

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 199528912 B2
(10) Patent No. 712397

(54) Title
Streptogramins and method for preparing same by mutasynthesis

(51)⁶ International Patent Classification(s)
C12N 015/52 C07D 295/135
A61K 038/08 C07K 007/06
C07C 229/36 C12N 001/21
C07C 229/42 C12N 015/76
C07C 321/28

(21) Application No: 199528912 (22) Application Date: 1995 .07 .04

(87) WIPO No: W096/01901

(30) Priority Data

(31) Number	(32) Date	(33) Country
94/08478	1994 .07 .08	FR

(43) Publication Date : 1996 .02 .09
(43) Publication Journal Date : 1996 .03 .21
(44) Accepted Journal Date : 1999 .11 .04

(71) Applicant(s)
Rhone-Poulenc Rorer S.A.

(72) Inventor(s)
Veronique Blanc; Denis Thibaut; Nathalie Bamas-Jacques; Francis
Blanche ; Joel Crouzet; Jean-Claude Barriere; Laurent
Debussche ; Alain Famechon; Jean-Marc Paris; Gilles Dutruc-Rosset

(74) Agent/Attorney
DAVIES COLLISON CAVE,1 Little Collins Street,MELBOURNE VIC 3000

(56) Related Art
AU 38934/93
WO 94/08014

OPI DATE 09/02/96 APPLN. ID 28912/95
AOJP DATE 21/03/96 PCT NUMBER PCT/FR95/00889



AU9528912

ETS (PCT)

(51) Classification internationale des brevets ⁶ : C12N 15/52, 15/76, C07K 7/06, C12N 1/21, A61K 38/08, C07C 229/36, 229/42, 321/28, C07D 295/135 // (C12N 1/21, C12R 1:465)	A1	(11) Numéro de publication internationale: WO 96/01901 (43) Date de publication internationale: 25 janvier 1996 (25.01.96)
(21) Numéro de la demande internationale: PCT/FR95/00889 (22) Date de dépôt international: 4 juillet 1995 (04.07.95) (30) Données relatives à la priorité: 94/08478 8 juillet 1994 (08.07.94) FR (71) Déposant (pour tous les Etats désignés sauf US): RHONE-POULENC RORER S.A. [FR/FR]; 20, avenue Raymond-Aron, F-92160 Antony (FR). (72) Inventeurs; et (75) Inventeurs/Déposants (US seulement): BLANC, Véronique [FR/FR]; 210, avenue Daumesnil, F-75012 Paris (FR). THIBAUT, Denis [FR/FR]; 28, rue Jean-Colly, F-75013 Paris (FR). BAMAS-JACQUES, Nathalie [FR/FR]; 16, rue Plumet, F-75015 Paris (FR). BLANCHE, Francis [FR/FR]; 41, rue des Solitaires, F-75019 Paris (FR). CROUZET, Joël [FR/FR]; 48-52, rue des Meuniers, F-75012 Paris (FR). BARRIERE, Jean-Claude [FR/FR]; Résidence des Hauts-de-Bures, 24, rue Max-Ernst, F-91440 Bures-sur-Yvette (FR). DEBUSSCHE, Laurent [FR/FR]; 112, avenue Jean-Jaurès, F-91200 Athis-Mons (FR). FAMECHON, Alain [FR/FR]; 10, rue Bouray-sur-Juine, F-91510 Janville-sur-	Juine (FR). PARIS, Jean-Marc [FR/FR]; 8, rue des Acacias, F-77360 Vaires-sur-Marne (FR). DUTRUC-ROSSET, Gilles [FR/FR]; 21, avenue du Docteur-Arnold-Netter, F-75012 Paris (FR). (74) Mandataire: LE COUPANEC, Pascale; Rhône-Poulenc Rorer S.A., Direction Brevets, 20, avenue Raymond-Aron, F-92160 Antony (FR). (81) Etats désignés: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, UG, US, UZ, VN, brevet européen (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), brevet OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Publiée Avec rapport de recherche internationale.	
(54) Title: STREPTOGRAMINES AND METHOD FOR PREPARING SAME BY MUTASYNTHESIS (54) Titre: STREPTOGRAMINES ET PROCEDE DE PREPARATION DE STREPTOGRAMINES PAR MUTASYNTHESE (57) Abstract Novel group B streptogramin-like compounds of general formula (I), and a method for preparing streptogramins by mutasynthesis using a mutated micro-organism to influence the biosynthesis of at least one of the precursors of group B streptogramins, are disclosed. Novel nucleotide sequences involved in the biosynthesis of said precursors, and their uses, are also disclosed. (57) Abrégé La présente invention se rapporte à de nouveaux composés apparentés aux streptogramines du groupe B, de formule générale (I) et à un procédé de préparation de streptogramines par mutasynthèse mettant en œuvre un micro-organisme muté de manière à altérer la biosynthèse d'au moins un des précurseurs des streptogramines du groupe B. Elle concerne également des nouvelles séquences nucléotidiques impliquées dans la biosynthèse de ces précurseurs et leurs utilisations. (I)		

Streptogramins and a process for preparing
streptogramins by mutasynthesis

The present invention relates principally to novel compounds which are related to the group B streptogramins, and to a process for preparing streptogramins by mutasynthesis. It also relates to novel genes which are involved in the biosynthesis of precursors of the group B streptogramins, and to their uses.

10 The streptogramins form a homogeneous group of antibiotics consisting of an association of two types of chemically different molecules; on the one hand polyunsaturated macrolactones (group A components) and, on the other hand, depsipeptides (group B components). This group comprises numerous antibiotics which are known under different names according to their origin and includes pristinamycins, mikamycins and virginiamycins (Cocito 1979, 1983).

20 The A and B components have a synergistic antibacterial activity which can amount to 100 times that of the separate components and which, contrary to that of each component, is bactericidal (Cocito 1979). This activity is more particularly effective against Gram-positive bacteria such as Staphylococci and Streptococci (Cocito 1979, Videau 1982). Components A and B inhibit protein synthesis by binding to the 50S

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subunit of the ribosome (Cocito 1979; Di Giambattista et al., 1989).

While knowledge of the routes by which each of the components is biosynthesized still remains partial to date, earlier studies, presented in Patent Application PCT/FR93/0923, have made it possible to identify several proteins, and the corresponding structural genes, which are involved in the biosynthesis of the two types of component.

Two parts can be distinguished in the process for biosynthesizing group B streptogramins:

1) Biosynthesis of the precursors, or their analogues, of the macrocycle: 3-hydropicolinic acid, L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine, L-pipecolic acid and L-phenylglycine.

2) Formation of the macrocycle from the precursors listed above, from L-threonine and from L-proline, or their analogues, with (a) possible subsequent modification(s) of the peptide N-methylation, epimerisation, hydroxylation and oxidation type.

Patent Application PCT/FR93/0923 relates, in particular, to the enzymes which catalyse incorporation of the precursors into the peptide chain of B streptogramins in the process of elongation, and also to their structural genes. These results have demonstrated the non-ribosomal peptide synthesis

character of the type B components.

The present invention relates, more particularly, to novel compounds which are related to group B streptogramins and, more precisely, to novel
5 compounds of the pristinamycin I family (Figures 1 and 2), termed PI below, or of the virginiamycin S family (Figure 3).

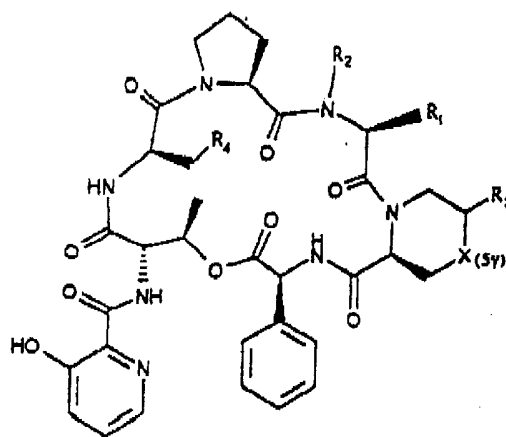
The major constituent of the I pristinamycins (PI) is PI_A (Figure 1), which represents approximately
10 94% of the PI, with the remaining approximately 6% being represented by minor constituents of the depsipeptide (PI_B to PI_I) whose structures are depicted in Figure 2. PI results essentially from the condensation of amino acids, certain of which are
15 essential for protein synthesis (threonine and proline) and others of which are novel and themselves considered to be secondary metabolites (L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid and L-phenylglycine for PI_A), and also of an
20 aromatic precursor, 3-hydroxypicolinic acid.

The virginiamycin S derivatives result from condensation of the same acids as in the case of PI, apart from 4-DMPAPA, which is replaced by a phenylalanine (see Figure 3).

25 Production of these different compounds by biosynthesis therefore requires preliminary synthesis, by a producer strain, of the novel precursors identified above.

The present invention results specifically from a novel process for preparing streptogramins which employs, as a strain for producing streptogramins, a microorganism strain which is mutated so as to alter the biosynthesis of the precursors of the group B streptogramins. According to this process, the said mutant strain is cultured in a medium which is supplemented with a precursor which is different from the precursor whose biosynthesis is altered. Unexpectedly, this results in the production of novel compounds which are related to the group B streptogramins and which are of value in the therapeutic sphere.

More precisely, the present invention provides compounds of the general formula I:



1

in which:

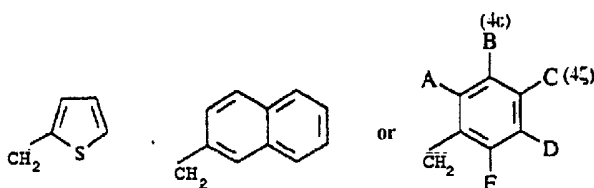


- R_2 and R_4 represent, independently of each other, a hydrogen atom or a methyl group,

- R_3 represents a hydrogen atom or a hydroxyl group,

5 - X represents a CO, CHOH or CH_2 group, and

- R_1 represents:



with

- for the meta derivatives:

A, C, D and E representing a hydrogen atom, and

10 B being able to represent

- a halogen, and preferably a fluorine atom,

- a monoalkylamino or dialkylamino group,

with alkyl preferably representing a methyl or ethyl group,

15 - an ether group; more particularly an OR group with R being preferably selected from among the methyl, ethyl, trifluoromethyl and allyl groups,

- a thioether group, preferably represented by an alkylthio group with alkyl preferably

20 representing a methyl group,

- a C_1 to C_3 alkyl group, or

- a trihalogenomethyl group, preferably

trifluoromethyl

- for the para derivatives

A, B, D and E representing a hydrogen atom, and
C being able to represent:

- a halogen,
- 5 - an NR_1R_2 group with R_1 and R_2 representing,
independently of each other, a group selected from
among
 - hydrogen,
 - a straight-chain or branched C_1 to C_4
 - 10 alkyl group where, when one of the substituents R_1 or R_2 ,
represents a methyl group, the other necessarily
represents an ethyl group,
 - an alkyl-cycloalkylmethyl group with a
 C_3 to C_4 cycloalkyl,
 - 15 - an optionally substituted C_3 to C_4
cycloalkyl group,
 - a straight-chain or branched C_1 to C_4
 - alkenyl group where, when one of the substituents R_1 or
 R_2 represents an alkenyl group, the other is different
 - 20 from a methyl group or a C_3 to C_6 cycloalkyl,
 - a substituted or unsubstituted N-
pyrrolidinyl group,
 - an ether group; preferably an OR group with
R preferably being selected from among the methyl and
 - 25 ethyl groups, where appropriate substituted by a
chlorine atom, or trifluoromethyl and alkenyl groups
 - a thioether group, preferably represented
by an alkylthio group with alkyl preferably

representing a C₁ to C₃ alkyl group,

- an acyl or alkoxycarbonyl group and, more particularly, a COR group with R preferably representing a C₁ to C₃ alkyl group or a C₁ to C₃ alkoxy

5 group,

- a C₁ to C₆ alkyl group which is straight-chain or branched and which is preferably selected from among the methyl, isopropyl and tert-butyl groups,

- an alkylthiomethyl group and, more preferably, a CH₂SR group with R preferably representing a C₁ to C₃ alkyl group,

10

- an aryl group, preferably a phenyl group,

or

- a trihalogenomethyl group, preferably trifluoromethyl

15

- for the meta-para disubstituted derivatives:

A, D and E representing a hydrogen atom, and

B being able to represent:

- a halogen, preferably a fluorine atom,

20 - a monoalkylamino or dialkylamino group with alkyl preferably representing a methyl or ethyl group,

- an ether group and preferably an OR group with R preferably selected from among the methyl, ethyl and trifluoromethyl groups,

25 - a thioether group and preferably alkylthio with alkyl preferably representing an ethyl group, or

- a C₁ to C₃ alkyl group, and

C being able to represent:

- a halogen, preferably a fluorine atom,
- an amino, monoalkylamino or dialkylamino group with alkyl preferably representing a methyl group with the proviso that B is different from a bromine or chlorine atom, or a substituted or unsubstituted allyl group,
- an ether group and preferably an OR group with R preferably selected from among the methyl, ethyl and trifluoromethyl groups,
- a thioether group and preferably an alkylthio group with alkyl preferably representing a methyl group,
- a C₁ to C₆ alkyl group, or
- a trihalogenomethyl group, preferably trifluoromethyl, and
- for the ortho-para disubstituted derivatives:
B, E and D representing a hydrogen atom and A and C a methyl group.

The following may be more particularly mentioned as preferred compounds:

- 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_A,
- 4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_B,
- 5γ-hydroxy-4ζ-methylthio-de(4ζ-dimethylamino)pristinamycin I_B,
- 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin I_A,

- 4ζ-methyl-de(4ζ-dimethylamino)pristinamycin
 I_H,
- 4ζ-methoxy-de(4ζ-dimethylamino)pristinamycin
 I_A,
- 5 4ζ-methoxycarbonyl-de(4ζ-dimethylamino)pristinamycin I_A,
- 4ζ-chloro-de(4ζ-dimethylamino)pristinamycin
 I_A,
- 10 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_A,
- 4ζ-bromo-de(4ζ-dimethylamino)pristinamycin I_H,
- 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_A,
- 4ζ-iodo-de(4ζ-dimethylamino)pristinamycin I_H,
- 4ζ-trifluoromethyl-de(4ζ-dimethylamino) -
 pristinamycin I_A,
- 15 4ζ-trifluoromethyl-de(4ζ-dimethylamino) -
 pristinamycin I_H,
- 4ζ-tert-butyl-de(4ζ-dimethylamino) -
 pristinamycin I_A,
- 4ζ-isopropyl-de(4ζ-dimethylamino) -
 20 pristinamycin I_A,
- 4ζ-isopropyl-de(4ζ-dimethylamino) -
 pristinamycin I_E,
- 4ε-methylamino-de(4ζ-dimethylamino) -
 pristinamycin I_A,
- 25 4ε-methoxy-de(4ζ-dimethylamino)pristinamycin
 I_A,
- 4ε-methoxy-de(4ζ-dimethylamino)pristinamycin
 I_H,

- 4ε-fluoro 4ζ-methyl-de(4ζ-dimethylamino)-
 pristinamycin I_A,
- 4ζ-amino-de(4ζ-dimethylamino)pristinamycin I_A,
- 4ζ-ethylamino-de(4ζ-dimethylamino)-
- 5 pristinamycin I_A,
- 4ζ-diethylamino-de(4ζ-dimethylamino)-
 pristinamycin I_A,
- 4ζ-allylamino-de(4ζ-dimethylamino)-
 pristinamycin I_A,
- 10 4ζ-diallylamino-de(4ζ-dimethylamino)-
 pristinamycin I_A,
- 4ζ-allylethylamino-de(4ζ-dimethylamino)-
 pristinamycin I_A,
- 4ζ-ethylpropylamino-de(4ζ-dimethylamino)-
- 15 pristinamycin I_A,
- 4ζ-ethylisopropylamino-de(4ζ-dimethylamino)-
 pristinamycin I_A,
- 4ζ-ethylmethylcyclopropylamino-de(4ζ-
 dimethylamino)pristinamycin I_A,
- 20 4ζ-(1-pyrrolidinyl)-de(4ζ-dimethylamino)-
 pristinamycin I_A,
- 4ζ-trifluoromethoxy-de(4ζ-dimethylamino)-
 pristinamycin I_A,
- 4ζ-allyloxy-de(4ζ-dimethylamino)pristinamycin
- 25 I_A,
- 4ζ-ethoxy-de(4ζ-dimethylamino)pristinamycin
- I_A,
- 4ζ-ethylthio-de(4ζ-dimethylamino)-

pristinamycin I_A,

4β-methylthiomethyl-de(4β-dimethylamino)-

pristinamycin I_A,

4β-(2-chloroethoxy)-de(4β-dimethylamino)-

5 pristinamycin I_A,

4β-acetyl-de(4β-dimethylamino)pristinamycin

I_A,

4β-ethyl-de(4β-dimethylamino)pristinamycin I_A,

4β-ethyl-de(4β-dimethylamino)pristinamycin I_A,

10

4ε-dimethylamino-de(4β-dimethylamino)-

pristinamycin I_A,

4ε-methylthio-de(4β-dimethylamino)-

pristinamycin I_A,

4ε-ethoxy-de(4β-dimethylamino)pristinamycin

15

I_A.

The present invention is also directed towards a process which is particularly useful for preparing the compounds of the general formula I.

The invention provides a process for
20 preparing streptogramins, characterized in that it employs a streptogramin-producing microorganism strain which possesses at least one genetic modification which affects the biosynthesis of a precursor of the group B streptogramins, and in that the said mutant strain is
25 cultured in a culture medium

which is supplemented with at least one precursor which is different from that whose biosynthesis is altered which precursor is selected from derivatives or analogues of amino acids and alpha-ketocarboxylic acids, and in that the said streptogramins are

recovered.

The strains which are employed within the scope of the present invention are therefore strains which produce streptogramins and which are mutated. The
5 genetic modification(s) can be located either within one of the genes which is involved in the biosynthesis of the said precursors or outside the coding region, for example in the regions responsible for the expression and/or the transcriptional or post-
10 transcriptional regulation of the said genes, or in a region belonging to the transcript containing the said genes.

According to one particular embodiment of the invention, the mutant strains possess one or more
15 genetic modifications within at least one of their genes which is/are involved in the biosynthesis of the group B streptogramin precursors.

This or these genetic modification(s) alter(s) the expression of the said gene, that is
20 render(s) this gene, and, as the case may be, another of the genes involved in the biosynthesis of the precursors, partially or totally incapable of encoding the natural enzyme which is involved in the biosynthesis of at least one precursor. The inability
25 of the said genes to encode the natural proteins may be manifested either by the production of a protein which is inactive due to structural or conformational modifications, or by the absence of production, or by

the production of a protein having an altered enzymic activity, or else by the production of the natural protein at an attenuated level or in accordance with a desired mode of regulation. The totality of these possible manifestations is expressed by an alteration of, or perhaps a blockage in, the synthesis of at least one of the group B streptogramin precursors.

The genes which are capable of being mutated within the scope of the present invention are preferably the genes which are involved in the biosynthesis of the following precursors:

L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid, L-phenylglycine and/or 3-hydroxypicolinic acid (3-HPA).

These genes are more preferably the papA, papM, papB (SEQ ID No. 3), papC (SEQ ID No. 2), hpaA (SEQ ID No. 8), snbF (SEQ ID No. 6) and pipA (SEQ ID No. 5) genes described below.

The papA and papM genes have already been described in Patent Application PCT/FR93/0923. They are present on the cosmid PIBV2. The papA gene appears to correspond to a gene for biosynthesizing 4-amino-L-phenylalanine from chorismate. The 4-amino-L-phenylalanine is then dimethylated by the product of the papM gene, an N-methyltransferase, in order to form 4-dimethylamino-L-phenylalanine, DMPAPA, which is then incorporated into pristinamycin I_A. These two genes are more particularly involved, therefore, in the synthesis

of the precursor termed DMPAPA.

The other genes, papB, papC, pipA, snbF and hpaA, have been identified and characterized within the scope of the present invention. They are grouped
5 together with the snbA, papA and papM genes on a chromosomal region of approximately 10 kb (Figure 7).

The sequence homologies demonstrated for the PapB and PapC proteins show that these proteins are also involved, jointly with the papA and papM proteins,
10 in the biosynthesis of the DMPAPA precursor. The two corresponding novel genes, papB and papC, were isolated and identified by subcloning which was carried out using cosmid pIBV2, described in Patent Application PCT/FR93/0923, and a plasmid, pVRC900, which is derived
15 from pIBV2 by means of a HindIII deletion and is also described in Patent Application PCT/FR93/0923.

The comparison of the protein encoded by the papC gene with the protein sequences contained in the Genpro library shows a 27% homology with the region
20 which is involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of E. coli (Hudson and Davidson, 1984) and Erwinia herbicola (EMBL data library, 1991). This region of TyrA catalyses aromatization of the prephenate to form
25 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, which proceeds from 4-deoxy-4-aminoprephenate and leads to 4-aminophenylpyruvate is very probably involved in the synthesis of

DMPAPA. It would be catalysed by the PapC protein (SEQ ID No. 2).

PapB possesses a 24 to 30% homology with the region which is involved in the chorismate mutase activity of the TyrA and PheA bifunctional proteins of *E. coli* (Hudson and Davidson, 1984) and of the TyrA protein of *Erwinia herbicola*. This region catalyses isomerization of the chorismate to form prephenate in the biosynthesis of tyrosine and of phenylalanine. The PapB protein (SEQ ID No. 3) is probably involved in a similar isomerization which proceeds from 4-deoxy-4-aminochorismate and leads to 4-deoxy-4-aminoprephenate in the synthesis of DMPAPA.

The pipA, snbF and hpaA genes have been located in the regions which are contained between the snbA gene, which encodes 3-hydroxypicolinic acid AMP ligase and is described in Patent Application PCT/FR93/0923, and the papA or snbR genes. They were located accurately by means of subcloning, which was carried out using the plasmid pVRC900 and the cosmid pIBV2, which are described in Patent Application PCT/FR93/0923.

On comparing the protein encoded by the hpaA gene and the protein sequences contained in the Genpro library, a homology of from 30 to 40% was detected with a group of proteins which are probably involved (Thorson *et al.*, 1993) in the transamination of intermediates in the biosynthesis of various

antibiotics (DnrJ, EryC1, TylB, StrS and PrgL).

Synthesis of the 3-HPA precursor, which appears to derive from lysine by another route than that of cyclodeamination (see examples 1-2 and 2-1), probably
5 requires a transamination step which can be catalysed by the product of this gene termed hpaA (SEQ ID No. 8). Furthermore, the results of mutating this gene demonstrate unequivocally that it is involved in the synthesis of the 3-HPA precursor.

10 Comparison of the product encoded by the gene termed pipA with the protein sequences contained in the Genpro library shows a 30% homology with the ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final
15 step of the catabolism of octopine; it converts L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of incorporating labelled lysine, that 4-oxopipicolinic acid and 3-hydroxypicolinic acid, which are found both
20 in PI₁ and in virginiamycin S1, derived from lysine (Molinero et al., 1989, Reed et al., 1989). Cyclodeamination of lysine, in a similar manner to that described for ornithine, would lead to the formation of
25 this product was termed PipA (SEQ ID No. 5). The results of mutating the pipA gene, presented in the examples below, demonstrate that it is involved solely in the synthesis of pipicolinic acid. It is noted, in

particular, that this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.

5 Finally, on comparing the product of the gene termed snbF with the protein sequences contained in the Genpro library, a 30 to 40% homology was noted with several hydroxylases of the cytochrome P450 type, which are involved in the biosynthesis of secondary
10 metabolites (Omer *et al.*, 1990. Trower *et al.*, 1992). Several hydroxylations can be envisaged in the biosynthesis of the precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of
15 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The corresponding protein was termed SnbF (SEQ ID No. 6).

 The results of mutating the pipA gene, with polar effects on the expression of the snbF gene,
20 demonstrate the involvement of the snbF gene in the hydroxylation of the pipecolic acid residue of group B streptogramins. The expression of the snbF gene is thus altered by the expedient of effecting a genetic modification of the pipA gene.

25 Preferentially, the genetic modification(s) render(s) the said gene partially or totally incapable of encoding the natural protein.

 Genetic modification should be understood to

mean, more particularly, any suppression, substitution, deletion, or addition of one or more bases in the gene(s) under consideration. Such modifications may be obtained in vitro (on the isolated DNA) or in situ, for example, by means of genetic engineering techniques, or else by exposing the said microorganisms to a treatment using mutagenic agents. Examples of mutagenic agents which may be cited are physical agents such as high-energy rays (X, γ , ultra violet, etc. rays), or chemical agents which are able to react with different functional groups of the DNA bases, and, for example, alkylating agents [ethyl methanesulphonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine, and N-nitroquinoline-1-oxide (NQO)], bialkylating agents, intercalating agents, etc. Deletion is understood to mean any suppression of a part for all of the gene under consideration. This deletion can, in particular, be of a part of the region encoding the said proteins, and/or of all or part of the promoter region for transcription or translation, or else of the transcript.

The genetic modifications may also be obtained by means of gene disruption, for example using the protocol initially described by Rothstein [Meth. Enzymol. 101 (1983) 202] or, advantageously, by means of double homologous recombination. In this case, the integrity of the coding sequence will preferentially be disrupted in order to permit, if need be, replacement,

by means of homologous recombination, of the wild-type genomic sequence with a non-functional or mutant sequence.

According to another option of the invention,
5 the genetic modifications can consist of placing the gene(s) encoding the said proteins under the control of a regulated promoter.

The mutant microorganism strains according to the present invention may be obtained from any
10 microorganism which produces streptogramins (cf. Table V). According to one particular embodiment of the invention, the mutant strain is a strain which is derived from *S. pristinaespiralis* and, more particularly, from *S. pristinaespiralis* SP92.

15 Mutant strains which are preferred within the scope of the present invention and which may more particularly be mentioned are the strain SP92::pVRC508, which is mutated in the biosynthesis of the DMPAPA precursor by disrupting the papA gene by means of
20 simple crossing over, or else, more preferably, the strain SP212, which is mutated in the biosynthesis of the DMPAPA precursor by disrupting the papA gene by means of double homologous recombination. These strains no longer produce PI unless they are supplemented with the
25 DMPAPA precursor. Unexpectedly, when a novel precursor, which is different from DMPAPA and which is capable, after, in this case, metabolization, of being incorporated by PI synthetase III (SnbD protein which

is responsible for incorporating L-proline and DMPAPA residues) is added to the production medium, these two strains then become able to produce novel I pristinamycins or virginiamycins, or else mainly to produce a component which is normally a minor component of PI, in particular PI₁ (Figure 2).

Two other mutant strains have been prepared within the scope of the present invention. These are, respectively, the strain SP92

pipA:Qam^r, in which the pipA gene is disrupted by homologous recombination, and the strain SP92

hpaA:Qam^r, in which the hpaA gene is disrupted. While strain SP92

pipA:Qam^r no longer produces PI under standard fermentation conditions, it strongly produces, in the presence of L-pipecolic acid, a component, which was initially a minor component among the B streptogramin components, in which 4-oxopipecolic acid is replaced by L-pipecolic acid. While strain S. pristinaspinalis SP92

hpaA:Qam^r no longer produces PI under standard fermentation conditions, it is able to produce novel group B streptogramins in the presence of novel precursors.

By supplementing the medium for culturing mutant strains according to the invention with at least one precursor as hereinbefore defined, it turns out that it is possible to orient biosynthesis either towards novel streptogramins, or towards a minor form of the streptogramins, or else to favour formation of one of the streptogramins.

The precursors which are employed within the scope of the present invention are derivatives or analogues of amino acids and, more particularly of phenylalanine, as well as organic acids and, in particular, alpha-cetocarboxylic acids and, more particularly, derivatives of phenylpyruvic acid.

Naturally, the precursor is such that it caters for the alteration or blockage, which is induced in accordance with the invention, within the biosynthesis of one of the natural precursors of the group B streptogramins and leads to the synthesis of streptogramins. According to one particular embodiment of the invention, this precursor is selected such that it is related to the precursor whose biosynthesis is altered. Thus, in the specific case of the mutant which is blocked in the biosynthesis of DMPAPA, the precursor is preferably a derivative of phenylalanine.

The following precursors are preferred:

phenylalanine, 4-dimethylaminophenylalanine, 4-methylaminophenylalanine, 4-aminophenylalanine, 4-diethylaminophenylalanine, 4-ethylaminophenylalanine, 4-methylthiophenylalanine, 4-methylphenylalanine, 4-methoxyphenylalanine, 4-trifluoromethoxyphenylalanine, 4-methoxycarbonylphenylalanine, 4-chlorophenylalanine, 4-bromophenylalanine, 4-iodophenylalanine,

- 4-trifluoromethylphenylalanine, 4-tert-butylphenylalanine, 4-isopropylphenylalanine, 3-methylaminophenylalanine, 3-methoxyphenylalanine, 3-methylthiophenylalanine, 3-fluoro-
- 5 4-methylphenylalanine, L-pipecolic acid, 4-tert-butylphenylpyruvic acid, 4-methylaminophenylpyruvic acid, 2-naphthylphenylalanine, 4-fluorophenylalanine, 3-fluorophenylalanine, 3-ethoxyphenylalanine, 2,4-dimethylphenylalanine, 3,4-dimethylphenylalanine,
- 10 3-methylphenylalanine, 4-phenylphenylalanine, 4-butylphenylalanine, 2-thienyl-3-alanine, 3-trifluoromethylphenylalanine, 3-hydroxyphenylalanine, 3-ethylaminophenylalanine, 4-allylaminophenylalanine, 4-diallylaminophenylalanine,
- 15 4-allylethylaminophenylalanine, 4-ethylpropylaminophenylalanine, 4-ethylisopropylaminophenylalanine, 4-ethylmethylcyclopropylaminophenylalanine, 4-(1-pyrrolidinyl)phenylalanine, 4-O-allyltyrosine,
- 20 4-O-ethyltyrosine, 4-ethylthiophenylalanine, 4-ethylthiomethylphenylalanine, 4-O-(2-chloroethyl)tyrosine, 4-acetylphenylalanine, 4-ethylphenylalanine, 3-dimethylaminophenylalanine,
- 25 and 4-aminomethylphenylalanine.

Among these precursors,

- 4-trifluoromethoxyphenylalanine, 3-methylaminophenylalanine, 3-methylthiophenylalanine,

3-fluoro-4-methylphenylalanine,
4-methylaminophenylpyruvic acid, 3-ethoxyphenylalanine,
4-allylaminophenylalanine, 4-diallylaminophenylalanine,
4-allylethylaminophenylalanine,
5 4-ethylpropylaminophenylalanine,
4-ethylisopropylaminophenylalanine,
4-ethylmethylcyclopropylaminophenylalanine,
4-(1-pyrrolidinyl)phenylalanine,
4-ethylthiomethylphenylalanine,
10 4-O-(2-chloroethyl)tyrosine,
3-dimethylaminophenylalanine and
3-ethylaminophenylalanine are novel and were prepared
and characterized within the scope of the present
15 invention. They are found to be particularly useful for
preparing streptogramins according to the invention.

The claimed process turns out to be
particularly advantageous for preparing novel group B
streptogramins or else for favouring formation of
20 particular streptogramins. As such, it is particularly
useful for preparing PIB.

The present invention also relates to a
nucleotide sequence which is selected from among:

25 (a) all or part of the genes papC (SEQ ID
No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF
(SEQ ID No. 6) and hpaA (SEQ ID No. 8) and which
encode a polypeptide which is involved in the
biosynthesis of streptogramins, and

30 (b) sequences which hybridize with all or
part of the (a) genes and which encode a polypeptide
which is involved in the biosynthesis of
streptogramins, and

(b) sequences on account of the degeneracy of the genetic code.

In the particular case of the hybrid sequences according to (b), these sequences preferably
5 encode a polypeptide which is involved in the biosynthesis of the streptogramins.

Still more preferably, the invention relates to the nucleotide sequences which are represented by the genes papC (SEQ ID No. 2), papB (SEQ ID No. 3),
10 pipA (SEQ ID No. 5), snbF (SEQ ID No. 6), and hpaA (SEQ ID No. 8).

The invention furthermore relates to any recombinant DNA which encompasses a papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ
15 ID No. 6) or hpaA (SEQ ID No. 8) gene.

Naturally, the nucleotide sequences defined above can be part of a vector of the expression vector type, which can be an autonomously replicating vector, an integrated vector or a suicide vector. The present
20 invention is also directed to these vectors as well as to any use of a sequence according to the invention or of a corresponding vector for, in particular, preparing metabolites of interest. It furthermore relates to any polypeptide which results from the expression of a
25 claimed sequence.

The present invention also relates to any mutated S. pristinaespiralis strain which possesses at least one genetic modification within one of the papC

(SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) and hpaA (SEQ ID No. 8) genes, and, more preferably, to strains SP92pipA:: Ω am^R and SP92hpaA:: Ω am^R, as well as any *S. pristinaespiralis* strain, such as SP212, which possesses a genetic modification which consists of a disruption of the papA gene by means of double homologous recombination.

Combinations of a component of the group A streptogramins and of a compound of the general formula I, according to the invention, constitute compositions which are particularly advantageous in the therapeutic sphere. They are employed, in particular, for treating ailments which are due to Gram-positive bacteria (of the genre *Staphylococci*, *Streptococci*, *Pneumococci* and *Enterococci*) and Gram-negative bacteria (of the genre *Haemophilus*, *Gonococci*, *Meningococci*). Thus, the compounds according to the invention have a synergistic effect on the antibacterial action of pristinamycin IIB on *Staphylococcus aureus* IP8203 in mice in vivo, at oral doses which are principally between 30 mg/kg and 100 mg/kg, when they are combined in PI/PII proportions of the order of 30/70.

The present invention extends to any pharmaceutical composition which contains at least one compound of the general formula I which is or is not combined with a group A streptogramin.

The examples appearing below are presented by way of illustrating the present invention and do not

limit it.

LIST OF FIGURES.

- Figure 1: Structure of pristinamycin I_A.
- Figure 2: Structure of the minor components of
5 pristinamycin I.
- Figure 3: Other examples of structures of B
components of streptogramins.
- Figure 4: Depiction of the PstI-XhoI region of
2.9 kb.
- 10 Figure 5: Depiction of the XhoI-PstI region of
4.5 kb.
- Figure 6: Depiction of the HindIII-BglIII region of
1.6 kb.
- Figure 7: Depiction of the BglIII-XhoI region of
15 approximately 10 kb.
- Figure 8: Depiction of plasmid pVRC415.
- Figure 9: Depiction of plasmid pVRC420.
- Figure 10: Depiction of plasmid pVRC411.
- Figure 11: Depiction of plasmid pVRC421.
- 20 Figure 12: Depiction of plasmid pVRC414.
- Figure 13: Strategy for constructing SP212.

**EXAMPLE 1: Sequencing and identification of
genes involved in the biosynthesis of pristinamycin I
and its precursors.**

- 25 Identification, by means of sequencing, of
the genes situated downstream and upstream of the gene
which encodes the enzyme PapA and which is described in
Patent PCT/FR93/0923, as well as of a gene which is

situated downstream of the gene which encodes the enzyme *SnbA* and which is also described in Patent PCT/FR93/0923.

This example describes how, using cosmid
5 pIBV2, which is described in Patent PCT/FR93/0923 and which contains the structural genes for the enzymes *PapA* and *PapM*, which are involved in the synthesis of the 4-dimethylamino-L-phenylalanine (DMPAPA) precursor of pristinamycin I, and the structural gene for the
10 enzyme *SnbA*, which is responsible for activating the aromatic precursor, 3-hydroxypicolinic acid (3-HPA), of pristinamycin I, it proved possible to identify, by sequencing around these genes and studying the corresponding mutants, other genes which are involved
15 in the biosynthesis of the DMPAPA precursor or in the biosynthesis of other precursors of pristinamycin I.

With this aim in mind, subclonings were carried out using cosmid pIBV2 and plasmid pVRC900, which is derived from pIBV2 by means of a HindIII
20 deletion and which is also described in Patent PCT/FR93/0923.

This example illustrates how the nucleotide sequences of fragments situated downstream and upstream of the *papA* and *snbA* genes of *S. pristinaespiralis* can
25 be obtained.

The techniques for cloning DNA fragments of interest in the M13mp18 and 19 vectors (Messing et al. 1981) are standard techniques for cloning in

Escherichia coli and are described in Maniatis et al. (1989).

1-1 Sequencing and analysis of the region downstream of the papA gene

5 In order to sequence this region, which is contained between the papA and papM genes, the PstI-PstI fragment of 1.5 kb, the PstI-XhoI fragment of 0.7 kb, and the XhoI-XhoI fragment of 0.7 kb were subcloned into the M13mp18 and M13mp19 vectors
10 proceeding from plasmid pVRC900. The cloning sites were sequenced through by sequencing on double-stranded DNA using plasmids pVRC900 and pVRC409, which are described in Patent PCT/FR93/0923.

The clonings were carried out as follows.

15 Approximately 2 µg of plasmid pVRC900 were cut with restriction enzymes PstI and/or XhoI (New England Biolands) under the conditions recommended by the supplier. The restriction fragments thus obtained were separated on a 0.8% agarose gel, and the 1.5 kb
20 PstI-PstI, 0.7 kb PstI-XhoI and 0.7 kb XhoI-XhoI fragments of interest were isolated and purified using GeneClean (Bio101, La Jolla, California). For each cloning, approximately 10 ng of M13mp19 and/or M13mp18, cut with PstI and/or XhoI, were ligated to 100 ng of
25 the fragment to be cloned under the conditions described by Maniatis et al. 1989. After transforming the strain TG1 (K12, Δ(lac-pro) *supE thi hsd* ΔS F' *traD36 proA⁺B⁺ lacI^q lacZ* Δ M15; Gibson, 1984) and

selecting lysis plaques on an LB + X-gal + IPTG medium in accordance with the technique described by Maniatis et al. (1989), the phage carrying the desired fragments were isolated. The different inserts were sequenced by the chain termination reaction using, as the synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. The reactions were carried out using fluorescent dideoxynucleotides (PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit-Applied Biosystem) and analysed on a model 373 A Applied Biosystems DNA sequencer. The overlap between these different inserts was such that it was possible to establish the entire nucleotide sequence between the papA and papM genes (SEQ ID No. 1).

With the aid of this nucleotide sequence, it is possible to determine the open reading frames and thereby identify genes which are involved, in S. pristinaespiralis, in the biosynthesis of PI or its precursors, as well as the polypeptides encoded by these genes.

We looked for the presence of open reading frames within the 2.9 kb PstI-XhoI fragment, which contains the nucleotide sequence between the papA and papM genes, making use of the fact that *Streptomyces* DNA displays a high percentage of G and C bases as well as a strong bias in the use of codons which make up the

coding frames (Bibb et al. 1984). The method of Staden and McLachlan (1982) makes it possible to calculate the probability of coding frames in terms of the codon usage of *Streptomyces* genes which have already been sequenced and which are assembled in a data file which contains 19673 codons and which was obtained using the BISANCE (Dessen et al. 1990) computer server.

Using this method, it was possible to characterize four highly probable open reading frames within the 2.9 kb PstI-XhoI fragment, which reading frames are depicted in the table below (TABLE I). They are designated frames 1 to 4 according to their position starting from the PstI site. The length of each reading frame in bases, has been indicated, as has its position within the fragment (the PstI site being situated at position 1); the number of amino acids in the encoded polypeptide has also been indicated for open reading frames 2 and 3. Frames 1, 3 and 4 are encoded by the same strand, while frame 2 is encoded by the complementary strand (Figure 4). Frames 1 and 4 correspond, respectively, to the C-terminal region of the PapA protein and to the N-terminal region of the PapM protein, which proteins were previously identified and described in Patent PCT/FR93/00923.

Frame number and/or gene name	Position	Number of nucleotides	Number of amino acids
1 (PapA)	1-684	684	-
5 2 (PapC) (inv)	949-1836	888	296
3 (PapB)	1873-2259	387	129
4 (PapM)	2259-2887	629	-

TABLE I

Comparison of the product of frame 2 (TABLE I) with the protein sequences contained in the Genpro library shows a 27% homology with the region involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of *E. coli* (Hudson and Davidson, 1984) and of *Erwinia herbicola* (EMBL data library, 1991). This region of TyrA catalyses aromatization of prephenate to form 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, proceeding from 4-deoxy-4-aminoprephenate and leading to 4-aminophenylpyruvate is very probably involved in the synthesis of DMPAPA. This reaction will be catalysed by the product of frame 2, termed PapC (SEQ ID No. 2).

Comparison of the product of frame 3 (TABLE I) with the protein sequences contained in the

Genpro library shows a 24 to 30% homology with the region involved in the chorismate mutase activity of the bifunctional TyrA and PheA proteins of E. coli (Hudson and Davidson, 1984) and of the TyrA protein of Erwinia herbicola. This region catalyses isomerization of chorismate to form prephenate in the biosynthesis of tyrosine and phenylalanine. A similar isomerization, proceeding from 4-deoxy-4-amino chorismate and leading to 4-deoxy-4-aminoprephenate, is very probably involved in the synthesis of DMPAPA. This reaction would be catalysed by the product of frame 3, termed PapB (SEQ ID No. 3).

In the case of TyrA and PheA, the chorismate mutase and prephenate dehydratase, or prephenate dehydrogenase, activities are catalysed by the same protein. In S. pristinaespiralis, the chorismate mutase and prephenate dehydrogenase enzyme activities are catalysed by two separate proteins, i.e. PapB and PapC, respectively.

The sequence homologies demonstrated for the PapB and PapC proteins demonstrate that these two proteins are involved, jointly with the PapA and PapM proteins, in the biosynthesis of the aromatic derivative DMPAPA. In the same way as for papA, disruption of the papB and papC genes should lead to the construction of S. pristinaespiralis strains which are incapable of producing PI but which are able, in the presence of novel precursors, to produce new PIs

which are modified at the level of the DMPAPA residue.

1-2. Sequencing and analysis of the region upstream of the papA gene

This region is contained between the snbA gene, which encodes 3-hydroxypicolinic acid AMP ligase and which is described in Patent PCT/FR93/00923, and the papA gene.

The clonings were carried out as described in Example 1-1, proceeding from plasmid pVRC900 and cosmid pIBV2, which are described in Patent PCT/FR93/00923.

The 1.3 kb XhoI-XhoI, 0.2 kb XhoI-XhoI, 3.3 kb XhoI-XhoI, 1.1 kb HindIII-PstI and 2.2 kb PstI-PstI fragments were subcloned into the M13mp18 and M13mp19 vectors. These different clonings made it possible to pass through all the cloning sites. The different inserts were sequenced as described in 1-1 using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence in the insert to be sequenced.

The overlap between these different inserts enabled the entire nucleotide sequence which is present between the snbA and papA genes (SEQ ID No. 4) to be established.

On the basis of this nucleotide sequence, it is possible to determine the open reading frames and to identify genes which are involved, in S. pristinaespiralis, in the biosynthesis of precursors of PI, as well as the polypeptides encoded by these

genes.

We have looked for the presence of open reading frames within the 4.5 kb XhoI-PstI fragment, which contains the nucleotide sequence between the snbA and papA genes, as described in Example 1.1. Using this method, it was possible to characterize four highly probable open reading frames within the 4.5 kb XhoI-PstI fragment, which frames are depicted in the table below (TABLE II). They are designated frames 1 to 4 in accordance with their position starting from the XhoI site. Their length in bases, and their position within the fragment (the XhoI site being situated at position I) has been indicated for each fragment; the number of amino acids within the encoded polypeptide has also been indicated for open reading frames 2 and 3. Frames 2, 3 and 4 are encoded by the same strand, and frame 1 is encoded by the complementary strand (Figure 5). Frames 1 and 4 correspond, respectively, to the N-terminal regions of the SnbA and PapA proteins, which were previously identified and described in patent PCT/FR93/00923.

	Frame number and/or gene name	Position	Number of nucleotides	Number of amino acids
	1 (SnbA) (inv)	1-329	329	-
5	2 (PipA)	607-1671	1065	355
	3 (SnbF)	1800-2993	1194	398
	4 (PapA)	3018-4496	1479	-

TABLE II

Comparison of the product of frame 2

- 10 (TABLE II) with the protein sequences contained in the Genpro library shows a 30% homology with ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final step in the catabolism of octopine; it converts
- 15 L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of the incorporation of labelled lysine, that 4-oxopipicolinic acid and 3-hydroxypicolinic acid, which are found both in PI₁ and in virginiamycin S1, derived
- 20 from lysine (Molinero et al., 1989; Reed et al., 1989). A reaction in which lysine was cyclodeaminated, similar to that described for ornithine, would lead to the formation of pipecolic acid. Taking this hypothesis into account, the product of frame 2 was termed PipA

(SEQ ID No. 5). The results of mutating the pipA gene, presented in 2-1, demonstrate that the pipA gene is involved solely in the synthesis of pipecolic acid, since this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.

Comparison of the product of frame 3 (TABLE II) with the protein sequences contained in the Genpro library shows a 30 to 40% homology with several hydroxylases of the cytochrome P450 type, which hydroxylases are involved in the biosynthesis of secondary metabolites (Omer *et al.*, 1990, Trower *et al.*, 1992). Several hydroxylations can be envisaged in the biosynthesis of precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The results of mutating the pipA gene, presented in 2-1-3, demonstrate that the product of frame 3 is involved in hydroxylation of the pipecolic acid residue of PI₂. The corresponding gene has therefore been termed snbF, and the corresponding protein SnbF (SEQ ID No. 6).

1-3. Sequencing the region downstream of the snbA gene.

This region is included between the snbA gene, which encodes 3-hydroxypicolinic acid adenylate

ligase, and the snbR gene, which encodes a membrane protein which is probably responsible for transport and for resistance to PI, with both genes having been described in Patent PCT/FR93/00923. Sequencing of this region was carried out using a fragment which was isolated from cosmid pIBV2, as described in Example 1-1.

The 1.6 kb HindIII-BglII fragment was subcloned into the M13mp18 and M13mp19 vectors, proceeding from cosmid pIBV2. The insert was sequenced as described in 1-1, using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. On the basis of the nucleotide sequence thus obtained (SEQ ID No. 7), it is possible to determine the open reading frames and to identify, in S. pristinaespiralis, genes which are involved in the biosynthesis of the precursors of PI, as well as the polypeptides encoded by these genes. We looked for the presence of open reading frames within the 1.6 kb HindIII-BglII fragment, which corresponds to the end of the snbA gene and its downstream region, as described in Example 1-1. A complete open coding frame, encoded by the same strand as the snbA gene (Figure 6), was detected. Relative to position 1, corresponding to the HindIII site, this frame starts at nucleotide 249, i.e. 30 nucleotides after the end of the snbA gene, and terminates at nucleotide 1481. It is 1233 nucleotides

in size, corresponding to a protein of 411 amino acids.

Comparison of the product of this open frame with the protein sequences contained in the Genpro library shows a 30 to 40% homology with a group of
5 proteins which are probably involved (Thorson et al., 1993) in the transamination of intermediates in the biosynthesis of various antibiotics (DnrJ, EryC1, TylB, StrS and PrgL). Synthesis of the 3-HPA precursor, which appears to derive from lysine by a route other than
10 cyclodeamination (see Examples 1-2 and 2-1), could necessitate a transamination step which can be catalysed by the product of this frame 3, termed HpaA (SEQ ID No. 8). The results of mutating this gene, presented in 2-2, demonstrate unequivocally that this
15 gene is involved in synthesis of the 3-HPA precursor and confirm our hypothesis.

The genes papB, papC, pipA, snbF and hpaA, which are described in the present invention, are grouped together with the snbA, papA and papM genes on
20 a chromosomal region of approximately 10 kb (Figure 7). This confirms the presence of a cluster of genes which are involved in the biosynthesis of PI and its precursors. Studying regions upstream and downstream of this cluster should enable the other genes involved in
25 the biosynthesis of PI precursors, in particular L-phenylglycine and L-2-aminobutyric acid, to be identified.

EXAMPLE 2: Construction of recombinant strains by means of disrupting identified genes.

This example illustrates how it is possible to demonstrate involvement of the genes described in Example 1 in the biosynthesis of pristinamycin precursors, and also to construct S. pristinaespiralis strains which are able to produce novel pristinamycins. These strains are obtained by disrupting the genes which are involved in the biosynthesis of the residue which it is desired to replace, and the novel pristinamycins are produced by supplementing these mutants with novel precursors.

Strain SP92::pVRCC508, which is employed in the present invention to produce novel derivatives of PI by replacing the precursor DMPAPA with other molecules, is described in Patent PCT/FR93/0923. It is obtained by disrupting, by means of simple crossing over, the papA gene, which is involved in the biosynthesis of the precursor of DMPAPA and is thought to participate in an early step relating to the transamination of chorismate. This disruption has a polar character since, in this mutant, expression of the papM gene (PCT/FR93/0923), which is situated 1.5 kb downstream of the papA gene and is involved in the double methylation of 4-amino-L-phenylalanine to form DMPAPA, is very reduced. Thus, assaying the activity of the SAM-dependant methylation enzyme for converting 4-amino-L-phenylalanine (PAPA) into DMPAPA indicates that

mutant SP92::pVRC508 has an activity which is less than 5% of the activity of the wild-type strain.

In the present invention, this strain, SP92::pVRC508, can be used, under appropriate
5 fermentation conditions and supplementation conditions, to produce novel pristinamycins which are modified at the level of the DMPAPA residue, as will be explained in Example 3. Mutants having the same phenotype can be obtained by disrupting the papB or papC genes described
10 in the present invention.

Another type of S. pristinaespiralis strain, whose papA gene is disrupted and which possesses the same phenotype as strain SP92::pVRC508, was obtained in a similar manner by disrupting the papA gene by means
15 of double crossing over. This construction was carried out starting with a 4.6 kb SphI-HindIII fragment, which fragment was isolated from cosmid pIBV2 and contains the 3' region of the pipA gene, the entire snbF and papA genes and the 3' part of the papC gene. This
20 fragment was cloned into the suicide vector pDH5, which vector is only able to replicate in *E. coli* but carries a resistance marker which is expressed in *Streptomyces* (the gene for resistance to thiostrepton or to nohiheptide, tsr). This vector, pDH5, was developed by
25 Wohleben et al (1991 Nucleic Acid Res. 19, 727-731). A BclI-BclI deletion of 1.1 kb was then made in the papA gene, and a 2.2 kb HindIII-HindIII fragment, carrying the amR gene (resistance to geneticin and to

apramycin), was introduced after the cohesive ends had been filled in. The recombinant vector was termed pVRC414 and is depicted in Figure 12. After transforming the pristinamycin-producing strain with plasmid pVRC414, transformants which were resistant to geneticin and sensitive to thiostrepton were isolated and analysed. These clones are the result of a double homologous recombination between the *S. pristinaespiralis* DNA regions of plasmid pVRC414 and the corresponding chromosomal region of *S. pristinaespiralis*, as described in Figure 13. One of these clones was termed SP212. Its phenotype is identical to that of strain SP92::pVRC508 as regards the absence of any production of PI and the ability of the strain to produce new antibiotics in the presence of novel precursors. Advantageously, this type of strain, which is obtained by double crossing over, is more stable than the strains which are obtained by simple crossing over.

2-1. Construction of a mutant of *S. pristinaespiralis* SP92 whose *pipA* gene is disrupted.

This example illustrates how it is possible, by means of disrupting the *pipA* gene, to construct a strain of *S. pristinaespiralis* SP92 which no longer produces PI under standard fermentation conditions and which is able to produce new pristinamycins, which are modified at the level of the 4-oxopipicolinic acid residue of PIA, when novel precursors are added to the

fermentation.

It was constructed using a suicide vector, the vector pUC1318, which only replicates in E. coli. This vector does not carry any resistance marker which
5 is expressed in Streptomyces. Its presence in the genome of Streptomyces can only be detected by colony hybridization.

2-1-1. Construction of plasmid pVRC420:

This example illustrates how it is possible
10 to construct a plasmid which does not replicate in S. pristinaespiralis SP92 and which can be employed to disrupt the pipA gene by means of double homologous recombination.

Plasmid pVRC420 was constructed in order to
15 produce the chromosomal mutant of SP92 in which the pipA gene is disrupted, proceeding from cosmid pIBV2, which is described in Patent PCT/FR93/0923. Cosmid pIBV2 was cut with the restriction enzyme PstI and, after the fragments, thus generated, had been separated
20 by electrophoresis on a 0.8% agarose gel, a 2.8 kb PstI-PstI fragment, containing the start of the snbA and snbF genes and the whole of the pipA gene, was isolated and purified using Geneclean (Bio101, La Jolla, California). 50 ng of vector pUC1318, which had
25 been linearized by digesting with PstI, were ligated to 200 ng of the 2.8 kb fragment, as described in Example 1. A clone carrying the desired fragment was isolated following transformation of the strain TG1 and

selection on LB + 150 µg/ml ampicillin + X-gal + IPTG medium. The recombinant plasmid was termed pVRC415 (Figure 8). A cassette containing the am^R gene, encoding resistance to apramycin or to geneticin (Kuhstoss et al., 1991), was then introduced into the unique HindIII site of plasmid pVRC415, this site being situated 530 bp downstream of the start of the pipA gene. This construction was effected as follows. A 2.5 kb DNA fragment, containing the am^R gene, the PerME promoter (Bibb et al., 1985) and the first 158 amino acids of the gene for resistance to erythromycin, ermE, was isolated by means of a SalI-BglIII double digestion of a plasmid which was derived from plasmids pIU4026 (plasmid carrying the ermE gene under the control of the PerME promoter) and pHP45Ωam^R. After filling in the SalI and BglIII protruding 5' cohesive ends using Klenow enzyme in accordance with the protocol described by Maniatis et al., 1989, the fragment containing the am^R gene was cloned into the HindIII site of plasmid pVRC415, whose protruding 5' cohesive ends had also been filled in with Klenow enzyme as previously described. The recombinant plasmid thus obtained was designated pVRC420. Its restriction map is depicted in Figure 9.

2-1-2. Isolation of mutant SP92pipA::Ωam^R, whose pipA gene is disrupted by homologous recombination.

This example illustrates how the mutant of

S. pristinaespiralis SP92 whose pipA gene is disrupted was constructed.

This mutant was isolated by transforming strain SP92 with the suicide plasmid pVRC420.

5 The preparation of protoplasts, their transformation and extraction of the total DNA from the recombinant strains were all effected as described by Hopwood et al. (1985).

10 The strain SP92 was cultured, at 30°C for 40 hours, in YEME medium (Hopwood et al., 1985), 34% sucrose, 5 mM MgCl₂, and 0.25% glycine. The mycelium was protoplasted in the presence of lysozyme, and 5 × 1 µg of pVRC420 were used to transform (by the method employing PEG) the protoplasts. After one night in
15 which the protoplasts were regenerated on R2YE medium (D. Hopwood et al. 1985), the recombinants were selected by spreading on 3 ml of SNA medium (D. Hopwood et al. 1985) containing 1,500 µg/ml geneticin.

20 100 clones which were resistant to geneticin were isolated from the 5 transformations that were carried out. These recombinants arise from integration, by means of simple or double homologous recombination between the pipA gene which is carried by the chromosome of strain SP92 and the parts of the pipA
25 gene which are contained in the 5.3 kb fragment carried by the suicide plasmid pVRC420. In order to select the recombinants which were obtained by double crossing over (that is which did not contain the pUC1318 part of

plasmid pVRC420 in their genome), colony hybridizations were carried out on 90 clones using pUC19 labelled with [α - ^{32} P]dCTP as the probe, as described in Maniatis et al (1989). 10 clones were selected which were resistant to

5 geneticin but which did not hybridize the vector pUC19. The spores of the recombinants were isolated by streaking and growing on HT7 medium containing 10 μ g/ml geneticin, and restreaked on the same medium in order to obtain isolated colonies. In order to verify the

10 position at which plasmid pVRC420 was integrated, various Southernblots of the total DNA from several recombinant clones, purified as described by Hopwood et al. 1985, were carried out, with hybridization to the 2.8 kb PstI-PstI fragment, which was used as a probe

15 after having been labelled with [α - ^{32} P]dCTP. The results confirm that these recombinants were obtained by double crossing over between vector pVRC420 and the chromosome of strain SP92, resulting in replacement of the 2.8 kb PstI-PstI fragment, containing the pipA gene, by a

20 5.3 kb PstI-PstI fragment containing the pipA gene which is disrupted by introduction of the am^R gene. One of these mutants was designated SP92pipA::am^R.

2-1-3. Production of pristinamycins using mutant SP92pipA::am^R.

25 This example illustrates how it is established that the mutant of S. pristinaespiralis SP92 whose pipA gene is disrupted by integration of plasmid pVRC420 on the one hand no longer produces PI

under standard fermentation conditions and on the other hand exhibits a high level of production of a minor form of the B components of streptogramins in which 4-oxopipicolinic acid is replaced by pipicolinic acid.

5 Mutant SP92Qam^R, as well as strain SP92

in the role of a control strain, were cultured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the abovementioned strain are added, under sterile

10 conditions, to 40 ml of inoculum medium in a 300 ml baffled Erlenmeyer flask. The inoculum medium is made up of 10 g/l corn steep, 15 g/l sucrose, 10 g/l (NH₄)₂SO₄, 1 g/l K₂HPO₄, 3 g/l NaCl, 0.2 g/l MgSO₄·7H₂O and 1.25 g/l CaCO₃. The pH is adjusted to 6.9 using

15 sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 fold, are added under sterile conditions to 30 ml of

20 production medium in a 300 ml Erlenmeyer flask. The production medium is made up of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before

25 introducing the calcium carbonate. The Erlenmeyer flasks are shaken for 24, 28 and 32 hours at 27°C. At each time point, 10 g of must are weighed into a smooth Erlenmeyer flask to which 20 ml of mobile phase,

consisting of 34% of acetonitrile and 66% of a solution of 0.1 M KH_2PO_4 (adjusted to pH 2.9 with concentrated H_3PO_4) are added for extracting the pristinamycins. After shaking, the whole is centrifuged and the

5 pristinamycins contained in the supernatant are assayed by HPLC by means of injecting 150 μl of the centrifugation supernatant onto a Nucleosil 5-C8 column of 4.6 x 150 mm, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9.

10 The I pristinamycins are detected by means of their UV absorbance at 206 nm.

The results demonstrated that, under the fermentation conditions employed, mutant SP92pipA:: Ωam^R did not produce PI at 24, 28 or 32 hrs of fermentation,

15 while control strain SP92 produced a quantity of PI which was standard for the 3 times which were tested. The quantity of PII which was produced remained the same for the two strains. Mutant SP92pipA:: Ωam^R is definitely blocked at a step in the biosynthesis of PI.

20 Fermentation complementation tests were carried out by adding different precursors of PI, separately or together, to the culture in production medium after 16 hours. The results of these complementations demonstrated that when 100 mg/l pipecolic acid and

25 100 mg/l DMPAPA are added simultaneously to the fermentation medium, the mutant produces what is normally a minor derivative of PI, i.e. PI_g (which is produced by SP92 in a quantity which is less than 5%)

at a level which is equivalent to the production of PI_A by the control strain. This production does not take place if the pipecolic acid and the DMPAPA are added separately. PI_x differs from PI_A (major component of PI) in the absence of the keto function in the 4 position on the pipecolic acid. The fact that mutant SP92Qam^R can only be complemented by adding pipecolic acid and DMPAPA simultaneously indicates that the papA, and probably the papB and papM genes were disrupted by a polar effect of the construct. Thus, all these genes are situated downstream of pipA and are probably cotranscripts together with pipA. Disruption of the latter therefore leads to disruption of the pap genes and, consequently, absence of DMPAPA synthesis.

The fact that complementation of mutant SP92Qam^R with pipecolic acid results in the production of PI_x and not PI_A leads to two conclusions: the first is that construction of the PI cycle is achieved by incorporating pipecolic acid and not 4-oxopipecolic acid and that a hydroxylation generating the keto function in the 4 position then takes place subsequently. The second is that this hydroxylation is probably carried out by the enzyme SnbF whose structural gene is situated directly downstream of the pipA gene. Thus, the obvious polarity of the disruption of the pipA gene on the pap genes probably involves a polar effect on the snbF gene, which is situated between pipA and the pap genes, which is manifested in

inhibition of the function of hydroxylation of the
pipecolic acid residue of PI₈ to form 4-hydroxypipecolic
acid, which is found in PI₇ and PI₆ (Figure 2) and then
oxidized to 4-oxopipecolic acid in PI₄.

5 Preparing a mutant of this nature made it
possible to construct a strain of S. pristinaespiralis
which is unable to produce PI except in the presence of
the PI precursors DMPAPA and pipecolic acid, using
which it is able to produce, in a quantity equivalent
10 to that of the starting strain, what is normally a
minor derivative of PI within the pristinamycin
mixture. Similarly, in the presence of novel
precursors, or of a mixture of novel precursors and of
precursors which are normally present in PI, this
15 strain will be able to produce new pristinamycins which
are modified in either DMPAPA or 4-oxopipecolic acid or
in both these residues.

2-2. Construction of a mutant of
S. pristinaespiralis SP92 whose hpaA gene is disrupted.

20 This example illustrates how it is possible,
by means of disrupting the hpaA gene, to construct a
strain of S. pristinaespiralis SP92 which no longer
produces PI under standard fermentation conditions and
which is able to produce new pristinamycins, which are
25 modified at the level of the 3-HPA precursor, when
novel precursors are added to the fermentation.

This mutant was constructed using a plasmid
which does not replicate in S. pristinaespiralis SP92

and which can be used for disrupting the hpaA gene by means of double homologous recombination.

2-2-1. Construction of the suicide plasmid pVRC421

5 Plasmid pVRC421 was constructed using a suicide vector which, while only being able to replicate in E. coli, carries a resistance marker which is expressed in Streptomyces, i.e. the gene for resistance to thiostrepton or to nosiheptide, tsr. This
10 vector, pDH5, was developed by Hillemann et al. (1991).

Plasmid pVRC421 was constructed in order to produce the chromosomal mutant of SP92 whose hpaA gene is disrupted, making use of cosmid pIBV2, which is described in Patent PCT/FR93/0923. pIBV2 was digested
15 with the restriction enzyme SphI and, after having separated the fragments, thus generated, by means of electrophoresis on a 0.6% agarose gel, a 4.8 kb SphI-SphI fragment, containing the whole of the hpaA gene and virtually the whole of the snbA gene, was
20 isolated and purified using Geneclean as described above. 50 ng of the vector pDH5, linearized by digesting with SphI, were ligated to 200 ng of the 4.8 kb fragment, as subsequently described. A clone harbouring the desired fragment was isolated after
25 transforming the strain TG1 and selecting on LB + 150 µg/ml ampicillin + IPTG + X-gal medium. The recombinant plasmid was designated pVRC411 (Figure 10). A cassette containing the gene am^R, encoding resistance

to apramycin or to geneticin, was then introduced into the unique PflmI site of plasmid pVRC411, this site being situated 610 bp downstream of the start of the hpaA gene. This construct was produced as follows. A
 5 2.2 kb DNA fragment, containing the am^R gene, was isolated following digestion of the plasmid pHP45Ωam^R, containing the am^R gene, with HindIII. After filling in the HindIII protruding 5' cohesive ends using Klenow enzyme according to the protocol described by Maniatis
 10 et al. 1989, the fragment containing the am^R gene was cloned into the PflmI site of plasmid pVRC411, whose protruding 3' cohesive ends had been rendered blunt using the enzyme T4 polymerase as described in Maniatis et al. 1989. The recombinant plasmid thus obtained was
 15 termed pVRC421. Its restriction map is depicted in Figure 11.

2-2-2. Isolation of mutant SP92hpaA::Ωam^R, whose hpaA gene is disrupted by means of homologous recombination.

20 This example illustrates how the mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted was constructed.

This mutant was isolated by transforming strain SP92 with the suicide plasmid pVRC421.

25 The protoplasts were prepared and transformed as described previously.

Strain SP92 was cultured, at 30°C for 40 hours, in YEME medium, 34% sucrose, 5 mM MgCl₂, 0.25%

glycine. The mycelium was protoplasted in the presence of lysozyme, and $5 \times 1 \mu\text{g}$ of pVRC421 were employed for transforming (by the method using PEG) the protoplasts. After one night for regenerating the protoplasts on
5 R2YE medium, the recombinants were selected by spreading on 3 ml of SNA medium containing $1,500 \mu\text{g/ml}$ geneticin.

600 clones which were resistant to geneticin were isolated from the 5 transformations which were
10 carried out. These recombinants result from integration by means of simple or double homologous recombination between the hpaA gene carried by the chromosome of strain SP92 and the 6 kb fragment of the suicide plasmid pVRC421. In order to select the recombinants
15 obtained by double crossing over (that is, the clones which no longer contain, in their genome, the pDH5 moiety of plasmid pVRC421), the clones were subcultured on HT7 medium containing $400 \mu\text{g/ml}$ thiostrepton.
6 clones which were resistant to geneticin but
20 sensitive to thiostrepton were selected. The spores of the recombinants were selected by streaking and growth on HT7 medium containing $10 \mu\text{g/ml}$ geneticin, and restreaked on the same medium in order to obtain isolated colonies. In order to verify the position of
25 integration of plasmid pVRC421, various Southern blots of the total DNA from the 6 recombinant clones, purified as described by Hopwood et al. 1985, were carried out with hybridization to the 4.8 kb SphI-SphI fragment,

which was used as the probe after having been labelled with [α - ^{32} P]dCTP. The results confirm that these recombinants were obtained by double crossing over between the vector pVRC421 and the chromosome of the SP92 strain, resulting in replacement of the 4.8 kb SphI-SphI fragment, containing the hpaA gene, by a 6 kb SphI-SphI fragment which contains the hpaA gene disrupted by the am^R gene. One of these mutants was designated SP92hpaA:: Ω am^R.

10 2-2-3. Production of pristinamycins by mutant SP92hpaA:: Ω am^R.

 This example illustrates how it is established that the mutant of S. pristinaespiralis SP92 whose hpaA gene is disrupted by integration of
15 plasmid pVRC421 no longer produces PI under the standard fermentation conditions.

 Mutant SP92hpaA:: Ω am^R, and also strain SP92 in the role of control strain, were cultured in liquid production medium. The fermentation was carried out as
20 described in Example 2-1-3, and the pristinamycins were then extracted and assayed as previously described. The results demonstrated that, under the fermentation conditions employed, mutant SP92hpaA:: Ω am^R did not produce PI, either at 24, 28 or 32 hrs of fermentation,
25 whereas the control strain produced a quantity of PI which was standard for the 3 time points tested. The quantity of PII produced remained the same for the two strains. Mutant SP92hpaA:: Ω am^R is definitely blocked at

a step in the biosynthesis of PI. Complementary fermentation tests were carried out by adding different precursors of PI, separately or together, to the culture in production medium after 16 hours. When
5 100 mg/l 3-hydroxypicolinic acid are added to the fermentation medium, the mutant then produces PI_A at a level which is equivalent to the production of PI by the control strain. The fact that mutant SP92~~hpaA~~::Qam^R can only be complemented by adding 3-hydroxypicolinic
10 acid demonstrates that the hpaA gene is involved in the synthesis of this precursor.

Construction of this mutant made it possible to produce a strain of S. pristinaespiralis which is mutated as regards its production of PI but which, in
15 the presence of the precursor 3-HPA, is capable of producing PI in a quantity equivalent to that produced by the starting strain. In the same way as in the preceding examples, it can be envisaged that it should be possible, using a mutant of this nature in the
20 presence of novel precursors, to produce new pristinamycins which are modified at the level of the 3-hydroxypicolinic acid residue.

EXAMPLE 3: Production of compounds of the general formula I by the mutant SP92::pVRC508.

25 This example illustrates how the mutant of S. pristinaespiralis SP92 whose papA gene is disrupted by integration of plasmid pVRC508 is able to synthesize new streptogramins in the presence of precursors which

are added to the production medium. These precursors can be derivatives of amino acids and, more particularly, of phenylalanine, but also of α -ketocarboxylic acids and, more particularly, of phenylpyruvic acid.

The mutant SP92::pVRC508 was cultured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the previously mentioned strain is added, under sterile conditions, to 40 ml of inoculum medium in a 300 ml baffled Erlenmeyer flask. The inoculum medium is made up of 10 g/l corn steep, 15 g/l sucrose, 10 g/l $(\text{NH}_4)_2\text{SO}_4$, 1 g/l K_2HPO_4 , 3 g/l NaCl, 0.2 g/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.25 g/l CaCO_3 . The pH is adjusted to 6.9 with sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 h old, are added, under sterile conditions, to 30 ml of production medium in a 300 ml Erlenmeyer flask. The production medium consists of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C on a rotating shaker at a speed of 325 rpm. After 16 h, 1 ml of a solution of one of the precursors listed in Table 3 (generally 5 or

10 g/l) is added to the culture. The latter is terminated 8 or 24 h later. The volume of the must is measured immediately, and 2 volumes of mobile phase, consisting of 34% acetonitrile and 66% of a solution of 5 0.1 M KH_2PO_4 (adjusted to pH 2.9 with concentrated H_3PO_4) are added to it for extracting the pristinamycins. After shaking, the whole is centrifuged and the pristinamycins contained in the supernatant are extracted and purified as described in Example 4. They 10 are also assayed by HPLC by means of injecting 150 μl of the centrifugation supernatant onto a Nucleosil 5-C8 4.6 $\times$ 150 mm column, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9. The new I pristinamycins are detected by means of 15 their UV absorbance at 206 nm and, where appropriate, by means of their fluorescence emission (370 nm filter, excitation at 306 nm).

	PRECURSOR	ORIGIN
	phenylalanine	Janssen
	4-dimethylaminophenylalanine	Example 33
	4-methylaminophenylalanine	Example 34-1
5	4-aminophenylalanine	Janssen 22.794.96
	4-diethylaminophenylalanine	Example 33
	4-ethylaminophenylalanine	Example 33
	4-methylthiophenylalanine	Example 33
	4-methylphenylalanine	J.P.S101-312-4/ Example 33
10	4-methoxyphenylalanine	Janssen 16.975.97
	4-trifluoromethoxyphenylalanine	Example 34-8
	4-methoxycarbonylphenylalanine	Example 33
	4-chlorophenylalanine	Janssen 15.728.14
	4-bromophenylalanine	Janssen 22.779.81
15	4-iodophenylalanine	Bachem F 1675
	4-trifluoromethylphenylalanine	P.C.R. Inc. 12 445-3
	4-tert-butylphenylalanine	Example 35-1

5	4-isopropylphenylalanine	Example 36-1
	3-methylaminophenylalanine	Example 35-3
	3-methoxyphenylalanine	J.P.S. 101-313-2
	3-methylthiophenylalanine	Example 34-11
	3-fluoro-4-methylphenylalanine	Example 34-5
10	4-tert-butylphenylpyruvic acid	Example 33
	4-methylaminophenylpyruvic acid	Example 34-4
	2-naphthylphenylalanine	Bachem F 1865
	4-fluorophenylalanine	Bachem F 1535
	PRECURSOR	ORIGIN
15	3-fluorophenylalanine	Bachem F 2135
	3-ethoxyphenylalanine	Example 37-1
	2,4-dimethylphenylalanine	Example 33
	3,4-dimethylphenylalanine	Example 33
	3-methylphenylalanine	Example 33
20	4-phenylphenylalanine	Example 33
	4-butylphenylalanine	Example 36-3
	2-thienyl-3-alanine	Aldrich 28.728.8
	3-trifluoromethylphenylalanine	Example 33
	3-hydroxyphenylalanine	Aldrich T 9.039.5
	3-ethylaminophenylalanine	Example 35-6

	4-aminomethylphenylalanine	Example 33
	4-allylaminophenylalanine	Example 38-2
	4-diallylaminophenylalanine	Example 38-1
	4-allylethylaminophenylalanine	Example 39-4
5	4-ethylpropylaminophenylalanine	Example 39-6
	4-ethylisopropylaminophenylalanine	Example 39-1
	4-ethylmethylcyclopropylamino- phenylalanine	Example 39-8
	4-(1-pyrrolidinyl)phenylalanine	Example 40-1
10	4-O-allyltyrosine	Example 33
	4-O-ethyltyrosine	Example 33
	4-ethylthiophenylalanine	Example 33
	4-ethylthiomethylphenylalanine	Example 41-1
	4-O-(2-chloroethyl)tyrosine	Example 42-1
15	4-acetylphenylalanine	Example 33
	4-ethylphenylalanine	Example 33
	3-dimethylaminophenylalanine	Example 35-10

TABLE III

The following table (TABLE IV) indicates the
 20 relative retention times of the new PI which are
 produced, taking PI₁ as the reference. The absolute

retention times were determined at 25°C in the HPLC system described above; they vary slightly from one injection to another and also in accordance with temperature.

5	Precursor	t_r (relative retention time) of the new PI (Neo PI)		
		Neo PI _A	Neo PI _B	Other neo PI
	4-methylaminophenylalanine	0.85		
	4-aminophenylalanine	0.64		
	4-methylthiophenylalanine	1.93	2.73	1.63
	4-methylphenylalanine	1.77	2.65	
10	4-methoxyphenylalanine	1.46		
	4-methoxycarbonylphenylalanine	1.49		
	4-chlorophenylalanine	2.04		
	4-bromophenylalanine	2.16		
15	4-iodophenylalanine	2.42		
	4-trifluoromethylphenylalanine	2.56	3.74	
	4-tert-butylphenylalanine	3.34		

	4-isopropylphenylalanine	2.80		4.35
	3-methylaminophenylalanine	1.15		
	3-methoxyphenylalanine	1.49	2.04	
5	3-fluoro-4-methylphenylalanine	2.93		
	4-tert-butylphenylpyruvic acid	3.34		
	4-methylaminophenylpyruvic acid	0.85		
10	4-ethylaminophenylalanine	0.94		
	4-diethylaminophenylalanine	0.61		
	4-allylaminophenylalanine	1.83		
	4-diallylaminophenylalanine	2.64		
15	4-allylethylaminophenylalanine	2.4		
	4-ethylpropylaminophenylalanine	1.06		
	4-ethylisopropylamino-phenylalanine	0.89		
20	4-ethylmethylcyclopropylaminophenylalanine	1.1		

5	4-(1-pyrrolidinyl)phenyl- alanine	2.0		
	4-O-trifluoromethyltyrosine	2.42		
	4-O-allyltyrosine	2.62		
	4-O-ethyltyrosine	2.2		
	4-ethylthiophenylalanine	1.96		
10	4-methylthiomethylphenyl- alanine	1.98		
	4-O-(2-chloroethyl)tyrosine	2.45		
	4-acetylphenylalanine	1.61		
	4-ethylphenylalanine	1.86	2.40	
	3-dimethylaminophenyl- alanine	1.49		
15	3-methylthiophenylalanine	1.93		
	3-O-ethyltyrosine	1.78		

TABLE IV

The new PI, with a t_R of 4.35, for
4-isopropylphenylalanine corresponds to a neo PI_R which
is described in Example 14.

20 The new PI, with a t_R of 1.63, for
4-methylthiophenylalanine corresponds to a 5 γ -hydroxy

neo PI_H, which is described in Example 5.

The mutant SP92::pVRC508 was otherwise fermented in the presence of 4-dimethylaminophenylalanine. Under these conditions of
5 complementation, mutant SP92::pVRC508 produces a quantity of I_A pristinamycins which is equivalent to that produced by strain SP92.

EXAMPLE 4: Preparation of pristinamycin I_B
[4 ζ -methylamino-de(4 ζ -dimethylamino)pristinamycin I_A]
10 and of 4 ζ -amino-de(4 ζ -dimethylamino)pristinamycin I_A
4.1: Preparation of pristinamycin I_B [4 ζ -methylamino-de(4 ζ -dimethylamino)pristinamycin I_A]

The strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks as
15 described in Example 3, with 1 ml of a 10 g/l aqueous solution of (R,S)-4-methylaminophenylalanine, synthesized as in Example 34-1, being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted
20 with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then
25 combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is successively eluted

with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing pristinamycin I₈ are combined and evaporated. The dry residue is taken up in 6 ml of a mixture of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10 $\times$ 250 mm column (Macherey Nagel), which is eluted with a mixture of 65% 100 mM phosphate buffer, pH 2.9, and 35% acetonitrile. The fractions containing pristinamycin I₈ are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried on sodium sulphate and then evaporated. 52 mg of pristinamycin I₈ are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.71 (dd, J=16 and 6 Hz, 1H, 5 β_2), 0.92 (t, J=7.5 Hz, 3H: CH₃ 2 γ), from 1.10 to 1.40 (mt, 2H: 3 β_2 and 3 γ_2), 1.34 (d, J=7.5 Hz, 3H: CH₃ 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH₂ 2 β), 2.03 (mt, 1H, 3 β_1), 2.22 (mt, 1H, 5 δ_2), 2.33 (broad d, J=16 Hz, 1H: 5 δ_1), 2.40 (d, J=16 Hz, 1H: 5 β_1), 2.82 (mt, 1H: 5 ϵ_2), 2.81 (s, 3H: 4 NCH₃ in the para position of the phenyl), 2.90 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.29 (s, 3H: 4 NCH₃) from 3.20 to 3.45 and 3.60 (2 mts, 1H each: CH₂ 3 δ), 3.40 (t, J=12 Hz, 1H: 4 β_1), 4.57 (dd, J=7 and 8 Hz, 1H, 3 α), 4.75 (broad dd, J=13 and 7 Hz, 1H: 5 ϵ_1), 4.83 (mt, 1H: 2 α), 4.89 (broad d, J=10 Hz, 1H: 1 α), 5.24 (dd, J=12 and 4 Hz, 1H: 4 α), 5.32 (broad d, J=6 Hz, 1H: 5 α), 5.89 (d, J=9 Hz, 1H: 6 α), 5.90 (broad q, J = 7.5 Hz, 1H: 1 β), 6.53 (d, J=9 Hz, 1H: NH 2), 6.53

(d, J=8 Hz, 2H: 4e), 7.03 (d, J=8 Hz, 2H: 4δ), from
7.10 to 7.35 (mt, 5H: aromatic H 6), 7.46 (mt, 2H: 1'H₅
and 1'H₄), 7.85 (dd, J=5.5 and 2 Hz, 1H: 1'H₆), 8.44
(d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9 Hz, 1H: NH 6),
5 11.63 (s, 1H: OH).

4.2: Preparation of 4'-amino-de(4'-dimethylamino)pristinamycin I_a

Strain SP92::pVRC508 is cultured in production medium, using 60 Erlenmeyer flasks as described in Example 3, with 1 ml of a 5 g/l aqueous solution of (S)-4-aminophenylalanine being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9 and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_a are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 65% 100 mM phosphate buffer, pH 2.9, and 35% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is

washed with water, dried over sodium sulphate and then evaporated. 5 mg of 4 ζ -amino-de(4 ζ -dimethylamino)pristinamycin I_a are obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm,
 5 ref. TMS): 0.72 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.90
 (t, J=7.5 Hz, 3H: CH₃ 2 γ), from 1.10 to 1.40 (mt, 2H:
 3 β_2 and 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH₃ 1 γ), from
 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH₂ 2 β), 2.02 (mt, 1H,
 3 β_1), 2.19 (mt, 1H, 5 δ_2), 2.33 (broad d, J=16 Hz, 1H:
 10 5 δ_1), 2.42 (d, J=16 Hz, 1H: 5 β_1), 2.81 (dt, J=13 and
 4 Hz, 1H: 5 ϵ_2), 2.90 (dd, J=12 and 4 Hz, 1H: 4 β_2),
 3.24 (s, 3H: NCH₃ 4), from 3.20 to 3.40 and 3.54 (2 mts,
 1H each: CH₂ 3 δ), 3.30 (t, J=12 Hz, 1H: 4 β_1), 3.72
 (unres.comp., 2H: ArNH₂), 4.54 (dd, J=7.5 and 7 Hz, 1H,
 15 3 α), 4.73 (broad dd, J=13 and 8 Hz, 1H: 5 ϵ_1), 4.82
 (mt, 1H: 2 α), 4.89 (broad d, J=10 Hz, 1H: 1 α), 5.22
 (dd, J=12 and 4 Hz, 1H: 4 α), 5.32 (broad d, J=5.5 Hz,
 1H: 5 α), 5.89 (mt, 2H: 6 α and 1 β), 6.51 (d, J=9.5 Hz,
 1H: NH 2) 6.61 (d J=8 Hz, 2H: 4 ϵ), 6.98 (d, J=8 Hz, 2H:
 20 4 δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.45
 (dd, J=8.5 and 1.5 Hz, 1H: 1' H_4), 7.48 (dd, J=8.5 and
 4 Hz, 1H: 1' H_5), 7.82 (dd, J=4 and 1.5 Hz, 1H: 1' H_6),
 8.43 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H:
 NH 6), 11.63 (s, 1H: OH).

Example 5: Preparation of 4 ζ -methylthio-de(4 ζ -dimethylamino)pristinamycin I_A, of 4 ζ -methylthio-de(4 ζ -dimethylamino)pristinamycin I_B and of 5- γ -hydroxy-4 ζ -methylthio-de(4 ζ -dimethylamino)pristinamycin I_B

5 Strain SP92::pVRC508 is cultured in
production medium using 60 Erlenmeyer flasks, as
described in Example 3, with 1 ml of a 10 g/l solution
of (R,S)-4-methylthiophenylalanine, synthesized as in
Example 33, in 0.1N sodium hydroxide solution being
10 added at 16 h. At the end of 40 h of culture, the
1.8 litres of must recovered from the 60 Erlenmeyer
flasks are extracted with 2 volumes of a mixture
consisting of 66% 100 mM phosphate buffer, pH 2.9, and
34% acetonitrile, and then centrifuged. The supernatant
15 is extracted with 2 times 0.5 volumes of
dichloromethane. The chloromethylene phases are washed
with water and then combined, dried over sodium
sulphate and evaporated. The dry extract is taken up in
20 ml of dichloromethane and injected onto a silica
20 (30 g) column which is mounted in dichloromethane and
is eluted successively with plateaus of from 0 to 10%
methanol in dichloromethane. The fractions containing
the new derivative of pristinamycin I_A are combined and
evaporated. 65 mg of dry residue are obtained. This is
25 taken up in 6 ml of a mixture consisting of 60% water
and 40% acetonitrile and injected in two batches onto a
semi-preparative Nucleosil 7 μ C8 10x250 mm column
(Macherey Nagel), which is eluted with a mixture

consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 45 mg of 4'-methylthio-de(4'-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.68 (dd, J=16 and 5.5 Hz, 1H 5 β₂), 0.93 (t, J=7.5 Hz, 3H: CH₃, 2 γ), 1.13 (mt, 1H: 3 β₂), from 1.25 to 1.40 (mt, 1H: 3 γ₂), 1.33 (d, J=7.5 Hz, 3H: CH₃ 1 γ), from 1.55 to 1.85 (mt, 3H: 3 γ₁, and CH₂ 2 β), 2.02 (mt, 1H, 3 β₁), 2.18 (mt, 1H, 5 δ₂), 2.38 (broad d, J=16.5 Hz, 1H: 5 δ₁), 2.46 (s, 3H: SCH₃), 2.48 (d, J=16 Hz, 1H, 5 β₁), 2.85 (dt, J=13.5 and 4 Hz, 1H: 5 ε₂), 3.00 (dd, J=12 and 5 Hz, 1H: 4 β₂), 3.23 (s, 3H: NCH₃, 4), 3.37 (t, J=12 Hz, 1H: 4 β₁), 3.37 and 3.58 (2 mts, 1H each: CH₂ 3 δ), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.77 (broad dd, J=13.5 and 8 Hz, 1H: 5 ε₁), 4.86 (mt, 1H: 2α), 4.89 (dd, J=10 and 1.5 Hz, 1H: 1α), 5.30 (broad d, J=5.5 Hz, 1H: 5 α), 5.32 (dd, J=12 and 5 Hz, 1H: 4 α), 5.90 (d, J=9.5 Hz, 1H: 6 α), 5.92 (dq, J=7.5 and 1.5 Hz, 1H: 1β), 6.55 (d, J=9.5 Hz, 1H: NH 2), 7.13 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.19 (d, J=8 Hz, 2H: 4ε), 7.45 (mt, 2H: 1'H₄ and H₅), 7.76 (t, J=5 Hz, 1'H₆), 8.42 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9.5 Hz, 1H: NH 6), 11.65 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the novel derivative of pristinamycin I_R, 10 mg of 4 ζ -methylthio-de(4 ζ -dimethylamino)pristinamycin I_R are isolated by means of semi-preparative column chromatography as described above but bringing the proportion of acetonitrile in the eluent phase to 50%.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.32 (mt, 1H, 5 β_2), 0.93 (t, J=7.5 Hz, 3H: CH₃ 2 γ), from 1.20 to 1.35 (mt, 2H: 3 β_2 and 3 γ_2), 1.30(d, J=7.5 Hz, 3H: CH₃ 1 γ), from 1.35 to 2.05 (mt, 9H: 3 γ_1 - 3 β_1 - CH₂ 2 β - CH₂ 5 δ - CH₂ 5 γ and 5 β_1), 2.44 (dt, J=13.5 and 1.5 Hz, 1H: 5 ϵ_2), 2.49 (s, 3H: SCH₃), 2.99 (dd, J=12 and 5 Hz, 1H: 4 β_2), 3.09 (dd, J=12.5 and 12 Hz, 1H: 4 β_1), 3.54 and 3.64 (2 mts, 1H each: CH₂ 3 δ), 4.17 (dd, J=7 and 6 Hz, 1H: 3 α), 4.49 (broad d, J=13.5 Hz: 1H: 5 ϵ_1), from 4.70 to 4.80 (mt, 3H: 2 α - 5 α and 4 α), 4.84 (dd, J=10 and 1.5 Hz, 1H: 1 α), 5.51 (d, J=7 Hz, 1H: 6 α), 5.73 (mt, 1H: 1 β), 6.65 (d, J=9.5 Hz, 1H: NH 2), 7.10 (d, J=8 Hz, 2H: 4 δ), 7.22 (d, J=8 Hz, 2H: 4 ϵ), from 7.20 to 7.40 (mt, 7H: aromatic H 6 = 1' H₄ and 1' H₅), 7.87 (d, J=4 Hz, 1H: 1' H₆), 8.55 (unres.comp., 1H: NH 6), 8.55 (d, J=10 Hz, 1H: NH 1), 11.70 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the novel derivative of pristinamycin I, 3 mg of 5 γ -hydroxy-4 ζ -methylthio-de(4 ζ -dimethylamino)pristinamycin I_R are

isolated by carrying out semi-preparative column chromatography as described above and maintaining the proportion of acetonitrile in the eluent phase at 45%.

NMR spectrum: ^1H (400 MHz, CDCl_3 , δ in ppm, ref. TMS): a markedly preponderant isomer is observed: the -OH in the 5 γ position in an axial position. 0.37 (d mt, $J=16$ Hz, 1H, 5 β_2), 0.93 (t, $J=7.5$ Hz, 3H: CH_3 2 γ), from 1.20 to 1.45 (mt, 2H: 3 β_2 and 3 γ_2) 1.31 (d, $J=7.5$ Hz, 3H: CH_3 1 γ), from 1.40 to 1.85 (mt, 5H: 3 γ_1 - CH_2 2 β and CH_2 5 δ), 1.98 (mt, 1H, 3 β_1), 2.17 (d, $J=16$ Hz, 1H: 5 β_1), 2.50 (s, 3H: SCH_3), 2.77 (dt, $J=13.5$ and 2 Hz, 1H: 5 ϵ_2), 2.99 (dd, $J=12$ and 4 Hz, 1H: 4 β_2), 3.11 (t, $J=12$ Hz, 1H: 4 β_1), from 3.45 to 3.70 (mt, 2H: CH_2 3 δ), 3.73 (mt, 1H: 5 γ in an equatorial position), 4.13 (t, $J=7$ Hz, 1H, 3 α), 4.37 (broad d, $J=13.5$ Hz, 1H: 5 ϵ_1), from 4.75 to 4.95 (mt, 3H: 2 α , 4 α and 5 α), 4.89 (dd, $J=10$ and 1 Hz, 1H: 1 α), 5.70 (d, $J=8$ Hz, 1H: 6 α), 5.80 (dq, $J=7.5$ and 1 Hz, 1H: 1 β), 6.37 (d, $J=5$ Hz, 1H: NH 4), 6.71 (d, $J=10$ Hz, 1H: NH 2), 7.10 (d, $J=8$ Hz, 2H: 4 δ), 7.22 (d, $J=8$ Hz, 2H: 4 ϵ), from 7.20 to 7.40 (mt, 5H: aromatic H 6), 7.43 (dd, $J=8.5$ and 1.5 Hz, 1H: 1' H_4), 7.46 (dd, $J=8.5$ and 4 Hz, 1H: 1' H_5), 7.89 (dd, $J=4$ and 1.5 Hz, 1H: 1' H_6), 8.55 (d, $J=10$ Hz, 1H: NH 1), 9.15 (d, $J=8$ Hz, 1H: NH 6), 11.70 (s, 1H: OH).

EXAMPLE 6: Preparation of 4 β -methyl-de(4 β -dimethylamino)pristinamycin I_a and of 4 β -methyl-de(4 β -dimethylamino)pristinamycin I_b

Strain SP92::pVRC508 is cultured in

5 production medium, using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (R,S)-4-methylphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from

10 the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed

15 with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10%

20 methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_a are combined and evaporated. 49 mg of dry residue are obtained. This residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected, in two

25 batches, onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing

the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 44 mg of 4'-methyl-de(4'-

5 dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.52 (dd, J=16 and 6 Hz, 1H, 5 β₂), 0.93 (t, J=7.5 Hz, 3H: CH₃ 2 γ), 1.15 (mt, 1H: 3 β₂), from 1.20 to 1.40 (mt, 1H: 3 γ₂), 1.35 (d, J=7.5 Hz, 3H: CH₃ 1 γ),
 10 from 1.50 to 1.85 (mt, 3H: 3 γ₁ and CH₂ 2 β), 2.04 (mt, 1H, 3 β₁), 2.18 (mt, 1H, 5 δ₂), from 2.25 to 2.45 (mt, 2H: 5 δ₁ and 5 β₁), 2.36 (s, 3H: ArCH₃), 2.83 (dt, J=13 and 4 Hz, 1H: 5 ε₂), 2.99 (dd, J=13 and 4 Hz, 1H: 4 β₂),
 15 3.28 (s, 3H: NCH₃4), 3.31 and 3.59 (2 mts, 1H each: CH₂ 3 δ), 3.40 (t, J=13 Hz, 1H: 4 β₁), 4.59 (t, J=7.5 Hz, 1H, 3 α), 4.74 (broad dd, J=13 and 7 Hz, 1H: 5 ε₁), 4.85 (mt, 1H: 2α), 4.89 (broad d, J=10 Hz, 1H: 1α), from 5.25 to 5.35 (mt, 2H: 5 α and 4 α), from 5.85 to 5.95 (mt, 2H: 6 α and 1β), 6.52 (d, J=9.5 Hz, 1H: NH 2),
 20 7.14 (AB limit, J=9 Hz, 4H: 4δ and 4ε), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.50 (mt, 2H: 1'H₄ and 1'H₅), 7.81 (dd, J=4 and 2Hz, 1H: 1'H₆), 8.41 (d, J=10 Hz, 1H: NH 1), 8.74 (d, J=9 Hz, 1H: NH 6), 11.63 (s, 1H:OH).

25 Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 21 mg of 4'-methyl-de(4'-dimethylamino)pristinamycin I_H (mass spectrometry:

M+H⁺=810) are isolated by carrying out semi-preparative column chromatography as described above.

EXAMPLE 7: Preparation of 4'-methoxy-de(4'-dimethylamino)pristinamycin I₁.

5 Strain SP92::pVRC508 is cultured in
production medium using 12 Erlenmeyer flasks, as
described in Example 3, with 1 ml of a 5 g/l solution
of (RS)-4-methoxyphenylalanine in 0.1 N sodium
hydroxide solution being added at 16 h. At the end of
10 40 h of culture, the 0.35 litres of must recovered from
the 12 Erlenmeyer flasks is extracted with 2 volumes of
a mixture consisting of 66% 100 mM phosphate buffer,
pH 2.9, and 34% acetonitrile, and then centrifuged. The
supernatant is extracted with 2 times 0.5 volumes of
15 dichloromethane. The chloromethylene phases are washed
with water and then combined, dried over sodium
sulphate and evaporated. The dry extract is taken up in
20 ml of dichloromethane and injected onto a silica
(30 g) column which is mounted in dichloromethane and
20 is eluted successively with plateaus of from 0 to 10%
methanol in dichloromethane. The fractions containing
the new derivative of pristinamycin I₁ are combined and
evaporated. 14 mg of dry residue are obtained. This
residue is taken up in 3 ml of a mixture consisting of
25 60% water and 40% acetonitrile and injected onto a
semi-preparative Nucleosil 7 μ C8 10x250 mm column
(Machery Nagel), which is eluted with a mixture
consisting of 60% 100 mM phosphate buffer, pH 2.9, and

40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then
 5 evaporated. 12 mg of 4'-methoxy-de(4'-dimethylamino)pristinamycin I_a are obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, d in ppm, ref. TMS): 0.63 (dd, J=16 and 5.5 Hz, 1H, 5 β₂), 0.96 (t, J=7.5 Hz, 3H: CH₃ 2 γ), 1.17 (mt, 1H: 3 β₂), from
 10 1.30 to 1.45 (mt, 1H: 3 γ₂), 1.38 (d, J=7.5 Hz, 3H: CH₃ 1 γ) from 1.55 to 1.85 (mt, 3H: 3 γ₁ and CH₂ 2 β), 2.05 (mt, 1H, 3 β₁), 2.20 (mt, 1H, 5 δ₂), 2.40 (broad d, J=16 Hz, 1H: 5 δ₁), 2.47 (d, J=16 Hz, 1H: 5 β₁), 2.88 (dt, J=13 and 4 Hz, 1H: 5 ε₂), 2.99 (dd, J=12.5 and
 15 5 Hz, 1H: 4 β₂), 3.30 (s, 3H: NCH₃ 4), 3.32 and 3.60 (2 mts, 1H each: CH₂ 3 δ), 3.40 (t, J=12.5 Hz, 1H: 4 β₁), 3.80 (s, 3H: OCH₃), 4.60 (t, J=7.5 Hz, 1H, 3 α), 4.80 (broad dd, J=13 and 8.5 Hz, 1H: 5 ε₁), 4.88 (mt, 1H: 2α), 4.92 (broad d, J=10 Hz, 1H: 1α), 5.31 (dd, J=12.5 and 5 Hz, 1H: 4 α), 5.34 (broad d, J=5.5 Hz, 1H: 5 α),
 20 5.90 (d, J=9 Hz, 1H: 6 α), 5.93 (broad q, J=7.5 Hz, 1H: 1β), 6.54 (d, J=9 Hz, 1H: NH 2), 6.87 (d, J=8 Hz, 2H: 4ε), 7.16 (d, J=8 Hz, 2H: 4δ), from 7.15 to 7.40 (mt, 5H: aromatic H 6), 7.50 (mt, 2H: 1'H₅ and 1'H₄), 7.80
 25 (dd, J=4 and 2.5 Hz, 1H: 1'H₆), 8.43 (d, J=10 Hz, 1H: NH 1), 8.78 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H: OH).

EXAMPLE 8: Preparation of 4 β -methoxycarbonyl-de(4 β -dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-methoxycarbonylphenylalanine, synthesized as in Example 33, being added at 16 h. At the end of 24 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 14 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one

volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 9 mg of 4 β -methoxycarbonyl-de(4 β -dimethylamino)pristinamycin I_a are obtained.

- 5 NMR spectrum: ^1H (400 MHz, CDCl_3 , δ in ppm, ref. TMS): 0.70 (dd, $J=16$ and 6 Hz, 1H, 5 β_2), 0.93 (t, $J=7.5$ Hz, 3H: CH_3 2 γ), 1.08 (mt, 1H: 3 β_2), from 1.30 to 1.40 (mt, 1H: 3 γ_2), 1.33 (d, $J=7.5$ Hz, 3H: CH_3 1 γ) from 1.55 to 1.85 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 10 1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.40 (broad d, $J=16.5$ Hz, 1H: 5 δ_1), 2.48 (d, $J=16$ Hz, 1H, 5 β_1), 2.89 (dt, $J=14.5$ and 4.5 Hz, 1H: 5 ϵ_2), 3.10 (dd, $J=13.5$ and 6 Hz, 1H: 4 β_2), 3.24 (s, 3H: NCH_3 4), 3.38 and 3.61 (2 mts, 1H each: CH_2 3 δ), 3.47 (t, $J=13.5$ Hz, 1H: 4 β_1), 15 3.96 (s, 3H: COOCH_3), 4.55 (t, $J=7.5$ Hz, 1H, 3 α), 4.78 (broad dd, $J=14.5$ and 8 Hz, 1H: 5 ϵ_1), 4.86 (mt, 1H: 2 α), 4.89 (broad d, $J=10$ Hz, 1H: 1 α), 5.33 (broad d, $J=6$ Hz, 1H: 5 α), 5.42 (dd, $J=13.5$ and 6 Hz, 1H: 4 α), 5.92 (d, ($J=9.5$ Hz) and mt, 1H each: 6 α and 1 β 20 respectively), 6.52 (d, $J=10$ Hz, 1H: NH 2), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.28 (d, $J=8$ Hz, 2H: 4 δ), 7.43 (dd, $J=9$ and 1.5 Hz, 1H: 1' H_4), 7.47 (dd, $J=9$ and 5 Hz, 1H: 1' H_5), 7.66 (d, $J=5$ and 1.5 Hz, 1H: 1' H_6), 7.98 (d, $J=8$ Hz, 2H: 4 ϵ), 8.38 (d, $J=10$ Hz, 1H: 25 NH 1), 8.76 (d, $J=9.5$ Hz, 1H: NH 6), 11.70 (s, 1H: OH).

EXAMPLE 9: Preparation of 4'-chloro-de(4'-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-chlorophenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is

washed with water, dried over sodium sulphate and then evaporated. 1 mg of 4 ζ -chloro-de(4 ζ -dimethylamino)pristinamycin I_A is obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm,
 5 ref. TMS): 0.93 (t, J=7.5 Hz, 3H: CH₃ 2 γ), 0.95 (dd, J=16 and 5 Hz, 1H, 5 β_2), 1.09 (mt, 1H: 3 β_2), from 1.20 to 1.40 (mt, 1H: 3 γ_2), 1.35 (d, J=7.5 Hz, 3H: CH₃ 1 γ) from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH₂ 2 β), 2.02 (mt, 1H, 3 β_1), 2.17 (mt, 1H, 5 δ_2), 2.43 (broad d, J=16 Hz, 10 1H: 5 δ_1), 2.59 (d, J=16 Hz, 1H: 5 β_1), 2.90 (dt, J=13.5 and 4 Hz, 1H: 5 ϵ_2), 3.04 (dd, J=13 and 6 Hz, 1H: 4 β_2), 3.21 (s, 3H: 4 NCH₃), 3.36 (t, J=13 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH₂ 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13.5 and 8 Hz, 1H: 5 ϵ_1),
 15 4.86 (mt, 1H: 2 α), 4.87 (broad d, J=10 Hz, 1H: 1 α), 5.38 (mt, 2H: 5 α and 4 α), 5.93 (mt, 2H: 6 α and 1 β), 6.52 (d, J=10 Hz, 1H: NH 2), 7.12 (d, J=8 Hz, 2H: 4 δ) from 7.15 to 7.35 (mt, 7H: aromatic H 6 and 4 ϵ), 7.38 (dd, J=9 and 4.5 Hz, 1H: 1' H_5), 7.43 (broad d, J=9 Hz, 20 1H: 1' H_4), 7.68 (dd, J=4.5 and 1 Hz, 1H: 1' H_6), 8.36 (d, J=10 Hz, 1H: NH 1), 8.75 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

EXAMPLE 10: Preparation of 4 ζ -bromo-de(4 ζ -dimethylamino)pristinamycin I_A and of 4 ζ -bromo-de(4 ζ -
 25 dimethylamino)pristinamycin I_B.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution

of (R,S)-4-bromophenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a
5 mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium
10 sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing
15 the new derivative of pristinamycin I_a are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel),
20 which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium
25 sulphate and then evaporated. 6 mg of 4 β -bromo-de(4 β -dimethylamino)pristinamycin I_a are obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.93 (J=7.5 Hz, 3H: CH₃ 2 γ), 0.95 (dd, J=16

and 5 Hz, 1H, 5 β_2), 1.10 (mt, 1H: 3 β_2), 1.35 (d, J=7.5 Hz, 3H: CH₃ 1 γ) 1.36 (mt, 1H: 3 γ_2), from 1.50 to 1.85 (mt, 3H, 3 γ_1 and CH₂ 2 β), 2.02 (mt, 1H, 3 β_1), 2.18 (mt, 1H: 5 δ_2), 2.43 (broad d, J=16 Hz, 1H: 5 δ_1), 2.59 (d, J=16 Hz, 1H: 5 β_1), 2.90 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 3.02 (dd, J=13 and 5.5 Hz, 1H: 4 β_2), 3.21 (s, 3H: 4 NCH₃), 3.33 (dd, J=13-11 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH₂ 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.76 (broad dd, J=13 and 7 Hz, 1H: 5 ϵ_1), 4.86 (mt, 1H, 2 α), 4.89 (d broad, J=10 Hz, 1H: 1 α), 5.37 (broad d, J=5 Hz, 1H: 5 α), (dd, J=11 and 5.5 Hz, 1H: 4 α), 5.92 (mt, 2H: 6 α and 1 β), 6.56 (d, J=9.5 Hz, 1H: NH 2), 7.08 (d, J=8 Hz, 2H: 4 δ), from 7.15 to 7.35 (mt, 5H: aromatic H 6), 7.40 (mt, 4H: 1'H₄ - 1'H₅ and 4 ϵ), 7.70 (broad d, J=5 Hz, 1H: 1'H₆), 8.40 (d, J=10 Hz, 1H: NH 1), 8.77 (d, J=9 Hz, 1H: NH 6), 11.68 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_B, 3 mg of 4 ζ -bromo-de(4 ζ -dimethylamino)pristinamycin I_B (mass spectrometry: M+H⁺=874) are isolated by carrying out semi-preparative column chromatography as described above.

EXAMPLE 11: Preparation of 4 ζ -iodo-de(4 ζ -dimethylamino)pristinamycin I_A and of 4 ζ -iodo-de(4 ζ -dimethylamino)pristinamycin I_B.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution

of (RS)-4-iodophenylalanine in sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a
5 mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate
10 and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new
15 derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two batches onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel),
20 which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium
25 sulphate and then evaporated. 12 mg of 4 ζ -iodo-de(4 ζ -dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, CDCl_3 , δ in ppm, ref. TMS): 0.93 (J=7.5 Hz, 3H: CH_3 , 2 γ), 0.95 (dd, J=16

and 5.5 Hz, 1H: 5 β_2), 1.10 (mt, 1H: 3 β_2), 1.35 (d, J=7.5 Hz, 3H: CH₃ 1 γ), 1.38 (mt, 1H: 3 γ_2), from 1.55 to 1.85 (mt, 3H, 3 γ_1 and CH₂ 2 β), 2.02 (mt, 1H, 3 β_1), 2.17 (mt, 1H: 5 δ_2), 2.43 (broad d, J=16.5 Hz, 1H: 5 δ_1), 2.60 (d, J=16 Hz, 1H: 5 β_1), 2.89 (dt, J=14 and 4.5 Hz, 1H: 5 ϵ_2), 3.02 (dd, J=13 and 5.5 Hz, 1H: 4 β_2), 3.21 (s, 3H: NCH₃ 4), 3.31 (dd, J=13 and 11 Hz, 1H: 4 β_1), 3.39 and 3.59 (2 mts, 1H each: CH₂ 3 δ), 4.53 (t, J=7.5 Hz, 1H, 3 α), 4.75 (broad dd, J=14 and 8 Hz, 1H: 5 ϵ_1), 4.83 (mt, 1H: 2 α), 4.88 (broad d, J=10 Hz, 1H: 1 α), 5.37 (broad d, J=5.5 Hz, 1H: 5 α), 5.39 (dd, J=11 and 5.5 Hz, 1H: 4 α), 5.92 (mt, 2H: 6 α and 1 β), 6.54 (d, J=9.5 Hz, 1H: NH 2), 6.94 (d, J=7.5 Hz, 2H: 4 δ), from 7.15 to 7.50 (mt, 5H: aromatic H 6), 7.36 (dd, J=9 and 4 Hz, 1H: 1'H₅), 7.43 (broad d, J=9 Hz, 1H: 1'H₄), 7.62 (d, J=7.5 Hz, 2H: 4 ϵ), 7.68 (d, J=4 Hz, 1H: 1'H₆), 8.38 (d, J=10 Hz, 1H: NH 1), 8.76 (d, J=9 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_R, 6 mg of 4 ζ -iodo-de(4 ζ -dimethylamino)pristinamycin I_R (mass spectrometry: M+H⁺=922) are isolated by carrying out semi-preparative column chromatography as described above.

EXAMPLE 12 Preparation of 4 ζ -trifluoromethyl-de(4 ζ -dimethylamino)pristinamycin I_A and of 4 ζ -trifluoromethyl-de(4 ζ -dimethylamino)pristinamycin I_R.

Strain SP92::pVRC508 is cultured in

production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (S)-4-trifluoromethylphenylalanine in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 5 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of 10 dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted 15 successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile 20 and injected onto a semi-preparative Nucleosil 7 μ C8 10 $\times$ 250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with 25 one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 5 mg of 4 β -trifluoromethyl-de(4 β -dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ^1H (400 MHz, CDCl_3 , δ in ppm, ref. TMS): 0.86 (dd, $J=16$ and 5.5 Hz, 1H, 5 β_2), 0.91 (t, $J=7.5$ Hz, 3H: CH_3 2 γ), 1.13 (mt, 1H: 3 β_2), 1.31 (d, $J=7.5$ Hz, 3H: CH_3 1 γ) 1.42 (mt, 1H: 3 γ_2), from 1.55 to 5 1.80 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.02 (mt, 1H, 3 β_1), 2.15 (mt, 1H, 5 δ_2), 2.40 (broad d, $J=16.5$ Hz, 1H: 5 δ_1), 2.55 (d, $J=16$ Hz, 1H: 5 β_1), 2.88 (dt, $J=14$ and 4 Hz, 1H: 5 ϵ_2), 3.18 (s, 3H: NCH_3 4), 3.20 and 3.31 (2 dd, respectively $J=13$ and 6 Hz and $J=13$ and 10 Hz, 10 1H each: 4 β_2 and 4 β_1), 3.42 and 3.60 (2 mts, 1H each: CH_2 3 δ), 4.50 (t, $J=7.5$ Hz, 1H, 3 α), 4.73 (broad dd, $J=14$ and 7.5 Hz, 1H: 5 ϵ_1), 4.83 (mt, 1H: 2 α), 4.91 (broad d, $J=10$ Hz, 1H: 1 α), 5.40 (broad d, $J=5.5$ Hz, 1H: 5 α), 5.55 (dd, $J=10$ and 6 Hz, 1H: 4 α), 5.87 (d, 15 $J=9.5$ Hz, 1H: 6 α), 5.90 (broad q, $J=7.5$ Hz, 1H: 1 β), 6.68 (d, $J=9.5$ Hz, 1H: NH 2), from 7.15 to 7.40 (mt, 9H: 4 δ -aromatic H 6 - 1' H_5 and 1' H_4), 7.52 (d, $J=8$ Hz, 2H: 4 ϵ), 7.68 (d, $J=4$ and 1.5 Hz, 1H: 1' H_6), 8.43 (d, $J=10$ Hz, 1H: NH 1), 8.76 (d, $J=9.5$ Hz, 1H: NH 6), 11.70 20 (s, 1H: OH).

Using the fractions derived from the silica column described above which contain the new derivative of pristinamycin I_H, 4 mg of γ -trifluoromethyl-de(4 γ -dimethylamino)pristinamycin I_H (mass spectrometry: 25 $M+H^+=864$) are isolated by carrying out semi-preparative column chromatography as described above.

EXAMPLE 13: Preparation of 4'-tert-butyl-de(4'-dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution of (R,S)-4-tert-butylphenylalanine, synthesized as in Example 35-1, in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyers are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried on sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 2 batches onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with

one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 30 mg of 4 ζ -tert-butyl-de(4 ζ -dimethylamino)pristinamycin I_A are obtained.

- 5 NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS, ref. TMS): 0.21 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH₃ 2 γ), 1.17 (mt, 1H: 3 β_2), from 1.20 to 1.40 (mt, 1H: 3 γ_2), 1.33 (s, 9H: CH₃ of tert-butyl), 1.35 (d, J=7.5 Hz, 3H: CH₃ 1 γ), from 10 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH₂ 2 β), 2.04 (mt, 1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.30 (mt, 2H: 5 δ_1 and 5 β_1), 2.80 (dt, J=13 and 4 Hz, 1H: 5 ϵ_2), 3.00 (dd, J=12 and 4 Hz, 1H: 4 β_2), 3.29 (s, 3H: NCH₃4), 3.31 and 3.59 (2 15 mts, 1H each: CH₂ 3 δ), 3.40 (t, J=12 Hz, 1H: 4 β_1), 4.57 (t, J=7.5 Hz, 1H, 3 α), 4.74 (broad dd, J=13 and 7 Hz, 1H: 5 ϵ_1), 4.85 (mt, 1H: 2 α), 4.90 (broad d, J=10 Hz, 1H: 1 α), 5.21 (broad d, J=5.5 Hz, 1H: 5 α), 5.25 (dd, J=12 and 4 Hz, 1H: 4 α), 5.87 (d, J=9 Hz, 1H: 6 α), 5.92 (broad q, J=7.5 Hz, 1H: 1 [lacuna] 1H: 1' H_6), 20 8.45 (d, J=10 Hz, 1H: NH 1), 8.74 (d, J=9 Hz, 1H: NH 6), 11.65 (s, 1H:OH).

EXAMPLE 14: Preparation of 4 ζ -isopropyl-de(4 ζ -dimethylamino)pristinamycin I_A and of 4 ζ -isopropyl-de(4 ζ -dimethylamino)pristinamycin I_B.

Strain SP92::pVRC508 is cultured in

5 production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-4-isopropylphenylalanine, synthesized as in Example 36-1, in 0.1 N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the

10 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of

15 dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and

20 eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 61 mg of the dry residue are obtained. This residue is taken up in 9 ml of a mixture consisting of

25 60% water and 40% acetonitrile and injected in 3 batches onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer,

pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then
 5 evaporated. 51 mg of 4 ζ -isopropyl-de(4 ζ -dimethylamino)pristinamycin I $_A$ are obtained.

NMR spectrum: ^1H (250 MHz, CDCl_3 , δ in ppm, ref. TMS, ref. TMS): 0.31 (dd, $J=16$ and 5.5 Hz, 1H , $5\beta_2$), 0.91 (t, $J=7.5$ Hz, 3H : CH_3 2 γ), from 1.00 to 1.45
 10 (mt, 2H : $3\beta_2$ and $3\gamma_2$), 1.25 (d, $J=7.5$ Hz, 6H : CH_3 of isopropyl), 1.35 (d, $J=7.5$ Hz, 3H : CH_3 1 γ), from 1.50 to 1.85 (mt, 3H : $3\gamma_1$ and CH_2 2 β), from 1.95 to 2.20 (mt, 2H , $3\beta_1$ and $5\delta_2$), 2.30 (mt, 2H : $5\delta_1$ and $5\beta_1$), 2.80 (dt, $J=13$ and 4 Hz, 1H : $5\epsilon_2$), 2.88 (mt, 1H : CH of
 15 isopropyl), 2.98 (dd, $J=12$ and 4 Hz, 1H : $4\beta_2$), 3.30 (s, 3H : NCH_3 4), 3.32 and 3.55 (2 mts, 1H each: CH_2 3 δ), 3.38 (t, $J=12$ Hz, 1H : $4\beta_1$), 4.55 (t, $J=7.5$ Hz, 1H , 3 α), 4.72 (broad dd, $J=13$ and 7 Hz, 1H : $5\epsilon_1$), 4.85 (mt, 1H : 2α), 4.88 (broad d, $J=10$ Hz, 1H : 1α), 5.21 (broad
 20 d, $J=5.5$ Hz, 1H : 5α), 5.25 (dd, $J=12$ and 4 Hz, 1H : 4α), 5.87 (d, $J=9$ Hz, 1H : 6α), 5.90 (broad q, $J=7.5$ Hz, 1H : 1β), 6.50 (d, $J=9.5$ Hz, 1H : NH 2), from 7.05 to 7.35 (mt, 9H : aromatic H 6 - 4ϵ and 4δ), 7.50 (mt, 2H : $1'\text{H}_5$ and $1'\text{H}_4$), 7.86 (dd, $J=4$ and 1.5 Hz, 1H :
 25 $1'\text{H}_6$), 8.40 (d, $J=10$ Hz, 1H : NH 1), 8.72 (d, $J=9$ Hz, 1H : NH 6), 11.60 (s, 1H : OH).

Using the same fractions derived from the silica column described above, which fractions also

contain the new derivative of pristinamycin I₈, 5 mg of ζ -isopropyl-de(4 ζ -dimethylamino)pristinamycin I₈ are isolated by carrying out semi-preparative column chromatography as described above.

5 NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.20 (mt, 1H, 5 β_2), 0.92 (t, J=7.5 Hz, 3H: CH₃ 2 γ), from 1.15 to 1.40 (mt, 2H: 3 β_2 and 3 γ_2), 1.24 (d, J=7.5 Hz, 6H: CH₃ of isopropyl), 1.34 (d, J=7.5 Hz, 3H: CH₃ 1 γ), from 1.35 to 2.05 (mt, 9H: 3 γ_1
10 - 3 β_1 - CH₂ 2 β - CH₂ 5 δ - CH₂ 5 γ and 5 β_1), 2.45 (dt, J=13 and 1.5 Hz, 1H: 5 ϵ_2), 2.89 (mt, 1H: ArCH), 3.09 (dd, J=14 and 7 Hz, 1H: 4 β_2), 3.17 (s, 3H: NCH₃ 4), 3.25 (dd, J=14 and 9 Hz, 1H: 4 β_1), 3.32 and 3.52 (2 mts, 1H each: CH₂ 3 δ), 4.55 (mt, 2H: 3 α and 5 ϵ_1),
15 4.80 (mt, 1H: 2 α), 4.89 (dd, J=10 and 1.5 Hz, 1H: 1 α), 4.90 (mt, 1H: 5 α), 5.35 (dd, J=9 and 7 Hz, 1H: 4 α), 5.60 (d, J=8 Hz, 1H: 6 α), 5.89 (dq, J=7.5 and 1.5 Hz, 1H: 1 β), 6.65 (d, J=9.5 Hz, 1H: NH 2), 7.08 (d, J=8 Hz, 2H: 4 δ), 7.14 (d, J=8 Hz, 2H: 4 ϵ), from 7.20 to 7.40
20 (mt, 7H: aromatic H 6 - 1'H₄ and 1'H₅), 7.77 (broad d, J=4 Hz, 1H: 1'H₆), 8.46 (d, J=10 Hz, 1H: NH 1), 8.48 (d, J=8 Hz, 1H: NH 6), 11.70 (s, 1H: OH).

EXAMPLE 15: Preparation of 4 ϵ -methylamino-de(4 ζ -dimethylamino)pristinamycin I₁.

25 Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-3-methylaminophenylalanine, synthesized as in

Example 35-3, in water being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% of 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and is eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I₁ are combined and evaporated. 19 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 8 mg of 4 ϵ -methylamino-de(4 γ -dimethylamino)pristinamycin I₁ are obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm,

ref. TMS): 0.93 (t, $J=7.5$ Hz, 3H: CH_3 2 γ), 1.00 (dd, $J=16$ and 6 Hz, 1H, 5 β_2), 1.17 (mt, 1H: 3 β_2), from 1.25 to 1.40 (mt, 2H: 3 γ_2), 1.35 (d, $J=7.5$ Hz, 3H: CH_3 1 γ), from 1.55 to 1.80 (mt, 3H: 3 γ_1 and CH_2 2 β), 2.03 (mt, 1H, 3 β_1), 2.23 (mt, 1H, 5 δ_2), 2.39 (broad d, $J=16$ Hz, 1H: 5 δ_1), 2.52 (d, $J=16$ Hz, 1H: 5 β_1), 2.78 (s, 3H: ArNCH_3 4), 2.85 (dt, $J=13$ and 4 Hz, 1H: 5 ϵ_2), 2.99 (dd, $J=13$ and 4.5 Hz, 1H: 4 β_2), 3.23 (s, 3H: NCH_3 4), 3.25 (t, $J=13$ Hz, 1H: 4 β_1), 3.38 and 3.58 (2 mts, 1H each: CH_2 3 δ), 4.05 (unres. comp., 1H: ArNH), 4.58 (dd, $J=6.5$ and 7.5 Hz, 1H, 3 α), 4.76 (broad dd, $J=13$ and 8 Hz, 1H: 5 ϵ_1), 4.85 (mt, 1H: 2 α), 4.87 (broad d, $J=10$ Hz, 1H: 1 α), 5.35 (dd, $J=13$ and 4.5 Hz, 1H: 4 α), 5.38 (broad d, $J=6$ Hz, 1H: 5 α), 5.90 (d, $J=9.5$ Hz, 1H: 6 α), 5.91 (mt, 1H: 1 β), 6.36 (broad s, 1H: H 2 of the aromatic moiety at position 4), from 6.45 to 6.55 (mt, 2H: H4 and H6 of the aromatic moiety in position 4), 6.53 (d, $J=10$ Hz, 1H: NH 2), 7.12 (t, $J=8$ Hz, 1H: H 5 of the aromatic moiety in position 4), from 7.15 to 7.45 (mt, 5H: aromatic H 6), 7.35 (mt, 2H: 1' H_4 and 1' H_5), 7.75 (t, $J=3$ Hz, 1H: 1' H_6), 8.40 (d, $J=10$ Hz, 1H: NH 1), 8.78 (d, $J=9.5$ Hz, 1H: NH 6), 11.60 (s, 1H: OH).

EXAMPLE 16: Preparation of 4 ϵ -methoxy-de(4'-dimethylamino)pristinamycin I_a and of 4 ϵ -methoxy-de(4'-dimethylamino)pristinamycin I_b .

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 5 g/l solution

of (S)-3-methoxyphenylalanine in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a
5 mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium
10 sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing
15 the new derivative of pristinamycin I_A are combined and evaporated. 41 mg of dry residue are obtained. This residue is taken up in 6 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in 2 batches onto a semi-preparative Nucleosil 7 μ C8
20 10x250 mm column (Macherey Nagel), which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is
25 washed with water, dried over sodium sulphate and then evaporated. 28 mg of 4 ϵ -methoxy-de(4 ζ -dimethylamino)pristinamycin I_A are obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm,

ref. TMS): 0.52 (dd, $J=16$ and 5.5 Hz, $1H$, $5\beta_2$), 0.90
 (t, $J=7.5$ Hz, $3H$: CH_3 2γ), from 1.10 to 1.34 (mt, $2H$: $3\beta_2$ and $3\gamma_2$), 1.34 (d, $J=7.5$ Hz, $3H$: CH_3 1γ), from 1.50
 to 1.80 (mt, $3H$: $3\gamma_1$ and CH_2 2β), 2.40 (mt, $1H$, $3\beta_1$),
 5 2.20 (mt, $1H$, $5\delta_2$), 2.35 (broad d, $J=16$ Hz, $1H$: $5\delta_1$),
 2.38 (d, $J=16$ Hz, $1H$: $5\beta_1$), 2.83 (dt, $J=13$ and 4 Hz,
 $1H$: $5\epsilon_2$), 2.97 (dd, $J=12$ and 4 Hz, $1H$: $4\beta_2$), 3.28 (s,
 $3H$: NCH_3 4), 3.28 and 3.56 (2 mts, $1H$ each: CH_2 3δ),
 3.40 (t, $J=12$ Hz, $1H$: $4\beta_1$), 3.80 (s, $3H$: OCH_3), 4.58
 10 (t, $J=7.5$ Hz, $1H$, 3α), 4.76 (broad dd, $J=13$ and 8 Hz,
 $1H$: $5\epsilon_1$), 4.85 (mt, $1H$: 2α), 4.90 (broad d, $J=10$ Hz,
 $1H$: 1α), 5.27 (dd, $J=12$ and 4 Hz, $1H$: 4α), 5.30 (broad
d, $J=5.5$ Hz, $1H$: 5α), 5.89 (d, $J=9.5$ Hz, $1H$: 6α),
5.91 (broad q, $J=7.5$ Hz, $1H$: 1β), 6.51 (d, $J=10$ Hz, $1H$:
15 NH 2), from 6.80 to 6.90 (mt, $3H$: H 2 - H 4 and H 6 of
the aromatic moiety in position 4), from 7.15 to 7.40
(mt, $6H$: H 5 of the aromatic moiety in position 4 and
aromatic H 6), 7.45 (broad d, $J=9$ Hz, $1H$: $1'H_4$), 7.50
(dd, $J=9$ and 4 Hz, $1H$: $1'H_5$), 7.80 (broad d, $J=4$ Hz,
20 $1H$: $1'H_6$), 8.40 (d, $J=10$ Hz, $1H$: NH 1), 8.73 (d,
 $J=9.5$ Hz, $1H$: NH 6), 11.62 (s, $1H$: OH).

Using the fractions derived from the silica
column described above which contain the new derivative
of pristinamycin I_H , 7 mg of 4 ϵ -methoxy-de(4 ζ -
25 dimethylamino)pristinamycin I_H (mass spectrometry: $M+H^+$
= 826) are isolated by carrying out semi-preparative
column chromatography as described above.

EXAMPLE 17: Preparation of 4 ϵ -fluoro-4 ζ -methyl-de(4 ζ -dimethylamino)pristinamycin I_A.

Strain SP92::pVRC508 is cultured in production medium using 60 Erlenmeyer flasks, as described in Example 3, with 1 ml of a 10 g/l solution of (R,S)-3-fluoro-4-methylphenylalanine, synthesized as in Example 34-5, in 0.1N sodium hydroxide solution being added at 16 h. At the end of 40 h of culture, the 1.8 litres of must recovered from the 60 Erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the new derivative of pristinamycin I_A are combined and evaporated. 15 mg of dry residue are obtained. This residue is taken up in 3 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10 $\times$ 250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% acetonitrile. The fractions containing the new

pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 9 mg of 4 ϵ -fluoro-4 ζ -methyl-de(4 ζ -

5 dimethylamino)pristinamycin I_a are obtained.

NMR spectrum: ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.60 (dd, J=16 and 5.5 Hz, 1H, 5 β_2), 0.91 (t, J=7.5 Hz, 3H: CH₃ 2 γ), 1.12 (mt, 1H: 3 β_2), from 1.25 to 1.35 (mt, 1H: 3 γ_2), 1.33 (d, J=7.5 Hz, 3H: CH₃ 1 γ), from 1.50 to 1.85 (mt, 3H: 3 γ_1 and CH₂ 2 β), 2.02 (mt, 1H, 3 β_1), 2.13 (mt, 1H, 5 δ_2), 2.27 (s, 3H: ArCH₃), 2.36 (broad d, J=16 Hz, 1H: 5 δ_1), 2.45 (d, J=16 Hz, 1H: 5 β_1), 2.85 (dt, J=13 and 4.5 Hz, 1H: 5 ϵ_2), 2.97 (dd, J=12.5 and 4.5 Hz, 1H: 4 β_2), 3.23 (s, 15 3H: NCH₃ 4), 3.30 and 3.56 (2 mts, 1H each: CH₂ 3 δ), 3.37 (t, J=12.5 Hz, 1H: 4 β_1), 4.55 (t, J=7.5 Hz, 1H, 3 α), 4.75 (broad dd, J=13 and 8 Hz, 1H: 5 ϵ_1), 4.83 (mt, 1H: 2 α), 4.89 (broad d, J=10 Hz, 1H: 1 α), 5.29 (dd, J=12.5 and 4.5 Hz, 1H: 4 α), 5.32 (broad d, J=5.5 20 Hz, 1H: 5 α), 5.89 (d J=9.5 Hz, 1H: 6 α), 5.92 (mt, 1H: 1 β), 6.49 (d, J=10 Hz, 1H: NH 2), 6.90 (mt, 2H: H 2 and H 6 of the aromatic moiety in position 4), 7.11 (t, J=8 Hz, 1H: H 5 of the aromatic moiety in position 4), from 7.10 to 7.30 (mt, 5H: aromatic H 6), 7.43 (dd, 25 J=8.5 and 1 Hz, 1H: 1'H₄), 7.49 (dd, J=8.5 and 4.5 Hz, 1H: 1'H₅), 7.75 (dd, J=4.5 and 1 Hz, 1H: 1'H₆), 8.48 (d, J=10 Hz, 1H: NH 1), 8.70 (d, J=9.5 Hz, 1H: NH 6), 11.60 (s, 1H: OH).

EXAMPLE 18: Preparation of 4'-ethylamino-de(4'-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 50 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylaminophenylalanine dihydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.5 litres of must recovered from the 50 erlenmeyer flasks are extracted with 2 volumes of a mixture of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica column (30 g) which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4'-ethylamino-de(4'-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 65% water and 35% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% acetonitrile. The fractions containing 4'-ethylamino-de(4'-

dimethylamino)pristinamycin I_a are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 10 mg of 4 ζ -ethylamino-
 5 de(4 ζ -dimethylamino)pristinamycin I_a are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.72 (dd, J = 16 and 6 Hz, 1H: 1H of the CH₂ in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 1.15 (mt, 1H: 1H of the CH₂ in 3 β); from 1.20 to 1.40 (mt, 1H: 1H of the CH₂ in 3 γ); 1.27 (t, J = 7.5 Hz, 3H: CH₃ of the ethyl); 1.33 (d, J = 7 Hz, 3H: CH₃ in 1 γ); from 1.50 to 1.65 (mt, 1H: the other H of the CH₂ in 3 γ); 1.60 and 1.74 (2 mts, 1H each: CH₂ in 2 β); 2.02 (mt, 1H: the other H of CH₂ in 3 β); 2.21 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH₂ in 5 δ); 2.40 (d, J = 16 Hz, 1H: the other H of the CH₂ in 5 β); 2.82 (dt, J = 13 and 4.5 Hz, 1H: 1H of the CH₂ in 5 ϵ); 2.89 (dd, J = 12 and 4 Hz, 1H: 1H of the CH₂ in 4 β); 3.10 (mt, 2H: NCH₂ of the ethyl); from 3.20 to 3.35 (mt, 1H: 1H of the CH₂ in 3 δ); 3.26 (s, 3H: NCH₃); 3.31 (t, J = 12 Hz, 1H: the other H of the CH₂ in 4 β); 3.54 (mt, 1H: the other H of the CH₂ in 3 δ); 3.67 (unres. comp., 1H: NH); 4.56 (dd, J = 6.5 and 7 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH₂ in 5 ϵ); 4.84
 25 (mt, 1H: 2 α); 4.90 (broad d, J = 10 Hz, 1H : 1 α) ; 5.24 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 6 Hz, 1H: 5 α); 5.88 (d, J = 9.5 Hz, 1H : 6 α); 5.90 (mt, 1H : 1 β); 6.52 (d, J = 8 Hz, 3H : NH in 2 and

aromatic H in 4 ϵ); 7.00 (d, J = 8 Hz, 2H : aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.46 (limiting AB, 2H: 1'H₄ and 1'H₅); 7.84 (dd, J = 4 and 1 Hz, 1H: 1'H₆); 8.45 (d, J = 10 Hz, 1H: NH in 1);
5 8.77 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H: OH).

EXAMPLE 19: Preparation of 4 ζ -diethylamino-de(4 ζ -dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 50 erlenmeyer flasks, as
10 described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-diethylaminophenylalanine dihydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.5 litres of must recovered from the 50
15 erlenmeyer flasks are extracted with 2 volumes of a mixture of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and
20 then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica column (30 g) which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in
25 dichloromethane. The fractions containing 4 ζ -diethylamino-de(4 ζ -dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in

7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected in two portions onto a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of
 5 68% 100 mM phosphate buffer, pH 2.9, and 32% acetonitrile. The fractions containing 4 ζ -diethylamino-de(4 ζ -dimethylamino)pristinamycin I $_A$ are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium
 10 sulphate and then evaporated. 50 mg of 4 ζ -diethylamino-de(4 ζ -dimethylamino)pristinamycin I $_A$ are obtained.

NMR spectrum. ^1H (400 MHz, CDCl_3 , δ in ppm, ref. TMS): 0.65 (dd, $J = 16$ and 6 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, $J = 7.5$ Hz, 3H: CH_3 in 2 γ); 1.14 (t, $J = 7$ Hz, 6H: CH_3 of the ethyl); 1.15 (mt, 1H: 1H of the CH_2 in 3 β); 1.26 (mt, 1H: 1H of the CH_2 in 3 γ); 1.32 (d, $J = 6.5$ Hz, 3H: CH_3 in 1 γ); 1.55 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.75 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β);
 20 2.22 and 2.31 (respectively, mt and broad d, $J = 16.5$ Hz, 1H each: CH_2 in 5 δ); 2.37 (d, $J=16$ Hz, 1H: the other H of the CH_2 in 5 β); 2.80 (dt, $J = 13$ and 4.5 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.89 (dd, $J = 12.5$ and 4 Hz, 1H : 1H of the CH_2 in 4 β); from 3.20 to 3.40 (mt, 6H: NCH_2 ,
 25 of the ethyl - 1H of the CH_2 in 3 δ and the other H of the CH_2 in 4 β); 3.27 (s, 3H: NCH_3); 3.55 (mt, 1H: the other H of the CH_2 in 3 δ); 4.58 (dd, $J = 8$ and 6 Hz, 1H: 3 α); 4.76 (broad dd, $J = 13$ and 7.5 Hz, 1H: the

other H of the CH₂ in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α) ; 5.21 (dd, J = 12.5 and 4 Hz, 1H: 4 α); 5.28 (broad d, J = 6 Hz, 1H : 5 α); 5.87 (d, J = 9.5 Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.52 (d, J = 9.5 Hz, 1H : NH in 2); 6.60 (d, J = 8 Hz, 2H: aromatic H in 4 ϵ); 7.02 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.46 (limiting AB, 2H: 1'H₄ and 1'H₅); 7.88 (dd, J = 4.5 and 2.5 Hz, 1H: 1'H₆); 8.43 (d, J = 10 Hz, 1H: NH in 1); 8.76 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

EXAMPLE 20: Preparation of 4 ζ -diallylamino-de(4 ζ -dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 94 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-diallylaminophenylalanine dihydrochloride, synthesized as in Example 38-1, in water being added at 16h. At the end of 40h of culture, the 2.8 litres of must recovered from the 94 erlenmeyer flasks are extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is

mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 ζ -diallylamino-de(4 ζ -dimethylamino)pristinamycin I $_A$ are combined and

5 evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10 $\times$ 250 mm (Machery Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH

10 2.9, and 48% acetonitrile. The fractions containing 4 ζ -diallylamino-de(4 ζ -dimethylamino)pristinamycin I $_A$ are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated.

15 15 mg of 4 ζ -diallylamino-de(4 ζ -dimethylamino)pristinamycin I $_A$ are obtained.

NMR spectrum. ^1H (400 MHz, CDCl_3 , δ in ppm, ref.TMS): 0.55 (dd, $J = 16$ and 6 Hz, 1H : 1H of the CH_2 in 5 β); 0.93 (t, $J = 7.5$ Hz, 3H: CH_3 in 2 γ); 1.18 (mt, 20 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H : 1H of the CH_2 in 3 γ); 1.34 (d, $J = 6.5$ Hz, 3H: CH_3 in 1 γ); 1.59 (mt, 1H: the other H of the CH_2 in 3 γ); 1.68 and 1.78 (2 mts, 1H each: CH_2 in 2 β); 2.04 (mt, 1H: the other H of CH_2 in 3 β); 2.25 and 2.34 (respectively, mt and broad 25 d, $J = 16.5$ Hz, 1H each: CH_2 in 5 δ); 2.40 (d, $J = 16$ Hz, 1H: the other H of the CH_2 in 5 β); 2.83 (dt, $J = 13$ and 4.5 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.92 (dd, $J = 12$ and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.20 to 3.30

(mt, 1H: 1H of the CH₂ in 3 δ); 3.29 (s, 3H: NCH₃); 3.33 (t, J = 12 Hz, 1H: the other H of the CH₂ in 4 β); 3.57 (mt, 1H: the other H of the CH₂ in 3 δ); 3.93 (limiting AB, 4H: NCH₂ of the allyl); 4.60 (dd, J = 8 and 6.5 Hz, 1H: 3 α); 4.78 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH₂ in 5 ε); 4.87 (mt, 1H: 2 α); 4.92 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.10 to 5.25 (mt, 5H: 4 α and =CH₂ of the allyl); 5.28 (broad d, J = 6 Hz, 1H: 5 α); 5.85 (mt, 2H: CH= of the allyl); 5.92 (d, J = 9.5 Hz, 1H: 6 α); 5.94 (mt, 1H : 1 β); 6.54 (d, J = 10 Hz, 1H: NH in 2); 6.65 (d, J = 8 Hz, 2H : aromatic H in 4 ε); 7.05 (d, J = 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.51 (limiting AB, 2H: 1'H₄ and 1'H₅); 7.88 (dd, J = 4 and 2 Hz, 1H: 1'H₆); 8.43 (d, J = 10 Hz, 1H: NH in 1); 8.77 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H : OH).

EXAMPLE 21: Preparation of 4'-allylethyl-amino-de(4'-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 26 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-allylethylaminophenylalanine dihydrochloride, synthesized as in Example 39-4, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 0.78 litre of must recovered from the 26 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM

phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane.

10 The fractions containing 4 ζ -allylethylamino-de(4 ζ -dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8

15 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 ζ -allylethylamino-de(4 ζ -dimethylamino)pristinamycin I_A are combined and extracted with one volume of

20 dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 20 mg of 4 ζ -allylethylamino-de(4 ζ -dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.58 (dd, J = 16 and 6 Hz, 1H: 1H of CH₂ in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 1.16 (t, J = 7 Hz, 3H: CH₃ of the ethyl); 1.16 (mt, 1H: 1H of the CH₂ in 3 β); 1.25 (mt, 1H: 1H of CH₂ in 3 γ); 1.32

(d, $J = 6.5$ Hz, 3H: CH_3 in 1 γ); 1.54 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.75 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.23 and 2.31 (respectively, mt and broad d, $J = 16.5$ Hz, 1H each: CH_2 in 5 δ); 2.37 (d, $J = 16$ Hz, 1H: the other H of the CH_2 in 5 β); 2.80 (dt, $J = 13$ and 4.5 Hz, 1H : 1H of CH_2 in 5 ϵ); 2.87 (dd, $J = 12$ and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.15 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.26 (s, 3H: NCH_3); 3.30 (t, $J = 12$ Hz, 1H: the other H of CH_2 in 4 β); 3.36 (mt, 2H: NCH_2 of the ethyl); 3.54 (mt, 1H: the other H of the CH_2 in 3 δ); 3.90 (limiting AB, 2H: NCH_2 of the allyl); 4.57 (dd, $J = 8$ and 6 Hz, 1H: 3 α); 4.76 (broad dd, $J = 13$ and 7.5 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.89 (dd, $J = 10$ and 1 Hz, 1H: 1 α); from 5.05 to 5.20 (mt, 3H: 4 α and $=\text{CH}_2$ of the allyl); 5.27 (broad d, $J = 6$ Hz, 1H : 5 α); 5.83 (mt, 1H: $\text{CH}=\text{}$ of the allyl); 5.88 (d, $J = 9.5$ Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.50 (d, $J = 10$ Hz, 1H: NH in 2); 6.60 (d, $J = 8$ Hz, 2H: aromatic H in 4 ϵ); 7.02 (d, $J = 8$ Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.47 (limiting AB, 2H: $1'\text{H}_4$ and $1'\text{H}_5$); 7.88 (dd, $J = 4$ and 2 Hz, 1H: $1'\text{H}_6$); 8.41 (d, $J = 10$ Hz, 1H: NH in 1); 8.75 (d, $J = 9.5$ Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

EXAMPLE 22: Preparation of the 4 ζ -ethyl-propylamino-de(4 ζ -dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylpropylaminophenylalanine dihydrochloride, synthesized as in Example 39-6, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litre of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 ζ -ethylpropylamino-de(4 ζ -dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 63% 100 mM phosphate buffer, pH 2.9, and 37% of acetonitrile. The fractions containing

4 ζ -ethylpropylamino-de(4 ζ -dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated.

- 5 16 mg of 4 ζ -ethylpropylamino-de(4 ζ -dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.67 (dd, J = 16 and 6 Hz, 1H: 1H of the CH₂ in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 0.95 (t, J = 7.5 Hz, 3H: CH₃ of propyl); 1.14 (t, J = 7 Hz, 3H: CH₃ of the ethyl); 1.15 (mt, 1H: 1H of the CH₂ in 3 β); 1.25 (mt, 1H: 1H of the CH₂ in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH₃ in 1 γ); from 1.45 to 1.65 (mt, 3H: the other H of the CH₂ in 3 γ and CH₂ propyl); 1.63 and 1.75 (2 mts, 1H each: CH₂ in 2 β); 2.02 (mt, 1H: the other H of the CH₂ in 3 β); 2.23 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH₂ in 5 δ); 2.37 (d, J = 16 Hz, 1H: the other H of the CH₂ in 5 β); 2.80 (dt, J = 13 and 5 Hz, 1H: 1H of the CH₂ in 5 ϵ); 2.89 (dd, J = 12 and 4 Hz, 1H: 1H of the CH₂ in 4 β); from 3.10 to 3.25 (mt, 3H: 1H of the CH₂ in 3 δ and NCH₂ of the propyl); 3.26 (s, 3H: NCH₃); from 3.25 to 3.40 (mt, 2H: NCH₂ of the ethyl); 3.34 (t, J = 12 Hz, 1H: the other H of the CH₂ in 4 β); 3.54 (mt, 1H: the other H of the CH₂ in 3 δ); 4.57 (dd, J = 7.5 and 6 Hz, 1H: 3 α); 4.76 (broad dd, J = 13 and 7.5 Hz, 1H: the other H of the CH₂ in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.21 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.28 (broad

d, $J = 6$ Hz, 1H: 5 α); 5.88 (d, $J = 9.5$ Hz, 1H: 6 α);
 5.91 (mt, 1H: 1 β); 6.48 (d, $J = 10$ Hz, 1H: NH in 2);
 6.60 (d, $J = 8$ Hz, 2H: aromatic H in 4 ϵ); 7.03 (d, $J =$
 8 Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt,
 5 5H: aromatic H in 6); 7.47 (limiting AB, 2H: 1'H₄ and
 1'H₅); 7.89 (mt, 1H: 1'H₆); 8.42 (d, $J = 10$ Hz, 1H : NH
 in 1); 8.76 (d, $J = 9.5$ Hz, 1H: NH in 6); 11.62 (s, 1H:
 OH).

EXAMPLE 23: Preparation of the 4'-trifluoro-
 10 **methoxy-de(4'-dimethylamino)pristinamycin I_A**

Strain SP92::pVRC508 is cultured in
 production medium using 60 erlenmeyer flasks, as
 described in Example 3, with 1 ml of a 20 g/l solution
 of (R,S)-4-O-trifluoromethyltyrosine hydrochloride,
 15 synthesized as in Example 34-8, in water being added at
 16h. At the end of 40h of culture, the 1.8 litres of
 must recovered from the 60 erlenmeyer flasks is
 extracted with 2 volumes of a mixture consisting of 66%
 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile,
 20 and then centrifuged. The supernatant is extracted with
 2 times [lacuna] volumes of dichloromethane. The
 chloromethylene phases are washed with water and then
 combined, dried over sodium sulphate and evaporated.
 The dry extract is taken up in [lacuna] ml of
 25 dichloromethane and injected onto a silica (30 g)
 column which is mounted in dichloromethane and eluted
 successively with plateaus of from 0 to 10% methanol in

dichloromethane. The fractions containing 4'-trifluoromethoxy-de(4'-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected in two portions onto a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4'-trifluoromethoxy-de(4'-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 46.5 mg of 4'-trifluoromethoxy-de(4'-dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.77 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH₂ in 5 β); 0.92 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 1.08 (mt, 1H: 1H of the CH₂ in 3 β); from 1.30 to 1.40 (mt, 1H: 1H of the CH₂ in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH₃ in 1 γ); from 1.55 to 1.70 (mt, 1H: the other H of the CH₂ in 3 γ); 1.65 and 1.76 (2 mts, 1H each: CH₂ in 2 β); 2.02 (mt, 1H: the other H of the CH₂ in 3 β); 2.11 and 2.40 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH₂ in 5 δ); 2.54 (d, J = 16 Hz, 1H: the other H of the CH₂ in 5 β); 2.88 (dt, J = 13 and 4 Hz, 1H: 1H of the CH₂ in 5 ϵ); 3.08 (dd, J = 12 and 5 Hz, 1H: 1H of the CH₂ in 4 β); 3.22 (s, 3H: NCH₃); from 3.30 to 3.45

(mt, 1H: 1H of the CH₂ in 3 δ); 3.39 (t, J = 12 Hz, 1H: the other H of the CH₂ in 4 β); 3.59 (mt, 1H: the other H of the CH₂ in 3 δ); 4.53 (t, J = 7.5 Hz, 1H : 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH₂ in 5 ε); 4.85 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1.5 Hz, 1H: 1 α); 5.35 (broad d, J = 5.5 Hz, 1H: 5 α); 5.41 (dd, J = 12 and 5 Hz, 1H: 4 α); 5.92 (d, J = 10 Hz, 1H : 6 α); 5.93 (mt, 1H: 1 β); 6.53 (d, J = 9.5 Hz, 1H: NH in 2); from 7.15 to 7.35 (mt, 5H: aromatic H in 6); 7.16 (d, J = 8 Hz, 2H: aromatic H in 4 ε); 7.26 (d, J = 8 Hz, 2H: aromatic H in 4 δ); 7.37 (dd, J = 8.5 and 4 Hz, 1H: 1'H₅); 7.42 (dd, J = 8.5 and 1.5 Hz, 1H: 1'H₄); 7.70 (dd, J = 4 and 1.5 Hz, 1H: 1'H₆); 8.37 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H: NH in 6); 11.66 (s, 1H: OH).

EXAMPLE 24: Preparation of 4'-allyloxy-de(4'-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 90 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-allyltyrosine hydrochloride, synthesized as in Example 33, in 0.1N hydrochloric acid being added at 16h. At the end of 40h of culture, the 2.7 litres of must recovered from the 90 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with

2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 ζ -allyloxy-de(4 ζ -dimethyl-amino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 ζ -allyloxy-de(4 ζ -dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 29 mg of 4 ζ -allyloxy-de(4 ζ -dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.63 (dd, J = 16 and 6 Hz, 1H: 1H of CH₂ in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 1.13 (mt, 1H: 1H of CH₂ in 3 β); 1.29 (mt, 1H: 1H of CH₂ in 3 γ); 1.33 (d, J = 6.5 Hz, 3H: CH₃ in 1 γ); 1.57 (mt, 1H: the other H of the CH₂ in 3 γ); 1.65 and 1.74 (2 mts, 1H each: CH₂ in 2 β); 2.02 (mt, 1H: the other H of the CH₂ in 3 β);

2.14 and 2.34 (respectively, mt and broad d, $J = 16.5$ Hz, 1H each: CH_2 in 5 δ); 2.43 (d, $J = 16$ Hz, 1H: the other H of the CH_2 in 5 β); 2.85 (dt, $J = 13$ and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.95 (dd, $J = 12$ and 4 Hz, 5 1H: 1H of the CH_2 in 4 β); 3.25 (s, 3H: NCH_3); 3.33 (mt, 1H: 1H of the CH_2 in 3 δ); 3.36 (t, $J = 12$ Hz, 1H: the other H of the CH_2 in 4 β); 3.56 (mt, 1H: the other H of the CH_2 in 3 δ); 4.51 (limiting AB, 2H: OCH_2 of the allyl); 4.56 (t, $J = 7.5$ Hz, 1H: 3 α); 4.75 (broad dd, 10 $J = 13$ and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.88 (dd, $J = 10$ and 1 Hz, 1H: 1 α); 5.27 (dd, $J = 12$ and 4 Hz, 1H: 4 α); 5.32 (broad d, $J = 6$ Hz, 1H: 5 α); 5.30 and 5.40 (respectively, mt and dd, $J = 17$ and 1.5 Hz, 1H each: $=\text{CH}_2$ of the allyl); 5.89 15 (d, $J = 9.5$ Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.02 (mt, 1H: $\text{CH}=\text{}$ of the allyl); 6.50 (d, $J = 10$ Hz, 1H: NH in 2); 6.85 (d, $J = 8$ Hz, 2H: aromatic H in 4 ϵ); 7.12 (d, $J = 8$ Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.45 (dd, $J = 8.5$ and 1.5 20 Hz, 1H: 1' H_4); 7.57 (dd, $J = 8.5$ and 4 Hz, 1H: 1' H_5); 7.77 (dd, $J = 4$ and 1.5 Hz, 1H: 1' H_6); 8.41 (d, $J = 10$ Hz, 1H: NH in 1); 8.74 (d, $J = 9.5$ Hz, 1H: NH in 6); 11.63 (s, 1H: OH).

EXAMPLE 25: Preparation of 4'-ethoxy-de(4'-
25 dimethylamino)pristinamycin I₁

Strain SP92::pVRC508 is cultured in
production medium using 90 erlenmeyer flasks, as

described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-O-ethyltyrosine hydrochloride, synthesized as in Example 33, in 0.1N hydrochloric acid being added at 16h. At the end of 40h of culture, the 2.7 litres of
5 must recovered from the 90 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The
10 chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with
15 plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 ζ -ethoxy-de(4 ζ -dimethyl-amino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto
20 a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 ζ -ethoxy-de(4 ζ -dimethylamino)pristinamycin I_A are combined
25 and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 29 mg of 4 ζ -ethoxy-de(4 ζ -dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, CDCl_3 , δ in ppm, ref. TMS): 0.64 (dd, $J = 16$ and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, $J = 7.5$ Hz, 3H: CH_3 in 2 γ); 1.12 (mt, 1H: 1H of the CH_2 in 3 β); 1.25 (mt, 1H: 1H of the CH_2 in 3 γ); 1.33 (d, $J = 7$ Hz, 3H: CH_3 in 1 γ); 1.42 (t, $J = 7$ Hz, 3H: CH_3 of the ethyl); 1.57 (mt, 1H: the other H of the CH_2 in 3 γ); 1.63 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.16 and 2.35 (respectively mt and broad d, $J = 16.5$ Hz, 1H each: CH_2 in 5 δ); 2.43 (d, $J = 16$ Hz, 1H: the other H of the CH_2 in 5 β); 2.83 (dt, $J = 13$ and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.93 (dd, $J = 12$ and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.15 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.24 (s, 3H: NCH_3); 3.35 (t, $J = 12$ Hz, 1H: the other H of the CH_2 in 4 β); 3.55 (mt, 1H: the other H of the CH_2 in 3 δ); 3.95 (limiting AB, 2H: OCH_2 of the ethyl); 4.56 (dd, $J = 7.5$ and 6 Hz, 1H: 3 α); 4.75 (broad dd, $J = 13$ and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.87 (dd, $J = 10$ and 1 Hz, 1H: 1 α); 5.26 (dd, $J = 12$ and 4 Hz, 1H: 4 α); 5.32 (broad d, $J = 5.5$ Hz, 1H: 5 α); 5.88 (d, $J = 10$ Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.48 (d, $J = 10$ Hz, 1H: NH in 2); 6.83 (d, $J = 8$ Hz, 2H: aromatic H in 4 ϵ); 7.10 (d, $J = 8$ Hz, 2H: aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.44 (dd, $J = 8.5$ and 1.5 Hz, 1H: $1'\text{H}_4$); 7.57 (dd, $J = 8.5$ and 4.5 Hz, 1H: $1'\text{H}_5$); 7.77 (dd, $J = 4.5$ and 1.5 Hz, 1H: $1'\text{H}_6$); 8.38 (d, $J = 10$ Hz, 1H: NH in 1); 8.75 (d, $J = 10$ Hz, 1H :

NH in 6); 11.60 (s, 1H: OH).

EXAMPLE 26: Preparation of 4'- (2-chloro-ethoxy) -de(4'-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in
5 production medium using 60 erlenmeyer flasks, as
described in Example 3, with 1 ml of a 20 g/l solution
of (S)-4-O-(2-chloroethyl)tyrosine hydrochloride,
synthesized as in Example 42-1, in water being added at
16h. At the end of 40h of culture, the 1.8 litres of
10 must recovered from the 60 erlenmeyer flasks is
extracted with 2 volumes of a mixture consisting of 66%
100 mM phosphate buffer, pH 2.9, and 34% acetonitrile,
and then centrifuged. The supernatant is extracted with
2 times 0.5 volumes of dichloromethane. The
15 chloromethylene phases are washed with water and then
combined, dried over sodium sulphate and evaporated.
The dry extract is taken up in 20 ml of dichloromethane
and injected onto a silica (30 g) column which is
mounted in dichloromethane and eluted successively with
20 plateaus of from 0 to 10% methanol in dichloromethane.
The fractions containing 4'- (2-chloroethoxy) -de(4'-
dimethylamino)pristinamycin I_A are combined and
evaporated. The dry residue is taken up in 7 ml of a
mixture consisting of 60% of water and 40% acetonitrile
25 and injected onto a semi-preparative Nucleosil 7 μ C8
10x250 mm (Macherey Nagel) column, which is eluted with
a mixture consisting of 60% 100 mM phosphate buffer, pH

2.9, and 40% of acetonitrile. The fractions containing 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 3.2 mg of 4ζ-(2-chloroethoxy)-de(4ζ-dimethylamino)-pristinamycin I_A are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.66 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH₂ in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 1.13 (mt, 1H: 1H of the CH₂ in 3 β); 1.28 (mt, 1H: 1H of the CH₂ in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH₃ in 1 γ); 1.57 (mt, 1H: the other H of the CH₂ in 3 γ); 1.66 and 1.76 (2 mts, 1H each: CH₂ in 2 β); 2.02 (mt, 1H: the other H of the CH₂ in 3 β); 2.16 and 2.37 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH₂ in 5 δ); 2.47 (d, J = 16 Hz, 1H: the other H of the CH₂ in 5 β); 2.86 (dt, J = 13 and 4 Hz, 1H: 1H of the CH₂ in 5 ε); 2.95 (dd, J = 12 and 4 Hz, 1H: 1H of the CH₂ in 4 β); 3.23 (s, 3H: NCH₃); 3.32 (mt, 1H: 1H of the CH₂ in 3 δ); 3.37 (t, J = 12 Hz, 1H: the other H of the CH₂ in 4 β); 3.57 (mt, 1H: the other H of the CH₂ in 3 δ); 3.82 (t, J = 6 Hz, 2H: CH₂Cl); 4.19 (limiting AB, 2H: OCH₂ of the ethyl); 4.55 (dd, J = 7.5 and 7 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH₂ in 5 ε); 4.84 (mt, 1H: 2 α); 4.87 (broad d, J = 10 Hz, 1H: 1 α); 5.28 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.32 (broad d, J = 5.5 Hz, 1H: 5 α); 5.88 (d, J = 10 Hz, 1H: 6α); 5.90 (mt, 1H :

1 β); 6.50 (d, $J = 10$ Hz, 1H: NH in 2); 6.86 (d, $J = 8$
 Hz, 2H: aromatic H in 4 ϵ); 7.13 (d, $J = 8$ Hz, 2H:
 aromatic H in 4 δ); from 7.10 to 7.35 (mt, 5H: aromatic
 H in 6); 7.45 (limiting AB, 2H: 1'H₄ and 1'H₅); 7.75
 5 (dd, $J = 4$ and 2 Hz, 1H: 1'H₆); 8.38 (d, $J = 10$ Hz, 1H:
 NH in 1); 8.74 (d, $J = 10$ Hz, 1H: NH in 6); 11.62 (s,
 1H: OH).

**EXAMPLE 27: Preparation of 4'-acetyl-de 4'-
 dimethylamino)pristinamycin I_A**

10 Strain SP92::pVRC508 is cultured in
 production medium using 60 erlenmeyer flasks, as
 described in Example 3, with 1 ml of a 20 g/l solution
 of (S)-4-acetylphenylalanine, synthesized as in Example
 33, in 0.1N sodium hydroxide solution being added at
 15 16h. At the end of 40h of culture, the 1.8 litres of
 must recovered from the 60 erlenmeyer flasks is
 extracted with 2 volumes of a mixture consisting of 66%
 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile,
 and then centrifuged. The supernatant is extracted with
 20 2 times 0.5 volumes of dichloromethane. The
 chloromethylene phases are washed with water and then
 combined, dried over sodium sulphate and evaporated.
 The dry extract is taken up in 20 ml of dichloromethane
 and injected onto a silica (30 g) column which is
 25 mounted in dichloromethane and eluted successively with
 plateaus of from 0 to 10% methanol in dichloromethane.
 The fractions containing 4'-acetyl)-de(4'-dimethyl-

amino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey
 5 Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing 4 ζ -acetyl-de(4 ζ -dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The
 10 organic phase is washed with water, dried over sodium sulphate and then evaporated. 4.2 mg of 4 ζ -acetyl-de(4 ζ -dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.73 (dd, J = 16 and 6 Hz, 1H: 1H of the CH₂
 15 in 5 β); 0.93 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 1.12 (mt, 1H: 1H of the CH₂ in 3 β); from 1.25 to 1.45 (mt, 1H: 1H of the CH₂ in 3 γ); 1.33 (d, J = 7 Hz, 3H: CH₃ in 1 γ); 1.62 (mt, 1H: the other H of the CH₂ in 3 γ); from 1.60 to 1.85 (mt, 2H: CH₂ in 2 β); 2.02 (mt, 1H: the other H
 20 of the CH₂ in 3 β); 2.20 and 2.42 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH₂ in 5 δ); 2.52 (d, J = 16 Hz, 1H: the other H of the CH₂ in 5 β); 2.60 (s, 3H: ArCOCH₃); 2.88 (dt, J = 13 and 4.5 Hz, 1H: 1H of CH₂ in 5 ϵ); 3.13 (dd, J = 13.5 and 5.5 Hz, 1H: 1H of the CH₂
 25 in 4 β); 3.21 (s, 3H: NCH₃); from 3.30 to 3.50 (mt, 1H: the other H of the CH₂ in 4 β); from 3.30 to 3.50 and 3.63 (2 mts, 1H each: CH₂ in 3 δ); 4.53 (t, J = 7.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the

other H of the CH₂ in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.88 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.35 (broad d, J = 6 Hz, 1H: 5 α); 5.43 (dd, J = 10.5 and 4 Hz, 1H: 4 α); 5.90 (d, J = 9.5 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.56 (d, J = 9.5 Hz, 1H: NH in 2); from 7.10 to 7.35 (mt, 5H: aromatic H in 6); 7.28 (d, J = 8 Hz, 2H: aromatic H in 4 δ); 7.38 (dd, J = 8.5 and 2 Hz, 1H: 1'H₄); 7.42 (dd, J = 8.5 and 4.5 Hz, 1H: 1'H₅); 7.66 (dd, J = 4.5 and 2 Hz, 1H: 1'H₆); 7.88 (d, J = 8 Hz, 2H: aromatic H in 4 ϵ); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.74 (d, J = 9.5 Hz, 1H: NH in 6); 11.65 (s, 1H: OH).

EXAMPLE 28: Preparation of 4 ϵ -dimethylamino-de(4 ζ -dimethylamino)pristinamycin I₁.

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-3-dimethylaminophenylalanine dihydrochloride, synthesized as in Example 35-10, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in

20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and evaporated. The dry residue is taken up in 3 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7μ C8 10×250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 57% 100 mM phosphate buffer, pH 2.9, and 43% of acetonitrile. The fractions containing 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 1.1 mg of 4ε-dimethylamino-de(4ζ-dimethylamino)pristinamycin I_A are obtained.

NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.63 (dd, J = 16 and 5 Hz, 1H: 1H of the CH₂ in 5 β); 0.91 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 1.13 (mt, 1H: 1H of the CH₂ in 3 β); from 1.20 to 1.35 (mt, 1H: 1H of the CH₂ in 3 γ); 1.32 (d, J = 6.5 Hz, 3H: CH₃ in 1 γ); 1.57 (mt, 1H: the other H of the CH₂ in 3 γ); 1.63 and 1.76 (2 mts, 1H each: CH₂ in 2 β); 2.02 (mt, 1H: the other H of the CH₂ in 3 β); 2.08 and 2.31 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH₂ in 5 δ); 2.35 (d, J = 16 Hz, 1H: the other H of the CH₂ in 5 β); 2.81 (dt, J = 13 and 4 Hz, 1H: 1H of the CH₂ in 5 ε); 2.90

(s, 6H: $N(CH_3)_2$); 2.97 (dd, $J = 12$ and 4 Hz, 1H: 1H of the CH_2 in 4 β); from 3.20 to 3.30 (mt, 1H: 1H of the CH_2 in 3 δ); 3.28 (s, 3H: NCH_3); 3.37 (t, $J = 12$ Hz, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 4.58 (t, $J = 7.5$ Hz, 1H: 3 α); 4.74 (broad dd, $J = 13$ and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.86 (mt, 1H: 2 α); 4.89 (broad d, $J = 10$ Hz, 1H: 1 α); 5.27 (dd, $J = 12$ and 4 Hz, 1H: 4 α); 5.29 (broad d, $J = 5$ Hz, 1H: 5 α); 5.89 (d, $J = 9.5$ Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.50 (d, $J = 10$ Hz, 1H: NH in 2); from 6.50 to 6.70 (mt, 3H: aromatic Hs in the ortho and in the para positions with respect to the dimethylamino); from 7.15 to 7.35 (mt, 5H: aromatic Hs in 6); 7.20 (t, $J = 8$ Hz, 1H: aromatic H in the meta position with respect to the dimethylamino); 7.43 (limiting AB, 2H: 1' H_4 and 1' H_5); 7.82 (mt, 1H: 1' H_6); 8.38 (d, $J = 10$ Hz, 1H: NH in 1); 8.73 (d, $J = 9.5$ Hz, 1H: NH in 6); 11.61 (s, 1H: OH).

EXAMPLE 29: Preparation of 4 ϵ -methylthio-de(4'-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 56 erlenmeyer flasks, as described in Example 3, with 1

ml of a 20 g/l solution of (R,S)-3-methylthiophenyl-alanine hydrochloride, synthesized as in Example 34-11, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.68 litres of must
5 recovered from the 56 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The
10 chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with
15 plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the novel derivative of pristinamycin I_A are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 54% of water and 46% acetonitrile and injected onto a
20 semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 55% 100 mM phosphate buffer, pH 2.9, and 45% of acetonitrile. The fractions containing the novel pristinamycin are combined and extracted with one
25 volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 20 mg of 4 ϵ -methylthio-de(4 ζ -dimethyl-amino)pristinamycin I_A are obtained.

NMR spectrum. ^1H (400 MHz, CDCl_3 , δ in ppm, ref. TMS): 0.56 (dd, $J = 16$ and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.90 (t, $J = 7.5$ Hz, 3H: CH_3 in 2 γ); 1.13 (mt, 1H: 1H of the CH_2 in 3 β); 1.28 (mt, 1H: 1H of the CH_2 in 3 γ); 1.32 (d, $J = 6.5$ Hz, 3H: CH_3 in 1 γ); 1.58 (mt, 1H: the other H of the CH_2 in 3 γ); 1.62 and 1.74 (2 mts, 1H each: CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.25 and 2.35 (respectively, mt and broad d, $J = 16.5$ Hz, 1H each: CH_2 in 5 δ); 2.39 (d, $J = 16$ Hz, 1H: the other H of the CH_2 in 5 β); 2.43 (s, 3H: SCH_3); 2.82 (dt, $J = 13$ and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.98 (dd, $J = 12$ and 4.5 Hz, 1H: 1H of the CH_2 in 4 β); 3.26 (s, 3H: NCH_3); 3.30 (t, $J = 12$ Hz, 1H: 1H of CH_2 in 3 δ); 3.38 (mt, 1H: the other H of the CH_2 in 4 β); 3.57 (mt, 1H: the other H of the CH_2 in 3 δ); 4.56 (t, $J = 7.5$ Hz, 1H: 3 α); 4.74 (broad dd, $J = 13$ and 8 Hz, 1H: the other H of the CH_2 in 5 ϵ); 4.84 (mt, 1H: 2 α); 4.89 (dd, $J = 10$ and 1 Hz, 1H: 1 α); 5.29 (dd, $J = 12$ and 4.5 Hz, 1H: 4 α); 5.32 (broad d, $J = 5.5$ Hz, 1H: 5 α); 5.88 (d, $J = 9.5$ Hz, 1H: 6 α); 5.90 (mt, 1H: 1 β); 6.51 (d, $J = 10$ Hz, 1H: NH in 2); 6.99 (broad d, $J = 8$ Hz, 1H: aromatic H in the para position with respect to the methylthio); 7.10 and 7.15 (respectively, broad s and broad d, $J = 8$ Hz, 1H each: aromatic Hs in the ortho position with respect to the methylthio); from 7.15 to 7.35 (mt, 6H: aromatic Hs in 6 and aromatic Hs in the meta position with respect to the methylthio); 7.43 (broad d, $J = 8$ Hz, 1H: 1' H_4);

7.52 (dd, $J = 8$ and 4 Hz, $1H: 1'H_5$); 7.79 (broad d, $J = 4$ Hz, $1H: 1'H_6$); 8.38 (d, $J = 10$ Hz, $1H: NH$ in 1); 8.73 (d, $J = 9.5$ Hz, $1H: NH$ in 6); 11.62 (s, $1H: OH$).

EXAMPLE 30: Preparation of 4 ϵ -ethoxy-de(4 ζ -dimethylamino)pristinamycin I $_A$.

Strain SP92::pVRC508 is cultured in production medium using 60 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-3-O-ethyltyrosine hydrochloride, synthesized as in Example 37-1, in 0.2N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 1.8 litres of must recovered from the 60 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing the novel derivative of pristinamycin I $_A$ are combined and evaporated. 19 mg of dry residue are obtained. The latter is taken up in 3 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a

semi-preparative Nucleosil 7 μ C8 10 $\times$ 250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 60% 100 mM phosphate buffer, pH 2.9, and 40% of acetonitrile. The fractions containing the novel
 5 pristinamycin are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 15.8 mg of 4 ϵ -O-ethoxy-de(4 ζ -dimethyl-amino)pristinamycin I_A are obtained.

10 NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm, ref. TMS): 0.55 (dd, J = 16 and 5.5 Hz, 1H: 1H of the CH₂ in 5 β); 0.90 (t, J = 7.5 Hz, 3H: CH₃ in 2 γ); 1.12 (mt, 1H: 1H of the CH₂ in 3 β); 1.20 (mt, 1H: 1H of the CH₂ in 3 γ); 1.31 (d, J = 6.5 Hz, 3H: CH₃ in 1 γ); 1.49
 15 (t, J = 7 Hz, 3H: CH₃ of the ethyl); 1.54 (mt, 1H: the other H of the CH₂ in 3 γ); 1.63 and 1.73 (2 mts, 1H each: CH₂ in 2 β); 2.02 (mt, 1H: the other H of the CH₂ in 3 β); 2.22 and 2.33 (respectively, mt and broad d, J = 16.5 Hz, 1H each: CH₂ in 5 δ); 2.46 (d, J = 16 Hz, 1H: 20 the other H of the CH₂ in 5 β); 2.83 (dt, J = 13 and 4 Hz, 1H: 1H of the CH₂ in 5 ϵ); 2.95 (dd, J = 12 and 4 Hz, 1H: 1H of the CH₂ in 4 β); 3.22 (mt, 1H: 1H of the CH₂ in 3 δ); 3.27 (s, 3H: NCH₃); 3.39 (t, J = 12 Hz, 1H: the other H of the CH₂ in 4 β); 3.53 (mt, 1H: the other
 25 H of the CH₂ in 3 δ); 3.93 and 4.03 (2 mts, 1H each: OCH₂ of the ethyl); 4.56 (dd, J = 7 and 5.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 8 Hz, 1H: the other H of the CH₂ in 5 ϵ); 4.82 (mt, 1H: 2 α); 4.88 (dd, J = 10

and 1 Hz, 1H: 1 α); 5.23 (dd, J = 12 and 4 Hz, 1H: 4 α); 5.23 (broad d, J = 5.5 Hz, 1H: 5 α); 5.87 (d, J = 9.5 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β); 6.47 (d, J = 10 Hz, 1H: NH in 2); 6.80 (mt, 3H: aromatic H in the ortho and in the para positions with respect to the ethoxy); from 7.10 to 7.35 (mt, 6H: aromatic Hs in 6 and aromatic Hs in the meta position with respect to the ethoxy); 7.43 (dd, J = 8 and 1 Hz, 1H: 1'H₄); 7.50 (dd, J = 8 and 4 Hz, 1H: 1'H₅); 7.77 (dd, J = 4 and 1 Hz, 1H: 1'H₆); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.70 (d, J = 9.5 Hz, 1H: NH in 6); 11.60 (s, 1H: OH).

EXAMPLE 31: Preparation of 4'-ethylthio-de (4'-dimethylamino)pristinamycin I_a

Strain SP92::pVRC508 is cultured in production medium using 2 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (S)-4-ethylthiophenylalanine hydrochloride, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 60 ml of must recovered from the 2 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times 0.5 volumes of dichloromethane. The chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in

20 ml of dichloromethane and injected onto a silica (30 g) column which is mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 ζ -ethylthio-de(4 ζ -dimethylamino)pristinamycin I $_A$ are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting of 60% of water and 40% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 ζ -ethylthio-de(4 ζ -dimethylamino)-pristinamycin I $_A$ are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 7 mg of 4 ζ -ethylthio-de(4 ζ -dimethylamino)-pristinamycin I $_A$ are obtained.

NMR spectrum. ^1H (400 MHz, CDCl_3 , δ in ppm):
 0.68 (dd, $J = 16$ and 6 Hz, 1H: 1H of the CH_2 in 5 β);
 0.92 (t, $J = 7.5$ Hz, 3H: CH_3 in 2 γ); from 1.10 to 1.40 (mt, 5H: 1H of the CH_2 in 3 β and 1H of the CH_2 in 3 γ and CH_3 of the ethyl); 1.32 (d, $J = 7$ Hz, 3H: CH_3 in 1 γ); from 1.45 to 1.85 (mt, 3H: the other H of the CH_2 in 3 γ and CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.18 and 2.37 (respectively, mt and broad d, $J = 16.5$ Hz, 1H each: CH_2 in 5 δ); 2.45 (broad d, $J = 16$ Hz, 1H: the other H of the CH_2 in 5 β); 2.85 (dt, $J = 13$ and 4 Hz, 1H: 1H of the CH_2 in 5 ϵ); 2.90 (mt, 2H:

ArSCH₂ ethyl); 2.98 (dd, J = 12 and 4 Hz, 1H: 1H of the CH₂ in 4 β); 3.25 (s, 3H: NCH₃); 3.35 (mt, 1H: 1H of the CH₂ in 3 δ); 3.39 (t, J = 12 Hz, 1H: the other H of the CH₂ in 4 β); 3.57 (mt, 1H: the other H of the CH₂ in 3 δ); 4.55 (t, J = 7.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and 7.5 Hz, 1H, : the other H of the CH₂ in 5 ε); 4.85 (mt, 1H: 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); from 5.25 to 5.40 (mt, 2H: 5 α and 4 α); 5.88 (d, J = 9.5 Hz, 1H: 6 α); 5.91 (mt, 1H: 1 β); 6.55 (d, J = 9.5 Hz, 1H: NH in 2); 7.10 (d, J = 8 Hz, 2H: aromatic Hs in 4 δ); from 7.10 to 7.35 (mt, 7H: aromatic Hs in 6 and 4 ε); 7.44 (limiting AB, 2H: 1'H₄ and 1'H₅); 7.74 (mt, 1H: 1'H₆); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 9.5 Hz, 1H: NH in 6); 11.62 (s, 1H: OH).

15 **EXAMPLE 32: Preparation of 4{*ε*-ethyl-de(4{*ε*-dimethylamino)pristinamycin I₁**

Strain SP92::pVRC508 is cultured in production medium using 2 erlenmeyer flasks, as described in Example 3, with 1 ml of a 20 g/l solution of (R,S)-4-ethylphenylalanine, synthesized as in Example 33, in 0.1N sodium hydroxide solution being added at 16h. At the end of 40h of culture, the 60 ml of must recovered from the 2 erlenmeyer flasks is extracted with 2 volumes of a mixture consisting of 66% 100 mM phosphate buffer, pH 2.9, and 34% acetonitrile, and then centrifuged. The supernatant is extracted with 2 times [lacuna] volumes of dichloromethane. The

chloromethylene phases are washed with water and then combined, dried over sodium sulphate and evaporated. The dry extract is taken up in 20 ml of dichloromethane and injected onto a silica (30 g) column which is
 5 mounted in dichloromethane and eluted successively with plateaus of from 0 to 10% methanol in dichloromethane. The fractions containing 4 ζ -ethyl-de(4 ζ -dimethyl-amino)pristinamycin I $_A$ are combined and evaporated. The dry residue is taken up in 7 ml of a mixture consisting
 10 of 52% of water and 48% acetonitrile and injected onto a semi-preparative Nucleosil 7 μ C8 10x250 mm (Macherey Nagel) column, which is eluted with a mixture consisting of 52% 100 mM phosphate buffer, pH 2.9, and 48% of acetonitrile. The fractions containing 4 ζ -ethyl-
 15 de(4 ζ -dimethylamino)pristinamycin I $_A$ are combined and extracted with one volume of dichloromethane. The organic phase is washed with water, dried over sodium sulphate and then evaporated. 0.50 mg of 4 ζ -ethyl-de(4 ζ -dimethylamino)pristinamycin I $_A$ are obtained.

20 NMR spectrum. ^1H (400 MHz, CDCl_3 , δ in ppm, ref. TMS): 0.42 (dd, $J = 16$ and 5.5 Hz, 1H: 1H of the CH_2 in 5 β); 0.92 (t, $J = 7.5$ Hz, 3H: CH_3 in 2 γ); from 1.10 to 1.40 (mt, 2H: 1H of the CH_2 in 3 β and 1H of the CH_2 in 3 γ); 1.23 (t, $J = 7.5$ Hz, 3H: CH_3 of the ethyl);
 25 1.35 (d, $J = 7$ Hz, 3H: CH_3 in 1 γ); from 1.45 to 1.85 (mt, 3H: the other H of the CH_2 in 3 γ and CH_2 in 2 β); 2.02 (mt, 1H: the other H of the CH_2 in 3 β); 2.15 and from 2.25 to 2.40 (2 mts, 1H each: CH_2 in 5 δ); from

2.25 to 2.40 (mt, 1H: the other H of the CH₂ in 5 β);
 2.60 (q, J = 7.5 Hz, 2H: ArCH₂ of the ethyl); 2.83 (dt,
 J = 13 and 4 Hz, 1H: 1H of the CH₂ in 5 ε); 2.98 (dd, J
 = 12 and 4 Hz, 1H: 1H of the CH₂ in 4 β); from 3.25 to
 5 3.35 (mt, 1H: 1H of the CH₂ in 3 δ); 3.27 (s, 3H: NCH₃);
 3.39 (t, J = 12 Hz, 1H: the other H of the CH₂ in 4 β);
 3.59 (mt, 1H: the other H of the CH₂ in 3 δ); 4.58 (dd,
 J = 7 and 6.5 Hz, 1H: 3 α); 4.75 (broad dd, J = 13 and
 8 Hz, 1H: the other H of the CH₂ in 5 ε); 4.87 (mt, 1H:
 10 2 α); 4.89 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.24 (broad
 d, J = 5.5 Hz, 1H: 5 α); 5.29 (dd, J = 12 and 4 Hz, 1H:
 4 α); 5.88 (d, J = 10 Hz, 1H: 6 α); 5.92 (mt, 1H: 1 β);
 6.73 (d, J = 10 Hz, 1H: NH in 2); from 7.10 to 7.35
 (mt, 9H: aromatic Hs in 6 - 4 ε and 4 δ); 7.44 (dd, J =
 15 8.5 and 1.5 Hz, 1H: 1'H₄); 7.50 (dd, J = 8.5 and 4.5 Hz,
 1H: 1'H₅); 7.80 (dd, J = 4.5 and 1.5 Hz, 1H: 1'H₆); 8.38
 (d, J = 10 Hz, 1H: NH in 1); 8.75 (d, J = 10 Hz, 1H: NH
 in 6); 11.66 (s, 1H: OH).

Using the same fractions derived from the
 20 silica column described above, which fractions also
 contain the novel pristinamycin I_R derivative, 0.3 mg of
 {*ε*-ethyl-de(4*ζ*-dimethylamino)pristinamycin I_R is isolated
 by carrying out semi-preparative column chromatography
 as described above.

25 NMR spectrum. ¹H (400 MHz, CDCl₃, δ in ppm):
 0.04 (mt 1H: 1H of the CH₂ in 5 β); 0.92 (t, J = 7.5 Hz,
 3H: CH₃ in 2 γ); from 1.10 to 1.40 (mt, 2H: 1H of the

CH₂ in 5 δ and 1H of the CH₂ in 5 γ); 1.18 (t, J = 7.5 Hz, 3H: CH₃ of the ethyl); 1.30 (d, J = 6.5 Hz, 3H: CH₃ in 1 γ); from 1.45 to 1.85 (mt, 7H: the other H of the CH₂ in 5 γ - the other H of the CH₂ in 5 δ - 1H of the CH₂ in 3 β - CH₂ in 3 γ and CH₂ in 2 β); 1.81 (broad d, J = 13 Hz, 1H: the other H of the CH₂ in 5 β); 2.02 (mt, 1H: the other H of the CH₂ in 3 β); 2.40 (dt, J = 13 and 4 Hz, 1H: 1H of the CH₂ in 5 ϵ); 2.65 (q, J = 7.5 Hz, 2H: ArCH₂ of the ethyl); 2.97 and 3.09 (respectively, dd and t, J = 12 and 5 Hz and J = 12 Hz, 1H each: CH₂ in 4 β); 3.50 and 3.60 (2 mts, 1H each: CH₂ in 3 δ); 4.13 (dd, J = 8 and 5 Hz, 1H: 3 α); 4.49 (broad d, J = 13 Hz, 1H: the other H of the CH₂ in 5 ϵ); 4.70 (mt, 2H: 5 α and 4 α); 4.77 (mt, 1H: 2 α); 4.83 (dd, J = 10 and 1 Hz, 1H: 1 α); 5.50 (d, J = 7 Hz, 1H: 6 α); 5.74 (mt, 1H: 1 β); 6.09 (d, J = 4 Hz, 1H: NH in 4); 6.72 (unres. comp., 1H: NH in 2); 7.07 (d, J = 8 Hz, 2H: aromatic Hs in 4 ϵ); 7.15 (d, J = 8 Hz, 2H: aromatic Hs in 4 δ); from 7.15 to 7.35 (mt, 5H: aromatic Hs in 6); 7.40 (dd, J = 8 and 1 Hz, 1H: 1'H₄); 7.45 (dd, J = 8 and 4 Hz, 1H: 1'H₅); 7.92 (dd, J = 4 and 1 Hz, 1H: 1'H₆); 8.40 (unres. comp., 1H: NH in 6); 8.50 (d, J = 10 Hz, 1H: NH in 1); 11.72 (s, 1H: OH).

EXAMPLE 33: Preparation of derivatives of phenylalanine and of phenylpyruvic acid which have already been described.

Phenylalanine, and its derivatives

4-methoxyphenylalanine, 4-bromophenylalanine,
4-chlorophenylalanine, 4-iodophenylalanine,
4-trifluoromethylphenylalanine, 4-aminophenylalanine
and 3-methoxyphenylalanine, which are employed in this
5 work, are commercially available.

The following derivatives of phenylalanine
can be prepared in accordance with methods described in
the literature.

(RS)-4-dimethylaminophenylalanine

10 D.F. Elliott, A.T. Fuller, C.R. Harrington,
J. Chem. Soc., 1948, 85-89.

(RS)-4-diethylaminophenylalanine

Moldaver B.L., Pushkareva Z.V., Zhur.
Obshechi Khim., 31, 1560-1569 (1961); C.A. 1961,
15 22226f.; J.A. Stock, J. Chem. Soc, 1959, 90-97

(RS)-4-ethylaminophenylalanine

F. Bergel, J.A. Stock, J. Chem. Soc, 1959,
90-97.

(RS)-4-phenylphenylalanine

20 J.V. Braun, J. Nelles, Berichte, 66B, 1933,
1464-1470.

(RS)-4-methylphenylalanine

R.R., Herr, T. Enjoki, J.P. Dailey,
J. Am. Chem. Soc, 1957, 79, 4229-4231.

25 (RS)-4-methylthiophenylalanine and (R,S)-4-
ethylthiophenylalanine

R.L. Colescott, R.R. Herr, J.P. Dailey
J. Am. Chem. Soc, 1957, 79, 4232-4235.

(RS)-4-methoxycarbonylphenylalanine

H. Cleland, J. Org. Chem., 1969, 34, 747.

(RS)-2,4-dimethylphenylalanine

R.R., Herr, T. Enjoki, J.P. Dailey,

5 J. Am. Chem. Soc, 1957, 79, 4229-4231.

(RS)-3,4-dimethylphenylalanine

R.R., Herr, T. Enjoki, J.P. Dailey,

J. Am. Chem. Soc, 1957, 79, 4229-4231.

(RS)-3-trifluoromethylphenylalanine

10 hydrochloride

R. Filler and H. Novar. J. Org. Chem, 1960,

25, 733-736.

(S)-4-aminomethylphenylalanine

G.E. Stokker, W.F. Hoffman and C.F. Homnick,

15 J. Org. Chem., 1993, 58, 5015-5017.

(R,S)-3-methylphenylalanine

J.H. Burckhalter, V.C. Stephens, J.A.C.S.

1951, 73, 56-58.

(R,S)-4-acetylphenylalanine

20 J.I. Degaw et coll., J. Med. Che., 1969, 11,

225-227

(S)-4-O-allyltyrosine

A. Loffet, H. Zang, Int. J. Pept. Protein.

Res., 1993, 42, 346

25 **(S)-4-O-ethyltyrosine**

Y. Sasaki et coll., Chem. Pharm, Bull., 1982,

30, 4435

(RS)-4-ethylphenylalanine

A. Zhuze et coll., Coll., Czech. Chem.

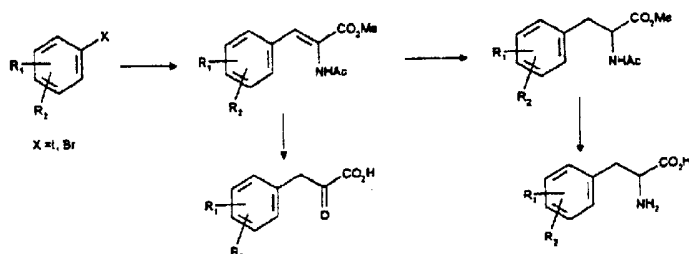
Commun., 1965, 62, 2648

4-tert-butylphenylpyruvic acid can be

- 5 prepared in accordance with R. Breslow, J.W. Canary, M. Varney, S.T. Waddell and D. Yang, J. Am. Chem. Soc., 1990, 112, 5212-5219.

The other derivatives of phenylalanine were prepared in accordance with Examples 34 to 42 which are
 10 given below. In these examples, flash chromatography was carried out under a mean nitrogen pressure of 50 kPa using a silica of granule size 40-53 μm , in accordance with Still et al., J. Org. Chem., 43, 2923, (1978).

- 15 **EXAMPLE 34: Preparation of derivatives of phenylalanine and of a derivative of phenylpyruvic acid using method A.**

**34-1 (RS)-4-methylaminophenylalanine,****hydrochloride**

- 20 37 ml of 12 N hydrochloric acid are added to 3.70 g of methyl N-acetyl-4-methylaminophenylalaninate, and the mixture is heated to reflux, while stirring,

for 8 h. After one night at room temperature, the reaction medium is concentrated to dryness under reduced pressure (50 kPa), and the residue is taken up in a mixture of 50 ml of toluene and 50 ml of ethanol, and this mixture is concentrated once again. After drying in a desiccator under reduced pressure (2.6 kPa), 4.18 g (100%) of (RS)-4-methylaminophenylalanine dihydrochloride are obtained in the form of a hygroscopic light beige solid which melts at 158°C.

34-2: Methyl (RS)-N-acetyl-4-methylaminophenylalaninate

0.4 g of 10% palladium on charcoal, and then 50 ml of absolute ethanol, are added to 4 g of methyl 4-methylamino-2-acetamidocinnamate which is placed under a nitrogen atmosphere in an autoclave. The mixture is placed under a pressure of 5.5 bar of hydrogen and heated at 50°C for 15 h with stirring. After stabilizing the temperature at 26°C, and returning the pressure to atmospheric, the medium is filtered through Clarcel®, washed with ethanol and then concentrated to dryness under reduced pressure (2.6 kPa). This results in 3.73 g of methyl N-acetyl-4-methylaminophenylalaninate in the form of white crystals which melt at 118°C.

34-3: Methyl 4-methylamino-2-acetamidocinnamate

5.75 g of methyl 2-acetamidoacrylate, 0.185 g

of palladium acetate, 8.1 g of tetrabutylammonium chloride and 6.03 g of sodium hydrogen carbonate are added to a 3-necked flask which is placed under nitrogen, and then 6.5 g of 4-iodo-N-methylalanine, in solution in 200 ml of DMF, are added to this mixture. The mixture is heated at 82°C for 16 h 30 min and then, after having been cooled down, is poured into 1000 ml of distilled water. The medium is extracted with 250 ml of CH₂Cl₂, and the organic phase is separated off; the aqueous phase is then washed twice with 250 ml of CH₂Cl₂. The organic phases are combined, dried over sodium sulphate, filtered and concentrated under reduced pressure (50 kPa) at 70°C to yield a brown oil which is purified by flash chromatography (eluent, AcOEt/cyclohexane and then pure AcOEt).

In this way, 4 g of methyl 4-methylamino-2-acetamidocinnamate is obtained in the form of a yellow solid (Merck Silica 5719, $R_f = 0.48$), which is employed in this form.

N-Methyl-p-iodoaniline can be prepared in accordance with: S. Krishnamurthy, Tetrahedron Letters, 33, 3315-3318, 1982.

34-4: 4-methylaminophenylpyruvic acid

2.4 g of methyl 4-methylamino-2-acetamidocinnamate and 32 ml of 12 N hydrochloric acid are placed in a round-bottomed flask. The mixture is heated to reflux for 3 h and then cooled down and washed twice with 20 ml of diethyl ether. The aqueous

phase is cooled down to -10°C and the precipitate which is obtained is filtered and then rinsed with a minimum of cold hydrochloric acid. The solid which is obtained is dried in a desiccator under reduced pressure in order to yield 1.1 g of 4-methylaminophenylpyruvic acid in the form of a light beige solid which melts at 210°C .

34-5: (R,S)-3-Fluoro-4-methylphenylalanine hydrochloride

0.6 g of (R,S)-3-fluoro-4-methylphenylalanine hydrochloride is obtained in the form of white crystals which melt at a temperature greater than 260°C by proceeding as in Example 34-1 but using 1.6 g of methyl N-acetyl(3-fluoro-4-methyl)phenylalaninate.

34-6: Methyl (R,S)-N-acetyl-(3-fluoro-4-methyl)phenylalaninate

1.6 g of methyl N-acetyl-(3-fluoro-4-methyl)phenylalaninate are obtained in the form of a colourless oil (Merck Silica 5719, $R_f = 0.46$; eluent $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ 50/50), by proceeding as in Example 34-2 but using 1.9 g of methyl (4-methyl-3-fluoro)-2-acetamidocinnamate and 0.2 g of 10% palladium on charcoal in 230 ml of ethanol.

34-7: Methyl (3-fluoro-4-methyl)-2-acetamidocinnamate

2.6 g of methyl (3-fluoro-4-methyl)-2-acetamidocinnamate are obtained in the form of a white solid which melts at 163°C by proceeding as in Example

34-3 but using 3.6 g of methyl 2-acetamidoacrylate,
0.12 g of palladium acetate, 5.2 g of
tetrabutylammonium chloride, 3.8 g of sodium hydrogen
carbonate and 4 g of 2-fluoro-4-bromotoluene in
5 solution in 120 ml of anhydrous DMF.

34-8: (R,S)-4-Trifluoromethoxyphenylalanine
hydrochloride or (R,S)-O-trifluoromethyltyrosine
hydrochloride

1.5 g of (R,S)-4-trifluoromethoxyphenyl-
10 alanine hydrochloride are obtained in the form of white
crystals which melt at 260°C by proceeding as in
Example 34-1 but using 3 g of methyl N-acetyl-(4-
trifluoromethoxy)phenylalaninate and 30 ml of 12 N
hydrochloric acid.

15 34-9: Methyl (R,S)-N-acetyl-(4-
trifluoromethoxy)phenylalaninate

3 g of methyl N-acetyl-(4-trifluoroethoxy)-
phenylalaninate are obtained in the form of a white
solid which melts at 80°C by proceeding as in Example
20 34-2 but using 3.1 g of methyl (4-trifluoromethoxy)-2-
acetamidocinnamate and 0.3 g of 10% palladium on
charcoal in 50 ml of ethanol.

34-10: Methyl 4-trifluoromethoxy-2-
acetamidocinnamate

25 3.1 g of methyl (4-trifluoromethoxy)-2-
acetamidocinnamate are obtained in the form of a white
solid which melts at 135°C by proceeding as in Example
34-3 but using 4.3 g of methyl 2-acetamido acrylate,

0.14 g of palladium acetate, 6.1 g of tetrabutylammonium chloride, 4.6 g of sodium hydrogen carbonate and 5 g of 4-trifluoromethoxybromobenzene in solution in 150 ml of anhydrous DMF.

5 34-11: (R,S)-3-Methylthiophenylalanine hydrochloride

1.38 g of (R,S)-3-methylthiophenylalanine hydrochloride are obtained in the form of white crystals which melt at 190°C by proceeding as in
10 Example 34-1 but using 3.3 g of methyl N-acetyl-3-methylthiophenylalaninate and 40 ml of 12 N hydrochloric acid.

34-12: Methyl (RS)-N-acetyl-3-methylthiophenylalaninate

15 3.72 g of methyl 3-methylthio-2-acetamidocinnamate, dissolved in 100 ml of methanol, and 30 ml of tetrahydrofuran are placed in a round-bottomed flask, and 1.4 g of magnesium are then added. After reacting for 20 min, the mixture is cooled in an
20 ice bath and a further 1.4 g of magnesium are then added. The mixture is stirred at room temperature for 18 h and then poured into 1.4 l of distilled water and 300 ml of CH₂Cl₂; this mixture is then filtered through Clarcel®. The aqueous phase is adjusted to pH 6 by
25 adding 12 N hydrochloric acid and then separated off and washed with 100 ml of CH₂Cl₂. The organic phases are collected, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in

order to yield 3.42 g of methyl N-acetyl-3-methylthiophenylalaninate in the form of a colourless oil (Merck Silica 5719, $R_f=0.5$; AcOEt).

34-13: Methyl 3-methylthio-2-

5 acetamidocinnamate

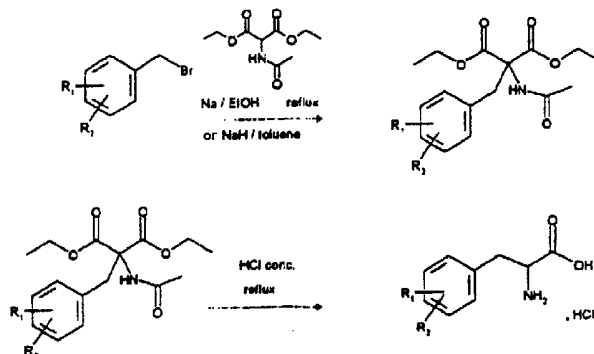
4.8 g of methyl (3-methylthio)-2-acetamidocinnamate are obtained in the form of a white solid which melts at 139°C by proceeding as in Example 34-3 but using 5.6 g of methyl 2-acetamidoacrylate,
10 0.18 g of palladium acetate, 8.2 g of tetrabutylammonium chloride, 5.86 g of sodium hydrogen carbonate and 6.5 g of 3-iodo-1-methylthiobenzene dissolved in 160 ml of anhydrous DMF.

34-14: 3-Iodomethylthiobenzene

15 20 ml of distilled water and 20 ml of 12 N hydrochloric acid are placed, with stirring, in a three-necked flask, and 10 ml of 3-methylthioaniline are then added using a dropping funnel. The mixture is warmed to ensure dissolution and is then cooled down to
20 5°C. 5.86 g of sodium nitrite dissolved in 15 ml of water are subsequently added slowly, using a dropping funnel, while maintaining the temperature between 5 and 8°C. 20 min after having completed the addition, 13.57 g of potassium iodide dissolved in 15 ml of water
25 are added over a period of 10 min and the mixture is then stirred at room temperature for 15 h. The oil which forms is separated from the aqueous phase by decantation, and an aqueous solution of sodium

thiosulphate is then added to it. The aqueous phase is decanted and the product is extracted with 100 ml of dichloromethane. The organic phase is washed with 100 ml of water, and the aqueous phase is adjusted to pH 9 with concentrated sodium hydroxide solution, and then separated off. The organic phase is washed with 2 times 100 ml of water, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure (50 kPa) at 40°C. The resulting product is purified by flash chromatography (eluent, cyclohexane) in order to yield 13 g of 3-iodo-1-methylthiobenzene in the form of a yellow liquid (Merck Silica 5719, $R_f=0.8$ /cyclohexane).

EXAMPLE 35: Preparation of derivatives of phenylalanine using method B.



35-1: (RS)-4-tert-butylphenylalanine

25 g of diethyl 4-(tert-butyl)benzylacetamidomalonate and 250 ml of 37% hydrochloric acid are added to a three-necked flask which is surmounted

by a condenser. The mixture is stirred and heated to reflux until there is no further evolution of gas. After the reaction medium has been cooled down, the precipitate which is obtained is filtered and then
5 recrystallized in acetonitrile to yield 25.6 g of (R,S)-4-tert-butylphenylalanine hydrochloride in the form of a white solid which melts at 234°C.

35-2: Diethyl 4-(tert-butyl)benzylacetamidomalonate

10 25 g of 4-(tert-butyl)benzyl bromide, 50 ml of anhydrous toluene and 3.1 g of sodium hydride in 80% suspension in oil are added to a three-necked flask which is surmounted by a condenser, followed by 21.8 g of diethyl acetamidomalonate. The mixture is heated at
15 110°C for 17 h. After it has been cooled down, 15 ml of absolute ethanol, then 15 ml of 50% ethanol and then 50 ml of water are added slowly to it using a dropping funnel. The organic phase is decanted and the aqueous phase is washed with 3 times 50 ml of diethyl ether.
20 The organic phases are combined, washed with water and then dried over sodium sulphate. Following filtration and concentration under reduced pressure, the product is crystallized in petroleum ether in order to yield 25 g of diethyl 4-(tert-butyl)benzylacetamidomalonate
25 in the form of a white solid which melts at 80°C.

35-3: (R,S)-3-Methylaminophenylalanine dihydrochloride

1.03 g of a yellow-beige solid are obtained

by proceeding as in Example 35-1 but using 1.17 g of diethyl 3-methylaminobenzylacetamidomalonate and 20 ml of 12 N hydrochloric acid. This yellow-beige solid is dissolved in 20 ml of absolute ethanol, and 0.4 g of animal charcoal is added to this solution. The solution is filtered through Clarcel and then filtered and concentrated under reduced pressure (50 kPa). The same procedure is repeated starting with 1 g of animal charcoal, and the solid which is obtained is triturated in 20 ml of ether. Following filtration and drying under reduced pressure (2.7 kPa) at 50°C, 0.65 g of (R,S)-3-methylaminophenylalanine dihydrochloride is obtained in the form of a white powder which melts at a temperature approaching 135°C (decomposition).

35-4: Diethyl 3-methylaminobenzylacetamidomalonate

3.11 ml of acetic anhydride are placed in a three-necked flask which is maintained under a nitrogen atmosphere. 1.51 ml of formic acid are subsequently added within 3 min at 0°C, and the mixture is then heated at 50°C for 2 hours. The mixture is allowed to return to room temperature, while shaking for 3 h 20 min, and 4 ml of anhydrous THF are added under nitrogen; the mixture is then cooled to -20°C. A solution of 4 g of diethyl 3-aminobenzylacetamidomalonate in a mixture of 15 ml of anhydrous THF and 15 ml of anhydrous dichloromethane is added within 10 min. Stirring is continued for 1 h

10 min at -20°C and then for 16 h at 20°C . The reaction mixture is concentrated to dryness under reduced pressure (50 kPa) at 30°C and then co-evaporated with 30 ml of anhydrous toluene in order to yield a white solid, which is dissolved in a mixture of 10 ml of anhydrous THF and 20 ml of anhydrous 1,2-dichloroethane, which solution is then placed in a three-necked flask under nitrogen.

The medium is cooled down to -5°C , and 1.55 ml of borane-dimethyl sulphide complex (2M solution in THF) are then added within 10 min. The mixture is allowed to return to room temperature, and the solution is heated to reflux for 3 h and then stirred at room temperature for 15 h. The reaction medium is cooled to 0°C , and 10 ml of MeOH are then added within 25 min. The mixture is stirred for 45 min at 0°C and then for 30 min at room temperature. It is then cooled to 0°C and HCl gas is bubbled in until a pH of 2 is reached. The mixture is heated at reflux for 1 h and is then concentrated to dryness under reduced pressure at 30°C in order to yield 5 g of a product which is taken up in 30 ml of an aqueous solution of NaHCO_3 and 30 ml of CH_2Cl_2 . The organic phase is decanted and the aqueous phase is washed with 20 ml of water. The organic phases are pooled, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure (2.6 kPa) in order to yield 3.43 g of a yellow oil, which is purified by

flash chromatography (eluent, AcOEt/cyclohexane 50/50). After drying under reduced pressure (2.7 kPa) at 20°C, 1.18 g of diethyl 3-methylaminobenzylacetamidomalonate are thus obtained in the form of a light beige solid which melts at 122°C.

35-5: Diethyl 3-aminobenzylacetamidomalonate

Diethyl 3-aminobenzylacetamidomalonate can be prepared as described in:

T.S. Osdene, D.N. Ward, W.H. Chapman and
10 H. Rakoff, J. Am. Chem. Soc., 81, 1959, 3100-3102.

35-6: (R,S)-3-Ethylaminophenylalanine
dihydrochloride

1.7 g of (R,S)-3-ethylaminophenylalanine dihydrochloride are obtained in the form of a
15 hygroscopic light beige solid, which contains
10 molar % of (R,S)-3-diethylaminophenylalanine dihydrochloride, by proceeding as in Example 34-1 but using 2 g of ethyl (R,S)-N-acetyl-3-ethylaminophenylalaninate and 30 ml of 12N hydrochloric acid.

20 35-7: (R,S)-N-acetyl-3-ethylaminophenylalaninate

3 g of ethyl (R,S)-N-acetyl-3-aminophenylalaninate, 40 ml of ethanol and 14 g of Raney nickel, which has previously been washed with distilled water
25 and ethanol, are placed in a round-bottomed flask under a nitrogen atmosphere. The mixture is heated to reflux for 19 h, cooled down, filtered through Clarcel®, and then concentrated to dryness under reduced pressure

(50 kPa) in order to yield 3.07 g of a colourless oil, which is purified by flash chromatography (eluent, AcOEt) in order to yield 2.1 g of ethyl (R,S)-N-acetyl-3-ethylaminophenylalaninate in the form of a colourless oil (Merck Silica 5719, $R_f=0.6$: AcOEt) which contains

5 10% ethyl (R,S)-N-acetyl-3-diethylaminophenylalaninate.

35-8: Ethyl (R,S)-N-acetyl-3-aminophenylalaninate

25 g of a mixture of ethyl (R,S)-N-acetyl-3-nitrophenylalaninate (75 mol %/mol) and diethyl

10 3-nitrobenzylacetamidomalonate (25 mol %/mol) are placed under nitrogen in an autoclave. 2.5 g of 10% palladium on charcoal and then 200 ml of dichloromethane are added. The mixture is placed under

15 a hydrogen pressure of 9 bar and then stirred at 18°C for 4 h. After returning the pressure to atmospheric, the reaction medium is filtered through Clarcel®, washed with dichloromethane and then concentrated to dryness under reduced pressure (50 kPa) in order to

20 yield a solid, which is recrystallized in 450 ml of distilled water under reflux and in the presence of 4 g of 3S animal charcoal. Following hot filtration through Clarcel®, the mixture is left to crystallize at 4°C, with the crystals being filtered and then dried in

25 order to yield 9.9 g of ethyl (R,S)-N-acetyl-3-aminophenylalaninate in the form of a light beige solid which melts at 106°C and which contains 5% of diethyl 3-aminobenzylacetamidomalonate.

35-9: Ethyl (R,S)-N-acetyl-3-nitrophenyl-
alaninate and diethyl 3-nitrobenzylacetamidomalonate

600 ml of absolute ethanol and then 7.9 g of sodium are placed, under a nitrogen atmosphere, in a three-necked flask which is surmounted by a condenser. Once dissolution is complete, 74.5 g of diethyl acetamidomalonate and then 60 g of 4-nitrobenzyl chloride in 200 ml of anhydrous ethanol are added. The mixture is heated to reflux for 16 h 30 min. After cooling, the reaction medium is concentrated under reduced pressure (50 kPa) and then taken up in a mixture of 500 ml of CH_2Cl_2 and 500 ml of water. The pH is adjusted to 7 by adding 0.5N sulphuric acid, and the organic phase is then separated off and the aqueous phase is washed with 2 times 200 ml of CH_2Cl_2 . The organic phases are pooled, washed with 200 ml of water saturated with sodium bicarbonate, separated off and then dried over magnesium sulphate. Following filtration and concentration under reduced pressure (50 kPa), the product is recrystallized in 600 ml of ethanol at reflux in order to yield, after crystallizing at ambient temperature, filtering and drying, 70.4 g of diethyl 3-nitrobenzylacetamidomalonate in the form of white crystals which melt at 156°C. The mother liquors are concentrated and then purified by flash chromatography (eluent, AcOEt) in order to yield 25.6 g of a mixture of ethyl N-acetyl-3-nitrophenylalaninate (75 mol %/mol) and diethyl

3-nitrobenzylacetamidomalonate (25 mol %/mol) in the form of a light beige solid, which is used in this form in the following step.

35-10: (RS)-3-Dimethylaminophenylalanine
5 dihydrochloride

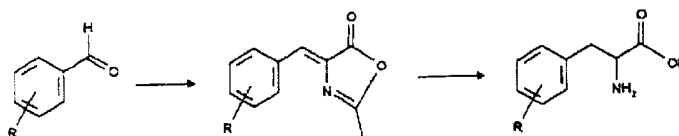
A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 0.72 g of ethyl (RS)-N-acetyl-3-dimethylaminophenylalaninate and 8.6 ml of 10N hydrochloric acid; the solid is subsequently
10 triturated in 50 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 0.68 g (93%) of (RS)-3-dimethylaminophenylalanine dihydrochloride is obtained in the form of a white solid which melts in the region of 120°C (decomposition).

15 35-11: Ethyl (RS)-N-acetyl-3-
dimethylaminophenylalaninate

4 g of ethyl (RS)-N-acetyl-3-aminophenylalaninate, prepared as described in Example 35-8, in 15 ml of DMF are placed in a three-
20 necked flask under a nitrogen atmosphere, and 5.5 ml of triethylamine, and then 2.5 ml of methyl iodide and 4 ml of dichloromethane, are added while maintaining the temperature in the region of 30°C using an icebath. The mixture is then warmed at 35°C for 18h. 1 ml of
25 methyl iodide dissolved in 1 ml of DMF is then added slowly while maintaining the temperature in the region of 30°C; 2.2 ml of triethylamine are then added and the mixture is subsequently warmed for a further 5h at

35°C. The mixture is brought to room temperature and then extracted with 100 ml of ethyl acetate and 150 ml of distilled water. The aqueous phase is separated off after settling and then rewashed with 2 times 70 ml of ethyl acetate. The organic phases are combined, washed with 2 times 80 ml of distilled water and then with 50 ml of distilled water which is saturated with NaCl. The organic phase is separated off after settling, dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure in order to yield 2.4 g of a product which is purified by flash chromatography (dichloromethane, MeOH 90/10). 0.72 g (16%) of ethyl (RS)-3-N-acetyl-3-dimethylamino phenylalaninate is thus obtained in the form of yellow crystals.

EXAMPLE 36: Preparation of derivatives of phenylalanine using method C.



36-1: (R,S)-4-Isopropylphenylalanine

7 g of red phosphorus and 8 g of 4-(isopropylbenzylidene)-2-methyl-5-oxazolone, in 45 ml of acetic anhydride, are placed in a three-necked flask, and then 35 ml of 57% hydriodic acid are added slowly, with stirring, using a dropping funnel. Once

the addition is complete, the mixture is heated to reflux for 3 h 30 min and then left at room temperature for 3 days. The reaction mixture is filtered and the solid which is obtained is rinsed twice with 10 ml of acetic acid on each occasion, and the filtrate is then concentrated to dryness under reduced pressure. The residue which is obtained is taken up in 100 ml of distilled water, and this solution is concentrated to dryness under reduced pressure in order to yield a solid which is taken up in 50 ml of distilled water; this solution is then extracted with 3 times 50 ml of diethyl ether after 0.5 g of sodium sulphite have been added. The ether is separated off and the aqueous phase is placed under reduced pressure in order to eliminate traces of diethyl ether. 2 g of animal charcoal are added to the aqueous phase, which is heated at 40-50°C, and then filtered through Clarcel®; rinsing then takes place with a minimum of water. The pH is adjusted to 5 by adding 32% ammonia at 4°C. The precipitate which is obtained is filtered in the cold, rinsed with 2 times 10 ml of water, with 10 ml of ethanol and then with 2 times 10 ml of ether in order to yield, after drying under reduced pressure at 20°C, 3.97 g of (R,S)-4-isopropylphenylalanine in the form of a white solid which melts at a temperature greater than 260°C. (See also Journal of the Takeda Research Laboratories, vol. 43; nos. 3/4, Dec. 1984, pp 53-76).

36-2: 4-(Isopropylbenzylidene)-2-methyl-5-oxazolone

18.52 g of N-acetylglycine, 10.6 g of sodium acetate, 20 ml of 4-isopropylbenzaldehyde and 57 ml of
5 acetic anhydride are placed in a round-bottomed flask which is provided with a condenser. The mixture is stirred for 30 min and then stirred for 1 h at 110°C and subsequently for 15 h at room temperature. The reaction medium is poured into 600 ml of water and
10 400 ml of petroleum ether which has previously been heated to 50°C. The organic phase is separated off and the aqueous phase is washed with 2 times 150 ml of petroleum ether.

The organic phases are combined, dried over
15 magnesium sulphate, filtered and concentrated under reduced pressure until the volume is 100 ml and a precipitate is obtained. The latter is filtered and washed with 2 times 50 ml of pentane in order to yield 8.2 g of 4-(isopropylbenzylidene)-2-methyl-5-oxazolone
20 in the form of a yellow solid which melts at 77°C.

36-3: (R,S)-4-Butylphenylalanine

0.35 g of (R,S)-4-butylphenylalanine is obtained in the form of a light beige solid which melts at a temperature greater than 260° by proceeding as in
25 Example 36-1 but using 1.49 g of red phosphorus, 1.8 g of 4-(butylbenzylidene)-2-methyl-5-oxazolone, in 9.23 ml of acetic anhydride, and 7.39 ml of 57% hydriodic acid.



36-4: 4-(Butylbenzylidene)-2-methyl-5-oxazolone

1.89 g of 4-(butylbenzylidene)-2-methyl-5-oxazolone are obtained in the form of a yellow solid which melts at 74°C by proceeding as in Example 36-2 but using 8.43 g of N-acetylglycine, 4.92 g of sodium acetate, 9.8 g of 4-butylbenzaldehyde and 26 ml of acetic anhydride.

EXAMPLE 37: Preparation of a derivative of phenylalanine using method D.

37-1: (R,S)-3-Ethoxyphenylalanine hydrochloride (or (R,S)-3-O-ethyltyrosine hydrochloride)

1 g of (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalanine, dissolved in 3.6 ml of hydrochloric dioxane, is placed in a round-bottomed flask, and the mixture is then stirred at room temperature for 5 h. The precipitate which forms is filtered, rinsed with dioxane and then ether, and then dried under reduced pressure (2.7 kPa) at 40°C to yield 0.65 g of (R,S)-3-ethoxyphenylalanine hydrochloride in the form of a white solid which melts at 200°C.

37-2: (R,S)-N-tert-Butoxycarbonyl-3-ethoxyphenylalanine

1.33 g of ethyl (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalaninate, dissolved in 8 ml of methanol, are placed in a round-bottomed flask, and 8 ml of 1N sodium hydroxide solution are then added. After the



mixture has been stirred at room temperature for 18 h, it is evaporated under reduced pressure and then acidified with 8.56 ml of 1N hydrochloric acid. The product is extracted with 2 times 10 ml of ethyl acetate, and the organic phases are pooled, washed with 2 times 10 ml of water, dried, filtered and then concentrated to dryness under reduced pressure to yield 1 g of (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalanine in the form of a yellow oil (Merck Silica 5719, $R_f=0.7$, eluent: toluene 80/MeOH 10/diethylamine 10).

37-3: (R,S)-N-tert-Butoxycarbonyl-3-ethoxyphenylalaninate

1.5 g of (R,S)-N-tert-butoxycarbonyl-3-tyrosine, dissolved in 7.5 ml of dry DMF, are placed in a three-necked flask under a nitrogen atmosphere, and 0.508 g of sodium hydride, as a 50% dispersion in oil, is then added. After the mixture has been stirred at room temperature for 2 h, 0.86 ml of iodoethane is added and the mixture is then stirred at room temperature for 4 h. The medium is filtered and the resulting solid is washed with 3 times 10 ml of water and then 2 times 10 ml of petroleum ether to yield, after drying under reduced pressure (2.7 kPa) at 30°C, 1.33 g of ethyl (R,S)-N-tert-butoxycarbonyl-3-ethoxyphenylalaninate in the form of a white solid.

37-4: (R,S)-N-tert-Butoxycarbonyl-3-tyrosine

18 g of (R,S)-3-tyrosine, dissolved in 180 ml



of dioxane, are placed, with stirring, in a three-necked flask, and 99 ml of 1N sodium hydroxide solution, followed by 26 g of di-tert-butyl dicarbonate, dissolved in 160 ml of dioxane, are then
5 added. After the mixture has been stirred for 36 h, it is concentrated under reduced pressure at 30°C and the residue is taken up in 100 ml of distilled water; this solution is acidified to pH 5 with 1N hydrochloric acid and then extracted with 2 times 200 ml of ethyl
10 acetate. The organic phase is dried over magnesium sulphate, filtered and then concentrated to dryness under reduced pressure at 30°C to yield 30 g of (R,S)-N-tert-butoxycarbonyl-3-tyrosine in the form of a white solid (Merck Silica 5719, $R_f=0.25$, eluent: toluene
15 80, MeOH 10, diethylamine 10).

EXAMPLE 38: Preparation of derivatives of phenylalanine using method E.

38-1: (RS)-4-Diallylaminophenylalanine dihydrochloride

20 A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 5.8 g of diethyl 4-diallylaminobenzylacetamido malonate and 48 ml of 10N hydrochloric acid; the solid is then triturated in 50 ml of acetone, filtered, then
25 triturated in 10 ml of dichloromethane, filtered and then rinsed with 3 times 10 ml of ethyl ether. After drying under reduced pressure (2.7 kPa) at 40°C, 4.41 g



of (RS)-4-diallylaminophenylalanine dihydrochloride are obtained in the form of an off-white solid which melts in the region of 135°C (decomposition).

5 38-2: (RS)-4-Allylaminophenylalanine
 dihydrochloride

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 3.27 g of diethyl 4-allylaminobenzylacetamidomalonate and 30 ml of 10N hydrochloric acid; the solid is then triturated
10 in 50 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2.3 g of (RS)-4-allylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 134°C (decomposition).

15 38-3: Diethyl 4-diallylaminobenzylacetamido-
 malonate and diethyl 4-allylaminobenzylacetamido-
 malonate

10 g of diethyl 4-aminobenzylacetamido-malonate dissolved in 150 ml of DMF are placed in a
20 three-necked flask which is surmounted with a dropping funnel and maintained under a nitrogen atmosphere. 6.57 ml of allyl bromide, and then 10.76 ml of triethylamine, are added slowly, at room temperature and while stirring. After stirring for 19h, a further
25 1.31 ml of allylbromide and 2.15 ml of triethylamine are then added and the mixture is stirred for 26h. The reaction medium is poured onto 1.5 l of distilled water and this mixture is extracted with 1 l of ethyl

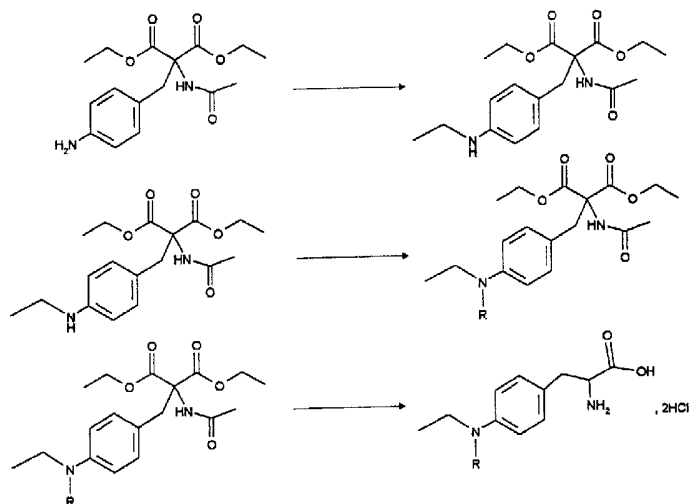


acetate. The aqueous phase is separated off after settling and washed with 2 times 500 ml of ethyl acetate. The organic phases are combined, washed with 500 ml of distilled water and then with 500 ml of water which is saturated with sodium chloride, separated off, dried over magnesium sulphate, filtered and then concentrated to dryness in order to yield a chestnut oil; this oil is purified by flash chromatography (eluant, CH_2Cl_2 90/AcOEt 10) in order to yield 6.66 g of diethyl 4-diallylaminobenzylacetamidomalonate in the form of a beige solid which melts at 94-96°C (R_f = 0.6, AcOEt 50/cyclohexane 50) and 3.49 g of diethyl 4-allylaminobenzylacetamidomalonate in the form of a beige solid which melts at 104-106°C (R_f = 0.45 AcOEt 50/cyclohexane 50).

The diethyl 4-aminobenzylacetamidomalonate can be prepared as described in J.B. Burckhalter, VC Stephens, J. Am. Chem. Soc. 56, 1951, 73.



EXAMPLE 39: Preparation of derivatives of phenylalanine using method F



39-1: (RS)-4-ethylisopropylphenylalanine dihydrochloride

- 5 A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 2.9 g of diethyl 4-ethylisopropylbenzylacetamidomalonate and 24.6 ml of 10N hydrochloric acid; the solid is then trituated in 20 ml of acetone, filtered and then dried
- 10 under reduced pressure (2.7 kPa) at 40°C. 2 g of (RS)-4-ethylisopropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 147°C (decomposition).



39-2: Diethyl 4-ethylisopropylaminobenzyl-
acetamidomalonate

15 g of diethyl 4-ethylaminobenzylacetamido-
malonate in 70 ml of THF are placed in a three-necked
5 flask which is maintained under a nitrogen atmosphere;
6.4 ml of 2-iodopropane, and then 8.4 ml of 1,5-
diazabicyclo[4.3.0]non-5-ene are added and the mixture
is then heated at 60°C for 24h. 2.13 ml of 2-
iodopropane, and then 8.4 ml of 1,5-
10 diazabicyclo[4.3.0]non-5-ene, are subsequently added
and the mixture is then heated for a further 24h at
60°C. The mixture is brought to room temperature and
then extracted with 50 ml of dichloromethane and 50 ml
of distilled water. The aqueous phase is separated off
15 after settling and then rewashed with 2 times 30 ml of
dichloromethane. The organic phases are combined,
washed with 60 ml of distilled water and then with
50 ml of distilled water which is saturated with NaCl.
The organic phase is separated off after settling,
20 dried over magnesium sulphate, filtered and then
concentrated to dryness under reduced pressure in order
to yield 16.2 g of a product which is purified by flash
chromatography (dichloromethane, MeOH 90/10). This
results in 4.59 g of a product which is recrystallized
25 in 45 ml of cyclohexane in order to yield 3.44 g of
diethyl 4-ethylisopropylaminobenzylacetamidomalonate in
the form of white crystals which melt at 80°C.



39-3: Diethyl 4-ethylaminobenzylacetamido-
malonate

Diethyl 4-ethylaminobenzylacetamidomalonate
can be prepared by proceeding as in Example 35-7 but
5 using 22 g of diethyl 4-aminobenzylacetamidomalonate,
500 ml of ethanol and 70 g of Raney nickel. This
results in 23.8 g of diethyl 4-ethylaminobenzyl-
acetamidomalonate in the form of an off-white solid
which melts at 136°C.

10 39-4: (RS)-4-Allylethylaminophenylalanine
dihydrochloride

A solid is obtained, after evaporation, by
proceeding as in Example 35-1 but using 4.54 g of
diethyl 4-allylethylbenzylacetamidomalonate and 37.9 ml
15 of 10N hydrochloric acid; the solid is then dried under
reduced pressure (2.7 kPa) at 40°C. 3.67 g of (RS)-4-
allylethylaminophenylalanine dihydrochloride are
obtained in the form of a brown solid which melts in
the region of 130°C (decomposition).

20 39-5: Diethyl 4-allylethylaminobenzyl-
acetamidomalonate

5.6 g of a solid are obtained, after
purification by flash chromatography (eluant, CH₂Cl₂/
AcOET 90-10 by volume), by proceeding as in Example
25 39-2 but using 8 g of diethyl 4-ethylaminobenzyl-
acetamidomalonate, 4 ml of allyl bromide and 5.82 ml of
1,5-diazabicyclo[4.3.0]non-5-ene in 50 ml of THF; the
solid is then recrystallized in 35 ml of cyclohexane.



This results in 5.43 g of diethyl 4-allylethylamino-benzylacetamidomalonate in the form of a white solid which melts at 86°C.

39-6: (RS)-4-Ethylpropylaminophenylalanine
5 dihydrochloride

A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 2.5 g of diethyl 4-ethylpropylaminobenzylacetamidomalonate and 21 ml of 10N hydrochloric acid;. The solid is then
10 dried under reduced pressure (2.7 kPa) at 40°C. 2 g (97%) of (RS)-4-ethylpropylaminophenylalanine dihydrochloride are obtained in the form of a white solid which melts in the region of 147°C (decomposition).

15 39-7: Diethyl 4-ethylpropylaminobenzyl-acetamidomalonate

2.8 g of a solid are obtained, after reacting for 36 hours and then purifying by flash chromatography (eluant, CH₂Cl₂/MeOH 97-3 by volume), by proceeding as
20 in Example 39-2 but using 10 g of diethyl 4-ethylamino-benzylacetamidomalonate, 5.6 ml of 1-iodopropane and 7.2 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 70 ml of THF; the solid is then recrystallized in 26 ml of cyclohexane. This results in 2.9 g of diethyl 4-ethyl-
25 propylaminobenzylacetamidomalonate in the form of a white solid which melts at 84-86°C.



39-8: (RS)-4-Ethylmethylcyclopropylamino-phenylalanine dihydrochloride

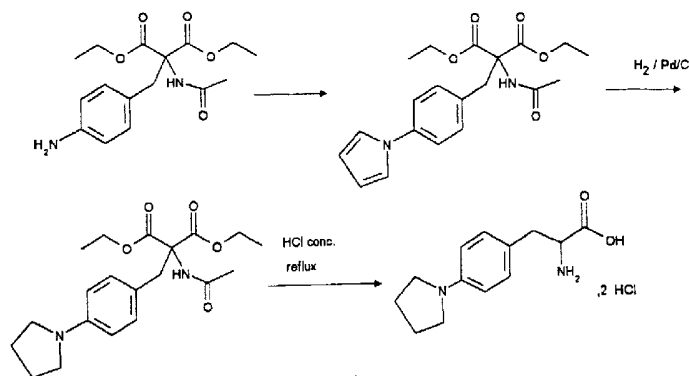
A solid is obtained, after reacting for 3 days and then evaporating, by proceeding as in Example 5 35-1 but using 3 g of diethyl 4-ethylmethylcyclopropylaminobenzylacetamidomalonate and 25 ml of 10N hydrochloric acid; the solid is then triturated in 40 ml of acetone, filtered and then dried under reduced pressure (2.7 kPa) at 40°C. 2.24 g of (RS)-4-ethylmethylcyclopropylaminophenylalanine dihydro- 10 chloride are obtained in the form of a white solid which melts in the region of 140°C (decomposition).

39-9: Diethyl 4-ethylmethylcyclopropylamino-benzylacetamidomalonate

15 By proceeding as in Example 39-2, but using 8 g of diethyl 4-ethylaminobenzylacetamidomalonate, 2.6 ml of bromomethylcyclopropane and 2.97 ml of 1,5-diazabicyclo[4.3.0]non-5-ene in 50 ml of THF, 3.3 g of diethyl 4-ethylmethylcyclopropylaminobenzylacetamido- 20 malonate are obtained, after reacting for 3 days and then purifying by flash chromatography (eluant CH₂Cl₂/AcOEt 90-10 by volume), in the form of a white solid which melts at 112-114°C.



EXAMPLE 40: Preparation of derivatives of phenylalanine using method G



40-1: (RS)-4-(1-Pyrrolidinyl)phenylalanine dihydrochloride

5 A solid is obtained, after evaporation, by proceeding as in Example 35-1 but using 1.5 g of diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate and 40 ml of 5N hydrochloric acid; the solid is then trituated in 15 ml of acetone, filtered and then dried

10 under reduced pressure (2.7 kPa) at 40°C. 0.6 g of (RS)-4-(1-pyrrolidinyl)phenylalanine dihydrochloride is obtained in the form of an off-white solid.

40-2: Diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate

15 4 g of diethyl 4-(1-pyrrolyl)benzylacetamidomalonate, dissolved in 100 ml of MeOH, and 1 g of 10% palladium on charcoal are placed in an autoclave. After having purged the autoclave 3 times with nitrogen, the



product is hydrogenated at 19°C under a pressure of 14 bars of hydrogen. After stirring for 25 hours, the hydrogenation is stopped and the product is filtered through Clarcel® and rinsed with dichloromethane; the solution is then concentrated under reduced pressure in order to yield 3.85 g of a solid which is triturated in a mixture of 50 ml of heptane and 10 ml of ethyl ether. The resulting solid is filtered, dried and then purified by flash chromatography (eluant CH₂Cl₂/acetone 90/10 by volume) in order to yield 1.6 g of diethyl 4-(1-pyrrolidinyl)benzylacetamidomalonate in the form of a white solid which melts at 132°C.

40-3: Diethyl 4-(1-pyrrolyl)benzylacetamido-malonate

4,6 g of diethyl 4-aminobenzylacetamidomalonate in 104 ml of acetic acid are placed in a three-necked flask which is maintained under nitrogen. 7.02 g of sodium acetate are added, followed by 1.87 ml of 2,5-dimethoxytetrahydrofuran. The mixture is heated at 65°C for 1h 15 min, then cooled down and extracted with 100 ml of dichloromethane and 100 ml of distilled water. The aqueous phase is separated off after settling and then washed with 3 times 100 ml of dichloromethane. The organic phases are combined, washed with 100 ml of water and then with 100 ml of a saturated solution of NaCl, separated off after settling and then dried over magnesium sulphate; the phases are filtered and then evaporated to dryness



under reduced pressure (50 kPa) in order to yield 6.2 g of a solid which is purified by flash chromatography (eluent CH₂Cl₂/acetone 75/25 by volume). This results in 3.57 g of diethyl 4-(1-pyrrolyl)benzylacetamidomalonate in the form of a beige solid which melts at 110°C.

EXAMPLE 41: Preparation of derivatives of phenylalanine using method H

41-1: (RS)-4-Ethylthiomethylphenylalanine

10 300 ml of anhydrous methanol are placed in a three-necked flask which is maintained under nitrogen; subsequently, 1.72 g of sodium methoxide, and then 5.55 ml of ethyl mercaptan, are added while stirring. The solvent is concentrated under reduced pressure at 15 40°C in order to yield 8.5 g of the sodium salt of ethyl mercaptan, which is dissolved in 100 ml of anhydrous THF. 3.6 g of (RS)-4-chloromethylphenylalanine are added at room temperature and the mixture is then heated to reflux for 18h. The solvent is 20 evaporated under reduced pressure at 40°C and the residue is taken up in 100 ml of distilled water. The turbid solution which is obtained is acidified with 5 ml of acetic acid. The resulting precipitate is 25 60°C under reduced pressure in order to yield 3.6 g of a solid which is purified by flash chromatography (eluant AcOEt 60, AcOH 12, water 10). This results in



256 mg of (RS)-4-ethylthiomethylphenylalanine in the form of a white solid which melts at 251°C.

The (RS)-4-chloromethylphenylalanine can be obtained by analogy with (S)-4-

- 5 chloromethylphenylalanine as described in: R.Gonzalez-Muniz, F. Cornille, F. Bergeron, D. Ficheux, J. Pothier, C. Durieux and B. Roques, Int. J. Pept. Protein. Res., 1991, 37 (41), 331-340.

10 **EXAMPLE 42: Preparation of derivatives of phenylalanine using method I**

42-1: (S)-4-O-(2-Chloroethyl)tyrosine hydrochloride

- 5 g of (S)-N-tert-butoxycarbonyl-4-O-(2-chloroethyl)tyrosine, dissolved in 50 ml of hydrochloric dioxane, are placed in a round-bottomed flask. After having been stirred for 28h, the mixture is concentrated to dryness under reduced pressure. The resulting residue is taken up in 50 ml of ether and this solution is then stirred and filtered. The resulting solid is washed with 2 times 25 ml of ether and then dried under reduced pressure in order to yield 1.58 g of (S)-4-O-(2-chloroethyl)tyrosine hydrochloride in the form of a white solid which melts at 260°C.

- 25 42-2: (S)-N-tert-Butoxycarbonyl-4-O-(2-chloroethyl)tyrosine

14 g of (S)-N-tert-butoxycarbonyltyrosine, dissolved in 140 ml of DMF, are placed in a three-



necked flask under a nitrogen atmosphere. 4.8 g of 50% sodium hydride in oil are added slowly using a spatula. 16.87 g of 1-tosyl-2-chloroethanol are added after the mixture has been stirred for 2h at room temperature.

- 5 2.4 g of 50% sodium hydride in oil, and a further 8.4 ml of 1-tosyl-2-chloroethanol, are added after the mixture has been stirred for 2 days. The same procedure is carried out after 24h and the stirring is continued for a further 24h. The reaction is stopped by adding
- 10 100 ml of distilled water, and the reaction mixture is concentrated to dryness under reduced pressure. The residue which is obtained is taken up in 100 ml of distilled water and then extracted with 3 times 100 ml of ethyl acetate. The aqueous phase is separated off
- 15 after settling and acidified to pH3 with 50 ml of 1N HCl, and the product is extracted with 3 times 100 ml of ethyl acetate. The organic phases are combined, washed with 2 times 50 ml of water, separated off, dried over magnesium sulphate, filtered and then
- 20 concentrated to dryness under reduced pressure in order to yield 13.51 g of (S)-N-tert-butoxycarbonyl-4-O-(2-chloroethyl)tyrosine in the form of a chestnut oil (Rf = 0.5, toluene 70%/methanol 20%/diethylamine 10%), which is used as such in the following step.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

TABLE V

	MICROORGANISMS	ANTIBIOTICS
	FUNGI	
	<u>Micromonospora</u> sp.	Vernamycins
5	STREPTOMYCES	
	<u>S. alborectus</u>	Virginiamycins
	<u>S. conganensis</u> (ATCC13528)	F1370 A, B
	<u>S. diastaticus</u>	Plauracins, Streptogramins
	<u>S. graminofasciens</u>	Streptogramins
10	<u>S. griseus</u> (NRRL2426)	Viridogrisein (Etamycin)
	<u>S. griseoviridus</u>	Griseoviridin
	<u>S. griseoviridus</u> (FERMP3562)	Neoviridogriseins
	<u>S. lavendulae</u>	Etamycins
	<u>S. loidensis</u> (ATCC11415)	Vernamycins
15	<u>S. mitakaensis</u> (ATCC15297)	Mikamycins
	<u>S. olivaceus</u> (ATCC12019)	Synergistins (PA 114 A, B)
	<u>S. ostreogriseus</u> (ATCC27455)	Ostreogrycins
	<u>S. pristinaespiralis</u> (ATCC25486)	Pristinamycins
	<u>S. virginiae</u> (ATCC13161)	Virginiamycins (Staphylomycins)
20	ACTINOMYCETES	
	<u>A. auranticolor</u> (ATCC31011)	Plauracins
	<u>A. azureus</u> (ATCC31157)	Plauracins
	<u>A. daghestanicus</u>	Etamycin
	<u>A. philippinensis</u>	A-2315 A,B,C
25	<u>Actinoplanes</u> sp. (ATCC3302)	A15104
	<u>Actinoplanes</u> sp.	A17002 A,B,C,F
	<u>Actinomadura flava</u>	Madumycins



Abbreviations employed:

	AcOEt	ethyl acetate
	DNA	deoxyribonucleic acid
	AMP	adenosine 5'-monophosphate
5	HPLC	high-performance liquid chromatography
	dCTP	deoxycytosine 5'-triphosphate
	DMF	dimethylformamide
	DMPAPA	4-dimethylamino-L-phenylalanine
	HCl	hydrochloric acid
10	HT7	Hickey Tresner solid medium
	3-HPA	3-hydroxypicolinic acid
	IPTG	isopropyl- β -D-thiogalactopyranoside
	kb	kilobase
	LB	Luria broth (rich growth medium for
15		<u>E. coli</u>)
	MeOH	methanol
	MMPAPA	4-methylamino-L-phenylalanine
	NaOH	sodium hydroxide
	PAPA	4-amino-L-phenylalanine
20	PEG	polyethylene glycol
	P I	pristinamycin I
	P II	pristinamycin II
	bp	base pair
	SAM	S-adenosylmethionine
25	TE	10 mM Tris-HCl buffer, 1 mM EDTA, pH 7.5
	THF	tetrahydrofuran
	Tris	2-amino-2-(hydroxymethyl)-1,3-



		propanediol
UV		ultraviolet rays
X-gal		5-bromo-4-chloro-3-indoyl- β -D-galactoside
5	YEME	yeast extract-malt extract medium (rich growth medium for <i>Streptomyces</i>)

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- 5 (A) NAME: RHONE-POULENC RORER S.A.
(B) STREET: 20, avenue Raymond ARON
(C) CITY: ANTONY
(E) COUNTRY: FRANCE
(F) POSTAL CODE: 92165

(ii) TITLE OF INVENTION: NOVEL STREPTOGRAMINS

- 10 AND PROCESS FOR PREPARING STREPTOGRAMINS BY
MUTASYNTHESIS.

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:

- 15 (A) MEDIUM TYPE: Tape
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: Release #1.0, Version
#1.25 (OEB)

(2) INFORMATION FOR SEQ ID NO: 1:

- 20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2888 base pairs
(B) TYPE: nucleic acid



(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

5 (iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces

pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CTGCAGTTCC	CCGGGGCCAC	CGTGCTCAGC	TCCTCACCAG	AACGGTTCTT	GCGCATCGGC
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GCCCAGGACG	CCGCCGTCAA	GGCCTCCCTC	GCCGCGGCGC	AGAAGGACCG	CAGCGAGAAC
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CTGATGATCG	TCGACCTGGT	CCGCAACGAC	CTCGGCCAGG	TCTGCGACAT	CGGCTCCGTC
250	260	270	280	290	300
CACGTACCGG	GCCTGTTCGA	GGTGGAGACC	TACGCCACCG	TCCACCAGCT	CGTCAGCAGC
310	320	330	340	350	360
GTCCGCGGCC	GCCTGGCGGC	CGACGCTCTC	CGCCCCCGCG	CGGTACGGGC	CGCCTTCCCC



370	380	390	400	410	420
GGCGGGTCGA	TGACCGGGGC	GCCCAAGGTC	CGCACCATGC	AGTTCATCGA	CCGGCTCGAG
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AAGGGCCGCG	GCGGCGTGTA	CTCGGGCGCG	CTGGGGTACT	TGCCCCCTCAG	CGGCGCGGCC
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GACCTCAGCA	TCGTATCCG	CACCATCGTC	GCCACCGAGG	AGGCCGCCAC	CATCGGCGTG
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GCGCAGACCA	CCCTCGCGGC	CCTGCGCCAG	GCACACGCGG	GCGCCACCGC	CTCGGACCGT
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GTGCCCGCGC	GCCGGCATCT	CCACCACCCG	GGCCCCCACC	CCGGCCACCA	GCTCCACCAG
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1630	1640	1650	1660	1670	1680
CACCACTGCC	GCCGCCGCCA	GCGCCGCGAC	CGCCTCCGGC	CCCGGCGGCC	GCACATCACC
1690	1700	1710	1720	1730	1740



GGCCACCAACC CGCACCCCGT CGCCCGCACC GGGCCCGGCC ACGTCCAGCC AGGTACACGC
1750 1760 1770 1780 1790 1800
CACCCCGGAA CGCACCAACC AGTGGGTGAA CATGCGGCC ACCGCAACCG CCCCGCCAC
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CACCACACAA CGCCCGAACA CCGAACCAACC CCTCATCCGC GTTCCCGATC CCCCCGGTAC
1870 1880 1890 1900 1910 1920
GGAGGAAGAA CCATGACCCC GCGCGCCATC CCGCGCGCC CGCCCGCCAC CGGGCCCGCC
1930 1940 1950 1960 1970 1980
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1990 2000 2010 2020 2030 2040
CTGGACGCGC TCCGCACAGC CCTGGACATC TGCCTGCGCA TCGCGAGTA CAAGGCCCTC
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2110 2120 2130 2140 2150 2160
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TCGCCCCAGG GCTGGACGAG GCGACCGGGC AGCTGACCGG CGCGGGGATC ACCCGCGAGC
2350 2360 2370 2380 2390 2400
CGCGCCGGGC CGACACCCGG CTGCTGGCGG CCGACGCTG CCAGGTGCGC CCGGGGGACC
2410 2420 2430 2440 2450 2460
TCGACACCTG CCTGGCCGCG CCGGTGCCCC CCGGTTCTG GCACTACGTC CGGCGCCGTC
2470 2480 2490 2500 2510 2520
TGACCCGCGA ACCCGCGGAA CGCATCGTGG GCGACGCTA CTTCATGGGC CACCGCTTCG
2530 2540 2550 2560 2570 2580
ACCTGGCCCC CGCGGTCTTC GTCCCAAAAC CCGAGACCGA GGAGATCACC CGGGACGCCA
2590 2600 2610 2620 2630 2640
TCGCCCCGCT GGAGGCCCTC GTCCGCGCGG GCACCAACCG ACCCTTGGTC GTGACCTGT
2650 2660 2670 2680 2690 2700
GCGCGGACCG GGGCACCATG GCGGTACCCC TGGCCGCGCA CGTACCGGCC GCGCGCTCC
2710 2720 2730 2740 2750 2760
TGGGCATCGA ACTCTCCAG GCGCGCGCCC GCGCGGCGCG GCGCAACGCC CGCGGCACCG
2770 2780 2790 2800 2810 2820
GCGCGCGCAT CGTGAGGGG GACGCGCGCG ACGCTTCCC CGAACTGAGC GGCACCGTCG
2830 2840 2850 2860 2870 2880
ACCTCGTCTG CACCAACCG CCTACATCC CCATCGACT GCGCACCTCC GCACCCGAG
TGCTCGAG

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 888 base pairs
(B) TYPE: nucleic acid



(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

5 (iv) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Streptomyces*
pristinaespiralis

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

ATG AGG GGT GGT TCG GTG TTC GGG CGT TGT GTG CTG GTG GGC GGG GCC GGT GCG	54
Met Arg Gly Gly Ser Val Phe Gly Arg Cys Val Val Val Gly Gly Ala Gly Ala	18
GTG GGC CGC ATG TTC AGC CAC TGG CTG GTG CGT TCG GGG GTG GCG GTG ACC TGG	108
Val Gly Arg Met Phe Ser His Trp Leu Val Arg Ser Gly Val Ala Val Thr Trp	36
CTG GAC GTG GCC GGG GCC GGT GCG GCG GAC GGG GTG CCG GTG GTG GCC GGT GAT	162
Leu Asp Val Ala Gly Ala Gly Ala Ala Asp Gly Val Arg Val Val Ala Gly Asp	54
GTG CCG CGG CCG GGG CCG GAG GCG GTC GCG GCG CTG GCG GCG GCG GAC GIG GTG	216
Val Arg Arg Pro Gly Pro Glu Ala Val Ala Ala Leu Ala Ala Ala Asp Val Val	72
GTG CTG GCG GTG CCG GAG CCG GTG GCG TGG GAG GCG GTG GAG GTG CTG GCG GGG	270
Val Leu Ala Val Pro Glu Pro Val Ala Trp Glu Ala Val Glu Val Leu Ala Gly	90
GTG ATG CCG CCC GGT GCG CTG CTC GCG GAC ACC TTG TCG GTC AAG AGC CCG ATC	324
Val Met Arg Pro Gly Ala Val Leu Ala Asp Thr Leu Ser Val Lys Ser Arg Ile	108
GCC GGG CGG CTG CGT GAG GCG GCG CCG GGG CTG CAG GCG GTG GGG CTG AAC CCG	378
Ala Gly Arg Leu Arg Glu Ala Ala Pro Gly Leu Gln Ala Val Gly Leu Asn Pro	126
ATG TTC GCC CCC TCG CTG GGT CTT CAG GGG CCG CCG GTG GCG GCG GTG CTG GTC	432
Met Phe Ala Pro Ser Leu Gly Leu Gln Gly Arg Pro Val Ala Ala Val Val Val	144
ACC GAC GGG CCC GGT GTG CCG GCC CTG GTG GAG CTG GTG GCC GGG TGG GGG GCC	486
Thr Asp Gly Pro Gly Val Arg Ala Leu Val Glu Leu Val Ala Gly Trp Gly Ala	162
CGG GTG GTG GAG ATG CCG GCG CCG CCG CAC GAC GAC CTG ACC GCC GCG CAG CAG	540
Arg Val Val Glu Met Pro Ala Arg Arg His Asp Glu Leu Thr Ala Ala Gln Gln	180
GCC GC ACG CAT GCC GCG GTG CTG GCC TTC GGG CTG GGC CTG GGT GAG CTG TCG	594
Ala Ala Thr His Ala Ala Val Leu Ala Phe Gly Leu Gly Leu Gly Glu Leu Ser	198



GTG GAC CTG GGG GCG CTG CCG GAC AGT GCC CCG CCG CCG CAT CTG GCG ATG CTG	648
Val Asp Val Gly Ala Leu Arg Asp Ser Ala Pro Pro Pro His Leu Ala Met Leu	216
GCG CTG CTG GCC CCG ATC GCC GCG GGG ACG CCG GAG GTG TAT TTC GAC ATC CAG	702
Ala Leu Leu Ala Arg Ile Ala Gly Gly Thr Pro Glu Val Tyr Phe Asp Ile Gln	234
GCC GCC AAC CCC GCG GCG CCG GCC GCG CCG CAG GCG CTG GCG CCG GCG CTG GTG	756
Ala Ala Asn Pro Gly Ala Pro Ala Ala Arg Gln Ala Leu Gly Arg Gly Leu Val	252
GCG CTG GGG CAG GCC GTC GAG AGG GCG GAC GAG GAG ACG TTC GCC GCC CTG TTC	810
Arg Leu Gly Gln Ala Val Glu Arg Gly Asp Glu Glu Thr Phe Ala Ala Leu Phe	270
GCC GAA CTG CCG GGT GTG CTG GCG GAG CAC GCC GCG GAG CTG GAA CCG CTG TGC	864
Ala Glu Leu Arg Gly Val Leu Gly Glu His Gly Ala Glu Leu Glu Arg Leu Cys	288
GCG CCG ATG TTC ACC GCC CTG CAC	888
Ala Arg Met Phe Thr Ala Leu His	296

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 387 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Streptomyces

pristinaespiralis



(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

ATG ACC CCG CCC GCC ATC CCC GCC CCG CCC GCC ACC GGG CCC GCC CCC GCC	54
Met Thr Pro Pro Ala Ile Pro Ala Ala Pro Pro Ala Thr Gly Pro Ala Ala Ala	18
ACC GAC CCC CTC GAC GCG CTG CGC GCC CGC CTG GAC GCC GCG GAC GCC GCC CTG	108
Thr Asp Pro Leu Asp Ala Leu Arg Ala Arg Leu Asp Ala Ala Asp Ala Ala Leu	36
CTG GAC GCC GTC CGC ACA CGC CTG GAC ATC TGC CTG CGC ATC GGC GAG TAC AAG	162
Leu Asp Ala Val Arg Thr Arg Leu Asp Ile Cys Leu Arg Ile Gly Glu Tyr Lys	54
CGC CTC CAC CAG GTG CCG ATG ATG CAG CCC CAC CGG ATC GCC CAG GTC CAC GCC	216
Arg Leu His Gln Val Pro Met Met Gln Pro His Arg Ile Ala Gln Val His Ala	72
AAC GCC GCC CGC TAC GCC GCC GAC CAC GGC ATC GAC CCC GCC TTC CTG CGC ACC	270
Asn Ala Ala Arg Tyr Ala Ala Asp His Gly Ile Asp Pro Ala Phe Leu Arg Thr	90
CTG TAC GAC ACG ATC ATC ACC GAG ACC TGC CGC CTC GAG GAC GAG TGG ATC GCC	324
Leu Tyr Asp Thr Ile Ile Thr Glu Thr Cys Arg Leu Glu Asp Glu Trp Ile Ala	108
TCC GGC GGC GCC CCC GTC CCC ACG CCC GTG CAC GCG TCC GCG TCC GCG CGG GGG	378
Ser Gly Gly Ala Pro Val Pro Thr Pro Val His Ala Ser Ala Ser Ala Arg Gly	126
GCC GTG TCG	387
Ala Val Ser	129

INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

5

- (A) LENGTH: 4496 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no



(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Streptomyces*
pristinaespiralis

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

10	20	30	40	50	60
CTCGAGCAGG	TGCCCCACCT	CGGCGGCACG	GTGCGCGGGC	AGCGCGAACA	CCGGCAGGCG
70	80	90	100	110	120
GCCCAGACGG	AACAGCGCGA	AGCACACCGC	GACGAACTCG	GGCGTGTTCG	GCAGCTGCAC
130	140	150	160	170	180
CAGCACCCGC	TGCGCGGCGC	CGATCCCGCG	CGCGCGGAAC	CCCGCCGCCA	GCCGGTCGCA
190	200	210	220	230	240
CCAGCGGTCC	AGGGCACGGT	AGGTGACACG	GGAGCACCCG	TCCGCGCCGA	CCAGCGCCTC
250	260	270	280	290	300
CCGCTCGCCG	TACTGCTCCG	CCCAGCGGCC	CAGCAGCATG	CCCAGCGGCT	CGCCCCGCCA
310	320	330	340	350	360
GTAGCCGGCC	GCCCCGTACT	TGCGGGCCAC	ATCCTCGGGC	CAGGGAACGC	ATCCGTCCAG
370	380	390	400	410	420



CATCGTTGGT CCTTTCGGG TTCGTCTCG CGTCGSCCC AGTGTCCGCA GCGCCGTTGA
 430 440 450 460 470 480
 CACGCCGCTG ATGCCGCGG CCGCGCGCC GCCGCTCCGT CAGGAGCCGA TCAGGGCGGC
 490 500 510 520 530 540
 GTCAGCCGGG CCGGACAGGA TGCCGCCAC GGGGCCCGG ACACCGGGCC GCGCGACAG
 550 560 570 580 590 600
 CGGGCCGGG ACCGGCAGG CGACACCAG CACGGACGAG AAGAAACAAC ACAAGGGGAG
 610 620 630 640 650 660
 CACCCGATGG AGACCTGGGT CCTGGGCGG CGCGACGTG CCGAGGTGGT GGGCCGCGTC
 670 680 690 700 710 720
 GGGCCGCGAG AACTCATGCG CGGCATCAG GACCGCTCA CCGGCGGACT GGGCGAGATC
 730 740 750 760 770 780
 GGGCCGCGG AGCGGCACCT GTCCCGCTG CGCGCGGAC TGGAACGCG CGAACCCGTC
 790 800 810 820 830 840
 CCGCGCATCT GGGAAATGGAT GCGCACCGG GAACCCGGG ACCACATCAC CCTCAAGACC
 850 860 870 880 890 900
 GTCCGCTACA GCGCCGCCAA CCGCGCGCG TTCGGCTGC CGACCATCT GGGCACCGTC
 910 920 930 940 950 960
 GCGCGCTACG ACGACACCAC CGGCGCGCTG ACCGCGCTGA TGGACGGCGT GGTGCTCACC
 970 980 990 1000 1010 1020
 GCGCTGCGCA CCGCGCGCG CTCCGCGCTC GCCTCCCGCC TGCTGGCCCG CCGCGACAGC
 1030 1040 1050 1060 1070 1080
 CACACCTTGG GACTGATCG CACCGCGCG CAGGCGCTCA CCAACTGCA CGCCCTGTCC
 1090 1100 1110 1120 1130 1140
 CTGGTACTGC CCGTGAACG GCGCTGCTG TGGGACACCG ACCCGCGCCA CCGGGAAGC
 1150 1160 1170 1180 1190 1200
 TTCGCGCGG GCGCGCGTT CACCGCGCTC AGCGTCGAGA TCGCCGAGCC CCGCCGATC
 1210 1220 1230 1240 1250 1260
 GCGCGCGAGG CCGACGTCAT CTCCACCGC ACCTCGGTAG CCGTCGGCCA GGGCCCGGTC
 1270 1280 1290 1300 1310 1320
 CTGCCGACA CCGCGCTCG CGAGCACCTG CACATCAAC CCGTCGGCGG GAGCTCGTC
 1330 1340 1350 1360 1370 1380
 GGCAAGACGG AACTGCCGCT CCGCTGCTC GAGCGGGCGT TCCTCACCGC CGACCACCCC
 1390 1400 1410 1420 1430 1440
 GAGCAGGCGG TGCGCGAGG CGAGTGCCAG CAACTCTCG CCGACCGGCT CGGCCCGCAG
 1450 1460 1470 1480 1490 1500
 CTGGCCCAAC TGTGCGCGA CCGCGCGGCG GCGCGCGGCG GGCAGGACAC CCTGAGCGTC
 1510 1520 1530 1540 1550 1560
 TTCGACTCCA CCGGCTTCG CTTCGAGGAC GCGCTGGCGA TGGAGGTGT CCTCGAGGCC
 1570 1580 1590 1600 1610 1620
 GCGCGCGAAC GGGACCTGG CATCCGGTG GGCATCGAAC ACCACCGCGG CGACGCGCTG
 1630 1640 1650 1660 1670 1680
 GACCCCTACG CCGTCCAGC CCTGCGCTG CCGCTGGCG CCGCGCGCCA CTGACCCCCC
 1690 1700 1710 1720 1730 1740
 CCTTTTTTCG GGACCCCGC TCTTTTCGA GACCCCGCG CCGCGCGGCC GGGCCCTCTC



1750	1760	1770	1780	1790	1800
CCGCCGGGCC	CCATGCCCGG	CCGGGCGGGG	GCACCCACGA	CGCCCTCGCG	AGGAGAGAGA
1810	1820	1830	1840	1850	1860
TGCCCCCAC	CCCCCGGCC	ACCACCGACG	ACGGCGGGCG	TGAACGTCTC	GCCTGGCTGC
1870	1880	1890	1900	1910	1920
GCGAGATGCG	CCACCACCAC	CCCGTCCACG	AGGACGAATA	CGGTGCCTTC	CACGTCTTCC
1930	1940	1950	1960	1970	1980
GGCACGCCGA	CGTCCTCACC	GTGCGCTCGG	ACCCCGGGGT	CTACTCCTCC	CAGCTCAGCC
1990	2000	2010	2020	2030	2040
GGCTACGGCC	CGGCTCCAG	GCGTTGAGCG	AACAGATCCT	GTGCGTCATC	GACCCGCCGA
2050	2060	2070	2080	2090	2100
TGCACCGCAC	CCTGCGCCGC	CTGGTCAGCC	AGGCCTTCAC	CCCCCGCACC	GTGCGCGACC
2110	2120	2130	2140	2150	2160
TGGAACACG	CGTCACCGAA	CTGGCCGGGC	AACTGCTCGA	CGCCGTGCGAC	GGCGACACGT
2170	2180	2190	2200	2210	2220
TGCACCTCGT	CGCGACTTTC	GCCTACCCGC	TGCCCGTGAT	CGTGATCGCC	GAACCTCTCG
2230	2240	2250	2260	2270	2280
GCGTGCCGCC	CGCCGACCGC	ACCCCTGTCC	GCTCCTGGTC	CGACCGGATG	CTGCAGATGC
2290	2300	2310	2320	2330	2340
AGGTGCGCGA	CCCGGCGGAC	ATGCAGTTCC	GCGACGACGC	CGACGAGGAC	TACCAACGCC
2350	2360	2370	2380	2390	2400
TCGTCAAAGA	ACCCATGCGC	GCCATGCACG	CCTACCTCCA	CGACCACGTC	ACCGACCGCC
2410	2420	2430	2440	2450	2460
GCGCCCGCCC	CGCGAACGAC	CTGATCTCCG	CACTCGTCCG	CGCCCGCGTG	GAGGGCGAAC
2470	2480	2490	2500	2510	2520
GACTCACCGA	CGAGCAGATC	GTGGAATTCC	GGGCGCTGCT	GCTGATGGCC	GGCCACGTCT
2530	2540	2550	2560	2570	2580
CCACCTCCAT	GCTGCTCGGC	AACACCGTGC	TGTGCCTCAA	GGACCACCCC	CGGGCCGAGG
2590	2600	2610	2620	2630	2640
CCGCCCGCCG	CGCCGACCGG	TCCCTGATCC	CGGCCCTGAT	CGAAGAAGTA	CTGCGGCTGC
2650	2660	2670	2680	2690	2700
GGCCGCCGAT	CACCGTCATG	GCCCCGCTCA	CCACCAAGGA	CACCGTCCTC	GCCGGCACCA
2710	2720	2730	2740	2750	2760
CCATCCCCGC	CGGACGCATG	GTGCTGCCCT	CCCTGCTGTC	CGCCAACCAC	GACGAACAGG
2770	2780	2790	2800	2810	2820
TCTTCACCGA	CCCCGACCAC	CTCGACCTCG	CCCGCGAAGG	CCGCCAGATC	GCCTTCGGCC
2830	2840	2850	2860	2870	2880
ACGGCATCCA	CTACTGCCTG	GCGCCCCGCG	TCGCCCGCCT	GGAGGGCCGC	ATCGCCCTGG
2890	2900	2910	2920	2930	2940
AAGCCCTCTT	CGACCGATTG	CCCGACTTCT	CGCCACCGGA	CGGGCGAAAA	CTGCGCTACC
2950	2960	2970	2980	2990	3000
ACCGCGACGG	ACTGTTCCGC	GTCAAGAACC	TGCCGCTGAC	CGTACGGCGC	GGCTGACACA
3010	3020	3030	3040	3050	3060
GACAAGGCGG	CCACCTGGTG	GCGACCGTGC	GAACCTGCT	GATCGACAAC	TACGACTCGT



3070	3080	3090	3100	3110	3120
TCACCTACAA	CCTCTTCCAG	ATGCTGGCCG	AGGTGAACGG	CGCCGCTCCG	CTCGTCGTCC
3130	3140	3150	3160	3170	3180
GCAACGACGA	CACCCGCACC	TGGCAGGCC	TGGCGCCGGG	CGACTTCGAC	AACGTCGTCC
3190	3200	3210	3220	3230	3240
TCTCAACCCG	CCCCGGCCAC	CCCGCCACCG	ACACCGACCT	GGCCCTCAGC	CGCCGGGTGA
3250	3260	3270	3280	3290	3300
TCACCGAATG	GGACCTGCCG	CTGCTCGGGG	TGTGCCTGGG	CCACCAGGCC	CTGTGCTTGC
3310	3320	3330	3340	3350	3360
TCGCCGGGCG	CGCCGTCTGC	CACGCACCCG	AACCCCTTCA	CGGCCGCACC	AGCGACATCC
3370	3380	3390	3400	3410	3420
GCCACGACGG	GCAGGGCCTG	TTCCGGAACA	TCCCTTCCCC	GCTGACCGTG	GTCCGCTACC
3430	3440	3450	3460	3470	3480
ACTCGCTGAC	CGTCCGGCAA	CTGCCCGCCG	ACCTGCGCGC	CACCCGCCAC	ACCGCGACGG
3490	3500	3510	3520	3530	3540
GGCAGCTGAT	GGCCGTCTGC	CACCGCCACC	TGCCCGCCTT	CGGCGTGCAG	TTCCGCTCCG
3550	3560	3570	3580	3590	3600
AATCGATCAG	CAGCGAACAC	GGCCACCGGA	TGCTCGCCAA	CTTCCGCGAC	CTGTCCCTGC
3610	3620	3630	3640	3650	3660
GCGCGGCCGG	CCACCGCCCC	CCGCACACCG	AACGCATACC	CGCACCCGCA	CCCGCCCCCG
3670	3680	3690	3700	3710	3720
CCCCCGCCCC	CGCACCGGCA	CCGCCCGCGT	CCCGCGCGGT	GGGGGAGTAC	CGGCTGCATG
3730	3740	3750	3760	3770	3780
TGCGCGAGGT	CGCCTGCGTG	CCCGACGCGG	ACGCCGCGTT	CACCGCCCTG	TTCCGCGACG
3790	3800	3810	3820	3830	3840
CCCGCGCCCC	GTTCTGGCTC	GACAGCAGCC	GCGTCGAGCC	GGGCCTCGCC	CGCTTCACCT
3850	3860	3870	3880	3890	3900
TCCTCGGCGC	CCCCGCCCGC	CCGCTCGGCG	AACAGATCAC	CTACGACGTC	GCCGACCGGG
3910	3920	3930	3940	3950	3960
CCGTGCGCGT	CAAGGACGGT	TCAGGGCGCG	AGACCCGCGG	GCCCGGCACC	CTCTTCGACC
3970	3980	3990	4000	4010	4020
ACCTGGAACA	CGAACTGGCC	GCCCCGCCCC	TGCCCGCCAC	CGGCCTGCCC	TTCCAGTTCA
4030	4040	4050	4060	4070	4080
ACCTCGGCTA	CGTCGGCTAC	CTCGGCTACG	AGACCAAGGC	CGACAGCGGC	GCCGAGGACG
4090	4100	4110	4120	4130	4140
CCACCGCGG	CGAACTGCCC	GACGGCGCCT	TCATGTTCCG	CGACCGGATG	CTCGCCCTCG
4150	4160	4170	4180	4190	4200
ACCACGAACA	GGGGCGGGCC	TGGCTCCTGG	CACTGAGCAG	CACCCGACGG	CCCGCCACCG
4210	4220	4230	4240	4250	4260
CACCCGCGCG	CGAACTGCTG	CTCACCAGCG	CCGCCCGGAC	CCTCGCCACC	ACCGCCCCCG
4270	4280	4290	4300	4310	4320
GCCCGCCCTT	CACCTGCTG	CCCGACGACC	AACTGCCCGC	CCTGGACGTC	CACTACCGCC
4330	4340	4350	4360	4370	4380
ACAGCCTGCC	CCGCTACCGG	GAACTGGTCG	AGGAATGCCG	CCGCTGATC	ACCGACGGCG
4390	4400	4 10	4420	4430	4440

AGACCTACGA	GGTGTGCTTG	ACGAACATGC	TCCGGGTGCC	CGGCCGGATC	GACCCGCTCA
4450	4460	4470	4480	4490	
CCGCTTACCG	CGCCCTGCGC	ACCGTCAGCC	CCGCCCCCTA	CGCCGCTTAC	CTGCAG



(6) INFORMATION FOR SEQ ID NO: 5

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 1065 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

10 (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Streptomyces pristinaespiralis*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5

ATG GAG ACC TGG GTC CTG GGC CGG CGC GAC GTC GCC GAG GTG GTG GCC GCC GTC	54
Met Glu Thr Trp Val Leu Gly Arg Arg Asp Val Ala Glu Val Val Ala Ala Val	18
GGC CGC GAC GAA CTC ATG CGC CGC ATC ATC GAC CGC CTC ACC GGC GGA CTG GCC	108
Gly Arg Asp Glu Leu Met Arg Arg Ile Ile Asp Arg Leu Thr Gly Gly Leu Ala	36
GAG ATC GGC CGC GGC GAG CGG CAC CTG TCC CCG CTG CGC GCC GGA CTG GAA CGC	162
Glu Ile Gly Arg Gly Glu Arg His Leu Ser Pro Leu Arg Gly Gly Leu Glu Arg	54
AGC GAA CCC GTG CCC GGC ATC TGG GAA TGG ATG CCG CAC CGC GAA CCC GGC GAC	216
Ser Glu Pro Val Pro Gly Ile Trp Glu Trp Met Pro His Arg Glu Pro Gly Asp	72
CAC ATC ACC CTC AAG ACC GTC GGC TAC AGC CCC GCC AAC CCC GGC CGC TTC GGC	270
His Ile Thr Leu Lys Thr Val Gly Tyr Ser Pro Ala Asn Pro Gly Arg Phe Gly	90
CTG CCG ACC ATC CTG GGC ACC GTC GCC CGC TAC GAC GAC ACC ACC GGC GCC CTG	324
Leu Pro Thr Ile Leu Gly Thr Val Ala Arg Tyr Asp Asp Thr Thr Gly Ala Leu	108
ACC GCC CTG ATG GAC GGC GTG CTG CTC ACC GCC CTG CGC ACC GGC GCC GCC TCC	378
Thr Ala Leu Met Asp Gly Val Leu Leu Thr Ala Leu Arg Thr Gly Ala Ala Ser	126
GCC GTC GCC TCC CGC CTG CTG GGC CGC CCC GAC AGC CAC ACC TG GGA CTG ATC	432
Ala Val Ala Ser Arg Leu Leu Ala Arg Pro Asp Ser His Thr Leu Gly Leu Ile	144
GGC ACC GGC GCC CAG GCC GTC ACC CAA CTG CAC GCC CTG TCC CTG GTA CTG CCC	486
Gly Thr Gly Ala Gln Ala Val Thr Gln Leu His Ala Leu Ser Leu Val Leu Pro	162



CTG CAA CGG GCC CTG GTG TGG GAC ACC GAC CCC GCC CAC CGG GAA ASC TTC GCC	540
Leu Gln Arg Ala Leu Val Trp Asp Thr Asp Pro Ala His Arg Glu Ser Phe Ala	180
CGG CGC GCC GCG TTC ACC GGC GTC AGC GTC GAG ATC GCC GAG CCC GCC CGG ATC	594
Arg Arg Ala Ala Phe Thr Gly Val Ser Val Glu Ile Ala Glu Pro Ala Arg Ile	198
GCC GCC GAG GCC GAC GTC ATC TCC ACC GCC ACC TCG GTA GCC GTC GCC CAG GGC	648
Ala Ala Glu Ala Asp Val Ile Ser Thr Ala Thr Ser Val Ala Val Gly Gln Gly	216
CCG GTC CTG CCC GAC ACC GGC GTC CGC GAG CAC CTG CAC ATC AAC GCC GTC GGC	702
Pro Val Leu Pro Asp Thr Gly Val Arg Glu His Leu His Ile Asn Ala Val Gly	234
CGG GAC CTC GTC GGC AAG ACG GAA CTG CCG CTC GGC CTG CTC GAG CGG GCG TTC	756
Ala Asp Leu Val Gly Lys Thr Glu Leu Pro Leu Gly Leu Leu Glu Arg Ala Phe	252
GTC ACC GCC GAC CAC CCC GAG CAG GCG CTG CGC GAG GGC GAG TGC CAG CAA CTC	810
Val Thr Ala Asp His Pro Glu Gln Ala Leu Arg Glu Gly Glu Cys Gln Gln Leu	270
TCC GCC GAC CGG CTC GGC CCG CAG CTG GCC CAC CTG TGC GCC GAC CCG GCG GCC	864
Ser Ala Asp Arg Leu Gly Pro Gln Leu Ala His Leu Cys Ala Asp Pro Ala Ala	288
GCC GCC GCG CGG CAG GAC ACC CTG AGC GTC TTC GAC TCC ACC GGC TTC GCC TTC	918
Ala Ala Gly Arg Gln Asp Thr Leu Ser Val Phe Asp Ser Thr Gly Phe Ala Phe	306
GAG GAC GCC CTG GCG ATG GAA GTG TTC CTC GAG GCC GCC GCC GAA CGG GAC CTG	972
Glu Asp Ala Leu Ala Met Glu Val Phe Leu Glu Ala Ala Ala Glu Arg Asp Leu	324
GGC ATC CGG GTG GGC ATC GAA CAC CAC CCC GGC GAC GCC CTG GAC CCC TAC GCC	1026
Gly Ile Arg Val Gly Ile Glu His His Pro Gly Asp Ala Leu Asp Pro Tyr Ala	342
CTC CAG CCC CTG CCC CTG CCC CTG GCC GCC CCC GCC CAC	1065
Leu Gln Pro Leu Pro Leu Pro Leu Ala Ala Pro Ala His	355

(7) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1194 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:



(A) ORGANISM: *Streptomyces pristinaespiralis*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

ATG CCC CCC ACC CCC CGG CCC ACC ACC GAC GAC GGC GGC CGT GAA CTG CTC GCC	54
Met Pro Pro Thr Pro Arg Pro Thr Thr Asp Asp Gly Gly Arg Glu Leu Leu Ala	18
TGG CTG CGC GAG ATG CGC CAC CAC CAC CCC GTC CAC GAG GAC GAA TAC GGT GCC	108
Trp Leu Arg Glu Met Arg His His His Pro Val His Glu Asp Glu Tyr Gly Ala	36
TTC CAC CTC TTC CGG CAC GCC GAC GTC CTC ACC GTC GCC TCC GAC CCC GGC GTC	162
Phe His Val Phe Arg His Ala Asp Val Leu Thr Val Ala Ser Asp Pro Gly Val	54
TAC TCC TCC CAG CTC AGC CGG CTA CGG CCC GGC TCC CAG GCG TTG AGC GAA CAG	216
Tyr Ser Ser Gln Leu Ser Arg Leu Arg Pro Gly Ser Gln Ala Leu Ser Glu Gln	72
ATC CTG TCG GTC ATC GAC CCG CCG ATG CAC CGC ACC CTG CGC CGC CTG GTC AGC	270
Ile Leu Ser Val Ile Asp Pro Pro Met His Arg Thr Leu Arg Arg Leu Val Ser	90
CAG GCC TTC ACC CCC CGC ACC GTC GCC GAC CTC GAA CCA CGC GTC ACC GAA CTG	324
Gln Ala Phe Thr Pro Arg Thr Val Ala Asp Leu Glu Pro Arg Val Thr Glu Leu	108
GCC GGC CAA CTG CTC GAC GCC GTC GAC GGC GAC ACG TTC GAC CTC GTC GCC GAC	378
Ala Gly Gln Leu Leu Asp Ala Val Asp Gly Asp Thr Phe Asp Leu Val Ala Asp	126
TTC GCC TAC CCG CTG CCC GTG ATC GTG ATC GCC GAA CTC CTC GGC GTG CCG CCC	432
Phe Ala Tyr Pro Leu Pro Val Ile Val Ile Ala Glu Leu Leu Gly Val Pro Pro	144
CCC GAC CGC ACC CTG TTC CGC TCC TGG TCC GAC CGG ATG CTG CAG ATG CAG GTC	486
Ala Asp Arg Thr Leu Phe Arg Ser Trp Ser Asp Arg Met Leu Gln Met Gln Val	162
GCC GAC CCG GCG GAC ATG CAG TTC GGC GAC GAC GCC GAC GAG GAC TAC CAA CGC	540
Ala Asp Pro Ala Asp Met Gln Phe Gly Asp Asp Ala Asp Glu Asp Tyr Gln Arg	180
CTC GTC AAA GAA CCC ATG CGC GCC ATG CAC GCC TAC CTC CAC GAC CAC GTC ACC	594
Leu Val Lys Glu Pro Met Arg Ala Met His Ala Tyr Leu His Asp His Val Thr	198
GAC CGC CGC GCC CGC CCC GCG AAC GAC CTG ATC TCC GCA CTC GTC GCC GCG CGC	648
Asp Arg Arg Ala Arg Pro Ala Asn Asp Leu Ile Ser Ala Leu Val Ala Ala Arg	216
GTG GAG GGC GAA CGA CTC ACC GAC GAG CAG ATC GTC GAA TTC GGG GCG CTG CTG	702
Val Glu Gly Glu Arg Leu Thr Asp Glu Gln Ile Val Glu Phe Gly Ala Leu Leu	234
CTG ATG GCC GGC CAC GTC TCC ACC TCC ATG CTG CTC GGC AAC ACC GTG CTG TGC	756
Leu Met Ala Gly His Val Ser Thr Ser Met Leu Leu Gly Asn Thr Val Leu Cys	252
CTG AAG GAC CAC CCC CGG GCC GAG GCC GCC GCC CGC GCC GAC CGG TCC CTG ATC	810
Leu Lys Asp His Pro Arg Ala Glu Ala Ala Ala Arg Ala Asp Arg Ser Leu Ile	270
CCC GCC CTG ATC GAA GAA GTA CTG CGG CTG CGG CCG CCG ATC ACC GTC ATG GCC	864
Pro Ala Leu Ile Glu Glu Val Leu Arg Leu Arg Pro Pro Ile Thr Val Met Ala	288



CGC GTC ACC ACC AAG GAC ACC CTC CTC GCC GGC ACC ACC ATC CCC GGC GGA CGC 918
Arg Val Thr Thr Lys Asp Thr Val Leu Ala Gly Thr Thr Ile Pro Ala Gly Arg 306

ATG GTC GTC CCC TCC CTG CTG TCC GCC AAC CAC GAC GAA CAG GTC TTC ACC GAC 972
Met Val Val Pro Ser Leu Leu Ser Ala Asn His Asp Glu Gln Val Phe Thr Asp 324

CCC GAC CAC CTC GAC CTC GCC CGC GAA GGC CGC CAG ATC GGC TTC GGC CAC GGC 1026
Pro Asp His Leu Asp Leu Ala Arg Glu Gly Arg Gln Ile Ala Phe Gly His Gly 342

ATC CAC TAC TGC CTG GGC GCC CCG CTC GCC CGC CTG GAG GGC CGC ATC GCC CTG 1080
Ile His Tyr Cys Leu Gly Ala Pro Leu Ala Arg Leu Glu Gly Arg Ile Ala Leu 360

GAA GCC CTC TTC GAC CGA TTC CCC GAC TTC TCG CCC ACC GAC GGC GCA AAA CTG 1134
Glu Ala Leu Phe Asp Arg Phe Pro Asp Phe Ser Pro Thr Asp Gly Ala Lys Leu 378

CGC TAC CAC CGC GAC GGA CTG TTC GGC GTC AAG AAC CTG CCG CTG ACC GTA CGG 1188
Arg Tyr His Arg Asp Gly Leu Phe Gly Val Lys Asn Leu Pro Leu Thr Val Arg 396

CGC GGC 1194
Arg Gly 398

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1561 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Streptomyces pristinaespiralis*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7

10 20 30 40 50 60
AAGCTTCCCG ACCGGGTGGA GGTCTCGAC GCGTTCCCGC TGACCGGCCT CAACAAGGTC

70 80 90 100 110 120
GACAAGAAGG CCTTGGCGGC CGACATCGCC GCCAAGACCG CCCCCACCG CCCACCACC

130 140 150 160 170 180
GCCGGCCACG GCCCGACCAC GGACGGCGAT ACGGCCGGTG GGGGTGGGTC CGCGGGCGGG

190 200 210 220 230 240
GTGACGGCGG CCGGTGGCGG GCGGGAGGAG GCGGCGTGAG CGGGCCCGGG CCCGAGGGCG

250 260 270 280 290 300
GCTACCGGGT GCCGTTCGCG CGACCGGTT CGGTGGTGGG CGAGGCGGAC CTGGCGGCGC



310	320	330	340	350	360
TGGGCGAACT	GGTCCGCTCG	GGCCGGTCCG	TGACGTCCGG	GGTGTGGCGG	GAGCGGTTCG
370	380	390	400	410	420
AGGAACAGTT	CGCCCGCCTG	ACCGGCGCCC	GGCACGCGCT	CAGTGTCAAC	AGCGGCACCG
430	440	450	460	470	480
TCGCGCTGGA	ACTGGCGGTG	CGGATGCTGG	ACCTGGCGCC	GGGCGACGAG	GTGATCGCCA
490	500	510	520	530	540
CCCGCGAGAC	GTTCAGGCG	ACGGTGACG	CGCTGCTCGA	CCACGACGTG	CGGCTGCGGT
550	560	570	580	590	600
TCTGCACAT	CGACCCGGAC	ACCTCAACC	TCGACCCGGC	GGTGTGGAG	ACGCTGATCA
610	620	630	640	650	660
CCGACCGCAC	CCGGGCGATC	CTGCTCGTCC	ACTACGGCGG	CAACCCGGCC	GACATGGACC
670	680	690	700	710	720
GCATCATGGC	CCTGGCCCGC	AAGCGCGGCA	TCATCGTCTG	CGAGSACAGC	GCGCACGCGC
730	740	750	760	770	780
TGGGCGCGGT	GTACCGGGGG	CGGGGCGCGG	GGGCACTGGC	GGACATCGGC	TGCTTCACTT
790	800	810	820	830	840
TCCACTCCAC	GAAGAACATC	ACCACCTCG	GCGAGGGCGG	CATGATCACC	CTGTGCGGTG
850	860	870	880	890	900
ACGAGTGGGC	CCAGCGGGTG	GGACGTATCC	GCGACAACGA	GGCCGACGGC	GTGTACGCGG
910	920	930	940	950	960
CGCTGCGGGA	CTCCGCGCGG	GCGGGTGCTC	CGGCGTGTCT	GCCGTGGATG	AAGTTGCGGG
970	980	990	1000	1010	1020
AGGGTGTGTA	CGGTCAACGG	GCGGTCGGGG	TCCGCGGGGC	GGGCACGAAC	GCGACGATGT
1030	1040	1050	1060	1070	1080
CGGAGGCGGC	GGCGGCGGTG	GGCGTGGTGC	AACTGGCGTC	GCTGGAGCGG	TTCGTGGCCC
1090	1100	1110	1120	1130	1140
GGGCGCGGAG	CATCGCGCAG	CGGCTGCACG	AGGCCGTGGC	CTCGGTGGCC	GGCACCCGGC
1150	1160	1170	1180	1190	1200
TGCACCGGGC	GGCGGCGGAC	AGTCTGCACG	CCTACCACCT	GTACACGTTT	TTCCTCACCG
1210	1220	1230	1240	1250	1260
GCGGCGGGCA	GGTCCGGGAG	CGGTTCGTGC	GCGCCCTGGA	CCGGCTGGGT	GTGGAGGTCC
1270	1280	1290	1300	1310	1320
AGTTGCGGTA	CTTCCCGCTC	CATCTGTGCG	CCGAGTGGCG	GCTGCGGGGC	CACGGGCGGG
1330	1340	1350	1360	1370	1380
GCGAGTGTCC	GACGGCCGAA	CGGGTCTGGT	TCGAGGAGCA	CATGAACCTG	CCGTGCCATC
1390	1400	1410	1420	1430	1440
CCGGTCTGAG	TCACGGCCAG	GTGACTACA	TGGTCGAGGC	GGTCACCCGC	GCCCTGCACG
1450	1460	1470	1480	1490	1500
AGGCCACCGG	CACGGGGACG	CGGGTGGCGG	CCGGGCACCT	GTGACACCGT	CCGCATCCGG
1510	1520	1530	1540	1550	1560
CCGGTGGTTT	TCCAAGACCG	AGGGAGAGGC	AGGCGTATGC	CGTTCATCGA	AGTGAAGATC

T



(9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1233 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: no

(iii) ANTISENSE: no

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Streptomyces pristinaespiralis*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8

GTG CCG TTC GCG CGA CGC GGT TCG GTG GTG GGC GAG GCG GAC CTG GCG GCG CTG	54
Val Pro Phe Ala Arg Arg Gly Ser Val Val Gly Glu Ala Asp Leu Ala Ala Leu	18
GGC GAA CTG CTC CCG TCG GGC CGG TCG CTG ACG TCG GCG GTG TGG CGG GAG CGG	108
Gly Glu Leu Val Arg Ser Gly Arg Ser Leu Thr Ser Gly Val Trp Arg Glu Arg	36
TTC GAG GAA CAG TTC GCC CGC CTG ACC GGC GCC CGG CAC GCG CTC AGT GTC ACC	162
Phe Glu Glu Gln Phe Ala Arg Leu Thr Gly Ala Arg His Ala Leu Ser Val Thr	54
AGC GGC ACC GTC GCG CTG GAA CTG GCG GTG CGG ATG CTG GAC CTG GCG CCG GGC	216
Ser Gly Thr Val Ala Leu Glu Leu Ala Val Arg Met Leu Asp Leu Ala Pro Gly	72
GAC GAG GTG ATC GCC ACC CCG CAG ACG TTC CAG GCG ACG CTG CAG CCG CTG CTC	270
Asp Glu Val Ile Ala Thr Pro Gln Thr Phe Gln Ala Thr Val Gln Pro Leu Leu	90
GAC CAC GAC GTG CCG CTG CCG TTC TGC GAC ATC GAC CCG GAC ACC CTC AAC CTC	324
Asp His Asp Val Arg Leu Arg Phe Cys Asp Ile Asp Pro Asp Thr Leu Asn Leu	108
GAC CCG GCG GTG CTG GAG ACG CTG ATC ACC GAC CCG ACC CCG GCG ATC CTG CTC	378
Asp Pro Ala Val Leu Glu Thr Leu Ile Thr Asp Arg Thr Arg Ala Ile Leu Leu	126
CTC CAC TAC GGC GGC AAC CCG GCC GAC ATG GAC CCG ATC ATG GCC CTG GCC CCG	432
Val His Tyr Gly Gly Asn Pro Ala Asp Met Asp Arg Ile Me Ala Leu Ala Arg	144
AAG CCG GGC ATC ATC GTC GTC GAG GAC AGC GCG CAC GCG CTG GGC GCC GTG TAC	486
Lys Arg Gly Ile Ile Val Val Glu Asp Ser Ala His Ala Leu Gly Ala Val Tyr	162
CGG GGC CCG CCG CCG GGC GCA CTG CCG GAC ATC GCG TGC TTC ACT TTC CAC TCC	540
Arg Gly Arg Arg Pro Gly Ala Leu Ala Asp Ile Gly Cys Phe Thr Phe His Ser	180

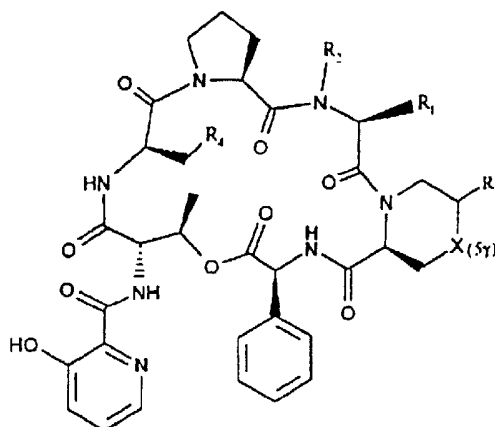


ACG AAG AAC ATC ACC ACC CTC GGC GAG GGC GGC ATG ATC ACC CTG TCG CGT GAC	594
Thr Lys Asn Ile Thr Thr Leu Gly Glu Gly Gly Met Ile Thr Leu Ser Arg Asp	198
GAG TGG GCC CAG CGG GTG GGA CGT ATC CGC GAC AAC GAG GCC GAC GGC GTG TAC	648
Glu Trp Ala Gln Arg Val Gly Arg Ile Arg Asp Asn Glu Ala Asp Gly Val Tyr	216
CGG GCG CTC CCG GAC TCC GCG CGG GCG GGT GGT CCG GCG CTG CTG CCG TGG ATG	702
Ala Ala Leu Pro Asp Ser Ala Arg Ala Gly Ala Pro Ala Leu Leu Pro Trp Met	234
AAG TTC GCG GAG GGT GTG TAC GGT CAC CGG GCG GTC GGG GTC CCG GCG GCG	756
Lys Phe Ala Glu Gly Val Tyr Gly His Arg Ala Val Gly Val Arg Gly Ala Gly	252
ACG AAC GCG ACG ATG TCG GAG GCG GCG GCG GCG GTG GGC GTG GTG CAA CTG GCG	810
Thr Asn Ala Thr Met Ser Glu Ala Ala Ala Val Gly Val Val Gln Leu Ala	270
TCG CTG GAG CGG TTC GTG GCC CGG CGC CGG AGC ATC GCG CAG CGG CTG GAC GAG	864
Ser Leu Glu Arg Phe Val Ala Arg Arg Arg Ser Ile Ala Gln Arg Leu Asp Glu	288
GCC GTG GCC TCG GTG GCC GGC ACC CGG CTG CAC CCG GCG GCG GCG GAC AGT CTG	918
Ala Val Ala Ser Val Ala Gly Thr Arg Leu His Arg Ala Ala Ala Asp Ser Leu	306
CAC GCC TAC CAC CTG TAC ACG TTC TTC CTC ACC GGC GGC CGG CAG GTG CGG GAG	972
His Ala Tyr His Leu Tyr Thr Phe Phe Leu Thr Gly Gly Arg Gln Val Arg Glu	324
CGG TTC GTG GCG GCC CTG GAC CGG CTG GGT GTG GAG GTC CAG TTG CCG TAC TTC	1026
Arg Phe Val Arg Ala Leu Asp Arg Leu Gly Val Glu Val Gln Leu Arg Tyr Phe	342
CCG CTC CAT CTG TCG CCC GAG TGG CGG CTG GCG GGC CAC GGG CCG GCG GAG TGT	1080
Pro Leu His Leu Ser Pro Glu Trp Arg Leu Arg Gly His Gly Pro Gly Glu Cys	360
CCG ACG GCC GAA CGG GTC TGG TTC GAG GAG CAC ATG AAC CTG CCG TGC CAT CCC	1134
Pro Thr Ala Glu Arg Val Trp Phe Glu Glu His Met Asn Leu Pro Cys His Pro	378
GGT CTG AGT GAC GGC CAG GTC GAC TAC ATG GTC GAG GCG GTC ACC CCG GCC CTG	1188
Gly Leu Ser Asp Gly Gln Val Asp Tyr Met Val Glu Ala Val Thr Arg Ala Leu	396
CAC GAG GCC CAC GCG ACG GGG ACG CGG GTG GCG GCC GGG CAC CTG	1233
His Glu Ala His Gly Thr Gly Thr Arg Val Ala Ala Gly His Leu	411



THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

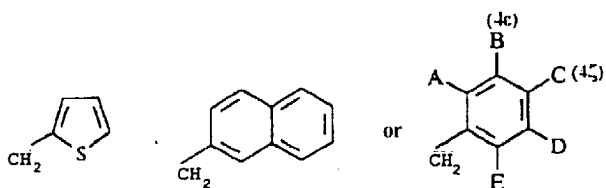
1. Compound characterized in that it is represented by the general formula I



I

in which:

- 5 - R₂ and R₄ represent, independently of each other, a hydrogen atom or a methyl group,
- R₃ represents a hydrogen atom or a hydroxyl group,
- X represents a CO, CHOH or CH₂ group, and
- 10 - R₁ represents:



with

- for the meta derivatives:

A, C, D and E representing a hydrogen atom, and

B being able to represent:

- a halogen, and preferably a fluorine atom,
- 5 - a monoalkylamino or dialkylamino group,

- an ether group,
- a thioether group,
- 10 - a C₁ to C₄ alkyl group, or
- a trihalogenomethyl group;

- for the para derivatives:

A, B, D and E representing a hydrogen atom, and

15 C being able to represent:

- a halogen,
- an NR₁R₂ group with R₁ and R₂ representing, independently of each other, a group selected from among

- 20 - hydrogen,
- a straight-chain or branched C₁ to C₄ alkyl group where, when one of the substituents R₁ or R₂ represents a methyl group, the other necessarily represents an ethyl group,

- 25 - an alkyl-cycloalkylmethyl group with a C₁ to C₄ cycloalkyl,

- an optionally substituted C₁ to C₄ cycloalkyl group,



- a straight-chain or branched C_1 to C_4 alkenyl group where, when one of the substituents R_1 or R_2 represents an alkenyl group, the other is different from a methyl group or a C_3 to C_6 cycloalkyl group,

5 - a substituted or unsubstituted N-pyrrolidinyl group,

- an ether group,
- a thioether group,
- an acyl or alkoxycarbonyl group,

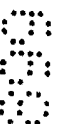
10 - a C_1 to C_6 alkyl group which is straight-chain or branched

- an alkylthiomethyl group,
- an aryl group,



15 or

- a trihalogenomethyl group;



20

- for the meta-para disubstituted derivatives:

A, D and E representing a hydrogen atom, and

B being able to represent:

- a halogen,
- a monoalkylamino or dialkylamino group with



alkyl

- an ether group,
- a thioether group,
- a C_1 to C_6 alkyl group, and

25

C being able to represent:

- a halogen,

- an amino, monoalkylamino or dialkylamino group with the proviso that B is different from a bromine or chlorine atom, or a substituted or unsubstituted allyl group,

- 5
- an ether group,
 - a thioether group
 - a C₁ to C₆ alkyl group, or
 - a trihalogenomethyl group, and

- for the ortho-para disubstituted derivatives:

B, E and D representing a hydrogen atom and A and C a methyl group.

10

2. A compound according to claim 1 wherein:

for the meta derivatives:

in the definition of B:

the alkyl groups in monoalkylamino or dialkylamino represent methyl or ethyl;

the trihalogenomethyl group is trifluoromethyl;

for the para derivatives:

in the groups represented by C:

the C₁ to C₆ alkyl group is methyl, isopropyl or tert-butyl;

the aryl group is phenyl;

the trihalogenomethyl is trifluoromethyl;

for the meta-para disubstituted derivatives:

in the groups represented by B:

the halogen atom is fluorine;

the alkyl groups in monoalkylamino or dialkylamino are methyl or ethyl; and

in the groups represented by C:

the halogen atom is fluorine;

the alkyl groups in monoalkylamino or dialkylamino are methyl;

the trihalogenomethyl is trifluoromethyl.

30

3. Compound according to claim 1, characterized in that it is:

4'-methylthio-

de(4'-dimethylamino)pristinamycin I_A,

4'-methylthio-

de(4'-dimethylamino)pristinamycin I_A,

5

5γ-hydroxy-4'-methylthio-

de(4'-dimethylamino)pristinamycin I_A,

4'-methyl-de(4'-dimethylamino)pristinamycin

I_A,

10

4'-methyl-de(4'-dimethylamino)pristinamycin

I_A,

4'-methoxy-de(4'-dimethylamino)pristinamycin

I_A,

4'-methoxycarbonyl-

9
9
9
9
9
9

- de (4 ζ -dimethylamino)pristinamycin I_A,
 4 ζ -chloro-de (4 ζ -dimethylamino)pristinamycin
 I_A,
 4 ζ -bromo-de (4 ζ -dimethylamino)pristinamycin I_A,
 5 4 ζ -bromo-de (4 ζ -dimethylamino)pristinamycin I_B,
 4 ζ -iodo-de (4 ζ -dimethylamino)pristinamycin I_A,
 4 ζ -iodo-de (4 ζ -dimethylamino)pristinamycin I_B,
 4 ζ -trifluoromethyl-de (4 ζ -dimethylamino) -
 pristinamycin I_A,
 10 4 ζ -trifluoromethyl-de (4 ζ -dimethylamino) -
 pristinamycin I_B,
 4 ζ -tert-butyl-de (4 ζ -dimethylamino) -
 pristinamycin I_A,
 4 ζ -isopropyl-de (4 ζ -dimethylamino) -
 15 pristinamycin I_A,
 4 ζ -isopropyl-de (4 ζ -dimethylamino) -
 pristinamycin I_B,
 4 ϵ -methylamino-de (4 ζ -dimethylamino) -
 pristinamycin I_A,
 20 4 ϵ -methoxy-de (4 ζ -dimethylamino)pristinamycin
 I_A,
 4 ϵ -methoxy-de (4 ζ -dimethylamino)pristinamycin
 I_B,
 4 ϵ -fluoro 4 ζ -methyl-de (4 ζ -dimethylamino) -
 25 pristinamycin I_A,
 4 ζ -am.no-de (4 ζ -dimethylamino)pristinamycin
 I_A,
 4 ζ -ethylamino-de (4 ζ -dimethylamino) -



- pristinamycin I_A,
 4'-diethylamino-de(4'-dimethylamino) -
 pristinamycin I_A,
 4'-allylamino-de(4'-dimethylamino) -
 5 pristinamycin I_A,
 4'-diallylamino-de(4'-dimethylamino) -
 pristinamycin I_A,
 4'-allylethylamino-de(4'-dimethylamino) -
 pristinamycin I_A,
 10 4'-ethylpropylamino-de(4'-dimethylamino) -
 pristinamycin I_A,
 4'-ethylisopropylamino-de(4'-dimethylamino) -
 pristinamycin I_A,
 4'-ethylmethylcyclopropylamino-
 15 de(4'-dimethylamino)pristinamycin I_A,
 4'-(1-pyrrolidinyl) -de(4'-dimethylamino) -
 pristinamycin I_A,
 4'-trifluoromethoxy-de(4'-dimethylamino) -
 pristinamycin I_A,
 20 4'-allyloxy-de(4'-dimethylamino)pristinamycin
 I_A,
 4'-ethoxy-de(4'-dimethylamino)pristinamycin
 I_A,
 4'-ethylthio-de(4'-dimethylamino) -
 25 pristinamycin I_A,
 4'-methylthiomethyl-de(4'-dimethylamino) -
 pristinamycin I_A,
 4'-(2-chloroethoxy) -de(4'-dimethylamino) -



pristinamycin I_A,

4'-acetyl-de(4'-dimethylamino)pristinamycin

I_A,

4'-ethyl-de(4'-dimethylamino)pristinamycin I_A,

4'-ethyl-de(4'-dimethylamino)pristinamycin I_B,

4'-dimethylamino-de(4'-dimethylamino) -

pristinamycin I_A,

4'-methylthio-de(4'-dimethylamino) -

pristinamycin I_A, and

4'-ethoxy-de(4'-dimethylamino)pristinamycin

I_A.

4. Process for preparing streptogramins, characterized in that it employs a streptogramin-producing microorganism strain which possesses at least one genetic modification which affects the biosynthesis of a precursor of the group B streptogramins, and in that the said mutant strain is cultured on a culture medium which is supplemented with at least one precursor which is different from that whose biosynthesis is altered the precursor being selected from derivatives or analogues of amino acids and alpha-ketocarboxylic acids, and in that the said streptogramins are recovered.

5. A process according to claim 4 in which the amino acid is phenylalanine and the alpha-ketocarboxylic acid is phenylpyruvic acid.

6. A process according to claim 4 in which the precursor is selected from phenylalanine, 4-dimethylaminophenylalanine, 4-methylaminophenylalanine, 4-aminophenylalanine, 4-diethylaminophenylalanine, 4-ethylaminophenylalanine, 4-methylthiophenylalanine, 4-methylphenylalanine, 4-methoxyphenylalanine, 4-trifluoromethoxyphenylalanine, 4-methoxycarbonylphenylalanine, 4-chlorophenylalanine, 4-bromophenylalanine, 4-iodophenylalanine, 4-trifluoromethylphenylalanine, 4-tert-butylphenylalanine, 4-isopropylphenylalanine,

3-methylaminophenylalanine, 3-methoxyphenylalanine, 3-methylthiophenylalanine, 3-fluoro-4-methylphenylalanine, L-pipecolic acid, 4-tert-butylphenylpyruvic acid, 4-methylaminophenylpyruvic acid, 2-naphthylphenylalanine, 4-fluorophenylalanine, 3-fluorophenylalanine, 3-ethoxyphenylalanine, 2,4-dimethylphenylalanine, 3,4-dimethylphenylalanine, 3-methylphenylalanine, 4-phenylphenylalanine, 4-butylphenylalanine, 2-thienyl-3-alanine, 3-trifluoromethylphenylalanine, 3-hydroxyphenylalanine, 3-ethylaminophenylalanine, 4-allylaminophenylalanine, 4-diallylaminophenylalanine, 4-allylethylaminophenylalanine, 4-ethylpropylaminophenylalanine, 4-ethylisopropylaminophenylalanine, 4-ethylmethylcyclopropylaminophenylalanine, 4-(1-pyrrolidinyl)phenylalanine, 4-O-allyltyrosine, 4-O-ethyltyrosine, 4-ethylthiophenylalanine, 4-ethylthiomethylphenylalanine, 4-O-(2-chloroethyl)tyrosine, 4-acetylphenylalanine, 4-ethylphenylalanine, 3-dimethylaminophenylalanine, and 4-aminomethylphenylalanine.

7. Process according to claim 4, 5 or 6 characterized in that the mutant strain possesses at least one genetic modification which is located within one of the genes involved in the biosynthesis of the group B streptogramin precursors.

8. Process according to claim 7 characterized in that the gene(s) whose expression is altered is/are selected from among the genes which are involved in the biosynthesis of L-2-aminobutyric acid, 4-dimethylamino-L-phenylalanine (DMPAPA), L-pipecolic acid, L-phenylglycine and/or 3-hydroxypicolinic acid.

9. Process according to claim 7 or 8, characterized in that at least one of the genes is selected from among the papA, papM, papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) and hpaA (SEQ ID No 8) genes.

10. Process according to any one of claims 4 to 9, characterized in that the said genetic modification renders at least one of the genes involved in the biosynthesis of the group B streptogramin precursors partially or totally incapable of encoding the natural enzyme.

5

11. Process according to any one of claims 4 to 10, characterized in that the genetic modification consists of a disruption of one of the genes involved in the biosynthesis of the group B streptogramin precursors.

10

12. Process according to any one of claims 4 to 11, characterized in that the mutant strain employed is derived from the strain S. pristinaespiralis.

13. Process according to claim 12 in which the strain is S. pristinaespiralis SP92.

14. Process according to claim 12, characterized in that the strain is SP92:pVRC508.

15. Process according to claim 12, characterized in that the strain is SP212.

16. Process according to claim 12, characterized in that the strain is SP92_{pipA}: :Ω_{am}^R.

17. Process according to claim 12, characterized in that the strain is SP92_{hpA}: :Ω_{am}^R.

18. Process according to any one of claims 4 to 17 characterized in that the precursor is selected such that it is related to the precursor whose biosynthesis is altered.

30

19. Process according to any one of claims 4 to 18, characterized in that the precursor is a derivative of phenylalanine when the gene whose expression is altered relates to the biosynthesis of DMPAPA.

20. Process according to any one of claims 4 to 19 which is useful for preparing pristinamycin IB.

21. Isolated nucleotide sequence, characterized in that it is selected from among:

(a) all or part of the genes papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) and hpaA (SEQ ID No. 8) and which encode a polypeptide which is involved in the biosynthesis of streptogramins, and

(b) sequences which hybridize with all or part of the (a) genes, and which encode a polypeptide which is involved in the biosynthesis of streptogramins, and

(c) sequences which are derived from (a) and (b) sequences on account of the degeneracy of the genetic code.

22. Isolated nucleotide sequence according to claim 21, characterized in that it is selected from among the papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) and hpaA (SEQ ID No. 8) genes.

23. Recombinant DNA encompassing a gene selected from among the papC (SEQ ID No. 2), papB (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) and hpaA (SEQ ID No. 8) genes.

24. Vector, characterized in that it encompasses a nucleotide sequence according to claim 21 or 22 or a recombinant DNA according to claim 23.

25. Use of a sequence according to claim 21 or 22 and/or of a vector according to claim 23 for preparing metabolites.

26. Isolated polypeptide which results from the expression of a sequence according to claim 21 or 22.

27. Isolated mutant *S. pristinaespiralis* strain, characterized in that it possesses at least one genetic modification within one of its papC (SEQ ID No. 2), papE (SEQ ID No. 3), pipA (SEQ ID No. 5), snbF (SEQ ID No. 6) and hpaA (SEQ ID No. 8) genes.

28. Isolated mutant strain according to claim 27, characterized in that it is the strain SP92 pipA: : Ω am^R.

29. Isolated mutant strain according to claim 27, characterized in that it is the strain SP92 hpaA: : Ω am^R.

30. Compound, characterized in that it is 4-trifluoromethoxyphenylalanine,
3-methylaminophenylalanine, 3-methylthiophenylalanine,
3-fluoro-4-methylphenylalanine,
4-methylaminophenylpyruvic acid, 3-ethoxyphenylalanine,
4-allylaminophenylalanine, 4-diallylaminophenylalanine,
4-allylethylaminophenylalanine,
4-ethylpropylaminophenylalanine,
4-ethylisopropylaminophenylalanine,
4-ethylmethylcyclopropylaminophenylalanine,
4-(1-pyrrolidinyl)phenylalanine,
4-ethylthiomethylphenylalanine,

4-O-(2-chloroethyl)tyrosine,
3-dimethylaminophenylalanine and
3-ethylaminophenylalanine.

5 31. Pharmaceutical composition, characterized in that it contains at least
one compound according to claim 1, 2 or 3 in association with a pharmaceutically
acceptable carrier or coating.

 32. A composition according to claim 31 which further comprises a group
0 A streptogramin.

 33. A compound of the general formula I as defined in claim 1
substantially as herein described with reference to any example thereof.

5 34. A process as claimed in claim 4 substantially as herein described with
reference to any example thereof.

 35. A nucleotide sequence as claimed in claim 21 substantially as herein
described with reference to any example thereof.

0 36. Recombinant DNA as claimed in claim 23 substantially as herein
described with reference to any example thereof.

5 37. A vector as claimed in claim 24 substantially as herein described with
reference to any example thereof.

 38. A mutant S. pristinaespiralis strain as claimed in claim 26
substantially as herein described with reference to any example thereof.

 DATED this 26th day of August 1999

Rhone-Poulenc Rorer SA.

By its Patent Attorneys

DAVIES COLLISON CAVE