Supplementary Information

for

One-pot three-enzyme synthesis of UDP-GlcNAc derivatives

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Cloning, expression, and purification of PmGlmU The gene sequence of *Pm1806* from *Pasteurella multocida* subsp. *multocida* strain Pm70 (GenBank accession no. AAK03890) was used as a reference for designing primers. The genomic DNA of *Pasteurella multocida* strain P-1059 (ATCC 15742) was used as a template for polymerase chain reaction (PCR). Full length *Pasteurella multocida N*-acetylglucosamine-1-phosphate uridylyltransferase (PmGlmU) was cloned in pET15b and pET22b(+) vectors as N-His_6- and C-His_6-tagged fusion proteins, respectively. For cloning into pET15b vector as an N-His_6-tagged protein, the primers used were: forward primer 5' GATC**CATATG** AAAGAGAAAGCATTAAGTATCGTG 3' (*Nde*I restriction site is bold and underlined) and reverse primer 5' CCG**CTCGAG**TTACTTTTTCGTTTGTTTAGTAGGGCG 3' (*Xho*I restriction site is bold and underlined). For cloning into pET22b(+) vector as a C-His_6-tagged protein, the primers used were: forward primer 5' GATC**CATATG**AAAGAGAAAGCATTAAGTATCGTG 3' (*Nde*I restriction site is bold and underlined) and reverse primer 5' CCG**CTCGAG** CTTTTTCGTTTGTTTAGTAGGGCGTTGC 3' (*Xho*I restriction site is bold and underlined). The resulting PCR products were digested with restriction enzymes, purified, and ligated with pET15b or pET22b(+) vector predigested with *Nde*I and *Xho*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.

Positive plasmids were transformed into *E. coli* BL21 (DE3) chemically competent cells. *E. coli* cells harboring the pET15b-PmGlmU or pET22b(+)-PmGlmU 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) until the $OD_{600\ nm}$ of the culture reached 0.8–1.0. Overexpression of the targeted proteins was achieved by adding 0.1 mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) followed by incubation at 25°C for 18 h with rigorous shaking at 250 rpm in a C25KC incubator shaker (New Brunswick Scientific, Edison, NJ).

His_6-tagged proteins were 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 h. 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 then added to the cell suspension. The mixture was incubated at 37 °C for 1 h 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 is 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 enzymes were collected and dialyzed against Tris-HCl (pH 7.5, 25 mM) buffer containing 10% glycerol. Dialyzed proteins were stored at 4 °C. On average, 170 mg of purified protein was obtained from 1 liter of cell culture.

DNA and protein sequences of PmGlmU cloned from *Pasteurella multocida* strain P-1059 (ATCC 15742) Compared to the sequences of GlmU (gene *Pm1806*) from *Pasteurella multocida* genomic strain Pm70 (GenBank accession numbers: AE004439 for gene and AAK03890 for protein), there are 13 base differences (C39A, T195C, A333G, G334A, T339C, G636A, G655A, G817C, T882A, A1006G, A1008T, G1071A, and G1266T) and four amino acid differences (E112K, D219N, E273Q, and T336A) (italicized and underlined) in *Pasteurella multocida* strain P-1059 (ATCC 15742).

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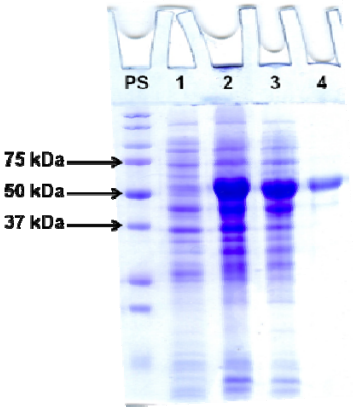


Figure S1. SDS-PAGE analysis of PmGlmU.

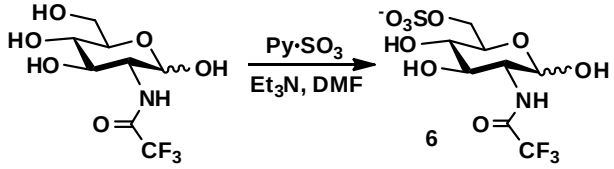
Lanes:
PS: Protein Standards
1. Whole cell extraction, before induction;
2. Whole cell extraction, after induction;
3. Lysate;
4. Purified protein.

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 600 MHz NMR spectrometer. High resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility in the University of California, Davis. Silica gel 60 Å (Sorbent Technologies) was used for flash column chromatography. Analytical thin-layer chromatography (Sorbent Technologies) was performed on silica gel plates 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. ATP, UTP, and GlcNAc (**1**) were purchased from Sigma. GlcNTFA (**2**)[1], $GlcN_3$ (**3**)[1], $GlcNAc6N_3$ (**4**)[2], GlcNAc6S (**5**)[1], GlcNS (**8**)[1] were synthesized as described previously. NanK_ATCC55813[3] and PmPpA[1] were overexpressed as reported.

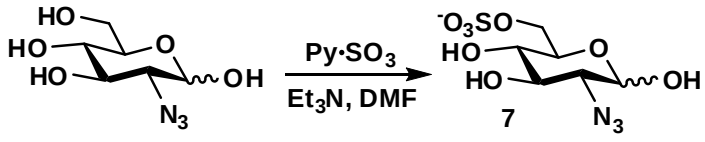
Chemical synthesis of GlcNAc derivatives

Synthesis of GlcNTFA6S (6)



GlcNTFA (**2**)[1] (300 mg, 1.09 mmol) was dissolved in 15 mL of anhydrous DMF. Anhydrous Et_3N (5 mL) and sulfur trioxide pyridine complex (1.2 eq.) were added at 0 °C. After being stirred at room temperature for overnight, the reaction was stopped by adding MeOH and concentrated. The residue was purified by flash column chromatography (EtOAc:MeOH:H_2O = 8:2:1, by volume) to afford 6-*O*-sulfo-GlcNTFA (243 mg, 63%). ^{1}H NMR (600 MHz, D_2O) δ 5.25 (d, *J* = 2.4 Hz, 0.6H), 4.84 (d, *J* = 8.4 Hz, 0.4H), 4.26–3.51 (m, 6H). ^{13}C NMR (150 MHz, D_2O) δ 159.75 (*J* = 37.5 Hz), 159.69 (*J* = 37.5 Hz), 117.01 (*J* = 284.7 Hz), 116.93 (*J* = 284.7 Hz), 94.46, 80.59, 74.03, 73.24, 70.21, 70.00, 69.84, 96.75, 67.22, 67.18, 57.34, 54.87.

Synthesis of $GlcN_3$6S (7)



6-*O*-Sulfo-$GlcN_3$ was synthesized from $GlcN_3$ (**3**)[1] (300 mg, 1.46 mmol) in 54% yield (224 mg) and the procedures were similarly as described above for **6**. ^{1}H NMR (600 MHz, D_2O) δ 5.35 (d, *J* = 2.9 Hz, 0.4H), 4.71 (d, *J* = 8.4 Hz, 0.6H), 4.21–3.82 (m, 3H), 3.65–3.28 (m, 3H). ^{13}C NMR (150 MHz, D_2O) δ 95.31, 91.39, 74.32, 73.94, 71.55, 69.80, 69.61, 69.27, 67.15, 67.12, 66.85, 63.55.

One-pot three-enzyme synthesis of UDP-sugar nucleotides 9–13

Glucosamine derivatives (50 to 300 mg, 1.0 eq.), ATP (1.2 eq.), and UTP (1.2 eq.) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.0) and $MgCl_2$ (10 mM). After the addition of appropriate amount of NanK_ATCC55813 (3.2–4.8 mg), PmGlmU (5–7.5 mg), and PmPpA (2.5–5 mg), water was added to bring the volume of the reaction mixture to 20 mL. The reaction was carried out by incubating the solution in an isotherm incubator for 24 to 48 h at 37 °C with gentle shaking. Product formation was monitored by TLC (EtOAc:MeOH:H_2O = 3:2:1 by volume) with *p*-anisaldehyde sugar staining. The reaction was stopped by adding the same volume of ice-cold ethanol and incubating at 4 °C for 30 min. The mixture was concentrated and passed through a BioGel P-2 gel filtration column to obtain the desired product. Silica gel column purification (EtOAc:MeOH:H_2O = 4:2:1) was applied when necessary to achieve further purification.

Uridine 5′-diphospho-2-acetamido-2-deoxy-α-D-glucopyranoside (UDP-GlcNAc, 9). Yield, 81% (445 mg); white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.97 (d, *J* = 8.4 Hz, 1H), 5.97–6.00 (m, 2H), 5.53 (dd, *J* = 6.6, 3.0 Hz, 1H), 4.37–4.40 (m, 2H), 4.21–4.31 (m, 3H), 3.81–3.75 (m, 5H), 3.58 (t, *J* = 9.0 Hz, 1H), 2.09 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 174.94, 166.39, 151.99, 141.82, 102.85, 94.68, 88.72, 83.40 (d, *J* = 8.7 Hz), 73.96, 73.20, 71.13, 69.83, 65.18, 65.15, 60.53, 53.88 (d, *J* = 8.4 Hz), 22.29. HRMS (ESI) *m/z* calcd for $C_{17}H_{27}N_3O_{17}P_2$ (M+H) 608.0894, found 608.0906.

Uridine 5′-diphospho-2-deoxy-2-trifluoroacetamido-α-D-glucopyranoside (UDP-GlcNTFA, 10). Yield, 97% (699 mg); white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.95 (d, *J* = 7.8 Hz, 1H), 5.97–5.98 (m, 2H), 5.64 (dd, *J* = 6.6, 3.0 Hz, 1H), 4.35–4.39 (m, 2H), 4.18–4.29 (m, 3H), 4.12 (d, *J* = 10.8 Hz, 1H), 3.93–3.98 (m, 2H), 3.91 (dd, *J* = 12.6, 1.8 Hz, 1H), 3.85 (dd, *J* = 12.0, 4.2 Hz, 1H), 3.61 (t, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 166.39, 159.73 (d, *J* = 37.5 Hz), 151.94, 141.83, 116.88 (d, *J* = 284.6 Hz), 102.79, 93.91, 88.79, 83.22(d, *J* = 9.0 Hz), 73.92, 73.23, 70.35, 69.76, 69.68, 65.12, 60.42, 54.53 (d, *J* = 8.9 Hz). HRMS (ESI) *m/z* calcd for $C_{17}H_{24}F_3N_3O_{17}P_2$ (M+H) 662.0611, found 662.0615.

Uridine 5′-diphospho-2-azido-2-deoxy-α-D-glucopyranoside (UDP-$GlcN_3$, 11). Yield, 54% (124 mg); white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.96 (d, *J* = 8.4 Hz, 1H), 5.97–5.96 (m, 2H), 5.68 (dd, *J* = 7.2, 3 Hz, 1H), 4.34–4.37 (m, 2H), 4.18–4.27 (m, 3H), 3.89–3.93 (m, 2H), 3.85 (dd, *J* = 12.6, 2.4 Hz, 1H), 3.79 (dd, *J* = 12.0, 4.2 Hz, 1H), 3.54 (t, *J* = 9.6 Hz, 1H), 3.38 (d, *J* = 10.2 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 166.39, 151.96, 141.84, 102.79, 94.60, 88.64, 83.34 (d, *J* = 9 Hz), 73.91, 73.07, 70.85, 69.77, 69.49, 65.07, 62.93 (d, *J* = 8.6 Hz), 60.29. HRMS (ESI) *m/z* calcd for $C_{15}H_{23}N_5O_{16}P_2$ (M+H) 633.0959, found 633.0960.

Uridine 5′-diphospho-2-acetamido-6-azido-2,6-dideoxy-α-D-glucopyranoside (UDP-GlcNAc6N_3, 12). Yield, 72% (462 mg); white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.93 (d, *J* = 7.8 Hz, 1H), 5.96-5.94 (m, 2H), 5.15 (s, 1H), 4.32–4.36 (m, 2H), 4.17–4.24 (m, 3H), 4.00–4.04 (m, 2H), 3.79 (t, *J* = 9.6 Hz, 1H), 3.72 (dd, *J* = 13.2, 2.4 Hz, 1H), 3.55–3.62 (m, 2H), 2.06 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 174.86, 166.33, 151.86, 141.82, 102.76, 94.53, 88.92, 83.15 (d, *J* = 8.9 Hz), 73.93, 71.85, 70.28, 70.28, 69.68, 65.16, 53.77 (d, *J* = 7.4 Hz), 50.71, 22.22. HRMS (ESI) *m/z* calcd for $C_{17}H_{26}N_6O_{16}P_2$ (M+H) 592.0693, found 592.0698.

Uridine 5′-diphospho-2-acetamido-2-deoxy-6-*O*-sulfo-α-D-glucopyranoside (UDP-GlcNAc6S, 13). Yield, 62% (70 mg); white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.96 (d, *J* = 7.8 Hz, 1H), 5.97–5.99 (m, 2H), 5.55 (dd, *J* = 7.2, 3.0 Hz, 1H), 4.35–4.38 (m, 3H), 4.26–4.30 (m, 3H), 4.18–4.22 (m, 1H), 4.12 (d, *J* = 9.6 Hz, 1H), 4.04 (d, *J* = 10.8 Hz, 1H), 3.84 (t, *J* = 9.6 Hz, 1H), 3.68 (t, *J* = 9.6 Hz, 1H), 2.09 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 174.84, 166.40, 151.93, 141.73, 102.76, 94.57, 88.72, 83.15 (d, *J* = 9.3 Hz), 73.89, 70.83, 69.70, 69.04, 66.56, 65.16, 65.13, 53.67 (d, *J* = 8.1 Hz), 22.17. HRMS (ESI) *m/z* calcd for $C_{17}H_{27}N_3O_{20}P_2S$ (M+H) 688.0462, found 688.0471.

Chemical synthesis of UDP-sugar nucleotides 16, 17, 19–23, 25–28

Uridine 5′-diphospho-2-amino-2-deoxy-α-D-glucopyranoside (UDP-GlcNH$_2$, 17). UDP-GlcNTFA **10** (150 mg, 0.22 mmol) was dissolved in 25 mL of methanol and 5 mL of H_2O. The pH of the solution was adjusted to 9.5 by adding K_2CO_3. After being vigorously stirred at r.t. for overnight, the reaction mixture was neutralized with DOWEX HCR-W2 (H^+) resin, filtered and concentrated. The residue was purified by flash column chromatography (EtOAc:MeOH:H_2O = 1:1:1, by volume) to afford **17** as white solid in 98% yield (122 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.90 (d, *J* = 7.8 Hz, 1H), 5.89–5.92 (m, 2H), 5.79 (dd, *J* = 6.0, 3.0 Hz, 1H), 4.30–4.32 (m, 2H), 4.16–4.24 (m, 3H), 3.86–3.90 (m 2H), 3.81 (dd, *J* = 12.6, 1.8 Hz, 1H), 3.77 (dd, *J* = 12.6, 4.2 Hz, 1H), 3.52 (t, *J* = 9.6 Hz, 1H), 3.33 (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) 566.0788, found 566.0791.

Uridine 5′-diphospho-2-sulfoamino-2-deoxy-α-D-glucopyranoside (UDP-GlcNS, 16). UDP-GlcNH$_2$ **17** (50 mg, 0.082 mmol) was dissolved in 30 mL of water. The pH of the solution was adjusted to 9.5 by adding 2 N NaOH (aq). Sulfur trioxide-pyridine complex (65 mg, 0.41 mmol) was added in three equal portions during 35 minutes intervals at room temperature, and the pH was maintained at 9.5 throughout the whole process using 2 N NaOH (aq). After being stirred at r.t. for overnight, the reaction mixture was neutralized with DOWEX HCR-W2 (H^+) resin, filtered, concentrated, and purified using silica gel column (EtOAc:MeOH:H_2O = 3:2:1, by volume) to obtain the UDP-GlcNS **16** in 86% yield (46 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.90 (d, *J* = 7.8 Hz, 1H), 5.92–5.93 (m, 2H), 5.71 (s, 1H), 4.31–4.33 (m, 2H), 4.16–4.23 (m, 3H), 3.73–3.86 (m, 3H), 3.66 (t, *J* = 9.6 Hz, 1H), 3.51 (t, *J* = 9.6 Hz, 1H), 3.24 (d, *J* = 9.6 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 166.49, 152.13, 141.99, 103.04, 95.50, 88.86, 83.53 (*J* = 8.9 Hz), 74.02, 73.06, 71.73, 69.98, 69.96, 65.38, 60.73, 58.11 (*J* = 9.2 Hz). HRMS (ESI) *m/z* calcd for $C_{15}H_{25}N_3O_{19}P_2S$ (M+H) 646.0356, found 646.0373.

Uridine 5′-diphospho-2-hydroxyacetamido-2-deoxy-α-D-glucopyranoside (19). To a solution of UDP-GlcNH$_2$ **17** (30 mg, 0.049 mmol) in CH_3CN-H_2O (30 mL, 1:1, v/v) in the presence of $NaHCO_3$ (40 mg, 0.49 mmol), the Acetoxyacetyl chloride (6.9 μL, 0.098 mmol) in CH_3CN (5 mL) was added. The reaction mixture was stirred for 4 hours at 0 °C and was neutralized with DOWEX HCR-W2 (H^+) resin, filtered, and concentrated. The residue was purified by flash column chromatography (EtOAc:MeOH:H_2O = 5:2:1, by volume) to afford UDP-GlcNGcAc **18** in 95% yield (31 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.99 (d, *J* = 7.8 Hz, 1H), 6.03–6.04 (m, 2H), 5.62 (dd, *J* = 6.6, 3.6 Hz, 1H), 4.41–4.45 (m, 2H), 4.24–4.35 (m, 3H), 4.13 (d, *J* = 10.2 Hz, 1H), 4.01 (d, *J* = 7.8 Hz, 1H), 3.86–3.96 (m, 3H), 3.63 (t, *J* = 9.6 Hz, 1H), 2.25 (s, 3H). Compound **18** was dissolved in dry methanol (50 mL) containing analytic amount of sodium methoxide. The resulted mixture was stirred at r.t. for overnight. The reaction mixture was then neutralized with DOWEX HCR-W2 (H+) resin, filtered, and

concentration to give product UDP-GlcNGc **19** in 98% yield (28 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.92 (d, *J* = 7.8 Hz, 1H), 5.93–5.95 (m, 2H), 5.52 (dd, *J* = 7.2, 3.0 Hz, 1H), 4.31–4.35 (m, 2H), 4.09–4.25 (m, 5H), 4.02 (d, *J* = 10.2 Hz, 1H), 3.83–3.91 (m, 3H), 3.79 (dd, *J* = 12.6, 4.2 Hz, 1H), 3.55 (t, *J* = 9.6 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 175.47, 166.37, 151.92, 141.74, 101.73, 94.36, 88.53, 83.28 (d, *J* = 8.4 Hz), 73.86, 73.09, 70.69, 69.71, 69.54, 65.05, 61.11, 60.36, 53.46 (d, *J* = 7.7 Hz). HRMS (ESI) *m/z* calcd for $C_{17}H_{27}N_3O_{18}P_2$ (M+H) 624.0843, found 624.0847.

Uridine 5′-diphospho-2-azidoxyacetamido-2-deoxy-α-D-glucopyranoside (20). Sodium azide (62 mg, 0.98 mmol) was dissolved in 5 mL of distilled H_2O and the mixture was cooled to 0 °C. Bromoacetic acid (68 mg, 0.49 mmol) was then added over 10 min and the reaction was allowed to slowly warm up to r.t. for overnight. The reaction was acidified to pH 1.0 and extracted three times with 5 mL of diethyl ether. The organic portions were combined, dried over $MgSO_4$ and concentrated. The crude mixture was dissolved in 10 mL of CH_2Cl_2 and two drops of DMF and cooled to 0 °C. Oxalyl chloride (54 μL, 0.64mmol) was slowly added over 15 min using a syringe. The reaction was allowed to warm up to r.t. for overnight. The solvent was removed under reduced pressure to afford the crude oil azidoacetyl chloride.[4] To a solution of UDP-GlcNH$_2$ **17** (30 mg, 0.049 mmol) in CH_3CN-H_2O (30 mL, 1:1, v/v) in the presence of $NaHCO_3$ (40 mg, 0.49 mmol), the azidoacetyl chloride in CH_3CN (5 mL) was added. The reaction mixture was stirred for 4 hours at 0 °C and was neutralized with DOWEX HCR-W2 (H^+) resin, filtered, and concentrated. The residue was purified by flash column chromatography (EtOAc:MeOH:H_2O = 5:2:1, by volume) to afford UDP-GlcNAz **20** in 68% yield (22 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.92 (d, *J* = 8.4 Hz, 1H), 5.91–5.94 (m, 2H), 5.49 (dd, *J* = 7.2, 3.6 Hz, 1H), 4.30–4.36 (m, 2H), 4.00–4.24 (m, 6H), 3.75–3.89 (m, 4H), 3.53 (t, *J* = 9.6 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 171.13, 166.41, 151.98, 141.86, 102.84, 94.59, 88.80, 83.34 (d, *J* = 9.0 Hz), 73.94, 73.25, 71.02, 69.81, 69.66, 65.24, 60.51, 53.94 (d, , *J* = 9.0 Hz), 52.69, 51.80. HRMS (ESI) *m/z* calcd for $C_{17}H_{26}N_6O_{17}P_2$ (M+H) 649.0908, found 649.0917.

Uridine 5′-diphospho-2-phenylacetamido-2-deoxy-α-D-glucopyranoside (21).
2-Phenylacetyl acid (33 mg, 0.25 mmol) was dissolved in 10 mL of CH_2Cl_2 and two drops of DMF. The mixture was cooled to 0 °C. Oxalyl chloride (28 μL, 0.33 mmol) was slowly added over 15 min using a syringe. The reaction was allowed to warm up to r.t. for overnight. The solvent was then removed under reduced pressure to afford 2-phenylacetyl chloride as a light pink solid. To a solution of UDP-GlcNH$_2$ **17** (30 mg, 0.049 mmol) in CH_3CN-H_2O (30 mL, 1:1, v/v) in the presence of $NaHCO_3$ (40 mg, 0.49 mmol), the 2-phenylacetyl chloride in CH_3CN (5 mL) was added. The reaction mixture was stirred for 4 hours at 0 °C and was neutralized with DOWEX HCR-W2 (H^+) resin, filtered, and concentrated. The residue was purified by flash column chromatography (EtOAc:MeOH:H_2O = 6:2:1, by volume) to afford white solid **21** in 79% yield (26 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.83 (d, *J* = 8.4 Hz, 1H), 7.32–7.35 (m, 2H), 7.26–7.29 (m, 3H), 5.90 (d, *J* = 3.6 Hz, 1H), 5.83 (d, *J* = 7.8 Hz, 1H), 5.54 (dd, *J* = 6.6, 3.0 Hz, 1H), 4.16–4.30 (m, 5H), 3.77–4.00 (m, 5H), 3.67 (s, 2H), 3.54 (t, *J* = 9.6 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 175.33, 166.16, 151.75, 141.64, 135.13, 129.36, 128.90, 127.26, 102.74, 94.77, 88.80, 83.08 (d, *J* = 9 Hz), 73.80, 73.25, 70.97, 69.74, 69.65, 65.07, 60.50, 53.89 (d, *J* = 8.7 Hz), 42.21. HRMS (ESI) *m/z* calcd for $C_{23}H_{31}N_3O_{17}P_2$ (M+H) 684.1207, found 684.1215.

Uridine 5′-diphospho-2-(1,1'-biphenyl-4-yl)acetamido-2-deoxy-α-D-glucopyranoside (22).
Compound **22** was synthesized from UDP-GlcNH$_2$ **17** using a similar procedure as described above for **21** except that the reagent 2-phenylacetyl acid was replaced by 2-([1,1'-biphenyl]-4-yl)acetic acid. Compound **22** was obtained as a white solid in 82% yield (31 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.69 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 2H), 7.59 (d, *J* = 7.2 Hz, 2H), 7.45–7.47 (m, 2H), 7.34–7.41 (m, 3H), 5.79 (d, *J* = 4.2 Hz, 1H), 5.64 (d, *J* = 7.2 Hz, 1H), 5.53 (dd, *J* = 6.6, 3.0 Hz, 1H) 4.14–4.19 (m,

5H), 4.01 (d, *J* = 10.2 Hz, 1H), 3.92 (d, *J* = 9.6 Hz, 1H), 3.65–3.85 (m, 5H), 3.53 (t, *J* = 9.0 Hz, 1H). ^{13}C NMR (150 MHz, D_2O) δ 175.19, 165.92, 151.44, 141.31, 140.08, 139.12, 134.51, 129.90, 129.16, 127.75, 127.14, 126.80, 102.49, 94.76, 88.77, 82.87 (d, *J* = 8.6 Hz), 73.82, 73.22, 71.01, 69.70, 69.44, 64.89, 60.46, 53.88 (d, *J* = 8.4 Hz), 41.80. HRMS (ESI) *m/z* calcd for $C_{29}H_{35}N_3O_{17}P_2$ (M+H) 760.1520, found 760.1534.

Uridine 5′-diphospho-2-acetamido-6-amino-2,6-dideoxy-α-D-glucopyranoside (UDP-GlcNAc6NH$_2$, 23). UDP-GlcNAc6N$_3$ **12** (100 mg, 0.16 mmol) was dissolved in MeOH-H_2O (10 mL, 1:1, v/v) and 20 mg of Pd/C was added. The mixture was shaken under H_2 gas (4 Bar) for 1 hour, filtered, and concentrated. The residue was purified by flash column chromatography (EtOAc:MeOH:H_2O = 3:2:1, by volume) to afford UDP-GlcNAc6NH$_2$ **23** in 96% yield (93 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.90 (d, *J* = 8.4 Hz, 1H), 5.89–5.93 (m, 2H), 5.48 (dd, *J* = 6.6, 3.0 Hz, 1H), 4.30–4.32 (m, 2H), 4.20–4.23 (m, 2H), 4.08–4.15 (m, 2H), 3.99 (d, *J* = 10.8 Hz, 1H), 3.77 (t, *J* = 9.6 Hz, 1H), 3.45 (d, *J* = 13.2 Hz, 1H), 3.40 (t, *J* = 9.6 Hz, 1H), 3.11 (t, *J* = 12.6 Hz, 1H), 2.02 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 174.94, 166.39, 151.96, 141.84, 102.79, 94.33, 88.82, 83.29 (*J* = 9.0 Hz), 73.92, 71.82, 69.80, 69.28, 65.30, 53.64 (*J* = 8.9 Hz), 40.70, 22.21. HRMS (ESI) *m/z* calcd for $C_{17}H_{28}N_4O_{16}P_2$ $(M+H)^-$ 607.1054, found 607.1068.

Uridine 5′-diphospho-2-acetamido-6-hydroxyacetamido-2,6-dideoxy-α-D-glucopyranoside (25). Compound **24** was synthesized from UDP-GlcNAc6NH$_2$ **23** using the same process as described above for **18**. Compound **24** was obtained as a white solid in 91% yield (31 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.91 (d, *J* = 7.8 Hz, 1H), 5.91–5.93 (m, 2H), 5.46 (dd, *J* = 6.6, 3.0 Hz, 1H), 4.62 (s, 2H), 4.30–4.34 (m, 2H), 4.14–4.24 (m, 3H), 3.95 (m, 2H), 3.76 (t, *J* = 9.0 Hz, 1H), 3.61 (dd, *J* = 14.4, 6.0 Hz, 1H), 3.54 (dd, *J* = 14.4, 2.4 Hz, 1H), 3.35 (dd, *J* = 14.4, 4.2 Hz, 1H), 2.15 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 174.85, 173.43, 170.60, 166.44, 151.95, 141.74, 102.70, 94.40, 88.70, 83.16, 73.87, 71.33, 70.89, 70.68, 69.66, 65.05, 62.88, 53.67, 39.59, 22.14, 20.13. Compound **25** was synthesized from compound **24** using the same process as described above for **19** and obtained as a white solid in 98% yield (29 mg). ^{1}H NMR (600 MHz, D_2O) δ 8.09 (d, *J* = 7.8 Hz, 1H), 6.11–6.13 (m, 2H), 5.66 (dd, *J* = 6.6, 3.0 Hz, 1H), 4.50–4.53 (m, 2H), 4.33–4.43 (m, 3H), 4.26 (s, 2H), 4.14–4.16 (m, 2H), 3.95 (t, *J* = 9.9 Hz, 1H), 3.82 (d, *J* = 14.4 Hz, 1H), 3.73 (dd, *J* = 13.8, 6 Hz, 1H), 3.56 (t, *J* = 10.2 Hz, 1H), 2.22 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 175.55, 175.05, 166.60, 152.20, 141.97, 102.99, 94.62, 88.99, 83.50 (d, *J* = 8.9 Hz), 74.06, 71.47, 71.06, 69.97, 65.33, 61.35, 53.98 (d, *J* = 8.4 Hz), 39.76, 22.42. HRMS (ESI) *m/z* calcd for $C_{19}H_{30}N_4O_{18}P_2$ (M+H) 665.1109, found 665.1113.

Uridine 5′-diphospho-2-acetamido-6-azidoacetamido-2,6-dideoxy-α-D-glucopyranoside (26). Compound **26** was synthesized from UDP-GlcNAc6NH$_2$ **23** using the same process as described above for **20**. Compound **26** was obtained as a white solid in 61% yield (21 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.89 (d, *J* = 7.8 Hz, 1H), 5.91 (m, 2H), 5.43 (dd, *J* = 6.6, 3.0 Hz, 1H), 4.32–4.29 (m, 2H), 4.19–4.22 (m, 3H), 4.00 (s, 2H), 3.92–3.95 (m, 2H), 3.74 (t, *J* = 10.2 Hz, 1H), 3.54 (s, 1H), 3.34 (t, *J* = 9.6 Hz, 1H), 2.01 (s, 3H). ^{13}C NMR (150 MHz, D2O) δ 174.85, 170.93, 166.40, 151.92, 141.75, 104.99, 102.71, 94.42, 88.71, 83.16 (*J* = 8.9 Hz), 73.87, 71.27, 71.01, 70.71, 69.67, 65.10, 53.72 (*J* = 8.1 Hz), 51.80, 39.92, 22.14. HRMS (ESI) *m/z* calcd for $C_{19}H_{29}N_7O_{17}P_2$ (M+H) 690.1173, found 690.1180.

Uridine 5′-diphospho-2-acetamido-6-phenylacetamido-2,6-dideoxy-α-D-glucopyranoside (27). Compound **27** was synthesized from UDP-GlcNAc6NH$_2$ **23** using the same way as described above for **21**. Compound **27** was obtained as a white solid in 86% yield (30 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.87 (d, *J* = 8.4 Hz, 1H), 7.36–7.38 (m, 2H), 7.29–7.32 (m, 3H), 5.87–5.89 (m, 2H), 5.48 (dd, *J* = 6.6, 2.4 Hz, 1H), 4.16–4.29 (m, 5H), 3.92–3.98 (m, 2H), 3.78 (t, *J* = 9.6 Hz, 1H), 3.53–3.65 (m, 4H), 3.30

(t, *J* = 9.6 Hz, 1H), 2.05 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 175.37, 174.89, 166.26, 151.75, 141.64, 135.34, 129.22, 129.05, 127.38, 102.66, 94.50, 88.93, 83.12 (*J* = 8.6 Hz), 73.94, 71.48, 71.06, 70.60, 69.51, 64.99, 53.79 (*J* = 8.3 Hz), 42.43, 40.01, 22.19. HRMS (ESI) *m/z* calcd for $C_{25}H_{34}N_4O_{17}P_2$ (M+H) 725.1473, found 725.1484.

Uridine 5′-diphospho-2-acetamido-6-(1,1'-biphenyl-4-yl)-acetamido-2,6-dideoxy-α-D-glucopyranoside (28). Compound **28** was synthesized from UDP-GlcNAc6NH_2 **23** using the same way as described above for **22**. Compound **28** was obtained as a white solid in 88% yield (35 mg). ^{1}H NMR (600 MHz, D_2O) δ 7.75 (d, *J* = 7.8 Hz, 1H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.44–7.45 (m, 2H), 7.34–7.45 (m, 3H), 5.77–5.80 (m, 2H), 5.44 (dd, *J* = 7.2, 3.6 Hz, 1H), 4.03–4.18 (m, 5H), 3.89–3.96 (m, 2H), 3.75 (t, *J* = 9.6 Hz, 1H), 3.49–3.63 (m, 4H), 3.28 (t, *J* = 9.0 Hz, 1H), 2.01 (s, 3H). ^{13}C NMR (150 MHz, D_2O) δ 175.17, 174.79, 166.28, 151.63, 141.34, 140.12, 139.31, 134.70, 129.74, 129.18, 127.75, 127.30, 126.88, 102.44, 94.39, 88.87, 82.62 (d, *J* = 8.7 Hz), 73.93, 71.18, 70.54, 69.27, 64.80, 53.77 (d, *J* = 8.4 Hz), 42.03, 40.16, 22.09. HRMS (ESI) *m/z* calcd for $C_{31}H_{38}N_4O_{17}P_2$ (M+H) 801.1785, found 801.1807.

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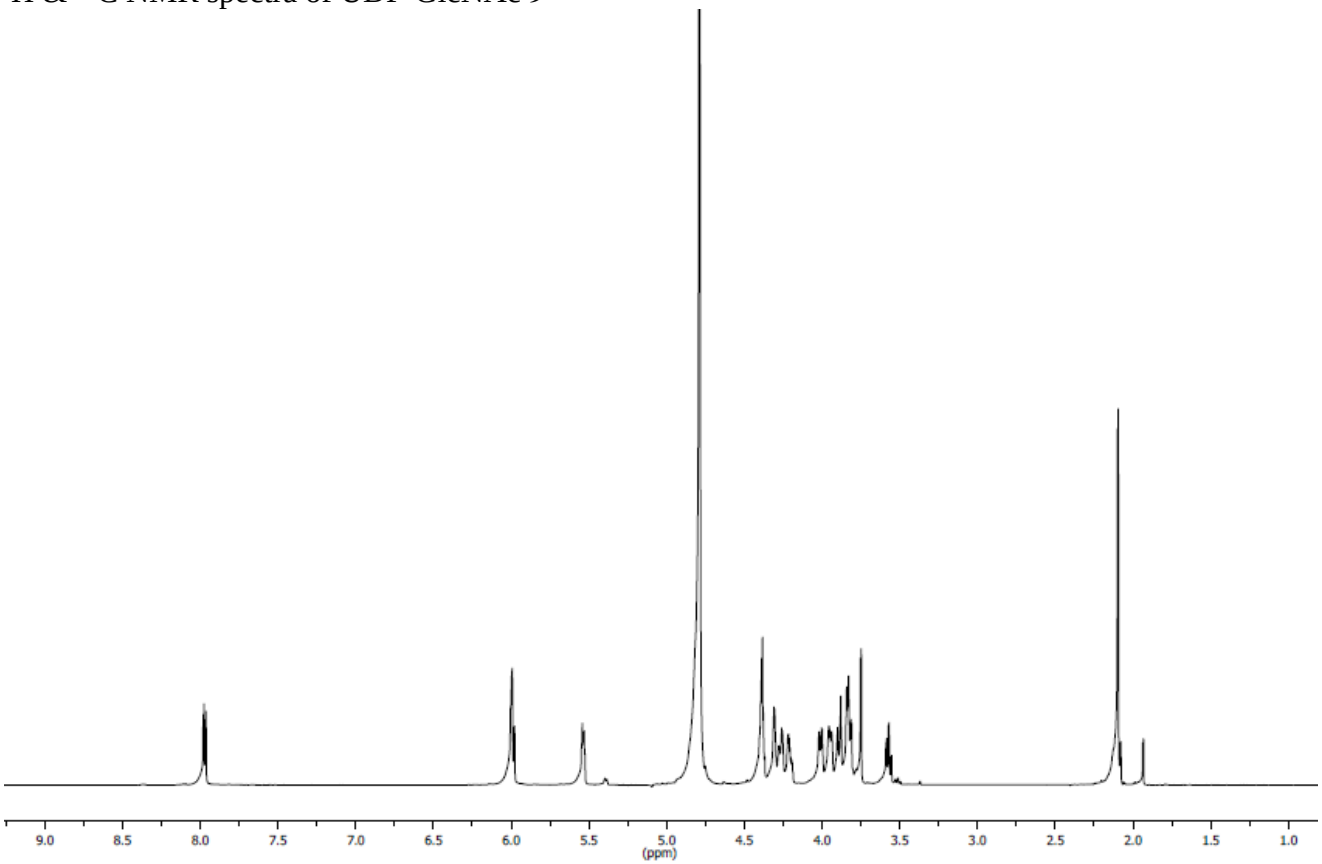
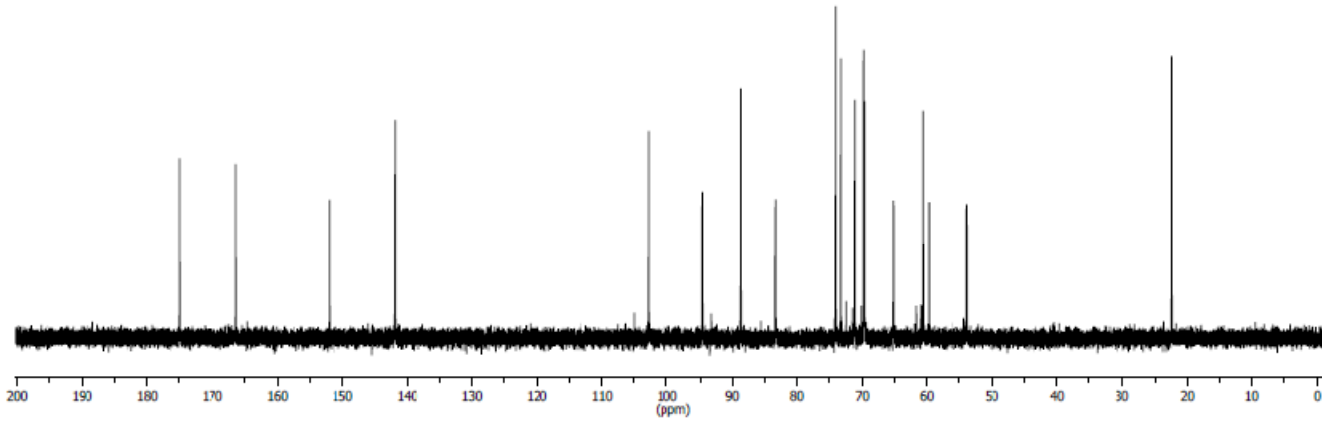
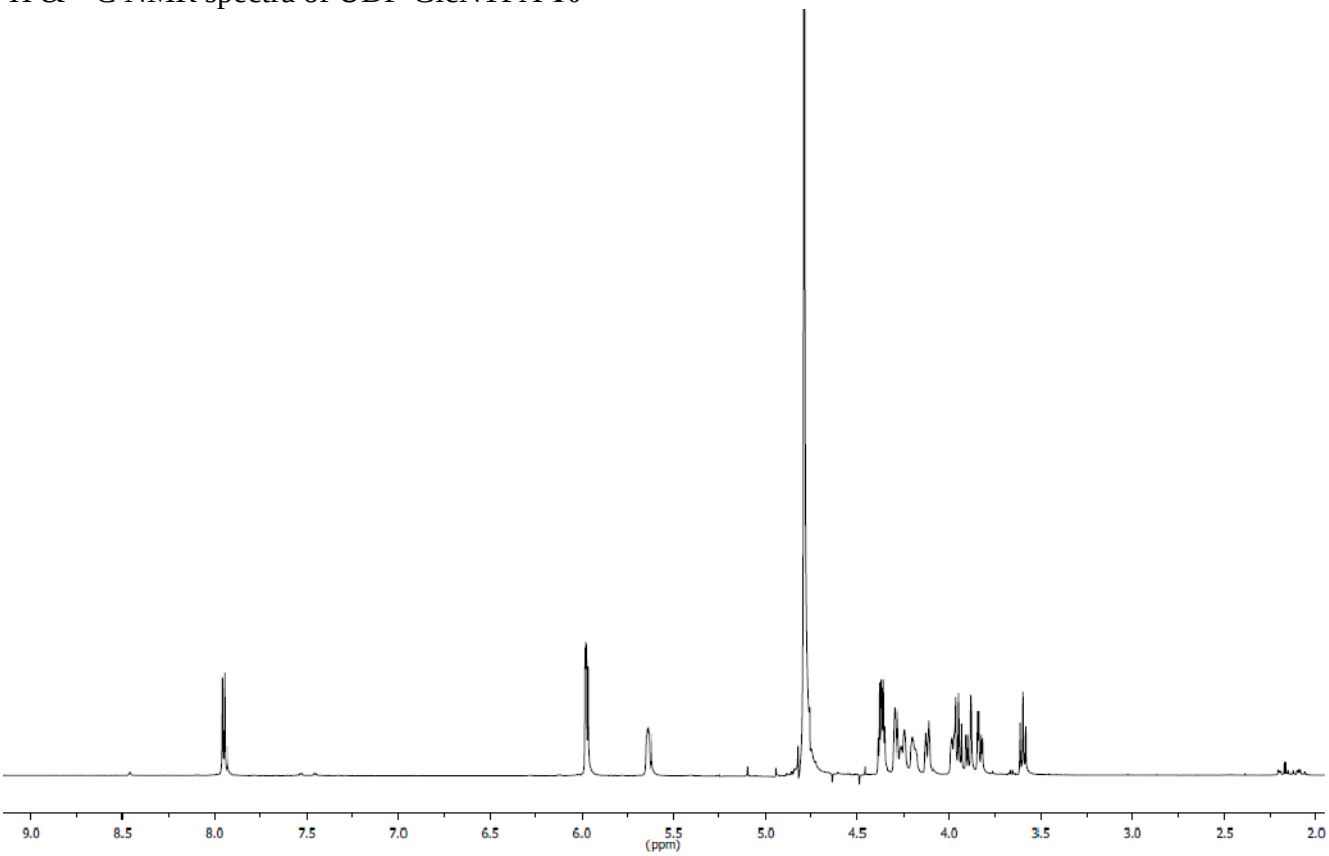
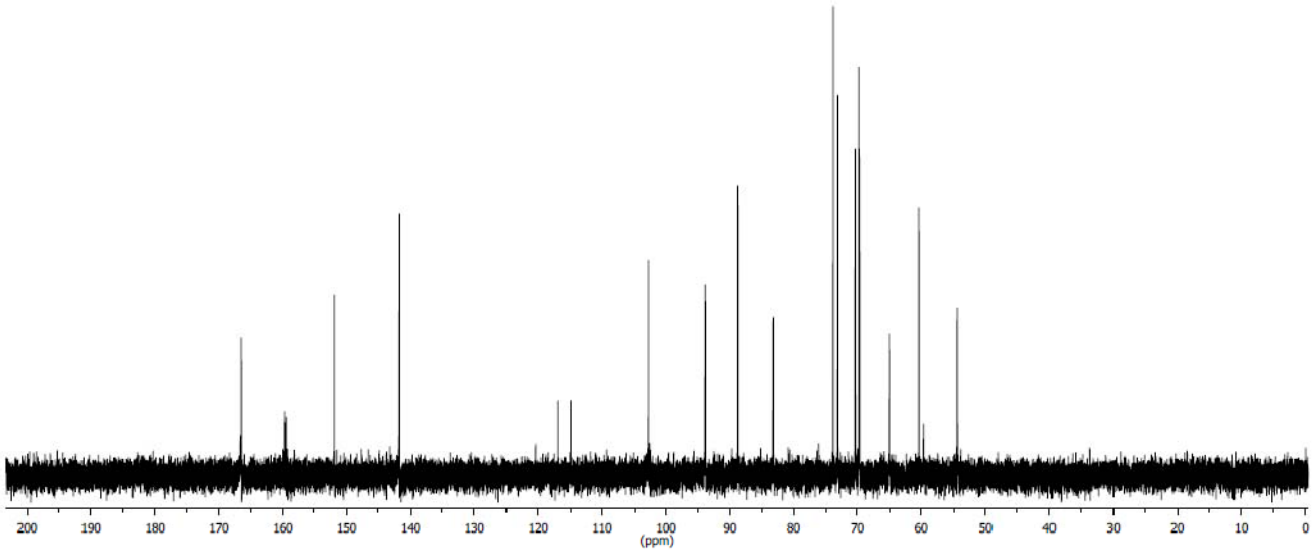
^{1}H & ^{13}C NMR spectra of UDP-GlcNAc **9**



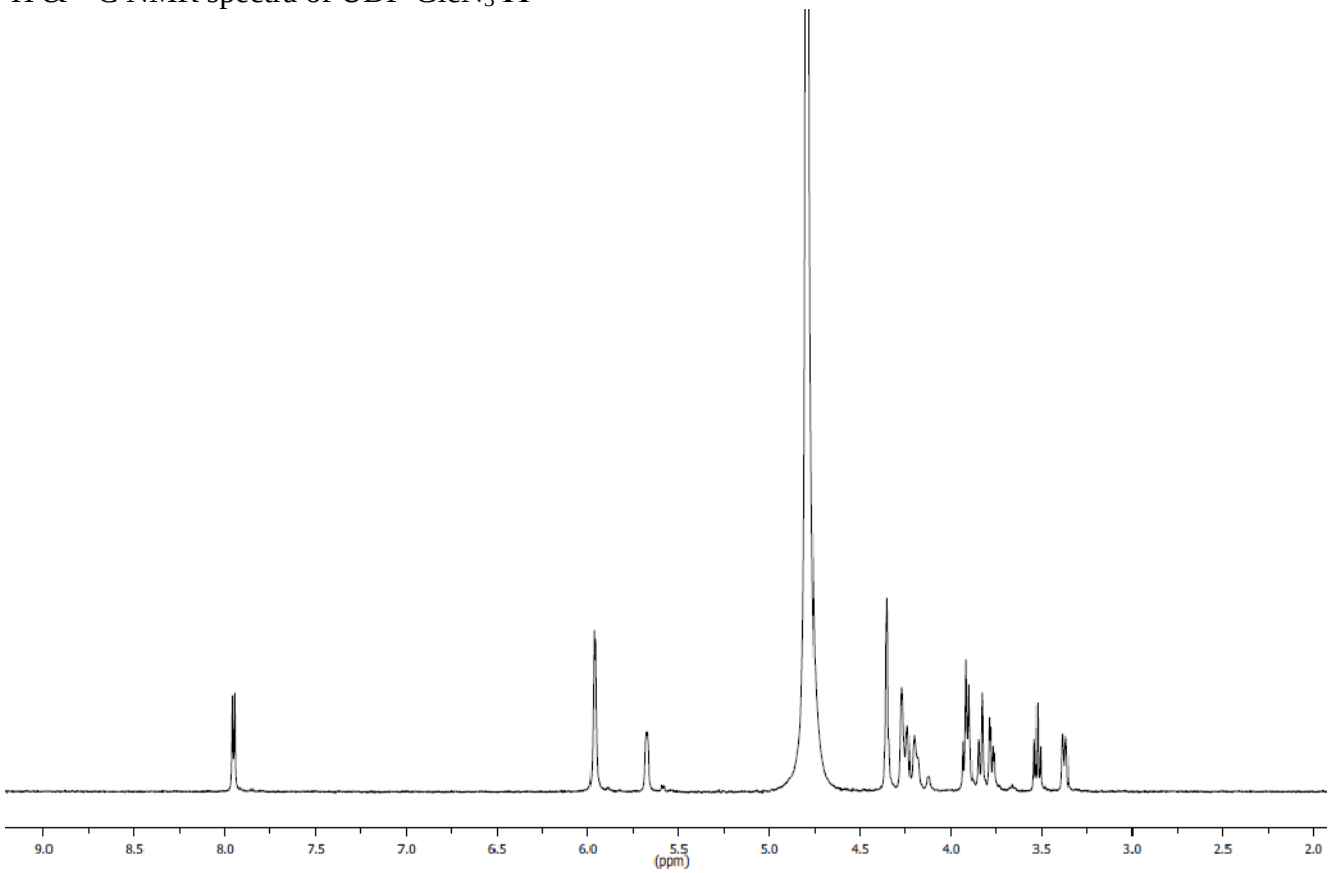


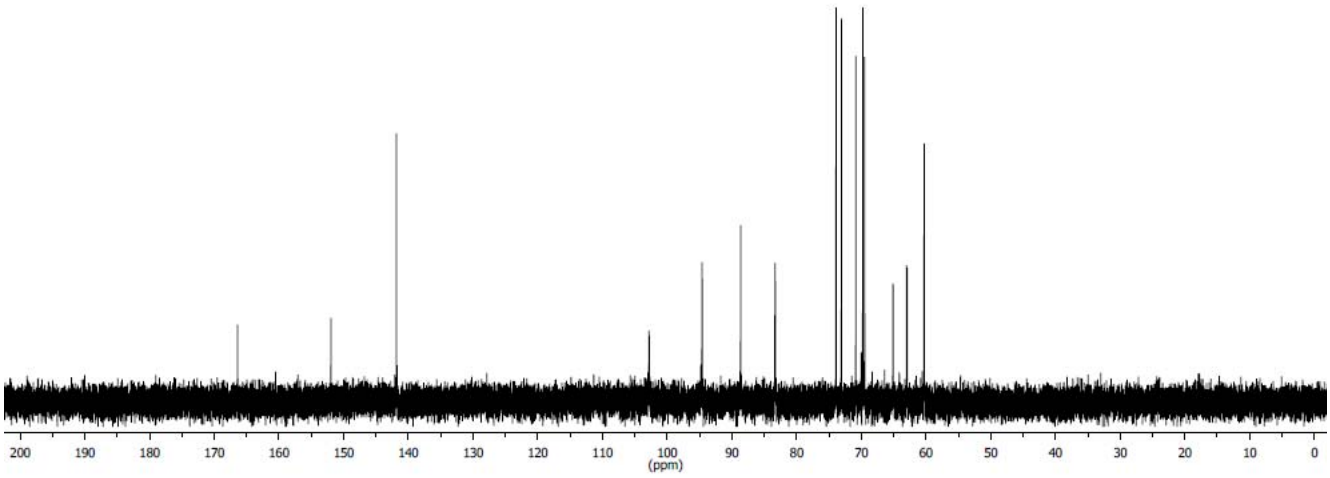
^{1}H & ^{13}C NMR spectra of UDP-GlcNTFA **10**



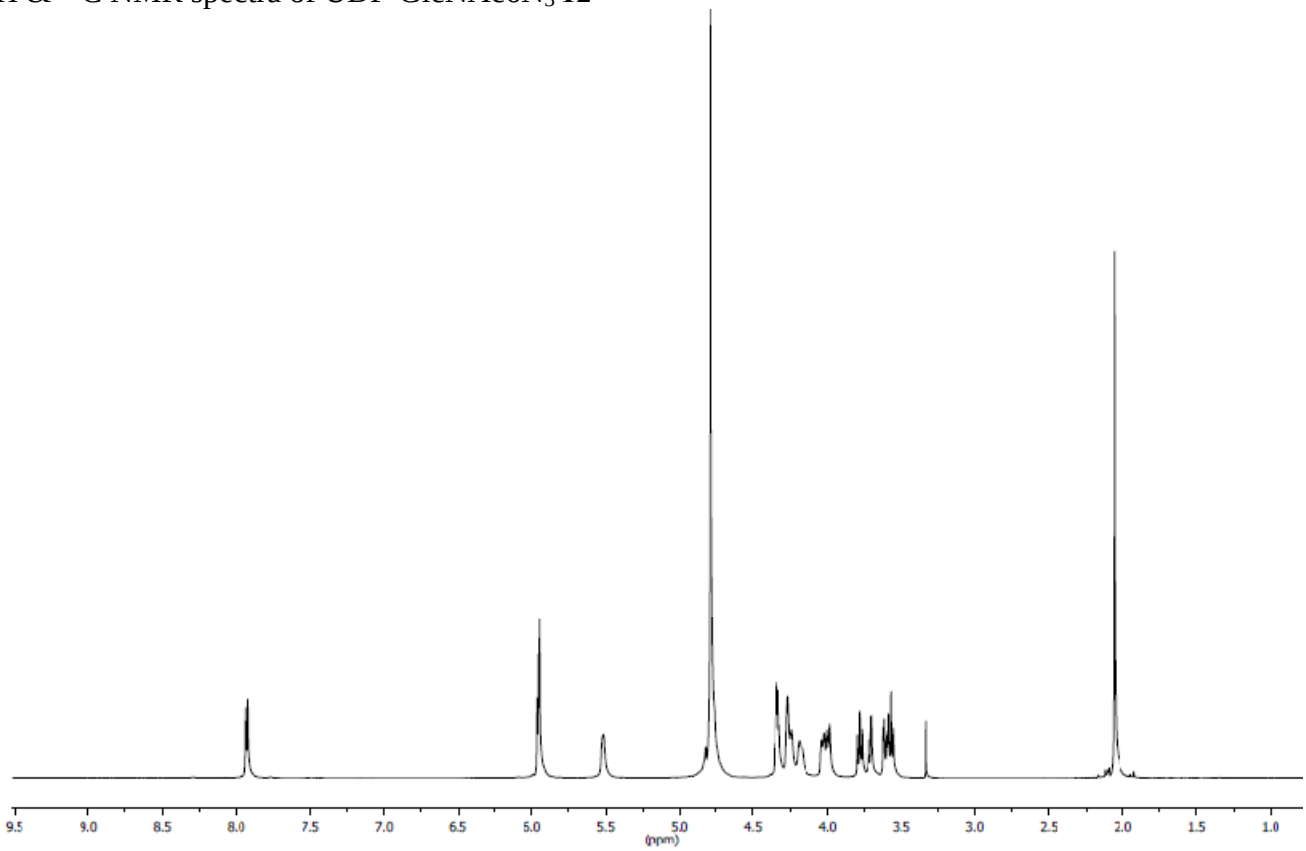


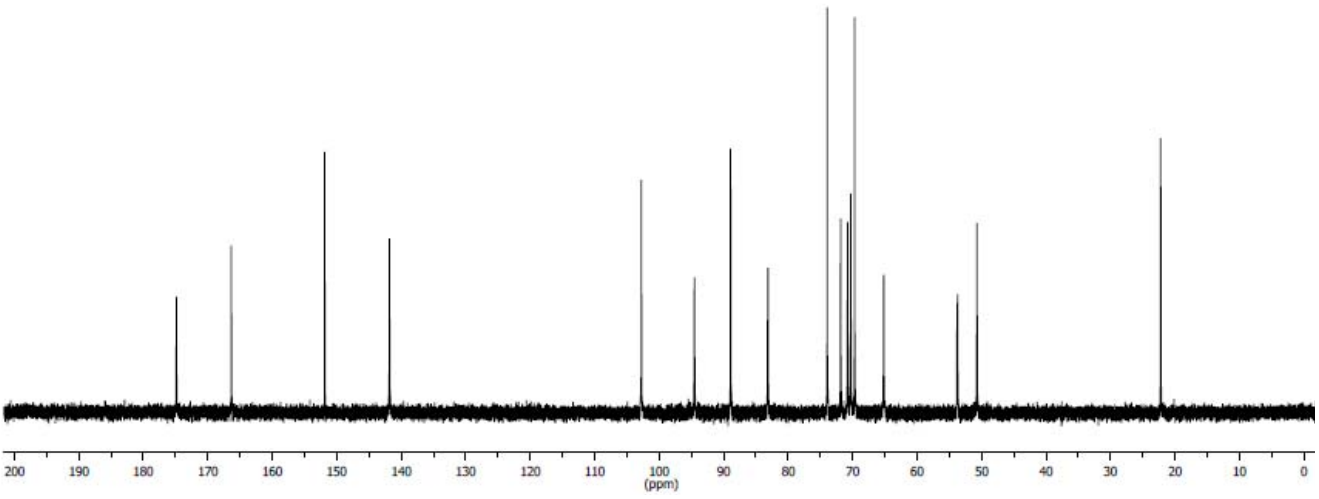
^{1}H & ^{13}C NMR spectra of UDP-$GlcN_3$ **11**



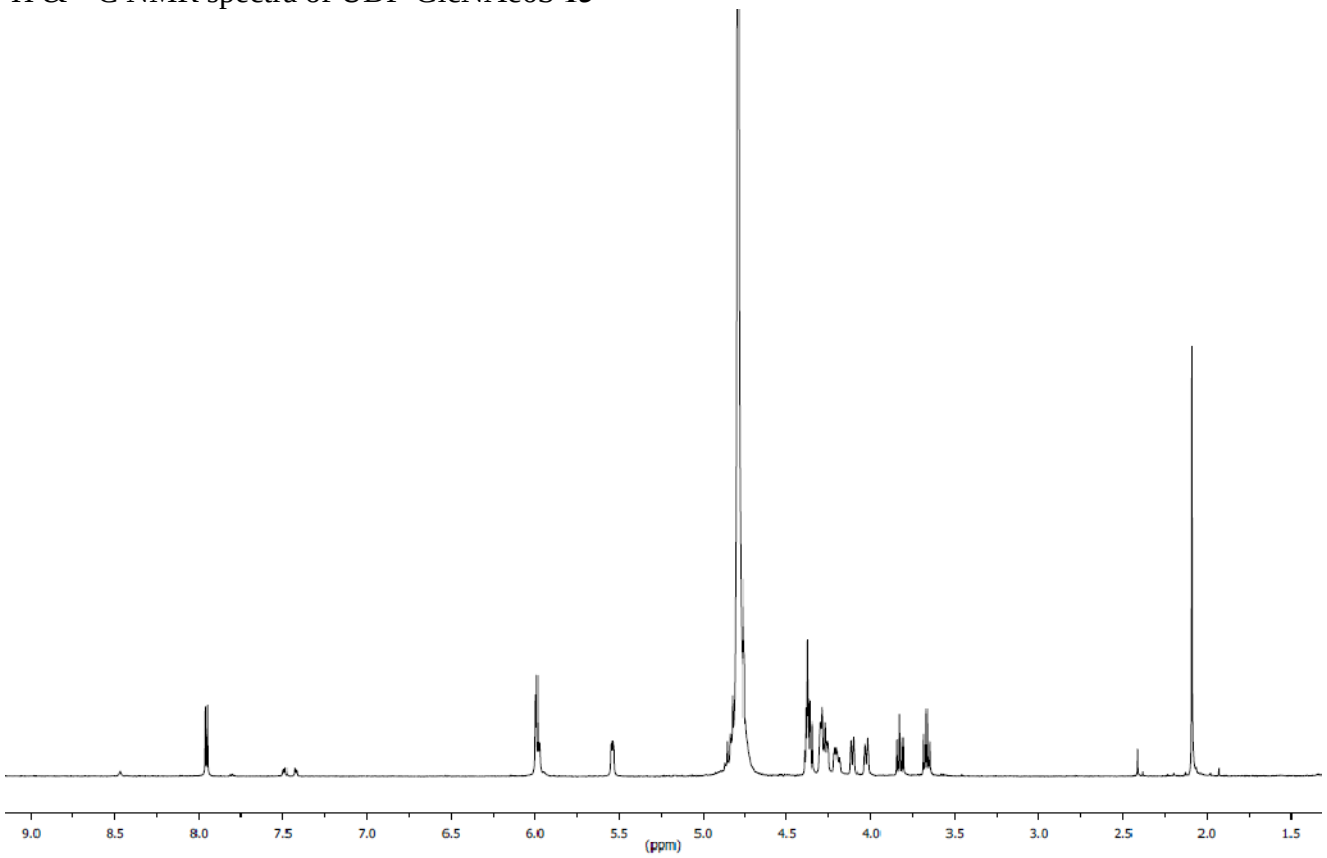


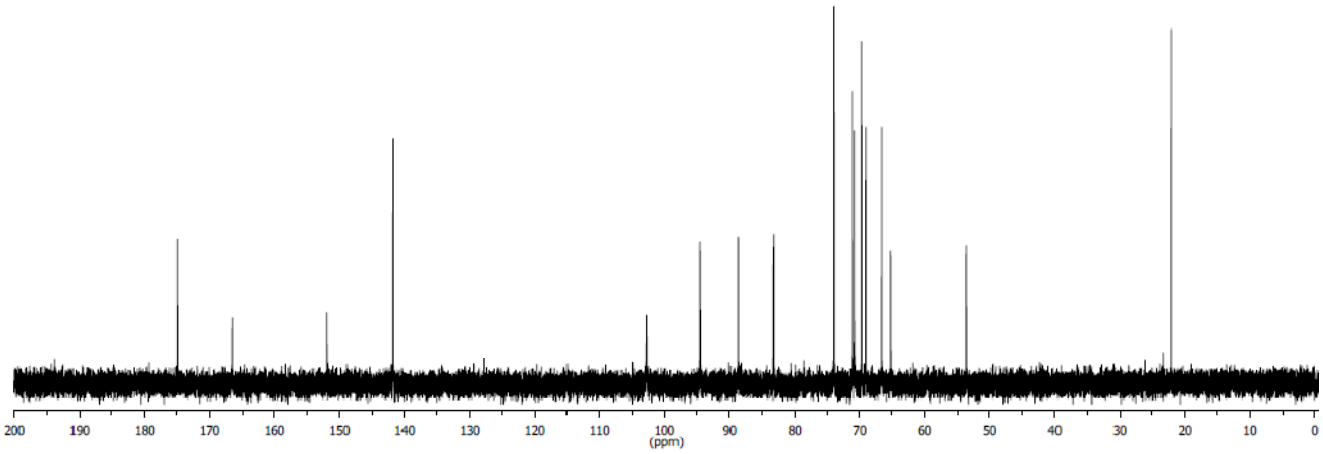
^{1}H & ^{13}C NMR spectra of UDP-GlcNAc6N_3 **12**



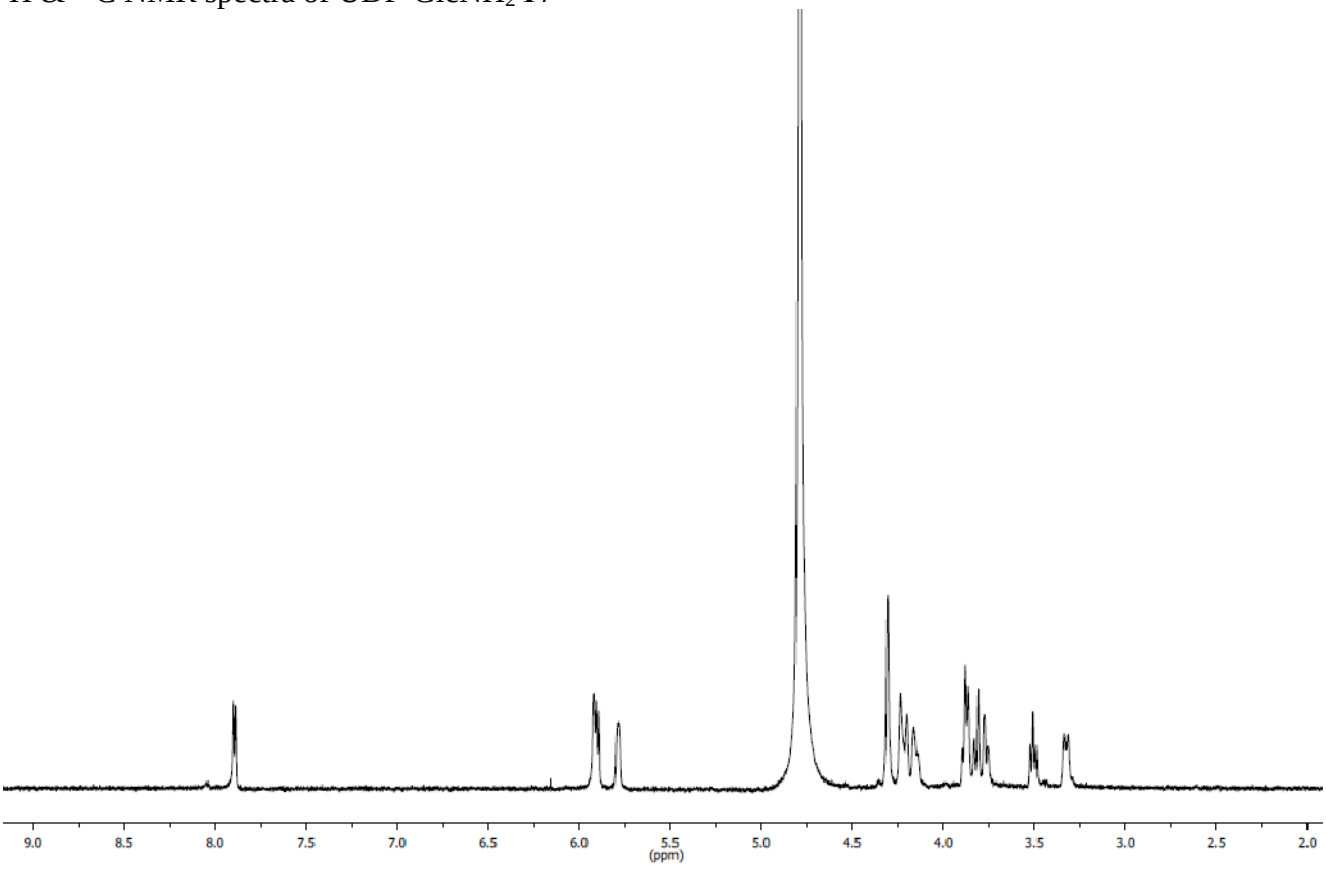


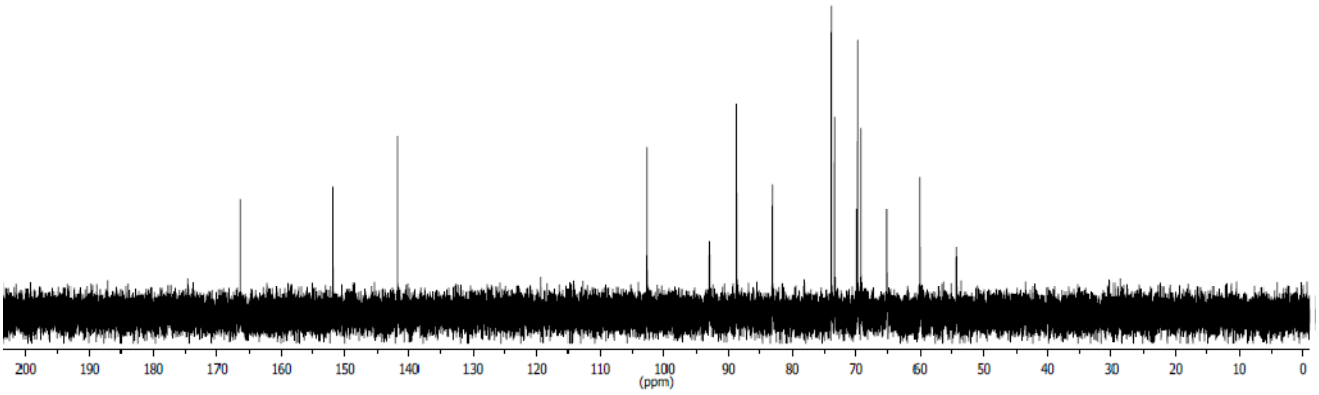
^{1}H & ^{13}C NMR spectra of UDP-GlcNAc6S **13**



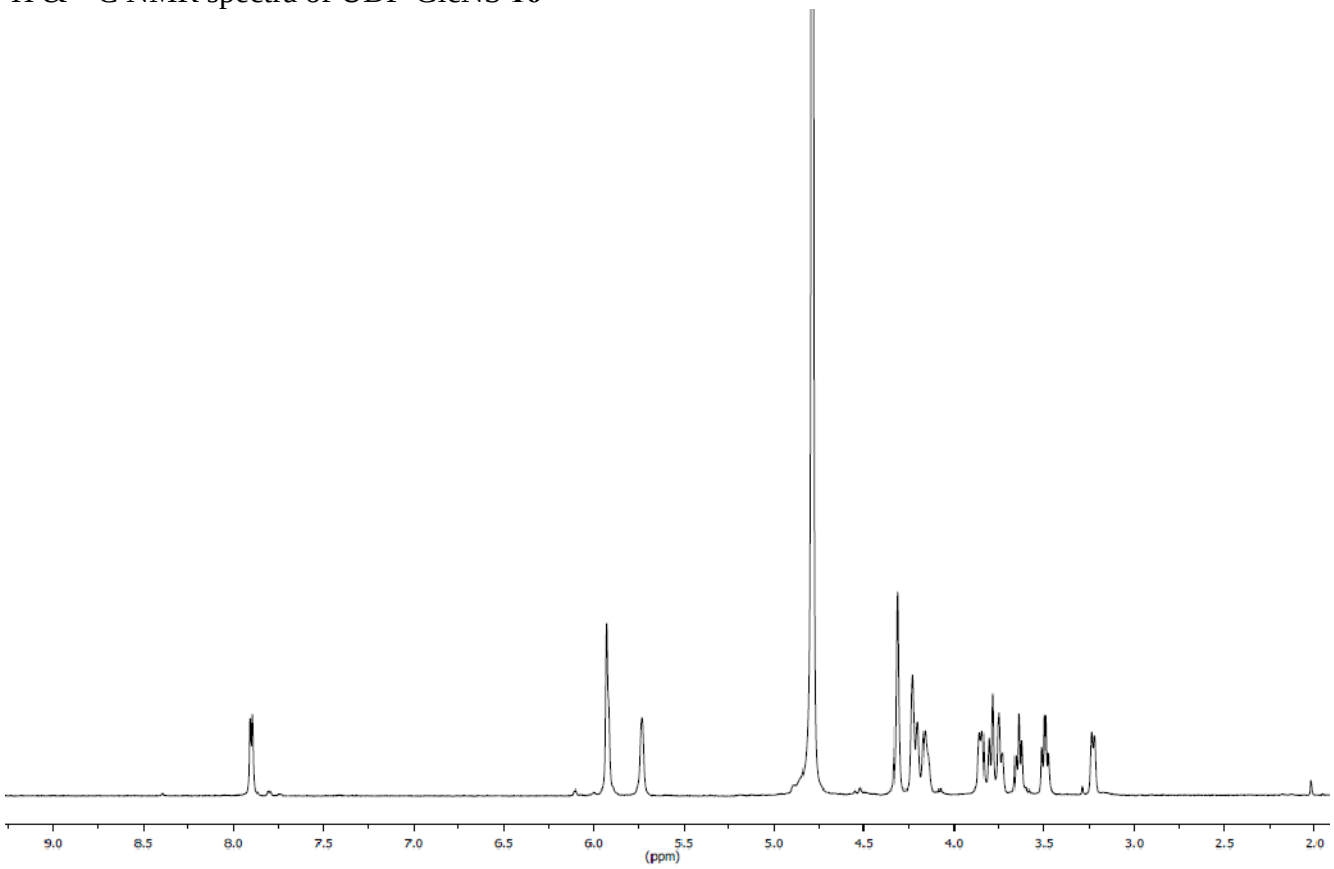


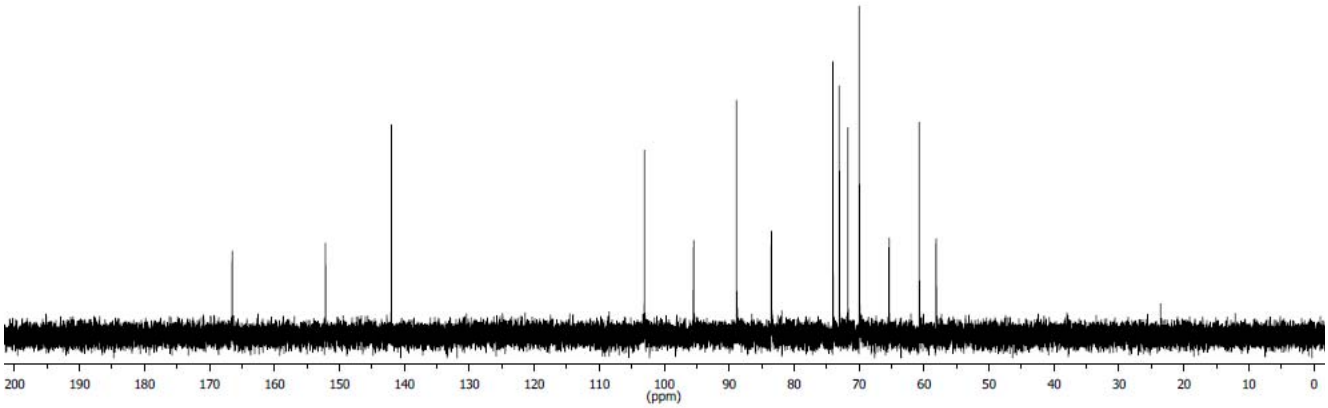
^{1}H & ^{13}C NMR spectra of UDP-GlcNH_2 **17**



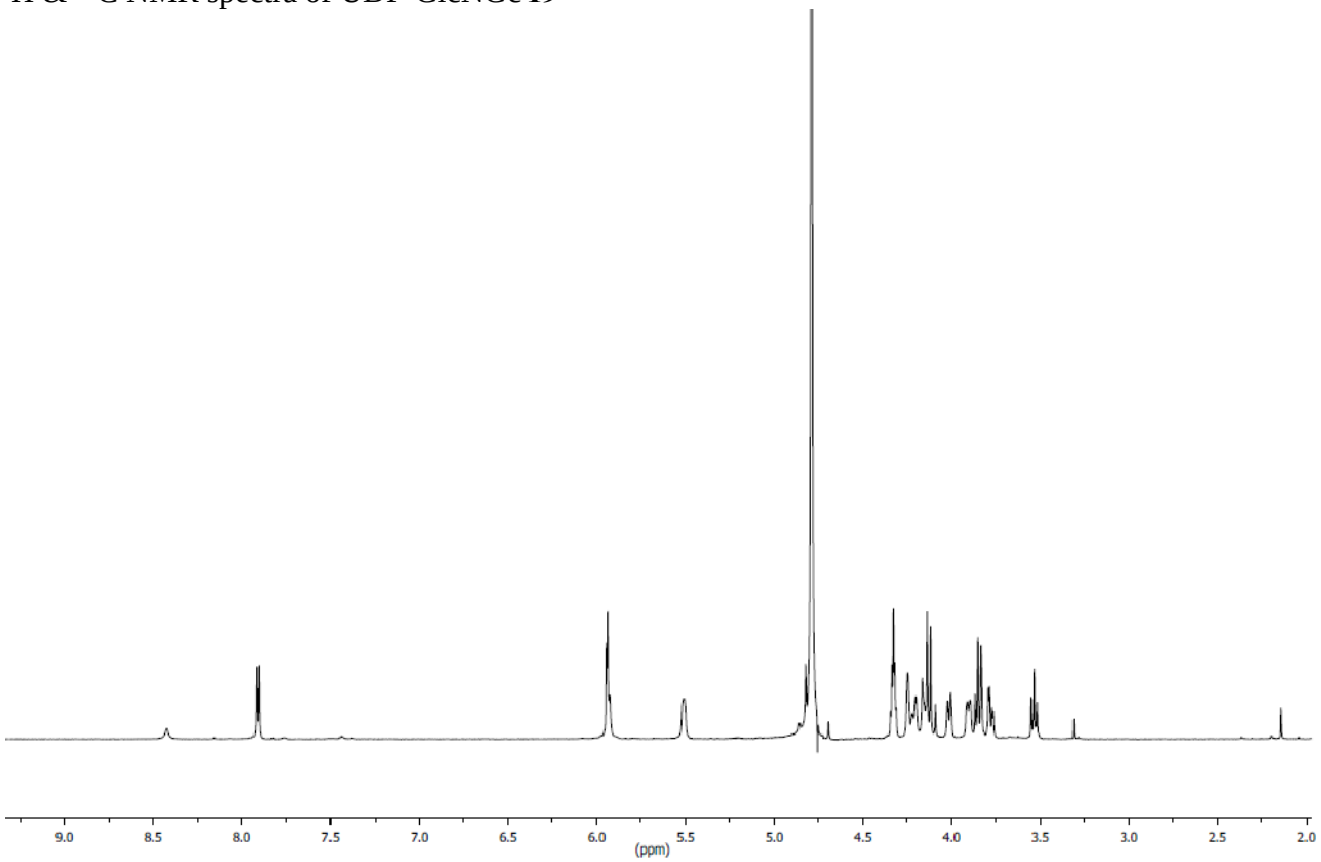


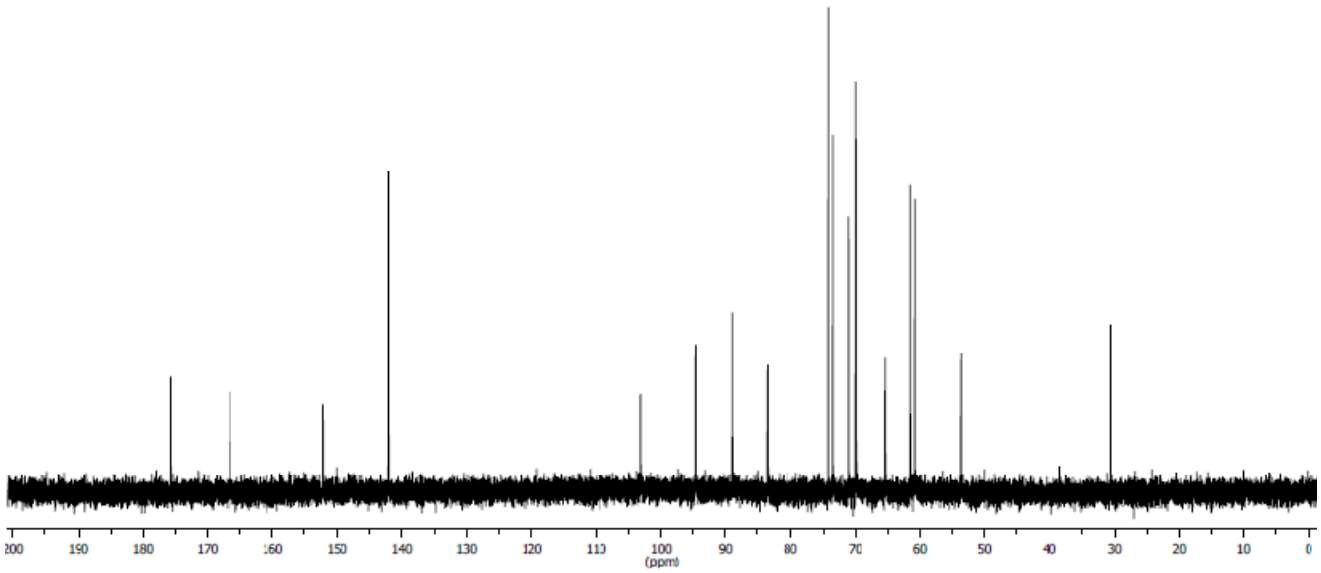
^{1}H & ^{13}C NMR spectra of UDP-GlcNS **16**



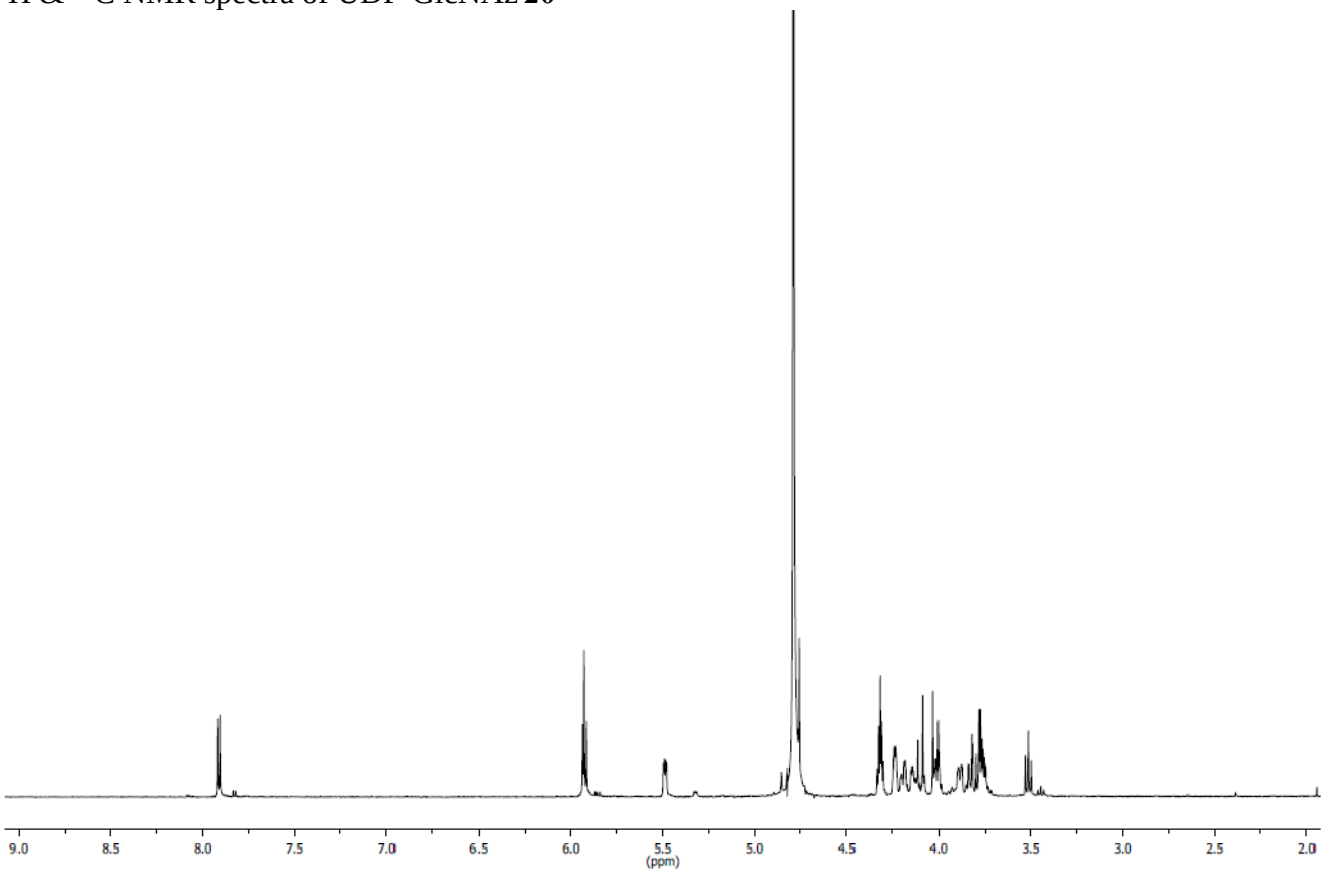


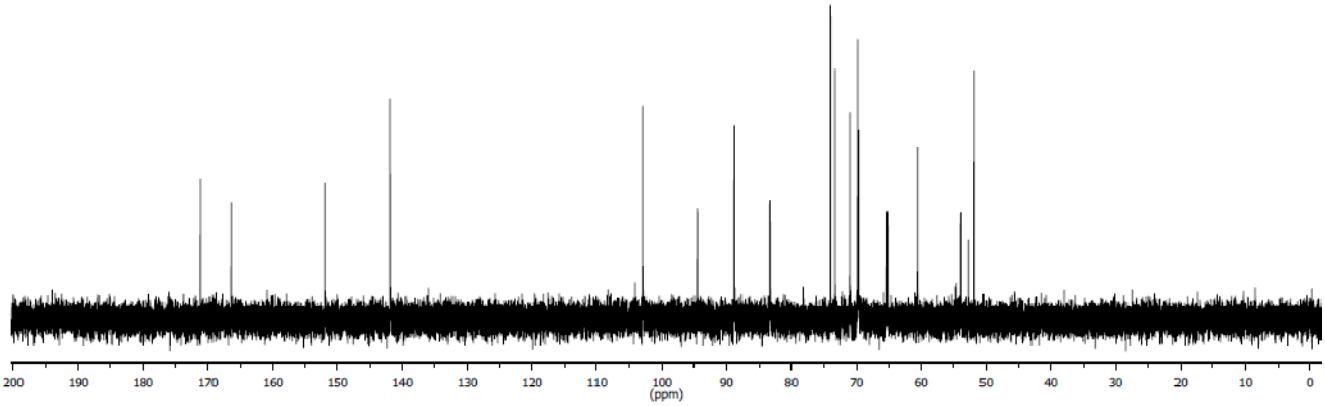
^{1}H & ^{13}C NMR spectra of UDP-GlcNGc **19**



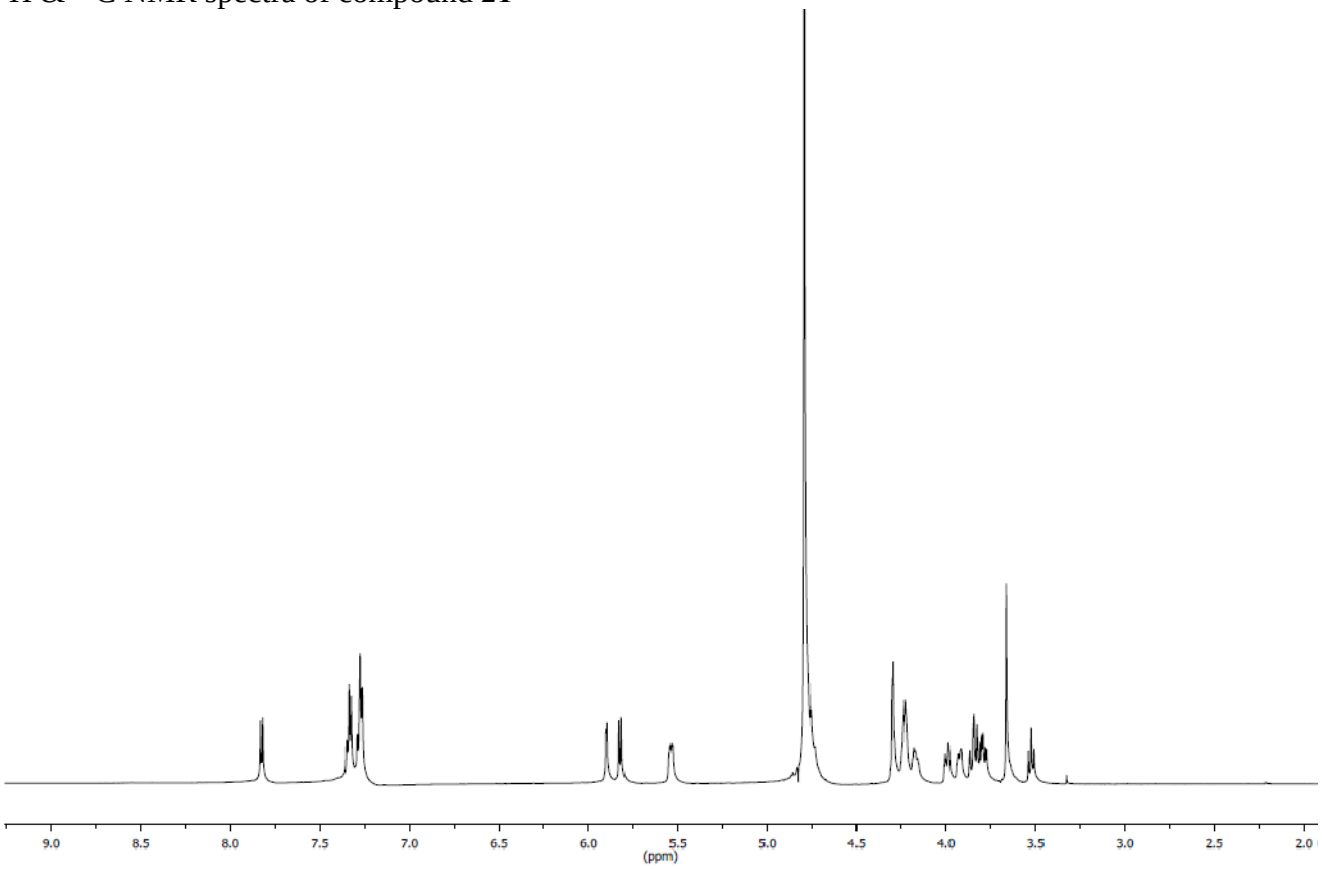


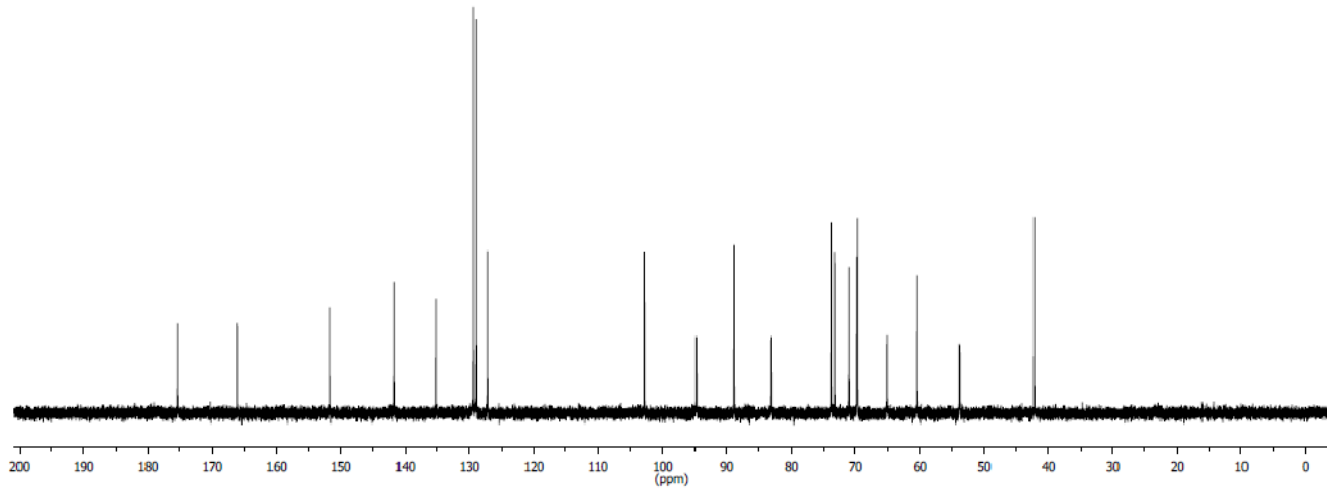
^{1}H & ^{13}C NMR spectra of UDP-GlcNAz **20**



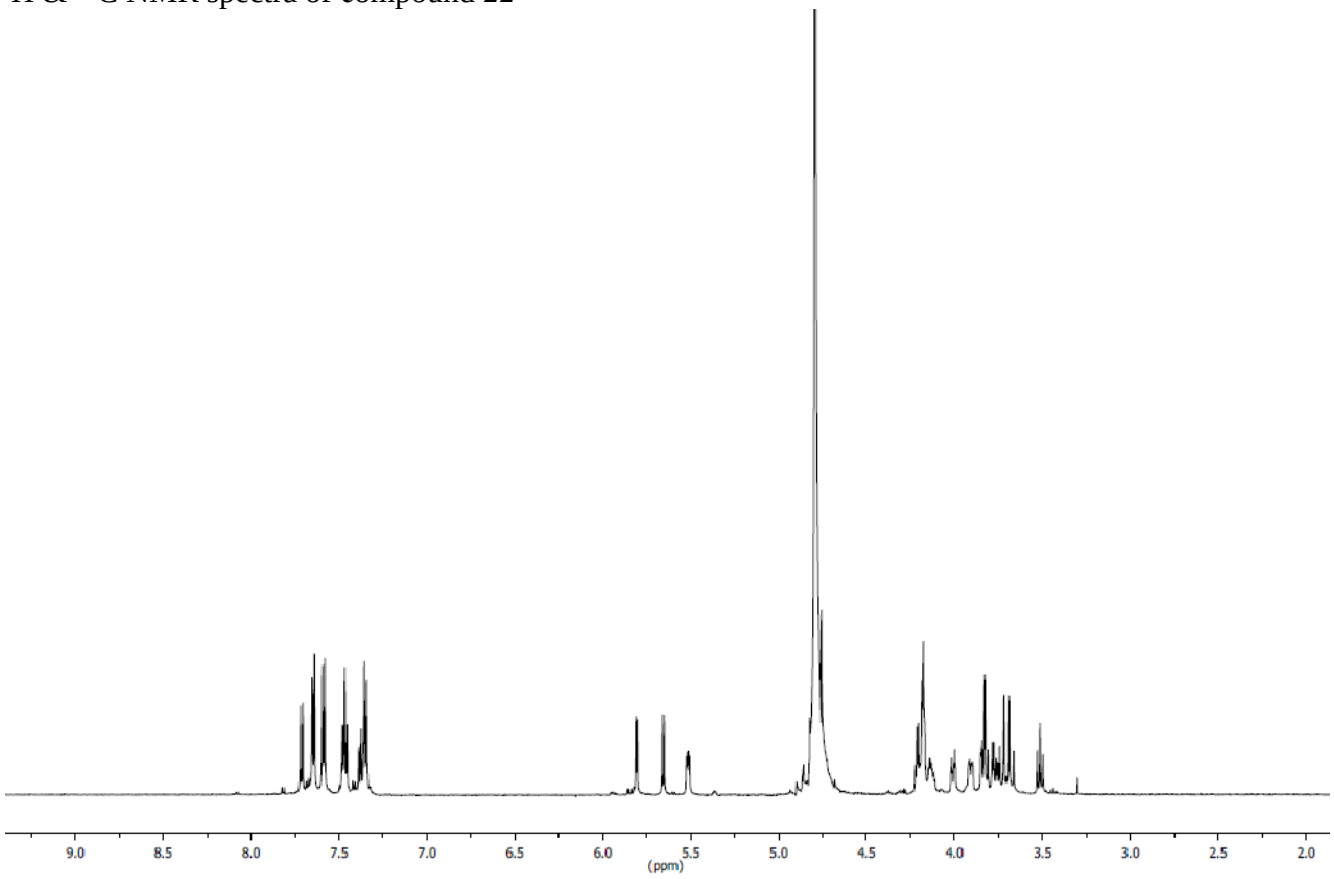


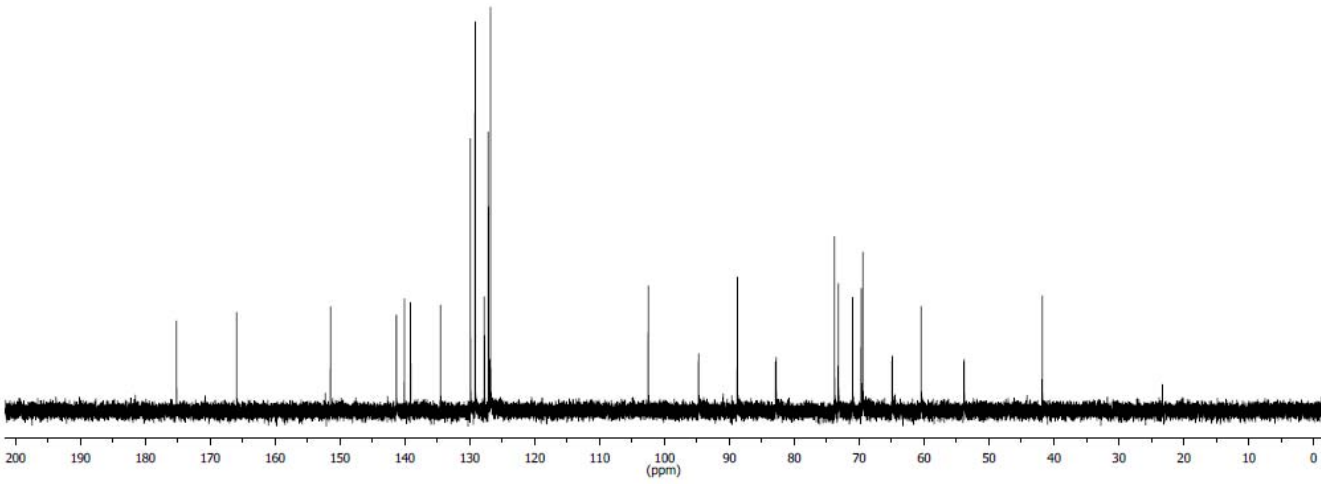
^{1}H & ^{13}C NMR spectra of compound **21**



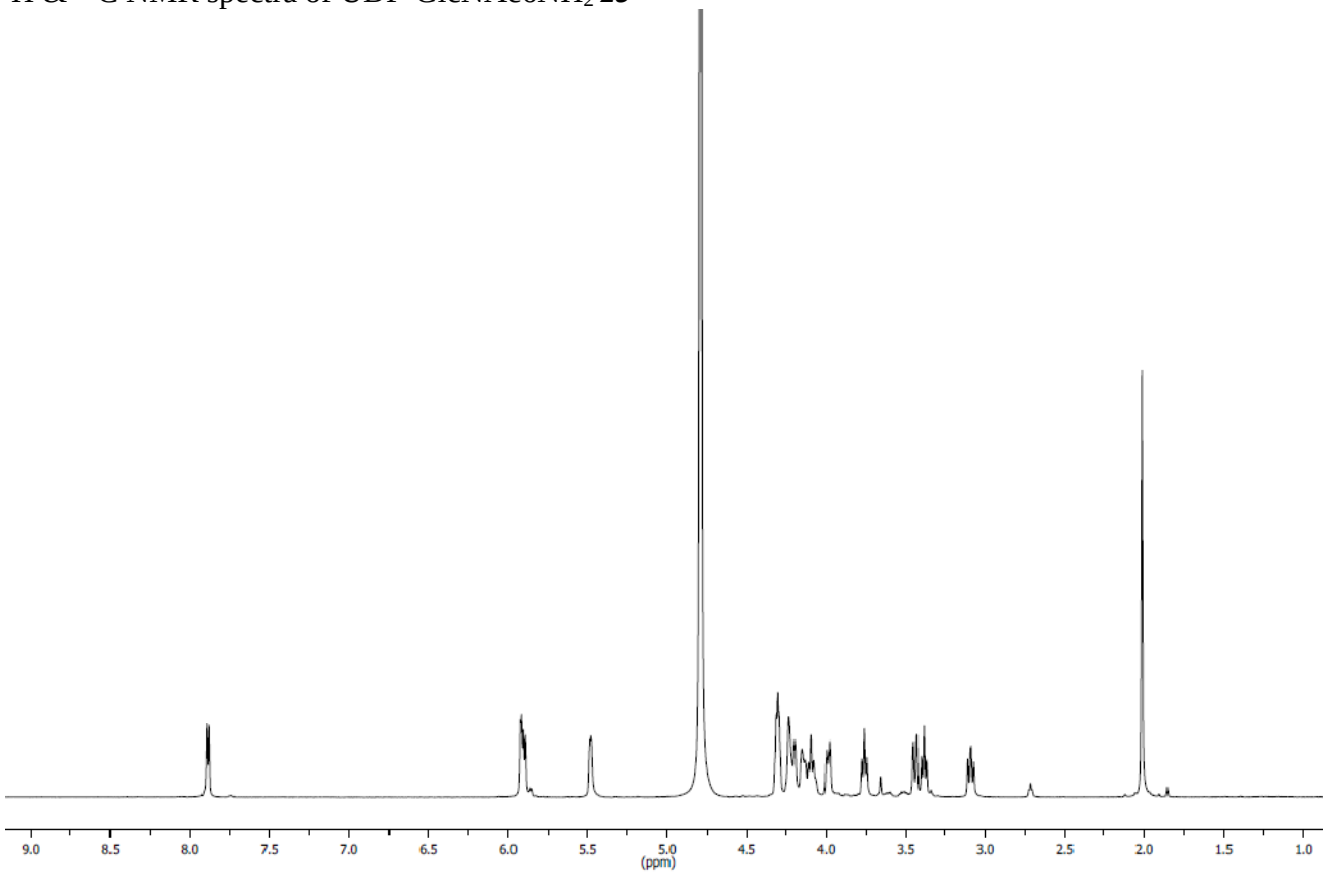


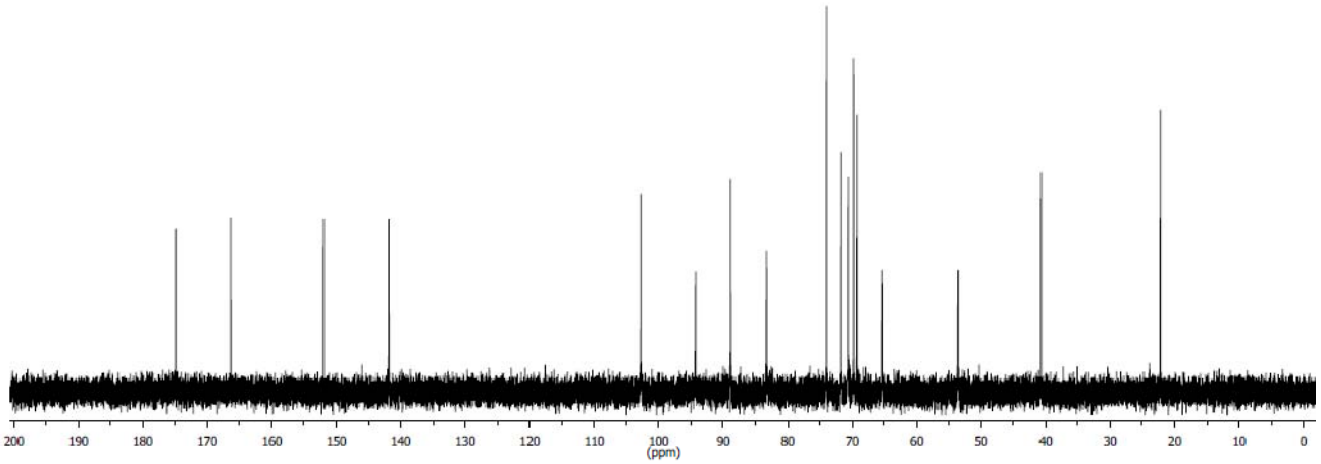
^{1}H & ^{13}C NMR spectra of **c**ompound **22**



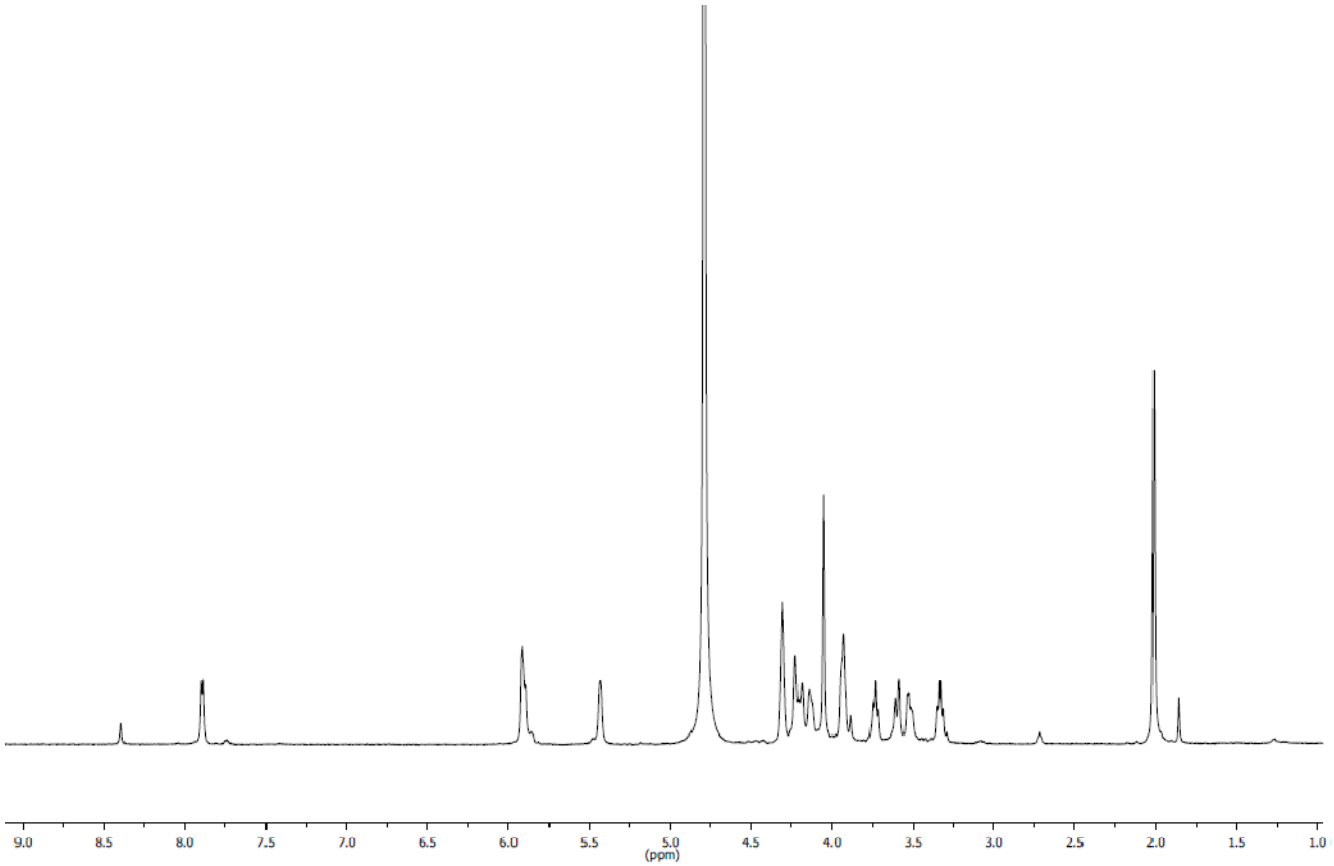


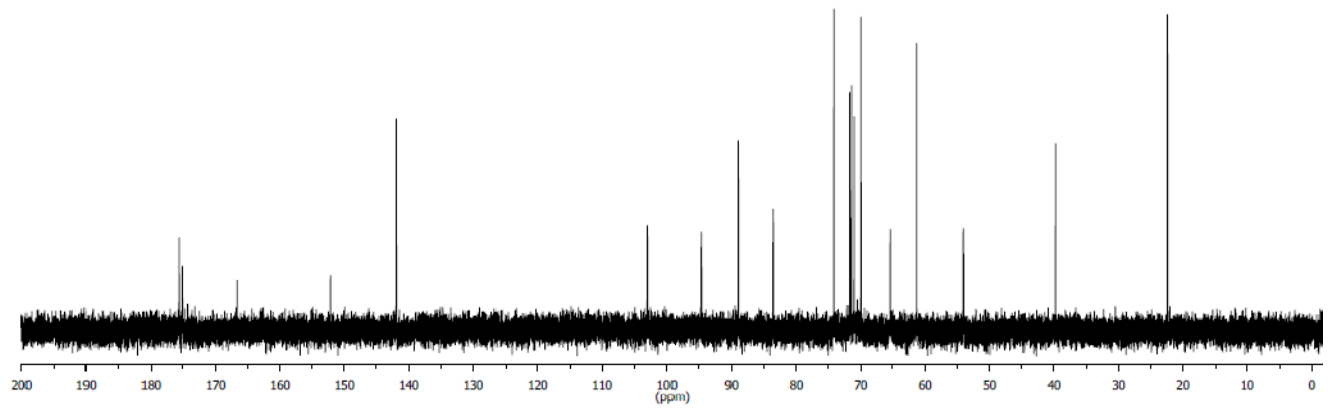
^{1}H & ^{13}C NMR spectra of UDP-GlcNAc6NH_2 **23**



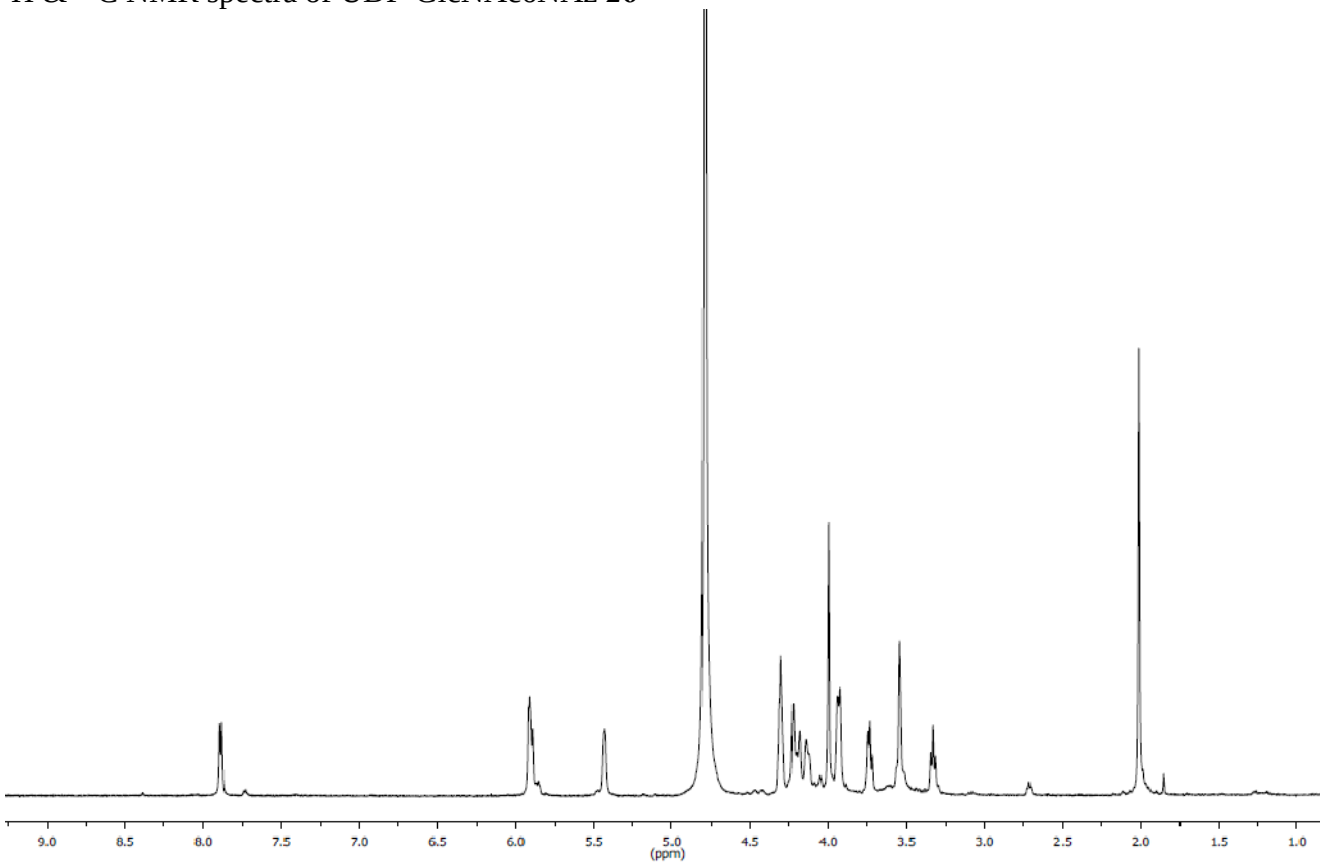


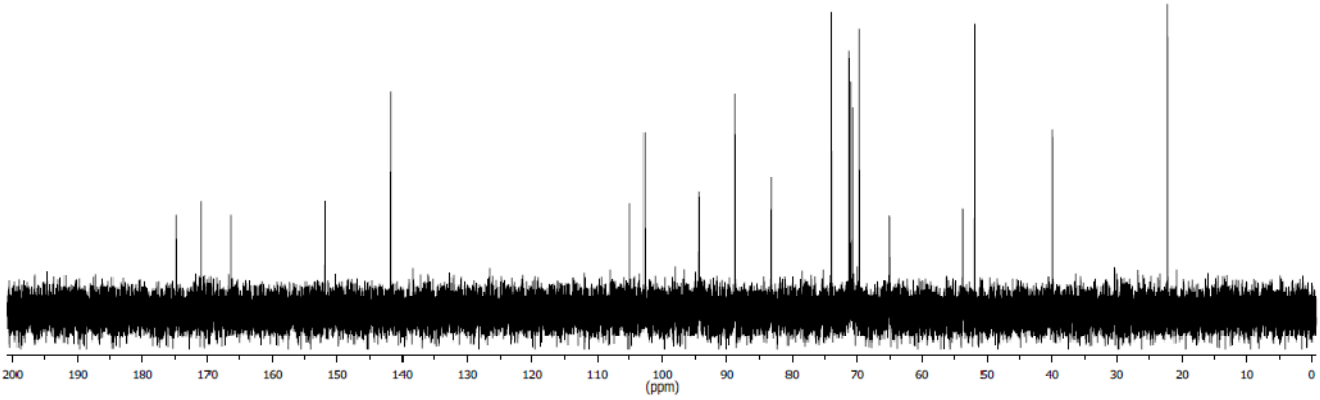
^{1}H & ^{13}C NMR spectra of UDP-GlcNAc6NGc **25**



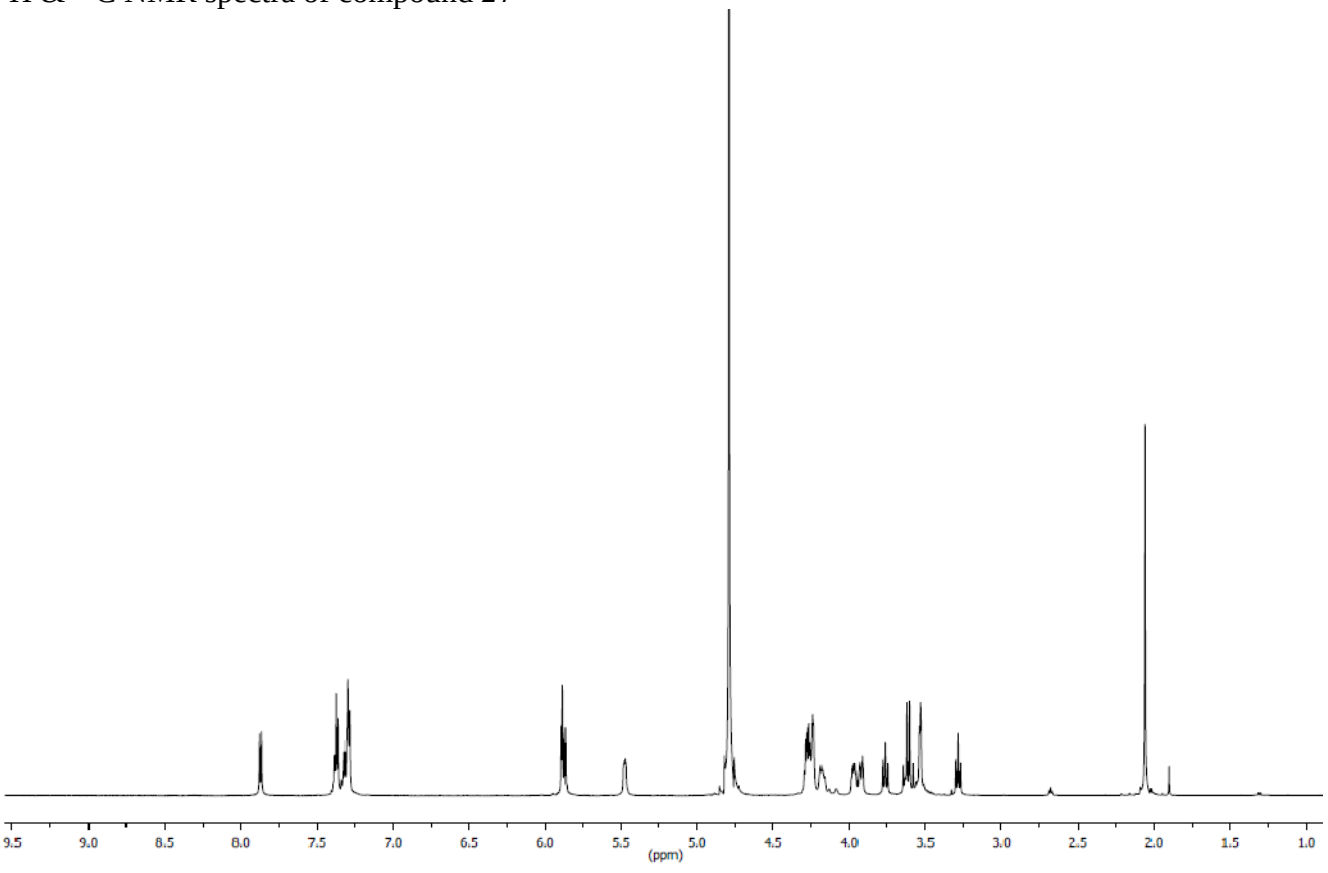


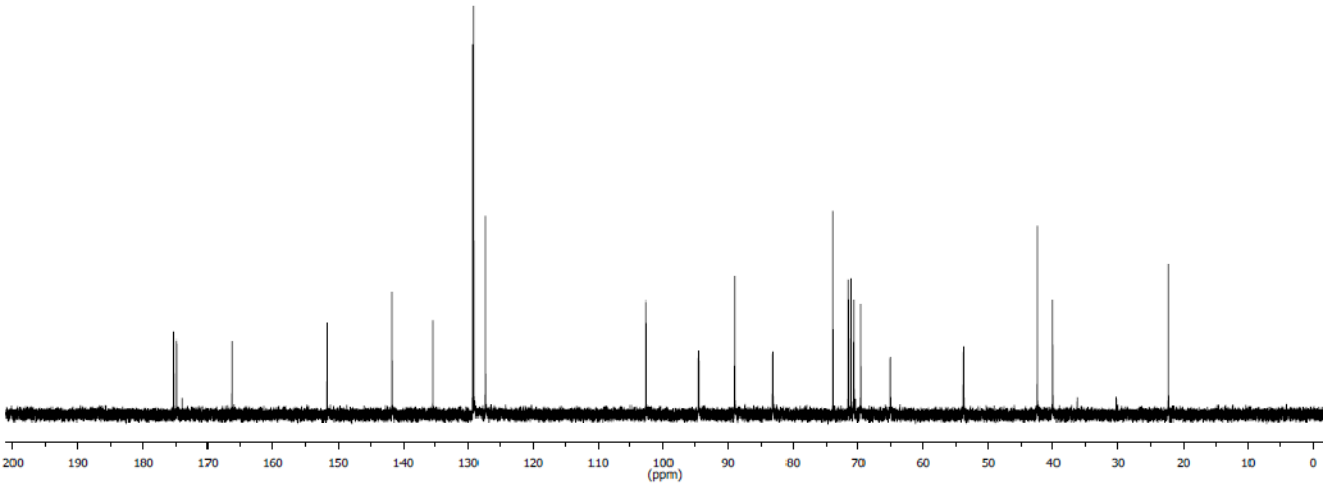
^{1}H & ^{13}C NMR spectra of UDP-GlcNAc6NAz **26**





^{1}H & ^{13}C NMR spectra of compound **27**





^{1}H & ^{13}C NMR spectra of **c**ompound **28**