The Transition-State in DNA Polymerase β Catalysis: Rate-limiting Chemistry Altered by Base-Pair Configuration

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SUPPORTING INFORMATION

DNA synthesis, purification, radiolabeling, and annealing

Primer (5´-TAT TAC CGC GCT GAT GCG C), template, (5´-GCG TTG TTC CGA C**M**G CGC ATC AGC GCG GTA ATA, **M** = C or T for dGTP analogs; **M** = A or G for dTTP analogs), and 5´-phosphorylated downstream (5´-GTC GGA ACA ACG C) oligomers were synthesized on a solid phase DNA synthesizer and purified by 16% denaturing polyacrylamide gel electrophoresis, followed by desalting using oligonucleotide purification cartridges. Radiolabeling reactions consisted of 1 mol equiv primer was 5´-end labeled with 0.4 U/μL T4 polynucleotide kinase and 0.7 mol equiv [γ-^{32}P]ATP with the supplied buffer at 37 °C for 30 minutes, followed by heat inactivation at 95 °C for 10 minutes. The primer was purified by size exclusion chromatography using a Bio-Spin 6 column, then annealed by mixing with 1.2 mol equiv template and 1.5 mol equiv downstream oligomers to yield a 1 nt gapped DNA substrate. The mixture was heated to 95 °C and cooled slowly to room temperature.

Buffer and protein preparation

The reaction buffer consisted of 50 mM Tris-Cl, 20 mM KCl, 20 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, and 6% glycerol at pH 8.0. Wild type pol β was purified as previously reported[1].

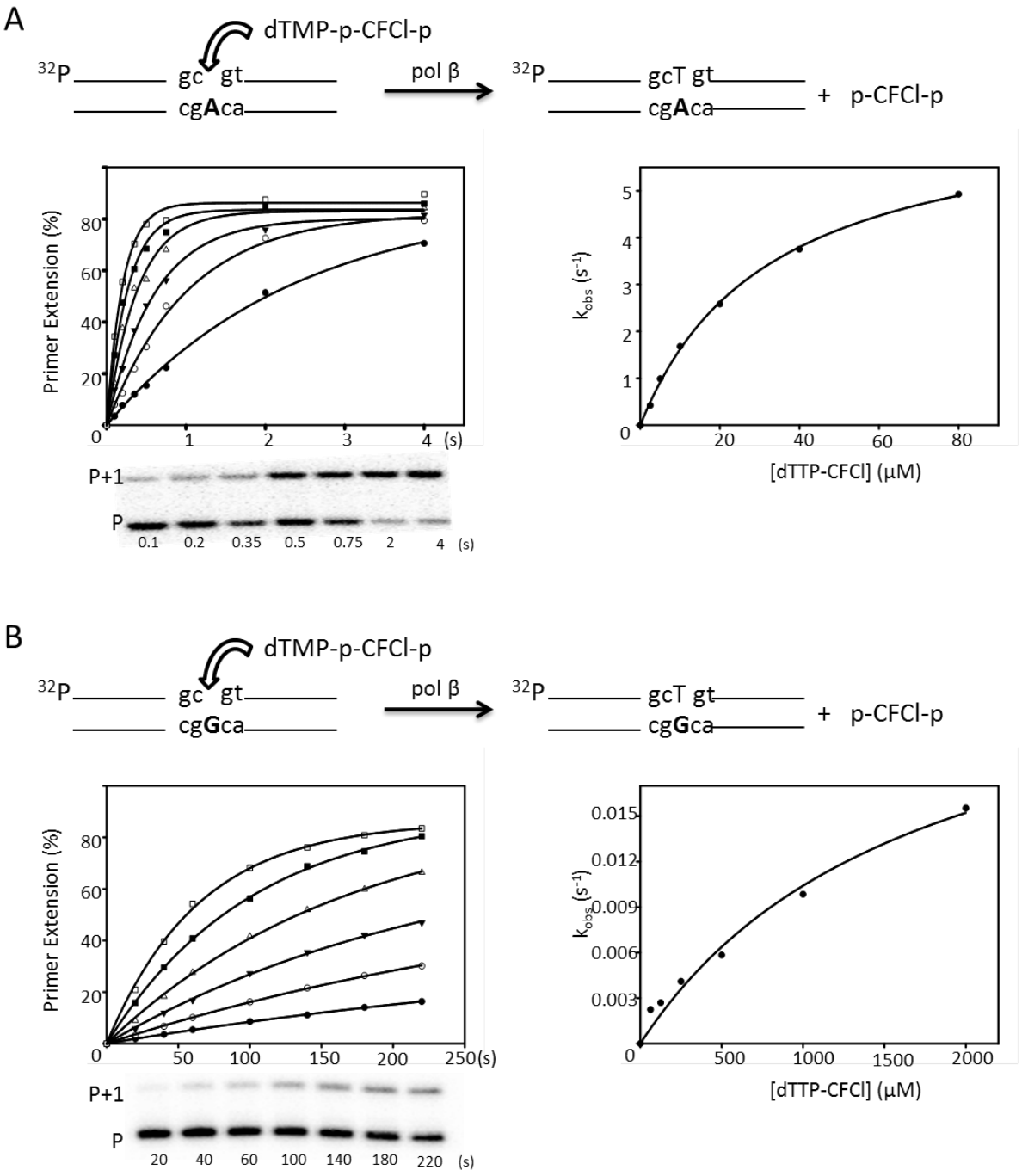

Figure S1. Presteady state incorporation of β,γ-CFCl-dTTP (**8b**) into single-gapped DNA. (A) Correct incorporation opposite dA. The cartoon shows the generalized scheme for the reaction. A plot of the percentage of primer extension vs. time for six concentrations of the analog is shown on the left for one of three repeats of each reaction (2.5 μM, filled circles; 5 μM, open circles; 10 μM, filled triangles; 20

μM, open triangles; 40 μM, filled squares; 80 μM, open squares). Below the plot is a time course shown on a representative gel for the first concentration (2.5 μM), where P represents the unextended primer and P+1 the extension of the primer by a single incorporation of dTMP from β,γ-CFCl-dTTP (**8b**). The corresponding reaction time is shown below each lane. To the right is a plot of the observed rate constant vs. the corresponding analog concentration. (B) Misincorporation opposite dG of β,γ-CFCl-dTTP (**8b**). As for the correct pairing, the percentage of primer extension vs. time for each concentration of analog is shown on the left (62.5 μM, filled circles; 125 μM, open circles; 250 μM, filled triangles; 500 μM, open triangles; 1000 μM, filled squares; 2000 μM, open squares) with a representative gel; the corresponding reaction time is shown below each lane. The observed rate constant is plotted vs. the corresponding analog concentration (right plot). The reactions were carried out in triplicate and have an SEM of ± 15%; the figure shows a representative data set.

-CXY-	pK_{a4}	N•M	k_{pol} (s^{-1})	K_d (μM)		N•M	k_{pol} (s^{-1})	K_d (μM)
CF_2 (**3a**)	7.8	G•C	21.9 ± 0.9	2.9 ± 0.3		G•T	0.76 ± 0.01	380 ± 50
CFCl (**8a**)	8.4	G•C	10.6 ± 1.6	9.6 ± 2.9		G•T	0.17 ± 0.01	660 ± 40
CCl_2 (**5a**)	8.8	G•C	8.4 ± 1.0	3.7 ± 1.3		G•T	0.05 ± 0.00	800 ± 70
O	8.9	G•C	14.9 ± 2.3	1.7 ± 0.7		G•T	1.34 ± 0.10	200 ± 7
CHF (**2a**)	9.0	G•C	11.6 ± 1.5	10.1 ± 1.1		G•T	1.34 ± 0.07	1300 ± 100
CBr_2 (**7a**)	9.3	G•C	3.1 ± 0.2	11.4 ± 1.4		G•T	0.02 ± 0.01	500 ± 200
CHN_3 (**12a**)	9.3	G•C	8.1 ± 0.6	5.3 ± 0.1		G•T	0.34 ± 0.05	540 ± 90
CHCl (**4a**)	9.5	G•C	7.6 ± 1.1	16.4 ± 7.0		G•T	0.22 ± 0.03	630 ± 160
CHBr (**6a**)	9.9	G•C	6.9 ± 0.8	2.1 ± 0.2		G•T	0.14 ± 0.02	470 ± 130
$CFCH_3$ (**11a**)	10.2	G•C	3.1 ± 0.2	1.0 ± 0.2		G•T	0.22 ± 0.02	110 ± 10
CH_2 (**1a**)	10.5	G•C	1.7 ± 0.2	0.9 ± 0.2		G•T	0.12 ± 0.03	470 ± 110
CCH_3N_3 (**13a**)	10.6	G•C	2.1 ± 0.1	55 ± 7		G•T	0.042 ± 0.007	3300 ± 1000
$CHCH_3$ (**9a**)	11.6	G•C	0.28 ± 0.01	0.87 ± 0.12		G•T	0.012 ± 0.001	750 ± 180
$C(CH_3)_2$ (**10a**)	12.3	G•C	0.30 ± 0.09	2.8 ± 1.0		G•T	0.0042 ± 0.0002	1050 ± 70

Table S1. Kinetic parameters for the entire β,γ-CXY-dGTP series. For each analog, the k_{pol} and K_d data are given as the average ± standard deviation of at least three replicates for both the correct pairing (N•M = G•C, left side) and mispairing (N•M = G•T, right side). Analogs with substituents that are not the same (X ≠ Y) were used as *R*/*S* mixtures.

-CXY-	pK_{a4}	N•M	k_{pol} (s^{-1})	K_d (μM)		N•M	k_{pol} (s^{-1})	K_d (μM)
CF_2 (**3b**)	7.8	T•A	18.1 ± 1.0	15.2 ± 2.0		T•G	0.088 ± 0.018	370 ± 190
CFCl (**8b**)	8.4	T•A	5.6 ± 0.6	33.0 ± 0.6		T•G	0.024 ± 0.003	1300 ± 230
CCl_2 (**5b**)	8.8	T•A	2.0 ± 0.3	98 ± 15		T•G	0.00082 ± 0.00002	170 ± 50
O	8.9	T•A	20.2 ± 1.1	8.2 ± 2.6		T•G	0.55 ± 0.5	910 ± 130
CHF (**2b**)	9.0	T•A	23.8 ± 1.8	19.1 ± 3.7		T•G	0.27 ± 0.05	360 ± 90
CBr_2 (**7b**)	9.3	T•A	0.42 ± 0.09	180 ± 60		T•G	n.d.	n.d.
CHCl (**4b**)	9.5	T•A	9.1 ± 1.6	31.7 ± 5.0		T•G	0.092 ± 0.009	1100 ± 100
CHBr (**6b**)	9.9	T•A	7.1 ± 0.9	19.2 ± 2.0		T•G	0.028 ± 0.005	500 ± 170
CH_2 (**1b**)	10.5	T•A	2.0 ± 0.0	8.3 ± 1.9		T•G	0.014 ± 0.001	310 ± 30

Table S2. Kinetic parameters for the β,γ-CXY-dTTP series. For each analog, the k_{pol} and K_d data are given as the average ± standard deviation of at least three replicates for both the correct pairing (N•M = T•A, left side) and mispairing (N•M = T•G, right side). Analogs with non-equivalent substituents (X ≠ Y) were used as *R*/*S* mixtures.

Synthetic Procedures

2'-Deoxythymidine 5'-phosphoromorpholidate, **14b**. Procedure adapted from the original synthesis of nucleoside 5'-phosphoromorpholidates[2,3]. Thymidine 5'-monophosphate, acid form (200 mg, 0.621 mmol), was dissolved in 7 mL *t*-BuOH:H_2O 1:1. Morpholine (162 mg, 1.86 mmol) was added to the solution which was set to reflux. DCC (384 mg, 1.86 mmol) was dissolved in *t*-BuOH 4 mL and 1/8 of this solution was added drop-wise to the reaction mixture every 15 min for 2 h. After 2.5 h, the reaction was complete (single peak in the ^{31}P NMR spectrum). The solvent was then removed and the resulting oil dissolved in water. The solution was filtered and

water was removed under reduced pressure to yield **14b** as an amorphous yellow solid which was used without further purification. ^{31}P (D_2O, pH neutral, 162 MHz): δ 8.1 (s).

2'-Deoxycytosine 5'-phosphoromorpholidate, **14c**. dCMP (0.285 mmol, 87.5 mg) was treated following the procedure for the synthesis of **14b** to afford compound **14c**. (95% by ^{31}P NMR): ^{31}P NMR (202 MHz; D_2O, pH 7.0): δ 7.9 (s).

General coupling procedure. As previously reported for β,γ–CXY dGTP analogues[4-7], the phosphoromorpholidate, **14b, 14c**, (1 equiv, ~100 mg) was dissolved in 2 mL anhydrous DMSO. Tri-*n*-butyl ammonium bisphosphonic acid (3 equiv) **1e-8e** was dissolved in anhydrous DMSO and added by syringe at rt. After 48 h, volatiles were removed on the rotovap and the reaction mixture was dissolved in 0.5 N TEAB buffer, pH 7.5. The solution was then purified by dual-pass preparative HPLC (SAX then RP) to yield the final compounds (~20%). Analytical SAX HPLC analysis demonstrated that their purity was ≥ 99% (UV detection). Retention times for **1b**–**8b** are presented in Table **S3**.

Compound	RT (min)
1b (CH_2)	9.1
2b (CHF)	10.5
3b (CF_2)	11.8
4b (CHCl)	9.8
5b (CCl_2)	9.9
6b (CHBr)	9.4
7b (CBr_2)	9.8

Table S3. Analytical SAX HPLC retention times.

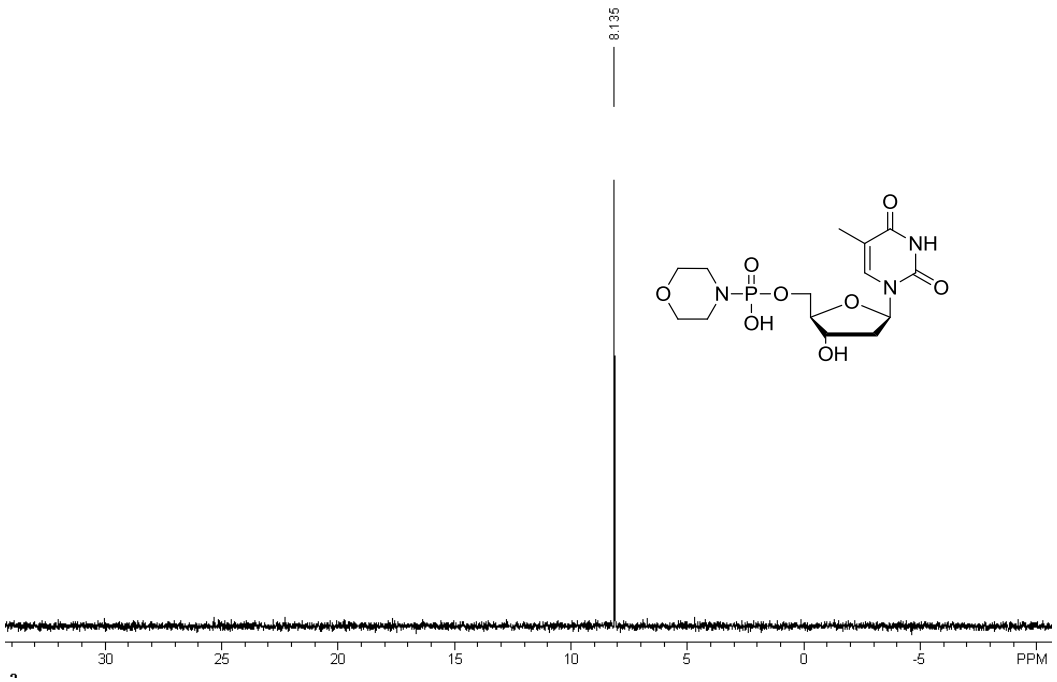

Figure S2. ^{31}P {^{1}H} NMR spectrum (202 MHz, D_2O, pH 8) of thymidine 5'-phosphoromorpholidate, **14b**.

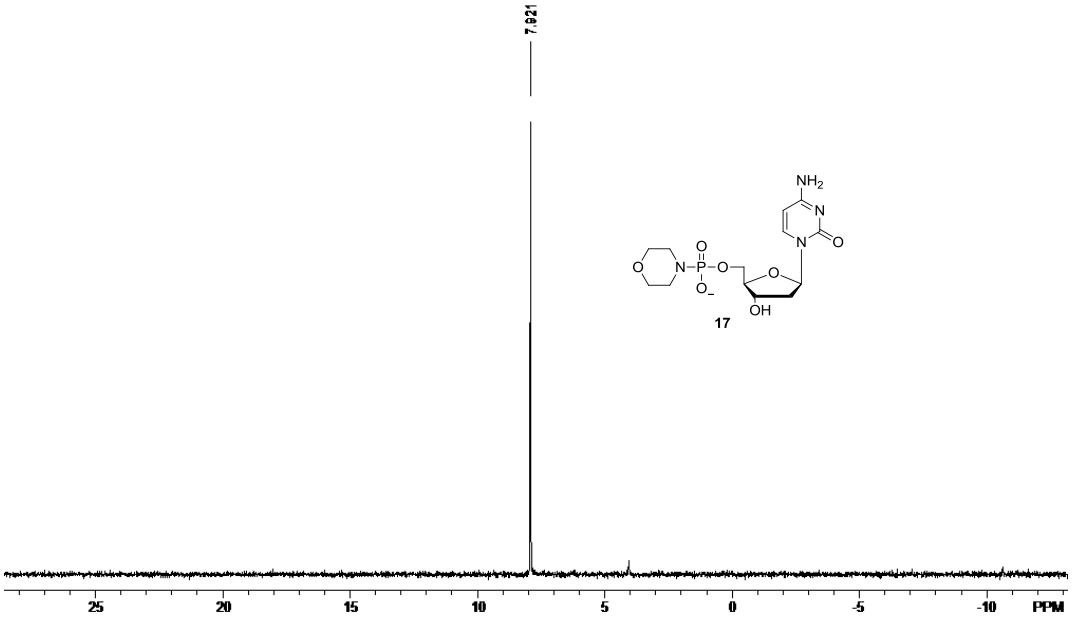

Figure S3. ^{31}P {^{1}H} spectrum (202 MHz, D_2O, pH 7.0) of dCMP-morpholidate, **14c**.

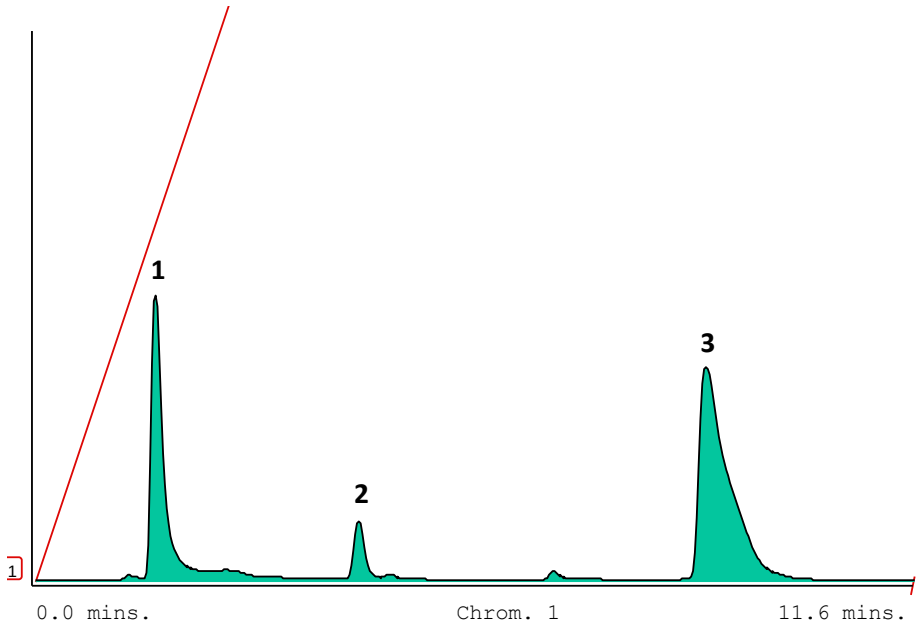

Figure S4. Representative HPLC trace. Shown: reaction of **6e** with **14b** after 48 h. Peak 1 = **14b,** peak 2 = TMP**,** peak 3 = **6b**. Conditions: Analytical HPLC analysis was conducted on a Varian PureGel SAX 10 mm x 100 mm 7 µL column eluted with A: H_2O, B: 0.5 M (0-50% linear) LiCl gradient over 30 min at a 4 mL/min flow rate.

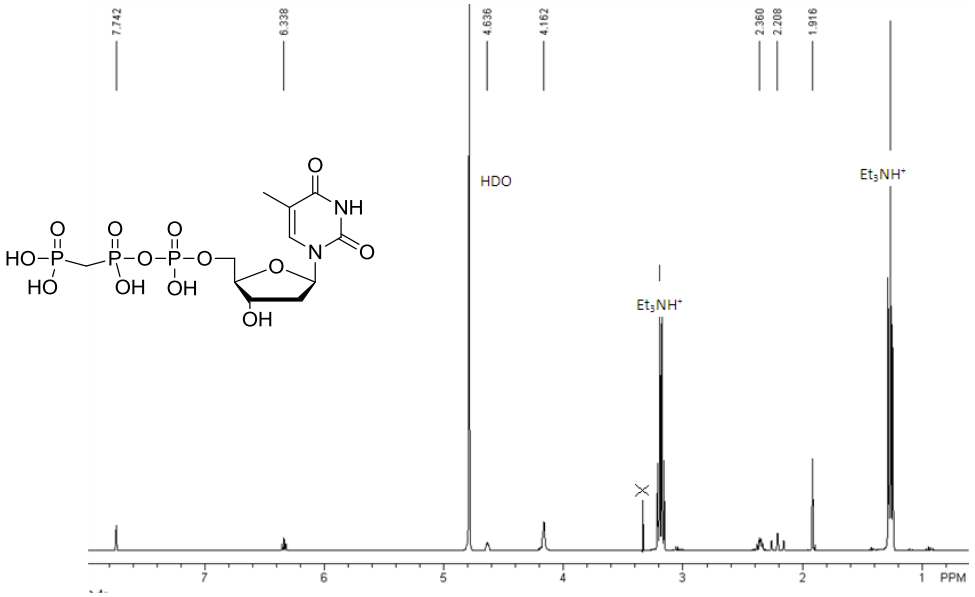

Figure S5. ^{1}H NMR spectrum (400 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CH_2, **1b**.
Note: products obtained as the triethylammonium salts**.**

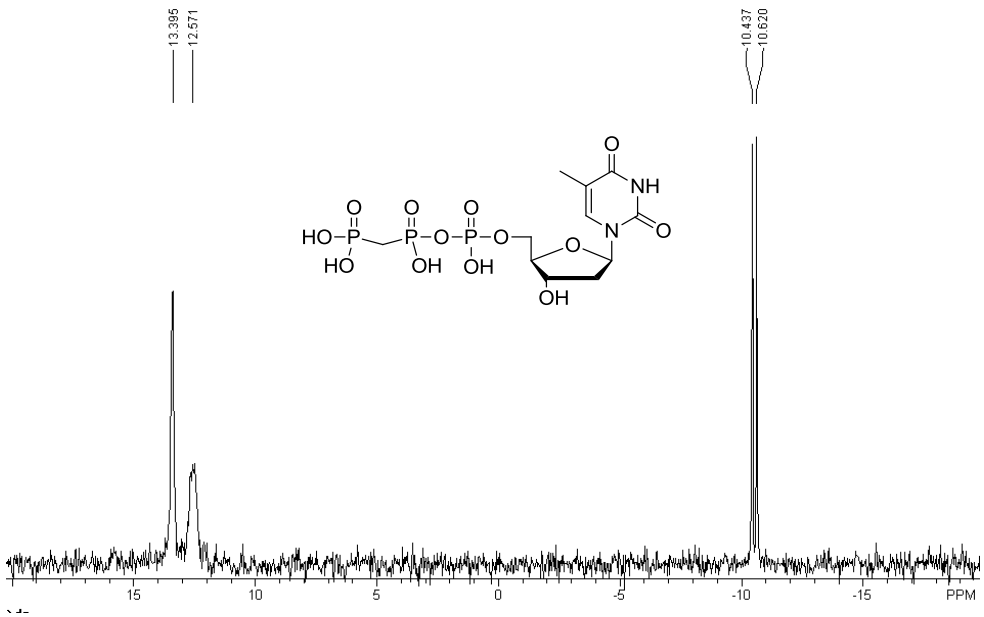

Figure S6. ^{31}P $\{^{1}H\}$ NMR spectrum (162 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CH_2, **1b.**

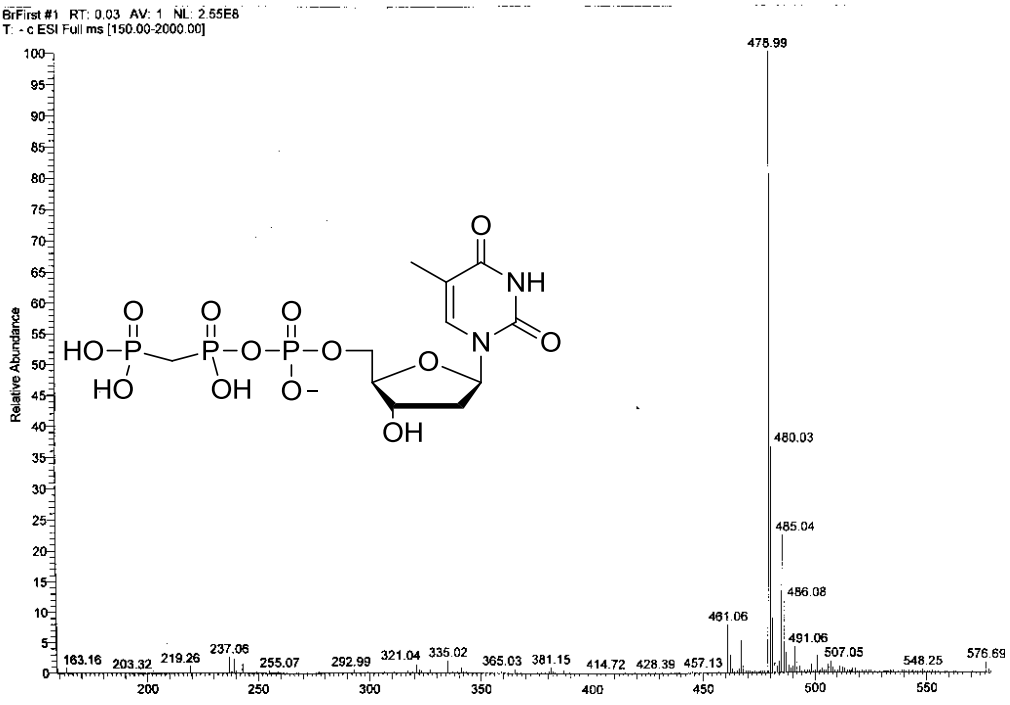

Figure S7. LRMS $[M-H]^-$ of thymidine 5'-triphosphate β,γ-CH_2, **1b.** Calc for $C_{12}H_{20}N_2O_{13}P_3$: 479.0; found: 479.0.

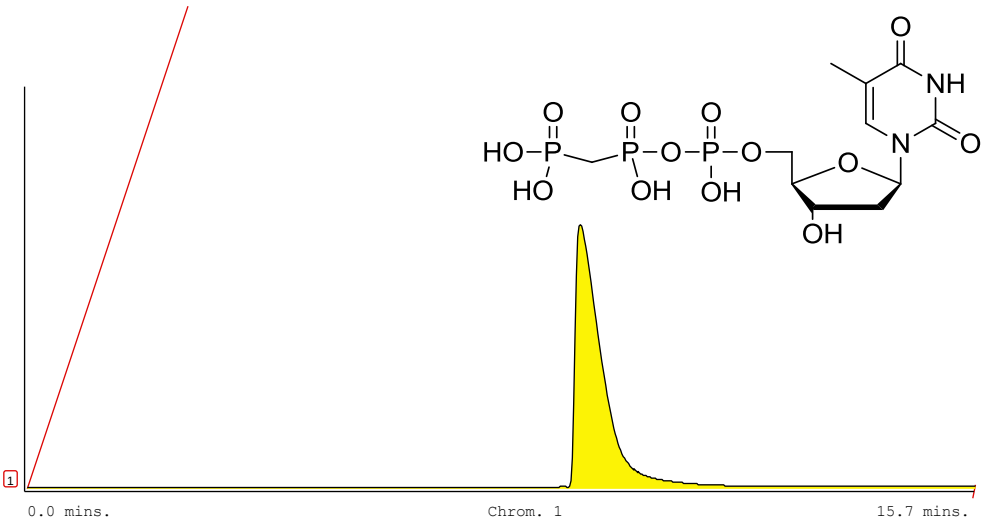

Figure S8. Analytical SAX HPLC trace for thymidine 5'-triphosphate β,γ-CH_2, **1b**. RT = 9.1 min.

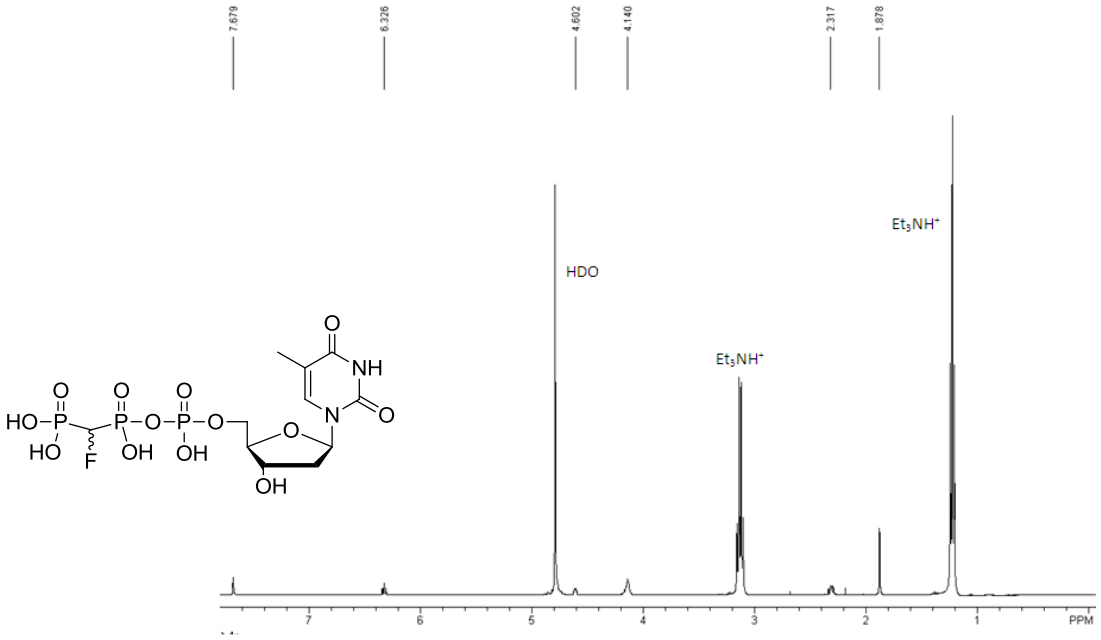

Figure S9. ^{1}H NMR spectrum (400 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CHF, **2b.**

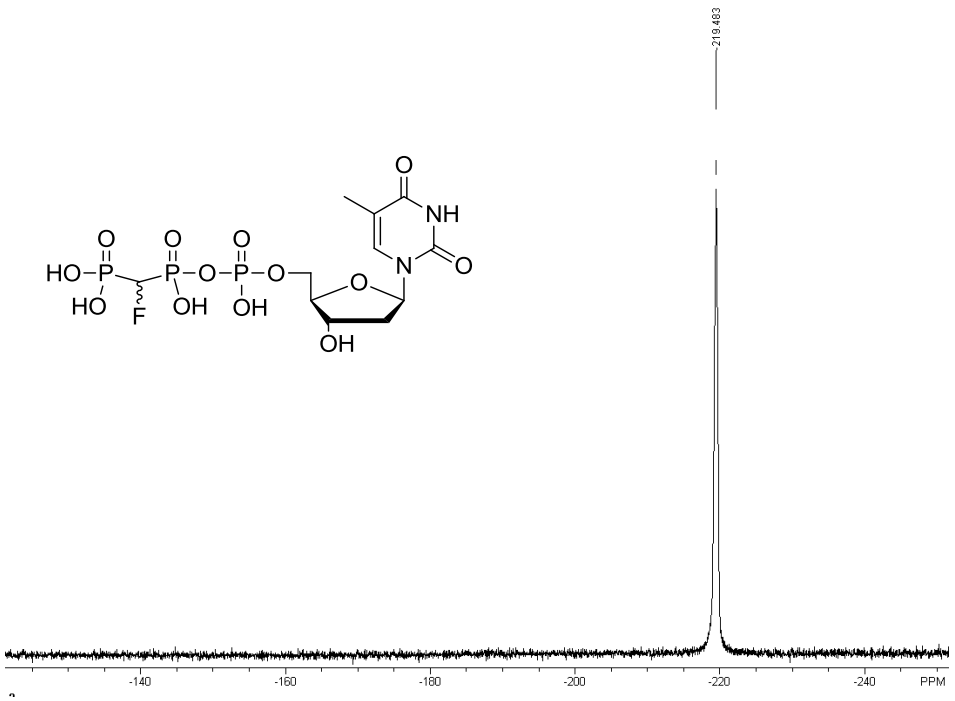

Figure S10. ^{19}F NMR spectrum (376 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CHF, **2b.**

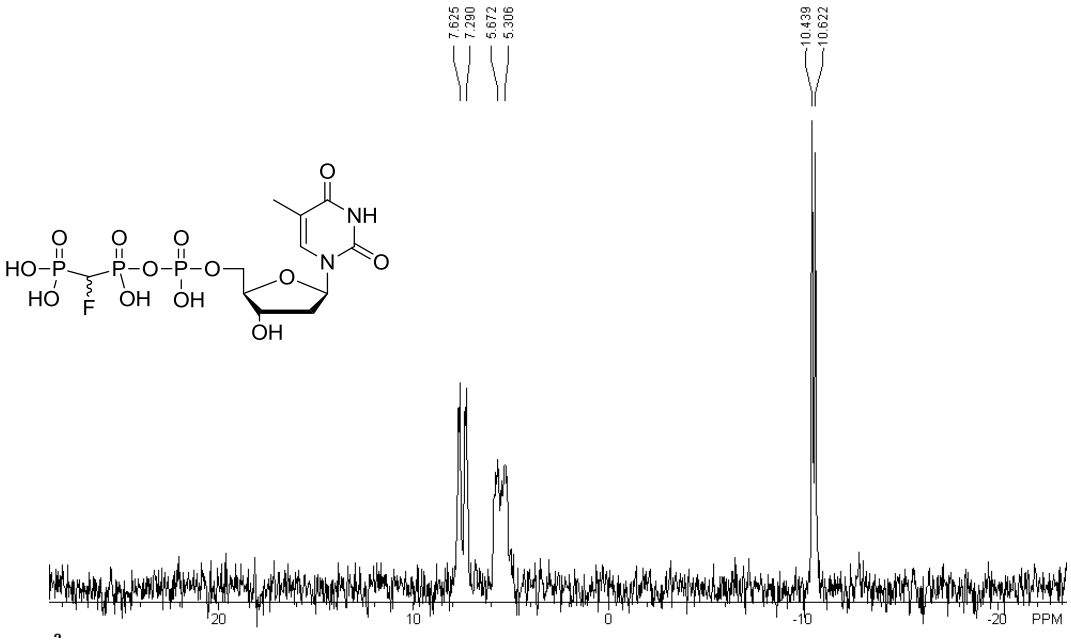

Figure S11. ^{31}P $\{^1H\}$ NMR spectrum (162 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CHF, **2b.**

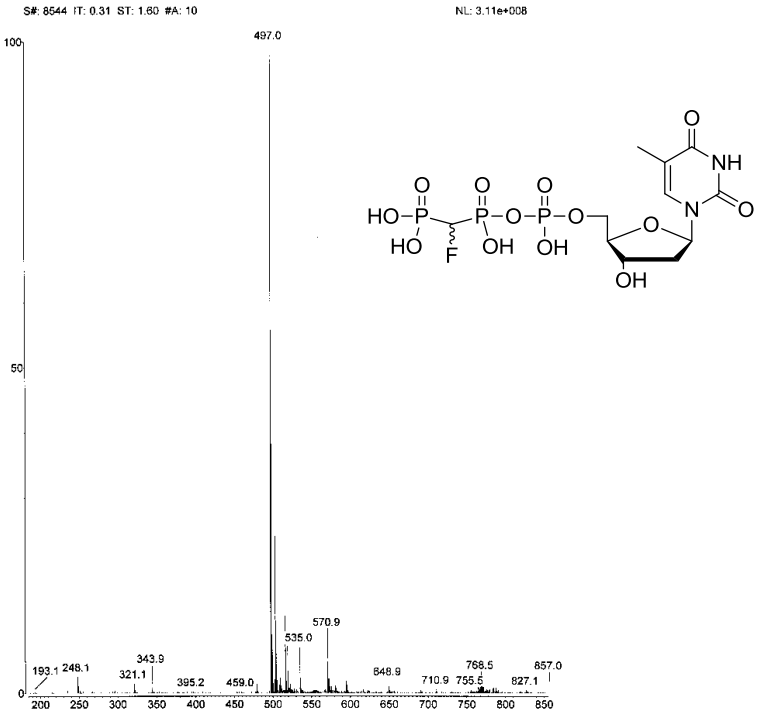

Figure S12. LRMS $[M-H]^-$ of thymidine 5'-triphosphate β,γ-CHF, **2b.** Calc for $C_{11}H_{17}FN_2O_{13}P_3$: 497.0; found 497.0.

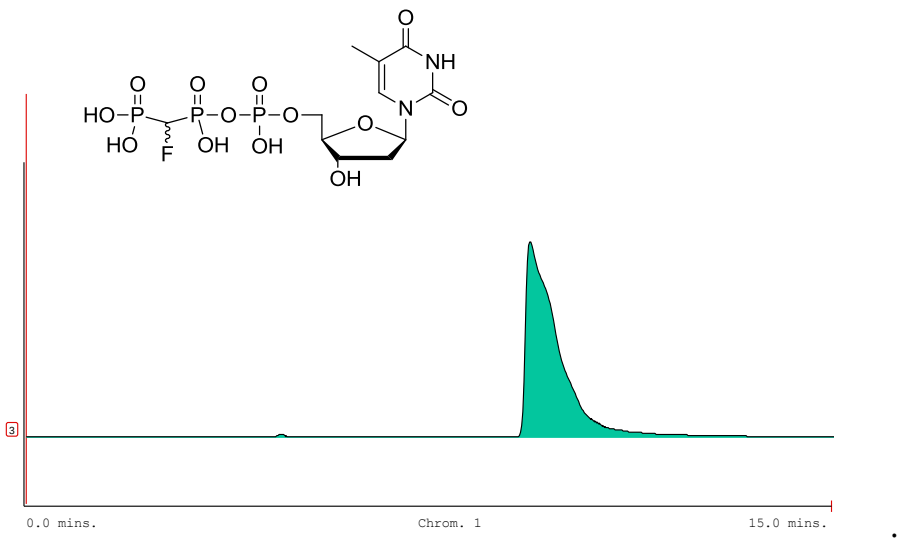

Figure S13. Analytical SAX HPLC trace for thymidine 5'-triphosphate β,γ-CHF, **2b.** RT = 10.5 min.

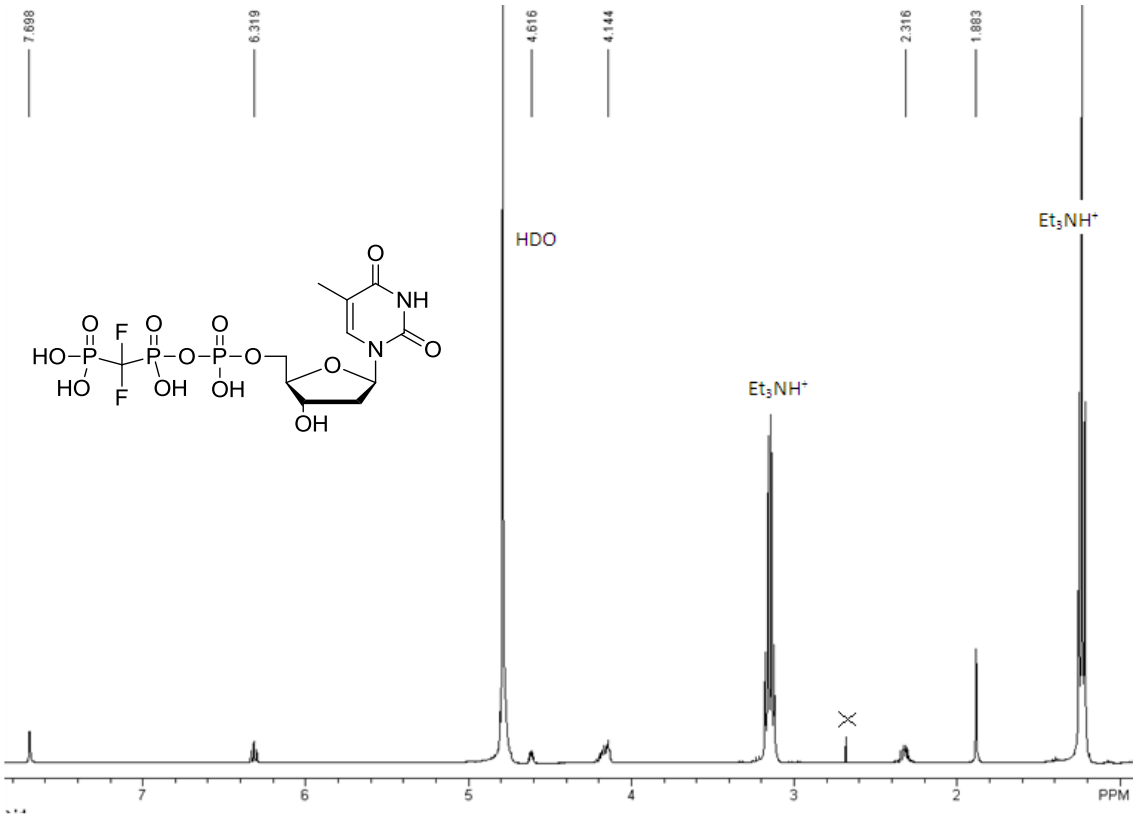

Figure S14. ^{1}H NMR spectrum (400 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CF_2, **3b**.

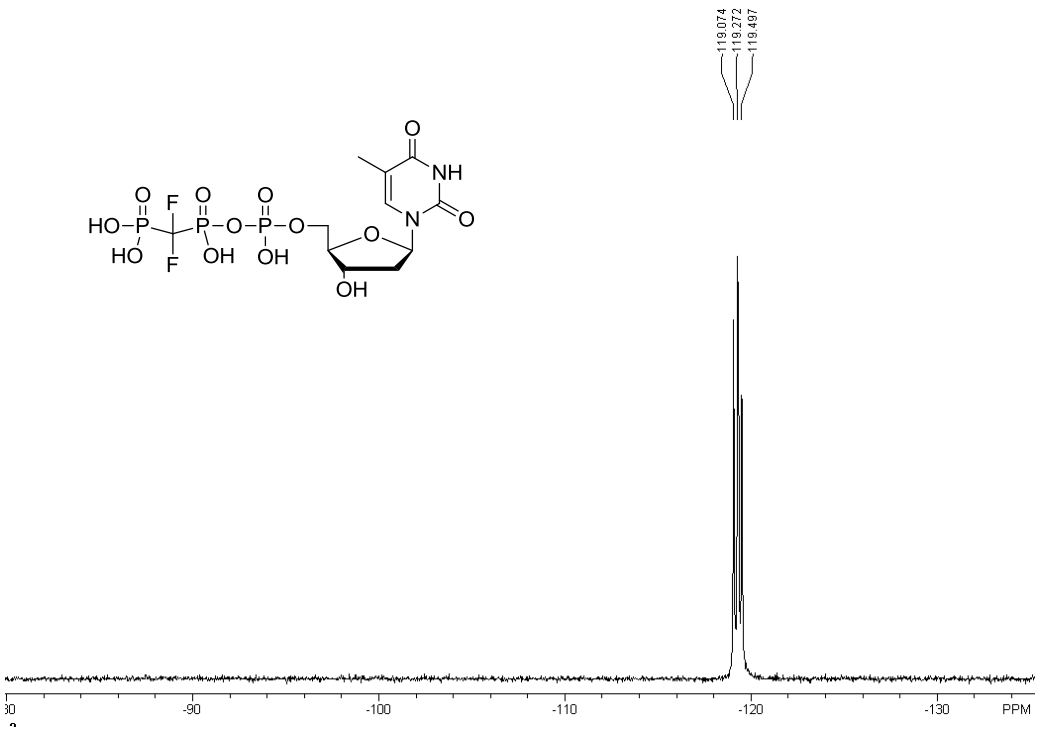

Figure S15. ^{19}F NMR spectrum (376 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CF_2, **3b.**

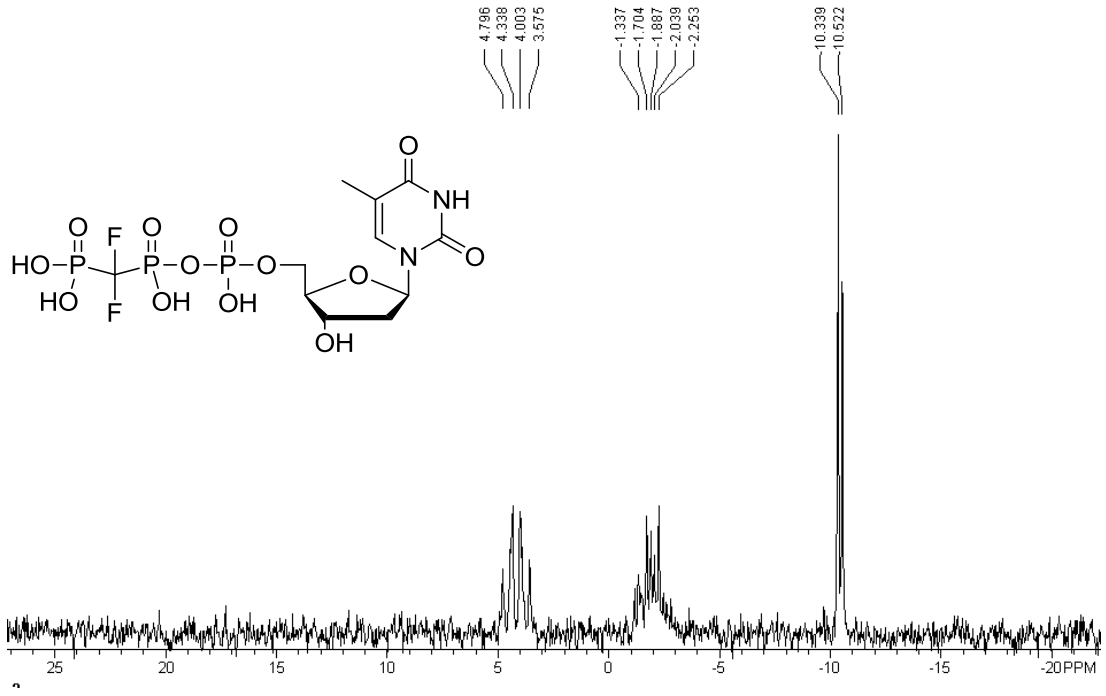

Figure S16. ^{31}P $\{^1H\}$ NMR spectrum (162 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CF_2, **3b.**

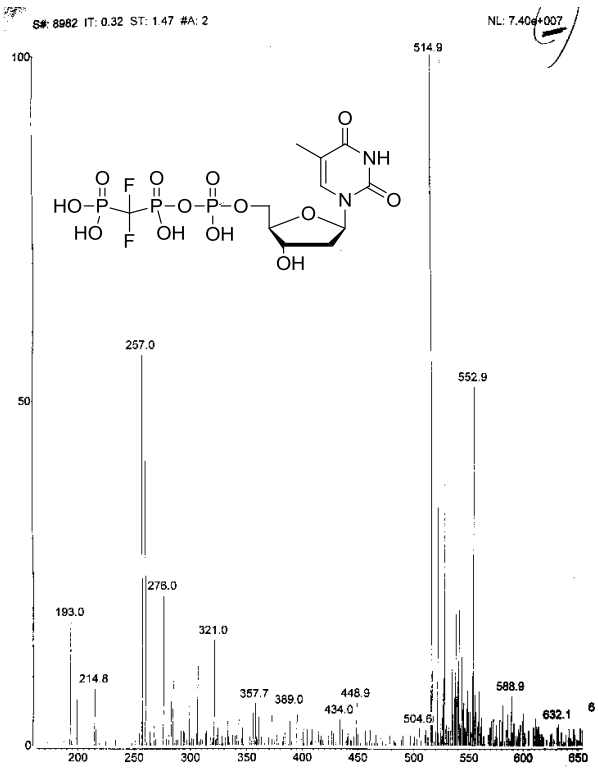

Figure S17. LRMS $[M-H]^-$ of thymidine 5'-triphosphate β,γ-CF_2, **3b.** Calc for $C_{11}H_{17}F_2N_2O_{13}P_3$: 515.0; found 514.9.

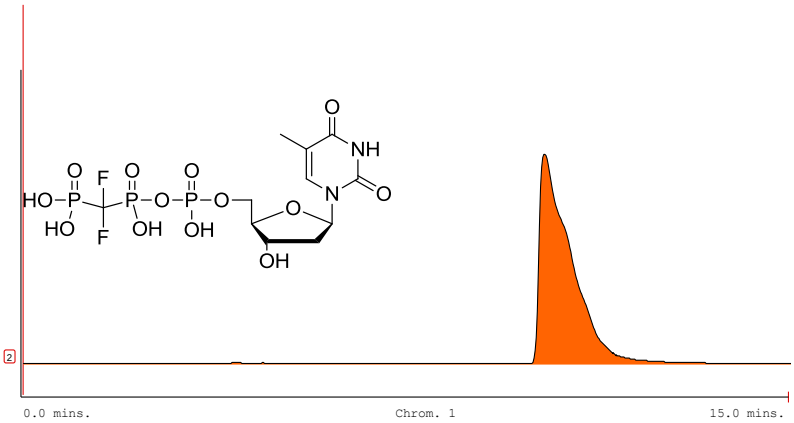

Figure S18. Analytical SAX HPLC trace for thymidine 5'-triphosphate β,γ-CF_2, **3b.** RT = 11.8 min.

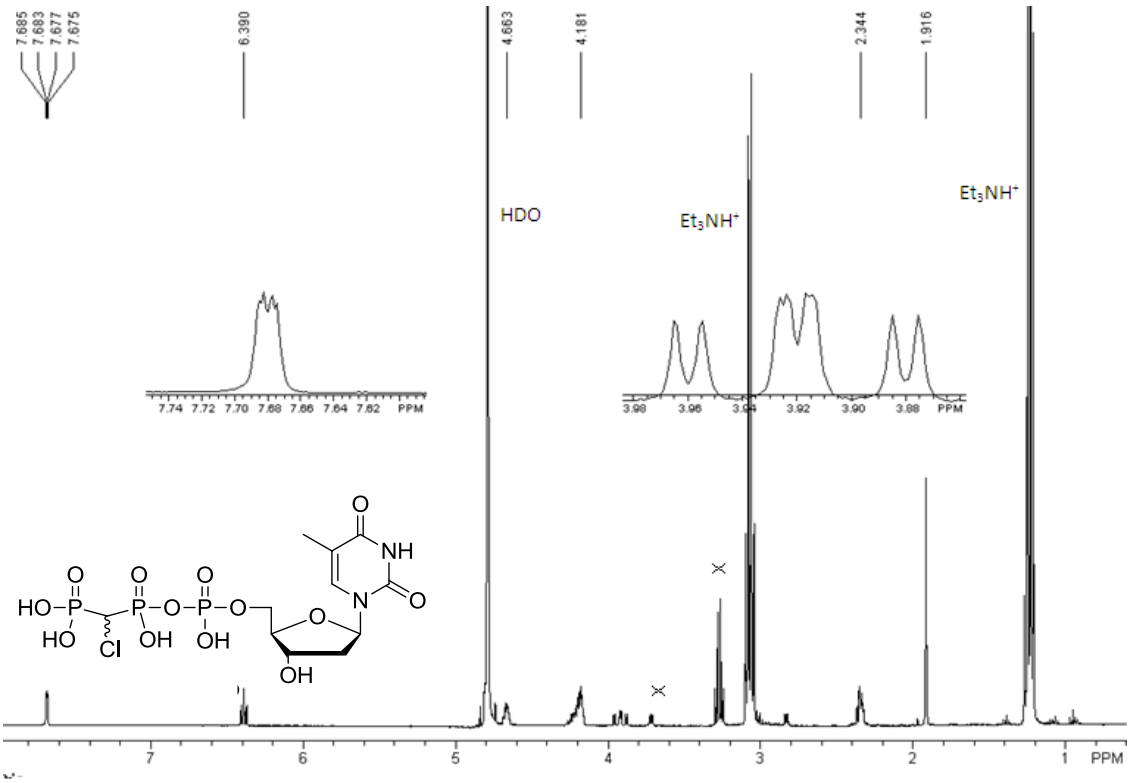

Figure S19. ^{1}H NMR spectrum (400 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CHCl, **4b.**

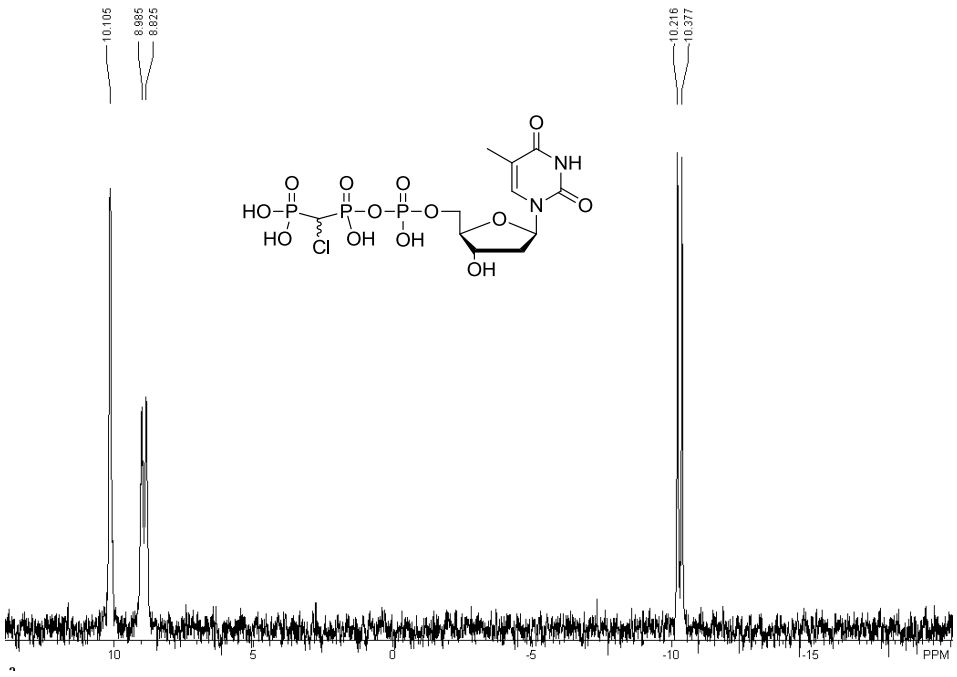

Figure S20. ^{31}P {^{1}H} NMR spectrum (162 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CHCl, **4b.**

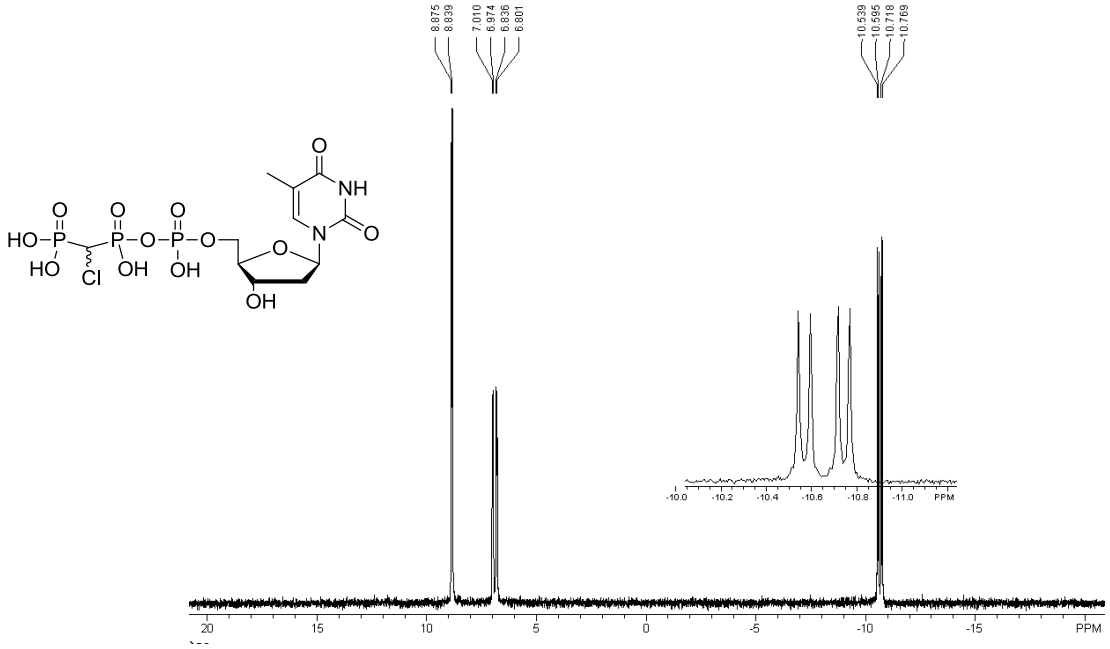

Figure S21. ^{31}P {^{1}H} NMR spectrum (162 MHz, D_2O, pH >10) of thymidine 5'-triphosphate β,γ-CHCl after treatment with chelex, **4b.**

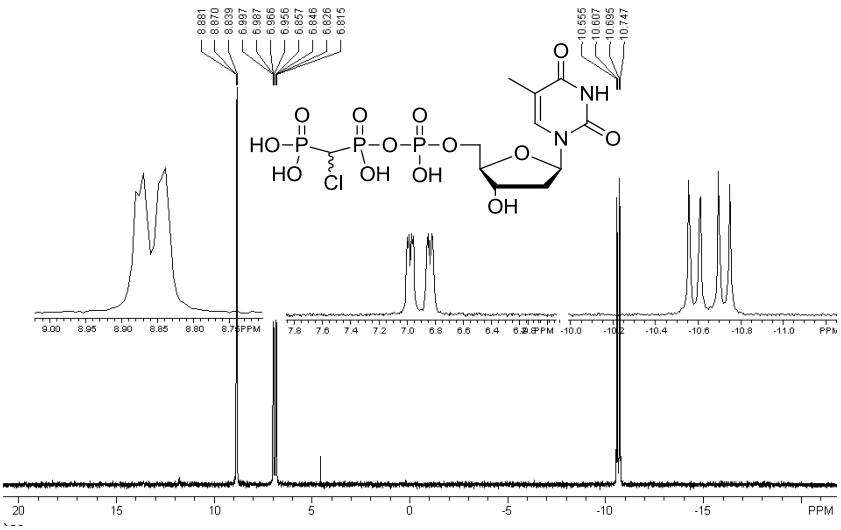

Figure S22. ^{31}P {^{1}H} NMR spectrum (202 MHz, D_2O, pH >10) of thymidine 5'-triphosphate β,γ-CHCl after treatment with chelex, **4b.**

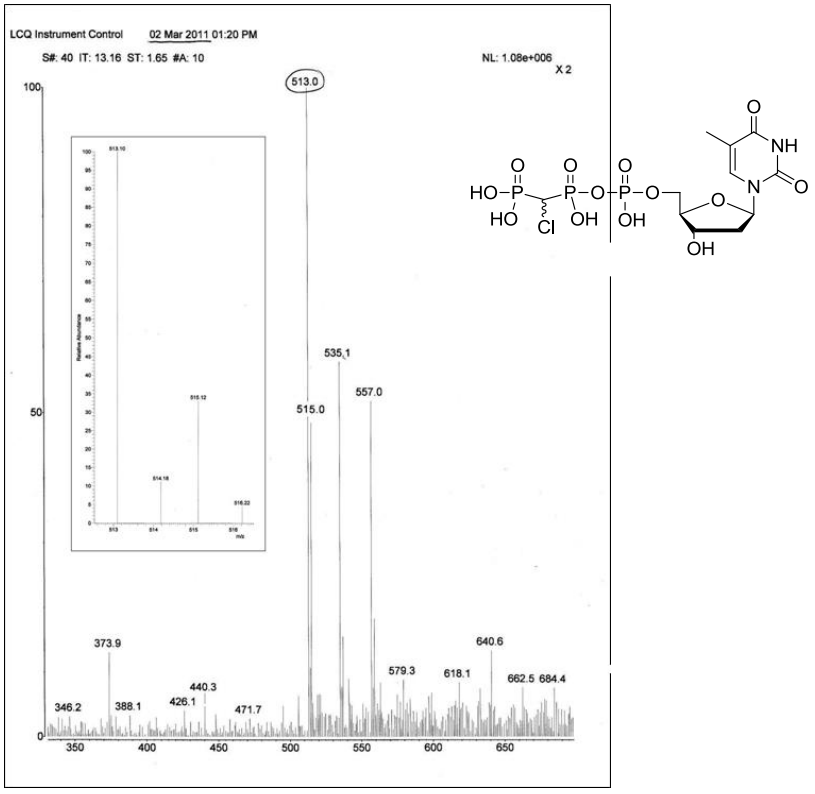

Figure S23. LRMS $[M-H]^-$ of thymidine 5'-triphosphate β,γ-CHCl, **4b**. Calc for $C_{11}H_{17}ClN_2O_{13}P_3$: 513.0 (100%), 515.0 (32%); found 513.0 (100%), 515.0 (33%). *Inset*: $[M-H]^-$ isotopic pattern confirming the presence of Cl.

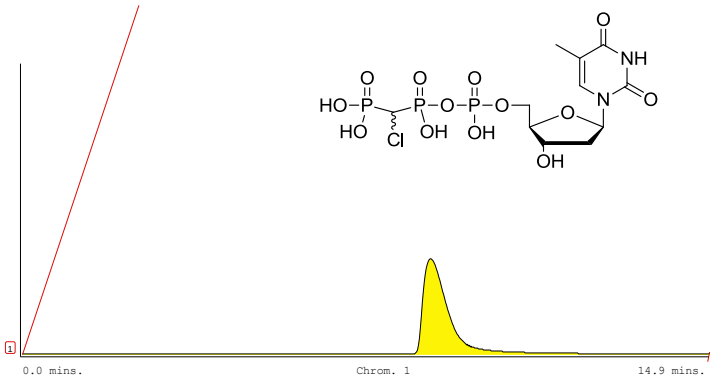

Figure S24. Analytical SAX HPLC trace for thymidine 5'-triphosphate β,γ-CHCl, **4b.** RT = 9.8 min.

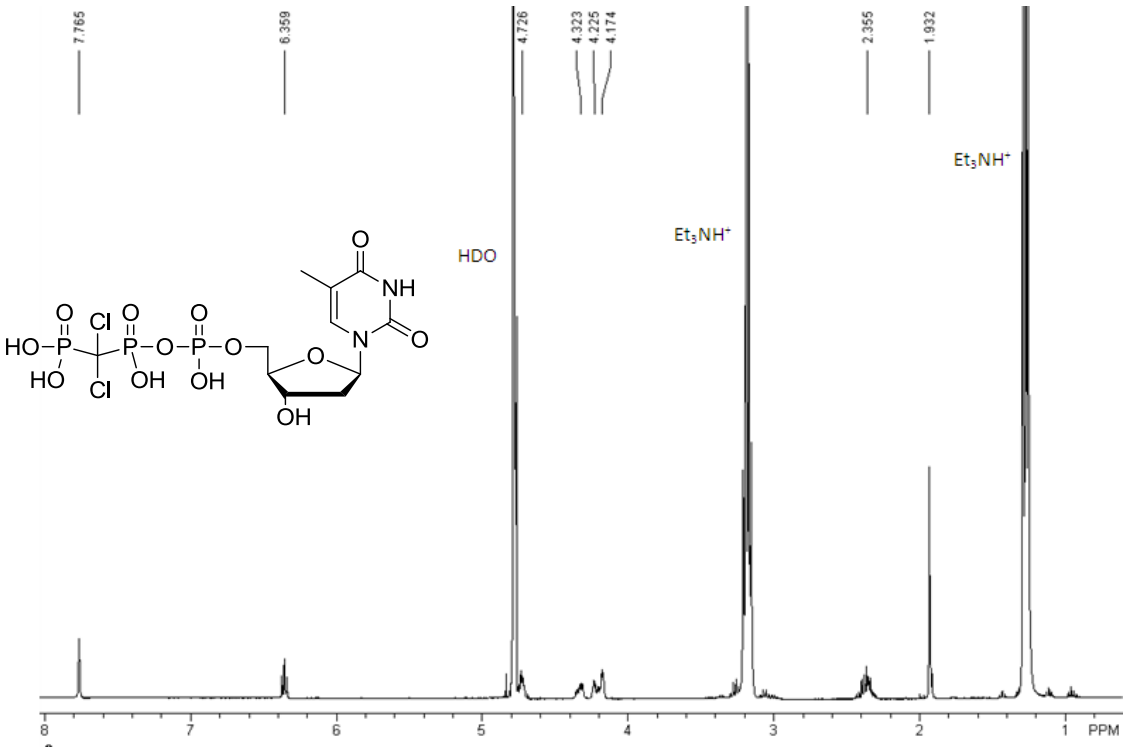

Figure S25. ^{1}H NMR spectrum (400 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CCl_2, **5b.**

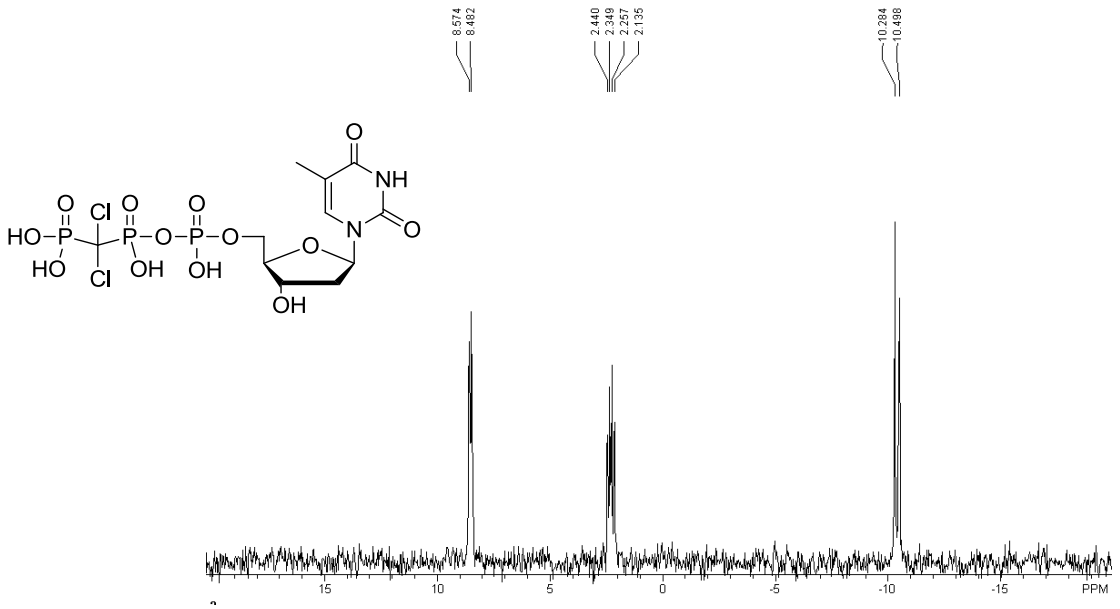

Figure S26. ^{31}P {^{1}H} NMR spectrum (162 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CCl_2, **5b.**

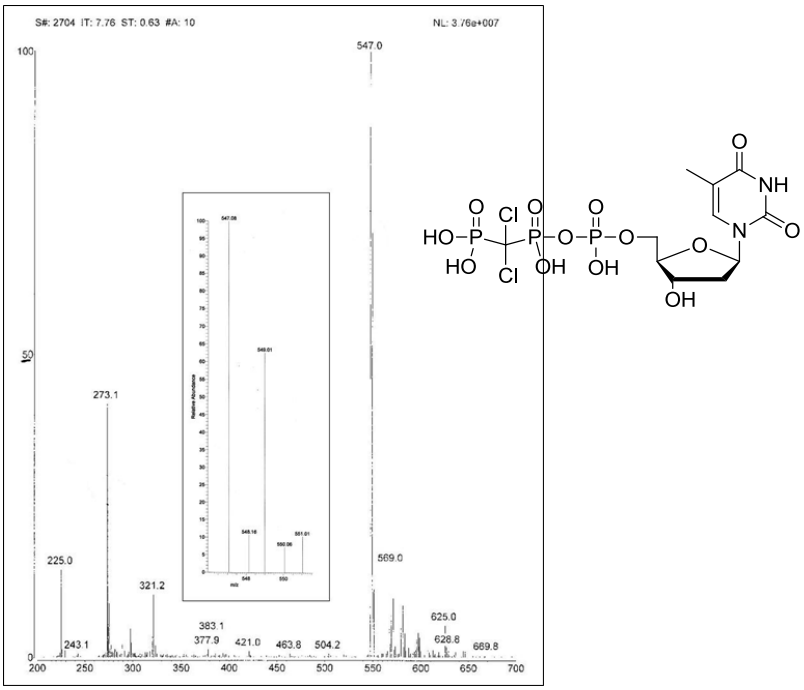

Figure S27. LRMS $[M-H]^-$ of thymidine 5'-triphosphate β,γ-CCl_2, **5b.** Calc for $C_{11}H_{16}Cl_2N_2O_{13}P_3$: 546.9 (100%), 548.9 (64%) ; found: 547.0 (100%), 549.0 (63%). *Inset*: $[M-H]^-$ isotopic pattern confirming the presence of Cl.

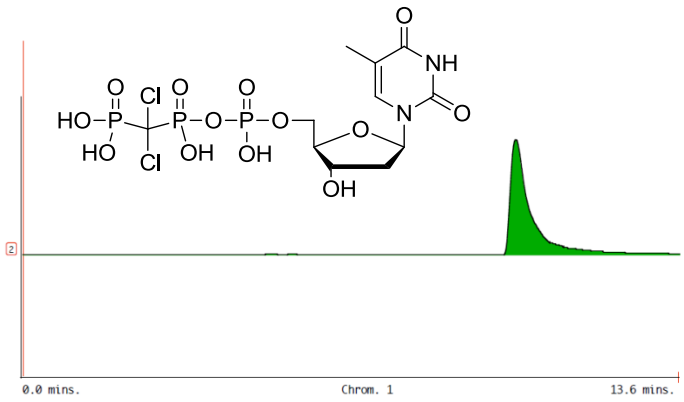

Figure S28. Analytical SAX HPLC trace for thymidine 5'-triphosphate β,γ-CCl_2, **5b**. RT = 9.9 min.

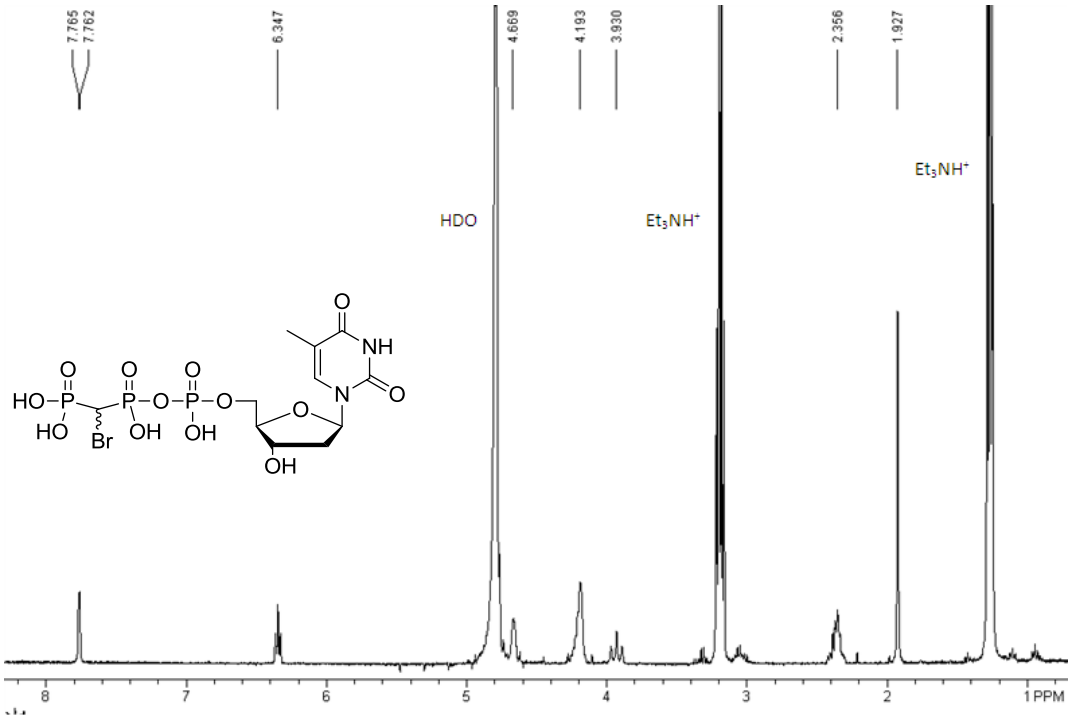

Figure S29. ^{1}H NMR spectrum (400 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CHBr, **6b.**

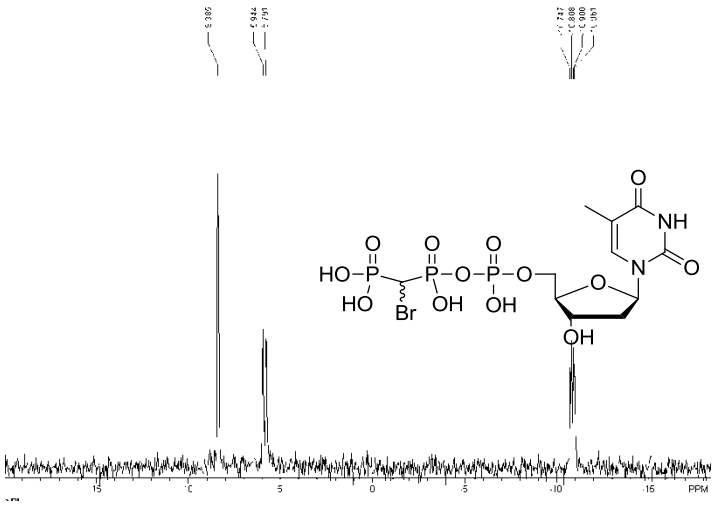

Figure S30. ^{31}P {^{1}H} NMR spectrum (162 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CHBr, **6b.**

Figure S31. LRMS $[M-H]^-$ of thymidine 5'-triphosphate β,γ-CHBr, **6b.** Calc for $C_{11}H_{17}BrN_2O_{13}P_3$: 556.9 (100%), 558.9 (97%); found 556.9 (100%), 558.9 (96%). *Inset*: $[M-H]^-$ isotopic pattern confirming the presence of Br.

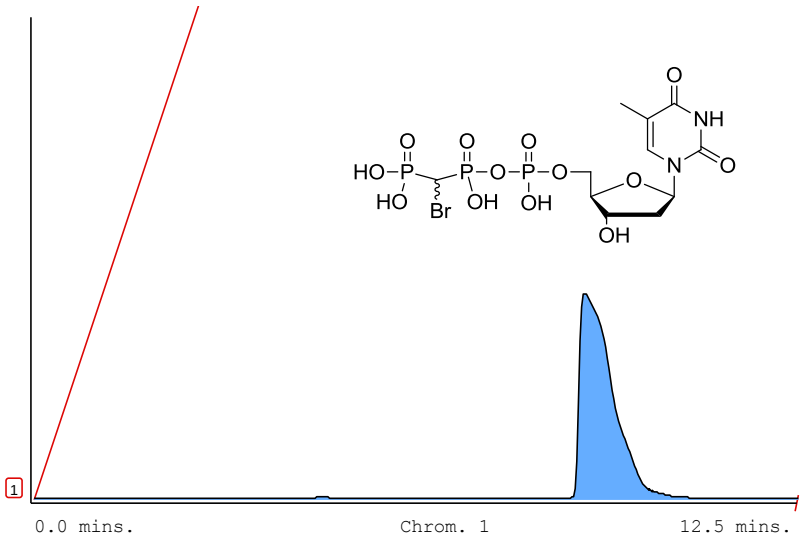

Figure S32. Analytical SAX HPLC trace for thymidine 5'-triphosphate β,γ-CHBr, **6b.** RT = 9.4 min.

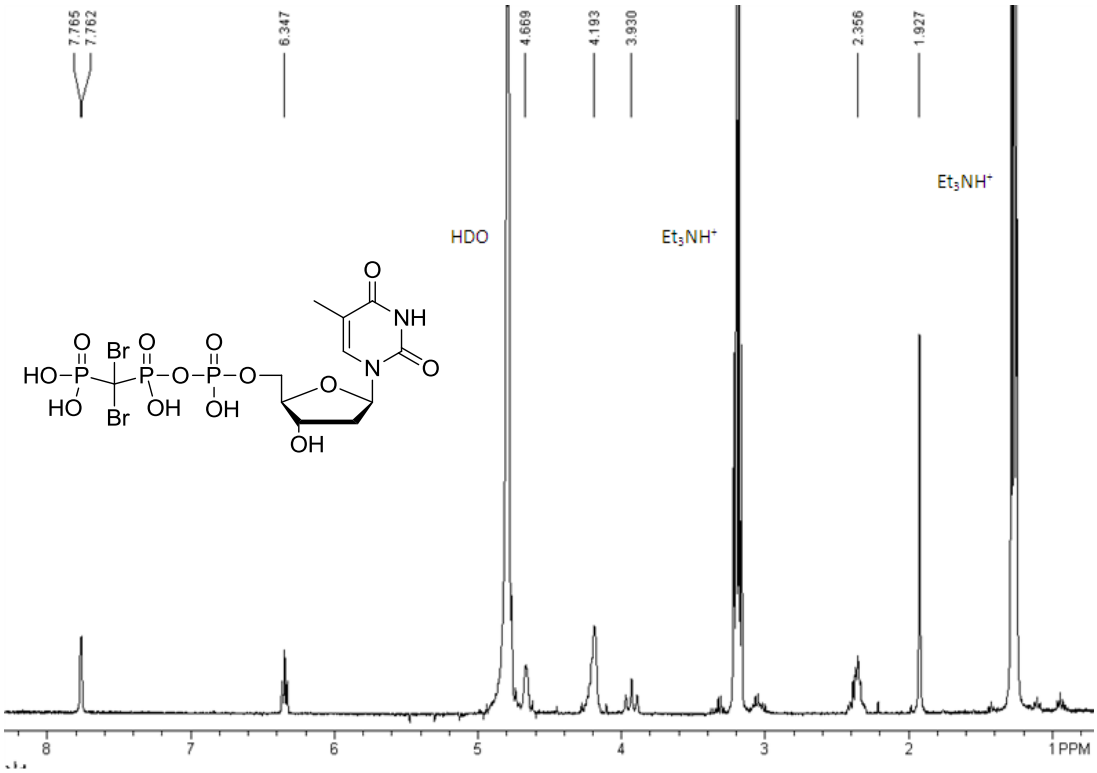

Figure S33. ^{1}H NMR spectrum (400 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CBr_2, **7b.**

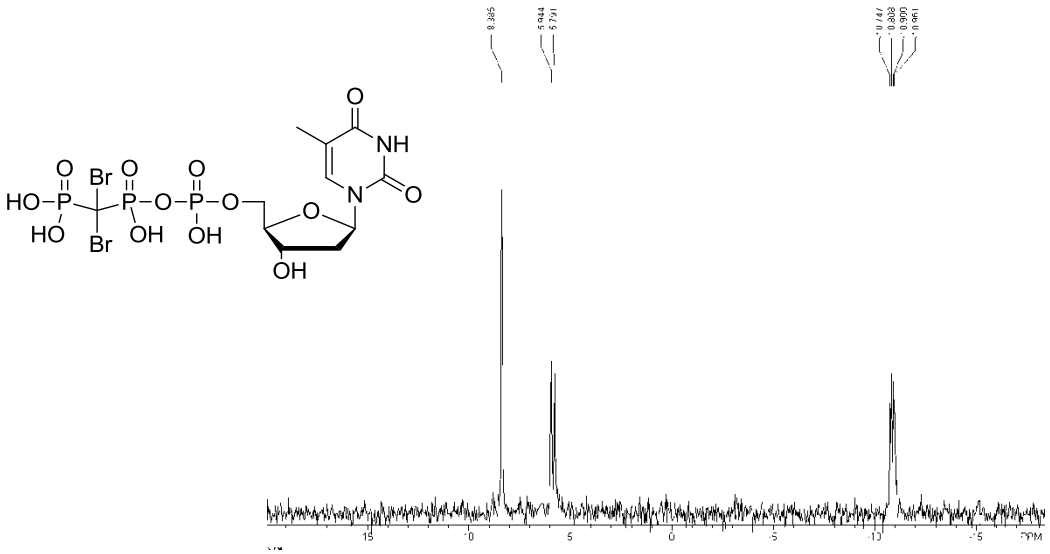

Figure S34. ^{31}P {^{1}H} NMR spectrum (162 MHz, D_2O, pH neutral) of thymidine 5'-triphosphate β,γ-CBr_2, **7b**.

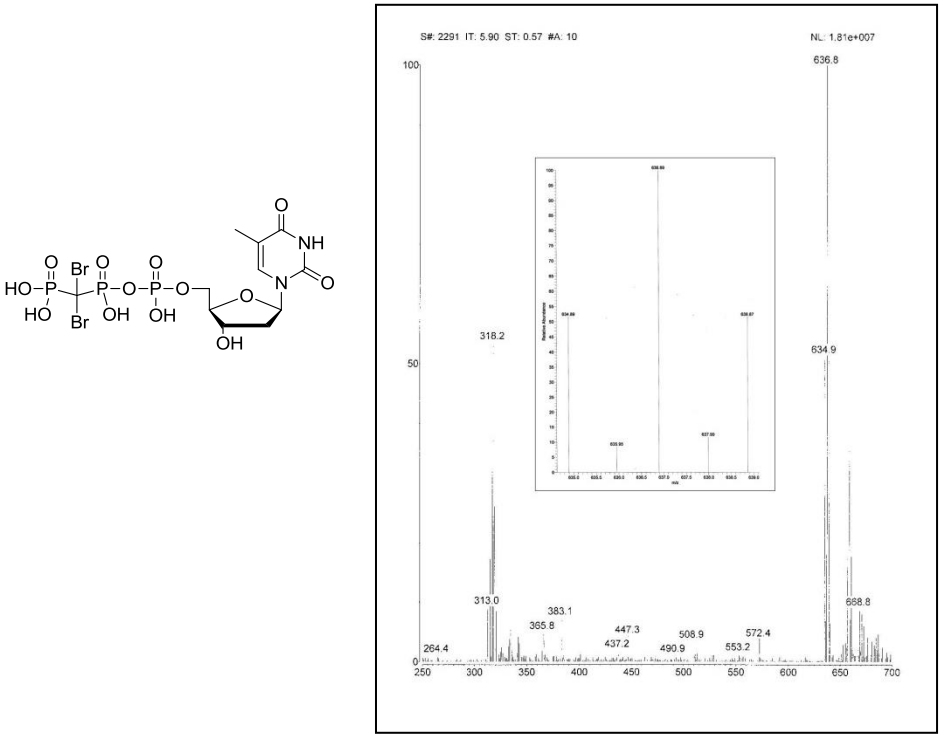

Figure S35. LRMS $[M-H]^-$ of thymidine 5'-triphosphate β,γ-CBr_2, **7b**. Calc for $C_{11}H_{16}Br_2N_2O_{13}P_3$: 636.8 (100%), 634.8 (51%), 638.8 (49%); found: 636.8 (100%), 634.9 (51%), 638.8 (52%). *Inset*: $[M-H]^-$ isotopic pattern confirming the presence of Br.

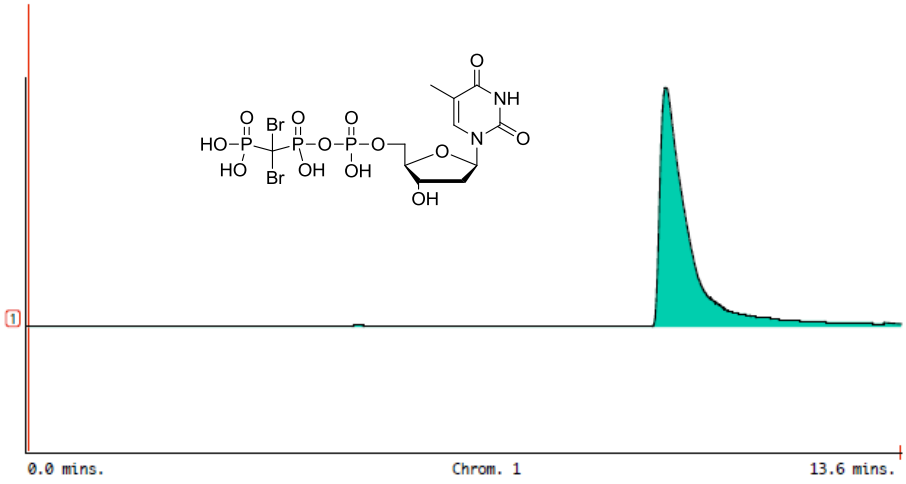

Figure S36. Analytical SAX HPLC trace for thymidine 5'-triphosphate β,γ-CBr_2, **7b**. RT = 9.8 min.

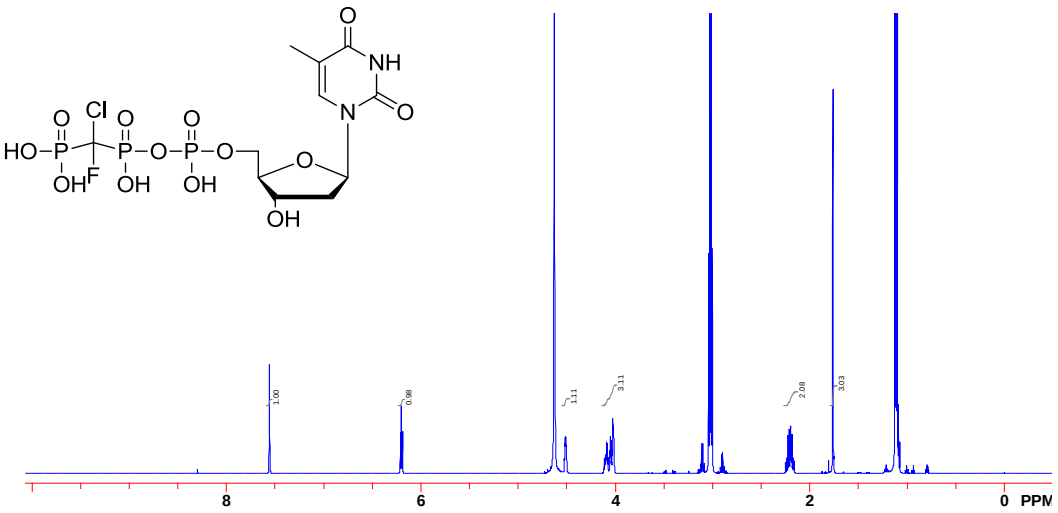

Figure S37. ^{1}H spectrum (D_2O, pH > 10) of dTTP-CFCl, **8b.**

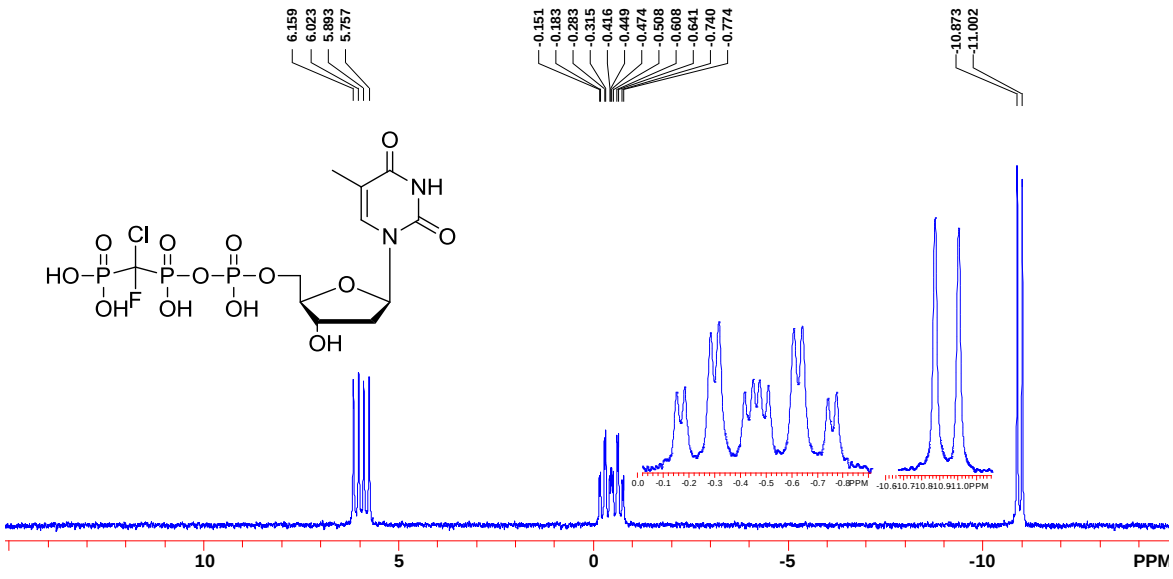

Figure S38. ^{31}P {^{1}H} spectrum (202 MHz, D_2O, pH 10.0) of dTTP-CFCl, **8b.**

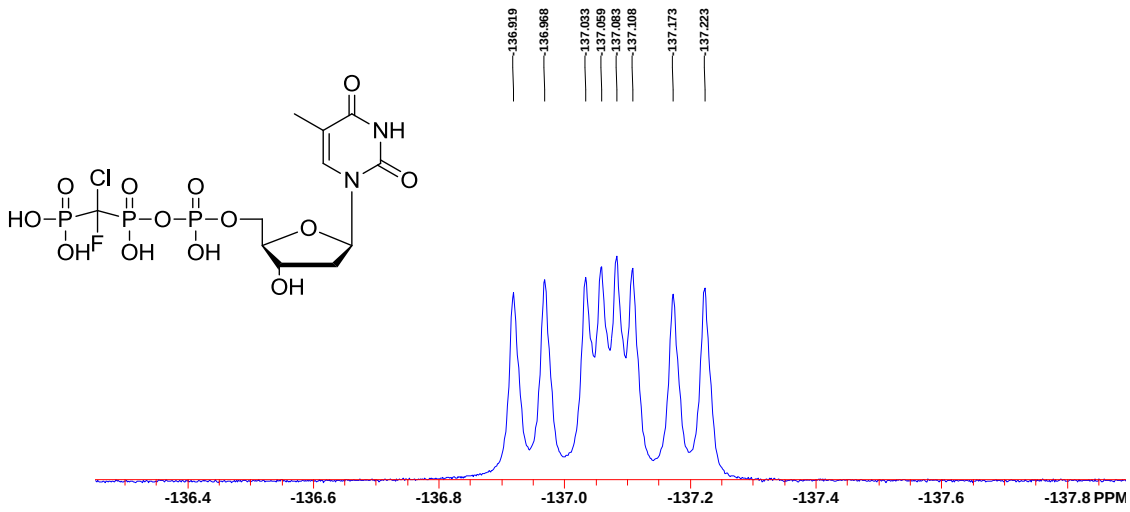

Figure S39. ^{19}F NMR spectrum (D_2O, pH>10) of thymidine 5'-triphosphate β,γ-CFCl **8b.**

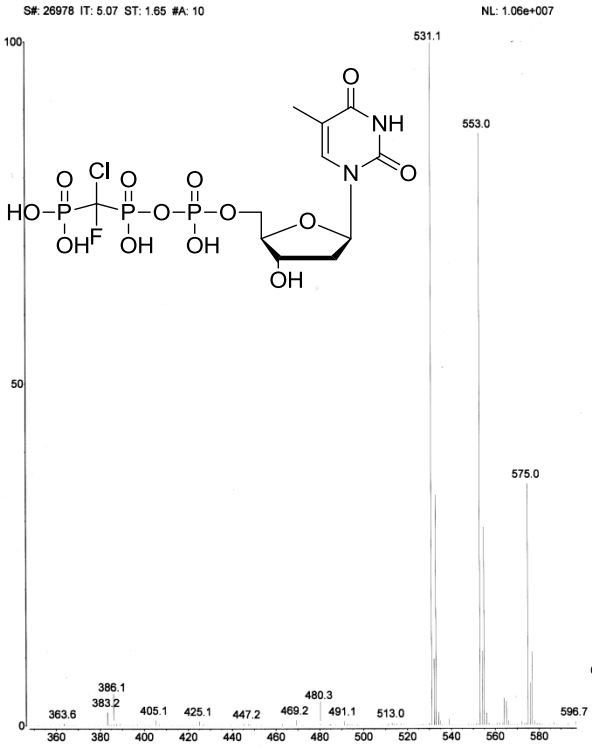

Figure S40. LRMS $[M-H]^-$ of dTTP-CFCl, **8b**. Calc for $C_{11}H_{16}ClFN_2O_{13}P_3$: 531.0; found 531.1.

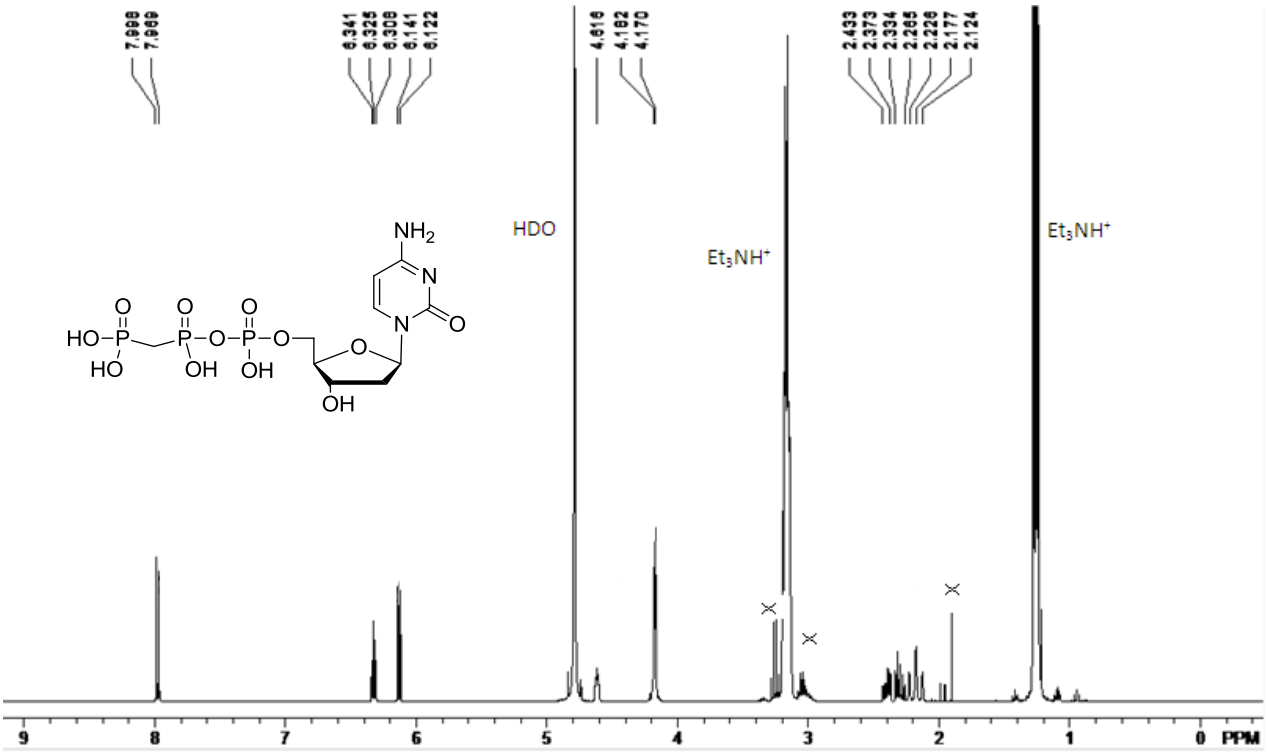

Figure S41. ^{1}H spectrum (400 MHz, D_2O, pH 8.9) of dCMP-MBP, **1c.**

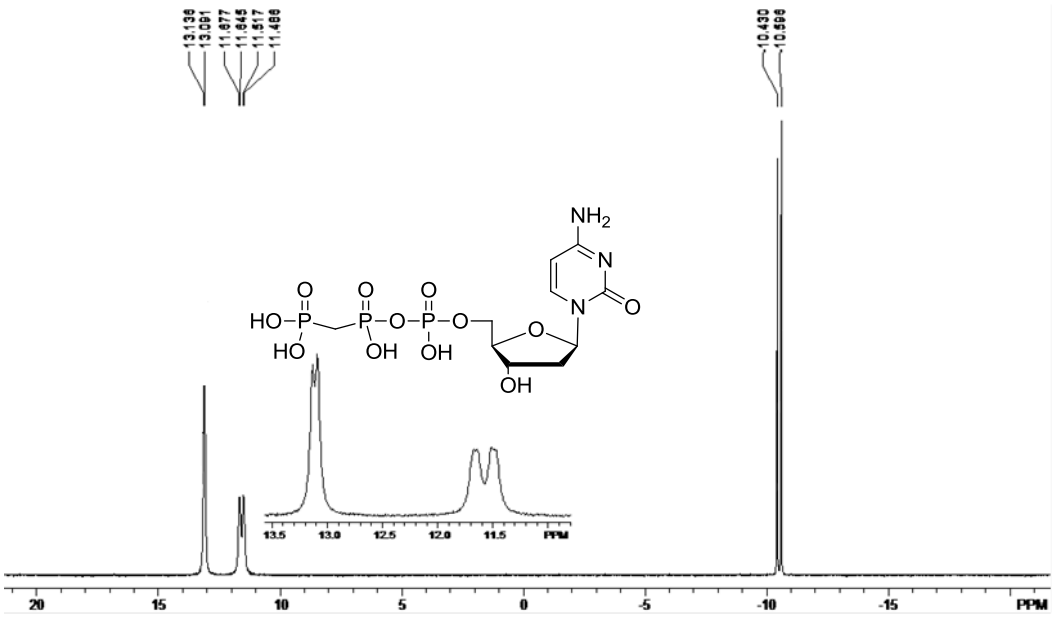

Figure S42. ^{31}P {^{1}H} spectrum (202 MHz, D_2O, pH 8.9) of dCMP-MBP, **1c.**

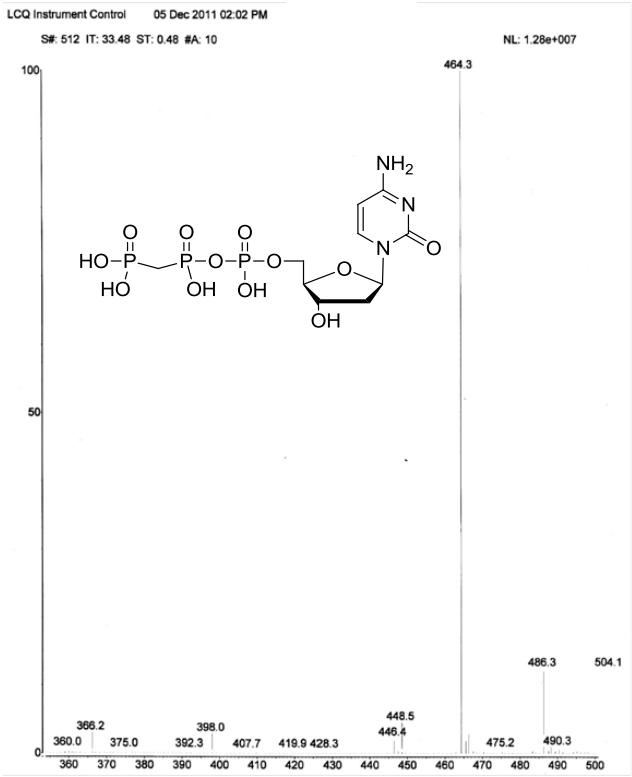

Figure S43. LRMS $[M-H]^-$ of dCMP-MBP, **1c**. Calc for $C_{10}H_{17}N_3O_{12}P_3$: 464.2; found 464.3.

2'-Deoxythymidine 5'-triphosphate β,γ–CH_2, 1b. ^{1}H (D_2O, pH 8, 400 MHz): δ 1.92 (s, 3H), 2.21 (2H, t, J_{HP} 20.0), 2.30–2.42 (2H), 4.13–4.21 (3H), 4.62-4.65 (1H), 6.34 (1H, t, *J* 7.2), 7.74 (d, 1H)**;** ^{31}P (D_2O, pH 9.5, 202 MHz): δ -10.6 (d, $J_{\alpha\beta}$ 26.9, P_α), 12.1 (dd, $J_{\beta\gamma}$ 7.5, $J_{\beta\alpha}$ 27.0, P_β), 12.9 (d, $J_{\gamma\beta}$ 7.9, P_γ); MS $[M-H]^-$: calcd for $C_{12}H_{20}N_2O_{13}P_3$: 479.0; found: 479.0 m/z.

2'-Deoxythymidine 5'-triphosphate β,γ–CHF, 2b. ^{1}H (D_2O, pH 8, 400 MHz): δ 1.88 (s, 3H), 2.25–2.37 (2H), 4.11–4.20 (3H), 4.61 (1H), 6.33 (1H, *J* 7.2), 7.68 (1H); ^{19}F (D_2O, pH 8, 376 MHz): δ –219 (bs); ^{31}P (D_2O, pH 8, 162 MHz): δ –10.5 (d, $J_{\alpha\beta}$ 29, P_α), 5.1 (m, P_β), 7.9 (dd, $J_{\beta\gamma}$ 11.9, J_{PF} 54.3, P_γ); MS $[M-H]^-$: calcd for $C_{11}H_{17}FN_2O_{13}P_3$: 497.0; found: 497.0 m/z. Lit Ref[8] (pH not reported): ^{31}P: δ –10.3 (d, *J* 27.3), 2.64 m, 8.9 (dt, *J* 60.2).

2'-Deoxythymidine 5'-triphosphate β,γ–CF_2, 3b. ^{1}H (D_2O, pH 8, 400 MHz): δ 1.88 (s, 3H), 2.27–2.38 (2H), 4.13–4.22 (3H), 4.60–4.63 (1H), 6.32 (1H, t, *J* 7.2), 7.70 (d, 1H); ^{19}F (D_2O, pH 8, 376 MHz): δ -119 (dd, J_{FP1} 90.4, J_{FP2} 75.3); ^{31}P (D_2O, pH 8, 162 MHz): δ –10.4 (d, *J* 32, P_α), –2.0 (m, P_β), 4.2 (dt, $J_{\gamma\beta}$ 56, J_{PF} 71, P_γ); MS $[M-H]^-$: calcd for $C_{11}H_{17}F_2N_2O_{13}P_3$: 515.0; found: 514.9 m/z. Lit Ref[8] (pH not reported): ^{31}P: δ –10.2 (d, *J* 30.1), –3.1 m, 4.3 (dt, *J* 66.0).

2'-Deoxythymidine 5'-triphosphate β,γ–CHCl, 4b. ^{1}H (D_2O, pH 8, 400 MHz): 1.92 (s, 3H), 2.33–2.37 (2H), 3.92 (2 dd, 1H), 4.17–4.28 (3H), 4.65–4.69 (1H), 6.39 (1H, t, *J* 7.2), 7.68 (1H, 2 d); ^{31}P (D_2O, 162 MHz, pH 8): –10.3 (d, $J_{\alpha\beta}$ 26, P_α), 8.9 (m, P_β), 10.1 (bs, P_γ); MS $[M-H]^-$: calcd for $C_{11}H_{17}ClN_2O_{13}P_3$: 513.0 (100%), 515.0 (32%); found: 513.0 (100%), 515.0 (33%) m/z. **After Chelex treatment and pH change:** ^{31}P (D_2O, pH 10.3, 202 MHz): –10.63 (d, $J_{\alpha\beta}$ 28.3, $P_{\alpha,}$), –10.68 (d, $J_{\alpha\beta}$ 28.3. $P_{\alpha,}$), 6.9 (dd, $J_{\alpha\beta}$ 28.3, $J_{\beta\gamma}$ 6.2, P_β), 8.9 (d, $J_{\beta\gamma}$ 6.2, P_γ).

2'-Deoxythymidine 5'-triphosphate β,γ–CCl_2, 5b. ^{1}H (D_2O, pH 8, 400 MHz): δ 1.93 (s, 3H), 2.30–2.43 (2H), 4.17–4.25 (2H), 4.31–4.36 (1H), 4.71–4.75 (1H), 6.36 (1H, t, *J* 7.2), 7.77 (d, 1H); ^{31}P (D_2O, pH 8, 162 MHz): δ –10.4 (d, $J_{\alpha\beta}$ 32, P_α), 2.3 (dd, $J_{\alpha\beta}$ 32, $J_{\beta\gamma}$ 17, P_β), 8.5 (d, $J_{\beta\gamma}$ 17, P_γ); MS $[M-H]^-$: calcd for $C_{11}H_{16}Cl_2N_2O_{13}P_3$: 546.9 (100%), 548.9 (64%); found: 547.0 (100%), 549.0 (63%) m/z.

2'-Deoxythymidine 5'-triphosphate β,γ–CHBr, 6b. ^{1}H (D_2O, pH 8, 400 MHz): δ 1.93 (s, 3H), 2.30–2.43 (2H), 3.93 (1H, t, J_{HP} 15.6), 4.18–4.26 (3H), 4.65–4.68 (1H), 6.35 (1H, t, *J* 7.2), 7.77 (1H, 2 doublets); ^{31}P (D_2O, 162 MHz, pH 8): δ −10.8 (d, $J_{\alpha\beta}$ 26, P_α), −10.9 (d, $J_{\alpha\beta}$ 26, P_α), 5.9 (d, $J_{\alpha\beta}$ 26, P_β), 8.4 (bs, P_γ); MS $[M-H]^-$: calcd for $C_{11}H_{17}BrN_2O_{13}P_3$: 556.9 (100%), 558.9 (97%); found: 556.9 (100%), 558.9 (96%) m/z.

2'-Deoxythymidine 5'-triphosphate β,γ–CBr_2, 7b. ^{1}H (D_2O, pH 8, 400 MHz): δ 1.91 (s, 3H), 2.30–2.40 (2H), 4.16 (s, 1H), 4.21–4.26 (1H), 4.34–4.40 (1H), 4.72–4.75 (1H), 6.39 (1H), 7.68 (1H); ^{31}P (D_2O, 162 MHz, pH 8): δ −10.4 (d, $J_{\alpha\beta}$ 32, P_α), 2.2 (dd, $J_{\alpha\beta}$ 32, $J_{\beta\gamma}$ 13, P_β), 8.1 (d, $J_{\beta\gamma}$ 13, P_γ); MS $[M-H]^-$: calcd for $C_{11}H_{16}Br_2N_2O_{13}P_3$: 636.8 (100%), 634.8 (51%), 638.8 (49%); found: 636.8 (100%), 634.9 (51%), 638.8 (52%) m/z. Lit Ref[9] (pH not reported): ^{1}H (D_2O): δ 7.63 (1H, q, $J_{6,5\text{-Me}}$ 1, H−6), 6.23 (1H, t, $J_{1'-2'}$ 7, H−1'), 4.56 (1H, m, H−3'), 4.11 (3H, m, H−4', 5'), 2.26 (2H, m, H−2'), 1.81 (3H, d, 5−CH_3). ^{31}P: δ −11.0 (d, P_α), −0.9 (dd, $J_{\beta\alpha}$ 24), 7.4 (d, $J_{\gamma\beta}$ 14, P_γ).

2'-Deoxycytosine 5'-triphosphate β,γ–CH_2, 1c. ^{1}H NMR (D_2O, pH 8.9, 202 MHz): δ 2.17 (t, J_{HP} 20.5, 2H), 2.33–2.27 (m, 1H), 2.43–2.37 (m, 1H), 4.18–4.17 (m, 2H), 4.62 (m, 1H),6.13 (d, *J* 7.5, 1H), 6.32 (t, *J* 6.6, 1H), 7.99 (d, *J* 7.7, 1H); ^{31}P NMR (D_2O, pH 8.9, 202 MHz): δ −10.5 (d, $J_{\alpha\beta}$ 26.9, P_α), 11.6 (d, $J_{\gamma\beta}$ 7.1, P_γ), 13.1 (dd, $J_{\beta\gamma}$ 7.1, $J_{\beta\alpha}$ 27.4, P_β); MS $[M-H]^-$: calcd for $C_{10}H_{17}N_3O_{12}P_3^-$: 464.2; found: 464.3 m/z.

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