Toward Enediyne Mimics: Methanolysis of Azoesters and a Bisazoester

Veeraraghavan Srinivasan, David J. Jebaratnam,† and David E. Budil*

Department of Chemistry, Northeastern University, Boston, Massachusetts 02115

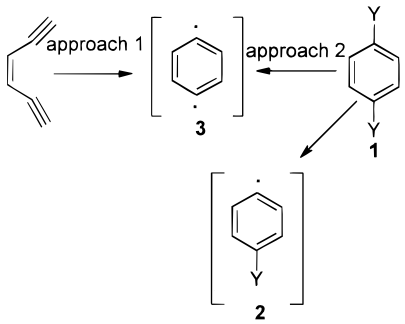
Received May 5, 1999

Enediyne anticancer antibiotics have attracted tremendous interest in the past decade. The inherent difficulty in synthesizing these structurally complex natural products with the strained enediyne moiety has motivated a search for simpler molecules that mimic enediyne chemistry. The ultimate objective is to identify molecules that produce 1,4-benzenoid diradicals, which are known to induce DNA cleavage in the natural products. Toward this goal, several aromatic azoesters have been synthesized, and EPR reveals the presence of radical intermediates in their methanolysis. A 1,4-bisazoester has also been synthesized, and its methanolysis products have been studied by reversed-phase HPLC. The formation of 1,2-dicyanobenzene from the 1,4-bisazoester is consistent with the existence of a 1,4-diradical intermediate.

Introduction

With the discovery of neocarzinostatin in 1985,[1] DNA cleavage by the so-called "enediyne" natural products has emerged as an important research area.[2] In addition to impressive advances in the total synthesis of these natural products,[3] many structurally simpler enediyne analogues capable of generating 1,4-benzenoid diradicals and hence cleaving DNA under physiological conditions have also been reported.[4] A few years ago, intrigued by the fact that most attempts to generate 1,4-benzenoid diradicals have relied on enediyne precursors (Scheme 1, approach 1),[5] we initiated research on a nonenediyne strategy (Scheme 1, approach 2). Our work has identified several new classes of compounds,[6−9] including azoesters that cleave DNA under physiologically relevant conditions.[7,8]

Scheme 1



One question that remains to be answered is the extent to which such compounds actually do mimic the mechanism of DNA cleavage by enediynes. Kosower's observation that azoesters cause membrane damage in cells that is attributable to phenyl radicals[10] has led us to speculate that radical intermediates might also be involved in the observed cleavage of DNA by azoesters. However, we have obtained no direct, independent evidence for the existence of radical intermediates in the DNA-cleavage reaction.

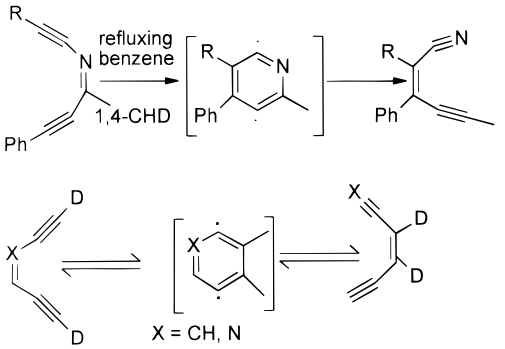
Even if radicals are involved in azoester cleavage of DNA, the probability is very low for two C−Y bonds (cf. Scheme 1) to break simultaneously. In other words, compounds represented by **1** (Scheme 1) are presumed to generate monoradicals **2** first, rather than generating the diradicals **3** directly; thus, they are not strictly analogous to enediyne sytems, which generate 1,4-benzenoid diradicals in a concerted fashion. On the other hand, DNA cleavage itself is almost certainly not a concerted reaction and should not depend on whether the 1,4 benzenoid diradical involved was generated via a concerted mechanism. Therefore, it should be possible to mimic the action of enediynes by generating a diradical through sequential formation of two radical sites. This

* To whom correspondence should be addressed. Phone: (617) 373-2369. Fax: (617) 373-8795. E-mail: dbudil@neu.edu.
† Original principal investigator.

(1) Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331.
(2) For recent reviews, see: (a) Borders, D. B., Doyle, T. W., Eds. *Enediyne Antibiotics As Antitumor Agents*; Marcel Dekker Inc.: New York, 1995. (b) Sugiyama, H.; Yamashita, K.; Fujiwara, T.; Saito, I. *Tetrahedron* **1994**, *50*, 1311.
(3) (a) Danishefsky, S. J.; Shair, M. D. *J. Org. Chem.* **1996**, *61*, 16. (b) Myers, A. G.; Fraley, M. E.; Tom, N. J.; Cohen, S. B.; Madar, D. J. *J. Chem. Biol.* **1995**, *2*, 33. (c) Hitchcock, S. A.; Boyer, S. H.; Chu-Moyer, M. Y.; Olson, S. H.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 858. (d) Nicolaou, K. C.; Hummel, C. W.; Pitsinos, E. N.; Nakada, M.; Smith, A. L.; Shibayama, K.; Saimoto, H. *J. Am. Chem. Soc.* **1992**, *114*, 8082. (e) Nicolaou, K. C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1377.
(4) For some notable examples, see: (a) Nicolaou, K. C.; Dai, W. M.; Tsay, S. C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172. *(b)* Semmelhack, M. F.; Gallagher, J. J.; Ding, W. D.; Krishnamurthy, G.; Babine, R.; Ellestad, G. A. *J. Org. Chem.* **1994**, *59*, 4357.
(5) For some recent exceptions. see: (a) Wender, P. A.; Touami, S. M.; Alayarc, C.; Philipp, U. C. *J. Am. Chem. Soc.* **1996**, *118*, 6522. (b) Bregant, T. M.; Groppe, J.; Little, R. D. *J. Am. Chem. Soc.* **1994**, *116*, 3635. (c) Sullivan, R. W.; Coghlan, V. M.; Maunk, S. A.; Reed, M. W.; Moore, H. W. *J. Org. Chem.* **1994**, *59*, 2276. (d) Griffiths, J.; Murphy, J. A. *J. Chem. Soc., Chem. Commun.* **1992**, 24. (e) Schottelius, M. J.; Chen, P. *J. Am. Chem. Soc.* **1996**, *118*, 4896.
(6) Arya, D. P.; Warner, P. W.; Jebaratnam, D. J. *Tetrahedron Lett.* **1993**, *34*, 7823.
(7) Jebaratnam, D. J.; Arya, D. P.; Chen, H.; Kugabalasooriar, S.; Vo, D. *BioMed. Chem. Lett.* **1995**, *5*, 1191.
(8) Jebaratnam, D. J.; Kugabalasooriar, S.; Chen, H.; Arya, D. P. *Tetrahedron Lett.* **1995**, *36*, 3123.
(9) Jebaratnam, D. J.; Arya, D. P. *Tetrahedron Lett.* **1995**, *36*, 4369.
(10) (a) Huang, P. C.; Kosower, E. M. *J. Am. Chem. Soc.* **1968**, *90*, 2354. (b) Huang, P. C.; Kosower, E. M. *J. Am. Chem.* Soc. **1968**, *90*, 2362. (c) Huang, P. C.; Kosower, E. M. *J. Am. Chem. Soc.* **1968**, *90*, 2367. Wax, R.; Rosenberg, E.; Kosower, N. S.; Kosower, E. M. *J. Bacteriol.* **1970**, *81*, 892. Huang, P. C.; Kosower, E. M. *Biochim. Biophys. Acta* **1968**,*165*, 483.

10.1021/jo990750v CCC: $18.00
Published on Web 07/09/1999

Scheme 2



mechanism would require the first radical formed to be relatively long-lived.

A strategy for generating diradicals in this way is suggested by recent investigations of the heteroatom version of the Bergman[11] cyclization. In particular, Chen[12] has observed that radicals and diradicals generated from nitrogen-containing heterocycles abstract hydrogen more slowly and hence may be more selective in DNA cleavage. The possibility that ring nitrogens could exert a stabilizing influence leading to longer radical lifetimes has stimulated our interest in heteroaromatic azoesters as potential enediyne mimics.

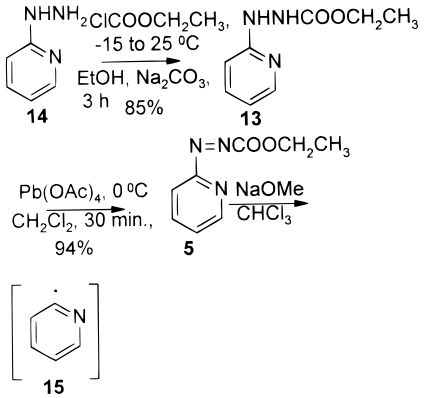
In this paper, we describe the synthesis of a new series of compounds aimed at clarifying our understanding of DNA cleavage by azoesters. The ultimate goal of this work is to investigate whether radical intermediates play an essential role in DNA cleavage. On the basis of earlier findings,[10c] we started with phenyl azoester **4** to verify its ability to generate aryl radicals upon methanolysis in chloroform.[10c] To investigate the effect of ring nitrogens on radical stability as proposed by Chen,[12] we also synthesized and methanolyzed the azoesters of pyridine **5** and pyrimidine **6**.

Finally, a 1,4-bisazoester **7** (Y = azoester in Scheme 1, approach 2) was synthesized in an effort to generate a 1,4-diradical from a precursor other than an enediyne. We find evidence from EPR that radicals are indeed formed by methanolysis of each of the monoazoesters **4**−**6**. Although no EPR signal was observed from methanolysis of the bisazoester **7**, the reaction mixture was analyzed by HPLC for possible degradation products of a diradical. Diradicals similar to **8** have been proposed as intermediates in cycloaromatization reactions, as shown in Scheme 2.[13] Evidence suggests that they are quite unstable and readily undergo retro-Bergman cyclization to form the corresponding nitriles in very high yields.[13a−c] The analogous decomposition product for the diradical that would be formed by methanolysis of **7** is 1,2-dicyanobenzene **9,** which is found in the reaction mixture in 25% yield. Taken together, these results suggest that a 1,4-diradical may be formed in significant amounts by methanolysis of the bisazoester.

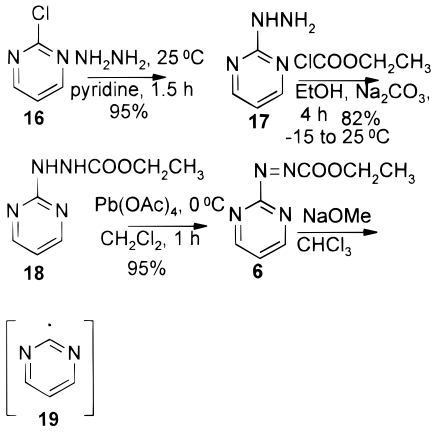
Results and Discussion

Phenyl azoester **4** was synthesized in two steps from the corresponding hydrazine **10** following the published

Scheme 3



Scheme 4



procedure.[10] The basic methanolysis of the azoester was carried out in an EPR tube, and the EPR spectrum was recorded immediately at 200 K. The pyridine and pyrimidine azoesters **5** and **6** were synthesized from readily available starting materials in very high yields (Schemes 3 and 4), and their methanolyses were carried out in an analogous fashion.

Parts a−c of Figure 1 show the EPR spectra obtained after methanolysis of phenylazoester **4**, pyridyl-2-azoester **5,** and pyrimidyl-2-azoester **6**, respectively. By analogy with the mechanism proposed by Huang and Kosower,[10c] these reactions should produce sp^2 radicals of six-membered aromatic rings, respectively, containing 0, 1, and 2 nitrogens adjacent to the radical site. However, we found that these spectra were not directly comparable to published spectra of the phenyl and pyridyl radicals.

EPR spectra of phenyl and 2-pyridyl free radicals formed in matrixes at 4 K do allow assignments of the hyperfine constants of the ring hydrogens.[14,15] The spectrum of the 2-pyridyl radical further exhibits the hyperfine interaction with the ring nitrogen, which produces an overall triplet with a splitting of approximately 27 G. The two equivalent ring nitrogens of the 2-pyrimidyl radical should presumably produce a 1:2:3:2:1 quintet with a splitting comparable to that of the 2-pyridyl radicals, although we are unaware of any published spectrum of the 2-pyrimidyl radical.

The spectra shown in Figure 1 are not comparable to published low-temperature spectra for a number of

(11) Bergmann, R. *G. Acc. Chem. Res.* **1973**, *6*, 625.
(12) (a) (a) Logan, C. F.; Chen, P. *J. Am. Chem. Soc.* **1996**, *118*, 2313. (b) Chen, P. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1478.
(13) (a) Shi, C.; Wang, K. K. *J. Org. Chem.* **1998**, *63*, 3517. (b) David, W. M.; Kerwin, S. M. *J. Am. Chem. Soc.* **1997**, *119*, 1464. (c) Nam, H. H.; Leroi, G. E.; Harrison, J. F. *J. Phys. Chem.* **1991**, *95*, 6514.
(14) Kasai, P. H.; Hedaya, E.; Whipple, E. B. *J. Am. Chem. Soc.* **1969**, *91*, 4364.
(15) Kasai, P. H.; McLeod, D., Jr. *J. Am. Chem. Soc.* **1972**, *94*, 720.

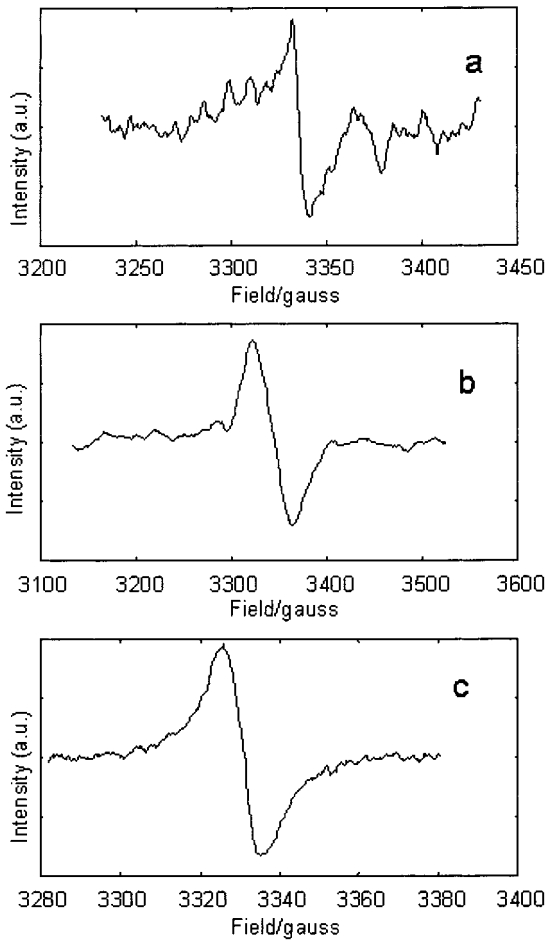


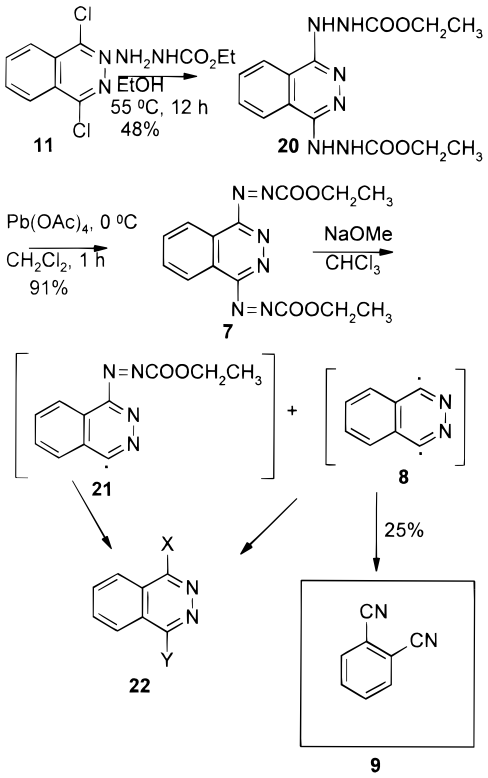
Figure 1. EPR spectra of the hydrolysis products of (a) phenylazoester **4**, (b) pyridine-2-azoester **5**, and (c) pyrimidine-2-azoester **6** at 200 K.

reasons. First, the radicals are generated chemically and trapped by freeze-quenching instead of being formed in situ at low temperatures. The very short lifetime of the radical intermediate thus requires relatively high concentrations of reactants to produce sufficient amounts of the radical to be observable by EPR. Second, the available experimental apparatus only allowed spectra to be obtained down to about 145 K. Thus, the spectra shown in Figure 1 are significantly narrower than those reported at 4 K, most likely because of spectral averaging due to rotation of the molecule in the ring plane. The line width we observe for the phenyl radical is consistent with the known narrowing of the phenyl radical spectrum at higher temperatures.[16] Presumably, similar narrowing would occur in the other two radicals under the same experimental conditions. However, although the EPR spectra are consistent with previous observations of aryl radicals at higher temperatures, they are not sufficiently well-resolved to assign a structure to the observed species, and we cannot rule out even the possibility that we are observing paramagnetic products of secondary radical reactions.

Nevertheless, the EPR results do confirm that radicals are formed under the experimental conditions, and control experiments allow us to attribute radical formation to the methanolysis of the azoesters. No detectable

(16) Ohnishi, S. I.; Tanei, T.; Nitta, I. *J. Chem. Phys.* **1962**, *37*, 2402.

Scheme 5



EPR signal was seen when 0. 1 mL of 0.27 M NaOMe was added to neat chloroform in the absence of azoesters. However, signals similar to those shown in Figure 1 were observed when 0.1 mL of 0.27 M NaOMe was added to a 200 mM solution of each azoester in acetonitrile. These results indicate that radical formation depends on the presence of the azoester precursor but does not depend on the solvent present. Since a phenyl azoester very similar to **4** has been shown to produce radical intermediates,[10c] the mere observation of radicals upon methanolysis of the other aryl azoesters strongly suggests that they cleave via a similar mechanism.

Phthalazine-1,4-bisazoester (7). The 1,4-bisazoester **7** was prepared from the corresponding dichloride **11** in two steps (Scheme 5). The dichloride was reacted with ethylcarbazate to provide roughly equal yields of the mono- and bishydrazo esters, from which the disubstituted compound was separated. This was immediately oxidized to the bisazoester in the usual manner in very high yield, and its methanolysis was carried out in the EPR tube as described above for the monoazoesters.

No EPR signal was observed after methanolysis of the bisazoester **7** at temperatures as low as 145 K. It is unclear whether a spectrum characteristic of a diradical intermediate should in fact have been observable. For a triplet diradical ground state, one expects an EPR spectrum that is severely broadened by strong electronic dipolar interactions. Hirama et al. have reported observing such a spectrum, which they attribute to *p*-benzyne, in a footnote found in refs 17 and 18, but the spectrum has not been published to the best of our knowledge. Moreover, a recent computational study[19] has concluded

(17) Hirama, M. *Pure Appl. Chem.* **1997**, *69*, 525.
(18) Iida, K.; Hirama, M. *J. Am. Chem. Soc.* **1995**, *117*, 8875.
(19) Cramer, C. J.; Nash, J. J.; Squires, R. R. *Chem. Phys. Lett.* **1997**, *277*, 311

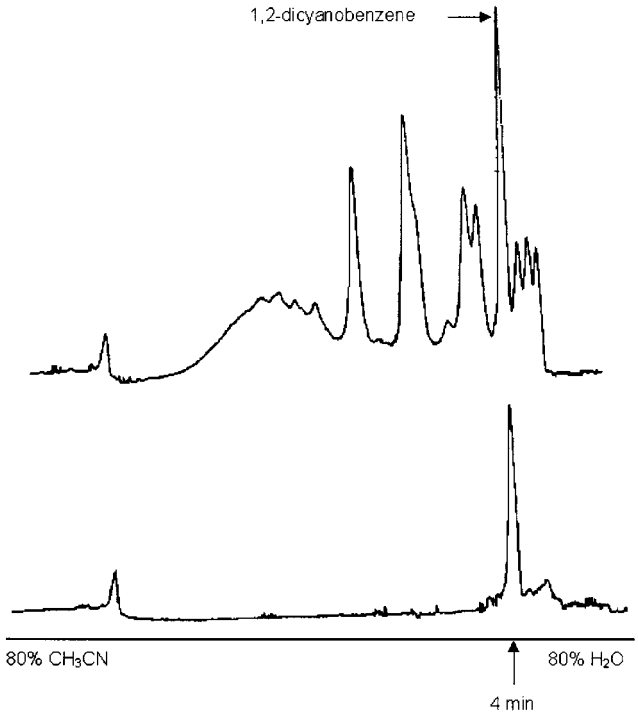


Figure 2. HPLC trace of the hydrolysis products of phthalazine-1,4-bisazoester **7**: (a) crude reaction mixture after hydrolysis; (b) dicyanobenzene fraction after isolation.

that all three isomers of benzyne should be singlet ground states, which would make them EPR-inactive.

In lieu of any direct EPR evidence, we searched for chemical indications of a diradical intermediate in the methanolysis of **7**. The bisazoester was methanolyzed under the same conditions that produced EPR-observable radical intermediates from the monoazoester, and the reaction mixture was examined by reversed-phase HPLC in a specific attempt to identify degradation products consistent with a diradical intermediate.

Figure 2 shows the HPLC trace of the crude reaction mixture. Although we did not individually characterize the many reaction products found, the results are generally consistent with the generation of reactive radicals followed by various secondary reactions such as hydrogen abstraction, formation of solvent adducts, and radical recombination. Since presumed intermediates similar to **8** have proved difficult to trap chemically,[20] it is likely that the diradical undergoes a concerted decomposition that is rapid compared with competing radical reactions (cf. Scheme 2). We therefore focused on searching for possible decomposition products consistent with a diradical intermediate rather than attempting to characterize all of the reaction products.

One possibility for this decomposition is a rapid retro-Bergman reaction to form the corresponding bisnitrile, namely 1,2-dicyanobenzene.[13a,c,20] 1,2-Dicyanobenzene was identified as a product in the reaction mixture by spiking experiments with commercially available bisnitrile. The corresponding fraction was separated by reversed-phase HPLC, collected, and characterized further by GC−MS. This was then quantified as described in the Experimental Section, and the calculated yield of the bisnitrile was 25%.

Although such a significant yield of 1,2 dicyanobenzene is consistent with the putative intermediate **8**, its appearance could conceivably be explained by other mechanisms that do not involve radical intermediates. For example, formation of a diazene from the azoester precursor can in some cases lead to the corresponding carbanion, as summarized in ref 10c, with subsequent rearrangement to form the nitrile. One may also imagine consecutive concerted shifts of the hydrogens on the diazene groups via six-membered-ring transition states that ultimately produce the bisnitrile.[21]

Although such mechanisms can explain the formation of the bisnitrile, they do not account for the EPR signals observed upon methanolysis of the corresponding monoazoesters. Since the methanolysis of the bisazoester was carried out under the same conditions that produce EPR signals from the analogous monoazoesters, it is most likely that the methanolysis of each individual azoester in this reaction proceeds by a similar mechanism, i.e., produces a radical site. Thus, although our results do not conclusively rule out alternative mechanisms, the EPR evidence that azoester cleavage produces radicals, combined with the observation of a bisnitrile product from diazeoster methanloysis, does give a reasonable indication that a 1,4-diradical species may be formed in appreciable amounts. We are presently carrying out EPR spin-trapping studies that will allow direct spectroscopic identification of the radical intermediates, which will be reported shortly.

Conclusion

A series of heteroatomic aryl azoesters with different numbers of ring nitrogens adjacent to the azoester group have been synthesized and their methanolysis in chloroform studied. EPR spectroscopy confirms that that these azoesters produce radicals under the given experimental conditions. By analogy with earlier findings,[10c] the most likely products are the corresponding sp^2 aryl radicals with 0, 1, and 2 nitrogens adjacent to the radical site.

A 1,4-bisazoester has also been synthesized and its methanolysis studied under the same experimental conditions as the monoazoesters. The degradation product 1,2-dicyanobenzene was found in significant yield, consistent with a 1,4-diradical intermediate that undergoes a retro-Bergman cyclization. Although alternative mechanisms for the formation of this product are possible, the observation that azoesters do produce radicals under the given conditions suggests that a significant amount of diradical is formed from the bisazoester. Having established DNA cleavage by azoesters, and the possibility that bisazoesters generate 1,4-diradicals, our results indicate that such compounds are good candidates for diradical generators based on a nonenediyne strategy (Scheme 1, approach 2). Efforts to make a more direct spectroscopic identification of the radical intermediates are underway and will be reported shortly.

Experimental Section

General Methods. All reactions were performed under a positive pressure of dry nitrogen atmosphere. ^{1}H NMR spectra were obtained in a 300 MHz spectrometer and ^{13}C NMR at

(20) Hoffner, J.; Schottelius, M. J.; Feichtinger, D.; Chen, *P. J. Am. Chem. Soc.* **1998**, *120*, 376.

(21) We acknowledge one of the reviewers of this manuscript for pointing out this possibility.

75.2 MHz. Chemical shifts are relative to TMS (when used) or DMSO-d_6. Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet(q), broad (br), and multiplet (m). EPR spectra were recorded on an ER-200 spectrometer at a frequency of 9.438 GHz over a 100 G field range centered on 3370 G. For all EPR spectra shown in this paper, the field sweep time was 100 s, and a gain of 2.5×10^5 was used with a modulation amplitude of 0.10 G. GC−MS was carried using helium as the carrier gas. Solvents were freshly distilled prior to use: pyridine, ethanol, and dichloromethane from calcium hydride. Reaction product solutions were concentrated by using a rotary evaporator.

EPR Measurements. Solutions (200 mM) of all azoesters were made in chloroform and thoroughly degassed by freeze−thaw−pump cycles (four to five times) on a Schlenk line using a glass adapter with a septum access. The solutions were then cooled to ∼200 K, 0. 1 mL of 0.27 M NaOMe (made from Na metal and methanol) was added via a syringe, and the resulting reddish brown solutions were thoroughly mixed. The reaction was performed in the EPR tube and then transferred to the cavity, and the spectrum was recorded immediately.

HPLC Analysis. An HPLC system equipped with a UV detector and a strip chart recorder using a reversed-phase C-18 analytical column was employed for all HPLC experiments. For making up the samples, a prefiltration of the crude mixture on a C-18 cartridge followed by ultracentrifugation was carried out. This crude mixture was subjected to a gradient elution using an acetonitrile/water mixture. Samples of commercially available 1,2-dicyanobenzene were used for spiking experiments and for GC−MS analyses. A calibration curve was used to estimate the amount of 1,2-dicyanobenzene formed from the methanolysis of phthalazine-1,4-bisazoester **7**.

Ethyl Phenylhydrazocarboxylate (12). A 1.0 g (0.91 mL, 0.0092 mol) sample of phenylhydrazine **10** was dissolved in 10 mL of pyridine. The solution was cooled in an ice bath, and 1.0035 g (0.88 mL, 0.0092 mol) of ethyl chloroformate was added dropwise over a period of 30 min. The mixture was stirred at 0 °C for 15 min and at room temperature for an additional 30 min. After addition of water (20 mL) and ether (100 mL), the ether layer was separated and concentrated (to a volume of approximately 10 mL). Phenyl hydrazoester began to crystallize upon leaving the concentrated solution in the refrigerator for a few hours. The crystals were collected by filtration, washed with ether, and dried under vacuum to obtain pure phenyl hydrazoester **12** (1.29 g, 78%): mp = 71−72 °C; ^{1}H NMR (DMSO) δ 8.9 (br, 1H), 7.6 (br, 1H), 7.1 (m, 2H), 6.7 (m, 3H), 4.05 (q, 2H, J = 7.1 Hz), 1. 18 (t, 3H, J = 7.2 Hz); ^{13}C NMR (DMSO) δ 157.11, 149.5, 128.8, 118.3, 111.7, 60.1, 14.5; IR (KBr) cm^{-1} 3370 (NH), 3225 (NH), 3050 (aromatic CH), 2975 (aliphatic CH), 1745 (carbonyl).

Ethyl Phenyldiazenecarboxylate (4). Phenyl hydrazoester **12** (0.25 g, 1.39 mmol) was taken up in 10 mL of dichloromethane. Lead tetraacetate (0.62 g, 1.39 mmol) was added all at once to the above solution, which was maintained at 0 °C. A red color, indicative of phenyl azoester **4**, appeared almost immediately. The reaction mixture was stirred at 0 °C for an extra 30 min and then filtered to remove solid lead salts, and the filtrate was partitioned between water and dichloromethane. The aqueous layer was further extracted with dichloromethane, and the combined organic layers were dried over anhydrous sodium sulfate and concentrated in vacuo to obtain phenyl azoester **4** (0.23 g, 91%) as a red oil judged to be pure by NMR analysis: ^{1}H NMR ($CDCl_3$) δ 7.9 (m, 2H), 7.5 (m, 3H), 4.5 (q, 2H, J = 7.1 Hz), 1.4 (t, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 162.0, 151.4, 133.6, 129.1, 123.5, 64.2, 13.9; IR ($CHCl_3$) cm^{-1} 3050 (aromatic CH), 2950 (aliphatic CH), 1750 (carbonyl).

2-Ethylpyridylhydrazocarboxylate (13). 2-Hydrazinopyridine **14** (0.17 g, 1.56 mmol) was dissolved in 10 mL of anhydrous ethanol to give a red solution. Anhydrous sodium carbonate (0.082 g, 0.78 mmol) was added, and the mixture was cooled to −15 °C. A solution of ethyl chloroformate (0.169 g, 0.149 mL, 1.56 mmol) in 8 mL of ethanol was added dropwise and the reaction stirred at −15 °C for 1 h and at room temperature for 2 h. Excess ethanol was removed at the pump, and the residue was partitioned between water and ethyl acetate. The aqueous layer was further extracted with ethyl acetate and the combined organic layer dried over anhydrous sodium sulfate. After most of the ethyl acetate was removed by concentration in vacuo, traces were removed at the pump to leave behind a yellow oil. This was recrystallized from ether to give pyridine-2-hydrazoester **13** (0.24 g, 85%) as yellow solid: ^{1}H NMR (DMSO) δ 9.0 (br, 1H), 8.2 (br, 1H,), 8.0 (m, 1H), 7.5 (m, 1H), 6.6 (m, 1H), 6.5 (m, 1H), 4.0 (q, 2H, J = 7.0 Hz), 1.0 (t, 3H, J = 7.0 Hz); ^{13}C NMR (DMSO) δ 159.9, 156.9, 147.6, 137.4, 114.3, 105.9, 60.3,14.6; IR (KBr) cm^{-1} 3380 (NH), 3200 (NH), 1740 (carbonyl).

2-Ethylpyridyldiazenecarboxylate (5). Pyridine-2-hydrazoester **13** (0.15 g, 0.83 mmol) was dissolved in 10 mL of dichloromethane. It was then cooled to 0 °C in an ice bath, and 0.36 g (0.83 mmol) of $Pb(OAc)_4$ was added. A red color characteristic of pyridine-2-azoester **5** was observed immediately. The reaction mixture was stirred at 0 °C for an extra 30 min and then filtered to remove solid lead salts, and the filtrate partitioned between water and dichloromethane. The aqueous layer was further extracted with dichloromethane, and the combined organic layers dried over anhydrous sodium sulfate and concentrated in vacuo to obtain pyridine-2-azoester **5** (0.14 g, 94%) as a red oil judged to be pure by NMR analysis: ^{1}H NMR ($CDCl_3$) δ 8.6 (m, 1H), 7.9 (m, 1H), 7.7 (m, 1H), 7.4 (m, 1H), 4.4 (q, 2H, J = 7.1 Hz), 1. 4 (t, 3H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) δ 162.1, 161.2, 149.6, 138.5, 127.1, 117.7, 64.5, 13.9; IR ($CHCl_3$) cm^{-1} 3040 (aromatic CH), 1754 (carbonyl).

2-Hydrazinopyrimidine (16). 2-Chloropyrimidine **17** (0.7 g, 6.1 mmol) was dissolved in 15 mL of pyridine. Anhydrous hydrazine (2.53 g, 78.9 mmol) followed by an extra 8 mL of pyridine were added dropwise. After 1.5 h at room temperature, pyridine was removed by concentration in vacuo and the residue suspended in 4 mL of water. Filtration followed by washing of the solid with methanol (2 × 25 mL) and drying at the pump gave pure 2-hydrazinopyrimidine **15** (0.64 g, 95%) as a white solid: mp = 109−110 °C (lit.[22] mp 110−111 °C); ^{1}H NMR (DMSO) δ 8.3 (d, 2H, J = 4.6 Hz), 8. 1 (br, 1H), 6.6 (t, 1H, J = 4.8 Hz), 4.1 (br, 2H); ^{13}C NMR (DMSO) δ 164.4, 157.8, 18.4; IR (KBr) cm^{-1} 3400−2400 (br).

2-Ethyl Pyrimidylhydrazocarboxylate (18). To a solution of 2-hydrazinopyrimidine **16** (0.14 g, 1.27 mmol) in 8 mL of anhydrous ethanol was added sodium carbonate (0.067 g, 0.635 mmol), and the solution was cooled to −15 °C. A solution of ethyl chloroformate (0.138 g, 0.121 mL, 1.27 mmol) in 8 mL of anhydrous ethanol was then added dropwise over a period of 25 min via an addition funnel. The resulting mixture was stirred at −15 °C for 2 h and at room temperature for an additional 2 h. After removal of ethanol in vacuo, the residue was partitioned between water and ethyl acetate and the aqueous layer extracted further with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, and ethyl acetate was distilled off at reduced pressure to leave behind a white solid. The crude product was recrystallized from ether to obtain pyrimidine-2-hydrazoester **18** (0.188 g, 82%): mp = 103−104 °C; ^{1}H NMR (DMSO) δ 9.1 (br, 1H), 8.9 (br, 1H), 8.4 (d, 2H, J = 4.7 Hz), 6.6 (t, 1H, J = 4.6 Hz), 4.1 (q, 2H, J = 7.2 Hz), 1.2 (t, 3H, J = 6.9 Hz); ^{13}C NMR (DMSO) δ 163.3, 158.2, 156.9, 112.5, 60.2, 14.5; IR (KBr) cm^{-1} 3450 (NH), 3250 (NH), 1745 (carbonyl).

2-Ethyl Pyrimidyldiazenecarboxylate (6). Pyrimidine-2-hydrazoester **18** (0.2 g, 1.1 mmol) was dissolved in 10 mL of dichloromethane. To this clear and colorless solution was added 0.487 g (1.1 mmol) of $Pb(OAc)_4$. The resulting yellow solution was stirred for I h at 0 °C. After separation of the byproducts (inorganic lead compounds) by filtration, the filtrate was partitioned between dichloromethane and water. The aqueous layer was further extracted with dichloromethane, and the combined organic layers were dried over anhydrous sodium sulfate. Concentration in vacuo gave pyrimidine-2-

(22) Vanderhaeghe, C. *Bull. Soc. Chim. Belg.* ***1959****, 68*, 30.

azoester **6** (0.185 g, 95%) judged to be pure as evidenced by ^{1}H and ^{13}C NMR analyses: ^{1}H NMR ($CDCl_3$) δ 9.0 (d, 2H, J = 4.7 Hz), 7.5 (t, 1H, J = 4.7 Hz), 4.5 (q, 2H, J = 7.0 Hz), 1.5 (t, 3H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 165.6, 161.7, 159.1, 122.5, 65.0, 14.1; IR ($CHCl_3$) cm^{-1} 3050 (aromatic CH), 1748 (carbonyl).

1,4-Diethyl Phthalazinylhydrazocarboxylate (20). 1,4-Dichlorophthalazine **11** (0.275 g, 1.3 mmol) was added, all at once, to a stirred solution of ethyl carbazate (0.28 g, 2.7 mmol) in 15 mL of ethanol. After addition of another 8 mL of ethanol, the reaction mixture was heated to 55 °C and stirred overnight (12 h). TLC analysis of the crude mixture after 12 h showed two spots corresponding to the mono- and bishydrazoesters. The desired phthalazine-1,4-bishydrazoester **20** (0.21 g, 48%) was obtained as an off-white solid upon chromatographic purification (R_f = 0. 13 in 95:5 ethyl acetate/methanol; mp = 185−186 °C). The yield of the corresponding monohydrazoester, a yellow solid, was 50%. Due to oxidative instability, lead tetraacetate oxidation of phthalazine-1,4-bishydrazoester **20** had to be carried out immediately (described below): ^{1}H NMR (DMSO) δ 8.9 (br, 2H), 8.7 (br, 2H), 8.2 (br, 2H), 7.8 (br, 2H), 4.1 (q, 4H, J = 7.0 Hz), 1.2 (t, 6H, J = 7.0 Hz); ^{13}C NMR (DMSO) δ 157.1, 150.0, 131.2, 122.1, 118.7, 59.8, 14.5.

1,4-Diethylphthalazinyldiazenecarboxylate(7). Phthalazine-1,4-bishydrazoester **20** (0.11 g, 0.32 mmol) was dissolved in dichloromethane and the solution cooled in an ice bath to 0 °C. Lead tetraacetate (0.28 g, 0.64 mmol) was added, and the appearance of deep red color characteristic of azoesters was virtually instantaneous. After the mixture was stirred at 0 °C for 1 h, solid lead salts were removed by filtration and the filtrate was partitioned with water (washed a number of times with water to remove brown lead compound) and dichloromethane. The aqueous layer was extracted further with dichloromethane, and the combined organic layers were dried over anhydrous sodium sulfate and concentrated in vacuo. The red oily film was then chromatographed using hexanes/ethyl acetate (R_f = 0.5 in 1:1 hexanes/ethyl acetate) to obtain pure phthalazine-1,4-bisazoester **7** as a dark red oil (0.096 g, 91%): ^{1}H NMR ($CDCl_3$) δ 8.6 (m, 2H), 8.2 (m, 2H), 4.6 (q, 4H, J = 7 Hz), 1.5 (t, 6H, J = 7 Hz); ^{13}C NMR ($CDCl_3$) δ 161.6, 160.6, 134.6, 125.2, 124.4, 65.6, 14.4; IR ($CHCl_3$) cm^{-1} 3025 (aromatic) 1745 (carbonyl).

Acknowledgment. This work was supported by grants NSF CHE-9406469 (D.J.) and NSF MCB-9600940 (D.E.B.). We are grateful to Profs. David A. Forsyth and Graham Jones for helpful discussions and to Dr. Stephen V. Kolaczkowski for assistance with the EPR freeze-quenching experiments.

Supporting Information Available: A listing of the 75 MHz ^{13}C NMR spectra of compounds **12**, **4**, **13**, **5**, **17**, **18**, **6**, **20**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO990750V