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Synthesis and properties of benzothioxanthene dicarboximide hydroperoxide: an efficient 'time-resolved' DNA photocleaver with long-wavelength

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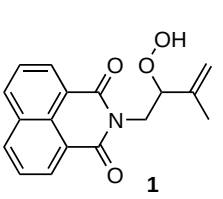
Abstract—A novel hydroperoxide of benzothioxanthene dicarboximide **B4** was synthesized through naphthyl radical-induced aromatic 1,5-hydrogen transfer in an unusual Pschorr cyclization and photooxygenation. It was evaluated as an excellent 'time-resolved' DNA photocleaver with long-wavelength absorption, which can photonick the duplex DNA at a micromolar concentration of 5 μM upon irradiation at 450 nm. © 2002 Published by Elsevier Science Ltd.

In recent years, the development of artificial photonucleases has received increasing interest due to their significant importance in molecular biology and human medicine.[1–4] Many kinds of DNA cleavage agents have been developed. Organic hydroperoxides that can efficiently generate hydroxyl radicals[5] by photoirradiation with long-wavelength light (>300 nm) without using metal ions is an extremely useful hydroxyl radical source, particularly for the design of DNA-cleaving molecules.[5] Long-wavelength UV-light (>350 nm) or more preferably visible light irradiation is an attractive cofactor, since it is safer and easy to manipulate. While up until now, most DNA photocleavers releasing oxygen-centered radicals showed photo-bioactivity around 350 nm,[6–9] few of them showed photocleavage under irradiation by visible light, and then only in the presence of metal cations.[10–12] In addition, those photocleavers with 'time-resolved' properties were very rare.[10–13] In fact, no organic hydroperoxide, with 'time-resolved' photocleaving properties or with photocleaving ability under irradiation with long-wavelength visible light, has been reported.

The hydroperoxides **1** and **2** were reported as novel and efficient intercalating DNA cleavers.[9,14] The high DNA cleavage efficiencies of these reagents might be attributed to the large conjugated planarity of these compounds. Encouraged by these results and special promotion action of sulfur to DNA intercalation or photocleavage,[9e,13c] benzothioxanthene hydroperoxide **B4** was synthesized, and its DNA photocleaving properties were evaluated.



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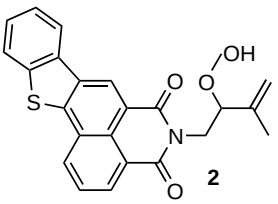

2

Compound **B4**, as an isomer of **2**, was obtained via three reaction steps: (1) 3-diazonium-4-phenylthionaphthalic anhydride **A** afforded isomers **A2** and **B2**[15,16] through an unusual Pschorr cyclization; (2) the imidation of **B2** to give **B3**; (3) the photooxygenation of **B3** to produce **B4**. The structures of all compounds were established via ^{1}H NMR, IR, EI MS and elemental analyses.[20] FT-IR spectra of hydroperoxide **B4** displayed the characteristic absorption peaks of the O–O band vibration around 800 cm^{-1}.

Usually, in Pschorr cyclization reactions a new bond is formed between two aromatic rings next to a heteroatom, at the diazonium substituted positions to give multinuclear aromatic hydrocarbons, especially for the derivatives of phenanthrene. Only one example of the

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formation of isomers in a Pschorr cyclization was known previously in benzophenone derivatives, where a phenyl radical-induced intramolecular aromatic 1,5-hydrogen transfer gave two five-membered ring isomers in a ratio of 55:45.[17] No other similar situations have been found, as aromatic compounds are usually poor hydrogen donors in radical transformations.[17] In our case, naphthyl radical-induced intramolecular aromatic hydrogen transfer in naphthalene derivatives (from **A1** to produce **B1**) gave the five-membered **A2** and the six-membered **B2** (Scheme 1). The isomeric ratio of **A2** and **B2** was at 86:14 (via ^{1}H NMR and HPLC) with **A** as starting material and 25:75 with **B** as starting material. The isomeric ratio of thio-compounds **A2** and **B2** was different from that of their oxo-counterpart (90:10 and 7:93, respectively) in the same reaction.[18] It suggests that the radical-induced intramolecular aromatic 1,5-hydrogen transfer in the thio-compounds is more efficient compared with their oxo-counterpart and that the presence of the sulfur atom promotes intramolecular migration of a hydrogen atom.

It can be seen in Table 1 that, because of the extended conjugated planarity, the UV–vis absorption and fluorescence maxima of **B4**, **B2** and **B3** are at longer wavelength compared with those of compound **2**. It can also be seen in Table 1 that the fluorescence quantum yield of compound **B4** is approximately three times higher than that corresponding to compound **2**, although they are isomers. In addition, **B4** was also very stable and did not give a decomposition product (with a hydroxyl group) by comparison with the photochemical behavior of **2** in chloroform solution under scattered daylight.[14] These differences imply possible differences in photo-biology.

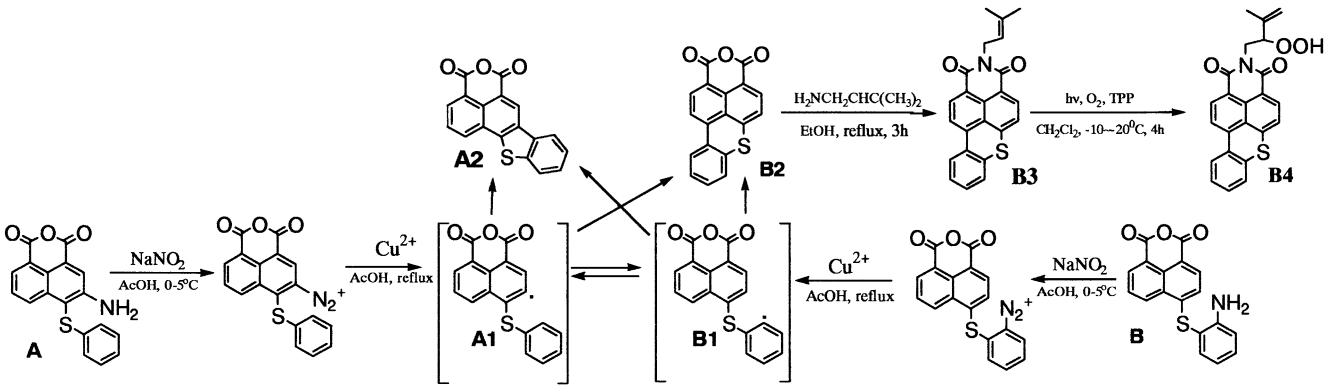
The DNA photocleavage of **B4** was evaluated using supercoiled circular pBR322 (form I) DNA (50 μM/base pair) under photoirradiation at a distance of 20 cm at 0°C and then analyzed on a 1% agarose gel. The efficiency was defined as the degree of relaxation of the supercoiled DNA, relaxed circular DNA (single-strand cleavage) as form II. When **B4** and DNA were mixed and photoirradiated with a transluminator (366 nm), no obvious cleavage was observed at as high as 50 μM concentration of **B4** (Fig. 1a), as the hydroxyl radicals cannot be efficiently generated due to the mismatch between the maximum absorption of **B4** and the photoirradiation wavelength. When the photoirradiation wavelength was changed to 450 nm through a light filter, **B4** showed effective photocleavage at a concentration of 5 μM (Fig. 1b).

The photocleavage activity of **B4** increased remarkably with prolonged photoirradiation time (Fig. 1c), which had not been seen in the case of **2** and the other organic hydroperoxides.[9] It was different from DNA photocleavers involving metal complexes and classical Fenton reactions, where radicals are produced in a rapid burst.[19] This continuous generation of reactive hydroxyl radicals provided a novel alternative to the well-known Fenton-based chemistry. Therefore, **B4** might be more attractive for 'time-resolved' DNA cleavage studies and for in vivo biomedical application involving 'photodynamic therapy'. In addition, the photocleavage ability of compound **B4** was increased with the pH values of buffers, it was sensitive to the outer environment and it gave good photocleaving results at pH 8.5. While compound **2** showed no difference in photonicking efficiency in the pH-dependent experiments.

The apparent association constant K_a (for the interaction between DNA and compound) of the hydroperoxides **B4** (4.26×10^3 M^{-1}) was smaller than that of **2** (3.82×10^5 M^{-1}), and this might also have a negative effect on the photocleavage of the former, but, it should be stressed that for efficient photonick DNA at μM level the photosensitizing wavelength of **B4** was much longer than that of **2** (at 366 nm, match with its absorption).

Table 1. UV–vis and fluorescence spectral data for benzo-(thio)xanthene dicarboximides

Compound	UV–vis $\lambda_{max/nm}$ (log ε)	FL $\lambda_{max/nm}$ (ϕ)	Stoke shift (nm)
2	379 (4.05)	457 (0.045)	78
B4	455 (4.24)	524 (0.12)	69
B2	456 (3.78)	521 (0.21)	65
B3	458 (4.40)	524 (0.11)	66



Scheme 1. The synthesis of **B4** and the Pschorr cyclization to give isomers **A2** and **B2**.

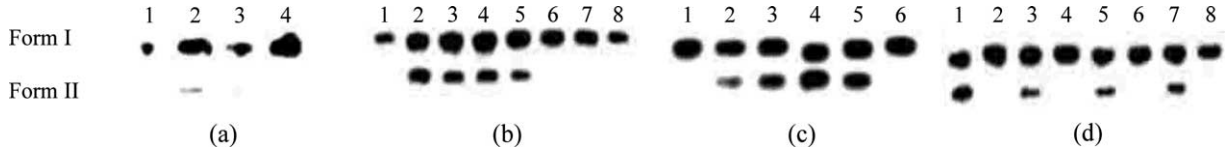


Figure 1. The photocleavage to DNA by B4. (a) **The photocleavage of DNA under irradiation with light at 366 nm.** Lane 1: DNA alone (no *hv*); Lane 2: DNA and **B4** at concentrations of 50 μM; Lane 3: DNA and **B4** at concentrations of 20 μM; Lane 4: DNA alone (*hv*, 75 min). (b) **Effect of concentrations on the photocleavage of DNA under the irradiation of light at 450 nm.** Lane 1: DNA alone (no *hv*); Lane 2–7: DNA and **B4** at concentrations of 50, 20, 10, 5, 1 and 0.5 μM, respectively; Lane 8: DNA alone (*hv*, 75 min). (c) **Effect of irradiation time on the photocleavage of DNA under the irradiation of light at 450 nm.** Lane 1: DNA alone (no *hv*); Lane 2: **B4** and DNA (*hv*, 30 min); Lane 3: **B4** and DNA (*hv*, 60 min); Lane 4: **B4** and DNA (*hv*, 75 min); Lane 5: **B4** and DNA (*hv*, 90 min); Lane 6: DNA alone (*hv*, 90 min). (d) **Effect of pH of the aqueous buffer on the photocleavage of DNA at 450 nm**. Lanes 1, 3, 5, 7: DNA and **B4** at 50 μM, buffer pH 8.5, 8.0, 7.5, 7.0, respectively; Lanes 2, 4, 6, 8: DNA alone, buffer pH 8.5, 8.0, 7.5, 7.0, respectively; *hv*, 75 min.

In conclusion, the above study on benzothioxanthene dicarboximide hydroperoxide **B4** probably provides the first example of an organic hydroperoxide, which has 'time-resolved' photocleavage properties on DNA and demonstrated photocleavage under irradiation with visible light (450 nm). And it also provided a novel example of naphthyl radical-induced intramolecular aromatic hydrogen transfer during Pschorr cyclization in the preparation of **B4**.

Acknowledgements

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20. Compound **A2**: yellow solid, mp 284–286°C, δ_H (DMSO-d_6, 300 MHz): δ 9.4 (s, 1H, 7-H), 8.75 (m, 2H, 3-H, 1-H), 8.59 (dd, $J_1=1.0$ Hz, $J_2=7.4$ Hz, 1H, 11-H), 8.28 (m, 1H, 2-H), 8.04 (dd, $J_1=8.2$ Hz, $J_2=7.4$ Hz, 1H, 8-H), 7.68 (m, 2H, 9-H, 10-H); EI MS (m/z, %): 306 ([M+2]$^+$, 11.4), 304 (M^+, 100). Compound **B2**: orange solid, mp >300°C, δ_H (DMSO-d_6, 300 MHz): δ 8.55 (m, 3H, 2-H, 6-H, 7-H), 8.36 (d, $J=8.0$ Hz, 1H, 2-H), 7.83 (d, $J=8.1$ Hz, 1H, 9-H), 7.56–7.70 (m, 3H, 10-H, 11-H, 12-H); EI MS (m/z, %): 306 ([M+2]$^+$, 8.7), 304 (M^+, 100). Compound **B3**: δ_H (500 MHz, $CDCl_3$): δ 1.74 (s, 3H, 3′-CH_3), 1.77 (s, 3H, 3′-CH_3), 4.73 (d, $J=7.5$Hz, 2H, –NCH_2–), 5.35 (t, $J_1=J_2=7.5$ Hz, 1H, 2′-H), 7.39 (m, 3H, 10-12H), 7.49 (d, $J=8.0$ Hz, 1H, 9-H), 8.20 (m, 2H, 1-H, 7-H), 8.42 (d,

$J=8.0$ Hz, 1H, 6-H), 8.62 (d, $J=8.0$ Hz, 1H, 2-H); EI MS (m/z, %): 373 ([M+2]$^+$, 5.1), 371 (M^+, 41.3), 302 ([M+H–CH_2CH=CMe_2]$^+$, 100); IR (KBr): 3080, 2960, 2930, 1690, 1645, 1585, 1380. Anal. calcd for $C_{23}H_{17}NO_2S$: C, 74.39; H, 4.58; N, 3.77. Found: C, 74.08; H, 4.89; N, 3.79%. Compound **B4**: δ_H (500 MHz, $CDCl_3$): 2.01 (s, 3H, 3′-CH_3), 4.70 (m, 3H, N–CH_2–CH–), 5.12 (d, $J=1.4$ Hz, 2H, 3′-CH_2=), 7.56 (m, 2H, 9-H, 10-H), 7.75 (m, 1H, 2-H), 7.92 (d, $J=7.6$ Hz, 1H, 8-H), 8.19 (d, $J=7.6$ Hz, 1H, 11-H), 8.32 (d, $J=8.0$ Hz, 1H, 1-H), 8.53 (d, $J=7.1$ Hz, 1H, 3-H), 9.04 (s, 1H, 7-H), 10.17 (br, 1H, –OOH); EI MS (m/z, %): 386 ([M–OH]$^+$, 5.7); IR (KBr): 3258, 1688, 1640, 1587, 1335, 906, 799, 780 cm^{-1}; FT-Raman: 3065, 1689, 1587, 1400, 1377, 799 cm^{-1}.