A Core System That Simulates the Cycloaromatization and DNA Cleavage Properties of Calicheamicin–Esperamicin: A Correlation Experiment

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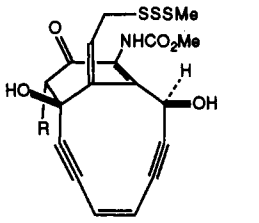
Summary: The synthesis and cycloaromatization reaction of core analogues of calicheamicin–esperamicin are described. Two derivatives were shown to undergo the cycloaromatization reaction at differing rates. Qualitatively, a similar rate relationship was reflected in the DNA-damaging properties of these derivatives.

Sir: The advent of the calicheamicin[1]–esperamicin[2] enediyne antibiotics offers exciting opportunities for interactive research among several branches of science and medicine. Among the challenges that these highly active and novel compounds implicitly pose are those of total synthesis, biosimulation, and new strategies for drug targeting. It was the synthetic component of the problem that first engaged our attentions. As recently described,[3] we have developed a concise route to the core skeleton of the enediyne antibiotics. While the entire aglycon has not yet been assembled in the laboratory, the progress that we have achieved already offers many interesting possibilities for probing substantive issues of biomechanism in the context of clearly relevant substructures.

The consensus of current thinking[1,2,4,5] is that the drugs suffer reductive cleavage of the trisulfide linkage (see structure i). This cleavage is then followed by intramolecular Michael addition to the bridgehead enone, generating a system (cf. structure ii) in which the enediyne linkage is free to undergo a Bergman type cyclization.[6] The diradical thus produced (cf. structure iii) has DNA-damaging properties. The remarkable base specificities for DNA cleavage manifested by the presumed diradical arising from calicheamicin have been identified by a Lederle group.[7]

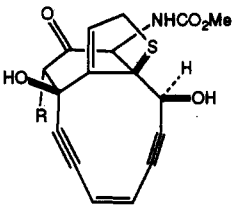
Our first goal was to construct a system wherein Bergman cyclization would be constrained by a bridgehead double bond. Intermolecular addition to this double bond would generate a substrate where cycloaromatization might occur, and its connectivity to DNA cleavage could be evaluated. Our results are described herein.

The starting material for our investigation was compound 1 whose synthesis we recently described.[3a] A variety of attempts to deliver a range of nucleophiles to this bridgehead enone were unsuccessful.[3b] It was necessary to find a substrate in this structural domain that would lend itself to saturation of the double bond. We proceeded as follows. Solvolysis of 1 with ammonium formate/formic acid gave an 80% yield of the enone tetrol 2. This compound, upon treatment with periodic acid dihydrate in THF, afforded the novel enedione 3.[8,9] Unlike the situation with compound 1, the bridgehead double bond in 3 is amenable to chemoselective operations. Thus, upon reaction with zinc and acetic acid, diol–dione 4 was obtained in 82% overall yield from 2 (Scheme I).

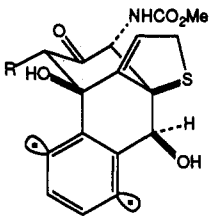


R = OH esperamicin aglycone
R = H calicheamicin aglycone

I



II



iii

The possibility of a Bergman reaction of compound 4 was first studied in a nonaqueous medium in the presence of a hydrogen atom donating source. *Thermolysis of a solution of 4 in acetonitrile in the presence of 1,4-cyclohexadiene (82 °C, 6 h) afforded a 40% yield of the dihydro product 5.* Compound 4 was also treated with sodium borohydride in methanol. This reaction did not lead to the isolation of the expected tetrol 6, but to a complex mixture of materials. We suspected that compound 6 was being produced but was decomposing through a room temperature Bergman reaction.[6] Accordingly, in another experiment the sodium borohydride reduction was carried out in a methanol solution containing 1,4-cyclohexadiene. *Under those conditions there was produced a 50% yield of a tetrol, 7.* Treatment of 7 with acetic anhydride–triethylamine–DMAP in CH_2Cl_2 afforded the triacetate 8 (under these conditions in tertiary alcohol resists acetylation).

(1) Lee, M.; Dunne, T.; Siegel, M.; Chang, C.; Morton, G.; Border, D. *J. Am. Chem. Soc.* **1987**, *109*, 3464, 3466.

(2) Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. *J. Am. Chem. Soc.* **1987**, *109*, 3462.

(3) (a) Danishefsky, S. J.; Mantlo, N. B.; Yamashita, D. S.; Schulte, G. *J. Am. Chem. Soc.* **1988**, *110*, 6890. (b) Among the species that we attempted to add to this double bond are cyanide, cuprate, and various thiolates (unpublished results, Nathan Mantlo).

(4) (a) Magnus, P.; Lewis, R. T.; Huffman, J. C. *J. Am. Chem. Soc.* **1988**, *110*, 6921. (b) Magnus, P.; Carter, P. A.; *J. Am. Chem. Soc.* **1988**, *110*, 1626.

(5) Nicolaou, K. C.; Ogawa, Y.; Zuccarello, G.; Kataoka, H. *J. Am. Chem. Soc.* **1988**, *110*, 7247.

(6) Bergman, R. G. *Acc. Chem. Res.* **1973**, *6*, 25.

(7) Zein, N.; Sihha, A. M.; McGahren, W. J.; Ellestad, G. A. *Science (Washington, D.C.)* **1988**, *240*, 1198.

(8) Spectral evidence (UV and ^{1}H NMR) together with the ease of reduction by zinc and acetic acid (10 min at room temperature) of 3 suggests that the enedione component has a relatively high degree of effective electronic conjugation. Particularly supportive of this view are the following comparisons between enedione 3 and its closely related "simple" enone 2. ^{1}H NMR (C=CHC=O) for 2, δ 6.02; for 3, δ 6.47.[9a] UV (π–π^*) for 2, $\lambda_{max}^{CH_3CN}$ = 222 nm (ϵ = 13 400); for 3, $\lambda_{max}^{CH_3CN}$ = 232 nm (ϵ = 10 900).[9b]

(9) (a) ^{1}H NMR for cyclohexenone (C=CHC=O) δ 6.0; for 1,4-cyclohexenedione (O=CH=CHC=O) δ 6.7. (b) UV (π–π^*) for cyclohexenone, λ_{max}^{MeOH} = 225 nm (ϵ = 7900); for 1,4-cyclohexenedione,[9c] λ_{max}^{EtOH} = 233 nm (ϵ = 15 000). (c) Garbisch, E. W., Jr. *J. Am. Chem. Soc.* **1965**, *87*, 4971.

(10) The stereochemical course of the reduction could not be assessed by ^{1}H NMR or NOE studies.

(11) These data are meant to represent trends and as such are not rigorously quantified. The values come directly from densitometry readings and are not corrected for the presence of form II in the starting DNA or for the differential stainability of the forms.

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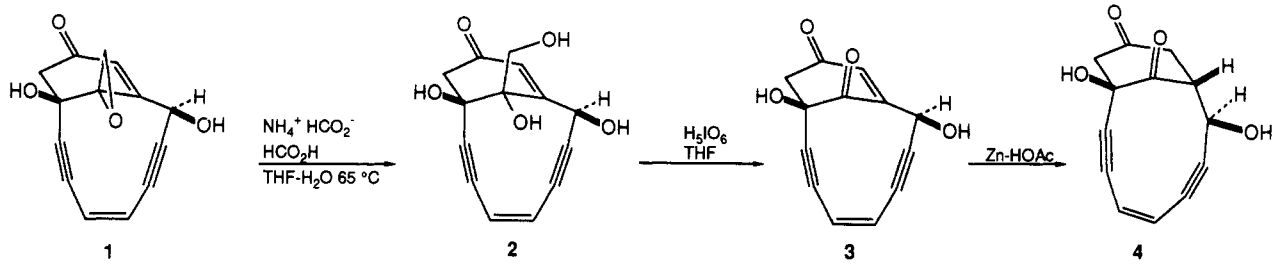
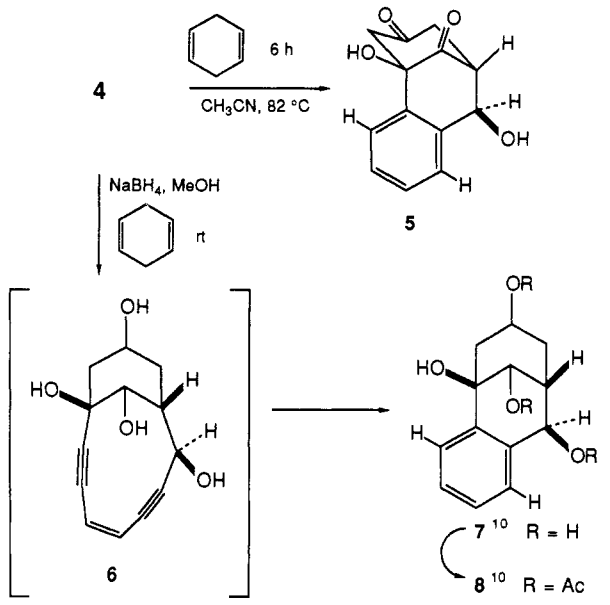
Scheme I



These cycloaromatization results are very much in keeping with discoveries of Magnus and co-workers[4a,b] in related model systems. In the Magnus experiment, reduction of a ketone to an alcohol at the one-carbon bridge exerts an accelerating effect on the rate of cycloaromatization. Our substrate 4 had, at the same time, undergone reduction at the ketone of the three-carbon bridge in going to the presumed 6. Though the contribution of the individual reductions in triggering cycloaromatization is not known, it seems likely that the bulk of the effect arises from reduction of the C1-bridged ketone.



A dramatic demonstration of DNA cleavage by a simple cyclic enediyne equipped with water-solubilizing hydroxyl groups was provided by Nicolaou and co-workers.[5] Armed with this precedent, and with the two related systems which undergo Bergman reaction at strikingly different rates, we probed the relationship between cycloaromatization and DNA degradation. When a solution of ΦX174 form I DNA (supercoiled, circular) was treated with varying concentrations of 4 at 37 °C for 12 h, single-strand and, to a smaller extent, double-strand breaks were observed as evidenced by the production of form II (relaxed, circular) and form III (linear) DNA, respectively (Table I).

We next turned our attention to an assessment, albeit necessarily qualitative, of the relationship between proclivity for Bergman cycloaromatization and DNA-damaging capacity. Since tetrol 6 was too unstable to be isolated in pure form and analyzed as such, we resorted to its in situ generation by reduction of 4 with sodium borohydride in a buffered aqueous solution containing ΦX174 rf I DNA. *Indeed, a marked increase in the amount of DNA cleavage was observed after 2 h at room temperature* (Table II). The degree of cleavage was also shown to decrease

Table I. Cleavage of ΦX174 Form I DNA by 4[a]

concn of 4, M	% form[11] I	II	III
5×10^{-5}	44	51	5
1×10^{-4}	42	48	10
5×10^{-4}	0	76	24
1×10^{-3}	0	78	22
DNA alone	76	24	0

[a] ΦX174 form I DNA[b] (0.02 μg/μL) was incubated with 4 in 40 mM trisacetate/1 mM EDTA (TAE buffer, pH 8) at 37 °C for 12 h and analyzed by 1% agarose gel electrophoresis. Forms were obtained from densitometry readings after ethidium bromide staining and photography. [b] The DNA used was contaminated by a small amount of form II.

Table II. Cleavage of ΦX174 Form I DNA by Activation of 4 with $NaBH_4$

conditions[a]	% form[11] I	II	III
4 (1×10^{-3} M) alone	71	22	7
4 (1×10^{-3} M) + $NaBH_4$ (2×10^{-2} M)	4	74	22
DNA alone	88	11	0
DNA + $NaBH_4$ (2×10^{-2} M)[b]	84	16	0

[a] Reactions were run at 20 °C for 2 h in 40 mM trisacetate/1 mM EDTA (TAE buffer, pH 8) using a DNA (ΦX174 form I)[c] concentration of 0.02 μg/μL. [b] $NaBH_4$ was added last as a 0.5 M stock solution in diglyme. [c] The DNA used was contaminated by a small amount of form II. The mixture was analyzed as in Table I.

Table III. Addition of ΦX174 Form I DNA[b] to 4 at Various Times after $NaBH_4$ Activation: A Reduction in Cleaving Activity with Time[a]

time after activation, h	% form[11] I	II	III
0	3	85	12
0.5	16	74	10
1.5	41	54	5
4.0	75	25	0
12	75	25	0
DNA + $NaBH_4$ in the absence of 4	77	23	0

[a] $NaBH_4$ (2 μL of a 0.5 M solution in diglyme) was added to 47 μL of a 1.06 mM buffer solution (described in Table I) of 4 at 20 °C. After the specified time, the DNA (1 μL of a 1 μg/μL solution) was added, and the mixture was agitated at room temperature for 2 h and analyzed as in Table I. [b] The DNA used was contaminated by a small amount of form II.

with the length of time that elapsed between exposure of 4 to sodium borohydride and exposure of that mixture to DNA (Table III). These data speak to the generation and disappearance of an intermediate (cf. 6) which is responsible for the damage to the nucleic acid. In a further control experiment, it was demonstrated that treatment of the aqueous DNA solution with sodium borohydride alone led to no cutting. The chemical fate of compounds 4 and 6 in these DNA-cleavage experiments has not been determined. Also to be determined in the future is whether

the damage arises from a direct reaction between a benzenoid diradical and DNA or through the intermediacy of other species.

In summary, compounds in the enediyne antibiotic manifold with demonstrated independent propensity for both Bergman cycloaromatization and DNA cleavage have been synthesized. Qualitatively, a direct relationship between the proclivities of these two processes has been demonstrated. Further experiments in this area are in progress.

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Supplementary Material Available: Experimental procedures and documentations of this work (3 pages). Ordering information is given on any current masthead page.

A Simple Highly Stereospecific Preparation of Vinylphosphonium Salts: Palladium-Catalyzed Vinylation of Triphenylphosphine via Vinyl Triflates

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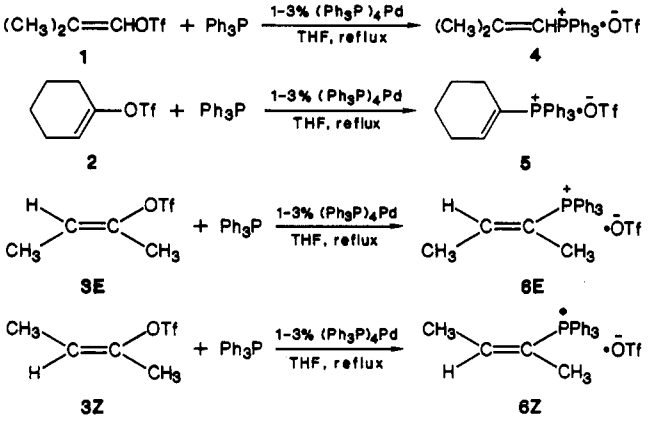
Summary: Interaction of vinyl triflates with Ph_3P in the presence of 1–3% $(Ph_3P)_4Pd$ results in vinylphosphonium salts in 62–89% isolated yields. The reaction is stereospecific, thereby providing a simple means to either (*E*)- or (*Z*)-vinylphosphonium salts.

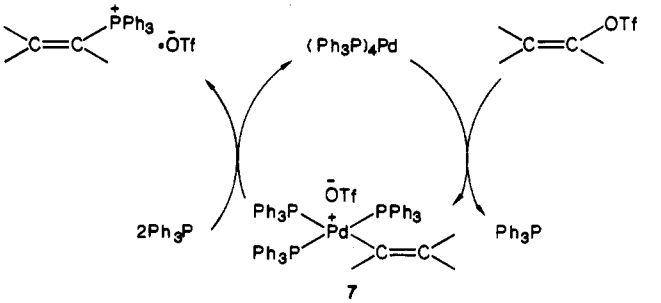
Sir: Vinylphosphonium salts are valuable synthetic reagents commonly used in cycloadditions, Michael additions, and the synthesis of heterocyclic systems.[1-7] In spite of their importance, there are no known methods for the preparation of stereodefined isomeric vinylphosphonium salts. Current methods of preparation include additions to alkynylphosphonium salts,[8] reaction of allyl bromides with triphenylphosphine, and subsequent base-catalyzed prototropic rearrangement to the vinyl compound,[9] as well as the high-temperature nickel-catalyzed fusion reaction of vinyl bromides with triphenylphosphine.[10] However, all these procedures result either in mixtures of stereoisomers or the exclusive formation of *E* isomers.[8,11] Hence, in this paper we report our preliminary results for the stereospecific preparation of vinylphosphonium salts via a simple new procedure employing vinyl triflates.

Interaction of the readily available[12] vinyl triflates **1–3** with a 5% excess of Ph_3P in refluxing THF in the presence of catalytic (1–3 mol %) $(Ph_3P)_4Pd$ results in the corresponding vinylphosphonium triflates **4–6** in 62–89% isolated yields as shown in Scheme I.

Scheme I



Scheme II



Phosphonium salts **4–6** are stable, crystalline, albeit slightly hygroscopic, solids that are fully characterized[13] by spectral means as summarized in Table I.

As the data show the reaction is applicable to the formation of cyclic as well as acyclic vinylphosphonium salts. Particularly noteworthy is the fact that the reaction is

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(13) Compounds **4** and **5** gave satisfactory elemental analyses and **6E** and **6Z** gave correct HRMS.

0022-3263/89/1954-2783$01.50/0