655190

AUSTRALIA

PATENTS ACT 1990

NOTICE OF ENTITLEMENT

We, **Rhone-Poulenc Rorer S.A.**, the applicant/Nominated Person in respect of Application No. 82219/91 state the following:-

The Nominated Person is entitled to the grant of the patent because the Nominated Person would, on the grant of a patent for the invention to the inventors, be entitled to have the patent assigned to the Nominated Person.

The Nominated Person is entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Person under its former name Rhone-Poulenc Sante, made the application listed in the declaration under Article 8 of the PCT, and because that application was the first application made in a Convention country in respect of the invention.

DATED this TWENTY NINTH day of MARCH 1993

.....

a member of the firm of
DAVIES COLLISON
CAVE for and on behalf
of the applicant(s)

(DCC ref: 1561201)

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NEW DERIVATIVES OF STREPTOGRAMINES AND PREPARATION THEREOF

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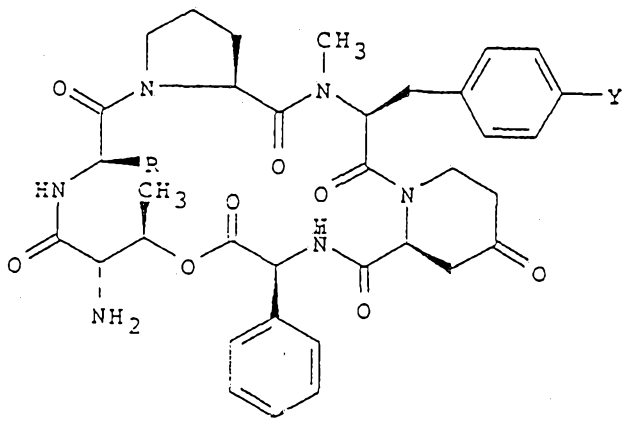
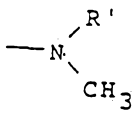
(57) Claim

1. Process for preparing a streptogramin derivative of general formula:



(I)

in which Y is a hydrogen atom or a methylamino or dimethylamino radical or a radical of structure:



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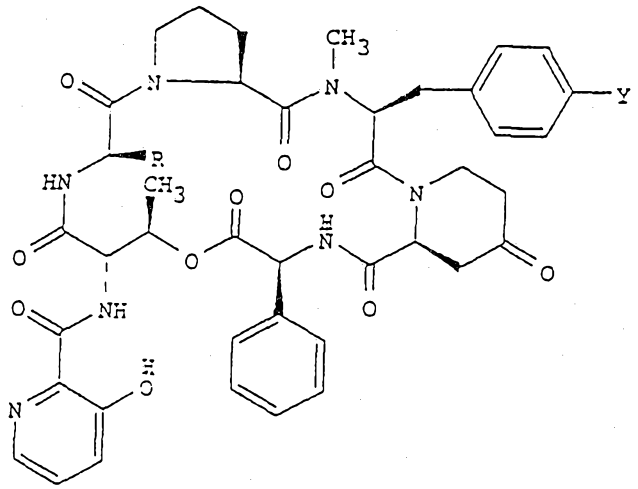
in which R' is an amino-protecting radical and R is a methyl or ethyl radical, characterised in that the reductive cleavage of a streptogramin derivative of general formula:



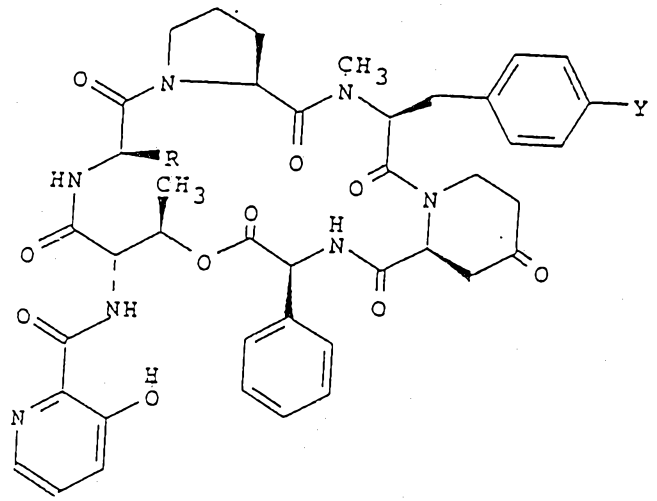
in which Y and R are defined as above, is performed in an acid medium, and the product obtained is then optionally converted to an addition salt with an acid.

2. Process according to Claim 1, characterised in that the reductive cleavage is performed by treatment in an acid medium in the presence of a reducing metal.

3. Process according to one of Claims 1 and 2, characterised in that the reaction is performed in the presence of a reducing metal whose redox potential is less than -0.94 V (s.c.e.).

4. Process for preparing a streptogramin derivative according to Claim 1, characterised in that the reductive cleavage is an electrochemical cleavage in an acid medium of the amide of the streptogramin

derivative of general formula:



in which Y and R are defined as in Claim 1, optionally followed by conversion of the product obtained to an addition salt with an acid.

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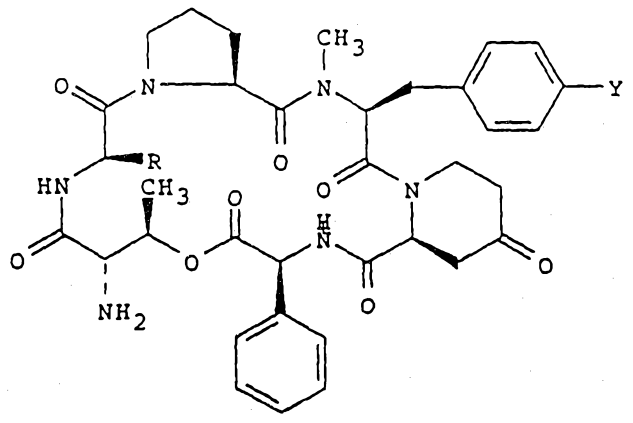
(54) Titre: NOUVEAUX DERIVES DE STREPTOGRAMINES ET LEUR PREPARATION



(I)

(57) Abstract

Method for the preparation of a streptogramine derivative having general formula (I) wherein the symbol Y is a hydrogen atom or a methyl amino or dimethyl amino radical, or a protected methyl amino radical, and the symbol R represents a methyl or ethyl radical.

(57) Abrégé

Procédé de préparation d'un dérivé de streptogramine de formule générale (I) dans laquelle le symbole Y représente un atome d'hydrogène ou un radical méthylamino ou diméthylamino, ou un radical méthylamino protégé, et le symbole R représente un radical méthyle ou éthyle.

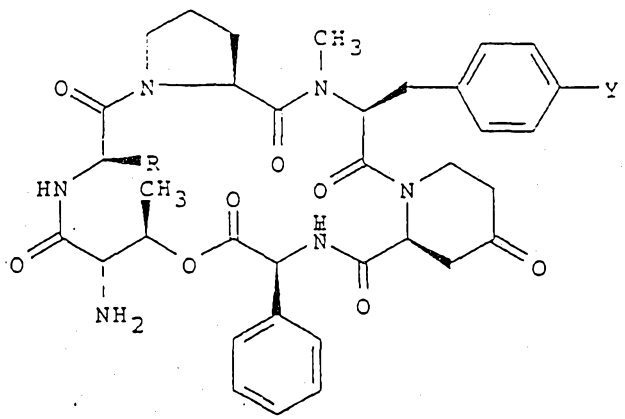
NEW STREPTOGRAMIN DERIVATIVES AND THEIR PREPARATION

Streptogramins are known products mentioned, in particular, by J. Preud'homme et al., Bull. Soc. Chim. Fr., 2, 585-91 (1968) or by C. Cocito, Antibiotics, 296 (1983).

The total synthesis of virginiamycin S has been described in Liebigs Ann. Chem., 21-31 (1986).

In Patents US 4,618,599 and US 4,798,827, soluble derivatives of pristinamycin I_A and of virginiamycin S have been described.

The present invention relates to the preparation of streptogramin derivatives of general formula:

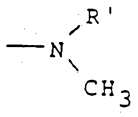


(I)

and to their salts, their preparation and their use.

In the general formula (I), the symbol R represents a methyl or ethyl radical and the symbol Y represents a hydrogen atom or a methylamino or

dimethylamino radical or a radical of structure:

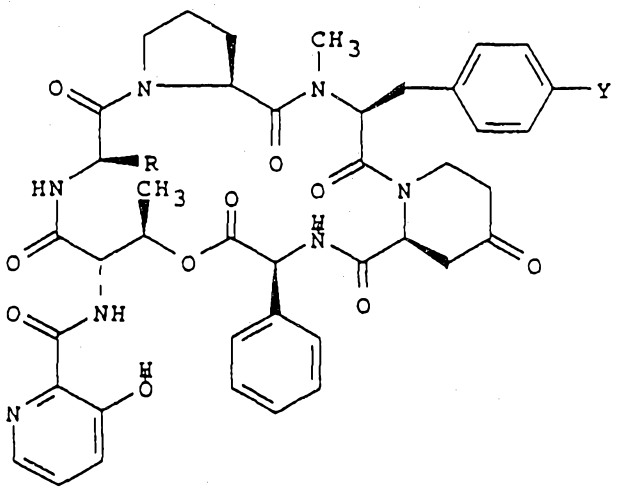


(Ia)

in which R' is an amino-protecting radical.

As an example, R' may advantageously be selected from trifluoroacetyl, benzyloxycarbonyl, 2-propenyloxycarbonyl, nitrobenzyloxycarbonyl, 9-fluorenylmethyloxycarbonyl or o-nitrobenzyloxyacetyl.

According to the invention, the streptogramin derivatives of general formula (I) may be obtained by reductive cleavage of a streptogramin of general formula:



(II)

in which Y and R are defined as above.

The reductive cleavage is carried out by treatment in an acid medium in the presence of a reducing metal.

The reaction is performed in a strong acid medium, at a pH below 2, in the presence of a reducing metal whose redox potential is less than -0.94V (s.c.e.). The procedure is carried out in an aqueous medium, or in an aqueous-alcoholic medium (for example in a water/methanol or water/ethanol mixture), at a

temperature of between -10 and 60°C. The acid may be selected from sulphuric, hydrochloric, hydrobromic, trifluoroacetic or methanesulphonic acid.

As an example, the reducing metal may be advantageously selected from zinc, magnesium, aluminium or sodium amalgam. It is preferable to work under nitrogen.

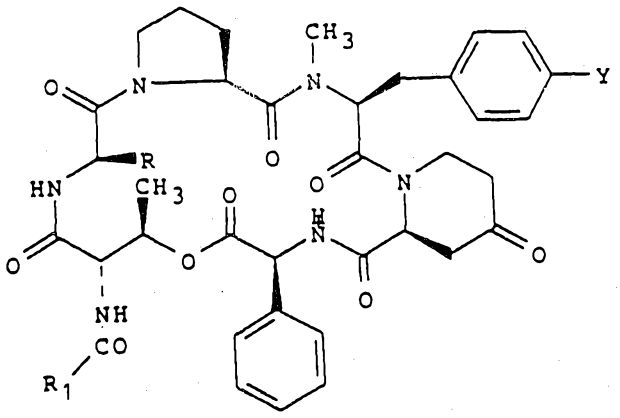
According to the invention, the streptogramin derivatives of general formula (I) may also be prepared by electrochemical reduction in an acid medium of a streptogramin derivative of general formula (II).

Controlled-potential electrolysis is carried out in aqueous or aqueous-alcoholic acid solution containing up to 50 % of alcohol (for example methanol or ethanol), at a temperature of between 0 and 60°C, with constant stirring and under a nitrogen atmosphere, in an electrolysis cell in which the cathode consists of a bed of mercury. The potential of the working electrode E is such that $-0.9 > E > -1.1$ V (s.c.e.).

The acid is advantageously selected from hydrochloric, hydrobromic or sulphuric acid.

The products obtained by the process according to the invention are especially advantageous as intermediates for the preparation of biologically active streptogramin derivatives.

More specifically, they serve as intermediates for obtaining new streptogramin derivatives of general formula:



(III)

in which Y and R are defined as above and R_1 represents a phenyl or pyridyl radical monosubstituted with a linear or branched alkyl radical containing 2 to 6 carbon atoms or with a trifluoromethyl radical, or represents a phenyl radical disubstituted with linear or branched alkyl radicals containing 1 to 6 carbon atoms or with nitro radicals, or represents a naphthyl radical or a quinolyl radical substituted with a halogen atom, which enable the resistance of tumours to anticancer substances to be eliminated, and are especially advantageous as agents associated with cancer treatment.

The products of general formula (III) may be obtained by the action of an acid of general formula:

$$R_1 \text{ —— } COOH \qquad \text{(IV)}$$

in which R_1 is defined as above, or a reactive derivative of this acid, on a product of general formula (I).

When a reactive derivative is reacted, the latter may be selected from the anhydride, a mixed

anhydride, an acid halide or a reactive ester.

The reaction is performed in an organic medium, optionally in the presence of an acid-acceptor such as a nitrogenous organic base (for example a trialkylamine, a pyridine, N-methylmorpholine, 1,8-diazabicyclo[5.4.0]undec-7-ene, 1,5-diazabicyclo-[4.3.0]non-5-ene), in an organic solvent such as a chlorinated solvent (for example methylene chloride, dichloroethane, chloroform), an amide (for example dimethylformamide), an oxide (for example dimethyl sulphoxide), a ketone (for example acetone) or an ether (for example tetrahydrofuran), at a temperature of between -20 and 60°C. It is also possible to work in the presence of a condensing agent such as a carbodiimide (for example dicyclohexylcarbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) and optionally in the presence of a catalyst such as hydroxybenzotriazole, in a solvent mentioned above, or in the presence of an alkali metal carbonate or bicarbonate or alkaline earth metal carbonate or bicarbonate, at the temperature defined above.

The products of general formula (I) or the products of general formula (III) may be purified by known methods such as crystallisation or chromatography.

The products obtained according to the invention may be converted to addition salts with acids

according to the usual methods. As an example, the salts can be addition salts with inorganic acids, such as hydrochlorides, hydrobromides, sulphates, nitrates or phosphates, or with organic acids, such as acetates, propionates, succinates, maleates, fumarates, methanesulphonates, tartrates, camphorsulphonates or substitution derivatives of these compounds.

The products of general formula (III), prepared from the products according to the invention, are agents capable of maintaining the chemosensitivity of tumours, or of restoring the chemosensitivity of tumours which have become resistant.

Their activity was demonstrated on a doxorubicin-resistant P388 cell line (P388/DOX) [R.K. JOHNSON et al., Canc. Treat. Rep., 62, 1535-1547 (1978)].

On day 0, tubes are inoculated with 3.6 cm^3 of a suspension of P388/DOX cells (2×10^5 cells/cm^3 in RPMI 1640 medium containing 10 % of foetal calf serum). The tubes are incubated with the test products at different concentrations and at 37°C (3 tubes per concentration); the products are solubilised in complete medium and added in a final volume of 0.4 cm^3. Another series of tubes is also incubated with the test products at different concentrations, but in the presence of 1 $\mu g/cm^3$ of doxorubicin.

On day 4, the cells are counted. The results are expressed as the IC_{50} (μM). The IC_{50} corresponds to

the concentration of product enabling 50 % cytotoxicity due to doxorubicin to be obtained, that is to say at a concentration where the product is not in itself cytotoxic.

In this technique, the products of general formula (III) were shown to be active at concentrations of between 0.2 and 2 μM.

In addition, the streptogramin derivatives are of low toxicity: they were generally shown to be non-toxic at subcutaneous doses of 200 mg/kg in mice.

The examples which follow, given without implied limitation, illustrate the present invention.

In the examples which follow, except where specifically stated, the NMR spectra were recorded at 250 MHz in deuterochloroform; the chemical shifts are expressed in ppm. Flash chromatography is performed under an average pressure of 50 kPa, using a silica of particle size 40-53 μm, according to W.C. STILL et al., J. Org. Chem., 43, 2923 (1978).

Example 1

200 g of pristinamycin IA are added to 5 litres of 1N aqueous hydrochloric acid solution stirred under a nitrogen atmosphere. A cloudy solution is obtained, the pH of which is in the region of 0.5 and the temperature of which is 27°C. 150 g of powdered zinc are then added in the course of 5 minutes; the temperature of the reaction mixture rises to 32°C. The reaction mixture is stirred for 1 hour at a temperature

in the region of 30°C; the pH of the mixture is then in the region of 1. After the addition of 2 litres of dichloromethane, the pH of the reaction mixture is adjusted to a value in the region of 4 by the slow addition of 110 cm^3 of 10N aqueous sodium hydroxide solution. The organic phase is separated after settling has taken place, the aqueous phase is extracted with 500 cm^3 of dichloromethane and the combined organic phases are then filtered through a bed of Supercel. The filtrate is washed with 3 times 100 cm^3 of distilled water, dried over sodium sulphate, filtered and then concentrated to a volume of 500 cm^3 under reduced pressure (2.7 kPa) at a temperature in the region of 30°C. The solution obtained is placed on a column of 7 kg of silica gel (diameter: 15 cm, height: 92 cm). The column is eluted with a dichloromethane/methanol(97:3 by volume) mixture, producing 1.5-litre fractions. Fractions 7 to 13 are concentrated to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 30°C, and the residue obtained is ground for 2 hours in 400 cm^3 of pentane, filtered off and then dried under reduced pressure (0.27 kPa) at a temperature in the region of 20°C. 66 g of de(3-hydroxypicolinoyl)pristinamycin IA are thereby obtained in the form of a white powder, melting at about 206°C.

NMR spectrum:

δ (ppm)	Form	Assignment
0.35	dd	5 β2
0.9	t	2γ
1.1-1.3	m	3 γ2 and 3 β2
1.38	d	1 γ
1.52	m	3 β1
1.65 and 1.8	2m	2 β1 and 2β2
2.02	m	3 β1
2.1 to 2.35	m	5 δ2, 5β1 and 5δ1
2.74	dt	5 ϵ2
2.87	s	$N(CH_3)_2$
2.98	dd	4 β2
3.3	s	NCH_3
3.2 to 3.4	m	3 δ2, 4 β1 and 1α
3.52	m	3 δ1
4.6 to 4.9	m	3 α, 5ϵ1 and 2α
4.97	d	5 α
5.24	dd	4 α
5.75	q (broad)	1 β
5.83	d	6 α
6.63	d	4 ϵ
7.1 to 7.35	m	4 δ and aromatic
8	d	2 NH
8.63	d	6 NH

Example 2

5 g of powdered zinc are added to a solution, maintained under a nitrogen atmosphere, of 10 g of

virginiamycin S1 in a mixture of 200 cm^3 of methanol and 50 cm^3 of 5N aqueous hydrochloric acid solution. The grey suspension obtained is stirred for 1 hour at a temperature in the region of 20°C. The pH of the reaction mixture is then 1.2; it is thereafter adjusted to 5 by adding 100 cm^3 of 1N aqueous sodium hydroxide solution. The mixture is extracted with 3 times 300 cm^3 of dichloromethane; the combined organic phases are dried over sodium sulphate, filtered and then concentrated to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 30°C. 8.1 g of residue are thereby obtained, which residue is purified by flash chromatography (eluant: dichloromethane/methanol, 98:2 by volume), collecting 80-cm^3 fractions. Fractions 10 to 30 are concentrated to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 30°C. 3.9 g of de(3-hydroxypicolinoyl)virginiamycin S1 are thereby obtained in the form of a white powder, melting at about 175°C.

NMR spectrum:

δ (ppm)	Form	Assignment
0.11	dd	5 β2
0.9	t	2 γ
1.13	m	3 γ2 and 3 β2
1.35	d	1 γ
1.5	m	3 γ1
1.64 and 1.8	2m	2 β1 and 2 β2
1.99	m	3 β1 and 5 δ2

2.08	m	5 β1
2.22	m	5 δ1
2.72	dt	5 ϵ2
3.04	dd	4 β2
3.22	s	3 δ2
3.26	s	NCH_3
3.3	m	1 α
3.38	m	4 β1
3.51	m	3 δ1
1.58	dd	3 α
4.65	m	5 ϵ1
4.75	dt	2 α
4.97	d	5 α
5.3	dd	4 α
5.73	dq	1 β
5.83	d	6 α
7.05 to 7.3	m	aromatic at positions 4 and 6
8	d	2 NH
8.61	d	6 NH

Example 3

Electrolysis is carried out by means of a 3-electrode set-up.

The electrolysis cell consists of an assembly of ground-necked glassware, the anode and cathode compartments being concentric and separated by a porosity 7 sintered-glass wall. A Tacussel PJT 120V-1A potentiostat-galvanostat and a Tacussel IG5 N

integrator complete the circuit.

The working electrode is a bed of mercury whose area is equal to 60 cm^2. The auxiliary electrode is a platinum strip. The reference electrode is a calomel electrode containing saturated potassium chloride solution (s.c.e.).

The electrolysis of a solution of 0.35 g of pristinamycin IA in 200 cm^3 of 1N sulphuric acid is performed under a nitrogen atmosphere, at 25°C, over a bed of mercury whose potential is set at -1.0 V s.c.e. At the end of the electrolysis, when the current intensity has become negligible (2 mA) compared with the initial current intensity (120 mA), the pH of the electrolysis solution is taken to around 6.0 by means of 5M potassium carbonate solution. The resulting solution is then extracted with 200 cm^3 of dichloromethane. The organic phase is dried over sodium sulphate and concentrated to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 30°C. The residue is taken up with 1 cm^3 of dichloromethane and the solution obtained is placed on a column of 15 g of silica gel (diameter: 1.5 cm, height: 60 cm). The column is eluted with a dichloromethane/methanol (98:2 by volume) mixture, collecting 5-cm^3 fractions. Fractions 22 to 32 are concentrated to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 30°C. 0.15 g of de(3-hydroxypicolinoyl)pristinamycin IA, the

characteristics of which are identical to those of the product obtained in Example 1, is obtained.

Example 4

Using the procedure described above in Example 3, but at a temperature of 10°C, the electrolysis of 0.35 g of pristinamycin I_A is performed. 0.105 g of de(3-hydroxypicolinoyl)pristinamycin IA, the characteristics of which are identical to those of the product obtained in Example 1, is obtained.

Example 5

Using the procedure described above in Example 3, but at a temperature of 40°C, the electrolysis of 0.35 g of pristinamycin I_A is performed. 0.09 g of de(3-hydroxypicolinoyl)pristinamycin IA, the characteristics of which are identical to those of the product obtained in Example 1, is obtained.

Example 6

Using the procedure described above in Example 3, but over a bed of mercury whose potential is set at -0.9 V s.c.e., the electrolysis of 0.35 g of pristinamycin I_A in 200 cm^3 of 1N hydrochloric acid is performed. 0.09 g of de(3-hydroxypicolinoyl)-pristinamycin IA, the characteristics of which are identical to those of the product obtained in Example 1, is obtained.

Example 7

De(3-hydroxypicolinoyl)pristinamycin I_C may be obtained as described in Example 1, but from 1 g of

pristinamycin I_C, 25 cm^3 of 1N aqueous hydrochloric acid solution and 0.5 g of powdered zinc.

0.8 g of a residue is thereby obtained, which residue is purified by flash chromatography (eluant: methylene chloride/methanol, 98:2 by volume), collecting 10-cm^3 fractions. Fractions 19 to 40 are combined and then concentrated to dryness under reduced pressure (2.7 kPa) at 30°C. 0.3 g of de(3-hydroxy-picolinoyl)pristinamycin I_C is thereby obtained in the form of a white powder.

NMR spectrum

1.34	d	2β
7.1 to 7.3	mt	aromatic and 4δ
8.1	d	>NH at position 2
8.65	d	>NH at position 6

Example 8

De(3-hydroxypicolinoyl)pristinamycin I_B may be obtained as described in Example 1, but from 1 g of pristinamycin I_B, 25 cm^3 of 1N aqueous hydrochloric acid solution and 0.5 g of powdered zinc. 0.8 g of a residue is thereby obtained, which residue is purified by flash chromatography (eluant: methylene chloride/methanol, 98:2 by volume), collecting 10-cm^3 fractions. Fractions 18 to 36 are combined and then concentrated to dryness under reduced pressure (2.7 kPa) at 30°C. 0.2 g of de(3-hydroxy-picolinoyl)pristinamycin I_B is thereby obtained in the form of a white powder.

NMR spectrum

1.6 to 1.9	mt	2β1 and 2β2
1.9 to 2.3	mt	5β1, 5γ1, 5γ2, 3β1 and NH_2
2.74	s and mt	$ArNHCH_3$ and 5ϵ_2

Example 9

De(3-hydroxypicolinoyl)-N^4-trifluoroacetyl-pristinamycin I_B may be obtained as described in Example 2, but from 0.68 g of N^4-trifluoroacetylpristinamycin I_B, 2.8 cm^3 of 5N aqueous hydrochloric acid solution in 14 cm^3 of methanol and 0.34 g of powdered zinc in 14 cm^3 of methanol.

0.52 g of a residue is obtained, which residue is purified by flash chromatography (eluant: methylene chloride/methanol, 97:3 by volume), collecting fractions of volume 5 cm^3. Fractions 6 to 16 are combined and concentrated to dryness under reduced pressure (2.7 kPa) at 30°C. 0.19 g of de(3-hydroxypicolinoyl)-N^4-trifluoroacetylpristinamycin I_B is thereby obtained in the form of a white powder.

Mass spectrum produced on a VG Autospec apparatus with FAB using a Cs gun in an NBA matrix:

$(M+H)^+ = 828$

$(M-H_2O+H)^+ = 810$

NMR spectrum

1.55 to 1.85	mt	3γ1, 2β1 and 2β2
3.23 and 3.3	2s	>N-CH_3 at position 4 [$ArN(CH_3)COCF_3$+-CO-$N(CH_3)$-]

N^4-Trifluoroacetylpristinamycin I_B is obtained

in the following manner:

6 mg of trifluoroacetic anhydride are added to 25 mg of pristinamycin I_B dissolved in 1 cm^3 of anhydrous dichloromethane. The mixture is left stirring for 12 hours. 6 mg of trifluoroacetic anhydride are added again. The mixture is stirred for 4 hours under reflux, then cooled and adjusted to pH 7-8 with aqueous sodium bicarbonate solution. The organic phase is separated off until settling has taken place, and the aqueous phase is washed with twice 2 cm^3 of water. The organic phases are combined, dried over sodium sulphate, filtered and then evaporated to dryness under reduced pressure (2.7 kPa) at 30°C. 25 mg of N^4-trifluoroacetylpristinamycin I_B are thereby obtained in the form of a white powder.

The products according to the invention may be used in the following manner:

Application Example 1

0.43 cm^3 of 4-tert-butylbenzoyl chloride dissolved in 5 cm^3 of methylene chloride and 0.34 cm^3 of triethylamine are simultaneously added dropwise to a solution, maintained at 5°C, of 1.5 g of de(3-hydroxypicolinoyl)pristinamycin IA in 25 cm^3 of methylene chloride. The reaction mixture is then stirred for 2 hours at a temperature in the region of 20°C and 20 cm^3 of distilled water are thereafter added. The organic phase is separated after settling has taken place, the aqueous phase is extracted twice with 25 cm^3 of

methylene chloride and the combined organic phases are washed with 20 cm^3 of distilled water and then dried over magnesium sulphate. After filtration and then concentration to dryness of the organic phases under reduced pressure (2.7 kPa) at 30°C, 2.2 g of a residue are obtained, which residue is purified by flash chromatography (eluant: ethyl acetate), collecting 10-cm^3 fractions. Fractions 8 to 11 are combined and concentrated to dryness under reduced pressure (2.7 kPa) at 30°C. 1.65 g of 1-(4-tert-butylbenzoyl)de(3-hydroxypicolinyl)pristinamycin IA is thereby obtained in the form of a white powder, melting at about 206°C.

NMR spectrum:

0.24 (dd, 5 β2)

1.34 (s, $C(CH_3)_3$)

2 to 2.3 (m, 5 δ2, 5 β1 and 5 δ1)

benzene ring at position 1:

7.57 (d, H3 and H5)

7.88 (d, H2 and H6)

4-tert-Butylbenzoyl chloride may be prepared according to the method described by F. Bell and R.D. Wilson, J. Chem. Soc. 2340 (1956).

Application Example 2

0.42 g of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride dissolved in 25 cm^3 of methylene chloride is added dropwise to a solution, maintained at 5°C, of 1.5 g of de(3-hydroxypicolinoyl)-

pristinamycin IA, 0.42 g of 3,4-dinitrobenzoic acid and 0.027 g of 1-hydroxybenzotriazole in 35 cm^3 of methylene chloride. The reaction mixture is then stirred for 2 hours at a temperature in the region of 5°C, and thereafter for 2 hours at a temperature in the region of 20°C. 20 cm^3 of distilled water are added;

The organic phase is separated after settling has taken place, the aqueous phase is extracted twice with 20 cm^3 of methylene chloride and the combined organic phases are washed with 20 cm^3 of distilled water and then dried over sodium sulphate. After filtration and then concentration to dryness of the organic phases under reduced pressure (2.7 kPa) at 30°C, 2.07 g of a residue are obtained, which residue is purified by flash chromatography (eluant: ethyl acetate), collecting 10-cm^3 fractions. Fractions 61 to 131 are combined and concentrated to dryness under reduced pressure (2.7 kPa) at 30°C. 1.25 g of 1-(3,4-dinitrobenzoyl)de(3-hydroxypicolinoyl)pristinamycin IA is thereby obtained in the form of a white powder, melting at about 212°C.

NMR spectrum:

0.25 (dd, 5 β2)

2 to 2.3 (m, 5 δ2, 5 β1 and 5 δ1)

benzene ring at position 1:

8.05 (d, H5)

8.38 (dd, H6)

8.65 (d, H2)

Application Example 3

0.285 cm^3 of isobutyl chloroformate is added to a solution, maintained at -10°C, of 0.394 g of 5-butylpicolinic acid and 0.22 g of N-methylmorpholine in 20 cm^3 of dichloromethane, and the reaction mixture is then stirred for 1 hour at -10°C. A solution of 1.5 g of de(3-hydroxypicolinoyl)pristinamycin IA in 15 cm^3 of dichloromethane is then added at -5°C. The mixture obtained is stirred for 16 hours at a temperature in the region of 20°C, and 50 cm^3 of distilled water are then added. The organic phase is separated after settling has taken place and washed with 50 cm^3 of saturated aqueous sodium chloride solution. After drying over magnesium sulphate, filtration and then concentration to dryness under reduced pressure (2.7 kPa) at a temperature in the region of 30°C, 1.8 g of residue is obtained, which residue is purified by flash chromatography (eluant: 1,2-dichloroethane/methanol, 97:3 by volume), collecting 60-cm^3 fractions. The residue obtained after concentration to dryness of fractions 12 to 16 under reduced pressure (2.7 kPa) at a temperature in the region of 30°C is ground in a mixture of 50 cm^3 of ethyl ether and 100 cm^3 of pentane. The solid is filtered off and dried at a temperature in the region of 20°C; 0.75 g of 1-(5-butylpicolinoyl)de(3-hydroxypicolinoyl)pristinamycin IA is thereby obtained in the form of a white powder, melting at 165°C.

NMR spectrum (400 Hz):

0.7 (dd, 5 β2)

1.04 (t, CH_3 of the butyl chain)

1.47 (m, CH_3CH_2 of the butyl chain)

1.74 (m, $CH_3CH_2CH_2$ of the butyl chain and 2 β1)

2.4 (d, 5 β1)

2.75 to 2.9 (m, $CH_3CH_2CH_2CH_2$ of the butyl chain and 5 ϵ2)

pyridine ring at position 1:

7.8 (dd, H4)

8.23 (d, H3)

8.28 (d, H6)

Application Example 4

Using a procedure similar to that described in Application Example 1, but starting with 1.5 g de(3-hydroxypicolinoyl)pristinamycin I_A and 0.37 g of 2,4-dimethylbenzoyl chloride, and after purification by flash chromatography (eluant: 1,2-dichloroethane/methanol, 97.5:2.5 by volume), collecting 30-cm^3 fractions, and concentration to dryness of fractions 15 to 19 under reduced pressure (2.7 kPa) at a temperature in the region of 30°C, 0.99 g of 1-(2,4-dimethylbenzoyl)de(3-hydroxypicolinoyl)-pristinamycin IA is obtained in the form of a white powder, melting at about 170°C.

NMR spectrum:

0.21 (dd, 5 β2)

2.1 (d, 5 β1)

2.4 and 2.56 (2s, CH_3 groups on the benzene ring at position 1)

benzene ring at position 1:

7.11 (d(broad), H5)

7.20 (s(broad), H3)

7.48 (d, H6)

Application Example 5

Using a procedure similar to that described in Application Example 1, but starting with 1.5 g de(3-hydroxypicolinoyl)pristinamycin IA and 0.33 cm^3 of 4-trifluoromethylbenzoyl chloride, and after purification by flash chromatography (eluant: dichloromethane/ethanol, 98:2 by volume), collecting 20-cm^3 fractions, and concentration to dryness of fractions 10 to 15 under reduced pressure (2.7 kPa) at a temperature in the region of 30°C, 0.69 g of 1-(4-trifluoromethylbenzoyl)de(3-hydroxypicolinoyl)pristinamycin IA (0.69 g) is obtained in the form of a white powder, melting at about 178°C.

NMR spectrum (400 MHz):

0.25 (dd, 5 β2)

2.05 to 2.3 (m, 5 δ2, 5 β1 and 5 δ1)

benzene ring at position 1:

7.79 (d, H3 and H5)

8.03 (d, H2 and H6)

Application Example 6

Using a procedure similar to that described in Application Example 1, but starting with 1.5 g de(3-hydroxypicolinoyl)pristinamycin IA, 0.63 cm^3 of triethylamine and 0.91 g of 2-chloro-4-quinolinecarbonyl chloride, and after purification by flash chromatography (eluant: dichloromethane/methanol, 98:2 by volume), collecting 30-cm^3 fractions, and concentration to dryness of fractions 10 to 15 under reduced pressure (2.7 kPa) at a temperature in the region of 30°C, 1.02 g of 1-(2-chloro-4-quinolylcarbonyl)de(3-hydroxypicolinoyl)-pristinamycin IA is obtained in the form of a white powder, melting at a temperature above 260°C.

2-Chloro-4-quinolinecarbonyl chloride may be prepared according to the method described by B. Mulert, Chem. Ber., 39, 1901 (1906).

NMR spectrum (400 MHz):

-0.1 (dd, 5 β2)

1.97 (d, 5 β1)

quinoline ring-system:

7.91 (s, H3)

7.78, 7.95, 8.18, 8.30 (2t, 2d, H_5, H_6, H_7 and H8)

Application Example 7

Using a procedure similar to that described in Application Example 1, but starting with 1.5 g de(3-hydroxypicolinoyl)pristinamycin IA and 0.42 g of 1-naphthoyl chloride, and after purification by flash chromatography (eluant: ethyl acetate/methanol, 96:4 by volume), collecting 25-cm^3 fractions, and concentration to dryness of fractions 12 to 20 under reduced pressure (2.7 kPa) at a temperature in the region of 30°C, 1.06 g of 1-(1-naphthoyl)de(3-hydroxy-picolinoyl)-pristinamycin IA is obtained in the form of a greenish powder, melting at about 170°C.

NMR spectrum:

0 (dd, 5 β2)

1.9 to 2.25 (m, 3 β1, 5 β1, 5 δ2 and 5 δ1)

naphthyl ring-system:

7.5 to 7.8 (m, H3, H6, H7)

7.85 to 8.1 (m, H4, H5, H8)

8.46 (d(broad), H2)

Application Example 8

0.21 g of 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride dissolved in 25 cm^3 of methylene chloride is added dropwise to a solution, maintained at 5°C, of 0.7 g of de(3-hydroxypicolinoyl)-virginiamycin S1, 0.21 g of 3,4-dinitrobenzoic acid and 0.014 g of 1-hydroxybenzotriazole in 20 cm^3 of methylene

chloride. The reaction mixture is then stirred for 2 hours at a temperature in the region of 5°C, and thereafter for 2 hours at a temperature in the region of 20°C. 20 cm^3 of distilled water are added; The organic phase is separated after settling has taken place, the aqueous phase is extracted twice with 20 cm^3 of methylene chloride and the combined organic phases are washed with 20 cm^3 of distilled water and then dried over sodium sulphate. After filtration and then concentration to dryness of the organic phases under reduced pressure (2.7 kPa) at 30°C, 0.9 g of a residue is obtained, which residue is purified by flash chromatography (eluant: dichloromethane/methanol, 97.5:2.5 by volume), collecting 8-cm^3 fractions. Fractions 36 to 60 are combined and concentrated to dryness under reduced pressure (2.7 kPa) at 30°C. 0.5 g of 1-(3,4-dinitro-benzoyl)de(3-hydroxypicolinoyl)virginiamycin S1 is thereby obtained in the form of a white powder, melting at about 190°C.

NMR spectrum:

0.05 (dd, 5 β2)

1.9 to 2.3 (m, 3 β, 5 δ2, 5 β1 and 5 δ1)

benzene ring at position 1:

8.03 (d, H5)

8.41 (dd, H6)

8.66 (d, H2)

Application Example 9

Using a procedure similar to that described in Application Example 1, but starting with 1 g de(3-hydroxypicolinoyl)virginiamycin S1, 0.31 cm^3 of triethylamine and 0.64 g of 2-chloro-4-quinoline-carbonyl chloride, and after purification by flash chromatography (eluant: dichloromethane/methanol, 98:2 by volume), collecting 10-cm^3 fractions, and concentration to dryness of fractions 15 to 35 under reduced pressure (2.7 kPa) at a temperature in the region of 30°C, 0.85 g of 1-(2-chloro-4-quinolylcarbonyl)de(3-hydroxypicolinoyl)virginiamycin S1 is obtained in the form of a white powder, melting at a temperature above 260°C.

NMR spectrum:

-0.4 (dd, 5 β2)

2 to 2.2 (m, 5 δ2, 5δ1 and 5 β1)

quinoline ring-system:

7.8 (s, H3)

7.71, 7.93, 8.18, 8.30 (2dt, and d, H5, H6, H7, H8)

Application Example 10

Using the procedure described in Application Example 2, but starting with 0.3 g of de(3-hydroxy-picolinoyl)pristinamycin I_C, 0.09 g of 3,4-dinitro-benzoic acid, 0.006 g of 1-hydroxybenzotriazole and 0.09 g of 1-ethyl-3-(3-dimethylamino-

propyl)carbodiimide hydrochloride, 0.3 g of a residue is obtained, which residue is purified by flash chromatography (eluant: methylene chloride/methanol, 97:3 by volume), collecting 5-cm^3 fractions. Fractions 31 to 45 are combined and concentrated to dryness under reduced pressure (2.7 kPa) at 30°C. 0.24 g of 1-(3,4-dinitrobenzoyl)de(3-hydroxypicolinoyl)pristinamycin I_C is thereby obtained in the form of a white powder, melting at about 200-210°C.

NMR spectrum:

1.26 and 1.32 (2d, 1γ and 2β)

7.10 to 7.4 (mt, aromatic)

8.06 (d, 1'H_5)

8.45 (dd, 1'H_6)

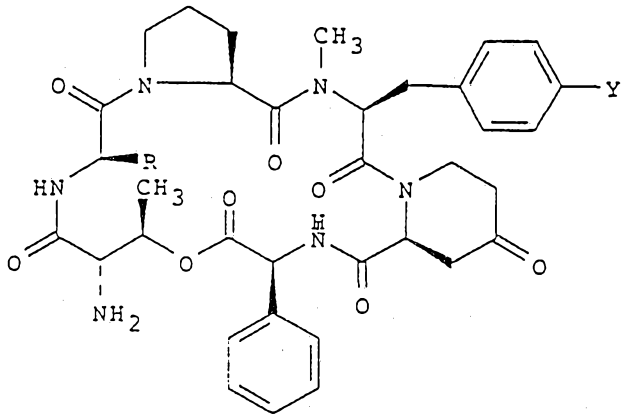
8.63 (d, >NH at position 1)

8.66 (d, 1'H_2)

8.85 (d, >NH at position 6)

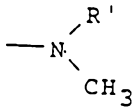
CLAIMS

1. Process for preparing a streptogramin derivative of general formula:

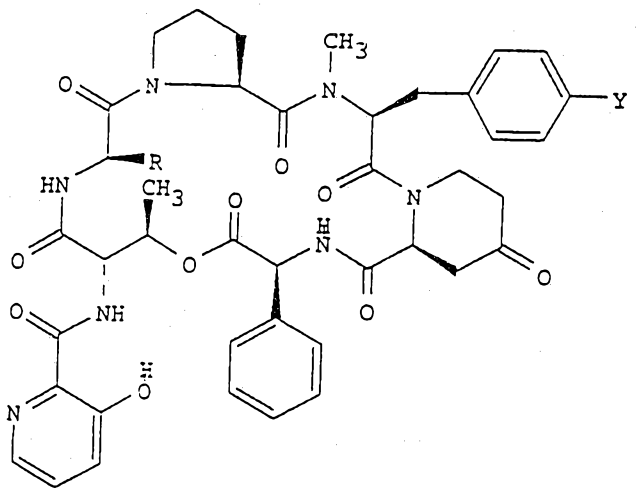


(I)

in which Y is a hydrogen atom or a methylamino or dimethylamino radical or a radical of structure:



in which R' is an amino-protecting radical and R is a methyl or ethyl radical, characterised in that the reductive cleavage of a streptogramin derivative of general formula:



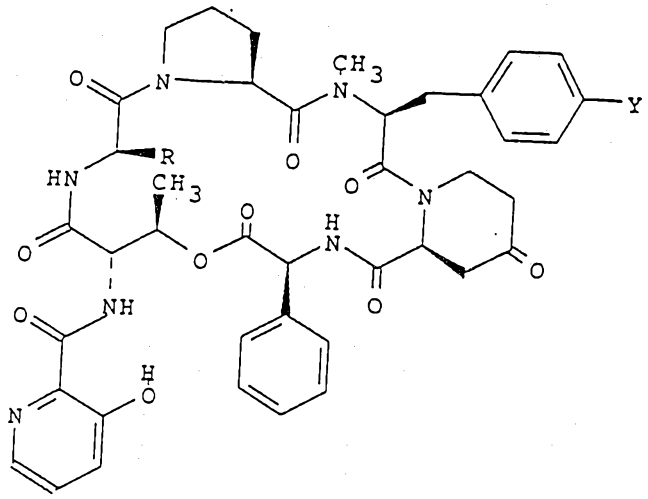
in which Y and R are defined as above, is performed in an acid medium, and the product obtained is then optionally converted to an addition salt with an acid.

2. Process according to Claim 1, characterised in that the reductive cleavage is performed by treatment in an acid medium in the presence of a reducing metal.

3. Process according to one of Claims 1 and 2, characterised in that the reaction is performed in the presence of a reducing metal whose redox potential is less than -0.94 V (s.c.e.).

4. Process for preparing a streptogramin derivative according to Claim 1, characterised in that the reductive cleavage is an electrochemical cleavage in an acid medium of the amide of the streptogramin

derivative of general formula:



in which Y and R are defined as in Claim 1, optionally followed by conversion of the product obtained to an addition salt with an acid.

Dated this 21st day of February, 1994

Rhone-Poulenc Rorer S.A.
By its Patent Attorneys
Davies Collison Cave

INTERNATIONAL SEARCH REPORT

International Application No PCT/FR 91/00590

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] C 07 D 498/14 C 07 K 5/06 C 07 K 7/06 //
(C 07 D 498/14 C 07 D 273:00 C 07 D 221:00 C 07 D 209:00)

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
Int. Cl.[5]	C 07 D 498/00 C 07 K 5/00 C 07 K 7/00

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]
A	Liebigs Annalen der Chemie, No. 1, 1986, VCH Verlagsgesellschaft mbH, (Weinheim, DE) H. Kessler et al.: "Synthese von Virginiamycin S1 und Virginiamycin S4", pages 21-31, see page 21, abstract; page 28, lines 8-12	1
A	Bulletin de la Société Chimique de France, No. 2, 1068 (Paris, FR) J. Preud'homme et al.: "Pristinamycine. Isolement, caractérisation et identification des constituants", pages 585-591, see page 587, chemical formula (cited in the application)	1
A	EP, A, 0133097 (RHONE-POULENC SANTE) 13 February 1985, see claim 1, & US, A, 4618599 (cited in the application)	1

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* 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
17 October 1991 (17.10.91)	11 December 1991 (11.12.91)
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	EP, A, 0248703 (RHONE-POULENC SANTE) 9 December 1987, see claim 1, & US, A, 4798827 (cited in the application)	1
A	GB, A, 2206879 (MAY AND BAKER LTD) 18 January 1989, see page 41, formula (XVI)	1
A	FR, A, 1505434 (E.R. SQUIBB AND SONS, INC.) 15 December 1967, see page 1, formula (I)	1

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. FR 9100590 SA 49788

This annex lists the patent family *members* relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 10/12/91
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0133097	13-02-85	FR-A- 2549063	18-01-85
		AU-B- 567570	26-11-87
		AU-A- 3047284	17-01-85
		CA-A- 1213583	04-11-86
		JP-A- 60038386	27-02-85
		SU-A- 1421260	30-08-88
		SU-A- 1445560	15-12-88
		SU-A- 1456019	30-01-89
		US-A- 4618599	21-10-86
EP-A- 0248703	09-12-87	FR-A- 2599036	27-11-87
		AU-B- 601426	13-09-90
		AU-A- 7323987	26-11-87
		CA-A- 1271297	03-07-90
		JP-A- 63022095	29-01-88
		SU-A- 1616520	23-12-90
		US-A- 4798827	17-01-89
		ZA-A- 8703639	17-11-87
GB-A- 2206879	18-01-89	None	
FR-A- 1505434		None	

EPO FORM P0479

RAPPORT DE RECHERCHE INTERNATIONALE

Demande Internationale No PCT/FR 91/00590

I. CLASSEMENT DE L'INVENTION (si plusieurs symboles de classification sont applicables, les indiquer tous) [7]

Selon la classification internationale des brevets (CIB) ou a la fois selon la classification nationale et la CIB

Int.Cl.5 C 07 D 498/14 C 07 K 5/06 C 07 K 7/06 //
(C 07 D 498/14 C 07 D 273:00 C 07 D 221:00 C 07 D 209:00)

II. DOMAINES SUR LESQUELS LA RECHERCHE A PORTE

Documentation minimale consultee[8]

Systeme de classification	Symboles de classification
Int.Cl.5	C 07 D 498/00 C 07 K 5/00 C 07 K 7/00

Documentation consultee autre que la documentation minimale dans la mesure ou de tels documents font partie des domaines sur lesquels la recherche a porte[9]

III. DOCUMENTS CONSIDERES COMME PERTINENTS[10]

Categorie °	Identification des documents cites, avec indication, si necessaire,[12] des passages pertinents [13]	No. des revendications visees [14]
A	Liebigs Annalen der Chemie, no. 1, 1986, VCH Verlagsgesellschaft mbH, (Weinheim, DE) H. Kessler et al.: "Synthese von Virginiamycin S1 und Virginiamycin S4", pages 21-31, voir page 21, abrégé; page 28, lignes 8-12 ---	1
A	Bulletin de la Société Chimique de France, no. 2, 1968 (Paris, FR) J. Preud'homme et al.: "Pristinamycine. Isolement, caractérisation et identification des constituants", pages 585-591, voir page 587, formule chimique (cité dans la demande) ---	1
A	EP,A,0133097 (RHONE-POULENC SANTE) 13 février 1985, voir revendicaton 1, & US, A, 4618599 (cité dans la demande) --- -/-	1

° Categories speciales de documents cites:[11]

"A" document définissant l'état général de la technique, non considéré comme particulièrement pertinent

"E" document anterieur, mais publié à la date de dépôt international ou apres cette date

"L" document pouvant jeter un doute sur une revendication de priorité ou cité pour déterminer la date de publication d'une autre citation ou pour une raison spéciale (telle qu'indiquée)

"O" document se référant à une divulgation orale, à un usage, à une exposition ou tous autres moyens

"P" document publié avant la date de dépôt international, mais postérieurement à la date de priorité revendiquée

"T" document ultérieur publié postérieurement à la date de depôt international ou à la date de priorité et n'appartenenant pas à l'état de la technique pertinent, mais cité pour comprendre le principe ou la théorie constituant la base de l'invention

"X" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme nouvelle ou comme impliquant une activité inventive

"Y" document particulièrement pertinent; l'invention revendiquée ne peut être considérée comme impliquant une activité inventive lorsque le document est associé à un ou plusieurs autres documents de même nature, cette combinaison étant evidente pour une personne du metier.

"&" document qui fait partie de la même famille de brevets

IV. CERTIFICATION

Date a laquelle la recherche internationale a ete effectivement achevee	Date d'expedition du present rapport de recherche internationale
17-10-1991	11. 12. 91
Administration chargee de la recherche internationale	**Signature du fonctionnaire autorise**
OFFICE EUROPEEN DES BREVETS	Daniels van der Haas

III. DOCUMENTS CONSIDERES COMME PERTINENTS [14] (SUITE DES RENSEIGNEMENTS INDIQUES SUR LA DEUXIEME FEUILLE)

Categorie °	Identification des documents cites, [16] avec indication, si necessaire des passages pertinents [17]	No. des revendications visees [18]
A	EP,A,0248703 (RHONE-POULENC SANTE) 9 décembre 1987, voir revendication 1, & US, A, 4798827 (cité dans la demande)	1
A	GB,A,2206879 (MAY AND BAKER LTD) 18 janvier 1989, voir page 41, formule (XVI)	1
A	FR,A,1505434 (E.R. SQUIBB AND SONS, INC.) 15 décembre 1967, voir page 1, formule (I)	1

ANNEXE AU RAPPORT DE RECHERCHE INTERNATIONALE RELATIF A LA DEMANDE INTERNATIONALE NO.

FR 9100590
SA 49788

La présente annexe indique les membres de la famille de brevets relatifs aux documents brevets cités dans le rapport de recherche internationale visé ci-dessus.
Lesdits membres sont contenus au fichier informatique de l'Office européen des brevets à la date du 10/12/91
Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsabilité de l'Office européen des brevets.

Document brevet cité au rapport de recherche	Date de publication	Membre(s) de la famille de brevet(s)	Date de publication
EP-A- 0133097	13-02-85	FR-A- 2549063	18-01-85
		AU-B- 567570	26-11-87
		AU-A- 3047284	17-01-85
		CA-A- 1213583	04-11-86
		JP-A- 60038386	27-02-85
		SU-A- 1421260	30-08-88
		SU-A- 1445560	15-12-88
		SU-A- 1456019	30-01-89
		US-A- 4618599	21-10-86
EP-A- 0248703	09-12-87	FR-A- 2599036	27-11-87
		AU-B- 601426	13-09-90
		AU-A- 7323987	26-11-87
		CA-A- 1271297	03-07-90
		JP-A- 63022095	29-01-88
		SU-A- 1616520	23-12-90
		US-A- 4798827	17-01-89
		ZA-A- 8703639	17-11-87
GB-A- 2206879	18-01-89	Aucun	
FR-A- 1505434		Aucun	

EPO FORM P0472