Supporting Information for

Structures of *KlenTaq* DNA Polymerase Caught While Incorporating C5-Modified Pyrimidine and C7-Modified 7-Deazapurine Nucleoside Triphosphates

Konrad Bergen,[‡] Anna-Lena Steck,[‡] Stefan Strütt, Anna Baccaro, Wolfram Welte, Kay Diederichs, and Andreas Marx*

Departments of Chemistry and Biology, Konstanz Research School Chemical Biology, University of Konstanz, Universitätsstr. 10, 78457 Konstanz (Germany)

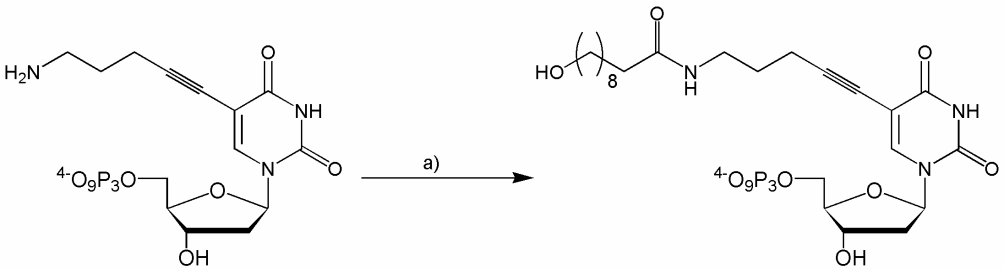
Table of contents

Chemical Synthesis of modified nucleotides

General experimental details

2',3'-Dideoxy-cytidine-5'-O-triphosphate was purchased from *JenaBioscience*. Succinimidyl 10-hydroxydecanoate[1] were prepared according to literature. 5-(aminopentinyl)-2'-deoxyuridinetriphosphate **dT*TP**, 5-(aminopentinyl)-2'-deoxycytidinetriphosphate **dC*TP**, 7-(aminopentinyl)-7-deaza-2'-deoxyadenosinetriphosphate **dA*TP** and 7-(aminopentinyl)-7-deaza-2'-deoxyguanosinetriphosphate **dG*TP** were synthesized according to known procedures.[2] All reagents are commercially available and were used without further purification. Solvents were stored over molecular sieves (*Fluka*) and used directly without further purification, unless otherwise noted. All synthetic reactions were performed under an inert atmosphere. Flash chromatography was done using *Merck* silica gel G60 (230–400 mesh) and *Merck* precoated plates (silica gel 60 F254) were used for TLC. NMR spectra were recorded on *Bruker* Avance 400 (^{1}H: 400 MHz, ^{13}C: 101 MHz, ^{32}P: 162 MHz) spectrometer and *Bruker* AVIII 600 (^{1}H: 600 MHz). The solvent signals were used as references and the chemical shifts converted to the TMS scale and are given in ppm (δ). HRMS spectra were recorded on a *Bruker* mircOTOF II in the negative mode.

Synthesis of 2'-deoxythymidine analogue dT**TP



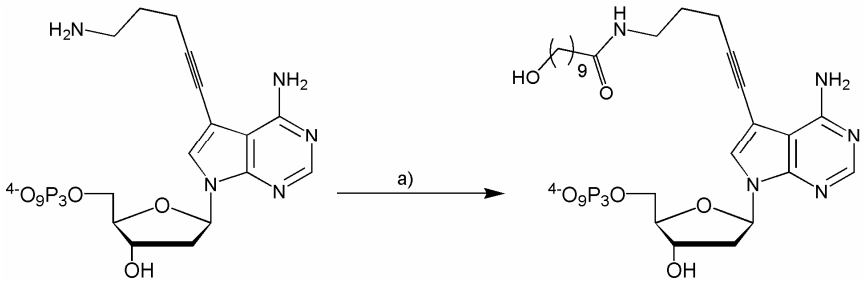
dT*TP **dT**TP**

Scheme S1: Synthesis of 2'-deoxythymidine analogue dT**TP, a) succinimidyl 10-hydroxydecanoate, DMSO, 5 h, rt.

5-(N-(10-hydroxydecanoyl)-aminopentinyl)-2'-deoxyuridinetriphosphate ***dT**TP***

5-(aminopentinyl)-2'-deoxyuridinetriphosphate **dT*TP** (548µg, 1µmol) and succinimidyl 10-hydroxydecanoate (2.85 mg, 10 µmol) were dissolved in DMSO (0.5 ml). After shaking at room temperature for five hours, the solvent was removed *in vacuo*. The residue was suspended in water and filtered. The solution was purified by RP-HPLC (Nucleosil 100-5 C18 PPN, 5-100 % acetonitrile/ 0.1 M TEAA buffer (0.1 M acetic acid, 0.1 M triethylamine, (pH 7.0)) to give triphosphate **dT**TP** in quantitative yield. ^{1}H-NMR (600 MHz, MeOD) δ = 8.02 (s, 1H, H-6), 6.26 (t, ^{3}J = 6.8 Hz, 1H, H-1'), 4.66 – 4.62 (m, 1H, H-3'), 4.36 – 4.31 (ddd, ^{2}J = 11.3 Hz, ^{3}J = 7.1 Hz, ^{3}J = 3.8 Hz, 1H, H-5'a), 4.23 – 4.18 (ddd, ^{2}J = 11.3 Hz, ^{3}J = 5.3 Hz, ^{3}J = 4.5 Hz, 1H, H-5'b), 4.10 – 4.07 (m, 1H, H-4), 3.55 (t, ^{3}J = 6.8 Hz, 2H, -CH_2CH_2OH), 3.35 – 3.31 (m, 2H, -CH_2NH-, superimposed by MeOH), 3.22 – 3.14 (m, 24H, Et_3N), 2.46 (t, ^{3}J = 6.8 Hz, 2H, -C≡CCH_2-), 2.32 – 2.26 (m, 2H, H-2'a/b), 2.24 – 2.20 (m, 2H, -COCH_2-), 1.80 (p, ^{3}J = 6.8 Hz, 2H, -$CH_2CH_2CH_2$NH-), 1.64 – 1.58 (m, 2H, -$COCH_2CH_2$-), 1.57 – 1.51 (m, 2H, -CH_2CH_2OH), 1.38 – 1.28 ppm (m, 48H, Et_3N, -CH_2-); ^{31}P-NMR (162 MHz, MeOD) δ = -10.20 (d, ^{2}J = 21.3 Hz, 1P, P_γ), -11.23 (d, ^{2}J = 21.6 Hz, 1P, P_α), -23.55 ppm (t, ^{2}J = 22.9 Hz, 1P, P_β).; HRMS (negative mode): *m/z*: calcd for $[C_{24}H_{39}N_3O_{16}P_3]^-$: 718.1549; found: 718.1545.

Synthesis of 2'-deoxyadenosine analogue dA**TP



dA*TP **dA**TP**

Scheme S2 Synthesis of 2'-deoxythymidine analogue dA**TP, a) succinimidyl 10-hydroxydecanoate, DMSO, 5 h, rt.

7-(N-(10-hydroxydecanoyl)-aminopentinyl)-7-deaza-2'-deoxyadenosinetriphosphate ***dA**TP***

7-(aminopentinyl)-7-deaza-2'-deoxyadenosinetriphosphate **dA*TP** (570 µg, 1µmol) and succinimidyl 10-hydroxydecanoate (2.85 mg, 10 µmol) were dissolved in DMSO (0.5 ml). After shaking at room temperature for five hours, the reaction mixture was freeze-dried. The residue was suspended in water and filtered. The solution was purified by RP-HPLC (Nucleosil 100-5 C18 PPN, 5-100 % acetonitrile/0.1 M TEAA buffer (0.1 M acetic acid, 0.1 M triethylamine, (pH 7.0)) to give triphosphate **dA**TP** in quantitative yield. ^{1}H-NMR (400 MHz, MeOD): δ = 8.20 (br, 1H, H-2), 7.72 (s, 1H, H-8), 6.64 (t, ^{3}J = 6.2 Hz, 1H, H-1'), 4.72 (br, 1H, H-3'), 4.32 – 4.20 (m, 2H, H-5'a/b), 4.14 (br, 1H, H-4'), 3.58 – 3.53 (m, 2H, -CH_2CH_2OH), 3.35 (m, 2H, -CH_2NH-, superimposed by MeOH), 3.24 – 3.19 (m, 13H, Et_3N), 2.61 – 2.50 (m, 3H, H-2'a, -C≡CCH_2-), 2.38 – 2.32 (m, 1H, H-2'b), 2.24 – 2.20 (m, 2H, -COCH_2-), 1.87 – 1.79 (m, 2H, -$CH_2CH_2CH_2$NH-), 1.65 – 1.63 (m, 2H, -$COCH_2CH_2$-), 1.58 – 1.52 (m, 2H, -CH_2CH_2OH), 1.35 ppm (m, 36H, Et_3N, -CH_2-); ^{31}P-NMR (162 MHz, MeOD): δ = -10.09 (d, J = 21.3 Hz, 1P, P_γ), -10.99 (d, J = 21.3 Hz, 1P, P_α), -22.99 - -23.58 ppm (m, 1P, P_β). HRMS (negative mode): *m/z*: calcd for $[C_{26}H_{41}N_5O_{14}P_3]^-$: 740.1868, found: 740.1891.

Enzymes, oligodeoxynucleotides, nucleotides

KlenTaq DNA polymerase was expressed and purified as described before.[3] T4 polynucleotide kinase PNK was purchased from *Fermentas*. Primer and templates were purchased from *Metabion* and *ThermoFisher*. [γ-^{32}P]ATP was purchased from *Hartmann Analytics* and natural dNTPs from *Roche*.

5' Radioactive labeling of ODNs

DNA oligonucleotide primers were radioactively labeled at the 5' terminus by a ^{32}P containing phosphate group using T4 PNK which transfers the γ-phosphate group from [γ-^{32}P]ATP to the 5' hydroxyl group. The reactions contained primer (0.4 μM), PNK reaction buffer (1 ×), [γ-^{32}P]ATP (0.8 μCi/μl) and T4 PNK (0.4 U/μl) in a total volume of 50 μl and were incubated for 1 h at 37 °C. The reaction was stopped by denaturing the T4 PNK for 2 min at 95 °C and buffers and excess [γ-^{32}P]ATP were removed by gel filtration (MicroSpin Sephadex G-25). Addition of unlabeled primer (20 μl, 10 μM) led to a final concentration of 3 μM of diluted radioactive labeled primer.

Gel electrophoresis

Denaturing polyacrylamide gels (12 %) were prepared by polymerization of a solution of urea (8.3 M) and bisacrylamide/acrylamide (12 %) in TBE buffer using ammonium peroxodisulfate (APS, 0.08 %) and N,N,N',N'-tetramethylethylene-diamine (TEMED, 0.04 %). Immediately after addition of APS and TEMED the solution was filled in a sequencing gel chamber (*Bio-Rad*) and left for polymerization for at least 45 min. After addition of TBE buffer (1 ×) to the electrophoresis unit, gels were prewarmed by electrophoresis at 100 W for 30 min and samples were added and separated during electrophoresis (100 W) for approx. 1.5 h. The gel was transfered to *Whatman* filter paper, dried at 80 °C, *in vacuo*, using a gel dryer (model 583, *Bio-Rad*) and exposed to a imager screen. Readout was performed with a molecular imager (FX, *Bio-Rad*).

DNA sequences

radioactive-labeled primer: 5' d(GTG GTG CGA AAT TTC TGA CAG ACA)
template (incorporation of dTMP): 5' d(GTG CGT CTG TC**A** TGT CTG TCA GAA ATT TCG CAC CAC)
template (incorporation of dAMP): 5' d(GTG CGT CTG TC**T** TGT CTG TCA GAA ATT TCG CAC CAC)
template (incorporation of dCMP): 5' d(GTG CGT CTG TC**G** TGT CTG TCA GAA ATT TCG CAC CAC)
template (incorporation of dGMP): 5' d(GTG CGT CTG TA**C** TGT CTG TCA GAA ATT TCG CAC CAC)

Primer extension reaction

A typical primer extension reaction (10 μL) employing *KlenTaq* DNA polymerase contained 1 x *KlenTaq* reaction buffer (50 mM Tris HCl (pH 9.2), 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1% Tween 20), 50 nM ^{32}P-labeled primer, 75 nM template, 200 μM dN*TP/dNTP mixture, and 100 nM *KlenTaq* DNA polymerase. First primer and template were annealed. Afterwards the primer/template complex, nucleotides and DNA polymerase were incubated (60°C; 10 sec). The reactions were quenched by addition of 30 μL PAGE gel loading buffer (80% formamide, 20 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanole FF) and the product mixtures were analyzed by 12% denaturing polyacrylamide gel and subjected to autoradiography. Quantification was done by using the *Bio-Rad* Quantity One software. The conversion in % was plotted *versus* the concentration using the program *GraphPad* Prism4. All reactions were done in duplicates.
dNTP mixture: 0/1, 1/1, 2/1, 4/1, 10/1, 20/1, 50/1, 100/1, 1/0 (dT*TP,dT**TP, dC*TP)
dNTP mixture: 0/1, 1/10, 1/4, 1/2, 1/1, 2/1, 4/1, 10/1, 1/0 (dA*TP, dA**TP, dG*TP)

Crystallization, data collection and analysis

The protein was overexpressed and purified as described earlier[3a] and concentrated to 18 mg/ml for storage. For crystallization, a 11-nt primer (5'-d(GAC CAC GGC GC)) and a 16-nt template (5'-d(AAA XNG CGC CGT GGT C; N: canonical template for incorporation of the dideoxy-nucleotide to terminate the primer, and X: canonical template for pairing with the incoming modified nucleotide) were used. The primer/template constructs were terminated by the incorporation of ddCMP (by incubation with ddCTP) in case of dA*, dG*, dT*, dA** and dT**. For dC* a ddGTP and appropriate template was used. Primer/template constructs were annealed and added to the DNA polymerase (protein to DNA ratio 1:1.2, final protein concentration of ~6.2 mg/ml). Dideoxy-terminated primer ends were created by the addition of a 5 molar excess of 2',3'-dideoxy-cytidine-5'-triphosphate. The solution was set to final concentration of 20 mM $MgCl_2$ and incubated for 1 hour at 30°C.

Crystals of the binary complex of the *KlenTaq* DNA polymerase with dideoxy-terminated primer strands were grown in hanging drop plates (*Qiagen*) against 1 ml reservoir (crystallization condition derived from NucPro HTS Screen, *JenaBioScience* (100 mM Tris·HCl pH 8, 200mM Mg-formate, 18% PEG 8000)) in a 1:1 ratio protein/DNA to reservoir. Grown crystals were harvested after 6 days of growth and transferred into a 2 µl drops of stabilizing solution consisting of the crystallization reservoir with additional 20% ethylene glycol, which was pre-equilibrated for 2 h. Soaking was performed by diluting the modified dNTPs in the stabilizing solution before equilibration. Best results were found at final concentrations between 0.5 to 2 mM of the modified dNTPs followed by 30 minutes of incubation. After soaking, crystals were transferred in liquid nitrogen.

Crystallographic data sets were collected at the beamlines PXI and PXIII at the Swiss Light Source (SLS), Paul-Scherrer Institute, Villigen, Switzerland. Data integration and reduction was performed using XDS[4]. For dC*TP and dT*TP multiple datasets were merged using XSCALE. Structure solution was done using difference Fourier-Methods with the PHENIX suite.[5]

Generation of library and geometry files was performed using the program Sketcher in the CCP4i suite[6]. Subsequent refinement of the data was done employing Coot[7] and ML refinement methods of the PHENIX suite (see Table S1). Omit maps for the triphosphates are shown in Figure S4. The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.rcsb.org (PDB ID codes 4DFM, 4DFP, 4DFJ, 4DF8, 4DFK and 4DF4).

Table S1 Data collection and refinement

PDB ID	**4DFM**	**4DFP**	**4DFJ**	**4DF8**	**4DFK**	**4DF4**
Data collection	**KlenTaq dC***	**KlenTaq dG***	**KlenTaq dT***	**KlenTaq dA***	**KlenTaq dT****	**KlenTaq dA****
Spacegroup	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$	$P3_121$
Cell dimensions						
***a, b, c* (Å)**	a,b=108.2 c=90.1	a,b=108.3 c=90.3	a,b=108.9 c=90.4	a,b=108.6 c=90.5	a,b=107.8 c=89.7	a,b=107.8 c=89.7
α, β, γ (°)	α,β=90 γ=120	α,β=90 γ=120	α,β=90 γ=120	α,β=90 γ=120	α,β=90 γ=120	α,β=90 γ=120
Resolution*	47.15- (1.94) 1.89	46.96- (2.12) 2.00	46.96- (1.95) 1.90	47.03- (2.12) 2.00	46.86- (1.75) 1.65	46.20- (2.22) 2.09
R_{meas}* +	19.3 (95.4)	13.5 (129.2)	14.4 (88.5)	14.2 (153.4)	7.3 (134.5)	14.1 (108.1)
***I/σI* ***	12.77 (1.83)	12.36 (1.69)	14.86 (1.61)	14.43 (1.78)	16.14 (1.65)	11.33 (1.73)
Completeness (%)*	98.6 (82.0)	99.8 (98.9)	94.1 (62.1)	99.9 (99.4)	99.8 (98.5)	99.7 (98.0)
Refinement						
Resolution (Å)*	47.15-1.89	46.96-2.00	46.96-2.00	47.03-2.00	46.86-1.65	46.20-2.20
No.unique reflections*	48759	41788	46509	41984	73657	30932
R_{work}/R_{free} *	18.6/21.5	18.5/22.4	18.0/20.4	19.5/23.4	17.0 /19.5	17.7/22.7
B-factors						
DNA	35.0	38.2	32.9	39.6	34.8	27.6
Protein	34.8	38.7	34.9	36.6	37.9	29.4
R.m.s deviations						
Bond lengths (Å)	0.008	0.007	0.007	0.007	0.009	0.007
Bond angles (°)	1.42	1.30	1.39	1.38	1.55	1.33

Ramachandran[#]						
Favored	97.7	97.4	97.4	97.6	97.8	97.2
Allowed	2.1	2.4	2.4	2.2	1.8	2.6
outlier	0.2	0.2	0.2	0.2	0.4	0.2

*Numbers in brackets refer to the highest resolution shell

+ for definition of R_{meas}, see [8]

#as determined by phenix.model_vs_data

R.m.s deviations Cα-atoms (Å)	0.34 (3KTQ)	0.34 (1QSS)	0.33 (1QTM)	0.38 (1QSY)	0.30 (1QTM)	0.54 (1QSY)

Deviation of Cα-atoms to the corresponding unmodified structures (the related PDB IDs are noted in brackets)

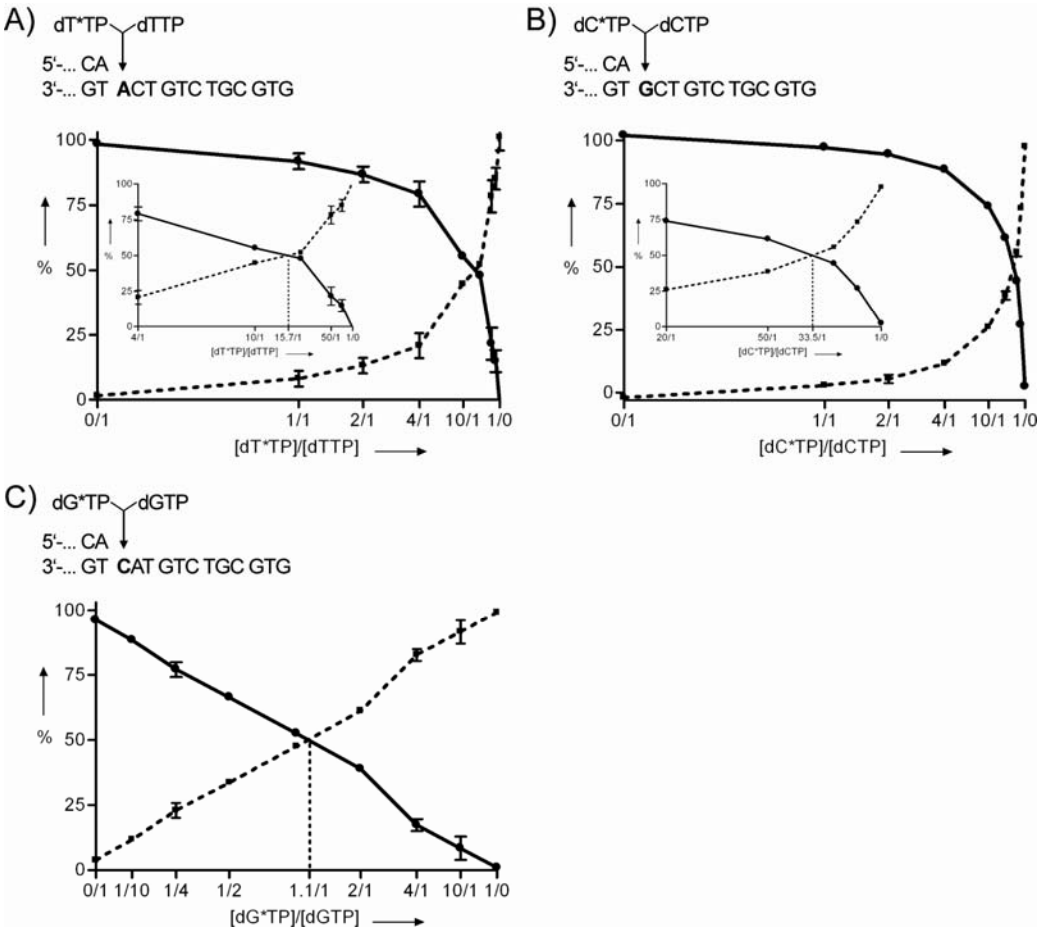


Figure S1: Competition experiments of dN*TP *versus* dNTP. The conversion in % was plotted *versus* the concentration using the program GraphPad Prism4. The dotted line marks the approximate ratio where both nucleotides are equally incorporated. A) Partial DNA sequences of primer and template for the incorporation of dT*TP and dTTP. Evaluation of the incorporation efficiency using dT*TP (■, dashed line)/dTTP (•, solid line) mixtures and *KlenTaq* DNA polymerase. B) Partial DNA sequences of primer and template for the incorporation of dC*TP and dCTP. Evaluation of the incorporation efficiency using dC*TP (■, dashed line)/dCTP (•, solid line) mixtures and *KlenTaq* DNA polymerase. C) Partial DNA sequences of primer and template for the incorporation of dG*TP and dGTP. Evaluation of the incorporation efficiency using dG*TP (■, dashed line)/dGTP (•, solid line) mixtures and *KlenTaq* DNA polymerase.

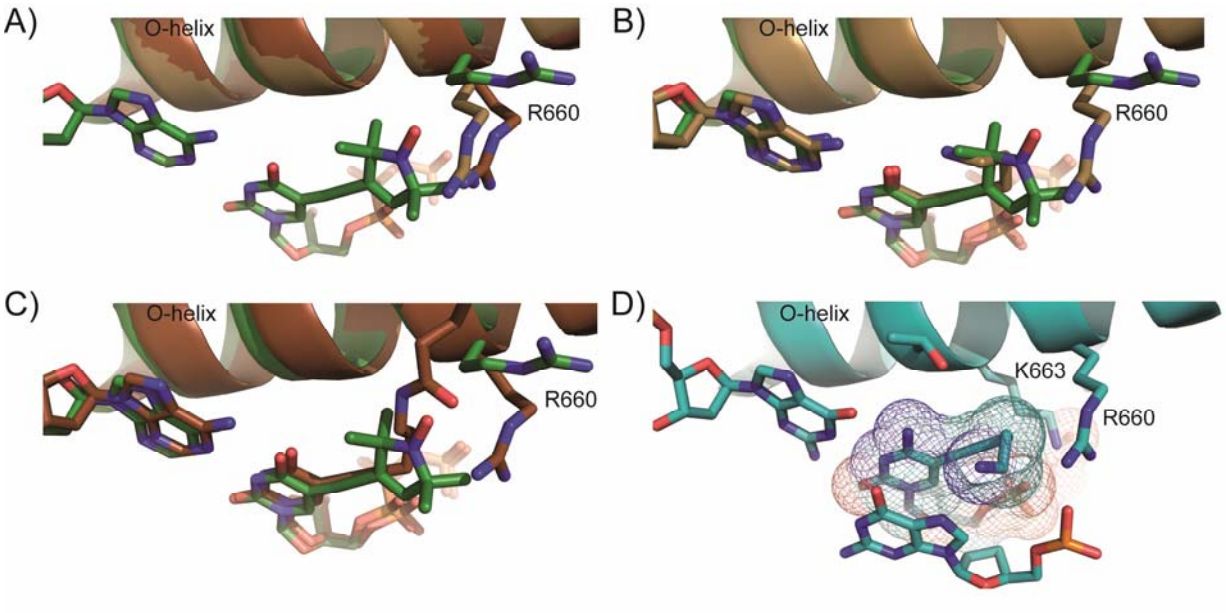


Figure S2 A) Overlay of the O-helices from pdb ID 3OJU (*KlenTaq* DNA polymerase in complex with C5-modified thymidines, spin label, in green), dT*TP (bronze) and dT**TP (brown), depicted are the positions of R660. B) Overlay of the C5-spinlabelled dTTP and dT*TP as well as the orientations of R660. C) Overlay of dT**TP and the C5-spinlabelled dTTP, depicted are the positions of R660. D) Simple surface mesh of dC*TP, repulsive electrostatics by the depicted amino acids and the nucleobase forcing an unstabilized orientation of the linker towards upstream base pair.

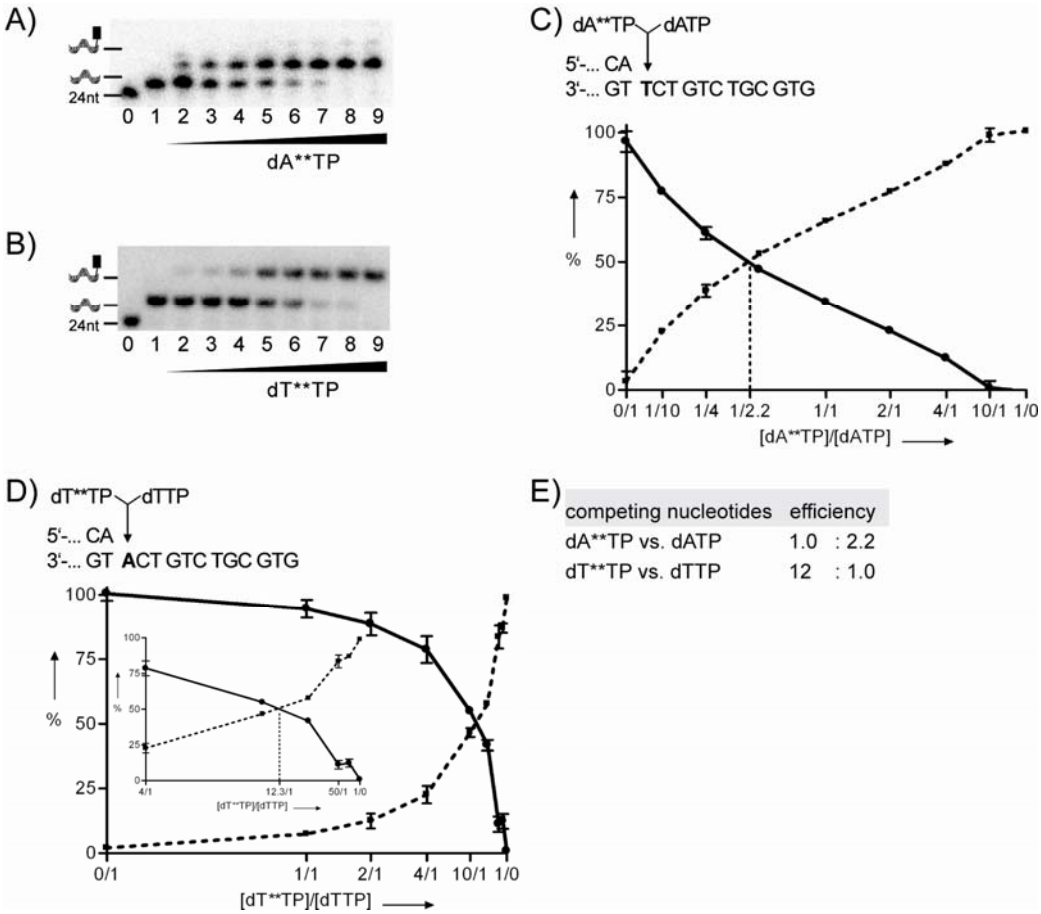


Figure S3: Competition experiments of dN**TP *versus* dNTP. The conversion in % was plotted *versus* the concentration using the program GraphPad Prism4. The dotted line marks the approximate ratio where both nucleotides are equally incorporated. A) PAGE analysis of a competition experiments employing *KlenTaq* DNA polymerase. The ratio of dA**TP/dATP was varied. Lane 0: 5'-^{32}P-labeled primer; lane 1: ratio: 0/1; lane 2: ratio: 1/10; lane 3: ratio: 1/4; lane 4: ratio: 1/2; lane 5: ratio: 1/1; lane 6: ratio: 2/1; lane 7: ratio: 4/1; lane 8: ratio: 10/1; lane 9: ratio: 1/0. B) PAGE analysis of a competition experiments employing *KlenTaq* DNA polymerase. The ratio of dT**TP/dTTP was varied. Lane 0: 5'-^{32}P-labeled primer; lane 1: ratio: 0/1; lane 2: ratio: 1/1; lane 3: ratio: 2/1; lane 4: ratio: 4/1; lane 5: ratio: 10/1; lane 6: ratio: 20/1; lane 7: ratio: 50/1; lane 8: ratio: 100/1; lane 9: ratio: 1/0.C) Partial DNA sequences of primer and template for the incorporation of dA**TP and dATP. Evaluation of the incorporation efficiency using dA**TP (■, dashed line)/dATP (•, solid line) mixtures and *KlenTaq* DNA polymerase. D) Partial DNA sequences of primer and template for the incorporation of dT**TP and dTTP. Evaluation of the incorporation efficiency using dT**TP (■, dashed line)/dTTP (•, solid line) mixtures and *KlenTaq* DNA polymerase. E) Overview of the efficiencies of the presented modified nucleotides in competition with their natural counterparts.

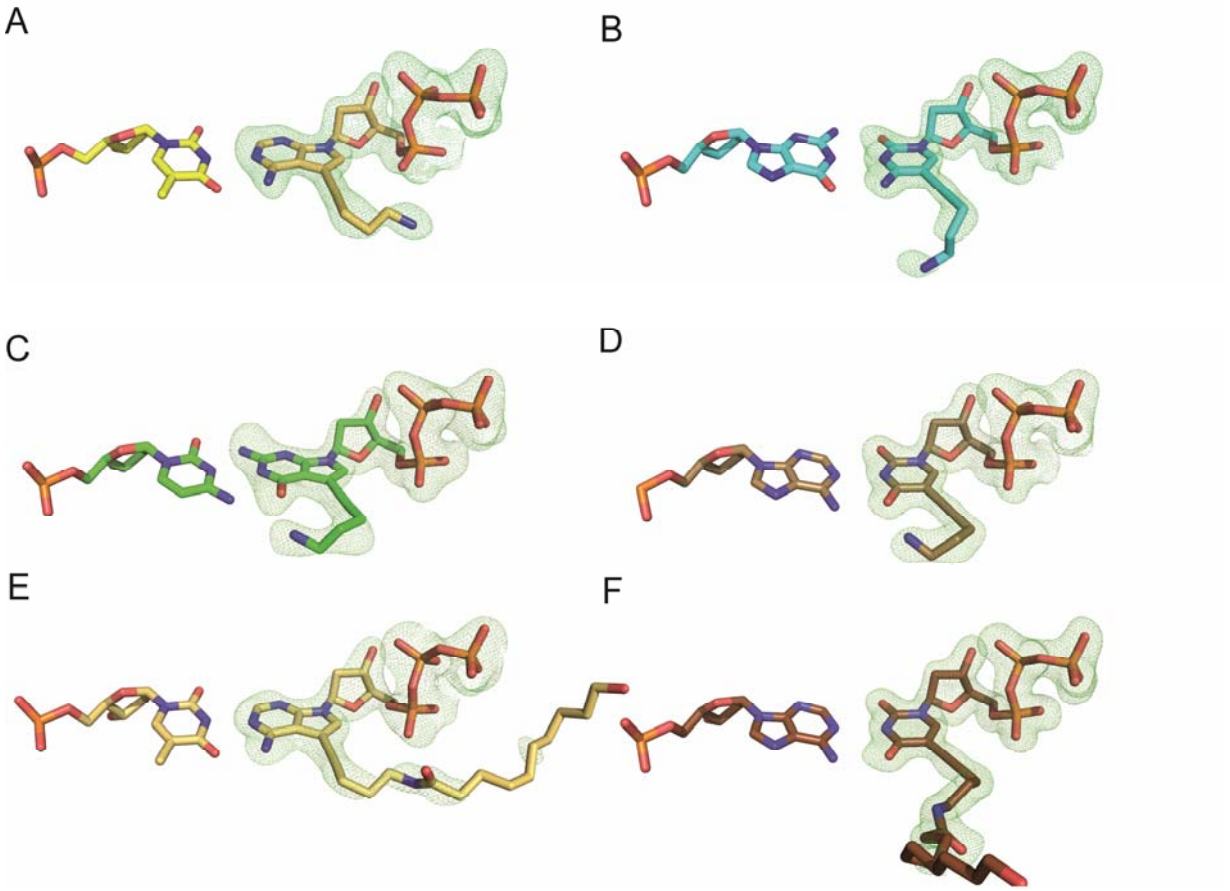


Figure S4 Omit maps for all crystallized triphosphates A) dA*TP, B) dC*TP C) dG*TP D) dT*TP E) dA**TP F) dT**TP. Shown are simulated annealing omit maps at 3σ.

References from the manuscript

(1)(a) Bentley, D. R.; Balasubramanian, S.; Swerdlow, H. P.; Smith, G. P.; Milton, J.; Brown, C. G.; Hall, K. P.; Evers, D. J.; Barnes, C. L.; Bignell, H. R.; Boutell, J. M.; Bryant, J.; Carter, R. J.; Keira Cheetham, R.; Cox, A. J.; Ellis, D. J.; Flatbush, M. R.; Gormley, N. A.; Humphray, S. J.; Irving, L. J.; Karbelashvili, M. S.; Kirk, S. M.; Li, H.; Liu, X.; Maisinger, K. S.; Murray, L. J.; Obradovic, B.; Ost, T.; Parkinson, M. L.; Pratt, M. R.; Rasolonjatovo, I. M.; Reed, M. T.; Rigatti, R.; Rodighiero, C.; Ross, M. T.; Sabot, A.; Sankar, S. V.; Scally, A.; Schroth, G. P.; Smith, M. E.; Smith, V. P.; Spiridou, A.; Torrance, P. E.; Tzonev, S. S.; Vermaas, E. H.; Walter, K.; Wu, X.; Zhang, L.; Alam, M. D.; Anastasi, C.; Aniebo, I. C.; Bailey, D. M.; Bancarz, I. R.; Banerjee, S.; Barbour, S. G.; Baybayan, P. A.; Benoit, V. A.; Benson, K. F.; Bevis, C.; Black, P. J.; Boodhun, A.; Brennan, J. S.; Bridgham, J. A.; Brown, R. C.; Brown, A. A.; Buermann, D. H.; Bundu, A. A.; Burrows, J. C.; Carter, N. P.; Castillo, N.; Chiara, E. C. M.; Chang, S.; Neil Cooley, R.; Crake, N. R.; Dada, O. O.; Diakoumakos, K. D.; Dominguez-Fernandez, B.; Earnshaw, D. J.; Egbujor, U. C.; Elmore, D. W.; Etchin, S. S.; Ewan, M. R.; Fedurco, M.; Fraser, L. J.; Fuentes Fajardo, K. V.; Scott Furey, W.; George, D.; Gietzen, K. J.; Goddard, C. P.; Golda, G. S.; Granieri, P. A.; Green, D. E.; Gustafson, D. L.; Hansen, N. F.; Harnish, K.; Haudenschild, C. D.; Heyer, N. I.; Hims, M. M.; Ho, J. T.; Horgan, A. M. *Nature* **2008**, *456*, 53; (c) Harris, T. D.; Buzby, P. R.; Babcock, H.; Beer, E.; Bowers, J.; Braslavsky, I.; Causey, M.; Colonell, J.; Dimeo, J.; Efcavitch, J. W.; Giladi, E.; Gill, J.; Healy, J.; Jarosz, M.; Lapen, D.; Moulton, K.; Quake, S. R.; Steinmann, K.; Thayer, E.; Tyurina, A.; Ward, R.; Weiss, H.; Xie, Z. *Science* **2008**, *320*, 106.

(3)(a) Guo, J.; Xu, N.; Li, Z.; Zhang, S.; Wu, J.; Kim, D. H.; Sano Marma, M.; Meng, Q.; Cao, H.; Li, X.; Shi, S.; Yu, L.; Kalachikov, S.; Russo, J. J.; Turro, N. J.; Ju, J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 9145.

References

(1) Lebedev, A. V.; Combs, D.; Hogrefe, R. I. *Bioconjug. Chem.* **2007**, *18*, 1530.

(2) (a) Baccaro, A.; Steck, A.-L.; Marx, A. *Angew. Chem., Int. Ed.* **2012**, *51*, 254-257; (b) Seela, F.; Feiling, E.; Gross, J.; Hillenkamp, F.; Ramzaeva, N.; Rosemeyer, H.; Zulauf, M. *J. Biotechnology* **2001**, *86*, 269; (c) Seela, F.; Zulauf, M. *Helv. Chim. Acta* **1999**, *82*, 1878; (d) Ramzaeva, N.; Mittelbach, C.; Seela, F. *Helv. Chim. Acta* **1997**, *80*, 1809.

(3) (a) Betz, K.; Streckenbach, F.; Schnur, A.; Exner, T.; Welte, W.; Diederichs, K.; Marx, A. *Angew. Chem. Int., Ed.* **2010**, *49*, 5181; (b) Summerer, D.; Rudinger, N. Z.; Detmer, I.; Marx, A. *Angew. Chem. Int., Ed.* **2005**, *44*, 4712.

(4) Kabsch, W. *Acta Crystallogr.* **2010**, *D 66*, 125.

(5) Adams, P. D.; et. al. *Acta Crystallogr.* **2010**, *D 66*, 213.

(6) Collaborative Computer Project, Number 4, *Acta Crystallogr.* **1994**, *D 50*, 760.

(7) Emsley, P.; Cowtan, K. *Acta Crystallogr.* **2004**, *D 60*, 2126.

(8) Diederichs, K.; Karplus, P. A. *Nat. Struct. Biol.* **1997**, *4*, 269.

(9) Obeid, S.; Baccaro, A.; Welte, W.; Diederichs, K.; Marx, A. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 21327