Supplementary Material (ESI) for Chemical Communications

Discrimination of N6-methyl adenine in a specific DNA sequence

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Supporting Information

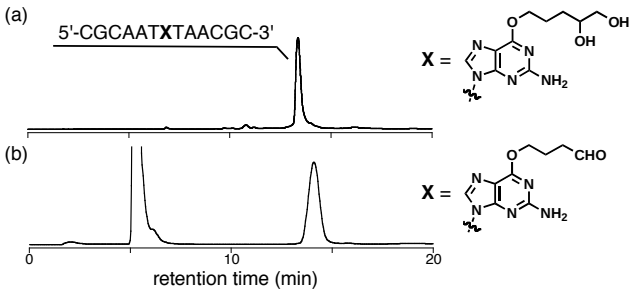


Figure S1. Synthesis of ^{f}G-containing DNA. A 13-mer oligonucleotide, 5′-CGCAATXTAACGC-3′, containing a vicinal diol moiety was converted to ^{f}G-containing DNA by treatment with sodium periodate ($NaIO_4$). HPLC charts before (a) and after (b) $NaIO_4$ treatment are shown. A peak eluted at 5 min is attributed from $NaIO_4$.

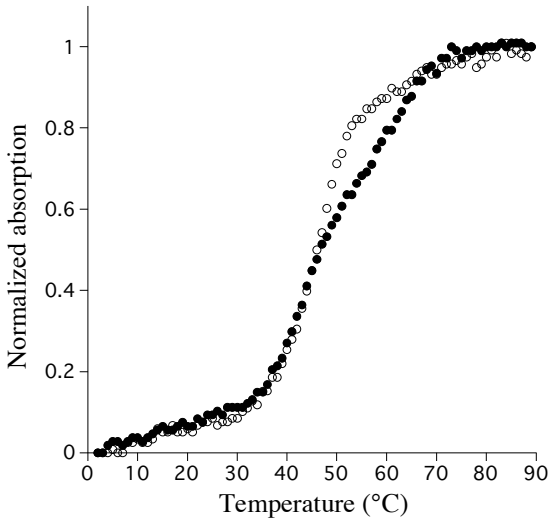


Figure S2. Thermal melting curves of duplex DNA (2.5 μM), 5′-(CGCAAT^{f}**G**TAACGC)-3′/ 3′-(GCGTTAC**A**TTGCG)-5′, in 50 mM Na cacodylate buffer (pH 6.0) containing 100 mM NaCl: before (open circles, T_m = 44 °C) and after incubation at 30 °C for 24 h (filled circles). The formation of ICL produced an additional melting transition at around 60 °C, indicating the ICL-induced duplex stabilization. Under the condition, the yield of ICL was about 27%.

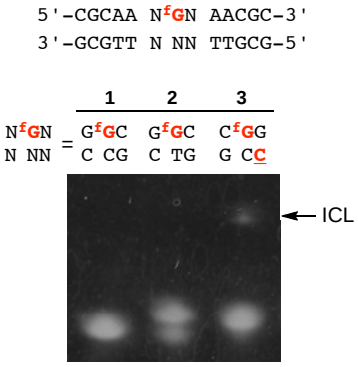


Figure S3. Electrophoretic mobility shift assay to monitor the ICL. DNA duplexes containing the sequences indicated above the lanes were incubated at 30 °C for 8 h in 50 mM Na cacodylate (pH 6.0) and 100 mM NaCl. Samples were subjected to electrophoresis through 20% polyacrylamide containing 7 M urea gel and stained with SYBR Gold nucleic acid gel stain.

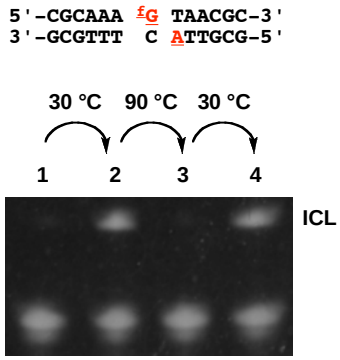


Figure S4. Reversibility of the ICL formation was judged by denaturing PAGE. Before incubation (lane 1); after incubation at 30 °C for 24 h (lane 2); subsequent heating at 90 °C for 5 min (lane 3); subsequent incubation at 30 °C for 24 h (lane 4). Samples were subjected to electrophoresis through 20% polyacrylamide containing 7 M urea gel and stained with SYBR Gold nucleic acid gel stain.

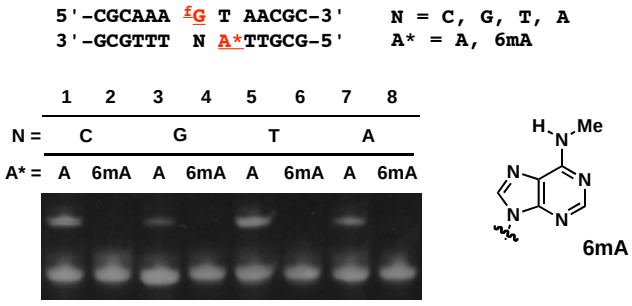


Figure S5. Discrimination of 6mA and the effects of the opposite base of [f]G. DNA duplexes containing a target nonmethylated or methylated adenine base were incubated at 30 °C for 24 h. Sample were subjected to electrophoresis through 20% polyacrylamide containing 7 M urea gel and stained with SYBR Gold nucleic acid gel stain. Relative ICL yields in Figure 3a were obtained by the calculation of (band intensity of ICL)/(total band intensity).

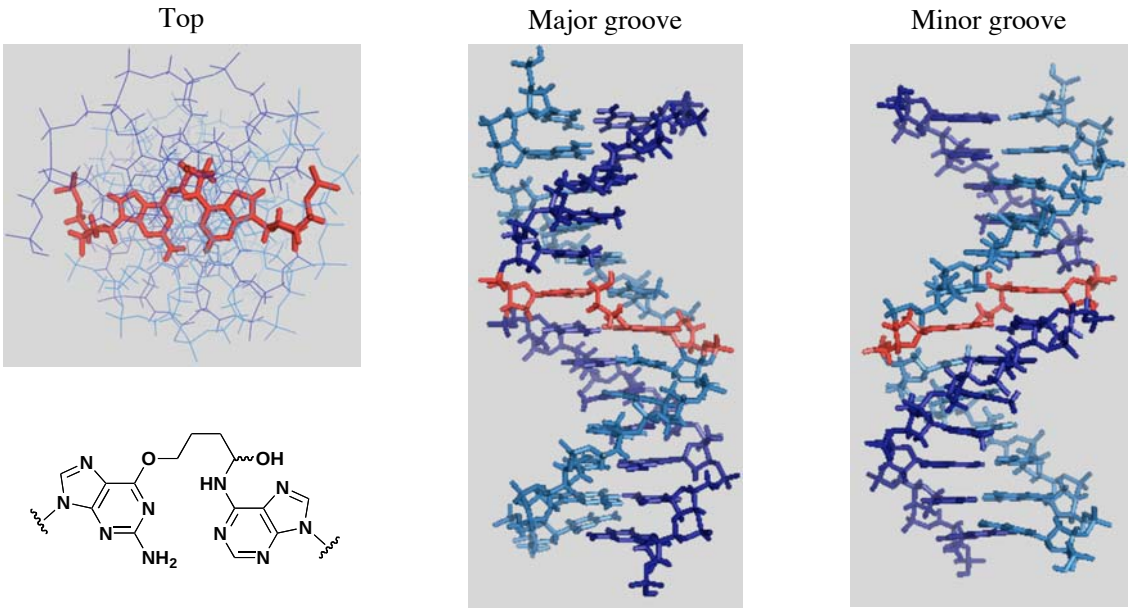


Figure S6. Molecular modeling of the conformations of the duplex containing a carbinolamine linkage, 5′-CGCAAT^{f}GTAACGC-3′/3′-GCGTTACATTGCG-5′. The model structure was optimized by use of the AMBER* force field in water with Macromodel version 9.1. The cross-linked structure of ^{f}G-A is represented in red color.

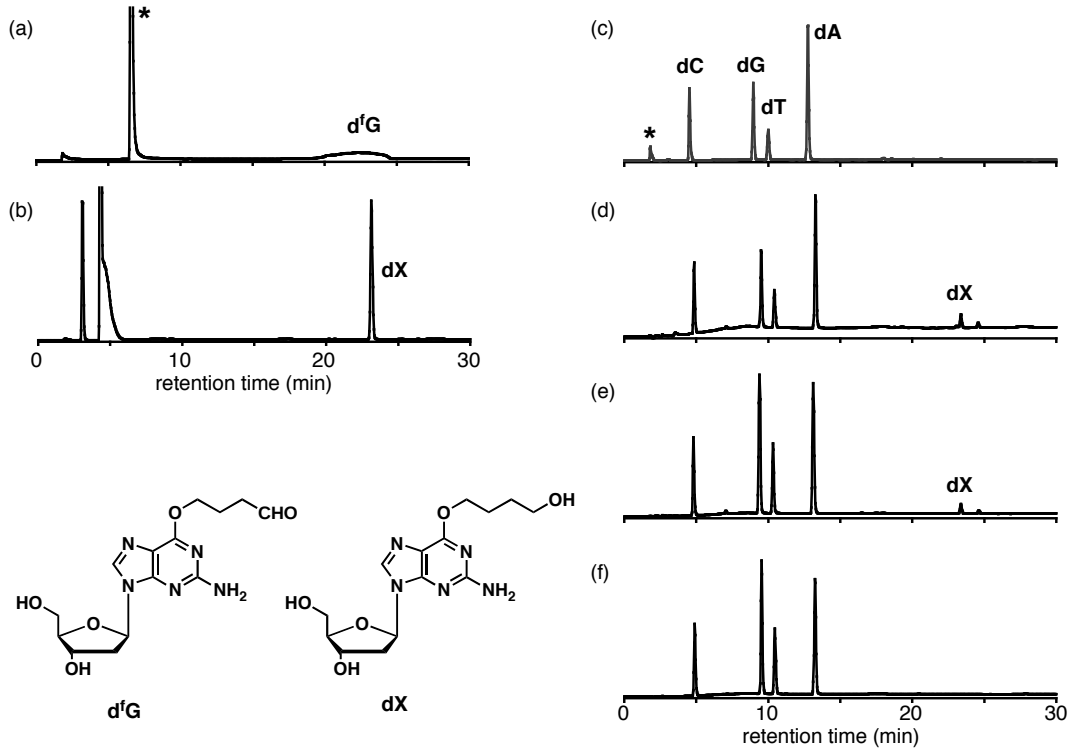
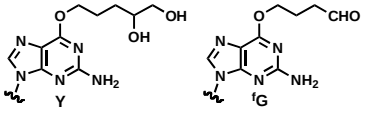


Figure S7. HPLC profiles of nucleosides mixtures obtained by enzymatic digestion of single stranded ^{f}G-containing DNA (5′-CGCAAT^{f}GTAACGC-3′), double stranded ^{f}G-containing DNA, (5′-CGCAAT^{f}GTAACGC-3′/3′-GCGTTACATTGCG-5′), and ICL. DNAs before and after $NaBH_4$ treatment were digested with a cocktail of three enzymes, calf intestinal alkaline phosphatase (Promega), Crotalus adamanteus venom phosphodiesterase I (USB), and Penicillium citrinum nuclease P1 (Roche). (a) $d^{f}G$. (b) Reduced-^{f}G (dX) obtained after $NaBH_4$ treatment of (a). (c) Nucleosides mixture obtained by enzymatic digestion of single stranded ^{f}G-containing DNA. (d) Single stranded ^{f}G-containing DNA after $NaBH_4$ treatment. (e) Double stranded ^{f}G-containing DNA after $NaBH_4$ treatment. (f) Isolated ICL after $NaBH_4$ treatment. A peak marked by asterisk is attributed from $NaIO_4$. Neither reduced-^{f}G (dX) nor new additional products was detected for the isolated ICL (f). Since ^{f}G nucleoside has a difficulty in the HPLC analysis probably due to the hydration of the aldehyde ((a) and (c)), these results suggested that ^{f}G in the ICL was resistant to $NaBH_4$ reduction. MALDI-tof MS measurements of the ICL also showed a recovery of the ^{f}G-containing DNA.

Table S1. Melting temperature (T_m/°C) of Y or ^{f}G-containing duplexes[a]

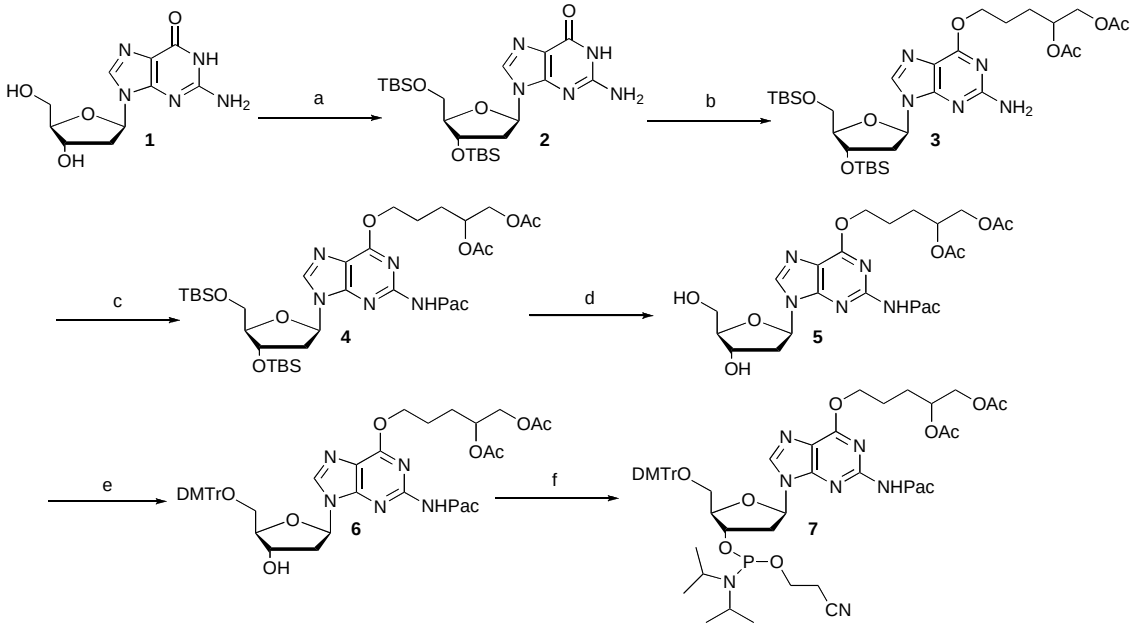
duplexes	T_m (°C)	duplexes	T_m (°C)
ODN-1 (Y) / ODN-2 (C)	45.3	ODN-1 (fG) / ODN-2 (C)	43.9
ODN-1 (Y) / ODN-2 (G)	42.1	ODN-1 (fG) / ODN-2 (G)	41.9
ODN-1 (Y) / ODN-2 (T)	43.1	ODN-1 (fG) / ODN-2 (T)	43.1
ODN-1 (Y) / ODN-2 (A)	43.6	ODN-1 (fG) / ODN-2 (A)	43.2

ODN-1(X):5'-CGCAA TXT AACGC-3'
ODN-2(N):3'-GCGTT ANA TTGCG-5'
X = Y,fG
N = C,G,T,A



[a]Thermal melting curves were measured for duplexes (2.5 μM) in 50 mM sodium cacodylate buffer (pH 6.0) containing 100 mM NaCl. The absorbance of the sample was monitored at 260 nm from 2 to 80 ˚C with a heating rate of 1 ˚C/min. T_m values (˚C) were calculated by median method.

Scheme 1[a]



[a]Reagent and condition: (a) TBSCl, imidazole, DMF, 73%; (b) 1, 2, 5-pentanetriol 1,2-diacetate, DEAD, PPh_3, 1,4-dioxane, 65%; (c) Phenoxyacetyl chloride, pyridine, 0 °C, 81%; (d) TBAF, AcOH, THF, 93%; (e) DMTrCl, DMAP, NEt_3, pyridine, 60 °C, 68%; (f) $(i\mathrm{Pr}_2\mathrm{N})_2\mathrm{PO}(\mathrm{CH}_2)_2\mathrm{CN}$, 1*H*-tetrazole, acetonitrile.

General. Reagent and solvents were purchased from standard suppliers and used without further purification. Reactions were monitored with TLC plates precoated with Merck silica gel 60 F_{254}. Spots were visualized with UV light or anisaldehyde. Wakogel C-200 was used for silica gel flash chromatography. ^{1}H-NMR spectra were measured with JEOL JNM-LA-400 and JEOL LA600. Coupling constant (*J* values) are represented in hertz. The chemical shifts are expressed in ppm relative to residual solvent as an internal standard. ^{13}C-NMR spectra were measured with JNM-ESC400 and JEOL LA600. ESI mass spectra were recorded on a JEOL AccuTOF JMS-T 100N mass spectrometer. DNA oligomers (ODNs) containing 2-aminopurine were purchased from JBioS. The reagents for DNA synthesis including *N*6-methyl-adenine phosphoramidite were purchased from Glen research. ODNs were synthesized by a conventional phosphoramidite method by using an Applied Biosystems 3400 DNA/RNA synthesizer. Masses of ODNs were determined with a MALDI-TOFF mass (Perseptive Voyager Elite, acceleration voltage 21 kV, negative mode) with 2',3',4'-trihydroxyacetophenone as a matrix, using dT_8 ($[M–H]^-$ 2370.61), dT_{17} ($[M–H]^-$ 5108.37) and dT_{27} ($[M–H]^-$ 8150.15) as internal standards. Reversed-phase HPLC was performed on COSMOSIL 5C_{18}-MS-II columns (4.6 × 150 mm, 10 × 150 mm) with a

Gilson chromatograph using a UV detector at 260 nm.

O^6-(4,5-diacetoxypentyl)-3',5'-*O*-di-*tert*-butyldimethylsilyl-2'-deoxyguanosine (3)

To a solution of 3',5'-*O*-di-*tert*-butyldimethylsilyl-2'-deoxyguanosine (495 mg, 1 mmol), triphenyl phosphine (262 mg, 1 mmol), and 1,2,5-pentanetriol 1,2-diacetate (204 mg, 1 mmol) in 1,4-dioxane (10 mL) at 60 °C was slowly added diethyl azodicarboxylate (0.46 mL, 1 mmol) via syringe. After stirring for 2 h, the solvent was concentrated to dryness, the reaction mixture was dissolved in diethyl ether (5 mL) and left to stand at –20 °C overnight. The precipitated white solid was removed by filtration, the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (ethyl acetate : hexane = 1 : 1) to give O^6-(4,5-diacetoxypentyl)-3',5'-*O*-di-*tert*-butyldimethylsilyl-2'-deoxyguanosine (444 mg, 65%)as a colorless oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.87 (s, 1 H), 6.29 (t, 1 H, J = 6.6 Hz), 5.15 (dq, 1 H, J = 6.6, 3.3 Hz), 4.83 (s, 2 H), 4.56–4.54 (m, 1 H), 4.51–4.44 (m, 2 H), 4.23 (dd, 1 H, J = 12.0, 3.3 Hz), 4.02 (dd, 1 H, J = 12.0, 6.6 Hz), 3.96–3.94 (m, 1 H), 3.78 (dd, 1 H, J = 10.9, 4.4 Hz), 3.73 (dd, 1 H, J = 10.9, 3.1 Hz), 2.54 (ddd, 1 H, J = 13.0, 7.14, 5.9 Hz), 2.34–2.30 (m, 1 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 1.92–1.74 (m, 4 H), 0.88 (s, 18 H), 0.07 (s, 6 H), 0.05 (s, 6 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.2, 170.1, 160.7, 159.1, 153.2, 136.9, 115.1, 87.1, 83.1, 71.5, 70.6, 65.3, 64.6, 62.4, 40.6, 26.8, 25.5, 25.3, 24.3, 20.6, 20.3, 18.0, 17.5, –5.0, –5.1, –5.7, –5.8; HRMS (ESIMS, positive-ion mode, MeOH): calcd. for $C_{31}H_{55}N_5O_8Si_2$ $[M + Na]^+$ 704.3486; found 704.3484.

O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-3',5'-*O*-di-*tert*-butyldimethylsilyl-2'-deoxyguanosine (4)

To a solution of O^6-(4,5-diacetoxypentyl)-3',5'-*O*-di-*tert*-butyldimethylsilyl-2'-deoxyguanosine (1.2 g, 1.8 mmol) in pyridine (15 mL) at 0 °C was slowly added phenoxyacetyl chloride (0.74 mL, 5.4 mmol) dropwise. After stirring for 2 h, the reaction mixture was quenched by adding saturated $NaHCO_3$, extracted with ethyl acetate, washed with brine. The organic layer was dried over Na_2SO_4 and the solvent was evaporated. The crude residue was purified by silica gel column chromatography (ethyl acetate : hexane = 1 : 1) to give O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-3',5'-*O*-di-*tert*-butyldimethylsilyl-2'-deoxyguanosine (1.2 g, 81%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 8.16 (s, 1 H), 7.33 (t, 2 H, J = 7.7 Hz), 7.03 (t, 1 H, J = 7.3 Hz)

7.00 (d, 2 H, *J* = 8.4 Hz), 6.42 (t, 1 H, *J* = 6.4 Hz), 5.14 (dq, 1 H, *J* = 6.6, 3.3 Hz), 4.59–4.58 (m, 3 H), 4.23 (dd, 1H, *J* = 11.7 ,3.3 Hz), 4.03 (dd, 1 H, *J* = 11.7, 6.2 Hz), 3.98 (q, 1 H, *J* =3.3 Hz), 3.85 (dd, 1 H, *J* = 11.2, 4.02 Hz), 3.76 (dd, 1 H, *J* =11.2, 3.3 Hz), 2.62–2.58 (m, 1 H), 2.44–2.40 (m, 1 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 1.98–1.87 (m, 2 H), 1.82–1.78 (m, 2 H), 0.88 (s, 18 H), 0.08 (s, 6 H), 0.06 (s, 6 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.3, 170.2, 166.1, 160.7, 157.2, 152.3, 150.9, 140.2, 129.5, 122.1, 118.7, 114.8, 87.8, 84.1, 71.7, 70.9, 68.0, 66.7, 64.7, 62.6, 41.0, 27.1, 25.8, 25.6, 24.5, 20.7, 20.4, 18.2, 17.8, -4.8, -4.9, -5.5, -5.6; HRMS (ESIMS, positive-ion mode, MeOH): calcd. for $C_{39}H_{61}N_5O_{10}Si_2$ $[M + Na]^+$ 838.3854; found 838.3853.

O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-2'-deoxyguanosine (5)

A mixture of 1.0 M solution of tetra-*n*-butylammonium fluoride in THF (4.4 mL, 4.4 mmol), acetic acid (0.25 mL, 4.4 mmol) and O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-3',5'-*O*-di-*tert*-butyldimethylsilyl-2'-deoxyguanosine (1.2 g, 1.5 mmol) in THF (8 mL) was stirred for 36 h at room temperature. The reaction mixture was evaporated, and the crude residue was purified by silica gel column chromatography (chloroform : methanol = 30 : 1) to give O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-2'-deoxyguanosine (800 mg, 93%) as a white solid: ^{1}H NMR ($CDCl_3$, 600 MHz); δ 8.95 (s, 1 H), 7.98 (s, 1 H), 7.33 (t, 2 H, *J* = 7.8 Hz), 7.03 (t, 1 H, *J* = 7.3 Hz), 7.01 (d, 2 H, *J* = 8.0 Hz), 6.30 (t, 1 H, *J* = 6.9 Hz), 5.15-5.11 (m, 1 H), 4.96 (s, 1 H), 4.67 (s, 2 H), 4.56 (t, 2 H, *J* = 6.2 Hz), 4.23 (dd, 1 H, *J* = 5.9, 3.1 Hz), 4.14 (d, 1 H, *J* = 2.1 Hz), 4.03 (dd, 1 H, *J* = 11.9, 6.6 Hz), 3.95 (dd, 1 H, *J* = 12.6, 2.0 Hz), 3.84 (dd, 1 H, *J* = 12.4, 2.2 Hz), 3.04-2.99 (m, 1 H), 2.40-2.36 (m, 1 H) 2.04 (s, 3 H), 2.02 (s, 3 H), 1.97-1.86 (m, 2 H), 1.82-1.77 (m, 2 H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 170.7, 170.5, 166.3, 161.2, 157.1, 151.8, 150.7, 141.6, 129.8, 122.4, 119.8, 115.0, 88.5, 86.4, 72.2, 71.0, 67.8, 67.1, 65.0, 62.7, 40.4, 27.2, 24.6, 20.9, 20.6; HRMS (ESIMS, positive-ion mode, MeOH): calcd. for $C_{27}H_{33}N_5O_{10}$ $[M + Na]^+$ 610.2125; found 610.2124.

O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (6)

To a solution of O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-2'-deoxyguanosine (727 mg, 1.2 mmol) in pyridine (12 mL) was added 4,4'-dimethoxytrityl chloride (508 mg, 1.5 mmol), *N*,*N*-dimethyl-4-aminopyridine (18 mg, 0.15 mmol) and triethylamine (0.42

mL, 3 mmol), and the mixture was stirred for 6 h at 60 °C. The reaction mixture was concentrated and purified by silica gel column chromatography (chloroform : methanol : triethylamine = 40 : 1 : 0.1) to give O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (751 mg, 68%) as a white foam: ^{1}H NMR ($CDCl_3$, 400 MHz); δ 8.79 (s, 1 H), 8.00 (s, 1 H), 7.38–7.15 (m, 11 H), 7.04 (t, 1 H, *J* = 7.3 Hz), 6.98 (d, 2 H, *J* = 8.5 Hz), 6.76 (dd, 4H, *J* = 8.8, 1.2 Hz), 6.61 (t, 1 H, *J* = 5.6 Hz), 5.15 (dq, 1 H, *J* = 6.4, 3.6 Hz), 4.80–4.78 (m, 1 H), 4.63 (s, 2 H), 4.57 (t, 2 H, *J* = 6.3 Hz), 4.23 (dd, 1 H, *J* = 12.0, 3.4 Hz), 4.18 (br, 1 H), 4.03 (dd, 1 H, *J* = 12.0, 6.3 Hz), 3.73 (s, 6 H), 3.43 (dd, 1 H, *J* = 10.2, 4.6 Hz), 3.32 (dd, 1 H, *J* = 10.2, 4.2 Hz), 2.76–2.70 (m, 1 H), 2.64–2.59 (m, 1 H), 2.05 (s, 3 H), 2.03 (s, 3 H), 1.99–1.87 (m, 2 H), 1.83–1.77 (m, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) 171.1, 168.6, 160.7, 158.6, 158.5, 158.0, 152.0, 151.6, 145.0, 141.6, 135.8, 135.7, 130.0, 129.8, 129.3, 127.9, 127.3, 126.4, 121.4, 118.0, 114.5, 112.6, 112.5, 87.0, 86.1, 85.1, 71.2, 67.7, 66.7, 64.8, 64.2, 54.4, 39.2, 26.8, 24.4, 19.6, 19.3; HRMS (ESI, positive-ion mode, MeOH): calcd. for $C_{48}H_{51}N_5O_{12}$ $[M + Na]^+$ 912.3431; found 912.3432.

O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-3'-*O*-[(*N*,*N*-diisopropylamino)(β-cyanoethoxy)phosphinyl]-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (7)

A mixture of O^6-(4,5-diacetoxypentyl)-N^2-phenoxyacetyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyguanosine (53 mg, 60 μmol) and 1*H*-tetrazole (6.3 mg, 90 μmol) was dried twice by evaporating off acetonitrile and dissolved in acetonitrile (600 μL). 2-Cyanoethyl *N*,*N*,*N*',*N*'-tetraisopropyl-diphosphoramidite (28.5 μL, 90 μmol) was added to the resulting solution. After stirring for 1.5 h, the reaction mixture was filtrated and evaporated to give the crude residue. The crude product was used for automated DNA synthesizer without further purification.

Synthesis of ^{f}G-Containing ODN.

The synthesis of ^{f}G-containing ODNs were accomplished by a postsynthetic modification of ODN containing O^6-(4,5-dihydroxypentyl)guanine with $NaIO_4$. ODNs containing O^6-(4,5-dihydroxypentyl)guanosine were synthesized on a DNA synthesizer. After the automated synthesis, the ODNs were cleaved from the resign and deprotected by treating with concentrated ammonia at 55 °C for 8 h. The ODNs were purified by reverse-phase HPLC on a COSMOSIL 5C_{18}-MS-II column (10 × 150 mm) eluting with

4-25% (30 min) acetonitrile in 0.1 M triethylammonium acetate (TEAA), pH 7.0, at a flow rate 3.0 mL/min. The resulting ODNs containing vicinal diol moieties were treated with excess $NaIO_4$ for 5 min. The ^{f}G-containing ODNs were desalted by gel filtration using G-10 columns (GE Healthcare) and identified by MALDI-tof-MS.

DNA cross-linking reaction between ^{f}G-containing ODNs and complementary strands.

^{f}G-containing ODN precursors possessing vicinal diol moieties and their complementary strands (7.5 μM each) were mixed in 50 mM Na cacodylate (pH 6.0) and 100 mM NaCl containing 150 μM $NaIO_4$. The solution was incubated at 30 °C for indicated times, typically 24 h for adenine. The ICL was monitored by reverse-phase HPLC on a COSMOSIL $5C_{18}$-MS-II column (4.6 × 150 mm) eluting with 4-19% (30 min) acetonitrile in 0.1 M triethylammonium acetate (TEAA), pH 7.0, at a flow rate 1.0 mL/min at 40 °C. For PAGE experiment, the samples (5 μL) were mixed with loading buffer (2 μL) and were subjected to electrophoresis through 20% polyacrylamide/7 M urea gel at 200 V for 1 h. The gels were stained for 10 min with SYBR Gold nucleic acid gel stain (invitrogen). Images of the gels were collected by using a Safe Imager Transluminator (invitrogen).

Thermal denaturing profiles.

All melting temperatures (T_m) of the ODNs (2.5 μM, final duplex concentration) were taken in 50 mM Na cacodylate (pH 6.0) containing 100 mM NaCl. Absorbance vs temperature profiles were measured at 260 nm using a Shimadzu UV-2550 spectrometer equipped with a temperature controller using a 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 2 to 90 °C with a heating rate of 1 °C/min.