Supplementary Data

Biochemical Behavior of N-Oxidized Cytosine and Adenine Bases in DNA Polymerase-Mediated Primer Extension Reactions

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1H-NMR of dC°TP

1P-NMR of dC°TP

ESI-MS Data of dC°TP

1H-NMR of dA°TP

31P-NMR of dA°TP

ESI-MS Data of dA°TP

MALDI-TOF of Template 7

MALDI-TOF of Template 8

MALDI-TOF of Template 13

MALDI-TOF of Template 14

References
Summary of This Study

(A) Low incorporation efficiency

(B) Low incorporation efficiency

(C) Low incorporation efficiency

(D) Low incorporation efficiency

(E) Low incorporation efficiency

(F) Low incorporation efficiency

DNA polymerase

C$^\circ$ recognize G residue

Inhibition of incorporation

A$^\circ$ recognize T residue

Inhibition of incorporation

KF (exo$^\prime$) DNA polymerase

C$^\circ$ recognize G residue

Inhibition of incorporation

Elongation after the incorporation of dGTP opposite C$^\circ$

Vent (exo$^\prime$) DNA polymerase

C$^\circ$ recognize G residue

Inhibition of elongation strongly

KF (exo$^\prime$) DNA polymerase

A$^\circ$ recognize A residue

Inhibition of elongation strongly

Vent (exo$^\prime$) DNA polymerase

A$^\circ$ recognize T residue

Elongation after the incorporation of dTTP opposite A$^\circ$
Table S1. \(T_m\) values of DNA–DNA 14-mer duplexes containing 2′-deoxyribonucleoside \(N\)-oxides

| DNA-DNA duplex | Y = C | Y = C\(^0\) | d(AAA AAA XAA AAA AA)-5′ | 5′-d(ITT TTT YTT TTT TT) | Y = A | Y = A\(^0\) |
|----------------|-------|------------|--------------------------|---------------------------|-------|------------|
| X              | \(T_m\) (°C)\(^a\) | \(T_m\) (°C)\(^a\) | \(ΔT_m\) (°C)\(^b\) | \(T_m\) (°C)\(^a\) | \(T_m\) (°C)\(^a\) | \(ΔT_m\) (°C)\(^b\) |
| G              | 47.1  | 30.5       | -16.6                    | 35.6                      | 31.6  | -4.0       |
| T              | 33.7  | 30.4       | -3.3                     | 45.0                      | 33.5  | -11.5      |
| A              | 35.3  | 34.7       | -0.6                     | 34.6                      | 34.6  | 0          |
| C              | 27.4  | 24.3       | -3.1                     | 30.3                      | 25.0  | -5.3       |

\(^a\)The \(T_m\) values are accurate within ±0.5°C. The \(T_m\) measurements were carried out in a buffer containing 10 mM sodium phosphate (pH 7.0), 1 M NaCl, 0.1 mM EDTA and 2 μM duplex.

\(^b\)\(ΔT_m\) is the difference in the \(T_m\) value between the unmodified DNA oligomer and the modified DNA oligomer.

Table S2. \(T_m\) values of DNA–RNA 14-mer duplexes containing 2′-deoxyribonucleoside \(N\)-oxides

| DNA–RNA duplex | Y = C | Y = C\(^0\) | r(AAA AAA XAA AAA AA)-5′ | 5′-d(ITT TTT YTT TTT TT) | Y = A | Y = A\(^0\) |
|----------------|-------|------------|--------------------------|---------------------------|-------|------------|
| X              | \(T_m\) (°C)\(^a\) | \(T_m\) (°C)\(^a\) | \(ΔT_m\) (°C)\(^b\) | \(T_m\) (°C)\(^a\) | \(T_m\) (°C)\(^a\) | \(ΔT_m\) (°C)\(^b\) |
| G              | 46.4  | 31.1       | -15.3                    | 30.5                      | 29.4  | -1.1       |
| U              | 24.4  | 24.3       | -0.1                     | 38.1                      | 25.3  | -12.8      |
| A              | 32.8  | 33.1       | +0.3                     | 31.3                      | 31.9  | +0.6       |
| C              | 23.5  | 21.4       | -2.1                     | 25.4                      | 20.5  | -4.9       |

\(^a\)The \(T_m\) values are accurate within ±0.5°C. The \(T_m\) measurements were carried out in a buffer containing 10 mM sodium phosphate (pH 7.0), 1 M NaCl, 0.1 mM EDTA and 2 μM duplex.

\(^b\)\(ΔT_m\) is the difference in the \(T_m\) value between the unmodified DNA oligomer and the modified DNA oligomer.
Synthesis of dC\textsuperscript{O}TP and dA\textsuperscript{O}TP

To a solution of 0.4 M 2′-deoxyribonucleoside 5′-triphosphate/H\textsubscript{2}O (50 µL, 20 µmol) and saturated NaHCO\textsubscript{3} aqueous solution (50 µL), 1.2 M mCPBA/MeOH (100 µL, 120 µmol) was added. The reaction mixture was stirred for 6 h at room temperature, and H\textsubscript{2}O (500 µL) was added. After 10 min at 0 °C, precipitates were removed by filtration. The filtrate was purified by DEAE-HPLC (eluted by a linear gradient of 50–500 mM triethylammonium bicarbonate buffer) to yield the fractions containing the desired nucleoside 5′-triphosphate. The fractions were collected and concentrated in vacuo. The residue of the triethylammonium nucleoside triphosphate was dissolved in HPLC grade methanol (100 µL) and precipitated by adding 0.6 M NaClO\textsubscript{4} solution in HPLC grade acetone (600 µL). The precipitated sodium salt was collected by centrifugation, washed with acetone (1.0 mL × 4), and dried under vacuum.

2′-Deoxycytidine N\textsuperscript{3}-oxide 5′-triphosphate (dC\textsuperscript{O}TP)

The compound was obtained in 42% yield. UV (H\textsubscript{2}O) \(\lambda_{\text{max}}\) 272 nm, \(\lambda_{\text{max}}\) 224 nm. \(^1\text{H}\) NMR (D\textsubscript{2}O) \(\delta\) 1.14 (t, 27H, \(J = 7.5\) Hz), 2.20–2.30 (m, 1H), 2.33–2.43 (m, 1H), 3.03–3.11 (m, 18H), 4.10–4.11 (m, 3H), 4.48–4.53 (m, 1H), 6.20 (t, 1H, \(J = 6.4\) Hz), 6.27 (d, 1H, \(J = 7.9\) Hz), 7.84 (d, 1H, \(J = 7.9\) Hz); \(^{31}\text{P}\) NMR (D\textsubscript{2}O) \(\delta\) −22.6 (t, \(J = 21.0\) Hz, \(J = 20.0\) Hz), −10.9 (d, \(J = 20.0\) Hz), −10.1 (d, \(J = 21.0\) Hz). HRMS (ESI): calcd for C\textsubscript{9}H\textsubscript{15}N\textsubscript{3}O\textsubscript{14}P\textsubscript{3}, 481.9772 (M−H); found, 481.9771 (M−H)\textsuperscript{−}.

2′-Deoxyadenosine N\textsuperscript{1}-oxide 5′-triphosphate (dA\textsuperscript{O}TP)

The compound was obtained in 48% yield. UV (H\textsubscript{2}O) \(\lambda_{\text{max}}\) 261 nm, \(\lambda_{\text{max}}\) 232 nm. \(^1\text{H}\) NMR (D\textsubscript{2}O) \(\delta\) 1.09 (t, 27H, \(J = 7.4\) Hz), 2.38–2.46 (m, 1H), 2.63–2.73 (m, 1H), 2.97–3.05 (m, 1H), 6.36 (t, 1H, \(J = 6.6\) Hz), 8.41–8.42 (2s, 2H); \(^{31}\text{P}\) NMR (D\textsubscript{2}O) \(\delta\) −22.3 (t, \(J = 19.5\) Hz), −10.7 (d, \(J = 19.5\) Hz), −7.9 (d, \(J = 19.5\) Hz). HRMS (ESI): calcd for C\textsubscript{10}H\textsubscript{15}N\textsubscript{5}O\textsubscript{13}P\textsubscript{3}, 505.9885 (M−H)\textsuperscript{−}; found, 505.9884 (M−H)\textsuperscript{−}.
Preparation of ODNs

Sequences of ODNs using enzyme reactions were shown in Table S3. The templates 7, 8, 13, and 14 were synthesized by the post-synthetic oxidation method (38).

**Table S3. Sequences of template and primer ODNs using enzyme reactions**

| ODN          | Sequence                             |
|--------------|--------------------------------------|
| Template 1   | 5'-TAAGACAGTAACCCCGGTCTTCGCCGCG-3'    |
| Template 2   | 5'-TAAGACCGTAACCCCGGTCTTCGCCGCG-3'    |
| Template 3   | 5'-TAAGACCGTAACCCCGGTCTTCGCCGCG-3'    |
| Template 4   | 5'-TAAGACTGTAAACCCCGGTCTTCGCCGCG-3'   |
| Template 5   | 5'-TAAGACAGTAACCCCGGTCTTCGCCGCGT-3'   |
| Template 6   | 5'-TAAGACCGTAACCCCGGTCTTCGCCGCGT-3'   |
| Template 7   | 5'-TAAGACA^0GTAACCCCGGTCTTCGCCGCGT-3' |
| Template 8   | 5'-TAAGACCGTAACCCCGGTCTTCGCCGCGT-3'   |
| Template 9   | 5'-TGATACAGTAACCCCGGTCTTCGCCGCGT-3'   |
| Template 10  | 5'-TGATACCGTAACCCCGGTCTTCGCCGCGT-3'   |
| Template 11  | 5'-TGATACCGTAACCCCGGTCTTCGCCGCGT-3'   |
| Template 12  | 5'-TGATACGTGAACCCCGGTCTTCGCCGCGT-3'   |
| Template 13  | 5'-TGATACG^0GTAACCCCGGTCTTCGCCGCGT-3' |
| Template 14  | 5'-TGATACC^0GTAACCCCGGTCTTCGCCGCGT-3' |
| 5'-FAM labeled primer | 5'-FAM-CGCCGCGAAGACCGGTTAC-3' |

*aThe mass spectral data of synthesized N-oxide ODNs are as follows. Template 7: MALDI-TOF Mass (M + H) C_{253}H_{320}N_{98}O_{155}P_{25}^+ 7984.4, found 7992.0. Template 8: MALDI-TOF Mass (M + H) C_{252}H_{320}N_{96}O_{156}P_{25}^+ 7960.3, found 7963.8. Template 13: MALDI-TOF Mass (M + H) C_{253}H_{321}N_{95}O_{157}P_{25}^+ 7975.3, found 7976.4. Template 14: MALDI-TOF Mass (M + H) C_{252}H_{321}N_{95}O_{158}P_{25}^+ 7951.3, found 7956.7.*
Single dNTP insertion reaction
For base recognition of modified nucleosides in polymerase-mediated chain elongations on DNA templates see the recent papers (44-52).

Single dNTP insertion reaction using Vent (exo⁻) DNA polymerase
The reaction mixture (10 µL) contained 20 mM Tris-HCl (pH 8.8), 10 mM (NH₄)₂SO₄, 10 mM KCl, 2 mM MgSO₄, 0.1% Triton X-100, 100 nM 5´-FAM-labeled primer/template, 0.1 unit enzyme, and 10 µM dNTP (N = A⁰, C⁰, A, G, C, or T). The mixture was incubated at 74 °C for 10 min, and the reactions were terminated by adding 30 µL of stop solution (98% formamide, 20 mM EDTA). After gently vortexing, the samples were separated by electrophoresis using 20% denaturing polyacrylamide gel containing 7 M urea and visualized by Fujifilm FLA-2000G or FLA-7000. These results are shown in Figures S2 and S3.

Single dNTP insertion reaction using Klenow fragment (exo⁺) DNA polymerase
The reaction mixture (10 µL) contained 10 mM Tris-HCl (pH 7.5), 70 mM MgCl₂, 1 mM DTT, 100 nM 5´-FAM-labeled primer/template, 0.1 unit enzyme, and 10 µM dNTP (N = A⁰, C⁰, A, G, C, or T). The mixture was incubated at 37 °C for 10 min, and the reactions were terminated by adding 30 µL of stop solution (98% formamide, 20 mM EDTA). After gently vortexing the samples, they were separated by electrophoresis with 20% denaturing polyacrylamide gel containing 7 M urea and visualized by Fujifilm FLA-2000G. These results are shown in Figures S2 and S3.

Single dNTP insertion reaction using Pyrobest DNA polymerase
The reaction mixture (10 µL) contained Pyrobest buffer II, 100 nM 5´-FAM-labeled primer/template, 0.1 unit enzyme, and 10 µM dNTP (N = A⁰, C⁰, A, G, C, or T). The mixture was incubated at 74 °C for 10 min, and the reactions were terminated by adding 30 µL of stop solution (98% formamide, 20 mM EDTA). After gently vortexing the samples, they were separated by electrophoresis using 20% denaturing polyacrylamide gel containing 7 M urea and visualized by Fujifilm FLA-2000G or FLA-7000. These results are shown in Figures S2 and S3.
**Figure S1.** Single dNTP insertion reaction by Klenow fragment (exo−). (A) Sequences of 5′-FAM labeled 18-nt primer and 25-nt templates. (B) PAGE analysis of single-insertion reactions using dC<sup>0</sup>TP (left) and dA<sup>0</sup>TP (right).
Figure S2. Single dNTP insertion reactions using various DNA polymerases. (A) Sequences of 5′-FAM labeled 18-nt primer and 25-nt templates. PAGE analysis of single-insertion reactions using (B) Vent (exo−), (C) KF (exo+), and (D) Pyrobest.
Figure S3. Single dNTP insertion reactions using various DNA polymerases. (A) Sequences of 5'-FAM labeled 18-nt primer and 26-nt templates. PAGE analysis of single-insertion reactions using (B) KF (exo⁻), (C) Vent (exo⁻), (D) KF (exo⁺), and (E) Pyrobust.
Steady-state kinetics methods in reactions using oxidized dNTPs and natural templates

A reaction mixture (10 µL) of 10 mM MgSO₄, 0.1 mM DTT, 100 nM 5´-FAM-labeled primer/template duplex having the sequence [5´-FAM-(CGCGCGAAGACCGGTTAC)-3´/5´-(TAAGACXGTAACCGGTCTTCGC GCG)-3´, X = A, G, C, and T], DNA polymerase Klenow fragment (exo⁻), dNTP (N = A°, C°, A, or C) in 50 mM Tris-HCl (pH 7.2) was incubated at 37 °C for the appropriate times. The concentration of dNTP (0.2-50 µM) and the reaction time were adjusted for each reaction so as to give the product yield of 25% or less. The details of the enzyme concentrations and times used are summarized in Table S4.

Table S4. Range of enzyme concentration and time in this assay

| dNTP (N) | Template base (X) | enzyme concentration (units / µL) | time (min) |
|----------|-------------------|----------------------------------|------------|
| C°       | G                 | 0.005                            | 3          |
| C°       | A                 | 0.1                              | 5          |
| C        | G                 | 0.005                            | 0.5        |
| A°       | T                 | 0.005                            | 3          |
| A°       | G                 | 0.05                             | 1          |
| A°       | C                 | 0.1                              | 3          |
| A        | T                 | 0.005                            | 0.5        |

The reactions were terminated by adding 30 µL of a stop solution (98% formamide, 20 mM EDTA). After being vortexed gently, the reactions were analyzed by electrophoresis with 20% denaturing polyacrylamide gel containing 7 M urea. The reactions were monitored with Fujifilm FLA-2000G. Relative velocity v was measured as the ratio of the extended product (I_ext) to the remaining primer (I_prim) using $v = \frac{I_{\text{ext}}}{I_{\text{prim}}}$ t, where t represents the reaction time, and normalized for the lowest enzyme concentration used. The kinetic parameters ($K_m$ and $V_{max}$) were obtained from Hanes-Woof plots. Each parameter was averaged from three data sets. These results are shown in Table 1 in the text.
Steady-state kinetics methods in the reactions of natural dNTPs and oxidized templates using Klenow fragment (exo-) DNA polymerase

A reaction mixture (10 µL) of 10 mM MgSO₄, 0.1 mM DTT, 100 nM 5’-FAM-labeled primer/template duplex having the sequence [5’-FAM-(CGCGCGGAAGACCAGGTAC)-3’/5’-(TAAGACXGTAAACCGGTCTTGCGCGT)-3’, X = A⁰, C⁰, A, and C], DNA polymerase Klenow fragment (exo-) (0.005 unit / µL), dNTP (N = A, G, C, or T) in 50 mM Tris-HCl (pH 7.2) was incubated at 37 °C for the appropriate times (0.25-10 min). The concentration of dNTP (0.1-500 µM) and the reaction time were adjusted for each reaction so as to give the product yield of 25% or less. The reactions were terminated by adding 30 µL of a stop solution (98% formamide, 20 mM EDTA). After being vortexed gently, the reactions were analyzed by electrophoresis with 20% denaturing polyacrylamide gel containing 7 M urea. The reactions were monitored with Fujifilm FLA-2000G or FLA-7000. Relative velocity \( v \) was measured as the ratio of the extended product \( (I_{\text{ext}}) \) to the remaining primer \( (I_{\text{prim}}) \) using \( v = \frac{I_{\text{ext}}}{I_{\text{prim}}} \), where \( t \) represents the reaction time. The kinetic parameters \( (K_m \text{ and } V_{\text{max}}) \) were obtained from Hanes-Woolf plots. Each parameter was averaged from three data sets. These results are shown in Table 2 in the text.

Steady-state kinetics methods in the reactions of natural dNTPs and oxidized templates using Vent (exo-) DNA polymerase

A reaction mixture (10 µL) of 10 mM (NH₄)₂SO₄, 10 mM KCl, 2 mM MgSO₄, 0.1% Triton X-100, 100 nM 5’-FAM-labeled primer/template duplex having the sequence [5’-FAM-(CGCGCGGAAGACCAGGTAC)-3’/5’-(TAAGACXGTAAACCGGTCTTGCGCGT)-3’, X = A⁰, C⁰, A, and C], Vent (exo-) DNA polymerase (0.01 unit / µL), dNTP (N = A, G, C, or T) in 20 mM Tris-HCl (pH 8.8) was incubated at 74 °C for the appropriate times (0.25-10 min). The concentration of dNTP (0.05-50 µM) and the reaction time were adjusted for each reaction so as to give the product yield of 25% or less. The reactions were terminated by adding 30 µL of a stop solution (98% formamide, 20 mM EDTA). After being vortexed gently, the reactions were analyzed by electrophoresis with 20% denaturing polyacrylamide gel containing 7 M urea. The reactions were monitored with Fujifilm FLA-2000G or FLA-7000. Relative velocity \( v \) was measured as the ratio of the extended product \( (I_{\text{ext}}) \) to the remaining primer \( (I_{\text{prim}}) \) using \( v = \frac{I_{\text{ext}}}{I_{\text{prim}}} \), where \( t \) represents the reaction time. The kinetic parameters \( (K_m \text{ and } V_{\text{max}}) \) were obtained from Hanes-Woolf plots. Each parameter was averaged from three data sets. These results are shown in Table 2 in the text.
Theoretical calculations

The geometries of cytosine $N^3$-oxide and adenine $N^4$-oxide were optimized at the HF/6-31+G** level using Gaussian 03 software (53). The initial structures of the base pairs were generated by placing the two geometry-optimized isolated bases on a plane maintaining the distances between the hydrogen-bonded O and H, and N and H within 1.96–1.99 Å. The geometries of the base pair were optimized at the HF/6-31+G** level and the final energies were calculated at the MP2/6-31+G** level.

$$\Delta E = E(\text{base pair}) - \{E(\text{isolated base 1}) + E(\text{isolated base 2})\}.$$  

The basis set superposition errors (BSSE) were estimated by the counterpoise method (54). All structural figures were prepared using PyMOL (55), and shown in Figure S4.

![Figure S4](image_url)

**Figure S4.** Optimized structures of the oxidized and natural base pairs. (A) C–G and C$^O$–G base pairs. (B) A–T and A$^O$–T base pairs.

Additionally, to investigate other types of oxidized base pairs in DNA duplexes, the simulations were carried out using the AMBER 10 program package with the parm99SB. The initial structures of ODNs were derived from NUCGEN module.
embedded in AMBER. The atomic charges of the modified nucleobases were calculated by using the RESP/6-31G(d) charges (56-58). DNA duplexes were solvated in a periodic box with a 10 Å buffer of water molecules explicitly described by the TIP3P model and neutralized, resulting in a concentration of added NaCl of approximately (59).

An initial optimization of 1000 cycles, the first 500 by steepest descent and the rest with a conjugate gradient method, was performed with the duplex constrained 500 kcal/(mol·Å²), to relax the solvent. Then a further optimization of 5000 cycles with no constraints on the whole system was carried out to lead to a final relaxed geometry. The first equilibrations were carried out with a 10 kcal/(mol·Å²) constraint on the duplex for 100 ps at constant volume, constantly increasing the temperature from 0 to 300 K. Next, the equilibrations were continued to 200 ps at a constant pressure of 1 atm, and the temperature were kept constant with the Langevin algorithm. The production simulations were performed for 2 ns with the Berendsen algorithm to maintain the temperature (60). During the MD calculation, hydrogen vibrations were removed using SHAKE bond constraints, allowing a longer time step of 2 fs (61). Long-range electrostatic interactions were treated using the particle mesh Ewald approach and 10 Å cutoff (62).

Molecular graphics images were produced using PyMOL (55). The sequences of ODNs used in these simulations and the structures of the C⁻G and A⁻T base pairs obtained are shown in Figure S5, S6.

The C⁻G base pair at 2300 ps (Figure S5B, point i) was the main structure, and its hydrogen bonding manner and C1'-C1' distances were almost similar to those obtained by the ab initio calculations. The structure at 1883 ps (point ii) was also observed as one of several structures in this MD calculation and was a transient structure. Since the C1'-C1' distance of this base pair was shorter than one of the main structure shown in Figure C, the base pair was not a planar structure but had a distortional structure. In the A⁻T base pair, the structure at 2300 ps (Figure S6B, point iii) was the main structure obtained by the MD calculation of the duplex containing an A⁻T base pair. This A⁻T base pair structure was also comparable with the structure resulted from the ab initio calculations.
Figure S5. Structure of the C\(^0\)-G base pair obtained by the MD simulation. (A) Sequence of a DNA duplex containing C\(^0\). (B) Distance (Å) of C1'-C1' during the MD simulation (C) Structure of the C\(^0\)-G base pair at 2300 ps (point i: red). (D) Structure of the C\(^0\)-G base pair at 1883 ps (point ii: blue).

Figure S6. Structure of the A\(^0\)-T base pair obtained by the MD simulation. (A) Sequence of a DNA duplex containing A\(^0\). (B) Distance (Å) of C1'-C1' during the MD simulation (C) Structure of the A\(^0\)-T base pair at 2300 ps (point iii: green).
Figure S7. Atom types and atomic charges of the modified C^O base.

Table S5. Bond and angle parameters for the modified C^O base

| bond^a | $K_r$ | $r_{eq}$ |
|-------|------|--------|
| N2-O2 | 416.20 | 1.394 |

| angle^b | $K_\theta$ | $\theta_{eq}$ |
|---------|------------|-------------|
| CA-NC-O2 | 68.407 | 125.410 |
| C-NC-O2 | 68.247 | 112.750 |

a) $E_{bond} = K_r(r-r_{eq})^2$; b) $E_{angle} = K_\theta(\theta-\theta_{eq})^2$
Figure S8. Atom types and atomic charges of the modified A\textsuperscript{O} base.

Table S6. Bond and angle parameters for the modified A\textsuperscript{O} base

| bond\textsuperscript{a} | $K_r$ (kcal mol\textsuperscript{-1} Å\textsuperscript{2}) | $r_{eq}$ (Å) |
|------------------------|-----------------|-------------|
| N2-O2                  | 564.00          | 1.303       |
| CA-H5                  | 344.30          | 1.087       |

| angle\textsuperscript{b} | $K_\theta$ (kcal mol\textsuperscript{-1} deg\textsuperscript{2}) | $\theta_{eq}$ (deg) |
|---------------------------|-----------------|-------------|
| CA-N2-CA                 | 64.300          | 127.460     |
| CA-N2-O2                 | 68.407          | 125.410     |
| N2-CA-H5                 | 52.400          | 113.540     |
| H5-CA-NC                 | 52.400          | 120.540     |

\textsuperscript{a} $E_{bond} = K_r(r-r_{eq})_2$; \textsuperscript{b} $E_{angle} = K_\theta(\theta-\theta_{eq})^2$
d[TAAAGACA0GTAACCGGTCTTCGCACG]

Template 7

Bruker Daltonics flexAnalysis

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Template 13
5'-TGATACA\textsuperscript{o}GTAACCGGTTCTTCGCGCGT-3'
Template 14
5' TGATACC^O GTAACCGGTTTCGCGGT-3'
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Legends

**Figure S1.** Single dNTP insertion reaction by Klenow fragment (exo⁻).

**Figure S2.** Single dNTP insertion reactions using various DNA polymerases.

**Figure S3.** Single dNTP insertion reactions using various DNA polymerases.

**Figure S4.** Optimized structures of the oxidized and natural base pairs.

**Figure S5.** Structure of the C⁰-G base pair obtained by the MD simulation.

**Figure S6.** Structure of the A⁰-T base pair obtained by the MD simulation.

**Figure S7.** Atom types and atomic charges of the modified C⁰ base.

**Figure S8.** Atom types and atomic charges of the modified A⁰ base.

**Table S1.** \( T_m \) values for DNA–DNA 14-mer duplexes containing 2´-deoxyribonucleoside N-oxides

**Table S2.** \( T_m \) values for DNA–RNA 14-mer duplexes containing 2´-deoxyribonucleoside N-oxides

**Table S3.** Sequences of template and primer ODNs using enzyme reactions

**Table S4.** Range of enzyme concentration and time in this assay

**Table S5.** Bond and angle parameters for the modified C⁰ base

**Table S6.** Bond and angle parameters for the modified A⁰ base