

## Electronic Supplementary Information

### Phosphamide nucleotide analog: A substrate for polymerase synthesis of DNA

Jiong Meng, Qiaqia Guo, Xiaona Zhai, Song Yang, Shuai Wang, Pengcheng Wang and Debin Ji\*

Institute of Frontier Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Qingdao, 266237, PR China.

\*Corresponding author Email: [debinji@sdu.edu.cn](mailto:debinji@sdu.edu.cn)

### Table of Contents

Materials and Methods .....	S2
Characterizations of dNTP $\gamma$ NH <sub>2</sub> .....	S5
NMR Spectra of dNTP $\gamma$ NH <sub>2</sub> .....	S6
DNA Sequences.....	S10
Stability.....	S10
Gel Intensity.....	S10
Decomposition of dNTPs causes PCR failure .....	S11
Supplemental Figures .....	S12
Fig. S1 .....	S12
Fig. S2.....	S13
Fig. S3.....	S14
Fig. S4.....	S15
Fig. S5.....	S16
Fig. S6.....	S17
Fig. S7.....	S18
Fig. S8.....	S19
References.....	S19

## Materials and Methods

### *Reagents.*

dATP, dTTP, dGTP and dCTP disodium salt was purchased from Solarbio Life Sciences (Beijing, China). dNTPs mixture was acquired from GENVIEW Scientific (Beijing, China), Sangong Biotech (Shanghai, China), and BBI Life Sciences (Shanghai, China). *N,N*-Dimethylformamide (DMF) and Dowex-50W ion exchange resin were from Thermo Fisher Scientific (Waltham, MA, USA). *N,N*-Dicyclohexylcarbodiimide (DCC), tetrabutylammonium hydroxide (25% in H<sub>2</sub>O), and ammonia methanol solution (4 M) were acquired from Aladdin Scientific (Shanghai, China). Pyridine hydrochloride was from Alfa Aesar (Ward Hill, MA, USA). PicoGreen was acquired from Fushenbio Life Sciences (Shanghai, China).  $\lambda$  DNA, Taq, Vent<sup>®</sup>, Phusion<sup>®</sup> High-Fidelity, Long Amp<sup>®</sup> Taq DNA polymerase and their buffers were purchased from New England Biolabs (Ipswich, MA, USA). PET-28a-FMO plasmid donated by other laboratories. Pfu DNA polymerase and DNA primers were obtained from Sangong Biotech (Shanghai, China). Nucleic Acid Stain was from Dingguo Biotech (Beijing, China). Nucleotide concentration was measured by NanoPhotometer-N50 ultramicro spectrophotometer (Implen, Munich, Germany). Separation and purification of substances through high-performance liquid chromatography (HPLC, LC-20AT, Shimadzu, Japan). ESI-MS analyses were performed on a Bruker Impact HD high-resolution quadrupole time-of-flight mass spectrometry (QTOF-MS, Ettlingen, Germany). <sup>1</sup>H NMR, and <sup>31</sup>P NMR spectra were recorded on a Bruker Ascend-600 spectrometer (Ettlingen, Germany).

### *Nucleotide decomposition analysis*

PCR amplification was performed using 40 ng PET-28a-FMO plasmid, 200  $\mu$ M dNTPs, 0.4  $\mu$ M PET-28a-FMO-1kb primers (DNA Sequence in Supplementary Information), 1 $\times$  Taq polymerase buffer, 1  $\mu$ L Taq DNA polymerase (2 U/ $\mu$ L). The PCR thermal cycles were used as follows: 1 min at 94 °C for DNA initial denaturation, followed by 36 cycles for 30 s at 94 °C, 30 s at 55 °C and 1 min at 72 °C, and a final extension step at 72 °C for 10 min. The PCR products were analyzed by gel electrophoresis on a 0.8% agarose gel containing Nucleic Acid Stain. The gel was run in 1 $\times$  Tris-borate-EDTA (TBE) buffer at 150 V for 0.5 h, after which it was visualized by GenoSens 2000 gel imaging system (Clinx Science Instruments, Shanghai, China). HPLC conditions: column (Shim-pack GIST 5 $\mu$ m C18 10 $\times$ 250 mm), UV detector (253 and 271 nm), flow rate (4.0 mL/min), and buffer (A: 0.025 M triethylammonium acetate, B: acetonitrile, B concentration 2 to 6%/22 min).

### *Synthesis of dNTP $\gamma$ NH<sub>2</sub>*

The dTTP disodium salt (21 mg, 40  $\mu$ mol) was converted into tetrabutylammonium salt by using Dowex-50W ion exchange resin and tetrabutylammonium hydroxide. The resulting tetrabutylammonium salt was co-evaporated with anhydrous DMF (1 mL) twice and then dissolved in 1 mL of anhydrous DMF. Subsequently, DCC (33 mg, 160  $\mu$ mol) and pyridine hydrochloride (3.3 mg, 30  $\mu$ mol) were added, and the formed mixture was incubated for 24 h at room temperature.<sup>1</sup>

Next, 100  $\mu$ L of ammonia methanol solution (4 M) was added, and the mixture was stirred at room temperature for 24 h prior to being quenched with 3 mL of distilled water. The resulting aqueous solution was purified by HPLC after chloroform extraction (three times). The HPLC

conditions were as follows: column (Shim-pack GIST 5  $\mu$ m C18 10 $\times$ 250 mm), buffer (A: 0.025 M triethylammonium acetate, B: acetonitrile, B concentration 2 to 7%/23 min for dATP, dCTP, dGTP and 3 to 6%/19 min for dTTP, linear gradient), flow rate (4.0 mL/min), and UV detector (260 nm). The fraction with dNTP $\gamma$ NH<sub>2</sub> was lyophilized, and analyzed by NMR and ESI-MS.

#### *Thermal stability analysis*

The experiment was conducted with dATP and dATP $\gamma$ NH<sub>2</sub> as representatives. The dATP or dATP $\gamma$ NH<sub>2</sub> was dissolved in 1 $\times$  Taq DNA polymerase buffer solution at 20 ng/ $\mu$ L concentration and incubated at 75  $^{\circ}$ C for 48 h. Aliquots of 20  $\mu$ L were taken out every 6 h and analyzed by HPLC. HPLC conditions: column (Inertsil ODS-3 3  $\mu$ m C18 4.6 $\times$ 150 mm), UV detector (260 nm), flow rate (0.8 mL/min), buffer (A: 0.025 M triethylammonium acetate, B: acetonitrile, B concentration 2 to 7%/20 min).

#### *DNA polymerases using dNTP $\gamma$ NH<sub>2</sub>s as substrates*

DNA of 1k bp was amplified using a 50  $\mu$ L reaction mixture consisting of 40 ng PET-28a-FMO plasmid, 0.4  $\mu$ M PET-28a-FMO-1kb primers (DNA Sequence in Table S1), 1 $\times$  Taq polymerase buffer, 1  $\mu$ L Taq DNA polymerase (2 U/ $\mu$ L), and deoxynucleotide substrates. The substrates are either four canonical dNTPs (each 200  $\mu$ M), or one of four dNTP $\gamma$ NH<sub>2</sub> and other three canonical dNTPs (each 200  $\mu$ M), or four dNTP $\gamma$ NH<sub>2</sub>s (each 200  $\mu$ M).

Polymerase screening was taken using assay mixture as follows: 2 units of DNA polymerase (Vent<sup>®</sup>, Pfu, Taq, Phusion<sup>®</sup> High-Fidelity or Long Amp<sup>®</sup>), 200  $\mu$ M dNTP $\gamma$ NH<sub>2</sub>s or dNTPs, 0.4  $\mu$ M PET-28a-FMO-1kb primers (DNA Sequence in Table S1), and 40 ng PET-28a-FMO plasmid in the buffer suggested by the manufacturer. The PCR thermal cycles were the same as the Nucleotide decomposition analysis. The resultant PCR products were analyzed by gel electrophoresis on a 0.8% agarose gel containing Nucleic Acid Stain. The gel was run in 1 $\times$ TBE buffer at 150 V for 0.5 h and visualized by GenoSens 2000 gel imaging system (Clinx Science Instruments, Shanghai, China). The gel band intensity was analyzed using ImageJ software (National Institutes of Health, USA).

#### *Using dNTP $\gamma$ NH<sub>2</sub>s in PCR at different conditions*

Assay of PCR extension time. The PCR mixture comprises 40 ng PET-28a-FMO plasmid, 200  $\mu$ M four dNTP $\gamma$ NH<sub>2</sub>s or four dNTPs, 0.4  $\mu$ M PET-28a-FMO-3kb primers (DNA Sequence in Table S1), 1  $\mu$ L Vent<sup>®</sup> DNA polymerase (2 U/ $\mu$ L), and 1 $\times$  Vent<sup>®</sup> DNA polymerase buffer. The thermal cycles started with 30 s of incubation at 95  $^{\circ}$ C, followed by 36 cycles of 30 s at 94  $^{\circ}$ C, 30 s at 53  $^{\circ}$ C, and 1/2/4 min for dNTPs (1/2/4/8 min for dNTP $\gamma$ NH<sub>2</sub>s) at 72  $^{\circ}$ C, and a final extension step at 72  $^{\circ}$ C for 10 min.

Long PCR Amplifications (6 kb and 10 kb). Amplification of 6 kb fragment: the reaction mixture contains 40 ng PET-28a-FMO plasmid, 200  $\mu$ M four dNTP $\gamma$ NH<sub>2</sub>s, 0.4  $\mu$ M PET-28a-FMO-6kb primers (DNA Sequence in Table S1), 1  $\mu$ L Long Amp<sup>®</sup>/Vent<sup>®</sup>/Phusion<sup>®</sup> High-Fidelity DNA polymerase and respective supporting 1 $\times$  buffer. The PCR conditions: 95  $^{\circ}$ C for 30 s, 36 cycles at 95  $^{\circ}$ C for 30 s, 58  $^{\circ}$ C for 30 s, 72  $^{\circ}$ C for 10 min, followed by final extension at 72  $^{\circ}$ C for 10 min. Amplification of 10 kb fragment: the dNTP $\gamma$ NH<sub>2</sub>s or dNTPs mixture was stored at 90  $^{\circ}$ C for 6 h, then used for 10 kb amplification. The reaction mixture consists of 4 ng  $\lambda$ DNA, 200  $\mu$ M four dNTP $\gamma$ NH<sub>2</sub>s (200  $\mu$ M four dNTPs), 0.4  $\mu$ M  $\lambda$  DNA 10 kb primers (DNA Sequence in Table S1), 1  $\mu$ L Long Amp<sup>®</sup> DNA polymerase and 1 $\times$  buffer. The PCR conditions

were: 94 °C for 30 s, 36 cycles at 94 °C for 30 s, 55 °C for 30 s, 65 °C for 20 min followed by final extension at 65 °C for 20 min. The PCR products were analyzed by gel electrophoresis on a 0.8% agarose gel containing Nucleic Acid Stain. The gel was run in 1×TBE buffer at 150 V for 0.5 h and visualized by GenoSens 2000 gel imaging system. Comparison between dNTP $\gamma$ NH<sub>2</sub>s and dNTPs in amplification of 1k bp DNA was performed using former mentioned condition. The gel band intensity was analyzed using ImageJ software (National Institutes of Health, USA).

#### *Steady-state kinetic analyses*

Steady-state kinetic analyses were performed in 1× Taq polymerase buffer following reported procedures.<sup>2</sup> The reaction mixture consisted of 100 nM annealed DNA template, 10 nM Taq DNA polymerase and equal quantities of each dNTP or dNTP $\gamma$ NH<sub>2</sub> (combined concentrations are given in the text). Reactions were initiated by the addition of the polymerase and incubated at 72 °C. Discontinuous assays were stopped with 3  $\mu$ M PicoGreen and measurements were made within 10 min of stopping via a microplate reader (BioTek, USA) using excitation wavelength at 480 nm. The sequence of DNA template is 5'-TACGGATCCATGCTAGTCCATTAG CAG GTGCCC-3', and the primer sequence is 5'-TAATACGACTCACTATAGGG-3'.

#### *Quantitative PCR (qPCR) Analysis*

The cDNA was donated from another group, which was reverse transcribed from RNA extracted from mouse oocytes. Real-time PCR was conducted on an Applied Biosystems 7500 Fast (ThermoFisher, USA). The reaction mixture contained 0.2  $\mu$ M primer, 0.4 U Taq DNA polymerase, 1× buffer, 0.4× SYBR Green I, and 200  $\mu$ M dNTPs or dNTP $\gamma$ NH<sub>2</sub>s. The PCR conditions were: 95 °C for 60 s, 40 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s.

Primer forward: 5'-TCAGGCTACAGAAGAGGCTTGC-3'

Primer reverse: 5'-ATCAGCCCATCCTTGATCAGC-3'

## Characterizations of dNTP $\gamma$ NH<sub>2</sub>

### dATP $\gamma$ NH<sub>2</sub>

<sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>NH<sup>+</sup> form)  $\delta$  8.42 (s, 1H), 8.18 (s, 1H), 6.44 (t,  $J$  = 6.8 Hz, 1H), 4.72 (s, 1H), 4.22 (s, 1H), 4.16 – 4.05 (m, 2H), 3.12 (q,  $J$  = 7.3 Hz, 14H), 2.99 (q,  $J$  = 7.3 Hz, 1H), 2.79 – 2.72 (m, 1H), 2.52 (ddd,  $J$  = 14.0, 6.3, 3.4 Hz, 1H), 1.20 (t,  $J$  = 7.3 Hz, 23H). <sup>31</sup>P NMR (D<sub>2</sub>O, 243 MHz, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>NH<sup>+</sup> form)  $\delta$  -1.19 (d,  $J$  = 18.7 Hz), -11.48 (d,  $J$  = 19.8 Hz), -22.80 (t,  $J$  = 19.0 Hz). HRMS (ESI) calcd for C<sub>10</sub>H<sub>17</sub>N<sub>6</sub>O<sub>11</sub>P<sub>3</sub> [M-H]<sup>-</sup> 489.0084, found 489.0077.

### dTTP $\gamma$ NH<sub>2</sub>

<sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>NH<sup>+</sup> form)  $\delta$  7.59 (s, 1H), 6.18 (t,  $J$  = 7.0 Hz, 1H), 4.49 (dt,  $J$  = 6.2, 3.2 Hz, 1H), 4.07 – 3.99 (m, 3H), 3.03 (q,  $J$  = 7.3 Hz, 15H), 2.92 – 2.88 (m, 1H), 2.25 – 2.15 (m, 2H), 1.76 (s, 3H), 1.11 (t,  $J$  = 7.3 Hz, 24H). <sup>31</sup>P NMR (D<sub>2</sub>O, 243 MHz, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>NH<sup>+</sup> form)  $\delta$  -1.24 (d,  $J$  = 19.1 Hz), -11.75 (d,  $J$  = 20.3 Hz), -22.88 (t,  $J$  = 19.7 Hz). HRMS (ESI) calcd for C<sub>10</sub>H<sub>18</sub>N<sub>3</sub>O<sub>13</sub>P<sub>3</sub> [M-H]<sup>-</sup> 479.9969, found 479.9968.

### dCTP $\gamma$ NH<sub>2</sub>

<sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>NH<sup>+</sup> form)  $\delta$  7.99 (dd,  $J$  = 16.3, 7.7 Hz, 1H), 6.23 (t,  $J$  = 6.6 Hz, 1H), 6.16 – 6.10 (m, 1H), 4.57 – 4.52 (m, 1H), 4.18 – 4.08 (m, 3H), 3.12 (q,  $J$  = 7.3 Hz, 11H), 2.99 (q,  $J$  = 7.4 Hz, 1H), 2.40 – 2.34 (m, 1H), 2.30 – 2.23 (m, 1H), 1.20 (t,  $J$  = 7.3 Hz, 18H). <sup>31</sup>P NMR (D<sub>2</sub>O, 243 MHz, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>NH<sup>+</sup> form)  $\delta$  -1.31 (d,  $J$  = 19.1 Hz), -11.54 (d,  $J$  = 20.0 Hz), -22.84 (t,  $J$  = 19.5 Hz). HRMS (ESI) calcd for C<sub>9</sub>H<sub>17</sub>N<sub>4</sub>O<sub>12</sub>P<sub>3</sub> [M-H]<sup>-</sup> 464.9972, found 464.9943.

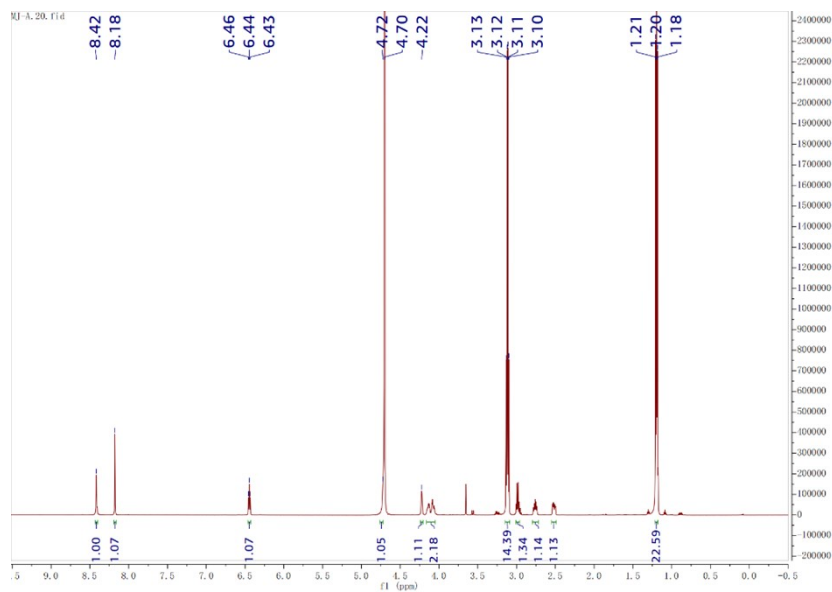
### dGTP $\gamma$ NH<sub>2</sub>

<sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>NH<sup>+</sup> form)  $\delta$  7.94 (s, 1H), 6.13 (t,  $J$  = 6.9 Hz, 1H), 4.61 – 4.58 (m, 1H), 4.08 (q,  $J$  = 3.8 Hz, 1H), 4.05 – 3.97 (m, 2H), 3.02 (qd,  $J$  = 7.3, 1.2 Hz, 34H), 2.92 – 2.87 (m, 1H), 2.66 – 2.60 (m, 1H), 2.34 (dddd,  $J$  = 14.1, 6.5, 3.5, 1.4 Hz, 1H), 1.10 (t,  $J$  = 6.9 Hz, 52H). <sup>31</sup>P NMR (D<sub>2</sub>O, 243 MHz, (C<sub>2</sub>H<sub>5</sub>)<sub>3</sub>NH<sup>+</sup> form)  $\delta$  -1.03 – -1.53 (m), -11.53 (d,  $J$  = 20.3 Hz), -22.68 – -23.14 (m). HRMS (ESI) calcd for C<sub>10</sub>H<sub>17</sub>N<sub>6</sub>O<sub>12</sub>P<sub>3</sub> [M-H]<sup>-</sup> 505.0034, found 505.0041.

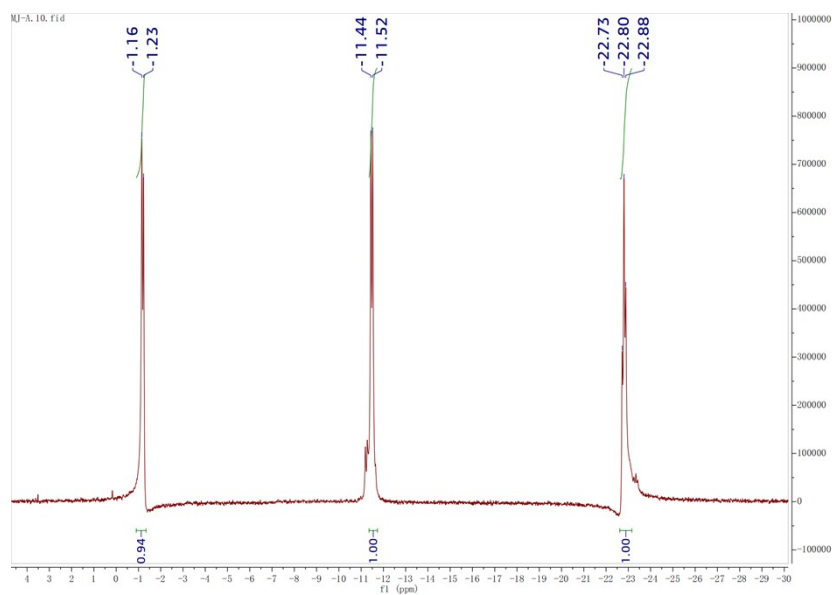
# NMR Spectra of dNTP $\gamma$ NH<sub>2</sub>

dATP $\gamma$ NH<sub>2</sub>

<sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz)

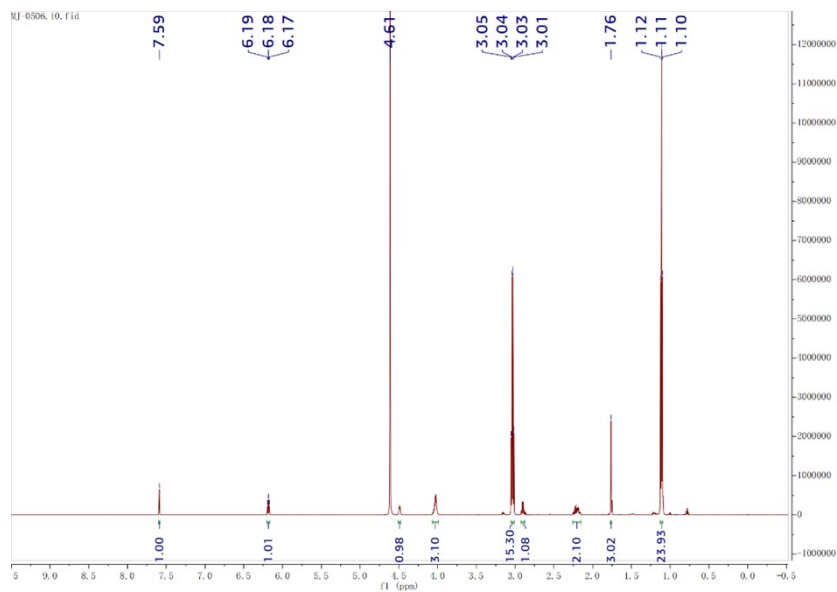


<sup>31</sup>P NMR (D<sub>2</sub>O, 243 MHz)

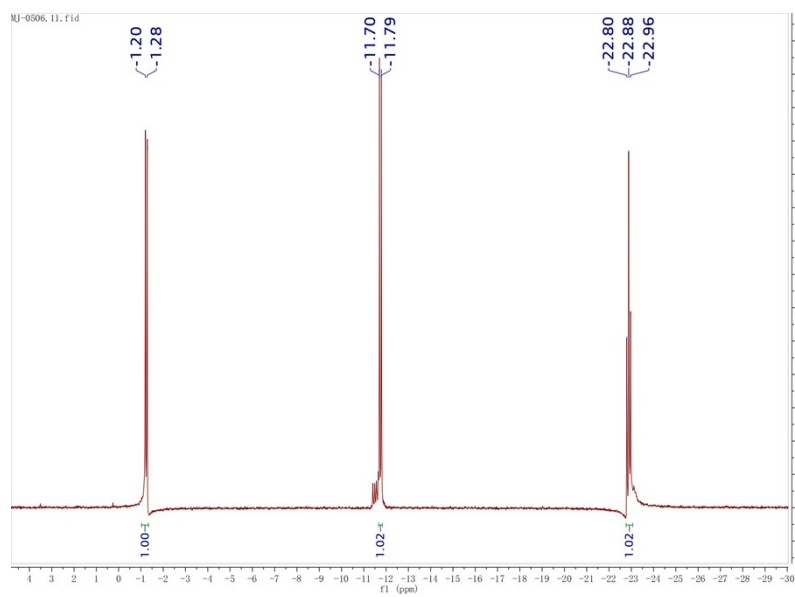


dTTP $\gamma$ NH<sub>2</sub>

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 600 MHz)

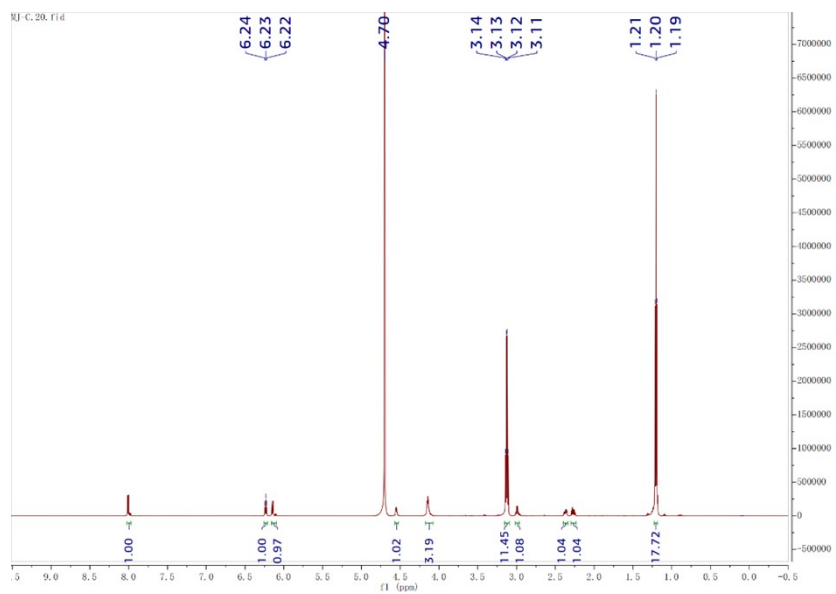


$^{31}\text{P}$  NMR ( $\text{D}_2\text{O}$ , 243 MHz)

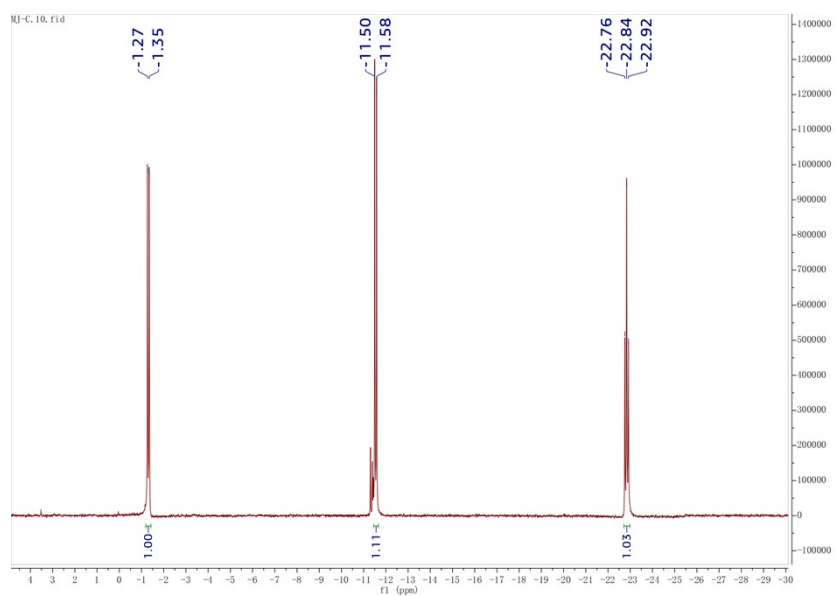


dCTP $\gamma$ NH<sub>2</sub>

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 600 MHz)



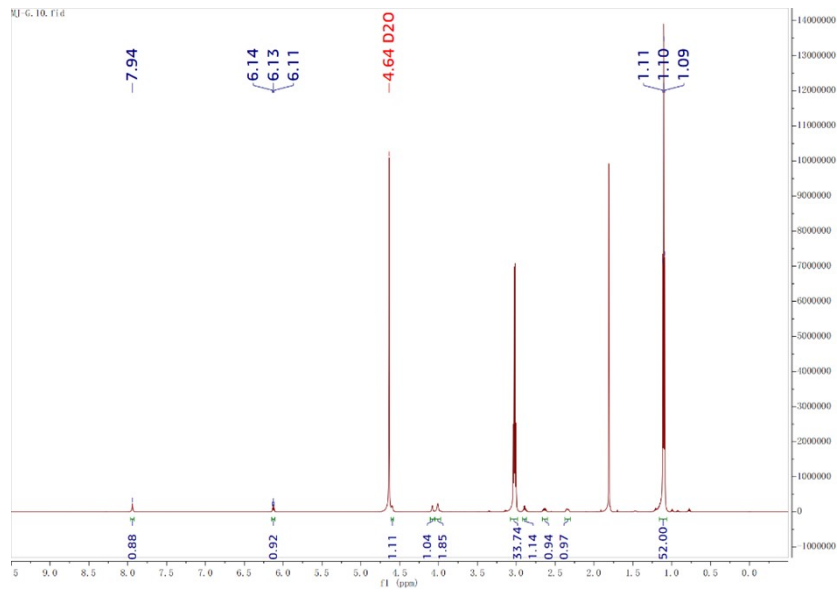
<sup>31</sup>P NMR (D<sub>2</sub>O, 243 MHz)



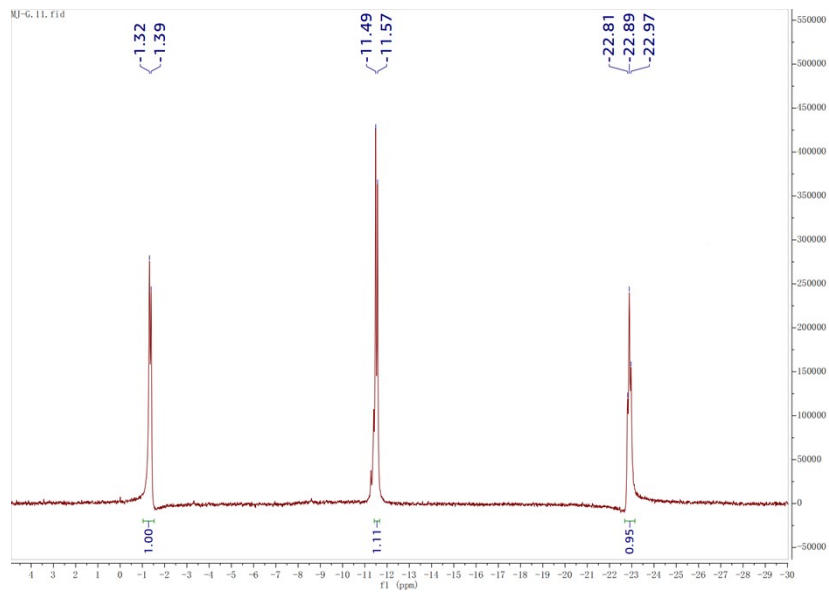
dGTPγNH<sub>2</sub>

<sup>1</sup>H NMR (D<sub>2</sub>O, 600 MHz)





<sup>31</sup>P NMR (D<sub>2</sub>O, 243 MHz)



## DNA Sequences

Plasmid	Primer Sequence
PET-28a-FMO-1kb	5'-AACAGTCGTTGCTGATTGGCGTTGC-3' 5'-CGGAAGGAGCTGACTGGGTTGAAGG-3'
PET-28a-FMO-3kb	5'-CGCCAGGGTGGTTTTTCTTTTCACC-3' 5'-CAGCAGCCAACCTCAGCTTCCTTTCG-3'
PET-28a-FMO-6kb	5'-GAAATGTGCGCGGAACCCCTATTTG-3' 5'-GCTTCTTTTGCAACCGGAATTCGC-3'
$\lambda$ DNA-10kb	5'-TCCTCATATAAAAAGTATTCGTTCACTTCC-3' 5'-ATTATATGGATCATAGCTGGCAACTAATTC-3'

## Stability

**Table S1. Stability of dNTP and dNTP $\gamma$ NH<sub>2</sub>**

dNTP	75 °C-48 h Residue (%)	dNTP $\gamma$ NH <sub>2</sub>	75 °C-48 h Residue (%)
dATP	15	dATP $\gamma$ NH <sub>2</sub>	69
dTTP	9	dTTP $\gamma$ NH <sub>2</sub>	64
dCTP	13	dCTP $\gamma$ NH <sub>2</sub>	61
dGTP	12	dGTP $\gamma$ NH <sub>2</sub>	65

## Gel Intensity

**Table S2. Intensity of gel band in figures**

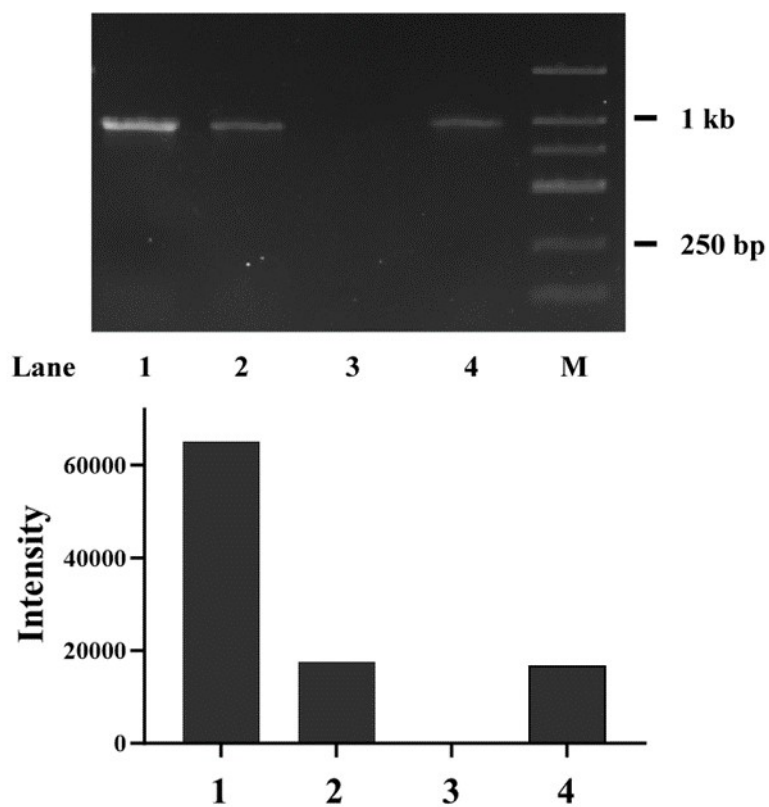
Lane	Fig. 3A	Fig. 3B	Fig. 4A	Fig. 4B	Fig. 5A	Fig. 5B	Fig. 5C	Fig. 5D	Fig. 5E
1	16986	18978	3355	34187	15907	0	4919	4485	11084
2	12333	17398	13776	35387	34662	19225	35714	19713	24780
3	63760	18914	18653	28232	—	—	—	—	0
4	29807	11987	4766	—	—	—	—	—	14850
5	22210	19920	6551	—	—	—	—	—	—
6	—	28822	3995	—	—	—	—	—	—
7	—	27267	15123	—	—	—	—	—	—
8	—	38208	—	—	—	—	—	—	—
9	—	27642	—	—	—	—	—	—	—
10	—	35567	—	—	—	—	—	—	—

### **Decomposition of dNTPs causes PCR failure**

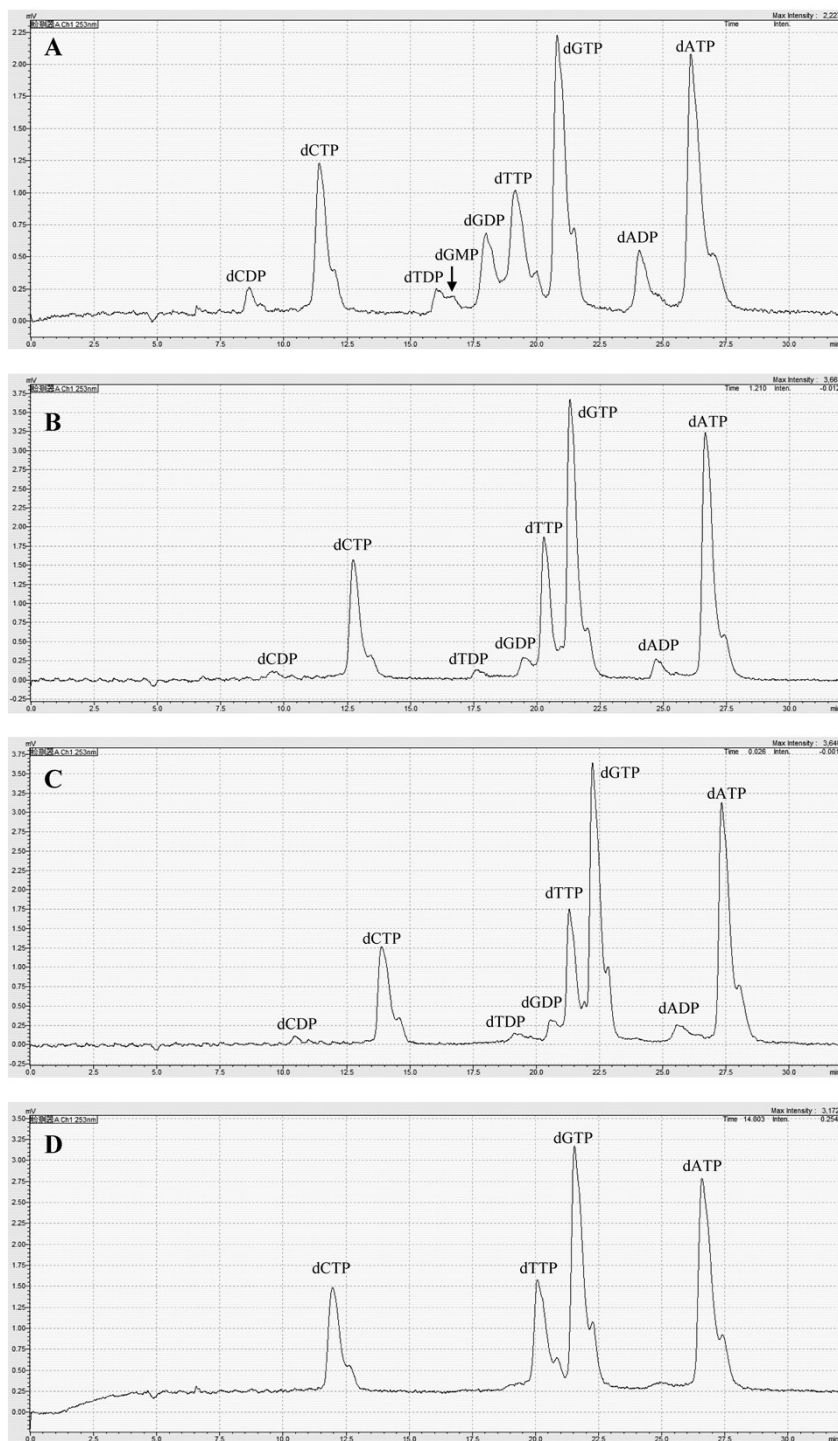
To evaluate the impact of dNTP decomposition on DNA amplification, we carried out experiments to amplify a 1 kb fragment using four batches of dNTPs stored at  $-20\text{ }^{\circ}\text{C}$  with different purchase time. As shown in Fig. S1 in the Supplementary Information, the PCR was unsuccessful with the initial batch of dNTPs (Lane 3), while the most recent batch afforded the highest yield (Lane 1). This confirmed that dNTPs indeed decomposed during storage and usage, directly leading to the failure of DNA synthesis.

To further probe the hydrolysis of dNTPs, we conducted HPLC and LC-MS analysis of four dNTPs from GENVIEW (Fig. S2, S3, and S4A). We found that dATP, dCTP, and dTTP undergo hydrolysis by releasing one phosphate group, resulting in the formation of corresponding diphosphate compounds. On the other hand, dGTP decomposes into a mixture of diphosphate and monophosphate compounds. This is consistent with the fact that dNTPs tend to hydrolyze into deoxynucleotide diphosphates (dNDPs) in aqueous solutions.<sup>3</sup> Combining MS and HPLC data, we also found that the first monophosphate compound (dNMP) generated was dGMP. This suggests that dGTP has the most unstable  $\alpha$ - $\beta$  phosphate diester bond among the four dNTPs. In addition, our results confirmed that the  $\gamma$ -phosphate of dNTP is initially involved in the hydrolysis process, resulting in the production of corresponding dNDP upon its removal. Subsequently, another phosphate is removed from the dNDP, leading to the formation of the corresponding dNMP.

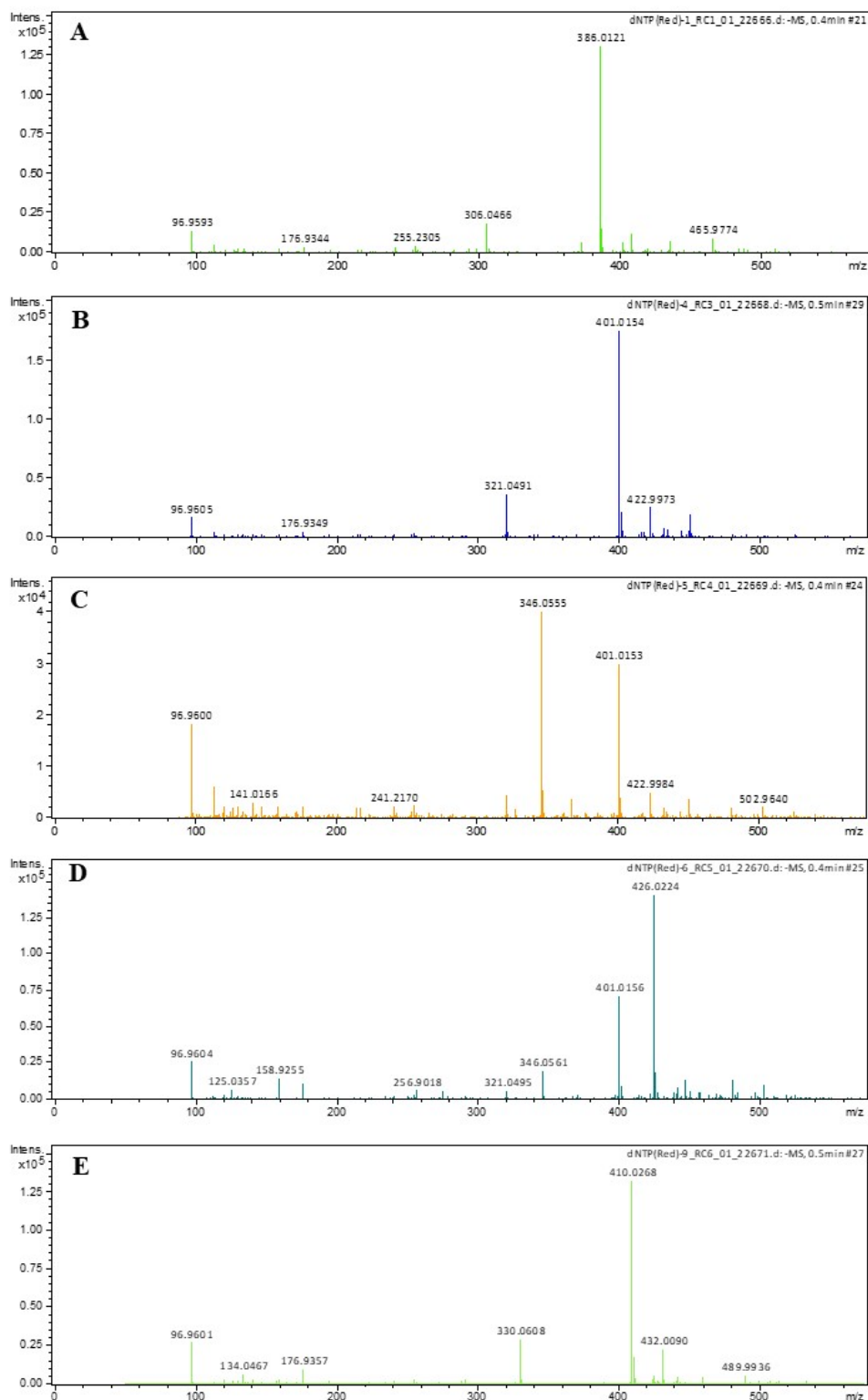
## Supplemental Figures



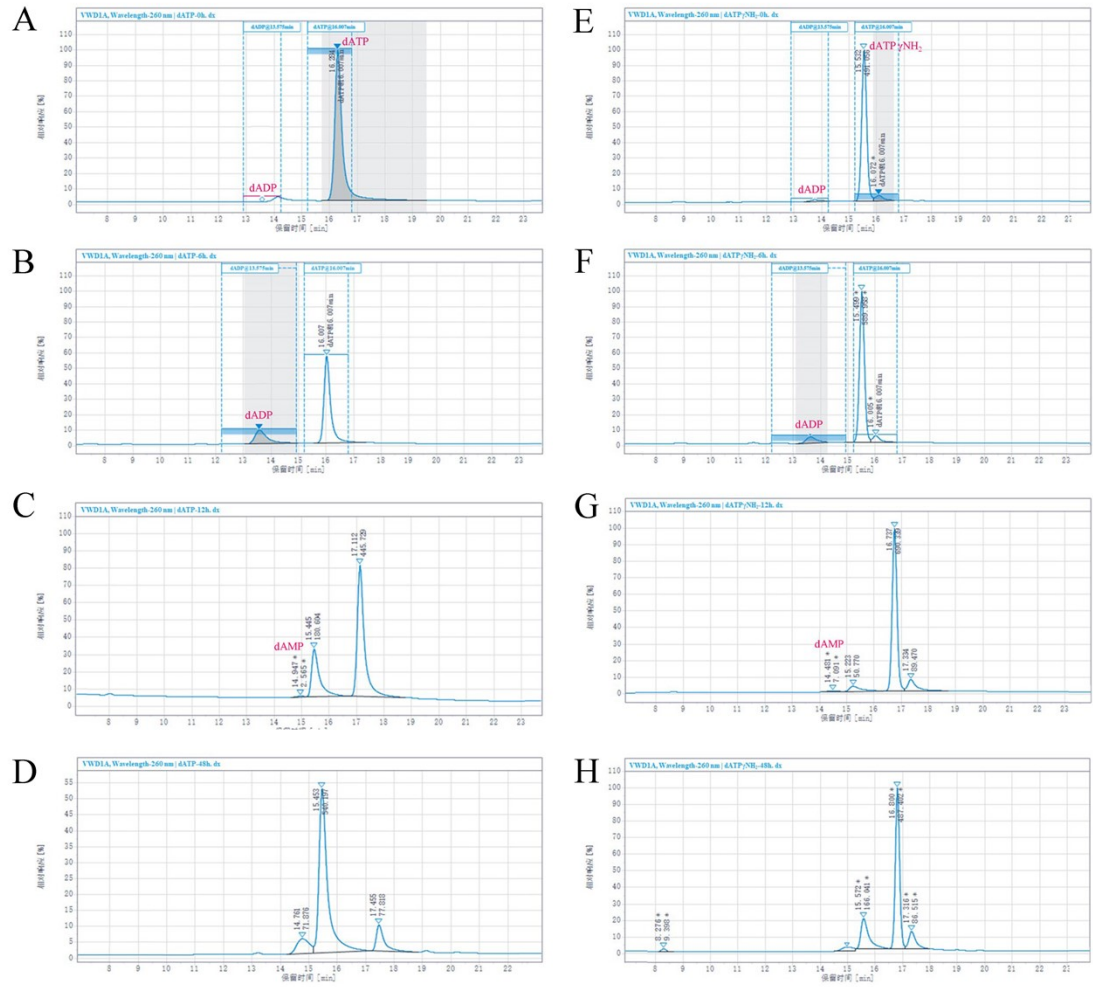
**Fig. S1** Gel image (upper half) and band intensity (lower half) of 1-kb PCR using different dNTPs. Lane 1, dNTPs 10 mM Mixture from Sangon (May 31, 2022); Lane 2, dNTP 10mM Mixture from BBI (Aug 17, 2020); Lane 3, 10mM dNTPs from GENVIEW (Feb 12, 2019); Lane 4, dNTP 10mM Mixture from BBI (Nov 17, 2020). The experiment was performed on Mar 27, 2023.



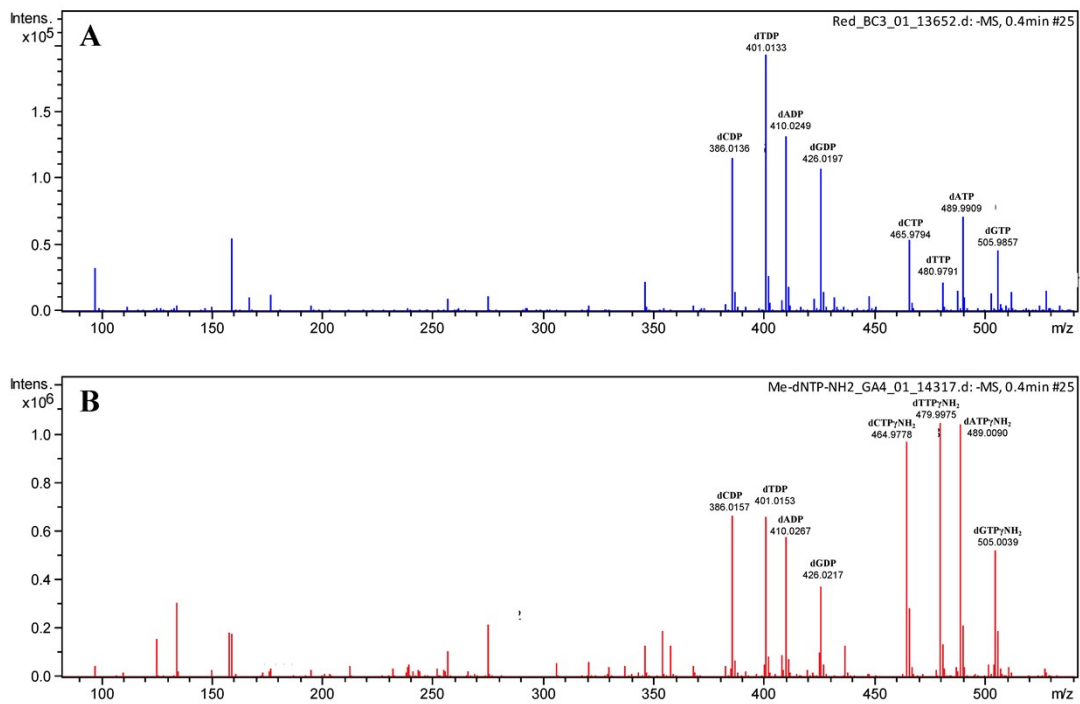
**Fig. S2.** HPLC chromatogram of (A) 10 mM dNTPs from GENVIEW (Feb 12, 2019), (B) 10 mM dNTP from BBI (Aug 17, 2020), (C) 10 mM dNTP from BBI (Nov 17, 2020), and (D) 10 mM dNTPs from Sangon (May 31, 2022). Experiments were performed on Mar 30, 2023.



**Fig. S3.** MS spectra of peaks with different retention time using GENVIEW dNTPs. (A) retention time: 8.652 min (B) retention time: 16.141 min (C) retention time: 16.707 min (D) retention time: 17.997 min and (E) retention time: 24.132 min (Refer to Fig. S2A).

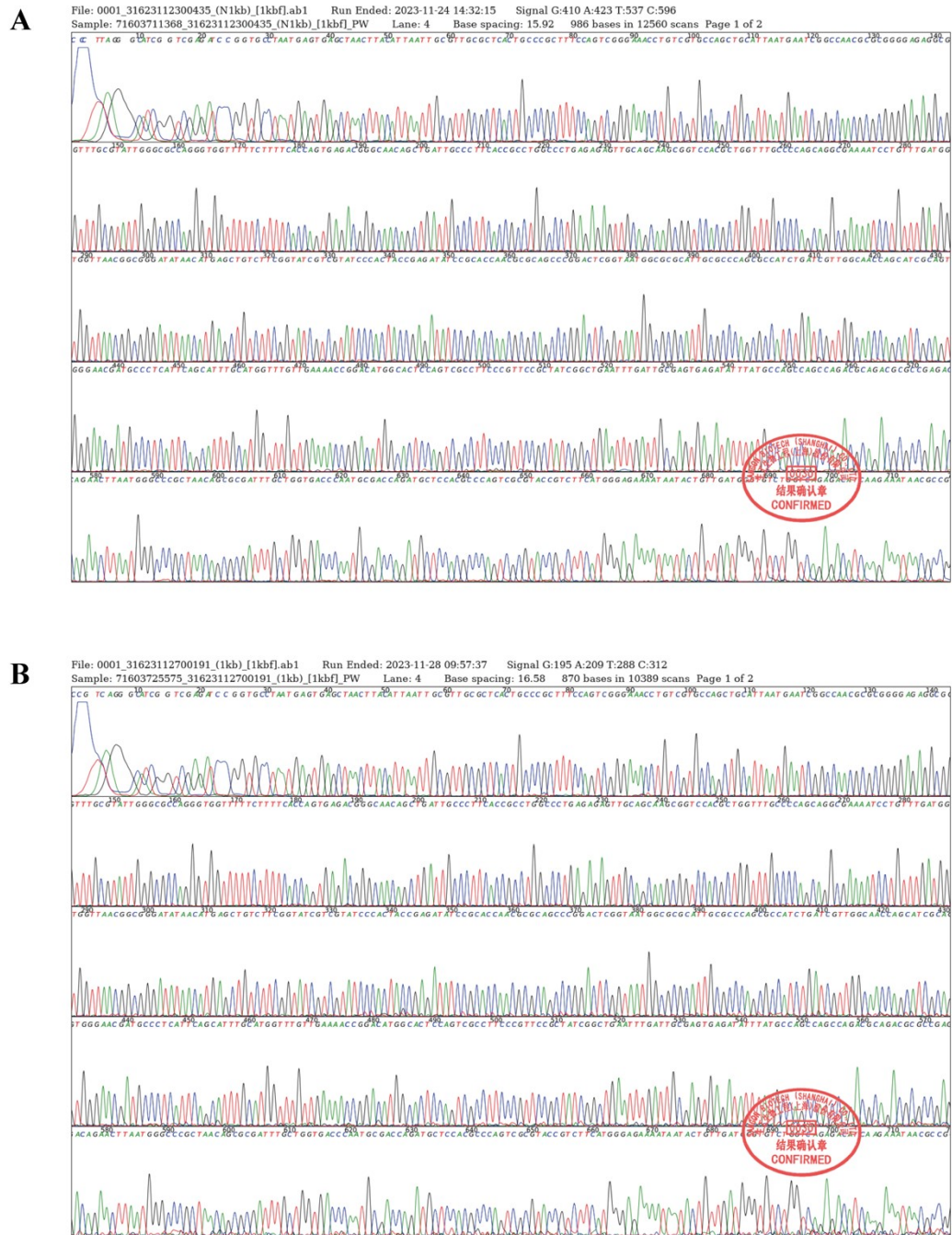


**Fig. S4.** HPLC chromatogram of dATP or dATP $\gamma$ NH $_2$  after incubation at 75 °C. (A) dATP without incubation, (B) dATP after 6-h incubation, (C) dATP after 12-h incubation, (D) dATP after 48-h incubation, (E) dATP $\gamma$ NH $_2$  without incubation, (F) dATP $\gamma$ NH $_2$  after 6-h incubation, (G) dATP $\gamma$ NH $_2$  after 12-h incubation, and (E) dATP $\gamma$ NH $_2$  after 48-h incubation.

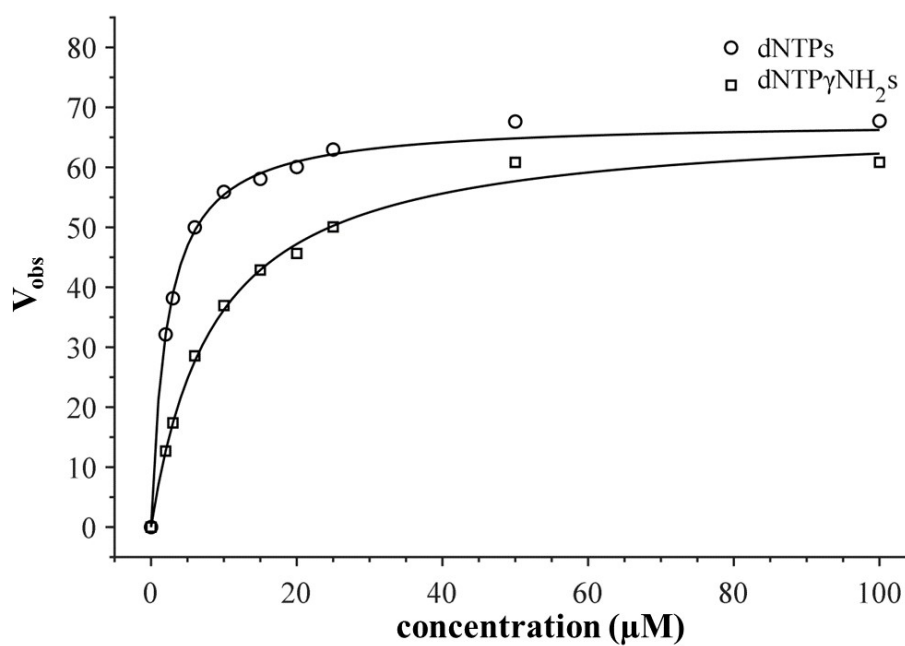


**Fig. S5.** MS spectra of (A) dNTPs and (B) dNTP $\gamma$ NH<sub>2</sub>s.

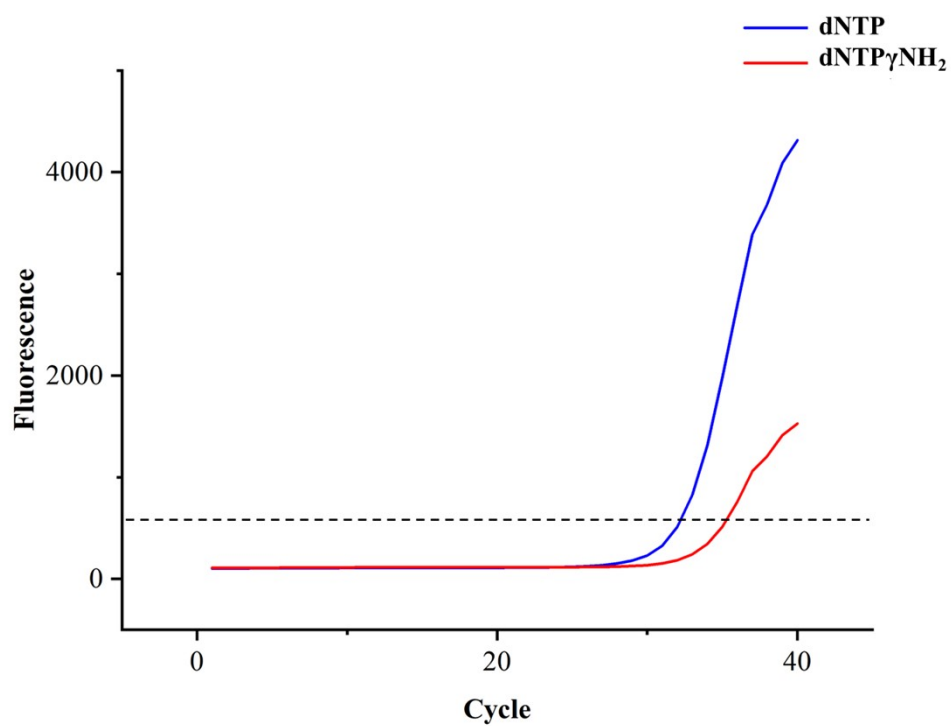




**Fig. S6.** Sanger sequencing read of 719 nt amplicon produced by PCR. (A) PCR with dNTP $\gamma$ NH<sub>2</sub> and (B) PCR with dNTP.



**Fig. S7.** Michaelis–Menten steady-state data. The dNTP or dNTP $\gamma$ NH $_2$  dependence of the observed initial reaction velocity data extracted from assay of Taq DNA polymerase (10 nM).



**Fig. S8.** Amplification plot of qPCR using dNTPs or dNTP $\gamma$ NH<sub>2</sub>s

### References

1. M. Stumber, C. Herrmann, S. Wohlgemuth, H. R. Kalbitzer, W. Jahn and M. Geyer, *Eur. J. Biochem.*, 2002, **269**, 3270-3278.
2. M. D. Driscoll, J. Rentergent and S. Hay, *FEBS J*, 2014, **281**, 2042-2050.
3. Y. Shigemori, *Nucleic Acids Res.*, 2005, **33**, e126.