New Class of DNA-Cleaving Agents Based on Trimethylenemethane[1]

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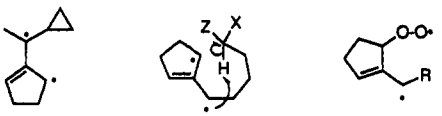
A common mode of action for the highly reactive σ-diyls derived from the enediyne class of antitumor antibiotics involves hydrogen atom abstraction from the sugar–phosphate backbone of DNA.[2] Abstraction triggers a series of events that ultimately leads to cleavage of the DNA and cell death. In contrast, the soft, π-diyls related to trimethylenemethane (TMM), while known for their ability to undergo cycloaddition to electron deficient alkenes,[3] are expected to be much less reactive toward hydrogen atom abstraction.[4] Such an event would require a loss in delocalization energy attending the conversion of a planar, four-π-electron triplet diyl, such as **1**, to an allyl radical.[4] In contrast, the short-lived bisected singlet **2** should be more reactive since one of the four electrons resides in a p-orbital which is orthogonal to the allyl unit.

1 2

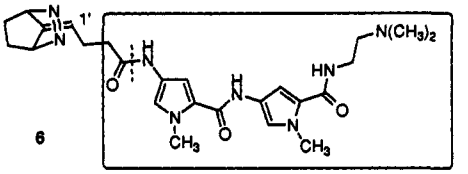
Given this uncertainty, one wonders whether a TMM diyl in any of its forms is reactive enough to cleave DNA in a manner reminiscent of the natural products. It is interesting to note that TMM, in spite of its lower reactivity toward hydrogen transfer, is capable of this reaction when special circumstances make it competitive with reactions such as diyl dimerization. For example, Adam and Finzel have shown that in the presence of 0.1 M cyclohexadiene, a good hydrogen atom donor, products resulting from intermolecular hydrogen abstraction are formed from diyl **3** (*ca.* 28%).[5] We have also shown intramolecular hydrogen transfer to be an efficient process in certain cases analogous to that illustrated in structure **4**.[6] Thus, it is not unreasonable to assume that if hydrogen abstraction is made competitive, TMM diyls may be capable of initiating DNA cleavage in this manner. In addition, triplet TMM diyls are known to react with molecular dioxygen;[7] the more reactive oxygen-centered radical **5** might engage in hydrogen atom abstraction from the sugar–phosphate backbone, thereby initiating the cleavage event.



3 4 5

If the diyl precursor were bound to the DNA, hydrogen abstraction or other pathways leading to cleavage might become kinetically accessible. In other words, the newly created diyl or the corresponding oxygen-centered radical (i) would have access to reactive sites on the double helix and (ii) would be physically separated from other diyls,[8] thereby suppressing the main side reaction possible, dimerization. Furthermore, selective binding to the double helix would allow one to map the intensities and sites of DNA cleavage relative to the expected binding sites.

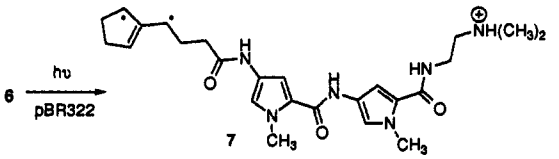
We chose the binding unit highlighted in **6**, since it resembles the known minor groove binding agent distamycin.[9] Each of the three amide N–H bonds is available for hydrogen bonding to AT base pairs, with a binding capacity of four base pairs. The terminal



N,N-dimethylamino group, when protonated, facilitates access to the negatively charged environment of the DNA double helix. Two methylene units were inserted between the diazene and pyrrole subunits to allow the molecule flexibility in reaching reactive sites in the minor groove. Diazene **6** was synthesized in a convergent manner by coupling the diazene and dipyrrole halves at the location indicated by the dashed line.[10]

Diyl generation from diazenes such as **6** can be accomplished either thermally or photochemically.[3,4] The temperatures required for the thermally initiated process vary markedly, ranging from *ca.* −20 °C to refluxing acetonitrile, depending upon the nature of the substituents appended to C1′ of a given diazene (numbering shown in structure **6**).[3,4] For compound **6**, 75 °C was selected since it promised to deliver the diyl at a convenient rate. Not unexpectedly, however, this temperature proved too high to allow meaningful experiments to be conducted in the presence of DNA.

In contrast, photogeneration of the diyl **7** in the presence of 517 and 167 base pair restriction fragments of 5′-^{32}P end-labeled pBR322 provided clear and unambiguous evidence indicating that TMM diyls are indeed capable of cutting DNA.



An autoradiogram illustrating the results obtained from a Pyrex-filtered irradiation conducted for 3 h at room temperature using the 517 base pair fragment is shown in Figure 1.[11] Notice particularly lane 2. It portrays the outcome of the important control experiment which shows that the restriction fragment is not affected when irradiated in the absence of compound **6**. Lane

(1) Preliminary account: Bregant, T. M.; Groppe, J.; Little, R. D. *Abstracts of Papers*, 206th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society; Washington, DC, 1993; ORGN 414.

(2) (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) Lee, M. D.; Ellestad, G. A.; Borders, D. B. *Acc. Chem. Res.* **1991**, *24*, 235. (d) Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311.

(3) (a) Little, R. D.; Masjedizadeh, M. R.; Moeller, K. D.; Dannecker-Doerig, I. *Synlett* **1992**, 107. (b) Masjedizadeh, M. R.; Dannecker-Doerig, I.; Little, R. D. *J. Org. Chem.* **1990**, *55*, 2742.

(4) Berson, J. A. In *Diradicals*; Borden, W. T., Ed.; Wiley: New York, 1982; Chapter 4.

(5) Adam, W.; Finzel, R. *J. Am. Chem. Soc.* **1992**, *114*, 4563.

(6) Little, R. D.; Billera, C. F. *Abstracts of Papers*, 205th National Meeting of the American Chemical Society, Denver, CO; American Chemical Society: Washington, DC, 1993; ORGN 103. A report detailing these findings has been submitted to *J. Am. Chem. Soc.*

(7) Little, R. D.; Losinski-Dang, L.; Venegas, M. G.; Merlic, C. *Tetrahedron Lett.* **1983**, *24*, 4499.

(8) Both distamycin and netropsin are known to bind two drugs per binding site,[9] but this binding is *antiparallel*. If this form of binding takes place with **6**, it should keep the two diyls away from each other. Finally, the initial concentration of diazene in solution is much lower than that associated with the typical conditions of diyl trapping.[3,4]

(9) For example, see: Pelton, J. G.; Wemmer, D. E. *J. Am. Chem. Soc.* **1990**, *112*, 1393.

(10) Complete synthetic details will be provided in our full paper. ^{1}H NMR (diazene **6**, $CDCl_3$, 200 MHz): δ 7.93 (s, 1H, CON*H*-pyrrole), 7.66 (s, 1H, pyrrole-CON*H*-pyrrole), 7.13, 7.09, 6.55, 6.39 (4d, 4H, pyrrole-*H*), 6.73 (t, 1H, pyrrole-CON*H*-alkyl), 5.41 (s, 1H, bridgehead), 5.14 (t, 1H, $R_2C{=}CRH$), 5.09 (d, 1H, bridgehead), 3.85 (s, 6H, pyrrole NCH_3), 3.44 (apparent quartet, 2H, $CONHCH_2$), 2.47 (t, 2H, CH_2NMe_2), 2.34 (m, 4H, $R_2C{=}CHCH_2CH_2$), 2.24 [s, 6H, $N(CH_3)_2$], 1.6, 1.1 (2d, 4H, C_5–C_6 bridge H's of diazene).

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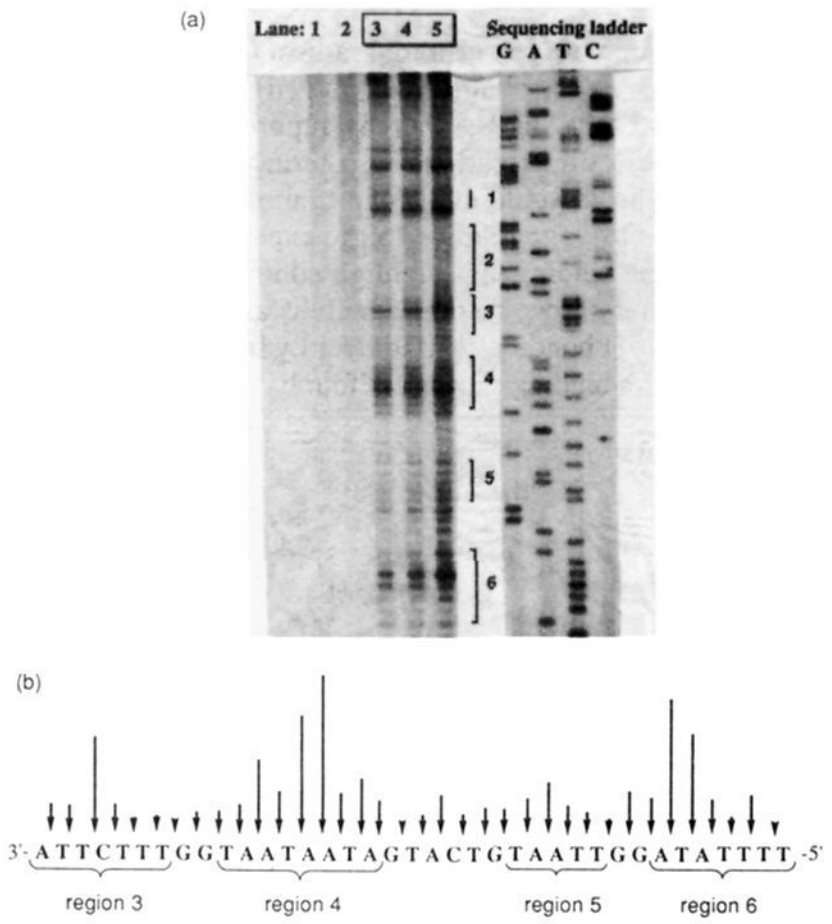

Figure 1. (a) Autoradiogram: "region 1", 3′-TTT-5′; "region 2", 3′-GGTGGACTGCAG-5′. (b) Densitometry analysis for run conducted at 25 μM **6**. The length of each arrow is proportional to the intensity of the band illustrated in the autoradiogram.

1 was loaded with a restriction fragment that had been stored at −20 °C and was not treated with the test compound, **6**. Lanes 3–5 illustrate the effect of increasing the concentration of diazene (25, 50, and 100 μM **6**, respectively, in a solution of 50 mM NaCl and 10 mM Tris-HCl at a pH of 8.9). While not illustrated, concentrations of diazene **6** as low as 5 μM afforded cleavage.

The location of diyl-induced cuts was determined by reading across the gel to the sequencing ladder, which was constructed using the Sanger dideoxy method.[12] As expected for a cutter bound to a distamycin analog, the cleavage intensities are highest in AT-rich regions of the DNA. Note that there is very little damage in the sequence labeled "region 2", it being a portion of the restriction fragment which is devoid of distamycin binding sites. This is evidence that DNA cutting takes place while diyl **7** is bound to the nucleic acid in a manner similar to distamycin.[13]

An analysis of the cutting pattern illustrated in Figure 1b may also provide information concerning the *direction* of binding. Notice, for example, the appreciable cut at the 5′-end of the 3′-ATT sequence of "region 3". That there is no such cut at the opposing 3′-end implies directionality in the binding of diazene **6**. At first glance, it seems that this cut could be attributed to the molecule binding to the 3′-TTT sequence of the same region. It is unlikely, however, given the short tether length between the diyl precursor and the binding unit, that **7** is capable of reaching sites that would lead to cutting between base pairs located beyond the perimeter of the binding site (for example, at the large arrow located between T and C of region 3, when bound to 3′-TTT). This idea is supported by computer modeling.[14]

As shown in Figure 1b, other potential binding sequences are quite long, thereby allowing the diyl/diazene more freedom of movement. This leads to a decrease in the cutting selectivity. Notice, however, how the cutting patterns differ significantly from the symmetrical arrangement which is associated with a diffusible, oxygen-centered radical.[15] The cuts in regions 4 and 6 tend to favor one side of the binding region or the other, also implying directionality to the binding.[16]

What is the mechanism associated with cleavage? At this time, we do not have evidence favoring *any* particular mechanism, nor do we wish to convey the impression that hydrogen atom abstraction constitutes the only pathway by which cleavage can occur.[17] We are currently focusing attention on efforts to uncover mechanistic detail and intend to report the results when they become available.

Acknowledgment. We are grateful to the Elsa U. Pardee Foundation and the National Institutes of Health for their support of this research. We wish to express our gratitude to Professors T. C. Bruice and N. Reich of the UCSB Chemistry Department for allowing us to use several key pieces of equipment. We are grateful to Mark Surby and Karen Maegley for their patient and much needed assistance. Finally, we thank the reviewers for their constructive criticism.

Supplementary Material Available: ^{1}H NMR spectrum for diazene **6** (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(11) In an assay testing for single- *vs* double-stranded cutting, **6** (100, 50, and 10 μM) was irradiated in the presence of form I (supercoiled) ΦX174 plasmid DNA. In the 100 and 50 μM runs, both form II (closed-circular) and form III (linear) were formed after 3 h of irradiation, as evidenced by agarose gel electrophoresis. The 10 μM reaction afforded form II only. This data suggests single-stranded cutting. At higher concentrations, separate cleavage events are thought to be responsible for the conversion to form III. To show that the diazene itself has no cutting activity, form I ΦX174 was treated, in the dark, with 100 μM **6**. After 3 h, no reaction was evident by gel electrophoresis.

(12) Adams, R. L. P.; Knowler, J. T.; Leader, D. P. *The Biochemistry of the Nucleic Acids*, 10th ed; Chapman & Hall: London, 1986; pp 473–476.

(13) Preliminary footprinting data for compound **6** in the presence of plasmid shows significant protection from DNase cleavage in those areas of the plasmid known to bind distamycin.

(14) Hyperchem software; Silicon Graphics computer; Dickerson dodecamer; diazene **6**.

(15) For example, see: Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457.

(16) We do not have a good explanation for the relatively small amount of cutting observed in region 5.

(17) Little, R. D.; Brown, L. M.; Masjedizadeh, M. R. *J. Am. Chem. Soc.* **1992**, *114*, 3071. Nicolaou suggested that a tetramethyleneethane (TME) diyl might be capable of cutting DNA. This notion was discarded in favor of a dipolar pathway. See ref 2a and the following: Nicolaou, K. C.; Skokotas, G.; Maligres, P.; Zuccarella, G.; Schweiger, E. J.; Toshima, K.; Wendeborn, S. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1272.