Molecular Design, Chemical Synthesis, and Biological Action of Enediynes

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Over the past few years much of our research has been concerned with an examination of molecules inspired by a new class of antibiotics, the enediynes, which are some of the most potent antitumor agents ever discovered.[1] These antibiotics, represented by calcheamicin γ_1^I (1),[2] esperamicin A_1 (2),[3] neocarzinostatin chromophore (3),[4] and dynemicin A (4)[5] in Chart I, have at their core an unprecedented confluence of acetylenic and olefinic linkages: a structural assembly which lies at the heart of the remarkable activity of these molecules. With the exception of neocarzinostatin chromophore (3), all of these molecules contain a 1,5-diyn-3-ene unit embedded within a strained 10-membered ring which, upon suitable chemical triggering, is capable of undergoing a cycloaromatization process to generate a benzenoid diradical. It is this diradical which is capable of abstracting hydrogen atoms from the sugar phosphate backbone of DNA, initiating double-stranded DNA cleavage leading to cell death.

Remarkably, Bergman had designed, observed, and studied this very cycloaromatization reaction some 15 years prior to the discovery of the enediyne antibiotics, clearly demonstrating the cyclization of model enediynes and the formation of 1,4-benzenoid diradicals (Figure 1).[6,7] However, this information lay buried and largely forgotten in the literature until Nature came along to show us the way to what now looks so obvious. Thus in 1987 we initiated a program directed at the synthesis of novel enediynes aimed at an understanding of their chemistry and the design of new structurally simple compounds which mimic the biological activity of the natural products. The potential of these molecules in cancer chemotherapy and other biomedical applications was not far from our thoughts.

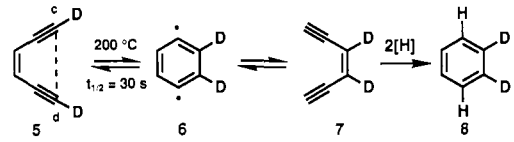
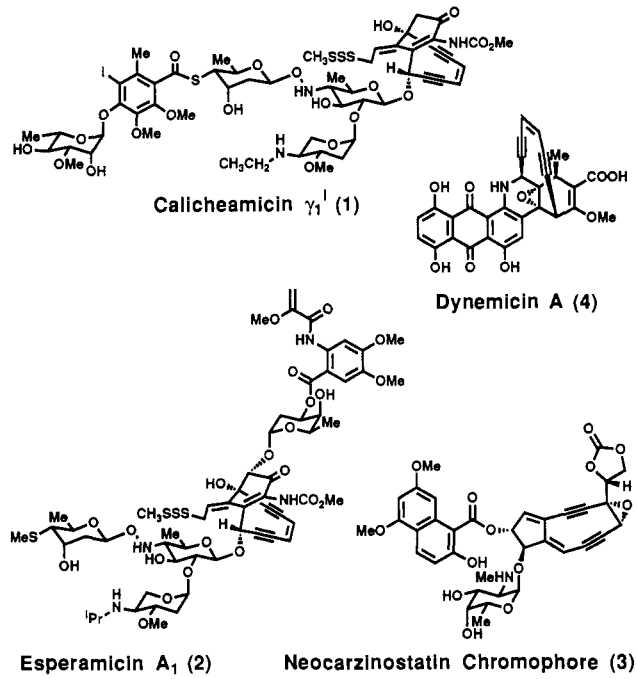


Figure 1.

Chart I



Investigation of the Bergman Reaction

The mechanism of action of calicheamicin γ_1^I (1),[2] shown in Scheme I, postulates binding of the oligosaccharide portion within the minor groove of DNA. Nucleophilic attack at the central sulfur atom of the

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(1) For a review, see: Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387.

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(3) Konishi, M.; Ohkuma, H.; Saitoh, K.; Kawaguchi, H.; Golik, J.; Dubay, G.; Groenewold, G.; Krishnan, B.; Doyle, T. W. *J. Antibiot.* **1985**, *38*, 1605. Golik, J.; Clardy, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. soc.* **1987**, *109*, 3461. Golik, J.; Dubay, G.; Groenewold, G.; Kawaguchi, H.; Konishi, M.; Krishnan, B.; Ohkuma, H.; Saitoh, K.; Doyle, T. W. *J. Am. Chem. Soc.* **1987**, *109*, 3462.

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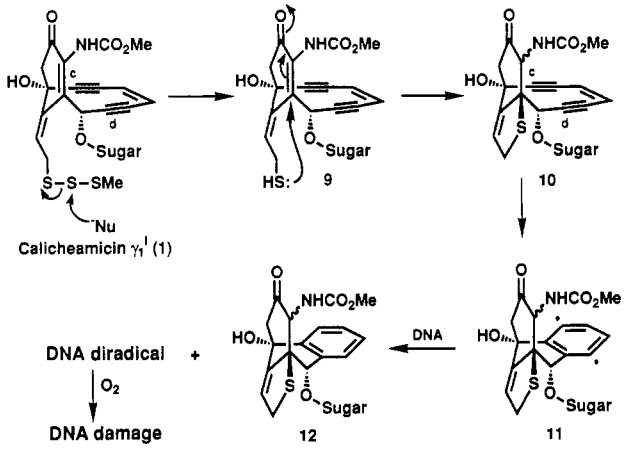
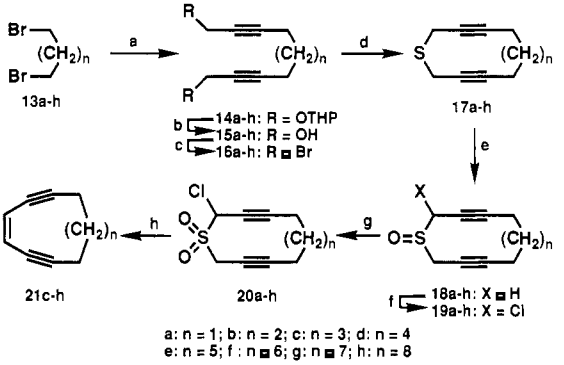
Scheme I



trisulfide then leads to the formation of a highly reactive thiol (**9**), which is perfectly positioned for 1,4-addition to the proximal α,β-unsaturated ketone, leading to compound **10**. This transformation, converting a trigonal bridgehead position to a tetragonal center, opens the way for a Bergman reaction leading to the benzenoid diradical **11**, the reactive species for DNA cleavage and hence cytotoxicity. Noteworthy is the change in the distance cd between the termini of the enediyne unit in calcheamicin γ_1^I (**1**) itself (cd = 3.35 Å)[8] and in the triggered enediyne **10** (cd = 3.16 Å). This suggested to us the idea that this distance may be used, at least in simple systems, as a guide for the strain energy of the enediyne system and the degree of p orbital overlap leading to bond formation, and hence its thermal stability toward Bergman cyclization.[9] It should be emphasized, however, that any such theoretical treatment would not necessarily translate well for more complicated systems, such as calicheamicin itself, such a treatment would ignore anti-Bredt strain energies associated with a bridgehead double bond which could, to a large extent, account for the thermal stability of the enediyne of calicheamicin prior to triggering.[10] With these considerations in mind, we embarked upon a systematic study of the Bergman reaction in simple monocyclic systems to probe the validity of this hypothesis.

The synthesis of the monocyclic 10-membered-ring enediyne and its homologues was the initial focus of this program. After several abortive attempts to construct the ring system via the palladium-catalyzed coupling of terminal acetylenes with vinyl halides, our efforts focused on the Ramberg–Bäcklund reaction[11]

Scheme II[a]



[a] Reagents and conditions: (a) $THPOCH_2C{\equiv}CH$, *n*-BuLi, HMPA, THF, −78 °C; (b) PPTS, MeOH; (c) *n*-Bu_3P, CBr_4, Et_2O, 0 °C; (d) $Na_2S{\cdot}9H_2O$, EtOH, H_2O, 25 °C; (e) *m*-CPBA, CH_2Cl_2, −78 °C; (f) SO_2Cl, Pyr, CH_2Cl_2, −78 °C; (g) *m*-CPBA, CH_2Cl_2, 0 °C; (h) 1.2 equiv of KO^{t}Bu, THF, −78 °C.

as the key process to form the enediyne moiety within these ring systems. Scheme II outlines the sequence by which this parent series of monocyclic conjugated enedynes were constructed.[9] In the key sequence of reactions, the propargylic dibromides **16a–h** were treated with sodium sulfide under high-dilution conditions to give the cyclic sulfides **17a–h** in varying yields (9% for **17a**, up to 72% for **17f**). These sulfides were then first oxidized to the corresponding sulfoxides **18a–h** with stoichiometric amounts of *m*-CPBA (50–85% yield) and then monochlorinated using sulfuryl chloride, forming **19a–h**. The crude products were further oxidized with excess *m*-CPBA to give chloro sulfones **20a–h** in 37–82% overall yields for the two steps. Finally, treatment of chloro sulfones **20c–h** with potassium *tert*-butoxide at −78 °C led to the formation of the desired cyclic enediynes **21c–h** in 32–52% isolated yields. Treatment of the 10- and 11-membered-ring sulfones **20a** and **20b** with potassium *tert*-butoxide led to the formation of products thought to be allenes, with no enediyne being formed. However, treatment of **20b** with methyllithium at −78 °C led to the desired 10-membered-ring enediyne **21b** (12% yield) along with the acyclic enediyne **25b** (12%, Scheme III) while treatment of the corresponding 10-membered cyclic sulfide **20a** under similar conditions failed to give the nine-membered enediyne **21a**, giving **25a** as the only isolated product (24% yield). Products **25a** and **25b** are presumed to arise from one of two mechanisns as outlined in Scheme III. Thus the initial base-induced formation of the episulfones **22a** and **22b** may be followed by chelatropic sulfur dioxide elimination to give the desired cyclic enediyne (path a), or it may undergo a Cope-type rearrangement (path b) to give the bis(allene) sulfones **23a** or **23b**. Spontaneous rearrangement of these highly strained bis(allenic) compounds accompanied by loss of sulfur dioxide then leads to the observed products **25a** or **25b**. The different partitioning between the two pathways can then be explained by the increased ring strain in **22a** compared with **22b**. The alternative pathway to **25a** and **25b** involving the intermediacy of the Bergman intermediates **24a** or **24b** is unlikely, since isolated **21b** does not convert into **25b**.

(4) Napier, M. A.; Holmquist, B.; Strydom, D. J.; Goldberg, I. H. *Biochem. Biophys. Rev. Commun.* **1979**, *89*, 635. Edo, K.; Mizugaki, M.; Koide, Y.; Seto, H.; Furihata, K.; Otake, N.; Ishida, N. *Tetrahedron Lett.* **1985**, *26*, 331. Myers, A. G. *Tetrahedron Lett.* **1987**, *28*, 4493.

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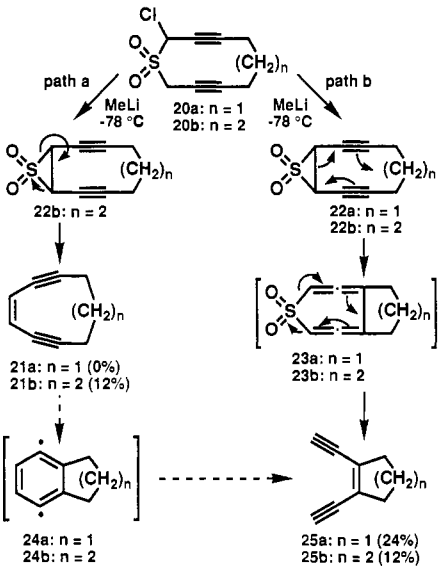
(8) Except where noted to the contrary, cd distances refer to MM2 minimized structures using W. C. Still's Macromodel.

(9) Nicolaou, K. C.; Zuccarello, G.; Ogawa, Y.; Schweiger, E. J.; Kumazawa, T. *J. Am. Chem. Soc.* **1988**, *110*, 4866.

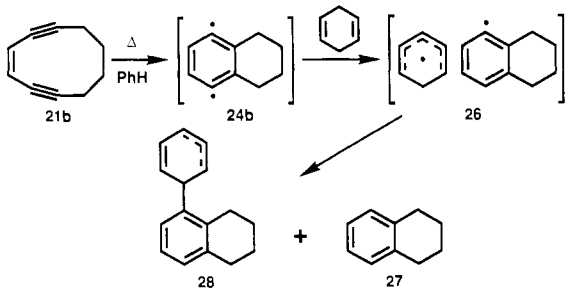
(10) Magnus, P.; Fortt, S.; Pitterna, T.; Snyder, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 4986. *Snyder*, J. P. *J. Am. Chem. Soc.* **1990**, *112*, 6367.

(11) Paquette, L. A. *Org. React.* **1977**, *25*, 1.

Scheme III



Scheme IV



While cyclononenediyne (**21a**) proved elusive and cyclodecenediyne (**21b**) exhibited reactivity toward Bergman cyclization at ambient temperature, the higher homologues **21c–h** were found to be thermally quite stable. Cycloundecenediyne (**21c**) crystallized from pentane, enabling an X-ray crystallographic analysis to be undertaken in order to compare the experimentally derived molecular parameters with the calculated ones, and a high degree of agreement was found, enhancing our confidence in using MM2 and related programs for these systems. In particular, the calculated cd value of 3.61 Å compared reasonably well with the experimental value of 3.66 Å.

The parent cyclodecenediyne system **21b** (cd = 3.25 Å), while being stable enough at ambient temperatures for purification and characterization purposes, slowly decomposes upon standing in solution. Thus **21b** underwent smooth Bergman cyclization in the presence of excess 1,4-cyclohexadiene (a good H atom donor) in benzene solution at 50 °C, leading to tetralin (**27**, 55%) and a mixture of cross-coupling products **28** (Scheme IV).

Subsequent to the early studies shown in Schemes II–IV, a collaboration with Bergman was undertaken in which the 10-membered cyclic enediynes **30** and **31** were prepared and studied (Scheme V).[12] Thus treatment of **29** with SmI_2 in THF at 25 °C gave a 42% yield of compounds **30** and **31** (1:20 ratio) while $TiCl_3$/Zn–Cu couple in DME at 25 °C gave a 45% yield in a reversed ratio of 2.6:1. Both **30** and **31** were observed to undergo

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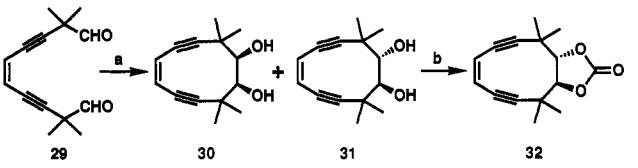
Scheme V[a]



[a] Reagents and conditions: (a) 5.0 equiv of SmI_2, THF, 25 °C, 1 h, 42% (trans/cis, ~20:1); or excess Ti(0) (from $TiCl_3$·$^3/_2$DME and Zn–Cu couple), DME, 25 °C, 12 h, 45% (trans/cis, ~1:2.6); (b) 1.1 equiv of $(COCl)_2$, 2.1 equiv of Et_3N, CH_2Cl_2, 0 °C, 15 min, 85%.

Table I. Calculated cd Distances and Stabilities of Cyclic Enediynes

compd	ring size	cd distance (Å)[a]	stability
1	10	3.35	stable at 25 °C
10	10	3.16	cyclizes at 25 °C
21a	9	2.84	unknown
21b	10	3.25	$t_{1/2}$ = 18 h at 25 °C
21c	11	3.66[b]	stable at 25 °C
21d	12	3.90	stable at 25 °C
21e	13	4.14	stable at 25 °C
21f	14	4.15	stable at 25 °C
21g	15	4.33	stable at 25 °C
21h	16	4.20	stable at 25 °C
30	10	3.29	$t_{1/2}$ = 4 h at 50 °C
31	10	3.34	$t_{1/2}$ = 2 h at 50 °C
32	10	3.42[b]	stable at 25 °C
33	10	3.20	$t_{1/2}$ = 11.8 h at 37 °C

[a] MM2 calculated values, except where indicated. [b] X-ray derived value.

slow decomposition at ambient temperatures, with measured half-lives of 4 and 22 h at 50 °C for the cis and trans isomers **30** and **31**, respectively (calculated cd distances: **30**, 3.29 Å; **31**, 3.34 Å). Introducing the cyclic carbonate in **32** provided a conformational lock for the trans isomer, and it is instructional to note that this compound (cd distance = 3.424 Å, X-ray) is thermally stable at 100 °C for 12 h.

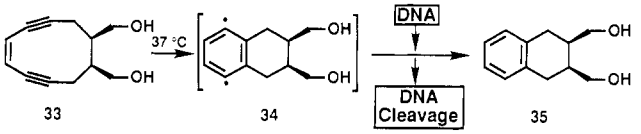
The stability data for the monocyclic compounds described above, summarized in Table I, shows a clear correlation between the cd distance and the stability of the enediyne with respect to Bergman cyclization such that compounds with a cd distance of less than 3.20 Å rapidly cyclize in 25 °C in while those with a cd distance of greater than about 3.35 Å are thermally stable at 25 °C. Those compounds which fall between this range display limited stability at ambient temperatures. These results therefore provide support for our hypothesis that the cd distance may be a useful guide for the prediction of stability of simple enediynes.

Biological Activity of the Simple Monocyclic Enediynes

Armed with the knowledge that simple 10-membered enediynes undergo Bergman cyclization at physiological temperatures with reasonable rates, we proceeded to attempt mimicking the DNA-cleaving action of the calicheamicins and esperamicins. In order to endow the projected molecule with at least partial water solubility and provide for the option of attachment to deliver systems, the diol **33** (Scheme VI) was designed and prepared via the previously describing Ramberg–Bäcklung strategy.[13] The crucial expectation was that it would be sufficiently stable for isolation and handling

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

Scheme VI



at ambient temperatures, but that it would undergo Bergman cyclization at physiological temperatures at useful rates to cause DNA cleavage. These expectations were based upon a calculated cd distance of 3.20 Å and the experimental data obtained with the parent cyclodecenediyne **21b**. The DNA-cleaving properties of **33** were explored with ΦX174 double-stranded supercoiled DNA (Scheme VI). According to our expectations, this enediyne caused noticeable DNA cleavage in the absence of any additives at concentrations as low as 10 μmol at 37 °C, with the extent of cleavage being dependent upon concentration, incubation time, and temperature. As a control, it was demonstrated that the corresponding Bergman cyclized product caused no DNA cleavage. Similarly, the diols **30** and **31** were found to cause DNA cleavage while the "conformationally locked" enediyne **32** failed to effect DNA cleavage. The cleavage data were consistent with a Bergman cyclization of **33** leading to a diradical species **34** which proceeds to abstract hydrogen atoms from DNA in the mechanistic mode similar to the one proposed for the calicheamicins and esperamicins.[2,3] These results thus constituted the first designed DNA-cleavage agents based upon the mechanism of action of the calicheamicins/esperamicins.

Designed Enediynes Inspired by Dynemicin A

In 1989 the structure of dynemicin A (**4**) was published.[5] This antibiotic exhibits very potent antibacterial activity against Gram-positive bacteria and antitumor activity with IC_{50} values of ~4–5 ng/mL against a number of human cell lines and prolongs the life span of mice inoculated with P388 leukemia and B16 melanoma. Unlike the calicheamicin/esperamicin antibiotics, dynemicin A (**4**) displays significant in vivo antibacterial activity and low toxicity. Structural studies revealed that dynemicin A (**4**), like the calcheamicins/esperamicins, belongs to the class of antibiotics containing a 1,5-diyn-3-ene bridging ring; however, dynemicin A (**4**) is unique in combining the enediyne unit with the anthraquinone chromophore of the anthracycline antibiotics.[14]

A mechanism for the antitumor activity of dynemicin A (**4**) was proposed which combines elements of the mechanisms of action of the calicheamicin/esperamicin, neocarzinostatin, and anthracycline classes of antibiotics and which is supported by the observation that DNA cleavage by dynemicin A (**4**) is enhanced by the presence of reducing agents such as NADPH and thiols.[15,16] In this mechanism (Scheme VII), the anthraquinone nucleus intercalates with DNA and undergoes bioreduction to give the 9,10-anthraquinol **36**. This rearranges via epoxide opening to give the quinone methide **37**, which is trapped by a nucleophile (e.g., H_2O) to give a cis-opened epoxide such as **38**. The strategically located nitrogen atom may also facilitate epoxide opening, either directly by electron donation or indirectly by acting as a base to deprotonate the adjacent acidic phenol in **36**. Opening of the epoxide moiety causes a dramatic conformational change in the molecule such that the distance between the termini of the 1,5-diyn-3-ene system (cd distance) is reduced from 3.54 Å (X-ray) in dynemicin A (**4**) to 3.17 Å (MM2) in the *cis*-diol **38**. This facilitates a rapid Bergman cyclization to give the DNA-damaging benzenoid diradical **39**. The proposed mechanism thus involves opening of the epoxide as a trigger for Bergman cyclization of the enediyne and the DNA cleavage/antitumor activity.

The promising antitumor activity of dynemicin A (**4**), combined with its synthetically interesting structure, prompted us to explore the synthesis and properties of dynemicin A models not only with a view to synthesizing dynemicin A (**4**) itself but also to shed further light upon the mechanism of action of this fascinating molecule. Scheme VIII summarizes the synthesis of the first functioning models of dynemicin A, **50** and **52**.[17] Key features of the strategy include regiospecific functionalization of the saturated ring at C-10 through an *N*-oxide rearrangement (**40** → **41**),[18] regiospecific introduction of the acetylenic unit (**43** → **44**),[19] a Pd(0)–Cu(I)-catalyzed coupling reaction to introduce the enediyne (**47** → **48**),[20] and a base-induced closure of the enediyne ring (**49** → **50**). Thus treatment of the model **52** with *p*-toluenesulfonic acid in benzene/1,4-cyclohexadiene at 25 °C for 24 h cleanly produced the aromatized product **55**, presumably through the intermediacy of the diol **53**. It is interesting to note the change in the cd distance in going from **52** to **53** (3.59 and 3.18 Å, respectively), fitting in well with our previous hypothesis.[9] Similarly, the use of HCl in CH_2Cl_2 also resulted in triggering epoxide opening and Bergman cyclization of **52**, this time trapping the benzyl cation formed with chloride to give the chlorinted product **56**. These results are analogous to those obtained with dynemicin A (**4**) itself.[15] For the dynemicin A model **50**, treatment under similar conditions also effected also opening and Bergman cyclization, but was further complicated by a pinacol-type rearrangement.[17]

While the dynemicin models such as **50** and **52** (Scheme VIII) in which the nitrogen was protected as the carbamate were robust and displayed no tendency to undergo the Bergman cyclization in the absence of fairly strong acid, the corresponding free amine **57** was rather labile (estimated half-life at 37 °C, ca. 4 h) and, significantly, caused notable DNA cleavage when incubated with ΦX174 DNA.[17] Figure 2 shows the DNA-cleaving action of the related free amine compound **76** (Scheme XII, vide infra). Furthermore, these results clearly indicated that **57** caused cleavage of double-stranded DNA (giving linear form III) like dynemicin A (**4**) itself.[15] These results suggested a DNA-cleavage mode of action as outlined in Scheme IX. Opening of the epoxide moiety of **57** is initiated

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(16) Snyder, J. P.; Tipsworth, G. E. *J. Am. Chem. Soc.* **1990**, *112*, 4040.
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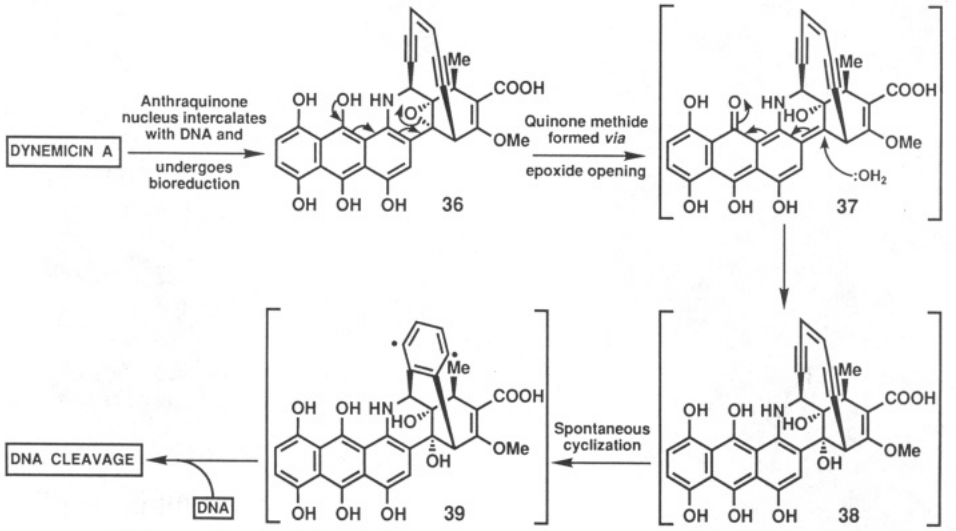
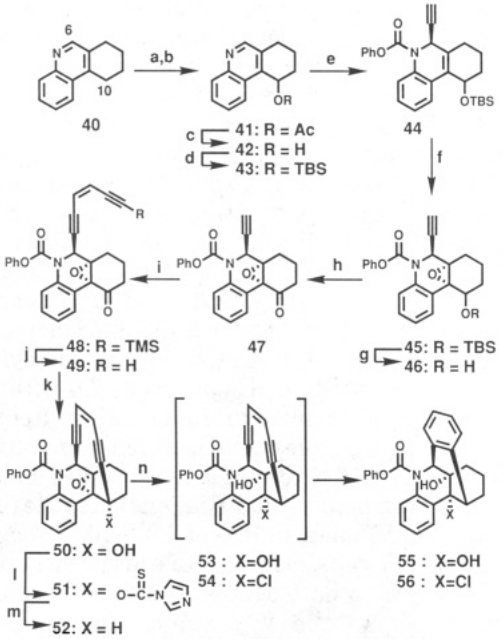
Scheme VII



Scheme VIII[a]



[a] Reagents and conditions: (a) 1.0 equiv of *m*-CPBA, CH_2Cl_2, 25 °C, 1 h, 80%; (b) Ac_2O, reflux, 20 h, 77%; (c) K_2CO_3 (catalytic), MeOH, 25 °C, 1 h, 100%; (d) 1.2 equiv of $^tBuMe_2SiOTf$, 1.4 equiv of 2,6-lutidine, CH_2Cl_2, 0.5 h, 92%; (e) 3.0 equiv of ethynylmagnesium bromide, 3.0 equiv of PhOCOCl, THF, −78 → 25 °C, 1 h, 92%; (f) 2.0 equiv of *m*-CPBA, CH_2Cl_2, 25 °C, 3 h, 85%; (g) 1.2 equiv of TBAF, THF, 42 °C, 3 h, 95%; (h) 3.0 equiv of PCC, CH_2Cl_2, 4-Å molecular sieves, 25 °C, 1 h, 81%; (i) 1.4 equiv of (*Z*)-(4-chloro-3-buten-1-ynyl)trimethylsilane, 1.5 equiv of *n*-$BuNH_2$, 0.25 equiv of PPh_3, 0.05 equiv of $Pd(OAc)_2$, 0.2 equiv of CuI, PhH, 25 °C, 4 h, 88%; (j) 4.0 equiv of $AgNO_3$, 7.0 equiv of KCN, H_2O, EtOH, THF, 25 °C, 10 min, 90%; (k) 1.1 equiv of LDA, toluene, −78 °C, 1 h, 80% based on 25% recovery of **49**; (l) 3 equiv of (thiocarbonyl)bis(imidazole), 0.5 equiv of DMAP, CH_2Cl_2, 25 °C, 48 h, 91%; (m) 2 equiv of *n*-Bu_3SnH, AIBN (catalytic), toluene, 75 °C, 2 h, 75%; (n) (i) 0.05 M in benzene–1,4-cyclodiene (4:1), 1.2 equiv of TsOH·H_2O, 24 h, 25 °C, 86% (X = OH); or (ii) HCl(g), 40 equiv of 1,4-cyclohexadiene, CH_2Cl_2, 1 min, 25 °C, (X = Cl).

by the lone pair of electrons on the nitrogen (which is unavailable due to delocalization in the case of the carbamates) to give the *o*-quinone methide-type intermediate **58**. Nucleophilic trapping gives the cis-opened epoxide **59** (cd = 3.15 Å), which undergoes the Bergman cyclization to give the DNA-damaging benzenoid diradical **60**.

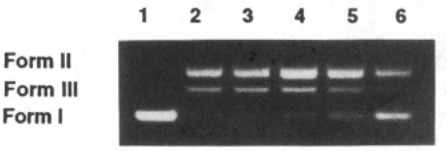
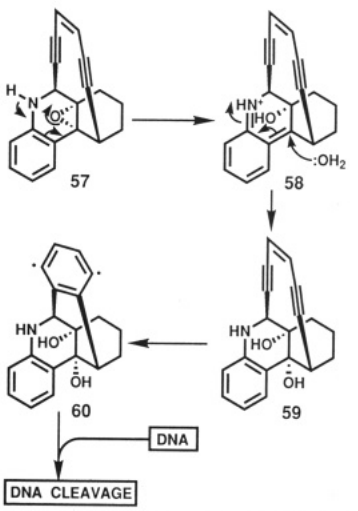


Figure 2. ΦX174 form I DNA (50 μM per base pair) was incubated for 4 h at 37 °C with compound **76** (in 10% EtOH in phosphate buffers, pH 7.4, 50 mM) and analyzed by gel electrophoresis (1% agarose, ethidium bromide stain). Lane 1, control; lanes 2–6, 5000, 2000, 1000, 500, 100 μM **76**, respectively.

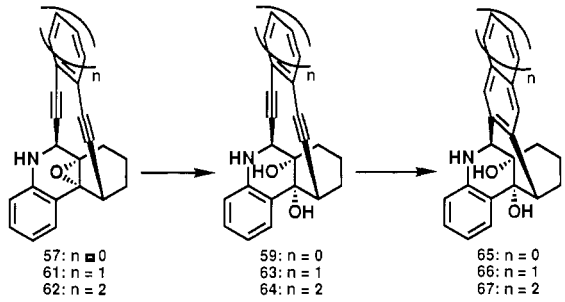
Scheme IX. Proposed DNA Cleavage Mode of Action of Dynemicin A Model 57



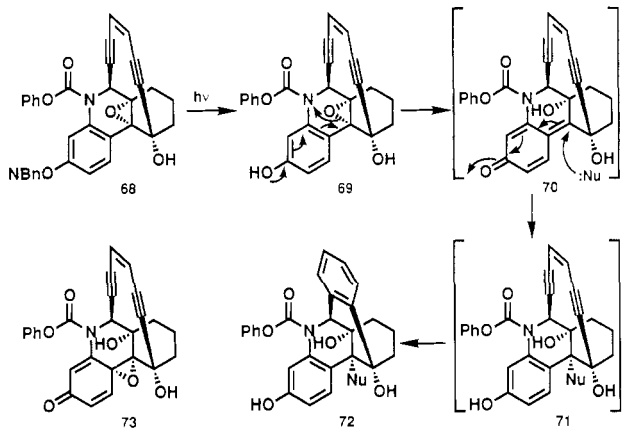
The instability of the enediyne-containing compounds in which the epoxide had been opened (e.g., **57**, Scheme X) led us to seek a means by which we could tame the reactivity of the *cis*-diols sufficiently to observe them.[21] Recalling the resonance energies of benzene (36 kcal/mol), naphthalene (61 kcal/mol), and anthracene (84 kcal/mol), we reasoned that there should be less of a driving force for cycloaromatization of the diols **63** and **64** compared with **59**. Indeed, treatment of **61** and **62** with silica gel in wet benzene led smoothly to the *cis*-diols **63** and **64**. The benzene diyne **63** was stable enough to be detected by TLC and ^1H NMR spectroscopy, but cyclized readily upon standing at

(21) Nicolaou, K. C.; Hong, Y.-P.; Torisawa, Y.; Tsay, S.-C.; Dai, W.-M. *J. Am. Chem. Soc.* **1991**, *113*, 9878.

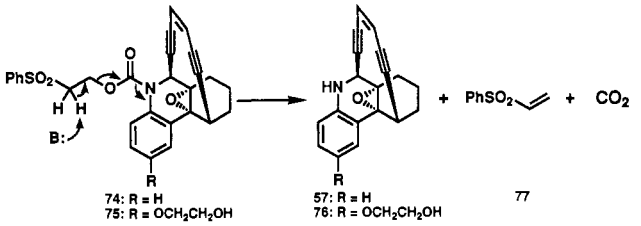
Scheme X



Scheme XI



Scheme XII



ambient temperature ($t_{1/2}$ ~2.5 h at 20 °C). On the other hand, the naphthalene diyne **64** exhibited enhanced stability compared to **63** and could be purified by chromatography and characterized by the usual means. Its half-life at 37 °C was determined to be ca. 44 h.

The ability of the free amine in these molecules to provide sufficient electron-donating power to unlock the epoxide prompted us to explore other novel triggering devices for the system (Figure 3). It was reasoned that other electron-donating substituents suitably positioned on the aromatic ring may provide a means of triggering the molecule, and to this end the system **68** (Scheme XI) was designed and synthesized.[22] Indeed, irradiation of the *o*-nitrobenzyl ether **68** resulted in its clean conversion to **69** (observed by TLC and ^{1}H NMR), although attempts to isolate it resulted on decomposition. Treatment of crude **69** with nucleophiles (e.g., EtOH, EtSH, *n*-PrNH$_2$) furnished the aromatized products **72**. An important observation in these reactions was the isolation of the novel quinone epoxide structure **73** from the reaction with ethanol in air as a sensitive but thermally stable molecule under neutral conditions. The isolation of **73** provides direct

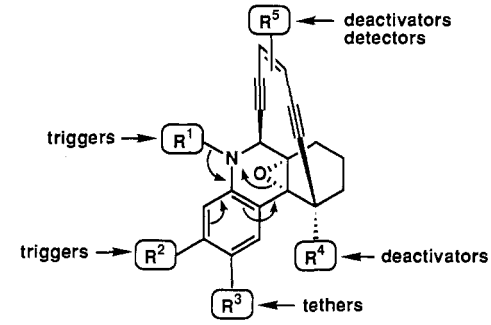


Figure 3.

evidence for the intermediacy of quinone methide **70**, being formed by trapping of **70** with molecular oxygen.[23]

Antitumor Activity of the Dynemicin Models

Given the potent antitumor activity of dynemicin A (**4**) (IC_{50} = 10^{-7}–10^{-11} M against 10 different tumor cell lines), the cytotoxicities of our dynemicin model compounds were tested.[24] Thus, while the phenyl carbamate **52** displayed a modest IC_{50} of 3.1×10^{-6} M against the sensitive Molt-4 leukemia cell line, the free amine **57** had a 10 000-fold increase in activity with an IC_{50} of 1.6×10^{-10} M against the same cell line, strongly reflecting the role of the carbamate protecting group in acting as a "lock" for the epoxide and the ability of the molecule to undergo the Bergman cyclization. This led to the design of the 2-(phenylsulfonyl)ethyl carbamate as a "lock" which could potentially be removed under mild, physiological conditions due to its susceptibility to cleavage in mildly basic media (Scheme XII). Indeed, slow release of the free amine and phenyl vinyl sulfone was observed at pHs as low as 7.4 with these compounds. This had a profound effect upon the cytotoxicity in the compounds, which compound **74** having an IC_{50} of 2.5×10^{-11} M against Molt-4 leukemia cells. Further structural modifications led to the finding that compound **75** had an IC_{50} of 2.0×10^{-14} M against Molt-4 leukemia cells, making it one of the most potent agents reported to date against tumor cell lines.

Table II shows the IC_{50} values determined for enediyne **75** with 21 different cell lines. There are significant differences in cytotoxicities, ranging from 10^{-6} M for the highly resistant melanoma cell lines to 10^{-14} M for the highly sensitive leukemia cell line. Particularly important is the high cytotoxicity against the multiple-drug-resistant TCAF-DAX cell line (IC_{50} = 1.7×10^{-9} M). Another striking feature is its relatively low cytotoxicity against a number of normal cell lines, and preliminary in vivo studies with animals infected with leukemia and solid tumors show encouraging results.[25]

In order to confirm that the remarkable cytotoxicity of enediyne **75** is indeed due to DNA damage, Molt-4 leukemia cells were treated with ethidium bromide, which intercalated into the DNA, rendering it fluorescent. Exposure of these cells to enediyne **75** at a concentration of 10^{-5} M led to rapid DNA strand breakage as determined by fluorimetry, resulting in 95% destruction after 4 h at 37 °C. Cell death showed an

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Table II. Cytotoxicities of Designed Enediyne 75 against a Panel of 21 Tumor Cell Lines (Top) and Four Normal Cell Lines (Bottom)[a]

cell type	cell line	IC_{50} (M)
melanoma	SK-MeI-28	3.1×10^{-6}
melanoma	M-14	1.6×10^{-6}
melanoma	M-21	1.6×10^{-6}
colon carcinoma	HT-29	1.6×10^{-6}
ovarian carcinoma	Ovcar-3	7.8×10^{-7}
ovarian carcinoma	Ovcar-4	7.8×10^{-7}
astrocytoma	U-87 UG	7.8×10^{-7}
glioblastoma	U-251 MG	3.9×10^{-7}
breast carcinoma	MCF-7	7.8×10^{-7}
lung carcinoma	H-322	3.9×10^{-7}
lung carcinoma	H-358	2.0×10^{-7}
lung carcinoma	H-522	9.8×10^{-8}
lung carcinoma	UCLA P-3	9.8×10^{-8}
pancreatic carcinoma	Capan-1	3.1×10^{-9}
T-cell leukemia	TCAF	1.1×10^{-9}
T-cell leukemia[a]	TCAF-DAX	1.7×10^{-9}
myeloma	RPMI-8226	7.7×10^{-9}
mouse leukemia	P-388	4.6×10^{-9}
mouse leukemia	L-1210	1.3×10^{-9}
promyeocytic leukemia	HL-60	3.6×10^{-11}
T-cell leukemia	Molt-4	2.0×10^{-14}
bone marrow	HNBM	5.0×10^{-5}
human mammary epithelial cells	HMEC	6.3×10^{-6}
normal human dermal fibroblast	NHDF	5.0×10^{-6}
Chinese hamster ovary	CHO	3.1×10^{-6}

[a] Multiple drug resistant cell line.

approximately 2 h delay relative to DNA strand breakage, implicating DNA strand cleavage as the direct cause of cell destruction in these experiments. It was also shown that compound **75** severely impairs the ability of Molt-4 leukemia cells to synthesize DNA (inhibition of [^{3}H]thymidine uptake), RNA (inhibition of [^{3}H]uracil uptake), and protein (inhibition of [^{3}H]-leucine uptake).

These results raise intriguing questions of how enediyne **75** is triggered intracellularly and what allows it to be so selective against different cell types. The projection that the chemical machinery of the cell would be capable of triggering the Bergman cyclization by removing the nitrogen-bound protecting device was supported by chemical studies. However, the selectively issue is not yet fully understood. Speculations include the possibility of the presence of tumor-associated factors which may activate these systems, differences in the permeability of membranes in normal and transformed cells regarding these molecules, and different abilities of normal and transformed cells to repair DNA damage caused by these agents.

Future Developments

The isolation of the enediyne antibiotics has prompted a great deal of activity by synthetic chemists, much of it directed at the total synthesis of these molecules and the synthesis of aglycon portions of calicheamicin γ_1^I (**1**) (calicheamicinone) and esperamicin A_1 (**2**), in particular.[26] Notable successes have included the first racemic synthesis of calicheamicinone by Danishefsky[27] and the first enantioselective syntheses of (−)-calicheamicinone[28] and the calicheamicin γ_1^I oligosaccharide[29] by us. These results make it also certain that the total synthesis of calicheamicin γ_1^I (**1**) will soon be achieved.

Perhaps more exciting, however, is the potential these molecules have provided us for developing structurally simpler mimics which have comparable, or even enhanced, properties over the natural products. It is intriguing to think that Bergman provided us with the necessary information to develop this field 20 years ago, but it took Nature to show us the way. This should serve as a constant reminder to those with little appreciation for natural products chemistry that this discipline continues to provide valuable leads which become the guiding forces in our search for new scientific ventures and the "wonder drugs" of tomorrow. Who knows what our treatures lie buried in our libraries waiting to be rediscovered through Nature, and what riches await those few individuals with the imagination to see past the bare facts and find the gold mine hidden beneath?

We thank our associates, whose names appear in the original articles upon which this Account is based, for their invaluable contributions to this research effort. This work was financially supported by the National Institutes of Health, the National Science Foundation, and The Scripps Research Institute.

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