SUPPORTING INFORMATION

Incorporation of 4'-*C*-aminomethyl-2'-*O*-methylthymidine into DNA by thermophilic DNA polymerases

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General methods

Dry solvents (THF, DMF) were obtained from commercial sources. CH_3CN and DCM were dried using calcium hydride. Thin layer chromatography (TLC) was performed on silica gel plates pre-coated with fluorescent indicator with visualization by UV light or by dipping into a solution of 5% (v/v) conc. H_2SO_4 in ethanol and heating. Column chromatography was performed by using silica gel (100-200 mesh). ^{1}H NMR (300 or 400 MHz), ^{13}C NMR (100 MHz), ^{19}F NMR (376 MHz), and ^{31}P NMR (121 MHz) were recorded on 300 or 400 MHz instruments. The chemical shifts in parts per million (δ) are reported downfield from TMS (0 ppm), or referenced to methanol-d4 (3.31 ppm) or D_2O (4.75) for ^{1}H NMR spectra, and $CDCl_3$ (77.2 ppm) or methanol-d4 (49.1 ppm) for ^{13}C NMR spectra. Multiplicities of ^{1}H NMR spin couplings are reported as s for singlet, bs for broad singlet, d for doublet, t for triplet, q for quartet, dd for doublet of doublet, ABq for AB quartet, AXq for AX quartet, or m for multiplet and overlapping spin systems. Values for apparent coupling constants (*J*) are reported in Hz. High resolution mass spectra (HRMS) for nucleoside intermediates were obtained in positive ion electrospray ionization (ESI) mode. ESI-MS for triphosphates were obtained in negative ion mode. Anion exchange chromatography was performed on a self-packed DEAE Sephadex A25 column (3 x 22 cm). Reversed-phase HPLC was performed on a EC 250/4 Nucleosil 100-5 C18 HD column (4 x 250mm). Chromatography was monitored by UV absorbance at 280 nm. The primer and template oligonucleotides were from Sigma Aldrich. [γ-^{32}P]-ATP (~3000 Ci/mmol) was from Perkin Elmer. T4 polynucleotide kinase and Pfu DNA polymerase (recombinant; from *E. coli* with a cloned gene from of *Pyrococcus furiosus*) were from Fermentas. Taq DNA polymerase (recombinant; from *E.coli* with a cloned gene from *Thermus aquatius*), Vent exo$^-$ polymerase (genetically engineered form of polymerase from *Thermococcus litoralis*), Klenow fragment (from *E.coli* DNA polymerase I), Therminator and Therminator III polymerases (engineered forms of DNA polymerase from *Thermococcus species* 9°N-7) were from New England Biolabs. KOD (recombinant form of *Thermococcus kodkaraensis*) DNA polymerase was from Novagen. Phusion polymerase (*Pyrococcus*-like enzyme with processivity-enhancing domain) was from Finnzymes. Phosphodiesterase I Type IV from Crotalus atrox (Snake venom phosphodiesterase, SVPD) was from Sigma Aldrich.

Synthesis procedures

2'-*O*-Acetyl-4'-*C*-azidomethyl-3',5'-di-*O*-benzyl-3-(4-methoxybenzyl)thymidine (3)

To a mixture of compound **2** (2.84 g, 5.32 mmol) and 4-methoxybenzyl chloride (1.32 mL, 9.47 mmol) in CH_3CN (40 mL), was added DBU (1.51 mL, 10.09 mmol) at 0 °C and the mixture was stirred at room temperature for 7 h. After cooling to 0 °C, the mixture was diluted with 5% $NaHSO_4$ solution (10 mL). The organic phase was separated, and the aqueous phase was extracted with DCM (3 x 100 mL). The combined organic layer was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give a light yellow residue. The residue was purified by column chromatography (12 % ethyl acetate in pet ether) to give compound **3** as white oily liquid. Yield: 3.14 g (90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43−7.24 (m, 13H), 6.82−6.79 (m, 2H), 6.25 (d, *J* = 5 Hz, 1H), 5.45 (t, *J* = 5 Hz, 1H), 5.06, 4.97 (ABq, *J* = 13.6 Hz, 2H), 4.63, 4.46 (AXq, *J* = 11.6 Hz, 2H), 4.43 (d, *J* = 5.8 Hz, 1H), 4.51, 4.43 (ABq, *J* = 11.3 Hz, 2H), 3.77, 3.49 (AXq, *J* = 10.3 Hz, 2H), 3.76 (s, 3H), 3.67, 3.37 (AXq, *J* = 13.3 Hz, 2H), 2.10 (s, 3H), 1.55 (d, *J* = 1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$)δ: 170.1, 163.4, 159.2, 151.2, 137.3, 137.2, 133.9, 130.9, 130.8, 129.2, 128.9, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 127.9, 113.8, 110.9, 87.9, 87.2, 75.1, 74.7, 73.9, 71.4, 55.4, 52.9, 44.1, 20.9, 13.0. ESI-HRMS (m/z): $[M + H]^+$ calcd. for $C_{35}H_{39}N_5O_8$ = 656.2720, found = 656.2737 (Δm + 0.0017, error +2.6 ppm).

4'-*C*-Azidomethyl-3',5'-di-*O*-benzyl-3-(4-methoxybenzyl)-2'-*O*-methylthymidine (4)

Compound **3** (3.14 g, 4.79 mmol) was dissolved in dry methanol (31.4 mL), to which 1 N solution of NaOMe in methanol (8.94 mL) was added, and stirred at room temperature for 3 h. The solvent was partially evaporated under reduced pressure and extracted with DCM (3 x 100 mL). The combined organic phase was washed with brine (100 mL), dried over anhydrous Na_2SO_4 and concentrated to get crude hydroxy nucleoside. To a stirred solution of crude hydroxy compound in dry THF (45 mL) at 0 ^oC was added NaH (270 mg, 11.25 mmol), and the reaction mixture was stirred for 5 min at 0 ^oC under nitrogen atmosphere. Methyl iodide (0.47 mL, 7.51 mmol) was added drop wise under nitrogen atmosphere, stirred for 3 h at room temperature. Saturated $NaHCO_3$ solution (100 mL) was added to the reaction mixture and organic phase was separated. The aqueous phase was extracted with ethyl acetate (3 x 100 mL). The combined organic layer was washed with brine (50 mL), and dried over anhydrous Na_2SO_4, concentrated under reduced pressure and purified by column chromatography (12% ethyl acetate in pet ether) to get compound **4** as yellow oily liquid. Yield: 2.90 g (90%). ^1H NMR(400 MHz, $CDCl_3$): δ 7.62 (d, *J* = 1.3 Hz, 1H), 7.45−7.19 (m, 12H), 6.83−6.79 (m, 2H), 6.11 (d, *J* = 2.3 Hz, 1H), 5.08, 4.98 (ABq, *J* = 13.6 Hz, 2H), 4.72, 4.46 (AXq, *J* = 11.7 Hz, 2H), 4.55, 4.47 (ABq, *J* = 11.4 Hz, 2H), 4.33 (d, *J* = 5.6 Hz, 1H), 3.93, 3.53 (AXq, *J* = 10.6 Hz, 2H), 3.92, 3.31 (AXq, *J* = 13.4 Hz, 2H), 3.82 (dd, *J* = 13.4 Hz, 2.3 Hz, 1H), 3.76 (s, 3H), 3.57 (s, 3H) 1.46 (d, *J* = 1.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.4, 159.1, 150.9, 137.4, 137.2, 133.8, 130.9, 129.3, 128.7, 128.6, 128.3, 127.9, 127.8, 113.8, 110.2, 89.2, 87.2, 83.7, 76.1, 73.8, 73.4, 70.4, 59.5, 55.3, 53.3, 43.9,12.8. ESI-HRMS (m/z): $[M + H]^+$ calcd. for $C_{34}H_{38}N_5O_7$ = 628.2771, found = 628.2749 (Δm −0.0022, error −3.5 ppm).

4'-*C*-Azidomethyl-3',5'-di-*O*-benzyl-2'-*O*-methylthymidine (5)

Compound **4** (2.9 g, 4.63 mmol) was dissolved in CH_3CN-H_2O mixture (60 mL, 9:1), and ceric ammonium nitrate (10.35 g, 18.9 mmol) was added. The reaction mixture was stirred at room temperature for 50 h. Saturated aqueous $NaHCO_3$ (100 mL) was added to the reaction mixture, stirred for an additional 20 min, then filtered and extracted with DCM (4 x 100 mL). The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography (25% ethyl acetate in pet ether) to give compound **5** as white solid. Yield: 1.5 g (64 %). ^1H NMR (400 MHz, $CDCl_3$): δ 9.20 (bs, 1H), 7.67 (d, *J* = 1.5 Hz, 1H), 7.44−7.20 (m, 10), 6.06 (d, *J* = 2.2 Hz, 1H), 4.73, 4.49 (AXq, *J* = 11.6 Hz, 2H), 4.57, 4.49 (ABq, *J* = 11.6 Hz, 2H), 4.34 (d, *J* = 5.6 Hz, 1H), 3.95, 3.57 (AXq, *J* = 10.6 Hz, 2H), 3.93, 3.32 (AXq, *J* = 13.6 Hz, 2H), 3.85 (dd, *J* = 5.9 Hz, 2.2 Hz, 1H), 3.56 (s, 3H), 1.44 (d, *J* = 1.1 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.9, 161.9, 150.2, 149.5, 146.6, 137.4, 137.2, 135.9, 130.9, 128.8, 128.7, 128.6, 128.4, 128.2, 127.9, 110.9, 88.6,87.4, 83.6, 76.1, 73.8, 73.4, 70.1, 59.4, 53.2, 12.0. ESI−HRMS (m/z): $[M + H]^+$ calcd. for $C_{26}H_{30}N_5O_6$ = 508.2196, found = 508.2191 (Δm −0.0005, error −1.0 ppm).

3',5'-Di-*O*-benzyl-4'-*C*-(trifluoroacetyl)aminomethyl-2'-*O*-methylthymidine (6)

Nucleoside **5** (100 mg, 0.19 mmol) was dissolved in THF (1.2 mL) and then H_2O (0.017 mL) and PPh_3 (103 mg, 0.39 mmol) were added. The reaction mixture was heated at 45 °C for 4 h, and subsequently cooled to 0 °C. Then $EtOCOCF_3$ (0.12 mL, 0.99 mmol) and Et_3N (0.14 mL, 0.99 mmol) were added, and the reaction mixture was stirred at room temperature for 16 h. The solvent was evaporated under reduced pressure. The crude compound was purified by column chromatography (1% MeOH in DCM) to get compound **6** as white solid. Yield: 80 mg (78 %). ^1H NMR (400 MHz, $CDCl_3$): δ 9.6 (bs, 1H), 7.61 (bs, 1H), 7.54 (d, *J* = 1 Hz, 1H), 7.40−7.30 (m, 10H), 5.92 (d, *J* = 1 Hz 1H), 4.73, 4.46 (AXq, *J* = 11.6 Hz, 2H), 4.52, 4.47 (ABq, *J* = 11.6 Hz, 2H), 4.46 (d, *J* = 6.1 Hz, 1H), 3.94 (dd, *J* = 6.3 Hz, 1.5 Hz, 1H), 3.79, 3.74 (ABq, *J* = 14.2 Hz, 2H), 3.71, 3.52 (AXq, *J* = 10.5 Hz, 2H), 3.57 (s, 3H), 1.47 (s, 3H). ^{13}C NMR (100

MHz, $CDCl_3$): δ 163.9, 157.8, 150.2, 137.0, 136.3, 133.1, 128.8, 128.8, 128.6, 128.7, 128.6, 128.4, 128.3, 128.1, 127.9, 111.0, 90.4, 86.9, 83.2, 76.2, 73.8, 73.4, 71.0, 59.5, 41.6, 12.0. ^{19}F NMR (376.4 MHz, $CDCl_3$): δ −75.9. HRMS: (m/z): $[M + H]^+$ calcd. for $C_{28}H_{31}F_3N_3O_7$ = 578.2114, found = 578.2133 (Δm +0.0019, error +3.3 ppm).

4'-*C*-(Trifluoroacetyl)aminomethyl-2'-*O*-methylthymidine (7)

Compound **6** (100 mg, 0.173 mmol) was dissolved in EtOH (2.8 mL), then 10% Pd/C (42.4 mg) was added and the mixture was heated at 70 °C for 48 h under H_2 atmosphere. The reaction mixture was filtered through celite pad and washed with MeOH (50 mL). The MeOH layer was evaporated and purified by column chromatography (6% MeOH in DCM) to give compound **7** as white solid compound. Yield: 46 mg (67 %). ^{1}H NMR (400 MHz, CD_3OD): δ 7.87 (s, 1H), 6.05 (d, *J* = 4.8Hz, 1H), 4.52 (d, *J* = 5.9 Hz, 1H), 4.03 (t, *J* = 5.1 Hz, 1H), 3.66, 3.62 (ABq, *J* = 11.6 Hz, 2H), 3.66, 3.59 (ABq, *J* = 14.5 Hz, 2H), 3.49 (s, 3H), 1.87 (d, *J* = 0.8 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 166.4, 159.5, 152.4, 138.4, 117.7, 111.8, 89.0, 88.9, 85.0, 71.2, 64.4, 59.2, 42.1, 12.5. ^{19}F NMR (376.4 MHz, CD_3OD): δ −76.9. ESI−HRMS (m/z): $[M + H]^+$ calcd. for $C_{14}H_{19}F_3N_3O_7$ = 398.1175, found = 398.1182 (Δm +0.0007, error +1.8 ppm).

4'-*C*-(Trifluoroacetyl)aminomethyl-2'-*O*-methylthymidine 5'-triphosphate triethylammonium salt (8)

The nucleoside **7** (35 mg, 0.09 mmol) and proton sponge (28.4 mg, 0.13 mmol) were dried together overnight under high vacuum, and dissolved in trimethyl phosphate (1.5 mL). The reaction mixture was cooled to −20 °C, and freshly distilled $POCl_3$ (0.014 mL, 0.15 mmol) was added. The temperature was maintained at −13 °C to −20 °C for 3 h. Then a solution of bis(tetra-n-butylammonium) dihydrogen pyrophosphate in anhydrous DMF (1 M, 0.60 mL) and tributylamine (0.15 mL, 0.63 mmol) were added, while the reaction mixture was kept on ice. After 1.5 h, the reaction was quenched by addition of triethylammonium bicarbonate (TEAB) buffer (12 mL, 1 M, pH 7.5), warmed up to room temperature and washed with ethyl acetate (3 x 15 mL). The aqueous phase was concentrated, and the residue was purified by anion exchange chromatography using a linear gradient from 0.1 to 1 M of TEAB buffer (pH 7.5). The product-containing fractions were pooled and evaporated, and further purified by RP-HPLC at 35 °C using the following mobile phases: A: 25 mM triethylammonium acetate (TEAA) buffer (pH 6.8), B: CH_3CN:TEAA buffer (20 mM, pH 6.8), 4:1. Flow rate: 1 mL/min. Gradient: 0% − 20% B in A in 60 min. The product was lyophilized overnight to remove the volatile components, resulting in the triethylammonium salt of triphosphate **8** as white residue. Yield: 0.016 mmol (17%, determined by UV absorbance at 260 nm, using ε^{260} 8800 $l.mol^{-1}.cm^{-1}$). ^{1}H NMR (400 MHz, D_2O): δ 7.72 (bs, 1H), 6.17 (d, *J* = 5.6 Hz, 1H), 4.85 (d, *J* = 6 Hz, 1H), 4.29, 4.15 (AXq, *J* = 11.2 Hz, 2H), 4.19 (d, *J* = 5.3 Hz, 1H), 4.15−4.08 (m, 1H), 4.05, 3.61 (AXq, *J* = 14.6 Hz, 2H), 3.56 (s, 3H), 2.0 (d, *J* = 4.8 Hz, 3H). ^{31}P NMR (121.5 MHz, D_2O): δ −9.48 (d, *J* = 17.1 Hz, 1P(γ)), −11.7 (d, *J* = 19.7 Hz, 1P(α)), −22.51 (t, *J* = 19.6 Hz, 1P(β)). ESI−MS (m/z): $[M-H]^-$ calcd. for m/z $C_{14}H_{21}F_3N_3O_{16}P_3$ 636.2, found 636.2.

4'-*C*-aminomethyl-2'-*O*-methylthymidine 5'-triphosphate triethylammonium salt (1)

The nucleoside triphosphate **8** (1.4 μmol) was dissolved in a mixture of H_2O/MeOH/NEt_3 (7/3/1 (v/v), 750 μL) and incubated at room temperature for 14 h. The volume was reduced to 100 μL by a flow of nitrogen, and the deprotected product was isolated by RP-HPLC at 35 °C using the same mobile phase as described above for the isolation of **8**. A: 25 mM TEAA, pH 6.8, B: CH_3CN:TEAA (20 mM, pH 6.8), 4:1. The product-containing fractions were lyophilized overnight to remove the volatile components, resulting in the triethylammonium salt of triphosphate **1** as white residue. Yield: 1.0 μmol (71%, determined by UV absorbance at 260 nm, using ε^{260} 8800 $l.mol^{-1}.cm^{-1}$). ^{1}H NMR (400 MHz, D_2O): δ 7.60 (d, *J* = 1.2 Hz, 1H), 6.11 (d, *J* = 7.0 Hz, 1H), 4.71 (d, *J* = 5.5 Hz, 1H), 4.33 (dd, *J* = 6.7 Hz, 5.5 Hz, 1H),

4.15 (dd, *J* = 4.5 Hz, 1.4 Hz, 2H), 3.53, 3.33 (ABq, *J* = 16 Hz, 2H), 3.45 (s, 3H), 1.92 (d, *J* = 1.2 Hz, 3H). ^{31}P NMR (162 MHz, D_2O): δ −10.43 (d, *J* = 16 Hz, 1P(γ)), −12.15 (d, *J* = 19.5 Hz, 1P(α)), −23.02 (t, *J* = 19.7 Hz, 1P(β)). ESI−HRMS (m/z): $[M-H]^-$ calcd. for m/z $C_{12}H_{21}N_3O_{15}P_3$ = 540.0186, found 540.0191 (Δm +0.0005, error +0.9 ppm).

Primer extension assays

The DNA primer (35 pmol) was 5'-^{32}P labelled by [γ-^{32}P]-ATP (10 μCi) using T4 polynucleotide kinase (PNK) (5 units) in PNK buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM each spermidine and EDTA, pH 7.6) in a final volume of 10 μL for 1 h at 37 °C. The labelled product was purified on a 20% denaturing polyacrylamide gel (7 M urea), extracted using TEN buffer (10mM Tris.HCl, pH 8.0, 1 mM EDTA, 300 mM NaCl) and isolated by ethanol precipitation. The 5'-labelled primer and the template were mixed in a molar ratio of 1:2, and annealed by heating to 95 °C for 2 min, followed by slow cooling to room temperature. The primer extension reactions were performed in a total volume of 10 or 20 μl, containing 0.3 μM primer and 0.6 μM template, 1x polymerase buffer (as provided by the vendors; e.g Thermopol buffer for Therminator III contained 20 mM Tris.HCl, pH 8.8, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 0.1% (v/v) Triton X-100, 2 mM $MgSO_4$), the desired concentration of NTPs or analogs (standard conditions used 200 μM, exceptions are noted in Figures or Figure captions). The primer extension reaction was initiated by the addition of the desired amount of DNA polymerase (final concentrations in u/μL given in the Fig captions). The reaction mixtures were heated to 72 °C for the indicated amount of time, followed by immediate chilling on ice and quenched by addition of stop solution (80% formamide, 0.025% each bromophenol blue, xylene cyanol and 50 mM EDTA). The extension products were separated by 7 M urea denaturing PAGE (15%, 0.4 mm, 1x TBE, 35 W). The gels were dried at 80 °C for 30 min, and analysed by phosphor imaging (using a Storm 820 and ImageQuant software).

The preparative primer extension reaction for isolation of ammT-terminated DNA was performed with 500 pmol of DNA primer P1 and 700 pmol of template T2 in a volume of 600 μL, containing 50 μM ammTTP (**1**), 1x Thermopol buffer and 10 units of Therminator III DNA polymerase. The mixture was incubated at 72 °C for 1.5 h, followed by phenol/chloroform/isoamyl alcohol extraction (600 μL of P:C:I = 25:24:1) and ethanol precipitation (1800 μL of EtOH, -80°C, 2 h). The pellet was dissolved in TEN buffer (300 μL, 10 mM Tris.HCl, pH 8.0, 1 mM EDTA, 300 mM NaCl) and re-precipitated with EtOH. Finally the product was desalted on a HiTrap desalting column (Sephadex G25). The extended primer – template complex was analyzed by ESI-MS.
ESI−MS calcd. ammT-P1: 7282.6, found 7283.7 (T2: 10902.0, found M+Na 10924.7).

Snake venom phosphodiesterase (SVPD) digestion assay

The 5'-^{32}P-labeled DNA of interest (0.5 pmol) was dissolved in digestion buffer (final concentration: 50 mM Tris.HCl, pH 8.0, 1 mM $MgCl_2$) and supplemented with 10^{-3} units of snake venom phosphodiesterase (SVPD) in a final volume of 10 μL (5x 10^{-3} units were used for experiment in Fig S4b). The reaction mixture was incubated at 22°C. Aliquots of 1.5 μL were removed at the indicated times, quenched by addition of 4.5 μL of stop solution (80% formamide, 0.025% each bromophenol blue, xylene cyanol and 50 mM EDTA) and analyzed by 7 M urea denaturing PAGE (15%, 0.4 mm, 1x TBE, 35 W). The gels were dried at 80 °C for 30 min, and analysed by phosphor imaging.

Sequences of primer and template DNA oligonucleotides

P1: 5'- GAC CCA CTC CAT CGA GAT TTC TC-3'

P2: 5'- ACT CCA TCG AGA TTT CTC AGG CTA GC-3'

T1: 5'-GCG CAG GCT CGG GAG AAA TCT CGA TGG AGT GGG TC-3'

T2: 5'-GCG CAG GCT CCA GAG AAA TCT CGA TGG AGT GGG TC-3'

T3: 5'-GCG CAG GCT CCG GAG AAA TCT CGA TGG AGT GGG TC-3'

T4: 5'-GCG CAG GCT CCC GAG AAA TCT CGA TGG AGT GGG TC-3'

T5: 5'-GCG CAG GCT CCT GAG AAA TCT CGA TGG AGT GGG TC-3'

T6: 5'-CGG TGG TCG TCG TTG TAG CTA GCC TGA GAA ATC TCG ATG GAG p-3'

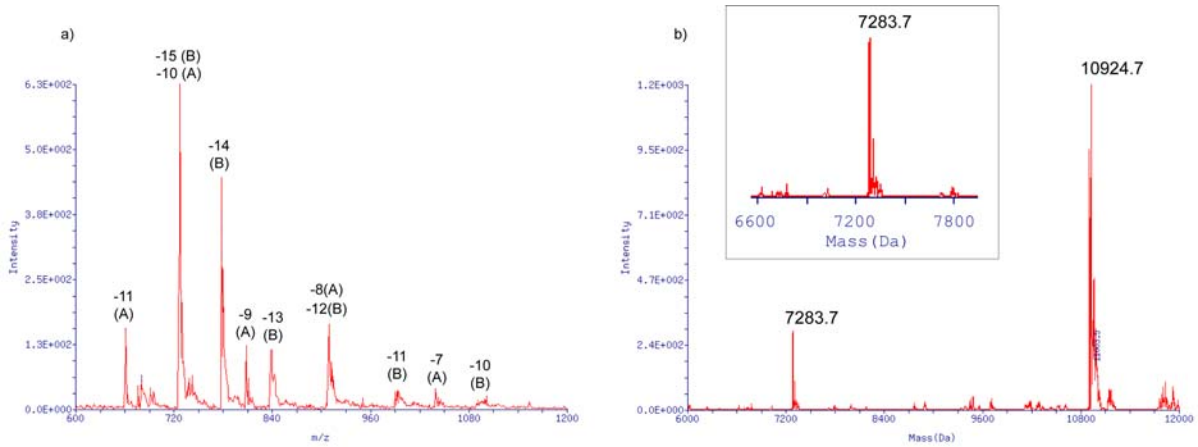


Figure S1. ESI-MS analysis of primer extension product (primer P1/template T2) with ammTTP **1** and Therminator III DNA polymerase. a) ESI-MS spectrum: m/z of extended primer (product A) and template (B) are marked. b) Deconvoluted mass spectrum. Inset shows mass region for extended primer. Expected mass: 7282.6, found 7283.7 (35-nt template B: expected 10902.0, found 10902.0, 10924.7 (+Na)).

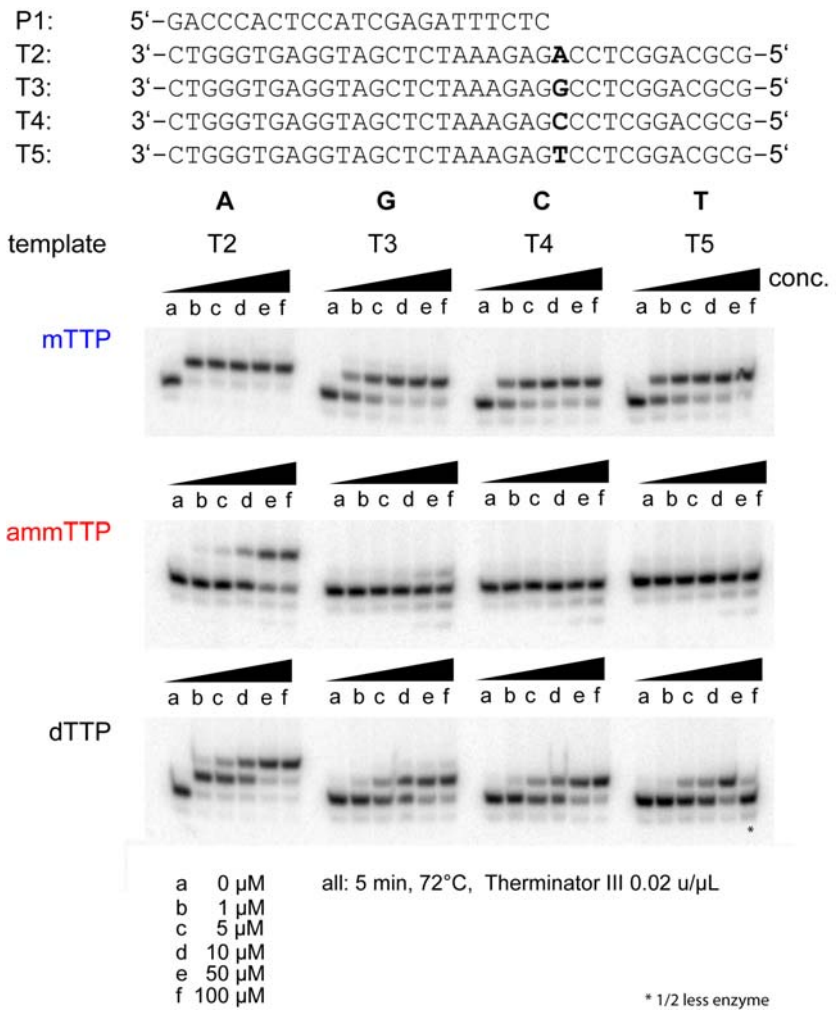


Figure S2. Single-nucleotide primer extension experiments with dTTP, mTTP and ammTTP (1), using matched and mismatched templates, with 0-100 µM thymidine analogs.

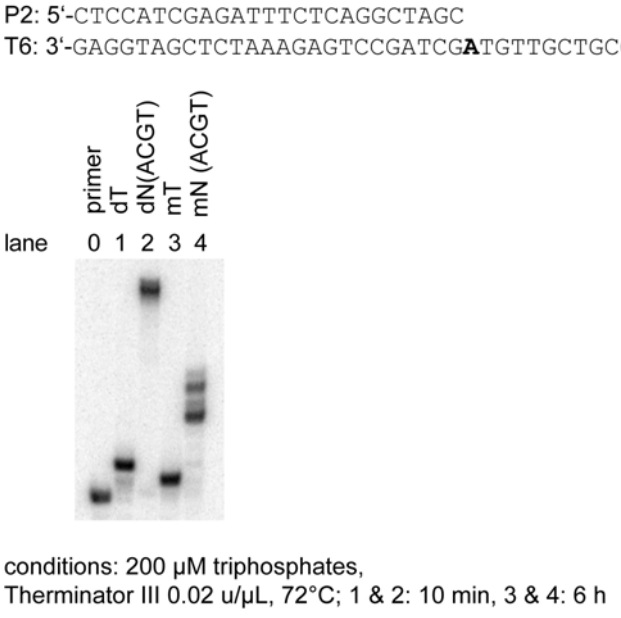


Figure S3. Primer extension using all mNTPs.

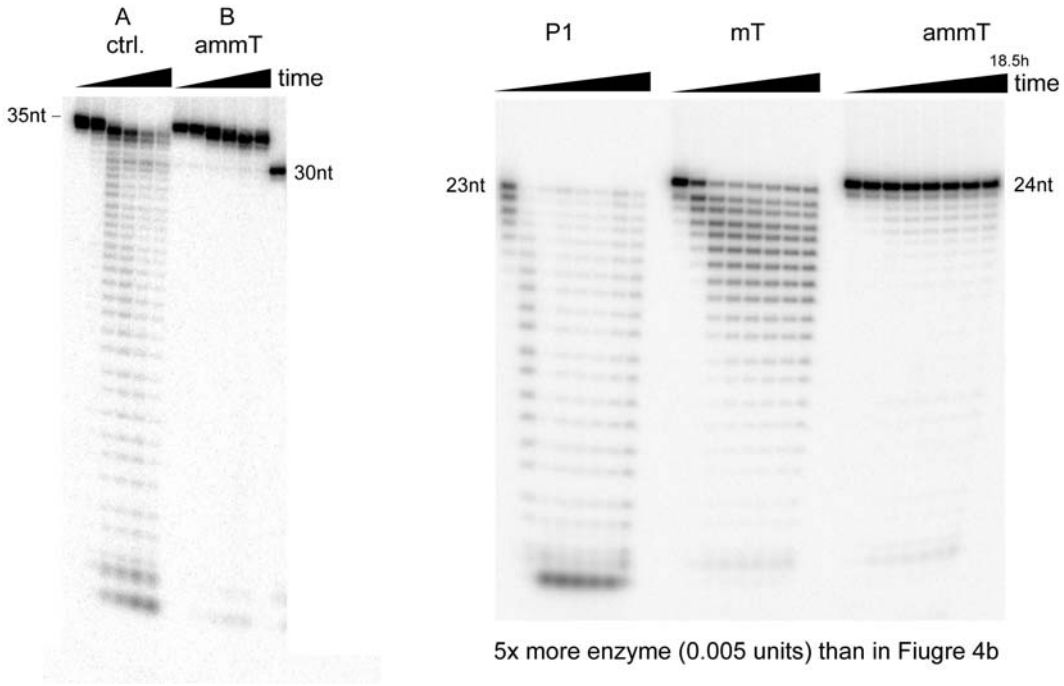


Figure S4. left: SVPD digestion of ummodified and internally ammT-modified DNA, isolated from 20 µL hot primer extension reactions with Pfu polymerase. right: longer time course experiment for dataset shown in Figure 4b. Here up to 18.5 hours, with 5-fold higher SVPD concentration.

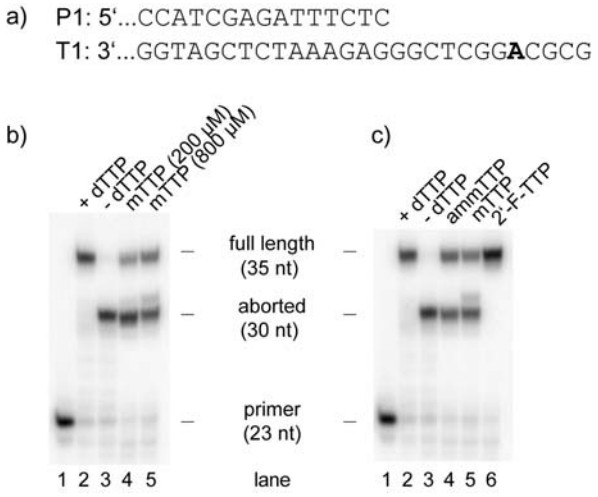
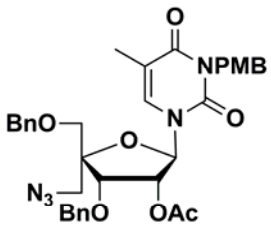


Figure S5. Comparison of TTP analog incorporation with Pfu polymerase. a) Partial sequences of primer P1 and template T1 (same as for Figure 2). b) Testing concentration dependence for mTTP. lanes 1: primer only, 2: pos. ctrl. (all dNTPs), 3: neg. ctrl. (no dTTP), 4: 200 µM mTTP, 5: 800 µM mTTP. c) lanes 1-3: same as in b), 4: 800 µM **1**, 5: 800 µM mTTP, 6: 200 µM 2'-F-TTP. All reactions in b) and c) were run with 0.1 u/µL Pfu, 50 mM LiCl, 72 °C, 8 h.

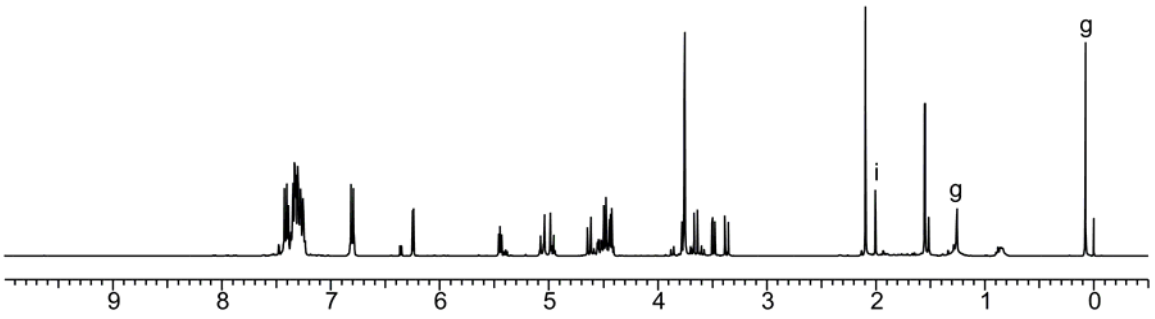
NMR spectra (^{1}H, ^{13}C, ^{31}P & ^{19}F)

(g- grease, i- impurity)

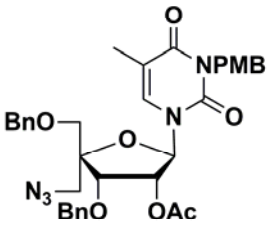
^{1}H NMR spectrum of compound **3**



3

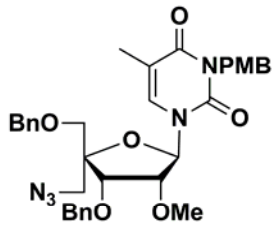


^{13}C NMR spectrum of compound **3**

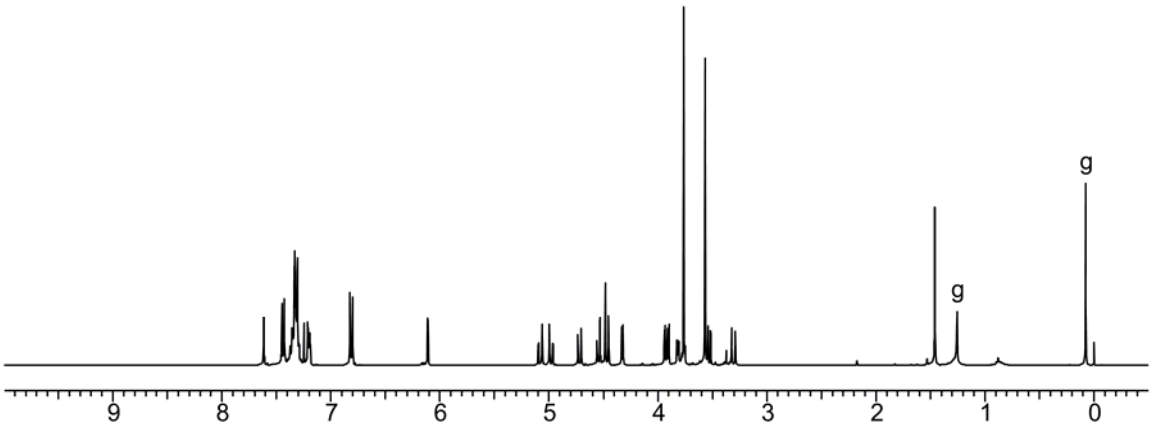


3

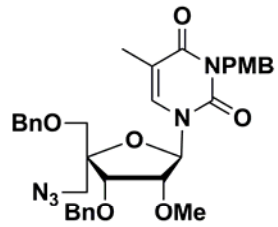
^{1}H NMR spectrum of compound **4**



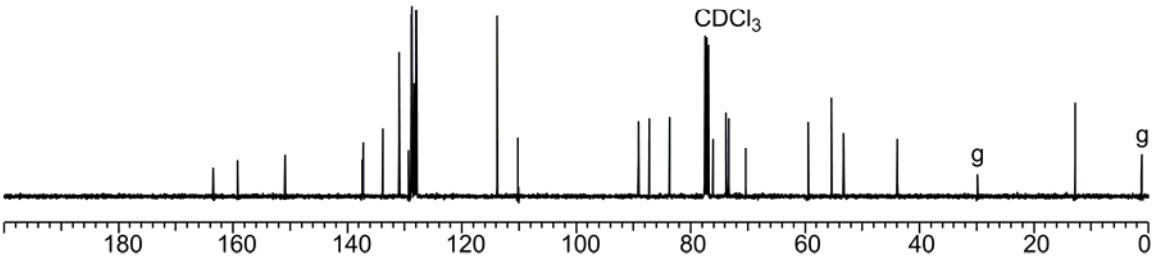
4



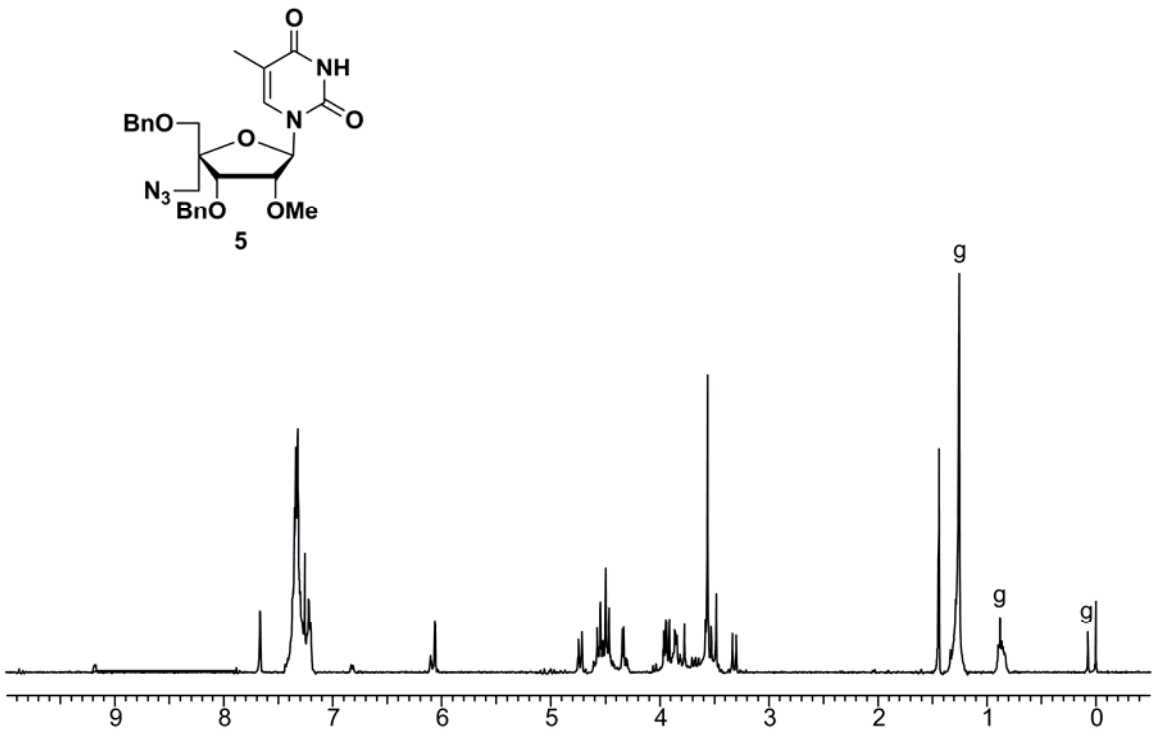
^{13}C NMR spectrum of compound **4**



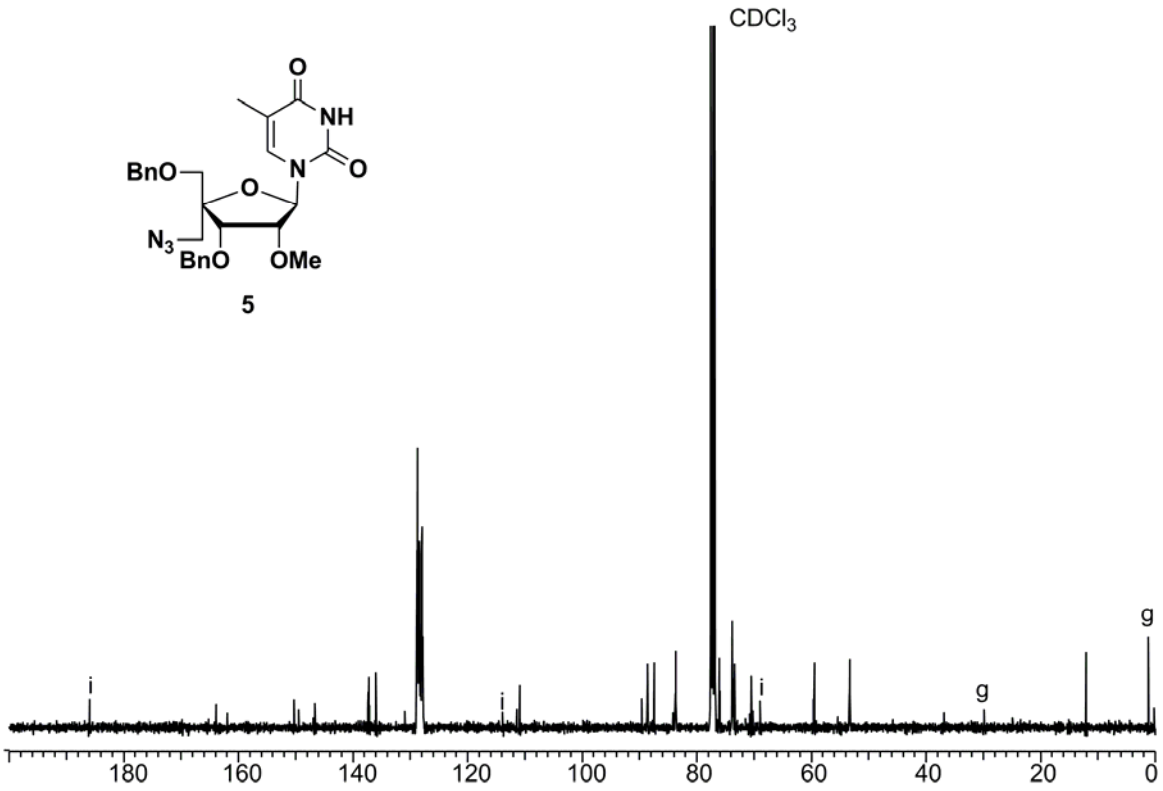
4



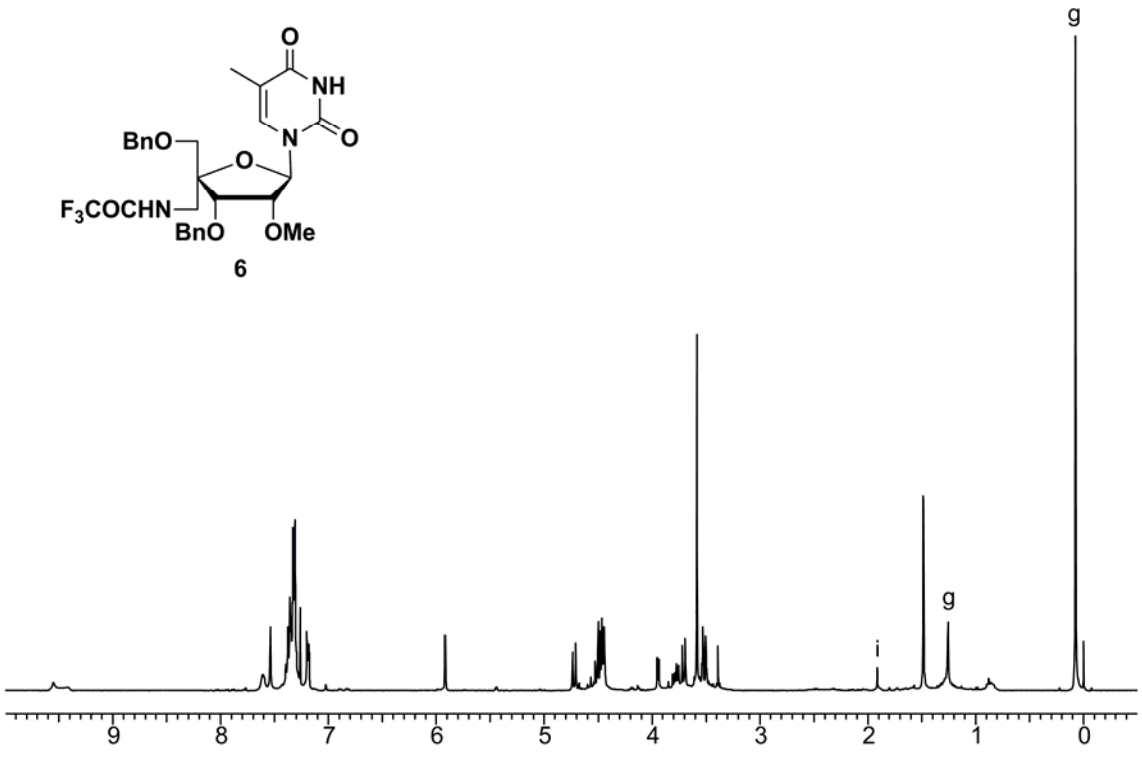
^{1}H NMR spectrum of compound **5**



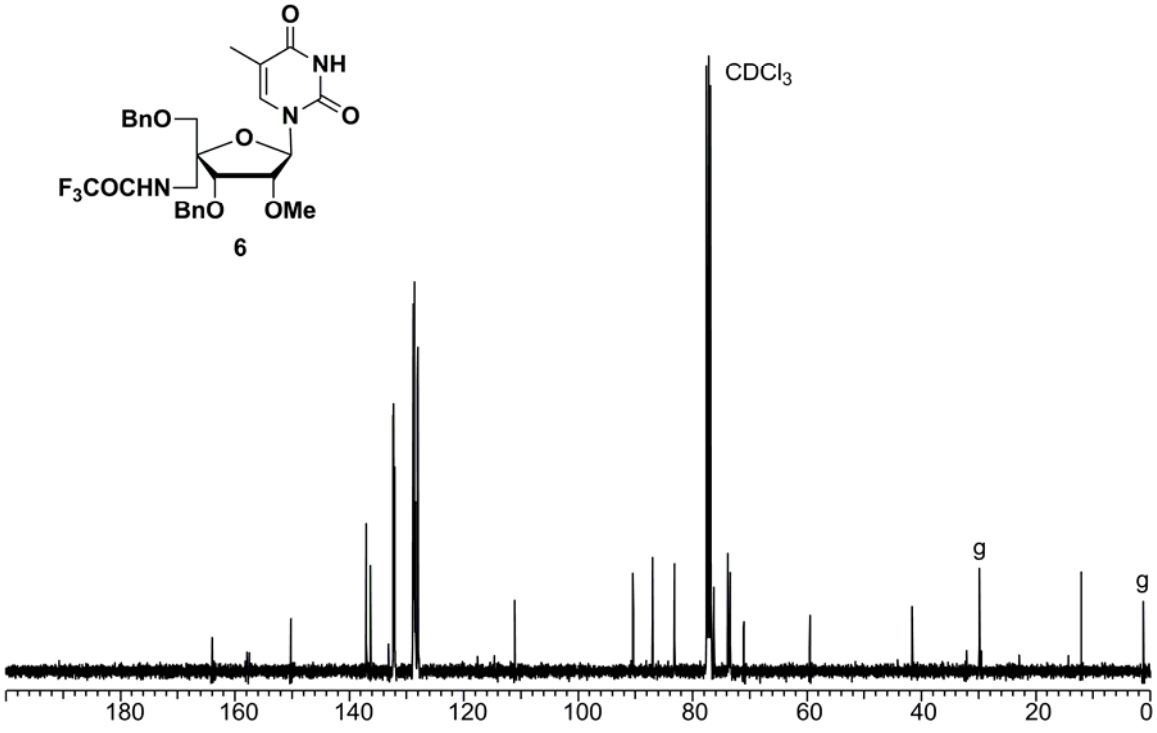
^{13}C NMR spectrum of compound **5**



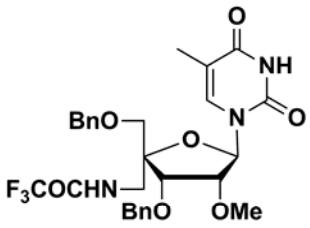
^{1}H NMR spectrum of compound **6**



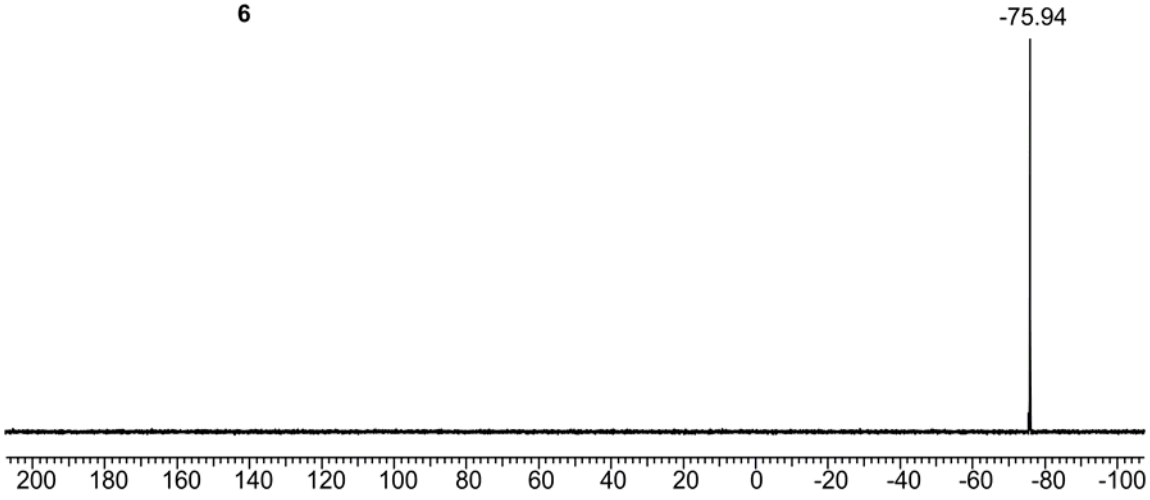
^{13}C NMR spectrum of compound **6**



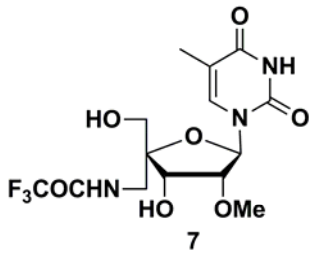
^{19}F NMR spectrum of compound **6**



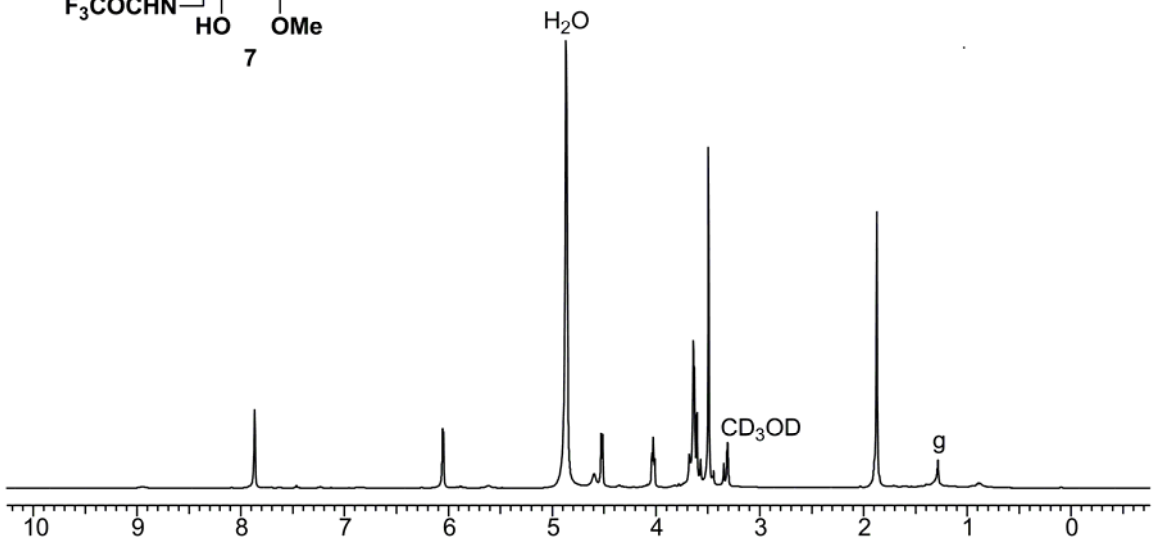
6



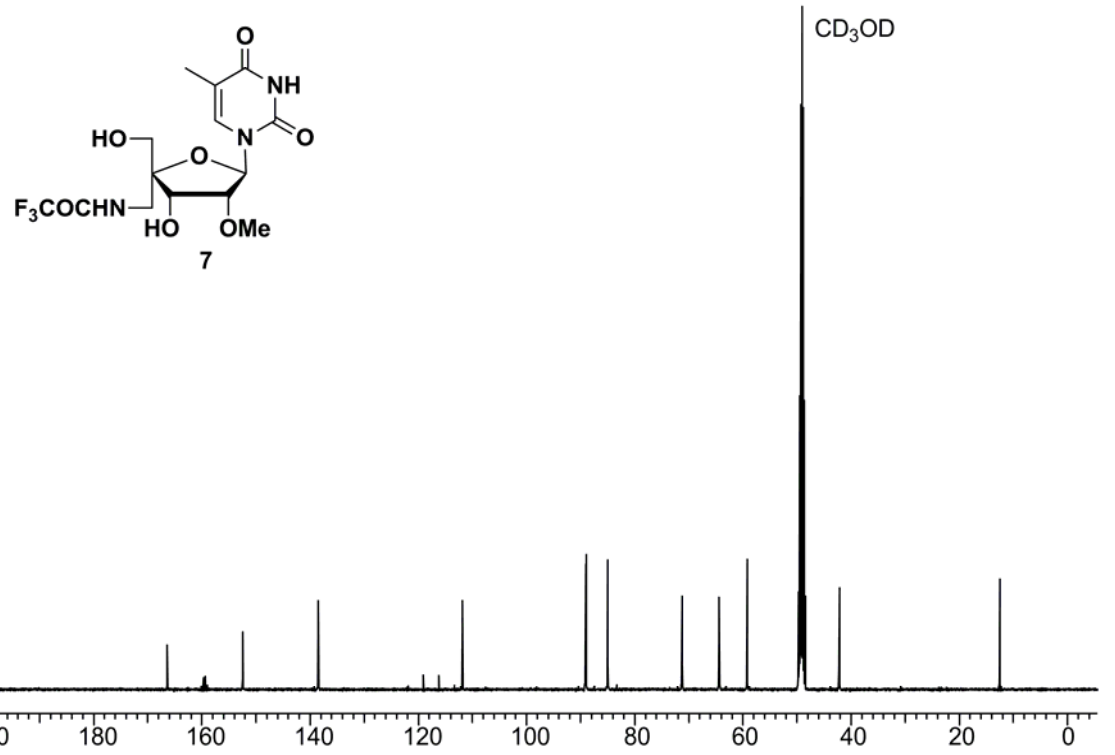
^{1}H NMR spectrum of compound **7**



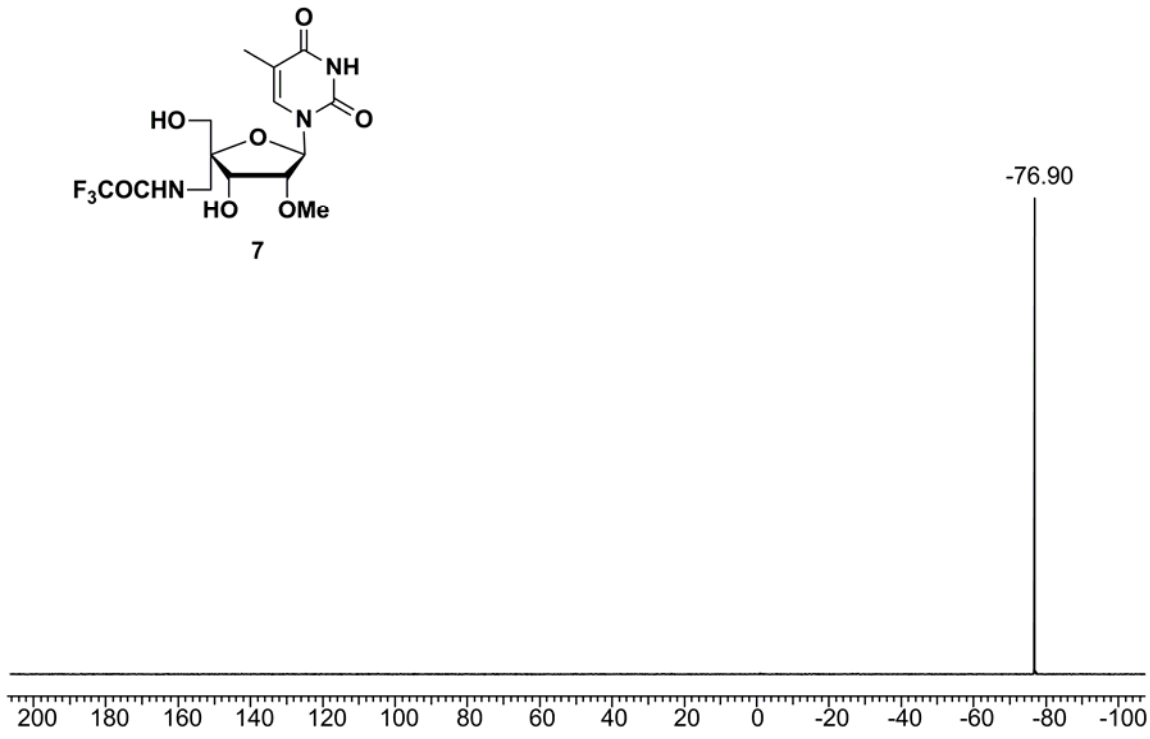
7



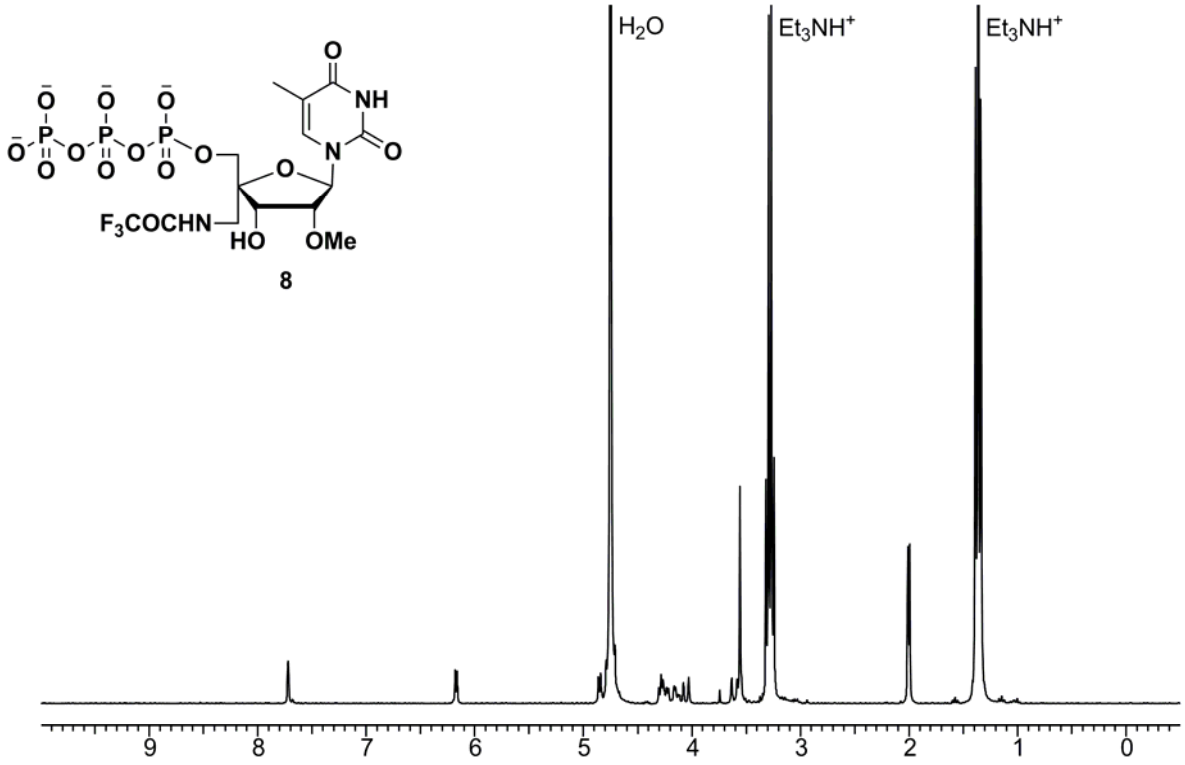
^{13}C NMR spectrum of compound **7**



^{19}F NMR spectrum of compound **7**

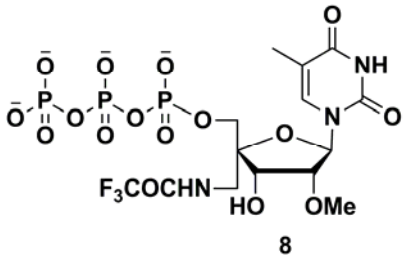


^{1}H NMR spectrum of compound **8**

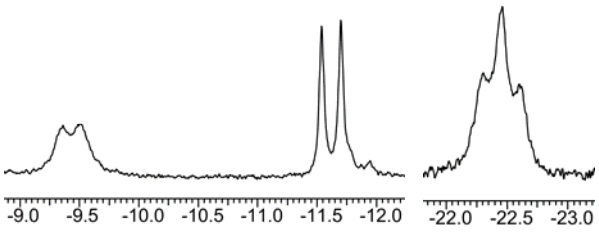


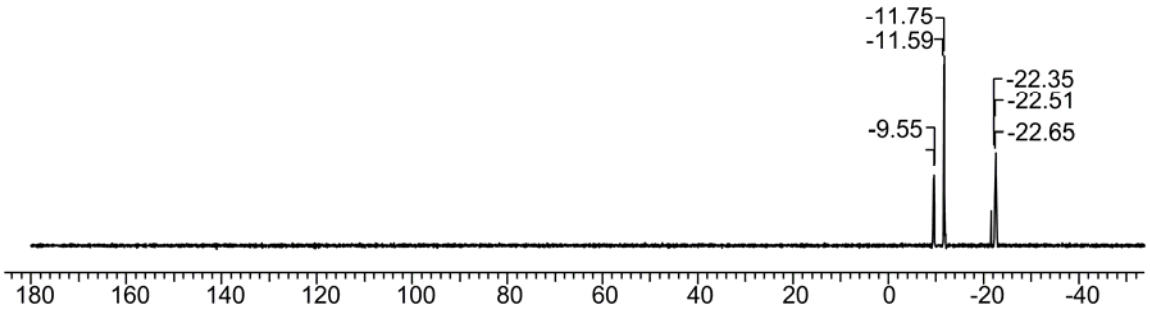
8

^{31}P NMR spectrum of compound **8**

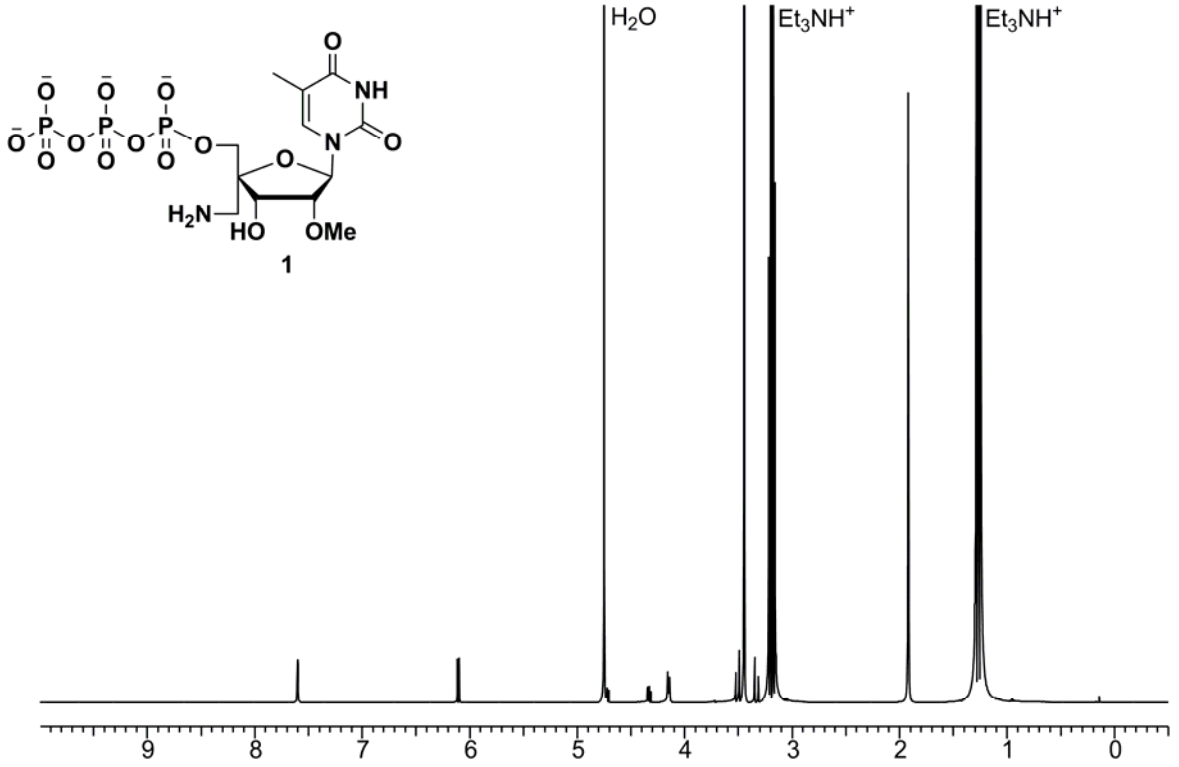


8

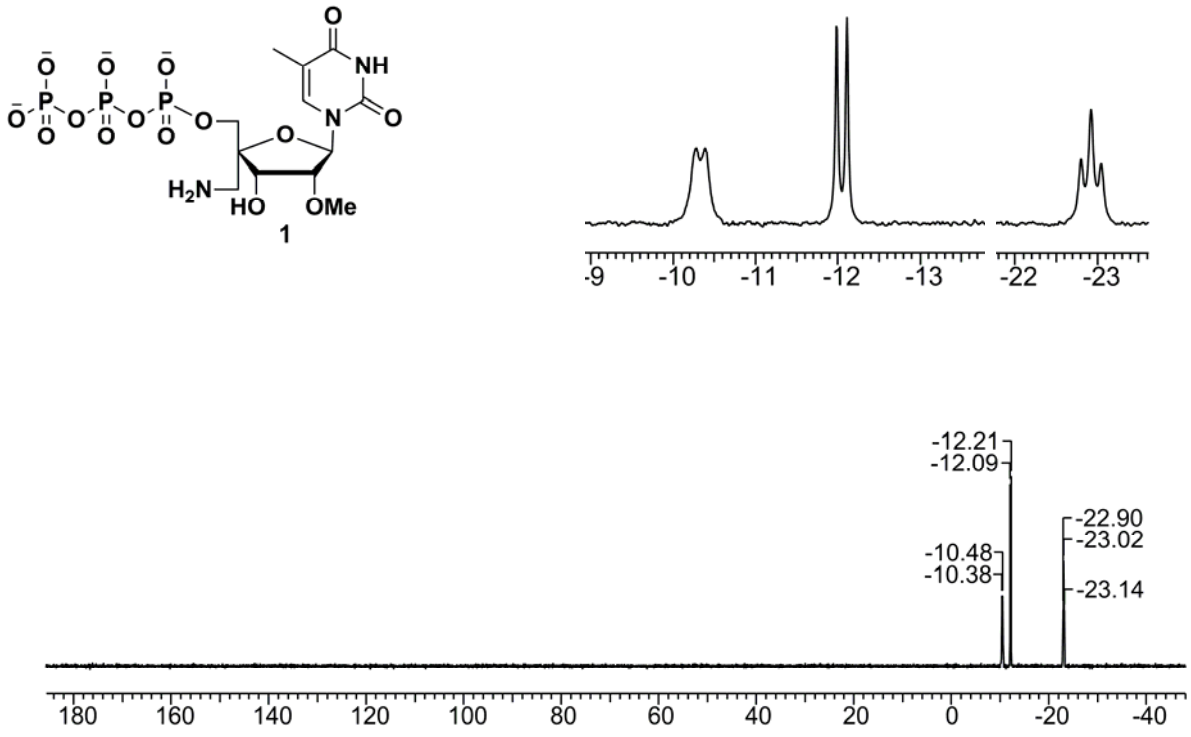




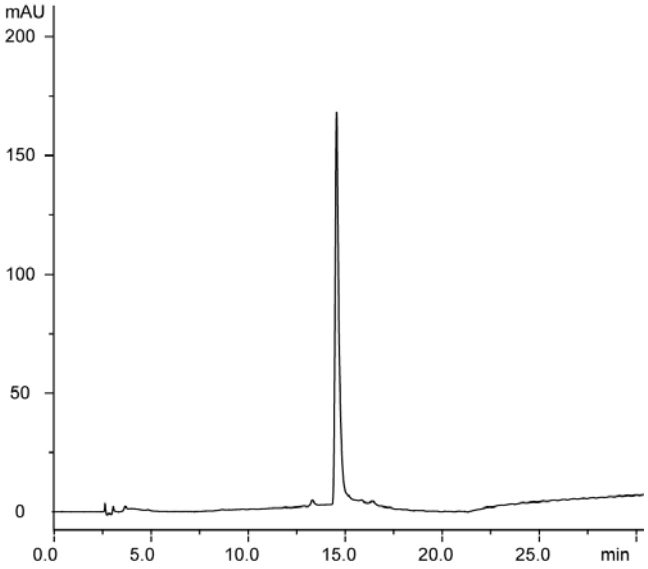
^{1}H NMR spectrum of compound **1**



^{31}P NMR spectrum of compound **1**



RP-HPLC Chromatogram of 4'-*C*-aminomethyl-2'-*O*-methyl thymidine triphosphate 1



Reversed-phase HPLC chromatogram of purified ammTTP **1**. Column and conditions: RP EC 250/4 Nucleosil 100-5 C18 HD at 35 ^{o}C; Mobile phase A: 25 mM TEAA buffer (pH 6.8), mobile phase B: acetonitrile: TEAA buffer (20 mM, pH 6.8) (4:1). Gradient: 0-10% B in 30 min. Flow rate: 1 mL/min.