Supporting Information

3'-Protected Derivatives of 2'-Deoxynucleoside 5'-Triphosphates as a Novel Tool for Heat-Triggered Activation of PCR

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Synthesis of 3'-protected 2'-deoxynucleosides

General procedure for the synthesis of 3'-O-tetrahydrofuranyl derivatives of 2'-deoxynucleosides

To a solution of 1 mmole of 5'-O-levulinyl protected N^6-benzoyl-2'-deoxyadenosine, or N^2-isobutyryl-2'-deoxyguanosine, or N^4-benzoyl-2'-deoxycytidine or 2'-deoxythymidine, respectively, in 6 mL of dioxane, 0.5 mmol (1.0 mmol for N^2-isobutyryl-2'-deoxyguanosine) of *p*-toluenesulfonic acid monohydrate was added as 0.5M dioxane solution followed by addition of 10 mmoles of 2,3-dihydrofuran (0.76 mL). After 10 min the reaction mixture was poured into 20 mL of saturated $NaHCO_3$ solution and extracted with 20 mL of DCM. Organic layer was evaporated and the resulting residue was treated with 20 mL of 7M methanolic ammonia overnight at room temperature. The mixture was evaporated under reduced pressure and the crude product was purified on silica gel column using DCM:MeOH:TEA mixture as described below. Fractions containing pure product were evaporated at reduced pressure at room temperature, co-evaporated once with MeOH and dried under high vacuum to give a white solid. In the case of 3'-THF-2'-deoxyadenosine and 3'-THF-2'-deoxyguanosine the product after silica gel chromatography was further purified by preparative reverse phase HPLC on Waters µBondaPak C18 column (47x300 mm) using a 0-30% linear gradient of acetonitrile in 50 mM TEAB over 40 min at flow rate of 50 mL/min. Fractions containing pure product were evaporated and dried as above.

Purification and analytical

3'-O-Tetrahydrofuranyl-2'-deoxyadenosine. Initial silica gel purification of crude product was performed on short column using DCM:MeOH:TEA=94.9:5:0.1 v/v/v mixture. Isolated yield after PR HPLC was ~ 50 %. ^{1}H NMR (DMSO-d_6), δ (two diastereomers): 8.34 (s,1H, H-8 of adenine),8.14 (s, 1H, H-2 of adenine), 7.32 (br s,2H,6-NH_2 of adenine), 6.27 (dd,1H, H-1'), 5.32 (br, 1H, 5'-OH), 5.28 (d,1H, H-2 of THF); 4.45 (br m, 1H, H-3'), 4.00 (m, 1H, H-4'), 3.81 (m,2H, H-5A and H-5B of THF), 3.63 and 3.53 (2 m, 2H, H-5' and H5"), 2.80 and 2.43 (2 m, 2H, H-2' and H-2''), 1.90 (m, 2H, H-3A, H-3B); 1.80 (1 m, 2H, H-4A,H-4B of THF). M = 321. LRMS m/z 320$(M-H)^-$, 344 $(M+Na)^+$. UV: λ_{max} (MeOH) 259 nm.

3'-O-Tetrahydrofuranyl-2'-deoxyguanosine. Initial silica gel purification of crude product was performed on short column using DCM:MeOH:TEA=89.9:10:0.1 v/v/v mixture. Isolated yield after PR HPLC was ~ 35 %. ^{1}H NMR (DMSO-d_6), δ (two diastereomers): 10.60 (br s, 1H,1-NH of guanine) 7.92 (two s,1H,H-8 of guanine), 6.46 (s,2H,2-NH_2 of guanine), 6.04 (m,1H, H-1'), 5.25 (m,1H, H-2 of THF), 5.01 (two t,1H, 5'-OH), 4.37 (m,1H,H-3'), 3.92 (m, 1H,H-4'), 3.78 (m, 2H,H-5A and H-5B of THF), 3.53 (m,2H,H-5' and H-5''), 2.52 and 2.35 (2 m, 1H each, H-2' and H-2''), 1.89 (m,2H, H-3A,H-3B of THF), 1.78 (m, 2H, H-4A,H-4B of THF). M = 337. LRMS m/z 336 $(M-H)^-$, 360 $(M+Na)^+$. UV: λ_{max} (MeOH) 253 nm.

3'-O-Tetrahydrofuranyl-2'-deoxycytidine. The product was purified on silica gel column using a gradient of MeOH from 0 to 10% in DCM containing 0.1% of TEA. Isolated yield was ~75%. ^{1}H NMR (DMSO-d_6), δ (two diastereomers): 7.78 (two d,1H, H-6 of cytosine), 7.14 (two br s,2H,4-NH_2 of cytosine), 6.08 (two m,1H, H-1'), 5.72 (two d, 1H,H-5 of cytosine), 5.20 (two s, 1H, H-2 of THF), 5.03 (two br s, 1H, 5'-OH), 4.26 and 4.23 (two m, 1H, H-3'), 3.88 (m, 1H, H-4'), 3.77 (m,2H, H-5A and H-5B of THF), 3.55 (br m, 2H, H-5' and H5"), 2.22 and 1.97 (2 m, 2H, H-2' and H-2''), 1.88 (m, 2H, H-3A, H-3B); 1.78 (1 m, 2H, H-4A,H-4B of THF). M = 297. LRMS m/z 296 $(M-H)^-$, 298 $(M+H)^+$, 320 $(M+Na)^+$. UV: λ_{max} (MeOH) 271 nm.

3'-O-Tetrahydrofuranyl-2'-deoxythymidine. The product was purified on silica gel column using a gradient of MeOH from 0 to 10% in DCM containing 0.1% of TEA. Isolated yield was ~65%. ^{1}H NMR (DMSO-d_6), δ (two diastereomers): 11.30 (s,1H, 3-NH of thymine), 7.70 (two s,1H, H-6 of thymine), 6.09 (m,1H, H-1'), 5.22 (two s, 1H, H-2 of THF), 5.10 and 5.07 (two t, 1H, 5'-OH), 4.30 and 4.27 (two m, 1H, H-3'), 3.87 (m, 1H, H-4'), 3.77 (m,2H, H-5A and H-5B of THF), 3.59 (m, 2H, H-5' and H5"), 2.19 and 2.12 (2 m, 2H, H-2' and H-2''), 1.88 (m, 2H, H-3A, H-3B); 1.77 (1 m, 2H, H-4A,H-4B of THF), 1.77 (s, 3H, 5-CH3 of thymine). M = 312. LRMS m/z 311 $(M-H)^-$, 313 $(M+H)^+$, 335 $(M+Na)^+$. UV: λ_{max} (MeOH) 266 nm.

Synthesis of 3'-O-tetrahydropyranyl and 3'-O-(4-methoxy)tetrahydropyranyl derivatives of 2'-deoxythymidine

3'-O-Tetrahydropyranyl-2'-deoxythymidine and 3'-O-(4-Methoxy)tetrahydropyranyl-2'-deoxythymidine. To a solution of 5'-O-acetyl-2'-deoxythymidine (17) (0.2 g, 0.70 mmol) in 3 mL of dioxane 67 mg (0.35 mmol) of *p*-toluenesulfonic acid monohydrate in 1 mL of dioxane was added followed by 383 µL (4.2 mmol) of 3,4-dihydro-*2H*-pyran or 470 µL (4.2 mmol) of 4-methoxy-5,6-dihydro-*2H*-pyran), respectively. After one hour the reaction mixture was poured into 25 mL of saturated $NaHCO_3$ and the resulting aqueous mixture was extracted with dichloromethane (2 x 10 mL). The organic layers were combined and evaporated to dryness. The resulting residue was dissolved in 10 mL of methanolic ammonia and left to stand at room temperature overnight. The volatiles were removed under reduced pressure and the resulting residue was purified on a silica gel column (DCM:MeOH:TEA = 94.9:5:0.1, v/v/v). Yields were ~ 60% each.

3'-O-Tetrahydropyranyl-2'-deoxythymidine: ^{1}H NMR (DMSO-d_6), δ (two diastereomers): 11.30 (s, 1H, 3-NH of thymine), 7.70 and 7.69 (two d,1H, H-6 of thymine), 6.12 (m,1H, H-1'), 4.69 (two t, 1H, H-2 of THF), 5.10 and 5.09 (two t, 1H, 5'-OH), 4.37 and 4.32 (two m, 1H, H-3'), 3.95 and 3.91 (two m, 1H, H-4'), 3.75 and 3.60 (m,2H, H-6A and H-6B of THP), 2.21 and 2.10 (2 m, 2H, H-2' and H-2''), 1.77 (s, 3H, 5-CH3 of thymine), 1.72, 1.64, 1.47 (three m, 6H, H-3A, H-3B, H-4A, H-4B, H-5A, H-5B). M = 326; LRMS m/z 325 $(M-H)^-$, 349 $(M+Na)^+$. UV: λ_{max} (MeOH) 266 nm.

3'-O-(4-Methoxy)tetrahydropyranyl-2'-deoxythymidine: ^{1}H NMR (DMSO-d_6), δ: 11.30 (s,1H, 3-NH of thymine), 7.68 (d,1H, H-6 of thymine), 6.14 (m,1H, H-1'), 5.10 (t, 1H, 5'-OH), 4.46 (m, 1H, H-3'), 3.90 (m, 1H, H-4'), 3.46-3.64 (three m, 6H, H-3A , H-3B, H-5A and H-5B of MTHP; H-5' and H5"), 3.13 (s, 3H, OCH_3 of MTHP), 2.17 (m, 2H, H-2' and H-2''), 1.77 (s, 3H, 5-CH3 of thymine). 1.75 and 1.67 (two m, 4H, H-2A, H-2B, H-6A, and H-4B of MTHP); M = 356; LRMS m/z 355 $(M-H)^-$, 389 $(M+Na)^+$. UV: λ_{max} (MeOH) 266 nm.

Synthesis of 3'-ester derivatives of 2'-deoxythymidine

3'-O-Methoxyacetyl-2'-deoxythymidine. A solution of 5'-O-[4,4'-dimethoxytrityl]-2'-deoxythymidine (0.3 g, 0.55 mmoles) and triethylamine (75 µL, 0.55 mmoles) in 3 mL of pyridine was treated with 55 µL (0.6 mmol) of methoxyacetylchloride overnight at room temperature. Pyridine was removed under reduced pressure and the resulting residue was dissolved in 10 mL of DCM and extracted with 10 mL of $NaHCO_3$. The organic layer was dried down with sodium sulfate, evaporated and the resulting foam was treated with 50 mL of 2% trifluoroacetic acid in DCM for 1 hour. The reaction mixture was extracted with 50 mL of $NaHCO_3$, the organic layer was dried with anhydrous sodium sulfate and purified on silica gel column (2 x 10 cm) using DCM:MeOH=95:5 as eluent to yield the product as a white solid (120 mg; 69% yield). ^{1}H NMR (DMSO-d_6), δ: 11.34 (s, 1H, 3-NH of thymine); 7.73 (m, 1H, H6 of thymine); 6.18 (m, 1H, H-1'); 5.31 (m, 1H,H-3'); 5.23 (t, 1H, 5'-OH); 4.10 (s, 2H, CH_2 of methoxyacetyl group); 4.00 (m, 1H, H-4'); 3.63 (m, 2H,H-5' and H-5''), 3.32 (s, 3H, CH_3 of methoxyacetyl group); 2.24 – 2.51 (mm, 2H, H-2' and H-2''); 1.78 (d, 3H, CH_3 of thymine). M = 314. LRMS m/z 313 $(M-H)^-$, 337 $(M+Na)^+$. UV: λ_{max} (MeOH) 266 nm.

3'-O-Phenoxyacetyl-2'-deoxythymidine. A solution of 5'-O-[4,4'-dimethoxytrityl]-2'-deoxythymidine (2.7 g, 5 mmole) and triethylamine (700 µL, 5 mmole) in 20 mL of pyridine was treated with 1.5 g (5.2 mmole) of phenoxyacetyl anhydride overnight at room temperature. Pyridine was removed under reduced pressure and the resulting residue was dissolved in 50 mL

of DCM and extracted with 50 mL of saturated solution of $NaHCO_3$. The DCM layer was evaporated and the resulting foam was treated with 150 mL of 2% trifluoroacetic acid in DCM for 1 hour. The reaction mixture was extracted with 150 mL of of saturated solution of $NaHCO_3$, the organic layer was dried with anhydrous sodium sulfate and purified on silica gel column (3 x 30 cm) using DCM:MeOH=95:5 as eluent to give 1.0 g (2.7 mmole, 53 % yield) of product. ^{1}H NMR (DMSO-d_6), δ: 11.35 (s, 1H, 3-NH of thymine); 7.73 (m, 1H, H6 of thymine); 7.29 and 6.96 (m, 2H and m, 3H, C_6H_5 of phenoxyacetyl group); 6.20 (m, 1H, H-1'); 5.34 (m,1H, H-3'); 5.23 (t,1H, 5'-OH); 4.85 (s, 2H, CH_2 of phenoxyacetyl group); 4.01 (m, 1H, H-4'); 3.63 (m, 2H,H-5' and H-5''), 2.26 – 2.51 (mm, 2H, H-2' and H-2''); 1.78 (d, 3H, CH_3 of thymine). M = 376. LRMS m/z, 375 $(M-H)^-$, 399 $(M+Na)^+$. UV: λ_{max} (MeOH) 267 nm.

5'-Triphosphorylation of 3'-THF 2'-deoxynucleosides

The 5'-triphosphorylation of 3'-THF derivatives of 2'-deoxynucleosides was performed at 0.5 mmole scale according to the procedure described by Ludwig and Eckstein [1]. All reactions were performed in pyridine:dioxane (1:1 mixture) with exception of 3'-THF-2'-deoxyguanosine which was reacted in pyridine:DMF (1:1 mixture). The crude products were purified on 2.6 x 40 cm column packed with DEAE Sephadex-A25 using a linear gradient of TEAB pH 8.5 (0-1M; 2L). The fractions with >98% purity (by analytical anion exchange and reverse phase HPLC) were pooled and purified from inorganic pyrophosphate by reverse phase HPLC on Waters µBondaPak C18 15 µ column (47x300 mm) using linear gradient of acetonitrile in 100 mM TEAB pH 8.5 (0-50% over 40 min) with flow rate of 50.0 mL/min. Fractions containing product were pooled and evaporated once to remove acetonitrile and most of the TEAB. The conversion of $TEAH^+$ salt of the 3'-THF dNTPs to a sodium salt form was performed using the same µBondaPak column. Each compound was loaded onto the column equilibrated in 100 mM NaCl, washed with two column volumes of 100 mM NaCl and eluted from the column using water. All compounds were concentrated on Rotavap at reduced pressure to 50 mM solution in water, filtered and stored at –20^{o}C. Structures and purity of synthesized 3'-THF 2'-deoxynucleoside 5'-triphosphates were confirmed by NMR, MS, UV and HPLC analyses. Isolated yields of 3'-THF dNTPs were from 25 to 40%.

3'-O-Tetrahydrofuranyl-2'-deoxyadenosine 5'-triphosphate. Isolated yield: 28%. ^{1}H NMR (D_2O), δ (two diastereomers): 8.49 and 8.46 (two s,1H, H-8 of adenine), 8.21 (s, 1H, H-2 of adenine), 6.45(m,1H, H-1'), 5.44 (two d,1H, H-2 of THF); 4.70 and 4.67 (two br m, 1H, H-3'), 4.41 and 4.37 (two br m, 1H, H-4'), 4.17 (m,2H, H-5A and H-5B of THF), 4.00 and 3.91 (two m, 2H, H-5' and H5"), 2.80 and 2.69 (two m, 2H, H-2' and H-2''), 1.92 – 2.05 (br m, 4H, H-3A, H-3B, H-4A,H-4B of THF). ^{31}P NMR (D_2O) δ (two diastereomers): -5.02 (d, P_γ); -10.37 and -10.38 (two d, P_α); -21.19 and -21.17 (two t, P_β) M = 561.3. LRMS m/z 560 $(M-H)^-$, 583 $(M-2H+Na)^-$. UV: λ_{max} (water) 259 nm.

3'-O-Tetrahydrofuranyl-2'-deoxyguanosine 5'-triphosphate. Isolated yield: 35%. ^{1}H NMR (D_2O), δ (two diastereomers): 8.07 and 8.047 (two s,1H, H-8 of guanine), 6.26 ((m,1H, H-1'), 5.45 and 5.42 (two d,1H, H-2 of THF), 4.67 and 4.65 (two m,1H,H-3'), 4.37 and 4.33 (br m, 1H, H-4'), 4.17(m, 2H, H-5A and H-5B of THF), 4.01 and 3.91 (two m, 2H each, H-5' and H-5''), 2.77 and 2.59 (two m, 1H each, H-2' and H-2''), 2.04 and 1.96 (two m, 2H each, H-3A, H-3B, H-4A, H-4B of THF). ^{31}P NMR (D_2O) δ (two diastereomers): -4.79 and -4.82 (two d, P_γ); -10.15 and -10.17 (two d, P_α); -20.64 and -20.69 (two t, P_β) M = 577.3. LRMS m/z 577 $(M-H)^-$, 610 $(M-2H+Na)^-$. UV: λ_{max} (water) 252 nm.

3'-O-Tetrahydrofuranyl-2'-deoxythymidine 5'-triphosphate. Isolated yield: 39%. ^{1}H NMR (D_2O), δ (two diastereomers): 7.76 and 7.72 (two s,1H, H-6 of thymine), 6.29 (m,1H, H-1'), 5.41 and 5.38 (two d, 1H, H-2 of THF), 4.59 and 4.55 (two br m, 1H, H-3'), 4.31 and 4.26 (two br s, 1H, H-4'), 4.20 (br m,2H, H-5A and H-5B of THF), 3.98 and 3.90 (two m, 2H, H-5' and H5"), 2.33 - 2.45 (two m, 2H, H-2' and H-2''), 2.03 (m, 2H, H-3A, H-3B); 1.92 (m, 2H, H-4A,H-4B of THF), 1.92 (s, 3H, 5-CH3 of thymine): ^{31}P NMR (D_2O) δ (two diastereomers): -5.32 (d, P_γ); -10.61 and -10.63 (two d, P_α); -20.10 and -20.12 (two t, P_β) M = 552. LRMS m/z 551 $(M-H)^-$, 573 $(M-2H+Na)^-$. UV: λ_{max} (water) 266 nm.

3'-O-Tetrahydrofuranyl-2'-deoxycytidine 5'-triphosphate. Isolated yield: 26%. ^{1}H NMR (D_2O), δ (two diastereomers): 7.96 and 7.94 (two d, 1H, H-6 of cytosine), 6.28 and 6.27 (two t,1H, H-1'), 6.14 and 6.13 (two d, 1H, H-5 of cytosine),5.38 and 5.37 (two m, 1H, H-2 of THF), 4.54 and 4.51 (two m, 1H, H-3'), 4.32 and 4.27 (two m, 1H, H-4'), 4.19 (m, 2H, H-5A and H-5B of THF), 3.97 and 3.89 (two m, 2H, H-5' and H5"), 2.50 and 2.27 (two m, 2H, H-2' and H-2''), 2.00 (m, 2H, H-3A, H-3B of THF); 1.92 (m, 2H, H-4A,H-4B of THF). ^{31}P NMR (D_2O) δ (two diastereomers): -6.32 (d, P_γ); -10.47 (d, P_α); -21.43 (t, P_β). M = 537. LRMS m/z 536 $(M-H)^-$, 558 $(M-2H+Na)^-$. UV: λ_{max} (water) 271 nm.

5'-Triphosphorylation of 3'-protected 2'-deoxythymidine derivatives

The 5'-triphosphorylation of other 3' derivatives of 2'-deoxythymidine was performed at 100 µmole scale as described above for 3'-THF protected 2'-deoxynucleosides.

The crude 3'-ether protected dTTPs were purified on Waters DeltaPak C18 15 µ column (19x300 mm) using linear gradient of acetonitrile in 100 mM TEAB pH 8.5 (0-50% over 40 min) with flow rate of 8.0 mL/min. The fractions with >98% purity (by analytical anion exchange and reverse phase HPLC) were converted to a sodium salt form on the same DeltaPak column (19x300 mm) using similar conditions as described above for 3'-THF dNTPs.

The crude 3'-ester protected dTTPs were purified on Waters DeltaPak C18 15 µ column (19x300 mm) using linear gradient of acetonitrile in 50 mM KH_2PO_4 pH 5.5 (0-50% over 40 min) with flow rate of 8.0 mL/min. The fractions with >98% purity (by analytical anion exchange and reverse phase HPLC) were converted to a sodium salt form on the same DeltaPak column (19x300 mm) using similar conditions as described above for 3'-THF dNTPs while keeping a pH of all solutions at 6.0. The compounds were eluted from the column with 30% acetonitrile in water, pH 6.0.

All compounds were concentrated on Rotavap at reduced pressure to 50 mM solution in water, filtered through 0.45 µ membrane filter and stored at –20°C. Structures and purity of synthesized 3'-protected 2'-deoxythymidine 5'-triphosphate derivatives were confirmed by MS, UV and HPLC analyses. The isolated yields of 3'-protected dTTPs were 20-30%.

3'-O-Tetrahydropyranyl-2'-deoxythymidine 5'-triphosphate M = 566. LRMS m/z 565 $(M-H)^-$, 587 $(M-2H+Na)^-$. UV: λ_{max} (MeOH) 266 nm.

3'-O-(4-Methoxytetrahydropyranyl)-2'-deoxythymidine 5'-triphosphate. M = 596. LRMS m/z 595 $(M-H)^-$, 617 $(M-2H+Na)^-$. UV: λ_{max} (MeOH) 266 nm.

3'-O-Acetyl-2'-deoxythymidine 5'-triphosphate. M = 524. LRMS m/z 523 $(M-H)^-$, 545 $(M-2H+Na)^-$. UV: λ_{max} (MeOH) 266 nm.

3'-O-Methoxyacetyl-2'-deoxythymidine 5'-triphosphate. M = 554. LRMS m/z 553 $(M-H)^-$, 575 $(M-2H+Na)^-$. UV: λ_{max} (MeOH) 266 nm.

3'-O-Phenoxyacetyl-2'-deoxythymidine 5'-triphosphate. M = 616 LRMS m/z 615 $(M-H)^-$, 637 $(M-2H+Na)^-$. UV: λ_{max} (MeOH) 267 nm.

Reference

1. Ludwig, J. and Eckstein, F. (1989) Rapid and efficient synthesis of nucleoside 5'-O-(1-thiotriphosphates), 5'-triphosphates and 2',3'-cyclophosphorothioates using 2-chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one. *J. Org. Chem.,* **54**, 631-635.

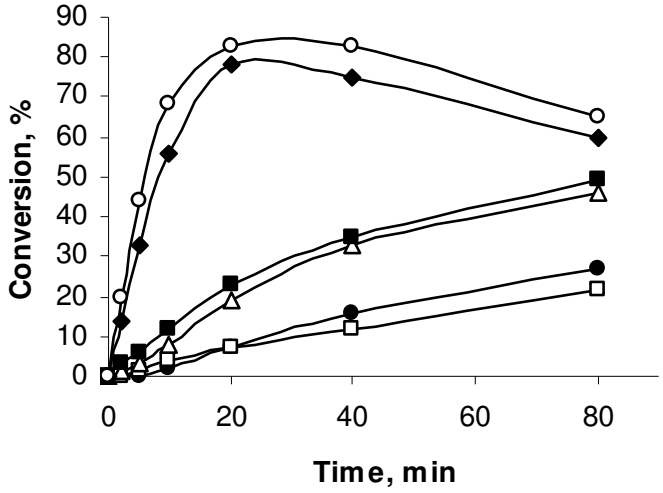

Figure S-1. Kinetics of accumulation of unprotected dTTP in PCR buffer at 95°C during incubation of 3'-protected dTTPs at 1 mM starting concentration: 3'-Pac dTTP(○), 3'-Mac dTTP(♦), 3'-THF dTTP(■), 3'-MTHP dTTP(Δ), 3'-Ac dTTP(●) and 3'-THP dTTP(□). Estimated half-reaction times: 6 min (Pac), 8 min (Mac), 80 min (THF), 100 min (MTHP), 200 min (Ac), 250 min (THP). PCR buffer: 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.4 at 25 ºC.

Estimation of the number of unprotected dNTP molecules generated from 3'-THF dNTPs during typical PCR amplification. The estimation is based on the kinetic data for 3'-THF dTTP obtained as described above at 95°C and at simulated PCR conditions (Figure 2 and Table). Since the PCR is not an isothermal process, the following calculations are dependent on the instrumentation and actual PCR cycle sequence used and should be considered as an approximation only. Assume that amount of DNA target molecules present in initial PCR sample is 10 copies. The upper theoretical limit of the amplicon molecules produced during the PCR would be: 10^4 molecules after 10 cycles; 10^7 molecules after 20 cycles; 10^{10} molecules after 30 cycles; 10^{13} molecules after 40 cycles. If a typical size of the DNA target sequence is 500 bp then the number of total dNTP molecules needed for the synthesis of 500 base long amplicon would be: $5x10^6$ molecules for 10 PCR cycles; $5x10^9$ for 20 cycles; $5x10^{12}$ for 30 cycles and $5x10^{15}$ for 40 cycles. A typical starting concentration of the dNTP in the PCR reaction mixture is 800 µM (200 µM each) or $2.4x10^{16}$ molecules. If at time "zero" all dNTP molecules contained 3'-THF protecting group then after 5 min initial activation step (at 95°C) there will be generated ~$1.6x10^{15}$ molecules of unprotected dNTPs available for the amplicon synthesis at the start of the first PCR cycle. That number of dNTP molecules is sufficient to support PCR amplification for at least 30 cycles. During following 30 PCR cycles another ~$7.2x10^{16}$ molecules of the unprotected dNTPs is generated. This amount of dNTPs should support PCR amplification up to 40 cycles and is expected to be sufficient for most PCR applications.