α-Diazo Ketones as Photochemical DNA Cleavers: A Mimic for the Radical Generating System of Neocarzinostatin Chromophore

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Abstract: The α-diazo ketones **1**, **10**, and **11** were designed as mimics for the radical-generating system of neocarzinostatin chromophore (**7**). These α-diazo ketones are able to generate diradicals under thermal or photoirradiation conditions in toluene via the cyclization of the ene−yne−ketene intermediates. *Ab initio* MO calculations revealed that the efficiency of the radical generation is highly dependent on the conformation of the α-diazo ketones which is controlled by the substituent on the carbon directly attached to the diazo group. Two α-diazo ketones **10** and **11** having a DNA binding group considerably improved the DNA-cleaving activity compared to that for **1** under photoirradiation at 366 nm. The absence of an appreciable amount of cyclized indanol **23** in the photoirradiated solution of **10** in the presence of pBR322 DNA strongly suggests that the diradical is not responsible for the observed DNA cleavage. Likewise, photoirradiation of **1** in a 50% aqueous acetonitrile solution did not produce indanol **5** but gave a novel furan derivative **33**. The increased yield of **33** under aerobic conditions suggested that the mechanism producing **33** involves the trapping of photogenerated α-keto carbene **29** with molecular oxygen. These experiments together with theoretical calculations indicated that α-keto carbenes generated by photoirradiation of α-diazo ketones may be the principal DNA-cleaving species.

Recent investigations on naturally occurring enediyne antitumor antibiotics[1] such as calicheamicin,[2] esperamicin,[3] dynamicin,[4] and neocarzinostatin chromophore (**7**)[5,6] have stimulated the research for the design of artificial systems that can effectively generate σ-sp^2 diradicals under mild physiological conditions.[7] Such radical species have been shown to be responsible for the DNA cleavage as well as for the biological activities observed for these natural antitumor antibiotics.[1−4,6a−c] Radical-generating systems for artificial DNA-cleaving molecules so far reported rely on either a spontaneous cyclization[8] or a nucleophilic,[9] photochemical,[10] or pH-dependent[11] triggered reaction. We have been particularly interested in the generation of DNA-cleaving species by phototriggered activation of a physiologically stable chromophore and designed σ-diazo ketones containing an ene−yne structure such as **1** as a radical-generating system.[12−14] Upon photoirradiation, these α-diazo ketones are expected to undergo Wolff rearrangement[15] (WR) to produce ene−yne−ketene intermediate **3** which may mimic the ene−yne−cumulene **8**, a key intermediate in the generation of σ-sp^2 diradical **9** from neocarzinostatin chromophore (**7**) via Myers cyclization (eq 2).[6] The ene−yne−ketene system such as **3** has already been suggested to undergo spontaneous cyclization to produce diradical **4** in organic solvents.[16−18]

In a preliminary communication,[12] we reported that both thermal and photochemical reactions **1** and **2** in the presence of 1,4-cyclohexadiene as a hydrogen donor in toluene produced

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(1) For reviews of the enediyne antibiotics, see: (a) Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387−1416. (b) Nicolaou, K. C.; Smith, A. L.; Yue, E. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5881−5888.

(2) (a) Lee, M. D.; Dunne, T. S.; Siegel, M. M.; Chang, C. C.; Morton, G. O.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3464−3466. (b) Lee, M. D.; Dunne, T. S.; Chang, C. C.; Ellestad, G. A.; Siegel, M. M.; Morton, G. O.; McGahren, W. J.; Borders, D. B. *J. Am. Chem. Soc.* **1987**, *109*, 3466−3468.

(3) (a) Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3461−3462. (b) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462−3464.

(4) (a) Konishi, M.; Ohkuma, H.; Matsumoto, K.; Tsuno, T.; Kamei, H.; Miyaki, T.; Ohki, T.; Kawaguchi, H.; VanDuyne, G. D.; Clardy, J. *J. Antibiot.* **1989**, *42*, 1449−1452. (b) Sugiura, Y.; Shiraki, T.; Masataka, K.; Oki, T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3831−3835.

(5) (a) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Ohtake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331−334. (b) Edo, K.; Akiyama, Y.; Saito, K.; Mizugaki, M.; Koide, Y.; Ishida, N. *J. Antibiot.* **1986**, *39*, 1615−1619. (c) Myers, A. G.; Proteau, P. J.; Handel, T. M. *J. Am. Chem. Soc.* **1988**, *110*, 7212−7214.

(6) For the cyclization of neocarzinostatin chromophore, see: (a) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493−4496. (b) Myers, A. G.; Proteau, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 1146−1147. (c) Myers, A. G.; Cohen, S. B.; Kwon, B.-M. *J. Am. Chem. Soc.* **1994**, *116*, 1670−1682. For an alternative cyclization mode, see also: (d) Sugiyama, H.; Yamashita, K.; Fujiwara, T.; Saito, I. *Tetrahedron* **1994**, *50*, 1311−1325 and references cited therein.

(7) Enediyne: (a) Nicolaou, K. C.; Zuccarello, G.; Ogawa, Y.; Schweiger, E. J.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4866−4868. (b) Nicolaou, K. C.; Smith, A. L. *Acc. Chem. Res.* **1992**, *25*, 497−503. (c) Magnus, P.; Carter, P.; Elliot, J.; Lewis, R.; Harling, J.; Pittema, T.; Bauta, E.; Fortt, S. *J. Am. Chem. Soc.* **1992**, *114*, 2554−2559. Eneynecumullene: (d) Hirama, M.; Fujiwara, K.; Shigematsu, K.; Fukazawa, Y. *J. Am. Chem. Soc.* **1989**, *111*, 4120−4122. (e) Myers, A. G.; Finney, N. S.; *J. Am. Chem. Soc.* **1992**, *114*, 10986−10987. Eneyneallene: (f) Nagata, R.; Yamanaka, H.; Okazaki, E.; Saito, I. *Tetrahedron Lett.* **1989**, *30*, 4995−4998. (g) Myers, A. G.; Kuo, E. Y.; Finney, N. S. *J. Am. Chem. Soc.* **1989**, *111*, 8057−8059. See also the references cited in refs 1 and 8−11.

(8) (a) Nicolaou, K. C.; Ogawa, Y.; Zuccarello, G.; Kataoka, H. *J. Am. Chem. Soc.* **1988**, *110*, 7247−7248. (b) Nagata, R.; Yamanaka, H.; Murahashi, E.; Saito, I. *Tetrahedron Lett.* **1990**, *31*, 2907−2910. (c) Nicolaou, K. C.; Maligres, P.; Shin, J.; Leon, E.; Rideout, D. *J. Am. Chem. Soc.* **1990**, *112*, 7825−7826.

(9) (a) Hirama, M.; Gomibuchi, T.; Fujiwara. K.; Sugiura, Y.; Uesugi, M. *J. Am. Chem. Soc.* **1991**, *113*, 9851−9853. (b) Tokuda, M.; Fujiwara, K.; Gomibuchi, T.; Hirama, M.; Uesugi, M.; Sugiura, Y. *Tetrahedron Lett.* **1993**, *34*, 669−672.

(10) Wender, P. A.; Zercher, C. K.; Beckham, S.; Haubold, E.-M. *J. Org. Chem.* **1993**, *58*, 5867−5869.

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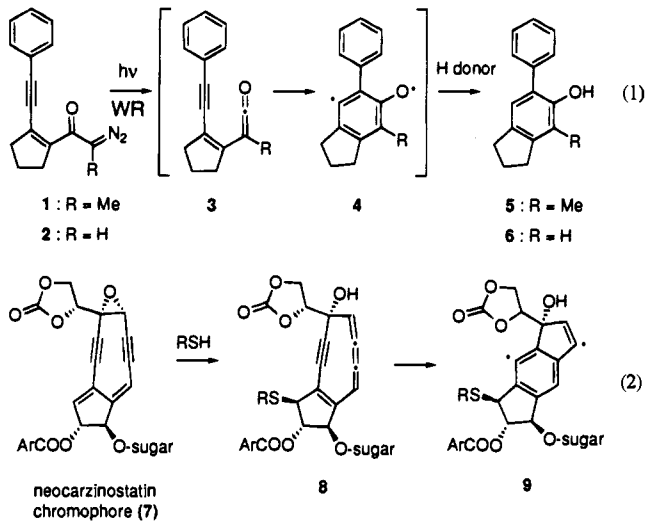


indanols **5** and **6**, respectively, wherein the efficiency of the cyclization is highly dependent on the substituent R on the carbon attached to the diazo group. The DNA-cleaving assay using supercoiled circular DNA (form I) under photoirradiation

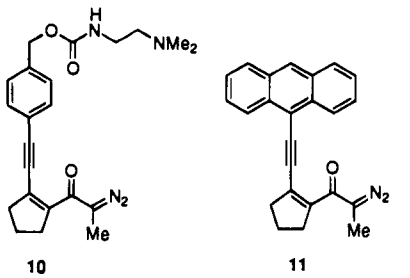


Figure 1. Structures of designed α-diazo ketones **10** and **11** as photochemical DNA cleavers.

at 366 nm light showed that **1** cleaves form I DNA to nicked circular DNA (form II) at a concentration of 100 μM. Less reactive diazo ketone **2** comparing to **1** in the photoirradiation in toluene showed only modest DNA-cleaving activity in aqueous media. Furthermore, no significant DNA cleavage was observed with α-diazo ketones that did not possess ene−yne functionality. These results prompted us to further examine the DNA-cleaving activities of **1** and its derivatives **10** and **11** possessing a DNA binder under the influence of UV light. In contrast to the results in organic solvents, it was found that photogeneration of diradical **4** from α-diazo ketones is only a minor pathway in aqueous solutions with the formation of a novel furan derivative **33** as a major product. Thus, it is highly likely that the photoinduced DNA cleavage by α-diazo ketone **1** results from the direct reaction of DNA with α-keto carbene **29**, a precursor of ene−yne−ketene **3**. We describe herein the synthesis of novel α-diazo ketones which show an enhanced DNA-cleaving activity and discuss the structure−reactivity relationship of these α-diazo ketones in the light of theoretical calculations.

Results and Discussion

Synthesis of α-Diazo Ketones. Two different types of α-diazo ketones **10** and **11** were designed in order to enhance DNA-cleaving activity (Figure 1). The former possesses an amino alkyl side chain on the phenyl ring to increase affinity toward DNA, whereas the latter has an anthracene moiety as an intercalator. Both compounds were synthesized according to the synthetic routes shown in Schemes 1 and 2. The introduction of the amino alkyl side chain into the reactive α-diazo ketone molecule was effectively achieved using aldehyde **17** as a precursor (Scheme 1). Thus, 4-ethynylbenzaldehyde[19] was coupled with enol triflate **12**[20] under standard Pd-catalyzed cross-coupling conditions[21] to yield ester **15**, which was hydrolyzed to acid **16**. Successive treatment of **16** with oxalyl chloride and ethereal diazoethane produced diazo ketone **17**. The formyl group of **17** was selectively reduced with $NaBH_4$ in ethanol at −78 °C to yield alcohol **18**, which was converted to **10** by successive treatment with disuccinimidyl carbonate (DSC) and *N,N*-dimethylethylenediamine.

The introduction of an anthracene ring into the ene−yne unit was achieved by an alternative synthetic route featuring successive Pd-catalyzed couplings as shown in Scheme 2. The enol triflate **12** was coupled at first with trimethylsilylacetylene to produce ene−yne **19**, which was desilylated to alkyne **20**. The Pd-catalyzed coupling of **20** with 9-bromoanthracene under typical Castro−Stephans coupling conditions[22] produced ester

(11) (a) Nicolaou, K. C.; Skokotas, G.; Maligres, P.; Zuccarello, G.; Schwiger, E. J.; Toshima, K.; Wendebom, S. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1272−1275. (b) Nicolaou, K. C.; Wendebon, S.; Maligres, P.; Isshiki, K.; Zein, N.; Ellested, G. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 418−420. (c) Nicolaou, K. C.; Dai, W.-M.; Wendebom, S. V.; Smith, A. L.; Torisawa, Y.; Maligres, P.; Hwang, C.-K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1032−1036. (d) Nicolaou, K. C.; Smith, A. L.; Wendebom, S. V.; Hwang, C.-K. *J. Am. Chem. Soc.* **1991**, *113*, 3106−3114. (e) Nicolaou, K. C.; Hong, Y.-P.; Torisawa, Y.; Tsay, S.-C.; Dai, W.-M. *J. Am. Chem. Soc.* **1991**, *113*, 9878−9880. (f) Nicolaou, K. C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; Wrasidlo, W. *Science*, **1992**, *256*, 1172−1178. (g) Sakai, Y.; Bando, Y.; Shishido, K.; Shibuya, M. *Tetrahedron Lett.* **1992**, *33*, 957−960. (h) Toshima, K.; Ohta, K.; Ohashi, A.; Ohtsuka, A.; Nakata, M.; Tatsuta, K. *J. Chem. Soc., Chem. Commun.* **1992**, *1306*−1308. (i) Toshima, K.; Ohta, K.; Ohtsuka, A.; Matsumura, S.; Nakata, M. *J. Chem. Soc., Chem. Commun.* **1993**, 1406−1407. (j) Toshima, K.; Ohta, K.; Ohashi, A.; Nakamura, T.; Nakata, M.; Matsumura, S. *J. Chem. Soc., Chem. Commun.* **1993**, 1525−1527. (k) Toshima, K.; Ohta, K.; Ohashi, A.; Nakamura, T.; Nakata, M.; Tatsuta, K.; Matsuura, S. *J. Am. Chem. Soc.* **1995**, *117*, 4822−4831.

(12) Nakatani, K.; Isoe, S.; Maekawa, S.; Saito, I. *Tetrahedron Lett.* **1994**, *35*, 605−608.

(13) Contributions from these laboratories for the photoinduced DNA cleavage using other systems, see: (a) Saito, I.; Takayama, M.; Matsuura, T.; Matsugo, S.; Kawanishi, S. *J. Am. Chem. Soc.* **1990**, *112*, 883−884. (b) Matsugo, S.; Kawanishi, S.; Yamamoto, K.; Sugiyama, H.; Matsuura, T.; Saito, I. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1351−1353. (c) Saito, I. *Pure Appl. Chem.* **1992**, *64*, 1305−1310. (d) Sugiyama, H.; Tsutsumi, K.; Fujimoto, K.; Saito, I. *J. Am. Chem. Soc.* **1993**, *115*, 4443−4448. (e) Saito, I.; Takayama, M.; Sakurai, T. *J. Am. Chem. Soc.* **1994**, *116*, 2653−2654. (f) Saito, I.; Sakurai, T.; Kurimoto, T.; Takayama, M. *Tetrahedron Lett.* **1994**, *35*, 4797−4800. (g) Saito, I.; Takayama, M.; Kawanishi, S. *J. Am. Chem. Soc.* **1995**, *117*, 5590−5591. (h) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406−6407. (i) Nakatani, K.; Shirai, J.; Tamaki, R.; Saito, I. *Tetrahedron Lett.* **1995**, *36*, 5363−5366.

(14) For a review on photoinduced DNA cleavage, see: *Bioorganic Photochemistry, Photochemistry and the Nucleic Acids*; Morrison, H., Ed.; John Wiley and Sons: New York, 1990; Vol. 1. For recent references of photoinduced DNA cleavage, see the references cited in ref 13g.

(15) For a review, see: Meier, H.; Zeller, K.-P. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 32−43.

(16) (a) Karlsson, J. O.; Nguyen, N. V.; Moore, H. W. *J. Am. Chem. Soc.* **1985**, *107*, 3392−3393. (b) Perri, S. T.; Foland, L. D.; Decker, O. H. W.; Moore, H. W. *J. Org. Chem.* **1986**, *51*, 3067−3068. (c) Foland, L. D.; Karlsson, J. O.; Perri, S. T.; Schwabe, R.; Xu, S. L.; Patil, S.; Moore, H. W. *J. Am. Chem. Soc.* **1989**, *111*, 975−989. (d) Chow, K.; Nguyen, N. V.; Moore, H. W. *J. Org. Chem.* **1990**, *55*, 3876−3880. (e) Moore, H. W.; Yeraxa, B. R. *Chemtracts* **1992**, *5*, 273−313.

(17) (a) Padwa, A.; Austin, D. J.; Chiacchio, U.; Kassir, J. M. *Tetrahedron Lett.* **1991**, *32*, 5923−5926. (b) Padwa, A.; Chiacchio, U.; Fairfax, D. J.; Kassir, J. M.; Litrico, A.; Semones, M. A.; Xu, S. L. *J. Org. Chem.* **1993**, *58*, 6429−6437.

(18) For DNA cleavage by the related ene−yne−ketene system, see: Sullivan, R. W.; Coghlan, V. M.; Munk, S. A.; Reed, M. W.; Moore, H. W. *J. Org. Chem.* **1994**, *59*, 2276−2278.

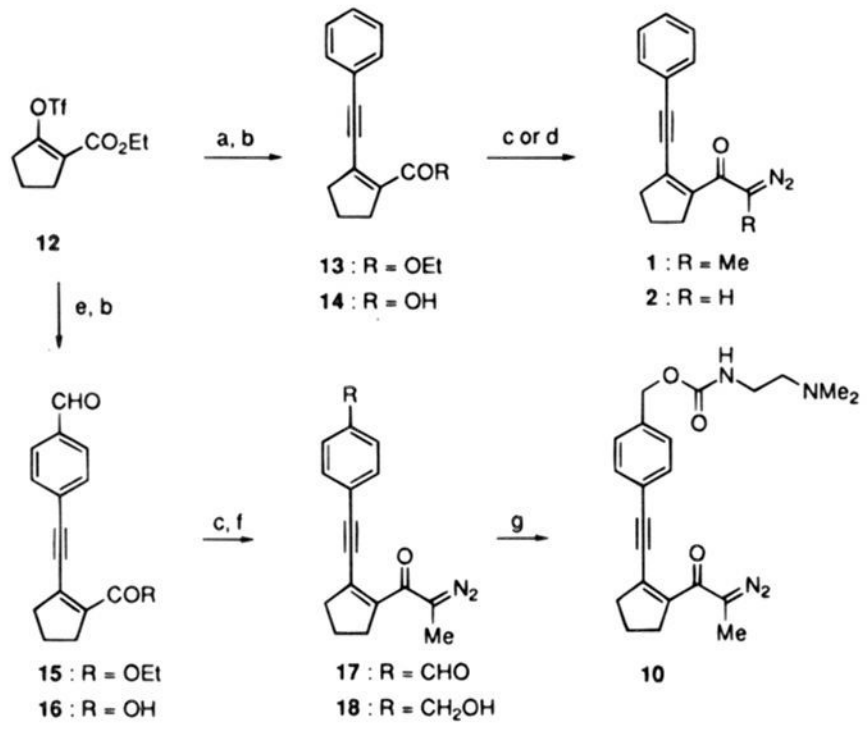
(19) Austin, W. B.; Bilow, N.; Kelleghan, W. J.; Lau, K. S. Y. *J. Org. Chem.* **1981**, *46*, 2280−2286.

(20) Houpis, I. N. *Tetrahedron Lett.* **1991**, *32*, 6675−6678.

(21) The use of 2,6-lutidine is essential for the coupling reactions of **13**. Conventional bases like diethylamine efficiently reacted with **13** giving the addition−elimination product.

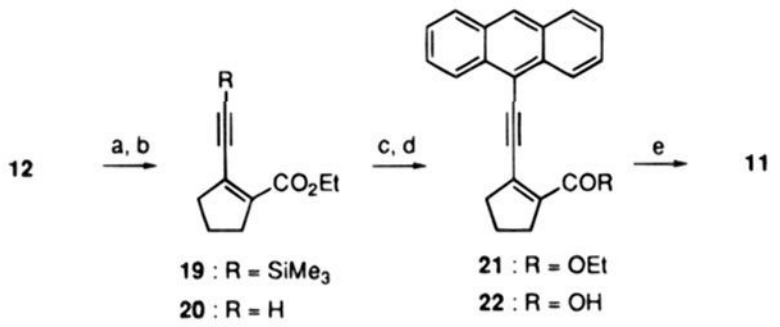
(22) (a) Castero, C. E.; Stephans, R. D. *J. Org. Chem.* **1963**, *28*, 2163. (b) Stephans, R. D.; Castero, C. E. *J. Org. Chem.* **1963**, *28*, 3313−3315.

Scheme 1[a]



[a] Reagents: (a) phenylacetylene, $PdCl_2(PPh_3)_2$, CuI, 2,6-lutidine; (b) aqueous NaOH/MeOH; (c) $(COCl)_2$ then CH_3CHN_2; (d) $(COCl)_2$ then CH_2N_2; (e) 4-ethynylbenzaldehyde, $PdCl_2(PPh_3)_2$, CuI, 2,6-lutidine; (f) $NaBH_4$, EtOH, −78 °C; (g) (i) disuccinimidyl carbonate, (ii) *N*,*N*-dimethylethylenediamine.

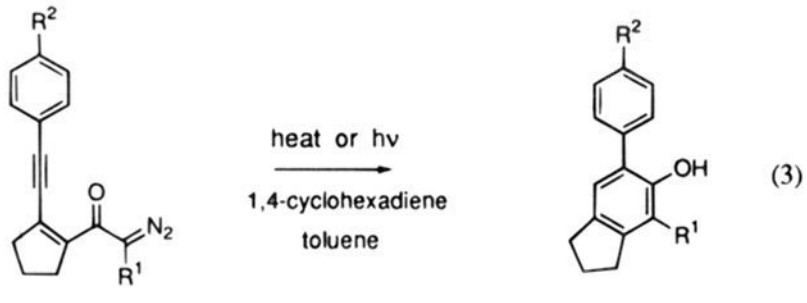
Scheme 2[a]



[a] Reagents: (a) trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, CuI, 2,6-lutidine; (b) *n*-Bu_4NF, AcOH; (c) 9-bromoanthracene, $PdCl_2(PPh_3)_2$, PPh_3, CuI, Et_3N; (d) aqueous NaOH/MeOH; (e) $(COCl)_2$, then CH_3CHN_2.

21. Hydrolysis of **21** followed by acid chloride formation from the resulting **22** and subsequent reaction with diazoethane afforded diazo ketone **11**. While α-diazo ketones **10** and **11** slowly decomposed in an aqueous acidic medium at ambient temperature, they were stable in neutral or basic aqueous solutions.

Thermal and Photochemical Reactions of α-Diazo Ketones. With α-diazo ketones **1**, **2**, **10**, and **17** in hand, their thermal and photochemical reactions in toluene were investigated (eq 3). Due to its low solubility in toluene, the reactio



(3)

1	R^1 = Me, R^2 = H	**5**
2	R^1 = H, R^2 = H	**6**
10	R^1 = Me, R^2 = $CH_2OCONH(CH_2)_2NMe_2$	**23**
17	R^1 = Me, R^2 = CHO	**24**

of **11** was not examined. In the presence of 1,4-cyclohexadiene as a hydrogen donor, both thermal and photochemical reactions of **10** and **17** proceeded smoothly to produce indanols **23** and **24** (Table 1, runs 5, 6, 7, and 8), respectively, in an efficiency comparable to that for **1** (runs 1 and 2), although no isolable product was obtained in the absence of 1,4-cyclohexadiene. The yields for the thermal reactions were superior to those for the photoreactions in general. The effect of the substituent (R^1) α to the diazo group on the cyclization is noteworthy. Thus, the methyl-substituted diazo ketones **1**, **10**, and **17** underwent a clean reaction under thermal conditions at 140 °C (runs 1, 5, and 7), whereas the reaction of unsubstituted **2** was very slow and was accompanied by the formation of side products. It was essential to carry out the reaction at 170 °C for complete conversion of **2** (run 3). A complex mixture was obtained in the photoirradiation of **2** with the formation of a trace amount of **6** (run 4).

Table 1. Thermal and Photochemical Reactions of α-Diazo Ketones **1**, **2**, **10**, and **17**[a]

run no.	diazo ketone	conditions	indanol	yield (%) (conv)
1	**1**	140 °C	**5**	58[b]
2	**1**	*hν*	**5**	36[b]
3	**2**	170 °C	**6**	38[b]
4	**2**	*hν*	**6**	*c*
5	**10**	140 °C	**23**	67[d] (80)
6	**10**	*hν*	**23**	29[d] (39)
7	**17**	140 °C	**24**	56[b]
8	**17**	*hν*	**24**	21[d]

[a] A toluene solution (0.01 M) of the diazo ketone containing 1,4-cyclohexadiene (10 equiv) was either heated at the indicated temperature in a sealed tube for 30 min or irradiated with a transilluminator (366 nm) for 2 h in a Pyrex vessel at room temperature. [b] Isolated yield. [c] A complex mixture containing **6**. [d] Yield determined by HPLC.

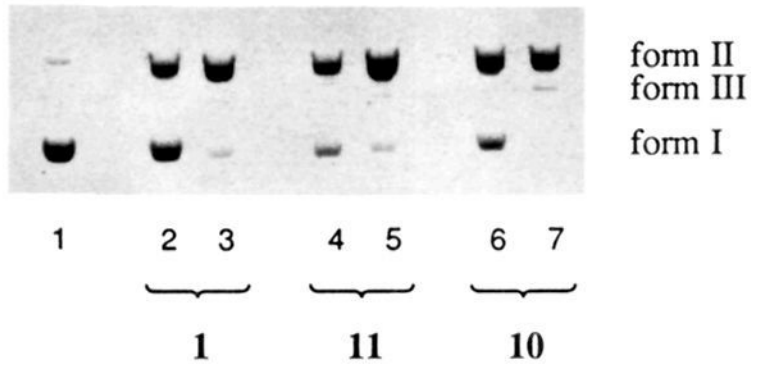


Figure 2. DNA cleavage by diazo ketones **1**, **10**, and **11** under 366-nm irradiation. pBR 322 DNA (40 μM) was irradiated at 366 nm at 0 °C (pH 7.0, Na cacodylate) for 1 h with or without drugs (added as an acetonitrile solution, final concentration of acetonitrile was 10%) and analyzed by agarose gel electrophoresis (ethidium bromide staining). Lane 1, control; lane 2, **1** (100 μM); lane 3, **1** (1 mM); lane 4, **11** (100 μM); lane 5, **11** (1 mM); lane 6, **10** (100 μM); lane 7, **10** (400 μM).

DNA-Cleaving Activities of Diazo Ketones. We have already reported that methyl-substituted diazo ketone **1** is able to cleave DNA under the photoirradiation conditions at a concentration of 100 μM to 1 mM, whereas diazo ketone **2** showed a considerably weaker DNA-cleaving activity under identical conditions.[12] By comparing DNA-cleaving activities of diazo ketones **10** and **11** with those of **1**, it is clear that **10** and **11** exhibit a greatly improved DNA-cleaving activity. The DNA cleavage was monitored by relaxation of supercoiled circular pBR322 DNA (form I) into nicked circular (form II) and linear (form III) DNA (Figure 2). The anthracene-equipped diazo ketone **11** exhibited the highest DNA-cleaving activity among these three α-diazo ketones **1**, **10**, and **11** at 100 μM concentration (lane 4, *cf.* lanes 2 and 6). The relative DNA-cleaving activity of these α-diazo ketones was in the following order: **11** > **10** > **1** ≫ **2**. While diazo ketone **11** was designed with an expectation of the intercalation of the anthracene moiety into DNA, the hypochromism and the red shift of the absorption in UV titration of **11** with calf thymus DNA were only negligible.

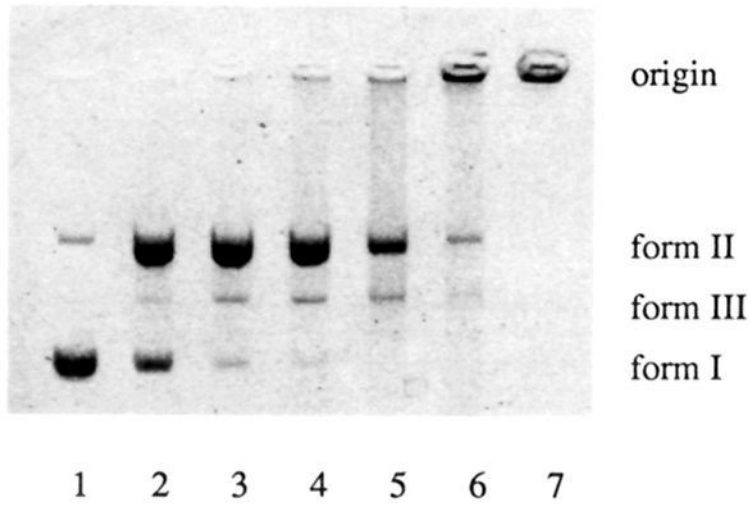


Figure 3. DNA cleavage by diazo ketone **10** under 366-nm irradiation. pBR 322 DNA (40 μM) was irradiated at 366 nm at 0 °C (pH 7.0, Na cacodylate) for 1 h at various concentrations of **10** (added as an acetonitrile solution, final concentration of acetonitrile was 10%) and analyzed by agarose gel electrophoresis (ethidium bromide staining). Lane 1, control; lane 2, 100 μM; lane 3, 200 μM; lane 4, 400 μM; lane 5, 600 μM; lane 6, 800 μM; lane 7, 1 mM.

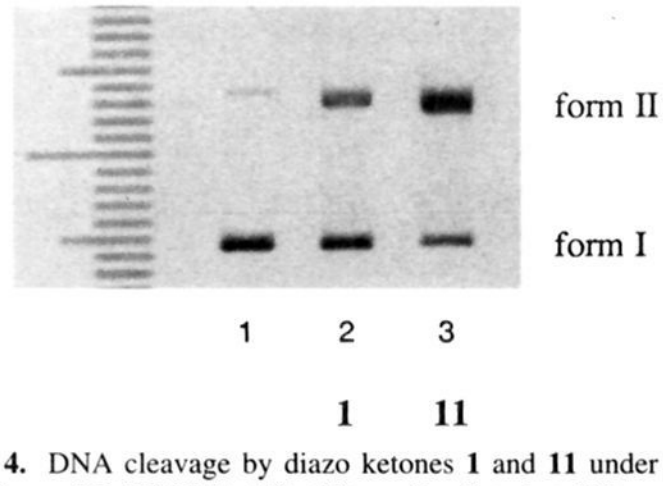


Figure 4. DNA cleavage by diazo ketones **1** and **11** under 425-nm irradiation. pBR 322 DNA (40 μM) was irradiated at 425 nm at room temperature (pH 7.0, Na cacodylate) for 2 h with drugs (added as an acetonitrile solution, final concentration of acetonitrile was 10%) and analyzed by agarose gel electrophoresis (ethidium bromide staining). Lane 1, control; lane 2, **1** (100 μM); lane 3, **11** (100 μM).

α-Diazo ketone **10** possessing an amino alkyl side chain cleaved DNA more efficiently than **1** at 100 μM concentration (Figure 2, lane 6, *cf.* lane 2). The DNA cleavage by **10** (100 μM) was not affected by the presence of sodium azide (10 mM) and mannitol (10 mM). Thus, singlet oxygen and hydroxyl radical are not likely to be involved in the DNA cleavage. It was also found that the DNA cleavage bands became smeared as the concentration of **10** increased (Figure 3). At a concentration of 800 μM or more, the band was observed only at the origin (lanes 6 and 7), suggesting that a covalent modification of DNA by a photochemically generated species probably occurs at high drug concentrations of **10**.

Another interesting feature of the DNA cleavage was observed when a longer wavelength light was used (Figure 4). Thus, photoirradiation of anthracene-containing **11** with pBR322 DNA at 425 nm isolated by a monochromator resulted in an effective DNA cleavage, with the DNA-cleaving efficiency being comparable to that observed at 366-nm light. In the photoreaction of **1** at 425 nm, the incident light was absorbed directly by the diazo group ($\epsilon = 60$ at 425 nm), whereas more than 99% of 425-nm light was absorbed by the anthracene moiety in **11** ($\epsilon = 9800$ at 425 nm). The anthracene ester **21** having no diazo group showed a strong fluorescence emission at 455 nm (emission intensity 0.175, excited at 407 nm), whereas the fluorescence intensity of **11** was substantially reduced at the same concentration (emission intensity 0.045, excited at 403 nm). Thus, at 425-nm irradiation to **11** the light is absorbed by anthracene and the excitation energy of anthracene is transferred to the diazo ketone chromopohore to result in a more efficient DNA cleavage compared to that observed for **1**.

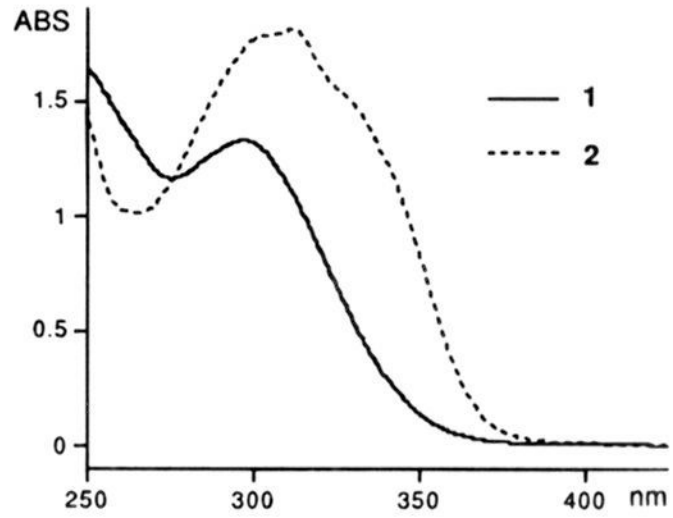


Figure 5. UV−visible absorption spectra of diazo ketones **1** and **2**. The concentration of each sample is 100 μM in acetonitrile.

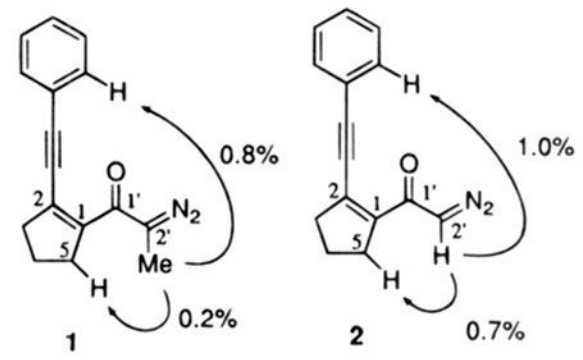
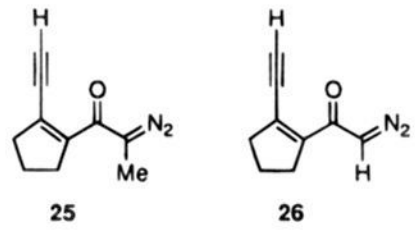


Figure 6. The NOEs for **1** and **2**.

Conformations of α-Diazo Ketones. To gain more insight into the difference in the reactivities of **1** and **2** during the thermal and photochemical reactions, spectroscopic and theoretical studies have been carried out. The absorption spectra of **1** and **2** were considerably different from each other as shown in Figure 5. While the absorption maximum of **2** was observed at 313 nm ($\epsilon = 18\,170$) with a shoulder at about 340 nm, it was found at 298 nm for **1** ($\epsilon = 13\,340$). These observations strongly suggest that the conformation of the α-diazocarbonyl group of **1** would be quite different from that of **2** in its ground state. In order to gain further insight into the conformations of **1** and **2**, the differential NOE spectra were measured. The C2′ H at the carbon attached to the diazo group of **2** showed the NOEs to the C5 methylene of the cyclopentene ring and to the ortho hydrogen of the phenyl ring as shown in Figure 6. On the other hand, the methyl group of **1** has the NOE to the phenyl hydrogen but only weakly to the C5 methylene.

In order to estimate the most stable conformations for **1** and **2**, theoretical calculations on the model systems **25** and **26** were carried out.[23] The molecular orbital calculations were initially performed at a semiempirical level using a PM3 model to get all possible conformational isomers for **25** and **26** within 10 kcal/mol from the most stable isomer, which were then optimized at the *ab initio* HF/3-21G(*) level and finally at the HF/6-31G* level (Figure 7).



Two conformational isomers *s-trans-syn*-**26** and *s-cis-syn*-**26** were found for **26**, with the former being more stable by

(23) All theoretical calculations were performed using the semiempirical and *ab initio* modules incorporated in SPARTAN molecular modeling software (version 3.1).

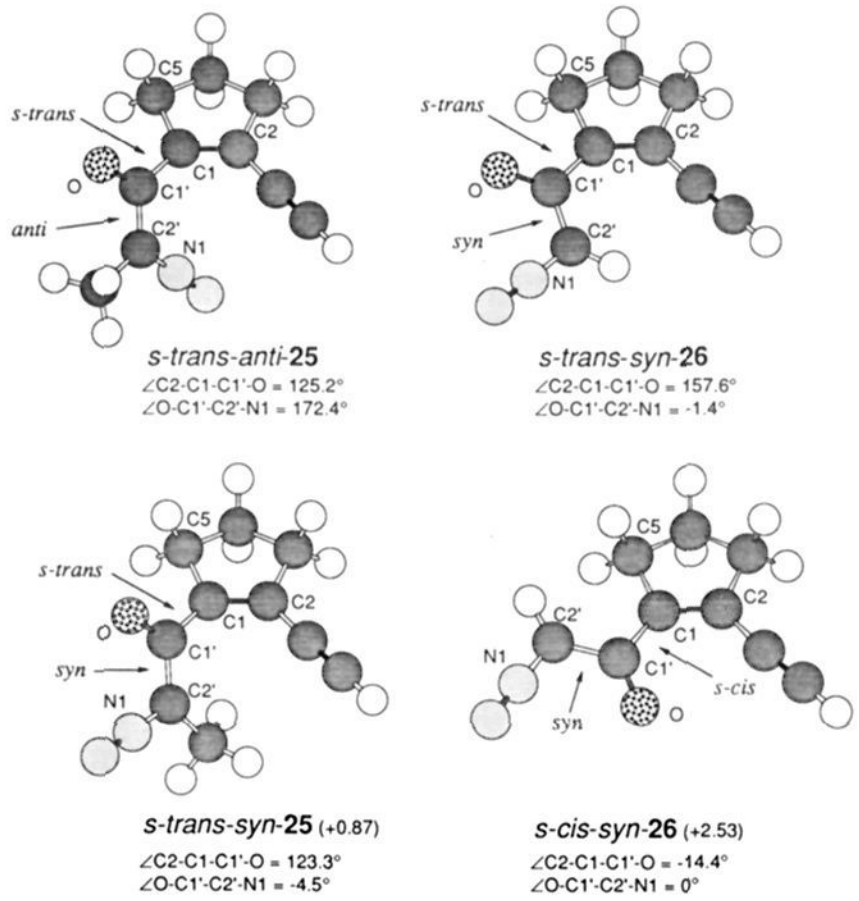


Figure 7. Energy minimized structures for conformational isomers of **25** and **26** at the HF/6-31G* level. The global energy minima for **25** and **26** are *s-trans-anti*-**25** and *s-trans-syn*-**26**, respectively. The numbers in parentheses denote the relative energy (kcal/mol) from the global energy minimum. The *s-trans-syn*-**25** and *s-cis-syn*-**26** are less stable than *s-trans-anti*-**25** and *s-trans-syn*-**26** in 0.87 and 2.53 kcal/mol, respectively.

2.53 kcal/mol. To avoid confusion on the definition of the conformation of α-diazo ketones, the expression of *s-cis* and *s-trans* was temporarily used for the conformation around the C1−C1′ single bond. For the assignment of the conformation of α-diazo ketone moiety, *syn* and *anti* representation was used. The most stable conformational isomer of **25** has an *anti* conformation (*s-trans-anti*-**25**). The corresponding *syn* conformer (*s-trans-syn*-**25**) is 0.87 kcal/mol less stable than the *s-trans-anti*-**25**. The rotational energy barrier from *anti* to *syn* conformation is estimated to be 17.7 kcal/mol by the single point energy calculation (HF/6-31G*) of diazo ketone **25** by fixing the dihedral angle of O−C1′−C2′−N1 to be 90°.[24] The dihedral angles between the cyclopentene and the carbonyl group in *s-trans-anti*-**25** (∠C2−C1−C1′−O = 125°) and *s-trans-syn*-**25** (123°) are considerably larger than those for *s-trans-syn*-**26** (157°) and *s-cis-syn*-**26** (−14°), respectively, which results in a decrease of the conjugation of the two functional groups (Figure 8). These structural features may well rationalize the differences in the UV absorption maximums of **1** and **2**. Based on the experimental results and the theoretical calculations of the model compounds, the C1−C1′ bond of **2** seems to rotate by maintaining the *syn* conformation for the C1′−C2′ bond, while the rotation of both C1−C1′ and C1′−C2′ bonds of **1** is moderately restricted.

The reactivity of α-diazo carbonyl compounds is known to be highly dependent on the conformation of the α-diazo carbonyl moiety, and the thermal Wolff rearrangement has been proposed to proceed concertedly from the *syn* conformation.[24] While the α-diazo ketone **1** is suggested to exist mainly in an *anti* conformation by calculations, it undergoes smooth thermal Wolff rearrangement giving indanol **5** (Table 1, run 1). Thus,

(24) The ratio of *syn* and *anti* conformers of RCO−CHN$_2$ determined by variable-temperature NMR studies is as follows: 92:4 (R = Me), 94:6 (R = Et), and >99:1 (R = *t*-Bu) in $CDCl_3$ (Kaplan, F.; Meloy, G. K. *J. Am. Chem. Soc.* **1966**, *88*, 950−956). The rotational energy barriers for *syn* and *anti* conformers of these diazo ketones were determined to be approximately 15−18 kcal/mol.

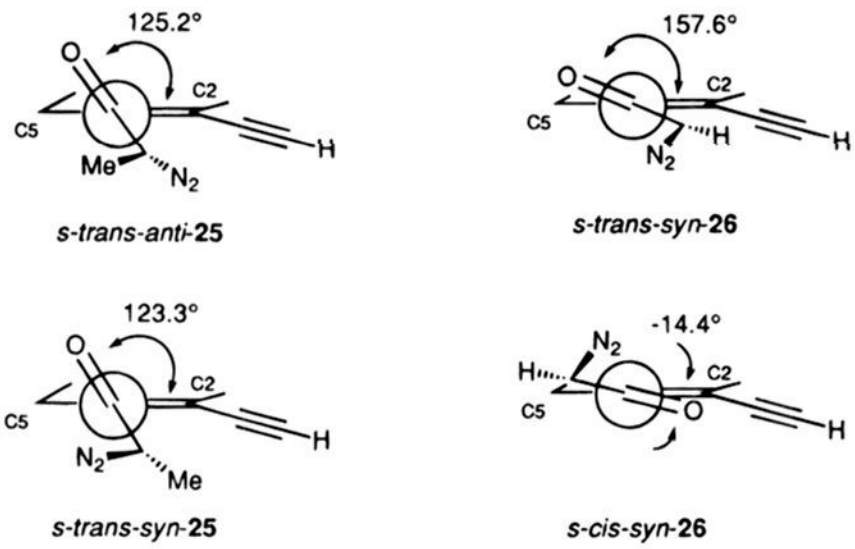
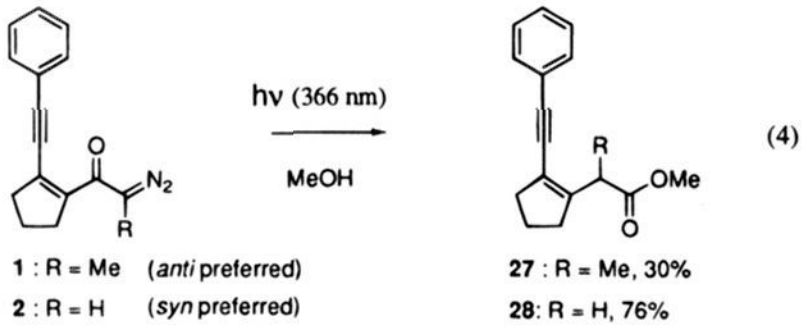


Figure 8. Newman projections of stable conformational isomers for **25** and **26** viewed from the C1′−C1 axis. The numbers with arrow represent the dihedral angle of C2−C1−C1′−O. For clarity the C2′−N bond was drawn as a single bond.

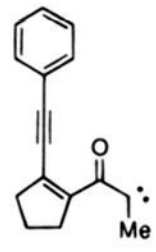
under these conditions facile conformational change from *anti* to *syn* conformation would be feasible. On the other hand, the thermal reaction of **2** requires an elevated temperature for complete conversion, suggesting that the activation energy for the Wolff rearrangement of **2** is much higher than that for **1**.

In contrast to the thermal Wolff rearrangement, the formation of ketenes from α-diazo carbonyl compounds under photoirradiation conditions was proposed to proceed mainly via the excited singlet state of the *syn* conformer and partially from the singlet α-keto carbene.[25] Photoirradiation of **2** in methanol afforded the methyl ester **28** in 76% yield (eq 4), while the same



(4)

irradiation of **1** produced **27** only in a lower yield (30%) accompanied with the formation of unidentified side products. These results may be rationalized by considering that the most stable conformations for **1** and **2** were *anti* and *syn* conformations, respectively, and suggested that besides Wolff rearrangement, other reactions like the formation of α-keto carbene **29** may be involved in the photoreaction of **1**.



29

The photoreaction of **2** giving a high yield of **28** in methanol suggests the efficient formation of the ene−yne−ketene intermediate, whereas the yield of cyclized indanol **6** from **2** was exceedingly low compared to that for **5** from **1** (Table 1, run

(25) (a) Tomioka, H.; Okuno, H.; Izawa, Y. *J. Org. Chem.* **1980**, *45*, 5278−5283. (b) Tomioka, H.; Kondo, M.; Izawa, Y. *J. Org. Chem.* **1981**, *46*, 1090−1094. (c) Tomioka, H.; Hayashi, N.; Asano, T.; Izawa, Y. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 758−761. For recent discussions, see also: (d) Toscano, J. P.; Platz, M. S.; Nikolaev, V.; Popic, V. *J. Am. Chem. Soc.* **1994**, *116*, 8146−8151. (e) Wang, J.-L.; Toscano, J. P. Platz, M. S.; Nikolaev, V.; Popik, V. *J. Am. Chem. Soc.* **1995**, *117*, 5477−5483 and references cited therein.

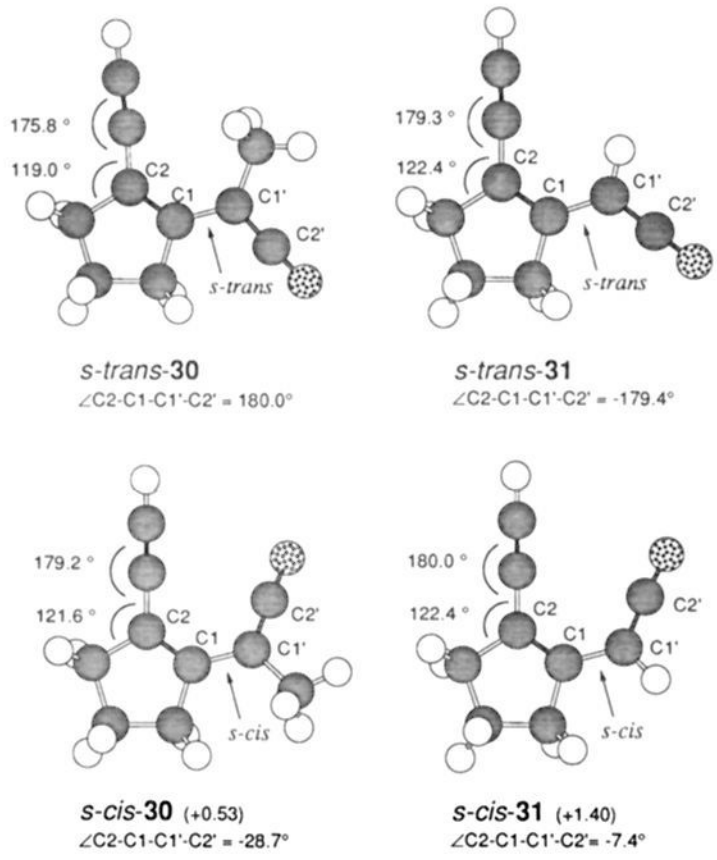
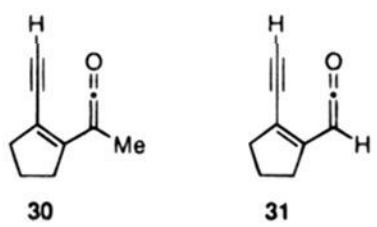


Figure 9. Geometry optimized structures for *s-cis* and *s-trans* conformers of **30** and **31** at the HF/6-31G* level. In both compounds the *s-trans* conformer (*s-trans*-**30** and *s-trans*-**31**) is the global energy minimum. The numbers in parentheses denote the relative energy (kcal/mol) from the global minimum.

4). These results suggest that the ene−yne−ketene intermediate generated from **2** would exist in the *s-trans* (with regards to the C1−C1′ single bond) conformation preferentially. Conformational analyses of simple ene−yne−ketene models **30** and **31** calculated at the HF/6-31G* level showed that the *s-trans* conformation is more stable in both cases (Figure 9). Analysis of the optimized geometry of *s-trans*-**30** showed that the acetylene bond is bent against the methyl group, implying that there is a substantial steric repulsion between the methyl and acetylene group. This structural feature of *s-trans*-**30** seems to be the reason why the energy gap between *s-trans*-**30** and *s-cis*-**30** (0.53 kcal/mol) is much smaller than that for *s-trans*-**31** and *s-cis*-**31** (1.40 kcal/mol). Thus, the proportion of the existing *s-cis* conformer in **30** is much higher than that in the case of **31**, allowing higher yield cyclization of **1** to indanol **5** compared to that for **2**.



30 **31**

The Reaction of α-Diazo Ketones in Aqueous Solutions. As we discussed, the thermal and photochemical Wolff rearrangement of diazo ketones produces ene−yne−ketene intermediates, which spontaneously cyclize to diradicals. However, the results of the photoirradiation of **1** in methanol suggest a concomitant formation of α-keto carbene besides the ketene formation. In order to examine the role of diradicals in the DNA cleavage by these α-diazo ketones, detection of the indanol derivatives resulting from diradicals like **4** under DNA-cleaving conditions was undertaken. We have previously attempted to detect indanol **5** in the photoirradiated solution of **1** in the presence of pBR322 DNA by HPLC and found that the amount of **5** produced in the photoreaction was very small. It was anticipated that the weak DNA-cleaving activity of **1** might be the reason for a minor formation of **5**. Therefore, α-diazo ketone **10** exhibiting much stronger DNA-cleaving activity than

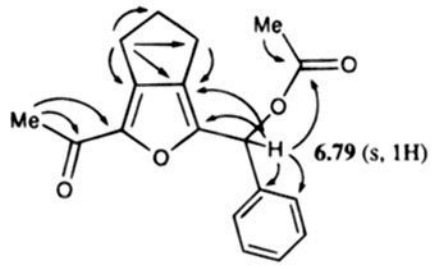
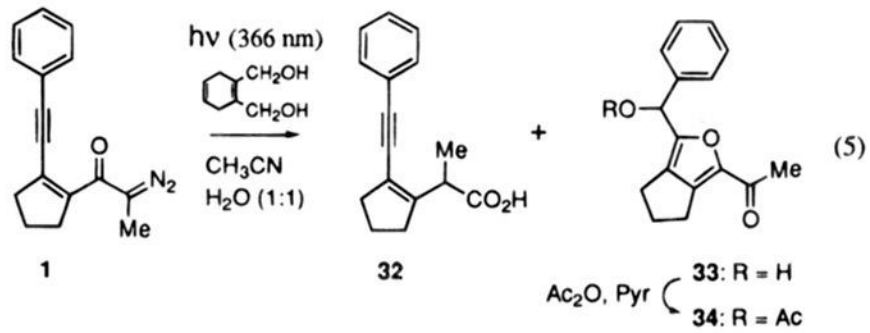


Figure 10. The correlations observed in the HMBC spectrum of **34**.

1 was used for the present study. After conventional DNA-cleaving experiments, the DNA solution was extracted with chloroform. DNA cleavage was confirmed by analyzing the aqueous phase by agarose gel electrophoresis, and the chloroform extracts were then subjected to reverse phase HPLC analysis. By comparing HPLC profiles of α-diazo ketone **10** (retention time, 13.6 min, HPLC conditions: linear gradient of acetonitrile and ammonium formate (50 mM), 50:50 to 75:25 in 25 min) and the authentic sample of indanol **23** (18.1 min), it was clear that under the photoirradiation conditions in the aqueous acetonitrile solution cyclized indanol **23** was not produced in an appreciable amount even after **10** was consumed completely. While the designed α-diazo ketones can produce diradicals by cyclization of the photochemically or thermally generated ene−yne−ketene intermediates in toluene, such cyclization leading to diradicals is less likely to occur in an aqueous system.

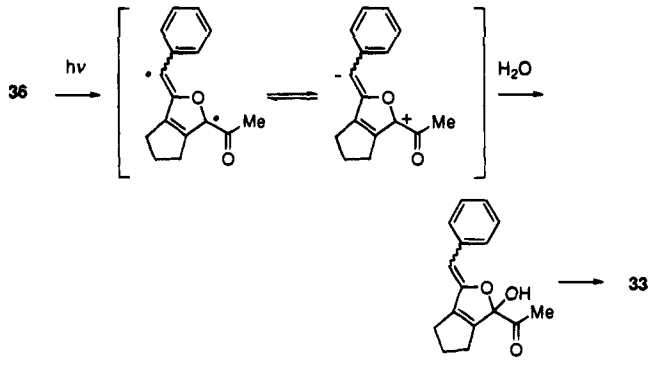
We next focused our attention on the photoreaction of α-diazo ketone **1** in aqueous solutions. Thus, when a 50% aqueous acetonitrile solution of **1** in the presence of 1,4-cyclohexadiene-1,2-dimethanol[6c] was irradiated with 366-nm light for 2 h under nitrogen, two isolable products were obtained along with the recovery of the starting material **1** (42%) (eq 5). One product



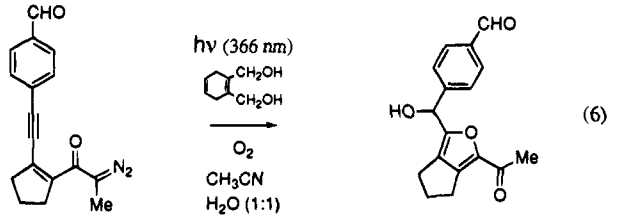
was identified as the carboxylic acid **32** (39%) resulting from water addition to the ene−yne−ketene intermediate. The other product **33** (8%) showed the molecular formula to be $C_{16}H_{16}O_3$ by HRMS. The presence of one hydroxyl group in **33** was confirmed by converting it to monoacetate **34** ($C_{18}H_{18}O_4$ by HRMS). The acetate **34** was reverted to **33** by treatment with sodium methoxide in methanol. The ^{1}H NMR spectrum of **34** showed two signals of singlet methyl at 2.14 and 2.36 ppm, suggesting the presence of a methyl ketone group besides the acetate group. The ^{13}C NMR spectrum of **34** indicated that there was no acetylenic carbon but a characteristic carbon signal at 70.5 ppm. The HMQC spectrum of **34** revealed that this carbon bears only one hydrogen which appears at 6.79 ppm in ^{1}H NMR. The structure was unambiguously determined from a combination of HMQC and HMBC spectroscopy as the 1-acetylcyclopentenofuran derivative **34** (Figure 10).

The formation of **33** and carboxylic acid **32** from **1** under DNA-cleaving conditions was confirmed by HPLC analysis. While the precise mechanism of the formation of **33** was not clear at this moment, the trapping of the initially formed α-keto carbene **29** with molecular oxygen was suggested by the photoreaction of **1** under an oxygen atmosphere. Thus, under the aerobic conditions the isolated yield of **33** increased to 24%. The formation of such furan derivatives from the diazo ketones having ene−yne functionality seems to be quite general under

Scheme 3

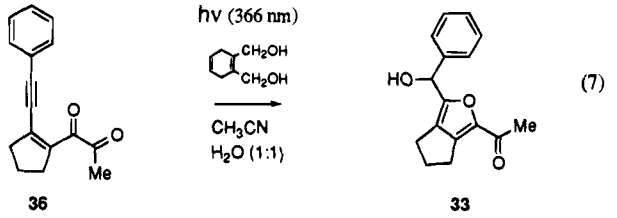


oxygen atmosphere in aqueous media. For example, formyl-substituted **35** was obtained from diazo ketone **17** in 15% yield (eq 6).



(6)

In order to determine whether **33** is formed from **1** via diketone **36**, we independently synthesized **36**[26] and examined its photoreaction. Irradiation of **36** in aqueous acetonitrile under aerobic conditions gave **33** in 44% yield (eq 7). A possible



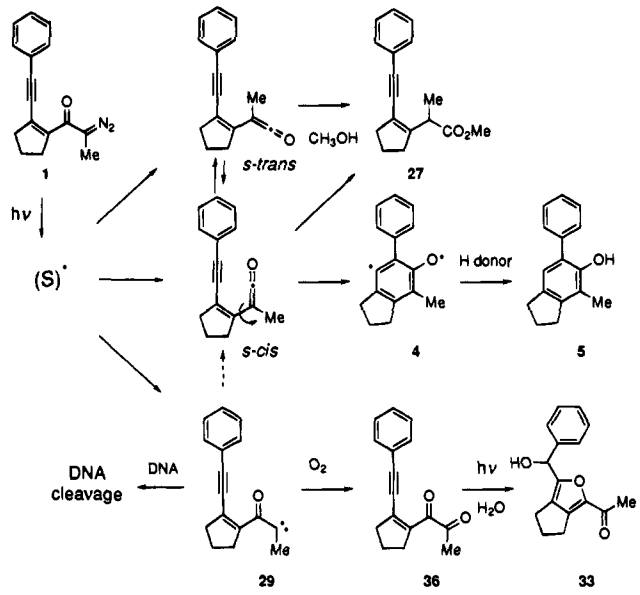
(7)

mechanism of the photocyclization is proposed as shown in Scheme 3. Excitation of the diketone chromophore of **36** could result in the formation of a cyclized five-membered diradical or zwitterion which is trapped with solvent water to ultimately give **33** via rearrangement. The detail mechanism and the scope and limitation of this novel photoreaction are now under active investigation. This result suggested that the photoreaction of **1** in an aqueous solution produces α-keto carbene **29** which is immediately trapped with molecular oxygen giving diketone **36**. It was also confirmed by DNA-cleaving experiments that the DNA-cleaving activity of **36** was substantially lower than that of **1** under 366−nm photoirradiation. These results imply that the photochemically generated α-keto carbene **29** would be a principal reactive species responsible for the observed DNA cleavage. The formation of diketone **36** in the photoreaction of **1** in aqueous solutions suggests triplet α-keto carbene, which may abstract hydrogen from the DNA sugar backbone to result in a spontaneous DNA strand cleavage.[27] Besides hydrogen abstraction by keto carbene, the modification of nucleobases by ene−yne−ketene intermediates may be responsible for the DNA cleavage in part. A general overview of the photoreaction of α-diazo ketone **1** is summarized in Scheme 4.

(26) Bestmann, H. J.; Klein, O.; Göthlich, L.; Buckschewski, H. *Chem. Ber.* **1963**, *96*, 2259−2265.

(27) For a reference describing photoinduced DNA cleavage by a diazo compound at 300 nm irradiation, see: Nielsen, P. E.; Jeppesen, C.; Egholm, M.; Buchardt, O. *Nucleic Acids Res.* **1988**, *16*, 3877−3888.

Scheme 4



Conclusion

α-Diazo ketones **1**, **10**, and **11** were designed as mimics for the radical-generating system of neocarzinostatin chromophore (**7**). These α-diazo ketones can generate diradicals under thermal or photoirradiation conditions in toluene via cyclization of the ene−yne−ketene intermediates. *Ab initio* MO calculations revealed that the efficiency of the diradical generation is highly dependent on the conformation of the α-diazo ketones which is controlled by the substituents on the carbon directly attached to the diazo group. The absence of indanol **23** in an appreciable amount in the photoirradiated mixture of **10** in the presence of pBR322 DNA in aqueous system strongly suggests that the observed DNA cleavage does not involve a diradical such as **4**. The photoreactions of **1** in methanol and aqueous acetonitrile suggest that **1** undergoes two major reactions, the Wolff rearrangement and the formation of α-keto carbene **29**. These experiments together with theoretical calculations suggest that α-keto carbene **29** is the principal DNA-cleaving species. From the standpoint for the design of efficient photochemical DNA cleavers, α-diazo ketones are showm to be potentially useful DNA-cleaving agents. Most importantly, these studies revealed that the reactivity and the DNA-cleaving activity of α-diazo ketones are highly dependent on their conformations. It also should be pointed out that the thermal and photochemical reactions of α-diazo ketones in organic solvents are considerably different from those observed in aqueous buffered solvents under DNA-cleaving conditions.

Experimental Section

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured with Varian GEMINI 200 (200 MHz), JEOL JNM α-400 (400 MHz), or JEOL JNM α-500 (500 MHz) spectrometers. Coupling constants (*J* values) are reported in hertz. ^{13}C NMR spectra were measured with Varian GEMINI 200 (50 MHz), JEOL JNM α-400 (100 MHz), or JEOL JNM α-500 (125 MHz) spectrometers. The chemical shifts are expressed in ppm downfield from tetramethylsilane ($\delta = 0$) or residual chloroform ($\delta = 7.25$) and benzene ($\delta = 7.15$) as an internal standard. IR spectra were recorded on a JASCO FT-IR-5M spectrophotometer. A JASCO 660 spectrophotometer and a FP-770 spectrofluorometer were used for absorption and emission spectra measurements, respectively. Mass spectra were recorded on a JEOL JMS DX-300 or a JEOL JMS SX-102A spectrometer. Photoirradiation at 366 nm was carried out using a Funakoshi TEL-33 transilluminator. A JASCO CRM-FD monochromator was used for irradiation at 425 nm. A Pharmacia

GNA100 and a GPS200/400 power supply were used for agarose gel electrophoresis. The gel was photographed with Polaroid 665 film under photoillumination. Wakogel C-200 was used for silica gel flash chromatography. Precoated TLC Merck silica gel 60 F_{254} plates were used for monitoring the reactions and also for preparative TLC. HPLC was performed on cosmosil 5C_{18}AR columns with Gilson Chromatography Model 305 using UV detector Model 118 at 254 nm. The conditions used for analysis of **10** and **23** were as follows: linear gradient of acetonitrile and ammonium formate (50 mM), 50:50 to 75:25 in 25 min. Anhydrous reactions were performed under N_2 atmosphere. Ether and tetrahydrofuran (THF) were distilled under N_2 from sodium/benzophenone ketyl prior to use. Calculations were performed on SGI INDY (R4000SC personal workstation) with SPARTAN molecular modeling software (version 3.1).

2-Diazo-1-[2-(phenylethynyl)cyclopentenyl]-1-propanone (1) (General Procedure for Diazo Ketone Preparation). To a mixture of ether (40 mL) and 40% aqueous potassium hydroxide solution (10 mL) in an Erlenmeyer flask cooled at 0 °C was added *N*-nitroso-*N*-ethylurea (0.86 g, 7.34 mmol) in small portions with rapid stirring. The deep yellow ethereal solution of diazoethane was separated from the aqueous layer by decantation and dried over pellets of sodium hydroxide. To a solution of **14** (0.18 g, 0.85 mmol) in dry benzene (3 mL) was added oxalyl chloride (0.26 g, 2.05 mmol) and the mixture was stirred for 2 h. Concentration of the reaction mixture *in vacuo* afforded acid chloride. To the ethereal solution of diazoethane prepared above was added an ether solution (10 mL) of the acid chloride at 0 °C and the mixture was stirred overnight. After concentration of the solvent to one-third of the volume in water bath at 50 °C, the residual solvent was removed *in vacuo*. The residue was purified by flash chromatography (SiO_2, 5% ethyl acetate/toluene) to give **1** (0.15 g, 71%) as a yellow oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 7.45−7.40 (2H), 6.99−6.92 (3H), 2.71 (tt, 2H, J = 7.6, 2.2 Hz), 2.39 (tt, 2H, J = 7.7, 2.2 Hz), 1.67 (s, 3H), 1.44 (quint, 2H, J = 7.6 Hz); ^{13}C NMR (C_6D_6, 100 MHz) δ 186.4, 147.1, 131.9, 128.9, 128.6, 127.9 (signal obscured by C_6D_6), 126.5, 123.4, 98.5, 85.6, 38.6, 35.2, 22.4, 8.8; IR (neat) 2955, 2070, 1600, 1485, 1375 cm^{-1}; UV (CH_3CN) 298 nm (ϵ 13 340); MS *m/z* (%) 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)$^+$] 222.1047, found 222.1058.

2-Diazo-1-[2-(phenylethynyl)cyclopentenyl]-1-ethanone (2). To a dry benzene solution (3 mL) of **14** (0.30 g, 1.41 mmol) was added oxalyl chloride (0.44 g, 3.47 mmol) and the mixture was stirred for 2 h. Concentration of the reaction mixture *in vacuo* afforded the acid chloride, which was dissolved in dry ether (10 mL) and treated with diazomethane prepared from *N*-methyl-*N*-nitrosourea (1.15 g, 11.2 mmol) as for the preparation of **1**. The crude product was purified by flash chromatography (SiO_2, 5% ethyl acetate/toluene) to give **2** (0.26 g, 78%) as a yellow oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 7.39−7.34 (2H), 6.99−6.95 (3H), 6.11 (s, 1H), 2.71 (tt, 2H, J = 7.6, 2.4 Hz), 2.45 (tt, 2H, J = 7.4, 2.4 Hz), 1.42 (quint, 2H, J = 7.7 Hz); IR (neat) 3120, 2955, 2100, 1605, 1380 cm^{-1}; UV (CH_3CN) 313 nm (ϵ 18 170); MS *m/z* (%) 236 (M$^+$) (1.2), 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.

4-Methyl-6-phenyl-5-indanol (5). (a) Under Thermal Conditions: A toluene solution (24 mL) of **1** (60.2 mg, 0.24 mmol) and 1,4-cyclohexadiene (0.20 g, 2.5 mmol) was heated at 140 °C in a sealed tube for 30 min. After the solution was cooled and concentrated *in vacuo*, the crude product was purified by flash chromatography (SiO_2, 5% ethyl acetate/toluene) to give **5** (31.4 mg, 58%) as a yellow oil. **(b) Under Photoirradiation Conditions:** A de-aerated toluene solution (12.8 mL) of **1** (32.0 mg, 0.13 mmol) and 1,4-cyclohexadiene (0.10 g, 1.3 mmol) was irradiated with a transilluminator (366 nm) for 2 h. After concentration *in vacuo* the crude product was purified by flash chromatography (SiO_2, 5% ethyl acetate/toluene) to give **5** (10.2 mg, 36%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.50−7.41 (4H), 7.37 (m, 1H), 6.93 (s, 1H), 5.11 (s, 1H), 2.88 (q, 4H, J = 7.3 Hz), 2.21 (s, 3H), 2.08 (quint, 2H, J = 7.3 Hz); IR (neat) 3555, 2950, 1600, 1460, 1220 cm^{-1}; MS *m/z* (%) 224 (M$^+$) (100), 209 (20), 147 (8), 69 (5); HRMS calcd for $C_{16}H_{16}O$ (M$^+$) 224.1201, found 224.1183.

6-Phenyl-5-indanol (6). A toluene solution (13 mL) of **2** (30.9 mg, 0.13 mmol) and 1,4-cyclohexadiene (0.11 g, 1.37 mmol) was heated at 170 °C in a sealed tube for 30 min. After the solution was cooled and concentrated *in vacuo* the residue was purified by flash chromatography (SiO_2, 5% ethyl acetate/toluene) to give **6** (10.5 mg, 38%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.50−7.43 (4H), 7.37 (m, 1H), 7.08 (s, 1H), 6.85 (s, 1H), 5.08 (br, 1H), 2.91 (t, 2H, J = 7.9 Hz), 2.87 (t, 2H, J = 7.9 Hz), 2.10 (quint, 2H, J = 7.3 Hz); IR ($CHCl_3$) 3600−3250, 2950, 1478, 760 cm^{-1}; MS *m/z* (%) 210 (M$^+$) (100), 181 (8), 165 (9), 133 (18); HRMS calcd for $C_{15}H_{14}O$ (M$^+$) 210.1044, found 210.1058.

2-Diazo-1-{2-{4-{[*N*-[2-(dimethylamino)ethyl]carbamoyloxy]-methyl}phenylethynyl}cyclopentenyl}-1-propanone (10). To a solution of **18** (36.2 mg, 0.13 mmol) and *N,N'*-disuccinimidyl carbonate (66.2 mg, 0.26 mmol) in CH_3CN (3 mL) and CH_2Cl_2 (3 mL) was added 2,6-lutidine (27.6 mg, 0.26 mmol) at 0 °C and the mixture was stirred at ambient temperature for 24 h. To the resulting mixture was added *N,N*-dimethylethylenediamine (24.1 mg, 0.27 mmol) and the mixture was stirred at ambient temperature for 1 h. The reaction mixture was diluted with saturated NH_4Cl and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 50% MeOH/ethyl acetate) to give **10** (26.7 mg, 52%) as a yellow oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 7.38 (d, 2H, J = 8.2 Hz), 7.08 (d, 2H, J = 8.1 Hz), 5.05 (br, 1H), 4.99 (s, 2H), 3.09 (q, 2H, J = 5.6 Hz), 2.70 (tt, 2H, J = 7.8, 2.3 Hz), 2.38 (tt, 2H, J = 7.7, 2.5 Hz), 1.91 (t, 2H, J = 5.6 Hz), 1.80 (s × 2, 6H), 1.69 (s, 3H), 1.44 (quint, 2H, J = 7.7 Hz); ^{13}C NMR (C_6D_6, 100 MHz) δ 186.1, 156.0, 147.1, 138.5, 131.9, 128.5, 128.4, 128.1, 127.9, 126.5, 122.7, 98.4, 85.7, 65.9, 58.0, 44.8, 38.6, 35.2, 22.4, 8.8; IR ($CHCl_3$) 3445, 3035, 2078, 1715, 1575, 1508 cm^{-1}; UV (CH_3CN) 299.2 (ϵ 12 180), 249.6 nm (ϵ 14 340).

2-Diazo-1-[2-(9-anthrylethynyl)cyclopentenyl]-1-propanone (11). To a solution of **21** (0.21 g, 0.62 mmol) in methanol (20 mL) and water (10 mL) was added sodium hydroxide (30.0 mg, 0.75 mmol) and the mixture was stirred at 50 °C for 24 h. After concentration *in vacuo*, the residue was diluted with water and washed with ethyl acetate. The water phase was acidified with 10% HCl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give **22** (0.19 g, 98%) as a yellow solid. This acid was used for the next step without further purification. According to the method detailed for **1** the reaction of **22** (0.10 g, 0.32 mmol) gave **11** (49.1 mg, 0.14 mmol, 44%) as a yellow solid: mp 117.4−118.6 °C (recrystallized from hexane−benzene); ^{1}H NMR (C_6D_6, 400 MHz) δ 8.73 (d, 2H, J = 8.6 Hz), 8.05 (s, 1H), 7.71 (d, 2H, J = 8.0 Hz), 7.33 (ddd, 2H, J = 8.3, 6.6, 1.2 Hz), 7.22 (ddd, 2H, J = 8.1, 6.6, 1.1 Hz), 2.76 (tt, 2H, J = 7.5, 2.4 Hz), 2.50 (tt, 2H, J = 7.6, 2.4 Hz), 1.80 (s, 3H), 1.55 (quint, 2H, J = 7.6 Hz); ^{13}C NMR (C_6D_6, 100 MHz) δ 186.3, 146.8, 133.3, 131.6, 129.1, 129.0, 127.9, 127.2, 127.1, 126.8, 125.9, 117.2, 96.8, 95.2, 38.5, 35.3, 22.7, 9.0; IR ($CDCl_3$) 3024, 2079, 1602, 767 cm^{-1}; UV (CH_3CN) 425.2 (ϵ 9 800), 403.6 (ϵ 10 200), 260.8 nm (e 60 400); MS *m/z* (%) 324 [(M − N_2 + H_2)$^+$] (82), 322 [(M − N_2)$^+$] (15), 295 (6), 141 (12), 137 (15), 113 (21), 97 (32), 85 (64), 71 (87), 57 (100); HRMS calcd for $C_{24}H_{20}O$ [(M − N_2 + H_2)$^+$] 324.1514, found 324.1505.

Ethyl 2-(Phenylethynyl)cyclopentenecarboxylate (13). To a mixture of **12** (1.02 g, 3.54 mmol), phenylacetylene (0.39 g, 3.82 mmol), bis(triphenylphosphine)palladium(II) chloride (0.12 g, 0.17 mmol), and 2.6-lutidine (0.75 g, 7.00 mmol) in de-aerated, anhydrous DMF (3 mL) was added cuprous iodide (78.0 mg, 0.41 mmol) and the mixture was heated at 65 °C for 5 h under nitrogen. The reaction mixture was diluted with saturated NH_4Cl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 2% ethyl acetate/hexane) to give **13** (0.83 g, 97%) as a yellow solid: mp 70.0−71.0 °C (recrystallized from hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.54−7.47 (2H), 7.36−7.31 (3H), 4.26 (q, 2H, J = 7.1 Hz), 2.75 (t, 4H, J = 7.6 Hz), 1.97 (quint, 2H, J = 7.7 Hz), 1.34 (t, 3H, J = 7.1 Hz); IR ($CHCl_3$) 3000, 1696, 1256, 1216, 750 cm^{-1}; MS *m/z* (%) 240 (M$^+$) (100), 211 (49), 195 (33), 165 (29), 152 (17). Anal. Calcd for $C_{16}H_{16}O_2$: C, 79.97; H, 6.71. Found: C, 80.13; H, 6.74.

2-(Phenylethynyl)cyclopentenecarboxylic Acid (14). To a solution of **13** (0.84 g, 3.50 mmol) in methanol (15 mL) and water (7 mL) was added sodium hydroxide (0.20 g, 5.00 mmol) and the mixture was stirred at 50 °C for 7 h. After concentration *in vacuo*, the resulting

mixture was diluted with water and washed with ethyl acetate. The water phase was acidified with 10% HCl and extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give **15** (0.69 g, 93%) as a yellow solid: mp 126.5−127.5 °C (recrystallized from hexane−ethyl acetate); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.53−7.48 (2H), 7.38−7.29 (3H), 2.79 (t, 4H, J = 7.6 Hz), 2.00 (quint, 2H, J = 7.6 Hz); IR ($CHCl_3$) 3600−2750, 1675, 1440, 1272 cm^{-1}; MS *m/z* (%) 212 (M^+) (100), 183 (14), 165 (38), 152 (19), 139 (15), 115 (14), 77 (12). Anal. Calcd for $C_{14}H_{12}O_2$: C, 79.23; H, 5.70. Found: C, 79.08; H, 5.67.

Ethyl 2-(4-Formylphenylethynyl)cyclopentenecarboxylate (15). To a mixture of **12** (9.93 g, 34.5 mmol), 4-ethynylbenzaldehyde (3.98 g, 30.6 mmol), bis(triphenylphosphine)palladium(II) chloride (1.78 g, 2.54 mmol), and 2,6-lutidine (7.36 g, 68.7 mmol) in de-aerated anhydrous DMF (10 mL) was added cuprous iodide (1.51 g, 7.93 mmol) and the mixture was stirred at ambient temperature for 1 h under nitrogen. The reaction mixture was diluted with saturated NH_4Cl and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 10% ethyl acetate/hexane) to give **15** (6.01 g, 73%) as a white solid: mp 62.5−64.5 °C (recrystallized from hexane) ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.99 (s, 1H), 7.83 (d, 2H, J = 8.3 Hz), 7.63 (d, 2H, J = 8.4 Hz), 4.25 (q, 2H, J = 7.1 Hz), 2.75 (m, 4H), 1.97 (quint, 2H, J = 7.7 Hz), 1.31 (t, 3H, J = 7.1 Hz); IR (KBr) 2970, 1688, 1597, 1024 cm^{-1}; MS *m/z* (%) 268 (M^+) (100), 240 (35), 223 (39), 211 (12), 195 (27), 165 (65), 152 (28), 139 (28), 115 (15), 81 (19), 69 (50), 55 (28); HRMS calcd for $C_{17}H_{16}O_3$ (M^+) 268.1100, found 268.1115. Anal. Calcd for $C_{17}H_{16}O_3$: C, 76.10; H, 6.01. Found: C, 75.96; H, 6.07.

2-(4-Formylphenylethynyl)cyclopentenecarboxylic Acid (16). To a solution of **15** (0.14 g, 0.52 mmol) in methanol (10 mL) and water (5 mL) was added sodium hydroxide (0.25 g, 6.25 mmol) and the mixture was stirred at 30 °C for 10 h. After concentration *in vacuo*, the residue was diluted with water and washed with ethyl acetate. The water phase was acidified with 10% HCl and then extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo* to give **16** (0.11 g, 88%) as a white solid: mp 147.0−152.0 °C (recrystallized from hexane−benzene); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.99 (s, 1H), 7.81 (d, 2H, J = 8.4 Hz), 7.63 (d, 2H, J = 8.3 Hz), 2.79 (t, 4H, J = 7.7 Hz), 2.01 (quint, 2H, J = 7.7 Hz); IR (KBr) 3250−2800, 1701, 1668, 1602 cm^{-1}; MS *m/z* (%) 240 (M^+) (100), 212 (12), 195 (10), 183 (14), 165 (18), 155 (20), 149 (15); HRMS calcd for $C_{15}H_{12}O_3$ (M^+) 240.0787, found 240.0799.

2-Diazo-1-[2-(4-formylphenylethynyl)cyclopentenyl]-1-propanone (17). According to the method detailed for **1** the reaction of **16** (79.0 mg, 0.33 mmol) gave **17** (80.0 mg, 88%) as a yellow oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 9.49 (s, 1H), 7.33 (m, 2H), 7.26 (m, 2H), 2.67 (tt, 2H, J = 7.4, 2.4), 2.37 (tt, 2H, J = 7.7, 2.4 Hz), 1.67 (s, 3H), 1.44 (quint, 2H, J = 7.7 Hz); ^{13}C NMR (C_6D_6, 50 MHz) δ 190.6, 186.1, 148.4, 136.3, 132.2, 129.6, 128.3, 125.7, 97.0, 88.6, 64.6, 38.1, 35.1, 22.1, 8.4; IR (neat) 2954, 2077, 1703, 1604, 1582 cm^{-1}; UV (CH_3CN) 309.6 (ε 19 620), 221.2 nm (ε 15 320); MS *m/z* (%) 250 [$(M - N_2)^+$] (40), 223 (21), 178 (15), 149 (100), 85 (18), 76 (20), 71 (28), 57 (40); HRMS calcd for $C_{17}H_{14}O_2$ [$(M - N_2)^+$] 250.0993, found 250.0965.

2-Diazo-1-[2-(4-(hydroxymethyl)phenylethynyl)cyclopentenyl]-1-propanone (18). To a solution of **17** (77.0 mg, 0.28 mmol) in ethanol (3 mL) was added $NaBH_4$ (3.1 mg, 82.0 μmol) at 0 °C and the mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with saturated NH_4Cl and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 15% ethyl acetate/hexane) to give **18** (73.1 mg, 93%) as a yellow oil: ^{1}H NMR (C_6D_6, 400 MHz) δ 7.43 (m, 2H), 7.00 (m, 2H), 4.16 (br, 2H), 2.71 (tt, 2H, J = 7.5, 2.3 Hz), 2.41 (tt, 2H, J = 7.6, 2.4 Hz), 1.68 (s, 3H), 1.45 (quint, 2H, J = 7.6 Hz); IR (neat) 3750−3200, 2282, 2073, 1604 cm^{-1}; UV (CH_3CN) 478.4 (ε 440), 301.2 (ε 16 340), 252.8 nm (ε 19 280); MS *m/z* (%) 252 [$(M - N_2)^+$] (100), 236 (56), 225 (80), 193 (22), 178 (45), 165 (25), 149 (19), 84 (30), 71 (20), 57 (29); HRMS calcd for $C_{17}H_{16}O_2$ [$(M - N_2)^+$] 252.1149, found 252.1099.

Ethyl 2-((Trimethylsilyl)ethynyl)cyclopentenecarboxylate (19). To a mixture of **12** (1.44 g, 5.00 mmol), bis(triphenylphosphine)-palladium(II) chloride (0.17 g, 0.24 mmol), and 2,6-lutidine (1.07 g, 9.99 mmol) in de-aerated anhydrous DMF (5 mL) were added (trimethylsilyl)acetylene (0.59 g, 6.00 mmol) and cuprous iodide (94.2 mg, 0.49 mmol) and the mixture was heated at 55 °C for 5 h under nitrogen. After dilution with saturated NH_4Cl the reaction mixture was extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 30% hexane/toluene) to give **19** (0.91 g, 77%) as a brown oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.22 (q, 2H, J = 7.2 Hz), 2.71−2.61 (4H), 1.88 (quint, 2H, J = 7.7 Hz), 1.30 (t, 3H, J = 7.2 Hz), 0.21 (s, 9H); IR (neat) 2960, 2142, 1720, 1260, 860 cm^{-1}; MS *m/z* (%) 236 (M^+) (15), 221 (20), 207 (18), 193 (19), 177 (100), 162 (39), 75 (26); HRMS calcd for $C_{13}H_{20}O_2Si$ (M^+) 236.1232, found 236.1224.

Ethyl 2-Ethynylcyclopentenecarboxylate (20). To a solution of **19** (0.43 g, 1.82 mmol) and acetic acid (0.55 g, 9.16 mmol) in THF (5 mL) was added TBAF (2.2 mL, 1.0 M in THF, 2.20 mmol) at 0 °C and the mixture was stirred at ambient temperature for 4 h. The reaction mixture was diluted with saturated $NaHCO_3$ and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 5% ethyl acetate/hexane) to give **20** (0.25 g, 84%) as a brown oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.21 (q, 2H, J = 7.1 Hz), 3.53 (s, 1H), 2.72−2.62 (4H), 1.91 (quint, 2H, J = 7.7 Hz), 1.29 (t, 3H, J = 7.1 Hz); IR (neat) 3270, 2970, 2100, 1710, 1610 cm^{-1}; MS *m/z* (%) 164 (M^+) (64), 136 (65), 119 (100), 108 (28), 91 (64), 65 (26); HRMS calcd for $C_{10}H_{12}O_2$ (M^+) 164.0837, found 164.0840.

Ethyl 2-(9-Anthrylethynyl)cyclopentenecarboxylate (21). To a mixture of 9-bromoanthracene (0.74 g, 2.88 mmol), **20** (0.21 g, 1.28 mmol), bis(triphenylphosphine)palladium(II) chloride (0.10 g, 0.14 mmol) and triphenylphosphine (0.27 g, 1.03 mmol) in de-aerated anhydrous triethylamine (30 mL) was added cuprous iodide (0.27 g, 1.42 mmol) and the mixture was heated at reflux for 12 h. The reaction mixture was cooled to room temperature and filtered through a Celite pad. The filtrate was concentrated *in vacuo* and the crude product was purified by flash chromatography (SiO_2, 20% toluene/hexane) to give **21** (0.37 g, 85%) as a yellow solid: mp 155.7−156.5 °C (recrystallized from hexane−benzene): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.68 (dd, 2H, J = 8.5 Hz), 8.44 (s, 1H), 7.99 (d, 2H, J = 8.5 Hz), 7.58 (ddd, 2H, J = 8.6, 6.5, 1.2 Hz), 7.49 (ddd, 2H, J = 8.3, 6.6, 1.2 Hz), 4.38 (q, 2H, J = 7.1 Hz), 2.99 (tt, 2H, J = 7.7, 2.5 Hz), 2.85 (tt, 2H, J = 7.5, 2.6 Hz), 2.06 (quint, 2H, J = 7.7 Hz), 1.34 (t, 3H, J = 7.1 Hz); IR ($CDCl_3$) 3094, 2187, 1693, 771 cm^{-1}; MS *m/z* (%) 340 (M^+) (100), 311 (16), 207 (10), 149 (18), 119 (14), 105 (11), 85 (13), 71 (20), 57 (33); HRMS calcd for $C_{24}H_{20}O_2$ (M^+) 340.1464, found 340.1472. Anal. Calcd for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92. Found: C, 84.39; H, 5.83.

6-{4-{[*N*-[2-(dimethylamino)ethyl]carbamoyloxy]methyl}phenyl}-4-methyl-5-indanol (23). A toluene solution (3.2 mL) of **10** (11.8 mg, 0.03 mmol) and 1,4-cyclohexadiene (25.6 mg, 0.32 mmol) was heated at 140 °C in a sealed tube for 30 min. After the solution was cooled to ambient temperature the reaction mixture was examined by RPHPLC showing the yield of **23** to be 67% with 19% of unreacted starting material **10**. The authentic **23** was prepared from **24** as follows: To a solution of **24** (46.8 mg, 0.19 mmol) in EtOH (3 mL) was added $NaBH_4$ (5.3 mg, 0.14 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 1 h. The mixture was diluted with saturated NH_4Cl and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 5% ethyl acetate/toluene) to give 6-(4-(hydroxymethyl)phenyl)-4-methyl-5-indanol (34.1 mg, 72%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.47−7.41 (4H), 6.92 (s, 1H), 5.09 (s, 1H), 4.73 (s, 2H), 2.89 (t, 2H, J = 7.2 Hz), 2.87 (t, 2H, J = 7.2 Hz), 2.21 (s, 3H), 2.09 (quint, 2H, J = 7.5 Hz), 1.76 (br, 1H); IR ($CHCl_3$) 3031, 1712, 1506, 766 cm^{-1}; MS *m/z* (%) 254 (M^+) (100), 239 (10), 224 (21), 209 (13), 147 (8), 93 (5); HRMS calcd for $C_{17}H_{18}O_2$ (M^+) 254.1307, found 254.1316. To a solution of the above alcohol (18.0 mg, 70.8 mmol) and *N,N'*-disuccinimidyl carbonate (90.2 mg, 0.35 mmol) in CH_3CN (3 mL) and CH_2Cl_2 (3 mL) was added 2,6-lutidine (14.7 mg, 0.14 mmol) at 0 °C and the mixture was stirred at ambient temperature for 24 h. To the

resulting mixture was added *N,N*-dimethylethylenediamine (13.2 mg, 0.15 mmol) and the mixture was stirred at ambient temperature for 1 h. The reaction mixture was diluted with saturated NH_4Cl and extracted with ethyl acetate. The extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 50% MeOH/ethyl acetate) to give **23** (17.8 mg, 68%) as a colorless oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.47−7.40 (4H), 6.91 (s, 1H), 5.37 (br, 1H), 5.13 (s, 2H), 3.28 (q, 2H, J = 5.8 Hz), 2.88 (t, 2H, J = 7.2 Hz), 2.86 (t, 2H, J = 7.2 Hz), 2.41 (t, 2H, J = 5.8 Hz), 2.21 (s × 2, 6H), 2.20 (s, 3H), 2.08 (quint, 2H, J = 7.6 Hz); IR ($CHCl_3$) 3559, 3025−2846, 1616, 1342, 1274 cm^{-1}; MS *m/z* (%) 368 (M^+) (22), 254 (100), 236 (17), 224 (20), 209 (11), 147 (7), 58 (100); HRMS calcd for $C_{22}H_{28}N_2O_3$ (M^+) 368.2099, found 368.2057.

6-(4-Formylphenyl)-4-methyl-5-indanol (24). A toluene solution (34 mL) of **17** (94.5 mg, 0.34 mmol) and 1,4-cyclohexadiene (0.28 g, 3.49 mmol) was heated at 140 °C in a sealed tube for 30 min. After the solution was cooled to ambient temperature and concentrated *in vacuo*, the crude product was purified by flash chromatography (SiO_2, 2% ethyl acetate/toluene) to give **24** (48.8 mg, 56%) as a yellow oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 10.04 (s, 1H), 7.95 (dt, 2H, J = 8.5, 1.8 Hz), 7.64 (dt, 2H, J = 8.1, 1.8 Hz), 6.96 (s, 1H), 4.92 (s, 1H), 2.90 (t, 2H, J = 7.6 Hz), 2.88 (t, 2H, J = 7.6 Hz), 2.22 (s, 3H), 2.10 (quint, 2H, J = 7.5 Hz); IR ($CHCl_3$) 3576, 1702, 1604, 775 cm^{-1}; MS *m/z* (%) 252 (M^+) (100), 237 (19), 147 (13); HRMS calcd for $C_{17}H_{16}O_2$ (M^+) 252.1149, found 252.1141.

Methyl 2-[2-(Phenylethynyl)cyclopentenyl]propionate (27). A methanol solution (10 mL) of **1** (25.2 mg, 0.10 mmol) was purged with nitrogen and irradiated with a transilluminator (313 nm) through a Pyrex filter. After 2 h the mixture was concentrated *in vacuo*, and the residue was purified by flash chromatography (SiO_2, 5% ethyl acetate/toluene) to give **27** (7.6 mg, 30%) as a yellow oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.45−7.41 (2H), 7.32−7.26 (3H), 3.88 (q, 1H, J = 7.4 Hz), 3.67 (s, 3H), 2.62−2.53 (2H), 2.52−2.34 (2H), 1.90 (m, 2H), 1.30 (d, 3H, J = 7.4 Hz); IR (neat) 3030, 2940, 1730, 730 cm^{-1}; MS *m/z* (%) 254 (M^+) (53), 195 (100), 179 (12), 167 (30), 91 (15), 69 (15); HRMS calcd for $C_{17}H_{18}O_2$ (M^+) 254.1306, found 254.1285.

Methyl 2-(Phenylethynyl)cyclopentenylacetate (28). The preparation of **28** was conducted from the diazo ketone **2** (0.10 g, 0.42 mmol) as detailed for **27** to give **28** (77.0 mg, 76%) as a yellow oil. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.45−7.39 (2H), 7.32−7.26 (3H), 3.68 (s, 3H), 3.40 (s, 2H), 2.58 (m, 2H), 2.51 (m, 2H), 1.93 (quint, 2H, J = 7.7 Hz); IR (neat) 2950, 2850, 1740, 750 cm^{-1}; MS *m/z* (%) 240 (M^+) (52), 181 (100), 165 (24), 57 (9); HRMS calcd for $C_{16}H_{16}O_2$ (M^+) 240.1150, found 240.1129.

1-Acetyl-3-(α-hydroxybenzyl)-4,5-cyclopentenofuran (33). (a) Under Aerobic Conditions: A solution of **1** (17.2 mg, 0.07 mmol) in CH_3CN (3.5 mL) and water (2.1 mL) in the presence of 1,4-cyclohexadiene-1,2-dimethanol (0.5 M in water, 1.4 mL, 0.69 mmol) was irradiated with a transilluminator (366 nm) through a Pyrex filter for 2 h at room temperature. After concentration of the reaction mixture, the residue was purified by flash chromatography (SiO_2, 30% ethyl acetate/hexane) to give carboxylic acid **32** (3.7 mg, 22%, conv. 39%), **33** (0.8 mg, 4.5%, conv. 8%), and recovery of **1** (7.2 mg, 42%). **(b) Under Oxygen Atmosphere:** A solution of **1** (26.1 mg, 0.10 mmol) in CH_3CN (5 mL) and water (3 mL) in the presence of 1,4-cyclohexadiene-1,2-dimethanol (0.5 M in water, 2.0 mL, 1.0 mmol) was purged with oxygen and irradiated with a transilluminator (366 nm) through a Pyrex filter. After 1.5 h of irradiation, the reaction mixture was concentrated and the residue was purified by flash chromatography (SiO_2, 30% ethyl acetate/hexane) to give **33** (5.2 mg, 20%, conv. 24%) and recovery of **1** (4.1 mg, 16%). **32:** ^1H NMR (C_6D_6, 400 MHz) δ 7.48−7.42 (2H), 7.00−6.90 (3H), 4.08 (q, 1H, J = 7.2 Hz), 2.51−2.43 (2H), 2.40 (m, 1H), 2.26 (m, 1H), 1.67−1.50 (2H), 1.21 (d, 3H, J = 7.2 Hz); IR ($CHCl_3$) 3340−2700, 1709, 1260, 767 cm^{-1}; MS *m/z* (%) 240 (M^+) (41), 212 (17), 195 (66), 178 (36), 165 (100), 152 (48), 139 (33), 128 (35), 115 (75), 102 (23), 91 (48), 77 (43), 69 (70), 57 (26); HRMS calcd for $C_{16}H_{16}O_2$ (M^+) 240.1151, found 240.1165. **33:** ^1H NMR ($CDCl_3$, 200 MHz) δ 7.41−7.25 (5H), 5.76 (br s, 1H), 2.72 (t, 2H, J = 7.2 Hz), 2.30 (s, 3H), 2.28−1.94 (4H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 186.8, 150.0, 144.4, 143.1, 140.1, 128.7, 128.3, 126.6, 69.7, 31.2, 26.3, 25.8, 23.5; IR ($CHCl_3$) 3600, 3540−3320, 3022, 1660, 1564 cm^{-1}; UV (CH_3CN) 285.6 nm (ϵ 10 020); MS *m/z* (%) 256 (M^+) (51), 238 (98), 213 [(M − Ac)$^+$] (100), 195 (40), 185 (35), 167 (31), 151 (38), 105 (36), 55 (26); HRMS calcd for $C_{16}H_{16}O_3$ (M^+) 256.1100, found 256.1146.

1-Acetyl-3-(α-acetoxybenzyl)-4,5-cyclopentenofuran (34). To a solution of **33** (3.2 mg, 12.5 mmol) in pyridine (2 mL) was added acetic anhydride (2.0 mg, 19.6 mmol) at 0 °C and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was diluted with toluene and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 20% ethyl acetate/toluene) to give **34** (3.3 mg, 89%) as a colorless oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.41−7.29 (5H), 6.79 (s, 1H), 2.83−2.76 (2H), 2.36 (s, 3H), 2.34−2.15 (4H), 2.14 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 186.7, 169.6, 145.7, 143.6, 143.3, 136.8, 134.4, 128.7, 127.1, 70.5, 31.4, 26.5, 25.9, 23.7, 21.0; IR ($CHCl_3$) 1737, 1666, 1567, 1233 cm^{-1}; MS *m/z* (%) 298 (M^+) (22), 256 (100), 238 (38), 213 (24), 195 (65), 84 (16); HRMS calcd for $C_{18}H_{18}O_4$ (M^+) 298.1206, found 298.1224.

1-Acetyl-3-[hydroxy(4-formylphenyl)methyl]-4,5-cyclopentenofuran (35). A solution of **17** (11.1 mg, 0.04 mmol) in CH_3CN (2 mL) and water (2 mL) in the presence of 1,4-cyclohexadiene-1,2-dimethanol (56.2 mg, 0.40 mmol) was purged with oxygen and irradiated with a transilluminator (366 nm) through a Pyrex filter. After 2 h of irradiation, the reaction mixture was concentrated and the residue was purified by flash chromatography (SiO_2, 20% ethyl acetate/hexane) then by preparative TLC to give **35** (0.8 mg, 7.5%, conv. 15%) and recovered **17** (3.9 mg, 35%). **35:** ^1H NMR ($CDCl_3$, 200 MHz) δ 10.01 (s, 1H), 7.88 (d, 2H, J = 8 Hz), 7.61 (d, 2H, J = 8 Hz), 5.91 (br, 1H), 2.79 (t, 2H, J = 6 Hz), 2.38 (s, 3H), 2.35−2.00 (4H); IR ($CHCl_3$) 3352, 1725, 1681, 1668, cm^{-1}; MS *m/z* (%) 284 (M^+) (5), 241 [(M − Ac)$^+$] (5), 120 (100); HRMS calcd for $C_{17}H_{16}O$ (M^+) 284.1047, found 284.1031.

[2-(Phenylethynyl)cyclopentenyl]-1,2-propanedione (36). To a dry ether solution (3 mL) of **1** (96.4 mg, 0.40 mmol) was added a solution of triphenylphosphine (144 mg, 0.55 mmol) in dry ether (1 mL) and the mixture was stirred for 5 h at ambient temperature. To the resulting mixture a THF solution (3 mL) of sodium nitrite (65.2 mg, 0.94 mmol) and 2 N HCl (0.72 mL, 1.44 mmol) was added successively at 0 °C and the mixture was stirred for 30 min at that temperature. The reaction mixture was diluted with EtOAc and washed with water. The organic extract was washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by flash chromatography (SiO_2, 4.7% ethyl acetate/hexane) to give **36** (85.4 mg, 90%) as a yellow oil: ^1H NMR (C_6D_6, 400 MHz) δ 7.50−7.47 (2H), 6.96−6.92 (3H), 2.54 (dt, 2H, J = 7.7, 2.3 Hz), 2.34 (dt, 2H, J = 7.7, 2.3 Hz), 1.35 (quint, 2H, J = 7.7 Hz); IR ($CHCl_3$) 2199, 1712, 1640, 1577, 1209 cm^{-1}; MS *m/z* (%) 238 (M^+) (4), 195 [(M − Ac)$^+$] (100); HRMS calcd for $C_{14}H_{11}O$ [(M − Ac)$^+$] 195.0810, found 195.0816.

Photoreaction of 36 Giving 33. A solution of **36** (26.4 mg, 0.11 mmol) in CH_3CN (5 mL) and water (5 mL) in the presence of 1,4-cyclohexadiene-1,2-dimethanol (154 mg, 1.1 mmol) was purged with oxygen and irradiated with a transilluminator (366 nm) through a Pyrex filter. After 1.5 h of irradiation, the reaction mixture was concentrated and the residue was purified by flash chromatography (SiO_2, 25% ethyl acetate/hexane) to give **33** (12.2 mg, 44%).

DNA-Cleaving Experiments under Photoirradiation Conditions. The solution (total volume 10 μL) containing pBR322 DNA (40 μM phosphate concentration), varying concentrations of drug as an acetonitrile solution (maximum concentration of acetonitrile is 10%), and 50 mM sodium cacodylate buffer (pH 7.0) was irradiated with a transilluminator (366 nm) at a distance of 10 cm at 0 °C for 1 h. Before loading on agarose gel, 2.5 μL of a solution containing 50% glycerol (v/v), 40 mM EDTA, and 0.05% bromophenol blue (w/v) was added. Electrophoresis with agarose gel (1.0%) containing 5 μg/mL of ethidium bromide was run at 50 V/10 cm for 2 h in 40 mM Tris-borate buffer (pH 8.4). The agarose gel was photographed under illumination of 312-nm light.

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