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Supporting Information

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Disaccharides as unusual sialic acid aldolase substrates: Novel synthesis of disaccharides containing a sialic acid at the reducing end

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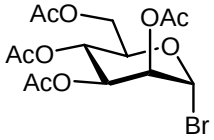
General methods:

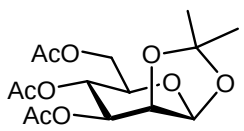
^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-300, a Varian Inova-400, or a Varian Inova-600 spectrometer. Assignment of ^{1}H NMR spectra was achieved by using 2D NMR (COSY) when necessary. Low and high resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility at the Ohio State University. 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) and anisaldehyde stain was used for detection. Gel filtration chromatography was performed on a column (100 cm × 2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad, Hercules, CA). All reagents were of analytical grade and were used as supplied without further purification unless indicated. Solvents used in organic reactions were distilled under an inert argon atmosphere. The recombinant *E. coli* K-12 sialic acid aldolase was cloned and expressed as described.[1] Thiogalactose donor **1**,[2] thioglucose donor **5**,[3] trichloroacetimidate donors **2**,[4] **3**,[5] and **4**[6] as well as glycosylation acceptors **6**,[7] **8**,[8,9] and **10**[10] were prepared as reported.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide (38).



D-Mannose (10 g, 55.5 mmol) was added to acetic anhydride (100 mL) and the mixture was heated to 180 °C, followed by the addition of NaOAc (5 g). After refluxing for 1 hour, the mixture was poured into 500 mL ice water. The solid was collected and dried to afford peracetylated mannose (16.2 g, 75%) as white power. The peracetylated mannose (2.5 g, 6.41 mmol) was dissolved in dry dichloromethane (50 mL) and cooled to 0 °C. HBr/HOAc (5 mL, 33% solution) was added to the mixture and stirred for overnight. The reaction solution was diluted by water and washed by water, aq. $NaHCO_3$, and brine. The organic layer was concentrated and applied to silica gel column (Hexane:EtOAc = 2:1, by volume) to obtain peracetylated mannosyl bromide **38** (2.29 g, 87%) as colorless syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (d, 1 H, J = 1.2 Hz), 5.71 (dd, 1 H, J = 10.0 Hz and 3.2 Hz), 5.44 (dd, 1 H, J = 3.2 Hz and 1.6 Hz), 5.36 (t, 1 H, J = 10.0 Hz), 4.32 (dd, 1 H, J = 8.4 Hz and 5.0 Hz), 4.22 (ddd, 1 H, J = 10.0 Hz, 5.0 Hz and 2.0 Hz), 4.13 (dd, 1 H, J = 12.4 Hz and 2.0 Hz), 2.17 (s, 3 H), 2.10 (s, 3 H), 2.07 (s, 3 H), 2.00 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.72, 169.90, 169.77, 83.26, 73.06, 72.36, 68.15, 65.53, 61.67, 20.98, 20.89, 20.86, 20.79.

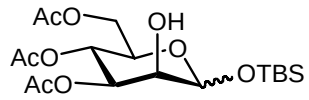
3,4,6-Tri-*O*-acetyl-1,2-*O*-ethylidene-β-D-mannopyranose (39).



Compound **38** (2.2 g, 5.35 mmol), sodium borohydride (100 mg, 2.67 mmol), tetrabutylamonium iodide (100 mg) were dissolved in anhydrous acetonitrile (10 mL) and stirred for 24 h at room

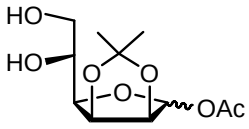
temperature. The solution was diluted by dichloromethane (50 mL) and washed by water and brine. The organic layer was dried and applied to silica gel column chromatography (Hexane:EtOAc = 3:2, by volume) to afford mannose derivative **39** (1.60 g, 90%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.31-5.24 (m, 1 H), 5.18 (dd, 1 H, *J* = 9.6 Hz and 3.6 Hz), 4.22 (dd, 1 H, *J* = 3.6 Hz and 2.4 Hz), 4.17 (dd, 1 H, *J* = 10.8 Hz and 5.4 Hz), 4.10-4.08 (m, 1 H), 3.65 (ddd, 1 H, *J* = 10.8 Hz, 5.4 Hz and 2.4 Hz), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.00 (s, 3 H), 1.48 (d, 6 H, *J* = 4.8 Hz). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.90, 170.52, 169.72, 105.00, 96.70, 77.54, 71.73, 70.77, 69.20, 62.69, 21.77, 20.95, 20.93, 20.88.

1-*O*-((1,1-Dimethylethyl)dimethylsilyl)-3,4,6-tri-*O*-acetyl-D-mannopyranose (7).



Compound **39** (1.50 g, 4.51 mmol) was dissolved in 90% trifluoroacetic acid (15 mL) and stirred for 3 hours at 50 °C. The mixture was concentrated *in vacuo* and purified by flash column chromatography to afford a diol derivative **40** (1.04 g, 75%). Compound **40** (1.0 g, 3.27 mmol) was dissolved in anhydrous THF (20 mL) and cooled to 0 °C, followed by the addition of imidazole (0.45g, 6.54 mmol) and TBSCl (0.55 g, 3.60 mmol). After stirred for 10 hours, the reaction mixture was concentrated and applied to silica gel column to afford **7** (1.02 g, 74%, α:β = 1:1) as white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.40 (t, 1 H, *J* = 9.6 Hz), 5.29 (t, 1 H, *J* = 9.6 Hz), 5.25 (dd, 1 H, *J* = 9.6 Hz and 2.7 Hz), 5.07 (dd, 1 H, *J* = 3.3 Hz and 2.1 Hz), 4.96 (dd, 1 H, *J* = 9.6 Hz and 3.3 Hz), 4.20-4.09 (m, 5 H), 4.06 (t, 1 H, *J* = 1.6 Hz), 4.00 (d, 1 H, *J* = 1.2 Hz, H-1α-isomer), 3.61 (ddd, 1 H, *J* = 9.6 Hz, 7.2 Hz and 4.0 Hz), 3.41 (d, 1 H, *J* = 3.0 Hz, H-1β-isomer), 2.09 (s, 3 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 0.91 (s, 9 H), 0.89 (s, 9 H), 0.14 (s, 3 H), 0.12 (s, 3 H), 0.05 (s, 3 H), 0.03 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.18, 170.96, 170.75, 170.53, 169.97, 169.85, 95.17, 94.74, 73.26, 72.28, 71.51, 70.44, 70.30, 69.12, 66.52, 66.25, 62.97, 62.86, 25.84, 25.79, 21.21, 21.09, 20.95, 20.92, 18.23, -4.05, -4.59, -4.80, -5.13.

Acetyl 2,3-*O*-isopropylidene-α-D-mannofuranoside (41).

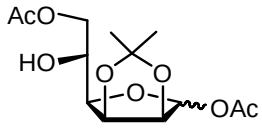


To a suspension of D-mannose (5 g, 27.8 mmol) and 2,2-dimethoxylpropane (30 mL), was added D-(+)-camphor-10-sulfonic acid (150 mg). The mixture was refluxed at 70 °C for 1 hour and then cooled to room temperature. The reaction was neutralized by adding Et_3N (0.2 mL) and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (Hexane:EtOAc = 3:2, by volume) to afford 2,3:5,6-di-*O*-isopropylidene-D-mannofuranose (5.13 g, 71%). The product was dissolved in pyridine (50 mL) and cooled to 0 °C and acetic anhydride (5 mL) was added. The mixture was stirred for overnight and the reaction was quenched by adding

methanol. The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography (Hexane:EtOAc = 3:1, by volume) to afford mannose derivative (5.85 g, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.04 (s, 1 H), 4.78 (dd, 1 H, *J* = 6.0 Hz and 4.0 Hz), 4.63 (d, 1 H, *J* = 6.0 Hz), 4.34-4.30 (m, 1 H), 4.04-3.94 (m, 3 H), 2.00 (s, 3 H), 1.41 (s, 3 H), 1.39 (s, 3 H), 1.30 (s, 3 H), 1.27 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.56, 113.39, 109.48, 100.92, 85.20,8 2.36, 79.46, 73.05, 66.96, 27.17, 26.10, 25.30, 24.81, 21.24.

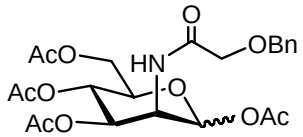
The above compound (4 g, 13.2 mmol) was dissolved in 64% HOAc (30 mL) and acetone (30 mL). After refluxing at 55 ^{o}C for 3 hours, the TLC (CH_2Cl_2:MeOH = 20:1, by volume) showed the completion of the reaction. The mixture was condense and purified by flash column chromatography to afford diol mannose derivative **41** (3.28 g, 95%).^{1}H NMR (400 MHz, $CDCl_3$) δ 6.01 (s, 1 H), 4.87 (dd, 1 H, *J* = 6.0 Hz and 4.0 Hz), 4.65 (d, 1 H, *J* = 6.0 Hz), 4.02 (dd, 1 H, *J* = 8.4 Hz and 4.0 Hz), 3.97-3.95 (m, 3 H), 3.80 (dd, 1 H, *J* = 12.0 Hz and 3.2 Hz), 3.64 (dd, 1 H, *J* = 12.0 Hz and 6.4 Hz), 2.02 (s, 3 H), 1.44 (s, 3 H), 1.30 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.78, 113.43, 100.87, 84.95, 81.45, 79.90, 70.03, 64.23, 26.17, 24.95, 21.27.

1,6-Di-*O*-acetyl-2,3-*O*-isopropylidene-α-D-mannofuranose (9).



Compound **41** (3.62 g, 13.8 mmol) was dissolved in pyridine (25 mL) and cooled to -20 °C. Acetyl chloride (1.08 mL, 16.0 mmol) was added dropwisely. The mixture was stirring at -20 °C for 2 hours and then at room temperature for overnight. TLC (Hexane:EtOAc = 1:2, by volume) showed that the reaction was completed. The mixture was concentrated *in vacuo*, the residue was purified by flash column chromatography to afford compound **9** (3.49 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.08 (s, 1 H, H-1), 4.86 (dd, 1 H, *J* = 6.0 Hz and 3.6 Hz, H-3), 4.64 (d, 1 H, *J* = 6.0 Hz, H-2), 4.34-4.31 (m, 1 H, H-6a), 4.12-4.08 (m, 2 H, H-5 and H-6b), 3.97 (dd, 1 H, *J* = 8.0 Hz and 3.6 Hz, H-4), 2.04 (s, 3 H), 2.00 (s, 3 H), 1.42 (s, 3 H), 1.28 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.53, 169.54, 113.51, 100.80, 84.91, 81.23, 79.80, 68.38, 66.50, 26.14, 24.89, 21.20, 21.03.

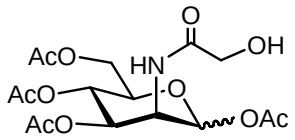
1,3,4,6-Tetra-*O*-acetyl-*N*-benzyloxyacetate-D-mannosamine (42).



Mannosamine hydrochloride salt (0.36 g, 1.65 mmol) was dissolved in dry MeOH (10 mL) under argon atmosphere. Triethylamine (0.23 mL) was added to the solution. The mixture was stirred for 10 min until the solution turned clear. Benzylglycolic acid *N*-hydroxysuccinimide ester (0.52 g, 1.98 mmol) was then added and the resulted solution was stirred at room temperature for overnight. The reaction mixture was concentrated and the residue was dissolved in dry Pyridine:Ac_2O (6 mL, 2:1 by volume) and stirred for 4 hours. The reaction mixture was

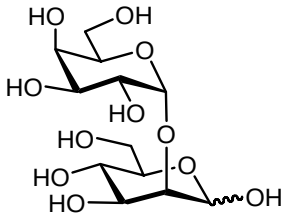
concentrated and purified by flash column chromatography (Acetone:Hexane = 1:2) to afford a ManNGc derivative **42** as a white solid (0.727 g, 89%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.34-7.25 (m, 5H), 6.98 (d, 0.2H, *J* = 9.0 Hz, NH), 6.86 (d, 0.8H, *J* = 9.6 Hz, NH), 5.98 (s, 0.8H, H-1β), 5.87 (d, 0.2H, *J* = 1.8 Hz, H-1α), 5.29 (dd, 1H, *J* = 4.2 and 10.2 Hz, H-3β), 5.20 (t, 0.8H, *J* = 10.2 Hz, H-4β), 5.14 (t, 0.2H, *J* = 9.6 Hz, H-4α), 5.04 (dd, 0.2H, *J* = 4.2 and 10.2 Hz, H-3α), 4.74-3.77 (m, 6H), 2.12, 2.01, 1.93, 1.85 (s, $4CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.74, 170.72, 170.58, 170.31 169.99, 169.73, 169.68, 168.59, 168.37, 136.86, 136.69, 128.95, 128.90, 128.56, 128.50, 128.15, 128.11, 128.07, 91.75, 90.62, 73.83, 73.68, 73.38, 71.57, 70.37, 69.46, 69.32, 69.19, 65.34, 65.25, 61.87, 49.13, 21.04, 20.96, 20.94, 20.89, 20.87, 20.84, 20.66, 20.59.

1,3,4,6-Tetra-*O*-acetyl-2-glycolylamido-*2-deoxy*-D-mannosamine (11).



Compound **42** (705 mg, 1.45 mmol) was dissolve in dry MeOH (10 mL) and Pd/C (100 mg) was added. The mixture was stirred at H_2 atmosphere for overnight. When TLC indicated the completion of the reaction, the reaction mixture was filtered and concentrated. The residue was purified by flash column chromatography (Acetone:Hexane = 1:2, by volume) to afford compound **11** (0.57 g, 99%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.10 (d, 1H, *J* = 9.6 Hz, NH), 5.98 (d, 1H, *J* = 1.8 Hz, H-1), 5.29 (dd, 1H, *J* = 4.2 and 10.2 Hz, H-3), 5.16 (t, 1H, *J* = 10.2 Hz, H-4), 4.61 (m, 1H, H-2), 4.17 (dd, 1H, *J* = 4.8 and 12.6 Hz, H-6a), 4.08 (d, 2H, *J* = 6.6 Hz, CH_2OH), 4.04 (dd, 1H, *J* = 3.0 and 12.6 Hz, H-6b), 4.00 (m, 1H, H-5), 2.14, 2.04, 2.01, 1.95 (s, $4CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.80, 170.97, 170.40, 170.03, 168.50, 91.79, 70.45, 69.21, 65.68, 62.19, 48.94, 31.13, 21.05, 20.90, 20.85, 20.82.

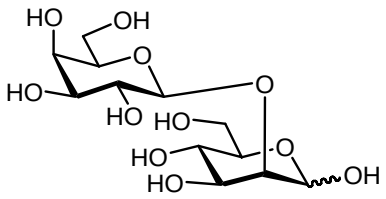
α-D-Galactopyranosyl-(1→2)-D-mannopyranose (12).



A solution of acceptor **6**[7] (0.90 g, 2.01 mmol) and thiogalactose donor **1**[2] (0.80 g, 1.37 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred with activated 4 Å molecular sieves (1.70 g) under argon for 30 min. The reaction mixture was cooled to -30 °C and NIS (368 mg, 1.64 mmol) was added. After 10 min, TfOH (30 μL) was added. The reaction mixture was quenched with Et_3N (0.5 mL) after 1 hour and filtered through celite. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane:EtOAc = 4:1, by volume) to afford an α-linked disaccharide derivative as amorphous solid (0.74 g, 56%). ^{1}H NMR (600Hz, $CDCl_3$) δ 87.55-7.19 (m, 35 H), 5.62 (d, 1 H, *J* = 3.6 Hz), 5.52 (S, 1 H), 4.5-4.98 (m, 2 H), 4.94-4.91(m, 2 H), 4.81-4.78 (m, 3 H), 4.74 (d, 1 H, *J* = 11.4 Hz), 4.68 (d, 1 H, *J* = 13.2 Hz), 4.61 (d, 1 H, *J* = 11.4 Hz), 4.50-4.47 (m, 2 H), 4.42-4.49 (m, 4 H), 4.22 (dd, 1 H, *J* = 9.0 Hz and 3.6 Hz), 4.12 (dd, 1 H, *J* = 9.0 Hz and 3.0 Hz), 4.09-4.06 (m, 1 H), 4.09-4.06 (m, 1 H), 4.00-3.99 (m, 3 H), 3.90-3.86 (m, 2 H), 3.52 (d, 1 H, *J* = 6.0 Hz). ^{13}C NMR (75MHz, $CDCl_3$) δ 139.34, 139.21, 138.88, 138.79, 138.34, 138.05, 137.42, 129.08, 128.95, 128.77, 128.71, 128.67, 128.61, 128.56, 128.55, 128.48, 128.42, 128.32, 128.21, 128.13, 128.05, 128.03, 127.99, 127.96, 127.86, 127.81, 127.68, 127.62, 127.47, 126.35, 126.31, 101.48, 99.92, 98.32, 79.61, 78.53, 77.75, 76.62, 75.38, 75.03, 74.14, 73.72, 73.42, 71.67, 70.11, 69.74, 69.56, 69.02, 64.91.

The protected disaccharide (740 mg, 0.76 mmol) was dissolved in 22 mL of MeOH:EtOAc:HOAc = 5:5:1 (by volume) and 150 mg of Pd/C was added. The mixture was stirred at H_2 (4 Bar) atmosphere for overnight. When TLC indicated that the reaction was completed, the mixture was filtered and concentrated. Purification of the residue by silica gel purification (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded **12** (231 mg, 89%). ^{1}H NMR (600 MHz, D_2O) δ 5.43 (d, 1 H, *J* = 1.2 Hz), 5.11 (d, 1 H, *J* = 4.2 Hz), 4.08-4.05 (m, 1 H), 3.94-3.60 (m, 11 H). ^{13}C NMR (75 MHz, D_2O) δ 101.29, 92.62, 80.31, 75.32, 72.54, 71.50, 70.42, 69.35, 68.94, 67.23, 61.36, 60.96.

β-D-Galactopyranosyl-(1→2)-D-mannopyranose (13).

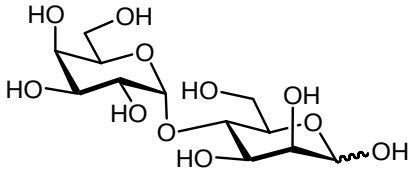


A solution of acceptor **7** (0.50 g, 1.19 mmol) and trichloroacetimidate donor **2**[4] (0.70 g, 1.42 mmol) in anhydrous CH_2Cl_2 (15 mL) was stirred with activated 4 Å molecular sieves (1.20 g)

under argon atmosphere for 30 min. The reaction mixture was cooled to -20 °C and TMSOTf (10 μL) was added dropwisely. The reaction mixture was quenched with Et_3N (0.5 mL) after 10 hours and filtered through celite. The filtrate was concentrated *in vacuo*. The residue was purified by flash column chromatography (Hexane:EtOAc = 2:1, by volume). The coupled product was treated by TBAF (2 mL, 1 M in THF) in THF (10 mL) for 2 hours. The TLC (Hexane:EtOAc = 1:2, by volume) showed the TBS group was removed. After purification, a β-linked disaccharide derivative was obtained (0.58 g, 78%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.35 (d, 1 H, *J* = 7.2 Hz), 5.31 (t, 1 H, *J* = 8.7 Hz), 5.24 (dd, 1 H, *J* = 9.6 Hz and 6.0 Hz), 5.11 (dd, 1 H, *J* = 15.6 Hz and 5.1 Hz), 4.98 (dd, 1 H, *J* = 15.6 Hz and 5.4 Hz), 4.40 (d, 1 H, *J* = 11.4 Hz), 4.19 (t, 1 H, *J* = 3.3 Hz), 4.14-4.06 (m, 5 H), 3.86 (t, 1 H, *J* = 9.9 Hz), 3.46 (d, 1 H, *J* = 5.4 Hz), 2.18 (m, 3 H), 2.12 (m, 3 H), 2.05 (m, 3 H), 2.04 (m, 3 H), 2.03 (m, 3 H), 2.02 (m, 3 H), 1.97 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.43, 170.80, 170.77, 170.61, 170.47, 169.65, 169.36, 100.99, 92.45, 75.41, 71.08, 70.89, 70.18, 68.96, 68.52, 67.25, 65.83, 62.45, 61.63, 21.14, 20.98, 20.92, 20.89, 20.84, 20.81, 20.77.

The protected disaccharide derivative (580 mg, 0.91 mmol) was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight and neutralized with Dowex-50 (H^+) resin and evaporated to dryness. Purification of the residue by silica gel chromatography (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded the target compound **13** (302 mg, 92%). ^{1}H NMR (600 MHz, D_2O) δ 5.27 (d, 1 H, *J* = 1.2 Hz), 4.96 (s, 0.3 H), 4.53 (d, 0.3 H, *J* = 7.8 Hz), 4.43 (d, 1 H, *J* = 7.8 Hz), 4.16 (d, 0.3 H, *J* = 3.0 Hz), 4.09 (dd, 1 H, *J* = 3.3 Hz and 1.5 Hz), 3.89-3.61 (m, 13 H), 3.56-3.52 (m, 1.3 H). ^{13}C NMR (150 MHz, D_2O) δ 102.37, 92.37, 78.16, 75.35, 72.59, 72.47, 70.71, 69.66, 68.78, 67.16, 61.32, 60.63.

α-D-Galactopyranosyl-(1→4)-D-mannopyranose (14).

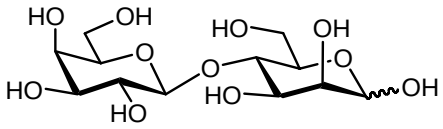


Glycosylation of acceptor **8** (Prepared from 2,3-di-O-benzyl-4,6-O-benzylidene-mannose[8] using the reported procedure[9]) (0.73 g, 1.25 mmol) and thiogalactose donor **1** (1.09 g, 2.02 mmol) was achieved using the similar condition as that described for preparing compound **12** to give α-linked disaccharide derivative (0.69 g, 52%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.11 (m, 40 H), 5.04 (d, 1 H, *J* = 2.4 Hz), 4.89 (d, 1 H, *J* = 11.4 Hz), 4.80 (d, 1 H, *J* = 12 Hz), 4.76 (d, 1 H, *J* =11.4 Hz), 4.68-4.51 (m, 12 H), 4.39-4.37 (m, 2H), 4.28 (d, 1 H, *J* = 12.0 Hz), 4.11 (dd, 1 H, *J* = 7.8 Hz and 3.3 Hz), 4.05-4.00 (m, 3 H), 3.95 (d, 1 H, *J* = 1.2 Hz), 3.90 (t, 1 H, *J* = 7.2 Hz), 3.88 (t, 1 H, *J* = 2.7 Hz), 3.82-3.80 (m, 2 H), 3.52 (t, 1 H, *J* = 8.4 Hz), 3.46 (dd, 1 H, *J* = 9.0 Hz and 5.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.12, 138.99, 138.66, 138.54, 138.43, 137.72, 128.71-127.03, 97.64, 97.59, 80.57, 79.12, 76.00, 75.08, 75.01, 74.60, 73.70, 73.44, 73.11, 73.10, 72.81, 71.65, 71.03, 70.92, 70.41, 70.13, 69.35, 68.99.

The protected disaccharide (690 mg, 0.65 mmol) was dissolved in 22 mL of MeOH:EtOAc:HOAc = 5:5:1 (by volume) and 150 mg of Pd/C was added. The mixture was stirred at H_2 (4 Bar)

atmosphere for overnight. When TLC indicated the completion of the reaction, the mixture was filtered and concentrated. Purification of the residue by silica gel purification (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded **14** (189 g, 85%). ^{1}H NMR (600 MHz, D_2O) δ 5.31 (s, 1 H), 5.13 (d, 1 H, *J* = 2.4 Hz), 4.05 (dd, 1 H, *J* = 9.0 Hz and 3.0 Hz), 3.98-3.95 (m, 2 H), 3.91-3.85 (m, 3 H), 3.82-3.74 (m, 3 H), 3.73-3.67 (m, 3 H). ^{13}C NMR (100 MHz, D_2O) δ 100.26, 93.93, 75.55, 74.84, 73.70, 71.85, 71.12, 70.84, 69.47, 68.86, 61.33, 61.13.

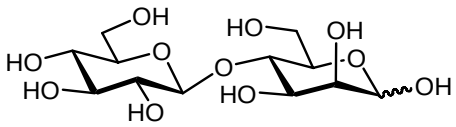
β-D-Galactopyranosyl-(1→4)-D-mannopyranose (15).



Glycosylation of acceptor **8** (0.75 g, 1.39 mmol) and trichloroacetimidate donor **2** (0.90 g, 1.83 mmol) was achieved using the similar condition as that described for preparing compound **13** to give β-linked disaccharide derivative (0.72 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39-7.22 (m, 20 H), 5.25 (d, 1 H, *J* = 3.2 Hz), 5.11 (dd, 1 H, *J* = 10.4 Hz and 4.0 Hz), 4.94 (d, 1 H, *J* = 1.6 Hz), 4.84 (dd, 1 H, *J* = 10.4 Hz and 3.2 Hz), 4.80-4.60 (m, 6 H), 4.48 (t, 2 H, *J* = 11.4 Hz), 4.27 (t, 1 H, *J* = 9.4 Hz), 4.00-3.90 (m, 2 H), 3.83-3.75 (m, 4 H), 3.63 (d, 1 H, *J* = 9.6 Hz), 3.52 (t, 1 H, *J* = 7.2 Hz), 2.07 (s, 3 H), 1.95 (s, 3 H), 1.91 (s, 3 H), 1.90 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.49, 170.39, 170.35, 169.72, 139.13, 138.64, 138.48, 137.45, 128.67-127.08, 101.10, 97.63, 78.75, 75.46, 75.22, 73.90, 73.06, 72.29, 71.84, 71.41, 70.59, 69.93, 69.38, 68.66, 67.07, 61.03, 21.00, 20.87, 20.82, 20.80.

The protected disaccharide (720 mg, 0.83 mmol) was dissolved in 22 mL of MeOH:EtOAc:HOAc = 5:5:1 (by volume) and 150 mg of Pd/C was added. The mixture was stirred at H_2 (4 Bar) atmosphere for overnight. When TLC indicited the completion of the reaction, the mixture was filtered and concentrated. The residue was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. Purification of the residue by silica gel (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded target compound **38** (279 mg, 99%). ^{1}H NMR (600 MHz, D_2O) δ 5.01 (d, 1 H, *J* = 1.8 Hz), 4.88 (d, 0.5 H, *J* = 1.2 Hz), 4.41 (d, 1 H, *J* = 7.8 Hz), 4.40 (d, 0.5 H, *J* = 7.8 Hz), 3.96-3.69 (m, 13.5 H), 3.67-3.61 (m, 3 H), 3.53-3.49 (m, 1.5 H). ^{13}C NMR (100 MHz, D_2O) δ 103.18, 93.93, 93.77, 76.67, 75.51, 75.09, 72.63, 72.51, 71.94, 71.35, 71.17, 71.10, 70.78, 70.31, 69.16, 68.75, 66.97, 61.27, 61.09, 60.47.

β-D-Glucopyranosyl-(1→4)-D-mannopyranose (16).

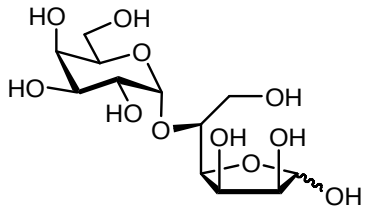


Glycosylation of acceptor **8** (0.58 g, 1.06 mmol) and trichloroacetimidate donor **3**[5] (0.69 g, 1.42 mmol) was achieved using the similar condition as that described for preparing compound **13** to give β-linked disaccharide derivative (0.49 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.21 (m,

20 H), 5.26 (d, 1 H, *J* = 0.8 Hz), 5.02-5.00 (m, 2 H), 4.92-4.88 (m, 2 H), 4.79-4.75 (m, 2 H), 4.70-4.64 (m, 3 H), 4.58 (t, 1 H, *J* = 12 Hz), 4.47 (t, 2 H, *J* = 12.8 Hz), 4.25 (t, 1 H, *J* = 9.2 Hz), 4.11-4.03 (m, 2 H), 3.88 (dd, 1 H, *J* = 8.8 Hz and 2.8 Hz), 3.81 (dd, 1 H, *J* = 12.4 Hz and 1.6 Hz), 3.77-3.68 (m, 3 H), 3.61 (d, 1 H, *J* = 10.8 Hz), 1.97 (m, 3 H), 1.96 (m, 3 H), 1.90 (m, 3 H), 1.89 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.91, 170.45, 169.65, 169.62, 139.23, 138.52, 138.45, 137.45, 128.69-127.12, 100.72, 97.64, 78.49, 75.80, 75.51, 73.87, 73.41, 73.03, 72.51, 72.23, 71.85, 71.71, 69.43, 68.55, 68.34, 61.89, 20.89, 20.81.

The protected disaccharide (490 mg, 0.56 mmol) was dissolved in 22 mL of MeOH:EtOAc:HOAc = 5:5:1 (by volume) and 150 mg of Pd/C was added. The mixture was stirred at H_2 (4 Bar) atmosphere for overnight. When TLC indicated the completion of the reaction, the mixture was filtered and concentrated. The residue was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. Purification of the residue by silica gel (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded the target compound **16** (183 mg, 96%). ^{1}H NMR (600 MHz, D_2O) δ 5.16 (d, 1 H, *J* = 1.8 Hz), 4.90 (s, 0.4 H), 4.49 (d, 1 H, *J* = 8.4 Hz), 4.48 (d, 0.4 H, *J* = 8.4 Hz), 3.97-3.89 (m, 5.6 H), 3.85-3.68 (m, 5.6 H), 3.52-3.48 (m, 2.8 H), 3.40-3.37 (m, 1.4 H), 3.33-3.27 (m, 1.4 H). ^{13}C NMR (75 MHz, D_2O) δ 102.82, 93.94, 77.02, 76.16, 75.65, 73.36, 71.21, 70.45, 69.72, 69.15, 60.86, 60.49.

α-D-Galactopyranosyl-(1→5)-D-mannopyranose (17).

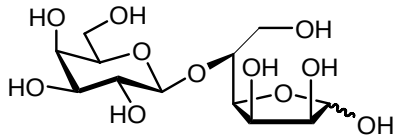


Glycosylation of acceptor **9** (1.20 g, 2.05 mmol) and thiogalactose donor **1** (0.59 g, 1.94 mmol) was achieved using the similar condition as that described for preparing compound **12** to give α-linked disaccharide derivative (0.89 g, 67%). ^{1}H NMR (600Hz, $CDCl_3$) δ 7.40-7.23 (m, 20 H), 5.13 (s, 1 H), 5.26 (d, 1 H, *J* = 4.2 Hz), 4.92 (d, 1 H, *J* = 10.2 Hz), 4.84-4.74 (m, 5 H), 4.55-4.50 (m, 5 H), 4.41 (d, 1 H, *J* = 12.0 Hz), 4.26 (dd, 1 H, *J* = 9.6Hz and 3.6 Hz), 4.14 (t, 1 H, *J* = 6.3 Hz), 4.06 (dd, 1 H, *J* = 10.2 Hz and 3.6 Hz), 3.99-3.95 (m, 3 H), 3.90 (d, 1 H, *J* = 1.8 Hz), 3.76 (dd, 1 H, *J* = 10.2 Hz and 3.6 Hz), 3.54 (dd, 1 H, *J* = 9.6 Hz and 7.8 Hz), 3.37 (dd, 1 H, *J* = 9.3 Hz and 5.1 Hz), 1.43 (s, 3 H), 1.21 (s, 3 H). ^{13}C NMR (150MHz, $CDCl_3$) δ 139.0, 138.75, 138.70, 137.80, 128.70-127.70, 112.50, 101.30, 98.70, 85.70, 79.90, 79.00, 78.40, 78.20, 76.40, 75.20, 74.80, 73.80, 73.30, 73.20, 69.90, 63.40, 26.50, 25.40.

The protected disaccharide (890 mg, 1.20 mmol) was dissolved in 22 mL of MeOH:EtOAc:HOAc = 5:5:1 (by volume) and 150 mg of Pd/C was added. The mixture was stirred at H_2 (4 Bar) atmosphere for overnight. When TLC indicited the completion of the reaction, the mixture was filtered and concentrated. The intermediate was dissolved in 90% trifluoroacetic acid and heated at 50 ^{o}C for 3 hours. The mixture was condensed and applied to silica gel purification (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) to afford compound **17** (364 mg, 89%) as white solid.

^{1}H NMR (600 MHz, D_2O) δ 5.22 (d, 1 H, *J* = 4.8 Hz), 5.20 (d, 0.5 H, *J* = 4.2 Hz), 5.18 (d, 0.5 H, *J* = 4.2 Hz), 5.15 (d, 1 H, *J* = 4.2 Hz), 4.32-4.28 (m, 1.5 H), 4.20 (t, 0.5 H, *J* = 4.8 Hz), 4.18-4.15 (m, 1 H), 3.95-3.92 (m, 1 H), 3.81-3.69 (m, 13.5 H). ^{13}C NMR (100 MHz, D_2O) δ 102.41, 100.80, 100.02, 95.54, 78.67, 78.54, 77.99, 77.73, 77.54, 77.46, 71.90, 71.69, 71.47, 71.42, 71.34, 69.95, 69.69, 69.35, 68.51, 61.68, 61.58, 61.24.

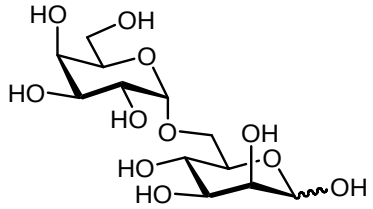
β-D-Galactopyranosyl-(1→5)-D-mannopyranose (18).



Glycosylation of acceptor **9** (0.34 g, 1.12 mmol) and trichloroacetimidate donor **2** (0.58 g, 1.18 mmol) was achieved using the similar condition as that described for preparing compound **13** to gave β-linked disaccharide derivative (0.34 g, 48%). ^{1}H NMR (600Hz, $CDCl_3$) δ 6.05 (s, 1 H), 5.36 (d, 1 H, *J* = 3.2 Hz), 5.15 (dd, 1 H, *J* = 10.6 Hz and 7.8 Hz), 4.98 (dd, 1 H, *J* = 10.2 Hz and 7.4 Hz), 4.91 (dd, 1 H, *J* = 6.2 Hz and 3.1 Hz), 4.64 (d, 1 H, *J* = 14.8 Hz), 4.62 (d, 1 H, *J* = 8.4 Hz), 4.35 (dd, 1 H, *J* = 12.0 Hz and 2.0 Hz), 4.24 (dt, 1 H, *J* = 7.2 Hz and 2.0 Hz), 4.16-4.06 (m, 4 H), 3.87 (t, 1 H, *J* = 7.8 Hz), 2.12 (s, 3 H), 2.04 (s, 3 H), 2.03 (s, 6 H), 2.01 (s, 3 H), 1.95 (s, 3 H), 1.43 (s, 3 H), 1.31 (s, 3 H). ^{13}C NMR (75MHz, $CDCl_3$) δ 170.7, 170.5, 170.4, 169.6, 169.5, 113.2, 102.1, 100.6, 85.0, 81.5, 79.4, 75.8, 71.1, 70.9, 69.1, 67.2, 65.1, 61.2, 26.4, 25.4, 21.2, 21.0, 20.92, 20.87, 20.78.

The protected disaccharide (340 mg, 0.54 mmol) was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. This residue was dissolved in 90% trifluoroacetic acid and heated at 50 ^{o}C for 3 hours. The mixture was condensed and applied to silica gel purification (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) to afford compound **18** (183 mg, 95%) as white solid. ^{1}H NMR (600 MHz, D_2O) δ 5.25 (d, 1 H, *J* = 6.0 Hz), 5.20 (d, 0.5 H, *J* = 6.0 Hz), 4.60-4.56 (m, 3 H), 4.37-4.33 (m, 1.5 H), 4.26 (dd, 1 H, *J* = 9.6 Hz and 2.4 Hz), 4.23 (t, 1 H, *J* = 5.1 Hz), 4.20 (t, 0.5 H, *J* = 4.5 Hz), 4.03-3.87 (m, 5 H), .78-3.62 (m, 6 H), 3.53-3.49 (m, 1.5 H). ^{13}C NMR (75 MHz, D_2O) δ 102.69, 102.37, 102.00, 101.26, 78.27, 77.75, 77.33, 77.08, 76.52, 75.55, 75.36, 72.64, 71.69, 71.36, 70.96, 69.68, 69.47, 68.70, 68.59, 61.34, 61.13, 60.64, 60.51.

α-D-Galatopyranosyl-(1→6)-D-mannopyranose (19).

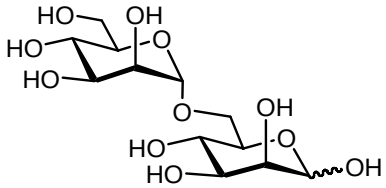


Glycosylation of acceptor **10**[10] (0.38 g, 1.09 mmol) and thiogalactose donor **1** (0.60 g, 1.03 mmol) was achieved using the similar condition as that described for preparing compound **12** to give α-linked disaccharide derivative (0.58 g, 65%). ^{1}H NMR (600Hz, $CDCl_3$) δ 7.37-7.25(m, 20 H),

6.05 (d, 1 H, J = 2.4 Hz), 5.45 (dd, 1 H, *J* = 9.3 Hz and 3.3 Hz), 5.28 (dd, 1 H, *J* = 9.6 Hz and 8.4 Hz), 5.17 (d, 1 H, *J* = 12.0 Hz), 4.92 (d, 1 H, *J* = 11.4 Hz), 4.80-4.71 (m, 3 H), 4.79 (d, 1H, *J* = 12.0 Hz), 4.74 (d, 1 H, *J* = 13.8 Hz), 4.66 (d, 1 H, *J* = 12.0 Hz), 4.54 (d, 1 H, *J* = 11.4 Hz), 4.49 (d, 2 H, *J* = 12.0 Hz), 4.40 (d, 1 H, *J* = 11.4 Hz), 4.27 (dd, 1 H, *J* = 12.6 Hz and 4.8 Hz), 4.17 (t, 1 H, *J* = 9.6 Hz), 4.07-4.04 (m, 2 H), 3.99 (d, 1 H, *J* = 1.2Hz), 3.95 (t, 1 H, *J* = 6.6Hz), 3.88 (dd, 1 H, *J* = 10.2 Hz and 2.4 Hz), 3.54-3.49 (m ,2 H), 2.16(s, 3 H), 2.14(s, 3 H), 2.02(s, 3 H), 1.88(s, 3H). ^{13}C NMR (75MHz, $CDCl_3$) δ 170.73, 169.97, 169.91, 138.74, 138.71, 138.49, 138.07, 128.66, 128.65, 128.59, 128.50, 128.47, 128.45, 128.40, 128.30, 128.07, 128.01, 127.84, 127.72, 98.91, 90.82, 76.43, 75.04, 74.96, 74.26, 73.79, 73.32, 71.61, 71.26, 70.97, 70.88, 69.01, 68.92, 63.33, 21.22, 21.17, 21.02, 20.99.

The protected disaccharide (580 mg, 0.67 mmol) was dissolved in 22 mL of MeOH:EtOAc:HOAc = 5:5:1 (by volume) and 150 mg of Pd/C was added. The mixture was stirred at H_2 (4 Bar) atmosphere for overnight. When TLC indicated the completion of the reaction, the mixture was filtered and concentrated. The residue was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. Purification of the residue by silica gel chromatography (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded target compound **19** (216 mg, 95%). ^{1}H NMR (600 MHz, D_2O) δ 5.14 (s, 1 H), 4.95 (d, 1 H, *J* = 4.2 Hz), 4.42-4.40 (m, 0.56 H), 4.19-4.13 (m, 1 H), 4.01-3.99 (m, 17.8 H), 3.54-3.49 (m, 1 H). ^{13}C NMR (150 MHz, D_2O) δ 103.47, 98.29, 98.26, 94.39, 75.28, 74.54, 73.32, 72.73, 71.48, 71.03, 70.92, 70.76, 70.57, 70.27, 69.61, 69.34, 68.94, 68.77, 68.60, 68.57, 66.70, 66.05, 61.27, 61.16.

α-D-Mannopyranosyl-(1→6)-D-mannopyranose (20).

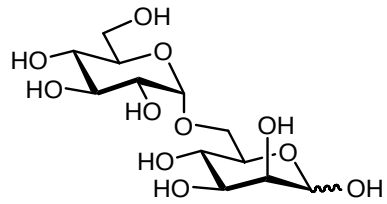


Glycosylation of acceptor **10** (0.44 g, 1.26 mmol) and trichloroacetimidate donor **4**[6] (1.15 g, 2.33 mmol) was achieved using the similar condition as that described for preparing compound **13** to give α-linked disaccharide derivative (0.65 g, 76%). ^{1}HNMR (600Hz, $CDCl_3$) δ 6.01 (d, 1 H, *J* = 1.8 Hz), 5.29-5.22 (m, 4 H), 5.09 (t, 1 H, *J* = 2.4 Hz), 5.04 (d, 1 H, *J* = 2.4 Hz), 4.41 (dd, 1 H, *J* = 12.0 Hz and 1.8 Hz), 4.22 (t, 1 H, *J* = 4.8 Hz), 4.20 (t, 1 H, *J* = 4.8 Hz), 4.10-4.07 (m, 2 H), 4.02-3.96 (m, 2 H), 2.16 (s, 3 H), 2.12 (s, 3 H), 2.10 (s, 3 H), 2.08 (s, 3 H), 2.07 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H), 1.97 (s, 3 H). ^{13}C NMR (150MHz, $CDCl_3$) δ 170.78, 170.74, 170.26, 170.06, 170.01, 169.82, 169.72, 148.41, 99.89, 90.58, 74.23, 71.31, 71.15, 70.13, 69.81, 68.65, 68.53, 65.93, 63.04,62.35, 21.10, 21.03, 20.98, 20.91, 20.88, 20.84.

The disaccharide analogue (650 mg, 0.96 mmol) was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. Purification of the residue by silica gel (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded target compound **20** (308 mg, 94%). ^{1}HNMR (600Hz, D_2O) δ 5.20 (s, 1 H), 5.13 (s, 1 H), 4.02-4.00 (m, 1 H), 3.94 (dd, 1H, *J* = 9.0 Hz and 3.0 Hz), 3.85-3.70 (m, 8 H), 3.66-3.60 (m, 2 H). ^{13}C NMR (75MHz, $CDCl_3$) δ 101.59, 93.94, 74.45, 73.79, 71.30, 71.08, 70.82,

70.44, 70.38, 66.67, 61.18, 61.04.

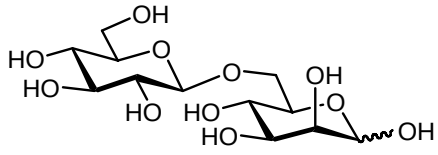
α-D-Glucopyranosyl-(1→6)-D-mannopyranose (21).



Glycosylation of acceptor **10** (0.48 g, 1.38 mmol) and thioglucose donor **5**[3] (0.78 g, 1.33 mmol) was achieved using the similar condition as that described for preparing compound **12** to give α-linked disaccharide derivative (0.89 g, 67%). ^{1}H NMR (600Hz, $CDCl_3$) δ 7.35-7.26 (m, 20 H), 6.06 (d, 1 H, *J* = 2.4 Hz), 5.49 (dd, 1 H, *J* = 9.0 Hz and 3.0 Hz), 5.30 (dd, 1 H, *J* = 3.6 Hz and 2.4 Hz), 5.16 (d, 1 H, *J* = 3.0 Hz), 4.90 (d, 1 H, *J* = 10.8 Hz), 4.83 (dd, 2 H, *J* = 10.8 Hz and 1.2 Hz), 4.75 (d, 1 H, *J* = 12.0 Hz), 4.66 (d, 1 H, *J* = 12.0 Hz), 4.60 (d, 1 H, *J* = 12.6 Hz), 4.51-4.46 (m, 3 H), 4.31 (dd, 1 H, *J* = 12.0 Hz and 4.5 Hz), 4.17 (t, 1 H, *J* = 9.6 Hz), 4.06-4.04 (m, 1 H), 3.92 (t, 1 H, *J* = 9.3 Hz), 3.78 (dt, 1 H, *J* = 9.0 Hz and 2.4 Hz), 3.72 (dd, 1 H, *J* = 10.5 Hz and 3.3 Hz), 3.68 (t, 1 H, *J* = 9.6 Hz), 3.63 (dd, 1 H, *J* = 10.8 Hz and 1.8 Hz), 3.56 (dd, 1 H, *J* = 10.2 Hz and 3.6 Hz), 2.18 (s, 3 H), 2.17 (s, 3 H), 2.04 (s, 3 H), 1.93 (s, 3 H). ^{13}C NMR (150MHz, $CDCl_3$) δ 170.6, 169.94, 169.85, 168.60, 138.80, 138.30, 138.00, 129.30, 128.76, 128.67, 128.66, 128.61, 128.48, 128.27, 128.20, 128.12, 128.06, 128.03, 127.96, 127.91, 125.55, 98.63, 90.78, 81.75, 80.18, 77.65, 75.94, 75.31, 74.05, 73.80, 71.61, 71.11, 68.91, 68.39, 63.33, 21.21, 21.06, 21.00.

The disaccharide derivative (800 mg, 0.92 mmol) was dissolved in 22 mL of MeOH:EtOAc:HOAc = 5:5:1 (by volume) and 150 mg of Pd/C was added. The mixture was stirred at H_2 (4 Bar) atmosphere for overnight. When TLC indicated the completion of the reaction, the mixture was filtered and concentrated. The residue was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. Purification of the residue by silica gel (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded target compound **21** (298 mg, 95%). ^{1}H NMR (600Hz, $CDCl_3$) δ 5.30 (d, 1 H, *J* = 3.0 Hz, H-1'), 5.13 (d, 1 H, H-1), 4.06 (dd, 1 H, *J* = 9.0 Hz and 2.4 Hz), 3.89-3.62 (m, 8 H), 3.56-3.53 (m, 1 H), 3.47 (t, 1H, *J* =9.3 Hz), 3.38 (t, 1 H, *J* = 9.6 Hz). ^{13}C NMR (150MHz, D_2O) δ 99.99, 93.94, 75.39, 73.00, 72.78, 71.90, 71.07, 70.86, 70.83, 69.45, 61.11, 60.60.

β-D-Glucopyranosyl-(1→6)-D-mannopyranose (22).

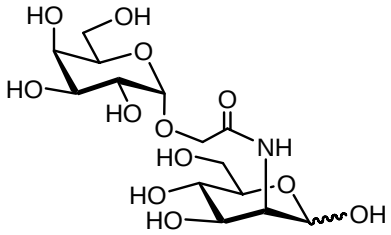


Glycosylation of acceptor **10** (0.42 g, 1.21 mmol) and trichloroacetimidate donor **3** (1.09 g, 2.21 mmol) was achieved using the similar condition as that described for preparing compound **13** to

give β-linked disaccharide derivative (0.59 g, 72%). ^{1}H NMR (600Hz, $CDCl_3$) δ 5.99 (d, 1 H, *J* = 1.8 Hz), 5.33 (dd, 1 H, *J* = 8.4 Hz and 3.6 Hz), 5.19 (dd, 1 H, *J* = 3.6 Hz and 1.8 Hz), 5.14 (t, 1 H, *J* = 9.3Hz), 5.06 (t, 1 H, *J* = 9.6 Hz), 4.91 (dd, 1 H, *J* =9.3 Hz and 8.1 Hz), 4.56 (d, 1 H, *J* = 7.8 Hz), 4.37 (dd, 1 H, *J* = 11.7 Hz and 1.5 Hz), 4.32 (dd, 1 H, *J* = 12.3 Hz and 4.5 Hz), 4.13 (dd, 1 H, *J* = 12.0 Hz and 4.2 Hz), 4.09 (q, 1 H, *J* = 7.2 Hz), 4.01 (dd, 1 H, *J* = 12.0 Hz and 2.4 Hz), 3.93-3.89 (m, 1 H), 3.68-3.65 (m,1 H), 2.14 (s, 3 H), 2.12 (s, 3 H), 2.09 (s, 3 H), 2.06 (s, 3 H), 2.02 (s, 3 H), 2.00 (s, 3 H), 1.99 (s, 3 H), 1.97 (s, 3 H). ^{13}C NMR (75MHz, $CDCl_3$) δ 170.71, 170.61, 170.44, 169.70, 169.66, 169.51, 169.43, 168.50, 101.06, 90.57, 74.21, 73.01, 71.99, 71.84, 71.26, 68.95, 68.82, 68.07, 62.31, 62.00, 21.12, 21.02, 20.95, 20.84, 20.80, 20.76.

The disaccharide derivative (590 mg, 0.87 mmol) was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. Purification of the residue by silica gel (EtOAc:CH_3OH:H_2O = 4:2:1, by volume) afforded target compound **22** (283 mg, 95%). ^{1}HNMR (600Hz, D_2O) δ 5.14 (d, 1 H, *J* = 1.8 Hz), 5.13 (d, 0.4 H, *J* = 1.8Hz), 4.46 (d, 1 H, *J* = 8.4 Hz), 4.45 (d, 0.4 H, *J* = 7.8 Hz), 3.95-3.94 (m, 1.4 H), 3.92-3.87 (m, 6.4 H), 3.48-3.35 (m, 3.2 H), 3.38-3.34 (m, 1.8 H), 3.29-3.25 (m, 1.40 H). ^{13}C NMR (75MHz, D_2O) δ 102.76, 93.89, 76.94, 76.10, 75.57, 73.27, 71.13, 70.37, 69.63, 69.08, 60.76, 60.40.

***N*-(α-D-Galactopyranosyl)oxyacetate-D-mannosamine (23).**

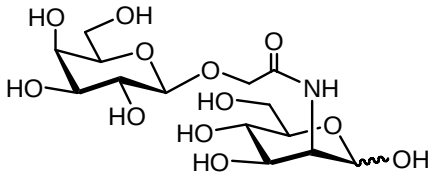


Glycosylation of acceptor **11** (0.26 g, 0.64 mmol) and thiogalactose donor **1** (0.57 g, 0.97 mmol) was achieved using the similar condition as that described for preparing compound **12** to give α-linked disaccharide analogue (0.46 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.22 (m, 25H), 6.10 (d, 1H, *J* = 1.6 Hz), 5.42 (dd, 1H, *J* = 4.4 and 10.0 Hz), 5.23 (t, 1H, *J* = 10.0 Hz), 5.00 (d, 1H, *J* = 12.4 Hz), 4.91 (d, 1H, *J* = 11.2 Hz), 4.84 (d, 1H, *J* = 11.6 Hz), 4.77 (d, 1H, *J* = 11.6 Hz), 4.74 (d, 1H, *J* = 12.4 Hz), 4.69 (m, 1H), 4.61 (d, 1H, *J* = 3.2 Hz), 4.54 (d, 1H, *J* = 11.2 Hz), 4.46 (d, 1H, *J* = 12.0 Hz), 4.37 (d, 1H, *J* = 12.0 Hz), 4.20-3.78 (m, 11H), 3.49 (d, 1H, *J* = 6.8 Hz), 2.19, 2.05, 2.02, 1.96 (s, 4CH_3). ^{13}C NMR (100 MHz): δ 170.72, 170.29, 169.96, 169.67, 168.40, 138.79, 138.75, 138.73, 138.07, 128.73, 128.68, 128.63, 128.58, 128.48, 128.41, 128.16, 128.02, 127.97, 127.86, 127.78, 99.51, 91.90, 78.90, 76.12, 75.05, 74.08, 73.68, 73.34, 70.59, 70.45, 69.24, 68.83, 67.84, 66.27, 62.81, 49.25, 21.17, 21.02, 20.87, 20.79.

The protected disaccharide analogue (410 mg, 0.442 mmol) was dissolved in 22 mL of MeOH:EtOAc:HOAc = 5:5:1 (by volume) and 300 mg of Pd/C was added. The mixture was stirred at H_2 (4 Bar) atmosphere for overnight. When TLC indicited the completion of the reaction, the mixture was filtered and concentrated. The residue was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50

(H^+) resin, and evaporated to dryness. Purification of the residue by Bio-gel P2 column (eluted with water) afforded **23** (0.146 g, 83%). ^{1}H NMR (400 MHz, D_2O) δ 5.09 (d, 0.7 H, *J* = 3.2 Hz), 5.01 (s, 0.3 H), 4.86 (d, 1H, *J* = 3.6 Hz), 4.17-4.01 (m, 2 H), 3.85-3.31 (m, 12 H). ^{13}C NMR (100 MHz, D_2O) δ 172.82, 172.55, 99.19, 99.08, 94.84, 90.91, 76.14, 75.52, 73.83, 71.76, 71.60, 70.82, 70.15, 70.03, 69.41, 69.32, 68.23, 66.49, 66.34, 61.32, 60.88, 60.72, 56.57, 53.95.

***N*-(β-D-Galactopyranosyl)oxyacetate-D-mannosamine (24).**

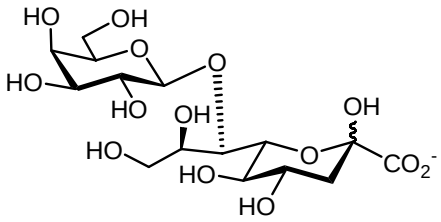


Glycosylation of acceptor **11** (0.17 g, 0.42 mmol) and trichloroacetimidate donor **2** (0.33 g, 0.63 mmol) was achieved using the similar condition as that described for preparing compound **13** to give β-linked disaccharide analogue (0.268 g, 87%). The protected disaccharide analogue (250 mg, 0.34 mmol) was dissolve in dry MeOH (10 mL) and NaOMe (50 mg) was added. The reaction was stirred for overnight, neutralized with Dowex-50 (H^+) resin, and evaporated to dryness. Purification of the residue by Bio-gel P2 column (eluted with water) afforded target compound **24** (127 mg, 94%). ^{1}H NMR (600 MHz, D_2O) δ 5.07 (d, 0.7 H, *J* = 3.6 Hz), 5.02 (s, 0.3 H), 4.37-4.17 (m, 5 H), 3.99-3.26 (m, 10 H). ^{13}C NMR (75 MHz, D_2O): δ 173.02, 172.68, 103.33, 94.87, 90.98, 76.13, 75.53, 73.79, 72.71, 72.10, 71.76, 70.89, 70.76, 70.15, 70.02, 68.73, 68.27, 66.83, 61.85, 61.09, 60.89, 60.72, 56.63, 53.86.

General procedures for aldolase-catalyzed reaction:

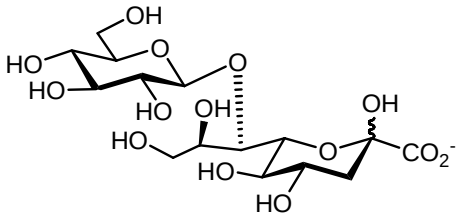
A disaccharide (50 to 100 mg, 1 equiv) and sodium pyruvate (5 equiv) were dissolved in 5 mL H_2O. Tris-HCl buffer (1 mL, 1 M, pH = 7.5) was added followed by the addition of *E. coli* aldolase (5 mg). The reaction solution was brought to 10 mL by adding H_2O. The reaction mixture was incubated at 37 °C with agitation at 140 rpm. The reaction was monitored by thin-layer chromatographic analysis with developing solvent EtOAc:MeOH:H_2O:HOAc = 5:3:2:0.1 (by volume) and stained with *p*-anisaldehyde sugar stain. When no additional products detected (general reaction time is 24 h), an equal volume (10 mL) of 95% EtOH was added to the reaction mixture. The precipitates were separated by centrifugation and discarded. The supernatant was concentrated by rotary evaporation and the residue was purified by a Bio-Gel P-2 gel filtration column.

Sodium β-D-galactopyranosyl-(1→7)-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Galβ1,7KDN, 28).



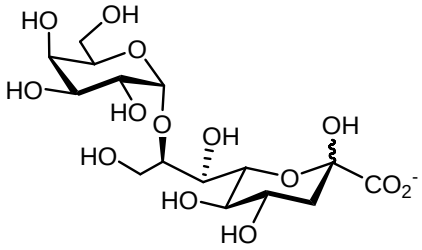
Yield, 38%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.43 (d, 1 H, *J* = 7.8 Hz, H-1'), 4.08 (dd, 1 H, *J* = 8.4 Hz and 1.5 Hz), 3.95-3.89 (m, 4 H), 3.85 (d, 1 H, *J* = 3.0 Hz), 3.78 (t, 1 H, *J* = 9.9 Hz), 3.76-3.61 (m, 5 H), 3.51 (dd, 1 H, *J* = 10.2 Hz and 7.8 Hz), 2.13 (dd, 1 H, *J* = 13.2 Hz and 4.8 Hz, H-3_{eq}), 1.79 (t, 1 H, *J* = 12.6 Hz, H-3_{ax}). ^{13}C NMR (75 MHz, D_2O) δ 176.54, 103.75, 96.58, 77.03, 75.13, 72.94, 71.59, 70.87, 70.48, 69.98, 68.80, 62.38, 61.34, 39.27. HRMS (ESI) calculated for $C_{15}H_{25}O_{14}$ (M-Na), 429.1244, found 429.1227.

Sodium β-D-glucopyranosyl-(1→7)-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Glcβ1,7KDN, 29).



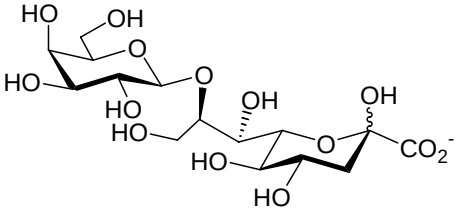
Yield, 35%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.52 (d, 1 H, *J* = 7.8 Hz, H-1'), 4.11 (d, 1 H, *J* = 8.4 Hz), 3.96-3.88 (m, 4 H), 3.49-3.46 (m, 2 H), 3.36-3.30 (m, 2 H), 2.14 (dd, 1 H, *J* = 12.9 Hz and 5.4 Hz, H-3_{eq}), 1.79 (t, 1 H, *J* = 12.9 Hz, H-3_{ax}). ^{13}C NMR (75 MHz, D_2O) δ 181.79, 102.73, 96.63, 76.54, 75.80, 75.71, 73.24, 71.55, 70.42, 70.26, 70.01, 69.70, 68.77, 62.32, 39.16. HRMS (ESI) calcd. for $C_{15}H_{25}O_{14}$ (M-Na), 429.1244, found 429.1238.

Sodium α-D-galactopyranosyl-(1→8)-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Galα1,8KDN, 30).



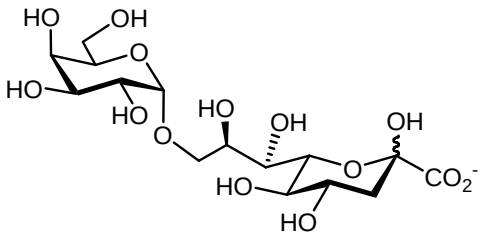
Yield, 62%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.06 (d, 1 H, *J* = 3.6 Hz, H-1'), 4.11-4.07 (m, 2 H), 4.02 (d, 1 H, *J* = 9.6 Hz), 3.98-3.85 (m, 4 H), 3.80-3.69 (m, 4 H), 3.63-3.62 (m, 1 H), 3.59 (t, 1 H, *J* = 9.3 Hz), 2.15 (dd, 1 H, *J* = 12.6 Hz and 5.1 Hz, H-3_{eq}), 1.77 (t, 1 H, *J* = 12.6 Hz, H-3_{ax}). ^{13}C NMR (75 MHz, D_2O) δ 176.92, 100.05, 96.73, 78.85, 71.47, 71.25, 70.59, 69.72, 69.35, 69.22, 68.68, 66.39, 61.46, 61.24, 39.43. HRMS (ESI) calculated for $C_{15}H_{25}O_{14}$ (M-Na), 429.1244, found 429.1253.

Sodium β-D-galactopyranosyl-(1→8)-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Galβ1,8KDN, 31).



Yield, 85%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.48 (d, 1 H, *J* = 7.8 Hz, H-1'), 4.07-3.92 (m, 5 H), 3.86 (d, 1 H, *J* = 3.6 Hz), 3.80 (dd, 1 H, *J* = 12.9 Hz and 3.9 Hz), 3.75-3.68 (m, 2 H), 3.63-3.61 (m, 2 H), 3.57-3.50 (m, 2 H), 2.16 (dd, 1 H, *J* = 12.6 Hz and 4.8 Hz, H-3_{eq}), 1.74 (t, 1 H, *J* = 12.6 Hz, H-3_{ax}). ^{13}C NMR (100 MHz, D_2O) δ 176.99, 102.21, 96.72, 78.28, 75.16, 72.81, 71.33, 71.25, 70.53, 69.34, 68.76, 66.85, 61.10, 60.74, 39.37. HRMS (ESI) calculated for $C_{15}H_{25}O_{14}$ (M-Na), 429.1244, found 429.1255.

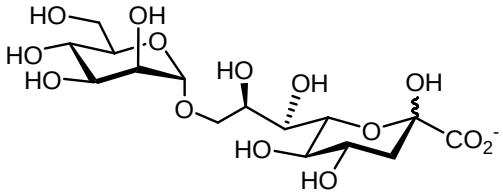
Sodium α-D-galactopyranosyl-(1→9)-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Galα1,9KDN, 32).



Yield, 81%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.94 (d, 1 H, *J* = 3.6 Hz, H-1'), 3.97-3.88 (m,

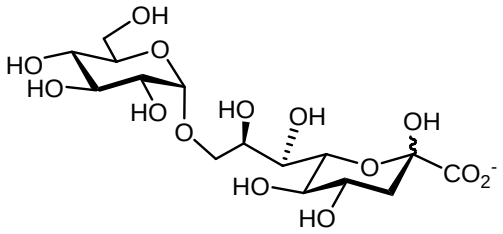
7 H), 3.87 (dd, 1 H, *J* = 10.2 Hz and 3.0 Hz), 3.79 (dd, 1 H, *J* = 10.2 Hz and 3.6 Hz), 3.71-3.63 (m, 5 H), 3.54 (t, 1 H, *J* = 9.6 Hz), 2.14 (dd, 1 H, *J* = 13.2 Hz and 7.2 Hz, H-3_{eq}), 1.73 (t, 1 H, *J* = 13.2 Hz, H-3_{ax}). ^{13}C NMR (75 MHz, D_2O) δ 177.10, 98.47, 96.66, 71.57, 71.02, 70.51, 69.79, 69.61, 69.36, 69.22, 68.94, 68.68, 67.80, 61.27, 39.25. HRMS (ESI) calculated for $C_{15}H_{25}O_{14}$ (M-Na), 429.1244, found 429.1233.

Sodium α-D-mannopyranosyl-(1→9)-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Manα1,9KDN, 33).



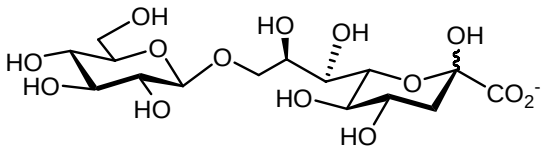
Yield, 78%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.06 (d, 1 H, *J* = 1.8 Hz, H-1'), 4.16 (dd, 1 H, *J* = 6.0 Hz and 1.8 Hz), 4.08 (dd, 1 H, *J* = 4.8 Hz and 1.8 Hz), 4.01 (dd, 1 H, *J* = 10.2 Hz and 1.8 Hz), 3.94-3.61 (m, 9 H), 3.42 (t, 1 H, *J* = 9.6 Hz), 2.15 (dd, 1 H, *J* = 12.9 Hz and 5.1 Hz, H-3_{eq}), 1.80 (d, 1 H, *J* = 12.9 Hz, H-3_{ax}). ^{13}C NMR (75 MHz, D_2O) δ 176.34, 102.97, 96.77, 77.82, 73.94, 72.67, 72.31, 71.24, 70.56, 70.26, 69.62, 66.73, 63.01, 61.00, 39.20. HRMS (ESI) calculated for $C_{15}H_{25}O_{14}$ (M-Na), 429.1244, found 429.1261.

Sodium α-D-glucopyranosyl-(1→9)-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Glcα1,9KDN, 34).



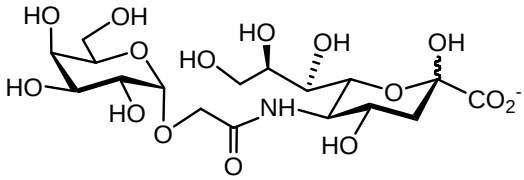
Yield, 65%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.16 (d, 1 H, *J* = 4.2 Hz, H-1'), 4.17 (dd, 1 H, *J* = 4.2 Hz and 1.2 Hz), 3.96-3.87 (m, 3 H), 3.84-3.55 (m, 6 H), 3.49-3.46 (m, 1 H), 3.42 (t, 1 H, *J* = 9.3 Hz), 3.36-3.31 (m, 1 H), 2.16 (dd, 1 H, *J* = 13.2 Hz and 5.1 Hz, H-3_{eq}), 1.78 (t, 1 H, *J* = 13.2 Hz, H-3_{ax}). ^{13}C NMR (75 MHz, D_2O) δ 176.34, 100.47, 96.74, 76.67, 76.19, 74.01, 73.52, 72.76, 72.53, 71.83, 70.43, 69.75, 69.49, 62.83, 39.15. HRMS (ESI) calculated for $C_{15}H_{25}O_{14}$ (M-Na), 429.1244, found 429.1230.

Sodium β-D-glucopyranosyl-(1→9)-3-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Glcβ1,9KDN, 35).



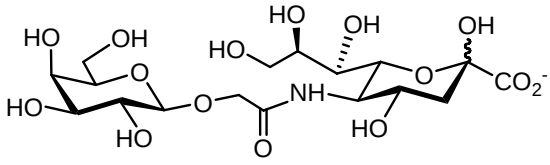
Yield, 83%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.53 (d, 1 H, *J* = 8.4 Hz, H-1'), 4.11 (dd, 1 H, *J* = 8.4 Hz and 0.6 Hz), 3.98-3.91 (m, 4 H), 3.78-3.67 (m, 4 H), 3.50-3.47 (m, 2 H), 3.36 (t, 1 H, *J* = 9.3 Hz), 3.33 (t, 1 H, *J* = 8.7 Hz), 2.15 (dd, 1 H, *J* = 12.6 Hz and 5.1 Hz, H-3_{eq}), 1.81 (t, 1 H, *J* = 12.6 Hz, H-3_{ax}). ^{13}C NMR (75 MHz, D_2O) δ 176.68, 102.87, 96.64, 76.81, 75.88, 75.74, 73.29, 71.59, 70.49, 70.31, 70.09, 69.79, 68.83, 62.39, 39.22. HRMS (ESI) calculated for $C_{15}H_{25}O_{14}$ (M-Na), 429.1244, found 429.1252.

Sodium
5-(((α-D-galactopyranosyl)oxy)acetyl)-5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Galα1,5Neu5Gc, 36).



Yield, 36%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.84 (d, 1H, *J* = 4.2 Hz, H-1'), 4.14 (d, 1H, *J* = 15.6 Hz), 3.99 (d, 1H, *J* = 16.2 Hz), 3.97-3.39 (m, 13H), 2.08 (dd, 1H, *J* = 4.8 and 13.2 Hz, H-3_{eq}), 1.67 (t, 1H, *J* = 12.6 Hz, H-3_{ax}). ^{13}C NMR (100 MHz) δ 176.92, 172.78, 99.10, 96.74, 71.53, 70.74, 70.16, 69.72, 69.31, 69.29, 68.28, 67.07, 66.46, 63.11, 61.31, 52.11, 39.54. HRMS (ESI) calcd. for $C_{17}H_{28}NO_{15}$ (M-Na), 486.1459, found 486.1447.

Sodium
5-(((β-D-galactopyranosyl)oxy)acetyl)-5-deoxy-D-glycero-α-D-galacto-2-nonulopyranosonate (Galβ1,5Neu5Gc, 37).



Yield, 34%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.31 (d, 1H, *J* = 7.8 Hz, H-1'), 4.28 (d, 1H, *J* = 15.6 Hz), 4.16 (d, 1H, *J* = 15.6 Hz), 3.97-3.39 (m, 13H), 2.07 (dd, 1H, *J* = 4.8 and 13.2 Hz, H-3_{eq}), 1.67 (t, 1H, *J* = 12.6 Hz, H-3_{ax}). ^{13}C NMR (100 MHz) δ 176.92, 172.85, 103.16, 96.65, 75.48, 72.60, 70.72, 70.15, 69.72, 68.69, 68.46, 68.29, 67.09, 63.27, 61.10, 52.15, 39.52. HRMS (ESI) calcd. for $C_{17}H_{28}NO_{15}$ (M-Na), 486.1459, found 486.1443.

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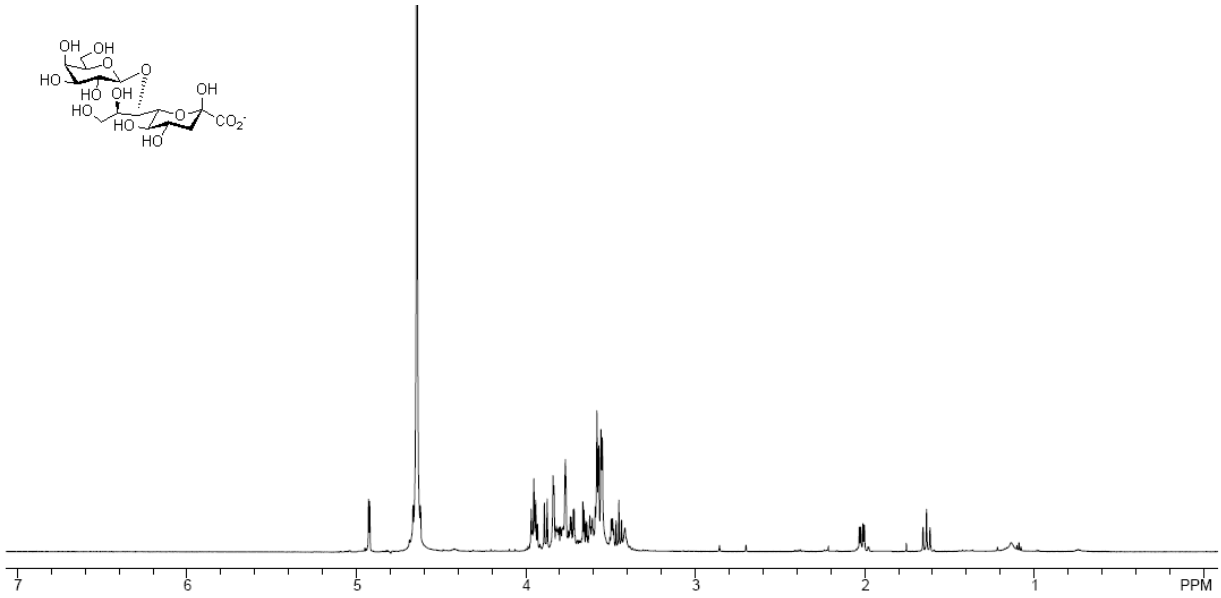
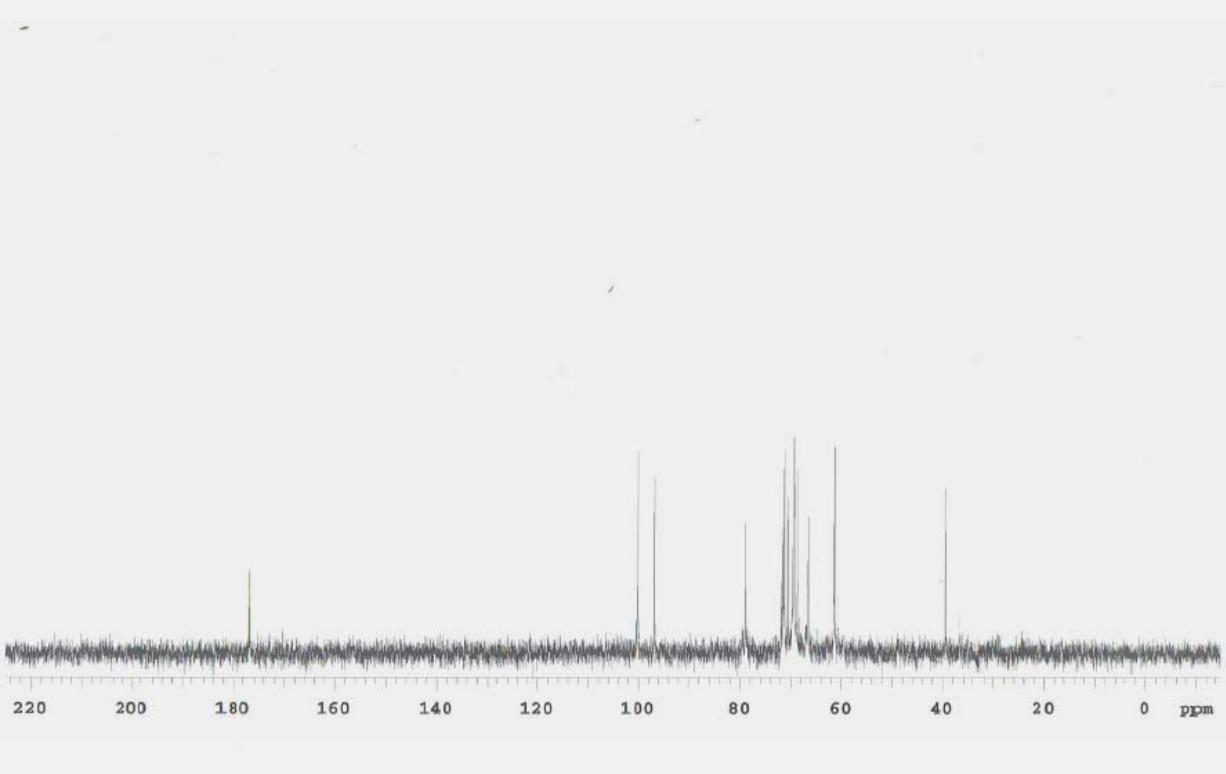
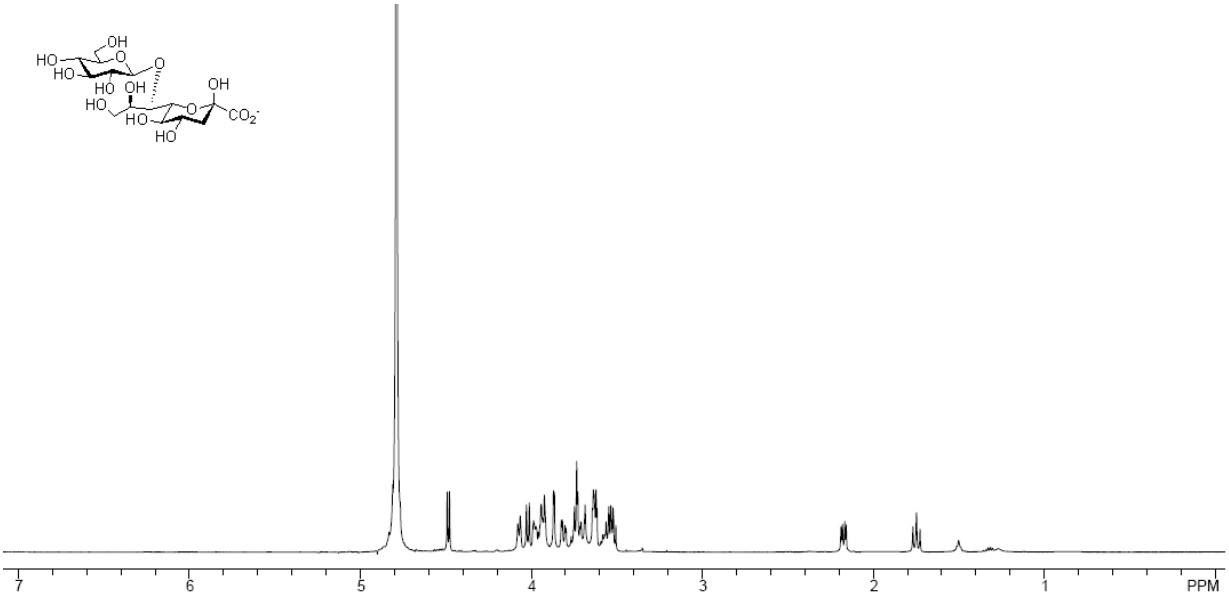
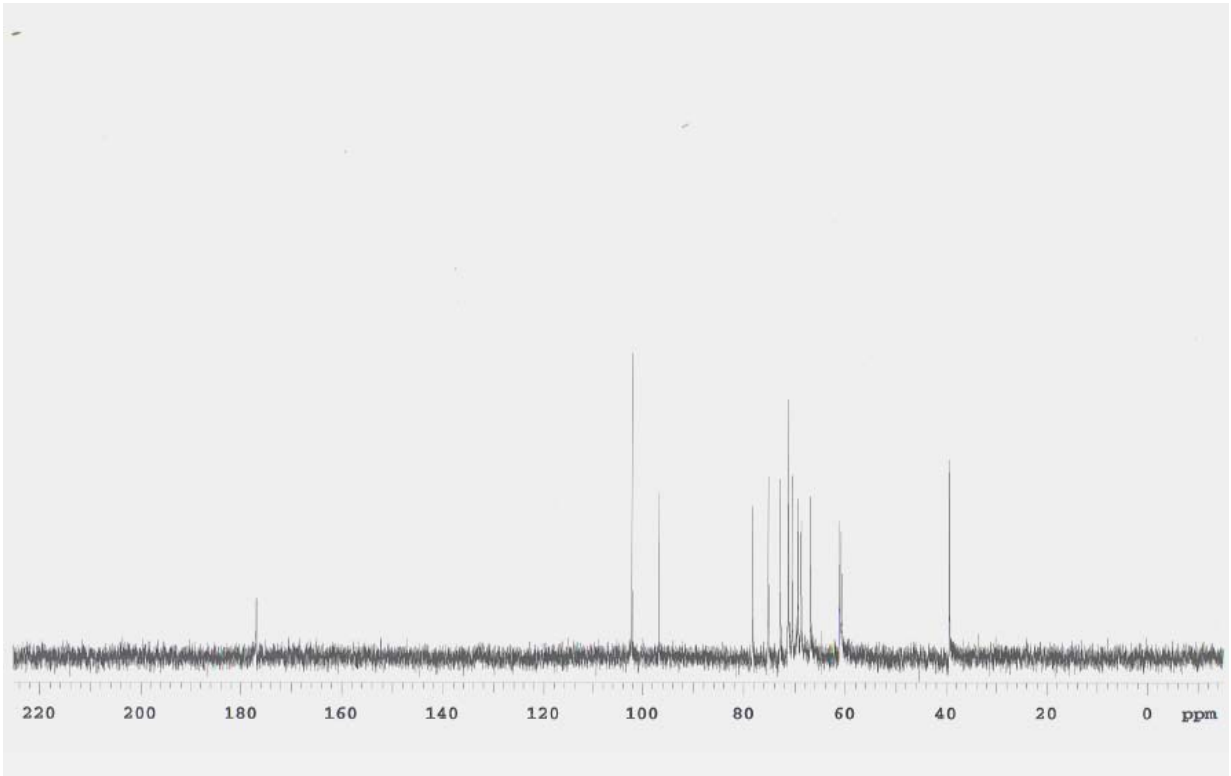
Galβ1,7KDN, 28



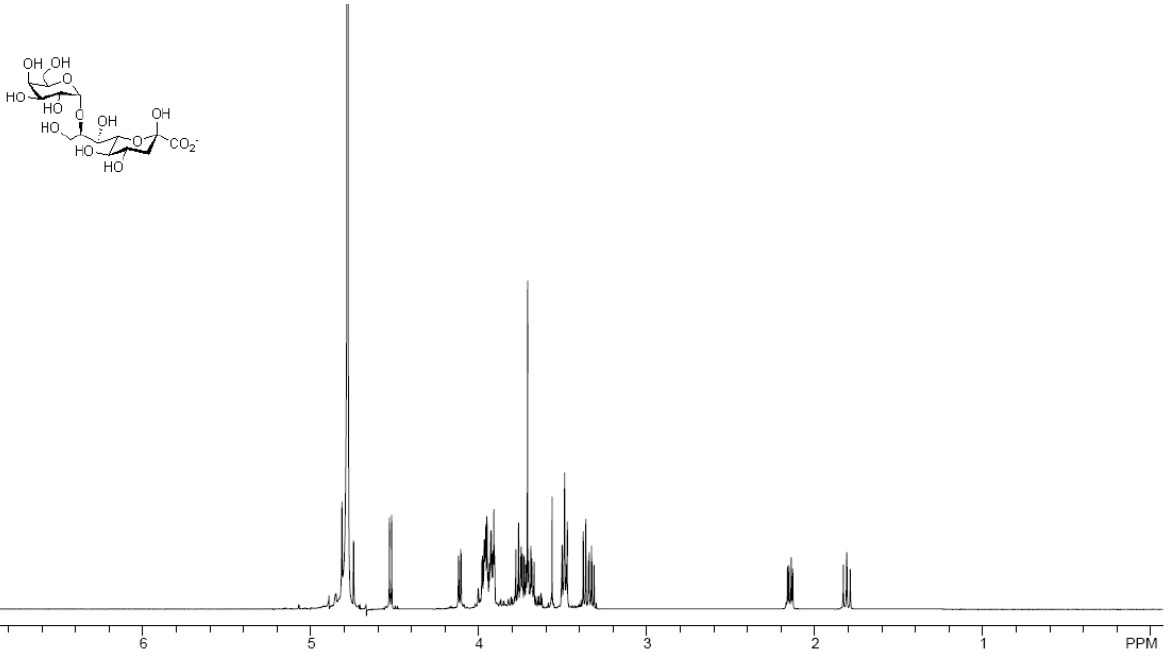


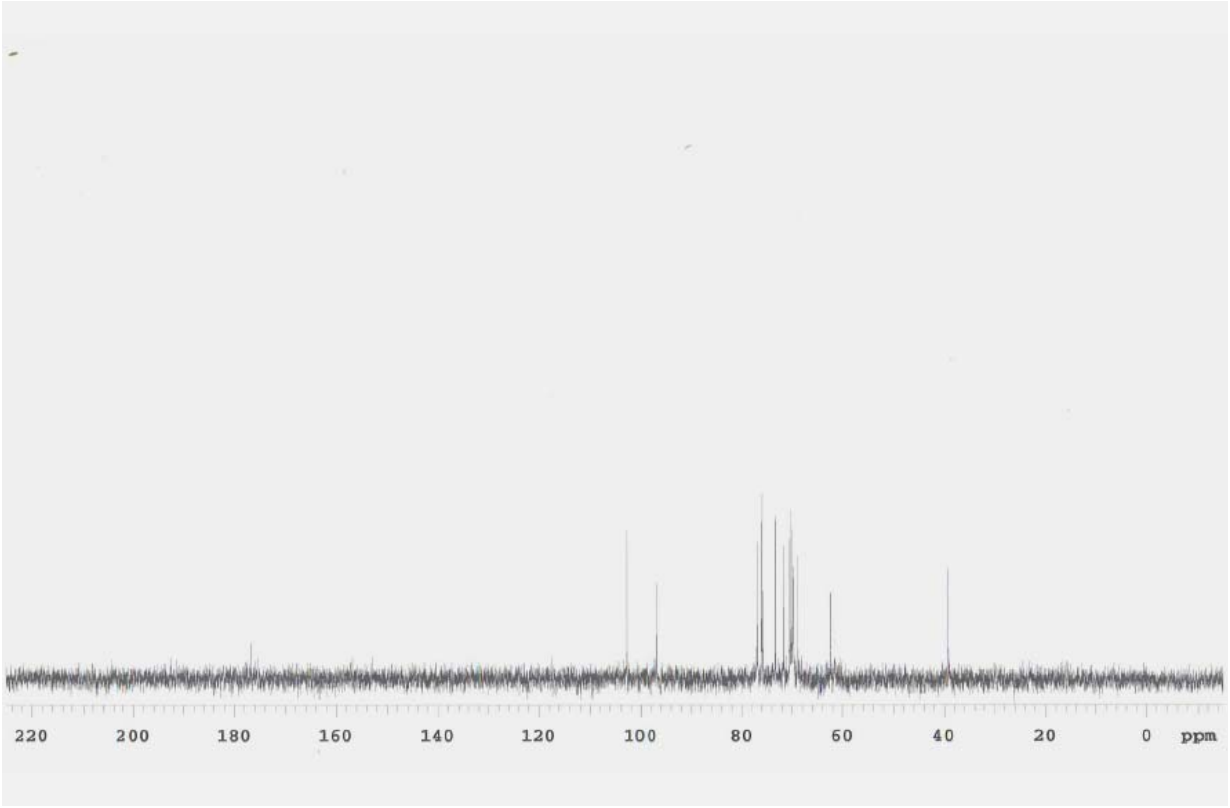
Glcβ1,7KDN, 29



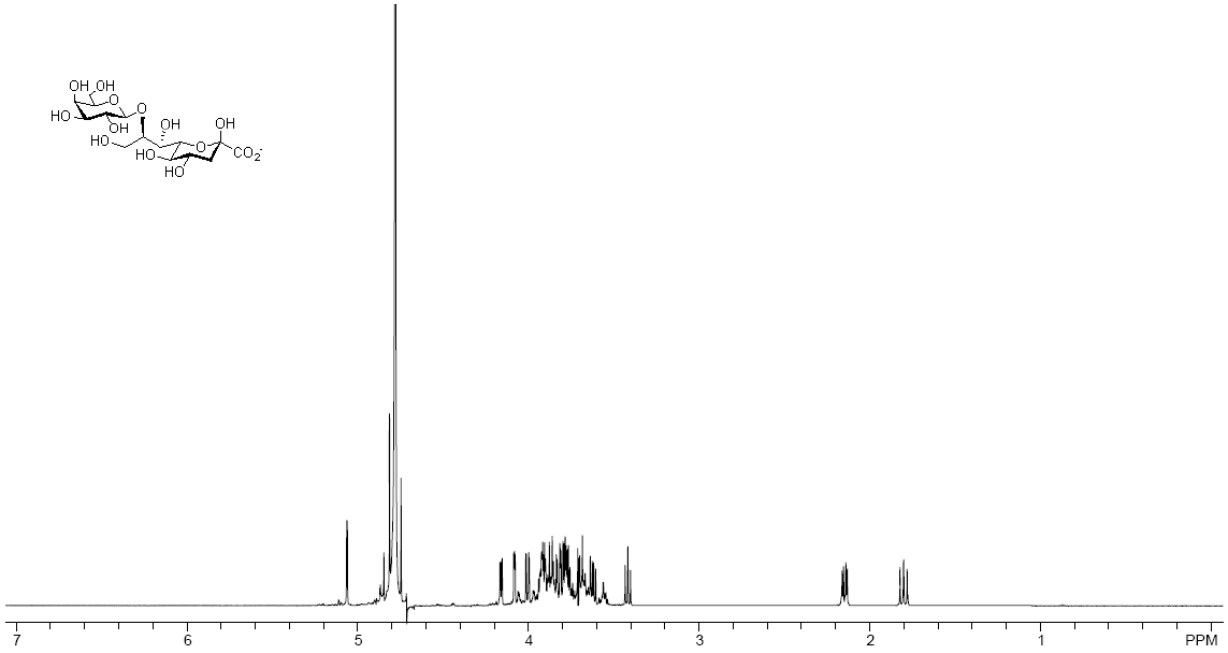


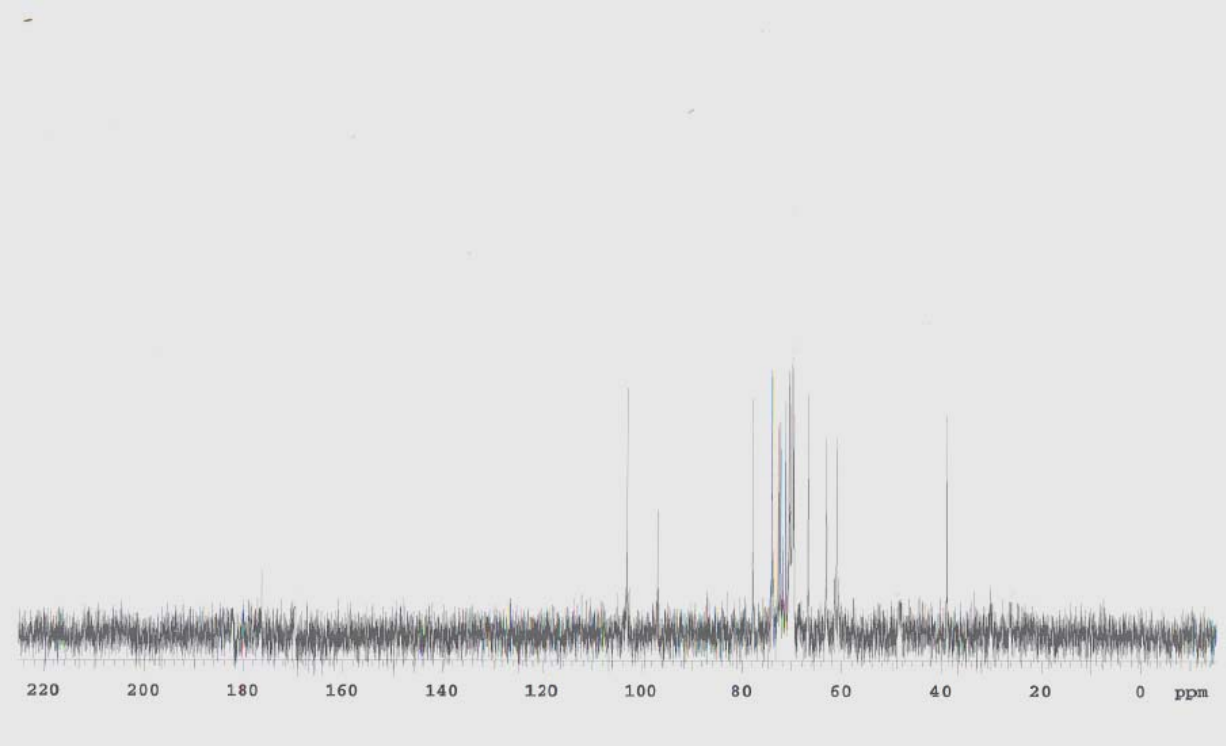
Galα1,8KDN, 30



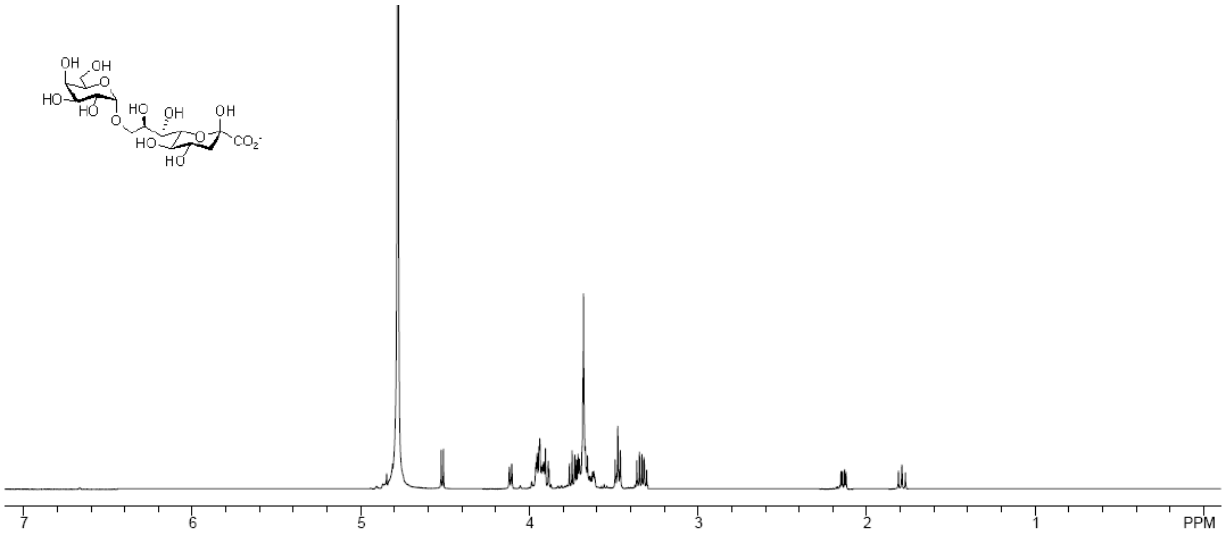


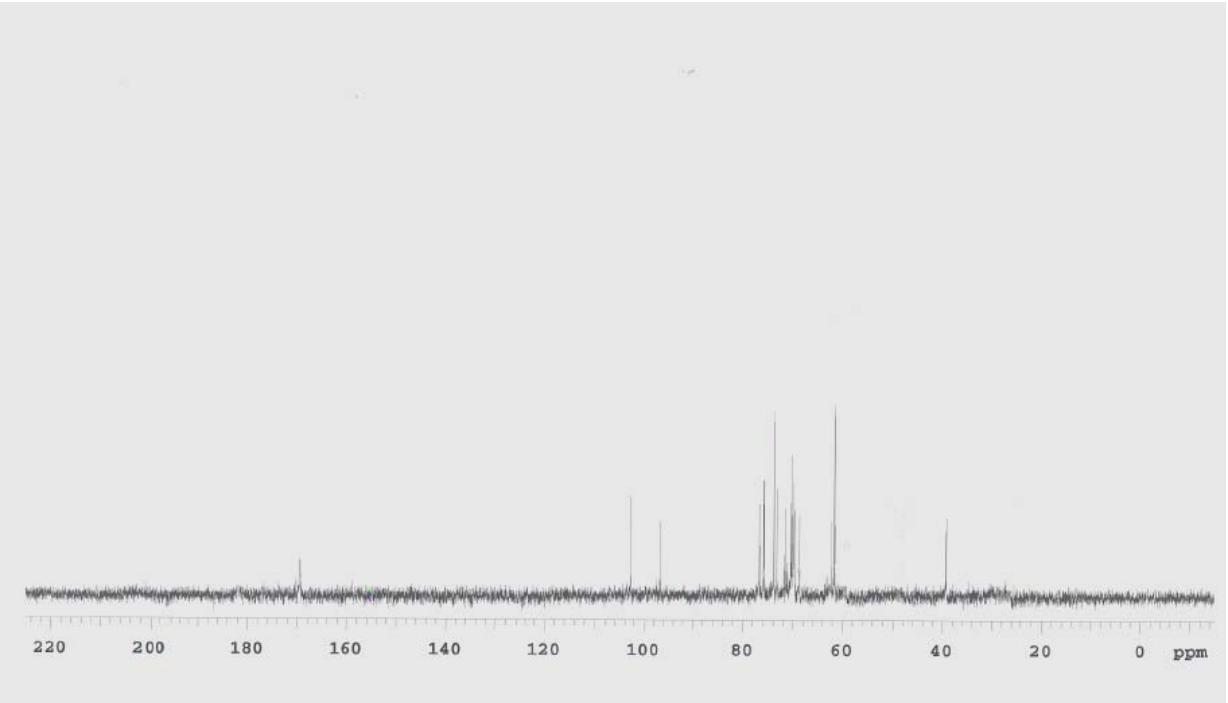
Galβ1,8KDN, 31



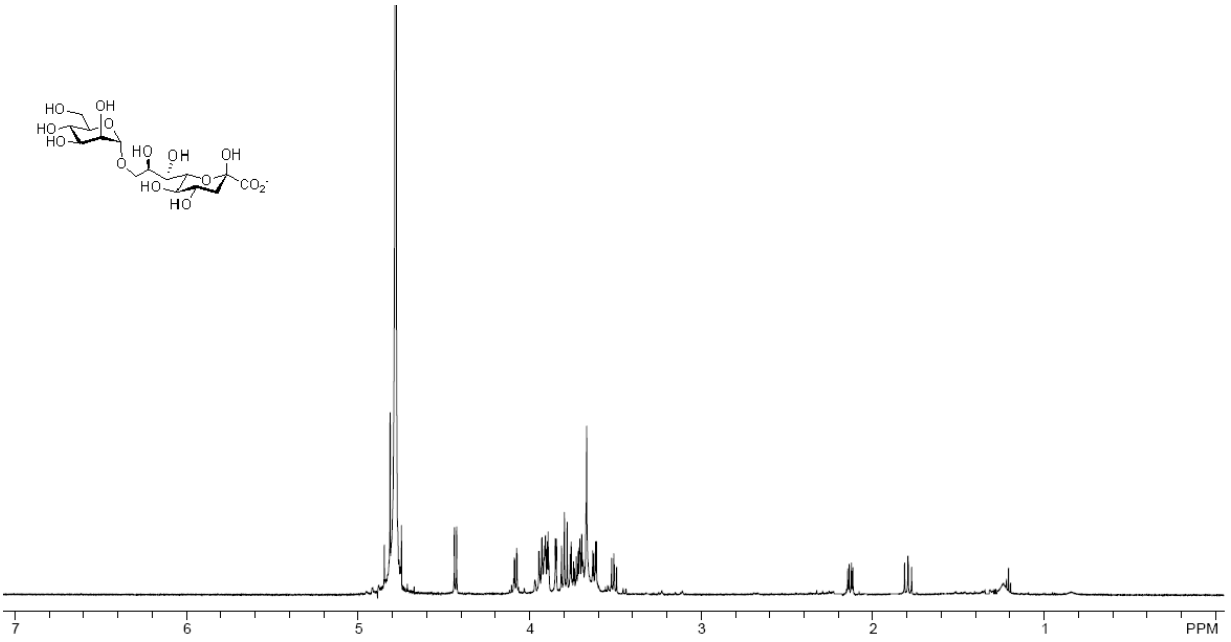


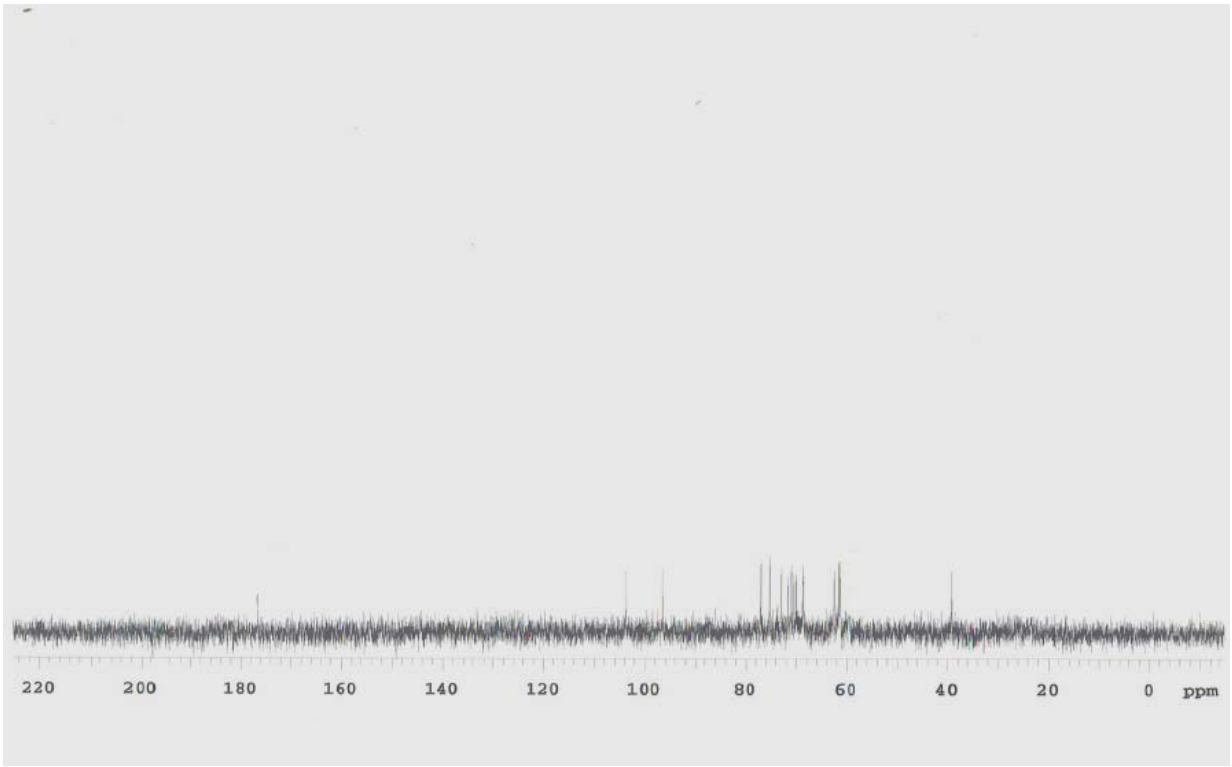
Galα1,9KDN, 32



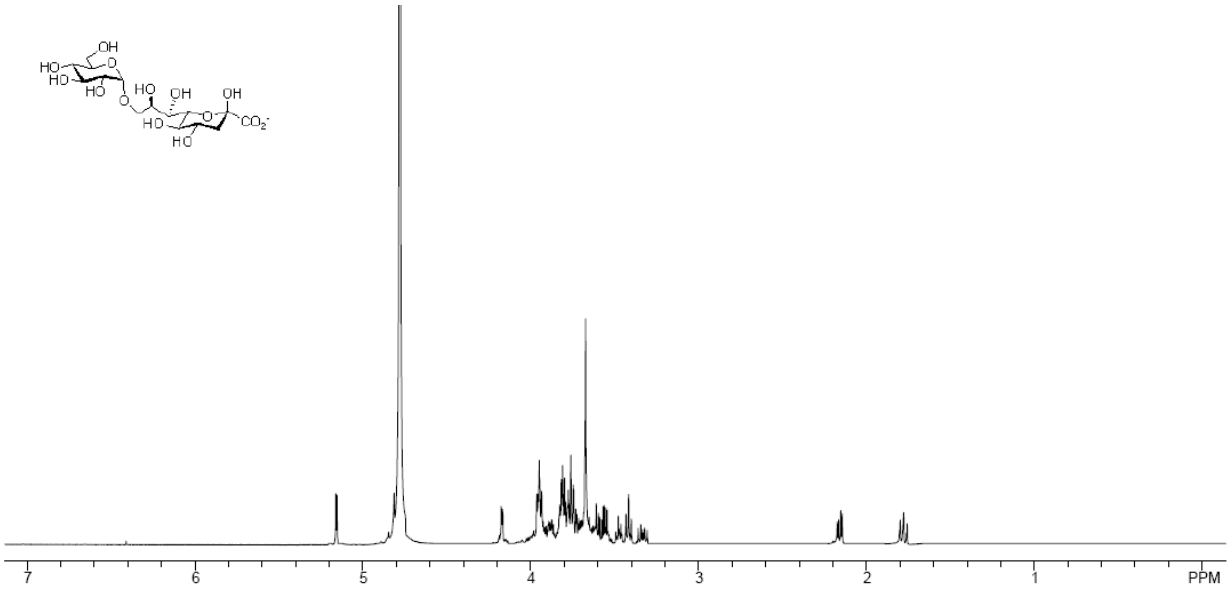


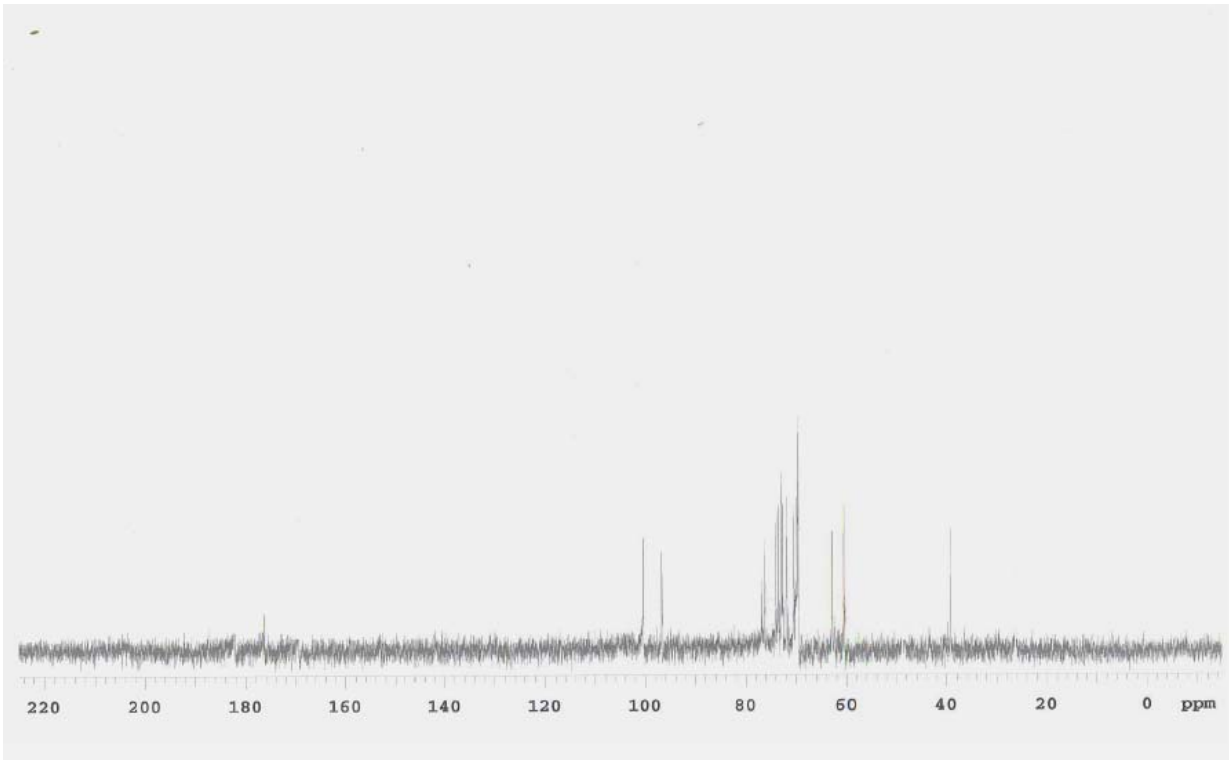
Manα1,9KDN, 33



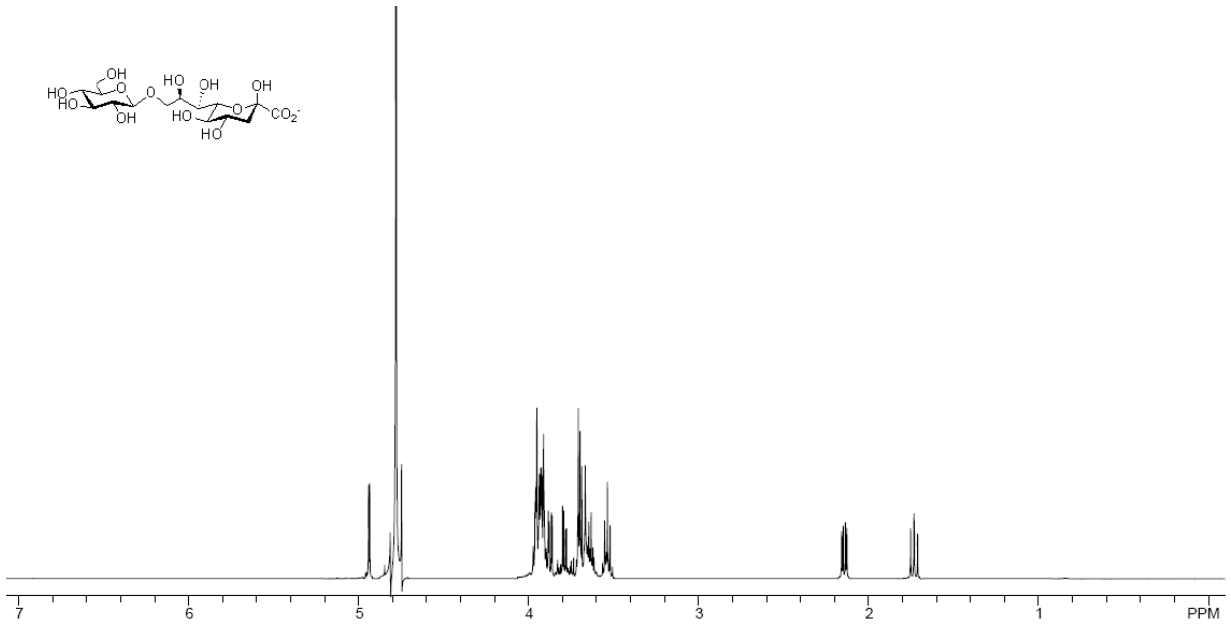


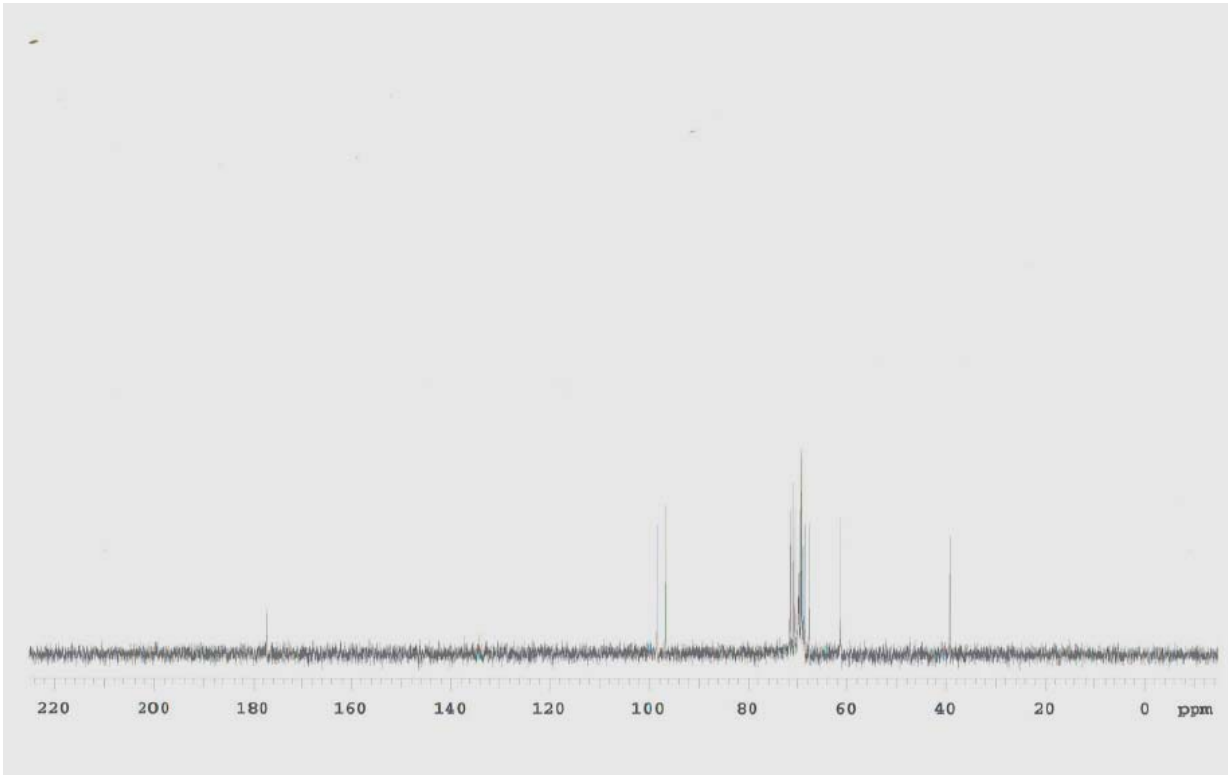
Glcα1,9KDN, 34



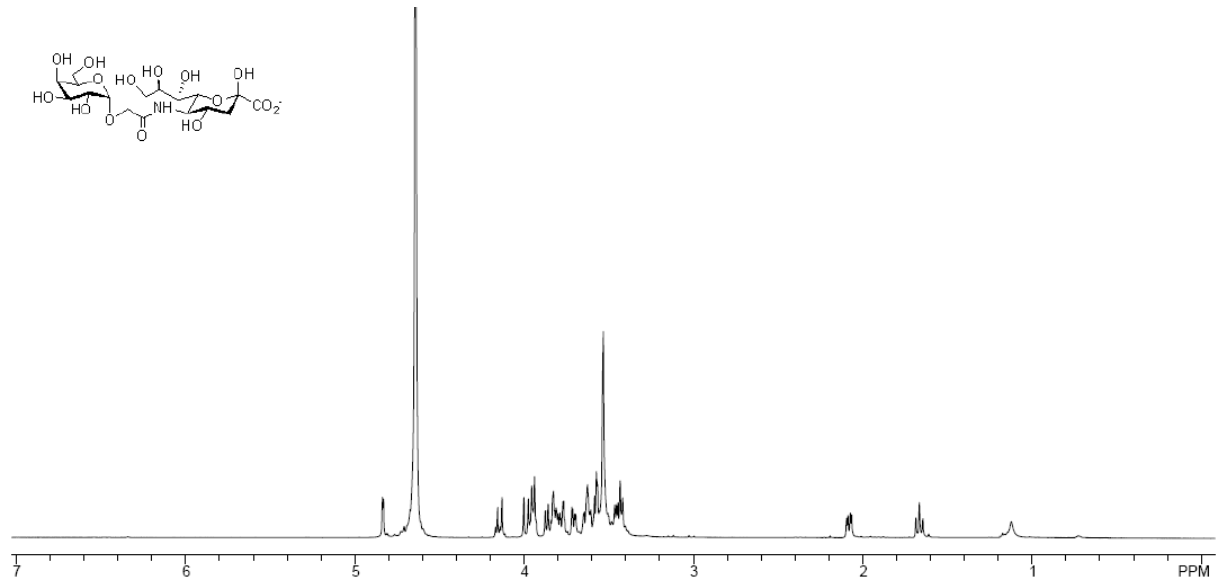


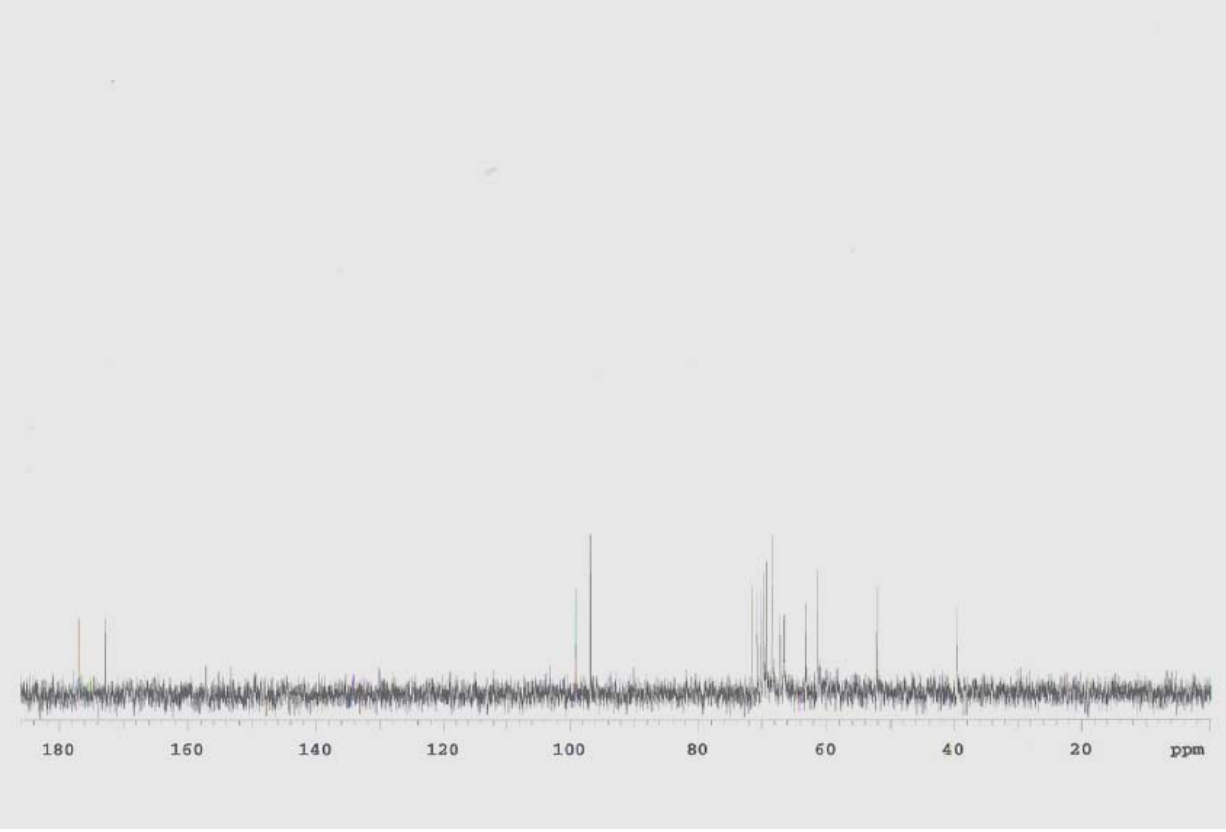
Glcβ1,9KDN, 35





Galα1,5Neu5Gc, 36





Galβ1,5Neu5Gc, 37