Photogeneration of Carbocation via Intramolecular Electron Transfer: Photoinduced DNA Alkylation

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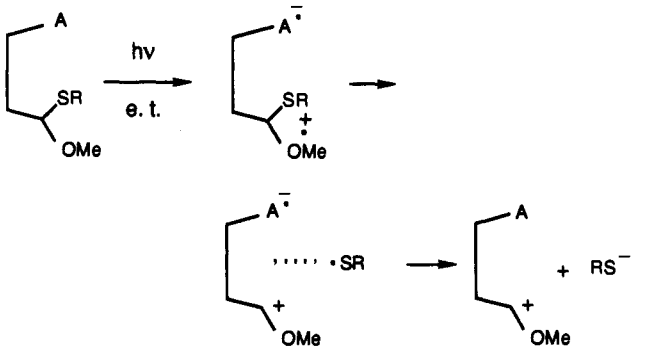
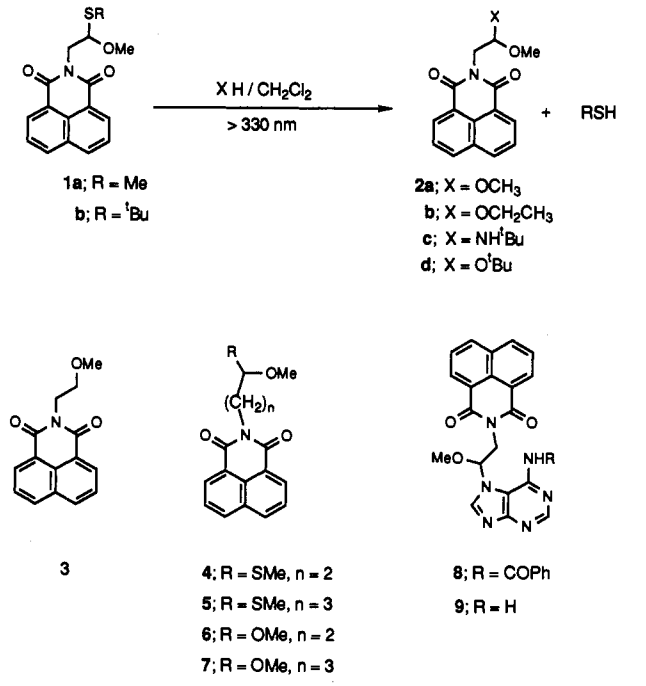
There has been much current interest in the design and synthesis of sequence-specific DNA-alkylating agents as potential antitumor agents or prothetic groups for antisense oligonucleotides.[1] Our laboratory has been interested in the design of photochemical DNA-cleaving molecules,[2] particularly light-inducible DNA-alkylating agents. We report herein an intriguing example of light-triggered generation of a carbocation capable of alkylating DNA under the influence of low-energy UV light (>330 nm). Our strategy for the photogeneration of carbocation is based on an electron-transfer-initiated fragmentation of monothioacetals as illustrated in Scheme 1.[3,4] The monothioacetal radical cation produced by intramolecular electron transfer to the excited acceptor (A) is expected to undergo rapid fragmentation to carbocation with release of alkylthio radical, which would be immediately reduced by the radical anion $A^{\bullet-}$, thus furnishing heterolytic cleavage of the C–S bond.[4]

Photoirradiation of 1,8-naphthalimide **1a** (λ_{max} 332 nm, log ϵ 4.12) (10 mM) in dichloromethane–methanol (9:1) by a high-pressure mercury lamp (cutoff filter < 330 nm) under nitrogen resulted in an efficient and clean formation of methanol adduct **2a** (98%) with a quantum efficiency of 0.038 (Scheme 2).[5] Photoirradiation in the presence of other nucleophiles such as ethanol and *tert*-butylamine provided the corresponding trapping products **2b** (85%) and **2c** (52%), respectively. In order to determine the fate of the thioalkyl group in the photoreaction, **1b** was exposed to UV light under the same conditions to result in an efficient formation of *tert*-butyl mercaptan (30%) together with **2a** (34%). Formation of di-*tert*-butyl disulfide, resulting from coupling of the alkylthio radical, was never observed, implicating an ionic dissociation of the C–S bond in preference to cleavage of the C–O bond. The photolysis of **1a**,**b** occurred via the triplet manifold, as evidenced by quenching of product formation with piperylene (Stern–Volmer slope of 56.2 M^{-1}) and by sensitization experiments using acetophenone or benzophenone as a sensitizer. For example, irradiation of **1a** (1 mM) with

Scheme 1



Scheme 2



>366-nm light in the presence of acetophenone (260 mM) in acetonitrile–*tert*-butanol (9:1) gave **2d** efficiently, where acetophenone absorbs more than 99% of the incident light. Inertness of 1,8-naphthalimides **2a** and **3**, both possessing less electron-rich side chains, toward photoirradiation under the above conditions suggests an intramolecular electron transfer from the highly electron-donating monothioacetal group on the side chain to the triplet state of the 1,8-naphthalimide chromophore. The presence of the alkylthio group is essential for the efficient photogeneration of carbocation from **1**. In support of the electron-transfer mechanism, the quantum yield of the photoreaction of **1a** was enhanced approximately 2 times in the presence of 0.1 M $Mg(ClO_4)_2$.[3,4b,7] Of special interest is that a more efficient photogeneration of carbocations has been observed with **4** and **5** possessing longer side chains (n = 2, 3). Thus, photoreactions of **4** and **5** in dichloromethane–methanol (9:1) gave the corresponding acetals **6** (ϕ = 0.049) and **7** (ϕ = 0.041), respectively, through intramolecular electron transfer from the remote monothioacetal group to the triplet naphthalimide.

We have next examined the DNA-cleaving properties of **1a** under the influence of UV light. Incubation of supercoiled circular pBR322 (form I) DNA with drug **1a** under light illumination (366 nm) induced the transformation of form I DNA into nicked circular (form II) DNA at 10 μM drug concentration (lane 3),

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(2) (a) Saito, I.; Morii, T.; Sugiyama, H.; Matsuura, T.; Meares, C. F.; Hecht, C. M. *J. Am. Chem. Soc.* **1989**, *111*, 2307. (b) Saito, I.; Takayama, M.; Matsuura, T.; Matsugo, S.; Kawanishi, S. *J. Am. Chem. Soc.* **1990**, *112*, 883. (c) Matsugo, S.; Kawanishi, S.; Yamamoto, K.; Sugiyama, H.; Matsuura, T.; Saito, I. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1351. (d) Saito, I. *Pure Appl. Chem.* **1992**, *64*, 1305. (e) Sugiyama, H.; Tsutsumi, K.; Fujimoto, K.; Saito, I. *J. Am. Chem. Soc.* **1993**, *115*, 4443.

(3) For recent reviews on electron-transfer photochemistry, see: (a) Fox, M. A.; Chanon, M., Eds. *Photoinduced Electron Transfer*; Elsevier: Amsterdam, 1988. (b) Mattay, J. *Synthesis* **1989**, 233. (c) Kavarnos, G. J.; Turro, N. J. *Chem. Rev.* **1986**, *86*, 401.

(4) Photoinduced cleavage reactions initiated by electron transfer have been reported for acetals [(a) Mella, M.; Fasani, E.; Albini, A. *J. Org. Chem.* **1992**, *57*, 3051] and dithioacetals [(b) Epling, G. A.; Wang, Q. *Tetrahedron Lett.* **1992**, *33*, 5909].

(5) Quantum yield measurements were carried out at 0 °C in a merry-go-round apparatus by using phenylglyoxylic acid (ϕ = 0.70 at 334 nm)[6] as an actinometer.

(6) Kuhn, H. J.; Defoin, A. *EPA News Lett.* **1986**, *26*, 23.

(7) Mizuno, K.; Ichinose, N.; Otsuji, Y. *Chem. Lett.* **1985**, 455.

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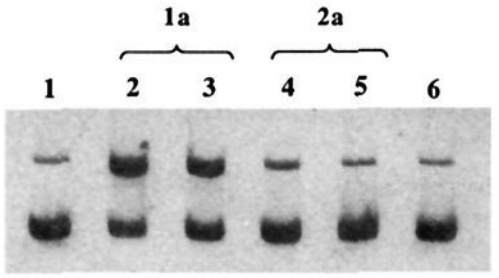


Figure 1. Light-induced cleavage of supercoiled circular pBR322 DNA (form I) into nicked circular DNA (form II) in the presence of **1a**. The reaction mixtures containing 50 μM DNA (form I) and varing concentrations of **1a** or **2a** in 10 mM sodium cacodylate buffer (pH 7.0) were irradiated at a distance of 10 cm from the transilluminator (366 nm) at 0 °C for 1.5 h. Lane 1, DNA control; lane 2, DNA + **1a** (100 μM); lane 3, DNA + **1a** (10 μM); lane 4, DNA + **2a** (100 μM); lane 5, DNA + **2a** (10 μM); lane 6, DNA + **1a** (100 μM) without light.

whereas irradiation without **1a** (lane 1) or incubation with **1a** without irradiation (lane 6) caused no DNA cleavage (Figure 1).[8]

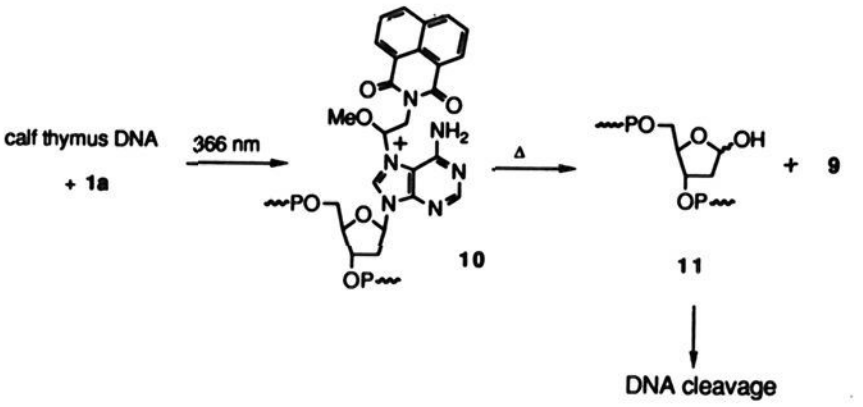
In order to study the DNA-alkylating properties of the photogenerated carbocation, a solution of **1a** (0.1 mM) and *N*-benzoyl-2′-deoxyadenosine (1 mM) in acetonitrile was irradiated under nitrogen, resulting in formation of N7-alkylated adenine adduct **8** (16%) together with a few unidentified products after heating of the photolysate at 90 °C for 10 min followed by column chromatographic separation. Deprotection of the benzoyl group (NH_3/methanol) provided adenine adduct **9**.[9] Encouraged by the isolation of stable adenine adduct **9**, we have examined the photoreaction of **1a** in the presence of calf thymus DNA. A solution of **1a** (1.0 mg, 3.3 mM) and sonicated calf thymus DNA (10 mg) in sodium cacodylate buffer at pH 7.0 (1.0 mL) was irradiated at 0 °C under nitrogen. The modified DNA was recovered by ethanol precipitation and heated at 90 °C for 20 min. HPLC analysis of the mixture revealed the presence of adenine adduct **9**, with retention time of 28.0 min,[10] among several other products, as evidenced by comigration with authentic **9** under different HPLC conditions and by identical UV spectral data obtained from diode array assay. These results imply that

(8) Photoinduced DNA cleavage by **2a** proceeded very sluggishly at much higher drug concentrations, but the cleavage was inhibited by addition of singlet oxygen quencher such as sodium azide, whereas the photocleavage by **1a** was not inhibited by addition of sodium azide.

(9) Mp 282–284 °C; UV (CH_3CN) λ_{max} 260 nm (log ϵ 3.77), 342 nm (3.68); ^{1}H NMR ($CDCl_3$) δ 2.69 (br s, 2H, $-NH_2$), 3.35 (s, 3H), 4.79–4.83 (m, 2H), 6.22 (dd, 1H, J = 5.8, 7.6 Hz), 7.72 (dd, 2H, J = 7.8, 8.0 Hz), 8.00 (s, 1H), 8.18 (s, 1H), 8.19 (dd, 2H, J = 8.4, 1.0 Hz), 8.52 (dd, 2H, J = 8.3, 1.0 Hz); MS *m/e* (relative intensity) 388 (M^+, 0.5), 254 (100); HRMS calcd for $C_{20}H_{16}N_6O_3$ 388.1227, found 388.1255.

(10) HPLC conditions: Cosmosil 5C_{18} AR ODS column (4.6 × 150 mm^2); 0.05 M ammonium formate containing 0–20% acetonitrile, linear gradient 1–20 min, and 20–100% acetonitrile, linear gradient 20–60 min; flow rate of 1.5 mL/min; 254-nm detection.

Scheme 3



the carbocation generated from drug **1a** is capable of undergoing DNA alkylation at adenine N7 as one of the DNA alkylation routes to produce alkylated DNA **10**, which upon heating at 90 °C releases adenine adduct **9** via hydrolytic cleavage of the N-glycoside bond, along with the formation of abasic site **11** (Scheme 3).[11] Detection of **9** as one of the DNA alkylation products in the photoreaction mixture of calf thymus DNA demonstrates the usefulness of this photogenerated carbocation as a DNA-alkylating agent at neutral pH.

In summary, we have demonstrated that (i) a carbocation is effectively generated from monothioacetals through photochemical intramolecular electron transfer and (ii) the monothioacetal group is an extremely good precursor for photochemical generation of carbocations under completely neutral conditions, which may find applications in many other photochemical processes requiring carbocation generation. Furthermore, phototriggered DNA alkylation has been first accomplished in the present work. Experiments designed to elucidate the details of the mechanisms, particularly direct detection of transient species, and further studies on DNA alkylation are underway in our laboratory.

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Supplementary Material Available: Experimental details, characterization data for new compounds, and HPLC profile of the photoreaction of **1a** with calf thymus DNA (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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