# Photochemical Cycloaromatization Reactions of *ortho*-Dialkynylarenes: A New Class of DNA Photocleaving Agents

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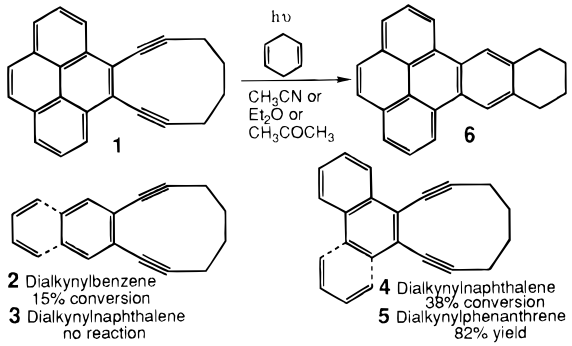
The fascinating class of enediyne-containing antitumor agents, in particular, calicheamicin $\gamma_1^I$,[1] dynemicin A, and neocarzinostatin chromophore have been the subject of extensive research efforts primarily directed toward their synthesis and the elucidation of their mechanism of action.[1] It is now widely appreciated that (1) DNA is targeted as a consequence of the various recognition domains and that (2) evolutionary pressure has ingeniously engineered these natural products to require a nucleophilic- or redox-based bioactivation step in order to trigger a facile, thermal cycloaromatization reaction leading to a lethal biradical intermediate.

We are intrigued by the possibility of consolidating the functional elements of the enediyne anticancer antibiotics, thereby simplifying the task of synthesizing potential chemotherapeutic agents which operate by DNA cleavage pathways. Thus, it was envisaged that certain polycyclic *ortho*-dialkynyl*arenes* would intercalate into DNA and, moreover, the planar $\pi$ systems might be further exploited by facilitating photochemical, as opposed to thermal, cycloaromatization/cleavage reactions.[2] We report herein[3] on the viability of this photochemical transformation and the resultant new class of DNA photocleaving agents.[4]

The dialkynylarenes **1**−**5** (Scheme 1) were prepared in order to determine whether the photochemical counterpart of the Bergman reaction was equally as effective as the thermal variant.[5] Indeed, all of the dialkynylarenes except **3**[6] underwent a photochemical cycloaromatization upon irradiation (Hanovia 450 W, Pyrex, $Et_2O$, 18 h) in the presence of 1,4-cycloheaxdiene (30 equiv). The cyclization of the dialkynylpyrene **1** to the benzpyrene **6** was the most efficient of the substrates **1**−**5** and, moreover, could be effected in sunlight ($CH_3CN$, 3 h, Pyrex)

**Scheme 1**




in quantitative yield. These transformations proceeded more slowly or not at all if lesser quantites of 1,4-cyclohexadiene (10−0 equiv) in cyclohexane were employed, a result which is consistent with a *reversible* cycloaromatization.[7] Irradiation of dialkynylpyrene **1** in THF-$d_8$ (99.5%) afforded **6** with 33% $D_2$, 37% $D_1$, and 30% $D_0$ incorporation (mass spectral analysis) and an attenuation of the intensity for the new aromatic (singlet) resonance. A strained, cyclic dialkynylarene facilitates the cycloaromatization, but is not obligate. Thus, irradiation of 9,-10-dipropynylphenanthrene afforded 1,2-dimethyltriphenylene (61% conversion) under conditions (*hν*, dioxane, cyclohexadiene, Pyrex, 18 h) similar to those shown for the cyclization of **5**.[8] However, the photochemical cycloaromatizations of the terminal acetylenic compounds, 9,10-diethynylphenanthrene and 4,5-diethynylpyrene, were unsuccessful. Finally, the benzylidene acetal of dialkynylphenanthrene **10**, which possesses a conformationally locked 10-membered ring, undergoes a smooth photochemical cycloaromatization (*hν*, 18 h, 30 equiv 1,4-cyclohexadiene, acetone, 47%) whereas the rate of the analogous thermal cycloaromatization is markedly attenuated (190 °C, 17 h, 69% conversion) relative to the rate of thermal cyclization of **5** (98 °C, $t_{1/2}$, 5 h).

We next considered the preparation of a water soluble dialkynylphenanthrene and/or dialkynylpyrene in order to examine the DNA photocleaving properties of these compounds.

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(1) For reviews, see: (a) Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387. (b) Nicolaou, K. C.; Smith, A. L. *Acc. Chem. Res.* **1992**, *25*, 497. (c) Maier, M. E. *Synlett* **1995**, 13.

(2) DNA cleavage by *bona fide* photocycloaromatization reactions have not yet been reported. However, for DNA cleavage by enediyne and related compounds by photochemically triggered, thermal cycloaromatization reactions, see: (a) Nicolaou, K. C.; Dai, W. M.; Wendeborn, S. V.; Smith, A. L.; Torisawa, Y.; Maligres, P.; Hwang, C. K. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1032. (b) Wender, P. A.; Zercher, C. K.; Beckham, S.; Haubold, E.-M. *J. Org. Chem.* **1993**, *58*, 5867. (c) Wender, P. A.; Beckham, S.; O'Leary, J. G. *Synthesis* **1994**, 1278. (d) Nakatani, K.; Isoe, S.; Maekawa, S.; Saito, I. *Tetrahedron Lett.* **1994**, *35*, 605. DNA photocleavage by dynemicin A involves an initial photoreduction step, see: (e) Shiraki, T.; Sugiura, Y. *Biochemistry* **1990**, *29*, 9795. DNA photocleavage by esperamicin and neocarzinostatin chromophore has also been reported, see: (f) Sugiura, Y.; Kuwahara, J.; Vesawa, Y. *Biochem. Biophys. Res. Comm.* **1989**, *164*, 903. The latter undergoes a Norrisch Type II cleavage to produce a fulvene derivative which may be responsible for the DNA cleavage, see: (g) Hirama, M.; Nehira, T.; Fujiwara, K.; Gomibuche, T. *Tetrahedron Lett.* **1993**, *34*, 5753.

(3) This and related work was presented at the following: 204th National ACS Meeting Washington, D.C., August, 1992. 33rd National Organic Chemistry Symposium Bozeman, MO, June, 1993. 34th National Organic Chemistry Symposium Williamsburg, VA, June, 1995.

(4) Selected examples of DNA photocleaving agents include the following. Radical precursors: (a) Hecht, S. M.; Levy, M. J.; Quada, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 12171. (b) Little, R. D.; Groppe, J.; Bregant, T. M. *Ibid.* **1994**, *116*, 3635. (c) Saito, I.; Sakurai, T.; Kurimoto, T.; Takayama, M. *Tetrahedron Lett.* **1994**, *35*, 4797 and references therein. Poly(hetero)cyclic aromatic compounds: (d) Perrouault, L.; Asseline, U.; Rivalle, C.; Thuong, N. T.; Bisagni, E.; Giovannangeli, C.; Le Doan, T.; Helene, C. *Nature* **1990**, *344*, 358. (e) Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918. (f) Schuster, G. B.; Armitage, B.; Yu, C.; Devadoss, C. *Ibid.* **1994**, *116*, 9847. (g) Saito, I.; Takayama, M.; Kawanishi, S. *Ibid.* **1995**, *117*, 5590 and references therein. Porphyrins: (h) Magda, D.; Wright, M.; Miller, R. A.; Sessler, J. L.; Sansom, P. I. *Ibid.* **1995**, *117*, 3629 and references therein. Metal Complexes: (i) Nielsen, P. E.; Hiort, C.; Sonnichsen, S. H.; Buchardt, O.; Dahl, O.; Norden, B. *Ibid.* **1992**, *114*, 4967. (j) Mascharak, P. K.; Farinas, E.; Tan, J. D.; Baidya, N. *Ibid.* **1993**, *115*, 2996. (k) Barton, J. K.; Sitlani, A.; Dupureur, D. M. *Ibid.* **1993**, *115*, 12589 and references therein. (l) Riordan, C. G.; Wei, P. *Ibid.* **1994**, *116*, 2189.

(5) The preparation and thermal cycloaromatization reactions of dialkynylarenes **1**−**5** will be reported separately. We thank Kay M. Brummond and Kim S. Para for the initial preparation of **2**, **3**, and **4**, **5**, respectively. All new compounds reported herein exhibit satisfactory spectral (IR, UV, NMR, HRMS) characteristics.

(6) Semiempirical calculations (PM3) show that the coefficients and nodal properties of the HOMO/LUMO's for **2**, **4**, and **5** are significantly different than those for **3**. Arene analogs of stilbenes also show this contrasting photochemical behavior. For a theoretical explanation, see: Tinnemans, A. H. A.; Laarhoven, W. H.; Sharafi-Ozeri, S.; Muszkat, K. A. *Recl. Trav. Chim. Pays-Bas* **1975**, *94*, 239.

(7) For related observations in thermal cycloaromatizations, see: (a) Semmelhack, M. F.; Neu, T.; Foubelo, F. *J. Org. Chem.* **1994**, *59*, 5038. (b) Yoshida, K.-i.; Minami, Y.; Otani, T.; Tada, Y.; Hirama, M. *Tetrahedron Lett.* **1994**, *35*, 5253.

(8) The photochemical cycloaromatization of 1,2-di(1-pentynyl)benzene has recently been reported to proceed in low yield (10−40%), see: Turro, J. J.; Evenzahav, A.; Nicolaou, K. C. *Tetrahedron Lett.* **1994**, *35*, 8089.

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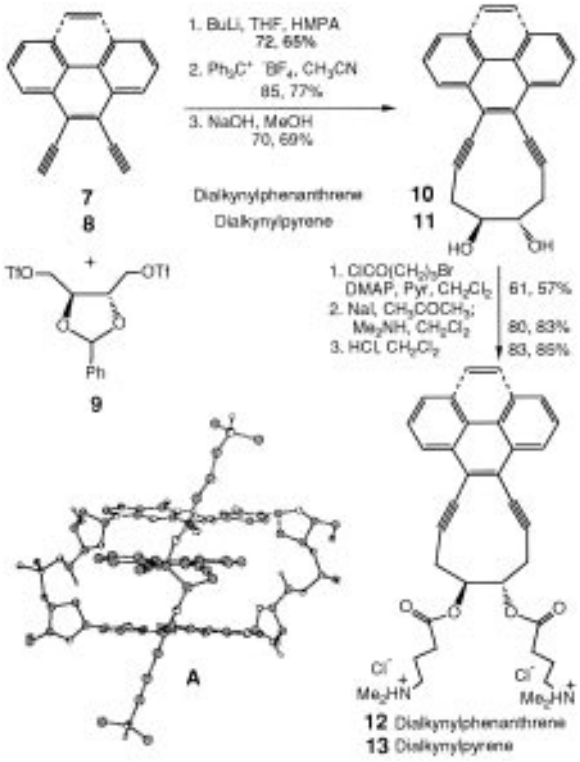
**Scheme 2**




The attachment of water-solubilizing side chains at the homopropargylic positions of **5** and **1**, e.g., the (*S*,*S*) enantiomers **12** and **13** (Scheme 2), respectively, seemed ideal for this purpose. As illustrated in the model **A**, it is feasible that these compounds would, upon binding to DNA, prefer to adopt the psuedodiaxial conformers and thereby place the polar, positively-charged appendages in the minor groove since they possess the correct orientation (approximately a 70° angle relative to the plane of the aromatic ring system) and curvature to complement the right-handed helical twist of B-DNA. To that end, the dilithio derivatives of **7** and **8** underwent facile cyclization reactions upon treatment with the homochiral ditriflate **9**.[9] The resulting benzylidine acetals could not be hydrolyzed by standard protocols and required treatment with trityl fluoroborate[9] to afford an intermediate hydroxy benzoate which was saponified to afford the desired diols **10** and **11**. The water-solubilizing side chains were then introduced in a straightforward manner by esterification of **10** and **11** with 4-bromobutyryl chloride and subsequent transformation of the resulting 4-bromobutyrate esters to 4-(dimethylamino)butyrate esters which were converted to the corresponding hydrochloride salts **12** and **13**, respectively.

DNA photocleavage by dialkynylarenes **12** and **13** was investigated using supercoiled plasmid pUC19 DNA (38 $\mu$M in base pairs) in Pyrex reaction vessels with a Hanovia 450 W light source. As shown in Figure 1, both dialkynylarenes **12** and **13** give rise to predominantly single-strand breaks (conversion to form II DNA) upon irradiation (lanes 6−13) but are ineffective in the dark (lanes 4 and 5). However, the dialkynylpyrene **13** is more effective in this regard since DNA cleavage could be accomplished at a lower concentration (2 $\mu$M, lane 6 vs lane 7) and rapidly consumed the DNA at 20 $\mu$M

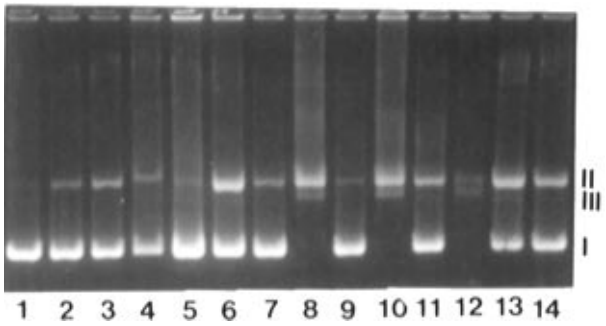



**Figure 1.** Photocleavage of supercoiled plasmid pUC19 DNA by dialkynylarenes **12** and **13**. Reaction mixtures were prepared by addition of appropriate stock solutions to a total volumne of 10 $\mu$L containing .25 $\mu$g of plasmid DNA buffered to pH 8 with 10 mM Tris and 1 mM EDTA. Irradiations were performed through a Pyrex filter at room temperature with a Hanovia 450 W light source. The mixtures were analyzed on a 1.2% agarose gel at 80 V for approximately 1.5 h. Lane 1: DNA, dark. Lane 2: DNA, *hν*, 60 min. Lane 3: 20 $\mu$M water soluble dialkylphenanthrene,[12] *hν*, 60 min. Lane 4: 20 $\mu$M **13**, dark, 60 min. Lane 5: 20 $\mu$M **12**, dark, 60 min. Lane 6: 2 $\mu$M **13**, *hν*, 60 min. Lane 7: 2 $\mu$M **12**, *hν*, 60 min. Lanes 8, 10, 12: 20 $\mu$M **13**, *hν*, 15, 30, 60 min, respectively. Lanes 9, 11, 13: 20 $\mu$M **12**, *hν*, 15, 30, 60 min, respectively. Lane 14: 20 $\mu$M **12**, 40 $\mu$M water soluble dialkylphenanthrene,[10] *hν*, 10 min.

concentration producing both form II and form III cleavage products (compare lanes 8, 10, and 12 to 9, 11, and 13) which presumably reflects either the superior binding affinity (better intercalator) and/or the more efficient photochemistry of dialkynylpyrene **13**. Moreover, the negligible photocleavage of DNA with a water soluble *dialkyl*phenanthrene[10] (lane 3) demonstrates that alkynyl substituted arenes are required for efficient photocleavage. (A similar result was obtained from the DNA photocleavage by the hydrochloride salt of 4,5-bis-[5-(dimethylamino)-1-pentynyl]pyrene *vis-a-vis* the hydrochloride salt of 4,5-bis[4-(dimethylamino)butyl]pyrene, see supporting information.) Finally, attenuation of DNA photocleavage by dialkynylphenanthrene **12** in the presence of a water soluble dialkylphenanthrene[10] was observed (lane 13 vs lane 14) which suggests that cleavage arises from bound (intercalated) **13**.

The new DNA photocleaving agents reported herein are novel in that the binding and photoactive domains are *consolidated*[11] and, moreover, generate two reactive sites for subsequent ribose hydrogen atom abstraction(s). Efforts are currently underway to improve upon the double-stranded to single-stranded cleavage ratio for this new class of DNA photocleaving agents.

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**Supporting Information Available:** Spectroscopic data and experimental details for the preparation of compounds **10**−**13** and a gel which provides a comparison of the DNA photocleavage by a dialkynylpyrene and a dialkylpyrene (see text) (6 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(9) For the cyclization of the ditosylate with the dilithio salt of 1,2-diethynylcyclohexene, see: Masamune, S.; Takada, S.; Shelton, K. W.; Salaun, J. A.; Kim, C. U.; Darby, N. *J. Chem. Soc., Chem. Commun.* **1971**, 1516.

(10) The water soluble dialkylphenanthrene was prepared by subjecting 9,10-di(4-hydroxybutyl)phenanthrene to the same three-step sequence used to convert **10**/**11** to **12**/**13**.

(11) Most DNA-cleaving agents possess separate binding and photoactive domains, see ref 4.