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Photogeneration of Highly Electrophilic Benzoylketene from Dibenzoyldiazomethane in Aqueous Solvents: Reaction with Amino Acids and DNA Cleavage

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Abstract: Photoirradiation of dibenzoyldiazomethane in the presence of amino acid derivatives in aqueous solutions efficiently produced the addition products through a reaction with photogenerated benzoylketene. Efficient DNA cleavage was observed with a dibenzoyldiazomethane derivative, possessing a cationic side chain under photoirradiation conditions.

Methods generating highly electrophilic species in aqueous solutions under physiological conditions have drawn considerable attention due to their inherent possibility of modifying biomolecules such as polypeptides and polynucleotides. Photo-triggered generation of such super electrophiles under mild conditions at the target site in a biopolymer is particularly attractive.¹ As such a photoinducible electrophile we examined the photochemistry of α -diazocarbonyl compounds.² Herein we disclose that highly electrophilic benzoylketene 2 generated by photoinduced Wolff rearrangement (WR)³ of dibenzoyldiazomethane 1 undergoes efficient acylation of amino acid derivatives in aqueous solvents. Efficient photoinduced DNA cleavage was also accomplished by dibenzoyldiazomethane derivative **3** possessing a cationic side chain.



Since the most abundant nucleophile in aqueous solutions is water itself, electrophiles if generated in an aqueous system would immediately react with water. Thus, when 1 (10 mM) was irradiated with transilluminator (366 nm) in 50% aqueous acetonitrile for 8 h, benzylphenylketone 5 was obtained in 71% yield as a single product with a quantum efficiency of 0.037 (Scheme 1). The formation of 5 indicates the water addition to the highly electrophilic ketene 2 produced from 1 by WR, followed by decarboxylation of the resulting β -keto acid 4.⁴ The trapping of 2 with amino compounds, especially amino acid derivatives, also proceeded quite effectively in competition with water addition. Thus, photoirradiation of 1 in the presence of the glycin ethyl ester (Gly-OEt, 2 eq) produced amide 6⁵ in 77 % yield with the formation of 5



(12%) and dibenzoylmethane 9 (9%) produced via dibenzoyl carbene 8.6.7 Likewise, photoirradiation of 1 with N^{α} -Boc-L-Lys-OMe (2 eq) in 50% aqueous acetonitrile gave amide 7 in 55% yield, implying that efficient trap of 2 in aqueous solvents with amino acid derivatives is a general phenomena.⁸

The trapping reactions with other nucleophiles showed that primary, secondary, and even tertiary amines can react with 2 in competition with water addition. In the presence of *t*-butylamine (2 eq) amide 10 was obtained in 33% yield, with the yield of 10 being improved to 50% using excess amine. Diethylamine (2 eq) reacted with 2 producing diethyl amide 11 in 25% yield, which was also the product from the reaction with Et₃N (10 eq, 20%).



The decrease of the reaction efficiency with increasing amine basisity was rationalized by the fact that 1 was not stable in basic aqueous solvents giving rise to benzoic acid and α -diazoacetophenone. The half life of 1 in the presence of *t*-butylamine (10 eq) in 50% aqueous acetonitrile is about 40 min. Although photoirradiation of 1 in the presence of methanol, ethanol, and *t*-butanol in acetonitrile produced 12 (R = Me, 25.5 h, 37%; R = Et, 25.5 h, 43%; R = ^tBu, 17 h, 90%) together with a small amount of insertion product 13, such trapping reactions with alcohols were completely suppressed in 50% aqueous acetonitrile.



Scheme 2

We next examined the reaction of dibenzoyldiazomethanes with DNA under the influence of light illumination. Supercoiled circular pBR322 DNA (form I) (40 µM) in Na cacodylate buffer (5 mM, pH 7.0) was mixed with 1 (added as an acetonitrile solution, final concentration of acetonitrile was 10% (v/v)) and the solution was irradiated with transilluminator (366 nm) for 2 h at 0 °C. The DNA was analyzed by gel electrophoresis in 1% agarose containing ethidium bromide in TBE buffer (90 mM Tris-HCl, 2 mM EDTA, pH 8.0) (Fig. 1). Formation of nicked circular (form II) DNA was observed on irradiation at concentration of 100 µM of 1 (lane 3), while incubation with 1 at 37 °C for 2 h in the dark did not cause strand cleavage significantly (lane 7). Since the DNA-cleaving activity of 1 is not so high due to the lack of DNA binder, dibenzoyldiazomethane derivative 39 possessing a cationic side chain was synthesized in order to improve the solubility in water and to enhance the binding to DNA (Scheme 2).¹⁰ As expected, 3 showed much stronger DNA-cleaving activity (lanes 5 and 6) than 1 (cf. with lanes 3 and 4). At concentration of $100 \,\mu M$ of 3 (lane 5) the disappearance of form I DNA and a faint formation of linear (form III) DNA were observed. Preliminary examinations of DNA cleavage by 3 using 5'-32P-endlabeld DNA indicated Gselective cleavage on treatment with piperidine. Thus, the DNA-cleaving species generated from 3 under the photoirradiation conditions would be benzoylketene, although hydrogen abstraction by dibenzoylcarbene is conceivable.^{11,12} Detailed study on the reaction of 3 with DNA under photoirradiation conditions is now under investigation in these laboratories.



Figure 1. The relaxation of supercoiled (form I) pBR322 DNA (40 μ M) in Na cacodylate buffer (5 mM, pH 7.0) treated with 1 and 3 was analyzed by electrophoresis with agarose gel containing ethidium bromide (1.7 μ g/mL). lane 1; DNA alone, lane 2; DNA with photoirradiation, lane 3; DNA+1 (100 μ M), lane 4; DNA+1 (50 μ M), lane 5; DNA+3 (100 μ M), lane 6; DNA+3 (50 μ M), lane 7; DNA+1 (100 μ M) dark, lane 8; DNA+3 (100 μ M) dark. Photoirradiation at 366 nm at 0 °C for 2 h for lanes 2 - 6. Incubation in the dark for 2 h at 37 °C for lanes 7 and 8.

In conclusion, photoirradiation of 1 can produce highly reactive electrophile 2 in aqueous solutions under physiological conditions, which is efficiently trapped with amino acid derivatives in competition with water addition. The efficient DNA cleavage was observed with 3 possessing a cationic side chain.

References and Notes

- (a) Henriksen, U.; Larsen, C.; Karup, G.; Jeppesen, C.; Nielsen, P. E.; Buchardt, O. Photochem. Photobiol. 1991, 53, 299-305. (b) Saito, I.; Takayama, M.; Sakurai, T. J. Am. Chem. Soc. 1994, 116, 2653-2654, and references cited therein.
- (2) For our previous studies using α-diazocarbonyl compounds in aqueous solutions, see: Nakatani, K.; Isoe, S.; Maekawa, S.; Saito, I. Tetrahedron Lett. 1994, 35, 605-608.
- (3) For reviews on WR, see: (a) Meier, H.; Zeller, K.-P. Angew. Chem. Int. Ed. Engl. 1975, 14, 32–43. (b) Gill, G. R. In Comprehensive Organic Synthesis, Trost, B. M.; Fleming, I. Eds.; Pergamon Press, 1991; Vol. 3, pp 887–912.
- (4) The half-life of 4 was reported to be 14 h at 59 °C (Meier, H.; Wengenroth, H.; Lauer, W.; Krause, V. *Tetrahedron Lett.* 1989, 30, 5253-5256). Therefore, decarboxylation of 4 to 5 should involve a photochemical (Norrish type II) process.
- (5) Satisfactory spectral data were obtained for all new compounds.
- (6) In all cases using 2 eq of amino compounds the photoirradiation of 1 was conducted for $8 \sim 10$ hr.
- (7) (a) Baumann, N. Helv. Chim. Acta 1972, 55, 2716-2723. (b) Tomioka, H.; Hayashi, N.; Asano, T.; Izawa, Y. Bull. Chem. Soc. Jpn. 1983, 56, 758-761.
- (8) Acetylation of amino group in aqueous solutions was well studied. For example, see: Kikugawa, Y.; Mitsui, K.; Sakamoto, T.; Kawase, M.; Tamiya, H. Tetrahedron Lett. 1990, 31, 243-246.
- (9) Mp 78.0-79.5 °C; ¹H NMR (CD₃OD): δ 3.16 (t, 2H, *J* = 6.0 Hz), 3.65 (t, 2H, *J* = 6.0 Hz), 7.38 (m, 2H), 7.50 (m, 1H), 7.65 (m, 2H), 7.70 (m, 2H), 7.84 (m, 2H); FABMS: 338 (M+H-CF₃CO₂-). Anal. Calcd. for C₂₀H₁₇F₃N₄O₅·3/4H₂O: C, 51.78; H, 4.02; N, 12.08%, Found C, 51.80; H, 3.65; N, 11.72%.
- (10) While the α -diazoketone group is acid sensitive, it is noteworthy that the dibenzoyldiazomethane functionality can survive under the Boc deprotection conditions (e.g., $16 \rightarrow 3$, TFA, CH₂Cl₂, rt, quantitative).
- (11) DNA cleavage by singlet oxygen and/or hydroxy radical is ruled out. Photoirradiation of 3 in the presence of NaN₃ or manitol did not effect the DNA-cleaving activity of 3.
- (12) DNA cleavage by photogenerated carbene has been reported. Nielsen, P.E.; Jeppesen, C.; Egholm, M.; Buchardt, O. Nucleic Acids Res. 1988, 16, 3877-3888.

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