Studies on Dynemicin. A Nonradical Cycloaromatization Pathway for the Azabicyclo[7.3.1] Enediyne Core Structure Initiated by Thiolate Addition

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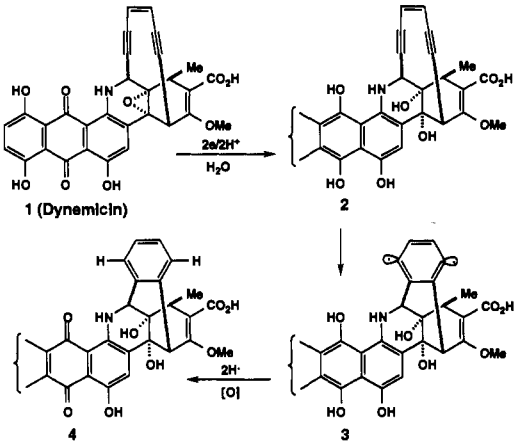
It has been speculated that the potent antitumor agent dynemicin A (**1**) exerts its *in vitro* biological activity through the formation of the diradical **3**.[1] Studies to design models that mimic dynemicin have been based upon this working hypothesis.[2] Scheme I outlines the notion that bioreduction of **1** triggers epoxide opening, followed by hydration to give the intermediate **2**, which can cycloaromatize (Bergman reaction) to the diyl **3** and hydrogen atom abstract from the backbone of DNA to give **4**, resulting in DNA cleavage. It has been generally assumed that the formation of a diradical intermediate is a prerequisite for biological activity.[3] In this paper we report that the simple azabicyclo[7.3.1] enediyne dynemicin core analogue **9** undergoes cycloaromatization *via* a polar nonradical pathway and exhibits both *in vitro* and *in vivo* antitumor activity.

During the course of our studies on the synthesis and mechanism of action of **1** we have developed a short synthetic route to the azabicyclo[7.3.1] enediyne core structure **5** (Scheme II).[4] A variety of carbamate nitrogen protecting groups have been employed that, in principle, can be removed using either acidic or basic conditions. Surprisingly, it was found that treatment of **5** with PhS^-Na^+/THF at 0 °C, with the expectation of producing **6**, gave a completely aromatized product provisionally formulated as **10**. Similarly, **7**, **8**, and **9** gave the adducts **11**, **12**, and **13**, respectively.

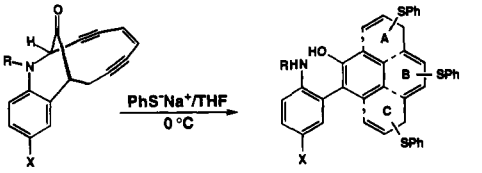
To enable characterization of the product(s) from this unexpected transformation we focused on the adamantyl carbamate **8**, since this compound can be readily deprotected to give the secondary amine **9** by treatment with CF_3CO_2H (TFA)/CH_2Cl_2/room temperature. Treatment of **8** with sodium 3,5-dimethylthiophenolate/THF at 0 °C gave a mixture of two compounds **14** (ca. 1:1; Scheme III), which upon deprotection (TFA, 95%) gave a single completely aromatized adduct **15** (structure by X-ray).[5] Conducting the above reaction in THF-d_8 did not result in any deuterium incorporation into **14** or **15**, thus precluding a radical intermediate in the conversion of **8** into **14**. Treatment of **8** with sodium 3,5-dimethylthiophenolate/THF/excess NaH at 0 °C gave the naphthol **16** (44%). Carrying out the same transformation in the presence of MeOD gave **16a** with the incorporation of two deuterium atoms in the positions shown. Excess NaH converted **14** into **16** (72%). Irradiation of **8** with PhSSPh/benzene resulted in slow decomposition to an intractable mixture.

A plausible mechanistic explanation for this unprecedented reaction involves thiolate addition to the enediyne **8** to give the cumulene **8a**, which can undergo further thiolate addition resulting in the enolate **8b**.[6] Enolate anion ring closure to **8c** followed by

Scheme I



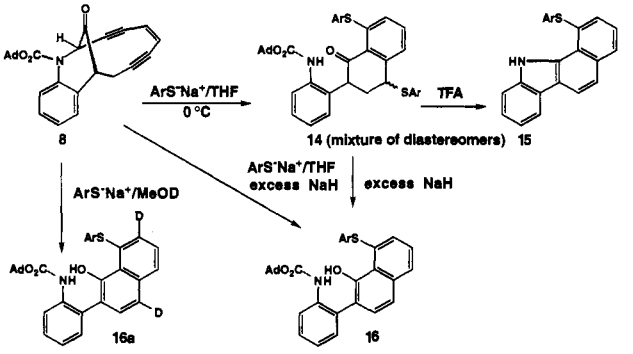
Scheme II



5 (R = $CO_2CH_2CH_2Cl$)
6 (R = $CO_2CH_2CH_2SPh$)
7 (R = CO_2Me)
8 (R = CO_2Ad)
9 (R = H)

10 (R = $CO_2CH_2CH_2SPh$)
11 (R = CO_2Me)
12 (R = CO_2Ad)
13 (R = H)

Scheme III



8
14 (mixture of diastereomers)
15
16a
16

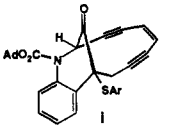
(1) Structure of dynemicin: Konishi, M.; Ohkuma, H.; Tsuno, T.; Oki, T.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1990**, *112*, 3715. Bioreductive diradical formation: Semmelhack, M. F.; Gallagher, J.; Cohen, D. *Tetrahedron Lett.* **1990**, *31*, 1521. Acid-catalyzed ring-opening of the epoxide in **1** also leads to cycloaromatization.

(2) Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, 1387. For recent synthetic studies, see: Porco, J. A., Jr.; Schoenen, F. J.; Stout, T. J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1990**, *112*, 7410. Nicolaou, K. C.; Hwang, C.-K.; Smith, A. L.; Wendeborn, S. V. *J. Am. Chem. Soc.* **1990**, *112*, 7416. Nicolaou, K. C.; Smith, A. L.; Hwang, C.-K.; Wendeborn, S. V. *J. Am. Chem. Soc.* **1991**, *113*, 3114. Wender, P. A.; Zercher, C. K. *J. Am. Chem. Soc.* **1991**, *113*, 2311. Wood, J. D.; Porco, J. A., Jr ; Taunton, J.; Lee, A. Y.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc.* **1992**, *114*, 5898. Isobe, M.; Nishikawa, T.; Yamamoto, N.; Tsukiyama, T.; Ino, A.; Okita, T. *J. Heterocycl. Chem.* **1992**, *29*, 619.

(3) Nicolaou, K. C.; Smith, A. L. *Acc. Chem. Res.* **1992**, *25*, 497.

(4) For the η^2 $Co_2(CO)_6$ mediated approach, see: Magnus, P.; Fortt, S. M. *J. Chem. Soc., Chem. Commun.* **1991**, 544.

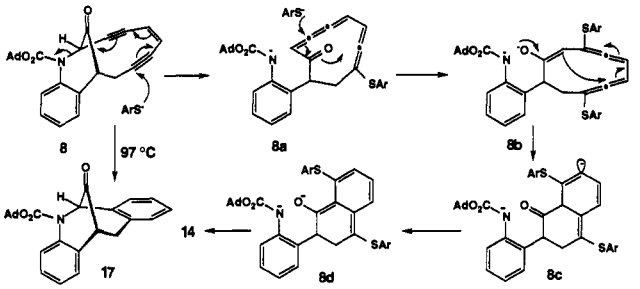
(5) The reaction mixture contains a small amount of the bridgehead sulfenylated compound **i**. An authentic sample of **i** was made by treatment of **8** with LiHMDS/THF/$(ArS)_2$.



i

0002-7863/93/1515-12627$04.00/0

Scheme IV



protonation and tautomerism results in **8d**, which gives **14**. It should be noted that **8** (X = OMe) does not undergo the normal Bergman cycloaromatization to give **17** at an appreciable rate until it is heated to at least 97 °C ($t_{1/2}$ = 8.26 h).[7] The mechanism shown in Scheme IV is consistent with the MeOD experiment, although it is possible that the deuterium *para* to the OH was introduced by base-catalyzed exchange after elimination of ArS.

Myers has shown that neocarzinostatin chromophore undergoes thiol addition to trigger cycloaromatization. The actual cycloaromatization reaction involves a diradical which has been trapped by THF-d_8.[8] It has been shown by Saito that there is a second pathway available for the cycloaromatization of neocarzinostatin. Under physiological conditions (D_2O/buffered 2-mercaptoethanol), neocarzinostatin cycloaromatizes with the incorporation of one deuterium atom (80%) in the aromatic ring.[9] This duality of cycloaromatization mechanisms, diradical and polar, has not been seen in any other enediynes. This study shows that the dynemicin core analogue **8** can undergo cycloaromatization to **17** *via* the "normal" thermal (97 °C) diradical cycloaromatization pathway, and in the presence of thiolate (0 °C), a polar cycloaromatization pathway intervenes to give **14**/**16**. The secondary amine **9** on treatment with ArS$^-$Na$^+$/THF, followed by acidification, gave the benzocarbazole **15**.

The core azabicyclo[7.3.1] enediyne compounds **9** (X = H and OMe) showed good *in vivo* potency and activity (efficacy, T/C > 125%) in P388 leukemia assays using CDF1 mice (2 mg/kg gave T/C values of 175% and 170%, respectively). Kedarcidin gave a T/C of 175% at 2.4 mg/kg. In a distal solid tumor model, which measured delay in tumor growth of a subcutaneous M109 lung carcinoma,[10] **9** (X = OMe) was active (T–C = 7.5 days) when administered intravenously every 2 days, beginning on the day of tumor implant for a total of five doses of 1.2 mg kg^{-1} dose^{-1}. Using the same model and schedule, **9** (X = H) was found to be marginally active (T–C = 3.0 days) while esperamicin (T–C = 11.0 days at 0.05 mg kg^{-1} dose^{-1}) and neocarzinostatin (T–C = 19.3 days at 0.6 mg kg^{-1} dose^{-1}) were more active. *In vitro* cytotoxicity, assessed in HCT116 human colon carcinoma cells, showed that **9** (X = H) was 350 times more potent than **8** (X = H) (IC_{50}'s of 0.21 and 75 μM, respectively).

It can be concluded that diradical formation is not a prerequisite for biological activity.

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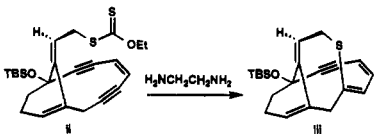
Supplementary Material Available: NMR, IR, and mass spectral data for adducts **14**–**16** and crystallographic details for $C_{24}H_{19}NS$ including fractional coordinates, thermal parameters, and bond lengths and angles (15 pages). This material is contained in many libraries on microfiche, immediately follows this artible in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(6) We have previously observed intramolecular thiolate addition to an acetylene during the construction of the trisulfide functionality of calicheamicin: Magnus, P.; Lewis, R.; Bennett, F. *J. Am. Chem. Soc.* **1992**, *114*, 2560. Treatment of **ii** with ethylenediamine gave the cyclic sulfide **iii** in >80% yield.



(7) P. Magnus and R. Fairhurst, unpublished results.

(8) Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493. Myers, A. G.; Dragovich, P. S. *J. Am. Chem. Soc.* **1989**, *111*, 9130. Myers, A. G.; Proteau, P. J. *J. Am. Chem. Soc.* **1989**, *111*, 1146. Nicolaou, K. C.; Skokotas, G.; Furuya, S.; Suemune, H.; Nicholaou, C. *Angew. Chem., Int. Ed. Engl.* **1990**, 1064. Recently Hensens and Goldberg have shown that the hydroxynaphthoate ester of neocarzinostatin participates in its cycloaromatization. Hensens, O. D.; Helms, G. L.; Zink, D. L.; Chin, D.-M.; Kappen, L. S.; Goldberg, I. H. *J. Am. Chem. Soc.* **1993**, *115*, 11030.

(9) Sugiyama, H.; Yamashita, K.; Nishi, M.; Saito, I. *Tetrahedron Lett.* **1992**, *33*, 515. Hirama, M.; Fujiwara, K.; Shigematu, K.; Fukazawa, Y. *J. Am. Chem. Soc.* **1989**, *111*, 4120. Fujiwara, K.; Kurisaki, A.; Hirama, M. *Tetrahedron Lett.* **1990**, *31*, 4329.

(10) Rose, W. C. *Cancer Treat. Rep.* **1981**, *65*, 299. Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *2*, 1.