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Photoinduced DNA Cleavage by Designed Molecules with Conjugated Ene-Yne-Ketene Functionalities

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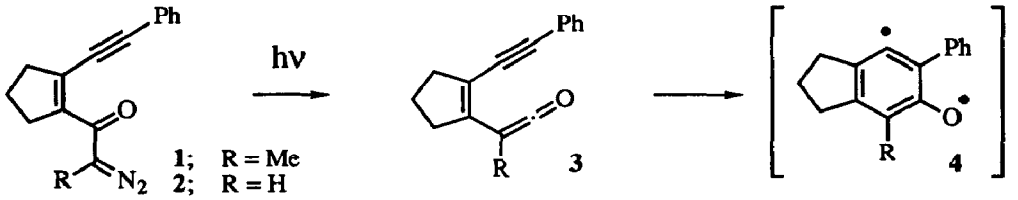
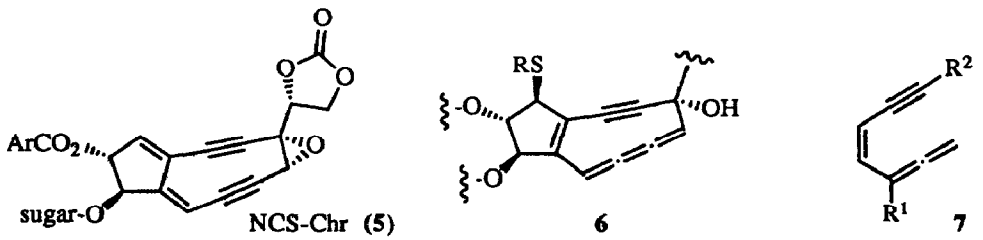
Abstract: *Upon photoirradiation, diazoketone 1 was found to induce single strand cleavage of plasmid pBR322 DNA at concentration of 100 μM. Conjugate ene-yne moiety was essential for DNA-cleaving activities.*

Recent understanding of the activation mechanism of ene-diyne antibiotics such as neocarzinostatin (NCS), calicheamicin-esperamicin and dynemicin has stimulated the design of novel artificial DNA-cleaving molecules.[2] We are particularly interested in the DNA cleavage reaction induced by photo-triggered activation of physiologically stable molecules.[3] Such molecules would have great potentials in tumor photodynamic therapy. We now disclose herein a novel type of photoinduced DNA cleavage by diazoketones (**1** and **2**) which are designed as a model of neocarzinostatin chromophore (NCS-Chr) (**5**).



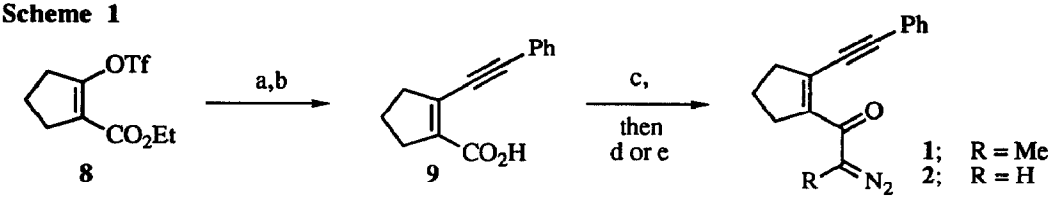


It has been postulated that labile NCS chromophore **5**, being responsible for antitumor activity of NCS, generates σ-sp^2-diradical by nucleophilic attack of thiol *via* ene-yne-cumulene **6**.[4] It was also demonstrated that ene-yne-allene systems, *e.g.*, **7**, cyclize to σ-sp^2-diradical, which eventually causes DNA strand cleavage.[5] We, thus, anticipated that σ-sp^2-diradical **4** generated by a spontaneous cyclization of ene-

yne-ketene 3[6] would cleave DNA effectively. Accordingly, we have designed diazoketones **1** and **2** in order to generate ene-yne-ketene functionalities *in situ* by photoinduced Wolff rearrangement.

Synthesis of **1** and **2** was accomplished in a straightforward manner as shown in Scheme 1. Palladium catalyzed coupling of enol triflate **8** with phenylacetylene afforded ene-yne ester which was hydrolyzed to the corresponding acid **9**. Acid chloride formation from **9** followed by treatment with diazoethane and diazomethane yielded **1**[7] and **2**[8] in moderate yields, respectively.

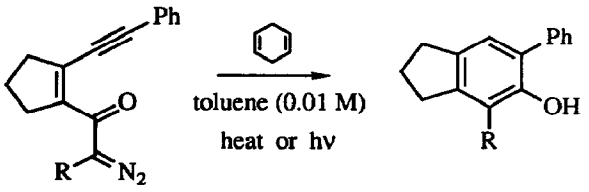
Scheme 1



(a) PhC≡CH, $PdCl_2(PPh_3)_2$, CuI, 2,6-lutidine, DMF, 88%; (b) NaOH, MeOH-H_2O, 83%; (c) $(COCl)_2$, PhH; (d) CH_3CHN_2, ether, 64% for **1** (2 steps); (e) CH_2N_2, ether, 50% for **2** (2 steps)

The results of their thermal and photochemical reactions in toluene in the presence of 1,4-cyclohexadiene as hydrogen donor were summarized in Scheme 2. It was shown that **1** bearing methyl group at α position to the diazo group was more reactive than **2** to produce radical cyclyzation product **10**[9] in a good yield by heating or as an only isolable product by photoirradiation. Generation of radical (*e.g.*, **4**, R=Me) as a transient species would also be supported by the fact that **10** was obtained only in a trace amount together with a considerable amount of complex polymeric products when the reaction was conducted without hydrogen donor. These observations were in good agreement with those reported in the related diazoketone systems.[10] The UV spectra of **1** and **2** were considerably different in each other as found λ_{max} 295 nm (ε = 12,800) for **1** and 313 nm (ε = 18,700) for **2**. This difference may be explained in terms of conformational difference as supported by calculations (PM3 in SPARTAN ver.2), showing that in the lowest energy conformation of **1** two planes of cyclopentene ring and carbonyl group were almost orthogonal (dihedral angle of ∠C5-C1-C1'-C2' is 93.8°) (Figure 1), while this was not the case for diazoketone **2**.[11]

Scheme 2

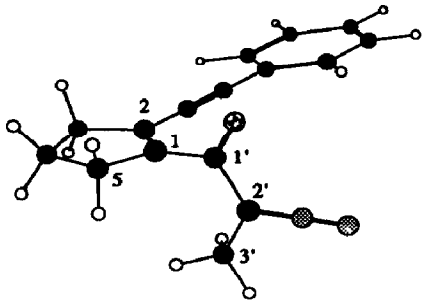


1 → **10** (R = Me)
140 °C,[a] 30 min, 51%[c]
hν,[b] 0 °C, 30 min, 36%[c]

2 → **11** (R = H)
140 °C,[a] very slow
170 °C,[a] 30 min, 38%[c]
hν,[b] 0 °C, 30 min, complex mixture

[a] in a seald tube, [b] high pressure Hg lamp through Pyrex filter, [c] isolated yields

We have examined the DNA-cleaving activity of **1** and **2** under photoirradiation conditions. Thus, supercoiled pBR322 plasmid DNA was irradiated in the presence of **1** or **2** with 366 nm light at 0 °C. DNA cleavage was analyzed by agarose gel electrophoresis (Figures 2 and 3). Upon irradiation, quite effective single strand cleavage was observed in the presence of **1** at concentration of 100 μM (lanes 6 - 8), while without irradiation **1** was quite ineffective toward DNA cleavage even at concentration of 1 mM (lane 2).



∠C5-C1-C1'-C2' = 93.8 °

Figure 1. Calculated conformation of **1**

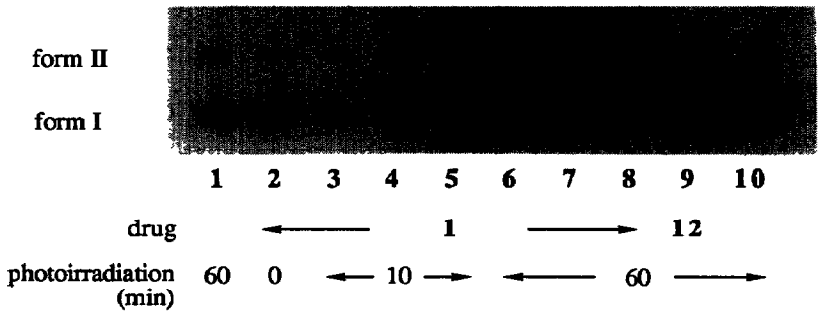


Figure 2. pBR322 DNA (40 μM) was irradiated at 366 nm at 0 °C (pH 7.0, Na cacodylate) in the presence of drugs (added as CH_3CN solution, maximum concentration of CH_3CN in a final solution was 10%) and analyzed by agarose gel electrophoresis (ethidium bromide staining). lanes 1, 10; DNA alone, lane 2; **1** (1 mM) without photoirradiation, lanes 3, 6; **1** (10 μM), lanes 4, 7; **1** (100 μM), lanes 5, 8; **1** (1 mM), lane 9; **12** (1 mM), Photoirradiation for 10 min (lanes 3 - 5) or for 60 min (lane 1 and lanes 6 - 10).

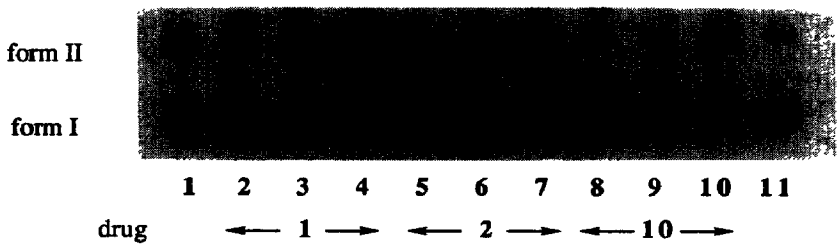
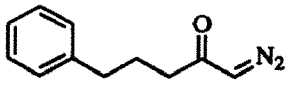


Figure 3. pBR322 DNA (40 μM) was irradiated at 366 nm at 0 °C for 60 min (pH 7.0, Na cacodylate) in the presence of drugs and analyzed by agarose gel electrophoresis. lanes 1, 11; DNA alone, lane 2; **1** (10 μM), lane 3; **1** (100 μM), lane 4; **1** (1 mM) lane 5; **2** (10 μM), lane 6; **2** (100 μM), lane 7; **2** (1 mM), lane 8; **10** (10 μM), lane 9; **10** (100 μM), lane 10; **10** (1 mM).

Of particular interest is that the ene-yne functionalities of **1** were indispensable for the photoinduced DNA cleavage by observing that simple diazoketone **12** was totally ineffective even at concentration of 1 mM under the same photoirradiation conditions (lane 9). On the other hand, DNA-cleaving activity of **2** was substantially weaker than that of **1** (lanes 2 - 4 for **1** *vs.* lanes 5 - 7 for **2** in Figure 3). Furthermore, phenol **10** did not cause DNA strand scission at comparable concentrations (lanes 8 - 10).



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It was apparent from these data that ene-yne-ketene functionalities produced in the photoirradiation of **1** play an essential role in the DNA cleavage. Since the difference in photochemical reactivity between **1** and **2** in toluene well reflects the DNA-cleaving properties and DNA was neither treated with base nor heating after photoirradiation, the observed DNA cleavage is due to the hydrogen abstraction from DNA sugar backbone by σ-sp^2-diradical **4** spontaneously formed from ene-yne-ketene. In fact, small but significant amount of **10** was actually detected by HPLC after photoirradiation of **1** in the presence of pBR322 DNA.[12]

References and Notes

(1) Present Address; Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606-01, Japan

(2) For recent reviews, see a) Nicolaou, K.C.; Dai, W.-M. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1387; b) Nicolaou, K.C.; Smith, A.L. *Acc. Chem. Res.* **1992**, *25*, 497. c) For a recent NCS-Chr model, see Tokuda, M.; Fujiwara, K.; Gomibuchi, T.; Hirama, M.; Uesugi, M.; Sugiura, Y. *Tetrahedron Lett.* **1993**, *34*, 669 and references therein.

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(6) For a related system, see a) Xia, H.; Moore, H.W. *J. Org. Chem.* **1992**, *57*, 3765. b) Foland, L.D.; Karlsson, J.; Perri, S. T.; Schwabe, R.; Xu, S.L.; Patil, S.; Moore, H.W. *J. Am. Chem. Soc.* **1989**, *111*, 975.

(7) **1**; yellow oil, ^{1}H-NMR (C_6D_6) δ 1.50 (m, 2H), 1.73 (s, 3H), 2.44 (m, 2H), 2.75 (m, 2H), 6.98-7.04 (3H), 7.44-7.48 (2H); IR (neat); 2956, 2929, 2849, 2080, 1597, 1375 cm^{-1}; MS (m/e) (%); 222 [(M-N_2)$^+$] (89), 195 (100), 165 (33), 152 (18), 139 (11), 105 (9); HRMS calcd. for $C_{16}H_{14}O$ (M-N_2)$^+$; 222.1045, found; 222.1058.

(8) **2**; yellow oil, ^{1}H-NMR (C_6D_6) δ 1.50 (m, 2H), 2.54 (m, 2H), 2.80 (m, 2H), 6.20 (br, 1H), 7.07-7.08 (3H), 7.43-7.47 (2H); IR (neat); 3120, 2956, 2846, 2105, 1606, 1485, 1381 cm^{-1}; MS (m/e) (%); 236 (M$^+$) (1), 208 [(M-N_2)$^+$] (82), 180 (100), 165 (52), 152 (63), 139 (22), 78 (26); HRMS calcd. for $C_{15}H_{12}O$ (M-N_2)$^+$; 208.0888, found; 208.0904.

(9) **10**; colorless oil, ^{1}H-NMR ($CDCl_3$) δ 2.09 (quint, 2H, J=7.3 Hz), 2.21 (s, 3H), 2.88 (q, 4H, J=7.3 Hz), 5.11 (s, 1H), 6.93 (s, 1H), 7.38 (m, 1H), 7.41-7.50 (4H); IR ($CHCl_3$); 3556, 2947, 2842, 1600, 1220 cm^{-1}; MS (m/e) (%); 224 (M$^+$) (100), 209 [(M-Me)$^+$] (35), 165 (14), 147 (17); HRMS calcd. for $C_{16}H_{16}O$ (M$^+$); 224.1202, found; 224.1198.

(10) For a related diazoketone system, see Padwa, A.; Austin, D.J.; Chiacchio, U.; Kassir, J.M.; Rescifina, A.; Xu, S.L. *Tetrahedron Lett.* **1991**, *32*, 5923.

(11) NMR experiments showing no NOE effect between C-3' methyl and C-5 methylene group of **1** would also support that in solution **1** exists in a conformation as predicted by calculations.

(12) While both **1** and **10** were ineffective against human oral epidermoid carcinoma KB and human lung carcinoma A-549 when subjected to *in vitro* cytotoxic assay, **1** would be a potentially useful compound for tumor photodynamic therapy.

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