Supporting Information

Chemoenzymatic Synthesis of Size-Defined Polysaccharides by Sialyltransferase-Catalyzed Block Transfer of Oligosaccharides

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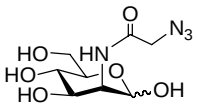
General Methods

Chemicals were purchased and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Mercury-300, Varian Inova-400, or Varian-600 spectrometer. Low and high resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility in the Ohio State University, or recorded on ABI 4700 MALDI TOF mass spectrometer. Silica gel 60 Å (200-425 mesh, Sorbent technologies) was used for flash column Chromatography. Thin-layer chromatography (TLC) was performed on silica gel plates 60 GF254 (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 BioGel P-2 Fine resins (Bio-Rad, Hercules, CA).

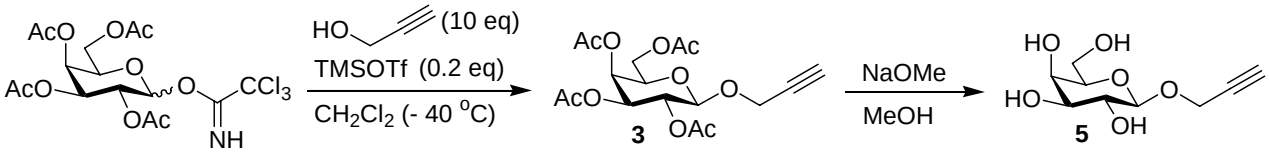
Experimental Procedures

N-Azidoacetyl-D-mannosamine 1



Mannosamine hydrochloride (1.80 g, 2.50 mmol) was dissolved in anhydrous MeOH (40 mL). Et_3N (4 mL) was added to the solution and the solution was allowed to stirred until solution turned clear. Azidoacetic acid *N*-hydroxysuccinimide ester[1] (1.38, 7.04 mmol) was added and the mixture was allowed to stir at room temperature overnight. The reaction mixture was then concentrated and purified by flash chromatography (EtOAc:MeOH = 4:1, v/v) to give *N*-azidoacetyl-D-mannosamine **1** as a white solid (1.47 g, 67%). ^{1}H NMR (600 MHz, D_2O) δ 4.98 (d, 0.5H, *J* = 1.2 Hz), 4.88 (d, 0.5H, *J* = 1.8 Hz), 4.33 (d, 0.5H, *J* = 3.0 Hz), 4.20 (d, 0.5H, *J* = 4.2 Hz), 3.94 (d, 1H, *J* = 3.0 Hz), 3.90 (s, 2H), 3.73- 3.66 (m, 4H), 3.43 (t, 0.5, *J* = 9.6 Hz), 3.33 (t, 0.5, *J* = 10.2 Hz); ^{13}C NMR (150 MHz, D_2O) δ 172.01, 171.11, 93.05, 93.03, 76.49, 72.11,72.08, 68.94, 66.83, 66.60, 60.46, 54.39, 53.47, 51.81, 51.73.

Propargyl β-D-galactopyranoside 5

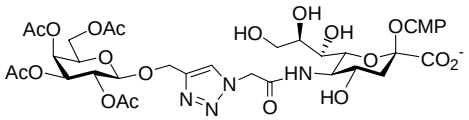


Galactosyl trichloroacetimidate[2] (2.56 g, 5.20 mmol) was dissolved in anhydrous CH_2Cl_2 (40 mL) with 4 Å molecular sieves (3 g) under argon. Propargyl alcohol (3 mL, 50.84 mmol) was then added and the reaction mixture was stirred for 30 min. TMSOTf (0.2 mL, 1.11 mmol) diluted with CH_2Cl_2 (1 mL) was slowly added at -40 °C. The reaction mixture was allowed to warm up to -20 °C and kept at -20 °C for 1 h. Et_3N (0.15 mL) was added to neutralize the reaction. After 10 min, the reaction mixture was filtered over celite and the filtrate was

concentrated in vacuo. The residue was purified by flash chromatography (Hexane:EtOAc = 2:1, v/v) to give the peracetylated propargyl galactoside **3** as a white solid (1.94 g, 97%).

Peracetylated galactoside **3** (1.57 g, 4.04 mmol) was dissolved in dry MeOH (30 mL) containing a catalytic amount of NaOMe (0.10 g). The mixture was stirred at room temperature for overnight, and neutralized with DOWEX (H^+) resin. After filtration and concentration, the propargyl-β-D-galactopyranoside **5** was afforded as a white solid (0.87 g, 99%). ^{1}H NMR (300 MHz, D_2O) δ 4.36 (d, 1H, *J* = 7.5 Hz, H-1), 4.26 (t, 2H, *J* = 2.1 Hz), 3.73 (d, 1H, *J* = 3.0 Hz), 3.59-3.44 (m, 4H), 3.36-3.30 (m, 1H), 2.75 (t, 1H, *J* = 2.7 Hz, C≡CH); ^{13}C NMR (75 MHz, D_2O) δ 101.22, 79.14, 76.54, 75.36, 72.86, 70.67, 68.70, 61.08, 56.64.

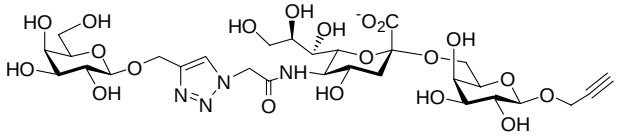
Synthesis of CMP-activated disaccharide 4



The one-pot two-enzyme reaction[3] was carried out in a 50 mL centrifuge tube in 20 mL of Tris-HCl buffer (100 mM, pH 8.5) containing *N*-azidoacetyl-D-mannosamine **1** (164 mg, 0.63 mmol), sodium pyruvate (346 mg, 3.14 mmol), CTP (498 mg, 0.94 mmol), and $MgCl_2$ (20 mM). Before adding the enzymes the pH of the mixture was adjusted to 8.5 with 2 M NaOH. The appropriate amount of *E. coli* sialic acid aldolase (1.3 mg) and *N. meningitidis* CMP-sialic acid synthetase (NmCSS, 2.9 mg) were added. The reaction mixture was incubated at 37 °C for 2-3 h with agitating at 125 rpm. The reaction was monitored by TLC with n-PrOH:H_2O:NH_4OH = 7:2:1 (v/v) and anisaldehyde stain solution. When no further product formed the reaction mixture was quenched with equal volume of 95% EtOH and centrifuged to remove proteins and insoluble precipitates. The supernatant was concentrated and dried under vacuum.

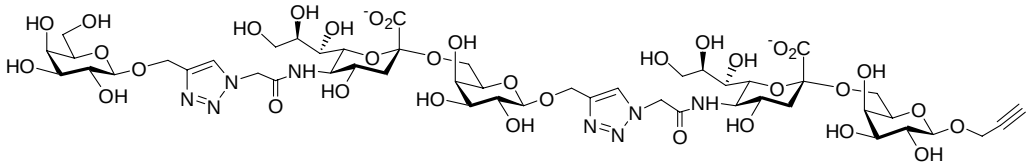
The crude product **2** (CMP-Neu5NAz) and peracetylated propargylgalactoside **3** (1.2 equiv.) were dissolved in CH_3CN/H_2O (1:1, by volume). Diisopropylethylamine (0.23 mL, 2 equiv.) and CuI (60 mg, 0.5 equiv.) were added to the mixture and the reaction was allowed to stir for 1-2 h at room temperature. The reaction was monitored by TLC (n-PrOH:H_2O:NH_4OH = 7:2:1, v/v) and anisaldehyde stain solution. When the reaction completed the reaction mixture was filtered over celite and concentrated in vacuo. The residue was purified by flash chromatography (n-PrOH:H_2O = 4:1, v/v) and then filtered through a BioGel P-2 gel filtration column with water to obtain the CMP-activated disaccharide **4** as a white solid (550 mg, 83%). ^{1}H NMR (600 MHz, D_2O) δ 8.09 (s, 1 H), 7.97 (d, *J* = 7.8 Hz, 1 H), 5.97 (d, *J* = 4.8 Hz, 1 H), 5.45 (d, *J* = 3.6 Hz, 1 H), 5.34 (d, *J* = 1.8 Hz, 2 H), 5.19 (dd, *J* = 3.3 and 10.5 Hz, 1 H), 5.05 (dd, *J* = 7.8 and 10.2 Hz, 1 H), 4.97-4.87 (m, 3 H), 4.34-4.19 (m, 9 H), 4.14-4.09 (m, 1 H), 4.00 (t, *J* = 10.2 Hz, 1 H), 3.93-3.91 (m, 1 H), 3.86 (dd, *J* = 2.4 and 12.0 Hz, 1 H), 3.62 (dd, *J* = 11.7 Hz and 5.7 Hz, 1 H), 3.48 (d, *J* = 5.4 Hz, 1 H), 2.49 (dd, *J* = 4.8 and 13.2 Hz, 1 H), 2.20 (s, 3 H), 2.09 (s, 3 H), 1.98 (s, 6 H), 1.68-1.62 (m, 1 H); ^{13}C NMR (75 MHz, D_2O) δ 173.68, 173.42, 173.03, 172.80, 168.26, 165.71, 157.04, 141.94, 126.97, 99.82, 89.33, 83.12, 83.01, 74.43, 71.76, 71.25, 70.79, 69.77, 68.87, 68.15, 66.86, 64.94, 62.99, 62.34, 62.06, 61.57, 59.45, 52.30, 52.21, 41.20, 41.07, 20.32, 20.27, 20.12; HRMS (ESI) *m/z* calculated for $C_{37}H_{50}N_7Na_3O_{26}P$ (M+Na) 1108.2236, measured 1108.2245.

Synthesis of trisaccharide 6



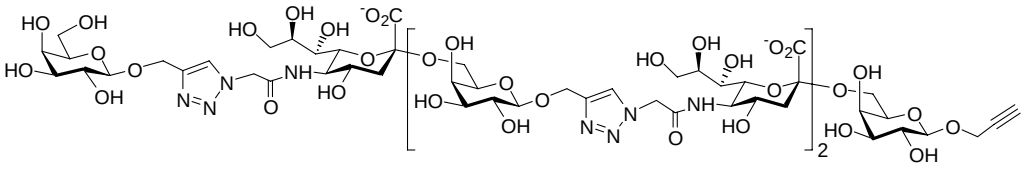
In a 50 mL centrifuge tube, the propargyl-β-galactoside **5** (70 mg, 0.32 mmol), CMP-activated disaccharide **4** (410 mg, 1.2 equiv.) and $MgCl_2$ (20 mM) were dissolved in 10 mL of Tris-HCl buffer (100 mM, pH 7.5). *Photobacterium damsela* α2,6-sialyltransferase (Pd2,6ST, 0.5 mg) was added, and the reaction mixture was incubated at 37 °C for 2 h with agitating at 140 rpm. The reaction was monitored by TLC (n-PrOH:H_2O:NH_4OH = 7:2:1, v/v) and anisaldehyde stain solution. When no further product detected the reaction was quenched with equal volume of 95% EtOH and centrifuged to remove proteins and insoluble precipitates. The supernatant was concentrated in vacuo and dried under vacuum. The crude mixture was dissolved in MeOH (30 mL) containing a catalytic amount of NaOMe. The mixture was stirred at room temperature overnight. The reaction mixture was then neutralized with DOWEX (H^+) resin, filtered, and concentrated in vacuo. The residue was filtered through a Bio-gel P-2 gel filtration column with water, a silica gel column with n-PrOH/ H_2O (4:1) to remove salts, and another BioGel P-2 gel filtration column with water to obtain the trisaccharide **6** as a white solid (242 mg, 95%). ^{1}H NMR (600 MHz, D_2O) δ 8.10 (s, 1 H), 5.31 (s, 2 H), 5.00 (d, *J* = 12.6 Hz, 1 H), 4.88 (d, *J* = 12.6 Hz, 1 H), 4.53 (d, *J* = 7.8 Hz, 1 H), 4.47 (d, *J* = 7.8 Hz, 1 H), 4.45-4.43 (dd, *J* = 2.4 and 10.2 Hz, 2 H), 3.93-3.69 (m, 11 H), 3.64-3.57 (m, 6 H), 3.52-3.47 (m, 2 H), 2.88 (t, *J* = 2.4 Hz, 1 H), 2.72 (dd, *J* = 4.8 and 12.3 Hz, 1 H), 1.67 (t, *J* = 12.3 Hz, 1 H); ^{13}C NMR (75 MHz, D_2O) δ 173.56, 168.62, 143.88, 126.96, 102.02, 101.41, 100.59, 79.08, 76.31, 75.38, 73.69, 72.82, 72.65, 72.46, 71.91, 70.77, 70.58, 68.73, 68.70, 68.32, 68.26, 63.52, 62.69, 61.87, 61.10, 56.94, 52.29, 52.20, 40.31; HRMS (ESI) *m/z* calculated for $C_{29}H_{43}N_4Na_2O_{20}$ (M+Na) 813.2266, measured 813.2258.

Synthesis of pentasaccharide 7



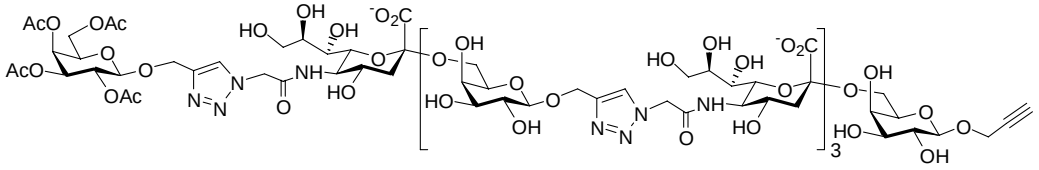
Pentasaccharide **7** was synthesized in a similar method described for preparing **6** by sialylation of the trisaccharide **6** using CMP-activated disaccharide analogue **4** as donor under the catalysis of Pd2,6ST. Yield, 91%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 8.10 (s, 1 H), 8.09 (s, 1 H), 5.30 (s, 4 H), 4.99 (dd, *J* = 4.2 and 12.6 Hz, 2 H), 4.52 (d, *J* = 7.8 Hz, 1 H), 4.47-4.43 (m, 4 H), 3.92-3.66 (m, 18 H), 3.63-3.57 (m, 10 H), 3.51-3.46 (m, 6 H), 2.88 (t, *J* = 2.4 Hz, 1 H), 2.73-2.69 (m, 2 H), 1.72-1.65 (m, 2 H); ^{13}C NMR (150 MHz, D_2O) δ 173.61, 168.64, 143.87, 126.99, 102.21, 102.03, 101.41, 100.64, 79.09, 76.32, 75.39, 73.69, 72.83, 72.66, 72.47, 71.94, 70.78, 70.69, 70.59, 69.70, 68.74, 68.34, 68.30, 63.49, 62.69, 62.14, 61.88, 61.11, 56.94, 52.31, 52.22, 40.28; HRMS (ESI) *m/z* calculated for $C_{49}H_{72}N_8Na_3O_{34}$ (M+Na) 1385.3844, measured 1385.3850.

Synthesis of heptasaccharide 8



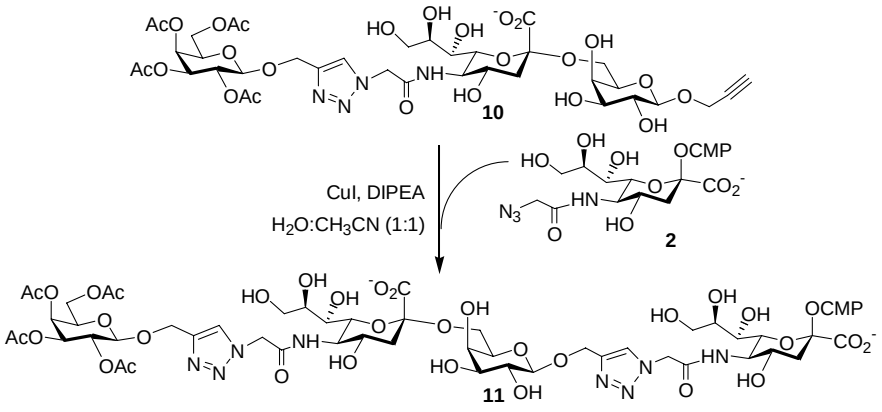
Heptasaccharide **8** was synthesized in a similar method described for preparing **6** by sialylation of the pentasaccharide **7** using CMP-activated disaccharide analogue **4** as donor under the catalysis of Pd2,6ST. Yield, 81%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 8.10-8.09 (m, 3 H), 5.31-5.30 (m, 6 H), 5.00 (dd, *J* = 3.6 and 12.6 Hz, 3 H), 4.89-4.85 (m, 3 H), 4.53 (d, *J* = 8.4 Hz, 1 H), 4.48-4.44 (m, 5 H), 3.93-3.58 (m, 41 H), 3.52-3.47 (m, 4 H), 2.88 (t, *J* = 2.4 Hz, 1 H), 2.74-2.69 (m, 3 H), 1.73-1.65 (m, 3 H); ^{13}C NMR (150 MHz, D_2O) δ 173.62, 168.65, 143.89, 127.00, 102.24, 101.18, 102.04, 101.42, 100.64, 76.34, 75.39, 73.67, 72.84, 72.63, 72.48, 71.95, 70.78, 70.70, 70.59, 69.71, 68.73, 68.35, 63.50, 62.72, 62.16, 61.89, 61.12, 56.94, 52.33, 52.22, 40.29; HRMS (ESI) *m/z* calculated for $C_{69}H_{101}N_{12}Na_4O_{48}$ (M+Na) 1957.5416, measured 1957.5322.

Synthesis of nonasaccharide 9



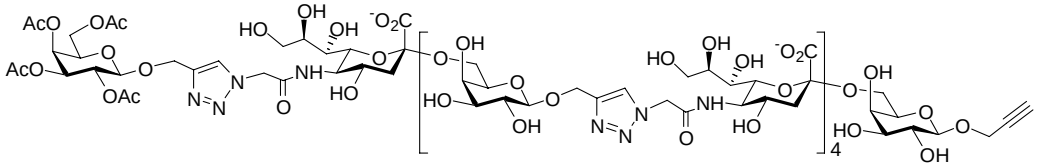
A small scale of nonasaccharide **9** was synthesized in a similar method described for preparing **6** by sialylation of the heptasaccharide **8** using CMP-activated disaccharide analogue **4** as donor under the catalysis of Pd2,6ST. The formation of the product was monitored by TLC and confirmed by mass spectrometry. Yield > 80% (estimated by TLC). MALDI-TOF *m/z* calculated for $C_{97}H_{138}N_{16}Na_5O_{66}$ (M+Na) 2697.74, measured 2697.72.

Synthesis of CMP-activated tetrasaccharide 11



The CMP-activated tetrasaccharide **11** was prepared in a similar method described for preparing **4**. Tetra-acetylated trisaccharide **10** (98 mg, 0.102 mmol) and CMP activated sialic acid derivative **2** (79 mg, 1.2 equiv) were dissolved in CH_3CN/H_2O (1:1, by volume). Diisopropylethylamine (0.04 mL, 2 equiv.) and CuI (10 mg, 0.5 equiv.) were added to the mixture and allowed to stir for 1 h at room temperature. The reaction was monitored by TLC (n-PrOH:H_2O:NH_4OH = 7:2:1, v/v) and anisaldehyde stain solution. When the reaction completed the reaction mixture was filtered over celite and concentrated in vacuo. The residue was purified by flash chromatography (n-PrOH:H_2O = 4:1, v/v) to obtain the CMP-activated tetrasaccharide **11** as a white solid (167 mg, 98%). ^{1}H NMR (600 MHz, D_2O) δ 8.12 (s, 1 H), 8.09 (s, 1 H),7.94 (d, *J* = 7.8 Hz, 1 H), 5.96 (d, *J* = 3.6 Hz, 1 H), 5.46 (d, *J* = 3.6 Hz, 1 H), 5.35 (d, *J* = 4.8 Hz, 2 H), 5.33 (s, 2 H), 5.20 (dd, *J* = 3.6 and 10.2 Hz, 1 H), 5.07-4.87 (m, 6 H), 4.48 (d, *J* = 7.8 Hz, 1 H), 4.35-3.49 (m, 32 H), 2.75 (dd, *J* = 4.8 and 12.6 Hz, 1 H), 2.49 (dd, *J* = 4.2 and 13.2 Hz, 1 H), 2.20 (s, 3 H), 2.10 (s, 3 H), 2.00 (s, 3 H), 1.99(s, 3 H) 1.81-1.70 (m, 2 H); ^{13}C NMR (150 MHz, D_2O) δ 173.68, 173.41, 173.03, 172.80, 168.62, 168.48, 165.89, 141.82, 141.73, 127.00, 102.17, 99.87, 89.66, 82.90, 82.85, 74.40, 73.66, 72.64, 72.52, 72.03, 71.94, 71.25, 70.79, 70.71, 70.04, 69.71, 69.60, 69.45, 69.38, 68.72, 68.34, 68.32,68.22, 68.15, 66.72, 64.88, 63.67, 63.49, 62.75, 62.59, 62.38, 62.10, 62.05 59.48, 52.37, 52.28, 52.21, 52.18, 52.07, 40.67, 40.61 40.25, 20.33, 20.28, 20.13; MALDI (TOF) *m/z* calculated for $C_{56}H_{79}N_{11}NaO_{40}P$ (M-H) 1610.4042, measured 1610.3905.

Synthesis of undecasaccharide 12

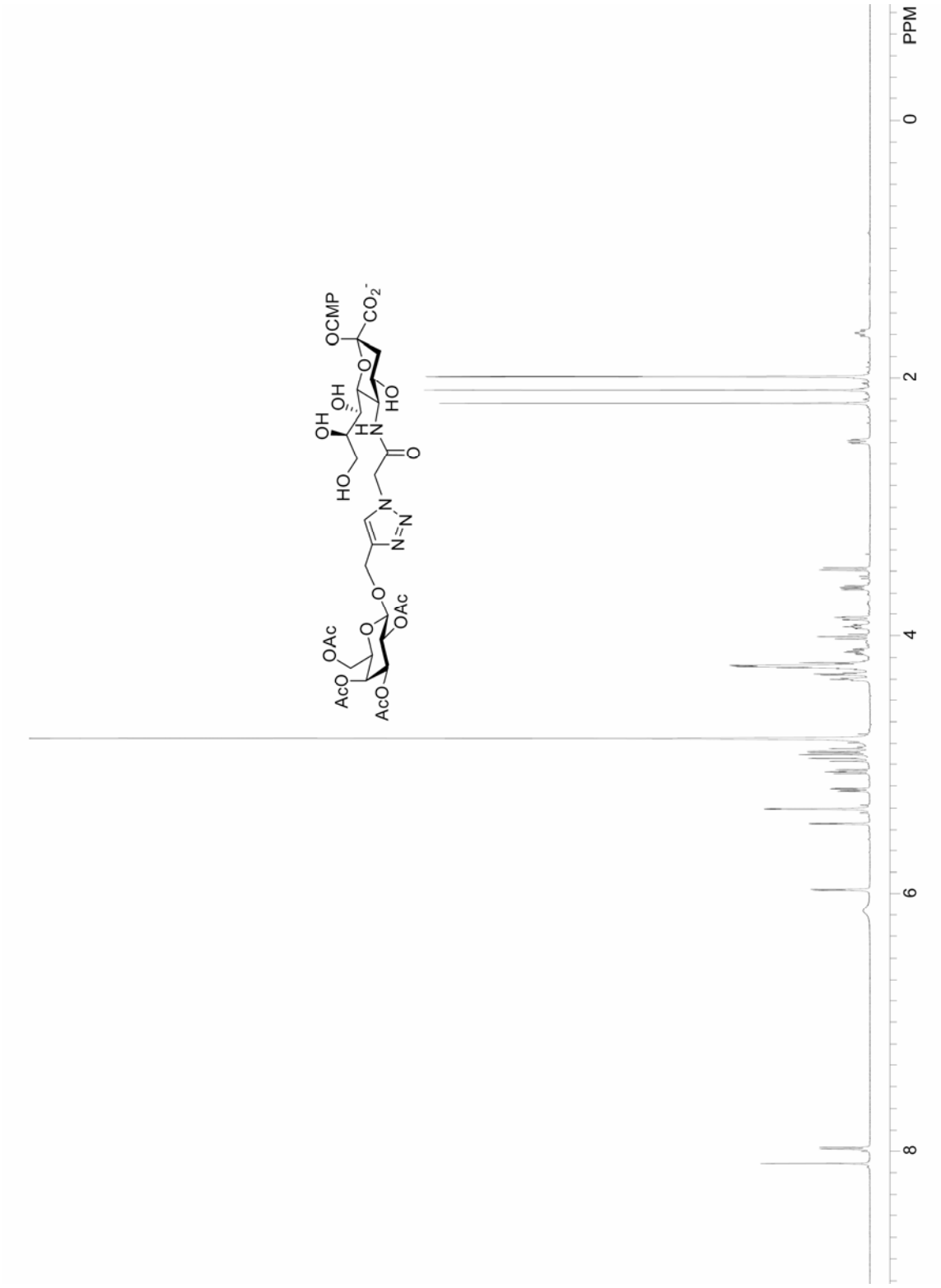


Undecasaccharide **12** was synthesized in a similar method described for preparing **6** by using CMP-activated tetrasaccharide analogue **11** as donor and heptasaccharide **8** as the acceptor. Yield, 65%, white foam. ^{1}H NMR (600 MHz, D_2O) δ 8.12-8.09 (m, 5 H), 5.46 (d, 3.0 Hz, 1 H), 5.34-5.29 (s, 8 H), 5.20 (dd, *J* = 4.2 and 10.2 Hz, 1 H), 5.06-4..86 (m, 12 H), 4.54 (d, *J* = 7.8 Hz, 1 H), 4.49-4.45 (m, 6H), 4.25-4.21 (m, 5 H), 4.06-4.04 (m, 2 H), 3.93-3.77 (m, 30 H), 3.66-3.60 (m, 17 H), 3.52-3. 48 (m, 5 H), 2.88 (t, *J* = 2.4 Hz, 1 H), 2.75-2.71 (m, 5 H) 2.20 (s, 3 H), 2.10 (s, 3 H), 1.99(s, 6 H), 1.74-1.66 (m, 5 H); ^{13}C NMR (150 MHz, D_2O) δ 173.62, 168.66, 143.89, 126.98, 102.19, 101.43, 100.67, 100.60, 99.86, 73.70, 73.65, 72.64, 72.50, 71.98, 71.85, 70.70, 70.60, 69.80, 69.58, 68.35, 63.50, 62.73, 62.61, 62.36, 62.11, 61.45, 61.39, 60.49, 56.93, 52.33, 52.22, 40.27, 20.31, 20.25, 20.11; HRMS (ESI) *m/z* calculated for $C_{117}H_{168}N_{20}O_{80}$ (M+H) 3132.97, measured 3133.00.

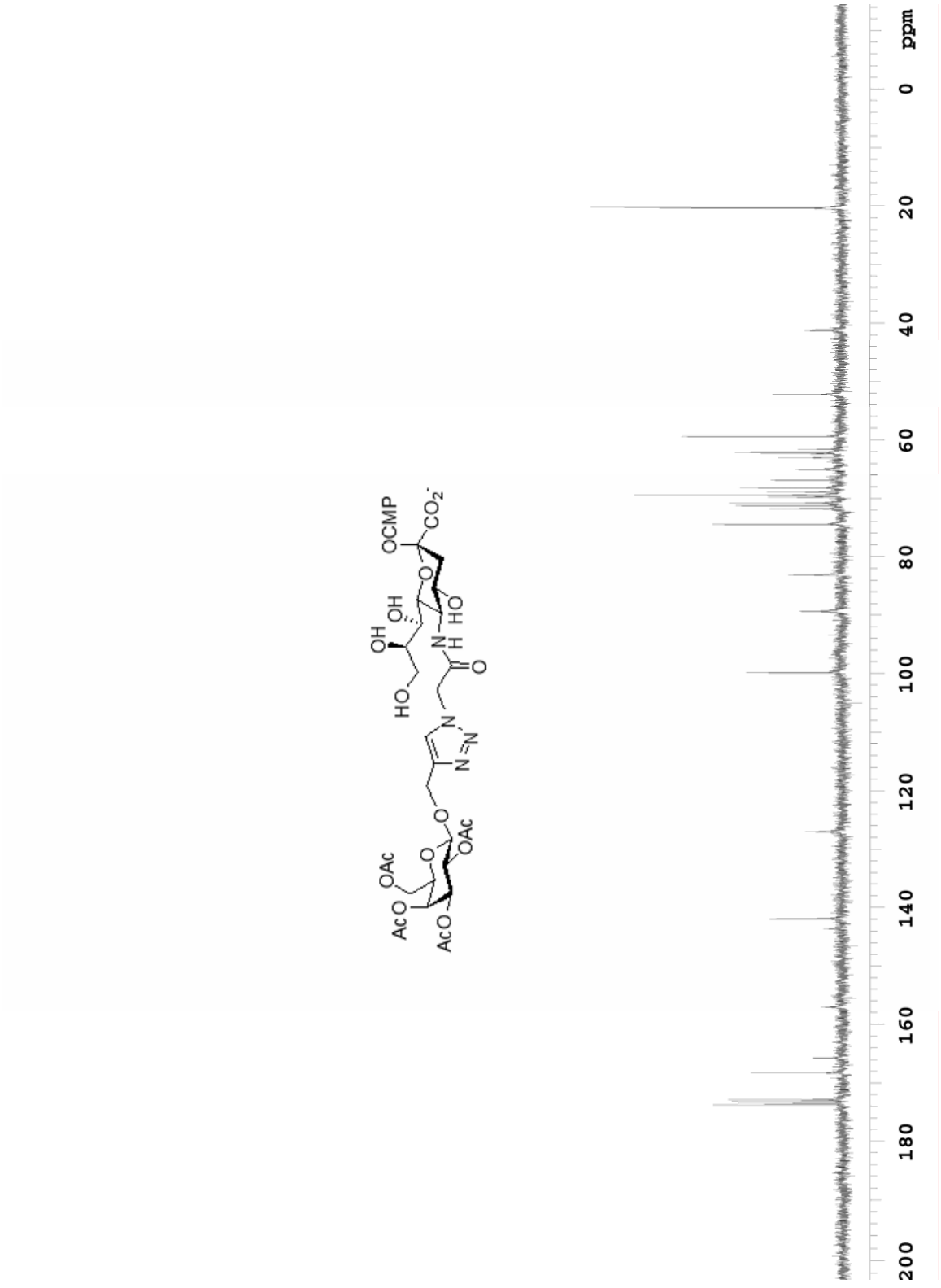
References:

(1) Choi, S. K.; Lee, S.; Whitesides, G. M. *J. Org. Chem.* **1996**, *61*, 8739-8745.
(2) Ren, T.; Zhang, G.; Liu, D. *Tetrahedron Lett.* **2001**, *42*, 1007-1010.
(3) Yu, H.; Yu, H.; Karpel, R.; Chen, X. *Bioorg. Med. Chem.* **2004**, *12*, 6427-6435.

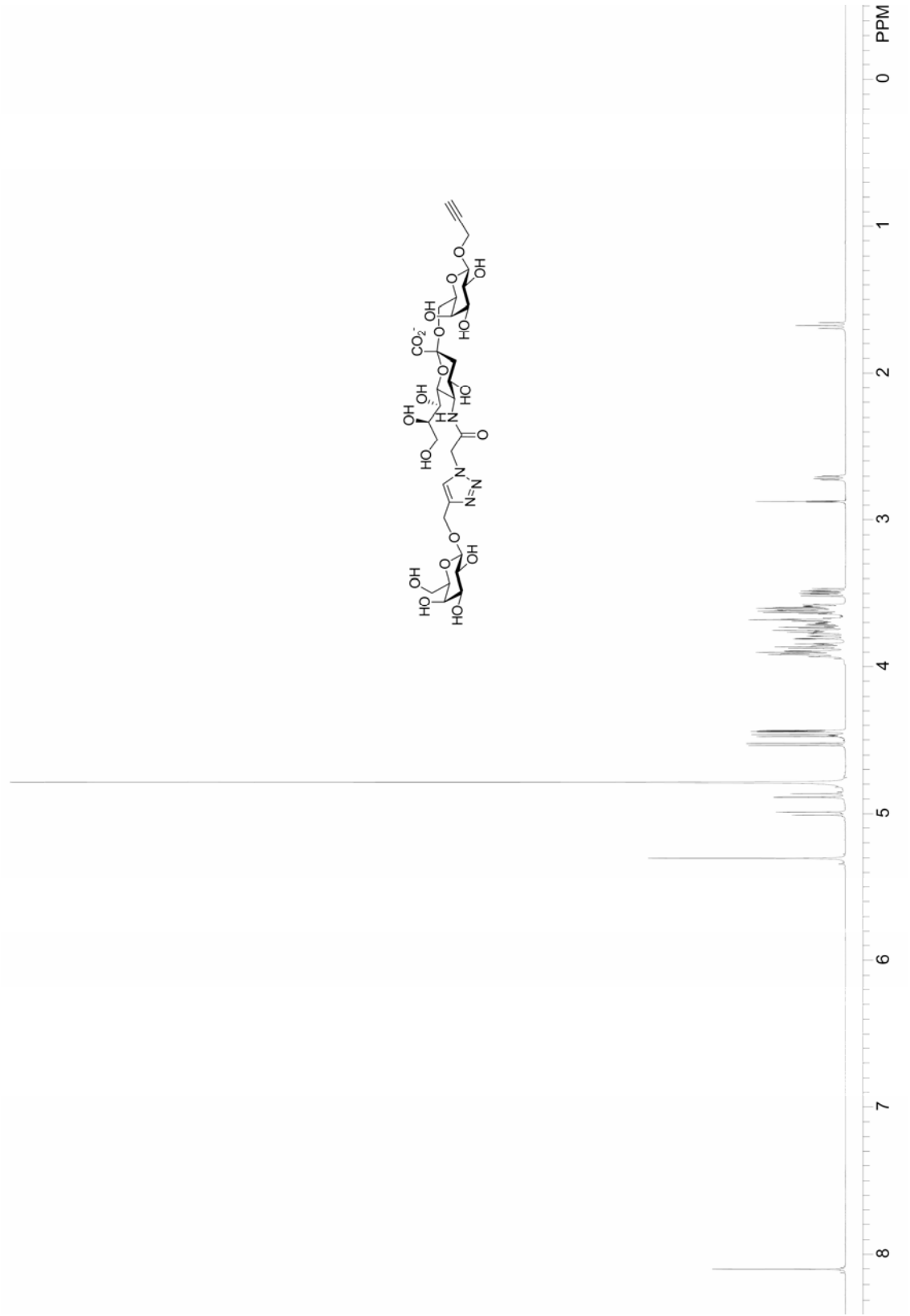
^{1}H NMR of CMP-disaccharide analogue **4**



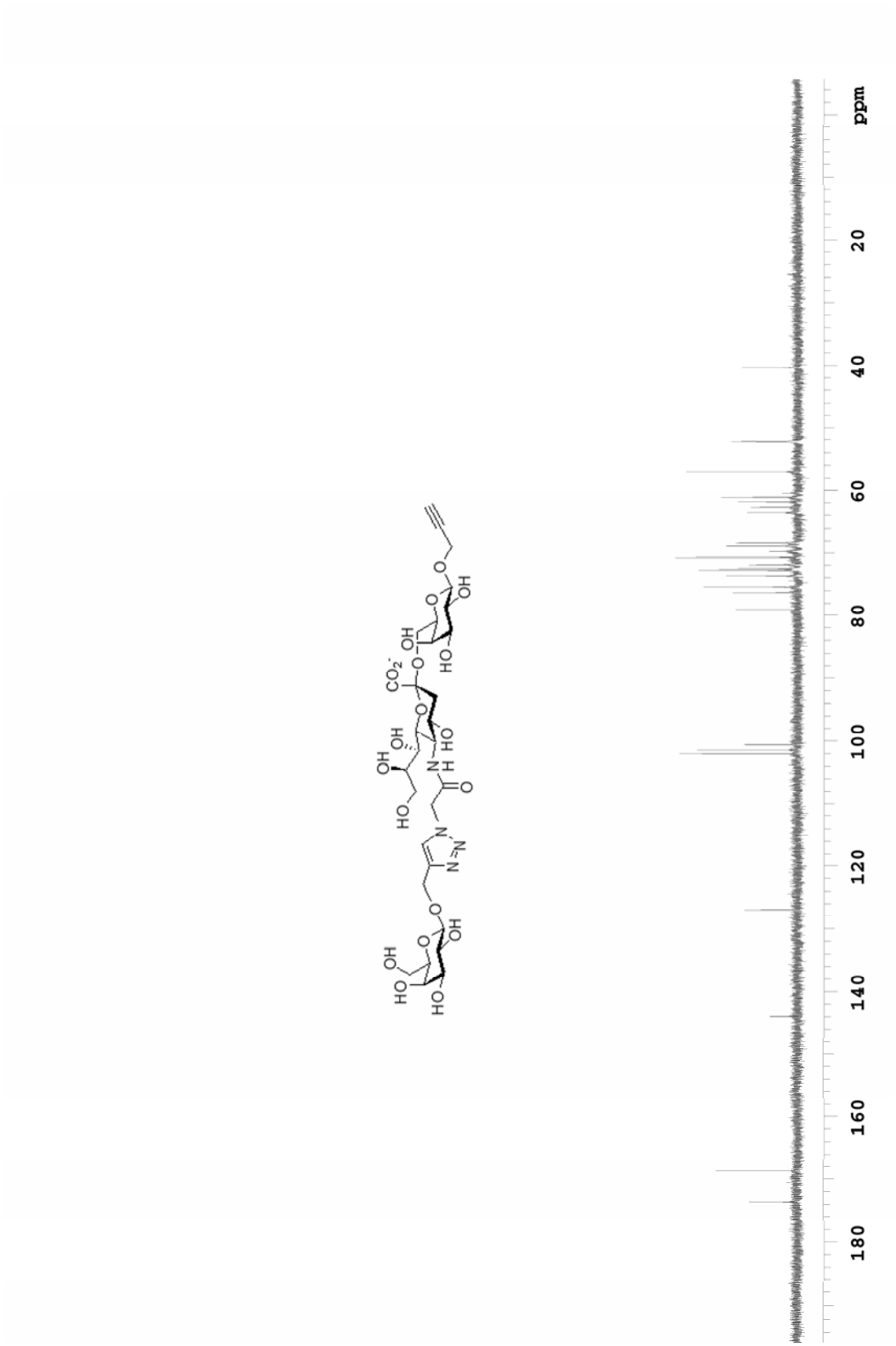
^{13}C NMR of CMP-disaccharide analogue **4**



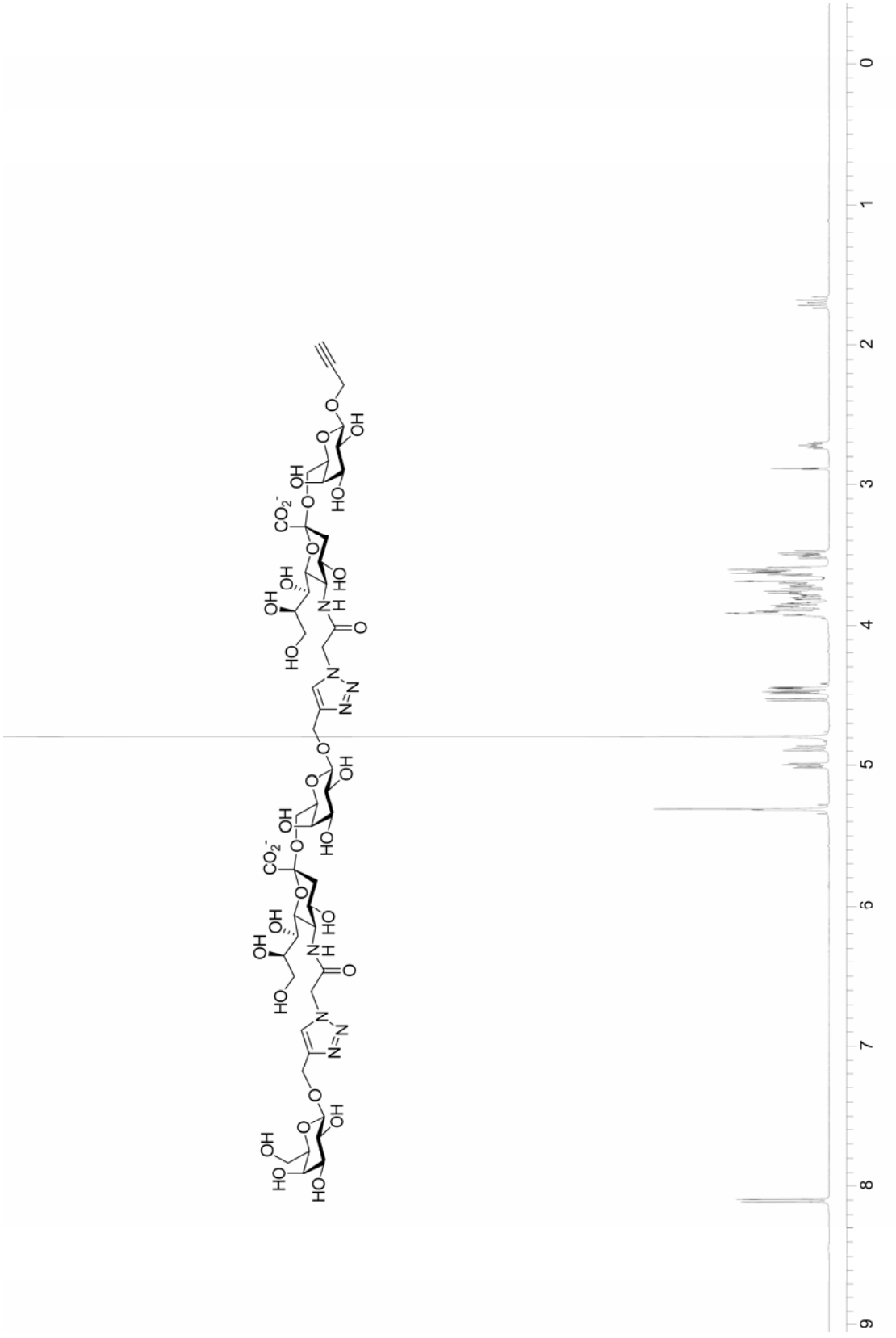
^{1}H NMR of trisaccharide **6**



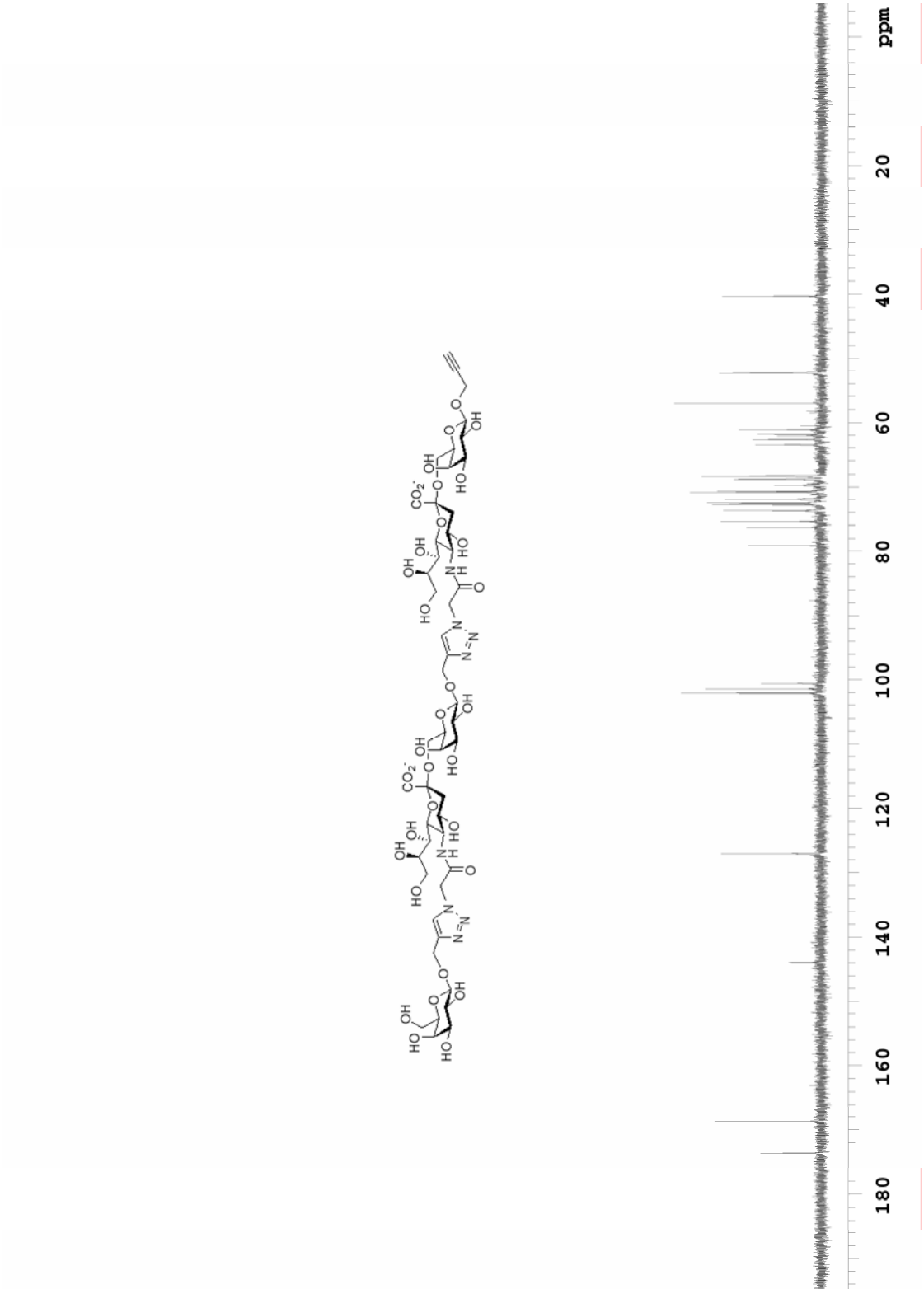
^{13}C NMR of trisaccharide **6**



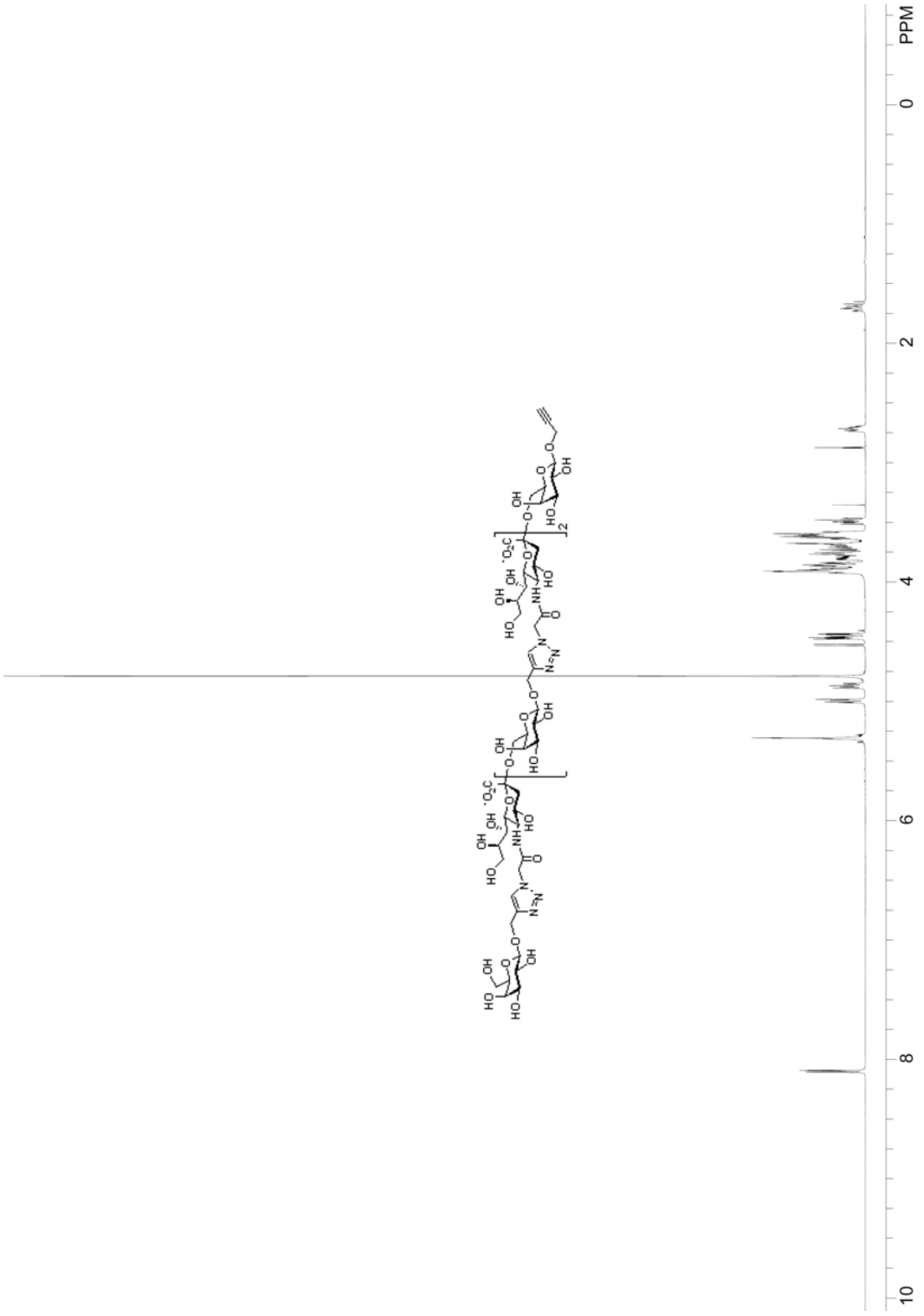
^{1}H NMR of pentasaccharide **7**



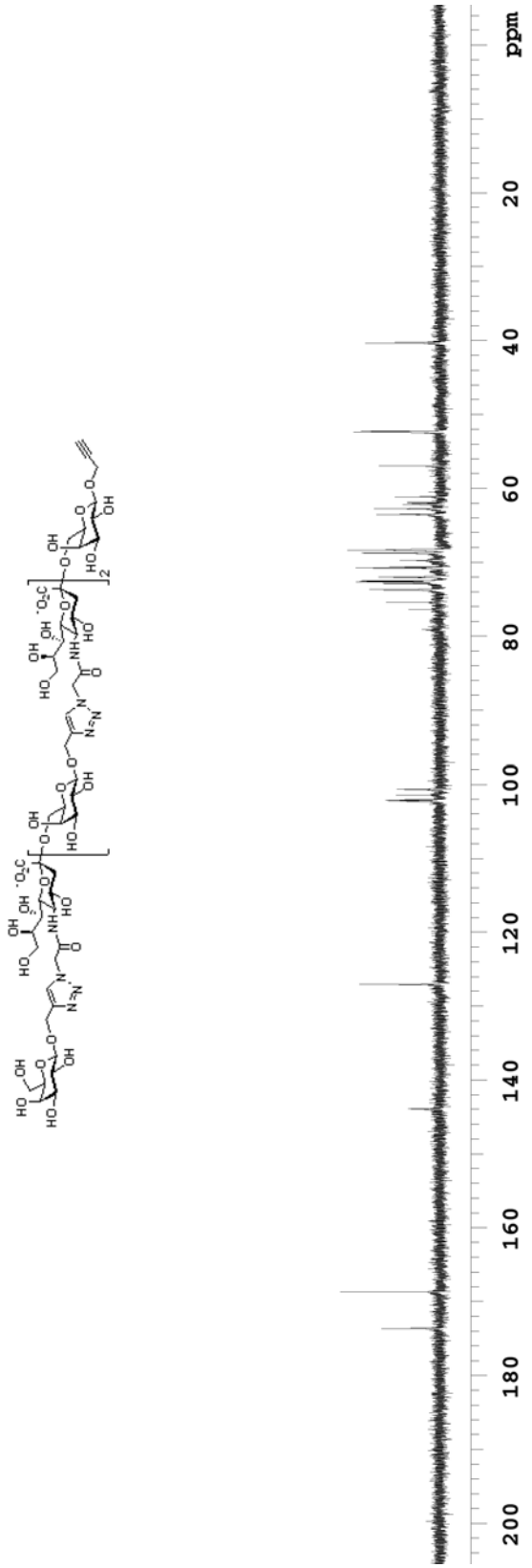
^{13}C NMR of pentasaccharide **7**



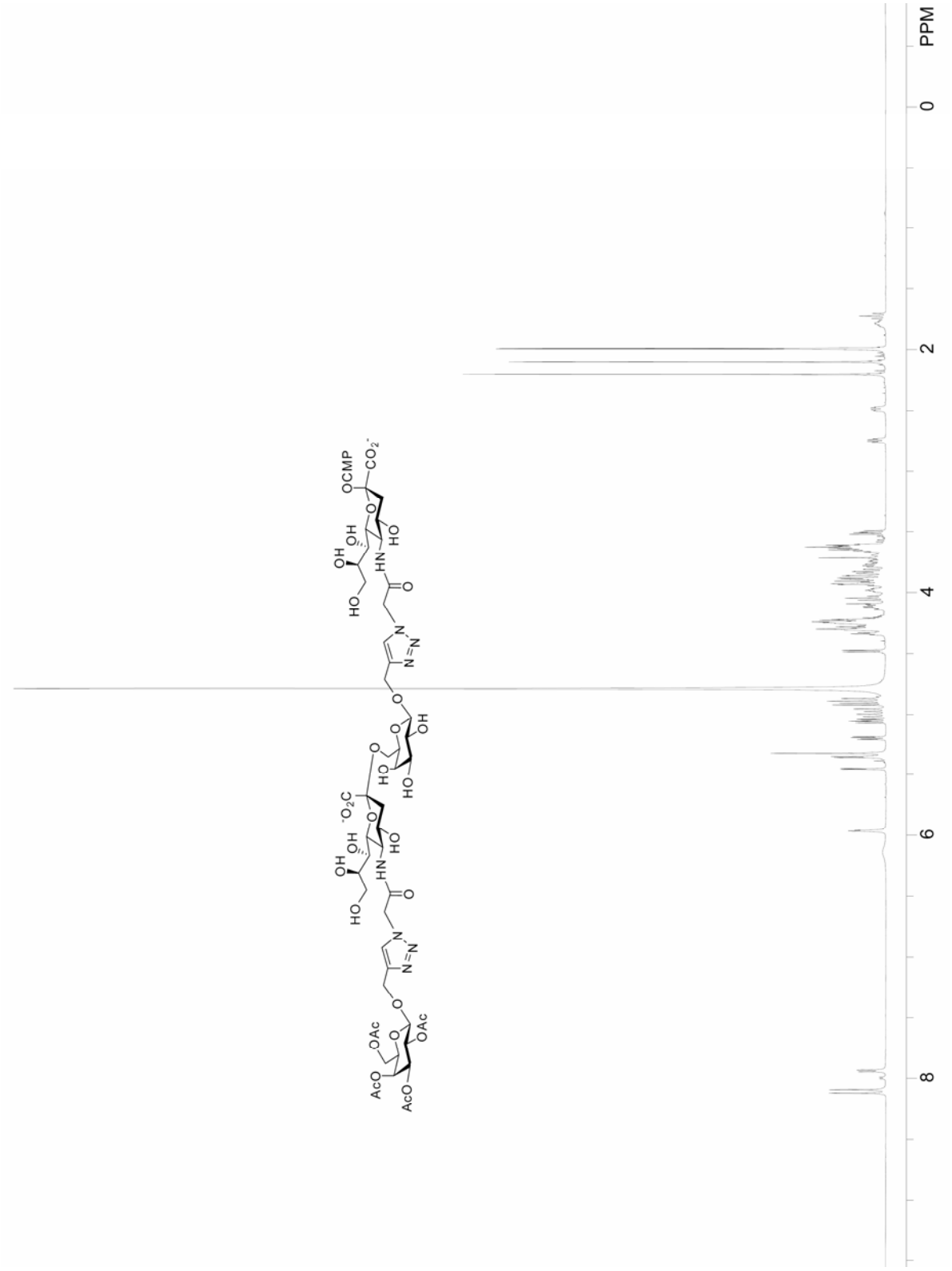
^{1}H NMR of heptasaccharide **8**



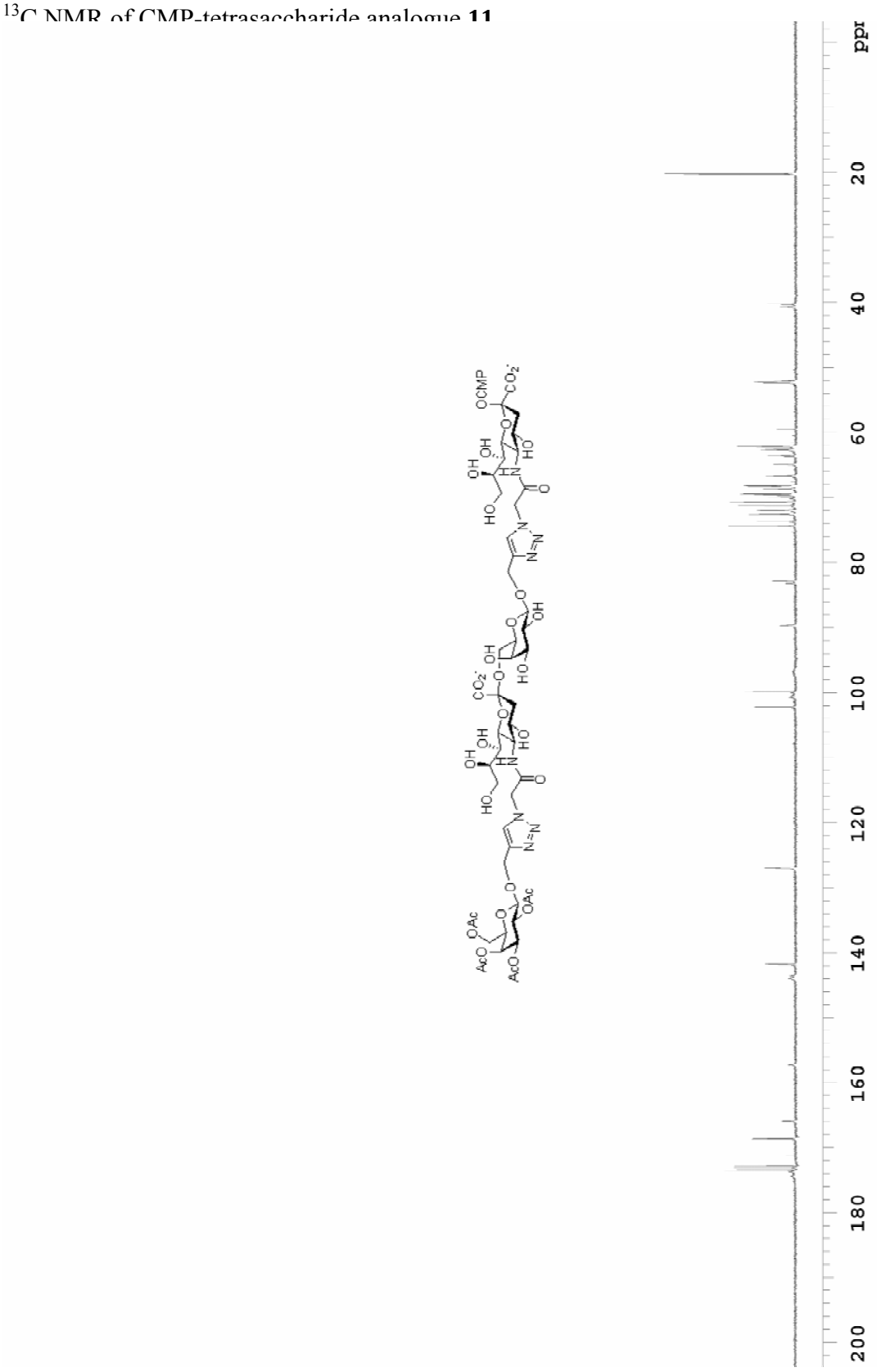
^{13}C NMR of heptasaccharide **8**



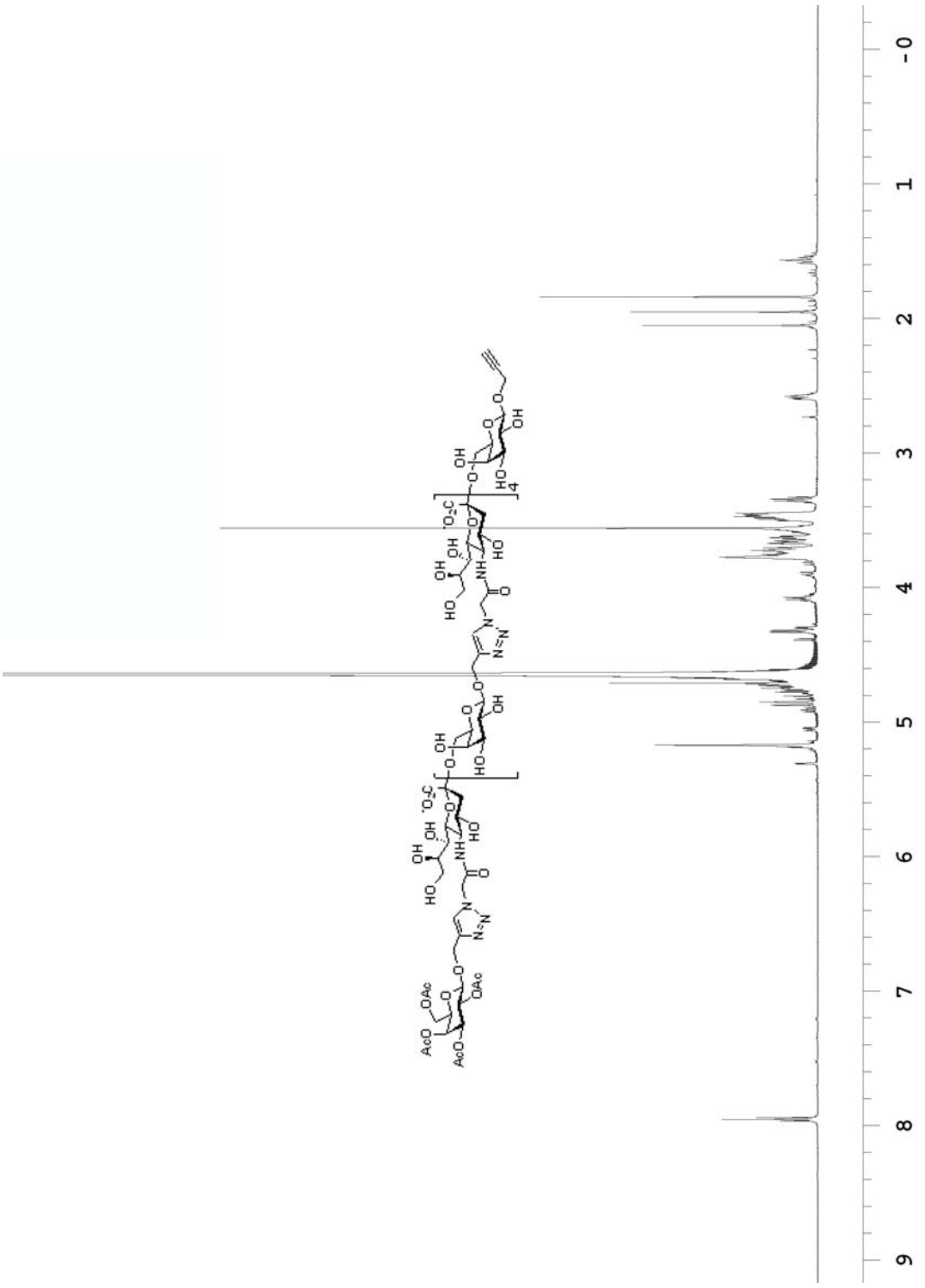
^{1}H NMR of CMP-tetrasaccharide analogue **11**



^{13}C NMR of CMP-tetrasaccharide analogue **11**



^{1}H NMR of undecasaccharide **12**



^{13}C NMR of undecasaccharide **12**