Guanine Specific DNA Cleavage by Photoirradiation of Dibenzoyldiazomethane−Oligonucleotide Conjugates

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Abstract: Photoirradiation of dibenzoyldiazomethane (DBDM) produced highly electrophilic benzoylketene via Wolff rearrangement. DBDM derivative possessing an aminoalkyl side chain induced a DNA cleavage selectively at guanine (G) residues upon photoirradiation and subsequent piperidine treatment. In order to devise photochemical DNA cleavers that can specifically alkylate a guanine residue proximal to the target sequence of long DNA fragments, a new reagent, DBDM-OSu, which facilitates the connection of DBDM unit to various DNA binders, was developed. DBDM-oligonucleotide (ODN) conjugates **5** and **6** were obtained by the coupling of 5′-aminohexyl 8-mer [H_2N-$(CH_2)_6$-d(ACGTCAGG)-3′] and 15-mer [H_2N-$(CH_2)_6$-d(ACGTCAGGTGGCACT)-3′], respectively, with DBDM-OSu in aqueous acetonitrile in the presence of sodium bicarbonate. Photoirradiation of **5** and **6** in the presence of 25-mer 5′-d(AGTGCCACCTGACGTCTG$_{18}$CTCTCTC)-3′ having a complementary sequence induced cross-linking of both oligomers. A distinct cleavage band at guanine residue (G_{18}) was observed upon heating the cross-linked oligomers with piperidine. A similar DNA cleavage reaction of 5′-d(AGTGCCACCTGACG$_{14}$TG$_{16}$CG$_{18}$TG$_{20}$CG$_{22}$-TCT)-3′ having multiple guanine sites in the presence of DBDM-ODN conjugate **6** indicated that the most effectively cleaved site is G_{16}. These results demonstrated that DBDM-oligonucleotide conjugates can serve as a new class of photonucleases that can cleave single-stranded DNA at predetermined guanine sites. Furthermore, the reagent DBDM-OSu can be used as a convenient and effective photoinducible electrophile for the cross-linking or the modification of biopolymers.

Introduction

Much current interest has been focused on the design of artificial DNA-cleaving molecules that are chemically stable and activatable by photoirradiation, particularly by a pulse of light.[1] The advantage of such photoactivatable DNA-cleaving molecules is that their action can be controlled within time and space by choosing proper irradiation methods. Various strategies for photoinduced DNA cleavage have been intensively investigated. These included a generation of active oxygen species[2−4] or photoreactive metal center,[5] an electron transfer from DNA nucleobases,[6−12] hydrogen abstraction from DNA sugar backbone by photogenerated radicals,[13−19] and a photoinduced DNA alkylation.[20−22] Among these approaches DNA

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(1) For recent reviews on photoinduced DNA cleavage, see: (a) Saito, I.; Nakatani, K. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 3007−3019. (b) *Bioorganic Photochemistry, Photochemistry and the Nucleic Acids;* Morrison, H., Ed.; John Wiley and Sons: New York, 1990; Vol. 1.

(2) (a) Kawanishi, S.; Inoue, S.; Sano, S.; Aiba, H. *J. Biol. Chem.* **1986**, *261*, 6090−6095. (b) Blazek, E. R.; Peak, J. G.; Peak, M. J. *Photochem. Photobiol.* **1989**, *49*, 607−613. (c) Schneider, J. E.; Price, S. Maidlt, L.; Gutteridge, J. M. C.; Floyd, R. A. *Nucleic Acids Res.* **1990**, *18*, 631−635. (d) Buchko, G. W.; Cadet, J.; Berger, M.; Ravanat, J.-L. *Nucleic Acids Res.* **1990**, *18*, 4847−4851. (e) Devasagayam, T. P. A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, *30*, 6283−6289. (f) Sies, H.; Menck, C. F. M. *Mutat. Res.* **1992**, *275*, 367−375. (g) Ravanat, J.-L.; Cadet, J. *Chem. Res. Toxicol.* **1995**, *8*, 379−388.

(3) (a) Saito, I.; Takayama, M.; Matsuura, T.; Matsugo, S.; Kawanishi, S. *J. Am. Chem. Soc.* **1990**, *112*, 883−884. (b) Matsugo, S.; Kawanishi, S.; Yamamoto, K.; Sugiyama, H.; Matsuura, T.; Saito, I. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1351−1353. (c) Saito, I. *Pure Appl. Chem.* **1992**, *64*, 1305−1310.

(4) For reactions of oxygen radicals with DNA, see a review: Breen, A. P.; Murphy, J. A. *Free Radical Biol. Med.* **1995**, *18*, 1033−1077.

(5) (a) Barton, J. K. *Science* **1986**, *233*, 727−734. (b) Lee, I.; Barton J. K.; *Biochemistry*, **1993,** *32*, 6121−6127. (c) Sitlani, A; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303−2312.

(6) (a) Steenken, S. *Chem. Rev.* **1989**, *89*, 503−520. (b) Candeias, L. P.; Steenken, S, *J. Am. Chem. Soc.* **1993**, *115*, 2437−2440.

(7) Dunn, D. A.; Lin, V. H.; Kochevar, I. E. *Biochemistry* **1992**, *31*, 11620−11625.

(8) (a) Sugiyama, H.; Tsutsumi, K.; Fujimoto, K.; Saito, I. *J. Am. Chem. Soc.* **1993**, *115*, 4443−4448. (b) Saito, I.; Takayama, M.; S. Kawanishi, *J. Am. Chem. Soc.* **1995**, *117*, 5590−5591. (c) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406−6407. (d) Saito, I.; Takayama, M.; Sugiyama, H.; Nakamura, T. In *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*; Meunier, B.; Ed: NATO ASI Series; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; pp 163−176. (e) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063−7068.

(9) Ito, K.; Inoue, S.; Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1993**, *268*, 13221−13227.

(10) Cadet, J.; Berger, M.; Bunchko, G. W.; Joshi, R. C.; Raoul, S.; Ravanet, J.-L. *J. Am. Chem. Soc.* **1994**, *116*, 7403−7404.

(11) (a) Armitage, B.; Changjun, Y.; Devadoss, C.; Schuster, G. B. *J. Am. Chem. Soc.* **1994**, *116*, 9847−9859. (b) Breslia, D.; Schuster, G. B. *J. Am. Chem. Soc.* **1996**, *118*, 2313−2319. (c) Ly, D.; Kan, Y.; Armitage, B.; Schuster, G. B. **1996**, *118*, 8747−8748.

(12) Hall, D. B.; Holmlin, R. E.; Barton, J. K. *Nature* **1996**, *382*, 731−735.

(13) Perrouault, L.; Asseline, U.; Rivallet, C.; Thuong, N. T.; Bisagni, E.; Giovannangeli, C.; Le Doan, T.; Hélène, C. *Nature* **1990**, *344*, 358−360.

(14) Nielsen, P. E.; Hirort, C.; Sonnichsen, S. H.; Buchardt, O.; Dahl, O.; Norden, B. *J. Am. Chem. Soc.* **1992**, *114*, 4967−4975.

(15) Quadda, J. C.; Levy, M. J., Jr.; Hecht, S. M. *J. Am. Chem. Soc.* **1993**, *115*, 12171−12172.

(16) (a) Nakatani, K.; Isoe, S.; Maekawa, S.; Saito, I. *Tetrahedron Lett.* **1994**, *35*, 605−608. (b) Saito, I.; Sakurai, T.; Kurimoto, T.; Takayama, M. *Tetrahedron Lett.* **1994**, *35*, 4797−4800. (c) Nakatani, K.; Maekawa, S.; Tanabe, K.; Saito, I. *J. Am. Chem. Soc.* **1995**, *117*, 10635−10644.

(17) Condorelli, G.; Costanzo, L. L.; De Guidi, G.; Giuffrida, S.; Sortino, S. *Photochem. Photobiol.* **1995**, *62*, 155−161.

(18) Matsugo, S.; Nakano, S.; Adachi, K.; Konishi, T. *J. Chem. Soc., Chem. Commun.* **1995**, 311−312.

(19) Wender, P. A.; Touami, S. M.; Alayrac, C.; Philipp. U. C. *J. Am. Chem. Soc.* **1996**, *118*, 6522−6533.

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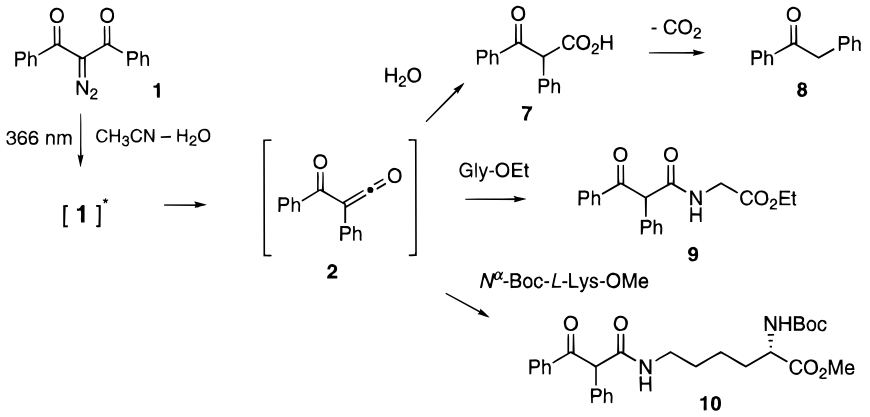
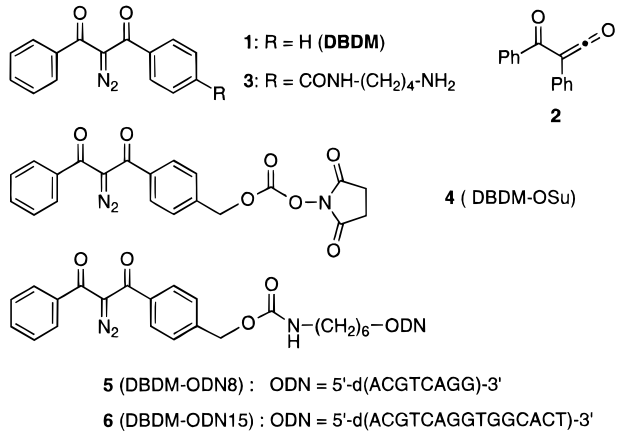
Scheme 1



alkylation by highly electrophilic species generated by light-triggered reactions is particularly attractive, since electrophilic species have an intrinsic reaction selectivity to nucleophilic nucleobases such as guanine and adenine. Photochemical methods for the generation of such electrophiles so far reported are (i) the photodecomposition of arylazide eventually producing azaheptatetraene via aryl nitrene,[20a] (ii) photoheterolysis of quinolylmethylisothioronium salt generating quinolylmethyl carbocation,[20b] (iii) photoenolization of 5-methylnaphthoquinone giving a quinone methide,[21] and (iv) the generation of oxonium cation from hemithioacetal by photoinduced electron transfer.[22]

We focused our attention on chemically stable α-diazocarbonyl compounds as the precursor for such photoinducible electrophile, because α-diazo ketones absorb light at UVA (320−400 nm) region and efficiently undergo Wolff rearrangement to produce electrophilic ketenes.[16a,c] We are especially interested in dibenzoyldiazomethane (**1**, DBDM) which can produce highly electrophilic benzoylketene by Wolff rearrangement.[23,24] It was already demonstrated that benzoylketene **2** produced by photoirradiation of **1** can be effectively trapped by amines in aqueous solvents.[23] Preliminary experiments for DNA cleavage by DBDM derivative **3** at 366 nm irradiation indicated an effective DNA cleavage at 50 μM drug concentration.[23] Encouraged by these results, we have attempted to design a novel type of photonuclease consisting of DBDM-oligonucleotide (ODN) conjugate, which may cleave DNA at predetermined guanine sites.[25,26] We herein disclosed that under photoirradiation conditions DNA cleavage by DBDM derivative **3** having an aminoalkyl side chain occurs selectively at guanine residues after hot piperidine treatment. Upon photoirradiation and subsequent hot piperidine treatment, DBDM-ODN conjugates **5** and **6** cleaved single-stranded DNA at the guanine residues proximal to the target sequence.



5 (DBDM-ODN8) : ODN = 5'-d(ACGTCAGG)-3'

6 (DBDM-ODN15) : ODN = 5'-d(ACGTCAGGTGGCACT)-3'

Results and Discussion

Photoreaction of DBDM with Guanosine Derivatives. Photoirradiation of DBDM **1** at 366 nm in aqueous solvent gave rise to 2-phenylacetophenone **8** by decarboxylation of β-keto acid **7** which was produced by water addition to the photogenerated benzoylketene **2** (Scheme 1).[23] In the presence of amines such as amino acid derivatives like glycine ethyl ester (Gly-OEt) and N^{α}-Boc-*L*-lysine methyl ester (N^{α}-Boc-*L*-Lys-OMe), benzoylketene **2** was shown to be effectively trapped to give the corresponding amides **9** and **10**, respectively.[23] Based on these previous results, we have examined the reaction of guanosine derivatives with ketene **2** produced by photoirradiation of **1**. Photoirradiation of **1** with 3,5-di-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxyguanosine (**11**) in benzene with high pressure Hg lamp through a Pyrex filter produced guanosine derivative **12** in 15% yield (Scheme 2). The thermal generation of ketene from **1** in refluxing benzene in the presence of **11** also produced **12** with an increased yield (58%). These experiments suggest that acylation of N-2 amino group of guanine base by photogenerated benzoylketene **2** would be a major reaction in the photoirradiation of DBDM in the presence of duplex DNA.

Synthesis of DBDM Derivative 3 and DBDM-Transferring Reagent DBDM-OSu (4). At first, DNA cleavage by DBDM derivative **3** possessing an aminoalkyl side chain was intended under photoirradiation with 366 nm light. The synthesis of **3** was accomplished as shown in Scheme 3. Ester hydrolysis of readily available dibenzoylmethane derivative **13**[16b] followed

(20) (a) Nielsen, P. E.; Jeepsen, C.; Egholm, M.; Buchardt, O. *Nucleic Acids Res.* **1988**, *16*, 3877−3888. (b) Henriksen, U.; Larsen, C.; Karup, G.; Jeepsen, C.; Nielsen, P. E.; Buchardt, O. *Photochem. Photobiol*. **1991**, *53*, 299−305.

(21) (a) Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1990**, *112*, 6397−6397. (b) Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1994**, *116*, 1690−1697.

(22) Saito, I.; Takayama, M.; Sakurai, T. *J. Am. Chem. Soc.* **1994**, *116*, 2653−2654.

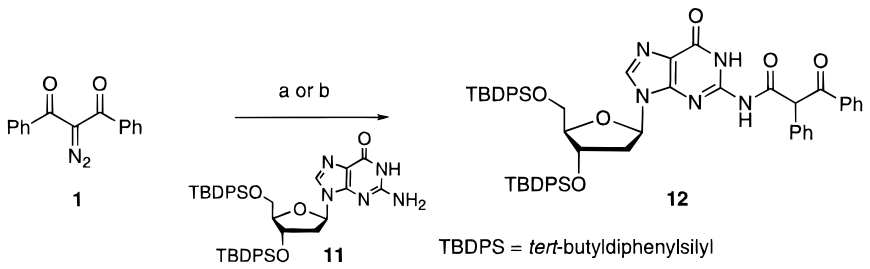
(23) Nakatani, K.; Shirai, J.; Tamaki, R.; Saito, I. *Tetrahedron Lett.* **1995**, *36*, 5363−5366.

(24) (a) Baumann, N. *Helv. Chim. Acta* **1972**, *55*, 2716−2723. (b) Tomioka, H.; Hayashi, N.; Asano, T.; Izawa, Y. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 758−761.

(25) For reviews on chemical nucleases, see: (a) Thuong, N. T.; Hélène, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 666−690 (b) Sigman, D. S.; Bruice, T. W.; Mazumder, A.; Sutton, C. L. *Acc. Chem. Res.* **1993**, *26*, 98−104.

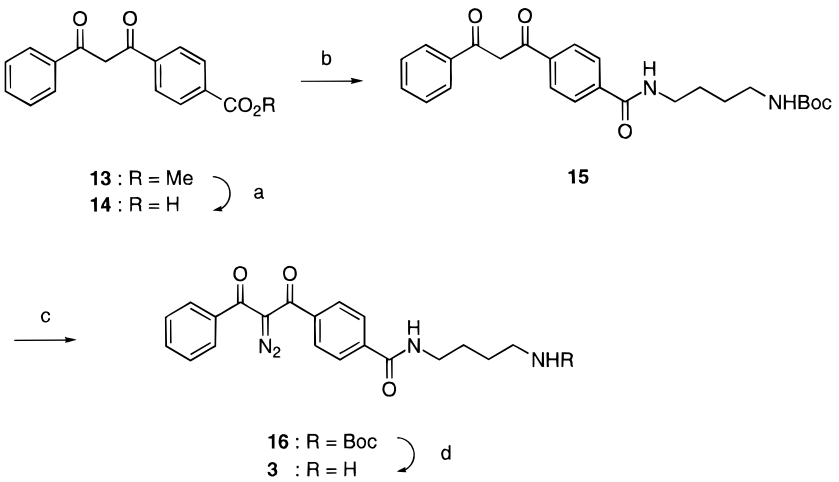
(26) For recent examples of photoactivatable oligonucleotide conjugates, see: (a) Boutorine, A. S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Hélène, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2462−2465. (b) Magda, D.; Wright, M.; Miller, R. A.; Sessler, J. L.; Sansom, P. I. *J. Am. Chem. Soc.* **1995**, *117*, 3629−3630. (c) Sessler, J. L.; Sansom, P. I.; Král, V.; O'Connor, D.; Iverson, B. *J. Am. Chem. Soc.* **1996**, *118*, 12322−12330, and references cited therein. See also ref 21b.

Scheme 2[a]



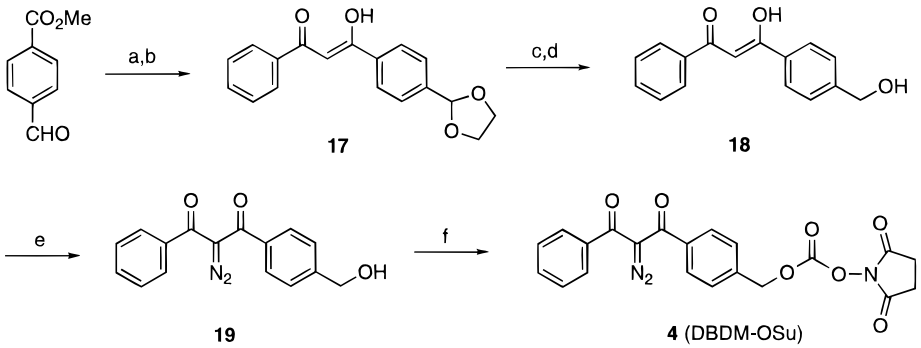
[a] Conditions: (a) high pressure Hg lamp, Pyrex, benzene (15%); (b) benzene reflux (58%).

Scheme 3[a]



[a] Reagents: (a) LiOH, H_2O, THF; (b) *N*-Boc-1,4-diaminobutane, PyBoP, DMF; (c) TsN_3, Et_3N, DMF; (d) HCl, EtOAc.

Scheme 4[a]



[a] Reagents: (a) ethylene glycol, *p*-TsOH; (b) NaH, acetophenone, THF reflux; (c) HCl, THF; (d) $NaBH_4$, EtOH, CH_2Cl_2; (e) TsN_3, Et_3N; (f) *N*,*N*′-disuccinimidyl carbonate, 2,6-lutidine, CH_3CN.

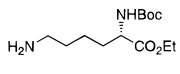
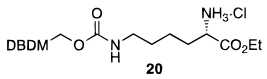
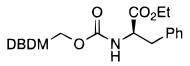
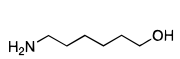
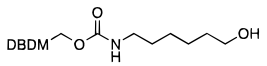
by coupling of the resulting acid **14** with *N*-Boc-1,4-diaminobutane produced amide **15**. Diazo transfer from tosyl azide to **15** and subsequent deprotection of the terminal amino group successfully gave **3**. It is noteworthy that DBDM can tolerate the highly acidic conditions for the deprotection of Boc group.

In order to devise photochemical DNA cleavers that can specifically alkylate a guanine residue proximal to the target site of long DNA fragments, we have designed a new reagent, DBDM-OSu (**4**), which facilitates the connection of the DBDM unit to various DNA binders, including DNA oligomers possessing an aminoalkyl linker. The synthesis of **4** was accomplished from methyl 4-formylbenzoate as depicted in Scheme 4. The utility of **4** was demonstrated by the reaction with amines in aqueous DMF in the presence of sodium bicarbonate to give DBDM adducts **20**−**22** in good yields (Table 1). In the case of N^{α}-Boc-*L*-Lys-OMe (entry 1), DBDM-lysine adduct **20** was isolated after deprotection of Boc group. These DBDM adducts were subjected to DNA cleavage assay using plasmid relaxation method, eventually showing that DBDM-lysine adduct **20** having a cationic side chain effectively cleaved DNA under photoirradiation conditions.

Synthesis of DBDM-Oligonucleotide Conjugates. DBDM was next tethered to oligonucleotides possessing hexamethyleneamino linker at the 5′-end (Scheme 5). Thus, two DBDM-oligonucleotide (ODN) conjugates **5** and **6** were synthesized by coupling of **4** with 5′-aminohexyl 8-mer [H_2N-$(CH_2)_6$-d(ACGTCAGG)-3′] (**ODN8**, Table 2) and 15-mer [H_2N-$(CH_2)_6$-d(ACGTCAGGTGGCACT)-3′] (**ODN15**), respectively, in 50% aqueous acetonitrile in the presence of sodium bicarbonate at ambient temperature. Structures of **5** and **6** were confirmed by enzymatic digestion with snake venom phosphodiesterase (sv PDE) and calf intestine alkaline phosphatase (AP) to give DBDM derivative **22** together with dA, dG, dC, and dT in a ratio of 2:3:2:1 and 3:5:4:3, respectively (Figure 1).

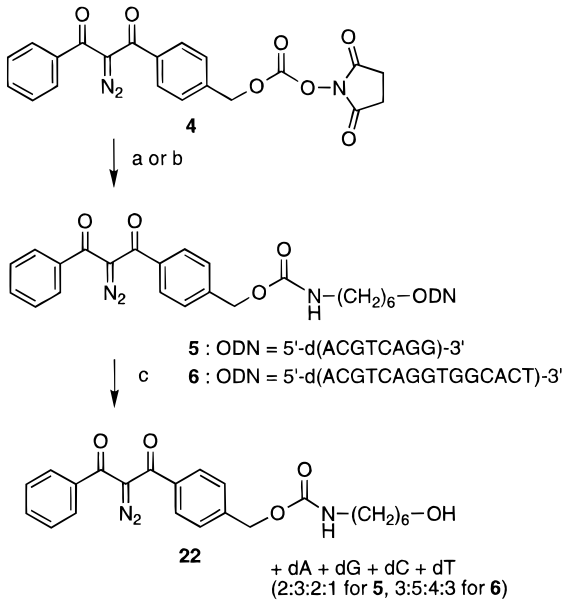
DNA Cleavage by DBDM Derivative 3 under Photoirradiation Conditions. Photoinduced DNA cleavage by DBDM

Table 1. Coupling Reactions of **4** with Various Amino Compounds in Aqueous Solvents[a]

entry	amine	product[b]	yield (%)[c]
1		 **20**	76
2		 **21**	99
3		 **22**	70

[a] An aqueous DMF solution of **4** and amine was stirred in the presence of sodium bicarbonate at ambient temperature for 1 h. [b] DBDM = $C_6H_5COC(N_2)COC_6H_4-$. [c] Isolated yield.

Scheme 5[a]



[a] Reagents and conditions: (a) **ODN8**, $NaHCO_3$, aqueous CH_3CN for **5**; (b) **ODN15**, $NaHCO_3$, aqueous CH_3CN for **6**; (c) sv PDE, AP.

derivative **3** was carried out using ^{32}P-labeled DNA fragment and analyzed by polyacrylamide gel electrophoresis. Thus, ^{32}P-5′-end-labeled *Eco*R I/*Rsa* I fragment of pBR322 DNA in sodium cacodylate buffer was illuminated with 366 nm-light in the presence of **3**. The DNA recovered by ethanol precipitation was heated at 90 °C for 30 min in the absence or presence of piperidine and analyzed by electrophoresis on a sequencing gel containing 8% polyacrylamide and 7 M urea.[27] The result of DNA cleavage by **3** was shown in Figure 2. Faint but characteristic DNA cleavage bands were observed for nonheated and heated DNAs (lanes 1 and 2). After heating with piperidine, the intensity of these bands considerably increased with each band being shifting to 5′-end (lane 3). By comparing the DNA cleavage pattern in lane 3 with Maxam−Gilbert A+G bands (lane 4), it was confirmed that the DNA cleavage occurred selectively at the guanine (G) residues after hot piperidine treatment. The DNA cleavage bands in lane 3 indicated that the G cleavage has no significant sequence selectivity. Band shift to 5′-end by heating with piperidine (lane 2 *vs* lane 3) strongly suggests that the photoinduced G cleavage proceeded via the modification of the guanine base by a photogenerated ketene from **3**.[28,29]

(27) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499−560.

Table 2. Sequences of Oligonucleotides (ODNs) and DBDM-ODN Conjugates

ODNs and DBDM-ODN conjugates	
ODN8	H_2N-$(CH_2)_6$-d(ACGTCAGG)-3′
ODN15	H_2N-$(CH_2)_6$-d(ACGTCAGGTGGCACT)-3′
5	DBDM-CH_2-O(CO)NH-$(CH_2)_6$-d(ACGTCAGG)-3′
6	DBDM-CH_2-O(CO)NH-$(CH_2)_6$-d(ACGTCAGGTGGCACT)-3′
23	5′-d(AGTGCCACCTGACGTCTGCTCTCTC)-3′
24	5′-d(AGTGCCACCTGACGTGCGTGCGTCT)-3′

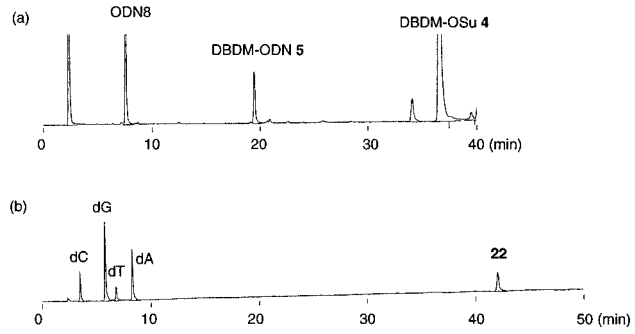


Figure 1. HPLC profiles for (a) a coupling of 5′-aminohexyl 8-mer H_2N-$(CH_2)_6$-d(ACGTCAGG)-3′ (**ODN8**) with DBDM-OSu **4** giving DBDM-ODN conjugate **5** and (b) product analysis of the mixture obtained by enzymatic digestion of **5** with snake venom phosphodiesterase (sv PDE) and calf intestine alkaline phosphatase (AP) giving four nucleosides and DBDM derivative **22**. Each HPLC peak was identified by comigration with authentic sample.

Photochemical G-selective cleavage is known to occur in one electron transfer oxidation[6−12] and in singlet oxygen oxidation.[2] Recently, we[8] and others[9−12] have demonstrated that photoinduced electron transfer from DNA occurred selectively at 5′-G of the 5′-GG-3′ step. The photoinduced DNA cleavage by **3** occurred almost equally at all Gs, suggesting that electron transfer from guanine base to photoexcited DBDM chromophore is at the very most a minor process. The reaction of guanine base with singlet oxygen produced various oxidation products, and this reaction is known to induce spontaneous cleavage at G with no sequence selectivity without piperidine treatment.[2g,30,31] Since photoirradiation of **3** with labeled DNA resulted in a weak but spontaneous strand cleavage at all Gs without piperidine treatment (Figure 2, lane 1), a considerable amount of singlet oxygen process may be involved in the G-selective DNA cleavage by **3** under the conditions.

In order to know the participation of singlet oxygen in the photoinduced DNA cleavage, we carried out the photoreaction of DBDM derivative **3** with DNA in the presence of singlet oxygen quencher, sodium azide (Figure 3). Upon addition of sodium azide, spontaneous G cleavage was no more detected (lane 1), implying that oxidation of G by singlet oxygen is suppressed under the conditions. Weak smear bands observed for heat-treated DNA (lane 2) became distinct and intense by hot piperidine treatment, being accompanied with a band shift to 5′-end (lane 3). Strong cleavage bands in lane 3 were in accordance with Maxam−Gilbert G-bands (lane 4). In addition to these intense G bands, weak nonselective bands were also observed at all bases. Since the DNA cleavage at G was enhanced by hot piperidine treatment with a band shift to 5′-

(28) Baker, B. F.; Dervan P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700−2712.

(29) (a) Maxam, A. M.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 560−564. (b) Nishikawa K.; Adams, B. L.; Hecht, S. M. *J. Am. Chem. Soc.* **1982**, *104*, 326−328 and references cited therein.

(30) Raoul, S.; Cadet, J. *J. Am. Chem. Soc.* **1996**, *118*, 1892−1898.

(31) (a) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 474−477. (b) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 6439−6442.

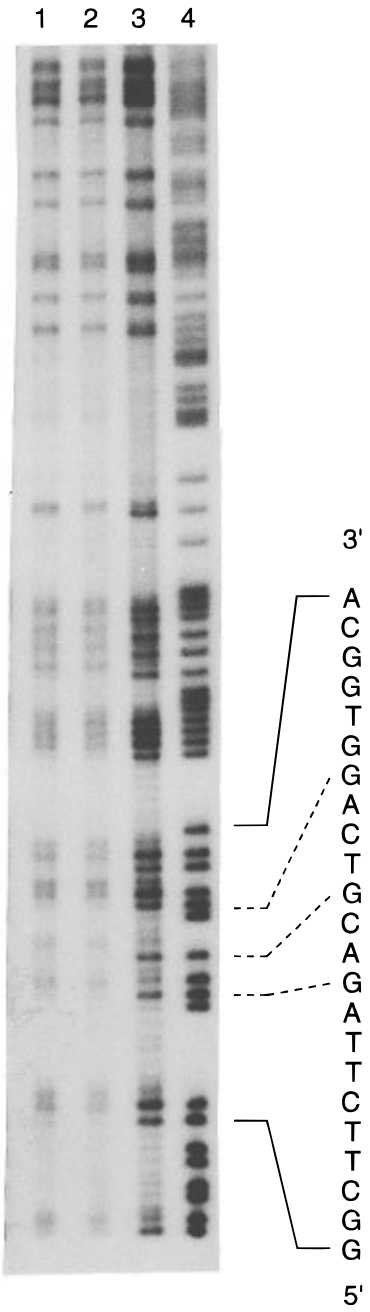


Figure 2. Photoinduced cleavage of ^{32}P-5′-end-labeled DNA by **3**. The reaction mixture containing **3** (50 μM) and ^{32}P-5′-end-labeled DNA (pBR322 DNA, *Eco*R I-*Rsa*I fragment, 514 bp) in sodium cacodylate buffer (5 mM, pH 7.0) in the presence of calf thymus DNA (1 μM) was photoirradiated with transilluminator (366 nm) at 0 °C for 1 h. Photoirradiated DNA recovered by ethanol precipitation was treated as described below and electrophoresed on a sequencing gel containing 8% polyacrylamide and 7 M urea: lane 1, nonheated DNA; lane 2, heated DNA (90 °C, 30 min); lane 3, heated DNA (90 °C, 30 min) in the presence of piperidine (10% v/v); lane 4, A+G sequencing reaction.

end, the G cleavage must proceed via the formation of alkali labile abasic sites which are produced by a photochemical modification of G base. Photogenerated benzoylketene is the most likely species responsible for the G modification, whereas the minor nonselective cleavage is probably due to the hydrogen abstraction from deoxyribose by photogenerated carbene from the DBDM chromophore.[20a]

Cleavage of Single-Stranded DNA by DBDM-ODN Conjugates 5 and 6. We next focused our attention to the specific cleavage of the single-stranded DNA by DBDM-ODN conjugates at predetermined guanine sites proximal to the target

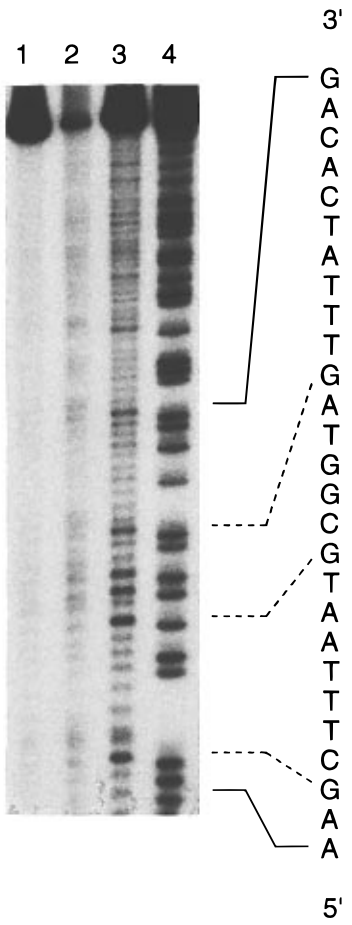


Figure 3. Photoinduced cleavage of ^{32}P-5′-end-labeled DNA by **3** in the presence of sodium azide. The reaction mixture containing **3** (50 μM) and ^{32}P-5′-end-labeled DNA (pBR322 DNA, *Eco*R I-*Rsa*I fragment, 167 bp) in sodium cacodylate buffer (5 mM, pH 7.0) in the presence of calf thymus DNA (1 μM) and sodium azide (100 mM) was photoirradiated with transilluminator (366 nm) at 0 °C for 1 h. Photoirradiated DNA recovered by ethanol precipitation was treated as described below and electrophoresed on a sequencing gel containing 8% polyacrylamide and 7 M urea: lane 1, nonheated DNA; lane 2, heated DNA (90 °C, 30 min); lane 3, heated DNA (90 °C, 30 min) in the presence of piperidine (10% v/v); lane 4, A+G sequencing reaction.

sequence. Single-stranded 25-mer 5′-d(AGTGCCA*CCTGA-CGT*CT**G$_{18}$**CTCTCTC)-3′ (**23**) has a complementary sequence (shown in italic) to 8-mer conjugate **5** and a targeted guanine (G_{18}) in the middle (Table 2). The site of the target G_{18} is three bases away from the 3′-end of the complementary sequence. A solution of **5** and ^{32}P-5′-end-labeled 25-mer **23** in sodium cacodylate buffer containing sodium azide to suppress singlet oxygen process and sonicated calf thymus DNA was irradiated with 366 nm light at 0 °C, and the DNA recovered by ethanol precipitation was analyzed by denatured polyacrylamide sequencing gel as indicated in Figure 4. While no appreciable DNA cleavage was observed upon only heating, intense cross-linked bands that migrated more slowly than **23** were detected (lanes 1 and 2). Upon heating with piperidine, the intensity of the cross-linked bands decreased with a concomitant appearance of a distinct cleavage band at G_{18} (lane 3). The results indicate that a major reaction occurred in the photoirradiation of **5** with **23** is the cross-linking of both oligomers. Heating the photoirradiated mixture containing cross-linked oligomers with piperidine led to the formation of the cleavage band at G_{18}, whereas a considerable portion of the cross-linked oligomer was stable to hot piperidine treatment. A major cross-linking reaction between **23** and **5** would probably be the acylation of guanine

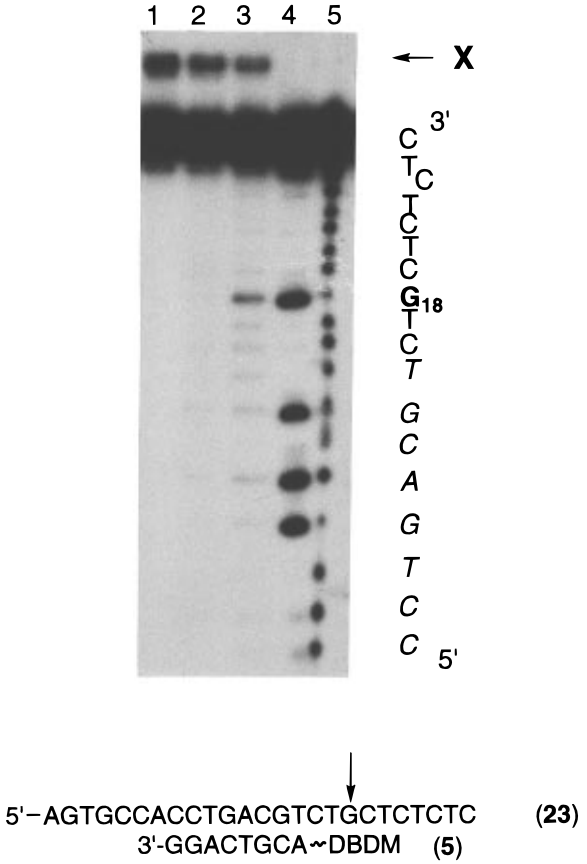


Figure 4. Photoinduced cleavage of ^{32}P-5′-end-labeled 25-mer **23** in the presence of DBDM-ODN conjugate **5**. The reaction mixture containing **5** (50 μM) and ^{32}P-5′-end-labeled **23** in sodium cacodylate buffer (10 mM, pH 7.0) in the presence of sodium azide (100 mM) and sonicated calf thymus DNA (10 μM base pair concentration) was photoirradiated with a transilluminator (366 nm) at 0 °C for 1 h. Photoirradiated DNA recovered by ethanol precipitation was treated as described below and electrophoresed on a sequencing gel containing 15% polyacrylamide and 7 M urea: lane 1, nonheated DNA; lane 2, heated DNA (90 °C, 30 min); lane 3, heated DNA (90 °C, 30 min) in the presence of piperidine (10% v/v); lanes 4 and 5, Maxam−Gilbert A+G and C+T sequencing reactions, respectively. Shown below are base sequences of **5** and **23** with the cleavage site being indicated by an arrow. Photoinduced cross-links are revealed by the appearance of the bands (as indicated **X**) migrating more slowly than the 25-mer.

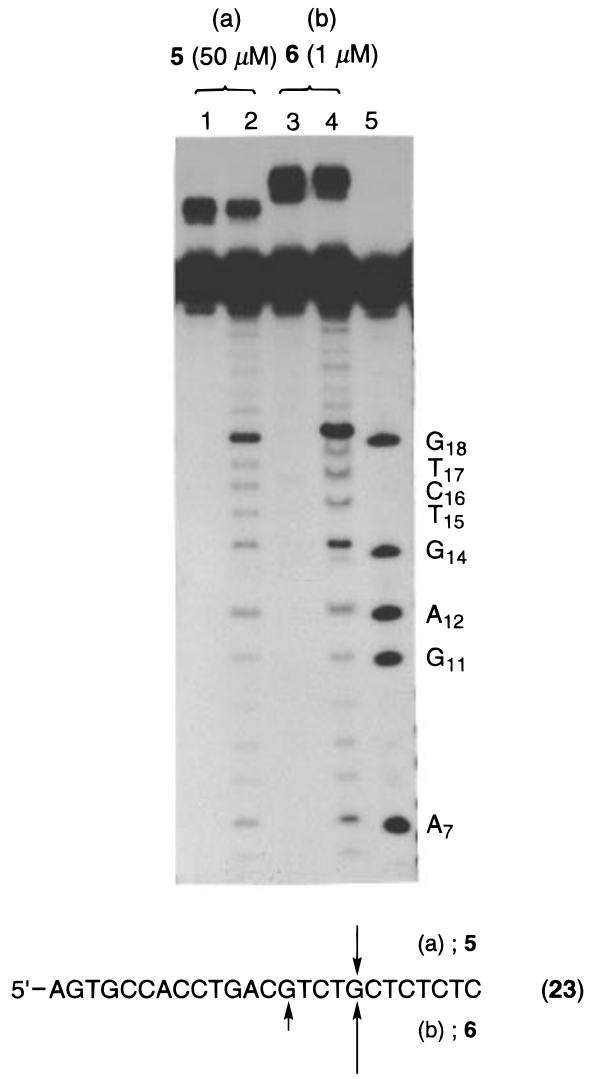


Figure 5. Photoinduced cleavage of ^{32}P-5′-end-labeled 25-mer **23** in the presence of (a) 8-mer DBDM-ODN conjugate **5** and (b) 15-mer DBDM-ODN conjugate **6**. The reaction mixture containing DBDM-ODN conjugate **5** (50 μM) or **6** (1 μM) and ^{32}P-5′-end-labeled **23** in sodium cacodylate buffer (10 mM, pH 7.0) in the presence of sodium azide (100 mM) and sonicated calf thymus DNA (10 μM base pair concentration) was photoirradiated with a transilluminator (366 nm) at 0 °C for 1 h. Photoirradiated DNA recovered by ethanol precipitation was treated as described below and electrophoresed on a sequencing gel containing 15% polyacrylamide and 7 M urea: lane 1, **5**, nonheated DNA; lane 2, **5**, heated DNA (90 °C, 30 min) in the presence of piperidine (10% v/v); lane 3, **6**, nonheated DNA; lane 4, **6**, heated DNA (90 °C, 30 min) in the presence of piperidine (10% v/v); lane 5, Maxam−Gilbert A+G sequencing reaction.

N-2 amino group by photogenerated benzoylketene as indicated in the model photoreaction of DBDM **1** with guanosine derivative **11** giving **12**. While the exact nature of the reaction eventually resulting in a specific cleavage at G_{18} by hot piperidine treatment was not clear at this moment, we suggest that acylation of the most nucleophilic guanine N7 by electrophilic benzoylketene would be the origin for the G_{18} cleavage by hot piperidine treatment of the cross-linked oligomer.

It was expected that the efficiency for both cross-linking and strand cleavage would increase with increasing length of the complementary sequence of DBDM-ODN conjugates due to increased duplex stability. In order to compare the lengths of DBDM-ODN conjugates, we examined the photoreactions of 8-mer conjugate **5** and 15-mer conjugate **6** in the presence of **23** by polyacrylamide gel electrophoresis (Figure 5). Melting temperatures for duplexes **5**/**23** and **6**/**23** measured in 100 mM sodium chloride and 10 mM sodium cacodylate buffer (pH 7.0) at 100 μM base concentration were 37.0 and 64.5 °C, respectively. In each case, distinct cross-linked bands were observed, and hot piperidine treatment produced the intense cleavage band at G_{18}. Minor bands at T_{17}, C_{16}, and T_{15} probably due to the hydrogen abstraction by photogenerated carbene were also detected in both photoreactions. A relatively strong cleavage band was also observed at G_{14} in the photoreaction of **6**. The relative intensity of the cross-linked band and the G_{18} cleavage band to the band of unchanged **23** was compared for **5** and **6** (Table 3). As clear from Table 3, with increasing the length of the complementary strand of the conjugate, the efficiency for both cross-linking and G_{18} cleavage increased considerably.

In order to know the site selectivity for the G cleavage, photoinduced cleavage of 25-mer ODN 5′-d(*AGTGCCACC-TGACG$_{14}$T*G$_{16}$CG$_{18}$TG$_{20}$CG$_{22}$TCT)-3′ (**24**) having five guanine residues G_{14}, G_{16}, G_{18}, G_{20}, and G_{22} near the 3′-end of the complementary sequence (italic) was investigated. The photoreaction of **6** in the presence of **24** was carried out and analyzed by polyacrylamide gel electrophoresis (Figure 6). The site of

Table 3. Relative Intensity of Cross-linked Bands and G Cleavage Bands to the Band of Unchanged **23** Obtained from Densitometry of the Gel Shown in Figure 5[a]

	relative intensity (%)			
	5		**6**	
	lane 1	lane 2	lane 3	lane 4
cross-linked bands	32.0	23.3	47.4	36.8
G_{18} band		8.3		15.2
G_{14} band		0.8		3.9

[a] The relative intensity (%) of cross-linked bands and cleavage bands (G18, G14) to the band of unchanged **23** (100%) was calculated by using band density determined by densitometric analysis of the gel shown in Figure 5.

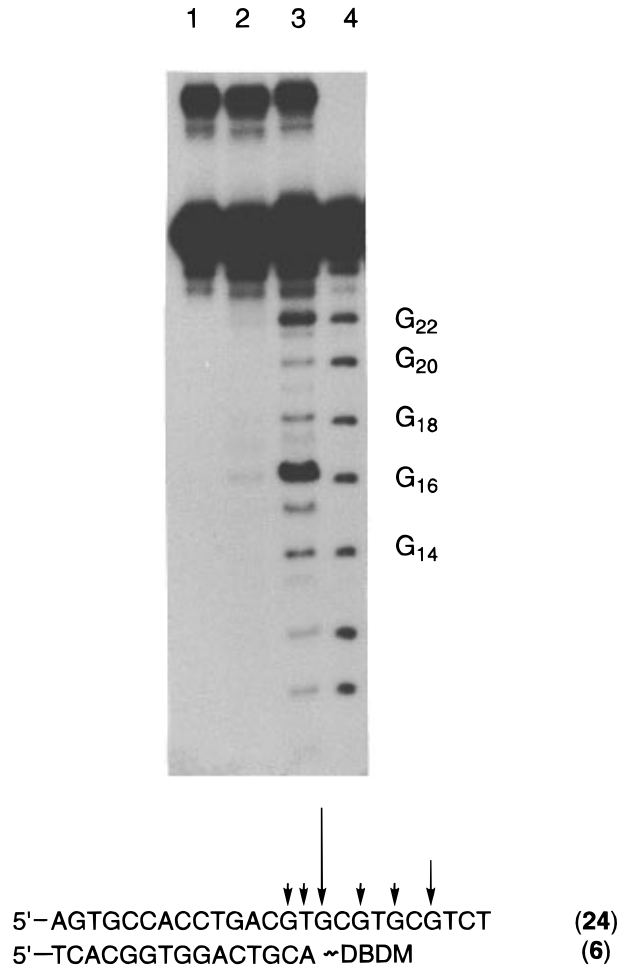


Figure 6. Photoinduced cleavage of ^{32}P-5′-end-labeled 25-mer **24** in the presence of DBDM-ODN conjugate **6**. The reaction mixture containing **6** (1 μM) and ^{32}P-5′-end-labeled **24** in sodium cacodylate buffer (10 mM, pH 7.0) in the presence of sodium azide (100 mM) and sonicated calf thymus DNA (10 μM base pair concentration) was photoirradiated with a transilluminator (366 nm) at 0 °C for 1 h. Photoirradiated DNA recovered by ethanol precipitation was treated as described below and electrophoresed on a sequencing gel containing 15% polyacrylamide and 7 M urea: lane 1, nonheated DNA; lane 2, heated DNA (90 °C, 30 min); lane 3, heated DNA (90 °C, 30 min) in the presence of piperidine (10% v/v); lane 4, Maxam−Gilbert A+G sequencing reaction.

the most effective guanine cleavage after hot piperidine treatment was G_{16}, the most proximal site to the 3′-end of the complementary sequence (lane 3). Cleavages at G_{14}, G_{18}, and G_{20} were very weak compared to G_{16}, although a moderate cleavage band was detected at G_{22} which is located on seven bases away from the 3′-end of the complementary sequence. The efficient cleavage at the most distal guanine, G_{22}, may suggest a hairpin-like conformation of the single-stranded region of the duplex comprised by **24** and **6**. A similar observation for the cleavage of single-stranded DNA at a distal base from the target sequence has been reported for oligonucleotide-phenazine di-*N*-oxide conjugate under nonphotochemical conditions.[32]

Conclusion

Photoinduced DNA cleavage by DBDM derivatives occurred selectively at guanine residues. Studies using DBDM-ODN conjugates indicated that the major reaction occurred between single-stranded DNA and the DBDM conjugate was the cross-linking between both oligomers. Acylation of N7 and N-2 amino group of guanine residues by photogenerated electrophilic ketene was suggested to be the major reaction for the cross-linking. Upon heating with piperidine, cross-linked oligomers at guanine N7 were immediately decomposed to result in a selective cleavage at the cross-linked site. DNA cleavage experiments using ODN having multiple guanine sites indicated that the most efficient cleavage site is the guanine which is next to the 3′-end of the complementary sequence.

In general, it was not easy for conventional photochemical DNA cleavers to cut DNA at specific single site, due to the diffusion of photogenerated reactive species.[13,26] The DBDM-linked ODN can effectively produce electrophilic ketene possessing an intrinsic reaction selectivity toward most nucleophilic guanine base among other nucleobases. Therefore, special emphasis is given to that DBDM-ODN conjugates are a very useful class of photonucleases that can cleave DNA at a predetermined guanine site. Furthermore, the reagent DBDM-OSu (**4**) can be used as a convenient and effective photoinducible electrophile for the cross-linking or modification of biomolecules.

Experimental Section

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured with Varian GEMINI 200 (200 MHz) or JEOL JNM α-400 (400 MHz) spectrometers. Coupling constants (*J* values) are represented in Hz. ^{13}C NMR spectra were measured with Varian GEMINI 200 (50 MHz) spectrometer. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual solvent as an internal standard. IR spectra were recorded on a JASCO FT-IR-5M spectrophotometer. A JASCO 660 spectrophotometer was used for absorption spectra measurements. Mass spectra were recorded on a JEOL JMS DX-300 or a JEOL JMS SX-102A spectrometer. Photoirradiation at 366 nm was carried out using a Funakoshi TEL-33 transilluminator. Sequence gel electrophoresis was carried out on a Gibco BRL Model S2 apparatus. Densitometric analysis of the gel was carried out using BIO-RAD GS-700 imaging densitometer with analytical software Molecular Analyst (ver. 2.1). Wakogel C-200 was used for silica gel flash chromatography. Precoated TLC plates Merck silica gel 60 F_{254} was used for monitoring reactions and also for preparative TLC. HPLC was performed on cosmosil $5C_{18}$AR and $5C_{18}$MS columns and DAISOPAC SP-120-5-ODS-AP column with Gilson Chromatography Model 305 using UV detector Model 118 at 254 nm. Gel permeation chromatography (GPC) was carried out on JAI LC-908 using polystyrene column. Ether and tetrahydrofuran (THF) were distilled under N_2 from sodium/benzophenone ketyl prior to use. All enzymes used in these studies were from commercial sources. [γ-^{32}P]ATP (6000 Ci/mmol) was obtained from Amersham.

Photoreaction of DBDM with Guanosine Derivative 11. A benzene solution (2 mL) of **1** (10 mg, 0.04 mmol) and 3′,5′-*O*-di(*tert*-butyldiphenylsilyl)-2′-deoxyguanosine (**11**) (10 mg, 0.01 mmol) was

(32) Nagai, K.; Hecht, S. M. *J. Biol. Chem.* **1991**, *266*, 23994−24002.
(33) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning. A Laboratory Mannual*, 2nd ed.; Cold Spring Harbor Laboratories Press: Cold Spring Harbor, NY, 1989.

irradiated with high pressure mercury lamp (400 W) through a Pyrex filter at 0 °C for 2 h. After concentration, the crude product was purified by silica gel column chromatography to give **12** (1.9 mg, 15%). Alternatively, a benzene solution of **1** (150 mg, 0.60 mmol) and **11** (150 mg, 0.20 mmol) was refluxed for 3 h to give **12** (116 mg, 58%) after concentration and purification by silica gel column chromatography: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 11.74 (major isomer) and 11.92 (minor isomer) (br, total 1H), 10.45 (major isomer) and 10.47 (br, total 1H), 7.15−7.75, 7.97 (m, 2H), 6.35 (dd, J = 8.1, 5.5 Hz, major isomer), 6.19 (dd, J = 8.2, 5.5 Hz, minor isomer), 5.78−5.80 (1H), 4.53 (minor isomer) and 4.59 (major isomer) (total 1H), 4.06 (minor isomer) and 4.11 (major isomer) (total 1H), 3.33−3.44 and 3.52−3.62 (2H), 2.09−2.43 (2H), 0.90−0.92 and 1.10−1.12 (9H, *tert*-Bu); FABMS (NBA) (relative intensity), *m*/*e* 966 [(M + H)$^+$] (10), 374 (100, base peak); HRMS calcd for $C_{20}H_{16}O_3N_5$ [(M + H − ribose with two TBDPS)$^+$] 374.1255, found 374.1239.

Methyl 4-(1,3-Dioxo-3-phenylpropyl)benzoate (13). To a suspension of sodium hydride (1.56 g, 60% oil dispersion, 39.0 mmol, washed with THF) in anhydrous THF (50 mL) was added a THF solution (20 mL) of acetophenone (3.65 g, 30.4 mmol) and dimethyl terephthalate (5.83 g, 30.0 mmol) for 30 min at room temperature, and the mixture was heated at reflux for 7 h. The reaction mixture was cooled on ice and quenched with addition of concentrated HCl (3.2 mL). The crude material obtained by concentration *in vacuo* was dissolved into CH_2Cl_2 and washed with water. The organic phase was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was roughly purified by flash chromatography (SiO_2, 100% CH_2Cl_2) to give a yellow solid (6.29 g) containing mainly **13**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 16.7 (br, 1H), 8.13−7.95 (7H), 7.57−7.44 (5H), 6.85 (s, 1H), 3.92 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 187.4, 183.7, 166.5, 139.6, 135.5, 133.3, 132.9, 129.9, 128.8, 127.4, 127.1, 93.8, 52.3. This material was used for the preparation of **15** without further purification.

***N*-(*N*′-*tert*-Butoxycarbonyl-4-aminobutyl)-4-(1,3-dioxo-3-phenylpropyl)benzamide (15).** To a solution of **7** (6.29 g, 22.3 mmol) in THF (100 mL) was added a solution of LiOH (8.15 g, 194 mmol) in water (100 mL) at 5 °C, and the mixture was stirred at room temperature for 18 h. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic phase was discarded. The alkaline aqueous layer was acidified with 1 N HCl, and a resulted pale green precipitate was filtered off. Recrystallization of the precipitate from ethanol gave 4-(1,3-dioxo-3-phenylpropyl)benzoic acid (**14**) (4.92 g, 83%) as a pale yellow solid. This material was used for the following reaction without further purification. To a solution of **14** (504 mg, 1.88 mmol), PyBOP (benzotriazole-1-yloxytri(pyrolidino)phosphonium hexafluorophosphonate) (1.63 g, 3.13 mmol), and triethylamine (580 mg, 5.74 mmol, 0.80 mL) in DMF (10 mL) was added *N*-*tert*-butoxycarbonyl-1,4-diaminobutane (530 mg, 2.81 mmol) at 0 °C, and the mixture was stirred at ambient temperature for 16 h. The reaction mixture was diluted with H_2O and extracted with ethyl acetate. The organic phase was washed with 5% $KHSO_4$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 0−5% methanol/EtOAc) to give **15** (706 mg, 86%) as a white solid: mp 160.0−161.5 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04−7.88 (6H), 7.58−7.46 (3H), 6.86 (s, 1H), 6.71 (br, 1H), 4.63 (br, 1H), 3.50 (q, 2H, J = 6.3 Hz), 3.16 (m, 2H), 1.66 (m, 2H), 1.60 (m, 2H), 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 187.0, 184.3, 166.9, 156.5, 138.1, 138.1, 135.6, 132.9, 128.9, 127.5, 127.4, 93.7, 79.4, 39.8, 39.7, 28.3, 27.8, 26.1; FABMS (NBA) *m*/*e* 439 [(M + H)$^+$]. Anal. Calcd for $C_{25}H_{30}O_5N_2$: C, 68.47; H, 6.90; N, 6.39. Found: C, 68.26; H, 6.87; N, 6.39.

***N*-(*N*′-*tert*-Butoxycarbonyl-4-aminobutyl)-4-(2-diazo-1,3-dioxo-3-phenylpropyl)benzamide (16).** To a solution of **15** (157 mg, 0.36 mmol) and triethylamine (80.2 mg, 0.79 mmol, 0.11 mL) in DMF (2 mL) was slowly added *p*-toluenesulfonyl azide (286 mg, 1.45 mmol) at 0 °C, and the mixture was stirred at ambient temperature for 4 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 0−5% methanol/CH_3Cl) to give **16** (134 mg, 80%) as a white foam: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.72 (m, 2H), 7.58−7.54 (4H), 7.44 (m, 1H), 7.31 (m, 2H), 6.76 (br, 1H), 4.67 (br, 1H), 3.43 (q, 2H, J = 6.3 Hz), 3.12 (q, 2H, J = 6.5 Hz), 1.60 (m, 2H), 1.54 (m, 2H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 186.0, 185.9, 166.3, 156.3, 139.3, 138.0, 136.8, 132.9, 128.54, 128.49, 128.3, 127.0, 84.5, 79.4, 39.8, 28.4, 27.8, 26.2; IR ($CHCl_3$) 1703, 1697, 1651, 1515, 1318, 1285, 1264, 1169 cm^{-1}; FABMS (NBA) *m*/*e* 465 [(M + H)$^+$].

***N*-(4-Aminobutyl)-4-(2-diazo-1,3-dioxo-3-phenylpropyl)benzamide Hydrochloride (3).** A solution of **16** (102 mg, 0.22 mmol) in HCl (3.2 N in EtOAc, 10 mL) was stirred at room temperature for 2 h. Solvent was removed *in vacuo*, and the crude product was purified by recrystallization from methanol−ether to give hydrochloride of **3** (46.9 mg, 53%) as a white solid: mp 133 °C dec; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.82−7.63 (6H), 7.49 (m, 2H), 7.38 (m, 2H), 3.44 (m, 2H), 2.97 (m, 2H), 1.72 (m, 4H); ^{13}C NMR (CD_3OD, 50 MHz) δ 188.3, 188.1, 169.4, 141.6, 139.0, 138.73, 134.1, 129.8, 129.7, 128.5, 86.1, 40.4, 40.1, 27.4, 25.9; UV (H_2O) 366.0 (ϵ 220) 287.6 (ϵ 3421); FABMS (NBA) *m*/*e* 365 [(M − Cl)$^+$]. Anal. Calcd for $C_{20}H_{21}O_3N_4Cl$: C, 59.93; H, 5.28; N, 13.98. Found: C, 60.01; H, 5.23; N, 13.68.

1-[4-(1,3-Dioxolan-2-yl)phenyl]-3-phenyl-1,3-propandione (17). To a mixture of methyl 4-formylbenzoate (2.53 g, 15.4 mmol) and ethylene glycol (5.00 g, 80.7 mmol) in benzene (50 mL) was added a catalytic amount of *p*-toluenesulfonic acid (28.8 mg, 0.15 mmol), and the mixture was refluxed for 6 h with azeotropical removal of H_2O. The reaction mixture was diluted with $CHCl_3$, washed with saturated $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude material was purified by bulb-to-bulb distillation to yield methyl 4-(1,3-dioxolan-2-yl)benzoate (3.04 g, 95%) as a colorless oil: bp 130−135 °C (5 mmHg); TLC R_f (hexane:ethyl acetate = 4:1) 0.41; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (dt, 2H, J = 8.6, 1.8 Hz), 7.53 (dt, 2H, J = 8.2, 1.8 Hz), 5.83 (s, 1H), 4.09 (m, 2H), 4.03 (m, 2H), 3.89 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 167.0, 142.9, 130.9, 129.8, 126.5, 103.0, 65.3, 52.0; IR (neat) 1730, 1719, 1436, 1280, 1113, 1085, 1019 cm^{-1}. Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.17; H, 5.87. To a suspension of washed NaH (576 mg, 23.9 mmol) in THF (25 mL) was added a solution of acetophenone (2.17 g, 18.0 mmol) and acetal (3.75 g, 18.0 mmol) in THF (25 mL) at room temperature for 2 h, and the resulting mixture was refluxed for 1.5 h. After the reaction mixture was cooled to ambient temperature, it was diluted with ethyl acetate and neutralized with 1 N HCl. The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (0−20% ethyl acetate/hexane gradient elution) to give **17** (2.63 g, 49%) as a colorless oil: bp 170−173 °C (0.5 mmHg); TLC R_f (hexane:ethyl acetate = 4:1) 0.29; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.96−8.00 (4H), 7.55−7.60 (2H), 7.53 (m, 1H), 7.46−7.50 (2H), 6.84 (s, 1H), 5.87 (s, 1H), 4.12 (m, 2H), 4.06 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 186.3, 185.3, 142.5, 136.3, 135.6, 132.6, 128.8, 127.3, 126.9, 103.0, 93.3, 65.3; IR (neat) 1601, 1564, 1302, 1229, 1086 cm^{-1}. Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 73.14; H, 5.54.

1-(4-Hydroxymethylphenyl)-3-phenyl-1,3-propandione (18). To a solution of **17** (2.63 g, 8.88 mmol) in THF (100 mL) was added concentrated HCl (4.6 mL, 56 mmol), and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was neutralized with 1 N NaOH (56 mL), diluted with $CHCl_3$ (100 mL), washed with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give 4-(1,3-dioxo-3-phenylpropyl)-benzaldehyde (2.15 g, 92%) as a yellow powder: TLC R_f (hexane:ethyl acetate = 4:1) 0.41; mp 141.0−142.0 °C (recrystallized from $CHCl_3$−hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.09 (s, 1H), 8.12 (m, 2H), 7.97−8.01 (4H), 7.58 (m, 1H), 7.47−7.52 (2H), 6.89 (s, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 191.8, 187.9, 183.1, 140.7, 138.8, 135.6, 133.1, 130.0, 128.9, 127.8, 127.5, 94.2; IR ($CHCl_3$) 1705, 1600, 1564, 1227 cm^{-1}. Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 75.92; H, 4.72. To a solution of the aldehyde (1.28 g, 5.08 mmol) in 30% ethanol in CH_2Cl_2 (83 mL) was added sodium borohydride (220 mg, 5.82 mmol) at −78 °C, and the mixture was stirred at −78 °C for 1 h. The reaction was quenched with addition of acetoaldehyde (20 mL), and the resulting mixture was warmed to ambient temperature, diluted with $CHCl_3$ (20 mL), washed with saturated $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by silica gel chromatography (0−40% ethyl

acetate/hexane gradient elution) to give **18** (641 mg, 50%) as colorless needles: TLC R_f (hexane:ethyl acetate = 1:1) 0.44; mp 105.0−106.0 °C (recrystallized from $CHCl_3$−hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 16.8 (m, 1H), 7.96−7.99 (4H), 7.54 (m, 1H), 7.45−7.50 (4H), 6.84 (s, 1H), 4.78 (s, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 186.0, 185.8, 145.7, 135.7, 134.9, 132.6, 128.8, 127.6, 127.3, 127.0, 93.1, 64.6; IR ($CHCl_3$) 1611, 1601, 1563, 1466, 1381, 1096 cm^{-1}. Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55. Found: C, 75.48; H, 5.32.

2-Diazo-1-(4-hydroxymethylphenyl)-3-phenyl-1,3-propandione (19). To a mixture of **18** (530 mg, 2.09 mmol) and triethylamine (275 mg, 2.72 mmol) in CH_2Cl_2 (10 mL) was slowly added *p*-toluenesulfonyl azide (1.24 g, 6.30 mmol), and the mixture was stirred at ambient temperature for 15 h. Solvent was removed under reduced pressure, and the crude product was purified by silica gel chromatography (25−50% ethyl acetate/hexane gradient elution) to give **19** (585 mg, 99%) as a pale yellow foam: TLC R_f (hexane:ethyl acetate = 1:1) 0.39; ^{1}H NMR ($CDCl_3$, 400 MHz); δ 7.53−7.58 (4H), 7.44 (m, 1H), 7.28−7.34 (4H), 4.66 (d, 2H, J = 3.8 Hz), 1.99 (brs, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 186.6, 186.5, 146.2, 137.0, 136.0, 132.8, 128.7, 128.5, 128.4, 126.4, 84.0, 64.2; FABMS (NBA), *m*/*e* 281 [(M + H)$^+$].

4-(2-Diazo-3-phenyl-1,3-propanedion-1-yl)phenylmethyl *N*-Hydroxysuccinimidyl Carbonate (DBDM-OSu) (4). To a mixture of *N*,*N*-disuccinimidyl carbonate (1.50 g, 5.84 mmol) and **19** (580 mg, 2.07 mmol) in DMF (7 mL) was added 2,6-lutidine (338 mg, 3.15 mmol), and the mixture was stirred at ambient temperature for 15 h. Solvent was removed *in vacuo* (below 35 °C), and the crude residue was purified by gel permeation chromatography (GPC) to give **4** (712 mg, 82%) as a yellow foam: TLC R_f (toluene:ethyl acetate = 2:1) 0.63; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.51−7.57 (4H), 7.43 (m, 1H), 7.28−7.34 (4H), 5.26 (s, 2H), 2.83 (s, 4H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 186.4, 186.2, 168.7, 151.7, 137.7×2, 136.9, 132.9, 128.9, 128.5, 128.4, 128.0, 84.9, 71.4, 25.2; UV (CH_3CN) 298.8 (ϵ 2700); IR ($CHCl_3$) 1813, 1789, 1743, 1643, 1263, 1220 cm^{-1}; FABMS (NBA), *m*/*e* 422 [(M + H)$^+$]; HRMS calcd for $C_{21}H_{16}O_7N_3$ [(M + H)$^+$] 422.0989, found 422.1023.

DBDM-Lysine Adduct 20. To a solution of **4** (81.6 mg, 0.19 mmol) and N^{α}-Boc-lysine methyl ester (278 mg, 1.1 mmol) in DMF (4 mL) and H_2O (4 mL) was added saturated $NaHCO_3$ (2 mL), and the mixture was stirred for 1 h. The reaction mixture was diluted with EtOAc and H_2O, and the organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by GPC to give adduct (118 mg, quantitative). This material was dissolved in EtOAc (20 mL) and treated with concentrated HCl (0.5 mL) at 0 °C for 10 min. Solvent was removed under reduced pressure to give **20** (72.1 mg, 76%) as a pale yellow foam: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60−7.63 (4H), 7.49 (m, 1H), 7.32−7.39 (4H), 5.07 (s, 2H), 4.02 (t, 1H, J = 6.4 Hz), 3.82 (s, 3H), 3.13 (t, 2H, J = 6.6 Hz), 1.85−2.00 (2H), 1.30−1.60 (4H); FABMS (NBA), *m*/*e* 467 [(M + H)$^+$]; HRMS calcd for $C_{24}H_{27}O_6N_4$ [(M + H)$^+$] 467.1931, found 467.1957.

DBDM-Phenylalanine Adduct 21. To a solution of **4** (84 mg, 0.20 mmol) and *L*-phenylalanine ethyl ester hydrochloride (154 mg, 0.67 mmol) in DMF (2 mL) and water (2 mL) was added saturated $NaHCO_3$ (1.2 mL) and stirred at ambient temperature for 1 h. The reaction mixture was diluted with EtOAc and H_2O, and the organic layer was washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by GPC to give **21** (100 mg, 99%) as a white solid: TLC R_f (toluene:ethyl acetate = 2:1) = 0.63; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53−7.57 (4H), 7.43 (m, 1H), 7.20−7.34 (7H), 7.10 (m, 2H), 5.23 (d, 1H, J = 8.2 Hz), 5.06 (s, 2H), 4.61 (dt, 1H, J = 8.2, 7.1 Hz), 4.16 (q, 2H, J = 7.0 Hz), 3.11 (dd, 1H, J = 13.9, 5.9 Hz), 3.08 (dd, 1H, J = 13.7, 5.9 Hz), 1.22 (t, 3H, J = 7.1 Hz); FABMS (NBA), *m*/*e* 500 [(M + H)$^+$]; HRMS calcd for $C_{28}H_{25}O_6N_3$ [(M + H)$^+$] 500.1823, found 500.1857.

DBDM−6-Amino-1-butanol Adduct 22. To a solution of **4** (25.2 mg, 0.06 mmol) and 6-amino-1-hexanol (14.6 mg, 0.13 mmol) in CH_3CN (1 mL) and H_2O (1 mL) was added saturated $NaHCO_3$ (100 μL), and the mixture was stirred for 30 min. The reaction mixture was concentrated and extracted with chloroform. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by GPC to give **22** (17.8 mg, 70%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56−7.57 (4H), 7.44 (m, 1H), 7.27−7.34 (4H), 5.05 (s, 2H), 4.80 (br, 1H), 3.60 (t, J = 6.4 Hz, 2H), 3.60 (t, J = 6.4 Hz, 2H), 3.17 (m, 2H), 1.46−1.58 (4H), 1.30−1.40 (4H); FABMS (NBA), *m*/*e* 424 [(M + H)$^+$]; HRMS calcd for $C_{23}H_{25}N_3O_5$ [(M + H)$^+$] 424.1872, found 424.1852.

Preparation of Oligonucleotides ODN8 and ODN15 Having an Aminoalkyl Linker. The hexamethyleneamino linker was attached in the last step of the synthesis of 5′-d(ACGTCAGGTGGCACT)-3′ or 5′-d(ACGTCAGG)-3′ using a monomethoxytrityl (MMTr) protected precursor (Clontech) that was designed for the routine protocols of automated coupling. The monomethoxytrityl protected amino arm linker-oligonucleotide was purified by reverse phase chromatography on a DAISOPAC column (6.0 × 150 mm, elution with a solvent mixture of 0.1 M triethylamine acetate, pH 7.0, linear gradient over 40 min from 5% to 45% acetonitrile at a flow rate of 1.5 mL/min, MMTr protected **ODN8**: t = 25.8 min, MMTr protected **ODN15**: t = 28.8 min). The protecting group was removed by treating the crude product with 80% acetic acid for 1 h under ambient temperature. The acetic acid was removed using vacuum rotary evaporator to dryness. The deprotected MMTr group was removed from the aqueous phase by extraction with diethyl ether. The purity of ODN was confirmed to be over 95% by HPLC analysis on a DAISOPAC column (6.0 × 150 mm, elution with a solvent mixture of 0.1 M triethylamine acetate, pH 7.0, linear gradient over 40 min from 5% to 45% acetonitrile at a flow rate of 1.5 mL/min, **ODN8**: t = 7.5 min, **ODN15**: t = 10.5 min).

DBDM-ODN Conjugates 5 and 6. To an acetonitrile solution (20 μL) of **4** (42 μg, 100 nmol) was added **ODN8** or **ODN15** (0.42 mM, 10 μL) and 10 μL of saturated $NaHCO_3$ and kept at ambient temperature for about 1 h. Monitor of the coupling reaction and purification of DBDM-ODN conjugate were carried out by HPLC on a DAISOPAC column (6.0 × 150 mm, elution with a solvent mixture of 0.1 M triethylamine acetate, pH 7.0, linear gradient over 30 min from 5% to 35% acetonitrile at a flow rate of 1.5 mL/min, **5**: t = 19.5 min, **6**: t = 23.4 min). Structures of **5** and **6** were confirmed by enzymatic digestion with calf intestine alkaline phosphatase and snake venom phosphodiesterase to produce four nucleosides and DBDM derivative **22** on HPLC (Figure 1) (DAISOPAC column, 6.0 × 150 mm, elution with a solvent mixture of 0.1 M triethylamine acetate, pH 7.0, linear gradient over 60 min from 5% to 65% acetonitrile at a flow rate of 1.5 mL/min, 2′-deoxycytidine: t = 3.1 min, 2′-deoxyguanosine: t = 4.8 min, thymidine: t = 5.8 min, 2′-deoxyadenosine: t = 7.2 min, **22**: t = 41.3 min). The concentrations of **5** and **6** were determined by comparison of the peak areas of the four nucleosides obtained by enzymatic digestion with those of authentic samples.

Preparation of ^{32}P-5′-End-Labeled DNA Restriction Fragments. Digestion of supercoiled pBR322 plasmid DNA with *Eco*R I restriction endonuclease followed by treatment with bacterial alkaline phosphotase gave a linearized pBR322 with hydroxyl terminus at its 5′-end. Labeling at the 5′-end of the linearized DNA was achieved by treatment with [γ-^{32}P]ATP and T4 polynucleotide kinase using standard procedure.[33] After labeling, the labeled DNA was further digested with *Rsa* I restriction endonuclease to yield two 5′-end-labeled DNA fragments (167 and 514 base pair) which were purified on 6% preparative nondenaturing polyacrylamide gel. The labeled DNA was recovered from the gel by a crush and soak method.[27]

Photoinduced Cleavage of ^{32}P-5′-End-Labeled DNA by 3. Cleavage reactions were carried out in a 100 μL total volume containing calf thymus DNA (1 μM base pair concentration), 3 × 10^4 cpm ^{32}P-5′-end-labeled DNA restriction fragment, and **3** (50 μM) in 5 mM sodium cacodylate buffer at pH 7.0. The solution was irradiated at a distance of 10 cm with transilluminator (366 nm) at 0 °C for 1 h. After irradiation, the reaction mixture was ethanol precipitated with addition of 3 M sodium acetate (20 μL) and ethanol (900 μL). The precipitated DNA was washed with 80% cold ethanol (100 μL) and dried *in vacuo*. The recovered DNA was dissolved in 100 μL of water or 10% (v/v) piperidine and heated at 90 °C for 30 min. The DNA was recovered by ethanol precipitation and resuspended in 10−15 μL of 80% formamide loading buffer (80% formamide, 1 mM EDTA, 0.1% xylene cyanole, and 0.1% bromophenol blue). All DNA samples obtained above and Maxam−Gilbert G+A and C+T sequencing markers[27] were heat denatured at 90 °C for 1 min and quick-chilled on ice. The samples (1−2 μL, 3×10^3 cpm) were loaded onto 8% polyacrylamide and 7 M urea sequencing gel and electrophoresed at 1900 V for approximately

2 h. The gel was dried and exposed to X-ray film with intensifying sheet at −70 °C.

Preparation of ^{32}P-5′-End-Labeled 25-Mer Oligomers 23 and 24. Single-stranded 25-mer DNA oligomers 5′-d(AGTGCCACCTGACGTCTCGTCTCT)-3′ (**23**) and 5′-d(AGTGCCACCTGACGTGCGTGCGTCT)-3′ (**24**) were purchased from Funakoshi CO., Ltd. Concentration of oligonucleotides was determined by HPLC analysis of 2′-deoxynucleosides after enzymatic digestion of the ODN. The 25-mer ODN (450 *p*mol/strand) was 5′-end-labeled by phosphorylation with 5 μL of [γ-^{32}P]ATP (Amersham, 370 MBq/μL) and 4 μL of T_4 polynucleotide kinase (Takara, 10 units/μL) using standard procedures.[33] The 5′-end-labeled DNA was recovered by ethanol precipitation and further purified by 15% preparative nondenaturing gel electrophoresis and isolated by the crush and soak method.

Photoinduced Cleavage of ^{32}P-5′-End-Labeled 25-Mers by DBDM-ODN Conjugates. Photoinduced cleavage of 25-mer ODN was carried out in a 50 μL total volume containing sonicated calf thymus DNA (10 μM base pair concentration), 3×10^4 cpm ^{32}P-5′-end-labeled 25-mer ODN, NaCl (100 mM), NaN_3 (100 mM), and **5** (50 μM) or **6** (1 μM) in 10 mM sodium cacodylate buffer at pH 7.0. The reaction mixture was irradiated with a transilluminator (366 nm) at a distance of 10 cm at 0 °C for 1 h. After irradiation, the reaction mixture was ethanol precipitated with addition of 50 μL of 3 M sodium acetate and 900 μL of ethanol. The precipitated DNA was washed with 200 μL of 80% cold ethanol and dried *in vacuo*. The precipitated DNA was dissolved in 100 μL of water or in 100 μL of 10% piperidine (v/v) and heated at 90 °C for 30 min. The solution was concentrated to dryness using vacuum rotary evaporator and resuspended in 10−15 μL of 80% formamide loading buffer (a solution of 80% v/v formamide, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). All DNA samples obtained above and Maxam−Gilbert G+A and C+T sequencing markers were heat denatured at 90 °C for 1 min and quick-chilled on ice. The samples (1−2 μL, $5-10 \times 10^3$ cpm) were loaded onto 15% (19:1) polyacrylamide and 7 M urea sequencing gels and electrophoresed at 1900 V for approximately 2 h. The gel was dried and exposed to X-ray film with intensifying sheet at −70 °C.

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