Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 5881–5888, July 1993

Review

Chemistry and biology of natural and designed enediynes

K. C. Nicolaou, A. L. Smith†, and E. W. Yue*

Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037; and Department of Chemistry, University of California at San Diego, La Jolla, CA 92093

ABSTRACT **Ever since the initial reports of the enediyne anticancer antibiotics in the late 1980s, researchers from a number of disciplines have been devoting increasing attention to their chemistry, biology, and potential medical applications. Synthetic chemists and molecular designers have been engaged in attempts to synthesize these molecules and to model their unique architecture. Considerable efforts have been directed at understanding and mimicking the various processes involved in the targeting, activation, and DNA cleavage associated with these natural products. This review summarizes the main contributions to the field, with particular emphasis on work from our laboratories. Highlights include studies of the Bergman reaction, which is central to the mechanism of action of enediynes, the design and chemical synthesis of a number of these systems, and biological studies with selected molecules. Finally, the total synthesis of calicheamicin γ_1^I, the most prominent member of this class of naturally occurring compounds, is discussed.**

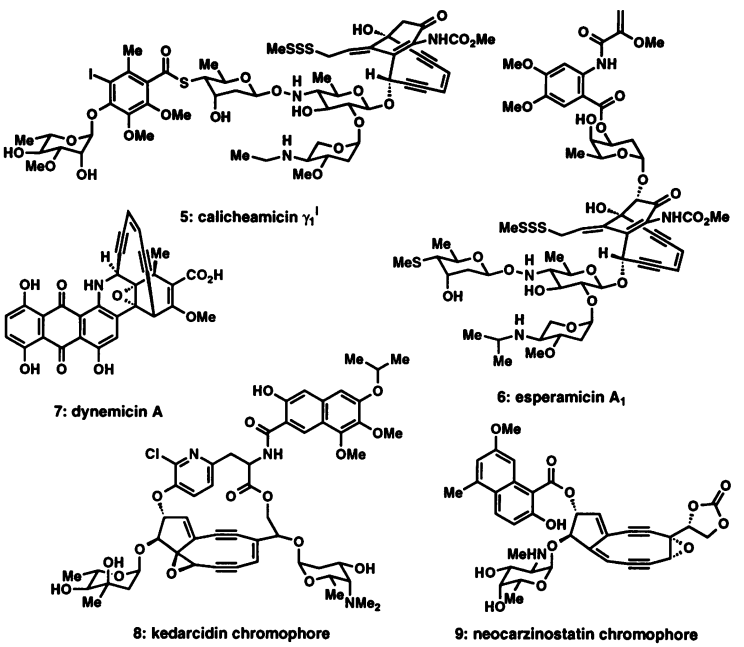
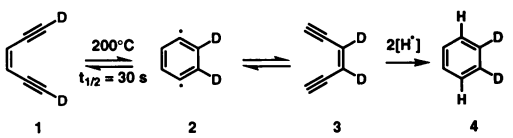


FIG. 1. Naturally occurring enediyne anticancer antibiotics.

Nature continually serves to provide scientists with new ideas, often provoking us to reexamine previous studies and leading us in new directions. An example of such an instance which has captured the imagination of many scientists, ourselves included, came in 1987 with the unveiling of a new class of natural products, the so-called "enediyne anticancer antibiotics" (Fig. 1), which possess potent anticancer activity and a hitherto unseen biological mode of action (for a previous review, see ref. 1). The story began some 15 years earlier, however, for it was in the laboratories of Robert Bergman that the intriguing chemical processes which provide the key to understanding the remarkable activity of these antibiotics were first studied (2). The findings of Bergman are summarized in Scheme I, in which the enediyne system **1** was observed to undergo cycloaromatization when heated to give the benzene ring **4** with the intermediacy of the highly reactive 1,4-benzenoid diradical species **2**. Masamune and coworkers (3) and Wong and Sondheimer (4) also observed the cycloaromatization of 10-membered ring enediynes. However, the full significance of these simple observations became apparent only when the structures of calicheamicin γ_1^I (**5**, Fig. 1) and esperamicin A_1 (**6**, Fig. 1), the first representatives of the enediyne antibiotics, were reported (5–8). For at the very heart of these molecules is contained the same enediyne unit; and therein lies the means by which these unprecedented molecules exert their remarkable biological properties. Dynemicin A (**7**, Fig. 1) (9, 10) and kedarcidin chromophore (**8**, Fig. 1) (11), representing structurally distinct classes of enediyne antibiotics, were subsequently reported. Neocarzinostatin chromophore (**9**, Fig. 1) (12–14) has also been grouped with the enediyne antibiotics due to the similarity of its structure and mode of action.



SCHEME I. Bergman's original design and observation regarding the thermal cycloaromatization of enediynes.

Molecular Structures, Biological Properties, and Mechanisms of Action of Naturally Occurring Enediynes

Calicheamicin γ_1^I (**5**), the most prominent member of the calicheamicins, was isolated from *Micromonospora echinospora* ssp. *calichensis* and is a remarkable piece of engineering by Nature, which has perfectly constructed the molecule to endow it with its extraordinary chemical and biological properties (15). These include activity in the biochemical prophage induction assay at concentrations < 1 pM, high antibacterial activity, and extreme potency against murine tumors such as P388 and L1210 leukemias and solid neoplasms such as colon 26 and B16 melanoma with optimal doses of 0.15–5 μg/kg. Calicheamicin γ_1^I (**5**), along with the

*To whom reprint requests should be addressed.
†Present address: Merck Sharp & Dohme Research Laboratories, Terlings Park, Harlow, Essex CM20 2QR, U.K.

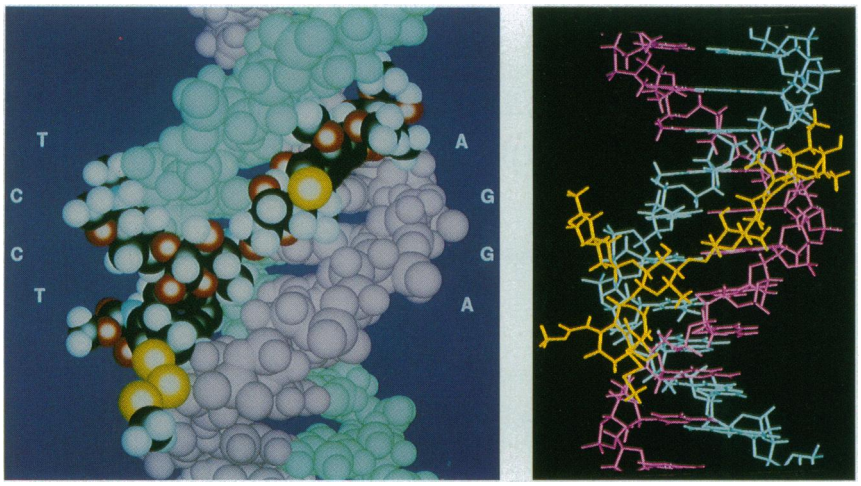
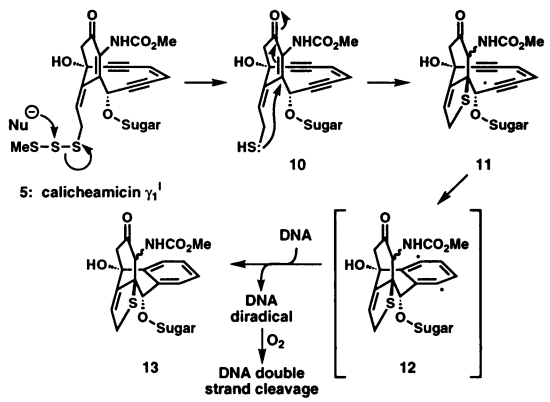


FIG. 2. Computer-generated model of DNA-bound calicheamicin γ_1^I (5) along a TCCT site. [Reprinted with permission from ref. 1 (copyright VCH, Weinheim, FRG).]

other enediyne antibiotics, is believed to exert its biological activity by damaging DNA. Indeed, it is a highly potent DNA-cleaving agent giving rise primarily to sequence-selective double-strand cuts (16).

The calicheamicin γ_1^I molecule contains two distinct structural regions. The larger of the two consists of an extended sugar residue comprising four monosaccharide units and one hexasubstituted benzene ring which are joined together through a highly unusual series of glycosidic, thioester, and hydroxylamine linkages. It is this aryloligosaccharide which serves to deliver the molecule to its target, binding tightly in the minor groove of double-helical DNA and displaying high specificity for sequences such as 5′-TCCT-3′ and 5′-TTTT-3′ (16, 17) through significant hydrophobic interactions and other forces (Fig. 2). This binding is thought to be facilitated by substantial preorganization of the oligosaccharide into a rigid, extended conformation (18). Molecular modeling calculations by Schreiber and coworkers (19) suggested that a significant portion of the sequence selectivity for 5′-TCCT-3′ arises from a favorable interaction between the large and polarizable iodo substituent of the hexasubstituted aromatic ring and the exocyclic amino substituents of the two guanines in the 3′-AGGA-5′ tract. Experimental evidence from our own laboratories supports the idea of an important role for the iodine in the binding affinity of this oligosaccharide to DNA (20).

The second region of calicheamicin γ_1^I is its aglycon, a rigid, highly functionalized bicyclic core termed calicheamicinone, which acts as the "warhead" of the molecule. The enediyne functionality of the molecule is locked within a rigid 10-membered bridged ring awaiting activation to undergo the Bergman reaction. Also forming part of the aglycon is a trisulfide, which serves as the trigger. Once the molecule is in the vicinity of DNA (whether prior or subsequent to binding is not known), a series of chemical events unfold which ultimately leads to DNA cleavage (Scheme II) (5–8). A nucleophile (e.g., glutathione) attacks the central sulfur atom of the trisulfide group, causing the formation of a thiolate or a thiol (10) which adds intramolecularly to the adjacent α,β-unsaturated ketone embedded within the framework of the aglycon. This reaction, converting a trigonal bridgehead position to a tetragonal center, causes a significant change in structural geometry which imposes a great deal of strain on the 10-membered ring. This strain is completely relieved by the enediyne undergoing the Bergman reaction, generating a highly reactive benzenoid diradical (12). The calicheamicin diradical abstracts hydrogen atoms from duplex DNA at the C-5′ position of the cytidine in 5′-TCCT-3′ and the C-4′ position of the nucleotide three base pairs removed on the 3′ side of the complementary strand (21, 22), leading to cleavage of both strands of DNA. The mechanism of action of the structurally similar esperamicins is thought to be essentially the same as that of the calicheamicins.



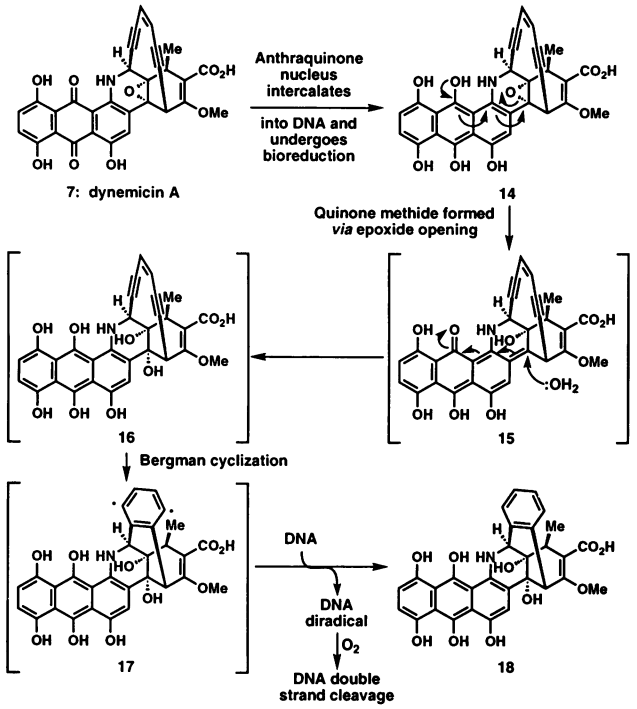
SCHEME II. Mechanism by which calicheamicin γ_1^I (5) cleaves DNA. Nu, nucleophile.

Dynemicin A (7), the first member of the dynemicin subclass of enediyne antibiotics to be reported (9, 10), was discovered in the fermentation broth of *Micromonospora chersina*. It exhibits very potent activity against a variety of cancer cell lines and significantly prolongs the lifespan of mice inoculated with P388 leukemia and B16 melanoma cells. Furthermore, it exhibits promising *in vivo* antibacterial activity with low toxicity. Like the calicheamicins and esperamicins, the dynemicins include in their molecular structures a 10-membered ring with a 1,5-diyne-3-ene bridge. They are, however, unique in combining the enediyne unit with the anthraquinone chromophore of the anthracycline anticancer antibiotics.

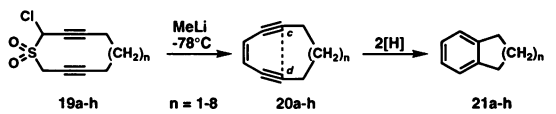
A mechanism for the antitumor activity of dynemicin A (7) has been proposed which combines elements of the mechanisms of action of the calicheamicin/esperamicin, neocarzinostatin, and anthracycline classes of antibiotics and which is supported by the observation that DNA strand cleavage by dynemicin A is enhanced by the presence of thiols. In this mechanism (Scheme III) (10), the anthraquinone nucleus intercalates into the DNA and undergoes bioreduction (not necessarily in that order), facilitating opening of the epoxide. This causes a significant conformational change in the molecule and introduces considerable strain into the enediyne system which is relieved by the new system undergoing the Bergman reaction, thus generating the DNA-damaging diradical species 17 (10).

Molecular Design, Chemical Synthesis, and Biological Actions of Enediynes

The reports of the structures of the calicheamicins and esperamicins, and their fascinating mode of action, prompted several groups to undertake investigation of the Bergman reaction in simple cyclic systems. The synthesis of the monocyclic 10-membered ring enediyne and its homologues was the initial focus of a



SCHEME III. Mechanism by which dynemicin A (7) cleaves DNA.



SCHEME IV. Synthesis and cycloaromatization of monocyclic enediynes.

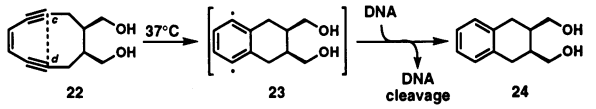
program in these laboratories (23, 24). The parent series of 10- through 16-membered ring enediynes (**20b–h**) were conveniently prepared via the Ramberg–Bäcklund reaction of the corresponding α-chlorosulfones **19b–h** (Scheme IV). The 10-membered ring enediyne **20b** readily underwent the Bergman reaction at room temperature with a half-life of 18 hr (Table 1), while the larger ring enediynes (**20c–h**) were found to be stable. By contrast, the 9-membered ring **20a** could not be prepared, although products formally arising from a Bergman reaction were identified. Comparison of the distances *cd* between the termini of the enediyne moiety of these systems and the ease with which they underwent the Bergman reaction (Table 1) showed a clear trend in which a decreased *cd* distance reflected, in addition to closer intimacy between the acetylenic groups, an increased ring torsion and hence an increased tendency to undergo the Bergman reaction in order to relieve the strain. For these simple systems a critical upper limit for the *cd* distance of around 3.2–3.3 Å appeared to be required for the Bergman reaction to occur at a measurable rate at ambient temperatures. This finding provided a useful means of predicting the thermal stability of compounds in subsequent work in our laboratories. More sophisticated calculations and kinetics experiments by Snyder (25) and Magnus *et al.* (26) later demonstrated that the crucial factor in determining the ease with which a particular system undergoes the Bergman reaction is the relative strain energies of the ground and transition states for the reaction. These findings should always be borne in mind when the empirical *cd* distance rule is applied.

Table 1. Calculated *cd* distances and stabilities of cyclic enediynes

Compound	*n*	Ring size	*cd* distance, Å	Stability
20a	1	9	2.84	Unknown
20b	2	10	3.25	$t_{1/2}$ = 18 hr at 25°C
20c	3	11	3.61	Stable at 25°C
20d	4	12	3.90	Stable at 25°C
20e	5	13	4.14	Stable at 25°C
20f	6	14	4.15	Stable at 25°C
20g	7	15	4.33	Stable at 25°C
20h	8	16	4.20	Stable at 25°C
22	—	10	3.20	$t_{1/2}$ = 11.8 hr at 37°C
25	—	10	3.29	$t_{1/2}$ = 4 hr at 50°C
26	—	10	3.34	$t_{1/2}$ = 2 hr at 50°C
27	—	10	3.42	Stable at 25°C

Given that the simple 10-membered ring enediyne **20b** underwent the Bergman reaction at physiological temperatures at a reasonable rate, we proceeded to attempt to mimic the DNA-cleaving action of the calicheamicins and esperamicins by using such simple systems. The diol **22** (Scheme V) was designed in order to endow the molecule with some degree of water solubility and also to provide for the option of attachment to delivery systems (24, 27). It was correctly predicted from the calculated *cd* distance of 3.20 Å that this molecule would be sufficiently stable for isolation and handling at ambient temperatures but would undergo the Bergman reaction at physiological temperature at a sufficient rate to cause DNA cleavage. Thus enediyne **22** caused significant cleavage of phage φX174 double-stranded supercoiled DNA in the absence of any additives at concentrations as low as 10 μM at 37°C, with the extent of cleavage being dependent upon concentration, incubation time, and temperature (24, 27). As a control, it was demonstrated that the corresponding Bergman cyclized product **24** (Scheme V) caused no DNA cleavage (24, 27). These molecules thus constituted the first designed DNA-cleaving agents based upon the mechanism of action of the calicheamicins/esperamicins. Later on, the thermally reactive diols **25** and **26** were similarly demonstrated to effect DNA cleavage whereas the conformationally locked and thermally stable derivative **27** (Fig. 3) failed to cleave DNA (28). Under basic conditions, however, compound **27** became active via the release of system **26** thus exhibiting both DNA cleavage and cytotoxic properties.

Since the naturally occurring enediyne antibiotics are triggered to exert their biological actions by bioreductive processes, a system was designed to control the Bergman cyclization by a hydroquinone ⇌ quinone redox process (29). It was postulated that hydroquinone systems such as **28** should be more stable toward cycloaromatization than the corresponding quinones **29** and **30** (Fig. 3). This was indeed found to be the case. Thus the hydroquinone **28** had a half-life of 74 hr at 110°C, while **29** and **30** had half-lives of 2.6 hr and 32 min, respectively at 55°C. These results were further emphasized by the finding that, while **28** exhibited no DNA-cleaving properties, **29** and **30** were able to cause DNA damage and death of tumor cells.



SCHEME V. Enediyne **22** as a designed DNA-cleaving agent.

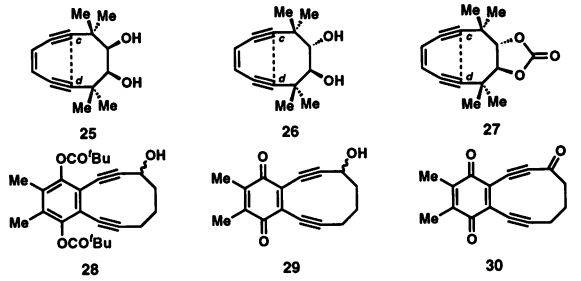
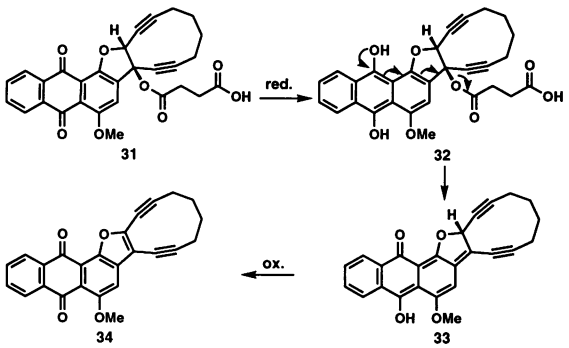


FIG. 3. Examples of designed enediynes with modulated reactivities.

This concept of activation of enediyne systems through redox processes was taken a step further by Myers and Dragovich (30), who designed the system shown in Scheme VI. Enzyme-mediated reduction of the anthraquinone **31** led to elimination of succinic acid followed by tautomerization and oxidation to reveal the enediyne system in **34**. This then slowly underwent the Bergman reaction at 37°C ($t_{1/2} \approx 2$ days).

Several groups have synthesized model systems of the calicheamicin/esperamicin enediyne core and studied their Bergman cycloaromatization. Notable among the early contributions are the studies of Danishefsky (e.g., **35**, Fig. 4) (31, 32), Kende (**36**, Fig. 4) (33), Magnus (e.g., **37**, Fig. 4) (34, 35), and Schreiber (**38**, Fig. 4) (36) and their coworkers. Particularly interesting were the observation of Danishefsky and collaborators (37, 38) that such simple enediyne systems can simulate the DNA cleaving properties of calicheamicin γ_1^I and the synthetic work of Magnus and coworkers (34, 35) which utilized acetylene cobalt complexes as intermediates to arrive at the targeted enediynes.

When the structure and mode of action of dynemicin A (**7**) were reported in 1989 (9), several groups, including ours, undertook programs directed toward the design and synthesis of models which would mimic the mechanism of action and facilitate the total synthesis of this highly unusual molecule. Work from these laboratories culminated in the molecular design, chemical synthesis, and biological evaluation of several dynemicin A mimics (39–47), including the highly potent systems **39** and **40** (Scheme VII) (42, 43). The latter system incorporated what was perceived to be the central structural features responsible for the biological actions of dynemicin A—namely, the strategic combination of the enediyne, epoxide, and nitrogen functionalities. Particularly important in these systems was the installation of the 2-(phenylsulfonyl)ethyl carbamate group on the nitrogen, which acts as an easily removable locking device for the epoxide functionality and thus stabilizes the system until activation. Indeed, removal of this triggering device under mild basic conditions released the parent compounds **41** and **42**, respectively, which were found to be rather labile, undergoing the cascade of reactions shown in Scheme VII (**41/42** → **43/44** → **45/46** → **47/48** → **49/50**). The intermediacy of the highly reactive 1,4-benzenoid diradicals **47** and **48** is presumably responsible for the high cytotoxicity of these compounds, although the precise target of this species within the cell has not been defined unambiguously. As expected,



SCHEME VI. Myers' approach to redox activation of enediyne systems.

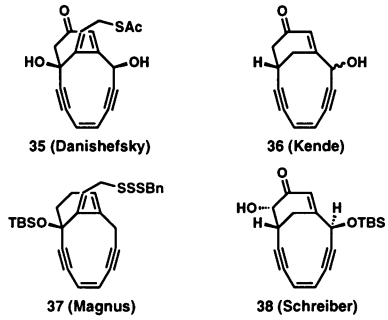
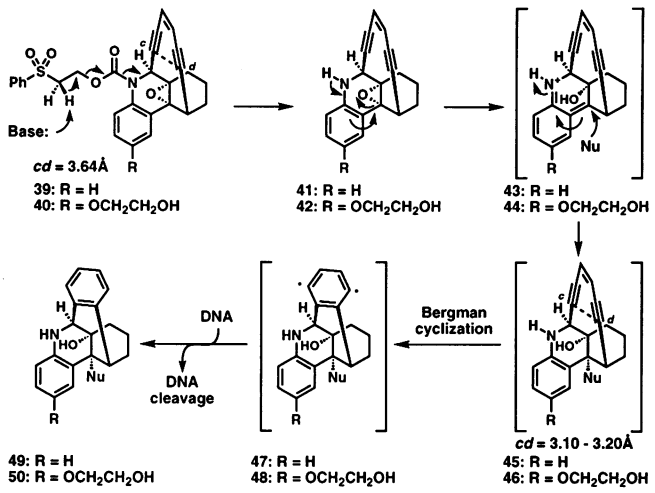


FIG. 4. Calicheamicin/esperamicin model systems.

however, compounds **39** and **40** were found to cleave DNA under basic conditions but failed to do so in acidic medium (Fig. 5) (42). The parent compound **42** also cleaved DNA at physiological pH (Fig. 6). Fig. 7 exhibits a number of other designed enediynes with triggering devices and modulated reactivity synthesized in these laboratories [**51** (44), **52** (unpublished results), **53** (45), **54** (45), **55** (43), and **56–58** (46)]. Some of these systems demonstrated significant DNA-cleaving properties (Fig. 5) (42). Particularly interesting is compound **52**, which contains a photosensitive triggering device (*o*-nitrobenzyl carbamate) and which has been demonstrated (unpublished results) to cleave DNA upon irradiation, as expected.

Table 2 (42) shows the cytotoxicity of compound **40** against a range of cell lines. Significant differences are observed, with cytotoxicities ranging from 1 μM for the highly resistant melanoma cell lines to 10 fM for the highly sensitive MOLT-4 leukemia cell line. Particularly significant is the high cytotoxicity of this compound against the multiple-drug-resistant TCAFDAX cell line (IC_{50} = 1.7 nM) and the relatively low cytotoxicity against a number of normal cell lines. Furthermore, preliminary *in vivo* studies using **40** and a tritiated analogue of **40** with mice infected with leukemia and solid tumors showed encouraging results (unpublished results).

Interestingly, treatment of MOLT-4 cells under appropriate conditions with enediyne **40** followed by observation of cell morphology and cell death revealed the phenomenon of programmed cell death (apoptosis) (48) as the prevailing cause of cell destruction (49). Furthermore, competition experiments using enediynes with relatively low toxicities resulted in the identification of certain inhibitors of apoptosis. Specifically, the methoxy enediyne **55** (Fig. 7), which displayed diminished tendency to undergo the Bergman reaction, exhibited inhibition of the cytotoxic action of compound **40**. Thus, when MOLT-4 cells were preincubated with enediyne **55** at 0.1 mM for 1 hr prior to treatment with the cytotoxic compound **40**, a dramatic reduction (fac-



SCHEME VII. Design of dynemicin models with triggering devices and their postulated mechanism of action.

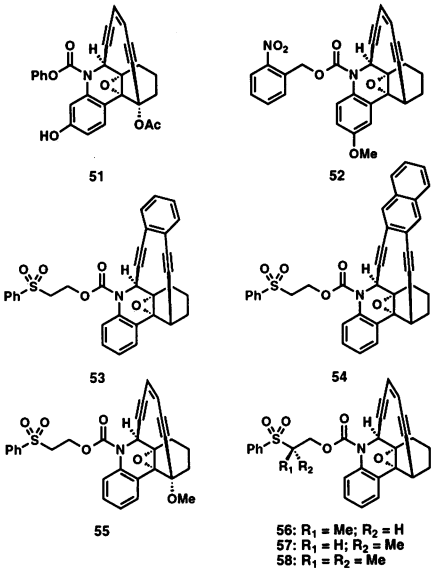


FIG. 7. Designed enediynes with triggering devices and modulated reactivity.

tor of $\approx 10^5$) in the cytotoxicity of **40** was observed. Similar reductions (factors of 10^2–10^4) were observed in the cytotoxicities of the naturally occurring enediynes dynemicin A (**9**) and calicheamicin γ_1^I (**5**) in the presence of the methoxy enediyne **55**. Particularly intriguing was the observation that **55** inhibited apoptotic morphology of cell death by powerful inducers of apoptosis such as actinomycin D and cycloheximide, although cell viability was not affected in these cases (49).

Further insight into the remarkable cell-type selectivity of these designed enediynes was obtained by comparing the cytotoxicities of enantiomerically pure compounds (+)-**39** and (−)-**39** (Scheme VII) (50) and the methyl-substituted compounds **56–58** (Fig. 7) (46). These experiments demonstrated dramatic differences in potencies depending on the enantiomeric form of the enediyne **39** and the degree and stereochemistry of methyl substitution in compounds **56–58** and raised intriguing questions: Is there an intracellular receptor for these enediynes other than DNA? Could this putative receptor serve as a capturing and delivery system for these enediynes to specific sequences of DNA? Is there a prevailing biological mechanism in certain cell types which facilitates the β-elimination? These questions raise even more interesting issues concerning the regulation of cell death. Exploitation of such observations and elucidation of the mechanism of action of these agents may lead to new approaches to drug design.

In addition to our own efforts in the dynemicin area, a number of other groups have contributed to the design and synthesis of model systems. Notable among these contributions are those of Schreiber (**59**, Fig. 8) (51), Wender (**60**, Fig. 8) (52), Magnus (**61**, Fig. 8) (53), and Isobe (**62**, Fig. 8) (54).

Inspired by the molecular architecture and the mechanism of action of neocarzinostatin chromophore (**9**), a number of groups have designed, synthesized, and studied a variety of model systems. A selection of these model compounds covering the rather wide spectrum of structural types synthesized in this field is shown in Fig. 9 [**63** (55), **64** (56), **65** (57), **66** (58), **67** (59), and **68** (60)].

Designed Enediynes Tethered to Delivery Systems

Compared with the naturally occurring compounds (e.g., calicheamicin γ_1^I), the designed enediynes reported so far exhibit significantly lower potencies as DNA-cleaving agents. In the absence of suitable delivery systems these observa-

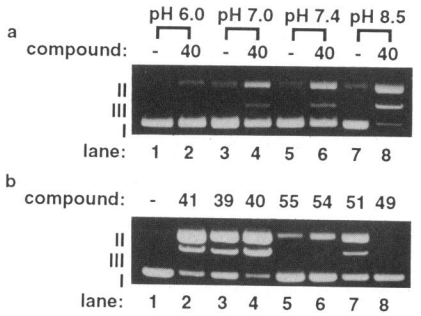


FIG. 5. (*a*) DNA cleavage by enediyne **40**. (*b*) DNA cleavage by enediynes **39–41**, **51**, **54**, and **55** and Bergman product **49**. For conditions, see ref. 42. [Reproduced with permission from ref. 42 (copyright 1992, AAAS, Washington, DC).]

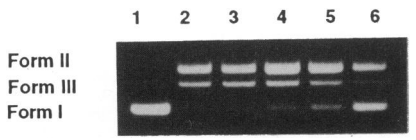


FIG. 6. DNA cleavage by enediyne **42**. For conditions see ref. 47. [Reproduced with permission from ref. 47 (copyright Am. Chem. Soc., Washington, DC).]

Table 2. Cytotoxicities of designed enediyne **40** against a panel of tumor cell lines and normal cell lines

Cell type	Cell line	IC_{50}, M
Tumor cell lines		
Melanoma	M-14	1.6×10^{-6}
Colon carcinoma	HT-29	1.6×10^{-6}
Ovarian carcinoma	Ovcar-3	7.8×10^{-7}
Breast carcinoma	MCF-7	7.8×10^{-7}
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}
	TCAF-DAX*	1.7×10^{-9}
Promyelocytic leukemia	HL-60	3.6×10^{-11}
T-cell leukemia	MOLT-4	2.0×10^{-14}
Normal cell lines		
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}

*Multiple-drug-resistant cell line.

tions are not surprising, but they did prompt several attempts to improve the DNA-cleaving properties of these molecules by tethering them to suitable molecules known to bind DNA. The hybrid molecules shown in Fig. 10 are representative examples of the work carried out in this area [**69** (1), **70** (1), **71** (61), **72** (62), **73** (63), and **74** (64)]. Despite these efforts, however, the increase in potency of these agents as DNA cleavers is not as dramatic as expected. Reasons for the lack of success in this area so far may be due to the requirement for precise docking of the enediyne into the DNA site in order for the generated radicals to abstract hydrogen from the DNA backbone. As in the case of the natural enediynes, much fine tuning and evolution needs to be done before we can reach sophisticated molecular designs with highly potent and efficient DNA-cleaving profiles. Such designs may be accelerated by molecular modeling and human ingenuity.

Total Synthesis of Naturally Occurring Enediynes: Calicheamicin γ_1^I

When the structures of calicheamicin γ_1^I (**5**) and esperamicin A_1 (**6**) were revealed to the scientific community, our group, like many other synthetic groups around the world, recognized the formidable synthetic challenge presented by these unprecedented molecular systems and undertook the task of achieving the total synthesis of calicheamicin γ_1^I. Such a daring endeavor would require careful planning in order to overcome the considerable difficulties inherent in constructing a molecule of such complexity. The total synthesis of calicheamicin γ_1^I would not only require control of the absolute stereochemistry at 19 chiral centers but also require insight into the potential instability and reactivity of the multitude of functionalities which comprise the molecule, in order to judge the correct timing for their introduction. Not unreasonably, the strategy adopted by our group was one in which suitably advanced precursors to the aglycon and oligosaccharide fragments of the molecule would be coupled together in the final stages of the synthesis (Fig. 11). However, degradation studies on the natural product by other groups had failed to furnish either the intact aglycon or oligosaccharide due to their inherent instabilities, and so there would be no opportunity for establishing the correct conditions necessary for achieving this final union of the two domains without first carrying out the mammoth task of their separate syntheses.

In tackling the synthesis of the oligosaccharide portion of calicheamicin γ_1^I, Nicolaou and coworkers were fortunate in being able to draw upon their experience in the field of sugar chemistry, enabling them to recognize the most challenging features of the fragment and design their synthetic strategy around the solution of these problems. They perceived that the B ring (Fig. 11), with its synthetically demanding 2-deoxy-β-hydroxylamino glycosidic linkage and 4-thio substituent, should be at the center of their thoughts. This led them to undertake several model studies for the construction of the B ring which culminated in providing a strategy (Scheme **VIII**) for the pivotal step of the synthesis of the oligosaccharide fragment (**65**). Thus, heating the thionoimidazolide **75** in refluxing toluene effected a clean allylic transposition of functionality on the B ring via a Ferrier-like rearrangement as illustrated by the arrows in Scheme **VIII**. This [3,3] sigmatropic rearrangement simultaneously introduced the 4-thio substituent at C-4 with the correct stereochemistry and deoxygenated the 2-position. Nicolaou *et al.* (65) were thus able to report the first total synthesis of the oligosaccharide portion of calicheamicin γ_1^I toward the end of 1990. Significantly, the efficient and convergent nature of this strategy provided a means of readily obtaining the multi-gram quantities of suitably advanced precursors to the oligosaccharide which would be needed in attempts to reach calicheamicin γ_1^I itself.

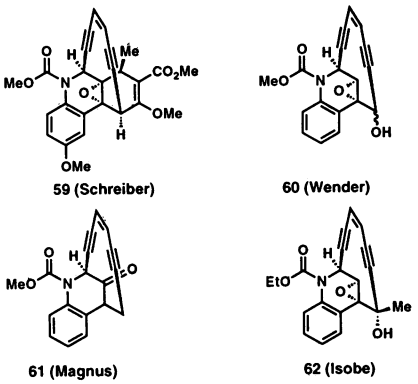


FIG. 8. Dynemicin model systems.

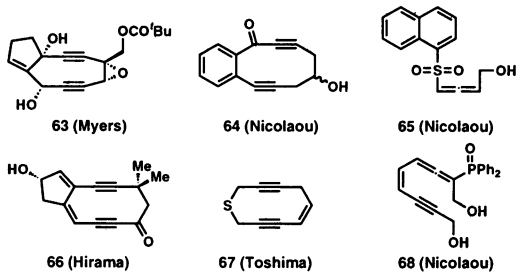


FIG. 9. Selected neocarzinostatin models and related systems.

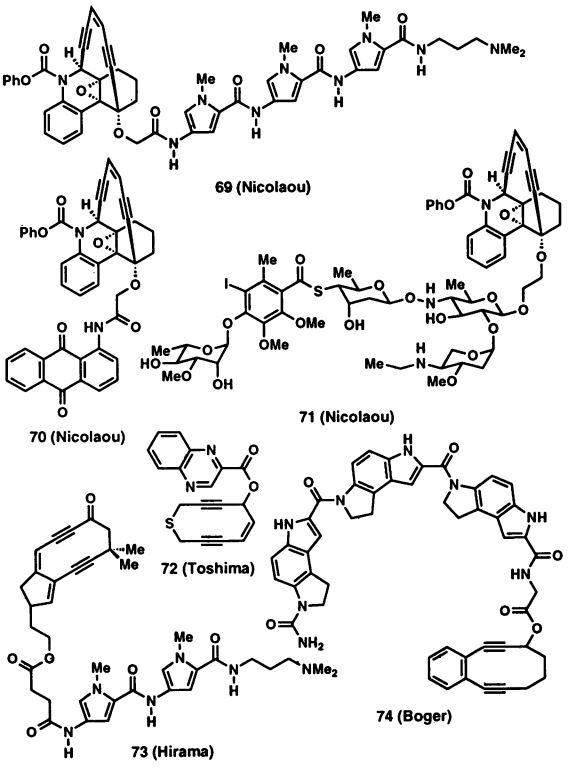


FIG. 10. Enediynes tethered to delivery systems.

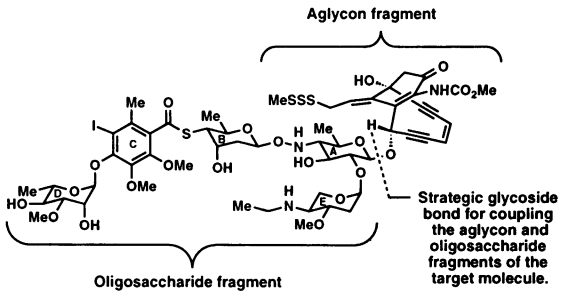
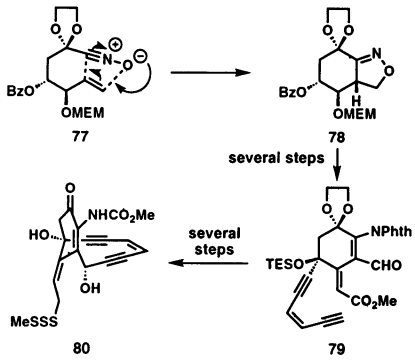


FIG. 11. Retrosynthetic analysis of calicheamicin γ_1^I (5).



SCHEME IX. Strategy for the enantioselective synthesis of calicheamicinone (80) and other calicheamicin precursors. MEM, 2-methoxyethoxymethyl; Phth, phthaloyl; Bz, benzoyl.

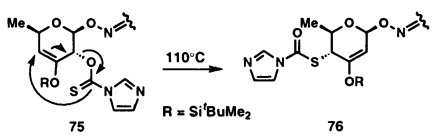
Furthermore, this strategy provided the means by which several modified oligosaccharides became available for binding studies with DNA fragments (20).

By this time thoughts were turning toward the synthesis of the other portion of calicheamicin γ_1^I, its aglycon. This synthesis would require a strategy which would provide large amounts of a suitably advanced precursor in homochiral form (i.e., as a single enantiomer). Several groups had spent the previous 4 years examining the chemistry of the aglycon and synthetic approaches toward its various structural features. Danishefsky (31, 32), Kende (33), Magnus (34, 35), and Schreiber (36) and their colleagues were among the first of many to demonstrate that the construction of the bicyclic framework of the aglycon is not as daunting as at first appeared. Meanwhile, Magnus *et al.* (66) had studied the chemistry of the allylic trisulfide group, and Danishefsky and coworkers (67), in a magnificent achievement, had succeeded in assembling the full functionality to achieve the first synthesis of calicheamicinone (**80**) as its racemate. Thus at the beginning of 1991, we embarked upon a new approach to the aglycon which would hopefully provide the molecule in enantiomerically pure form and in sufficient quantities for coupling to the oligosaccharide and completing the total synthesis of calicheamicin γ_1^I. Indeed, progress was rapid, since we were able to benefit during the latter stages of our synthesis from the lessons learned by our peers, allowing us to complete the first enantioselective synthesis of the aglycon towards the end of the same year (68). At the heart of our strategy was an intramolecular dipolar cycloaddition reaction of the nitrile oxide **77** (Scheme IX), in which chirality had already been established, to give the adduct **78**. This strategy not only led directly to the efficient installation of the full functionality of the aglycon but also allowed ultimate control of its absolute stereochemistry to provide calicheamicinone (**80**) as well as an advanced precursor, **82** (Scheme X), suitable for coupling with the oligosaccharide fragment and thus opening the way for further progress.

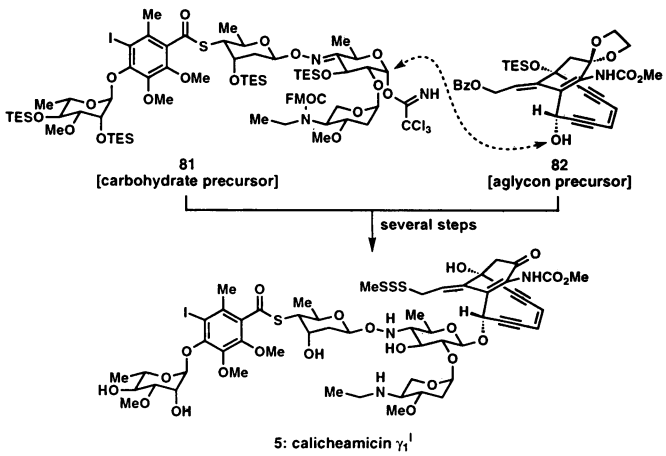
Now that both domains of calicheamicin γ_1^I were available, it remained for us to couple them together and complete the synthesis. By this time Nicolaou *et al.* (69) had already demonstrated the synthetic utility of the carbohydrate precursor **81** (Scheme X) as a coupling partner in model studies, but the question remained as to whether it would be possible to find a precursor to the aglycon which was suitable for coupling to the oligosaccharide and yet sufficiently advanced to allow the final unveiling of its features. Examination of molecular models indicated that it would be sterically demanding to bring the two pieces together, although only the required β-glycosidic linkage would be formed if it was possible at all. Indeed, at this very time Danishefsky and coworkers (70) were reporting difficulty in their attempts to couple a sufficiently advanced precursor of the aglycon to their oligosaccharide, which they had also synthesized by this time, although they were able to couple an immature version of the aglycon. However, our fears were unfounded, for the aglycon precursor **82** coupled smoothly and stereoselectively with **81** (Scheme X), and the careful choice we had made of protecting groups for the various sensitive functionalities of the entire molecule meant that several steps later we were able to unveil the final target in its full glory to complete the synthesis of calicheamicin γ_1^I (**5**) (71), the first total synthesis of any member of the enediyne class of natural products.

Concluding Remarks and Future Prospects

Much original work has been carried out in the area of enediynes since 1987, when the first structures of the naturally occurring calicheamicins and esperamicins were disclosed. Even though some of the fundamental chemistry had been known prior to the 1987 reports on these novel anticancer antibiotics, it took a bold lesson from Nature again to show us the way to bring together chemistry and biology in arriving at such elegant molecular systems and with such specific biological actions. Research in this field is continuing unabated in chemistry, biology, and



SCHEME VIII. Ferrier-like rearrangement strategy utilized in the synthesis of the calicheamicin γ_1^I oligosaccharide.



SCHEME X. Coupling of oligosaccharide and aglycon fragments and completion of the total synthesis of calicheamicin γ_1^I (**5**).

medicine, with some compounds already in clinical trials.

In chemistry, research has taken several directions, including molecular design and chemical synthesis. Several model systems related to the natural products have been synthesized, demonstrating new synthetic principles and strategies. Designed enediynes demonstrated abilities to cleave DNA and exhibited selective cytotoxicity against tumor cells versus normal cells (39, 42). Enediynes have been implicated in the puzzling but important phenomenon of programmed cell death (apoptosis) (49). And the total synthesis of the most prominent and complex member of the enediyne class, calicheamicin γ_1^I (5), has been achieved (71).

The next phase of research in the enediyne field will undoubtedly include further synthetic attempts at the naturally occurring targets, new designed enediynes with sophisticated mechanisms of *in vitro* and *in vivo* activation, and attachment of these systems to suitable delivery systems. Targeting devices may include antibodies, oligonucleotides, oligosaccharides, peptides and proteins, DNA intercalators, DNA groove binders, hormones, and other ligands. Hybrid molecules between enediyne "molecular warheads" and such delivery systems should provide new insights into biological phenomena and may facilitate drug design and development.

We thank our many talented associates, whose names appear in the original articles cited below, for their invaluable contributions to this research effort. Our work was financially supported by the National Institutes of Health, the National Science Foundation, The Scripps Research Institute, and the University of California, San Diego.

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