# Photoactivatable DNA-Cleaving Amino Acids: Highly Sequence-Selective DNA Photocleavage by Novel L-Lysine Derivatives

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There is much current interest in designing artificial DNA-cleaving molecules that are chemically stable and activatable by photoirradiation.[1,2] While the photochemical DNA cleavage has been observed with various types of DNA-binding molecules, the detailed chemistry associated with photoinduced DNA cleavage is not well understood.[1,2] We report herein novel water-soluble L-lysine derivatives possessing a naphthalimide chromophore that can induce efficient and highly sequence selective cleavage of double-stranded DNA upon photoirradiation at 320−380 nm. By incorporating a nitro group into the naphthalimide ring, the selectivity of DNA cleavage was dramatically changed from a specific cleavage at the 5′ side of guanine−guanine (-GG-) sequences to a thymine (T)-specific cleavage.

In our efforts to design practically useful DNA-cleaving amino acids, we incorporated a 1,8-naphthalimide chromophore into the $\epsilon$-amino group of L-lysine. Treatment of Boc-L-lysine methyl ester with commercially available 1,8-naphthalic anhydride followed by deprotection provided water-soluble L-lysine derivative **1**, having an intense UV absorption (log $\epsilon$ 3.70 at 366 nm). Nitro-substituted lysine derivatives **2** (log $\epsilon$ 3.61 at 366 nm) and **3** (log $\epsilon$ 3.68 at 366 nm) were synthesized in a similar fashion, since certain aromatic nitro compounds have been known to cleave DNA by photoirradiation.[3]

The DNA-cleaving properties of **1**, **2**, and **3** were preliminarily examined using supercoiled circular pBR322 (form I) DNA under photoirradiation with a transilluminator (366 nm) at 0 °C for 1 h. In each case, efficient single-strand breaks and a small amount of double-strand breaks were observed at 10 $\mu$M drug concentration. The base and sequence specificity of DNA cleavage were analyzed using $^{32}$P-end-labeled double-stranded DNA fragments obtained from human c-Ha-*ras*-1 protooncogene.[4] Lysine derivative **1** induced highly specific DNA cleavage at the 5′ side of 5′-GG-3′ steps with a very weak cleavage at the 5′ side of 5′-GA-3′ steps after piperidine treatment of the photoirradiated mixture: no cleavage was observed at other sites, including single G residues (Figure 1, lane 1). Control experiments established that photoirradiation, piperidine treatment, and double-helical structure of DNA are all indispensable for the specific 5′-GG-3′ cleavage.[5] In marked contrast, under the same conditions, 3-nitro derivative **2**

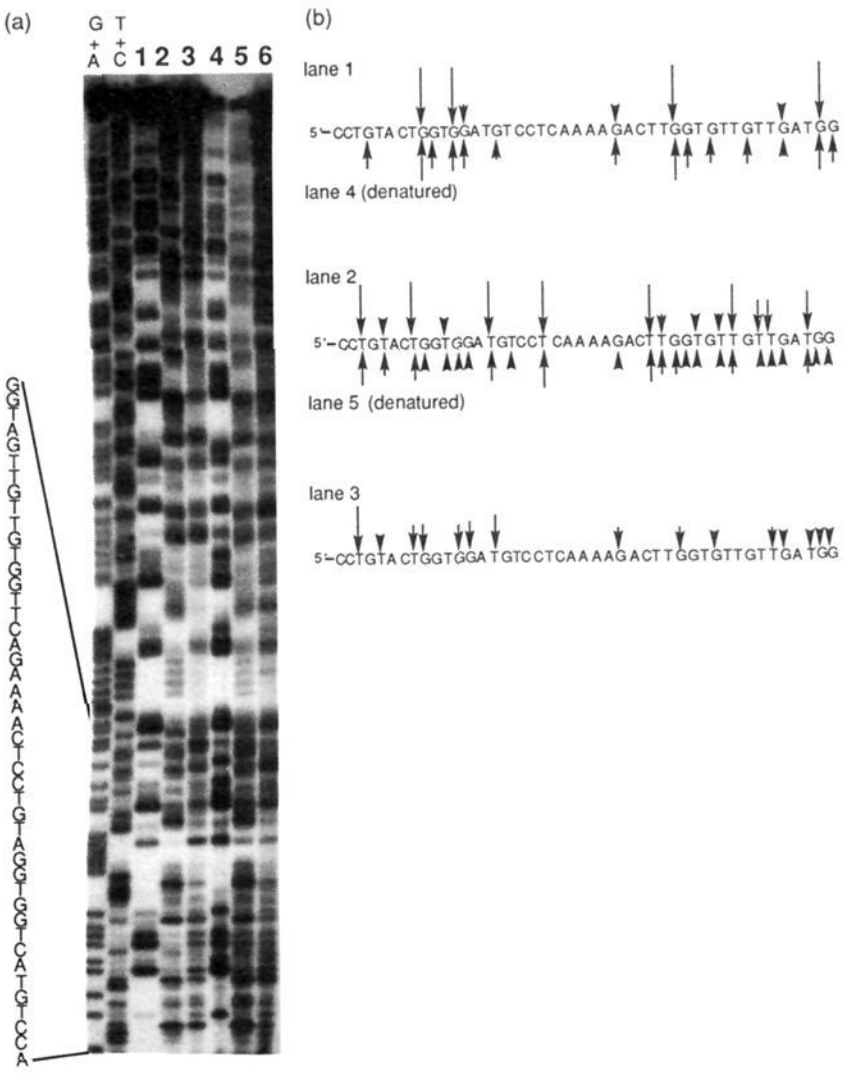
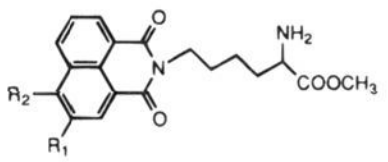



**Figure 1.** (a) Autoradiogram of photoirradiated $^{32}$P-5′-end-labeled DNA fragments in the presence of L-lysine derivatives **1**, **2**, and **3**. The reaction mixture containing **1**, **2**, or **3**, $^{32}$P-5′-end-labeled 341-base pair DNA fragment (*pst*-I2345 to *Aval* 2681) of human c-Ha-*ras*-1 protooncogene, and 50 mM sonicated calf thymus DNA in 50 mM sodium cacodylate buffer (pH 7.0) was photoirradiated with a transilluminator (366 nm) at 0 °C for 20 min under otherwise identical conditions. After piperidine treatment (90 °C, 20 min), the DNA fragment was analyzed by electrophoresis on an 8% polyacrylamide/8 M urea gel. Lane 1, **1** (50 $\mu$M); lane 2, **2** (20 $\mu$M); lane 3, **3** (25 $\mu$M); lane 4, **1** (50 $\mu$M), denatured DNA; lane 5, **2** (20 $\mu$M), denatured DNA; lane 6, **3** (25 $\mu$M), denatured DNA. (b) Histogram representation of the DNA cleavage data shown in Figure 1a. Arrow represents the extent of cleavage by photoirradiation followed by piperidine treatment. Lanes 1, 2, and 3 show the cleavage of double-stranded DNA, whereas lanes 4, 5, and 6 represent single-strand breaks using denatured DNA.




**1** $R_1 = R_2 = H$
**2** $R_1 = NO_2$, $R_2 = H$
**3** $R_1 = H$, $R_2 = NO_2$

(1) For a review, see: *Bioorganic Photochemistry, Photochemistry and the Nucleic Acid*; Morrison, H., Ed.; John Wiley and Sons: New York, 1990; Vol. 1.

(2) For recent references, see: (a) Bowler, B. E.; Hollis, L. S.; Lippard, S. *J. Am. Chem. Soc.* **1984**, *106*, 6102. (b) Barton, J. K. *Science* **1986**, *233*, 727. (c) Saito, I.; Takayama, M.; Matsuura, T.; Matsugo, S., Kawanishi, S. *J. Am. Chem. Soc.* **1990**, *112*, 883. (d) Perrouault, L.; Asseline, U.; Rivallet, C.; Thuong, N. T.; Bisagni, E.; Giovannangeli, C.; Le Doan, T.; Héléne, C. *Nature* **1990**, *344*, 358, (e) Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1990**, 112, 6397. (f) Matsugo, S.; Kawanishi, S.; Yamamoto, K.; Sugiyama, H.; Matsuura, T.; Saito, I. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1351. (g) Saito, I. *Pure Appl. Chem.* **1992**, *64*, 1305. (h) Siitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303. (i) Nielsen, P. E.; Hirort, C.; Sonnichsen, S. H.; Buchardt, O.; Dahl, O.; Norden, B. *J. Am. Chem. Soc.* **1992**, *114*, 4967. (j) Dunn, D. A.; Lin, V. H.; Kochevar, I. E. *Biochemistry* **1992**, *31*, 11620. (k) Quada, J. C.; Levy, M. J., Jr.; Hecht, S. M. *J. Am. Chem. Soc.* **1993**, *115*, 12171. (l) Sitlani, A.; Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 12589. (m) Ito, K.; Inoue, S.; Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1993**, *268*, 13221. (n) Wender, P. A.; Zercher, C. K.; Beckham, S.; Haubold, E.-M. *J. Org. Chem.* **1993**, *58*, 5867. (o) Saito, I.; Takayama, M.; Sakurai, T. *J. Am. Chem. Soc.* **1994**, *116*, 2653. (p) Saito, I.; Sakurai, T.; Kurimoto, T.; Takayama, M. *Tetrahedron Lett.* **1994**, *35*, 4797. (q) Nakatani, K.; Isoe, S.; Maekawa, S.; Saito, I. *Tetrahedron Lett.* **1994**, *35*, 605. (r) Armitage, B.; Changjun, Y.; Devadoss, C.; Schuster, G. B. *J. Am. Chem. Soc.* **1994**, *116*, 9847.

(3) (a) Buchardt, O.; Egholm, M.; Karup, G.; Nielsen, P. E. *J. Chem. Soc., Chem. Commun.* **1987**, 1696. (b) Nielsen, P. E.; Jeppesen, C.; Egholm, M.; Buchardt, O. *Biochemistry* **1988**, *27*, 6338. (c) Nishiwaki, E.; Matsumoto, T.; Toyooka, K.; Sakurai, H.; Shibuya, M. *Tetrahedron Lett.* **1990**, *31*, 1299. (d) Nielsen, P. E.; Egholm, M.; Koch, T.; Christensen, J. B.; Buchardt, O. *Bioconjugate Chem.* **1991**, 2, 57.

(4) Yamamoto, K.; Inoue, S.; Yamazaki, A.; Yamashita, T.; Kawanishi, S. *Chem. Res. Toxicol.* **1989**, *2*, 234.

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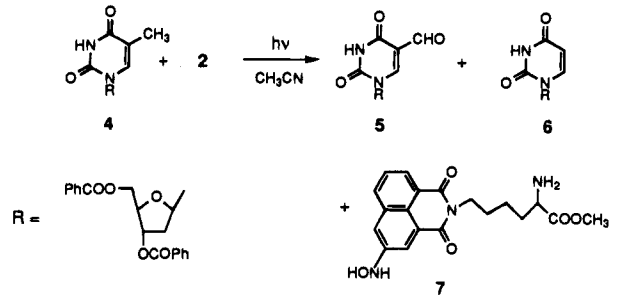
**Scheme 1**



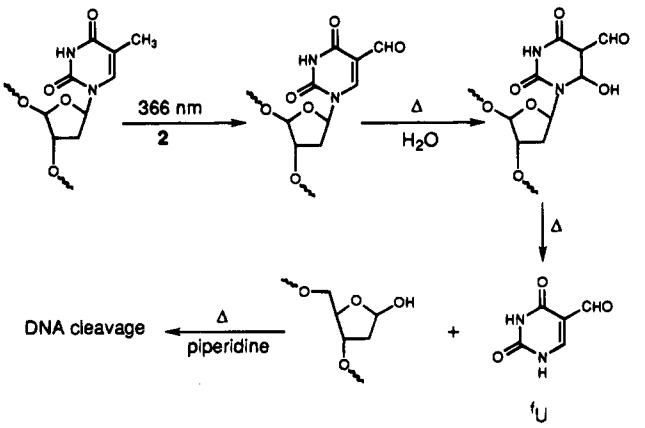

photonicked the double-stranded DNA preferentially at T residues with a relatively weak cleavage at 5′-GT-3′ steps after piperidine treatment: neither 5′-GG-3′ nor G cleavage has been observed in this case (lane 2). In the photoiradiation of 4-nitro derivative **3**, both 5′-GG-3′ and T cleavage occurred almost equally after piperidine treatment (lane 3). Thus, the sequence selectivity of the double-strand cleavage is highly dependent upon substituents and the substitution pattern on the aromatic ring. In none of these cases did the DNA cleavage occur without piperidine treatment.

The high sequence-selectivity observed for **1** and **2** was completely lost in the photoreaction of heat-denatured single-stranded DNA. Thus, almost equal cleavage occurred at all G residues in the photoreaction of **1** with single-stranded DNA (lane 4), whereas the cleavage at both T and G residues occurred in the presence of **2** (lane 5). The equal G cleavage of single-stranded DNA by **1** may be due to the oxidation of guanine base with singlet oxygen,[6] since the single-strand breaks induced by **1** were enhanced more than 2-fold in $D_2O$[2m] and inhibited by the addition of a singlet oxygen quencher, $NaN_3$ , whereas the 5′-GG-3′ specific cleavage of double-stranded DNA was not affected in $D_2O$.

To get insight into the mechanism of the T-specific cleavage by **2**, we examined the photoreaction of **2** with T monomer and T-containing deoxyoligonucleotides. Irradiation of **2** in the presence of 3′,5′-O-dibenzoylthymidine (**4**) (5 equiv) in acetonitrile gave 5-formyldeoxyuridine ($d^fU$) derivative **5**[7] (14%) and deoxyuridine **6** (17%), together with reduction product **7** (36%) (Scheme 1). Deoxyuridine **6** was proven to be derived from further photoirrdiation of **5** via photodecarbonylation.

Photoirradiation of **2** in the presence of deoxytrinucleotide d(ATA) (2 equiv) in sodium cacodylate buffer (pH 7.0) gave d(A$^f$UA) in 65% yield. The structure of d(A$^f$UA) was confirmed by converting it to $d^fU$ and dA (2 equiv) by enzymatic digestion with snake venom phosphodiesterase and alkaline phosphatase (AP). Treatment of isolated d(A$^f$UA) with 1 M piperidine at 90 °C for 20 min gave 5-formyluracil ($^fU$) (52%) and Ap plus pA. Likewise, photoirradiation of **2** (100 mM) and poly[dA]/poly[dT] (200 mM) in sodium cacodylate buffer (pH 7.0) at 0 °C for 1 h followed by enzymatic digestion with snake venom phosphodiesterase and AP produced $d^fU$ (55%) and deoxyuridine (11%). These results clearly indicate that the first step of the T-specific cleavage of double-stranded DNA by **2** is an oxidative transformation of the T methyl group into a formyl group initiated by the hydrogen abstraction from the T methyl group by a photoexcited nitro group, not via the hydrogen abstraction from the DNA sugar backbone or an electron transfer as suggested earlier.[3b,d] Heating $^fU$-containing sites in DNA (90 °C, 20 min) eventually resulted in a DNA strand scission via the mechanism shown in Scheme 2, as evidenced by the thermal degradation of d(A$^f$UA).[8,9]

**Scheme 2**




While the detailed mechanism of the 5′-GG-3′ specific cleavage by **1** is not clear at present, it is very likely that an electron transfer from the most electron-rich -GG- step to photoexcited naphthalimide **1** is an initial step for the photooxidative destruction of the guanine base at the 5′ side of 5′-GG-3′ steps.[2j,m,10,11] Further studies on the electron transfer from -GG- steps to triplet excited **1** by laser flash photolysis are underway and will be reported in due course.

In summary, novel L-lysine derivatives described here exhibited high DNA cleavage efficiency and selectivity upon photoirradiation at 320−380 nm. Such artificial amino acids should find widespread use as a water-soluble DNA-cleaving amino acid as well as a DNA-cleaving moiety for the design of photoactivatable DNA-cleaving polypeptides and hybrid molecules.

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**Supplementary Material Available:** Experimental details, photocleavage of supercoiled circular pBR322 DNA, $D_2O$ effect on the photocleavage of $^{32}P$-end-labeled DNA fragments, characterization data for new compouds, and HPLC profiles of the photoreactions of d(ATA) and poly[dA]/poly[dT] in the presence of **2** (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(5) Specific cleavage at 5′-GG-3′ sequences has previously been observed with other DNA-cleaving molecules.[2f,m]

(6) (a) Friedman, T.; Brown, D. M. *Nucleic Acids Res.* **1978**, *5*, 615. (b) Kawanishi, S.; Inoue, S.; Sano, S. *J. Biol. Chem.* **1986**, *261*, 6090. (c) Devasagayam, T. P. A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, *30*, 6283.

(7) (a) Mertes, M. P.; Shipchandler, M. T. *J. Heterocycl. Chem.* **1970**, *7*, 751. (b) Decarroz, C.; Wagner, J. R.; Van Lier, J. E.; Kroshna, C. M.; Riesz, P.; Cadet, J. *Int. J. Radiat. Biol.* **1986**, *50*, 491. (c) For the synthesis of $^fU$-containing oligonucleotides, see: Ono, A.; Okamoto, T.; Inada, M.; Nara, H.; Matsuda, A. *Chem. Pharm. Bull.* **1994**, *42*, 2231 and references therein.

(8) Instability of $d^fU$- and $^fU$-containing oligonucleotides under alkaline conditions, see ref 7c. See also: Armstrong V. W.; Dattagupta, J. K.; Eckstein, F.; Saenger, W. *Nucleic Acids Res.* **1976**, *3*, 1791.

(9) Other examples for T-specific cleavage, see: (a) Saito, I.; Matsuura, T. *Acc. Chem. Res.* **1985**, *18*, 134. (b) Welch, T. W.; Neyhart, G. A.; Goll, J. G.; Ciftan, S. A.; Thorp, H. H. *J. Am. Chem. Soc.* **1993**, *115*, 9311.

(10) Electron transfer from guanine base, see: (a) Steenken, S. *Chem. Rev.* **1989**, *89*, 503. (b) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1993**, *115*, 2437. (c) Cadet, J.; Berger, M.; Buchko, G. W.; Joshi, R. C.; Raoul, S.; Ravanet, J.-L. *J. Am. Chem. Soc.* **1994**, *116*, 7403, and references therein.

(11) Oxidation of guanine by metal complexes, see: (a) Pyle, A. M.; Barton, J. K. In *Progress in Inorganic Chemistry: Bioinorganic Chemistry*; Lippard, S. J., Ed.; Wiley: New York, 1990; Vol. 38, pp 413. (b) Billadeau, M. A.; Wood, K. V.; Morrison, H. *Inorg. Chem.* **1994**, *33*, 5780. (c) Johmston, D. H.; Cheng, C.-C.; Campbell, K. J.; Thorp, H. H. *Inorg. Chem.* **1994**, *33*, 6388. (d) Burrows, C. J.; Rokita, S. E. *Acc. Chem. Res.* **1994**, *27*, 295.