662122

CONVENTION

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

We, **GRUPPO LEPETIT S.p.A.** of Via Murat, 23, I-20159 Milano, Italy state the following in connection with Australian Application No. 11544/92:

1. We are the nominated person.

2. The nominated person is the assignee of the actual inventors.

3. The nominated person is the applicant of the basic application listed in the declaration under Article 8 of the PCT.

4. The basic application is the application first made in a Convention country in respect of the invention.

Dated: 26 March 1993

By **PHILLIPS ORMONDE & FITZPATRICK**
Patent Attorneys for the Applicant
By:

.....

To: The Commissioner of Patents

PHILLIPS ORMONDE & FITZPATRICK
367 Collins Street
Melbourne, Australia, 3000

Our Ref: 323265

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AMIDES OF ANTIBIOTIC GE 2270 FACTORS

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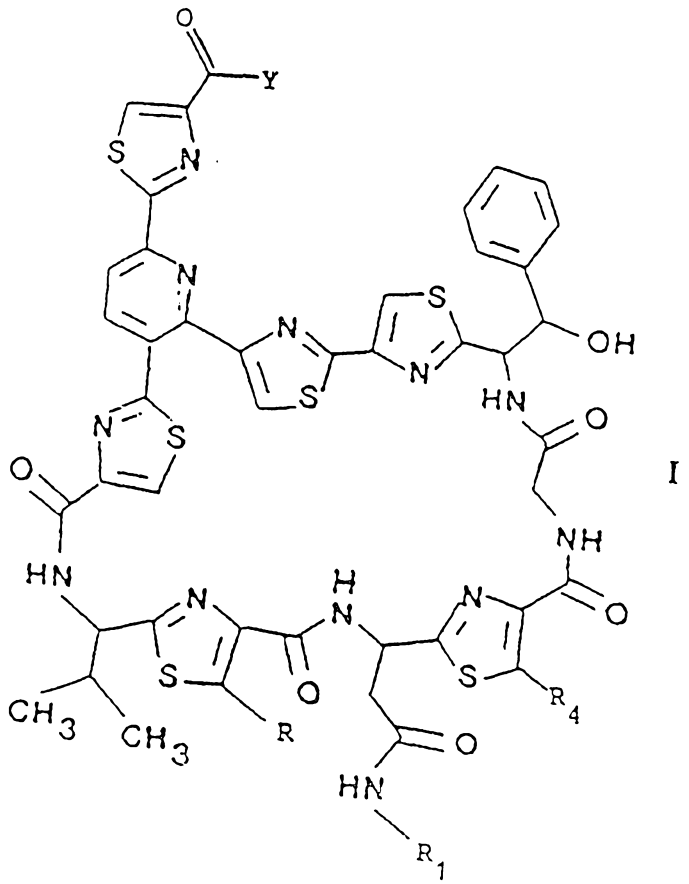
(71) Applicant(s)
GRUPPO LEPETIT S.P.A.

(72) Inventor(s)
PAOLO TAVECCHIA; SERGIO LOCIURO; ROMEO CIABATTI; ENRICO SELVA

(74) Attorney or Agent
PHILLIPS ORMONDE & FITZPATRICK , 367 Collins Street, MELBOURNE VIC 3000

(57) Claim

1. An amide derivative of antibiotic GE 2270 having the following formula I



I

.../2

wherein

R represents:

hydrogen,

hydroxymethyl, or

methoxymethyl;

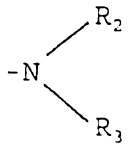
R_1 represents:

hydrogen, or

methyl;

Y represents:

a group of formula



wherein:

R_2 represents:

hydrogen,

(C_1-C_4)alkyl,

amino(C_2-C_4)alkyl,

(C_1-C_4)alkylamino-(C_1-C_4)alkyl, or

di-(C_1-C_4)alkylamino-(C_1-C_4)alkyl;

R_3 represents:

hydrogen;

a linear or branched (C_1-C_{14}) alkyl group bearing from 1 to 3 substituents selected from:

formyl,

carboxy,

sulfo,

phosphono,

amino which may be optionally protected with a lower alkoxycarbonyl or a benzyloxycarbonyl group,

(C_1-C_4)alkylamino wherein the alkyl moiety may be optionally substituted with a carboxy group,

di-(C_1-C_4)alkylamino,

hydroxy,

halo,

(C_1-C_4)alkoxy wherein the alkyl moiety may be optionally substituted with a carboxy group,

(C_1-C_4)alkoxycarbonyl,

mercapto,

(C_1-C_4)alkylthio wherein the alkyl moiety may be optionally substituted with a carboxy group,

phenyl which may be optionally substituted with 1 to 3 substituents selected from carboxy, sulfo, hydroxy, halo and mercapto,

carbamyl,

(C_1-C_6)alkylcarbamyl wherein the alkyl moiety may be optionally substituted with 1 or 2 substituents selected from carboxy, amino, (C_1-C_4)alkylamino and di-(C_1-C_4)alkylamino,

di-(C_1-C_4)alkylcarbamyl wherein the alkyl moieties together with the adjacent nitrogen atom may also represent a saturated 5-7 membered heterocyclic ring which may optionally be substituted with a carboxy or a carbamyl group on one of the ring carbons and may optionally contain a further heterogroup selected from O, S and N,

benzoylamino wherein the phenyl group may be substituted from 1 to 3 hydroxy group,

a nitrogen containing 5-6 membered heterocyclic ring which may be unsaturated, partially saturated or wholly saturated and may contain 1 to 3 further heteroatoms selected from N, S and O wherein one of the carbons of the ring may optionally bear a group carboxy, sulfo, carboxy(C_1-C_4)alkyl and sulfo (C_1-C_4)alkyl and

.../4

the ring nitrogen atom may optionally be substituted by (C_1-C_4)alkyl, carboxy(C_1-C_4)alkyl, sulfo(C_1-C_4)alkyl, and benzyl;
(C_3-C_6)alkenyl, optionally substituted by carboxy or sulfo;
1-deoxy-1-glucityl;
2-deoxy-2-glucosyl;
a fully saturated 5 to 7 membered nitrogen containing heterocyclic ring wherein the nitrogen atom may be optionally substituted by (C_1-C_4)alkyl or benzyl and one or two carbons of the ring skeleton may bear a substituent selected from (C_1-C_4)alkyl, carboxy and sulfo;

or R_2 and R_3

taken together with the adjacent nitrogen atom represent a fully saturated 5-7 membered heterocyclic ring which may optionally contain a further heteroatom selected from O, S and N, and may optionally bear one or two substituents on the ring carbons selected from (C_1-C_4)alkyl, benzyl, carboxy, sulfo, carboxy(C_1-C_4)alkyl, and sulfo(C_1-C_4)alkyl;

R_4 represents:
hydrogen,
methyl, or
hydroxymethyl;

with the proviso that when R_4 is hydrogen or hydroxymethyl, then simultaneously R is methoxymethyl and R_1 is methyl;

and the pharmaceutically acceptable addition salts thereof.

15. A pharmaceutical composition containing a compound of any of claims 1 to 10 as the active ingredient in admixture with a pharmaceutically acceptable carrier.

CORRECTED VERSION*

662122

PCT

WORLD | page 73, description, replaced by correct page 73

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(71) Applicant *(for all designated States except US)*: GRUPPO LEPETIT S.P.A. [IT/IT]; Via Murat, 23, I-20159 Milano (IT).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : TAVECCHIA, Paolo [IT/IT]; Via Buon Gesù, 1, I-20017 Rho (IT). LOCIURO, Sergio [IT/IT]; Via Troilo, 4, I-20136 Milano (IT). CIABATTI, Romeo [IT/IT]; Via Brodolini, 15/A, I-20026 Novate Milanese (IT). SELVA, Enrico [IT/IT]; Via Di Vittorio, 15, I-27027 Gropello Cairoli (IT).

(74) Agent: PIMPINELLI, Natale; Gruppo Lepetit S.p.A., Patent & Trademark Department, Via Roberto Lepetit, 34, I-21040 Gerenzano (IT).

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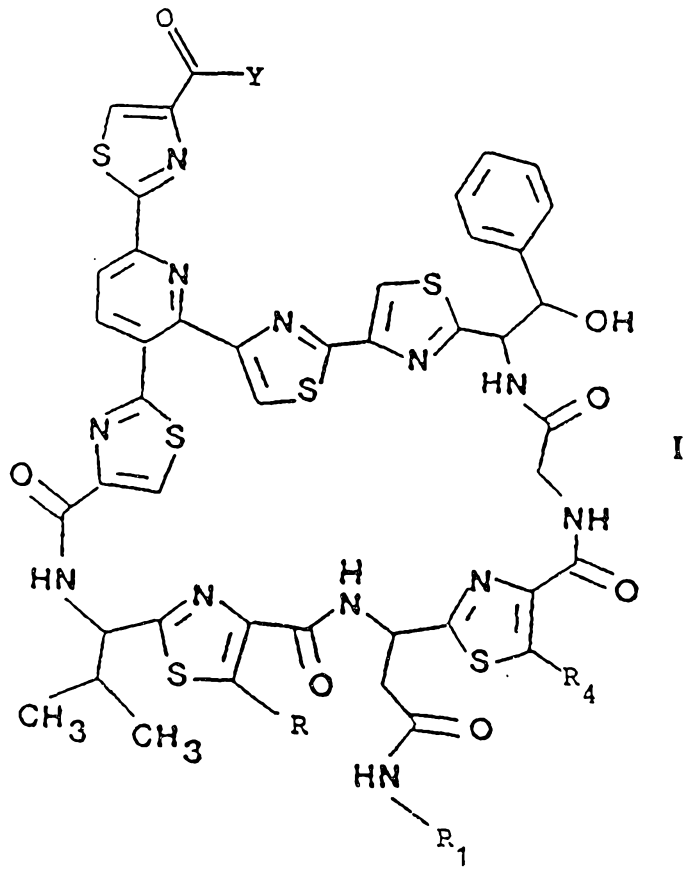
(54) Title: AMIDES OF ANTIBIOTIC GE 2270 FACTORS

(57) Abstract

The present invention is directed to novel amide derivatives of antibiotic GE 2270 compounds and a process for preparing them. Said amide derivatives are antimicrobial agents active against gram positive bacteria as well as gram negative bacteria.

AMIDES OF ANTIBIOTIC GE 2270 FACTORS

The present invention is directed to novel amide derivatives of antibiotic GE 2270 having the following formula I



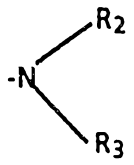
I

wherein

R represents:
hydrogen,
hydroxymethyl, or
methoxymethyl;

R_1 represents:
hydrogen, or
methyl;

Y represents:
a group of formula



wherein:

R_2 represents:
hydrogen,
(C_1-C_4)alkyl,
amino(C_2-C_4)alkyl,
(C_1-C_4)alkylamino-(C_1-C_4)alkyl, or
di-(C_1-C_4)alkylamino-(C_1-C_4)alkyl;

R_3 represents:
hydrogen,
a linear or branched (C_1-C_{14})alkyl group bearing
from 1 to 3 substituents selected from: carboxy,

sulfo, phosphono, amino which may be optionally protected with a lower alkoxycarbonyl or a benzyloxycarbonyl group, (C_1-C_4)alkylamino wherein the alkyl moiety may be optionally substituted with a carboxy group, di-(C_1-C_4)alkylamino, hydroxy, halo, (C_1-C_4)alkoxy wherein the alkyl moiety may be optionally substituted with a carboxy group, (C_1-C_4)alkoxycarbonyl, mercapto, (C_1-C_4)alkylthio wherein the alkyl moiety may be optionally substituted with a carboxy group, phenyl which may be optionally substituted with 1 to 3 substituents selected from carboxy, sulfo, hydroxy, halo and mercapto, carbamyl, (C_1-C_6)alkylcarbamyl wherein the alkyl moiety may be optionally substituted with 1 or 2 substituents selected from carboxy, amino, (C_1-C_4)alkylamino and di-(C_1-C_4)alkylamino, di-(C_1-C_4)alkylcarbamyl wherein the alkyl moieties together with the adjacent nitrogen atom may also represent a saturated 5-7 membered heterocyclic ring which may optionally be substituted with a carboxy or a carbamyl group on one of the ring carbons and may optionally contain a further heterogroup selected from O, S and N, benzoylamino wherein the phenyl group may be substituted from 1 to 3 hydroxy group, a nitrogen containing 5-6 membered heterocyclic ring which may be unsaturated, partially saturated or wholly saturated and may contain 1 to 3 further heteroatoms selected from N, S and O wherein one of the carbons of the ring may optionally bear a group carboxy, sulfo, carboxy(C_1-C_4)alkyl and sulfo(C_1-C_4)alkyl and

the ring nitrogen atom may optionally be substituted by (C_1-C_4)alkyl, carboxy(C_1-C_4)alkyl, sulfo(C_1-C_4)alkyl, and benzyl;

(C_3-C_6)alkenyl, optionally substituted by carboxy or sulfo;

1-deoxy-1-glucityl;

2-deoxy-2-glucosyl;

a fully saturated 5 to 7 membered nitrogen containing heterocyclic ring wherein the nitrogen atom may be optionally substituted by (C_1-C_4)alkyl or benzyl and one or two carbons of the ring skeleton may bear a substituent selected from (C_1-C_4)alkyl, carboxy and sulfo;

or R_2 and R_3

taken together with the adjacent nitrogen atom represent a fully saturated 5-7 membered heterocyclic ring which may optionally contain a further heteroatom selected from O, S and N, and may optionally bear one or two substituents on the ring carbons selected from (C_1-C_4)alkyl, benzyl, carboxy, sulfo, carboxy(C_1-C_4)alkyl, and sulfo(C_1-C_4)alkyl;

R_4 represents:

hydrogen,

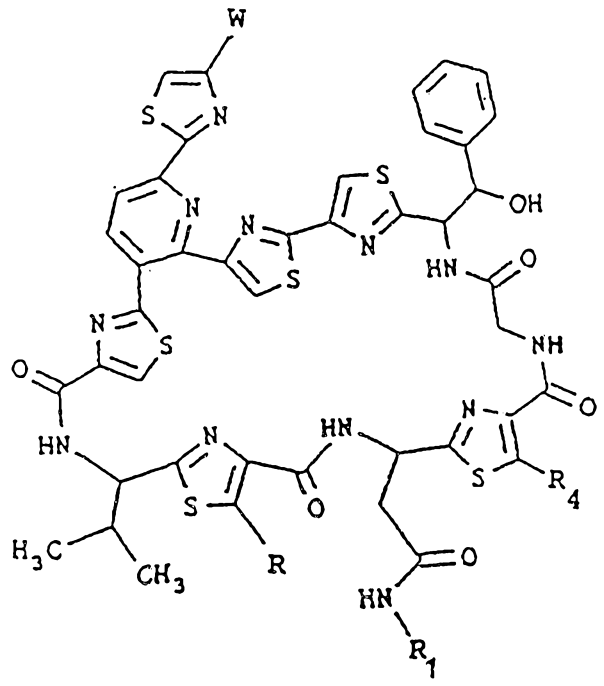
methyl, or

hydroxymethyl;

with the proviso that when R_4 is hydrogen or hydroxymethyl, then simultaneously R is methoxymethyl and R_1 is methyl;

and the pharmaceutically addition salts thereof.

This invention includes also a process for preparing the compounds of this invention from the corresponding starting compounds of formula (II)



II

wherein W is a carboxylic function or an activated ester thereof.

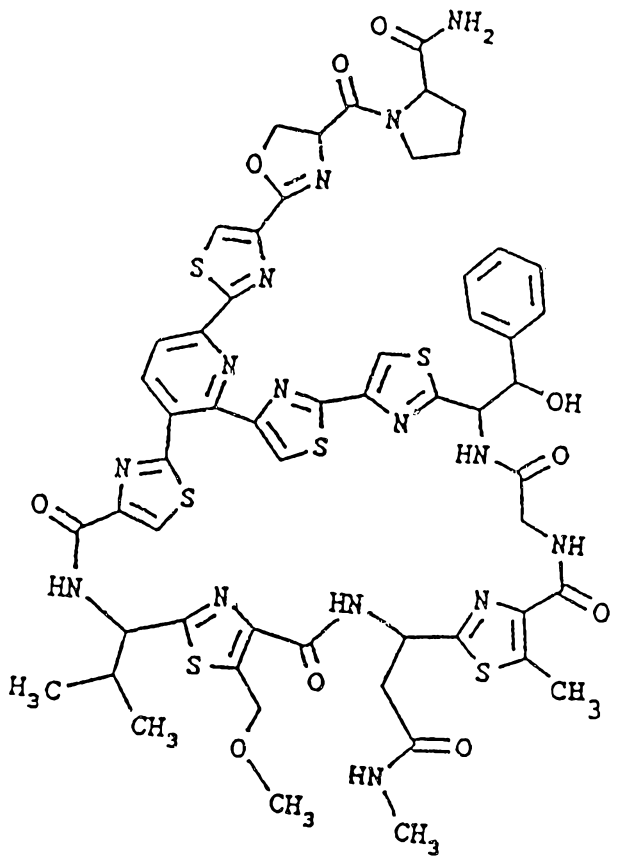
Antibiotic GE 2270 is prepared by culturing a sample of Planobispora rosea ATCC 53773 or a producing variant or mutant thereof and isolating the desired antibiotic substance from the mycelium and/or the fermentation broth. Planobispora rosea ATCC 53773 was isolated from a soil sample and deposited on June 14, 1988 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 Maryland, U.S.A., under the provisions of the Budapest Treaty.

The strain has been accorded accession number ATCC 53773.

Antibiotic GE 2270 factor A is the main component of the antibiotic GE 2270 complex.

Antibiotic GE 2270 factor A and Planobispora rosea ATCC 53773 are described in European Patent Application Publication No. 359062.

Recent studies showed that antibiotic GE 2270 factor A can be represented by the following general formula III



III

When antibiotic GE 2270 factor A is treated under selective hydrolysis conditions some derivatives named antibiotic GE 2270 factor A_1, A_2 and A_3 are obtained. Said factors A_1, A_2 and A_3 and the hydrolysis process for preparing them is disclosed in the European

Patent Application Publication No. 406745 and U.S. Patent Application No. 547,647.

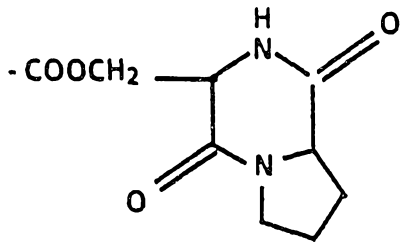
Generally, the above mentioned hydrolytic conditions involve the use of mixtures of buffered or unbuffered aqueous acid media and polar organic solvents. The reaction temperature varies depending on factors such as the strength and the concentration of the acid employed, and is generally comprised between -10°C and 90°C. Also the reaction time varies considerably depending on parameters such as the temperature, the acid strength and its concentration; generally, it may vary from a few minutes to several hours.

In general, when milder hydrolysis conditions are employed, e.g. shorter reaction time and lower temperature or lower acid strength or concentration, antibiotic GE 2270 factor A_1 is normally obtained, while stronger hydrolysis conditions yield antibiotic GE 2270 factor A_2. To obtain antibiotic GE 2270 factor A_3, still more drastic hydrolysis conditions are necessary.

While antibiotic GE 2270 factors A_2 and A_3 can be directly utilized as the starting materials for the production of the compounds of this invention, antibiotic GE 2270 factor A_1 is not suitable as the starting material for direct production of the compounds of this invention; however, it can be utilized as a precursor of the said starting materials as it will be explained further.

Antibiotic GE 2270 factors A_2 and factor A_3 are characterized by having an ester and a carboxy function respectively in the upper part of the molecule. In particular, it has been found that antibiotic GE 2270 factor A_2 and factor A_3 can be represented by the above defined formula II wherein:

W represents COOH (antibiotic GE 2270 factor A_3) or the ester moiety (antibiotic GE 2270 factor A_2)



R is methoxymethyl,
R_1 is methyl and
R_4 is methyl.

Both antibiotic GE 2270 factor A_2 and factor A_3 (and mixture thereof) can be used as suitable starting materials for the production of the compounds of the invention, even if factor A_3 is the preferred one. Factor A_2 may be employed directly as an activated ester or may be converted to factor A_3 by drastic acid hydrolysis conditions, as mentioned above, or by basic

hydrolysis with diluted alkali (as described in European Patent Application Publication No. 406745 and U.S. Patent Application No. 547,647).

It was recently found (European Patent Application Publication No.451486 and U.S. Patent Application No. 665,612) that other minor components can be isolated from the cultures of Planobispora rosea ATCC 53773 or an antibiotic GE 2270 producing variant or mutant thereof. In particular, they are found in the mycelium and also in the fermentation broths of the cultured microorganism.

A preferred procedure for recovering said minor components of antibiotic GE 2270 from the mycelium includes extracting the filtered or centrifugated mycelium with a water-miscible organic solvent, concentrating the extracts and recovering the crude antibiotic substance by precipitation, optionally with the addition of a precipitating agent, by extraction of the aqueous residue with a water-immiscible organic solvent or by adsorption chromatography followed by elution of the desired product from the absorption matrix.

It was recently found (European Patent Application No. 91114667.8) that a further minor component (factor C_{2a}) can be isolated from the same culture of Planobispora rosea ATCC 53773 described above.

The physico-chemical characteristics of antibiotic GE 2270 C_{2a} are the following:

A) The ultraviolet absorption spectrum recorded with a Perkin Elmer Model 320 spectrometer exhibit the following absorption maxima:

Solvent	UV max (nm)
0.1 M HCl	245-250 (shoulder) 300-315
0.1 M KOH	245-250 (shoulder) 300-315
Phosphate buffer pH 7.38	245-250 (shoulder) 300-315
Methanol	245-250 (shoulder) 300-315

B) The ^{1}H-NMR spectrum of antibiotic GE 2270 factor C_{2a} was recorded at 250 MHz with a Bruker spectrometer. The spectrum of the antibiotic in DMSO-d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm) exhibits the following groups of signals [δ, ppm, m] (s=singlet, d=doublet, t=triplet, m=multiplet, Py=pyridine, Tz=thiazole)

9.03, d, (NH); 8.70, d, (2NH's); 8.60, s, 8.54, s, 8.29, s, and 7.38, s, (Tz CH's); 8.48, m, (glycine NH); 8.43, d, and 8.27, d, (Py CH's);

7.35-7.20, m, (aromatic CH's and primary amide NH); 6.98, s (primary amide NH); 6.04, d, (OH); 5.80, t (OH); 5.35-5.15, m, (αCH's); 5.04, m, (phenylserine βCH); 4.98, s [$CH_2(OCH_3)$]; 4.87, d, [$CH_2(OH)$]; 4.81, m and 4.56, m, (oxazoline CH_2); 4.35-3.75, m, (CH_2 of glycine and prolineamide CH's); 3.39,s, (OCH_3); 2.71, m, and 1.30, m, (CH_2 of asparagine); 2.48, d, (NCH_3 of N-methylasparagine); 2.22-1.80, m, (isopropyl CH and prolineamide CH's); 0.88 and 0.84, d, (valine CH_3's)

C) Antibiotic GE 2270 factor C_{2a} shows retention time (R_t) of 12.6 min and retention time relative to antibiotic GE 2270 factor A (R_t 16.6 min) of 0.76 when analyzed with the following reverse phase HPLC system:

Column: Bakerbond® C8 (5 µm) 4.6x250 mm (Bakerbond® is a trade name for reverse phase octylsilyl silica gel HPLC columns supplied by J.T. Baker Research Product, Phillisburg, New Jersey 08865 USA)

Flow rate: 1.8 ml/min

Phase A: CH_3CN:tetrahydrofuran:40 mM $HCOONH_4$ 40:40:20

Phase B: CH_3CN:tetrahydrofuran:40 mM $HCOONH_4$ 10:10:80

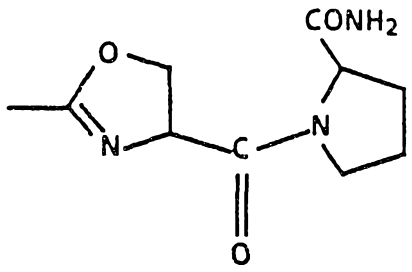
Elution: linear gradient from 20% to 30% of Phase A in 20 min

Detection: UV 254 nm

D) The main FAB-MS peak of antibiotic GE 2270 factor C_{2a} is 1306 daltons. This corresponds most likely to the lowest isotope of the protonated molecular ion. The analysis was performed on a Kratos MS-50 double focusing mass spectrometer, using 8 kV accelerating voltage and a saddle field atom gun with Xe gas ($2x10^{-5}$ torr pressure indicated on the source ion guage) at 6 kV voltage and 1 mA current. The antibiotic for the FAB-MS analysis was mixed with a thioglycerol matrix containing 0.1 M acetic acid.

Some of said minor components of antibiotic GE 2270 (i.e. factors B_1, B_2, C_1, C_2, C_{2a}, D_1, D_2 and E) may be represented by the general formula II mentioned above wherein

W represents the moiety:



R represents respectively hydrogen for GE 2270 factors C_1 and D_1, methyl for factor B_2, hydroxymethyl for factors D_2 and E and methoxymethyl for factors B_1, C_2 and C_{2a};

R_1 represents hydrogen for GE 2270 factors B_1, D_1 and E and methyl for GE 2270 factors B_2, C_1, C_2, C_{2a} and D_2; and

R_4 represents hydrogen for GE 2270 factor C_2, methyl for GE 2270 factors B_1, B_2, C_1, D_1, D_2 and E and hydroxymethyl for factor C_{2a}.

When antibiotic GE 2270 factors D_1, D_2 and E or mixture thereof are treated by the same hydrolytic process outlined above (and described in European Patent Application Publication No. 406745 and U.S. Patent Application No. 547,647) for preparing antibiotic

GE 2270 factors A_2 and A_3 from antibiotic GE 2270 factor A, the common moiety W cited above is hydrolyzed to a carboxy moiety leaving the substituents R, R_1 and R_4 unaltered.

Therefore, the derivatives of formula II wherein W is a carboxy or an activated ester function, R is hydrogen, hydroxymethyl or methoxymethyl, R_1 is hydrogen or methyl and R_4 is hydrogen, methyl or hydroxymethyl, with the proviso that when R_4 is hydrogen or hydroxymethyl then R is methoxymethyl and R_1 is methyl, can be used as starting material of the present invention. It has to be clear that as with other microorganisms, the characteristics of the GE 2270 producing strains are subject to variation. For example, artificial variants and mutants of the strain can be obtained by treatment with various known mutagens, such as U.V. rays, X-rays, high frequency waves, radioactive rays, and chemicals such as nitrous acid, N-methyl-N'-nitro-N-nitroso-guanidine, and many others. All natural and artificial variants and mutants which belong to a species of the genus Planobispora and produce antibiotic GE 2270 are deemed equivalent to strain Planobispora rosea ATCC 53773 for the purposes of this invention.

As used herein, the term "alkyl", either alone or in combination with other substituents, includes both straight and branched hydrocarbons groups; more particularly, "(C_1-C_{14})alkyl" represents a straight or branched aliphatic hydrocarbon chain of 1 to 14 carbon atoms such as methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 1,1-dimethylethyl, pentyl, 1-methylbutyl, 2-methylbutyl, 1-hexyl, 2-hexyl, 3-hexyl, 3,3-dimethyl-1-butyl, 4-methyl-1-pentyl and 3-methyl-1-pentyl, heptyl, octyl, nonyl, decyl, undecyl,

dodecyl, tridecyl and tetradecyl; likewise, "(C_1-C_4)alkyl" represents a straight or branched hydrocarbon chain of 1 to 4 carbon atoms such as those alkyl of 1 to 4 carbons exemplified above.

As described above the "(C_1-C_{14})alkyl" moiety may bear 1 to 3 substituents.

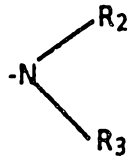
The term "halo" represents a halogen atom radical selected from fluoro, chloro, bromo and iodo.

As used herein, the term "(C_3-C_6)alkenyl" means an alkylene radical having three to six carbon atoms and a double bond; it comprises propenyl, 3-butenyl, 2-butenyl, 2-methylpropenyl, 2-pentenyl, 3-hexenyl and so on, which may be optionally substituted with a carboxy or a sulfo group.

The expression "a nitrogen containing 5-6 membered heterocyclic ring which may contain 1 to 3 further heteroatoms selected from N, S and O" according to the present invention includes unsaturated, partially saturated and wholly saturated ring systems such as pyridine, pyrimidine, pyrazine, pyrrolidine, piperidine, piperazine, oxazole, oxazoline, oxazolidine, pyrazoline, pyrazolidine, thiazolidine, morpholine, thiomorpholine, pyrrole, pyrroline, imidazole, imidazolidine, thiadiazole, oxadiazole and tetrazole.

In said "nitrogen containing 5-6 membered heterocyclic ring" 1 to 3 ring carbons may optionally bear a group carboxy, sulfo, carboxy(C_1-C_4)alkyl and sulfo(C_1-C_4)alkyl and the ring nitrogen atom may optionally be substituted by (C_1-C_4)alkyl, carboxy(C_1-C_4)alkyl, sulfo(C_1-C_4)alkyl, and benzyl.

The expression "fully saturated 5-7 membered nitrogen containing heterocyclic ring wherein the nitrogen atom may be optionally substituted by (C_1-C_4)alkyl or benzyl" identifies a fully saturated heterocycle of 5-7 members containing a nitrogen atom which can be optionally substituted by (C_1-C_4)alkyl or benzyl wherein the carbon skeleton may optionally bear one or two substituents selected from (C_1-C_4)alkyl, carboxy and sulfo. Said heterocyclic rings are connected with the nitrogen moiety of the rest



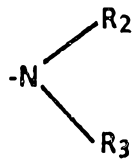
through a bond between the same nitrogen moiety and a carbon atom of the heterocyclic rest. Examples of said radicals are: 1-methyl-4-pyrrolidinyl, 3-piperidinyl, 1-ethyl-4-piperidinyl, 1-benzyl-2,6-dimethyl-4-piperidinyl, and 4-carboxy-1-methyl-2-piperidinyl.

When R_2 and R_3 taken together with the adjacent nitrogen atom represent "a fully saturated 5-7 membered heterocyclic ring which may optionally contain a further heteroatom selected from O, S and N" this expression includes, for instance, the following heterocyclic groups: pyrrolidino, morpholino, piperidino, piperazino, thiomorpholino, pyrazolidino, 1,3-oxazolidino, 1,3-thiazolidino and hexahydroazepino When the further heteroatom is N it may optionally bear a substituent selected from (C_1-C_4)alkyl, benzyl, carboxy, carboxy(C_1-C_4)alkyl, sulfo and sulfo(C_1-C_4)alkyl.

The term "1-deoxy-1-glucityl" identifies a compound of formula (I) wherein Y is a radical deriving from glucamine, i.e. 1-amino-1-deoxy-glucitol. The term "2-deoxy-2-glucosyl" identifies a compound of formula (I) wherein Y is a radical deriving from glucosamine, i.e. 2-amino-2-deoxyglucose.

A preferred group of compounds of the invention is represented by those compounds of formula I wherein R represents methoxymethyl, R_1 and R_4 represent a methyl group and the other substituents are as defined above.

A further preferred group of compounds of the invention are those compounds of formula I wherein R represents methoxymethyl, R_1 and R_4 represent a methyl group, and Y represents a group of formula



wherein R_2 is hydrogen and R_3 is defined as above.

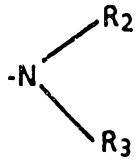
A further preferred group of compounds of the invention is represented by those compounds of formula I wherein R is methoxymethyl, R_1 and R_4 represent a methyl group and Y is an amino moiety which derive from a natural amino acid such as for example glycine, ornithine, serine, aspartic acid, tyrosine, leucine, phenylalanine, methionine, proline, threonine, lysine,

or a synthetic dipeptide such as glycyllysine, serylproline, glycylprolinamide, tyrosylprolinamide, threonylprolinamide, leucylprolinamide.

A further preferred group of compounds comprises those compounds of formula I wherein R is methoxymethyl, R_1 and R_4 are methyl, Y is a group NR_2R_3 wherein R_2 is hydrogen and R_3 is a linear alkyl chain preferably of 3 to 12 carbons, more preferably of 3 to 7 carbons substituted with a group selected from COOH, SO_3H and PO_3H_2.

The most preferred compound is represented by the formula I wherein R is methoxymethyl, R_1 and R_4 are methyl and Y is a group NR_2R_3 wherein R_2 is hydrogen and R_3 is $CH_2CH_2CH_2CH_2CH_2$-COOH.

A further preferred group of compounds of the invention are those compounds of formula I wherein R represents hydrogen, hydroxymethyl and methoxymethyl, R_1 represents hydrogen or a methyl group, and Y represents a group of formula



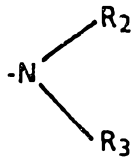
wherein R_2 is hydrogen and R_3 and R_4 are defined as above.

A further preferred group of compounds of the invention is represented by those compounds of formula I wherein R is hydrogen, hydroxymethyl or methoxymethyl, R_1 represents hydrogen or a methyl group, R_4 is hydrogen, methyl or hydroxymethyl with the proviso that when R_4 is hydrogen or hydroxymethyl then R is methoxymethyl and R_1 is methyl, and Y is an amino moiety which derive from a natural amino acid such as for example glycine, ornithine, serine, aspartic acid, tyrosine, leucine, phenylalanine, methionine, proline, threonine, lysine, or a synthetic dipeptide such as glycyllysine, serylproline, glycylprolinamide, tyrosylprolinamide, threonylprolinamide, leucylprolinamide.

A further preferred group of compounds comprises those compounds of formula I wherein R is hydrogen, hydroxymethyl or methoxymethyl, R_1 is hydrogen or methyl, R_4 is hydrogen, methyl or hydroxymethyl with the proviso that when R_4 is hydrogen or hydroxymethyl then R is methoxymethyl and R_1 is methyl, Y is a group NR_2R_3 wherein R_2 is hydrogen and R_3 is a linear alkyl chain preferably of 3 to 12 carbons, more preferably of 3 to 7 carbons substituted with a group selected from COOH, SO_3H and PO_3H_2.

The last preferred group of compounds is represented by the formula I wherein R hydrogen, hydroxymethyl or methoxymethyl, R_1 is hydrogen or methyl, R_4 is as defined above and Y is a group NR_2R_3 wherein R_2 is hydrogen and R_3 is $CH_2CH_2CH_2CH_2CH_2$-COOH.

Representative examples of the compounds of the invention, include those compounds of formula I wherein R, R_1, R_4 and Y are as defined above and



represents

$-NH_2$

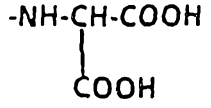
$-NHC_4H_9$

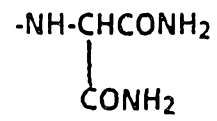
$-NH(CH_2)_4-PO_3H_2$

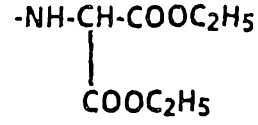
$-NHCH_2COOH$

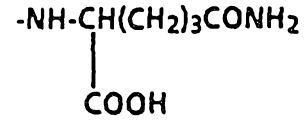
$-NH-CH_2CONH_2$

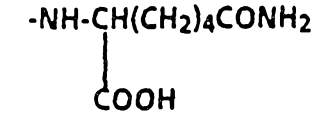
$-NH-CH_2-CON(C_2H_5)_2$

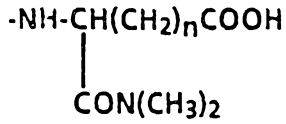




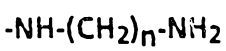


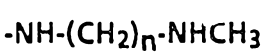


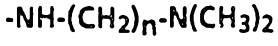


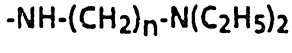


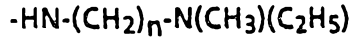
wherein n is 2, 3 or 4



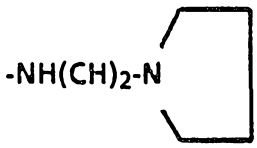


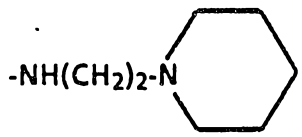


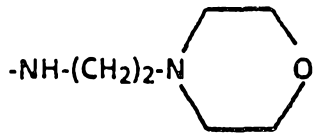


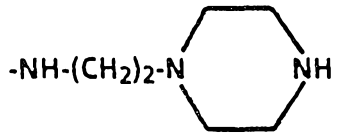


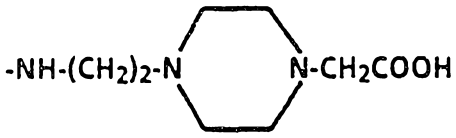
wherein n is 2, 3, 4, 5, 6, 7 or 8

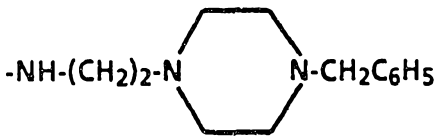


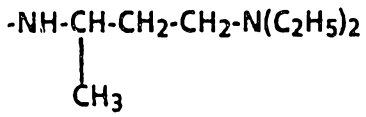


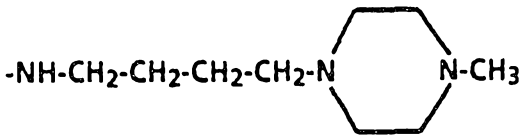


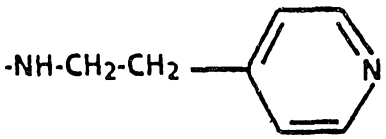


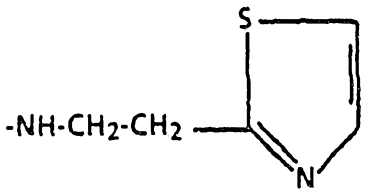


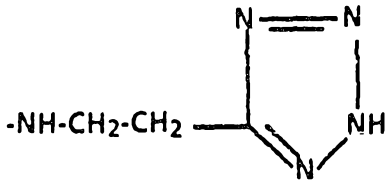


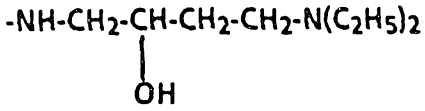


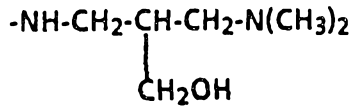


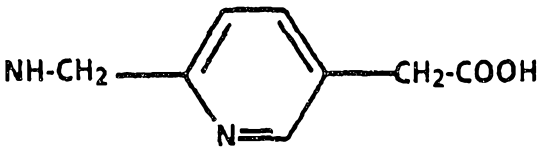


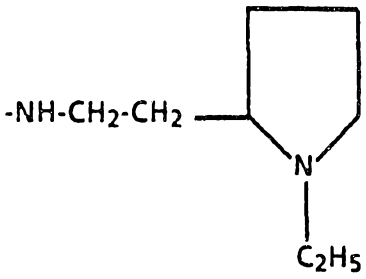


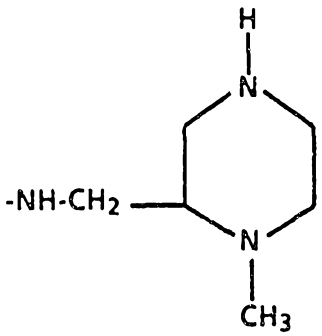


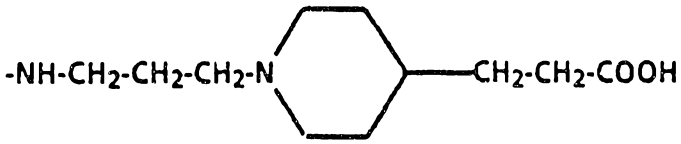


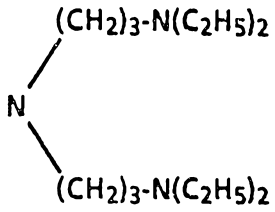


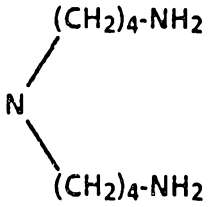


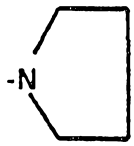

-NH-CH2-CH2
N
C2H5

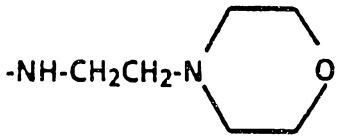

H
N
-NH-CH2
N
CH3

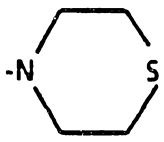

-NH-CH2-CH2-CH2-N
CH2-CH2-COOH

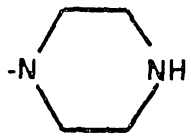


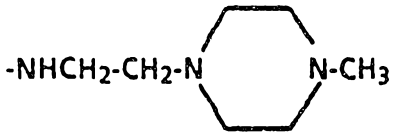









-N
NH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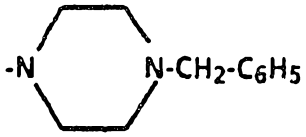
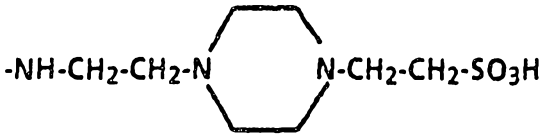
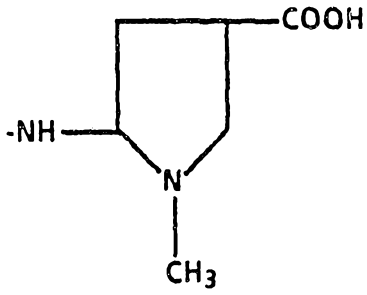
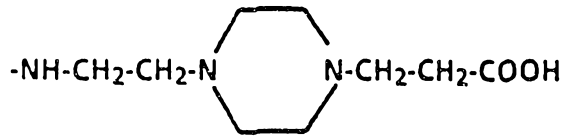
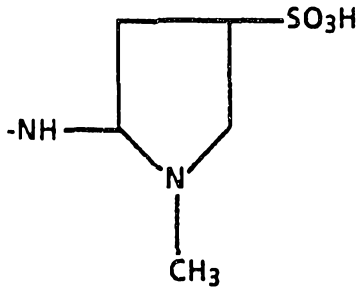
N-CH3

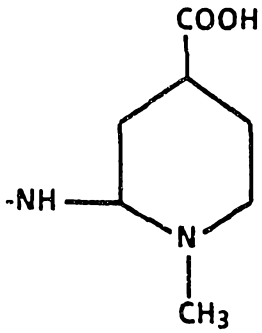

-N
N-CH2-C6H5

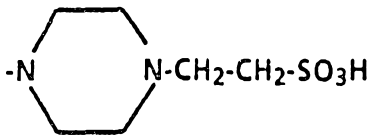

-NH-CH2-CH2-N
N-CH2-CH2-SO3H

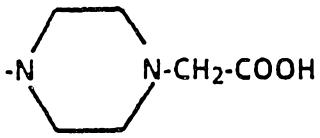

COOH
-NH
N
CH3


-NH-CH2-CH2-N
N-CH2-CH2-COOH


SO3H
-NH
N
CH3


COOH
-NH
N
CH3





$-NH-(CH_2)_n-NH-(CH_2)_m-COOH$

$-NH-(CH_2)_n-NH-(CH_2)_m-SO_3H$

$-NH-(CH_2)_n-O-(CH_2)_m-COOH$

$-NH-(CH_2)_n-O-(CH_2)_m-SO_3H$

$-NH-(CH_2)_n-S-(CH_2)_m-COOH$

$-NH-(CH_2)_n-S-(CH_2)_m-SO_3H$

wherein n is 2, 3, 4 or 5 and m is 1, 2, 3 or 4

$$-NH-(CH_2)_n-CH = CH-(CH_2)_m-COOH$$

$$-NH-(CH_2)_n-CH = CH-(CH_2)_m-SO_3H$$

wherein n is 1, 2 or 3 and m is 0, 1 or 2

The compounds of the invention can form salts according to conventional procedures.

In particular, those compounds of formula I wherein the group $-NR_2R_3$ contains further amine functions form acid addition salts.

In addition, those compounds of the invention which contain acid functions in the $-NR_2R_3$ moiety may also form base addition salts.

In general, those compounds of the invention which contain acid and basic functions can form internal salts. For the scope of the present invention the "internal salts" are encompassed by the definition of the "non-salt" form.

Preferred addition salts of the compounds of this invention are the pharmaceutically acceptable acid and/or base addition salts.

With the term "pharmaceutically acceptable acid and/or base addition salts" are intended those salts with acids and/or bases which from biological, manufacturing and formulation standpoint are compatible with the pharmaceutical practice as well as with the use in the animal growth promotion.

Representative and suitable acid addition salts of the compounds of formula I include those salts formed by standard reaction with both organic and inorganic acids such as, for example, hydrochloric, hydrobromic, sulfuric, phosphoric, acetic, trifluoroacetic, trichloroacetic, succinic, citric, ascorbic, lactic, maleic, fumaric, palmitic, cholic, pamoic, mucic, glutamic, camphoric, glutaric, glycolic, phthalic, tartaric, lauric, stearic, salicylic, methanesulfonic,

dodecylsulfonic acid (estolic acid), benzenesulfonic, sorbic, picric, benzoic, cinnamic and the like acids.

Representative examples of these bases are: alkali metal or alkaline-earth metal hydroxide such sodium, potassium, and calcium hydroxide; ammonia and organic aliphatic, alicyclic or aromatic amines such as methylamine, dimethylamine, trimethylamine, 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS), picoline and basic aminoacids such as lysine, ornithine, arginine and histidine.

The transformation of the free amino or non-salt compounds of the invention into the corresponding addition salts, and the reverse, i.e. the transformation of an addition salt of a compound of the invention into the non-salt or free amino form, are within the ordinary technical skill and are encompassed by the present invention.

For instance, a compound of formula I can be transformed into the corresponding acid or base addition-salt by dissolving the non-salt form in an aqueous solvent and adding a slight molar excess of the selected acid or base. The resulting solution or suspension is then lyophilized to recover the desired salt. Instead of lyophilizing, in some instances, it is possible to recover the final salt by extraction with an organic solvent, concentration to a small volume of the separated organic phase and precipitation by adding a non-solvent.

In case the final salt is unsoluble in an organic solvent where the non-salt form is soluble it is recovered by filtration from the organic solution of the non-salt form after addition of the stoichiometric amount or a slight molar excess of the selected acid or base.

The non-salt form can be prepared from a corresponding acid or base salt dissolved in an aqueous solvent which is then neutralized to free the non-salt form. This is then recovered for instance by extraction with an organic solvent or is transformed into another base or acid addition salt by adding the selected acid or base and working up as above.

When following the neutralization desalting is necessary, a common desalting procedure may be employed.

For example, column chromatography on controlled pore polydextrane resins (such as Sephadex LH 20) or silanized silica gel may be conveniently used. After eluting the undesired salts with an aqueous solution, the desired product is eluted by means of linear gradient or step-gradient of a mixture of water and a polar or apolar organic solvent, such as acetonitrile/water from 50:50 to about 100% acetonitrile.

As is known in the art, the salt formation either with pharmaceutically acceptable acids (bases) or non-pharmaceutically acceptable acids (bases) may be used as a convenient purification technique. After formation and isolation, salt form of a compound of formula I can be transformed into the corresponding non-salt or into a pharmaceutically acceptable salt.

In some instances the acid addition salt of a compound of formula I is more soluble in water and hydrophilic solvents and has an increased chemical stability.

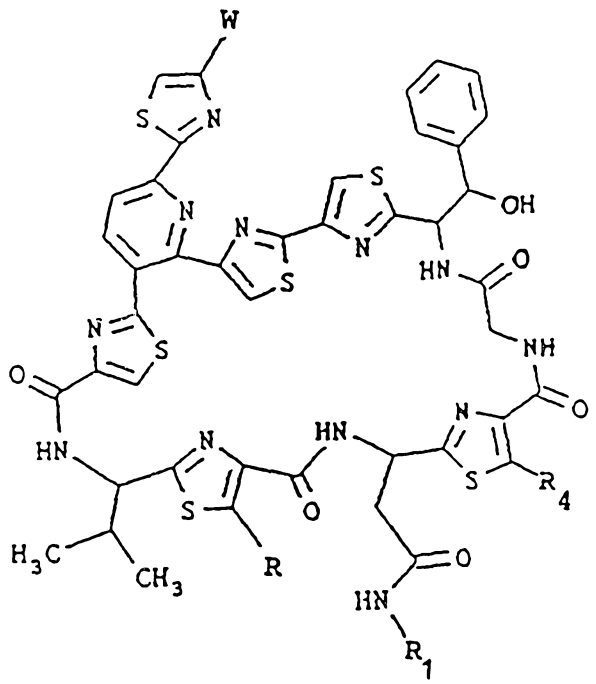
However, in view of the similarity of the properties of the compounds of formula I and their

salts, what is said in the present application when dealing with the biological activities of the compounds of formula I applies also to their pharmaceutically acceptable salts, and viceversa.

In view of their properties, the compounds of the invention can be used as active ingredients in the preparation of medicaments for human or animal treatment.

In particular, the amide derivatives of the antibiotic GE 2270 compounds of formula I are antimicrobial agents mainly active against gram positive bacteria and gram positive as well as gram negative anaerobes.

A general procedure for preparing a compound of this invention is represented by the reaction (amidation) of a suitable antibiotic GE 2270 compound having formula (II)



II

wherein

W represents a carboxy or an activated ester function;

R represents hydrogen, hydroxymethyl or methoxymethyl;

R_1 represents hydrogen or methyl;

R_4 represents hydrogen, methyl or hydroxymethyl,

with the proviso that, when R_4 represents hydrogen or hydroxymethyl, then simultaneously R is methoxymethyl and R_1 is methyl; with a selected amine of formula HNR_2R_3 wherein R_2 and R_3 have the same meanings as above in an inert organic solvent and, when W is carboxy, in the presence of a condensing agent.

In carrying out the amidation for preparing the compounds of this invention, sometimes, it is convenient to protect the functions of the reactants which are not involved in the amidation reaction but could result sensitive to the reaction conditions or negatively affect the reaction course, for instance, yielding undesired side-product.

Furthermore, when the amino acid contains further reactive functions such as amino, carboxy or mercapto groups which may interfere with the course of the amidation, these are protected by means of methods known per se in the art such as those described in references books like E. Gross and J. Meienhofer "The Peptides", Vol. 3, Academic Press, New York, 1981 and M. Bodanszky and A. Bodanszky "The Practice of Peptide Synthesis, Springer-Verlag, Berlin Heidelberg, 1984. These protecting groups must be stable at the conditions the amidation reaction takes place and must be easily removable at the end of the reaction without affecting

either the newly formed amide bond or any other part of the molecule.

Representative examples of N-protecting groups which may be advantageously used in the process of the invention for protecting an amino function are carbamate forming reagents characterized by the following oxycarbonyl groups: 1,1-dimethylpropynyl-oxycarbonyl, t-butyloxycarbonyl, vinyloxycarbonyl, aryloxycarbonyl, cinnamyloxycarbonyl, benzyloxycarbonyl, p-nitrobenzyloxycarbonyl-3,4-dimethoxy-6-nitrobenzyl-oxycarbonyl, 2,4-dichlorobenzyloxycarbonyl, 5-benzisoxazolylmethyloxycarbonyl, 9-anthranylmethyl-oxycarbonyl, diphenylmethyloxycarbonyl, isonicotinyloxycarbonyl, diphenylmethyloxycarbonyl, isonicotinyloxycarbonyl, S-benzyloxycarbonyl, and the like.

A suitable protection for reactive carboxylic acid function is, for instance, by forming an ester function.

The man skilled in the art is capable, also on the basis of the present disclosure, of deciding which functions of the amine HNR_2R_3 need to be protected, how they must be protected and the proper deprotection reaction which is necessary to free the final compound.

As it is appreciated by the skilled technician, the ultimate choice of the specific protecting group depends on the characteristics of the particular amide derivative which is desired. In fact, this amide function of the final compound should be stable at the condition of removal of the protecting group(s).

Since the conditions of removal of the different protecting groups are known, the skilled technician is capable of selecting the proper protecting group.

Inert organic solvents useful for the condensation reaction are those solvents which do not unfavorably interfere with the reaction course and are capable of at least partially solubilizing the antibiotic starting material.

Examples of said inert solvents are organic amides, ethers of glycols and polyols, phosphoramides, sulfoxides. Preferred examples of inert solvents are: dimethylformamide, dimethoxyethane, hexamethylphosphoramide, dimethylsulfoxide, dioxane, and mixtures thereof.

Sometimes, water is compatible with the reaction conditions.

The condensing agent in the process of the invention when W is carboxy is one suitable for forming amide bonds in organic compounds and in particular in peptide synthesis.

Representative and preferred examples of condensing agents are (C_1-C_4)alkyl, phenyl or heterocyclic phosphorazidates such as, diphenyl-phosphorazidate (DPPA), diethyl-phosphorazidate, di(4-nitrophenyl)phosphorazidate, dimorpholyl-phosphorazidate and diphenylphosphorochloridate or benzotriazol-1-yl-oxy-tripyrrolidinophosphoniumhexa-fluorophosphate (PyBOP). The preferred condensing agent is diphenyl phosphorazidate (DPPA).

In the process of the invention, the amine reactant HNR_2R_3 is normally used in a slight molar excess.

In general, a 1- to 2-fold molar excess is used while a 1.2- to 1.5-fold molar excess is preferred.

For the amidation to proceed, it is necessary that the amine HNR_2R_3 be capable of forming a salt with the carboxy function of the antibiotic starting material. In case the amine HNR_2R_3 is not strong enough to form such a salt in the selected reaction medium, it is necessary to add a salt-forming base to the reaction mixture at least in an equimolecular amount with the antibiotic starting material.

Examples of said salt-forming bases are tertiary organic aliphatic or alicyclic amines such as trimethylamine, triethylamine, N-methyl pyrrolidine or heterocyclic bases such as picoline, and the like.

The condensing agent is generally employed in a slight molar excess such as from 1.1 to 1.5 and preferably is 1.2 times the antibiotic GE 2270 starting compound.

In addition, the amine reactant HNR_2R_3 may also conveniently be introduced in the reaction medium as a corresponding acid addition salt, e.g. the hydrochloride. In this case, at least a double molar proportion and preferably a 2 to 3 fold molar excess of a strong base capable of freeing the HNR_2R_3 amine from its salts, is used. Also in this case, the suitable base is a tertiary organic aliphatic or alicyclic amine like those exemplified above. In fact, at least in some instances, the use of salt of the amine HNR_2R_3, which is then freed in situ with the above mentioned bases, is

greatly preferred especially when the salt is more stable than the corresponding free amine.

The reaction temperature will vary considerably depending on the specific starting materials and reaction conditions. In general, it is preferred to conduct the reaction at temperatures between 0-20°C.

Also the reaction time vary considerably depending on the other reaction parameters. In general the condensation reaction is completed in about 5-24 h.

In any case, the reaction course is monitored by TLC or preferably by HPLC according to methods known in the art.

On the basis of the results of these assays a man skilled in the art will be able to evaluate the reaction course and decide when to stop the reaction and start working up the reaction mass according to known per sè techniques which include, for instance, extraction with solvents, precipitation by addition of non-solvents, etc., in conjunction with further separations and purifications by column chromatography.

As already said, when protection of the HNR_2R_3 reactant is necessary, the protected final compound is then de-protected according to procedures which are known per sè and mainly depends on the protecting group involved.

When an activated ester is used as the GE 2270 starting material, said ester is one wherein the esterified alcohol is providing a leaving group which can be readily displaced and substituted by the amine HNR_2R_3 under reaction conditions which do not modify the other portions of the molecule. The amine reactant is

usually employed in a molar excess over the activated ester in a solvent which is selected from those mentioned above and the lower alkanols. The reaction temperature generally ranges between 0°C and 100°C. Examples of the activated ester include lower alkyl esters wherein the lower alkyl moiety is optionally substituted by cyano and nitro, phenyl esters substituted by halo and nitro groups as well as the ester moiety contained in GE 2270 factor A_2.

It is evident that in many instances a compound of the invention may be prepared in more than one way and that a compound of the invention may be transformed into another by means of known per se reactions.

For instance when the HNR_2R_3 amine contains a carboxy or an ester function which can be further converted into the corresponding amide derivative, a desired compound of formula I may be prepared by condensing first said amine with the selected GE 2270 starting material and then converting the carboxy or ester function to amide by reaction with the appropriate amine.

The following tables list the structure formulas of some representative compounds of the invention (TABLE I) and their methods of preparation, (described in details in the Experimental Section), starting materials and reaction yields (TABLE II).

In the following Table (TABLE I) the structure formulas of representative examples of compounds of the invention are reported.

TABLE I

COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
1	-NH CH_2COOH	CH_2O-CH_3	CH_3	CH_3
2	-NH $CH(CH_2CH_2CH_2NH_2)COOH$	CH_2O-CH_3	CH_3	CH_3
3	-NH $CH(CH_2OH)COOH$	CH_2O-CH_3	CH_3	CH_3
4	-NH $CH(CH_2COOH)COOH$	CH_2O-CH_3	CH_3	CH_3
5	-NH $CH(CH_2$-C_6H_4-$OH)COOH$	CH_2O-CH_3	CH_3	CH_3

TABLE I (continued)

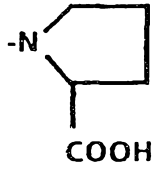
COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
6	-NH CHCOOH $CH_2CH(CH_3)_2$	CH_2O-CH_3	CH_3	CH_3
7	-NH CH-COOH CH_2 —	CH_2O-CH_3	CH_3	CH_3
8	-NH CHCOOH $CH_2CH_2SCH_3$	CH_2O-CH_3	CH_3	CH_3
9	 COOH	CH_2O-CH_3	CH_3	CH_3
10	-NH CHCOOH HO — CH — CH_3	CH_2O-CH_3	CH_3	CH_3

TABLE I (continued)

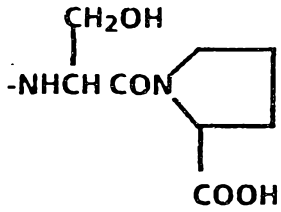
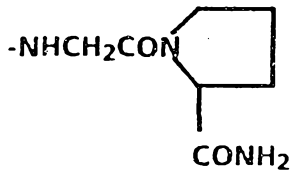
COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
11	-NH $CH_2CH_2CH_2CH_2CH$(NH$_2$)-COOH	CH_2O-CH_3	CH_3	CH_3
12	-NH $CH_2CONHCH$($CH_2CH_2CH_2CH_2NH_2$)COOH	CH_2O-CH_3	CH_3	CH_3
13		CH_2O-CH_3	CH_3	CH_3
14		CH_2O-CH_3	CH_3	CH_3

TABLE I (continued)

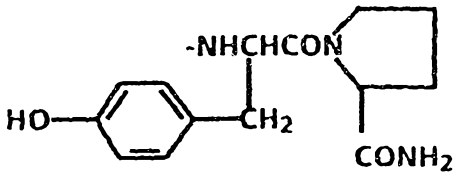
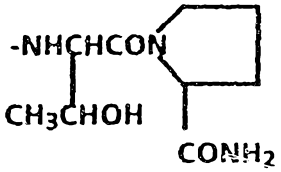
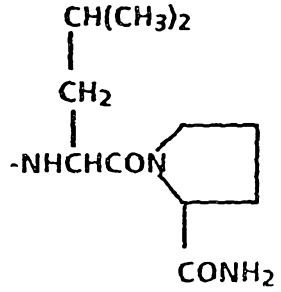
COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
15		$CH_2O\text{-}CH_3$	CH_3	CH_3
16		$CH_2O\text{-}CH_3$	CH_3	CH_3
17		$CH_2O\text{-}CH_3$	CH_3	CH_3

TABLE I (continued)

COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
18	-NH $CH_2CH_2CH_2COOH$	CH_2O-CH_3	CH_3	CH_3
19	-NH $CH_2CH_2CH_2CH_2CH_2COOH$	CH_2O-CH_3	CH_3	CH_3
20	-NH $CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$	CH_2O-CH_3	CH_3	CH_3
21	-NH $CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$	CH_2O-CH_3	CH_3	CH_3
22	-NH $CH_2CH_2SO_3H$	CH_2O-CH_3	CH_3	CH_3
23	-NH $CH_2CH_2CH_2SO_3H$	CH_2O-CH_3	CH_3	CH_3

TABLE I (continued)

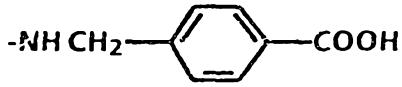
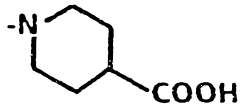
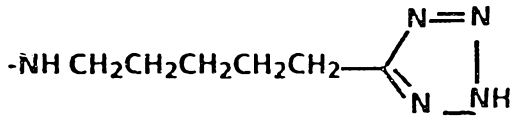
COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
24	-NH $CH_2CH_2CH_2PO_3H_2$	CH_2O-CH_3	CH_3	CH_3
25	-NH $CH_2CH_2CH_2CH_2CH_2PO_3H_2$	CH_2O-CH_3	CH_3	CH_3
26		CH_2O-CH_3	CH_3	CH_3
27		CH_2O-CH_3	CH_3	CH_3
28		CH_2O-CH_3	CH_3	CH_3

TABLE I (continued)

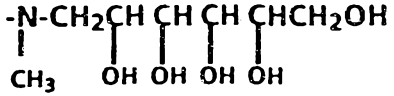
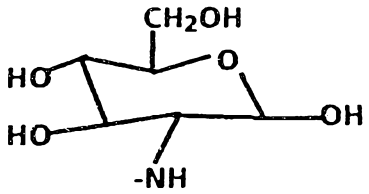
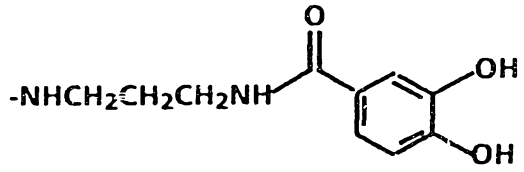
COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
29		CH_2O-CH_3	CH_3	CH_3
30		CH_2O-CH_3	CH_3	CH_3
31		CH_2O-CH_3	CH_3	CH_3
32	-NH $CH_2CH_2N(CH_3)_2$	CH_2O-CH_3	CH_3	CH_3

TABLE I (continued)

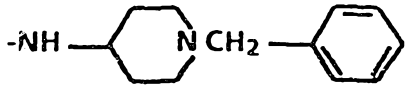
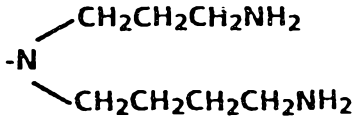
COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
33		CH_2O-CH_3	CH_3	CH_3
34		CH_2O-CH_3	CH_3	CH_3
35	$-NH_2$	CH_2O-CH_3	CH_3	CH_3
36	-NH $CH_2CH_2CH_2NH_2$	CH_2O-CH_3	CH_3	CH_3

TABLE I (continued)

COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
37	-NH CH_2CHO	CH_2O-CH_3	CH_3	CH_3
38	-NH $CH_2CH_2NHCH_2CH_2COOH$	CH_2O-CH_3	CH_3	CH_3
39	-NH $CH_2CH_2SCH_2CH_2COOH$	CH_2O-CH_3	CH_3	CH_3
40	-NH $CH_2CH_2CH_2CH = CHCOOH$	CH_2O-CH_3	CH_3	CH_3
41	-NH $CH_2CH_2OCH_2CH_2COOH$	CH_2O-CH_3	CH_3	CH_3

TABLE I (continued)

COMPOUND OF EXAMPLE NO.	Y	R	R_1	R_4
42	-NH $CH_2CH_2CH_2CH_2CH_2COOH$	CH_2O-CH_3	CH_3	CH_2OH
43	-NH $CH_2CH_2CH_2CH_2CH_2COOH$	H	H	CH_3
44	-NH $CH_2CH_2CH_2CH_2CH_2COOH$	CH_2OH	CH_3	CH_3

Compounds No. 2, 11, 12, 34, 36 were isolated as trifluoroacetate salts

TABLE II

COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
1	A_3 + $HCl.NH_2CH_2COOEt$	A_1	80%
2	A_3 + $HCl.NH_2CH(CH_2CH_2CH_2NH.Cbz)COOMe$	A_1	72%
3	A_3 + $HCl.NH_2CH(CH_2OH)COOMe$	A_1	70%
4	A_3 + $HCl.NH_2CH(CH_2COOMe)COOMe$	A_1	54%
5	A_3 + $HCl.NH_2CH(CH_2C_6H_4OH)COOMe$	A_1	70%
6	A_3 + $HCl.NH_2CH(CH_2CH(CH_3)_2)COOMe$	A_1	70%

TABLE II (continued)

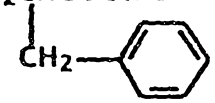
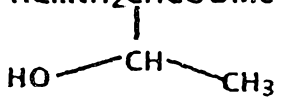
COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
7	A_3 + HCl.$NH_2CHCOOMe$ 	A_1	60%
8	A_3 + HCl.$NH_2CHCOOMe$ $CH_2CH_2SCH_3$	A_1	70%
9	A_3 + HCl.HN  COOMe	A_1	75%
10	A_3 + HCl.$NH_2CHCOOMe$ 	A_1	70%

TABLE II (continued)

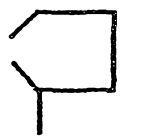
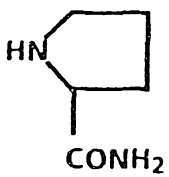
COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
11	A_3 + $NH_2CH_2CH_2CH_2CH_2CH(NH.Cbz)COOH$	B_1	64%
12	A_3 + $TFA.NH_2CH_2CONHCH(CH_2CH_2CH_2CH_2NH.Cbz)COOH$	B_1	74%
13	3 + 	C_1	70%
14	1 + 	C	83%

TABLE II (continued)

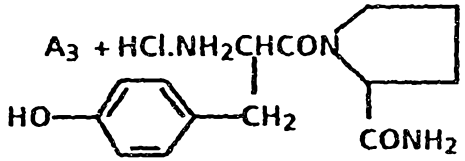
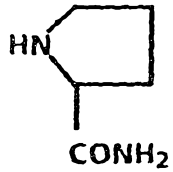
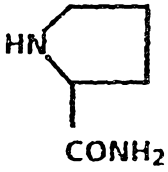
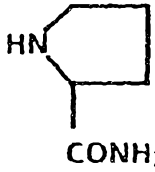
COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
15		A	60%
	or		
	5 + 	C	70%
16	10 + 	C	60%
17	6 + 	C	65%

TABLE II (continued)

COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
18	A_3 + HCl.$NH_2CH_2CH_2CH_2COOMe$	A_1	73%
19	A_3 + HCl.$NH_2CH_2CH_2CH_2CH_2CH_2COOMe$	A_1	77%
	A_3 + $NH_2CH_2CH_2CH_2CH_2CH_2COOH$	B	70%
20	A_3 + PTSA.$NH_2CH_2CH_2CH_2CH_2CH_2CH_2COOMe$	A_1	75%
21	A_3 + PTSA.$NH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOMe$	A_1	70%
22	A_3 + $NH_2CH_2CH_2SO_3H$	B	20%

TABLE II (continued)

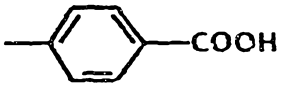
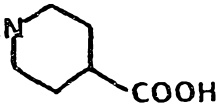
COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
23	A_3 + $NH_2CH_2CH_2CH_2SO_3H$	B	25%
24	A_3 + $NH_2CH_2CH_2CH_2PO_3H_2$	B	40%
25	A_3 + $NH_2CH_2CH_2CH_2CH_2CH_2PO_3H_2$	B	35%
26	A_3 + 	B	60%
27	A_3 + 	B	50%

TABLE II (continued)

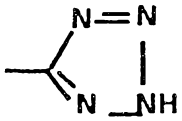
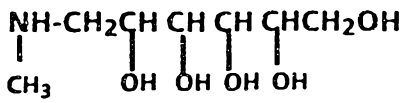
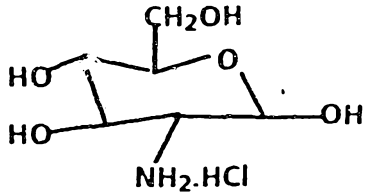
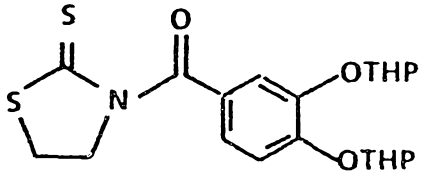
COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
28	A_3 + $NH_2CH_2CH_2CH_2CH_2CH_2$ 	B	65%
29	A_3 + 	A	20%
30	A_3 + 	A	80%
31	36 + 	C_1	80%

TABLE II (continued)

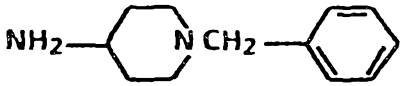
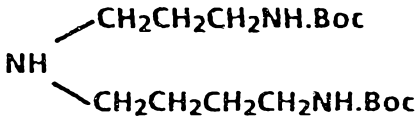
COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
32	A_3 + $NH_2CH_2CH_2N(CH_3)_2$	A	75%
33	A_3 + 	A	60%
34	A_3 + 	A_1	50%
35	A_3 + NH_3 in MeOH	D	83%
36	A_3 + $NH_2CH_2CH_2CH_2NH.Boc$	A_1	70%

TABLE II (continued)

COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
37	$A_3 + NH_2CH_2CH(OCH_3)_2$	A_1	65%
38	37 + $HCl.NH_2CH_2CH_2COOCH_2CH_3$	C_1	20%
39	A_3 + $TFA.NH_2CH_2CH_2SCH_2CH_2COOCH_3$	A_1	33%
40	A_3 + $TFA.NH_2CH_2CH_2CH_2CH=CH\text{-}COOH$	B	51%

TABLE II (continued)

COMPOUND OF EXAMPLE NO.	STARTING MATERIALS (GE 2270 FACTOR + AMINE REACTANT)	METHOD	OVERALL YIELD
41	A_3 + TFA.$NH_2CH_2CH_2OCH_2CH_2COOH$	B	37%
42	C_{2a} + HCl.$NH_2CH_2CH_2CH_2CH_2CH_2COOCH_3$	F	40%
	$NH_2CH_2CH_2CH_2CH_2CH_2COOH$	G	35%
43	D_1 + HCl.$NH_2CH_2CH_2CH_2CH_2CH_2COOCH_3$	H	50%
	$NH_2CH_2CH_2CH_2CH_2CH_2COOH$	I	40%
44	D_2 + HCl.$NH_2CH_2CH_2CH_2CH_2CH_2COOCH_3$	J	35%
	$NH_2CH_2CH_2CH_2CH_2CH_2COOH$	K	30%

TFA = trifluoroacetic acid
PTSA = p-toluenesulfonic acid

HPLC Analysis

The following table (TABLE III) reports the R_t of representative examples of compounds of this invention.

Analysis were run with a Varian model 5000 LC pump equipped with a 10 µl loop injector and a Varian 2050 variable wavelength detector at 254 nm.

Columns: Pre-column LiChroCart-LiChrosorb RP-8 (5 µm) followed by a column LiChroCart 125-4 LiChrospher 100 RP-8 (5 µm)

Eluents: A 0.05 M aq. $HCOONH_4$
B CH_3CN
C THF

Method A: isochratic 44% of B in A
Flow rate: 0.7 ml/min

Method B: isochratic 40% of B in A
Flow rate 0.7 ml/min

Method C: isochratic 38% of B in A
Flow rate: 0.5 ml/min

Method D: isochratic 30% of B in A
Flow rate: 0.7 ml/min

Method E: isochratic 38% of B in A
Flow rate: 0.7 ml/min

Method F: gradient from 38 to 55% of B in A in 11 min according to the following program

Time (min)	%B in A
0	38
6	38
7	45
10	45
11	55

Flow rate: 0.7 ml/min

Method G: gradient from 38 to 55% of B in A in 25 min according to the following program

Time (min)	%B in A
0	38
6	38
10	44
15	44
25	55

Flow rate: 0.7 ml/min

Method H: isochratic 55% of B in A
Flow rate: 0.7 ml/min

Method I: isochratic 60% of B in A
Flow rate: 0.7 ml/min

Method L: isochratic 48% of B in A
Flow rate: 0.7 ml/min

Method M: gradient according to the following program:

Time (min)	%A	%B	%C
0	74	10	16
20	62	19	19

Flow rate: 0.7 ml/min

TABLE III
HPLC Analysis

Compound No.	Method	R_t (min)	K
1	A	2.56	0.92
2	B	4.09	1.15
3	C	6.21	1.12
4	D	14.70	1.79
5	E	5.99	1.28
6	A	4.05	1.46
7	A	4.52	1.63
8	A	3.44	1.24
9	E	5.32	1.18
10	E	4.22	0.90
11	F	14.30	3.05
12	F	5.99	1.28
13	E	4.88	1.04
14	G	14.09	3.01
15	G	17.60	3.76
16	G	13.77	2.94
17	G	23.75	5.07
18	G	7.49	1.60

K = Relative Retention time

TABLE III (continued)

HPLC Analysis

Compound No.	Method	R_t (min)	K
19	F	8.84	1.89
20	G	17.77	3.78
21	G	31.10	6.64
22	F	5.01	1.07
23	F	4.40	0.94
24	F	6.27	1.34
25	F	11.17	2.39
26	F	29.04	6.02
27	F	6.28	1.34
28	F	12.14	2.59
29	F	8.02	1.71
30	E	6.81-7.61 anomeric mixture	1.45-1.62 anomeric mixture
31	F	17.64	3.76
32	B	9.64	2.70
33	H	15.10	7.40
34	I	7.22	3.92
35	L	10.28	4.11
36	F	19.32	4.13

K = Relative Retention time

TABLE III (continued)
HPLC Analysis

Compound No.	Method	R_t (min)	K
37	F	14.56	3.11
38	F	11.00	2.35
39	F	9.48	2.02
40	F	6.84	1.46
41	F	3.95	0.84
42	M	17.23	1.32 *
43	M	15.76	1.52 **
44	M	16.64	1.50 ***
19	M	20.81	1.32

K = Relative Retention time

K = Relative Retention time = R_t amide/R_t GE 2270 proper starting material (i.e. the compound of formula II wherein W is COOH)

EXPERIMENTAL SECTION

TABLE IV - N.M.R.

The ^1H-NMR spectra were recorded with a Bruker spectrometer in DMSO-d_6 (hexadeuterodimethylsulfoxide) using TMS as the internal standard (0.00 ppm) [δ, ppm, m) at 250 MHz and/or 500 MHz (s=singlet, br s = broad singlet, d=doublet, dd=doublet of doublets, t=triplet, m=multiplet)

TABLE V - I.R.

The infrared spectra (IR) were recorded with a Perkin Elmer mod. 580 spectrophotometer in nujol mull.

TABLE VI - U.V.

The ultraviolet absorption spectra were recorded with a Perkin Elmer Model 320 spectrometer.

It will be clear to the skilled technician that the data represented in TABLES IV, V and VI below, do not represent all the values of the peaks obtained but only the values of those peaks which permit to characterize the single substance.

TABLE IV - N.M.R. Spectra

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
1	0.84 (d, 3H); 0.87 (d, 3H); 2.57 (s, 3H); 3.39 (s, 3H); 3.77 (dd, 1H); 3.99 (d, 2H); 4.25 (dd, 1H); 4.96 (s, 2H); 7.36-7.22 (m, 7H); 8.28 (s, 1H); 8.50 (s, 1H); 8.59 (s, 1H)
2	0.79 (d, 3H); 0.85 (d, 3H); 2.05-1.70 (m, 4H); 2.54 (s, 3H); 3.33 (s, 3H); 3.65 (m, 2H); 3.81 (dd, 1H); 4.10 (m, 1H), 4.35 (dd, 1H); 4.99 (s, 2H); 7.35-7.05 (m, 7H); 8.20 (s, 1H); 8.42 (s, 1H); 8.58 (s, 1H)
3	0.84 (d, 3H); 0.87 (d, 3H); 2.58 (s, 3H); 3.37 (s, 3H); 3.80 (dd, 2H); 3,84 (dd, 1H); 3.91 (dd, 1H); 4.26 (dd, 1H); 4.55 (m, 1H); 4.97 (s, 2H); 7.36-7.20 (m, 7H); 8.29 (s, 1H); 8.55 (s, 1H); 8.59 (s, 1H)
4	0.85 (d, 3H); 0.89 (d, 3H); 2.58 (s, 3H); 2.90 (m, 2H); 3.38 (s, 3H); 3.70 (dd, 1H); 4.29 (dd, 1H); 4.85 (m, 1H); 4.98 (s, 2H); 7.40-7.20 (m, 7H); 8.28 (s, 1H); 8.52 (s, 1H); 8.58 (s, 1H)
5	0.85 (d, 3H); 0.88 (d, 3H); 2.58 (s, 3H); 3.11 (m, 2H); 3.26 (br; s, 1H); 3.38 (s, 3H); 3.78 (dd, 1H); 4.28 (dd, 1H) 4.64 (m, 1H); 4.97 (s, 2H); 6.68 (d, 1H); 7.09 (d, 1H); 7.40-7.20 (m, 7H); 8.27 (s, 1H); 8.47 (s, 1H); 8.59 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
6	0.84 (d, 3H). 0.87 (d, 3H); 0.92 (d, 3H); 0.95 (d, 3H); 1.69 (m, 2H); 1.86 (m, 1H); 2.57 (s, 3H); 3.37 (s, 3H); 3.78 (dd, 1H); 4.26 (dd, 1H); 4.53 (m, 1H); 4.97 (s, 2H); 7.38-7.20 (m, 7H); 8.28 (s, 1H); 8.46 (s, 1H); 8.59 (s, 1H)
7	0.84 (d, 3H); 0.88 (d, 3H); 2.58 (s, 3H); 3.20 (m, 2H); 3.37 (s, 3H); 3.77 (dd, 1H); 4.25 (dd, 1H); 4.73 (m, 1H); 7.40-7.2 (m, 12H); 8.28 (s, 1H); 8.47 (s, 1H) 8.59 (s, 1H)
8	0.85 (d, 3H); 0.89 (d, 3H); 2.08 (s, 3H); 2.16 (m, 2H); 2.56 (m, 2H); 2.57 (s, 3H); 3.40 (s, 3H); 3.79 (dd, 1H); 4.27 (dd, 1H); 4.61 (m, 1H); 5.00 (s, 2H); 7.37-7.20 (m, 7H); 8.29 (s, 1H); 8.52 (s, 1H); 8.60 (s, 1H)
9	0.84 (d, 3H); 0.88 (d, 3H); 2.45-1.70 (m, 4H); 2.58 (s, 3H); 3.37 (s, 3H); 3.68 (m, 2H); 3.78 (dd, 1H); 4.10 (m, 1H); 4.27 (dd, 1H); 4.49 (m, 1H); 7.35-7.22 (m, 7H); 8.27 (s, 1H); 8.50 (s, 1H); 8.59 (s, 1H)
10	0.85 (d, 3H); 0.88 (d, 3H); 1.19 (d, 3H); 2.59 (s, 3H); 3.39 (s, 3H); 3.78 (dd, 1H); 4.30 (m, 2H); 4.48 (dd, 1H); 4.99 (s, 2H); 7.4-7.2 (m, 7H); 8.33 (s, 1H); 8.49 (s, 1H); 8.60 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
11	0.84 (d, 3H); 0.88 (d, 3H): 1.55-1.35 (m, 2H); 1.61 (m, 2H); 1.83 (m, 2H); 2.58 (s, 3H); 3.34 (m, 2H); 3.38 (s, 3H); 3.79 (dd, 1H); 3.91 (br; s, 1H); 4.29 (dd, 1H); 4.97 (s, 2H); 7.35-7.13 (m, 7H); 8.27 (s, 1H); 8.43 (s, 1H); 8.59 (s, 1H)
12	0.85 (d, 3H); 0.88 (d, 3H); 1.37 (m, 2H); 1.70-1.49 (m, 3H); 1.75 (m, 1H); 2.58 (s, 3H); 2.76 (m, 2H); 3.38 (s, 3H); 3.78 (dd, 1H); 4.03 (m, 2H); 4.28 (m, 2H); 4.97 (s, 2H); 7.35-7.20 (m, 7H); 8.28 (s, 1H); 8.49 (s, 1H); 8.59 (s, 1H)
13	0.84 (d, 3H); 0.88 (d, 3H); 1.98-1.82 (m, 2H); 2.18 (m, 2H); 2.56 (s, 3H); 2.69 (dd, 2H); 3.36 (s, 3H); 3.85-3.62 (m, 3H); 4.31 (m, 2H); 4.85 (m, 1H); 4.96 (s, 2H); 7.38-7.19 (m, 7H); 8.24 (s, 1H); 8.55 (s, 1H); 8.63 (s, 1H)
14	0.85 (d, 3H); 0.88 (d, 3H); 1.99-1.82 (m, 3H); 2.06 (m, 1H); 2.58 (s, 3H); 3.58 (m, 1H); 3.67 (m, 1H); 3.79 (dd, 1H); 4.18 (d, 2H); 4.28 (dd, 1H); 4.97 (s, 2H); 6.93 (s, 1H); 7.36-7.28 (m, 8H); 8.28 (s, 1H); 8.52 (s, 1H); 8.59 (s, 1H)
15	0.85 (d, 3H); 0.88 (d, 3H); 2.07-1.63 (m, 4H); 2.58 (s, 3H); 2.99 (dd, 1H); 3.09 (dd, 1H); 3.38 (s, 3H); 3.51 (m, 1H); 3.77 (m, 2H); 4.30 (m, 2H); 4.89 (m, 1H); 4.98 (s, 2H); 6.66 (d, 1H); 6.95 (br; s, 1H); 7.16 (d, 1H); 7.39-7.20 (m, 8H); 8.23 (s, 1H); 8.42 (s, 1H); 8.58 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
16	0.84 (d, 3H); 0.87 (d, 3H); 1.20 (d, 3H); 1.98-1.80 (m, 3H); 2.08 (m, 1H); 2.56 (s, 3H); 3.36 (s, 3H); 3.85-3.71 (m, 2H); 4.13 (m, 1H); 4.28 (dd, 1H); 4.31 (dd, 1H); 4.73 (m, 1H); 5.05 (d, 1H); 6.89 (br; s, 1H); 7.15 (br; s, 1H); 7.38-7.19 (m, 7H); 8.26 (s, 1H); 8.51 (s, 1H); 8.56 (s, 1H)
17	0.84 (d, 3H); 0.87 (d, 3H); 0.94 (d, 3H); 0.98 (d, 3H); 2.10-1.62 (m, 7H); 2.56 (s, 3H); 3.36 (s, 3H); 3.65 (m, 1H); 3.88-3.70 (m, 2H); 4.31 (m, 2H); 4.88 (m, 1H); 4.96 (s, 2H); 6.79 (br, s, 1H); 7.18 (br; s, 1H); 7.35-7.20 (m, 7H); 8.25 (s, 1H); 8.48 (s, 1H); 8.56 (s, 1H)
18	0.84 (d, 3H); 0.88 (d, 3H); 1.81 (m, 2H); 2.30 (t, 2H); 2.58 (s, 3H); 3.35 (m, 2H); 3.37 (s, 3H); 3.78 (dd, 1H); 4.28 (dd, 1H); 4.97 (s, 2H); 7.35-7.20 (m, 7H); 8.27 (s, 1H); 8.46 (s, 1H); 8.59 (s, 1H)
19	0.84 (d, 3H); 0.87 (d, 3H); 1.35 (m, 2H); 1.56 (m, 4H); 2.22 (t, 2H); 2.58 (s, 3H); 3.36 (m, 2H); 3.38 (s, 3H); 3.80 (dd, 1H); 4.29 (dd, 1H); 4.97 (s, 2H); 7.42-7.22 (m, 7H); 8.29 (s, 1H); 8.45 (s, 1H); 8.62 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
20	0.84 (d, 3H); 0.88 (d; 3H); 1.31 (br; s, 6H); 1.51 (m, 2H); 1.57 (m, 2H); 2.19 (t, 2H); 2.58 (s, 3H); 3.32 (m, 2H); 3.37 (s, 3H); 3.79 (dd, 1H); 4.28 (dd, 1H); 4.97 (s, 2H); 7.38-7.19 (m, 7H); 8.27 (s, 1H); 8.45 (s, 1H); 8.59 (s, 1H)
21	0.84 (d, 3H); 0.88 (d, 3H); 1.41-1.20 (m, 12H); 1.47 (m, 2H); 1.57 (m, 2H); 2.17 (t, 2H); 2.58 (s, 3H); 3.29 (m, 2H); 3.38 (s, 3H); 3.79 (dd, 1H); 4.28 (dd, 1H); 4.97 (s, 2H); 7.38-7.19 (m, 7H); 8.27 (s, 1H); 8.43 (s, 1H); 8.59 (s, 1H)
22	0.85 (d, 3H); 0.87 (d, 3H); 2.57 (s, 3H); 2.79 (t, 2H); 3.37 (s, 3H); 3.59 (t, 2H); 3.78 (dd, 1H); 4.28 (dd, 1H); 4.97 (s, 1H); 7.41-7.20 (m, 7H); 8.27 (s, 1H); 8.44 (s, 1H); 8.57 (s, 1H)
23	0.84 (d, 3H); 0.87 (d, 3H); 1.67 (m, 2H); 2.53 (t, 2H); 2.57 (s, 3H); 3.26 (t, 2H); 3.37 (s, 3H); 3.78 (dd, 1H); 4.28 (dd, 1H); 4.97 (s, 2H); 7.41-7.26 (m, 7H); 8.26 (s, 1H); 8.44 (s, 1H); 8.57 (s, 1H)
24	0.85 (d, 3H); 0.88 (d, 3H); 1.58 (m, 2H); 1.79 (m, 2H); 2.58 (s, 3H); 3.38 (s, 3H); 3.50 (m, 2H); 3.78 (dd, 1H); 4.28 (dd, 1H); 4.97 (s, 2H); 7.38-7.21 (m, 7H); 8.27 (s, 1H); 8.45 (s, 1H); 8.59 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
25	0.84 (d, 3H); 0.88 (d, 3H); 1.65-1.35 (m, 8H); 4.58 (s, 3H); 3.38 (s, 3H); 3.78 (dd, 1H); 4.28 (dd, 1H); 4.97 (s, 2H); 7.40-7.20 (m, 7H); 8.28 (s, 1H); 8.43 (s, 1H); 8.59 (s, 1H)
26	0.85 (d, 3H); 0.89 (d, 3H); 2.56 (s, 3H); 3.36 (s, 3H); 3.80 (dd, 1H); 4.31 (dd, 1H); 4.62 (br; s, 2H); 4.96 (s, 2H); 7.39-7.15 (m, 7H); 7.47 (d, 2H); 7.90 (d, 2H); 8.26 (s, 1H); 8.41 (s, 1H); 8.58 (s, 1H)
27	0.84 (d 3H); 0.88 (d, 3H); 1.62 (br, s, 2H); 1.92 (br, s, 2H); 2.58 (s, 3H); 2.60 (m, 1H); 3.38 (s, 3H); 3.79 (dd, 1H); 4.16 (m, 2H); 4.29 (dd, 1H); 4.38 (m, 2H); 7.35-7.19 (m, 7H); 8.25 (s, 1H); 8.29 (s, 1H); 8.57 (s, 1H)
28	0.85 (d, 3H); 0.89 (d, 3H); 1.39 (m, 2H); 1.61 (m, 2H) 1.76 (m, 2H); 2,58 (s, 3H); 2.88 (t, 2H); 3.33 (m, 2H); 3.80 (dd, 1H); 4.29 (dd, 1H); 4.98 (s, 2H); 7.34-7.20 (m, 7H) 8.26 (s, 1H); 8.45 (s, 1H); 8.58 (s, 1H)
29	0.84 (d, 3H); 0.88 (d, 3H); 2.58 (s, 6H); 3.38 (s, 3H); 3.70-3.41 (m, 5H); 3.89-3.75 (m, 2H); 3.98 (br; s, 1H); 4.35-4.26 (m, 2H); 4.97 (s, 2H); 7.35-7.21 (m, 7H); 8.26 (s, 1H); 8.28 (s, 1H); 8.58 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
30	0.84 (d, 3H); 0.88 (d, 3H); 2.58 (s, 3H); 3.29-3.14 (m, 2H); 3.38 (s, 3H); 3.90-3.49 (m, 4H); 4.29 (dd, 1H); 4.92 (m, 1H); 4.97 (s, 2H); 5.12 (t, 1H); 7.35-7.18 (m, 7H); 8.26 (s, 1H); 8.51 (s, 1H); 8.58 (s, 1H)
31	0.86 (d, 3H); 0.89 (d, 3H); 1.81 (m, 2H); 2.59 (s, 3H); 3.32 (m, 4H); 3.39 (s, 3H); 3.80 (dd, 1H); 4.30 (dd, 1H); 4.99 (s, 2H); 6.75 (d, 1H); 7.41-7.18 (m, 9H); 8.28 (s, 1H); 8.46 (s, 1H); 8.59 (s, 1H)
32	0.85 (d, 3H); 0.88 (d, 3H); 2.21 (s, 6H); 2.59 (s, 3H); 3.38 (s, 3H); 3.43 (m, 4H); 3.81 (dd, 1H); 4.31 (dd, 1H); 4.98 (s, 2H); 7.45-7.19 (m, 7H); 8.28 (s, 1H); 8.45 (s, 1H); 8.61 (s, 1H)
33	0.86 (d, 3H); 0.90 (d, 3H); 1.91-1.70 (m, 2H); 2.26-2.05 (m, 2H); 2.60 (s, 3H); 2.91-2.69 (m, 4H); 3.40 (s, 3H); 3.51 (br; s, 2H); 3.95-3.75 (m, 2H); 4.30 (dd, 1H); 4.99 (s, 2H); 7.41-7.18 (m, 12H); 8.28 (s, 1H) 8.45 (s, 1H); 8.66 (s, 1H)
34	0.85 (d, 3H); 0.89 (d, 3H); 1.81-1.49 (m, 4H); 2.01-1.88 (m, 2H); 2.59 (s, 3H); 2.98-2.65 (m, 4H); 3.39 (s, 3H); 3.80-3.51 (m, 4H); 3.81 (dd, 1H); 4.31 (dd, 1H); 4.99 (s, 2H); 7.41-7.18 (m, 7H); 7.90-7.65 (m, 6H); 8.25 (s, 1H); 8.36 (s, 1H); 8.61 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
35	0.85 (d, 3H); 0.88 (d, 3H); 2.59 (s, 3H); 3.39 (s, 3H); 3.79 (dd, 1H); 4.29 (dd, 1H); 4.98 (s, 2H); 7.40-7.19 (m, 7H); 7.72 (br, s, 1H); 8.03 (br, s, 1H); 8.28 (s, 1H); 8.47 (s, 1H); 8.60 (s, 1H)
36	0.85 (d, 3H); 0.88 (d, 3H); 1.87 (m, 2H); 2.54 (s, 3H); 2.89 (m, 2H); 3.37 (s, 3H); 3.42 (m, 2H); 3.79 (dd, 1H); 4.29 (dd, 1H); 4.98 (s, 2H); 7.38-7.20 (m, 7H); 7.69 (br; s, 3H); 8.29 (s, 1H); 8.49 (s, 1H); 8.61 (s, 1H)
37	0.83 (d, 3H); 0.87 (d, 3H); 1.32 (m, 1H); 2.16 (m, 1H); 2.46 (d, 3H); 2.57 (s, 3H); 2.71 (m, 1H); 3.37 (s, 3H); 3.78 (dd, 1H); 4.16 (d, 1H); 4.26 (dd, 1H); 4.67 (m, 1H); 4.96 (s, 2H); 6.02 (d, 1H); 6.35 (dd, 1H); 7.35-7.20 (m, 7H); 8.28 (s, 1H); 8.49 (s, 1H); 8.60 (s, 1H); 9.61 (s, 1H)
38	0.83 (d, 3H); 0.87 (d, 3H); 1.25 (m, 1H); 2.2 (m, 1H); 2.5 (s, 3H); 2.70 (m, 3H); 3.35 (s, 3H); 3.63 (m, 1H); 3.79 (d, 1H); 4.27 (dd, 1H); 4.97 (s, 2H); 7.4-7.15 (m, 7H); 8.28 (s, 1H); 8.53 (s, 1H); 8.61 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
39	0.83 (d, 3H); 0.87 (d, 3H); 1.32 (m, 1H); 2.16 (m, 1H); 2.47 (d, 3H); 2.57 (s, 3H); 2.72 (m, 4H); 3.37 (s, 3H); 3.50 (m, 2H); 3.78 (dd, 1H); 4.27 (dd, 1H); 4.97 (s, 2H); 7.40-7.20 (m, 7H); 8.28 (s, 1H); 8.49 (s, 1H); 8.60 (s, 1H)
40	0.83 (d, 3H); 0.87 (d, 3H); 1.32 (m, 1H); 1.71 (m, 2H); 2.25-2.14 (m, 3H); 2.46 (d, 3H); 2.57 (s, 3H); 2.7 (m, 1H); 3.37 (s, 3H); 3.76 (dd, 1H); 4.27 (dd, 1H); 4.97 (s, 2H); 5.81 (d, 1H, J=15.7 Hz); 6.78 (m, 1H); 7.39-7.12 (m, 7H); 8.28 (s, 1H); 8.45 (s, 1H); 8.60 (s, 1H)
41	0.86 (d, 3H); 0.89 (d, 3H); 1.43 (m, 1H); 2.19 (m, 1H); 2.47 (d, 3H); 2.59 (s, 3H); 2.72 (m, 1H); 3.39 (s, 3H); 3.50 (t, 2H); 3.58 (t, 2H); 3.68 (t, 2H); 3.79 (dd, 1H); 4.99 (s, 2H); 7.42-7.20 (m, 7H); 8.27 (s, 1H); 8.47 (s, 1H); 8.59 (s, 1H)
42	0.83 (d, 3H); 0.85 (d, 3H); 1.2-1.4 (m, 3H); 1.5-1.65 (m, 4H); 2.22 (t, 3H); 2.60 (d, 1H); 2.69 (d, 1H); 3.37 (s, 3H); 3.79 (dd, 1H); 4.27 (dd, 1H); 4.86 (d, 2H); 4.97 (s, 2H); 5.00 (dd, 1H); 5.1-5.4 (m, 3H); 5.74 (t, 1H); 6.00 (d, 1H); 7.2-7.4 (m, 7H); 8.27 (s, 1H); 8.44 (s, 1H); 8.62 (s, 1H)

TABLE IV - N.M.R. Spectra (continued)

COMPOUND NO.	^{1}H-NMR (DMSOd_6) δ(ppm)
43	0.84 (d, 3H); 0.89 (d, 3H); 1.4-1.2 (m, 3H); 1.65-1.50 (m, 4H); 2.23 (t, 3H); 2.59 (s, 3H); 2.79 (m, 1H); 3.87 (m, 1H); 4.25 (m, 1H); 5.04 (t, 1H); 5.35-5.20 (m, 3H); 6.09 (d, 1H); 6.67 (br, s, 1H); 7.04 (br, s, 1H); 7.35-7.15 (m, 6H); 8.24 (s ,1H); 8.26 (s, 1H); 8.45 (s, 1H); 8.61 (s, 1H)
44	0.84 (d, 3H); 0.88 (d, 3H); 1.4-1.25 (m, 3H); 1.65-1.50 (m, 4H); 2.23 (t, 3H); 2.58 (s, 3H); 2.75 (m, 1H); 3.78 (dd, 1H): 4.28 (dd, 1H); 4.98 (m, 3H); 5.35-5.15 (m, 3H); 6.03 (m, 2H); 7.42-7.15 (m, 7H): 8.30 (s, 1H): 8.45 (s, 1H): 8.62 (s, 1H)

TABLE V - I.R. Spectra

COMPOUND NO.	I.R. (nujol cm $^{-1}$)
1	3370; 3110; 1730; 1655; 1545; 1520
2	3350; 3110; 1720; 1650; 1535; 1500
3	3340; 3105; 1720; 1645; 1535; 1500
4	3360; 1725; 1640; 1535
5	3350; 3110; 1725; 1650; 1535; 1510
6	3370; 3105; 1725; 1655; 1535; 1500
7	3360; 3100; 1725; 1655; 1535; 1490
8	3370; 3105; 1725; 1655: 1535; 1505
9	3370; 3110; 3100; 1725; 1657; 1550; 1530; 1505
10	3370; 3105; 1730; 1655; 1540; 1510
11	3359; 3115; 1653; 1551; 1510;
12	3360; 3113; 1720; 1662; 1547; 1510

TABLE V - I.R. Spectra (continued)

COMPOUND NO.	I.R. (nujol cm $^{-1}$)
13	3370; 3110; 1720; 1655; 1530; 1505
14	3350; 3120; 1655; 1535; 1500
15	3350; 3100; 1650; 1530; 1510;
16	3340; 3105; 1650; 1530
17	3340; 3100; 1655; 1530
18	3350; 3100; 1710; 1645; 1540
19	3360; 3115; 1720; 1665; 1540; 1506;
20	3350; 3113; 1720; 1659; 1549; 1506
21	3340; 1710; 1645; 1540; 1500
22	3304; 1653; 1540
23	3333; 1657; 1547; 1092; 1038;
24	3354; 3113; 1653; 1550; 1506; 1245

TABLE V - I.R. Spectra (continued)

COMPOUND NO.	I.R. (nujol cm $^{-1}$)
25	3348; 3111; 1660; 1548; 1507; 1245
26	3315; 1653; 1539; 1238
27	3361; 3113; 1720; 1653; 1531; 1507; 1092
28	3333; 1653; 1547; 1494; 1243
29	3356; 3114; 1653; 1508; 1088
30	3360; 1670; 1505; 1200
31	3351; 3115; 1653; 1549; 1509; 1250
32	3370; 3110; 1655; 1545; 1500; 1245
33	3350; 1655; 1530; 1490; 1220
34	3360; 3105; 1650; 1545; 1510; 1240
35	3320; 1747; 1650; 1540; 1225
36	3330; 1662; 1547; 1496; 1201

TABLE V - I.R. Spectra (continued)

COMPOUND NO.	I.R. (nujol cm $^{-1}$)
37	3327; 1730; 1653; 1464; 1377
38	3355; 1720; 1657; 1543; 1377
39	3321; 1717; 1652; 1545
40	3337; 1665; 1549
41	3341; 1721; 1653; 1548; 1377
42	3335; 1722; 1647; 1543
43	3317; 1665; 1539
44	3317; 1720; 1649; 1545

TABLE VI
U.V. DATA λmax ($E^{1\%}_{1cm}$)

Compound No.	MeOH	HCl 0.1N	Phosphate Buffer pH 7.38	KOH 0.1N
1	309 (290.9)	312	309 (247.9)	309 (252.7)
2	309 (257.5)	310 (222.6)	311	309 (226.3)
3	309 (297.5)	312	309 (229.6)	309 (235.8)
4	309 (245.1)	312	308 (234.7)	308 (234.1)
5	308 (173.8)	312	309 (150.0)	305 (181.2)
6	309 (277.1)	313	309 (229.8)	309 (236.7)
7	309 (258.6)	313	309 (207.9)	309 (218.9)
8	309 (279.8)	311	309 (225.9)	309 (229.4)
9	309 (261.9)	313	308 (228.1)	309 (235.0)
10	309 (279.3)	314	309 (241.8)	309 (251.1)
11	309 (216.8)	310 (178.5)	312	310 (194.9)
12	309 (226.2)	310 (188.3)	311	309 (202.4)

TABLE VI (continued)
U.V. DATA λmax ($E^{1\%}_{1cm}$)

Compound No.	MeOH	HCl 0.1N	Phosphate Buffer pH 7.38	KOH 0.1N
13	308 (237.9)	314	308 (247.4)	308 (260.3)
14	309 (263.4)	313	313	314
15	309 (222.6)	313	314	304 (169.8)
16	309 (235.6)	313	312	312
17	309 (230.3)	312	312	312
18	309 (288.8)	313	309 (239.4)	309 (248.0)
19	309 (283.2)	312	309 (220.3)	309 (230.1)
20	309	314	309	309
21	309 (271.6)	313	311 (221.6)	309 (221.6)
22	309 (190.9)	309 (152.2)	308 (160.4)	309 (165.6)
23	309 (242.2)	310 (182.2)	309 (200.9)	309 (200.9)
24	309	312	310	309

TABLE VI (continued)
U.V. DATA λmax ($E^{1\%}_{1cm}$)

Compound No.	MeOH	HCl 0.1N	Phosphate Buffer pH 7.38	KOH 0.1N
25	309	312	310	309
26	309 (260.0)	313	310 (197.7)	310 (208.6)
27	310 (264.6)	313	310 (227.4)	310 (232.1)
28	309 (260.5)	314	310 (186.8)	310 (203.6)
29	309 (243.4)	311	312	311
30	309 (248.5)	311	311	309
31	305 (253.7)	310	310	313 (249.5)
32	309 (267.9)	310 (234.9)	312	312
33	309 (247.5)	311 (234.4)	314	312
34	310 (224.0)	309 (198.2)	310	312
35	308 (269.8)	314	313	313
36	309 (243.9)	309 (205.5)	312	313

TABLE VI (continued)
U.V. DATA λmax ($E^{1\%}_{1cm}$)

Compound No.	MeOH	HCl 0.1N	Phosphate Buffer pH 7.38	KOH 0.1N
37	309 (255.1)	312	314	312
38	308	308	308	308
39	308 (247.9)	312	308 (201.3)	308 (215)
40	309 (304.3)	312	309 (235.9)	309 (262.0)
41	309 (256.4)	312	309 (215.1)	309 (228.6)
42	309	312	309	307
43	309 (253.6)	313	309 (208.4)	309 (235.4)
44	309 (264.9)	314	309 (208.1)	309 (223.7)

The antimicrobial activity of the compounds of the invention can be demonstrated by a series of standard tests in vitro.

MIC for Propionibacterium acnes, and Bacteroides fragilis are determined by agar dilution (inocula $10^4/10^5$ CFU/spot). MIC for other organisms are determined by microbroth dilution (inocula 10^4 to 10^5 CFU/ml). Incubation times are 18-24 h, except for Haemophilus influenzae, P. acnes, B. fragilis (48 h). All organisms are incubated at 37°C; H. influenzae is incubated in a 5% CO_2 atmosphere, anaerobes in an anaerobic gas mixture. Media used are: Iso-Sensitest broth (Oxoid) (Staphylococci, Streptococcus faecalis, Escherichia coli, Proteus vulgaris; brain heart infusion broth (Difco) + 1% Supplement C (Difco) (H. influenzae);

The minimal inhibitory concentrations (MIC, microgram/ml) for some microorganisms are reported below in Table VII.

TABLE VII - (MIC, microgram/ml)

STRAIN	COMPOUND OF EXAMPLE No.				
	1	2	6	7	8
Staph. aureus L165 Tour	0.5	0.13	<0.13	0.25	0.25
Staph. epidermidis L147 ATCC 12228	1	0.25	1	0.5	2
Staph. haemolyticus L602	4	16	1	4	2
Strep. pneumoniae L44 UC41	8	>128	2	4	4
Strep. faecalis L149 ATCC 7080	0.25	0.06	<0.13	<0.13	0.25
Prop. acnes L1014 ATCC 6919	<0.13	0.06	<0.13	<0.13	<0.13
Bact. fragilis L1010 ATCC 23745	8	>128	>128	>128	32
Haemophilus Influenzae type B	8	>128	32	128	64
Esch. coli L47 SKF 12140	>128	>128	>128	>128	>128
Prot. vulgaris ATCC 881	>128	>128	>128	>128	>128

TABLE VII - (MIC, microgram/ml) (continued)

STRAIN	COMPOUND OF EXAMPLE No.				
	14	18	19	20	26
Staph. aureus L165 Tour	0.06	0.25	0.06	0.25	0.25
Staph. epidermidis L147 ATCC 12228	0.13	0.25	0.06	0.25	0.25
Staph. haemolyticus L602	0.25	1	0.25	0.25	0.5
Strep. pneumoniae L44 UC41	>128	1	0.25	2	2
Strep. faecalis L149 ATCC 7080	0.06	0.13	0.06	<0.13	0.13
Prop. acnes L1014 ATCC 6919	0.03	0.016	0.008	0.03	0.008
Bact. fragilis L1010 ATCC 23745	>128	2	4	>128	32
Haemophilus Influenzae type B	>128	2	2	>128	8
Esch. coli L47 SKF 12140	>128	>128	>128	>128	>128
Prot. vulgaris ATCC 881	>128	>128	>128	>128	>128

TABLE VII - (MIC, microgram/ml) (continued)

STRAIN	COMPOUND OF EXAMPLE No.			
	27	28	32	35
Staph. aureus L165 Tour	0.25	0.13	0.5	0.13
Staph. epidermidis L147 ATCC 12228	0.5	0.5	0.5	0.13
Staph. haemolyticus L602	1	1	0.5	0.5
Strep. pneumoniae L44 UC41	8	2	1	>128
Strep. faecalis L149 ATCC 7080	1	0.06	0.25	0.06
Prop. acnes L1014 ATCC 6919	0.03	0.008	0.13	0.004
Bact. fragilis L1010 ATCC 23745	64	>128	>128	>128
Haemophilus Influenzae type B	8	>128	>128	>128
Esch. coli L47 SKF 12140	>128	>128	>128	>128
Prot. vulgaris ATCC 881	>128	>128	>128	>128

TABLE VII - (MIC, microgram/ml) (continued)

STRAIN	COMPOUND OF EXAMPLE No.				
	36	37	38	39	40
Staph. aureus L165 Tour	0.13	0.03	32	0.13	0.25
Staph. epidermidis L147 ATCC 12228	0.13	0.06	32	0.25	0.25
Staph. haemolyticus L602	0.13	0.13	64	2	1
Strep. pneumoniae L44 UC41	4	>128	>128	4	1
Strep. faecalis L149 ATCC 7080	0.13	0.06	16	≤0.13	≤0.13
Prop. acnes L1014 ATCC 6919	0.06	0.004	0.25	≤0.13	-
Bact. fragilis L1010 ATCC 23745	>128	>128	>128	8	4
Haemophilus Influenzae type B	>128	>128	>128	1	1
Esch. coli L47 SKF 12140	>128	>128	>128	>128	>128
Prot. vulgaris ATCC 881	>128	>128	>128	>128	>128

TABLE VII - (MIC, microgram/ml) (continued)

STRAIN	COMPOUND OF EXAMPLE No.			
	41	42	43	44
Staph. aureus L165 Tour	0.25	0,13	0.13	0.13
Staph. epidermidis L147 ATCC 12228	0.5	0,13	0.5	0.5
Staph. haemolyticus L602	1	0.5	0.5	1
Strep. pneumoniae L44 UC41	2	1	0.5	1
Strep. faecalis L149 ATCC 7080	≤0.13	0.13	0.13	0.06
Prop. acnes L1014 ATCC 6919	≤0.13	0.016	0.016	0.016
Bact. fragilis L1010 ATCC 23745	4	>128	>128	>128
Haemophilus influenzae type B	1	4	>128	>128
Esch. coli L47 SKF 12140	>128	>128	>128	>128
Prot. vulgaris ATCC 881	>128	>128	>128	>128

In view of their properties, the compounds of the invention can be used as active ingredients in the preparation of medicaments for human or animal treatment

In particular, the amide derivatives of the antibiotic GE 2270 compounds of formula I are antimicrobial agents mainly active against gram positive bacteria and gram positive as well as gram negative anaerobes.

The main therapeutic indication of the antibiotic substances of the invention is thus in the treatment of infections related to the presence of microorganisms susceptible to them.

The term "treatment" is intended to encompass also prophylaxis, therapy and cure.

The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

The compounds of the invention can be administered as such or in admixture with pharmaceutically acceptable carriers and can also be administered in conjunction with other antimicrobial agents. Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active compounds in a way that the therapeutical effects of the first administered one is not entirely disappeared when the subsequent is administered.

A preferred pharmaceutical formulation is represented by a formulation suitable for a topical application on an intact or damaged skin or mucous membrane. Examples of such formulations are powders, ointments, creams and lotions. The excipients in these

formulations are the usual pharmaceutically acceptable vehicles such oleaginous ointment bases (e.g. cetyl esters wax, oleic acid, olive oil, paraffin, spermaceti, starch glycerite); absorbent ointment bases (e.g. anhydrous lanolin, hydrophilic petrolatum), emulsion ointment bases (e.g. cetyl alcohol, glyceryl monostearate, lanolin, stearic acid), water-soluble ointment bases (e.g. glycol ethers and their derivatives which include polyethylene glycols, poly(oxy-1,2-ethan ediyl)-alpha-hydro-omega-hydroxy-octadecanoate, polysorbates, and polyethylene glycols mono-stearates).

These formulations may contain other known excipients, such as preservatives and are prepared as known in the art and reported in reference handbooks such as Remington's Pharmaceutical Sciences, Seventeenth edition, 1985, Mack Publishing Co.

The compounds of the invention can also be formulated into formulation suitable for parenteral administration according to procedures known per se in the art. For instance, a compound of the invention is formulated with polypropylene glycol or dimethylacetamide and a surface-active agent such as polyoxyethylene sorbitan mono-oleate or polyethoxylated castor oil.

A preferred formulation for parenteral administration includes the following excipients: Cremophor® EL (polyoxyl 35 castor oil USP/NF) 20%, propylene glycol 5-10%.

Preferably, this formulation is used for i.v. administration in the treatment of any infection involving a microorganism susceptible to an antibiotic of the invention.

An example of a suitable formulation used for I.V. is the following

compound No. 19	100 mg
propylene glycol	1 ml
water for injection q.s.	5 ml
phosphate buffer pH 8-8.5	

In the treatment of pseudomembranous colitis or other diseases attributable to the presence of anaerobes in the gastrointestinal tract, an effective dose of the compounds of the invention may be administered orally in suitable pharmaceutical form such as a capsule or an aqueous suspension.

The dosage of the active ingredient depends on many factors which include type, age and conditions of the patient, specific active ingredient and formulation selected for administration, administration schedule, etc.

In general, effective antimicrobial dosages are employed per single unit dosage form.
Repeated applications of these dosage forms, e.g. from 2 to 6 times a day, are in general preferred. An effective dosage may be in general in the range 0.5-50 mg/kg body weight/day.

A preferred topic preparation is an ointment containing from 1% to 10% of a compound of the present invention.

Anyway, the prescribing physician will be able to determine the optimal dosage for a given patient in a given situation.

Besides their use as medicaments in human and veterinary therapy, the compounds of the invention can also be used as animal growth promoters.

For this purpose, a compound of the invention is administered orally in a suitable feed. The exact concentration employed is that which is required to provide for the active agent in a growth promotant effective amount when normal amounts of feed are consumed.

The addition of the active compound of the invention to animal feed is preferably accomplished by preparing an appropriate feed premix containing the active compound in an effective amount and incorporating the premix into the complete ration.

Alternatively, an intermediate concentrate or feed supplement containing the active ingredient can be blended into the feed. The way in which such feed premixes and complete rations can be prepared and administered are described in reference books (such as "Applied Animal Nutrition", W.H. Freedman and CO., S. Francisco, USA, 1969 or "Livestock Feeds and Feeding" O and B books, Corvallis, Oregon, USA, 1977).

The following examples further illustrate the invention and should not be interpreted as limiting it in any way.

EXAMPLES OF THE INVENTION

PROCEDURE A - Reaction of GE 2270 factor A_3 starting material with the selected amine

Example 1:

Preparation of compound no. 15, 29, 30, 32, 33

To a stirred solution of 1 mmol of GE 2270 factor A_3 (prepared as described in European Patent Application Publication No. 406745) in 10 ml of dimethylformamide (DMF), 1.2 mmols of the selected amine, 1.4 mmols of triethylamine (TEA) and 1.2 mmols of di-phenylphosphorazidate (DPPA) were added at 0°C. (If the salt (chloride, p-toluenesulfonate, etc) of the selected amine was used, a double amount of TEA had to be used). The temperature was allowed to rise to room temperature and stirring was continued for about 4 h. The reaction mixture was then acidified with 1N aq HCl to about pH 3 and then diluted with water to complete precipitation of the product. The wet solid was dried in air and then purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 3 to 5% methanol in chloroform. Fractions containing the title compound were pooled together and the solvent evaporated. Trituration of the solid with ethyl ether yielded the title compound as a fine powder.

PROCEDURE A1 - Reaction of GE 2270 factor A_3 starting material with the selected amine containing

further reactive functional group(s), all of which protected, and subsequent deprotection of the protecting group(s).

Example 2:
Preparation of compound no. 34, 36

The reaction was substantially carried out as described in Example 1. Once the reaction product had been purified by flash chromatography, 1 mmol of the solid obtained was treated with 7 ml of cold trifluoroacetic acid (TFA). The suspension was swirled for a few minutes until a solution was obtained and TFA was evaporated "in vacuo" in the cold. The gummy product still containing traces of TFA was then treated with ethyl ether and the title compound was obtained as the trifluoroacetate salt in the form of a fine powder.

Example 3:
Preparation of compound no. 1, 3 to 10, 18 to 21, 39

The reaction was substantially carried out as described in Example 1. Once the reaction product had been purified by flash chromatography, 1 mmol of the solid obtained was dissolved in 20 ml of dioxane and 1.2 ml of 1N aq NaOH were added under stirring at room temperature. After 5 h the solution was acidified with 1N aq HCl to pH 2 and diluted with water to complete precipitation of the title compound which was filtered off and allowed to dry in air yielding the title compound as a fine powder.

Example 4:

Preparation of compound no. 2

The reaction was carried out as described in Example 3. Once hydrolysis of the ester function had been accomplished and the compound had been allowed to dry in air, 1 mmols of the solid obtained was dissolved in 20 ml of TFA and 50 mmols of thioanisole were added under stirring at room temperature as described by Y. Kiso et al., Chem. Pharm. Bull. 28, 673, 1980. After 3.5 h, TFA was evaporated "in vacuo" in the cold and the residue taken up in a minimum amount of 1% methanol in chloroform. Addition of ethyl ether induced the precipitation of the title compound which was filtered, washed with more ethyl ether and dried "in vacuo" to yield the trifluoroacetate salt of the title compound as a fine powder.

Example 4bis:

Preparation of compound no. 37

The reaction was substantially carried out as described in Example 1. Once the starting material had disappeared from the reaction mixture, water was added and the precipitate obtained was filtered off, washed with additional water and allowed to dry in air. The crude material was then dissolved in 3 ml of THF and stirred overnight at room temperature in the presence of 10% aq. HCl. Dilution with water provided complete precipitation of the product which was filtered off and allowed to dry in air. The solid was then purified by

flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 2 to 4% methanol in chloroform. Fractions containing the title compound were pooled together and the solvent evaporated yielding a pale yellow powder.

PROCEDURE B - Reaction of GE 2270 factor A_3 starting material with the selected amine containing unprotected acid moieties.

Example 5

Preparation of compound no. 19, 22 to 28, 40, 41

1.1 mmols of DPPA were added at 0°C to a stirred solution of 1 mmol of GE 2270 factor A_3 and 1.5 mmols of TEA in 10 ml of DMF. The temperature was allowed to rise to room temperature and stirring was continued for 4.5 more hours. 1.5 Mmols of the selected amine and 2 mmols of TEA were then added to the solution at room temperature and stirring was continued at the same temperature for 5 more hours. (If the selected amine contained more than one acid function, the amount of TEA was adjusted so to free the amino group). The reaction mixture was then acidified with 1N aq HCl to about pH 2 and then diluted with water to complete precipitation of the product. The wet solid was dried in air and then purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 5 to 10% methanol in chloroform. Fractions containing the title compound were pooled together and the solvent evaporated. Trituration

of the solid with ethyl ether yielded the title compound as a fine powder.

PROCEDURE B1 - Reaction of GE 2270 factor A_3 starting material with the selected amine containing reactive functional group(s), all of which are variously protected, in addition to the unprotected acid group(s) and subsequent deprotection of the protecting group(s).

Example 6:
Preparation of compound no. 11, 12

The reaction was substantially carried out as described in Example 5. Once the reaction product had been purified by flash chromatography, 1 mmol of the solid obtained was dissolved in 20 ml of TFA and 50 mmols of thioanisole were added under stirring at room temperature. After 3.5 h, TFA was evaporated "in vacuo" in the cold and the residue taken up in a minimum amount of 1% methanol in chloroform. Addition of ethyl ether induced the precipitation of the title compound which was filtered, washed with more ethyl ether and dried "in vacuo" to yield the trifluoroacetate salt of the title compound as a fine powder.

PROCEDURE C - Reaction of selected amide derivatives of GE 2270 factor A_3 as starting material with the selected reagent.

Example 7

Preparation of compound no. 14, 15, 16, 17 from compound no. 1, 5, 10, 6 respectively

To a stirred solution of 1 mmol of the appropriate amide derivative of GE 2270 factor A_3 (prepared as described in the previous examples) in 10 ml of DMF, 1.2 mmols of the selected amine, 1.4 mmols of TEA and 1.2 mmols of DPPA were added at 0°C. (If the salt (chloride, p-toluenesulfonate, etc) of the selected amine was used, a double amount of TEA had to be used). The temperature was allowed to rise to room temperature and stirring was continued for about 4 h. The reaction mixture was then acidified with 1N aq HCl to about pH 3 and then diluted with water to complete precipitation of the product. The wet solid was dried in air and then purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 3 to 5% methanol in chloroform. Fractions containing the title compound were pooled together and the solvent evaporated. Trituration of the solid with ethyl ether yielded the title compound as a fine powder.

PROCEDURE C1 - Reaction of the selected amide derivative of the GE 2270 factor A_3 as starting material with the selected reagent which contains further reactive functional group(s), all of which protected, and subsequent deprotection of the protecting group(s).

Example 8:

Preparation of compound no. 13 from compound no.3

The reaction was carried out as described in Example 7. Once the reaction product had been purified by flash chromatography, 1 mmol of the solid obtained was dissolved in 20 ml of dioxane and 1.2 ml of 1N aq NaOH were added under stirring at room temperature. After 5 h the solution was acidified with 1N aq HCl to pH 2 and diluted with water to complete precipitation of the title compound which was filtered off and allowed to dry in air yielding the title compound as a fine powder.

Example 9:

Preparation of compound no. 31 from compound no. 36

To a stirred solution of 1 mmol of the appropriate amide derivative of GE 2270 factor A_3 (prepared as described in the previous examples) in 10 ml of 10% methanolic chloroform, 1.2 mmols of TEA and 1.1 mmols the selected reagent (see table -) were added at room temperature. After 20 min the solvent was evaporated "in vacuo" and the residue treated with 5% aq Na_2CO_3. The solid obtained was filtered off, washed with more 5% Na_2CO_3 and water and finally redissolved in 10 ml of methanol. To this solution, 0.5 ml of water and 0.1 mmols of p-toluenesulfonic acid were added and the reaction mixture was stirred at room temperature overnight. The solution was then reduced to a small volume (about 2 ml) under vacuum and water was added to

precipitate the title compound which, after drying in air, was obtained as a fine powder.

Example 9bis:

Preparation of compound no. 38 from compound no. 37

To a stirred solution of 0.23 mmols of the appropriate amide derivative of GE 2270 factor A_3 (prepared as described in the previous examples) in 40 ml of ethanol, 9.2 mmols of acetic acid, 9.2 mmols of sodium acetate and 0.506 mmols of the selected reagent (see table II) were added at room temperature. After 2 hours 0.46 mmols of $NaBH_4$ (Fluka) were added and stirring was continued overnight at the same temperature. Evaporation of the solvent provided a crude material which was washed with 10 ml of 1N HCl, filtered and allowed to dry in air. The solid was then purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 0 to 10% methanol in dichloromethane. The fractions containing the methyl ester of the title compound (intermediate) were pooled together and the solvent evaporated providing a solid which was redissolved in 2 ml dioxane and treated overnight with a 1.2 molar excess of 1N NaOH at room temperature. Evaporation of the solvent gave a solid which was further purified by trituration with a 1:1 mixture of ethyl acetate:methanol yielding the title compound as a fine powder.

PROCEDURE D - Reaction of GE 2270 factor A_2 starting material with the selected amine

Example 10:
Preparation of compound No. 35

1 mmol of GE 2270 factor A_2 (prepared as described in European Patent Application Publication No. 406745) were dissolved in 10 ml of a saturated solution of methanolic ammonia. The solution was allowed to stand for 3 days at room temperature and then evaporated "in vacuo". The residue was taken up in 2 ml of methanol and the title compound precipitated with water, filtered off and allowed to dry in air. Trituration with ethyl ether yielded the title compound as a fine powder.

PROCEDURE E - Preparation of a salt of a compound of the invention.

Example 11:
Preparation of the arginine salt of compound No. 19

To a suspension of 3 g of compound No. 19 (2.42 mmols) 180 ml of dioxane, a solution of 423 mg of L-arginine (2.42 mmols) in 120 ml of water were added under stirring and the non clear solution was thus lyophilized to recovered the desired salt.

PROCEDURE F - Reaction of GE 2270 component C_{2a} starting material (i.e. the compound of formula II wherein R is methoxymethyl, R_1 is methyl, R_4 is hydroxymethyl and W is COOH) with the selected amine containing further reactive functional group(s), all of which protected, and subsequent deprotection of the protecting group(s).

Example 12:
Preparation of compound no. 42

The reaction was carried out as described in Example 3 using GE 2270 component C_{2a} starting material instead of factor A_3.

PROCEDURE G - Reaction of GE 2270 component C_{2a} starting material as described in procedure F with the selected amine containing unprotected acid moieties.

Example 13:
Preparation of compound no.42

The reaction was carried out as described in Example 5 using GE 2270 component C_{2a} starting material instead of factor A_3.

PROCEDURE H - Reaction of GE 2270 component D_1 starting material (i.e. the compound of formula II wherein R and R_1 are hydrogen, R_4 is methyl and W is COOH) with the selected amine containing further reactive functional group(s), all of which protected, and subsequent deprotection of the protecting group(s).

Example 14:
Preparation of compound no. 43

The reaction was carried out as described in Example 3 using GE 2270 component D_1 starting material instead of factor A_3.

PROCEDURE I - Reaction of GE 2270 component D_1 starting material as described in procedure H with the selected amine containing unprotected acid moieties.

Example 15:
Preparation of compound no.43

The reaction was carried out as described in Example 5 using GE 2270 component D_1 starting material instead of factor A_3.

PROCEDURE J - Reaction of GE 2270 component D_2 (i.e. the compound of formula II wherein R is hydroxymethyl, R_1 and R_4 are methyl and W is COOH)

starting material with the selected amine containing further reactive functional group(s), all of which protected, and subsequent deprotection of the protecting group(s).

Example 16:
Preparation of compound no. 44

The reaction was carried out as described in Example 3 using GE 2270 component D_2 starting material instead of factor A_3.

PROCEDURE K - Reaction of GE 2270 component D_2 starting material as described in procedure J with the selected amine containing unprotected acid moieties.

Example 17:
Preparation of compound no.44

The reaction was carried out as described in Example 5 using GE 2270 component D_2 starting material instead of factor A_3.

PROCEDURE L - Reaction of a mixture of minor components (C_{2a}, D_1, D_2 and E) of antibiotic GE 2270 (starting material) with the selected amine containing further reactive functional group(s), all of which

protected, and subsequent deprotection of the protecting group(s).

Example 18:

The reaction was carried out as described in Example 3 using a mixture of minor components (C_{2a}, D_1, D_2 and E) of antibiotic GE 2270 starting material instead of factor A_3 and methyl 6-aminocaproate hydrochloride (Fluka). R_t (min) refer to HPLC method M reported in the HPLC analysis section.

When Y=-NH $CH_2CH_2CH_2CH_2CH_2COOCH_3$, R_t (min) are respectively 43.43 for GE 2270 factor C_{2a}, 39.42 for GE 2270 factor D_1, 42.29 for GE 2270 factor D_2 and 37.41 for GE 2270 factor E.

When Y=-NH $CH_2CH_2CH_2CH_2CH_2COOH$, R_t (min) are respectively 17.23 for GE 2270 factor C_{2a}, 15.76 for GE 2270 factor D_1, 16.64 for GE 2270 factor D_2 and 15.13 for GE 2270 factor E.

PROCEDURE M - Reaction of a selected mixture of minor components (C_{2a}, D_1, D_2 and E) of antibiotic GE 2270 starting material with the selected amine containing unprotected acid moieties.

Example 19:

The reaction was carried out as described in Example 5 using a selected mixture of minor components (C_{2a}, D_1, D_2 and E) of antibiotic GE 2270 starting

material instead of factor A_3 and 6-aminocaproic acid (Fluka).

R_t (min) refer to Method M reported in the HPLC analysis section and are respectively 17.23 for GE 2270 factor C_{2a}, 15.76 for GE 2270 factor D_1, 16.64 for GE 2270 factor D_2 and 15.13 for GE 2270 factor E.

PREPARATION OF THE STARTING MATERIALS

1. The following starting materials have been purchased from Fluka (Fluka, Chemika-Biochemika, Buchs, Switzerland):
Glycine ethyl ester hydrochloride,
L-threonine methyl ester hydrochloride,
L-tyrosine methyl ester hydrochloride,
L-leucine methyl ester hydrochloride,
L-phenylalanine methyl ester hydrochloride,
L-methionine methyl ester hydrochloride,
L-proline methyl ester hydrochloride,
L-threonine methyl ester hydrochloride,
Nα-Cbz-L-lysine,
methyl 4-aminobutyrate hydrochloride,
methyl 6-aminocaproate hydrochloride,
6-aminocaproic acid,
4-(methylamino)benzoic acid,
piperidine-4-carboxylic acid,
N-methyl-D-glucamine,
D(+)-glucosamine hydrochloride,
2-dimethylaminoethylamine,
amino acetaldehyde dimethylacetal,
β-alanine ethyl ester hydrochloride.

2. The following starting materials have been purchased from Sigma (Sigma, Biochemicals Organic Compounds, St. Louis, U.S.A.):
Nδ-Cbz-L-ornithine,
L-aspartic acid dimethyl ester hydrochloride.

3. The following starting materials have been purchased from Aldrich (Aldrich, Catalogo Prodotti di Chimica Fine, Milano, Italy):
L-Prolinamide,
taurine,
3-amino-1-propanesulfonic acid,
3-aminopropylphosphonic acid,
4-amino-1-benzylpiperidine.

4. Production of antibiotic GE 2270 for preparing antibiotic GE 2270 factors A, B_1, B_2, C_1, C_2, C_{2a}, D_1, D_2, and E

A culture of Planobispora rosea ATCC 53773 is grown on an oatmeal agar slant for two weeks at 28-30°C and then used to inoculate 500 ml flasks containing 100 ml of a seed medium of the following composition:

Starch	20 g/l
Polypeptone	5 g/l
Yeast extract	3 g/l
Beef extract	2 g/l
Soybean meal	2 g/l
Calcium carbonate	1 g/l
Distilled water q.s.	100 ml

(adjusted to pH 7.0 before sterilization)

The flask is incubated on a rotary shaker (200 rpm) at 28-30°C for 92 h. The obtained culture is then used to inoculate a jar fermenter containing 4 liters of the same medium and the culture is incubated at 28-30°C for 48 hours with stirring (about 900 rpm) and

aeration (about one standard liter of air per volume per minute).

The obtained broth is transferred to a fermenter containing 50 l of the following production medium:

Starch	20	g/l
Peptone	2.5	g/l
Hydrolyzed casein	2.5	g/l
Yeast extract	3	g/l
Beef extract	2	g/l
Soybean meal	2	g/l
Calcium carbonate	1	g/l
Distilled water	q.s.	

(adjusted to pH 7.0 before sterilization) and incubated for about 72 hours at 28-30°C.

Antibiotic production is monitored by paper disc agar assay using B. subtilis ATCC 6633 grown on minimum Davis medium. The inhibition zones are evaluated after incubation overnight at 35°C.

4a) Recovery of crude antibiotic GE 2270

The fermentation mass (50 l) obtained above is harvested and submitted to filtration in the presence of a filter aid (Clarcell).

Antibiotic GE 2270 is found mainly in the mycelium, even if a certain amount of it can be recovered also from the filtrates.

The filtrate is adjusted to about pH 7.0 and extracted with ethyl acetate (50 l). The organic phase is separated by centrifugation and concentrated to a small volume under reduced pressure. The obtained oily residue is then treated with petroleum ether to precipitate crude antibiotic GE 2270 that is collected by filtration and dried. 415 mg of crude antibiotic GE 2270 complex is obtained.

The mycelium is extracted twice with 20 l of methanol and the pooled extracts are concentrated under reduced pressure to give an aqueous residue which is extracted twice with ethyl acetate. Crude antibiotic GE 2270 (6.06 g) is precipitated by addition of petroleum ether from the concentrated organic phase.

4b) Isolation of antibiotic GE 2270 factor A

The crude obtained from the mycelium according to the procedure described above (3 g) is dissolved in tetrahydrofuran and concentrated under reduced pressure in the presence of silica gel (230-400 mesh). The obtained solid residue is collected and applied to a chromatography column containing 300 g of silica gel (230-400 mesh) prepared in methylene chloride (CH_2Cl_2). The column is developed first with methylene chloride (2 l) and then sequentially with 1.5 l mixtures of methylene chloride and methanol in the following ratios: 98/2; 96/4, 94/6, 92/8, 90/10 and 88/12 (v/v).

Fractions are collected, analyzed by TLC, HPLC or microbiologically against B. subtilis and pooled according to their antibiotic content.

The pooled fractions containing antibiotic GE 2270 factor A are concentrated under reduced pressure to give an oily residue which is solubilized with tetrahydrofuran.

From this solution, antibiotic GE 2270 factor A (600 mg) is precipitated by adding petroleum ether.

4bis) Isolation of mixtures of minor components of antibiotic GE 2270

A representative mixture particularly enriched in the minor components C_{2a}, D_1, D_2 and E was established by HPLC comparison with analytical samples of each single component.

R_t (min) refer to HPLC method M reported in the HPLC analysis section and are 20.55 for GE 2270 factor C_{2a}, 17.43 for GE 2270 factor D_1, 18.17 for GE 2270 factor D_2, and 16.61 for GE 2270 factor E.

Concentration of this fraction under reduced pressure produced an oily residue which was redissolved in tetrahydrofuran and precipitated with petroleum ether as whitish powder.

4c) Separation and isolation of antibiotic GE 2270 factors B_1, B_2, C_1, C_2, D_1, D_2, and E

Antibiotic GE 2270 factors D_1, D_2 and E are separated and purified from the above obtained crude mixture by preparative HPLC using a 250x20 mm column packed with Nucleosil® C18 (silica gel functionalized with octadecylsilane groups) (5 µm) and eluted with mixtures of Phase A: CH_3CN:tetrahydrofuran:40 mM $HCOONH_4$ (40:40:20); Phase B: CH_3CN:tetrahydrofuran:40 mM $HCOONH_4$ (10:10:80). The antibiotic mixture (6 mg) was solubilized in 3 ml of Phase B and 1 ml of Phase A and was injected into the HPLC column which was eluted at a flow rate of 14 ml/min with a 26:74 mixture of Phase A and B. The eluted fractions were collected according to the UV adsorption profile at 254 nm. The fractions of subsequent chromatographic runs having homogeneous content were pooled and concentrated under reduced pressure to eliminate CH_3CN. The residual solution showed antibacterial activity against *Staphylococcus aureus* Tour L165 by paper disc assay. These solutions were lyophilized at least three times to remove completely the $HCOONH_4$ buffer residue from the HPLC phases.

The yields were as follows: antibiotic GE 2270 factor E, 11 mg; antibiotic GE 2270 factor D_1, 12 mg; antibiotic GE 2270 factor D_2, 10 mg.

4d) Isolation of a purified mixture containing antibiotic GE 2270 factor C_{2a} in mixture with other GE 2270 factors

The preparations of crude GE 2270 factors from 6 repeated fermentations were pooled and solubilized

into 12 l of CH_2Cl_2:methanol (93:7). The insoluble material was removed by filtration and the solution, containing the antibiotic complex, was applied to a 13 kg (230-400 mesh) silica gel column equilibrated in CH_2Cl_2:methanol (93:7). Antibiotic GE 2270 factor C_{2a} was eluted from the column by eluting with CH_2Cl_2 : methanol (93:7). The fractions containing the antibiotic of the invention (HPLC analysis) were pooled, were concentrated under reduced pressure and were dried to yield 23.5 g of antibiotic GE 2270 factor C_{2a} in mixture with other minor factors.

A portion (5.5 g) of this preparation was again purified by flash chromatography on a column containing 400 g of silica gel (230-400 mesh) equilibrated in methylene chloride (CH_2Cl_2). The column was developed first with methylene chloride (1 liter) and then sequentially with a series of mixtures of methylene chloride / methanol in the following ratios (v/v): 96/4 (3 liters); 94/6 (1 liter); 92/8 (2 liters); 90/10 (6 liters) and 88/12 (4 liters).

The fractions containing mainly GE 2270 factor C_{2a} (HPLC analysis) were pooled and were concentrated. The antibiotic preparation (646 mg) was precipitated upon addition of petroleum ether.

4e) Isolation of pure antibiotic GE 2270 factor C_{2a}

The purified mixture containing mainly antibiotic GE 2270 factor C_{2a} was further purified by preparative HPLC from the above described preparation.

A portion of the above described preparation of the antibiotic (10 mg) was solubilized in 1 ml of Phase A (CH_3CN : tetrahydrofuran : 40 mM $HCOONH_4$ - 40:40:20)

and 1 ml of Phase B (CH_3CN : tetrahydrofuran : 40 mM $HCOONH_4$ -10:10:80) and was injected into a HPLC 250x20 mm Hibar column (E. Merck; Darmstadt F.R. Germany) packed with 7 µm Nucleosil®C18 (silica gel functionalized with octadecylsilane groups) which was equilibrated with a mixture of 40% Phase A and 60% Phase B. The column was eluted at 15 ml/min flow rate with a 22 minutes linear gradient from 40% to 50% of Phase A. The UV detection was 254 nm. The fractions of 10 subsequent chromatographic runs containing the pure antibiotic of the invention were pooled and were concentrated under reduced pressure to eliminate CH_3CN. Antibiotic GE 2270 factor C_{2a} precipitated from water. The precipitate was collected by centrifugation, was washed twice with distilled water and was dried under vacuum yielding 66 mg of the pure antibiotic.

5. Preparation of antibiotic GE 2270 factor A_2

Antibiotic GE 2270 factor A (prepared as described above) (86 mg) is dissolved in 17 ml of 95% ethanol and 1.7 ml of acetic acid. After incubation at 60°C for 24 h, the resulting solution is diluted with 0.1M sodium phosphate buffer pH 7.5 (100 ml) and adjusted to pH 7.5 with 1M sodium hydroxide. Ethanol is removed by evaporation under reduced pressure and the aqueous residue is extracted twice with ethyl acetate (100 ml). The organic phase is concentrated under reduced pressure to obtain a solid residue which is solubilized with tetrahydrofuran and then precipitated by adding petroleum ether. Antibiotic GE 2270 factor A_2 (62 mg) is obtained with minor amounts of antibiotic

GE 2270 factors A and A_1. Pure antibiotic GE 2270 factor A_2 is obtained by preparative HPLC as follows:

10 Mg of the above crude product is solubilized in tetrahydrofuran, diluted to the solubility limit with water and then injected into a HPLC system with a column (250 x 20 mm) packed with Nucleosil^R C18 (5 micrometer) reverse phase silica gel by Stacroma^R, eluting with a linear gradient from 64% to 93% of phase B in phase A, in 20 min, at a flow rate of about 15 ml/min. In this system, phase A is a 90:10 (v/v) mixture of 18 mM sodium phosphate pH 7.2 and acetonitrile, while phase B is a 40:60 (v/v) mixture of 18 mM sodium phosphate pH 7.2 and acetonitrile. Fractions of five consecutive runs are collected and UV monitored at 330 nm. Fractions which contain substantial amounts of antibiotic GE 2270 factor A_2, which correspond to the major peaks of the UV elution profile, are pooled and concentrated under reduced pressure to an aqueous phase which is extracted twice with ethyl acetate. This organic layer is then washed with distilled water to remove the residual inorganic salts and concentrated to precipitate a solid residue that is then dissolved in tetrahydrofuran and re-precipitated with petroleum ether, to obtain pure antibiotic GE 2270 factor A_2 (45 mg).

In European Patent Application Publication No. 406745 are described other alternative method for preparing antibiotic GE 2270 factor A_2 as main reaction product of antibiotic GE 2270 factor A.

6. Preparation of antibiotic GE 2270 factor A_3

Antibiotic GE 2270 factor A_2 is incubated for 1 h at room temperature in 0.5M sodium carbonate. The reaction mixture is then diluted with cold water and brought to pH 6.5 with hydrochloric acid. The neutralized solution contains antibiotic GE 2270 factor A_3 as the main reaction product. This antibiotic is extracted from the aqueous phase with ethyl acetate and then is precipitated from the concentrated organic phase by adding petroleum ether.

Pure antibiotic GE 2270 factor A_3 is obtained by column chromatography as described below:

1.5 Grams of crude GE 2270 A_3 is dissolved in 60 ml of a 1/1 (v/v) mixture of methanol and dichloromethane and adsorbed on silica gel (75-230 mesh) by evaporation of the solvents under reduced pressure. The solid residue is then put on the top of a silica gel (75-230 mesh) column (bed height 40 cm) equilibrated with dichloromethane. The column is then eluted with mixtures of methanol in dichloromethane in the order: 1) 2% methanol (450 ml); 2) 5% methanol (500 ml); 3) 10% methanol (600 ml); 4) 15% methanol (500 ml); 5) 20% methanol (500 ml); 6) 30% methanol (250 ml).

Fractions are collected and monitored by TLC and a microbiological assay on B. subtilis ATCC 6633. Antibiotic GE 2270 factor A_3 is normally present in the eluates which contain about 15-20% methanol.

The fractions containing the desired product are pooled and concentrated under reduced pressure. Upon addition of petroleum ether to the residue, antibiotic GE 2270 factor A_3 precipitates (854 mg of pure product).

7. Preparation of the proper starting material from antibiotic factors D_1, D_2, E and C_{2a}

By substantially following the same procedure described at points 5 and 6 above but starting from the single factors D_1, D_2, E and C_{2a} of antibiotic GE 2270 instead of factor A, the proper starting materials of formula III wherein W is COOH or an activated ester, R is hydrogen or CH_2OH, R_1 is CH_3 or hydrogen and R_4 is hydroxymethyl or methyl, are obtained.

7a) Preparation of proper starting material from a mixture of minor components (C_{2a}, D_1, D_2 and E) of antibiotic GE 2270

By substantially following the same procedure described at point 5 and 6 above but starting from a mixture of minor components (C_{2a}, D_1, D_2 and E) of antibiotic GE 2270 instead of the single factor A, the proper starting material of formula III wherein W is COOH or an activated ester and R, R_1 and R_4 are respectively methoxymethyl, methyl and hydroxymethyl for C_{2a}, hydrogen, hydrogen and methyl for D_1, hydroxymethyl, methyl and methyl for D_2 and hydroxymethyl, hydrogen and methyl for E are obtained.

R_t (min) refer to HPLC method M reported in the HPLC analysis section.

When W is an activated ester, R_t (min) are respectively 22.51 for GE 2270 factor C_{2a}, 19.80 for GE 2270 factor D_1, 20.41 for GE 2270 factor D_2 and 18.92 for GE 2270 factor E.

When W is COOH, R_t (min) are respectively 12.99 for GE 2270 factor C_{2a}, 10.38 for GE 2270 factor D_1, 11.08 for GE 2270 factor D_2 and 9.03 for GE 2270 factor E.

8. Preparation of glycyl-Nε-Cbz-L-lysine trifluoroacetate

4.8 ml of DPPA (22 mmols) was added at 0°C to a well stirred solution of 3.5 g of BOC-glycine (Fluka) (20 mmols) and 7.28 g of Nε-Cbz-L-lysine methyl ester hydrochloride (Fluka) (22 mmols) in 50 ml of dry DMF. To this solution, a solution of 5.8 ml of TEA (42 mmols) in 50 ml of dry DMF was added at 0°C over a 10 - 15 min period. Stirring was continued for 2 more hours at 0°C and then overnight at room temperature. The reaction mixture was diluted with 250 ml of toluene and 500 ml of ethyl acetate and washed with 1N aq. HCl (x3), water, a saturated solution of $NaHCO_3$ and brine. Drying over Na_2SO_4 and evaporation of the solvent yielded 9.7 g of a thick oil which resisted any attempt of crystallization. NMR of this oil was in perfect agreement with the structure of BOC-glycyl-Nε-Cbz-L-lysine methyl ester.

The oil was dissolved in 200 ml of acetone/dioxane 1:1 and 22 ml of 1N aq. NaOH were added over a 30 min period at 0°C under stirring. The

reaction was then stirred for 45 min at room temperature, diluted with 300 ml of cold water, acidified with 25 ml of 1N aq HCl and extracted with chloroform (x3) and ethyl acetate (x3). Drying over Na_2SO_4 and evaporation of the solvent yielded 9.4 g of a gum which resisted any attempt of crystallization. NMR of this gum was in perfect agreement with the structure of BOC-glycyl-N$\underline{\varepsilon}$-Cbz-L-lysine.

The gummy compound was treated with 20 ml of cold trifluoroacetic acid (TFA). The reaction mixture was swirled at room temperature until all the compound went in solution. The solution was reduced to a small volume under vacuum in the cold and then ethyl ether was added to induce precipitation of the title compound. 9.6 g of glycyl-N$\underline{\varepsilon}$-Cbz-L-lysine trifluoroacetate were obtained as a white powder. NMR was in perfect agreement with the structure.

9. Preparation of L-tyrosyl-L-prolinamide

0.48 ml of DPPA (2 mmols) were added at 0°C to a well stirred solution of 538 mg of BOC-L-tyrosine (Fluka) (2 mmols), 228.3 mg of L-prolinamide (Aldrich) (2 mmols) and 168 mg of $NaHCO_3$ in 5 ml of dry DMF. The reaction was stirred for 24 h at room temperature and then diluted with 50 ml of water and extracted with chloroform (x3). The organic phase was washed water, dried over Na_2SO_4 and the solvent evaporated to yield an oil which was purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with hexane/acetone 2:3. 420 mg of BOC-L-tyrosyl-L-

prolinamide were in this way obtained as a white solid. NMR was in agreement with the structure.

The solid obtained was dissolved in 6 ml of ethyl acetate and stirred for 48 h at room temperature in the presence of 4 ml of 3N aq. HCl. The reaction mixture was then evaporated to dryness in vacuo and the residue redissolved in ethanol was precipitated with ethyl ether. 302 mg of L-tyrosyl-L-prolinamide were obtained as a white powder. NMR was in perfect agreement with the structure.

10. Preparation of methyl 8-aminocaprylate and methyl 11-aminoundecanoate p-toluenesulfonates

A solution of 40 mmols of the selected amino acid (Fluka) and 15.2 g of p-toluenesulfonic acid monohydrate (Fluka) (80 mmols) in 200 ml of methanol was refluxed overnight. The solvent was then evaporated in vacuo and the residue redissolved in ethyl ether. After sometime the title compounds crystallized out quantitatively. The NMR of both compounds was in agreement with their structure.

11. Preparation of 5-aminopentylphosphonic acid

3.48 g of 5-amino-1-pentanol (Fluka) (33.7 mmols) and 5.0 g of phthalic anhydride (Fluka) (33.7 mmols) were melted together at 180°C. This temperature was maintained for 90 min until no more

water developed. The reaction was allowed to cool to room temperature and the oily mixture was chromatographed on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 2% methanol in chloroform. 5.9 g of a pure oil were obtained. NMR was in agreement with the structure.

To the 5.9 g of the oily intermediate (25 mmols), 1.6 ml of PBr_3 (17 mmols) were added portionwise so to control the exothermic reaction. The reaction mixture was heated at 100°C for 1.5 h and then poured into crushed ice. The solid material that separated was filtered and allowed to dry in air overnight. 6.6 g of the pure bromo intermediate were obtained. The mass was in agreement with the expected molecular weight.

500 mg of the pure bromo intermediate (1.69 mmols) and 140 mg of triethyl phosphite (Fluka) (0.84 mmols) were heated together at 150°C for about 1 h. Other three portions of 140 mg of triethyl phosphite were then added at 30 min interval at the same temperature. When all the starting material had disappeared, the excess of triethyl phosphite was distilled off and the crude material purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 2% methanol in dichloromethane. 468 mg of the expected diethyl phosphonate were obtained as a thick oil. NMR confirmed the structure.

468 mg of the diethyl phosphonate intermediate were treated overnight with 3 ml of a 0.2 M solution of hydrazine in methanol at room temperature. The precipitated phthalhydrizide was filtered off and the

remaining solution was evaporated to dryness in vacuo. The residue was taken up in 1 N aq. HCl and the solution was washed with ethyl acetate, basified with NaOH and extracted several times with n-butanol. The butanolic phase was dried over Na_2SO_4 and evaporated to dryness to yield 175 mg of a thick oil whose NMR was in agreement with the product expected.

175 mg of diethyl 5-aminopentylphosphonate were refluxed for 20 h in 0.6 ml of conc. HCl. The acid solution was then evaporated to dryness by azeotropic distillation in vacuo in the presence of n-butanol. The NMR of the glassy oil obtained confirmed it to be the 5-aminopentylphosphonic acid.

12. Preparation of 5-(5-aminopentyl)tetrazole

To a solution of 10 ml of 6-aminocapronitrile (Fluka) (80 mmols) and 13.3 ml of TEA (96 mmols) in 80 ml of tetrahydrofuran, 12.48 ml of benzyl chloroformate (Fluka) (88 mmols) were added dropwise at 0°C under stirring. Stirring was continued for 2 h at room temperature and the solvent was evaporated in vacuo. The residue was dissolved in ethyl acetate, washed with 1 N aq. HCl, water and then dried over Na_2SO_4 and the solvent evaporated to yield 19.6 g of a syrup whose NMR was in agreement with the structure.

1 g of the protected 6-aminocapronitrile (4.06 mmols) in 40 ml of 1-methyl-2-pyrrolidone was heated at 150°C under argon in the presence of 793 mg of sodium azide (12.2 mmols) and 834 mg triethylamine

hydrochloride (6.1 mmols). After 4 h the reaction mixture was diluted with 120 ml of water and then carefully acidified to pH 1 with 10% aq. HCl (attention: azotidric acid forms!). The solution was extracted with ethyl acetate, the organic phase re-extracted with 10% aq. NaOH (x2) and the basic solution washed with ethyl ether, acidified with conc. HCl and extracted with ethyl acetate (x3). Drying and evaporation of the organic phase yielded a syrup that crystallized from methanol/water. 260 mg of a fine powder were obtained. NMR and mass confirmed the structure.

250 mg of the N-protected amino tetrazole (0.86 mmols) were treated at room temperature with 5ml of thioanisole (43.25 mmols) and 17.5 ml of trifluoroacetic acid for 3 h. Trifluoroacetic acid was concentrated in vacuo in the cold and ethyl ether was added to precipitate the title compound as its trifluoroacetate salt. NMR and mass confirmed the structure.

13. Preparation of N-[3,4-di-(O-tetrahydropyranyl)benzoyl]-thiazolidin-2-thione

A solution of 4.62 g of 3.4-dihydroxybenzoic acid (Fluka) (30 mmols) in 40 ml of methanol was refluxed for 24 h in the presence of 0.325 ml of conc. H_2SO_4. After cooling the solution to room temperature some solid $NaHCO_3$ was added and the solvent evaporated in vacuo. The residue was taken up in ethyl acetate, washed with water, dried over Na_2SO_4 and the solvent

evaporated to yield a syrup which was crystallized from ethyl acetate/hexane. 3.53 g of white crystals were obtained.

9.1 ml of dihydropyrane (Fluka) (0.1 mol) and 250 mg of pyridinium p-toluenesulfonate (1 mmol) were added at room temperature to a stirred solution of 1.68 g of methyl 3,4-dihydroxybenzoate (10 mmols) in 4 ml of ethyl acetate and 25 ml of dichloromethane. After 4 d the reaction mixture was washed with a saturated solution of $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness to obtain 3.36 g of an oil which was used for the next step without further purification.

The crude from the previous reaction was dissolved in 40 ml of acetone and to the stirred solution 20 ml of water, 2.76 g of K_2CO_3 (20 mmols) and 10 ml of 1N aq. NaOH (10 mmols) were added and stirring was continued for 7 d at room temperature. Acetone was evaporated in vacuo and the residual water phase was washed with ethyl acetate. The aqueous phase was transferred to an E. flask containing an equal volume of chloroform, cooled to 0°C and carefully acidified under vigorous stirring with 50 ml of 1 N aq. HCl. The water phase was then extracted 3 more times with chloroform and the combined organic layers were washed with 0.2% ammonium formate, dried over Na_2SO_4 and evaporated to dryness to yield a syrup which crystallized after hexane addition. 2.34 g of a white solid were obtained. The NMR was in agreement with the structure.

333 mg of 2-thiazoline-2-thiol (Fluka) (2.8 mmols), 577 mg of N,N'-dicyclohexylcarbodiimide

(Fluka) (2.8 mmols) and 35 mg of 4-dimethylamino-pyridine were added in the order at 0°C to a stirred solution of 644 mg of the benzoic acid intermediate (2 mmols) in 14 ml ethyl acetate/dichloromethane 5:2. Stirring was continued overnight at room temperature, the precipitated dicyclohexylurea was filtered off and the yellow solution was evaporated in vacuo to yield a yellow oil which was purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting from 25% acetone in hexane. 700 mg of yellow crystals were obtained from acetone/hexane. NMR and IR confirmed the compound to be the title compound.

14. Preparation of N^1-N^8-di-tert-butoxycarbonylspermidine

A solution of 19.72 g of BOC-ON (Aldrich) (80 mmols) in 60 ml of degassed tetrahydrofuran (THF) was added dropwise over a 1 h period under argon to a stirred solution of 5.8 g of spermidine (Aldrich) (40 mmols) in 40 ml of degassed THF cooled at 0°C. The reaction was then stirred at room temperature overnight and then evaporated to dryness. The residue was taken up in ethyl ether, washed with 1 N aq. NaOH (x4) and water (x4), dried over Na_2SO_4 and the solvent concentrated to a small volume in vacuo. Upon addition of ethyl ether 11 g of a white powder precipitated. NMR confirmed it to be the title compound.

15. Preparation of N-tert-butoxycarbonylpropilendiamine

4.2 g of BOC-ON (Aldrich) (17.2 mmols) were added at room temperature to a stirred solution of 2 g of 3-aminopropionitrile fumarate (Aldrich) (15.6 mmols) dissolved in a mixture of 10 ml of dioxane, 10 ml of water and 3.3 ml of triethylamine. After 3 h the reaction mixture was diluted with more water and extracted with dichloromethane (x3). The combined organic layers were washed with 1 N aq. NaOH (x3) and water (x3), dried over Na_2SO_4 and evaporated to dryness. The residual oil was taken up in ethyl ether and precipitated with hexane to yield 2.2 g of a white powder.

1 g of N-BOC-protected intermediate (5.9 mmols) in 7 ml of 1 N ethanolic NaOH was hydrogenated at 40 psi in the presence of 130 mg of Raney nickel (50% slurry in water, pH>9) (Aldrich) for 40 h. Raney nickel was filtered off and the solvent was evaporated to dryness. The residue was taken up in ethyl acetate and washed with 1 N aq. NaOH, dried over Na_2SO_4 and the solvent removed in vacuo yielding 950 mg of a colorless oil which solidified on standing. NMR confirmed it to be the title compound.

16. Preparation of 3-(2-aminoethylthio)propanoic acid methyl ester trifluoroacetate

To a solution of 0.5 g of cysteamine (Fluka) (6.48 mmols) in 5 ml of CH_2Cl_2, 1.4 g of di-tert-butyl dicarbonate (Aldrich) (6.48 mmols) in 5 ml of CH_2Cl_2 were added at room temperature under stirring. After 30

min the organic solvent was evaporated and the crude material dissolved in 5 ml of absolute ethanol. To the ethanolic solution, 2.7 ml of TEA (19.1 mmols) and 1.07 ml of methyl 3-bromopropionate (Fluka) (9.57 mmols) were added in the order. The reaction was completed in about 30 min. Ethanol was removed in vacuo and replaced by 15 ml of chloroform. The organic phase was then washed with water, anidrified on Na_2SO_4 and the solvent evaporated to yield an oil which was finally treated with 1 ml of trifluoroacetic acid at 0°C for 5 min. Evaporation to dryness gave 270 mg of a pale yellow oil. NMR and IR confirmed it to be the title compound.

17. Preparation of 6-amino-2(E)-hexenoic acid

To a stirred solution of 2 ml of 4-amino-butyraldehyde diethyl acetal (Fluka) (11.6 mmols) and 3.6 ml of TEA (25.6 mmols) in 5 ml of CH_2Cl_2, a solution of 1.5 ml of benzoyl chloride (Fluka) (12.9 mmols) in 5 ml CH_2Cl_2 was added in 30 min. at room temperature. After 1 hour the reaction was diluted with 10 more ml of CH_2Cl_2, washed with water and the organic phase dried over Na_2SO_4 and the volume adjusted to 20 ml. The new solution was allowed to react for three days under argon in the presence of 1.6 ml of TEA (11.5 mmols), 10.2 g of di-tert-butyl dicarbonate (Aldrich) (46.8 mmols) and 1.4 g of 4-dimethylamino-pyridine (Fluka) (11.5 mmols) at room temperature. Removal of the solvent gave a brown oil that was purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 20% ethyl

acetate in n-hexane yielding 1.6 g of the N,N deprotected 4-aminobutyraldehyde diethyl acetal as a colorless oil. NMR confirmed the structure.

The obtained oil was then dissolved in 5 ml of THF and treated with 5 ml of 1N HCl at room temperature for three hours. THF was removed in vacuo and the remaining solution was washed with chloroform (2 ml x3). The organic phase was then washed with a solution of Na_2CO_3, water, dried over Na_2SO_4 and evaporated to dryness yielding an oil that was used in the next step without further purification.

To a suspension of 160 mg of 60% NaH (4mmols) in 5 ml of dry THF at 0°C under argon, 0.837 ml of triethylphosphonoacetate (Fluka) (4.3 mmols) were added. After 30 min. a dry THF (2 ml) solution of the previously obtained aldehyde (1.17 g) (4.02 mmols) was added and the temperature was allowed to rise to room temperature. The reaction was stirred overnight and then 50 more mg of 60% NaH were added at 0 C. After two more hours at room temperature the reaction mixture was treated with diluted HCl (10 ml) and extracted with ethyl acetate (5ml x3). The combined organic phase was washed with water, dried over Na_2SO_4 and evaporated to dryness. The crude material was purified by flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 15% ethyl acetate in n-hexane yielding 765 mg of a syrup. NMR confirmed it to be the expected product with the double bond in E configuration (J = 16Hz).

6.15 ml of 1N LiOH (6.15 mmols) were added to a solution of 739 mg of the unsaturated ester previously

obtained (2.05 mmols) in 10 ml of THF under stirring at room temperature. When the starting material had disappeared the reaction mixture was concentrated in vacuo at 30°C (bath temperature). The aqueous solution was acidified at pH 2 with 1N HCl and then extracted with ethyl acetate. The combined organic phase was dried over Na_2SO_4, filtered and the solvent evaporated yielding an oil that solidified upon standing under vacuum. NMR and MS confirmed it to be 6-N-BOC-amino-2(E)-hexenoic acid.

Removal of the N-BOC protection to obtain the title compound was carried out in neat trifluoroacetic acid at 0°C just before the coupling with the appropriate GE 2270 starting material.

18. Preparation of 3-(2-aminoethoxy)propanoic acid trifluoroacetate

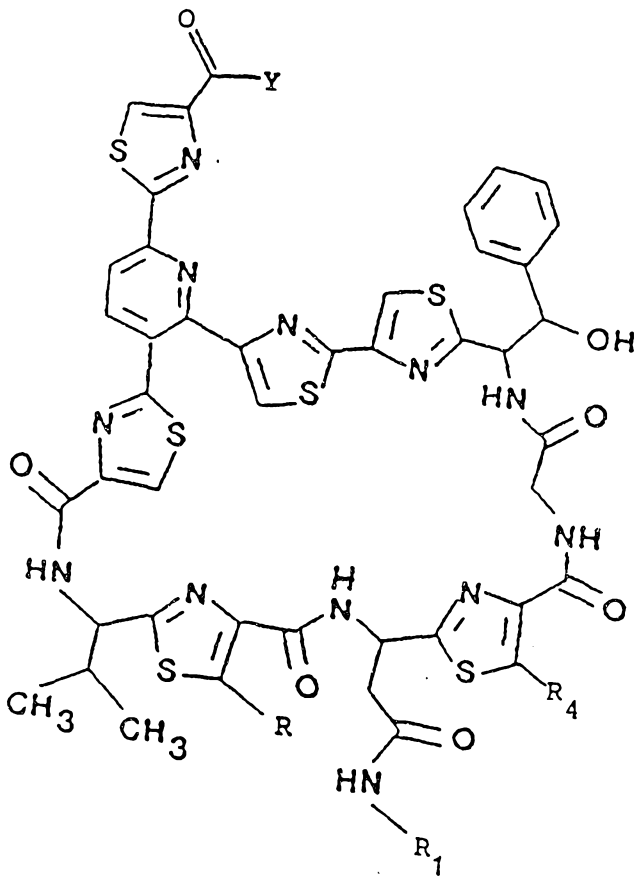
To a stirred solution of 1 g of N-BOC-ethanolamine (6.22 mmols) [prepared according to classical methodologies from ethanolamine (Fluka)] in 10 ml of dry THF at -78 C, 3.88 ml of 1.6 M solution of butyllithium (Fluka) (6.22 mmols) were added under argon. After 30 min. 1.3 g of t-butyl 3-bromo propanoate [prepared according to classical methodologies from 3-bromo propanoic acid (Fluka)] (6.22 mmols) were added, the temperature allowed to rise to room temperature and the resulting mixture stirred for 20 hours at that temperature. After dilution with water the reaction mixture was extracted with n-hexane (5ml x2). Removal of the solvent gave a crude material that was purified by

flash chromatography on silica gel 60 (230 - 400 mesh ASTM - Merck) eluting with 20% ethyl acetate in n-hexane yielding 1.43 g of an oil. NMR confirmed it to be the coupled compound.

The total deprotection of the coupled compound was carried out immediately before addition to the appropriate GE 2270 starting material by stirring it in trifluoroacetic acid for about 5 min at room temperature. Removal of trifluoroacetic acid in vacuo yielded the title compound.

CLAIMS

1. An amide derivative of antibiotic GE 2270 having the following formula I



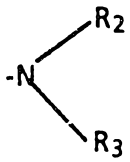
I

wherein

R represents:
hydrogen,
hydroxymethyl, or
methoxymethyl;

R_1 represents:
hydrogen, or
methyl;

Y represents:
a group of formula



wherein:

R_2 represents:
hydrogen,
(C_1-C_4)alkyl,
amino(C_2-C_4)alkyl,
(C_1-C_4)alkylamino-(C_1-C_4)alkyl, or
di-(C_1-C_4)alkylamino-(C_1-C_4)alkyl;

R_3 represents:
hydrogen,
a linear or branched (C_1-C_{14})alkyl group bearing from 1 to 3 substituents selected from: carboxy,

sulfo, phosphono, amino which may be optionally protected with a lower alkoxycarbonyl or a benzyloxycarbonyl group, (C_1-C_4)alkylamino wherein the alkyl moiety may be optionally substituted with a carboxy group, di-(C_1-C_4)alkylamino, hydroxy, halo, (C_1-C_4)alkoxy wherein the alkyl moiety may be optionally substituted with a carboxy group, (C_1-C_4)alkoxycarbonyl, mercapto, (C_1-C_4)alkylthio wherein the alkyl moiety may be optionally substituted with a carboxy group, phenyl which may be optionally substituted with 1 to 3 substituents selected from carboxy, sulfo, hydroxy, halo and mercapto, carbamyl, (C_1-C_6)alkylcarbamyl wherein the alkyl moiety may be optionally substituted with 1 or 2 substituents selected from carboxy, amino, (C_1-C_4)alkylamino and di-(C_1-C_4)alkylamino, di-(C_1-C_4)alkylcarbamyl wherein the alkyl moieties together with the adjacent nitrogen atom may also represent a saturated 5-7 membered heterocyclic ring which may optionally be substituted with a carboxy or a carbamyl group on one of the ring carbons and may optionally contain a further heterogroup selected from O, S and N, benzoylamino wherein the phenyl group may be substituted from 1 to 3 hydroxy group, a nitrogen containing 5-6 membered heterocyclic ring which may be unsaturated, partially saturated or wholly saturated and may contain 1 to 3 further heteroatoms selected from N, S and O wherein one of the carbons of the ring may optionally bear a group carboxy, sulfo, carboxy(C_1-C_4)alkyl and sulfo(C_1-C_4)alkyl and

the ring nitrogen atom may optionally be substituted by (C_1-C_4)alkyl, carboxy(C_1-C_4)alkyl, sulfo(C_1-C_4)alkyl, and benzyl;

(C_3-C_6)alkenyl, optionally substituted by carboxy or sulfo;

1-deoxy-1-glucityl;

2-deoxy-2-glucosyl;

a fully saturated 5 to 7 membered nitrogen containing heterocyclic ring wherein the nitrogen atom may be optionally substituted by (C_1-C_4)alkyl or benzyl and one or two carbons of the ring skeleton may bear a substituent selected from (C_1-C_4)alkyl, carboxy and sulfo;

or R_2 and R_3

taken together with the adjacent nitrogen atom represent a fully saturated 5-7 membered heterocyclic ring which may optionally contain a further heteroatom selected from O, S and N, and may optionally bear one or two substituents on the ring carbons selected from (C_1-C_4)alkyl, benzyl, carboxy, sulfo, carboxy(C_1-C_4)alkyl, and sulfo(C_1-C_4)alkyl;

R_4 represents:

hydrogen,

methyl, or

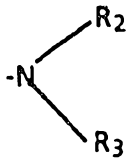
hydroxymethyl;

with the proviso that when R_4 is hydrogen or hydroxymethyl, then simultaneously R is methoxymethyl and R_1 is methyl;

and the pharmaceutically addition salts thereof.

2. A compound according to claim 1 wherein R represents methoxymethyl and the other substituents are defined as in claim 1.

3. A compound as claimed in claim 1 wherein R represents methoxymethyl, R_1 and R_4 represent methyl and Y represents a group of formula



wherein R_2 is hydrogen and R_3 is defined as in claim 1.

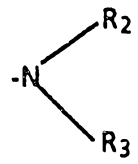
4. A compound as claimed in claim 1 wherein R is methoxymethyl, R_1 and R_4 represent a methyl group and Y is an amino moiety which derive from a natural amino acid such as for example glycine, ornithine, serine, aspartic acid, tyrosine, leucine, phenylalanine, methionine, proline, threonine, lysine, or a synthetic dipeptide such as glycyllysine, serylproline,

glycylprolinamide, tyrosylprolinamide, threonylprolinamide, leucylprolinamide.

5. A compound as claimed in claim 1 wherein R is methoxymethyl, R_1 and R_4 are methyl, Y is a group NR_2R_3 wherein R_2 is hydrogen and R_3 is a linear alkyl chain preferably of 3 to 12 carbons, more preferably of 3 to 7 carbons substituted with a group selected from COOH, SO_3H and PO_3H_2.

6. A compound as claimed in claim 1 wherein R is methoxymethyl, R_1 and R_4 are methyl, Y is a group NR_2R_3 wherein R_2 is hydrogen and R_3 is $CH_2CH_2CH_2CH_2CH_2$-COOH.

7. A compound as claimed in claim 1 wherein R represents hydrogen, hydroxymethyl or methoxymethyl, R_1 represents hydrogen or methyl, R_4 represents hydrogen, methyl or hydroxymethyl and Y represents a group of formula



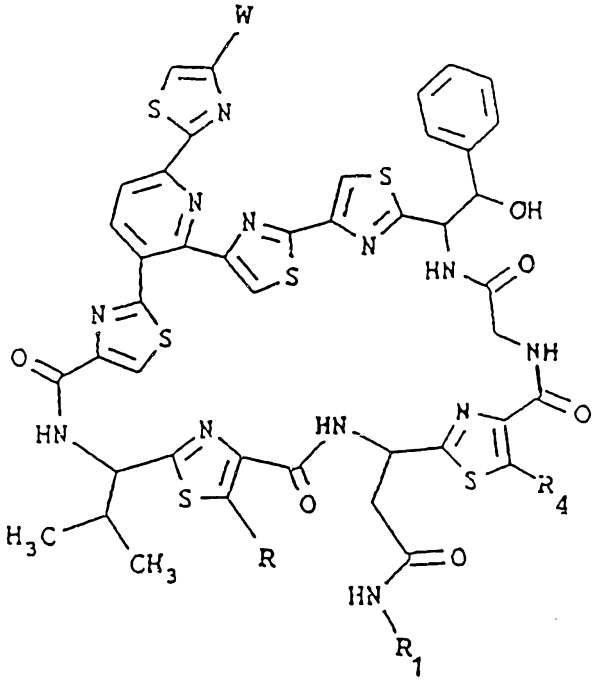
wherein R_2 is hydrogen and R_3 is defined as in claim 1.

8. A compound as claimed in claim 7 wherein Y is an amino moiety which derive from a natural amino acid such as for example glycine, ornithine, serine, aspartic acid, tyrosine, leucine, phenylalanine, methionine, proline, threonine, lysine, or a synthetic dipeptide such as glycyllysine, serylproline, glycylprolinamide, tyrosylprolinamide, threonylprolinamide, leucylprolinamide.

9. A compound as claimed in claim 1 wherein R is hydrogen, hydroxymethyl or methoxymethyl, R_1 is hydrogen or methyl, R_4 is hydrogen, methyl or hydroxymethyl and Y is a group NR_2R_3 wherein R_2 is hydrogen and R_3 is a linear alkyl chain preferably of 3 to 12 carbons, more preferably of 3 to 7 carbons substituted with a group selected from COOH, SO_3H and PO_3H_2.

10. A compound as claimed in claim 1 wherein R is hydrogen, hydroxymethyl or methoxymethyl, R_1 is hydrogen or methyl, R_4 is hydrogen, methyl or hydroxymethyl and Y is a group NR_2R_3 wherein R_2 is hydrogen and R_3 is $CH_2CH_2CH_2CH_2CH_2$-COOH.

11. A process for preparing a compound of claim 1 which comprises reacting an antibiotic GE 2270 compound having formula II:



II

wherein

W represents a carboxy or an activated ester function;

R represents hydrogen, hydroxymethyl or methoxymethyl;

R_1 represents hydrogen or methyl;

R_4 represents hydrogen, methyl or hydroxymethyl;

with the proviso that when R_4 is hydrogen or hydroxymethyl, then simultaneously R is methoxymethyl and R_1 is methyl, with a selected amine of formula HNR_2R_3 wherein R_2 and R_3 have the same meanings as in

claim 1, in an inert organic solvent and, when W is carboxy, in the presence of a condensing agent.

12. A process according to claim 11 wherein the condensing agents are selected from (C_1-C_4)alkyl, phenyl or heterocyclic phosphorazidates such as, diphenyl-phosphorazidate (DPPA), diethyl-phosphorazidate, di(4-nitrophenyl)phosphorazidate, dimorpholyl-phosphorazidate and diphenylphosphorochloridate.

13. A process according to claims 11 and 12 wherein the amine reactant HNR_2R_3 is used in a 1 to 2 fold molar excess with respect on the antibiotic starting material and the reaction temperature is comprised between 0 and 20°C.

14. A compound of any of claims 1 to 10 for use as a medicine.

15. A pharmaceutical composition containing a compound of any of claims 1 to 10 as the active ingredient in admixture with a pharmaceutically acceptable carrier.

16. Use of a compound according to any of claims 1 to 10 for preparing a medicament for use as an antibiotic.

INTERNATIONAL SEARCH REPORT

International Application PCT/EP 92/00002

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K7/56; C07K5/06; A61K35/66

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07K ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
	No relevant documents disclosed ---	

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"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)

"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

"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

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"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.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18 MARCH 1992	27.03.92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	KORSNER S.E.