Process for the Manufacture of Cyclic Undecapeptides

Field of the invention

The invention relates to novel process(es), novel process step(s) and novel intermediate(s) useful for the opening of Cyclosporin derivatives and subsequently for the preparation of cyclic polypeptides, more specifically, cyclic undecapeptides, such as alisporivir (also known as DEB025, Debio025, or Debio).

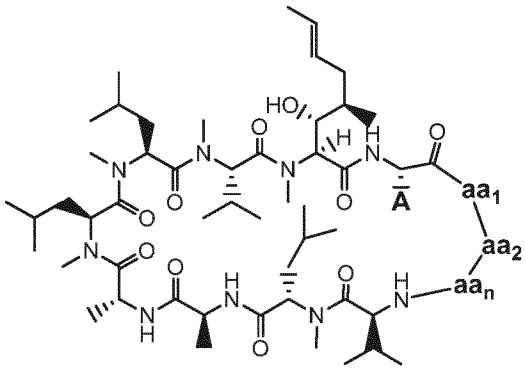
Background of the invention

The present invention relates to processes for the preparation of cyclic polypeptides, such as, for example, cyclic undecapeptides, such as alisporivir.
Alisporivir is a cyclophilin (Cyp) inhibitor used for the treatment of hepatitis C virus (HCV) infection or HCV induced disorders as described in WO 2006/038088.Furthermore, WO2009/042892 describes methods for the use of alisporivir in the treatment of multiple sclerosis; WO2009/098577 describes methods for the use of alisporivir in the treatment of muscular dystrophy; WO2008/084368 describes methods for the use of alisporivir in the treatment of Ullrich congenital muscular dystrophy.

Alisporivir and a synthesis thereof are described in WO 00/01715. Alisporivir has been attributed the CAS Registry Number 254435-95-5.

Processes for the preparation of Alisporivir on laboratory scale are described by J.F. Guichoux in “De nouveaux analogues de Cycloposrine A comme agent anti-HIV-1” PhD thesis, Faculte des Sciences de L’Universite de Lausanne, 2002, in WO2006/038088, and in WO2008/084368.

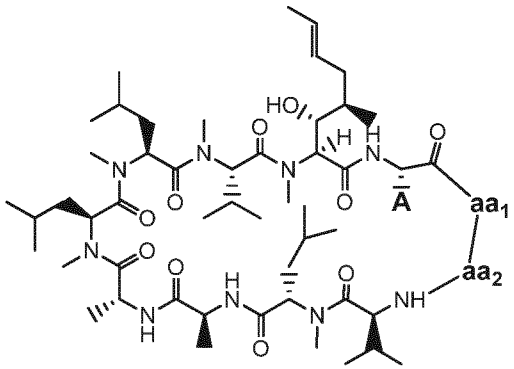
Cyclic undecapeptides, as represented below, are cyclic polypeptides of Formula (Ia), wherein n=2.
Alisporivir (Formula I) is a cyclic undecapeptide of Formula (Ib) wherein n=2, aa1 is D-MeAla and aa2 is EtVal.



CYCLIC POLYPEPTIDES

A= Alkyl substituent
aa$_n$= amino acids

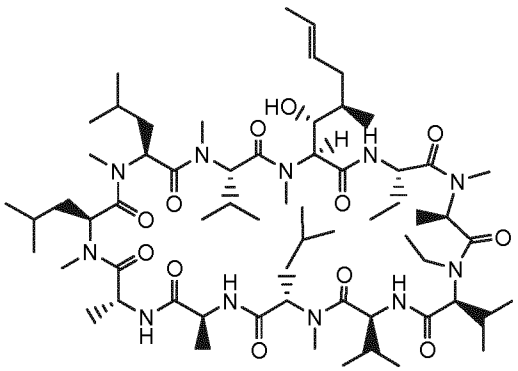
(Formula Ia)



CYCLIC UNDECAPEPTIDES

A= Alkyl substituent
aa$_n$= amino acid

(Formula Ib)



ALISPORIVIR

n = 2, aa_1 = D-MeAla, aa_2= EtVal

(Formula I)

GENERIC FORMULA:

Cyclo-(AXX1-AXX2-AXX3-AXX4-AXX5-AXX6-AXX-7-AXX8-AXX9-AXX10-AXX11), should cover examples from case WO2010/052559 A1 as fragmentation made at key Sar fragment

AXX1= MeBmt, Bmt, MeLeu, Desoxy-MeBmt, Methylaminooctanoic acid

AXX2= Abu, Ala, Thr, Val, Nva

AXX3= Sar

AXX4= MeLeu, Val

AXX5= Val, Nva
AXX6= MeLeu, Leu
AXX7= Ala, Abu
AXX8= D-Ala
AXX9= MeLeu, Leu
AXX10= MeLeu, Leu
AXX11= MeVal, Val, D-MeVal

And all other combinations covered in WO 2010/052559 A1

Over the last several years, cyclosporin A (CyA) has been used as a raw material for a variety of synthetic cyclic undecapeptides which are useful for the treatment of inflammatory or viral diseases. Cyclic undecapeptides may be obtained bystrain selection, however obtaining most un-natural derivatives requires a chemical transformation which relies on opening of the cyclic polypeptide, for example, of Formula (Ia) or of Formula (Ib) and subsequent amino acid replacement.

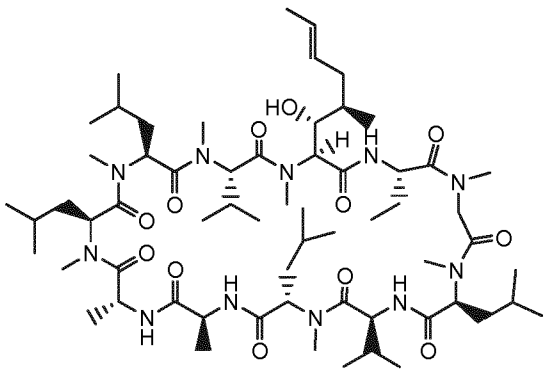
Traditionally, cyclic polypeptide, for example of Formula (Ia) are opened in a highly selective process and an amino acid residue is removed via the Edman degradation to access the opened cyclic polypeptide as a key intermediate (Wenger, R. M. In Peptides 1996; Ramage, R.; Epton, R., Eds.; The European Peptides Society, 1996; pp. 173; Wenger, R. M. *et al*. *Tetrahedron Letters* **2000**, *41*, 7193.). Numerous scientists and companies have used this reliable and selective strategy wherein pure cyclosporin A and purification by column chromatography have been used to obtain cyclic undecapeptides.

Furthermore, purification of products, such as opened cyclosporin A, involve several steps of purification by liquid chromatography on silica. Beside the moderate overall obtained yield, the major drawback of this purification scheme is the very high costs for the chromatography steps. Large-scale purification processes of such products derived from cyclosporin A or its structural analogues described in the literature generally involve a chromatographic purification or at least a pre-purification by adsorption chromatography. Such pre-purification may be followed, for instance, by extraction, counterflow extraction, and/or supercritical fluid extraction.

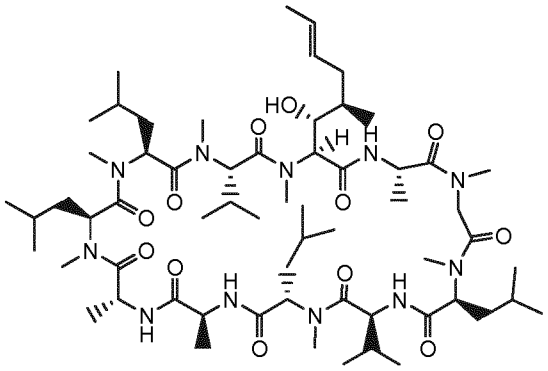
However, none of these techniques appear to be fully satisfactory for obtaining the key opened intermediates with the desired quality requirements, with an acceptable overall yield, and at an acceptable cost for an industrial scale production, as costly precursors of high quality were required.

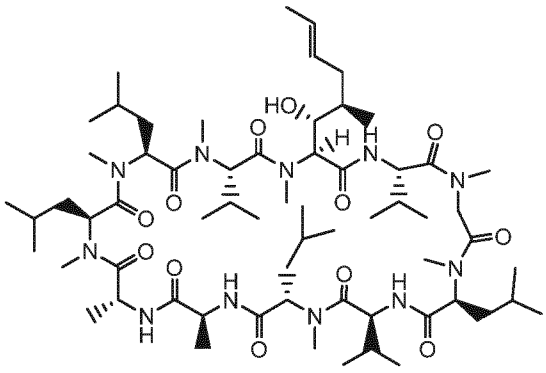
We identified that dimethoxycarbenium ions (described in Novartis patent application EP 0 908 461 A1 for the methylation of Cephalosporine derivatives), do the same chemistry as oxonium ions (trimethyl or triethyloxonium Meerwein salts) in the opening of the macrocyclic polypeptide. The new conditions can advantageously be prepared *in situ*, thus avoiding the handling of hazardous and hygroscopic substance, can proceed in a variety of solvents such as for example toluene, xylene, anisole, by-passing the need for using the undesirable chlorinated solvents such as dichloromethane or dichloroethane, and avoid the use of oxonium Meerwein salts originating from the genotoxic epichlorhydrin. Either the dedicated carbenium tetrafluoroborate salt or the *in situ* generated reactive species made by the reaction of boron trifluoride and an orthoester derivative, preferably trimethyl orthoformate, will result in the desired opened polypeptides such as compound **3** below.

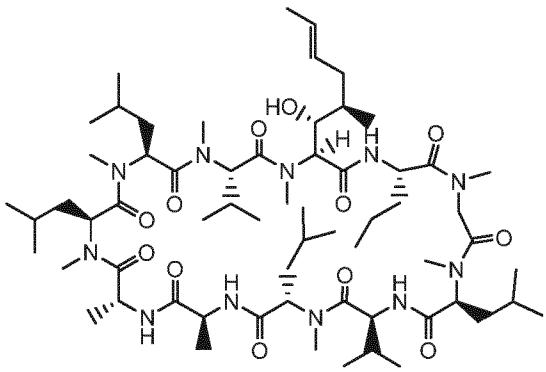
We identified an improved process which maintains the advantage of a highly selective Edman degradation strategy while taking full advantage of newly identified crystalline intermediates.

The following disclosure presents newly isolated and crystalline intermediates derived from the opening of cyclosporin A 

,

cyclosporin B ,

cyclosporin D ,

or cyclosporin G 

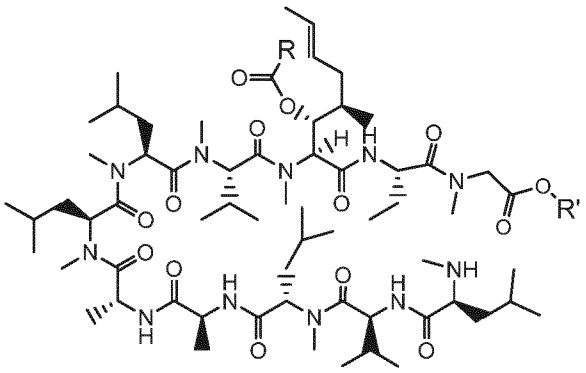
and a process to generate and purify them, via methods such as crystallizations. This approach allows for a rapid, practical and much more effective access to opened cyclosporin A, cyclosporin B, cyclosporin D or cyclosporin G and can be used to produce cyclic undecapeptides, such as alisporivir. Furthermore, the process according to the present disclosure may also be applied to other cyclosporins that can be opened via the same sequence. It was found that opened cyclosporin salts, such as hydrochloric acid (HCl), fluoroboric acid (HBF_4), or hexafluorophosphoric acid (HPF_6), can be formed at several stages.

The present invention provides novel crystalline intermediates, such as cylosporine esters, such as acetate, pivaloate, and opened cyclosporin A, cyclosporin B, cyclosporin D or

cyclosporin G salts such as the HCl salt, the HBF_4 salt, or the HPF_6 salt, and processes to generate them.

Summary of the Invention

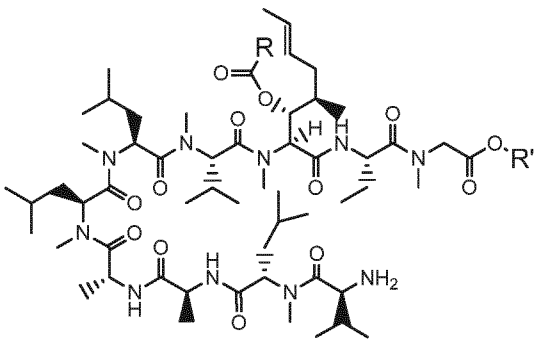
A process for preparing a compound of formula **3** or a salt thereof is provided,



(3)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl. The method includes the steps of acylation of cyclosporin A, to form acetyl-Cyclosporin A; ring opening of the acetyl-Cyclosporin A; and crystallizing the ring opened acetyl-Cyclosporin A to obtain the compound of formula **3**.

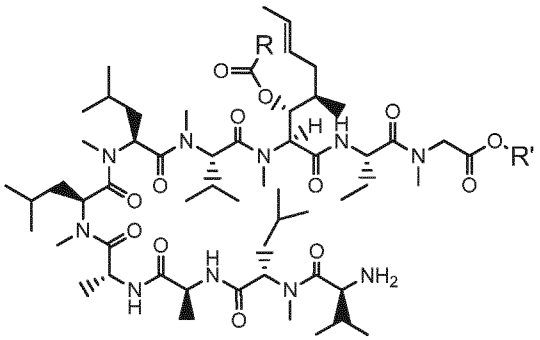
A process for preparing a compound of formula **4** or a salt thereof is provided,



(4)

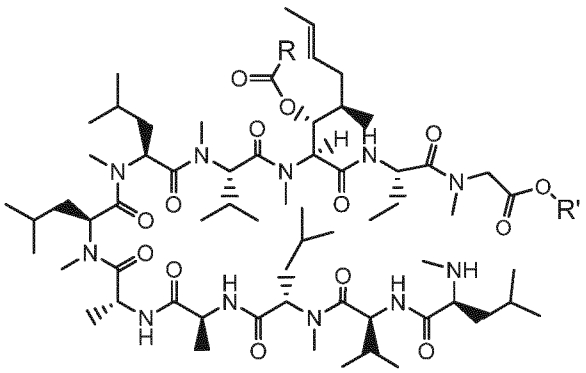
wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl. The method includes the steps of Edman degradation of compound of formula **3**; and then crystallizing the compound to obtain the compound of formula **4**.

A process for preparing a compound of formula **4** or a salt thereof is provided,



(4)

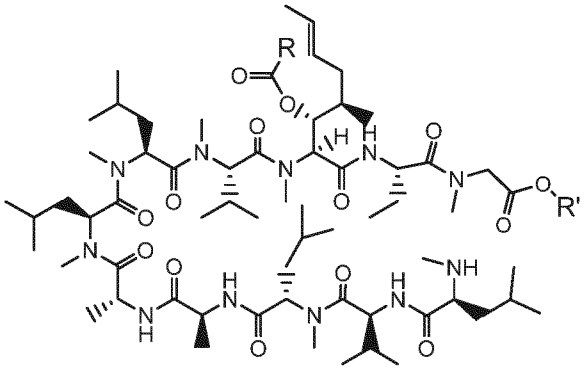
wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl. The method includes the steps of: acylation of cyclosporin A to form acetyl-Cyclosporin A; ring opening of the acetyl-Cyclosporin A; and crystallizing the ring opened acetyl-Cyclosporin A to obtain the compound of formula **3**



(3) ;

Edman degradation of the compound of formula **3**; and then crystallizing the compound to obtain the compound of formula **4** or a salt thereof.

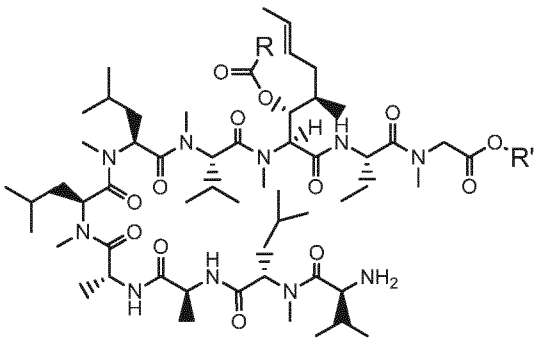
A compound of formula **3** or a salt thereof is provided



(3)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl.

A compound of formula **4** or a salt thereof is provided



(4)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl.

Brief Description of the Drawings

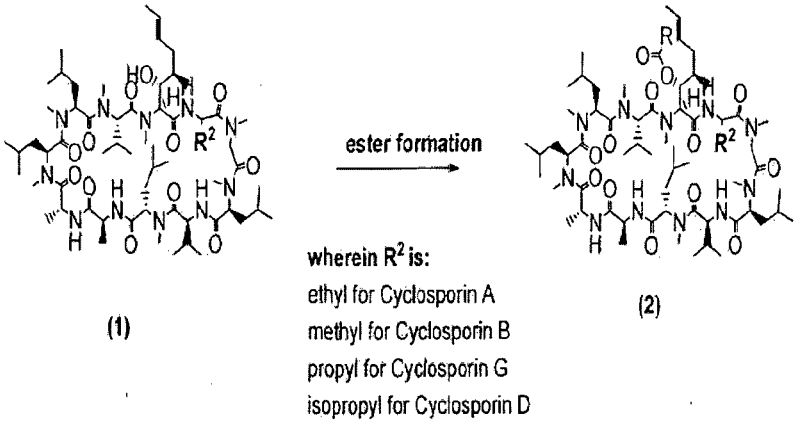
Figure 1 is a proton NMR spectra for compound **3**.

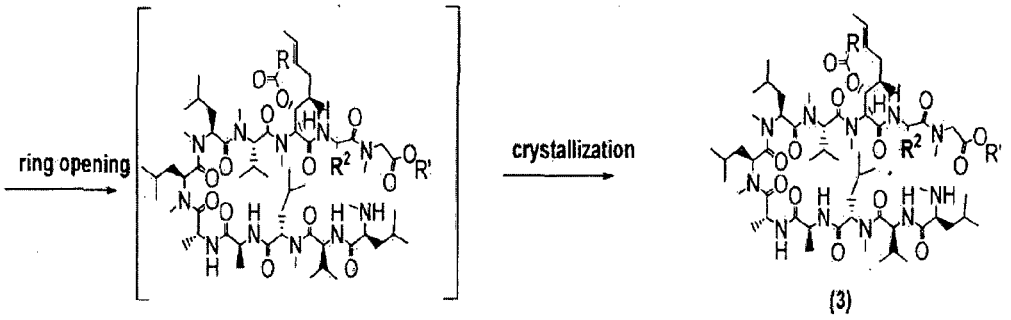
Figure 2 is a proton NMR spectra for compound **4**.

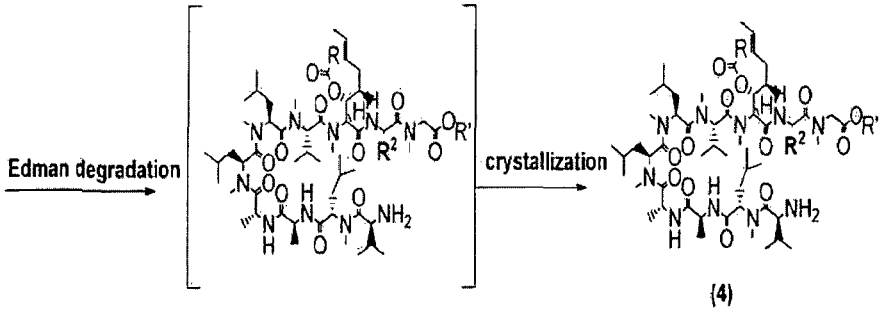
Detailed Description of the Invention

The general process according to the present invention for producing cyclic polypeptides, more specifically, cyclic undecapeptides, such as Alisporivir, is shown in the scheme below; however,

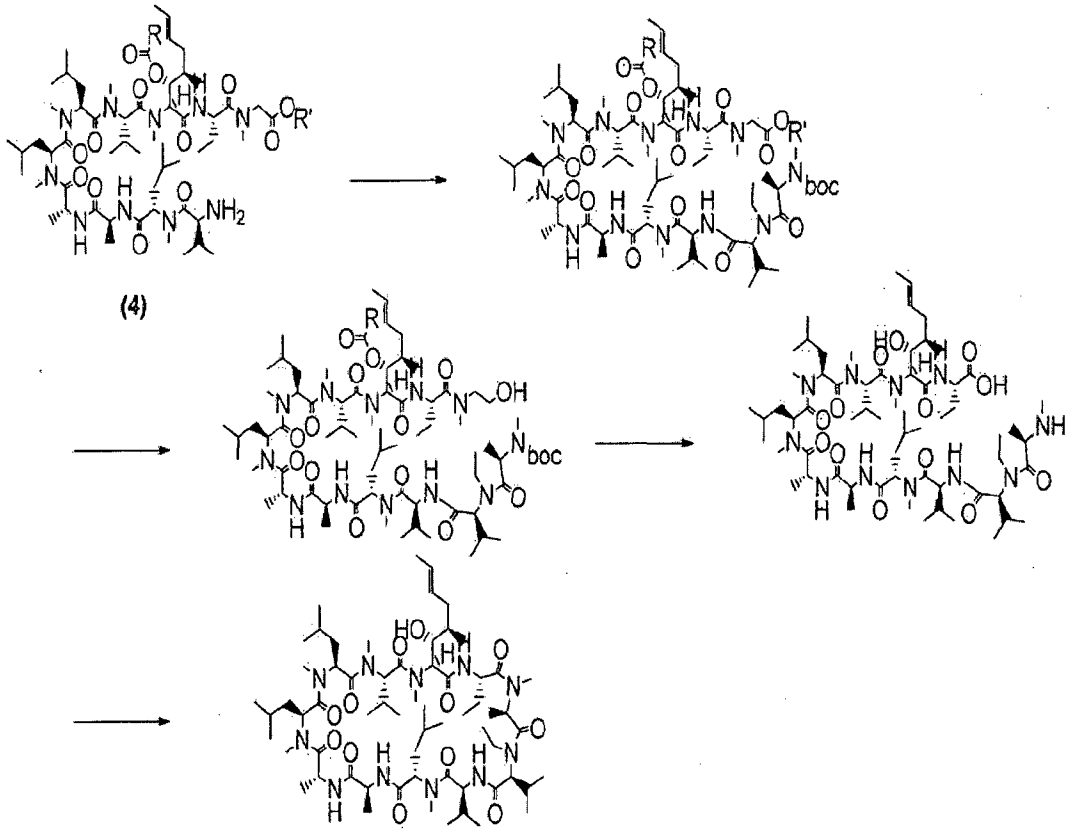
this general scheme can also be used to make cyclic polypeptides, more specifically, cyclic undecapeptides, derived from cyclosporine A, B, D, or G.







Specifically, alisporivir can be made by converting cyclosporin A (compound **(1)** wherein R^2 is ethyl) into a compound of formula **4** as shown above by acylation of cyclosporin A, to form acetyl-Cyclosporin A **(2)**; ring opening; crystallization to obtain a compound **3**, Edman degradation of compound **3**; crystallization to obtain a compound **4** and then cyclizing compound **4** to form alisporivir (as shown below).



(4)

ALISPORIVIR

The invention specially relates to the processes described in each section. The invention likewise relates, independently, to every single step described in a process sequence within the corresponding section. Therefore, each and every single step of any process, consisting of a sequence of steps, described herein is itself a preferred embodiment of the present invention. Thus, the invention also relates to those embodiments of the process, according to which a compound obtainable as an intermediate in any step of the process is used as a starting material.

The invention also relates to intermediates which have been specifically developed for the preparation of the compounds according to the invention, to their use and to processes for their preparation.

It is noted that in the present application, explanations made in one section may also be applicable for other sections, unless otherwise stated.

Cyclosporin A, cyclosporin B, cyclosporin D or cyclosporin G or salts thereof, may be prepared, for example by fermentation.

In one embodiment the present invention relates to a method for preparing compound of formula **3**, comprising the steps of acylation of cyclosporin A, cyclosporin B, cyclosporin D or cyclosporin G to form acetyl-Cyclosporin A, B, D, or G; ring opening; and crystallization.

In one embodiment the present invention relates to a method for preparing compound of formula **4** or a salt thereof, comprising Edman degradation, a reaction well known in the art, of a compound of formula **3** and crystallization thereof to obtain compound of formula **4**.

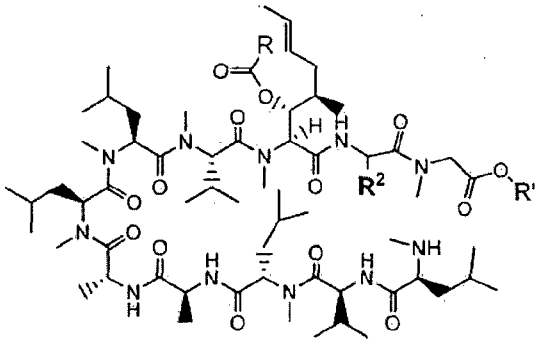
Another embodiment of the present invention relates to a method for preparing a compound of formula **3** or formula **4** wherein the purity of the Cyclosporin A starting material is >80% by weight

Another embodiment of the present invention relates to a method for preparing a compound of formula **3** or formula **4** wherein the purity of the Cyclosporin A starting material is >85% by weight.

Another embodiment of the present invention relates to a method for preparing a compound of formula **3** or formula **4** wherein the purity of the Cyclosporin A starting material is 60 to 80%, weight % assay.

In the processes shown above, novel and inventive compounds are involved. Consequently, further subjects of the present invention are the compounds shown below.

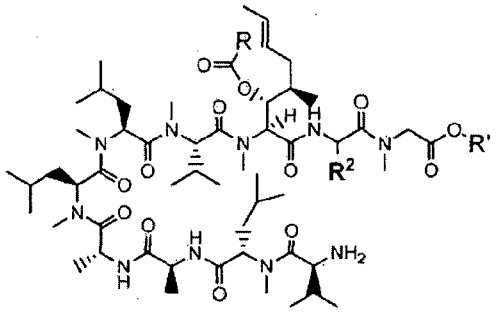
Compounds of formula **3** or salts thereof,



(3)

wherein R is methyl, ethyl, propyl or phenyl, R' is methyl or ethyl, and R_2 is methyl, ethyl, or propyl.

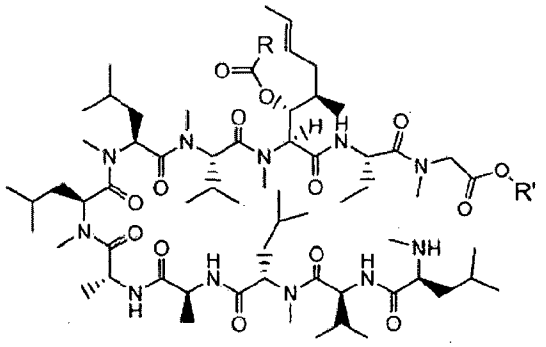
Compounds of formula **4** or salts thereof,



(4)

wherein R is methyl, ethyl, propyl or phenyl, R' is methyl or ethyl, and R^2 is methyl, ethyl, or propyl.

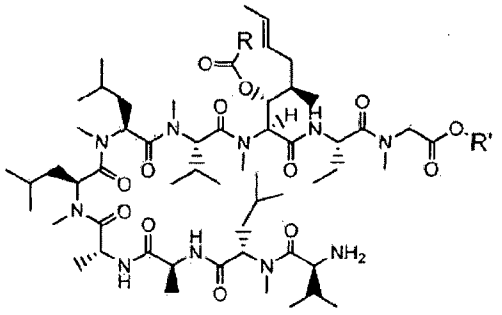
Compounds of formula **3** or salts thereof,



(3)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl.

Compounds of formula **4** or salts thereof,



(4)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl.

The following Examples represent preferred embodiments of the reaction steps, intermediates and/or the process of the present invention and serve to illustrate the invention without limiting the scope thereof.

Preparation of Compound 3 HBF_4 Salt with Merwein Salt

Acetyl-Cyclosporin A (100g as is) was reacted with trimethyloxonium tetrafluoroborate (32 g) at 20-25°C in dichloromethane (180 mL). After 20 h, acetonitrile (200 mL) and water (650 mL) were added to perform the hydrolysis. After 3 h, at 20-25°C, the phases were separated and the reaction mixture was dried by azeotropic distillation with 2-Methyl-Tetrahydrofuran (solvent exchange dichloromethane / 2-Methyl-Tetrahydrofuran). The desired product was then

crystallized from 2-Methyl-Tetrahydrofuran (900 mL) and 2-Methoxy-2-methylpropane (400 mL) to provide compound **3** HBF_4 as a white crystalline powder (63.9 g, after drying, purity >92%). 0.69, (3H,d,J=6.6Hz); 0.71, (3H,d,J=6.5Hz); 0.81, (6H,m); [0.82 .. 0.89], (24H,m); 0.90, (3H,d,J=6.6Hz); 0.93, (3H,d,J=6.6Hz); 1.16, (6H,m); [1.23 .. 1.50], (4H,m); 1.52, (1H,m); [1.32 .. 1.73], (8H,m); 1.59, (3H,d,J=6.0Hz); 1.65, (2H,m); 1.65, 2.13, (2H,m); 1.93, 1.94, (3H,s); 2.03, (1H,m); 2.19, (1H,m); 2.45, (3H,s); 2.72, (3H,s); 2.84, (3H,s); 2.86, (3H,s); 2.99, (3H,s); 3.02, (3H,s); 3.06, (3H,s); 3.62, 3.68, (3H,s); 3.78, (1H,m); 3.87, 4.53, (1H,d,J=17.2Hz,18.6Hz); 4.10, 4.26, (1H,d,J=18.6Hz,16.8Hz); 4.23, (1H,m); 4.60, (1H,m); 4.62, (1H,m); 4.66, (1H,m); 5.02, (1H,m); 5.13, (1H,dd,J=11.3Hz,4.7Hz); 5.26, (1H,m); 5.29, (1H,m); 5.32, (1H,m); 5.36, (1H,m); 5.39, (2H,m); 7.72, (1H,d,J=7.3Hz); 8.14, (1H,d,J=7.3Hz); 8.21, 8.35, (1H,d, J=7.3Hz,8.1Hz); 8.85, (2H,s,br); 8.96, (1H,d,J=8.4Hz).

Preparation of Compound 3 HBF_4 Salt with Use of Trimethylorthoformate and Borontrifluoride Etherate

A solution of Acetyl-Cyclosporin A (10g) in dichloromethane (20 mL) was added at -15°C to a slurry of dimethoxycarbenium tetrafluoroborate generate at -20°C by a slow addition of borontrifluoride (2ml) to a solution of trimethylorthoformate (2ml) in dichloromethane (20 mL). After the addition, the slurry was allowed to warm up to room temperature and was kept stirring for 20 h. Afterward, Acetonitrile (10 ml) and water (10 ml) were added. After 2 h stirring at 0°C, phases were split. Then, after having washed the organic phase with water, solvent switched to 2-Methyl-Tetrahydrofuran and saturation with 2-Methoxy-2-methylpropane, compound **3** was obtained as a white solid which was dried under vacuum (5.1 g, >90 % purity) (see Figure 1)

Preparation of Compound HBF_4 Salt:

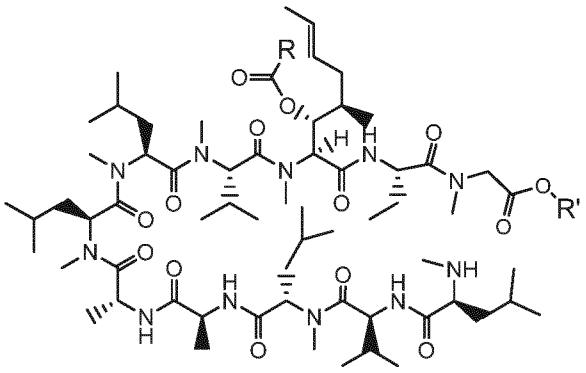
The previously prepared salt of compound **3** (34.62 g) was charged to a reactor along with sodium carbonate (4.8 g), Toluene (50 mL) and water (50 mL). The resulting mixture was stirred at 20-25°C for 30 minutes, and the phases were separated. Phenylisothiocyanate (3.81 g) was added drop wise in 1 h at 20-25°C and the resulting reaction mixture was stirred until completion. Then methanol (20 mL), and 48% fluoroboric acid in water (2.5 g) was added and the mixture was stirred for an additional 1h. Then water (25 mL) was added, and the phases were split. The aqueous layers were extracted once more with toluene (50 mL) and then extracted with 2-Methyl-Tetrahydrofuran (100 mL). The organic extract was dried azeotropically and the desired product was crystallized from 2-Methyl-Tetrahydrofuran (100 mL) and 2-

Methoxy-2-methylpropane (50 mL) to provide compound 4 HBF_4 as a white crystalline powder (*ca.* 30 g, after drying, >93% purity). (see Figure 2)
0.69, (3H,d,J=6.2Hz); 0.73, (3H,d,J=7.0Hz); 0.81, (3H,t, J=7.3Hz, 7.3Hz); 0.82, (3H,m); 0.85, (9H,m); 0.88, (6H,m); 0.91, (3H,d,J=7.0Hz); 0.93, (3H,d,J=6.6Hz); 0.99, (3H,d,J=7.0Hz); 1.17, (6H,d,J=6.6Hz); [1.30 .. 1.55], (9H,m); 1.60, (3H,d,J=5.5Hz); [1.56 .. 1.72], (4H,m); 1.93, 1.95 (3H,s); 2.09, (1H,m); 2.14, (1H,m); 2.20, (1H,m); 2.74, (3H,s); 2.82, 3.06, (3H,s); 2.84, (3H,s); 2.87, (3H,s); 2.94, (3H,s); 3.02, (3H,s); 3.63, 3.68, (3H,s); 3.88, 4.52, (1H,d,J=17.2Hz,18.6Hz); 4.10, 4.24, (1H,d,J=18.7Hz,m); 4.24, (2H,m); 4.39, 4.62, (1H,m); 4.66, (1H,m); 5.02, (1H,m); 5.08, (1H,m); 5.26, (2H,m); 5.32, (1H,m); 5.37, (1H,m); 5.39, (2H,m); 7.84, 8.51 (1H,d,J=7.3Hz, 8.1Hz); 7.98, (3H,s,br); 8.07, 8.18 (1H,d,J=7.7Hz, 7.3Hz); 8.13, 8.27, (1H,d,J=7.3Hz,8.1Hz).

The "undecapeptide amino acid" precursor (5 to 13% to the overall end mass) dissolved in dichloromethane and the DCC dissolved into dichloromethane were added continuously in parallel in ca. 10 h to a mixture of Cl-HOBT, and NMM in dichloromethane at 40 ^{o}C. At the end of the addition, the mixture was stirred for an additional 2h, filtered to remove the DCU salt and concentrated to give Alisporivir as a crude product.

Claims:

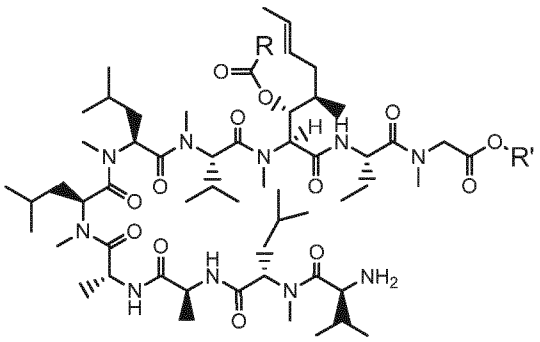
1. A process for preparing a compound of formula **3** or a salt thereof,



(3)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl, the method comprising the steps of acylation of cyclosporin A, to form acetyl-Cyclosporin A;
ring opening of the acetyl-Cyclosporin A ; and
crystallizing the ring opened acetyl-Cyclosporin A to obtain a compound of formula **3**.

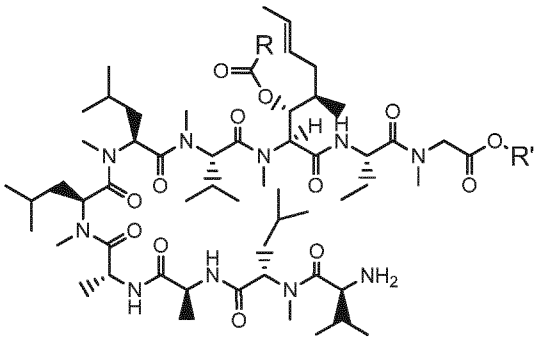
2. A process according to claim 1 for preparing a compound of formula 4 or a salt thereof,



(4)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl, the method comprising the steps of Edman degradation of compound of formula **3**; and then
crystallizing the compound to obtain a compound of formula **4**.

3. A process for preparing a compound of formula **4** or a salt thereof,



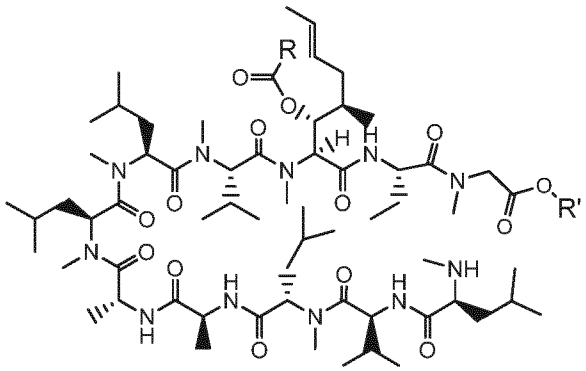
(4)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl, the method comprising the steps of:

i) acylation of cyclosporin A to form acetyl-Cyclosporin A;

ii) ring opening of the acetyl-Cyclosporin A; and ;

iii) crystallizing the ring opened acetyl-Cyclosporin A to obtain a compound of formula **3**

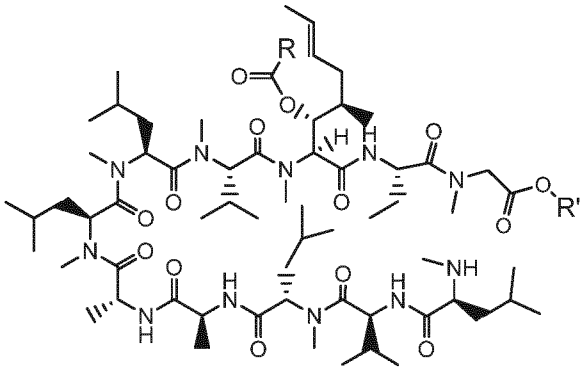


(3) or salt thereof;

iv) Edman degradation of the compound of formula **3**; and then

v) crystallizing the compound to obtain a compound of formula **4** or a salt thereof.

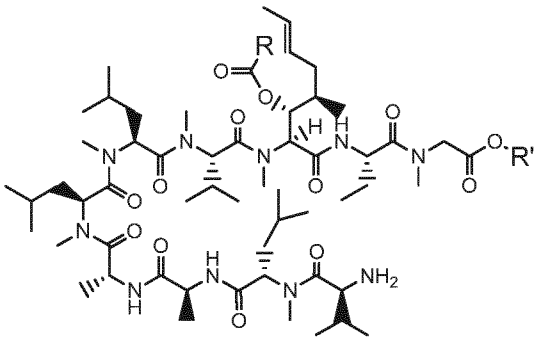
4. A compound of formula **3** or a salt thereof



(3)

wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl.

5. A compound of formula **4** or a salt thereof



(4)

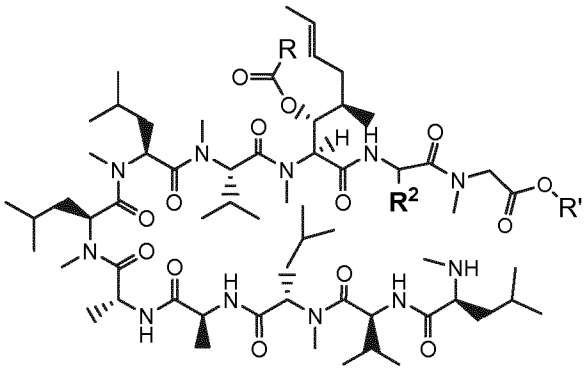
wherein R is methyl, ethyl, propyl or phenyl and R' is methyl or ethyl.

6. A process according to claim 1 or 3 wherein the purity of the starting material is >80%, by weight, Cyclosporin A.

7. A process according to claim 6 wherein the purity of the starting material is >85%, by weight, Cyclosporin A.

8. A process according to claim 7 wherein the purity of the starting material is 60 to 80%, weight % assay, of Cyclosporin A).

9. A process for preparing a compound of formula **3** or a salt thereof from from Cyclosporin A, Cyclosporin B, or from Cyclosporin D, or from Cyclosporin G,



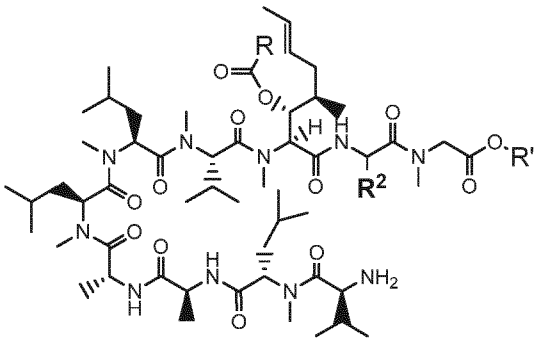
(3)

wherein R is methyl, ethyl, propyl or phenyl, R' is methyl or ethyl, and R^2 is methyl, ethyl, or propyl, the method comprising the steps of acylation of cyclosporin A , B, D, or G, to form acetyl-Cyclosporin A, B, D, or G;

ring opening of the acetyl-Cyclosporin A, B, D, or G; and

crystallizing the ring opened acetyl-Cyclosporin A, B, D, or G to obtain a compound of formula **3**.

10. A process according to claim 9 for preparing a compound of formula **4** or a salt thereof,

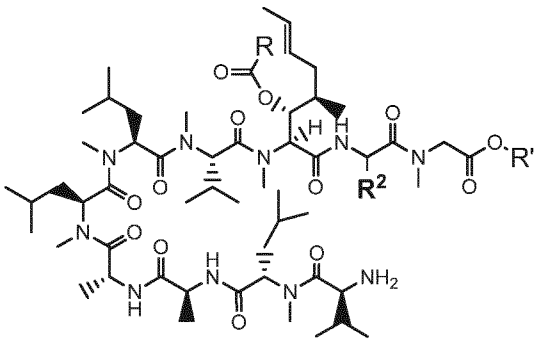


(4)

wherein R is methyl, ethyl, propyl or phenyl, R' is methyl or ethyl, and R^2 is methyl, ethyl, or propyl, the method comprising the steps of Edman degradation of compound of formula **3**; and then

crystallizing the compound to obtain a compound of formula **4**.

11. A process for preparing a compound of formula **4** or a salt thereof,



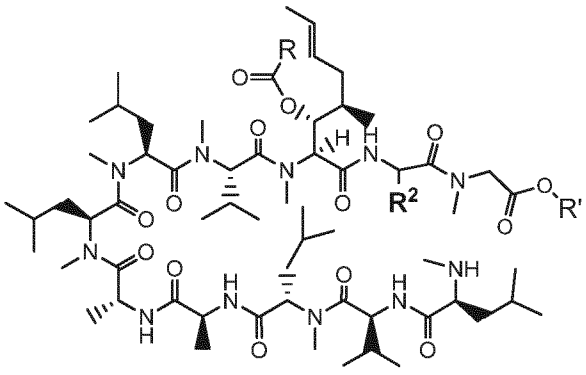
(4)

wherein R is methyl, ethyl, propyl or phenyl, R' is methyl or ethyl, and R^2 is methyl, ethyl, or propyl, the method comprising the steps of:

vi) acylation of cyclosporin A , B, D, or G, to form acetyl-Cyclosporin A, B, D, or G;

vii) ring opening of the acetyl-Cyclosporin A, B, D, or G; and

viii) crystallizing the ring opened acetyl-Cyclosporin A, B, D, or G to obtain a compound of formula **3** or salt thereof



(3)

ix) Edman degradation of compound of formula **3**; and then

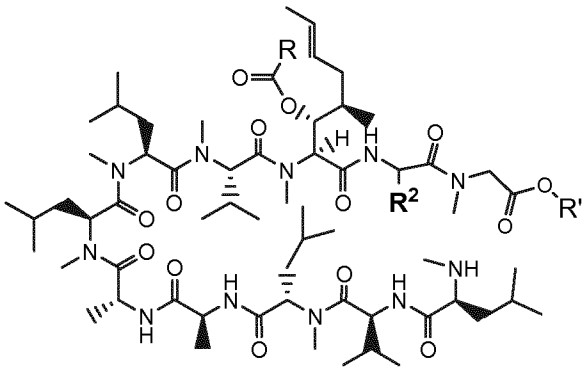
x) crystallizing the compound to obtain a compound of formula **4** or a salt thereof.

12. A process according to claim 9 or 11 wherein the purity of the starting material is >90%, by weight, Cyclosporin A.

13. A process according to claim 12 wherein the purity of the starting material is >92%, by weight, Cyclosporin A.

14. A process according to claim 13 wherein the purity of the starting material is 60 to 80%, weight % assay, of Cyclosporin A).

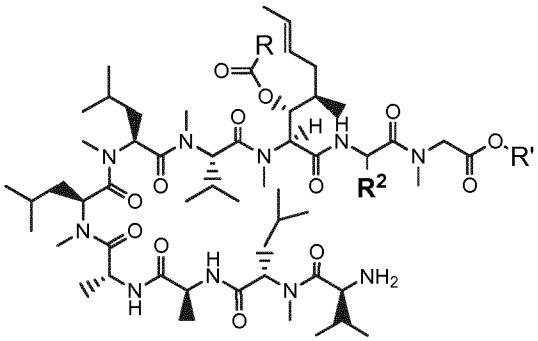
15. A compound of formula **3** or a salt thereof



(3)

wherein R is methyl, ethyl, propyl or phenyl, R' is methyl or ethyl, and R^2 is methyl, ethyl, or propyl.

16. A compound of formula **4** or a salt thereof



(4)

wherein R is methyl, ethyl, propyl or phenyl, R' is methyl or ethyl, and R^2 is methyl, ethyl, or propyl.