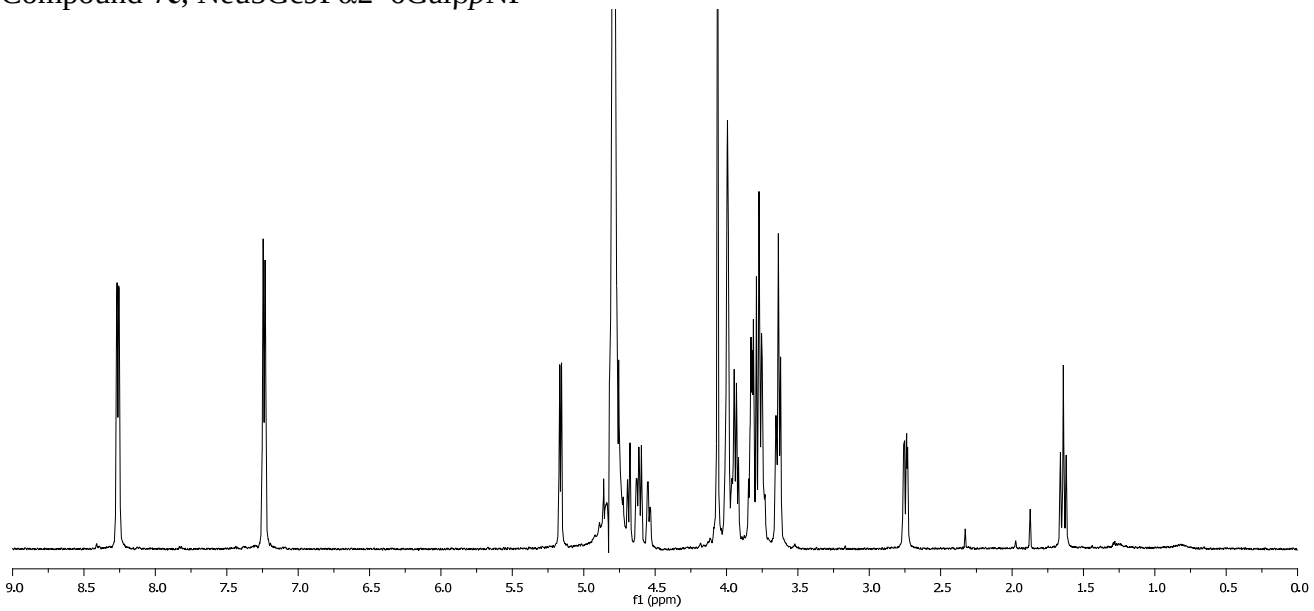


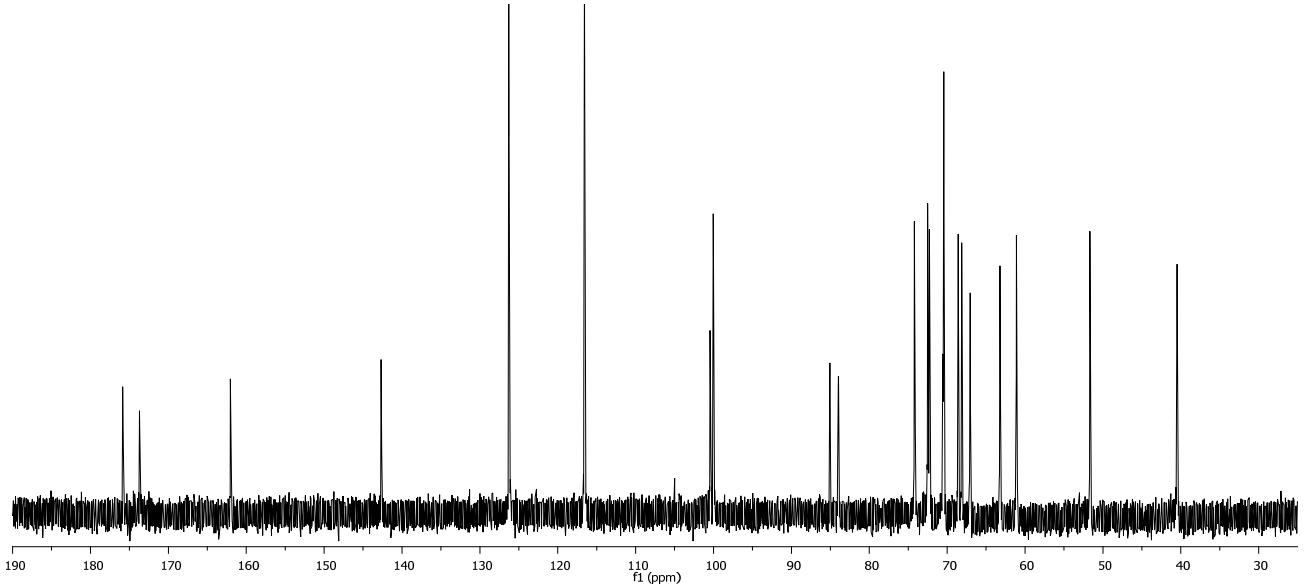
Compound **5c**, Neu5Ac9N$_3$α2–6Galβ*p*NP

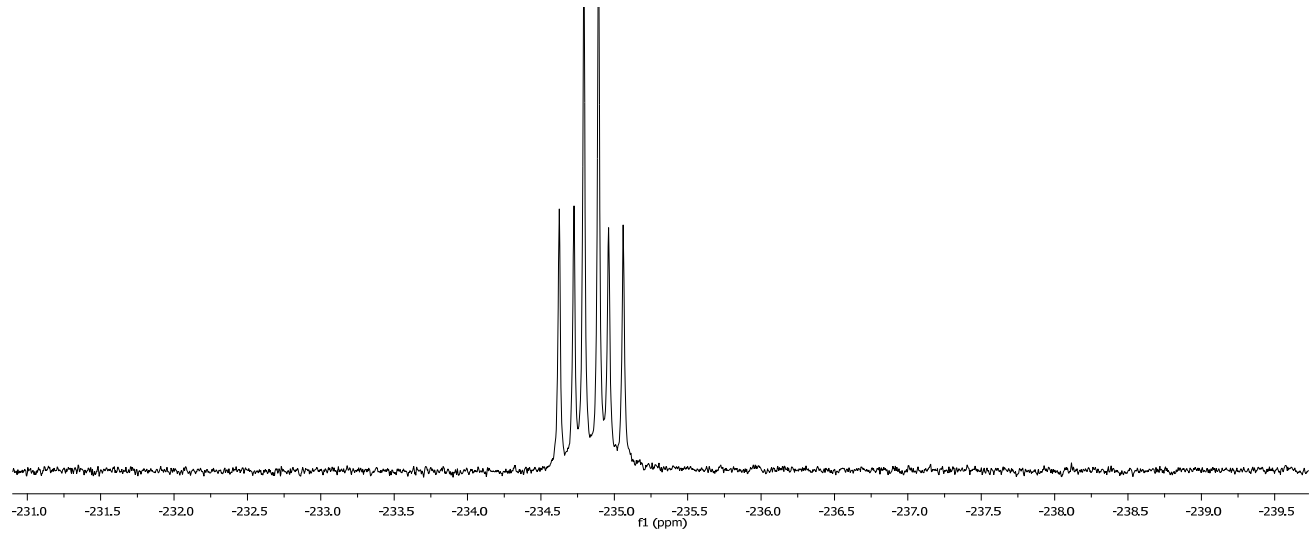




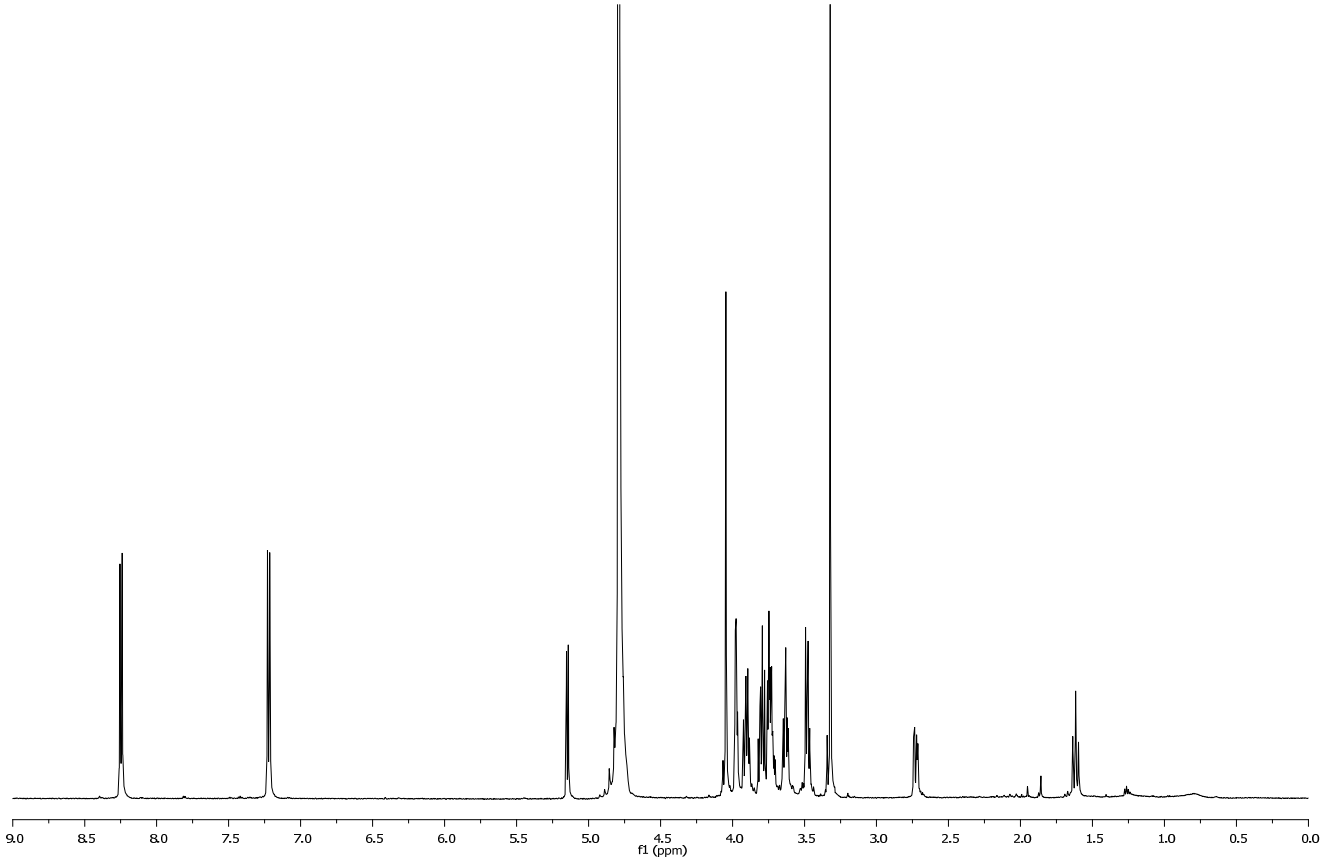
Compound **7c**, Neu5Gc9Fα2–6Galβ*p*NP

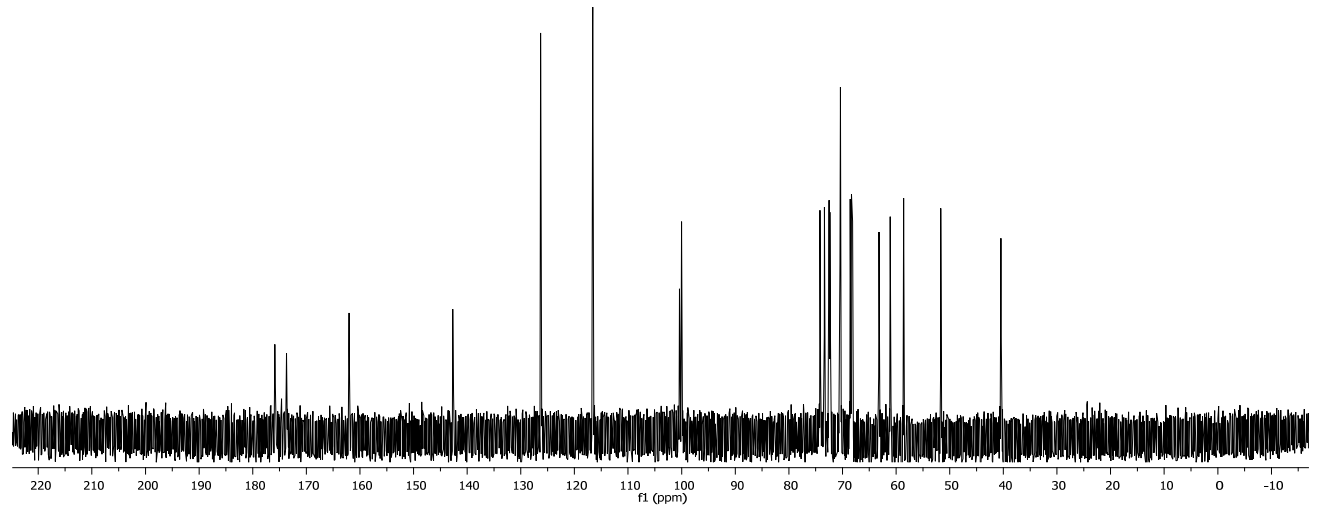




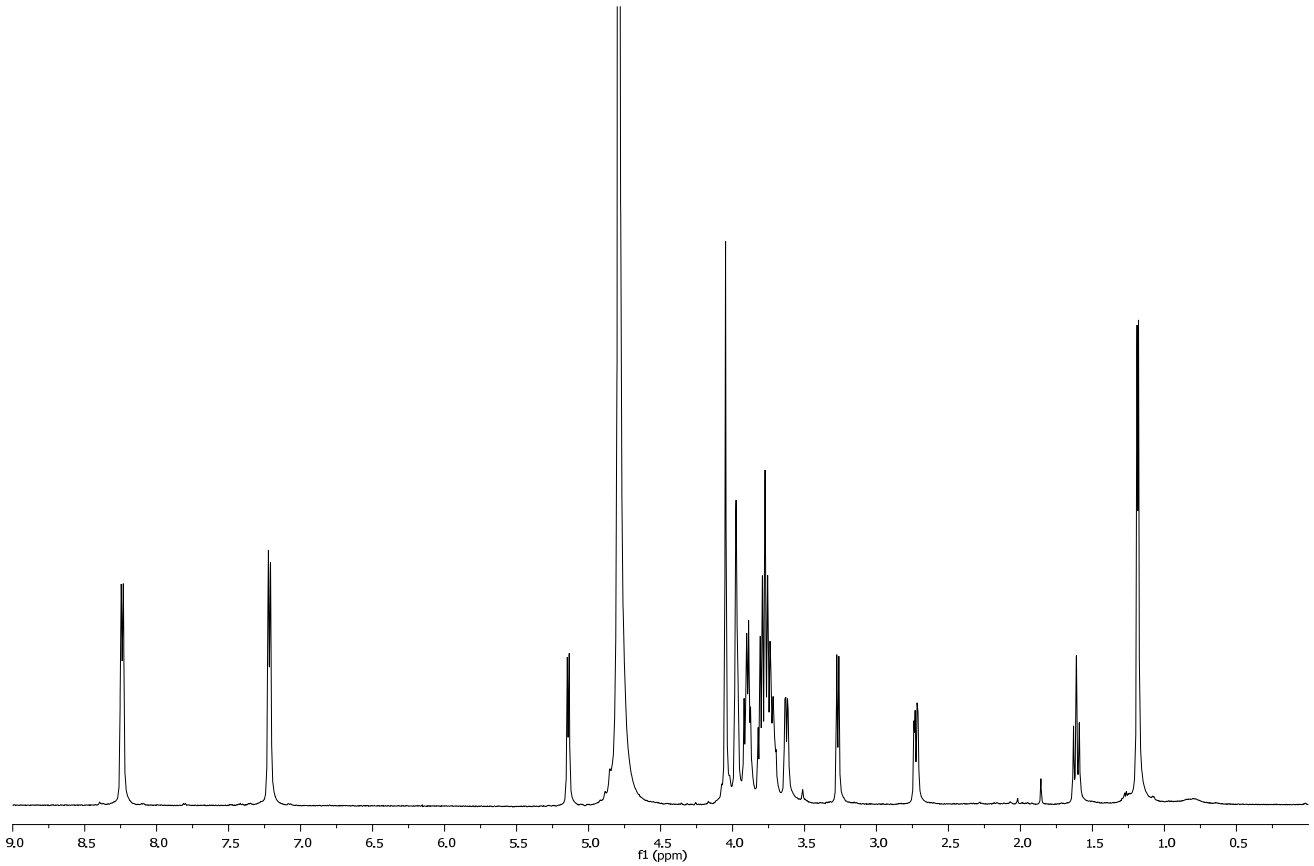


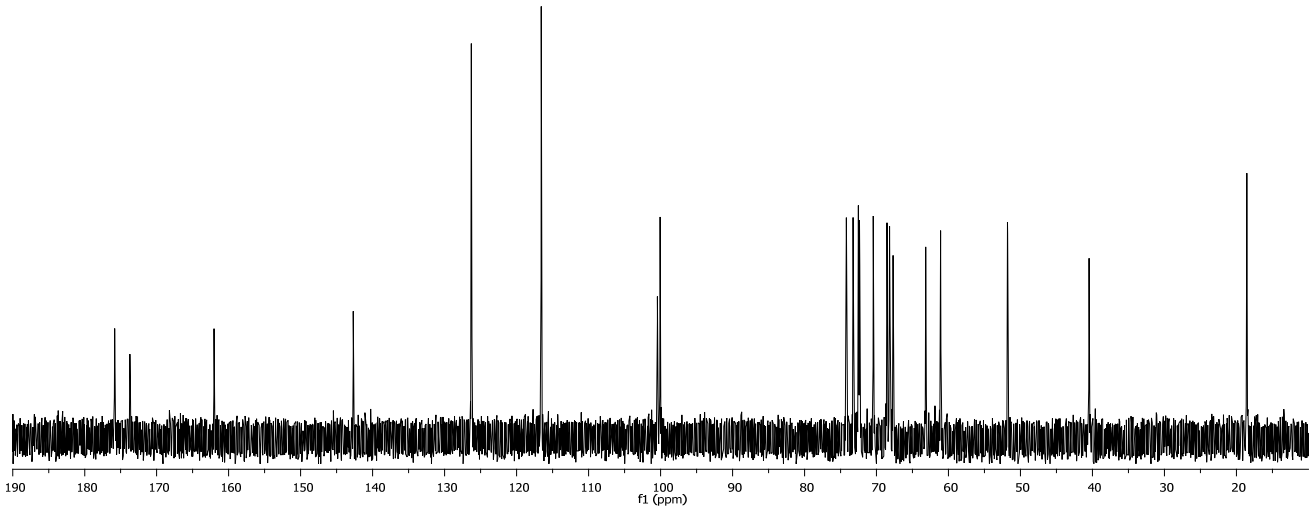
Compound **8c**, Neu5Gc9OMeα2–6Galβ*p*NP



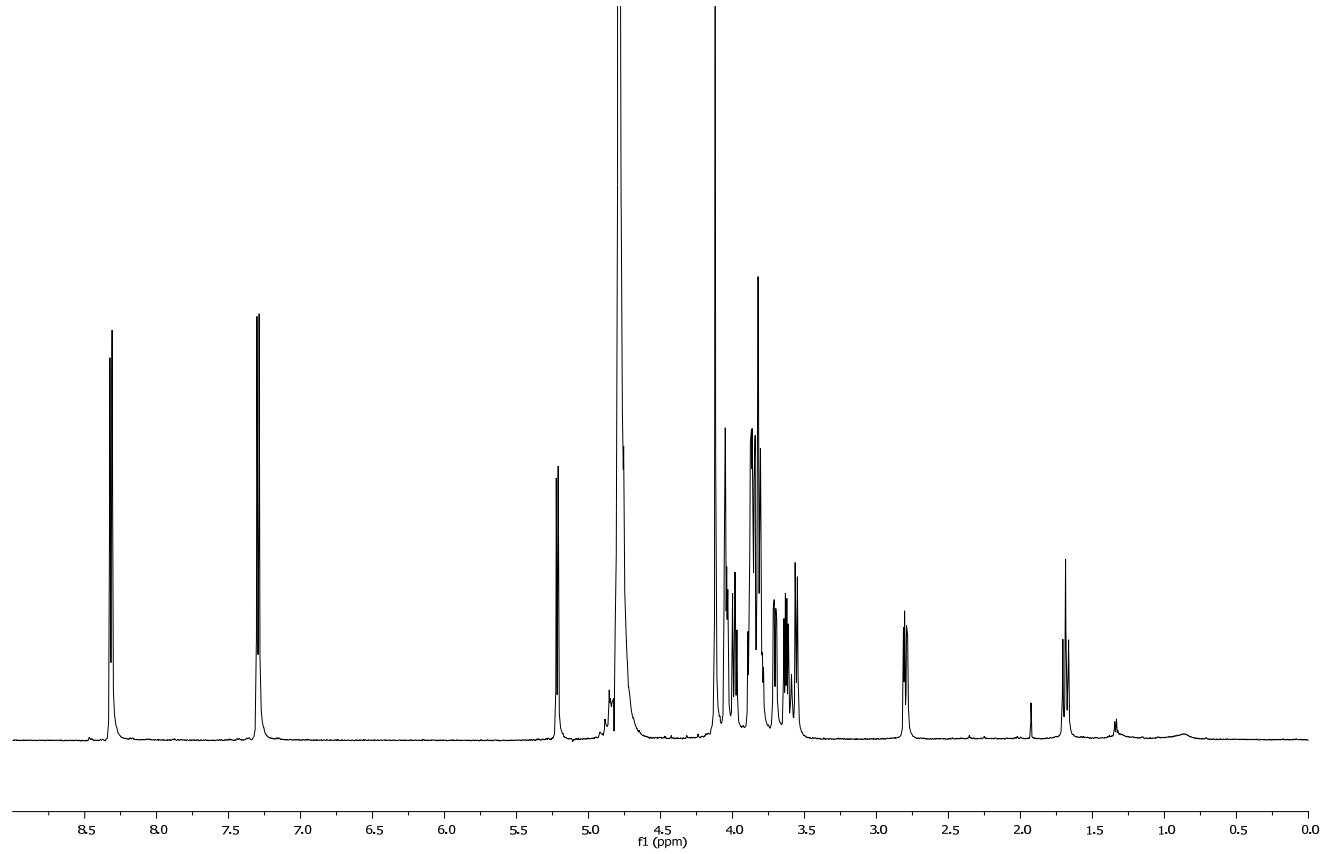


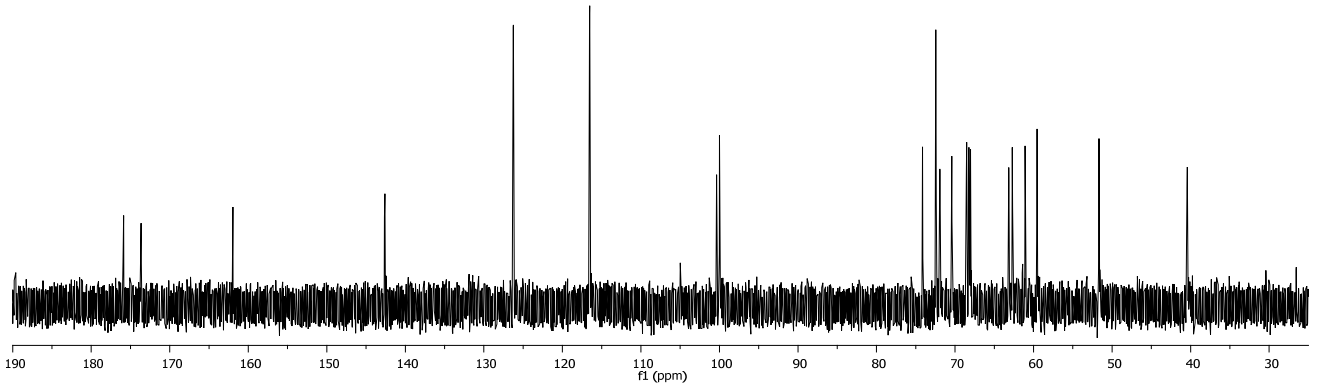
Compound **9c**, Neu5Gc9Deoxyα2–6Galβ*p*NP



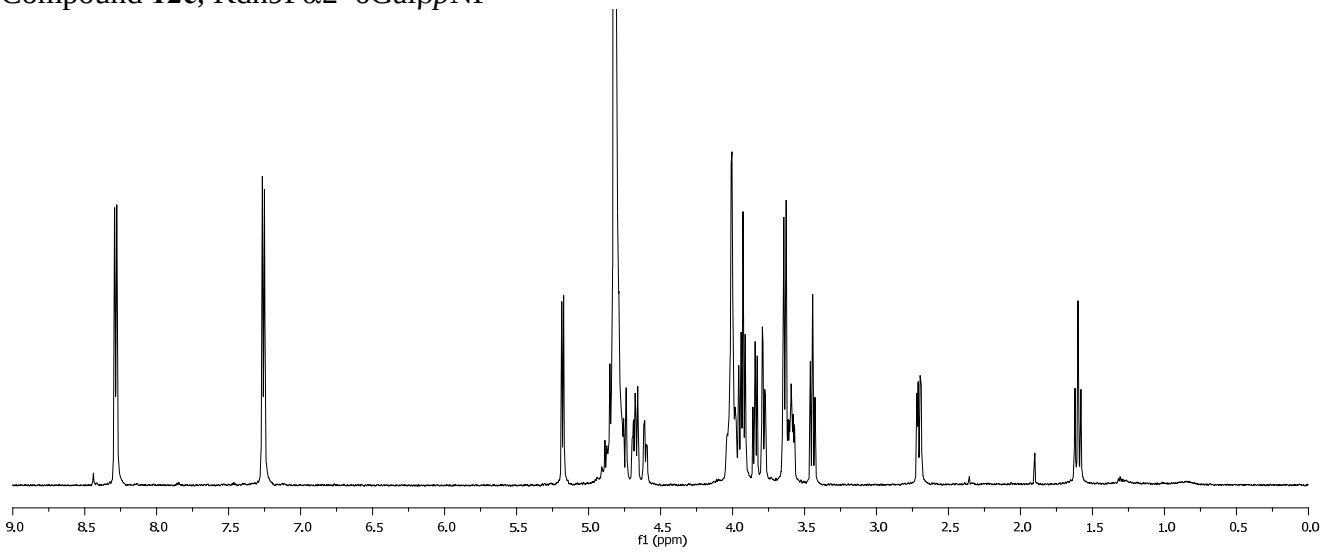


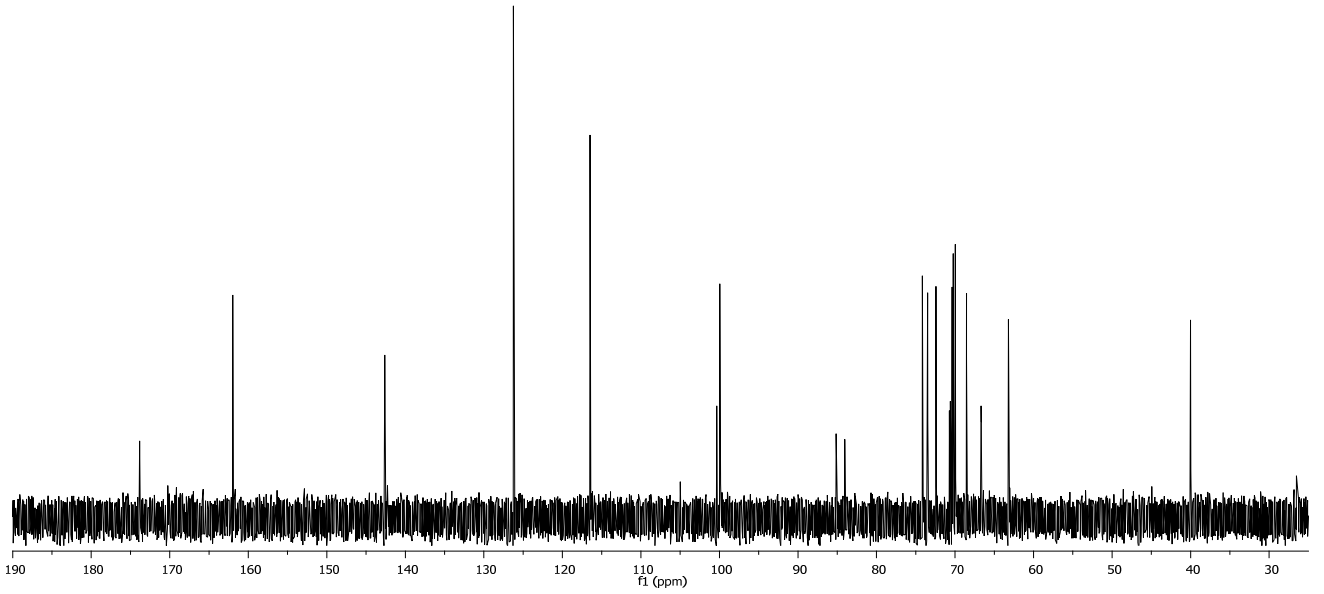
Compound **10c**, Neu5Gc9N$_3$α2–6Galβ*p*NP

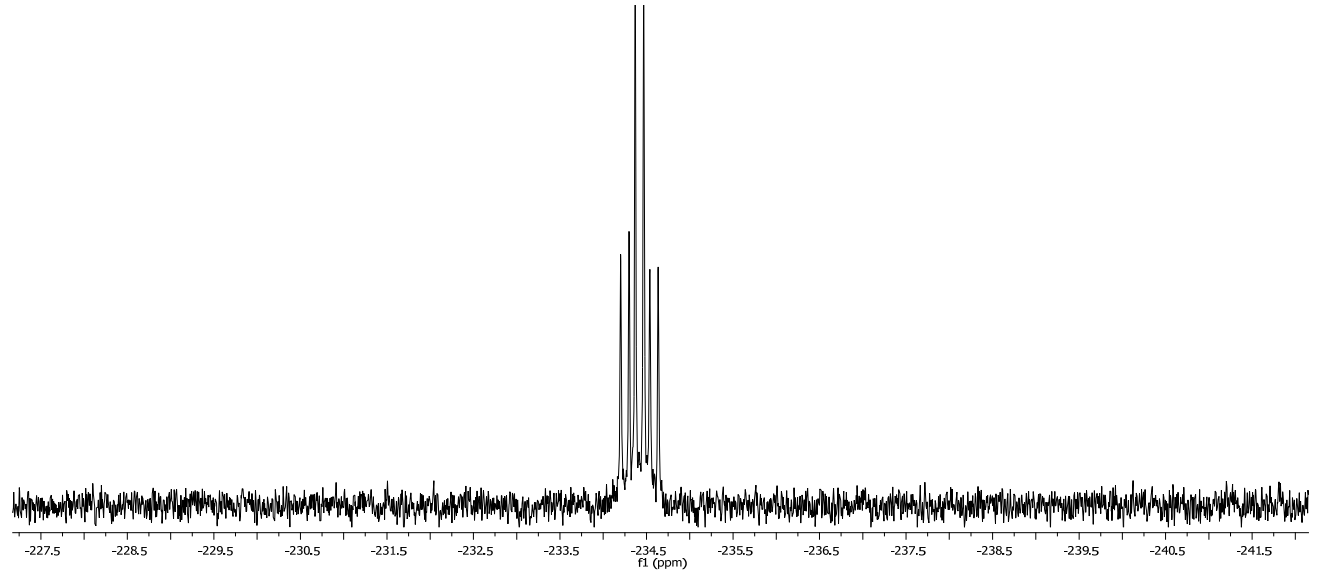




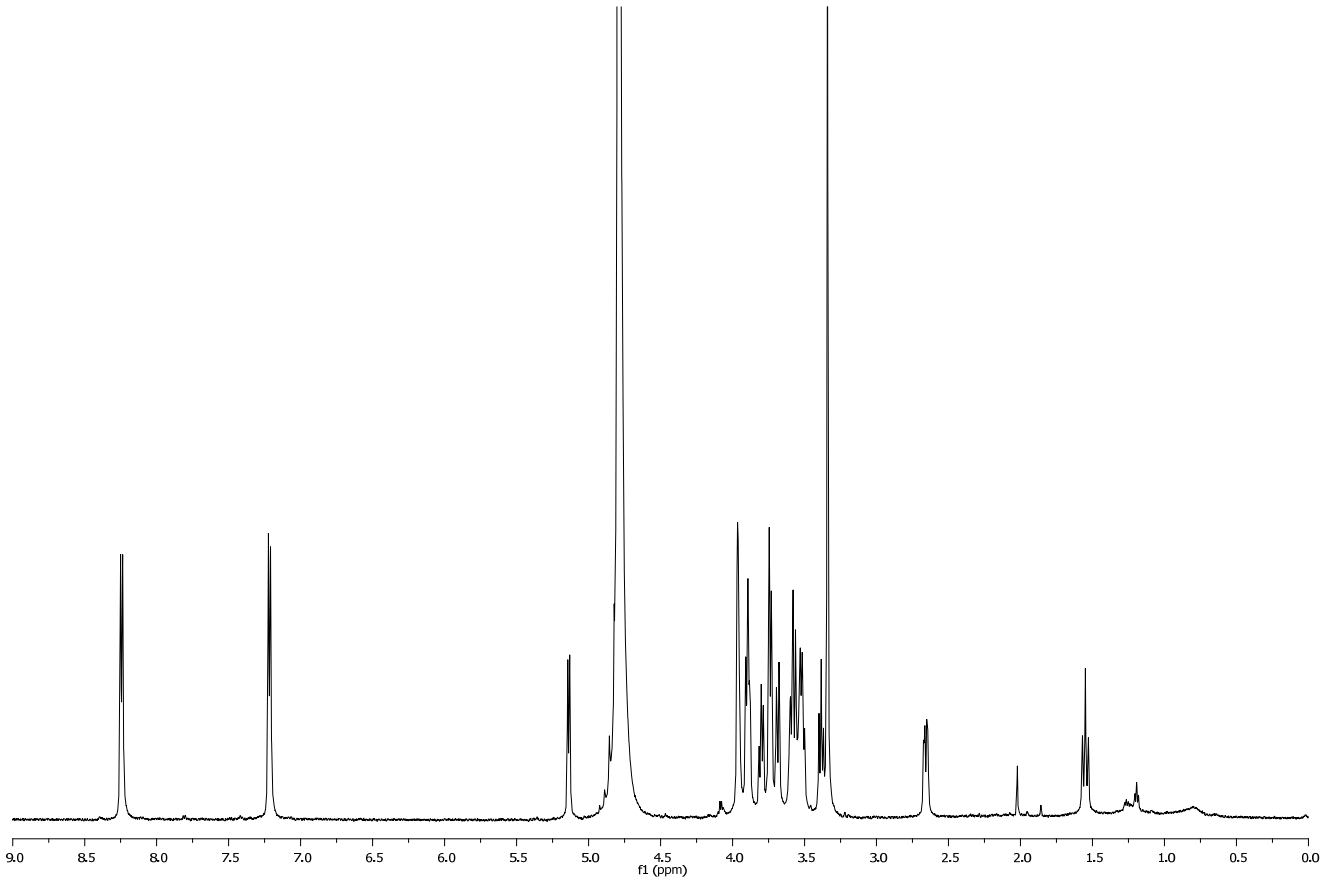
Compound **12c**, Kdn9Fα2–6Galβ*p*NP

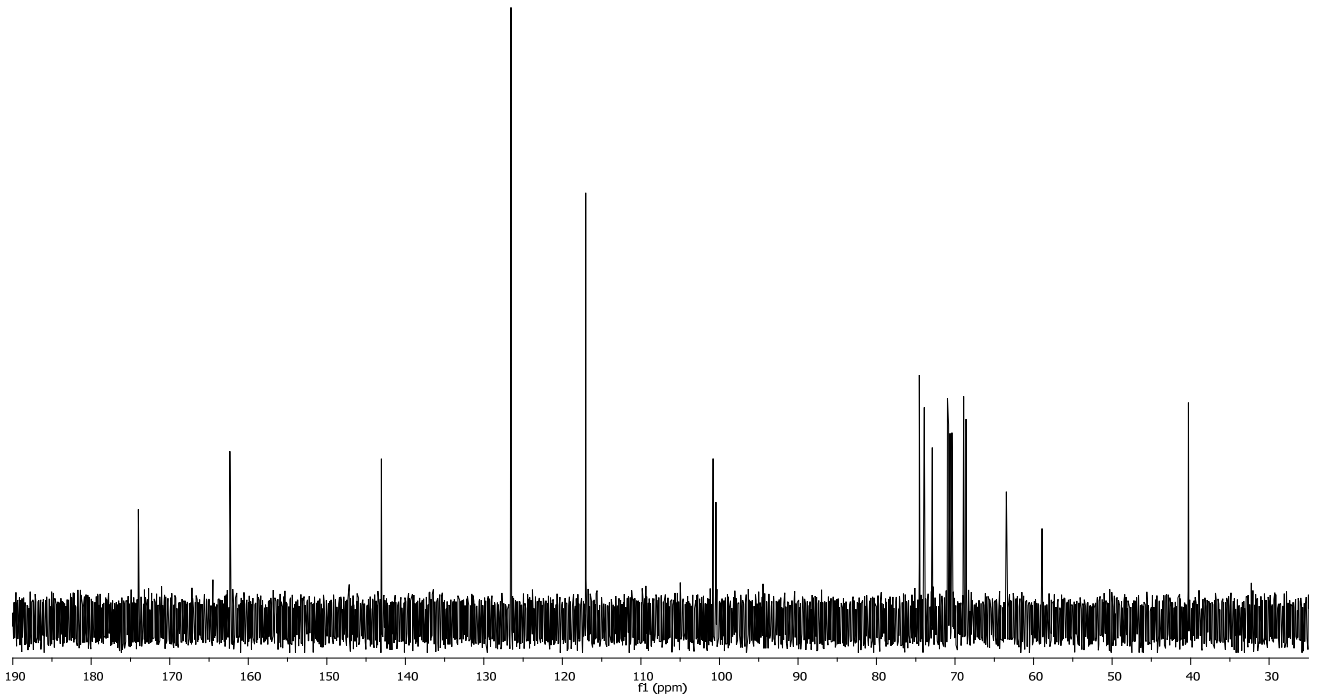




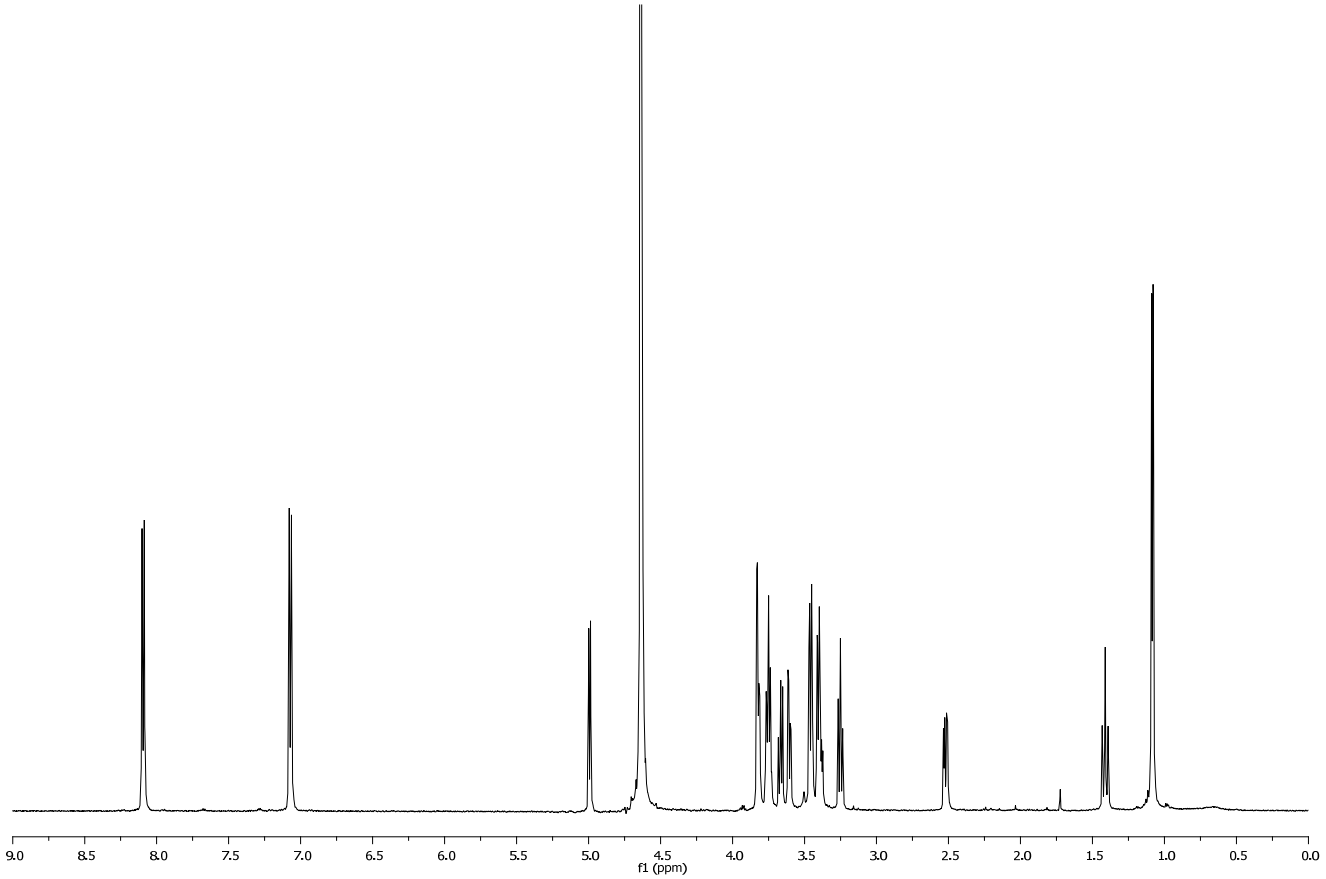


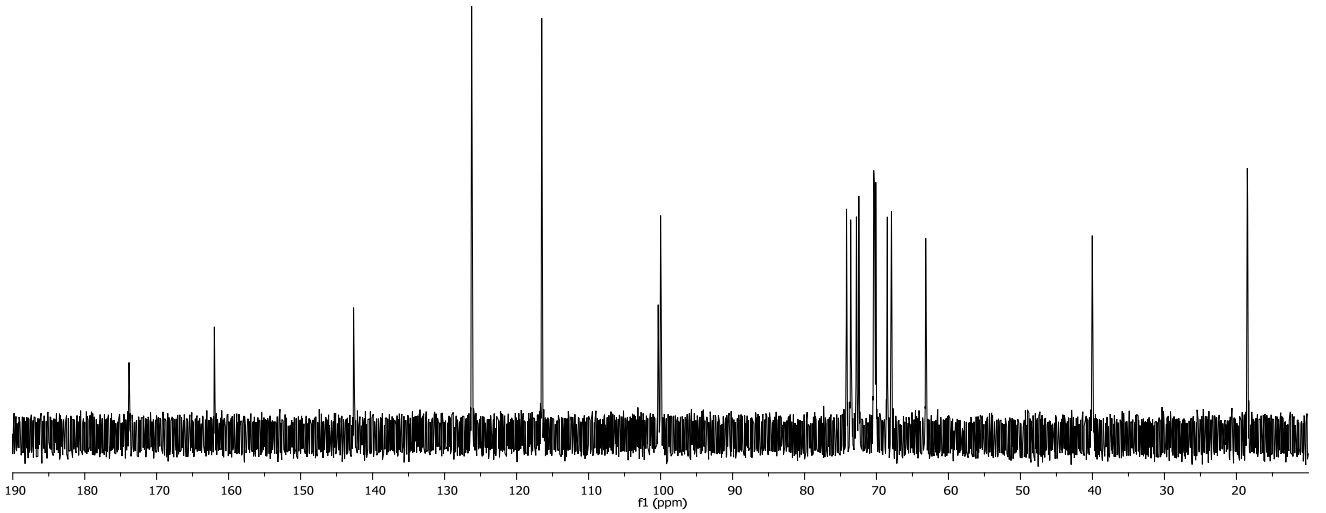
Compound **13c**, Kdn9OMeα2–6Galβ*p*NP





Compound **14c**, Kdn9Deoxyα2–6Galβ*p*NP





Compound **15c**, Kdn9N$_3$α2–6Galβ*p*NP