Design and Synthesis of Dynemicin Analogs

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Dynemicin A is a member of the family of enediyne natural products. It is unique in that it combines a ten-membered enediyne with an anthraquinone substructure. These features stimulated the development of synthetic approaches to the natural product itself and of analogs thereof. This review summarizes the total syntheses of dynemicin A. In addition, an overview of the known analogs is presented. The analogs can be classified according to the designed trigger mechanism. Most of the analogs contain a removable carbamate on the nitrogen atom. Others are quite similar to the natural lead in that they contain a quinone substructure, which upon reduction causes opening of the oxirane ring. In addition, there are analogs that contain an aromatic sector, the enediyne, and the oxirane ring but lack the nitrogen heterocycle. In these compounds the aryl ring assumes a different conformation from that in dynemicin A. Many of the simplified analogs proved to be quite active in vitro as well in vivo against murine tumor models. A highlight is compound **30** which is much more active than dynemicin A itself. However, looking at all analogs there is no clear-cut correlation between the DNA-cleaving ability at neutral pH and the in vitro results. From this one might conclude that there are possibly two mechanisms for antitumor activity. One involves diradical formation whereas the other might be due to a ligand-receptor interaction.

Introduction

In a number of antitumor compounds, the transition of a quinone substructure to an aromatic derivative is a key step in mediating cytotoxic effects (Scheme 1). Examples are the mitosenes,[1] the cyclopropyl ring containing CC-1065,[2,3] duocarmycin,[3,4] and certain anthracyclinones.[5−7] These compounds act as potent DNA alkylating agents.[8] In the case of the mitosenes, two-electron reduction and loss of the leaving group generates the highly electrophilic quinone methide species. A related compound is FR900482[8,9] which also causes DNA cross-linking. Interestingly, this compound already contains an aromatic ring. In this case reductive activation is needed to cleave the N−O bond.[10] CC-1065 and duocarmycin, on the other hand aromatize by nucleophilic attack, that is by DNA, to the electrophilic cyclopropane ring without prior activation.

For about ten years now, the structure of dynemicin A (**1**) has been known.[11] It was isolated from the fermentation broth of the microorganism *Micromonospora chersina*.[11] This very potent antitumor antibiotic combines an anthraquinone[12] part with an enediyne substructure. However, in contrast to the above-mentioned compounds, dyne-

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Martin E. Maier (center), born in 1956, studied chemistry at the University of Konstanz. He obtained his Diploma in 1981 and completed his Ph.D. in 1885 with Prof. R. R. Schmidt on hetero Diels−Alder reactions. After a post-doctoral stay with Prof. S. J. Danishefsky at Yale University (USA), he returned to the University of Konstanz, to obtain his habilitation in 1992. In 1993 he joined the chemistry department at the University of Braunschweig. From 1994 until 1997 he worked as Professor of Organic Chemistry at the Martin-Luther-University of Halle/Saale. Since then he is a full Professor of Organic Chemistry at the University of Tübingen (Germany). His research interest includes the synthesis of bioactive molecules with an emphasis on antitumor compounds.

Folkert Boße (left), born in 1967, studied chemistry at the University of Braunschweig (Germany). In 1995 he finished his Diploma with Prof. M. E. Maier on cross-coupling reactions of electron-rich arylmetal compounds with α-iodoenones. Currently, he is completing his doctorate with Prof. M. E. Maier on the synthesis of dynemicin analogs.

André J. Niestroj (right), born in 1968, studied chemistry in Halle/Saale at the Martin-Luther-University (Germany). In 1994 he finished his Diploma with Prof. H. Jeschkeit on the regio- and stereoselective addition of amines to amino acid esters. Currently, he is completing his doctorate with Prof. M. E. Maier on cross-coupling approaches to dynemicin analogs.

MICROREVIEWS: This feature introduces the readers to the authors' research through a concise overview of the selected topic. Reference to important work from others in the field is included.

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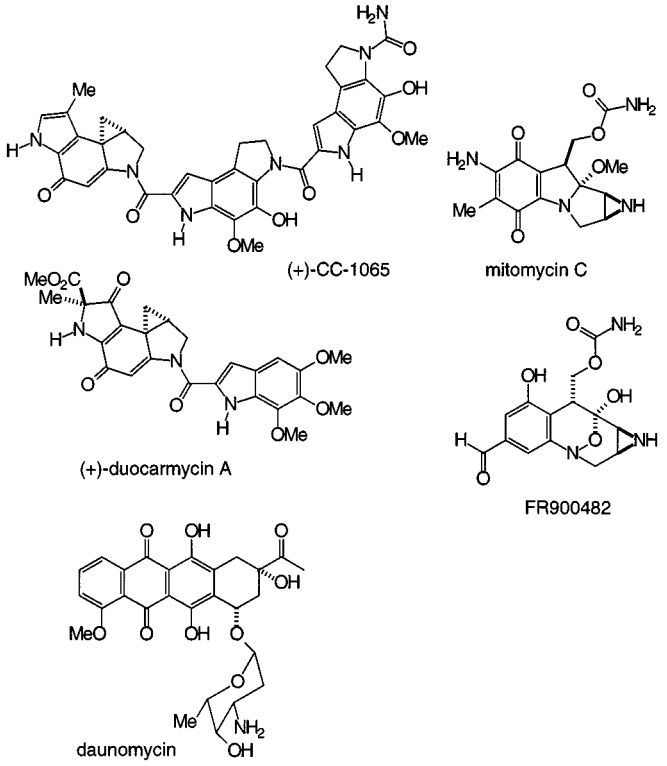
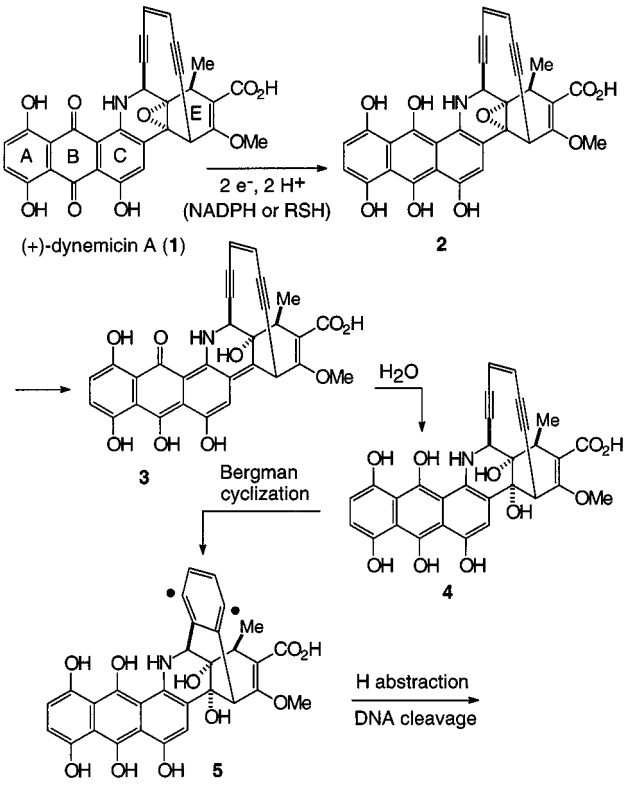


Scheme 1



Scheme 2

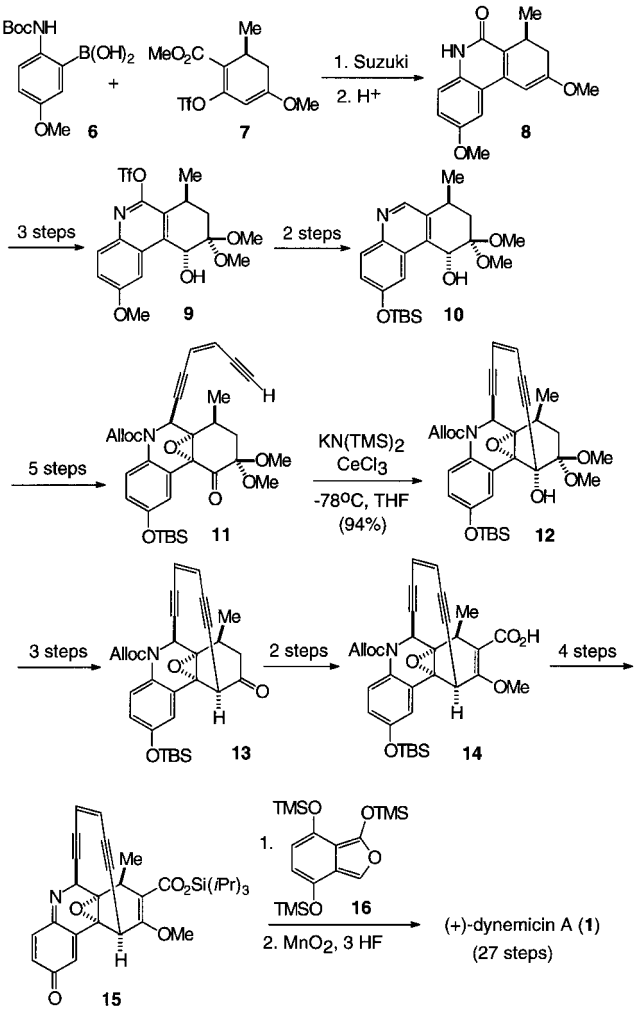
micin A does not alkylate but rather cleaves DNA. Nevertheless, the activation of dynemicin A is triggered by two-electron reduction to provide the anthraquinol **2**. This in turn rearranges to the quinone methide **3** with simultaneous opening of the epoxide. Most likely, the electrophilic quinone methide is trapped with water giving the *cis*-diol **4**. The epoxide opening is crucial for the mode of action because it enables the enediyne to undergo a rapid Bergman cyclization. Basically, the epoxide serves as a blocking device because the Bergman cycloaromatization of dynemicin A (**1**) is seriously handicapped due to a highly strained transition-state.[13] Particularly, the epoxide opening is accompanied with a reduction of the distance between the enediyne termini from 354 pm (X-ray) down to 317 pm (MM2). The aromatic diradical **5** is the species that ultimately cleaves the DNA by hydrogen abstraction from the sugar backbone (Scheme 2).[14,15] It should be noted that besides dynemicin A other enediyne-containing antitumor antibiotics are known.[16,17] Common to the enediynes are three functional units:[17] 1) a delivery system which is responsible for penetration of cellular membranes and binding to DNA, 2) a triggering device that serves to remove the blocking group, and 3) the enediyne which also has been termed the warhead.

It is clear that with the modular structure dynemicin A and the other enediynes are suitable lead compounds for development of less toxic analogs. As in the anthracyclines[11c][18] the aromatic part provides room for ample modifications. In addition, the nitrogen containing ring, the blocking group, or the functional group in the E-ring present itself for changes. It would make sense to leave the ten-membered enediyne intact. Experiments[19] and calculations[13] indicate that such enediynes cyclize with a reasonable half-life at physiological temperatures. As in the natural products, bridging of the enediyne-containing ring, for example to give a [7.3.1]enediyne enhances the reactivity towards the Bergman cyclization. The following review article summarizes the synthesis and biological studies of such dynemicin A analogs.

Total Syntheses of Dynemicin A

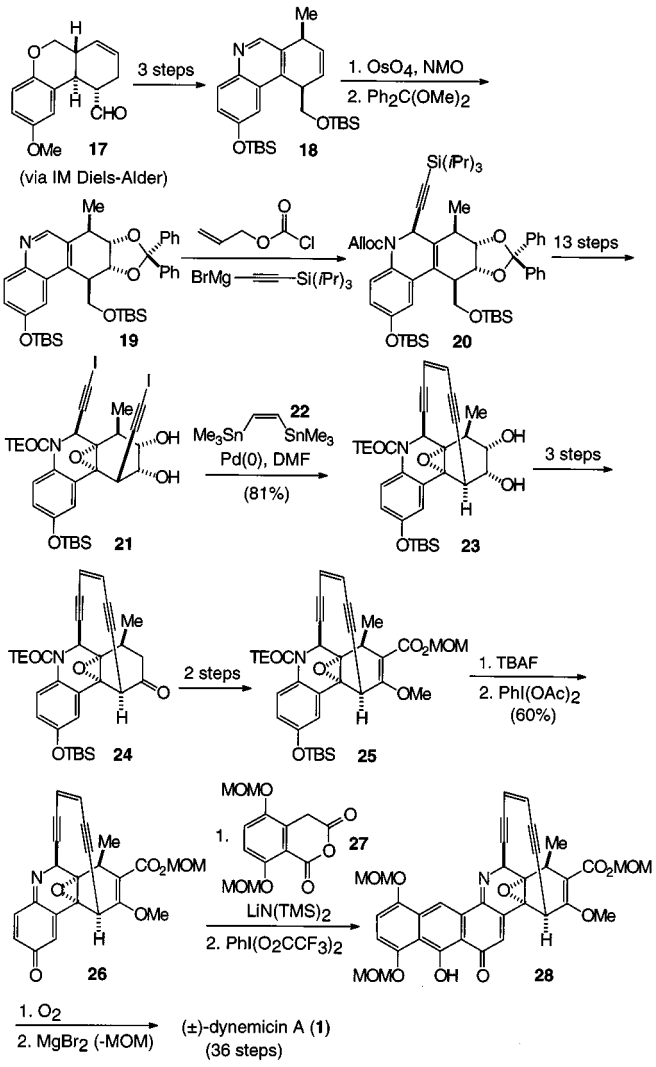
One rationale behind a total synthesis of a natural product is to demonstrate a synthetic strategy that can be easily modified to provide analogs. Therefore, it seems appropriate to briefly discuss the known total syntheses of dynemicin A (**1**). Both syntheses have as a common subgoal the synthesis of a phenanthridine structure which is subsequently bridged with the enediyne, supplied with the carboxylate and finally extended in the aromatic sector. In the Myers synthesis (Scheme 3), the C−C bond between the two carbocyclic rings is formed by a Suzuki cross-coupling reaction (arylboronic acid + enol triflate).[20] Subsequently, lactam formation closed the heterocyclic ring. Reduction of the amide was accomplished after formation of the quinolyl triflate followed by treatment with formic acid and a catalytic amount of $Pd(PPh_3)_4$. The enediyne was attached in one step to the quinoline **10**. Acylation and activation of the imine was followed by addition of the enediyne anion.

Conversion of the hydroxyl group to the corresponding magnesium alkoxide turned out to be crucial for obtaining the desired *cis* adduct. The ring closing reaction was performed on the enediyne ketone **11** using $KN(TMS)_2$ in the presence of cerium(III) chloride. From **12** the cyclic thionocarbonate could be obtained, whose reductive cleavage afforded the ketone **13**. After carboxylation, liberation of the phenol, oxidation of the latter with iodosobenzene and removal of the allyl carbamate the quinone imine **15** was obtained as a stable, yellow semisolid. In the final steps the anthraquinone ring was constructed. This was realized by a Diels–Alder reaction between the isobenzofuran **16** and quinone imine **15**. Deprotection and oxidation were accomplished with manganese dioxide and triethylamine hydrofluoride to provide (+)-dynemicin A (**1**) as a violet solid. This route involves 26 steps from an optically pure diketone and delivered 15 mg of the target molecule. Through variation of the isobenzofuran component, many other analogs could be obtained.



Scheme 3

The Danishefsky synthesis[21] is similar in the overall strategy but differs very much in the execution (Scheme 4). For example, the C–C bond between the aryl and the carbocyclic ring is fashioned through an intramolecular Diels–Alder reaction (structure **17**). Oxidation gave the quinone which upon treatment with ammonium acetate lead to the quinoline **18**. Again, activation of the imine and nucleophilic addition was used to introduce an alkyne. In this case, stereochemical control was provided by a bulky diol protecting group that blocked the α-face. Compound **20** was further converted by routine steps to the bis-iodoalkyne **21**. The enediyne bridge could be closed by a stitching cyclization using the distannylene **22** as the ethylene fragment. The two hydroxyl groups were differentiated by selective triflation of the OH next to the methyl group. Subsequent oxidation of the other one and $CrCl_2$-mediated reductive removal of the triflate function gave a ketone that could be subjected to α-carboxylation. Removal of the silicon protecting groups and oxidation of the intermediate aminophenol gave quinone imine **26**. The synthesis of dynemicin A was completed by attachment of a naphthoquinone ring through addition of the anion of **27**. The resulting annulation product gave, after oxidation and deprotection, racemic dynemicin A (**1**). This synthesis involves 36 steps from 5-methoxysalicyl aldehyde and provided 2.3 mg of dy-



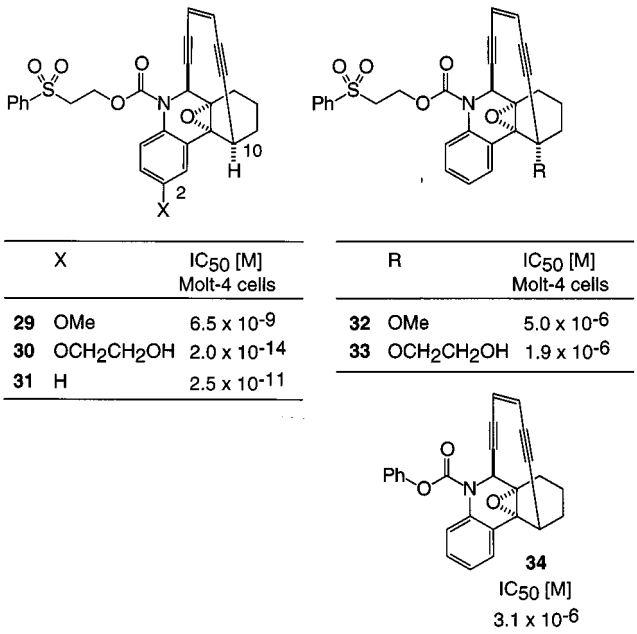
Scheme 4

nemicin A. It can be seen that the choice of protecting groups proved to be crucial since in the end the ultimate precursor carried only one type of a protecting group.

Another route to the structure of dynemicin A was reported by Schreiber et al. The key step in this synthesis is a transannular Diels–Alder reaction that establishes the D and E rings in one step. However, the synthesis did not lead to the natural product but gave instead the fully methylated derivative.[22,23]

Analogs

Since the structure of dynemicin A has been known, a large number of analogs have been prepared. These analogs might be classified according to their structural features or their similarities regarding the mode of activation. It can be seen that most of the analogs retain the C and D rings of dynemicin A. Of course, all of them feature the ten-membered enediyne whereas they lack various functional groups or rings. Some even come without the epoxide.



	X	IC_{50} [M] Molt-4 cells
29	OMe	6.5×10^{-9}
30	OCH_2CH_2OH	2.0×10^{-14}
31	H	2.5×10^{-11}

	R	IC_{50} [M] Molt-4 cells
32	OMe	5.0×10^{-6}
33	OCH_2CH_2OH	1.9×10^{-6}

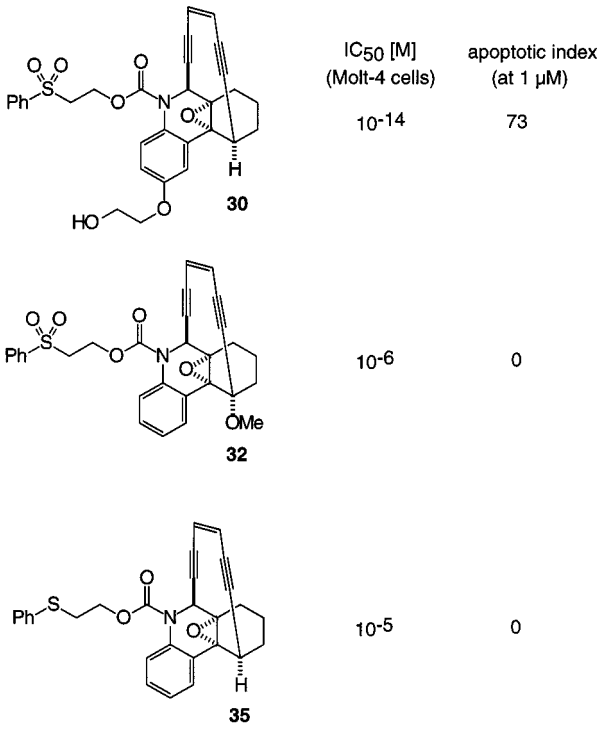
34
IC_{50} [M]
3.1×10^{-6}

Scheme 5

Analogs with a Removable Carbamate Moiety on the Nitrogen

In the course of the activation of dynemicin A an electron-rich aromatic system is produced. That means in the resulting anthraquinol the amino function plus the two neighboring hydroxy groups can assist in the epoxide opening. Instead of deactivating the aromatic portion in the form of a quinone, the electron-donating properties of the C-ring can also be tamed by engaging the nitrogen atom in a carbamate. This concept was first demonstrated by the Nicolaou group.[24] The most active compound of this series turned out to be the enediyne **30** (Scheme 5). The in vitro cytotoxicity (IC_{50}) against various cell types spans a range from 10^{-5} to 10^{-14} M (cf. Dynemcin A:[25] IC_{50} 10^{-7} to 10^{-11} M). This high activity of **30** was attributed to the slow release of the free amine with concomitant formation of phenyl vinyl sulfone.[24][25] The β-elimination occurs already at a very low pH of 7.4. In comparison, the phenyl carbamate **34** displayed a modest IC_{50} of $3.1 \cdot 10^{-6}$ M in the assay with the sensitive Molt-4 leukemia cell line, indicating a higher stability of the nitrogen protecting group. The study of several related dynemicin A analogs carrying also a phenylsulfone ethylene carbamate moiety on the nitrogen revealed the following: 1) The substituent at the 2-position of the phenanthridine system slightly modulates the activity in the order OCH_2CH_2OH > H > OMe.[25] 2) On the other hand, an electronegative substituent at position 10 considerably reduces the activity (cf. **32** and **33**).

In a related study[26] enediynes **32** and **35** were used to shed light on the cell-specific mechanisms of cytotoxicity. If the "stable" enediynes **32** or **35** were used together with the highly toxic **30**, inhibition of apoptotic cell death was observed (Scheme 6). It was therefore proposed that there are cellular targets for these synthetic enediynes and that there is a specific receptor-ligand interaction. Interestingly, the stable enediynes could even block the apoptotic morphology normally induced by structurally unrelated cytotoxic agents, such as calicheamicin or actinomycin D. The apoptosis (that is, suicide of a cell) was quantified by microscopic inspection of the cells. In the apoptotic process cells undergo morphological changes due to chromatin condensation and degradation of DNA into small fragments.

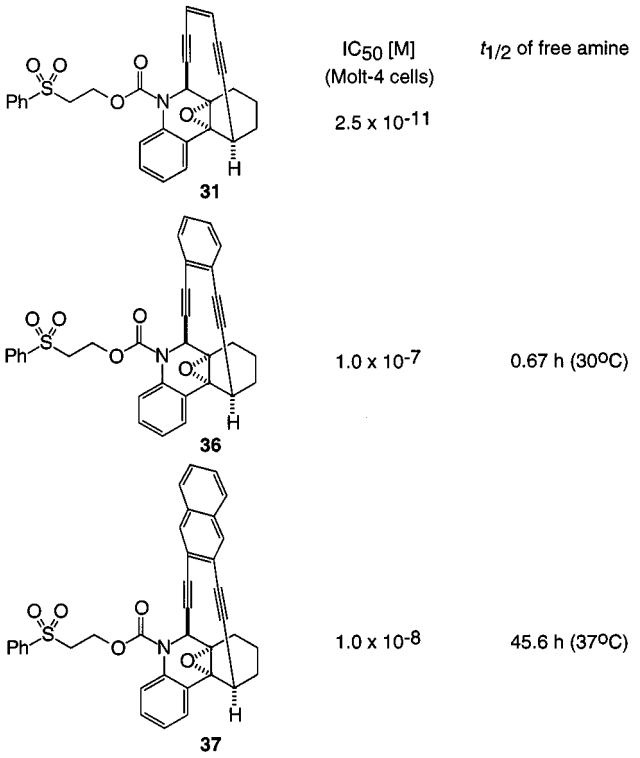


	IC_{50} [M] (Molt-4 cells)	apoptotic index (at 1 μM)
30	10^{-14}	73
32	10^{-6}	0
35	10^{-5}	0

Scheme 6

Other analogs in this series contained annelated aryl systems on the enediyne (Scheme 7). This caused reduction of

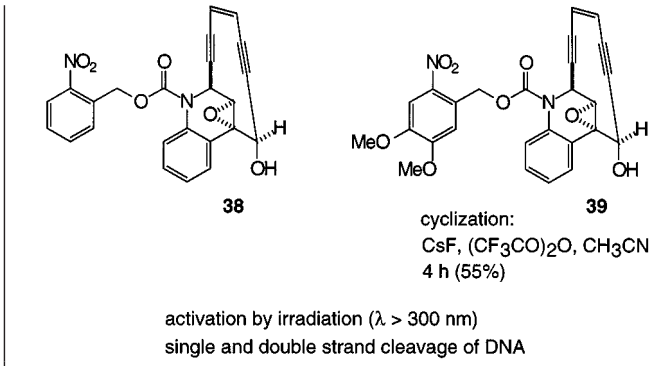
the cycloaromatization rate of the corresponding free amine. In line with this finding is the reduced cytotoxicity of the enediynes **36** and **37**.[27]



Scheme 7

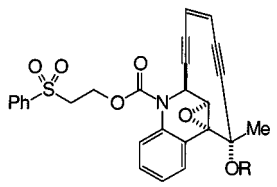
Besides modulating the electron-donating power of the aryl ring through a protecting group on the nitrogen, substituents on the *para*-position with regard to the epoxide could also be incorporated in a trigger mechanism. For example, Nicolaou et al. prepared analogs that carry a pivaloate or a (2-nitrobenzyl) group on a phenolic OH. Removal of the protecting group and exposure of the resulting enediyne to basic conditions induced the Bergman cycloaromatization.[26,28]

More with a view toward using enediynes as DNA cleaving agents,[14,29] the Wender group synthesized dynemicin analogs that contain the C and D rings. Noteworthy in this case are the conditions for the macrocyclization. In the case at hand an intramolecular cesium fluoride induced condensation between a silylated enediyne and an aldehyde proved to be advantageous since it avoids the use of strong base. The use of an additive such as trifluoroacetic anhydride prevents an unwanted reaction of the initially formed alkoxide with the *N*-protecting group. The trifluoroacetate hydrolyzes during the aqueous work-up. This way yields for the cyclization step improved to 55%. In the analogs **38** and **39** the electron-donating properties of the nitrogen atom are temporarily locked by a nitro benzyl carbamate (Scheme 8). Irradiation with wavelengths greater than 300 nm removed the nitrogen protecting group and led to the formation of cycloaromatized products. Comparing **38** and **39** reveals that **38** damages DNA more effectively than **39** although the dimethoxynitrobenzyl is cleaved more easily.[30−32]

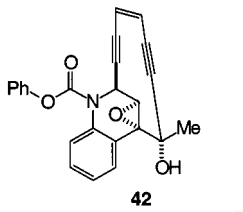


Scheme 8

The most thorough structure activity study of dynemicin analogs equipped with aryl carbamate trigger devices was performed by the Sanwa Kagaku Kenkyusho company in cooperation with the group of Isobe (Scheme 9). Using conceptually similar synthetic routes as Nicolaou and Wender, residues on various positions were systematically varied. Not unexpectedly, the character of the carbamate moiety is important. The sulfones **40** and **41** showed both potent DNA cleaving ability and potent in vitro cytotoxicity against various tumor cell lines. Surprisingly however, these sulfones proved to be essentially inactive against murine P388 leukemia in mice. In contrast, the enediyne **42** with a stable N-protecting phenyl carbamate showed effective antitumor activity in vitro as well as in vivo.[33]



	R	IC50 [M] (Molt-4 cells)	T/C (%) P388 (2 mg/kg)
40	H	4.1 x 10-8	108
41	Me	2.6 x 10-7	94

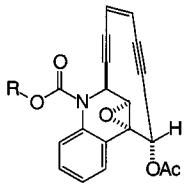


IC50 [M] (Molt-4 cells)	T/C (%) P388 (2 mg/kg)
6.8 x 10-6	165

Scheme 9

Among various phenyl carbamates, **43** and **45** (with a 4-chlorophenyl aryl group) were found to be the most active ones, prolonging the survival times of P388 leukemia infected mice significantly (**45**: T/C 256% at a daily dosage of 4.0 mg/kg). Both compounds also were effective against Meth A sarcoma, a solid tumor, causing substantial inhibition of tumor growth (**43**: dosage 2.0 mg/kg, 71% inhibition; **45**: dosage 3.0 mg/kg, 77% inhibition) (Scheme 10).[34] The T/C represents the ratio of mean survival time

of the treated to the control mice. T/C values over 125% are indicative of significant activity.

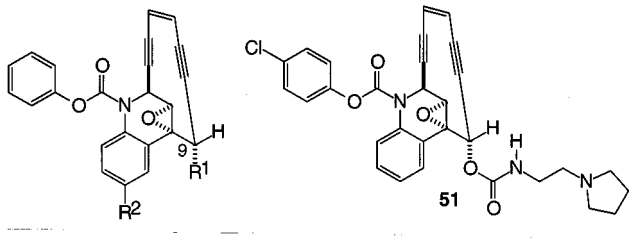


compound	R	*in vitro*; IC_{50} [M] (KB cells)	*in vivo* activity (P388) dose (mg/kg)	T/C (%)
43	Ph	2.3×10^{-6}	2.0	202
44	2-Cl-Ph	3.1×10^{-6}	2.0	154
45	4-Cl-Ph	3.6×10^{-6}	4.0	256
46	2-NO_2-Ph	1.7×10^{-7}	2.0	151

Scheme 10

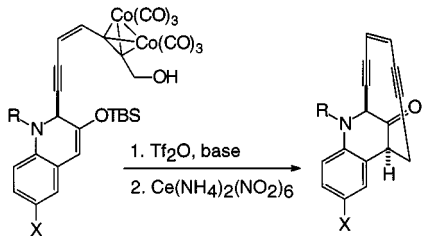
In a separate study the phenyl carbamate was kept constant and the effect of substituents R^1-R^2 was examined (Scheme 11).[35] Thus, an oxygen-containing functional group (R^2 = OMe) on the benzene ring abolishes both the in vitro and the in vivo potencies. Varying the residues R^1 and R^2 the following trend emerged: Compound **48** (R^1 = R^2 = H) with no bulky groups at C-9 showed significant in vivo activity with a T/C of 170% at a daily dosage of 2.0 mg/kg for 4 days. The corresponding 4-chlorophenyl carbamate even had a T/C value of 215% at that dosage. It is interesting to note that the stereochemistry at C-9 has a certain effect on the activity. Thus it was found that the (9*R**)-isomers are more active than the corresponding (9*S**)-isomers. For example, the isomer of **49** (R^1 and H exchanged) has an IC_{50} of $2.3 \cdot 10^{-6}$ M and in vivo activity of T/C = 183%.[35] The antitumor activity of the analogs could be further improved by attaching an aliphatic amino group at the C-9 position making them water-soluble. Among them compound **51** showed the most enhanced in vivo antitumor activity (T/C = 222% at a daily dosage of 1.25 mg/kg for 4 days). This is paralleled with a decreased toxicity. It is proposed that this is due to the improved bioavailability of these compounds.[36] With the rather stable aryl carbamates at the nitrogen, compounds **42**−**51** hardly showed any DNA-cleaving ability under neutral conditions. From this it is concluded that the biological activity is not due to DNA-cleavage by radical generation.

A similar conclusion was reached in a paper of the Magnus group.[37,38] The analogs **52**−**55** are devoid of an epoxide (Scheme 12). Because of strain effects caused by the sp^2-carbon in the one-carbon bridge, they are quite stable towards the Bergman cycloaromatization (**52**: $t_{1/2}$ = 324 d, **53**: $t_{1/2}$ = 196 d; 37°C). Nevertheless, both **53** and **54** proved to be quite active in vitro and in vivo (X = H, T/C = 175%; X = OMe, T/C = 170%) at a daily dosage of 2.0 mg/kg. The authors state that diradical formation is not a prerequisite for biological activity. Although in the case at hand biological reduction of the carbonyl function with concomitant activation might occur.



compound	R^1	R^2	IC_{50} [M] (KB cells)	*in vivo* activity (P388, dose 2.0 mg/kg) T/C (%)
43	OAc	H	2.3×10^{-6}	202
47	OAc	OMe	1.0×10^{-5}	140
48	H	H	1.0×10^{-6}	170
49	OBz	H	1.0×10^{-5}	133
50	O-2-pyrazinyl	H	7.4×10^{-6}	131

Scheme 11



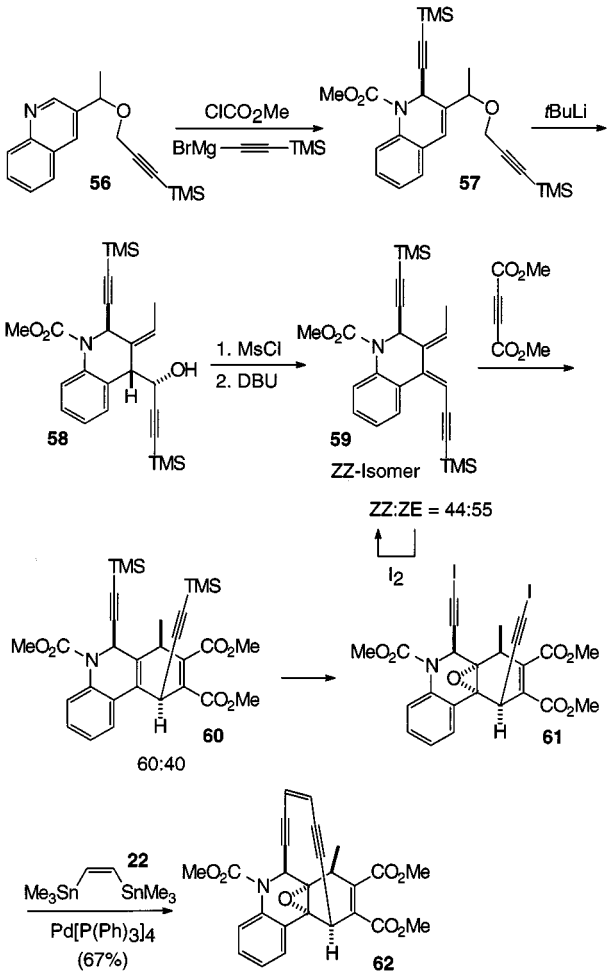
	R	X	$t_{1/2}$ (37°C)	*in vitro*; IC_{50} [M] (HCT116 cells)	*in vivo* activity (P388, dose 2.0 mg/kg) T/C (%)
52	CO_2Ad	OMe	324 d	-	-
53	H	OMe	196 d	-	170
54	H	H	-	2.1×10^{-7}	175
55	CO_2Ad	H	-	7.5×10^{-5}	-

Scheme 12

A conceptually interesting extension of the use of a blocking group on the nitrogen atom was reported by Denny et al.[39] The aim was to find a group that would come off through the action of an enzyme. They chose a 4-nitrobenzyloxycarbonyl group (cf. compound **34**). Upon enzymatic reduction by a nitroreductase, the nitro group is converted into a hydroxylamino function. Supposedly, the corresponding 4-hydroxylaminobenzyloxycarbonyl derivatives fragment readily to an active enediyne. Indeed, in the presence of the enzyme and NADH the cytotoxicity increases 90-fold. Thus, this compound has potential as a prodrug for antibody-directed enzyme prodrug therapy (ADEPT).[40]

Although the analog **62** has not been examined with regard to its biological activity, the synthetic route to this molecule is highly original (Scheme 13). Starting from 3-bromoquinoline the propargyl ether **57** was constructed. With the use of a [2,3]-Wittig rearrangement a further substituent was introduced into the heterocyclic ring. Mesylation of the alcohol and eliminatienes, of which the wrong isomer could be isomerized to the desired one. The Diel-

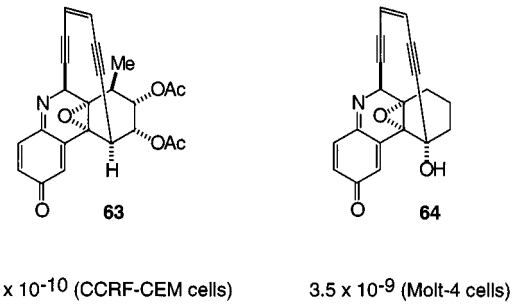
s−Alder reaction of **59** with dimethyl acetylenedicarboxylate gave a mixture of two diastereomeric cyclohexadienes. The one with the correct stereochemistry was subjected to an epoxidation and further converted to the bis(iodoalkyne) **61**. The missing ethylene was introduced by a palladium(0)-catalyzed coupling reaction of **61** with bis(trimethylstannyl)ethylene **22**. Activation, that is epoxide opening followed by Bergman cyclization, could be achieved with *p*TsOH in aqueous THF (Scheme 13).[41,42]



Scheme 13

Analogs with a Quinone Trigger

The endeavors toward the total synthesis of dynemicin A did indeed provide access to a number of analogs.[20,21] Actually all synthetic intermediates in Scheme 3 and Scheme 4 that contain the macrocyclic enediyne and the epoxide are interesting analogs themselves. With regard to the mode of action, quinone-containing analogs are able to imitate the natural leads. That is, they are activated by two-electron reduction of the quinone. Indeed, some of these analogs showed impressive activities. The Danishefsky group synthesized two quinone imines **63** and **64** that were also tested (Scheme 14). Although one would expect a reduced binding affinity to the DNA because of the smaller aryl part, the in vitro activities span a range from $2.0 \cdot 10^{-8}$ to $5 \cdot 10^{-10}$ M. The in vivo results are also very good. At a daily dosage of 0.5 mg/kg administered for 7−10 d they caused reduction of the tumor volume by 42−63%. In comparison, the well-established and clinically used mitomycin C is less effective in these studies (29% reduction in tumor mass).[21,43]

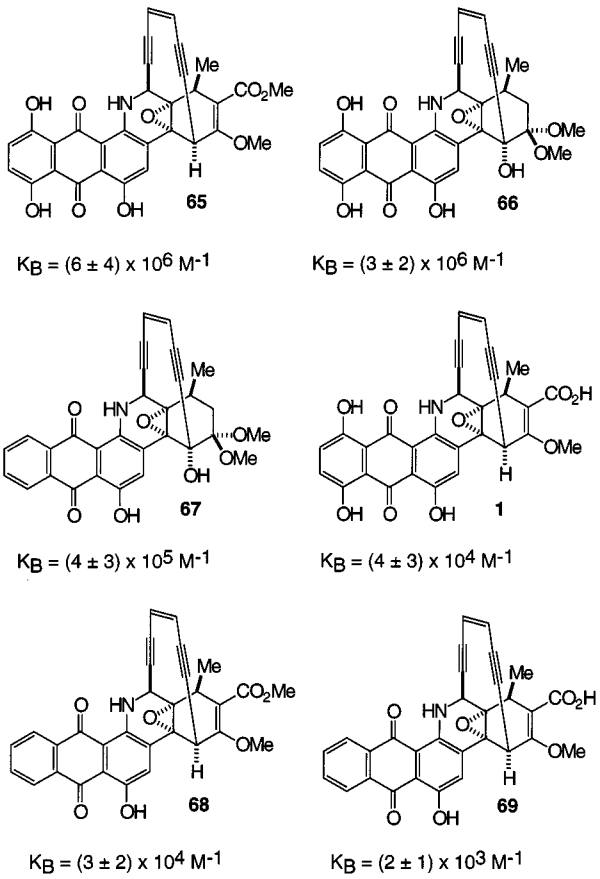


Scheme 14

Using the Diels−Alder reaction between quinone imines and suitable isobenzofurans the Myers group was able to prepare a number of anthraquinone-containing analogs.[20c] In studying these analogs the emphasis was put more on DNA binding and cleaving studies. It was found that some analogs bind more tightly to DNA than dynemicin A (Scheme 15).[44,45] It was suggested that the carboxylate of dynemicin A destabilizes the DNA−drug complex due to electrostatic repulsion. On the other hand, the hydroxyl groups in ring A make a positive contribution to the binding. These studies revealed interesting insights into the activating process. A striking finding was that the tight-binding analogs **65** and **66** ($K_B \geq 10^6$ M^{-1}) were devoid of DNA cleaving ability in the presence of the reducing agents GSH (20 mM) or NADPH (20 mM). Based on these findings it was proposed that reductive activation does not occur at any appreciable rate when the analogs are bound to DNA. However, in further studies it was found that flavin-based enzymatic reductants are able to substantially accelerate the rate of DNA cleavage by all analogs, including the tight binders **68** and **69**. Thus, enzyme-catalyzed processes might be a likely pathway for the in vivo activation of dynemicin A. The question of whether the enzyme-based reductants activate the DNA-bound or free forms of the analogs still remains unsolved. Unfortunately, in vitro and in vivo activities for these analogs are not yet reported.

Analogs without the Nitrogen Heterocycle

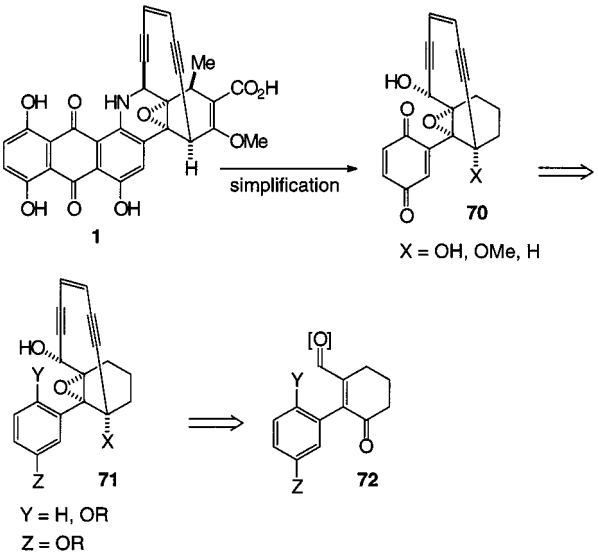
In designing structurally simple analogs of dynemicin A one could reduce the natural lead compound to the essential functional units − that is, a ten-membered enediyne carrying the epoxide in a *trans*-annulation. Furthermore, a quinone would be needed to assist in the epoxide opening via the corresponding hydroquinone. This way several key questions could be answered: Which functional groups on the aryl system or ring E are important in binding to DNA? What is the role of the nitrogen heterocycle? The nitrogen



Scheme 15

atom could have a threefold function: a) It might act as an intra- or intermolecular hydrogen donor. – b) It might contribute in the epoxide opening by pushing electron density to the benzylic position. Indeed, some designed analogs (cf. Schemes 5–9) rely on the electron-donating effect of the unprotected nitrogen to induce epoxide opening. – c) Finally, the role of the nitrogen atom, or the nitrogen heterocycle, might be to fix the arrangement of the anthraquinone part with regard to the E ring and the macrocyclic enediyne. This way the planes of the anthraquinone and the enediyne are nearly perpendicular. This arrangement is perfect for intercalation into the DNA and at the same time aligns the enediyne or the corresponding 1,4-diradical along the minor groove.[46]

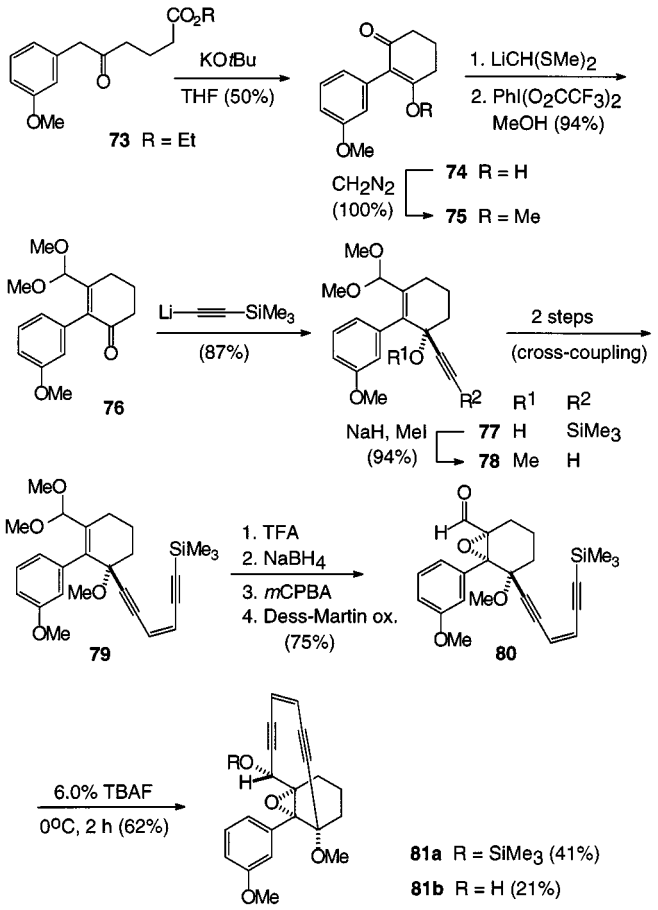
To answer some of these questions we designed compounds that lack the nitrogen heterocycle. Just like dynemicin A itself these analogs should undergo activation upon reduction of the quinone (Scheme 16). In the last step of the synthesis, the quinone will be generated from an electron-rich aromatic precursor through an oxidation reaction. Clearly, the choice of the aryl ring is crucial since it should not be too electron-rich otherwise it might succumb to an unwanted epoxide opening during the synthesis. At the same time it should be easy to oxidize in the final step. Even the enediynes with an electron-rich aryl system might be worth studying in biological systems, since epoxide opening might occur upon protonation of the epoxide with assistance by the aryl ring.



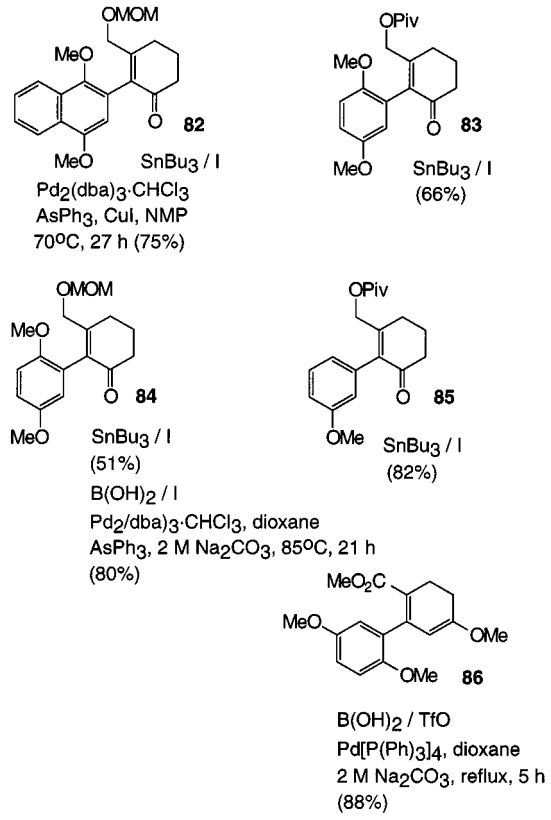
Scheme 16

These analogs should be available from functionalized cyclohexenone derivatives by bridging with an enediyne bow. For the synthesis of compounds corresponding to **71** we developed two routes. In the first approach a cyclohexenone ring was constructed through a Dieckmann condensation of a substrate that already contained an electron-rich aromatic ring (Scheme 17). This synthesis started from the ketoester **73** which is available by coupling the arylacetic acid chloride with the zinc reagent derived from 4-iodobutyrate. Cyclization with potassium *tert*-butoxide provided the vinylogous acid which was converted to the corresponding ester **75**. Addition of a thioacetal anion as a C_1-building block gave after acidic workup the cyclohexenone. Transacetalization converted the thioacetal into the dimethylacetal **76**. Attachment of the enediyne began with the lithium acetylide addition and protection of the hydroxyl group as methyl ether. Basic workup delivered the acetylene that was extended to the enediyne **79** by two coupling reactions. Cleavage of the acetal, followed by $NaBH_4$ reduction of the resulting aldehyde provided an allylic alcohol whose epoxidation gave a single oxirane diastereomer. After oxidation to the enediyne aldehyde **80**, cyclization could be accomplished by using a catalytic amount of TBAF in THF. These conditions furnished a mixture of the two enediynes **81a** and **81b** (Scheme 17). Each of these compounds displayed in the ^{1}H-NMR spectrum several signals as pairs. The temperature dependent ^{1}H-NMR spectrum of **81a** indicated that this is due to the presence of rotamers.[47]

For example, at room temperature two aromatic methoxy signals (δ = 3.75/3.81) are visible, whereas at 60°C coalescence is observed. The two rotamers are present in about equal amounts. From these studies a rotational barrier of about 70–75 kJ mol^{-1} can be calculated. One conclusion from this finding is that the nitrogen atom serves to restrain the rotation of the anthraquinone.



Scheme 17



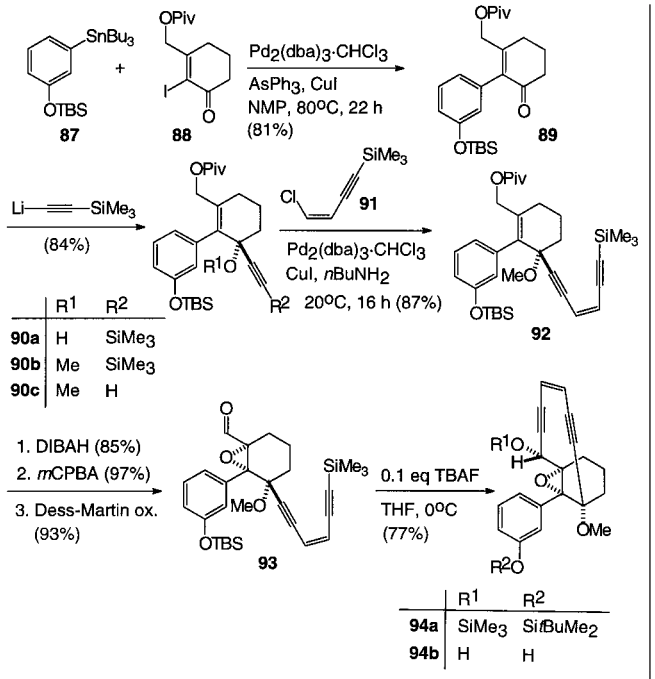
Scheme 18

Unfortunately, several attempts to oxidize the methoxyphenyl substituent to the quinone resulted in destruction of the analog **81a**. However, in vitro tests with **81a** indicated some cytotoxicity. Using various tumor cell lines, such as HT15, CCRF-CEM, and HeLa cells an IC_{50} value of about $1.2 \cdot 10^{-4}$ M was found. This points to the fact that oxirane opening and activation might occur in vitro.

The problem with the final oxidation led us to choose different, more electron-rich aryl groups. At the same time, a more flexible and convergent approach was sought. We therefore examined the palladium-catalyzed cross-coupling reaction of arylmetal compounds with electrophilic cyclohexenyl building blocks. For example, we could show that electron-rich arylstannanes couple with β-substituted α-iodoenones in the presence of $Pd_2(dba)_3 \cdot CHCl_3$, and a weak coordinating ligand such as triphenylarsane or tris(2-methylphenyl)phosphane. Other crucial parameters were the use of a polar solvent and the addition of copper(I) iodide (0.75−1.0 equivalents). Some representative examples are shown in Scheme 18. The functional groups in the starting materials are indicated next to the coupling products. It can be seen that even sterically demanding coupling products are accessible. Alternatively, compound **84** was prepared by a Suzuki coupling reaction with the arylboronic acid.[48,49] These results underscore the importance of the metal that mediates the transmetalation step of the aryl group to the palladium.

Based on this cross-coupling strategy some other dynemicin analogs were synthesized. In the first instance a 3-*tert*-butyldimethylsilyloxyphenyl group was chosen as a potential quinone precursor. The synthesis involved the palladium-catalyzed cross-coupling reaction of the arylstannane **87** with the iodoenone **88** on a 10-g scale.[50] The iodoenone **88** was obtained from the corresponding enone by reaction with trimethylsilyl azide and iodine. Following the construction of the aryl-cyclohexenone bond, the enediyne was attached. In contrast to the previous synthesis (Scheme 17), the enediyne was fashioned from the alkyne **90c** by coupling with the vinyl chloride **91**. Routine steps, that is removal of the pivaloyl protecting group, epoxidation, and oxidation of the alcohol provided the cyclization substrate **93**. Despite the fact that **93** contains two silyl groups, treatment of **93** with catalytic amounts of anhydrous TBAF[51] provided the macrocycle **94a** in 77% yield. Again, the intramolecular carbonyl addition proceeded in a stereospecific manner. The stereochemistry could be assigned on the basis of an upfield shift of the methine proton due to the aromatic ring current. Complete removal of the silicon groups gave the dynemicin analog **94b** as a colorless solid (Scheme 19). Although, preliminary experiments to oxidize **94b** indicated the formation of a quinone structure, these reactions were of low yield and not reproducible. However, just like compound **81a** the enediyne **94b** did show weak antitumor activity. For example, with Molt-4 leukemia cells **94b** has

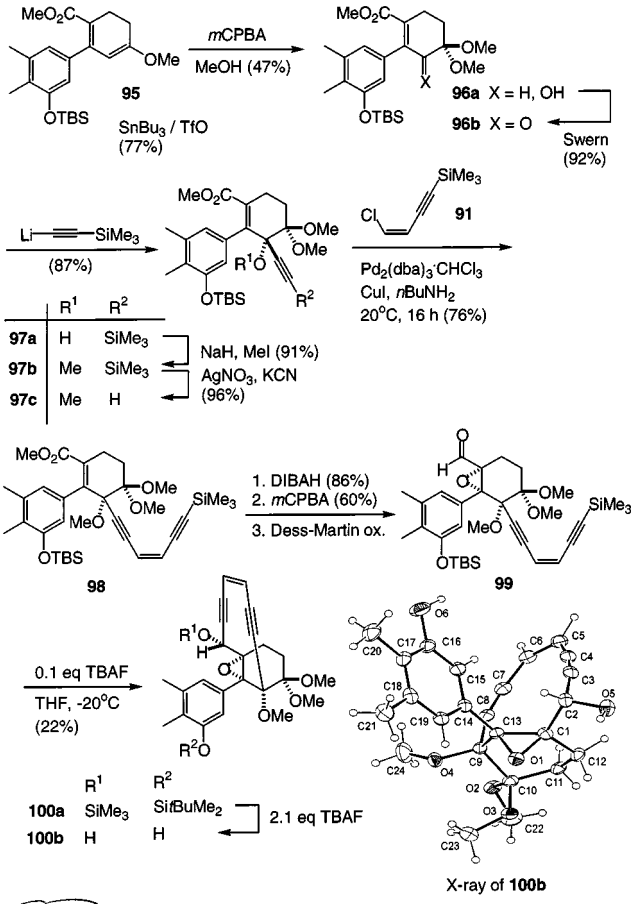
an IC_{50} of $9 \cdot 10^{-5}$ M. Electrochemical studies and also the chemical experiment led us to suspect that the oxidation might produce aryl dimers.



Scheme 19

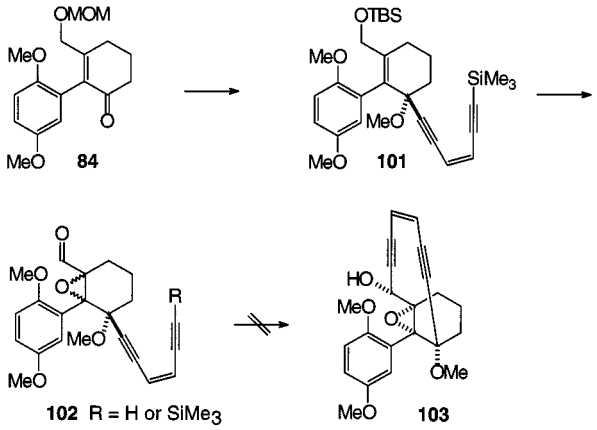
Therefore we used a dimethyl-substituted phenol residue instead. By a similar overall strategy the analog **100b** was constructed. Beginning with 5-bromo-2,3-dimethylphenol[52] the substituted 1,3-cyclohexadiene carboxylate **95** was fashioned by cross-coupling with the triflate.[20b] Following conversion of the enol ether to an α-acetal ketone **96b**, the enediyne was attached in the usual way. This included acetylide addition to the ketone, protection of the tertiary alcohol and cross-coupling reaction of **97c** with the vinyl chloride **91**. Subsequent reduction of the ester group to the allylic alcohol, epoxidation of the double bond and Dess–Martin oxidation[53,54] provided the enediyne aldehyde **99**. The cyclization to the macrocyclic enediyne was possible with catalytic amounts of TBAF. It should be noted that base-induced macrocyclization reactions on the unprotected enediyne did not give any cyclized product. The structure of **100b** was supported by NMR and MS data. A final structural proof came from the X-ray analysis of **100b** (Scheme 20). This revealed a distance between the enediyne ends (cd-distance) of 362 pm. This can be compared with the corresponding distance in dynemicin A which amounts to 354 pm.[11b] The most striking feature is that the aryl ring is rotated with regard to the aryl system in dynemicin A. It is obvious that in this conformation the aryl ring is not able to directly assist in the epoxide opening. Currently we are studying the oxidation of this enediyne to the corresponding quinone.

As shown earlier the cross-coupling strategy (Scheme 18) even allowed the preparation of sterically hindered aryl substituted cyclohexenes. Using the cyclohexenone **84**[48] we



Scheme 20

performed a sequence of steps that led to the enediyne aldehyde **102**. Despite the fact that various bases and reaction conditions were screened, we never were able to isolate the desired macrocycle. Only a dimer could be identified. It was not clear whether the additional *ortho*-methoxy substituent on the aryl group was obstructing the cyclization reaction

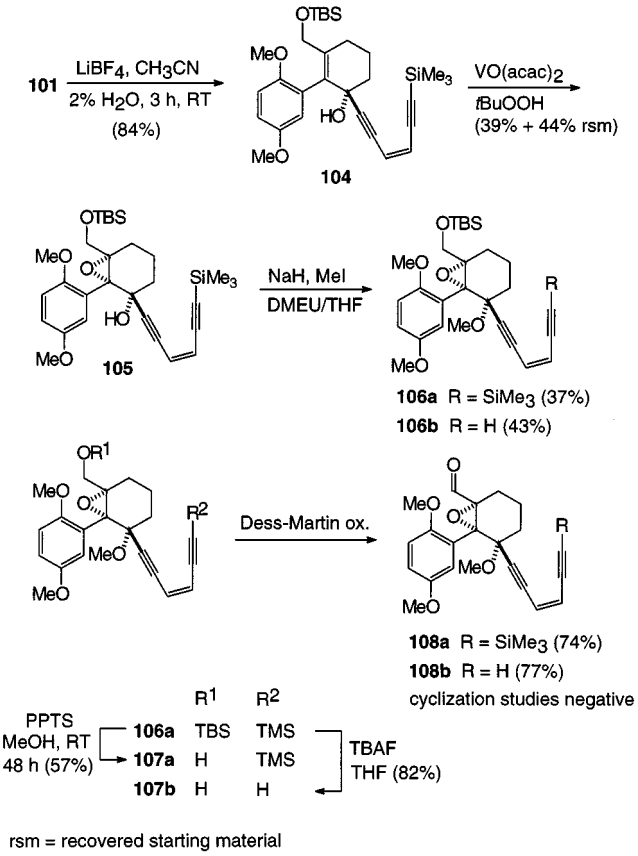


Scheme 21

or whether the epoxide was on the wrong side (Scheme 21).

Therefore the synthesis was modified in such a way to allow for a substrate-directed epoxidation.[55] On other oc-

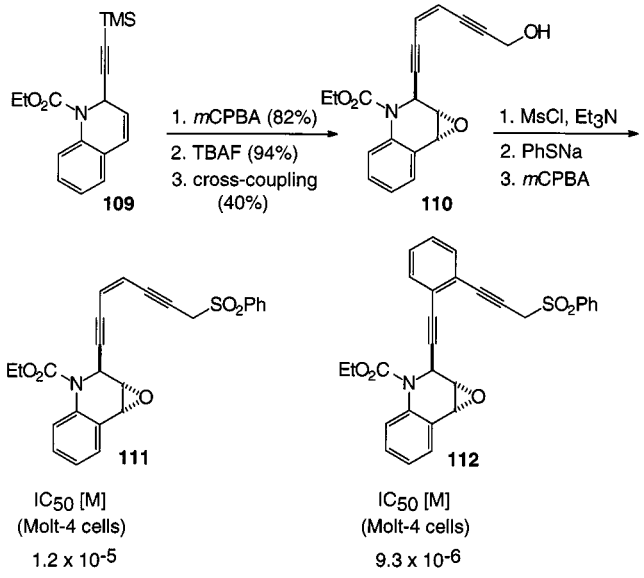
casions we had noted that the tertiary ether function in compounds of type **101** is very sensitive to acid. Taking advantage of this fact, ether **101** was hydrolyzed to the tertiary alcohol with lithiumtetrafluoro borate in aqueous acetonitrile. Under these conditions both silicon groups survived. Indeed, epoxidation of **104** with *tert*-butylhydroperoxide in the presence of a vanadium(V) catalyst followed by methylation of the hydroxy group gave rise to an epoxide that was different from the one obtained by the other route (Scheme 22). However, many attempts to induce macrocyclization of **108a** and **108b** proved to be fruitless. This shows once more that this reaction is very difficult and strongly influenced by the aryl group.



Scheme 22

Other Analogs

Another kind of a simplified version of dynemicin A has been reported by Wu et al.[56] These analogs contain a (*Z*)-7-sulfonyl-3-hepten-1,5-diyne attached to a quinoline moiety related to dynemicin A. It was expected that the quinoline core would deliver the molecule to DNA and that the epoxide and keto group might be engaged in a hydrogen bond to the DNA. Indeed these molecules (cf. **111** and **112**) proved to be active against the growth of various tumor cell lines (Scheme 23). Mechanistically, the diradical formation is due to a so-called Myers cyclization[57] of the intermediate enyne-allene system. Related compounds lacking the enediyne are inactive which supports a diradical-based mode of action.



Scheme 23

Other enediynes that contain a dihydropyridine ring have also been reported.[58] Because of the high tendency of these compounds to polymerize, in vitro assays were not performed.

Summary

This review article summarizes the syntheses of the antitumor antibiotic dynemicin A (**1**) and some analogs thereof. Key steps in most of the syntheses include a Yamaguchi reaction[59–61] to introduce an acetylene to the flat heteroaromatic ring containing skeleton. Cross-coupling reactions are used to establish the enediyne subunit. The macrocyclic ten-membered enediyne ring system is closed by intramolecular acetylide addition to a carbonyl group or alternatively by a stitching cyclization that introduces the ethylene fragment between the diyne. The enediyne itself is stabilized against the Bergman cycloaromatization by the *trans*-annulated epoxide. Thus, opening of the epoxide, mediated by the electron-rich aromatic sector triggers the DNA and cell-damaging diradical formation of the enediyne.

In order to shed light on the role of the nitrogen atom, we targeted analogs that lack this particular heterocyclic ring. A series of such analogs was prepared. As evidenced from an X-ray structure analysis of compound **100b**, the aromatic ring is turned relative to that in dynemicin A. Moreover, NMR studies indicate restricted rotation of the aryl ring around the single bond that connects the aryl system to the remainder of the molecule. One can thus conclude that the nitrogen heterocycle serves to fix the conformation of the anthraquinone part of dynemicin A with respect to the enediyne. This allows intercalation of the anthraquinone and at the same time positions the enediyne into the minor groove of the DNA. So far the oxidation of

the aryl ring to the corresponding quinone was not possible or still has to be tried. Nevertheless, in vitro studies with analogs **81a** and **94b** did show cytotoxic effects. If we assume a diradical mechanism then the epoxide must have opened, possibly with the assistance of an acidic enzymatic group.

Most of the analogs listed in this review article are the ones that contain the nitrogen heterocycle. With a removable carbamate the epoxide opening can be triggered. Indeed, some of the analogs show extremely potent antitumor activity, surpassing even the natural lead itself. However, in vivo studies revealed some puzzling results. First, it seems that stable enediynes (**36** or **37**) are able to block the apoptotic activity of otherwise highly toxic dynemicin analogs. Second, analogs with a rather stable aryl carbamate at the nitrogen proved to be very active in vivo. If however, the prodrug is a quinone then the corresponding dynemicin A analogs are generally very active.

Although it is difficult to compare in vivo results from several groups, one can conclude that there are probably two mechanisms for antitumor activity. One would seem to involve the typical diradical formation. The other mechanism might rely on a ligand-receptor interaction possibly without diradical formation through Bergman cyclization. It might therefore be worthwhile to prepare an affinity column carrying dynemicin A or a suitable analog.

Altogether dynemicin A represents a challenging synthetic target. At the same time it proved to be an ideal lead compound for optimization studies. We hope that this review stimulates further developments in this field.

Acknowledgments

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