Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information for

Synthesis of activated 3′-amino-3′-deoxy-2-thio-thymidine, a superior substrate for the nonenzymatic copying of nucleic acid templates

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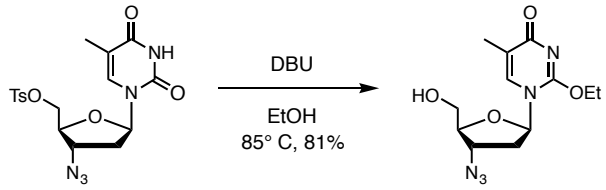
1. General Materials and Methods

Pyridine (pyr), dimethylformamide (DMF), trimethyl phosphate ($PO(OMe)_3$), Phosphorus(V) oxychloride ($POCl_3$), acetic anhydride and piperidine were purchased as anhydrous grade from Sigma-Aldrich. Ammonia solution (7 N) in methanol, tetramethylguanidine (TMG), 1-(2-hydroxyethyl)-imidazole (HEI), 4-morpholineethane-sulfonic acid sodium salt (MES), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were also purchased from Sigma-Aldrich. A hydrogen sulfide (H_2S) gas tank equipped with an H_2S pressure gauge was purchased from Airgas. Primer (DNA) and template (RNA) oligonucleotide strands were obtained from IDT (Coralville, IA). The primer strand was then enzymatically modified with a 3′-amino-2′,3′-dideoxyguanosine triphosphate, which was purschased from TriLink Biotechnologies (San Diego, CA). Silica gel chromatographic purification of the synthesized molecules was performed using Teledyne Isco combiflash instrument equipped with a C18Aq high performance RediSep® column. All primer extension reactions were carried out with solutions prepared in nuclease-free water and at 4 °C. Nuclear Magnetic Resonance (NMR) spectroscopic analyses were carried out on either a Varian INOVA 400 MHz or a Bruker Ascend 400 MHz spectrometer. Chemical shifts (δ) for ^{1}H NMR spectra were referenced to TMS at δ = 0.00 ppm, to CHD_2OD at δ = 3.31 ppm, to $CHD_2S(O)CD_3$ at δ = 2.50 ppm, or to HDO at δ = 4.80 ppm. ^{13}C NMR spectra were referenced to $CDCl_3$ at δ = 77.23 ppm, to CD_3OD at δ = 49.15 ppm, or to $CD_3S(O)CD_3$ at δ = 39.51 ppm. The ^{31}P NMR spectra were referenced externally to $P(OMe)_3$ (dissolved in $CDCl_3$) at δ = 140.0 ppm using a co-axial NMR tube. The following abbreviations are used to describe NMR resonances: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), and nfom (non-first order multiplet). Coupling constants (*J*) are reported in Hz. Infrared spectra were recorded on an FT-IR spectrometer; the most intense and/or diagnostic peaks are reported. Low-resolution mass spectroscopy (LRMS) analyses were performed using a Bruker Daltonics Esquire 6000 mass spectrometer. Liquid chromatography followed by high-resolution mass spectroscopy (LC-HRMS) analysis in the ESI mode was carried out on an Agilent 6520 Q-TOF LC/MS-MS instrument.

2. Experimental Procedures

1-((2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-ethoxy-5 methyl-pyrimidin-4(1*H*)-one: Compound 3.

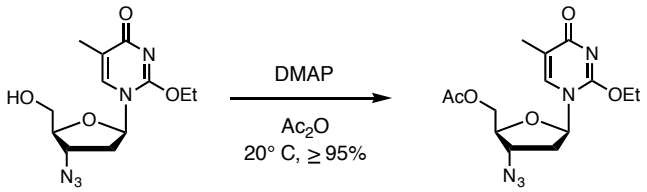


2 *3*

To a solution of **2**[1] (10 g, 23.8 mmol, 1 equiv) in absolute EtOH (650 mL), was added freshly distilled DBU (20 g, 133.0 mmol, 4 equiv) at room temperature. The reaction mixture was purged with Ar for 15 min and a vertical condenser was attached to the reaction flask, which was then placed in a 90 °C-oil bath. The reaction mixture was stirred for 24 h, then cooled to room temperature. The precipitate was filtered off and the filtrate was concentrated by rotary evaporation. The resulting material was re-dissolved in CH_2Cl_2 (500 mL) washed with water (250 mL) and saturated aqueous NaCl solution (250 mL). The extracted organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude product was purified by combiflash silica-gel chromatography with a CH_2Cl_2-MeOH elution mixture (0-to-10% MeOH gradient) to afford the nucleoside **3** (7.0 g, 81% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 6.15 (t, *J* = 6.5 Hz, 1H), 4.77 (br m, 1H), 4.48 (q, *J* = 7.0 Hz, 2H), 4.46 (br dd, *J* = 5.5, 5.0 Hz, 1H), 4.07–4.04 (br ddd, *J* = 10.0, 6.0, 3.5 Hz, 1H), 4.03 (dd, *J* = 4.0, 3.0 Hz, 1H), 3.94–3.89 (br ddd, *J* = 10.0, 6.0, 3.5 Hz, 1H), 2.46–2.35 (m, 2H), 1.91 (s, 3H), and 1.38 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (100 MHz, CD_3OD) δ 172.9, 155.0, 134.2, 117.3, 85.9, 85.6, 65.3, 61.7, 60.4, 39.1, 14.4, and 13.9; **LRMS** (ESI): calcd for $C_{12}H_{17}N_5O_4$•H^+ 296.1, found 295.8; calcd for 2($C_{12}H_{17}N_5O_4$)•Na^+ 613.2, found 613.1.

((2*S*,3*S*,5*R*)-3-azido-5-(2-ethoxy-5-methyl-4-oxopyrimidin-1(4*H*)-yl)tetrahydrofuran-2-yl)methyl acetate: Compound 4.

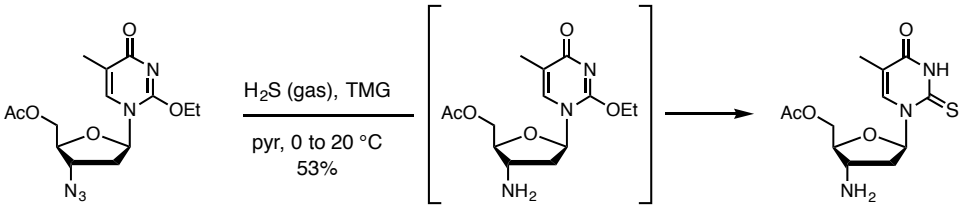


3 *4*

To a stirred solution of **3** (7.0 g, 23.7 mmol, 1 equiv) in acetic anhydride (200 mL) was added 4-dimethylaminopyridine (DMAP) (57 mg, 0.5 mmol, 0.02 equiv) at room temperature. The reaction mixture was stirred for 12 h, then the bulk of the solvent was removed by successive rounds of azeotropic co-evaporation with toluene. The resulting oil was re-dissolved in CH_2Cl_2 (500 mL), then washed with water (250 mL) and saturated aqueous NaCl solution (250 mL). The extracted organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was then purified by combiflash silica gel chromatography with a CH_2Cl_2-MeOH elution mixture (0-to-10% MeOH gradient) to afford the 5′-*O*-acetylated nucleoside **4** (7.5 g, 95% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (q, *J* = 1.5 Hz, 1H), 6.12 (t, *J* = 6.5 Hz, 1H), 4.53 (q, *J* = 7.0 Hz, 2H), 4.40 (dd, *J* = 12.5, 4.0 Hz, 1H), 4.36 (dd, *J* = 12.5, 4.0 Hz, 1H), 4.18 (ddd, *J* = 7.5, 5.5, 5.0 Hz, 1H), 4.13 (ddd, *J* = 5.5, 4.0, 4.0 Hz, 1H), 2.51 (ddd, *J* = 14.0, 6.5, 5.0 Hz, 1H), 2.33 (ddd, *J* = 14.0, 7.5, 6.5 Hz, 1H), 2.16 (s, 3H), 2.00 (d, *J* = 1.5 Hz, 3H); and 1.41 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (100 MHz, CD_3OD) δ 171.7, 170.3, 154.8, 131.9, 118.0, 85.6, 82.1, 65.4, 63.2, 60.4, 38.6, 20.9, 14.4, and 14.2; **LRMS** (ESI): calcd for $C_{14}H_{19}N_5O_5$•Na^+ 360.128, found 360.13; calcd for $2(C_{14}H_{19}N_5O_5)$•Na^+ 697.27, found 697.31.

((2*S*,3*S*,5*R*)-3-amino-5-(5-methyl-4-oxo-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl acetate: Compound 6.



4 ***5*** ***6***

A representative reaction setup is depicted in Fig. S1 below. A two-neck reaction flask containing a solution of **4** (1.0 g, 3.0 mmol, 1 equiv) in anhydrous pyridine (30 mL) was purged with Ar (or N_2) for 30 min at room temperature. To this solution was added freshly distilled TMG (1.5 mL, 11.7 mmoles, 4 equiv), and the reaction mixture was cooled in an ice-bath. H_2S gas was then gently bubbled through a glass pipette into the stirred reaction solution, which turned to a deep green color over 5-10 mins. After 1 h, the H_2S gas source was closed, the remaining H_2S in the system was bubbled out, and the glass pipette was raised above the reaction liquid. After the pressure was equalized, valves connecting the tygon tubes to the reaction flask were closed, and

the reaction mixture was stirred at room temperature for 16 hrs. At the end of this mixing period, Ar (or N_2) was bubbled into the mixture for ca. 1 h to remove any residual volatile sulfur species. The reaction mixture was poured into a flask containing chloroform (300 mL). While being stirred vigorously, 1N HCl was added dropwise until the pH decreased to around 4. The chloroform layer was removed, and the remaining aqueous layer was treated with fresh chloroform. The stirred emulsion was neutralized by addition of saturated aqueous $NaHCO_3$ solution, and the organic part was then extracted and washed with water and saturated aqueous NaCl solution. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting crude material was purified by combiflash silica gel chromatography with a CH_2Cl_2-MeOH elution mixture (0-to-15% MeOH gradient) to afford the 2-thio modified nucleoside **6** (470 mg, 53% yield).

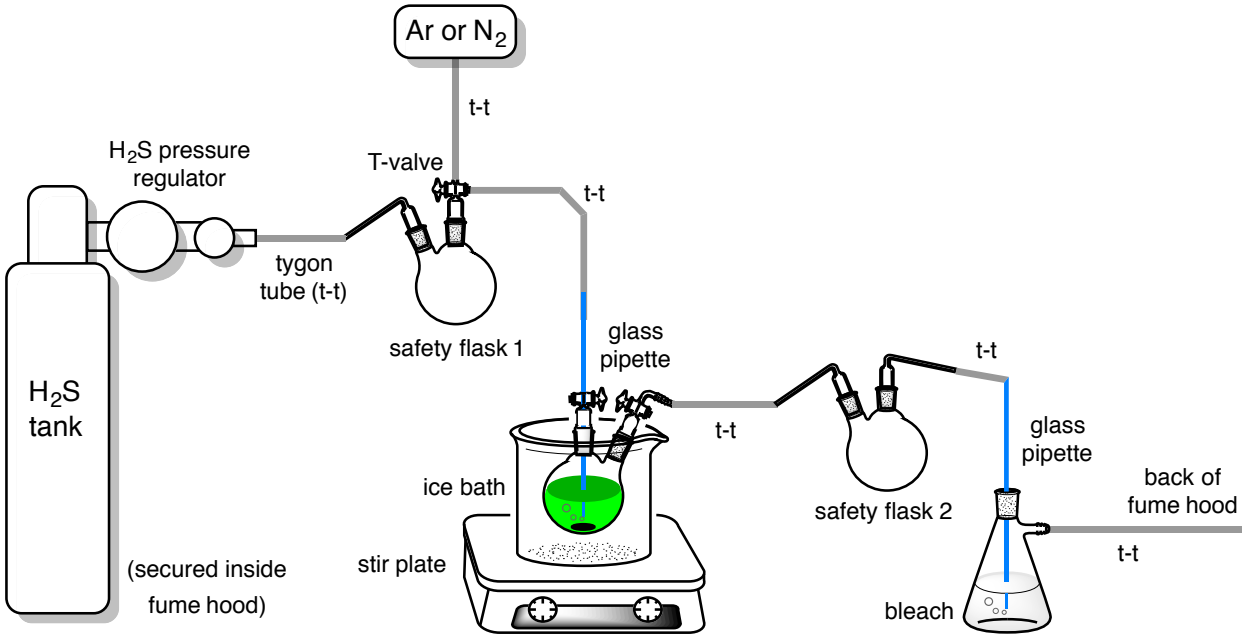


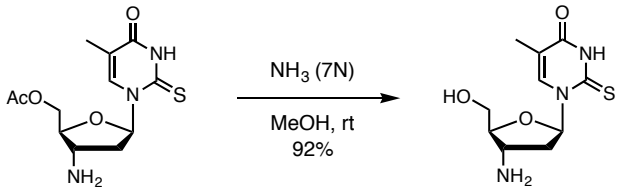
Figure S1. Experimental Setup for the Sulfuration Reaction. Safety flask 1 prevents the reaction mixture from reaching into the H_2S source in case of an unexpected downstream pressure increase. Similarly, safety flask 2 prevents bleach from mixing into the reaction mixture in case of an unexpected pressure buildup.

Intermediate 5: ^{1}**H NMR** (400 MHz, CD_3OD) δ 7.58 (q, J = 1.0 Hz, 1H), 6.17 (dd, J = 6.5, 4.5 Hz, 1H), 4.49^{+} (dq, J = 2.5, 7.0 Hz, 1H), 4.49^{-} (dq, J = 2.5, 7.0 Hz, 1H), 4.43 (dd, J = 12.5, 5.0 Hz, 1H), 4.35 (dd, J = 12.5, 7.5 Hz, 1H), 3.95 (ddd, J = 7.5, 7.5, 5.0 Hz, 1H), 3.47 (q, J = 7.5 Hz, 1H), 2.37 (ddd, J = 14.0, 7.5, 4.5 Hz, 1H), 2.31 (ddd, J = 14.0, 7.5, 6.5 Hz, 1H), 2.10 (s, 3H), 1.97 (d, J = 1.0 Hz, 3H), and 1.42 (t, J = 7.0 Hz, 3H); ^{13}**C NMR** (100 MHz, CD_3OD) δ 175.1, 172.5, 156.8,

136.3, 117.5, 88.0, 86.5, 66.7, 65.0, 52.0, 42.1, 20.9, 14.6, and 13.9; **LRMS** (ESI): calcd for $C_{14}H_{21}N_3O_5 \bullet H^+$ 312.16, found 311.95.

Product 6: **^{1}H NMR** (400 MHz, CD_3OD) δ 7.74 (q, *J* = 1.5 Hz, 1H), 6.78 (dd, *J* = 7.0, 4.0 Hz, 1H), 4.51 (dd, *J* = 12.5, 5.0 Hz, 1H), 4.35 (dd, *J* = 12.5, 3.0 Hz, 1H), 3.95 (ddd, *J* = 7.5, 5.0, 3.0 Hz, 1H), 3.41 (q, *J* = 7.5 Hz, 1H), 2.40 (ddd, *J* = 14.0, 7.5, 7.0 Hz, 1H), 2.30 (ddd, *J* = 14.0, 7.5, 4.0 Hz, 1H), 2.13 (s, 3H), and 1.95 (d, *J* = 1.5 Hz, 3H); **^{13}C NMR** (100 MHz, CD_3OD) δ 176.0, 172.5, 163.4, 138.0, 116.6, 90.7, 86.6, 64.4, 51.8, 42.1, 21.0, and 13.0.; **LRMS** (ESI): calcd for $C_{12}H_{17}N_3O_4S \bullet H^+$ 300.10, found 299.53; calcd for $C_{12}H_{17}N_3O_4S \bullet H^+$ 322.08, found 321.75.

1-((2*R*,4*S*,5*S*)-4-amino-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one: Compound S1.

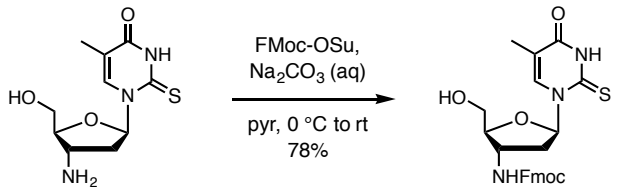


6 ***S1***

An NH_3 solution (7 N) in MeOH (8 mL) was introduced into a pressure reaction vessel containing a solution of **6** (450 mg, 1.5 mmol, 1 equiv) in MeOH (8 mL) at room temperature. The reaction solution was then stirred for 16 hours, and the solvent was removed by rotary evaporation and the residue was then left under high-vacuum for 2 hours. Dried deacetylated nucleoside **S1** (350 mg, 92% yield) was sufficiently pure (by ^{1}H NMR analysis) and was used directly in the next step without any purification.

^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (q, *J* = 1.5 Hz, 1H), 6.77 (dd, *J* = 6.5, 4.0 Hz, 1H), 3.94 (dd, *J* = 12.5, 3.0 Hz, 1H), 3.83 (dd, *J* = 12.5, 3.0 Hz, 1H), 3.75 (ddd, *J* = 7.5, 3.0, 3.0 Hz, 1H), 3.51 (q, *J* = 7.5 Hz, 1H), 3.35 (s, 1H), 2.35 (ddd, *J* = 14.0, 7.5, 6.5 Hz, 1H), 2.30 (ddd, *J* = 14.0, 7.5, 4.0 Hz, 1H), and 1.92 (d, *J* = 1.5 Hz, 3H); **^{13}C NMR** (100 MHz, CD_3OD) δ 175.8, 163.6, 139.1, 116.2, 90.7, 89.5, 61.2, 50.5, 42.7, and 12.9; **LRMS** (ESI): calcd for $C_{10}H_{15}N_3O_3S \bullet H^+$ 258.09, found 257.86; calcd for $[C_{10}H_{15}N_3O_3S - (H^+)]^-$ 256.07, found 255.97.

1-((2*R*,4*S*,5*S*)-4-amino-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one: Compound 7.

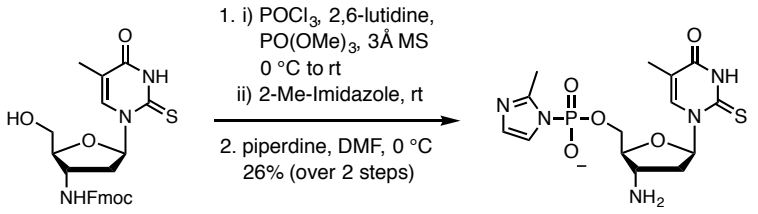


S1 ***7***

To a stirred solution of **S1** (300 mg, 1.2 mmoles, 1 equiv) and 1 M aqueous Na_2CO_3 (0.7 mL, 0.6 equiv) in pyridine (12 mL) was added 9-fluorenylmethyl *N*-succinimidyl carbonate (Fmoc-OSu) (530 mg, 1.6 mmoles, 1.3 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 6 h and then filtered through a PTFE filter (pore size 0.22 μm). The filtrate was concentrated to remove the bulk pyridine, and the residue was then dissolved with $CHCl_3$, and then washed with a minimum amount of water. The organic layer was concentrated under reduced pressure to give an oil, which was triturated with the HPLC-grade acetonitrile. The resulting suspension was centrifuged and the supernatant was removed to provide the 3′-Fmoc protected nucleoside **7** (440 mg, 78% yield).

^{1}H NMR [400 MHz, $CD_3S(O)CD_3$] δ 12.2 (br s, 1H), 8.09 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 6.78 (br t, *J* = 6.0 Hz, 1H), 5.25 (br s, 1H), 4.35 (d, *J* = 6.5 Hz, 2H), 4.23 (t, *J* = 6.5 Hz, 1H), 4.15 (br ddd, *J* = 7.5, 6.5, 6.5 Hz, 1H), 3.88 (br m, 1H), 3.75–3.69 (nfom, 1H), 3.61–3.54 (nfom, 1H), 2.35–2.26 (m, 1H), 2.23–2.16 (m, 1H), and 1.82 (s, 3H); **^{1}H NMR** [400 MHz, CD_3OD] δ 12.6 (br s, 1H), 8.11 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 6.77 (br t, *J* = 6.0 Hz, 1H), 5.25 (br t, *J* = 5.0 Hz, 1H), 4.35 (d, *J* = 6.5 Hz, 2H), 4.23 (t, *J* = 6.5 Hz, 1H), 4.15 (br ddd, *J* = 7.5, 6.5, 6.5 Hz, 1H), 3.88 (br m, 1H), 3.75–3.69 (br m, 1H), 3.61–3.54 (br m, 1H), 2.35–2.26 (m, 1H), 2.23–2.16 (m, 1H), and 1.83 (s, 3H); **^{13}C NMR** [100 MHz, $CD_3S(O)CD_3$] δ 174.1, 160.6, 156.0, 143.8 (2C), 140.7 (2C), 136.7, 127.6 (2C), 127.0 (2C), 125.1 (2C), 120.1 (2C), 115.1, 88.5, 85.1, 65.4, 60.1, 49.4, 46.7, 38.0, and 12.5; **LRMS** (ESI): calcd for $C_{25}H_{25}N_3O_5S{\bullet}Na^+$ 502.14, found 501.98; calcd for $C_{25}H_{25}N_3O_5S{\bullet}Cl^-$ 514.12, found 514.10.

3′- amino-3′-deoxy-2-thio-thymidine-5′-phosphor-2-methylimidazolide: 3′-NH_2-2-MeImpdds^{2}T.



To a stirred solution of **7** (400 mg, 0.8 mmol, 1 equiv) in $PO(OMe)_3$ (8 mL) was added freshly activated 3Å molecular sieves and the mixture was stirred under Ar for 30 min. To this mixture was then added freshly distilled 2,6-lutidine (200 μL, 1.6 mmol, 2 equiv) and $POCl_3$ (120 μL, 1.2 mmol, 1.5 equiv) sequentially at 0 °C. The resulting white suspension was stirred for 2 hours and treated with 2-methylimidazole (260 mg, 3.2 mmol, 4 equiv). Resulting reaction mixture was allowed to warm to room temperature and stirred for an additional 2 hours. The mixture was then added into a pre-chilled mixture of acetone/diethyl ether/triethylamine/saturated sodium perchlorate solution in acetone (400:200:20:1 mL) in order to precipitate the desired product as a sodium salt. Most of the supernatant was removed by pipette-suction, and the remaining suspension was transferred into two falcon tubes, which were centrifuged to pellet the solid material. The supernatant was removed and a mixture of acetone/diethyl ether (1:1) was added into the tubes. After another centrifugation, the isolated solid material was purified by combiflash column (C18Aq) chromatography with an acetonitrile/TEAB buffer (pH ca. 7.5) elution mixture. The aqueous product fraction, which was determined by LR-ESI analysis, was placed into a falcon tube and freeze-dried under high-vacuum at –15 °C to afford the triethylammonium salt of the Fmoc protected 3′-amino-5′-phosphoroimidazolide intermediate. **^{31}P NMR** [161 MHz, $CD_3OD/CD_3S(O)CD_3$]: δ –9.2; **LRMS** (ESI): calcd for $C_{29}H_{30}N_5O_7PS{\bullet}Et_3NH^+$ 725.29, found 725.3; also calcd for $[C_{29}H_{30}N_5O_7PS - (H^+)]^-$ 622.15, found 622.1. To a stirred solution of this intermediate (100 mg, 0.13 mmol, 1 equiv) in DMF (2 mL), was added piperidine (130 μL, 1.3 mmol, 10 equiv) at 0 °C. The reaction mixture was stirred for 30 min at the same temperature, then directly concentrated in an ice-bath under high-vacuum. The resulting oil was immediately treated with a chilled mixture (21 mL) of acetone/diethyl ether/saturated sodium perchlorate solution in acetone (20:20:1 volume ratio) in order to precipitate the desired product. Most of the supernatant was removed by pipette-suction, and the remaining suspension was transferred into two falcon tubes, which were vortexed and then centrifuged to pellet the solid. The supernatant was removed

again by suction and a mixture of acetone/diethyl ether (1:1) was added into the tubes. After another vortexing and centrifugation, the liquid was removed and the solid material was purified by reverse-phase HPLC with an acetonitrile/TEAB buffer (pH ca. 7.5) elution mixture. The product fraction was transferred into a falcon tube and freeze-dried under high-vacuum at –15 °C to afford **3′-NH$_2$-2-MeImpdds^{2}T** (43 mg, 26% overall yield from nucleoside **7**). The ^{1}H and ^{31}P NMR spectroscopic data matched the previously reported results.[2] **LRMS** (ESI): calcd for $[C_{14}H_{20}N_5O_5PS - (H^+)]^-$ 400.09, found 399.96.

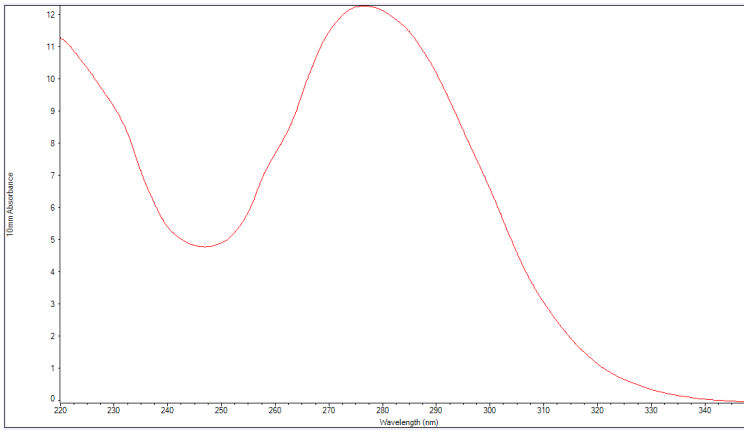
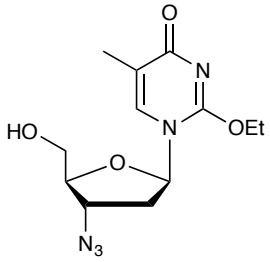


Figure S2. UV-VIS Spectrum of **3′-NH$_2$-2-MeImpdds^{2}T**



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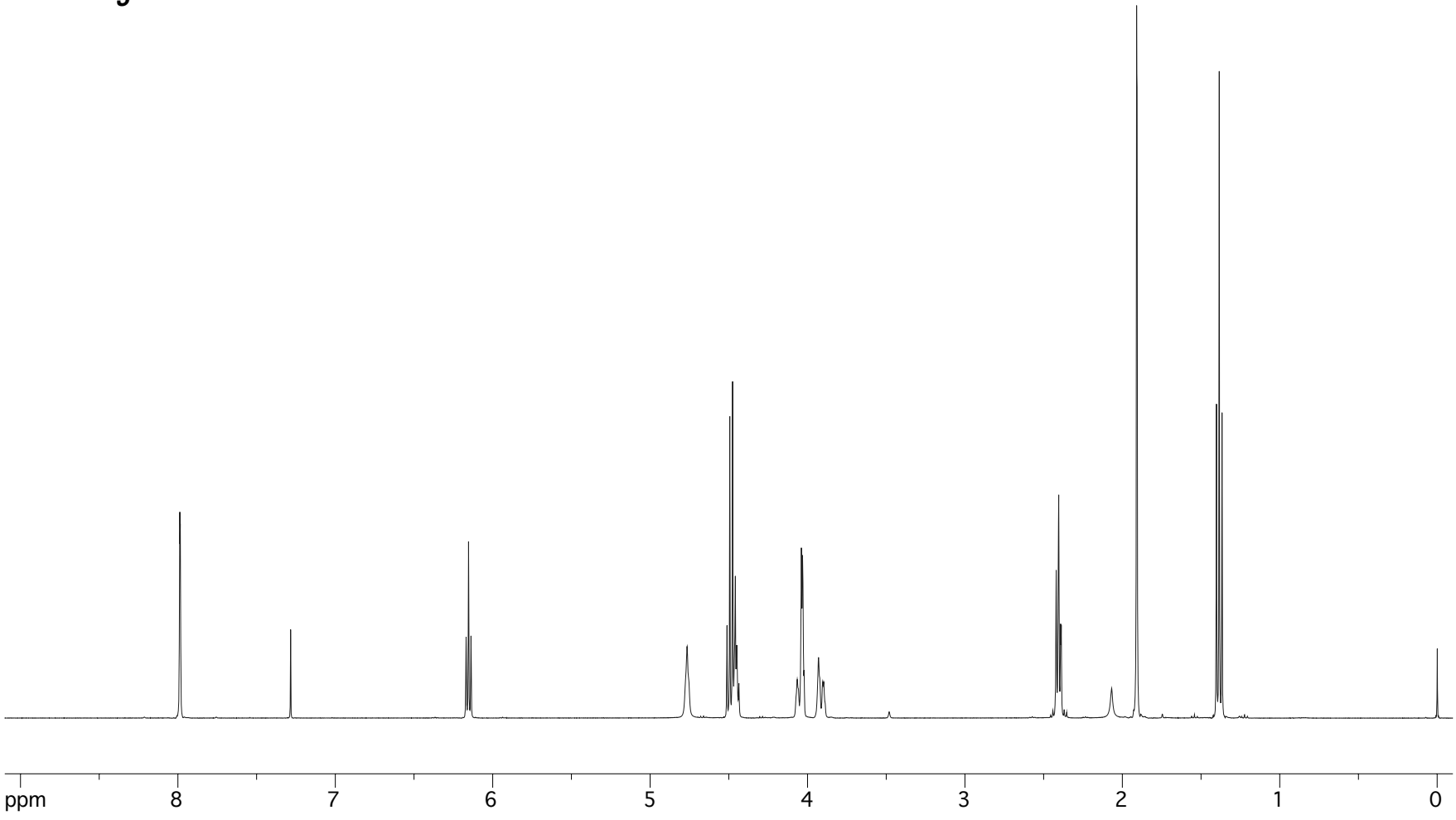

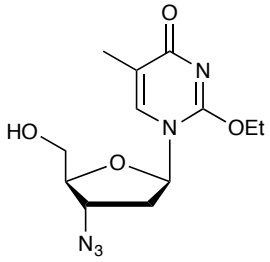

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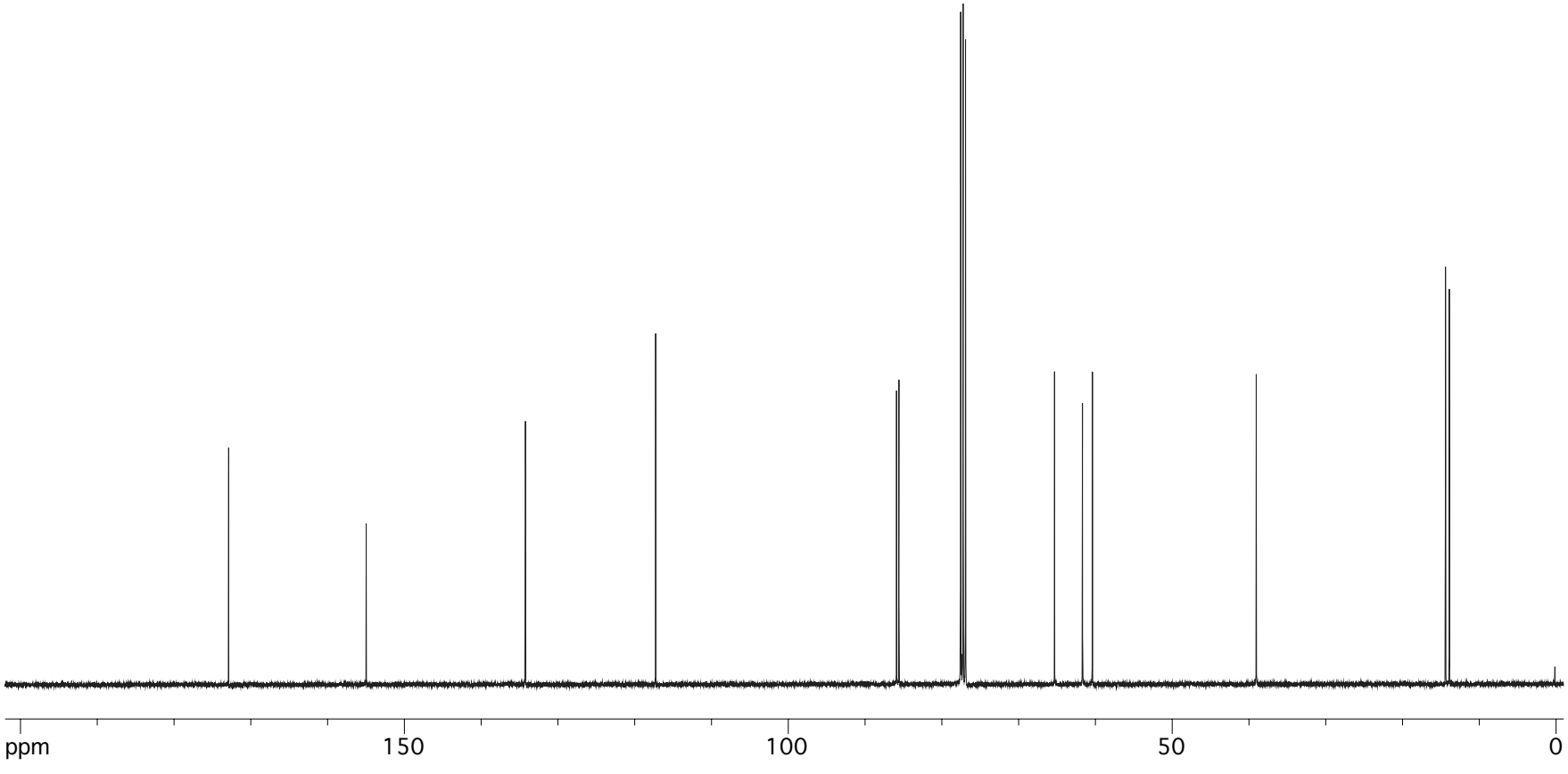

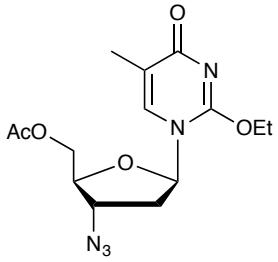

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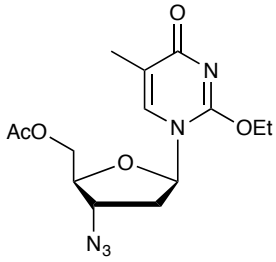

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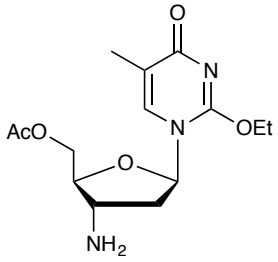

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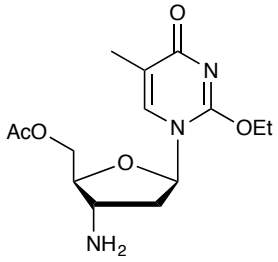

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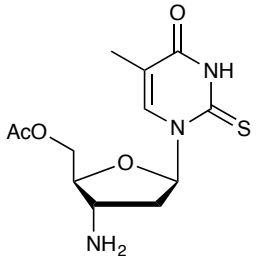

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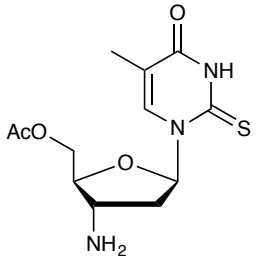

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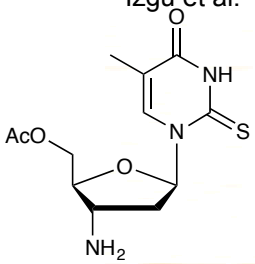
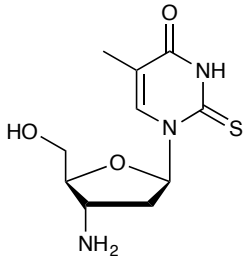
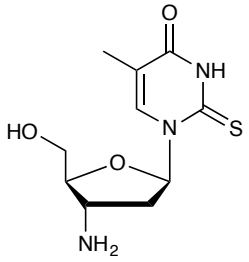


6

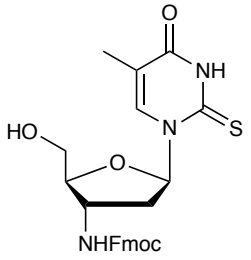
in $CDCl_3$



S1



S1



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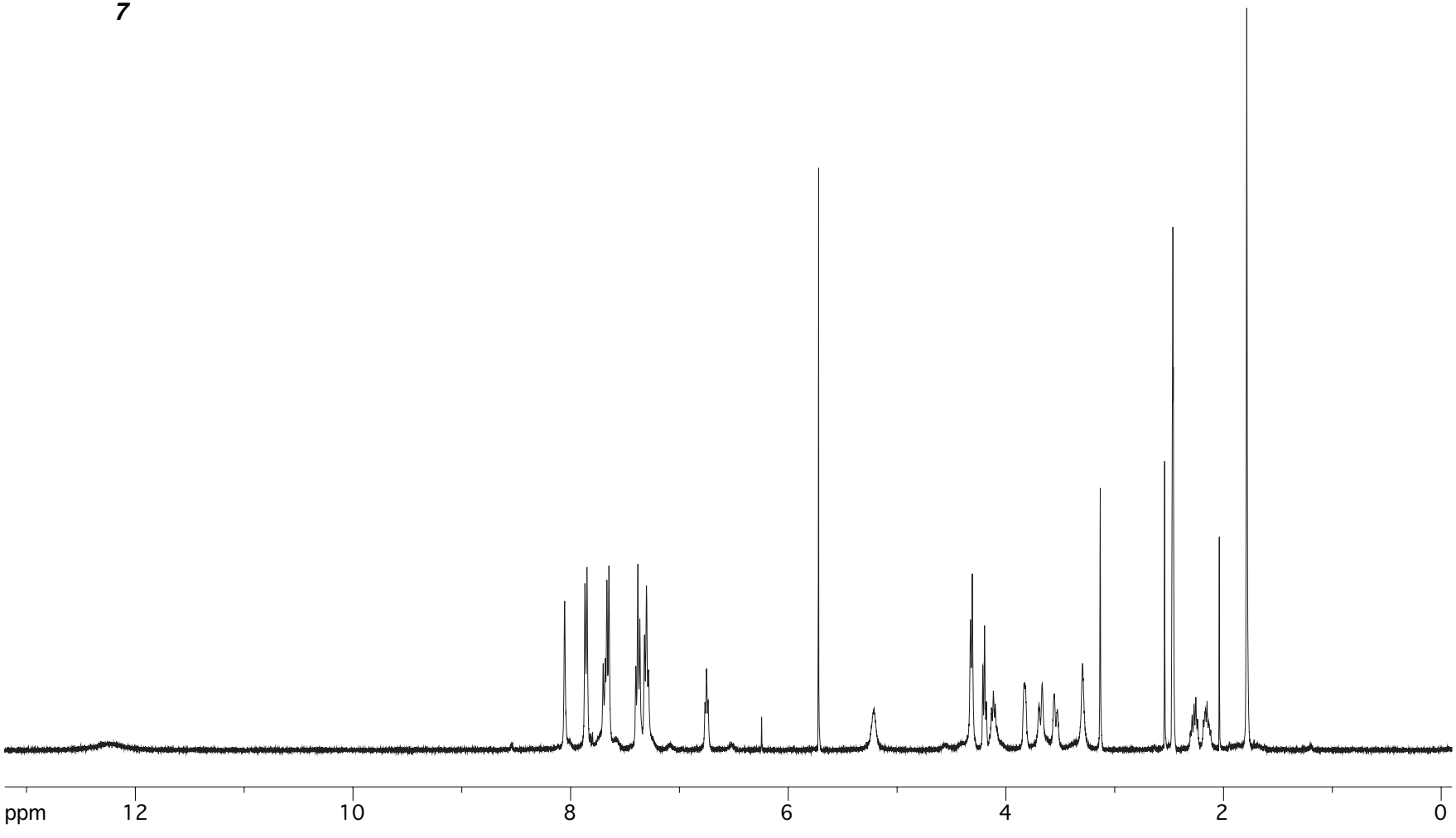
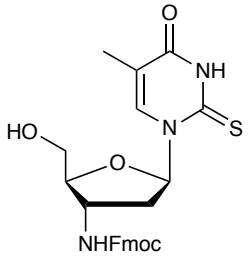




7

4. Monomer Decay-^{31}P NMR Experiment

To determine the rate of decomposition of 3′-NH_2-2-MeImpdds^{2}T under our primer extension reaction conditions, we have carried out a monomer decay-^{31}P NMR experiment (Fig. S3).

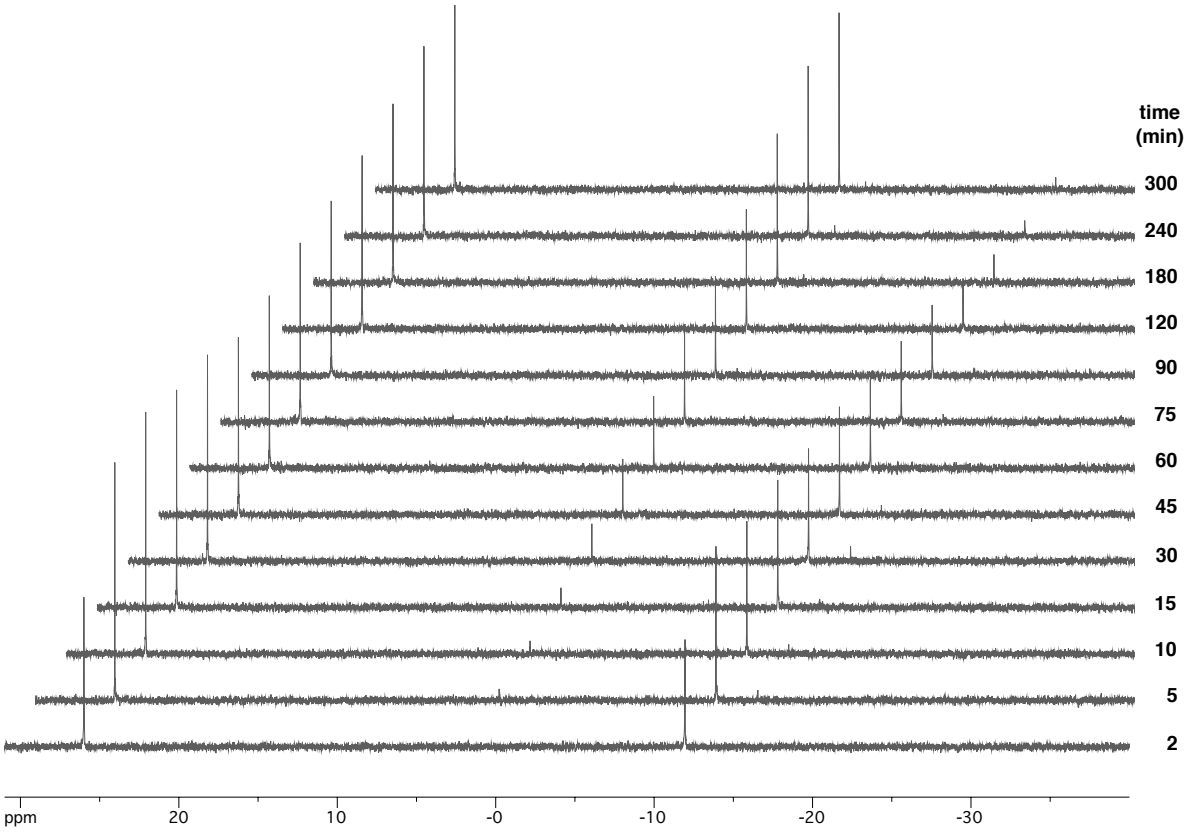


Figure S3. Monomer decay-^{31}P NMR spectral overlay of 3′-NH_2-2-MeImpdds^{2}T. This nucleotide (10 mM) was dissolved in a solution (pH 7.5) of NaCl (150 mM), HEI (100 mM), CAPS:MES:HEPES (100 mM each) prepared using H_2O/D_2O (4:1). Each spectrum was acquired at 4 °C and referenced externally — by means of a co-axial NMR tube — to Ph_3***P***O dissolved in $CDCl_3$ at 26 ppm. Chemical shifts of 3′-NH_2-2-MeImpdds^{2}T and its 5′,3′-cyclization product appear at –11.9 and 1.7 ppm, respectively.

Results revealed that the half-life of 3′-NH_2-2-MeImpdds^{2}T is 77 min, or 1.3 h (Fig. S4), which is similar to that (1.2 h) we previously measured for the unmodified analog, 3′-NH_2-2-MeImpddT.[3] This suggests that the 2-thio modification does not affect the rate of cyclization of activated T monomer.

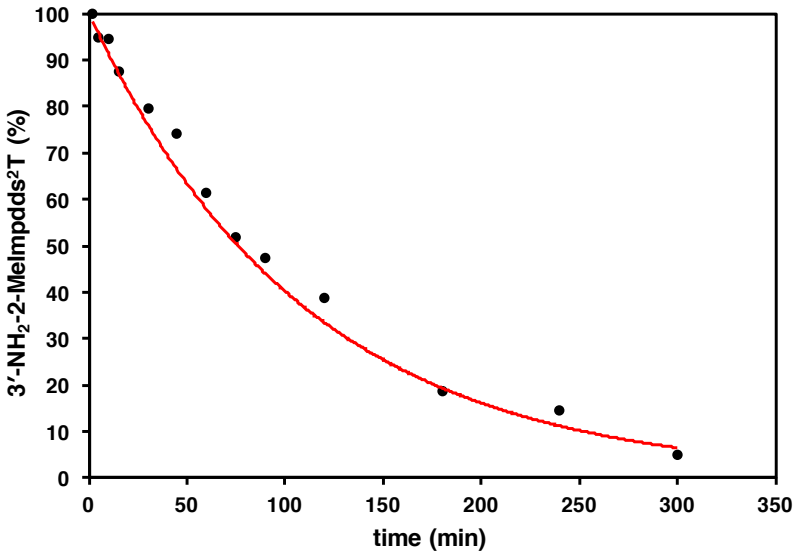


Figure S4. Plot of the percent decay of 3′-NH_2-2-MeImpdds^{2}T. $R^2 = 0.982$.

5. Variable pH-^{13}C NMR Experiment

We have carried out a variable pH-^{13}C NMR spectroscopy experiment (Fig. S5) on 3′-amino-2′,3′-dideoxy-2-thio-thymidine (**S1**) to measure the pK_a of the protonated form of 3′-amine. We have monitored the resonance of C3′ of **S1**, while gradually increasing the pH of the NMR sample.

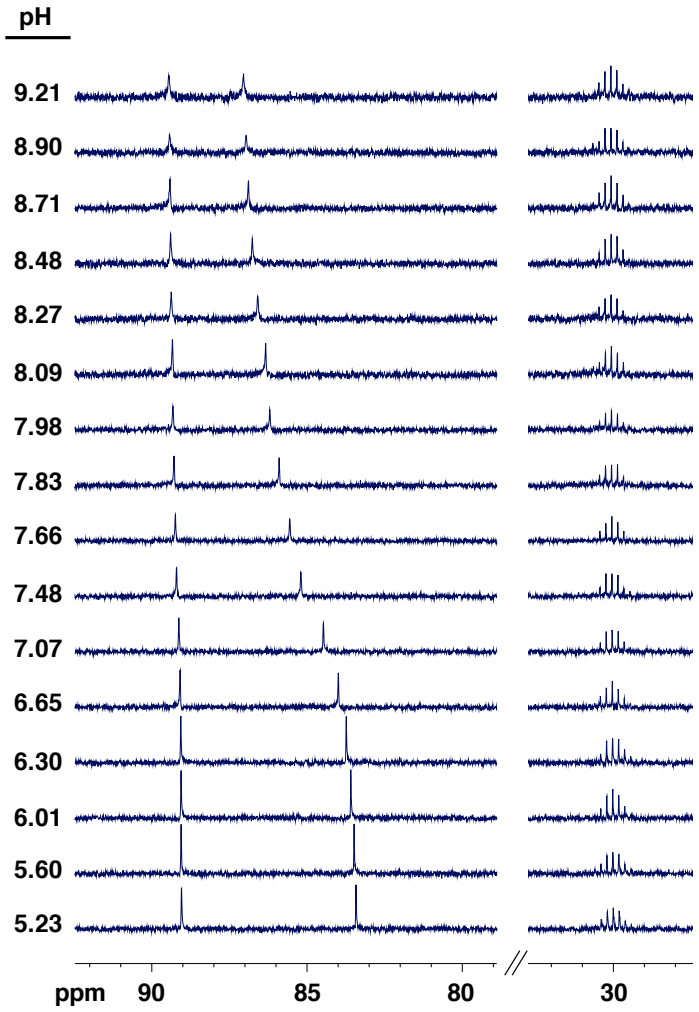
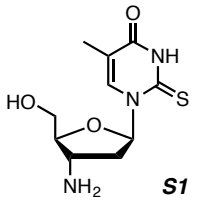


Figure S5. Variable pH-^{13}C NMR spectral overlay of 3′-amino-2′,3′-dideoxy-2-thio-thymidine (**S1**). This nucleoside (50 mM) was dissolved in a phosphate buffer (300 mM) prepared using H_2O/D_2O (4:1). Each spectrum was acquired at 100 MHz and referenced externally — by means of a co-axial NMR tube — to C(O)(***C***$D_3)_2$ at 30.0 ppm (middle peak of the septet). The C3′ resonance is observed to shift downfield from 83.4 to 87.0 ppm as the pH increases from 5.23 to 9.21, whereas the C4′ resonance shifts minimally, from 89.0 to 89.5 ppm.



S1

We have plotted the C3′ chemical shift values (δ) against pH (Fig. S6) and fit to equation 1,[4] derived from the Henderson-Hasselbalch equation,

$$\delta = \delta_{acid} + \frac{\delta_{base}\, 10^{(\text{pH-p}Ka)}}{1 + 10^{(\text{pH-p}Ka)}} \qquad (1)$$

where δ_{acid} and δ_{base} correspond to the extrapolated C3′ chemical shift values at the low and high

extremes of pH, respectively. With an excellent correlation coefficient (R^2 = 0.999), we have determined the midpoint of pH value as 7.51, which corresponds to pK_a of the 3′-amino group.

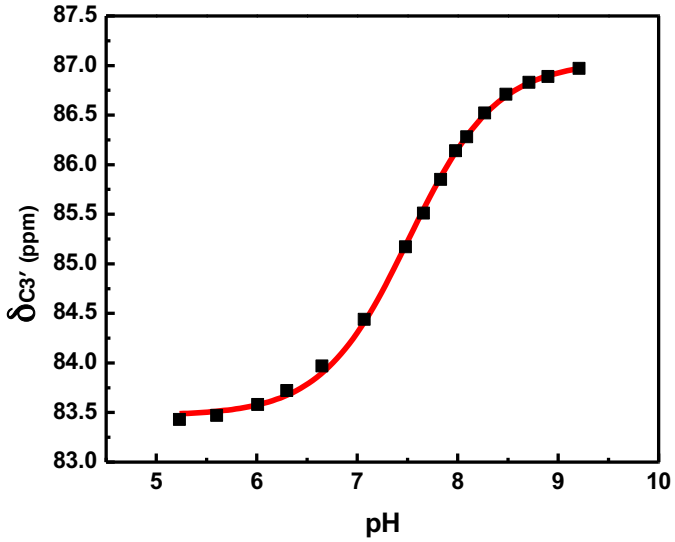
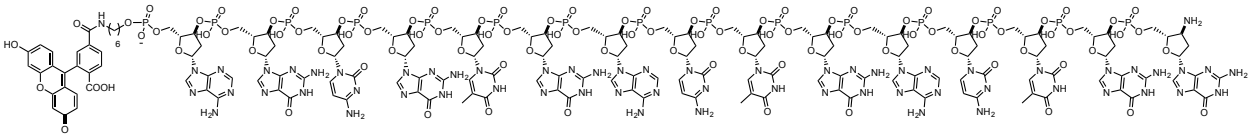


Figure S6. Plot of the chemical shift for C3′ resonance of 3′-amino-2′,3′-dideoxy-2-thio-thymidine with respect to pH.

6. LC-HRMS Analysis of the DNA Primer Strand.



5′-(FAM)-AGC-GTG-ACT-GAC-TGG-(NH$_2$)-3′

Exact mass (Neg. mode): 5165.937; measured accurate mass: 5165.925, error: -2.3 ppm.

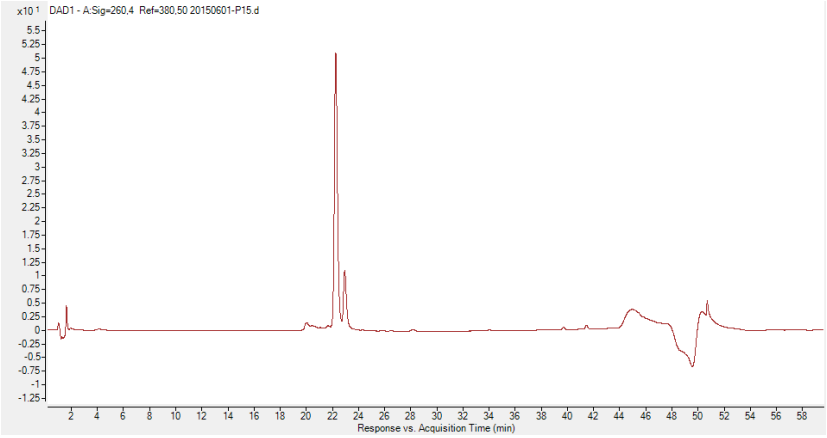


Figure S7. LC trace of the DNA primer strand.

7. Kinetic Studies of Primer Extension Reactions

Primer and template oligonucleotide strands were re-annealed by a heat-cool cycle in a PCR instrument. Primer extension reactions on poly(A) RNA templates (Fig. S8), which were performed in a 4 °C cold room, were initiated by addition of the monomer solution (for a total monomer concentration of 10 or 30 mM). Each reaction aliquot was quenched by 3x volume of formamide, and the quenched samples were stored at –80 °C until the gel electrophoresis.

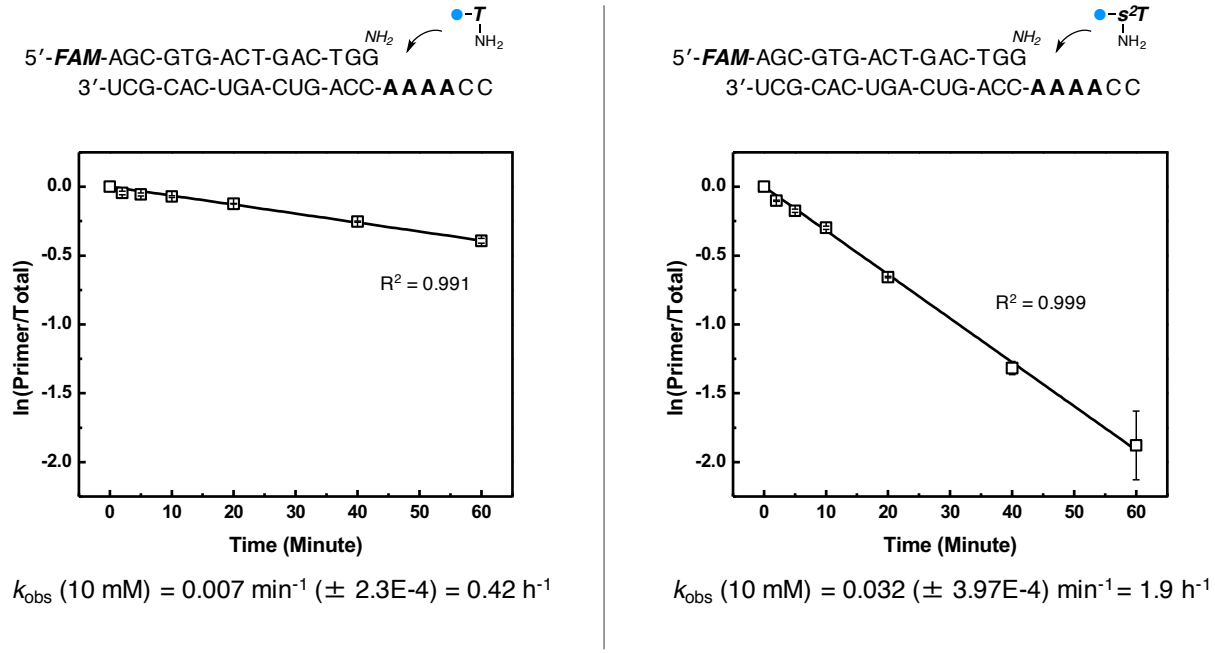


Figure S8. Primer extension kinetics.

8. X-Ray Crystallography

Nucleoside **6** was crystallized from chloroform. A crystal was mounted on a diffractometer and data was collected at 100 K. The intensities of the reflections were collected by means of a Bruker APEX II DUO CCD diffractometer (Cu_{K_α} radiation, λ=1.54178 Å), equipped with an Oxford Cryosystems nitrogen flow apparatus. The collection method involved 1.0° scans in ω at -30°, -55°, -80°, 30°, 55°, 80° and 115° in 2θ. Data integration down to 0.84 Å resolution was carried out using SAINT V8.34 C with reflection spot size optimization.[5] Absorption corrections were made with the program SADABS.[5] The structure (Fig. S9) was solved by the Intrinsic Phasing methods and refined by least-squares methods against F^2 using SHELXT-2014[6] and SHELXL-2014 with OLEX 2 interface.[7] Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were allowed to ride on the respective atoms. Crystal data as well as details of data collection and refinement are summarized in Table S1, and geometric parameters are shown in Table S2. The Ortep plots produced with SHELXL-2014 program, and the other drawings were produced with Accelrys DS Visualizer 2.0.[8]

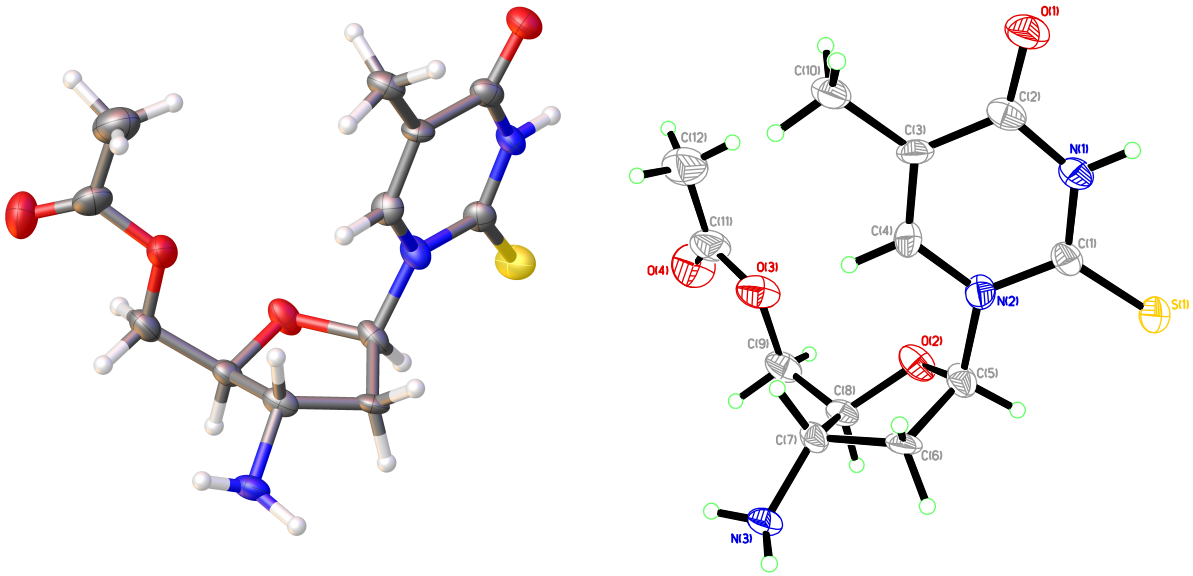


Figure S9. Crystal structure of nucleoside **6.** (Right) Perspective views showing 50% probability displacement.

Table S1. Experimental details for the X-ray analysis.

Crystal data	
Chemical formula	$C_{12}H_{17}N_3O_4S$•$CHCl_3$
M_r	418.71
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	100
a, b, c (Å)	4.9957 (3), 17.7935 (9), 20.4746 (10)
V (Å^3)	1820.01 (17)
Z	4
Radiation type	Cu $K\alpha$
μ (mm^{-1})	5.85
Crystal size (mm)	0.12 × 0.01 × 0.01
Data collection	
Diffractometer	Bruker D8 goniometer with CCD area detector diffractometer
Absorption correction	Multi-scan *SADABS*
T_{min}, T_{max}	0.683, 0.815
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	15691, 3129, 2776
R_{int}	0.091
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.595
Refinement	
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.079, 0.214, 1.08
No. of reflections	3129
No. of parameters	233
No. of restraints	6
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.73, -0.43
Absolute structure	Flack x determined using 930 quotients [(I+)-(I-)]/[(I+)+(I-)] [9]
Absolute structure parameter	0.03 (3)

Table S2. Geometric parameters (Å, º). Most relevant measurements are listed.

C1—N1:	1.342 (12)		N1—C1—S1:	120.2 (7)
C1—N2:	1.365 (12)		N2—C1—S1:	124.2 (7)
C1—S1:	1.684 (10)		O2—C5—N2:	107.2 (7)
C2—O1:	1.239 (11)		O2—C5—C6:	108.0 (7)
C2—N1:	1.394 (13)		N2—C5—C6:	114.6 (8)
C2—C3:	1.443 (13)		N3—C7—C6:	112.2 (7)
C3—C4:	1.337 (13)		N3—C7—C8:	111.4 (8)
C3—C10:	1.503 (14)		O3—C9—C8:	109.3 (8)
C5—N2:	1.485 (11)		S1—C1—N2—C5:	0.2 (13)
C7—N3:	1.470 (11)		O2—C5—N2—C4:	64.6 (10)

9. References

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