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(54) Title: ENANTIOMERIC DYNEMICIN ANALOGS, PREPARATION AND USE THEREOF

(57) Abstract

An enantiomer of a fused ring system compound is disclosed that contains an epoxide group on one side of the fused rings and an enediyne macrocyclic ring on the other side of the fused rings. The enantiomeric compounds have DNA-cleaving, antimicrobial and tumor growth-inhibiting properties that are enhanced over their racemates. Chimeric compounds having the enantiomeric fused ring system compound as an aglycone bonded to (i) a sugar moiety as the oligosaccharide portion or (ii) a monoclonal antibody or antibody combining site portion thereof that immunoreacts with target tumor cells are also disclosed. Compositions containing an enatiomeric compound or an enantiomeric chimer are disclosed, as are methods of preparing an enantiomeric compound.

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ENANTIOMERIC DYNEMICIN ANALOGS, PREPARATION AND USE THEREOF

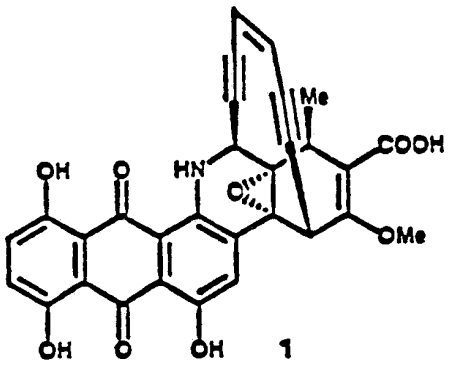
Description

Technical Field

The present invention relates to novel DNA-cleaving, cytotoxic and anti-tumor compounds, and particularly to enantiomeric fused ring compound systems that contain an enediyne macrocyclic ring and also an epoxide ring, as well as chimeras that contain such a fused ring compound system.

Background Art

Dynemicin A (Compound 1 shown below),



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where Me is methyl, is a potent antibacterial and anticancer agent recently isolated from Micromonospora chersina [(a) Konishi et. al, J. Am. Chem. Soc., 112:3715-3716 (1990); (b) Konishi et al., J. Antibiot., 42:1449-1452 (1989)]. Its striking molecular structure combines characteristics of both the enediyne [Golik et al., J. Am. Chem. Soc., 109:3461-3462 (1987); Golik et al., J. Am. Chem. Soc., 109:3462-3464 (1987); Lee et al., J. Am. Chem. Soc., 109:3464-3466 (1987); Ellestad

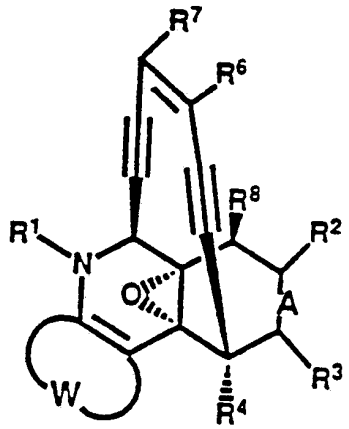
et al., J. Am. Chem. Soc., 109:3466-3468 (1987)] and the anthracycline ["Anthracycline Antibiotics", H.S. El Khadem, ed., Academic Press, New York (1982) and "Recent Aspects in Anthracyclinone Chemistry", Tetrahedron Symposia-in-Print No. 17, T.R. Kelly, ed., Tetrahedron; 40:4537-4794 (1984)] classes of antibiotics, and presents a considerable challenge to organic synthesis as well as a unique opportunity for the development of new synthetic technology and therapeutic agents.

The calicheamicin and esperamicin derivatives are perhaps the best known of the enediyne compounds. For a key paper describing the first synthesis of calicheamicinone, see: (a) Cabal et al., J. Am. Chem. Soc., 112:3253 (1990). For other selected studies of model systems in the area of calicheamicins-esperamicins, see: (b) Nicolaou et al, J. Am. Chem. Soc., 110:4866-4868 (1988); (c) Nicolaou et al., J. Am. Chem. Soc., 110:7247-7248 (1988); (d) Schoenen et al., Tetrahedron Lett., 30:3765-3768 (1989); (e) Magnus et al., J. Am. Chem. Soc., 110:6921-6923 (1988; (f) Kende et al., Tetrahedron Lett., 29:4217-4220 (1988).

Brief Summary of the Invention

The present invention relates to novel enantiomeric fused ring compound systems that contain an epoxide ring and an enediyne macrocyclic ring, and thus have structural features similar to dynemicin A. A contemplated enantiomeric fused ring compound is substantially free of the other enantiomer. The compounds have DNA-cleaving, antibiotic and antitumor activities. Compositions and methods of making and using the compounds are disclosed.

An entiomeric fused ring compound of the invention has a structure that corresponds to the formula



wherein A is a double or single bond;

R^1 is selected from the group consisting of H, C_1-C_6 alkyl, phenoxycarbonyl, benzyloxycarbonyl, C_1-C_6 alkoxycarbonyl, substituted C_1-C_6 alkoxycarbonyl (particularly substituted ethoxycarbonyl where the substituent is phenylsulfonyl or naphthylsulfonyl, with phenylsulfonyl most particularly preferred), o-nitrobenzyloxycarbonyl and 9-fluorenylmethyloxycarbonyl;

R^2 is selected from the group consisting of H, carboxyl, hydroxylmethyl and carbonyloxy C_1-C_6 alkyl;

R^3 is selected from the group consisting of H and C_1-C_6 alkoxy;

R^4 is selected from the group consisting of H, hydroxyl, C_1-C_6 alkoxy, oxyacetic acid, oxyacetic C_1-C_6 hydrocarbyl or benzyl ester, oxyacetic amide, oxyimidazilthiocarbonyl and C_1-C_6 acyloxy;

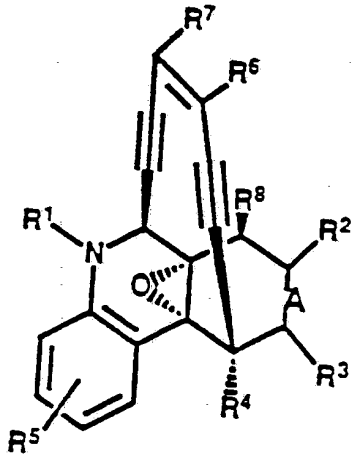
R^6 and R^7 are each H or together with the unsaturated carbon atoms of the intervening vinylene group form a one, two or three fused aromatic six-membered ring system;

W together with the carbon atoms of the depicted, intervening vinylene group forms an aromatic hydrocarbyl ring system containing 1, 2 or 3 six-

membered rings such that the fused ring compound contains 3, 4 or 5 fused rings, all but two of which are aromatic, and in which that aromatic hydrocarbyl ring system, W, is joined [a, b] to the structure shown (i.e., W is joined [a,b] to the nitrogen-containing rings of the structure shown); and

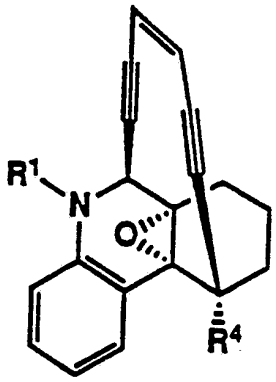
R^8 is hydrogen or methyl, with the proviso that R^8 is hydrogen when W, together with the carbon atoms of the intervening vinylene group is 9,10-dioxoanthra.

In preferred practice, W together with the a intervening vinylidene group forms a benzo ring so that a contemplated enantiomeric compound has the structural formula shown below.

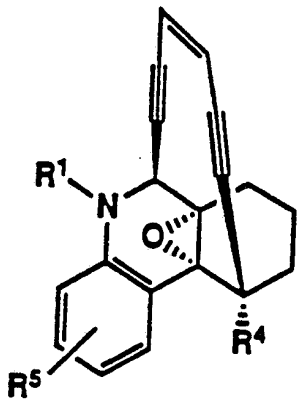


wherein R^5 is selected from the group consisting of hydrogen, C_1-C_6 alkoxy, hydroxyl, C_1-C_6 acyloxy, oxyethanol, oxyacetic acid, oxyacetic acid amide, oxyacetic C_1-C_6 hydrocarbyl ester, oxyethanol tertiary amino- or quaternary ammonium-substituted C_2-C_3 alkyl carboxylate, 3-hydroxyprop-1-ynyl, o-nitrobenzyloxy and halo, and A and the remaining R groups are as before described.

More particularly, in one embodiment, R^2, R^3, R^5, R^6, R^7 and R^8 are hydrogen so that a contemplated enantiomeric compound of the invention corresponds to the structural formula shown below, where R^1 and R^4 are as previously defined.



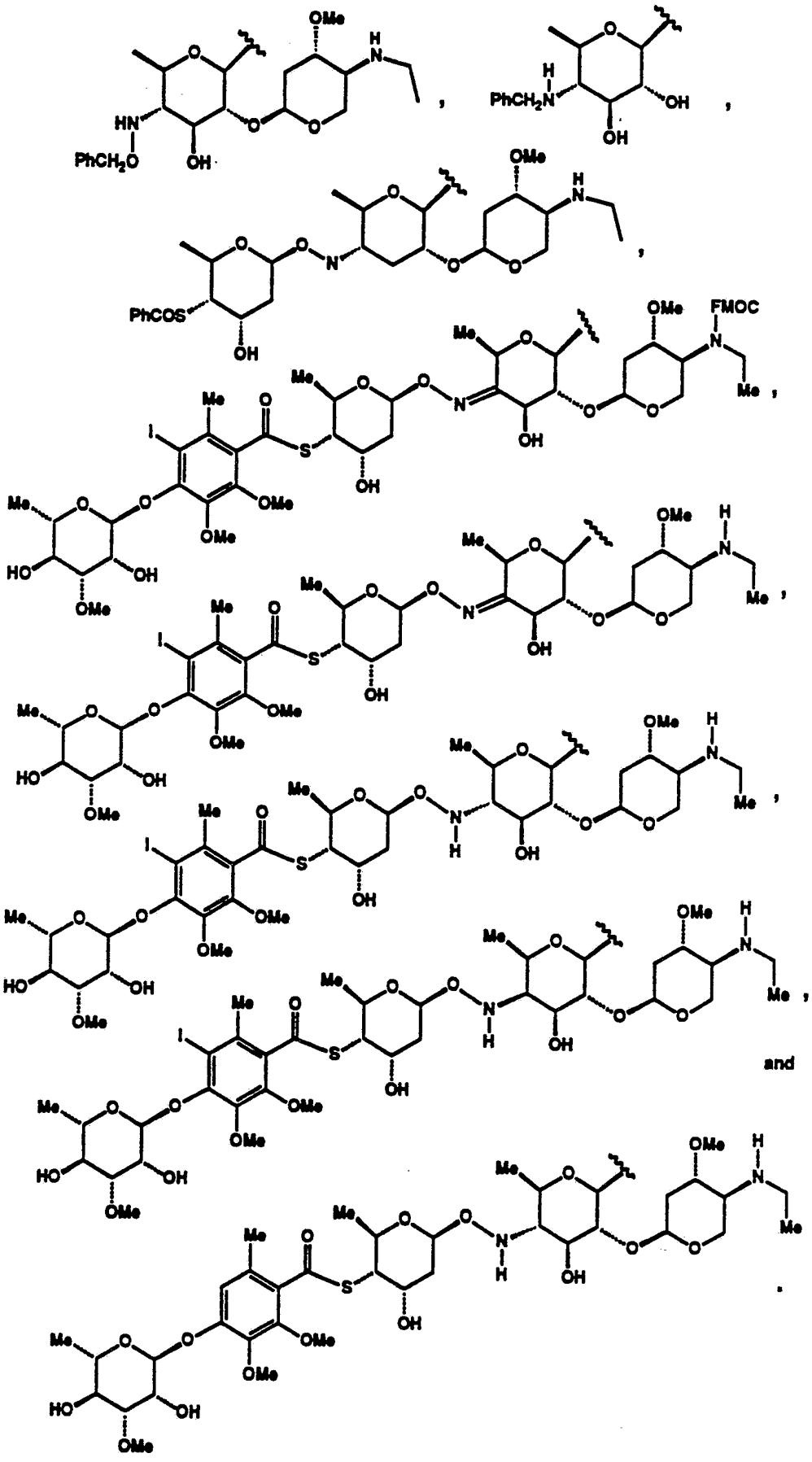
More preferably, R^5 is C_1-C_6 alkoxy, hydroxyl, C_1-C_6 acyloxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, oxyethanol, oxyacetic acid, oxyacetic acid amide, oxyethanol tertiary amino- or quaternary ammonium-substituted C_2-C_3 alkyl carboxylate or 3-hydroxyprop-1-ynyl and R^4 is hydrogen (H) or hydroxyl so that a fused ring compound has the structural formula shown below.



Any of the above fused ring enediyne compounds can be prepared as a single enantiomer. Where the R^1 group is also prepared from an individual chiral compound, as where R^1 is a 2-mono- or di-C_1-C_6 alkyl-substituted-2-phenylsulfonyl ethoxycarbonyl group, any of the above compounds can be present as a further enantiomer.

Also contemplated is a chimeric compound (also referred to as a chimer or chimera) that is comprised of a before-described enantiomeric fused ring compound as an aglycone portion bonded to (i) an oligosaccharide portion or (ii) a monoclonal antibody or antibody combining site portion thereof that immunoreacts with target tumor cells.

The oligosaccharide portion comprises a sugar moiety selected from the group consisting of ribosyl, deoxyribosyl, fucosyl, glucosyl, galactosyl, N-acetylglucosaminyl, N-acetylgalactasaminyl, a saccharide whose structure is shown below, wherein a wavy line adjacent a bond indicates the position of linkage.



and

A monoclonal antibody or binding site portion thereof is bonded to the enantiomeric fused ring compound aglycone portion through an R^4 oxyacetic acid amide or ester bond, an oxyacetic acid amide or ester bond or oxyethanol ester bond from W such as from an R^5 group. An oligosaccharide portion is glycosidically bonded to the aglycone portion through the hydroxyl of an R^4 oxyethanol group or the hydroxyl of an oxyethanol-substituent of W, e.g. an R^5 group.

A pharmaceutical composition is also contemplated. That pharmaceutical composition contains a DNA cleaving, antibiotic or tumor cell growth-inhibiting amount of a before-defined enantiomeric compound or chimera as active agent dissolved or dispersed in a physiologically tolerable diluent.

An enantiomeric compound, chimera or a pharmaceutical composition of either is also useful in a method for cleaving DNA, for inhibiting tumor growth and as an antimicrobial. In accordance with such a method, the DNA to be cleaved, target tumor cells whose growth is to be inhibited or target microbial cells are contacted with a composition of the invention. That contact is maintained for a time period sufficient for the desired result to occur. Multiple administrations of a pharmaceutical composition can be made to provide the desired contact.

Brief Description of the Drawings

In the drawings forming a portion of this disclosure,

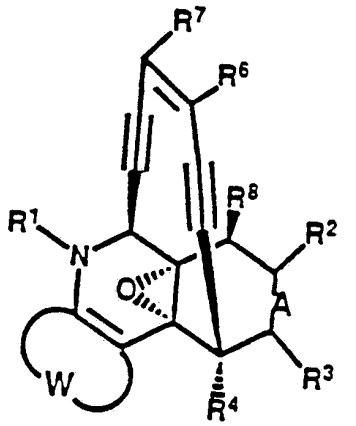
Figure 1 in two panels as Figs. 1a and 1b are photographs of ethidium bromide stained 1 percent agrose gel that illustrates the effect on ϕX174 Form I DNA by the following compounds in 50 mM Tris-HCl buffer (Fig. 1a = pH 8.5, Fig. 1b = pH 9.0) after 48 hours at

37°C. Lane 1 (1.0 mM) is the DNA control; lane 2 is DNA plus Compound **21** (1.0 mM); lane 3 is DNA plus Compound **427** (5.0 mM); lane 4 is DNA plus Compound **428** (5.0 mM); lane 5 is DNA plus Compound **429** (5.0 mM); lane 6 is DNA plus 2-(phenylsulfonyl)propanol (5.0 mM); lane 7 is DNA plus phenyl isopropenyl sulfone (5.0 mM); and lane 8 is DNA plus phenyl vinyl sulfone (5.0 mM). Form I is supercoiled DNA; Form II is nicked DNA; and Form III is linear DNA.

Detailed Description of the Invention

I. The Compounds

An enantiomeric compound of the invention contains an enediyne macrocycle linked to a fused ring compound system that corresponds to structural Formula I



I

wherein A is a double or single bond;

R^1 is selected from the group consisting of H, C_1-C_6 alkyl, phenoxycarbonyl, benzyloxycarbonyl, C_1-C_6 alkoxycarbonyl, substituted C_1-C_6 alkoxycarbonyl (particularly a substituted ethoxycarbonyl where the substituent is phenylsulfonyl or naphthylsulfonyl with phenylsulfonyl most particularly preferred), o-nitrobenzyloxycarbonyl and 9-fluorenylmethyloxycarbonyl;

R^2 is selected from the group consisting of H, carboxyl, hydroxylmethyl and carbonyloxy-C_1-C_6 alkyl;

R^3 is selected from the group consisting of H and C_1-C_6 alkoxy;

R^4 is selected from the group consisting of H, hydroxyl, C_1-C_6 alkoxy, oxyacetic acid ($-OCH_2CO_2H$), C_1-C_6 hydrocarbyl or benzyl oxyacetic acid ester, oxyacetic amide, oxyethanol ($-OCH_2CH_2OH$), oxyimidazylthiocarbonyl and C_1-C_6 acyloxy;

R^6 and R^7 are each H or together with the intervening vinylene group form a one, two or three fused aromatic six-membered ring system;

W together with the bonded, intervening, vinylene group (i.e., the unsaturated carbon atoms bonded to W) forms a substituted aromatic hydrocarbyl ring system containing 1, 2 or 3 six-membered rings such that said fused ring compound contains 3, 4 or 5 fused 6-membered rings all but two of which rings are aromatic, and in which that aromatic hydrocarbyl ring system, W, is joined [a, b] to the structure shown; and

R^8 is hydrogen or methyl with the proviso that R^8 is hydrogen when W together with the intervening vinylidene group is 9,10-dioxoanthra.

A compound of Formula I and the other fused ring enediyne compounds disclosed herein are chiral, and are prepared as a single or individual enantiomer that is substantially free of the other enantiomer. Only one of the enantiomeric pair is shown in Formula I and most of the other formulas depicted herein. For ease in depiction, the depicted enantiomeric fused ring enediyne compounds are shown having the absolute stereochemistry of dynemicin A [Landley et al., J. Am. Chem. Soc., 113:4395 (1991) and Wender, Proc. Natl. Acad. Sci. USA, 88:8835 (1991)], which absolute stereochemistry is preferred.

The syntheses and assays using the racemic forms of the compounds disclosed herein are disclosed in International Application WO 92/02522, published on February 20, 1992 and in the published literature. See, for example, Nicolaou et al., Science, 256:1172-1178 (1992) and the citations therein. A compound contemplated here is one or the other or both of the two separate enantiomers that constitute those racemates.

A contemplated enantiomer is synthesized using similar reactions to those disclosed in WO 92/02522 with different intermediate steps that permit a stereocontrolled synthesis. These different steps are outlined hereinafter.

Racemates are useful as the data of WO 92/02522 and the literature indicate. However, separate enantiomers have also been prepared and a compound having the same absolute stereochemistry as that of dynemicin A, the (+) isomer, has been found to be more potent against some cancer cell lines, e.g. Molt-4 T cell leukemia and Capan-1 pancreatic carcinoma, than the other, (-), enantiomer. Separated (+) and (-) isomers also exhibit similar potencies against other cancer cell lines such as SK-MEL-28 melanoma. Thus, a fused ring enediyne disclosed herein is contemplated as either or both of the separated (+) and (-), single, enantiomeric molecules (enantiomers).

Exemplary R^6 and R^7 groups other than hydrogen, which is preferred for both, are discussed hereinafter.

As noted above, the bond, A, between the R^2 and R^3 substituents can be a double or single bond. The bond A is preferably a single bond.

A C_1-C_6 alkyl group, as can be present in R^1 is exemplified by methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, pentyl, 2-methylpentyl, hexyl, cyclohexyl, cyclopentyl and the like. A substituted C_1-C_6 alkyl group is also contemplated as an R^1 group. Such substituted alkyl groups include hydroxyalkyl groups such as 2-hydroxyethyl, 4-hydroxyhexyl and 3-hydroxypropyl, haloalkyl groups such as 2-chlorobutyl, 3-halopentyl such as 3-fluoropentyl, and the like. The above C_1-C_6 alkyl and substituted C_1-C_6 alkyl groups are further contemplated as the C_1-C_6 alkyl portion of a carbonyloxy C_1-C_6 alkyl group of R^2; i.e., a C_1-C_6 alkyl ester of a R^2 carboxyl group, and of a R^1 urethane group. Those same alkyl groups can constitute the alkyl portion of a C_1-C_6 alkoxy group of R^3 or R^4. A C_1-C_6 acyloxy group as is present in R^4 or R^5 (discussed hereinafter) is a carboxylic acid derivative of an appropriate alkyl group, above, except for, for example, cyclohexyl and iso-propyl, and is limited to a cyclopentylcarboxyl group for the cyclopentane derivatives. Examples of such C_1-C_6 acyloxy groups include formyloxy, acetoxy, propionoxy, butyryloxy, iso-butyryloxy, pentanoyloxy, 2-methylbutyryloxy, pivaloyloxy, hexanoyloxy, and the like.

The alcohol-carbonyl portion of a urethane R^1 is typically formed by the reaction of a corresponding halo formate derivative, such as a chloroformate like phenylchloroformate, with the secondary amine nitrogen atom that is formed by addition of an acetylenic group-containing moiety to the 6-position or a correspondingly numbered position of a fused ring system such as that shown in Scheme II hereinafter. Such groups can also be prepared by base-catalyzed exchange from a formed carbamate using the substituted ethyl alcohol as is illustrated hereinafter.

Exemplary C_1-C_6 alkoxycarbonyl groups and substituted C_1-C_6 alkoxycarbonyl groups contain a before-described C_1-C_6 alkoxy group or substituted C_1-C_6 alkoxy group linked to the carbonyl group and can be formed by reaction of a C_1-C_6 alkylchloroformate. Exemplary substituted ethoxycarbonyl groups that are a particularly preferred group of substituted C_1-C_6 alkoxycarbonyl group have a substituent other than hydrogen at the 2-position of the ethoxy group, and include 2-(trimethylsilyl)ethoxycarbonyl, 2-(phenylsulfonyl)ethoxycarbonyl, α- or β-2-(naphthylsulfonyl)ethoxycarbonyl, α- or β-2-(anthracylsulfonyl)ethoxycarbonyl, 2-propenoxycarbonyl, 2-hydroxyethoxycarbonyl, 2-(triphenylphosphoniume)thoxycarbonyl halide (e.g., chloride, bromide or iodide) and 2-(trimethylammonium)ethoxycarbonyl halide (as before).

It is particularly preferred that R^1 be a group that can be enzymatically or otherwise removed intracellularly to provide the resulting secondary amine free of a substituent group. A compound where R^1 contains a 2-substituted-ethoxycarbonyl group such as a 2-(phenylsulfonyl)-, 2-(naphthylsulfonyl)- and 2-(anthracylsulfonyl)- as are shown in Scheme III (shown as R_1 therein) can form the free secondary amine compound via a β-elimination under relatively mild conditions. An ethoxy carbonyl group can also be named an ethylene oxycarbonyl group. Phenylsulfonylethoxycarbonyl, α-naphthyl- and β-naphthylsulfonylethoxycarbonyl (collectively referred to as naphthylsulfonylethoxycarbonyl) are particularly preferred R^1 groups, with phenoxycarboxyl being a preferred R^1 group. When an R^1 group is o-nitrobenzyloxycarbonyl, UV light-irradiation (about

290-400 nm) provides cleavage of that group from a fused ring system, thereby providing a free amine group.

The absolute stereochemistry of 2-(phenyl)- or 2-(naphthylsulfonyl)ethoxycarbonyl R^1 group can also lead differences in potency when the ethoxy portion of that group is also substituted at its 2-position by one or two C_1-C_6 alkyl groups discussed before such as methyl. The presence of a chiral, enantiomeric 2-(phenylsulfonyl)-2-(mon- or di-C_1-C_6 alkyl)ethoxycarbonyl R^1 group in an otherwise racemic fused ring enediyne compound provides a pair of diastereomers, whereas a single enantiomer is formed when both parts of the molecule are themselves chiral enantiomers.

Thus, the 2-(S)-(-)-methyl derivative was more potent against several cancer cells lines than was the 2-(R)-(+)-methyl derivative. Both were more potent than was the 2,2-dimethyl derivatives.

Each of the 2-(phenylsulfonyl)-2-(mono- or di-C_1-C_6 alkyl)-ethoxy carbonyl-containing fused ring enediyne compounds was less potent than the very potent un-allylated derivatives. These differences in potency can be used to adjust the potency and selectivity of a contemplated compound.

Exemplary R^6 and R^7 that together with the intervening vinylene group form a one, two or three fused aromatic six-membered ring system that includes benzo, naphtho and anthra rings, as well as 6,8-dimethoxynaphtho and 6,8-diazanaphtho. As noted before, it is preferred that both R^6 and R^7 be hydrogen.

An R^8 group can be methyl or hydrogen with the proviso that R^8 is hydrogen when W along with the intervening vinylene group carbon atoms forms a 9,10-dioxoanthra ring. It is particularly preferred that R^8 be methyl when W forms a benzo ring.

R^4 groups that are hydrogen, hydroxyl, oxyethanol ($-OCH_2CH_2OH$), oxyacetic acid ($-OCH_2CO_2H$), oxyacetic C_1-C_6 hydrocarbyl esters such as the before-discussed C_1-C_6 alkyl groups such as ethyl oxyacetate ($-OCH_2CO_2CH_2CH_3$), as well as C_1-C_6 unsaturated esters such as the allyl, propargyl, 2-butenyl and the like, as well as the benzyl ester and oxyacetic amides constitute particularly preferred embodiments of the invention.

A pharmaceutically acceptable non-toxic salt of the oxyacetic acid such as sodium, potassium, ammonium, calcium and magnesium is also contemplated. An oxyacetic acid amide corresponds to the chemical formula $-OCH_2CONR^{13}R^{14}$ wherein R^{13} is hydrogen (H) or C_1-C_6 alkyl (as before) and R^{14} is independently hydrogen, C_1-C_6 alkyl, phenyl, 1- or 2-napthyl, 1- or 2-anthryl, or a peptide having 1 to about six amino acid residues; or R^{13} and R^{14} together with the nitrogen atom form a 5- or 6-membered ring as is present in pyrrolidine, piperidine, morpholine, imidazole or pyrrole.

A particularly contemplated peptide is distamycin, or a derivative thereof as discussed in Taylor et al., Tetrahedron, 40:457 (1984) and Baker et al., J. Am. Chem. Soc., 111:2700 (1989). Distamycin derivatives are themselves known DNA-cleaving agents.

Another particularly preferred peptide is -Ala-Ala-Ala-, [$(-Ala-)_3$] which sequence is recognized and cleaved by an intracellular lysosomal enzyme. Further suitable peptide linkers that are cleaved enzymatically in vivo are well known to skilled workers. See, for example, Reisfeld et al., Human Cancer Immunology II, 11(2):341 (1991) and the citations therein. So-called acid-cleavable linkers such as cis-aconitate and the like as are also well known can also be used alone or in conjunction with a cleavable peptide linker. See for example, Reisfeld et al., Human Cancer

Immunology II, 11(2): 341 (1991) and Mueller et al., Bioconjugate Chem., 2:325 (1990), and the citations therein.

Appropriate diamine and dicarboxylic acid groups can be added at the carboxy- and amino-termini of the peptides or acid labile linkers, respectively, to join the fused ring enediyne to the Mab, as is discussed below. Exemplary diamines are the α,ω-C_2-C_6 alkylene diamines such as ethylene diamine, 1,3-propylene diamines and 1,6-hexylene diamine. Exemplary α,ω-C_4-C_6 dicarboxylic acids include succinic, maleic, glutaric and adipic acids.

An R^4 group that contains a derivatized oxyacetic acid amide or ester can also include a peptidyl spacer containing zero to about 6 residues such as $(\text{-Ala-})_3$ that links the compound to a monoclonal antibody or an antibody binding site portion thereof, collectively referred to herein as a "Mab". An R^5 group as discussed in detail hereinafter as a substituent of W as in a compound of Formula XIb can also constitute a useful spacer for bonding to a Mab.

The Mab utilized immunoreacts substantially only with target tumor cells; i.e., is tumor cell specific, and thereby provides further specificity to the drug molecules. Such a Mab-linked fused ring enediyne is one type of chimeric molecule of the invention.

The spacer portion of the enantiomeric compound-Mab construct serves to link the two portions of the molecule together. When there are zero peptide residues present, a lysine epsilon-amino group of the Mab forms the amido bond with an R_3 group as spacer. The spacer peptide chain, when present, is typically comprised of amino acid residues having small side chains such as glycine or alanine, or relatively

hydrophilic side chains such as serine, glutamine and aspartic acid. A peptide spacer is typically free of cysteine residues, but can contain cystine residues and otherwise can have substantially any structure that does not interfere with bonding between the two portions of the chimeric compound. A peptide can be prepared by an one of several synthetic methods as are well known. A particularly preferred peptide spacer includes an amine acid residue sequence that is recognized and cleaved by an enzyme such as a lysosomal or other proteolytic enzyme present within a target neoplastic cell so that the fused ring enediyne can be freed from the Mab after endocytosis, as is well known.

The Mab portion of the above chimeric construct can constitute an intact antibody molecule of IgG or IgM isotype, in which case, a plurality of compounds can be present per antibody molecule. The binding site portions of an antibody can also be utilized, in which case, at least one compound is linked to the proteinaceous antibody binding site portion.

An antibody binding site portion is that part of an antibody molecule that immunoreacts with an antigen, and is also sometimes referred to as a paratope. Exemplary antibody binding site portions include F(ab), F(ab'), $F(ab')_2$ and F_v portions of an intact antibody molecule, and can be prepared by well known methods. An intact monoclonal antibody and a portion that includes its antibody combining site portion can be collectively referred to as a paratope-containing molecule.

Exemplary anti-tumor Mabs are noted in the table below, listed by the name utilized in a publication, along with its deposit accession number at the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852 U.S.A., and

the tumor antigen with which the Mab paratope is reported to react. A citation to a discussion of each Mab and its immunoreactivity is provided by the footnote under the antigen listing.

5

Exemplary Anti-Tumor Mabs

Mab	ATCC No.	Antigen
B 3.6	HB 8890	GD3[1]
14.8	HB 9118	GD2[2]
11C64	--	GD3[3]
9.2.27	--	Condritin sulfate proteoglycan[4]
R24	--	GD3[5]
HT29/26	HB 8247	colon cancer glycoprotein gp 29[6]
HT29/36	HB 8248	colon cancer glycoprotein gp29[6]
CLT85	HB 8240	colon cancer[6]
F64.5	--	mammary carcinoma[7]
R38.1	--	pan carcinoma 70Kd protein[7]
F36/22	HB 8215	human breast carcinoma[8]
T16	HB 8279	human bladder tumor, glycoprotein gp48[9]
T43	HB 8275	human bladder tumor[9]
T101	HB 8273	human bladder tumor[9]
116-NS-19-1	HB 8059	colorectal carcinoma monosialoganglioside[10]
126	HB 8568	GD2[11]
CLH 6	HB 8532	colon cancer[12]
CLG 479	HB 8241	colon cancer[12]
19.9	CRL 8019	CEA[13]
CLNH5	--	lung carcinoma[14]
16-88	--	colon carcinoma[15]
KS1/4	--	lung adenocarcinoma[16]
LM609	--	vitronectin receptor[17]

[1] Cheresch et al., Proc. Natl. Acad. Sci., USA, 82:5155-5159 (1985): Ibid, 81:5767-5771 (1984)

[2] Cheresch et al. Cancer Res. 44:5112-5118 (1986)

[3] Cheresch et al., J. Cell. Biol., 102:688(1986)

[4] Bumol et al., Proc. Natl. Acad. Sci., USA, 79:1245 (1982); Harper et al., J. Immunol., 132:2096 (1984)

[5] U.S. Patent No. 4,507,391

[6] U.S. Patent No. 4,579,827

[7] U.S. Patent No. 4,522,918

[8] European Patent Application No. 84400420.0, publication No. 0 118 365, published September 12, 1984

[9] European Patent Application No. 84102517.4, publication No. 0 118 891, published September 19, 1984

[10] U.S. Patent No. 4,471,057

[11] Cheresch et al., J. Cell. Biol., 102:688 (1986); U.S. Patent No. 4,675,287

[12] U.S. Patent No. 4,579,827

[13] U.S. Patent No. 4,349,528

[14] Patent Application PCT/US83/00781, WO 83/04313

[15] European Patent Application No. 85300610.4, publication No. 0 151 030, published August 7, 1985

[16] Varki et al., Cancer Res., 44:681 (1984); Bumol et al., Hybridoma, 7:407 (1988)

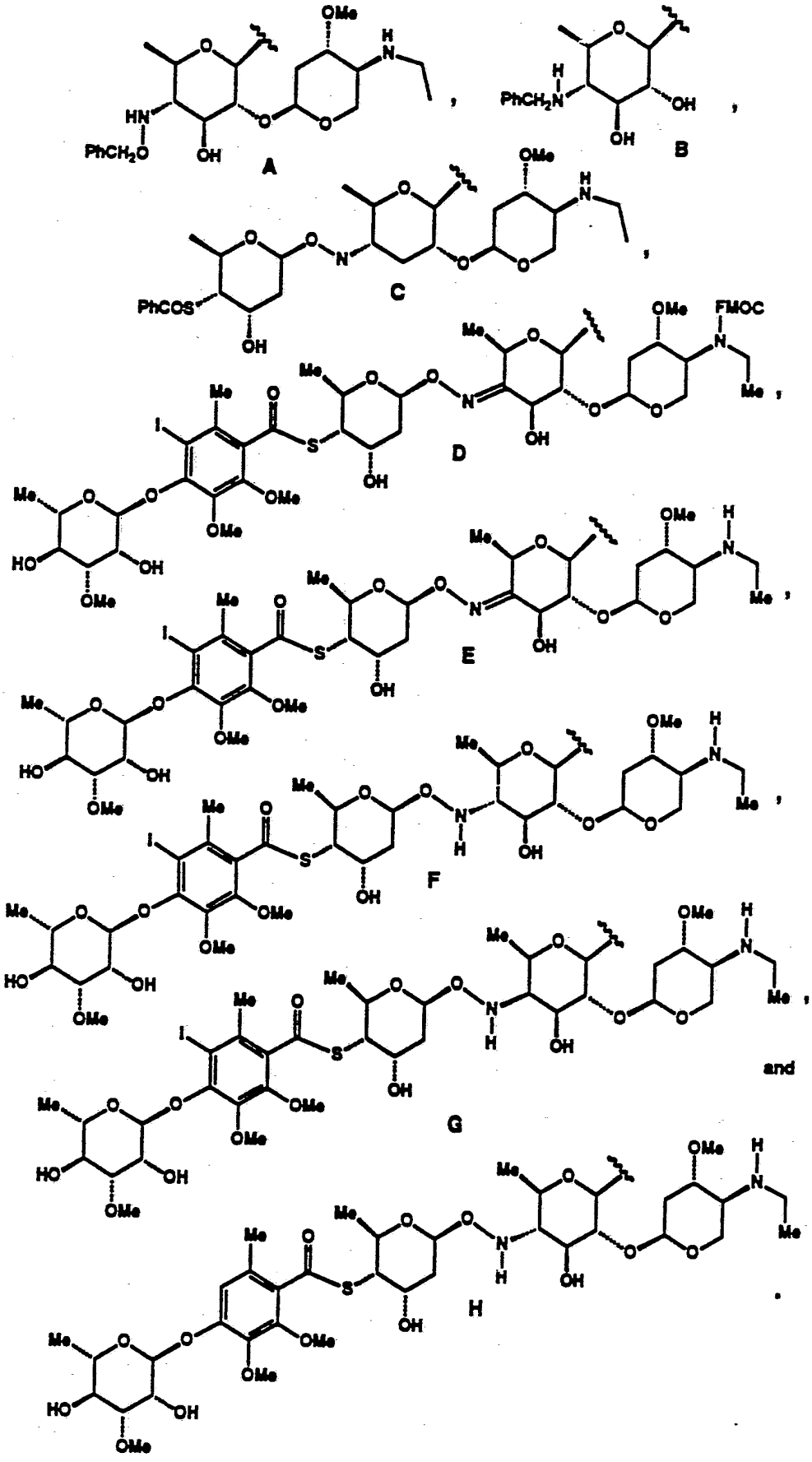
[17] Cheresch et al., J. Biol. Chem., 262:17703 (1987); Smith et al., J. Biol. Chem., 265:2168 (1990)

An enantiomeric fused ring enediyne compound of the invention can also be glycosidically linked to a sugar moiety to form a second type chimeric molecule. In such a chimer, the enantiomeric fused ring enediyne compound takes the place of the aglycone as in an

antibiotic molecule such as doxorubicin, calicheamicin or esperamicin, with the sugar moiety taking the place of the oligosaccharide portion. Bonding between the enantiomeric fused ring enediyne compound aglycone and oligosaccharide is typically via a hydroxyl group of a spacer group that is itself linked to the fused ring enediyne through a reacted hydroxyl group. A preferred spacer group is an oxyethanol group that can be an R^4 group or can be an R^5 substituent of W as is discussed and illustrated hereinafter. The glycosidically bonded saccharide thus forms an ether bond via the hydroxyl group of the oxyethanol group.

The oligosaccharide portion of the molecule is typically added after the synthesis of the fused ring enediyne compound (aglycone) portion is complete, except for any blocking groups on otherwise reactive functionalities of the aglycone that are typically removed after addition of the oligosaccharide portion. A sugar moiety is added by standard techniques as are discussed hereinafter.

A glycosidically-linked sugar moiety can be a monosaccharide such as a ribosyl, deoxyribosyl, fucosyl, glucosyl, galactosyl, N-acetylglucosaminyl, N-acetylgalactosaminyl moiety or the more preferred saccharides whose structures are shown below, wherein a wavy line adjacent a bond indicates the position of linkage.


A
B
C
D
E
F
G
H
and

The position of the glycosyl bond to be formed in the sugar moiety used for forming a chimeric compound is typically activated prior to linkage to the fused ring enediyne compound. For example, the 1-position hydroxyl group of an otherwise protected sugar (as with $^{t}BuMe_2Si$ or Et_3Si groups) is reacted with diethylaminosulfur trifluoride (DAST) in THF and in the presence of 4Å molecular sieves at -78°C to form the 1-fluoroderivative. The enantiomeric enediyne having a free hydroxyl group is then reacted with the 1-fluro-protected saccharide in the presence of silver perchlorate and stannous chloride to provide a protected desired, typically blocked, enantiomeric chimer molecule.

Similarly, treatment of 1-position hydroxyl of an otherwise protected saccharide with sodium hydride and trichloracetonitrile [Grandler et al., Carbohydr. res., 135:203 (1985); Schmidt, Angew. Chem. Int. Ed., Engl., 25:212 (1986)] in methylene chloride at about room temperature provides a 1-α-trichloroacetimidate group to activate the saccharide for coupling with the fused ring enediyne (aglycon) hydroxyl. Coupling is then carried out in boron trifluoride-etherate in methylene chloride to provide the protected desired chimer compound.

Once the enantiomeric aglycone and oligosaccharide are coupled, the protecting groups that are present are removed to provide the desired compound, which is then recovered using standard techniques. Exemplary syntheses are discussed hereinafter.

The 1, 2 or 3 six-membered ring fused rings that along with the depicted vinylene group constitute the structure W are aromatic hydrocarbyl rings. Such rings can thus be benzo, naphtho and anthra rings, using fused ring nomenclature. The anthra (anthracene)

derivative rings contemplated here contain 9,10-dioxo groups (are derivatives of anthraquinone) and are therefore referred to as 9,10-dioxoanthra rings.

Where a benzo, naphtho or 9,10-dioxoanthra ring forms part of the fused ring system, those fused rings are bonded to the remaining fused ring system through the carbon atoms of the 1- and 2-positions or are (a, b). A benzo, naphtho or 9,10-dioxoanthra fused ring portion can also contain one or more substituents at the ring positions remaining for substitution. Those substituent groups are selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, oxo, C_1-C_6 acyloxy and halo (chloro, bromo or iodo).

For a benzo ring, one or two substituents can be present at one or two of the remaining positions of the radical. Symmetrical substitution by the same substituent is preferred because of the lessened possibility for isomer formation. When a single substituent is present on a benzo ring, that substituent is referred to as R^5, which designation for convenience includes hydrogen. R^5 is thus selected from the group consisting of hydrogen (no substituent), C_1-C_6 alkoxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, benzyloxy, o-nitrobenzyloxy, hydroxy, C_1-C_6 acyloxy, oxyethanol, oxyethanol tertiary amino or quaternary ammonium C_2-C_6 alkyl carboxylic acid ester, oxyacetic acid, oxyacetic acid C_1-C_6 hydrocarbyl ester, oxyacetic acid amide, 3-hydroxyprop-1-ynyl and halo.

It is preferred that a hydroxyl group or a group that can form a hydroxyl group intracellularly be present, such that a hydroxyl group be present intracellularly at a position meta to the nitrogen in the adjacent ring. When two substituents are present on a benzo ring, they are referred to as R^{10} and R^{11} and are

selected from the group consisting of C_1-C_6 alkoxy, benzyloxy, oxo, C_1-C_6 acyloxy, hydroxyl and halo.

W is more preferably a benzo group that contains a single substituent R^5. In one particularly preferred embodiment, R^5 is situated in the benzo ring meta or para to the nitrogen atom bonded to R^1. That R^5 group is more preferably selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, benzyloxy, o-nitrobenzyloxy, C_1-C_6 acyloxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, oxyethanol, oxyacetic acid, oxycacetic C_1-C_6 hydrocarbyl ester, oxyacetic acid amide, oxyethanol tertiary amino- or quaternary ammonium-substituted C_2-C_6 alkyl carboxylate or 3-hydroxyprop-1-ynyl. An R^5 oxyacetic acid or oxyethanol or 3-hydroxyprop-1-ynyl group is useful for linking the aglycone to an oligosaccharide or antibody combining site portion via an ether or ester group, as discussed previously for R^4.

When R^5 is meta to the above nitrogen atom, it is preferred that the R^5 group be an electron releasing group such as hydroxyl or a C_1-C_6 acyloxy group that can provide a hydroxyl group intracellularly. A C_1-C_6 acyloxy group is believed to be a pro-drug form of the hydroxyl group that is cleaved intracellularly by an endogenus esterase or the like to provide the hydroxyl group. The presence of such an electron releasing group appears to assist in enhancing the potency of the compound against target tumor cells. It is believed that the enhanced potency is due to enhanced triggering of the epoxide opening and cyclization reactions.

When R^5 is para to the above nitrogen atom, it is preferred that the R^5 group be an o-nitrobenzyloxy group, oxyethanol, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, oxyacetic acid or oxyacetic acid C_1-C_6

hydrocarbyl ester. Those groups are particularly useful for the preparation of chimeras.

The presence of an R^5 substituent para to the nitrogen that is an oxyethanol, oxyacetic acid or oxyacetic acid amide as discussed for an R^4 group before, is also useful for providing enhanced water solubility to a fused ring enediyne compound discussed herein. One particularly preferred compound contains an oxyethanol R^5 group para to the nitrogen atom.

Another particularly prefered R^5 substituent that is para to the nitrogen atom is an oxyethanol ester of a tertiary or quaternary amine substituted C_2-C_6 alkyl carboxylic acid (carboxylate). These substituents provide still further enhancements to water solubility because of the formal charge of a quaternary ammonium group or the protonation of the tertiary amine at physiological pH values, e.g. pH 7.2-7.4.

Exemplary C_2-C_6 alkyl carboxylic acids are those discussed in conjunction with a C_1-C_6 acyloxy group. The amine substituent is preferably bonded to the carbon atom farthest down the alkyl chain from the carboxyl group and is therefore an ω-(omega) substituent. Thus, tertiary amine and quaternary ammonium derivatives of ω-amino acids such as glycine, β-alanine, γ-aminobutyric acid and 6-aminocaproic acid are preferred.

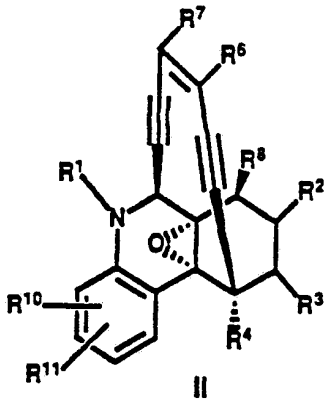
The amine portion of an oxyethanol tertiary amine- or quaternary ammonium-substituted C_2-C_6 alkyl carboxylic acid ester has the structure $-NR^{20}R^{21}$ or $-^{+}NR^{20}R^{21}R^{22}$ wherein R^{20}, R^{21} and R^{22} are each independently C_1-C_6 alkyl, or R^{20} and R^{21} together with the nitrogen atom form a 5- or 6-membered ring, or R^{20}, R^{21} and R^{22} (R^{20-22}) together with the nitrogen atom form a pyridinium or pyrazinium group. Exemplary C_1-C_6 alkyl groups have already been discussed, and methyl is preferred for each

of R^{20-22}. Exemplary 5- and 6-membered ring compounds formed by R^{20}, R^{21} and the nitrogen atom include piperidine, morpholine, pyrrolidine, imidazole, pyrrole and piperazine. Thus, exemplary $-NR^{20}R^{21}$ portions of tertiary amine groups include dimethylamino, diethylamino, hexyliso-propylamino, di-sec-butylamino, N-morpholinyl, N-piperidyl and N-imidazyl. Exemplary quaternary ammonium groups include trimethylammonium, ethyldimethylammonium, ethyliso-propylhexylammonium, N-methylmorpholinium, N-butylpiperidinium, pyridinium and pyrazinium. A suitable anion for the quaternary group is of course contemplated and includes halide ions such as chloride and bromide, sulfate, acetate or another C_1-C_6 acyloxy group anion. An N,N,N-trimethylglycine chloride ester of an oxyethanol substituent is particularly preferred.

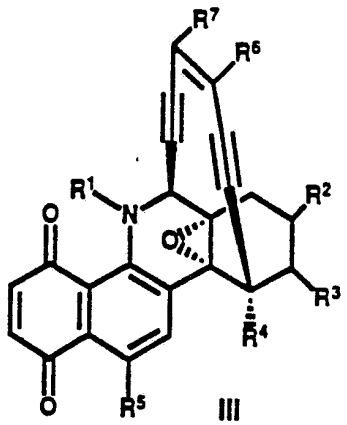
A particularly preferred compound has a structure corresponding to Formula XIb, hereinafter.

A naphtho ring can have three substituents. This ring can have a 4-position radical, R^5, selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, benzyloxy, C_1-C_6 acyloxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, and halo, and substituents at the 5-(R^{10}) and 8-positions (R^{11}) that are selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, benzyloxy, C_1-C_6 acyloxy, oxo and halo radicals. A 9,10-dioxoanthra ring can have three substituents at the 4-(R^5), 5- (R^9) and 8-positions (R^{12}) that are independently selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, benzyloxy, C_1-C_6 acyloxy and halo. Thus, R^5, R^9 and R^{12} can define the same groups, and all three groups can be written as either R^5, R^9 or R^{12}, but they are shown separately herein.

Exemplary structural formulas for a contemplated enantiomeric fused ring compound are illustrated below by structural Formulas II-IX, wherein each of the R groups is as discussed before.



II



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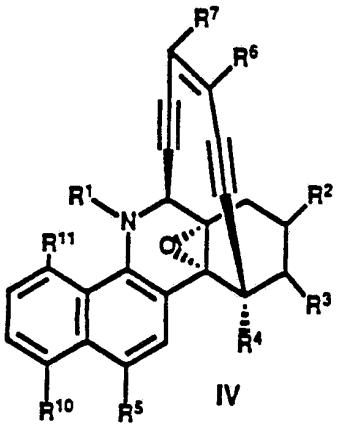

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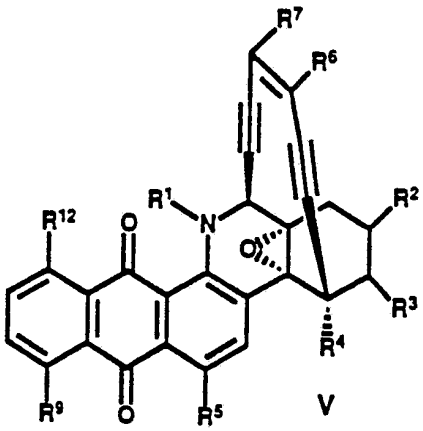

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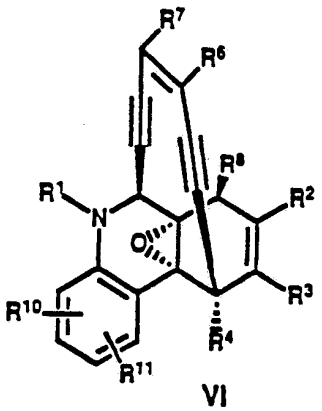

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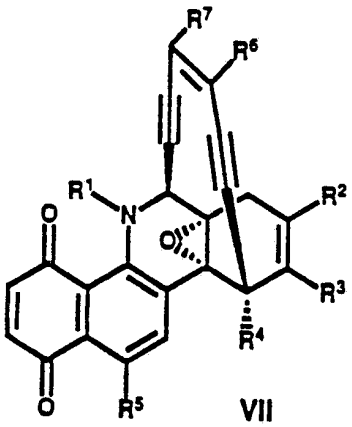

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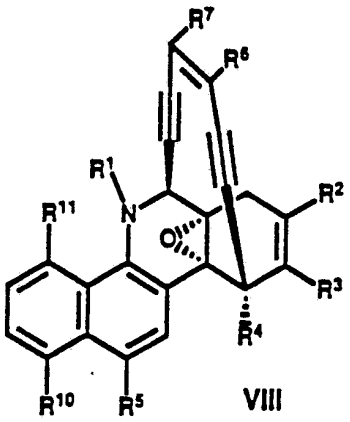
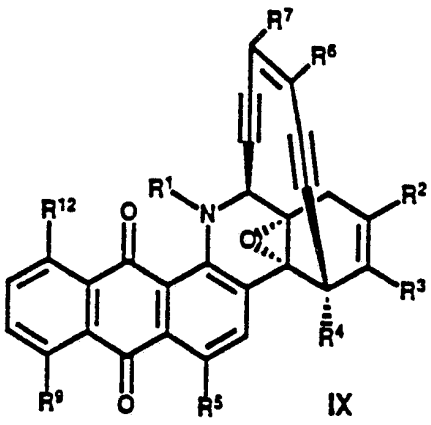


VIII

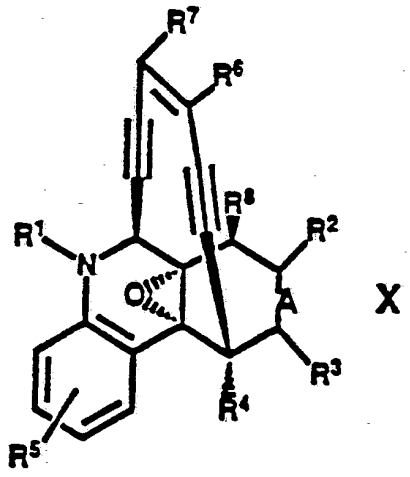


IX

In addition to the before-stated preference regarding R^8 and that bond A be a single bond, several other structural features and substituents are preferred.

Thus, it is preferred that R^2 and R^3 be hydrogen, and that R^6 and R^7 be hydrogen. It is also preferred that the fused ring system W together with the depicted vinylene group be substituted benzo, or an unsubstituted benzo, naphtho or 9,10-dioxoanthra ring. It is further preferred that the fused ring compound contain a total of 3-fused six-membered rings so that W together with the depicted vinylene group forms a benzo ring.

One particularly preferred group of enantiomeric compounds of the invention in which W is an R^5-substituted benzo ring corresponds to structural Formula X.



wherein A is a double or single bond;

R^1 is selected from the group consisting of H, C_1-C_6 alkyl, phenoxycarbonyl, benzoxycarbonyl, C_1-C_6 alkoxycarbonyl, substituted C_1-C_6 alkoxycarbonyl (particularly substituted ethoxycarbonyl where the substituent is phenylsulfonyl or naphthylsulfonyl, with

phenylsulfonyl most particularly preferred), o-nitrobenzyloxycarbonyl, and 9-fluorenylmethyloxycarbonyl;

R^2 is selected from the group consisting of H, carboxyl, hydroxylmethyl and carbonyloxy C_1-C_6 alkyl;

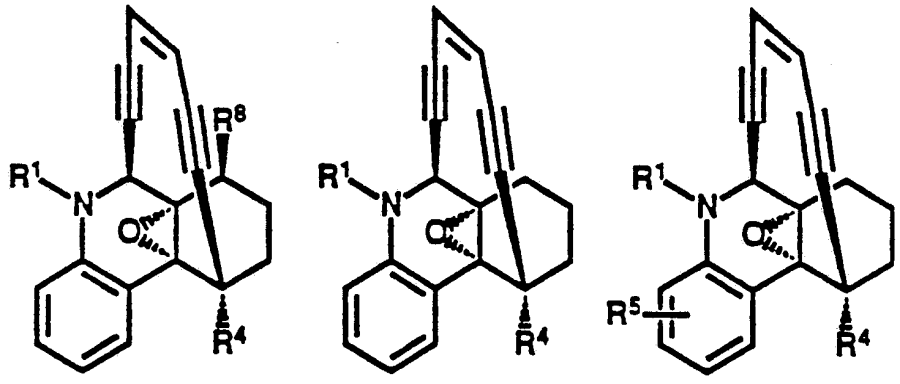
R^3 is selected from the group consisting of H and C_1-C_6 alkoxy;

R^4 is selected from the group consisting of H, hydroxyl, oxyacetic acid ($-OCH_2CO_2H$), oxyacetic C_1-C_6 hydrocarbyl or benzyl ester, oxyacetic amide, oxyethanol, oxyimidazylthiocarbonyl and C_1-C_6 acyloxy;

R^5 is selected from the group consisting of hydrogen, C_1-C_6 alkoxy, benzyloxy, o-nitrobenzyloxy, hydroxyl, C_1-C_6 acyloxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, oxyethanol, oxyacetic acid, oxyacetic acid C_1-C_6 hydrocarbyl ester, halo, oxyacetic acid amide, oxyethanol tertiary amino- or quaternary ammonium-substituted C_2-C_6 alkyl carboxylate and 3-hydroxyprop-1-ynyl; and

R^6 and R^7 are each H or together form with the intervening vinylidine group form a one, two or three fused aromatic ring system, and R^8 is methyl or hydrogen.

A still more preferred group of enantiomeric compounds of the invention correspond to structural Formulas XI, XIa and XIb.



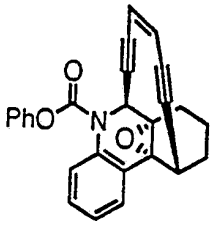
XI XIa XIb

wherein R^1, R^4, R^5 and R^8 are as previously defined.

Of the individual enantiomeric compounds corresponding to structural Formulas XI, XIa and XIb, there are further preferences for R^1, R^4 and R^5. These preferences also relate to the previously discussed compounds.

Thus, R^1 is most preferably phenoxycarbonyl 2-(phenylsulfonyl)ethoxycarbonyl, 2-(naphthylsulfonyl)ethoxycarbonyl or hydrogen. R^8 is most preferably hydrogen (H) to provide a compound of Formulas XIa or XIb. R^4 is most preferably H, hydroxyl, imidazylthiocarbonyloxy, benzyl oxyacetate and C_1-C_6 hydrocarbyl oxyacetate such as ethyl oxyacetate. R^5 in Formulas XI and XIa is H, but is more preferably hydroxyl, C_1-C_6 alkoxy, benzyloxy, C_1-C_6 acyloxy, oxyethanol, oxyacetic acid, oxyacetic acid C_1-C_6 hydrocarbyl or benzyl ester and o-nitrobenzyloxy, oxyacetic acid amide, oxyethanol tertiary amino- or quaternary ammonium-substituted C_2-C_6 alkyl carboxylate or 3-hydroxyprop-1-ynyl as in Formula XIb. It is noted that an R^5 o-nitrobenzyloxy group is not usually used in a pharmaceutical composition discussed hereinafter.

The structural formulas of particularly preferred enatiomeric compounds are shown below, generally as the preferred (+)-stereoisomers, along with compound numbers as utilized in WO 92/02522. Syntheses for the racemates of those compounds are disclosed in WO 92/02522, as well as in Nicolaou et al., Science, 256:1172-1178 (1992), and the citations therein, as are analytical data. In the formulas below and elsewhere herein, Ph = phenyl, Me = methyl, NBnO = o-nitrobenzyloxy and $^tBuCO_2$ = pivaloyl.



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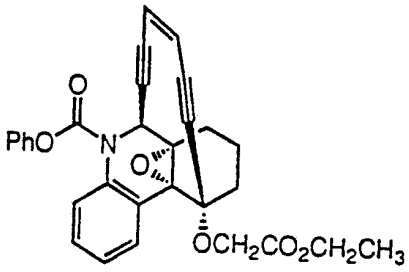

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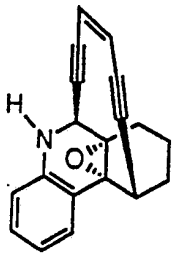

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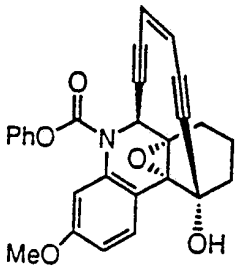

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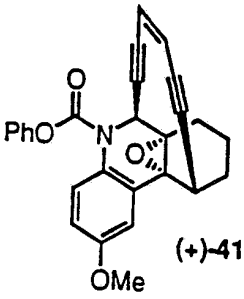

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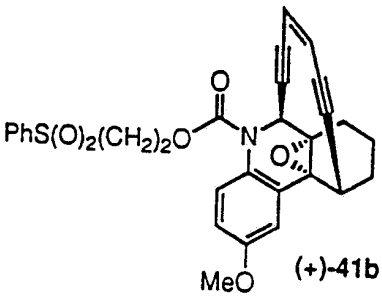

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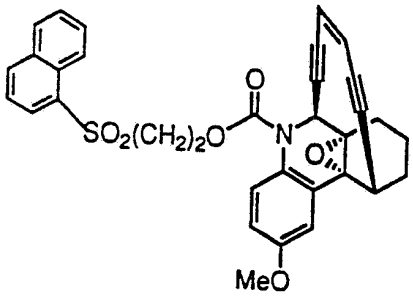

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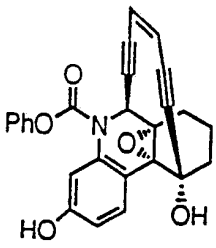

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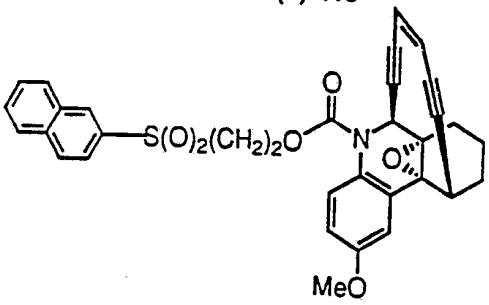

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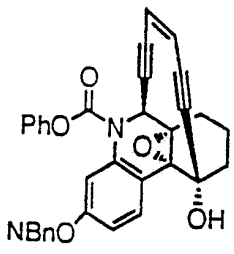

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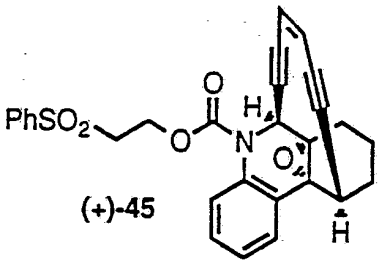

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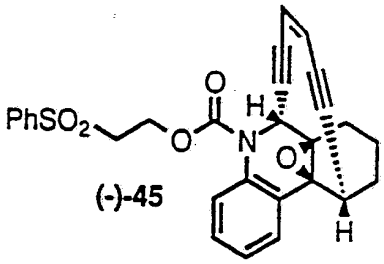
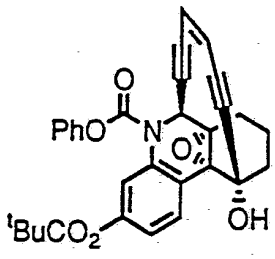
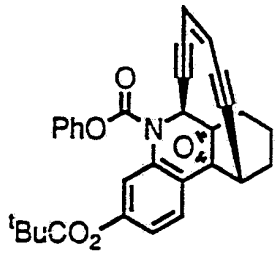


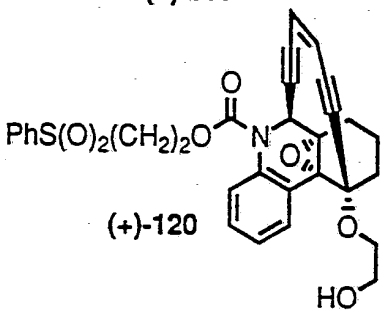
(-)-45



(+)-59a



(+)-59b



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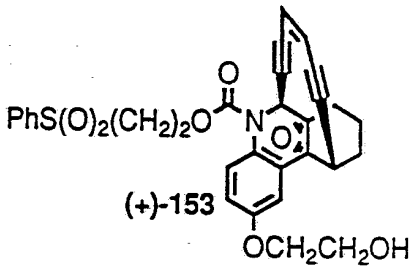

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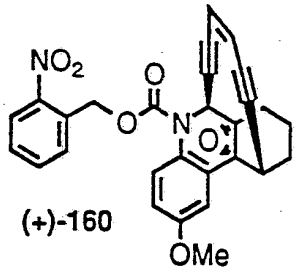

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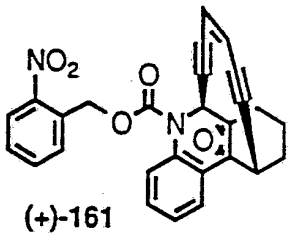
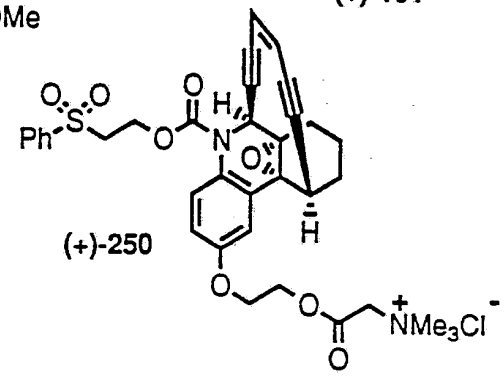


(+)-161



(+)-250

A before-discussed compound is chiral, and as such can exist in two enantiomeric forms (+) and (-). The compounds are generally shown in the absolute stereoconfiguration of dynemicin A [Landley et al., J. Am. Chem. Soc., 113:4395 (1991), and Wender et al., Proc. Natl. Acad. Sci. USA, 88:8835 (1991)].

A contemplated enantiomeric fused ring enediyne compound can be synthesized as a racemic mixture of the enantiomers as discussed in WO 92/02522 and resolved into single enantiomers for use herein, or can be synthesized as an optically pure single enantiomer, which is preferred. The synthesis of single enantiomeric Compounds (+)- and (-)-45 are discussed hereinafter, and analogous syntheses can be applied to the preparation of an enantiomer of any of the other compounds disclosed herein. As is also discussed hereinafter, the enantiomeric Compounds 45 exhibited similar DNA cleaving activities to eaoh other and to the racemate, but exhibited some startling differences between themselves and the known racemate in cytotoxicity when assayed against cancer cell lines.

II. Pharmaceutical Compositions

An enantiomeric compound or chimera of the invention is useful as a DNA cleaving agent, and also as an antimicrobial and a cytoxic (antitumor) agent, as are dynemicin A, calicheamicin, esperamicin and neocarzinostatin. A compound of the invention can also therefore be referred to as an "active agent" or "active ingredient".

DNA cleavage can be assayed using the techniques described hereinafter as well as those described by Mantlo et al., J. Org. Chem., 54:2781 (1989); Nicolaou et al., J. Am. Chem. Soc., 110:7147 (1989); Nicolaou et al., J. Am. Chem. Soc., 110:7247

(1988) or Zein et al., Science, 240:1198 (1988) and the citations therein.

An enantiomeric compound or chimer of the invention is useful against Gram-positive bacteria such as S. aureus and epidermis, Micrococcus luteus and Bacillus subtillis as is dynemicin A. Such a compound or chimer also exhibits antimicrobial activity against E. coli, Pseudomonas aeruginos, Candida albucans and Aspergillis fumigatus. Activity of an enantiomeric compound of the invention against the above microorganisms can be determined using various well known techniques. See, for example, Konishi et al., J. Antibiotics, XLII:1449 (1989). Antimicrobial and antitumor assays can also be carried out by techniques described in U.S. Patent No. 4,837,206, whose disclosures are incorporated by reference, as well as by the procedures described hereinafter.

A before-described enantiomeric compound can also be shown to undergo a Bergman cycloaromatization reaction in the presence of benzyl mercaptan, triethylamine and 1,4-cyclohexadiene as discussed in Haseltine et al., J. Am. Chem. Soc., 111:7638 (1989). This reaction forms a tetracyclic reaction as is formed during DNA cleavage, and can be used as a co-screen to select more active compounds.

A pharmaceutical composition is thus contemplated that contains a before-described enantiomeric compound or chimer of the invention as active agent. A pharmaceutical composition is prepared by any of the methods well known in the art of pharmacy all of which involve bringing into association the active compound and the carrier therefor. For therapeutic use, a compound or chimer of the present invention can be administered in the form of conventional pharmaceutical compositions. Such

compositions can be formulated so as to be suitable for oral or parenteral administration, or as suppositories. In these compositions, the enantiomeric agent is typically dissolved or dispersed in a physiologically tolerable carrier.

A carrier or diluent is a material useful for administering the active compound and must be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. As used herein, the phrases "physiologically tolerable" and "pharmaceutically acceptable" are used interchangeably and refer to molecular entities and compositions that do not produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a mammal. The physiologically tolerable carrier can take a wide variety of forms depending upon the preparation desired for administration and the intended route of administration.

As an example of a useful composition, an enantiomeric compound or chimer of the invention (active agent) can be utilized, dissolved or dispersed in a liquid composition such as a sterile suspension or solution, or as isotonic preparation containing suitable preservatives. Particularly well-suited for the present purposes are injectable media constituted by aqueous injectable buffered or unbuffered isotonic and sterile saline or glucose solutions, as well as water alone, or an aqueous ethanol solution. Additional liquid forms in which these compounds or chimers can be incorporated for administration include flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, peanut oil, and the like, as well as elixirs and similar pharmaceutical vehicles. Exemplary further liquid

diluents can be found in Remmington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA (1980).

An active agent can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain stabilizers, preservatives, excipients, and the like in addition to the agent. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic.

Methods of forming liposomes are known in the art. See, for example, Prescott, Ed., Methods in cell Biology, Vol. XIV, Academic press, New York, N.Y. (1976), p.33 et seq.

An active agent can also be used in compositions such as tablets or pills, preferably containing a unit dose of the enantiomeric compound or chimer. To this end, the agent (active ingredient) is mixed with conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate, gums, or similar materials as non-toxic, physiologically tolerable carriers. The tablets or pills can be laminated or otherwise compounded to provide unit dosage forms affording prolonged or delayed action.

It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical formulation described herein can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents,

binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

The tablets or pills can also be provided with an enteric layer in the form of an envelope that serves to resist disintegration in the stomach and permits the active ingredient to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, including polymeric acids or mixtures of such acids with such materials as shellac, shellac and cetyl alcohol, cellulose acetate phthalate, and the like. A particularly suitable enteric coating comprises a styrene-maleic acid copolymer together with known materials that contribute to the enteric properties of the coating. Methods for producing enteric coated tablets are described in U.S. Patent 4,079,125 to Sipos, which is herein incorporated by reference.

The term "unit dose", as used herein, refers to physically discrete units suitable as unitary dosages for administration to warm blooded animals, each such unit containing a predetermined quantity of the agent calculated to produce the desired therapeutic effect in association with the pharmaceutically acceptable diluent. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, pills, powder packets, granules, wafers, cachets, teaspoonfuls, dropperfuls, ampules, vials, segregated multiples of any of the foregoing, and the like.

A previously noted preferred or particularly preferred compound or chimer is preferred or particularly preferred for use in a pharmaceutical composition.

An enantiomeric compound or chimer of the invention is present in such a pharmaceutical composition in an amount effective to achieve the desired result. For example, where in vitro DNA cleavage is the desired result, a compound or chimer of the invention can be utilized in an amount sufficient to provide a concentration of about 1.0 to about 5000 micromolar (μM) with a DNA concentration of about 0.02 μg/μL. As a cytotoxic (antitumor) agent, an effective amount of an enantiomeric compound or chimer of the invention is about 0.05 to about 50 mg per kilogram, and more preferably about 0.1 to about 15 mg per kilogram of body weight or an amount sufficient to provide a concentration of about 0.01 to about 50 μg/mL to the bloodstream. A compound or chimer of the invention exhibits antimicrobial activity in a concentration range of about 0.01 mg to about 50 μg/mL. The above concentrations and dosages vary with the particular compound of the invention utilized as well as with the target, e.g., DNA, tumor, microbe, as is well known. Lower dosages are preferred when multiple administration is utilized.

III. Methods

An enantiomeric compound or chimer of the invention is useful in cleaving DNA, as a cytotoxic agent and also in inhibiting the growth of neoplastic cells, and is utilized in a method for effecting such a result. An enantiomeric compound or chimer of the invention is typically utilized in a before-described composition.

In accordance with such a method, DNA to be cleaved or target cells to be killed or whose growth is to be inhibited are contacted with a compound or chimer of the invention (active ingredient), typically in a

composition as before, in an amount effective or sufficient for such a purpose, as discussed before, dissolved or dispersed in a physiologically tolerable (pharmaceutically acceptable) diluent. That contact is maintained for a time sufficient for the desired result to be obtained; i.e., DNA cleaved, cells killed or neoplastic cell growth inhibited.

As is discussed hereinafter, preliminary studies indicate that the principal mechanism of cytotoxicity exhibited by an enantiomeric fused ring enediyne disclosed herein is DNA cleavage within the contacted cells. Without being bound by theory, it is nevertheless believed that both DNA cleavage in vitro and cytotoxity or cell growth inhibition by contact with a disclosed fused ring enediyne operate substantially similarly.

Where the desired result is carried out in vitro, contact is maintained by simply admixing the DNA or target cells with the composition and maintaining them together under the appropriate conditions of temperature and for cell growth to occur, as for control, untreated cells. Thus, a single admixing and contacting is typically sufficient for in vitro purposes.

The above method is also useful in vivo, as where a mammal such as a rodent like a rat, mouse, or rabbit, a farm animal like a horse, cow or goat, or a primate like a monkey, ape or human is treated. Here, contact of a composition and the cells to be killed or whose growth is to be inhibited is achieved by administration of the composition to the mammal by oral, nasal or anal administration or by introduction intravenously, subcutaneously or intraperitoneally. Thus, contact in vivo is achieved via the blood or lymph systems.

Although a single administration (admixture) and its resulting contact is usually sufficient to maintain the required contact and obtain a desired result in vitro, multiple administrations are typically utilized in vivo. Thus, because of a body's breakdown and excreting pathways, contact between an active ingredient of a composition and the target cells is typically maintained by repeated administration of a compound of the invention over a period of time such as days, weeks or months, or more, depending upon the target cells.

Exemplary methods of the invention for DNA cleavage and inhibition of MIA PaCa-2 human pancreatic carcinoma (ATCC CRL 1420) and MB49 murine bladder carcinoma target cells (obtained from Dr. Lan Bo Chen of the Dana Farber Cancer Institute, Boston, MA) as well as several other neoplastic cell lines are discussed in WO 92/02522, and in Nicolaou et al., Science, 256:1172-1178 (1992), and in the citations therein.

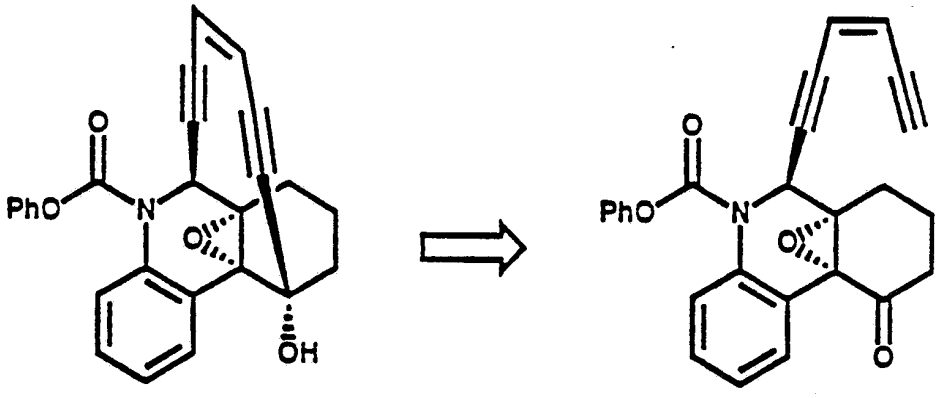
Exemplary concentrations for in vitro cytoxicity studies vary with the cells to be killed, and can range from about 10^{-5}M to about 10^{-15}M, as is seen from the data in Tables 1-2 hereinafter. Exemplary concentrations and dosages for in vivo use can be those used for dynemicin A or calicheamicin γ_1^I. Typical in vivo dosages are about 1 to about 100 mg/kg body weight of the recipient mammal. Exemplary concentrations useful for in vitro cleavage of DNA range from about 0.1 to about 5 mM.

IV. Compound Syntheses

An enantiomeric contemplated compound of the invention can be prepared by a number of routes, several of which are illustrated in WO 92/02522, as well as in Nicolaou et al., Science, 256:1172-1178 (1992) and the

citations therein. The retrosynthetic plan for those syntheses is illustrated below in Scheme I.

Scheme I

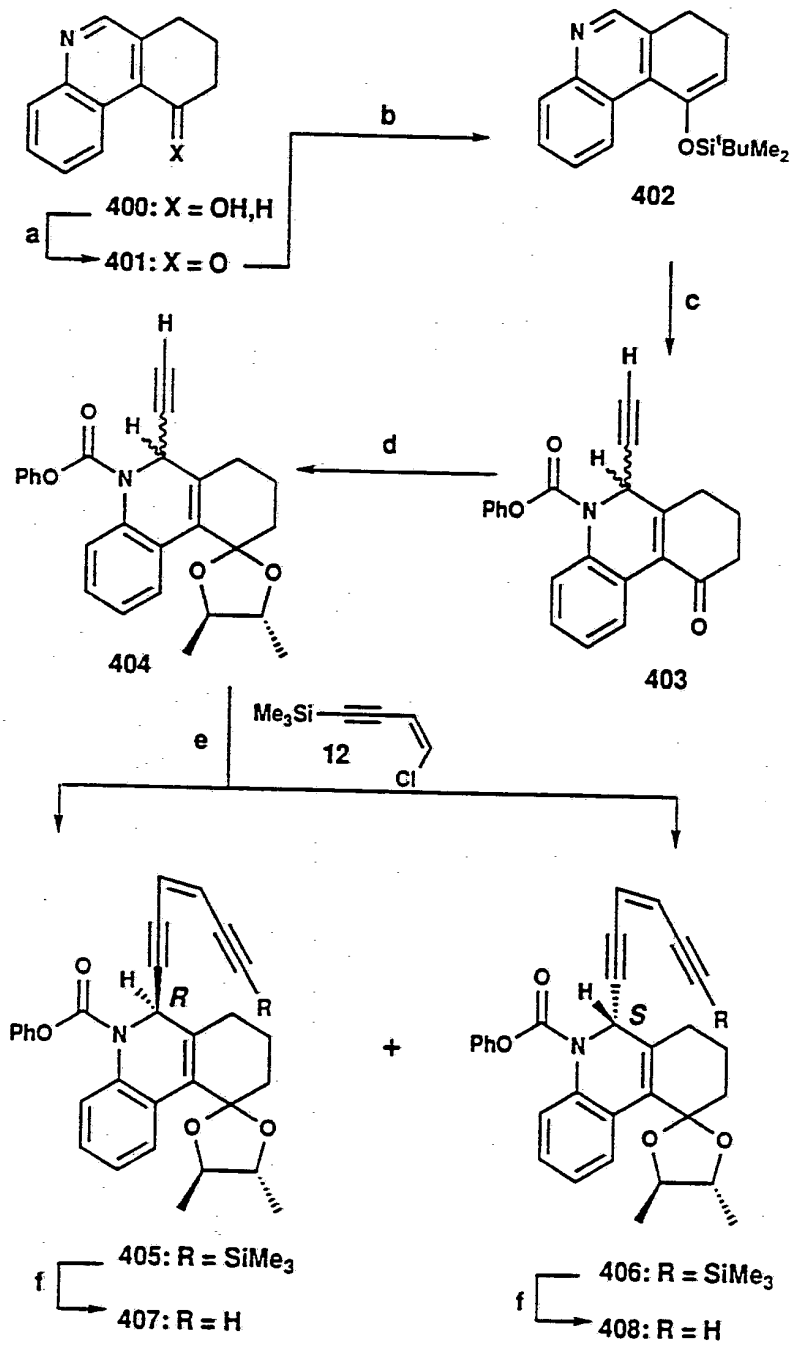


2 3

In the reactions shown in WO 92/02522, the compounds prepared such as Compound 3 were racemates, even though only one enantiomer was shown. As is shown in the schemes that follow, a pair of diastereomeric precursor molecules to Compound 3 were prepared, separated and then converted to a single enantiomer from which enantiomeric Compound 3 were prepared. After preparation of a Compound 3 enantiomer or an analogue thereof having one or more substituents discussed before, that enantiomer is converted to a desired enantiomeric fused ring compound that of Formulas I-XI, XIa or XIb.

Briefly, the basic hydroxyl-substituted 3-, 4- or 5-fused six-membered ring system is first formed such as Compound 6 discussed in WO 92/02522, or Compound 400 that is shown in Scheme II, below.

Scheme II


N
X
400: X = OH,H
a
401: X = O
b
OSi^{t}BuMe$_2$
402
c
H
O
PhO
N
O
O
404
d
403
e
Me$_3$Si
12
Cl
R
S
+
405: R = SiMe$_3$
f
407: R = H
406: R = SiMe$_3$
408: R = H

Thus, hydroxy quinoline Compound **400** was oxidized to ketone Compound **401** using Jones reagent [1.3 equivalents of Jones' reagent, 1.0 equivalents of H_2SO_4, AcOH-acetone (1:1), zero → 25°C, 30 minutes, 98 percent], as step **a**, and then converted to enol silyl ether Compound **402** in high yield in step **b** by treatment with 1.2 equivalents of $^tBuMeSiOTf$, 1.5 equivalents of ET_3N in CH_2Cl_2 at 25°C for three hours 99 percent.

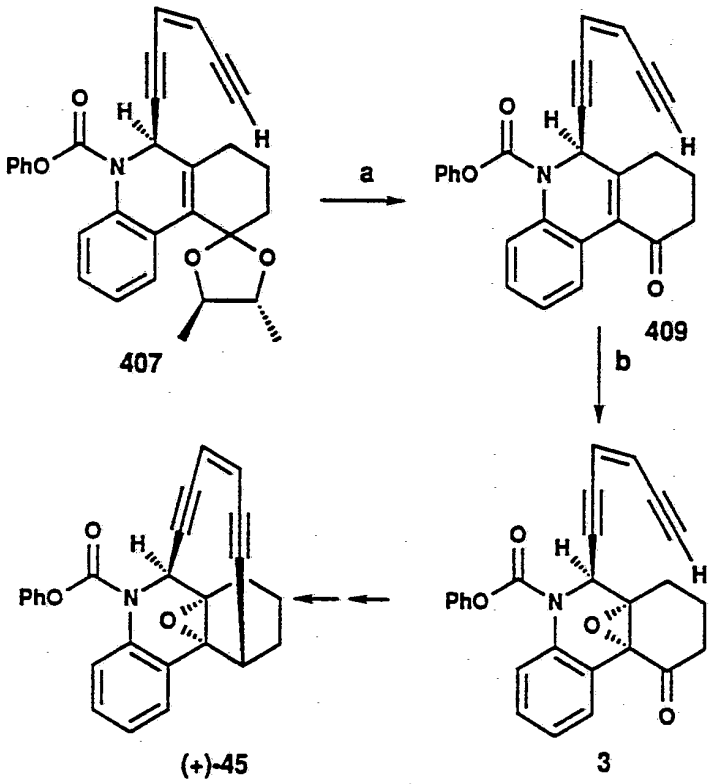
Sequential treatment of Compound **402** with ethynylmagnesium bromide (1.1 equivalents) and phenyl chloroformate (1.1 equivalents) and in THF at -78 → 25°C, for one hour, and then 10 percent HCl at 25°C for ten minutes as step **c** afforded acetylenic Compound **403** in 92 percent overall yield. Ketalization of Compound **403** with (2R,3R)-2,3-butanediol (1.5 equivalents, plus 0.2 equivalents of $TsOH \cdot H_2O$ in refluxing benzene for 20 hours) gave an inseparable mixture of diastereomers Compound **404** (about 1:1 by ^1H NMR) in 95 percent yield as step **d**. That mixture was coupled with vinyl chloride Compound **12** under the influence of Pd(0)-Cu(I) catalysis [1.5 equivalents of Compound **12**, 0.05 equivalents of $Pd(PPh_3)$, 0.2 equivalents of CuI, and 1.5 equivalents of $^nBuNH_2$ in benzene at 25°C for two hours] afforded a 1:1 mixture of enediyne Compounds **405** and **406** (63 percent yield) in step **e**. Flash column chromatography (silica gel, 0.25 percent ethyl acetate in benzene) led to pure diastereoisomeric Compounds **405** [R_f=0.22 (silica gel, 0.25 percent ethyl acetate in benzene); $[\alpha]_D^{25}$+427° (c 0.88, benzene)] and **406** [R_f=0.20 (silica gel, 0.25 percent ethyl acetate in benzene); $[\alpha]_D^{25}$-397° (c 0.95, benzene)] in 45 and 42 percent yield, respectively.

Separate removal of the trimethylsilyl group from Compounds **405** and **406** [4.0 equivalents of $AgNO_3$ in ETOH:THF:H_2O (1:1:1) at 25°C for two hours and then 7.0

equivalents of NaCN at 25° for 30 minutes) led to enediyne Compounds **407** and **408** in high yields as step f. Assignment of absolute stereochemistry in this series was based on X-ray crystallographic analysis of Compound **408**.

Transformation of the diastereomeric Compounds **407** and **408** to the targeted Compounds (+)-**45** and (-)-**45** was carried out as illustrated in Scheme III, below, for the synthesis of Compound (+)-**45**.

Scheme III

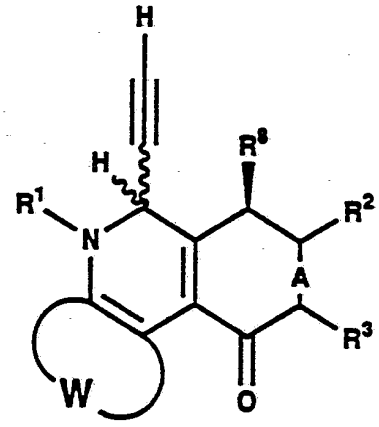


Thus, acid hydrolysis of ketal Compound **407** [0.2 equivalents of $TsOH \cdot H_2O$ in benzene:acetone: H_2O (100:1:1) at reflux for six hours) afforded enone Compound **409** (85 percent) in step **a**, which was converted to epoxyketone Compound **3** in step **b** using mCPBA under basic conditions [2.0 equivalents of mCPBA in aqueous $NaHCO_3$:CH_2Cl_2 (1:1) at 25°C for 1.5 hours; 43 percent yield based on 87 percent conversion]. Steps for transforming Compound **3** into Compound **45** followed the pathway for synthesis of the racemic Compound **45** discussed in relation to Scheme II and steps **a** and **b** of Scheme VIII of WO 92/02522. Enantiomer (-)**45** was prepared similarly.

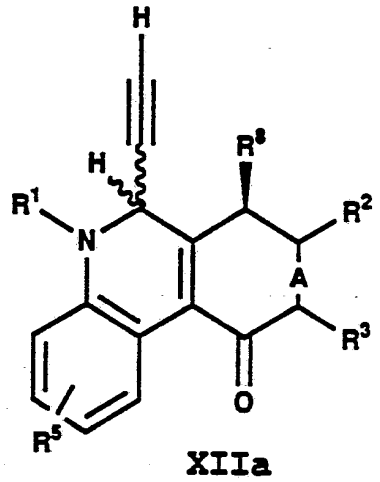
It is noteworthy that enantiomer Compound (+)-**45** had the same absolute stereochemistry [the absolute stereochemistry of dynemicin A was suggested based on a working model of its interaction with DNA, see: Landley et al., J. Am. Chem. Soc., 113:4395 (1991) and Wender, Proc. Natl. Acad. Sci. USA, 88:8835 (1991)] and sign of optical rotation as dynemicin A [(+)-**45**:$[\alpha]_D^{25}$+586° (c 0.56, benzene), dynemicin A (**1**):$[\alpha]_D^{24}$+270° (c 0.01, DMF [Konishi et al., J. Antibiot., 42:1449 (1989); Konishi et al., J. Am. Chem. Soc., 112:3715 (1990)]. Enantiomer (-)-**45** [$[\alpha]_D^{25}$-562° (c 0.50, benzene].

The above-discussed enantioselective syntheses of Compounds (+)-**45** and (-)-**45** are general for any of the enantiomeric dynemicin analogs discussed herein. Thus, either enantiomer of any desired fused ring dynemicin analog can be readily prepared via diastereometric ketalization of a compound such as Compound **403**, or more generally, a compound of structural Formulas XII and XIIa, below, wherein R^1,R^2,R^3,R^5,R^8, A and W are as before described, to form a compound of structural Formulas XIII and XIIIa, below,

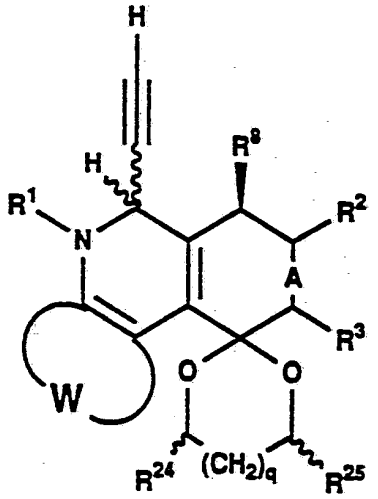
wherein R^{24} and R^{25} are independently C_1-C_3 alkyl or phenyl, q is zero or 1 such that the parenthesized CH_2 group is absent or present, respectively, and ketalization forms at least two diastereomers.



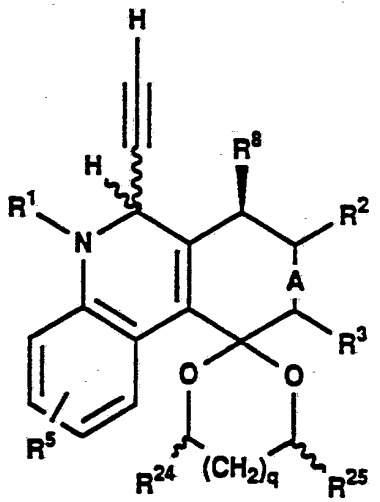
XII



XIIa



XIII



XIIIa

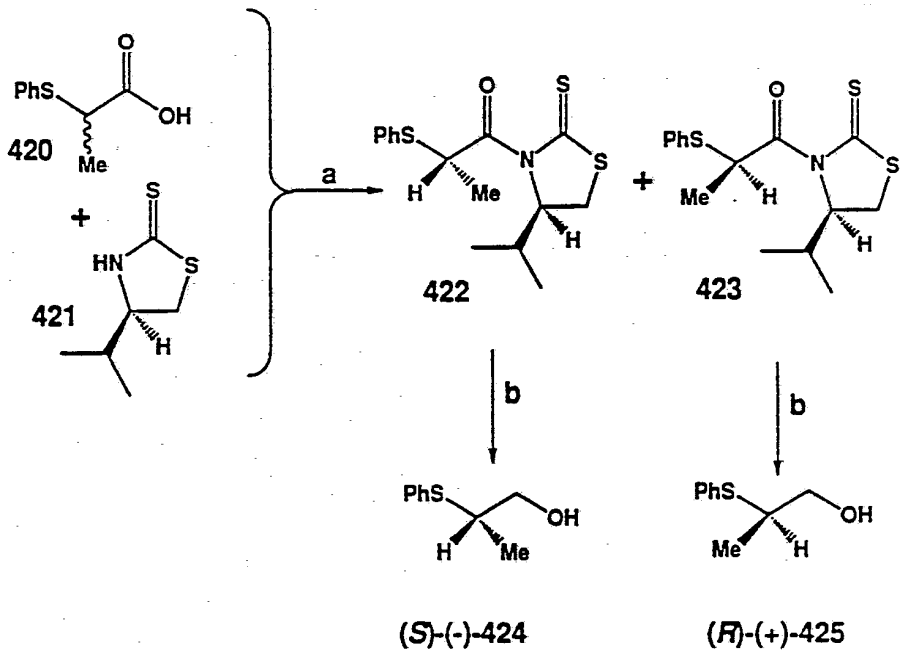
It should, of course, be understood that (2R,3R)-2,3-butanediol as used herein is not the only useful diol. Any chiral diol that contains unreactive substituents in the above reactions and can form a 5- or 6-membered ring ketal can be used. For example, (2S,3S)-2,3-butanediol, (2R,4R)-2,4-pentanediol,

(2S,4S)-pentanediol, (S)- or (R)-1,2-propanediol, (S)- or (R)-2-phenyl-1,2-propanediol, (S)- or (R)-1-phenyl-1,2-ethanediol and the like can be used. Symmetrical diols such as the chiral 2,3-butanediols and 2,4-pentanediols are preferred. Diastereomeric compounds of Formulas XIII and XIIa are also contemplated as are separated enantiomers of Formulas XII and XIIa.

As was discussed earlier, the stereochemistry of a 2-phenylsulfonyl-2-mono- or di-C_1-C_6 alkyl ethoxycarbonyl R^1 substituent can also have an effect on the potency of a contemplated enantiomer. The discussion below illustrates an exemplary stereoselective synthesis for exemplary chiral R^1 groups.

Racemic Compound **21** was used as the basis for the synthesis of racemic Compound **45** as discussed in regard to Scheme VIII of WO 92/02522, and was also used as a starting material for synthesis of 2-(C_1-C_6 alkyl)-2-(phenylsulfonyl)ethoxycarbonyl derivatives. The required chiral 2-phenylthio-1-propanols were prepared as illustrated in Scheme IV, below, by an asymmetric reduction method based on the chemistry of 4(R)-isopropyl-1,3-thiazolidine-2-thione), Compound **421** [Fujita et al., in "Advances in Heterocyclic Chemistry", 45:1-36 (1989); Nagao et al., J. Org. Chem., 51:2391 (1986)], that was coupled with racemic 2-methylphenylthioacetic acid (Compound **420**). Compound **420** was itself prepared by reaction of phenylthioacetic acid methyl ester and methyl iodide in the presence of lithium diisopropylamide (LDA) at -78°C.

Scheme IV



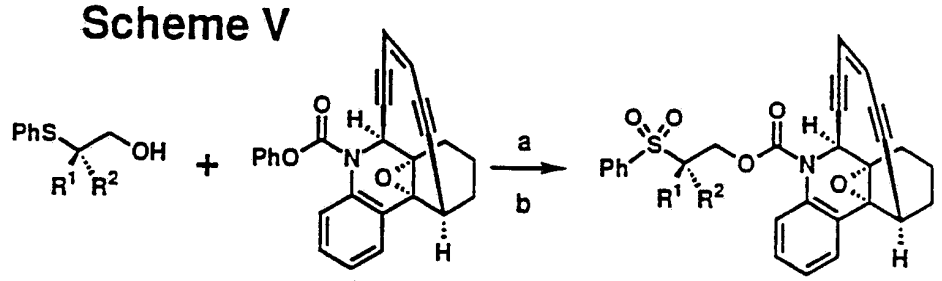
Thus, the racemic acid (Compound **420**) and Compound **421** were coupled in step **d** in the presence of 1.2 equivalents of DCC and 0.2 equivalents of DMAP in CH_2Cl_2 at 25°C for one hour to provide the diastereomeric imides, Compounds **422** and **423**. Those compound were separated by flash column chromatography to provide 42 and 22 percent yields, respectively. [Compound **422** $[\alpha]_D^{25}$-565.0° (c 0.1, ETOH), R_f = 0.41 (silica, 10 percent ET_2O in petroleum ether); Compound **423** $[\alpha]_D^{225}$-268.0° (c 0.1, ETOH), R_f = 0.27 (silica, 10 percent ET_2O in petroleum ether).]

Separate reductions of Compound **422** and **423** with $LiAlH_4$ (one equivalent in THF at zero degrees C for two minutes) provided Compounds **424** and **425** as the (S)-(-)- and (R)-(+)-isomers in 73 and 79 percent yields, respectively, in step **b**. Compound **424**: $[\alpha]_D^{25}$ = -10.3° (c 0.6, ETOH); Compound **425**: $[\alpha]_D^{25}$ = 9.9° (c 0.87, ETOH). The assignment of absolute stereochemistry was made by an independent synthesis of Compound **425**.

Compound **426**, 2,2-(dimethyl)-2-phenylthioethanol was prepared by reaction of ethyl isobutyrate with diphenyldisulfide in the presence of LDA, followed by reduction with $LiAlH_4$.

Compounds **424**, **425** and **426** were then reacted with racemic Compound **21** as an exemplary compound to demonstrate the reaction and to form the fused ring enediynes **427**, **428** and **429**, after oxidation. These reactions are illustrated below in Scheme V.

Scheme V



424: R^1 = H, R^2 = Me
425: R^1 = Me, R^2 = H
426: R^1 = R^2 = Me

427: R^1 = H, R^2 = Me
428: R^1 = Me, R^2 = H
429: R^1 = R^2 = Me

Thus, 1.2 equivalents of each of Compounds **424-426** was separately reacted with one equivalent of WO 92/02522 Compound **21** and 1.2 equivalents of NaH in THF at 25°C for 0.5 hours in step **a**. The compounds so prepared were then separately reacted in step **b** with 2.5 equivalents of mCPBA in CH_2Cl_2 at zero degrees C for 0.5 hours to provide Compounds **427**, **428** and **429** in 79, 79 and 66 percent yields, respectively. Each of Compounds **427** and **428** was an inseparable pair of diastereomers (single enantiomers at R^1 linked to a racemate).

DNA cleaving properties of Compounds **427-429** at 5.0 mM each were assayed and compared to Compound **21** at 1.0 mM using ϕX174 DNA (50 μM per base pair) at pH values of 8.5 and 9.0 at 37°C for 48 hours. See Figs. 1a and 1b.

As seen in Figs. 1a and 1b, Compounds **427** and **428** (lanes 3 and 4) exhibited greatly reduced in vitro DNA cleaving ability at basic pH values relative to racemic Compound **21** (lane 5), whereas Compound **429** (lane 6) exhibited no DNA cleaving ability. Phenyl isopropenyl sulfone (lane 6) and 2-(phenylsulfonyl)propanol (lane 7) used as controls confirmed that Compounds **427** and **428** cleaved DNA by benzenoid diradicals generated from the enediyne core (the fused ring enediyne freed of the R^1 group). Because phenyl vinyl sulfone (lane 8) is an alkylating agent, it was not surprising to see Form II DNA at the concentration used for these studies. Separate studies at 1.0 mM showed no DNA cleavage. The increased DNA damage from Compounds **427** and **428** at the higher pH value supports the concept of a base-catalyzed β-elimination of the R^1 group leading to formation of the DNA-cleaving material.

Further biological evaluation data for Compound **427**, **428** and **429** are provided hereinafter in Tables 1 and 2.

Best Mode for Carrying out the Invention

Methods

DNA cleavage studies, and cytotoxicity studies were carried out as discussed in WO 92/02522, Nicolaou et al., Science, 256:1172-1178 (1992) and the citations therein. Compound data for a contemplated fused ring dynemicin A analogue racemates are provided in WO 92/02522 or in the above published literature.

The cell lines assayed were obtained from the American Type Culture Collection (ATCC) of Rockville, Maryland, except for normal human dermal fibroblasts

(NHDF) that were obtained from Clonetics Corporation, San Diego, California.

Control studies were also carried out using the following well known anticancer drugs with the following IC_{50} values for NHDF and cancer cells. The results of these studues are shown below.

	Range of Average IC_{50} Values (Molarity)	
Drug	NHDF	Cancer Cells
Doxorubicin	--	$1.6X10^{-10}$ - $9.8X10^{-8}$
Dynemicin A	10^{-8}	$1.6X10^{-8}$ - $9.8X10^{-10}$*
Calicheamicin	$2.5X10^{-9}$	$5X10^{-5}$ - 10^{-12}**
Morpholinodoxorubicin	--	$1.6X10^{-7}$ - $9.8X10^{-9}$
Taxol	10^{-8}	10^{-7} - 10^{-9}
Methotrexate	$5X10^{-5}$	$>10^{-4}$ - 10^{-8}
Cis-Platin	$5X10^{-5}$	10^{-4} - 10^{-6}
Melphelan	10^{-4}	10^{-4} - 10^{-6}

* UCLA-P3 cells were susceptible at 10^{-12}M. All other cells were susceptible at $1.56X10^{-10}$ M or higher concentrations.

** Molt-4 cells were susceptible at 10^{-12}M. All other cells were susceptible at $3.9X10^{-9}$M or higher concentrations.

Compounds (±)-45, (+)-45, and (-)-45 cleaved φX174 supercoiled DNA under basic conditions (pH 8.5) with comparable potencies (at 1000 and 100 μM concentrations). [These results may arise from the lack of an extended aromatic ring skeleton in these compounds as compared to dynemicin A, which was proposed to intercalate into DNA prior to drug activation, see: Sugiura et al., Proc. Natl. Acad. Sci. USA, 87:3831 (1990)].

The data in Table 1, below, show that the enantiomer utilized can result in dramatically differing

cytotoxicites. Thus, against some cancer cell lines, there was no difference in cytotoxicity among the racemate and the two enantiomers, e.g. SK-Mel-28 cells, whereas with other cells such as Molt-4 T-cell leukemia cells, the (+)-enantiomer was about one million times more potent than was the (-)-enantiomer.

Table 1

Cytotoxicites of Enediynes (±)-45, (+)-45 and (-)-45

		IC_{50}(M)		
Cell Type	Cell line	(±)-45	(+)-45	(-)-45
Melanoma	SK-Mel-28	$6.3X10^{-6}$	$6.3X10^{-6}$	$6.3X10^{-6}$
Pancreatic carcinoma	Capan-1	$1.6X10^{-6}$	$3.9X10^{-7}$	$1.6X10^{-6}$
Breast carcinoma	MCF-7/ADR*	$1.6X10^{-6}$	$7.8X10^{-7}$	$1.6X10^{-6}$
Promyeocytic leukemia	HL-60	$3.9X10^{-6}$	$>9.8X10^{-8}$	$7.8X10^{-7}$
T-cell leukemia	Molt-4	$1.0X10^{-11}$	$1.0X10^{-13}$	$1.0X10^{-7}$

*Adriamycin resistant cell line.

Cytotoxicity studies using Compounds **21** and **427-429** were conducted as discussed before. The reduced potency in cell killing by Compounds **427-429** again reflected that the C2 methyl group(s) attached next to the sulfone residue hindered the activation of these agents via a β-elimination process. As shown in Table 2, below, significant differences were obtained with the most sensitive Molt-4 leukemia cell line (10^3 to 10^6-fold less active by attaching a methyl group at the C2 position; 10^8-fold less active by attaching two methyl groups at the same position). The differential in cytotoxicities for Compounds **427** and **428** was intriguing

in that it suggests the invovlement of chiral molecules in the activation of these agents in living cells. The reduced cytotoxicity of Compound 427 against normal cell lines while maintaining considerable activity against cancer cell lines is noteworthy in the context of selective thereapeutic agents.

Table 2

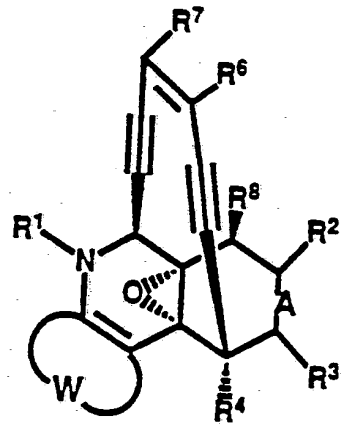
Cytotoxicity (IC_{50}) of Designed Enediynes Containing β-Sulfone Triggers

	Compound			
Cell Line	21	427	428	429
NHDF	$6.3X10^{-6}$	$<10^{-4}$	$<10^{-4}$	Non-Toxic
CHO	$6.3X10^{-6}$	$<10^{-4}$	$<10^{-4}$	Non-Toxic
Molt-4	10^{-12}	10^{-9}	10^{-6}	10^{-4}
HL-60	$9.8X10^{-8}$	$7.8X10^{-7}$	$1.6X10^{-6}$	$2.5X10^{-5}$
Capan-1	$7.8X10^{-8}$	$3.1X10^{-6}$	$6.3X10^{-6}$	$5.0X10^{-5}$
P-388	$9.8X10^{-8}$	$1.6X10^{-6}$	$1.6X10^{-6}$	$1.3X10^{-5}$
Ovcar-3	$7.8X10^{-7}$	$3.1X10^{-6}$	$1.3X10^{-5}$	$5.0X10^{-5}$
HT-29	$3.9X10^{-7}$	$7.8X10^{-7}$	$1.3X10^{-5}$	$2.5X10^{-5}$
UCLA-P3	$7.8X10^{-7}$	$3.1X10^{-6}$	$1.3X10^{-5}$	$5.0X10^{-5}$
MCF-7	$3.1X10^{-6}$	$2.5X10^{-5}$	$<10^{-4}$	$2.5X10^{-5}$
H-322	$3.1X10^{-6}$	$1.3X10^{-5}$	Non-Toxic	$5.0X10^{-5}$
SK-Mel-28	$6.3X10^{-5}$	$5.0X10^{-5}$	$<10^{-4}$	$<10^{-4}$

Although the present invention has now been described in terms of certain preferred embodiments, and exemplified with respect thereto, one skilled in the art will readily appreciate that various modifications, changes, omissions and substitutions may be made without departing from the spirit thereof.

CLAIMS:

1. An enantiomer of a fused ring compound corresponding to the structural formula



wherein A is a double or single bond;

R^1 is selected from the group consisting of H, C_1-C_6 alkyl, phenoxycarbonyl, benzyloxycarbonyl, C_1-C_6 alkoxycarbonyl, substituted C_1-C_6 alkoxycarbonyl, o-nitrobenzyloxycarbonyl, and 1-9-fluorenylmethyloxycarbonyl;

R^2 is selected from the group consisting of H, carboxyl, hydroxylmethyl and carbonyloxy-C_1-C_6 alkyl;

R^3 is selected from the group consisting of H and C_1-C_6 alkoxy;

R^4 is selected from the group consisting of H, hydroxyl, C_1-C_6 alkoxy, oxyacetic acid, oxyacetic C_1-C_6 hydrocarbyl or benzyl ester, oxyacetic amide, oxyethanol, oxyimidazilthiocarbonyl and C_1-C_6 acyloxy;

R^6 and R^7 are each H or together form with the intervening vinylene group form a one, two or three fused aromatic six-membered ring system;

W together with the bonded vinylene group forms an aromatic hydrocarbyl ring system containing 1,

2 or 3 six-membered rings such that said fused ring compound contains 3, 4 or 5 fused rings, all but two of which are aromatic, and in which W is joined [a, b] to the nitrogen-containing ring of the structure shown; and

R^8 is hydrogen or methyl, with the proviso that R^8 is hydrogen when W together with the intervening vinylene group is 9,10-dioxoanthra.

2. The enantiomer according to claim 1 wherein R^6 and R^7 are H, or together with the intervening vinylene group form a benzo or naphtho ring system.

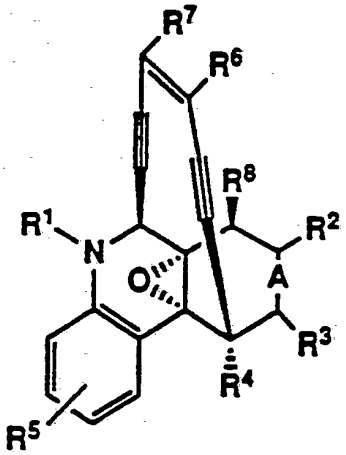
3. The enantiomer according to claim 1 wherein said aromatic hydrocarbyl ring system W is selected from the group consisting of a benzo ring, a naphtho ring and a 9,10-dioxoanthra ring.

4. The enantiomer according to claim 3 wherein the formed aromatic hydrocarbyl ring system is a benzo ring.

5. The enantiomer according to claim 4 wherein the benzo ring is substituted at one or two of the remaining positions by a radical selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, o-nitrobenzyloxy, benzyloxy, C_1-C_6-acyloxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, oxyethanol, oxyacetic acid, oxyacetic acid amide, oxyethanol tertiary amino or quaternary ammonium C_2-C_6 alkyl carboxylate, 3-hydroxyprop-1-ynyl, an oxyacetic C_1-C_6 hydrocarbyl or benzyloxy ester and halo.

6. The enantiomer according to claim 1 wherein A is a single bond.

7. An enantiomer of a fused ring compound corresponding in structure to the formula



wherein A is a double or single bond;

R^1 is selected from the group consisting of H, C_1-C_6 alkyl, phenoxycarbonyl, benzyloxycarbonyl, C_1-C_6 alkoxycarbonyl, substituted ethoxycarbonyl, o-nitrobenzyloxycarbonyl, and 9-fluorenylmethyloxycarbonyl;

R^2 is selected from the group consisting of H, carboxyl, hydroxylmethyl and carbonyloxy-C_1-C_6 alkyl;

R^3 is selected from the group consisting of H and C_1-C_6 alkoxy;

R^4 is selected from the group consisting of H, hydroxyl, oxyacetic acid, oxyacetic C_1-C_6 hydrocarbyl or benzyl ester, oxyacetic amide, oxyethanol, oxyimidazilthiocarbonyl and C_1-C_6 acyloxy;

R^5 is selected from the group consisting of H, hydroxyl, C_1-C_6 alkoxy, o-nitrobenzyloxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, oxyethanol, oxyacetic acid, oxyacetic acid, oxyacetic acid amide, oxyethanol tertiary amino or quaternary ammonium C_2-C_6 alkyl carboxylate, 3-hydroxyprop-1-ynyl, benzyloxy, and C_1-C_6 acyloxy;

R^6 and R^7 are each H or together form with the intervening vinylene group form a one, two or three fused aromatic six-membered ring system; and

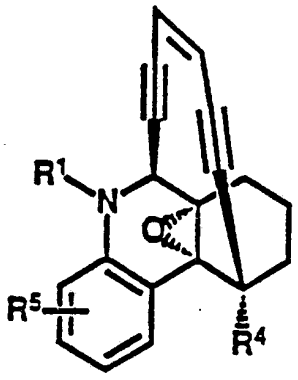
R^8 is methyl or hydrogen.

8. The enantiomer according to claim 7 wherein R^2, R^3, R^5, R^6 and R^7 are H.

9. The enantiomer according to claim 8 wherein R^1 is phenoxycarbonyl, 2-(phenylsulfonyl)ethoxycarbonyl, 2-(phenylsulfonyl)-2(C_1-C_6 alkyl)ethoxycarbonyl or 2-(naphthylsulfonyl)ethoxycarbonyl.

10. The enantiomer according to claim 9 wherein R^4 is selected from the group consisting of H, hydroxyl, C_1-C_6 alkoxy, oxyacetic acid, imidazylthiocarbonyloxy, oxyacetic amide and oxyacetic C_1-C_6 hydrocarbyl or benzyl esters.

11. An enantiomer of a fused-ring compound corresponding to the formula



wherein R^1 is selected from the group consisting of H, phenoxycarbonyl, benzyloxycarbonyl,

2-(phenylsulfonyl)ethoxycarbonyl, 2-(phenylsulfonyl)-2-(C_1-C_6 alkyl)ethoxycarbonyl, 2-(naphthylsulfonyl)ethoxycarbonyl and o-nitrobenzyloxycarbonyl;

R^4 is selected from the group consisting of H, hydroxyl, oxyacetic acid, oxyacetic amide, oxyacetic C_1-C_6 hydrocarbyl or benzyl ester and oxyethanol; and

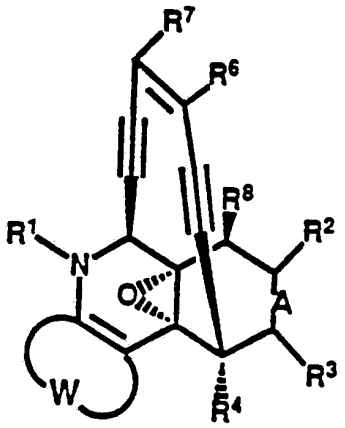
R^5 is situated meta or para to the nitrogen atom bonded to R^1 and is selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, benzyloxy, C_1-C_6 acyloxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, oxyethanol, oxyacetic acid, oxyacetic acid amide, oxyethanol tertiary amino or quaternary ammonium C_2-C_6 alkyl carboxylate, 3-hydroxyprop-1-ynyl, an oxyacetic C_1-C_6 hydrocarbyl or benzyloxy ester and o-nitrobenzyloxy.

12. The fused ring compound according to claim 11 wherein R^1 is 2-(phenylsulfonyl)ethoxycarbonyl, 2-(phenylsulfonyl)-2-(C_1-C_6 alkyl)ethoxycarbonyl, phenoxycarbonyl or 2-(naphthylsulfonyl)ethoxycarbonyl.

13. The fused ring compound according to claim 12 wherein R^4 is H.

14. The fused ring compound according to claim 24 wherein R^5 is hydroxyl, oxyethanol or C_1-C_6 acyloxy.

15. A pharmaceutical composition that comprises a DNA-cleaving or cytotoxic amount of an enantiomer of a fused ring compound having the structural formula shown below dissolved or dispersed in a physiologically tolerable diluent



wherein A is a double or single bond;

R^1 is selected from the group consisting of H, C_1-C_6 alkyl, phenoxycarbonyl, benzyloxycarbonyl, C_1-C_6 alkoxycarbonyl, substituted C_1-C_6 alkoxycarbonyl, o-nitrobenzyloxycarbonyl, and 9-fluorenylmethyloxycarbonyl;

R^2 is selected from the group consisting of H, carboxyl, hydroxylmethyl and carbonyloxy-C_1-C_6 alkyl;

R^3 is selected from the group consisting of H and C_1-C_6 alkoxy;

R^4 is selected from the group consisting of H, hydroxyl, C_1-C_6 alkoxy, oxyacetic acid, oxyacetic C_1-C_6 hydrocarbyl or benzyl ester, oxyacetic amide, oxyethanol, oxyimidazilthiocarbonyl and C_1-C_6 acyloxy;

R^6 and R^7 are each H or together form with the intervening vinylene group form a one, two or three fused aromatic six-membered ring system;

W together with the bonded vinylene group forms an aromatic hydrocarbyl ring system containing 1,

2 or 3 six-membered rings such that said fused ring compound contains 3, 4 or 5 fused rings, all but two of which are aromatic, and in which W is joined [a, b] to the nitrogen-containing ring of the structure shown; and

R^8 is hydrogen or methyl, with the proviso that R^8 is hydrogen when W together with the intervening vinylene group is 9,10-dioxoanthra.

16. The composition according to claim 15 wherein R^6 and R^7 are H, or together with the intervening group form a benzo or naphtho ring system, and R^2, R^3 and R^8 are H.

17. The composition according to claim 16 wherein said aromatic hydrocarbyl ring system W is selected from the group consisting of a benzo ring, a naphtho ring and a 9,10-dioxoanthra ring.

18. The composition according to claim 16 wherein the formed aromatic hydrocarbyl ring system is a benzo ring substituted at one or two of the remaining positions by a radical selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, benzyloxy, C_1-C_6-acyloxy, carboxyl, C_1-C_6 hydrocarbyl or benzyl carboxylate, oxyethanol, oxyacetic acid, oxyacetic acid amide, oxyethanol tertiary amino or quaternary ammonium C_2-C_6 alkyl carboxylate, 3-hydroxyprop-1-ynyl, oxyacetic C_1-C_6 hydrocarbyl or benzyloxy ester and halo.

19. The composition according to claim 16 wherein A is a single bond.

20. The composition according to claim 17 wherein R^1 is phenoxycarbonyl, 2-(phenylsulfonyl)ethoxycarbonyl, 2-(phenylsulfonyl)-2-

(C_1-C_6 alkyl)ethoxycarbonyl, or 2-(naphthylsulfonyl)ethoxycarbonyl.

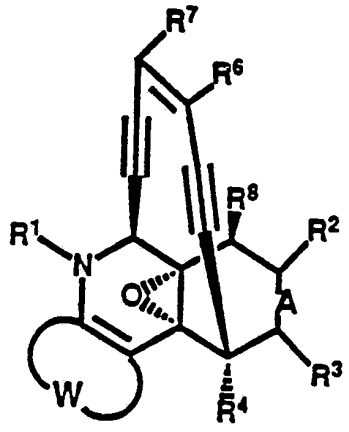
21. The composition according to claim 20 wherein W is benzo.

22. The composition according to claim 17 wherein R^6 and R^7 are both H.

23. The composition according to claim 17 wherein said benzo group, W, is substituted meta or para to the nitrogen atom bonded to R^1 with a moiety selected from the group consisting of hydroxyl, C_1-C_6 alkoxy, benzyloxy, C_1-C_6 acyloxy, oxyethanol, oxyacetic acid, oxyacetic C_1-C_6 hydrocarbyl ester, oxyacetic acid amide, oxyethanol tertiary amino or quaternary ammonium C_2-C_6 alkyl carboxylate and 3-hydroxyprop-1-ynyl.

24. A chimeric compound comprised of an aglycone portion bonded to (i) an oligosaccharide portion or (ii) a monoclonal antibody or antibody binding site portion thereof that immunoreacts with target tumor cells,

wherein said aglycone portion is an enantiomer of a fused ring compound corresponding to the structural formula



wherein A is a double or single bond;

R^1 is selected from the group consisting of H, C_1-C_6 alkyl, phenoxycarbonyl, benzoxycarbonyl, C_1-C_6 alkoxy carbonyl, substituted C_1-C_6 alkoxycarbonyl, o-nitrobenzyloxycarbonyl, and 9-fluorenylmethyloxycarbonyl;

R^2 is selected from the group consisting of H, carboxyl, hydroxylmethyl and carbonyloxy-C_1-C_6 alkyl;

R^3 is selected from the group consisting of H and C_1-C_6 alkoxy;

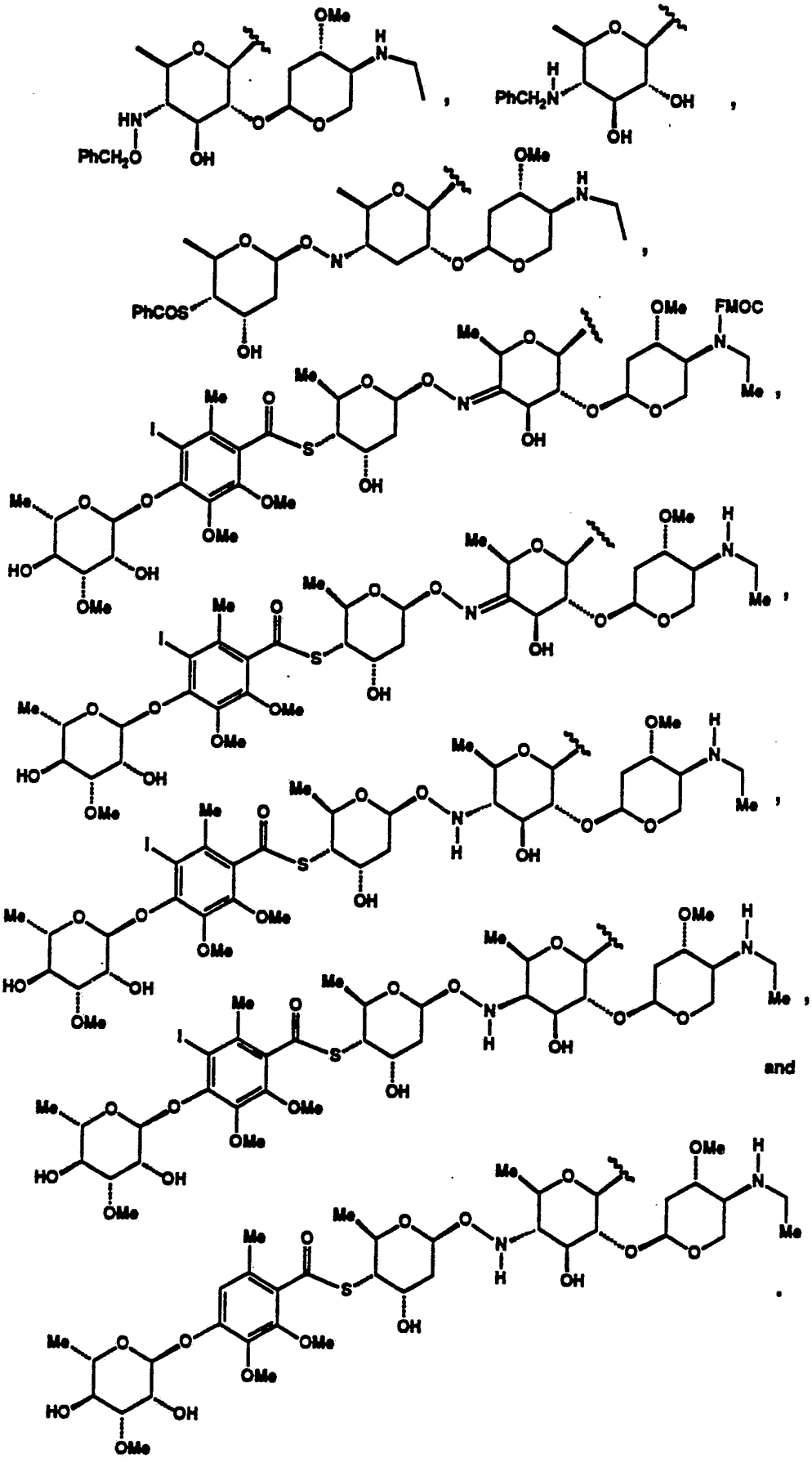
R^4 is selected from the group consisting of H, hydroxyl, C_1-C_6 alkoxy, oxyacetic acid, oxyacetic C_1-C_6 hydrocarbyl or benzyl ester, oxyacetic amide, oxyethanol, oxyimidazilthiocarbonyl and C_1-C_6 acyloxy;

R^6 and R^7 are each H or together form with the intervening vinylene group form a one, two or three fused aromatic six-membered ring system;

W together with the bonded vinylene group forms an aromatic hydrocarbyl ring system containing 1, 2 or 3 six-membered rings such that said fused ring compound contains 3, 4 or 5 fused rings, all but two of which are aromatic, and in which W is joined [a, b] to the nitrogen-containing ring of the structure shown; and

R^8 is hydrogen or methyl, with the proviso that R^8 is hydrogen when W together with the intervening vinylene group is 9,10-dioxoanthra;

said oligosaccharide portion comprising a sugar moiety selected from the group consisting of ribosyl, deoxyribosyl, fucosyl, glucosyl, galactosyl, N-acetylglucosaminyl, N-acetylgalactasaminyl, a saccharide whose structure is shown below, wherein a wavy line adjacent a bond indicates the position of linkage



and

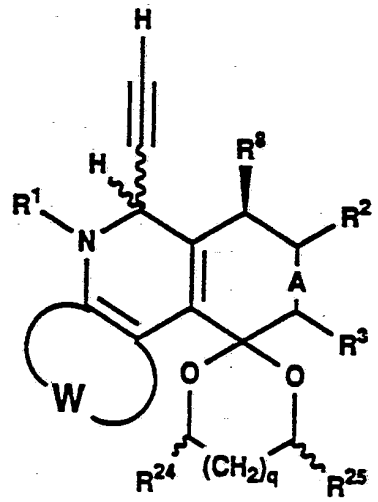
said monoclonal antibody or combining site portion thereof being bonded to said fused ring compound aglycone portion through an R^4 oxyacetic acid amide or ester bond, or an oxyacetic acid amide or ester bond from W, and said oligosaccharide portion being glycosidically bonded to the aglycone portion through the hydroxyl of an R^4 oxyethanol group or the hydroxyl of an oxyethanol-substituted W.

25. The chimeric compound according to claim 24 wherein A is a single bond, R^2, R^3, R^6, R^7 and R^8 are hydrogen, and W is benzo.

26. The chimeric compound according to claim 25 wherein said aglycone portion is bonded to an oligosaccharide portion.

27. The chimeric compound according to claim 25 wherein R^1 is phenoxycarbonyl, 2-(phenylsulfonyl)ethoxycarbonyl, 2-(phenylsulfonyl)-2-(C_1-C_6)ethoxycarbonyl or 2-(naphthylsulfonyl)ethoxycarbonyl.

28. A compound of the formula



wherein A is a double or single bond;

R^1 is selected from the group consisting of H, C_1-C_6 alkyl, phenoxycarbonyl, benzoxycarbonyl, C_1-C_6 alkoxy carbonyl, substituted C_1-C_6 alkoxycarbonyl, o-nitrobenzyloxycarbonyl, and 9-fluorenylmethyloxycarbonyl;

R^2 is selected from the group consisting of H, carboxyl, hydroxylmethyl and carbonyloxy-C_1-C_6 alkyl;

R^3 is selected from the group consisting of H and C_1-C_6 alkoxy;

R^8 is hydrogen or methyl;

W together with the bonded vinylene group forms an aromatic hydrocarbyl ring system containing 1, 2 or 3 six-membered rings such that said fused ring compound contains 3, 4 or 5 fused rings, all but two of which are aromatic, and in which W is joined [a, b] to the nitrogen-containing ring of the structure shown;

R^{24} and R^{25} are independently C_1-C_3 alkyl or phenyl; and

q is zero or 1.

29. The compound according to claim 28 wherein A is a single bond and R^2, R^3 amd R^8 are H.

30. The compound according to claim 29 wherein W together with the bonded vinylene group forms a benzo ring.

31. The compound according to claim 30 wherein R^1 is phenoxycarbonyl.

FIG. 1A

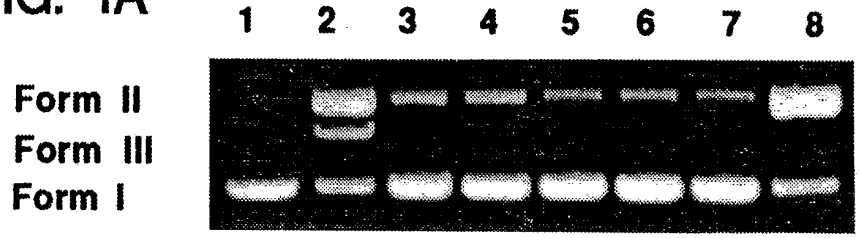
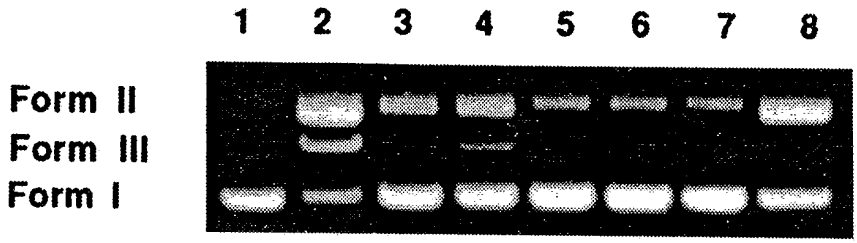

1 2 3 4 5 6 7 8
Form II
Form III
Form I

FIG. 1B


1 2 3 4 5 6 7 8
Form II
Form III
Form I

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04708

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 31/44, 31/70; CO7H 15/04, CO7D 491/08, 491/113.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 546/14, 18, 34, 39, 43; 536/4.1, 16.8, 17.3, 17.6, 17.9; 514/25, 279, 281

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS on line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	U.S.A. 4,837,206 (Golik) 6 June 1989. See entire document.	24-27
A	J. Am. Chem. Soc. 1990, 112, 3715-3716. Konishi et al., "Crystal and Molecular Structure of Dynemicin A: A novel 1, 5-Diyn-3-ene Antitumor Antibiotic. See structures 1-5.	1-23
A	J. Am. Chem. Soc. Vol. 113, no. 8, 1991, pp. 3106-3114. Nicolaou et al. "Synthesis and Chemistry of Dynemicin A Models".	1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 04 AUGUST 1993	Date of mailing of the international search report 18 AUG 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer CECILIA TSANG Telephone No. (703) 308-1235

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04708

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. Am. Chem. Soc. 1990 112, pp. 8193-8195. K.C. Nicolaou et al. "Total Synthesis of the Oligosaccharide Fragment of Calicheamicin Vi"	24-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04708

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

546/14, 18, 34, 39, 43; 536/4.1, 16.8, 17.3, 17.6, 17.9; 514/25, 279, 281

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-23, drawn to compounds and compositions, classified in 546/14, 34, 39, 43 and 514/279, 281.

II. Claims 24-27, drawn to chimeric compound comprised of an aglycone portion (group I) bond to (i) an oligosaccharide portion, or (ii) a monoclonal antibody or antibody binding site portion thereof that immuno reacts with target tumor cells, classified in 536/4.1, 16.8, 17.3, 17.6, 17.9; 514/25.

III. Claims 28-31, drawn to spiro intermediates, classified in 546/18.

I, II and III are directed to different inventions which are not so linked as to form a single general inventive concept. I can be prepared via other intermediates as discussed in the instant specification. I can be used independently from II or vice versa. Art which may anticipate or render obvious I would not necessarily do the same for II or III. Each group can support a patent.

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II. Claims 24-27, drawn to chimeric compound comprised of an aglycone portion (group I) bond to (i) an oligosaccharide portion, or (iii) a monoclonal antibody or antibody binding site portion thereof that immunoreacts with target tumor cells, classified in 536/4.1, 16.8, 17.3, 17.5, 17.6, 17.9.

III. Claims 28-31, drawn to spiro intermediates, classified in 546/18.

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