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54. **TÍTULO** " SÍNTESIS REGIOESPECÍFICA DE DERIVADOS 42-ÉSTER DE RAPAMICINA"

51. **CLASIFICACIÓN INTERNACIONAL** C07D 498/18 // A61K 47/4

71. **SOLICITANTE** WYETH

DOMICILIO NUEVA JERSEY, ESTADOS UNIDOS DE AMERICA

74. **APODERADO** ALVARO CORREA ORDOÑEZ
T-P-20281

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① SOLICITUD DE :

[X]	Patente de Invención	[ ]	Patente de Modelo de Utilidad
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Número _____

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Número 79.143.366 de Usaquén TP 20.281

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(10) **ANEXOS**

00002

- [X] Comprobante de pago de la tasa de presentación de la solicitud
- [ ] Documento que acredita la existencia y representación legal cuando el solicitante sea persona jurídica
- [ ] Poderes, si fuere el caso
- [ ] Documento de cesión del inventor al solicitante o a su causante
- [ ] Declaraciones
- [X] Copia de la solicitud internacional. (Resumen, descripción, reivindicaciones, dibujos)
- [X] Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- [ ] Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen preliminar (IPEA)
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- [ ] De ser el caso, copia del contrato de acceso
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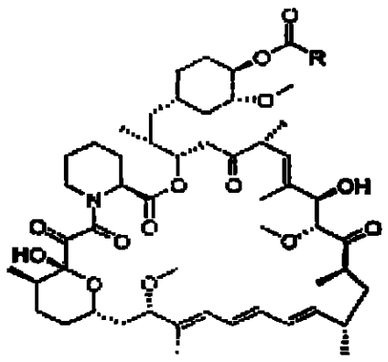
- [ ] De ser el caso, modificaciones efectuadas a la solicitud internacional.
- [X] Otros: Reporte de Búsqueda Internacional, Opinión Escrita, Extractos

(11) **FIGURA CARATERISTICA**



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(54) Title: REGIOSPECIFIC SYNTHESIS OF RAPAMYCIN 42-ESTER DERIVATIVES

(57) Abstract: A method for the regiospecific synthesis of rapamycin 42-ester derivatives is described. The method involves lipase-catalyzed acylation of 42 hydroxy rapamycin with an acyl donor such as a vinyl ester, an isopropenyl ester or an anhydride in a suitable organic solvent.

WO 2005/105811 A1

REGIOSPECIFIC SYNTHESIS OF RAPAMYCIN 42-ESTER DERIVATIVES

BACKGROUND OF THE INVENTION

Rapamycin (Rapamune®) is an immunosuppressant derived from nature, which has a novel mechanism of action. CCI-779 (rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid) is an ester of rapamycin, which has demonstrated significant inhibitory effects on tumor growth in both *in vitro* and *in vivo* models.

Modification of rapamycin has mainly focused on production of its 42-hydroxy ester derivatives. These 42-hydroxy rapamycin ester derivatives are useful for inducing immunosuppression, and in the treatment of transplantation rejection, autoimmune diseases, diseases of inflammation, adult T-cell leukemia/lymphoma, solid tumors, fungal infections, et al.

Esterification of rapamycin at the 42-position has been performed by directly reacting rapamycin with acylating agents. However, as rapamycin contains two secondary hydroxyl groups at position 31 and 42, attempts to discriminate between these two hydroxyl groups in order to achieve a regioselective synthesis of 42-monoacylated products, posed a difficult challenge.

A number of patents involving the synthesis of 42-acylated derivatives have been issued, such as alkyl ester (US Patent No. 4,316,885), aminoalkyl esters (US Patent No. 4,650,803), fluorinated esters (US Patent No. 5,100,883), amide esters (US Patent No. 5,118,677), carbamate esters (US Patent No. 5,118,678), alkoxyesters (US Patent No. 5,233,036), carbonate esters (US Patent No. 5,260,300), hydroxyesters (US Patent Nos. 5,362,718 & 6,277,983). However, none of the patents described methods that are stereospecific. Further, the yields for 42-monoesters of rapamycin produced by these methods are typically poor to moderate due to the poor regioselectivity and instability of rapamycin molecule in basic or acidic conditions. High performance liquid chromatography (HPLC) separation is usually required in order to get the pure product. One solution proposed for improving the regioselectivity is the use of 31-silyl protected

rapamycin as an intermediate. However, this method adds several more steps of manipulation.

What is needed is an efficient method for synthesis of rapamycin esters.

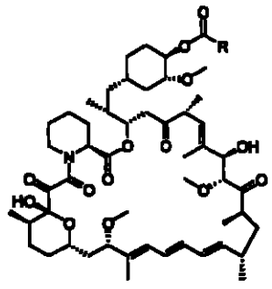
SUMMARY OF THE INVENTION

The invention provides a lipase-catalyzed synthesis of rapamycin 42-ester derivatives. The remarkable features of this simple process are regiospecificity and excellent yield under mild conditions.

Other aspect and advantages of the invention will be readily apparent to one of skill in the art.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a process for preparing rapamycin 42-esters of the general formula (I) in a regiospecific fashion using lipase in the presence of a rapamycin and an acyl donor.



(I)

wherein, R is a linear or cyclic, aliphatic or aromatic, saturated or unsaturated hydrocarbon which optionally contains a hydroxyl, halogen and/or thio substituent(s). In one embodiment, the halogen is Cl, Br, I or F.

Rapamycin can be prepared as previously described. See, *e.g.*, US Patent No. 3,929,992, issued December 30, 1975. Alternatively, rapamycin may be purchased from commercial sources [*e.g. Rapamune*®, Wyeth] or prepared using alternative methods. The means of preparing, purifying and/or obtaining the rapamycin starting material are not a limitation of the present invention.

In one embodiment, the invention provides a regiospecific route for the production of ketal-protected rapamycin 42-esters, useful in the produciton of a rapamycin 42-ester. In one embodiment, the invention provides for the production of isopropylidene ketal protected rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid, a precursor of CCI-779 (example 7).CCI-779 is an ester of rapamycin which has demonstrated significant inhibitory effects on tumor growth in both *in vitro* and *in vivo* models. The use of this and other hydroxyesters of rapamycin are described in US Patent Nos. 5,362,718 and 6,277,983, and US Patent Publication No. US 2005-0033046 A1 (US Patent Application No. 10/903,062).

The removal of the ketal protecting group can be accomplished under mildly acidic conditions. In general, the procedure published in US Patent No. 6,277,983 and documents cited therein can be followed. In one embodiment, the deprotection is carried out in a single phase aqueous acid/organic solvent system, *e.g.*, diluted sulfuric acid in tetrahydrofuran (THF), such as 2 N H_2SO_4/THF at about 0 to 5°C. However, this reaction can take about 3 days or more to complete and solvent extraction is needed to recover the product from aqueous media after the reaction is complete. Other procedures for removal of the ketal protecting group would be known to one of ordinary skill in the art, such as those described in the International Patent Application entitled *Proline CCI-779, Production and Uses Therefor, and Two Step Enzymatic Synthesis of Proline CCI-779 and CCI-779* (Chew, et al., based on US Provisional Patent Application Nos.: 60/562,069 (filed April 14, 2004) and 60/623,594 (filed October 29, 2004)).

As used herein, "microbial lipases" include enzymes which catalyze the hydrolysis and formation of ester bonds, which were originally isolated from a non-eukaryotic source, such as, *Aspergillus niger, Candida antarctica, Candida rugosa, Mucor miehei, Pseudomonas cepacia, Pseudomonas fluorescens, Rhizopus delemar, inter alia.* However, the enzyme selected for use in the invention need not be directly isolated and purified from the original source, but can be prepared synthetically or recombinantly, or through other suitable means. A variety of these enzymes are available from some commercial sources, further, these enzyme preparations can be used as crude, partially purified, purified or immobilized from different microbial origin under different trade names by various suppliers.

The lipase from *Candida antarctica, type B* is found to be very particularly suitable in the practice of this invention. Of all the lipases studied to date, this one provides the highest conversion rates and highest isolated yields. *C. antarctica* lipase is commercially available, *e.g.*, under the product designation NOVO SP435 or NOVOZYME 435 from Novo Nordisk, or CHIRAZYME L-2 from Roche Molecular Biochemicals and BioCatalytics.

Lipase PS, from *Pseudomonas cepacia*, particularly its immobilized form, lipase PS-C [e.g., which is available as "Amano" II lipase from Amano], can perform the reaction equally well as NOVOZYM 435 lipase from the synthetic point of view, although at slower reaction rate. For the process of the invention, the microbial (e.g., *C. antarctica (type B))* lipase is combined with a suitable solvent for catalysis of the reaction between an acyl donor and rapamycin. One of skill in the art can readily select a suitable solvent from among, *e.g.*, toluene, *tert*-butyl methyl ether (TBME), ethyl ether, THF, MeCN, CH_2Cl_2, $CHCl_3$, hexane, dioxane, or mixtures including these solvents. In one embodiment, TBME (*tert*-butyl methyl ether) is used. The acyl donor utilized in the method of the invention is selected from among several activated esters such as vinyl esters, isopropenyl esters and anhydrides.

In one embodiment, the vinyl esters are selected from among esters of the formula CH_2=CH-O-COR^1, where R^1 is an alkyl, alkenyl, aryl, benzyl, either unsubstituted or substituted with hydroxyl, halogen (F, Cl, Br, I) and thio. Suitable vinyl esters include vinyl acetate, vinyl propionate, vinyl chloroacetate, vinyl crotonate, vinyl benzoate, and vinyl decanoate. However, other suitable vinyl esters can be readily selected by one of skill in the art.

In one embodiment, isopropenyl esters are selected from among esters of the formula CH_2=C(CH_3)-OCOR^2, where R^2 is an alkyl, alkenyl, aryl, benzyl, either unsubstituted or substituted with hydroxyl, halogen (F, Cl, Br, I) and thio. In another embodiment, the acyl donor is isopropenyl acetate.

The term "alkyl" is used herein to refer to both straight- and branched-chain saturated aliphatic hydrocarbon groups having one to ten carbon atoms, preferably one to eight carbon atoms and, most preferably, one to six carbon atoms.

The term "alkenyl" is intended to include both straight- and branched-chain alkyl group with at least one carbon-carbon double bond and two to eight carbon atoms, preferably two to six carbon atoms.

The term "aryl" is used herein to refer to a carbocyclic aromatic system, which may be a single ring, or multiple aromatic rings fused or linked together such that at least one part of the fused or linked rings forms the conjugated aromatic system e.g. of 6-14 carbon atoms. The aryl groups include, but are not limited to, phenyl, naphthyl, biphenyl, anthryl, tetrahydronaphthyl, phenanthryl, and indane.

The term "benzyl" is used herein to refer to a group of formula $C_6H_5CH_2$.

Suitable anhydrides are readily selected from among alkanoic anhydrides (i.e., C_1, C_2, C_3, C_4, C_5, C_6, C_7 and C_8 anhydrides), which may be branched or straight-chained, or substituted with halogen, hydroxyl.

In one embodiment, the enzymatic process of the invention can be carried out in the range of about 20°C to about 75°C, or about 25°C, 27°C, 30°C, 40°C to about 70°C, or about 32°C or about 37°C to about 65°C. In another embodiment, the temperature is about 30°C to about 55°C. In yet another embodiment, the temperature is about room temperature to about 45°C. Typically, the reaction is performed under N_2 until all starting material is consumed. The reaction can be monitored by various techniques such as thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Alternatively, other monitoring methods can be used by one of skill in the art.

In a reaction utilizing a vinyl ester or isopropenyl ester as the acyl donor, the enzyme (lipase) is filtered off and washed with a suitable solvent. The solvent may be the same as selected for use in the reaction, or may differ from the solvent in the reaction. Where the solvent differs, it can be chosen from among the solvents defined above, or other commonly-used solvents, such as acetone, ethyl acetate, methanol, ethanol, isopropanol, among others. The combined organic solvent can then be evaporated off under suitable conditions, e.g., reduced pressure. The residue is then purified by suitable means, e.g., by silica gel column chromatography, eluting with a suitable solvent, or recrystallized with a suitable solvent (e.g., hexane-acetone, hexane-ethyl acetate, ethyl ether, among others). Other suitable purification means are known to those of skill in the

art. Further, other suitable solvent mixtures and ratios can be readily determined by one of skill in the art.

In another embodiment, anhydrides are used as acyl donors in the enzyme-catalyzed preparation of 42-ester derivatives. Yields are usually high, around 95%. (example 9-11). In such an embodiment, the anhydride and suitable amount of enzyme are mixed in a suitable solvent with rapamycin, and stirred for about 16 to 96 hours, and more preferably, about 24 h to 48 h in the presence of N_2, protected from light. Suitably, the reaction is performed at about room temperature to about 50 °C. The amount of enzyme (w/w) to rapamycin can vary based on the kind of anhydride and length of reaction, e.g., from approximately equivalent amounts (on a weight basis) of rapamycin and enzyme (w/w) to excess amounts of enzyme in order to drive the reaction more quickly. Optionally, if the reaction does not finish after certain period time as stated above, additional enzyme can be added, and the mixture stirred for a further period of time until the reaction was completed as judged by TLC or HPLC. After the enzyme is removed via filtration, the solvent is then removed under reduced pressure. The residue is purified using suitable techniques, *e.g.*, by silica gel column chromatography or recrystallization.

The regiospecific rapamycin 42-derivatives of the invention are useful in pharmaceutical compositions. Thus, the rapamycin 42-derivatives of the invention can be formulated by any suitable method described in the art for rapamycin or derivatives thereof.

Oral formulations containing the active compounds of this invention may comprise any conventionally used oral forms, including tablets, capsules, buccal forms, troches, lozenges and oral liquids, suspensions or solutions. Capsules may contain mixtures of the active compound(s) with inert fillers and/or diluents such as the pharmaceutically acceptable starches (*e.g.*, corn, potato or tapioca starch), sugars, artificial sweetening agents, powdered celluloses, such as crystalline and microcrystalline celluloses, flours, gelatins, gums, etc. Useful tablet formulations may be made by conventional compression, wet granulation or dry granulation methods and utilize pharmaceutically acceptable diluents, binding agents, lubricants, disintegrants, surface modifying agents (including surfactants), suspending or stabilizing agents, including, but

not limited to, magnesium stearate, stearic acid, talc, sodium lauryl sulfate, microcrystalline cellulose, carboxymethylcellulose calcium, polyvinylpyrrolidone, gelatin, alginic acid, acacia gum, xanthan gum, sodium citrate, complex silicates, calcium carbonate, glycine, dextrin, sucrose, sorbitol, dicalcium phosphate, calcium sulfate, lactose, kaolin, mannitol, sodium chloride, talc, dry starches and powdered sugar. Preferred surface modifying agents include nonionic and anionic surface modifying agents. Representative examples of surface modifying agents include, but are not limited to, poloxamer 188, benzalkonium chloride, calcium stearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, magnesium aluminum silicate, and triethanolamine. Oral formulations herein may utilize standard delay or time release formulations to alter the absorption of the active compound(s). The oral formulation may also consist of administering the active ingredient in water or a fruit juice, containing appropriate solubilizers or emulsifiers as needed.

In one embodiment, oral formulations for rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid are described in US Patent Publication No. US 2004-0077677 A1 (also US Patent Application No. 10/663,506). Such an oral formulation contains a granulation prepared using a wet granulation process.

In some cases it may be desirable to administer the compounds directly to the airways in the form of an aerosol.

The compounds may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxy-propylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of

manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

In one embodiment, injectable formulations for rapamycin 42-ester with 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid are described in US Patent Publication No. US 2004-0167152 A1 (also US Patent Application No. 10/626,943).

The parenteral formulations useful in this invention can be used to produce a dosage form that is suitable for administration by either direct injection or by addition to sterile infusion fluids for intravenous infusion.

Transdermal administrations are understood to include all administrations across the surface of the body and the inner linings of bodily passages including epithelial and mucosal tissues. Such administrations may be carried out using the present compounds, or pharmaceutically acceptable salts thereof, in lotions, creams, foams, patches, suspensions, solutions, and suppositories (rectal and vaginal).

Transdermal administration may be accomplished through the use of a transdermal patch containing the active compound and a carrier that is inert to the active compound, is non-toxic to the skin, and allows delivery of the agent for systemic absorption into the blood stream via the skin. The carrier may take any number of forms such as creams and ointments, pastes, gels, and occlusive devices. The creams and ointments may be viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type. Pastes comprised of absorptive powders dispersed in petroleum or hydrophilic petroleum containing the active ingredient may also be suitable. A variety of occlusive devices may be used to release the active ingredient into the blood stream such as a semi-permeable membrane covering a reservoir containing the active ingredient with or without a carrier, or a matrix containing the active ingredient. Other occlusive devices are known in the literature.

Suppository formulations may be made from traditional materials, including cocoa butter, with or without the addition of waxes to alter the suppository's melting point, and glycerin. Water soluble suppository bases, such as polyethylene glycols of various molecular weights, may also be used.

The present invention further provides packaging and kits containing the regiospecific rapamycin 42-derivatives produced according to the present invention and formulated for administration by a suitable delivery method. In one embodiment, the regiospecific rapamycin 42-derivatives are present in unit dosage form. A variety of suitable containers, including bottles, vials, blister packs, and the like are known to those of skill in the art. Such packaging and kits may further contain other components, including, e.g., instructions for use, syringes, applicators, and the like.

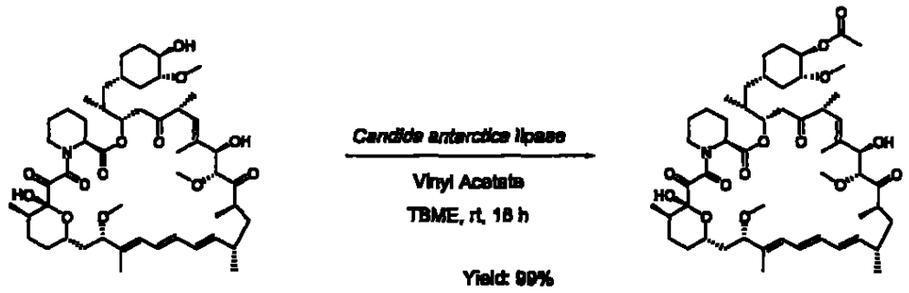
The following examples are illustrative of the methods of the invention for regiospecific production of rapamycin 42-ester derivatives. As illustrated in the following examples, *Candida antarctica* lipase is particularly well suited in its ability to catalyze transesterification of rapamycin to its 42-acyl derivative using vinyl acetate as acyl donor. However, as stated above, the invention is not so limited and other suitable lipases of microbial origin can be utilized. For example, lipase PS, from *Pseudomonas cepacia* and its immobilized form, lipase PS-C "Amano"II, Lipase PS-D the reaction conditions can include higher temperature with more enzyme. For example, in one embodiment utilizing immobilized lipase PS-C, double the amount of lipase (i.e., 200% of rapamycin (W/W) is required to achieve the conversion rate of Novozym 435 lipase at room temperature; alternatively, the temperature can be raised to about 45 °C when less amount of enzyme (100% (w/w) to rapamycin) is used].

EXAMPLES

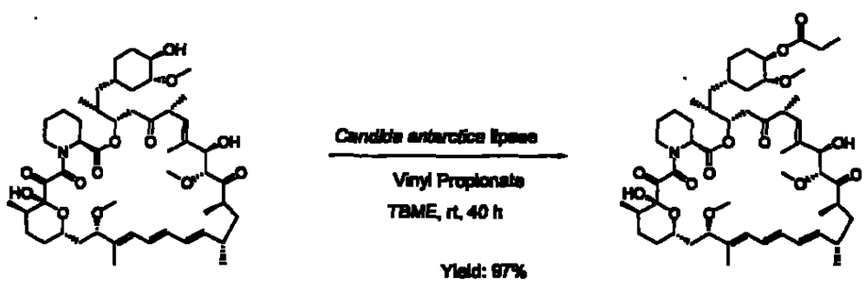
The following examples illustrate the process of the invention, using vinyl ester (examples 1-8), isopropenyl ester (example 9) or an anhydride (examples 10-12).

In one embodiment, a mixture of rapamycin (20 mg, 0.022 mmol), vinyl ester (50 μL) and NOVOZYM 435 lipase (20 mg) in TBME (0.5 mL) was stirred at room temperature (rt) or 45 °C under N_2 until all starting material was consumed monitored by TLC. The enzyme was filtered off and washed with TBME. The combined organic solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography eluting with hexane-acetone (2:1, v/v) or recrystallized from hexane-acetone. Additional examples are illustrated in the schemes below.

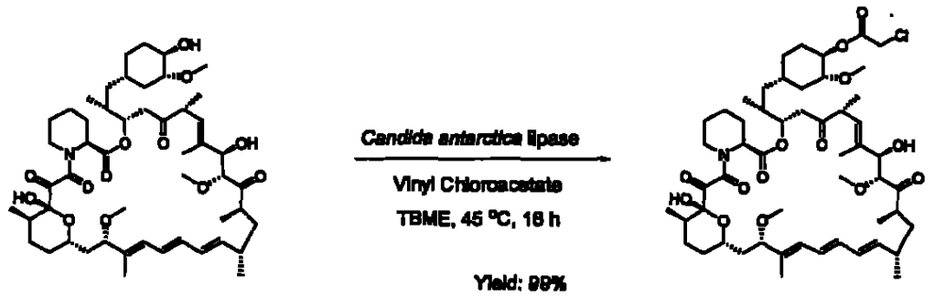
Example 1



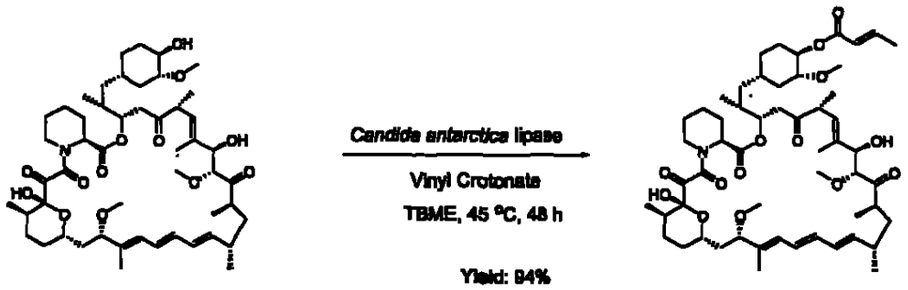
Example 2



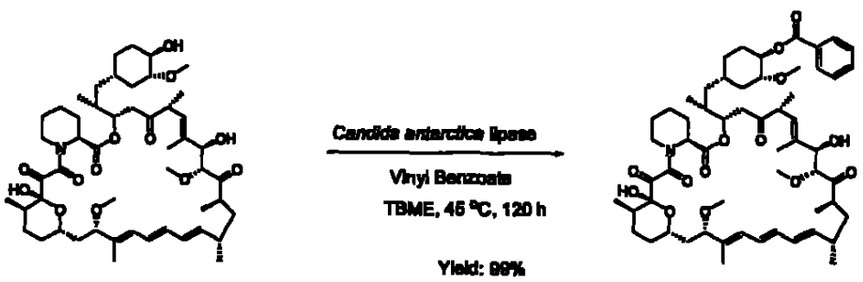
Example 3



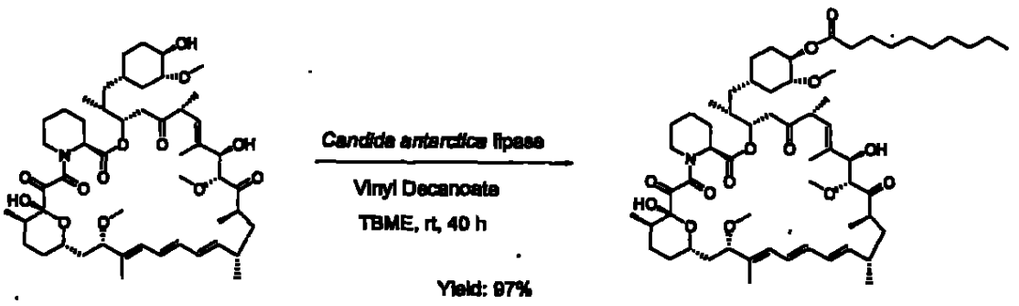
Example 4



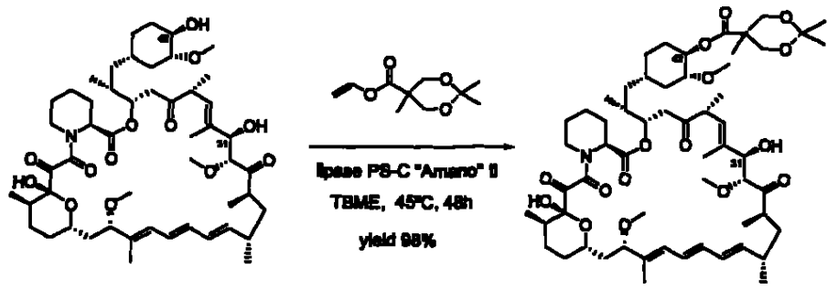
Example 5



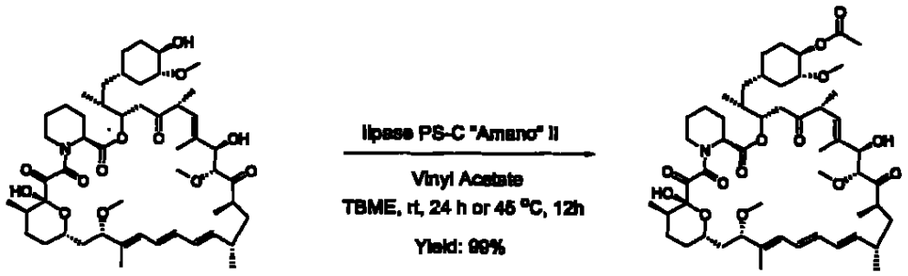
Example 6



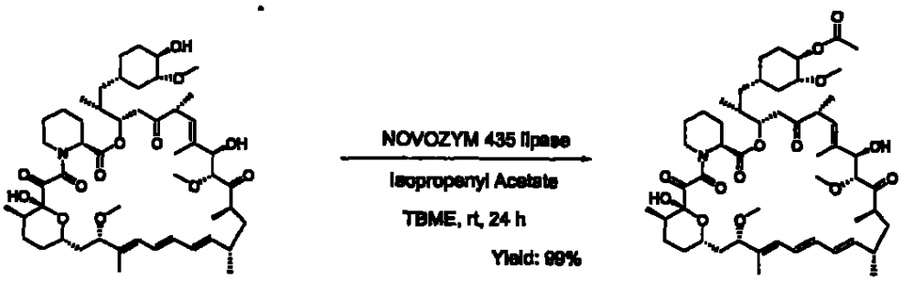
Example 7



Example 8



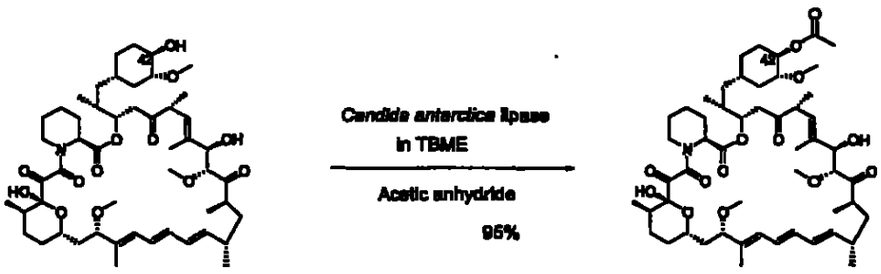
Example 9



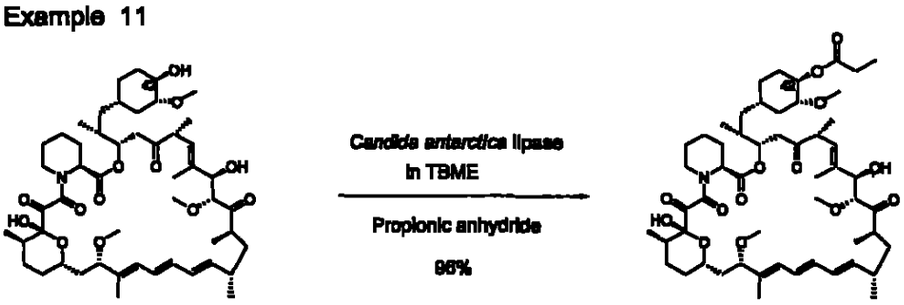
Rapamycin 42-ester derivatives using anhydrides as the acyl donor are prepared according to the invention as follows.

A mixture of rapamycin (20 mg, 0.022 mmol), anhydride (30 mg) and NOVOZYM 435 lipase (20 mg) in TBME (0.5 mL) were stirred at room temperature for 48 h (N_2, protected from light). [In the case of acetic anhydride or propionic anhydride, after 48 h, another portion of NOVOZYM 435 lipase (20 mg) and TBME (0.1 mL) was added and the mixture was stirred another 48 h before the reaction was quenched]. The solvent was then removed by flushing with N_2 gas. The residue was purified by silica gel column chromatography eluting with hexane-acetone (2:1, v/v). The product was isolated as a white solid.

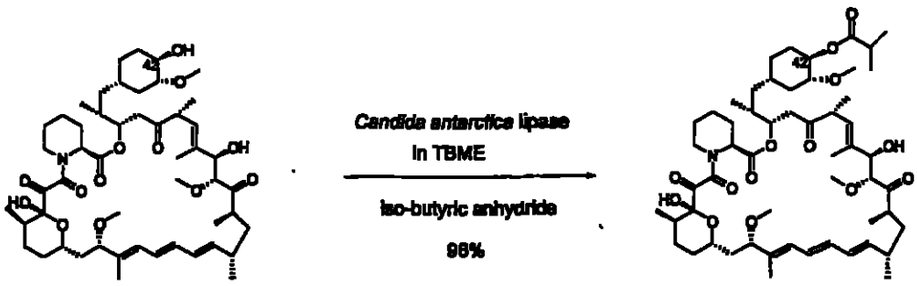
Example 10



Example 11



Example 12



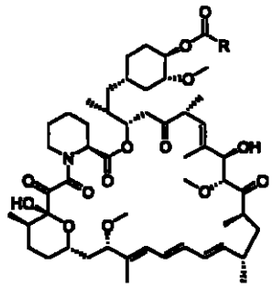
The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

It is further to be understood that values are approximate, and are provided for description.

Patents, patent applications, publications, procedures, and the like are cited throughout this application, the disclosures of which are incorporated herein by reference in their entireties. To the extent that a conflict may exist between the specification and a document listed herein, the language of the disclosure made herein controls.

CLAIMS:

1. A method for the regiospecific preparation of rapamycin 42-ester derivatives of the formula (I)



(I)

wherein R is a linear or cyclic, aliphatic or aromatic, saturated or unsaturated hydrocarbon which optionally contains hydroxyl, halogen and/or thio, said method comprising acylating a 42-hydroxy rapamycin with an acyl donor in the presence of a lipase.

2. The method according to claim 1, wherein the lipase used is a microbial lipase from microorganisms such as *Aspergillus niger, Candida antarctica, Candida rugosa, Mucor miehei, Pseudomonas cepacia, Pseudomonas fluorescens, Rhizopus delemar.*

3. The method according to claim 2, wherein the lipase used is from *Candida antarctica type B* (NOVOZYM 435 lipase) or *Pseudomonas cepacia* (lipase PS-C "Amano" II).

4. The method according to any one of claims 1 to 3, wherein the acyl donor is a vinyl ester, an isopropenyl ester or an anhydride.

5. The method according to claim 4 wherein the vinyl ester has the formula CH_2=CH-O-COR^1, wherein R^1 is selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_6-C_{14} aryl, benzyl, optionally substituted with a group independently selected from hydroxyl, halogen and SH.

6. The method according to claim 5 wherein the vinyl ester is selected from the group consisting of vinyl acetate, vinyl propionate, vinyl chloroacetate, vinyl crotonate, vinyl benzoate, and vinyl decanoate.

7. The method according to claim 4 wherein the vinyl ester is isopropylidene protected vinyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropionate.

8. The method according to claim 4 wherein the isopropenyl ester has the formula CH_2=C(CH_3)-OCOR^2, wherein R^2 is selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_6-C_{14} aryl, benzyl, optionally substituted with a group independently selected from hydroxyl, halogen and SH.

9. The method according to claim 8 wherein the isopropenyl ester is isopropenyl acetate.

10. The method according to claim 4 wherein the anhydride is a C_1-C_8 straight-chain or branch-chain alkanoic anhydride, optionally substituted with a group independently selected from halogen and hydroxyl.

11. The method according to any one of claims 1 to 10, wherein the reaction takes place in an organic solvent selected from the group consisting of toluene, *tert*-butyl methyl ether (TBME), ethyl ether, THF, MeCN, CH_2Cl_2, $CHCl_3$, hexane, dioxane or mixtures thereof.

12. The method according to any one of claims 1 to 11, wherein the reaction is conducted in the range of 20 °C to 75 °C.

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13. A regiospecific preparation of rapamycin 42-ester with isopropylidene ketal protected 3-hydroxy-2-(hydroxymethyl)-2-methylpropionic acid, a precursor of CCI-779.

14. A composition comprising a regiospecific rapamycin 42-derivative produced according to the method of any one of claims 1 to 12.

15. A composition comprising the regiospecific rapamycin 42-derivative preparation according to claim 13 .

16. The composition according to claims 14 or 15 further comprising a physiologically compatible carrier.

17. A product comprising a composition according to any one of claims 14 to 16, and a container for said composition.

18. A pharmaceutical kit comprising units of a regiospecific rapamycin 42-derivative produced according to any one of claims 1 to 12 in unit dosage form.

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22

SÍNTESIS REGIOESPECÍFICA DE DERIVADOS 42-ÉSTER DE RAPAMICINA

ANTECEDENTES DE LA INVENCIÓN

La Rapamicina (Rapamune®) es un derivado inmunosupresor de la naturaleza, que tiene un mecanismo novedoso de acción. El CCI-779 (42-éster de rapamicina con ácido de 3-hidroxi-2-(hidroximetil)-2-metilpropiónico) es un éster de rapamicina, que ha demostrado significativos efectos inhibidores en el crecimientote tumores en modelos in Vitro e in vivo.

La modificación de la rapamicina se ha enfocado principalmente den la producción de sus derivados 42-hidroxi éster. Estos derivados de éster de rapamicina 42 hidroxi son útiles para inducir inmunosupresión, y en el tratamiento de rechazo de trasplantes, enfermedades autoinmunes, enfermedades de inflamación, leucemia/linfoma de células T adultas, tumores sólidos, infecciones fúngicas, et al.

La esterificación de la rapamicina en la posición 42 se ha desarrollado al hacer reaccionar directamente la rapamicina con agentes de acilación. Sin embargo, como la rapamicina contiene dos grupos hidroxilos secundarios en la posición 31 y 42, intentos para discriminar entre estos dos grupos hidroxilo con el fin de lograr una síntesis regioselectiva de productos 42-monoacilados, poseen un reto difícil.

Un número de patentes que involucran la síntesis de derivados de 42-acilados se han publicado, tales como éster de alquilo (Patente US No. 4,316,885),ésteres de aminoalquilo (Patente US No. 4,650,803), ésteres fluorinados (Patente US No. 5,100,883), ésteres de amida (Patente US No. 5,118,677), ésteres de carbamato (Patente US No. 5,118,678), alcoxiésteres (Patente US No. 5,233,036), ésteres de carbonato (Patente US No. 5,260,300), hidroxiésteres (Patente US Nos. 5,362,718 & 6,277,983). Sin embargo, ninguna de las patentes describe métodos que sean estereoespecíficos. Adicionalmente, los productos para 42-monoésteres de rapamicina producidos por estos métodos son típicamente pobres a moderados debido a la pobre regioselectividad e inestabilidad de la molécula de rapamicina en condiciones acídicas o básicas. La separación por cromatografía líquida de alto desempeño (HPLC) se requiere usualmente con el fin de conseguir el producto puro. Una solución propuesta para mejorar la regioselectividad es el uso de rapamicina 31-sililo protegida como un intermedio. Sin embargo, este método agrega varias etapas más de manipulación.

Lo que se necesita es un método eficiente para la síntesis de ésteres de rapamicina.

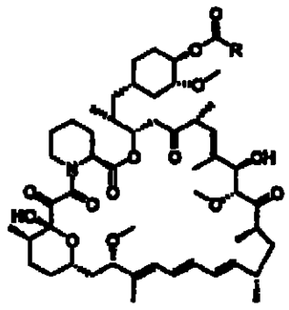
RESUMEN DE LA INVENCIÓN

La invención suministra una síntesis catalizada por lipasa de derivados 42-éster de rapamicina. Las destacadas características de este proceso simple son la regioespecificidad y excelente producción bajo condiciones suaves.

Otro aspecto y ventajas de la invención serán fácilmente evidentes para un experto en la técnica.

DESCRIPCIÓN DETALLADA DE LA INVENCIÓN

La presente invención suministra un proceso para preparar 2-ésteres de rapamicina de la fórmula general (I) en una forma regioespecífica utilizando lipasa en la presencia de una rapamicina y un donante acilo.



(I)

En donde, R es un hidrocarburo lineal o cíclico, alifático o aromático, saturado o insaturado que opcionalmente contiene un hidroxilo, halógeno y/o sustituyente tio(s). En una modalidad, el halógeno es Cl, Br, I o F.

La rapamicina se puede preparar como se describió previamente. Ver, por ejemplo, la Patente Estadounidense No. 3,929,992, publicada en diciembre 30, 1975. Alternativamente, la rapamicina se puede comprar de fuentes comerciales [por ejemplo Rapamune®, Wyeth] o preparada utilizando métodos alternativos. Los medios de preparación, purificación y/o obtención de la

materia de partida de la rapamicina no son una limitación de la presente invención.

En una modalidad, la invención suministra una ruta regioespecifica para la producción de 42-ésteres de rapamicina protegidos de cetal, útiles en la producción de un 42-éster de rapamicina. En una modalidad, la invención suministra la producción de 42-éster de rapamicina protegida de cetal de isopropilideno con ácido 3-hidroxi-2-(hidroximetil)-2-metilpropiónico, un precursor de CCI-779 (ejemplo 7).CCI-779 es un éster de rapamicina que ha demostrado efectos inhibidores significativos sobre el crecimiento de tumor en modelos in Vitro e in vivo. El uso de este y otros hidroxiésteres de rapamicina se describen en la Patente Estadounidense Nos. 5,362,718 y 6,277,983, y la publicación de Patente Estadounidense No. US 2005-0033046 A1 (Solicitud de Patente Estadounidense No. 10/903,062).

La remoción del grupo protector cetal se puede acompañar bajo condiciones acídicas suaves. En general, el procedimiento publicado el la Patente Estadounidense No. 6,277,983 y los documentos citados en esta se pueden seguir. En una modalidad, la desprotección se lleva acabo en un sistema solvente de ácido/orgánico acuoso de fase simple, por ejemplo, acido sulfúrico diluido en tetrahidrofurano (THF), tal como H_2SO_4 2 N/THF en aproximadamente 0 a 5° C. Sin embargo, esta reacción puede tomar cerca de 3 días o más para completase y la extracción del solvente es necesaria para recuperar el producto del medio acuosos después de que se completa la reacción. Otros

procedimientos para la remoción del grupo protector cetal serán de conocimiento de un experto en la técnica, tal como aquellos descritos en la Solicitud de Patente Internacional titulada *Proline CCI-779, Production and Uses Therefor, and Two Step Enzymatic Synthesis of Proline CCI-779 and CCI-779* (Chew, et al., based on US Provisional Patent Application Nos.: 60/562,069 (filed April 14, 2004) and 60/623,594 (filed October 29, 2004)).

Como se utilia aquí "lipasas microbiales" incluyen enzimas que catalizan la hidrólisis y la formación de enlaces éster, que se aislan originalmente de una fuente no eucariotica, tal como, *Aspergillus niger, Candida antarctica, Candida rugosa, Mucor miehei, Pseudomonas cepacia, Pseudomonas fluorescens, Rhizopus delemar, inter alia*. Sin embargo, la enzima seleccionada para uso en la invención no necesita ser aislada directamente y purificada de la fuente original, pero se puede preparar sintéticamente o recombinantemente, o a través de otros medios adecuados. Una variedad de estas enzimas están disponibles de algunas fuentes comerciales, adicionalmente, estas preparaciones de enzima se pueden utilizar como crudas, parcialmente purificadas, purificadas o inmovilizadas de diferente origen microbial bajo diferentes nombres comerciales por varios proveedores.

La lipasa de *Candida antarctica, tipo B* se encuentra que es particularmente adecuada en la práctica de esta invención. De todas las lipasas estudiadas hasta la fecha, esta suministra el índice de conversión más alto y los productos aislados más altos. La lipasa *C. antarctica* está disponible comercialmente, por ejemplo, bajo la designación del producto NOVO SP435 o NOVOZYME

435 de Novo Nordisk, o CHIRAZYME L-2 de Roche Molecular Biochemicals and BioCatalytics.

La lipasa PS, de *Pseudomonas cepacia*, particularmente su forma inmovilizada, la lipasa PS-C [por ejemplo, la que está disponible como lipasa II "Amano" de Amano], puede desarrollar la reacción igualmente bien como la lipasa NOVOZYM 435 desde el punto de vista sintético, aunque en una proporción de reacción más lenta. Para el proceso de la invención, la lipasa microbial (por ejemplo, *C. antarctica (tipo B)*) se combina con un solvente adecuado para catálisis de la reacción entre un donante acilo y rapamicina. Un experto en la técnica puede seleccionar fácilmente un solvente adecuado de entre, por ejemplo, tolueno, terc-butil éter de metilo, (TBME), éter de etilo, THF, MeCN, CH_2Cl_2, $CHCl_3$, hexano, dioxano, o mezclas que incluyen estos solventes. En una modalidad, se utiliza TBME (terc-butil metil éter). El donante acilo utilizado en el método de la invención se selecciona entre varios ésteres activados tales como ésteres de vinilo, isopropenil ésteres y anhídridos.

En una modalidad, los ésteres de vinilo se seleccionan de ésteres de la fórmula CH_2=CH-O-COR^1, en donde R^1 en donde es un alquilo, alquenilo, arilo, bencilo, sustituido o no sustituido con hidroxilo, halógeno (F, Cl, Br, I) y tio. Los ésteres de vinilo adecuados incluyen acetato de vinilo, propionato de vinilo, cloroacetato de vinilo, crotonato de vinilo, benzoato de vinilo, y decanoato de vinilo. Sin embargo, otros ésteres de vinilo adecuados se pueden seleccionar fácilmente por un experto en la técnica.

En una modalidad, los ésteres de isopropenilo se seleccionan de entre ésteres de la fórmula $CH_2=C(CH_3)-OCOR^2$, en donde R^2 es un alquilo, alquenilo, arilo, bencilo, ya sea sustituido o no sustituido con hidroxilo, halógeno (F, Cl, Br, I) y tio. En otra modalidad, el donante acilo es acetato de isopropenilo.

El término "alquilo" se utiliza aquí para referirse a grupos de hidrocarburo alifático saturados de cadena recta y cadena ramificada que tienen uno a diez átomos de carbono, preferiblemente uno a ocho átomos de carbono y, más preferiblemente, uno a seis átomos de carbono.

El término "alquenilo" se pretenda que incluya el grupo alquilo de cadena recta y cadena ramificada con al menos un doble enlace carbono-carbono y dos a ocho átomos de carbono, preferiblemente dos a seis átomos de carbono.

El término "arilo" se utiliza aquí para referirse a un sistema aromático carbocíclico, que puede ser un anillo sencillo, o múltiples anillos aromáticos fusionados o ligados juntos de tal forma que al menos una parte de los anillos ligados o fusionados forma el sistema aromático conjugado, por ejemplo de 6-14 átomos de carbono. Los grupos arilo incluyen pero no están limitados a, fenilo, naftilo, bifenilo, antrilo, tetrahidronafthilo, fenantrilo, e indano.

El término "bencilo" se utiliza aquí para referirse a un grupo de la fórmula $C_6H_5CH_2$.

29

Los anhídridos adecuados seleccionan fácilmente de entre anhídridos alcanóicos (es decir., anhídridos C_1, C_2, C_3, C_4, C_5, C_6, C_7 y C_8), que pueden ser de cadena recta o ramificada o sustituidos con halógeno, hidroxilo.

En una modalidad, el proceso enzimático de la invención se puede llevar a cabo en el rango de aproximadamente 20° C a aproximadamente 75° C, o aproximadamente 25° C, 27° C, 30° C, 40° C a aproximadamente 70° C, o aproximadamente 32° C o aproximadamente 37° C a aproximadamente 65° C. en otra modalidad, la temperatura es de aproximadamente 30° C a aproximadamente 55° C. En aún otra modalidad, la temperatura es de aproximadamente temperatura ambiente a aproximadamente 45° C. Típicamente, la reacción se desarrolla bajo N_2 hasta que se consume todo el material de partida. La reacción se puede monitorear por varias técnicas tales como cromatografía de capa delgada (TLC) y cromatografía líquida de alto desempeño (HPLC). Alternativamente, otros métodos de monitoreo se pueden utilizar por un experto en la técnica.

En una reacción que utiliza éster de vinilo o éster de isopropenilo como el donante acilo, se filtra la enzima (lipasa) y se lava con un solvente adecuado. El solvente puede ser el mismo seleccionado para uso en la reacción, o puede diferir del solvente en la reacción.

Cuando el solvente difiere, este se puede escoger de entre los solventes definidos anteriormente, u otros solventes

utilizados comúnmente, tal como acetona, acetato de etilo, metanol, etanol, isopropanol, entre otros. El solvente orgánico combinado se puede evaporar bajo condiciones adecuadas, por ejemplo, presión reducida. El residuo se puede purificar entonces por medios adecuados, por ejemplo, por cromatografía de columna de gel de sílice, eluyendo con un solvente adecuado, o recristalizado con un solvente adecuado (por ejemplo, hexano-acetona, hexano-acetato de etilo, etil éter, entre otros). Otros medios de purificación adecuados son conocidos por aquellos expertos en la técnica. Adicionalmente, otras mezclas de solvente adecuados y proporciones se pueden determinar fácilmente por un experto en la técnica.

En otra modalidad, se utilizan anhídridos como donantes acilo en la preparación enzima-catalizada de derivados 42-éster. Los productos usualmente son altos, cerca del 95%. (Ejemplo 9-11). En tal una modalidad, el anhídrido y la cantidad adecuada de enzima se mezclan en un solvente adecuado con rapamicina, y se agitan aproximadamente 16 a 96 horas, y más preferiblemente, cerca de 24 h a 48 h en la presencia de N_2, protegida de la luz. De forma adecuada, la reacción se desarrolla en aproximadamente la temperatura ambiente a aproximadamente 50 ° C. la cantidad de enzima (p/p) a rapamicina puede variar en la clase de anhídrido y la longitud de la reacción, es decir, desde aproximadamente cantidades equivalentes (en una base en peso) de rapamicina y enzima (p/p) a exceso de cantidades de enzima con el fin de controlar la reacción más rápidamente. Opcionalmente, si la reacción no termina después de cierto periodo de tiempo como se estableció anteriormente, se puede agregar enzima adicional y la

31

mezcla se agita durante un periodo adicional de tiempo hasta que se completa la reacción como se juzga por TLC o HPLC. Después que se remueve la enzima por filtración, el solvente se remueve bajo presión reducida. El residuo se purifica utilizando técnicas adecuadas, por ejemplo, cromatografía de columna de gel de sílice o recristalización.

Los derivados 42 de rapamicina regioespecíficos de la invención son útiles en composiciones farmacéuticas. Así, los derivados 42 de rapamicina de la invención se pueden formular por cualquier método adecuado descrito en la técnica para rapamicinas o derivados de estas.

Formulaciones orales que contienen los compuestos activos de esta invención pueden comprender cualquier forma oral utilizada convencionalmente, incluyendo tabletas, cápsulas, formas bucales, píldoras, rombos y líquidos orales, suspensiones o soluciones. Las cápsulas pueden contener mezclas de los compuestos activos con llenadotes inertes y/o diluyentes tales como almidones farmacéuticamente aceptables (por ejemplo, maíz, papa, o almidón de tapioca), azúcares, agentes endulzantes artificiales, celulosas en polvo, tal como celulosa cristalina y microcristalina, harinas, gelatinas, gomas, etc. formulaciones de tabletas útiles se pueden hacer por compresión convencional, granulación en húmedo o métodos de granulación en seco o utilizar diluyentes farmacéuticamente aceptables, agentes ligadores, lubricantes, desintegrantes, agentes modificadores de superficie (que incluyen tensoactivos), agentes de suspensión o estabilizantes, que incluyen, pero no están limitados a,

estearato de magnesio, ácido esteárico, talco, sodio, laurel sulfato de sodio, celulosa microcirstalina, carboximeticelulosa de calcio, polivinilpirrolidona, gelatina, ácido algínico, goma de acacia, goma xantan, citrato de sodio, silicatos complejos, carbonato de calcio, glicina, dextrina, sucrosa, sorbitol, fosfato dicalcio, sulfato de calcio, lactosa, caolín, manitol, cloruro de sodio, talco, almidón seco y azúcar en polvo. Agentes modificadores de superficie preferidos incluyen agentes modificadores de superficie aniónica y no iónico. Ejemplos representativos de agentes modificadores de superficie incluyen, peo no están limitados a, poloxámero 188, cloruro de benzalconio, estearato de calcio, cetostearil alcohol, cera emulsificadora de cetomacrogol, ésteres de sorbitan, dióxido de silicio coloidal, fosfatos, dodecil sulfato de sodio, silicato de magnesio aluminio, y trietanolamina. Las formulaciones orales aquí pueden utilizar formulaciones de liberación en el tiempo o retardadas estándar para alterar la absorción de los compuestos. La formulación oral puede consistir de administrar el ingrediente activo en agua o un jugo de frutas que contiene los solubilizadores apropiados o emulsificadores según se necesite.

En una modalidad, las formulaciones orales pata 42-éster de rapamicina con ácido 3-hidroxi-2-(hidroximetil)-2-metilpropiónico se describen en Publicación de Patente Estadounidense No. US 2004-0077677 A1 (también Solicitud de Patente Estadounidense No. 10/663,506). Tal una formulación oral contiene una granulación preparada utilizando un proceso de granulación en húmedo.

En algunos casos puede se deseable administrar los compuestos directamente a las vías aéreas en la forma de un aerosol.

Los compuestos también se pueden administrar parenteralmente o intraperitonealmente. Las soluciones o suspensiones de estos compuestos activos como una base libre o sal farmacológicamente aceptable se pueden preparar en agua mezclando adecuadamente con tensoactivos tales como hidroxi-propilcelulosa. Las dispersiones se pueden preparar en glicerol, polietilenglicoles líquidos y mezclas de estos en aceites. Bajo condiciones ordinarias de almacenamiento y uso, estas preparaciones contienen un preservativo para evitar el crecimiento de los microorganismos.

Las formas farmacéuticas adecuadas para el uso inyectable incluyen soluciones acuosas estériles o dispersiones y polvos estériles para la preparación extemporánea de soluciones inyectables estériles o dispersiones. En todos los casos, la forma debe ser estéril y debe ser fluida para que exista fácil suministro con jeringa. Esta debe ser estable bajo las condiciones de fabricación y almacenamiento y se deben preservar contra la acción contaminante de los microorganismos tales como bacterias y hongos. El portador puede ser un solvente o medio de dispersión que contiene, por ejemplo, agua, etanol, poliol (por ejemplo, glicerol, propilenglicol y polietilenglicol líquido), mezclas adecuadas de estos, y aceites vegetales.

En una modalidad, las formulaciones inyectables para 42-éster de rapamicina con ácido 3-hidroxi-2-(hidroximetil)-2-

34

metilpropiónico se describen en la Publicación de Patente Estadounidense No. US 2004-0167152 A1 (también Solicitud de Patente Estadounidense No. 10/626,943).

Las formulaciones parenterales útiles en esta invención se pueden utilizar para Los compuestos de esta invención se pueden producir una forma de dosis que es adecuada para administración por inyección directa o adición de fluidos de infusión estéril para infusión intravenosa.

Las administraciones transdérmicas se entiende que incluyen todas las administraciones a través de la superficie del cuerpo y los recubrimientos interiores de pasajes del cuerpo que incluyen tejidos epiteliales y mucosos. Tales administraciones pueden ser llevadas a cabo utilizando los presentes compuestos o las sales farmacéuticamente aceptables de estos, en lociones, cremas, espumas, parches, suspensiones, soluciones, y supositorios (rectal y vaginal).

La administración transdérmica se puede acompañar a través del uso de un parche transdérmico que contiene el compuesto activo y un portador que se inserta al compuesto activo, que no es toxico a la piel, y que permite el suministro del agente por absorción sistémica dentro del torrente sanguíneo por vía de la piel. El portador puede tomar cualquier número de formas tales como cremas y ungüentos, pastas, geles y dispositivos oclusivos. Las cremas y los ungüentos pueden ser líquidos viscosos o emulsiones semisólidas del tipo de aceite en agua o agua en aceite. Las pastas comprenden de polvos absorptivos dispersos en

petróleo o petróleo hidrofílico que contiene el ingrediente activo también pueden ser adecuadas. Una variedad de dispositivos oclusivos se pueden utilizar para liberar el ingrediente activo dentro del torrente sanguíneo, tal como una membrana semipermeable que cubre un reservorio que contiene el ingrediente activo con o sin un vehículo, o una matriz que contiene el ingrediente activo. Otros dispositivos oclusivos se conocen en la literatura.

Formulaciones de supositorios se pueden hacer a partir de materiales tradicionales que incluyen manteca de cacao, con o sin la adición de ceras para alterar el punto de fusión del supositorio, y glicerina. Las bases de supositorio soluble, tales como polietilenglicoles de varios pesos moleculares, también se pueden utilizar.

La presente invención suministra adicionalmente empaques y kits que contienen los derivados 42 de rapamicina regioespecíficos producidos de acuerdo con la presente invención y formulados para administración por un método de suministro adecuado. En una modalidad, los derivados 42 de rapamicina regioespecíficos están presentes en forma de dosis unitaria. Una variedad de contenedores adecuados que incluyen botellas, frascos, empaques blister, y similares se conocen por aquellos expertos en la técnica. Tales empaques y kits pueden contener adicionalmente otros componentes, que incluyen, por ejemplo, instrucciones para uso, jeringas, aplicadores y similares.

Los siguientes ejemplos son ilustrativos de los métodos de la invención para la producción regioespecífica de derivados 42-éster de rapamicina. Como se ilustra en los siguientes ejemplos, la lipasa *Candida antarctica* es particularmente bien adecuada en su capacidad para catalizar la transesterificación de rapamicina a su derivado 42-acilo utilizando acetato de vinilo como el donante acilo. Sin embargo, como se estableció anteriormente la invención no es limitada así y otras lipasas adecuadas de origen microbiano se puede utilizar. Por ejemplo, la lipasa PS, de *Pseudomonas cepacia* y su forma inmovilizada, la lipasa PS-C "Amano" II, la lipasa PS-D las condiciones de reacción puede incluir la más alta temperatura con más enzima. Por ejemplo en una modalidad utilizando lipasa PS-C inmovilizada, dobla la cantidad de lipasa, (es decir., 200% de rapamicina (p/p) se requiere para alcanzar la taza de conversión de Novozym 435 lipasa a temperatura ambiente; alternativamente la temperatura se puede elevar a aproximadamente 45° C cuando menos cantidad de enzima se utiliza (100% p/p) a rapamicina)].

EJEMPLOS

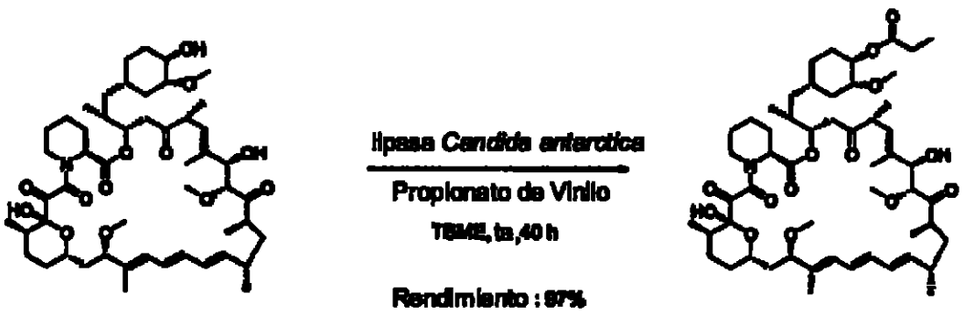
Los siguientes ejemplos ilustran el proceso de la invención, utilizando éster de vinilo (ejemplos 1-8), éster de isopropenilo (ejemplo 9) o un anhídrido (ejemplos 10-12).

En una modalidad, una mezcla de rapamicina (20 mg, 0.022 mmol), éster de vinilo (50 µL) y lipasa NOVOZYM 435 (20 mg) en TBME (0.5 mL) se agita a temperatura ambiente (ta) o 45 ° C bajo N_2 hasta que todo el material de partida se consume monitoreado

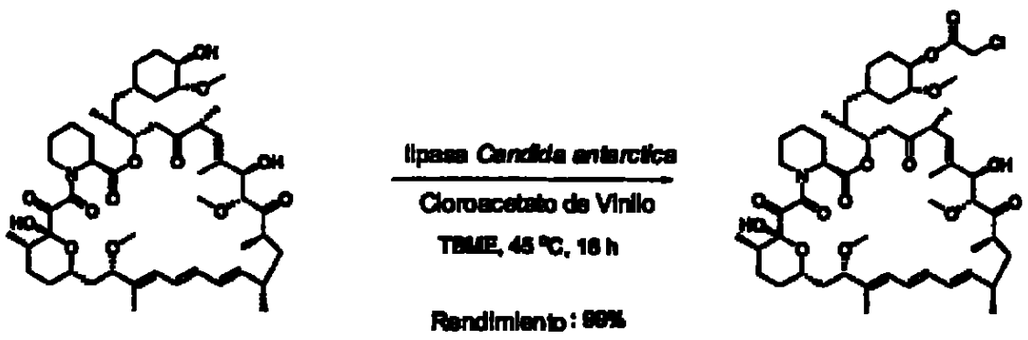
por TLC. La enzima se filtra y se lava con TBME. El solvente orgánico combinado se evapora bajo presión reducida. El residuo se purifica por cromatografía de columna de gel de sílice eluyendo con hexano-acetona (2: 1, v/v) o recristalizado de hexano-acetona. Ejemplos adicionales se ilustran en los esquemas de adelante.

Ejemplo 1

Ejemplo 2

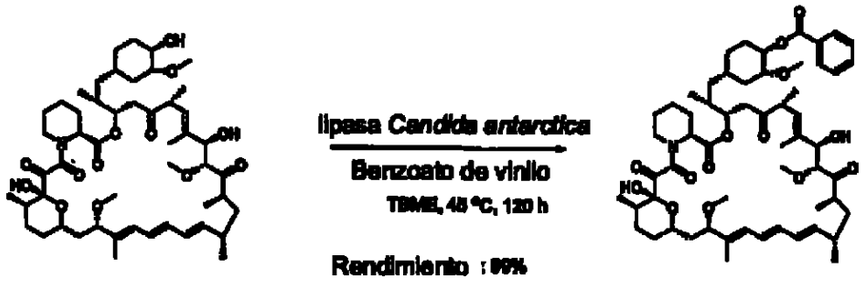


Ejemplo 3

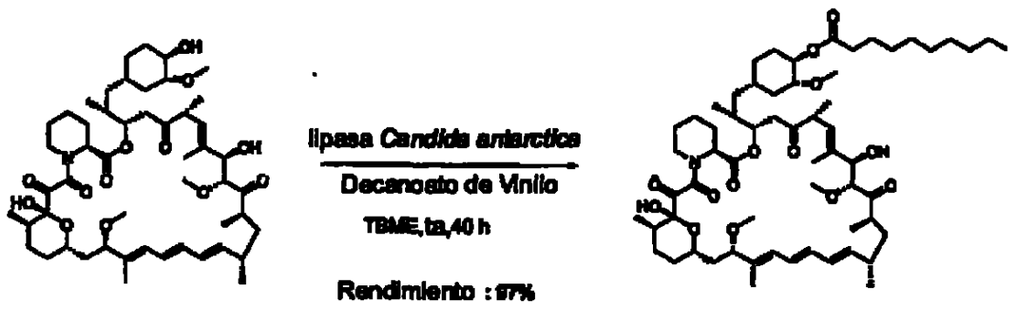


Ejemplo 4

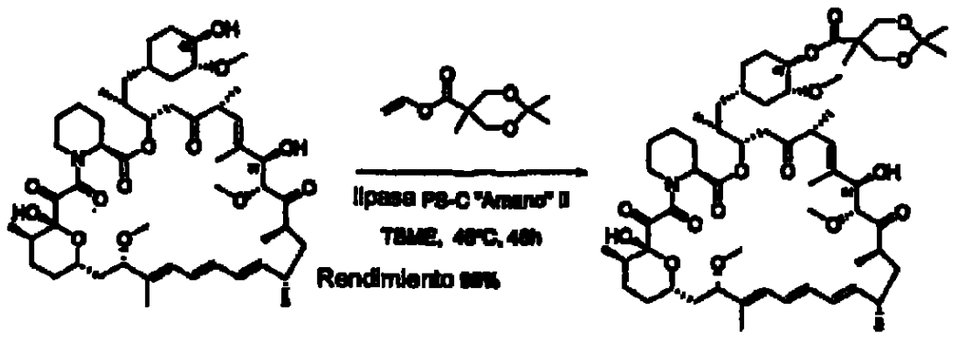
Ejemplo 5



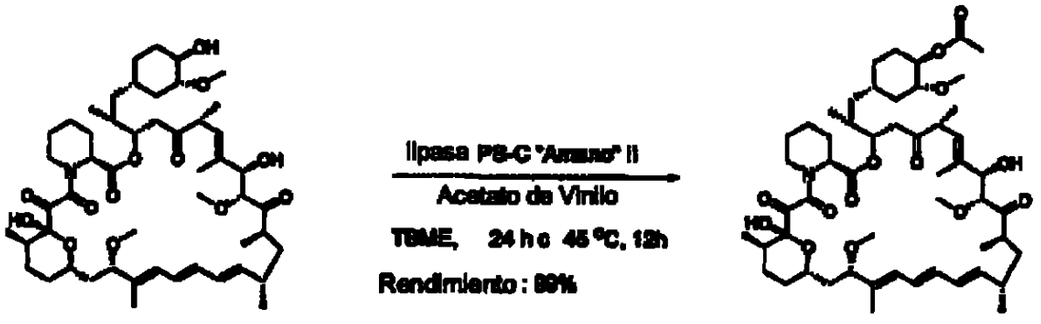
Ejemplo 6



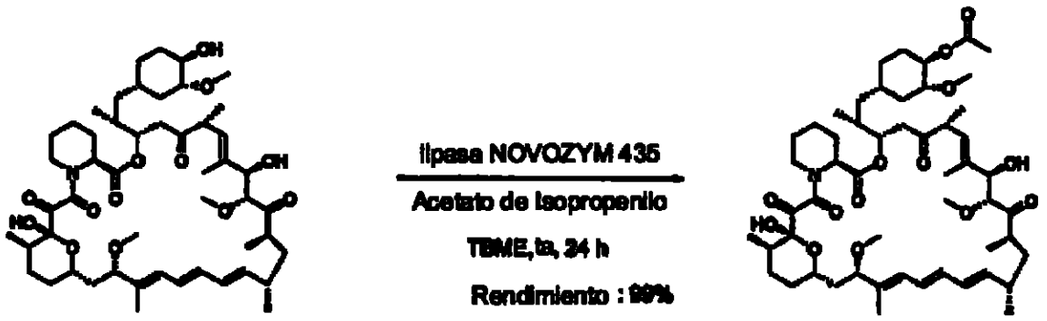
Ejemplo 7



Ejemplo 8



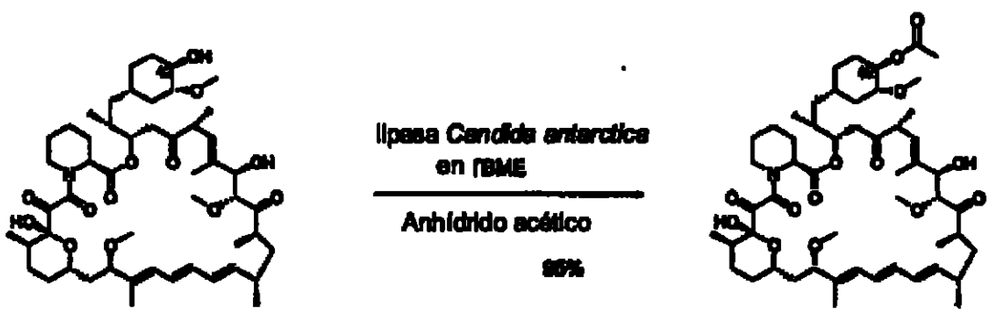
Ejemplo 9



Los derivados 42-éster de rapamicina utilizan anhídridos compuesto el donante acilo se preparan de acuerdo a la invención como sigue.

Una mezcla de rapamicina (20 mg, 0.022 mmol), anhídrido (30 mg) y lipasa NOVOZYM 435 (20 mg) en TBME (0.5 mL) se agitan a temperatura ambiente durante 48 h (N_2, protegido de la luz). [En el caso del anhídrido acético o anhídrido propiónico, después de 48 h, otra porción de lipasa NOVOZYM 435 (20 mg) y se agrega TBME (0.1 mL) y la mezcla se agita otras 48 h antes la reacción se apaga]. El solvente entonces se remueve por chorro gas N_2. El residuo se purifica por cromatografía de columna de gel de sílice eluyendo con hexano-acetona (2:1, v/v). El producto se aísla como un sólido blanco.

Ejemplo 10



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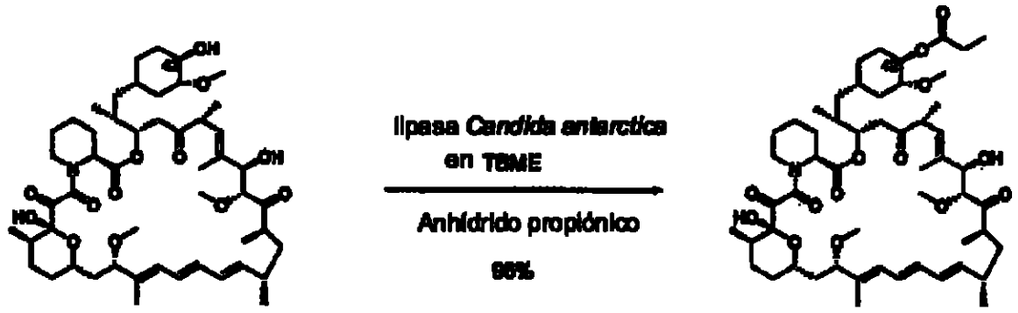
Ejemplo 11



Ejemplo 12

La presente invención no se limita en alcance por las modalidades específicas descritas aquí. En cambio varias modificaciones de la invención en adición a aquellas descritas aquí serán evidentes para aquellos expertos en la técnica de la anterior descripción y de las figuras que lo acompañan. Se pretende que tales modificaciones caigan dentro del alcance de las reivindicaciones adjuntas.

Adicionalmente se entiende que los valores son aproximados, y se suministran para descripción.

Patentes, solicitudes de patente, publicaciones, procedimientos, y similares se citan a través de esta solicitud, la descripción de las cuales se incorpora aquí como referencia en su totalidad. Para el caso que pueda existir un conflicto entre la especificación y un documento listado aquí, el idioma de la descripción hecha aquí domina.

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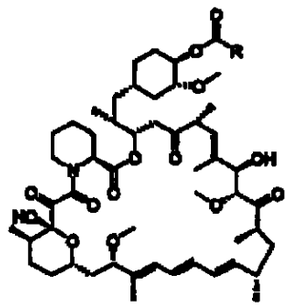
REIVINDICACIONES

1. Un método para la preparación regioespecífica de DERIVADOS 42-ÉSTER DE RAPAMICINA de la fórmula (I)



(I)

en donde R es un hidrocarburo lineal o cíclico, alifático o aromático, saturado o insaturado que opcionalmente contiene hidroxilo, halógeno y/o tio, dicho método comprende acilataruna rapamicina hidroxi-42 con un donante acilo en la presencia de una lipasa.

2. El método de acuerdo a la reivindicación 1, en donde la lipasa utilizada es una lipasa microbiana de microorganismos tales como *Aspergillus niger, Candida antarctica, Candida rugosa, Mucor miehei, Pseudomonas cepacia, Pseudomonas fluorescens, Rhizopus delemar.*

3. El método de acuerdo a la reivindicación 2, en donde la lipasa utilizada es de *Candida antarctica tipo B* (lipasa NOVOZYM 435) o *Pseudemonas cepacia* (lipasa PS-C "Amano" II).

44

4. El método de acuerdo a una cualquiera de las reivindicaciones 1 a 3, en donde el donante acilo es un éster de vinilo, un éster de isopropenilo o un anhídrido.

5. El método de acuerdo a la reivindicación 4 en donde el éster de vinilo tiene la fórmula $CH_2=CH-O-COR^1$, en donde R_1 se selecciona del grupo que consiste de alquilo C_1-C_6, alquenilo C_2-C_6, arilo c_6-C_{14}, bencilo, opcionalmente sustituido con un grupo independientemente seleccionado de hidroxilo, halógeno y SH.

6. El método de acuerdo a la reivindicación 5 en donde el éster de vinilo se selecciona del grupo que consiste de acetato de vinilo, propionato de vinilo, cloroacetato de vinilo, crotonato de vinilo, benzoato de vinilo, y decanoato de vinilo.

7. El método de acuerdo a la reivindicación 4 en donde el éster de vinilo es protegido 3-hidroxi-2-(hidroximetil)-2-metilpropionato de vinilo protegido por isopropilideno.

8. El método de acuerdo a la reivindicación 4 en donde el éster de isopropenilo tiene la fórmula $CH_2=C(CH_3)-OCOR^2$, en donde R^2 se selecciona del grupo que consiste de alquilo C_1-C_6, alquenilo C_2-C_6, arilo C_6-C_{14}, bencilo, opcionalmente sustituido con un grupo independientemente seleccionado de hidroxilo, halógeno y SH.

9. El método de acuerdo a la reivindicación 8 en donde el éster de isopropenilo es acetato de isopropenilo.

10. El método de acuerdo a la reivindicación 4 en donde el anhídrido es un anhídrido alcanóico C_1-C_8 de cadena lineal o cadena ramificada, opcionalmente sustituido con un grupo independientemente seleccionado de halógeno e hidroxilo.

11. El método de acuerdo a una cualquiera de las reivindicaciones 1 a 10, en donde la reacción tiene lugar en un solvente orgánico seleccionado del grupo que consiste de tolueno, éter de terc-butil metilo (TBME), éter de etilo, THF, MeCN, CH_2Cl_2, $CHCl_3$, hexano, dioxano o mezclas de estos.

12. El método de acuerdo a una cualquiera de las reivindicaciones 1 a 11, en donde la reacción se conduce en el rango de 20° C a 75° C.

13. Una preparación regioespecífica de 42-éster de rapamicina con ácido 3-hidroxi-2-(hidroximetil)-2-metilpropiónico protegido por cetal isopropilideno, un precursor de CCI-779.

14. Una composición que comprende un derivado-42 de rapamicina regioespecífico producido de acuerdo al método de una cualquiera de las reivindicaciones 1 a 12.

15. Una composición que comprende el derivado-42 de rapamicina regioespecífico se prepara de acuerdo a la reivindicación 13.

16. La composición de acuerdo a las reivindicaciones 14 o 15 comprende adicionalmente un portador fisiológicamente compatible.

17. Un producto que comprende una composición de acuerdo con una cualquiera de las reivindicaciones 14 a 16, y un envase para dicha composición.

18. Un kit farmacéutico que comprende unidades de un derivado-42 de rapamicina regioespecífico que se produce de acuerdo con una cualquiera de las reivindicaciones 1 a 12 en forma de dosis unitaria.

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SÍNTESIS REGIOESPECÍFICA DE DERIVADOS 42-ÉSTER DE RAPAMICINA

RESUMEN

Se describe un método para la síntesis regioespecífica de derivados 42-éster de rapamicina. El método involucra acilación de lipasa-catalizada de 42 hidroxi de rapamicina con un donante acilo tal como un éster de vinilo, un éster de isopropenilo o un anhídrido en un solvente orgánico adecuado.

PATENT COOPERATION TREATY

PCT

nnnx8

98

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
AM-100906PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US2005/012266	12/04/2005	14/04/2004
Applicant		
WYETH		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
      [ ] The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
   b. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. [ ] Certain claims were found unsearchable (See Box II).

3. [X] Unity of invention is lacking (see Box III).

4. With regard to the title,
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established by this Authority to read as follows:

5. With regard to the abstract,
   [X] the text is approved as submitted by the applicant.
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,
   a. the figure of the drawings to be published with the abstract is Figure No. _____
      [ ] as suggested by the applicant.
      [ ] as selected by this Authority, because the applicant failed to suggest a figure.
      [ ] as selected by this Authority, because this figure better characterizes the invention.
   b. [ ] none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2005/012266

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D498/18 A61K47/48 C12P17/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, BEILSTEIN Data, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 464 895 A (MERCK & CO. INC) 8 January 1992 (1992-01-08) see whole document, especially claim 1 -----	1-12,14, 16-18
A	ADAMCZYK M ET AL: "Lipase mediated hydrolysis of rapamycin 42-hemisuccinate benzyl and methyl esters" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 35, no. 7, 14 February 1994 (1994-02-14), pages 1019-1022, XP002218069 ISSN: 0040-4039 cited in the application the whole document -----	1-12,14, 16-18
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 June 2005	06.09.05

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Scruton-Evans, I

Form PCT/ISA/210 (second sheet) (January 2004)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/05179 A (AMERICAN HOME PRODUCTS CORPORATION) 2 April 1992 (1992-04-02) cited in the application see examples	14,16-18
X	WO 95/28406 A (AMERICAN HOME PRODUCTS CORPORATION) 26 October 1995 (1995-10-26) cited in the application see whole document and examples. the whole document	14,16-18
X	WO 01/23395 A (AMERICAN HOME PRODUCTS CORPORATION) 5 April 2001 (2001-04-05)	14,16-18
Y	see whole document	1-12,14, 16-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/012266
000051

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see annex

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International Application No. PCT/ US2005/ 012266

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-12,14,16(part),17 (part), 18

   Process for the regiospecific preparation of a rapamycin 42-ester using a lipase, compositions and pharmaceutical kit containing a rapamycin 42-derivative prepared according to the process

   ---

2. claims: 13,15,16(part),17(part)

   A regiospecific preparation of rapamycin 42-isopropylidene ketal protected 3-hydroxy-2-(hydroxymethyl)-2-methyl propionic acid.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No PCT/US2005/012266 00053

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 0464895	A	08-01-1992	US	5210030 A	11-05-1993
			CA	2045421 A1	26-12-1991
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			JP	4252191 A	08-09-1992
WO 9205179	A	02-04-1992	AU	653175 B2	22-09-1994
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			CA	2051781 A1	20-03-1992
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			IE	913302 A1	25-02-1992
			JP	6501012 T	27-01-1994
			MX	9101139 A1	04-05-1992
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			US	5358944 A	25-10-1994
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WO 9528406	A	26-10-1995	US	5362718 A	08-11-1994
			AT	234307 T	15-03-2003
			AU	679854 B2	10-07-1997
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			CA	2187024 A1	26-10-1995
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			DE	69529897 D1	17-04-2003
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			EP	0763039 A1	19-03-1997
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			AU	782234 B2	14-07-2005
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		WO 0123395 A2	05-04-2001
		ZA 200201918 A	09-06-2003

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

00055

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	FOR FURTHER ACTION See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2005/012266	12.04.2005	14.04.2004

International Patent Classification (IPC) or both national classification and IPC
C07D498/18, A61K47/48, C12P17/18

Applicant
WYETH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Scruton-Evans, I Telephone No. +49 89 2399-8272

Form (PCT/ISA/237) (Cover Sheet) (January 2004)

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 14,16-18

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 14,16-18

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-12,14,16(part),17 (part), 18(part)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	14,16-18
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-12,14,16-18
Industrial applicability (IA)	Yes:	Claims	1-12,14,16-18
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 14,16-18 all refer to compositions and kits containing a rapamycin derivative prepared according to the process of claims 1-12. In the absence of any distinguishing feature of these products by process claims, they are to be seen as products per se. So many of these esters are known that a meaningful search was not possible over the whole of the scope, and only a selection of the documents prejudicial to the novelty of these products is cited.

Re Item IV

Lack of unity of invention

This Authority considers that there are two inventions covered by the claims indicated as follows:

I: Claims 1-12,14,16-18 (part) directed to a process for the preparation of a 42-ester of rapamycin using a lipase, compositions and pharmaceutical kit

II: Claims 13,15,16-18 (part) directed to a regiospecific preparation of rapamycin 42-ester with isopropylidene ketal protected 3-hydroxy-2-(hydroxymethyl)-2-methyl propionic acid

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

Invention 1 describes the process of preparation of rapamycin 42-ester derivatives using an acyl donor and a lipase. The problem underlying this invention was the provision of a further novel process for the preparation of known 42-ester derivatives, the solution provided being the use of a lipase.

Invention 2 is independent, i.e. describes only the regiospecific preparation of the ketal compound, without the use of a lipase, or, indeed any reaction conditions. It is already known from WO-A-0123395, Scheme 1, to prepare the compound B (corresponding to the compound prepared in invention 2) regioselectively.
According to Rule 13 PCT, an international application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept, and their should be a technical relationship among the inventions involving one or more of the same or corresponding special technical features. Such "special technical features" shall mean those that define a contribution which each of the claimed inventions, considered as a whole, makes over the prior art. In the present application, it is considered that the special technical feature of invention 1 is the use of a lipase. Invention 2 is not so linked to invention 1 as to form a single general inventive concept, as the use of a lipase is not mentioned in invention 2, and thus their is a lack of unity according to Rule 13. PCT

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents cited in the search report are referred to in this communication;

D1: EP-A-0 464 895 (MERCK & CO. INC) 8 January 1992 (1992-01-08)

D2: ADAMCZYK M ET AL: "Lipase mediated hydrolysis of rapamycin 42-hemisuccinate benzyl and methyl esters" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 35, no. 7, 14 February 1994 (1994-02-14), pages 1019-1022, XP002218069 ISSN: 0040-4039

D3: WO 92/05179 A (AMERICAN HOME PRODUCTS CORPORATION) 2 April 1992 (1992-04-02)

D4: WO 95/28406 A (AMERICAN HOME PRODUCTS CORPORATION) 26 October 1995 (1995-10-26)

D5: WO 01/23395 A (AMERICAN HOME PRODUCTS CORPORATION) 5 April 2001 (2001-04-05)

WRITTEN PINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.

PCT/US2005/012266

With regard to the requirement for novelty (Article 33(2) PCT), D1 is concerned with FK-506 derivatives, not rapamycin. D2 discloses the lipase hydrolyis of esters, but not the lipase mediated preparation of the esters per se. D3 and D4 disclose 42-esters of rapamycin which are prejudicial to the novelty of claims 14,16-18, but the process for their preparation does not involve lipases. D5 also discloses a regioselective synthesis of 42-esters, but again without lipases, although it is prejudicial to the novelty of claims 14,16-18. Article 33(2) of the PCT is thus not satisfied for claims 14,16-18.

With regard to the requirement for inventive step (Article 33(3) of the PCT), the problem underlying the present application was the provision of further novel process for the regiospecific preparation of 42-esters of rapamycin. The solution provided by the application is the use of an acyl donor in the presence of a lipase. The man skilled in the art, faced with this problem, would have been aware from D5 that the problem had been addressed by other prior arts, and would also have been aware from D1, in the related FK-506 field, that lipases and an acyl donor had been used to carry out regioselective acylation, and would have considered the use of lipases in this related filed, given the teaching of D1. The present claims thus are considered to represent an obvious solution to the problem, in the light of D1, and thus lack an inventive step.

From the INTERNATIONAL BUREAU 00052

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

KODROFF, Cathy. A.
Howson and Howson
321 Norristown Road. Suite 200
Spring House Corporate Center
P.O. Box 457
Spring House, PA 19477
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 06 July 2005 (06.07.2005)	
Applicant's or agent's file reference AM-100906PCT	IMPORTANT NOTIFICATION
International application No. PCT/US2005/012266	International filing date *(day/month/year)* 12 April 2005 (12.04.2005)
International publication date *(day/month/year)*	Priority date *(day/month/year)* 14 April 2004 (14.04.2004)
Applicant WYETH et al	

1. By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. *(If applicable)* The letters "NR" appearing in the right-hand column denote a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

3. *(If applicable)* An asterisk (*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b) (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as the priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
14 April 2004 (14.04.2004)	60/561,826	US	20 May 2005 (20.05.2005)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Maurice COVINO Facsimile No. (41-22) 338.87.40 Telephone No. +41 22 338 8455

Industria y Comercio
SUPERINTENDENCIA

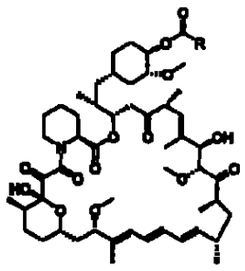
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
EXTRACTO PARA PUBLICACIÓN
SOLICITUD DE PATENTE DE INVENCIÓN PCT

(21) N° de solicitud: 06-100244	(51) Int Cl: C07D 498/18; A61K 47/48; C12P 17/18
22) Fecha de solicitud: 04/10/06	(71)Solicitante(s) WYETH
(30) Prioridad (31) *No.* 60/561,926 32) *Fecha* 14/04/2004 (33) *País* US	(72) Inventor(es) GU, Jianxin Otros
	(74) Apoderado: ALVARO CORREA ORDOÑEZ
(85) Fecha límite inicio fase nacional: *14/10/06* (86) Datos relativos a la presentación de la solicitud PCT Fecha de presentación de la solicitud: 12/04/2005 No. de solicitud PCT/US2005/012266	(87) Publicación internacional Fecha: 10/11/2005 N° publicación: WO 2005/105811

(54) Título: **SÍNTESIS REGIOESPECÍFICA DE DERIVADOS42-ÉSTER DE RAPAMICINA**

Reivindicación(es):

1. Un método para la preparación regioespecífica de DERIVADOS 42-ÉSTER DE RAPAMICINA de la fórmula (I)



(I)

en donde R es un hidrocarburo lineal o cíclico, alifático o aromático, saturado o insaturado que opcionalmente contiene hidroxilo, halógeno y/o tio, dicho método comprende acilataruna rapamicina hidroxi-42 con un donante acilo en la presencia de una lipasa.

13. Una preparación regioespecífica de 42-éster de rapamicina con ácido 3-hidroxi-2-(hidroximetil)-2-metilpropiónico protegido por cetal isopropilideno, un precursor de CCI-779.

folios 84 y 85
Tarjetas Zenaitrans

Industria y Comercio
SUPERINTENDENCIA

REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT

PATENTE DE INVENCION [X] MODELO DE UTILIDAD [ ]

- Indicación de que se solicita la concesión de una patente [X]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [X]
- Descripción de la Invención [X]
- Dibujos de ser estos pertinentes [X]
- Comprobante de pago de las tasas establecidas [X]

COMPLETA [X] INCOMPLETA [ ]

DISEÑO INDUSTRIAL [ ]

- Indicación de que se solicita el registro de Diseño Industrial [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

ESQUEMA DE TRAZADO [ ]

- Indicación de que se solicita el registro de un Esquema de Trazado [ ]
- Datos de identificación del solicitante o de la persona que presenta la solicitud [ ]
- Representación gráfica del esquema trazado [ ]
- Comprobante de pago de las tasas establecidas [ ]

COMPLETA [ ] INCOMPLETA [ ]

Fdo B
Firma Responsable

Fecha 04-oct-06

Carrera 13 No. 27-00 Piso 5, 7 y 10
Conmutador: 3 82 08 40
Fax: 350 52 20
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

87
66

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
CORREA ORDOÑEZ ALVARO

REFERENCIA	Radicación No.	06 100244
	Solicitud internacional	PCT/US2005/012266
	Fecha inicio fase nacional	14/11/2006
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	13893

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

1. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.

2. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsiguientes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 01 DIC. 2006
aalvarado