Synthesis, Miscoding Specificity, and Thermodynamic Stability of Oligodeoxynucleotide Containing 8-Methyl-2′-deoxyguanosine

Kohfuku Kohda,*,† Hirotaka Tsunomoto,† Yasushi Minoura,‡ Kazushi Tanabe,‡ and Shinya Shibutani§

Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabedori, Mizuho-ku, Nagoya 467, Japan, Laboratory of Biochemistry, Aichi Cancer Center Research Institute, Kanokoden, Chikusa-ku, Nagoya 467, Japan, and Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, New York 11794-8651

Received June 26, 1996⊗

8-Methyl-2′-deoxyguanosine (8-MedG) was synthesized by reacting dG under the methyl radical generating system and incorporated into oligodeoxynucleotides using phosphoramidite techniques. The site-specifically modified oligodeoxynucleotide containing a single 8-MedG was then used as a template for primer extension reactions catalyzed by the 3′→5′ exonuclease-free (exo⁻) Klenow fragment of *Escherichia coli* DNA polymerase I and mammalian DNA polymerase α. Primer extension catalyzed by the exo⁻ Klenow fragment readily passed the 8-MedG lesion in the template while that catalyzed by pol α was retarded opposite the lesion. The fully extended products formed during DNA synthesis were analyzed to quantify the miscoding specificities of 8-MedG. Both DNA polymerases incorporated primarily dCMP, the correct base opposite the lesion, along with small amounts of incorporation of dGMP and dAMP. In addition, two-base deletion was observed only when the exo⁻ Klenow fragment was used. The thermodynamic stability of 8-MedG in the duplex was also studied. The duplex containing 8-MedG:dG was more thermally and thermodynamically stable than that of dG:dG. The duplex containing 8-MedG:dA was more thermodynamically stable than that of dG:dA. We conclude that 8-MedG is a miscoding lesion and capable of generating G → C and G → T transversions and deletion in cells.

Introduction

DNA damage is an initiating event in cancer (*1*). 1,2-Dimethylhydrazine (DMH)[1] has been known to induce colon cancer in rodents (*2*) and form several methylated DNA adducts, including *N*7-methylguanine and O^6-methylguanine, in tissue DNA of rodents (*3*−*5*). Such DNA adducts are also formed by the typical methylating carcinogens, *N*-methyl-*N*-nitrosourea (MNU) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (*6*). MNU and MNNG are metabolically activated to produce electrophilic methanediazonium ions as a reactive species (*6, 7*) while DMH forms methyl radicals *in vitro* (*8*−*10*) and *in vivo* (*7*) in addition to electrophilic methanediazonium ions. Chemical (*11*) and biochemical (*12*) studies on radical methylation reveal that the C8-position of the guanine moiety is the most reactive site to form 8-methylguanine (8-MeGua). 8-MeGua has been detected in the liver and colon DNA of rats treated with DMH (*13*). O^6-Methylguanine has been reported to be a mutagenic and carcinogenic lesion (*14*). However, the mutagenic properties of 8-MedG have not been investigated. Recently, various antioxidants, such as vitamin E (*15*) and selenium (*16*), decreased the incidence of DMH-induced colon cancer. Thus, methyl radicals induced by DMH may play a role in mutagenesis and carcinogenesis.

We have described the synthesis of 8-MedG using the radical methylation system and the preparation of 8-MedG-modified oligodeoxynucleotides by phosphoramidite techniques. The site-specifically modified oligodeoxynucleotide was used as a template in primer extension reactions catalyzed by DNA polymerases. Miscoding specificities of 8-MedG were determined quantitatively using an established *in vitro* experimental system (*17*−*19*). We also investigated the effect of 8-MedG lesions on the thermodynamic stabilities to characterize the impact of the lesions on the physicochemical properties of DNA duplex. These results indicate that 8-MedG is a mutagenic lesion, generating G → C and G → T transversions and deletion.

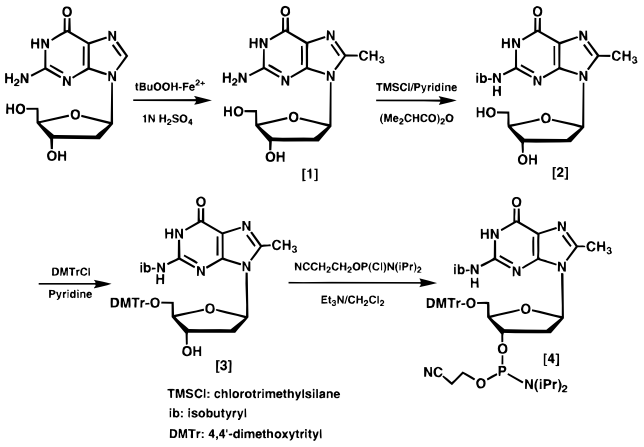
Experimental Procedures

Synthesis of 8-MedG Derivatives. General. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL EX 270 or GSX 400 spectrometer (Tokyo, Japan), and chemical shifts were reported in parts per million (ppm) using tetramethylsilane as the internal standard. Mass spectra were measured by a JEOL DX-300 spectrometer (Tokyo, Japan). UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer (Kyoto, Japan). Melting points were measured with a Yanagimoto micro-melting point apparatus (Kyoto, Japan). Merck silica gel 60 (70-230 mesh, 63-200 μm) was used for column chromatography. HPLC

* To whom correspondence should be addressed. Telephone: Japan (81)-52-836-3408; Telefax: Japan (81)-52-834-9309; E-mail: kohda@phar.nagoya-cu.ac.jp.
† Nagoya City University.
‡ Aichi Cancer Center Research Institute.
§ State University of New York at Stony Brook.
⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1996.
[1] Abbreviations: DMH, 1,2-dimethylhydrazine; 8-MedG, 8-methyl-2′-deoxyguanosine; MNU, *N*-methyl-*N*-nitrosourea; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; 8-oxodG, 7,8-dihydro-8-oxo-2′-deoxyguanosine or 8-hydroxydeoxyguanosine; 8-MeGua, 8-methylguanine; dG, 2′-deoxyguanosine; Gua, guanine; dNTP, 2′-deoxynucleoside triphosphate; exo⁻, 3′→5′ exonuclease-free Klenow fragment; pol α, DNA polymerase α; T_m, melting temperature; $\Delta H°$, van't Hoff transition enthalpy; $\Delta S°$, entropy; $\Delta G°$, free energy; C_t, oligonucleotide strand concentration; PAGE, polyacrylamide gel electrophoresis; HPLC, high performance liquid chromatography; t_R, retention time.

S0893-228x(96)00105-1 CCC: $12.00

Scheme 1



analyses were carried out using a Shimadzu LC10AD apparatus equipped with a photodiode array UV detector SPD-M6A (Kyoto, Japan). Oligodeoxynucleotides were synthesized by an Applied Biosystems automated solid phase DNA synthesizer Model 392 (CA).

8-Methyl-2′-deoxyguanosine (1) (Scheme 1). dG (2.0 g, 7.5 mmol) was added to an ice-cold solution of $FeSO_4 \cdot 7H_2O$ (8.3 g, 30 mmol) in 400 mL of 1 N H_2SO_4 with stirring. *tert*-Butyl hydroperoxide (80%, 2.5 g, 22 mmol) in 40 mL of H_2O was added dropwise to this solution during 4 min, and the mixture was left standing for 1 min. Aqueous 1 N NaOH was then added to the mixture to adjust neutral pH. For analysis of 8-MedG formation, LiChrospher 100 RP-18(e) column (Merck, 4 × 125 mm, 5 μm) was eluted with H_2O−MeOH (0−50% linear gradient of MeOH over 30 min) at a flow rate of 1 mL/min. Retention times (t_R) of Gua, 8-MeGua, dG, and 8-MedG were 4.3, 7.6, 9.6, and 12.7 min, respectively. Approximate yields of products were as follows: 8-MedG (**1**) (59%), 8-MeGua (9%), Gua (10%), and unreacted dG (22%). After solvent was removed by lyophilization, products were extracted from the residue with hot MeOH (200 mL × 2). 8-MedG was separated, using silica gel column chromatography (4.4 × 30 cm, $CHCl_3$ 0.5 L, $CHCl_3$/MeOH = 9/1, 2.5 L, and 17/3, 6 L), and further purified by Sephadex LH 20 column chromatography (2.5 × 30 cm, MeOH). After removal of solvent, white powder was obtained in 29% yield (606 mg). Recrystallization from MeOH gave analytically pure white powder of **1**. This is the first time that the mp (226−229 °C) of 8-MedG has been determined; ^{1}H NMR (Me_2SO-d_6) δ 10.59 (s, 1H, 1-NH), 6.33 (s, 2H, 2-NH_2), 6.13 (dd, 1H, $J_{1',2'a}$ = 8.5 Hz, $J_{1',2'b}$ = 6.3 Hz, 1′-H), 5.25 (d, 1H, $J_{3',3'\text{-OH}}$ = 4.2 Hz, 3′-OH), 4.98 (t, 1H, $J_{5'a,5'\text{-OH}} = J_{5'b,5'\text{-OH}}$ = 5.6 Hz, 5′-OH), 4.34 (dddd, 1H, $J_{2'a,3'}$ = 6.6 Hz, $J_{2'b,3'}$ = 2.7 Hz, $J_{3',4'}$ = 3.2 Hz, 3′-H), 3.77 (dt, 1H, $J_{4',5'a} = J_{4',5'b}$ = 4.9 Hz, 4′-H), 3.62−3.57 (ddd, 1H, $J_{5'a,5'b}$ = 11.7, 5′-Ha), 3.54−3.48 (ddd, 1H, 5′-Hb), 2.84−2.77 (ddd, 1H, $J_{2'a,2'b}$ = 13.9 Hz, 2′-Ha), 2.40 (s, 3H, 8-CH_3), 2.08−2.02 (ddd, 1H, 2′-Hb); UV λ_{max} nm (pH 1) 257 (ϵ = 13 300), 277 (sh) (9700), (H_2O) 251 (14 200), 274 (sh) (9100), (pH 12) 257 (12 500). Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 42.86; H, 5.39; N, 22.72. Found: C, 42.68; H, 5.27; N, 22.93. FAB-MS: *m*/*z* 282 $(M + H)^+$, 166 $(M - \text{sugar} + H)^+$.

***N*²-Isobutyryl-8-methyl-2′-deoxyguanosine (2).** After 8-MedG (**1**, 500 mg, 1.77 mmol) was coevaporated with dry pyridine (5 mL) twice, it was suspended in 18 mL of dry pyridine. Chlorotrimethylsilane (1.12 mL, 8.85 mmol) was added to this solution, and the mixture was left standing for 30 min in an ice-cold water bath. Isobutyric anhydride (1.48 mL, 8.85 mmol) was then added, and the mixture was left standing at room temperature for 2 h. After the mixture was cooled again in an ice-cold water bath, 3.7 mL of H_2O was added, followed by addition of 3.7 mL of concentrated NH_4OH. After 30 min, solvent was removed by evaporation. The residue was dissolved in 15 mL of H_2O and washed with diethyl ether (30 mL × 3). Aqueous phase was reduced to two-thirds volume and left at 4 °C for 1 day. A white precipitate was collected in 77% yield (482 mg). ^{1}H NMR (Me_2SO-d_6) δ 10−11 (hump, 2H, 1-NH and 2-NH), 6.26 (t, 1H, J = 7.3 Hz, 1′-H), 5.28 (d, 1H, J = 4.3 Hz, 3′-OH), 4.84 (t, 1H, J = 5.4 Hz, 5′-OH), 4.39 (m, 1H, 3′-H), 3.78 (m, 1H, 4′-H), 3.60−3.52 (m, 2H, 5′-CH_2), 2.86−2.75 (m, 2H, 2′-Ha and C*H*$(CH_3)_2$), 2.51 (s, 3H, 8-CH_3), 2.15−2.06 (m, 1H, 2′-Hb), 1.13 (d, 6H, J = 6.6 Hz, $C(CH_3)_2$).

5′-*O*-(4,4′-Dimethoxytrityl)-*N*²-isobutyryl-8-methyl-2′-deoxyguanosine (3). After *N*²-isobutyryl-8-methyl-2′-deoxyguanosine (**2**, 476 mg, 1.35 mmol) was coevaporated with dry pyridine (10 mL) twice, it was suspended in 10 mL of dry pyridine. 4,4′-Dimethoxytrityl chloride (95%, 578 mg, 1.62 mmol), triethylamine (263 μL, 1.89 mmol), and 4-(dimethylamino)pyridine (8.3 mg, 0.068 mmol) were added to the solution, and the mixture was stirred at room temperature for 2 h. After 10 mL of H_2O was added, the product was extracted with diethyl ether (50 mL × 3). The combined organic extracts were dried with Mg_2SO_4, filtered, and concentrated at reduced pressure. The crude materials were purified by silica gel column chromatography (2.8 × 10 cm, $CHCl_3$ (500 mL), then $CHCl_3$/MeOH = 19/1, (800 mL)). The yield of **3** was 75% (664 mg). ^{1}H NMR (Me_2SO-d_6) δ 12.0 and 11.3 (each br s, each 1H, 1-NH and 2-NH), 7.31−6.72 (m, 13H, pheny of trityl), 6.28 (t, 1H, J = 7.0 Hz, 1′-H), 5.24 (d, 1H, J = 4.9 Hz, 3′-OH), 4.45 (m, 1H, 3′-H), 3.96 (m, 1H, 4′-H), 3.71 and 3.70 (each s, each 3H, O-CH_3), 3.38−3.08 (m, 2H, 5′-CH_2), 2.99 (m, 1H, 2′-H), 2.73 (m, 1H, C*H*$(CH_3)_2$), 2.48 (s, 3H, 8-CH_3), 2.20 (m, 1H, 2′-H), 1.12 and 1.11 (each d, each 3H, J = 6.8 Hz, $C(CH_3)_2$).

3′-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5′-*O*-(4,4′-dimethoxytrityl)-*N*²-isobutyryl-8-methyl-2′-deoxyguanosine (4). *N*²- and 5′-*O*-diprotected 8-methyl-2′-deoxyguanosine (**3**, 102 mg, 0.16 mmol) was dried over P_2O_5. After compound **3** was coevaporated with dry CH_2Cl_2−benzene, CH_2Cl_2 (1 mL), triethylamine (54 μL, 0.388 mmol, 2.5 equiv mol), and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (52 μL, 0.233 mmol, 1.5 equiv mol) were added, and the mixture was stirred at room temperature for 30 min. Formation of products was monitored by TLC (silica gel, $CHCl_3$/MeOH = 19/1, R_f values of products (*R*/*S*) were 0.42 and 0.51 and that of **3** was 0.19). After solvent was removed by evaporation, 5 mL of tetrahydrofuran−benzene (1:4) was added and the mixture was stirred for 10 min. The precipitate was removed by filtration and evaporated to remove the solvent. Coevaporation of the residue with dry benzene (5 mL × 2) gave **4** in 96% (128 mg). Without further purification, the product was dried over P_2O_5 and used for subsequent oligodeoxynucleotide synthesis.

Synthesis of Oligodeoxynucleotides Containing 8-MedG. The following oligodeoxynucleotides containing 8-MedG were prepared by the phosphoramidite method using an automated solid phase DNA synthesizer: $^{5'}$CCTTCXCTACTTTCCTCTC-CATTT (sequence 2 in Table 2) and $^{5'}$GCGCCXGCGGTG, where X is 8-MedG. Purification of oligodeoxynucleotides was carried out using an OPC column (Applied Biosystems). The following procedure was used for base analysis. A 20 μL aliquot of nuclease P1 (1 mg/mL) was added to a solution of oligodeoxynucleotides (1 OD_{260} unit) in 280 μL of 20 mM sodium acetate buffer (pH 4.8) and incubated at 37 °C for 30 min. To this solution, 200 μL of Tris-HCl buffer (pH 7.5) and alkaline phosphatase (3.5 units/100 μL) were added, and the mixture was incubated at 37 °C for another 30 min for 12-mer and 10 min for 24-mer. Composition of nucleosides was examined by HPLC using an ODC column (4 × 250 mm). Elution was performed with a linear gradient of MeOH in 0.67 M phosphate buffer (pH 7.0) at a flow rate of 0.8 mL/min (0−50% MeOH with an elution time of 120 min for 12-mer, and 0−5% MeOH with an elution time of 5 min, then 5−100% MeOH with an elution time of 115 min for 24-mer). The data of base composition for each 8-MedG-modified oligodeoxynucleotide were consistent with the calculated values (data not shown).

Experiments for Miscoding Specificity. General. Organic chemicals used for the synthesis of oligodeoxynucleotides were supplied by Aldrich Chemical (Milwaukee, WI). [γ-^{32}P]ATP (specific activity >6000 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL). Cloned exo$^-$ Klenow fragment

of *Escherichia coli* DNA polymerase I (21 200 units/mg of protein) was purchased from United States Biochemical Corp. (Cleveland, OH), calf thymus DNA pol α (30 000 units/mg of protein) was from Molecular Biology Resources, Inc. (Milwaukee, WI), and T4 DNA ligase (400 units/μL) was from New England BioLabs (Beverly, MA). HPLC grade acetonitrile, triethylamine, and distilled water were purchased from Fisher Chemical (Pittsburgh, PA). A Waters 990 HPLC instrument equipped with a photodiode array detector (Milford, MA) was used for the separation and purification of oligodeoxynucleotides.

Synthesis of Oligodeoxynucleotides. Unmodified DNA templates (sequences 1 and 2, X = dG), primer, and standard markers listed in Table 2 were prepared by the phosphoramidite method using an automated DNA synthesizer (*20*). These oligonucleotides and modified DNA template (sequence 2, X = 8-MedG) were purified on a Waters reverse-phase μBondapak C_{18} (3.9 × 300 mm) using a linear gradient of 0.05 M triethylammonium acetate (pH 7.0) containing 10−20% acetonitrile with an elution time of 60 min at a flow rate of 1.0 mL/min as described previously (*21*). DNA templates and primers were further purified by electrophoresis on 20% polyacrylamide gel in the presence of 7 M urea (35 × 42 × 0.04 cm) (*22*). The oligomers recovered from PAGE were again subjected to HPLC to remove urea. Oligonucleotides were labeled at the 5′ terminus by treating with T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP (*23*) and subjected to electrophoresis to establish homogeneity. The position of the oligomers was established by autoradiography using Kodak Xomat XAR film.

Primer Extension Reactions. Using a ^{32}P-labeled 10-mer (0.5 pmol, sequence 5 in Table 2) primed with an unmodified or 8-MedG-modified 24-mer template (1.0 pmol, sequence 2), primer extension reactions catalyzed by exo$^-$ Klenow fragment or pol α were carried out at 25 °C in 10 mL of a buffer containing all four dNTPs (100 μM each) (*19*). The reaction buffer for exo$^-$ Klenow fragment contained 50 mM Tris-HCl (pH 8.0), 8 mM $MgCl_2$, and 5 mM 2-mercaptoethanol. The buffer for pol α contained 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 2 mM dithiothreitol, and BSA (0.5 μg/μL). Reactions were stopped by adding formamide dye and heating to 95 °C for 3 min. Samples were subjected to electrophoresis on a 20% polyacrylamide gel containing 7 M urea (35 × 42 × 0.04 cm). Bands were identified by autoradiography and excised from the gel, and the radioactivities were measured by a Wallac liquid scintillation counter.

Quantitation of Miscoding Specificity. One microgram of 8-MedG-modified 24-mer (sequence 2 in Table 2) was phosphorylated at the 5′ terminus using 7 μL of T4 kinase and 10 mM ATP (*23*) and then ligated to a 14-mer (1.2 μg, sequence 3) at 8 °C overnight using 3 μL of T4 DNA ligase, 2 μL of 10 mM ATP, and an 18-mer template (1.5 μg, sequence 4). The resultant 38-mer (sequence 1) was isolated by HPLC as described above (*21*) and used as a DNA template. Using a 38-mer template (1.0 pmol) primed with a ^{32}P-labeled 12-mer (0.5 pmol, sequence 6), primer extension reactions were conducted at 30 °C for 1 h in the presence of four dNTPs, using 0.05 unit of exo$^-$ Klenow fragment or 2.4 unit of pol α as described above. The molar ratios of the exo$^-$ Klenow fragment:template and of pol α:template were estimated to be approximately 1:29 and 1:2, respectively. The reaction samples were subjected to 20% PAGE containing 7 M urea (35 × 42 × 0.04 cm). The fully-extended products were recovered from the gel, annealed with an unmodified 38-mer (sequence 1), and incubated with *Eco*RI restriction enzyme (100 units) at 30 °C for 1 h and subsequently at 15 °C for 1 h to cleave completely as shown in Figure 2. To quantify all base substitutions and deletions, the samples were subjected to electrophoresis on two-phase 20% polyacrylamide gels (15 × 72 × 0.04 cm) containing 7 M urea in the upper phase and no urea in the lower phase (*17*).

Measurement of T_m. Melting temperature (T_m) of oligodeoxynucleotide duplex containing 8-MedG was measured under the three different concentrations (1.1, 2.2, and 3.3 μM), using 12-mer oligodeoxynucleotide (Table 3). The sequence of the 12-mer was selected from the c-Ha-*ras* gene (*24*); 8-MedG is inserted in the first position of codon 12. Oligodeoxynucleotides were dissolved in a 10 mM sodium cacodylate buffer (pH 7.0) containing 100 mM NaCl and 1 mM EDTA, heated at 80 °C for 10 min with complementary strand where the nucleotide opposite 8-MedG was dC, dA, dT, or dG, and then cooled gradually to room temperature for the annealing. Thermal denaturation curves were recorded at 260 nm on a UV spectrometer with a temperature controller, and the melting temperature was evaluated. van't Hoff transition enthalpies ($\Delta H°$), entropies ($\Delta S°$), and free energies ($\Delta G°$) were determined by calculations based on the slope and intercept of a $1/T_m$ versus $\ln(C_t/4)$ plot of the following equations (*25*):

$$1/T_m = (R/\Delta H°) \ln(C_t/4) + \Delta S°/\Delta H°$$

$$\Delta G° = \Delta H° - T\Delta S°$$

where C_t is the total concentration of single strands.

Table 1. Product Analysis

condition[a]	% [b] dG	8-MedG	Gua	8-MeGua
1 N H_2SO_4				
0 °C, 5 min	22.3	59.4	9.8	8.5
0 °C, 7.5 min	12.5	52.6	15.2	19.7
0 °C, 10 min	9.4	44.8	17.2	28.7
25 °C, 5 min	4.6	35.0	19.5	40.9
0.1 N H_2SO_4				
0 °C, 5 min	92.5	7.5	0	0
0 °C, 10 min	91.1	8.9	0	0
0 °C, 35 min	88.0	5.3	6.7	0
25 °C, 5 min	75.4	14.2	7.8	2.6

[a] To the solution of dG (20 mg) and $FeSO_4 \cdot 7H_2O$ (83 mg) in 4 mL of 1 or 0.1 N H_2SO_4 with stirring was added dropwise a solution of *tert*-butyl hydroperoxide (80%, 25 mg) in 0.4 mL of H_2O during 5 min at 0 or 25 °C. The mixture was then left standing for 0−30 min at the same temperature. At the indicated time (5 min for addition plus standing time), an aliquot was taken and neutralized with aqueous 1 N NaOH. The formation of products formed was analyzed by HPLC. [b] Yield was expressed as mol %.

Results

Synthesis of 8-MedG Derivatives. 8-Methylguanosine was reported to be formed directly from Gua by radical methylation using the *tert*-butyl hydroperoxide−$FeSO_4$ system in 1 N H_2SO_4 at room temperature for about 30 min (*11*). When the same reaction conditions were used to prepare 8-MedG, the glycosidic bond of 8-MedG was readily cleaved (data not shown). To obtain optimum conditions for the synthesis of 8-MedG, the formation of 8-MedG was measured under various reaction conditions using HPLC (Table 1). When 1 N H_2SO_4 was used, the maximum yield (60%) of 8-MedG was observed at 0 °C for 5 min incubation. Longer reaction times and higher reaction temperatures decreased the yield of 8-MedG. Weakly acidic reaction conditions also decreased the amount of 8-MedG formation. Purification using column chromatography resulted in a tolerable yield of 8-MedG (29%).

Preparation of Oligomer Containing 8-MedG. 8-MedG (**1**) was isobutyrylated at the 2-NH_2 group to give a 77% yield of N^2-isobutyryl-8-methyl-2′-deoxyguanosine (**2**), and the subsequent dimethoxytritylation at the 5′-*O*-position gave 5′-*O*-(4,4′-dimethoxytrityl)-N^2-isobutyryl-8-methyl-2′-deoxyguanosine (**3**) (75%). The phosphoramidite of compound **3** (**4**) was prepared by the established protocol (*26*). Compound **4** was used for the synthesis of the following 8-MedG-modified oligomers by solid phase DNA synthesizer: 5′CCTTCXCTACTTTCCTCTCCATTT (sequence 2 in Table 2) and $\overline{5'}$GCGCCXGCGGTG, where X is 8-MedG. The 24-mer was used for the experiment

Table 2. Sequence of Oligodeoxynucleotides[a]

Number	Sequence
	5′ 3′
1	CATGCTGATGAATTCCTTCXCTACTTTCCTCTCCATTT
2	CCTTCXCTACTTTCCTCTCCATTT
3	CATGCTGATGAATT
4	GTAGCGAAGGAATTCATC
5	AGAGGAAAGT
6	AGAGGAAAGTAG
7	AGAGGAAAGTAGNGAAGG
8	AGAGGAAAGTAGGAAGG
9	AGAGGAAAGTAGAAGG

[a] Sequence of templates, primers, and standard markers. X = 8-MedG or dG; N = dC, dA, dG, or dT.

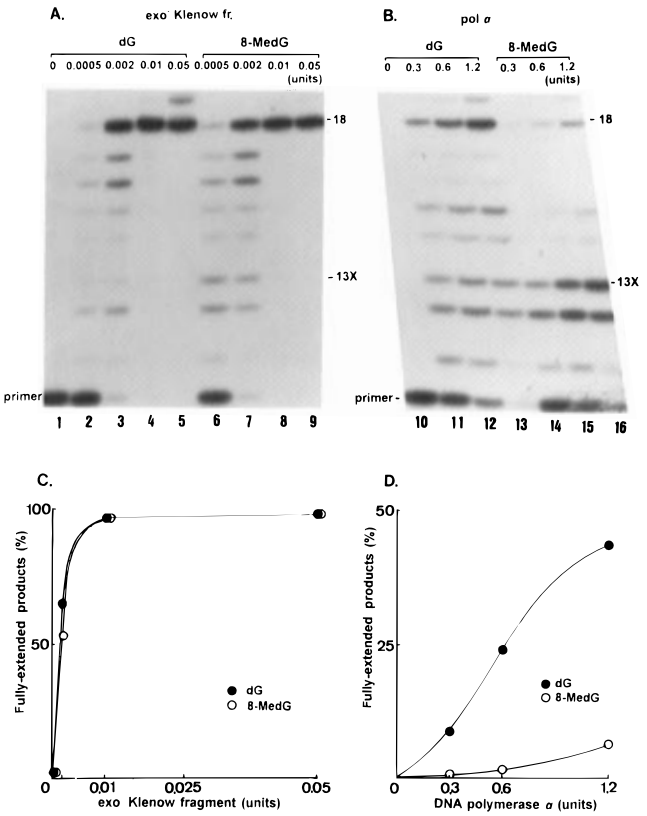


Figure 1. Primer extension reactions catalyzed by DNA polymerases on 8-MedG-modified template. Using an unmodified or 8-MedG-modified 24-mer template (sequence 2 in Table 2) primed with a ^{32}P-labeled 10-mer (sequence 5), primer extension reactions were conducted at 25 °C for 1 h using a variable amount of the exo$^-$ Klenow fragment (A) or pol α (B) as described under Experimental Procedures. One-third of the reaction mixture was subjected to denaturing 20% polyacrylamide gel electrophoresis (35 × 42 × 0.04 cm). The bands of fully-extended products observed in (A) and (B) were cut from PAGE, and the radioactivities were measured by a liquid scintillation counter. Exo$^-$ Klenow fragment (C) and pol α (D).

of the miscoding specificity, and the 12-mer was for the measurements of T_m.

Miscoding Specificity of Oligodeoxynucleotide Containing 8-MedG. Primer extension reactions catalyzed by the exo$^-$ Klenow fragment of *E. coli* DNA polymerase I were conducted in the presence of four dNTPs (Figure 1A). Using an unmodified template (sequence 2, X = dG in Table 2), primer extension occurred smoothly to form the fully-extended products. On the 8-MedG-modified template (sequence 2, X = 8-MedG), the primer extension was not blocked at the lesion and readily formed the fully-extended products. The amount of fully-extended products formed on an

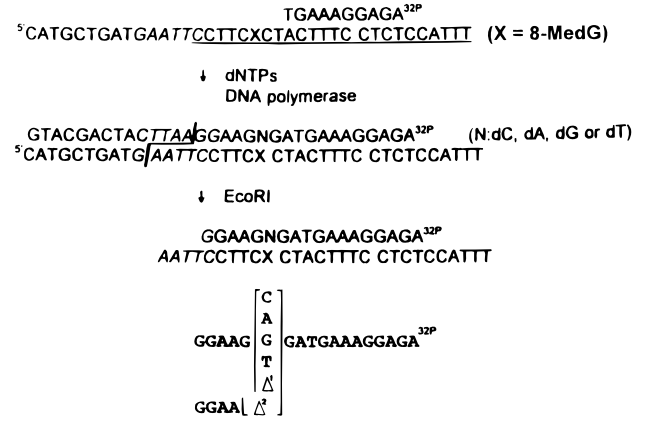


Figure 2. Diagram of the primer extension methods and analysis of reaction products.

unmodified template was similar to that of the 8-MedG template (Figure 1C). In contrast, when pol α, a mammalian replicative enzyme, was used, the primer extension reactions were retarded at the position of the 8-MedG lesion (Figure 1B); a portion of the primers passed the lesion forming the fully-extended products. When 0.6 and 1.2 units of pol α were used, the amounts of fully-extended products formed on the 8-MedG-modified template were 14 and 6.8 times lower, respectively, than that of unmodified template (Figure 1D).

When a large amount of enzyme was used for the primer extension reaction, a blunt-end addition reaction (*27*, *28*) occurred at the 3′ terminus, as observed in Figure 1A,B (lanes 5, 13, and 16). The formation of blunt-end products failed to quantify base substitutions and deletions (*17*). Using a 38-mer template (sequence 1 in Table 2) instead of 24-mer (sequence 2), fully-extended reaction products were cleaved with *Eco*RI restriction enzyme and subjected to a two-phase gel electrophoresis for quantifying the miscoding specificity of 8-MedG (Figure 2). The standard mixture of six ^{32}P-labeled oligodeoxynucleotides containing dC, dA, dG, or dT opposite the lesion and one- or two-base deletion are completely resolved by this method (Figure 3A, lanes 1 and 4). When the exo$^-$ Klenow fragment (lane 2) or pol α (data not shown) was used, DNA synthesis on an unmodified template leads to the expected incorporation of dCMP opposite dG. Using the exo$^-$ Klenow fragment, dCMP (77.0%) was preferentially incorporated opposite 8-MedG, along with the incorporation of dGMP (1.10%), dAMP (0.41%), and one- (0.38%) and two-base (0.81%) deletions (lane 3). When pol α was used, the incorporation of dCMP (78.3%) opposite 8-MedG was also detected predominantly with small amounts of incorporation of dGMP (0.82%) and dAMP (0.38%) (lane 5).

Thermodynamic Stability of Base Pairs Containing 8-MedG. An unmodified or 8-MedG-modified 12-mer was annealed with complementary strands (Table 3). The melting temperatures (T_ms) were measured under the three different concentrations (1.1, 2.2, and 3.3 μM). Plots of $1/T_m$ versus $\ln(C_t/4)$ showed a straight line (data not shown), and the thermodynamic parameters ($\Delta H°$, $\Delta S°$, and $\Delta G°$) were calculated using the equation reported previously (*25*). The T_ms and thermodynamic parameters obtained are summarized in Table 3. The correct base pair, dG:dC, showed the highest T_m (67.3 °C). The 8-MedG:dC showed a higher T_m than that of the other pairs with 8-MedG. The T_m of 8-MedG:dG was 2.5 °C higher than that of the mismatched dG:dG pair.

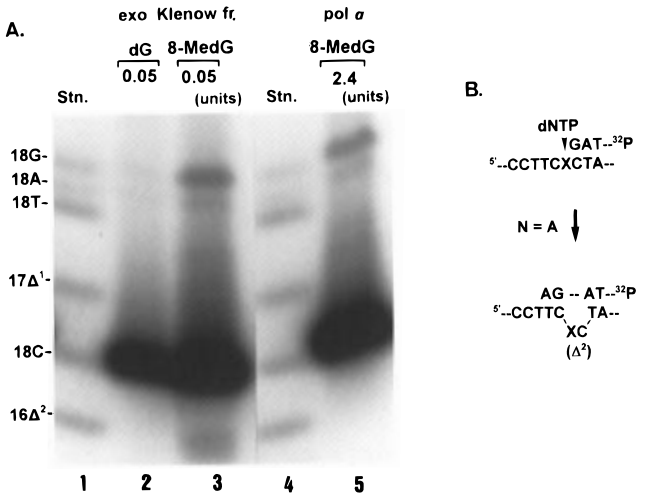


Figure 3. Quantitation of miscoding specificities. (A) Using a 38-mer template (sequence 1 in Table 2) primed with a 5′-^{32}P-labeled 12-mer (sequence 6), primer extension reactions were conducted for 1 h at 30 °C, using 0.05 unit of exo⁻ Klenow fragment for the unmodified and 8-MedG-modified templates and 2.4 unit of pol α for 8-MedG-modified template. The fully-extended products recovered from PAGE were cleaved by *Eco*RI restriction enzyme at 30 °C for 1 h and subsequently at 15 °C for 1 h as described under Experimental Procedures. The reaction samples were subjected to a two-phase 20% polyacrylamide gel electrophoresis (15 × 72 × 0.04 cm). Mobilities of reaction products were compared with those of 18-mer standards (sequences 7−9) containing dC, dA, dG, or dT opposite the lesion and one-base (Δ^1) or two-base (Δ^2) deletions (lanes 1 and 4). (B) Proposed mechanism for two-base deletion.

Table 3. Melting Temperatures (T_m) and Thermodynamic Parameters for Modified and Unmodified Oligodeoxynucleotide Duplexes

5′GCGCCXGCGGTG3′
3′CGCGGNCGCCAC5′
(X = 8-MedG or dG, N = dC, dA, dT or dG)

base pair[a]	T_m[b] (°C)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/Kmol)	$\Delta G°_{298}$ (kcal/mol)
GC	67.3	−110.4	−299	−21.4
GA	60.4	−58.4	−149	−14.1
GT	58.5	−66.3	−174	−14.5
GG	55.4	−68.5	−182	−14.3
G̲C	61.8	−90.3	−244	−17.8
G̲A	55.6	−86.4	−236	−16.1
G̲T	53.1	−79.5	−218	−14.6
G̲G	57.9	−86.4	−234	−16.6

[a] G̲ = 8-MedG. [b] The concentration of duplexes was 3.3 μM.

However, the other pairs with 8-MedG showed lower T_ms than the corresponding pairs with unmodified dG. Although the $\Delta G°$ value of 8-MedG:dC was lower than that of the other pairs with 8-MedG, the $\Delta G°$ values of 8-MedG:dG and 8-MedG:dA were 2.3 and 2.0 kcal/mol lower than that of mismatched dG:dG and dG:dA, respectively.

Discussion

8-MedG was reported to be prepared from 8-methylguanine by enzymatic deoxyribosylation (*29*, *30*), but the yield was too low to characterize it unequivocally. We have prepared 8-MedG directly from dG using a modification of the radical methylation system (*11*). The phosphoramidite of the N^2- and 5′-*O*-diprotected 8-MedG was used for the synthesis of oligodeoxynucleotides containing 8-MedG using a solid phase DNA synthesizer. These results are consistent with a recently reported synthesis of oligodeoxynucleotides containing 8-MedG, where 3′,5′-di-*O*-acetyl-2′-deoxyguanosine (*31*) or N^2-isobutyryl-2′-deoxyguanosine (*32*) was used as a substrate for radical methylation.

Primer extension reactions catalyzed by the exo⁻ Klenow fragment of *E. coli* DNA polymerase I or mammalian pol α were conducted in the presence of all four dNTPs. When the exo⁻ Klenow fragment was used, the primer was extended readily past 8-MedG, forming fully-extended products, similar to that observed with the unmodified template (Figure 1A,C). However, primer extension by pol α was retarded at the position opposite 8-MedG (Figure 1B,D). These results indicate that the extension process past 8-MedG is different depending on the DNA polymerase used.

Using a modified *in vitro* experimental system (Figure 2), base substitutions and deletions induced by 8-MedG were quantified (*17*). Both exo⁻ Klenow fragment and pol α incorporated predominantly dCMP, the correct base, opposite 8-MedG. In addition, small amounts of incorporation of dGMP (0.82−1.10%) and dAMP (0.38−0.41%) opposite the lesion were detected (Figure 3A). The polymerase activity of DNA polymerase III, an *E. coli* replicative enzyme, is carried out by the α subunit (*33*). When the α subunit of pol III was used, dCMP only was incorporated opposite 8-MedG (data not shown). Using a similar experimental condition with 0.05 unit of the exo⁻ Klenow fragment, one of the authors (S.S.) reported that O^6-methyl-2′-deoxyguanosine (O^6MedG) directed incorporation of dTMP (75.5%), along with small amounts of incorporation of dCMP (5.27%) (*17*). When pol α (2.5 units) was used, incorporation of dTMP (24.2%) and dCMP (4.78%) opposite O^6MedG was observed (*17*). Thus, the miscoding specificity of 8-MedG differs from that of O^6MedG. The miscoding frequencies of 8-MedG were 25−50 times less than that of O^6MedG. Since the amount of 8-MedG induced by 1,2-dimethylhydrazine in tissue DNA of rats was similar to that of O^6MeG (*13*), 8-MedG may be weakly mutagenic predicting the generation of G → C and G → T transversions in cells.

Two-base deletion was observed opposite 8-MedG when the exo⁻ Klenow fragment was used (Figure 3A). Based on studies with several DNA adducts, a general mechanism of frameshift deletion was proposed (*18*). When the extension of the newly inserted dNMP opposite the lesion is blocked, the newly inserted dNMP and/or its 5′ flanking bases in the primer could pair with bases 5′ to the lesion in the template to form deletions (*18*). Since a small amount of dAMP was inserted opposite 8-MedG under the reaction conditions containing a single dNTP (data not shown), the newly inserted dAMP and its 5′ flanking G could pair with C 5′ to the lesion in the template to form two-base deletion (Figure 3B).

Methyl radicals and hydroxy radicals attack at the C8-position of dG in duplex DNA to create 8-MedG (*12*, *34*) and 7,8-dihydro-8-oxodG (*35*, *36*), respectively. 7,8-Dihydro-8-oxodG is a mutagenic lesion (*37*−*40*) and promotes the incorporation of dCMP and dAMP opposite the lesion during DNA synthesis; the ratio of dCMP and dAMP varies depending on the DNA polymerase used (*19*). Since this adduct exists predominantly as the 6,8-diketo form (8-oxodG) under physiological conditions (*41*, *42*), 8-oxodG could be in the *anti* conformation to pair with dCMP, forming a Watson−Crick base pair (*43*); alternatively, 8-oxodG could be in the *syn* conformation to pair with dAMP, forming a Hoogsteen base pair (*44*). However, since 8-MedG can not exist as the other

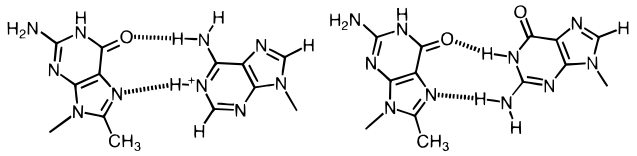


Figure 4. Proposed structures of 8-MedG:dG and 8-MedG:dA pairs.

tautomers, the miscoding specificities of 8-MedG may differ from that of 8-oxodG and the miscoding frequencies of 8-MedG may be less than that of 8-oxodG.

The miscoding properties of 8-MedG are quite similar to that of *N*-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF), which permitted incorporation of dCMP only in reactions catalyzed by exo$^-$ and exo$^+$ Klenow fragment (*17*, *18*, *45*) and promoted predominant incorporation of dCMP, along with small amounts of dAMP, dGMP, and dTMP, and two-base deletions in reactions catalyzed by pol α (*46*). Based on NMR and computational characterization, dG-C8-AF is in the *anti* conformation to pair with dCMP, forming a Watson−Crick base pair (*47*, *48*), while dG-C8-AF is in the *syn* conformation to pair with dAMP (*49*) or dGMP (*50*). 8-MedG may exist primarily in the *anti* conformation to pair with dCMP. Since 8-MedG can also be in the *syn* conformation in duplex oligomers (*32*), the *syn* conformation of 8-MedG may pair with dAMP or dGMP, as proposed in Figure 4.

The pair of 8-MedG:dG showed higher T_m and lower $\Delta G°$ values than the corresponding dG:dG (Table 3). Although the T_m of 8-MedG:dA was lower than that of dG:dA, the $\Delta G°$ value of 8-MedG:dA was lower than that of dG:dA. These data indicate that 8-MedG:dA and 8-MedG:dG are thermodynamically more stable than the corresponding mismatched base pairs. Since the T_m of 8-MedG:dC was the highest and the $\Delta G°$ value of 8-MedG:dC was the lowest among the duplexes containing 8-MedG (Table 3), 8-MedG:dC is thermodynamically more stable than the other pairs with 8-MedG. Although the sequence context of oligomer used for thermodynamic study was different from that used for the *in vitro* mutagenesis study, the $\Delta G°$ values inversely parallel the frequency of base incorporation opposite 8-MedG as follows: dCMP > dGMP > dAMP ≫ dTMP. Thus, the thermodynamic stabilities may correlate to the miscoding specificities induced by 8-MedG.

Small amounts of misincorporation of dGMP and dAMP were observed opposite 8-MedG. The thermodynamic data support the pairing of 8-MedG with dGMP and dAMP. Our results demonstrate that 8-MedG is a miscoding lesion, predicting G → C and G → T transversions and deletion in cells.

Acknowledgment. We thank Professor Y. Kawazoe of Nagoya City University for his encouragement.

References

(1) Lawley, P. D. (1994) From fluorescence spectra to mutational spectra, a historical overview of DNA-reactive compounds. In *DNA Adducts: Carcinogen & Mutagenic Agents: Chemistry, Identification and Biological Significance* (Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerback, D., and Bartsch, H., Eds.) IARC Scientific Publication No. 125, pp 3−22, IARC, Lyon, France.

(2) Druckrey, H. (1970) Production of colonic carcinomas by 1,2-dialkylhydrazines and azoalkanes. In *Carcinoma of the Colon and Antecedent Epithelium* (Burdette, W. J., Ed.) pp 267−279, Charles C. Thomas Publisher, Springfield, IL.

(3) Hawks, A., and Magee, P. N. (1974) The alkylation of nucleic acids of rat and mouse *in vivo* by the carcinogen 1,2-dimethylhydrazine. *Br. J. Cancer* **30**, 440−447.

(4) Rogers, K. J., and Pegg, A. E. (1977) Formation of O^6-methylguanine by alkylation of rat liver, colon, and kidney DNA following administration of 1,2-dimethylhydrazine. *Cancer Res.* **37**, 4082−4087.

(5) Herron, D. C., and Shank, R. C. (1981) *In vivo* kinetics of O^6-methylguanine and 7-methylguanine formation and persistence in DNA of rats treated with symmetrical dimethylhydrazine. *Cancer Res.* **41**, 3967−3972.

(6) Singer, B. (1975) The chemical effects of nucleic acid alkylation and their relation to mutagenesis and carcinogenesis. *Prog. Nucleic Acid Res. Mol. Biol.* **15**, 219−284.

(7) Kang, J. O., Slater, G., Aufses, A. H., Jr., and Cohen, G. (1988) Production of ethane by rats treated with the colon carcinogen, 1,2-dimethylhydrazine. *Biochem. Pharmacol.* **37**, 2967−2971.

(8) Augusto, O., Du Plessis, L. R., and Weingrill, C. L. (1985) Spin-trapping of methyl radical in the oxidative metabolism of 1,2-dimethylhydrazine. *Biochem. Biophys. Res. Commun.* **126**, 853−858.

(9) Netto, L. E., Leite, L. C., and Augusto, O. (1988) Hemoglobin-mediated oxidation of the carcinogen 1,2-dimethylhydrazine to methyl radicals. *Arch. Biochem. Biophys.* **266**, 562−572.

(10) Albano, E., Tomasi, A., Goria-Gatti, L., and Iannone, A. (1989) Free radical activation of monomethyl and dimethyl hydrazine in isolated hepatocytes and liver microsomes. *Free Radical Biol. Med.* **6**, 3−8.

(11) Maeda, M., Nushi, K., and Kawazoe, Y. (1974) Studies on chemical alterations of nucleic acids and their components VII. C-Alkylation of purine bases through free radical process catalyzed by ferrous ion. *Tetrahedron* **30**, 2677−2682.

(12) Augusto, O., Cavalieri, E. L., Rogan, E. G., RamaKrishna, N. V. S., and Kolar, C. (1990) Formation of 8-methylguanine as a result of DNA alkylation by methyl radicals generated during horseradish peroxidase-catalyzed oxidation of methylhydrazine. *J. Biol. Chem.* **265**, 22093−22096.

(13) Netto, L. E. S., RamaKrishna, N. V. S., Kolar, C., Cavalieri, E. L., Rogan, E. G., Lawson, T. A., and Augusto, O. (1992) Identification of C^8-methylguanine in the hydrolysates of DNA from rats administered 1,2-dimethylhydrazine: evidence for *in vivo* alkylation by methyl radicals. *J. Biol. Chem.* **267**, 21524−21527.

(14) Loveless, A. (1969) Possible relevance of O-6 alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. *Nature* **223**, 206−207.

(15) Cook, M. G., and McNamara, P. (1980) Effect of dietary vitamin E on dimethylhydrazine-induced colonic tumors in mice. *Cancer Res.* **40**, 1329−1331.

(16) Jacobs, M. M., Forst, C. F., and Beams, F. A. (1981) Biochemical and clinical effects of selenium on dimethylhydrazine-induced colon cancer in rats. *Cancer Res.* **41**, 4458−4465.

(17) Shibutani, S. (1993) Quantitation of base substitutions and deletions induced by chemical mutagens during DNA synthesis *in vitro*. *Chem. Res. Toxicol.* **6**, 625−629.

(18) Shibutani, S., and Grollman, A. P. (1993) On the mechanism of frameshift (deletion) mutagenesis *in vitro*. *J. Biol. Chem.* **268**, 11703−11710.

(19) Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* **349**, 431−434.

(20) Takeshita, M., Chang, C.-N., Johnson, F., Will, S., and Grollman, A. P. (1987) Oligodeoxynucleotides containing synthetic abasic sites. *J. Biol. Chem.* **262**, 10171−10179.

(21) Shibutani, S., Gentles, R., Johnson, F., and Grollman, A. P. (1991) Isolation and characterization of oligodeoxynucleotides containing dG-N^2-AAF and oxidation products of dG-C8-AF. *Carcinogenesis* **12**, 813−818.

(22) Shibutani, S., Bodepudi, V., Johnson, F., and Grollman, A. P. (1993) Translesional synthesis on DNA templates containing 8-oxo-7,8-dihydrodeoxyadenosine. *Biochemistry* **32**, 4615−4621.

(23) Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

(24) Koizume, S., Kamiya, H., Inoue, H., and Ohtsuka, E. (1994) Synthesis and thermodynamic stabilities of damaged DNA involving 8-hydroxyguanine (7,8-dihydro-8-oxoguanine) in a *ras* gene fragment. *Nucleosides Nucleotides* **13**, 1517−1534.

(25) Marky, L. A., and Breslauer, K. J. (1987) Calculating thermodynamic data for transitions of any molecularity from equilibrium melting curves. *Biopolymers* **26**, 1601−1620.

(26) Beaucage, S. L. (1993) Oligodeoxyribonucleotides synthesis. Phosphoramidite approach. In *Methods in Molecular Biology, Vol. 20, Protocols for Oligonucleotides and Analogs* (Agrawal, S., Ed.) pp 33−61, Humana Press, Totawa, NJ.

(27) Clark, J. M., Joyce, C. M., and Beardsley, G. P. (1987) Novel blunt-end addition reactions catalyzed by DNA polymerase I of *Escherichia coli*. *J. Mol. Biol.* **198**, 123−127.
(28) Shibutani, S., Takeshita, M., and Grollman, A. P. Translesional synthesis on DNA templates containing a single abasic site. (Submitted to *J. Biol. Chem.*)
(29) Chapeau, M.-C., and Marnett, L. J. (1991) Enzymatic synthesis of purine deoxynucleoside adducts. *Chem. Res. Toxicol.* **4**, 636−638.
(30) Humphreys, W. G., Kadlubar, F. F., and Guengerich, F. P. (1992) Mchanism of C8 alkylation of guanine residues by activated arylamines: evidence for initial adduct formation at the N7 position. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8278−8282.
(31) Krawczyk, S. H., Bischofberger, N., Griffin, L. C., Law, V. S., Shea, R. G., and Swaminathan, S. (1995) Structure−activity study of oligodeoxynucleotides which inhibit thrombin. *Nucleosides Nucleotides* **14**, 1109−1116.
(32) Sugiyama, H., Kawai, K., Matsunaga, A., Fujimoto, K., Saito, I., Robinson, H., and Wang, A. H.-J. (1996) Synthesis, structure and thermodynamic properties of 8-methylguanine-containing oligonucleotides: Z-DNA under physiological salt conditions. *Nucleic Acids Res.* **24**, 1272−1278.
(33) Maki, H., and Kornberg, A. (1985) The polymerase subunit of DNA polymerase III of Escherichia coli. *J. Biol. Chem.* **260**, 12987−12992.
(34) Hix, S., Morais, M. S., and Augusto, O. (1995) DNA methylation by *tert*-butyl hydroperoxide−iron(II). *Free Radical Biol. Med.* **19**, 293−301.
(35) Kasai, H., and Nishimura, S. (1984) Hydroxylation of deoxyguanosine at the C-8 position by ascorbic acid and other reducing agents. *Nucleic Acids Res.* **12**, 2137−2145.
(36) Halliwell, B., and Aruoma, O. I. (1991) DNA damage by oxygen-derived species: its mechanism and measurement in mammalian systems. *FEBS Lett.* **281**, 9−19.
(37) Wood, M. L., Dizdaroglu, M., Gajewski, E., and Essigmann, J. M. (1990) Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. *Biochemistry* **29**, 7024−7032.
(38) Moriya, M., Ou, C., Bodepudi, V., Johnson, F., Takeshita, M., and Grollman, A. P. (1991) Site-specific mutagenesis using a gapped duplex vector: a study of translesional synthesis past 8-oxodeoxyguanosine in *E. coli*. *Mutat. Res.* **254**, 281−288.
(39) Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G → T and A → C substitutions. *J. Biol. Chem.* **267**, 166−172.
(40) Moriya, M. (1993) Single strand shuttle phagemid for mutagenesis studies in mammalian cells: 8-oxoguanine in DNA induces targeted G:C → T:A transversion in simian kidney cells. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 1122−1126.
(41) Aida, M., and Nishimura, S. (1987) An ab initio molecular orbital study on the characteristics of 8-hydroxyguanine. *Mutat. Res.* **192**, 83−89.
(42) Culp, S. J., Cho, B. P., Kadlubar, F. F., and Evans, F. E. (1989) Structural and conformational analyses of 8-hydroxy-2′-deoxyguanosine. *Chem. Res. Toxicol.* **2**, 416−422.
(43) Oda, Y., Uesugi, S., Ikehara, M., Kawase, Y., and Ohtsuka, E. (1991) NMR studies of a DNA containing 8-hydroxydeoxyguanosine. *Nucleic Acids Res.* **19**, 5263−5267.
(44) Kouchakdjian, M., Bodepudi, V., Shibutani, S., Eisenberg, M., Johnson, F., Grollman, A. P., and Patel, D. J. (1991) NMR structural studies of the ionizing radiation adduct 7-hydro-8-oxodeoxyguanosine (8-oxo-7H-dG) opposite deoxyadenosine in a DNA duplex: 8-oxo-7H-dG(syn)-dA(anti) alignment at lesion site. *Biochemistry* **30**, 1403−1412.
(45) Michaels, M. L., Reid, T. M., King, C. M., and Romano, L. J. (1991) Accurate *in vitro* translesion synthesis by *Escherichia coli* DNA polymerase I (large fragment) on a site-specific aminofluorene-modified oligonucleotide. *Carcinogenesis* **12**, 1641−1646.
(46) Shibutani, S., and Grollman, A. P. Molecular mechanisms of mutagenesis by aromatic amines and amides. *Mutat. Res.* (in press).
(47) Cho, B. P., Beland, F. A., and Marques, M. M. (1994) NMR structural studies of a 15-mer DNA duplex from a ras protooncogene modified with the carcinogen 2-aminofluorene: conformational heterogeneity. *Biochemistry* **33**, 1373−1384.
(48) Eckel, L. M., and Krugh, T. R. (1994) Structural characterization of two interchangeable conformations of N-2-acetylaminofluorene modified DNA oligomer by NMR and energy minimization. *Biochemistry* **33**, 13611−13624.
(49) Norman, D., Abuaf, P., Hingerty, B. E., Live, D., Grunberger, D., Broyde, S., and Patel, D. J. (1989) NMR and computational characterization of the *N*-(deoxyguanosin-8-yl)aminofluorene adduct [(AF)G] opposite adenosine in DNA: (AF)G[syn]:A[anti] pair formation and its pH dependence. *Biochemistry* **28**, 7462−7476.
(50) Abuaf, P., Hingerty, B. E., Broyde, S., and Grunberger, D. (1995) Solution conformation of the *N*-(deoxyguanosin-8-yl)aminofluorene adduct opposite deoxyinosine and deoxyguanosine in DNA by NMR and computational characterization. *Chem. Res. Toxicol.* **8**, 369−378.

TX9601059