Synthesis of (+)-dynemicin A and analogs of wide structural variability: establishment of the absolute configuration of natural dynemicin A

Andrew G Myers*, Mark E Fraley, Norma J Tom, Scott B Cohen and David J Madar

The Arnold and Mabel Beckman Laboratories of Chemical Synthesis, California Institute of Technology, Pasadena, CA 91125, USA

Background: Dynemicin A is an exceedingly potent antitumor antibiotic derived from microbial fermentation that cleaves double-stranded B-form DNA *in vitro* in the presence of activating factors such as NADPH or glutathione. Because of the structural complexity, high reactivity, and scarcity of natural dynemicin A, it has not been feasible to modify the structure to any significant extent. Previous studies have not determined the absolute configuration of the natural product.

Results: A multistep route for the preparation of enantiomerically pure, synthetic dynemicin A was developed. The absolute configuration of natural dynemicin was determined by comparing the synthetic drug with dynemicin A derived from fermentation. The route that was developed is highly convergent, as the result of a late-stage coupling reaction that combines two complex synthetic fragments, and has been shown to provide access to non-natural dynemicins of wide structural variability by modifications of these fragments. In this way, several nonnatural dynemicins, unavailable by any other means, were synthesized and shown to have DNA-cleaving activity in the presence of glutathione or NADPH.

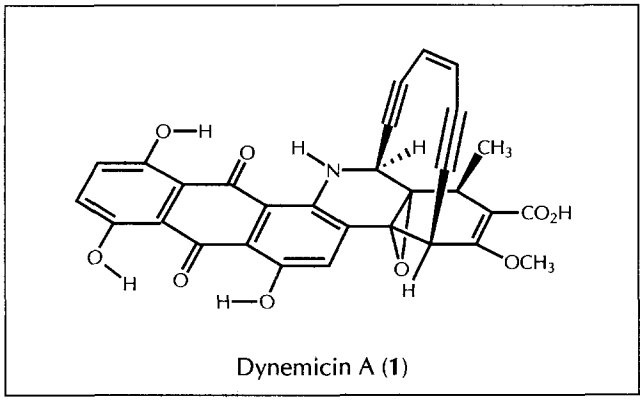
Conclusions: Enantiomerically pure dynemicin A is now available by laboratory synthesis. The natural, (+)-enantiomer of dynemicin A is shown to possess the 2*S*, 3*S*, 4*S*, 7*R*, 8*R* configuration. A wide variety of heretofore unavailable, active analogs of dynemicin A have been prepared and are found to produce subtle variations in sequence specificity of DNA cleavage compared to the natural product and, of potentially greater significance, display variations in the efficiency of DNA cleavage as a function of the activating agent.

Chemistry & Biology January 1995, **2**:33–43

Key words: anthracycline antibiotics, chemical synthesis, DNA cleavage, dynemicin A, enediyne antibiotics

Introduction

Fermentation of the microorganism *Micromonospora chersina* produces a broth with potent antitumor and antibiotic properties. This bioactivity has been traced to a deep violet metabolite known as dynemicin A (Fig. 1). The relative, but not absolute, stereochemistry of this molecule was established by X-ray crystallographic analysis [1]. Dynemicin A (compound **1**) exhibits remarkable antitumor activity, with LD_{50} values in the picogram to nanogram per ml range against many tumor cell lines. This, in conjunction with its reportedly low acute toxicity, defines **1** as an important new chemotherapeutic lead [2]. Dynemicin A (**1**) is unique among natural antitumor agents, possessing features of both the anthracycline and enediyne antibiotic families. The highly reactive anthraquinone fragment imbues the molecule with its deep violet color and is characteristic of the anthracyclines, while the (*Z*)-enediyne bridge and epoxide ring classify it among the enediyne antibiotics [3]. In parallel with other members of the enediyne antibiotic family, dynemicin A has been proposed to function *in vivo* as a DNA-damaging agent. It has been amply demonstrated that compound **1** is capable of cleaving double-stranded B-form DNA *in vitro* in the presence of a reducing cofactor such as NADPH or a thiol, and Sugiura and co-workers [4–6] have provided convincing evidence that **1** is transformed into the biradical intermediate shown in Fig. 2 upon reductive activation. This proposal requires initial reaction at the anthraquinone site, followed sequentially by epoxide



Dynemicin A (**1**)

Fig. 1. The structure of dynemicin A (compound **1**).

*Corresponding author.

© Current Biology Ltd ISSN 1074-5521

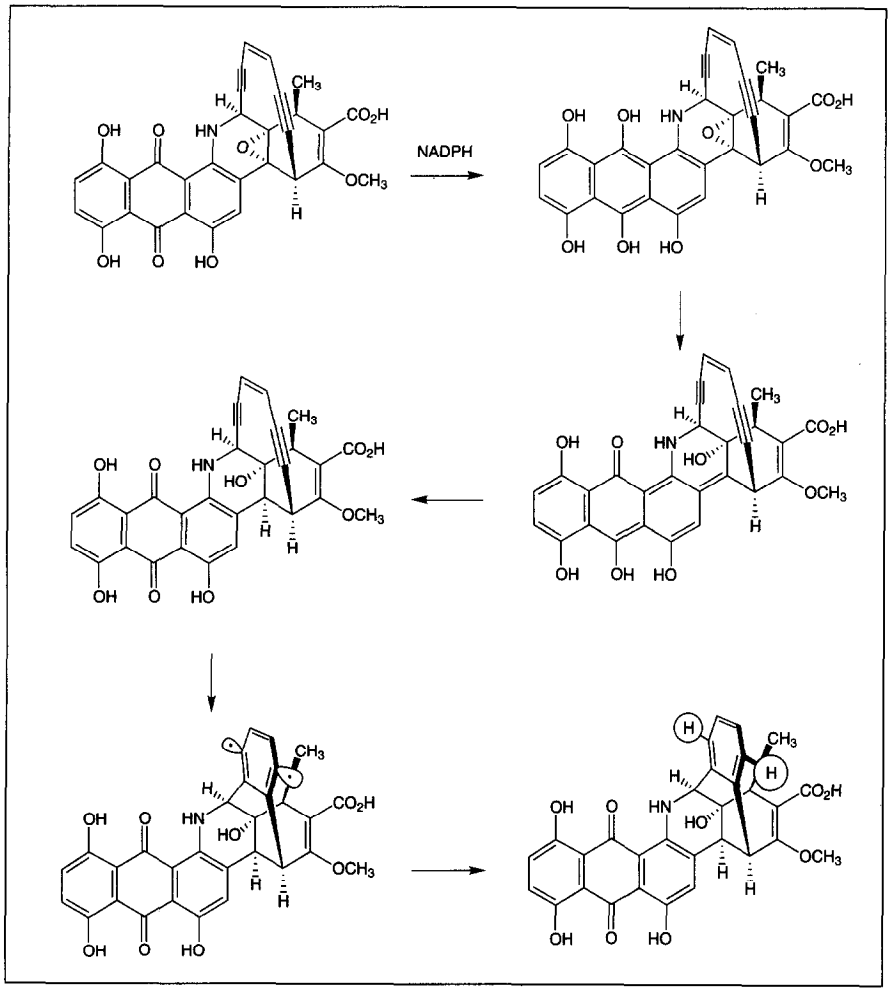


Fig. 2. Proposed mechanism of action of dynemicin A [4–6]. Reduction at the anthraquinone site is followed by opening of the epoxide ring, tautomerization and Bergman cyclization of the strained (*Z*)-enediyne group to produce a highly reactive biradical intermediate.

opening, tautomerization, and Bergman cyclization of the strained (*Z*)-enediyne group. The detailed interplay between complex functional groups can be seen to establish the unique reactivity profile of **1** and defines an exceptionally challenging problem in chemical synthesis. As a result of this reactivity profile, only limited chemical modifications of the natural product have been feasible. Consideration of the mechanism shown in Fig. 2 leads naturally to proposed studies involving more extensive modifications of the dynemicin structure, for example, nuclear substitution of the anthraquinone ring. Implicit in the design of dynemicin analogs of this type is a virtual requirement for the development of a laboratory synthetic route. Several research groups have reported notable advances toward the development of laboratory routes to dynemicin analogs of various kinds, both simple and complex [7–17]. With regard to synthetic design, perhaps less apparent, but no less important, is the need for a convergent approach, in which structural variability is introduced late in the synthetic route so as to achieve maximum structural divergence. The development of such a route has been the objective of our research for some time; its realization and application to the first preparation of enantiomerically pure dynemicin A and a wide variety of structural analogs is the subject of this report.

Results and discussion

Chemical synthesis

Both natural and nonnatural dynemicins were envisioned to arise from the retrosynthetic fragments illustrated in Fig. 3 for a generalized dynemicin target molecule. In this strategy, the anthraquinone moiety is introduced at a very late stage in the synthetic scheme, a

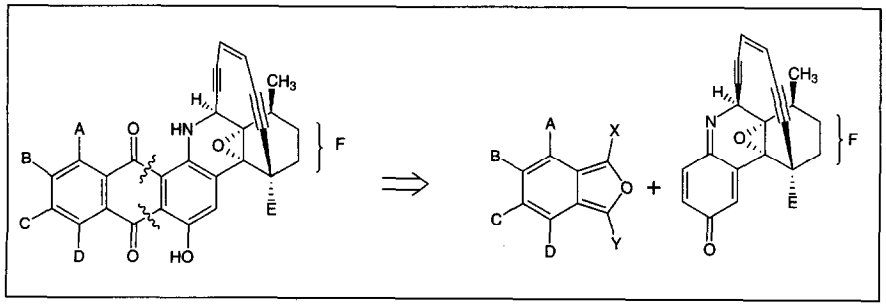


Fig. 3. Generalized retrosynthetic analysis of the dynemicins and structural analogs.

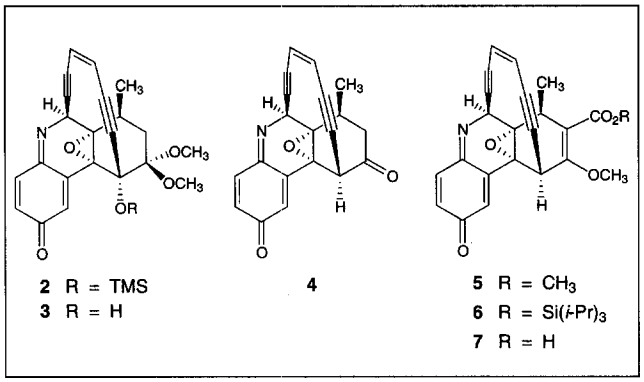


Fig. 4. Quinone imine precursors for dynemicin synthesis. Compounds **2–7**, and similar structures, have previously been synthesized in enantiomerically pure form [18].

highly desirable, if not essential, feature given the high reactivity of this functional group. In addition, this convergent approach allows for the preparation of an enormous variety of dynemicin analogs by modification of the two coupling components. In the generalized scheme, the anthraquinone portion of dynemicin is introduced by the coupling of a substituted isobenzofuran derivative with a quinone imine component that contains the (*Z*)-enediyne bridge and epoxide ring. We have previously synthesized several quinone imine precursors of this type in enantiomerically pure form, to include the structures **2–7** (Fig. 4) [18]. These quinone imines are stable materials, can be purified, stored, and otherwise handled as *bona fide* synthetic intermediates, and have been transformed into anthraquinones by two different methods. The first employs a Diels–Alder cycloaddition/oxidation reaction sequence and is illustrated with the preparation of the dideoxydynemicin analog **10** (Fig. 5). Heating a solution of excess 1,1-diethoxyphthalan (5 equiv) and glacial acetic acid (0.2 equiv) in toluene at reflux leads to *in situ* generation of the highly reactive diene 1-ethoxyisobenzofuran [19]. In the presence of the quinone imine **2**, Diels–Alder cycloaddition takes place to form a 1:1 mixture of the exo and endo adducts **8** (56 % combined yield). Both products are believed to result from diene addition to the π-face of the quinone imine that is opposite the (*Z*)-enediyne bridge, with the indicated orientation (a potentially valuable control element for the introduction of dissymetrical anthraquinone coupling partners). Stirring the Diels–Alder adducts **8** with excess pyridinium chlorochromate (PCC) [20] (10 equiv) in dichloromethane at 23 °C leads to oxidative cleavage of the bicyclic ketal to form the anthraquinone **9**; desilylation of the tertiary trimethylsilyl ether of **9** with triethylamine trihydrofluoride in acetonitrile then affords the purple dideoxydynemicin analog **10** in 30 % yield for the two steps after purification on silica gel. Hydrolysis of the dimethylketal group of **10** with *p*-toluenesulfonic acid in acetone at 23 °C produces the dynemicin analog **11** in 50 % yield. By using the Diels–Alder cycloaddition/oxidation methodology, the dideoxydynemicin analog **12** was prepared from the

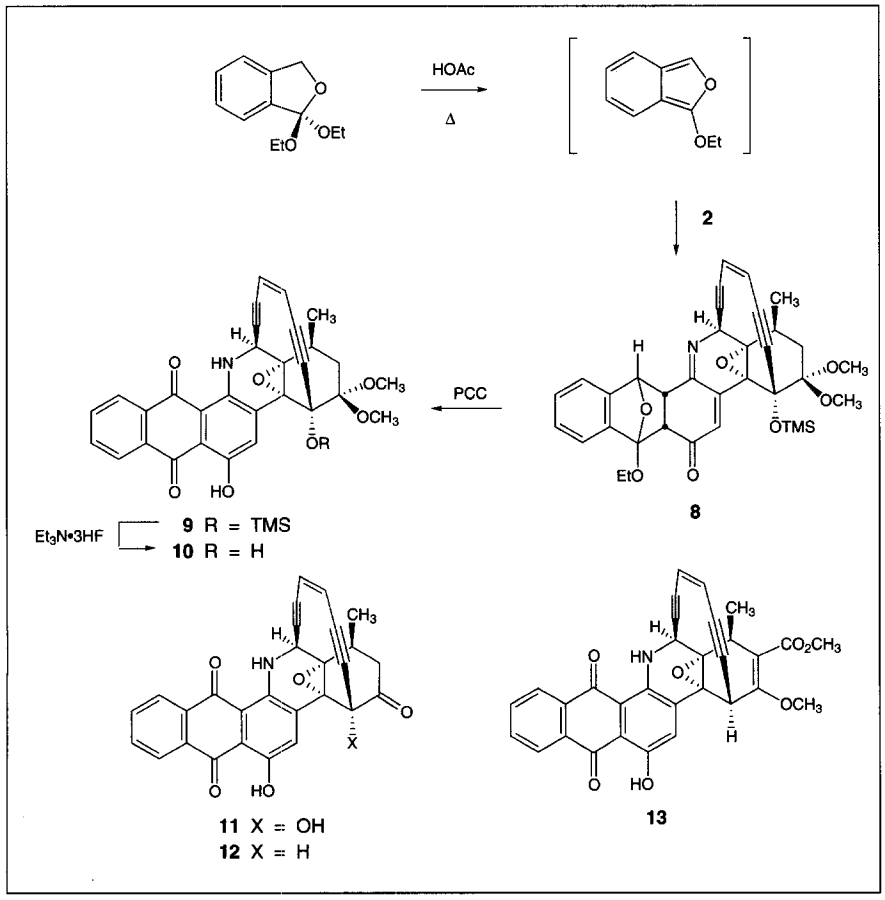


Fig. 5. Preparation of dideoxydynemicin analogs by a Diels–Alder cycloaddition/oxidation reaction sequence. Diels–Alder cycloaddition between the reactive diene 1-ethoxyisobenzofuran (generated *in situ* from 1,1-diethoxyphthalan [19]) and the quinone imine **2** (see Fig. 4) produces adducts **8**, which are transformed into the anthraquinone **9** with PCC (pyridinium chlorochromate [20]).

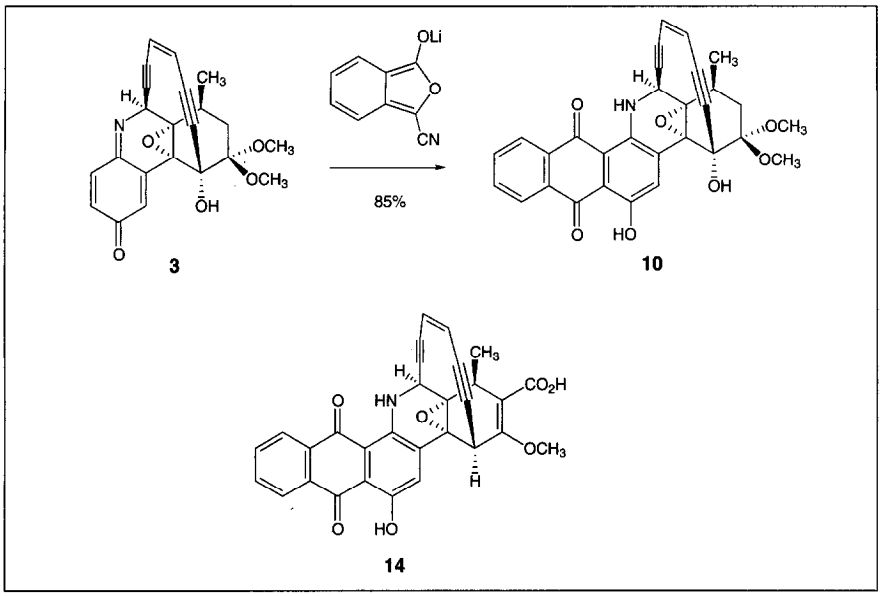


Fig. 6. Synthesis of dideoxydynemicin analogs from quinone imines by the phthalide addition method. The dideoxydynemicin analog **10** was synthesized in one step from the quinone imine **3**; similarly, dideoxydynemicin A (compound **14**) was synthesized in one step from the quinone imine **6** (see Fig. 4).

quinone imine **4** in 10 % yield for the two steps, and dideoxydynemicin methyl ester (**13**) was synthesized in 6 % yield from the quinone imine 5. The latter products (**12** and **13**) were unstable to purification on silica gel, but could be purified by reverse-phase high-pressure liquid chromatography (HPLC).

More recently, we have used a second protocol for the synthesis of anthraquinones that promises to proceed with substantially greater chemical efficiency (Fig. 6). Addition of 2.0 equivalents of lithiated cyanophthalide [21,22] to the quinone imine **3** at −78 °C in tetrahydrofuran and slow warming of the resultant solution to 23 °C produced the dideoxydynemicin analog **10** in 85 % yield after purification by chromatography on silica gel followed by Sephadex LH-20. Similarly, dideoxydynemicin A (**14**) was synthesized in a single step in 47 % yield by the addition of lithiated cyanophthalide to the quinone imine **6** at −78 °C, followed by warming to −30 °C and quenching with hydrogenfluoride pyridine complex.

To synthesize the more highly oxidized anthraquinone ring of dynemicin A itself, it was necessary to modify the Diels–Alder cycloaddition/oxidation strategy (Fig. 7).

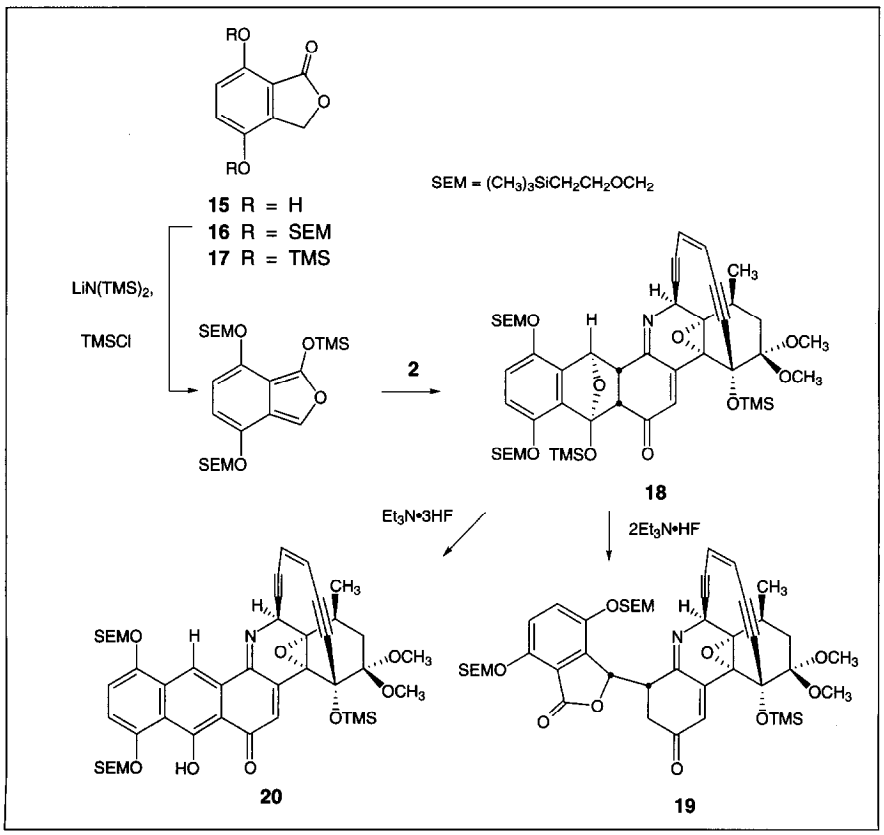


Fig. 7. Modification of the Diels–Alder cycloaddition/oxidation synthetic strategy directed toward the preparation of the highly oxidized anthraquinone moiety of dynemicin A.

The oxygenated phthalides **15** and **16** served as precursors to the requisite isobenzofurans using a deprotonation-silylation sequence [23]. Treatment of phthalide **16** with lithium *N,N*-bis(trimethylsilyl) amide in tetrahydrofuran at −78 °C and trapping of the resulting anion with trimethylsilyl chloride afforded the corresponding *O*-trimethylsilyloxyisobenzofuran derivative, which, without isolation, formed the exo-oriented cycloadduct **18** in 34 % yield upon brief warming with quinone imine **2**. Product **18** was a sensitive material, readily undergoing ring cleavage to form the phthalide **19** under even mildly basic conditions, a result that is reminiscent of transformations occurring within the tetracycline antibiotics [24,25]. Ring cleavage was avoided when the cycloadduct **18** was treated with triethylamine trihydrofluoride in acetonitrile at 23 °C, thereby forming the red anthrol derivative **20** in 44 % yield.

Although compound **20** lies only one oxidation state from the desired anthraquinone, a large series of oxidants failed to bring about this conversion. In reconsidering the problem, it was recognized that oxidation of the left-most ring of **18** from the hydroquinone to quinone level would produce an intermediate at the same level of oxidation as the desired anthraquinone, and one that is removed from that product only by the opening of the bicyclic ketal and tautomerization. With this in mind, the hydrolytically sensitive bis(trimethylsilyl)ether **17** was prepared from **15**, in quantitative yield, by heating **15** with *N,N*-bis(trimethylsilyl)amine in tetrahydrofuran at reflux containing a trace of concentrated sulfuric acid as catalyst (30 min), followed by the removal of volatiles *in vacuo* [26]. Treatment of the residue with potassium *N,N*-bis(trimethylsilyl)amide (1.04 equiv) in tetrahydrofuran and trapping of the resulting anion with trimethylsilyl chloride produced the tris(trimethylsilyloxy)isobenzofuran, as evidenced by the formation of the Diels–Alder adduct **21** (exo product) upon addition of quinone imine **2** and brief heating at 55 °C (Fig. 8).

Direct addition of this highly sensitive product to an emerald green solution of cuprous chloride in pyridine under an oxygen atmosphere followed by the addition of hydrogen fluoride pyridine complex afforded the dynemicin analog **22** directly, in 63 % yield for the two-step procedure. In the same way, the quinone imine **6** was transformed in two steps to dynemicin A (**1**) in 14 % yield. Synthetic dynemicin A was shown to be identical with an authentic sample of the natural product by spectroscopic comparison, reverse-phase HPLC analysis (by co-injection), and circular dichroism. The latter establishes for the first time the absolute configuration of natural dynemicin A as: 2*S*, 3*S*, 4*S*, 7*R*, 8*R*.

DNA cleaving studies

Preliminary evaluation of the DNA-cleaving activity of several of the synthetic dynemicin analogs described herein was undertaken by incubating each analog (50 μM) with a 3'-^{32}P-labeled 193-base pair DNA restriction fragment, double-stranded calf thymus DNA (1.0 mM base pairs), and, in separate experiments, with each of the reducing cofactors NADPH and glutathione (20 mM). The cleavage reactions were run for 12 h at 37 °C and the cleavage products were analyzed by gel electrophoresis after precipitation and washing. As illustrated in Fig. 9, DNA cleaving activity was observed with each analog tested (compounds **10**–**13**); for comparison, lanes 3 and 8 show DNA cleavage by dynemicin A (**1**) under identical conditions using NADPH and glutathione, respectively, as activating factors.

Analysis of the data shows that any variations in the sequence specificity of cleavage by the analogs examined thus far versus dynemicin A are modest at best. The evaluation is made difficult because dynemicin A itself exhibits little sequence specificity of cleavage within this 193-bp restriction fragment, raising questions as to whether the sequence specificity of DNA cleavage by **1** is important in determining its antitumor activity. Greater variance is observed when the cleavage efficiencies of natural and nonnatural dynemicins are compared.

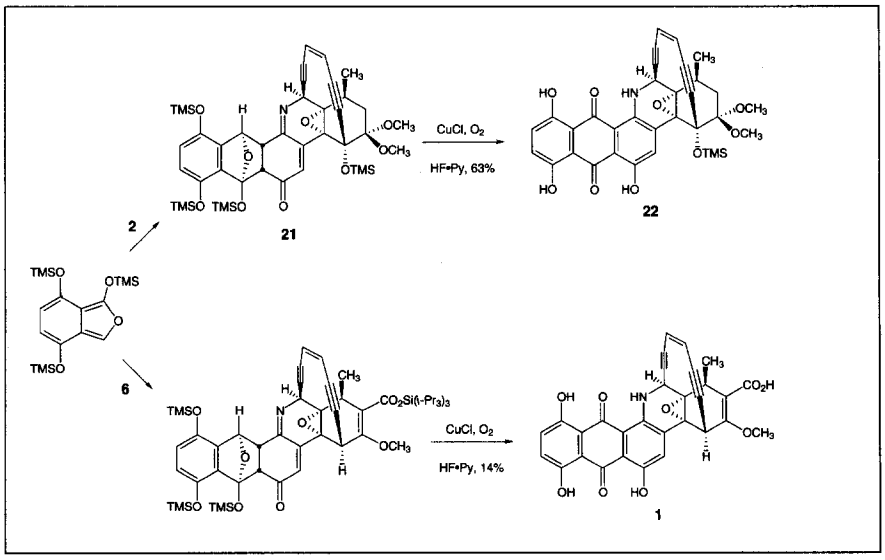


Fig. 8. Synthesis of the dynemicin analog **22** and dynemicin A by a modified Diels–Alder cycloaddition/oxidation strategy employing the tris(trimethylsilyloxy)isobenzofuran intermediate shown as the dienophile. Direct treatment of the Diels–Alder cycloadducts with hydrogen fluoride pyridine complex and cuprous chloride in pyridine under an atmosphere of oxygen then produces the target anthraquinones.

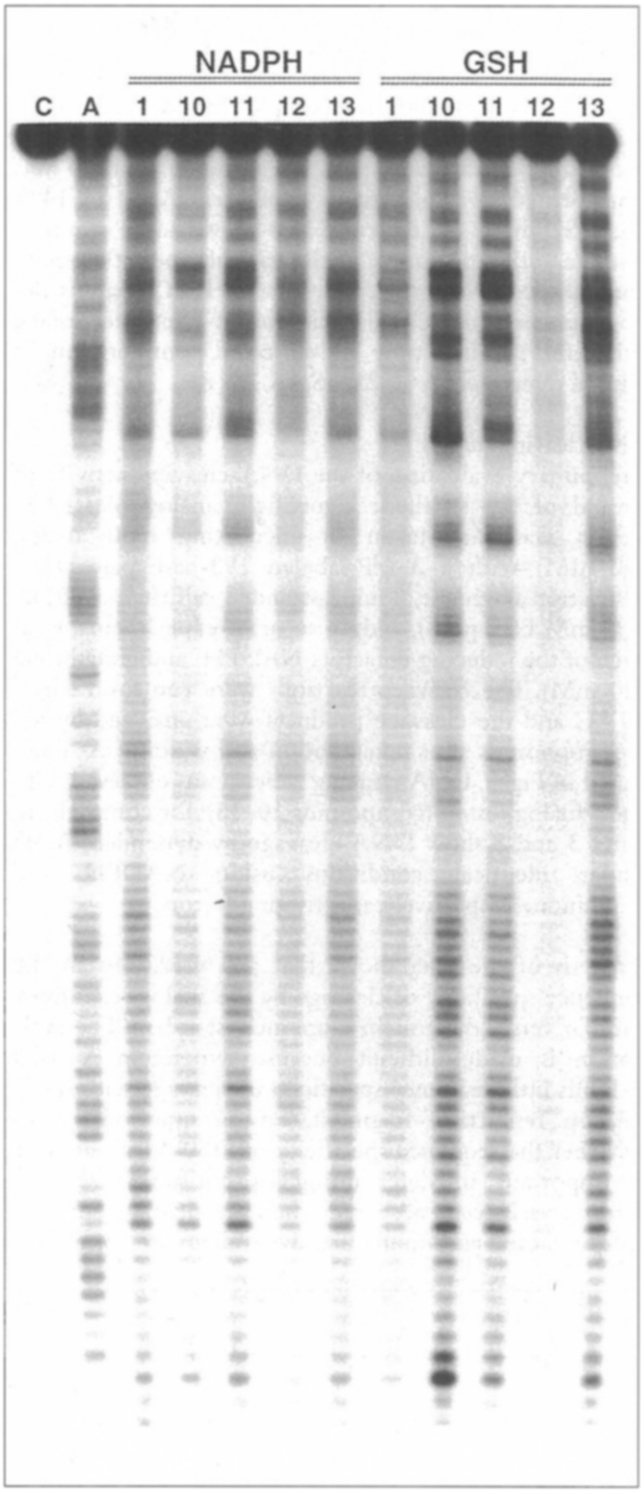


Fig. 9. DNA cleavage of a 3′-labeled 193 base-pair restriction fragment of pBR322 (*Eco* RI/*Ssp* I digests) from the reaction of dynemicin A or synthetic anthraquinones **10**, **11**, **12**, and **13** with glutathione (GSH) or NADPH. Reactions were performed on a volume of 50 µl and contained calf thymus DNA (1.0 mM bp), 193 base-pair restriction fragment (~10^5 cpm), tris-HCl buffer (30 mM, pH 7.5), sodium chloride (50 mM), dynemicin A or synthetic anthraquinone (0.05 mM), and either glutathione (GSH, 20 mM) or NADPH (20 mM), as indicated. Reactions were incubated at 37 °C for 12 h. Lane C: 193 bp restriction fragment alone. Lane A: products from an adenine-specific cleavage reaction as described in [29].

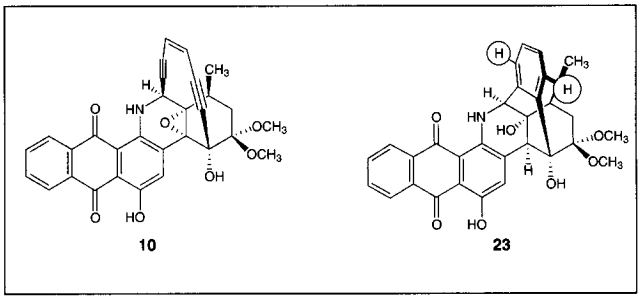


Fig. 10. Activation of the dideoxydynemicin analog **10** produces the expected Bergman cyclization product, compound **23**.

For example, whereas greater DNA cleavage is observed in the activation of **1** by NADPH than by glutathione (cleavage efficiencies: 5.7 and 4.0 %, respectively), the opposite is true with analog **10** (cleavage efficiencies: 3.5 and 7.2 %, respectively, Fig. 9). Reactivity differences such as these may provide the basis for variations in biological activity and, perhaps, therapeutic potential, and underscore the need for continued exploration of modified dynemicin structures. The latter contention is further supported by the fact that several of these analogs, and compound **10** in particular, have exhibited potent anticancer activity in preliminary *in vitro* evaluations conducted by the National Cancer Institute against a panel of 60 human tumor cell lines and have been selected for further evaluation *in vivo*.

In light of its biological activity and differential reactivity toward reducing cofactors versus dynemicin A (**1**), it was of interest to determine whether DNA cleavage by the analog **10** proceeded by a mechanism analogous to that of **1**. Treatment of a methanolic solution of analog **10** containing 1,4-cyclohexadiene with an aqueous solution of glutathione (20 equiv) led to clean formation of the Bergman cyclization product **23**, isolated in 50 % yield after purification by reverse-phase HPLC (Fig. 10). Incubation of the analog **10** with NADPH or glutathione in water in the presence of double-stranded calf thymus DNA produced the same product, **23**, in ~45 % yield in each case. Thus, it would appear that in terms of net transformation, compound **10** behaves as a perfect analog of **1**, although the details of these processes remain to be established.

Significance

Dynemicin A is a potent natural antitumor antibiotic and an important new chemotherapeutic lead. Because of its structural complexity and high reactivity, it has not been possible to substantially modify the natural product so as to produce analogs for study. Prior to this work, the absolute configuration of dynemicin A was unknown.

We have developed an unambiguous, enantioselective laboratory synthetic route to dynemicin A. By comparing enantiomerically pure, synthetic

dynemicin A with material derived from fermentation, the absolute configuration of the natural product was established. A key feature of the synthetic pathway we have developed is the fact that a wide variety of structural analogs of dynemicin A, compounds which could not have been prepared from the natural product, are now available for study, as demonstrated by the preparation of several active dynemicin analogs. These compounds have shown promising activity in DNA cleaving assays and *in vitro* screening against human tumor cell lines. It is anticipated that the availability of these and like molecules will lead to a greater understanding of the chemistry of dynemicin A and a more extensive exploration of its chemotherapeutic potential.

Materials and methods

*Synthesis of dideoxydynemicin anthraquinones by the Diels–Alder addition/PCC oxidation method. Preparation of dideoxydynemicin analog **10***

A 50-ml modified Schlenk flask equipped with a reflux condenser was charged with quinone imine **2** (160 mg, 0.35 mmol, 1 equiv), 1,1-diethoxyphthalan (370 mg, 1.78 mmol, 5 equiv), acetic acid (5 µl, 0.09 mmol, 0.2 equiv), and toluene (5 ml). The resulting solution was deoxygenated, then heated at reflux for 30 min. The reaction mixture was cooled to 23 °C, then was partitioned between saturated aqueous sodium bicarbonate solution (100 ml) and dichloromethane (100 ml). The organic layer was dried over sodium sulfate and was concentrated. The residue was purified by flash column chromatography (20 % ethyl acetate-hexanes) to afford the Diels–Alder adduct as a colorless oil (122 mg, 56 %, 1:1 mixture of exo:endo isomers). A solution of the latter product (115 mg, 0.19 mmol, 1 equiv) in dichloromethane (30 ml) at 23 °C was treated with solid pyridinium chlorochromate (390 mg, 1.90 mmol, 10 equiv) and the resulting purple solution was stirred at 23 °C for 10 min. The reaction mixture was partitioned between saturated aqueous sodium bicarbonate solution (500 ml) and dichloromethane (200 ml). The organic layer was treated with triethylamine (1 ml), then dried over sodium sulfate and concentrated to a volume of ~10 ml. The product was purified by flash column chromatography (20 % ethyl acetate-hexanes) to afford a dark purple film which was dissolved in acetonitrile (5 ml). The resulting purple solution was treated with triethylamine trihydrofluoride (610 µl, 3.8 mmol, 20 equiv), then stirred at 23 °C for 3 h. The reaction mixture was partitioned between saturated aqueous sodium bicarbonate solution (50 ml) and dichloromethane (50 ml). The organic layer was dried over sodium sulfate and concentrated. The residue was purified by flash column chromatography (20 % ethyl acetate-hexanes) to afford dynemicin analog **10** as a dark purple film (30 mg, 36 %).

*Synthesis of dideoxydynemicin anthraquinones by the phthalide addition method. Preparation of dideoxydynemicin analog **10***

A solution of *t*-butyllithium in pentane (1.7 M, 0.13 ml, 0.21 mmol, 2.5 equiv) was added to a solution of cyanophthalide (27 mg, 0.099 mmol, 2.0 equiv) in tetrahydrofuran (1.0 ml) at −78 °C. The resulting bright orange solution was stirred for 10 min at −78 °C whereupon a solution of quinone imine **3** (32 mg, 0.09 mmol, 1.0 equiv) in tetrahydrofuran (1.0 ml) was added, the cooling bath was removed, and the reaction mixture was allowed to warm to 23 °C. After 10 min, the purple reaction mixture was applied directly to a flash chromatography column loaded with ethyl acetate-hexanes (1:1) and the product anthraquinone was eluted using the same solvent mixture. Concentration of the appropriate column fractions and further purification of the residue by chromatography on Sephadex LH-20 (methanol) afforded the anthraquinone **10** as a dark purple oil (36 mg, 85 %).

*Dideoxydynemicin analog **10***

^{1}H NMR (500 MHz, C_6D_6): δ 13.76 (s, 1H, aryl O**H**), 10.54 (d, 1H, J = 4.1 Hz, N**H**), 9.17 (s, 1H, *m*-aryl **H**), 8.30 (d, 1H, J = 7.9 Hz, *o*-C(O)-aryl **H**), 8.10 (d, 1H, J = 7.2 Hz, *o*-C(O)-aryl **H**), 7.10 (td, 1H, J = 7.9, 1.2 Hz, *m*-C(O)-aryl **H**), 7.01 (td, 1H, J = 6.9, 1.2 Hz, *m*-C(O)-aryl **H**), 5.11 (s, 2H, **HC=CH**), 3.84 (d, 1H, J = 4.3 Hz, NC**H**), 3.33 (s, 3H, OCH_3), 2.93 (s, 3H, OCH_3), 2.20 (m, 1H, $CHCH_3$), 2.12 (t, 1H, J = 13.9 Hz, $CHCH_2$), 1.78 (dd, 1H, J = 11.9, 4.7 Hz, $CHCH_2$), 0.92 (d, 3H, J = 7.3 Hz, $CHCH_3$). Fourier-transform infra red spectroscopy (FTIR) (neat, cm^{-1}): 2944 (m), 2851 (w), 1621(m), 1590 (s), 1569 (m), 1492 (s), 1462 (m), 1251 (s), 1154 (m), and 1051 (s). HRMS (FAB): m/z calcd for $C_{30}H_{24}NO_7$ $(MH)^+$ 510.1553, found 510.1552.

*Synthesis of dynemicin anthraquinones by the Diels–Alder addition/oxidation method. Preparation of dynemicin analog **22***

A solution of potassium *N,N*-bis(trimethylsilyl)amide in toluene (0.5 M, 1.73 ml, 0.867 mmol, 26 equiv) was added to a solution of 4,7-bis(trimethylsilyloxy)phthalide (**17**, 259 mg, 0.834 mmol, 25 equiv) in tetrahydrofuran (5.0 ml) at −78 °C, and the resulting bright yellow solution was stirred for 25 min at −78 °C. During this time the reaction mixture darkened to yellow-brown. A 1:2 mixture of chlorotrimethylsilane and triethylamine (415 µl, 1.00 mmol in chlorotrimethylsilane, 30 equiv) was added. The reaction mixture was brought to −20 °C where the reaction solution became pale yellow within 5 min. At this point, a solution of the quinone imine **2** (15.0 mg, 33.4 µmol, 1 equiv) in tetrahydrofuran (1.5 ml) was transferred to the cold reaction mixture. The reaction flask was placed in an oil bath preheated to 55 °C and the reaction mixture was held at that temperature for 15 min. The flask was then allowed to cool to 23 °C and the reaction solution was concentrated to afford a light yellow residue. This residue was dissolved in pyridine (1 ml) and the resulting solution was transferred to an emerald green solution of copper(I) chloride (13.0 mg, 0.133 mmol, 4.0 equiv) in pyridine (1.0 ml) under an atmosphere of oxygen, producing an olive-green solution. To this solution was added a 600-µl aliquot of an ice-cold solution composed of hydrogen fluoride pyridine complex (1.0 ml), pyridine (4.0 ml), and tetrahydrofuran (10 ml). Oxygen was bubbled gently through the reaction mixture, which quickly turned black. After 10 min, the flow of oxygen gas was discontinued and the product solution was partitioned between ethyl acetate (50 ml) and water (50 ml). The aqueous layer was further extracted with ethyl acetate (50 ml) and the combined organic layers were washed sequentially with a saturated aqueous solution of EDTA (50 ml) and brine (3 x 50 ml). The aqueous layers were combined and extracted with ethyl acetate (50 ml). The combined ethyl acetate extracts were dried with sodium sulfate and were concentrated, and the residue was purified by chromatography on Sephadex LH-20 (methanol) to afford dynemicin analog **22** as a dark blue film (13.0 mg, 63 %).

*Dynemicin analog **22***
^{1}H NMR (500 MHz, C_6D_6): δ 13.58 (s, 1H, aryl O**H**), 12.84 (s, 1H, aryl O**H**), 12.45 (s, 1H, aryl O**H**), 9.96 (d, 1H, *J* = 3.9 Hz, N**H**), 9.01 (s, 1H, *m*-aryl **H**), 6.96 (d, 1H, *J* = 9.3 Hz, *o*-aryl *H*), 6.86 (d, 1H, *J* = 9.2 Hz, *o*-aryl **H**), 5.22 (d, 1H, J = 9.9 Hz, C≡C–C**H**=C), 5.18 (d, 1H, *J* = 10.0 Hz, C≡C–C**H**=C), 3.88 (d, 1H, *J* = 3.2 Hz, NC**H**), 3.47 (s, 3H, OC**H**$_3$), 3.06 (s, 3H, OC**H**$_3$), 2.27 (m, 1H, CH$_3$C**H**), 2.19 (t, 1H, *J* = 13.7 Hz, CHC**H**$_2$), 1.89 (dd, 1H, *J* = 13.8, 4.5 Hz, CHC**H**$_2$), 0.98 (d, 3H, *J* = 7.2 Hz, CHC**H**$_3$), 0.45 (s, 9H, OSi(C**H**$_3$)$_3$). FTIR (neat, cm^{-1}): 3600–3300 (br, m), 2959 (m), 2939 (m), 2851 (m), 1746 (w), 1579 (s), 1490 (w), 1450 (s), 1303 (s), 1249 (w), 1175 (s), 1057 (m). HRMS (FAB): *m/z* calcd for $C_{33}H_{31}NO_9Si$ $(M)^+$ 613.1768, found 613.1779.

*Synthesis of dideoxydynemicin analog **11***
Hydrolysis of dideoxydynemicin analog **10** (4 mg, 0.007 mmol, 1 equiv) in acetone (5 ml) containing *p*-toluenesulfonic acid (50 mg, 0.26 mmol, 40 equiv) at 23 °C for 2 h followed by aqueous work-up and purification by flash column chromatography (20 % ethyl acetate-hexanes) afforded the dideoxydynemicin analog **11** as a dark purple oil (2.0 mg, 50 %).

*Dideoxydynemicin analog **11***
^{1}H NMR (500 MHz, C_6D_6), δ: 13.59 (s, 1H, aryl O**H**), 10.31 (d, 1H, *J* = 4.2 Hz, N**H**), 9.06 (s, 1H, *m*-aryl **H**), 8.29 (d, 1H, *J* = 7.0 Hz, *o*-C(O)-aryl **H**), 8.07 (d, 1H, *J* = 7.9 Hz, *o*-C(O)-aryl **H**), 7.10 (td, 1H, *J* = 7.3, 1.3 Hz, *m*-C(O)-aryl **H**), 7.02 (td, 1H, *J* = 7.3, 1.1 Hz, *m*-C(O)-aryl **H**), 5.14 (dd, 1H, *J* = 10.6, 1.3 Hz, NCH–C≡C–C**H**), 5.09 (d, 1H, *J* = 10.1 Hz, C≡C–C**H**), 4.86 (s, 1H, O**H**), 3.66 (dd, 1H, *J* = 3.9, 1.2 Hz, NC**H**), 2.55 (dd, 1H, *J* = 9.4, 8.4 Hz, CHC**H**$_2$), 2.24 (m, 1H, C**H**CH$_3$), 2.18 (dd, 1H, *J* = 9.4, 7.4 Hz, CHC**H**$_2$), 0.80 (d, 3H, *J* = 7.1 Hz, CHC**H**$_3$). FTIR (neat, cm^{-1}): 3423 (w), 2922 (s), 2849 (w), 1742 (m), 1622 (m), 1585 (s), 1486 (m), 1351 (m), 1272 (s). HRMS (FAB): *m/z* calcd for $C_{28}H_{18}NO_6$ $(MH)^+$ 464.1134, found 464.1152.

*Synthesis of dideoxydynemicin analog **12***
Dideoxydynemicin analog **12** was prepared in 10 % yield (4.0-mg scale) following the Diels–Alder addition/PCC oxidation method described above for analog **10**.

*Dideoxydynemicin analog **12***
^{1}H NMR (500 MHz, C_6D_6), δ: 13.57 (s, 1H, aryl O**H**), 10.24 (d, 1H, *J* = 4.2 Hz, N**H**), 8.26 (d, 1H, *J* = 7.0 Hz, *o*-C(O)-aryl **H**), 8.10 (d, 1H, *J* = 7.6 Hz, *o*-C(O)-aryl **H**), 7.20 (s, 1H, *m*-aryl **H**), 7.10 (td, 1H, *J* = 7.3, 1.3 Hz, *m*-C(O)-aryl **H**), 7.02 (td, 1H, *J* = 7.3, 1.1 Hz, *m*-C(O)-aryl **H**), 5.14 (dd, 1H, *J* = 9.8, 1.1 Hz, C≡C–C**H**=C), 5.11 (dd, 1H, *J* = 10.1, 1.4 Hz, C≡C–C**H**=C), 3.92 (s, 1H, C≡C–C**H**), 3.58 (d, 1H, *J* = 3.9 Hz, NC**H**), 2.48 (dd, 1H, *J* = 15.9, 7.7 Hz, CHC**H**$_2$), 2.28 (m, 1H, C**H**CH$_3$), 2.21 (dd, 1H, *J* = 15.8, 4.4 Hz, CHC**H**$_2$), 1.00 (d, 3H, *J* = 7.4 Hz, CHC**H**$_3$). FTIR (neat, cm^{-1}): 2923 (w), 2848 (w), 1710 (m), 1620 (s), 1588 (s), 1483 (m), 1356 (m), 1257 (s). HRMS (FAB): *m/z* calcd for $C_{28}H_{18}NO_5$ $(MH)^+$ 448.1185, found 448.1155.

*Synthesis of dideoxydynemicin A methyl ester (**13**)*
Dideoxydynemicin A methyl ester (**13**) was prepared in 6 % yield (17.0-mg scale) following the Diels–Alder addition/PCC oxidation method described above for analog **10**.

*Dideoxydynemicin A methyl ester (**13**)*
^{1}H NMR (500 MHz, C_6D_6), d: 13.71 (s, 1H, aryl O**H**), 10.29 (d, 1H, *J* = 4.2 Hz, N**H**), 8.30 (d, 1H, *J* = 7.0 Hz, *o*-C(O)-aryl **H**), 8.12 (d, 1H, *J* = 7.6 Hz, *o*-C(O)-aryl **H**), 7.43 (s, 1H, *m*-aryl **H**), 7.20 (td, 1H, *J* = 7.3, 1.3 Hz, *m*-C(O)-aryl **H**), 7.10 (td, 1H, *J* = 7.3, 1.1 Hz, *m*-C(O)-aryl **H**), 5.17 (dd, 1H, *J* = 9.8, 1.1 Hz, C≡C–C**H**=C), 5.13 (dd, 1H, *J* = 10.0, 1.2 Hz, C≡C–C**H**=C), 3.90 (s, 1H, C≡C–C**H**), 3.76 (q, 1H, *J* = 7.2 Hz, C**H**CH$_3$), 3.58 (d, 1H, *J* = 4.0 Hz, NC**H**), 3.45 (s, 6H, CO$_2$CH$_3$, OC**H**$_3$), 1.45 (d, 3H, *J* = 7.3 Hz, CHC**H**$_3$). FTIR (neat, cm^{-1}): 2922 (m), 2850 (w), 1711 (m), 1693 (m), 1588 (s), 1484 (m), 1357 (m), 1257 (s). HRMS (FAB): *m/z* calcd for $C_{31}H_{22}NO_7$ $(MH)^+$ 520.1396, found 520.1434.

*Synthesis of dideoxydynemicin A (**14**)*
Dideoxydynemicin A was prepared following the phthalide addition method described above for dynemicin analog **10**, albeit employing the quinone imine **6** (8.0 mg, 0.015 mmol) as substrate. The reaction also differed in that hydrogen fluoride pyridine complex (75 μl) was used to quench the reaction (at −30 °C) in order to effect cleavage of the triisopropylsilyl ester. Dideoxydynemicin A was obtained as a dark purple oil (3.6 mg, 47 %) after purification on Sephadex LH-20 gel (methanol).

*Dideoxydynemicin A (**14**)*
^{1}H NMR (500 MHz, C_6D_6): δ 13.81 (s, 1H, aryl O**H**), 10.26 (d, 1H, *J* = 3.7 Hz, N**H**), 8.34 (d, 1H, *J* = 6.9 Hz, *o*-C(O)-aryl **H**), 8.15 (d, 1H, *J* = 7.2 Hz, *o*-C(O)-aryl **H**), 7.42 (s, 1H, *m*-aryl **H**), 7.25 (td, 1H, *J* = 6.9, 1.4 Hz, m-C(O)-aryl **H**), 7.05 (td, 1H, *J* = 7.2, 1.3 Hz, *m*-C(O)-aryl **H**), 5.23 (d, 1H, *J* = 9.9 Hz, C≡C–C**H**=C), 5.15 (d, 1H, *J* = 9.8 Hz, C≡C–C**H**=C), 3.95 (q, 1H, *J* = 7.3 Hz, C**H**CH$_3$), 3.72 (br s, 1H, C≡C–C**H**), 3.51 (d, 1H, *J* = 3.7 Hz, NC**H**), 2.75 (s, 3H, OC**H**$_3$), 1.49 (d, 3H, *J* = 7.3 Hz, CHC**H**$_3$). FTIR (neat, cm^{-1}): 3350 (br), 2964 (s), 2929 (m), 2848 (w), 1702 (s), 1621 (s), 1586 (m), 1388 (m), 1240 (m), 1120 (s), 1000 (s). MS (FAB): *m/z* calcd for $C_{30}H_{21}NO_7$ $(M)^+$ 505.1, found 505.1.

*Synthesis of dynemicin A (**1**)*
Dynemicin A was prepared following the procedure described above for dynemicin analog **22**, but employing the quinone imine **6** as substrate. In addition, the amount of isobenzofuran precursor was reduced from 25 to 7 equiv, heating at 55 °C was reduced from 15 to 5 min, and purification was achieved by chromatography on Sephadex LH-20 gel eluting with 20 % acetonitrile-methanol. In this manner, a 9.0-mg portion of the quinone imine **6** was transformed into 1.3 mg of pure dynemicin A (14 % yield). Synthetic dynemicin A was shown to be identical to an authentic sample by spectroscopic comparison (^{1}H NMR, infra-red, circular dichroism) and by chromatographic behavior (reverse-phase HPLC, co-injection).

*Dynemicin A (**1**)*
^{1}H NMR (400 MHz, C_6D_6): δ 13.45 (br s, 1H, aryl O**H**), 12.91 (br s, 1H, aryl O**H**), 12.43 (br s, 1H, aryl O**H**), 9.77 (d, 1H, *J* = 4.8 Hz, N**H**), 7.35 (s, 1H, *m*-aryl H), 6.95 (d, 1H, *J* = 9.2 Hz, *o*-aryl **H**), 6.85 (d, 1H, *J* = 9.2 Hz, *o*-aryl ***H***), 5.22 (dd, 1H, *J* = 10.0, 1.5 Hz, C≡C–C**H**=C), 5.14 (dd, 1H, *J* = 10.0, 1.5 Hz, C≡C–C**H**=C), 3.94 (q, 1H, *J* = 7.3 Hz, CH$_3$C**H**), 3.70 (d, 1H, *J* = 1.5 Hz, C≡C–C**H**), 3.53 (dd, 1H, *J* = 4.8, 1.5 Hz, NC**H**), 2.87 (s, 3H, OC**H**$_3$), 1.47 (d, 3H, *J* = 7.3 Hz, CHC**H**$_3$). ^{1}H NMR (400 MHz, DMSO): δ 13.13 (s, 1H, aryl O**H**), 12.73 (s, 1H, aryl O**H**), 12.14 (s, 1H, aryl O**H**), 9.85 (br s, 1H, N**H**), 8.04 (s, 1H, *m*-aryl **H**), 7.37 (br m, 2H, *o*-aryl **H**), 6.09 (br d, 1H, *J* = 10.0 Hz, C≡C–C**H**=C), 6.05 (br d, 1H, *J* = 10.0 Hz, C≡C–C**H**=C), 5.07 (br m, 1H, NC**H**), 4.89 (br s, 1H, C≡C–C**H**), 3.81 (s, 3H, OC**H**$_3$), 3.57 (m, 1H, CH$_3$C**H**),

1.26 (d, 3H, J = 7.0 Hz, CHC**H**$_3$). FTIR (neat, cm^{-1}): 3686-2730 (br, m), 3405 (br, m), 3285 (m), 2924 (w), 2854 (w), 1750-1500 (br, m), 1642 (m), 1585 (vs), 1471 (s), 1395 (s), 1295 (sh), 1274 (s), 1189 (s), 1169 (s), 1144 (m), 1099 (w), 1034 (m), 969 (w), 923 (w), 783 (w). MS(FAB): m/z calcd for $C_{30}H_{19}NO_9$ $(MH)^+$ 538.1, found 538.1.

Preparation of 3'-^{32}P-labeled 193-base pair restriction fragment

Plasmid pBR322 (40 µl, 0.25 µg ml^{-1}, Boehringer Mannheim) was precipitated by the addition of aqueous ammonium acetate buffer solution (20 µl, 8 M, pH 7) and ethanol (180 µl), followed by centrifugation at 2 °C (16 000 g, 30 min), then washed with aqueous ethanol (100 µl, 70 %). The DNA pellet was dried on a Savant rotary speed vac, then was dissolved in water (90 µl). Digestion buffer H (10 µl, Boehringer Mannheim) was added, and the plasmid was digested with *Eco* RI (4 µl, 40 units, Boehringer Mannheim) and *Ssp* I (4 µl, 40 units, Boehringer Mannheim) at 37 °C for 6 h. The digestion was quenched by extracting the reaction solution twice with phenol:chloroform (100 µl, 1:1 v/v), and the DNA was precipitated from the aqueous layer by the addition of aqueous ammonium acetate buffer solution (50 µl, 8 M, pH 7) and ethanol (400 µl), followed by centrifugation at 2 °C (16 000 g, 30 min). The DNA pellet was washed with aqueous ethanol (100 µl, 70 %), then dried. The DNA was dissolved in water (18 µl), then mixed with aqueous dithiothreitol solution (4 µl, 100 mM), Sequenase 2.0 buffer (8 µl, USB), and [α-^{32}P]-dATP (10 µl, 200 µCi, NEN, ≥6000 Ci mmol^{-1}). The restriction fragment was 3'-labeled with Sequenase Version 2.0 (2 µl, 25 units, USB) at 23 °C for 3 h, then treated with aqueous dATP solution (5 µl, 10 mM) and Sequenase Version 2.0 (2 µl, 25 units, USB) and incubated further at 23 °C for 2 h. The reaction solution was eluted through a NICK column (Pharmacia) to remove unincorporated ^{32}P-dATP, and the eluant containing the labeled fragment was concentrated to a volume of 100 µl. The labeled fragment was precipitated by the addition of aqueous ammonium acetate buffer solution (50 µl, 8 M, pH 7) and ethanol (400 µl), followed by centrifugation at 2 °C (16 000 g, 30 min), then was washed with aqueous ethanol (100 µl, 70 %) and dried. The 193-base pair fragment was dissolved in water (20 µl) and Ficoll loading buffer (5 µl), then purified over a non-denaturing polyacrylamide gel (8 %, 0.8 mm thickness) [27]. The band containing the 193-base pair fragment was located by autoradiography and was excised from the gel. The gel slice was crushed thoroughly and, after combination with aqueous Nonidet P-40 detergent solution (700 µl, 0.05 %, Sigma), was vortexed for 6 h at 23 °C. The resulting suspension was filtered through a Centrex filter (0.45 µm) and the filtrate was extracted twice with phenol:chloroform (400 µl, 1:1 v/v), then concentrated to a volume of 100 µl. The labeled product was precipitated by the addition of aqueous ammonium acetate buffer solution (50 µl, 8 M, pH 7) and ethanol (400 µl), followed by centrifugation at 2 °C (16 000 x g, 30 min), then washed with aqueous ethanol (100 µl, 70 %). The purified labeled fragment was stored frozen in tris-HCl aqueous buffer solution (10 mM, pH 7.4) containing EDTA (1 mM).

Analysis of DNA cleavage products, general

The products from a given DNA cleavage reaction were precipitated by the addition of aqueous ammonium acetate buffer solution (50 µl, 8 M, pH 7) and ethanol (250 µl), followed by centrifugation at 2 °C (16 000 g, 20 min). The resulting product pellet was washed with aqueous ethanol (100 µl, 70 %), dried, then dissolved in formamide loading buffer (10 µl), and the resulting solution transferred to a 0.65-ml Eppendorf tube. After assaying for radioactivity with a Beckman LS 6000SC scintillation counter, the solution was diluted with additional formamide loading buffer so as to produce a radiation density of 3000 cpm µl^{-1}. After heating at 80 °C for 3 min to induce denaturation, the solution (5 µl) was analyzed by gel electrophoresis. Cleavage products were loaded onto a denaturing polyacrylamide gel (8 %, 42 x 34 cm, 0.4 mm thickness) and were separated by electrophoresis in 1 x TBE buffer at 2000 V for 15 min and then at 1500 V until the bromophenol blue dye had migrated off the gel [27]. The gel was exposed to a storage phosphor plate and the DNA cleavage products were quantified with a Molecular Dynamics 400-S PhosphorImager.

*Analysis of DNA cleavage by dynemicin A and synthetic analogs **10**, **11**, **12**, and **13***

Reactions were performed at 37 °C in 1.5-ml Eppendorf tubes containing a total reaction volume of 50 µl. A 5-µl aliquot of a solution of dynemicin A (0.5 mM) in DMSO was combined with a solution of double-stranded calf thymus DNA (5 µl, 10 mM bp) in water, tris-HCl aqueous buffer solution (15 µl, 100 mM, pH 7.5), aqueous sodium chloride solution (5 µl, 500 mM), water (15 µl), and 3'-^{32}P-labeled restriction fragment (~100 000 cpm). The reaction was initiated at 23 °C by the addition of an aqueous solution of glutathione (5 µl, 200 mM, pH 7.5) to the remaining solution, thus producing the following concentrations of solution components at the onset of the reaction: dynemicin A, 0.05 mM; GSH, 20 mM; double-stranded calf thymus DNA, 1.0 mM bp; tris-HCl buffer, 30 mM; sodium chloride, 50 mM. The reaction solution was incubated at 37 °C for 12 h and the cleavage products were precipitated and analyzed as described above. DNA cleavage reactions employing the synthetic dynemicin analogues **10**, **11**, **12**, and **13** were conducted in an identical manner, employing a solution of each respective dynemicin analog in DMSO (0.5 mM) in lieu of dynemicin A. DNA cleavage reactions employing NADPH were conducted in an identical manner, employing an aqueous solution of NADPH (200 mM) in lieu of GSH.

*Reaction of **10** with GSH and NADPH in the presence of DNA; HPLC analysis*

The reaction of **10** with GSH was performed at 37 °C in a 1.5-ml Eppendorf tube containing a total reaction volume of 100 µl, as follows. A 10-µl aliquot of a solution of **10** (1.0 mM) in methanol containing 2,5-dimethoxybenzyl alcohol (1.0 mM, internal HPLC standard) was combined with a solution of double-stranded calf thymus DNA (10 µl, 10 mM bp) in water, tris-HCl aqueous buffer solution (30 µl, 100 mM, pH 7.5), aqueous sodium chloride solution (10 µl, 500 mM), aqueous 1,2-bis(hydroxymethyl)-1,4-cyclohexadiene [28] (10 µl, 500 mM, a hydrogen atom source), and water (10 µl). The reaction was initiated at 37 °C by the addition of an aqueous solution of GSH (20 µl, 100 mM, pH 7.5), thus producing the following concentrations of solution components at the onset of the reaction: **10**, 0.1 mM; GSH, 20 mM; double-stranded calf thymus DNA, 1.0 mM bp; tris-HCl buffer, 30 mM; sodium chloride, 50 mM; 1,2-bis(hydroxymethyl)-1,4-cyclohexadiene, 50 mM. The reaction solution was incubated at 37 °C for 16 h. HPLC analysis of the reaction product (40-µl injection volume) employed a Beckman HPLC system equipped with a Beckman Ultrasphere ODS (C_{18}, 5 µm) rp-HPLC

column, 4.6 x 250 mm, flow = 0.40 ml min^{-1} with a linear gradient of acetonitrile:aqueous ammonium acetate buffer solution (10 mM, pH 6.0): 10:90 v/v acetonitrile: aqueous ammonium acetate buffer to 100 % acetonitrile over a period of 50 min. Peaks were detected by ultraviolet absorption at 255 nm with a Beckman 168 Programmable Photodiode Detector. An initial ratio of **10** to 2,5-dimethoxybenzyl alcohol was established by HPLC analysis of the methanolic solution of **10**. Product **23** (retention time t_R ~ 44 min) was formed in ~45 % yield, as determined by integration against the internal standard. The identity of product **23** was established by co-injection with an authentic sample of **23** (see below). The reaction with NADPH was conducted in an identical manner, employing an aqueous solution of NADPH (200 mM) in lieu of GSH and formed product **23** in ~45 % yield, as determined by integration against the internal standard.

Preparation of product 23

A solution of dynemicin analog **10** (6 mg, 0.012 mmol, 1 equiv) in methanol (9 ml) containing 2,5-dimethoxybenzyl alcohol (1 mM, internal HPLC standard) was treated sequentially with 1,4-cyclohexadiene (1.0 ml, 10 mmol, 830 equiv), aqueous glutathione solution (200 mM, 1.0 ml, 0.20 mmol, 20 equiv), and triethylamine (0.30 ml, 220 mg, 2.2 mmol, 180 equiv), thus producing the following concentrations of solution components at the onset of the reaction: **10**, 1 mM; 1,4-cyclohexadiene, 1 M; glutathione, 20 mM; triethylamine, 200 mM. The solution was incubated at 37 °C for 12 h, during which time its color changed from violet to dark purple. The product solution was concentrated to a volume of ~0.5 ml, then was diluted with water (1 ml). Purification of the product was achieved by reverse-phase HPLC, using three separate 500-µl injection volumes, on a Beckman Ultrasphere ODS (C_{18}, 5 µm) rp-HPLC column, 10 x 250 mm, as part of a Beckman HPLC system, flow = 2.00 ml min^{-1}, with a linear gradient of acetonitrile:aqueous ammonium acetate buffer solution (10 mM, pH 6.0): 10:90 v/v acetonitrile:aqueous ammonium acetate buffer to 90:10 v/v acetonitrile:aqueous ammonium acetate buffer over a period of 50 min. Peaks were detected by ultraviolet absorption at 250 nm with a Beckman 168 Programmable Photodiode Detector. Fractions containing product **23** (retention time t_R ~45 min) were collected and pooled. Acetonitrile was removed by rotary evaporation and the resulting concentrated aqueous solution was lyophilized. Product **23** was obtained as a dark purple film (3 mg, 50 %, as determined by integration against the internal standard).

Product 23

^{1}H NMR (500 MHz, C_6D_6),: δ 13.80 (s, 1H, aryl O**H**), 10.48 (d, 1H, J = 4.3 Hz, N**H**), 8.27 (dd, 1H, J = 8.0, 1.0 Hz, *o*-C(O)-aryl **H**), 8.15 (dd, 1H, J = 7.8, 1.2 Hz, *o*-C(O)-aryl **H**), 7.41 (dd, 1H, J = 8.0, 1.1 Hz, aromatized core), 7.25 (s, 1H, *m*-aryl **H**), 7.12 (td, 1H, J = 7.8, 1.3 Hz, *m*-C(O)-aryl **H**), 7.04 (td, 1H, J = 8.5, 1.3 Hz, *m*-C(O)-aryl **H**), 6.96 (td, 1H, J = 7.4, 1.2 Hz, aromatized core), 6.92 (td, 1H, J = 7.3, 1.7 Hz, aromatized core), 6.85 (dd, 1H, J = 7.0, 1.3 Hz, aromatized core), 3.91 (dd, 1H, J = 4.4, 1.9 Hz, NC**H**), 3.50 (d, 1H, J = 1.7 Hz, C(OH)-C**H**-C(OH)), 3.11 (s, 3H, OC$\mathbf{H_3}$), 3.00 (s, 3H, OC$\mathbf{H_3}$), 2.93 (s, 1H, O**H**), 2.66 (s, 1H, O**H**), 2.43 (m, 1H, C**H**CH$_3$), 1.58 (dd, 1H, J = 14.5, 5.9 Hz, CHC$\mathbf{H_2}$), 0.96 (d, 3H, J = 6.8 Hz, CHC$\mathbf{H_3}$), 0.55 (t, 1H, J = 13.9 Hz, CHC$\mathbf{H_2}$). FTIR (neat, cm^{-1}): 3446 (m, br), 2954 (m), 2923 (s), 2851 (w), 1615 (m), 1585 (s), 1492 (m), 1354 (s), 1251 (s), 1051 (s). HRMS (FAB): m/z calcd for $C_{30}H_{28}NO_7$ $[MH]^+$ 514.1866, found 514.1869.

Acknowledgements: Generous financial support from the National Institutes of Health is gratefully acknowledged. We also express our gratitude to Dr Dolatrai M. Vyas of Bristol-Myers Squibb Pharmaceutical Research Institute for an authentic sample of dynemicin A. This is contribution No. 8976 from the Arnold and Mabel Beckman Laboratories of Chemical Synthesis.

References

1. Konishi, M., *et al.*, & Clardy, J. (1990). Crystal and molecular structure of dynemicin A: a novel 1,5-diyn-3-ene antitumor antibiotic. *J. Am. Chem. Soc.* **112**, 3715–3716.
2. Kamei, H., Nishiyama, Y., Takahashi, A., Obi, Y., & Oki, T. (1991). Dynemicins, new antibiotics with the 1,5-diyn-3-ene and anthraquinone subunit. II. Antitumor activity of dynemicin A and its triacetyl derivative. *J. Antibiot. (Tokyo)* **44**, 1306–1311.
3. Nicolaou, K.C. & Dai, W.-M. (1991). Chemistry and biology of the enediyne anticancer antibiotics. *Angew. Chem. Int. Ed. Engl.* **30**, 1387–1416.
4. Sugiura, Y., Shiraki, T., Konishi, M. & Oki, T. (1990). DNA intercalation and cleavage of an antitumor antibiotic dynemicin that contains anthracycline and enediyne cores. *Proc. Natl. Acad. Sci. USA* **87**, 3831–3835.
5. Semmelhack, M.F., Gallagher, J. & Cohen, D. (1990). Bioreductive alkylation as a trigger for toxic effects of dynemicin. *Tetrahedron Lett.* **31**, 1521–1522.
6. Sugiura, Y., *et al.*, & Konishi, M. (1991). Reductive and nucleophilic activation products of dynemicin A with methyl thioglycolate. A rational mechanism for DNA cleavage of the thiol-activated dynemicin A. *Biochemistry* **30**, 2989–2992.
7. Porco Jr., J.A., Schoenen, F.J., Stout, T.J., Clardy, J. & Schreiber, S.L. (1990). Transannular Diels–Alder route to systems related to dynemicin A. *J. Am. Chem. Soc.* **112**, 7410–7411.
8. Nicolaou, K.C., Hwang, C.-K., Smith, A.L. & Wendeborn, S.V. (1990). Synthesis of dynemicin A models. *J. Am. Chem. Soc.* **112**, 7416–7418.
9. Nishikawa, T., Isobe, M. & Goto, T. (1991). Synthetic studies on the bicyclo[7.3.1]tridecenediyne system in an antitumor antibiotic, dynemicin A. *Synlett.* 393–395.
10. Magnus, P. & Fortt, S.M. (1991). A rapid entry into the dynemicin core structure: remarkable solvent effect on an η^2-hexacarbonyldicobalt propargylic cation cyclization. *J. Chem. Soc., Chem. Commun.* 544–546.
11. Wender, P.A. & Zercher, C.K. (1991). Studies on DNA-cleaving agents: synthesis of a functional dynemicin analogue. *J. Am. Chem. Soc.* **113**, 2311–2313.
12. Nicolaou, K.C., Smith, A.L., Wendeborn, S.V. & Hwang, C.-K. (1991). Synthesis and chemistry of dynemicin A models. *J. Am. Chem. Soc.* **113**, 3106–3114.
13. Nicolaou, K.C., *et al.*, & Siegel, J.S. (1993). Molecular design, chemical synthesis, kinetic studies, calculations, and biological studies of novel enediynes equipped with triggering, detection, and deactivating devices. Model dynemicin A epoxide and *cis*-diol systems. *J. Am. Chem. Soc.* **115**, 7944–7953.
14. Taunton, J., Wood, J.L. & Schreiber, S.L. (1993). Total syntheses of di-and tri-*O*-methyl dynemicin A methyl esters. *J. Am. Chem. Soc.* **115**, 10378–10379.
15. Yoon, T., Shair, M.D., Danishefsky, S.J. & Shulte, G.K. (1994). Experiments directed toward a total synthesis of dynemicin A: a solution to the stereochemical problem. *J. Org. Chem.* **59**, 3752–3754.
16. Shair, M.D., Yoon, T. & Danishefsky, S.J. (1994). A remarkable cross coupling reaction to construct the enediyne linkage relevant to dynemicin A: synthesis of the deprotected ABC system. *J. Org. Chem.* **59**, 3755–3757.
17. Yoon, T., Shair, M.D., & Danishefsky, S.J. (1994). An advanced dynemicin A model: stabilization of the 3,8-epoxide by anthraquinone functionality in the absence of the bridging enediyne. *Tetrahedron Lett.* **35**, 6259–6262.
18. Myers, A.G., Fraley, M.E. & Tom, N.J. (1994). A highly convergent route to dynemicins of wide structural variability. Enantioselective synthesis of quinone imine precursors to natural and nonnatural dynemicins. *J. Am. Chem. Soc.* **116**, 11556–11557.
19. Contreras, L., Slemon, C.E. & MacLean, D.B. (1978). Polysubstituted naphthalenes from phthalide ortho esters and dienophiles.

Tetrahedron Lett. 4237–4240.
20. Corey, E.J. & Suggs, J.W. (1975). Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* 2647–2650.
21. Kraus, G.A. & Sugimoto, H. (1978). An annelation route to quinones. *Tetrahedron Lett.* 2263–2266.
22. Freskos, J.N., Morrow, G.W. & Swenton, J.S. (1985). Synthesis of functionalized hydroxyphthalides and their conversion to 3-cyano-1(3*H*)-isobenzofuranones. The Diels–Alder reaction of methyl 4,4-diethoxybutynoate and cyclohexadienes. *J. Org. Chem.* **50**, 805–810.
23. Iwao, M., Inoue, H. & Kuraishi, T. (1984). Generation and Diels–Alder reaction of 1-siloxy-3-arylisobenzofurans from 3-arylphthalides. *Chem. Lett.* 1263–1266.
24. Waller, C.W., *et al.*, & Williams, J.H. (1952). Degradation of auromycin. VI. Isoaureomycin and aureomycin. *J. Am. Chem. Soc.* **74**, 4981.
25. Stephens, C.R., *et al.*, & Woodward, R.B. (1954). The structure of aureomycin. *J. Am. Chem. Soc.* **76**, 3568–3575.
26. Barker, S.A. & Settine, R.L. (1979). The synthesis of olivetol. Trimethylsilation as a protective group during Grignard reactions. *Org. Prep. Proced. Int.* **11**, 87–92.
27. Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular Cloning — A Laboratory Manual.* (2nd ed), Cold Spring Harbor Laboratory Press, N.Y.
28. Myers, A.G., Cohen, S.B. & Kwon, B.-M. (1994). DNA cleavage by neocarzinostatin chromophore. Establishing the intermediacy of a chromophore-derived cumulene and biradical species and their role in sequence-specific cleavage. *J. Am. Chem. Soc.* **116**, 1670–1682.
29. Iverson, B.L. & Dervan, P.B. (1987). Adenine specific DNA chemical sequencing reaction. *Nucleic Acids Res.* **15**, 7823–7830.

Received: **22 Dec 1994**. Accepted: **28 Dec 1994**.