SUPPORTING INFORMATION

Stereoselective Chemical Synthesis of Sugar Nucleotides via Direct Displacement of Acylated Glycosyl Bromides

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1. General Methods. All chemicals were purchased from Sigma-Aldrich and used without further purification. Solvents were reagent grade unless otherwise noted. Analytical thin-layer chromatography was performed on glass-backed TLC plates pre-coated with silica gel (250 μm, Silicycle) and compounds were detected by UV absorbance (254 nm) and/or spraying with a $KMnO_4$ visualization solution (3 g $KMnO_4$, 20 g K_2CO_3, 5 mL aq. NaOH, 300 mL H_2O). Analytical HPLC of sugar nucleotide reaction mixtures and purified products was performed on a Hewlett Packard Series 1050 instrument using an Agilent Zorbax 5 μm Rx-C18 column (150 cm x 4.6 mm). Compounds containing a nucleoside base chromophore were monitored at an absorbance of 254 nm over a linear gradient from 90/10 A/B to 40/60 A/B over 8.0 min followed by a plateau at 40/60 A/B from 8.0 to 10.0 min at 1.0 mL/min where A was an aqueous buffer containing 12 mM Bu_4NBr, 10 mM KH_2PO_4, and 5% HPLC grade MeCN (pH 4.0) and B was HPLC grade MeCN. C18 reversed-phase chromatography was performed on a Biotage SP1™ automated flash chromatography system. NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer located in the Atlantic Region Magnetic Resonance Centre, Department of Chemistry, Dalhousie University. Chemical shifts are reported in parts per million (ppm) relative to a tetramethylsilane (TMS) internal standard at 0.00 ppm for samples in $CDCl_3$, while spectra recorded in D_2O were referenced to the solvent peak at 4.79 ppm. $^{31}P\{^1H\}$ spectra were referenced relative to an external 85% aqueous H_3PO_4 sample at 0.00 ppm. Mass spectra were obtained using a ThermoFinnigan LCQ duo ion trap mass spectrometer equipped with an electrospray ionization (ESI) source used in negative ion mode located in the Maritime Mass Spectrometry Laboratories, Department of Chemistry, Dalhousie University.

2. Synthetic Procedures and Spectral Data.

2.1. *1,2,3,4,6-penta-O-acetyl-α/β-D-mannopyranose* (**2**): D-Mannose (**1**) (5.00 g, 27.8 mmol), pyridine (23 mL), and acetic anhydride (26 mL, 278 mmol) were combined in a round-bottomed flask and stirred at RT for 4 h after which the reaction was deemed complete by TLC (35/65 EtOAc/hexanes, R_f product

2 = 0.30). The reaction mixture was diluted with ice-water (50 mL) and extracted with CH_2Cl_2 (3 x 50 mL). The organic extracts were combined and washed with 1 M aqueous HCl (3 x 150 mL), H_2O (150 mL), saturated aqueous $NaHCO_3$ (150 mL), and H_2O (150 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated to give a colorless syrup (10.12 g, 25.9 mmol, 93% yield) as a mixture of α and β anomers. This product was used in the next synthetic step without any further purification. ^{1}H NMR (500 MHz, $CDCl_3$): α/β = 1/3; α diastereomer δ (ppm) = 5.87 (d, $J_{1,2}$ = 1.0 Hz, 1 H, 1-H), 5.49 (dd, $J_{2,3}$ = 3.0 Hz, 1 H, 2-H), 5.30 (dd, $J_{3,4}$ = 10.0 Hz, $J_{4,5}$ = 10.0 Hz, 1 H, 4-H), 5.14 (dd, 1 H, 3-H), 4.31 (dd, $J_{5,6a}$ = 5.5 Hz, $J_{6a,6b}$ = 12.5 Hz, 1 H, 6a-H), 4.15 (dd, $J_{5,6b}$ = 2.5 Hz, 1 H, 6b-H), 3.81 (ddd, 1 H, 5-H), 2.22 (s, 3 H, C(O)CH_3), 2.12 (s, 3 H, C(O)CH_3), 2.10 (s, 3 H, C(O)CH_3), 2.06 (s, 3 H, C(O)CH_3), 2.01 (s, 3 H, C(O)CH_3); β diastereomer δ (ppm) = 6.09 (d, $J_{1,2}$ = 2.0 Hz, 1 H, 1-H), 5.35 (m, 2 H, 3-H, 4-H), 5.26 (dd, $J_{2,3}$ = 2.0 Hz, 1 H, 2-H), 4.28 (dd, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 12.5 Hz, 1 H, 6a-H), 4.11 (dd, $J_{5,6b}$ = 2.5 Hz, 1 H, 6b-H), 4.06 (ddd, 1 H, 5-H), 2.18 (s, 3 H, C(O)CH_3), 2.17 (s, 3 H, C(O)CH_3), 2.10 (s, 3 H, C(O)CH_3), 2.06 (s, 3 H, C(O)CH_3), 2.01 (s, 3 H, C(O)CH_3).

2.2. *2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide* (**3**): Acetylated D-mannopyranoside (**2**) (2.00 g, 5.12 mmol) was dissolved in CH_2Cl_2 (16 mL) in a round-bottomed flask, which was stoppered with a septum and cooled in an ice-water bath. PBr_3 (820 μL, 8.70 mmol) and H_2O (550 μL, 30.7 mmol) were added dropwise. After 10 min, the reaction mixture was warmed to RT and stirred for 3 h after which the reaction was deemed complete by TLC (35/65 EtOAc/hexanes, R_f product **3** = 0.43).[a] The reaction mixture was diluted with CH_2Cl_2 (25 mL) and extracted with H_2O (2 x 40 mL), saturated aqueous $NaHCO_3$ (40 mL), and saturated aqueous NaCl (2 x 40 mL). The organic layer was dried over Na_2SO_4 and concentrated to give a pale orange syrup (1.78 g, 4.33 mmol, 85% yield). This product was used in the next synthetic step without any further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 6.30 (d, $J_{1,2}$ = 1.0 Hz, 1 H, 1-H), 5.72 (dd, $J_{2,3}$ = 3.5 Hz, $J_{3,4}$ = 10.0 Hz, 1 H, 3-H), 5.45 (dd, 1 H, 2-H), 5.37

[a] It should be noted that bromide **3** breaks down on silica gel TLC plates, presumably to the hemiacetals, which had an R_f of 0.11 in 35/65 EtOAc/hexanes. Crude product **3** was pure by ^{1}H NMR.

(dd, $J_{4,5}$ = 10.0 Hz, 1 H, 4-H), 4.33 (dd, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 12.5 Hz, 1 H, 6a-H), 4.23 (ddd, $J_{5,6b}$ = 2.5 Hz, 1 H, 5-H), 4.14 (dd, 1 H, 6b-H), 2.18 (s, 3 H, $C(O)CH_3$), 2.11 (s, 3 H, $C(O)CH_3$), 2.08 (s, 3 H, $C(O)CH_3$), 2.01 (s, 3 H, $C(O)CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 170.5 (0), 169.7 (0), 169.6 (0), 169.5 (0), 83.1 (1), 72.9 (1), 72.2 (1), 68.0 (1), 65.4 (1), 61.5 (1), 20.74 (3), 20.66 (3), 20.63 (3), 20.55 (3).

2.3. *UDP-α-D-mannose* (**4**): Uridine 5'-diphosphate (Bu_4N^+ salt titrated to pH 6, 2.3 eq. Bu_4N^+ per eq. of UDP by ^1H NMR, 96 mg, 0.10 mmol) was dissolved in anhydrous MeCN (3 mL) under an N_2 atmosphere in a round-bottomed flask fitted with a condensor. Anhydrous triethylamine (14 μL, 0.10 mmol) was added and the solution was stirred at RT for 15 min with ~10 activated 3 Å molecular sieves. In a separate round-bottomed flask, acetylated D-mannosyl bromide (**3**) (41 mg, 0.10 mmol) was dissolved in anhydrous MeCN (2 mL) under an N_2 atmosphere and transferred via cannula to the round-bottomed flask containing UDP and triethylamine. The reaction mixture was heated at 80 °C for 30 min. After 30 min, TLC (35/65 EtOAc/hexanes) revealed that all glycosyl bromide had been consumed or degraded. Analysis via HPLC (using the conditions outlined in the General Methods section) revealed a new peak corresponding to the sugar nucleotide product (t_R = 5.43 min). The molecular sieves were filtered off and the reaction mixture was concentrated and re-dissolved in H_2O (3 mL). The pH of this solution was immediately adjusted to 8 using triethylamine. Alkaline phosphatase (50 EU[b]) was added to degrade residual UDP and simplify the purification protocol. The enzymatic reaction was stirred overnight at RT (~16 h) and the degradation process was monitored by HPLC using the conditions described above (t_R UDP = 6.16 min). Upon complete UDP degradation, the reaction mixture was concentrated and dissolved in 2/2/1 MeOH/H_2O/Et_3N (3 mL). This solution was stirred at RT for 24 h to remove the acetyl protecting groups from the monosaccharide. After 24 h, the reaction mixture was concentrated and re-dissolved in 10 mM aqueous tributylammonium bicarbonate buffer (~2

[b] 1 EU = the amount of enzyme needed to catalyze the transformation of 1 μmol of substrate per min

mL) in preparation for purification via C18 ion-pair reversed-phase chromatography. The 10 mM aqueous tributylammonium bicarbonate buffer was prepared by adding tributylamine (2.4 mL, 10 mmol / L) to H_2O and bubbling CO_2 (obtained from the sublimation of dry ice) through the solution, cooled in an ice bath, until all tributylamine appeared to be dissolved (~3 h). Automated C18 reversed-phase ion-pair chromatography was accomplished using a 25M (25 mm x 15 cm) Biotage C18 reversed-phase column. Compounds bearing nucleotide base chromophores were monitored at 254 nm. A solvent system of 100/0 A/B (2 CV) followed by a linear gradient to 60/40 A/B over 15 CV and a plateau at 60/40 A/B (2 CV) was employed where A represents 10 mM aqueous tributylammonium bicarbonate buffer and B represents HPLC MeOH at a flow rate of 25 mL/min. The C18 column was subsequently washed with 0/100 A/B for 3 CV. All UV active peaks were analyzed by HPLC and fractions containing the desired sugar nucleotide were combined and concentrated to ~10 mL in volume. This solution was then passed down a column filled with Dowex® 50W-X8 cation exchange resin (100-200 mesh, Na^+ form, 18 mm x 14 cm) to bind tributylammonium cations and generate the sodium salt of the desired sugar nucleotide. Fractions containing the product were concentrated and lyophilized to generate a white solid, which was pure by HPLC. NMR analysis revealed a small quantity of an organic *n*-propyl-containing counterion (not connected to the sugar nucleotide by COSY NMR analysis). It is postulated that this impurity was present in the tributylamine.[c] To generate a final product in one salt form for accurate yield analysis, the sugar nucleotide was re-dissolved in 0.1% aqueous NH_4HCO_3 and passed down a 12M (12 mm x 15 cm) Biotage C18 reversed-phase column. A solvent system of 100/0 A/B (4 CV) followed by a linear gradient to 0/100 A/B over 3 CV and a plateau at 0/100 A/B (4 CV) was employed where A represents 0.1% aqueous NH_4HCO_3 and B represents 0.1% NH_4HCO_3 in HPLC MeOH at a flow rate of 10 mL/min. After concentration and lyophilizaton, pure UDP-α-D-mannose (**4**) was obtained in its ammonium salt form (20 mg, 0.033 mmol, 33% yield by mass, 29% yield by UV absorbance at λ_{max} 261 nm = 1.01 x 10^4 $M^{-1}cm^{-1}$ over 2 steps). ^1H NMR (500 MHz, D_2O, pH 6): δ

[c] This chemical will be distilled before future purifications using this protocol in an attempt to circumvent this difficulty.

(ppm) = 7.93 (d, $J_{5,6}$ = 8.0 Hz, 1 H, 6-H), 5.98 (d, $J_{1',2'}$ = 3.5 Hz, 1 H, 1'-H), 5.95 (d, 1 H, 5-H), 5.49 (br d, $J_{1'',P}$ = 8.0 Hz, 1 H, 1''-H), 4.35 (m, 2 H, 2'-H, 3'-H), 4.27 (m, 1 H, 4'-H), 4.22, 4.17 (m, 2 H, 5a'-H, 5b'-H), 4.03 (br d, $J_{2'',3''}$ = 3.5 Hz, 1 H, 2''-H), 3.90 (dd, $J_{3'',4''}$ = 10.0 Hz, 1 H, 3''-H), 3.85 (m, 2 H, 5''-H, 6a''-H), 3.75 (dd, $J_{5'',6b''}$ = 5.5 Hz, $J_{6a'',6b''}$ = 12.5 Hz, 1 H, 6b''-H), 3.66 (dd, $J_{4'',5''}$ = 10.0 Hz, 1 H, 4''-H); ^{13}C NMR (126 MHz, D_2O, pH 6): δ (ppm) = 167.5 (0), 152.8 (0), 141.5 (1), 102.8 (1), 96.5 (1), 88.5 (1), 83.2 (1), 73.8 (1), 73.7 (1), 70.3 (1), 69.9 (1), 69.7 (1), 66.5 (1), 65.0 (2), 60.8 (2); ^{31}P{^{1}H} NMR (202 MHz, D_2O, pH 6): δ (ppm) = -11.55 (d, $J_{P,P}$ = 20.2 Hz), -13.85 (d); LRMS (ESI$^-$) for $C_{15}H_{24}N_2O_{17}P_2$ (free acid, 566.1) = *m/z* 565.1 $[M-H]^-$.[S1]

2.4. *GDP-α-D-mannose* (**5**): Guanosine 5'-diphosphate (Bu_4N^+ salt titrated to pH 6, 2.5 eq. Bu_4N^+ per eq. of GDP by ^{1}H NMR, 105 mg, 0.10 mmol) was dissolved in anhydrous MeCN (3 mL) under an N_2 atmosphere in a round-bottomed flask fitted with a condensor. Anhydrous triethylamine (14 mL, 0.10 mmol) was added and the solution was stirred at RT for 15 min with ~10 activated 3 Å molecular sieves. In a separate round-bottomed flask, acetylated D-mannosyl bromide (**3**) (41 mg, 0.10 mmol) was dissolved in anhydrous MeCN (2 mL) under an N_2 atmosphere and transferred via cannula to the round-bottomed flask containing GDP and triethylamine. The reaction mixture was heated at 80 °C for 30 min. After 30 min, TLC (35/65 EtOAc/hexanes) revealed that all glycosyl bromide had been consumed or degraded. Analysis via HPLC (using the conditions outlined in the General Methods section) revealed a new peak corresponding to the sugar nucleotide product (t_R = 5.26 min). The molecular sieves were filtered off and the reaction mixture was concentrated and re-dissolved in H_2O (3 mL). The pH of this solution was immediately adjusted to 8 using triethylamine. Alkaline phosphatase (50 EU) was added to degrade residual GDP and simplify the purification protocol. The enzymatic reaction was stirred overnight at RT (~16 h) and the degradation process was monitored by HPLC using the conditions described above (t_R GDP = 5.93 min). Upon complete GDP degradation, the reaction mixture was concentrated and dissolved in 2/2/1 MeOH/H_2O/Et_3N (3 mL). This solution was stirred at

RT for 24 h to remove the acetyl protecting groups from the monosaccharide. After 24 h, the reaction mixture was concentrated and re-dissolved in 10 mM aqueous tributylammonium bicarbonate buffer (~2 mL) in preparation for purification via C18 ion-pair reversed-phase chromatography. The protocol used to purify GDP-α-D-mannose (**5**) was the same as the procedure reported above for UDP-α-D-mannose (**4**). After lyophilizaton, pure GDP-α-D-mannose (**5**) was obtained in its ammonium salt form (24 mg, 0.038 mmol, 38% yield by mass, 35% yield by UV absorbance at λ_{max} 253 nm = 1.37 x 10^4 $M^{-1}cm^{-1}$ over 2 steps). ^{1}H NMR (500 MHz, D_2O, pH 6): δ (ppm) = 8.09 (s, 1 H, 8-H), 5.92 (d, $J_{1',2'}$ = 6.0 Hz, 1 H, 1'-H), 5.49 (br d, $J_{1'',P}$ = 7.5 Hz, 1 H, 1''-H), 4.75 (dd, $J_{2',3'}$ = 5.5 Hz, 1 H, 2'-H), 4.49 (dd, $J_{3',4'}$ = 4.0 Hz, 1 H, 3'-H), 4.34 (m, 1 H, 4'-H), 4.19 (m, 2 H, 5a'-H, 5b'-H), 4.03 (br d, $J_{2'',3''}$ = 3.3 Hz, 1 H, 2''-H), 3.90 (dd, $J_{3'',4''}$ = 10.0 Hz, 1 H, 3''-H), 3.84 (m, 2 H, 5''-H, 6a''-H), 3.73 (dd, $J_{5'',6b''}$ = 5.3 Hz, $J_{6a'',6b''}$ = 12.5 Hz, 1 H, 6b''-H), 3.66 (dd, $J_{4'',5''}$ = 10.0 Hz, 1 H, 4''-H); ^{13}C NMR (126 MHz, D_2O, pH 6): δ (ppm) = 160.5 (0), 160.1 (0), 154.9 (0), 137.4 (1), 116.5 (0), 96.5 (1), 86.8 (1), 83.8 (1), 73.7 (1), 70.5 (1), 70.3 (1), 70.2 (1), 69.9 (1), 66.5 (1), 65.3 (2), 60.8 (2); ^{31}P{^{1}H} NMR (202 MHz, D_2O, pH 6): δ (ppm) = -11.40 (d, $J_{P,P}$ = 20.2 Hz), -13.74 (d); LRMS (ESI^-) for $C_{16}H_{25}N_5O_{16}P_2$ (free acid, 605.1) = *m/z* 604.1 $[M-H]^-$.[S2]

2.5. *1,2,3,4-tetra-O-benzoyl-α/β-L-fucopyranose* (**7**): L-Fucose (**6**) (2.00 g, 12.2 mmol) and pyridine (40 mL) were combined in a round-bottomed flask and the mixture was cooled in an ice-water bath. Benzoyl chloride (7.20 mL, 62.1 mmol) was added dropwise and the ice-water bath was removed. The reaction mixture was stirred at RT for 3 h after which the reaction was deemed complete by TLC (35/65, R_f product **7** = 0.64). The reaction mixture was poured into an ice-water mixture (100 mL) and extracted with EtOAc (2 x 100 mL). The organic extracts were washed with cold 1 M aqueous HCl (3 x 200 mL), H_2O (200 mL), saturated aqueous $NaHCO_3$ (200 mL), and saturated aqueous NaCl (200 mL). The organic layer was dried over Na_2SO_4 and concentrated to give a light brown solid (7.03 g, 12.1 mmol, 99% yield). This product was used in the next synthetic step without any further purification. ^{1}H

NMR (500 MHz, $CDCl_3$): α/β = 10/1; α diastereomer δ (ppm) = 8.17-7.23 (m, 20 H, Ph), 6.87 (d, $J_{1,2}$ = 3.5 Hz, 1 H, 1-H), 6.08 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.3 Hz, 1 H, 3-H), 5.99 (dd, 1 H, H-2), 5.90 (dd, $J_{4,5}$ = 1.0 Hz, 1 H, 4-H), 4.64 (br q, $J_{5,6}$ = 6.5 Hz, 1 H, 5-H), 1.32 (d, 3 H, 6-H_3).[3] No spectral data was given in reference 3.

2.6. *2,3,4-tri-O-benzoyl-α-L-fucopyranosyl bromide* (**8**): Benzoylated L-fucopyranoside (**7**) (1.04 g, 1.79 mmol) was dissolved in CH_2Cl_2 (6 mL) in a round-bottomed flask, which was stoppered with a septum and cooled in an ice-water bath. PBr_3 (285 μL, 3.02 mmol) and H_2O (190 μL, 10.5 mmol) were added dropwise. After 10 min, the reaction mixture was warmed to RT and stirred for 2 h after which the reaction was deemed complete by TLC (35/65 EtOAc/hexanes, R_f product **8** = 0.78).[d] The reaction mixture was diluted with CH_2Cl_2 (25 mL) and extracted with H_2O (2 x 40 mL), saturated aqueous $NaHCO_3$ (40 mL), and saturated aqueous NaCl (2 x 40 mL). The organic layer was dried over Na_2SO_4 and concentrated to give a pale orange syrup (1.78 g, 4.33 mmol, 85% yield). This product was used in the next synthetic step without any further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 8.13-7.24 (m, 15 H, Ph), 6.94 (d, $J_{1,2}$ = 4.0 Hz, 1 H, 1-H), 6.01 (dd, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.5 Hz, 1 H, 3-H), 5.84 (dd, $J_{4,5}$ = 1.0 Hz, 1 H, 4-H), 5.61 (dd, 1 H, 2-H), 4.69 (br q, $J_{5,6}$ = 6.5 Hz, 1 H, 5-H), 1.36 (d, 3 H, 6-H_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) = 165.8 (0), 165.7 (0), 165.5 (0), 133.7 (1), 133.6 (1), 133.3 (1), 130.2 (0), 130.0 (1), 129.9 (1), 129.7 (1), 129.1 (0), 129,0 (0), 128.7 (1), 128.6 (1), 128.3 (1), 89.4 (1), 70.9 (1), 70.5 (1), 69.3 (1), 68.7 (1), 15.8 (3). [S3,e]

2.7. *UDP-β-L-fucose* (**9**): Uridine 5'-diphosphate (Bu_4N^+ salt titrated to pH 6, 2.3 eq. Bu_4N^+ per eq. of UDP by ^{1}H NMR, 96 mg, 0.10 mmol) was dissolved in anhydrous MeCN (3 mL) under an N_2 atmosphere in a round-bottomed flask fitted with a condensor. Anhydrous triethylamine (14 mL, 0.10 mmol) was added and the solution was stirred at RT for 15 min with ~10 activated 3 Å molecular

[d] It should be noted that bromide **8** breaks down on silica gel TLC plates, presumably to the hemiacetals, which had an R_f of 0.41 in 35/65 EtOAc/hexanes. Crude product **8** was pure by ^{1}H NMR.
[e] Not all phenyl ^{13}C resonances were observed, presumably due to spectral overlap in the 133.7 – 128.3 ppm region.

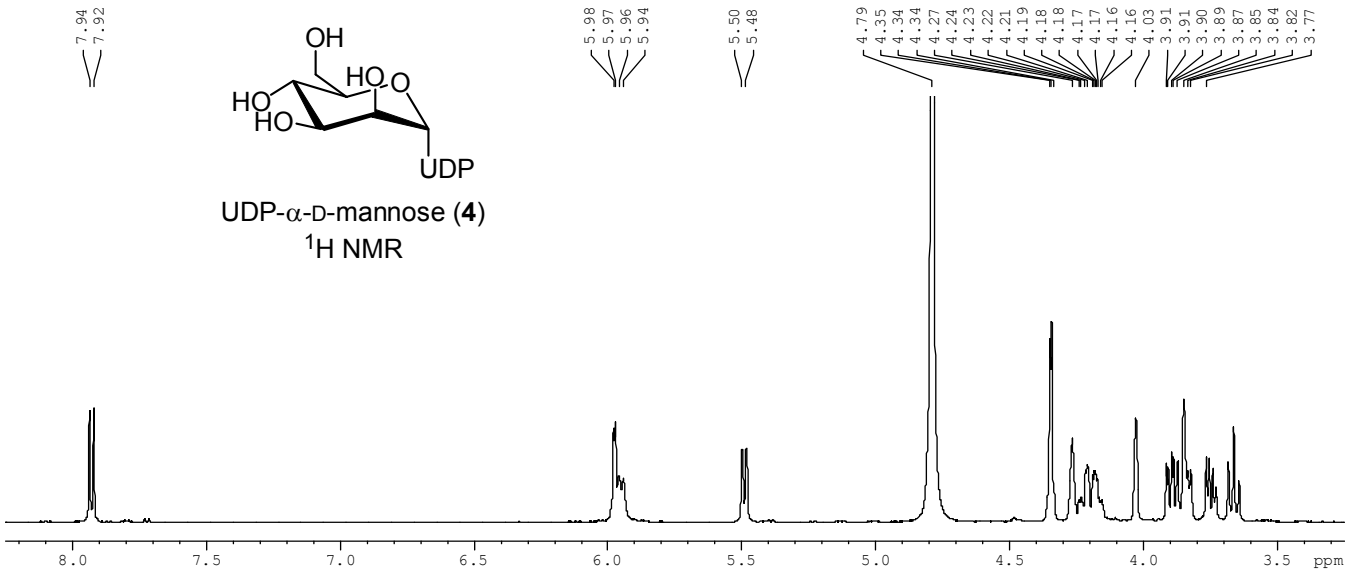
sieves. In a separate round-bottomed flask, benzoylated L-fucosyl bromide (**8**) (54 mg, 0.10 mmol) was dissolved in anhydrous MeCN (2 mL) under an N_2 atmosphere and transferred via cannula to the round-bottomed flask containing UDP and triethylamine. The reaction mixture was heated at 80 °C for 30 min. After 30 min, TLC (35/65 EtOAc/hexanes) revealed that all glycosyl bromide had been consumed or degraded. Analysis via HPLC (using the conditions outlined in the General Methods section) revealed a new peak corresponding to the sugar nucleotide product (t_R = 5.55 min). The molecular sieves were filtered off and the reaction mixture was concentrated and re-dissolved in H_2O (3 mL). The pH of this solution was immediately adjusted to 8 using triethylamine. Alkaline phosphatase (50 EU) was added to degrade residual UDP and simplify the purification protocol. The enzymatic reaction was stirred overnight at RT (~16 h) and the degradation process was monitored by HPLC using the conditions described above (t_R UDP = 6.16 min). Upon complete UDP degradation, the reaction mixture was concentrated and dissolved in 2/2/1 MeOH/H_2O/Et_3N (3 mL). This solution was stirred at RT for 24 h to remove the benzoyl protecting groups from the monosaccharide. After 24 h, the reaction mixture was concentrated and re-dissolved in 10 mM aqueous tributylammonium bicarbonate buffer (~2 mL) in preparation for purification via C18 ion-pair reversed-phase chromatography. The protocol used to purify UDP-β-L-fucose (**9**) was the same as the procedure reported above for UDP-α-D-mannose (**4**). After lyophilizaton, pure UDP-β-L-fucose (**9**) was obtained in its ammonium salt form (18 mg, 0.031 mmol, 31% yield by mass, 26% yield by UV absorbance at λ_{max} 261 nm = 1.01 x 10^4 $M^{-1}cm^{-1}$ over 2 steps). ^{1}H NMR (500 MHz, D_2O, pH 6): δ (ppm) = 7.84 (d, $J_{5,6}$ = 8.0 Hz, 1 H, 6-H), 5.91 (d, $J_{1',2'}$ = 4.5 Hz, 1 H, 1'-H), 5.88 (d, 1 H, 5-H), 4.84 (dd, $J_{1'',2''}$ = 8.0 Hz, $J_{1'', P}$ = 8.0 Hz, 1 H, 1''-H), 4.29 (m, 2 H, 2'-H, 3'-H), 4.19 (m, 1 H, 4'-H), 4.15, 4.11 (m, 2 H, 5a'-H, 5b'-H), 3.73 (br q, $J_{5'',6''}$ = 6.5 Hz, 1 H, 5''-H), 3.65 (br d, $J_{3'',4''}$ = 3.5 Hz, 1 H, 4''-H), 3.59 (dd, $J_{2'',3''}$ = 10.0 Hz, 1 H, 3''-H), 3.47 (dd, $J_{1'',2''}$ = 8.0 Hz, 1 H, 2''-H), 1.17 (d, 3 H, 6-H_3); ^{13}C NMR (126 MHz, D_2O, pH 6): δ (ppm) = 167.4 (0), 152.8 (0), 141.5 (1), 102.8 (1), 98.5 (1), 88.4 (1), 83.3 (1), 73.8 (1), 72.5 (1), 71.5 (1), 71.2 (1), 71.1 (1), 69.8 (1),

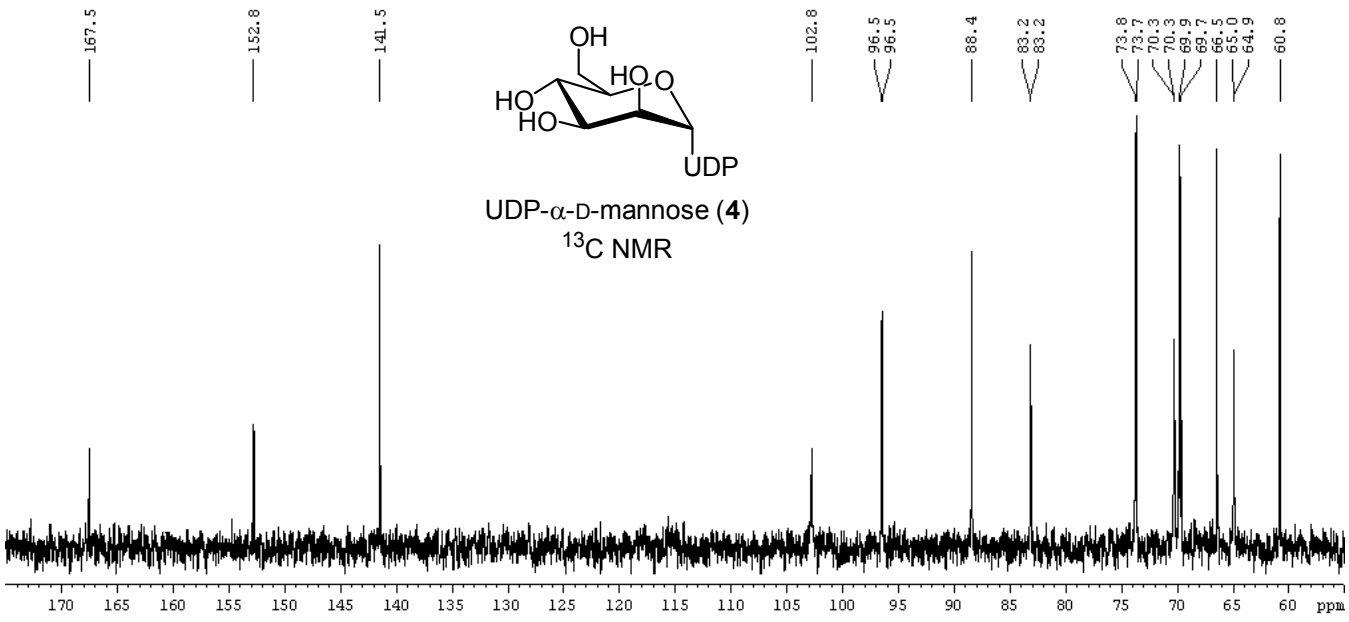
65.0 (2), 15.5 (3); $^{31}P\{^{1}H\}$ NMR (202 MHz, D_2O, pH 6): δ (ppm) = -11.17 (d, $J_{P,P}$ = 20.2 Hz), -12.93 (d); LRMS (ESI$^-$) for $C_{15}H_{24}N_2O_{16}P_2$ (free acid, 550.1) = *m/z* 549.1 $[M-H]^-$.[S4]

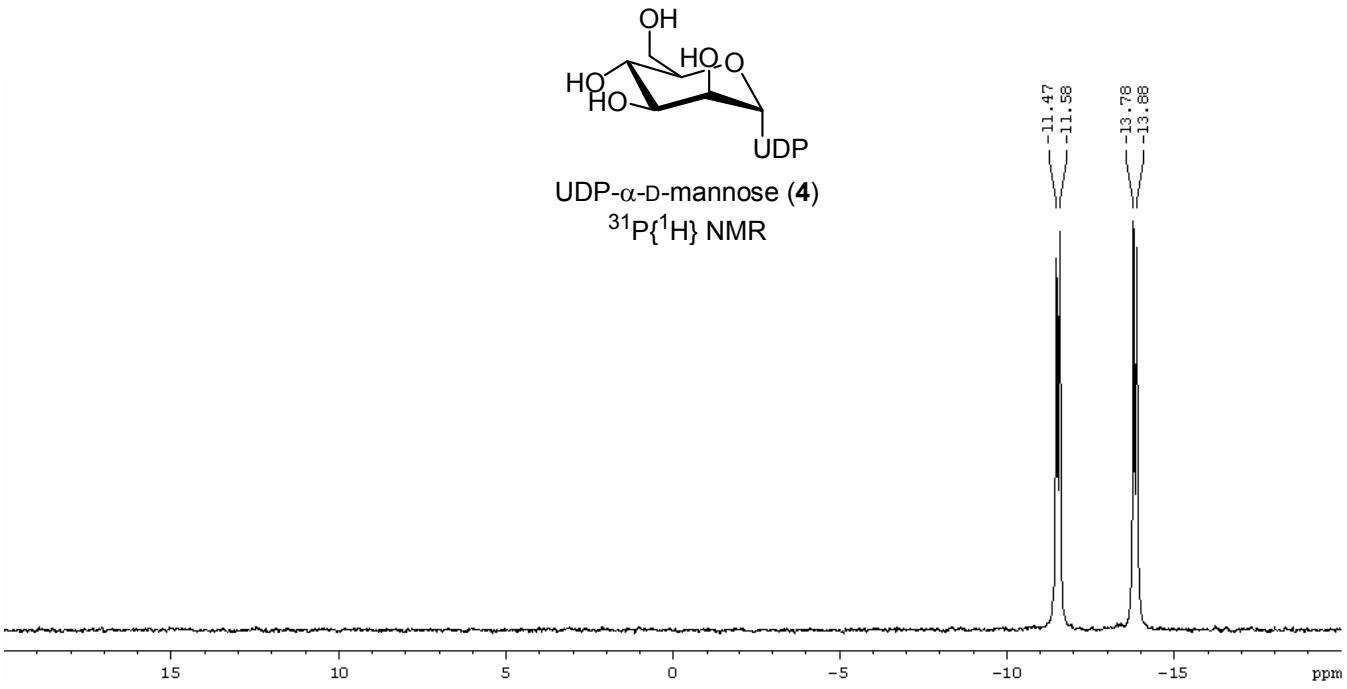
2.8. *GDP-β-L-fucose* (**10**): Guanosine 5'-diphosphate (Bu_4N^+ salt titrated to pH 6, 2.5 eq. Bu_4N^+ per eq. of GDP by ^1H NMR, 105 mg, 0.10 mmol) was dissolved in anhydrous MeCN (3 mL) under an N_2 atmosphere in a round-bottomed flask fitted with a condensor. Anhydrous triethylamine (14 mL, 0.10 mmol) was added and the solution was stirred at RT for 15 min with ~10 activated 3 Å molecular sieves. In a separate round-bottomed flask, benzoylated L-fucosyl bromide (**8**) (54 mg, 0.10 mmol) was dissolved in anhydrous MeCN (2 mL) under an N_2 atmosphere and transferred via cannula to the round-bottomed flask containing GDP and triethylamine. The reaction mixture was heated at 80 °C for 30 min. After 30 min, TLC (35/65 EtOAc/hexanes) revealed that all glycosyl bromide had been consumed or degraded. Analysis via HPLC (using the conditions outlined in the General Methods section) revealed a new peak corresponding to the sugar nucleotide product (t_R = 5.26 min). The molecular sieves were filtered off and the reaction mixture was concentrated and re-dissolved in H_2O (3 mL). The pH of this solution was immediately adjusted to 8 using triethylamine. Alkaline phosphatase (50 EU) was added to degrade residual GDP and simplify the purification protocol. The enzymatic reaction was stirred overnight at RT (~16 h) and the degradation process was monitored by HPLC using the conditions described above (t_R GDP = 5.93 min). Upon complete GDP degradation, the reaction mixture was concentrated and dissolved in 2/2/1 MeOH/H_2O/Et_3N (3 mL). This solution was stirred at RT for 24 h to remove the benzoyl protecting groups from the monosaccharide. After 24 h, the reaction mixture was concentrated and re-dissolved in 10 mM aqueous tributylammonium bicarbonate buffer (~2 mL) in preparation for purification via C18 ion-pair reversed-phase chromatography. The protocol used to purify GDP-β-L-fucose (**10**) was the same as the procedure reported above for UDP-α-D-mannose (**4**). After lyophilizaton, pure GDP-β-L-fucose (**9**) was obtained in its ammonium salt form (22 mg, 0.035 mmol, 35% yield by mass, 31% yield by UV absorbance at λ_{max} 253 nm = 1.37 x 10^4 $M^{-1}cm^{-1}$

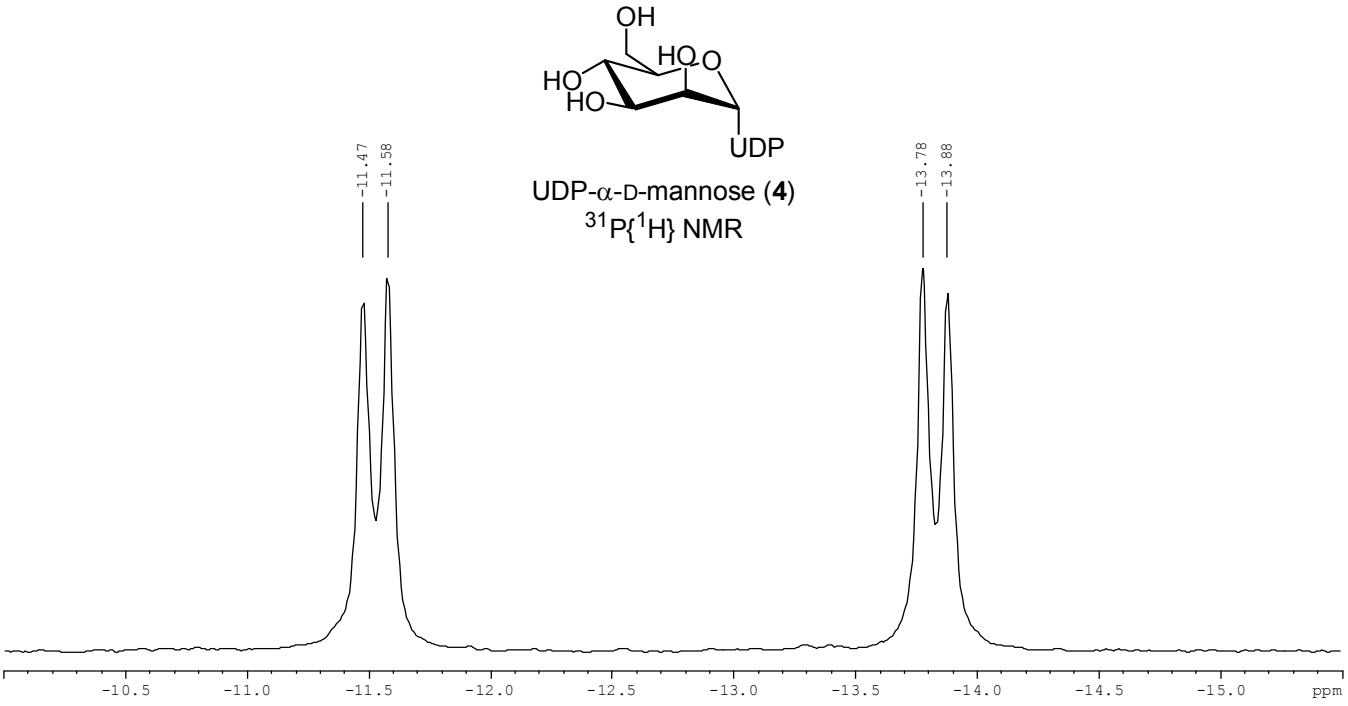
over 2 steps). ^{1}H NMR (500 MHz, D_2O, pH 6): δ (ppm) = 8.09 (s, 1 H, 8-H), 5.92 (d, $J_{1',2'}$ = 6.0 Hz, 1 H, 1'-H), 4.90 (dd, $J_{1'',2''}$ = 8.0 Hz, $J_{1'',P}$ = 8.0 Hz, 1 H, 1''-H), 4.77 (dd, $J_{2',3'}$ = 6.0 Hz, 1 H, 2'-H), 4.52 (dd, $J_{3',4'}$ = 4.0 Hz, 1 H, 3'-H), 4.34 (m, 1 H, 4'-H), 4.19 (m, 2 H, 5a'-H, 5b'-H), 3.74 (br q, $J_{5'',6''}$ = 6.5 Hz, 1 H, 5''-H), 3.69 (br d, $J_{3'',4''}$ = 3.5 Hz, 1 H, 4''-H), 3.63 (dd, $J_{2'',3''}$ = 10.0 Hz, 1 H, 3''-H), 3.54 (dd, $J_{1'',2''}$ = 8.0 Hz, 1 H, 2''-H), 1.20 (d, 3 H, 6-H_3); ^{13}C NMR (126 MHz, D_2O, pH 6): δ (ppm) = 160.5 (0), 154.7 (0), 151.9 (0), 137.5 (1), 116.5 (0), 99.4 (1), 86.7 (1), 83.8 (1), 73.6 (1), 72.5 (1), 71.5 (1), 71.2 (1), 71.1 (1), 70.5 (1), 65.3 (2), 15.4 (3); ^{31}P{^{1}H} NMR (202 MHz, D_2O, pH 6): δ (ppm) = -11.07 (d, $J_{P,P}$ = 20.2 Hz), -12.80 (d); LRMS (ESI$^-$) for $C_{16}H_{25}N_5O_{15}P_2$ (free acid, 589.1) = *m/z* 588.1 $[M-H]^-$.[S5,S6]

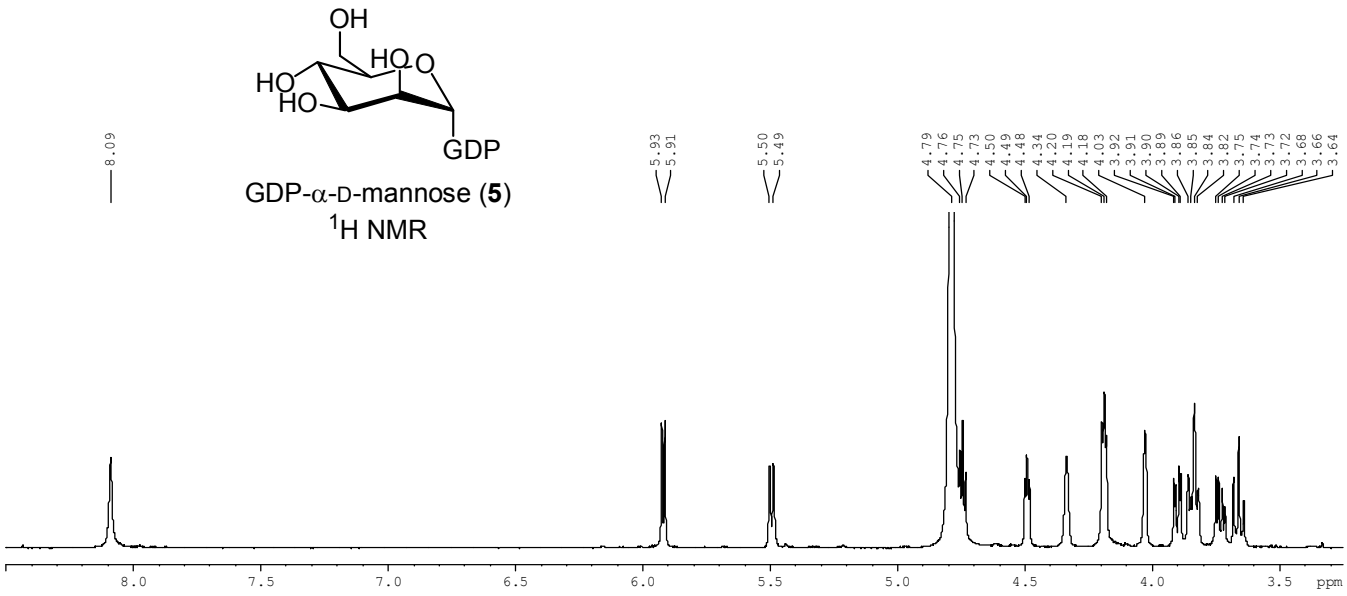
3. ^{1}H, ^{13}C, and $^{31}P\{^{1}H\}$ NMR Spectra of Sugar Nucleotides.






OH
HO
HO
HO
O
UDP
UDP-α-D-mannose (4)
31P{1H} NMR
-11.47
-11.58
-13.78
-13.88
15
10
5
0
-5
-10
-15
ppm


OH
HO
HO
HO
O
UDP
UDP-α-D-mannose (4)
31P{1H} NMR
-11.47
-11.58
-13.78
-13.88
-10.5
-11.0
-11.5
-12.0
-12.5
-13.0
-13.5
-14.0
-14.5
-15.0
ppm


OH
HO
O
HO
HO
GDP
GDP-α-D-mannose (5)
1H NMR
8.09
5.93
5.91
5.50
5.49
4.79
4.76
4.75
4.73
4.50
4.49
4.48
4.34
4.20
4.19
4.18
4.03
3.92
3.91
3.90
3.89
3.86
3.85
3.84
3.82
3.75
3.74
3.73
3.72
3.68
3.66
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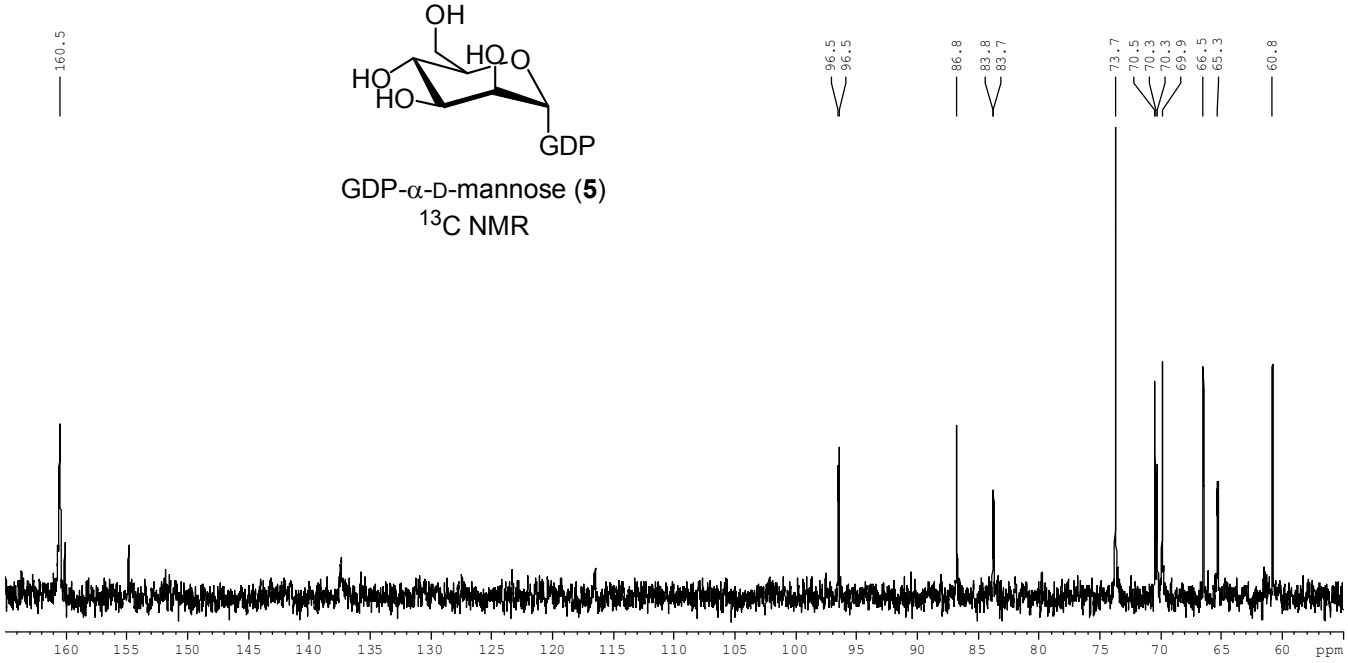
5.5
5.0
4.5
4.0
3.5
ppm


OH
HO
O
HO
HO
GDP
GDP-α-D-mannose (5)
13C NMR
160.5
96.5
96.5
86.8
83.8
83.7
73.7
70.5
70.3
70.3
69.9
66.5
65.3
60.8
160
155
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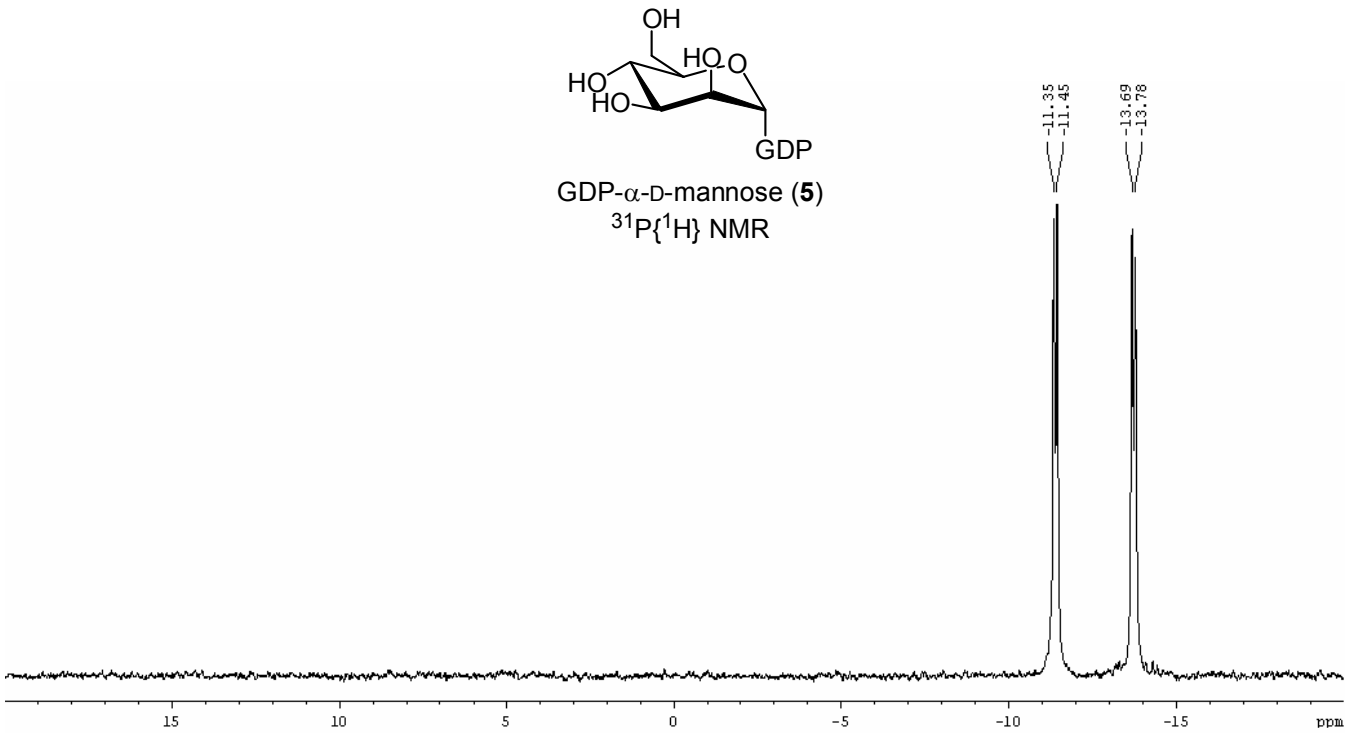
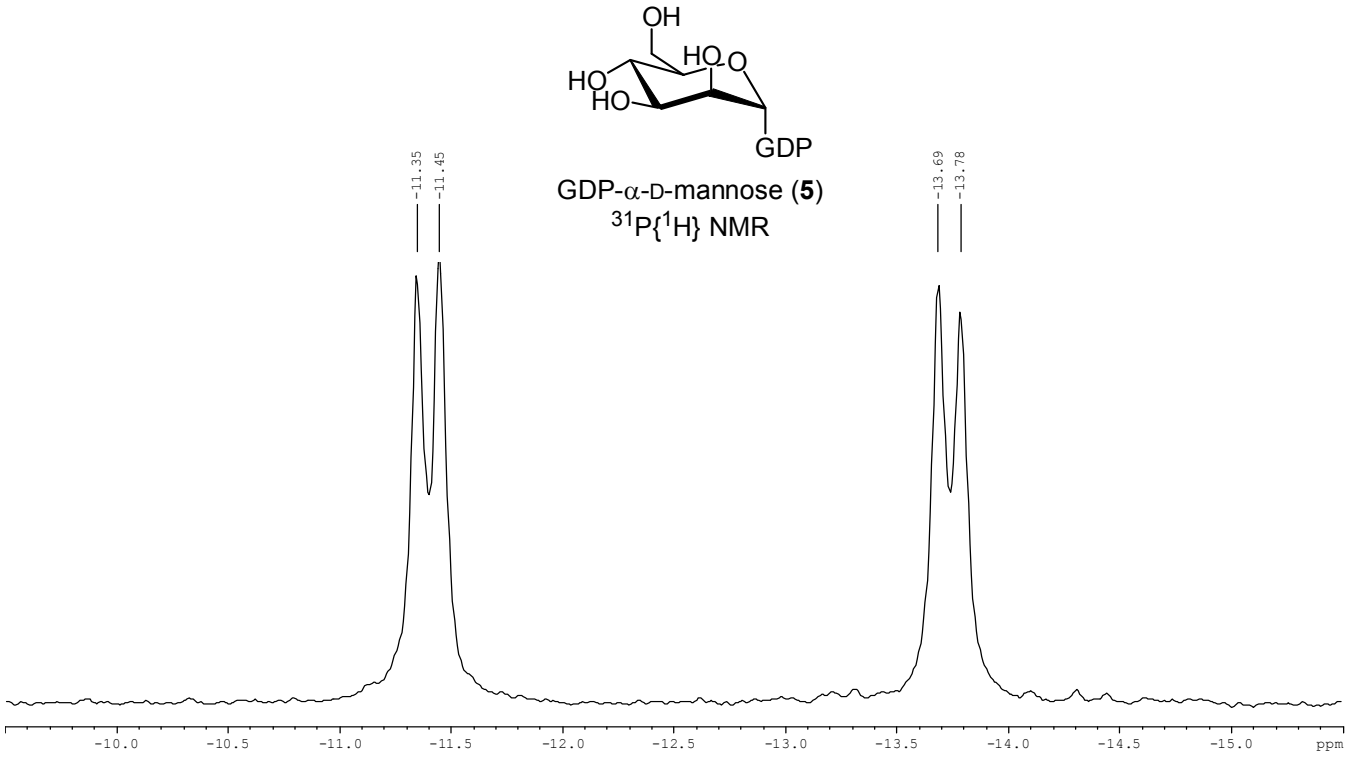
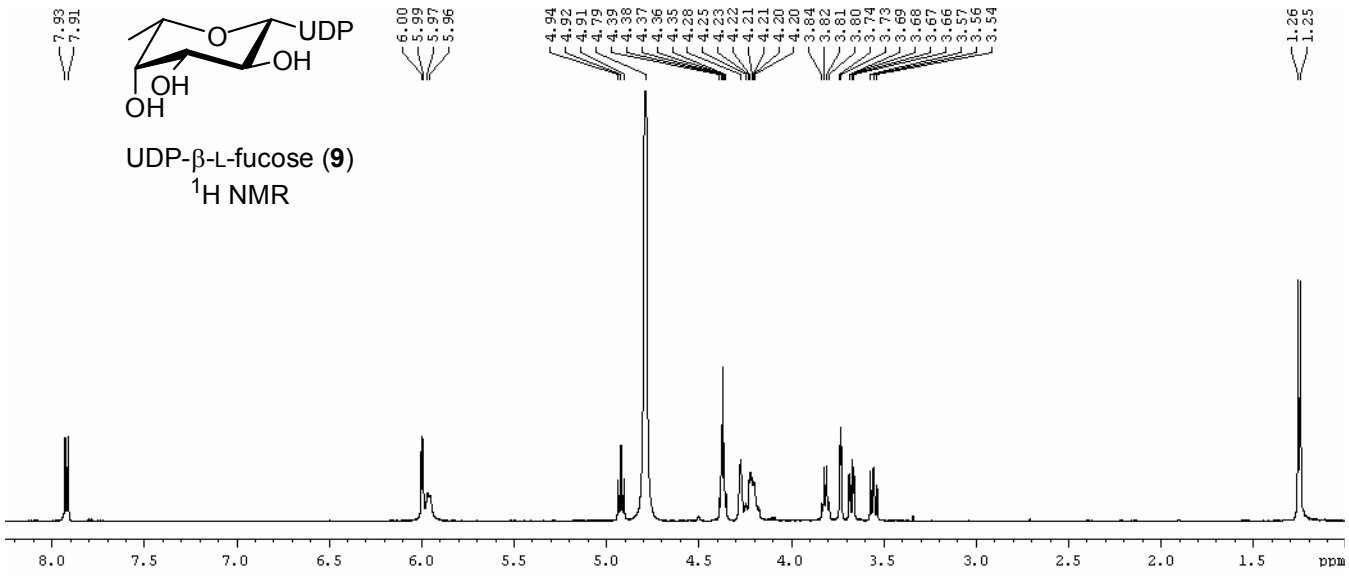
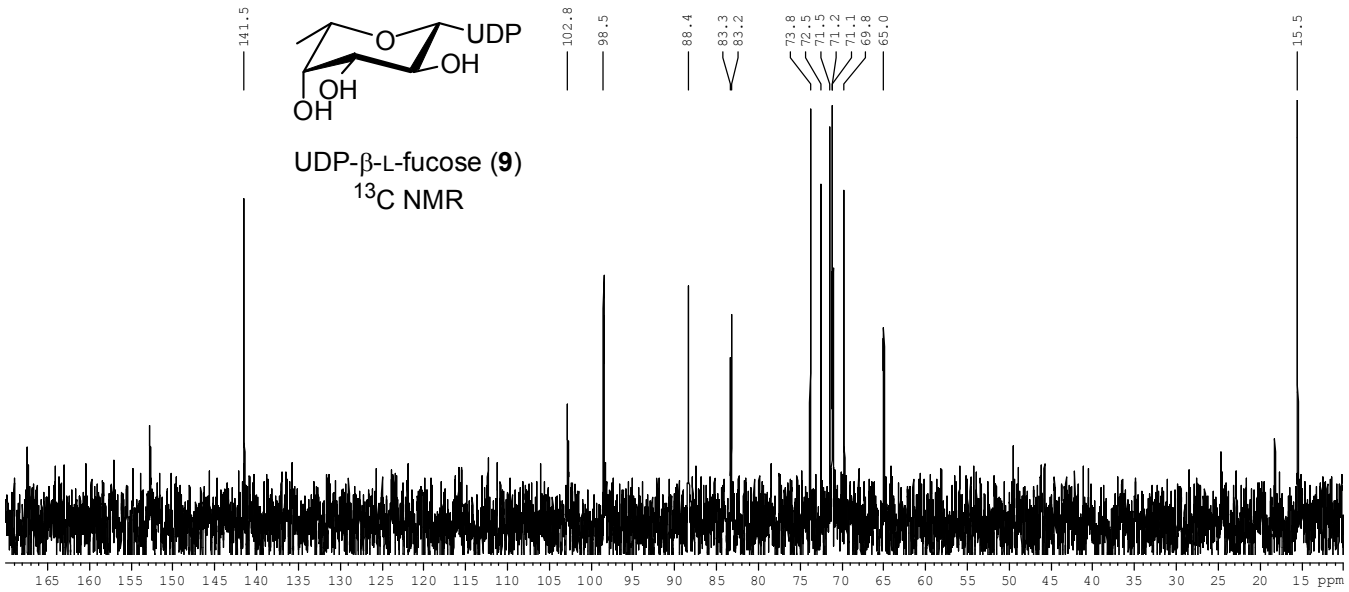
100
95
90
85
80
75
70
65
60
ppm


OH
HO
HO
HO
O
GDP
GDP-α-D-mannose (5)
31P{1H} NMR
-11.35
-11.45
-13.69
-13.78
15
10
5
0
-5
-10
-15
ppm

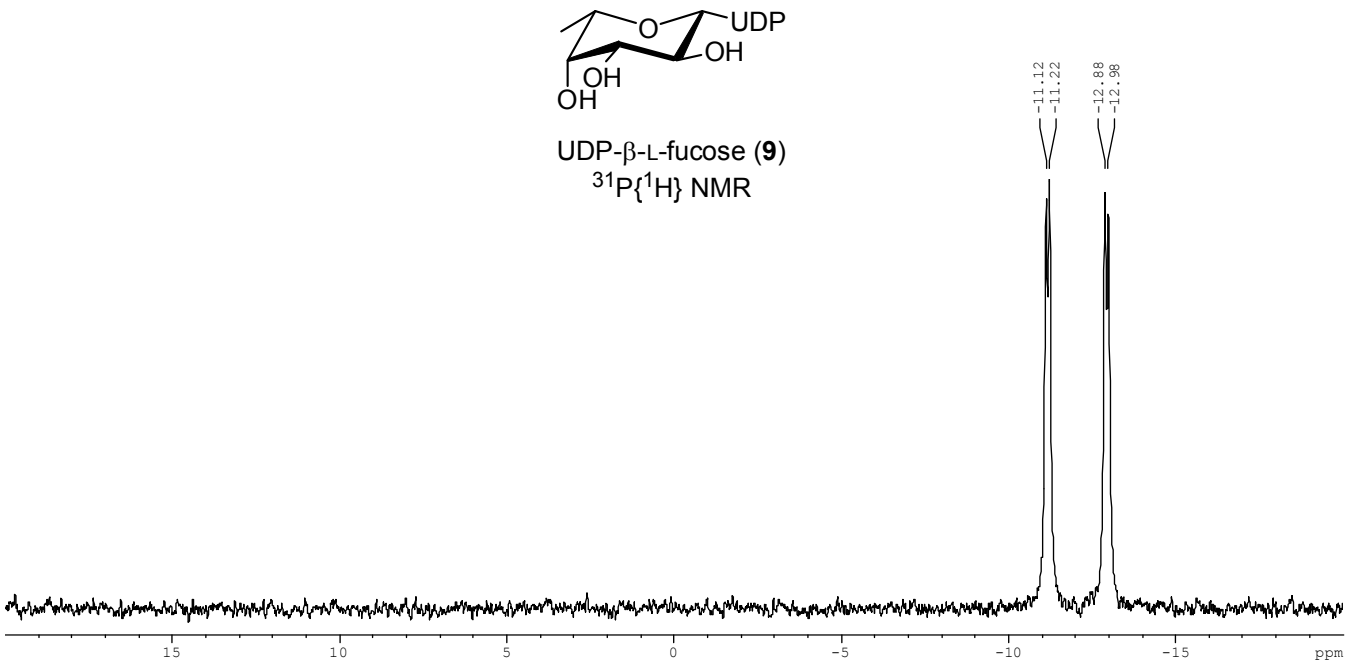

OH
HO
HO
HO
O
GDP
GDP-α-D-mannose (5)
31P{1H} NMR
-11.35
-11.45
-13.69
-13.78
-10.0
-10.5
-11.0
-11.5
-12.0
-12.5
-13.0
-13.5
-14.0
-14.5
-15.0
ppm

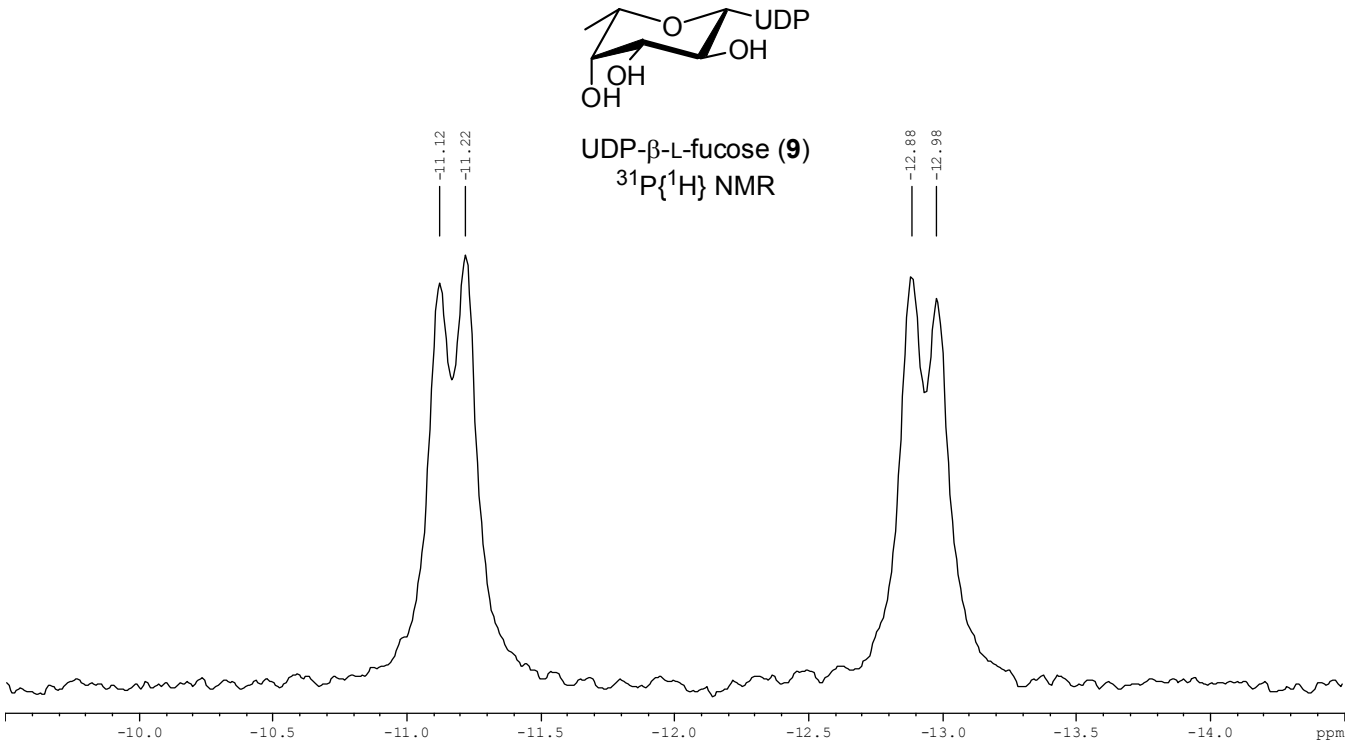


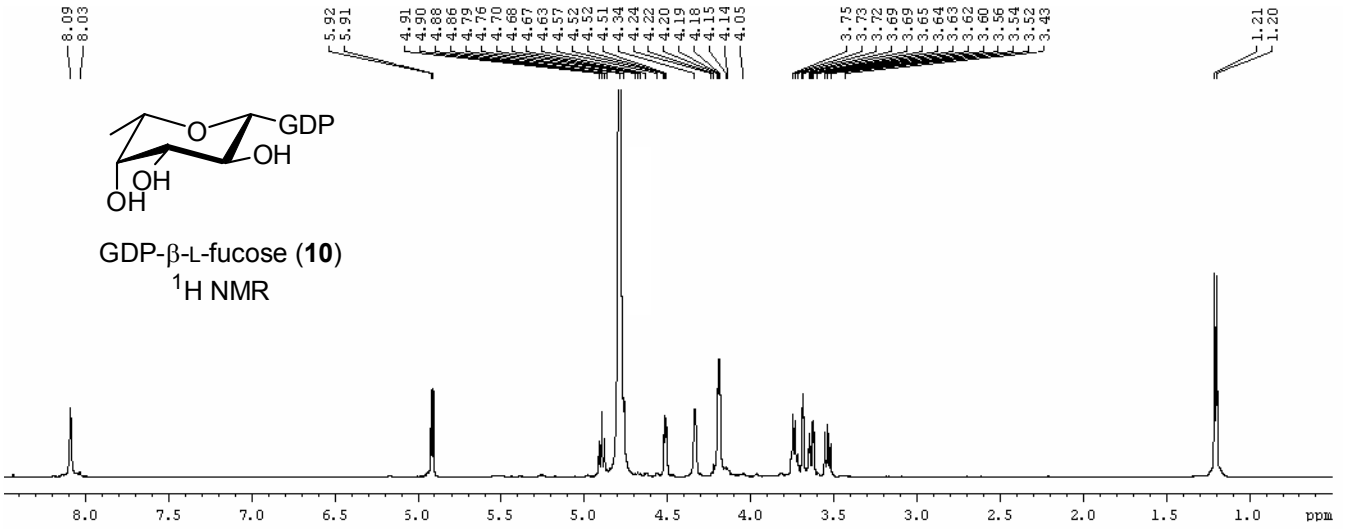
UDP-β-L-fucose (**9**)
^{1}H NMR

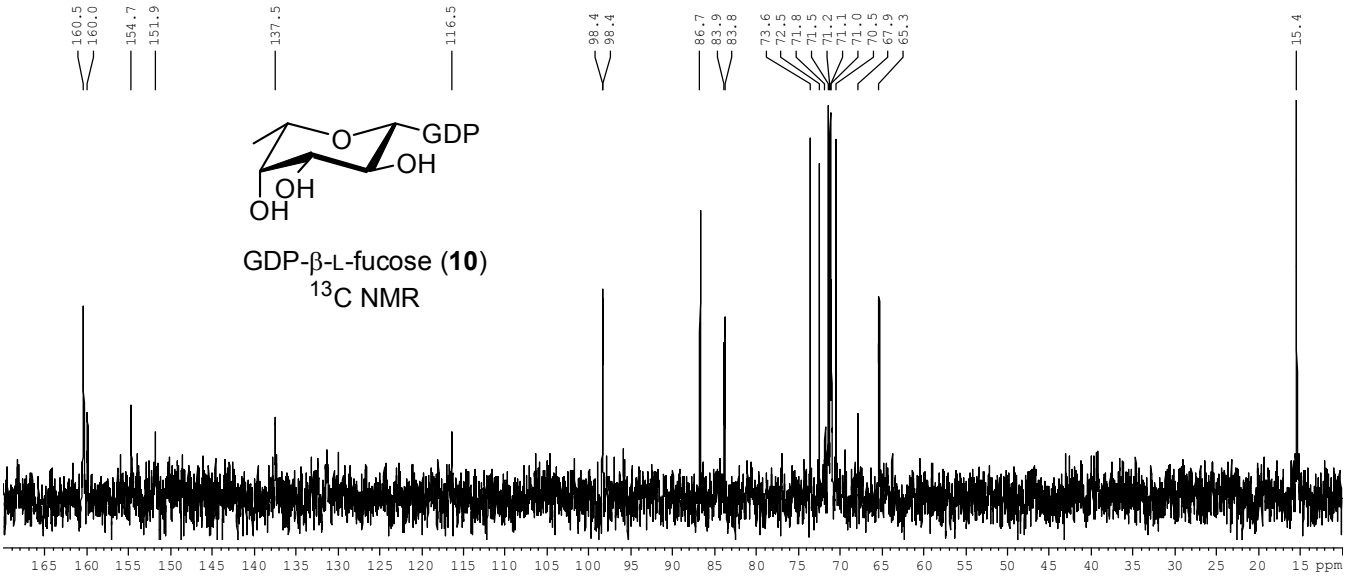


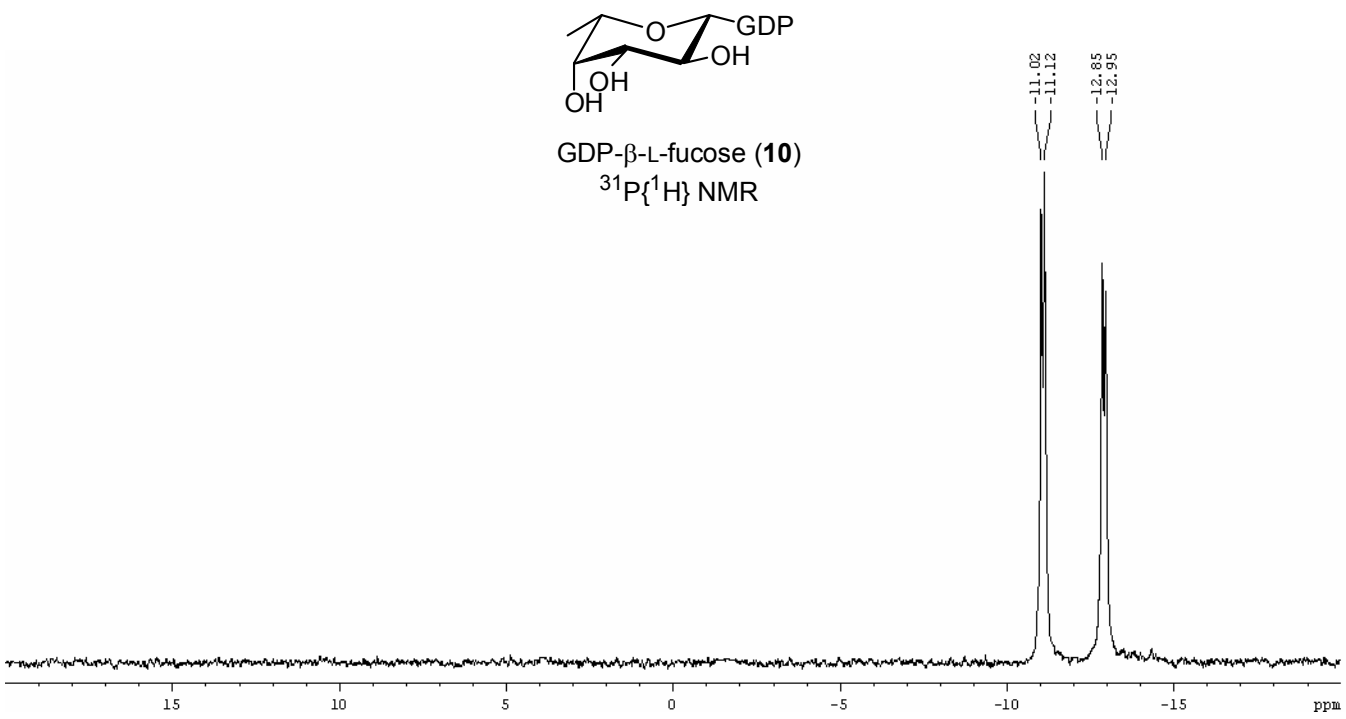
UDP-β-L-fucose (**9**)
^{13}C NMR


O
UDP
OH
OH
OH
UDP-β-L-fucose (9)
31P{1H} NMR
-11.12
-11.22
-12.88
-12.98
15
10
5
0
-5
-10
-15
ppm

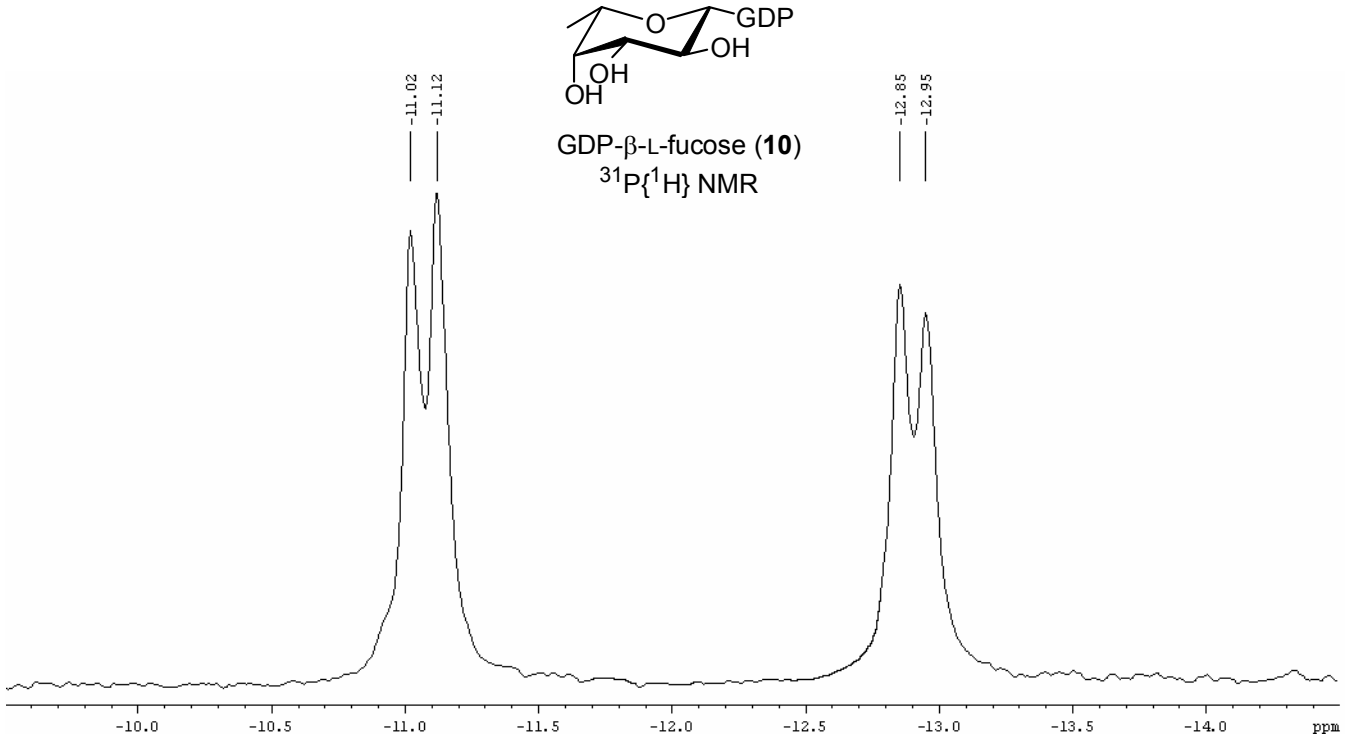

O
UDP
OH
OH
OH
UDP-β-L-fucose (9)
31P{1H} NMR
-11.12
-11.22
-12.88
-12.98
-10.0
-10.5
-11.0
-11.5
-12.0
-12.5
-13.0
-13.5
-14.0
ppm


8.09
8.03
5.92
5.91
4.91
4.90
4.88
4.86
4.79
4.76
4.70
4.68
4.67
4.63
4.57
4.52
4.51
4.34
4.24
4.22
4.20
4.19
4.18
4.15
4.14
4.05
3.75
3.73
3.72
3.69
3.69
3.65
3.64
3.63
3.62
3.60
3.56
3.54
3.52
3.43
1.21
1.20
O
GDP
OH
OH
OH
GDP-β-L-fucose (10)
1H NMR
8.0
7.5
7.0
6.5
5.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


160.5
160.0
154.7
151.9
137.5
116.5
98.4
98.4
86.7
83.9
83.8
73.6
72.5
71.8
71.5
71.2
71.1
71.0
70.5
67.9
65.3
15.4
O
GDP
OH
OH
OH
GDP-β-L-fucose (10)
13C NMR
165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 ppm



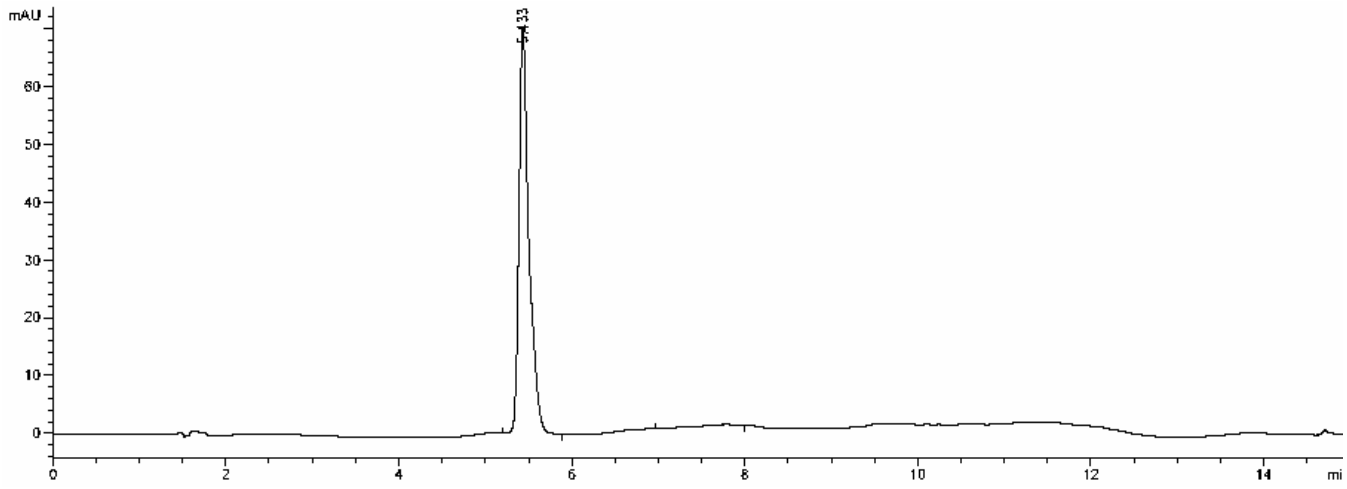
GDP-β-L-fucose (**10**)
$^{31}P\{^{1}H\}$ NMR



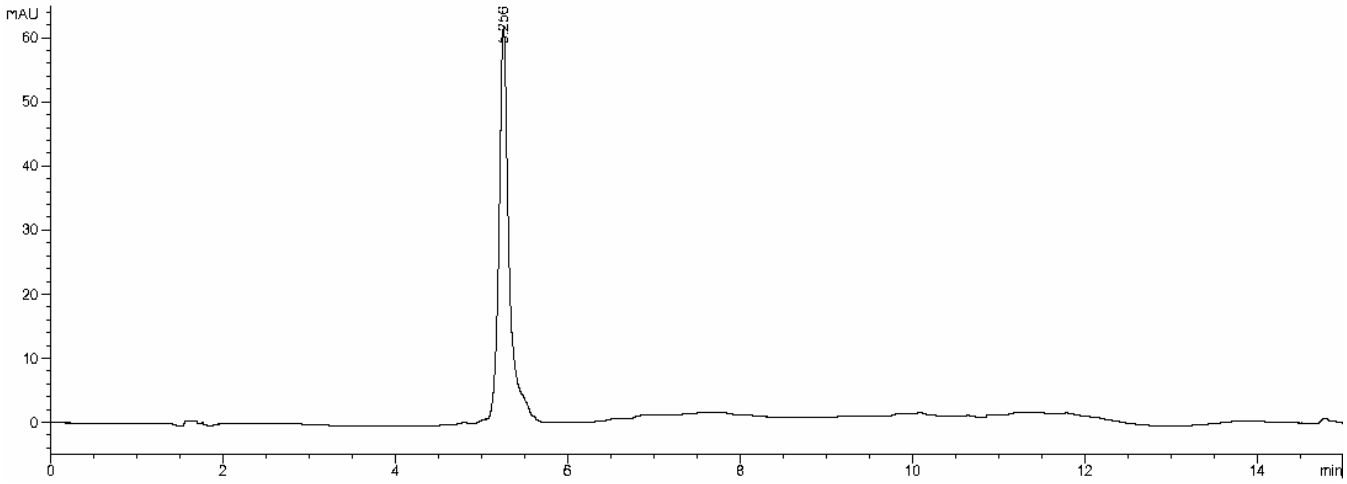
GDP-β-L-fucose (**10**)
$^{31}P\{^{1}H\}$ NMR

4. HPLC Traces of Sugar Nucleotides.

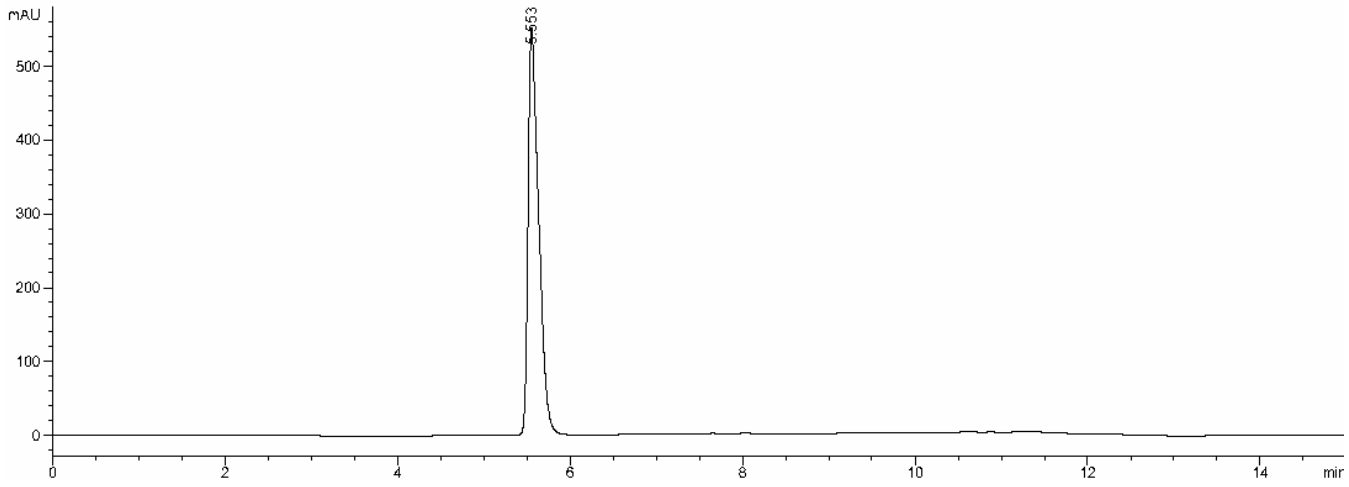
UDP-α-D-mannose (**4**):



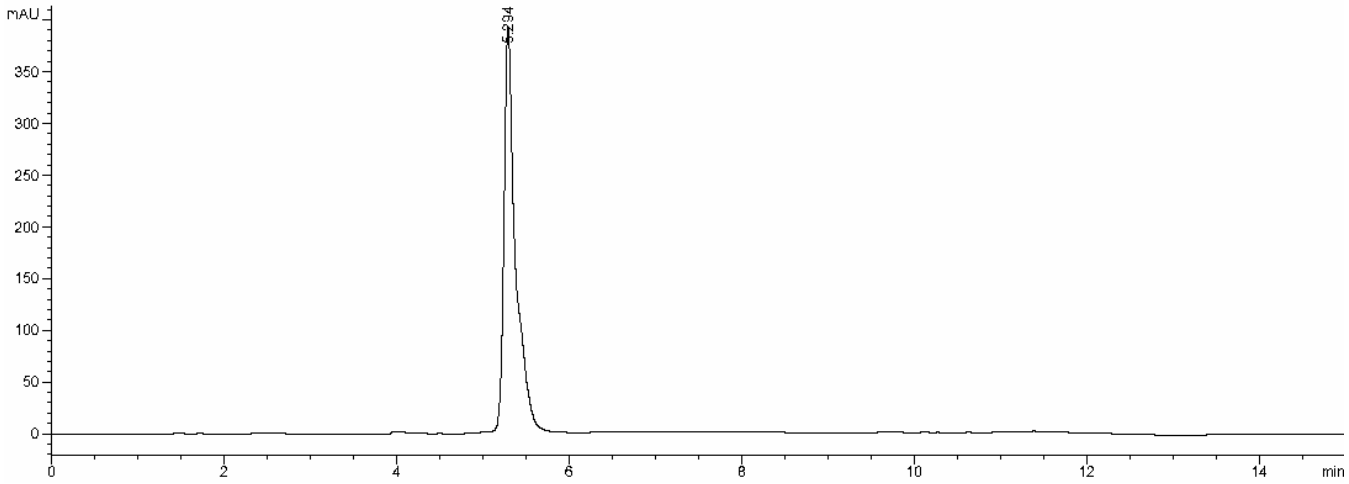
GDP-α-D-mannose (**5**):



UDP-β-L-fucose (**9**):



GDP-β-L-fucose (**10**):



5. References.

(S1) Bae, J.; Kim, K.-H.; Kim, D.; Choi, Y.; Kim, J. S.; Koh, S.; Hong, S.-I.; Lee, D.-S. *ChemBioChem* **2005**, *6*, 1963.

(S2) Pallanca, J. E.; Turner, N. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, *23*, 3017.

(S3) Ichikawa, Y.; Sim, M. M.; Wong, C.-H. *J. Org. Chem.* **1992**, *57*, 2943.

(S4) Khaled, A.; Ivannikova, T.; Augé, C. *Carbohydr. Res.* **2004**, *339*, 2641.

(S5) Gokhale, U. B.; Hindsgaul, O.; Palcic, M. M. *Can. J. Chem.* **1990**, *68*, 1063.

(S6) Nunez, H. A.; O'Connor, J. V.; Rosevear, P. R.; Barker, R. *Can. J. Chem.* **1981**, *59*, 2086.