Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

This journal is © The Royal Society of Chemistry 2014

Kinetic evaluation of glucose 1-phosphate analogues with a thymidylyltransferase using a continuous coupled assay

S. M. Forget,[a] A. Jee,[b] D. A. Smithen,[a] R. Jagdhane,[c] S. A. Beaton,[a] D. R. J. Palmer,[c] R. T. Syvitski,[a] and D. L. Jakeman*[a,b] ‡

[a] *Department of Chemistry, Dalhousie University, 6274 Coburg Rd., PO Box 15,000, Halifax, Nova Scotia, B3H 4R2, Canada.*

[b] *College of Pharmacy, Dalhousie University, 5968 College St., PO Box15,000, Halifax, Nova Scotia, B3H 4R2, Canada.*

[c] *Department of Chemistry, University of Saskatchewan, 110 Science Place, Saskatoon, Saskatchewan, S7N 5C9, Canada.*

david.jakeman@dal.ca

Table of Contents

Analytical enzyme assays

HPLC retention times and MS fragmentation

Table 1. Sugar nucleotide retention times and EPI fragmentation.

NDP-sugar product	HPLC retention time (min)[a]	EPI (*m/z*)
dTTP	7.52	-
UTP	7.43	-
dTDP-Gln **15**[b]	2.60[b]	562,383,357[b]
UDP-Gln **16**	2.34	564, 385, 320
dTDP-kan **17**	2.87	562, 321, 273
UDP-Kan **18**	2.34	564,385,323
dTDP-3AzGlc **19**	6.45	589, 321, 266
UDP-3azGlc **20**	5.75	590, 323
dTDP-*myo*-Ino **21**	5.46	563, 321, 241
dTDP-Glc1CP **22**[c]	5.47[c]	561,321[c]
UDP-Glc1CP **23**[c]	5.27[c]	563, 323[c]

[a]The provided retention times are representative, as some variability in the retention times (+/- 0.2 min) was observed due to instrument variability; [b,c]Previous work[1; 2]

1. Timmons, S. C.; Mosher, R. H.; Knowles, S. A.; Jakeman, D. L. *Org. Lett.* **2007**, *9*, 857-860.

2. Beaton, S. A.; Huestis, M. P.; Sadeghi-Khomami, A.; Thomas, N. R.; Jakeman, D. L. *Chem. Commun.* **2009**, 238-240.

HPLC traces for small scale enzyme assays

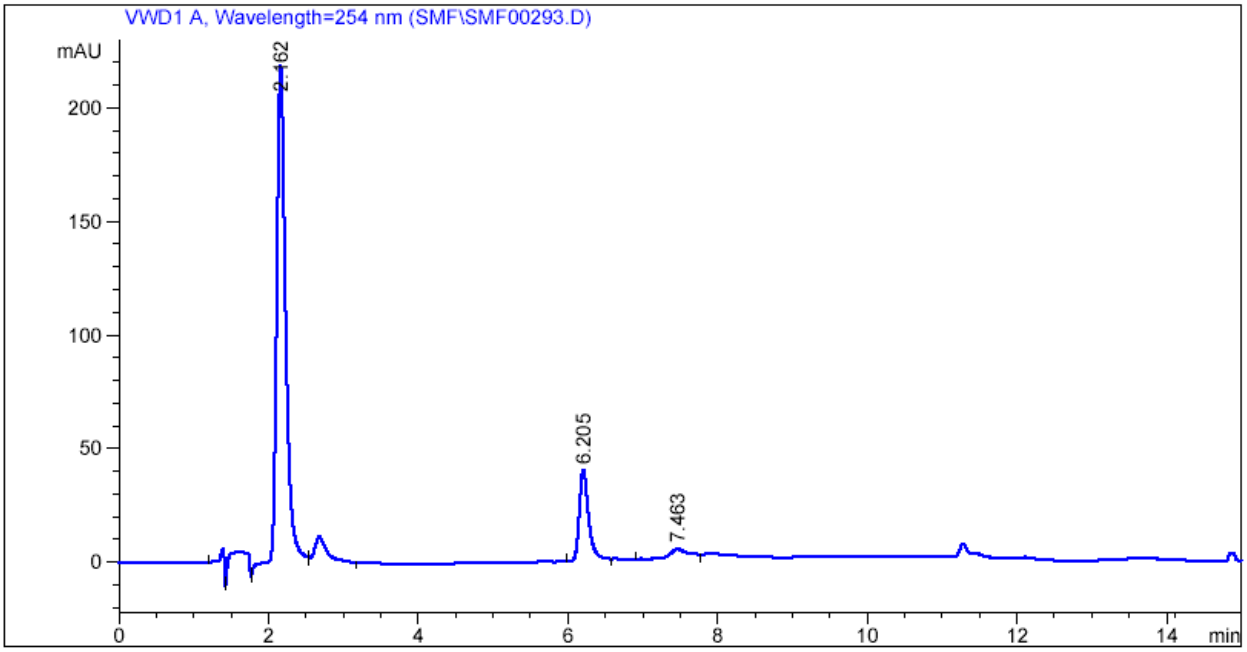


Figure 1. Small scale Cps2L (2 EU) assay containing Gln-1P **1** (2 mM) + **UTP** (1 mM) after 24h at 37 °C. The peak at a T_R 2.162 min is a result of the formation of dTDP-Gln **16.**

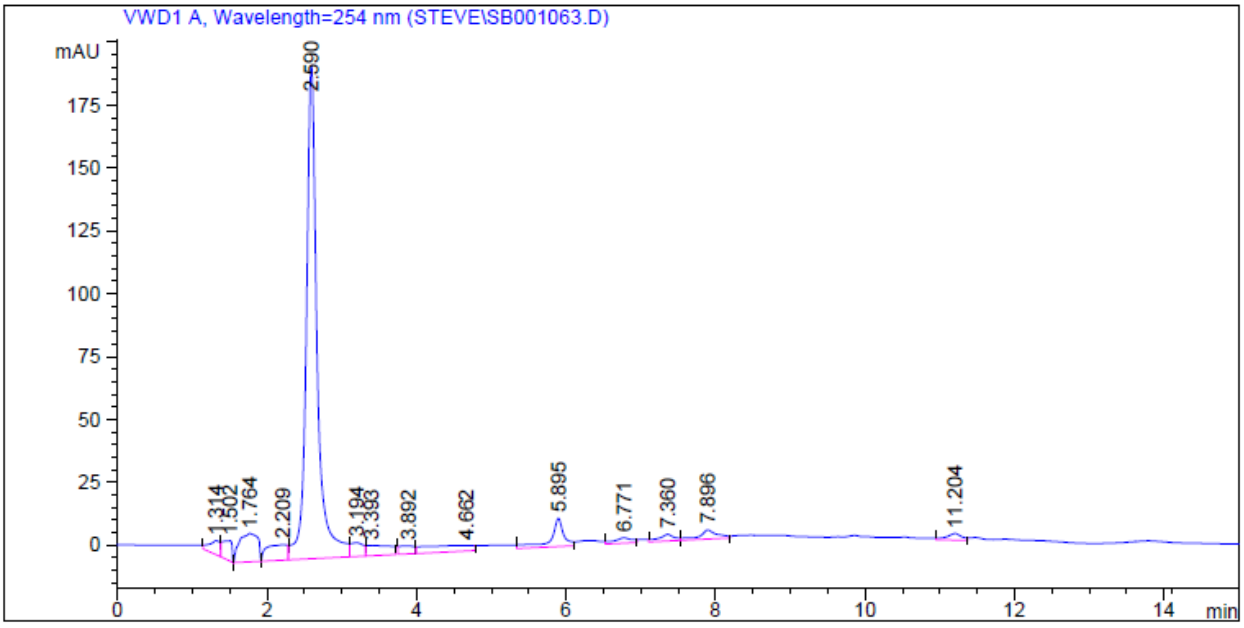


Figure 2. Small scale Cps2L (2 EU) assay containing Kan-1P **2** (2 mM) + **dTTP** (1 mM) after 30 min at 37 °C. The peak at a T_R 2.590 min is a result of the formation of dTDP-Kan **17**.

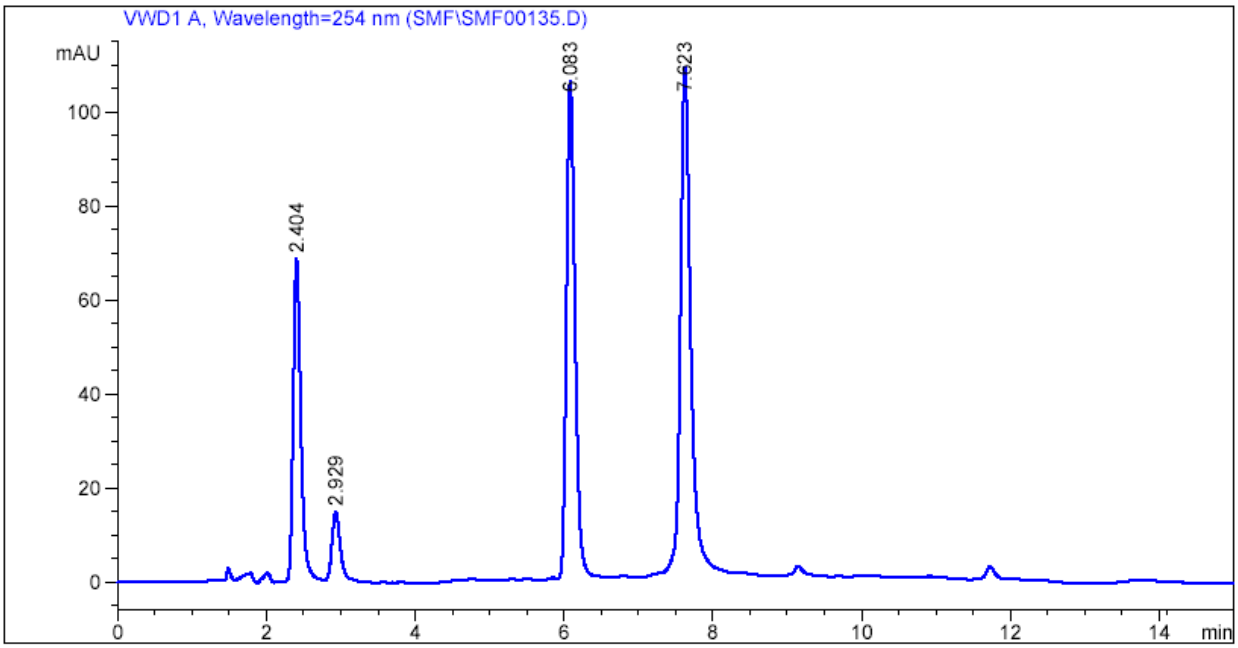


Figure 3. Small scale Cps2L (8 EU) assay containing Kan-1P **2** (2 mM) + **UTP** (1 mM) after 48 h at 37 °C. The peak at a T_R 2.404 min is a result of the formation of UDP-Kan**18**.

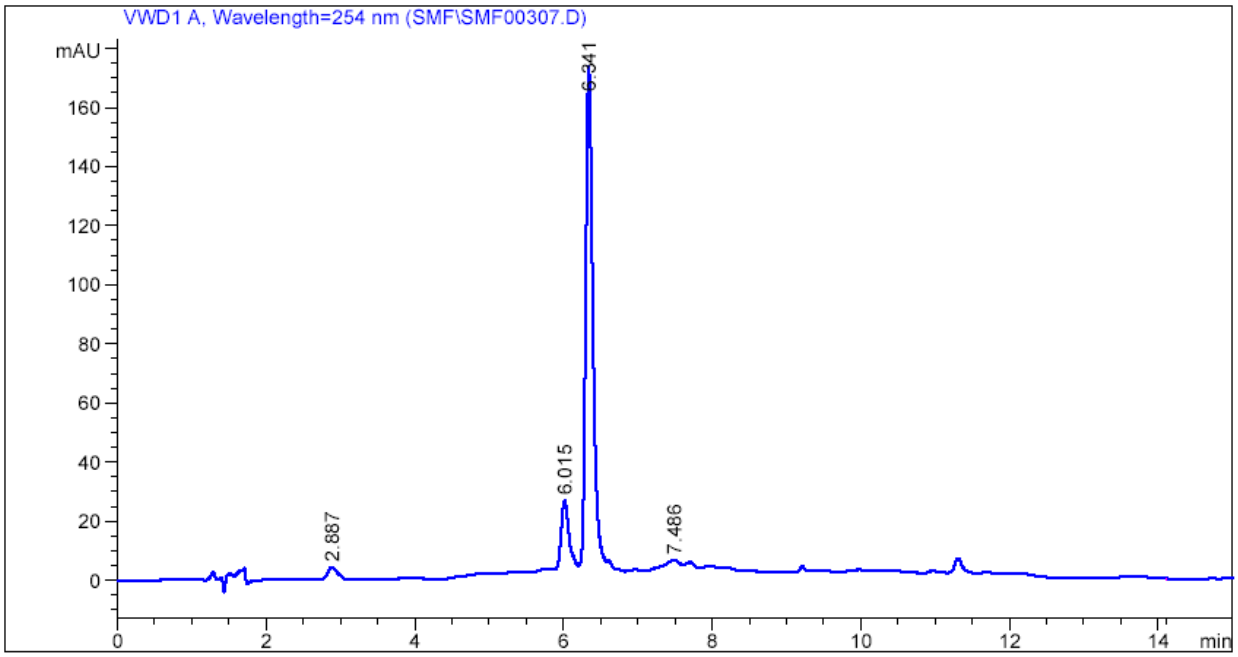


Figure 4. Small scale Cps2L (8 EU) assay containing 3AzGlc-1P **3** (2 mM) + **dTTP (**1 mM) after 17 h at 37 °C. The peak at a T_R 6.341 min is a result of the formation of dTDP-3AzGlc **19**.

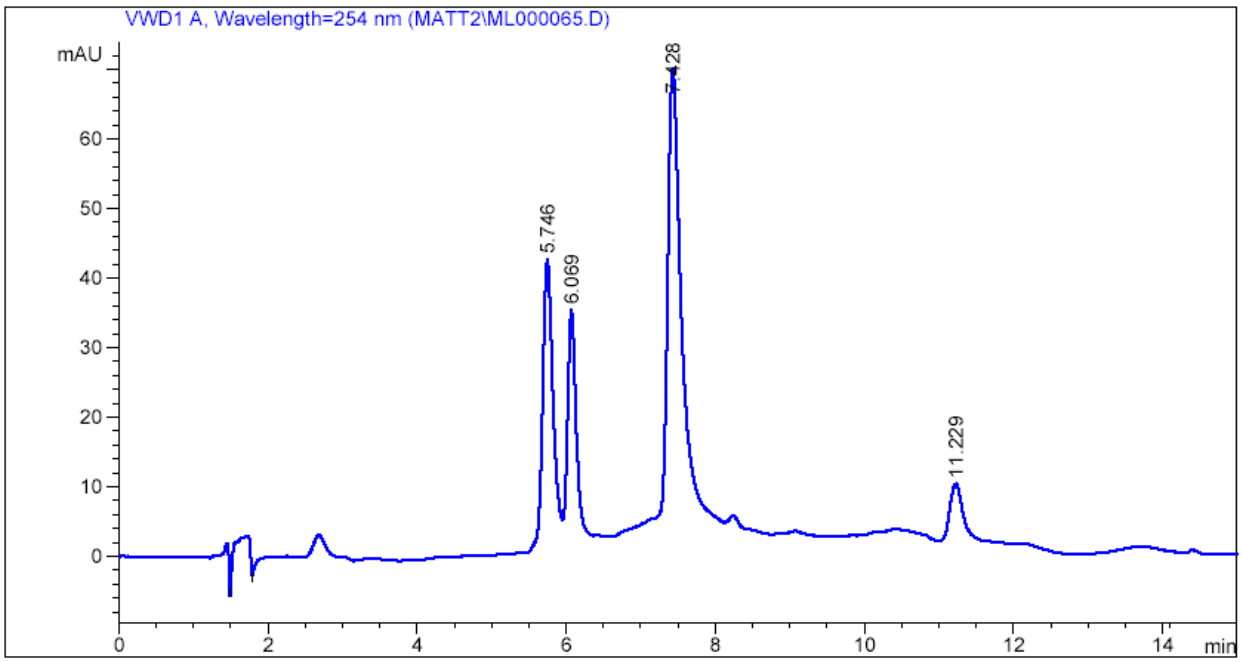


Figure 5. Small scale Cps2L (8 EU) assay containing 3AzGlc-1P **3** (2 mM) + **UTP** (1 mM) after 24 h at 37 °C. The peak at a T_R 5.746 min is a result of the formation of UDP-3AzGlc **20**.

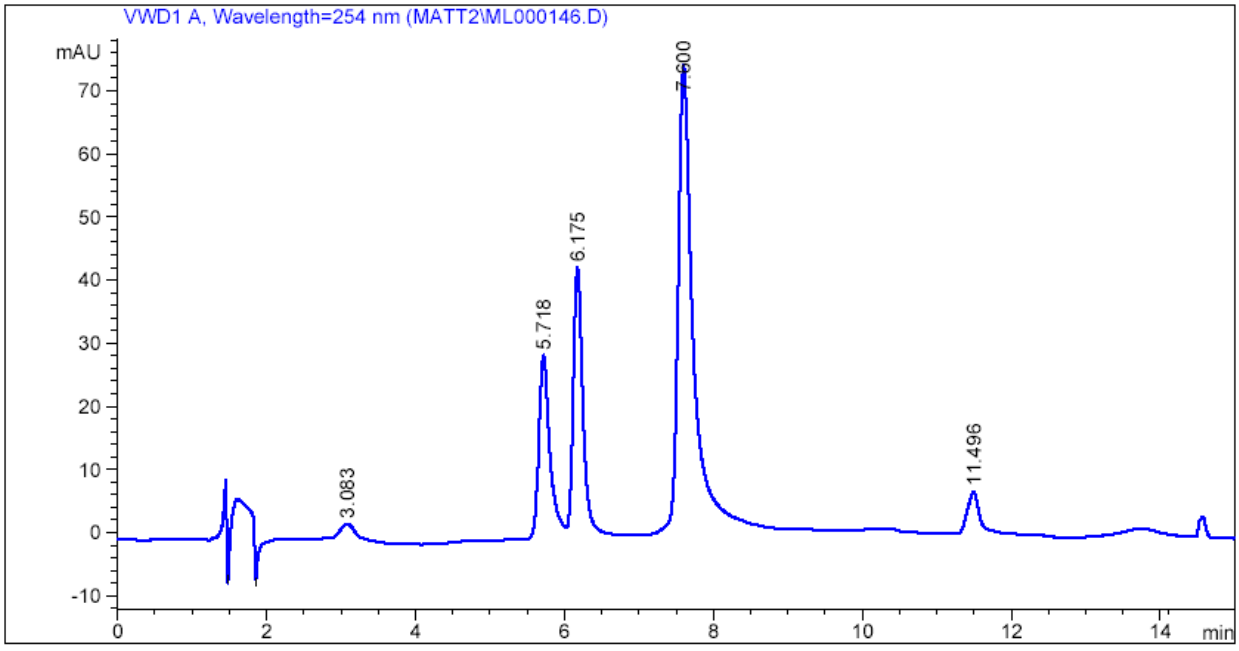


Figure 6. Small scale Cps2L (8 EU) assay containing *myo*-Ino-2P **4** (2 mM) + **dTTP (**1 mM) after 8 h at 37 °C. The peak at a T_R 5.718 min is a result of the formation of dTDP-*myo*-Ino **21**.

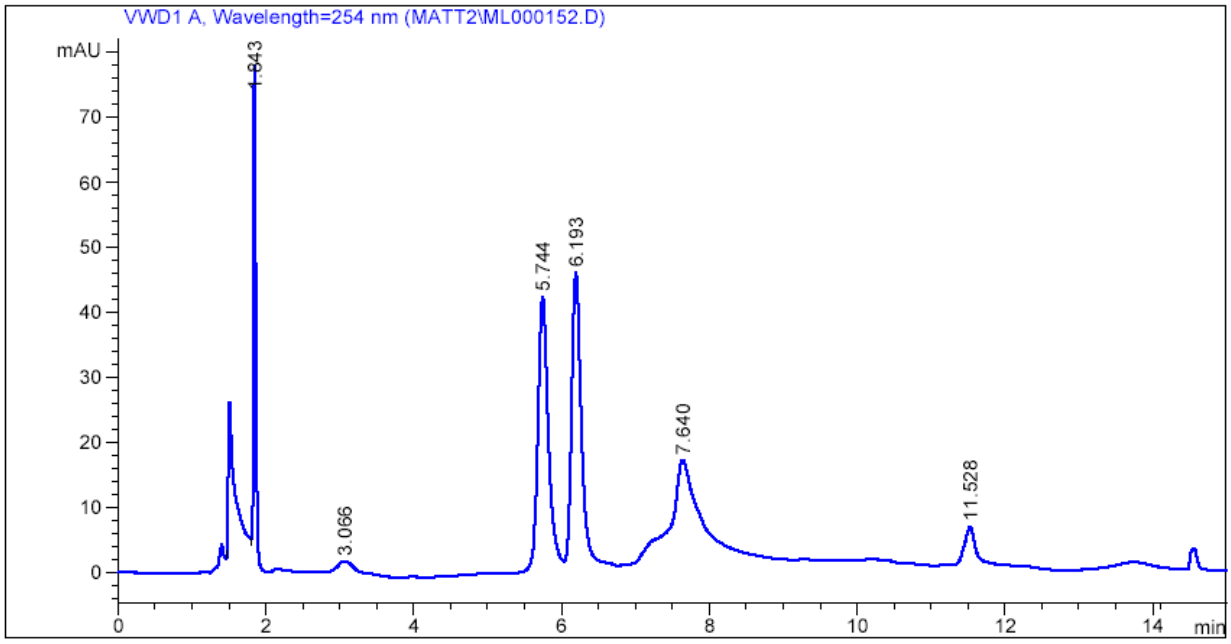


Figure 7. Small scale Cps2L (8 EU) assay containing *myo*-Ino-2P **4** (2 mM) + dTTP (1 mM) after 24 h at 37 °C. The peak at a T_R 5.744 min is a result of the formation of dTDP-*myo*-Ino **21**. Breakdown products are visible at 6.193 and 1.4-1.8 minutes.

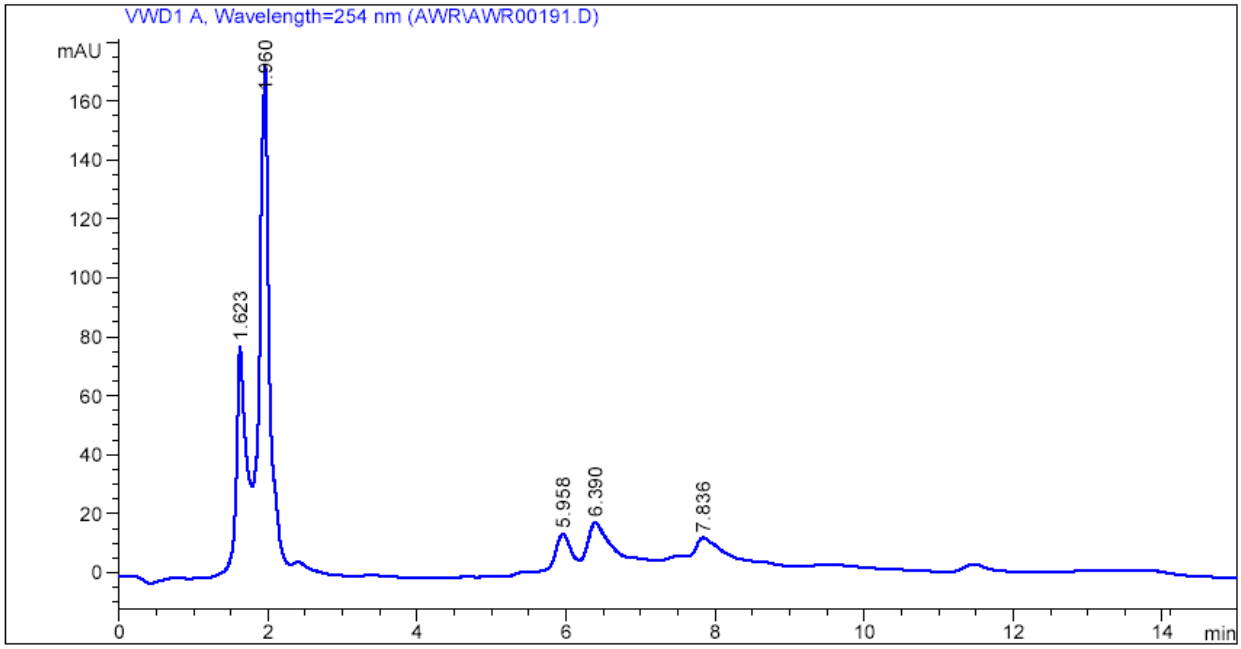


Figure 8. Small scale Cps2L (8 EU) assay containing *myo*-Ino-2P **4** (2 mM) + dTTP (1 mM) after 48 h at 37 °C. Breakdown products are visible at 6.193 and 1.4-1.8 minutes.

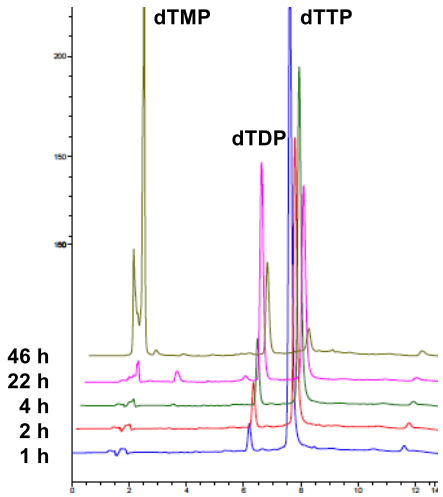
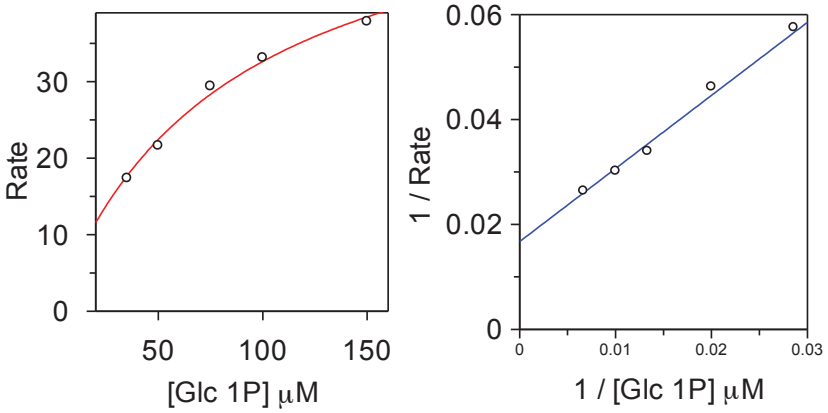


Figure 9. HPLC traces obtained at various time intervals to monitor the enzymatic reaction between dTTP and Glc-2CP **6** showing no conversion to product over 46 h**.**

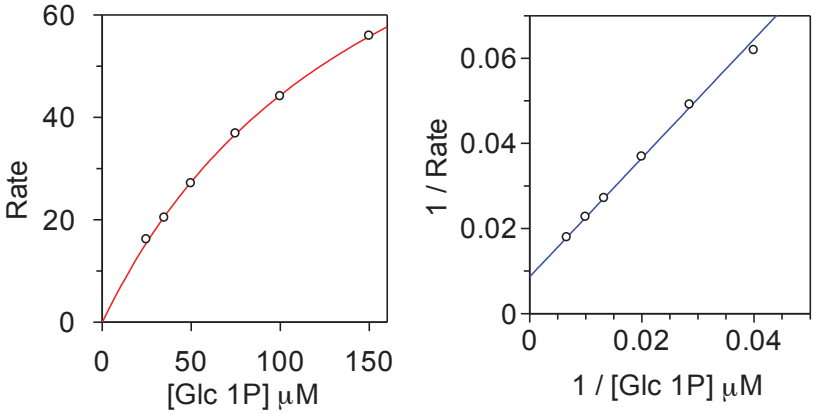
IPP-PNP-XO Kinetic Assays

Michealis-Menten and Lineweaver-Burk plots



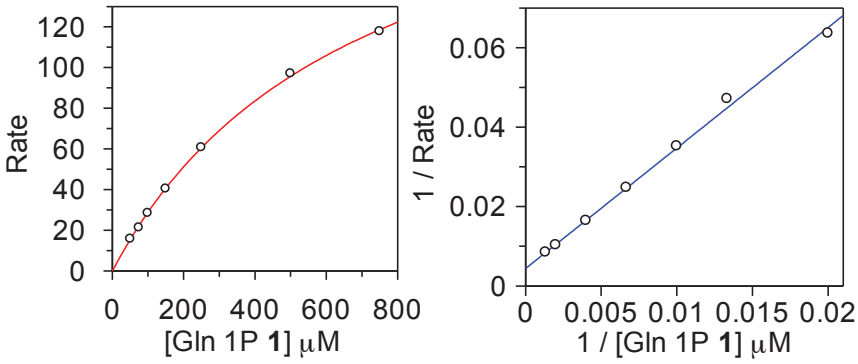
Parameter	Value	Std. Error
Vmax	59.7952	3.7389
Km	83.2866	10.7150

Figure 10. Variable Glc-1P in the presence of constant dTTP (1 mM) and 0.003 μM Cps2L.



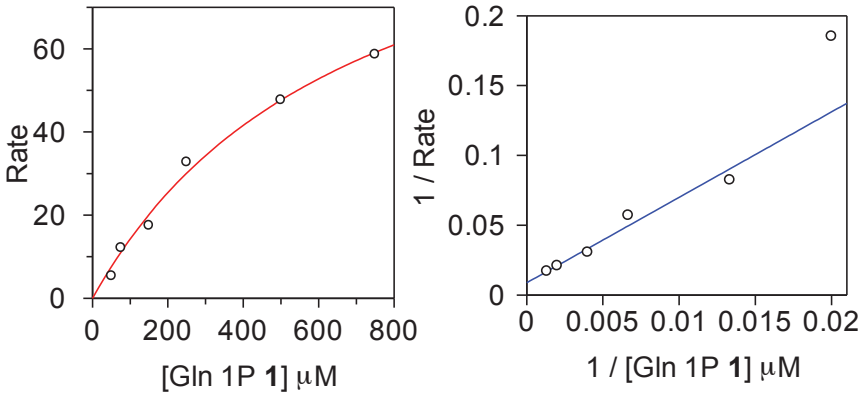
Parameter	Value	Std. Error
Vmax	116.0364	3.1190
Km	162.0003	6.8941

Figure 11. Variable Glc-1P in the presence of constant UTP (1 mM) and 0.0925 μM Cps2L.



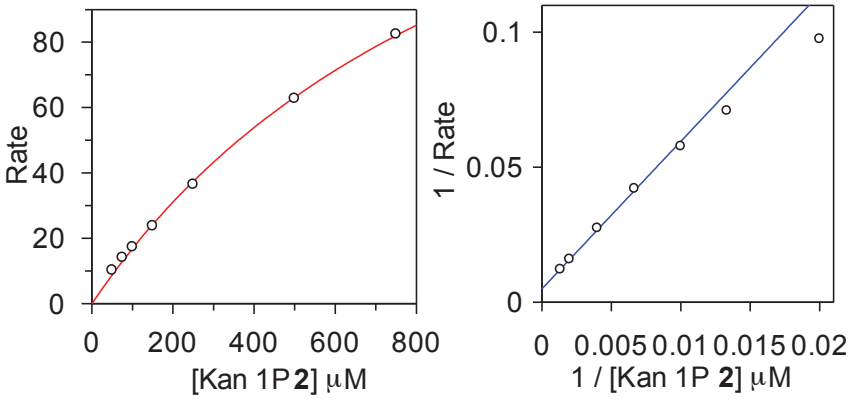
Parameter	Value	Std. Error
Vmax	228.0977	5.7181
Km	692.1244	29.1530

Figure 12. Variable Gln-1P **1** in the presence of constant dTTP (1 mM) and 0.06 μM Cps2L.



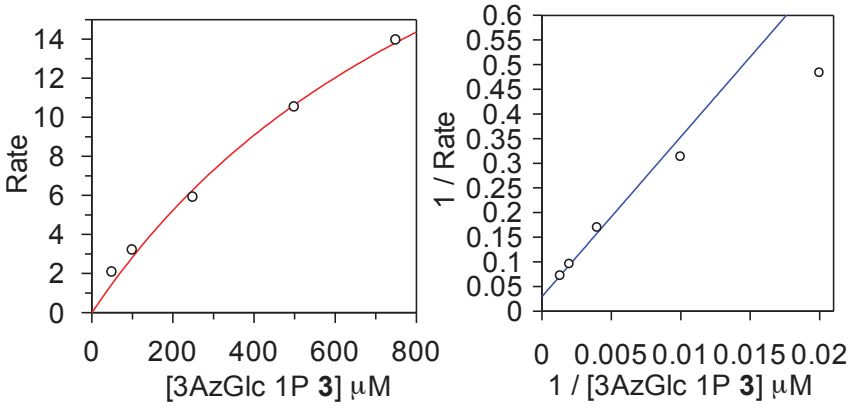
Parameter	Value	Std. Error
Vmax	114.3145	15.0980
Km	699.7641	157.2080

Figure 13. Variable Gln-1P **1** in the presence of constant UTP (1 mM) and 1.34 μM Cps2L.



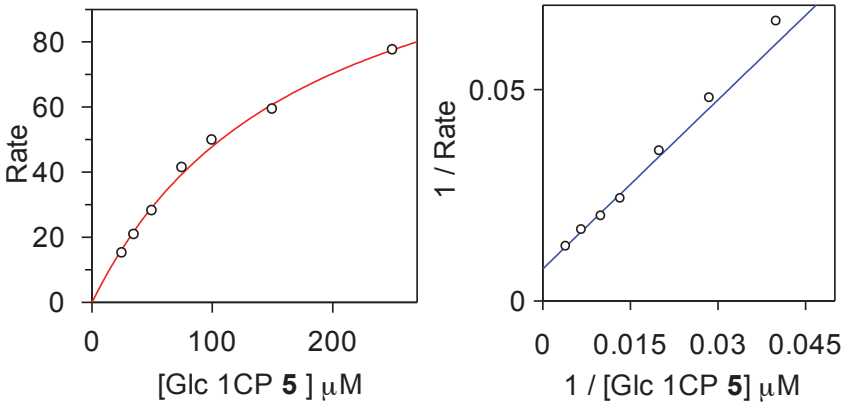
Parameter	Value	Std. Error
Vmax	203.7614	12.6594
Km	1113.1561	101.4656

Figure 14. Variable Kan-1P **2** in the presence of constant dTTP (1 mM) and 3 μM Cps2L.



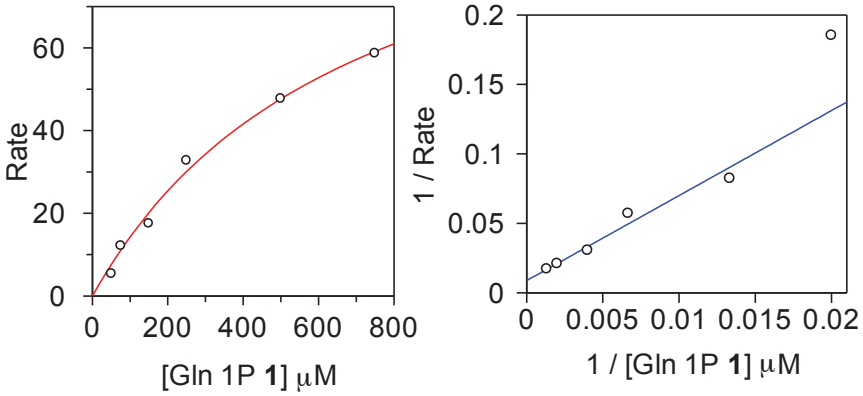
Parameter	Value	Std. Error
Vmax	34.3670	6.3967
Km	1114.7553	310.5301

Figure 15. Variable 3AzGlc-1P **3** in the presence of constant dTTP (1 mM) and 134 μM Cps2L.



Parameter	Value	Std. Error
Vmax	132.3376	7.2254
Km	176.5519	16.9727

Figure 16. Variable Glc-1CP **5** in the presence of constant dTTP (1 mM) and 0.03 μM Cps2L.



Parameter	Value	Std. Error
Vmax	114.3145	15.0980
Km	699.7641	157.2080

Figure 17. Variable Glc-1CP **5** in the presence of constant UTP (1 mM) and 1.85 μM Cps2L.

NMR Binding Studies

K_d Determination using WaterLOGSY NMR Spectroscopy with Glc-2CP (**6**)

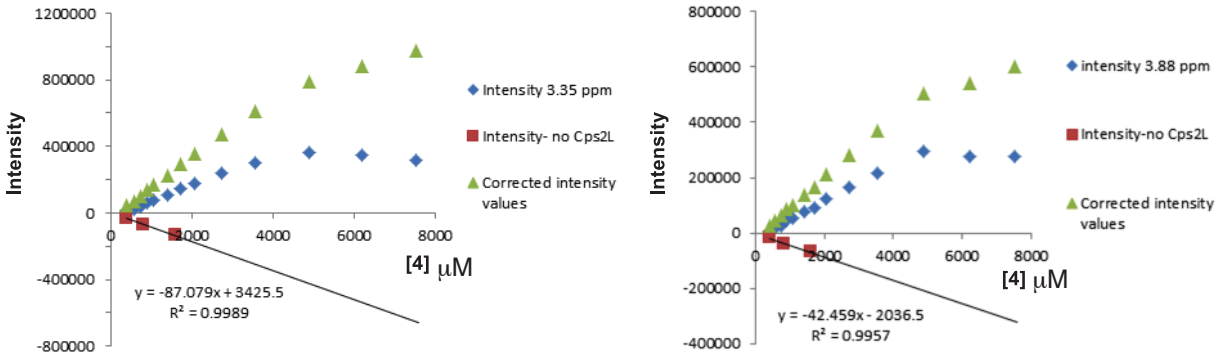


Figure 18. Determination of K_d for **6** binding to Cps2L in the presence of dTTP. Plot of signal intensity with (♦) and without (■) Cps2L and corrected signal intensity (▲) *vs* concentration of **6** for peaks at 3.35 ppm (left) and 3.88 ppm (right).

Chemoenzymatic synthesis of sugar nucleotides

HPLC traces for purified sugar nucleotides

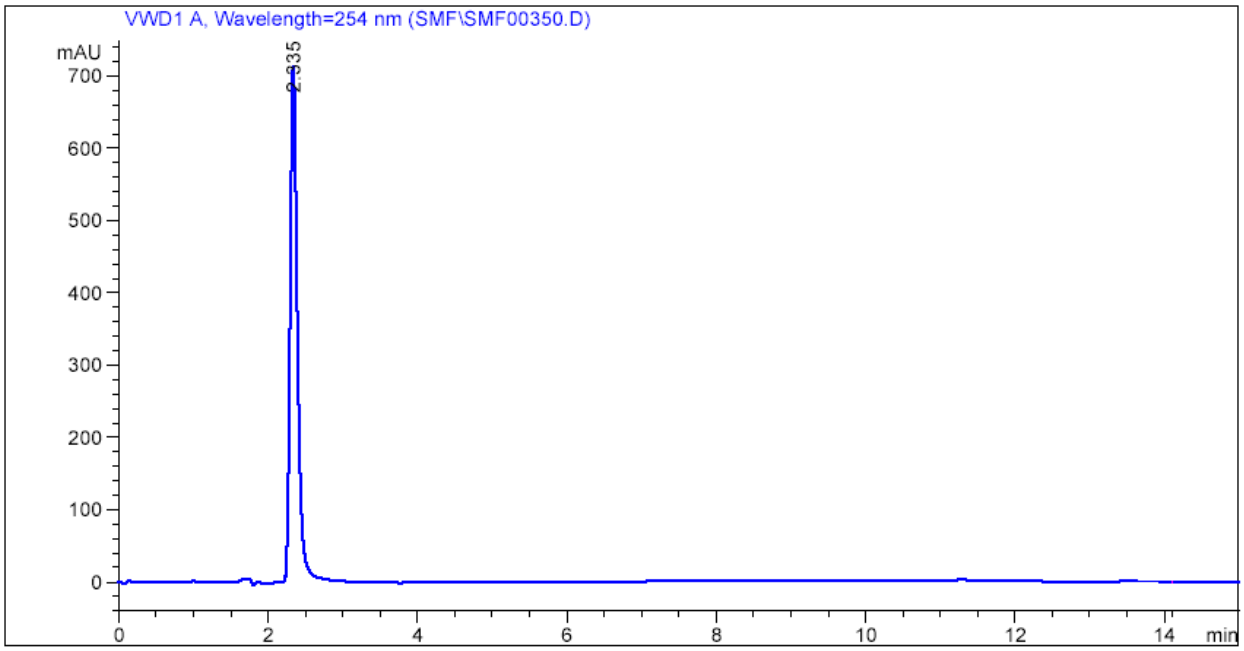


Figure 19. HPLC trace of purified UDP-Gln **16**.

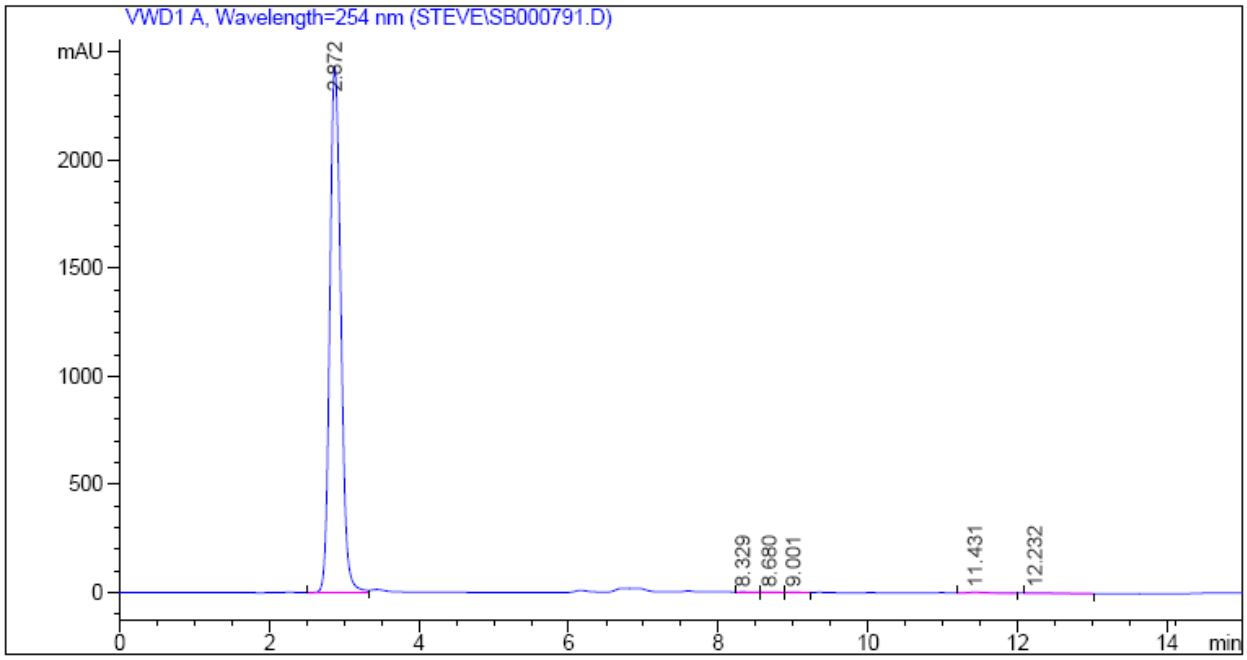


Figure 20. HPLC trace of purified dTDP-Kan **17.**

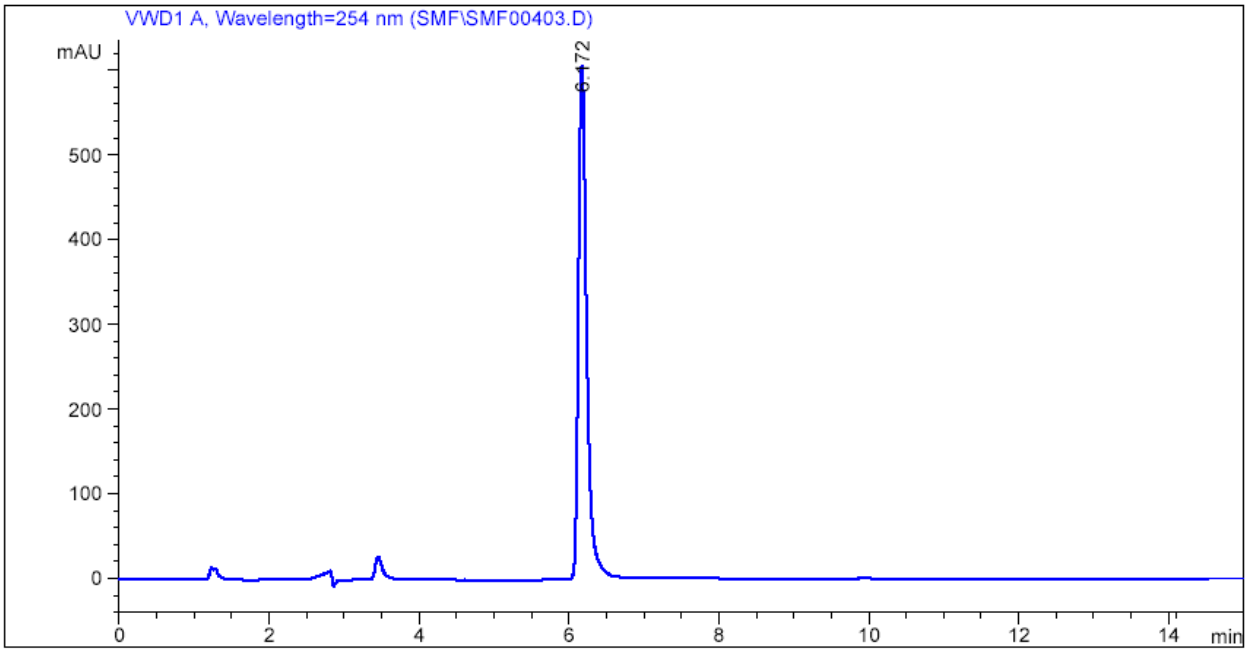
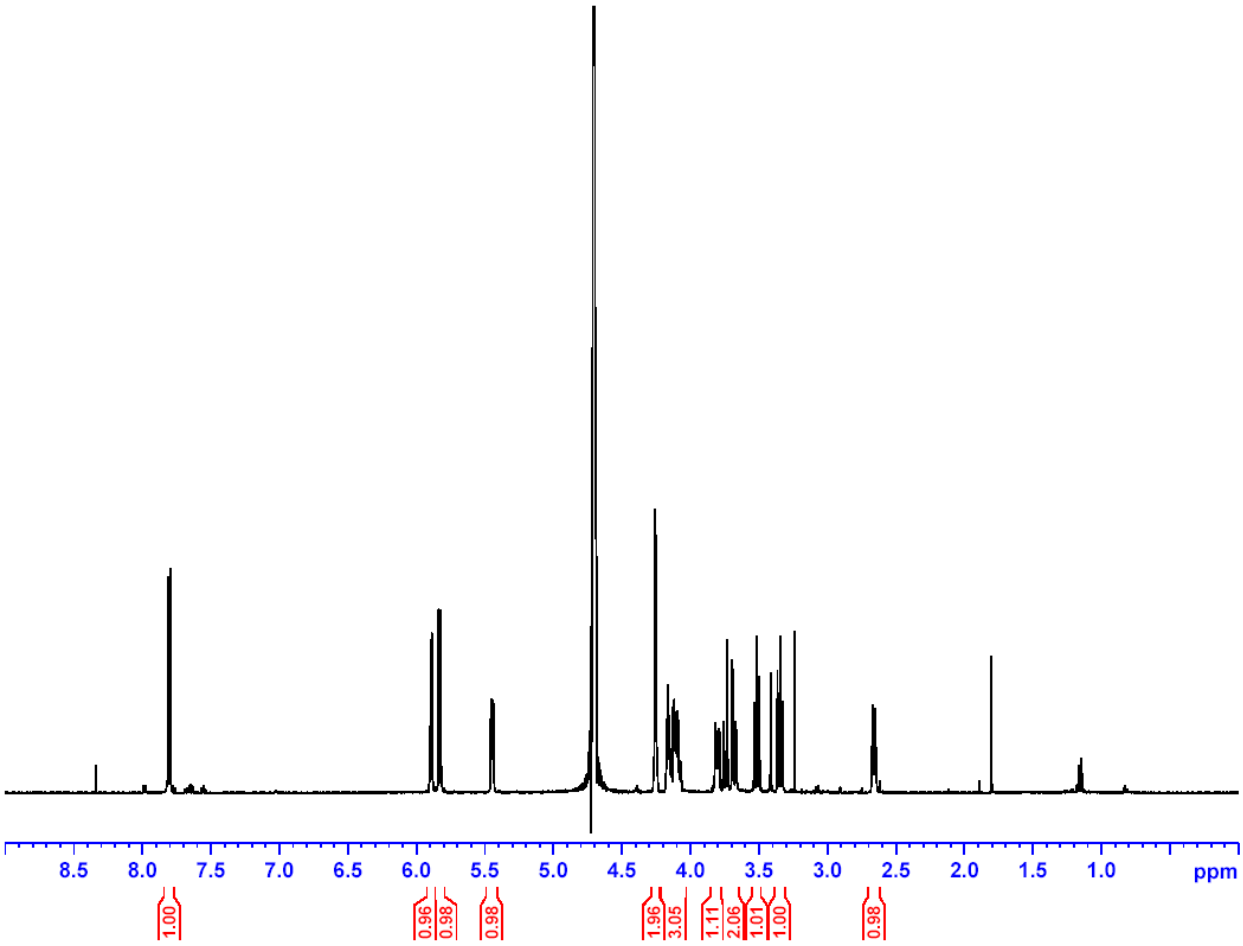


Figure 21. HPLC trace of purified dTDP-3AzGlc **19.**

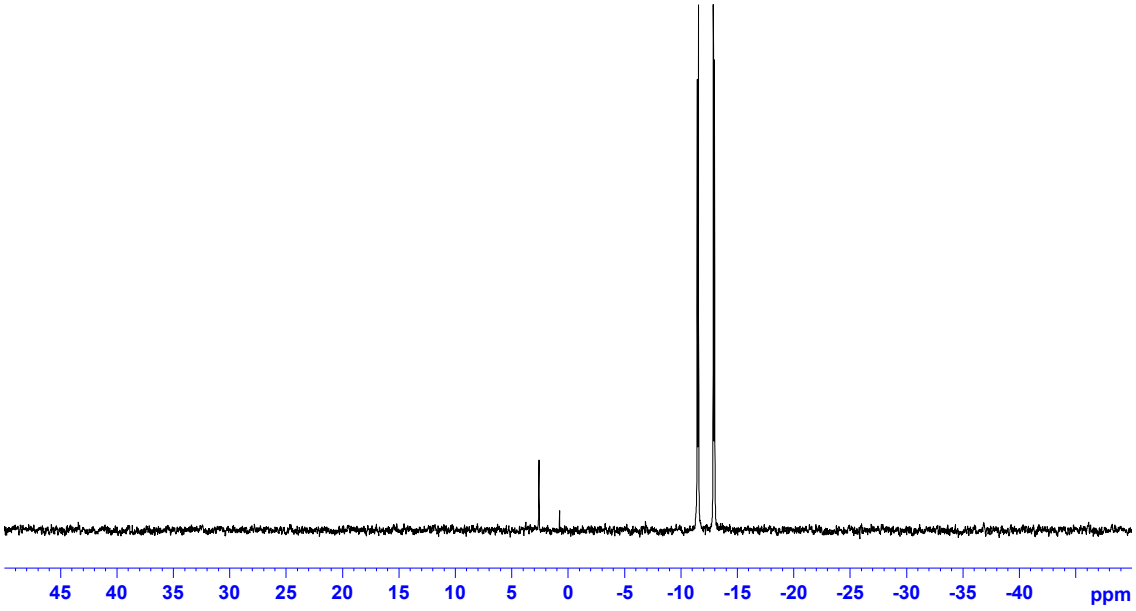
NMR spectra for final purified sugar nucleotides

*UDP-glucosamine (**16**)*

^{1}H NMR (D_2O, 500 MHz) of **16**:

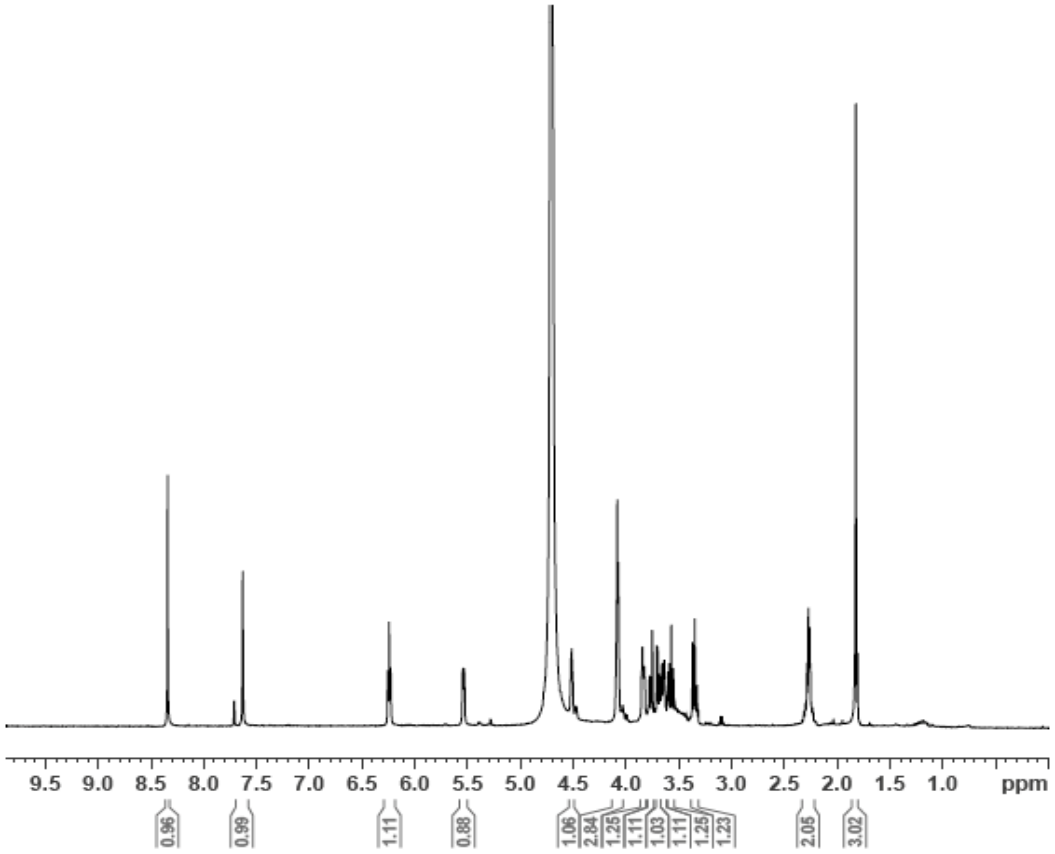


^{31}P $\{^{1}H\}$ NMR (D_2O, 202 mHz) of **16**:

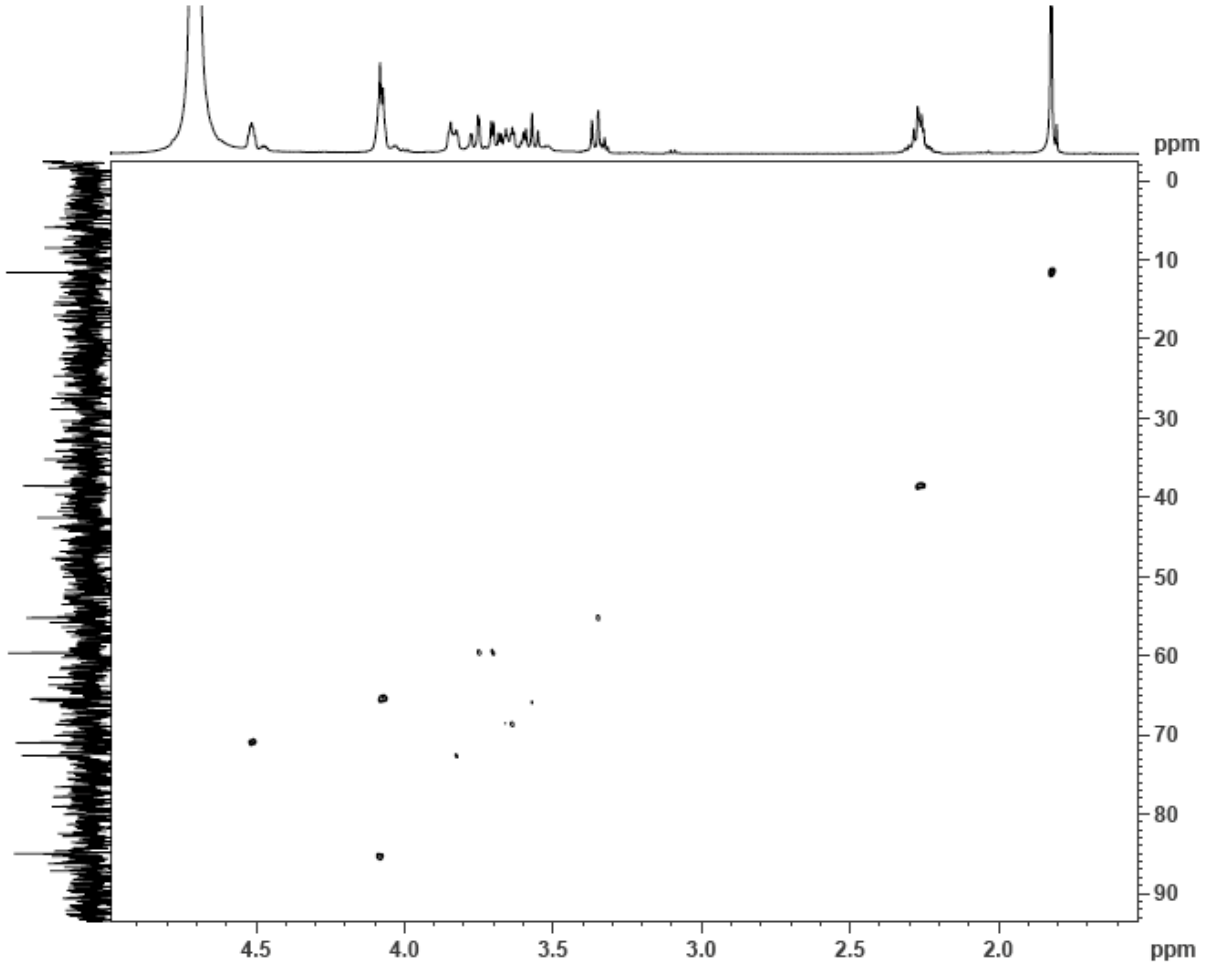


*dTDP-kanosamine (**17**)*

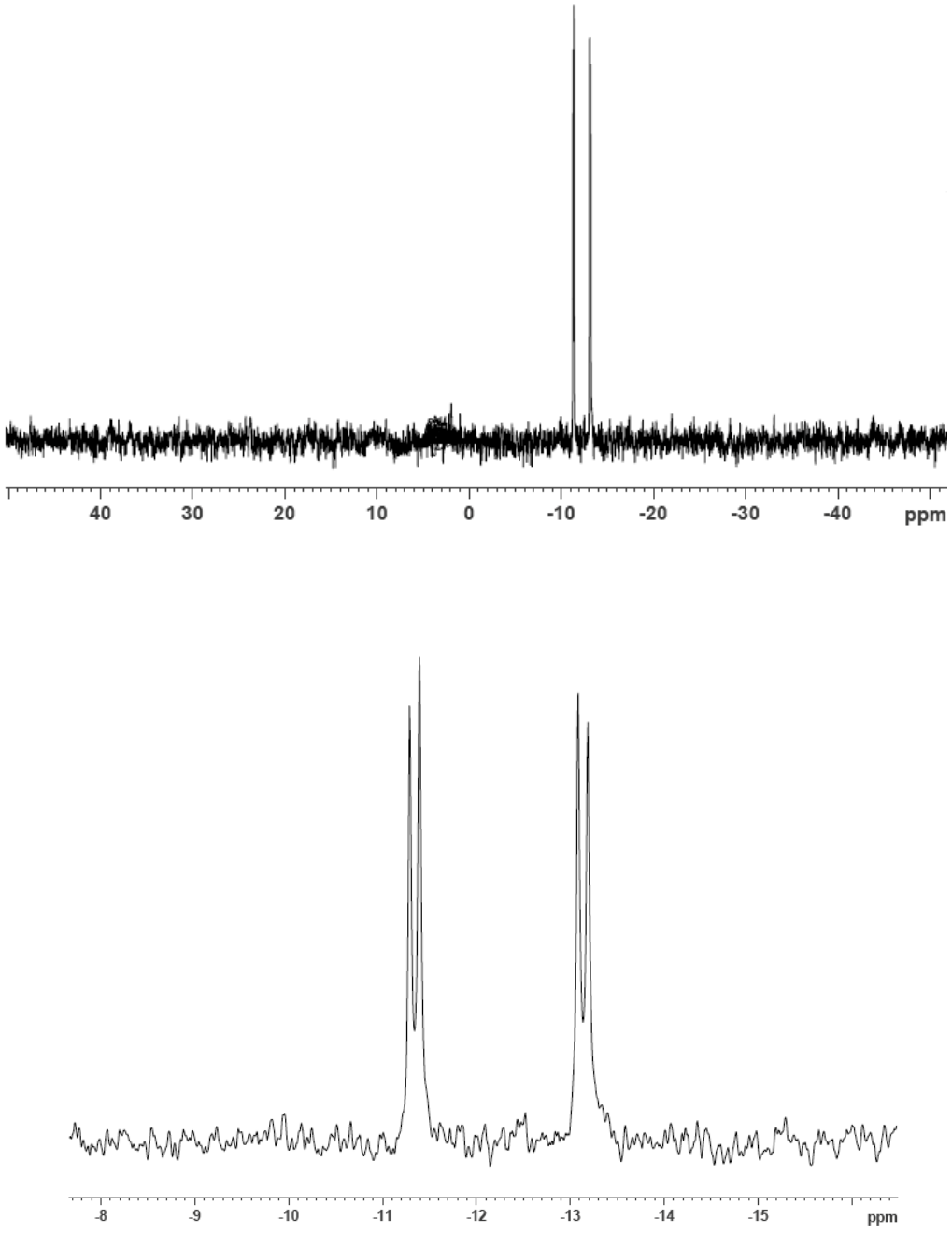
^{1}H NMR (D_2O. 500 mHz) of **17**



2D HSQC (D_2O) of **17**:

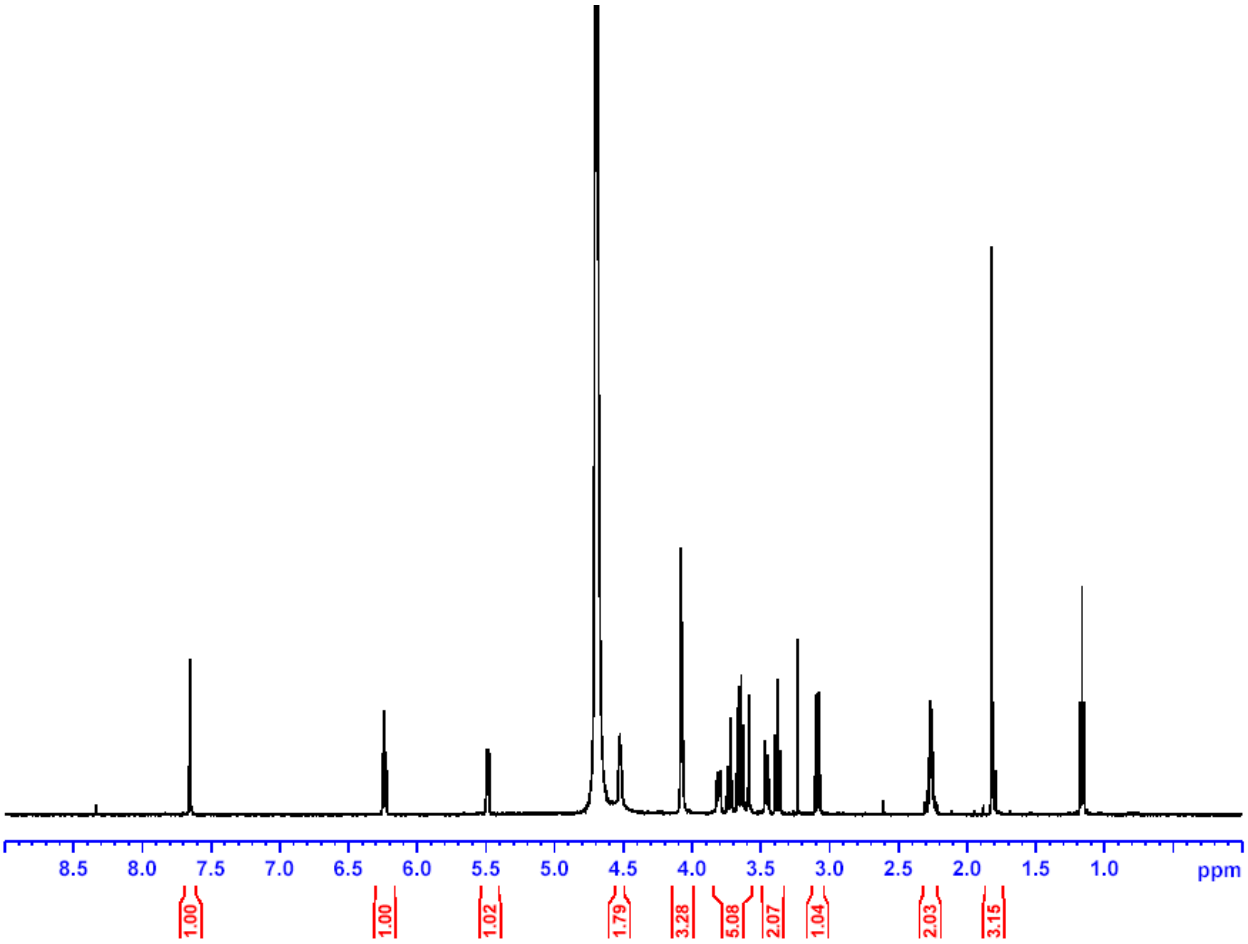


^{31}P $\{^{1}H\}$ NMR (D_2O, 202 mHz) of **17**:

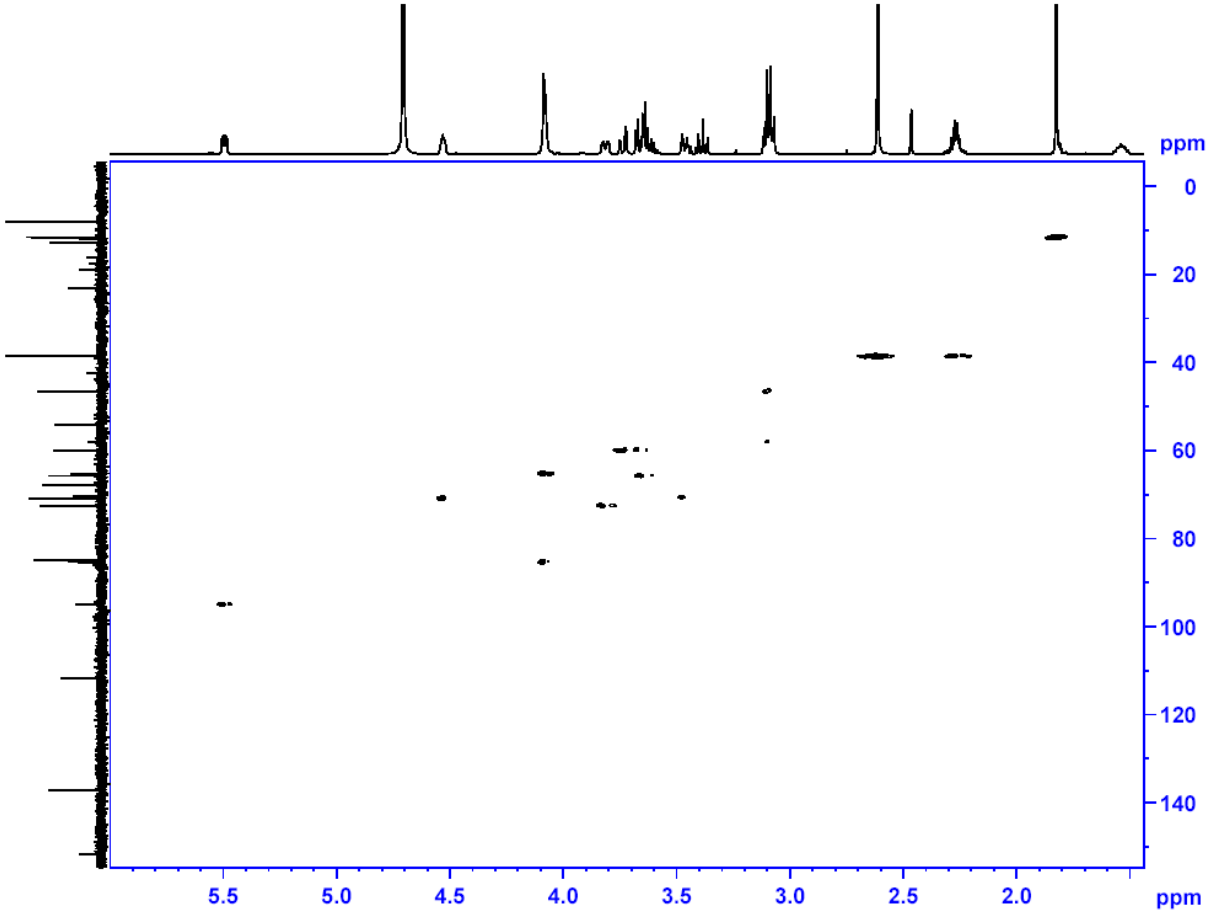


dTDP-3AzGlc ***(19)***

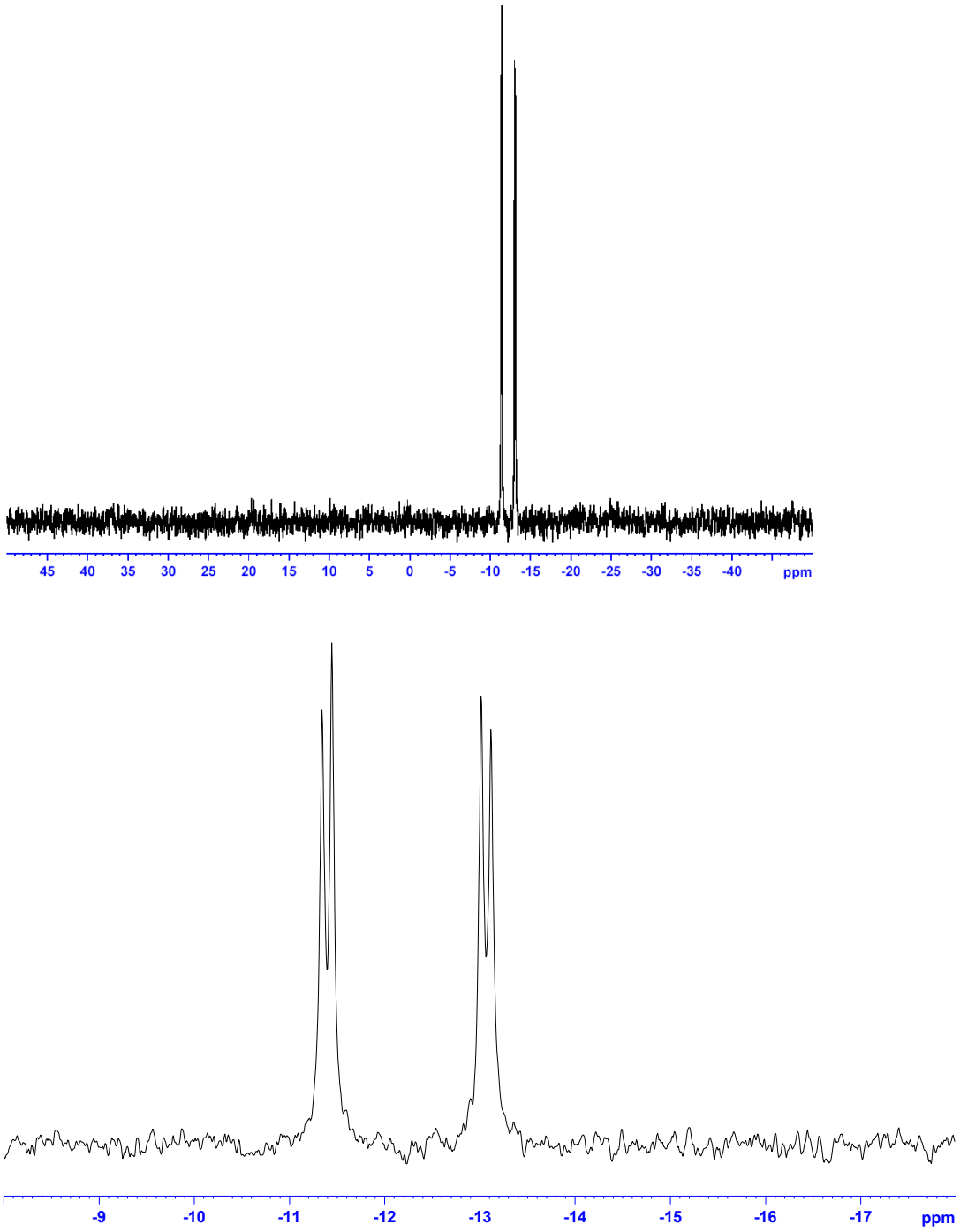
^{1}H NMR (D_2O. 500 mHz) of **19:**



HSQC (D_2O) of **19**:



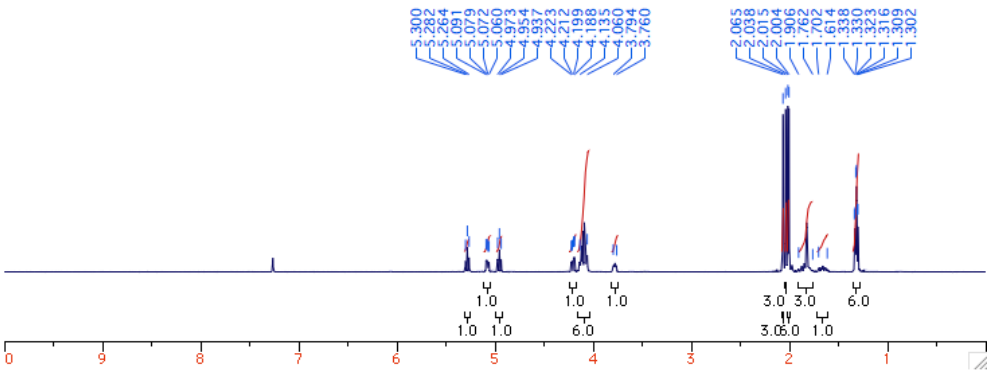
^{31}P $\{^{1}H\}$ NMR (D_2O, 202 mHz) of **19**:



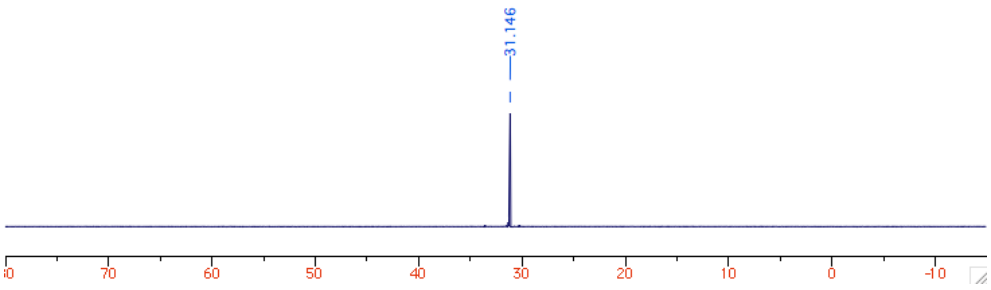
NMR data for synthetic compounds

*Diethyl-2-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl)-ethylphosphonate (**14**)*

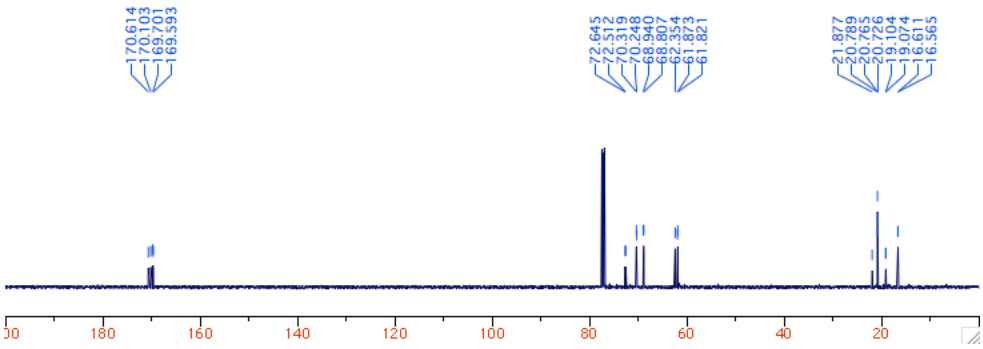
^{1}H NMR ($CDCl_3$, 500 MHz)



^{31}P {^{1}H } NMR ($CDCl_3$, 202 MHz)

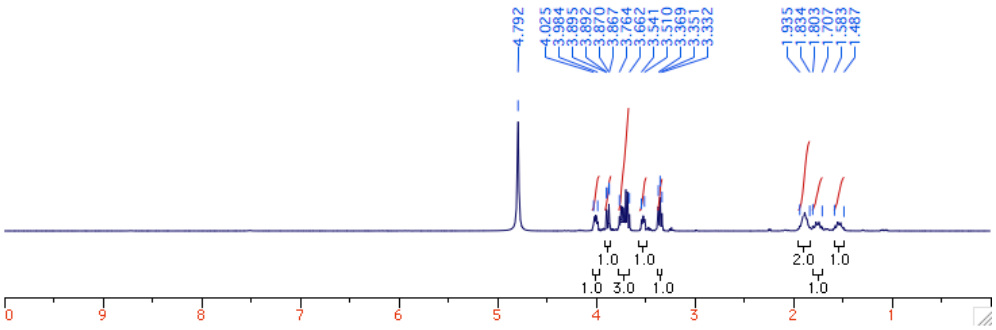


^{13}C NMR ($CDCl_3$, 125 MHz)

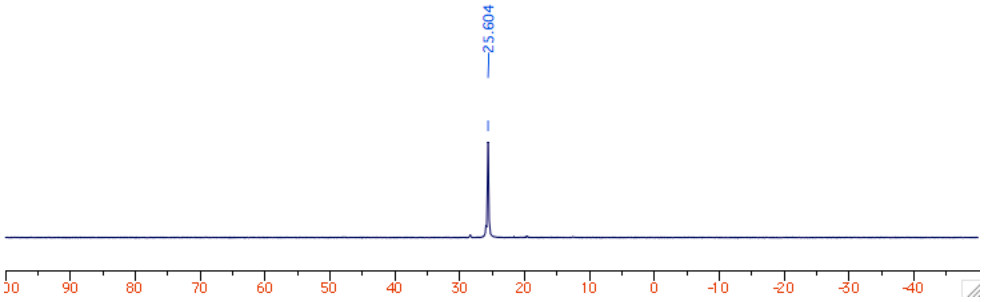


*Bis(ammonium)-2-(α-D-glucopyranosyl)-ethylphosphonate (**6**)*

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



^{31}P {^{1}H } NMR (D_2O, 202 MHz):



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