Supplementary Information

for

Efficient one-pot multienzyme synthesis of UDP-sugars using a promiscuous UDP-sugar pyrophosphorylase from *Bifidobacterium longum* (BLUSP)

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Cloning, expression, and purification of BLUSP Full length *Bifidobacterium longum* UDP-sugar pyrophosphorylase (EC 2.7.7.64) (BLUSP) (encoded by gene *ugpA*, DNA GenBank accession number: ACHI01000119, locus tag: HMPREF0175_1671; protein GenBank accession number: EEI80102) was cloned from the genomic DNA of *Bifidobacterium longum* strain ATCC55813 in pET15b vector as an N-His_6-tagged fusion protein. The primers used were: forward primer 5' GGAATTC**CATATG**ACAGAAATAAACGATAAGGCC 3' (*Nde*I restriction site is bold and underlined) and reverse primer 5' CGC**GGATCC**TCACACCCAATCGTCCG 3' (*BamH*I restriction site is bold and underlined). The resulting PCR products were digested with restriction enzymes, purified, and ligated with pET15b vector predigested with *Nde*I and *BamH*I restriction enzymes. The ligated product was transformed into electrocompetent *E. coli* DH5α cells. Selected clones were grown for minipreps and positive clones were verified by restriction mapping and DNA sequencing performed by Davis Sequencing Facility. The DNA sequence of the insert matched to *BL0739 (ugpA)* gene in the genomic sequence of *Bifidobacterium longum* NCC2705. Compared to the *BL0739 (ugpA)* gene sequence of *Bifidobacterium longum* NCC2705 (GenBank accession number: AE014295) which was annotated to encoding a hypothetic UTP:glucose-1-phosphate uridylyltransferase (GenBank accession number: AAN24556), there are 4 base differences (T35C, A47G, C228T, A465C) resulting in one amino acid difference (D16G) in the protein sequence of BLUSP.

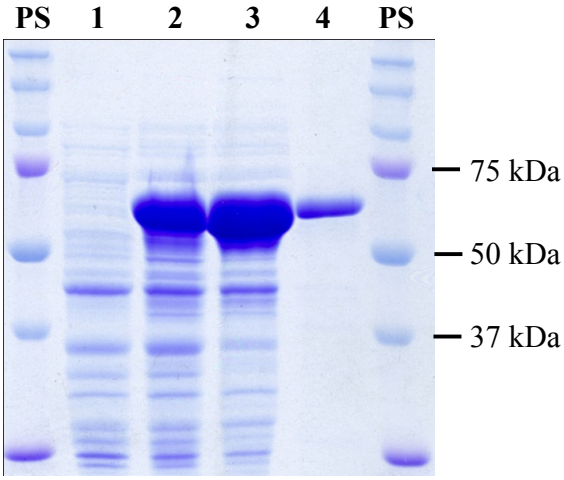
The plasmid was transformed into *E. coli* BL21 (DE3) chemically competent cells for protein expression. *E. coli* cells harboring the pET15b-BLUSP plasmid were cultured in LB medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) with ampicillin (100 μg/mL) at 37 °C with rigorous shaking at 250 rpm in a C25KC incubator shaker (New Brunswick Scientific, Edison, NJ) until the $OD_{600\ nm}$ of the culture reached 0.8–1.0. Overexpression of the targeted proteins was achieved by adding 0.15 mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) followed by incubation at 18 °C for 20 hr with rigorous shaking at 250 rpm.

His_6-tagged protein was purified from cell lysate using Ni^{2+}-NTA affinity column. To obtain cell lysate, cells were harvested by centrifugation at 4,000 rpm (Sorvall) at 4 °C for 2 hr. The cell pellet was resuspended in lysis buffer (pH 8.0, 100 mM Tris-HCl containing 0.1% Triton X-100). Lysozyme (100 μg/mL) and DNaseI (5 μg/mL) were added to the cell suspension. The mixture was incubated at 37 °C for 1 hr with vigorous shaking (200 rpm). Cell lysate was obtained as the supernatant by centrifugation at 11,000 rpm (Sorvall) at 4 °C for 45 min. Purification was performed by loading the supernatant onto a Ni^{2+}-NTA column pre-equilibrated with 10 column volumes of binding buffer (10 mM imidazole, 0.5 M NaCl, 50 mM Tris-HCl, pH 7.5). The column was wash with 10 column volumes of binding buffer and 10 column volumes of washing buffer (40 mM imidazole, 0.5 M NaCl, 50 mM Tris-HCl, pH 7.5). Protein of interest was eluted with Tris-HCl (pH 7.5, 50 mM) containing imidazole (200 mM) and NaCl (0.5 M). The fractions containing the purified enzyme were collected and dialyzed against Tris-HCl buffer (pH 7.5, 25 mM) containing 10% glycerol and 0.25 M NaCl. Dialyzed proteins were stored at 4 °C. Alternatively, fractions containing purified enzyme were dialyzed against Tris-HCl buffer (pH 7.5, 25 mM) and freeze dried. On average, 167 mg of purified protein was obtained from 1 liter of cell culture. Protein concentration was determined in a 96-well plate using bicinchoninic acid with BSA as standard. The absorbance was measured at 562 nm using a plate reader.

SDS-PAGE analysis of BLUSP

SDS-PAGE analysis (Figure S1) shows that the recombinant BLUSP has a very good expression level in *E. coli* and has a high solubility. It consists of about 90% of the total protein extracts from *E. coli* host cells and more than 90% of the soluble protein. The protein size observed is about 60 kDa which is close to 59.7 kDa calculated molecular weight.

Figure S1. SDS-PAGE analysis of BLUSP.



Lanes:
PS: Protein Standards
1. Whole cell extraction, before induction;
2. Whole cell extraction, after induction;
3. Lysate;
4. Purified protein.

pH profile study for BLUSP

Typical enzymatic assays for pH profile studies were carried out for 10 min at 37 °C in a total volume of 20 µL containing Glc-1-P (1 mM), UTP (1 mM), Mg^{2+} (20 mM), and BLUSP (10 ng) in a buffer (100 mM) with pH varying from 3.0 to 9.5. The reaction mixture was quenched by boiling for 5 min followed by adding 20 µL of pre-chilled 95% (v/v) ethanol. The samples were then kept on ice until analyzed by a Beckman Coulter P/ACE MDQ Capillary Electrophoresis system equipped with a UV detector and a 50 cm capillary tubing (75 µm I.D., Beckman Coulter). Assays were run at 25 kV with 25 mM sodium borate buffer (pH 9.8) for 22 min. Percent conversions were calculated from peak areas of UDP-sugar and UTP monitored by UV absorbance at 254 nm. All assays were carried out in duplicate.

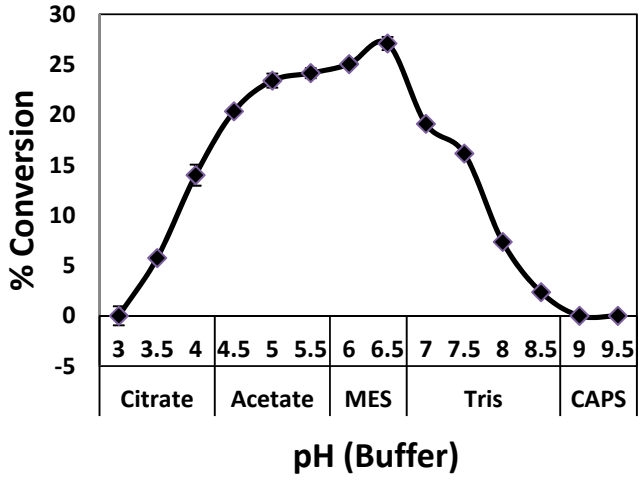
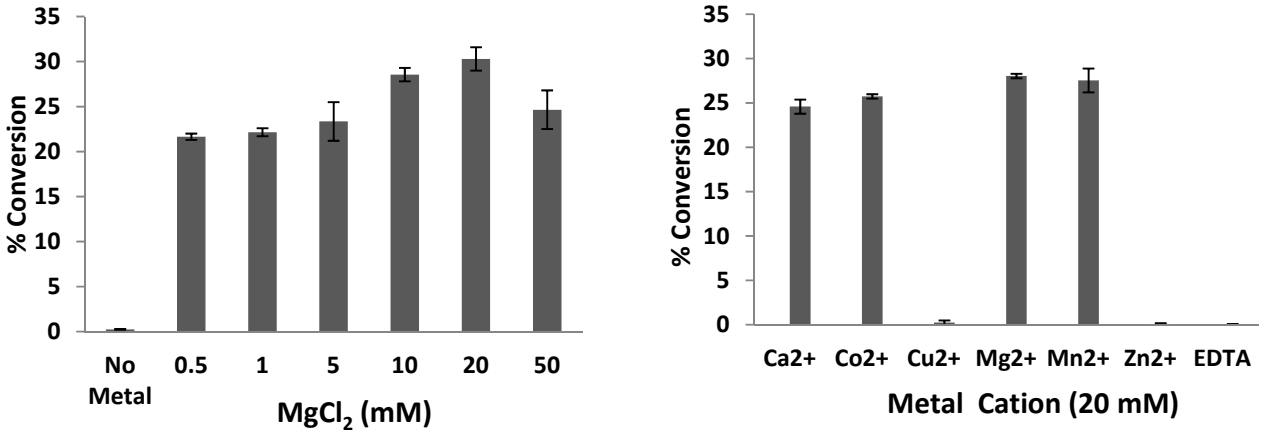


Figure S2. pH profile of BLUSP.

Effects of metal ions and EDTA

EDTA (5 mM), different concentrations (0.5, 1, 5, 10, 20, 50 mM) of $MgCl_2$, and various divalent metal cations ($CaCl_2$, $CoCl_2$, $CuSO_4$, $MnCl_2$, $ZnCl_2$) were used in a MES buffer (pH 6.5, 100 mM) to analyze their effects on the uridylyltransferase activity of BLUSP (10 ng in 20 µL total volume) using Glc-1-P (1 mM) as the acceptor. Other components are the same as those described for the pH profile studies. Reaction without EDTA or metal ions was used as a control.

Figure S3. Metal requirement by BLUSP.



Capillary electrophoresis and TLC assays for kinase reactions

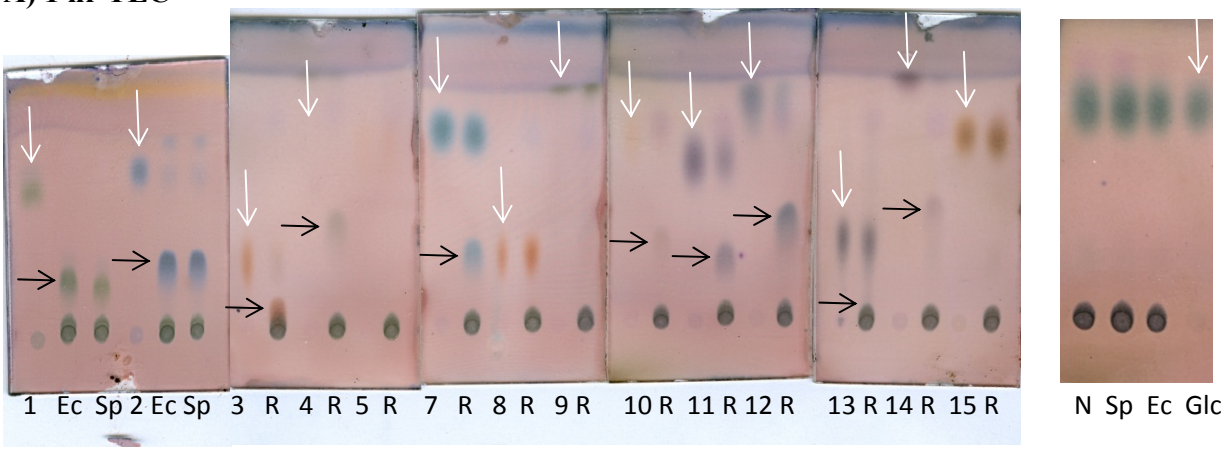
Kinase reactions were carried out at 37 °C in a total volume of 30 µL in Tris-HCl buffer (100 mM, pH 8.0) containing monosaccharide (15 mM), ATP (18 mM, 1.2 eq.), $MgCl_2$ (10 mM), and a kinase (6 µg). These conditions were similar to those used for preparative-scale synthesis. After 1 hr, 4 hr, and 24 hr, an aliquot of 8 µL was withdrawn from each reaction mixture, boiled in a water bath for 5 min and stored at -20 °C until being analyzed by capillary electrophoresis (CE) and TLC. For TLC analysis, 0.5 µL of each sample was directly spotted on TLC plates, developed using suitable developing solvents, and stained with anisaldehyde sugar stain. For CE analysis, 1.5 µL of each sample was diluted into 30 µL and subjected to CE analysis as described above for pH profile studies.

Table S1. Yields of the kinase reactions monitored by the conversion of ATP to ADP in capillary electrophoresis (CE) assays. Abbreviation: NA, not assayed.

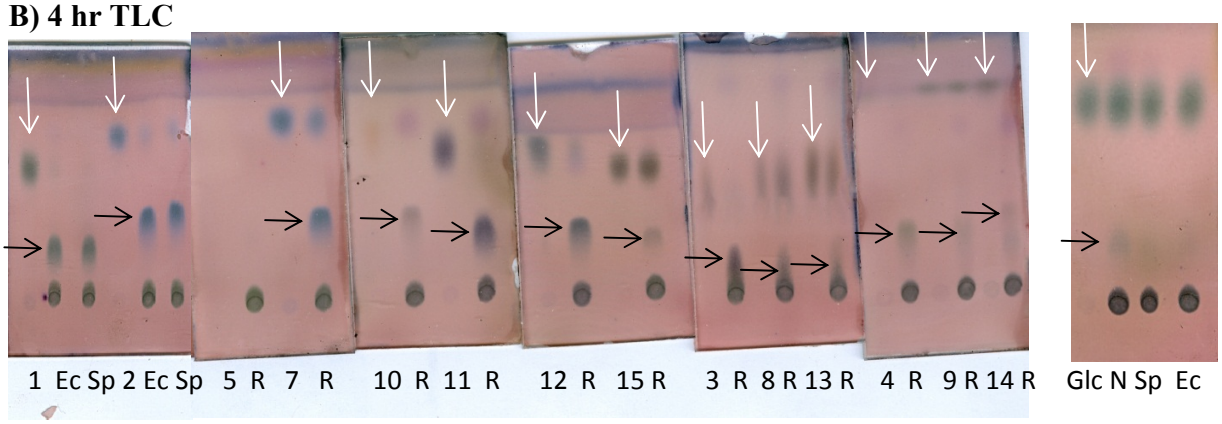
Substrate	Kinase	ATP Conversion (%)		
		1 hr	4 hr	24 hr
No	No	< 2	< 5	11.1
1 Gal	SpGalK	**92.2**	NA	NA
1 Gal	EcGalK	**90.3**	NA	NA
2 2-deoxyGal	SpGalK	80.3	**89.5**	NA
2 2-deoxyGal	EcGalK	78.5	**87.6**	NA
3 GalNH_2	EcGalK	**90.2**	NA	NA
4 GalN_3	SpGalK	45.7	**79.0**	**81.2**
5 GalNAc	SpGalK	11.8	24.7	**69.5**
Glc	EcGalK	8.2	13.0	**66.4**
Glc	SpGalK	6.9	13.8	**75.5**
Glc	NahK	10.3	18.6	**82.2**
7 2-deoxyGlc	NahK	36.8	**69.6**	**79.4**
8 GlcNH_2	NahK	11.9	28.0	**67.1**
9 GlcN_3	NahK	12.4	25.9	**71.2**
10 GlcNAc	NahK	72.6	**84.6**	**85.5**
11 Man	NahK	29.6	**69.3**	**75.1**
12 Man2F	NahK	57.9	**67.9**	**78.2**
13 ManNH_2	NahK	10.3	22.8	**58.0**
14 ManN_3	NahK	34.9	**65.9**	**76.4**
15 ManNAc	NahK	11.4	26.1	**73.8**

Figure S4. Kinase reactions monitored by the formation of monosaccharide-1-phosphates from monosaccharides by thin-layer chromotography (TLC) assays. Developing solvents: EtOAc:MeOH:H_2O:HOAc = 5 : 3 : 3 : 0.3 for reactions using substrates 1, 2, 5, 7, 10, 11, 12 and 15; EtOAc:MeOH:H_2O:HOAc = 3:3:3:0.3 for reactions using substrates 3, 8 and 13; EtOAc : MeOH : H_2O : HOAc = 5 : 2 : 2 : 0.3 for reactions using substrates 4, 9 and 14. #1–15: substrate standards; R: reaction mixtures using the corresponding substrate; Ec: EcGalK; Sp: SpGalK; N: NahK. White vertical arrows point to monosaccharides and black horizontal arrows point to their corresponding sugar-1-phosphate products.

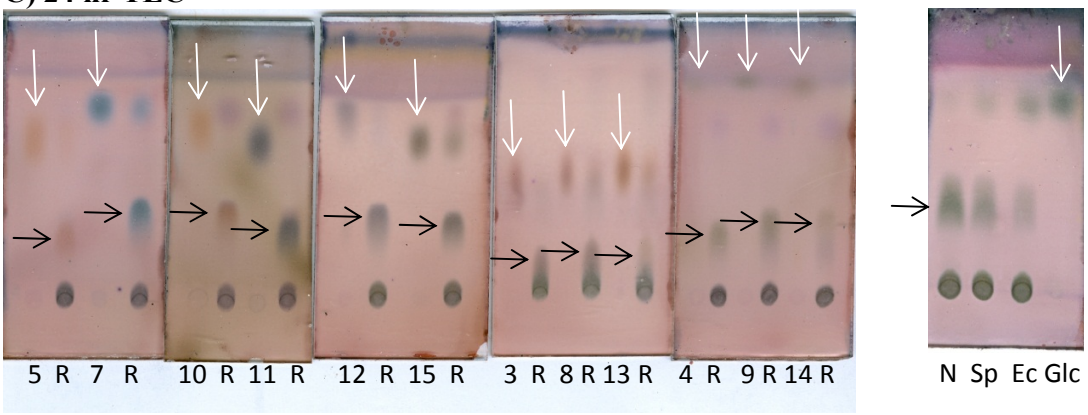
A) 1 hr TLC



B) 4 hr TLC



C) 24 hr TLC



General methods for compound purification and characterization

Chemicals were purchased and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 600 NMR spectrometer. High resolution electrospray ionization (ESI) mass spectra were obtained in negative mode using Thermo Electron LTQ-Orbitrap mass spectrometer. Silica gel 60 Å (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 for detection. Gel filtration chromatography was performed with a column (100 cm × 2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad). $GlcN_3$ (**9**)[1], ManF (**11**)[2, 3], $GalN_3$ (**4**)[4] and $ManN_3$ (**14**)[4] were previously synthesized using reported methods. NahK_ATCC15697,[5] EcGalK,[6] SpGalK,[7] and PmPpA[1] were overexpressed as reported previously.

One-pot multienzyme synthesis of UDP-sugar nucleotides

Monosaccharides and derivatives (30–100 mg, 1.0 eq.), ATP (1.2 eq.), and UTP (1.3 eq.) were dissolved in water in a 15 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.0) and $MgCl_2$ (10 mM). After the addition of appropriate amount of NahK_ATCC15697,[5] EcGalK,[6] or SpGalK (1.3–4.5 mg),[7] BLUSP (1.0–2.5 mg), and PmPpA (1.5–2.5 mg), millipore water was added to bring the total volume of the reaction mixture to 10 mL. The reaction was carried out by incubating the solution in an isotherm incubator for 24 hr at 37 °C with gentle shaking or without shaking. In the synthesis of UDP-Glc, commercially available Glc-1-P (55.2 mg), UTP (1.2 eq.), Tris-HCl buffer (100 mM, pH 8.0), and $MgCl_2$ (10 mM) were used along with BLUSP (1 mg) and PmPpA (1.5 mg). The reaction was left for 2 hr at 37 °C in isotherm with gentle shaking. Product formation was monitored by TLC (EtOAc:MeOH:H_2O:AcOH = 5:3:3:0.3 by volume) with *p*-anisaldehyde sugar staining. The reaction was terminated by adding the same volume of ice-cold ethanol and incubating at 4 °C for 30 min followed by centrifugation remove the enzymes. The supernatant was collected and concentrated and passed through a BioGel P-2 gel filtration column to afford the product. Silica gel column purification (EtOAc:MeOH:H_2O = 7:3:2) was applied when necessary to achieve further purification.

Uridine 5′-diphospho-α-D-galactopyranoside (UDP-Gal, 16). 135 mg. Yield, 86%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.93 (d, J = 8.4 Hz, 1H), 5.97–5.95 (m, 2H), 5.63 (dd, J = 7.2, 3.6 Hz, 1H), 4.37–4.35 (m, 2H), 4.28–4.18 (m, 3H), 4.16 (t, J = 6 Hz, 1H), 4.02 (d, J = 3 Hz, 1H), 3.90 (dd, J = 10.2, 3.6 Hz, 1H), 3.80 (dt, J = 10.2, 3.3 Hz, 1H), 3.76–3.71 (m, 2H). ^{13}C NMR (150 MHz, D_2O) δ 166.39, 151.96, 141.78, 102.80, 96.01 (d, J = 6.6 Hz), 88.65, 83.32 (d, J = 8.9 Hz), 73.93, 72.11, 69.78, 69.43, 69.24, 68.50 (d, J = 7.8 Hz), 65.15 (d, J = 5.0 Hz), 61.16. HRMS (ESI) *m/z* calcd for $C_{15}H_{24}N_2O_{17}P_2$ (M-H) 565.0472, found 565.0453.

Uridine 5′-diphospho-α-D-glucopyranoside (UDP-Glc, 21). 82 mg. Yield, 99%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.94 (d, J = 8.4 Hz, 1H), 5.98–5.96 (m, 2H), 5.59 (dd, J = 7.2, 3.6 Hz, 1H), 4.37–4.35 (m, 2H), 4.28–4.18 (m, 3H), 3.9–3.83 (m, 2H), 3.78–3.74 (m, 2H), 3.53 (dt, J = 9.6, 3.3 Hz, 1H), 3.46 (t, J = 9.6 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 166.20, 151.75, 141.52, 102.57, 95.51(d, J = 6.8 Hz), 88.35, 83.07 (d, J = 8.9 Hz), 73.67, 72.72 (2C), 71.45 (d, J = 8.4 Hz), 69.52, 69.05, 64.86 (d, J = 5.6 Hz), 60.20. HRMS (ESI) *m/z* calcd for $C_{15}H_{24}N_2O_{17}P_2$ (M-H) 565.0472, found 565.0458.

Uridine 5′-diphospho-2-deoxy-α-D-glucopyranoside (UDP-2-deoxyGlc, 22). 96 mg. Yield, 56%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.95 (d, *J* = 8.4 Hz, 1H), 5.96–5.95 (m, 2H), 5.70 (dd, *J* = 7.2, 1.8 Hz, 1H), 4.36–4.33 (m, 2H), 4.27–4.16 (m, 3H), 4.0–3.95 (m, 1H), 3.86–3.75 (m, 3H), 3.39 (t, *J* = 9.6 Hz, 1H), 2.28–2.24(m, 1H), 1.74–1.68 (m, 1H), (150 MHz, D_2O) δ 168.99, 154.54, 144.38, 105.38, 97.63(d, *J* = 5.7 Hz), 91.25, 85.86 (d, *J* = 9.0 Hz), 76.51, 76.13, 73.33, 72.32, 70.46, 67.63 (d, *J* = 5.0 Hz), 63.21, 40.18(d, *J* = 7.2 Hz), HRMS (ESI) *m/z* calcd for $C_{15}H_{24}N_2O_{16}P_2$ (M-H) 549.0523, found 549.0513.

Uridine 5′-diphospho-2-amino-2-deoxy-α-D-glucopyranoside (UDP-GlcNH$_2$, 23). 56 mg. Yield, 43%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.93 (d, *J* = 7.8 Hz, 1H), 5.97–5.94 (m, 2H), 5.82 (d, *J* = 6.0 Hz, 1H), 4.36–4.34 (m, 2H), 4.28–4.17 (m, 3H), 3.92–3.90 (m 2H), 3.86 (dd, *J* = 12.0, 2.4 Hz, 1H), 3.81 (dd, *J* = 12.6, 4.2 Hz, 1H), 3.55 (t, *J* = 9.9 Hz, 1H), 3.37 (d, *J* = 10.8 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 166.40, 151.93, 141.75, 102.71, 92.87, 88.74, 83.21 (d, *J* = 9 Hz), 73.91, 73.39, 69.85, 69.69, 69.16, 65.23, 60.09, 54.27 (d, *J* = 8.4 Hz). HRMS (ESI) *m/z* calcd for $C_{15}H_{25}N_3O_{16}P_2$ (M-H) 564.0632, found 564.0619.

Uridine 5′-diphospho-2-azido-2-deoxy-α-D-glucopyranoside (UDP-GlcN$_3$, 24). 88 mg, Yield, 61%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.95 (d, *J* = 8.4 Hz, 1H), 5.96–5.95 (m, 2H), 5.67 (dd, *J* = 7.2, 3 Hz, 1H), 4.36–4.33 (m, 2H), 4.27–4.18 (m, 3H), 3.93–3.88 (m, 2H), 3.85–3.76 (m, 2H), 3.53 (t, *J* = 9.6 Hz, 1H), 3.38 (d, *J* = 10.8 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 166.14, 151.75, 141.61, 102.79, 94.39 (d, *J* = 4.5 Hz), 88.47, 83.48 (d, *J* = 8.4 Hz), 73.68, 72.85, 70.61, 69.53, 69.24, 64.87, 62.71 (d, *J* = 7.8Hz), 60.05. HRMS (ESI) *m/z* calcd for $C_{15}H_{23}N_5O_{16}P_2$ (M-H) 590.0537, found 590.0524.

Uridine 5′-diphospho-α-D-mannopyranoside (UDP-Man, 26). 60 mg. Yield, 60%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.93 (d, *J* = 8.4 Hz, 1H), 5.96–5.94 (m, 2H), 5.51 (d, *J* = 7.2, 1H), 4.35–4.18 (m, 5H), 4.02 (m, 1H), 3.89–3.82 (m, 3H), 3.75 (dd, *J* = 12, 4.8 Hz, 1H), 3.67 (t, *J* = 9.9 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 166.40, 151.94, 141.77, 102.79, 96.64 (d, *J* = 5.5), 88.70, 83.24 (d, *J* = 8.7 Hz), 73.93, 73.91, 70.38 (d, *J* = 9.3 Hz), 69.98, 69.74, 66.56, 65.15 (d, *J* = 4.7 Hz), 60.92. HRMS (ESI) *m/z* calcd for $C_{15}H_{24}N_2O_{17}P_2$ (M-H) 565.0472, found 565.0467.

Uridine 5′-diphospho-2-fluoro-2-deoxy-α-D-mannopyranoside (UDP-ManF, 27). 142 mg. Yield, 92%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.94 (d, *J* = 8.4 Hz, 1H), 5.97–5.95 (m, 2H), 5.70 (t, *J* = 6.3 Hz, 1H), 4.39–4.35 (m, 2H), 4.36–4.33 (m, 2H), 4.28–4.16 (m, 3H), 4.00 (ddd, *J* = 30.6, 9.6, 2.4 Hz, 1H), 3.88–3.86 (m, 2H), 3.79 (d, *J* = 12.6, 4.8 Hz, 1H), 3.74 (t, *J* = 9.9 Hz, 1H),. ^{13}C NMR (150 MHz, D_2O) δ 166.42, 151.98, 141.80, 102.84, 93.75 (dd, *J* = 31.2, 5.7 Hz), 89.75 (dd, *J* = 173.6, 10.5 Hz), 88.75, 83.26 (d, *J* = 9.0 Hz), 73.95, 73.84, 69.76, 69.32 (d, *J* = 17.3 Hz), 66.46, 65.15 (d, *J* = 5.1 Hz) 60.46. HRMS (ESI) *m/z* calcd for $C_{15}H_{23}FN_2O_{16}P_2$ (M-H) 567.0429, found 567.0426.

Uridine 5′-diphospho-2-azido-2-deoxy-α-D-mannopyranoside (UDP-ManN$_3$, 29). 259 mg, Yield, 90%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.96 (d, *J* = 8.4 Hz, 1H), 6.00–5.98 (m, 2H), 5.62 (d, *J* = 7.2 Hz, 1H), 4.39–4.35 (m, 2H), 4.31–4.18 (m, 3H), 4.16–4.13 (m, 2H), 3.87–3.83 (m, 2H), 3.77 (dd, *J* = 12.6, 4.8 Hz, 1H), 3.70 (t, *J* = 9.6 Hz, 1H),. ^{13}C NMR (150 MHz, D_2O) δ 166.42, 151.97, 141.81, 102.84, 94.86 (d, *J* = 5.7 Hz), 88.80, 83.24 (d, *J* = 8.9 Hz), 73.96, 73.95, 70.09, 69.74, 66.49, 65.16 (d,

J = 5.0 Hz), 64.18 (d, *J* = 9.5 Hz) 60.64. HRMS (ESI) *m/z* calcd for $C_{15}H_{23}N_5O_{16}P_2$ (M-H) 590.0537, found 590.0532.

Uridine 5′-diphospho-2-acetamido-2-deoxy-α-D-mannopyranoside (UDP-ManNAc, 30). Yield for two steps from UDP-$ManN_3$ (**29**), 79%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.96 (d, *J* = 7.8 Hz, 1H), 5.98–5.95 (m, 2H), 5.44 (dd, *J* = 7.8, 1.8 Hz, 1H), 4.43 (dd, *J* = 4.8, 1.8 Hz, 1H), 4.37–4.34 (m, 2H), 4.28–4.22 (m, 2H), 4.19–4.15 (m, 1H), 4.11 (dd, *J* = 10.2, 4.8 Hz, 1H), 3.90 (dt, *J* = 10.2, 3.0 Hz, 1H), 3.85 (d, *J* = 3.6 Hz, 1H), 3.62 (t, *J* = 10.2 Hz, 1H), 2.03 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 175.59, 166.16, 151.75, 141.55, 102.57, 95.35, 88.23, 83.17 (d, *J* = 8.9 Hz), 73.73, 73.20, 69.58, 68.72, 66.29, 64.82, 59.23, 52.94 (d, *J* = 8.9 Hz), 21.85. HRMS (ESI) *m/z* calcd for $C_{17}H_{27}N_3O_{17}P_2$ (M-H) 606.0737, found 606.0723.

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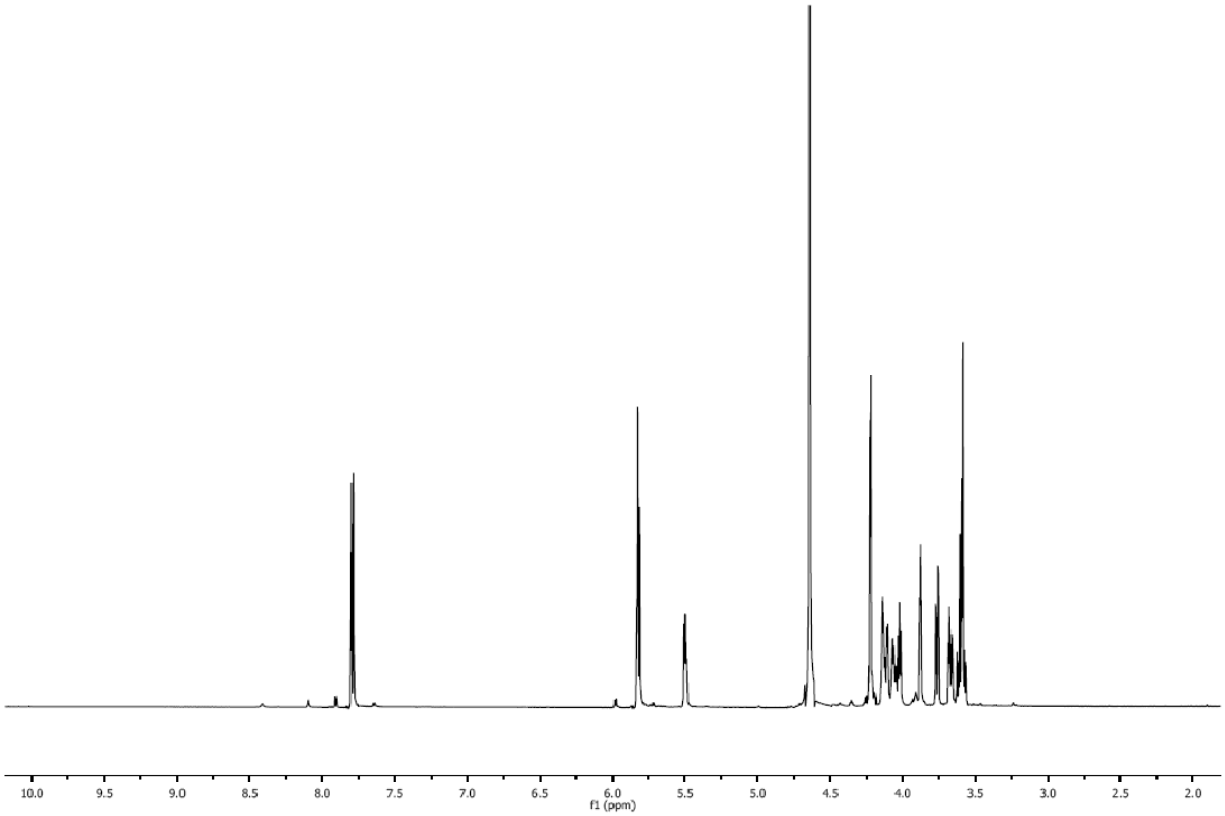
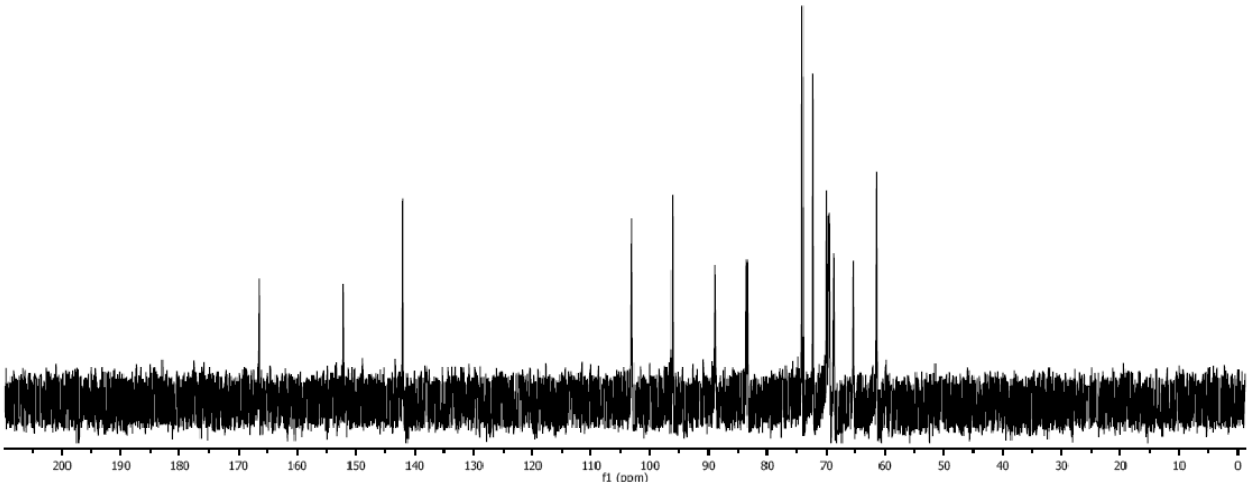
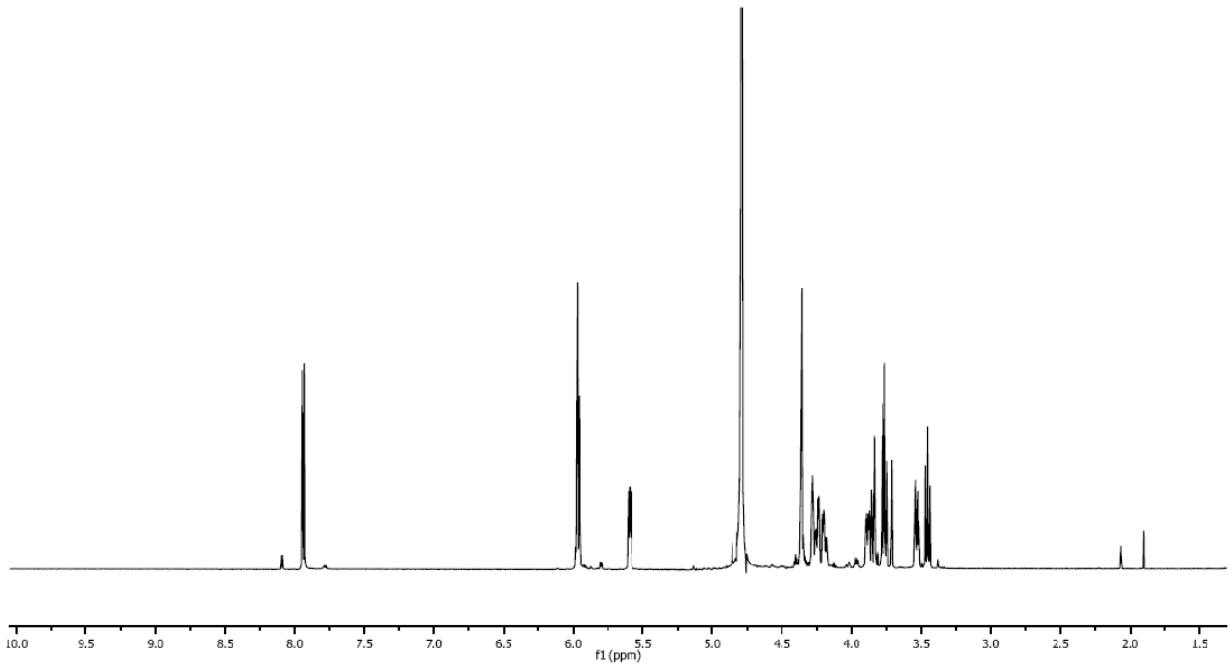
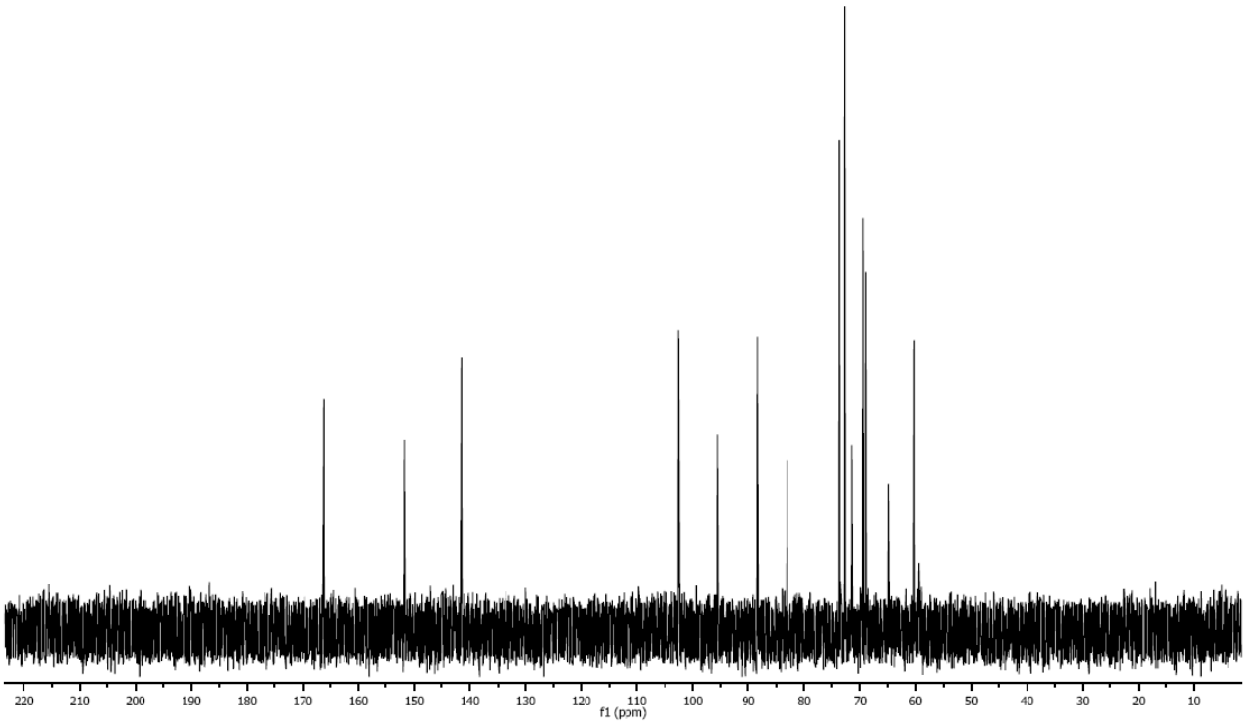
^{1}H & ^{13}C NMR spectra of UDP-Gal **16**



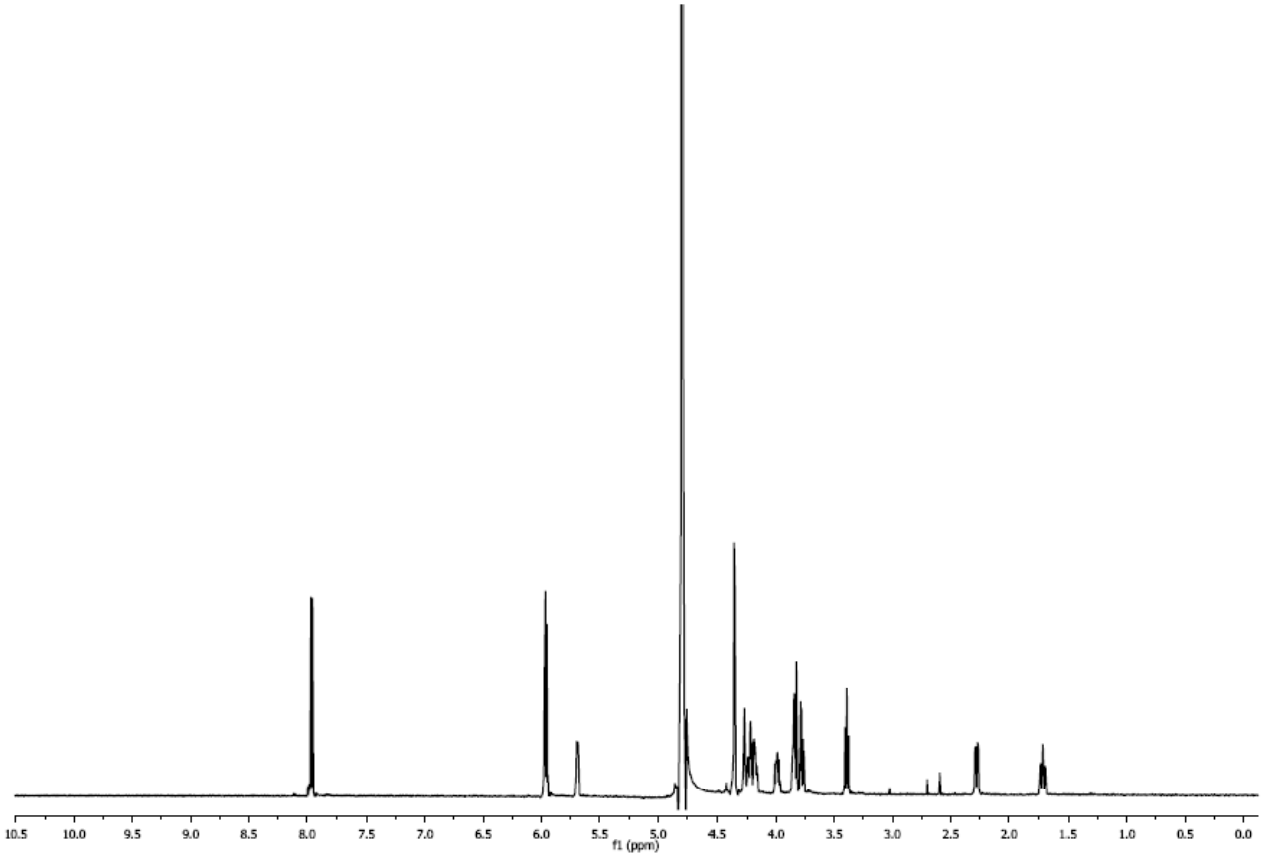


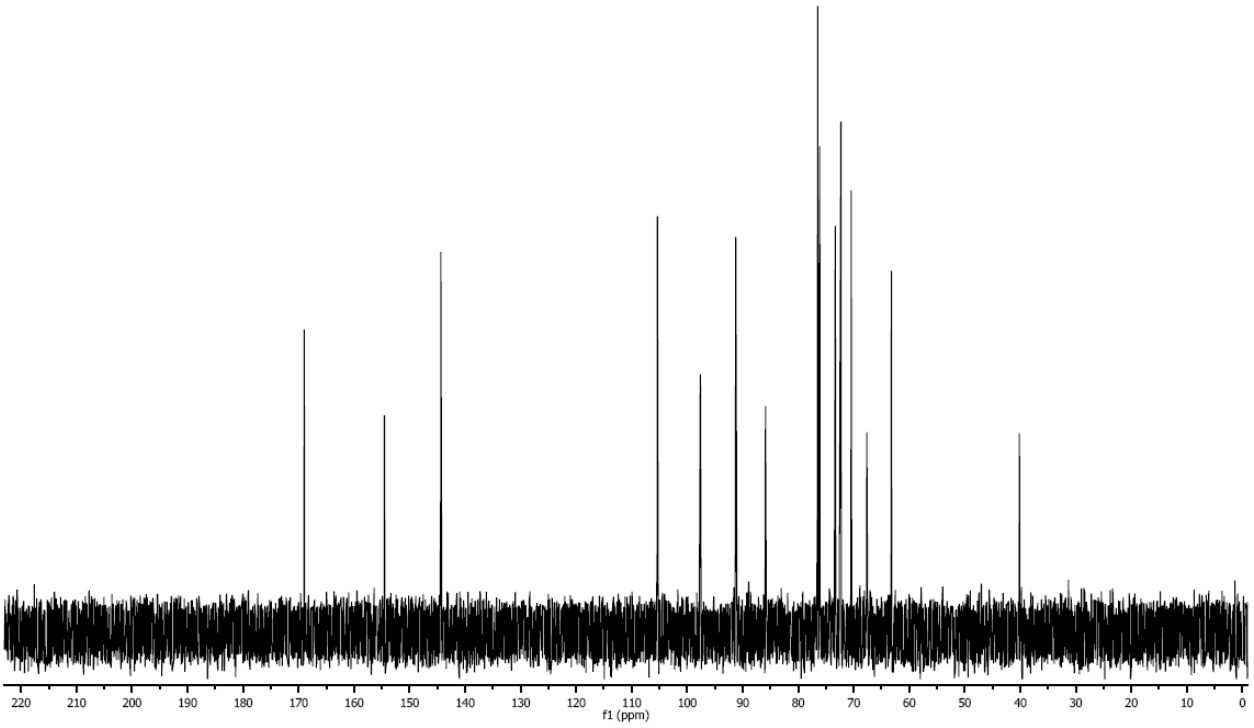
^{1}H & ^{13}C NMR spectra of UDP-Glc **21**



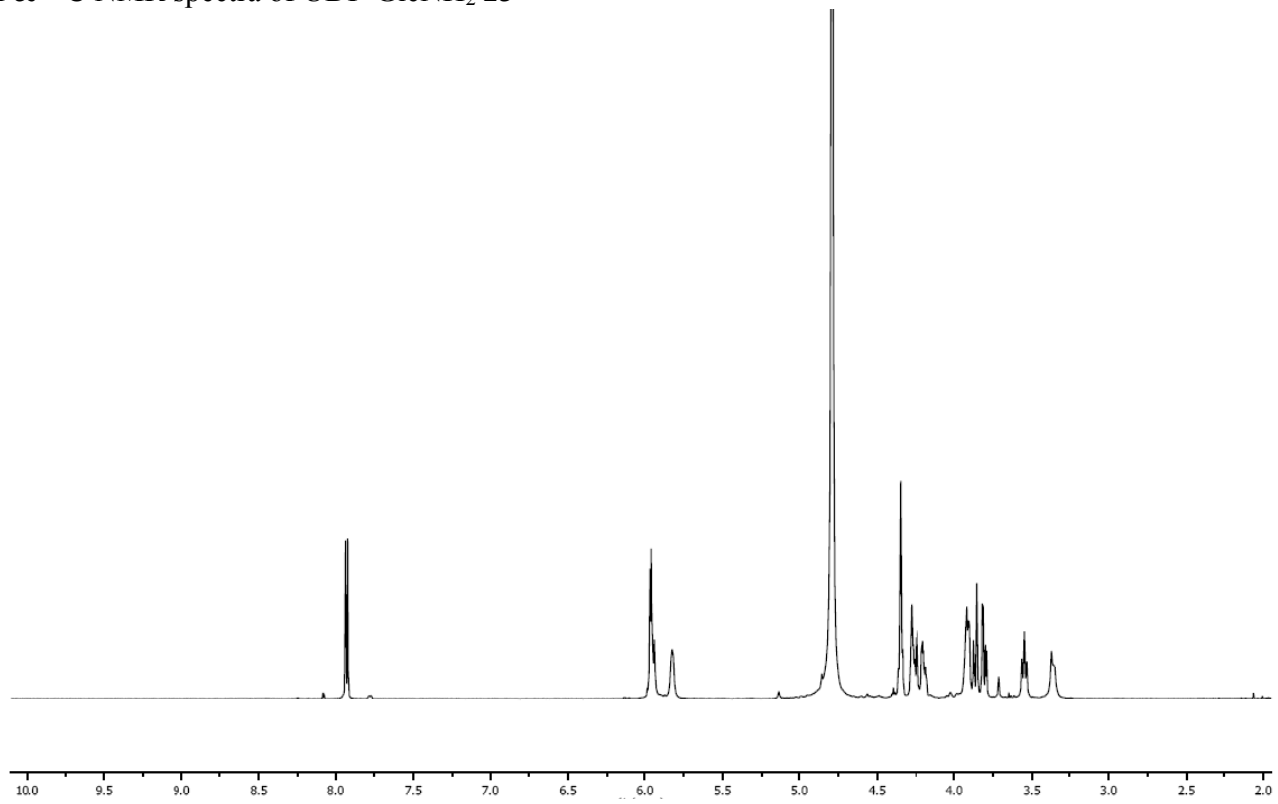


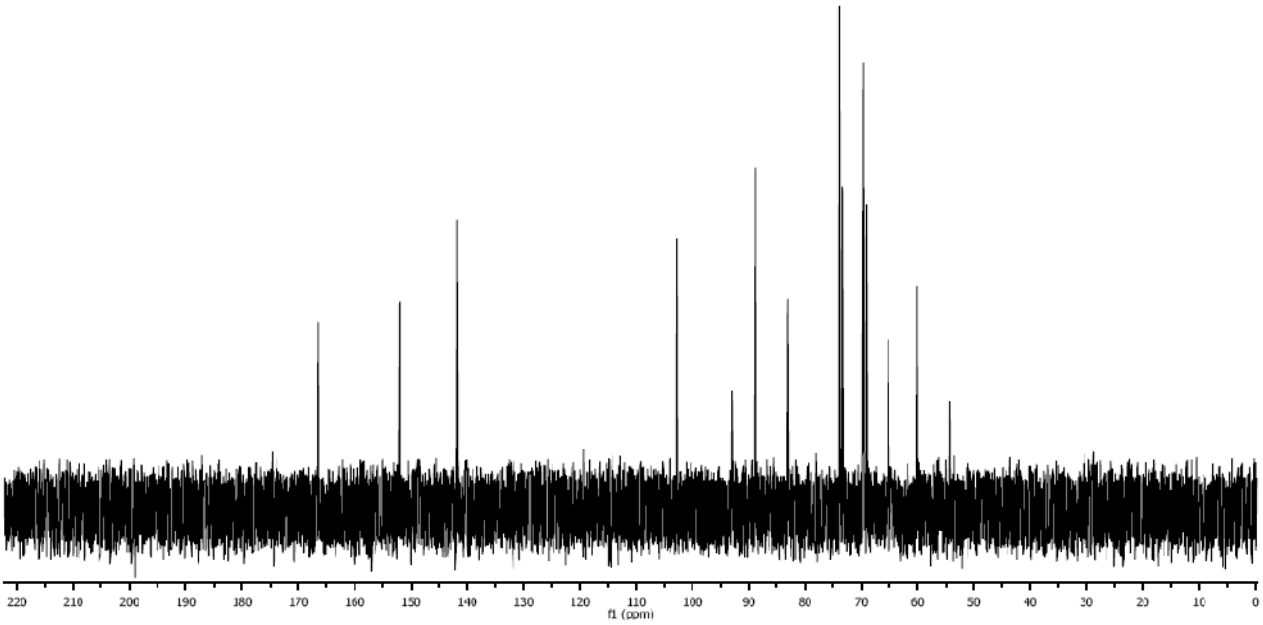
^{1}H & ^{13}C NMR spectra of UDP-2-deoxyGlc **22**



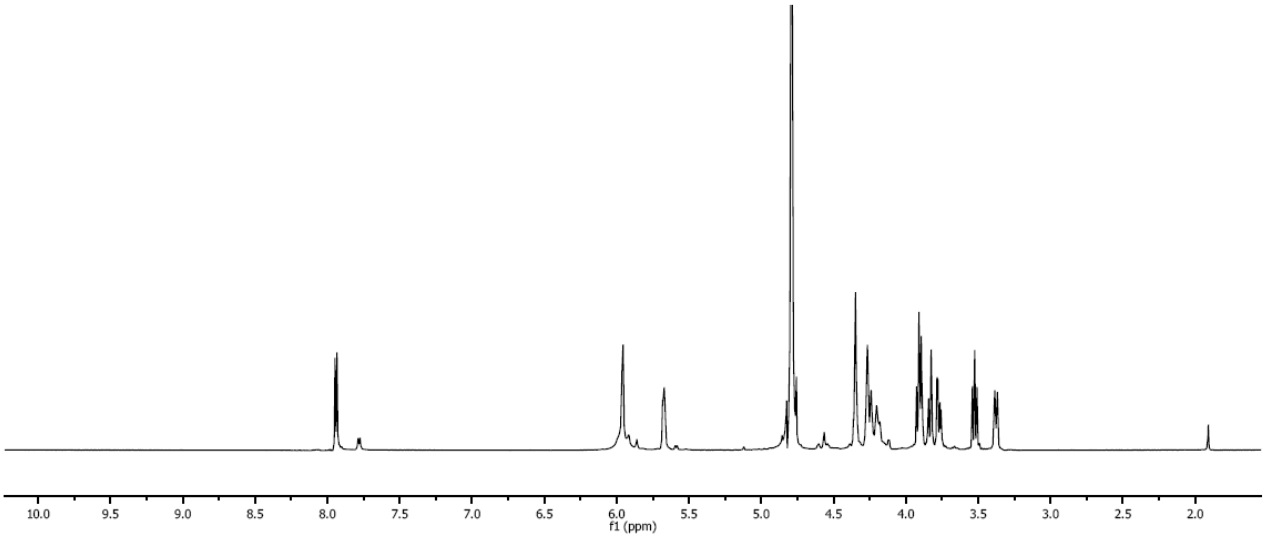


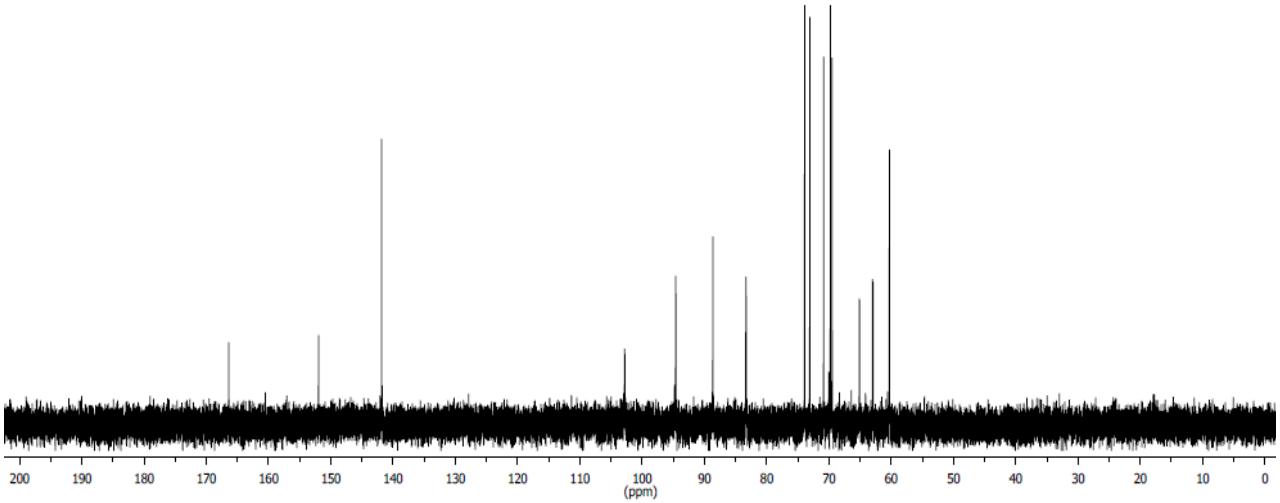
^{1}H & ^{13}C NMR spectra of UDP-GlcNH_2 **23**



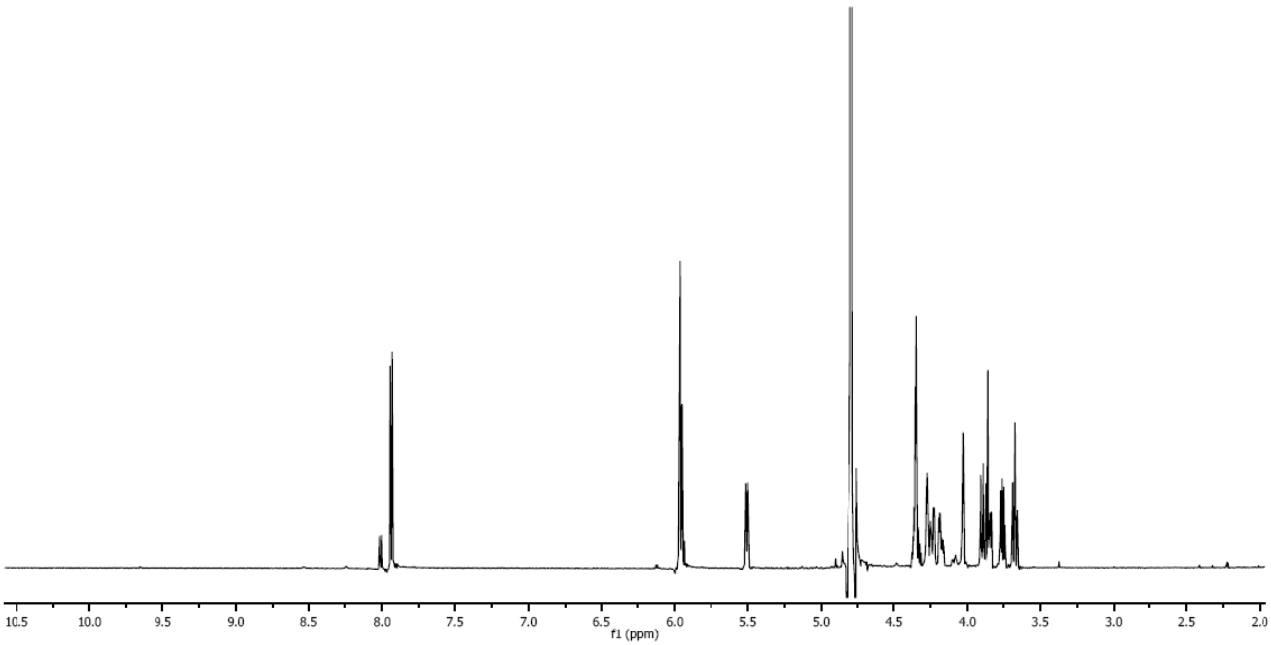


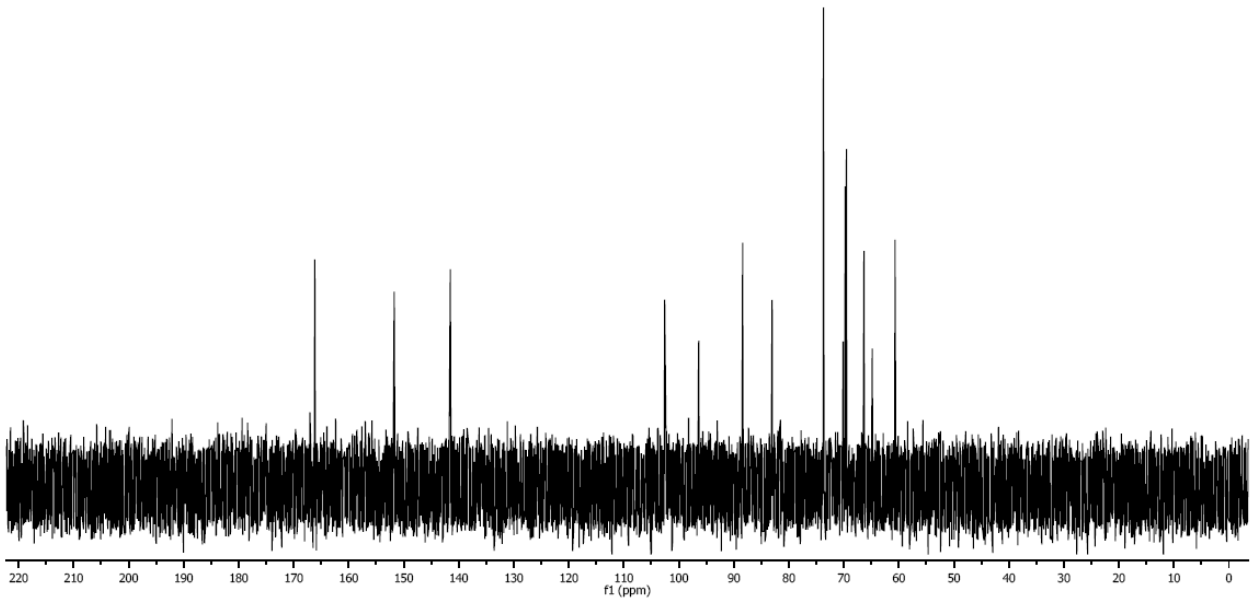
^{1}H & ^{13}C NMR spectra of UDP-GlcN_3 **24**



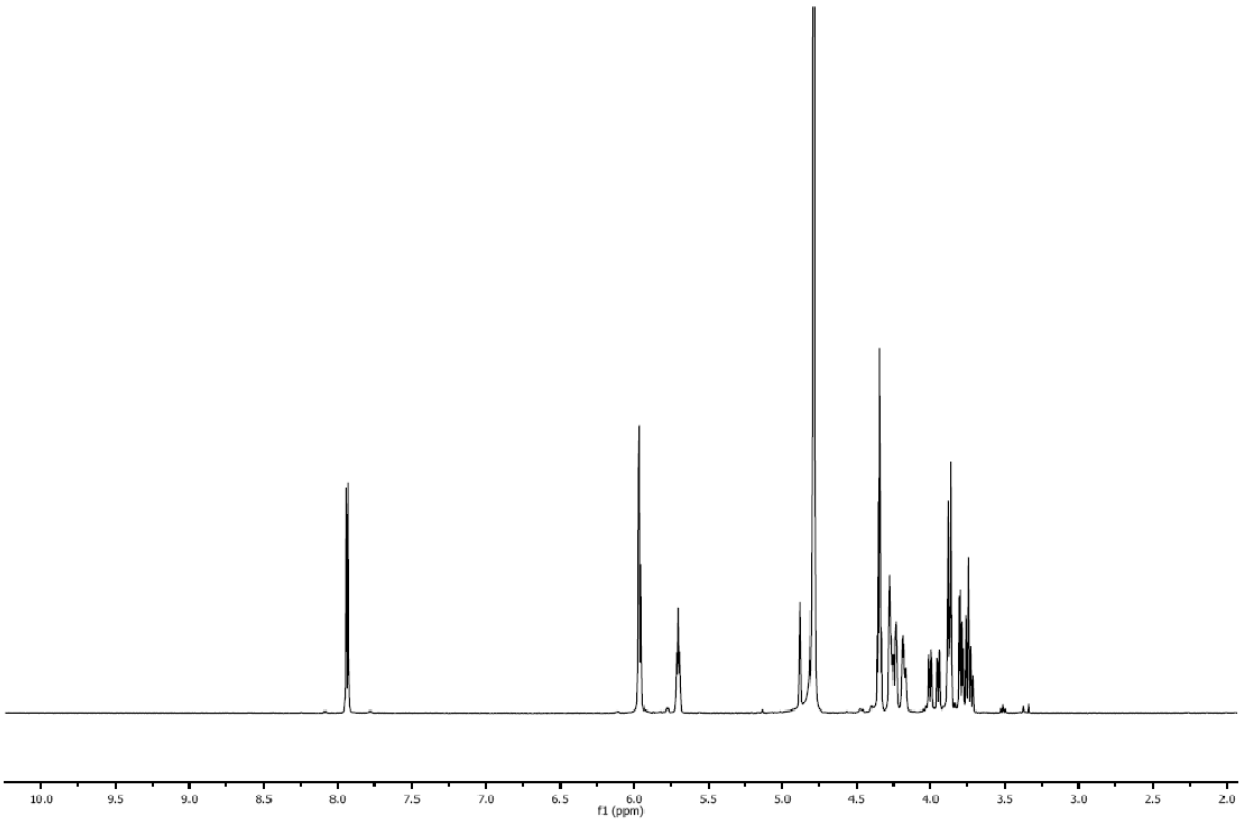


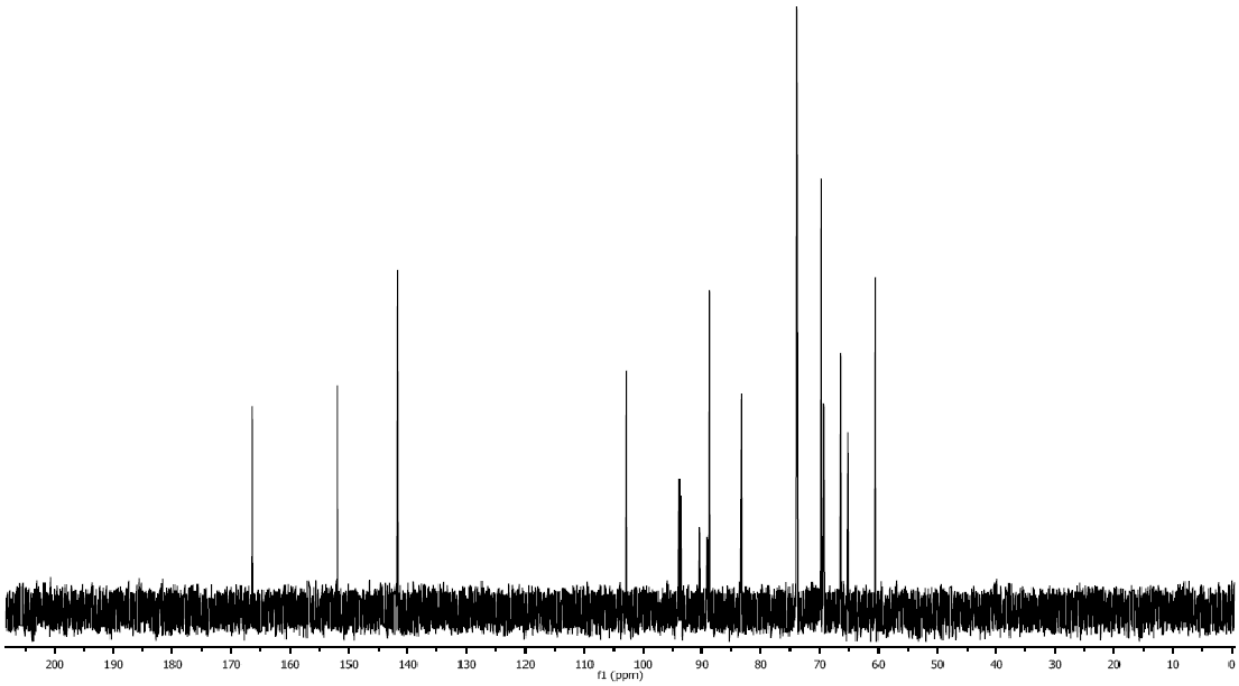
^{1}H & ^{13}C NMR spectra of UDP-Man **26**



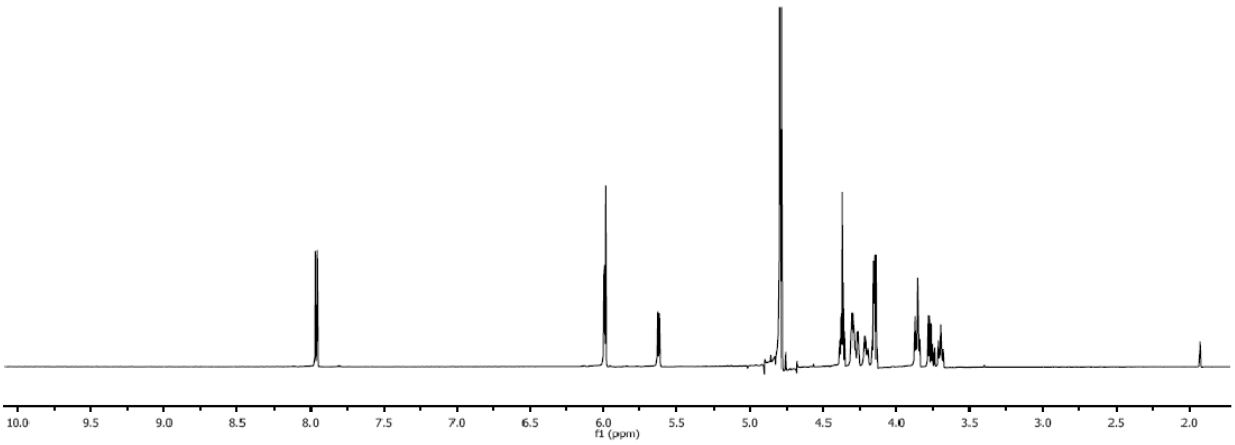


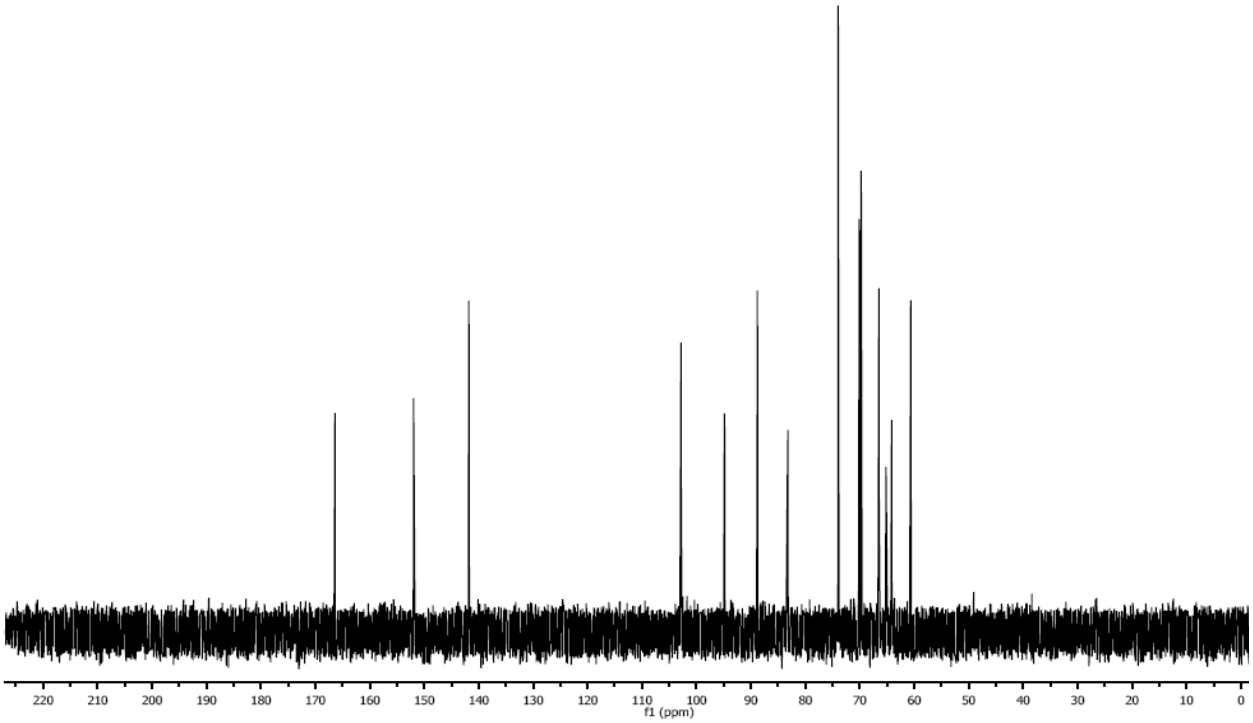
^{1}H & ^{13}C NMR spectra of UDP-ManF **27**





^{1}H & ^{13}C NMR spectra of UDP-ManN_3 **29**





^{1}H & ^{13}C NMR spectra of UDP-ManNAc **30**