Supporting Information for manuscript entitled

Synthesis of N3´-P5´-linked Phosphoramidate DNA by Nonenzymatic Template-Directed Primer Extension

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Contents:

1. General information for reagents and instrumentation

All solvents and reagents were reagent grade, purchased commercially, and used without further purification unless specified. All chemicals were purchased from Sigma-Aldrich unless other wise indicated. Oligonucleotides used as primers or templates were synthesized on an Expedite nucleic acid synthesizer (Applied BioSystems) or purchased from IDT (Coralville, IA) unless otherwise indicated. All the Nuclear Magnetic Resonance (NMR) real-time studies and NMR spectra were recorded on a Varian NMR spectrometer (Oxford AS-400). Chemical shifts are reported as parts per million (ppm) using tetramethylsilane (TMS) as internal standard or by reference to proton resonances resulting from incomplete deuteration of the NMR solvent. Data were reported as follows: (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, *J* = coupling constant in Hz, integration). Proton-decoupled ^{13}C NMR (100 MHz) spectra were reported in ppm from $CDCl_3$, CD_3OD, or DMSO-d6 (77.0, 49.0, or 39.5 ppm, respectively). Proton-decoupled ^{31}P NMR (161.8 MHz) spectra were reported in ppm using phosphate buffer as reference. Electrospray mass spectra were recorded on a Bruker Daltonics Esquire 6000 ESI-MS. LC-MS studies of oligonucleotides were carried out on Agilent Q-TOF LC/MS system.

Starting materials 3′-amino-2′,3′-dideoxyadenosine and 3′-amino-2′,3′-dideoxyguanosine were purchased from Metkinen Chemistry (Finland). 3′-amino-N^4-benzoyl-5′-O-DMTr-2′,3′-dideoxycytidine was purchased from R. I. Chemical Inc (Orange, CA). 7-Deaza-6-methoxy 2′-deoxyguanosine was purchase from ChemGenes Corporation (Wilmington, MA). 3′-azido-2′,3′-dideoxythymidine (AZT) was purchased from Berry & Associates (Dexter, MI). Phosphoramidites, reagents, and columns for oligonucleotide synthesis were purchased from Glen Research (Sterling, VA). The activated phosporimidazolide and phosphor-2-methylimidazolide nucleotide monomers were purified by reverse-phase preparative HPLC (Varian ProStar Preparative LC) on a Prep-C18 column (Varian Dynamax 250 × 21.4 mm) equilibrated with 25 mM triethylammonium bicarbonate, pH 8.0 and eluted with an acetonitrile linear gradient (0-60%). Synthetic oligonucleotides were purified by reverse-phase HPLC (Agilent 1100

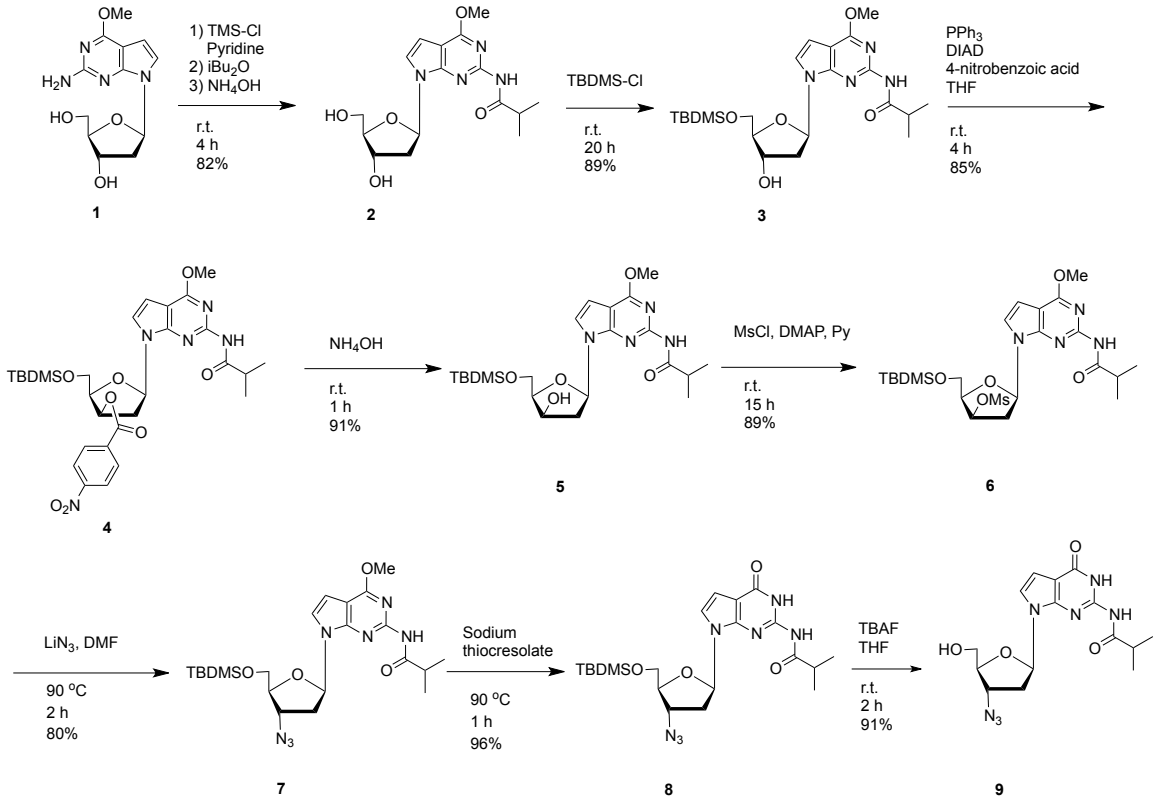
series LC) on a 50 × 4.6 mm C18 column (XTerra), mobile phase: A, 8.6 mM Et_3N/100 mM 1,1,1,3,3,3-hexafluoro-2-propanol in water (pH 8.0); B, methanol. Elution was performed from 100% A isocratic over 10 min followed by a linear gradient of 0 –50% B for 20 min and then 50% B isocratic over another 30 min.

NMR kinetic experiments were performed on a Varian 400 MHz NMR spectrometer (Oxford AS-400) equipped with a Varian 5 mm broadband PFG (z-gradient) probe. Spectra were collected at 4 °C or 25 °C unless otherwise indicated, and data was analyzed using the Varian VnmrJ 2.1B software. Proton decoupled one-dimensional ^{31}P spectra (161.8 MHz) were acquired with a spectral width of 5000 Hz, a 90° pulse of 8.5 μs, 256 scans, 2 s repetition delay, and 1.5 s acquisition time for each scan. All ^{31}P chemical shifts are reported relative to that of phosphate buffer as internal reference (0 ppm at 4 °C). Kinetic studies on the decay of activated monomers were accomplished by real-time ^{31}P NMR at a given reaction time. The signal intensities of all ^{31}P spectra are on a uniform arbitrary scale throughout the whole course of the reaction.

LC-MS analysis was performed using an Agilent 6520 Q-TOF mass analyzer and 1200 series HPLC with a Waters XBridge C18 column (3.5 μm, 1x100 mm). Mobile phase A was aqueous 200 mM HFIP and 3 mM TEA at pH 7.0, and mobile phase B was methanol. The HPLC method for 35 μL of a 2.5 μM solution was a linear increase of 5% to 20% B over 30 min at 0.1 mL/min, with the column heated to 60 ^{o}C. Sample elution was monitored by absorbance at 260 nm and the eluate was passed directly to an ESI source with 325 °C drying nitrogen gas flowing at 8.0 L/min, a nebulizer pressure of 30 psig and a capillary voltage of 3500 V. Agilent MassHunter Qualitative Analysis software was used for Q-TOF derived MS data.

2. Synthesis of activated phosphorimidazolide and phosphor-2-methylimidazolide nucleotide monomers

(1) Synthesis of 3′-NH_2-7-deaza-ImpddG



N^2-isobutyryl-7-deaza-6-methoxy-2′-deoxyguanosine (Compound **2***).*

The preparation of compound 2 was adapted from a previously reported procedure,[(1)] with minor modifications as follows.

To a stirred solution of 7-deaza-6-methoxy 2′-deoxy guanosine **1** (1.0 g; 3.57 mmol) in anhydrous pyridine (15 ml) cooled in an ice-bath, trimethylsilyl chloride (1.81 ml; 14.27 mmol) was added slowly. After stirring at room temperature for 45 minutes, isobutyric anhydride (2.22 ml; 14.27 mmol) was added dropwise, and the reaction mixture was stirred under a nitrogen atmosphere at room temperature for 4 h. The reaction mixture was then chilled in an ice-bath, 10 mL of cold water was added and stirred for 30 min. Concentrated aqueous NH_4OH was then added, and stirring for another 30 min. The solvent was removed under high vacuum by rotovaporation to give an oil with salts.

The crude product was purified by a silica gel column chromatography using methanol-chloroform (5%-20%) as the eluent to afford **2** (1.03 g; 82% yield) as a white foam. ^{1}H NMR δ (400 MHz, $CDCl_3$): 7.08 (d, *J* = 3.2 Hz, 1H), 6.49 (d, *J* = 3.6 Hz, 1H), 6.27 (t, *J* = 6.4 Hz, 1H), 4.85 (m, 1H), 4.14 (s, 3H), 3.95-3.93 (m, 1H), 3.92-3.85 (m, 1H), 2.93 (m, 1H), 2.46 (m, 1H), 1.28 (d, *J* = 6.8 Hz, 1H), 1.19 (m, 1H); ESI-MS calcd for $C_{16}H_{23}N_4O_5$ [$(M+H)^+$]: 351.17, found: 351.1.

*5′-O-(tert-butyldimethylsilyl)-N^2-isobutyryl-7-deaza-6-methoxy-2′-deoxyguanosine (Compound **3**).*

To a stirred solution of **2** (1.09 g; 3.11 mmol) and imidazole (508 mg; 7.47 mmol) in anhydrous DMF (15 ml) was added tert-butyldimethyl-silyl chloride (TBDMS-Cl) (563 mg; 3.73 mmol). The reaction mixture was stirred at room temperature for 20 h. Then most solvent was removed under vacuum, and the residue was extracted with $CHCl_3$ (150 ml). The organic layer was washed with saturated aqueous $NaHCO_3$ and NaCl, respectively, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by flash column chromatography over silica gel using methanol-chloroform (2%-15%) as the eluent to afford **3** (1.29 g; 89% yield) as a white foam. ^{1}H NMR δ (400 MHz, $CDCl_3$): 7.16 (d, *J* = 4.8 Hz, 1H), 6.62 (dd, *J* = 6.4 Hz, 7.2 Hz, 1H), 6.39 (d, *J* = 4.0 Hz, 1H), 4.56-4.52 (m, 1H), 3.98 (s, 3H), 3.80-3.71 (m, 2H), 2.47-2.35 (m, 2H), 1.20 (d, *J* = 6.8 Hz, 6H), 1.19 (m, 1H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C NMR δ (100 MHz, $CDCl_3$): 161.40, 160.80, 149.64, 121.25, 101.75, 99.34, 86.48, 83.40, 72.80, 64.26, 54.51, 41.76, 37.54, 32.57, 27.18, 20.74, 20.77, 19.79, -3.46, -3.58. ESI-MS calcd for $C_{22}H_{37}N_4O_5Si$ [$(M+H)^+$]: 465.25, found: 465.1.

*(2R,3R,5R)-2-(((tert-butyldimethylsilyl)oxy)methyl)-5-(2-isobutyramido-4-methoxy-7H-pyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3-yl 4-nitrobenzoate (Compound **4**).*

The preparation of compound 4 was adapted from a previously reported procedure,[(2)] with minor modifications as follows.

To a solution of **3** (1.45 g; 3.12 mmol) in anhydrous THF (20 ml) were added triphenylphosphine (1.23 g; 4.68 mmol) and diisopropyl azodicarboxylate (DIAD) (947 mg, 4.68 mmol) at room temperature. After 20 min, 4-nitrobenzoic acid (782 mg, 4.68 mmol) was added to the reaction mixture and the reaction mixture was stirred further

for 4 h. The solvent was removed under vacuum, and the resultant residue was extracted with $CHCl_3$ (150 ml). The organic layer was washed with saturated aqueous $NaHCO_3$ and NaCl, respectively, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by flash column chromatography over silica gel using methanol-dichloromethane (1%-10%) as the eluent to afford **4** (1.63 g, 85%) as a yellow foam. ^{1}H NMR δ (400 MHz, $CDCl_3$): 8.38 (d, *J* = 8.4 Hz, 2H), 8.20 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 4.0 Hz, 1H), 6.68 (dd, *J* = 3.2Hz, 4.4 Hz, 1H), 6.57 (d, *J* = 4.0 Hz, 1H), 5.94-5.91 (m, 1H), 4.41-4.37 (m, 1H), 4.16-4.07 (m, 1H), 4.13 (s, 3H), 3.11-3.03 (m, 1H), 2.83-2.78 (m, 1H), 1.37 (dd, *J* = 2.2 Hz, 4.4 Hz, 6H), 1.36 (m, 1H), 0.88 (s, 9H), 0.08 (s, 3H), 0.01 (s, 3H); ^{13}C NMR δ (100 MHz, $CDCl_3$): 161.76, 161.51, 161.49, 150.78, 149.89, 149.16, 133.84, 129.59, 122.75, 120.64, 101.71, 99.36, 83.08, 82.17, 73.95, 61.14, 54.48, 40.07, 27.19, 26.94, 20.72, 19.56, -3.51, -3.56. ESI-MS calcd for $C_{29}H_{40}N_5O_8Si$ [$(M+H)^+$]: 614.26, found: 614.1.

N-(7-((2R,4R,5R)-5-(((tert-butyldimethylsilyl)oxy)methyl)-4-hydroxytetrahydrofuran-2-yl)-4-methoxy-7H-pyrrolo[2,3-d]pyrimidin-2-yl)isobutyramide (Compound ***5****).*

A suspension of **4** (2.0 g, 3.26 mmol) in methanolic ammonia (20 ml) was stirred for 1 h at room temperature. The resulting homogeneous solution was concentrated under vacuum and the residue was purified by flash column chromatography over silica gel using methanol-chloroform (5%-15%) as the eluent to afford **3** (1.38 g; 91% yield) as a white foam. ^{1}H NMR δ (400 MHz, $CDCl_3$): 7.27 (d, *J* = 4.0 Hz, 1H), 6.39 (d, *J* = 4.0 Hz, 1H), 6.21 (dd, *J* = 3.2 Hz, 5.6 Hz, 1H), 5.22-5.20 (m, 1H), 4.08-3.96 (M, 2H), 4.03 (s, 3H), 3.89-3.86 (m, 1H), 2.75-2.67 (m, 1H), 2.51-2.47 (m, 1H), 1.21 (d, *J* = 6.4 Hz, 1H), 1.20 (m, 1H), 0.85 (s, 9H), 0.11 (s, 3H), 0.09 (s, 3H); ^{13}C NMR δ (100 MHz, $CDCl_3$): 161.43, 154.90, 150.10, 149.44, 127.72, 124.12, 102.52, 98.63, 84.34, 83.00, 71.62, 70.15, 62.54, 54.44, 41.51, 27.12, 23.21, 20.71, 19.64, -3.52, -3.55. ESI-MS calcd for $C_{22}H_{37}N_4O_5Si$ [$(M+H)^+$]: 465.25, found: 465.2.

(2R,3R,5R)-2-(((tert-butyldimethylsilyl)oxy)methyl)-5-(2-isobutyramido-4-methoxy-7H-pyrrolo[2,3-d]pyrimidin-7-yl)tetrahydrofuran-3-yl methanesulfonate (Compound ***6****).*

To a solution of **5** (970 mg; 2.09 mmol) in anhydrous pyridine (10 ml) were added MsCl (0.41 ml; 597 mg; 5.21 mmol) and DMAP (1.28 g; 10.44 mmol) at room temperature. The reaction mixture was quenched with MeOH after stirring for 15 h and concentrated. The

residue was dissolved in CH_2Cl_2 (100 ml) and washed with water (2×50 ml). The organic layer was dried (Na_2SO_4), concentrated under vacuum, and the residue was purified by flash column chromatography over silica gel using ethyl acetate-hexane (10%-50%) as the eluent to afford **6** (1.01 g; 89% yield) as a white foam. ^{1}H NMR δ (400 MHz, $CDCl_3$): 7.24 (d, *J* = 4.0 Hz, 1H), 6.59 (dd, *J* = 4.0 Hz, 5.2 Hz, 1H), 6.47 (d, *J* = 4.0 Hz, 1H), 5.29-5.27 (m, 1H), 4.06-4.01 (m, 1H), 4.02 (s, 3H), 3.90-3.88 (m, 2H), 3.05 (s, 3H), 2.94-2.87 (m, 1H), 2.71-2.66 (m, 1H), 1.24 (d, *J* = 8.0 Hz, 1H), 1.23 (m, 1H), 0.86 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR δ (100 MHz, $CDCl_3$): 161.47, 151.25, 149.97, 121.22, 101.49, 100.09, 81.82, 81.62, 79.46, 60.46, 54.45, 40.52, 39.31, 27.05, 20.74, 20.70, 19.63, -3.44, -3.51. ESI-MS calcd for $C_{23}H_{39}N_4O_7SSi$ [$(M+H)^+$]: 543.23, found: 543.1.

*3'-azido-5'-O-(tert-butyldimethylsilyl)-N^2-isobutyryl-7-deaza-6-methoxy-2',3'-dideoxyguanosine (Compound **7**).*

To a solution of **6** (840 mg, 1.55 mmol) in anhydrous DMF (12 ml) was added lithium azide (362 mg, 8.04 mmol). The reaction mixture was heated at 90 °C for 2 h. The solvent was evaporated under vacuum, and the residue was extracted with $CHCl_3$ (100 ml). The organic layer was washed with saturated aqueous $NaHCO_3$ and NaCl, respectively, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by flash column chromatography over silica gel using ethyl acetate-hexane (10%-60%) as the eluent to afford **7** (606 mg; 80% yield) as a white foam. ^{1}H NMR δ (400 MHz, $CDCl_3$): 7.18 (d, *J* = 4.0 Hz, 1H), 6.46 (t, *J* = 6.0 Hz, 1H), 6.45 (d, *J* = 3.6 Hz, 1H), 4.49 (m, 1H), 4.04 (s, 3H), 3.98-3.96 (m, 1H), 3.83-3.81 (m, 1H), 2.64 (m, 1H), 2.47 (m, 1H), 1.27 (d, *J* = 6.8 Hz, 1H), 1.26 (m, 1H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR δ (100 MHz, $CDCl_3$): 161.46, 150.78, 149.85, 121.10, 101.74, 99.41, 84.10, 83.34, 63.39, 61.20, 54.42, 38.75, 27.12, 20.70, 19.74, -3.48, -3.62. ESI-MS calcd for $C_{22}H_{36}N_7O_4Si$ [$(M-H)^-$]: 488.24, found: 488.1.

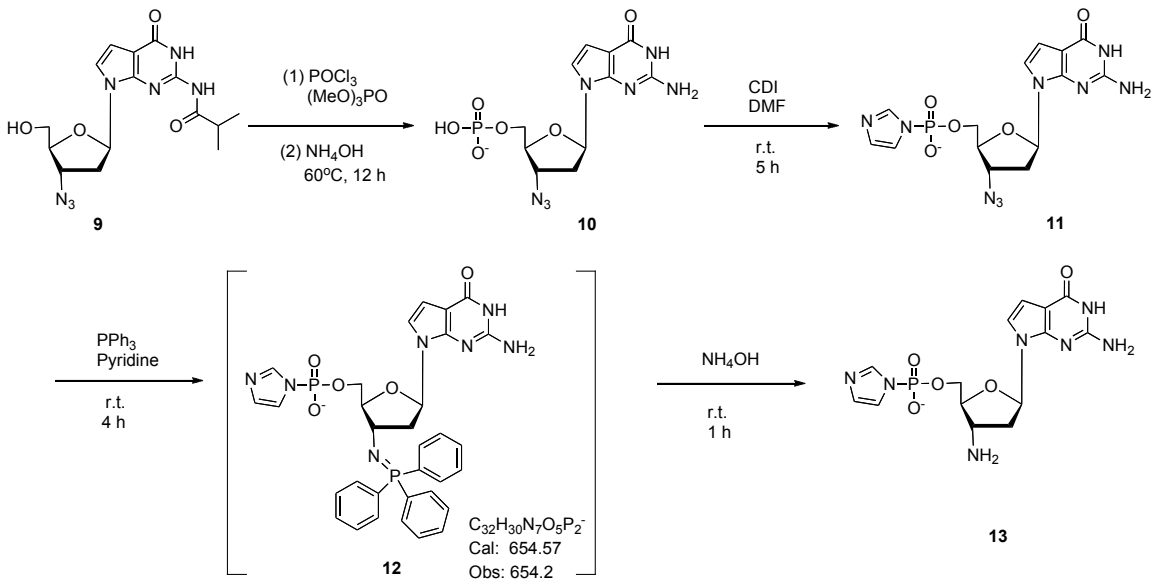
*3'-azido-5'-O-(tert-butyldimethylsilyl)-N^2-isobutyryl-7-deaza-2',3'-dideoxyguanosine (Compound **8**).*

To a solution of **7** (610 mg; 1.25 mmol) in anhydrous DMF (15 ml) was added sodium thiocresolate (1.09 g; 7.46 mmol). The reaction mixture was heated at 90 °C for 1 h. The solvent was evaporated under vacuum, and the residue was extracted with $CHCl_3$ (100 ml). The organic layer was washed with saturated aqueous $NaHCO_3$ and NaCl,

respectively, and dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by flash column chromatography over silica gel using ethyl acetate-hexane (10%-50%) as the eluent to afford **8** (569 mg; 96% yield) as a white foam. ^{1}H NMR δ (400 MHz, $CDCl_3$): 6.91(d, *J* = 3.6 Hz, 1H), 6.55 (d, *J* = 3.6 Hz, 1H), 6.24 (dd, *J* = 6.8 Hz, 6.0 Hz, 1H), 4.29-4.28 (m, 1H), 3.92-3.90 (m, 1H), 3.73-3.71 (m, 2H), 2.55-2.54 (m, 1H), 2.45-2.42 (m, 1H), 2.31-2.25 (m, 1H), 1.18 (d, *J* = 6.0 Hz, 1H), 1.14 (m, 1H), 0.83 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H); ^{13}C NMR δ (100 MHz, $CDCl_3$): 176.15, 160.89, 124.51, 120.58, 117.72, 112.98, 104.83, 103.51, 84.11, 82.81, 63.52, 61.55, 38.73, 37.56, 27.14, 20.46, 19.77, -3.43, -3.60. ESI-MS calcd for $C_{21}H_{32}N_7O_4Si$ [(M-H)$^-$]: 474.23, found: 474.2.

*3'-azido-N^2-isobutyryl-7-deaza-2',3'-dideoxyguanosine (Compound **9**)*.

To a solution of **8** (200 mg; 0.42 mmol) in anhydrous THF (6 ml) was added 1.0 M tetrabutylammonium fluoride (TBAF) in THF solution (0.84 mL; 0.84 mmol). After stirring at room temperature for 2 h, the reaction mixture was concentrated under reduced pressure and partitioned between H_2O and CH_2Cl_2. The organic layer was separated and dried over Na_2SO_4. After concentration, the residue was purified by flash column chromatography over silica gel using methanol-chloroform (5%-20%) as the eluent to afford **9** (138 mg; 91% yield) as a white foam. ^{1}H NMR δ (400 MHz, $CDCl_3$): 6.79 (d, *J* = 2.8 Hz, 1H), 6.57 (d, *J* = 3.8 Hz, 1H), 6.08 (dd, *J* = 6.8 Hz, 7.2 Hz, 1H), 4.49-4.47 (m, 1H), 4.09 (m, 1H), 3.99-3.96 (m, 1H), 3.75-3.72 (m, 1H), 2.90-2.86 (m, 1H), 2.65-2.61 (m, 1H), 2.40-2.34 (m, 1H), 1.24 (dd, *J* = 6.8 Hz, 6.0 Hz, 6H); ^{13}C NMR δ (100 MHz, $CDCl_3$): 176.18, 144.72, 120.80, 106.47, 103.07, 102.94, 87.26, 84.98, 63.61, 62.23, 38.77, 37.55, 30.88, 20.44, 20.34. ESI-MS calcd for $C_{15}H_{18}N_7O_4$ [(M-H)$^-$]: 360.14, found: 360.1.



*3′-azido-7-deaza-2′,3′-dideoxyguanosine-5′-phosphate (Compound **10**).*

Compound **9** (120 mg; 0.33 mmol) and proton sponge (86 mg; 0.39 mmol) were dried in a vacuum desiccator over P_2O_5 overnight before dissolving in trimethyl phosphate (1.0 ml). Then freshly distilled $POCl_3$ (38 μl; 0.39 mmol) was added dropwise at 0°C. After stirring at 0°C for 1.5 h, triethyl ammonium bicarbonate solution (TEAB) (0.1 M; pH 8.0; 1 ml) was added and the mixture was stirred for 10 min at room temperature. Then concentrated NH_4OH (5 ml) was added and stirred for 12 h at 60°C. The resulting mixture was concentrated under vacuum and the residue was diluted with 5 ml of water. The crude mixture was then purified by anion exchange chromatography on DEAE-Sephadex A-25 at 4°C using a gradient of TEAB (pH 8.0; 0.1–1.0 M) to afford **10** as a white powder. ^{1}H NMR δ (400 MHz, D_2O): 6.89 (d, *J* = 4.4 Hz, 1H), 6.38 (d, *J* = 4.4 Hz, 1H), 6.16 (dd, *J* = 4.4 Hz, 5.2 Hz, 1H), 4.49-4.47 (m, 1H), 4.09 (m, 1H), 3.89-3.84 (m, 1H), 3.58-3.48 (m, 2H), 2.66-2.62 (m, 1H), 2.41-2.35 (m, 1H); ^{31}P NMR δ (168.1 MHz, D_2O): 1.79; ESI-MS calcd for $C_{11}H_{13}N_7O_6P^-$ (M^-): 370.07, found: 370.0.

*3′-azido-7-deaza-2′,3′-dideoxyguanosine-5′-phosphorimidazolide (Compound **11**).*

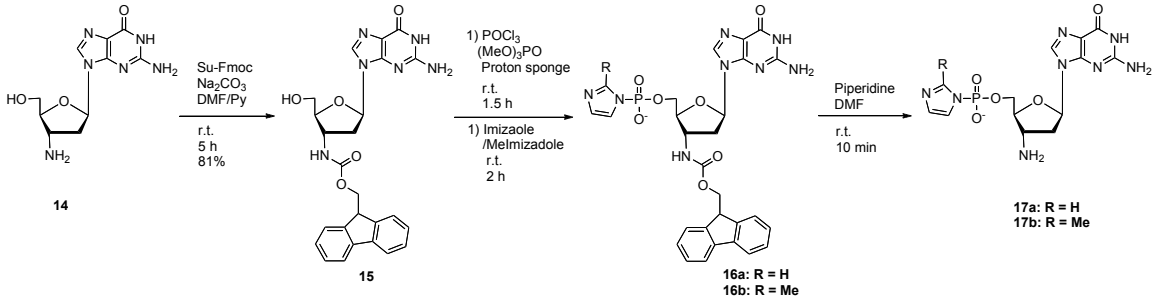
A solution of **10** (156 mg; 0.42 mmol) was co-evaporated with pyridine (10 ml × 3) and then dissolved in anhydrous DMF (3 ml). To the resulting solution, 1,1-carbonyldiimidazole (CDI) (102 mg; 0.63 mmol) was added and the mixture was stirred at room temperature for 5 h. After centrifuging the reaction, the supernatant was treated

with a solution of sodium perchlorate (478 mg) in acetone (15 ml). After cooling for 3 h in the refrigerator, the mixture was centrifuged and the supernatant was discarded. The precipitate was washed twice with acetone and dried over P_2O_5 to afford **11** as a white powder (143 mg; 81%). ^{1}H NMR δ (400 MHz, D_2O): 7.85 (s, 1H), 7.16 (s, 1H), 7.02 (s, 1H), 6.91 (d, *J* = 4.0 Hz, 1H), 6.49 (d, *J* = 4.0 Hz, 1H), 6.34 (t, *J* = 6.8 Hz, 1H), 4.49-4.47 (m, 1H), 4.09-4.08 (m, 1H), 3.99-3.97 (m, 2H), 2.74-2.71 (m, 1H), 2.52-2.50 (m, 1H); ^{31}P NMR δ (168.1 MHz, D_2O): -10.2; ESI-MS calcd for $C_{14}H_{15}N_9O_5P^-$ (M^-): 420.09, found: 420.1.

*3'-amino-7-deaza-2',3'-dideoxy guanosine-5'-phosphorimidazolide (3'-NH_2-7-deaza-ImpddG, Compound **13**).*

To a solution of **11** (20 mg; 0.04 mmol) in pyridine (1 ml) was added triphenylphosphine (21 mg; 0.08 mmol). The reaction mixture was stirred at room temperature for 4 h to afford intermediate compound **12** [ESI-MS calcd for $C_{32}H_{30}N_7O_5P_2^-$ (M^-): 654.57, found: 654.2]. Without further purification, 2 ml of concentrated NH_4OH was added to the reaction and stirred for 1 h at room temperature. The resulting mixture was concentrated under vacuum and the residue was diluted with 1 ml of DMSO for $NaClO_4$ precipitation as described above for compound **11**. The crude product was further purified by reverse-phase preparative HPLC as previously described to afford **13**. ^{1}H NMR δ (400 MHz, D_2O): 7.74 (s, 1H), 7.04 (s, 1H), 6.91 (s, 1H), 6.90 (d, *J* = 4.0 Hz, 1H), 6.44 (d, *J* = 4.0 Hz, 1H), 6.30 (dd, *J* = 6.8 Hz, 5.6 Hz, 1H), 3.98 (m, 1H), 3.48 (m, 1H), 3.25-3.20 (m, 2H), 2.62-2.54 (m, 1H), 2.33-2.26 (m, 1H); ^{31}P NMR δ (168.1 MHz, D_2O): -10.1; ESI-MS calcd for $C_{14}H_{17}N_7O_5P^-$ (M^-): 394.10, found: 394.1.

(2) Synthesis of 3′-NH_2-ImpddG and 3′-NH_2-2-MeImpddG



*3′-(9-fluorenylmethoxycarbonyl)-amino-2′,3′-dideoxyguanosine (Compound **15**).*

General Protocol **A**: The following protocol is representative for selective protection of aminonucleosides **14** and **26** to generate the aminonucleosides **15** and **27**, respectively.

3′-amino-2′,3′-dideoxy guanosine **14** (200 mg; 0.75 mmol) was dissolved in DMF(1.0 ml), pyridine (1.0 ml) and 1 M Na_2CO_3 aqueous solution (0.2 ml). Fmoc N-hydroxysuccinimide ester (304 mg, 0.90 mmol) was added slowly to the above reaction mixture and then stirred at room temperature for 5 h with exclusion of light. The crude product was washed with water, and precipitated from chloroform-acetonitrile to afford **15** (297 mg; 81% yield) as a white powder. ^{1}H NMR δ (400 MHz, CD_3OD): 7.92 (s, 1H), 7.80 (d, *J* = 7.6 Hz, 2 H), 7.66 (d, *J* = 7.2 Hz, 2 H), 7.39 (t, *J* = 6.4 Hz, 2 H), 7.32 (dd, *J* = 6.4 Hz, 7.6 Hz, 2 H), 6.18 (t, *J* = 6.4 Hz, 1H), 4.48-4.43 (m, 2H), 4.22(t, *J* = 6.0 Hz, 2 H), 3.92 (m, 1H), 3.82-3.79 (m, 1H), 3.72-3.68 (m, 1H), 2.78-2.71 (m, 1H), 2.40-2.34 (m, 1H). ESI-MS calcd for $C_{25}H_{23}N_6O_5$ [(M-H)$^-$]: 487.17, found: 487.1.

*3′-(9-fluorenylmethoxycarbonyl)-amino-2′,3′-dideoxyguanosine-5′-phosphor-2-methylimidazolide (Compound **16b**).*

General Protocol **B**: The following protocol is representative for the conversion of nucleoside **15** to the nucleotides **16a**/**16b**.

Compound **15** (120 mg; 0.24 mmol) and proton sponge (64 mg; 0.29 mmol) were dried in a vacuum desiccator over P_2O_5 overnight before dissolving in trimethyl phosphate (1.0 ml). Then freshly distilled $POCl_3$ (26 μl; 0.29 mmol) was added dropwise at 0°C. After stirring at 0°C for 1.5 h, 2-methylimidazole (99 mg; 1.2 mmol) (5 equivalents of imidazole was added for *Compound **16a***) was then added at 0°C. After stirring for an

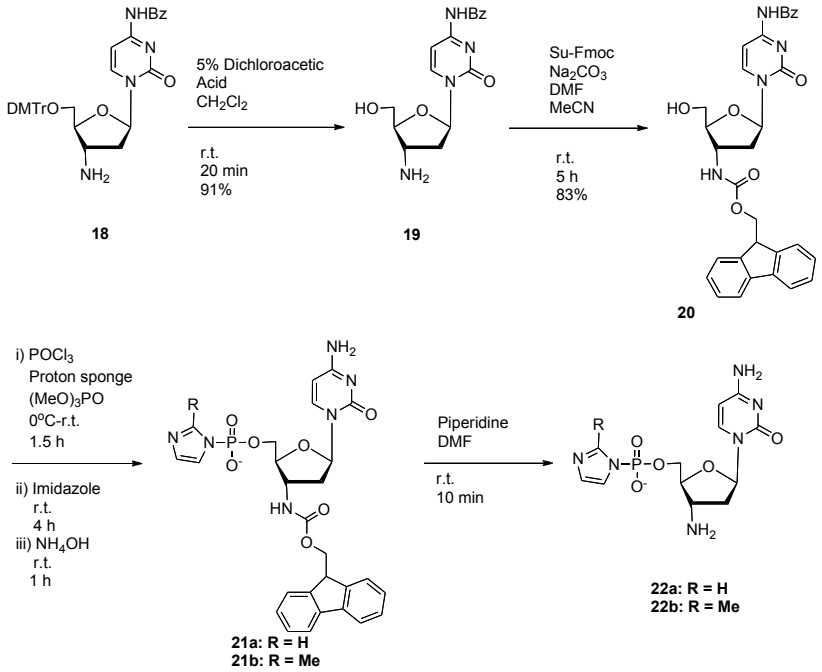
additional 2 h at room temperature, the reaction mixture was partitioned between H_2O and CH_2Cl_2. The crude aqueous product was further purified by reverse-phase preparative HPLC as previously described to afford **16b**. ^{1}H NMR δ (400 MHz, D_2O): 7.71 (d, *J* = 7.2 Hz, 2 H), 7.66 (s, 1H), 7.50 (d, *J* = 6.0 Hz, 2 H), 7.30 (t, *J* = 6.4 Hz, 2 H), 7.23 (t, *J* = 6.4 Hz, 2 H), 6.06 (t, *J* = 6.4 Hz, 1H), 4.28 (m, 2H), 4.13 (m, 1H), 3.82 (t, *J* = 7.2 Hz, 2 H), 3.63 (m, 2H), 2.33-2.25 (m, 2H), 2.20 (m, 1H). ^{31}P NMR δ (168.1 MHz, D_2O): -11.36; ESI-MS calcd for $C_{29}H_{28}N_8O_7P^-$ (M^-): 631.18, found: 631.1.

*3'-amino-2',3'-dideoxyguanosine-5'-phosphor-2-methylimidazolide (3'-NH_2-2-MeImpddG, Compound **17b**).*

General Protocol **C**: The following protocol is representative for conversion of FMoc protected aminonucleotides **16a/16b** to the aminonucleotides **17a/17b**.

To a solution of **16b** (20 mg; 0.03 mmol) in DMF (0.6 ml) was added piperidine (0.12 ml). The reaction mixture was stirred at room temperature for 10 min. The resulting mixture was concentrated under vacuum, and the crude product was further purified by reverse-phase preparative HPLC as previously described to afford **17b**. ^{1}H NMR δ (400 MHz, D_2O): 7.79 (s, 1H), 7.00 (s, 1H), 6.65 (s, 1H), 6.17-6.14 (m, 1 H), 4.03-3.93 (m, 2H), 3.89-3.84 (m, 1 H), 3.77-3.72 (m, 1H), 2.78-2.72 (m, 1H), 2.44-2.31 (m, 1H), 2.22 (s, 3H); ^{31}P NMR δ (168.1 MHz, D_2O): -11.23; ESI-MS calcd for $C_{14}H_{18}N_8O_5P^-$ (M^-): 409.11, found: 409.0.

(3) Synthesis of 3′-NH_2-ImpddC and 3′-NH_2-2-MeImpddC



*3′-amino-N⁴-benzoyl-2′,3′-dideoxycytidine (Compound **19**).*

To a solution of 3′-amino-N⁴-benzoyl-5′-O-DMTr-2′,3′-dideoxycytidine **18** (200 mg; 0.316 mmol) in anhydrous dichloromethane (3 ml) was dropwise added 0.6 ml of 5% dichloroacetic acid at room temperature in two portions. After stirring at room temperature for 20 min, the resulting red reaction mixture was concentrated under reduced pressure and partitioned between H_2O and $CHCl_3$. The organic layer was separated and dried over Na_2SO_4. After concentration, the residue was purified by flash column chromatography over silica gel using methanol-chloroform (2%-10%) as the eluent to afford **19** (95 mg; 91% yield) as a white foam. ^{1}H NMR δ (400 MHz, CD_3OD): 8.50 (d, *J* = 8.0 Hz, 1H), 7.94 (d, *J* = 7.2 Hz, 2H), 7.61 (dd, *J* = 7.6 Hz, 7.2 Hz, 1H), 7.52 (dd, *J* = 7.2 Hz, 6.8 Hz, 2H), 7.51 (d, *J* = 8.0 Hz, 1H), 6.24 (t, *J* = 6.0 Hz, 1H), 4.27-4.26 (m, 1H), 3.99-3.97 (m, 1H), 3.85-3.84 (m, 1H), 2.77-2.71 (m, 1H), 2.55 (m, 1H). ESI-MS calcd for $C_{16}H_{19}N_4O_4$ [$(M+H)^+$]: 331.14, found: 331.1.

*3′-(9-fluorenylmethoxycarbonyl)-amino-N⁴-benzoyl-2′,3′-dideoxycytidine (Compound **20**).*

3′-Amino-2′,3′-dideoxy guanosine **19** (80 mg; 0.24 mmol) was dissolved in DMF (2.0 ml) and 1 M Na_2CO_3 aqueous solution (0.2 ml). To the above reaction mixture was added Fmoc N-hydroxysuccinimide ester (158 mg; 0.47 mmol). After stirring at room temperature for 5h with exclusion of light, the reaction mixture was concentrated under reduced pressure and partitioned between H_2O and $CHCl_3$. The organic layer was separated and dried over Na_2SO_4. After concentration, the residue was purified by flash column chromatography over silica gel using methanol-chloroform (5%-15%) as the eluent to afford **20** (111 mg; 83% yield) as a white foam. ^{1}H NMR δ (400 MHz, CD_3OD): 8.37 (d, *J* = 7.6 Hz, 1H), 7.71 (d, *J* = 7.6 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.38 (d, *J* = 6.8 Hz, 2H), 7.37(t, *J* = 4.0 Hz, 1H), 7.29 (t, *J* = 7.6 Hz, 2H), 7.22 (t, *J* = 7.6 Hz, 2H), 7.12 (dd, *J* = 7.2 Hz, 7.6 Hz, 2H), 5.98 (dd, *J* = 3.0 Hz, 4.0 Hz, 1H), 5.30 (d, *J* = 7.2 Hz, 1H), 4.30-4.21 (m, 2H), 4.08 (m, 1H), 4.01-3.98 (m, 1H), 3.84-3.81 (m, 1H), 3.69-3.61 (m, 2H), 2.41-2.35 (m, 1H), 2.31-2.28 (m, 1H). ESI-MS calcd for $C_{31}H_{28}N_4NaO_6^+$ [$(M+Na)^+$]: 575.19, found: 575.1.

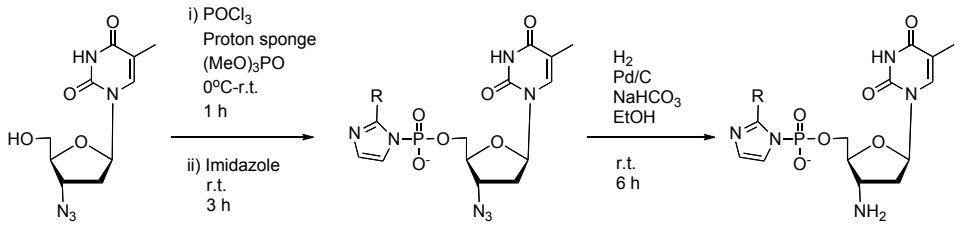
*3′-(9-fluorenylmethoxycarbonyl)-amino-N^4-benzoyl-2′,3′-dideoxycytidine-5′-phosphorimidazolide (Compound **21a**)*.

Compound **20** (120 mg; 0.22 mmol) and proton sponge (70 mg; 0.32 mmol) were dried in a vacuum desiccator over P_2O_5 overnight before dissolving in trimethyl phosphate (1.0 ml). Then freshly distilled $POCl_3$ (30 μl; 0.32 mmol) was added dropwise at 0°C. After stirring at 0°C for 1.5 h, imidazole (76 mg; 1.12 mmol) (5 equivalents of 2-methylimidazole was added for *Compound **21b***) was then added at 0°C. After stirring for an additional 4 h at room temperature, TEAB (0.1 M; pH 8.0; 0.5 ml) was added and the mixture was stirred for 10 min at 0°C. Then concentrated NH_4OH (5 ml) was added and stirred for 1 h at room temperature. The reaction mixture was partitioned between H_2O and $CHCl_3$. The aqueous layer was lyophilized and the dry crude product was further purified by reverse-phase preparative HPLC as previously described to afford **21a**. ^{1}H NMR δ (400 MHz, D_2O): 7.97 (s, 1H), 7.84 (s, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.65 (d, *J* = 5.6 Hz, 2H), 7.60 (d, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 1H), 7.33(dd, *J* = 5.2 Hz, 7.2 Hz, 2H), 7.25 (t, *J* = 7.2 Hz, 2H), 7.17 (t, *J* = 6.4 Hz, 2H), 7.14 (s, 1H), 6.87 (d, *J* = 6.4 Hz, 1H), 6.03 (dd, *J* = 5.6 Hz, 6.0 Hz, 1H), 3.80 (m, 1H), 3.41 (m, 1H), 3.18 (m, 1H), 3.01 (m, 2H), 2.96 (m, 1H), 2.83-2.75 (m, 2H), 2.07-2.04 (m, 1H); ^{31}P NMR δ (168.1 MHz, D_2O): -11.13; ESI-MS calcd for $C_{27}H_{26}N_6O_7P^-$ (M^-): 577.16, found: 577.1.

3′-amino-2′,3′-dideoxycytidine-5′-phosphorimidazolide (Compound ***22a****, 3′-NH_2-ImpddC) and 3′-amino-2′,3′-dideoxycytidine-5′-phosphor-2-methylimidazolide (Compound* ***22b****, 3′-NH_2-2-MeImpddC).*

The procedure for removal of the FMoc group for compounds **22a** and **22b** is similar to General Protocol **C** used in preparing compound **17b**. Compound **22a** ^{1}H NMR δ (400 MHz, D_2O): 7.86 (s, 1H), 7.32 (d, *J* = 6.2 Hz, 1H), 6.93 (s, 1H), 6.68 (s, 1H), 6.02 (dd, *J* = 4.2 Hz, 4.6 Hz, 1H), 5.73 (d, *J* = 6.2 Hz, 1H), 4.27 (m, 1H), 3.84 (m, 1H), 3.68-3.65 (m, 2H), 2.78-2.75 (m, 2H); ^{31}P NMR δ (168.1 MHz, D_2O): -10.37. ESI-MS calcd for $C_{12}H_{16}N_6O_5P^-$ (M^-): 355.09, found: 355.0.

(4) Synthesis of 3′-NH_2-ImpddT and 3′-NH_2-2-MeImpddT



*3′-azido-3′-deoxythymidine-5′-phosphorimidazolide (Compound **24a**).*

The preparation procedure for phosphorylation and activation was similar to General Protocol **B** using in synthesizing compound **16**.

^{1}H NMR δ (400 MHz, D_2O): 7.86 (s, 1H), 7.43 (s, 1H), 7.22 (s, 1H), 7.04 (s, 1H), 6.20 (t, *J* = 6.4, 1H), 4.30-4.29 (m, 1H), 4.03-3.96 (m, 3H), 2.40-2.36 (m, 2H), 1.82 (s, 3H); ^{31}P NMR δ (168.1 MHz, D_2O): -11.12. ESI-MS calcd for $C_{13}H_{15}N_7O_6P^-$ (M^-): 396.08, found: 396.0.

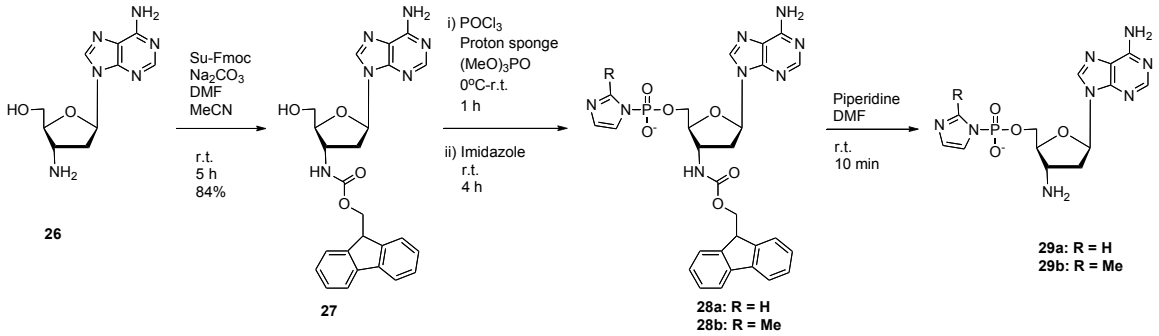
*3′-amino-3′-deoxythymidine-5′-phosphorimidazolide (Compound **25a**, 3′-NH_2-ImpddT).*

General Protocol **D**: The following protocol is representative for conversion of azidonucleotides **24a**/**24b** to the aminonucleotides **25a**/**25b**, as reported previously,[3] with minor modifications as follows.

To a solution of **24a** (28 mg; 0.07 mmol) in ethanol (2 ml) was added saturated aqueous $NaHCO_3$ solution (40 µl). The resulting solution was placed under an argon atmosphere, and Pd/C (2 mg) was added. After the argon was replaced with a hydrogen atmosphere, under the hydrostatic pressure of a 20 cm water column, the slurry was stirred, with the hydrogen atmosphere being replaced every 60 min, until TLC indicated complete conversion (about 6 h). The catalyst was removed by filtration over a bed of celite and washed with ethanol. The combined solutions were concentrated under vacuum, and the crude product was further purified by reverse-phase preparative HPLC as previously described to afford **25a**. ^{1}H NMR δ (400 MHz, D_2O): 7.60 (s, 1H), 7.49 (s, 1H), 7.28 (s, 1H), 7.13 (s, 1H), 6.14 (t, *J* = 6.8 Hz, 1H), 4.36-4.32 (m, 1H), 4.18-4.14 (m, 1H), 4.05-4.03 (m, 1H), 2.47-2.44 (m, 2H), 1.89 (s, 3H); ^{31}P NMR δ (168.1 MHz, D_2O): -11.15. ESI-MS calcd for $C_{13}H_{17}N_5O_6P^-$ (M^-): 370.09, found: 370.1.

For 3′-NH_2-2-MeImpddT **25b** ^{1}H NMR δ (400 MHz, D_2O): 7.47 (s, 1H), 7.15 (s, 1H), 7.12 (s, 1H), 6.80 (s, 1H), 6.15 (dd, *J* = 5.6 Hz, 6.8 Hz, 1H), 4.10-4.08 (m, 1H), 4.02-4.00 (m, 1H), 3.98-3.90 (m, 1H), 3.75-3.70 (m, 1H), 2.49-2.40 (m, 1H), 2.40 (s, 3H), 2.38-2.30 (m, 1H), 1.88 (s, 3H); ^{31}P NMR δ (168.1 MHz, D_2O): -11.16. ESI-MS calcd for $C_{14}H_{19}N_5O_6P^-$ (M^-): 384.11, found: 384.0.

(5) Synthesis of 3′-NH_2-ImpddA and 3′-NH_2-2-MeImpddA



3′-(9-fluorenylmethoxycarbonyl)-amino-2′,3′-dideoxyadenosine (Compound ***27****).*

The procedure for selectively protecting the 3′-amino group of compound **27** is similar to General Protocol **A** using in preparing compound **15**.

^{1}H NMR δ (400 MHz, CD_3OD): 8.39 (s, 1H), 8.19 (s, 1H), 7.81 (d, *J* = 6.8 Hz, 2H), 7.67 (d, *J* = 7.2 Hz, 2H), 7.40 (t, *J* = 8.0 Hz, 2H), 7.32 (t, *J* = 7.2 Hz, 2H), 6.37 (t, *J* = 6.4 Hz, 1H), 4.49-4.45 (m, 2H), 4.24-4.21 (m, 1H), 4.00 (m, 1H), 3.86-3.82 (m, 1H), 3.74-3.70 (m, 1H), 2.85-2.79 (m, 1H), 2.49-2.43 (m, 1H). ESI-MS calcd for $C_{25}H_{24}N_6NaO_4^+$ [$(M+Na)^+$]: 495.18, found: 495.1.

3′-(9-fluorenylmethoxycarbonyl)-amino-2′,3′-dideoxyadenosine-5′-phosphorimdazolide (Compound ***28a****).*

The procedure used for phosphorylation and activation was similar to General Protocol **B** used in synthesizing compound **16**.

^{1}H NMR δ (400 MHz, D_2O): 7.91 (s, 1H), 7.47 (d, *J* = 6.4 Hz, 2H), 7.28 (s, 1H), 7.19 (d, *J* = 6.4 Hz, 2H), 6.97 (t, *J* = 7.2 Hz, 2H), 6.69 (t, *J* = 8.0 Hz, 2H), 6.19 (t, *J* = 6.2 Hz, 1H), 4.57-4.45 (m, 2H), 4.08 (m, 1H), 3.78 (m, 1H), 3.59-3.42 (m, 2H), 2.58-2.52 (m, 1H), 2.24-2.19 (m, 1H); ^{31}P NMR δ (168.1 MHz, D_2O): -10.41. ESI-MS calcd for $C_{28}H_{26}N_8O_6P^-$ (M^-): 601.17, found: 601.2.

3′-amino-2′,3′-dideoxyadenosine-5′-phosphorimidazolide (Compound ***29a****, 3′-NH_2-ImpddA) and 3′-amino-2′,3′-dideoxyadenosine-5′-phosphor-2-methylimidazolide (Compound* ***29b****, 3′-NH_2-2-MeImpddA)*

The procedure for removal of the FMoc group to yield compounds **29a** and **29b** was similar to General Protocol **C** using in preparing compound **17b**.

Compound **29b** ^{1}H NMR δ (400 MHz, D_2O): 8.16 (s, 1H), 6.92 (s, 1H), 6.61 (s, 1H), 6.34 (m, 1H), 4.12-4.10 (m, 2H), 3.94-3.92 (m, 1H), 3.81-3.79 (m, 1H), 2.86-2.84 (m, 1H), 2.51-2.49 (m, 1H), 2.18 (s, 3H), 2.13 (s, 3H); ^{31}P NMR δ (168.1 MHz, D_2O): -10.57. ESI-MS calcd for $C_{14}H_{18}N_8O_4P^-$ (M^-): 393.12, found: 393.0.

References:

1. Challa, H.; Bruice, T. C. *Bioorg Med Chem* **2004**,12, 1475-1481.
2. Jain, M. L.; Bruice, T. C. *Bioorg Med Chem* **2006**,14, 7333-7346.
3. Eisenhuth, R.; Richert, C. *J Org Chem* **2009**, 74, 26-37.

Supporting Figures

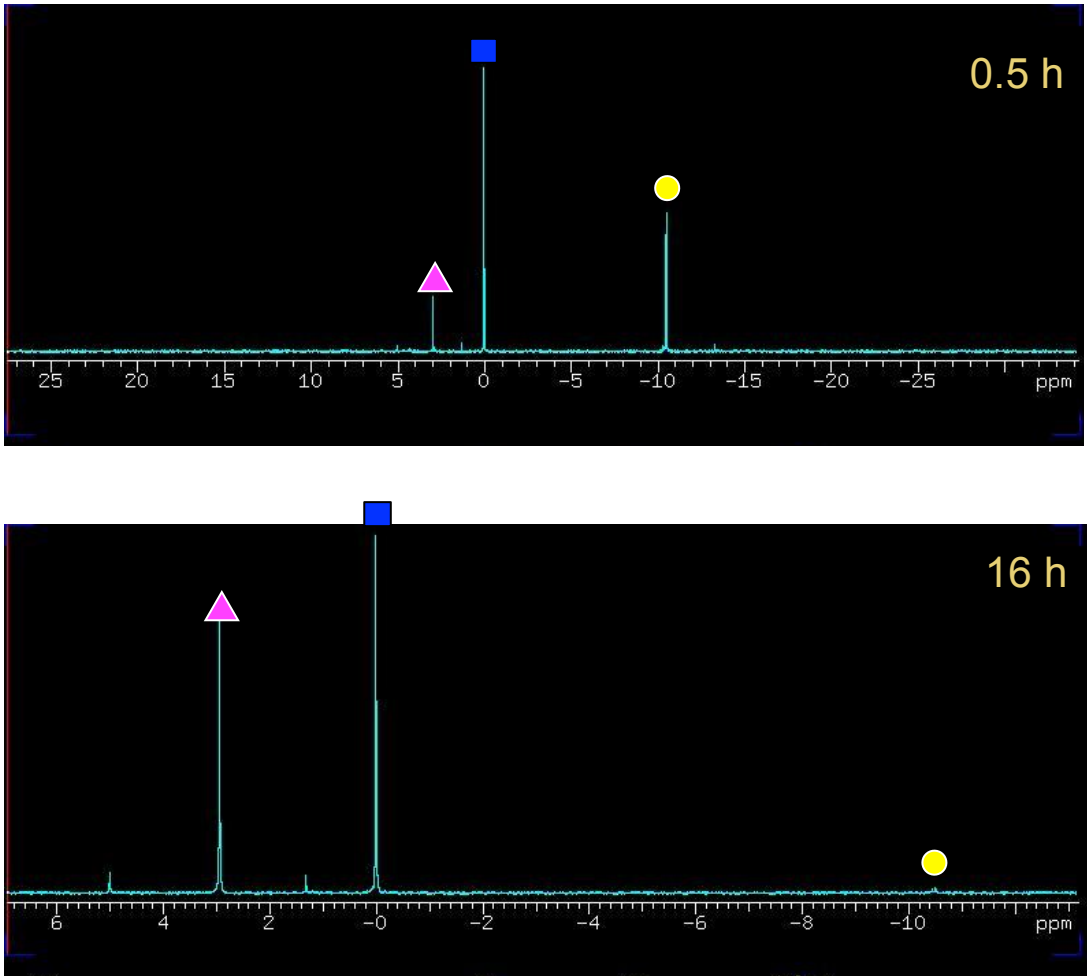


Figure S1. ^{31}P-NMR of 5 mM activated 3′-NH_2-ImpddT in a solution of 100 mM HEI, 100 mM MES-CAPS-HEPES, pH 7.5, 150 mM NaCl.
Upper panel: Measured at 4 °C after 30 min; Lower panel: Measured after 16 h at 4 °C. Activated 3′-NH_2-ImpddT **4a** (-10.58 ppm, yellow dot), hydrolyzed product (1.34 ppm), cyclized product **5** (2.96 ppm, pink triangle), cyclized dimer (5.00 ppm) and 10.0 mM phosphate buffer as a reference (blue square).

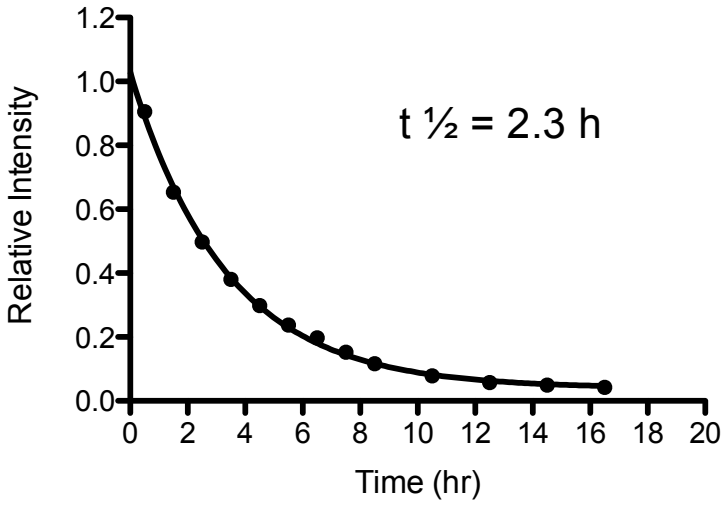
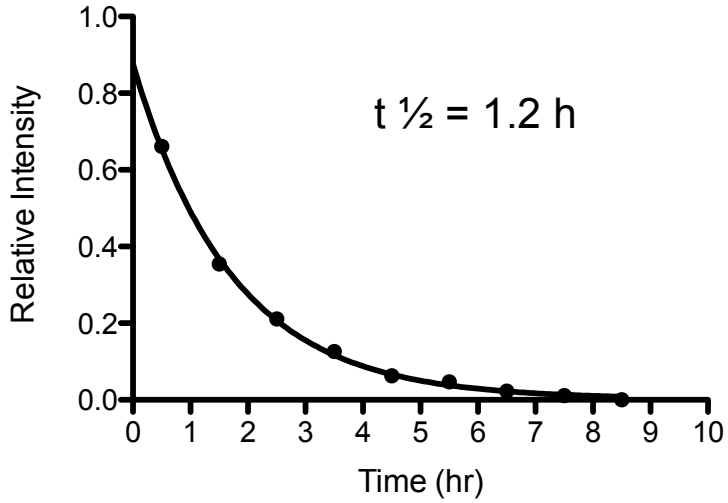


Figure S2. The decay diagram of 5.0 mM activated 3′-NH_2-ImpddT, as monitored by signal intensity at -10.58 ppm, from real-time ^{31}P-NMR spectra over 16 hours. Kinetic studies were performed at 4 °C in a solution of 100 mM HEI, 100 mM MES-CAPS-HEPES, pH 7.5, 150 mM NaCl with 10.0 mM phosphate buffer as a reference.

A)



B)

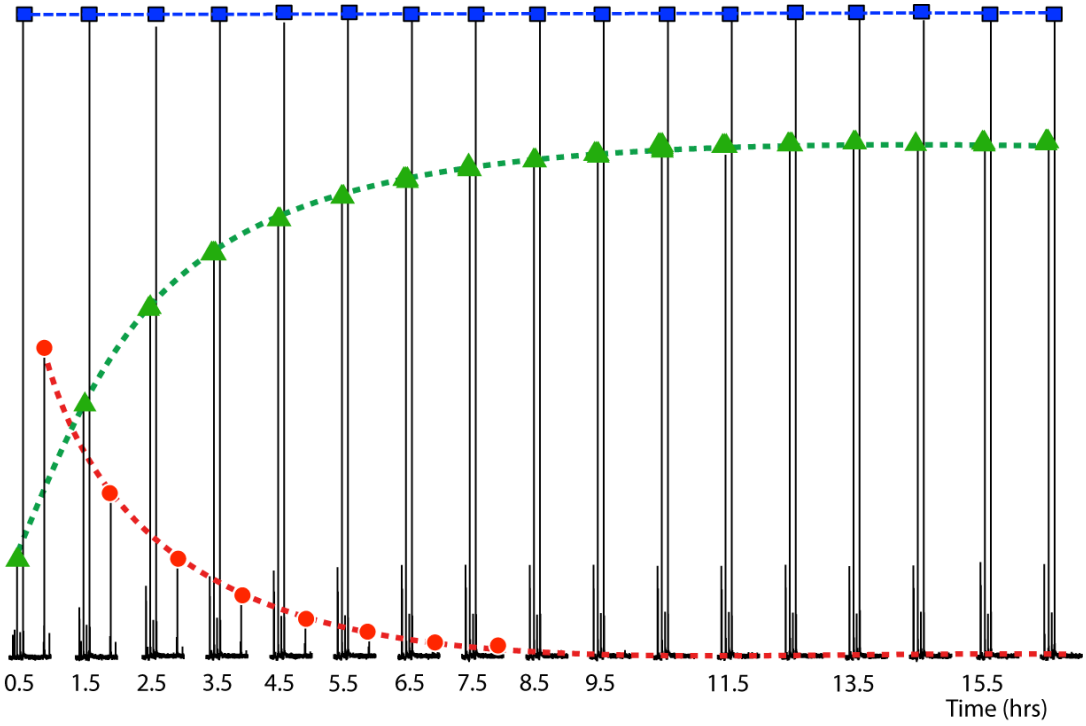


Figure S3. Real-time ^{31}P-NMR studies of the decay of 5.0 mM 3′-NH_2-2-MeImpddT **4b** over 16 hours

Kinetic studies were performed at 4 °C in a solution of 100 mM HEI, 100 mM MES-CAPS-HEPES, pH 7.5, 150 mM NaCl.

A) The decay diagram of activated 3′-NH_2-2-MeImpddT **4b** was monitored by signal intensity at -10.68 ppm.

B) Real-time ^{31}P-NMR of the decrease of activated 3′-NH_2-2-MeImpddT **4b** (-10.68 ppm, red circles) and the increase of cyclized product **5** (3.06 ppm, green triangles) in the above conditions with phosphate buffer as a reference (blue squares).

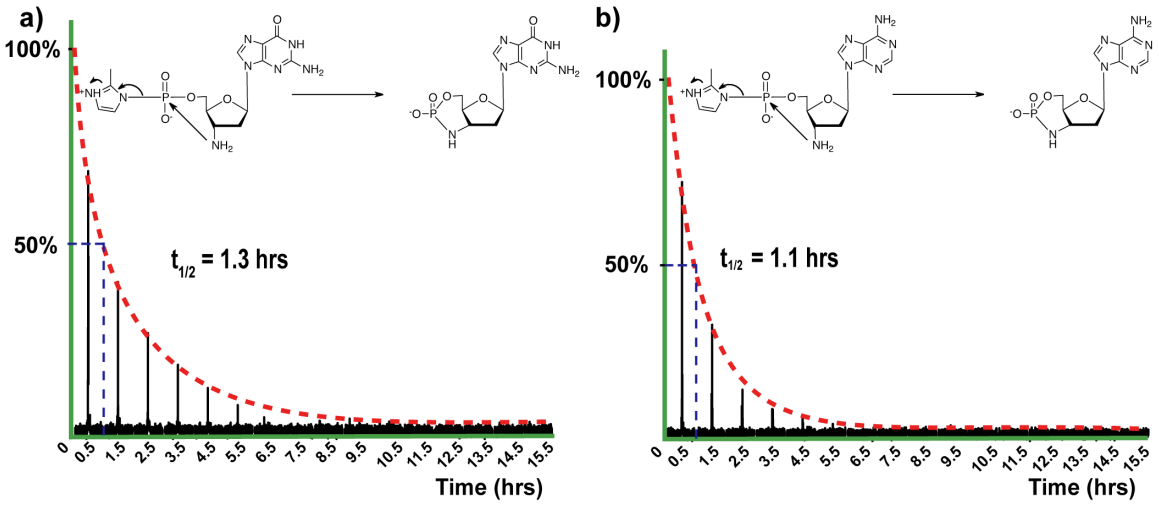


Figure S4. Real-time ^{31}P-NMR studies of the decay of 3′-NH_2-MeImpddNs over 15 h. **a**) 2.5 mM activated 3′-NH_2-MeImpddG monitored at δ = -10.45 ppm; and **b**) 2.5 mM activated 3′-NH_2-MeImpddA monitored at δ = -10.38 ppm.
Both reactions were performed at 4 °C in a solution of 100 mM HEI, 100 mM MES-CAPS-HEPES, pH 7.5, and 150 mM NaCl with 10.0 mM phosphate buffer (δ = 0 ppm as an internal reference).

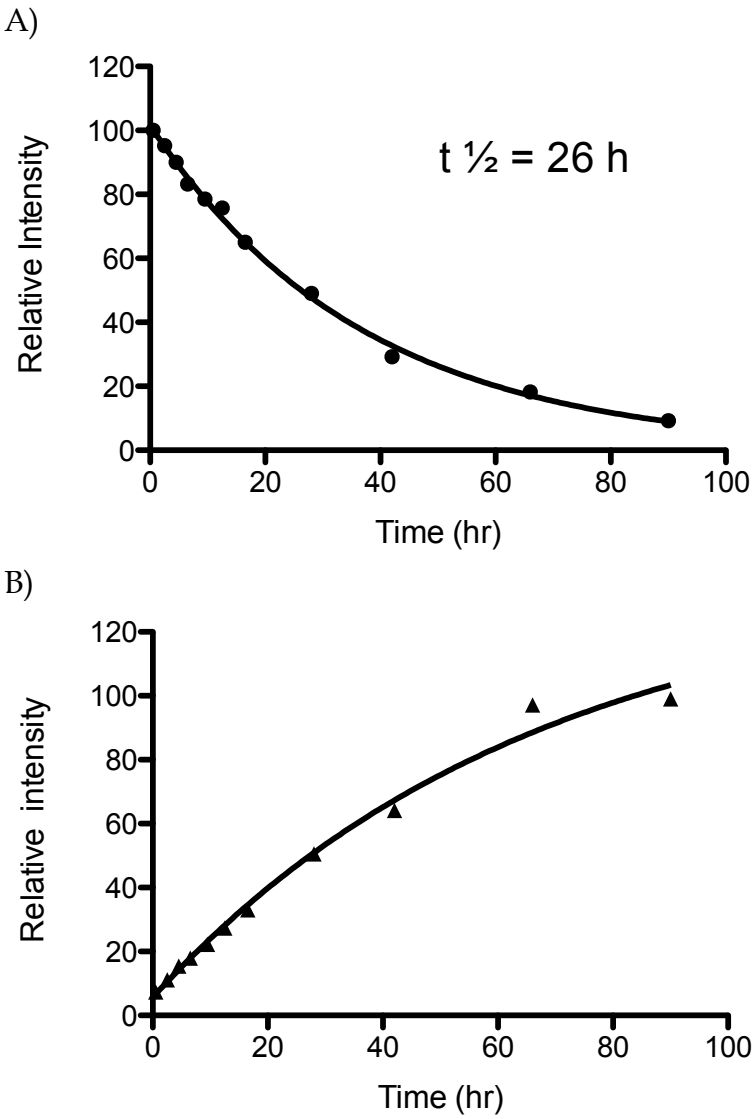


Figure S5. Kinetic study of the decay of 5.0 mM activated 3'-NH_2-2-MeImpddT over 90 hours in a solution of 100 mM MES-CAPS-HEPES, pH 7.5, 150 mM NaCl (in the absence of HEI), 10.0 mM phosphate buffer as a reference. A) The decay of activated 3'-NH_2-2-MeImpddT as monitored by signal intensity at -10.68 ppm, from real-time ^{31}P-NMR spectra; B) The appearance of cyclic-monomer by-product as monitored by signal intensity at 3.24 ppm from real-time ^{31}P-NMR spectra.

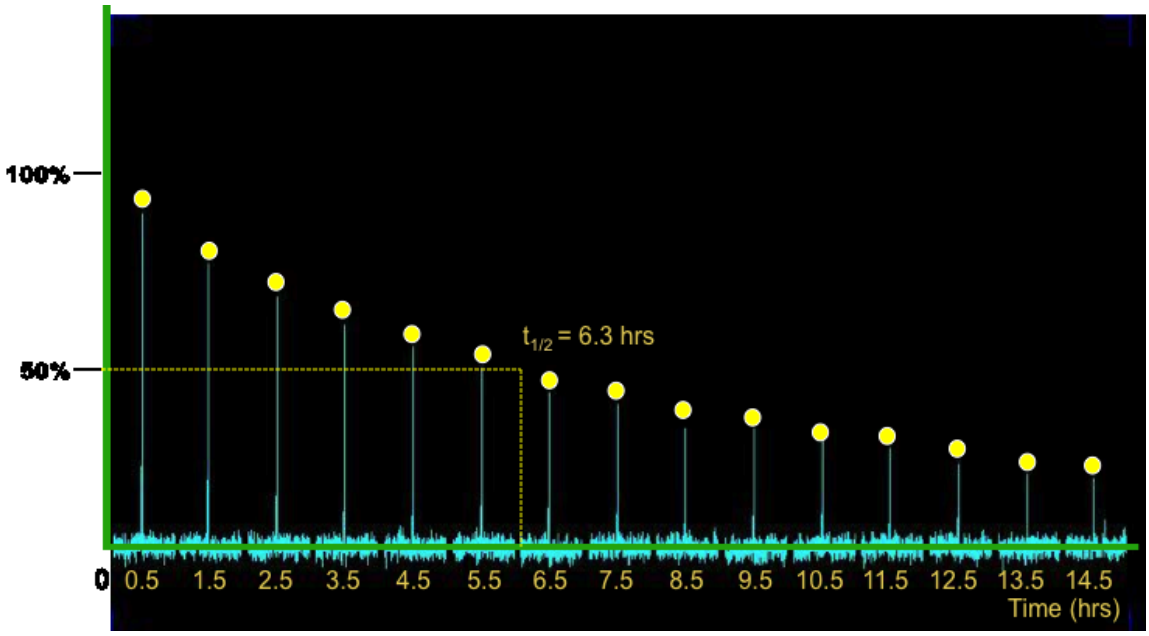


Figure S6. Real-time ^{31}P-NMR studies of 5 mM activated 3′-NH_2-ImpddT over 14 hours at 4 °C.

Intensity was monitored at -10.60 ppm. The experiment was performed in a solution of 100 mM HEI, 100 mM MES-CAPS-HEPES, pH 9.3, 150 mM NaCl.

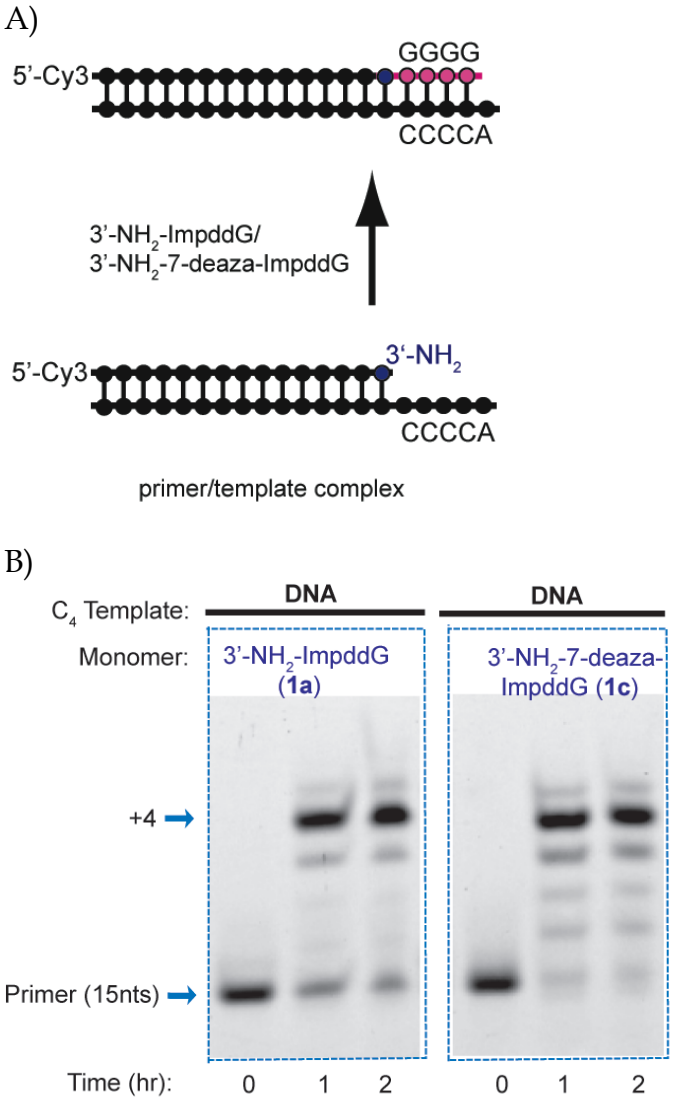


Figure S7. Non-enzymatic primer-extension reaction using 3′-NH_2-ImpddG (**1a**) /3′-NH_2-7-deaza-ImpddG (**1c**) as monomers.

(a) Primer-extension reaction scheme showing a 5′-Cy3-labeled 3′-amino-terminated DNA primer annealed to a complementary template. Both 3′-NH_2-ImpddG and 3′-NH_2-7-deaza-ImpddG monomers participate in a chemical chain reaction extending the primer by four (4) nucleotides on the complementary template forming a chimeric DNA/3′-NP-DNA polymer product. The line in red indicates new phosphoramidate bonds. (b) High-resolution gel electrophoresis analysis of primer-extension products on indicated templates. Primer-extension reactions contained 0.1 μM Cy3-labeled-3′-amino-terminated DNA primer, 0.5 μM template, 100 mM MES-CAPS-HEPES, pH 7.5, and 100 mM 1-(2-hydroxyethyl)imidazole. The reaction was initiated by addition of 5 mM 3′-NH_2-ImpddG/3′-NH_2-7-deaza-ImpddG. Arrows indicate primer and full-length product.

	template	Sequence
1	DNA	5' -A**CCCC**CCAGTCAGTCTACGC-3′

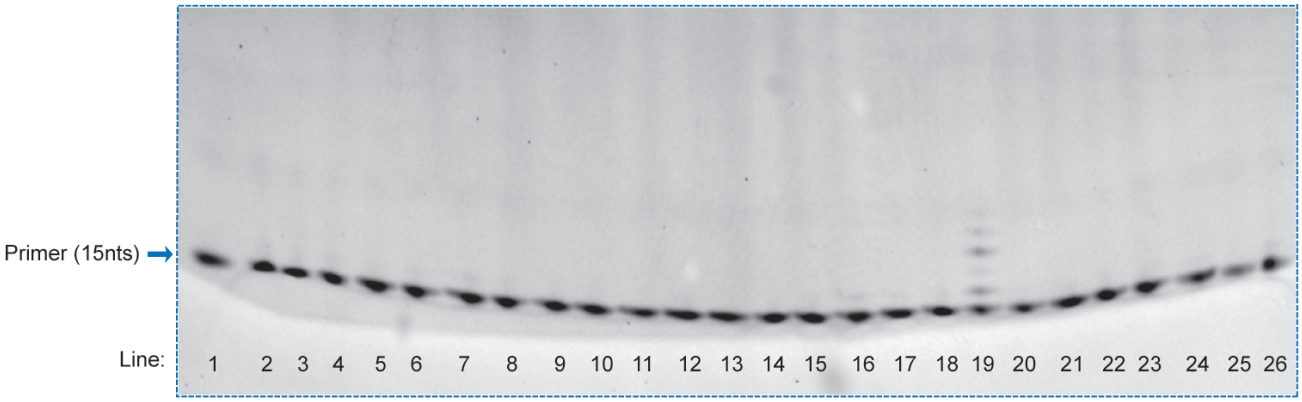


Figure S8. Control experiments on noncomplementary templates.
Primer-extension reactions contained 0.1 μM Cy3-labeled-3′-amino-terminated DNA primer, 0.5 μM non-complementary template, 100 mM MES-CAPS-HEPES, pH 7.5, 100 mM 1-(2-hydroxyethyl)-imidazole and 5 mM 3′-amino nucleotide monomers. The reaction was completed as previously described at 2 hours.

Lane	Monomers	Templates	Sequence
1	Primer only		
2	No template		
3	3′-NH_2-2-MeImpddG	DNA	5'-C**AAAA**CCAGTCAGTCTACGC-3′
4	3′-NH_2-2-MeImpddG	DNA	5'-T**GGGG**CCAGTCAGTCTACGC-3′
5	3′-NH_2-2-MeImpddG	DNA	5'-A**TTTT**CCAGTCAGTCTACGC-3′
6	3′-NH_2-2-MeImpddG	RNA	5'-C**AAAA**CCAGUCAGUCUACGC-3′
7	3′-NH_2-2-MeImpddG	RNA	5'-U**GGGG**CCAGUCAGUCUACGC-3′
8	3′-NH_2-2-MeImpddG	RNA	5'-A**UUUU**CCAGUCAGUCUACGC-3′
9	3′-NH_2-2-MeImpddC	DNA	5'-C**AAAA**CCAGTCAGTCTACGC-3′
10	3′-NH_2-2-MeImpddC	DNA	5'-A**CCCC**CCAGTCAGTCTACGC-3′
11	3′-NH_2-2-MeImpddC	DNA	5'-A**TTTT**CCAGTCAGTCTACGC-3′
12	3′-NH_2-2-MeImpddC	RNA	5'-C**AAAA**CCAGUCAGUCUACGC-3′
13	3′-NH_2-2-MeImpddC	RNA	5'-A**CCCC**CCAGUCAGUCUACGC-3′
14	3′-NH_2-2-MeImpddC	RNA	5'-A**UUUU**CCAGUCAGUCUACGC-3′
15	3′-NH_2-2-MeImpddT	DNA	5'-A**CCCC**CCAGTCAGTCTACGC-3′
16	3′-NH_2-2-MeImpddT	DNA	5'-T**GGGG**CCAGTCAGTCTACGC-3′
17	3′-NH_2-2-MeImpddT	DNA	5'-A**TTTT**CCAGTCAGTCTACGC-3′
18	3′-NH_2-2-MeImpddT	RNA	5'-A**CCCC**CCAGUCAGUCUACGC-3′
19	3′-NH_2-2-MeImpddT	RNA	5'-U**GGGG**CCAGUCAGUCUACGC-3′
20	3′-NH_2-2-MeImpddT	RNA	5'-A**UUUU**CCAGUCAGUCUACGC-3′
21	3′-NH_2-2-MeImpddA	DNA	5'-C**AAAA**CCAGTCAGTCTACGC-3′
22	3′-NH_2-2-MeImpddA	DNA	5'-A**CCCC**CCAGTCAGTCTACGC-3′
23	3′-NH_2-2-MeImpddA	DNA	5'-T**GGGG**CCAGTCAGTCTACGC-3′
24	3′-NH_2-2-MeImpddA	RNA	5'-C**AAAA**CCAGUCAGUCUACGC-3′
25	3′-NH_2-2-MeImpddA	RNA	5'-A**CCCC**CCAGUCAGUCUACGC-3′
26	3′-NH_2-2-MeImpddA	RNA	5'-U**GGGG**CCAGUCAGUCUACGC-3′

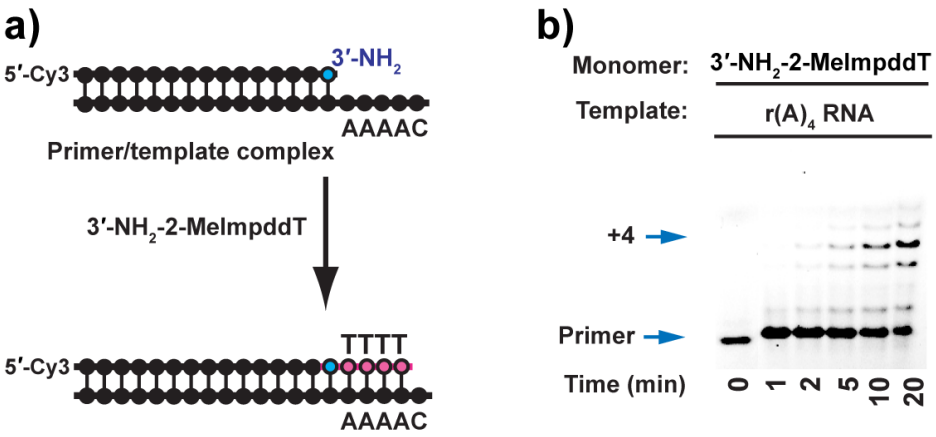


Figure S9. Non-enzymatic primer-extension reaction using 5.0 mM 3′-NH_2-2-MeImpddT copying an r(A)$_4$ RNA template. **a**) Primer-extension reaction scheme showing a 5′-Cy3-labeled 3′-amino-terminated DNA primer annealed to a complementary RNA template. 3′-NH_2-2-MeImpddT monomer participates in a chemical extension reaction extending the primer by four (4) nucleotides on the complementary template forming a chimeric DNA/3′-NP-DNA polymer product. The line in red indicates newly-formed phosphoramidate bonds. **b**) High-resolution gel electrophoresis analysis of primer-extension products on an r(A)$_4$ RNA template. Primer-extension reactions contained 0.1 μM Cy3-labeled 3′-amino-terminated DNA primer, 0.5 μM template, 100 mM MES-CAPS-HEPES, pH 7.5, 150 mM NaCl and 100 mM HEI. The reaction was initiated by addition of 5.0 mM 3′-NH_2-2-MeImpddT. Arrows indicate primer and full-length product.

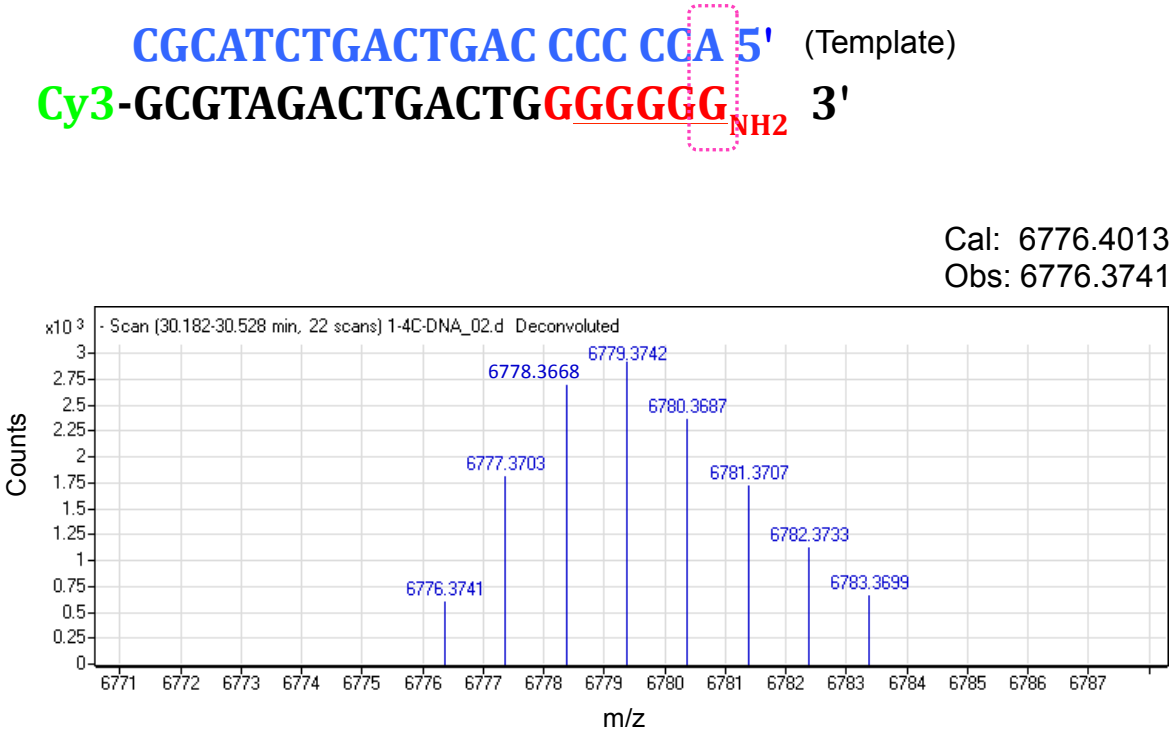


Figure S10. High-resolution LC-MS profile of the N+5 product resulting from copying a d(C)$_4$ DNA template using 3′-NH$_2$-2-MeImpddG

High resolution MS analysis of the primer-extension products from a reaction of 25 pmol 5′-Cy3-labeled 3′-amino-terminated primer extended on a d(C)$_4$ DNA template for 12 hours followed by ethanol precipitation. Letters in red indicate N3′-P5′ phosphoramidate bonds, and letters underlined in red indicate newly incorporated nucleobases. The monoisotopic mass for the chimeric DNA/3′-NP-DNA of Cy3-labled N+5 product: calculated mass 6776.4013 and observed mass 6776.3741.

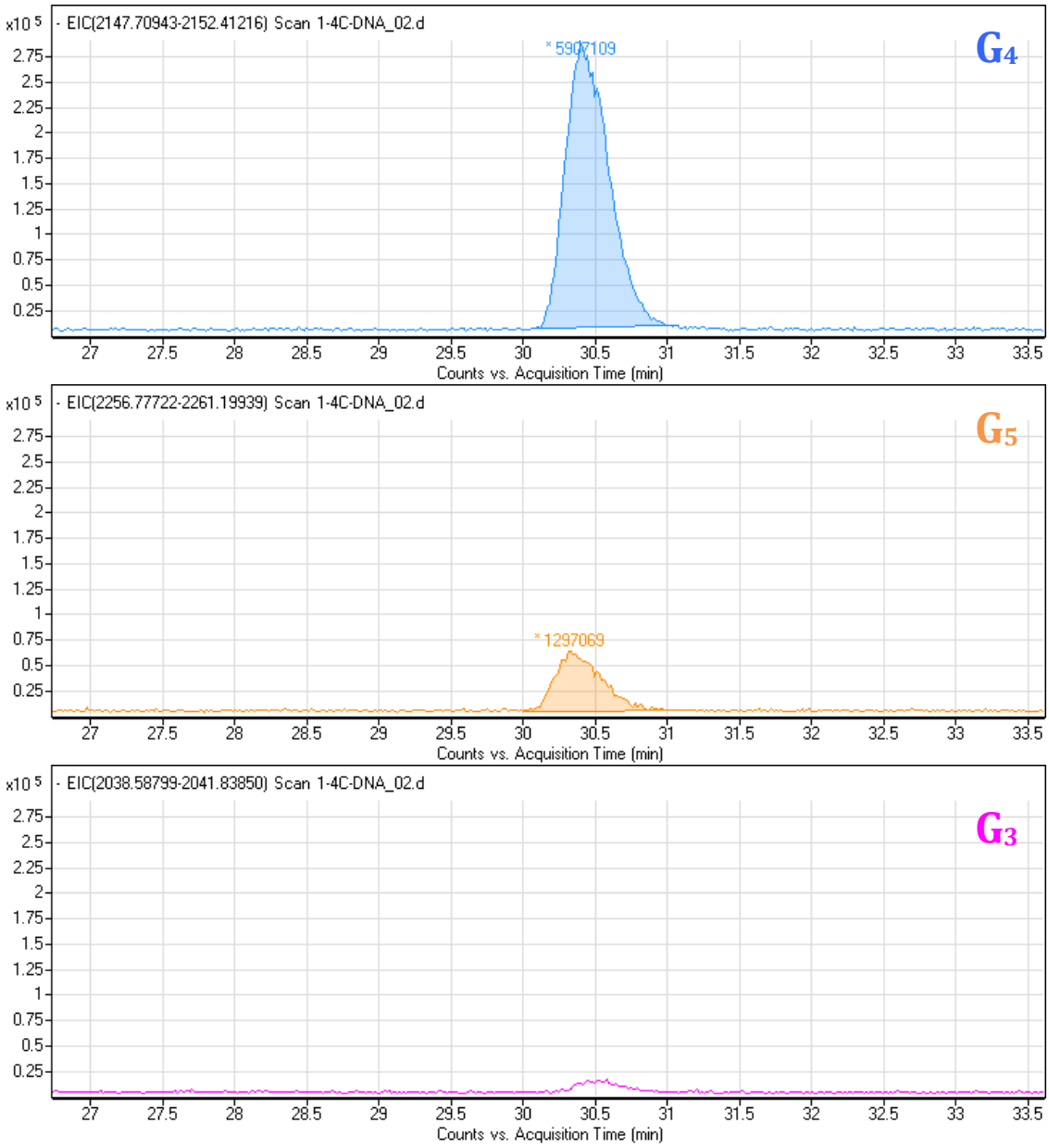


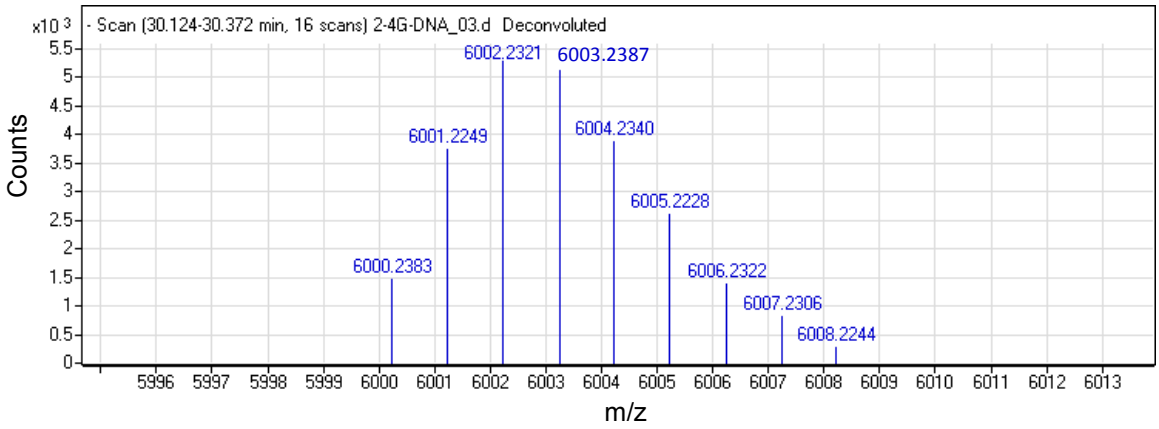
Figure S11. EIC[a] profile of three extension products from copying a $d(C)_4$ DNA template

For quantification, the m/z ions (3rd charge state) of all the products were extracted from the TIC[b]. EIC peaks of m/z 2148.4370 (N+4) and m/z 2257.7932 ions (N+5) were profiled, and both peaked at ~ 30.4 min (retention time). Their relative integrations were compared to obtain relative percentages (Table S1).

[a]EIC: extracted ion chromatogram; [b]TIC: total ion current.

Cy3-GCGTAGACTGACTGGCCC$_{NH2}$ 3'

Cal: 6000.2459
Obs: 6000.2383



Cy3-GCGTAGACTGACTGGCC$_{NH2}$ 3'

Cal: 5712.1835
Obs: 5712.1624

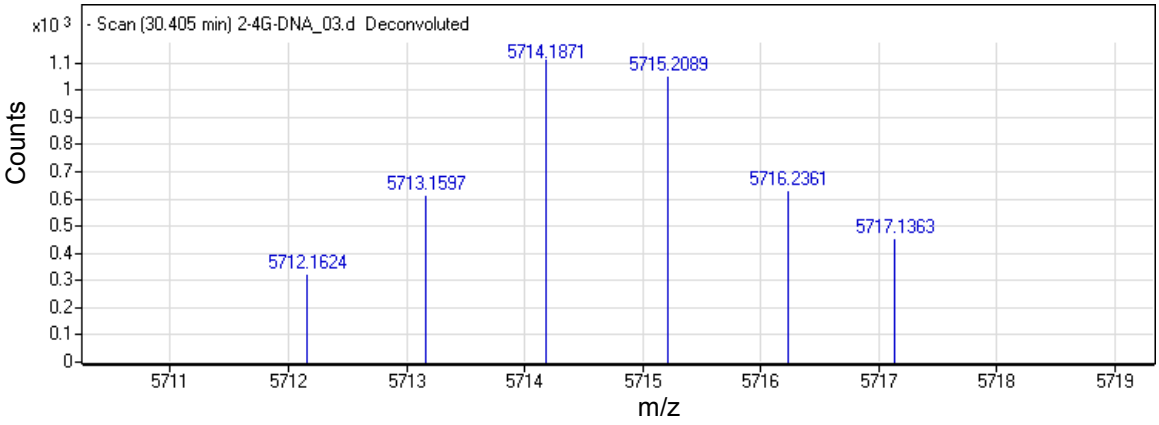


Figure S12. High-resolution LC-MS profile of N+3 and N+2 products resulting from copying a d(G)$_4$ DNA template using 3′-NH$_2$-2-MeImpddC

High resolution MS analysis of the primer-extension products from a reaction of 25 pmol 5′-Cy3-labeled 3′-amino-terminated primer extended on a d(G)$_4$ DNA template for 12 hours, followed by ethanol precipitation. Letters in red indicate N3′-P5′ phosphoramidate bonds, and letters underlined in red indicate newly incorporated nucleobases. Upper panel: the monoisotopic mass for the chimeric DNA/3′-NP-DNA of Cy3-labled N+3 product: calculated mass 6000.2459 and observed mass 6000.2383. Lower panel: the monoisotopic mass for the chimeric DNA/3′-NP-DNA of Cy3-labled N+2 product: calculated mass 5712.1835 and observed mass 5712.1624.

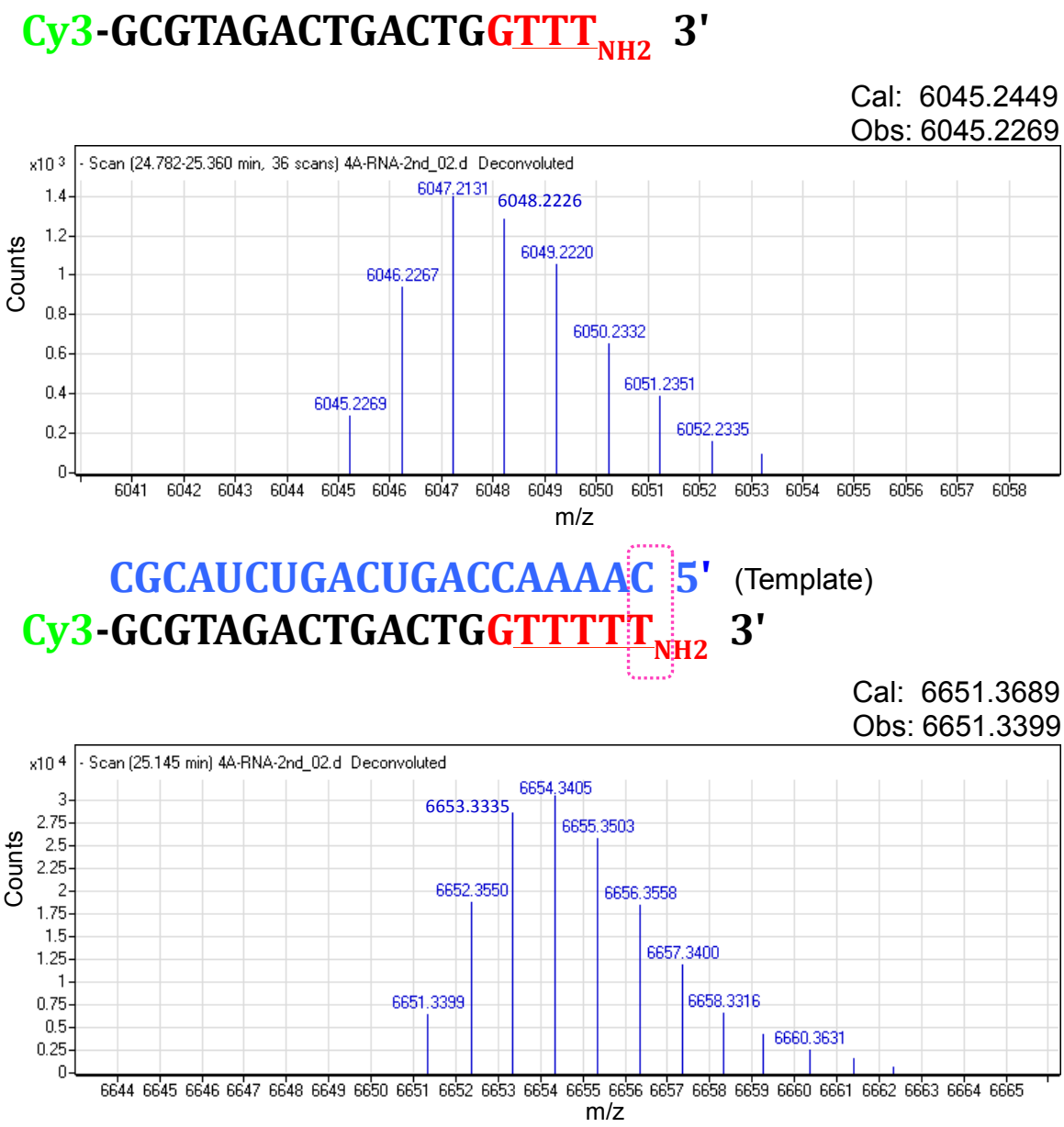


Figure S13. High-resolution LC-MS profile of N+3 and N+5 products resulting from copying an r(A)$_4$ RNA template using 3′-NH$_2$-2-MeImpddT

High resolution MS analysis of the primer-extension products from a reaction of 30 pmol 5′-Cy3-labeled 3′-amino-terminated primer extended on an r(A)$_4$ RNA template for 12 hours followed by ethanol precipitation. Letters in red indicate N3′-P5′ phosphoramidate bonds, and letters underlined in red indicate newly incorporated nucleobases. Upper panel: the monoisotopic mass for the chimeric DNA/3′-NP-DNA of Cy3-labled N+3 product: calculated mass 6045.2449 and observed mass 6045.2269. Lower panel: the monoisotopic mass for the chimeric DNA/3′-NP-DNA of Cy3-labled N+5 product: calculated mass 6651.3689 and observed mass 6651.3399.

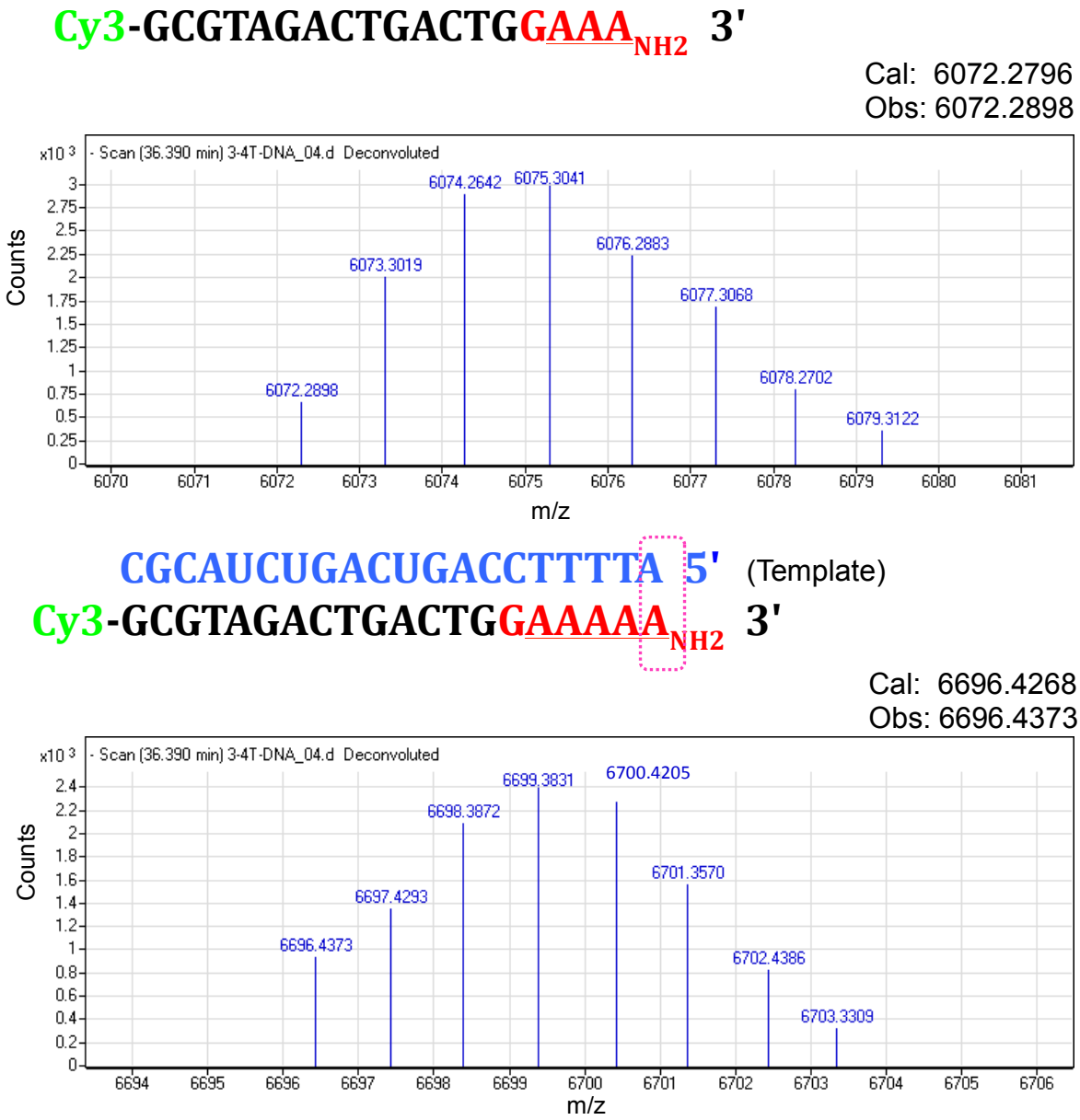


Figure S14. High-resolution LC-MS profile of N+3 and N+5 products resulting from copying a d(T)$_4$ DNA template using 3′-NH$_2$-2-MeImpddA

High resolution MS analysis of the primer-extension products from a reaction of 30 pmol 5′-Cy3-labeled 3′-amino-terminated primer extended on a d(T)$_4$ RNA template for 12 hours followed by ethanol precipitation. Letters in red indicate N3′-P5′ phosphoramidate bonds, and letters underlined in red indicate newly incorporated nucleobases. Upper panel: the monoisotopic mass for the chimeric DNA/3′-NP-DNA of Cy3-labled N+3 product: calculated mass 6072.2796 and observed mass 6072.2898. Lower panel: the monoisotopic mass for the chimeric DNA/3′-NP-DNA of Cy3-labled N+5 product: calculated mass 6696.4268 and observed mass 6696.4373.

Supporting Tables

CGCATCTGACTGAC CCC CCA 5' (Template)

Cy3-GCGTAGACTGACTGGGGGG$_{NH2}$ 3'

		G_1	G_2	G_3	G_4	G_5	G_6
1	3rd charge State	1820.3685	1929.7247	2039.0808	2148.4370	2257.7932	2367.1493
2	Exact MS	5464.1273	5792.1958	6120.2643	6448.3328	6776.4013	7104.4699
3	Observed	-	-	-	6448.3045	6776.3741	-
4	Diff (ppm)	-	-	-	4.39	4.02	-
5	Relative Integrations[a]	-	-	-	1	0.2196	-
6	Percentage	-	-	-	82.0%	18.0%	-

Table S1. High-resolution LC-MS Analysis of primer extension products from copying a $d(C)_4$ DNA template

[a]Relative integrations were obtained as previously described in Figure S11.

CGCATCTGACTGAC GGGGT 5' (Template)

Cy3-GCGTAGACTGACTGGCCCCC$_{NH2}$ 3'

		C_1	C_2	C_3	C_4	C_5
1	3rd charge State	1807.0331	1903.0539	1999.0747	2095.0955	2191.1163
2	Exact MS	5424.1212	5712.1835	6000.2459	6288.3083	6576.3706
3	Observed	-	5712.1624	6000.2383	6288.2864	-
4	Diff (ppm)	-	3.70	1.27	3.47	-
5	Relative Integrations	-	0.1012	0.6722	1	-
6	Percentage	-	5.71%	37.90%	56.39%	-

Table S2. High-resolution LC-MS Analysis of primer extension products from copying a d(G)$_4$ DNA template

CGCATCTGACTGAC CAA AAC 5' (Template)

Cy3-GCGTAGACTGACTGGTT TT$_{NH2}$ 3'

		T_1	T_2	T_3	T_4	T_5
1	3rd charge State	1812.0330	1913.0537	2014.0744	2115.0950	2216.1157
2	Exact MS	5439.1208	5742.1829	6045.2449	6348.3069	6651.3689
3	Observed	-	-	6045.2269	6348.2936	6651.3399
4	Diff (ppm)	-	-	2.98	2.10	4.36
5	Relative Integrations	-	-	0.3965	1	0.3410
6	Percentage	-	-	19.62%	57.55%	22.82%

Table S3. High-resolution LC-MS Analysis of primer extension products from copying an r(A)$_4$ RNA template

CGCATCTGACTGAC CTT TTA 5' (Template)

Cy3-GCGTAGACTGACTGGAA AA$_{NH2}$ 3'

		A_1	A_2	A_3	A_4	A_5
1	3rd charge State	1815.0369	1919.0614	2023.0859	2127.1105	2231.1350
2	Exact MS	5448.1324	5760.2060	6072.2796	6384.3532	6696.4268
3	Observed	-	-	6072.2898	6384.3454	6696.4373
4	Diff (ppm)	-	-	-1.68	1.22	-1.57
5	Relative Integrations	-	-	0.5922	1	0.3388
6	Percentage	-	-	30.67%	51.79%	17.55%

Table S4. High-resolution MS Analysis of primer extension products from copying a $d(T)_4$ DNA template