Designed Enediynes: A New Class of DNA-Cleaving Molecules with Potent and Selective Anticancer Activity

K. C. Nicolaou,* W.-M. Dai, S.-C. Tsay, V. A. Estevez, W. Wrasidlo

The rational design and biological actions of a new class of DNA-cleaving molecules with potent and selective anticancer activity are reported. These relatively simple enediyne-type compounds were designed from basic chemical principles to mimic the actions of the rather complex naturally occurring enediyne anticancer antibiotics, particularly dynemicin A. Equipped with locking and triggering devices, these compounds damage DNA in vitro and in vivo on activation by chemical or biological means. Their damaging effects are manifested in potent anticancer activity with remarkable selectivities. Their mechanism of action involves intracellular unlocking and triggering of a Bergman reaction, leading to highly reactive benzenoid diradicals that cause severe DNA damage. The results of these studies demonstrate the potential of these de novo designed molecules as biotechnology tools and anticancer agents.

The enediyne anticancer antibiotics (*1*) are a rapidly emerging class of naturally occurring substances that combine unusual molecular architecture, potent biological activities, and intricate modes of action. At present this family of compounds includes the neocarzinostatin chromophore (*2*), the calicheamicins (*3*), the esperamicins (*4*), and the dynemicins (*5*) (Fig. 1). Immediately after being characterized, these molecules became the focus of chemical, biological, and medical research and inspired considerable work in molecular design, synthetic chemistry, and clinical therapy (*1*, *6*, *7*). These compounds exert their biological actions through DNA cleavage, a property derived from their common ability to generate a benzenoid diradical on suitable activation. These highly reactive radicals are capable of abstracting hydrogen atoms from the DNA backbone, which initiates its rupture. The biological actions of these molecules are a result of at least three important functional domains. Each molecule comprises (i) an assemblage that consists of an enediyne moiety; (ii) a delivery system that conveys the enediyne moiety to its DNA target; and (iii) a triggering device that, when suitably activated, initiates the cascade of reactions that leads to generation of the reactive chemical species. The chemistry involved in the mode of action of these natural products centers around the ability of their enediyne moiety to generate reactive benzenoid diradicals after bioactivation at ambient temperature through the Bergman cycloaromatization reaction (*8*) (Fig. 2). Guided by this chemistry, we have designed, synthesized, and characterized chemical and biological mimics of these naturally occurring enediynes with the goal of attaining maximum biological activity with low molecular complexity (*9–12*).

We now describe our studies with relatively simple dynemicin A model systems that cleave DNA readily in aqueous buffer solutions and in tumor cells. Unlike many known anticancer agents, these compounds may be modulated to a fine degree through structural changes that take advantage of chemical principles and that capitalize on differences between normal and tumor cells such as cell permeability and enzymatic activity. Design of these enediyne compounds (**I**, Fig. 3) must provide a means to lock the enediyne core in a stable form that can be triggered and subsequently deactivated in a controlled fashion. Molecular triggering groups or "initiators" were placed either on the nitrogen or the benzene ring (such as structures **II** and **III**, Fig. 4). They are expected to be activated under basic conditions according to the mechanisms shown in Fig. 4 in the laboratory or within cells by taking advantage of intracellular chemical processes. It was hypothesized that different cells might possess varying degrees of activating power toward initiators and consequently result in DNA damage in a cell-specific manner. Tethering devices ("tethers") were included to allow subsequent attachment of ligands that aid in delivery to the target (such as tumor cells and DNA or RNA sequences). Deactivating groups ("deactivators") were introduced to modulate the activity of the enediyne compounds. Finally, detection devices ("detectors") were included to facilitate mechanistic studies. A series of compounds were designed and synthesized (*11–13*) in

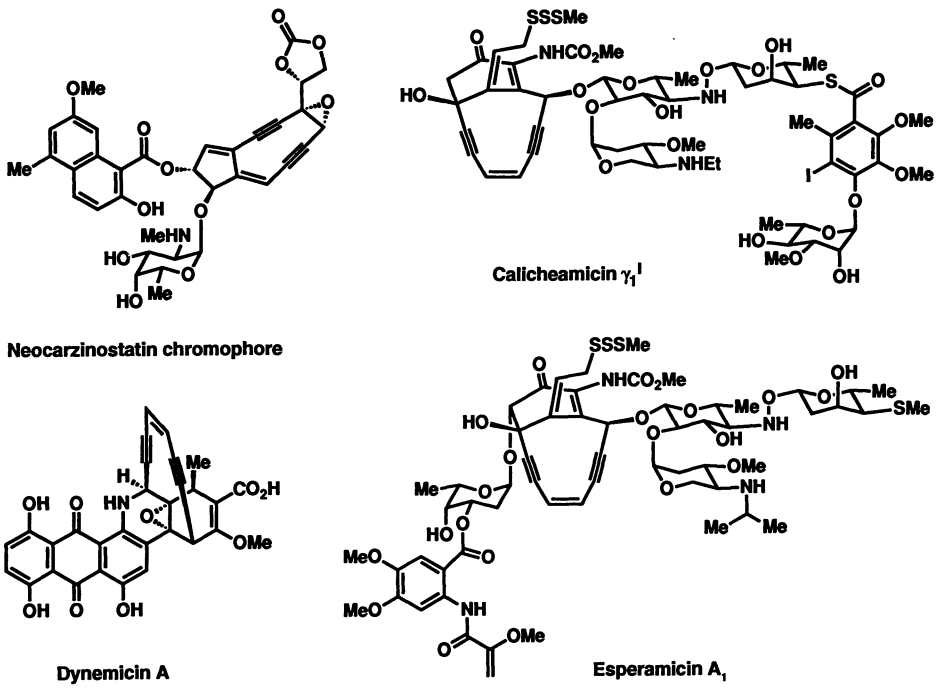

Fig. 1. Structures of naturally occurring enediyne anticancer antibiotics.

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order to test hypotheses regarding their chemical and biological properties.

The expectations of the rational design were met and led to a number of highly potent DNA-cleaving molecules with powerful and selective activities against tumor cell lines. Under neutral or acidic conditions, the designed enediynes showed little if any DNA-cleaving activity, whereas DNA damage was observed under basic conditions, as expected from their inherent chemical properties. Furthermore, incubation of compound **II** (R = OCH_2CH_2OH, Fig. 4) in basic buffer solution led to its decomposition and formation of phenyl vinyl sulfone and compound **IV** (R = OCH_2CH_2OH), supporting the activation mechanism shown in Fig. 4. The designed compounds, when tested with a variety of tumor and normal cell lines, revealed high toxicity against leukemia and other tumor cell lines but showed relatively low activity toward various normal cell lines. Furthermore, it was confirmed by fluorimetry that a large percentage of the genetic material of the cell was damaged, even before cell death occurred, implicating DNA cleavage as the direct mechanism of action of these compounds.

In vivo studies with the most potent compounds of the series demonstrated significant tumor growth inhibition in standard animal models (*14*). Unlike the naturally occurring enediyne antibiotics, some of these synthetic enediynes have relatively low toxicity (*14*).

Molecular design. The molecular structure and mode of action of dynemicin A (*5*) and the other naturally occurring enediynes (*1–4*), studies on the Bergman cycloaromatization reaction (*8*), and our initial studies on simple mimics (*15*) of these compounds guided the design of a new set of enediyne compounds that would fulfill the following requirements: (i) be stable under neutral conditions but also be endowed with the latent capacity to undergo the Bergman reaction upon suitable activation; (ii) be structurally as simple as possible to allow easy access by chemical synthesis; (iii) provide for tethering to suitable delivery systems and other desirable moieties through appropriate handles; (iv) be equipped with initiators that would be activated under mild chemical or biological conditions or both; and (v) allow modulation of reactivity through deactivators.

The general structure shown in Fig. 3 embodies molecular variations that were considered in order to test various chemical hypotheses pertaining to reactivity issues of these systems. Rational design based on chemical principles allowed the definition of distinct structural features that would be required for high biological activity. In particular, the following results were anticipated: (i) Because of strain effects, the epoxide moiety should "lock" the "enediyne core," preventing it from undergoing a Bergman cycloaromatization (*8*). (ii) Engaging the lone pair of electrons on the nitrogen with an electron-withdrawing group should ensure stability, whereas liberation of the free amine should allow the electrons to flow toward the epoxide site and trigger radical generation (Fig. 4). Thus, this position

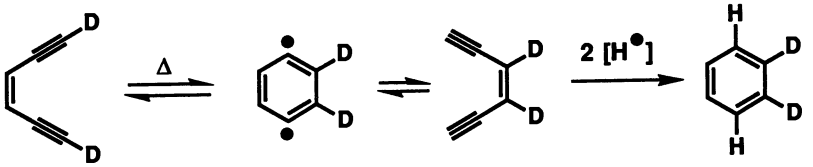


Fig. 2. The Bergman cycloaromatization reaction.

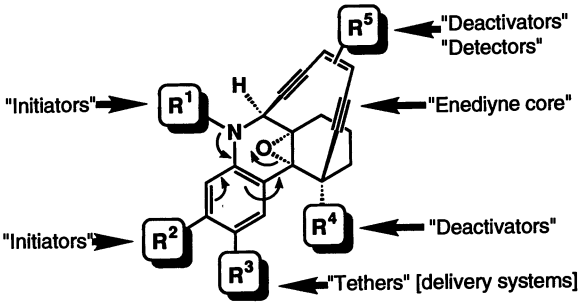


Fig. 3. Molecular design of enediyne compounds **I**.

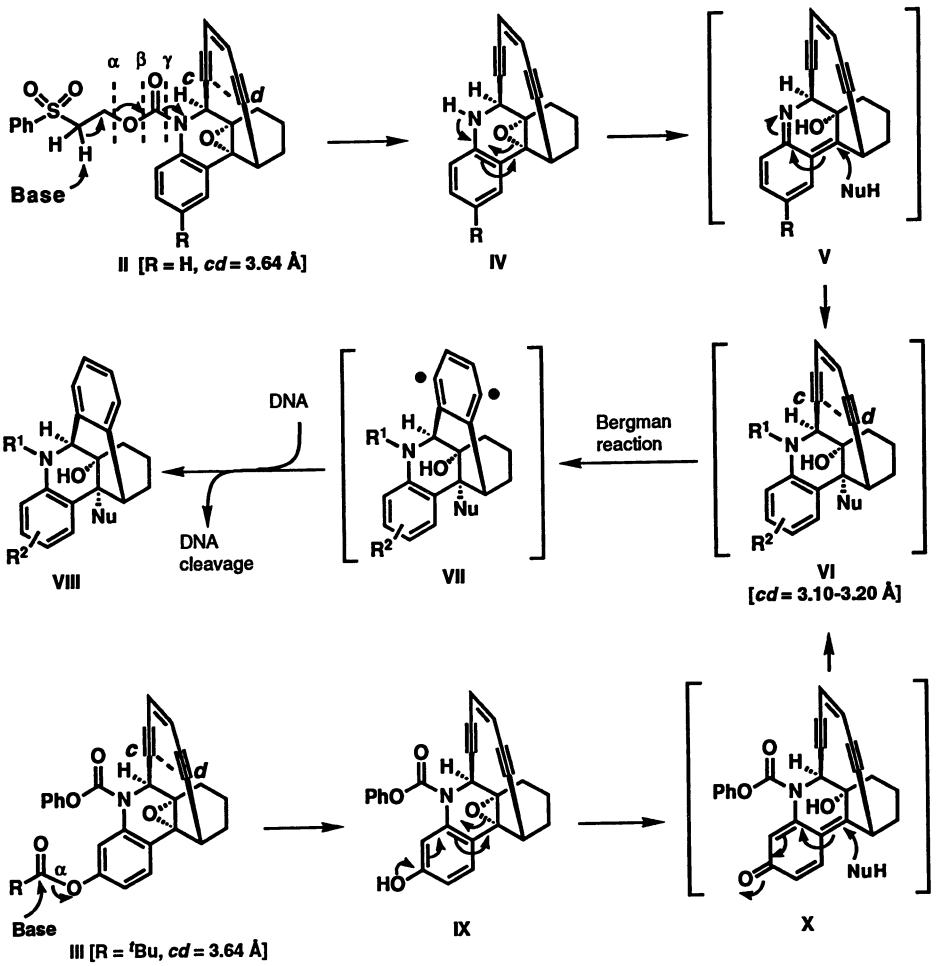
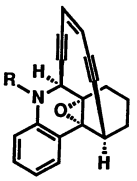
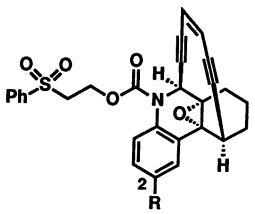
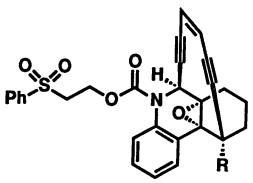
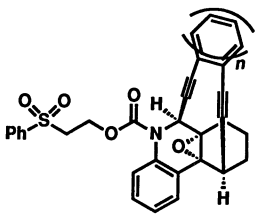
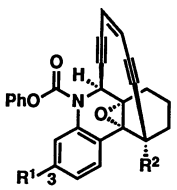
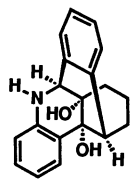


Fig. 4. Postulated mechanism of action of designed enediyne compounds; *cd* is defined as the distance between the remote acetylenic carbons.

could serve as the point of attachment for an initiator (R^1). (iii) Position R^2 was viewed as a second point at which a triggering device could be directed, considering its potential to donate electrons into the epoxide site. Substituent R^2, if endowed with electron density, could serve as an excellent initiator for the Bergman reaction (*8*). (iv) Substituent R^3, because of its position on the benzene ring, should be electronically neutral and sterically indifferent, and should, therefore, provide an ideal location for tethers. (v) Structures where R^4 = H should be more active than those containing electron-withdrawing substituents such as oxygen-attached groups because of destabilization of the incipient positive charge on the adjacent benzylic carbon encountered during epoxide opening, which must preceed radical formation. This position, therefore, may be used for deactivators. (vi) Position R^5 could be occupied by deactivators such as benzene or naphthalene (*16*) and should therefore serve to modulate the reactivity of these systems toward Bergman cyclization (*8*).

On the basis of the above considerations, two types of triggerable enediyne compounds represented by structures **II** and **III** (Fig. 4) were designed and synthesized. The first series (**II**) incorporates a phenylsulfone ethylene carbamate moiety [$PhSO_2CH_2CH_2OC(O)$-N] on the nitrogen, a group that, in principle, could be removed under mild chemical or biological conditions. Furthermore, removal of the triggering device from **II** would allow the formation of reactive compound **IV** (Fig. 4), which should follow the reaction path shown in Fig. 4 to furnish the cis-opened product **VI** through intermediate **V**. Molecular mechanics calculations (*17*) indicated a crucial structural change during conversion of the epoxide **II** (R = H) to the cis-opened product **VI** (R^1 = R^2 = H, nucleophile Nu = OH). The distance between the inner acetylenic carbons (*cd* distance) (*17*) is shortened considerably, from 3.64 to 3.13 Å, an event that should allow the Bergman cycloaromatization (*8*) to proceed spontaneously at ambient temperatures and lead to the benzenoid diradical **VII**. The reactive species (**VII**) should then result in DNA damage by hydrogen-atom abstraction, and in the process be converted to product **VIII**. The second series of projected triggerable enediynes (**III**) included an ester function on the benzene ring at the para position relative to the epoxide. The ester group was expected to serve as a triggering device by engaging the lone pair of electrons on the ring-bound oxygen until a hydrolysis event occurred. The compound so generated (**IX**) would then rearrange to intermediate **X** as shown in Fig. 4. Nucleophilic attack as shown in **X** should then lead to a similar cis-opened system **VI** [R^1 = PhOC(O)-, R^2 = Nu = OH], as in the reaction sequence above. Again, the *cd* distance change (*17*) in going from the locked epoxide system **III** [R = *tert*-butyl, *cd* = 3.64 Å] to the opened system **VI** [R^1 = PhOC(O)-, R^2 = Nu = OH, *cd* = 3.15 Å] would allow spontaneous cycloaromatization and radical formation [**VI** → **VII** → **VIII**] to take place with concomitant DNA cleavage. Chemical synthesis (*11–13, 16*) provided a number of compounds (Table 1) that proved to be triggerable, highly potent, and selective agents against DNA and cancer cells.

Table 1. Structure-activity correlation for enediynes **1** to **11** and Bergman product **12** against Molt-4 leukemia cells. Cytotoxicities were determined by the sulforhodamine B assay (*19*).

Compound	IC_{50} [M]
 1: R = H	1.6×10^{-10}
2: R = PhOC(O)	3.1×10^{-6}
3: R = $PhSO_2CH_2CH_2OC(O)$	2.5×10^{-11}
 4: R = OMe	6.5×10^{-9}
5: R = OCH_2CH_2OH	2.0×10^{-14}
 6: R = OMe	5.0×10^{-6}
7: R = OCH_2CH_2OH	1.9×10^{-6}
 8: *n* = 1	4.0×10^{-7}
9: *n* = 2	1.8×10^{-8}
 10: R^1 = OH, R^2 = OAc	6.0×10^{-8}
11: R^1 = $^tBuCO_2$ R^2 = H	3.5×10^{-11}
 12	$>10^{-4}$

DNA cleavage. The propensity of the synthetic enediyne compounds to enter the Bergman cyclization reaction, (*11–13, 15–17*) prompted us to test their ability to cleave DNA under various conditions. The ϕX174 supercoiled DNA was incubated with each of a selected group of compounds at different concentrations, pH values, and temperatures in buffer solutions. Cleavage of DNA was assayed by agarose gel electrophoresis (Fig. 5). Compounds **1, 3, 5, 6, 9, 10,** and **12,** (Table 1) demonstrate the expected DNA cleavage activity. These experiments also demonstrate that the highly reactive compound **1** is an effective DNA damaging agent and that the sulfone series of compounds (such as **3** and **5**, Table 1), which are capable of generating structures of type **1** under basic conditions, exhibit strong DNA cleaving properties at

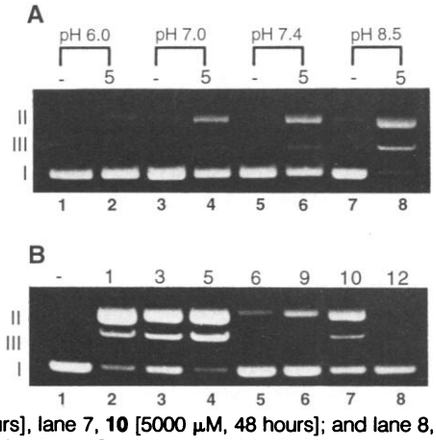


Fig. 5. (**A**) Interaction of supercoiled DNA with compound **5**. ϕX174 DNA (50 μM per base pair) was incubated for 24 hours at 37°C with **5** in various buffer solutions and analyzed by electrophoresis (1 percent agarose gel, ethidium bromide stain). Lanes 1, 3, 5, and 7, DNA controls at pH 6.0, 7.0, 7.4, and 8.5. Lanes 2, 4, 6, and 8, compound **5** [1000 μM] at pH 6.0, 7.0, 7.4, and 8.5. (**B**) Interaction of supercoiled DNA with compounds **1, 3, 5, 6, 9, 10**, and **12**. ϕX174 DNA (50 μM per base pair) was incubated for 24 or 48 hours at 37°C with compounds in buffer (50 mM tris-HCl, pH 8.5) and analyzed by electrophoresis (1 percent agarose gel, ethidium bromide stain). Lane 1, DNA control; lane 2, **1** [1000 μM, 24 hours]; lane 3, **3** [1000 μM, 24 hours]; lane 4, **5** [1000 μM, 24 hours], lane 5, **6** [5000 μM, 48 hours]; lane 6, **9** [5000 μM, 48 hours], lane 7, **10** [5000 μM, 48 hours]; and lane 8, **12** [5000 μM, 48 hours]. Key: I, supercoiled DNA; II, nicked DNA; and III, linear DNA.

basic pH. As expected, compounds such as **5** do not damage DNA at acidic pH (for example, at pH 6, Fig. 4); but they exhibit noticeable DNA cleaving activity in pH 7 buffer solution although this effect may be partly due to added ethanol or to local basicity on the DNA molecule. These compounds slowly decompose to form the labile free amine (structure **IV**, Fig. 4) in pH 7.0 buffer. The free phenolic compound **10** (Table 1) has considerable DNA cleaving activity (Fig. 5), showing that this type of molecule is also quite effective in damaging DNA. The *tert*-butyl ester derivative **11** (Table 1) did not cleave DNA even at pH 9, presumably because of its resistance toward hydrolysis to generate structures of type **10** (Table 1) under the incubation conditions. The methoxy compound **6** showed only slight DNA cleaving activity, whereas the naphthalenediyne **9** caused considerable DNA cleavage, albeit with somewhat diminished potency compared to the enediyne **3**, demonstrating the anticipated deactivating effect of these substituents.

Cytotoxicity. The compounds designed and synthesized in our study were tested for cytotoxicity against a broad spectrum of cell lines (typically 12 to 21) ranging from the highly resistant SK-Mel-28 melanoma to the sensitive Molt-4 leukemia cells in order to assess antitumor activity and cell-type selectivity. The results of these experiments revealed high potencies for a number of compounds against certain tumor cells and, most significantly, dramatic cell selectivities. Data representing the most significant findings are presented in Tables 1 to 3 for compounds **1** to **12**.

The IC_{50} values for a number of selected compounds (Table 1), reflect their activities against Molt-4 leukemia cells. The observed potencies of these compounds show definite correlation with molecular structure and reflect the expectations of the molecular design. Thus, the parent enediyne **1**, although rather labile (estimated half-life at 37°C, ~4 hours) showed high activity in this test. Engaging the lone pair of electrons on the nitrogen atom with an electron withdrawing group as in phenyl carbamate **2** resulted in a 10,000-fold loss in activity, reflecting the reduced ability of the molecule to unlock the epoxide and enter the Bergman pathway (*8*). A dramatic increase in potency was observed when the $PhSO_2CH_2CH_2OC(O)$-group was introduced on the nitrogen to serve as a triggering device (Fig. 4). The high activity associated with this group was maintained throughout the series of compounds in our studies. Thus, the $PhSO_2CH_2CH_2OC(O)$-group emerged as the preferred protecting-triggering device for these systems.

Compound **4** (Table 1) demonstrates that substitution at the C-2 position does not significantly disturb activity. In fact, this position may be used to enhance the activity with suitable groups such as in **5**, which proved to be the most potent compound we have studied. The very high potency of enediyne **5** ($IC_{50} < 10^{-12}$ M and as low as 10^{-14} M against Molt-4 leukemia cells) makes it one of the most potent agents reported to date against tumor cell lines (see Table 3 for comparisons with a number of well-known anticancer agents). The higher water solubility of **5** compared to other synthetic enediynes may account for its higher potency.

Compounds **6** and **7** (Table 1) exhibited greater chemical stability toward radical generation (*13*), even after liberation of the amino group, which was reflected in their much lower potencies ($IC_{50} = 10^{-6}$ M, Table 1) against Molt-4 leukemia cells. The deactivating effect of the benzene and naphthalene rings in compounds **8** and **9** (Table 1) was evident from the lower chemical propensity of these systems (*16*) to undergo the Bergman reaction, which manifests as lower cytotoxicity (10^{-7} and 10^{-8} M, respectively, Table 1). Any increase in potency due to enhanced DNA intercalation by these moieties may be offset by their higher chemical stability toward the Bergman reaction. The naphthalene diyne **9** may serve as a useful fluorescent mechanis-

Table 2. Cytotoxicities of designed enediyne **5** against a panel of 21 tumor cell lines and 4 normal cell lines.

Cell type	Cell line	IC_{50} [M]
	Cancer cell lines	
Melanoma	SK-Mel-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*	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}
	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.

tic probe because of its ability to generate a highly fluorescent anthracene ring upon cycloaromatization (*16*).

Enediyne **10** represents the active molecular warhead of type **II** structures (Fig. 4). This compound exhibited strong antitumor properties against the Molt-4 leukemia cell line (IC_{50} = 10^{-8} M, Table 1), although it is considerably deactivated by the acetoxy group. The parent compound generated from **11** (R^1 = OH, R^2 = H, Table 1) could not be isolated because of its rapid cycloaromatization. Compound **12** and phenyl vinyl sulfone ($PhSO_2CH$ = CH_2) were tested against Molt-4 leukemia cells in control experiments and found to be inactive, as expected, confirming the attribution of all biological activity to the enediyne moieties of these new compounds.

The IC_{50} values determined for enediyne **5** with 21 different cancer cell lines are shown in Table 2. There are significant differences in cytotoxicities, ranging from 1.6 × 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 of enediyne **5** against the multiple-drug resistant TCAF-DAX cell line (IC_{50} = 1.7 × 10^{-9} M, compared with 1.1 × 10^{-9} M for TCAF, Table 2). Another striking feature of enediyne **5** is its relatively low cytotoxicity against a number of normal cell lines, including HNBM, HMEC, NHDF, and CHO (Table 2). The selectivity of enediyne **5** against a number of cell lines and the dramatic ability of this compound to differentiate between Molt-4 leukemia cells and normal HMEC cells are shown in Fig. 6. This selectivity implies different intrinsic capacities of various cell types to interact with these compounds.

The mode of action and potency ranges of a number of selected, well-known anticancer agents are presented in Table 3 so that the activity profile of synthetic compounds represented by enediyne **5** can be placed in perspective. In certain cell lines, such as the Molt-4 leukemia cells, the designed enediyne **5** exhibited the highest potency amongst all agents tested, including taxol, doxorubicin, and bleomycin.

Mechanism of action. The chemical profiles of the synthetic enediynes under physiological pH and temperature, coupled with the requirement of Bergman reaction for activity, points to a cell-mediated cascade of events as one possibility responsible for their biological action. To address this issue, we assessed intracellular DNA damage and investigated the molecular basis of the triggering mechanism for the $PhSO_2CH_2CH_2OC(O)$-containing enediynes.

Molt-4 leukemia cells were treated with ethidium bromide, which led to uptake by DNA through intercalation and consequent fluorescence. Exposure of these cells to enediyne **5** led to rapid DNA strand breakage as determined by fluorimetry, resulting in 95% destruction after 4 hours at 37°C (Fig. 7). Cell death showed approximately a 2-hour delay relative to DNA strand breakage (Fig. 7). These observations implicate DNA strand cleavage as the direct cause of cell destruction in these experiments. Compound **5** severely impairs the ability of M-21 cells to synthesize DNA (inhibition of [^{3}H]thymidine uptake), RNA (inhibition of [^{3}H]uracil uptake), and

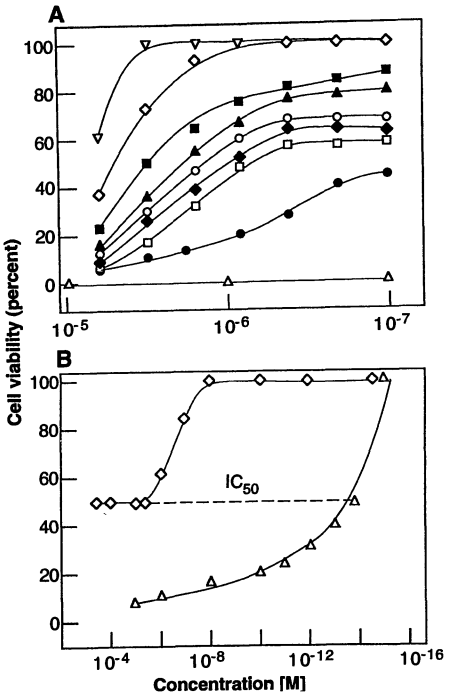


Fig. 6. Selectivity of cytotoxicity of enediyne **5** against various tumor and normal cell lines. (**A**) Plots of cell viability versus concentration of compound: (▽) NHDF; (◇) HMEC; (●) M21/SK; (▲) OVCAR-3; (□) P-3; (○) HT-29; (■) MCF-7/U 87; (◆) CAPAN-1; and (△) Molt-4. (**B**) Expanded plots of viability versus compound concentration for normal mammary epithelial cells (◇ HMEC) and leukemia (△ Molt-4) cells. The IC_{50} values of enediyne **5** for these two cell lines were determined from these plots to be 2.5 × 10^{-5} M for HMEC and 2.0 × 10^{-14} M for Molt-4. Tumor cells were cultured in 96-well plates with the compound in 200 μl of RPMI supplemented with 10 percent fetal bovine serum (Hyclone, Salt Lake City, Utah) at 37°C in a humidified atmosphere containing 5 percent CO_2 in air for 72 hours. Sulforhodamine B dissolved in 1 percent acetic acid was added, and the plates were incubated for 30 minutes at ambient temperature, washed with 1 percent acetic acid, and blotted. To the air-dried plates were added 100 μl of 10 μM unbuffered tris base (pH 10.5) with shaking. The optical densities of the plates were measured with a microplate reader (Molecular Devices Thermomax) at 540 nm.

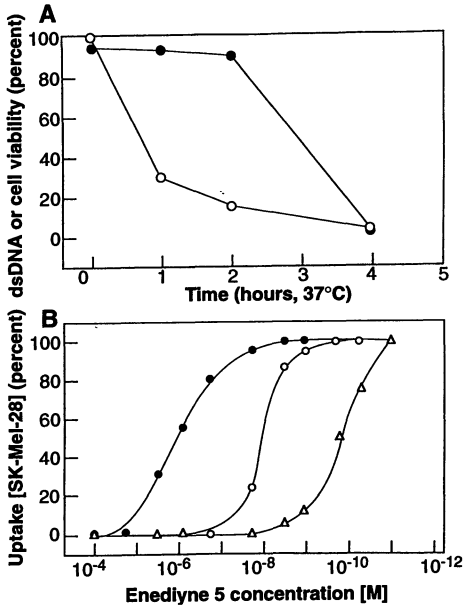


Fig. 7. Intracellular DNA strand breaking and inhibition of DNA, RNA, and protein syntheses by enediyne **5**. (**A**) DNA strand-breaking (○) and cell death (●) versus time. Intracellular tumor cell DNA strand breaking experiments were performed by adapting an earlier procedure developed for white blood cells (*20*). Enediyne-treated (10 μM) cell suspensions (10^7 cells per milliliter) in RPMI medium were centrifuged at 3000 rpm and resuspended in 10 μM phosphate buffer containing 0.25 M *meso*-inositol and 1 μM magnesium chloride. The suspensions were then subjected to the conditions described in (*20*). Finally, all of the samples were sonicated for 4 s at 30 W, the dye ethidium bromide was added, and the fluorescence was measured with a Perkin-Elmer LS 5B spectrophotometer at an excitation frequency of 520 nm and analyzed at 590 nm. The results were expressed in percent of double-stranded (ds) DNA remaining as a function of time. (**B**) Inhibition plots [uptake of tritiated thymidine (△), uracil (○), and leucine (●) versus concentration of compound] for enediyne **5**. Human M-21 tumor cells were grown in RPMI tissue culture media supplemented with 10 percent fetal bovine serum (FBS) and then plated at 10^4 cells in 100 μl of medium per well on 96-well tissue culture plates. The cells were allowed to adhere for 24 hours, and then dilutions of compound were added in 10-μl volumes and incubated for 72 hours at 37°C in a humidified atmosphere containing 5 percent CO_2. The plates were then washed with tissue culture media (three times) and again incubated for 24 hours under the above conditions. Each well received 1 μCi of ^{3}H-labeled substrate (leucine, uracil, or thymidine) and after 16 hours of additional incubation the cells were collected on glass fiber filters with a Skatron cell harvester. Finally, the filters were placed in Ecolume scintillation cocktail (ICN, Irvine, California), and radioactivity was measured with a Beckman scintillation counter. Incorporation of ^{3}H label was calculated as a percentage of untreated control cells.

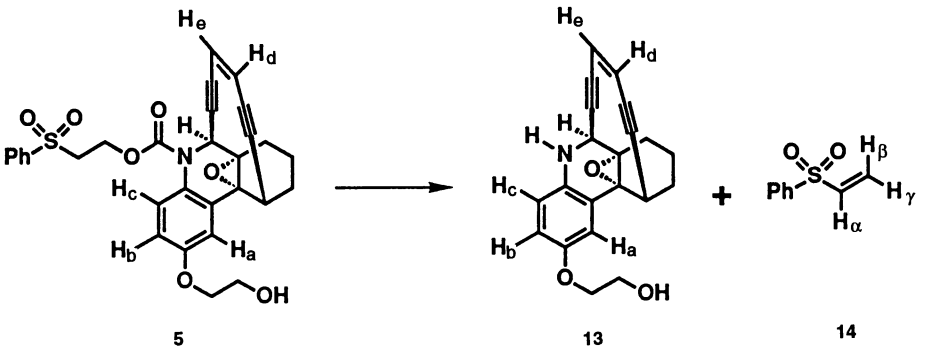
protein (inhibition of [^{3}H]leucine uptake) (Fig. 7).

There are several possible explanations for the mechanism by which the reaction cascade leading to the diradical species is initiated within the cell. These include: (i) direct enzymatic opening of the epoxide ring in **II** (Fig. 4); (ii) enzymatic rupture of either bond β or γ in structure **II** (Fig. 4), leading to the free amine **IV**; and (iii) biochemically induced β-elimination of phenyl vinyl sulfone ($PhSO_2CH{=}CH_2$), followed by CO_2 elimination and formation of species **IV**. Although differentiation among these possibilities requires further biochemical investigation, chemical studies with compound **5** confirm the postulated mechanism (Fig. 4) of triggering the Bergman cascade under basic conditions. The decomposition of **5** in various pH solutions was followed by high-performance liquid chromatography (HPLC) (Fig. 8) and ^{1}H nuclear magnetic resonance (NMR) spectroscopy (Fig. 9). As seen from Fig. 8, compound **5** remained intact at pH 7.0 after 1.5 hours, whereas it is cleanly decomposed to the rather labile free amine **13** and phenyl vinyl sulfone (**14**) at higher pH's (Fig. 8, B to D). Both compounds **13** and **14** were detected by ^{1}H NMR spectroscopy (Fig. 9). Careful procedures allowed the isolation and characterization (by ^{1}H NMR spectroscopy and mass spectrometry) of the labile enediyne **13** which, however, failed to give clean Bergman products under a variety of conditions.

Applications. Our results may allow further developments in the area of drug design and understanding of the mechanism of action of the enediynes. These compounds may find future application in the areas of DNA cleavage and cancer chemotherapy. Of significance is the higher potency of the designed enediynes against tumor cells compared to a number of well-established anticancer agents such as *cis*-platin, doxorubicin, vinblastine, and bleomycin. These compounds are also superior in potency to the highly promising anticancer agent taxol (*18*). Provided their selec-

Table 3. Comparison of cytotoxicities of designed enediynes with a number of potent experimental and clinically applied anticancer drugs; IC_{50} is the molarity at which 50 percent tumor cell viability was observed after 72 hours exposure under normal tissue culture conditions with the sulforhodamine B assay (*19*). The following tumor cell lines gave IC_{50} values within the indicated range: SK-Mel-28, M-14, M-21, Capan-1, Ovcar-3, Ovcar-4, UCLA-P-3, U-87, U-251, and Molt-4.

Compound	Mode of action	IC_{50} [M]
Designed enediyne **5**	Direct DNA cleavage	10^{-6}–10^{-14}
Dynemicin A	Direct DNA cleavage	10^{-7}–10^{-11}
Calicheamicin γ_1^I	Direct DNA cleavage	10^{-8}–10^{-12}
Taxol	Mitosis inhibitor	10^{-7}–10^{-9}
Vinblastine, vincristine	Mitosis inhibitor	10^{-7}–10^{-9}
Doxorubicin	DNA intercalator	10^{-6}–10^{-9}
Actinomycin D	RNA-DNA inhibitor	10^{-7}–10^{-9}
Bleomycin sulfate	Direct DNA cleavage	10^{-4}–10^{-5}



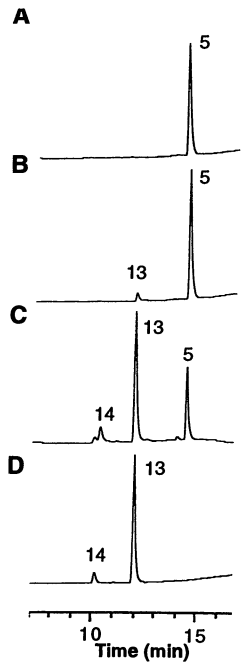


Fig. 8. Reaction of compound **5** in phosphate buffer (100 mM) at various pH at 37°C for 1.5 hours as monitored by HPLC. (**A**) Compound **5** at pH 7.0. (**B**) Compound **5** at pH 7.4. (**C**) Compound **5** at pH 8.5. (**D**) Compound **5** at pH 12. Numbers marked on each peak refer to compounds shown in Fig. 9. The HPLC analysis was performed on a Waters 600E HPLC instrument equipped with a Vydac reversed-phase C_{18} (1.0 cm by 25 cm) column with triethylammonium acetate buffer (100 mM, pH 7.0)–acetonitrile (linear gradient) as eluent (flow rate, 3.5 ml/min, ultraviolet detection at 254 nm).

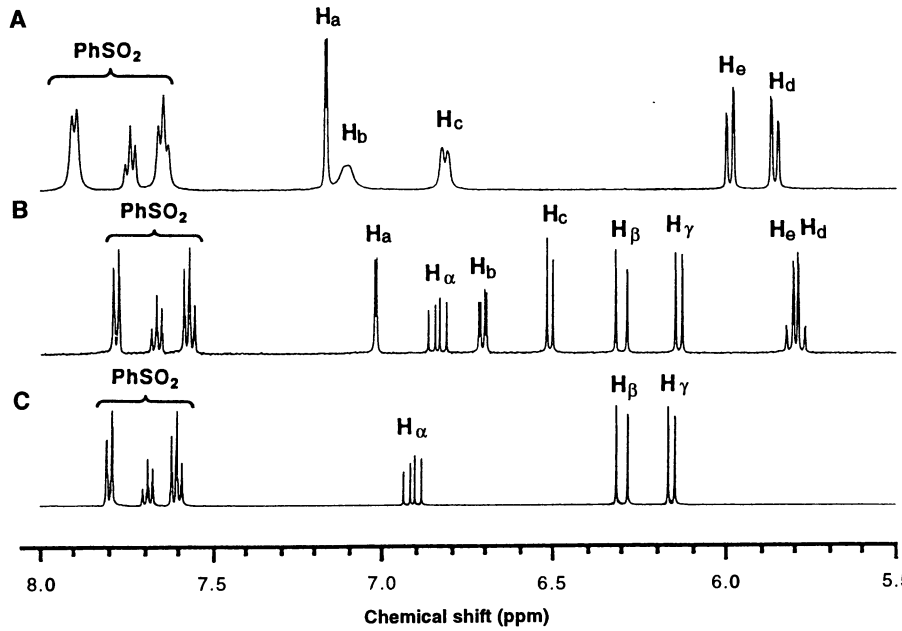


Fig. 9. Proton NMR spectra (aromatic and olefinic region only) of compounds **5**, **13**, and **14**. (**A**) Compound **5** in dimethyl sulfoxide-d_6 (DMSO-d_6). (**B**) Reaction mixture of compound **5** in 60 percent DMSO-d_6 and 40 percent phosphate buffer in D_2O (100 mM, final pH 12) at 37°C for 1.5 hours. (**C**) Proton NMR spectrum of phenyl vinyl sulfone (**14**) in DMSO-d_6. The small chemical shift differences for the phenyl vinyl sulfone signals in spectra B and C are due to the different media. Spectra were recorded on a Bruker AMX-500 instrument at 37°C.

tivity against tumor cells versus normal cells can be maintained and enhanced in animal models and humans, these molecules may emerge as powerful drugs against cancer. Attachment of the reported enediynes or related systems to suitable groups may improve properties such as solubility, membrane permeability, and cell specificity. Targeting these agents to specific sites with tumor-associated antibodies or certain receptor substrates may also prove useful against a number of cancer types. Furthermore, tethering the reported enediynes to appropriate delivery systems such as intercalators, oligonucleotides, oligosaccharides, or peptides may enhance their DNA or RNA binding properties and allow them to exhibit sequence specificity. Selective DNA cleaving agents and antisense tools may then become available. Delivering these reactive species to enzyme active sites or protein receptors by attaching them to the appropriate substrates may also prove to be useful.

Issues remaining to be elucidated include the precise mechanism by which these compounds are triggered intracellularly and what allows some of them to be so selective against certain cell types. Although evidence from chemical studies support the hypothesis that the chemical machinery of the cell could be capable of triggering the Bergman cyclization by removing the nitrogen-bound triggering group, the cell selectivity issue is not yet understood. Speculations include: (i) the possible existence of tumor-associated factors that may activate these systems preferentially; (ii) differences in the permeability of membranes for these compounds in various cell types; and (iii) differences in the ability of the machinery of the cell to repair DNA damage caused by these agents (*21*).

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