Regular Article

Effects of the 2-Substituted Adenosine-1,3-diazaphenoxazine 5′-Triphosphate Derivatives on the Single Nucleotide Primer Extension Reaction by DNA Polymerase

Yosuke Taniguchi,* Ikuko Sagara, Yusuke Nagata, Yoshiya Kikukawa, and Shigeki Sasaki*

Graduate School of Pharmaceutical Sciences, Kyushu University; 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan.

Received May 29, 2019; accepted July 1, 2019

The adenosine triphosphate derivatives of 2-oxo-1,3-diazaphenoxazine (dAdapTP) showed a significant discrimination ability for the template strand including that between 8-oxo-2′-deoxyguanosine (8-oxodG) and 2′-deoxyguanosine (dG) by the single nucleotide primer extension reaction using the Klenow Fragment. In this study, we synthesized new dAdapTP derivatives, *i.e.*, 2-amino-dAdapTP, 2-chloro-dAdapTP and 2-iodo-dAdapTP, to investigate the effect on the selectivity and efficiency of incorporation for the primer extension reaction using a variety of DNA polymerases. In contrast to the previously tested dAdapTP, the selectivity and efficiency of the 2-halo-dAdapTP incorporation were dramatically decreased using the Klenow Fragment. Moreover, the efficiency of the 2-amino-dAdapTP incorporation into the T-containing template was almost the same with that of dAdapTP. In the case of the Bsu DNA polymerase, the efficiency of all the dAdapTP derivatives decreased compared to that using the Klenow Fragment. However, the incorporation selectivity of dAdapTP had improved against the oxodG-containing template for all the template sequences including the T-containing template. Moreover, 2-amino-dAdapTP showed a better efficiency than dAdapTP using the Bsu DNA polymerase. The 2-amino group of the adenosine unit may interact with *syn*-oxodG at the active site of the Bsu DNA polymerase during the single primer extension reaction.

Key words DNA damage; 8-oxo-2′-deoxyguanosine (8-oxodG); enzymatic primer extension; adenosine-1,3-diazaphenoxazine (Adap) triphosphate derivative

Introduction

Genomic DNA is constantly damaged by external or internal stimuli. In particular, the reactive oxygen species (ROS) react at the 8-position of the guanine nucleobase (G) to produce a compound called 8-oxoguanine (oxoG) as the oxidative damage.[1] The 8-oxo-2′-deoxyguanosine (oxodG) can form hydrogen bonding, not only with 2′-deoxycytidine (dC), but also with 2′-deoxyadenosine (dA) in duplex DNA. This property induces the transversion mutation from a GC to a TA base pair during the DNA replication.[2,3] According to the results of several studies, the amount of oxoG in urine, blood or cell is associated with the various types of diseases such as neurodegenerative diseases, so it becomes a good biomarker.[4] Furthermore, the presence of the oxodG in the DNA sequences may play an important role in biological systems.[5] However, an innovative technique or simple method that can sequence oxodG in DNA does not presently exist. In addition, amplification is necessary due to the low amount of oxodG in the DNA. Thus, in order to identify it, the chemical modification or multistep operation is required.[6–16] Recently, we developed a novel nucleoside triphosphate, adenosine-1,3-diazaphenoxazine triphosphate (dAdapTP), which showed a discrimination ability for the template strand including between that 8-oxodG and dG using the Klenow Fragment.[17] However, dAdapTP consists of 2′-deoxyadenosine as the basic skeleton, thus it was also incorporated into the primer strands for the thymidine-containing template (T-template).[18–20] In order to solve this problem, we tested the properties of the 2-substituted adenosine derivatives of the dAdapTP derivatives (Fig. 1) and several commercially-available DNA polymerases.

The syntheses of the 2-chloro-Adap (**11**) and the 2-iodo-Adap (**12**) are summarized in Chart 1. These two compounds were synthesized from the 3′-*O*- and 5′-*O*-*tert*-butyldimethylsilyl (TBS) protected 2,4,6-triisopropylbenzenesulfonyl derivative (**4**).[15] Chlorination was carried out using *tert*-butyl nitrite and trimethylsilyl chloride (TMSCl) in dichloromethane at −10°C, and the chlorinated product **5** was obtained in a 43% yield.[21] On the other hand, iodination was carried out using isoamyl nitrite, copper(I) iodide, iodine and diiodomethane in acetonitrile under refluxed conditions, and the iodinated product **6** was obtained in a 61% yield.[22] A substitution reaction was done using the phenoxazine unit **7** and the corresponding 2-halogenated compounds (**5** or **6**) in the presence of diisopropylethylamine to give the TBS protected compound **8** or **9** in a 68 or 83% yield, respectively. The TBS groups at the 3′- and 5′-hydroxyl group of each compound were removed to produce the 2-chloro-Adap (**11**) or the 2-iodo-Adap (**12**) in a good yield. The conventional triphosphate synthesis method is shown in Chart 2.[17] Briefly, these diol compounds (**10**,[15] **11** and **12**) were converted into the 3′-*O*-Ac compounds *via* protection and deprotection at the 5′-hydroxyl group with the dimethoxytrityl (DMTr) group (**13**, **14** and **15**). The 5′-hydroxyl group was reacted with phosphorylated reagents. After deprotection under alkaline conditions, the target triphosphate compounds were purified by reverse phase HPLC. Although the isolated or reaction yields were not good because of the remaining corresponding diol material, the 2-amino-dAdapTP (**1**), 2-chloro-dAdapTP (**2**) or 2-iodo-dAdapTP (**3**) were identified by ^{1}H- and ^{31}P-NMR and high resolution mass spectra measurements.

* To whom correspondence should be addressed. e-mail: taniguch@phar.kyushu-u.ac.jp; sasaki@phar.kyushu-u.ac.jp

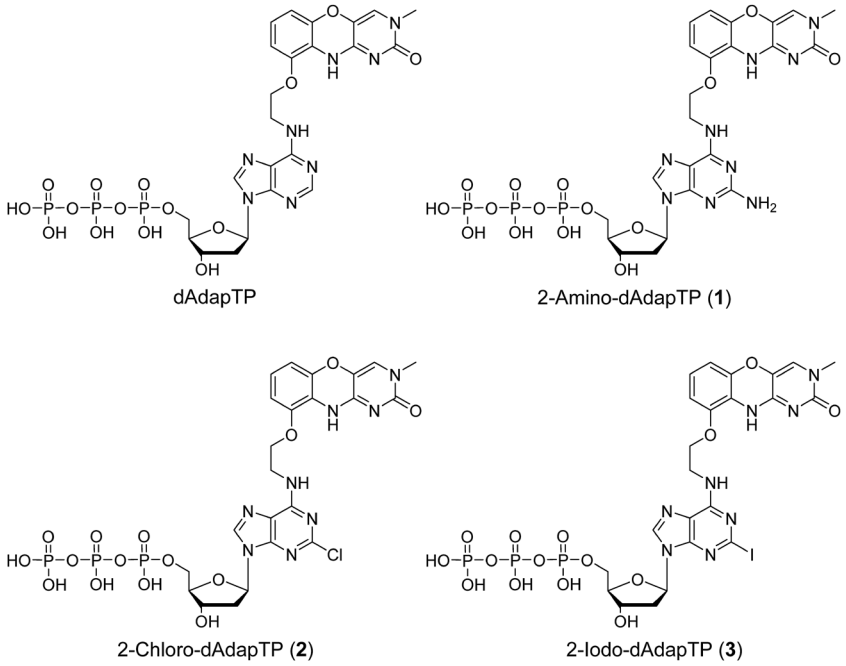
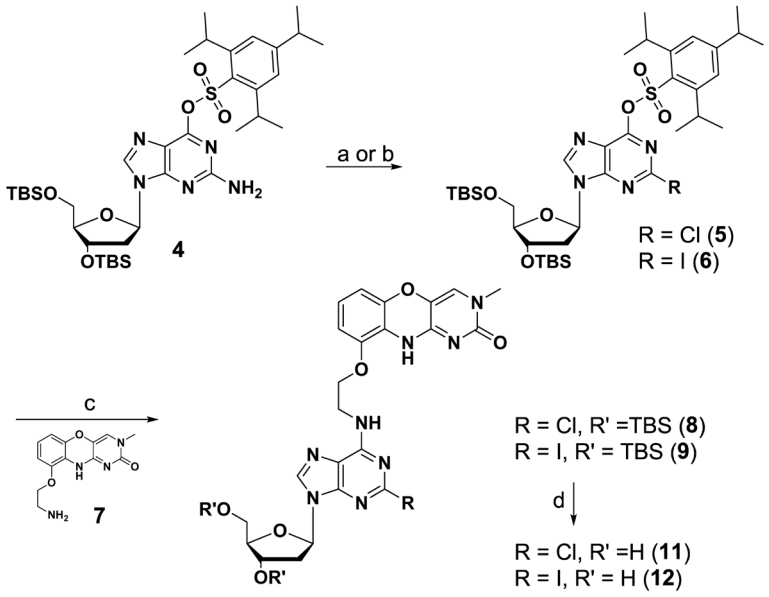


Fig. 1. The Structures of dAdapTP and 2-Substituted dAdapTP Derivatives (**1**, **2** and **3**)

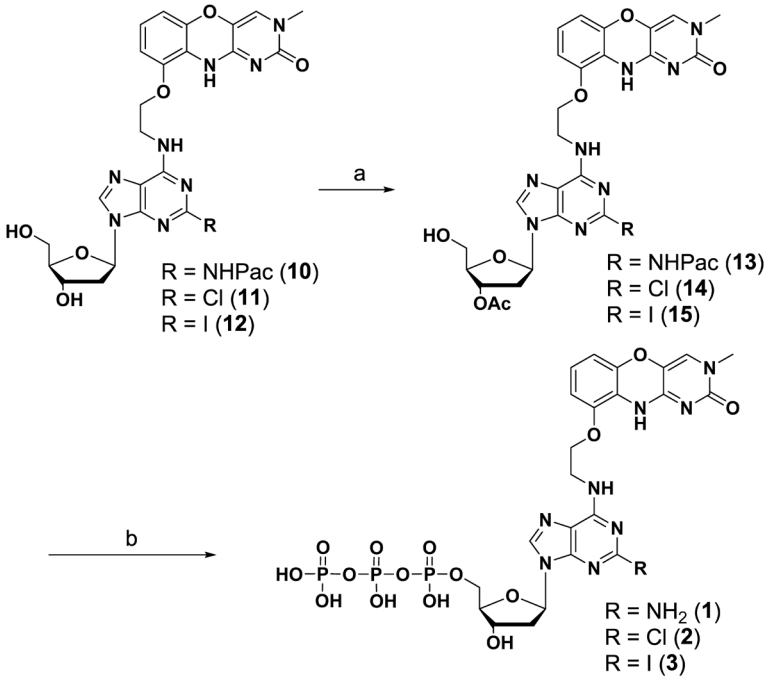


(a) *tert*-Butyl nitrite, TMSCl, CH_2Cl_2, −10°C, 43%; (b) Isoamyl nitrite, CuI, I_2, CH_2I_2, CH_3CN, reflux, 61%; (c) Phenoxazine unit **7**, DIPEA, 1-propanol, reflux, 68% for **8** and 83% for **9**; (d) Et_3N-3HF, TEA, pyridine, 93% for **11** and 73% for **12**.

Chart 1. The Syntheses of the 2-Chloro-Adap (**11**) and the 2-Iodo-Adap (**12**)

We first tested the single nucleotide extension reaction using the fluorescein (FAM)-labeled primer, template DNA (X) (X = oxodG, dG, dA, dC or T), dAdapTP or 2-substituted dAdapTP derivatives (**1**, **2** and **3**) and commercially-available DNA polymerases (Klenow Fragment (exo^-), Bsu DNA polymerase, KOD Dash, Bst DNA polymerase, Vent DNA polymerase (exo^-) and Taq DNA polymerase). After the single nucleotide extension reaction, the elongated FAM-labeled primer was separated by gel electrophoresis. These gel result pictures are depicted in Fig. 2. Among these polymerases, the elongation products were obviously confirmed using the Klenow Fragment, Bsu DNA polymerase and KOD Dash.

We next obtained the steady-state kinetic data for the single primer extension reaction using the following three polymerases: Klenow Fragment, Bsu DNA polymerase and KOD Dash. The results of the steady-state kinetics (V_{max} and K_M) are summarized in Tables 1, 2 and 3 for the Klenow Fragment, Bsu DNA polymerase and KOD Dash, respectively. In the case of the Klenow Fragment, the incorporation efficiency (V_{max}/K_M) of 2-amino-dAdapTP into the oxodG-containing template DNA was reduced while maintaining the incorporation efficiency into the T-containing template (Table 1, entries 1 *vs.* 6 and 5 *vs.* 10). The efficiency into the T-containing template of 2-amino-dAdapTP was slightly lower than that of the



(a) 1) DMTrCl, pyridine; 2) Acetic anhydride, pyridine; 3) CCl_3COOH, CH_2Cl_2, (3 steps, 87% for **13**, 80% for **14** and 80% for **15**). (b) 1) 2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one, pyridine, 1,4-dioxane; 2) Tributyl ammonium pyrophosphate, tributylamine, DMF; 3) 1% I_2, pyridine, H_2O, r.t.; 4) 28% ammonium solution, then HPLC purification (4 steps, 2% for **1**, 30% for **2** and 1% for **3**).

Chart 2. The Synthesis of the 2-Substituted dAdapTP Derivatives (**1**–**3**)

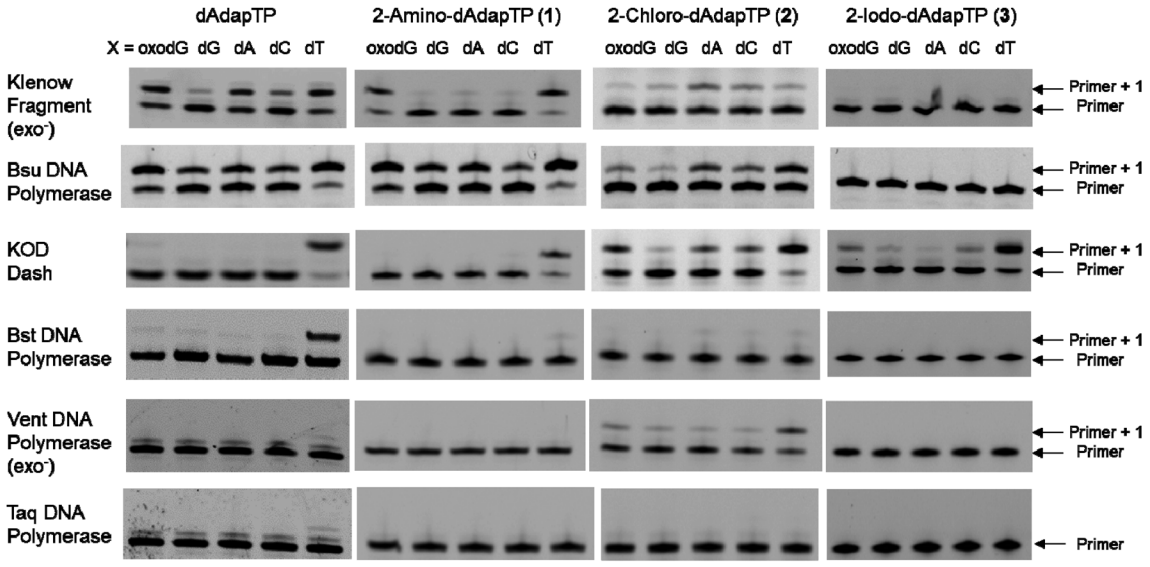


Fig. 2. Gel Results of the Evaluation of the Single Nucleotide Extension Reaction

Conditions: 1.0 μM FAM-labeled primer (15 mer), 1.0 μM template DNA (X) (25 mer), 0.1 unit/μL polymerase in the corresponding reaction buffer, 50 μM dAdapTP or 2-substituted dAdapTP derivatives, reaction in 10 μL for 30 min at 37°C, 15% denatured polyacrylamide gel.

natural dATP (Table 1, entry 25). Unfortunately, the selectivity and efficiency of the 2-chloro-dAdapTP and 2-iodo-dAdapTP incorporations were dramatically decreased (Table 1, entries 11–20). These results indicated that the 2-substitution of the adenosine unit of dAdapTP reduced the incorporation efficiency but the amino group was expected to interact with the 2-position of the carbonyl group of the thymine nucleobase at the complimentary position at the active site of the Klenow Fragment.

The Bsu DNA polymerase showed interesting kinetics results (Table 2). The incorporation efficiency of dAdapTP into the oxodG-containing template was slightly reduced, but the selectivity for the oxodG-containing template was improved (Table 2, entries 1–5). In particular, the incorporation efficiencies for the oxodG-containing template and T-containing template were almost the same (Table 2, entries 1 *vs.* 5). Interestingly, 2-amino-dAdapTP having an amino group at the 2-position of the adenosine skeleton slightly improved the incorporation efficiency over dAdapTP (Table 2, entries 1 *vs.* 6 and 5 *vs.* 10). Interestingly, the efficiency of 2-amino-dAdapTP for the oxodG-containing template was better than that of dATP, which is believed to be due to multiple hydrogen

Table 1. Steady-State Kinetic Parameter Using Klenow Fragment[a)]

Entry	dNTP	X	V_{max} [% min^{-1}]	K_M [μM]	V_{max}/K_M [% min^{-1} M^{-1}]	Relative [%]
1	dAdapTP	8-oxodG	2.35 (0.37)	0.78 (0.02)	3.02×10^6	100
2		dG	0.26 (0.01)	3.11 (0.25)	0.08×10^6	2.75
3		dA	0.99 (0.26)	6.35 (0.34)	0.16×10^6	5.17
4		dC	0.79 (0.01)	5.40 (0.47)	0.15×10^6	4.87
5		T	5.23 (0.43)	0.48 (0.14)	10.9×10^6	360
6	2-Amino-dAdapTP	8-oxodG	1.20 (0.40)	2.60 (1.10)	0.46×10^6	100
7		dG	0.09 (0.01)	0.64 (0.03)	0.14×10^6	29.7
8		dA	0.15 (0.02)	1.47 (0.23)	0.10×10^6	22.2
9		dC	0.15 (0.05)	1.86 (0.92)	0.08×10^6	18.0
10		T	3.29 (0.03)	0.30 (0.02)	10.8×10^6	2350
11	2-Chloro-dAdapTP	8-oxodG	0.82 (0.11)	6.17 (1.52)	0.13×10^6	100
12		dG	0.87 (0.08)	2.31 (0.22)	0.38×10^6	283
13		dA	0.65 (0.04)	2.30 (0.38)	0.28×10^6	212
14		dC	0.82 (0.09)	4.23 (0.53)	0.19×10^6	145
15		T	0.75 (0.10)	5.23 (0.05)	0.14×10^6	108
16	2-Iodo-dAdapTP	8-oxodG	0.03 (0.01)	1.65 (0.12)	0.02×10^6	100
17		dG	0.05 (0.02)	0.51 (0.22)	0.10×10^6	546
18		dA	0.14 (0.02)	1.29 (0.16)	0.11×10^6	612
19		dC	0.14 (0.03)	2.56 (0.83)	0.05×10^6	300
20		T	0.10 (0.02)	2.48 (0.51)	0.04×10^6	211
21	dATP[b)]	8-oxodG	14.9 (0.97)	4.42 (0.39)	3.30×10^6	100
22		dG	0.90 (0.06)	3.21 (0.29)	0.28×10^6	8.44
23		dA	0.85 (0.05)	2.75 (0.17)	0.31×10^6	9.37
24		dC	0.86 (0.13)	3.83 (0.74)	0.23×10^6	6.82
25		T	13.8 (1.42)	0.73 (0.06)	18.9×10^6	572

a) Conditions: 1.0 μM FAM-labelled primer-template duplex, 0.01–0.1 unit/μL Klenow Fragment (exo-), 50 mM NaCl, 10 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9, 0.1–35 μM dNTP, incubated at 37°C for 2–10 min in a reaction volume of 10 μL. Velocity is normalized for the lowest enzyme concentration used. *b*) ref 17.

bonds (Table 2, entries 6 *vs.* 21). Furthermore, the selectivity also improved compared to dATP (Table 2, entries 21–25). The 2-halogenated derivatives, especially the 2-iodo-dAdapTP, was not incorporated into the primer strand at all (Table 2, entries 11–20). These results indicated that the amino group at the 2-position of the adenosine unit might interact with the 8-carbonyl group of *syn*-oxodG at the active site of the Bsu DNA polymerase.

On the other hand, KOD Dash incorporated the 2-amino-dAdapTP into the primer strand better than dAdapTP for the T-containing template (Table 3, entries 5 *vs.* 10). The 2-amino group of 2-amino-dAdapTP could interact with the thymine nucleobase during the polymerase reaction of KOD Dash. Interestingly, the incorporation efficiency of the 2-halo-dAdapTP derivatives increased more than that of dAdapTP (Table 3, entries 11–20). These results also indicated that the 2-halo substituted derivative showed the some interaction such as the halogen-oxygen interaction with 2-cabonyl group of thymine or shape fitting in the active site of KOD dash DNA polymerase. However, they had reduced efficiency and selectivity compared to the incorporation of dATP into the template (Table 3, entries 21–25).

Conclusion

In this study, we susccesfully synthesized the 2-substetuted adenosine-1,3-diazaphenoxazine derivatives and evaluated the properties of their triphosphate for the single nucleotide primer extension reaction using DNA polymerase. Based on the value of the steady-state kinetic parameter, we found an interesting DNA polymerase, the Bsu DNA polymerase, concerning the base selectivity between the oxodG-containing template and T-containing template. Furthermore, the amino group substitution at the 2-position of the adenosine unit improved the uptake efficiency, and their selectivity using the Bsu DNA polymerase was better than the results using the Klenow Fragment. These results indicated that the 2-amino-dAdapTP can be successfully incorporated into the active site of the Bsu DNA polymerase to interact with *syn*-oxodG in the complimentary position. Unfortunately, the halogen substitution at the 2-position of adenosine has a negative effect on the enzymatic incorporation under these conditions. These results encourage us to further modify the dAdapTP derivatives and the functional evaluation of them using various polymerases, which will lead to the development of a new oxodG sequencing technology.

Experimental

General The ^{1}H-NMR (400 MHz, 500 MHz), ^{13}C-NMR (125 MHz) and ^{31}P-NMR (202 MHz) spectra were recorded by Varian UNITY-400 and Bruker Ascend-500 spectrometers. The high-resolution electrospray ionization (HR-ESI)-MS were recorded by a Bruker micrOTOF II. The FAM labelled primer and template DNAs were purchased from Gene Design, Inc., or Genenet Co., Ltd., Japan.

3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-6-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]-2-chloro-guanosine (5) Under

Table 2. Steady-State Kinetic Parameter Using Bsu DNA Polymerase[a)]

Entry	dNTP	X	V_{max} [% min^{-1}]	K_M [μM]	V_{max}/K_M [% min^{-1} M^{-1}]	Relative [%]
1	dAdapTP	8-oxodG	1.37 (0.21)	1.42 (0.22)	0.97×10^6	100
2		dG	0.20 (0.02)	3.30 (0.15)	0.06×10^6	6.26
3		dA	0.19 (0.02)	3.15 (0.63)	0.06×10^6	6.12
4		dC	0.14 (0.01)	2.85 (0.68)	0.05×10^6	4.91
5		T	2.12 (0.49)	2.13 (0.71)	0.99×10^6	103
6	2-Amino-dAdapTP	8-oxodG	2.02 (0.98)	1.52 (0.87)	1.33×10^6	100
7		dG	0.20 (0.04)	3.20 (1.54)	0.06×10^6	4.74
8		dA	0.28 (0.06)	3.47 (0.88)	0.08×10^6	6.08
9		dC	0.17 (0.02)	2.55 (0.52)	0.03×10^6	5.17
10		T	3.14 (0.52)	1.72 (0.84)	1.82×10^6	137
11	2-Chloro-dAdapTP	8-oxodG	0.16 (0.01)	1.24 (0.29)	0.13×10^6	100
12		dG	0.12 (0.04)	1.72 (0.72)	0.07×10^6	50.3
13		dA	0.21 (0.07)	1.14 (0.58)	0.19×10^6	140
14		dC	0.21 (0.01)	1.14 (0.07)	0.19×10^6	140
15		T	0.55 (0.06)	2.02 (0.49)	0.27×10^6	206
16	2-Iodo-dAdapTP	8-oxodG	—[b)]	—[b)]	—[b)]	—[b)]
17		dG	—[b)]	—[b)]	—[b)]	—[b)]
18		dA	—[b)]	—[b)]	—[b)]	—[b)]
19		dC	—[b)]	—[b)]	—[b)]	—[b)]
20		T	—[b)]	—[b)]	—[b)]	—[b)]
21	dATP	8-oxodG	2.10 (0.92)	3.65 (0.42)	0.58×10^6	100
22		dG	0.30 (0.03)	4.36 (0.45)	0.07×10^6	11.9
23		dA	0.09 (0.01)	0.73 (0.21)	0.12×10^6	21.3
24		dC	0.10 (0.01)	2.28 (0.27)	0.04×10^6	7.71
25		T	8.19 (0.94)	0.62 (0.16)	13.1×10^6	2278

a) Conditions: 1.0 μM FAM-labelled primer-template duplex, 0.01–0.1 unit/μL Bsu DNA polymerase), 50 mM NaCl, 10 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9, 0.1–35 μM dNTP, incubated at 37°C for 2–10 min in a reaction volume of 10 μL. Velocity is normalized for the lowest enzyme concentration used. *b*) Single nucleotide incorporation reaction did not occur, therefore, the parameters were not determined.

an argon atmosphere, to a solution of *tert*-butyl nitrite (310 μL, 2.62 mmol) in dry CH_2Cl_2 (13 mL) was added the solution of **4** (1.0 g, 1.31 mmol) in dry CH_2Cl_2 (13 mL) and TMSCl (330 μL, 2.61 mmol) at −10°C. After stirring for 90 min at the same temperature, the reaction was quenched by a saturated $NaHCO_3$ solution. The organic layer was washed with water and a saturated NaCl solution, then dried over Na_2SO_4. The solvent was removed under reduced pressure, then the residue was purified by silica gel column chromatography (kanto 60N, Hexane/EtOAc = 10/1) to obtain a yellow foam (445 mg, 0.56 mmol, 43%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.31 (1H, s), 7.20 (2H, s), 6.39 (1H, t, J = 6.4 Hz), 4.61–4.58 (1H, m), 4.33–4.26 (2H, m), 3.99 (1H, dt, J = 6.7, 3.4 Hz), 3.86 (1H, dd, J = 11.3, 4.0 Hz), 3.74 (1H, dd, J = 11.3, 3.1 Hz), 2.94–2.87 (1H, m), 2.60–2.54 (1H, m), 2.45–2.39 (1H, m), 1.27–1.24 (18H, m), 0.89 (9H, s), 0.88 (9H, s), 0.08 (6H, s), 0.06 (3H, s), 0.05 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 155.0, 154.8, 154.6, 152.2, 146.9, 131.0, 124.0, 122.0, 88.4, 85.1, 71.9, 62.8, 41.6, 34.5, 30.0, 26.1, 25.9, 24.8, 24.7, 23.7, 23.6, 18.6, 18.2, −4.5, −4.7, −5.3, −5.4; IR (neat, cm^{-1}) 2956.2, 1601.6, 1565.2, 1391.3, 1256.2; HRMS (ESI-time-of flight (TOF)) Calcd for $C_{37}H_{61}ClN_4O_6SSi_2Na$ $[M+Na]^+$: 803.3431, 805.3405. Found: 803.3445, 805.3405.

3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-6-*O*-[(2,4,6-triisopropylphenyl)sulfonyl]-2-iodo-guanosine (6) Under an argon atmosphere, the mixture of **4** (1.0 g, 1.31 mmol), CuI (300 mg, 1.58 mmol), I_2 (666 mg, 5.25 mmol), CH_2I_2 (700 μL, 7.82 mmol) and isoamyl nitrite (880 μL, 6.55 mmol) in dry CH_3CN (15 mL) was refluxed for 90 min. After cooling to room temperature, the reaction was quenched by a saturated $Na_2S_2O_3$ solution. The reaction products were extracted with EtOAc. The organic layer was then washed with a saturated NaCl solution and dried over Na_2SO_4. The solvent was removed under reduced pressure, then the residue was purified by silica gel column chromatography (kanto 60N, Hexane/EtOAc = 10/1) to obtain a white foam (698 mg, 0.80 mmol, 61%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.28 (1H, s), 7.22 (2H, s), 6.40 (1H, t, J = 6.4 Hz), 4.62–4.60 (1H, m), 4.26 (2H, sep, J = 6.7 Hz), 4.01–3.99 (1H, m), 3.87 (1H, dd, J = 11.3, 4.1 Hz), 3.76 (1H, dd, J = 11.3, 3.2 Hz), 2.93 (1H, sep, J = 6.9 Hz), 2.63–2.57 (1H, m), 2.45–2.40 (1H, m), 1.29–1.26 (18H, m), 0.91 (9H, s), 0.90 (9H, s), 0.11 (6H, s), 0.08 (3H, s), 0.07 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 154.6, 154.3, 153.4, 151.0, 143.5, 131.4, 124.0, 123.3, 115.7, 88.4, 85.1, 72.0, 62.8, 41.5, 34.5, 30.0, 26.1, 25.9, 25.0, 24.9, 23.7, 23.6, 18.6, 18.2, −4.5, −4.6, −5.2, −5.3; IR (neat, cm^{-1}) 2958.6, 1597.9, 1554.8, 1389.8, 1254.3; HRMS (ESI-TOF) Calcd for $C_{37}H_{61}IN_4O_6SSi_2Na$ $[M+Na]^+$: 895.2787. Found: 895.2818.

General Procedure of Coupling Reaction with Phenoxazine Unit Under an argon atmosphere, a reaction mixture of **5** or **6** (1.0 eq.), phenoxazine unit **7** (1.5 eq.) and DIPEA (1.5 eq.) in 1-propanol (0.1 M) was refluxed at 100°C for 5 h. The reaction was quenched by a saturated aqueous $NaHCO_3$. The 1-propanol was removed under reduced pressure. The res-

Table 3. Steady-State Kinetic Parameter Using KOD Dash[a)]

Entry	dNTP	X	V_{max} [% min^{-1}]	K_M [μM]	V_{max}/K_M [% min^{-1} M^{-1}]	Relative [%]
1	dAdapTP	8-oxodG	0.06 (0.01)	10.5 (4.12)	0.53×10^4	100
2		dG	—[b)]	—[b)]	—[b)]	—[b)]
3		dA	—[b)]	—[b)]	—[b)]	—[b)]
4		dC	—[b)]	—[b)]	—[b)]	—[b)]
5		T	4.85 (0.18)	13.9 (1.00)	34.9×10^4	6551
6	2-Amino-dAdapTP	8-oxodG	0.05 (0.01)	8.65 (1.70)	0.63×10^4	100
7		dG	—[b)]	—[b)]	—[b)]	—[b)]
8		dA	—[b)]	—[b)]	—[b)]	—[b)]
9		dC	0.07 (0.01)	4.34 (0.62)	1.66×10^4	264
10		T	3.94 (0.11)	5.89 (0.19)	66.8×10^4	10600
11	2-Chloro-dAdapTP	8-oxodG	0.57 (0.20)	12.9 (6.00)	4.42×10^4	100
12		dG	0.06 (0.01)	3.18 (0.18)	1.96×10^4	44.3
13		dA	0.28 (0.02)	22.3 (3.69)	1.26×10^4	28.4
14		dC	0.29 (0.02)	19.8 (1.40)	1.47×10^4	33.2
15		T	4.66 (0.30)	15.6 (1.51)	29.9×10^4	677
16	2-Iodo-dAdapTP	8-oxodG	0.27 (0.05)	3.36 (1.00)	8.07×10^4	100
17		dG	0.09 (0.01)	2.93 (0.14)	3.05×10^4	37.9
18		dA	0.03 (0.01)	4.38 (1.27)	0.76×10^4	9.38
19		dC	0.19 (0.02)	4.59 (1.59)	4.09×10^4	50.6
20		T	1.24 (0.13)	0.75 (0.07)	164×10^4	2031
21	dATP	8-oxodG	0.12 (0.92)	1.17 (0.40)	10.0×10^4	100
22		dG	0.12 (0.01)	2.94 (0.23)	4.11×10^4	41.0
23		dA	0.07 (0.01)	0.60 (0.06)	11.8×10^4	117
24		dC	0.08 (0.04)	1.73 (1.08)	4.91×10^4	49.0
25		T	126 (2.65)	1.71 (0.22)	7356×10^4	73450

a) Conditions: 1.0 μM FAM-labelled primer-template duplex, 0.01–0.1 unit/ μL KOD Dash, 20 mM Tirs–HCl, 8 mM $MgCl_2$, 7.5 mM DTT, 2.5 μg BSA, pH 7.5, 0.1–35 μM dNTP, incubated at 37°C for 5–50 min in a reaction volume of 10 μL. Velocity is normalized for the lowest enzyme concentration used. *b*) Single nucleotide incorporation reaction did not occur, therefore, the parameters were not determined.

idue was purified by silica gel column chromatography (kanto 60N, Hexane/EtOAc = 1/1 to 1/5) to give the corresponding coupling compounds **8** or **9**.

3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-6-*N*-{2-[(1,3-diaza-3-methyl-2-oxo-phenoxazine-9-yl)oxy]-ethyl}-2-chloro-adenosine (8) Yellow foam (260 mg, 0.34 mmol, 68%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.72 (1H, br), 8.23 (1H, s), 6.78 (1H, t, J = 8.2 Hz), 6.66 (1H, d, J = 8.1 Hz), 6.43 (1H, d, J = 8.0 Hz), 6.34 (1H, t, J = 6.7 Hz), 6.31 (1H, s), 4.63–4.61 (1H, m), 4.18 (2H, br), 4.02–3.99 (1H, m), 3.84 (2H, br), 3.83 (1H, dd, J = 10.9, 5.9 Hz), 3.74 (1H, dd, J = 11.0, 4.2 Hz), 3.18 (3H, s), 2.82–2.77 (1H, m), 2.37 (1H, ddd, J = 13.1, 5.9, 3.2 Hz), 0.92 (9H, s), 0.87 (9H, s), 0.11 (6H, s), 0.06 (3H, s), 0.05 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 155.1, 154.8, 151.9, 151.1, 149.4, 145.1, 139.9, 129.3, 124.4, 123.5, 118.4, 110.4, 88.3, 85.2, 77.4, 72.6, 63.2, 41.8, 40.1, 35.9, 29.8, 29.4, 26.1, 26.0, 18.5, 18.2, −4.5, −4.6, −5.3, −5.4; IR (neat, cm^{-1}) 2928.3, 1630.4, 1473.2, 1260.8; HRMS (ESI-TOF) Calcd for $C_{35}H_{51}ClN_8O_6Si_2Na$ $[M+Na]^+$: 793.3051, 795.3025. Found: 793.3068, 795.3075.

3′,5′-Bis-*O*-*tert*-butyldimethylsilyl-2′-deoxy-6-*N*-{2-[(1,3-diaza-3-methyl-2-oxo-phenoxazine-9-yl)oxy]-ethyl}-2-iodo-adenosine (9) Yellow foam (276 mg, 0.32 mmol, 83%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.46 (1H, br), 8.18 (1H, brs), 6.78 (1H, t, J = 8.2 Hz), 6.60 (1H, d, J = 7.4 Hz), 6.38 (1H, d, J = 7.9 Hz), 6.30 (1H, t, J = 6.4 Hz), 4.63–4.61 (1H, m), 4.14 (2H, br), 3.99–3.96 (1H, m), 3.84 (2H, br), 3.82 (1H, dd, J = 11.0, 6.0 Hz), 3.74 (1H, dd, J = 10.9, 4.4 Hz), 3.20 (3H, s), 2.80 (1H, br), 2.35–2.33 (1H, br), 0.91 (9H, s), 0.87 (9H, s), 0.11 (6H, s), 0.06 (3H, s), 0.05 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 154.0, 153.4, 151.4, 150.9, 148.4, 146.4, 141.6, 138.8, 126.9, 125.6, 125.0, 121.3, 118.8, 113.3, 88.4, 85.6, 74.2, 72.3, 63.1, 40.3, 39.9, 34.5, 29.4, 26.1, 25.9, 18.6, 18.1, −4.5, −4.6, −5.1, −5.2; IR (neat, cm^{-1}) 2928.1, 1701.4, 1622.8, 1567.0, 1472.5, 1257.9; HRMS (ESI-TOF) Calcd for $C_{35}H_{51}IN_8O_6Si_2Na$ $[M+Na]^+$: 885.2407. Found: 885.2444.

General Procedure of Deprotection Reaction of TBS Group Under an argon atmosphere, Et_3N-3HF (0.19 mL, 1.18 mmol) and triethylamine (0.18 mL, 1.29 mmol) were added to a solution of compound **8** or **9** (0.30 mmol) in dry pyridine (3.0 mL). After stirring for 20 h, the solvent was removed under reduced pressure. The residue was purified by amino-silica gel column chromatography ($CHCl_3$/MeOH = 30/1 to 15/1) to obtain the corresponding diol products.

2′-Deoxy-6-*N*-{2-[(1,3-diaza-3-methyl-2-oxophenoxazine-9-yl)oxy]-ethyl}-2-chloro-adenosine (2-Chloro-Adap) (11) Pale yellow powder (150 mg, 0.28 mmol, 93%). ^{1}H-NMR (500 MHz, dimethyl sulfoxide (DMSO)-d_6) δ: 9.94 (1H, br), 8.88 (1H, brs), 8.40 (1H, s), 7.49 (1H, br), 6.75 (1H, t, J = 8.2 Hz), 6.61 (1H, d, J = 7.8 Hz), 6.41 (1H, d, J = 8.2 Hz), 6.27 (1H, t, J = 6.6 Hz), 5.32 (1H, d, J = 4.2 Hz), 4.95 (1H, t, J = 5.5 Hz), 4.39 (1H, br), 4.05 (2H, br), 3.86 (3H, m), 3.61–3.57 (1H, m), 3.53–3.49 (1H, m), 3.19 (3H, s), 2.64 (1H, qui, J = 6.6 Hz), 2.31–2.29 (1H, m); ^{13}C-NMR (125 MHz,

DMSO-d_6) δ: 154.8, 154.6, 154.0, 153.2, 149.4, 146.0, 142.2, 139.9, 129.2, 125.9, 122.9, 118.6, 115.8, 107.9, 106.8, 88.0, 83.6, 70.7, 67.3, 61.6, 40.1, 39.6, 36.7; IR (neat, cm^{-1}) 3481.7, 2928.0, 1670.1, 1630.0, 1512.5, 1473.0, 1317.8; HRMS (ESI-TOF) Calcd for $C_{23}H_{23}ClN_8O_6Na$ $[M+Na]^+$: 565.1321, 567.1294. Found: 565.1324, 567.1294.

2′-Deoxy-6-*N*-{2-[(1,3-diaza-3-methyl-2-oxophenoxazine-9-yl)oxy]-ethyl}-2-iodo-adenosine (2-Iodo-Adap) (12) Pale yellow powder (140 mg, 0.22 mmol, 73%). ^{1}H-NMR (500 MHz, DMSO-*d*6) δ: 9.93 (1H, br), 8.72 (1H, brs), 8.32 (1H, s), 7.51 (1H, br), 6.77 (1H, t, J = 8.0 Hz), 6.64 (1H, d, J = 8.0 Hz), 6.42 (1H, d, J = 8.0 Hz), 6.26 (1H, t, J = 6.5 Hz), 5.32 (1H, d, J = 4.1 Hz), 4.92 (1H, t, J = 5.4 Hz), 4.38 (1H, br), 4.04 (2H, br), 3.85 (3H, m), 3.61–3.56 (1H, m), 3.52–3.48 (1H, m), 3.19 (3H, s), 2.62 (1H, qui, J = 6.6 Hz), 2.29–2.26 (1H, m); ^{13}C-NMR (125 MHz, DMSO-*d*6) δ: 154.1, 154.0, 153.8, 148.9, 146.0, 142.2, 139.2, 129.1, 125.9, 122.9, 120.8, 119.5, 107.9, 106.8, 88.0, 83.5, 70.7, 67.3, 61.6, 48.6, 39.6, 36.6; IR (neat, cm^{-1}) 3257.9, 2916.7, 2354.2, 1669.0, 1627.5, 1559.3, 1505.9, 1476.2, 1280.6; HRMS (ESI-TOF) Calcd for $C_{23}H_{23}IN_8O_6Na$ $[M+Na]^+$: 657.0677. Found: 657.0663.

General Procedure of Synthesis of 3′-*O*-Acetyl Compound Under an argon atmosphere, DMTrCl (135 mg, 0.40 mmol) was added to a solution of compound **10**,[15] **11** or **12** (0.20 mmol) in dry pyridine (1.5 mL). After stirring for 90 min, acetic anhydride (56.7 μL, 0.60 mmol) was added to the reaction mixture. After stirring for 1 h, the solvent was removed under reduced pressure, then the residue was dissolved in 3% trichloroacetic acid in CH_2Cl_2 (10 mL). After stirring for 1 h, the solvent was removed under reduced pressure, then the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 100/1 to 50/1) to obtain the corresponding 3′-*O*-acetyl compound.

3′-*O*-Acetyl-2′-deoxy-6-*N*-{2-[(1,3-diaza-3-methyl-2-oxophenoxazine-9-yl)oxy]-ethyl}-2-phenoxyacetylamino-adenosine (13) Pale yellow powder (121 mg, 0.17 mmol, 87%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 13.63 (1H, br), 8.81 (1H, br), 8.74 (1H, br), 8.17 (1H, brs), 7.33 (2H, dd, J = 8.2, 7.8 Hz), 7.05–7.02 (3H, m), 6.78 (1H, t, J = 8.1 Hz), 6.69 (1H, br), 6.45 (1H, d, J = 7.9 Hz), 6.25–6.22 (2H, m), 5.56 (1H, d, J = 5.5 Hz), 4.76 (2H, br), 4.23 (1H, br), 4.20–4.12 (2H, m), 3.97 (1H, dd, J = 12.7, 1.2 Hz), 3.91 (1H, dd, J = 12.7, 1.2 Hz), 3.86 (2H, br), 3.19 (3H, s), 3.17–3.12 (1H, m), 2.39 (1H, dd, J = 13.9, 5.5 Hz), 2.12 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 170.5, 157.4, 155.1, 152.1, 140.0, 131.1, 129.9, 124.5, 122.3, 117.6, 115.1, 110.6, 87.4, 87.2, 76.4, 68.0, 63.2, 42.1, 37.9, 35.7, 21.2; IR (neat, cm^{-1}) 2948.6, 1674.2, 1628.7, 1564.5, 1512.9, 1474.6, 1428.4, 1382.9, 1279.5, 1233.7; HRMS (ESI-TOF) Calcd for $C_{33}H_{33}N_9O_9Na$ $[M+Na]^+$: 722.2293. Found: 722.2284.

3′-*O*-Acetyl-2′-deoxy-6-*N*-{2-[(1,3-diaza-3-methyl-2-oxophenoxazine-9-yl)oxy]-ethyl}-2-chloro-adenosine (14) Pale yellow powder (94 mg, 0.16 mmol, 80%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.88 (1H, br), 8.16 (1H, brs), 6.79 (1H, t, J = 8.2 Hz), 6.66 (1H, d, J = 8.0 Hz), 6.44 (1H, d, J = 8.1 Hz), 6.30 (1H, brs), 6.23 (1H, dd, J = 9.4, 5.3 Hz), 5.53 (1H, d, J = 5.6 Hz), 4.24 (1H, s), 4.19–4.10 (2H, m), 4.01 (1H, dd, J = 12.8, 1.4 Hz), 3.92–3.87 (3H, m), 3.19 (3H, s), 3.08 (1H, ddd, J = 13.9, 9.8, 5.7 Hz), 2.40 (1H, dd, J = 13.9, 5.4 Hz), 2.12 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 170.5, 155.3, 154.7, 151.6, 151.3, 148.6, 145.1, 140.4, 129.5, 124.7, 119.6, 119.3, 116.1, 110.6, 87.6, 87.5, 76.4, 72.4, 63.4, 42.0, 38.1, 35.8, 21.2; IR (neat, cm^{-1}) 3354.7, 2342.6, 1672.5, 1565.7, 1512.0, 1475.5, 1354.1, 1229.1; HRMS (ESI-TOF) Calcd for $C_{25}H_{25}ClN_8O_7Na$ $[M+Na]^+$: 607.1427, 609.1400. Found: 607.1421, 609.1398.

3′-*O*-Acetyl-2′-deoxy-6-*N*-{2-[(1,3-diaza-3-methyl-2-oxophenoxazine-9-yl)oxy]-ethyl}-2-iodo-adenosine (15) Pale yellow powder (108 mg, 0.16 mmol, 80%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 13.35 (1H, br), 8.83 (1H, br), 8.14 (1H, brs), 6.79 (1H, t, J = 8.1 Hz), 6.69 (1H, d, J = 7.9 Hz), 6.46 (1H, dd, J = 8.1, 1.2 Hz), 6.23–6.20 (2H, m), 5.53 (1H, d, J = 5.5 Hz), 4.24 (1H, br), 4.18–4.09 (2H, m), 4.00 (1H, dd, J = 10.9, 1.8 Hz), 3.91 (1H, dt, J = 12.0, 1.7 Hz), 3.83 (2H, br), 3.19 (3H, s), 3.10 (1H, ddd, J = 14.1, 9.7, 5.5 Hz), 2.38 (1H, dd, J = 14.0, 5.4 Hz), 2.12 (3H, s); ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 170.4, 154.2, 151.3, 150.6, 148.0, 145.4, 140.0, 129.8, 124.7, 120.5, 120.1, 110.9, 87.7, 87.5, 76.5, 73.1, 63.3, 42.3, 38.0, 35.6, 21.2; IR (neat, cm^{-1}) 2354.3, 1669.8, 1627.0, 1563.1, 1512.4, 1473.0, 1281.9; HRMS (ESI-TOF) Calcd for $C_{25}H_{25}IN_8O_7Na$ $[M+Na]^+$: 699.0783. Found: 699.0770.

General Procedure of Synthesis of 5′-Triphosphate Compound 2-Chloro-4*H*-1,3,2-benzodioxaphosphorin-4-one (19.2 mg, 0.10 mmol) in 1,4-dioxane (0.4 mL) was added to the solution of **13**, **14** or **15** (0.05 mmol) in pyridine/1,4-dioxane (50/50, 0.5 mL), then the mixture was stirred for 30 min at room temperature. The reaction mixture was treated with a 0.38 M solution of tributylammonium pyrophosphate in DMF (0.25 mL, 0.10 mmol) and tributylamine (57 μL, 0.24 mmol) at room temperature for 30 min. The reaction mixture was then treated with 1% iodine in pyridine–water (98/2, 2.0 mL) for 5 min, which was treated with a 5% $NaHSO_3$ solution (1.3 mL) for 30 min. The solvent was evaporated under reduced pressure, then the residue was dissolved in a 28% ammonium solution (15 mL). After stirring for 12 h, the solvent was removed under reduced pressure. The residue was washed with 75% ethanol in water containing 0.07 M NaCl (1.3 mL), and the precipitate was dissolved in water and purified by HPLC. (HPLC conditions: Column (Shiseido CAPCELL PAK C18-MG), Buffer (A: 20 mM TEAA, B: CH_3CN, B conc. 10 to 50%/20 min linear gradient.), Flow rate (1.0 mL/min), UV-detector (254 nm), Column oven (35°C)). After lyophilization of the fraction, the residue was dissolved in deionized water. The resulting solution was treated with Dowex Resins (Na^+ form) to convert the counter cation to a sodium ion, whose purity and structure were determined by NMR and HR-ESI-MS measurements. Their concentrations were determined by NMR measurements with dATP at a known concentration as the internal standard.

2-Amino-dAdapTP (1) A solution was obtained as a colorless solution (1.0 μmol, 2%). ^{1}H-NMR (500 MHz, D_2O) δ: 7.99 (1H, brs), 7.24 (1H, s), 6.84 (1H, dd, J = 8.6, 7.3 Hz), 6.77 (1H, d, J = 8.5 Hz), 6.37 (1H, d, J = 7.9 Hz), 6.21 (1H, dd, J = 7.2, 6.5 Hz), 4.51 (2H, br), 4.27 (1H, br), 4.26–4.22 (1H, m), 4.18–4.15 (1H, m), 4.00 (2H, br), 3.37 (3H, s), 2.75–2.70 (1H, m), 2.54–2.49 (1H, m); ^{31}P-NMR (162 MHz, D_2O) δ: −10.9, −11.5, −23.2; HRMS (ESI-TOF) Calcd for $C_{23}H_{27}N_9O_{15}P_3$ $[M-H]^-$: 762.0834. Found: 762.0832.

2-Chloro-dAdapTP (2) A solution was obtained as a colorless solution (15 μmol, 30%). ^{1}H-NMR (400 MHz, D_2O) δ: 8.44 (1H, s), 7.07 (1H, s), 6.92 (1H, t, J = 8.2 Hz), 6.70 (1H, d, J = 8.2 Hz), 6.48 (1H, br), 6.43 (1H, d, J = 8.2 Hz), 4.45 (2H, br), 4.37 (1H, br), 4.33–4.29 (1H, m), 4.24–4.21 (1H, m), 3.74 (2H, br), 3.26 (3H, s), 2.78 (1H, br), 2.72 (1H, br); ^{31}P-NMR

(202 MHz, D_2O) δ: −6.6, −11.2, −21.9; HRMS (ESI-TOF) Calcd for $C_{23}H_{25}ClN_8O_{15}P_3$ $[M-H]^-$: 781.0335, 783.0309. Found: 781.0349, 783.0336.

2-Iodo-dAdapTP (3) A solution was obtained as a colorless solution (0.5 μmol, 1%). ^{1}H-NMR (500 MHz, D_2O) δ: 8.29 (1H, brs), 7.22 (1H, s), 6.85 (1H, t, J = 8.3 Hz), 6.76 (1H, d, J = 8.3 Hz), 6.38 (1H, d, J = 7.9 Hz), 6.37 (1H, br), 4.51 (2H, br), 4.31 (1H, br), 4.29–4.25 (1H, m), 4.22–4.19 (1H, m), 3.93 (1H, br), 3.79 (1H, br), 3.34 (3H, s), 2.74 (1H, br), 2.63 (1H, br); ^{31}P-NMR (202 MHz, D_2O) δ: −6.4, −10.8, −20.7; HRMS (ESI-TOF) Calcd for $C_{23}H_{25}IN_8O_{15}P_3$ $[M-H]^-$: 872.9691. Found: 872.9704.

Single Nucleotide Primer Extension Reaction The mixture of the template DNA (**X**) (final conc. 1.0 μM, 25 mer, 5′ CGA CAG TTA X GGT TAG GGT TAT GCG; X = 8-oxodG, dG dA, dC or T) and primer (final conc. 1.0 μM, 15 mer of FAM-labeled primer, 5′ FAM-CGC ATA ACC CTA ACC) in the corresponding buffer (Klenow Fragment (exo$^-$) and Bsu DNA polymerase: 10 mM Tris–HCl, 50 mM NaCl, 10 mM $MgCl_2$ and 1 mM DTT, pH 7.9, New England Biolabs Japan, Inc.; KOD Dash: KOD Dash 10× PCR Buffer, Toyobo Co., Ltd.; Bst DNA Polymerase: 20 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton®X-100, pH 8.8, pH 8.8, New England Biolabs Japan, Inc.; Vent DNA Polymerase (exo$^-$): 20 mM Tris–HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton®X-100, pH 8.8, New England Biolabs Japan, Inc.; Taq DNA polymerase: 10 mM Tris–HCl, 50 mM KCl, 1.5 mM $MgCl_2$, pH 8.3, New England Biolabs Japan, Inc.) was annealed at 90°C for 5 min. The corresponding dAdapTP derivatives (final concentration of 50 μM in 10 μL of reaction volume), and DNA polymerase (1.0 unit) were added, and the mixture (10 μL) was incubated at 37°C for 10 min. The reaction was quenched with loading buffer and analyzed by 15% denaturing polyacrylamide gel electrophoresis. The bands were visualized using a fluorescence imager (LAS4000).

Steady-State Kinetics Study The mixture of the template DNA (**X**) (X = oxodG, dG, dA, dC or T) (1.0 μM) and FAM-labeled primer (1.0 μM) in the corresponding buffer was annealed at 90°C for 5 min, and the corresponding polymerase was added to the mixture at 37°C. The reaction was initiated by the addition of an identical volume of the corresponding dAdapTP derivatives solution (0.2–70 μM) in the same buffer at 37°C. The enzyme concentrations (0.01–0.1 unit/mL) and reaction times (2–50 min) were adjusted in the different dAdapTP derivative reactions to achieve a 1–20% incorporation, then the reactions were quenched with loading buffer and analyzed by 15% denaturing polyacrylamide gel electrophoresis. The bands were visualized and quantified using a fluorescence imager (LAS4000). The relative velocity v was calculated from the ratio of the extended product (I_{ext}) to the remaining primer (I_{pri}) as follows: $v = I_{ext}/I_{pri}\ t$, where t represents the reaction time, which was normalized to the lowest enzyme concentration used. The apparent V_{max} and K_M values were obtained from the Hanes–Woolf plots using the data points of at least five deoxyribonucleotide triphosphate (dNTP) concentrations. The average values were obtained in three different independent experiments, and in parentheses showing the standard deviations.

Acknowledgments This study was supported by a Grant-in-Aid for Scientific Research (B) (Grant Number 19H03351 for Y.T.), a Grant-in-Aid for Scientific Research (B) (18H02558 for S.S.), and a Challenging Research (Exploratory) (Grant Number 17K19494 for Y.T.) from the Japan Society for the Promotion of Science (JSPS), and Takeda Science Foundation.

Conflict of Interest The authors declare no conflict of interest.

References

1) Kasai H., Nishimura S., Kurokawa Y., Hayashi Y., *Carcinogenesis*, **8**, 1959–1961 (1987).
2) Shibutani S., Takeshita M., Grollman A. P., *Nature* (London), **349**, 431–434 (1991).
3) Kino K., Hirao-Suzuki M., Morikawa M., Sakaga A., Miyazawa H., *Genes and Environment*, **39**, 21 (2017).
4) Nakabeppu Y., Tsuchimoto D., Yamaguchi H., Sakumi K., *J. Neurosci. Res.*, **85**, 919–934 (2007).
5) Ohno M., Miura T., Furuichi M., Tominaga Y., Tsuchimoto D., Sakumi K., Nakabeppu Y., *Genome Res.*, **16**, 567–575 (2006).
6) Poulsen H. E., Nadal L. L., Broedbaek K., Nielsen P. E., Weimann A., *Biochim. Biophys. Acta*, **1840**, 801–808 (2014).
7) Lim K. S., Cui L., Taghizadeh K., Wishnok J. S., Chan W., DeMott M. S., Babu I. R., Tannenbaum S. R., Dedon P. C., *J. Am. Chem. Soc.*, **134**, 18053–18064 (2012).
8) Jiang D., Malla S., Fu Y., Choudhary D., Rusling J. F., *Anal. Chem.*, **89**, 12872–12879 (2017).
9) Schibel A. E. P., An N., Jin Q., Fleming A. M., Burrows C. J., White H. S., *J. Am. Chem. Soc.*, **132**, 17992–17995 (2010).
10) Hong I. S., Greenberg M. M., *Bioorg. Med. Chem. Lett.*, **25**, 4918–4921 (2015).
11) Bajacan J. E. V., Hong I. S., Penning T. W., Greenberg M. M., *Chem. Res. Toxicol.*, **27**, 1227–1235 (2014).
12) Tang F., Liu S., Li Q.-Y., Yuan J., Li L., Wang Y., Yuan B.-F., Feng Y.-Q., *Chem. Sci.*, **10**, 4272–4281 (2019).
13) Taniguchi Y., Kawaguchi R., Sasaki S., *J. Am. Chem. Soc.*, **133**, 7272–7275 (2011).
14) Taniguchi Y., Koga Y., Fukabori K., Kawaguchi R., Sasaki S., *Bioorg. Med. Chem. Lett.*, **22**, 543–546 (2012).
15) Taniguchi Y., Fukabori K., Kikukawa Y., Koga Y., Sasaki S., *Bioorg. Med. Chem.*, **22**, 1634–1641 (2014).
16) Koga Y., Taniguchi Y., Kikukawa Y., Sasaki S., *Bioorg. Med. Chem.*, **24**, 1308–1313 (2016).
17) Taniguchi Y., Kikukawa Y., Sasaki S., *Angew. Chem. Int. Ed.*, **54**, 5147–5151 (2015).
18) Matyašovský J., Perlíková P., Malnuit V., Pohl R., Hocek M., *Angew. Chem. Int. Ed.*, **55**, 15856–15859 (2016).
19) Chollet A., Kawashima E., *Nucleic Acids Res.*, **16**, 305–317 (1988).
20) Hentosh P., McCastlain J. C., Blakley R. L., *Biochemistry*, **30**, 547–554 (1991).
21) Pottabathini N., Bae S., Pradhan P., Hahn H.-G., Mah H., Lakshman M. K., *J. Org. Chem.*, **70**, 7188–7195 (2005).
22) Pair V., Purdy D. F., *Tetrahedron*, **41**, 365–382 (1991).