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A NOVEL PHOTOCHEMICAL DNA-CLEAVING AGENT, BROMINATED DIBENZOYLMETHANES

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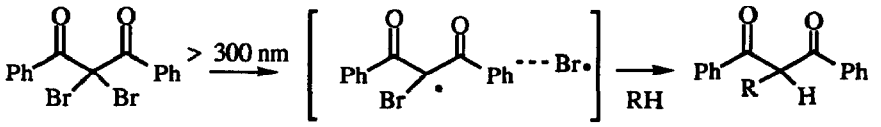
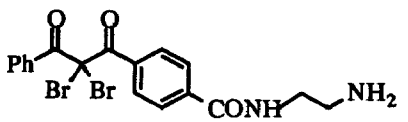
Abstract: Novel photochemical DNA-cleaving molecules possessing a dibenzoyldibromomethane chromophore have been prepared. The efficiency and the sequence selectivity for the DNA photocleavage have been described.

In recent years, synthetic DNA-cleaving molecules, particularly light-triggered DNA cleavers are of great interest for designing DNA-cleaving molecules directed at specific sequences.[1] Our laboratory has focused primarily upon exploitation of new methodologies for site selective DNA cleavage triggered by photochemical reactions.[2] Minimum requirements for designing practically useful photochemical DNA cleavers, including photofootprinting agents, are: i) high absorptivity in the UVA region (320-400 nm); ii) efficient generation of reactive species, preferably non-diffusible species capable of reacting with nucleic acid base or DNA deoxyribose backbone with high quantum yield; and iii) accessibility for tethering to DNA binders. We are particularly interested in the design of DNA cleavers that can generate non-diffusible radical species by irradiation with long wavelength UV light.[2e,f] Herein described is a novel type of photochemical DNA-cleaving molecules possessing a dibenzoyldibromomethane chromophore as exemplified by **6** and **9**.[3]

Dibenzoyldibromomethane (**1**) has an adequate absorption band between 300- 400 nm (366 nm, ε 67) and is susceptible to photolytic decomposition to **2** by irradiation with > 300 nm



1 **2** R = Br **3** R = H



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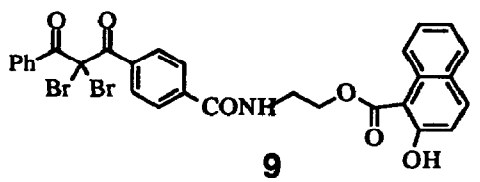
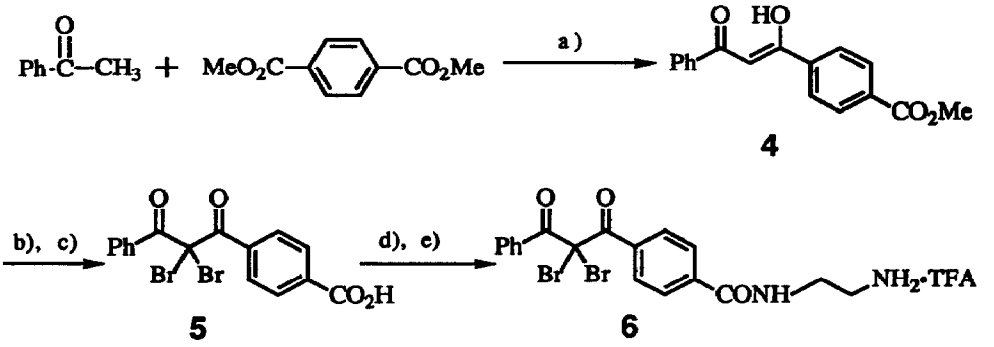


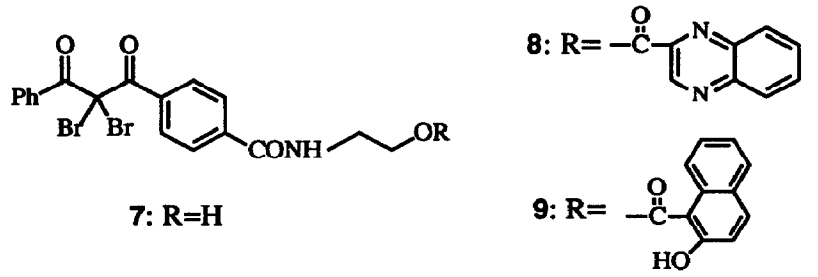
9

via a homolytic cleavage of the C-Br bond. For example, photoirradiation of **1** (10 mM) with a high-pressure Hg lamp through a $CuSO_4$ filter solution (cut off 330 nm) in benzene containing excess 1,4-cyclohexadiene as a hydrogen donor under nitrogen produced mono-debrominated product **2** almost quantitatively at low conversion (< 15 %) with a quantum yield of 0.47.[4] Further irradiation resulted in a formation of a mixture of products including **2** and dibenzoylmethane (**3**). We have then prepared brominated 1,3-diketones possessing a carboxyl group (**5**) and an alkyl amino side chain (**6**) in order to improve the solubility in water via the route shown in Scheme 1.[5] Irradiation of **5** and **6** also gave the corresponding mono-debrominated products in the presence of excess 1,4-cyclohexadiene at low conversion.

Scheme 1



a) NaH(1eq)/THF, reflux(62%); b) LiOH(5eq)/H_2O/THF(61%); c) $Br_2/CHCl_3$, 0 °C(62%); d) $SOCl_2$, reflux; $H_2NCH_2CH_2NHBoc$/pyridine, 0 °C(62%); e) 50%TFA/CH_2Cl_2(100%)



We have next examined the DNA-cleaving activities of these compounds (**1**, **5** and **6**) under the influence of light illumination. Incubation of supercoiled circular pBR322 DNA (form I) with these drugs under light illumination (366 nm) induced the transformation of form I DNA into nicked circular (form II) DNA (Figure 1). Particularly, efficient single strand cleavage was observed with **6** possessing cationic alkyl amino side chain at concentration of 10 μM (lane 8), whereas without irradiation no DNA cleavage occurred at all even at 1 mM drug concentration.

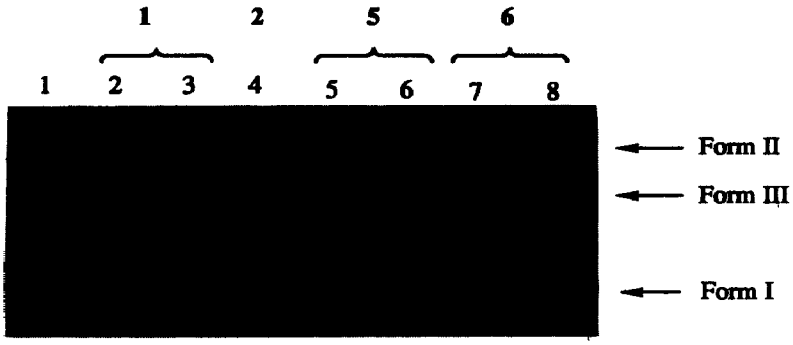


Figure 1: Light-induced cleavage of supercoiled circular pBR322 DNA (form I) into nicked circular DNA (form II) in the presence of 1,2,5 and 6. The reaction mixtures containing 50 μM DNA (form I) and varing concentrations of drug in 10 mM sodium cacodylate buffer (pH 7.0) were irradiated at a distance of 5 cm from transilluminator (366 nm) at 0 °C for 1 h. Lane 1, DNA control; lane 2, DNA + 1 (100 μM); lane 3, DNA + 1 (10 μM); lane 4, DNA + 2 (100 μM); lane 5, DNA + 5 (100 μM); lane 6, DNA + 5 (10 μM); lane 7, DNA + 6 (100 μM); lane 8, DNA + 6 (10 μM).

Encouraged by the observed high DNA-cleaving activities of **1** and **6**, we have then prepared hybrid molecules **8** and **9** both possessing an aromatic DNA binder from **7**.[5] DNA-cleaving activities of **7**, **8** and **9** are shown in Figure 2. Contrary to our expectation, hybrid molecules **8** and **9** exhibited only slightly higher DNA-cleaving activities at 366 nm irradiation. Sequence selectivity for the photocleavage by **9** was next examined by using 5'-^{32}P-end labeled 337 base pair DNA fragments at 366 nm irradiation. Faint DNA cleavage bands were observed but with no definite base selectivity (Figure 3, lower part). However, when the photolysate was treated with piperidine, intense cleavage bands appeared at all guanine (G) residues (Figure 3, upper peaks). The faint DNA cleavage bands without piperidine treatment are probably resulted from

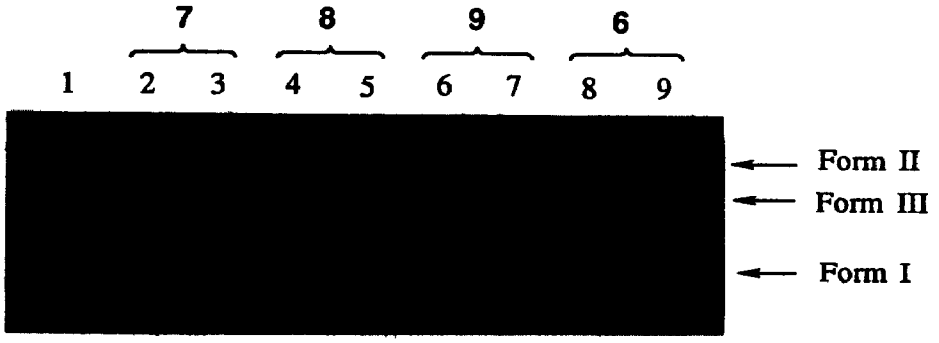


Figure 2: Light-induced cleavage of supercoiled circular pBR322 DNA (form I) into nicked circular DNA (form II) in the presence of 6,7,8 and 9. The reaction mixtures containing 50 μM DNA (form I) and varing concentrations of drug in 10 mM sodium cacodylate buffer (pH 7.0) were irradiated as described in Figure 1. Lane 1, DNA control; lane 2, DNA + 7 (100 μM); lane 3, DNA + 7 (10 μM); lane 4, DNA + 8 (100 μM); lane 5, DNA + 8 (10 μM); lane 6, DNA + 9 (100 μM); lane 7, DNA + 9 (10 μM); lane 8, DNA + 6 (100 μM); lane 9, DNA + 6 (10 μM).

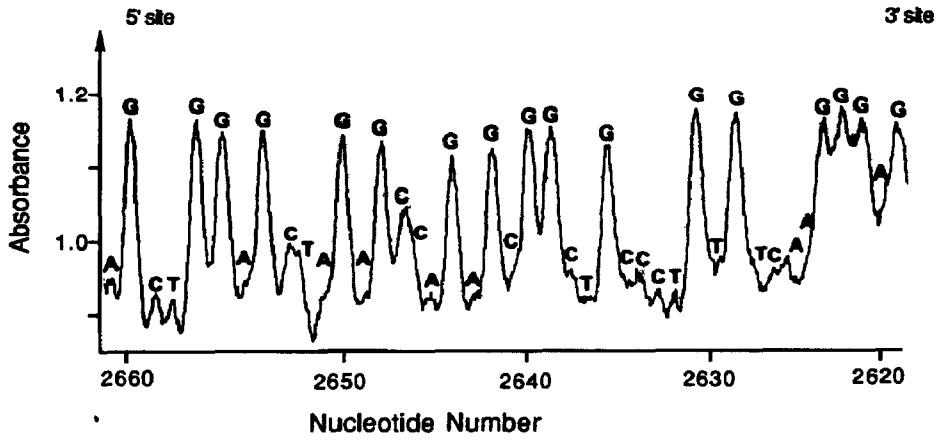


Figure 3: Site specificity of DNA cleavage induced by photoirradiation in the presence of **9**. The reaction mixture containing the ^{32}P-5'-end labeled 337-bp fragment (*Pst* I 2345 to *Ava* I *2681) of human c-Ha-*ras*-1 protooncogene, 50 μM of **9** and 5 μM/base sonicated calf thymus DNA in 10 mM sodium cacodylate buffer (pH 7.0) was irradiated at a distance of 5 cm from transilluminator (366 nm) at 0 °C for 20 min. After piperidine treatment (90 °C, 20 min) the DNA fragment was analyzed by electrophoresis on an 8 % polyacrylamide/8 M urea gel. The relative amounts of oligonucleotides produced by photoreaction and subsequent piperidine treatment were measured by a laser densitometer.

hydrogen abstraction from DNA sugar backbone by drug radical, whereas strong G bands only appeared after piperidine treatment with almost equal intensities would be ascribable to the photooxidation of guanine base under the aerobic conditions.[6]

In summary, the present work has demonstrated that readily available dibenzoyldibromomethane derivatives can be used as a convenient and useful photochemical DNA cleaver which would be potentially applicable to photofootprinting agents or photonucleases.[7]

References and Notes

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3. Photochemcal DNA cleavage by halogenated bithiazoles has been reported. Quada,J. C. Jr.; Levy, M. L.; Hecht S. M. *J. Am. Chem. Soc.* **1993**, 115, 12171.
4. Quantum yield measurements were carried out at 0 °C in a merry-go-round apparatus using phenylglyoxylic acid (ϕ = 0.70 at 334 nm) as an actinometer.
5. All new compounds were purified by silica-gel column chromatography and fully characterized by spectroscopic means.
6. For example, see a) Devasagayam, T. P.A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, 30, 6283. (b) Nielsen, P. E.; Egholm, M.; Koch, T.; Christensen, J. B.; Buchardt, O. Bioconjugate Chem. **1991**, 2, 57.
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