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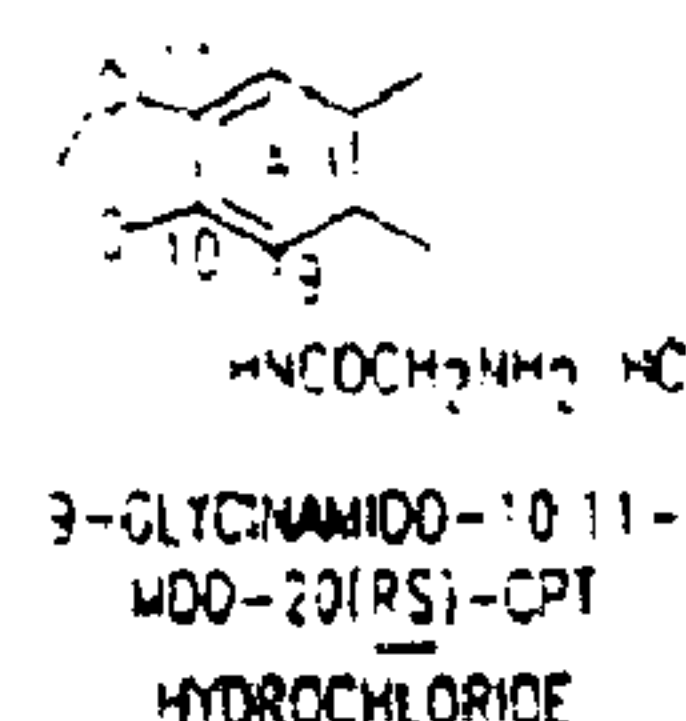
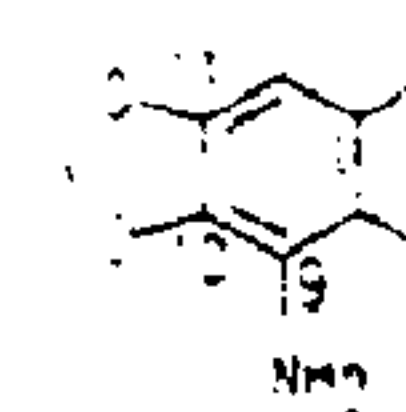
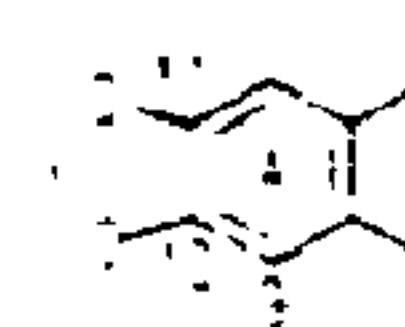
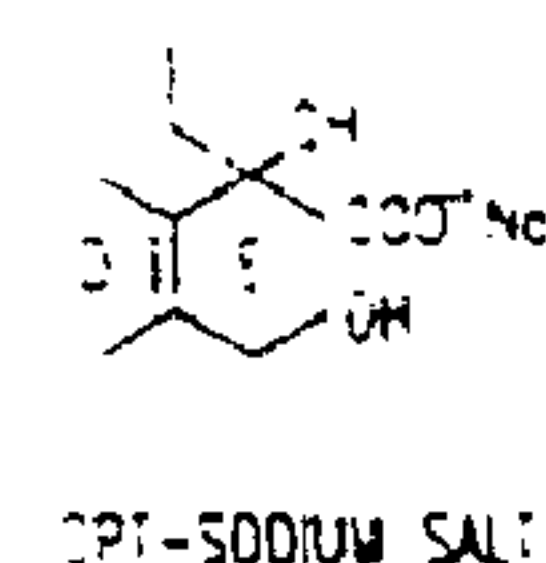
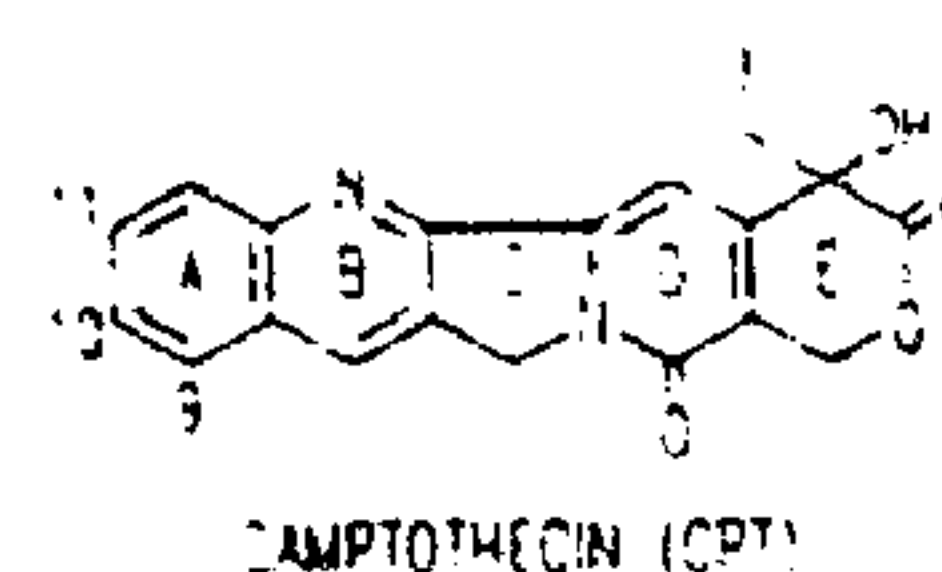
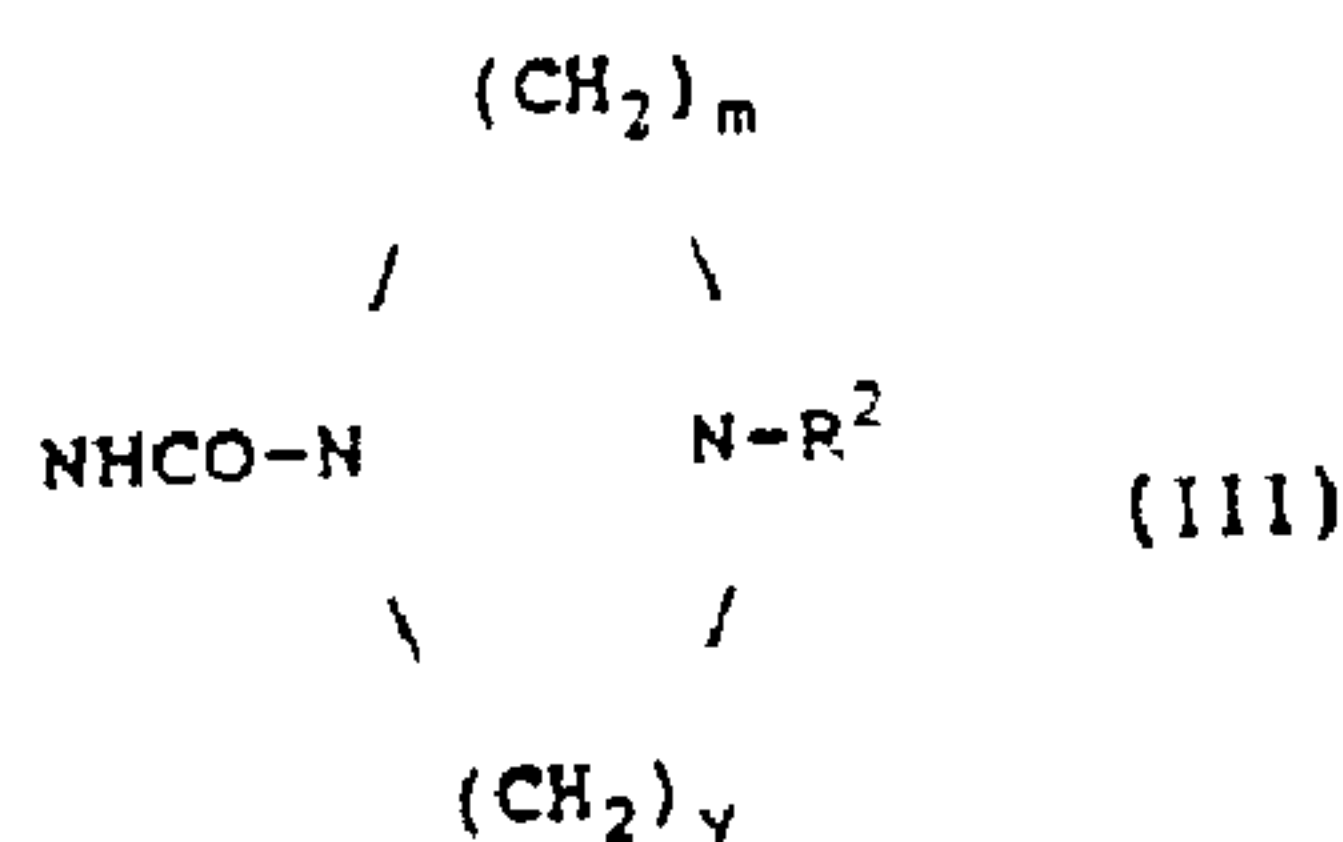
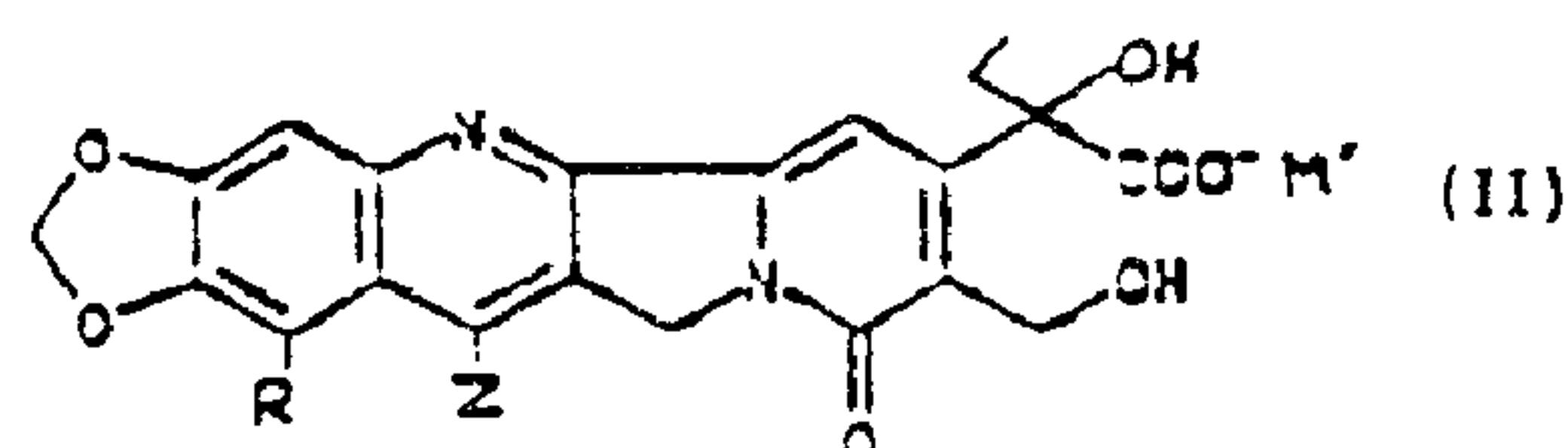
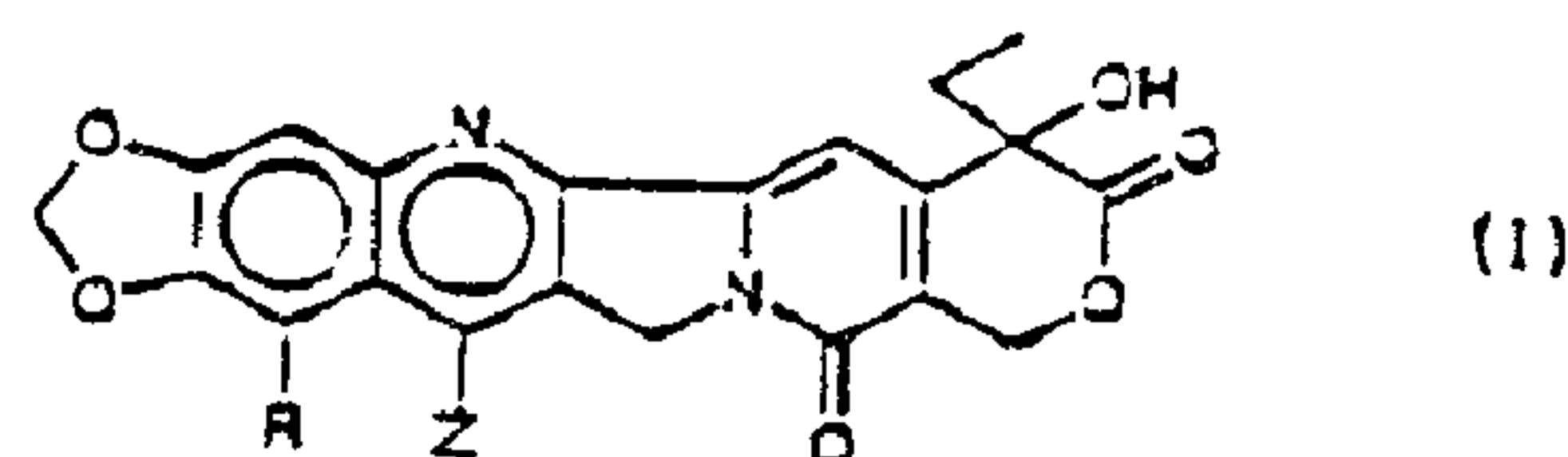
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(54) Titre : ANALOGUES DE LA 10,11-METHYLENEDIOXY-20(RS)-CAMPTOTHECINE ET DE LA 10,11-METHYLENEDIOXY-20(S)-CAMPTOTHECINE

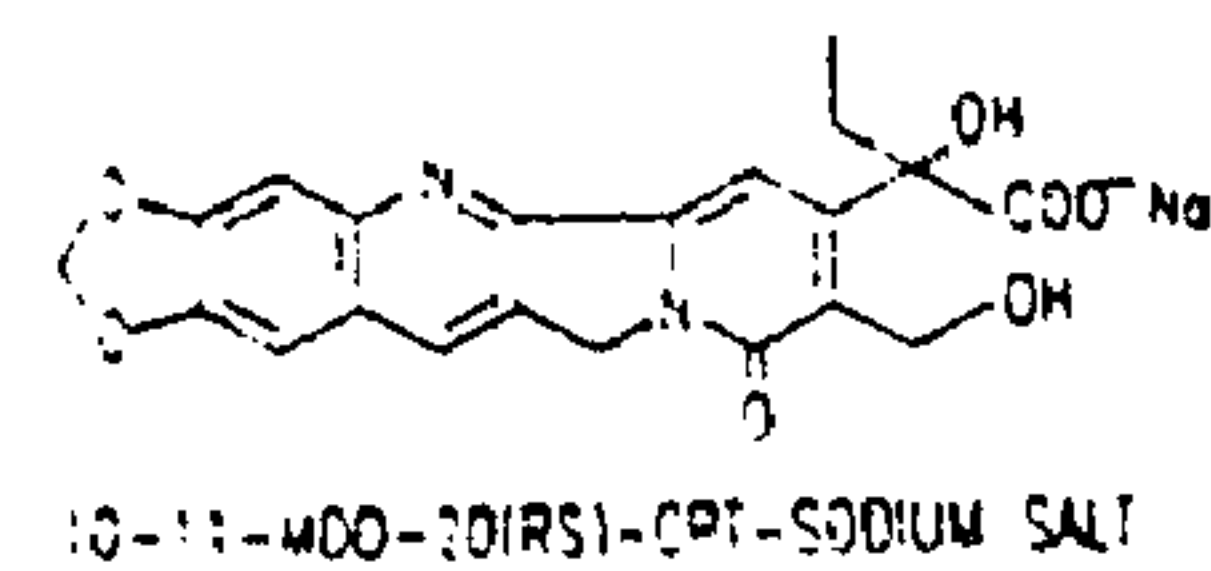
(54) Title: 10,11-METHYLENEDIOXY-20(RS)-CAMPTOTHECIN AND 10,11-METHYLENEDIOXY-20(S)-CAMPTOTHECIN ANALOGS



10,11-MDO-20(RS)-CPT

9-AMINO-10,11-MDO-20(RS)-CPT

9-GLUTYNAMIDO-10,11-MDO-20(RS)-CPT HYDROCHLORIDE



10,11-MDO-20(RS)-CPT-SODIUM SALT

(57) **Abrégé/Abstract:**

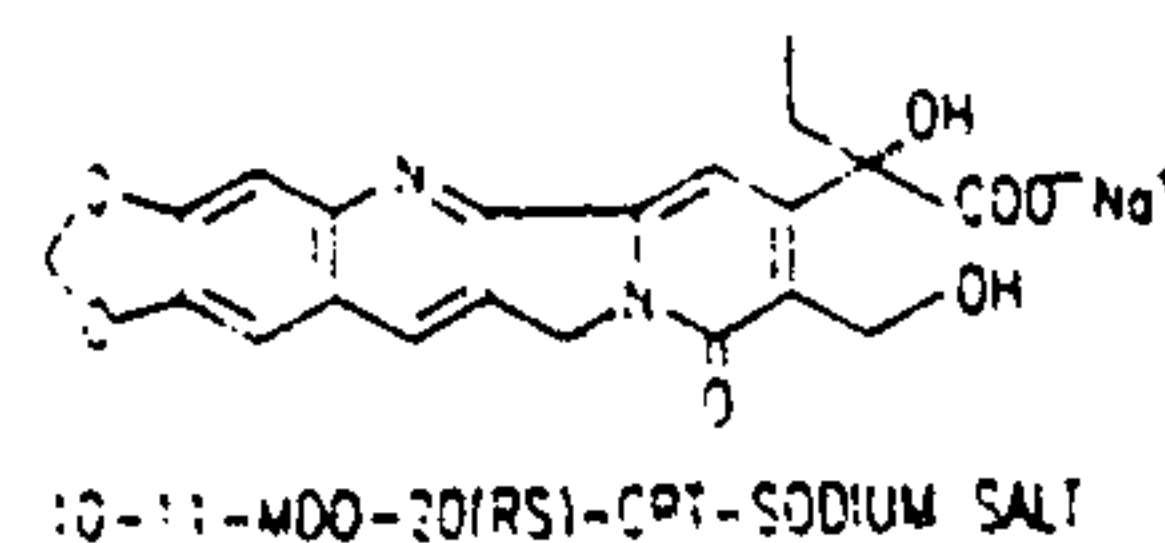
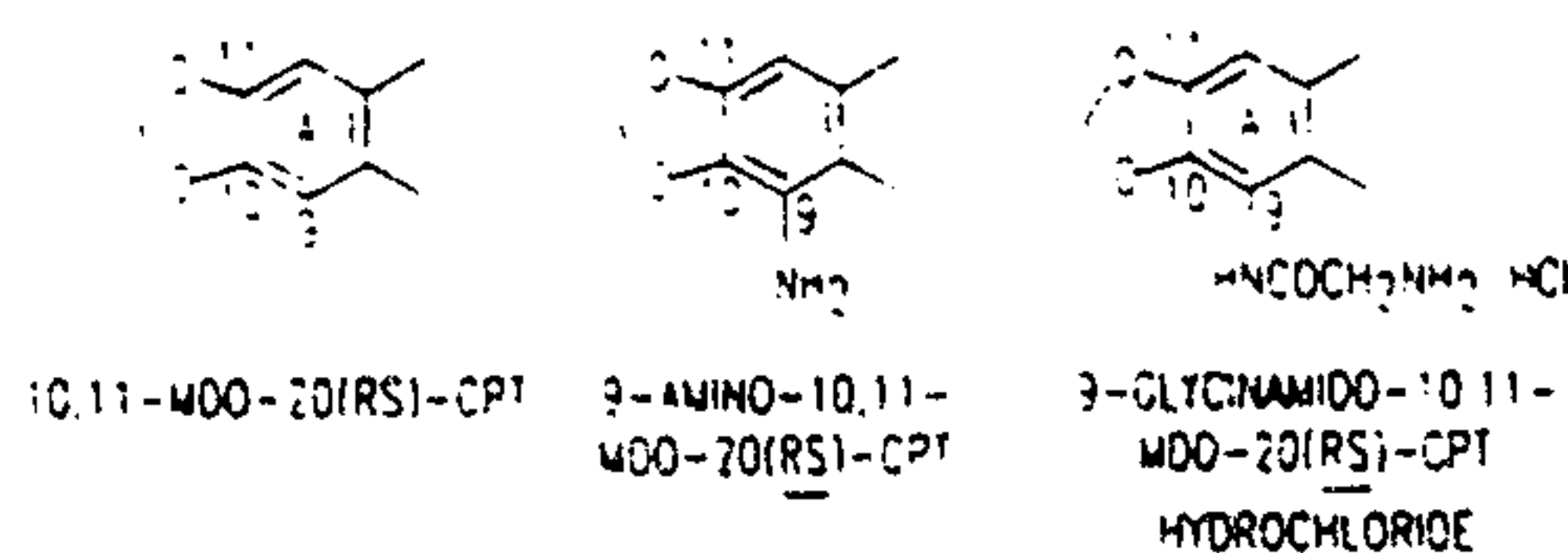
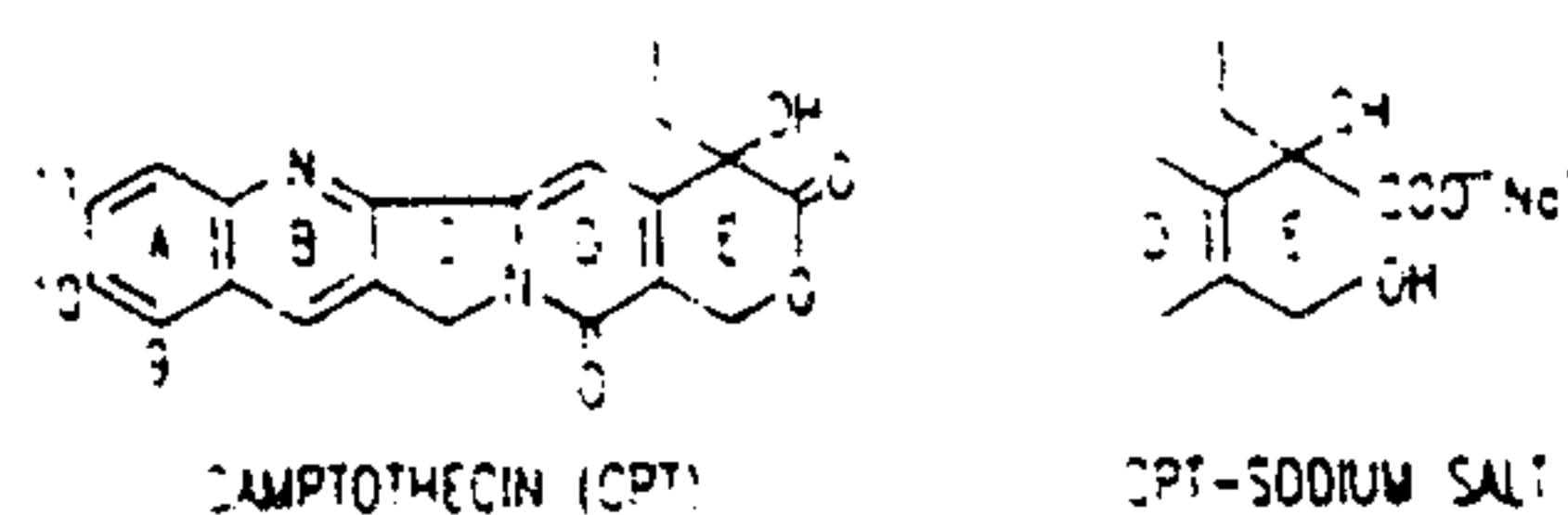
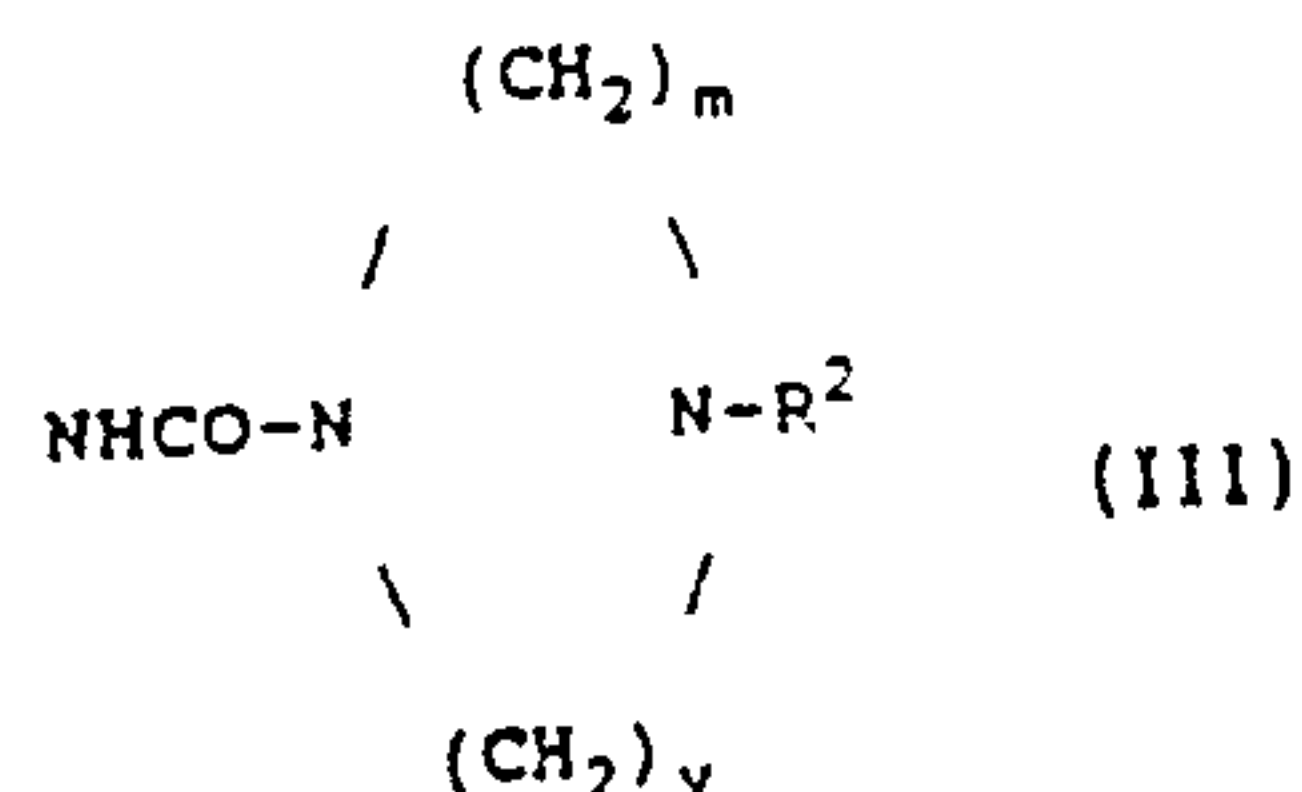
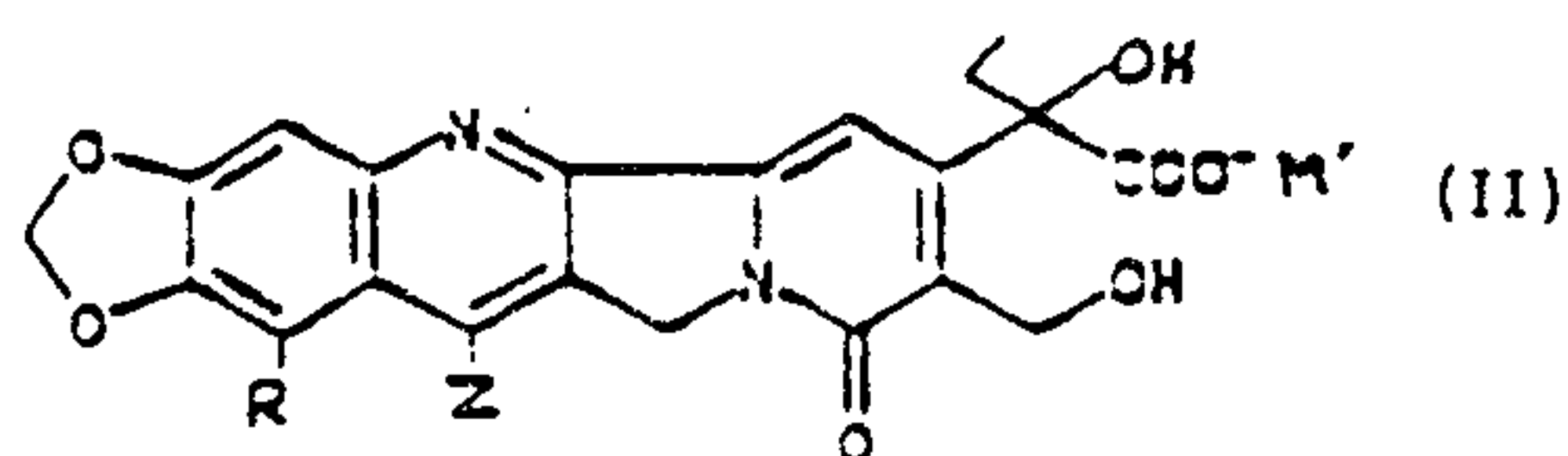
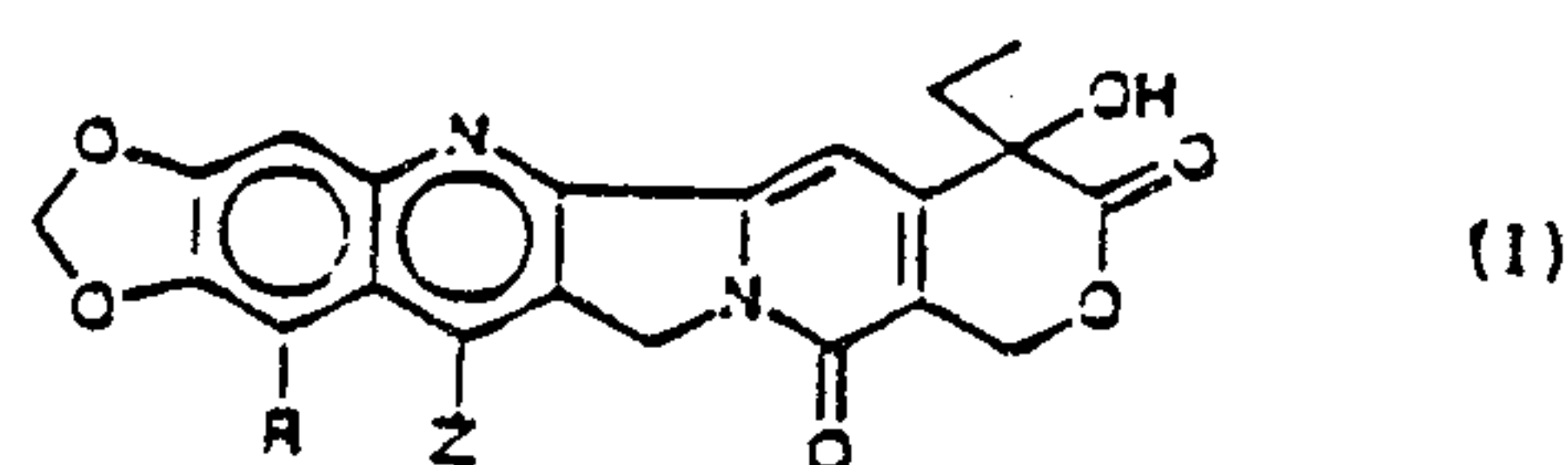
A camptothecin analog having structures (I) or (II), where Z is H or C₁₋₈ alkyl and R is NO₂, NH₂, N₃, hydrogen, halogen, COOH, OH, O-C₁₋₃ alkyl, SH, S-C₁₋₃ alkyl, CN, CH₂NH₂, NH-C₁₋₃ alkyl, CH₂-NH-C₁₋₃ alkyl, N(C₁₋₃ alkyl)₂, CH₂N(C₁₋₃ alkyl)₂, O-, NH- or S-CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂CH₂N(C₁₋₃ alkyl)₂, O-, NH- or S-CH₂CH₂CH₂N(C₁₋₃ alkyl)₂, CHO, C₁₋₃ alkyl or NHCOCHR¹NR²R³, where R¹ is the side-chain of an α-amino acid and R² and R³, independently are hydrogen or a lower alkyl group or R³ is a peptide unit containing 1-3 amino acid units bonded to the nitrogen through a peptide bond; NHCO-C₂₋₈-alkylene-X or NHCO-C₂₋₈-alkenylene-X, where X is COOH; CONR²-(CH₂)_n-NR²-R³, where n = 1-10 and R² and R³ are as defined above; NHCO-B-(CH₂)_n-NR²R³, where B = oxygen or NH, or structure (III), where m + y = 3-6, and salts thereof.



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(54) Title: 10,11-METHYLENEDIOXY-20(RS)-CAMPTOTHECIN AND 10,11-METHYLENEDIOXY-20(S)-CAMPTOTHECIN ANALOGS



(57) Abstract

A camptothecin analog having structures (I) or (II), where Z is H or C₁₋₈ alkyl and R is NO₂, NH₂, N₃, hydrogen, halogen, COOH, OH, O-C₁₋₃ alkyl, SH, S-C₁₋₃ alkyl, CN, CH₂NH₂, NH-C₁₋₃ alkyl, CH₂-NH-C₁₋₃ alkyl, N(C₁₋₃ alkyl)₂, CH₂N(C₁₋₃ alkyl)₂, O-, NH- or S-CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂N(C₁₋₃ alkyl)₂, O-, NH- or S-CH₂CH₂CH₂N(C₁₋₃ alkyl)₂, CHO, C₁₋₃ alkyl or NHCOCHR¹NR²R³, where R¹ is the side-chain of an α-amino acid and R² and R³, independently are hydrogen or a lower alkyl group or R³ is a peptide unit containing 1-3 amino acid units bonded to the nitrogen through a peptide bond; NHCO-C₂₋₈-alkylene-X or NHCO-C₂₋₈-alkenylene-X, where X is COOH; CONR²-(CH₂)_n-NR²R³, where n = 1-10 and R² and R³ are as defined above; NHCO-B-(CH₂)_n-NR²R³, where B = oxygen or NH, or structure (III), where m + y = 3-6, and salts thereof.

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10,11-Methylenedioxy-20(RS)-Camptothecin And
10,11-Methylenedioxy-20(S)-Camptothecin Analogs

Technical Field

5 The present invention relates to camptothecin analogs,
which are useful as antitumor agents. More specifically,
the invention is directed to water-insoluble and
water-soluble derivatives of
10,11-methylenedioxy-20(RS)-camptothecin and
10,11-methylenedioxy-20(S)-camptothecin. These compounds
10 are collectively referred to as 10,11-MDOCPT below.

Background Art

Camptothecin is a pentacyclic alkaloid initially
isolated from the wood and bark of Camptotheca acuminata by
Wall et al (M.E. Wall, M.C. Wani, C.E. Cook, K.H. Palmer,
15 A.T. McPhail, and G.A. Sim, J. Am. Chem. Soc., 94:388
(1966)).

Camptothecin is highly biologically active and
displays strong inhibitory activity toward the biosynthesis
of nucleic acids. Additionally, camptothecin exhibits
20 potent antitumor activity against experimentally
transplanted carcinoma such as leukemia L-1210 in mice or
Walker 256 tumor in rats.

Several methods for the synthesis of camptothecin and camptothecin analogs are known. These synthetic methods include (i) methods in which naturally occurring camptothecin is synthetically modified to produce a number of analogs and (ii) totally synthetic methods.

U.S. Patents 4,604,463; 4,545,880 and 4,473,692 as well as European Patent Application No. 74,256 published on March 16, 1983 are examples of the former type of synthetic strategy. Additional examples of this strategy can be found in Japanese Patents 84/46,284; 84/51,287 and 82/116,015. These methods require naturally occurring camptothecin which is difficult to isolate and hence these methods are not suitable for the production of large quantities of camptothecin or analogs.

Examples of a variety of totally synthetic routes to camptothecin and camptothecin analogs can be found in the following references: Sci. Sin. (Engl. Ed), 21(1), 87-98 (1978); Fitoterapia, 45(3), 87-101 (1974) ; Yakugaku Zasshi, 92(6), 743-6 (1972) ; J. Org. Chem., 40(14), 2140-1 (1975); Hua Hsueh Hsueh Pao, 39(2), 171-8 (1981); J. Chem. Soc., Perkin Trans 1, (5) 1563-8 (1981) ; Heterocycles, 14(7), 951-3 (1980) ; J. Amer. Chem. Soc., 94(10), 3631-2 (1972); J. Chem. Soc. D, (7), 404 (1970) and U.S. Patent 4,031,098.

Synthetic studies directed to camptothecin analogs have also been conducted by the present inventors and are disclosed in J. Med. Chem., 23(5), 554-560 (1980); J. Med. Chem., 29(8), 1553-1555 (1986) and J. Med. Chem., 29(11), 2358-2363(1986) for example.

Water-solubility is an important criterion in developing potential antitumor compounds for pharmaceutical use. Most camptothecin analogs known in the art have

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relatively poor water-solubility. A need exists for additional camptothecin compounds showing high anti-tumor activity and for water-soluble camptothecin analogs and methods for preparing the-same.

5 Disclosure of the Invention

Accordingly, one object of the present invention is to provide camptothecin analogs containing the 10,11-methylenedioxy moiety.

10 A further object is to provide camptothecin analogs which exhibit high cytotoxic activity and which can be readily prepared.

These and other objects which will become apparent from the following specification have been achieved by the process of the present invention and the compounds produced
15 thereby.

More specifically, the invention is directed to compounds which are derivatives of 10,11-methylenedioxy-20(RS)-camptothecin (also called 10,11-MDO-20(RS)-CPT) and 10,11-methylenedioxy-20(S)-
20 camptothecin (also called 10,11-MDO-20(S)-CPT) which are highly active camptothecin analogs.

Brief Description of the Drawings

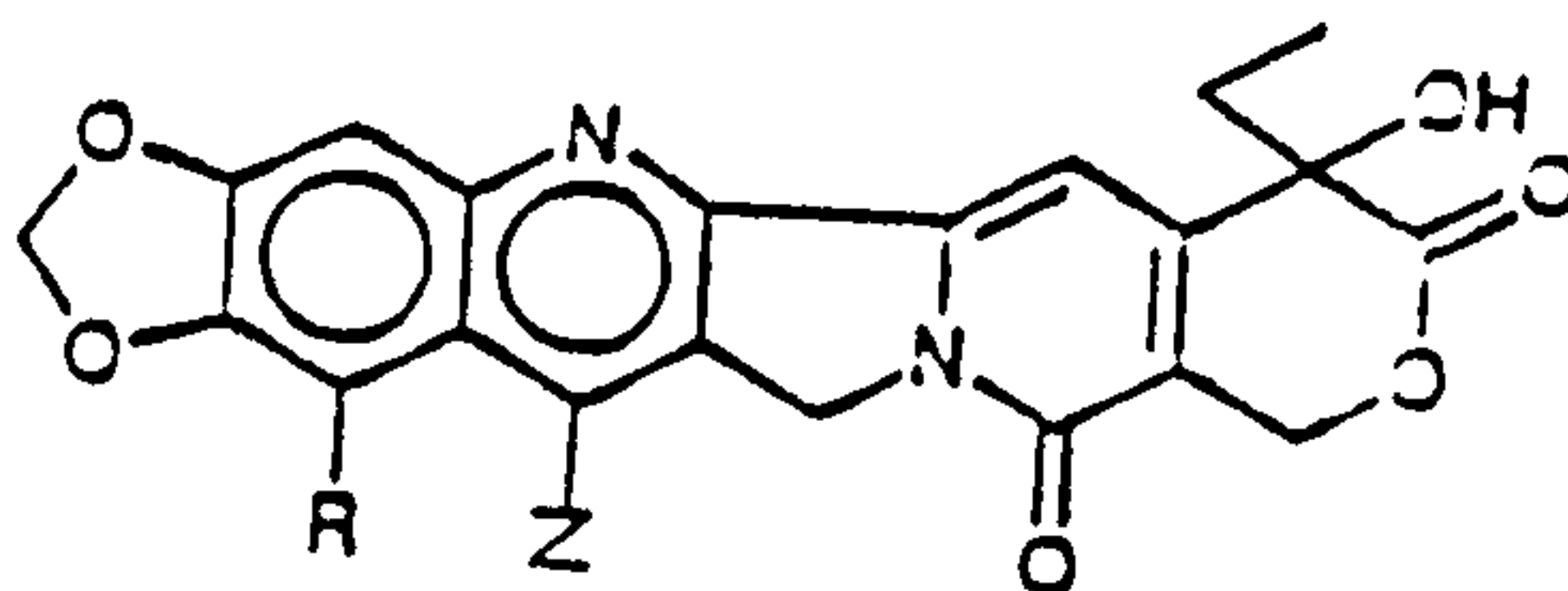
A more complete appreciation of the invention and many of the attendant advantages thereof will be obtained as the
25 same becomes better understood by reference of the following detailed description when considered in connection with the accompanying drawing, wherein:

Figure 1 shows the structure of CPT and derivatives thereof.

Best Mode for Carrying Out the Invention

10,11-MDO-20(S)-CPT is an extremely potent
 5 camptothecin analog and is one of the most potent
 inhibitors of the enzyme topoisomerase I known.
 10,11-MDO-20(S)-CPT is highly active in such in vitro
 cytotoxicity tests as the 9KB and 9PS tests and
 demonstrates ED₅₀ values equal to or more potent than
 10 camptothecin itself. 10,11-MDO-20(S)-CPT is also very
 potent in the L-1210 leukemia in vivo life prolongation
 assay. The synthesis of 10,11-MDO-20(RS)-CPT is known and
 described in Wani et al, J. Med. Chem., 29 (11), 2358-2363
 (1986) and in U.S. 4,894,456.

15 Novel analogs of camptothecin have been prepared, all
 of which contain the 10,11-methylenedioxy moiety. The
 structures of these compounds are shown below.



In the structure shown above, R is NO₂, NH₂, N₃,
 20 hydrogen, halogen (F, Cl, Br, I), COOH, OH, O-C₁₋₃ alkyl,
 SH, S-C₁₋₃ alkyl, CN, CH₂NH₂, NH-C₁₋₃ alkyl, CH₂-NH-C₁₋₃ alkyl,
 N(C₁₋₃ alkyl)₂, CH₂N(C₁₋₃ alkyl)₂, O-, NH- and
 S-CH₂CH₂N(CH₂CH₂OH)₂, CH₂CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- and
 S-CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- and S-CH₂CH₂CH₂N(CH₂CH₂CH₂OH)₂,
 25 O-, NH- and S-CH₂CH₂N(C₁₋₃ alkyl)₂, O-, NH- and
 S-CH₂CH₂CH₂N(C₁₋₃ alkyl)₂, CHO or C₁₋₃ alkyl. Preferred
 compounds are those in which R is halogen, nitro or amino.
 The compound in which R is a chlorine atom is particularly

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preferred.

Z in the structure shown above is H or C₁₋₈ alkyl with the proviso that R and Z are not both hydrogen.

5 Preferably, Z is H.

The structure shown above is understood to represent all isomers having the chemical structure indicated. The structure shown above, therefore, represents both 10,11-MDO-20(S)-CPT and 10,11-MDO-20(RS)-CPT compounds.

Compounds having the structure shown above are generally prepared by first synthesizing 10,11-MDO-20(S)-CPT or 10,11-MDO-20(RS)-CPT in which Z is hydrogen or C₁₋₈ alkyl. The synthesis of 10,11-MDO-20(RS)-CPT compounds is which Z is hydrogen or C₁₋₈ alkyl is possible by means of a Friedland condensation reaction between an appropriately substituted tricyclic compound representing rings C, D and E of the camptothecin structure with an ortho-amino benzaldehyde or ketone. Friedlander condensation with an ortho-amino benzaldehyde produces compounds in which Z is hydrogen. Condensation using corresponding ortho-amino ketones produces compounds in which Z is C₁₋₈ alkyl. Synthesis of the 10,11-MDO-20(RS)-CPT is fully described in U.S. Patent No. 4,894,456. The synthesis of 10,11-MDO-20(S)-CPT is described in U.S. Patent No. 5,053,512 which provides a complete description of the synthesis of the 10,11-MDO-20(S)-CPT starting compounds in which Z is hydrogen or C₁₋₈ alkyl.

The 9-substituted-10,11-MDO-20(RS)-CPT and 9-substituted-10,11-MDO-20(S)-CPT compounds of the present

invention can be synthesized from the 10,11-MDOCPT starting materials described above by preparing a diazonium salt at the 9-position. To prepare the diazonium salts, 10,11-MDO-20(S)-CPT or 10,11-MDO20(RS)-CPT is nitrated to form the corresponding 9-nitro compound. This nitro compound is then reduced to form the corresponding 9-amino compound which is used to prepare the diazonium salt.

Using known mixtures of H_2SO_4 and HNO_3 and standard nitration reaction conditions for the nitration of camptothecin (CPT) itself, one obtains a mixture of the 12-nitro and 9-nitro-camptothecin analogs with the 12-nitro analog present in considerable excess. A structure analysis of 10,11-MDO-20(S)-CPT and 10,11-MDO-20(RS)-CPT reveals that the 9- and 12-positions are available for nitration and the 10,11-methylenedioxy group appears to exhibit an analogous electronic influence on both the 9- and 12-positions. An analysis of the electronic and steric environments on the potential nitration positions of 10,11- leads to the expectation that both 10,11-MDO-20(S)-CPT and 10,11-MDO-20(RS)-CPT will nitrate in a manner similar to camptothecin itself and provide an excess of the 12-nitro analog. Quite unexpectedly, it was found that nitration of 10,11-MDO-20(S)CPT and 10,11-MDO-20(RS)-CPT gives substantially the 9-nitro-10,11-MDO-analogs with only trace amounts of the 12-nitro-10,11-MDO analogs. The present method, therefore, provides a surprisingly effective procedure for preparing the 9-nitro-10,11-MDOCPT analogs in high yield regioselectively.

The nitration reaction may be conducted using standard conditions for the nitration of aromatic compounds, and is generally conducted by dissolving/suspending the 10,11-MDOCPT in concentrated sulfuric acid with cooling and stirring followed by the addition of a slight excess of

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concentrated nitric acid. After stirring for a period of time sufficient to substantially complete the reaction, the solution is poured into water, ice or a ice/water mixture to provide the desired 9-nitro-10,11-MDOCPT compound.

5 Purification can be effected by standard extraction and recrystallization processes.

The 9-nitro-10,11-MDOCPT may then be catalytically reduced using hydrogen and a hydrogenation catalyst such as platinum, palladium, etc., or other conventional
10 hydrogenation reactions. Preferably, the hydrogenation catalyst is present on an inert support such as powdered carbon. Reduction of the 9-nitro analog to the 9-amino analog is conducted using standard hydrogenation solvents and hydrogen pressure conditions. Generally, the nitro
15 compound is dissolved/suspended in ethanol and contacted with a hydrogen atmosphere. The concentration of catalyst and of the nitro compound in the solvent is not critical. Concentrations of the nitro compound from about 1 mg/ml to 3 mg/ml may be used with catalyst concentrations ranging
20 from about 20-100 wt.%. The preferred solvent is absolute ethanol although other conventional inert solvents may be used.

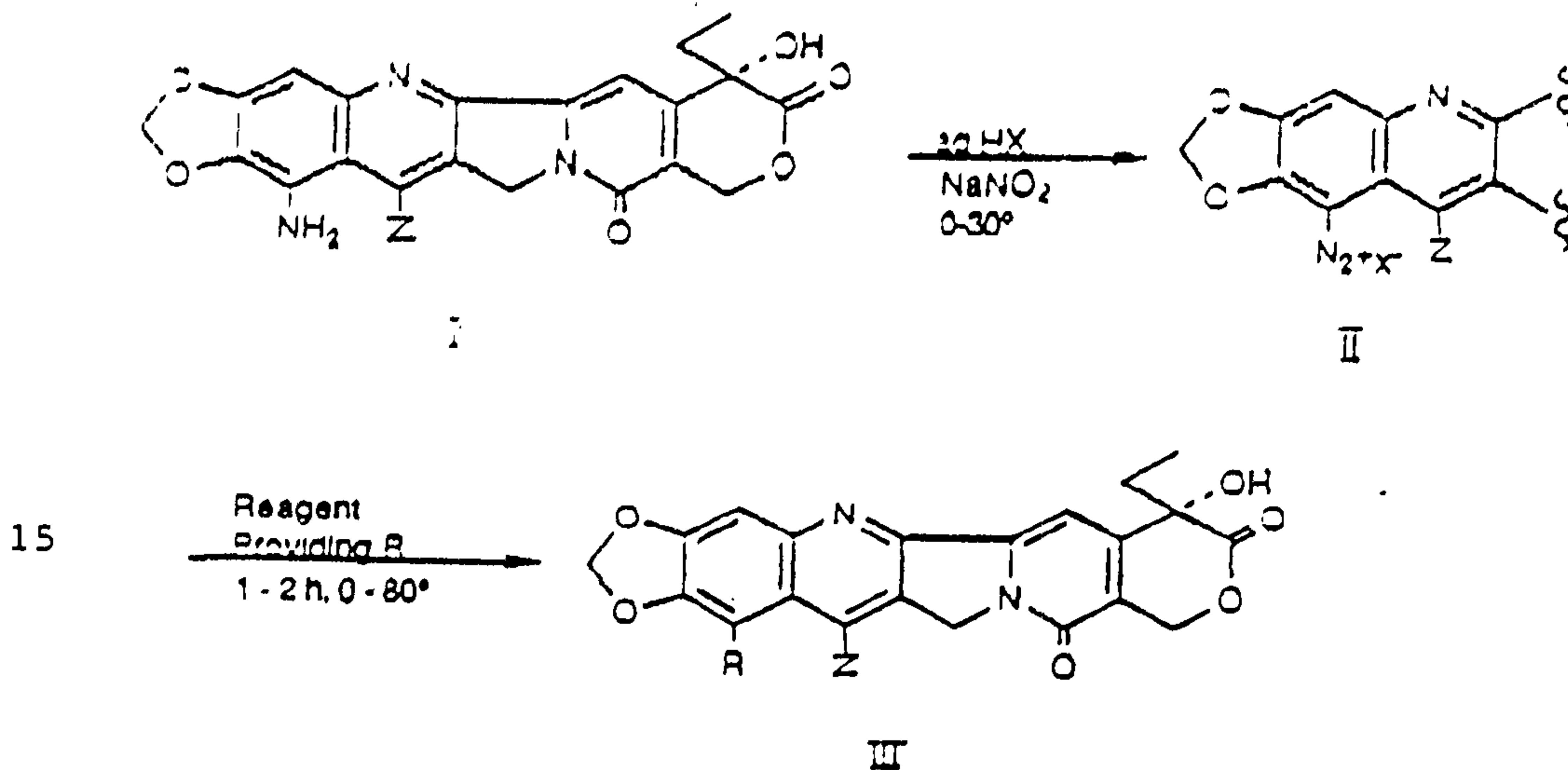
The hydrogenation reaction is generally conducted at ambient temperature although temperatures above or below
25 ambient temperature may be used so long as the camptothecin analog is not decomposed. Hydrogenation reaction times vary with the amount of nitro compound to be hydrogenated and can be easily determined by one skilled in the art. Generally, reaction times ranging from 2-30 hours are
30 sufficient to hydrogenate 9-nitro-10,11-MDOCPT.

Although catalytic hydrogenation is a preferred reduction method, other known chemical reductions such as

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$\text{FeSO}_4/\text{NH}_4\text{OH}$, Sn/HCl , etc. may also be employed to reduce the nitro group to an amino group.

The formation of diazonium salts is a general reaction undergone by primary aromatic amines upon treatment with sodium nitrite in acidic solution. Accordingly, the 9-amino-10,11-MDOCPT can be treated with sodium nitrite in acid solution to form the corresponding diazonium salt. These diazonium salts are then reacted with nucleophiles or free radicals to generate nitrogen gas (N_2) and the desired 9-substituted-10,11-MDOCPT compound. The overall reaction sequence is shown in scheme 1 below. In the scheme, the diazonium salt is shown as structure II where the counter anion X is derived from the acid HX.



Scheme 1

Non-limiting examples of suitable acids and reaction conditions to prepare a variety of 9-substituted-10,11-MDOCPT compounds are shown in Table A.

5

Table A

<u>Example</u>	<u>Reactant</u>	<u>HX</u>	<u>Other Reagents and Conditions</u>	<u>R in Product III</u>
	I	HBr	CuBr 80°C	Br
	I	HCl	CuCl 80°C	Cl
	I	HF ₄	Co, Pd(OAc) ₂ NaOAc, MeCN, 25°C	CO ₂ H
10	5	I	HCl	CHO
			H ₂ C=NOH, CuSO ₄ , Na ₂ SO ₃ , 25°C; aq. HCl, 80°C	
	6	I	H ₂ SO ₄ 80°C	OH
	7	I	HCl	CN
	8	I	HCl	N ₃
	9	I	HCl ≥ 120°C HF ₄	F
15	10	I	HCl	I
	11	I	HF ₄	NO ₂
			NaNO ₂ , Cu° 25°C	
	12	I	H ₂ SO ₄	H
			H ₃ PO ₂ , -10°C	
	13	I	HCl	SH
			1) KCS ₂ OEt, 40°C 2) KOH	
	14	I	HF ₄	CH ₃
			(CH ₃) ₄ Sn, Pd(OAc) ₂ MeCN, 25°C	

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Additional 10,11-MDOCPT compounds can be prepared by further reactions on the compounds shown in Table A or by analogous reactions. For example, the compound in which R is ethyl (C_2H_5) or propyl (C_3H_7) can be prepared by a reaction analogous to Example 14 using the reagent $(C_2H_5)_4Sn$ or $(C_3H_7)_4Sn$ in place of $(CH_3)_4Sn$. The compounds in which R is CN can be readily reduced by catalytic hydrogenation to obtain the compound in which R is CH_2NH_2 by hydrogenation processes analogous to the hydrogenation of 9-nitro-10,11-MDOCPT to 9-amino-10,11-MDOCPT discussed above or other known reduction reactions.

Alkylation reactions of compounds in which R is OH, SH, NH_2 or CH_2NH_2 yields compounds in which R is O- $C_{1.3}$ alkyl, S- $C_{1.3}$ alkyl, NH- $C_{1.3}$ alkyl or $CH_2NH-C_{1.3}$ alkyl. Dialkylation of the nitrogen-containing substituents is also possible to yield $N(C_{1.3} \text{ alkyl})_2$ and $CH_2N(C_{1.3} \text{ alkyl})_2$ substituents as R. Alkylation may be accomplished, for example, using C_1 - C_3 alkyl halides or tosylates (OTs). Preferred alkyl halides are the C_1 - C_3 alkyl chlorides and bromides. If desired, a base such as a tertiary amine may be added to facilitate the alkylation reaction.

It is possible to incorporate additional nitrogen and oxygen atoms into the substituent R by means of alkylation reactions. For example, alkylation with a reagent having the formula $(C_{1.3} \text{ alkyl})_2N-CH_2CH_2-X$ or $(C_{1.3} \text{ alkyl})_2N-CH_2CH_2CH_2-X$, where X is halogen or OTs yields the correspondingly alkylated products containing the di- $C_{1.3}$ alkylaminoethyl or di- $C_{1.3}$ alkylaminopropyl group. In a similar manner, introduction of an oxygen atom is possible using alkylating agents having the formula $(HOCH_2CH_2)_2N-(CH_2)_{2.3}-X$ and $(HOCH_2CH_2CH_2)_2N-(CH_2)_{2.3}-X$ to provide the corresponding diethanolaminoethyl, diethanolaminopropyl, dipropanolaminoethyl and dipropanolaminopropyl

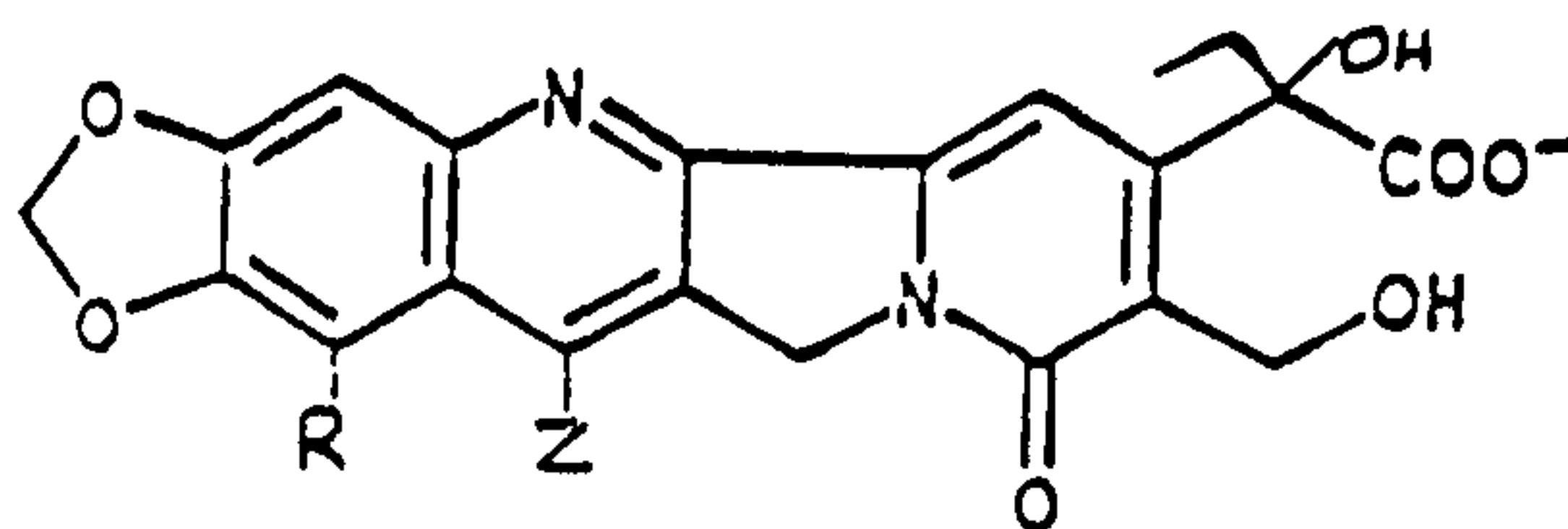
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groups. It may be necessary to protect the hydroxyl group in these latter alkylating agents using standard hydroxyl protecting groups such as THPO-. These hydroxyl protecting groups can be conveniently removed or deprotected after
5 alkylation by treatment with mild aqueous acid.

It has also been discovered that water-soluble analogs of 10,11-MDOCPT can be prepared by opening the lactone ring of 10,11-MDOCPT compounds to form water-soluble salts. These new derivatives exhibit substantially improved
10 water-solubility and retain a high level of cytotoxicity.

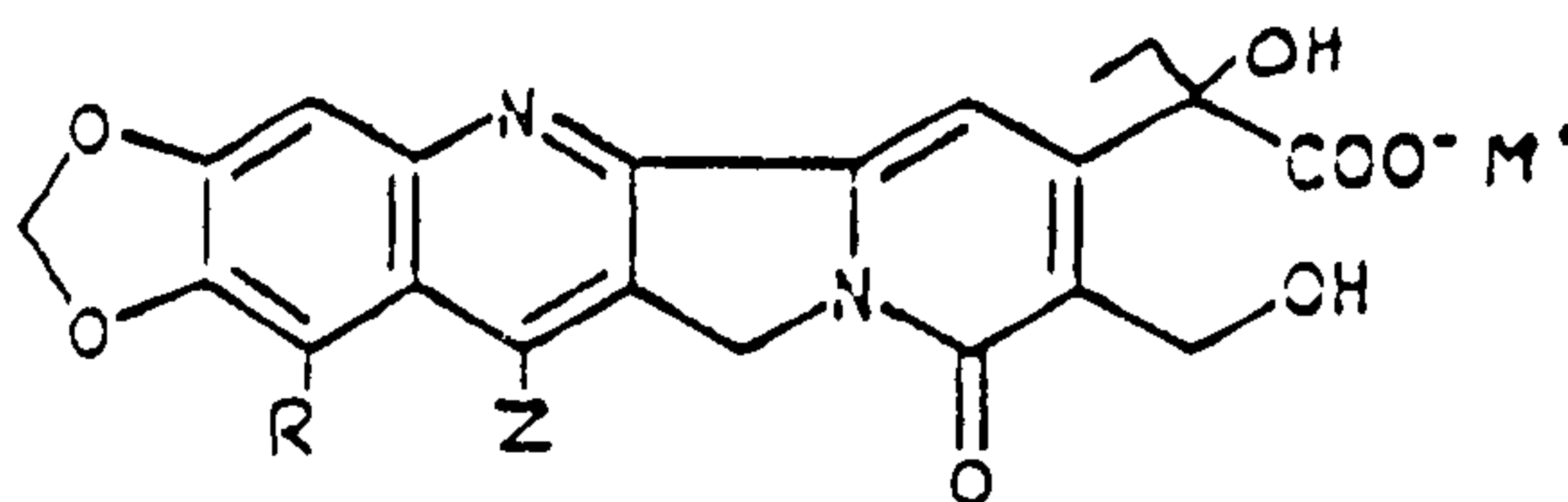
The interaction of pharmaceutical compounds with biological systems is highly specific and intimately related to the three-dimensional structure of a compound and the chemical functionality present on the
15 pharmaceutical compound. It is well known in the pharmaceutical art that structural changes as simple as the use of an opposite enantiomer can result in complete loss of biological activity and in some instances even opposite biological activity. Surprisingly, it has been discovered
20 that it is possible to hydrolyze the lactone ring of 10,11-MDOCPT and yet retain substantial biological activity while also enhancing water-solubility.

The open lactone compounds of the present invention have the structure shown below where R and Z have the same
25 definition as given above for the closed lactone compounds and further Z and R may both be hydrogen.



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The water-soluble analogs of the present invention are prepared by hydrolyzing the lactone ring of 10,11-MDOCPT or a 9-substituted-10,11-MDOCPT by utilizing one equivalent of an aqueous alkali metal hydroxide. The hydrolysis is preferably carried out in an aqueous solution. The resulting product is the alkali metal salt of 10,11-MDOCPT or 9-substituted-10,11-MDOCPT in which the lactone ring has been opened to form the corresponding hydroxyl and carboxylate functional groups, as shown below, where M^+ is a monovalent metal cation.



Preferred alkali metal hydroxides are potassium hydroxide and sodium hydroxide, with sodium hydroxide being particularly preferred.

Obviously, alkali metal hydroxide concentrations above or below one equivalent may be used in the present process. Concentrations below one equivalent result in incomplete formation of the metal salt.

The incomplete formation of the camptothecin salt provides a convenient purification method. Unreacted camptothecin (closed lactone form) is only slightly soluble in water and can be filtered off from the aqueous solution containing the camptothecin sodium salt in solution. This provides a convenient method for separating and purifying camptothecin salts.

The hydrolysis reaction may be conducted at any temperature which allows adequate reaction of the

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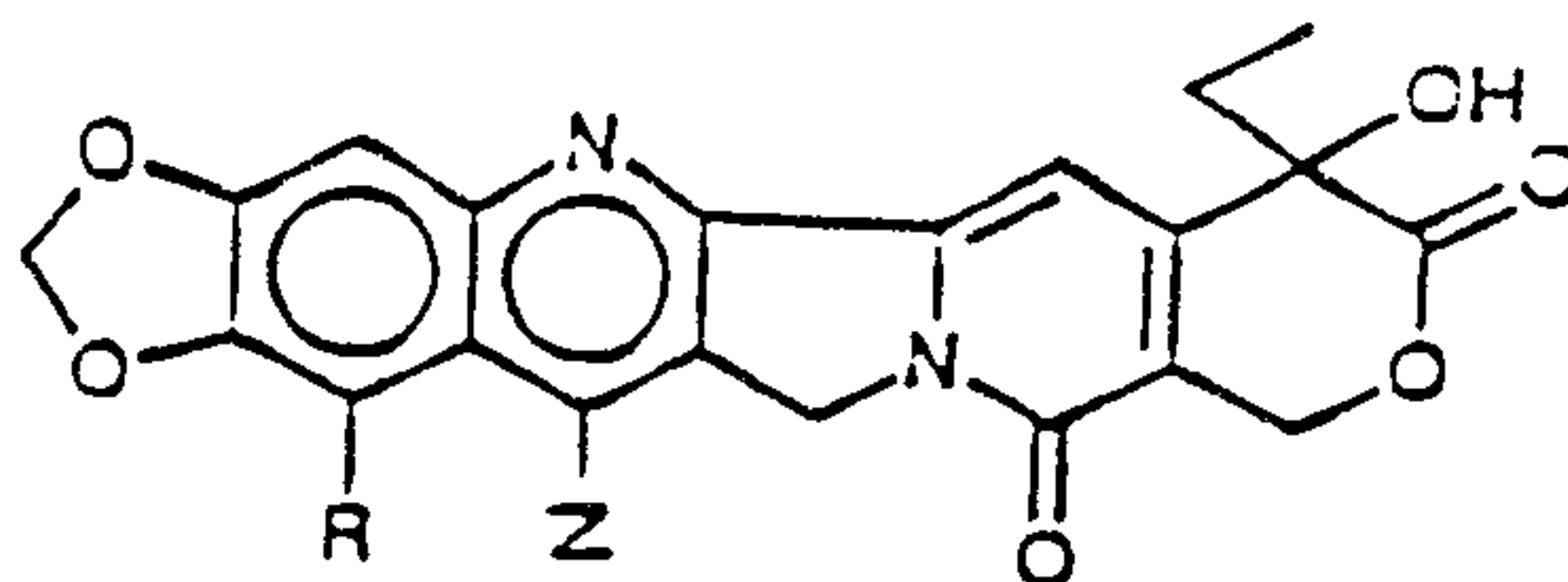
10,11-MDOCPT and alkali metal hydroxide so long as the temperature is sufficiently low to prevent decomposition of the starting materials. Suitable temperatures are from about 5-50°C with preferred temperatures being
5 approximately room temperature.

In the hydrolysis reaction, the 10,11-MDOCPT is generally, but not necessarily suspended in a suitable solvent such as methanol or aqueous methanol and treated with aqueous alkali metal hydroxide. To increase the rate
10 of reaction, the reaction mixture may be gently heated. After cooling, the 10,11-MDOCPT metal salt may be isolated by standard recrystallization or chromatographic processes following removal of the methanol and water solvents. Any water miscible solvent conventionally used with
15 camptothecin analogs may be used instead of methanol.

Alkali metal salts (open lactone compounds) of other 10,11-MDOCPT analogs such as 9-substituted-10,11-MDOCPT compounds may also be prepared by analogous reactions. For example, 9-nitro-10,11-MDOCPT, 9-amino-10,11-MDOCPT,
20 9-chloro-10,11-MDOCPT, 9-amido-10,11-MDOCPT or any other 9-substituted-10,11-MDOCPT derivative may also be hydrolyzed by a process analogous to the process described above for 10,11-MDOCPT to provide the corresponding monovalent metal salts of these derivatives.

25 Water-soluble derivatives of 10,11-MDOCPT can also be prepared by reacting the amino group of 9-amino-10,11-MDOCPT with appropriately protected amino acids and peptides, C₄₋₁₀ saturated or unsaturated carboxylic acid anhydrides, or the corresponding ester-acid halide
30 derivatives. For example, 9-amino-10,11-MDOCPT may be reacted with the carboxylic acid group of an α -amino acid to give compounds having the structure shown below:

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in which Z is as defined above and R is the group
-NHCOCHR¹NR²R³, where R¹ is the side-chain of an α-amino
acid, preferably the side chain of a D or L-isomer of one
of the naturally occurring amino acids, preferably one of
the 20 commonly occurring amino acids, and R² and R³ are,
independently, hydrogen or a lower alkyl group having 1-6
carbon atoms. Additionally, R³ may be a peptide unit
containing 1-3 amino acid units bonded to the nitrogen atom
through a peptide bond. These water-soluble analogs,
therefore, contain from 1-4 peptide units bonded to the
9-amino nitrogen atom by means of a peptide bond.
Obviously, amino acids which are not naturally occurring
may also be used to prepare water-soluble
9-amido-10,11-MDOCPT derivatives so long as the amino acid
has a carboxylic acid, acid halide or other reactive acyl
functionality to form the required peptide bond with the
9-amino group of 9-amino-10,11-MDOCPT. Other, preferred
side chains R¹ are alkyl and aralkyl groups containing 2-20,
preferably 2-10 carbon atoms.

Generally, these amino acid and peptide-containing derivatives are prepared using amino acids and peptides in which reactive functional groups such as amino groups and carboxylic acid groups are protected using standard amino acid and carboxylic protecting groups. For example, when preparing a derivative from an amino acid such as glycine, one can protect the amino group of glycine by reaction with tBOC chloride to prepare the reactive tBOC-protected amino acid. Appropriately protected amino acids are also

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available commercially. The protected amino acid is reacted with 9-amino-10,11-MDOCPT and the tBOC group is then removed to give the water-soluble salt of the 9-glycinamido derivative, for example.

5 If desired, free amino groups on the amino acids or peptides may be derivatized by known nitrogen alkylation reactions, i.e., reaction with alkyl halides, to provide mono or dialkylamino acid amido derivatives as shown above (R^2 and/or R^3 = alkyl). Preferably, free amino groups are
10 derivatized to form C_{1-3} mono or dialkylamino groups.

Dibasic amino acids such as arginine, histidine, lysine, etc., and dicarboxylic amino acids such as aspartic acid, glutamic acid, etc., may be used for one or more of the amino acids in the amino acid or peptide derivatives
15 described above. If desired, standard addition salts may be prepared by reacting the free amino groups of any amino acid with a mineral acid such as HCl, HBr, H_3PO_4 or organic acids such as malic, maleic or tartaric acids. Likewise, free carboxylic acid groups on any amino acid may be
20 derivatized by the formation of monovalent metal cation salts, ammonium salts or quaternary ammonium salts by the addition of monovalent metal hydroxides, ammonia or amines. Quaternary ammonium salts may be formed with primary, secondary or tertiary amines in which the nitrogen atom of
25 the amine contains 1, 2 or 3 lower alkyl or substituted lower alkyl groups. Substituted lower alkyl groups containing one or more hydroxyl groups are preferred. Sodium salts, triethylammonium and triethanol ammonium salts are particularly preferred.

30 Other water-soluble derivatives can also be prepared by reacting 9-amino-10,11-MDOCPT with a C_{4-10} saturated or unsaturated acid anhydride, the corresponding ester-acid

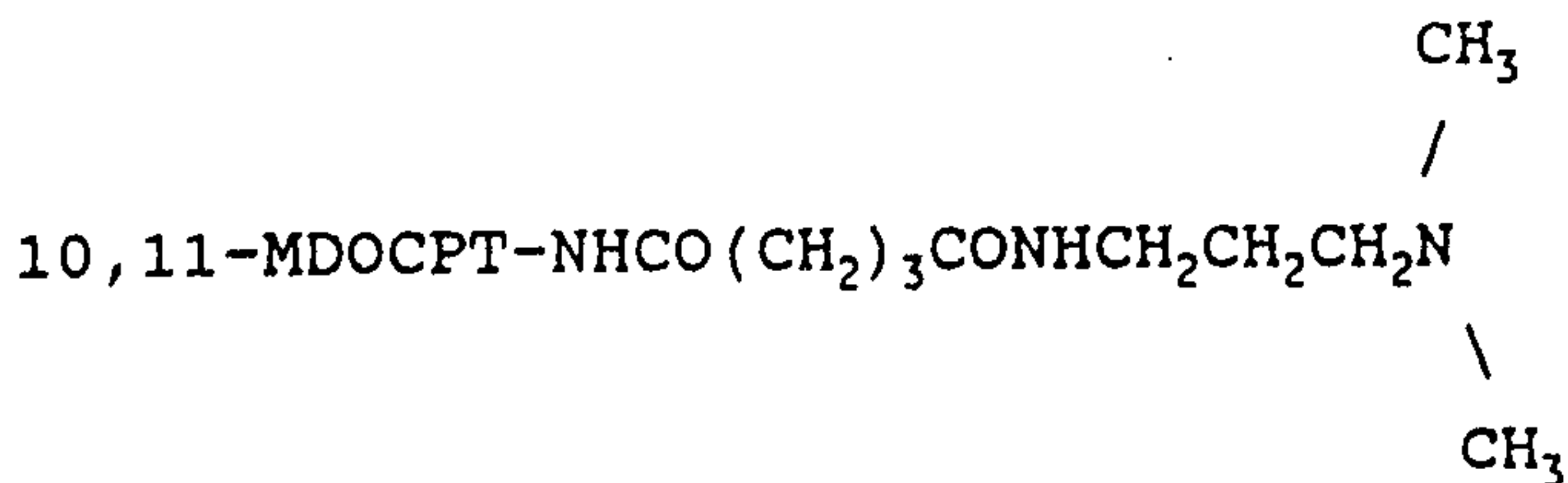
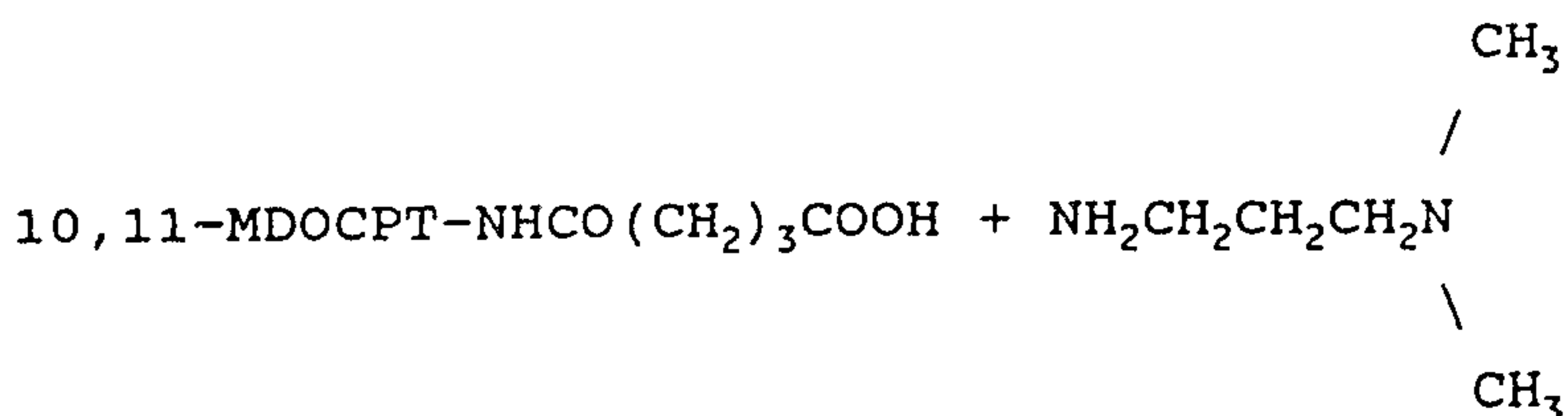
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halide or other reactive acyl derivatives to provide analogs having structure I in which R is $\text{NHCO-C}_{2-8}\text{-alkylene-X}$ and $\text{NHCO-C}_{2-8}\text{-alkenylene-X}$ where $\text{X} = \text{COOH}$. The reaction is optionally carried out in a suitable solvent and produces the corresponding half acid. For example, reaction of 9-amino-10,11-MDOCPT with glutaric anhydride gives the 9-glutaramide half acid. Likewise, reaction of 9-amino-10,11-MDOCPT with the C_{1-6} ester-acid halide corresponding to glutaric anhydride results in the 9-glutaramide half acid ester. Conventional hydrolysis of the ester produces the half acid. Water solubility may be imparted in each case by reaction with one equivalent of any of the bases noted above.

The reaction of 9-amino-10,11-MDOCPT with the anhydride or other reactive acyl compound is preferably carried out in the presence of a weak base such as a tertiary amine to facilitate the formation of the product amide. Suitable amines include cyclic amines such as pyridine as well as lower alkyl tertiary amines.

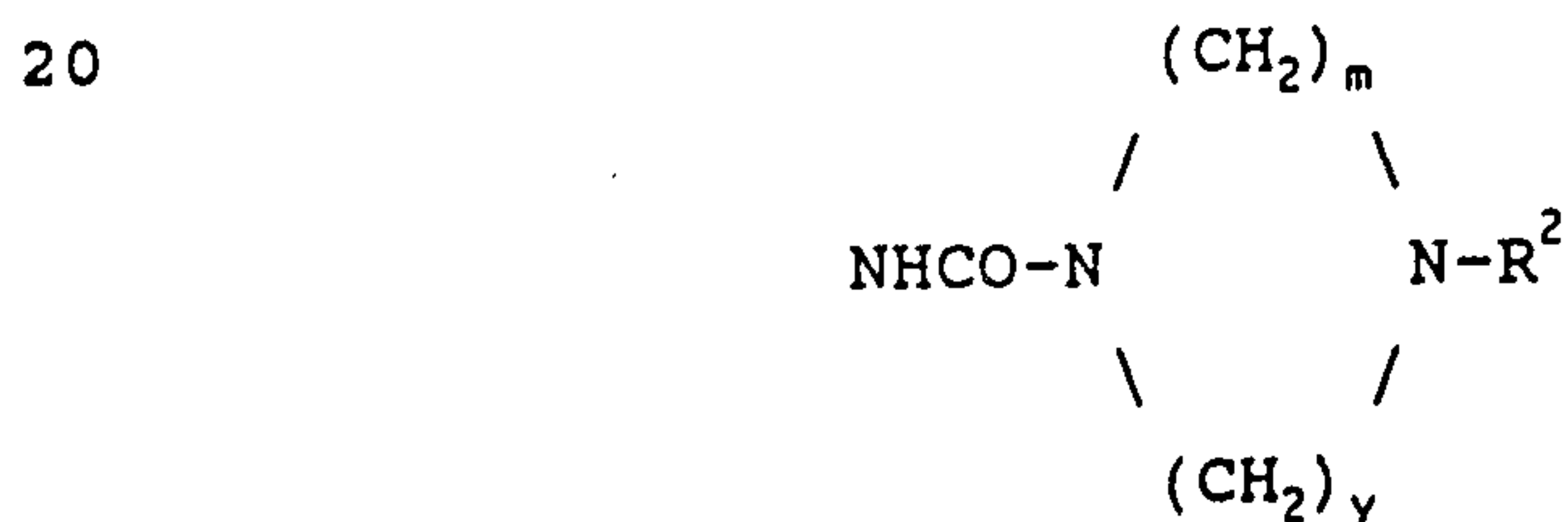
The free acid group of the amide half acid may be further coupled with a suitable alkylene diamine ($\text{NHR}^2\text{-(CH}_2)_n\text{-NR}^2\text{R}^3$) to give amino amides in which the R group in structure I is $\text{-NH-A'-NR}^2\text{-(CH}_2)_n\text{-NR}^2\text{R}^3$, where $n = 1-10$, preferably 2-6, and A' is a C_{4-10} acyl-alkylene-acyl or C_{4-10} acyl-alkenylene-acyl group, i.e., R is $\text{NHCO-C}_{2-8}\text{-alkylene-X}$ or $\text{NHCO-C}_{2-8}\text{-alkenylene-X}$ where X is COOH or $\text{CONR}^2\text{-(CH}_2)_n\text{-NR}^2\text{R}^3$. For example, the reaction of 9-glutaramido-10,11-MDOCPT with a suitable diamine such as 3-(dimethylamino)-1-propylamine gives the corresponding amino acid amide as shown below.

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Acid and base addition salts of these derivatives may also be prepared in a manner analogous to that described above.

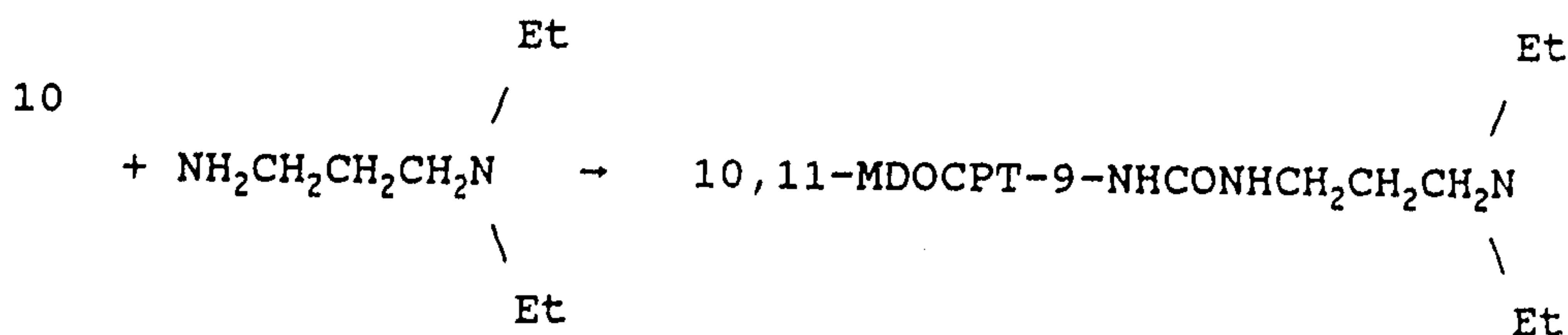
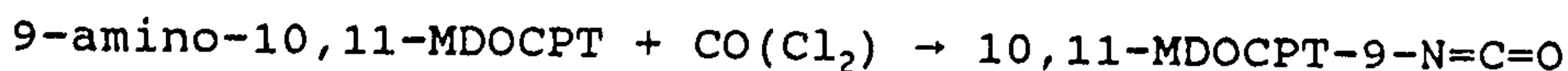
15 In another embodiment, water-soluble urea and urethane analogs can be prepared by reacting 9-amino-10,11-MDOCPT with phosgene followed by reaction with an appropriate diamine or tertiary-amino alcohol to give compounds having the formula I in which R is $\text{-NHCO-B-(CH}_2)_n\text{-NR}^2\text{R}^3$, where B is oxygen or NH, and compounds in which R is



25 where $m + y = 3-6$ and n , R^2 and R^3 are as defined above.

Suitable diamines are primary and secondary straight-chain, branched or cyclic diamines containing 3-15 carbon atoms. Examples of straight-chained and branched diamines include diaminoethane, 1,2- and 1,3-diaminopropane,

1,4-diaminobutane, etc. Examples of cyclic diamines included pyrazolidine, imidazolidine, piperazine, etc. Preferred diamines are diamines in which one of the amino groups is derivatized to form a di-lower-alkyl-amino group such as, for example, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{N}(\text{CH}_2\text{CH}_3)_2$. The reaction of 9-amino-10,11-MDOCPT with phosgene followed by a diamine is represented below.



Tertiary-amino alcohols for the preparation of urethane analogs include N,N-di- C_{1-6} -alkylamino alkanols prepared from straight chain or branched amino alkanols having 2-10 carbon atoms, for example, N,N-diethyl-aminoethanol.

Water soluble standard acid and base addition salts can be prepared from the urea and urethane analogs in a manner similar to that described above for other amino and carboxylic acid group-containing analogs.

Preferred derivatives within the scope of the present invention are 10,11-MDOCPT analogs having glycinamido, succinamido, glutaramido, (4-methylpiperazino) carbonylamino, N,N-dimethylaminopropylamido-glutaramido and (N,N-diethylaminoethoxy)carbonylamino substituents at the 9-position and the water soluble salts thereof.

The salts of the present invention exhibit

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substantially improved water-solubility relative to conventional camptothecin analogs and may be formulated into solid and aqueous pharmaceutical compositions by conventional methods. The compounds of the present invention are active in standard cytotoxicity tests and are inhibitors of topoisomerase I.

The 10,11-methylenedioxy (MDO) group confers striking and unexpected improvements on the in vitro and in vivo activity found in the camptothecin molecule with particular reference to anti-tumor activity. Thus, Jaxel et al., Cancer Res., 49, 1465-1469 (1989), and Hsiang et al., Cancer Res., 49, 4385-4389 (1989), have shown that 10,11-MDO-20(RS)-CPT has three to five times the potency of camptothecin in the inhibition of topoisomerase I. Inhibition of this enzyme has been shown by Jaxel et al. (loc. cit.) to be very well correlated with in vivo anti-tumor and anti-leukemic activity.

In contrast, a compound with quite similar structure, 10,11-dimethoxy-20(RS)-CPT, is totally inactive, Wani et al., J. Med. Chem., 92: 2360 (1986). Unlike 10,11-dimethoxy-20(RS)-CPT, the 10,11-MDO moiety is held rigidly in the plane of ring A of CPT (See the structure in Figure 1), and this is thought to contribute to the additional biological activity unexpectedly noted with all of these compounds.

Table B shown below shows the potent topoisomerase I inhibitory activity of the compounds of the present invention. The cleavable complex assay was performed according to the method described in Hsiang, Y-H. et al., J. Biol. Chem., 260:14873-14878 (1985). The cleavable complex assay correlates well with in vivo anti-tumor activity in animal models for camptothecin analogs. See

Hsiang et al., Cancer Research, 49:4385-4389 (1989) and Jaxel et al., Cancer Research, 49:1465-1469 (1989).

Table B - Cleavable Complex Assay of Camptothecin and Analogs

<u>Compound</u>		<u>Name</u> *	<u>EC₅₀</u> ** <u>μg/mL</u>
5	1	9-AMINO-10,11-MDO-20(S)-CPT	~.01 μg/mL
	2	10,11-MDO-20(S)-CPT	~.01 μg/mL
	3	10,11-MDO-20(RS)-CPT	~.02 μg/mL
	4	9-AMINO-10,11-MDO-20(RS)-CPT	~.02 μg/mL
	5	9-NITRO-10,11-MDO-20(RS)-CPT	~.09 μg/mL
10	6	10,11-MDO-20(S)-CPT, Na ⁺ SALT	~0.1 μg/mL
	7	9-GLA-10,11-MDO-20(RS)-CPT, HCl	~0.1 μg/mL
	8	10,11-MDO-20(RS)-CPT, Na ⁺ SALT	~0.2 μg/mL
	9	20(S)-CPT	~0.2 μg/mL
	10	20(RS)-CPT	~0.8 μg/mL
15	11	20(RS)-CPT, Na ⁺ SALT	~0.9 μg/mL
	12	9-AMINO-10,11-MDO20(S)-CPT, Na ⁺ SALT	~1 μg/mL
	13	9,10-MDO-20(RS)-CPT	~2 μg/mL
	14	9-AMINO-10,11-MDO-20(RS)-CPT, Na ⁺ SALT	~2 μg/mL
	15	9-AMINO-10,11-MDO-20(R)-CPT	>10 μg/mL
20	16	20(R)-CPT	>10 μg/mL

* Abbreviations

CPT = Camptothecin

MDO = Methylenedioxy

GLA = Glycinamido

25 ** EC₅₀ is the concentration of a compound which gives 50% topoisomerase I inhibition as revealed by cleavable complex formation. All EC₅₀

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values represent the mean of several independent assays; all values are normalized with respect to #9, 20(S)-CPT, which was always assayed as a control.

The present compounds are active against murine
5 tumors, such as lymphocytic leukemia L-1210, RAW117-H10
lymphosarcoma and K1735-M2 melanoma. Activity in one or
more of these tumor tests has been reported to be
indicative of anti-tumor activity in man (A. Goldin et al.,
in Methods in Cancer Research, ed. V.T. DeVita Jr. and H.
10 Busch, 16: 165, Academic Press, New York, 1979).

In tumor histioculture studies (See Table C) using
human cancers obtained by surgery or biopsy, the compounds
of the present invention demonstrate significant activity,
measured as inhibition of tumor cell proliferation during
15 treatment with the compounds of the present invention. As
used herein, the term "cancer" is synonymous with the terms
"malignant tumor" and more generally "tumor". The data
shown in Table C demonstrate the activity of the present
compounds against human colon cancer, which is well known
20 to be a very resistant cancer to chemotherapy. See H.L.
Davis, Chemotherapy of Large Bowel Cancer, Cancer (Phila.)
50: 2638-2646 (1982); J.R. Neefe and P.S. Schein, Chapter
43: The Management of Disseminated Large-Bowel Cancer in
Principals of Cancer Treatment, page 402, ed. S.K. Carter,
25 E. Glatstein and R.B. Livingston, McGraw-Hill Co., 1982; K.
Mekhail-Ishak, Cancer Research, 49: 4866-4869 (1989) and
P.J. Ferguson and Y.C. Cheng, Cancer Research, 49:
1148-1153 (1989).

Table C - HUMAN COLON TUMOR HISTIOCULTURE

Inhibition of Cell Proliferation

<u>Name</u>		**IC ₅₀ (<u>μg/mL</u>)
	20(S)-CPT	~0.02
5	10,11-MDO-20(S)-CPT	~0.003
	10,11-MDO-20(S)-CPT, Na ⁺ SALT	~0.005
	9-NH ₂ -10,11-MDO-20(S)-CPT	~0.002
	10,11-MDO-20(RS)-CPT	~0.005
	10,11-MDO-20(RS)-CPT, Na ⁺ SALT	~0.01
10	9-NH ₂ -10,11-MDO-20(RS)-CPT	~0.005
	9-NH ₂ -10,11-MDO-20(RS)-CPT, Na ⁺ SALT	~0.01

* Abbreviations

CPT = Camptothecin

MDO = Methylenedioxy

- 15 ** IC₅₀: concentration of compound required to inhibit by 50% the incorporation of ³[H]thymidine into DNA

- 20 Inhibition of tumor cell proliferation was performed in vitro on human colorectal tumors obtained from surgery or biopsy, as described by Vescio et al (Proc. Nat'l. Acad. Sci. USA 84:5029-5033, 1987) with the following modifications: Tumors were cultured 1 day prior to drug addition; tumors were exposed to compounds for 24 hours, washed, and then exposed to ³[H]thymidine for 3 days.

The compounds of the present invention exhibit

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antitumor activity against human colon cancer, which is known to exhibit de novo drug resistance, and thus be difficult to treat chemotherapeutically. Therefore, it is believed that the present compounds will be active against
5 a wide spectrum of mammalian (including human) cancers such as cancers of the oral cavity and pharynx (lip, tongue, mouth, pharynx), esophagus, stomach, small intestine, large intestine, rectum, liver and biliary passages, pancreas, larynx, lung, bone, connective tissue, skin, breast, cervix
10 uteri, corpus endometrium, ovary, prostate, testis, bladder, kidney and other urinary tissues, eye, brain and central nervous system, thyroid and other endocrine gland, leukemias (lymphocytic, granulocytic, monocytic), Hodgkin's disease, non-Hodgkin's lymphomas, multiple myeloma, etc.
15 Obviously, the present compounds may be used to treat other cancers not specifically named so long as antitumor activity is demonstrated by the present compounds in the particular cancer.

The present invention also includes pharmaceutical
20 compositions containing the camptothecin derivatives of the present invention. There may be included as part of the composition pharmaceutically acceptable binding agents, carriers and/or adjuvant materials. The active materials can also be mixed with other active materials which do not
25 impair the desired action and/or supplement the desired action. The active materials according to the present invention can be administered by any route, for example, orally, parenterally, intravenously, intradermally, subcutaneously, or topically, in liquid or solid form.

30 For the purposes of parenteral therapeutic administration, the active ingredient may be incorporated into a solution or suspension. The solutions or suspensions may also include the following components: a

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sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens;
5 antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in
10 ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Another mode of administration of the compounds of this invention is oral. Oral compositions will generally include an inert diluent or an edible carrier. They may be
15 enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the aforesaid compounds may be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, chewing gums and the like.

20 The tablets, pills, capsules, troches and the like may contain the following ingredients: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, corn starch and the like; a
25 lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; and a sweetening agent such as sucrose or saccharin or flavoring agent such as peppermint, methyl salicylate, or orange flavoring may be added. When the dosage unit form is a capsule, it may
30 contain, in addition to material of the above type, a liquid carrier such as a fatty oil. Other dosage unit forms may contain other various materials which modify the physical form of the dosage unit, for example, as coatings.

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Thus tablets or pills may be coated with sugar, shellac, or other enteric coating agents. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and
5 flavors. Materials used in preparing these various compositions should be pharmaceutically pure and non-toxic in the amounts used.

As known in this art, dosage values will vary with the specific cancer to be treated, the stage of tumor
10 development, tumor location, weight and physical condition of the patient being treated, etc. Good results should be achieved when the compounds described herein are administered to a subject requiring such treatment as an effective oral, parenteral or intravenous dose of from
15 about 0.1 to about 100 mg per day per patient. It is to be understood, however, that for any particular subject, specific dosage regimens should be adjusted to the individual need in view of the patient's response to treatment with the drug and the professional judgment of
20 the person administering or supervising the administration of the aforesaid compound. It is to be further understood that the dosages set forth herein are exemplary only and they do not limit the scope or practice of the invention. Dosages above or below the range cited above are within the
25 scope of the present invention and may be administered to the individual patient if desired and necessary. The dosages may be administered at once, or may be divided into a number of smaller doses to be administered at varying intervals of time.

30 Other features of the invention will become apparent from the following descriptions of preferred embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

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EXAMPLESExample 1 - Synthesis of 9-Amino-10,11-MDOCPT.

10,11-MDO-20(RS)-CPT and 10,11-MDO-20(S)-CPT were prepared according to Wani et al., J. Med. Chem. 29, 2358 (1986) and the process disclosed in U.S. Patent No. 5,053,512.

Conversion of 10,11-MDOCPT to 9-Nitro-10,11-MDOCPT.

10,11-MDOCPT (332 mg, 0.847 mmol) was dissolved/suspended in conc. H_2SO_4 (5 mL), stirred and cooled to 0°C , and treated over 5 min with conc. HNO_3 (25 drops). After 1 hr. the brown solution was poured onto ice/ H_2O (50 mL) to provide a yellow-orange precipitate which was collected by filtration (292 mg). Extraction of the filtrate with CHCl_3 (2 x 50 mL) provided additional material (83 mg) for a total yield of 375 mg (100%). Recrystallization from $\text{MeOH}/\text{CHCl}_3$ provided a 75% recovery of the title compound as a yellow powder: mp darkening above 255°C with no melting below 350°C : IR ν_{max} (KBr) 3430 (br), 2920, 1741 (lactone), 1654 (pyridone), 1596 (aromatic), 1525 (NO_2) $1450, 1343, 1242, 1191, 1154, 1043, 928, 785$ and 565 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$) δ 0.87 (t, 3, $J = 7\text{ Hz}$, H-18), 1.85 (m, 2, H-19), 5.21 (s, 2, H-5), 5.41 (s, 2, H-17), 6.52 (s, 2, $-\text{OCH}_2\text{O}-$), 7.24 (s, 1, H-14), 7.78 (s, 1, H-12), 8.96 (s, 1, H-7).

25 Conversion of 9-Nitro-10,11-MDOCPT to 9-Amino-10,11-MDOCPT.

A suspension of the nitro compound (139 mg) prepared above and 10% Pd/C (75 mg) in abs EtOH (40 mL) was stirred at ambient temperature under 1 atm H_2 for 20 hr. The mixture was filtered (Celite) and the pad washed profusely

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with MeOH/CHCl₃ and HCl. Evaporation of the solvents afforded the crude amine as an orange-brown solid (125 mg, 97%). Recrystallization from MeOH/CHCl₃ gave the title compound as a tan-orange powder (87 mg, 67%), mp darkening
5 above 250°C with no discreet melting below 350°C. ¹H NMR (DMSO-d₆) δ 0.88 (t, 3, J = 7 Hz, H-18), 1.87 (m, 2, H-19), 5.22 (s, 2, H-5), 5.41, (s, 2, H-- 17), 5.74 (s, 2, NH₂), 6.18 (s, 2, -OCH₂O-), 6.47 (s, 1, OH), 6.91 (s, 1, H-14), 7.23 (s, 1, H-12), 8.74 (s, 1, H-7).

10 Example 2 - Synthesis of 9-Bromo-10,11-MDO-20(S)-CPT (III, R=Br).

A stirred mixture of 9-amino-10,11-MDO-20(S)-CPT (10.0 mg, 25.5 μmol) in 48% aq HBr (0.5 mL) at 0°C was treated with a solution of NaNO₂ (2.1 mg, 30.6 μmol) in H₂O (25 μl).
15 The cooling source was removed, and after the addition of CuBr (4.0 mg, 35 μmol), the brown mixture was heated for 20 min at 80°C. The mixture was cooled and poured over ice (3 g). The resulting suspension was extracted with several 10 mL portions of CHCl₃, and the extract was dried (Na₂SO₄) and
20 evaporated under reduced pressure to afford an orange-yellow solid (10 mg) containing mostly the title compound III (R=Br) and, to a lesser extent, III (R=H). Purification was effected by flask column (1 g 230-400 mesh SiO₂, 0.25-1% MeOH in CHCl₃) to provide III (R=Br) as a pale
25 yellow solid (3.8 mg) and III (R=H) in later fractions as a cream colored solid (2.0 mg). 300 MHz ¹H NMR (DMSO-d₆) δ 0.84 (t, 3, J=7 Hz, H-18), 1.82 (m, 2, H-19), 5.24 (s, 2, H-5), 5.39 (s, 2, H-17), 6.36 (s, 2, -OCH₂O-), 6.49 (s, 1, OH), 7.24 (s, 1, H-14), 7.54 (s, 1, H-12) and 8.63 (s, 1,
30 H-7); HRMS: calcd. for C₂₁H₁₅N₂O₆Br, 470.0114; measured, 470.0115.

Example 3 - Synthesis of 9-Chloro-10,11-MDO-20(S)-CPT (III,

R=Cl).

The intermediate diazonium chloride II (X=Cl) is prepared as in Example 2 except that 39% aq HCl is used. Similarly, the substitution of CuCl leads to the expected 9-chloro compound III (R=Cl) after chromatography.

Example 4 - Synthesis of 9-Carboxy-10,11-MDO-20(S)-CPT (III, R=CO₂H).

The diazonium salt II (X=Cl) is prepared as in Example 2. After filtration of the aq HCl solution, aq HBF₄ is added to give a precipitate of II (X=BF₄). This salt is combined in a pressure reactor with Pd(OAc)₂ and NaOAc in MeCN. Carbon monoxide (1-2 atm) is introduced and the mixture is left for 1 hr at ambient temperature. The mixture is concentrated by evaporation and reconstituted in H₂O. Crude III (R=CO₂H) is isolated by extraction into CHCl₃, and purified by further extraction into dilute aq NaHCO₃ followed by precipitation with acid.

Example 5 - Synthesis of 9-Formyl-10,11-MDO-20(S)-CPT (III, R=CHO).

The diazonium salt II (X=Cl) is prepared as in Example 2. The salt solution is treated at room temperature with an aqueous solution of formaldoxime containing CuSO₄ and Na₂SO₃. After 1 hr, conc. HCl is added and the intermediate oxime is collected and hydrolyzed to the product aldehyde III (R=CHO) by refluxing in conc. HCl.

Example 6 - Synthesis of 9-Hydroxy-10,11-MDO-20(S)-CPT (III, R=OH).

The intermediate diazonium salt II (X=HSO₄) is prepared

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in a manner analogous to that of Example 2 by using aq H_2SO_4 instead of aq HBr . The mixture is then heated at 80°C for 1 hr whereby hydrolysis occurred. On cooling, the product III ($\text{R}=\text{OH}$) is isolated by extraction into CHCl_3 .

5 Example 7 - 9-Cyano-10,11-MDO-20(S)-CPT (III, $\text{R}=\text{CN}$).

The diazonium chloride II ($\text{X}=\text{Cl}$) is prepared as in Example 2 and treated at 20°C with CuCN after the pH has been adjusted to 7 with Na_2CO_3 . After 2 hr, the reaction mixture is extracted with CHCl_3 . The CHCl_3 extract is used
10 to isolate the title compound III ($\text{R}=\text{CN}$) by chromatography.

Example 8 - 9-Azido-10,11-MDO-20(S)-CPT (III, $\text{R}=\text{N}_3$).

The diazonium chloride II ($\text{X}=\text{Cl}$) is prepared as described in Example 2. The resulting mixture is treated with an aqueous solution of NaN_3 , and after 15 min at room
15 temperature, the azide III ($\text{R}=\text{N}_3$) results as a precipitate. Centrifugation provides the product as a pale solid which is purified by column chromatography.

Example 9 - 9-Fluoro-10,11-MDO-20(S)-CPT (III, $\text{R}=\text{F}$).

The diazonium chloride II ($\text{X}=\text{Cl}$) is prepared as before
20 (Example 2), and after filtration the stirred solution is treated at 0°C with a slight excess of HBF_4 , whereupon salt II ($\text{X}=\text{BF}_4$) precipitates. After collection and drying, this salt is pyrolyzed ($\geq 120^\circ$) over 1 hr to afford fluoro product III ($\text{R}=\text{F}$). Dark colored impurities can be removed
25 by a flash column chromatography.

Example 10 - 9-Iodo-10,11-MDO-20(S)-CPT (III, $\text{R}=\text{I}$).

A solution of chloride II ($\text{X}=\text{Cl}$, prepared as before,

Example 2) in aq HCl is treated with aq KI and heated for 1 hr. Upon cooling, the mixture is extracted with CHCl_3 , and the extract concentrated and subjected to column chromatography to provide III ($\text{R}=\text{I}$).

5 Example 11 - 9-Nitro-10,11-MDO-20(S)-CPT (III, $\text{R}=\text{NO}_2$).

The salt II ($\text{X}=\text{BF}_4$) is isolated as in Example 4 and treated at 25° with aq NaNO_2 solution followed by the addition of copper powder. After 1 hr, the mixture is extracted with CHCl_3 , which on evaporation gives crude III
10 ($\text{R}=\text{NO}_2$). Column chromatography affords pure III ($\text{R}=\text{NO}_2$).

Example 12 - 10,11-MDO-20(S)-CPT (III, $\text{R}=\text{H}$).

The solution of diazonium sulfate II ($\text{X}=\text{HSO}_4$), prepared as in Example 6, is maintained at -10° to 0° and treated with excess hypophosphorous acid (H_3PO_2). After 1 hr, the
15 unsubstituted product III ($\text{R}=\text{H}$) can be isolated in nearly pure form by extraction with a few portions of CHCl_3 .

Example 13 - 9-Mercapto-10,11-MDO-20(S)-CPT (III, $\text{R}=\text{SH}$).

A diazonium chloride II ($\text{X}=\text{Cl}$) solution, prepared as in Example 2, is treated at 40° with potassium ethyl
20 xanthate (KCS_2OEt). The intermediate ethyl xanthate is extracted into CHCl_3 , and after evaporation of the CHCl_3 , the xanthate is hydrolyzed with KOH in aq MEOH. The solution is neutralized with conc. HCl and the thiol III ($\text{R}=\text{SH}$) isolated by extraction with CHCl_3 .

25 Example 14 - 9-Methyl-10,11-MDO-20(S)-CPT (III, $\text{R}=\text{Me}$).

The diazonium tetrafluoroborate salt II ($\text{X}=\text{BF}_4$), prepared as in Example 4, is added to MeCN and to the

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resulting stirred mixture is added Me_4Sn and $\text{Pd}(\text{OAc})_2$ at room temperature. After 2 hr, the MeCN is evaporated and the residue partitioned between H_2O and CHCl_3 . The CHCl_3 is reserved and the aqueous portion is extracted twice more
5 with CHCl_3 . From this extract, III (R=Me) is isolated.

Example 15 - 9-Ethyl-10,11-MDO-20(S)-CPT (III, R=Et).

The diazonium tetrafluoroborate salt II ($\text{X}=\text{BF}_4$), prepared as in Example 4, is added to MeCN and to the resulting stirred mixture is added Et_4Sn and $\text{Pd}(\text{OAc})_2$ at
10 room temperature. After 2 hr, the MeCN is evaporated and the residue partitioned between H_2O and CHCl_3 . The CHCl_3 is reserved and the aqueous portion is extracted twice more with CHCl_3 . From this extract, III (R=Et) is isolated.

Example 16 - Conversion of 9-Amino-10,11-MDOCPT to 9-
15 Glycinamido-10,11-MDOCPT Hydrochloride.

A stirred mixture of the 9-amino compound (186 mg, 0.457 mmol) and BOC-glycine (150 mg, 0.85 mmol) in pyridine (1 mL) and DMF (15 mL) was chilled to 0°C and treated with DCC (200 mg, 0.971 mmol). The mixture was warmed to
20 ambient temperature and stirred for 65 hr. The solvents were evaporated and the residue dissolved in $\text{MeOH}/\text{CHCl}_3$. Celite (3 g) was added, the mixture evaporated, and the Celite-dispersed sample placed on a silica gel column (20 9). Elution (200 mL CHCl_3 , 500 mL 5% $\text{MeOH}/\text{CHCl}_3$, 500 mL 12%
25 $\text{MeOH}/\text{CHCl}_3$) and evaporation of appropriate fractions gave the intermediate BOC-protected derivative (98 mg, 38%). The derivative was treated with chilled conc $\text{HCl}/\text{dioxane}$ (1:9, 5 mL), and the resulting mixture was stirred at ambient temperature for 5 hr. The solvent was evaporated,
30 the residue sonicated in deionized H_2O (50 mL) and filtered (0.45 micron membrane). The clear yellow solution was

-32-

lyophilized to give an amber gummy solid which on trituration with abs EtOH gave the glycineamide hydrochloride salt as a yellow microcrystalline solid (57 mg, 73%), mp darkening above 230°C with no apparent melting below 340°C. IR $\nu_{\max}$ (KBr) 3680-2300 with maxima at 3220, 2990 and 2920 (OH, amide H, amine HCl), 1740 (lactone), 1700 (amide), 1655 (pyridone), 1585, 1492, 1447, 1390, 1249, 1160, 1108, 1075, 1041, 933 and 845 cm^{-1} ; ^1H NMR (DMSO- d_6) δ 0.89 (t, 3, $J = 7$ Hz, H-18), 1.87 (m, 2, H-19), 4.02 (d, 2, $J = 5.4$ Hz, $\text{COCH}_2\text{N-}$), 5.17 (s, 2, H-5), 5.42 (s, 2, H-17), 6.32 (s, 2, $-\text{OCH}_2\text{O-}$), 07.26 (s, 1, H-14), 7.47 (s, 1, H-12), 8.38 (br s, 3, $-\text{NH}_3$), 8.59 (s, 1, H-7), 10.75 (s, 1, amide H).

Example 17 - Synthesis of 9-Glutaramido-10,11-MDOCPT
Triethanolamine Salt.

The 9-glutaramido derivative was synthesized from 9-amino-10,11-MDOCPT by the following method:

9-Glutaramido-10,11-MDOCPT.

A stirred suspension of 9-amino-10,11-MDOCPT and glutaric anhydride in pyridine under nitrogen was heated at 95°C for 2 hr. The solvent was removed from the brown solution by high vacuum distillation to give the crude amide as a brown gum. Purification was effected by chromatography through silica gel employing a solvent gradient from 5% methanol/chloroform to 50% methanol/chloroform. Evaporation of the appropriate fractions gave the 9-glutaramide half acid.

Alternatively, the 9-glutaramido derivative can be prepared by hydrolysis of its ethyl ester which is prepared by the following general method: 9-Amino-10,11-MDOCPT in

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dry N,N-dimethylformamide containing pyridine is reacted at 0-10°C with a slight excess of ethylglutaryl chloride in N,N-dimethylformamide solution. After work-up and chromatography on silica gel, the 9-(ethyl)glutaramide
5 derivative is obtained.

Example 18 Synthesis of 9-(4-methylpiperazino)
carbonylamino-10,11-MDOCPT Hydrochloride.

The title compound was prepared from 9-amino-10,11-MDOCPT in the following manner:

10 9-(4-Methylpiperazino)carbonylamino-10,11-MDOCPT.

9-Amino-10,11-MDOCPT was added to chloroform (treated with alumina to remove hydroxylic components) containing triethylamine. The resulting solution was treated with phosgene gas and filtered to remove solids. The filtrate
15 containing the intermediate carbamoyl chloride was treated with N-methylpiperazine under nitrogen and left overnight. The turbid mixture was washed several times with aqueous sodium bicarbonate solution, dried and evaporated to afford the crude title compound. Chromatography on silica gel
20 provided 9-(4-methylpiperazino)carbonylamino-10,11-MDOCPT.

9-(4-Methylpiperazino)carbonylamino-10,11-MDOCPT
Hydrochloride.

The free base urea obtained above was suspended in methanol and treated with one equivalent of dilute aqueous
25 hydrochloric acid. The methanol was evaporated and the aqueous residue filtered through a membrane filter. The sample was lyophilized to provide the title compound.

Example 19 - Synthesis of 9-(N,N-Diethylaminoethoxy)

carbonylamino-10,11-MDOCPT.

The intermediate 9-carbamoyl chloride was prepared as in the preceding example. The resulting chloroform solution was treated with N,N-diethylaminoethanol under nitrogen. After standing overnight, the mixture was washed with aqueous sodium bicarbonate solution, dried and evaporated to afford the crude carbamate. Purification by silica gel chromatography gave the pure title carbamate as the free base.

10 Example 20 - 9-(N,N-Diethylaminoethoxy)carbonylamino-10,11-MDOCPT Hydrochloride.

The free base from Example 5 was suspended in methanol and treated with one equivalent of dilute aqueous hydrochloric acid. The methanol was evaporated and the aqueous solution filtered (membrane). Lyophilization afforded the water soluble title carbamate.

15 Example 21 - 10,11-MDO-20(RS)-camptothecin Sodium Salt

The title compound was prepared from 10,11-MDO-20(RS)-camptothecin (Wani et al., J. Med. Chem. 20 29, 2358 (1986)) by hydrolytic action of sodium hydroxide. Thus, 10,11-MDO-20(RS)-CPT (77 mg, 0.194 mmol) was suspended in 90% aqueous methanol (30 mL) and treated with 0.1 N aqueous sodium hydroxide (1.94 mL, 0.194 mmol). Upon heating at 50-60°C for 1 h under nitrogen a clear solution 25 resulted which was cooled to ambient temperature and evaporated to dryness. The residue was dissolved in distilled water (2 mL) and filtered (0.45 micron membrane), and the resulting solution evaporated. The residue was recrystallized from ethanol/ether to provide the title 30 compound as a pale yellow solid (53 mg, 65%), mp > 300°C;

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IR $\nu_{\max}$ (KBr) 3400 (br), 2970, 2920, 1640, 1610, 1560-1580, 1497, 1466, 1370, 1246, 1225, 1183, 1030, 1000, 947, 855, 810, 761, 708 and 560-580; ^1H NMR (DMSO- D_6) δ 0.85 (t, 3, $J = 7$ Hz, H-18), 2.09 (m, 2, H-19), 4.74 (ABq, 2, $\Delta\nu = 68$ Hz, $J = 12, 4$ Hz, H-17), 5.12 (s, 2, H-5), 5.64 (dd, 1, $J = 4, 7$ Hz, 17-OH), 6.17 (s, 1, 20-OH), 7.47 (s, 1, H-14), 7.54 (s, 1, H-9), 7.62 (s, 1, H-12), 8.41 (s, 1, H-7).

Example 22 - 9-Amino-10,11-MDO-20(RS)-Camptothecin Sodium Salt.

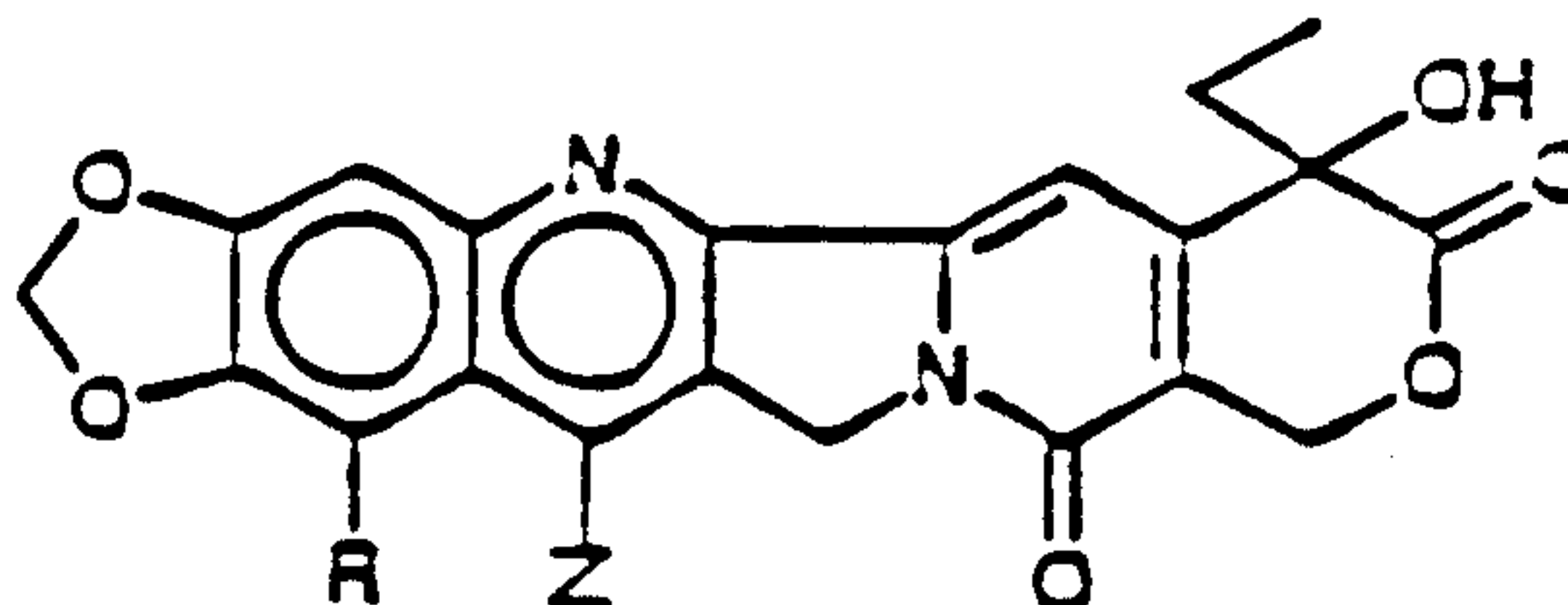
10 The title compound was prepared by an analogous alkaline hydrolysis of 9-amino-10,11-MDO-20(RS)CPT which was prepared as described above. Thus, a suspension of 9-amino-10,11-MDO-20(RS)CPT in aqueous methanol was warmed with one equivalent of aqueous sodium hydroxide to provide
15 a clear solution. Isolation as above provided the water soluble title compound as an orange-yellow solid.

The synthesis of 10,11-MDO-20(S)-CPT, a starting material for Example 1 is disclosed in J. Med. Chem., 1987, 30: 2317.

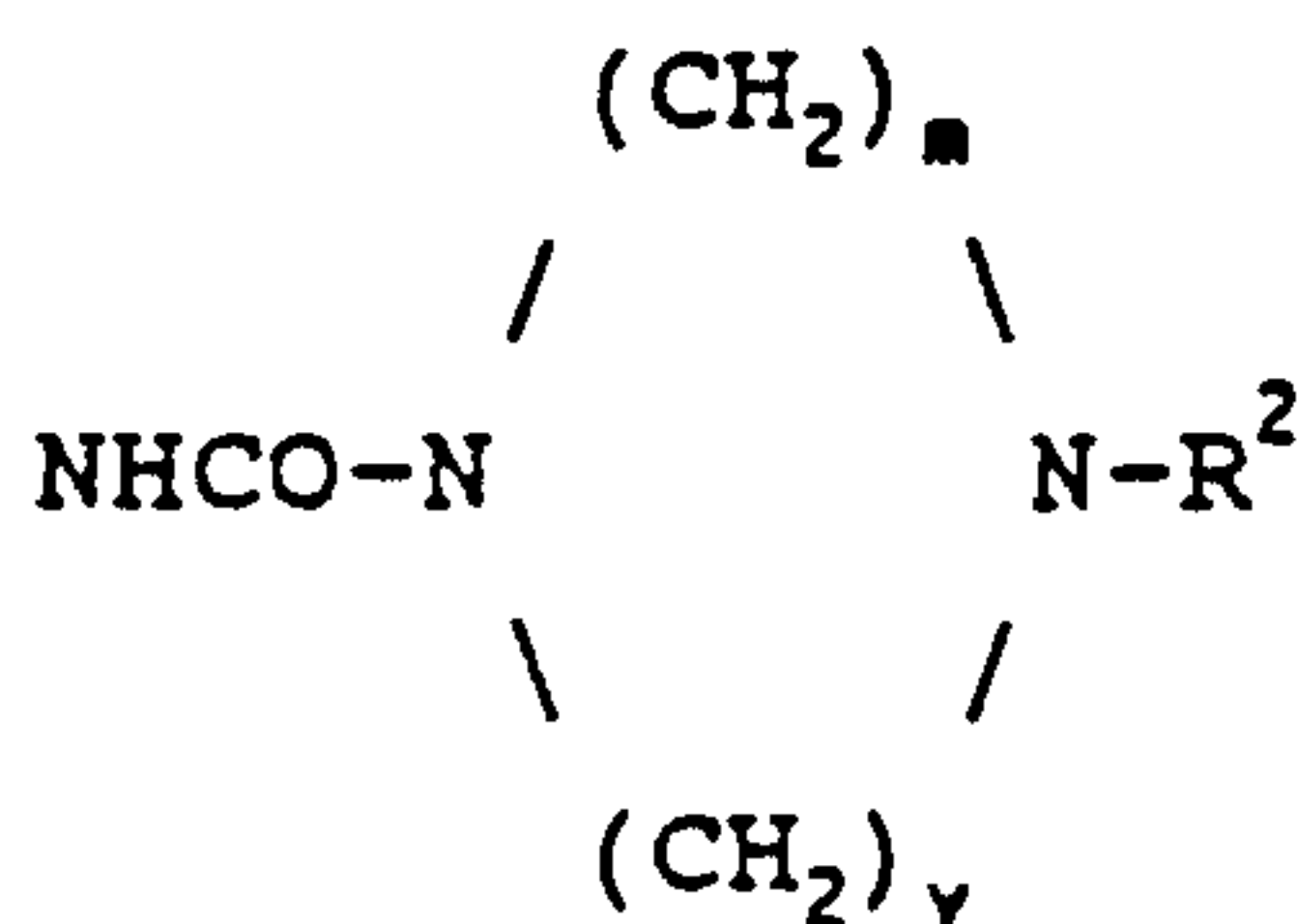
-36-

Claims

1. A 20(S) or 20(RS)-camptothecin having the structure shown below:



- wherein Z is hydrogen or C₁₋₈ alkyl, R is NO₂, NH₂, N₃,
 5 hydrogen, halogen, COOH, OH, O-C₁₋₃ alkyl, SH, S-C₁₋₃ alkyl, CN, CH₂NH₂, NH-C₁₋₃ alkyl, CH₂-NH-C₁₋₃ alkyl, N(C₁₋₃ alkyl)₂, CH₂N(C₁₋₃ alkyl)₂, O-, NH- or S-CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂CH₂N(CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂CH₂N(CH₂CH₂CH₂OH)₂, O-, NH- or S-CH₂CH₂N(C₁₋₃
 10 alkyl)₂, O-, NH- or S-CH₂CH₂CH₂N(C₁₋₃ alkyl)₂, CHO, C₁₋₃ alkyl or NHCOCHR¹NR²R³, where R¹ is a side-chain of an α-amino acid and R² and R³, independently, are hydrogen or a lower alkyl group or R³ is a peptide unit containing 1-3 amino acid units bonded to the nitrogen through a peptide bond;
 15 NHCO-C₂₋₈-alkylene-X or NHCO-C₂₋₈-alkenylene-X, where X is COOH; CONR²-(CH₂)_n-NR²R³, where n = 1-10 and R² and R³ are as defined above; NHCO-B-(CH₂)_n-NR²R³, where B = oxygen or NH;
 or



- 20 where m + y = 3-6, with the proviso that R and Z are not both hydrogen, and salts thereof.

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2. The camptothecin of Claim 1, wherein R is $\text{NHCOCHR}^1\text{NR}^2\text{R}^3$.

3. The camptothecin of Claim 2, wherein R^1 is the side chain of a naturally occurring α -amino acid.

5 4. The camptothecin of Claim 2, wherein R^2 and R^3 are, independently, hydrogen or a lower alkyl group having 1-6 carbon atoms.

5. The camptothecin of Claim 2, wherein R^3 is a peptide unit containing 1-3 amino acid units.

10 6. The camptothecin of Claim 2, wherein said side-chain of an α -amino acid is a C_{2-20} alkyl or aralkyl group.

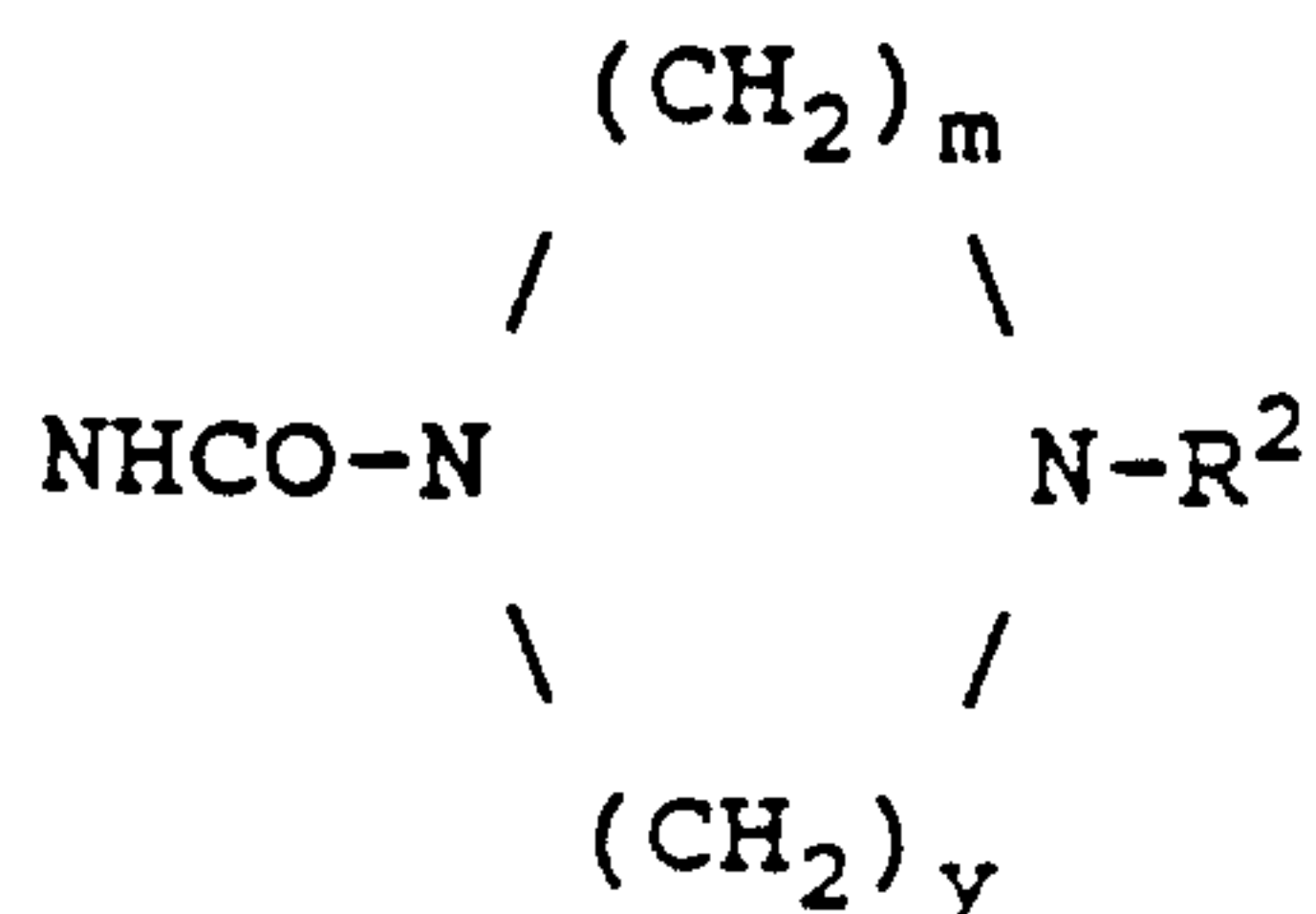
7. The camptothecin of Claim 1, wherein R is $\text{NHCO-C}_{2-8}\text{-alkylene-X}$ or $\text{NHCO-C}_{2-8}\text{-alkenylene-X}$.

15 8. The camptothecin of Claim 7, wherein X is COOH .

9. The camptothecin of Claim 7, wherein X is $\text{CONR}^2\text{-(CH}_2)_n\text{-NR}^2\text{R}^3$.

10. The camptothecin of Claim 1, wherein R is $\text{NHCO-B-(CH}_2)_n\text{-NR}^2\text{R}^3$ or

20



where B is oxygen or NH and $m + y = 3-6$.

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11. The camptothecin of Claim 10, wherein B is oxygen and $m + y = 3-4$.

12. The camptothecin of Claim 10, wherein B is NH and $m + y = 3-4$.

5 13. The camptothecin of Claim 1, wherein said salts are mineral acid or organic acid addition salts.

14. The camptothecin of Claim 1, wherein said salts are monovalent metal cation salts, ammonium salts or quaternary ammonium salts.

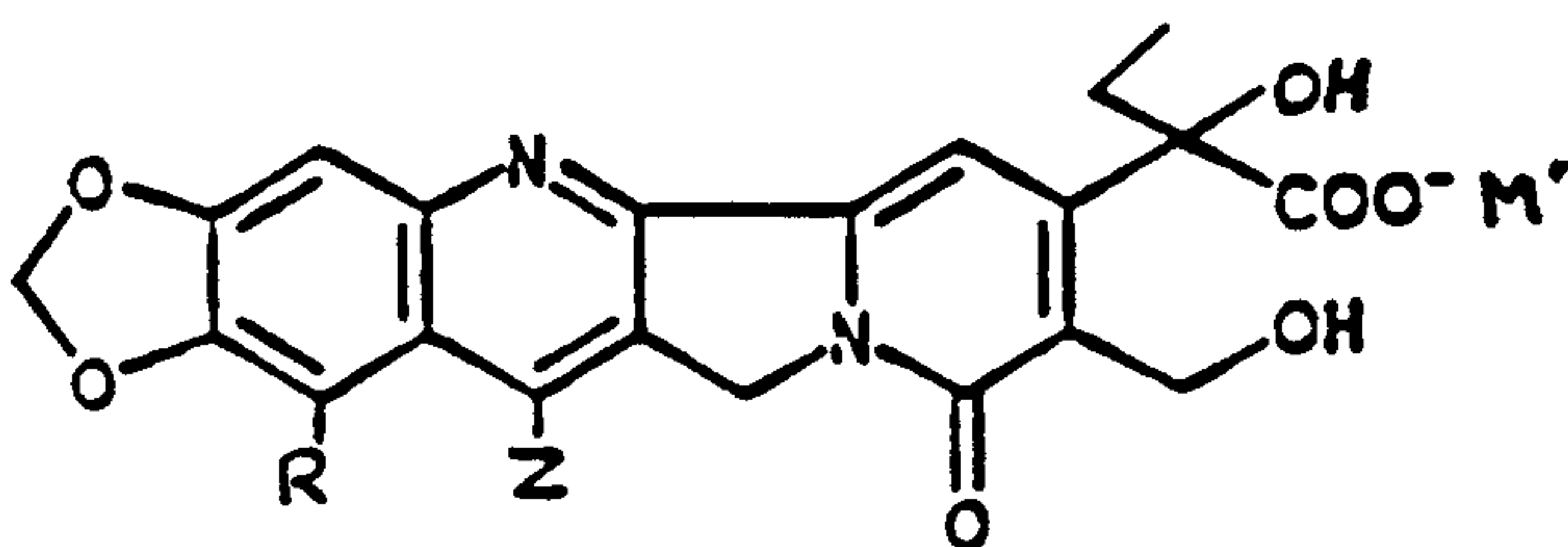
10 15. The camptothecin of Claim 1, wherein R is Cl and Z is H.

16. The camptothecin of Claim 1, wherein R is NH_2 or NO_2 and Z is H.

15 17. The camptothecin of Claim 1, wherein said camptothecin is a 20(S)-camptothecin.

18. A pharmaceutical composition comprising the camptothecin of Claim 1 and a pharmaceutically acceptable carrier.

20 19. A 20(S) or 20(RS)-camptothecin salt, wherein said salt has the structure shown below

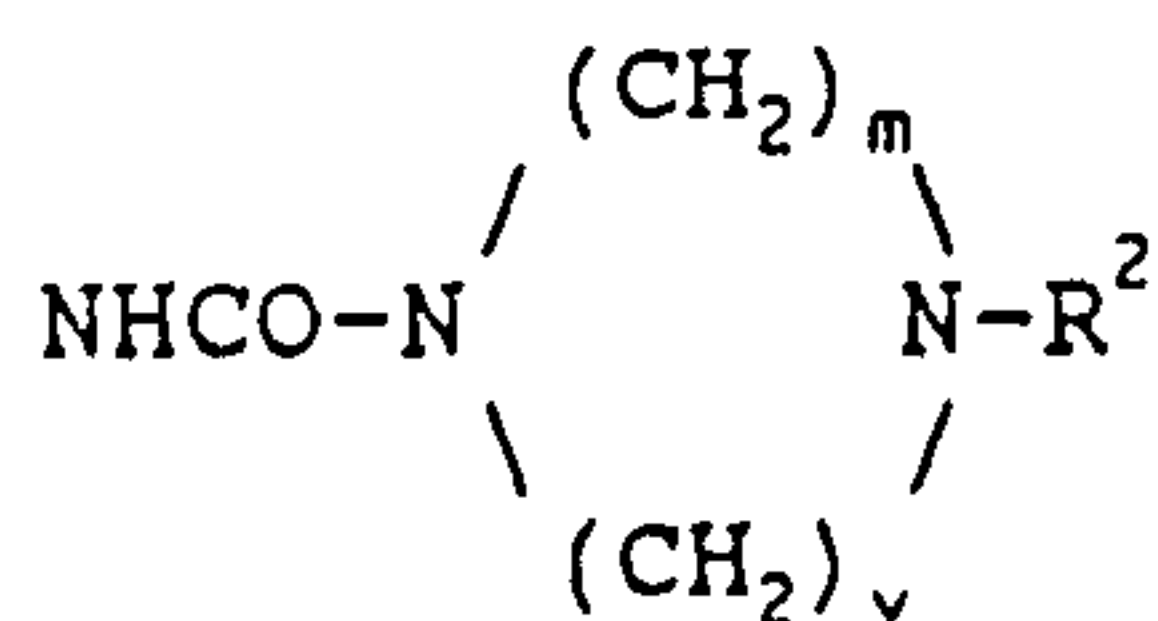


wherein Z is hydrogen or C_{1-8} alkyl, R is NO_2 , NH_2 , N_3 , hydrogen, halogen, COOH , OH , O-C_{1-3} alkyl, SH , S-C_{1-3} alkyl, CN , CH_2NH_2 , NH-C_{1-3} alkyl, $\text{CH}_2\text{-NH-C}_{1-3}$ alkyl, $\text{N(C}_{1-3}\text{ alkyl)}_2$, $\text{CH}_2\text{N(C}_{1-3}\text{ alkyl)}_2$ -, O- , NH- or $\text{S-CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2\text{OH)}_2$, O- , NH- or $\text{S-CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2\text{OH)}_2$, O- , NH- or $\text{S-CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2\text{CH}_2\text{OH)}_2$, O- , NH- or $\text{S-CH}_2\text{CH}_2\text{CH}_2\text{N(CH}_2\text{CH}_2\text{CH}_2\text{OH)}_2$, O- , NH- or $\text{S-CH}_2\text{CH}_2\text{N(C}_{1-3}\text{ alkyl)}_2$, O- , NH- or $\text{S-CH}_2\text{CH}_2\text{CH}_2\text{N(C}_{1-3}\text{ alkyl)}_2$, CHO , C_{1-3} alkyl or $\text{NHCOCHR}^1\text{NR}^2\text{R}^3$, where R^1 is a side-chain of an α -amino

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acid and R^2 and R^3 , independently, are hydrogen or a lower alkyl group or R^3 is a peptide unit containing 1-3 amino acid units bonded to the nitrogen through a peptide bond; NHCO-C₂₋₈-alkylene-X or NHCO-C₂₋₈-alkenylene-X, where X is
 5 COOH; CONR²-(CH₂)_n-NR²R³, where n = 1-10 and R^2 and R^3 are as defined above; NHCO-B-(CH₂)_n-NR²R³, where B = oxygen or NH; or

10



where $m + y = 3-6$, M^+ is a monovalent metal cation and salts thereof.

15

20. The camptothecin salt of Claim 19, wherein M^+ is a sodium cation.

21. The camptothecin salt of Claim 19, wherein R is NO₂.

20

22. The camptothecin salt of Claim 19, wherein R is NH₂.

23. The camptothecin salt of Claim 19, where R is H and Z is H.

24. The camptothecin salt of Claim 19, wherein said salt is a 20(S)-camptothecin salt.

25

25. A pharmaceutical composition, comprising the camptothecin salt of Claim 19 and a pharmaceutically acceptable carrier.

26. A method for preparing 9-nitro-10,11-methylenedioxy-20(S) or 20(RS)-camptothecin, comprising

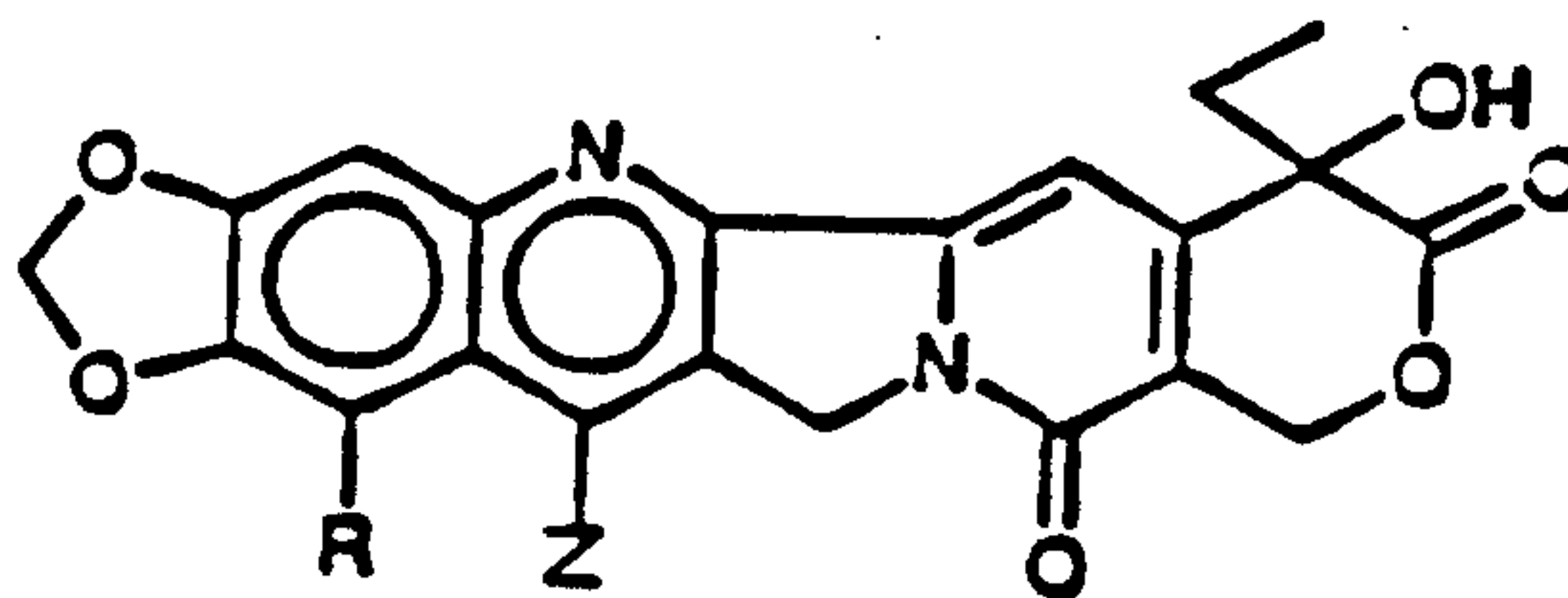
-40-

reacting 10,11-methylenedioxy 20(S) or 20(RS)- camptothecin
with a mixture of concentrated sulfuric acid and
concentrated nitric acid to obtain a product containing
substantially 9-nitro-10,11-methylenedioxy-20(S)- or
5 20(RS)-camptothecin.

27. A method for preparing 9-amino-10,11
methylenedioxy-20(S)- or 20(RS)-camptothecin, comprising
the steps of nitrating 10,11-methylenedioxy-20(S)- or
20(RS)-camptothecin to obtain 9-nitro-10,11-
10 methylenedioxy-20(S)- or 20(RS)-camptothecin, and then
reducing the 9-nitro group of said 9-nitro-10,11-
methylenedioxy-20(S)- or 20(RS)-camptothecin to obtain 9-
amino-10,11-methylenedioxy-20(S)- or 20(RS)camptothecin.

28. The method of Claim 27, wherein said reducing
15 step is conducted by catalytic hydrogenation.

29. A method for preparing a 20(S) or 20(RS)-
camptothecin having the structure shown below:

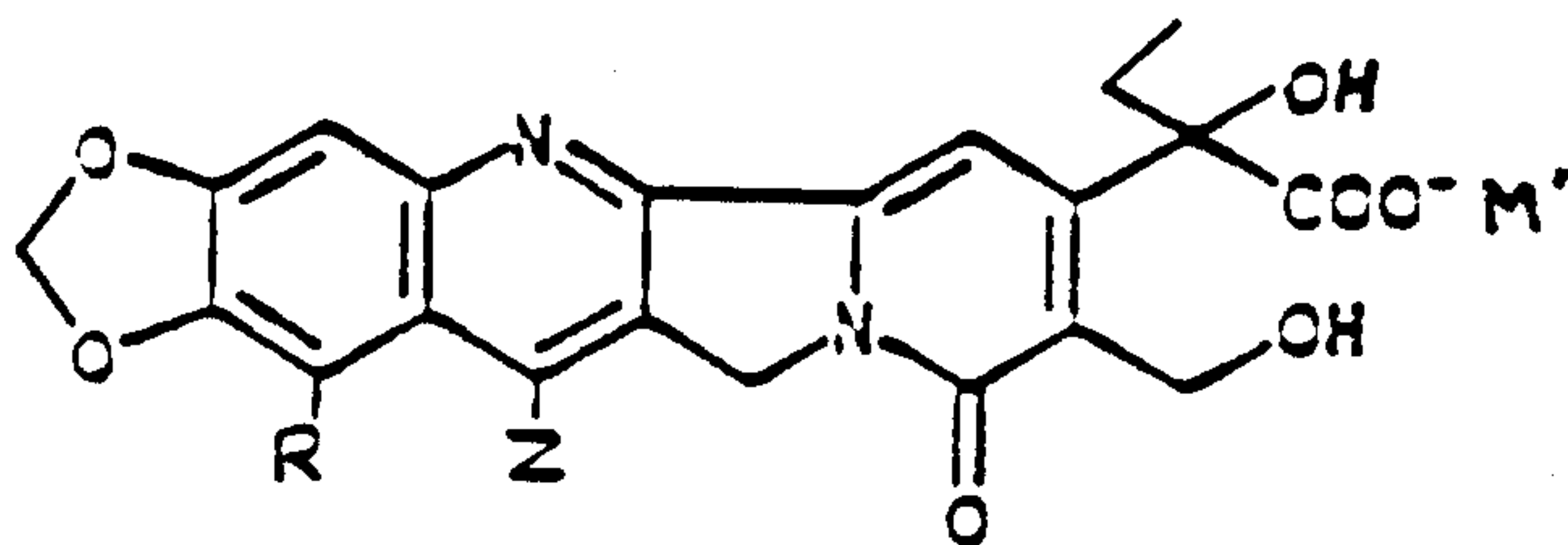


wherein Z is H or C₁₋₈ alkyl and R is NHCOCHR¹NR¹R³, where R¹
20 is a side-chain of an α-amino acid and R² and R³
independently, are hydrogen or a lower alkyl group or R³ is
a peptide unit containing 1-3 amino acid units bonded to

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the nitrogen through a peptide bond; $\text{NHCO-C}_{2-8}\text{-alkylene-X}$ or $\text{NHCOC}_{2-8}\text{-alkenylene-X}$, where X is COOH and $n = 1-10$, comprising reacting 9-amino- or 9-amino-7- C_{1-8} alkyl-10,11-methylenedioxy-20(S) or 20(RS)camptothecin with an amino
 5 group-protected amino acid or peptide containing 1-4 amino acid units, a C_{4-10} saturated or unsaturated carboxylic acid anhydride or with phosgene followed by the reaction with a primary or secondary straight-chain, branched or cyclic diamine or a tertiary-amino alcohol.

10 30. A method for preparing a 20(S) or 20(RS)-camptothecin salt having the structure shown below



wherein M^+ is a monovalent metal cation, comprising hydrolyzing the lactone ring in the camptothecin of Claim 1
 15 with an aqueous solution of a monovalent metal hydroxide.

31. Use of the camptothecin of Claim 1 for inhibiting the enzyme topoisomerase I.

32. Use of the camptothecin salt of Claim 19 for inhibiting the enzyme topoisomerase I.

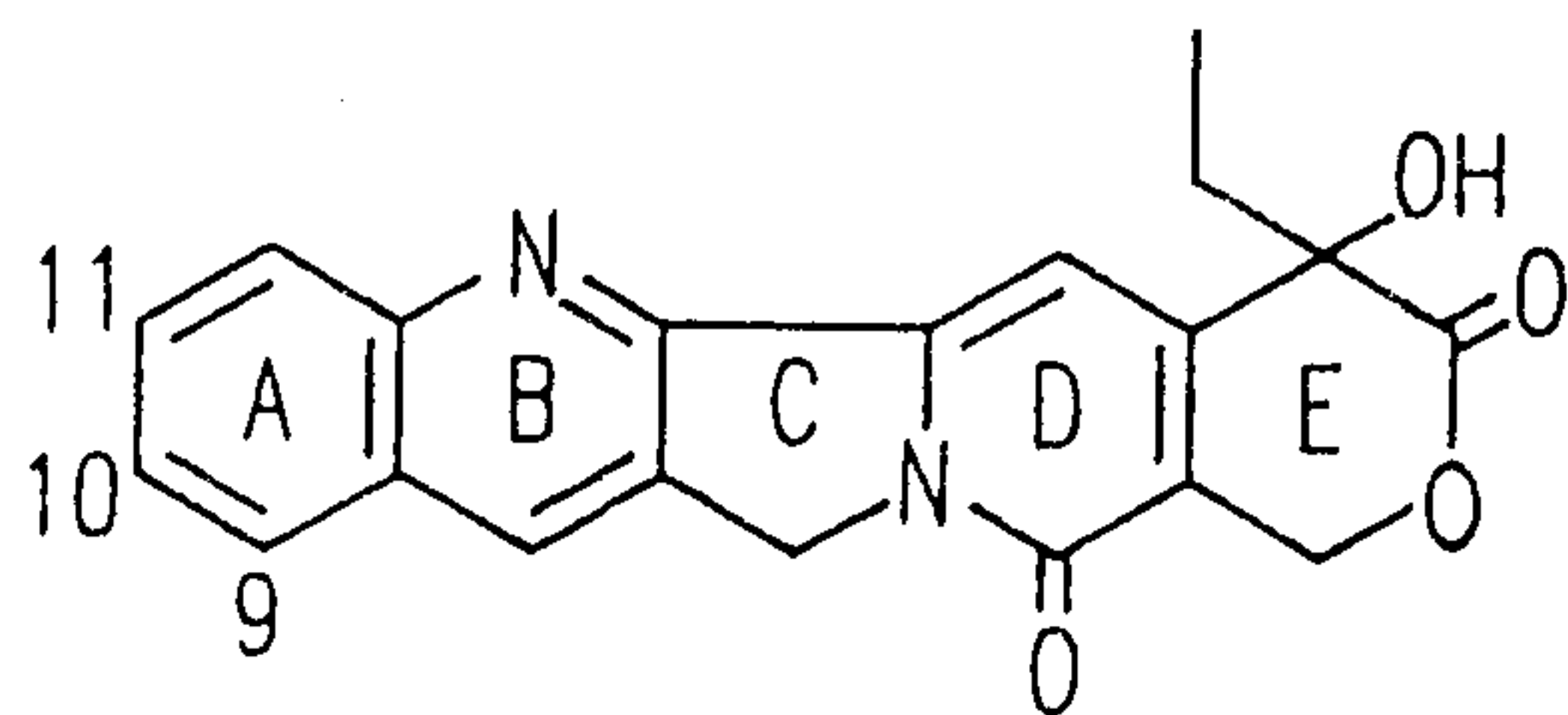
33. Use of the camptothecin of Claim 1 for treating cancer.

34. Use according to Claim 33, wherein said cancer is human colon cancer.

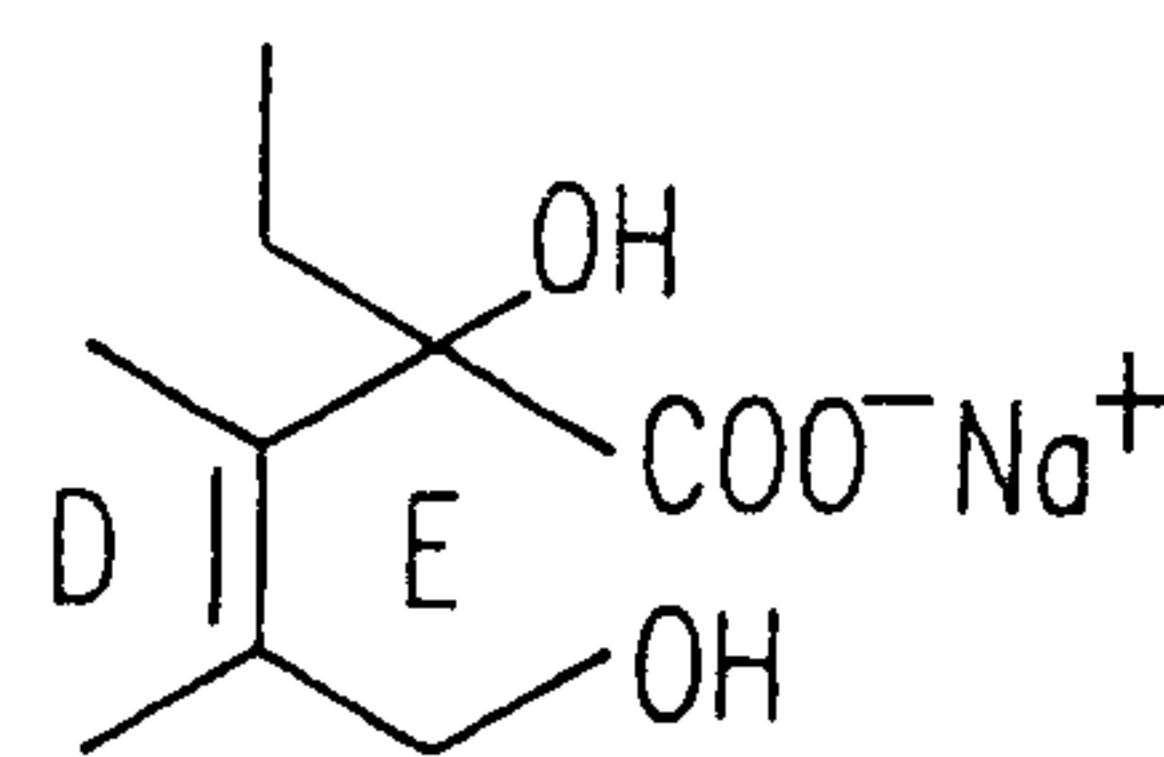
5 35. Use of the camptothecin salt of Claim 19 for treating cancer.

36. Use according to Claim 35, wherein said cancer is human colon cancer.

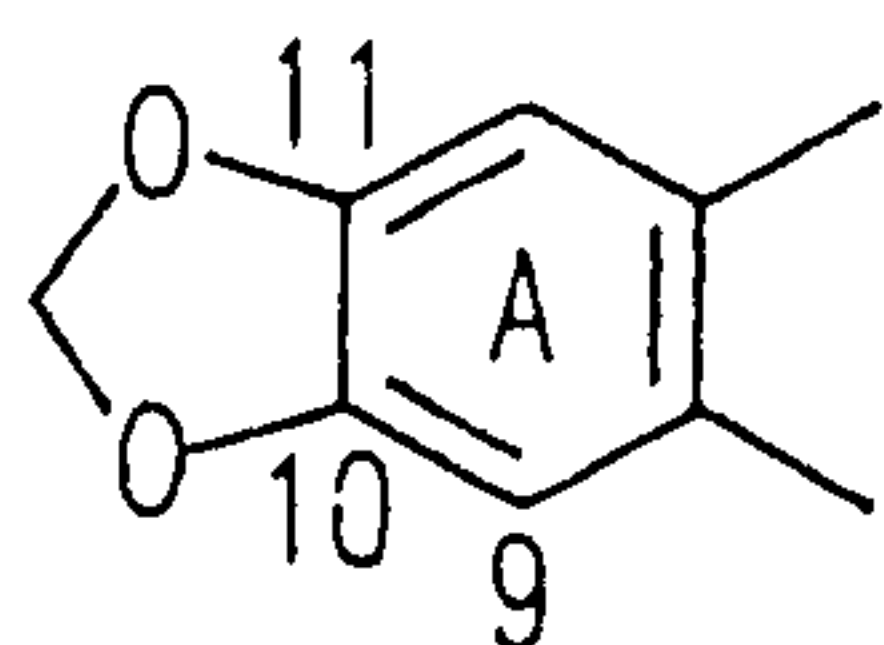
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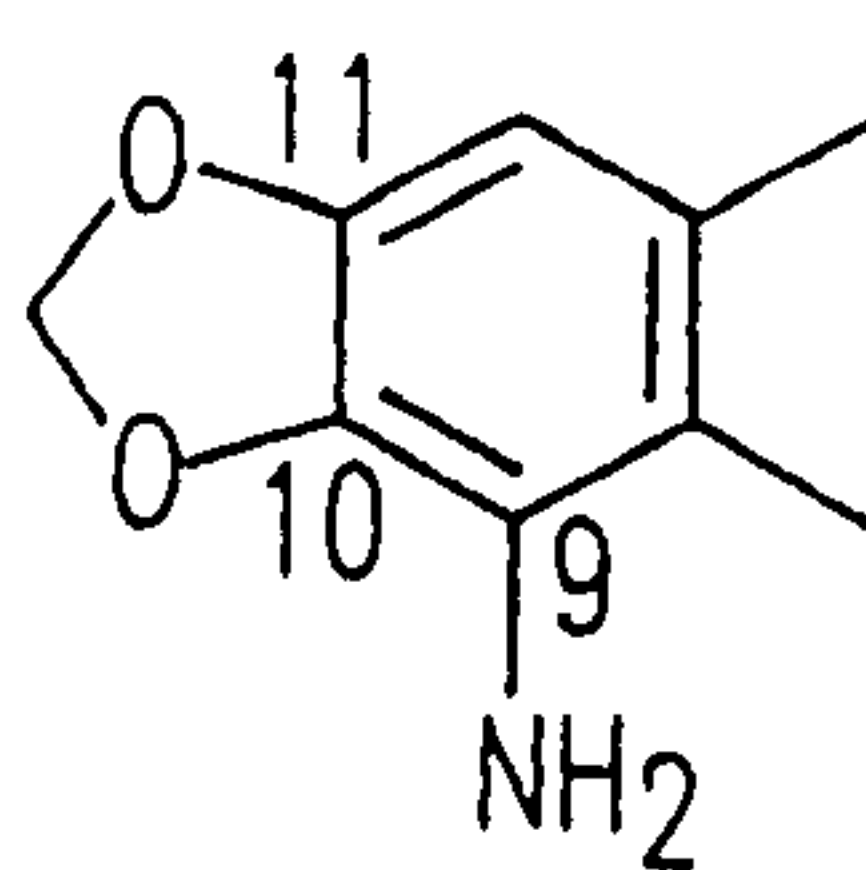
CAMPTOTHECIN (CPT)



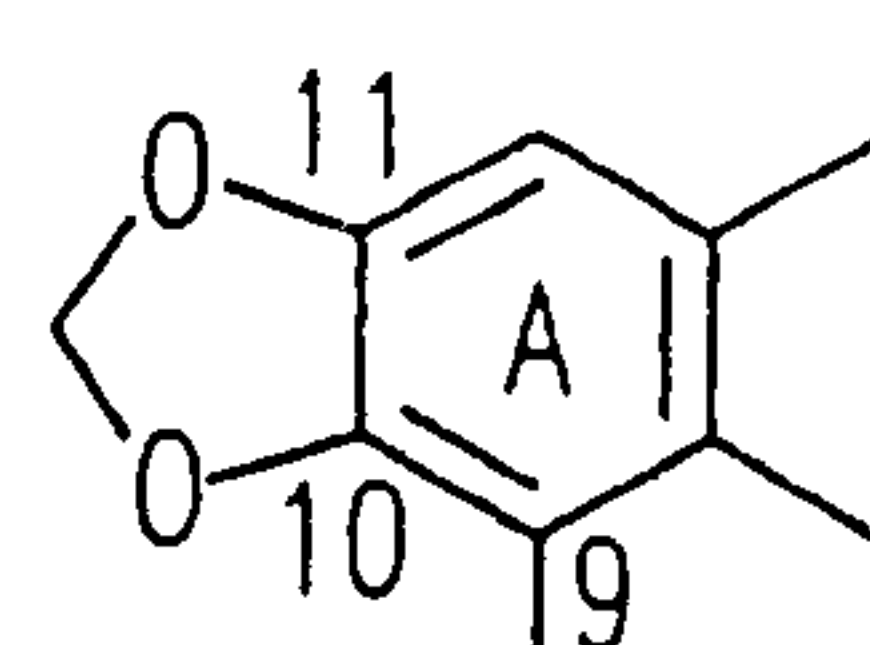
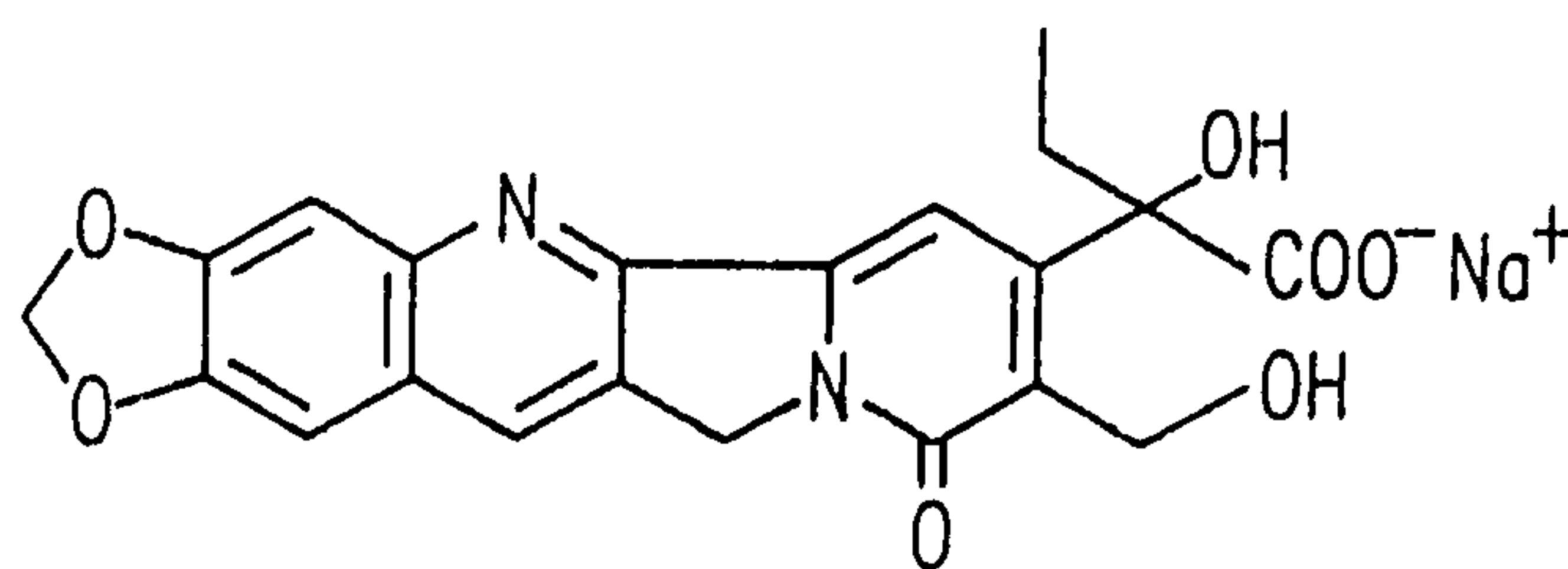
CPT-SODIUM SALT



10,11-MDO-20(RS)-CPT



9-AMINO-10,11-MDO-20(RS)-CPT

 $\text{HNC(O)CH}_2\text{NH}_2 \cdot \text{HCl}$ 9-GLYCINAMIDO-10,11-MDO-20(RS)-CPT
HYDROCHLORIDE

10-11-MDO-20(RS)-CPT-SODIUM SALT

FIG. 1
SUBSTITUTE SHEET

