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Dibenzoyldiazomethane-Acridine Conjugate: A Novel DNA Photofootprinting Agent

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Abstract: Dibenzoyldiazomethane derivative covalently attached to acridine effectively cleaves DNA by photoirradiation with long wavelength UV light (> 400 nm). DNA cleavage experiments using ^{32}P-5'-end-labeled DNA showed that the cleavage by the conjugate is neither sequence nor base selective, indicating that DBDM-acridine conjugate can be used as a photofootprinting agent.
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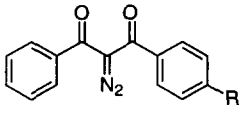
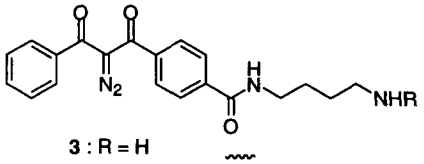
Footprinting method is a technique widely used for determining binding site of drugs and proteins to DNA. Enzymes like DNase I and II and chemical footprinting agents such as methidium-EDTA-iron (II)[1] and 1,10-phenanthroline copper (I)[2] are well known. Recently, photochemical footprinting agents have also been reported by several groups. These include $Rh(phi)_2(bpy)^{3+}$,[3] azido-9-aminoacridine derivatives,[4] uranyl (VI) salt,[5] and anionic diplatinum agent.[6] Such photofootprinting agents have an advantage over the enzymatic methods or non-photochemical footprinting agents in that the DNA cleavage is controllable by light. We have been interested in designing molecules which can cleave DNA upon photochemical activation but remain stable in the dark,[7] and focused our attention on the compounds having dibenzoyldiazomethane (DBDM, **1**) as a chromophore.[8] We herein report that a DBDM derivative covalently attached to acridine (DBDM-acridine conjugate **4**) is a novel type of DNA-cleaving agent which effectively cleaves DNA without any sequence or base selectivity under photoirradiation with long wavelength UV light.

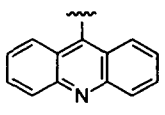


1 : R = H (**DBDM**)
2 : R = CO_2Me



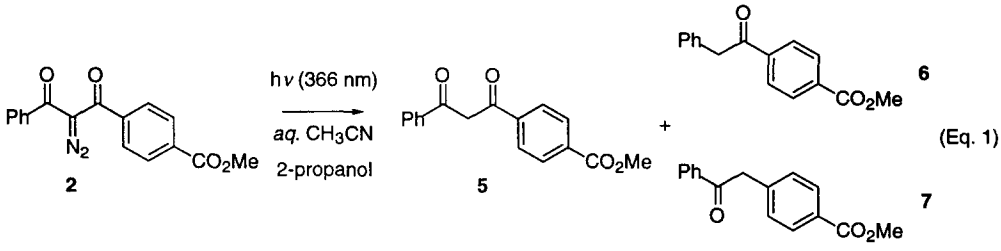
3 : R = H

4 : R =



Upon direct photoirradiation, DBDM is known to undergo Wolff rearrangement to produce electrophilic benzoylketene. We have previously reported that DBDM derivatives having aminoalkyl side chain, *e.g.*, **3**, effectively cleave DNA by photoirradiation at 366 nm.[8] The cleavage selectivity investigated

by high resolution denaturing gel electrophoresis using ^{32}P-5'end-labeled DNA indicated that DNA was selectively cleaved at guanine residues after hot piperidine treatment possibly via the electrophilic attack of benzoylketene on the guanine N7. In contrast, triplet sensitization of DBDM derivative **2** by Michler's ketone (MK) and 9-aminoacridine (AA) preferentially produced dibenzoylmethane derivative **5** via triplet carbene **8** over the formation of acetophenone derivatives **6** and **7** derived from benzoylketene derivative **9** (Eq. 1 and Table 1).[8]

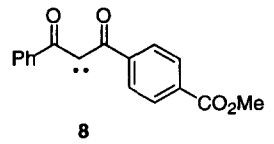


(Eq. 1)

Table 1. Photoreaction of **2** in 50% Aqueous Acetonitrile in the Presence and Absence of Sensitizer[a]

entry	sensitizer[b] (ratio)[c]	time (h)	isolated yield (%)[d]	product ratio[e] (**5** : **6**+**7**)
1	——	23.5	92	23 : 77
2	**MK** (0.1)	55.5	92	72 : 28
3	**AA** (5.0)	57.0	65	81 : 19

[a] A 50% aqueous acetonitrile solution of **2** (0.2 mmol, 0.2 mM, $\varepsilon_{366} \approx 1\times10^2$) was irradiated with transilluminator (366 nm) in the presence and absence of sensitizer. 2-Propanol (2 mM for entries 1 and 2, and 20 mM for entry 3) was added as a hydrogen donor. [b] **MK**: 4,4'-dimethylaminobenzophenone (Michler's ketone, $\varepsilon_{366} \approx 3\times10^4$); **AA**: 9-aminoacridine, ($\varepsilon_{366} \approx 3\times10^3$). [c] Relative molar ratio to **2**. [d] Combined yield of **5**, **6**, and **7**. [e] Determined by ^{1}H NMR.



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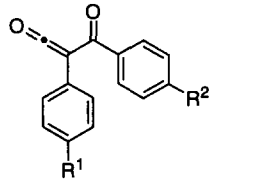
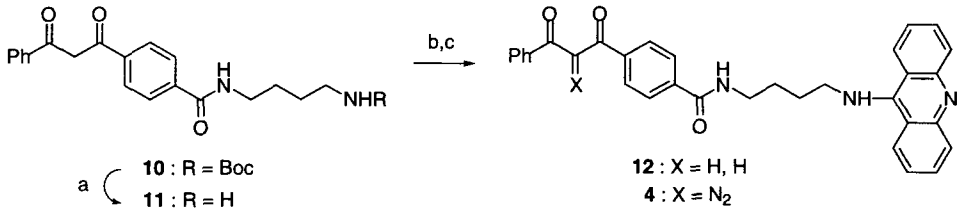


9: R^1, R^2 = H and CO_2Me

We have then examine the synthesis of DBDM derivatives covalantely attached to acridine sensitizer. Synthetic scheme for DBDM-acridine conjugate **4**[9] from **10**[8] having tetramethylene amino linker was shown in Scheme 1. Fluorescence intensity of **4** at 455 nm (excited at 366 nm) substantially decreased compared to that observed for 9-aminoacridine. In fact, fluorescence of 9-aminoacridine was quenched by external addition of **2** ($k_q\tau$ = 423 M^{-1}), suggesting an efficient energy transfer from triplet acridine to DBDM chromophore. The acridine moiety of **4** also played an important role as a DNA binder with association constant of 1.9×10^4 M^{-1} as determined by UV-Vis titration.[10]

Scheme 1[a]



[a] *Reagents*: (a) HCl, AcOEt; (b) aqueous NaOH, then 9-chloroacridine, PhOH; (c) TsN_3, Et_3N, DMF.

Photoinduced cleavage of supercoiled plasmid DNA by **4** was examined at 412 and 434 nm isolated from monochromator (Figure 1). As clear from the figure, DNA was effectively cleaved at concentration as low as 5 μM (lanes 2 and 4). While most of incident lights at 412 and 434 nm were absorbed by the acridine chromophore under the irradiation conditions, 9-aminoacridine alone did not induce DNA cleavage at this concentration.

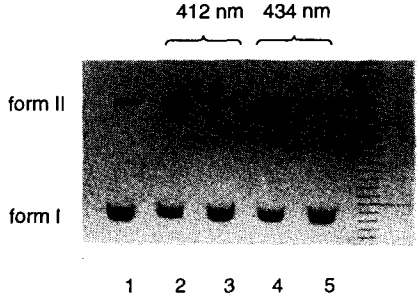


Figure 1. DNA cleavage by **4** under photoirradiation conditions. Buffered solutions (Na cacodylate, pH 7.0, 5 mM, 10 μL) containing supercoiled (form I) pBR322 DNA (40 μM) and **4** were irradiated at 412 and 434 nm at 0 °C for 1 h. Photoirradiated DNAs were analyzed by electrophoresis on 1% agarose gel containing ethidium bromide (0.6 μg/mL). lane 1, DNA control; lane 2, 5 μM, 412 nm; lane 3, 2.5 μM, 412 nm; lane 4, 5 μM, 434 nm; lane 5, 2.5 μM, 434 nm.

The cleavage selectivity for DBDM-acridine conjugate **4** investigated by high resolution denaturing gel electrophoresis indicated that the DNA cleavage was neither sequence nor base selective (Figure 2). While heating the photoirradiated DNAs with piperidine drastically enhanced the DNA cleavage (lane 5), weak smear DNA cleavage bands were also observed for both non-heated (lane 3) and heated (lane 4) DNAs, showing that spontaneous strand cleavage also occurred at every nucleotide. These results suggest that photoinduced DNA cleavage by **4** may proceed via a hydrogen abstraction reaction from DNA sugar backbone. While 9-aminoacridine induced guanine specific DNA cleavage at 366 nm due to selective oxidation of guanine residues by singlet oxygen,[11,12] the generation of singlet oxygen in the photoillumination of **4** was suppressed by efficient intramolecular energy transfer from triplet acridine to DBDM chromophore. The resulting triplet DBDM chromophore would eventually produce triplet carbene via nitrogen extrusion, a most likely DNA-cleaving species, as supported by triplet sensitization of **2** giving **5** preferentially.[13]

In summary, the present work has demonstrated that DBDM-acridine conjugate **4** effectively cleaves DNA under photoirradiation with long wavelength UV light. Observed highly efficient and base neutral

DNA cleavage indicates that DBDM-acridine conjugate **4** can be used as a convenient photofootprinting agent.

Figure 2. Sequencing gel for the photoreaction of **4** with ^{32}P-5'-end-labeled DNA. ^{32}P-5'-end-labeled DNA (pBR322 DNA, *Eco*R I–*Rsa* I fragment, 167 bp) with calf thymus DNA (1 μM) was irradiated at 366 nm for 1 h in the presence of **4** (50 μM) at pH 7.0 (5 mM, Na cacodylate) at 0 °C. Recovered DNA by ethanol precipitation was treated as below and analyzed on a sequencing gel containing 8% polyacrylamide and 7 M urea. lane 1, A+G reaction; lane 2, C+T reaction; lane 3, non-heated DNA; lane 4, heated DNA; lane 5, heated DNA with piperidine (10% v/v).

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(9) **4**: mp 105 °C (decomp.); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08–8.01 (2H), 7.95–7.93 (2H), 7.80 (m, 1H), 7.75–7.71 (2H), 7.58–7.51 (5H), 7.43 (m, 1H), 7.34–7.25 (4H), 6.69 (br, 1H), 3.86 (m, 2H), 3.49 (m, 2H), 1.89 (m, 2H), 1.77 (m, 2H); UV (H_2O) 434.0 (ε 4770), 412.4 (ε 5500), 395.4 (ε 3800), 366.0 (ε 2200); FABMS (NBA+glycerol) *m/z* 542 [$(M+H)^+$], 514 [$(M-N_2+H)^+$]; HRFABMS (NBA+glycerol) calcd for $C_{33}H_{28}O_3N_3$ [$(M-N_2+H)^+$], 514.2132; Found 514.2114.
(10) UV-Vis titration of **4** (100 μM) with calf thymus DNA (0~300 μM) was carried out in 5 mM sodium cacodylate buffer (pH 7.0). Absorbance change at 434 nm induced by the addition of DNA was analyzed by Scatchard plot.
(11) (a) Houba-Herin, N.; Calberg-Bacq, C. M.; Piette, J.; Van de Vorst, A. *Photochem. Photobiol.* **1982**, *36*, 297–306. (b) Iwamoto, Y.; Yoshioka, H.; Yanagihara, Y. *Chem. Pharm. Bull.* **1987**, *35*, 2478–2483.
(12) For a recent reference of guanine oxidation by singlet oxygen, see: Raoul, S.; Cadet, J. *J. Am. Chem. Soc.* **1996**, *118*, 1892–1898.
(13) Photoreaction of **4** in aqueous acetonitrile in the presence of 2-propanol actually produced dibenzoylmethane derivative **12** by hydrogen abstraction, but accompanied with the formation of unidentified complex mixtures, indicating that other processes besides triplet carbene formation are also involved in the photodecomposition of **4**.

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