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[54] **ANHYDRO- AND ISOMER-A-21978C CYCLIC PEPTIDES**

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[21] Appl. No.: **07/809,039**

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Related U.S. Application Data

[63] Continuation of application No. 07/670,375, Mar. 14, 1991, abandoned, which is a continuation of application No. 07/060,148, Jun. 10, 1987, abandoned.

[51] **Int. Cl.**[6] **A61K 38/12**; C07K 11/00

[52] **U.S. Cl.** **514/9**; 514/2; 530/317

[58] **Field of Search** 514/9, 2; 530/317

[56] **References Cited**

U.S. PATENT DOCUMENTS

Re. 32,311	12/1986	Debono	530/317
3,953,415	4/1976	Kistaludy et al.	530/306
4,399,067	8/1983	Debono	530/323
4,537,717	8/1985	Abbott et al.	530/317

OTHER PUBLICATIONS

Goodman et al, American Chemical Society, vol. 12, No. 1, (Jan. 1979), pp. 1–7.

Organic Chemistry, 3rd ed., Morrison & Boyd, p. 225.

T. Geiger et al., "Deamidation, Isomerization, and Racemization at Asparaginyl and Aspartyl Residues in Peptides," *J. Biol. Chem. 262* (2), 785–794 (1987).

M. Bodansky et al., "Side Reactions in Peptide Synthesis," *Synthesis 1981* (May), 333–338, 351–356.

E. A. Hagan et al., "Synthesis of Ac–Asp–Gly–Ser and Ac–Asp–Pro–Leu–Gly–NH_2," *Int. J. Peptide Protein Res. 23,* 642–649 (1984).

M. Bodanszky et al., "Side Reactions in Peptide Synthesis," *Int. J. Peptide Protein Res. 12,* 69–74 (1978).

B. A. Johnson et al., "Enzymatic Protein Carboxyl Methylation at Physiological pH: Cyclic Imide Formation Explains Rapid Methyl Turnover," *Biochemistry 24,* 2581–2586 (1985).

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[57] **ABSTRACT**

Two new groups of A-21978C cyclic peptides, anhydro- and isomer-A21978C peptide derivatives, have antibacterial activity and are useful as intermediates. The two groups are prepared via transpeptidation of the parent cyclic peptides. Pharmaceutical formulations containing the new peptides as active ingredients and methods of treating infections caused by susceptible Gram-positive bacteria with the formulations are also provided.

The invention also provides an antibacterial composition containing the new drug substance LY 146032 in substantially pure form.

15 Claims, No Drawings

ANHYDRO- AND ISOMER-A-21978C CYCLIC PEPTIDES

This application is a continuation of application Ser. No. 07/670,375, filed on Mar. 14, 1991, abandoned which is a continuation of application Ser. No. 07/060,148, filed on Jun. 10, 1987, abandoned.

SUMMARY OF THE INVENTION

This invention relates to two new groups of derivatives of A-21978C cyclic peptides, designated "anhydro-A-21978C peptide derivatives" (formula 1 compounds) and "isomer-A-21978C peptide derivatives" formula 2 compounds). Like the previously known A-21978C cyclic peptide derivatives (the parent cyclic peptides), the two new groups of derivatives and their salts are useful semi-synthetic antibacterial agents or are intermediates to such agents.

This invention also provides processes for preparing the anhydro- and isomer-derivatives by trans-peptidation of the parent peptides.

In another aspect, this invention provides an improved antibacterial composition comprising the new drug substance LY146032, or a pharmaceutically-acceptable salt thereof, in substantially pure form.

This invention further provides 1) methods of treating infections caused by susceptible Gram-positive bacteria which comprises administering a formula 1 or 2 compound to the animal to be treated, and 2) pharmaceutical formulations comprising a formula 1 or 2 compound or LY146032 in a pharmaceutically purified form as the active ingredient.

DETAILED DESCRIPTION OF THE INVENTION

In this specification the following abbreviations, most of which are commonly known in the art, are used:

Ala: alanine

Asn: asparagine

Asp: aspartic acid

Gly: glycine

Kyn: kynurenine

3-MG: L-threo-3-methylglutamic acid

Orn: ornithine

Ser: serine

Thr: threonine

Trp: tryptophan

t-BOC: tert-butoxycarbonyl

Cbz: benzyloxycarbonyl

DMF: dimethylformamide

THF: tetrahydrofuran

HPLC: high performance liquid chromatography

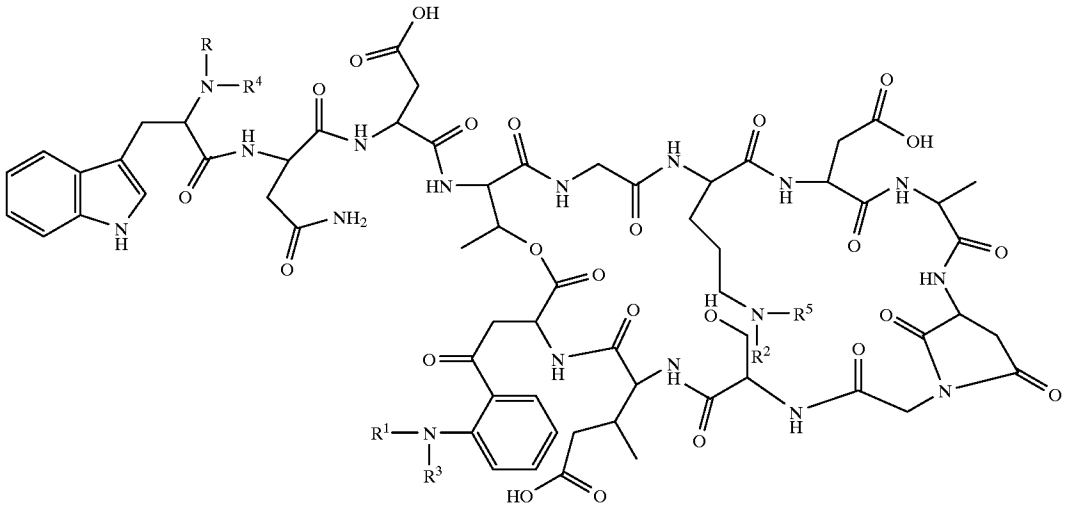
NMR: ^{1}H nuclear magnetic resonance

TLC: thin-layer chromatography

UV: ultraviolet

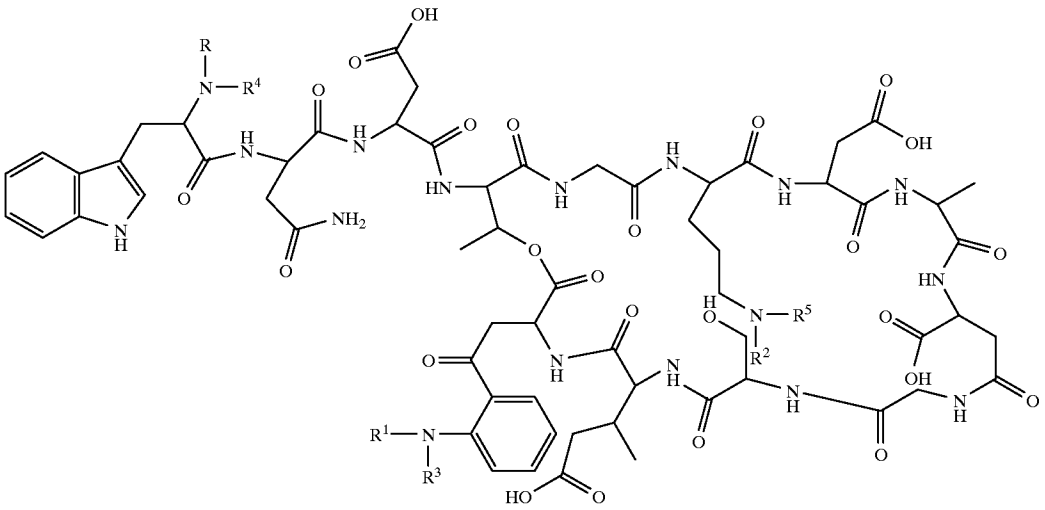
Despite the availability of antibacterial agents today, the need for improved antibiotics continues. Antibiotics differ in their effectiveness against specific pathogenic organisms. In addition, organism strains resistant to known antibiotics continue to develop. Furthermore, individual patients frequently suffer serious reactions to specific antibiotics, due to hypersensitivity and/or to toxic effects. There is, therefore, a continuing need for new and improved antibiotics.

This invention relates to new antibiotics and an improved form of the known antibiotic LY146032, which inhibit the growth of Gram-positive bacteria. In particular, the invention relates to two new groups of A-21978C cyclic peptide derivatives. The first group of derivatives, the anhydro-A-21978C peptide derivatives, are compounds which have formula 1:



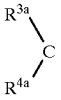
in which R, R^1 and R^2 are, independently, hydrogen, C_4–C_{14}-alkyl, optionally substituted C_2–C_{19}-alkanoyl, C_5–C_{19}-alkenoyl or an amino-protecting group; R^3, R^4 and R^5 are hydrogen or (i) R^3 and R^1 and/or (ii) R^4 and R and/or (iii) R^5 and R^2, taken together, may represent a C_4–C_{14} alkylidene group; provided that 1) at least one of R, R^1 or R^2 must be other than hydrogen or an amino-protecting group, 2) at least one of R^1 or R^2 must be hydrogen or an amino-protecting group, and 3) the R, R^1 and R^2 groups must together contain at least four carbon atoms; and their salts.

The second group of A-21978C cyclic peptide derivatives, the isomer-A-21978C peptide derivatives, are compounds which have formula 2:



in which R, R^1, R^2, R^3, R^4 and R^5 are as defined supra with the same provisos; and their salts.

The term "C_4–C_{14}-alkylidenyl" refers to a group of the formula



wherein R^{3a} and R^{4a} are hydrogen or an alkyl group of from 3 to 13 carbon atoms, provided that one of R^{3a} and R^{4a} must be other than hydrogen and further provided that the sum of the carbon atoms in R^{3a} and R^{4a} must be no greater than 13. Those compounds wherein one of R and R^4, R^1 and R^3 or R^2 and R^5 is C_4–C_{14}-alkylidenyl are known as Schiff's bases.

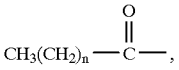
The term "C_4–C_{14}-alkyl" refers to a univalent saturated, straight- or branched-chain alkyl group containing from 4 to 14 carbon atoms. Those compounds wherein one of R, R^1 or R^2 are C_4–C_{14}-alkyl, referred to herein as "reduced Schiff's bases", are prepared by reduction of the corresponding compounds where R and R^4, R^1 and R^3 or R^2 and R^5 represent a C_4–C_{14}-alkylidenyl group.

The terms "optionally substituted C_2–C_{19}-alkanoyl" and "C_5–C_{19}-alkenoyl" refer to acyl groups derived from carboxylic acids containing from 2 to 19 and 5 to 19 carbon atoms, respectively. When the group is alkanoyl, the alkyl portion is a univalent saturated, straight-chain or branched-chain hydrocarbon radical which can optionally bear one hydroxyl, carboxyl, or C_1–C_3-alkoxy group or from one to three halo substituents selected from chlorine, bromine, and fluorine. When R is alkenoyl, the alkenyl portion is a univalent, unsaturated, straight-chain or branched-chain hydrocarbon radical containing not more than three double bonds. The double bond portion(s) of the unsaturated hydrocarbon chain may be either in the cis or trans configuration.

The term "amino-protecting group" refers to a recgonized amino-protecting group which is compatible with the other functional groups in the A-21978C molecule. Preferably, amino-protecting groups are those which can be readily removed from the subsequently acylated compound. Examples of suitable protecting groups can be found in "Protective Groups in Organic Synthesis" by Theodora W. Greene, John Wiley and Sons, New York, 1981, chapter 7. Especially preferably amino-protecting groups are the tert-butoxycarbonyl and benzyloxycarbonyl groups.

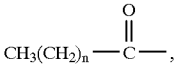
In subgeneric aspects, the invention contemplates the following preferred embodiments of the compound of formulas 1 and 2

(a) The compounds wherein R is alkanoyl of the formula



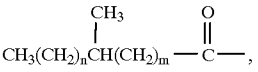
wherein n is an integer from 3 to 17;

(b) The compounds wherein R is alkanoyl of the formula



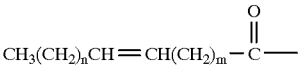
wherein n is 5 to 14;

(c) The compounds wherein R is alkanoyl of the formula



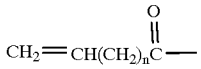
wherein n and m are each, independently, an integer from 0 to 14, provided that n+m must be no less than 1 and no greater than 15; and further provided that, when n is 0, m cannot be 8 and, when n is 1, m cannot be 6 or 8;

(d) The compounds wherein R is cis or trans alkenyl of the formula



wherein n and m are each, independently, an integer from 0 to 14, provided that n+m must be no less than 1 and no greater than 15;

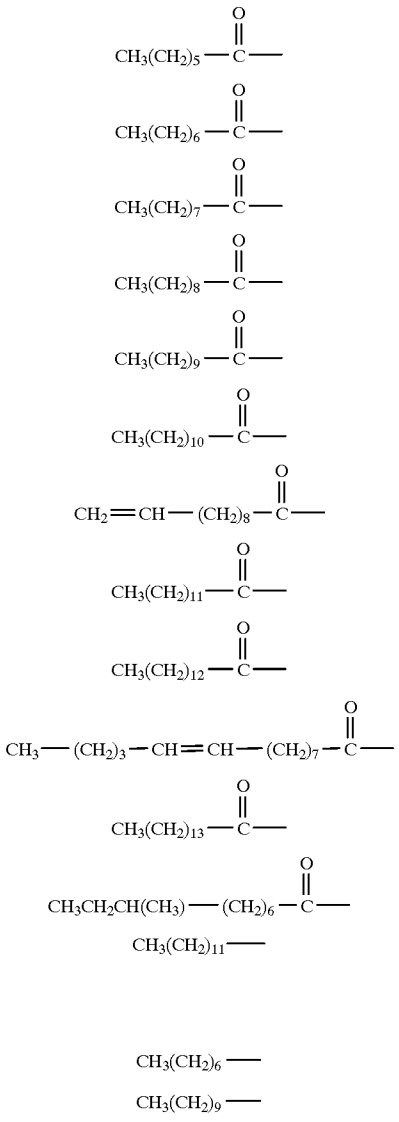
(e) The compounds where R is cis or trans alkenyl of the formula



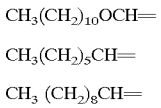
wherein n is an integer of from 4 to 15;

(f) The compounds where R is alkyl of the formula $CH_3(CH_2)_n$— and n is an integer from 5 to 12; and

(g) The compounds wherein R is:



(h) The compounds wherein R and R^4 together are:



The compounds of the formulas 1 and 2 are capable of forming salts. These salts are also part of this invention. Such salts are useful, for example, for separating and purifying the compounds.

For example, the compounds of formulas 1 and 2 have several free carboxyl groups which can form salts. Partial, mixed and complete salts of these carboxyl groups are, therefore, contemplated as part of this invention. In preparing these salts, pH levels greater than 10 should be avoided due to the instability of the compounds at such levels.

Representative and suitable alkali-metal and alkaline-earth metal salts of the compounds of formulas 1 and 2 include the sodium, potassium, lithium, cesium, rubidium, barium, calcium and magnesium salts.

The alkali-metal and alkaline-earth-metal cationic salts of the compounds for formula 1 and 2 are prepared according to procedures commonly used for the preparation of cationic salts. For example, the free acid form of a formula 1 or 2 compound is dissolved in a suitable solvent such as warm methanol or ethanol. A solution containing a stoichiometric quantity of the desired inorganic base in aqueous methanol is added to this solution. The salt thus formed can be isolated by routine methods, such as filtration or evaporation of the solvent. A convenient method of preparing salts is by the use of ion-exchange resins.

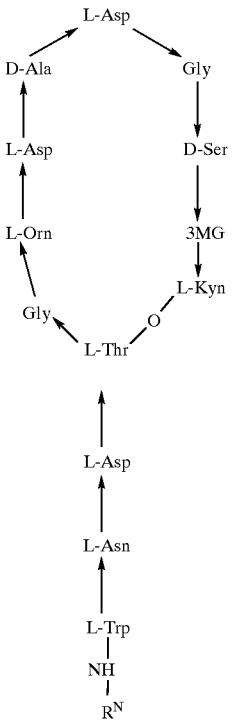
Suitable amine salts of the formula 1 and 2 compounds include the ammonium and the primary, secondary, and tertiary C_1–C_4-alkylammonium and hydroxy-C_2–C_4-alkylammonium salts. Illustrative amine salts include those formed by reaction of a formula 1 or 2 compound with ammonium hydroxide, methylamine, sec-butylamine, isopropylamine, diethylamine, di-isopropylamine, cyclohexylamine, ethanolamine, triethylamine, 3-amino-1-propanol and the like.

The salts formed with organic amines can also be prepared by well known procedures. For example, the gaseous or liquid amine can be added to a solution of a formula 1 or 2 compound in a suitable solvent such as ethanol. The solvent and excess amine can be removed by evaporation.

Because the compounds of this invention also have free amino groups, they can, therefore, form acid addition salts. Such salts are also part of this invention. Representative and suitable acid-addition salts of the compounds of formula 1 or 2 include those salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, sulfuric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, D-glutamic, d-camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic and like acids.

Pharmaceutically acceptable alkali-metal, alkaline-earth-metal, amine and acid-addition salts are a particularly useful group of compounds of this invention.

The formula 1 and 2 compounds are prepared from previously known A-21978C cyclic peptides, which in turn are prepared from the A-21978C antibiotics. The A-21978C antibiotics, a group of closely related, acidic peptide antibiotics, are described by Robert L. Hamill and Marvin M. Hoehn in U.S. Pat. No. 4,208,403, issued Jun. 17, 1980. As described in U.S. Pat. No. 4,208,403, the A-21978 antibiotic complex contains a major component, factor C, which is itself a complex of closely related factors. A-21978 factor C, which is called the A-21978C complex, contains individual factors C_0, C_1, C_2, C_3, C_4 and C_5. Factors C_1, C_2 and C_3 are major factors; and factors C_0, C_4 and C_5 are minor factors. The A-21978C factors have the structure shown in formula 3:



wherein R^N represents a specific fatty acid moiety. The specific R^N groups of the factors are as follows:

A-21978C Factor	R^N Moiety
C_1	8-methyldecanoyl
C_2	10-methylundecanoyl
C_3	10-methyldodecanoyl
C_0	C_{10}-alkanoyl*
C_4	C_{12}-alkanoyl**
C_5	C_{12}-alkanoyl**

*A-21978C_0 was later found to be a mixture of two compounds in approximately 2:1 ratio, the R^N of the major component being a branched C_{10}-alkanoyl, and the R^N of the minor component being n-decanoyl
**Identity not yet determined

The parent A-21978C cyclic peptides are prepared from the A-21978C antibiotics as described by Abbott, Manuel Debono and David S. Fukuda in U.S. Pat. No. 4,537,717. The preparation involves removing the fatty acid side chain (R^N) from the naturally occurring antibiotics with an enzyme produced by *Actinoplanes utahensis* NRRL 12052 to give the common A-21978C cyclic peptide (the A-21978C nucleus). The nucleus, or an appropriately substituted derivatives of the nucleus, is then reacylated with the desired acyl group to give the parent group of cyclic peptides.

An improved method for preparing the parent group of cyclic peptides is described by Floyd M. Huber, Richard L. Pieper and Anthony J. Tietz in the copending U.S. patent application Ser. No. 773,762, filed Sep. 9, 1985, entitled IMPROVED PROCESS FOR A-21978C DERIVATIVES.

In the parent group described by Abbott et al., one particular compound has been found to have especially outstanding activity, i.e. the compound wherein the reacylated side chain is n-decanoyl. This compound has been given the designation "LY146032".

The two groups of cyclic peptides of this invention were discovered during work with LY146032. During that work we found that the LY146032 material contained two impurities. The impurities were more pronounced when LY146032 was in solution in the pH range of 4 to 6. Our work led to the isolation of these materials and to the further discovery that they were closely related to LY146032. Like LY146032, the new compounds also have antibacterial activity. Identification of the two materials and subsequent studies showed that they were formed by a transpeptidation reaction. This reaction involves 3 compounds: 1) the starting α-aspartyl peptide (LY146032), 2) a stable intermediate and 3) the β-aspartyl isomer of LY146032.

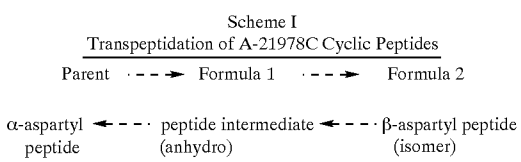
The stable intermediate was found to be the compound of formula 1 wherein R is n-decanoyl and R^1, R^2, R^3, R^4 and R^5 are hydrogen. In discussions herein, this compound is designated "anhydro-LY146032".

The third compound was found to be the β-aspartyl isomer of LY146032, i.e. the formula 2 compound wherein R is n-decanoyl and R^1, R^2, R^3, R^4, and R^5 are hydrogen. In discussions herein this compound is designated "isomer-LY146032".

Thus, the formula 1 and 2 compounds are formed by aspartyl transpeptidation of the parent cyclic peptides, which include LY146032. The transpeptidation involves two distinct, reversible steps: (1) formation of the compounds of formula 1 (the anhydro intermediates) from either the parent α-aspartyl peptide or from the formula 2 peptides (the β-aspartyl peptides) and (2) hydrolysis of the intermediate formula 1 compounds to either the parent α-aspartyl peptides or to the β-aspartyl peptides of formula 2.

The mechanism of transpeptidation involves formation of a succinimide intermediate, probably through intramolecular dehydration of the free carboxyl group of aspartic acid and the amino group of the neighboring glycine. This step is followed by nucleophilic hydroxide attack of either the α- or β-carbonyl of the succinimido intermediate which results in formation of the corresponding β- or α-aspartyl peptide. Formation of the β-aspartyl peptide predominates by a factor of 2–3, presumably because of the greater electrophilicity of the α-carbonyl of the succinimide intermediate. The transpeptidation reactions are shown in Scheme 1.

Scheme I
Transpeptidation of A-21978C Cyclic Peptides



In the preparation of formula 1 and 2 compounds, a pH range of 4-6 is optimum for the transpeptidation reactions. At pH levels below 4 and above 6, other degradation processes predominate.

In another aspect, this invention provides an improved antibacterial composition comprising the new drug substance LY146032 in substantially pure form. The term "new drug substance LY146032" refers to LY146032 in bulk pharmaceutical form prior to its formulation as a pharmaceutical. The term "in substantially pure form" refers to LY146032 which contains less than 2.5 percent of a combined total of anhydro-LY146032 and isomer-LY146032. Previously, the new drug substance LY146032 contained a combined total amount of anhydro- and isomer-LY146032 in amounts of at least 6 percent.

The new derivatives of this invention inhibit the growth of a broad spectrum of pathogenic bacteria, especially Gram-positive bacteria. Table I summarizes the minimal inhibitory concentrations (MIC's) at which the two illustrative

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