Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Material

Gram-scale production of sugar nucleotides and their derivatives

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I. Supplementary Figures

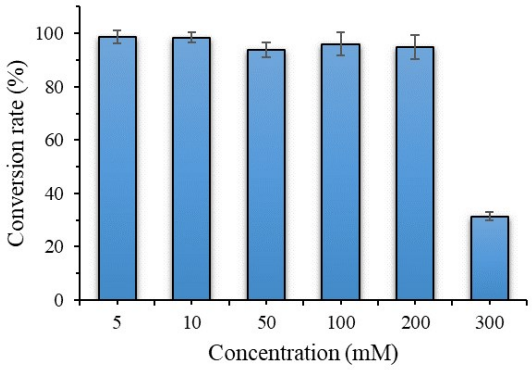


Figure S1. Effects of GlcNAc substrate concentration on conversion rate of UDP-GlcNAc.

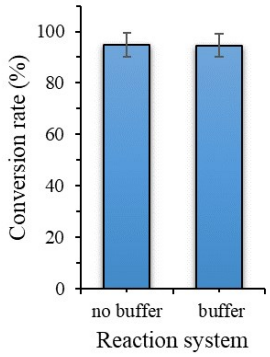


Figure S2. Effect of buffer sytem on enzymatic conversion rate of UDP-GlcNAc.

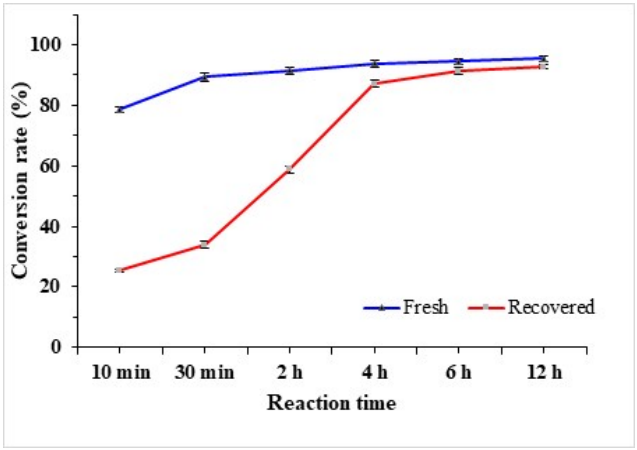


Figure S3. Evaluation of recovery and recyclability of enzymes for UDP-GlcNAc

Table S1. Enzymes used in this work

Enzyme	Source of the enzyme	E.C. number	Reference
BiNahK	*N*-acetyl hexosamine 1-kinase from *Bifidobacterium infantis*	2.7.1.162	Li, Y et al[1]
PmGlmU	UDP-*N*-acetylgalactosamine pyrophosphorylase from *Pasteurella multocida*	2.3.1.157& 2.7.7.23	Chen, Y et al[2]
AGX1	UDP-*N*-acetylgalactosamine pyrophosphorylase from human	2.7.7.83	Xue, M et al[3]
BiGalK	Galactokinase from *B. infantis*	2..7.1.6	Li, L.et al[4]
AtUSP	UDP-sugar pyrophosphorylase from *Arabidopsis thaliana*	2.7.7.9	Guo,Y et al [5]
AtGlcAK	Glucuronokinase from *A. thaliana*	2.7.1.43	Muthana, M. et al[6]
BfFKP	Bifunctional L-fucokinase/GDP-fucose pyrophosphorylase from *Bacteroides fragilis*	2.7.7.30& 2.7.1.52	Yi, W et al[7]
PfManC	GDP-Man pyrophosphorylase from *Pyrococcus furiosus* DSM3638	2.7.7.13	Li, L[8]
NmCSS	CMP-sialic acid synthetase from *Neisseria meningitidis*	2.7.7.43	Yu, H et.al[9]
PmPPA	inorganic pyrophosphatase from *P. multocida*	3.6.1.1	Lau, K[10]

Table S2. Reaction system for substrate concentration optimization.

Substance	Final concentration					
$MgCl_2$	5	10	50	100	200	300 mM
GlcNAc	5	10	50	100	200	300 mM
ATP	7.5	15	75	150	300	450 mM
UTP	7.5	15	75	150	300	450 mM
BiNahK	0.2	0.4	2	4	8	12 mg/mL
PmGlmU	0.2	0.4	2	4	8	12 mg/mL
PmPPA	0.1	0.2	1	2	4	6 mg/mL

Table S3. Initial data of AAS

Sample	Absorbance	RSD(%)	Concentrations of Na (μg/mL)
Standard 1	0.0013	0.02	0
Standard 2	0.0920	0.05	0.1
Standard 3	0.2094	0.08	0.2
Standard 4	0.3973	0.27	0.4
UDP-GlcNAc	0.2223	0.02	0.22228*

* The concentration of UDP-GlcNAc measured by AAS was 3 μg/mL

Table S4. Initial data of ICP-MS

Sample	Ba 138			Mg 24		
	Net Intensities (cps)	Concentrations (ng/mL)	Concentrations RSDs (%)	Net Intensities (cps)	Concentrations (ng/mL)	Concentrations RSDs (%)
Blank	8.328050291199 78			357.004471691 143		
Standard 1	3201.960084164 07	1	1.486432723535 22	8186.21661330 391	1	2.03977557440 522
Standard 2	16435.69220338 22	5.004988166807 68	1.806420852921 16	31668.8533274 198	4.94438117539 614	0.14251964856 748
Standard 3	33316.77058638 51	10.02970225748 09	3.294338757613 03	61095.2921935 402	9.90118472631 114	2.91512749069 333
Standard 4	183638.9084127 73	50.23031067109 2	1.367338591004 69	302692.529042 107	49.9538143208 804	1.65966449760 25
Standard 5	344936.3711353 24	98.76975816632 19	1.773916357326 84	572192.177754 403	98.7880115155 139	1.06141736373 478
UDP-GlcNAc	156381.1366206 12	44.77848188335 24	1.731068777374 05	77572.6859182 409	13.3927929945 763	1.80988441594 35

II. Experientials information

Materials

Adenosine 5'-triphosphate (ATP), uridine 5'-triphosphate (UTP), guanosine 5'-triphosphate (GTP), and cytidine 5'-triphosphate (CTP) were purchased from Hangzhou Meiya Pharmaceutical Co. Ltd. (Zhejiang, China). Monosaccharides were from Beijing Chemsynlab Co. Ltd. (Beijing, China). All other reagents unless otherwise stated were all of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Amberlite® IRC120 H were purchased from Sigma.

High-resolution electrospray ionization (ESI) mass spectra were obtained using a Bruker HPLC-Orbitrap Elite mass spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on an Agilent DD2 600-MHz NMR spectrometer (D_2O as the solvent). Atomic absorption spectra (AAS) were obtained using a Shimadzu Atomic Absorption Spectroscopy. Inductively coupled plasma mass spectra (ICP-MS) were obtained using a PerkinElmer NEXION Inductively Coupled Plasma Mass Spectrometer. ^{31}P ssNMR spectra were obtained using AVANCE III HD 600 MHz Wide Bore Solid State NMR spectrometer.

Expression and purification of enzymes

All recombinant strains harboring corresponding genes were cloned and stored in our laboratory (Table S1). Briefly, *Escherichia coli* BL21 (DE3) strains were cultured in lysogeny broth (LB) medium with 100 μg/mL ampicillin at 37 ℃ under vigorous shaking at 200 rpm. When OD_{600} reached 0.6-0.8, 0.2 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) was added and cultured at 16 ℃ for 20 h. Cells were harvested by centrifuging at 8, 000 rpm for 15 min. Protein purification were performed by utilizing Ni-NTA Sepharose affinity resin according to the protocol. The purified fraction was dialyzed with a Millipore Amicon ultra 10 K centrifugal filter to remove imidazole and other components.

Optimization of reaction system concentration

Enzymatic synthesis of UDP-GlcNAc was carefully studied to optimize the substrate concentration. Parallel reactions were carried out with GlcNAc (5 mM, 10 mM, 50 mM, 100 mM, 200 mM, 300 mM), ATP (1.5 eq.), UTP (1.5 eq.), and $MgCl_2$ (1 eq.) in the present of BiNahK, PmGlmU, and PmPPA for 24 h at 37 ℃ (Table S2). Reactions were quenched by adding equal volume ice-cooled cold ethanol. Formed UDP-GlcNAc was detected by HPLC using a C18 column (5 μm particle size, 250*4.6 mm, Agilent) with flow rate at 1 mL/min using UV detector at 254 nm.

Mobile phase A was 100 mM potassium phosphate buffer, 8 mM tetrabutylammonium bisulfate at pH 6.5. Mobile phase B was 70% deionized water and 30% acetonitrile. Gradient elution was used in the analysis, and the gradient change of the buffer was 0-13 min 100% Buffer A elution, 13-35 min 0-77% Buffer B, 35-39 min 77% Buffer B, 39-40 min Buffer B. Finally, equilibrate with 100% buffer A for 20min.

Optimization of buffer system

Reaction was carried out with 1 M Tris-HCl (pH 7.5), 200 mM GlcNAc, 300 mM ATP, 300 mM UTP, 200 mM $MgCl_2$. Parallel reaction without buffer system was carried out with 200 mM GlcNAc, 300 mM ATP, 300 mM UTP, 200 mM $MgCl_2$. The pH of the reaction mixture was monitored and adjusted by NaOH in the beginning 2 h. After incubation at 37 ℃ for 20 h, reactions were quenched by adding equal volume ice-cooled cold ethanol. The conversion rate of UDP-GlcNAc was measured by HPLC using a C18 column (5μm particle size, 250*4.6 mm, Agilent) with flow rate at 1 mL/min using UV detector at 254 nm.

Evaluation of recovery and recyclability of enzymes for UDP-GlcNAc

UDP-GlcNAc synthetic reaction was carried out in a 5 mL reaction system containing 200 mM GlcNAc, 300 mM ATP, 300 mM UTP, 200 mM $MgCl_2$, 2 mg/L BiNahK, 2 mg/L PmGlmU, and 2 mg/L PmPPA. The pH of the reaction mixture was monitored and adjusted by NaOH in the beginning 2 h. The formation of UDP-GlcNAc and consumption of reaction components were monitored by TLC and HPLC in a time curve. After incubation at 37 ℃ for 12 h, enzymes involved UDP-GlcNAc synthetic process were recovered by ultrafiltration with a 10 kD ultrafiltration tube for ~6 times until no substrate and product existed in the supernatant. To investigate the recyclability of enzymes, further UDP-GlcNAc synthetic reaction was carried out under standard conditions by using recovered enzymes. Reaction was monitored by TLC and HPLC, and other conditions were same as the above.

Precipitation efficiency of metal ion

A mixture containing 20 mM ATP, 20 mM UTP, and 20 mM UDP-GlcNAc was prepared. Then, 20 μL 1 M AgCl, 1 M $ZnCl_2$, 1 M $BaCl_2$, 1 M $CuCl_2$, 1 M $CaCl_2$, 1 M $NiSO_4$, 1 M $MnCl_2$, or deionized water (negative control) was added to 100 μL prepared mixture, respectively. The mixtures were vortexed and centrifuged to remove the formed precipitate. The absorbance of supernatants was measured by the UV absorbance at 254 nm. The precipitation efficiency of metal ion toward mixture was calculated by comparing with the untreated mixture.

Similarly, 20 μL 1 M AgCl, 1 M $BaCl_2$ solution or deionized water (negative control) were added to 20 mM UDP-GlcNAc, UDP-GlcA, UDP-GalA, CMP-Neu5Ac, GDP-Man, ATP, UTP, GTP, and CTP solution, respectively. The precipitation efficiency was measured as above.

XPS of formed precipitate

1 M barium chloride ($BaCl_2$) was mixed with 1 M ATP solution or 1 M UTP solution to generate corresponding precipitates, respectively. The resultant precipitates (ATP+Ba^{2+} and UTP+Ba^{2+}) were

collected and washed with deionized water for three times. Lyophilized precipitates were further characterized by XPS.

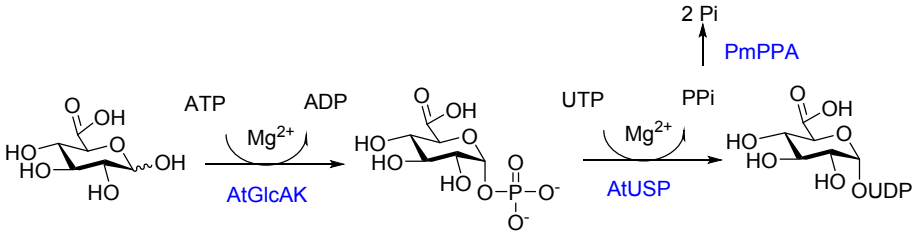
^{13}P ssNMR of formed precipitate

1 M barium chloride ($BaCl_2$) was mixed with 1 M ATP solution or 1 M UTP solution to generate corresponding precipitates, respectively. The resultant precipitates (ATP+Ba^{2+} and UTP+Ba^{2+}) were collected and washed with deionized water for three times. 100 mg ATP, 100 mg UTP, 100 mg ATP+Ba^{2+} and 100 mg UTP+Ba^{2+} precipitates were further characterized by VANCE III HD 600 MHz Wide Bore Solid State NMR spectrometer.

AAS and ICP-MS of UDP-GlcNAc

30 mg UDP-GlcNAc was dissolved in 1mL concentrated nitric acid, and digested overnight at 100 °C. Extra acid was removed by heating at 120 °C for 5 h. UDP-GlcNAc sample was adjusted to 1 L with deionized water. Ion concentration of Na^+, Ba^{2+}, Mg^{2+} was detected by AAS and ICP-MS, respectively.

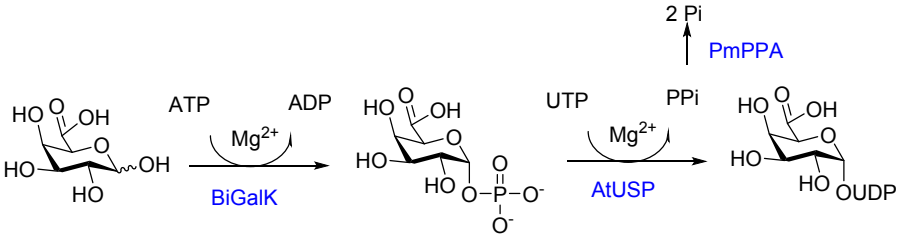
Synthesis of uridine 5′-diphosphate glucuronic acid (UDP-GlcA)



Reaction mixtures containing 200 mM GlcA (0.8 g), 300 mM ATP (2.2 g), 300 mM UTP (1.9 g), and 200 mM $MgCl_2$ was adjusted with 1 M NaOH to pH 7.5, followed by pre-incubated at 37 ℃ for 15min before addition of 2 mg/L AtGlcAK, 2 mg/L AtUSP and 1 mg/L PmPPA to a final volume of 20 mL. The reaction was incubated at 37 ℃ with shaking at 80 rpm for 24 h. pH value of the reaction system was monitored and adjusted with 1 M NaOH every 15 min at the beginning 2 h. Thin Layer Chromatography (TLC) was used to monitor the formation of UDP-GlcA and the consumption of ATP and UTP. The reaction was quenched by adding equal volume ice-cooled ethanol when starting monosaccharide GlcA was consumed thoroughly. Appropriate amounts 1 M barium chloride solution was drop wisely added to the reaction supernatant to remove unreacted nucleotides and byproduct. Formed insoluble precipitate was removed by centrifugation at 13, 000 rpm for 5 min. The above ion precipitation was repeated until no precipitation formed. 200 mL pretreated Amberlite® IRC120 H cation exchange resin (exchange capacity 2 mmol/mL) were added to the supernatant and mixed thoroughly to affinity positively charged impurities, such as Ba^{2+} and Mg^{2+} for 30 min at 4 ℃. A routine filtration process was performed to remove cation exchange resin and the supernatant was lyophilized to generate pure UDP-GlcA. Final product was characterized by ESI-MS and NMR, respectively.

UDP-GlcA: ^{1}H NMR (600 MHz, D_2O) δ 7.92 (d, *J* = 8.1 Hz, 1H), 5.98 – 5.91 (m, 2H), 5.59 (dd, *J* = 7.6, 3.5 Hz, 1H), 4.35 – 4.32 (m, 2H), 4.25 (s, 1H), 4.22 – 4.18 (m, 1H), 4.15 (ddd, *J* = 11.8, 5.4, 2.7 Hz, 1H), 4.10 (d, *J* = 10.2 Hz, 1H), 3.75 (t, *J* = 9.5 Hz, 1H), 3.54 (dt, *J* = 9.8, 3.1 Hz, 1H), 3.47 (t, *J* = 9.7 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 176.29, 166.13, 151.72, 141.41, 102.55, 95.23, 95.18, 88.09, 83.18, 83.12, 73.64, 72.87, 72.41, 71.69, 71.24, 71.19, 69.50, 64.82, 64.79. ESI-MS (negative ion): Calcd for $C_{15}H_{22}N_2O_{18}P_2$: 580.0343, $[M-H]^-$ 579.0265; Found $[M-H]^-$ 579.0156.

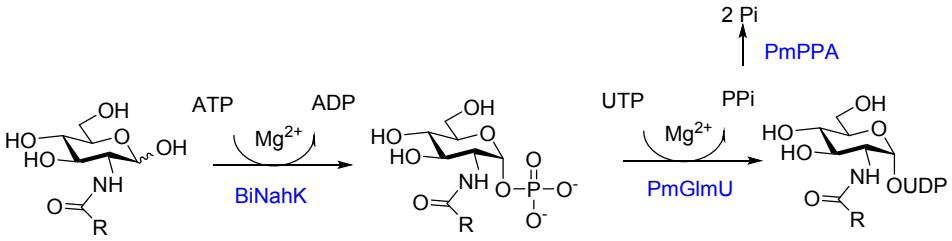
Synthesis of uridine 5′- diphosphate galacturonic acid (UDP-GalA)



Due to the reduced catalytic efficiency of BiGalK toward GalA even in 10 mM concentration, the reaction was optimized by conducting at 100 mM, with elongated incubation time and more enzymes. Reaction mixtures containing 100 mM GalA (0.8 g), 150 mM ATP (2.2 g), 150 mM UTP (1.9 g) and 100 mM $MgCl_2$ was adjusted with 1 M NaOH to pH 7.5, followed by pre-incubated at 37 ℃ for 15 min before addition of 2 mg/mL BiGalK, 2 mg/L AtUSP, and 1 mg/L PmPPA to a final volume of 40 mL. The reaction was incubated at 37 ℃ with shaking at 80 rpm for 24 h. The pH value of the reaction system was monitored and adjusted with 1 M NaOH every 15 min at the beginning 2 h. TLC was used to monitor the formation of UDP-GalA and the consumption of ATP and UTP. The reaction was quenched by addition of equal volume ice-cooled cold ethanol until all starting monosaccharide was consumed thoroughly. The purification process is the same as reported. Final product was characterized by ESI-MS and NMR, respectively.

UDP-GalA: ^{1}H NMR (600 MHz, D_2O) δ 7.96 (d, *J* = 8.1 Hz, 1H), 6.02 – 5.96 (m, 2H), 5.68 (dd, *J* = 7.2, 3.6 Hz, 1H), 4.51 (d, *J* = 1.2 Hz, 1H), 4.39 – 4.36 (m, 2H), 4.32 (dd, *J* = 3.4, 1.4 Hz, 1H), 4.28 (dt, *J* = 5.2, 2.6 Hz, 1H), 4.24 (ddd, *J* = 11.7, 4.6, 2.5 Hz, 1H), 4.18 (ddd, *J* = 11.8, 5.5, 2.8 Hz, 1H), 3.98 (dd, *J* = 10.3, 3.4 Hz, 1H), 3.84 (dt, *J* = 10.3, 3.2 Hz, 1H).^{13}C NMR (151 MHz, D_2O) δ 175.20, 166.22, 151.83, 141.58, 102.65, 95.72, 95.67, 88.31, 83.28, 83.22, 73.77, 72.76, 70.60, 69.62, 69.35, 68.06, 68.00, 64.93, 64.89. ESI-MS (negative ion): Calcd for $C_{15}H_{22}N_2O_{18}P_2$: 580.0343, $[M-H]^-$ 579.0265; Found $[M-H]^-$ 579.0150.

Synthesis of uridine 5′- diphosphate *N*-acetyl glucosamine (UDP-GlcNAc) and its derivatives



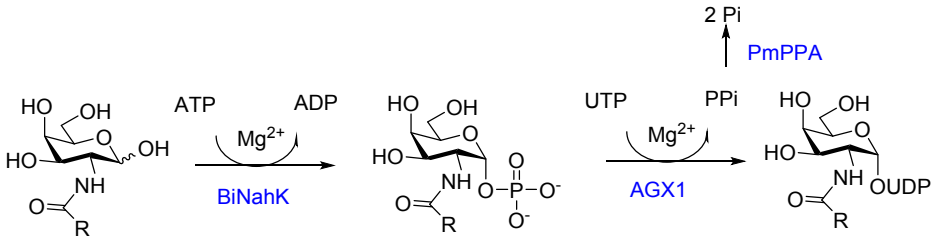
Reaction mixtures containing 200 mM GlcNAc (0.9 g) or GlcNTFA (1.1 g) or GlcNAz (1.0), 300 mM ATP (2.2 g), 300 mM UTP (1.9 g) and 200 mM $MgCl_2$ was adjusted with 1 M NaOH to pH 7.5, followed by pre-incubated at 37 ℃ for 15min before addition of 2 mg/L BiNahK, 2 mg/L PmGlmU and 1 mg/L PmPPA to a final volume of 20 mL. The reaction was incubated at 37 ℃ with shaking at 80 rpm for 24 h. The pH value of the reaction system was monitored and adjusted with 1 M NaOH every 15 min at the beginning 2 h. It should be note that when synthesizing of UDP-GlcNTFA, reaction mixture needs to maintain pH value to 7.0 as GlcNTFA could hydrolyze into glucosamine under alkaline condition. TLC was used to monitor the formation of UDP-sugars and the consumption of ATP and UTP. Synthetic reaction was quenched by addition of equal volume ice-cooled ethanol until all starting monosaccharide was consumed thoroughly. The purification process is the same as reported. Final product was characterized by ESI-MS and NMR, respectively.

UDP-GlcNAc: ^{1}H NMR (600 MHz, D_2O) δ 7.97 (d, *J* = 8.1 Hz, 1H), 5.98 (dd, *J* = 7.8, 6.4 Hz, 2H), 5.53 (dd, *J* = 7.3, 3.3 Hz, 1H), 4.40 – 4.36 (m, 2H), 4.31 – 4.28 (m, 1H), 4.25 (ddd, *J* = 11.7, 4.6, 2.6 Hz, 1H), 4.20 (ddd, *J* = 11.8, 5.6, 3.1 Hz, 1H), 4.00 (dt, *J* = 10.5, 3.0 Hz, 1H), 3.94 (ddd, *J* = 10.1, 4.4, 2.3 Hz, 1H), 3.90 – 3.86 (m, 1H), 3.84 – 3.80 (m, 2H), 3.56 (dd, *J* = 10.0, 9.3 Hz, 1H), 2.09 (s, 3H). ^{13}C NMR (151 MHz, D_2O) δ 174.75, 166.22, 151.80, 141.62, 102.63, 94.49, 94.45, 88.47, 83.21, 83.14, 73.76, 73.00, 70.91, 69.62, 69.49, 64.96, 64.92, 60.31, 53.68, 53.62, 22.05. ESI-MS (negative ion): Calcd for $C_{17}H_{27}N_3O_{17}P_2$: [M] 607.0816, $[M-H]^-$ 606.0737; Found $[M-H]^-$ 606.0629.

UDP-GlcNTFA: ^{1}H NMR (600 MHz, D_2O) δ 7.97 (d, *J* = 8.1 Hz, 1H), 5.98 (dd, *J* = 8.8, 6.4 Hz, 2H), 5.62 (dd, *J* = 7.0, 3.3 Hz, 1H), 4.37 (p, *J* = 5.2 Hz, 2H), 4.29 (dd, *J* = 5.5, 2.9 Hz, 1H), 4.24 (ddd, *J* = 11.7, 4.5, 2.6 Hz, 1H), 4.19 (ddd, *J* = 11.8, 5.6, 3.0 Hz, 1H), 4.11 (dt, *J* = 10.6, 2.9 Hz, 1H), 4.00 – 3.92 (m, 2H), 3.89 (dd, *J* = 12.5, 2.3 Hz, 1H), 3.83 (dd, *J* = 12.5, 4.3 Hz, 1H), 3.59 (dd, *J* = 9.9, 9.4 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 166.29, 159.58, 159.33, 151.85, 141.64, 114.80, 102.64, 93.76, 93.72, 88.41, 83.24, 83.18, 73.76, 72.98, 70.26, 69.62, 69.48, 64.90, 64.86, 60.18, 54.38, 54.32. ESI-MS (negative ion): Calcd for $C_{17}H_{24}F_3N_3O_{17}P_2$: [M] 661.0533, $[M-H]^-$ 660.0455, $[M-2H+Na]^-$ 682.0274; Found $[M-H]^-$ 660.0337, $[M-2H+Na]^-$ 682.0151.

UDP-GlcNAz: ^{1}H NMR (600 MHz, D_2O) δ 7.96 (d, *J* = 8.1 Hz, 1H), 6.02 – 5.96 (m, 2H), 5.55 (dd, *J* = 7.2, 3.3 Hz, 1H), 4.38 (td, *J* = 9.7, 5.2 Hz, 2H), 4.31 – 4.28 (m, 1H), 4.25 (ddd, *J* = 11.7, 4.6, 2.6 Hz, 1H), 4.19 (ddd, *J* = 11.8, 5.7, 3.3 Hz, 1H), 4.22 – 4.13 (m, 2H), 4.10 – 4.05 (m, 2H), 3.95 (ddd, *J* = 10.1, 4.4, 2.2 Hz, 1H), 3.85 (tdd, *J* = 12.5, 10.9, 3.4 Hz, 3H), 3.57 (dd, *J* = 10.0, 9.3 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 170.95, 166.29, 151.85, 141.65, 102.65, 94.38, 94.34, 88.53, 83.18, 83.12, 73.75, 73.02, 70.85, 69.62, 69.44, 65.01, 64.98, 60.28, 53.73, 53.67, 51.58. ESI-MS (negative ion): Calcd for $C_{17}H_{26}N_6O_{17}P_2$: [M] 648.0830, $[M-H]^-$ 647.0751, $[M-2H+Na]^-$ 669.0571; Found $[M-H]^-$ 647.0635, $[M-2H+Na]^-$ 669.0438.

Synthesis of uridine 5′- diphosphate *N*-acetyl galactosamine (UDP-GalNAc) and its derivatives



Reaction mixtures containing 200 mM GalNAc (0.9 g) or GalNTFA (1.1 g), GalNAz (1.0), 300 mM ATP (2.2 g), 300 mM UTP (1.9 g) and 200 mM $MgCl_2$ was adjusted with 1 M NaOH to pH 7.5, followed by pre-incubated at 37 ℃ for 15min before addition of 2 mg/mL BiNahK, 2 mg/L AGX1 and 1 mg/L PmPPA to a final volume of 20 mL. Reaction was incubated at 37 ℃ with shaking at 80 rpm for 24 h. The pH value of the reaction system was monitored and adjusted with 1 M NaOH every 15 min at the beginning 2 h. It should be note that when synthesizing of UDP-GalNTFA, reaction mixture needs to maintain pH value to 7.0 as GalNTFA could hydrolyze into galactosamine under alkaline condition. TLC was used to monitor the formation of UDP-sugars and the consumption of ATP and UTP. The reaction was quenched by addition of equal volume ice-cooled ethanol until all starting monosaccharide was consumed thoroughly. The purification process is the same as reported. The structures of the obtained products were confirmed by NMR and MS analysis.

UDP-GalNAc: ^{1}H NMR (600 MHz, D_2O) δ 7.91 (d, *J* = 8.1 Hz, 1H), 5.92 (dd, *J* = 10.4, 6.2 Hz, 2H), 5.50 (dd, *J* = 7.1, 3.2 Hz, 1H), 4.31 (t, *J* = 6.2 Hz, 2H), 4.24 (s, 1H), 4.20 (d, *J* = 11.1 Hz, 2H), 4.15 – 4.12 (m, 2H), 3.91 (dd, *J* = 11.0, 2.7 Hz, 1H), 3.75 – 3.67 (m, 3H), 2.03 (s, 3H). ^{13}C NMR (151 MHz, D_2O) δ 174.82, 166.11, 151.66, 141.51, 102.48, 94.56, 94.52, 88.35, 83.08, 83.02, 73.64, 71.95, 69.49, 68.21, 67.46, 64.86, 64.82, 60.91, 49.64, 49.58, 22.00. ESI-MS (negative ion): Calcd for $C_{17}H_{27}N_3O_{17}P_2$: [M] 607.0816, $[M-H]^-$ 606.0737; Found $[M-H]^-$ 606.0627.

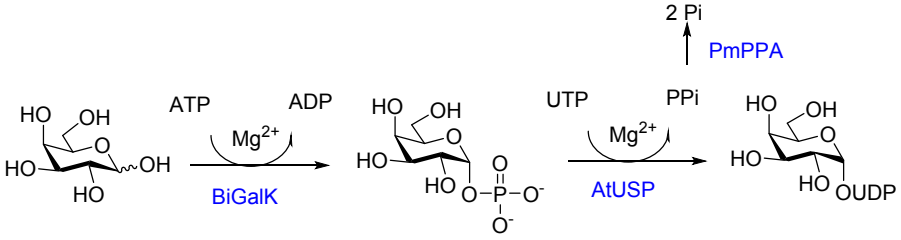
UDP-GalNTFA: ^{1}H NMR (600 MHz, D_2O) δ 7.96 (dd, *J* = 7.4, 3.4 Hz, 1H), 6.00 – 5.96 (m, 2H), 5.64 (dd,

J = 7.0, 3.4 Hz, 1H), 4.39 – 4.35 (m, 2H), 4.34 (t, *J* = 3.0 Hz, 1H), 4.30 – 4.28 (m, 1H), 4.24 (ddd, *J* = 7.0, 4.8, 2.9 Hz, 2H), 4.19 (ddd, *J* = 11.8, 5.7, 3.0 Hz, 1H), 4.12 (dd, *J* = 11.0, 3.2 Hz, 1H), 4.08 (d, *J* = 2.9 Hz, 1H), 3.78 (ddd, *J* = 17.0, 11.8, 6.2 Hz, 2H). ^{13}C NMR (151 MHz, D_2O) δ 166.24, 159.73, 151.81, 141.66, 114.81, 102.61, 93.91, 93.87, 88.44, 83.25, 83.18, 73.77, 72.03, 69.62, 68.31, 66.85, 64.91, 64.87, 60.91, 50.74, 50.69. ESI-MS (negative ion): Calcd for $C_{17}H_{24}F_3N_3O_{17}P_2$: [M] 661.0533, $[M-H]^-$ 660.0455; Found $[M-H]^-$ 660.0336, $[M-2H+Na]^-$ 682.0146.

UDP-GalNAz: ^{1}H NMR (600 MHz, D_2O) δ 7.97 – 7.95 (m, 1H), 6.02 – 5.95 (m, 2H), 5.58 (dd, *J* = 7.2, 3.5 Hz, 1H), 4.37 (dt, *J* = 9.4, 5.2 Hz, 2H), 4.31 (dtd, *J* = 9.3, 6.3, 2.9 Hz, 2H), 4.25 (ddd, *J* = 11.7, 4.6, 2.6 Hz, 1H), 4.22 – 4.14 (m, 3H), 4.10 – 4.05 (m, 2H), 4.00 (dd, *J* = 10.9, 3.1 Hz, 1H), 3.92 – 3.59 (m, 4H).

^{13}C NMR (151 MHz, D_2O) δ 171.13, 166.23, 151.80, 141.67, 102.62, 94.58, 94.53, 88.54, 83.19, 83.13, 73.75, 72.10, 69.63, 68.37, 67.51, 65.00, 61.02, 51.61, 49.92, 49.87. ESI-MS (negative ion): Calcd for $C_{17}H_{26}N_6O_{17}P_2$: [M] 648.0830, $[M-H]^-$ 647.0751; Found $[M-H]^-$ 647.0631, $[M-2H+Na]^-$ 669.0427.

Synthesis of uridine 5′- diphosphate galactose (UDP-Gal)

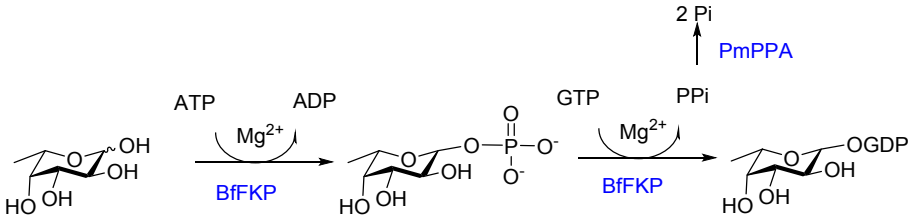


Reaction mixtures containing 200 mM Gal (0.7 g), 300 mM ATP (2.2 g), 300 mM UTP (1.9 g) and 200 mM $MgCl_2$ was adjusted with 1 M NaOH to pH 7.5, followed by pre-incubated at 37 ℃ for 15min before addition of 2 mg/L BiGalK, 2 mg/L AtUSP and 1 mg/L PmPPA to a final volume of 20 mL. The reaction was incubated at 37 ℃ with shaking at 80 rpm for 24 h. The pH value of the reaction system was monitored and adjusted with 1 M NaOH every 15 min at the beginning 2 h. TLC was used to monitor the formation of UDP-Gal and the consumption of ATP and UTP. The reaction was quenched by addition of equal volume ice-cooled ethanol until starting monosaccharide galactose was consumed thoroughly. The purification process is the same as reported. The structures of the obtained products were confirmed by NMR and MS analysis.

UDP-Gal: ^{1}H NMR (600 MHz, D_2O) δ 7.97 (d, *J* = 8.1 Hz, 1H), 6.00 (dd, *J* = 12.0, 6.2 Hz, 2H), 5.65 (dd, *J* = 7.2, 3.6 Hz, 1H), 4.40 – 4.37 (m, 2H), 4.32 – 4.29 (m, 1H), 4.26 (ddd, *J* = 11.7, 4.5, 2.6 Hz, 1H), 4.23 – 4.17 (m, 2H), 4.04 (d, *J* = 3.2 Hz, 1H), 3.93 (dd, *J* = 10.3, 3.3 Hz, 1H), 3.82 (dt, *J* = 10.3, 3.2 Hz, 1H), 3.75 (qd, *J* = 11.8, 6.2 Hz, 2H). ^{13}C NMR (151 MHz, D_2O) δ 166.32, 151.89, 141.60, 102.67, 95.84, 95.80, 88.37, 83.26, 83.20, 73.77, 71.90, 69.64, 69.30, 69.10, 68.43, 68.37, 64.95, 64.92, 60.99. ESI-MS (negative ion):

Calcd for $C_{15}H_{24}N_2O_{17}P_2$: [M] 566.0550, $[M-H]^-$ 565.0472, $[M-2H+Na]^-$ 587.0291; Found $[M-H]^-$ 565.0486, $[M-2H+Na]^-$ 587.0174.

Synthesis of guanosine 5′- diphosphate fucose (GDP-Fuc)

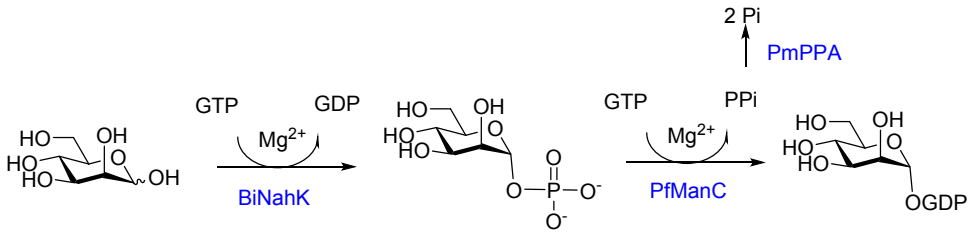


Reaction mixtures containing 200 mM fucose (0.7 g), 300 mM ATP (2.2 g), 300 mM GTP (3.1 g), and 200 mM $MgCl_2$ was adjusted with 1 M NaOH to pH 7.5, followed by pre-incubated at 37 ℃ for 15min before addition of 2 mg/L BfFKP and 1 mg/L PmPPA to a final volume of 20 mL. The reaction was incubated at 37 ℃ with shaking at 80 rpm for 24 h. The pH value of the reaction system was monitored and adjusted with 1 M NaOH every 15 min at the beginning 2 h. TLC was used to monitor the formation of GDP-Fuc and the consumption of ATP and GTP. The reaction was quenched by addition of equal volume of cold ethanol until starting monosaccharide fucose was consumed thoroughly. The purification process is the same as reported. The structures of the obtained products were confirmed by NMR and MS analysis.

GDP-Fuc: ^{1}H NMR (600 MHz, D_2O) δ 8.05 (s, 1H), 5.86 (d, J = 6.1 Hz, 1H), 4.87 (d, J = 8.0 Hz, 1H), 4.72 (t, J = 5.6 Hz, 1H), 4.49 – 4.47 (m, 1H), 4.30 (s, 1H), 4.16 (d, J = 4.1 Hz, 2H), 3.72 (d, J = 6.5 Hz, 1H), 3.66 (d, J = 3.1 Hz, 1H), 3.62 – 3.60 (m, 1H), 3.52 – 3.48 (m, 1H), 1.17 (d, J = 6.5 Hz, 3H).

^{13}C NMR (151 MHz, D_2O) δ 158.74, 153.74, 151.60, 137.42, 115.99, 98.29, 98.25, 86.65, 83.65, 83.59, 73.55, 72.31, 71.31, 71.00, 70.92, 70.87, 70.61, 70.30, 65.19, 65.16, 59.17, 15.29. ESI-MS (negative ion): Calcd for $C_{16}H_{25}N_5O_{15}P_2$: [M] 589.0822, $[M-H]^-$ 588.0744; Found $[M-H]^-$ 588.0652.

Synthesis of guanosine 5′- diphosphate mannose (GDP-Man)

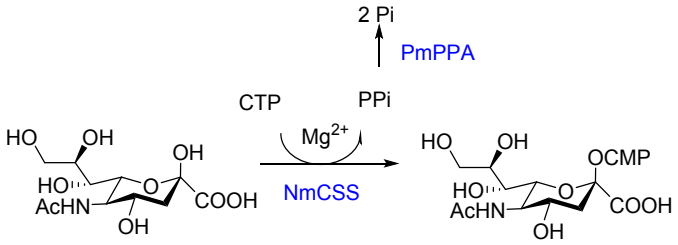


Reaction mixtures containing 200 mM mannose (0.7 g), 600 mM GTP (6.2 g), and 200 mM $MgCl_2$ was adjusted with 1 M NaOH to pH 7.5, followed by pre-incubated at 37 ℃ for 15min before addition of 2 mg/L BiNahK, 2 mg/L PfManC, and 1 mg/L PmPPA to a final volume of 20 mL. The reaction was incubated at

37 ℃ with shaking at 80 rpm for 24 h. The pH value of the reaction system was monitored and adjusted with 1 M NaOH every 15 min at the beginning 2 h. TLC was used to monitor the formation of GDP-Man and the consumption of GTP. The reaction was quenched by addition of equal volume of cold ethanol until starting monosaccharide mannose was consumed thoroughly. The purification process is the same as reported. The structures of the obtained products were confirmed by NMR and MS analysis.

GDP-Man: ^{1}H NMR (600 MHz, D_2O) δ 8.11 (s, 1H), 5.94 (d, *J* = 6.2 Hz, 1H), 5.52 (dd, *J* = 7.8, 1.7 Hz, 1H), 4.77 (s, 1H), 4.52 (dd, *J* = 5.1, 3.4 Hz, 1H), 4.36 (dd, *J* = 3.2, 2.0 Hz, 1H), 4.22 (dd, *J* = 5.4, 3.6 Hz, 2H), 4.06 (dd, *J* = 3.2, 2.1 Hz, 1H), 3.93 (dd, *J* = 9.8, 3.4 Hz, 1H), 3.87 (ddd, *J* = 9.8, 6.5, 2.2 Hz, 2H), 3.76 (dd, *J* = 12.4, 5.5 Hz, 1H), 3.68 (t, *J* = 10.0 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 159.02, 153.94, 151.83, 137.62, 116.29, 96.46, 96.43, 86.78, 83.82, 83.76, 73.67, 73.61, 70.43, 70.28, 70.22, 69.81, 66.42, 65.27, 65.23, 60.75. ESI-MS (negative ion): Calcd for $C_{16}H_{25}N_5O_{16}P_2$: [M] 605.0772, $[M-H]^-$ 604.0693; Found $[M-H]^-$ 604.0604.

Synthesis of cytidine 5'-monophosphate N-acetyl neuraminic acid (CMP-Neu5Ac)

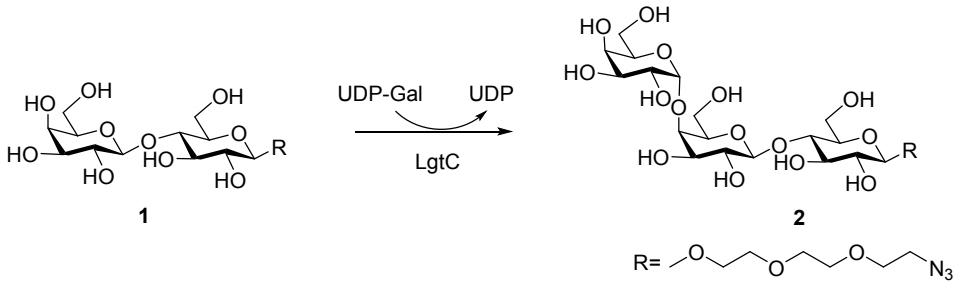


Reaction mixtures containing 200 mM Neu5Ac (1.2 g), 300 mM CTP (3.1 g), and 200 mM $MgCl_2$ was adjusted with 1 M NaOH to pH 9.0, followed by pre-incubated at 37 ℃ for 15min before addition of 2 mg/L NmCSS and 1 mg/L PmPPA to a final volume of 20 mL. The reaction was incubated at 37 ℃ with shaking at 80 rpm for 4 h. The pH value of the reaction system was monitored and adjusted with 1 M NaOH every 15 min at the beginning 2 h. Thin Layer Chromatography (TLC) was used to monitor the formation of CMP-Neu5Ac and the consumption of CTP. The reaction was quenched by addition of equal volume of cold ethanol until starting monosaccharide Neu5Ac was consumed thoroughly. The purification process is the same as reported. The structures of the obtained products were confirmed by NMR and MS analysis.

CMP-Neu5Ac: ^{1}H NMR (600 MHz, D_2O) δ 7.94 (d, *J* = 7.6 Hz, 1H), 6.09 (d, *J* = 7.6 Hz, 1H), 5.95 (d, *J* = 4.5 Hz, 1H), 4.31 (t, *J* = 4.7 Hz, 1H), 4.27 (t, *J* = 4.8 Hz, 1H), 4.20 (d, *J* = 4.7 Hz, 3H), 4.10 (d, *J* = 10.5 Hz, 1H), 4.02 (dd, *J* = 10.7, 4.7 Hz, 1H), 3.94 – 3.89 (m, 2H), 3.85 (dd, *J* = 11.8, 2.2 Hz, 1H), 3.58 (dd, *J* = 11.8, 6.6 Hz, 1H), 3.42 (d, *J* = 9.6 Hz, 1H), 2.45 (dd, *J* = 13.2, 4.7 Hz, 1H), 2.01 (s, 3H), 1.62 (td, *J* = 12.3, 11.9, 5.8 Hz, 1H). ^{13}C NMR (151 MHz, D_2O) δ 177.58, 177.26, 168.74, 160.30, 144.57, 103.00, 102.95, 99.45, 91.93, 85.84, 85.79, 77.17, 74.68, 72.54, 72.20, 71.69, 69.74, 65.77, 63.47, 62.18, 54.66, 43.97, 43.91,

24.97. ESI-MS (negative ion): Calcd for $C_{20}H_{31}N_4O_{16}P$: [M] 614.1473, $[M-H]^-$ 613.1394; Found $[M-H]^-$ 613.1363.

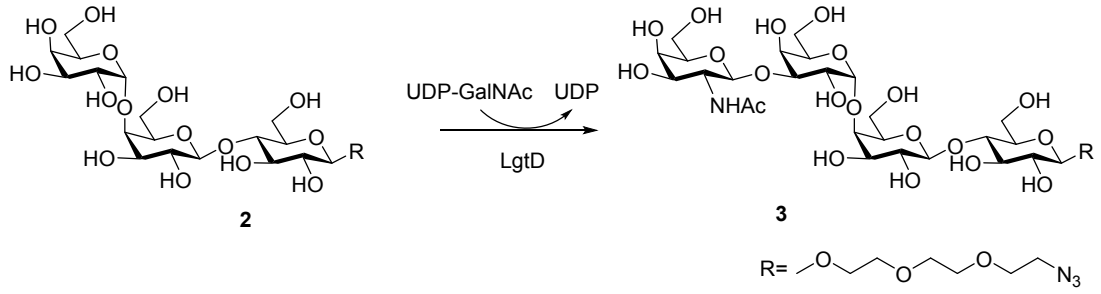
Enzymatic synthesis of compound 2: Galα1-4Galβ1-4Glcβ-PEG-N_3



The reaction was carried out in a 24 mL reaction system containing 50 mM pH 7.0 Tris-HCl, 20 mM $MgCl_2$, 20 mM Lactose-N_3 (Compound **1**), 30 mM UDP-Gal in the presence of LgtC at 25°C for 36 h. Reaction was monitored by TLC (ethyl acetate: methanol: water: acetic acid=5: 2: 1.5: 0.5) and quenched by boiling at 100 °C for 5 min. The supernatant centrifuged at 12,000 rpm for 5 min was purified by silica gel column (200-300 mesh). After lyophilization, **2** was obtained as white powder (165 mg, 96%).

^{1}H NMR (600 MHz, D_2O) δ 4.96 (d, *J* = 4.0 Hz, 1H), 4.55 – 4.51 (m, 2H), 4.37 (t, *J* = 6.7 Hz, 1H), 4.10 – 4.04 (m, 3H), 4.01 (dd, *J* = 12.3, 2.2 Hz, 1H), 3.96 – 3.91 (m, 2H), 3.88 – 3.83 (m, 4H), 3.82 – 3.76 (m, 4H), 3.75 – 3.72 (m, 8H), 3.69 – 3.65 (m, 2H), 3.61 (ddd, *J* = 14.9, 7.7, 5.0 Hz, 2H), 3.54 – 3.51 (m, 2H), 3.37 – 3.34 (m, 1H). ^{13}C NMR (151 MHz, D_2O) δ 103.27, 102.04, 100.31, 78.64, 77.36, 75.42, 74.80, 74.36, 72.90, 72.16, 70.91, 70.82, 69.69, 69.58, 69.49, 69.19, 69.12, 68.94, 68.73, 68.56, 60.51, 60.37, 60.04, 50.13. ESI-MS (negative ion): Calcd for $C_{24}H_{43}N_3O_{18}$: [M] 661.2542, $[M+Cl]^-$ 696.2236; Found $[M+Cl]^-$ 696.2205.

Enzymatic synthesis of compound 3: GalNAcβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3

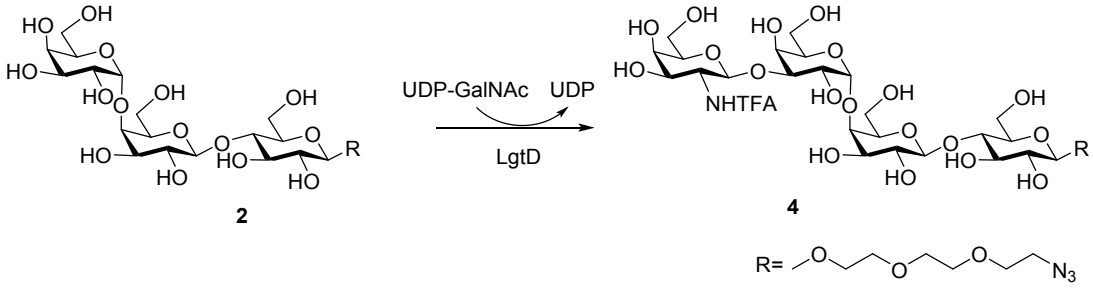


Compound **3** was synthesized starting from **2** in a 10 mL reaction solution containing 50 mM pH 6.5 Tris-HCl, 20 mM $MgCl_2$, 18 mM compound **2**, 30 mM UDP-GalNAc in the presence of LgtD at 30°C for 48 h.

Reaction was monitored by TLC and quenched by boiling at 100 °C for 5 min, followed by centrifugation at 12,000 rpm for 5 min. Final product was purified by DEAE Sepharose Fast Flow and desalted by Bio-gel P2 size-exclusion column chromatography. After lyophilization, **3** was obtained as white powder (113 mg, 97%).

^{1}H NMR (600 MHz, D_2O) δ 4.93 (t, *J* = 5.1 Hz, 1H), 4.65 (d, *J* = 8.4 Hz, 1H), 4.54 (dd, *J* = 7.9, 6.0 Hz, 2H), 4.40 (t, *J* = 6.7 Hz, 1H), 4.27 (d, *J* = 2.4 Hz, 1H), 4.08 (ddd, *J* = 13.0, 8.6, 3.5 Hz, 2H), 4.01 (dd, *J* = 12.3, 2.1 Hz, 1H), 3.98 – 3.89 (m, 5H), 3.88 – 3.83 (m, 3H), 3.82 – 3.73 (m, 14H), 3.72 – 3.65 (m, 5H), 3.63 – 3.58 (m, 2H), 3.54 – 3.51 (m, 2H), 3.38 – 3.33 (m, 1H), 2.05 (d, *J* = 4.3 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 175.19, 103.31, 103.25, 102.04, 100.42, 78.73, 77.23, 75.43, 74.93, 74.80, 74.39, 72.93, 72.12, 70.89, 70.79, 70.28, 69.69, 69.58, 69.49, 69.19, 69.19, 68.95, 68.73, 67.77, 67.63, 61.00, 60.39, 60.31, 60.05, 52.59, 50.13, 22.27.ESI-MS (positive ion): Calcd for $C_{32}H_{56}N_4O_{23}$: [M] 864.3335, $[M+H]^+$ 865.3414, $[M+Na]^+$ 887.3233; Found $[M+H]^+$ 865.3245, $[M+Na]^+$ 887.3061.

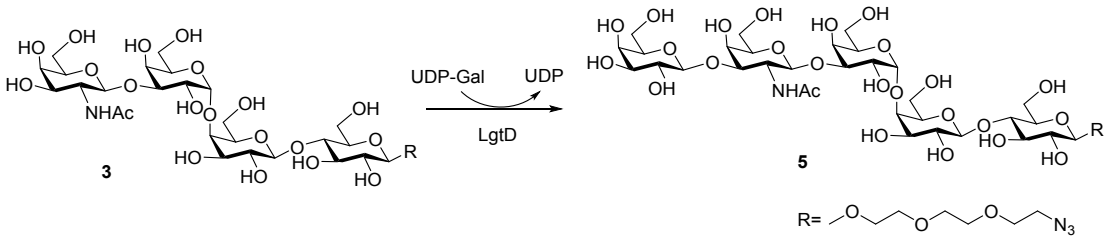
Enzymatic synthesis of compound 4: GalNTFAβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3



Compound **4** was synthesized in a 10 mL reaction system containing 50 mM Tris-HCl pH 6.5, 20 mM $MgCl_2$, 18 mM compound **2**, 30 mM UDP-GalNTFA at 30 °C for 4 days in the presence of LgtD. The reaction was monitored by TLC and quenched by boiling at 100 °C for 5 min. Final product was purified by DEAE Sepharose Fast Flow and desalted by Bio-gel P2 size-exclusion column chromatography. After lyophilization, **4** was obtained as white powder (95 mg, 90%).

^{1}H NMR (600 MHz, D_2O) δ 4.92 (d, *J* = 4.0 Hz, 1H), 4.77 (s, 1H), 4.54 (dd, *J* = 7.9, 5.7 Hz, 2H), 4.39 (t, *J* = 6.6 Hz, 1H), 4.29 (d, *J* = 2.5 Hz, 1H), 4.11 – 3.97 (m, 6H), 3.93 – 3.88 (m, 3H), 3.86 – 3.82 (m, 3H), 3.79 (ddd, *J* = 11.2, 8.1, 4.1 Hz, 5H), 3.76 – 3.69 (m, 10H), 3.68 – 3.65 (m, 2H), 3.61 (ddd, *J* = 18.1, 7.8, 5.0 Hz, 2H), 3.54 – 3.51 (m, 2H), 3.38 – 3.33 (m, 1H). ^{13}C NMR (151 MHz, D_2O) δ 159.64, 114.89, 103.34, 102.55, 102.02, 100.49, 79.06, 78.84, 77.34, 75.41, 75.04, 74.79, 74.40, 72.92, 72.13, 70.90, 70.29, 70.02, 69.69, 69.58, 69.49, 69.19, 68.89, 68.73, 67.70, 67.56, 60.94, 60.37, 60.29, 60.06, 53.40, 50.13. ESI-MS (positive ion): Calcd for $C_{32}H_{53}F_3N_4O_{23}$: [M] 918.3053, $[M+H]^+$ 919.3131, $[M+Na]^+$ 941.2950; Found $[M+H]^+$ 919.3010, $[M+Na]^+$ 941.2823.

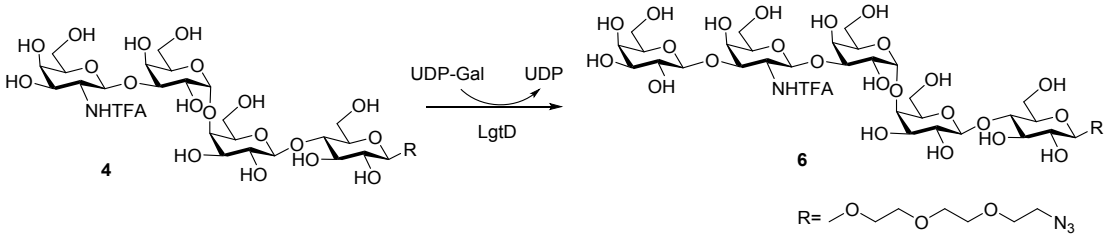
Enzymatic synthesis of compound 5: Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3



Compound **5** was synthesized in a 10 mL reaction system containing 50 mM Tris-HCl pH 7.5, 20 mM $MgCl_2$, 15 mM compound **3**, 30 mM UDP-Gal at 30 °C for 4 days in the presence of LgtD. The reaction quenched by boiling in water bath for 5 min followed by centrifuged at 12,000 rpm for 5 min. Final product was purified by DEAE Sepharose Fast Flow and desalted by Bio-gel P2 size-exclusion column chromatography. After lyophilization, **5** was obtained as white powder (126 mg, 94%).

^{1}H NMR (600 MHz, D_2O) δ 4.93 (d, *J* = 4.0 Hz, 1H), 4.70 (d, *J* = 8.5 Hz, 1H), 4.53 (dd, *J* = 7.9, 5.7 Hz, 2H), 4.47 (d, *J* = 7.8 Hz, 1H), 4.40 (t, *J* = 6.7 Hz, 1H), 4.26 (d, *J* = 3.0 Hz, 1H), 4.19 (d, *J* = 3.2 Hz, 1H), 4.10 – 4.04 (m, 3H), 4.02 – 3.97 (m, 2H), 3.92 (td, *J* = 7.9, 4.3 Hz, 4H), 3.88 – 3.82 (m, 3H), 3.80 – 3.73 (m, 14H), 3.72 – 3.59 (m, 9H), 3.56 – 3.51 (m, 3H), 3.38 – 3.34 (m, 1H), 2.04 (s, 3H). ^{13}C NMR (151 MHz, D_2O) δ 175.13, 104.80, 103.30, 102.95, 102.03, 100.40, 79.57, 78.72, 78.69, 77.19, 75.42, 74.98, 74.79, 74.59, 74.38, 72.92, 72.44, 72.10, 70.87, 70.58, 70.26, 69.68, 69.57, 69.48, 69.18, 68.93, 68.72, 68.56, 67.98, 67.61, 60.98, 60.94, 60.36, 60.29, 60.04, 51.46, 50.12, 22.26. ESI-MS (positive ion): Calcd for $C_{38}H_{66}N_4O_{28}$: [M] 1026.3864, $[M+H]^+$ 1027.3942; Found $[M+H]^+$ 1027.3930.

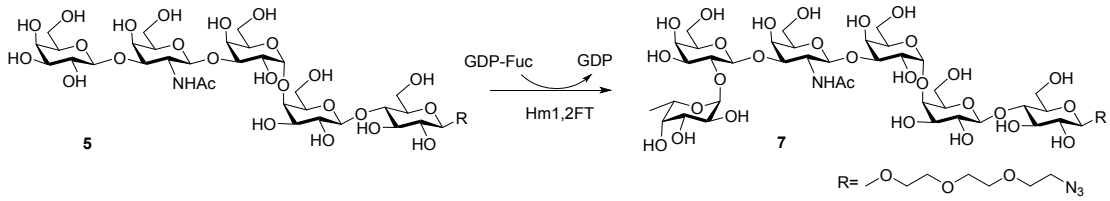
Enzymatic synthesis of compound 6: Galβ1-3GalNTFAβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3



Similarly, Compound **6** was prepared in a 10 mL system containing 50 mM Tris-HCl buffer, pH 7.0, 20 mM $MgCl_2$, 15mM compound **4**, 30 mM UDP-Gal in the presence of LgtD. The reaction was incubated at 30 °C for 3 days and monitored by TLC (ethyl acetate: methanol: water: acetic acid=5: 2: 2: 0.5). It is worth mentioning that LgtD has a higher catalytic efficiency toward compound **4**. Final product was purified by DEAE Sepharose Fast Flow and desalted by Bio-gel P2 size-exclusion column chromatography. After lyophilization, **6** was obtained as white powder (108 mg, 96%).

^{1}H NMR (600 MHz, D_2O) δ 4.92 (d, *J* = 4.0 Hz, 1H), 4.82 (d, *J* = 8.2 Hz, 1H), 4.53 (dd, *J* = 7.9, 5.4 Hz, 2H), 4.46 (d, *J* = 7.8 Hz, 1H), 4.39 (t, *J* = 6.7 Hz, 1H), 4.26 (dd, *J* = 20.2, 3.1 Hz, 2H), 4.16 (dd, *J* = 10.9, 8.5 Hz, 1H), 4.11 – 3.98 (m, 5H), 3.93 – 3.72 (m, 21H), 3.71 – 3.65 (m, 5H), 3.63 – 3.59 (m, 3H), 3.55 – 3.50 (m, 3H), 3.37 – 3.33 (m, 1H). ^{13}C NMR (151 MHz, D_2O) δ 159.45, 116.76, 104.84, 103.33, 102.30, 102.02, 100.48, 79.09, 78.83, 77.31, 75.40, 74.99, 74.74, 74.39, 72.90, 72.47, 72.12, 70.89, 70.51, 70.27, 69.75-69.38, 69.19, 68.87, 68.71, 68.55, 67.83, 67.53, 60.92, 60.30, 60.05, 52.19, 50.12. ESI-MS (positive ion): Calcd for $C_{38}H_{63}F_3N_4O_{28}$: [M] 1080.3581, $[M+H]^+$ 1081.3659; Found $[M+H]^+$ 1081.3624.

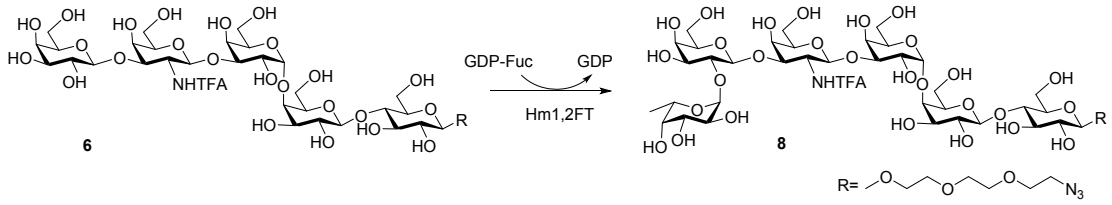
Enzymatic synthesis of compound 7: Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-N_3



Compound **7** was synthesized in a 10 mL reaction system containing 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM compound **5**, 20 mM GDP-Fuc at 37 °C for 24 h in the presence of Hmα1,2 FucT. The reaction quenched by boiling for 5 min followed by centrifuged at 12,000 rpm for 5 min. Final product was purified by DEAE Sepharose Fast Flow and desalted by Bio-gel P2 size-exclusion column chromatography. After lyophilization, **7** was obtained as white powder (140 mg, 97%, 85% overall yield).

^{1}H NMR (600 MHz, D_2O) δ 5.24 (d, *J* = 4.2 Hz, 1H), 4.90 (d, *J* = 4.0 Hz, 1H), 4.63 (d, *J* = 7.7 Hz, 1H), 4.57 – 4.51 (m, 3H), 4.40 (td, *J* = 6.3, 1.3 Hz, 1H), 4.27 – 4.23 (m, 2H), 4.12 (dd, *J* = 2.8, 1.0 Hz, 1H), 4.10 – 4.06 (m, 1H), 4.04 (d, *J* = 3.3 Hz, 1H), 4.03 – 3.94 (m, 4H), 3.93 – 3.89 (m, 2H), 3.88 – 3.82 (m, 5H), 3.81 – 3.76 (m, 8H), 3.74 (d, *J* = 8.9 Hz, 9H), 3.71 – 3.58 (m, 9H), 3.54 – 3.51 (m, 2H), 3.37 – 3.33 (m, 1H), 2.06 (s, 3H), 1.23 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 174.29, 103.99, 103.31, 102.04, 102.03, 100.44, 99.28, 78.74, 78.30, 77.15, 76.36, 76.12, 75.47, 75.06, 74.79, 74.62, 74.38, 73.57, 72.92, 72.10, 71.85, 70.86, 70.14, 69.68, 69.57, 69.51, 69.48, 69.19, 69.11, 68.72, 68.48, 68.03, 67.83, 66.78, 60.97, 60.94, 60.36, 60.32, 60.04, 51.63, 50.12, 22.24, 15.31. ESI-MS (positive ion): Calcd for $C_{44}H_{76}N_4O_{32}$: [M] 1172.4442, $[M+H]^+$ 1173.4515, $[M+H+Na]^{2+}$ 598.2204; Found $[M+H]^+$ 1173.4503, $[M+H+Na]^{2+}$ 598.2206.

Enzymatic synthesis of compound 8: Fucα1-2Galβ1-3GalNTFAβ1-3Galα1-4Galβ1-4Glcβ-N_3

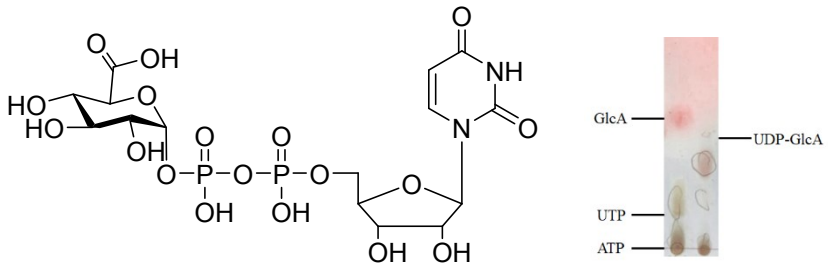


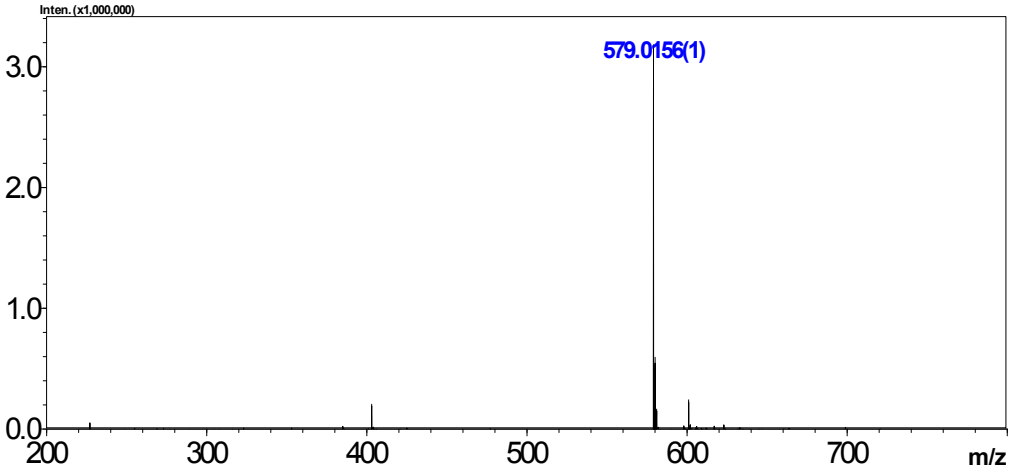
Compound **8** was synthesized in a 10 mL reaction system containing 50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM compound **6**, 20 mM GDP-Fuc at 37 °C for 24 h in the presence of Hmα1,2FucT. The reaction quenched by boiling for 5 min followed by centrifuged at 12,000 rpm for 5 min. Final product was purified by DEAE Sepharose Fast Flow and desalted by Bio-gel P2 size-exclusion column chromatography. After lyophilization, **8** was obtained as white powder (110 mg, 92%, 76% overall yield).

^{1}H NMR (600 MHz, D_2O) δ 5.20 (d, *J* = 4.1 Hz, 1H), 4.90 (d, *J* = 4.0 Hz, 1H), 4.68 (d, *J* = 7.7 Hz, 1H), 4.61 (d, *J* = 7.7 Hz, 1H), 4.53 (dd, *J* = 7.9, 5.4 Hz, 2H), 4.40 (t, *J* = 6.6 Hz, 1H), 4.28 (d, *J* = 2.8 Hz, 1H), 4.18 – 4.12 (m, 4H), 4.08 (dt, *J* = 11.5, 4.1 Hz, 1H), 4.04 (d, *J* = 3.1 Hz, 1H), 3.99 (ddd, *J* = 16.4, 11.4, 2.6 Hz, 2H), 3.91 – 3.73 (m, 23H), 3.71 – 3.59 (m, 10H), 3.54 – 3.50 (m, 2H), 3.36 (dd, *J* = 9.8, 7.3 Hz, 1H), 1.25 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 159.51, 114.92, 103.34, 103.20, 102.02, 101.58, 100.50, 99.67, 78.85, 78.79, 77.29, 76.77, 75.42, 75.18, 75.05, 74.78, 74.75, 74.39, 73.39, 72.91, 72.11, 71.73, 70.87, 70.17, 69.68, 69.57, 69.48, 69.22, 69.18, 69.07, 69.04, 68.72, 68.35, 68.05, 67.70, 66.79, 60.97, 60.85, 60.31, 60.31, 60.05, 52.53, 50.12, 15.30. ESI-MS (positive ion): Calcd for $C_{44}H_{73}F_3N_4O_{32}$: [M] 1226.4160, $[M+H]^+$ 1227.4138, $[M+2Na]^{2+}$ 636.1972; Found $[M+H]^+$ 1227.4225, $[M+2Na]^{2+}$ 636.1977.

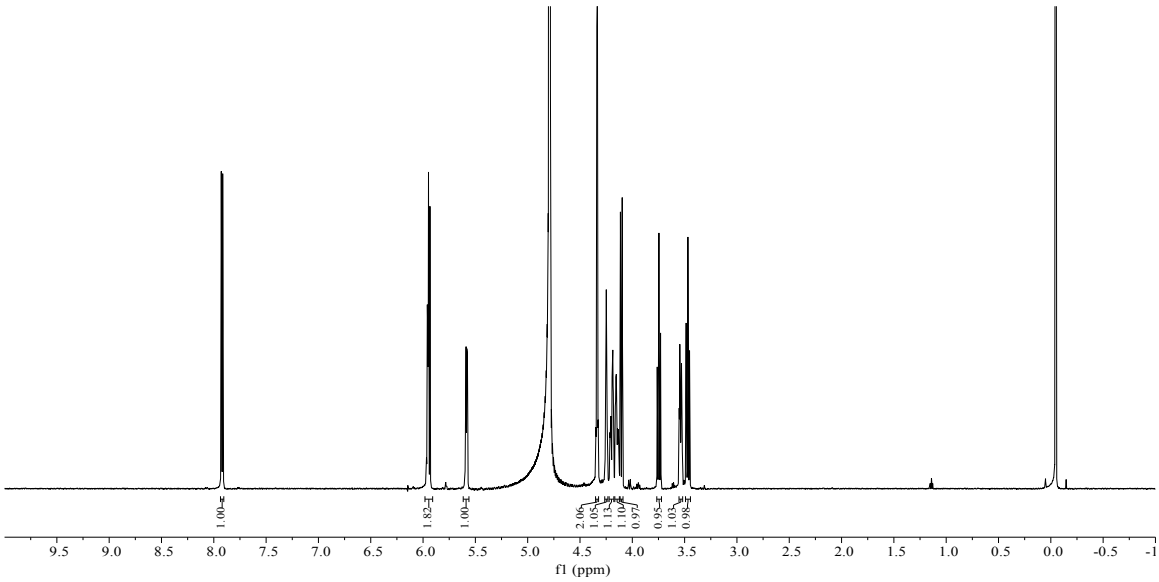
III. ESI and NMR spectrum

UDP-GlcA: uridine 5′-diphosphate glucuronic acid

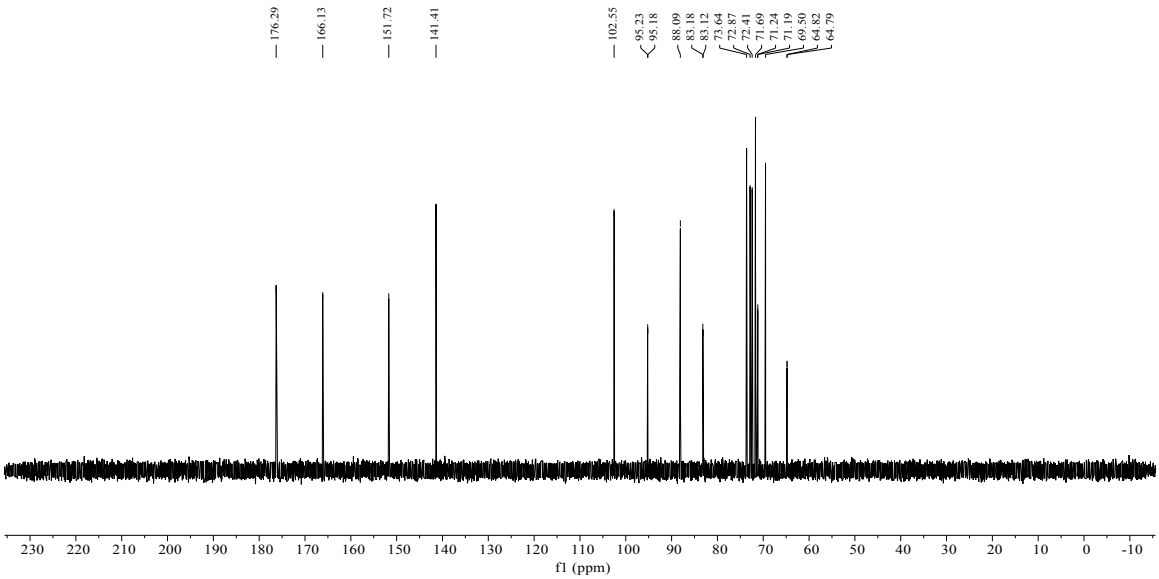




$^{-}$Q ESI-MS profile of UDP-GlcA

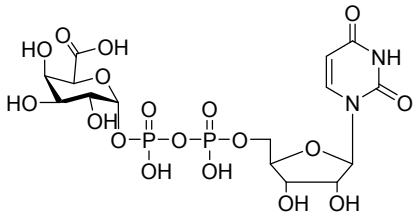


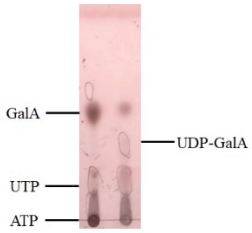
^{1}H NMR Spectrum of UDP-GlcA

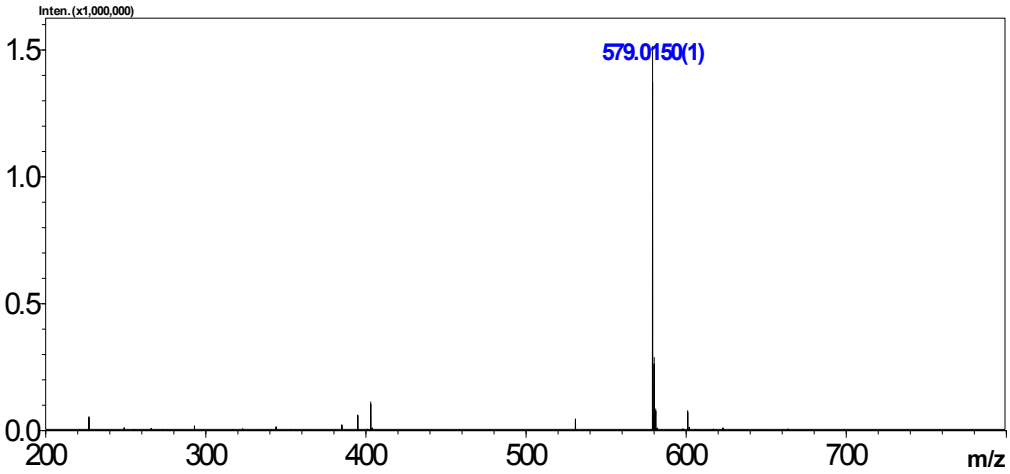


^{13}C NMR Spectrum of UDP-GlcA

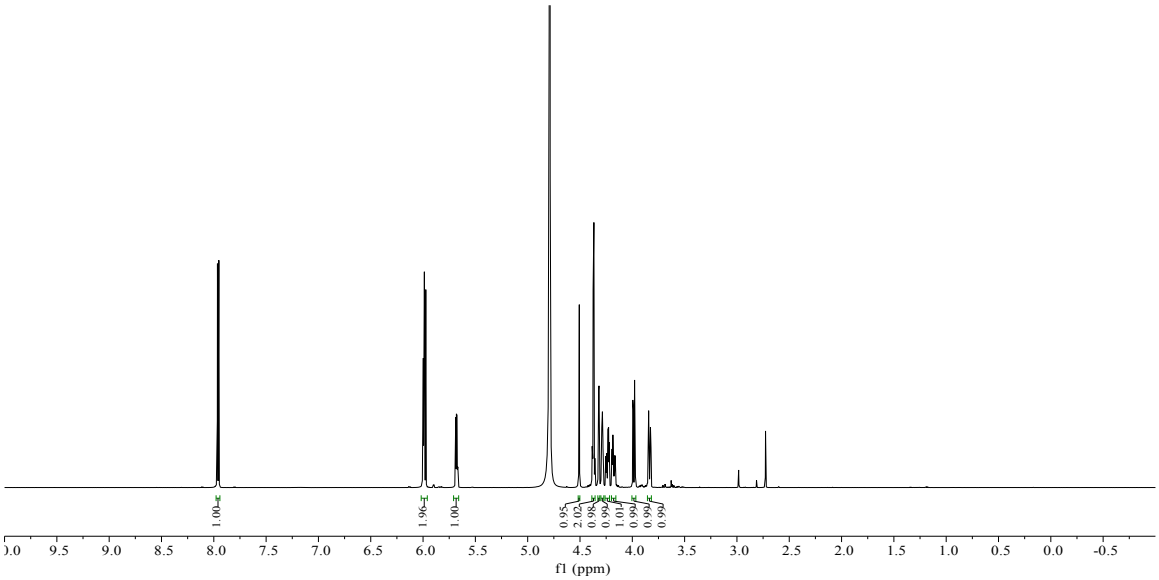
UDP-GalA: uridine 5′-diphosphate galacturonic acid



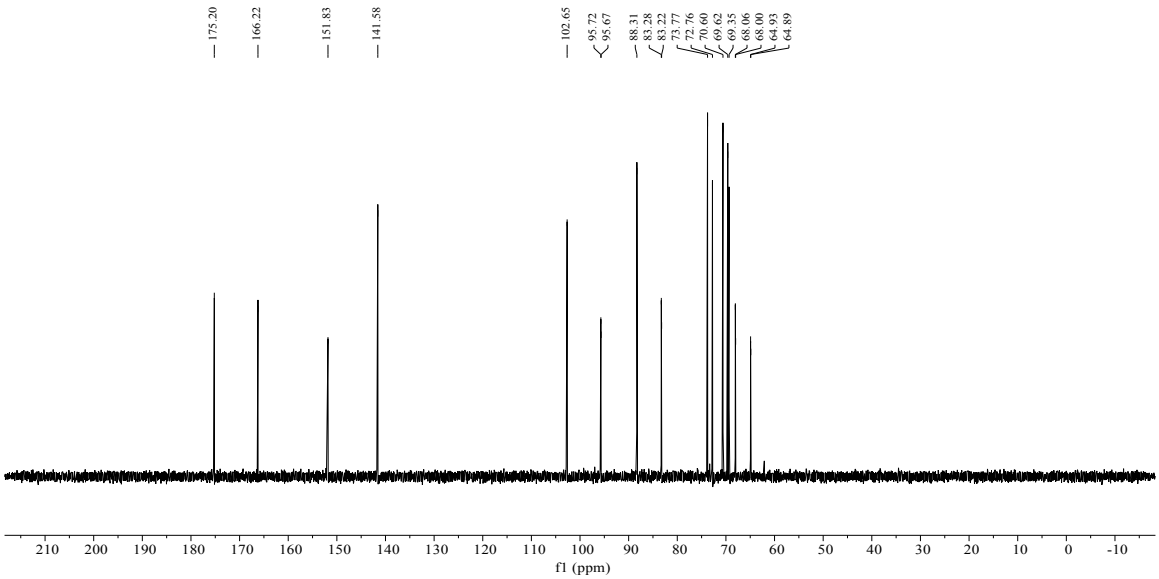




$^{-}$**Q ESI-MS profile of UDP-GalA**

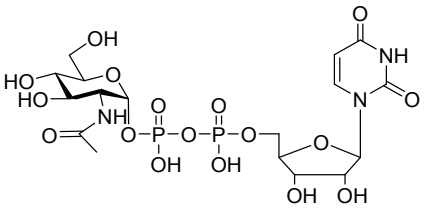


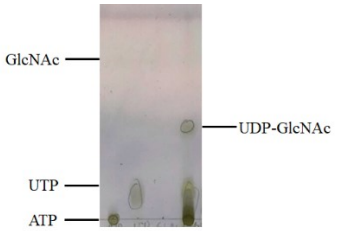
^{1}H NMR Spectrum of UDP-GalA

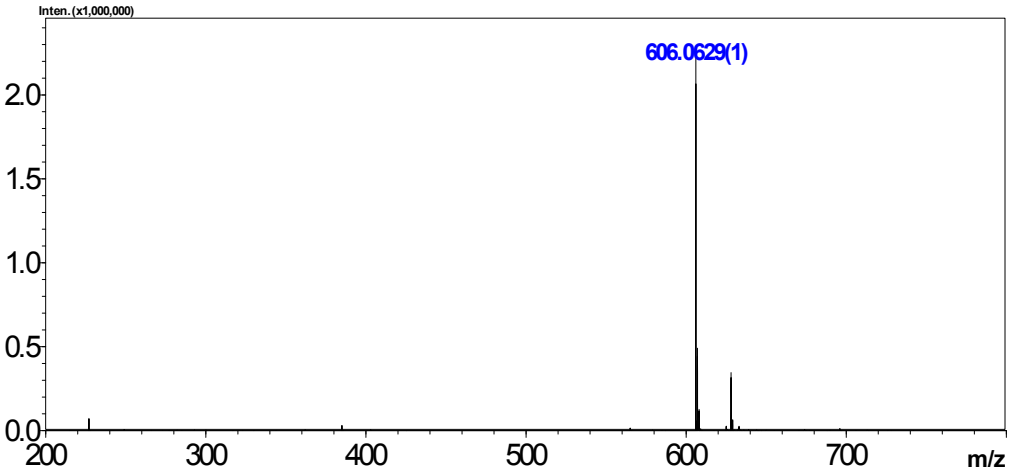


^{13}C NMR Spectrum of UDP-GalA

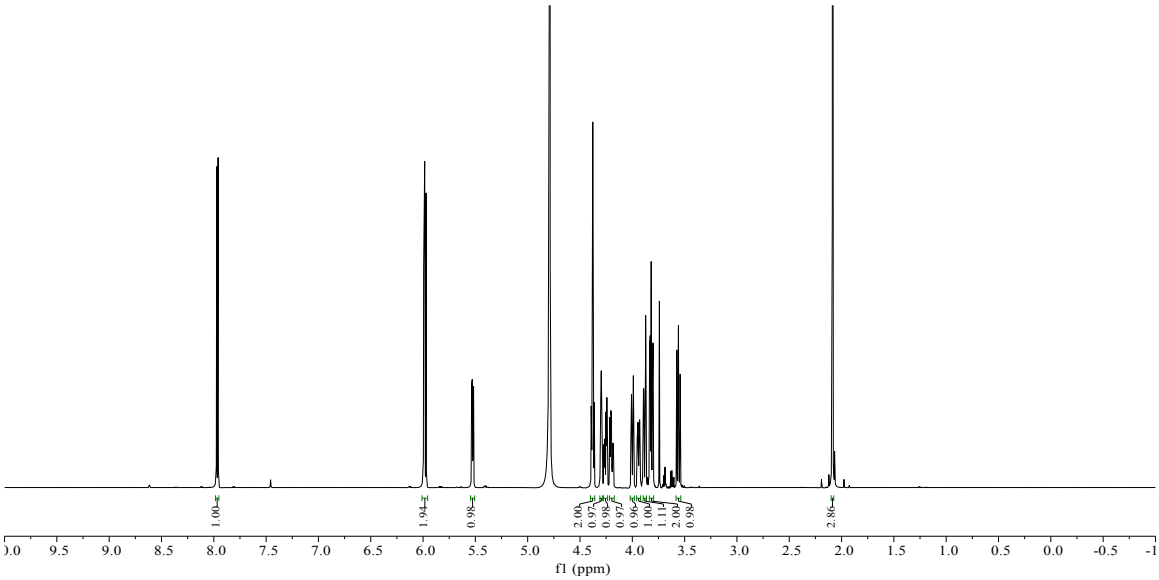
UDP-GlcNAc: uridine 5′-diphosphate *N*-acetyl glucosamine



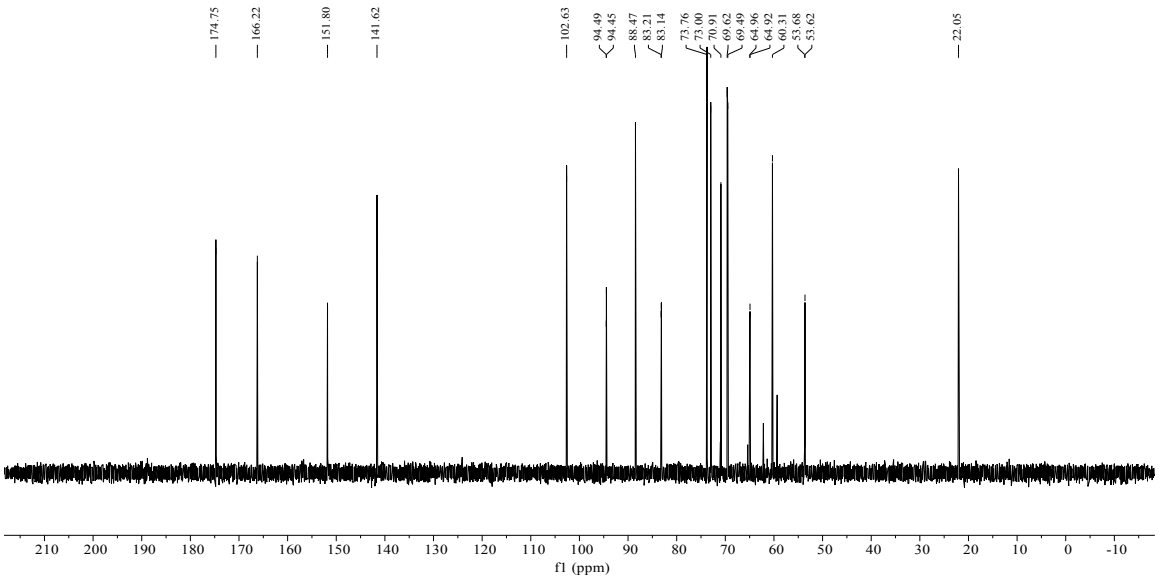




$^{-}$Q model ESI-MS profile of UDP-GlcNAc

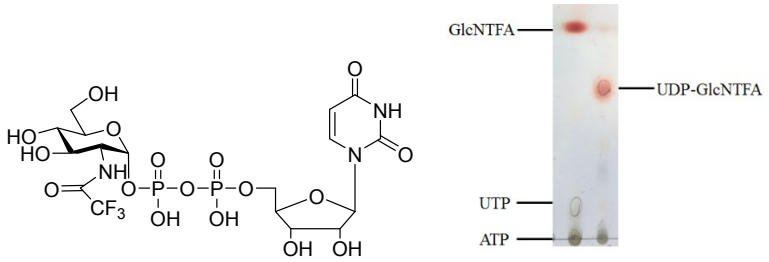


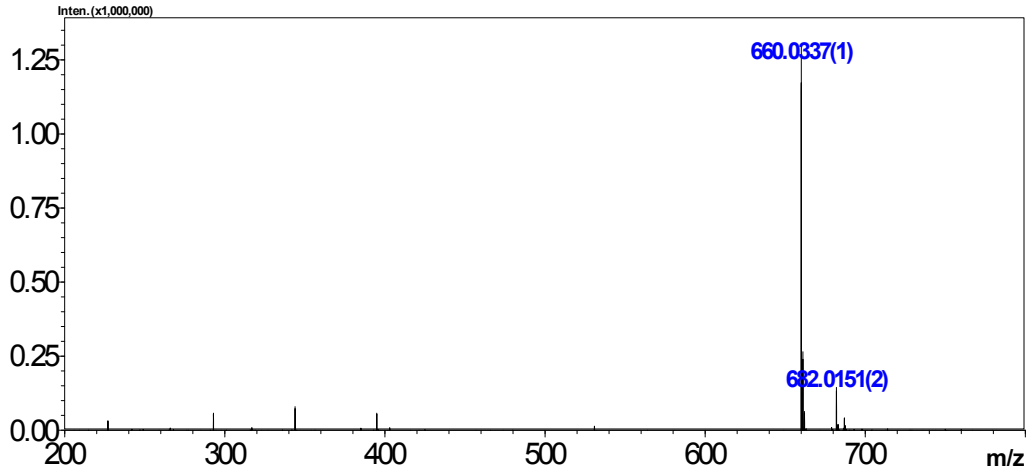
^{1}H NMR Spectrum of UDP-GlcNAc



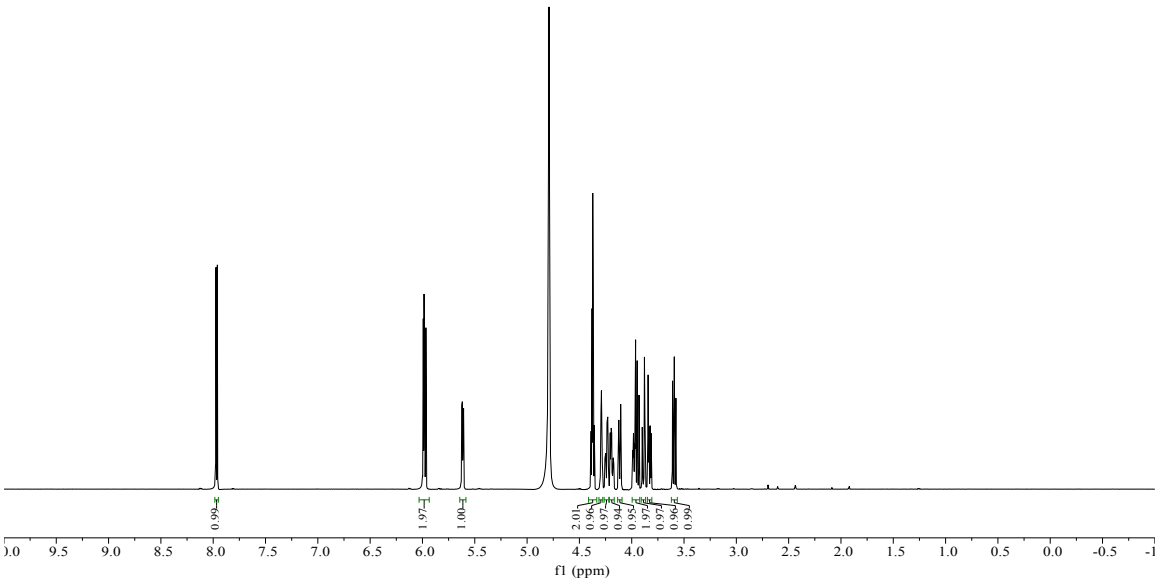
^{13}C NMR Spectrum of UDP-GlcNAc

UDP-GlcNTFA: uridine 5′-diphosphate *N*-trifluoacetyl glucosamine

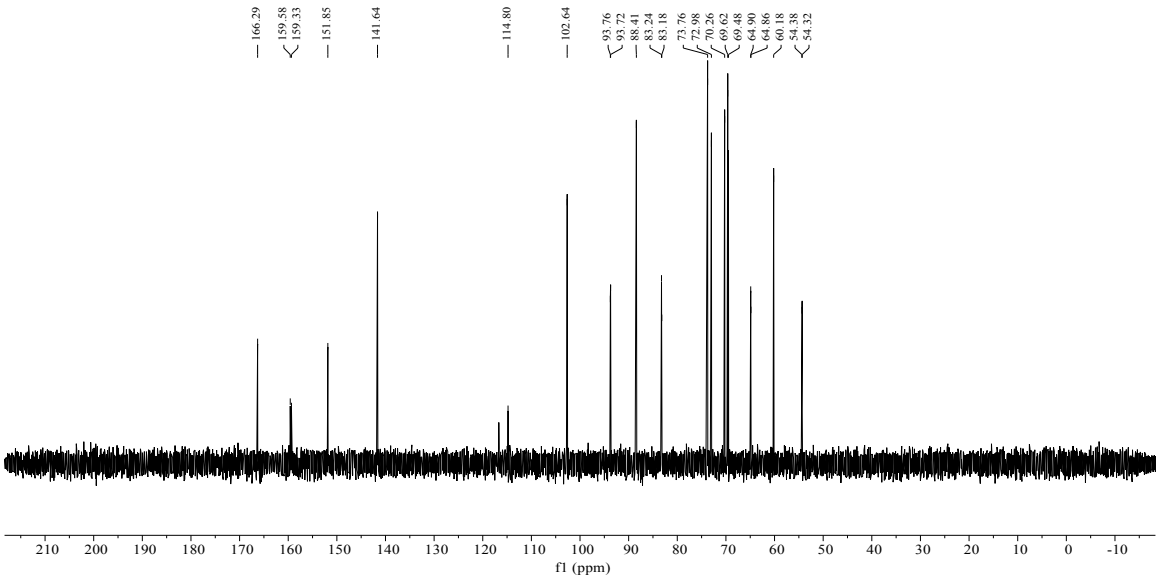




-Q model ESI-MS profile of UDP-GlcNTFA

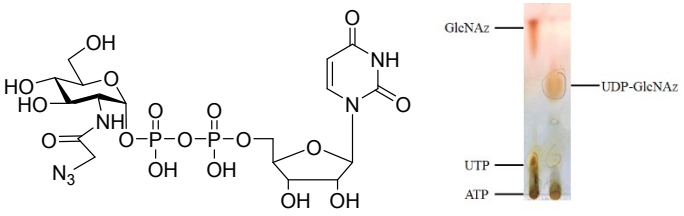


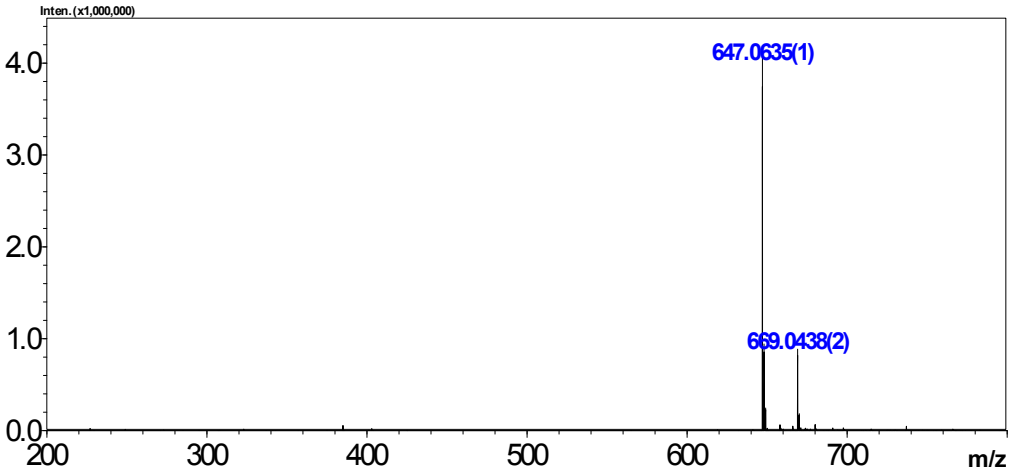
^{1}H NMR Spectrum of UDP-GlcNTFA



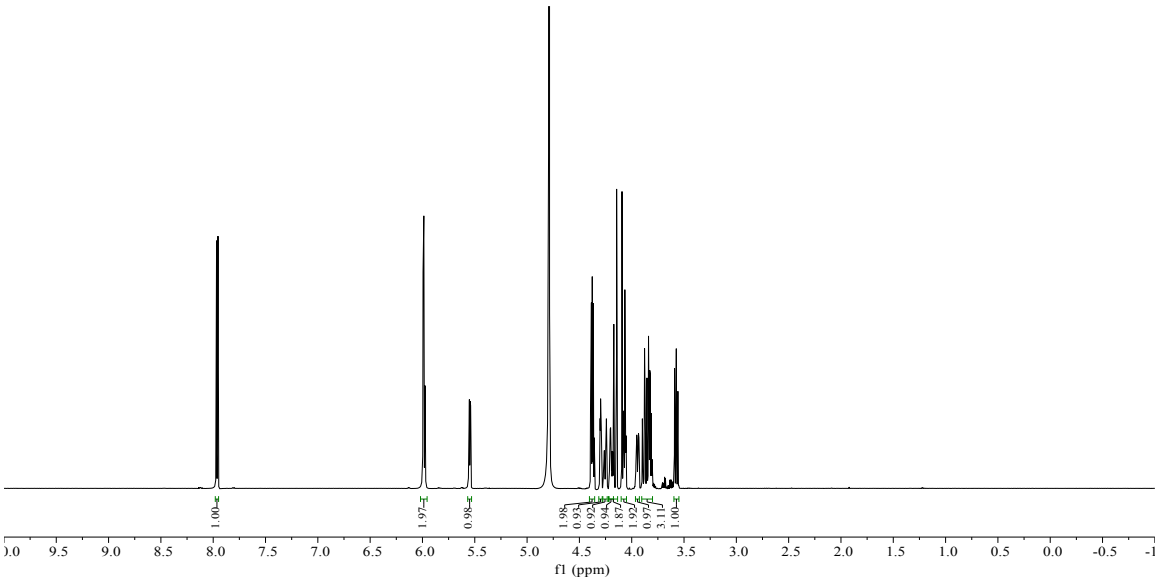
^{13}C NMR Spectrum of UDP-GlcNTFA

UDP-GlcNAz: uridine 5′-diphosphate *N*-azidoacetyl glucosamine

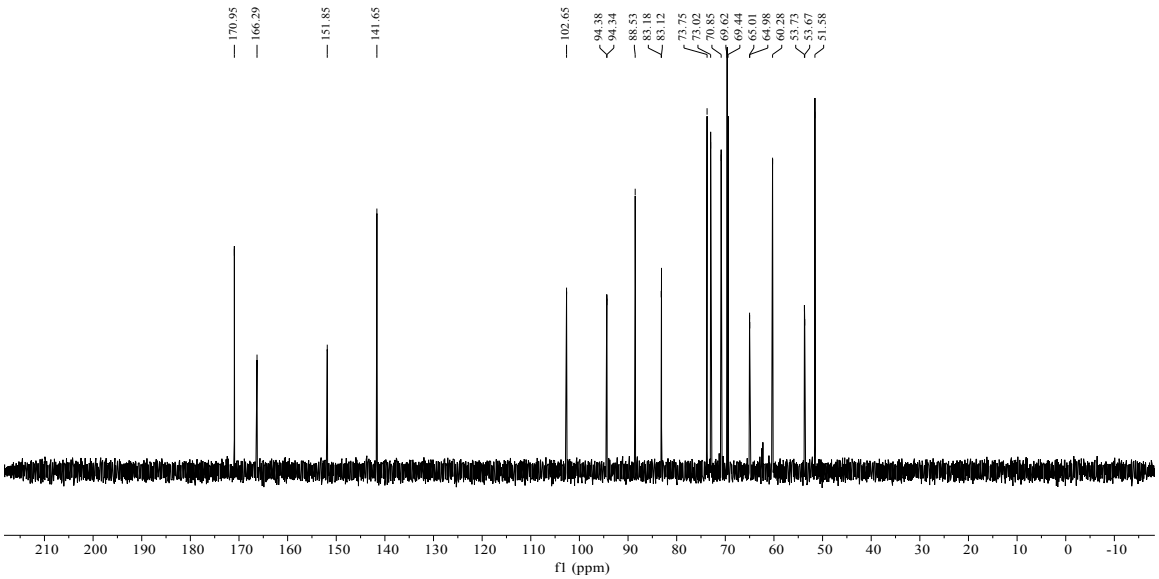




-Q model ESI-MS profile of UDP-GlcNAz

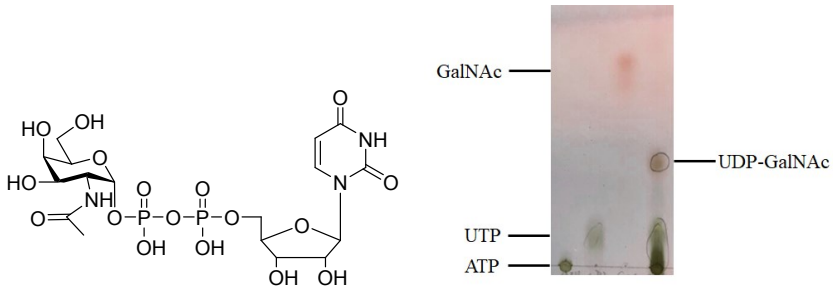


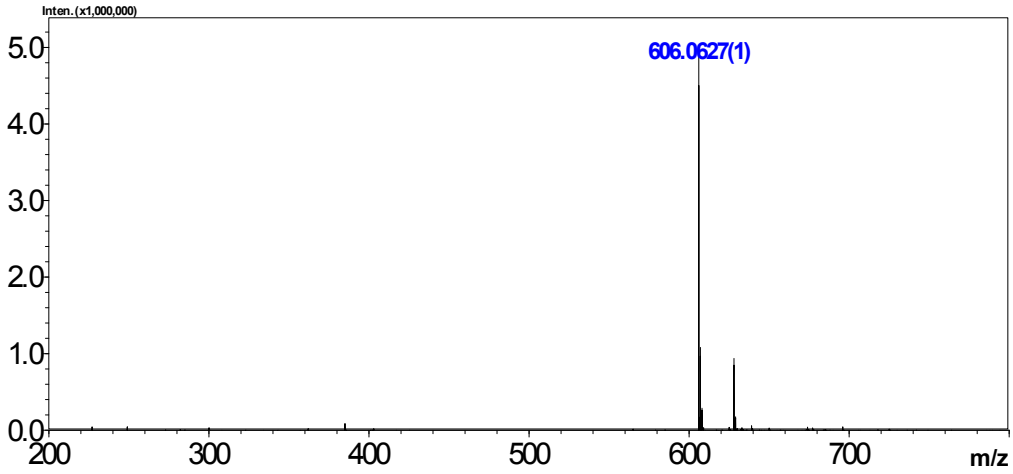
^{1}H NMR Spectrum of UDP-GlcNAz



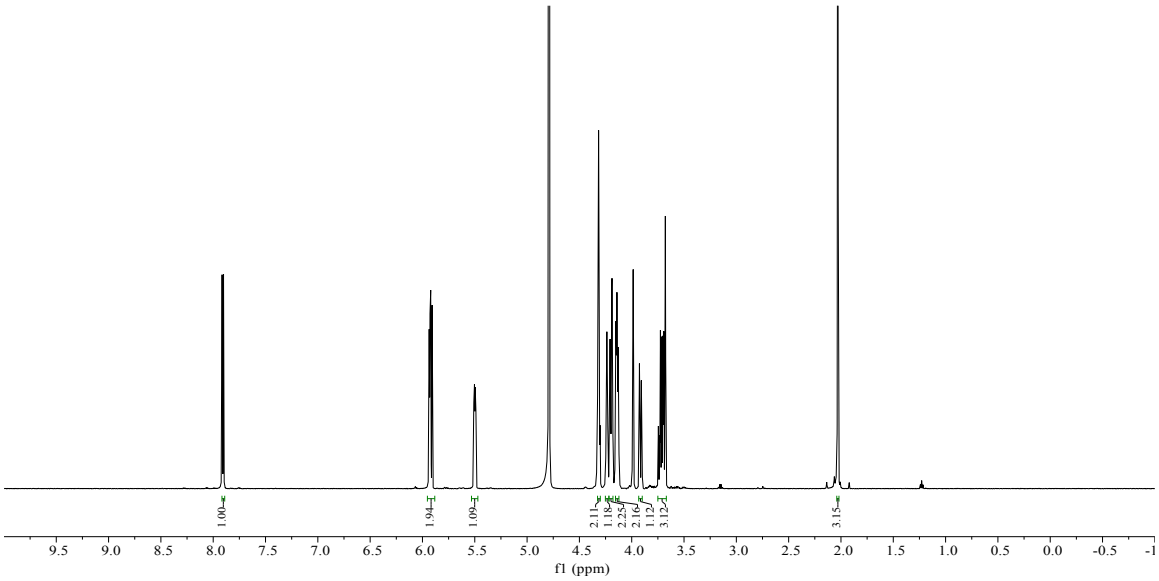
^{13}C NMR Spectrum of UDP-GlcNAz

UDP-GalNAc: uridine 5′-diphosphate *N*-acetyl galactosamine

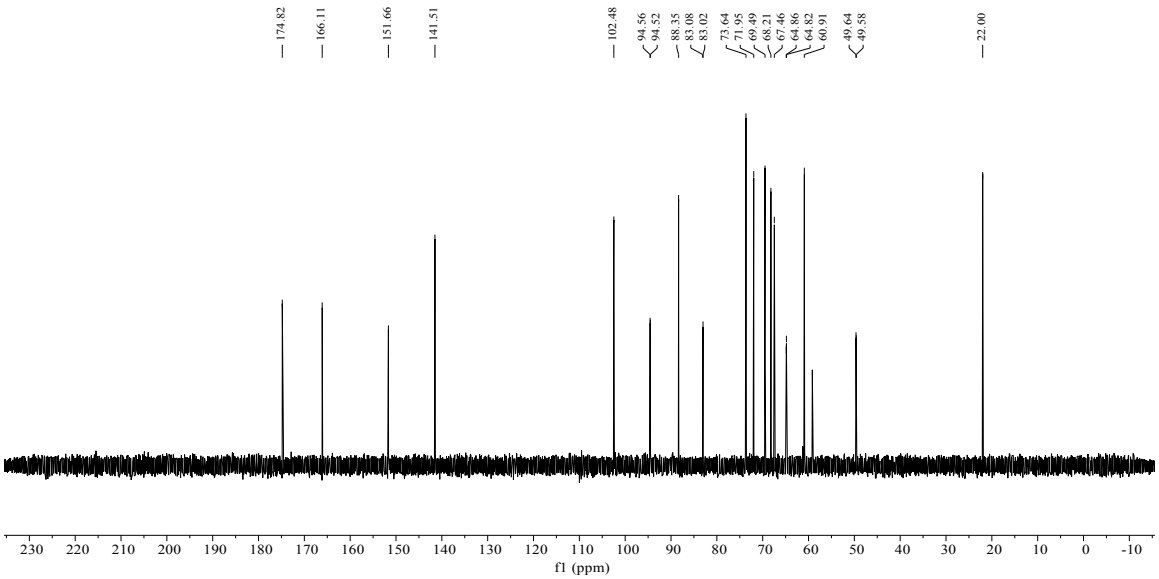




$^{-}$Q model ESI-MS profile of UDP-GalNAc

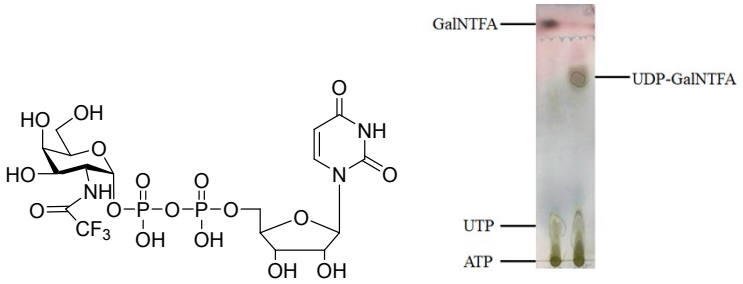


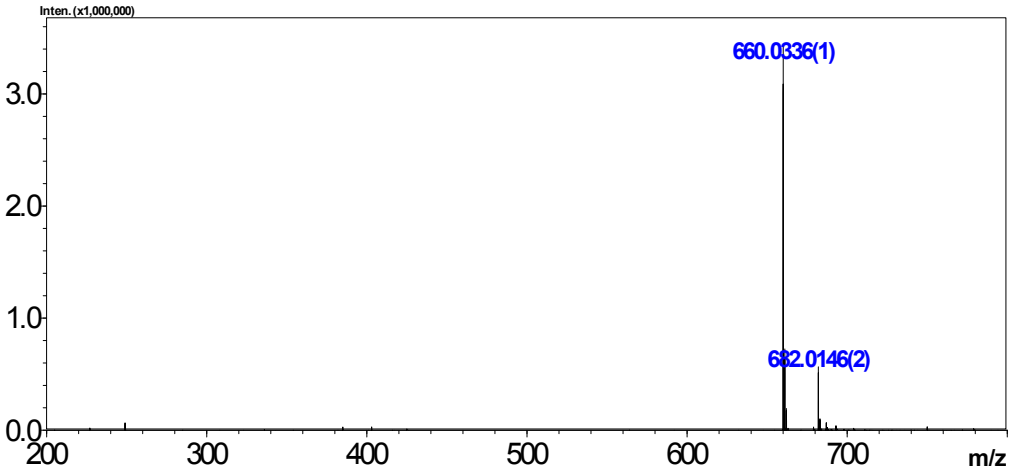
^{1}H NMR Spectrum of UDP-GalNAc



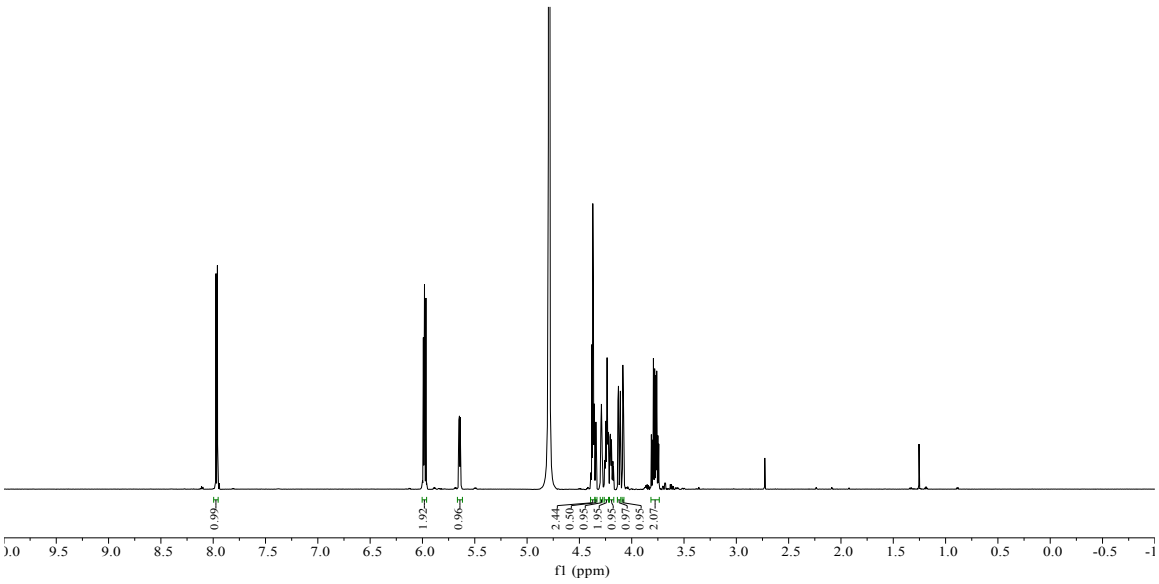
^{13}C NMR Spectrum of UDP-GalNAc

UDP-GalNTFA: uridine 5′-diphosphate *N*-trifluoacetyl galactosamine

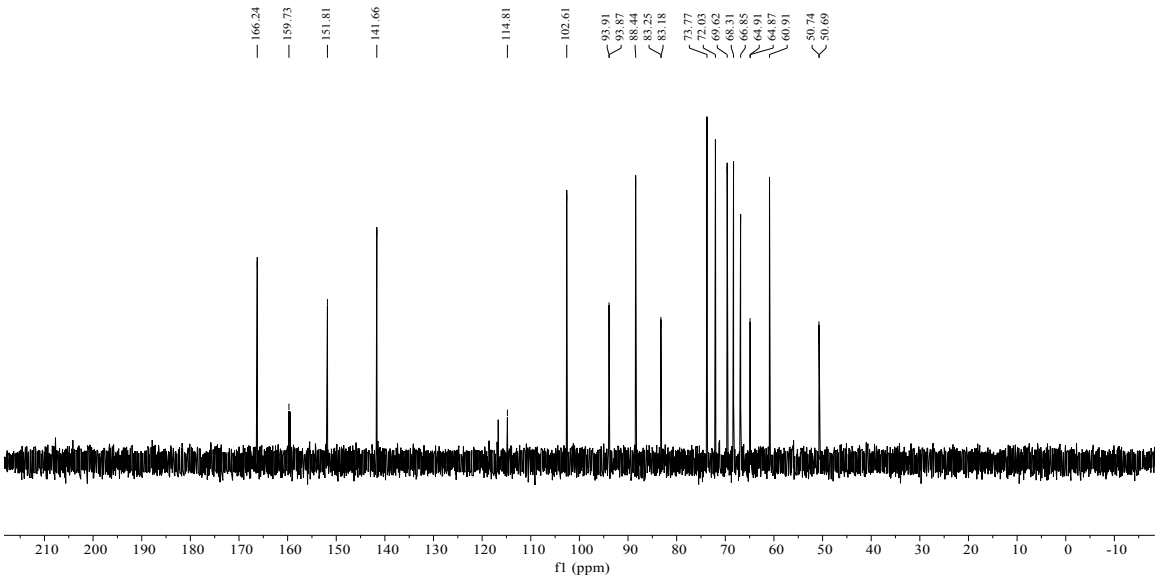




$^{-}$Q model ESI-MS profile of UDP-GalNTFA

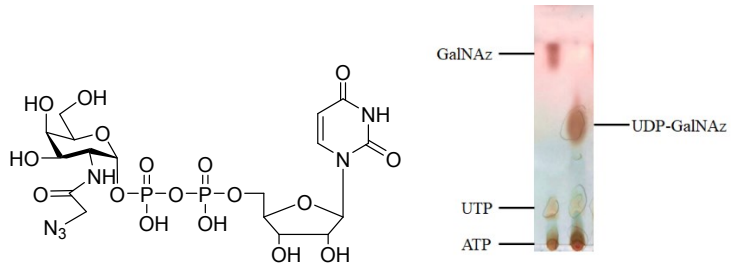


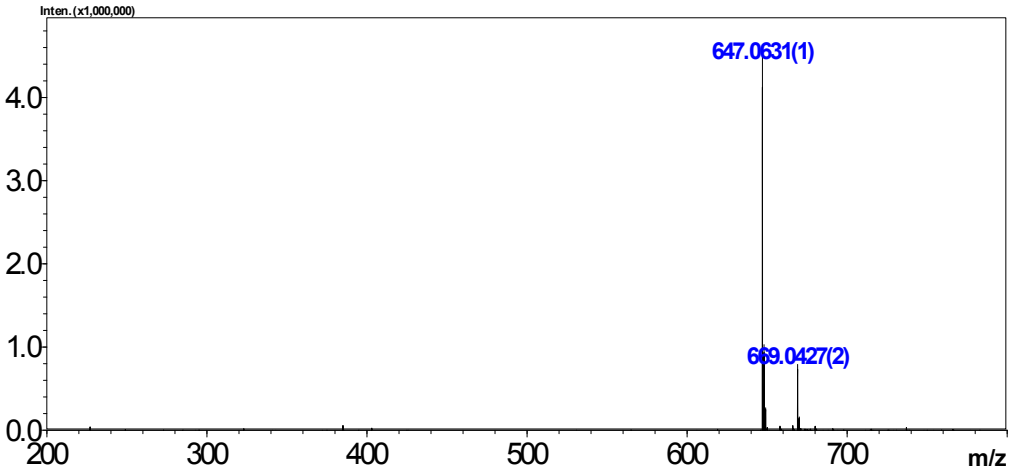
^{1}H NMR Spectrum of UDP-GalNTFA



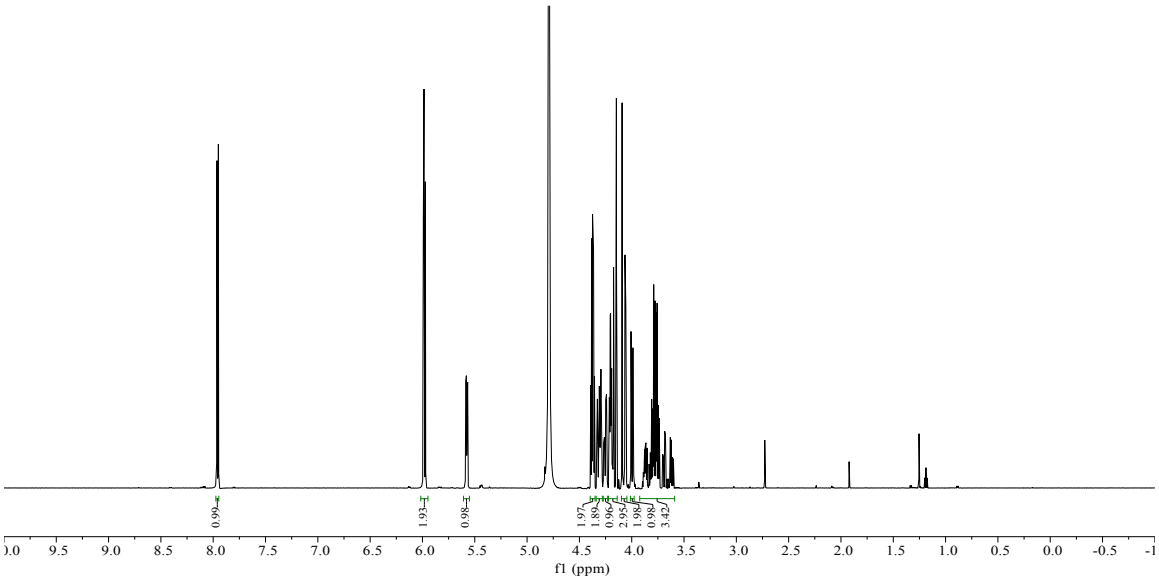
^{13}C NMR Spectrum of UDP-GalNTFA

UDP-GalNAz: uridine 5′-diphosphate *N*-azidoacetyl galactosamine

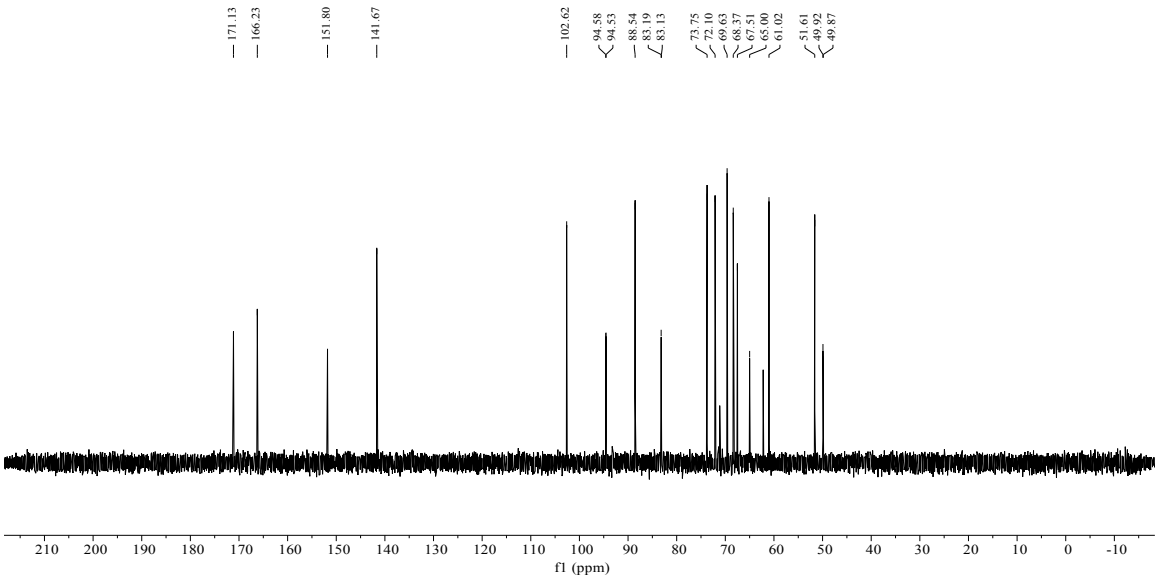




$^{-}$Q model ESI-MS profile of UDP-GalNAz

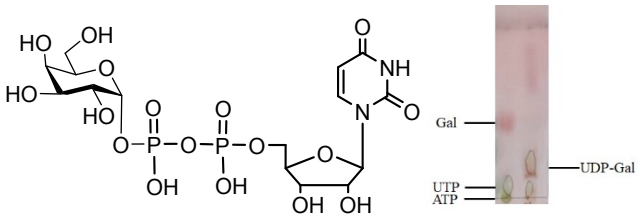


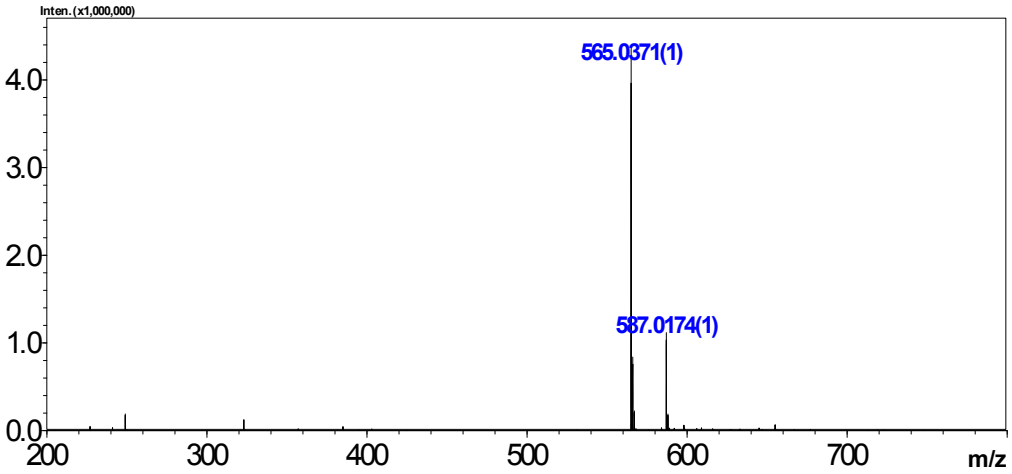
^{1}H NMR Spectrum of UDP-GalNAz



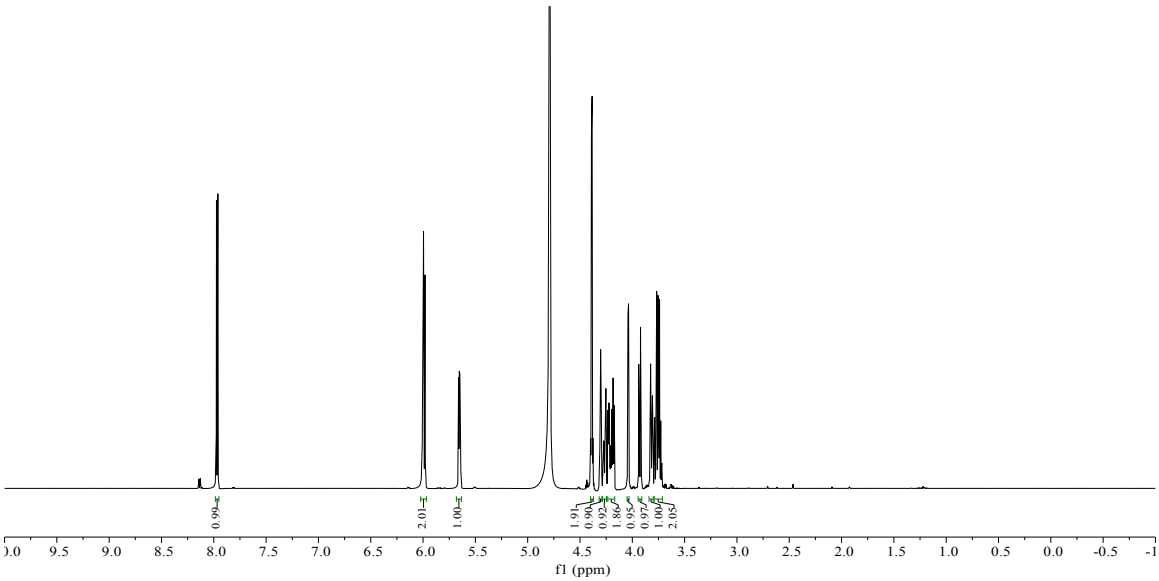
^{13}C NMR Spectrum of UDP-GalNAz

UDP-Gal: uridine 5′-diphosphate galactose

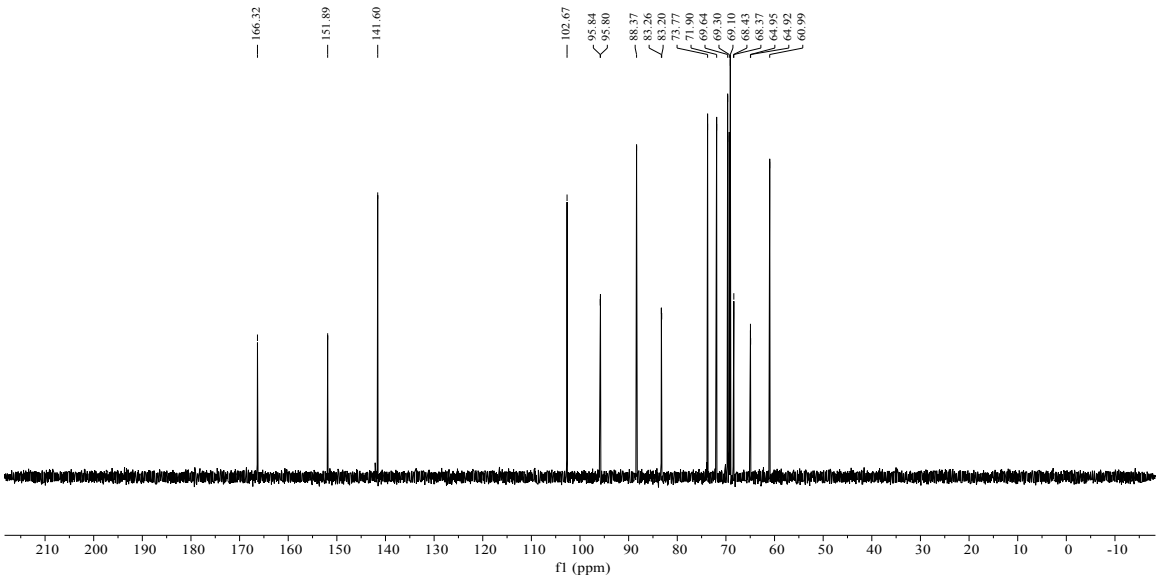




$^{-}$Q model ESI-MS profile of UDP-Gal

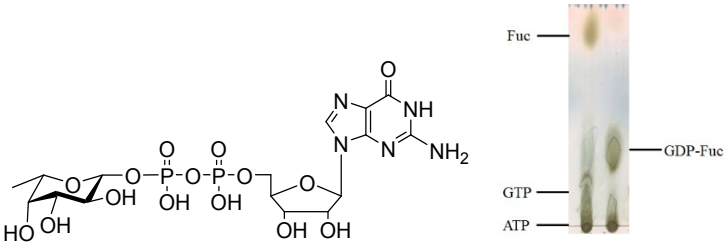


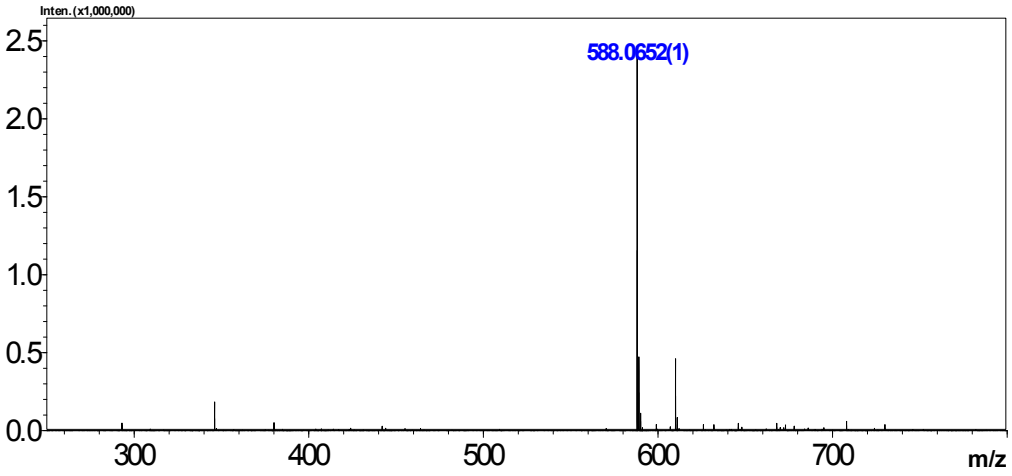
^{1}H NMR Spectrum of UDP-Gal



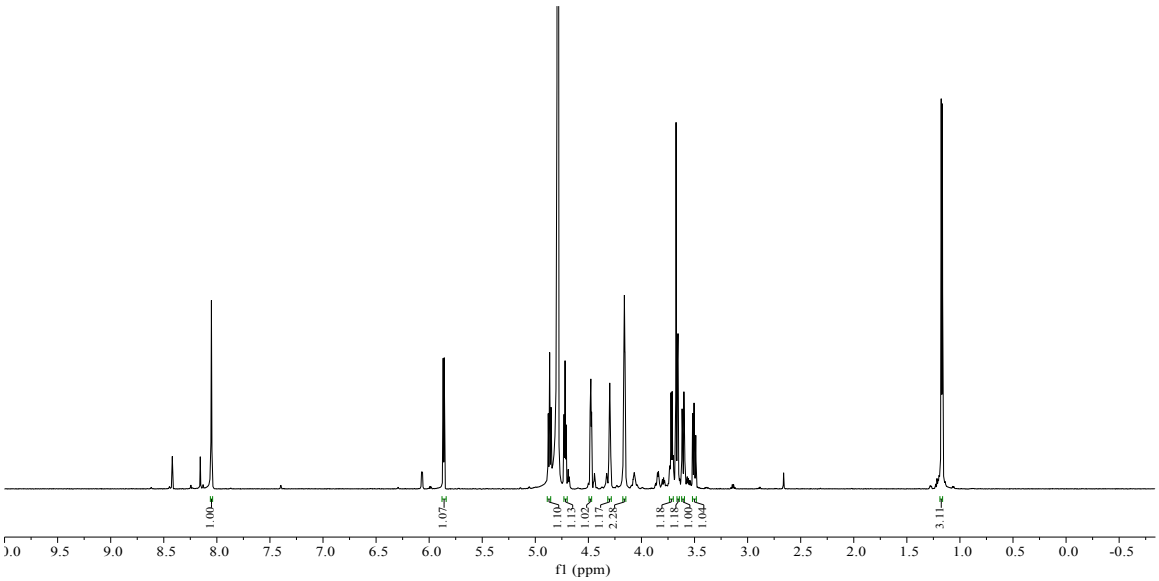
^{13}C NMR Spectrum of UDP-Gal

GDP-Fuc: guanosine 5'-triphosphate Fucose

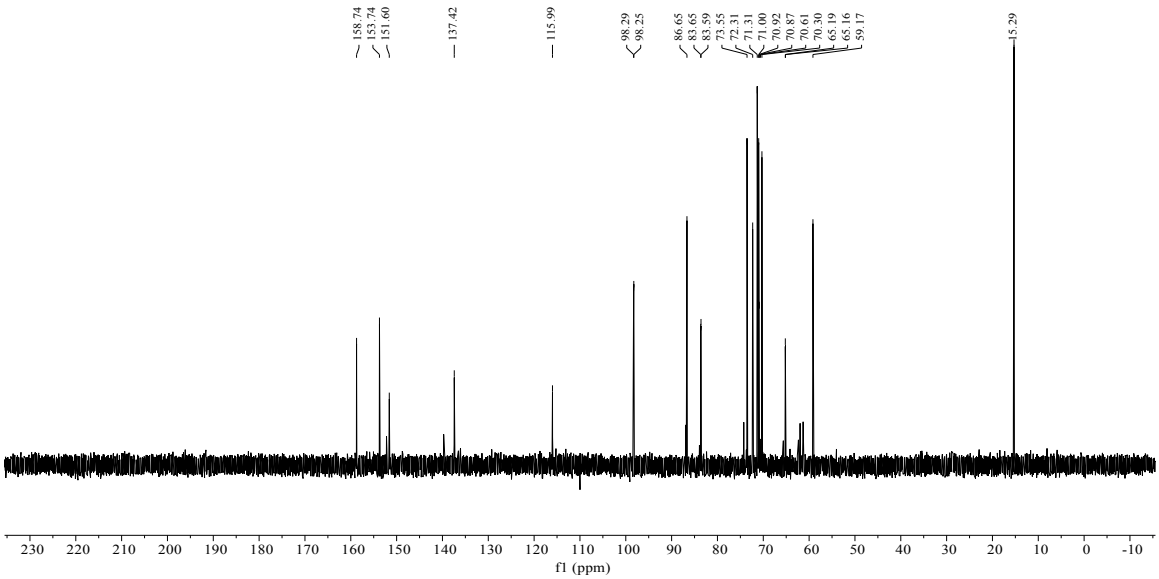




$^{-}$Q model ESI-MS profile of GDP-Fuc

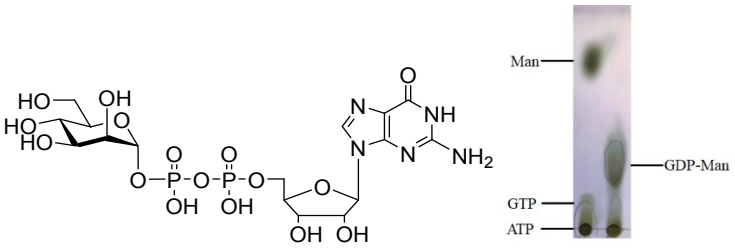


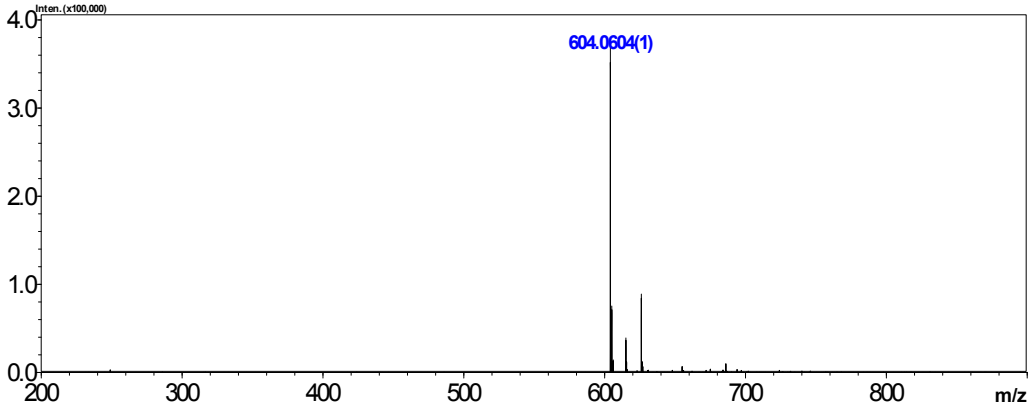
^{1}H NMR Spectrum of GDP-Fuc



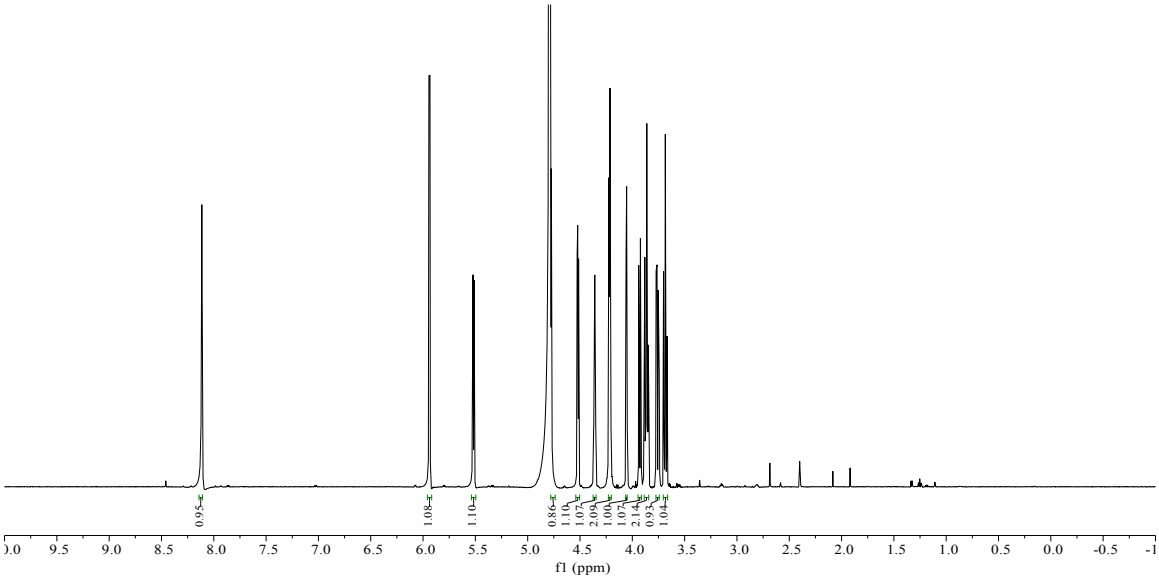
^{13}C NMR Spectrum of GDP-Fuc

GDP-Man: guanosine 5'-triphosphate Mannose

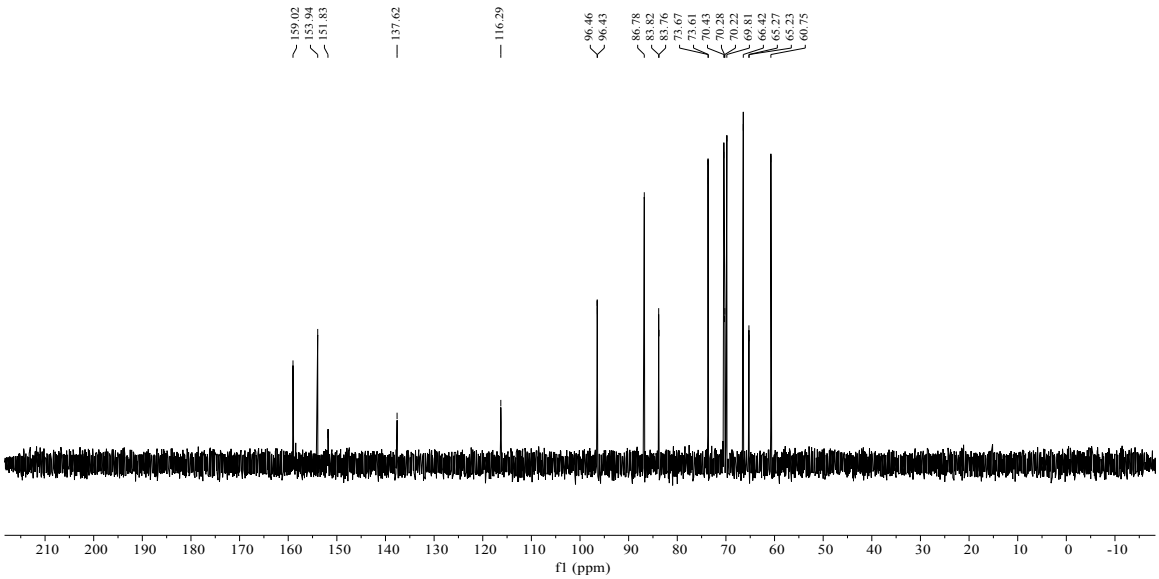




$^{-}$Q model ESI-MS profile of GDP-Man

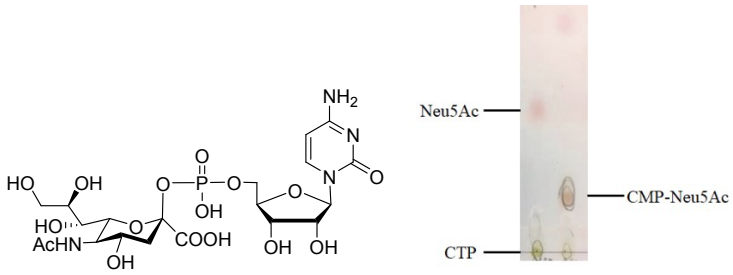


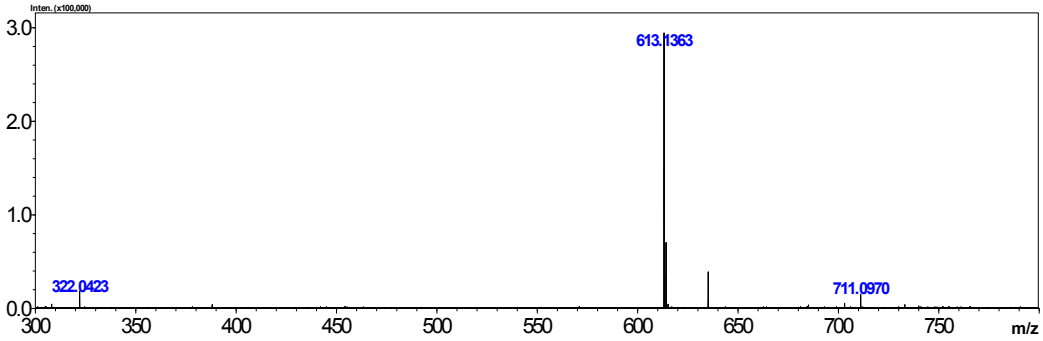
^{1}H NMR Spectrum of GDP-Man



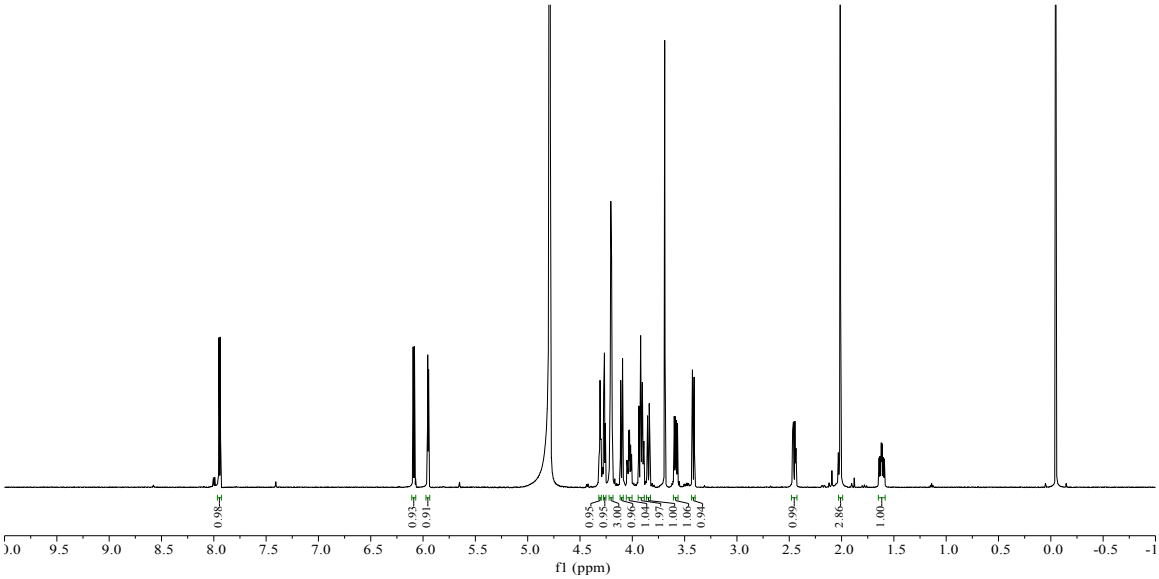
^{13}C NMR Spectrum of GDP-Man

CMP-Neu5Ac: cytidine 5'-monophosphate *N*-acetyl neuraminic acid

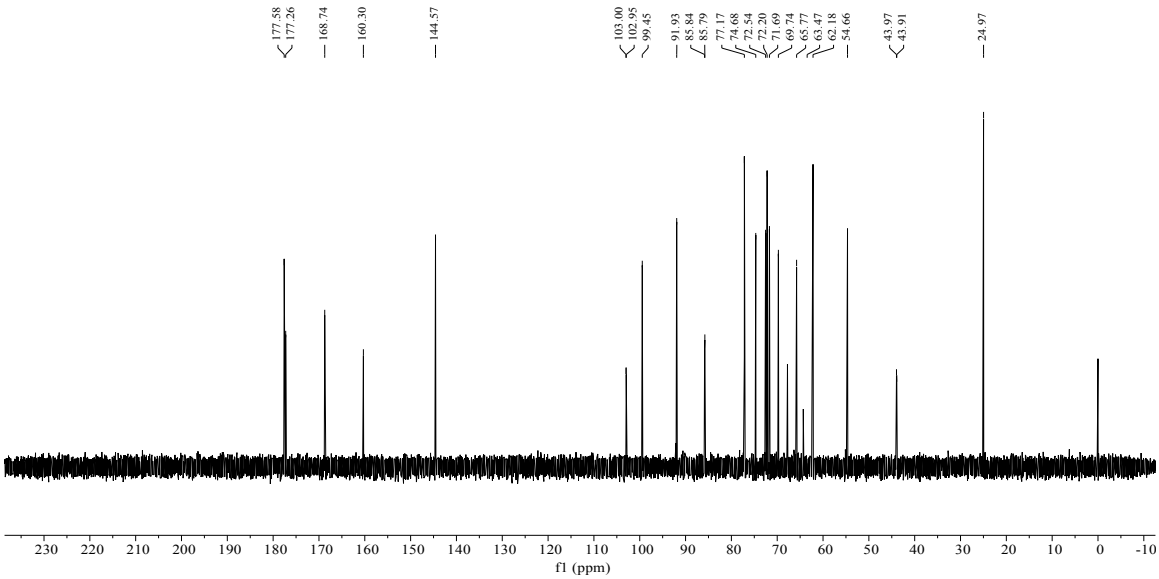




$^{-}$Q model ESI-MS profile of CMP-Neu5Ac

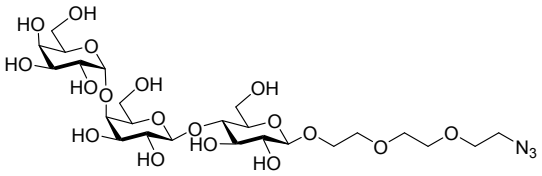


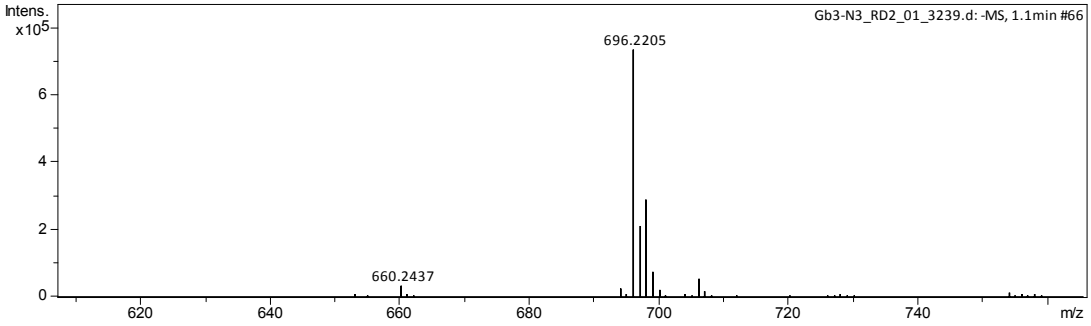
^{1}H NMR Spectrum of CMP-Neu5Ac



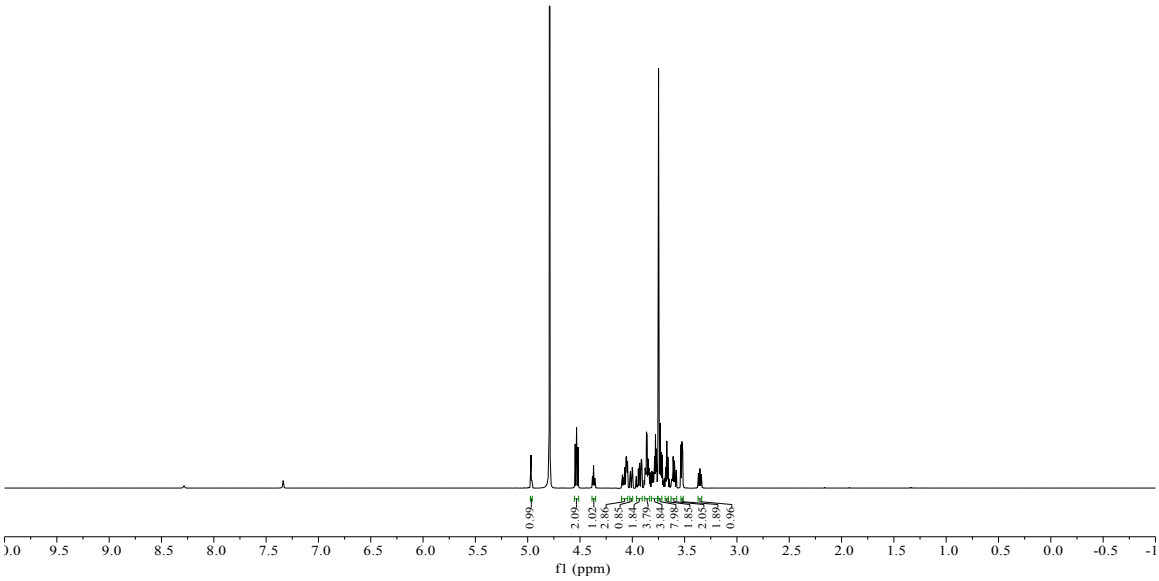
^{13}C NMR Spectrum of CMP-Neu5Ac

Compound 2: Galα1-4Galβ1-4Glcβ-PEG-N_3

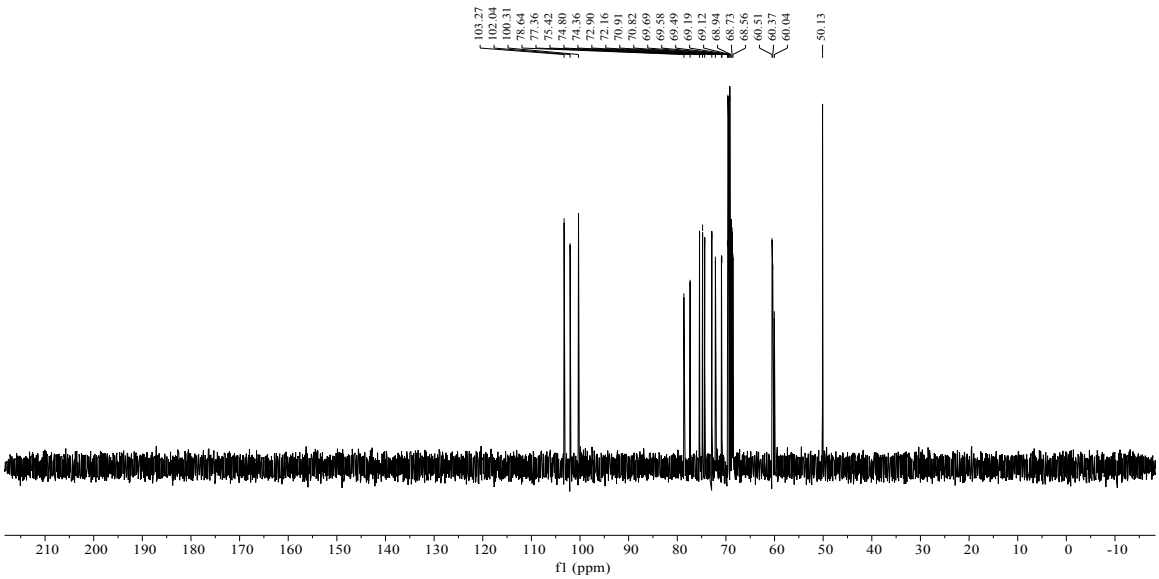




$^{-}$Q model ESI-MS profile of compound 2

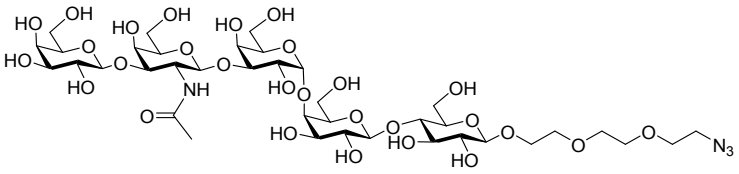


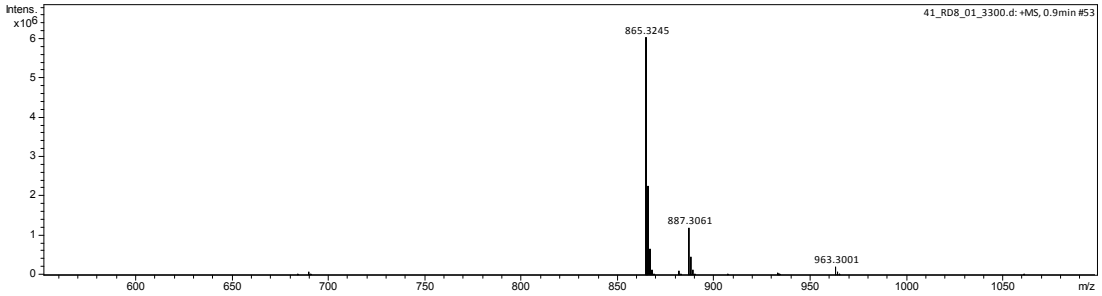
^{1}H NMR Spectrum of compound 2



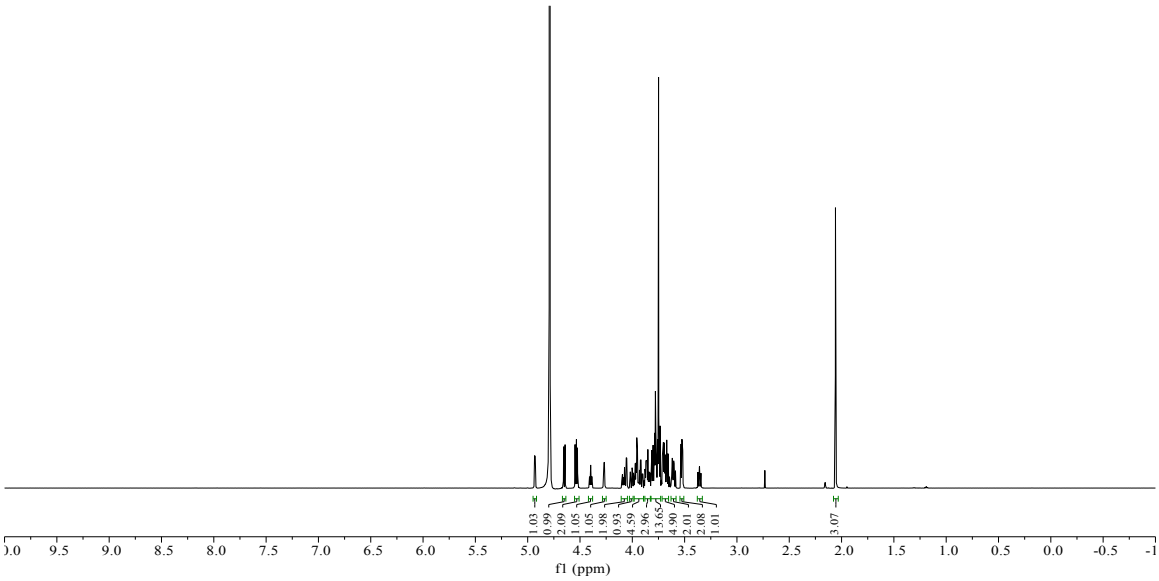
^{13}C NMR Spectrum of compound 2

Compound 3: GalNAcβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3

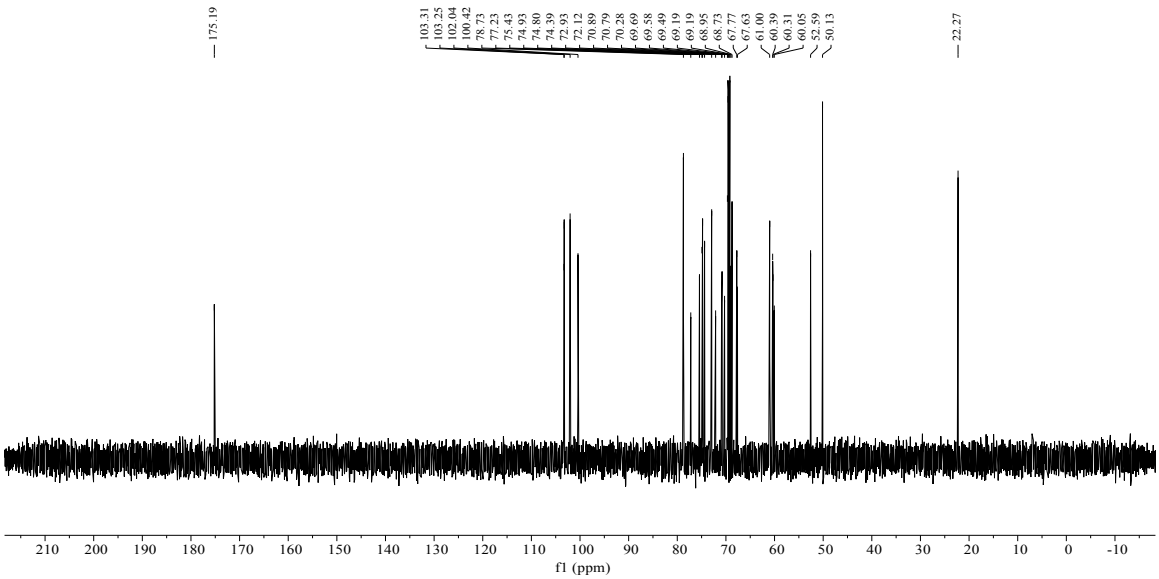




$^{+}$Q model ESI-MS profile of compound 3

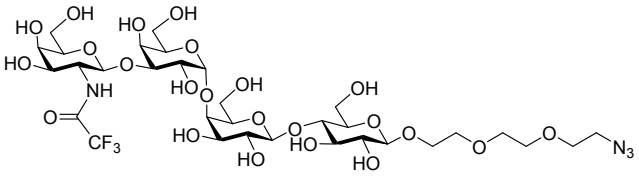


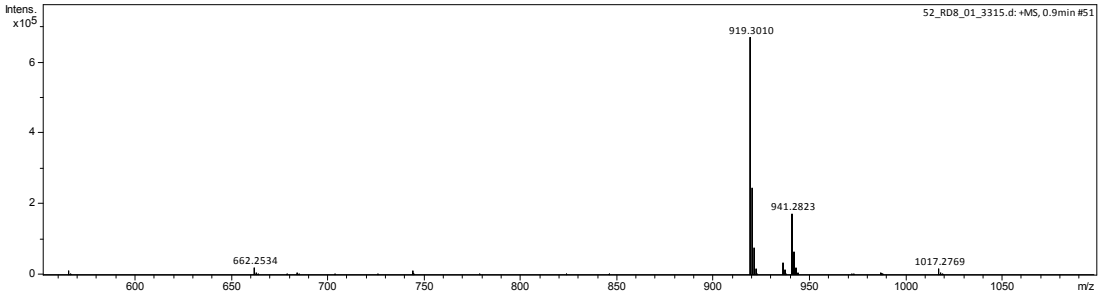
^{13}H NMR Spectrum of compound 3



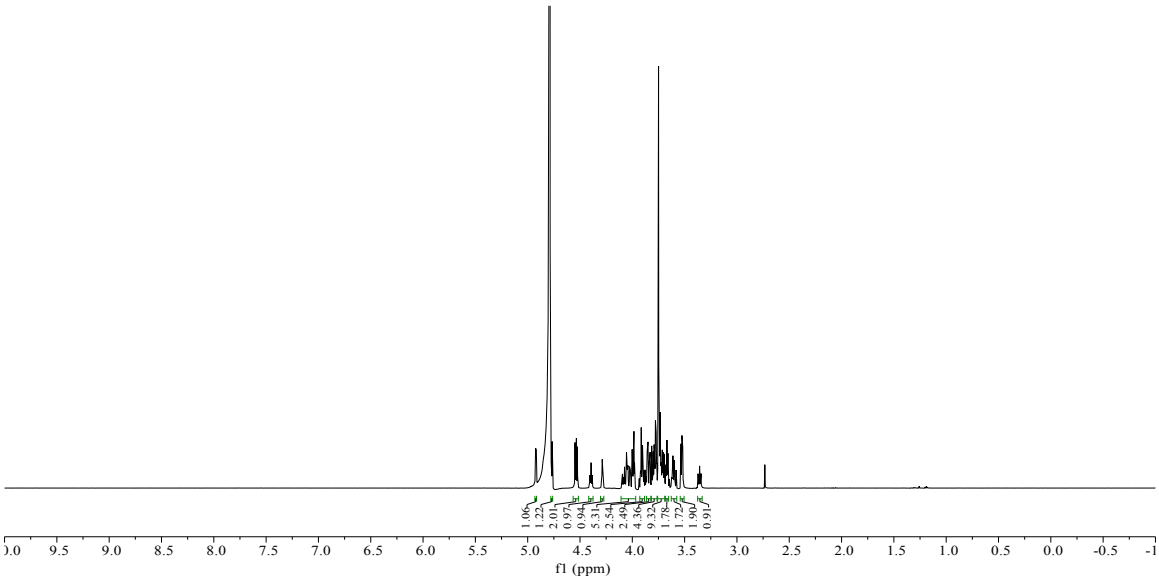
^{13}C NMR Spectrum of compound 3

Compound 4: GalNTFAβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3

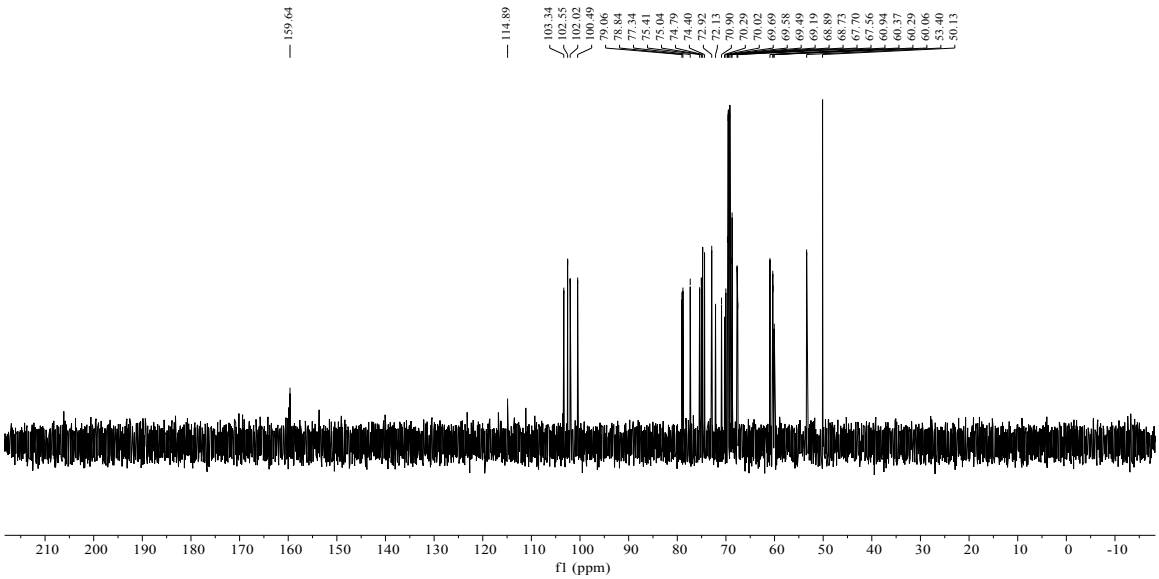




$^{+}$Q model ESI-MS profile of compound 4

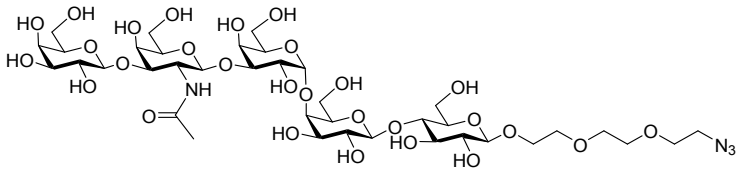


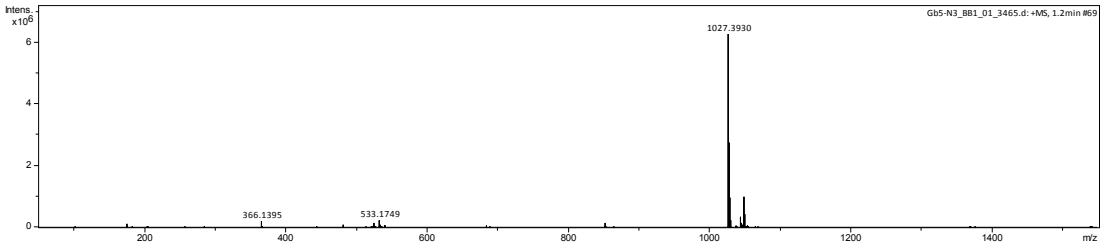
^{1}H NMR Spectrum of compound 4



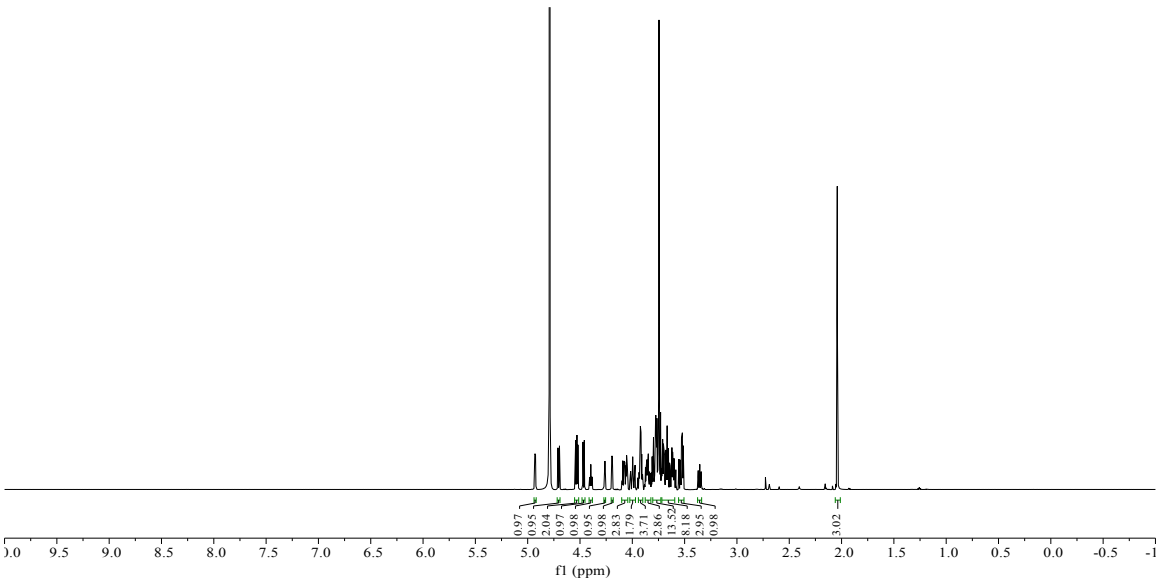
^{13}C NMR Spectrum of compound 4

Compound 5: Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3

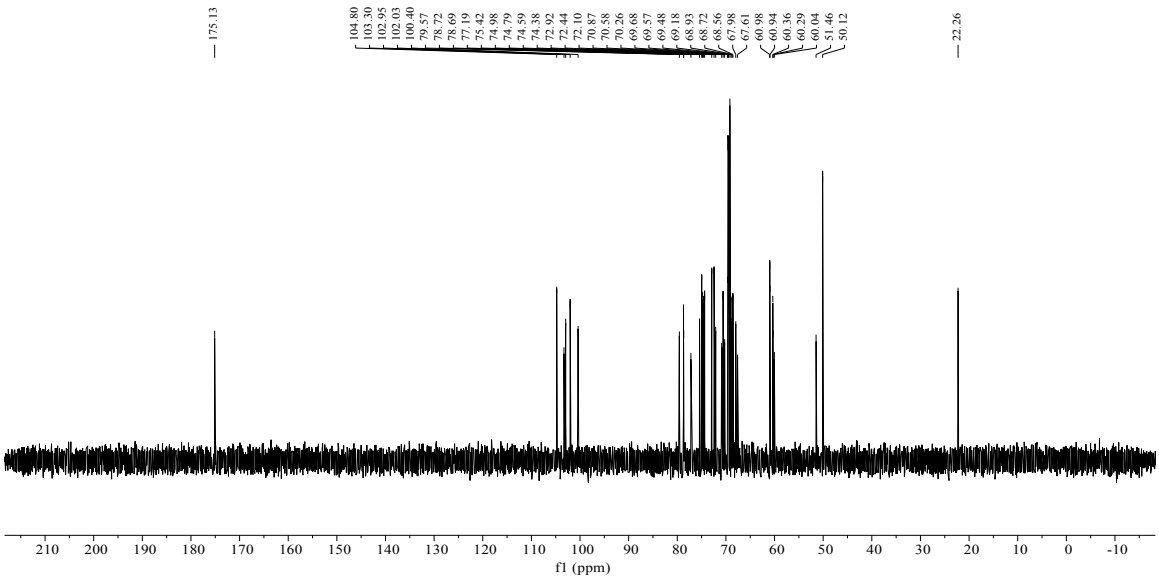




$^{+}$Q model ESI-MS profile of compound 5

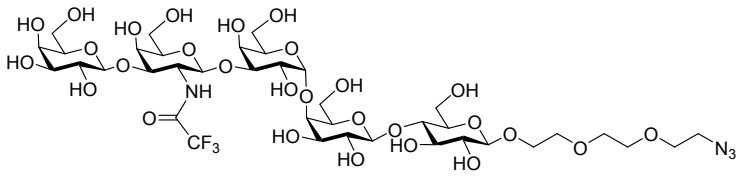


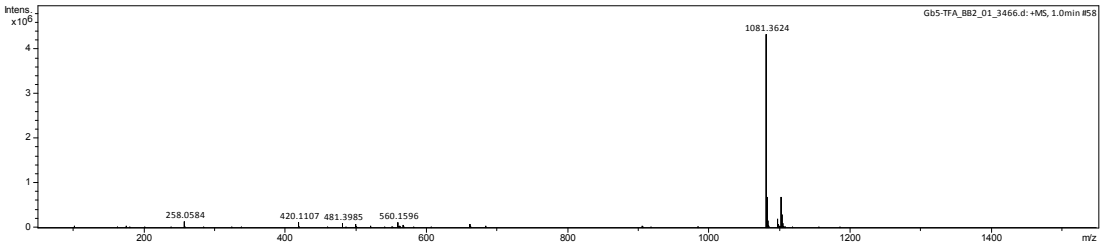
^{1}H NMR Spectrum of compound 5



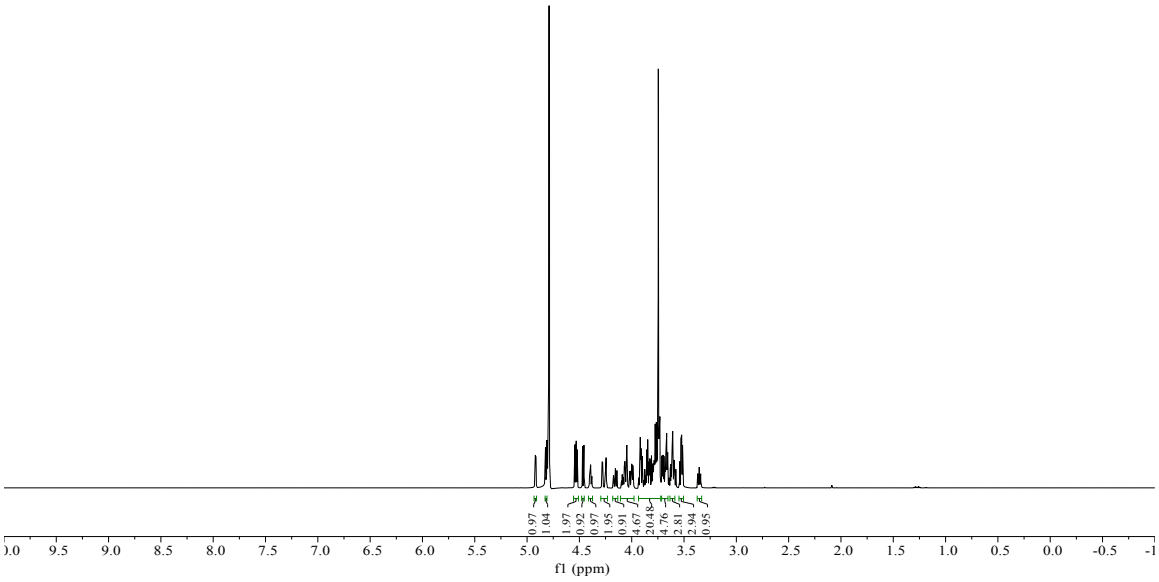
^{13}C NMR Spectrum of compound 5

Compound 6: Galβ1-3GalNFAβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3

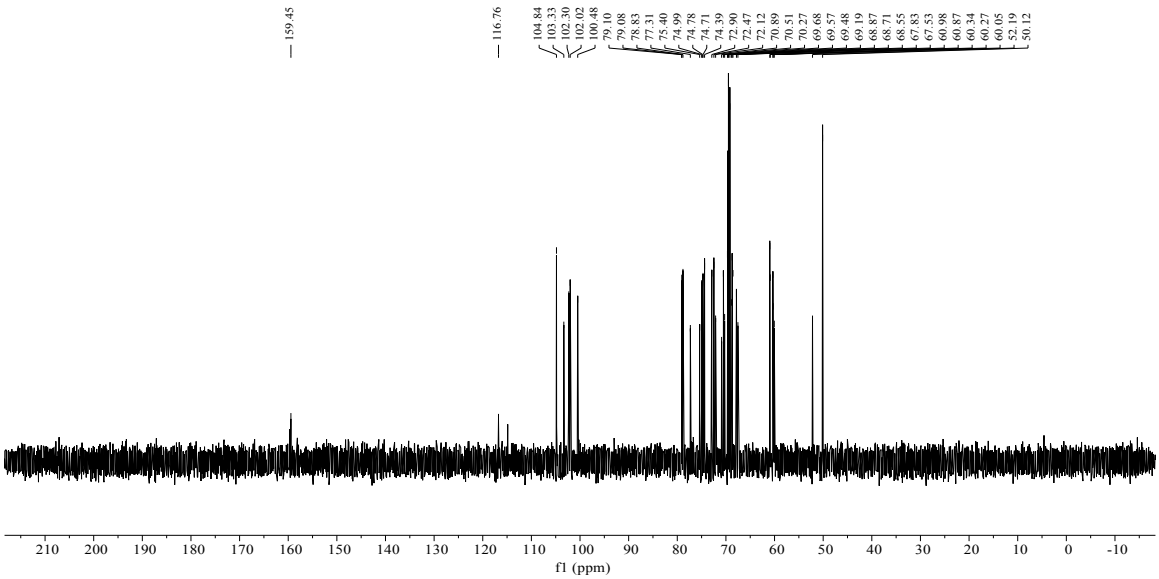




$^{+}$Q model ESI-MS profile of compound 6

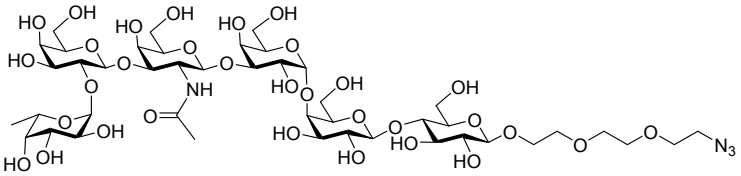


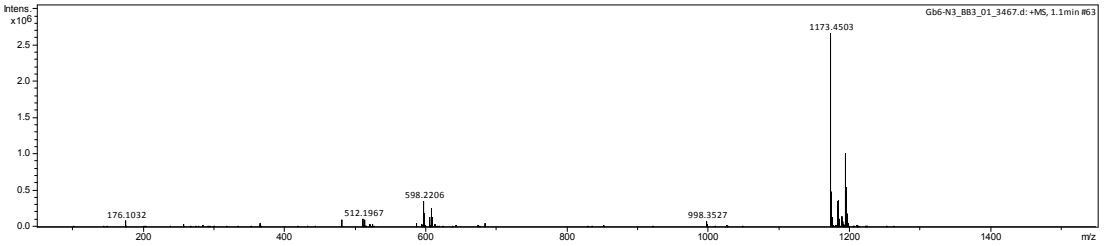
^{1}H NMR Spectrum of compound 6



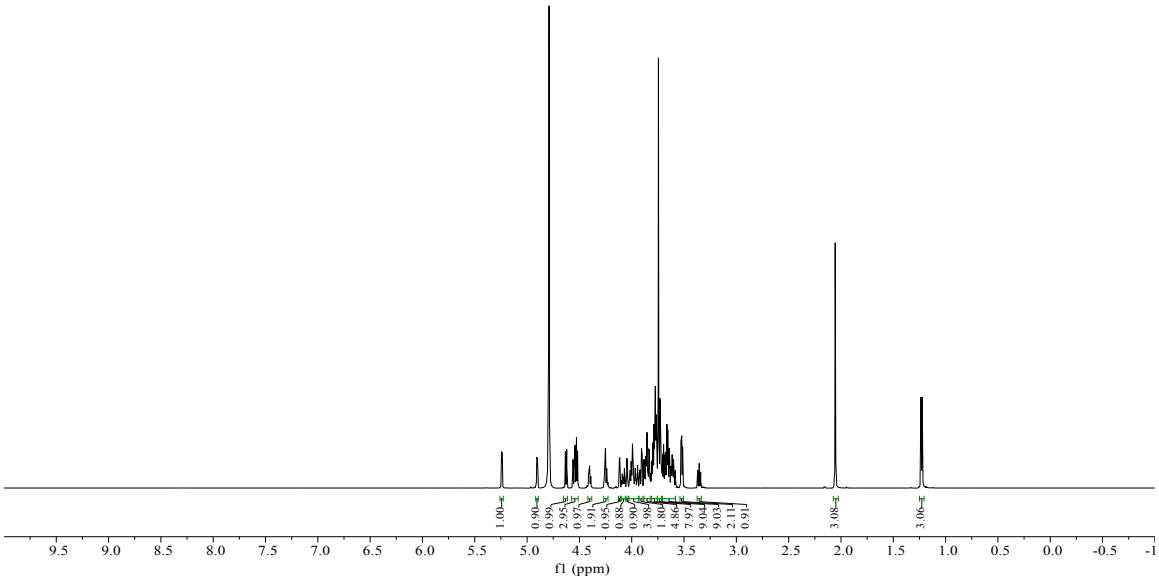
^{13}C NMR Spectrum of compound 6

Compound 7: Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3

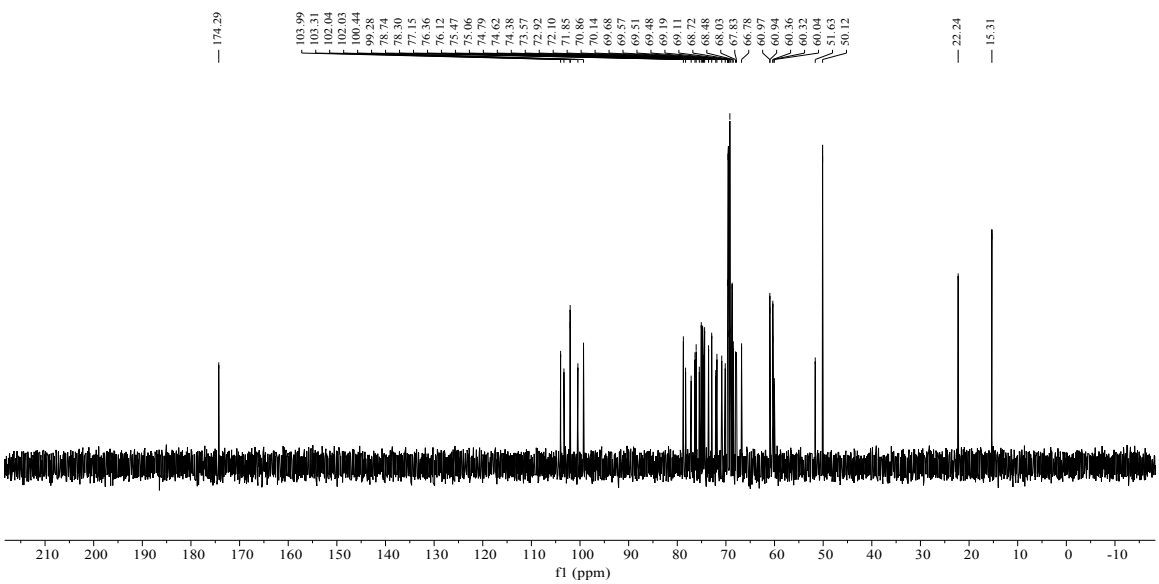




$^{+}$Q model ESI-MS profile of compound 7

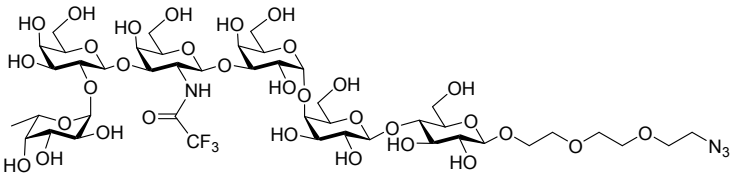


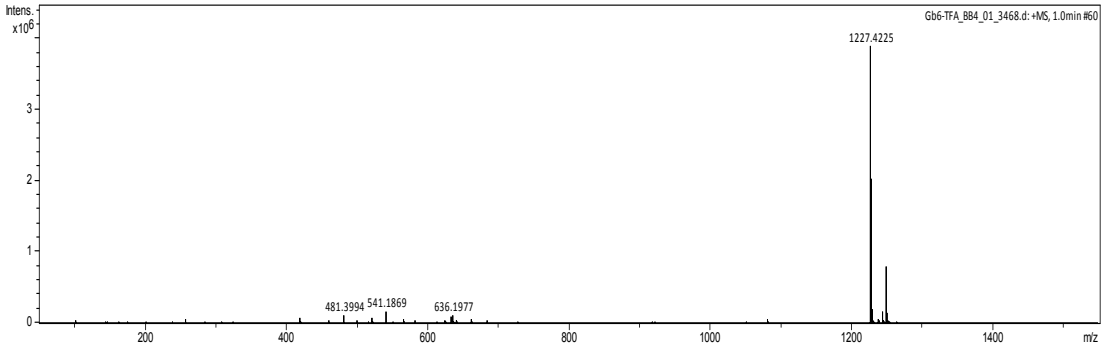
^{1}H NMR Spectrum of compound 7



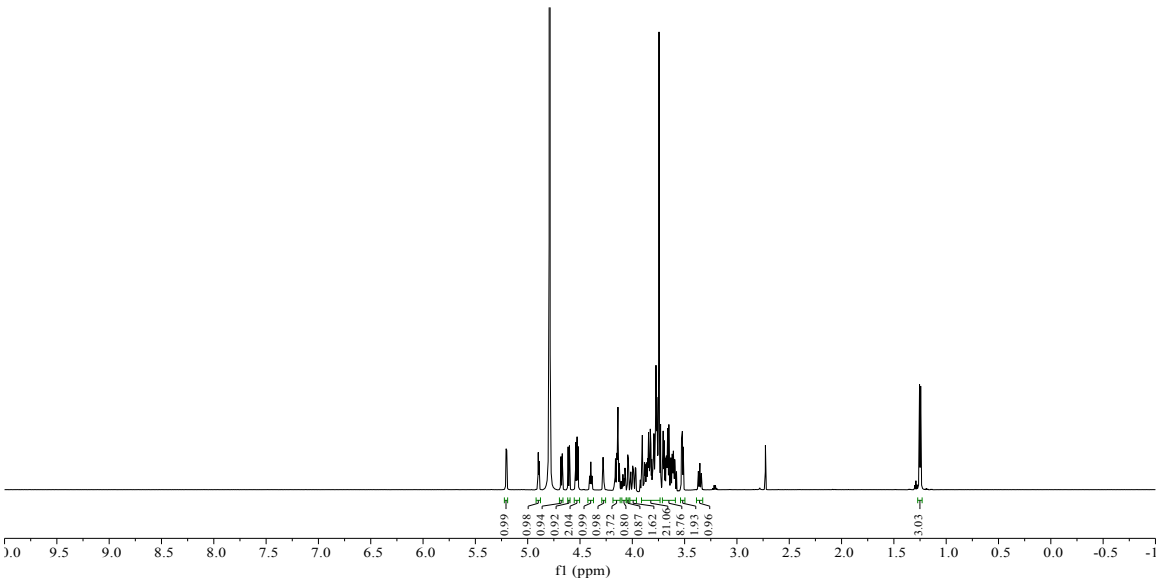
^{13}C NMR Spectrum of compound 7

Compound 8: Fucα1-2Galβ1-3GalNFAβ1-3Galα1-4Galβ1-4Glcβ-PEG-N_3

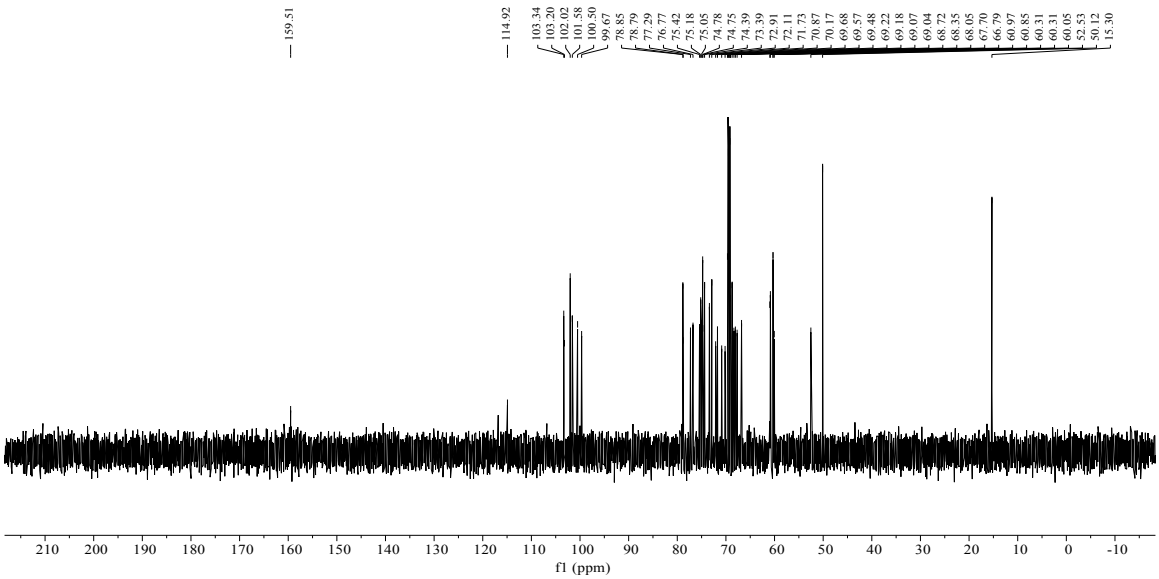




$^{+}$Q model ESI-MS profile of compound 8



^{1}H NMR Spectrum of compound 8



^{13}C NMR Spectrum of compound 8

Reference

[1] Y. Li, H. Yu, Y. Chen, K. Lau, L. Cai, H. Cao, V. K. Tiwari, J. Qu, V. Thon, P. G. Wang, X. Chen, *Molecules* **2011**, *16*, 6396-6407.

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