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p-Cyano Substituted Benzophenone as an Excellent Photophore for One-Electron Oxidation of DNA

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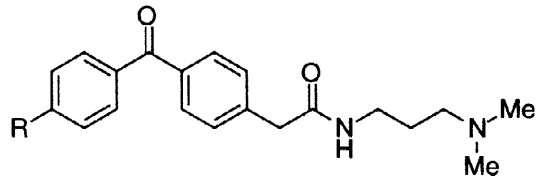
Abstract: Novel DNA-photocleaving molecule **1** consisting of a *p*-cyano substituted benzophenone photophore and an alkyl amino side chain was developed. DNA cleavage by **1** under photoirradiation conditions occurred selectively at 5' side G of 5'GG3' sequence after hot piperidine treatment. Photoirradiation of deoxyguanosine at 312 nm in the presence of **1** efficiently produced imidazolone as a predominant product.

There has been much current interest in the oxidative DNA damage occurred predominantly at 5' side guanine (G) of 5'GG3' doublet by one-electron transfer reactions.[1–4] Various types of photocleaving molecules including naphthalimide,[1] riboflavine,[2] anthraquinone derivatives,[3] and Rh(III)-intercalators[4] have been used for such photoinduced one-electron oxidations. In our studies to execute precise mapping of the "hot spots" for oxidative G damage by one-electron oxidation in long DNA and to investigate detail chemistry of such G oxidation, water-soluble and strongly electron-accepting photosensitizer that can bind to DNA duplex is highly desirable. We herein report that *p*-cyano substituted benzophenone **1** fulfills such requirement and efficiently cleaves DNA selectively at 5' side G of 5'GG3' doublet almost exclusively via one-electron oxidation.[5]

Synthesis of **1** was outlined in Scheme 1. Friedel-Crafts acylation of toluene with 4-bromobenzoyl chloride produced 4-bromobenzophenone **3**. Bromination of **3** at benzylic position produced a mixture of mono- and di-borominated products, which was subjected without separation to further reaction with sodium cyanide giving **5** in 53% yield after chromatographic purification. Acid hydrolysis of **5** produced carboxylic acid **6** in good yield. Substitution of bromide with cyanide was best accomplished by palladium catalyzed coupling reaction with potassium cyanide in the presence of 18-crown-6 to give **7**.[6] Condensation of activated ester **8** with *N,N*-dimethyl-1,3-propanediamine furnished the synthesis of **1**.[7] The corresponding benzophenone derivative **2** was also synthesized via a similar route.



1 : R = CN
2 : R = H

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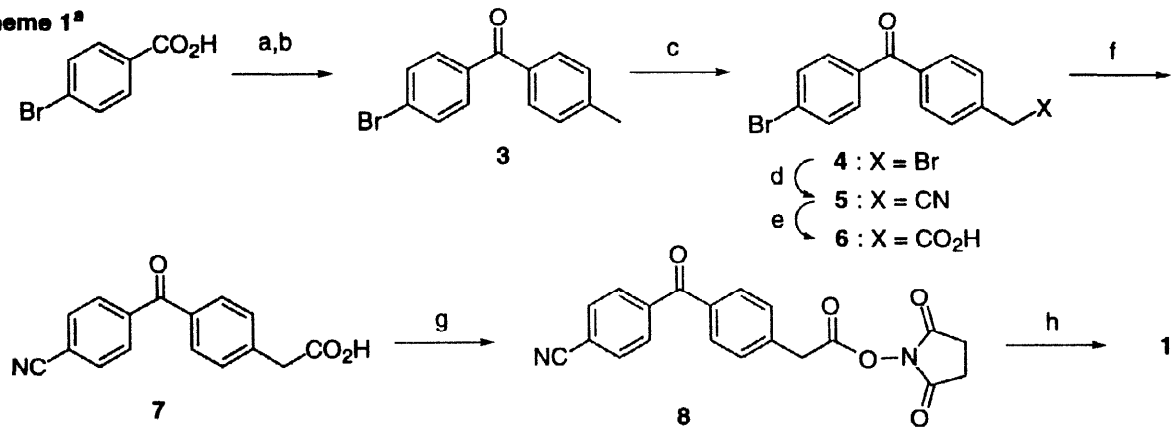
Scheme 1[a]



[a] *Reagents*: (a) $SOCl_2$, reflux; (b) toluene, $AlCl_3$, 92% (2 steps); (c) Br_2, 150 °C; (d) NaCN, 1,4-dioxane, H_2O, reflux, 53% (2 steps); (e) H_2SO_4, AcOH, H_2O, reflux, 95%; (f) KCN, 18-crown-6, $Pd(PPh_3)_4$, benzene, reflux, 79%; (g) DCC, *N*-hydroxysuccinimide, THF, 85%; (h) *N,N*-dimethyl-1,3-propanediamine, $NaHCO_3$, CH_3CN, H_2O, 78%.

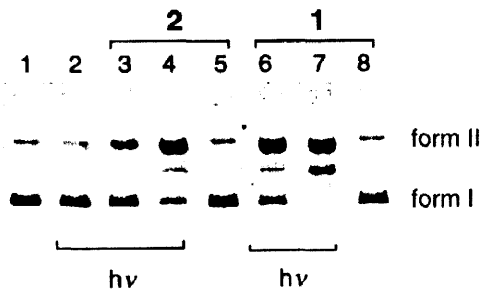


Figure 1. Cleavage of supercoiled DNA by benzophenone derivatives **1** and **2** under 312 nm irradiation. A buffered solution (Na cacodylate, 5 mM, pH 7.0) of supercoiled (form I) pBR322 DNA (40 μM) in the presence of drug was irradiated at 312 nm at 0 °C for 1 h (lanes 2, 3, 4, 6, and 7) or incubated in the dark at 0 °C for 1 h (lanes 1, 5, and 8). Formation of open circular (form II) DNA was analyzed by electrophoresis on 1% agarose gel containing ethidium bromide (0.6 μg/mL). lane 1, intact pBR322 DNA; lane 2, photoirradiated DNA without drug; lane 3, **2** (1 μM); lanes 4 and 5, **2** (10 μM); lane 6, **1** (1 μM); lanes 7 and 8, **1** (10 μM).

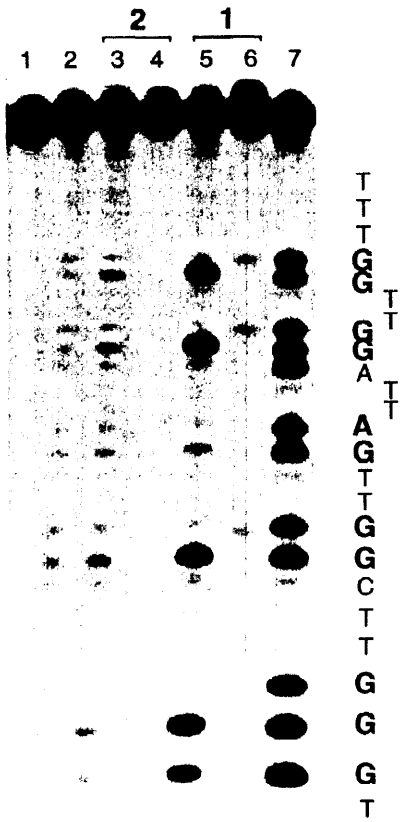
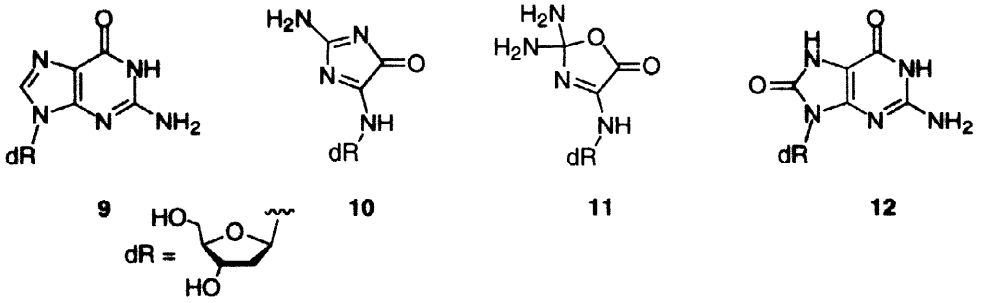


Figure 2. Base sequence selectivity for photoinduced cleavage of 41-mer ODN in the presence of **1** and **2**. ^{32}P-5′-end-labeled ODN was annealed with a complementary strand in a buffer (Na cacodylate, 10 mM, pH 7.0) and the solution of the duplex containing sonicated calf thymus DNA was irradiated at 312 nm for 10 min in the presence of **1** or **2** (each 25 μM) at 0 °C. Photoirradiated ODN recovered by ethanol precipitation was analyzed by electrophoresis on a sequencing gel containing 12% polyacrylamide and 7 M urea. ODNs for lanes 3 and 5 were heated in 10% piperidine at 90 °C for 20 min, and those for lanes 4 and 6 were heated without piperidine prior to gel electrophoresis. lane 1, intact ODN; lane 2, photoirradiated ODN without drug; lanes 3 and 4, **2** (25 μM); lanes 5 and 6, **1** (25 μM); lane 7, Maxam-Gilbert A+G reaction. Partial sequence of the ODN was shown in the right. G-containing sites were shown in bold face.



Photoinduced DNA cleavage by **1** and **2** was examined by relaxation assay of supercoiled plasmid DNA. As clear from Figure 1, cyano substituted benzophenone **1** cleaved DNA much more efficiently than unsubstituted derivative **2** under photoirradiation at 312 nm (cf. lane 3 *vs* lane 6, lane 4 *vs* lane 7). Base sequence selectivity for DNA cleavage by **1** and **2** was examined using 41-mer oligodeoxynucleotide (ODN) 5'-CGT ACT CTT GGG TTC GGT TGA TTA GGT TGG TTT CTT TCT AT-3' containing 5'GG3' doublet and 5'GGG3' triplet (Figure 2). Photoirradiation of ODN with **1** followed by hot piperidine treatment (lane 5) produced distinct cleavage bands which comigrated with Maxam-Gilbert G bands. The G cleavage occurred selectively at 5' side G of GG doublet with a very weak cleavage at GA sequence. In the case of GGG triplet, the cleavage occurred primarily at the middle G. Almost no cleavage without hot piperidine treatment (lane 6) indicated that photoirradiation of **1** with DNA never induced spontaneous strand cleavage but produced alkaline labile sites at 5' side G of GG. In marked contrast, only weak G bands even after hot piperidine treatment were detected for the photoreaction of **2** (lane 3). Under prolonged photoirradiation, DNA cleavage occurred at all nucleotides (data not shown), suggesting that some other reactions besides single electron transfer would be involved in the DNA cleavage induced by unsubstituted benzophenone **2**. These results clearly showed a prominent advantage of cyano substituted benzophenone derivative as a GG selective photocleaver over unsubstituted benzophenone derivatives in one-electron oxidation of DNA.

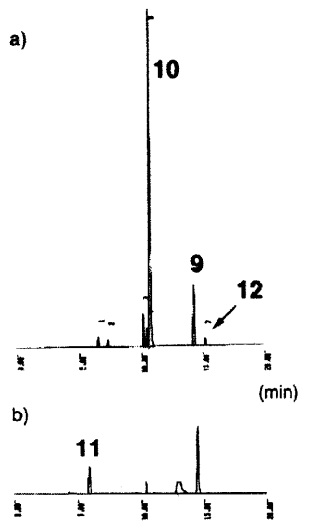


Figure 3. HPLC profiles of (a) the crude reaction mixture produced by photoirradiation of **9** (2 mM) in the presence of **1** (2 mM) in sodium cacodylate (10 mM, pH 7.0) at 312 nm for 2 h, and (b) the mixture further incubated at 37 °C for 24 h. HPLC analysis was carried out on a CHEMCOBOND 5-ODS-H column (10 X 150 mm) eluted with water containing 0–20% acetonitrile linear gradient over 20 min at a flow rate of 2.0 mL/min, detected at 254 nm.

It has been reported that benzophenone-photosenstitized oxidation of deoxyguanosine **9** resulted in a formation of imidazolone **10**, which spontaneously degraded upon incubation at 37 °C to oxazolone **11**.[8] The formation of **10** was proposed to proceed via one-electron transfer from **9** to triplet excited benzophenone. Photoirradiation of **9** in the presence of **1** in sodium cacodylate buffer at 312 nm actually produced **10** as a major product which comigrated with authentic **10** on HPLC (Figure 3a). 8-OxodG (**12**) was produced only in a small amount under the conditions. Upon incubation of the photolysate at 37 °C for 24 h, a new peak

corresponding to **11** appeared with concomitant disappearance of **10** (Figure 3b).[9] Selective cleavage at 5' side G of GG doublet and a predominant formation of imidazolone **10** strongly support that photoinduced DNA cleavage by cyano substituted benzophenone **1** proceeded almost exclusively via single electron transfer process. Other photochemical process does not seem to occur with **1** unlike the case of unsubstituted benzophenone.[8]

In conclusion, the present studies demonstrated that cyano substituted benzophenones like **1** possess a remarkably superior photochemical property as a DNA-photocleaver as compared with unsubstituted benzopheneones and can serve as an excellent photophore for one-electron oxidation of DNA. Furthermore, activated ester **8** is a very useful reagent for introducing cyano substituted benzophenone photophore into other DNA binding biomolecules. Study along this line is currently underway.

References and Notes

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(7) **1**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.58 (quintet, 2H, J = 6.0 Hz), 2.04 (s, 6H), 2.31 (t, 2H, J = 6.0 Hz), 3.32 (brq, 2H, J = 5.6 Hz), 3.59 (s, 2H), 7.41 (d, 2H, J = 8.0), 7.72–7.87 (6H); MS m/z (%) 349 (M^+) (94), 305 (18), 246 (6), 221 (48), 192 (64), 190 (28), 130 (47), 129 (49), 102 (42), 90 (42), 72 (35), 58 (100); IR ($CHCl_3$) 3310, 3015, 2234, 1661, 1607, 1521, 1276; HRMS calcd for $C_{16}H_{11}O_3N$ (M^+) 349.1789, found 349.1812.

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(9) Detailed chemistry of photochemical one-electron oxidation of GG-containing ODN will be published [Sugiyama, H.; Kino, K.; Saito, I. manuscript in preparation].