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Synthesis of 8-halogenated-7-deaza-2′-deoxyguanosine as an 8-oxo-2′-deoxyguanosine analogue and evaluation of its base pairing properties

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ABSTRACT

8-Halogenated-7-deaza-2′-deoxyguanosines (8-halo-7-deaza-dG) were designed to structurally mimic 8-oxo-2′-deoxyguanosine (8-oxo-dG), which is representative of an oxidized nucleoside. It has been shown by NMR that the conformation around the *N*-glycosidic bond of (8-halo-7-deaza-dG) is preferably *syn*, similar to 8-oxo-dG. The base pairing properties of 8-halo-7-deaza-dG were studied by measuring the thermal denaturation temperature of the duplexes, showing that their base pair with dC is destabilized compared with natural dG. These results also support their preference for *syn* conformation. Unlike 8-oxo-dG, 8-halo-7-deaza-dG did not form a stable base pair with dA, most likely due to the lack of N7-H hydrogen bonding with dA. In conclusion, the newly-designed 8-halo-7-deaza-dG analogs resemble 8-oxo-dG in its shape and preference for *syn* conformation, but they do not form Hoogsteen base pair with the opposing dA.

1. Introduction

Cellular DNA is continuously exposed to various types of damage, e.g., irradiation, oxidation, etc.[1–4] 8-Oxo-2′-deoxyguanosine (8-oxo-dG) is representative of a damaged nucleoside; it is formed by the reaction of 2′-deoxyguanosine (dG) with reactive oxygen species (ROS). Abundant 8-oxo-dG has been linked to aging and diseases, such as cancer.[5–7] 8-Oxo-dG forms stable base pairs with 2′-deoxycytidine (dC) and 2′-deoxyadenosine (dA), while dG forms a stable base pair with only dC.[8–13] 8-Oxo-dG and dC form a standard Watson–Crick base pair (Fig. 1A). Because 8-oxo-dG exists in the 8-keto form preferentially at physiological pH,[14] it forms a Hoogsteen base pair with dA (Fig. 1B). 8-Oxo-dG adopts an *anti* conformation in its Watson–Crick base pair, while occupying a *syn* conformation in its Hoogsteen base pair. Because of the steric effect of the 8-oxygen atom, the *anti* conformation of 8-oxo-dG is less stable than that of dG, decreasing the thermal stability of the 8-oxo-dG:dC base pair. In contrast, as the *syn* conformation of 8-oxo-dG is relatively stable, and the 8-oxo-dG:dA Hoogsteen base pair displays comparable thermal stability. Due to the base pairing properties of 8-oxo-dG, DNA polymerases incorporate 8-oxo-dGTP to opposing dA and dATP to opposing 8-oxo-dG, causing AT to CG and GC to TA transversion mutations.[15,16]

Specific repair enzymes suppress the promutagenicity and genotoxicity of 8-oxo-dG by excising 8-oxo-dG or dA from the 8-oxo-dG:dC or 8-oxo-dG:dA pairs, respectively. In a repair system,

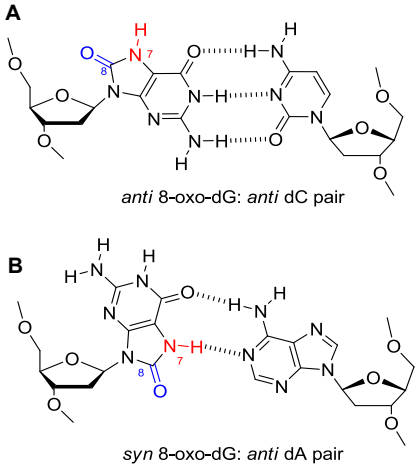

Fig. 1. Base paired structures of (A) 8-oxo-dG:*anti*-dC and (B) 8-oxo-dG:*syn*-dA.

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8-oxo-dGTP is enzymatically hydrolyzed to 8-oxo-dGMP to avoid its incorporation into DNA.[17,18] Preference in dCTP or dATP incorporation to opposing 8-oxo-dG at a replication step depends on the polymerase, most likely because *syn* or *anti* conformation in the base-paring with 8-oxo-dG is determined at the enzyme active site. To investigate the mechanism of DNA polymerases and repair enzymes, Hamm's group investigated effects of 7-deaza-dG, 9-deaza-dG, 8-thio-dG, and 8-halogenated-dG as mimics for 8-oxo-dG on their efficiency[19–24] It was revealed that Fpg (formamidopyrimidine [fapy]-DNA glycosylase) repairs 7-deaza-dG approximately equivalent to 8-oxo-dG.[24] However, 7-deaza-dG is an inferior substrate to 8-oxo-dG for hOGG1. Seela's group has reported 7-halogenated-7-deaza-dG and 8-methyl-7-deaza-dG derivatives. The former is not a mimic for the structural shape of 8-oxo-dG,[25] nor is the latter, in the sense that it adopted the *anti* conformation in X-ray and NMR studies.[26] However the octamer duplex constructed of continuing 8-methyl-7-deaza-dG and dC showed Z-DNA structure, suggesting that 8-methyl-7-deaza-dG might adopt a *syn* conformation in oligonucleotide.[27] It is clear from these studies that the 7-NH of 8-oxo-dG mimics is necessary for both hydrogen bonding and shape complementarity in its pairing with dA and that the 8-hetero atom is necessary for a preference of the *syn*- over the *anti*-conformation. To better imitate 8-oxo-dG by taking into account both the 7-NH and 8-oxygen atom, we designed 8-halogenated-7-deaza-dG derivatives (Fig. 2). It was expected that the 8-halogen atom might mimic the *C*8-oxygen in terms of the dipole interaction, that is, important for lesion discrimination[28–30] and that the 7-CH might resemble the shape of the 7-NH in 8-oxo-dG. Here, we describe the synthesis of 8-halogenated-7-deaza-2′-deoxyguanosines, the investigation of their conformation in solution, and the evaluation of their base pairing properties.

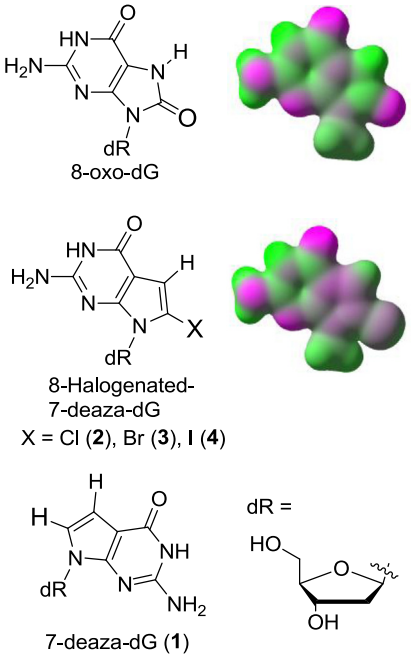
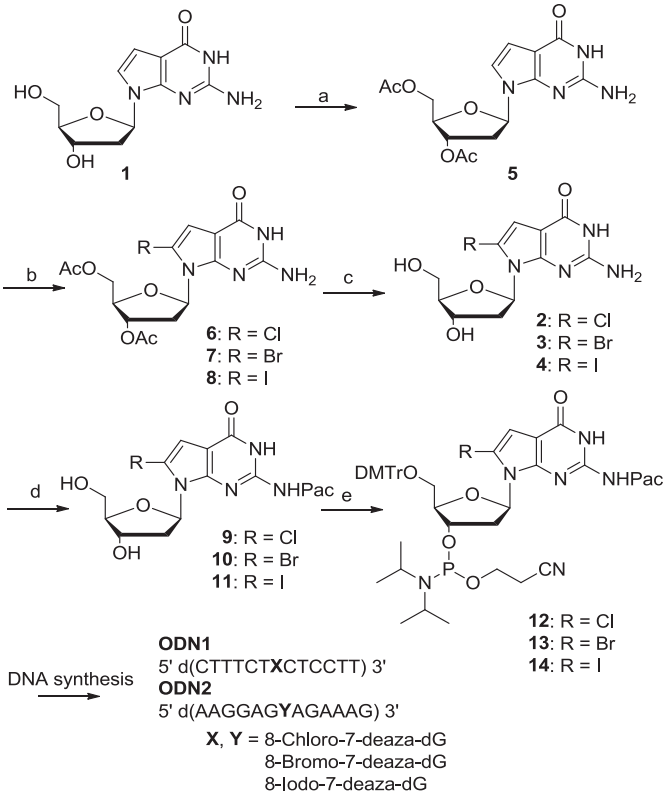


Fig. 2. Structures of oxo-dG and 8-halogenated-7-deaza-dG derivatives.

2. Results and discussion

The syntheses of the 8-halogenated-7-deaza-dG derivatives are presented in Scheme 1. The 5′- and 3′-hydroxyl groups of 7-deaza-dG (**1**) were acetylated using acetic anhydride in pyridine.[31] The regioselective halogenation reactions at the 8-position of the acetylated 7-deaza-guanine (**5**) were performed using *N*-halogenated-succinimides in DMF to furnish the corresponding halogenated nucleoside derivatives.[32,33] The use of *N*-chlorosuccinimide (NCS) produced 8-chlorinated 7-deaza-dG (**6**) in a good yield. 8-Brominated 7-deaza-dG (**7**) was obtained in a satisfactory yield using *N*-bromosuccinimide (NBS), however, iodination with *N*-iodosuccinimide (NIS) gave 8-iodinated 7-deaza-dG (**8**) in low yield due to the formation of biproducts. A number of synthetic attempts in which we changed reaction times, temperatures, reagent stoichiometry, etc. were unsuccessful. After removing the acetyl groups in 7 N ammonium in methanol at room temperature, the halogenated positions of the resulting diol compounds (**2**, **3**, and **4**) were confirmed by NMR spectroscopy, including 2D-COSY, NOESY, HMBC, and HMQC spectra in DMSO-d_6. Typically, the ^{1}H and ^{13}C signals of the 8C–H of the deazaguanine base disappeared and showed upfield shifts, respectively, due to the halogenation reactions. The hydroxyl groups were transiently protected with TMSCl and the 2-amino group was efficiently protected using phenoxyacetic anhydride to produce 2NH-Pac-protected diol compounds (**9**, **10**, and **11**) in good yields. These diol compounds were converted into the corresponding phosphoramidite derivatives (**12**, **13**, and **14**) using conventional methods, and incorporated into the middle position of the 13-*mer*-oligonucleotides (**ODN1** and **ODN2**, where X or Y were Cl-deaza-dG (**2**), Br-deaza-dG (**3**) or I-deaza-dG (**4**)). The synthesized ODNs were cleaved from the CPG and the Pac groups were removed with 50 mM K_2CO_3 in MeOH at room temperature. Subsequently, the products were purified using reverse-phase HPLC and carefully treated with 1–5% acetic acid to remove the DMTr protective group while retaining the halogen atom at the 8-position. Finally, the structure and purity of all synthesized ODNs were confirmed by MALDI-TOF mass measurements.

Because the conformation around the *N*-glycosidic bond of the



Scheme 1. Reagents and conditions: (a) acetic anhydride, pyridine, 88%. (b) NRS (R=Cl, Br, and I), DMF, 86%, 51%, and 21%, respectively. (c) 7 N ammonia in MeOH, 60–70%. (d) TMSCl, pyridine, followed by phenoxyacetic anhydride, pyridine, 70–80%. (e) (1) DMTrCl, pyridine. (2) 2-cyanoethyl-*N*,*N*′-diisopropyl-chlorophosphoroamidite, DIPEA, CH_2Cl_2, 39–61% over two steps.

8-halogenated-deaza-dG nucleosides are reflected in the chemical shifts if their ^{1}H and ^{13}C NMR spectra,[21] the NMR spectra of dG, oxo-dG, deaza-dG (**1**), Cl-deaza-dG (**2**), Br-deaza-dG (**3**), and I-deaza-dG (**4**) were measured in DMSO-d_6. The selected chemical shifts are summarized in Table 1. The differences in the chemical shifts of the signals corresponding to those of dG are summarized in Fig. 3A. 8-Oxo-dG showed medium and large upfield shifts of the C1′ and C2′ signals, respectively, and a small downfield shift of the H2′ signals.[21,34] These changes were interpreted as evidence of its *syn* conformation. In contrast, 7-deaza-dG (**1**) displayed the same chemical shifts as dG, except for the C8 signal, when its chemical shifts were calculated by assuming 100% *anti*-conformer. Remarkably, the large upfield shifts of the C2′ were commonly observed for all 8-halogenated-deaza-dG. The downfield shifts of the H2′ signal increased in the order of Cl, Br, and I, most likely reflecting the electron withdrawing nature of the C8-halogen atom. The signals of H1′ and H2′ did not show large chemical shift changes.

Table 1
Selected chemical shift (ppm) of corresponding diol compounds[a]

Base	dG	8-Oxo-dG	deazadG	Cl-deazadG	Br-deazadG	I-deazadG
C1′	82.5	81.2	82.2	83.0	84.2	86.8
C2′	39.5	35.7	39.2	37.2	37.3	37.5
C3	70.7	71.4	71.0	71.0	71.1	71.3
C4′	87.5	87.3	86.9	87.2	87.3	87.4
C5′	61.7	62.4	62.0	62.1	62.2	62.3
H2″	2.49	2.96	2.30	2.99	3.04	3.12
H2′	2.18	1.91	2.05	2.03	2.02	1.97
H1′	6.10	6.02	6.29	6.31	6.28	6.18
C7	—[b]	—[b]	102.2	101.4	105.4	112.9
C8	135.2	151.6	116.7	115.0	101.9	71.3
H7	—[b]	—[b]	6.25	6.32	6.41	6.54
H8	7.90	—[b]	6.89	—[b]	—[b]	—[b]

[a] ^{1}H and ^{13}C NMR shifts were recorded using 0.04 M solutions of the corresponding diol compounds in DMSO-d_6.
[b] Lack of the corresponding atom.

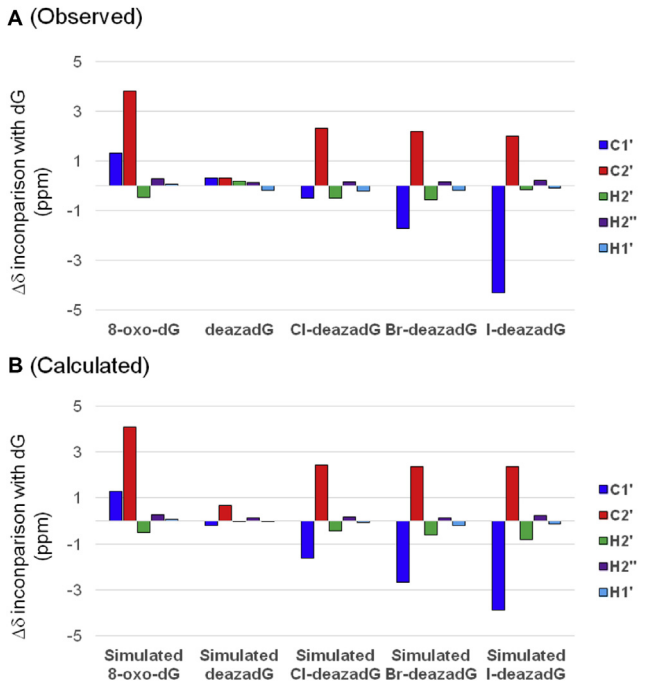


Fig. 3. The differences in the chemical shifts between dG and 8-oxo-dG, 7-deaza-dG, and 8-halogenated-7-deaza-dG derivatives. NMR data are shown in Table 1, and the simulated data were calculated using HF/6–311G(d,p)_DMSO or HF/LanL2DZ/6–311G(d,p)_DMSO, which were modified with the optimized *anti*- or *syn*-dG structure using B3LYP/6–31G(d)_DMSO. In order to match to the observed values, the simulated chemical shifts were calculated by the weighted average assuming 75% *syn*-conformer and 25% *anti*-conformer except for 7-deaza-dG, which was calculated assuming 100% *anti*-conformer.

To obtain insight into preference in *syn*- or *anti* conformation around the *N*-glycosidic bond of 8-halogenated-7-deaza-dG, each conformer was estimated by quantum mechanical calculation in vacuo. The reported crystal structures were utilized as the initial structures.[35] The calculated energies are summarized in Table 2, and the optimized structures are displayed in Fig. 4. For dG and deaza-dG, the relative energies indicated that the *anti* conformation is favored (ΔE are −3.09 kcal/mol and −3.48 kcal/mol, respectively). In contrast, the *anti* and *syn* conformations of oxo-dG are relatively isoenergetic (ΔE is 0.06 kcal/mol). Cl-deaza-dG slightly prefers the *anti* conformation, albeit with a small energetic difference. The *anti* conformation is favorable for Br-deaza-dG (the relative energies of Cl-deaza-dG and Br-deaza-dG are −0.54 and −1.75 kcal/mol, respectively). An intramolecular interaction between the bromide atom at the 8-position and the oxygen atom on the sugar may contribute to the stabilization of *anti* Br-deaza-dG, because the distance of these atoms is within the sum of their van der Waals radii (3.29–3.32 Å).[36] Notably, I-deaza-dG prefers the *syn* conformation (ΔE is 0.94 kcal/mol). These quantum chemical calculations indicate that the 8-halogenated-7-deaza-dG derivatives are more likely to prefer the *syn* conformation than is the non-substituted deaza-dG. The chemical shifts were predicted by HF/6–311G(d,p)_DMSO or HF/LanL2DZ/6–311G(d,p)_DMSO using the structures derived from

Table 2
DFT energies for the 8-halogenated-7-deaza-dG derivatives in vacuo[a]

Nucleoside	*E* (a.u.)	Δ*E* (kcal/mol)	Bond length of R-C8 (Å)
dG (*anti*)	−1041.848521		1.08271
dG (*syn*)	−1041.843600	−3.09	
oxodG (*anti*)	−1117.087880		1.21774
oxodG (*syn*)	−1117.087980	0.06	
deazadG (*anti*)	−1025.793500		1.08050
deazadG (*syn*)	−1025.787957	−3.48	
Cl-deazadG (*anti*)	−1485.388305		1.73135
Cl-deazadG (*syn*)	−1485.387439	−0.54	
Br-deazadG (*anti*)	−3596.900728		1.88166
Br-deazadG (*syn*)	−3596.897944	−1.75	
I-deazadG (*anti*)[b]	−1036.549415		2.11099
I-deazadG (*syn*)[b]	−1036.550920	0.94	1.08271

[a] A B3LYP/6-31G(d) geometric optimization and frequency analysis in vacuo.
[b] A B3LYP/LanL2DZ/6–31G(d) geometric optimization and frequency analysis in vacuo. Relative energies (Δ*E*) were determined as the difference between the *anti* and *syn* conformations (*anti*–*syn*); a positive difference indicates that the *syn* conformation is favored.

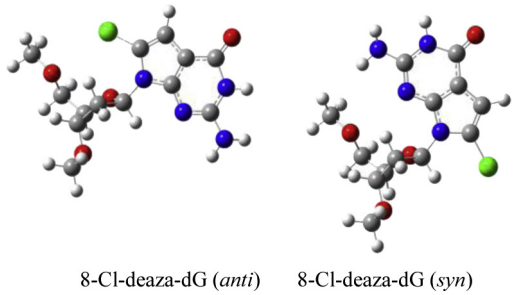


Fig. 4. Optimized structures of the 8-chloro-7-deaza-dG derivatives generated using B3LYP/6–31G(d) in vacuo.

modification of the *syn*- and *anti*-conformers of dG, and were corrected with the difference from the observed values for dG. When the chemical shifts were calculated by assuming weighted average of 75% *syn*- and 25% *anti*-conformer, the predicted chemical shift changes were similar to the observed values (Fig. 3A vs Fig. 3B). These results suggest that 8-halogenated-deaza-dG are similar to 8-oxo-dG with respect to their preference for the *syn*- or *anti*-conformation around the *N*-glycosidic bond.

To elucidate the base pairing properties of duplex DNA (**ODN1** and **2**) containing the 8-halogenated-7-deaza-dG derivatives, UV melting experiments were performed in a buffer containing 100 mM NaCl and 10 mM sodium phosphate at pH 7.0. The melting temperatures (T_m values) for 2 μM oligonucleotides and the thermodynamic parameters of the experiment are summarized in Table 3. The T_m values for each base pair were compared with the corresponding base pair with dG according to the equation $\Delta T_{m(Z)}=T_{m(Z)}-T_{m(dG)}$, where Z represents 8-oxo-dG, deaza-dG or 8-halogenated-7-deaza-dG. The ΔT_m values for the base pairs formed with dC and dA are displayed in Fig. 5. The ΔT_m values for the 8-oxo-dG:dC base pair are negative (Fig. 5A and C, red bars), indicating destabilization compared with the natural dG:dC base pair. In contrast, the ΔT_m value for 8-oxo-dG:dA are positive (Fig. 5B and D, red bars), representing stabilization compared with the dG:dA base pair. These results have been interpreted as *syn* 8-oxo-dG diminishing the stability of the base pair with dC and enhancing the stability with dA as shown in Fig. 1. The base pair of *syn* 8-oxo-dG with dA produces more stability than the non-canonical base pair between *anti*-dG and dA (Fig. 6A)[37,38] As discussed in the conformational analysis, the *syn* preference of 7-deaza-dG is similar to dG. Therefore, it is reasonable that the base pairs of 7-deaza-dG with dC and dA did not affect the duplex stability compared to the natural base pairs of dG:dC and dG:dA (blue bars in Fig. 5A and B). Although 8-halogenated-7-deaza-dG forms a Watson-Crick base pair with dC (Fig. 6B), the base pair of dC with Cl-deaza-dG, Br-deaza-dG, and I-deaza-dG were strongly destabilized (Fig. 5A and C, orange, yellow, and green bars). These results suggest that the *syn* preference of 8-halogenated-7-deaza-dG decreases the stability of base pair with dC.

Unlike deaza-dG, 8-halogenated-7-deaza-dG showed destabilizing effects for the base pair with dA (Fig. 5B and D, orange, yellow, and green bars). The non-canonical base pair with *anti* dA most likely destabilized by the *syn* preference. In addition, although the

Table 3
Melting temperatures and thermodynamic parameters for the duplexes containing 8-halogenated-7-deaza-dG[a]

ODN1: 5′ d(CTTTCT**X**CTCCTT) 3′						**ODN2**: 5′ d(AAGGAG**Y**AGAAAG) 3′					
X	Y	T_m (°C at 2 μM)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal K^{-1} mol^{-1})	$\Delta G°_{310\ K}$ (kcal/mol)	X	Y	T_m (°C at 2 μM)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal K^{-1} mol^{-1})	$\Delta G°_{310\ K}$ (kcal/mol)
dG	dC	48.2	−115±18	−328±55	−13±0.8	dC	dG	44.1	−101±6	−288±20	−11±0.2
dG	dA	33.1	−94.6±4	−280±13	−7.8±0.04	dA	dG	35.4	−71.1±8	−201±25	−8.7±0.2
dG	dG	35.7	−78.8±4	−226±12	−8.6±0.04	dG	dG	35.7	−78.8±4	−226±12	−8.6±0.04
dG	T	36.5	−76.3±1	−218±4	−8.8±0.01	T	dG	33.7	−68.4±1	−194±2	−8.2±0.01
oxodG	dC	44.3	−98.3±8	−281±13	−11±0.8	dC	oxodG	42.3	−96.8±8	−278±25	−10.6±0.2
oxodG	dA	39.8	−91.4±3	−263±9	−9.7±0.05	dA	oxodG	39.2	−96.1±7	−279±21	−9.6±0.1
oxodG	dG	32.7	−45.9±5	−122±15	−8.2±0.16	dG	oxodG	33.4	−80.0±16	−233±52	−7.9±0.6
oxodG	T	33.0	−95.4±5	−283±15	−7.8±0.06	T	oxodG	32.2	−73.2±5	−252±17	−7.8±0.07
deazadG	dC	46.9	−78.7±5	−217±15	−11±0.2	dC	deazadG	41.6	−72.6±1	−202±4	−10.0±0.1
deazadG	dA	31.9	−68.2±6	−195±20	−7.8±0.14	dA	deazadG	35.4	−106±11	−314±34	−8.4±0.15
deazadG	dG	34.1	−66.6±10	−189±35	−8.1±0.38	dG	deazadG	32.0	−68.2±5	−195±18	−7.8±0.12
deazadG	T	37.2	−117±20	−347±65	−9.2±0.46	T	deazadG	33.1	−85.9±5	−252±17	−7.8±0.07
Cl-deazadG	dC	41.9	−77.8±5	−218±15	−10.2±0.1	dC	Cl-deazadG	38.0	−88.2±13	−255±42	−9.3±0.34
Cl-deazadG	dA	30.0	−121±20	−370±65	−6.4±0.51	dA	Cl-deazadG	30.3	−74.2±8	−216±28	−7.2±0.24
Cl-deazadG	dG	30.7	−77.5±6	−226±20	−7.2±0.14	dG	Cl-deazadG	31.4	−294±58	−936±188-	−3.3±0.11
Cl-deazadG	T	34.5	−71.0±6	−202±18	−8.3±0.01	T	Cl-deazadG	30.8	−85.4±11	−252±35	−7.3±0.27
Br-deazadG	dC	41.0	−85.7±5	−244±16	−10.2±0.1	dC	Br-deazadG	37.0	−73.4±7	−208±22	−8.9±0.13
Br-deazadG	dA	28.2	−99.6±12	−301±40	−6.1±0.37	dA	Br-deazadG	30.1	−202±25	−637±83	−4.5±0.56
Br-deazadG	dG	30.0	−90.3±13	−268±42	−7.0±0.36	dG	Br-deazadG	31.0	−141±8	−435±27	−6.1±0.16
Br-deazadG	T	34.1	−81.0±2	−235±8	−8.2±0.02	T	Br-deazadG	30.5	−90.1±6	−268±20	−7.0±0.14
I-deazadG	dC	39.7	−113±8	−333±26	−10.0±0.1	dC	I-deazadG	35.3	−73.3±4	−209±13	−8.6±0.1
I-deazadG	dA	29.2	−79.9±10	−235±31	−6.9±0.25	dA	I-deazadG	29.4	−108±14	−328±46	−6.3±0.38
I-deazadG	dG	30.5	−66.1±8	−189±27	−7.6±0.25	dG	I-deazadG	31.4	−107±8	−322±25	−7.1±0.14
I-deazadG	T	34.7	−99.4±14	−295±45	−8.0±0.30	T	I-deazadG	29.3	−137±6	−424±19	−5.5±0.13

[a] Conditions: 1–8 μM duplex, 100 mM NaCl, and 10 mM sodium phosphate buffer, pH 7.0. The data were analyzed with the melt curve processing program, MeltWin 3.5. T_m values are for 2 μM oligonucleotides, and experimental errors are less than 0.5 °C.

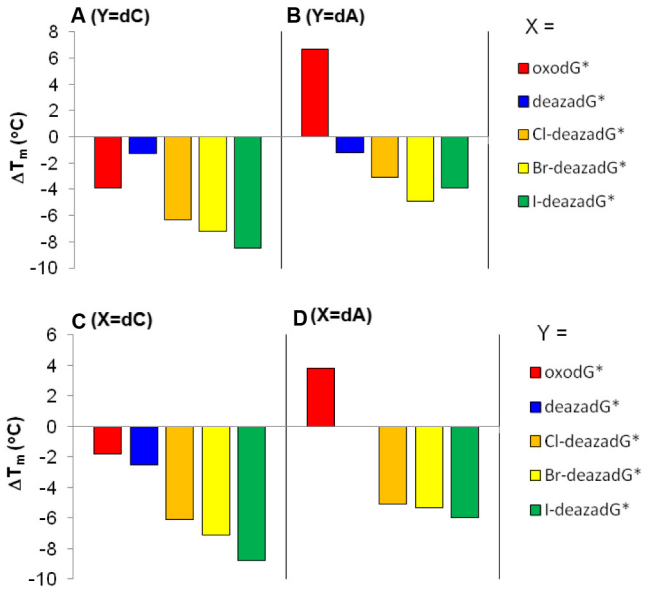


Fig. 5. The bar graphs for the ΔT_m values between the corresponding dG analogues and dG in 2 μM solutions of duplex DNA ($\Delta T_{m(Z)}=T_{m(Z)}-T_{m(dG)}$, Z represents modified dG). **ODN1**/**ODN2** duplexes were used, where X and Y represent 8-oxo-dG, 7-deaza-dG, and 8-halogenated-7-deaza-dG. **ODN1**: 5′-d(CTTTCTXCTCCTT). **ODN2**: 3′-d(GAAAGAYGAGGAA).

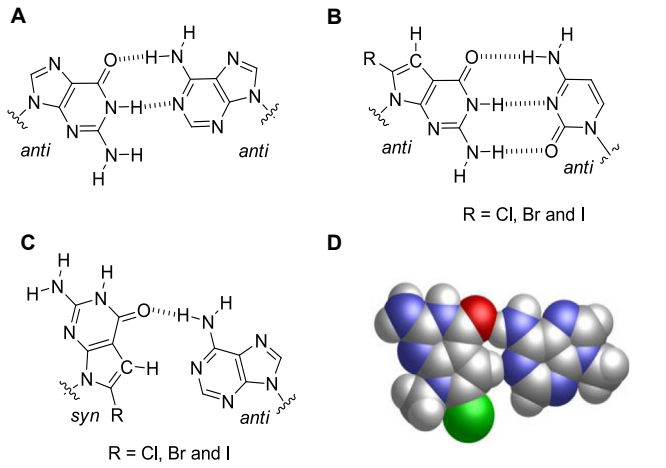


Fig. 6. Schematic structure of base pair formation (A) *anti*-dG or deazadG with dA, (B) *syn*-8-halogenated derivatives with *anti*-dC, (B) *syn*-8-halogenated derivatives with *anti* dA and computational models, (D) Cl-deazaG:A, calculated using Gaussian09 at the PM6 level.

shape of *syn* 8-halogenated-7-deaza-dG may fit with the Watson–Crick face of dA, a lack of hydrogen bonding with the 1 N of dA results in insufficient stabilization. Thus, the destabilizing effect exerted by 8-halogenated-deaza-dG on the base pair formation with dA might imply that 8-halogenated-7-deaza-dG is preferable in the *syn* conformation in DNA. CD spectra for duplexes formed with the 8-halogenated-7-deaza-dG derivatives indicate that these nucleoside derivatives did not disturb the canonical B-type duplex DNA formation (data not shown).

3. Conclusion

In summary, 8-halogenated-7-deaza-dG derivatives were designed to be 8-oxo-dG mimics in shape, and their syntheses were achieved via the reaction between 7-deaza-dG and *N*-halogenated-succinimides. These compounds were incorporated into 13-*mer*-oligonucleotides. It has been shown by NMR that the conformation around the *N*-glycosidic bond of 8-halogenated-7-deaza-dG is preferably in the *syn* conformation similar to 8-oxo-dG. The base-paring properties of 8-halogenated-7-deaza-dG in DNA were investigated by measuring the denaturation temperature, and it was shown that its base pair with dC destabilizes duplexes. These results imply that 8-halogenated-7-deaza-dG also prefer *syn* conformation when incorporated in DNA. The base pair between *syn* 8-halogenated-deaza-dG and *anti* dA did not stabilize the duplexes, most likely due to a lack of hydrogen bonding with 7C–H. Thus, 8-halogenated-7-deaza-dG analogs have been shown to be suitable candidates to investigate the effects on efficiency in the 8-oxo-dG repair system. Further study is now on-going along this line.

4. Experimental section

4.1. General conditions

The ^{1}H NMR (400 MHz, 500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded using solutions in $CDCl_3$ or DMSO-d_6. The ^{31}P NMR (161 MHz) spectra were recorded using 10% phosphoric acid in D_2O as an internal standard (0 ppm). The IR spectra were obtained with an FTIR spectrometer. The high-resolution mass spectra were recorded on the positive mode with an ESI-TOF mass spectrometer using bradykinin and angiotensin as the internal standards. The MALDI-TOF mass spectra were recorded on the negative mode. HPLC purification was carried out with reverse-phase C18 columns. The T_m measurements were taken with a UV spectrometer and the thermodynamic parameters were analyzed using the curve-fitting program, MeltWin (V3.5).

*4.1.1. 2′,5′-Di-O-acetyl-8-chloro-7-deaza-2′-deoxyguanosine (**6**).* 2′,5′-Di-*O*-acetyl-7-deaza-2′-deoxyguanosine **5** (500 mg, 1.43 mmol)was dissolved in anhydrous DMF (10 mL). The flask was covered before 210 mg (1.57 mmol) of *N*-chlorosuccinimide was added to the reaction mixture. The resulting solution was stirred at room temperature for 30 min. The reaction mixture was diluted with 20 mL of methanol and stirred for 40 min. The organic solvent was concentrated, and the residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH=20:1) to give **6** (470 mg, 86%) as a white powder. Mp 177–179 °C. IR (cm^{-1}): 3175, 1743, 1667, 1624, 1527, 1431, 1367, 1238, 1043. ^{1}H NMR (DMSO-d_6) δ 10.61 (br s, 1H), 6.35–6.29 (m, 4H), 5.39–5.35 (m, 1H), 4.40–4.36 (m, 1H), 4.18–4.10 (m, 2H), 3.39–3.31 (m, 1H), 2.29–2.35 (m, 1H), 2.07 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 170.1, 170.0, 157.5, 152.6, 150.4, 114.9, 101.6, 99.7, 82.8, 80.9, 74.2, 63.5, 34.1, 20.5, 20.4. HR-ESI-MS *m*/*z* $[M+H]^+$ calcd for $C_{15}H_{18}ClN_4O_6$ 385.0909, 387.0886, found 385.0941, 387.0935.

*4.1.2. 2′,5′-Di-O-acetyl-8-bromo-7-deaza-2′-deoxyguanosine (**7**).* 2′,5′-Di-*O*-acetyl-7-deaza-2′-deoxyguanosine **5** (500 mg, 1.43 mmol) was dissolved in anhydrous DMF (24 mL). The flask was covered and then 280 mg (1.62 mmol) of *N*-bromosuccinimide was added in several portions over 1 h. The reaction mixture was diluted with 20 mL of methanol and stirred for 40 min. The organic solvent was concentrated, and the residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH=30:1) to provide **7** (313 mg, 51%) as a white powder. Mp 150–152 °C. IR (cm^{-1}): 3168, 1741, 1669, 1625, 1547, 1430, 1365, 1235, 1049. ^{1}H NMR (DMSO-d_6) δ 10.60 (br s, 1H), 6.44 (s, 1H), 6.32 (s, 2H), 6.27 (t, 1H, *J*=7.4 Hz), 5.42–5.37 (m, 1H), 4.43–4.38 (m, 1H), 4.20–4.10 (m, 2H), 3.49–3.42 (m, 1H), 2.35–2.29 (m, 1H), 2.07 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.1, 170.0, 157.4, 152.4, 151.1, 105.6, 101.4, 101.2, 84.2, 80.9, 74.3, 63.6, 34.1, 20.7, 20.5. HR-ESI-MS *m*/*z* $[M+H]^+$ calcd for $C_{15}H_{18}BrN_4O_6$ 429.0404, 431.0386, found 429.0382, 431.0416.

*4.1.3. 2′,5′-Di-O-acetyl-8-iodo-7-deaza-2′-deoxyguanosine (**8**).* Two hundred milligrams (0.57 mmol) of 2′,5′-di-*O*-acetyl-7-deaza-2′-deoxyguanosine **5** was dissolved in anhydrous DMF (10 mL). The flask was covered and then 198 mg (0.88 mmol) of *N*-iodosuccinimide was added in several portions over 1 h. The reaction mixture was diluted with 10 mL of methanol and stirred for 40 min. The organic solvent was concentrated, and the crude product was purified using short silica gel column ($CHCl_3$/MeOH=5:1). The residue was separated by column chromatography (silica gel, $CHCl_3$/MeOH=50:1 to 40:1) to give **8** (58 mg, 21%) as a white powder. Mp 174–176 °C. IR (cm^{-1}): 3305, 1733, 1674, 1630, 1597, 1557, 1429, 1362, 1247, 1030. ^{1}H NMR (DMSO-d_6) δ 10.56 (br s, 1H), 6.56 (s, 1H), 6.27 (s, 2H), 6.18 (t, 1H, *J*=7.4 Hz), 5.44–5.40 (m, 1H), 4.46–4.42 (m, 1H), 4.22–4.10 (m, 2H), 3.54–3.47 (m, 1H), 2.33–2.27 (m, 1H), 2.07 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 170.1, 170.0, 157.2, 152.0, 151.5, 113.1, 103.6, 86.7, 80.9, 74.4, 71.4, 63.7, 34.3, 20.7, 20.5. HR-ESI-MS *m*/*z* $[M+H]^+$ calcd for $C_{15}H_{18}IN_4O_6$ 477.0266, found 477.0297.

*4.1.4. 8-Chloro-7-deaza-2′-deoxyguanosine (**2**).* Compound **6** (470 mg, 1.22 mmol) was suspended in 7 N ammonium in methanol solution (15 mL). The resulting solution was stirred at room temperature overnight. The organic solvent was concentrated, and the residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH=10:1) to give **2** (258 mg, 70%) as a white powder. Mp 188–190 °C. IR (cm^{-1}): 3232, 1675, 1646, 1608, 1553, 1519, 1437, 1368, 1100. ^{1}H NMR (DMSO-d_6) δ 10.45 (br s, 1H), 6.35–6.28 (m, 2H), 6.25 (s, 2H), 5.18 (d, 1H, *J*=4.0 Hz), 4.86 (m, 1H), 4.36–4.30 (m,

1H), 3.77–3.71 (m, 1H), 3.63–3.42 (m, 2H), 3.03–2.94 (m, 1H), 2.06–1.99 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 157.5, 152.5, 150.3, 115.0, 101.4, 99.6, 87.2, 83.0, 71.0, 62.1, 37.2. HR-ESI-MS m/z $[M+H]^+$ calcd for $C_{11}H_{14}ClN_4O_4$ 301.0698, 303.0673, found 301.0719, 303.0658.

*4.1.5. 8-Bromo-7-deaza-2′-deoxyguanosine (**3**).* Compound **7** (278 mg, 0.65 mmol) was suspended in 7 N ammonium in methanol solution (15 mL). The resulting solution was stirred at room temperature overnight. The organic solvent was concentrated, and the residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH=10:1) to give **3** (133 mg, 60%) as a white powder. Mp 197–199 °C. IR (cm^{-1}): 3230, 1651, 1606, 1504, 1434, 1368, 1099. ^{1}H NMR (DMSO-d_6) δ 10.66 (br s, 1H), 6.41 (s, 1H), 6.29–6.21 (m, 3H), 5.18 (d, 1H, J=4.0 Hz), 4.94 (m, 1H), 4.37–4.32 (m, 1H), 3.77–3.71 (m, 1H), 3.64–3.44 (m, 2H), 3.07–3.01 (m, 1H), 2.06–1.98 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 157.3, 152.3, 150.8, 105.4, 101.9, 101.3, 87.3, 84.2, 71.1, 62.2, 37.3. HR-ESI-MS m/z $[M+H]^+$ calcd for $C_{11}H_{14}BrN_4O_4$ 345.0193, 347.0174, found 345.0183, 347.0193.

*4.1.6. 8-Iodo-7-deaza-2′-deoxyguanosine (**4**).* Compound **8** (235 mg, 0.50 mmol) was suspended in 7 N ammonium in methanol solution (15 mL). The resulting solution was stirred at room temperature for overnight. The organic solvent was concentrated, and the residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH=10:1) to give **4** (126 mg, 65%) as a white powder. Mp 171–173 °C. IR (cm^{-1}): 3106, 1673, 1630, 1533, 1474, 1435, 1391, 1102. ^{1}H NMR (DMSO-d_6) δ 10.51 (br s, 1H), 6.54 (s, 1H), 6.21–6.09 (m, 3H), 5.16 (d, 1H), 4.91 (m, 1H), 4.38–4.32 (m, 1H), 3.77–3.72 (m, 1H), 3.66–3.46 (m, 2H), 3.16–3.08 (m, 1H), 2.01–1.93 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 157.1, 151.9, 151.2, 112.9, 103.7, 87.4, 86.8, 71.3, 62.3, 37.5. HR-ESI-MS m/z $[M+H]^+$ calcd for $C_{11}H_{14}IN_4O_4$ 393.0054, found 393.0075.

4.2. General procedure for synthesis of Pac-protected compounds

To a solution of **2**, **3** or **4** (0.33 mmol) in pyridine (10 mL) was added TMSCl (500 μL, 3.96 mmol). The reaction mixture was stirred for 1 h at room temperature, and phenoxyacetic anhydride (143 mg, 0.50 mmol) was added. After stirring overnight at room temperature, the reaction was quenched with water (10 mL) and extracted with $CHCl_3$. The organic layer was evaporated, and the residue was purified by column chromatography (silica gel, $CHCl_3$/MeOH=50:1 to 40:1 to 30:1).

*4.2.1. 2-N-Phenoxylacetyl-8-chloro-7-deaza-2′-deoxyguanosine (**9**).* This compound (115 mg, 80%) was isolated as a white powder. Mp 236–238 °C. IR (cm^{-1}): 3215, 1681, 1611, 1550, 1499, 1441, 1244, 1053, 1009. ^{1}H NMR (DMSO-d_6) δ 11.66 (br s, 1H), 11.52 (br s, 1H), 7.33–7.25 (m, 2H), 6.99–6.88 (m, 3H), 6.59 (s, 1H), 6.42 (t, 1H, J=7.6 Hz), 5.25 (s, 1H), 4.83 (s, 2H), 4.75 (s, 1H), 4.38 (m, 1H), 3.76–3.74 (m, 1H), 3.59–3.49 (m, 2H), 3.11–3.04 (m, 1H), 2.12–2.09 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 170.7, 157.7, 155.6, 147.2, 145.8, 129.5, 121.3, 118.6, 114.6, 104.1, 102.2, 87.2, 82.9, 70.7, 66.3, 61.9, 36.9. HR-ESI-MS m/z $[M+H]^+$ calcd for $C_{19}H_{20}ClN_4O_6$ 435.1066, 437.1045, found 435.1113, 437.1036.

*4.2.2. 2-N-Phenoxylacetyl-8-bromo-7-deaza-2′-deoxyguanosine (**10**).* This compound (115 mg, 73%) was isolated as a white powder. Mp 194–196 °C. IR (cm^{-1}): 3207, 1678, 1611, 1548, 1498, 1438, 1245, 1056, 1009, 754. ^{1}H NMR (DMSO-d_6) δ 11.66 (br s, 1H), 11.45 (br s, 1H), 7.33–7.29 (m, 2H), 7.00–6.96 (m, 3H), 6.68 (s, 1H), 6.39 (t, 1H, J=7.8 Hz), 5.24 (d, 1H, J=4.0 Hz), 4.85 (s, 2H), 4.74 (t, 1H, J=5.8 Hz), 4.40–4.39 (m, 1H), 3.77–3.75 (m, 1H), 3.66–3.48 (m, 2H), 3.22–3.12 (m, 1H), 2.12–2.07 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 170.7, 157.6, 155.5, 147.9, 145.5, 129.5, 121.3, 114.6, 106.1, 105.6, 105.3, 87.3, 84.2, 70.8, 66.3, 62.0, 37.0. HR-ESI-MS m/z $[M+H]^+$ calcd for $C_{19}H_{20}BrN_4O_6$ 479.0561, 481.0543, found 479.0592, 481.0593.

*4.2.3. 2-N-Phenoxylacetyl-8-iodo-7-deaza-2′-deoxyguanosine (**11**).* This compound (119 mg, 70%) was isolated as a white powder. Mp 204–206 °C. IR (cm^{-1}): 3196, 1682, 1611, 1547, 1498, 1433, 1243, 1058, 756. ^{1}H NMR (DMSO-d_6) δ 11.62 (br s, 1H), 11.36 (br s, 1H), 7.33–7.29 (m, 2H), 7.00–6.96 (m, 3H), 6.78 (s, 1H), 6.29 (t, 1H, J=7.6 Hz), 5.22 (d, 1H, J=4.0 Hz), 4.85 (s, 2H), 4.74 (t, 1H, J=5.8 Hz), 4.45–4.39 (m, 1H), 3.78–3.75 (m, 1H), 3.70–3.50 (m, 2H), 3.08–2.99 (m, 1H), 2.12–2.03 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 170.7, 157.6, 155.3, 148.4, 145.1, 129.5, 121.3, 114.6, 113.5, 107.7, 87.3, 86.5, 76.1, 70.9, 66.3, 62.0, 37.1. HR-ESI-MS m/z $[M+H]^+$ calcd for $C_{19}H_{20}IN_4O_6$ 527.0422, found 527.0421.

4.3. General procedure of synthesis for amidite compounds

To a solution of the appropriate Pac-protected compound in pyridine was added 4,4′-dimethoxytrityl chloride (1.5 equiv) at room temperature. After stirring for 1 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc three times. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under vacuum. The residue was purified using column chromatography. The resulting DMTr-protected compound was dissolved in CH_2Cl_2. To this solution was added diisopropylethylamine (6 equiv) and 2-cyanoethyl-*N*,*N*′-diisopropylchlorophosphorodiamidite (3 equiv) at 0 °C. After stirring for 2 h, the reaction mixture was quenched with saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated under vacuum. The residue was purified using column chromatography (silica gel, Hexane/EtOAc).

*4.3.1. The β-phosphoroamidite derivative of 2-N-phenoxylacetyl-8-chloro-7-deaza-2′-deoxyguanosine (**12**).* Compound **12** (39% for two steps) was isolated as a white powder. ^{1}H NMR ($CDCl_3$) δ 7.41–7.25 (m, 8H), 7.20–7.13 (m, 3H), 7.09–7.05 (m, 1H), 6.95–6.92 (m, 2H), 6.72–6.67 (m, 4H), 6.59 (s, 1H), 6.50 (t, 1H, J=7.0 Hz), 4.51 (t, 2H, J=3.0 Hz), 4.20–4.15 (m, 1H), 3.90–3.75 (m, 1H), 3.73 (s, 3H), 3.72 (s, 3H), 3.64–3.52 (m, 3H), 3.42–3.06 (m, 4H), 2.60–2.33 (m, 3H). 1.30–1.06 (m, 12H). ^{31}P NMR ($CDCl_3$) δ 149.23, 149.05. ESI-MS (m/z): 959.3, 961.4 $[M+Na]^+$.

*4.3.2. The β-phosphoroamidite derivative of 2-N-phenoxylacetyl-8-bromo-7-deaza-2′-deoxyguanosine (**13**).* Compound **13** (45% for two steps) was isolated as a white powder. ^{1}H NMR ($CDCl_3$) δ 7.41–7.25 (m, 8H), 7.18–7.12 (m, 3H), 7.09–7.05 (m, 1H), 6.94–6.91 (m, 2H), 6.73 (s, 1H), 6.71–6.66 (m, 4H), 6.48–6.46 (m, 1H), 4.49 (t, 2H, J=4.6 Hz), 4.20–4.17 (m, 1H), 3.80–3.76 (m, 1H), 3.73 (s, 3H), 3.72 (s, 3H), 3.70–3.52 (m, 3H), 3.44–3.10 (m, 4H), 2.60–2.32 (m, 3H). 1.30–1.06 (m, 12H). ^{31}P NMR ($CDCl_3$) δ 149.03, 148.88. ESI-MS (m/z): 1004.3, 1006.8 $[M+Na]^+$.

*4.3.3. The β-phosphoroamidite derivative of 2-N-phenoxylacetyl-8-iodo-7-deaza-2′-deoxyguanosine (**14**).* Compound **14** (61% for two steps) was isolated as a white powder. ^{1}H NMR ($CDCl_3$) δ 7.41–7.25 (m, 8H), 7.17–7.12 (m, 3H), 7.09–7.05 (m, 1H), 6.95 (s, 1H), 6.93–6.90 (m, 2H), 6.70–6.65 (m, 4H), 6.38 (t, 1H), 4.46 (t, 2H), 4.21–4.17 (m, 1H), 3.80–3.76 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.70–3.52 (m, 3H),

3.47–3.12 (m, 4H), 2.60–2.31 (m, 3H). 1.30–1.06 (m, 12H). ^{31}P NMR ($CDCl_3$) δ 149.27, 149.13. ESI-MS (m/z): 1051.3 $[M+Na]^+$

4.4. DNA synthesis

Oligonucleotides (ODNs) were synthesized using standard DNA synthesis procedures with an NTS-H6 DNA/RNA synthesizer (NIHON TECHNO SERVICE CO., Ltd). The synthesized ODNs were cleaved from the resin and deprotected at the nucleobase using 50 mM K_2CO_3 in methanol at room temperature for 4 h. The DMTr-ODN was purified by HPLC (nacalai tesque COSMOSIL C18-ARII) using a linear gradient (A: 0.1 M TEAA buffer, B: CH_3CN, B concd 10 to 40%/20 min). The DMTr-group on the ODNs was removed with 1–5% aqueous acetic acid at room temperature; the resulting DMTr-off ODNs were washed with ether. The purities and structures were confirmed with MALDI-TOF Mass measurements. 8-Oxo-dG and 7-deaza-dG containing ODNs were purchased from Gene Design Inc. or Genenet.

MALDI-TOF mass results of synthesized ODNs (m/z)

ODNs	Calcd	Found
ODN1 5′ d(CTTTCT X CTCCTT)		
X=8-chloro-7-deaza-dG (**2**)	3873.56	3872.61
X=8-bromo-7-deaza-dG (**3**)	3917.51	3916.70
X=8-iodo-7-deaza-dG (**4**)	3965.50	3964.46
ODN2 5′ d(AAGGAG Y AGAAAG)		
Y=8-chloro-7-deaza-dG (**2**)	4136.75	4135.65
Y=8-bromo-7-deaza-dG (**3**)	4180.70	4179.86
Y=8-iodo-7-deaza-dG (**4**)	4228.69	4227.74

4.5. T_m measurements

A 1–8 μM solution of each oligonucleotide with 100 mM NaCl, and 10 mM sodium phosphate buffer (pH 7) was heated for 5 min at 60 °C before being slowly cooled to room temperature overnight. Melting temperatures were determined with a DU-800 spectrometer. The absorbance at 260 nm was monitored from 20 to 70 °C at a rate of 1 °C/min. The melting temperatures (T_m) and thermodynamic parameters were analyzed using the curve-fitting program, MeltWin (v3.5).

4.6. CD measurements

The CD spectra were recorded using 5.0 μM solutions of each strand in buffer containing 100 mM NaCl, and 10 mM sodium phosphate buffer at 25 °C with a JASCO J-720W spectrometer in cylindrical quartz cell with a 0.1 cm path length.

4.7. Computational study

The NMR chemical shifts of *anti*- or *syn*-8-halogenated-deazadG derivatives were simulated using the Hartree–Fock with the 6–311G(d,p) or LanL2DZ/6–311G(d,p) basis set and were corrected by the difference of the measurement values between dG and 8-oxo-dG. The simulated chemical shifts were calculated by assuming weighted average of 75% *syn*-conformer and 25% *anti*-conformer. Geometric optimizations and frequencies were performed in vacuo and implemented in the Gaussian09 program using the B3LYP density functional with a 6–31G(d) basis set for dG, oxodG, deazadG, Cl-deazadG, and Br-deazadG, and the LanL2DZ/6–31G(d) basis set for I-deazadG. The experimental results for the crystal structures (PDB 183D and PDB 178D) were utilized to establish whether the starting structures had *anti* or *syn* geometries. The modeled base pair formations of the 9-methyl-8-halogenated nucleoside analogues with 9-methyl-adenine were calculated using the Gaussian09 program at PM6.

4.8. NMR study

Assignments of the anomeric stereochemistry and conformation about glycosyl bonds for the corresponding diol compounds were confirmed by NMR spectroscopy using a 0.04 M solution in DMSO-d_6.

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Supplementary data

Supplementary data of NMR spectra of the new products are available. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2014.01.047.

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