Polyphosphate-Containing Bisubstrate Analogues as Inhibitors of a Bacterial Cell Wall Thymidylyltransferase

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Supporting Information

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General Procedures and Instrumentation

Synthesis and Characterisation

General Experimental

All chemicals and reagents were purchased from commercial sources and were used as received, unless otherwise noted. HPLC grade chloroform and methanol was employed where stated. Anhydrous DMF was purchased from Sigma Aldrich. Normal phase gravity column chromatography was performed using 230-400 mesh Silicycle Ultra Pure Silica Gel. Reversed-phase column chromatography was performed using a Biotage SP1 Flash Chromatography Purification System over Silicycle SiliaSep C18 silica or Sephadex LH20 resin pre-swelled with water, as indicated. TLC was performed on silica gel plates and visualized using UV light (254 and/or 365 nm) and/or developed with Vanillin stain. Lyophilisation of samples was carried out using an Edward Freeze-Dryer. NMR spectra were recorded at the Nuclear Magnetic Resonance Research Resource (NMR^3) using a Bruker AVANCE 500 spectrometer. All ^{1}H, ^{13}C and ^{31}P chemical shifts are reported in ppm using the solvent signal [$CDCl_3$ (^{1}H 7.26 ppm; ^{13}C 77.16 ppm); D_2O (^{1}H 4.79 ppm); Acetone-*d6* (^{1}H 2.02 ppm; ^{13}C 29.84, 206.26)] as the internal reference or MeOD (^{13}C 49.50 ppm in D_2O) or 85% aq. H_3PO_4 (^{31}P 0.00 ppm) as an external reference. Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; at, apparent triplet; q, quartet; m, multiplet. All coupling constants (*J*) are reported in Hertz (Hz). All WaterLOGSY NMR Spectra were recorded on a 700 MHz spectrometer equipped with a 1.7 or 5 mm cryoprobe at the Biomolecular Magnetic Resonance Facility, National Council of Canada, Halifax. HPLC analysis was performed with a Hewlett Packard Series 1050 instrument using an Agilent Zorbax 5 μM Rx-C18 column (150 x 4.6 mm) and monitoring at an absorbance wavelength of 254 nm. 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 over 2.0 min at 1.0 mL/min^{-1} was used, where A is an aqueous buffer containing 12 mM Bu_4NBr, 10 mM KH_2PO_4 and 5% HPLC grade CH_3CN and B is HPLC grade CH_3CN.Mass spectra were recorded by Mr. Xiao Feng using ion trap (ESI TOF) instruments.

General Procedure for Nucleotide Salt Conversion (GP1).

1) ~50 g Amberlite (IR-120 plus (H)) resin was placed in a Buchner funnel and washed with HPLC grade methanol, until washings were colourless, followed by distilled water (3 x 50 mL). The resin was then transferred to a narrow column (1.5 cm Ø x min. 30 cm high (resin should be ~10 cm high within column)) and washed with distilled water until pH of eluent is neutral.

2) The nucleotide sodium salt (~0.35 mmol) was then dissolved in 1-3 mL of distilled water and applied directly to the column. The column was eluted with water and all acidic fractions were collected until the pH of the eluent returned to neutral. The acidic fractions were immediately combined and titrated with the aq. tetrabutylammonium hydroxide solution (30% w/v), with care taken to achieve a pH of 5–6 (salts with pH of ≤ 5 and ≥ 7 are unstable).

3) The resulting aq. solution was concentrated using a rotary evaporator, at a maximum temperature of 35 °C, until 1-3 mL remained, before freeze-drying overnight to give the desired salt as a white solid, which was stored in a desiccator in the freezer (-20 °C). The equivalents of tetrabutylammonium cation present was obtained using ^{1}H NMR in D_2O and used to calculate molecular weight. ^{31}P $\{^{1}H\}$ NMR also was obtained to confirm no degradation of the nucleoside during ion exchange.

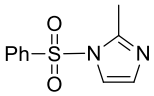
4) The resin was regenerated by washing column with 1 M aq. HCl (~100 mL), followed by distilled water until the pH of the eluent was neutral.

General Procedure for Nucleotide Coupling (GP2).

Separate solutions of Nucleotide·xNBu_4 (0.1 mmol) in anhydrous DMF (2.0 mL) and glucose 1-phosphate·xNBu_4 (0.15 mmol) in anhydrous DMF (2.0 mL) were prepared and dried over 4Å molecular sieves for 3 hours, at room temperature under an inert atmosphere. Anhydrous magnesium chloride (0.1 mmol) was then added to the Glc-1-P solution and allowed to stir for 2 minutes before cooling to 0 °C. Meanwhile, DIPEA (0.3 mmol) followed by imidazole coupling reagent **1** (0.12 mmol) were added to the nucleotide solution, which was stirred for 1 minute before being added drop-wise, over 1 minute, to the phosphate solution. The reaction mixture was allowed to stir for 40 minutes under nitrogen, warming to room temperature, before cooling back down to 0 °C and quenching with 50 mM aqueous triethylammonium acetate solution (2 mL, pH 7). The reaction mixture was then diluted with water (10 mL) and extracted with chloroform 3 x 10 mL). Chelex resin (100 mg) was added to the aqueous phase and stirred for 2 minutes before filtering through a cotton plug and concentrating to give the crude product.

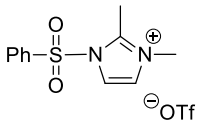
Experimental Procedures and Data

2-Methyl-1-(phenylsulfonyl)-1H-imidazole[1]



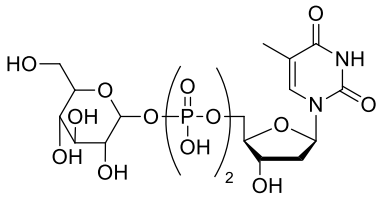
Benzenesulphonyl chloride (5.2 mL, 40.6 mmol) was added drop-wise, over 15 min, to a suspension of 2-methylimidazole (10.0 g, 0.12 mol, 3 eq.) in anhydrous dichloromethane (100 mL), with stirring at 0 °C under nitrogen for 5 hours, warming to room temperature. During the addition of benzenesulphonyl chloride, the 2-methylimidazole was noted to completely dissolve, though no precipitate was observed to form in this instance. Following completion of the reaction, the reaction mixture was washed with water (80 mL) and brine (80 mL), dried over anhydrous sodium sulfate and concentrated *in vacuo* to give to crude product, which was recrystallized from ethyl acetate/hexane to give the title compound (8.79 g, 97% yield) as a translucent white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.86 (d, 2H, J = 7.0 Hz, ArH), 7.64 (t, 1H, J = 7.5 Hz, ArH), 7.53 (t, 2H, J = 7.8 Hz, ArH), 7.40 (d, 1H, J = 1.5 Hz, PyH), 6.87 (d, 1H, J = 1.5 Hz, PyH) ppm. ^{1}H NMR matches reported data.[1]

2,3-Dimethyl-1-(phenylsulfonyl)-1H-imidazolium triflate **1**[1]



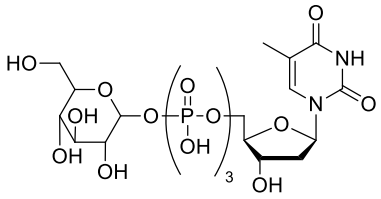
Methyl triflate (0.64 mL, 5.67 mmol, 1.05 eq.) was added drop-wise, over 10 minutes, to a solution of the preceding compound (1.2 g, 5.4 mmol) in anhydrous diethyl ether (45 mL), with stirring at room temperature under nitrogen for 3 hours. A white precipitate was observed to form during the reaction, which was collected by filtration, washing with diethyl ether, and dried in a vacuum oven to give **1** (2.03 g, 97% yield) as a white solid. ^{1}H NMR (Acetone-*d6*, 500 MHz) δ 9.88 (s, 1H, PyH), 8.32 (d, 2H, J = 7.5 Hz, ArH), 8.27 (at, 1H, J = 2.0 Hz, PyH), 8.00 (t, 1H, J = 7.8 Hz, ArH), 7.96 (at, 1H, J = 2.0 Hz, PyH), 7.83 (t, 1H, J = 8.0 Hz, ArH), 4.15 (s, 3H, NCH_3) ppm. ^{1}H NMR matches reported data.[1]

dTDP-Glucose **2**[2]



Compound **2** was synthesized from dTMP·NBu_4 and α-D-glucose 1-phosphate·NBu_4 using GP2. The crude product was purified over Sephadex LH20 resin, eluting with water, then over C18-silica, eluting with 20-70% methanol/10 mM tributylammonium bicarbonate buffer at 4 mL/min over 50 column volumes, whereby the UV-active fractions were combined and concentrated, before treating with alkaline phosphatase (4 µL, 10 EU/µL). Two further C18-silica columns were then run, over gradients of 20-55% and 20-45% methanol/buffer, respectively, and the product containing fraction was concentrated and lyophilised to give **2** (7 mg, 6% yield) as a white solid. ^{1}H NMR (D_2O, 500 MHz) δ 7.73 (s, 1H, C=CH), 6.33 (t, 1H, J = 7.0 Hz, C1'H), 5.58 (dd, 1H, J = 3.5, 7.5 Hz, Glucose C1-H), 4.62-4.60 (m, 1H), 4.16-4.15 (m, 3H), 3.89-3.82 (m, 2H), 3.77-3.73 (m, 2H), 3.51-3.48 (m, 1H), 3.43 (t, 1H, J = 9.5 Hz), 2.37-2.33 (m, 2H), 1.91 (s, 3H, CH_3) ppm; ^{31}P NMR (D_2O, 202.4 MHz) δ 11.4 (1P, d, J = 20.8 Hz), 13.0 (1P, d, J = 20.8 Hz) ppm; LRMS: 563.1 $(M-H)^-$; HRMS: 563.0680 Found, 563.0685 Calculated for $C_{16}H_{25}N_2P_2O_{16}$; HPLC: >95%.

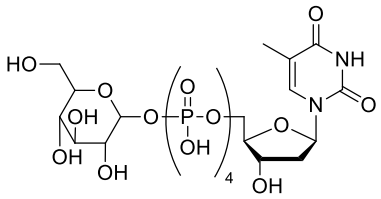
dTTP-Glucose **3**



Compound **3** was synthesized from dTDP·NBu_4 and α-D-glucose 1-phosphate·NBu_4 using GP2. The crude product was purified over Sephadex LH20 resin, eluting with water, then over C18-silica, eluting with 20-80% methanol/10 mM tributylammonium bicarbonate buffer at 4 mL/min over 50 column volumes, whereby the UV-active fractions were combined and concentrated, before treating with alkaline phosphatase (4 µL, 10 EU/µL). Two further C18-silica columns

were then run, over gradients of 25-70% and 30-55% methanol/buffer, respectively, and the product containing fraction was concentrated and lyophilised to give **3** (9.0 mg, 7% yield) as a white solid. ^{1}H NMR (D_2O, 500 MHz) δ 7.75 (s, 1H, C=CH), 6.33 (t, 1H, *J* = 7.0 Hz, C1'H), 5.60 (dd, 1H, *J* = 3.5, 7.0 Hz, Glucose C1-H), 4.66-4.63 (m, 1H), 4.22-4.15 (m, 3H), 3.92-3.89 (m, 1H), 3.86-3.84 (m, 1H), 3.80-3.73 (m, 2H), 3.50-3.47 (m, 1H), 3.41 (t, 1H, *J* = 9.8 Hz), 2.33-2.29 (m, 2H), 1.91 (s, 3H, CH_3) ppm; ^{31}P NMR (D_2O, 202.4 MHz) δ 11.9 (1P, d, *J* = 19.4 Hz), 13.1 (1P, d, *J* = 19.6 Hz), 23.2 (1P, t, *J* = 19.2 Hz) ppm; ^{13}C NMR (D_2O, 175 MHz) δ 167.8, 153.0, 138.7, 113.0, 96.8 (d, *J* = 6.3 Hz), 86.7 (d, *J* = 8.9 Hz), 86.1, 74.0 (2 x C), 72.9 (d, *J* = 8.6 Hz), 72.2, 70.5, 66.7 (d, *J* = 5.3 Hz), 61.6, 39.8, 12.9 ppm; LRMS: 643.0 $(M-H)^-$; HRMS: 643.0378 Found, 643.0348 Calculated for $C_{16}H_{26}N_2P_3O_{19}$; HPLC: >95%.

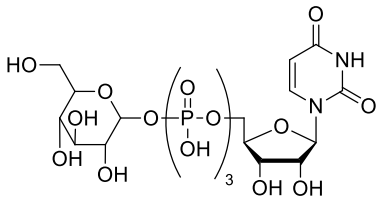
dTP$_4$-Glucose **4**



Compound **4** was synthesized from dTTP·NBu_4 and α-D-glucose 1-phosphate·NBu_4 using GP2. The crude product was purified over Sephadex LH20 resin, eluting with water, then over C18-silica, eluting with 20-80% methanol/10 mM tributylammonium bicarbonate buffer at 4 mL/min over 50 column volumes, whereby the UV-active fractions were combined and concentrated, before treating with alkaline phosphatase (4 µL, 10 EU/µL). Two further C18-silica columns were then run, over gradients of 25-65% and 30-55% methanol/buffer, respectively, and the product containing fraction was concentrated and lyophilised to give **4** (9 mg, 8% yield) as a white solid. ^{1}H NMR (D_2O, 500 MHz) δ 7.77 (s, 1H, C=CH), 6.34 (t, 1H, *J* = 7.0 Hz, C1'H), 5.61 (dd, 1H, *J* = 3.5, 7.5 Hz, Glucose C1-H), 4.68-4.65 (m, 1H), 4.25-4.21 (m, 1H), 4.18-4.16 (m 2H), 3.94-3.91 (m, 1H), 3.87-3.79 (m, 2H), 3.77-3.74 (m, 1H), 3.50-3.46 (m, 1H), 3.41 (t, 1H, *J* = 9.8 Hz), 2.37-2.31 (m, 2H), 1.91 (s, 3H, CH_3) ppm; ^{31}P NMR (D_2O, 202.4 MHz) δ −11.84 (d, 1P, *J* = 18.0 Hz), −13.02 (d, 1P, *J* = 18.0 Hz), −23.3 – −23.7 (m, 2P) ppm; ^{13}C NMR (D_2O, 175 MHz) δ 167.8, 153.0, 138.7, 113.1, 96.8, 86.9 (d, *J* = 9.1 Hz), 86.2, 74.04, 73.99, 73.0 (d, *J* = 9.4

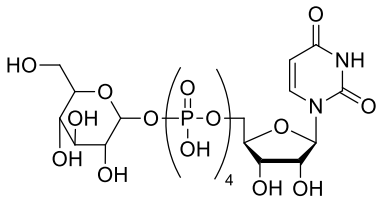
Hz), 72.3, 70.6, 66.9, 61.7, 39.8, 12.9 ppm LRMS: 723.0 $(M-H)^-$; HRMS: 723.0039 Found, 723.0011 Calculated for $C_{16}H_{27}N_2P_4O_{22}$; HPLC: >95%.

UTP-Glucose **5**[3]



Compound **5** was synthesized from UDP·NBu_4 and α-D-glucose 1-phosphate·NBu_4 using GP2. The crude product was purified over Sephadex LH20 resin, eluting with water, then twice over C18-silica, first eluting with 30-75% methanol in 10 mM aqueous tributylammonium bicarbonate buffer at 4 mL/min over 25 column volumes, then eluting with 35-60% methanol in buffer at 4 mL/min over 30 column volumes to give **5** (6 mg, 10% yield) as a white solid. ^{1}H NMR (D_2O, 500 MHz) δ 7.94 (d, 1H, *J* = 8.0 Hz, C=CH), 5.98 (d, 1H, *J* = 5.0 Hz, C=CH), 5.94 (d, 1H, *J* = 5.0 Hz, C1'H), 5.60 (dd, 1H, *J* = 3.0, 7.0 Hz, Glucose C1-H), 4.39-4.34 (m, 2H), 4.23-4.22 (m, 3H), 3.92-3.88 (m, 1H), 3.86-3.83 (m, 1H), 3.80-3.73 (m, 2H), 3.51-3.48 (m, 1H), 3.42 (t, 1H, *J* = 9.5 Hz); ^{31}P NMR (D_2O, 202.4 MHz) δ 10.80 (1P, d, *J* = 15.8 Hz, γP), 12.18 (1P, d, *J* = 15.8 Hz, αP), 22.15 (1P, brs, βP); LRMS: 645.0 $(M-H)^-$; HRMS: 645.0129 Found, 645.0141 Calculated for $C_{15}H_{24}N_2P_3O_{20}$; HPLC: >95%.

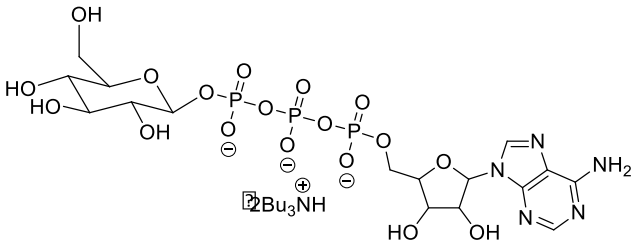
UP_4-Glucose **6**[4]



Compound **6** was synthesized from UTP·NBu_4 and α-D-glucose 1-phosphate·NBu_4 using GP2. The crude product was purified over Sephadex LH20 resin, eluting with water, then twice over C18-silica, first eluting with 30-75% methanol in 10 mM aqueous tributylammonium bicarbonate buffer at 4 mL/min over 25 column volumes, then eluting with 35-60% methanol in

buffer at 4 mL/min over 30 column volumes to give **6** (8.5 mg, 8.5% yield) as a white solid. ^{1}H NMR (D_2O, 500 MHz) δ 7.96 (d, 1H, *J* = 8.0 Hz, C=CH), 5.98 (d, 1H, *J* = 5.0 Hz, C=CH), 5.94 (d, 1H, *J* = 8.0 Hz, C1'H), 5.61 (dd, 1H, *J* = 3.5, 7.0 Hz, Glucose C1-H), 4.44-4.41 (m, 1H), 4.39 (t, 1H, *J* = 5.5 Hz), 4.27-4.21 (m, 3H), 3.93-3.90 (m, 1H), 3.87-3.84 (m, 1H), 3.81 (t, 1H, *J* = 9.5 Hz), 3.77-3.74 (m, 1H), 3.50-3.47 (m, 1H), 3.41 (t, 1H, *J* = 9.5 Hz); ^{31}P NMR (D_2O, 202.4 MHz) δ −11.64 (1P, d, *J* = 18.2 Hz, γP), 13.00 (1P, d, *J* = 18.0 Hz, αP), 23.2-23.7 (2P, m); LRMS: 362.0 $(M^{2-}/2)^{-}$; HRMS: 361.9874 Found, 361.9866 Calculated for $C_{15}H_{24}N_2P_4O_{23}$; HPLC: >95%.

ATP-Glucose **7**



Compound **7** was synthesized from ADP·NBu_4 and α-D-glucose 1-phosphate·NBu_4 using GP2. The crude product was purified over Sephadex LH20 resin, eluting with water, then twice over C18-silica, first eluting with 30-75% methanol in 10 mM aqueous tributylammonium bicarbonate buffer at 4 mL/min over 25 column volumes, then eluting with 35-60% methanol in buffer at 4 mL/min over 30 column volumes to give **7** (12 mg, 20% yield) as a white solid. ^{1}H NMR (D_2O, 500 MHz) δ 8.51 (s, 1H, ArH), 8.23 (s, 1H, ArH), 6.11 (d, 1H, *J* = 6.5 Hz, C1'H), 5.59 (dd, 1H, *J* = 3.3, 7.3 Hz, Glucose C1-H), 4.78-4.76 (m, 1H), 4.56-4.54 (m, 1H), 4.39-4.37 (m 1H), 4.27-4.23 (m, 1H), 4.21-4.17 (m, 1H), 3.91-3.87 (m, 1H), 3.85-3.82 (m, 1H), 3.78 (t, 1H, *J* = 9.5 Hz), 3.74-3.71 (m, 1H), 3.49-3.45 (m, 1H), 3.40 (t, 1H, *J* = 9.5 Hz); ^{31}P NMR (D_2O, 202.4 MHz) δ 10.78 (1P, d, *J* = 19.6 Hz, γP), 12.19 (1P, d, *J* = 19.2 Hz, αP), 22.24 (1P, t, *J* = 19.2 Hz, βP); ^{13}C NMR (D_2O, 175 MHz) δ 156.0, 153.0, 150.2, 141.4, 119.7, 96.8 (d, *J* = 6.8 Hz), 87.9, 85.4 (d, *J* = 8.9), 75.6, 74.00, 73.97, 72.9 (d, *J* = 8.9 Hz), 71.7, 70.5, 66.5, 61.6 ppm; LRMS: 668.0 $(M-H)^{-}$; HRMS: 668.0421 Found, 668.0413 Calculated for $C_{16}H_{25}N_5P_3O_{18}$; HPLC: >95%.

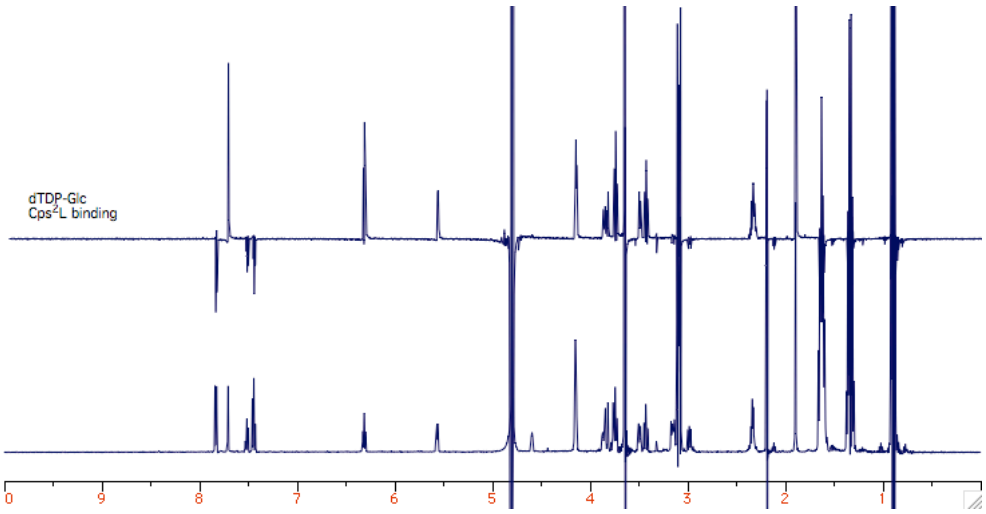
Binding Determination using WaterLOGSY NMR Spectroscopy[5]

WaterLOGSY NMR samples were composed of binding substrate (**2**-**7**, 4 mM), $MgCl_2$ co-factor (1.33 mM), benzoic acid non-binding control (4 mM), Cps2L (0.9 EU), D_2O (10% total volume, 6 µL), TRIS-*d11*·HCl buffer (pH 7.5, 20 mM) and H_2O to give a total sample volume of 60 µL.

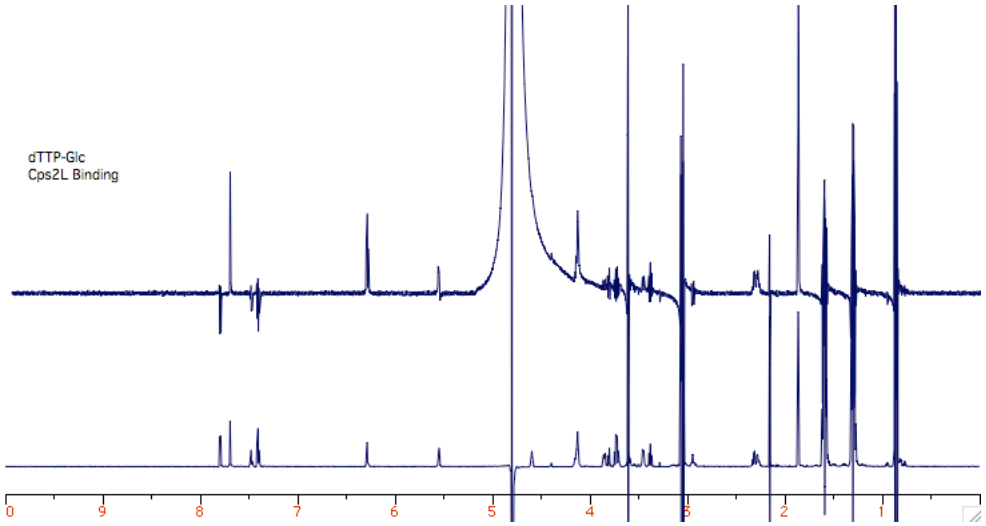
WaterLOGSY NMR Spectra

(10% D_2O/H_2O, 700 MHz)

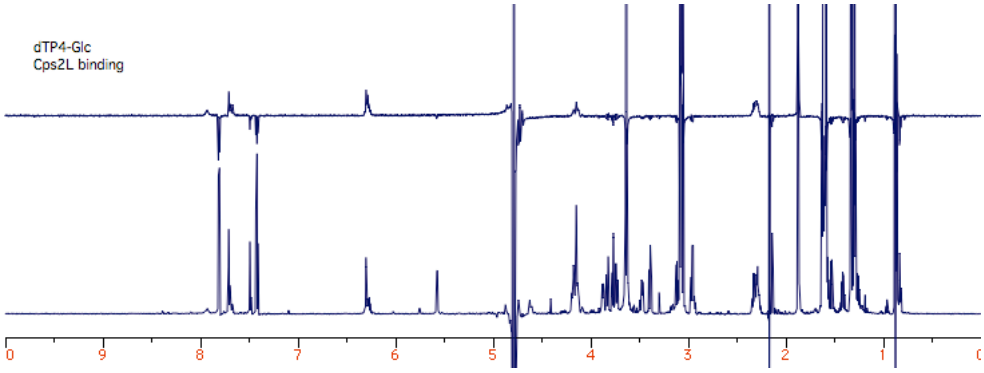
dTDP-Glc **2**



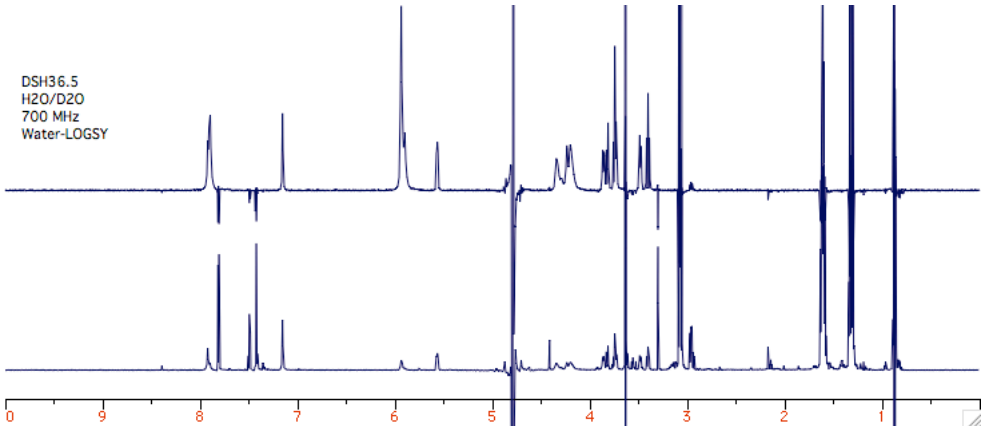
dTTP-Glc **3**



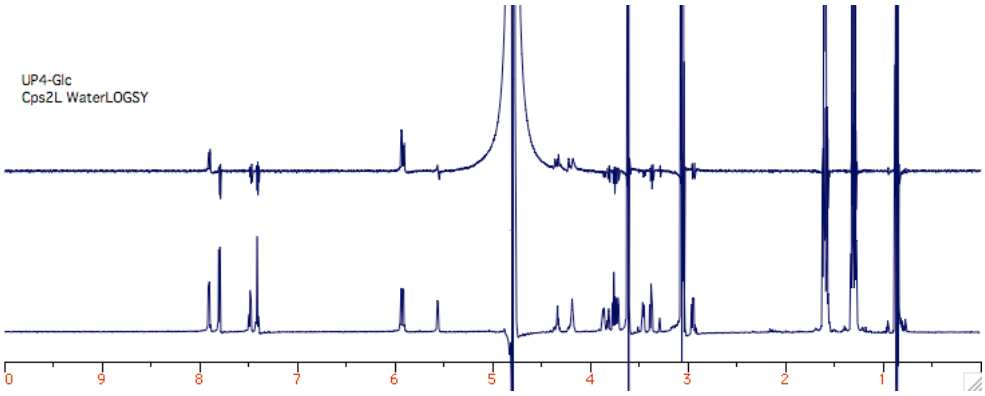
dTP$_4$-Glc **4**



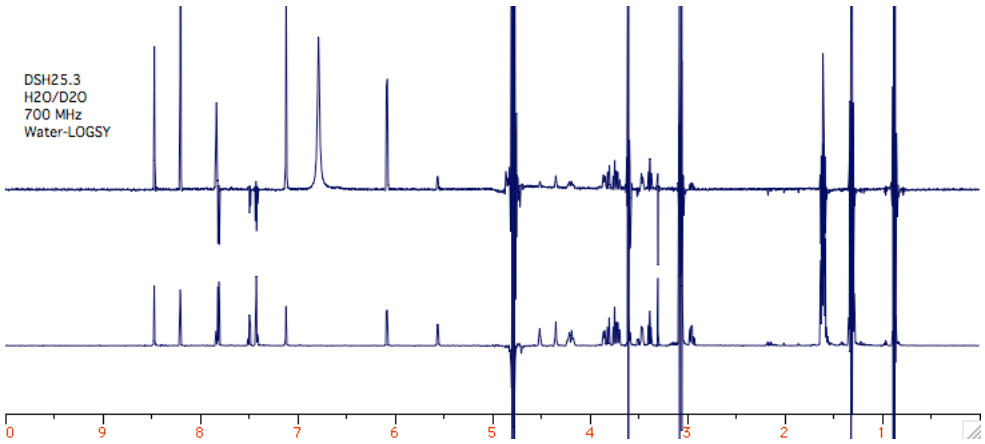
UTP-Glc **5**



UP$_4$-Glc **6**



ATP-Glc **7**



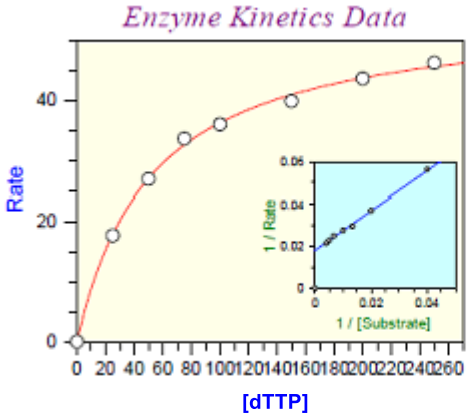
Coupled Spectrophotometric Enzyme Inhibition Assays

Cps2L[6] and human purine nucleotide phosphorylase[7] (hPNP) were overexpressed, isolated, and quantified as previously described. Recombinant inorganic pyrophosphatase (IPP) expressed in *Escherichia coli* was obtained from Sigma-Aldrich. IPP stock solutions (0.1 EU/μL) were prepared in Milllipore water; thawed aliquots were kept in a fridge and were used for up to 1 month after thawing. MESG was purchased from Berry and Associates and stock solutions (2 mM) were prepared in double distilled H_2O and stored at −30 °C; aliquots were used immediately after thawing. Stock solutions of dTTP at variable concentrations (0 – 500 μM) were prepared in Tris·HCl buffer (pH 7.5, 50 mM). All other stock solutions were prepared in Millipore water. Kinetic reactions were performed in 384-well plates and initial velocities were monitored continuously by UV spectrometry at λ 360 nm using a SPECTRAmax Plus^{384} Microplate Reader spectrophotometer with SoftMax Pro version 4.8. Non-linear regression analysis was performed using using GraFit 5.0.4., Erathacus Software.

General Procedure for Perfoming Enzyme Assay in the Absence of Inhibitor (GP3)

A stock solution was prepared consisting of Tris·HCl buffer (pH 7.5, 50 mM, 88 μL), $MgCl_2$ (500 mM, 9 μL), glucose 1-phosphate (100 mM, 8 μL), hPNP (282 μM, 25 μL), IPP (0.1 EU, 10 μL) and MESG (2 mM, 160 μL) to give a total volume of 300 μL. 40 μL of variable

concentrations of dTTP were added separatly to 8 epindorphs. 30 µL of the stock solution was then added to each epindorph and the mixture was left for 5 minutes to allow consumption of background phosphate (P*i*). The coupled enzymatic reaction was then initiated through the addition of Cps2L (10.7 nM, 10 µL) to each epindorph (going from low to high dTTP comcentrations) and 75 µL of each of the resulting solutions were immediately pippetted into separate wells of a 384-well plate. The plate was the placed in the plate-reader and monitored spectrophotometrically for 10 minutes, with readings taken every 6 seconds.



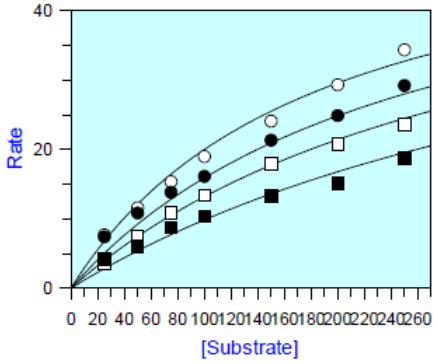
Parameter	Value	Std. Error
Vmax	55.1709	0.9877
Km	51.9635	2.9237

General Procedure for Perfoming Enzyme Inhibition Assays (GP4)

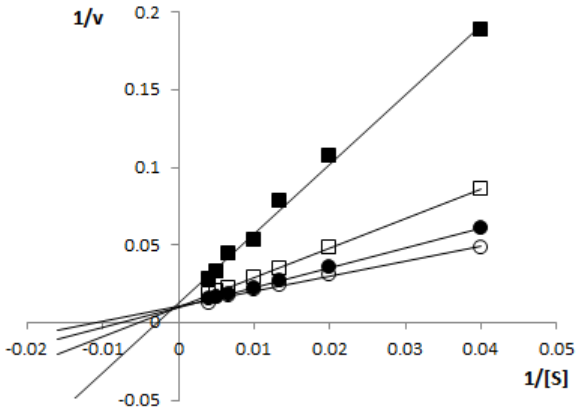
A stock solution was prepared that was 4.2 times the previous volumes, with the exception of Tris·HCl buffer: Tris·HCl buffer (pH 7.5, 50 mM, 202 µL (= 48 µL x 4.2)), $MgCl_2$ (500 mM, 38 µL), glucose 1-phosphate (100 mM, 34 µL), hPNP (282 µM, 105 µL), IPP (0.1 EU, 42 µL) and MESG (2 mM, 672 µL) to give a total volume of 1093 µL. 260 µL of the stock solution was added separately to 4 epindorphs. 40 µL of variable concentrations of an inhibitor (**2**-**7**, 0 – 200 µM in Tris·HCl buffer (pH 7.5, 50 mM)) was then added to each of the 4 epindorphs. Starting with the lowest concentration of inhibitor, each final stock was examined in turn. 30 µL of final stock was added separately to 8 epindorphs, followed by 40 µL of variable concentrations of dTTP and the mixture was left for 5 minutes to allow consumption of background phosphate (P*i*). The coupled enzymatic reaction was then initiated through the addition of Cps2L (10.7 nM, 10 µL) to each epindorph (going from low to high dTTP comcentrations) and 75 µL of each of the resulting solutions were immediately pippetted into separate wells of a 384-well plate. The plate was the placed in the plate-reader and monitored spectrophotometrically for 10 minutes, with readings taken every 6 seconds.

Michaelis-Menten and Lineweaver-Burk Plots

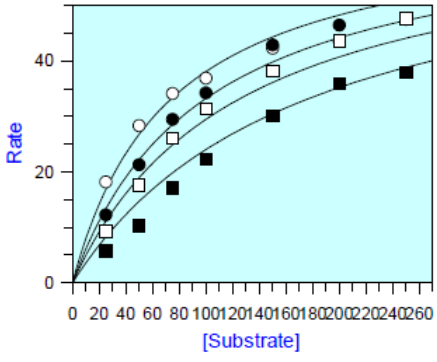
dTDP-Glc **2** (Competitive Inhibitor)



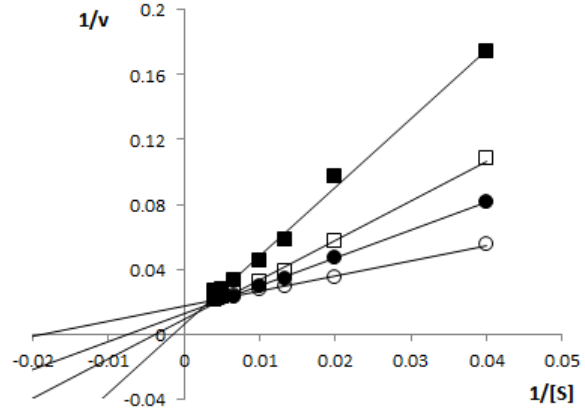
Parameter	Value	Std. Error
Vmax	56.8473	3.7827
Km	184.3822	22.8577
Ki	125.9161	11.9144



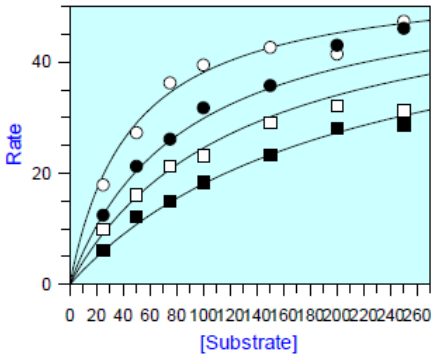
dTTP-Glc **3** (Competitive Inhibitor)



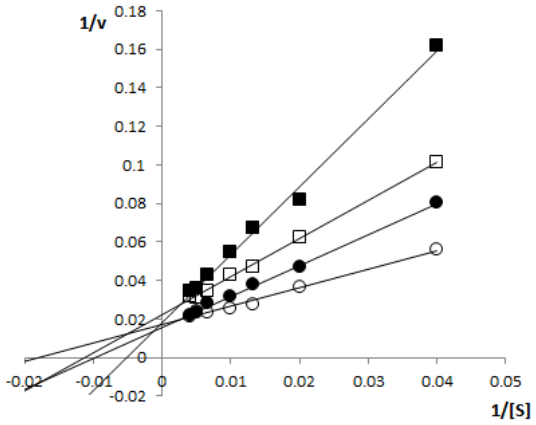
Parameter	Value	Std. Error
Vmax	65.9561	2.9405
Km	73.0679	9.1499
Ki	144.3283	22.1464



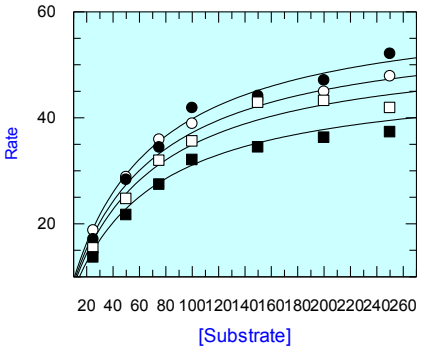
dTP_4-Glc **4** (Competitive Inhibitor)



Parameter	Value	Std. Error
Vmax	55.5917	2.2437
Km	45.6657	6.4603
Ki	56.5431	7.8046

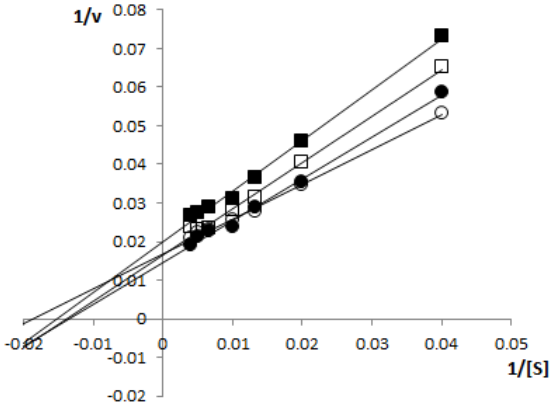


UTP-Glc **5** (Non-Competitive Inhibitor)*

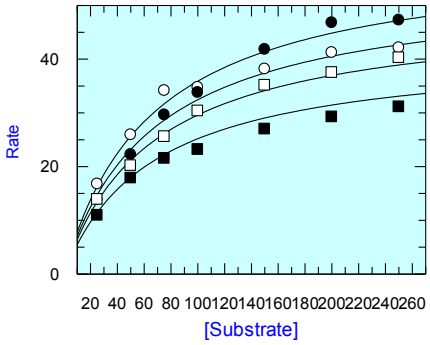


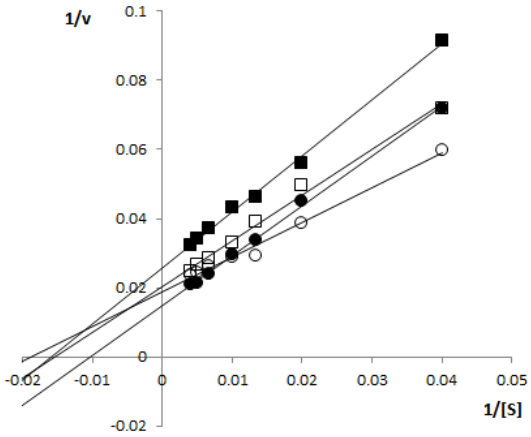
Reduced chi²: 113.4822

Parameter	Value	Std. Error
Vmax	61.8415	1.9560
Km	55.3063	4.8895
Ki	710.6808	102.9124



UP$_4$-Glc **6** (Non-Competitive Inhibitor)*

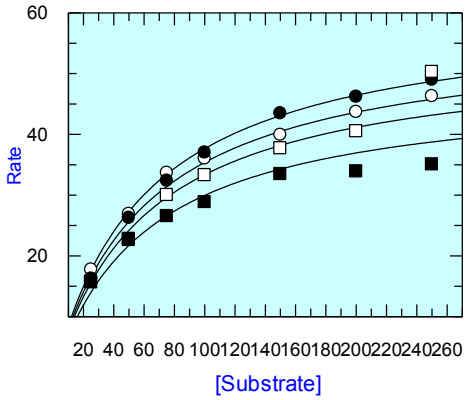


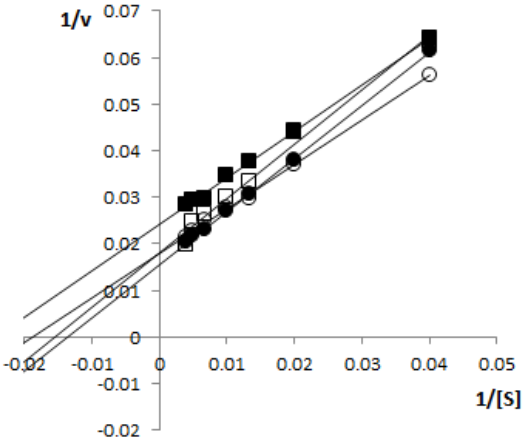


Reduced chi²: 164.0723

Parameter	Value	Std. Error
Vmax	59.6986	2.7501
Km	66.2640	7.9746
Ki	470.0322	69.4646

ATP-Glc **7** (Non-Competitive Inhibitor)*





Reduced chi²: 138.4844

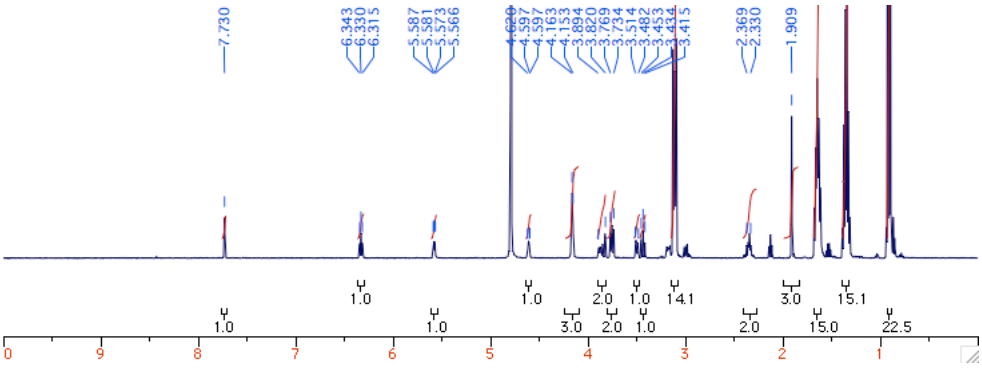
Parameter	Value	Std. Error
Vmax	60.5407	2.2792
Km	60.7731	6.1539
Ki	778.4164	139.4004

*Non-Competitive fit was chosen because it produced the lowest error (Chi2) upon fitting to the model

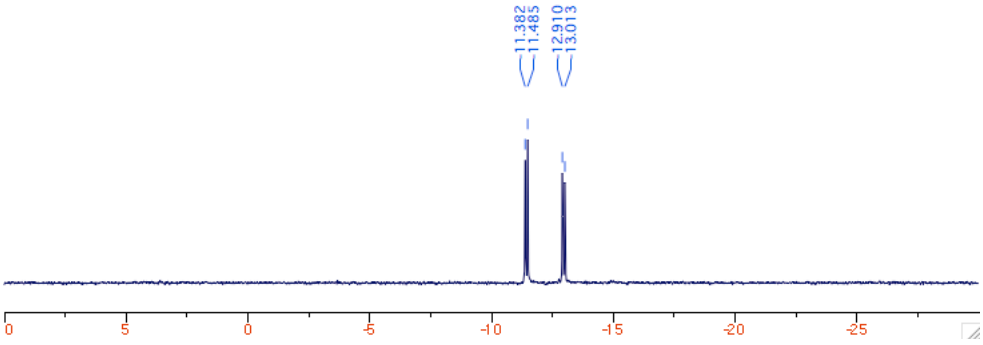
NMR and HPLC Spectra

dTDP-Glc **2**

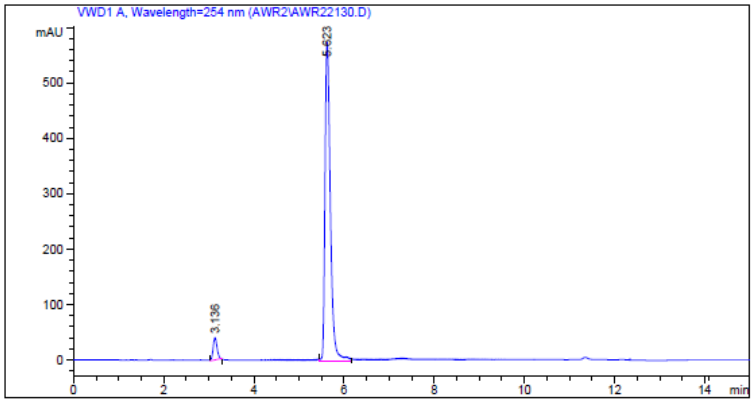
^{1}H NMR (D_2O, 500 MHz):



^{31}P NMR (D_2O, 202.4 MHz):

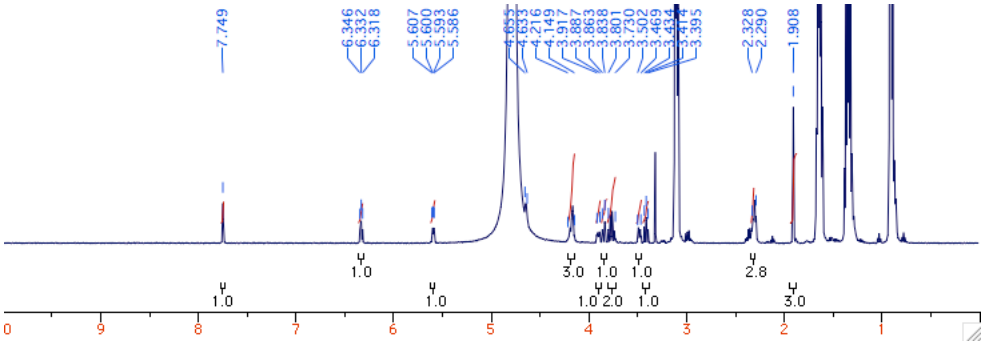


HPLC trace showing retention time of 5.62 min:

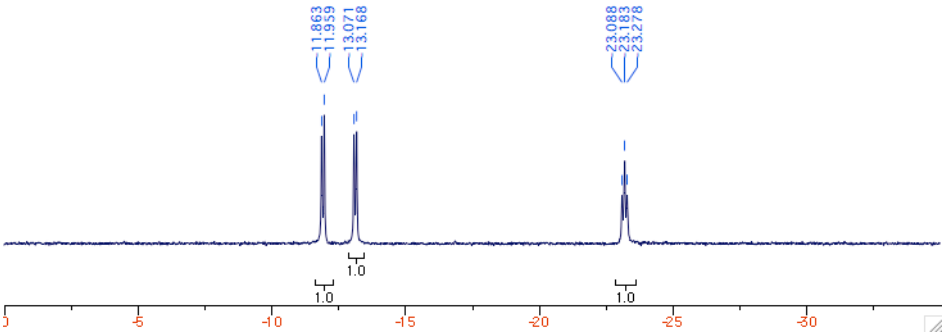


dTTP-Glc **3**

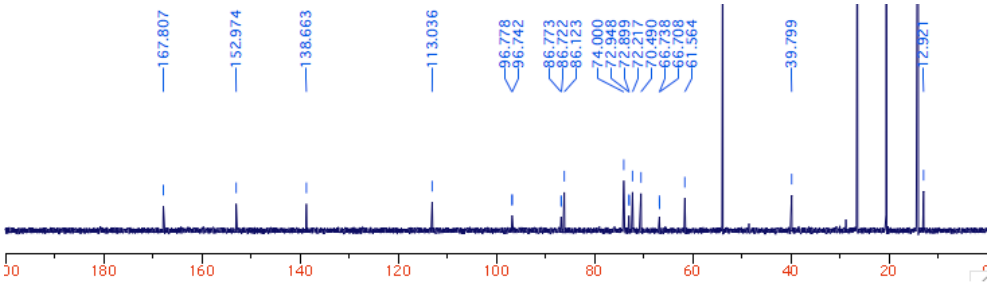
^{1}H NMR (D_2O, 500 MHz):



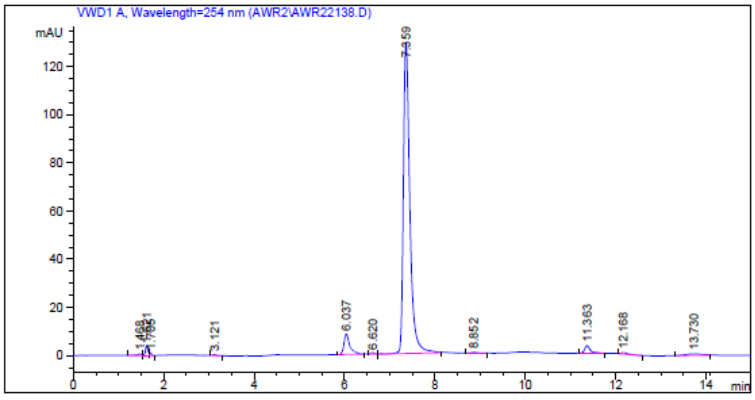
^{31}P NMR (D_2O, 202.4 MHz)



^{13}C NMR (D_2O, 175 MHz)

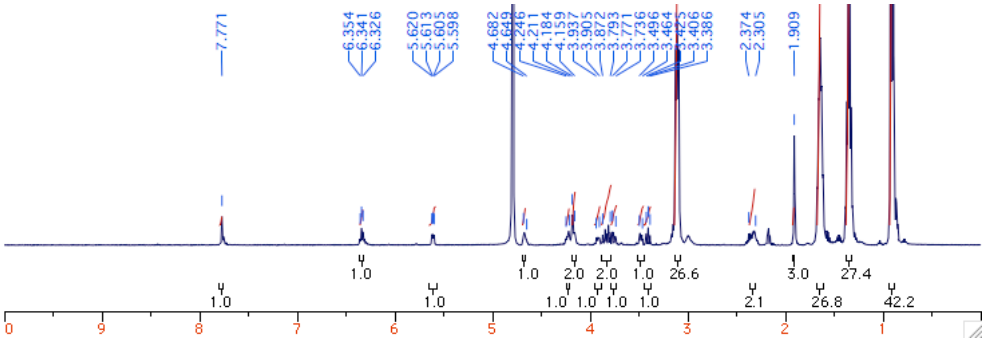


HPLC trace showing retention time of 7.36 min

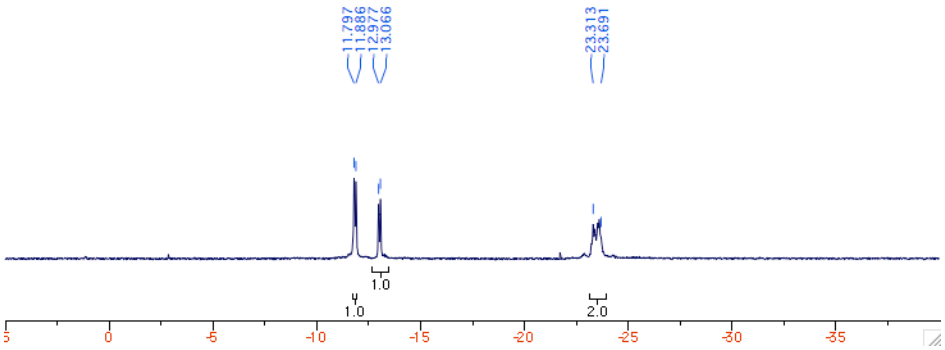


dTP_4-Glc **4**

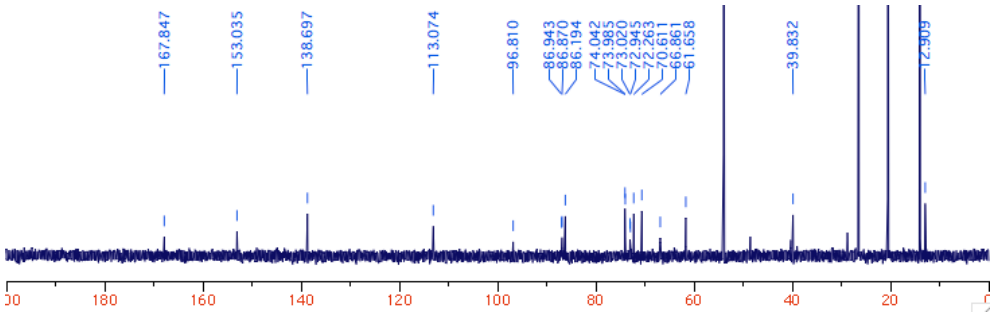
^{1}H NMR (D_2O, 500 MHz):



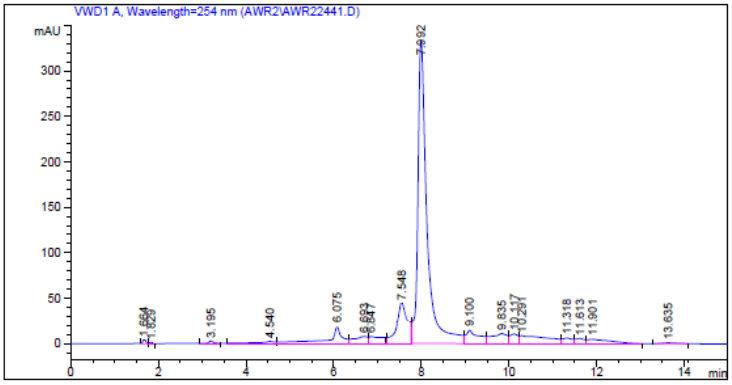
^{31}P NMR (D_2O, 202.4 MHz):



^{13}C NMR (D_2O, 125 MHz):

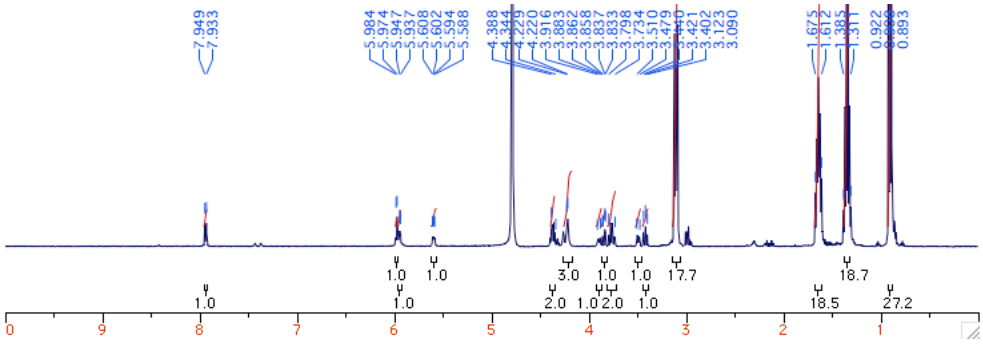


HPLC trace showing retention time of 7.99 min

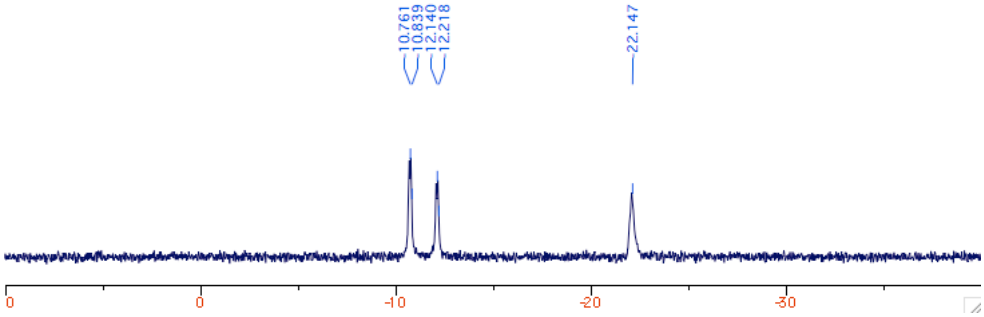


UTP-Glc **5**

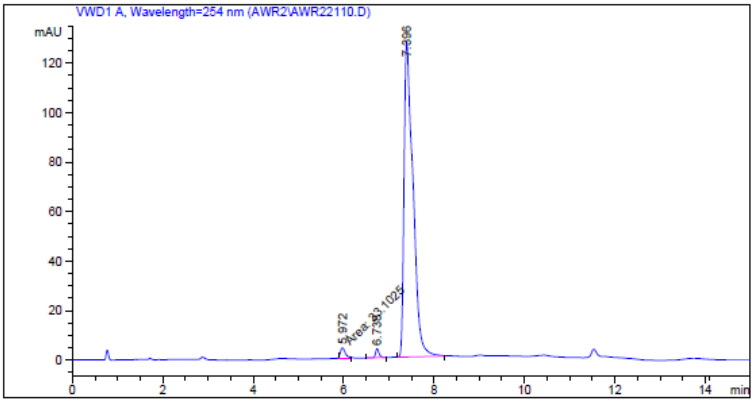
^{1}H NMR (D_2O, 500 MHz):



^{31}P NMR (D_2O, 202.4 MHz):

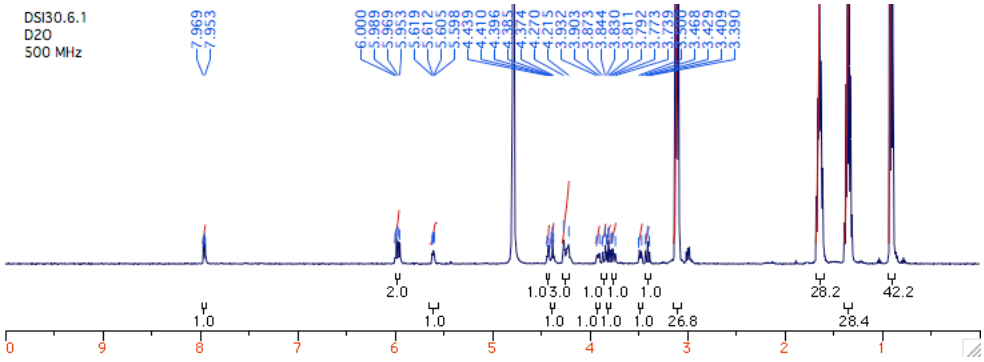


HPLC trace showing retention time of 7.40 min:

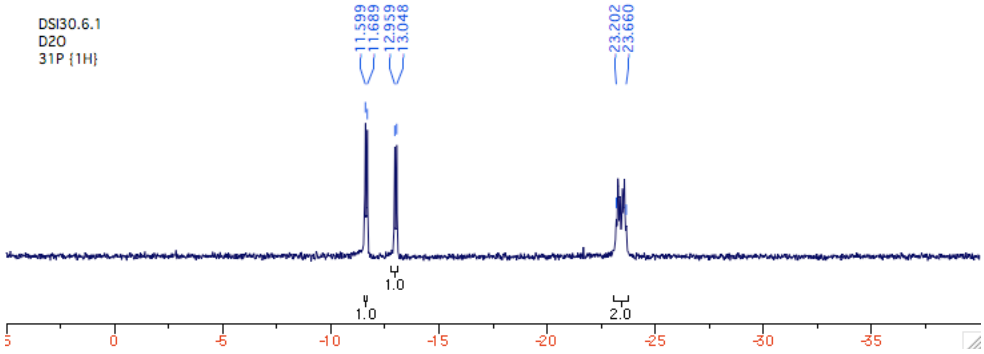


UP_4-Glc **6**

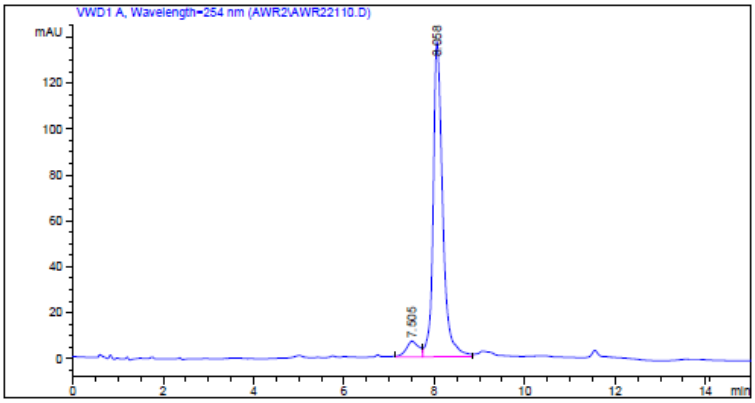
^{1}H NMR (D_2O, 500 MHz):



^{31}P NMR (D_2O, 202.4 MHz):

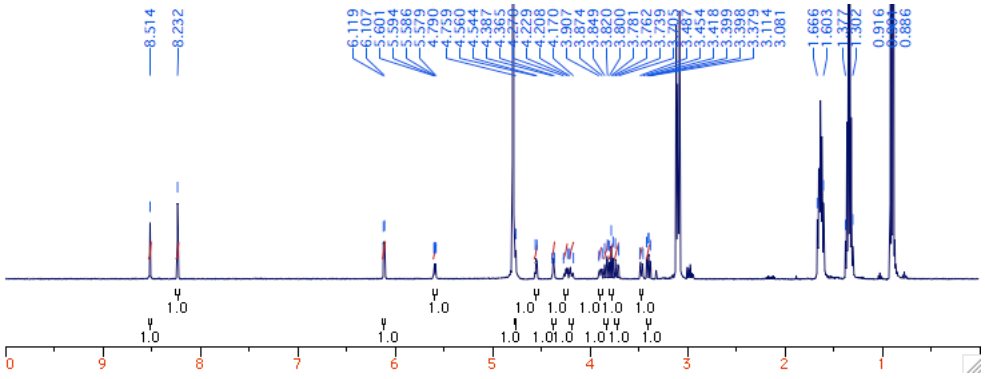


HPLC trace showing retention time of 8.09 min:

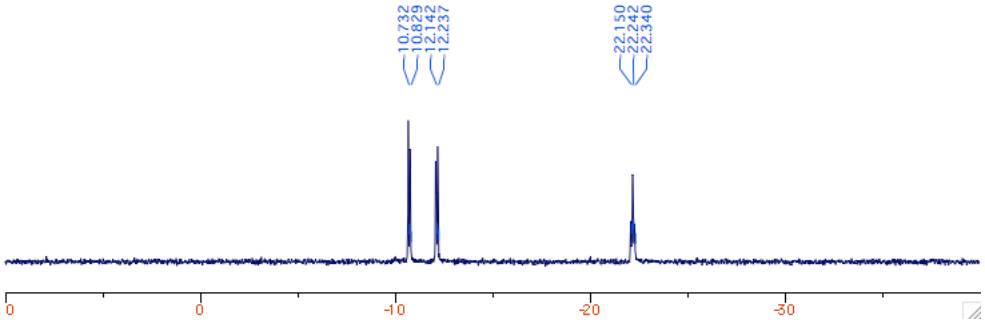


ATP-Glc **7**

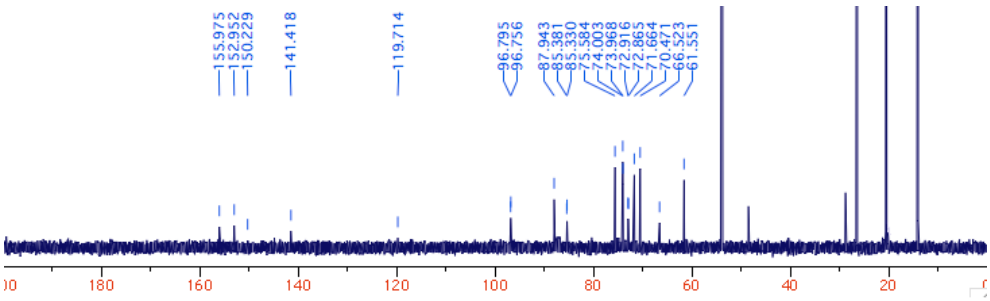
^{1}H NMR (D_2O, 500 MHz):



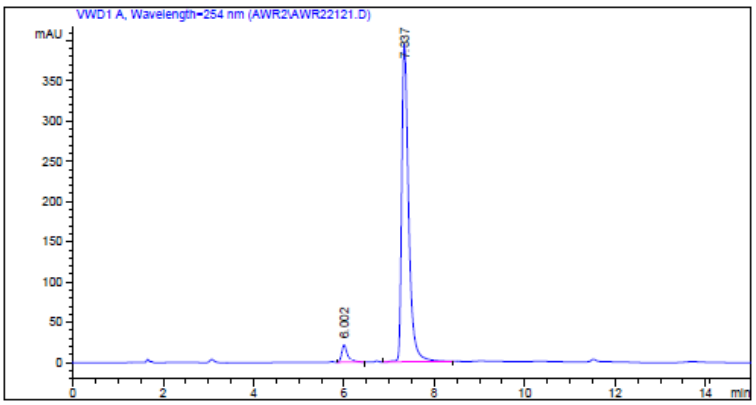
^{31}P NMR (D_2O, 202.4 MHz):



^{13}C NMR (D_2O, 175 MHz):



HPLC trace showing retention time of 7.34 min:



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