Industria y Comercio

SUPERINTENDENCIA

Delegatura de propiedad Industrial

División de Nuevas Creaciones

PCT

SOLICITUD

PATENTE DE INVENCIÓN

21. **EXPEDIENTE No.** 05-116201

54. **TÍTULO** DERIVADOS DE CARBOHIDRATOS DE RAPAMICINA

51. **CLASIFICACIÓN INTERNACIONAL** A61K 31/436

71. **SOLICITANTE** ISOTECHNIKA INC.

DOMICILIO ALBERTA, CANADA

74. **APODERADO** JAIME EDUARDO DELGADO VILLEGAS

22. **BOGOTÁ, D. C.,** _____

(FORMA P-10)

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PETITORIO

1

SUPERINDUSTRIA Y COMERCIO
Radicacion : 05116201 00000000 Folios: 293
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Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
PCT

ENTRADA EN FASE NACIONAL 16-12-2005

(1) **SOLICITUD DE:** folio 212

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

[X] Capitulo I [ ] Capitulo II

(2) **SOLICITANTE** '(71)

Nombre: **ISOTECHNIKA INC.**

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C.E. [ ] Otro [ ]

Cual: _____

Número:

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(4) **INVENTOR (ES)** (72)

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(5) **PCT '(86)**

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DERIVADOS DE CARBOHIDRATOS DE RAPAMICINA

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PCT/CA2004/000724
PUBLICACIÓN No. WO 2004/ 101583 A1 Fecha 25/11/2004

TITULO DE LA INVENCIÓN: **DERIVADOS DE CARBORHIDRATOS DE RAPAMICINA**

SOLICITANTE:

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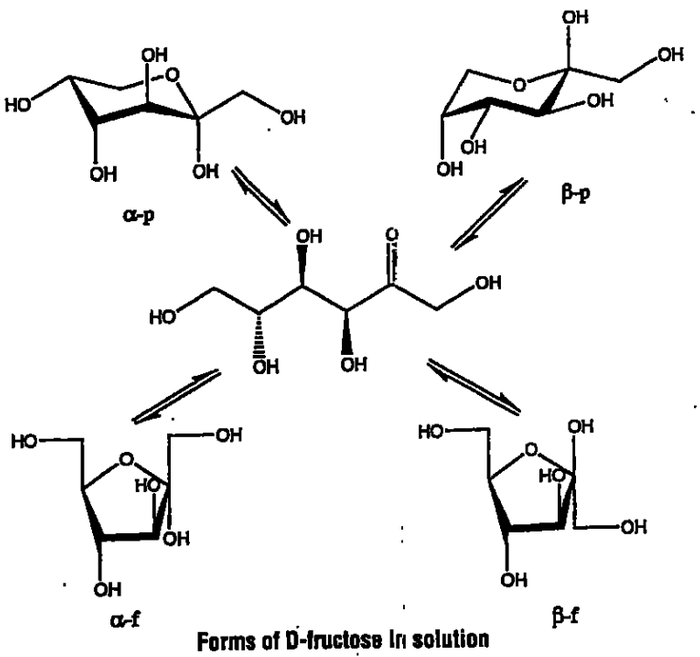
NACIONALIDAD : Canadiense

(10) **ANEXOS**

- [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, descripcion, reivindicaciones, dibujos)
- [X] Traducción de la solicitud internacional al castellano. (Resumen, descripcion, reivindicaciones, dibujos)
- [ ] Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA).
- [ ] Traduccion del examen preliminar internacional efectuado por la IPEA.
- [ ] De ser el caso, copia del contrato de acceso.
- [ ] De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas.
- [ ] De ser el caso, certificado de depósito del material biológico
- [X] Arte final 12X12 cm.
- [ ] De ser el caso, información de otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionadas total o parcialmente con la invención de esta solicitud.
- [ ] De ser el caso, modificaciones efectuadas a la solicitud internacional, Nuevas Reivindicaciones
- [X] Otros: Informe de Busqueda de Antecedentes Internacional

(11) **FIG**



Forms of D-fructose in solution

(12) **Sol**

NOMBRE: JAIME EDUARDO DELGADO VILLEGAS

FIRMA:

C.C. 19.270.955 Bogotá T.P. 43.308 C.S.J.

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página

4

ISOTECHNIKA INC

SUPERINTENDENCIA Y COMERCIO

Radicacion : 05116201 00000000 Folios: 293
Fecha (AMD): 2005-11-16 16:35:08
Tramite : 011 PET-PATENT 378-FASE NAC1 411 PRESENTAC
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 05 - 86,716
FECHA : NOVIEMBRE 10 DE 2005

***** C O N S I G N A C I O N *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PGO	Vr. PAGO
MARIO DELGADO ECHEVERRY	CONSIGNACION	BANCO POPULAR	050-00110-6	45330836	10/11/2005	1,772,000.00

***** C O N C E P T O *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	1 TRAMITES DE SOL. DE PATENTE DE IN	443,000.00
		TOTAL : $	443,000.00

SON: CUATROCIENTOS CUARENTA Y TRES MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No._____

PATENT COOPERATION TREATY

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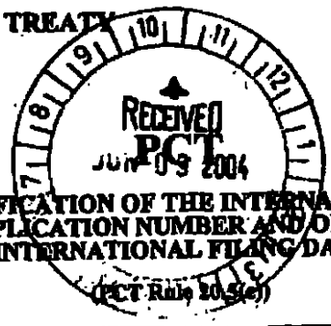

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TORYS LLP
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DUE DATE PROCESSED

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NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE
(PCT Rule 20.5(c))

Date of mailing (day/month/year): 04 June 2004 (04-06-2004)

Applicant's or agent's file reference: 31284-2007

IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/CA2004/000724	14 May 2004 (14-05-2004)	16 May 2003 (16-05-2003)

Applicant: ISOTECHNIKA INTERNATIONAL INC. ET AL

Title of the invention: RAPAMYCIN CARBOHYDRATE DERIVATIVES

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

[X] was transmitted to the International Bureau on 04 June 2004 (04-06-2004).

[ ] has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:

[ ] because the necessary national security clearance has not yet been obtained.

[ ] because (*reason to be specified*):

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Form PCT/RO/105 (July 1992; reprint January 2004)

RAPAMYCIN CARBOHYDRATE DERIVATIVES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Application Serial Nos. 60/471,367 filed May 16, 2003, 60/546,240 filed February 20, 2004 and 60/562,840 filed April 16, 2004.

FIELD OF THE INVENTION

[0002] This application relates to carbohydrate derivatives of rapamycin, a potent immunosuppressant, with structurally defined carbohydrate moieties attached to the rapamycin structure via a connective moiety. The rapamycin carbohydrate derivatives may act as prodrugs. That is, they may be substantially without immunosuppressive activity themselves, but *in vivo* be converted to rapamycin which then exhibits an immunosuppressive effect.

REFERENCES

[0003] The following references are related hereto or referred to herein by patent or application number or by author and year at the relevant portions of this specification.

[0004] C. Vezina , A. Kiudelski, S.N. Sehgal, "Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle," *J. Antibiot.* 28, 721 (1975).

[0005] S. N. Sehgal H. Baker, C. Vezina, "Rapamycin (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization," *J. Antibiot.* 28, 727 (1975).

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[0011] F. Streit, U. Christians, H.M. Schiebel, K.L. Napoli, L. Ernst, A. Linck, B.D. Kahan, K.F. Sewing, "Sensitive and specific quantification of sirolimus (rapamycin) and its metabolites in blood of kidney graft recipients by HPLC/electrospray-mass spectrometry," *Clin. Chem.* 42, 1417 (1996).

[0012] S. Miura, "Regulation of monosaccharide transporter proteins in the small intestine," *Journal of Gastroenterology* 37, 491 (2002).

[0013] Chem Sources USA and Chem Sources International; t e ACD electronic database; and Chemical Abstracts).

[0014] Advanced Organic Chemistry, Jerry March, John Wiley &Sons.

[0015] C.K. Wang et al., Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, 545 (1993).

[0016] M.J.M. Nickmilder , D. Latinne, R.K. Verbeeck, W. Janssens, D. Swoboda, G.J. Lhoest, "Isolation and identification of new rapamycin dihydrodiol metabolites from dexamethasone-induced rat liver microsomes," *Xenobiotica*, 27, 869 (1997).

[0017] U.S. Patent No. 3,929,992.

[0018] U.S. Patent No. 3,993,749.

[0019] U.S. Patent No. 4,885,171.

[0020] U.S. Patent No. 4,401,653.

[0021] U.S. Patent No. 4,316,885.

[0022] U.S. Patent No. 4,650,803.

[0023] PCT application No.WO 92/05179.

[0024] U.S. Patent No. 5,118,678.

[0025] U.S. Patent No. 5,260,300.

[0026] U.S. Patent No. 5,118,678.

[0027] U.S. Patent No. 5,118,678.

[0028] U.S. Patent No. 5,100,883.

[0029] U.S. Patent No. 5,151,413.

[0030] U.S. Patent No. 5,120,842.

[0031] U.S. Patent No. 5,120,725.

[0032] U.S. Patent No. 5,120,727.

[0033] U.S. Patent No. 5,258,389.

[0034] U.S. Patent No. 5,672,605.

[0035] U.S. Patent No. 5,583,139.

[0036] U.S. Patent No. 5,527,907.

[0037] U.S. Patent No. 5,457,111.

[0038] U.S. Patent No. 5,955,100.

[0039] U.S. Patent No. 6,146,658.

[0040] U.S. Patent No. 5,935,995.

[0041] U.S. Patent No. 5,665,728.

[0042] U.S. Patent No. 6,146,658.

BACKGROUND OF THE INVENTION

[0043] Rapamycin, also known as sirolimus, is a 31-membered macrolide lactone, $C_{51}H_{79}NO_{13}$, with a molecular mass of 913.6 Da. In solution, rapamycin forms conformational trans- and cis-isomers with a ratio of 4:1 (in chloroform solution) due to hindered rotation around the pipecolic acid amide bond. It is sparingly soluble in water, aliphatic hydrocarbons and diethyl ether, whereas it is soluble in alcohols, halogenated hydrocarbons and dimethyl sulfoxide. Rapamycin is unstable in solution; it degrades in plasma and in low and neutral pH buffers at 37°C with a half-life of less than 10 hours.

[0044] Produced by *Streptomyces hygroscopicus*, rapamycin has been shown to possess a number of valuable pharmacological attributes. The compound is a macrocyclic triene antibiotic that possesses antifungal activity, particularly against *Candida albicans*, both *in vitro* and *in vivo*. See, C. Vezina et al., *J. Antibiot.* 28, 721 (1975), S. N. Sehgal et al., *J. Antibiot.* 28, 727 (1975), H. A. Baker et al., *J. Antibiot.* 31, 539 (1978), and U.S. Patent Numbers 3,929,992; and 3,993,749. Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has also been shown to have antitumor activity. Futhermore, R. Martel et al. *Can. J. Physiol. Pharmacol.* 55, 48 (1977) disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

[0045] The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Rapamycin, Cyclosporin A, FK-506 (also known as tacrolimus), and other macrocyclic molecules, have been shown to be effective immunosuppressive agents and therefore are useful in preventing transplant rejection. See, FASEB 3, 3411 (1989), FASEB 3, 5256 (1989), and R. Y. Calne et al., *Lancet* 1183 (1978). Although rapamycin shares structural homology with the immunosuppressant tacrolimus (FK506) and binds to the same intracellular binding protein in lymphocytes, rapamycin and tacrolimus have been shown to have different mechanisms of immunospressive action. Rapamycin inhibits S6p70-kinase whereas Tacrolimus inhibits calcineurin. Rapamycin was found to prolong graft survival of different transplants in several species alone or in combination with other immunosuppressants. See, S.N. Sehgal et al., *Medicinal Research Reviews* 14, 1 (1994).

[0046] Rapamycin is also known as an mTOR inhibitor. These inhibitors are a class of immunosuppressive drugs that inhibit T cell activation at a later stage in the immune response than other types of inhibitors like calcineurin inhibitors and DNA synthesis inhibitors. In transplantation, mTOR inhibitors are typically used in combination with calcineurin inhibitors.

[0047] Unfortunately, the side effects (e.g., gastrointestinal effects, hyperlipidemia) of mTOR inhibitors currently limit their broader use in transplantation and the treatment of autoimmune diseases. And, while not having been shown to induce nephrotoxicity, rapamycin has been shown to induce a number of toxic side effects in animal model. Such toxic effects include, for example, impairment of glucose homeostasis, gastrointestinal tract ulceration, weight loss, diarrhea and thrombocytopenia.

[0048] Numerous rapamycin derivatives have been synthesized in the hopes of alleviating and improving some drawbacks that rapamycin retains, which include low and/or variable bioavailability and solubility, and high toxicity. Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat. No. 4,316,885) and used to make water soluble prodrugs (U.S. Pat. No. 4,650,803). Other derivatives include, carboxylic acid esters (PCT Publication No.WO 92/05179), carbamates (U.S. Pat. No. 5,118,678), carbonates (U.S. Pat. No. 5,260,300), amide esters (U.S. Pat. No. 5,118,678), fluorinated esters (U.S. Pat. No. 5,100,883), acetals (U.S. Pat. No. 5,151,413), silyl ethers (U.S. Pat. No. 5,120,842), bicyclic derivatives (U.S. Pat. No. 5,120,725), rapamycin dimers (U.S. Pat. No. 5,120,727) and O-aryl, O-alkyl, O-alkenyl and O-alkynyl derivatives (U.S. Pat. No. 5,258,389). Various rapamycin prodrugs have also been developed (U.S. Pat. Nos. 5,672,605, 5,583,139, 5,527,907, 5,457,111, 5,955,100, 6,146,658, and 5,935,995).

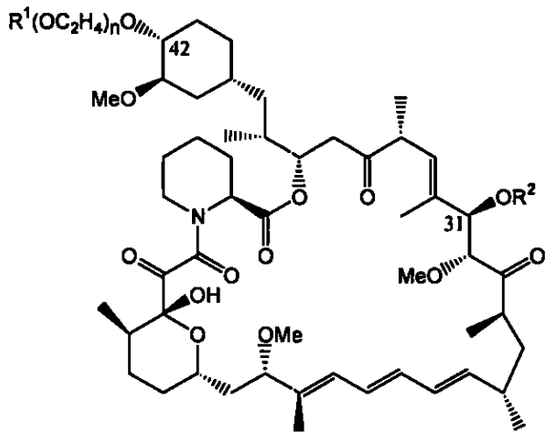
[0049] As rapamycin has been shown to possess excellent immunosuppressant, antifungal, antitumor, and other important biological activities, a need still exists for improved derivatives that increase solubility and improve the pharmakokinetic profile while decreasing its toxicity. The present invention addresses these needs.

SUMMARY OF THE INVENTION

[0050] The present invention is based in part on the recognition that carbohydrate derivatives of rapamycin in which the rapamycin molecule is modified at the 31- and/or 42- position, as defined

by the current Chemical Abstracts nomenclature, by attaching monosaccharide(s), oligosaccharide(s) or pseudosugars, have similar or enhanced pharmacokinetic and/or pharmacodynamic profiles compared to rapamycin. In addition, administration of the rapamycin carbohydrate derivatives may result in reduced toxicity with retention of the desired pharmacological effect. In addition, the rapamycin carbohydrate derivatives may render rapamycin more water soluble allowing for simplified drug formulation. Thus, the present invention provides compounds with characteristics that are distinct from other drugs in its class such as rapamycin.

[0051] In one aspect, the invention is directed to a rapamycin carbohydrate derivative having the structure of formula (I):



(I)

wherein

n = 0 or 1, R^1 and R^2 are independently hydrogen or-X-Z, wherein each X is a linker, and each Z is a carbohydrate moiety independently selected from the group consisting of a monosaccharide, an oligosaccharide and a pseudosugar wherein Z is attached to X through a hydroxyl oxygen atom of Z with the proviso that R^1 and R^2 are not both hydrogen. In an embodiment, R^1 can be hydrogen and R^2 can be X-Z or in another embodiment, R^2 is hydrogen and R^1 is X-Z. In a further embodiment, X can be selected from the group consisting of (i) $-R^3C(O)-$; (ii) $-C(O)R^3-$; iii) $-R^3S(O)_2-$; and (iv) $-S(O)_2R^3-$; wherein R^3 is selected from the group consisting of: (a) $-(CH_2)_p-$ where p is an integer from 1 to 18; (b) $-(CH_2)_n-O-(CH_2)_m-$ where n and m are each independently an integer from 2 to 6; and(c) a bond. In another embodiment, X can be selected from the group consisting of -C(O)- and $-SO_2-$. In an additional embodiment, X can be a single functional group. In another aspect, Z can be selected from the group consisting of fructose, fucitol and allose. In yet a further aspect, Z can be a monosaccharide derivative wherein at least one of the hydroxyl groups of the monosaccharide is replaced with a hydrogen, an alkoxy, an alkanoate or a halogen group.

[0052] Rapamycin carbohydrate derivatives having the structure of formula I that are within the scope of this invention include, for example, those set forth below (including pharmaceutically acceptable salts thereof):

42-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-[2-(Methyl-D-glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-D-fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-L-fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-D-fructosylcarbonyl)rapamycin;

31-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-L-fructosylcarbonyl)rapamycin;

42-O-(D-Allosylcarbonyl)rapamycin;

42-O-[2-(D-Allosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Allosylcarbonyl)rapamycin;

42-O-[2-(L-Allosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-allosylcarbonyl)rapamycin;

31-O-(L-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-allosylcarbonyl)rapamycin;

42-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-[2-(D-Fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-[2-(L-Fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fructoslylcarbonyl)rapamycin;

31-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fructoslylcarbonyl)rapamycin;

42-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-[2-(D-Fucitolylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-[2-(L-Fucitolylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fucitolylcarbonyl)rapamycin;

31-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fucitolylcarbonyl)rapamycin;

42-O-(D-Glucalylcarbonyl)rapamycin;

42-O-[2-(D-Glucalylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Glucosylcarbonyl)rapamycin;

42-O-[2-(D-Glucosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Glucosylcarbonyl)rapamycin;

42-O-[2-(L-Glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Glucalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucalylcarbonyl)rapamycin;

31-O-(D-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucosylcarbonyl)rapamycin;

31-O-(L-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-glucosylcarbonyl)rapamycin;

42-O-(L-Sorbosylcarbonyl)rapamycin;

42-O-(D-Sorbosylcarbonyl)rapamycin;

31-O-(L-Sorbosylcarbonyl)rapamycin;

31-O-(D-Sorbosylcarbonyl)rapamycin;

42-O-[2-(L-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-[2-(D-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sorbosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-sorbosylcarbonyl)rapamycin;

42-O-(D-Lactalylcarbonyl)rapamycin;

42-O-[2-(D-Lactalylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Lactalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-lactalylcarbonyl)rapamycin;

42-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-[2-(D-Sucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sucrosylcarbonyl)rapamycin;

42-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-[2-(D-Gentobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(D-gentobiosylcarbonyl)rapamycin

42-O-(D-Cellobiosylcarbonyl)rapamycin;

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31-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellobiosylcarbonyl)rapamycin;

42-O-(D-Turanosylcarbonyl)rapamycin;

42-O-[2-(D-Turanosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Turanosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-turanosylcarbonyl)rapamycin;

42-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-[2-(D-Palatinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-palatinosylcarbonyl)rapamycin;

42-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltosylcarbonyl)rapamycin;

42-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-[2-(D-Maltulosylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Maltosylcarbonyl)rapamycin;

42-O-[2-(D-Maltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltulosylcarbonyl)rapamycin;

31-O-(D-Maltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltosylcarbonyl)rapamycin;

42-O-(D-Lactosylcarbonyl)rapamycin;

42-O-[2-(D-Lactosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-lactosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-lactosylcarbonyl)rapamycin;

42-O-(D-Melibiosylcarbonyl)rapamycin;

31-O-(D-Melibiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-melibiosylcarbonyl)rapamycin;

42-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-[2-(D-Leucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-leucrosylcarbonyl)rapamycin;

42-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-[2-(D-Rafinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-rafinosylcarbonyl)rapamycin;

42-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltotriosylcarbonyl)rapamycin;

42-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-[2-(D-Cellotetraosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellotetraosylcarbonyl)rapamycin;

42-O-(Valiolylcarbonyl)rapamycin

42-O-[2-(D-Valiolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolylcarbonyl)rapamycin

42-O-(Valiolonylcarbonyl)rapamycin

42-O-[2-(D-Valiolonylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolonylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolonylcarbonyl)rapamycin

42-O-(Valienolylcarbonyl)rapamycin

42-O-[2-(D-Valienolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valienolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valienolylcarbonyl)rapamycin

42-O-(Valienoneylcarbonyl)rapamycin

42-O-[2-(D-Valienoneylcarbonyloxy)ethyl]rapamycin;

31-O-(Valienoneylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)- 31-O-(valienoneylcarbonyl)rapamycin

[0053] In another aspect, the invention is directed to a pharmaceutical composition comprising the aforementioned rapamycin carbohydrate derivative, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. In addition, an embodiment of the invention is a method for treating a disease treatable by rapamycin by administering a therapeutically effective amount of the rapamycin carbohydrate derivative to a subject in need thereof.

[0054] In yet another aspect of the current invention, a method is presented for treating a condition such as transplantation rejection, host vs. graft disease, graft vs. host disease, leukemia, lymphoma, hyperproliferative vascular disorders, autoimmune disease, diseases of inflammation, solid tumors, and fungal infections in a patient, said method comprising administering a

therapeutically effective amount of the aforementioned pharmaceutical composition to a patient in need thereof.

[0055] Still further, the invention provides a medical device where the medical device comprises a rapamycin carbohydrate derivative or a pharmaceutically acceptable salt thereof. In an embodiment, the device is coated with the rapamycin carbohydrate derivative.

[0056] And, the invention provides a method for treating a disease treatable by rapamycin, comprising coadministering a therapeutically effective amount of a rapamycin carbohydrate derivative of the present invention to a subject in need thereof with a pharmaceutical composition selected from the group consisting of a cyclosporine or cyclosporine derivative, a steroid, or an immunomodulatory compound.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] Figure 1 depicts fructose as it exists in several configurations in solution

[0058] Figure 2 depicts a general approach to the preparation of rapamycin carbohydrate derivatives of the present invention. "RAPA-OH" stands for rapamycin, wherein the hydroxyl group (-OH) may be any of the hydroxyl groups of rapamycin.

[0059] Figure 3 depicts the reaction pathway for the synthesis of 42-O-(D-fructosylcarbonyl)rapamycin.

[0060] Figure 4 depicts the reaction pathway for the synthesis of 31-O-(D-fructosylcarbonyl)rapamycin.

[0061] Figure 5 depicts the *in vitro* immunosuppressive activity of rapamycin, 42-O-(D-fructosylcarbonyl)rapamycin and a carbamate-linked analog of 42-O-(D-fructosylcarbonyl)rapamycin.

[0062] Figure 6, 7 and 8 depict pharmacokinetic profiles of selected rapamycin carbohydrate derivatives in rats.

[0063] Figure 9 depicts serum cholesterol levels in rats following treatment with 42-O-(D-fructosylcarbonyl)rapamycin and rapamycin.

[0064] Figure 10 shows the effect of two different doses of rapamycin on platelet aggregation.

[0065] Figure 11 compares the effect of 42-O-(D-fructosylcarbonyl)rapamycin and rapamycin on platelet aggregation.

[0066] Figure 12 illustrates the effect of 42-O-(D-fructosylcarbonyl)rapamycin and rapamycin on survival rates in a rat heart transplant model.

DETAILED DESCRIPTION OF THE INVENTION

[0067] This invention relates to the unexpected discovery that the claimed rapamycin carbohydrate derivatives have improved pharmacological properties as compared to underivatized rapamycin.

[0068] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural references unless context clearly dictates otherwise. Thus, for example, a reference to "a rapamycin carbohydrate derivative" includes a plurality of such derivatives; a reference to a "pharmaceutically acceptable carrier" is a reference to one or more carriers and to equivalents thereof known to those skilled in the art, and so forth.

[0069] Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can

be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described.

[0070] All publications cited herein are incorporated herein by reference in their entirety for the purpose of describing and disclosing the methodologies, reagents, and tools reported in the publications that might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0071] The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, cell biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. (See, e.g., Gennaro, A.R., ed. (1990) Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co.; Colowick, S. et al., eds., Short Protocols in Molecular Biology, 4th edition, John Wiley & Sons; Ream et al., eds. (1998) Molecular Biology Techniques: An Intensive Laboratory Course, Academic Press).

Definitions

[0072] When discussing rapamycin carbohydrate derivatives, compositions, or methods, the following terms have the following meanings unless otherwise indicated. Undefined terms have their art-recognized meanings.

[0073] The term "alkyl" refers to alkyl groups having from 1 to 10 carbon atoms, for example 1 to 6 carbon atoms, and includes both straight chain and branched chain alkyl groups. This term is exemplified by groups such as methyl, t-butyl, n-heptyl, octyl and the like.

[0074] The term "alkene" refers to an unsaturated alkyl group having at least one point of alkene unsaturation (i.e. -C=C-) and further having from 1 to 10 carbon atoms, for example from 1 to 6 carbon atoms, and includes both straight chain and branched chain alkyl groups.

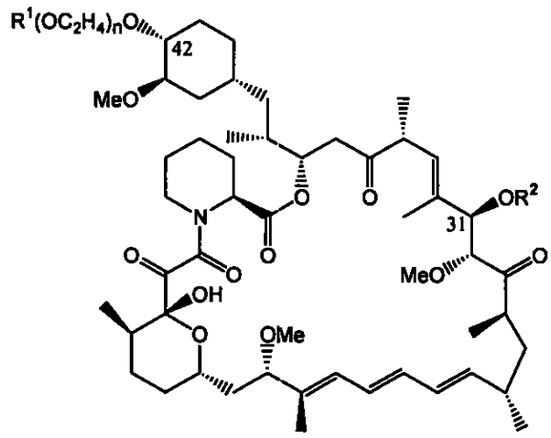
[0075] The term "alkyne" refers to an unsaturated alkyl group having at least one point of alkyne unsaturation (i.e. –C≡C-) and further having from 1 to 10 carbon atoms, for example from 1 to 6 carbon atoms, and includes both straight and branched chain alkyl groups.

[0076] The term "aromatic" group refers to an aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl or anthryl) which condensed rings may or may not be aromatic (e.g., 2-benzoxazolinone, 2H-1,4-benzoxazin-3(4H)-one-7-yl, and the like).

[0077] The term "protecting group" or "blocking group" refers to any group which, when bound to one or more hydroxyl group(s) of rapamycin or a sugar moiety, prevents reactions from occurring at these hydroxyl group(s) and which protecting groups can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl group(s). The particular removable protecting group employed is determined by the nature of the compounds and chemical processes being utilized. Removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidene, phenacyl, t-butyldimethylsilyl and trialkylsilyls such as triethylsilyl, triisopropylsilyl, trimethylsily, tributylsilyl and the like, and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

The Rapamycin Carbohydrate Derivatives

[0078] As discussed above, the rapamycin carbohydrate derivatives of the invention are compounds having the structure of formula (I):



(I)

wherein

n=0 or 1, R^1 and R^2 are independently hydrogen or-X-Z, wherein each X is a linker, and each Z is a carbohydrate moiety independently selected from the group consisting of a monosaccharide, oligosaccharide and pseudosugar, with the proviso that R^1 and R^2 are not both hydrogen.

[0079] As will be evident, the rapamycin derivatives can have sugar derivative moieties attached at the 42-position, the 31-position, or both the 42- and the 31-positions. In one embodiment, the sugar derivative is attached at the 42-position alone and in another embodiment the sugar derivative is attached at the 31-position alone.

[0080] It is important to note that in naturally-occurring rapamycin, the 41-methoxy and 42-hydroxy substituents exist in a *trans* configuration relative to each other. The 42-O-(glycosylcarbonyl) rapamycin compounds of the present invention are prepared in such a way as to retain the *trans* configuration of the 41- and 42-substituents. Consequently, upon hydrolysis of the carbonate linkage, the rapamycin will be released in its naturally-occurring configuration. Similarly, the 31-O-(glycosylcarbonyl)rapamycin compounds of the present invention are prepared in such a way as to retain the naturally-occurring stereochemistry of the 31-hydroxyl substitutent of rapamycin.

The Carbohydrate Moiety

[0081] The carbohydrate (or sugar) moiety, represented by the identifier "Z" in formula (I), is formed from a monosaccharide, oligosaccharide, pseudosugar or derivative thereof having a reactive functional group that can be coupled (i) directly to the hydroxyl group(s) at either or both of the 31- and 42- positions of rapamycin or (ii) to a reactive functional group(s) on an activated rapamycin to yield a rapamycin carbohydrate derivative of the present invention. The functional group is optionally attached to a linker that in turn is attached to the sugar, typically but not necessarily at the anomeric center. Such optional linker groups are discussed further below.

[0082] The sugars that comprise the sugar derivative can differ from each other in a multitude of ways. For example, they can exist in the pyranose or furanose forms to differing degrees. Certain sugars such as fucitol exist exclusively in the open chain form whereas many others (e.g. glucose, ribose, allose) exist predominantly in the cyclic form. Physicochemical properties of the sugar such as the geometry, composition, size, flexibility or rigidity, and the relative hydrophilicity can all affect the chemical characteristics of the rapamycin carbohydrate derivative.

[0083] In solution fructose, for example, can exist mostly in a 6-membered pyranose form and/or its 5-membered furanose form. (See Figure 1). And, each of these forms can exist in alpha or beta configurations. In addition, fructose can exist in an open-chain form. Therefore, fructose can exist in its α-fructopyranose, β-fructopyranose, α-fructofuranose or , β-fructofuranose or open chain forms as shown in Fig. 6. And, fructose can be attached to a drug in any of these configurations. In its 6-membered pyranose form, fructose is more likely to form a bond, to a drug, for example, through the single primary alcohol that is present at the 1 position in the pyranose form. In its 5-membered furanose form, fructose has two primary alcohols which are at the 1 and 6 position. In the furanose form, bonds are likely through either of these primary alcohols at the 1 or the 6 position.

[0084] The manner in which the body absorbs and processes the specific sugars varies. Many people have difficulty processing lactose, for instance. Such lactose intolerance could make the incorporation of lactose into the rapamycin carbohydrate derivative unsuitable. Further, oligosaccharides are not absorbed intact from the digestive tract and must first be digested to their monosaccharide constituents. Monosaccharides, however, are absorbed by transporter systems located in the brush border membrane of enterocytes. See, S. Miura, Journal of Gastroenterology 37, 491 (2002). For example, glucose and galactose are absorbed by the SGLT1 transporter system. Another transporter, GLUT2, facilitates mainly glucose transport, and yet another transporter known as GLUT5 transports fructose. The presence in the gastrointestinal tract of different monosaccharide transporter proteins with differing selectivities could lead to varied absorption of various rapamycin carbohydrate derivatives. That is, certain derivatives may be more readily able to take advantage of an available transporter in order to facilitate the passage of the rapamycin carbohydrate derivative out of the gastrointestinal tract and deliver it to the blood stream where the carbohydrate moiety can be cleaved off, thereby releasing rapamycin. It is believed that such a facilitated process may result in lowered gastrointestinal toxicity associated with localized exposure to rapamycin.

[0085] In view of the above, it is apparent that the appropriate selection of the carbohydrate moiety (monosaccharide, oligosaccharide, or pseudosugar) incorporated into the rapamycin carbohydrate derivative can have a major influence on the derivative's pharmacokinetic and/or pharmacodynamic properties. Accordingly, the carbohydrate moiety can be carefully chosen to optimize the pharmacokinetic and/or pharmacodynamic properties of the rapamycin carbohydrate derivative.

[0086] Suitable monosaccharides include, but are not limited to, any of several simple open or closed chain sugars (in the L or D configuration), typically having 5 or 6 carbons (a pentose monosaccharide or a hexose monosaccharide), as well as 7 carbons (heptose monosaccharide). Included are sugar derivatives in which the ring oxygen atom has been replaced by carbon, nitrogen or sulfur, amino sugars in which a hydroxyl substituent on the simple sugar is replaced

with an amino group or sugars having a double bond between two adjacent carbon atoms, (e.g. glucosamine, 5-thio-D-glucose, nojirimycin, deoxynojirimycin, 1,5-anhydro-D-sorbitol, 2,5-anhydro-D-mannitol, 2-deoxy-D-galactose, 2-deoxy-D-glucose, 3-deoxy-D-glucose, allose, arabinose, arabinitol, fucitol, fucose, galactitol, glucitol, iditol, lyxose, mannitol, levo-rhamnitol, 2-deoxy-D-ribose, ribose, ribitol, ribulose, rhamnose, xylose, xylulose, allose, altrose, fructose, galactose, glucose, gulose, idose, levulose, mannose, psicose, sorbose, tagatose, talose, galactal, glucal, fucal, rhamnal, arabinal, xylal, valienamine, validamine, valiolamine, valiol, valiolon, valienol, valienone, glucuronic acid, galacturonic acid, N-acetylneuraminic acid, gluconic acid D-lactone, galactonic acid γ-lactone, galactonic acid δ-lactone, mannonic acid γ-lactone, D-*altro*-heptulose, D-*manno*-heptulose, D-*glycero*-D-*manno*-heptose, D-*glycero*-D-*gluco*-heptose, D-*allo*-heptulose, D-*altro*-3-heptulose, D-*glycero*-D-*manno*-heptitol, D-*glycero*-D-*altro*-heptitol and the like), The hydroxyl groups on monosaccharides may optionally be replaced with hydrogen, alkoxy (e.g. 2-O-methyl-D-fructose), alkanoate or halogen groups. Included are sulfate and/or phosphate derivatives of monosaccharides as defined herein.

[0087] Suitable oligosaccharides include, but are not limited to, carbohydrates having from 2 to 10 or more monosaccharides linked together. The constituent monosaccharide unit may be, for example, a pentose monosaccharide, a hexose monosaccharide, or a pseudosugar (including a pseudoaminosugar). Oligosaccharides do not include bicyclic groups that are formed by fusing a monosaccharide to a benzene ring, a cyclohexane ring, or a heterocyclic ring.

[0088] Pseudosugars that may be used in the invention are members of the class of compounds wherein the ring oxygen atom of the cyclic monosaccharide is replaced by a methylene group. Pseudosugars are also known as "carba-sugars."

The Linker

[0089] As discussed above, the carbohydrate moiety is covalently attached to the rapamycin via a linker, indicated as "X" in formula I. In it's simplest form, the linker is a chemical functional group that is formed when the sugar derivative is covalently attached to rapamycin, but is itself

part of neither rapamycin nor the sugar molecule. In one embodiment, the nature of the linker is determined by the chemistry employed to covalently attach the sugar or sugar derivative to rapamycin. For example, if 42-O-(4-nitrophenyloxycarbonyl) rapamycin (an activated rapamycin) is reacted with a sugar the result is a rapamycin carbohydrate derivative wherein the 42-hydroxyl oxygen of rapamycin is covalently bonded to a carbonyl group which in turn is covalently bonded to a hydroxyl oxygen on the sugar. In this example, the linker is the carbonyl group (C=O). An example of a linker as defined herein is depicted in Figure 3. Examples of linkers include moieties such as carbonyl (C=O) and sulfonyl (O=S=O). Such a carbonyl or sulfonyl linker is recognized as a single functional group linker.

[0090] The linker together with the functionality through which the sugar and rapamycin are attached to that linker form a "linkage." For example, when a carbonyl linker is attached to a sugar via one of its hydroxyl oxygen atoms and then to a rapamycin hydroxyl oxygen, the resulting "linkage" is a carbonate (i.e. –OC(O)O-). An example of a linkage as defined herein in depicted in Figure 4. Examples of linkages include esters, ethers, carbonates, carbamates, sulfates and urethanes.

[0091] The linker and associated linkage are selected to provide a biocompatible, substantially non-immunogenic rapamycin carbohydrate derivative. While the present invention is based on the recognition that the presence of the sugar(s) will improve the effectiveness of rapamycin, its pharmacokinetic and/or pharmacodynamic properties may also be enhanced by the geometry, composition, size, flexibility or rigidity, the relative hydrophobicity or hydrophilicity, and similar properties of the linker and/or linkage. Accordingly, the linker or linkage can be chosen to optimize the pharmacokinetic and/or pharmacodynamic properties of the rapamycin carbohydrate derivative. For example, the rates of acid-catalyzed or enzymatic hydrolysis of the derivatives resulting in the release of free rapamycin will vary depending on which linkage is created. The linker or linkage may be biologically "neutral," i.e., not itself contribute any additional biological activity to the carbohydrate rapamycin derivative, or it may be chosen to further enhance the biological activity of the compound.

[0092] The reaction chemistries resulting in linkers and linkages employ conventional techniques. These techniques generally involve the use of complimentary reactive functional groups located on rapamycin or activated rapamycin and the sugar or sugar derivative. Examples of complimentary functional groups and the resulting linkages are found in Table 1.

TABLE 1. LINKAGES RESULTING FROM COMPLIMENTARY FUNCTIONALITIES

FIRST REACTIVE GROUP	SECOND REACTIVE GROUP	LINKAGE
hydroxyl	isocyanate	urethane
amine	epoxide	ß-aminohydroxy
sulfonyl halide	amine	sulfonamide
carboxyl acid	amine	amide
hydroxyl	alkyl/aryl halide	ether
aldehyde	amine/$NaCNBH_3$	amine
ketone	amine/$NaCNBH_3$	amine
amine	isocyanate	carbamate
carboxyl acid	hydroxyl	ester
chloroformate	hydroxyl	carbonate

[0093] If desired, the linker may contain more than a single functional group. Complex linkers may be used to provide different chemical properties into the rapamycin carbohydrate derivative. For example, different hydrophobic/hydrophilic characteristics may be imparted to the rapamycin carbohydrate derivative by manipulation of the linker. Similarly, charged moieties may also be introduced. Techniques for modification of the linker will be readily understood by those of skill in the art. For example, the hydrophobic nature of a linker derived from hexamethylene diamine or a related polyamine can be modifed to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group.

[0094] For example, glycosyl-Y[-C(=Y)-X-]p-W(R)n-X-C(=Y)-drug where W is an aromatic or heteroaromatic or aliphatic group with conjugated double bonds or an amino-acid derivative radical which cyclizes after elimination of the glycosyl radical was disclosed in U.S. Pat. 6,146,658. These complex linkers are designed to self-eliminate via cyclization subsequent to enzymatic removal of the glycosyl moiety.

[0095] Wide varieties of linkers are suitable for use in the invention. Ordinarily skilled artisans will recognize that a carbonyl (C=O) or a sulfonyl (O=S=O) linker, or a carbonyl or sulfonyl linker combined with a simple alkyl chain or a polyether chain (e.g. a small number of repeating ethylene oxide units) will have properties that are different from those of the more complex linkers described above. For example, it is contemplated that the linkers of the present invention will not undergo cyclization to self-eliminate. Further, the susceptibility of the linker/drug compound to enzymatic or hydrolytic cleavage will be highly dependent on the nature of the spacer or linker moiety. In addition, it is contemplated that compounds with complicated linkers may be more difficult and expensive to produce than compounds with only single functional group linkers, such as those of the present invention.

[0096] Wide varieties of linkers are commercially available (e.g., Chem Sources USA and Chem Sources International; the ACD electronic database; and Chemical Abstracts). Many of the linkers that are suitable for use in this invention fall into this category. Others can be readily synthesized by methods known in the art, and as described below. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples of commercially available linkers are peptides or polyamides, hydrocarbons, aromatics, heterocyclics, ethers, lipids, cationic or anionic groups, or a combination thereof.

[0097] It is contemplated that the properties of the linker of this invention can be modified by the addition or insertion of ancillary groups, for example, to change the solubility of the multibinding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more polyethylene glycol (PEG) groups onto the linker enhances the hydrophilicity and water

solubility of the rapamycin carbohydrate derivative, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further, PEG may decrease antigenicity and potentially enhances the overall rigidity of the linker.

[0098] Ancillary groups that enhance the water solubility/hydrophilicity of the linker, and accordingly, the resulting compounds, are useful in practicing this invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, small repeating units of ethylene glycols, alcohols, polyols, (e.g., glycerin, glycerol propoxylate, etc.) carboxylates (e.g., small repeating units of glutamic acid, acrylic acid, etc.), amines (e.g., tetraethylenepentamine), and the like to enhance the water solubility and/or hydrophilicity of the carbohydrate rapamycin derivatives of this invention. For example, the ancillary group used to improve water solubility/hydrophilicity may be a polyether containing a small number of repeating ethylene oxide (-CH_2CH_2O-) units.

[0099] The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the rapamycin carbohydrate derivatives is also within the scope of this invention. Lipophilic groups useful with the linkers of this invention include, but are not limited to, lower alkyl, aromatic groups, and polycyclic aromatic groups. The aromatic groups may be either unsubstituted or substituted with other groups, but are at least substituted with a group that allows their covalent attachment to the linker. As used herein the term "aromatic groups" incorporates both aromatic hydrocarbons and heterocyclic aromatics. Other lipophilic groups useful with the linker of this invention include fatty acid derivatives that may or may not form micelles in aqueous medium and other specific lipophilic groups that modulate interactions between the carbohydrate rapamycin derivative and biological membranes.

[00100] The flexibility of the linker can be manipulated by the inclusion of ancillary groups that are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker, or bonds between the linker and the ancillary group(s), or bonds between the linker and the functional groups. Rigid groups can include, for example, those

groups whose conformational freedom is restrained by the presence of rings and/or bonds, for example, aryl, heteroaryl and heterocyclic groups. Other groups that can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

[00101] Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the linker into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other, which is inversely related to the square of the distance between the groups, will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further, ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker in a conformation that allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, protected groups that bear a latent charge which is unmasked, following addition to the linker, by deprotection, a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art, is within the scope of this invention.

[00102] Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups, and structures incorporating one or more carbon-carbon bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers that are branched- or straight-chain species. Branched-species are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

[00103] In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art.

[00104] Linkers can be attached to rapamycin or a sugar by employing reactive functional groups. The reactive functional groups are selected relative to the functional groups available on rapamycin or the sugar for coupling, or which are introduced onto rapamycin or the sugar for this purpose. For example, reaction between a carboxylic acid of the linker and a primary or secondary amine of the sugar in the presence of suitable activating agents results in formation of an amide moiety covalently linking the sugar to the linker. Reaction between an amine group of the linker and a sulfonyl halide of the sugar results in formation of a sulfonamide moiety covalently linking the sugar to the linker. Reaction between an alkyl or aryl halide of the linker and an alcohol of the sugar results in formation of an ether moiety covalently linking the sugar to the linker.

[00105] Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as Advanced Organic Chemistry, Jerry March, John Wiley &Sons (5th Ed., 2000). The term linker embraces everything that is not considered to be part of the sugar or rapamycin. Linkers can be derived from linear compounds having reactive functional groups at the end of the linker.

[00106] Suitable divalent linkers include, by way of example, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates, diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the sugar and rapamycin to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the above-discussed Table 1.

[00107] In embodiments of the invention, the linker (X) is selected from the group consisting of: (i) $-R^3C(O)$; (ii) $-C(O)R^3$, (iii) $-R^3S(O)_2$; or (iv) $-S(O)_2R^3$ where R^3 is selected from the group consisting of: (i) $-(CH_2)_p-$ where p is an integer from 1 to 18, (ii) $-(CH_2)_n-O-(CH_2)_m-$ where n and m are each independently an integer from 2 to 6, or (iii) a bond. It is to be understood that the linker (X) may be the same or different at each occurrence, i.e., at

the 31 and 42 positions. In additional embodiments of the invention, the linker (X) is carbonyl (C=O), sulfonyl (O=S=O), or a single functional group. Carbonyl linkers may be in the 31 position, the 42 position, or both. In other embodiments, R^1 is –C(O)-Z and R^2 is H or R^2 is –C(O)-Z and R^1 is H.

[00108] Accordingly, rapamycin carbohydrate derivatives having the structure of formula I that are within the scope of this invention include, for example, those set forth below (including pharmaceutically acceptable salts thereof):

42-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-[2-(Methyl-D-glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-D-fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-L-fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-D-fructosylcarbonyl)rapamycin;

31-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-L-fructosylcarbonyl)rapamycin;

42-O-(D-Allosylcarbonyl)rapamycin;

42-O-[2-(D-Allosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Allosylcarbonyl)rapamycin;

42-O-[2-(L-Allosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-allosylcarbonyl)rapamycin;

31-O-(L-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-allosylcarbonyl)rapamycin;

42-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-[2-(D-Fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-[2-(L-Fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fructoslylcarbonyl)rapamycin;

31-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fructoslylcarbonyl)rapamycin;

42-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-[2-(D-Fucitolylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-[2-(L-Fucitolylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fucitolylcarbonyl)rapamycin;

31-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fucitolylcarbonyl)rapamycin;

42-O-(D-Glucalylcarbonyl)rapamycin;

42-O-[2-(D-Glucalylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Glucosylcarbonyl)rapamycin;

42-O-[2-(D-Glucosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Glucosylcarbonyl)rapamycin;

42-O-[2-(L-Glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Glucalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucalylcarbonyl)rapamycin;

31-O-(D-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucosylcarbonyl)rapamycin;

31-O-(L-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-glucosylcarbonyl)rapamycin;

42-O-(D-Lactalylcarbonyl)rapamycin;

42-O-[2-(D-Lactalylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Lactalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-lactalylcarbonyl)rapamycin;

42-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-[2-(D-Sucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sucrosylcarbonyl)rapamycin;

42-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-[2-(D-Gentobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(D-gentobiosylcarbonyl)rapamycin

42-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-[2-(D-Cellobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellobiosylcarbonyl)rapamycin;

42-O-(D-Turanosylcarbonyl)rapamycin;

42-O-[2-(D-Turanosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Turanosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-turanosylcarbonyl)rapamycin;

42-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-[2-(D-Palatinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-palatinosylcarbonyl)rapamycin;

42-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltosylcarbonyl)rapamycin;

42-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-[2-(D-Maltulosylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Maltosylcarbonyl)rapamycin;

42-O-[2-(D-Maltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltulosylcarbonyl)rapamycin;

31-O-(D-Maltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltosylcarbonyl)rapamycin;\

42-O-(L-Sorbosylcarbonyl)rapamycin;

42-O-(D-Sorbosylcarbonyl)rapamycin;

31-O-(L-Sorbosylcarbonyl)rapamycin;

31-O-(D-Sorbosylcarbonyl)rapamycin;

42-O-[2-(L-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-[2-(D-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sorbosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-sorbosylcarbonyl)rapamycin;

42-O-(D-Lactosylcarbonyl)rapamycin;

42-O-[2-(D-Lactosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-lactosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-lactosylcarbonyl)rapamycin;

42-O-(D-Melibiosylcarbonyl)rapamycin;

31-O-(D-Melibiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-melibiosylcarbonyl)rapamycin;

42-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-[2-(D-Leucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-leucrosylcarbonyl)rapamycin;

42-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-[2-(D-Rafinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-rafinosylcarbonyl)rapamycin;

42-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltotriosylcarbonyl)rapamycin;

42-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-[2-(D-Cellotetraosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellotetraosylcarbonyl)rapamycin;

42-O-(Valiolylcarbonyl)rapamycin

42-O-[2-(D-Valiolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolylcarbonyl)rapamycin

42-O-(Valiolonylcarbonyl)rapamycin

42-O-[2-(D-Valiolonylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolonylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolonylcarbonyl)rapamycin

42-O-(Valienolylcarbonyl)rapamycin

42-O-[2-(D-Valienolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valienolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valienolylcarbonyl)rapamycin

42-O-(Valienoneylcarbonyl)rapamycin

42-O-[2-(D-Valienoneylcarbonyloxy)ethyl]rapamycin;

31-O-(Valienoneylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)- 31-O-(valienoneylcarbonyl)rapamycin

[00109] In addition, the invention is directed to a rapamycin derivative having the structure of formula I wherein n=1, R^1 is H and R^2 is X-Z, wherein X is a linker, and Z is a carbohydrate moiety independently selected from the group consisting of a monosaccharide, an oligosaccharide and a pseudosugar.

[00110] The invention is also directed to a pharmaceutical composition comprising the aforementioned rapamycin carbohydrate derivative, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

Preparation of the Rapamycin Carbohydrate Derivatives

[00111] A general procedure for synthesizing rapamycin carbohydrate derivatives of the present invention involves the coupling of a monosaccharide, oligosaccharide, pseudosugar or sugar derivative to the 31- and/or 42- positions of activated rapamycin.

[00112] For example, as depicted in Figure 2, rapamycin (II) can be reacted with p-nitrophenyl chloroformate to yield an activated rapamycin (III). Under carefully controlled conditions, the reaction will preferentially take place at the 42-hydroxyl position. By altering the reaction conditions, both the 31- and 42-hydroxyl groups can be similarly activated. Selective activation of the 31-hydroxyl group can be accomplished by first protecting the 42-hydroxyl moiety with, for example an alkylsilyl group such as triethylsilyl, triisopropylsilyl or *tert*-butyldimethylsilyl, and then reacting with p-nitrophenyl chloroformate or other chloroformates. Removal of the protecting group then affords rapamycin activated at the 31-hydroxyl position. Thus, it is possible to prepare a rapamycin derivative that is selectively activated at the 31-position, the 42-position, or both.

[00113] Thereafter, in the second step, a sugar moiety or sugar derivative is reacted with an activated rapamycin (III) to give a rapamycin carbohydrate derivative. When the reactive functional group on the sugar moiety or sugar derivative is a hydroxyl group, the reaction with activated rapamycin may take place on a primary hydroxyl group to form a carbonate linkage to rapamycin. The resulting linker is a carbonyl group. When the reactive functional group on the sugar or sugar derivative is an amino group (which can be situated at any position of the sugar), a carbamate linkage to rapamycin results and the resulting linker is a carbonyl group. Amino substituted sugar derivatives, e.g, sugar-X-NH_2 or sugar-NH_2, can be prepared from precursors by conventional methods. For example, reduction of sugar-X-N_3 or de-phthaloylation of sugar-X-

NPhth, where Phth is phthalyl, yields the amino substituted sugar derivative. These precursors can be synthesized by glycosidation of linker moieties with appropriately activated glycosyl donors.

[00114] Thus, using the general approach described herein is it possible to prepare a wide range of rapamycin carbohydrate derivatives in which the 31- and/or 42-hydroxyl groups are modified with a wide range of sugars or sugar derivatives.

[00115] It is contemplated that hydroxyl groups of rapamycin or a rapamycin metabolite, including those that are located in positions other than 31- and 42-, can also be glycosylated as described herein, and the resultant rapamycin carbohydrate derivatives will also exhibit higher water-solubility and/or enhanced pharmacokinetic and/or pharmacodynamic properties compared to their unglycosylated counterparts.

[00116] Rapamycin metabolites are known in the art. For example, Streit *et. al.* structurally identified several rapamycin metabolites from human liver microsomes. See, F. Streit et al., Drug Metabol. Disp., 24, 1272 (1996). These include 41-demethyl rapamycin, 7-demethyl rapamycin, 11-hydroxy rapamycin, and a 24-hydroxy ester hydrolysis degradation product of rapamycin. It has also been shown that the metabolites of rapamycin can undergo this ester hydrolysis. Streit also partially identified di, tri, and tetra hydroxylated rapamycin metabolites. Wang *et. al.* found 16 hydroxylated and/or demethylated metabolites in the bile of rapamycin treated rats. See, C.K. Wang et al., Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, 545 (1993). Nickmilder *et. al.* identified a 3,4 and 5,6 dihydrodiol rapamycin metabolite in rat liver microsomes. See, M.J.M. Nickmilder et al., Xenobiotica, 27, 869 (1997). In trough whole blood, Streit *et. al.* have identified 41-demethyl, hydroxy, dihydroxy, and didemethyl rapamycin metabolites. See, F. Streit et al., Clin. Chem., 42, 1417 (1996). These metabolites accounted for 56% of total rapamycin derivatives measured. Finally, Leung *et. al.* looked at the disposition of [^{14}C]-rapamycin in healthy male volunteers. They found that rapamycin represented approximately 35% of the total radioactivity in blood and that 41-demethyl, 7-demethyl, and several hydroxy, hydroxydemethyl, and didemethyl rapamycin

metabolites individually represented between 1 and 12% of the total radioactivity. Rapamycin metabolites can be isolated from a number of various sources, including but not limited to blood, urine or feces samples, from liver microsomes or from microorganism cultures.

[00117] Accordingly, in addition to 31- and 42-, the hydroxyl groups of particular interest include those at the 27-, 41-, 3-, 4-, 5-, 6-, 7-, 11- and 24-positions of rapamycin and rapamycin metabolites.

Pharmaceutical Compositions and Utility

[00118] The compounds of this invention may be administered neat or with a pharmaceutical carrier to an animal, such as a warm blooded mammal, and especially humans, in need thereof. The pharmaceutical compositions may also contain other drugs, particularly drugs known to have different mechanisms of action. Thus, the pharmaceutical compositions may contain, in addition to the compounds of the present invention, at least one drug of another class, for example,a calcineurin inhibitor, a steroid or other immunomodulatory compounds which may interfere with DNA synthesis or intra- or inter- cellular signaling or other cell processes. Examples of calcineurin inhibitors include Cyclosporin A (available from Novartis as Sandimmune® and Neoral® and FK506 (also known as tacrolimus or Prograf® available from Fujisawa). Examples of cyclosporine derivatives are those disclosed in W099/18120. Examples of steroids include predisone, prednisolone or methylprednisolone. Examples of these immunomodulatory compounds include azothioprine, mycophenolic acid (mycophenolate mofitil or Cellcept® available from Roche), leflunomide available from Aventis, Brequinar, Mizoribine, antibodies including α-LFA-1, and α-ICAM-1, thimoglobuline, IL2R antagonists including basiliximab (Stimulect®), and daclizumab (Zenapax®), alemtuzumab (Campath 1H®, a humanized monoclonal antibody recognizing CD52), Orthoclone OKT3® or muromonab CD3, Atgam(R) lymphocyte immune globulin, ATG (antithymocyte globulin), and other compounds. The individual drugs, and the rapamycin carbohydrate derivative, may be formulated separately

as distinct components of the pharmaceutical composition, and administered together or separately.

[00119] The pharmaceutically effective carrier may be solid or liquid. A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid that is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets may contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

[00120] Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs, and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, surfactants, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, possibly sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

[00121] Liquid pharmaceutical compositions that are sterile solutions or suspensions are suitable for intramuscular, intraperitoneal, and subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form. Pulmonary administration is also contemplated.

[00122] The pharmaceutical composition can be in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form. The dosage to be used in the treatment must be subjectively determined by the attending physician.

[00123] In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles administered to an affected area.

[00124] The compounds of this invention can also be used in conjunction with a medical device. For example, a rapamycin carbohydrate derivative as a component of a drug-coated or impregnated intravascular stent may be used to inhibit neointimal tissue proliferation and thereby prevent restenosis (See, for example U.S. Pat. No. 5,665,728). Rapamycin carbohydrate derivatives can also be used as a component of other drug-coated or impregnated medical devices such as catheters, pumps, or drug-delivery medical devices such as beads or discs containing drugs. The presence of rapamycin, a potent immunosuppressant, may decrease inflammation, rejection, or other immune responses to the presence of these implantable medical devices in the body.

[00125] The following examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of the present invention.

EXAMPLES

[00126] In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

g	=	gram
mg	=	milligram
kg	=	kilogram
mmol	=	millimole
M	=	molar
N	=	Normal
mL	=	milliliter
min	=	minute
BzCl	=	benzoyl chloride
DMAP	=	4-dimethylaminopyridine
DMS	=	dimethyl sulfate
Py	=	Pyridine
DMF	=	N,N-dimethylformamide
Me	=	methyl
HOAt	=	1-hydroxy-7-azabenzotriazole
HOBT	=	1-hydroxybenzotriazole hydrate
TBDMSiCl	=	tert-butyldimethylsilyl chloride
AcOH	=	acetic acid
THF	=	tetrahydrofuran
LC/MS or LCMS	=	liquid chromatography/mass spectroscopy
HPLC	=	high performance liquid chromatography
conc.	=	concentrated
eq.	=	equivalents

[00127] In the following examples and procedures, the starting materials are available commercially from Aldrich Chemical Company, Inc., Milwaukee, WI 53233 USA; Lancaster Synthesis, Inc., NH 03087 USA; Sigma, St. Louis MO 63178 USA; Maybridge Chemical Co. Trevillett, Tintagel, Cornwall PL34 OHW United Kingdom; TCI America, Portland OR 97203; Frontier Scientific, Utah, USA; and Bachem, Torrance, California, USA.

EXAMPLE 1

Synthesis of 42-O-(D-Fructosylcarbonyl)rapamycin

[00128] Figure 3 depicts the method of synthesis for 42-O-(D-Fructosylcarbonyl)rapamycin. The detailed procedure is described below:

42-O-(4-nitrophenyloxycarbonyl)rapamycin

[00129] A solution of 10.0 g of rapamycin in 50mL of dichloromethane and 10 mL of dry pyridine was cooled to -78°C under a nitrogen atmosphere. To this solution 3.31g of 4-nitrophenyl chloroformate was added and the reaction mixture was stirred for 1 hour at -78°C, and then brought directly to room temperature. Reaction was complete after 2 hours. The mixture was diluted with water and extracted with dichloromethane. The organic phase was washed with water, dried over Na_2SO_4 and evaporated. Chromatography on a silica gel column (solvent: hexanes – ethyl acetate, 2:1) gave 9.66g of 42-O-(4-nitrophenyloxycarbonyl)rapamycin as a yellowish solid (freeze dried from benzene). $C_{58}H_{82}N_2O_{17}$, M=1078.6; MS(ES+): m/z=1101.7 $(M+Na)^+$

42-O-(D-Fructosylcarbonyl)rapamycin

[00130] To a solution of D-fructose (2.025g, 11.24mmol) and DMAP (250mg) in *N,N*-dimethylformamide (25mL) 42-O-(4-nitrophenyloxycarbonyl)rapamycin (4.05g, 3.757mmol) was added and the reaction mixture was stirred at room temperature for 24 hours. The solvent was evaporated under high vacuum and the residue was flash chromatographed on a silica gel column using dichloromethane – methanol (9:1) as eluent. Obtained product was re-purified on a preparative HPLC column (80% methanol – 20% water, flow 10mL/min.), yielding 1.461g of 42-O-(D-fructosylcarbonyl)rapamycin as white solid (freeze dried from benzene). $C_{58}H_{89}NO_{20}$, M=1119.6; MS(ES+): m/z=1142.7 $(M+Na)^+$ In the alternative HOBT (or HOAT) may be employed in place of DMAP as disclosed in example 3.

EXAMPLE 2

[00131] Following procedures analogous to that outlined in Example 1, and employing the appropriate sugars or sugar derivatives, the following compounds have been obtained:

42-O-(D-Glucosylcarbonyl)rapamycin
42-O-(Methyl-D-glucosylcarbonyl)rapamycin
42-O-(D-Allosylcarbonyl)rapamycin
42-O-(D-Fructosylcarbonyl)rapamycin
42-O-(L-Fructosylcarbonyl)rapamycin
42-O-(D-Fucitolylcarbonyl)rapamycin
42-O-(L-Fucitolylcarbonyl)rapamycin
42-O-(D-Glucalylcarbonyl)rapamycin
42-O-(L-Sorbosylcarbonyl)rapamycin
42-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin
42-O-(D-Lactalylcarbonyl)rapamycin
42-O-(D-Sucrosylcarbonyl)rapamycin
42-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-(D-Cellobiosylcarbonyl)rapamycin

42-O-(D-Turanosylcarbonyl)rapamycin

42-O-(D-Palatinosylcarbonyl)rapamycin

42-O-(D-Isomaltosylcarbonyl)rapamycin

42-O-(D-Maltulosylcarbonyl)rapamycin

42-O-(D-Maltosylcarbonyl)rapamycin

42-O-(D-Lactosylcarbonyl)rapamycin

42-O-(Methyl-D-lactosylcarbonyl)rapamycin

42-O-(D-Melibiosylcarbonyl)rapamycin

42-O-(D-Leucrosylcarbonyl)rapamycin

42-O-(D-Rafinosylcarbonyl)rapamycin

42-O-(D-Isomaltotriosylcarbonyl)rapamycin

42-O-(D-Cellotetraosylcarbonyl)rapamycin

EXAMPLE 3

Synthesis of 31-O-(D-Fructosylcarbonyl)rapamycin

[00132] Figure 4 depicts the method of synthesis for 31-O-(D-Fructosylcarbonyl)rapamycin. The detailed procedure is described below:

42-O-(tert-Butyldimethylsilyl)rapamycin

[00133] To a solution of rapamycin (10g) and imidazole (2.2g) in *N,N*-dimethylformamide (40mL) *tert*-butyldimethylsilyl chloride (1.76g) was added, and the reaction mixture was stirred at room temperature under nitrogen for 5 days. The solvent was evaporated under high vacuum and the residue was chromatographed on a silica gel column (solvent: hexanes – ethyl acetate,

3:2) yielding 5.84g of 42-O-(tert-butyldimethylsilyl)rapamycin as off-white foam. $C_{57}H_{93}NO_{13}Si$, M=1027.6; MS(ES+): m/z=1050.7 $(M+Na)^+$

42-O-(tert-Butyldimethylsilyl)-31-O-(4-nitrophenyloxycarbonyl)rapamycin

[00134] 42-O-(*tert*-Butyldimethylsilyl)rapamycin (5.84g) was dissolved in dichloromethane (30mL) and pyridine (6mL), 4-nitrophenyl chloroformate (2.582g) was added and the reaction mixture was stirred under nitrogen at room temperature for 2 hours. Solvents were evaporated and the residue purified on a silica gel column. Elution with hexanes – ethyl acetate (3:1) gave title compound as yellowish foam (5.4g). $C_{64}H_{96}N_2O_{17}Si$, M=1192.6; MS(ES+): m/z=1215.6 $(M+Na)^+$

31-O-(4-nitrophenyloxycarbonyl)rapamycin

[00135] 42-O-(*tert*-Butyldimethylsilyl)-31-O-(4-nitrophenyloxycarbonyl) rapamycin (5.4g) was dissolved in a mixture of acetic acid (30mL), tetrahydrofuran (10mL) and water (10mL). It was stirred at room temperature for 20 hours. The reaction mixture was diluted with water and extracted with ethyl acetate (3 x 200mL).The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated. Silica gel column chromatography (solvent: hexanes – ethyl acetate, 3:2) gave 2.1g of 31-O-(4-nitrophenyloxycarbonyl) rapamycin as yellowish solid (freeze dried from benzene). $C_{58}H_{82}N_2O_{17}$, M=1078.6; MS(ES+): m/z=1101.6 $(M+Na)^+$

31-O-(D-Fructosylcarbonyl)rapamycin

[00136] A mixture of 31-O-(4-nitrophenyloxycarbonyl)rapamycin (1.3g), D-fructose (0.434g), and 1-hydroxybenzotriazole (HOBT) (0.325g) in dry pyridine (20mL) was stirred at room temperature for 3 days. Then, the solvent was evaporated under reduced pressure and the

residue was chromatographed on a silica gel column. Elution with dichloromethane – methanol (10:1) provided 31-O-(D-fructosylcarbonyl)rapamycin (0.625g, 46%) as white solid (freeze dried from benzene). $C_{58}H_{89}NO_{20}$, M=1119.6; MS(ES+): m/z=1142.7 $(M+Na)^+$

[00137] In an analogous manner, 31-O-(D-allosylcarbonyl)rapamycin was also prepared. In addition, in the alternative, DMAP may be employed in place of HOBT as disclosed in example 1.

Example 4

Synthesis of 42-O-(2-O-methyl-β-D-fructosylcarbonyl)rapamycin

1,3,4,5-Tetra-O-benzoyl-β-D-fructopyranose

[00138] A mixture of anhydrous pyridine (52 mL), benzoyl chloride (51.5 mL, 0.444 mmol) and
anhydrous dichloromethane (125 mL) was cooled to -10°C. Finely powdered fructose (20 g, 0.111 mmol) was added portion wise and the reaction mixture was stirred at -10°C for 18 hours. The reaction mixture was quenched with ice water, diluted with dichloromethane and transferred to separatory funnel. Organic layer was separated and washed with 5 % citric acid, saturated sodium bicarbonate, water, and dried over sodium sulfate. It was filtered and the solvent evaporated. The residue was dissolved in diethyl ether (75 ml) then added slowly to hexanes (300 ml) to give a white solid which upon drying afforded 58.9 g (89 %) of 1,3,4,5-tetra-O-benzoyl-β-D-fructopyranose

1,3,4,5-Tetra-O-benzoyl-2-O-methyl-β-D-fructopyranose

[00139] To a solution of 1,3,4,5-tetra-O-benzoyl-β-D-fructopyranose (10.00g, 16.8mmol) in acetone (40 mL) dimethyl sulfate (2.4ml, 25.16 mmoles, 1.5 eq.) was added followed by potassium carbonate (3.48g, 25.16 mmoles) and the reaction mixture was stirred at 50°C under nitrogen for 18 hours. Solvents were removed under vacuo and the resulting residue was dissolved in ethyl acetate (150ml), washed with 5% citric acid, water, brine, dried over sodium sulfate, filtered and concentrated to an oily solid. Purification on a silica gel column (hexane: ethyl acetate, 4:1) afforded 10.0g (98%) of title compound as white foam.

2-O-methyl-β-D-fructopyranose

[00140] A solution of sodium methoxide (0.5 M in methanol, 10.0 ml) was added to a vigorously stirred solution of 1,3,4,5-tetra-O-benzoyl-2-O-methyl-β-D-fructopyranose (10.0g, 16.4 mmol) in anhydrous methanol. After stirring for 1.5 hrs at room temperature the reaction was complete. The pH was adjusted to 7.0 with Amberlite IRC-50 (~4.0g). The solids were removed by filtration and the filtrate was concentrated in vacuo. Purification on a silica gel column (methanol: dichloromethane, 4:1 and 3:1) provided the product as white foam 2.70 g (81%).

42-O-(2-O-methyl-β-D-fructosylcarbonyl)rapamycin

[00141] A mixture of 42-O-(4-nitrophenyloxycarbonyl)rapamycin (10.0 g, 9.3 mmol), 2-O-methyl-β-D-fructopyranose (6.2g, 28 mmol, 3 eq.), and 1-hydroxy-7-azabenzotriazole (HOAt) (2.5 g, 2 eq.) in dry pyridine (60mL) was stirred at room temperature for 4 days. Then, the solvent was evaporated under reduced pressure and the residue was re-dissolved in ethyl acetate, washed with water. Organic layer was evaporated and the residue chromatographed on a silica gel column. Elution with dichloromethane – methanol (100:5) gave 42-O-(2-O-methyl-β-D-fructosylcarbonyl)rapamycin

(4.7g,) as white solid (freeze dried from benzene). $C_{59}H_{91}NO_{20}$, M=1133.7; MS (ES+): m/z=1156.7 $(M+Na)^+$

EXAMPLE 5

Stability of Rapamycin Carbohydrate Derivatives Toward Hydrolysis in Acidic Media

[00142] The stability toward hydrolysis of various rapamycin carbohydrate derivatives in acidic media was investigated by dissolving the compounds in 70/30 methanol/0.1N HCl (pH 1.5) and then measuring by HPLC the amount of free rapamycin in the samples at 1 hour to determine the extent of hydrolysis. The results are summarized in Table 2.

TABLE 2. ACID CATALYZED HYDROLYSIS OF RAPAMYCIN CARBOHYDRATE DERIVATIVES

Compound	Extent of Hydrolysis (%)
42-O-(D-Fructosylcarbonyl)rapamycin	<1
42-O-(D-Glucosylcarbonyl)rapamycin	<1
42-O-(D-Maltulosylcarbonyl)rapamycin	<5
42-O-(L-Fucitolylcarbonyl)rapamycin	<1
42-O-(D-Lactalylcarbonyl)rapamycin	<1
31-O-(D-Fructosylcarbonyl)rapamycin	<1
42-O-(D-Allosylcarbonyl)rapamycin	<1

[00143] As can be seen in Table 2, all compounds exhibited good stability in acidic media with little or no observable hydrolysis.

EXAMPLE 6

Hydrolysis of Rapamycin Carbohydrate Derivatives in Human Whole Blood

[00144] The ability of various rapamycin carbohydrate derivatives to release free rapamycin via hydrolysis in whole blood was investigated by spiking the compounds in human whole blood and then measuring by HPLC the amount of free rapamycin in the samples at 1 hour to determine the extent of hydrolysis. The results are summarized in Table 3.

TABLE 3. HYDROLYSIS OF RAPAMYCIN CARBOHYDRATE DERIVATIVES IN WHOLE BLOOD

Compound	Extent of Hydrolysis (%)
42-O-(D-Fructosylcarbonyl)rapamycin	28±2
42-O-(D-Glucosylcarbonyl)rapamycin	5±1
42-O-(D-Maltulosylcarbonyl)rapamycin	13±4
42-O-(L-Fucitolylcarbonyl)rapamycin	31±4
42-O-(D-Lactalylcarbonyl)rapamycin	2±1
31-O-(D-Fructosylcarbonyl)rapamycin	23±0.5
42-O-(D-Allosylcarbonyl)rapamycin	39±4
31-O-(D-Allosylcarbonyl)rapamycin	34±2

[00145] As shown in Table 3, the extent of hydrolysis in human whole blood varied greatly depending on the nature of the carbohydrate moiety. The incorporation via carbonate linkages of sugars such as D-fructose, L-fucitol or D-allose generally led to a higher degree of hydrolysis than was observed when D-glucose, D-maltulose, and D-lactal were employed. The results also show that when a suitable sugar is chosen, both 31-O- and 42-O-rapamycin carbohydrate derivatives are effective at releasing free rapamycin in whole blood. Additionally, previous, similar experiments that investigated the hydrolysis in whole blood of rapamycin carbohydrate derivatives with carbamate linkages showed little or no hydrolysis in contrast to many of the compounds with carbonate linkages in Table 3.

Example 7

Comparison of the *In Vitro* Immunosuppressive Activity of Rapamycin Carbohydrate Derivatives with Carbonate and Carbamate Linkages

[00146] The immunosuppressive activity of rapamycin, 42-O-(D-Fructosylcarbonyl)rapamycin and a carbamate-linked analog of 42-O-(D-Fructosylcarbonyl)rapamycin (a non-hydrolyzable form of 42-O-(D-Fructosylcarbonyl)rapamycin) was assessed in primary blood lymphocyte cultures (PBMC) using alamar blue as detection of cell proliferation. The carbamate analog of 42-O-(D-Fructosylcarbonyl)rapamycin is formed from an amino sugar wherein the carbohydrate moiety is attached to the carbonyl linker through the amino nitrogen atom of the amino sugar, thereby forming a carbamate linkage. Figure 5 illustrates that 42-O-(D-Fructosylcarbonyl)rapamycin shows cell proliferation inhibition equivalent to rapamycin, while the non-hydrolyzable carbamate-linked analog does not possess any intrinsic immunosuppressive activity. This data indicates that the active species is rapamycin resulting from the hydrolysis of 42-O-(D-Fructosylcarbonyl)rapamycin over the course of the 3-day culturing, and not non-hydrolysed 42-O-(D-Fructosylcarbonyl)rapamycin. In other words, the prodrug does not appear to possess any intrinsic immunosuppressive activity and must be hydrolyzed to rapamycin in order to exhibit the desired pharmacological effect. This experiment also demonstrates the importance of the selection of the linkage between the carbohydrate moiety and rapamycin as, in this example, a carbonate linkage allows for the desired hydrolysis to occur whereas the carbamate linkage remains intact and little or no rapamycin is released.

EXAMPLE 8

Comparison of Pharmacokinetic Profiles of Rapamycin and Rapamycin Carbohydrate Derivatives in Rats

[00147] The pharmacokinetic profiles in rats of selected rapamycin carbohydrate derivatives were determined to investigate the ability of the derivatives to deliver free rapamycin

into the bloodstream *in vivo*. Briefly, Sprague Dawley rats were orally dosed with rapamycin and derivatives at 2.5 or 10 mg/kg. Whole blood was extracted via jugular bleed over 24 hours and frozen at -20 °C until analysis. Whole blood was analyzed by Liquid Chromatography Mass Spectrometry for the presence of rapamycin. The results are summarized in Figures 6,7 and 8.

[00148] As can be seen in Figures 6 and 7, upon oral administration of rapamycin a rapid rise in rapamycin concentration in the blood was observed with a maximum concentration reached at approximately 30 minutes. The level of rapamycin then dropped quite rapidly over the next several hours. A similar profile was observed for 42-O-(D-glucosylcarbonyl)rapamycin. Surprisingly, however, when 42-O-(D-fructosylcarbonyl)rapamycin or 42-O-(L-fucitolylcarbonyl)rapamycin were administered orally the level of rapamycin in the blood rose gradually to reach a maximum concentration at approximately 3 hours before gradually decreasing over time (Figure 6). A similar profile was observed for both 31-O-(D-fructosylcarbonyl)rapamycin and 31-O-(D-allosylcarbonyl)rapamycin (Figure 7) as well as 42-O-(D-allosylcarbonyl)rapamycin, 42-O-(D-sorbosylcarbonyl)rapamycin and 42-O-(2-O-methyl-D-fructosylcarbonyl)rapamycin (Figure 8),. The delayed kinetics observed for selected compounds may offer the advantage of allowing for less frequent dosing than that typical for rapamycin. Additionally, the gradual rise in rapamycin concentration associated with selected rapamycin carbohydrate derivatives may ameliorate toxic effects associated with the rapid rise in drug concentration when rapamycin itself is orally administered.

[00149] A second observation from Figures 6, 7 and 8 is that the variability in the concentration of rapamycin, as demonstrated by the standard deviations shown on the graphs, was considerably lower for the rapamycin carbohydrate derivatives than for rapamycin itself. Thus, the compounds of the present invention may also have the advantage of reduced inter-individual variability that could allow for more consistent, predictable dosing.

[00150] These experiments demonstrated that careful selection of the glycosyl substituent has a profound impact on the pharmacokinetic profiles of the rapamycin carbohydrate derivatives

and that the compounds of the present invention may possess considerable advantage, including pharmacokinetic advantages, over rapamycin itself.

EXAMPLE 9

Comparison of GI Tract Toxicity of Rapamycin and 42-O-(D-Fructosylcarbonyl)rapamycin in a Canine Model

[00151] Beagle dogs are considered to be a hypersensitive model of gastrointestinal tract toxicity associated with rapamycin. See, S.N. Sehgal et al., Medicinal Research Reviews 14, 1 (1994). Even short exposures of low dose rapamycin given orally to dogs is known to result in rapid weight loss due to ulceration occurring from the mouth to the colon secondary to necrotizing fibrinoid vasculitis. As summarized in Table 4, when 2 beagle dogs were given a single oral dose of rapamycin at 10 mg/kg both dogs became lethargic and lost over 30% of their body weight in less than 1 week resulting from reduced food intake. The animals did not recover. Another beagle dog given the same dose of 42-O-(D-fructosylcarbonyl)rapamycin showed no overt physical changes and maintained a normal food intake. All three dogs had similar blood levels of rapamycin as measured by LCMS. In a fourth dog a dose of 1 mg/kg 42-O-(D-fructosylcarbonyl)rapamycin resulted in slight lethargy but the dog quickly recovered. A subsequent dose of rapamycin (1mg/kg) one week later resulted in severe lethargy and weight loss, from which the animal did not recover.

TABLE 4. GASTROINTESTINAL TOLERABILITY IN BEAGLE DOGS

Dog #	Drug Treatment	Dose (mg/kg)	Observations
1	Rapamycin	10	Lethargic 30 % weight loss No recovery

Dog #	Drug Treatment	Dose (mg/kg)	Observations
2	Rapamycin	10	Lethargic 30 % weight loss No recovery
3	42-O-(D-Fructosylcarbonyl) rapamycin	10	Normal
4	42-O-(D-Fructosylcarbonyl) rapamycin	1	Lethargic Recovered
4	Rapamycin	1	30 % weight loss No recovery

[00152] This experiment clearly demonstrates that oral administration of 42-O-(D-fructosylcarbonyl)rapamycin resulted in little or no overt sign of gastrointestinal toxicity in a hypersensitive dog model as compared to rapamycin which led to signs of severe toxicity. This result further demonstrates the potential of the rapamycin carbohydrate derivatives of the present invention to improve the pharmacodynamic profile of rapamycin.

Example 10

Comparison of Serum Cholesterol Levels in Rats Treated with Rapamycin and 42-O-(D-Fructosylcarbonyl)rapamycin

[00153] 42-O-(D-fructosylcarbonyl)rapamycin and rapamaycin were tested head-to-head in Sprague-Dawley rats for changes in cholesterol level. Rats (n = 12) were dosed daily for 12 days with equivalent doses (2.5 mg/kg/day) of either rapamycin or 42-O-(D-fructosylcarbonyl)rapamycin. Cholesterol levels were measured at the 24-hour trough level on day 11. Results (Figure 9) indicate that rats treated with rapamycin displayed significantly increased levels of cholesterol relative to a vehicle control group, while cholesterol levels in the

42-O-(D-fructosylcarbonyl)rapamycin group were significantly lower ($p < 0.01$) than in the rapamycin group and not significantly different than the vehicle. It is important to note that both compounds showed equivalent efficacy in a heterotopic rat heart transplant model at the same 2.5 mg/kg/day dose used in this study (Example 12). This experiment demonstrates the ability of the rapamycin carbohydrate derivatives to improve the side effect profile of rapamycin while maintaining efficacy.

EXAMPLE 11

Comparison of Platelet Aggregation due to Rapamycin and 42-O-(D-Fructosylcarbonyl)rapamycin in a Washed Human Platelet Aggregation Assay

[00154] Platelet aggregation is suspected to be a side effect of rapamycin and has been implicated in increasing chronic rejection and other long term side effects of the use of rapamycin in transplantation. (Ann Babinska et. al., Enhancement of Human Platelet Aggregation and Secretion Induced by Rapamycin. (1998) Nephrology Dialysis Transplantation Vol. 13 pp. 3153-3159. These experiments were conducted using fresh washed human platelets stimulated with 2 μM ADP and spiked with either Rapamycin or 42-O-(D-Fructosylcarbonyl)rapamycin at time zero. The treated platelet aggregation was continuously read over a period of 8 to 10 minutes in a ChronoLog™ Platelet Aggregometer.

[00155] Figure 10 shows a plot of percent platelet aggregation versus time for two concentrations of rapamycin, 1 μg/ml and 25 μg/ml. Figure 10 illustrates that rapamycin induces platelet aggregation in a dose dependent manner. Rapamycin at the 1 μg/ml dose induced platelet aggregation by approximately 20% after 8 minutes. Rapamycin at the 25 μg/ml dose induced platelet aggregation by approximately 70% after 8 minutes. Figure 11 shows a plot of percent platelet aggregation versus time for washed human platelets, stimulated with 2 μM ADP dosed with 25 μg/ml rapamycin or 25 μg/ml 42-O-(D-Fructosylcarbonyl)rapamycin. Fig. 11 shows that while rapamycin induces nearly 80% platelet aggregation after 8 minutes, 42-O-(D-

Fructosylcarbonyl)rapamycin does not exhibit a measurable effect on platelet aggregation in the same time.

EXAMPLE 12

Graft Survival of Heterotopic Heart Allografts in Rats Receiving Oral 42-O-(D-Fructosylcarbonyl)rapamycin (2.5 and 10 mg/kg/day), Rapamycin (2.5 mg/kg/day), or Vehicle

[00156] Heterotopic transplants were administered to the abdominal aorta and inferior vena cava of a genetically unmatched (allogenic) heart from Wistar Furth rats to Lewis rats. The controls (vehicle and Rapamycin at 2.5 mg/kg/day) or 42-O-(D-Fructosylcarbonyl)rapamycin, at 2.5 and 10 mg/kg/day were administered once daily by oral gavage to the transplant recipients (6 rats per group) starting 3 days prior to transplantation and continuing for 30 days post-transplantation. If graft dysfunction was noted during the 30-day post-transplantation period, the animal was sacrificed. If the animal survived longer than 30 days post-transplantation, the test and control articles were discontinued and the animal was allowed to continue until graft dysfunction or up to 100 days post-transplantation. The average survival rates for each group of recipient animals are summarized in Table 5 and in Figure 12. As shown in Table 5, 42-O-(D-Fructosylcarbonyl)rapamycin prolonged survival of the graft at the 2.5 and 10 mg/kg/day dose level by 214 and 341% over the vehicle control. This was similar to the prolonged survival exhibited with rapamycin at the 2.5 mg/kg/day dose. Figure 12 illustrates that 42-O-(D-Fructosylcarbonyl)rapamycin prolonged survival of the graft over the vehicle as did rapamycin at the 2.5 mg/kg/day dose level. These data demonstrate the immunosuppressive activity of 42-O-(D-Fructosylcarbonyl)rapamycin in preventing graft rejection.

TABLE 5. AVERAGE SURVIVAL RATES AFTER HETEROTOPIC RAT HEART TRANSPLANTS (N=6)

Dose (mg/kg/day)	Average Survival Time (days post-transplant) Mean ± SEM		
	Vehicle Control	Rapamycin	42-O-(D-Fructosylcarbonyl)rapamycin
0	13 ± 2		
2.5		39 ± 4	41 ± 4*
10			57 ± 4

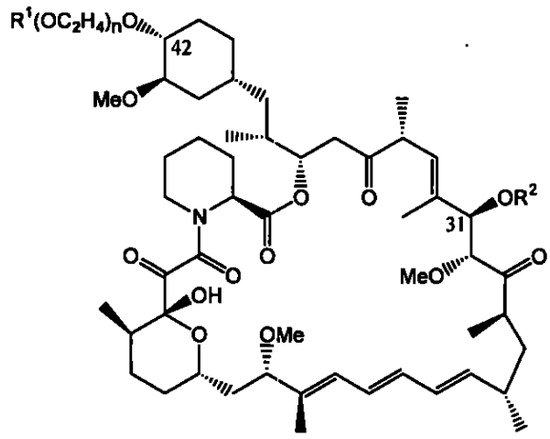
* n=5

[00157] Although only some embodiments of the invention are specifically disclosed and described above, it will be appreciated that many modifications and variations of the present invention are possible in light of the above teachings and within the purview of the appended claims without departing from the spirit and intended scope of the invention.

[00158] All of the above publications as well as any other references mentioned hereafter are herein incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated as incorporated herein by reference in its entirety.

We Claim:

1. A rapamycin carbohydrate derivative having the structure of formula (I):



(I)

wherein

R^1 and R^2 are independently hydrogen or-X-Z,

wherein n= 0 or 1; and

wherein each X is a linker, and each Z is a carbohydrate moiety independently selected from the group consisting of a monosaccharide, oligosaccharide and pseudosugar, wherein Z is attached to X through a hydroxyl oxygen atom of Z with the proviso that R^1 and R^2 are not both hydrogen.

2. The rapamycin carbohydrate derivative of claim 1, wherein R^1 is hydrogen and R^2 is –X-Z.

3. The rapamycin carbohydrate derivative of claim 2, wherein X is selected from the group consisting of:

(i) $-R^3C(O)-$;

(ii) $-C(O)R^3-$;

(iii) $-R^3S(O)_2-$; and

(iv) $-S(O)_2R^3-$;

wherein R^3 is selected from the group consisting of:

(a) $-(CH_2)_p-$ where p is an integer from 1 to 18;

(b) $-(CH_2)_n-O-(CH_2)_m-$ where n and m are each independently an integer from 2 to 6; and

(c) a bond.

4. The rapamycin carbohydrate derivative of claim 3, wherein X is selected from the group consisting of -C(O)- and $-SO_2-$.

5. The rapamycin carbohydrate derivative of claim 2, wherein X is a single functional group.

6. The rapamycin carbohydrate derivative of claim 2, wherein Z is selected from the group consisting of fructose, fucitol and allose.

7. The rapamycin carbohydrate derivative of claim 6, wherein Z is D-fructose.

8. The rapamycin carbohydrate derivative of claim 3 wherein Z is a monosaccharide derivative wherein at least one of the hydroxyl groups of the monosaccharide is replaced with a hydrogen, an alkoxy, an alkanoate or a halogen group.

9. The rapamycin carbohydrate derivative of claim 1, wherein R^1 is –X-Z and R^2 is hydrogen.

10. The rapamycin carbohydrate derivative of claim 9, wherein X is selected from the group consisting of:

(i) $-R^3C(O)-$;

(ii) $-C(O)R^3-$;

(iii) $-R^3S(O)_2-$; and

(iv) $-S(O)_2R^3-$;

wherein R^3 is selected from the group consisting of:

(a) $-(CH_2)_p-$ where p is an integer from 1 to 18;

(b) $-(CH_2)_n-O-(CH_2)_m-$ where n and m are each independently an integer from 2 to 6; and

(c) a bond.

11. The rapamycin carbohydrate derivative of claim 10, wherein X is selected from the group consisting of -C(O)- and $-SO_2-$.

12. The rapamycin carbohydrate derivative of claim 9, wherein X is a single functional group.

13. The rapamycin carbohydrate derivative of claim 9, wherein Z is selected from the group consisting of fructose, fucitol and allose.

14. The rapamycin carbohydrate derivative of claim 13, wherein Z is D-fructose.

15. The rapamycin carbohydrate derivative of claim 9 wherein Z is a monosaccharide derivative wherein at least one of the hydroxyl groups of the monosaccharide is replaced with a hydrogen, an alkoxy, an alkanoate or a halogen group.

16. A rapamycin carbohydrate derivative selected from the group consisting of:

42-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-[2-(Methyl-D-glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-D-fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-L-fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-D-fructosylcarbonyl)rapamycin;

31-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-L-fructosylcarbonyl)rapamycin;

42-O-(D-Allosylcarbonyl)rapamycin;

42-O-[2-(D-Allosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Allosylcarbonyl)rapamycin;

42-O-[2-(L-Allosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-allosylcarbonyl)rapamycin;

31-O-(L-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-allosylcarbonyl)rapamycin;

42-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-[2-(D-Fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-[2-(L-Fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fructoslylcarbonyl)rapamycin;

31-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fructoslylcarbonyl)rapamycin;

42-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-[2-(D-Fucitolylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-[2-(L-Fucitolylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fucitolylcarbonyl)rapamycin;

31-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fucitolylcarbonyl)rapamycin;

42-O-(D-Glucalylcarbonyl)rapamycin;

42-O-[2-(D-Glucalylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Glucosylcarbonyl)rapamycin;

42-O-[2-(D-Glucosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Glucosylcarbonyl)rapamycin;

42-O-[2-(L-Glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Glucalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucalylcarbonyl)rapamycin;

31-O-(D-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucosylcarbonyl)rapamycin;

31-O-(L-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-glucosylcarbonyl)rapamycin;

42-O-(L-Sorbosylcarbonyl)rapamycin;

42-O-(D-Sorbosylcarbonyl)rapamycin;

31-O-(L-Sorbosylcarbonyl)rapamycin;

31-O-(D-Sorbosylcarbonyl)rapamycin;

42-O-[2-(L-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-[2-(D-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sorbosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-sorbosylcarbonyl)rapamycin;

42-O-(D-Lactalylcarbonyl)rapamycin;

42-O-[2-(D-Lactalylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Lactalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-lactalylcarbonyl)rapamycin;

42-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-[2-(D-Sucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sucrosylcarbonyl)rapamycin;

42-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-[2-(D-Gentobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(D-gentobiosylcarbonyl)rapamycin

42-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-[2-(D-Cellobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellobiosylcarbonyl)rapamycin;

42-O-(D-Turanosylcarbonyl)rapamycin;

42-O-[2-(D-Turanosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Turanosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-turanosylcarbonyl)rapamycin;

42-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-[2-(D-Palatinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-palatinosylcarbonyl)rapamycin;

42-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltosylcarbonyl)rapamycin;

42-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-[2-(D-Maltulosylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Maltosylcarbonyl)rapamycin;

42-O-[2-(D-Maltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltulosylcarbonyl)rapamycin;

31-O-(D-Maltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltosylcarbonyl)rapamycin;

42-O-(D-Lactosylcarbonyl)rapamycin;

42-O-[2-(D-Lactosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-lactosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-lactosylcarbonyl)rapamycin;

42-O-(D-Melibiosylcarbonyl)rapamycin;

31-O-(D-Melibiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-melibiosylcarbonyl)rapamycin;

42-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-[2-(D-Leucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-leucrosylcarbonyl)rapamycin;

42-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-[2-(D-Rafinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-rafinosylcarbonyl)rapamycin;

42-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltotriosylcarbonyl)rapamycin;

42-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-[2-(D-Cellotetraosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellotetraosylcarbonyl)rapamycin;

42-O-(Valiolylcarbonyl)rapamycin

42-O-[2-(D-Valiolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolylcarbonyl)rapamycin

42-O-(Valiolonylcarbonyl)rapamycin

42-O-[2-(D-Valiolonylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolonylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolonylcarbonyl)rapamycin

42-O-(Valienolylcarbonyl)rapamycin

42-O-[2-(D-Valienolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valienolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valienolylcarbonyl)rapamycin

42-O-(Valienoneylcarbonyl)rapamycin

42-O-[2-(D-Valienoneylcarbonyloxy)ethyl]rapamycin;

31-O-(Valienoneylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)- 31-O-(valienoneylcarbonyl)rapamycin

17. A pharmaceutical composition comprising the rapamycin carbohydrate derivative of claim 1 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

18. A method for treating a disease treatable by rapamycin, comprising administering a therapeutically effective amount of a pharmaceutical composition of claim 17 to a subject in need thereof.

19. The method of claim 18, wherein the disease is selected from the group consisting of transplantation rejection, host vs. graft disease, graft vs. host disease, leukemia, lymphoma, hyperproliferative vascular disorders, autoimmune disease, diseases of inflammation, solid tumors, and fungal infections.

20. A medical device comprising a rapamycin carbohydrate derivative of claim 1 or a pharmaceutically acceptable salt thereof.

21. A medical device wherein said medical device is coated with a rapamycin carbohydrate derivative of claim 1.

22. A medical device of claim 21, wherein the medical device is selected from the group consisting of stents, grafts, and implants

23. The medical device of claim 20, wherein the medical device is selected from the group consisting of stents, grafts, and implants.

24. A method for treating a disease treatable by rapamycin, comprising coadministering a therapeutically effective amount of a pharmaceutical composition of claim 17 to a subject in need thereof with a pharmaceutical composition comprising a compound selected from the group consisting of a cyclosporine or cyclosporine derivative, a steroid, or an immunomodulatory compound.

RAPAMYCIN CARBOHYDRATE DERIVATIVES

ABSTRACT

This invention provides modified rapamycins that have specific monosaccharide(s), oligosaccharide(s), pseudosugar(s) or derivatives thereof attached through a linker to create rapamycin carbohydrate derivatives having enhanced pharmacokinetic and/or pharmacodynamic profiles. For example, administration of the rapamycin carbohydrate derivative results in altered pharmacokinetic profiles and reduced toxicities. Thus, the present invention provides compounds with characteristics that are distinct from other drugs in its class such as rapamycin.

PATENTE
Expediente No. 550

DERIVADOS DE CARBOHIDRATOS DE RAPAMICINA

REFERENCIA CRUZADA CON SOLICITUDES RELACIONADAS

[0001] La presente solicitud reivindica el beneficio de la Solicitud U.S. No. Serial 60/471,367 presentada en mayo 16 de 2003, 60/546,240 presentada en Febrero 20 de 2004 y 60/562,840 presentada en Abril 16 de 2004.

CAMPO DEL INVENTO

[0002] La presente solicitud se relaciona con derivados de carbohidrato del rapamicina, que es un inmunosupresor potente, con medios de carbohidrato estructuralmente definidos adheridos a la estructura de rapamicina vía medio conectivo. Los derivados de carbohidrato de rapamicina pueden actuar como prodrogas. Lo anterior implica que pueden aparecer sustancialmente sin actividad inmunosupresiva ellas mismas, pero *in vivo* se pueden convertir en rapamicina que luego muestra un efecto inmunosupresor.

REFERENCIAS

[0003] Las siguientes referencias se relacionan en este documento o se hace referencia a ellas mismas mediante número de patente o solicitud o por parte del autor indicándose el año en las secciones pertinentes de la presente especificación.

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[0005] S. N. Sehgal H. Baker, C. Vezina, "Rapamicina (AY-22,989), a new antifungal antibiotic. II. Fermentation, isolation and characterization," *J. Antibiot.* 28, 727 (1975).

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[0015] C.K. Wang et al., Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, 545 (1993).

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[0017] Patente U.S. No. 3,929,992.

[0018] Patente U.S. No. 3,993,749.

[0019] Patente U.S. No. 4,885,171.

[0020] Patente U.S. No. 4,401,653.

[0021] Patente U.S. No. 4,316,885.

[0022] Patente U.S. No. 4,650,803.

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[0023] Solicitud PCT No. WO 92/05179.

[0024] Patente U.S. No. 5,118,678.

[0025] Patente U.S. No. 5,260,300.

[0026] Patente U.S. No. 5,118,678.

[0027] Patente U.S. No. 5,118,678.

[0028] Patente U.S. No. 5,100,883.

[0029] Patente U.S. No. 5,151,413.

[0030] Patente U.S. No. 5,120,842.

[0031] Patente U.S. No. 5,120,725.

[0032] Patente U.S. No. 5,120,727.

[0033] Patente U.S. No. 5,258,389.

[0034] Patente U.S. No. 5,672,605.

[0035] Patente U.S. No. 5,583,139.

[0036] Patente U.S. No. 5,527,907.

[0037] Patente U.S. No. 5,457,111.

[0038] Patente U.S. No. 5,955,100.

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[0039] Patente U.S. No. 6,146,658.

[0040] Patente U.S. No. 5,935,995.

[0041] Patente U.S. No. 5,665,728.

[0042] Patente U.S. No. 6,146,658.

ANTECEDENTES DEL INVENTO

[0043] La rapamicina, conocida también como sirolimus, es una lactona macrólida de 31 miembros, $C_{51}H_{79}NO_{13}$, con masa molecular de 913.6 Da. En solución, la rapamicina forma trans- y cis-isómeros conformacionales con una proporción de 4:1 (en solución de cloroformo) debido a la rotación obstaculizada al proveedor del enlace de la amida del ácido pipecólico. Es bastante soluble en agua, hidrocarburos alifáticos y dietil éter, en tanto que es soluble en alcoholes, hidrocarbonos halogénados y dimetil sulfóxido. El rapamicina es inestable en solución; se degrada en plasma y en buffers de pH bajo y neutro a una temperatura de 37°C con una vida media de menos de 10 horas.

[0044] La rapamicina es producida por el *Streptomyces hygroscopicus,* y ha demostrado que posee una serie de atributos farmacológicos valiosos. El compuesto es un antibiótico triene macrocíclico que posee actividad antifungal, particularmente contra la *Candida albicans*, tanto *in vitro* como *in vivo*. Ver, C. Vezina et al., *J. Antibiot.* 28, 721 (1975), S. N. Sehgal et al., *J. Antibiot.* 28, 727

(1975), H. A. Baker et al., *J. Antibiot.* 31, 539 (1978), y las Patentes U.S. Números 3,929,992; y 3,993,749. La rapamicina sola (Patente U.S. No. 4,885,171) o en combinación con picibanil (Patente U.S. No. 4,401,653) también ha demostrado que tiene una actividad antitumoral. Por otra parte, autores como R. Martel et al. *Can. J. Physiol. Pharmacol.* 55, 48 (1977) divulgaron que la rapamicina es eficaz dentro del modelo experimental de encefalomielitis alérgica, modelo para esclerosis múltiple; en el modelo de artritis adyuvante, modelo para artritis reumatoide; y se inhibe eficazmente en la formación de anticuerpos similares al IgE.

[0045] Los efectos inmunosupresores de rapamicina se han divulgado en FASEB 3, 3411 (1989). La rapamicina, ciclosporin A, FK-506 (también conocida como tacrolimus), y otras moléculas macrocíclicas han demostrado ser eficaces como agentes inmunosupresores y por lo tanto son útiles para prevenir el rechazo de transplantes. Ver FASEB 3, 3411 (1989), FASEB 3, 5256 (1989), y R. Y. Calne et al., *Lancet* 1183 (1978). Aun cuando la rapamicina comparte una homología estructural con el tacrolimus inmunosupresor (FK506) y se une a la misma proteína aglutinante intracelular en linfocitos, la rapamicina y el tacrolimus han demostrado tener diferentes mecanismos de acción inmunosupresora. La rapamicina inhibe la S6p70-quinasa en tanto que el Tacrolimus inhibe la calcineurina. Se encontró que la rapamicina prolonga la sobrevivencia del injerto de distintos de transplantes en diversas especies por si solas o en combinación con otros inmunosupresores. Ver, S.N. Sehgal et al., *Medicinal Research Reviews* 14, 1 (1994).

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[0046] La rapamicina también se conoce como inhibidor del mTOR. Estos inhibidores son un tipo de medicamentos inmunosupresores que inhiben la activación de las células T en una etapa posterior de la respuesta inmune en comparación con otros tipos de inhibidores como los inhibidores calcineurin y los inhibidores de las síntesis del ADN. En transplantes, los inhibidores mTOR son básicamente utilizados en combinación con los inhibidores calcineurin.

[0047] Desafortunadamente, los efectos colaterales (por ejemplo, efectos gastrointestinales, hiperlipidemia) de los inhibidores mTOR limitan actualmente su uso más amplio en transplante y tratamiento de enfermedades autoinmunes. Y si bien no se ha demostrado que inducen una nefrotoxicidad, si se ha podido demostrar que la rapamicina induce una serie de efectos colaterales tóxicos en modelos animales. Estos efectos tóxicos son entre otros la obstaculización de la homeostasis de glucosa, ulceración del tracto gastrointestinal, pérdida de peso, diarrea y trombocitopenia.

[0048] Numerosos derivados de la rapamicina se han sintetizado con miras a aliviar y mejorar ciertas desventajas que conserva la rapamicina, entre las cuales están la baja y/o variable biodisponibilidad y solubilidad, y alta toxicidad. Los derivados mono y diacilatados de la rapamicina (esterificados en las posiciones 28 y 43) han demostrado ser útiles como agentes antifungales (Patente U.S. No. 4,316,885) y utilizados para realizar prodrogas solubles en agua (patente U.S. No. 4,650,803). Otros derivados son los esteres del ácido carboxílico (Publicación PCT No. WO 92/05179), carbamatos (Patente U.S. No. 5,118,678), carbonatos

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(Patente U.S. No. 5,260,300), esteres de amida (Patente U.S. No. 5,118,678), esteres fluorinatados (Patente U.S. No. 5,100,883), acetales (Patente U.S. No. 5,151,413), silil éteres (Patente U.S. No. 5,120,842), derivados bicíclicos (Patente U.S. No. 5,120,725), dimeros de rapamicina (Patente U.S. No. 5,120,727) y O-arilo, O-alquilo, O-alquenilo and O-alquinilo derivados (Patente U.S. No. 5,258,389). Se ha desarrollado también una serie de prodrogas de la rapamicina (Patentes U.S. Nos. 5,672,605, 5,583,139, 5,527,907, 5,457,111, 5,955,100, 6,146,658, y 5,935,995).

[0049] Se ha demostrado que debido a que la rapamicina posee excelentes actividades inmunosupresoras, antifungales, antitumorales y otras importantes de carácter biológico, sigue existiendo la necesidad de lograr derivados mejorados que incrementen la solubilidad y mejoren el perfil farmacocinético disminuyendo su toxicidad. El presente invento enfoca tales necesidades.

RESUMEN DEL INVENTO.

[0050] El presente invento se basa en parte en el reconocimiento de que los derivados de carbohidratos de la rapamicina en donde la molécula de rapamicina se modifica en la posición 31 y/o 42, según se define en la nomenclatura actual de los Resúmenes Químicos (Chemical Abstracts), adhiriéndose a los monosacáridos, oligosacáridos o pseudoazúcares, tienen perfiles similares o mejorados de carácter farmacocinético y/o farmacodinámicos en comparación con la rapamicina. En adición, la administración de los derivados del carbohidrato de la rapamicina pueden dar como resultado una reducción en el nivel de toxicidad con

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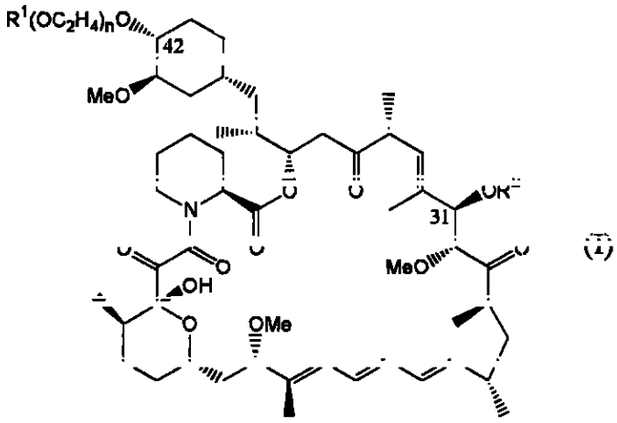
retención del efecto farmacológico deseado. Adicionalmente, los derivados de carbohidrato de la rapamicina pueden hacer que ésta sea más hidrosoluble permitiendo una formulación simplificada del medicamento. Así las cosas, el presente invento proporciona compuestos con características que son claramente diferenciadas de otros medicamentos de su clase tales como la rapamicina.

[0051] En un aspecto, el invento se dirige hacia un derivado de carbohidrato de rapamicina que tiene la estructura de la formula (I):



donde

n = 0 o 1, R^1 y R^2 son independientemente hidrógeno o -X-Z, donde cada X es un enlace, y cada Z es un medio carbohidrato seleccionado independientemente a partir del grupo conformado por un monosacárido, un oligosacárido y una pseudoazúcar en donde Z se adhiere a X a través de un átomo de hidróxilo oxígeno de Z con la condición de que R^1 y R^2 no sean ambos hidrógeno. En una presentación, R^1 puede ser hidrógeno y R^2 puede ser X-Z o en otra presentación, R^2 es hidrógeno y R^1 es X-Z. En otra presentación, X se puede seleccionar a partir

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del grupo conformado por (i) $-R^3C(O)-$; (ii) $-C(O)R^3-$; (iii) $-R^3S(O)_2-$; y (iv) $-S(O)_2R^3-$; donde R^3 se selecciona a partir del grupo conformado por: (a) $-(CH_2)_p-$ donde p es un entero de 1 a 18; (b) $-(CH_2)_n-O-(CH_2)_m-$ donde n y m son cada uno de ellos y de manera independiente un entero de 2 a 6; y (c) un enlace. En otra presentación, X se puede seleccionar del grupo conformado por -C(O)- y $-SO_2-$. En una presentación adicional, X puede ser un grupo funcional sencillo. En otro aspecto, Z se puede seleccionar a partir del grupo conformado por fructosa, fucitol y alosa. En otro aspecto más, Z puede ser un derivado del monosacárido en donde por lo menos uno de los grupos hidróxilos del monosacárido se reemplaza por un hidrógeno, un alcoxi, un alcanoato o un grupo halógeno.

[0052] Los derivados de carbohidrato de la rapamicina al tener la estructura de la formula I que se encuentra dentro del alcance del presente invento incluye por ejemplo los elementos que a continuación se mencionan (incluyendo sus sales farmacéuticamente aceptables):

42-O-(Metil-D-glucosilcarbonilo)rapamicina;

42-O-[2-(Metil-D-glucosilcarboniloxi)etil]rapamicina;

31-O-(Metil-D-glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(metil-D-glucosilcarbonilo)rapamicina;

42-O-(2-O-Metil-D-fructosilcarbonilo)rapamicina;

42-O-[2-(2-O-Metil-D-fructosilcarboniloxi)etil]rapamicina;

42-O-(2-O-Metil-L-fructosilcarbonilo)rapamicina;

42-O-[2-(2-O-Metil-L-fructosilcarboniloxi)etil]rapamicina;

31-O-(2-O-Metil-D-fructosilcarbonilo)rapamicina;

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42-O-(2-Hidroxietil)-31-O-(2-O-metil-D-fructosilcarbonilo)rapamicina;

31-O-(2-O-Metil-L-fructosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(2-O-metil-L-fructosilcarbonilo)rapamicina;

42-O-(D-Alosilcarbonilo)rapamicina;

42-O-[2-(D-Alosilcarboniloxi)etil]rapamicina;

42-O-(L-Alosilcarbonilo)rapamicina;

42-O-[2-(L-Alosilcarboniloxi)etil]rapamicina;

31-O-(D-Alosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-alosilcarbonilo)rapamicina;

31-O-(L-Alosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-alosilcarbonilo)rapamicina;

42-O-(D-Fructoslilcarbonilo)rapamicina;

42-O-[2-(D-Fructosilcarboniloxi)etil]rapamicina;

42-O-(L-Fructoslilcarbonilo)rapamicina;

42-O-[2-(L-Fructosilcarboniloxi)etil]rapamicina;

31-O-(D-Fructoslilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-fructoslilcarbonilo)rapamicina;

31-O-(L-Fructoslilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-fructoslilcarbonilo)rapamicina;

42-O-(D-Fucitolilcarbonilo)rapamicina;

42-O-[2-(D-Fucitolilcarboniloxi)etil]rapamicina;

42-O-(L-Fucitolilcarbonilo)rapamicina;

42-O-[2-(L-Fucitolilcarboniloxi)etil]rapamicina;

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31-O-(D-Fucitolilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-fucitolilcarbonilo)rapamicina;

31-O-(L-Fucitolilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-fucitolilcarbonilo)rapamicina;

42-O-(D-Glucalilcarbonilo)rapamicina;

42-O-[2-(D-Glucalilcarboniloxi)etil]rapamicina;

42-O-(D-Glucosilcarbonilo)rapamicina;

42-O-[2-(D-Glucosilcarboniloxi)etil]rapamicina;

42-O-(L-Glucosilcarbonilo)rapamicina;

42-O-[2-(L-Glucosilcarboniloxi)etil]rapamicina;

31-O-(D-Glucalilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-glucalilcarbonilo)rapamicina;

31-O-(D-Glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-glucosilcarbonilo)rapamicina;

31-O-(L-Glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-glucosilcarbonilo)rapamicina;

42-O-(L-Sorbosilcarbonilo)rapamicina;

42-O-(D-Sorbosilcarbonilo)rapamicina;

31-O-(L-Sorbosilcarbonilo)rapamicina;

31-O-(D-Sorbosilcarbonilo)rapamicina;

42-O-[2-(L-Sorbosilcarboniloxi)etil]rapamicina;

42-O-[2-(D-Sorbosilcarboniloxi)etil]rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-sorbosilcarbonilo)rapamicina;

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42-O-(2-Hidroxietil)-31-O-(L-sorbosilcarbonilo)rapamicina;

42-O-(D-Lactalilcarbonilo)rapamicina;

42-O-[2-(D-Lactalilcarboniloxi)etil]rapamicina;

31-O-(D-Lactalilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-lactalilcarbonilo)rapamicina;

42-O-(D-Sucrosilcarbonilo)rapamicina;

42-O-[2-(D-Sucrosilcarboniloxi)etil]rapamicina;

31-O-(D-Sucrosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-sucrosilcarbonilo)rapamicina;

42-O-(D-Gentobiosilcarbonilo)rapamicina

42-O-[2-(D-Gentobiosilcarboniloxi)etil]rapamicina;

31-O-(D-Gentobiosilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(D-gentobiosilcarbonilo)rapamicina

42-O-(D-Celobiosilcarbonilo)rapamicina;

42-O-[2-(D-Celobiosilcarboniloxi)etil]rapamicina;

31-O-(D-Celobiosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-celobiosilcarbonilo)rapamicina;

42-O-(D-Turanosilcarbonilo)rapamicina;

42-O-[2-(D-Turanosilcarboniloxi)etil]rapamicina;

31-O-(D-Turanosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-turanosilcarbonilo)rapamicina;

42-O-(D-Palatinosilcarbonilo)rapamicina;

42-O-[2-(D-Palatinosilcarboniloxi)etil]rapamicina;

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31-O-(D-Palatinosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-palatinosilcarbonilo)rapamicina;

42-O-(D-Isomaltosilcarbonilo)rapamicina;

42-O-[2-(D-Isomaltosilcarboniloxi)etil]rapamicina;

31-O-(D-Isomaltosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-isomaltosilcarbonilo)rapamicina;

42-O-(D-Maltulosilcarbonilo)rapamicina;

42-O-[2-(D-Maltulosilcarboniloxi)etil]rapamicina;

42-O-(D-Maltosilcarbonilo)rapamicina;

42-O-[2-(D-Maltosilcarboniloxi)etil]rapamicina;

31-O-(D-Maltulosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-maltulosilcarbonilo)rapamicina;

31-O-(D-Maltosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-maltosilcarbonilo)rapamicina;

42-O-(D-Lactosilcarbonilo)rapamicina;

42-O-[2-(D-Lactosilcarboniloxi)etil]rapamicina;

31-O-(Methil-D-lactosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(metil-D-lactosilcarbonilo)rapamicina;

42-O-(D-Melibiosilcarbonilo)rapamicina;

31-O-(D-Melibiosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-melibiosilcarbonilo)rapamicina;

42-O-(D-Leucrosilcarbonilo)rapamicina;

42-O-[2-(D-Leucrosilcarboniloxi)etil]rapamicina;

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31-O-(D-Leucrosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-leucrosilcarbonilo)rapamicina;

42-O-(D-Rafinosilcarbonilo)rapamicina;

42-O-[2-(D-Rafinosilcarboniloxi)etil]rapamicina;

31-O-(D-Rafinosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-rafinosilcarbonilo)rapamicina;

42-O-(D-Isomaltotriosilcarbonilo)rapamicina;

42-O-[2-(D-Isomaltosilcarboniloxi)etil]rapamicina;

31-O-(D-Isomaltotriosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-isomaltotriosilcarbonilo)rapamicina;

42-O-(D-Celotetraosilcarbonilo)rapamicina;

42-O-[2-(D-Celotetraosilcarboniloxi)etil]rapamicina;

31-O-(D-Celotetraosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-celotetraosilcarbonilo)rapamicina;

42-O-(Valiolilcarbonilo)rapamicina

42-O-[2-(D-Valiolilcarboniloxi)etil]rapamicina;

31-O-(Valiolilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valiolilcarbonilo)rapamicina

42-O-(Valiolonilcarbonilo)rapamicina

42-O-[2-(D-Valiolonilcarboniloxi)etil]rapamicina;

31-O-(Valiolonilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valiolonilcarbonilo)rapamicina

42-O-(Valienolilcarbonilo)rapamicina

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42-O-[2-(D-Valienolilcarboniloxi)etil]rapamicina;

31-O-(Valienolilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valienolilcarbonilo)rapamicina

42-O-(Valienoneilcarbonilo)rapamicina

42-O-[2-(D-Valienoneilcarboniloxi)etil]rapamicina;

31-O-(Valienoneilcarbonilo)rapamicina

42-O-(2-Hidroxietil)- 31-O-(valienoneilcarbonilo)rapamicina

[0053] En otro aspecto, el invento se dirige a una composición farmacéutica que comprende los derivados antes mencionados de carbohidrato de la rapamicina, o una de sus sales farmacéuticamente aceptables, y un portador farmacéuticamente aceptable. Adicionalmente, una presentación del invento consiste en un método para el tratamiento de una enfermedad la cual puede ser tratada por rapamicina mediante la administración de una cantidad terapéuticamente eficaz del derivado de carbohidrato de la rapamicina a un sujeto que lo requiera.

[0054] En otro aspecto más del invento actual, se presenta un método para el tratamiento de una condición tal como rechazo de transplante, enfermedad de huésped versus injerto, enfermedad de injerto versus huésped, leucemia, linfoma, trastornos vasculares hiperproliferativos, enfermedades autoinmunes, enfermedades de inflamación, tumores sólidos e infecciones fungales en un paciente, donde dicho método comprende la administración de una cantidad terapéuticamente eficaz de la composición farmacéutica antes mencionada a una paciente que lo requiera.

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[0055] Más adelante, el invento proporciona un instrumental médico en donde dicho instrumental comprende una derivado de carbohidrato de rapamicina o una de sus sales farmacéuticamente aceptables. En una presentación, el dispositivo está revestido con un derivado de carbohidrato de rapamicina.

[0056] Igualmente el método proporciona un método para el tratamiento de una enfermedad que puede ser tratada con rapamicina, que comprende la coadministración de una cantidad terapéuticamente eficaz de un derivado de carbohidrato de rapamicina del presente invento a un sujeto que lo requiera, con una composición farmacéutica seleccionada a partir del grupo consistente en un derivado de ciclosporina o ciclosporina propiamente, un esteroide, o un compuesto inmunomodulatorio.

BREVE DESCRIPCIÓN DE LOS DIBUJOS

[0057] La Figura 1 muestra la fructosa tal y como existe en diversas configuraciones en la solución.

[0058] La Figura 2 muestra un enfoque general a la preparación de los derivados del carbohidrato de rapamicina del presente invento. "RAPA-OH" significa rapamicina, en donde el grupo hidróxilo (-OH) puede ser cualquiera de los grupos hidróxilos de la rapamicina.

[0059] La Figura 3 muestra la trayectoria de reacción para la síntesis de 42-O-(D-fructosilcarbonilo)rapamicina.

[0060] La Figura 4 muestra la trayectoria de reacción para la síntesis de 31-O-(D-fructosilcarbonilo)rapamicina.

[0061] La Figura 5 muestra la actividad inmunosupresora *in vitro* de la rapamicina, 42-O-(D-fructosilcarbonilo)rapamicina y un análogo unido al carbamato de 42-O-(D-fructosilcarbonilo)rapamicina.

[0062] Las Figuras 6, 7 y 8 muestran perfiles farmacocinéticos de derivados de carbohidrato de rapamicina previamente seleccionados en ratas hembra.

[0063] La Figura 9 muestra niveles de colesterol en suero en ratas hembra con posterioridad al tratamiento con 42-O-(D-fructosilcarbonilo)rapamicina y rapamicina.

[0064] La Figura 10 muestra el efecto de dos dosis distintas de rapamicina en agregación plaquetaria.

[0065] La Figura 11 compara el efecto de 42-O-(D-fructosilcarbonilo)rapamicina y rapamicina en un agregado plaquetario.

[0066] La Figura 12 ilustra el efecto de 42-O-(D-fructosilcarbonilo)rapamicina y rapamicina en frecuencias de sobrevivencia en modelo de transplante de corazón de rata hembra.

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DESCRIPCIÓN DETALLADA DEL INVENTO

[0067] El presente invento se relaciona con el descubrimiento inesperado de que los derivados reivindicados del carbohidrato de rapamicina presentan propiedades farmacológicas en comparación con la rapamicina no derivatizada.

[0068] Debe tenerse en cuenta que tal y como se utiliza en este contexto y en las reivindicaciones anexas, las formas en singular "un" "una," y "el o la" incluyen referencias al plural a menos que el contexto dicte de una manera clara un procedimiento diferente. De esta manera, por ejemplo, una referencia a "un derivado de carbohidrato de rapamicina" incluye la pluralidad de tales derivados, y una referencia a "un portador farmacéuticamente aceptable" es referencia también a uno o varios portadores y a sus equivalentes conocidos por las personas expertas en la materia, y así sucesivamente.

[0069] A menos que se indique un procedimiento diferente, todos los términos técnicos y científicos utilizados en este documento tienen los mismos significados que se utilizan comúnmente entre las personas expertas en la materia a la que pertenece este trabajo inventivo. Aun cuando ciertos métodos y materiales similares o equivalentes a los descritos en este documento pueden utilizarse en la práctica o prueba del presente invento, los métodos preferidos, los dispositivos y materiales son materia de descripción en esta documentación.

[0070] Todas las publicaciones citadas aquí se incorporan a este documento por referencia en su totalidad para los propósitos de describir y divulgar las

metodologías, reactivos y herramientas reportadas en las publicaciones que podrían utilizarse en conexión con el invento. Nada de lo contenido en este documento deberá interpretarse como una admisión de que el invento no tiene derechos a antefecha tales como divulgación por virtud de invención previa.

[0071] La práctica del presente invento utilizará, a menos que se indique un procedimiento distinto, métodos convencionales de la química, bioquímica, biología molecular, biología celular, inmunología y farmacología, al interior de las destrezas de la materia. Estas técnicas se explican totalmente en la literatura. (Ver por ejemplo, Gennaro, A.R., ed. (1990) Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co.; Colowick, S. et al., eds., Short Protocols in Molecular Biology, 4th edition, John Wiley & Sons; Ream et al., eds. (1998) Molecular Biology Techniques: An Intensive Laboratory Course, Academic Press).

Definiciones

[0072] Cuando se analizan los derivados del carbohidrato de la rapamicina, las composiciones o métodos, los siguientes términos tienen los significados que se indican a continuación a menos que se señale una significación diferente. Los términos no definidos tienen sus significados reconocidos en la materia.

[0073] El término "alquilo" se refiere a grupos alquilo que tienen de 1 a 10 átomos de carbono, por ejemplo 1 a 6 átomos de carbono, y se incluye grupos alquilo de cadena recta y de cadena bifurcada. Este término se ejemplifica por grupos tales como el metilo, t-butilo, n-heptilo, octilo y similares.

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[0074] El término "alqueno" se refiere a un grupo alquilo no saturado que tiene por lo menos un punto de insaturación del alqueno (por ejemplo, -C=C-) y que además tiene de 1 a 10 átomos de carbono, por ejemplo de 1 a 6 átomos de carbono, e incluye grupos alquilo de cadera recta y cadena bifurcada.

[0075] El término "alquino" se refiere a un grupo alquilo insaturado que tiene por lo menos un punto de insaturación alquino (por ejemplo, –C≡C-) y que además tiene de 1 a 10 átomos de carbono, por ejemplo de 1 a 6 átomos de carbono, e incluye grupos alquilo de cadena recta y bifurcada.

[0076] El término grupo "aromático" se refiere a un grupo carbocíclico aromático de 6 a 14 átomos de carbono que tienen un solo anillo (por ejemplo, fenilo) o anillos múltiples condensados (por ejemplo, naftilo o antrilo) donde tales anillos condensados pueden ser o no ser aromáticos (por ejemplo, 2-benzoxazolinona, 2H-1,4-benzoxazin-3(4H)-ona-7-il, y similares).

[0077] El término "grupo protector" o "grupo bloqueador" se refiere a cualquier grupo que cuando se une a uno o varios grupos hidróxilos de rapamicina o un medio de azúcar, evita que ocurran reacciones en estos grupos hidróxilos y donde tales grupos protectores pueden ser retirados mediante pasos químicos o enzimáticos convencionales para restablecer el grupo o grupos hidróxilos. El grupo protector removible en particular utilizado se determina mediante la naturaleza de los compuestos y procesos químicos que se utilicen. Los grupos de bloqueo hidróxilos removibles incluyen sustitutos convencionales tales como alilo, bencilo, acetilo, cloroacetilo, tiobencilo, bencildeno, fenacilo, t-butildimetilsililo y trialquilsilils

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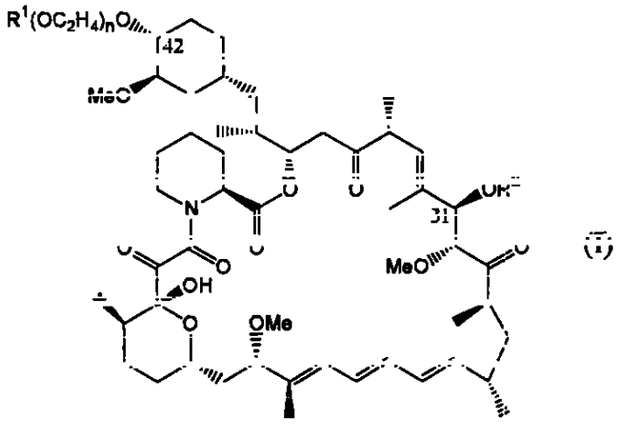
tales como el trietilsililo, triisopropilsililo, trimetilsilo, tributilsililo y similares, y cualquier otro grupo que pueda introducirse químicamente en una funcionalidad hidróxilo y posteriormente ser removido de manera selectiva bien por métodos químicos o enzimáticos en condiciones suaves compatibles con la naturaleza del producto.

Los Derivados de Carbohidrato de Rapamicina

[0078] Tal y como se analizó anteriormente, los derivados de carbohidrato de rapamicina del presente invento son compuestos que tienen la estructura de la formula (I):



donde

n=0 o 1, R^1 y R^2 son independientemente hidrógeno o -X-Z, donde cada X es un enlace, y cada Z es un medio de carbohidrato seleccionado independientemente a partir del grupo conformado por un

monosacárido, oligosacárido y pseudoazúcar, con la condición de que R^1 y R^2 no sean ambos hidrógeno.

[0079] Tal y como se evidenciará, los derivados de la rapamicina pueden tener medios derivados de azúcar adheridos en la posición 42, posición 31, o posiciones 42 y 31. En una presentación, el derivado del azúcar se adhiere en la posición 42 solamente y en otra presentación el derivado del azúcar se adhiere en la posición 31 solamente.

[0080] Es importante tener en cuenta que en la rapamicina que ocurre en la naturaleza, los sustitutos 41-metoxi y 42-hidroxi existen en una *trans* configuración relativa entre si. Los compuestos de rapamicina 42-O-(glicosilcarbonilo) del presente invento se preparan de tal manera que se pueda retener la *trans* configuración de los sustitutos 41 y 42. Por consiguiente, al hacer la hidrólisis de la unión de carbonato, la rapamicina se liberará en su configuración de ocurrencia natural. De manera análoga, los compuestos 31-O-(glicosilcarbonilo)rapamicina del presente invento se preparan de tal manera que se pueda retener la estereoquímica de ocurrencia natural del sustituto 31-hidróxilo de rapamicina.

El Medio Carbohidrato

[0081] El medio de carbohidrato (o azúcar), representado por el identificador "Z" en la formula (I), se forma a partir de un monosacárido, oligosacárido, pseudoazúcar o sus derivados que tienen un grupo funcional reactivo que se puede acoplar (i) directamente al grupo o grupos hidróxilos en cualquiera o ambos de los puntos 31

y 42 de la rapamicina, o (ii) a uno o varios grupos funcionales reactivos en una rapamicina activada con el fin de obtener un derivado de carbohidrato de rapamicina del presente invento. El grupo funcional se adhiere opcionalmente a un enlace que a su vez se adhiere al azúcar, típicamente pero no en forma necesaria en el centro anomérico. Estos grupos opcionales de enlace se analizan más adelante.

[0082] Los azúcares que comprenden los derivados del azúcar pueden diferir entre si de una serie de formas. Por ejemplo, pueden existir en la piranosa o en forma de furanosa a diversos grados. Ciertos azúcares tales como el fusitol existen única y exclusivamente en forma de cadena abierta en tanto que muchas otras (por ejemplo, glucosa, ribosa, alosa) existen predominantemente en forma cíclica. Las propiedades físico-químicas del azúcar tales como la geometría, composición, tamaño, flexibilidad o rigidez y la hidrofilicidad relativa pueden afectar todas las características químicas del derivado de carbohidrato de rapamicina.

[0083] En solución la fructosa por ejemplo puede existir principalmente en forma piranosa de 6 miembros y/o en su forma de furanosa de 5 miembros (ver Figura 1). Igualmente cada una de estas formas pueden existir en configuraciones alfa o beta. Adicionalmente, la fructosa puede existir en forma de cadena abierta. Por lo tanto, la fructosa puede existir en su forma de □-fructopiranosa, □-fructopiranosa, □-fructofuranosa o, □-fructofuranosa o cadena abierta tal y como se muestra en la Figura 6. La fructosa puede adherirse a un medicamento en cualquiera de estas configuraciones. En su forma de piranosa de 6 miembros, la fructosa tiene mas

probabilidades de formar un enlace, unida a un medicamento, por ejemplo, a través del alcohol primario sencillo que se presenta en la posición 1 de la forma de piranosa. En su forma de furanosa de 5 miembros, la fructosa tiene dos alcoholes primarios que son los que se encuentran en las posiciones 1 y 6. En la forma de furanosa, los enlaces tienen probabilidades de existir a través de cualquiera de estos alcoholes primarios en las posiciones 1 o 6.

[0084] La manera como el organismo absorbe y procesa los azúcares específicos varía según el caso. Muchas personas presentan dificultades en el procesamiento de la lactosa por ejemplo. Esta intolerancia a la lactosa podría hacer que la incorporación de la lactosa a los derivados de carbohidrato de la rapamicina sea inadecuada. Por otra parte, los oligosacáridos no son absorbidos de manera intacta desde el tracto digestivo y deben en primer término digerirse hasta conformar sus elementos constitutivos de monosacáridos. Los monosacáridos sin embargo son absorbidos por sistemas transportadores ubicados en la membrana de borde cepillo de los enterocitos. Consultar, S. Miura, Journal of Gastroenterology 37, 491 (2002). Por ejemplo, la glucosa y la galactosa se absorben por el sistema transportador SGLT1. Otro transportador, el GLUT2, facilita principalmente el transporte de glucosa, y otro transportador mas conocido como GLUT5 transporta la fructosa. La presencia en el tracto gastrointestinal de diferentes proteínas transportadoras de monosacáridos con diversas selectividades podría conducir a una variación de absorción de distintos derivados de carbohidratos de la rapamicina. Lo anterior significa que ciertos derivados pueden ser más rápidamente capaces de aprovechar un transportador disponible

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con el objeto de facilitar el paso del derivado de carbohidrato de rapamicina hacia el exterior del tracto gastrointestinal y descargarlo en el sistema vascular en donde el medio carbohidrato puede ser escindido, liberando de esta manera la rapamicina. Se considera que este proceso facilitado pueda producir una menor toxicidad gastrointestinal asociada con exposición localizada a la rapamicina.

[0085] En vista de lo anteriormente expuesto, es obvio que la selección apropiada del medio carbohidrato (monosácrido, oligosacárido o pseudoazúcares) incorporados en el derivado de carbohidrato de la rapamicina puedan ejercer una gran influencia sobre las propiedades farmacocinéticas y/o farmacodinámicas del derivado. De acuerdo con estos principios, el medio carbohidrato puede escogerse cuidadosamente para optimizar las propiedades farmacocinéticas y/o farmacodinámicas del derivado de carbohidrato de la rapamicina.

[0086] Entre los monosacáridos adecuados están los azúcares de cadenas abiertas o cerradas simples (en la configuración L o D), que básicamente tienen 5 o 6 carbonos (un monosacárido pentosa o un monosacárido hexosa), al igual que 7 carbonos (monosacárido de heptosa). Se incluyen los derivados del azúcar en donde el átomo de oxígeno anular se ha sustituido por carbono, nitrógeno o azufre, amino azúcares en donde un sustituto hidróxilo del azúcar simple se reemplaza por un grupo amino o azúcares que tienen un doble enlace entre dos átomos adyacentes de carbono, (por ejemplo, glucosamina, 5-tio-D-glucosa, nojirimicina, deoxinojirimicina, 1,5-anhidro-D-sorbitol, 2,5-anhidro-D-manitol, 2-deoxi-D-galactosa, 2-deoxi-D-glucosa, 3-deoxi-D-glucosa, alosa, arabinosa,

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arabinitol, fucitol, fucosa, galactitol, glucitol, iditol, lixosa, manitol, levo-ramnitol, 2-deoxi-D-ribosa, ribosa, ribitol, ribulosa, ramnosa, xilosa, xilulosa, alosa, altrosa, fructosa, galactosa, glucosa, gulosa, idosa, levulosa, manosa, psicosa, sorbosa, tagatosa, talosa, galactal, glucal, fucal, ramnal, arabinal, xalal, valienamina, validamina, valiolamina, valiol, valiolon, valienol, valienona, ácido glucorónico, ácido galacturónico, ácido N-acetilneuramínico, ácido glucónico D-lactona, ácido galactónico □-lactona, ácido galactónico □-lactona, ácido manónico □-lactona, D-*altro*-heptulosa, D-*manno*-heptulosa, D-*glicero*-D-*manno*-heptosa, D-*glicero*-D-*gluco*-heptosa, D-*allo*-heptulosa, D-*altro*-3-heptulosa, D-*glicero*-D-*manno*-heptitol, D-*glicero*-D-*altro*-heptitol y similares). Los grupos hidróxilo en monosacáridos pueden ser opcionalmente reemplazados por hidrógeno, alcoxi (por ejemplo 2-O-metil-D-fructosa), alcanoato o grupos halógenos, Se incluyen derivados del sulfato y/o fosfato de monosacáridos tal y como se define en este estudio.

[0087] Los oligosacáridos adecuados incluyen entre otros los carbohidratos que tienen 2 a 10 o más monosacáridos enlazados entre si. La unidad constitutiva del monosacárido puede ser por ejemplo un monosacárido pentosa, un monosacárido hexosa, o un pseudoazúcar (incluyendo la pseudoaminoazúcar), Los oligosacáridos no incluyen grupos bicíclicos que se formen mediante la fusión de un monosacárido hasta lograr un anillo de benceno, un anillo de ciclohexano, o un anillo heterocíclico.

[0088] Los pseudoazúcares que puedan ser utilizados en el invento son miembros de la clase de compuestos en donde el átomo de oxígeno anular del

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monosacárido cíclico es reemplazado por un grupo de metileno. Los pseudoazúcares son también conocidos como "carba-azúcares".

El Enlace

[0089] Tal y como se señaló anteriormente, el medio carbohidrato se adhiere de manera covalente a la rapamicina vía un enlace, indicado como una "X" en la formula I. En su forma más simple, el enlace es un grupo químico funcional que se forma cuando el derivado del azúcar se adhiere en forma covalente a la rapamicina, pero en sí no es parte ni de la rapamicina ni de la molécula de azúcar. En una presentación, la naturaleza del enlace se determina por la química empleada para adherir de manera covalente el azúcar o derivado del azúcar a la rapamicina. Por ejemplo, si 42-O-(4-nitropheniloxicarbonilo) rapamicina (rapamicina activa) se hace reaccionar con un azúcar, el resultado es un derivado de carbohidrato de rapamicina en donde el oxígeno 42-hidróxilo de la rapamicina se enlaza de manera covalente a un grupo carbonilo que a su vez se enlace de manera covalente a un oxígeno hidróxilo en el azúcar. De acuerdo con este ejemplo, el enlace es un grupo carbonilo (C=O). Un ejemplo del enlace tal y como se define en este estudio se muestra en la Figura 3. Los ejemplos de enlaces incluyen medios tales como el carbonilo (C=O) y el sulfonilo (O=S=O). Estos carbonilos o sulfonilos enlazados se reconocen como un enlace del grupo funcional sencillo.

[0090] El enlace conjuntamente con la funcionalidad a través de la cual se adhieren el azúcar y la rapamicina a dicho enlace forman una "unión". Por

ejemplo, cuando un enlace carbonilo se adhiere a un azúcar vía uno de sus átomos de oxígeno hidróxilo y luego a un oxígeno hidróxilo de rapamicina, la "unión" resultante es un carbonato (por ejemplo,–OC(O)O-). Un ejemplo de una unión tal y como se define en este documento se señala en la Figura 4. Los ejemplos de uniones son entre los esteres, éteres, carbonatos, carbamatos, sulfatos y uretanos.

[0091] El enlace y las uniones asociadas se seleccionan para lograr un derivado de carbohidrato de rapamicina biocompatible, sustancialmente no inmunogénico. Si bien el presente invento se basa en el reconocimiento de que la presencia de una o varios azúcares mejoraran la efectividad de la rapamicina, sus propiedades farmacocinéticas y/o farmacodinámicas pueden también mejorarse por la geometría, composición, tamaño, flexibilidad o rigidez, hidrofobicidad relativa e hidrofilicidad, y similares propiedades del enlace y/o unión. En este orden de ideas, el enlace o unión se pueden escoger para optimizar las propiedades farmacocinéticas y/o farmacodinámicas del derivado de carbohidrato de la rapamicina. Por ejemplo, las frecuencias de ácido catalizado o hidrólisis enzimática de los derivados dan como resultado la liberación de la rapamicina libre que variará de acuerdo con cual enlace es el que se origina. El enlace o unión pueden ser "neutros" desde un punto de vista biológico, es decir no contribuyen por si mismos a realizar ninguna actividad biológica adicional a la del derivado de rapamicina de carbohidrato, o bien se pueden escoger para mejorar aun más la actividad biológica del compuesto.

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[0092] Las químicas de reacción que dan como resultado enlazamientos emplean técnicas convencionales. Estas técnicas por lo general involucran el uso de grupos funcionales complementarios y reactivos ubicados en la rapamicina o rapamicina activada y derivados del azúcar o azúcares. Entre los ejemplos de grupos funcionales complementarios y sus enlaces resultantes están los que se muestran en la Tabla 1.

TABLA 1. ENLACES RESULTANTES DE FUNCIONALIDADES COMPLEMENTARIAS

PRIMER GRUPO REACTIVO	SEGUNDO GRUPO REACTIVO	UNIÓN
Hidróxilo	isocianato	uretano
Amina	epóxido	ß-aminohidroxi
Sulfonil hálida	amina	sulfonamida
Ácido carbóxilo	amina	amida
hidróxilo	alquil/aril hálida	éter
Aldehído	amina/$NaCNBH_3$	amina
Cetona	Amina/$NaCNBH_3$	amina
amina	isocianato	carbamato
Ácido carbóxilo	hidróxilo	ester
Cloroformato	hidróxilo	carbonato

[0093] Si se desea, el enlace puede contener más de un solo grupo funcional. Los enlaces complejos pueden utilizarse para lograr diversas propiedades químicas

dentro del derivado de carbohidrato de rapamicina. Por ejemplo, se pueden impartir diversas características hidrofóbicas/hidrofílicas al derivado carbohidrato de rapamicina mediante manipulación del enlace. De manera similar, los medios cargados también se pueden introducir. Las técnicas para modificación del enlace se podrán comprender rápidamente por parte de los expertos en la materia. Por ejemplo, la naturaleza hidrofóbica de un enlace derivado de la diamina hexametileno o una poliamina relacionada se puede modificar para que sea sustancialmente más hidrofílica reemplazando el grupo alquileno por un grupo poli(oxialquileno).

[0094] Por ejemplo, el glicosil-Y[-C(=Y)-X-]p-W(R)n-X-C(=Y)-medicamento en W es un grupo aromático o heteroaromático o alifático con dobles enlaces conjugados o un radical de derivado de amino ácido que se cicla después de la eliminación del radical glicosilo se divulgó en la Patente U.S. 6,146,658. Estos enlaces complejos se designan para que se auto-eliminen vía ciclización posterior a la remoción enzimática del medio glicosilo.

[0095] Hay diversas variedades de enlaces que resultan adecuados en el invento. Las personas conocedoras de este campo podrán reconocer que un carbonilo (C=O) o un enlace sulfonilo (O=S=O), o un enlace carbonilo o sulfonilo combinado con una cadena sencilla alquilo o una cadena polieter (por ejemplo, un pequeño número de unidades de óxido de etileno que se repiten) tendrán propiedades distintas de aquellas registradas en enlaces más complejos descritos anteriormente. Por ejemplo, se indica que los enlaces del presente invento no se

someterán a una ciclización para auto-eliminación. Por otro lado, la susceptibilidad del compuesto enlace/medicamento para la escisión enzimática o hidrolítica dependerán en gran medida de la naturaleza del espaciador o del medio de enlace. Adicionalmente, se indica que los compuestos con enlaces complicados pueden ser más difíciles y costos de producir que aquellos que compuestos que tienen tan solo enlaces sencillos de grupos funcionales, tales como los descritos en este invento.

[0096] En el comercio se puede obtener una amplia variación de enlaces (por ejemplo Chem Sources USA y Chem Sources International; base de datos electrónica ACD; y Abstracts Químicos). Muchos de los enlaces que presentan adecuabilidad para el uso en este invento aparecen dentro de esta categoría; en tanto que otros pueden sintetizarse rápidamente mediante métodos conocidos en la técnica, según se describe mas adelante. Entre los ejemplos de enlaces están los medios alifáticos, medios aromáticos, medios esteroidales, péptidos y similares. Entre los ejemplos específicos de enlaces comercialmente disponibles podemos citar los grupos péptidos o poliámidas, hidrocarbonos, aromáticos, heterocíclicos, éteres, lípidos, catiónicos o aniónicos, o una combinación de los mismos.

[0097] Se indica que las propiedades del enlace del presente invento se pueden modificar mediante la adición o inserción de grupos auxiliares, para cambiar por ejemplo la solubilidad del compuesto multi-enlace (en agua, grasas, lípidos, fluidos biológicos, etc.), hidrofobicidad, hidrofilicidad, flexibilidad de enlaces,

antigenicidad, estabilidad y similares. Por ejemplo, la introducción de uno o varios grupos polietileno glicol (PEG) en el enlace mejorará las condiciones de hidrofilicidad e hidrosolubilidad del derivado de carbohidrato de la rapamicina, incrementando tanto el peso el molecular como el tamaño molecular, y según la naturaleza del enlace unPEGilatado, se puede incrementar el tiempo de retención *in vivo*. Por otro lado, el PEG puede disminuir la antigenicidad y aumentar potencialmente la rigidez generalizada del enlace.

[0098] Los grupos auxiliares que mejoran la hidrosolubilidad/hidrofilicidad del enlace, y por consiguiente, los compuestos resultantes, son útiles en el ejercicio del presente invento. De esta forma, se encuentra dentro del alcance de este invento el uso de grupos auxiliares tales como pequeñas unidades de repetición de etileno glicoles, alcoholes, polioles, (por ejemplo, glicerina, propoxilato de glicerol, etc.), carboxilatos, (por ejemplo, pequeñas unidades de repetición del ácido glutámico, ácido acrílico, etc.), aminas (por ejemplo tetraetilenpentamina), y similares para mejorar la hidrosolubilidad y/o hidrofilicidad de los derivados de rapamicina del carbohidrato del presente invento. Por ejemplo, el grupo auxiliar utilizado para mejorar la hidrosolubilidad/hidrofilicidad pueden ser un polieter que contenga un pequeño número de unidades repetidas de óxido de etileno (-CH_2CH_2O-).

[0099] La incorporación de grupos auxiliares lipofílicos dentro de la estructura del enlace para mejorar la lipofilicidad y/o hidrofobicidad de los derivados del carbohidrato de la rapamicina también se encuentra dentro del alcance del

presente invento. Los grupos lipofílicos útiles junto con los enlaces del invento incluyen pero no se limitan a alquilo inferior, grupos aromáticos, o grupos aromáticos policíclicos. Los grupos aromáticos pueden ser insustituidos o sustituidos por otros grupos, pero son por lo menos sustituidos por un grupo que permita su adherencia covalente al enlace. Tal y como se utiliza en este documento el término "grupos aromáticos" incorpora tanto a los hidrocarbonos aromáticos como a los aromáticos heterocíclicos. Otros grupos lipofílicos útiles con el enlace de este invento incluyen los derivados de ácidos grasos que pueden o no formar micelles en un medio acuoso y otros grupos lipofílicos específicos que modulan las interacciones entre el derivado de rapamicina de carbohidrato y las membrana biológicas.

[00100] La flexibilidad del enlace se puede manipular mediante la inclusión de grupos auxiliares que sean voluminosos y/o rígidos. La presencia de grupos voluminosos o rígidos podrá obstaculizar la rotación libre alrededor de los enlaces en la unión, o enlaces entre la unión y los grupos auxiliares, o enlaces entre la unión y los grupos funcionales. Los grupos rígidos pueden incluir por ejemplo aquellos cuya libertad conformacional sea restringida por la presencia de anillos y/o enlaces, por ejemplo arilo, heteroarilo y grupos heterocíclicos. Otros grupos que pueden impartir rigidez son los grupos polipéptidos tales como las cadenas oligo- o poliprolina.

[00101] La rigidez también se puede impartir electroestáticamente. De esta manera, si los grupos auxiliares se encuentran cargados positiva o negativamente,

entonces los grupos auxiliares similarmente cargados forzarán la unión para producir una configuración que permita que se establezca la distancia máxima entre cada una de las cargas similares. El costo energético de acercar los grupos con carga similar entre si, lo cual se encuentra inversamente relacionado con el cuadrado de la distancia entre los grupos, tenderá a retener la unión dentro de una configuración que mantenga la separación entre los grupos auxiliares con carga similar. Por otro lado, los grupos auxiliares que lleven cargas opuestas tenderán a ser atraídos a sus contrapartes de carga opuesta y potencialmente podrán ingresar a los enlaces iónicos inter- e intra moleculares. Este mecanismo no covalente tenderá a retener el enlace en una conformación que permita la unión entre grupos con carga opuesta. La adición de grupos auxiliares que tenga carga, o alternativamente los grupos protegidos que lleven una carga latente que se encuentre desenmascarada, con posterioridad a la adición de la unión, por desprotección, cambio en pH, oxidación, reducción u otros mecanismos conocidos entre los expertos en la materia, se encontrará dentro del alcance del presente invento.

[00102] Los grupos voluminosos podrán incluir por ejemplo grandes átomos o iones (por ejemplo, yodo, azufre, iones metálicos, etc.), o grupos que contengan grandes átomos, grupos policíclicos, que incluyen grupos aromáticos, grupos no aromáticos y estructuras que incorporan uno o varios enlaces carbono-carbono (por ejemplo, alquenos y alquines). Los grupos voluminosos pueden también incluir oligómeros y polímeros que son especies de cadena bifurcada o recta. Las especies bifurcadas deberán incrementar la rigidez de la estructura mas por peso

molecular unitario de ganancia que lo que sucede con las especies de cadena recta.

[00103] En vista de lo expuesto anteriormente, es obvio que la selección apropiada de un grupo de unión que proporcione una orientación adecuada, propiedades de entropía y físico-químicas se ubique perfectamente bien dentro de la técnica aquí descrita.

[00104] Las uniones pueden adherirse a la rapamicina o al azúcar empleando grupos funcionales reactivos. Estos grupos funcionales reactivos se seleccionan en relación con los grupos funcionales disponibles en la rapamicina o en los azúcares para el acople, o introducidos en la rapamicina o en el azúcar para este mismo propósito. Por ejemplo, la reacción entre un ácido carboxílico de la unión y una amina primaria o secundaria del azúcar en presencia de agentes adecuados de activación dará como resultado la formación de un medio de amida que une de forma covalente el azúcar al enlace. La reacción entre el grupo de amina del enlace y un hálido sulfonilo del azúcar dará como resultado la formación de un medio de sulfonamida que une en forma covalente al enlace. La reacción entre un alquilo o aril hálido del enlace y un alcohol del azúcar dará como resultado la formación de un medio de éter que une en forma covalente el azúcar al enlace.

[00105] Cuando se carece de grupos funcionales, éstos pueden ser creados mediante el uso de químicas adecuadas que se describen en los textos estándar de química orgánica tales como Advanced Organic Chemistry, Jerry March, John Wiley &Sons (5th Ed., 2000). El término enlace (linker) se refiere a todo aquello

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que no se considera como parte del azúcar o la rapamicina. Los enlaces (linkers) se pueden derivar de compuestos lineales que tengan grupos funcionales reactivos en el extremo del enlace (linker).

[00106] Los enlaces divalentes adecuados incluyen a manera de ejemplo los que son derivados de los ácidos dicarboxílico, disulfonilhálidos, dialdehídos, dicetonas, dihálidos, diisocianatos, diaminas, dioles, mezclas de ácidos carboxílicos, sulfonilhálidos, aldehídos, cetonas, hálidos, isocianatos, aminas y dioles. En cada caso, el ácido carboxílico, sulfonilhálido, aldehído, cetona, hálido, isocianato, amina y grupos funcionales diol se hacen reaccionar con una funcionalidad complementaria sobre el azúcar y la rapamicina para formar un enlace covalente. Esta funcionalidad complementaria es bien conocida en la técnica tal y como se ilustra en la Tabla 1 analizada anteriormente.

[00107] En presentaciones del invento, el enlace (X) se selecciona partir del grupo conformado por: (i) $-R^3C(O)$; (ii) $-C(O)R^3$, (iii) $-R^3S(O)_2$; o (iv) $-S(O)_2R^3$ donde R^3 se selecciona a partir del grupo conformado por: (i) $-(CH_2)_p-$ donde p es un entero de 1 a 18, (ii) $-(CH_2)_n-O-(CH_2)_m-$ donde n y m son cada uno de ellos y en forma independiente un entero de 2 a 6, o (iii) un enlace. Debe entenderse que el enlace (X) puede ser igual o distinto en cada ocurrencia, es decir en las posiciones 31 y 42. En presentaciones adicionales del invento, el enlace (X) es carbonilo (C=O), sulfonilo (O=S=O), o un solo grupo funcional. Los enlaces (linkers) de carbonilo pueden estar en las posiciones 31, 42, o ambas. En otras presentaciones, R^1 es –C(O)-Z y R^2 es H o R^2 es –C(O)-Z y R^1 es H.

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[00108] De acuerdo con lo anterior, los derivados del carbohidrato de rapamicina tienen la estructura de la formula I que se encuentra dentro del alcance del presente invento e incluyen por ejemplo los elementos que se estipulan a continuación (incluyendo sus sales farmacéuticamente aceptables):

42-O-(Metil-D-glucosilcarbonilo)rapamicina;

42-O-[2-(Metil-D-glucosilcarboniloxi)etil]rapamicina;

31-O-(Metil-D-glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(metil-D-glucosilcarbonilo)rapamicina;

42-O-(2-O-Metil-D-fructosilcarbonilo)rapamicina;

42-O-[2-(2-O-Metil-D-fructosilcarboniloxi)etil]rapamicina;

42-O-(2-O-Metil-L-fructosilcarbonilo)rapamicina;

42-O-[2-(2-O-Metil-L-fructosilcarboniloxi)etil]rapamicina;

31-O-(2-O-Metil-D-fructosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(2-O-metil-D-fructosilcarbonilo)rapamicina;

31-O-(2-O-Metil-L-fructosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(2-O-metil-L-fructosilcarbonilo)rapamicina;

42-O-(D-Alosilcarbonilo)rapamicina;

42-O-[2-(D-Alosilcarboniloxi)etil]rapamicina;

42-O-(L-Alosilcarbonilo)rapamicina;

42-O-[2-(L-Alosilcarboniloxi)etil]rapamicina;

31-O-(D-Alosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-alosilcarbonilo)rapamicina;

31-O-(L-Alosilcarbonilo)rapamicina;

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42-O-(2-Hidroxietil)-31-O-(L-alosilcarbonilo)rapamicina;

42-O-(D-Fructoslilcarbonilo)rapamicina;

42-O-[2-(D-Fructosilcarboniloxi)etil]rapamicina;

42-O-(L-Fructoslilcarbonilo)rapamicina;

42-O-[2-(L-Fructosilcarboniloxi)etil]rapamicina;

31-O-(D-Fructoslilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-fructoslilcarbonilo)rapamicina;

31-O-(L-Fructoslilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-fructoslilcarbonilo)rapamicina;

42-O-(D-Fucitolilcarbonilo)rapamicina;

42-O-[2-(D-Fucitolilcarboniloxi)etil]rapamicina;

42-O-(L-Fucitolilcarbonilo)rapamicina;

42-O-[2-(L-Fucitolilcarboniloxi)etil]rapamicina;

31-O-(D-Fucitolilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-fucitolilcarbonilo)rapamicina;

31-O-(L-Fucitolilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-fucitolilcarbonilo)rapamicina;

42-O-(D-Glucalilcarbonilo)rapamicina;

42-O-[2-(D-Glucalilcarboniloxi)etil]rapamicina;

42-O-(D-Glucosilcarbonilo)rapamicina;

42-O-[2-(D-Glucosilcarboniloxi)etil]rapamicina;

42-O-(L-Glucosilcarbonilo)rapamicina;

42-O-[2-(L-Glucosilcarboniloxi)etil]rapamicina;

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31-O-(D-Glucalilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-glucalilcarbonilo)rapamicina;

31-O-(D-Glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-glucosilcarbonilo)rapamicina;

31-O-(L-Glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-glucosilcarbonilo)rapamicina;

42-O-(L-Sorbosilcarbonilo)rapamicina;

42-O-(D-Sorbosilcarbonilo)rapamicina;

31-O-(L-Sorbosilcarbonilo)rapamicina;

31-O-(D-Sorbosilcarbonilo)rapamicina;

42-O-[2-(L-Sorbosilcarboniloxi)etil]rapamicina;

42-O-[2-(D-Sorbosilcarboniloxi)etil]rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-sorbosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-sorbosilcarbonilo)rapamicina;

42-O-(D-Lactalilcarbonilo)rapamicina;

42-O-[2-(D-Lactalilcarboniloxi)etil]rapamicina;

31-O-(D-Lactalilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-lactalilcarbonilo)rapamicina;

42-O-(D-Sucrosilcarbonilo)rapamicina;

42-O-[2-(D-Sucrosilcarboniloxi)etil]rapamicina;

31-O-(D-Sucrosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-sucrosilcarbonilo)rapamicina;

42-O-(D-Gentobiosilcarbonilo)rapamicina

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42-O-[2-(D-Gentobiosilcarboniloxi)etil]rapamicina;

31-O-(D-Gentobiosilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(D-gentobiosilcarbonilo)rapamicina

42-O-(D-Celobiosilcarbonilo)rapamicina;

42-O-[2-(D-Celobiosilcarboniloxi)etil]rapamicina;

31-O-(D-Celobiosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-celobiosilcarbonilo)rapamicina;

42-O-(D-Turanosilcarbonilo)rapamicina;

42-O-[2-(D-Turanosilcarboniloxi)etil]rapamicina;

31-O-(D-Turanosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-turanosilcarbonilo)rapamicina;

42-O-(D-Palatinosilcarbonilo)rapamicina;

42-O-[2-(D-Palatinosilcarboniloxi)etil]rapamicina;

31-O-(D-Palatinosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-palatinosilcarbonilo)rapamicina;

42-O-(D-Isomaltosilcarbonilo)rapamicina;

42-O-[2-(D-Isomaltosilcarboniloxi)etil]rapamicina;

31-O-(D-Isomaltosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-isomaltosilcarbonilo)rapamicina;

42-O-(D-Maltulosilcarbonilo)rapamicina;

42-O-[2-(D-Maltulosilcarboniloxi)etil]rapamicina;

42-O-(D-Maltosilcarbonilo)rapamicina;

42-O-[2-(D-Maltosilcarboniloxi)etil]rapamicina;

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31-O-(D-Maltulosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-maltulosilcarbonilo)rapamicina;

31-O-(D-Maltosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-maltosilcarbonilo)rapamicina;

42-O-(D-Lactosilcarbonilo)rapamicina;

42-O-[2-(D-Lactosilcarboniloxi)etil]rapamicina;

31-O-(Methil-D-lactosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(metil-D-lactosilcarbonilo)rapamicina;

42-O-(D-Melibiosilcarbonilo)rapamicina;

31-O-(D-Melibiosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-melibiosilcarbonilo)rapamicina;

42-O-(D-Leucrosilcarbonilo)rapamicina;

42-O-[2-(D-Leucrosilcarboniloxi)etil]rapamicina;

31-O-(D-Leucrosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-leucrosilcarbonilo)rapamicina;

42-O-(D-Rafinosilcarbonilo)rapamicina;

42-O-[2-(D-Rafinosilcarboniloxi)etil]rapamicina;

31-O-(D-Rafinosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-rafinosilcarbonilo)rapamicina;

42-O-(D-Isomaltotriosilcarbonilo)rapamicina;

42-O-[2-(D-Isomaltosilcarboniloxi)etil]rapamicina;

31-O-(D-Isomaltotriosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-isomaltotriosilcarbonilo)rapamicina;

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42-O-(D-Celotetraosilcarbonilo)rapamicina;

42-O-[2-(D-Celotetraosilcarboniloxi)etil]rapamicina;

31-O-(D-Celotetraosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-celotetraosilcarbonilo)rapamicina;

42-O-(Valiolilcarbonilo)rapamicina

42-O-[2-(D-Valiolilcarboniloxi)etil]rapamicina;

31-O-(Valiolilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valiolilcarbonilo)rapamicina

42-O-(Valiolonilcarbonilo)rapamicina

42-O-[2-(D-Valiolonilcarboniloxi)etil]rapamicina;

31-O-(Valiolonilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valiolonilcarbonilo)rapamicina

42-O-(Valienolilcarbonilo)rapamicina

42-O-[2-(D-Valienolilcarboniloxi)etil]rapamicina;

31-O-(Valienolilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valienolilcarbonilo)rapamicina

42-O-(Valienoneilcarbonilo)rapamicina

42-O-[2-(D-Valienoneilcarboniloxi)etil]rapamicina;

31-O-(Valienoneilcarbonilo)rapamicina

42-O-(2-Hidroxietil)- 31-O-(valienoneilcarbonilo)rapamicina

[00109] Adicionalmente, el invento está dirigido a un derivado de la rapamicina que tiene la formula estructural I en donde n=1, R^1 es H y R^2 es X-Z. donde X es a enlace (linker), y Z es un medio carbohidrato independientemente

seleccionado a partir del grupo conformado por un monosacárido, un oligosacárido y una pseudoazúcar.

[00110] El invento está igualmente dirigido a una composición farmacéutica que comprende el derivado de carbohidrato de rapamicina antes mencionado, o una de sus sales farmacéuticamente aceptables, y un portador farmacéuticamente aceptable.

Preparación de los Derivados de Carbohidrato de la Rapamicina

[00111] El procedimiento general para sintetizar los derivados de carbohidrato de la rapamicina del presente invento implica el acoplamiento de un monosacárido, oligosacárido, pseudoazúcar o derivado del azúcar en las posiciones 31 y/o 42 de la rapamicina activada.

[00112] Por ejemplo, según se muestra en la Figura 2, la rapamicina (II) se puede hacer reaccionar con p-nitrofenil cloroformato para lograr una rapamicina activada (III). Bajo condiciones controladas cuidadosamente, la reacción tomará preferencialmente lugar en la posición del hidróxilo 42. Al alterar las condiciones de reacción, tanto los grupos 31 como 42 hidróxilos se podrán activar de manera análoga. La activación selectiva del grupo 31 hidróxilo se puede lograr protegiendo en primer término el medio 42 hidróxilo utilizando por ejemplo un grupo alquilsililo tal como el trietilsililo, triisopropilsililo o *tert*-butildimetilsililo, y luego haciendo reaccionar con p-nitrofenil cloroformato u otros cloroformatos. La remoción del grupo protector producirá entonces una rapamicina activada en la posición

hidróxilo 31. De esta manera, es posible preparar un derivado de la rapamicina que se active selectivamente en la posición 31, posición 42, o ambas.

[00113] Desde este momento en adelante, en un segundo paso, un medio de azúcar o un derivado del azúcar se hace reaccionar con una rapamicina activada (III) hasta lograr un derivado de carbohidrato de la rapamicina. Cuando el grupo funcional reactivo en el medio del azúcar o el derivado del azúcar sea un grupo hidróxilo, la reacción con la rapamicina activada podrá tener lugar en un grupo hidróxilo primario para formar un enlace de carbonato con la rapamicina. El enlace (linker) resultante será un grupo carbonilo. Cuando el grupo funcional reactivo en el azúcar o derivado del azúcar sea un grupo amino (el cual se puede ubicar en cualquier posición del azúcar), entonces un enlace del carbamato con la rapamicina se producirá y el enlace (linker) resultante será un grupo carbonilo. Los derivados del azúcar amino sustituida, por ejemplo el azúcar-X-NH_2 o el azúcar-NH_2, se podrán preparar a partir de precursores utilizando métodos convencionales. Por ejemplo, la reducción del azúcar -X-N_3 o la de-ftalilación del azúcar-X-NPhth, en donde Phth es un ftalilo, producirá el derivado del azúcar amino sustituido. Estos precursores se pueden sintetizar mediante la glicosidación de los medios del enlace (linker) con donantes del glicosilo activados apropiadamente.

[00114] De esta manera, utilizando el enfoque general descrito en este documento será posible preparar una amplia gama de derivados del carbohidrato

de la rapamicina en donde los grupos hidróxilos 31 y/o 42 se modifican con una amplia gama de azúcares o derivados de ésta.

[00115] Se indica que los grupos hidróxilos de la rapamicina o un metabolito de ésta, incluyendo los que se ubican en posiciones distintas de la 31 y 42, podrán también glicosilatarse tal y como se describe en este estudio, y los derivados resultantes del carbohidrato de la rapamicina también exhibirán condiciones más altas de hidrosolubilidad y/o farmacocinecia mejorada y/o mejores propiedades farmacodinámicas en comparación con sus contrapartes no glicosiladas.

[00116] Los metabolitos de la rapamicina son conocidos en la técnica. Por ejemplo, Streit *et. al.* identificaron estructuralmente varios metabolitos de la rapamicina tomados de microsomas de hígado humano. Ver, F. Streit et al., Drug Metabol. Disp., 24, 1272 (1996). Estos incluyen 41-demetil rapamicina, 7-demetil rapamicina, 11-hidroxi rapamicina, y un éster 24-hidroxi correspondiente a un grupo de degradación por hidrólisis de la rapamicina. Se ha demostrado igualmente que los metabolitos de la rapamicina pueden someterse a esta hidrólisis de éster. Streit también identificó parcialmente los di, tri, y tetra metabolitos hidroxilados de la rapamicina. Wang *et. al.* encontraron 16 metabolitos hidroxilados y/o demetilados en la bilis de ratas hembras tratadas con rapamicina. Ver C.K. Wang et al., Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, 545 (1993). Nickmilder *et. al.* identificaron un metabolito de la rapamicina 3,4 y 5,6 dihidrodiol en microsomas de hígado de rata hembra. Ver, M.J.M. Nickmilder et al., Xenobiotica, 27, 869 (1997).

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En sangre entera, Streit *et. al.* han identificado 41-demetil, hidroxi, dihidroxi y didemetil metabolitos de la rapamicina. Ver, F. Streit et al., Clin. Chem., 42, 1417 (1996). Estos metabolitos fueron responsables del 56% del total de derivados de la rapamicina medidos. Finalmente, Leung *et. al.* analizaron la disposición de [^{14}C]-rapamicina en voluntarios hombres saludables. Los investigadores encontraron que la rapamicina representaba aproximadamente el 35% de la radioactividad total en sangre y que 41-demetil, 7-demetil, y varios hidroxi, hidroxidemetil, y metabolitos de rapamicina didemetil representaban individualmente entre 1 y 12% de la radioactividad total. Los metabolitos de la rapamicina se pueden aislar a partir de una serie de puentes, que incluyen pero no se limitan a la sangre, orina o muestras de materias fecales, tomadas de microsomas de hígado o de cultivos de microorganismos.

[00117] De acuerdo con lo anterior, en adición a 31- y 42-, los grupos hidróxilos de interés particular incluyen aquellos que se encuentran en las posiciones 27-, 41-, 3-, 4-, 5-, 6-, 7-, 11- y 24- de la rapamicina y de los metabolitos de la rapamicina.

Composiciones Farmacéuticas y Utilidad

[00118] Los compuestos de este invento se pueden administrar en forma clara y nítida o con un portador farmacéutico a un animal, que puede ser un mamífero de sangre caliente, y especialmente a humanos, que lo requieran. Las composiciones farmacéuticas pueden también contener otros medicamentos, particularmente medicamentos de los cuales se sabe que presentan diversos

mecanismos de acción. De esta manera, las composiciones farmacéuticas pueden contener en adición a los compuestos del presente invento, por lo menos un medicamento de otra clase, por ejemplo, un inhibidor de la calcineurina, un esteroide u otros compuestos inmunomodulatorios, que puedan interferir con la síntesis del ADN o con la señalización intra- o inter- celular u otros procesos celulares. Entre los ejemplos de inhibidores de la calcineurina se pueden citar la Ciclosporina A (que la suministra Novartis como Sandimmune® y Neoral® y FK506 (que conoce también como tacrolimus o Prograf® disponible a partir de Fujisawa). Entre los ejemplos de derivados de la ciclosporina están los divulgados en W099/18120. Entre los ejemplos de esteroides están la predisona, prednisolona o metilprednisolona. Entre los ejemplos de estos compuestos inmunomoduladores están la azotioprina, ácido micofenólico (micofenolato mofitil o Cellcept® que suministra Roche), leflunomida que suministra Aventis, Brequinar, Mizoribina, anticuerpos como el □-LFA-1, y □-ICAM-1, timoglobulina, IL2R antagonistas que incluyen basiliximab (Stimulect®), y daclizumab (Zenapax®), alemtuzumab (Campath 1H®, anticuerpo monoclonal humanizado que reconoce el CD52), Ortoclone OKT3® o muromonab CD3, Atgam® globulina inmune de linfocitos, ATG (globulina antitimocita), y otros compuestos. Los medicamentos individuales, y el derivado de carbohidrato de la rapamicina, se pueden formular en forma independiente como componentes claramente diferenciados de la composición farmacéutica, y administrarse en forma conjunta o por separado.

[00119] El portador farmacéuticamente eficaz puede ser sólido o líquido. Un portador sólido puede incluir una o varias sustancias que pueden también actuar

como agentes saborizantes, lubricantes, solubilizadores, agentes suspensores, rellenos, glidantes, ayudas para compresión, aglutinantes o agentes desintegradores de tabletas, y puede ser también un material encapsulante. En los polvos, el portador es un sólido finamente dividido que se mezcla con el ingrediente activo finamente dividido. En tabletas, el ingrediente activo se mezcla con un portador que tiene las propiedades de compresión necesarias en proporciones adecuadas y que se compacta en la forma y tamaño deseados. Los polvos y tabletas pueden contener hasta el 99% del ingrediente activo. Entre los portadores sólidos adecuados están por ejemplo el fosfato de calcio, estearato de magnesio, talco, azúcares, lactosa, dextrina, almidón, gelatina, celulosa, metil celulosa, carboximetil celulosa sódica, polivinilpirrolidina, ceras de baja fusión y resinas de intercambio de iones.

[00120] Los portadores líquidos se utilizan en preparación de soluciones, suspensiones, emulsiones, jarabes, elixires y composiciones presurizadas. El ingrediente activo se puede disolver o suspender en un portador líquido farmacéuticamente aceptable como agua, solvente orgánico, mezcla de ambos o aceites o grasas farmacéuticamente aceptables. El portador líquido puede contener otros aditivos farmacéuticamente adecuados tales como solubilizadores, emulsificadores, surfactantes, buffers, preservativos, edulcorantes, agentes saborizantes, agentes de suspensión, agentes engrosadores, colores, reguladores de viscosidad, estabilizadores u osmo-reguladores. Entre los ejemplos adecuados de portadores líquidos para administración oral y parenteral están el agua (que parcialmente contiene aditivos como se señaló anteriormente, como derivados de

la celulosa, posiblemente solución de carboximetil celulosa sódica), alcoholes (que incluye alcoholes monohídricos y alcoholes polihídricos, como los glicoles) y sus derivados, y aceites (por ejemplo, aceite de coco fraccionado y aceite de araquis). Para la administración parenteral, el portador también puede ser un éster aceitoso como el etil oleato y el isopropil miristato. Los portadores líquidos estériles son útiles en líquidos estériles que son composiciones para administración por vía parenteral. El portador líquido para composiciones presurizadas puede ser un hidrocarbono halogenado u otro impulsor farmacéuticamente aceptable.

[00121] Las composiciones farmacéuticas líquidas que sean soluciones o suspensiones estériles son adecuadas para aplicación por medio de inyecciones intramusculares, intraperitoneales y subcutáneas. Las soluciones estériles también se pueden administrar por vía intravenosa. El compuesto también se puede administrar por vía oral ya sea en forma de composición líquida o sólida. La administración pulmonar también se contempla en este estudio.

[00122] La composición farmacéutica puede presentarse en forma de dosificación unitaria, por ejemplo como tabletas o cápsulas. En tales formas, la composición se subdivide en dosis unitarias que contiene cantidades apropiadas del ingrediente activo; formas de dosis unitarias que se pueden presentar como composiciones empacadas, por ejemplo, polvos empacados, vials, ampollas, jeringas prellenadas o sachets que contengan líquidos. La forma de dosificación unitaria puede ser por ejemplo una cápsula o una tableta en si misma, o bien puede ser un número adecuado de tales composiciones en forma paquetes. La

dosificación que debe utilizarse en el tratamiento deberá determinarse según criterio del médico tratante.

[00123] De manera adicional, los compuestos de este invento se pueden emplear como una solución, crema o loción mediante formulación con vehículos farmacéuticamente aceptables que se le administren a un área afectada.

[00124] Los compuestos del presente invento pueden ser también utilizados conjuntamente con un instrumental médico. Por ejemplo, un derivado de carbohidrato de la rapamicina como componente de medicamento revestido o stent intravascular impregnado pueden utilizarse para inhibir la proliferación de tejidos neoíntimos previniendo de esta manera la restenosis (ver por ejemplo la Patente U.S. No. 5,665,728). Los derivados del carbohidrato de la rapamicina también se pueden emplear como componente de otros dispositivos médicos de medicamentos revestidos o impregnados tales como catéteres, bombas, o dispositivos médicos para la administración de un medicamento tales como perlas (beads) o discos que contengan medicamentos. La presencia de la rapamicina, que es un potente inmunosupresor, puede disminuir la inflamación, rechazo u otras respuestas inmunes a la presencia de estos implementos médicos implantables en el organismo.

[00125] Los siguientes ejemplos se ofrecen para ilustrar el presente invento y no deben interpretarse de ninguna manera como límites al alcance de este trabajo.

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EJEMPLOS

[00126] En los ejemplos a continuación, las abreviaturas que allí aparecen tendrán los significados que se indican. Si no se define una abreviatura, ésta tendrá su significado aceptado de manera generalizada.

g	=	Gramos
mg	=	Miligramos
kg	=	Kilogramos
mol	=	Milimol
M	=	Molar
N	=	Normal
mL	=	Mililitro
min	=	Minuto
BzCl	=	Benzoil cloruro
DMAP	=	4-dimetilaminopiridina
DMS	=	Dimetil sulfato
Py	=	Piridina
DMF	=	N,N-dimetilformamida
Me	=	Metilo
HOAt	=	1-hidroxi-7-azabenzotriazola
HOBT	=	1-hidroxibenzotriazola hidrato
TBDMSiCl	=	Tert-butildimetilsilio cloruro
AcOH	=	Ácido acético

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THF	=	Tetrahidrofurano
LC/MS o LCMS	=	Espectroscopia de cromatografía líquida/masa
HPLC	=	Cromatografía líquida de alto desempeño
conc.	=	Concentrado
eq.	=	Equivalentes

[00127] En los siguientes ejemplos y procedimientos, los materiales de partida se obtienen en el comercio y son suministrados por Aldrich Chemical Company, Inc., Milwaukee, WI 53233 USA; Lancaster Synthesis, Inc., NH 03087 USA; Sigma, St. Louis MO 63178 USA; Maybridge Chemical Co. Trevillett, Tintagel, Cornwall PL34 OHW United Kingdom; TCI America, Portland OR 97203; Frontier Scientific, Utah, USA; and Bachem, Torrance, California, Estados Unidos.

EJEMPLO 1

Síntesis de 42-O-(D-Fructosilcarbonilo)rapamicina

[00128] La Figura 3 muestra el método de síntesis para 42-O-(D-Fructosilcarbonilo)rapamicina. El procedimiento detallado es como se describe a continuación:

42-O-(4-nitrofeniloxicarbonilo)rapamicina

[00129] Una solución de 10.0 g de en 50mL de diclorometano y 10 mL de piridina seca se enfrió a una temperatura de -78°C a una atmósfera de nitrógeno.

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A esta solución se le agregaron 3.31 g de 4-nitrofeinl cloroformato y la mezcla de reacción se agitó durante 1 hora a -78°C, y luego se pasó directamente a temperatura ambiente. La reacción quedó completa luego de 2 horas. La mezcla fue diluida con agua y extraída con diclorometano. La fase orgánica fue lavada con agua, secada en Na_2SO_4 y evaporada. La cromatografía sobre columna de silica gel (solvente: hexanos – etil acetato, 2:1) produjo 9.66g de 42-O-(4-nitrophenyloxycarbonilo)rapamicina en forma de sólido amarillento (se secó por congelación utilizando benceno). $C_{58}H_{82}N_2O_{17}$, M=1078.6; MS(ES+): m/z=1101.7 $(M+Na)^+$

42-O-(D-Fructosilcarbonilo)rapamicina

[00130] A una solución de D-fructosa (2.025g, 11.24mmol) y DMAP (250mg) en *N,N*-dimetilformamida (25mL) se agregó 42-O-(4-nitrofeniloxicarbonilo)-rapamicina (4.05g, 3.757mmol) y luego la mezcla de reacción se agitó a temperatura ambiente durante 24 horas. El solvente se evaporó a un alto vacío y luego el residuo fue cromatografiado con flash en una columna de silica gel utilizando diclorometano - metanol (9:1) como eluyente. El producto obtenido fue repurificado en una columna preparatoria HPLC (80% metanol – 20% agua, con flujo de 10mL/min.), arrojando 1.461g de 42-O-(D-fructosilcarbonilo)rapamicina en forma de sólido blanco (secado por congelación a partir del benceno). $C_{58}H_{89}NO_{20}$, M=1119.6; MS(ES+): m/z=1142.7 $(M+Na)^+$. De manera alterna el HOBT (o el HOAT) se pueden utilizar en lugar del DMAP como se describe en el ejemplo 3.

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EJEMPLO 2

[00131] De acuerdo con los procedimientos análogos al descrito en el Ejemplo 1, y empleando los azúcares o derivados de azúcares apropiados, se pudieron obtener los siguientes compuestos:

42-O-(D-Glucosilcarbonilo)rapamicina

42-O-(Metil-D-glucosilcarbonilo)rapamicina

42-O-(D-Alosilcarbonilo)rapamicina

42-O-(D-Fructosilcarbonilo)rapamicina

42-O-(L-Fructosilcarbonilo)rapamicina

42-O-(D-Fucitolilcarbonilo)rapamicina

42-O-(L-Fucitolilcarbonilo)rapamicina

42-O-(D-Glucalilcarbonilo)rapamicina

42-O-(L-Sorbosilcarbonilo)rapamicina

42-O-(2-O-Metil-D-fructosilcarbonilo)rapamicina

42-O-(D-Lactalilcarbonilo)rapamicina

42-O-(D-Sucrosilcarbonilo)rapamicina

42-O-(D-Gentobiosilcarbonilo)rapamicina

42-O-(D-Celobiosilcarbonilo)rapamicina

42-O-(D-Turanosilcarbonilo)rapamicina

42-O-(D-Palatinosilcarbonilo)rapamicina

42-O-(D-Isomaltosilcarbonilo)rapamicina

42-O-(D-Maltulosilcarbonilo)rapamicina

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42-O-(D-Maltosilcarbonilo)rapamicina

42-O-(D-Lactosilcarbonilo)rapamicina

42-O-(Metil-D-lactosilcarbonilo)rapamicina

42-O-(D-Melibiosilcarbonilo)rapamicina

42-O-(D-Leucrosilcarbonilo)rapamicina

42-O-(D-Rafinosilcarbonilo)rapamicina

42-O-(D-Isomaltotriosilcarbonilo)rapamicina

42-O-(D-Celotetraosilcarbonilo)rapamicina

EJEMPLO 3

Síntesis de 31-O-(D-Fructosilcarbonilo)rapamicina

[00132] La Figura 4 muestra el método de síntesis para 31-O-(D-Fructosilcarbonilo)rapamicina. El procedimiento en detalle se describe a continuación:

42-O-(tert-Butildimetilsililo)rapamicina

[00133] A una solución de rapamicina (10g) e imidazola (2.2g) en *N,N*-dimetilformamida (40mL) se agregó cloruro de *tert*-butildimetilsililo (1.76g) y luego la mezcla de reacción se agitó a temperatura ambiente bajo nitrógeno durante 5 días. El solvente fue evaporado a un vacío elevado y el residuo fue cromatografiado en una columna de silica gel (solvente: hexanos - etil acetato, 3:2) produciendo 5.84g de 42-O-(tert-butildimetilsililo)rapamicina como espuma blancuzca. $C_{57}H_{93}NO_{13}Si$, M=1027.6; MS(ES+): m/z=1050.7 $(M+Na)^+$

42-O-(tert-Butildimetilsililo)-31-O-(4-nitrofeniloxicarbonilo)rapamicina

[00134] El 42-O-(*tert*-Butildimetilsililo)rapamicina (5.84g) se disolvió en diclorometano (30mL) y se agregó piridina (6mL), 4-nitrofenil cloroformato (2.582g) y luego la mezcla de reacción se agitó bajo nitrógeno a temperatura ambiente durante 2 horas. Los solventes fueron evaporados y el residuo se purificó en una columna de silica gel. Las elusiones con hexanos - etil acetato (3:1) produjeron el compuesto del título en forma de espuma amarillenta (5.4g). $C_{64}H_{96}N_2O_{17}Si$, M=1192.6; MS(ES+): m/z=1215.6 $(M+Na)^+$

31-O-(4-nitrofeniloxicarbonilo)rapamicina

[00135] El 42-O-(*tert*-Butildimetilsilil)-31-O-(4-nitrofeniloxicarbonilo) rapamicina (5.4g) se disolvió en una mezcla de ácido acético (30mL), tetrahidrofurano (10mL) y agua (10mL). Luego se agitó a temperatura ambiente durante 20 horas. La mezcla de reacción se diluyó con agua y se extrajo con etil acetato (3 x 200mL). La fase orgánica se seco en sulfato sódico anhídrido, se filtro y evaporo. La cromatografía en columna de silica gel (solvente: hexanos – etil acetato, 3:2) produjo 2.1g de 31-O-(4-nitrofeniloxicarbonilo)rapamicina en forma de sólido amarillento (secado por congelación a partir del benceno). $C_{58}H_{82}N_2O_{17}$, M=1078.6; MS(ES+): m/z=1101.6 $(M+Na)^+$

31-O-(D-Fructosilcarbonilo)rapamicina

[00136] Una mezcla de 31-O-(4-nitrofeniloxicarbonilo)rapamicina (1.3g), D-fructosa (0.434g), y 1-hidroxibenzotriazola (HOBT) (0.325g) en piridina seca

(20mL) se agitó a temperatura ambiente durante 3 días. Luego, el solvente se hizo evaporar bajo una presión reducida y el residuo se cromatografió en columna de silica gel. La elusión con diclorometano - metanol (10:1) proporcionó 31-O-(D-fructosilcarbonilo)rapamicina (0.625g, 46%) en forma de sólido blanco (secado por congelación a partir del benceno). $C_{58}H_{89}NO_{20}$, M=1119.6; MS(ES+): m/z=1142.7 $(M+Na)^+$

[00137] De manera análoga, se preparó igualmente 31-O-(D-alosilcarbonilo)rapamicina. Adicionalmente, en forma alterna, se puede utilizar el DMAP en lugar del HOBT como se señala en el Ejemplo 1.

Ejemplo 4

Síntesis de 42-O-(2-O-metil-β-D-fructosilcarbonilo)rapamicina

1,3,4,5-Tetra-O-benzoil-β-D-fructopiranosa

Una mezcla de piridina anhídrida (52 mL), benzoil cloruro (51.5 mL, 0.444 mmol) y diclorometano anhídrido (125 mL) se enfrío a -10°C. Se agregó fructosa finamente pulverizada (20 g, 0.111 mmol) por porciones y luego la mezcla de reacción se agitó a -10°C durante 18 horas. La mezcla de reacción se neutralizó con agua helada, se diluyó con diclorometano y se transfirió a un embudo de separación. La capa orgánica fue separada y lavada con ácido cítrico al 5%, bicarbonato sódico saturado, agua, y se secó en una preparación de sulfato sódico. Luego se filtró y el solvente se evaporó. El residuo se disolvió en dietil éter (75 ml) luego se agregó lentamente a los hexanos (300 ml) hasta lograr un sólido blanco que al secarse produjo 58.9 g (89 %) de 1,3,4,5-tetra-O-benzoil-β-D-fructopiranosa.

1,3,4,5-Tetra-O-benzoil-2-O-metil-β-D-fructopiranosa

[00138] A una solución de 1,3,4,5-tetra-O-benzoil-β-D-fructopiranosa (10.00g, 16.8mmol) en acetona (40 mL) se agregó dimetil sulfato (2.4ml, 25.16 mmoles, 1.5 eq.) seguido de carbonato potásico (3.48g, 25.16 mmoles) y la mezcla de reacción se agitó a 50°C bajo nitrógeno durante 18 horas. Los solventes se removieron al vacío y el residuo resultante se disolvió en etil acetato (150ml), se lavó con ácido cítrico al 5%, agua, sal muera, se secó en sulfato sódico, se filtró y concentró hasta lograrse un sólido aceitoso. La purificación en columna de silica gel (hexano: etil acetato, 4:1) produjo 10.0g (98%) del compuesto de la titulación en forma de espuma blanca.

2-O-metil-β-D-fructopiranosa

[00139] Una solución de metóxido sódico (0.5 M en metanol, 10.0ml) se agregó a una solución agitada vigorosarnente de 1,3,4,5-tetra-O-benzoil-2-O-metil-β-D-fructopiranosa (10.0g, 16.4 mmol) en metanol anhídrido. Después de agitar durante 1.5 horas a temperatura ambiente la reacción quedo completa. El pH se ajustó a 7.0 con Amberlite IRC-50 (~4.0g). Los sólidos se removieron por filtración y el filtrado se concentró al vacío. La purificación en columna de silica gel (metanol: diclorometano, 4:1 y 3:1) permitió obtener el producto en forma de espuma blanca de 2.70 g (81%).

42-O-(2-O-metil-β-D-fructosilcarbonilo)rapamicina

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[00140] Una mezcla de 42-O-(4-nitrofeniloxicarbonilo)rapamicina (10.0 g, 9.3 mmol), 2-O-metil-β-D-fructopiranosa (6.2g, 28mmol, 3eq.), y 1-hidroxi-7-azabenzotriazola (HOAt) (2.5g, 2eq.) en piridina seca (60mL) se agitó a temperatura ambiente durante 4 días. Luego, el solvente se evaporó bajo presión reducida y el residuo fue redisuelto en etil acetato, y lavado con agua. La capa orgánica se evaporó y el residuo se cromatografió en una columna de silica gel. La elusión con diclorometano – metanol (100:5) produjo 42-O-(2-O-metil-β-D-fructosilcarbonilo)rapamicina (4.7g,) en forma de sólido blanco (secado por congelación a partir del benceno). $C_{59}H_{91}NO_{20}$, M=1133.7; MS (ES+): m/z=1156.7 $(M+Na)^+$

EJEMPLO 5

Estabilidad de Derivados del Carbohidrato de Rapamicina hacia una Hidrólisis en Medios Ácidos

[00141] La estabilidad hacia la hidrólisis de diversos derivados del carbohidrato de rapamicina en medios ácidos se investigó disolviendo los compuestos en 70/30 metanol/0.1N HCl (pH 1.5) y luego midiendo mediante el HPLC la cantidad de rapamicina libre en las muestras a 1 hora para determinar el alcance de la hidrólisis. Los resultados se resumen en la Tabla 2.

TABLA 2. HIDRÓLISIS CATALIZADA POR ÁCIDO DE DERIVADOS DE CARBOHIDRATO DE RAPAMICINA

Compuesto	Grado de Hidrólisis (%)

42-O-(D-Fructosilcarbonilo)rapamicina	<1
42-O-(D-Glucosilcarbonilo)rapamicina	<1
42-O-(D-Maltulosilcarbonilo)rapamicina	<5
42-O-(L-Fucitolilcarbonilo)rapamicina	<1
42-O-(D-Lactalilcarbonilo)rapamicina	<1
31-O-(D-Fructosilcarbonilo)rapamicina	<1
42-O-(D-Alosilcarbonilo)rapamicina	<1

[00142] Como se puede observar en la Tabla 2, todos los compuestos mostraron buena estabilidad en medios ácidos con poca hidrólisis o hidrólisis casi no observada.

EJEMPLO 6

Hidrólisis de Derivados de Carbohidrato de Rapamicina en Sangre Entera Humana

[00143] La capacidad de diversos derivados de carbohidrato de rapamicina de liberar rapamicina libre vía hidrólisis en sangre entera se investigó haciendo spiking de compuestos en sangre entera humana y luego midiendo por HPLC la cantidad de rapamicina libre en las muestras a 1 hora para determinar el alcance de la hidrólisis. Los resultados se resumen en la Tabla 3.

TABLA 3. HIDRÓLISIS DE DERIVADOS DE CARBOHIDRATO DE RAPAMICINA EN SANGRE ENTERA

Compuesto	Grado de Hidrólisis (%)
42-O-(D-Fructosilcarbonilo)rapamicina	28±2
42-O-(D-Glucosilcarbonilo)rapamicina	5±1
42-O-(D-Maltulosilcarbonilo)rapamicina	13±4
42-O-(L-Fucitolilcarbonilo)rapamicina	31±4
42-O-(D-Lactalilcarbonilo)rapamicina	2±1
31-O-(D-Fructosilcarbonilo)rapamicina	23±0.5
42-O-(D-Alosilcarbonilo)rapamicina	39±4
31-O-(D-Alosilcarbonilo)rapamicina	34±2

[00144] Como se observa en la Tabla 3, el grado de hidrólisis en sangre entera humana varió en gran medida de acuerdo con la naturaleza del medio de carbohidrato. La incorporación vía uniones de carbohidrato de azúcares tales como D-fructosa, L-fucitol o D-alosa por lo general condujeron a un grado mayor de hidrólisis de lo observado cuando se utilizó D-glucosa, D-maltulosa, y D-lactal. Los resultados muestran también que cuando se escoge un azúcar adecuado, tanto el 31-O- como el 42-O- de derivados del carbohidrato de rapamicina son eficaces para la liberación de la rapamicina libre en sangre entera. Adicionalmente, experimentos similares anteriores que investigaron la hidrólisis en sangre entera de derivados de carbohidrato de rapamicina con enlaces de

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carbamato mostraron poca o ninguna hidrólisis en contraposición con muchos de los compuestos con uniones de carbonato señalados en la Tabla 3.

Ejemplo 7

Comparición de la Actividad Inmunosupresora *In Vitro* de los Derivados de Carbohidrato de Rapamicina con Carbonato y Enlaces de Carbamato

[00145] La actividad inmunosupresora de la rapamicina, 42-O-(D-Fructosilcarbonilo)rapamicina y de un análogo enlazado con el carbamato de 42-O-(D-Fructosilcarbonilo)rapamicina (forma no hidrolizable de 42-O-(D-Fructosilcarbonilo)rapamicina) se evaluó en cultivos primarios de linfocitos sanguíneos (PBMC) utilizando azul alamar como un medio para detectar proliferación celular. El análogo de carbamato de 42-O-(D-Fructosilcarbonilo)-rapamicina se forma a partir de un amino azúcar en donde el medio de carbohidrato se adhiere al enlace (linker) carbonilo a través del átomo amino nitrógeno del amino azúcar, formando de esta manera un enlace de carbamato. La Figura 5 ilustra que la 42-O-(D-Fructosilcarbonilo)rapamicina muestra inhibición de proliferación celular equivalente a la rapamicina, en tanto que el análogo enlazado con el carbamato no hidrolizable no posee ninguna actividad inmunosupresora intrínseca. Estos datos indican que la espacie activa es rapamicina resultante a partir de la hidrólisis de 42-O-(D-Fructosilcarbonilo)rapamicina en el curso de un cultivo de 3 días, y no de la 42-O-(D-Fructosilcarbonilo)rapamicina no hidrolizada. En otras palabras, la prodroga no demuestra poseer ninguna actividad inmunosupresora intrínseca, y deberá hidrolizarse en rapamicina con el propósito

de que exhiba el efecto farmacológico deseado. Este experimento demuestra también la importancia de la selección del enlace entre el medio carbohidrato y la rapamicina como sucede para este ejemplo en particular, que un enlace de carbonato permite que ocurra la hidrólisis deseada, en tanto que el enlace de carbamato permanece intacto y no hay liberación de rapamicina o ésta se libera muy poco.

EJEMPLO 8

Comparación de los Perfiles Farmacocinéticos de la Rapamicina y Derivados de Carbohidrato de Rapamicina en Ratas Hembras

[00146] Los perfiles farmacocinéticos en ratas hembras de derivados de carbohidrato de rapamicina seleccionados se determinaron con el propósito de investigar la capacidad de los derivados de producir rapamicina libre dentro del flujo sanguíneo *in vivo*. Brevemente, se dosificaron por vía oral las ratas Sprague Dawley con rapamicina y derivados a 2.5 o 10 mg/kg. Se extrajo luego sangre entera vía sangrado yugular en el curso de 24 horas con congelación a -20°C hasta cuando se hizo el análisis. La sangre entera se analizó mediante espectrometría de masa de cromatografía líquida para detectar la presencia de rapamicina. Los resultados se resumen en las Figurar 6, 7 y 8.

[00147] Como se puede observar en las Figuras 6 y 7, mediante la administración oral de rapamicina se observó un rápido aumento en la concentración de la rapamicina en sangre y una concentración máxima se alcanzó

al cabo de 30 minutos aproximadamente. El nivel de rapamicina luego descendió en forma bastante rápida durante las siguientes horas. Se observó un perfil similar para el 42-O-(D-glucosilcarbonilo)rapamicina. Lo sorprendente en este caso es que cuando la 42-O-(D-fructosilcarbonilo)rapamicina o 42-O-(L-fucitolilcarbonilo)-rapamicina se administraron por vía oral el nivel de rapamicina en sangre se elevó gradualmente hasta alcanzar una concentración máxima al cabo de 3 horas aproximadamente antes de descender gradualmente con el paso del tiempo (Figura 6). Se observó un perfil similar para 31-O-(D-fructosilcarbonilo)rapamicina y para 31-O-(D-alosilcarbonilo)rapamicina (Figura 7) como también para 42-O-(D-alosilcarbonilo)rapamicina, 42-O-(D-sorbosilcarbonilo)rapamicina y 42-O-(2-O-metil-D-fructosilcarbonilo)rapamicina (Figura 8). La cinética retardada que se observó para compuestos seleccionados puede ofrecer la ventaja de permitir una menos frecuente dosificación que la se observa típicamente para la rapamicina. Adicionalmente, el aumento gradual en la concentración de rapamicina asociado con derivados seleccionados de carbohidrato de rapamicina puede aliviar los efectos tóxicos asociados con el rápido aumento en la concentración del fármaco cuando la rapamicina en sí misma se administra por vía oral.

[00148] Una segunda observación a partir de las Figuras 6, 7 y 8 es que la variabilidad en la concentración de la rapamicina, como se demostró mediante las desviación estándar indicadas en los gráficos, fue considerablemente menor para los derivados de carbohidrato de la rapamicina que para la rapamicina misma. Así las cosas, los compuestos del presente invento pueden también tener la ventaja

de una reducción en la variabilidad inter-individual que podría permitir una dosificación más consistente y predecible.

[00149] Estos experimentos demostraron que la selección cuidadosa del sustituto glicosilo tiene un impacto profundo sobre los perfiles farmacocinéticos de los derivados del carbohidrato de la rapamicina y que los compuestos del presente invento pueden poseer una considerable ventaja, incluyendo ventajas farmacocinéticas, sobre la rapamicina misma.

EJEMPLO 9

Comparación de Toxicidad del Tracto Gastrointestinal de la Rapamicina y 42-O-(D-Fructosilcarbonilo)rapamicina en Modelo Canino

[00150] Se considera que los perros Beagle son un modelo hipersensible de toxicidad del tracto gastrointestinal que se asocia con la rapamicina. Ver S.N. Sehgal et al., Medicinal Research Reviews 14, 1 (1994). Incluso las cortas exposiciones a bajas dosis de rapamicina aplicadas por vía oral a perros según se sabe dan como resultado una rápida pérdida de peso debida a la ulceración que ocurre desde la boca hasta el colon en forma secundaria a la vasculitis fibrinoide necrotizante. Según se resume en la Tabla 4, cuando a dos perros beagle se les aplicó una sola dosis oral de rapamicina a 10 mg/kg ambos perros se mostraron letárgicos y perdieron más del 30% de su peso corporal en menos de una semana dando como resultado una reducción en la ingesta alimentaria. Los animales no se recuperaron. A otro perro beagle que se le aplicó la misma dosis de 42-O-(D-

fructosilcarbonilo)rapamicina no mostró cambios físicos evidentes y mantuvo una ingesta normal del alimento. Todos estos tres perros tuvieron niveles similares en sangre de rapamicina según mediciones hechas por LCMS. En un cuarto perro una dosis de 1 mg/kg 42-O-(D-fructosilcarbonilo)rapamicina dio como resultado una ligera letargía pero el perro se recupero rápidamente. Una dosis posterior de rapamicina (1mg/kg) una semana más tarde dio como resultado una letargía severa y pérdida de peso corporal, de lo que el animal no se pudo recuperar.

TABLA 4. TOLERABILIDAD GASTROINTESTINAL EN PERROS BEAGLE

Perro #	**Tratamiento medicamentoso**	**Dosis (mg/kg)**	**Observaciones**
1	Rapamicina	10	Letárgico con pérdida de peso del 30% sin recuperación
2	Rapamicina	10	Letárgico con pérdida de peso del 30% sin recuperación
3	42-O-(D-Fructosilcarbonilo) rapamicina	10	Normal
4	42-O-(D-Fructosilcarbonilo) rapamicina	1	Letárgico con Recuperación

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Perro #	Tratamiento medicamentoso	Dosis (mg/kg)	Observaciones
4	Rapamicina	1	Pérdida de peso corporal del 30 % sin recuperación

[00151] Este experimento demuestra claramente que la administración oral de 42-O-(D-fructosilcarbonilo)rapamicina dio como resultado poco o ningún signo claro de toxicidad gastrointestinal en un modelo de perro hipersensible en comparación con la rapamicina que condujo a signos de severa toxicidad. Este resultado demuestra además el potencial de los derivados de carbohidrato de rapamicina del presente invento para mejorar el perfil farmacodinámico de la rapamicina.

Ejemplo10

Comparación de los Niveles de Colesterol en Suero en Ratas Hembras Tratadas con Rapamicina y 42-O-(D-Fructosilcarbonilo)rapamicina

[00152] El 42-O-(D-fructosilcarbonilo)rapamicina y la rapamicina se probaron cabeza con cabeza en ratas hembras Sprague-Dawley para detectar cambios en el nivel de colesterol. Las ratas (n = 12) fueron dosificadas diariamente durante 12 días con dosis equivalente (2.5 mg/kg/día) de rapamicina o 42-O-(D-fructosilcarbonilo)rapamicina. Los niveles de colesterol fueron medidos a las 24

horas para detectar el nivel en el día 11. Los resultados (Figura 9) indican que estas ratas tratadas con rapamicina desplegaron niveles significativamente aumentados de colesterol en relación con un grupo vehículo de control, en tanto que los niveles de colesterol en el grupo de 42-O-(D-fructosilcarbonilo)rapamicina fueron significativamente menores ($p < 0.01$) en comparación con el grupo de rapamicina y no presentaron diferencias significativas con el vehículo. Es importante tener en cuenta que ambos compuestos mostraron una eficacia equivalente en un modelo de transplante de corazón heterotrópico en rata hembra utilizando la misma dosis de 2.5 mg/kg/día que se empleo para este estudio (Ejemplo 12). Este experimento demuestra la capacidad que tienen los derivados de carbohidrato de rapamicina para mejorar el perfil de efectos colaterales de la rapamicina mientras se mantiene su eficacia.

EJEMPLO 11

Comparación de la Agregación Plaquetaria Debida a la Rapamicina y 42-O-(D-Fructosilcarbonilo)rapamicina en un Ensayo Lavado de Agregación Plaquetaria Humana

[00153] La agregación plaquetaria según se cree es un efecto colateral de la rapamicina y se ha implicado en el rechazo crónico cada vez mayor y otros efectos colaterales a largo plazo relacionados con el uso de la rapamicina para transplantes. (Ann Babinska et. al., Enhancement of Human Platelet Aggregation and Secretion Induced by Rapamycin. (1998) Nephrology Dialysis Transplantation Vol. 13 pp. 3153-3159. Estos experimentos se llevaron a cabo utilizando plaquetas

humanas recién lavadas estimuladas con 2 µM ADP y sometidas a spiked con Rapamicina o con 42-O-(D-Fructosilcarbonilo)rapamicina en un tiempo cero. La agregación plaquetaria tratada se pudo leer continuamente en un período de 8 a 10 minutos en un Agregómetro Plaquetario ChronoLog™.

[00154] La Figura 10 muestra un gráfico de agregación plaquetaria porcentual versus tiempo para dos concentraciones de rapamicina, 1 µg/ml y 25 µg/ml. La Figura 10 ilustra que la rapamicina induce la agregación plaquetaria de una manera dependiente de la dosis. La rapamicina en la dosis de 1 µg/ml indujo agregación plaquetaria en aproximadamente el 20% después de transcurridos 8 minutos. La rapamicina con la dosis de 25 µg/ml indujo una agregación plaquetaria de aproximadamente el 70% después de 8 minutos. La Figura 11 muestra un gráfico de agregación plaquetaria porcentual versus tiempo para plaquetas humanas lavadas, estimuladas con 2 µM ADP dosificadas con 25 µg/ml de rapamicina o de 25 µg/ml 42-O-(D-Fructosilcarbonilo)rapamicina. La Figura 11 muestra que si bien la rapamicina induce aproximadamente el 80% de agregación plaquetaria después de 8 minutos, la 42-O-(D-Fructosilcarbonilo)rapamicina no muestra ningún efecto medible en agregación plaquetaria durante el mismo período.

EJEMPLO 12

Sobrevivencia de Injertos de Aloingertos Cardíacos Heterotópicos en Ratas que recibieron por Vía Oral 42-O-(D-Fructosilcarbonilo)rapamicina (2.5 y 10 mg/kg/día), Rapamicina (2.5 mg/kg/día), o un Vehículo

[00155] Los transplantes heterotópicos fueron administrados en la aorta abdominal y en la vena cava inferior de un corazón genéticamente no acoplado (alogénico) tomado de ratas Wistar Furth y con aplicación a ratas Lewis. Los controles (vehículo y Rapamicina a 2.5 mg/kg/día) o 42-O-(D-Fructosilcarbonilo)rapamicina, a 2.5 y 10 mg/kg/día se administraron una vez al día mediante gavage oral a receptores de transplante (6 ratas por grupo) a partir de 3 días con anterioridad al transplante y continuando durante 30 días post-transplante. Si se registró alguna disfunción del injerto durante el período post-transplante de 30 días, el animal se sacrificaba. Si el animal sobrevivía más de 30 días post-transplante, los artículos de prueba y de control se descontinuaron y el animal se dejaba que continuara hasta cuando se registrara una disfunción de injerto, o hasta alcanzar los 100 días post-transplante. Las tasas promedio de sobrevivencia para cada grupo de animales receptores se resumen en la Tabla 5 y en la Figura 12. Según se observa en la Tabla 5, la 42-O-(D-Fructosilcarbonilo)rapamicina prolongo la sobrevivencia del injerto a niveles de dosificación de 2.5 y 10 mg/kg/día por 214 y 341% sobre el control de vehículo. Este valor fue similar a la sobrevivencia prolongada exhibida por la rapamicina a la dosis de 2.5 mg/kg/día. La Figura 12 ilustra que la 42-O-(D-Fructosilcarbonilo)rapamicina prolongo la sobrevivencia del injerto sobre el

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vehículo como sucedió con la rapamicina al nivel de dosis de 2.5 mg/kg/día. Estos datos demuestran la actividad inmunosupresora de la 42-O-(D-Fructosilcarbonilo)rapamicina para prevenir el rechazo de los injertos.

TABLA 5. TASAS PROMEDIO DE SOBREVIVENCIA DESPUÉS DE TRANSPLANTES CARDÍACOS EN RATAS HEMBRAS HETEROTÓPICAS (N=6)

Dosis (mg/kg/día)	**Tiempo Promedio de Sobrevivencia (días post-transplante) Medio ± SEM**		
	Control de Vehículo	**Rapamicina**	**42-O-(D-Fructosilcarbonilo)rapamicina**
0	13 ± 2		
2.5		39 ± 4	41 ± 4*
10			57 ± 4

* n=5

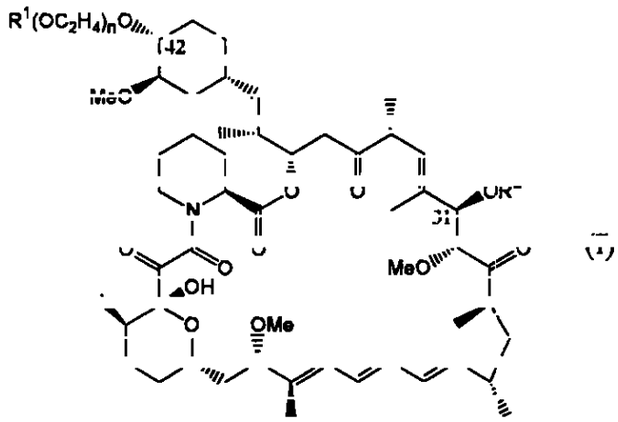
[00156] Aun cuando algunas presentaciones del invento se encuentran específicamente divulgadas y descritas en este documento, se podrán apreciar que muchas modificaciones y variaciones del presente invento son posibles a la luz de las enseñanzas anteriores y dentro del alcance de las reivindicaciones adjuntas sin separarse del espíritu y alcance del invento.

[00157] Todas las publicaciones anteriores al igual que las demás referencias que se mencionan en este documento se incorporan al mismo a manera de

referencia en su totalidad en la misma medida como si cada una de las publicaciones individuales se hubiese indicado de manera específica e individual como incorporada a este documento a manera de referencia en su totalidad.

Reivindicaciones:

1. Un derivado de carbohidrato de rapamicina que tiene la estructura de la formula (I):



donde

R^1 y R^2 son independientemente hidrógeno o -X-Z,

Donde n= 0 o 1; y

Donde cada X es un enlace (linker), y cada Z es un medio carbohidrato seleccionado independientemente a partir del grupo conformado por monosacárido, oligosacárido y pseudoazúcar, donde Z se adhiere a X a través de un átomo de oxígeno hidróxilo de Z con la condición de que R^1 y R^2 no sean ambos hidrógeno.

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2. El derivado de carbohidrato de rapamicina de la reivindicación 1, donde R^1 es hidrógeno y

R^2 es –X-Z.

3. El derivado de carbohidrato de rapamicina de la reivindicación 2, donde X se selecciona partir del grupo conformado por:

(i) $-R^3C(O)-$;

(ii) $-C(O)R^3-$:

(iii) $-R^3S(O)_2-$: y

(iv) $-S(O)_2R^3-$:

donde R^3 se selecciona a partir del grupo conformado por:

(a) $-(CH_2)_p-$ donde p es un entero de 1 a 18;

(b) $-(CH_2)_n-O-(CH_2)_m-$ donde n y m son cada uno de ellos de manera independiente un entero de 2 a 6; y

(c) un enlace.

4. El derivado de carbohidrato de rapamicina de la reivindicación 3, donde X se selecciona a partir del grupo conformado por -C(O)- y $-SO_2-$.

5. El derivado de carbohidrato de la rapamicina de la reivindicación 2, donde X es un solo grupo funcional.

6. El derivado de carbohidrato de rapamicina de la reivindicación 2, donde Z se selecciona a partir del grupo conformado por fructosa, fucitol y alosa.

7. El derivado de carbohidrato de rapamicina de la reivindicación 6, donde Z es D-fructosa.

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8. El derivado de carbohidrato de rapamicina de la reivindicación 3, donde Z es un derivado monosacárido en donde por lo menos uno de los grupos hidróxilos del monosacárido se reemplaza por hidrógeno, alcoxi, alcanoato o grupo halógeno.

9. El derivado de carbohidrato de rapamicina de la reivindicación 1, donde R^1 es –X-Z y R^2 es hidrógeno.

10. El derivado de carbohidrato de rapamicina de la reivindicación 9, donde X se selecciona a partir del grupo conformado por:

(i) $-R^3C(O)-$;

(ii) $-C(O)R^3-$;

(iii) $-R^3S(O)_2-$; y

(iv) $-S(O)_2R^3-$;

donde R^3 se selecciona a partir del grupo conformado por:

(a) $-(CH_2)_p-$ donde p es un entero de 1 a 18;

(b) $-(CH_2)_n-O-(CH_2)_m-$ donde n y m son cada uno de ellos de manera independiente un entero de 2 a 6; y

(c) un enlace.

11. El derivado de carbohidrato de rapamicina de la reivindicación 3, donde X se selecciona a partir del grupo conformado por -C(O)- y $–SO_2-$.

12. El derivado de carbohidrato de la rapamicina de la reivindicación 2, donde X es un solo grupo funcional.

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13. El derivado de carbohidrato de rapamicina de la reivindicación 2, donde Z se selecciona a partir del grupo conformado por fructosa, fucitol y alosa.

14. El derivado de carbohidrato de rapamicina de la reivindicación 6, donde Z es D-fructosa.

15. El derivado de carbohidrato de rapamicina de la reivindicación 3, donde Z es un derivado monosacárido en donde por lo menos uno de los grupos hidróxilos del monosacárido se reemplaza por hidrógeno, alcoxi, alcanoato o grupo halógeno.

[00158] 16. Un derivado de carbohidrato de rapamicina que se selecciona a partir del grupo conformado por:

42-O-(Metil-D-glucosilcarbonilo)rapamicina;

42-O-[2-(Metil-D-glucosilcarboniloxi)etil]rapamicina;

31-O-(Metil-D-glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(metil-D-glucosilcarbonilo)rapamicina;

42-O-(2-O-Metil-D-fructosilcarbonilo)rapamicina;

42-O-[2-(2-O-Metil-D-fructosilcarboniloxi)etil]rapamicina;

42-O-(2-O-Metil-L-fructosilcarbonilo)rapamicina;

42-O-[2-(2-O-Metil-L-fructosilcarboniloxi)etil]rapamicina;

31-O-(2-O-Metil-D-fructosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(2-O-metil-D-fructosilcarbonilo)rapamicina;

31-O-(2-O-Metil-L-fructosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(2-O-metil-L-fructosilcarbonilo)rapamicina;

42-O-(D-Alosilcarbonilo)rapamicina;

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42-O-[2-(D-Alosilcarboniloxi)etil]rapamicina;

42-O-(L-Alosilcarbonilo)rapamicina;

42-O-[2-(L-Alosilcarboniloxi)etil]rapamicina;

31-O-(D-Alosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-alosilcarbonilo)rapamicina;

31-O-(L-Alosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-alosilcarbonilo)rapamicina;

42-O-(D-Fructoslilcarbonilo)rapamicina;

42-O-[2-(D-Fructosilcarboniloxi)etil]rapamicina;

42-O-(L-Fructoslilcarbonilo)rapamicina;

42-O-[2-(L-Fructosilcarboniloxi)etil]rapamicina;

31-O-(D-Fructoslilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-fructoslilcarbonilo)rapamicina;

31-O-(L-Fructoslilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-fructoslilcarbonilo)rapamicina;

42-O-(D-Fucitolilcarbonilo)rapamicina;

42-O-[2-(D-Fucitolilcarboniloxi)etil]rapamicina;

42-O-(L-Fucitolilcarbonilo)rapamicina;

42-O-[2-(L-Fucitolilcarboniloxi)etil]rapamicina;

31-O-(D-Fucitolilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-fucitolilcarbonilo)rapamicina;

31-O-(L-Fucitolilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-fucitolilcarbonilo)rapamicina;

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Traductor e Intérprete Oficial
Res. 5906 de 1982 del Min. de Justicia
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42-O-(D-Glucalilcarbonilo)rapamicina;

42-O-[2-(D-Glucalilcarboniloxi)etil]rapamicina;

42-O-(D-Glucosilcarbonilo)rapamicina;

42-O-[2-(D-Glucosilcarboniloxi)etil]rapamicina;

42-O-(L-Glucosilcarbonilo)rapamicina;

42-O-[2-(L-Glucosilcarboniloxi)etil]rapamicina;

31-O-(D-Glucalilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-glucalilcarbonilo)rapamicina;

31-O-(D-Glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-glucosilcarbonilo)rapamicina;

31-O-(L-Glucosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-glucosilcarbonilo)rapamicina;

42-O-(L-Sorbosilcarbonilo)rapamicina;

42-O-(D-Sorbosilcarbonilo)rapamicina;

31-O-(L-Sorbosilcarbonilo)rapamicina;

31-O-(D-Sorbosilcarbonilo)rapamicina;

42-O-[2-(L-Sorbosilcarboniloxi)etil]rapamicina;

42-O-[2-(D-Sorbosilcarboniloxi)etil]rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-sorbosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(L-sorbosilcarbonilo)rapamicina;

42-O-(D-Lactalilcarbonilo)rapamicina;

42-O-[2-(D-Lactalilcarboniloxi)etil]rapamicina;

31-O-(D-Lactalilcarbonilo)rapamicina;

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Res. 5966 de 1982 del Min. de Justicia
Min. de Relaciones Exteriores

42-O-(2-Hidroxietil)-31-O-(D-lactalilcarbonilo)rapamicina;

42-O-(D-Sucrosilcarbonilo)rapamicina;

42-O-[2-(D-Sucrosilcarboniloxi)etil]rapamicina;

31-O-(D-Sucrosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-sucrosilcarbonilo)rapamicina;

42-O-(D-Gentobiosilcarbonilo)rapamicina

42-O-[2-(D-Gentobiosilcarboniloxi)etil]rapamicina;

31-O-(D-Gentobiosilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(D-gentobiosilcarbonilo)rapamicina

42-O-(D-Celobiosilcarbonilo)rapamicina;

42-O-[2-(D-Celobiosilcarboniloxi)etil]rapamicina;

31-O-(D-Celobiosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-celobiosilcarbonilo)rapamicina;

42-O-(D-Turanosilcarbonilo)rapamicina;

42-O-[2-(D-Turanosilcarboniloxi)etil]rapamicina;

31-O-(D-Turanosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-turanosilcarbonilo)rapamicina;

42-O-(D-Palatinosilcarbonilo)rapamicina;

42-O-[2-(D-Palatinosilcarboniloxi)etil]rapamicina;

31-O-(D-Palatinosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-palatinosilcarbonilo)rapamicina;

42-O-(D-Isomaltosilcarbonilo)rapamicina;

42-O-[2-(D-Isomaltosilcarboniloxi)etil]rapamicina;

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Min. de Relaciones Exteriores

31-O-(D-Isomaltosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-isomaltosilcarbonilo)rapamicina;

42-O-(D-Maltulosilcarbonilo)rapamicina;

42-O-[2-(D-Maltulosilcarboniloxi)etil]rapamicina;

42-O-(D-Maltosilcarbonilo)rapamicina;

42-O-[2-(D-Maltosilcarboniloxi)etil]rapamicina;

31-O-(D-Maltulosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-maltulosilcarbonilo)rapamicina;

31-O-(D-Maltosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-maltosilcarbonilo)rapamicina;

42-O-(D-Lactosilcarbonilo)rapamicina;

42-O-[2-(D-Lactosilcarboniloxi)etil]rapamicina;

31-O-(Methil-D-lactosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(metil-D-lactosilcarbonilo)rapamicina;

42-O-(D-Melibiosilcarbonilo)rapamicina;

31-O-(D-Melibiosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-melibiosilcarbonilo)rapamicina;

42-O-(D-Leucrosilcarbonilo)rapamicina;

42-O-[2-(D-Leucrosilcarboniloxi)etil]rapamicina;

31-O-(D-Leucrosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-leucrosilcarbonilo)rapamicina;

42-O-(D-Rafinosilcarbonilo)rapamicina;

42-O-[2-(D-Rafinosilcarboniloxi)etil]rapamicina;

GABRIEL RUEDA CHADID
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Res. 5996 de 1982 del Min. de Justicia
Min. de Relaciones Exteriores

31-O-(D-Rafinosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-rafinosilcarbonilo)rapamicina;

42-O-(D-Isomaltotriosilcarbonilo)rapamicina;

42-O-[2-(D-Isomaltosilcarboniloxi)etil]rapamicina;

31-O-(D-Isomaltotriosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-isomaltotriosilcarbonilo)rapamicina;

42-O-(D-Celotetraosilcarbonilo)rapamicina;

42-O-[2-(D-Celotetraosilcarboniloxi)etil]rapamicina;

31-O-(D-Celotetraosilcarbonilo)rapamicina;

42-O-(2-Hidroxietil)-31-O-(D-celotetraosilcarbonilo)rapamicina;

42-O-(Valiolilcarbonilo)rapamicina

42-O-[2-(D-Valiolilcarboniloxi)etil]rapamicina;

31-O-(Valiolilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valiolilcarbonilo)rapamicina

42-O-(Valiolonilcarbonilo)rapamicina

42-O-[2-(D-Valiolonilcarboniloxi)etil]rapamicina;

31-O-(Valiolonilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valiolonilcarbonilo)rapamicina

42-O-(Valienolilcarbonilo)rapamicina

42-O-[2-(D-Valienolilcarboniloxi)etil]rapamicina;

31-O-(Valienolilcarbonilo)rapamicina

42-O-(2-Hidroxietil)-31-O-(valienolilcarbonilo)rapamicina

42-O-(Valienoneilcarbonilo)rapamicina

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42-O-[2-(D-Valienoneilcarboniloxi)etil]rapamicina;

31-O-(Valienoneilcarbonilo)rapamicina

42-O-(2-Hidroxietil)- 31-O-(valienoneilcarbonilo)rapamicina

17. Una composición farmacéutica que comprende el derivado de carbohidrato de rapamicina de la reivindicación 1 o una de sus sales farmacéuticamente aceptables, y un portador farmacéuticamente aceptable.

18. Un método para el tratamiento de una enfermedad que puede ser tratada mediante aplicación de la rapamicina, que comprende la administración de una cantidad terapéuticamente eficaz de una composición farmacéutica de la reivindicación 17 a un sujeto que lo requiera.

19. El método de la reivindicación 18, donde la enfermedad se selecciona a partir del grupo conformado por rechazo de transplante, huésped versus enfermedad de injerto, injerto versus enfermedad de huésped, leucemia, linfoma, trastornos vasculares hiperproliferativos, enfermedad autoinmune, enfermedades de inflamación, tumores sólidos e infecciones fungales.

20. Un dispositivo médico que comprende un derivado de carbohidrato de rapamicina de la reivindicación 1 o una de sus sales farmacéuticamente aceptables.

21. Un dispositivo médico donde el mencionado dispositivo médico está revestido con un derivado de carbohidrato de rapamicina de la reivindicación 1.

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Res: 5998 de 1992 del Min. de Justicia
Min. de Relaciones Exteriores

22. Un dispositivo médico de la reivindicación 21 donde el dispositivo médico se selecciona a partir del grupo conformado por stents, injertos e implantes.

23. El dispositivo médico de la reivindicación 20, donde dicho dispositivo médico se selecciona a partir del grupo conformado por stents, injertos e implantes.

24. Un método para el tratamiento de una enfermedad que puede ser tratada por aplicación de rapamicina, que comprende la co-administración de una cantidad terapéuticamente eficaz de un compuesto farmacéutico de la reivindicación 17 a un sujeto que lo requiera, con composición farmacéutica que comprende un compuesto seleccionado a partir del grupo conformado por ciclosporina o derivado de la ciclosporina, un esteroide o un compuesto inmunomodulador.

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Traductor e Interprete Oficial
Res. 5296 de 1982 del Min. de Justicia
Min. de Relaciones Exteriores

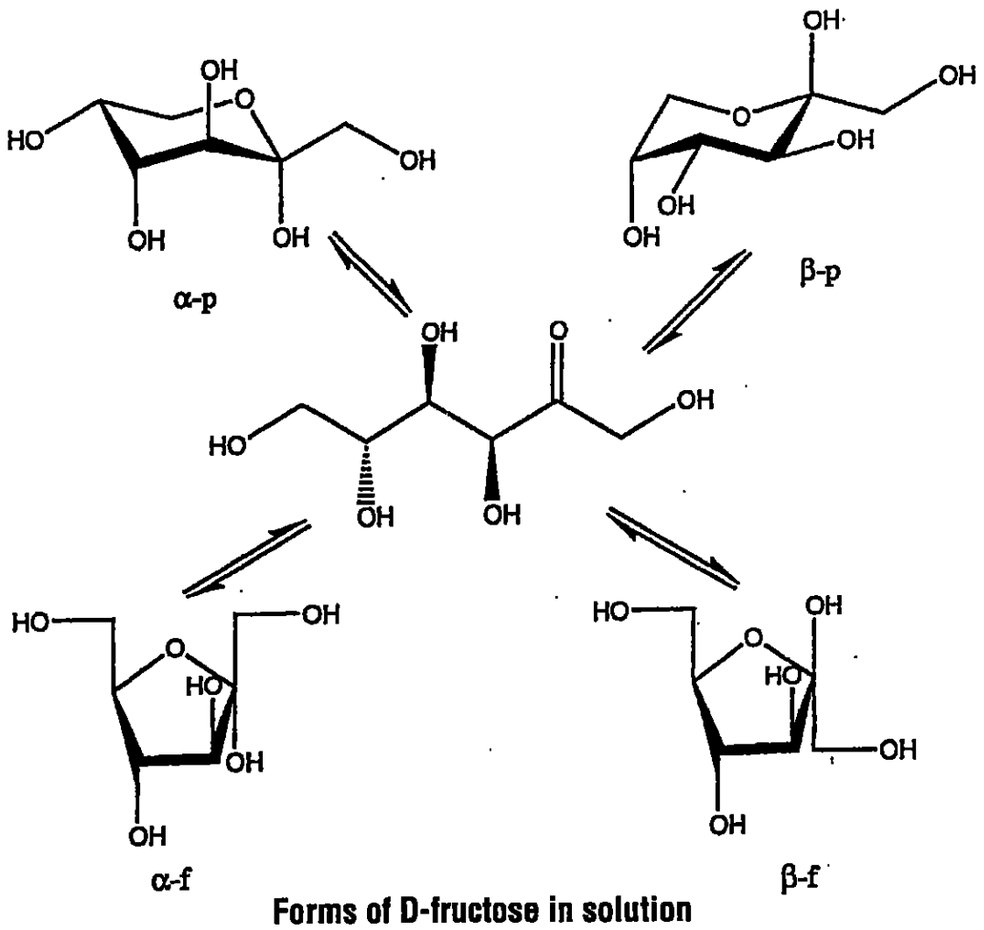
PATENTE
Expediente No. 550

DERIVADOS DE CARBOHIDRATOS DE RAPAMICINA

RESUMEN

El presente invento proporciona rapamicinas modificadas que tienen uno o varios monosacáridos, oligosacáridos, pseudoazúcares o sus derivados específicos que se adhieren a través de un enlace (linker) para crear derivados de carbohidrato de rapamicina que tienen perfiles mejorados farmacocinéticos y/o farmacodinámicos. Por ejemplo, la administración del derivado de carbohidrato de rapamicina da como resultado perfiles alterados farmacocinéticos y reducción en toxicidades. De esta manera, el presente invento proporciona compuestos con características que están claramente diferenciadas de otros fármacos en sus clases como son las rapamicinas.

GABRIEL RUEDA CHADID
Traductor e Interprete Oficial
Res. 5996 de 1982 del Min. de Justicia
Min. de Relaciones Exteriores



Forms of D-fructose in solution

FIG. 1

General Route of Synthesis for Rapamycin Carbohydrate Derivatives

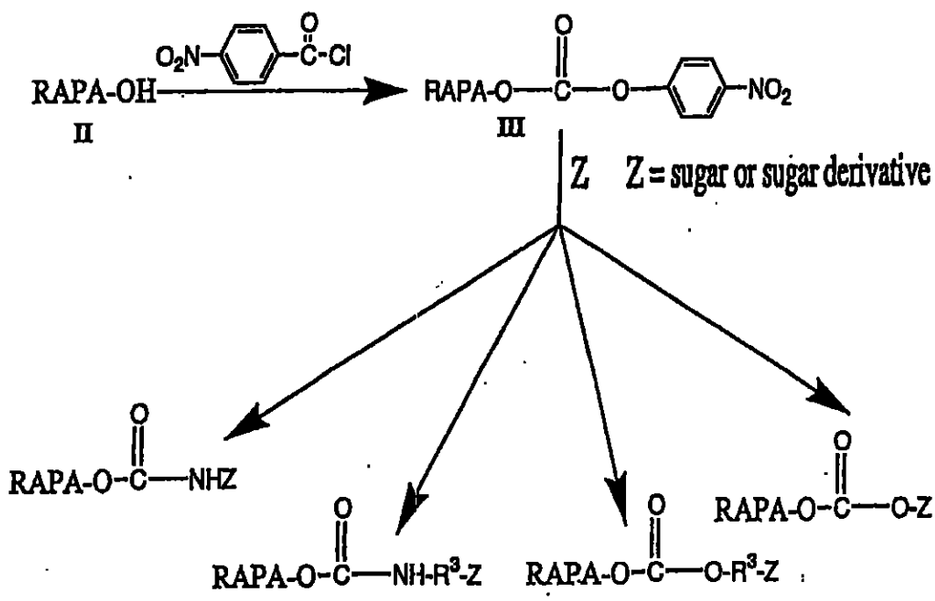
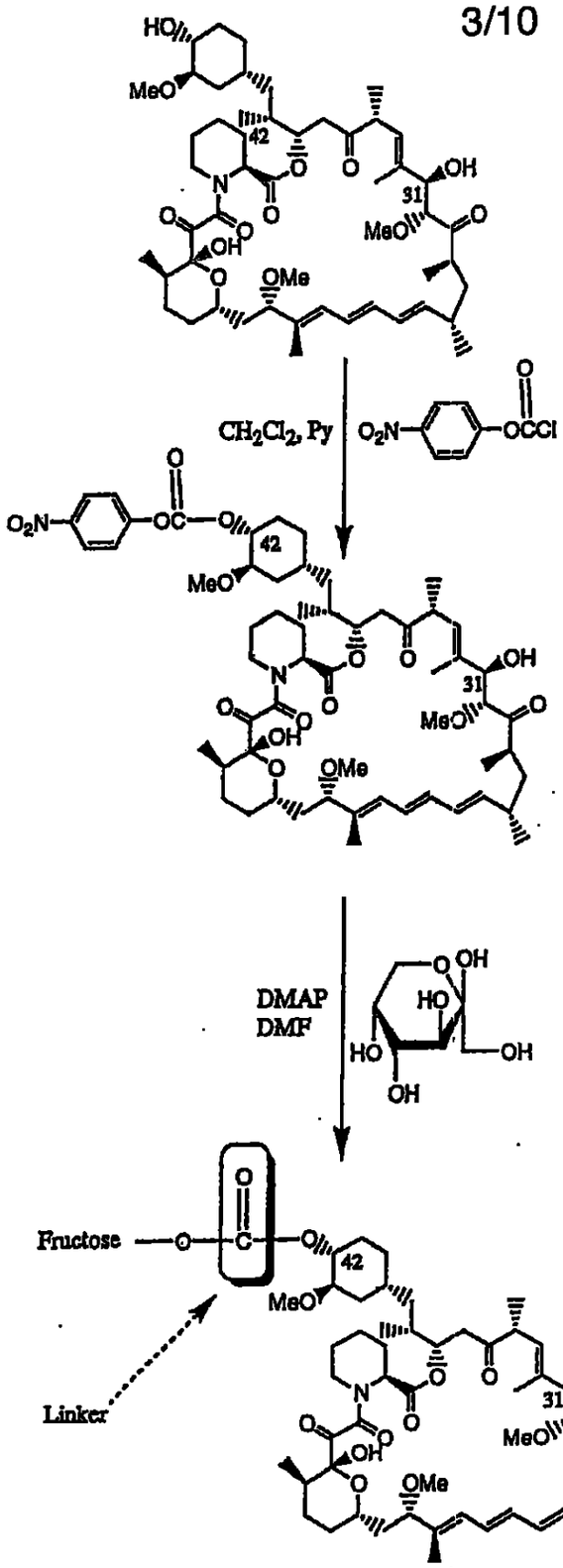


FIG. 2



Fructose = α-fructopyranose or β-fructopyranose or α-fructofuranose or β-fructofuranose attached through the 1 or 6 position.

FIG. 3

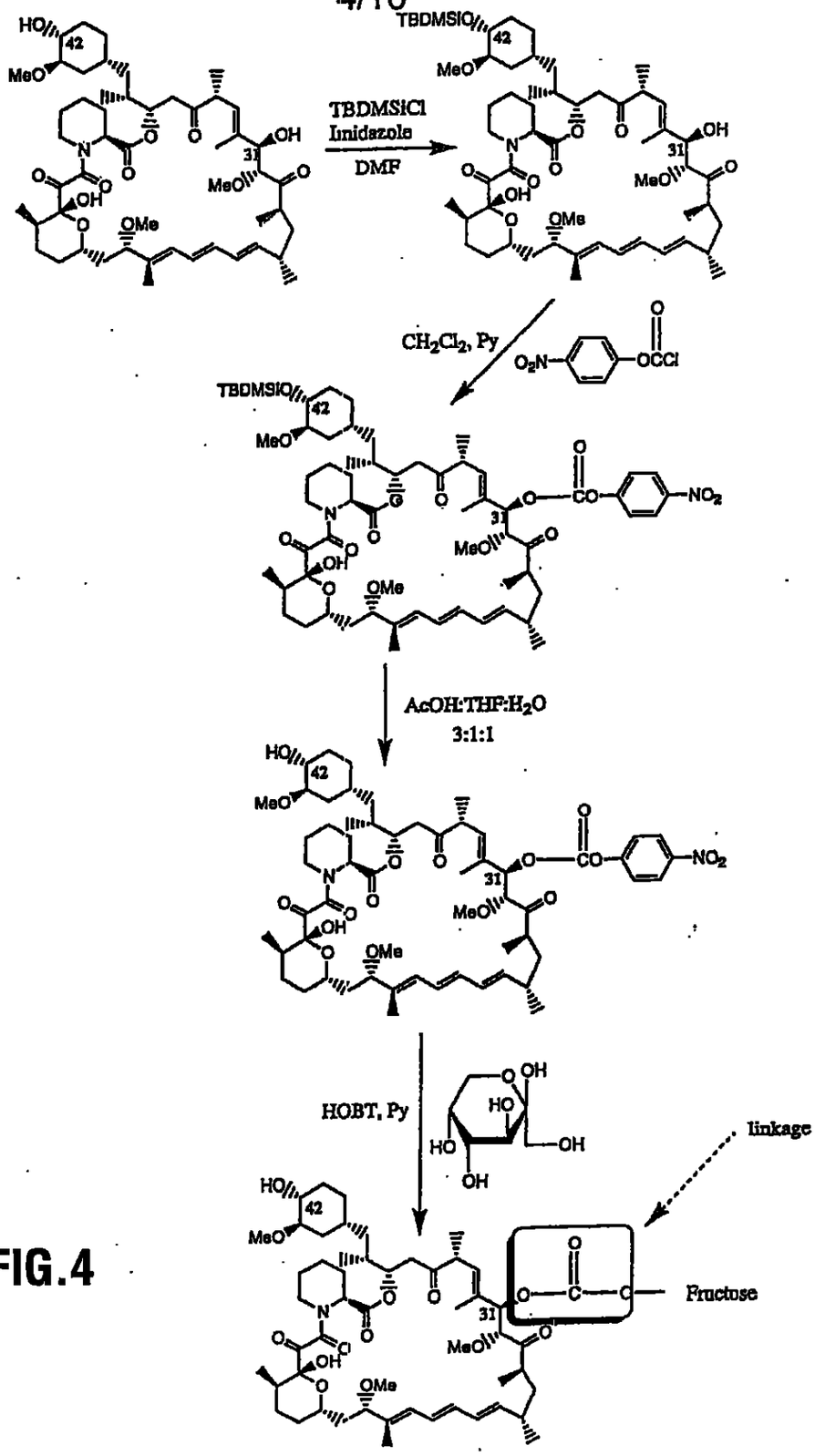

TBDMSiCl
Imidazole
DMF
CH_2Cl_2, Py
O_2N
OCCl
AcOH:THF:H_2O
3:1:1
HOBT, Py
linkage
Fructose

FIG.4

***In vitro* Immunosuppressive Activity of Rapamycin 42-O-(D-Fructosylcarbonyl)rapamycin, and a Carbamate-linked Analog in a Cell Proliferation assay.**

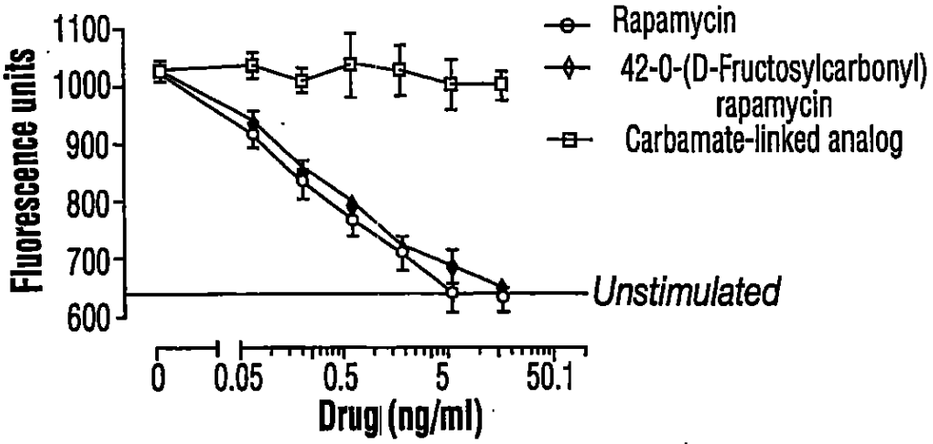


FIG. 5

Pharmacokinetic Profiles in the Rat of Rapamycin-42-O-Carbohydrate Derivatives

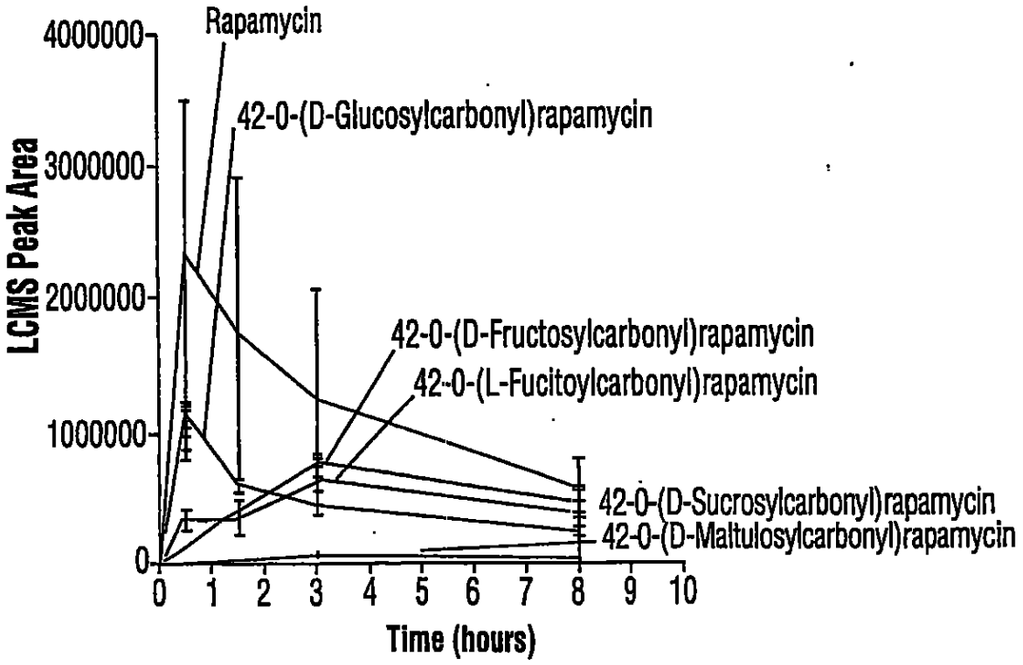


FIG. 6

Pharmacokinetic Profiles in the Rat of Rapamycin-31-O-Carbohydrate Derivitives

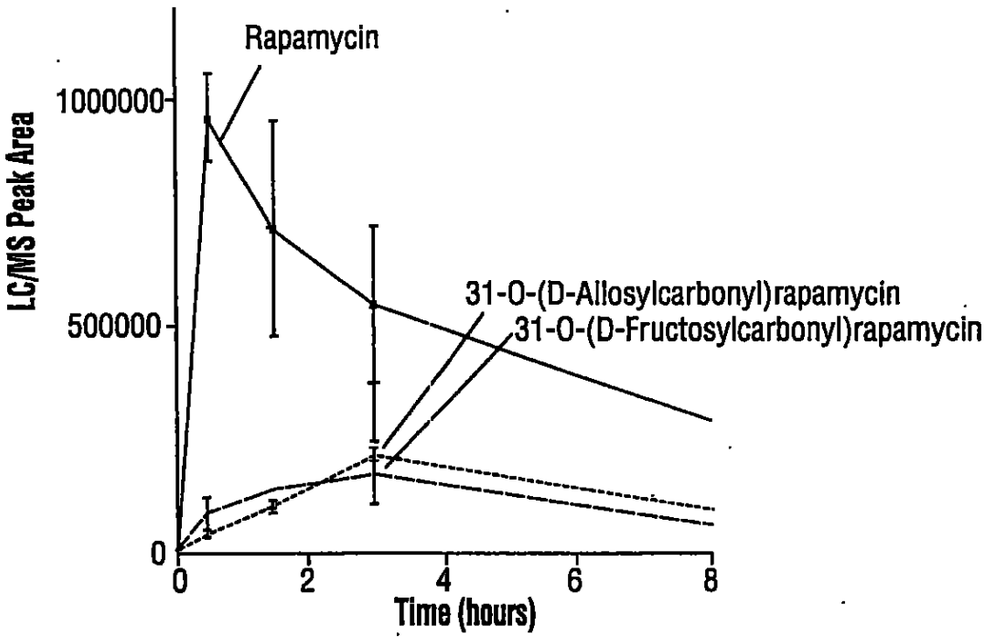


FIG. 7

Pharmacokinetic Profiles in the Rat of Rapamycin-42-O-Carbohydrate Derivitives

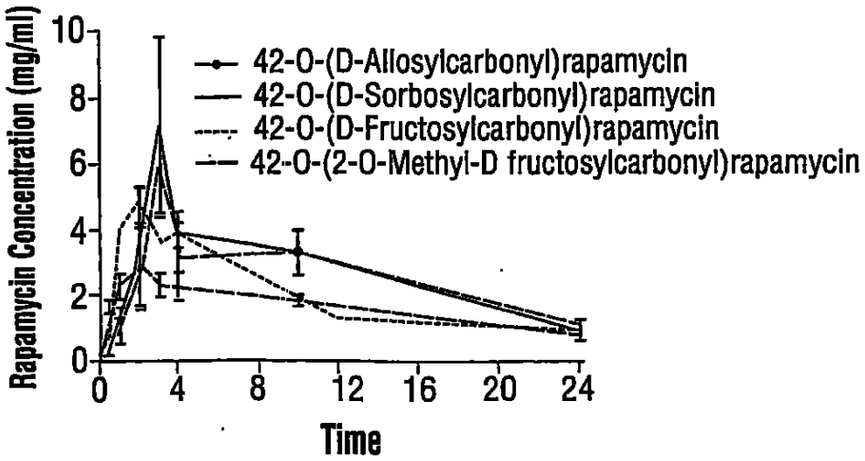
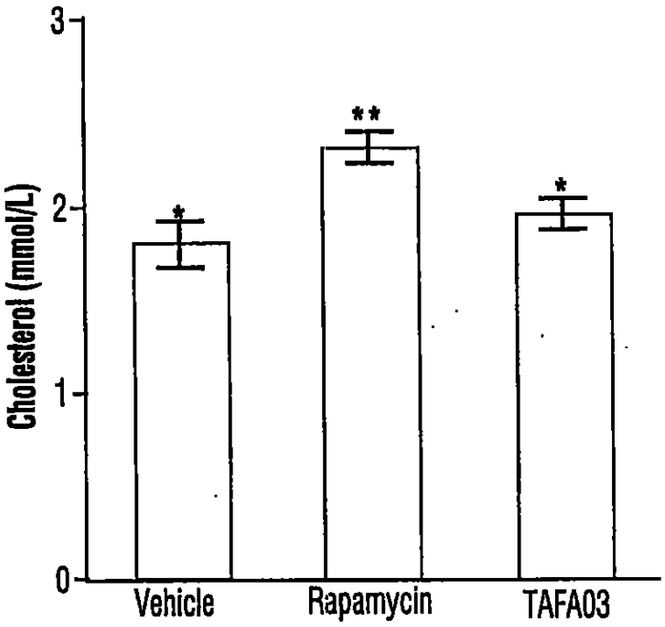


FIG. 8

Serum Cholesterol Levels Following 12-Day Dosing of 42-O-(D-Fructosylcarbonyl)rapamycin and Rapamycin in Sprague-Dawley Rats



* Significantly Lower than Rapamycin $p < 0.01$

** Significantly higher than Vehicle, $p < 0.01$

ANOVA, Dunnell's Post Hoc Test

FIG. 9

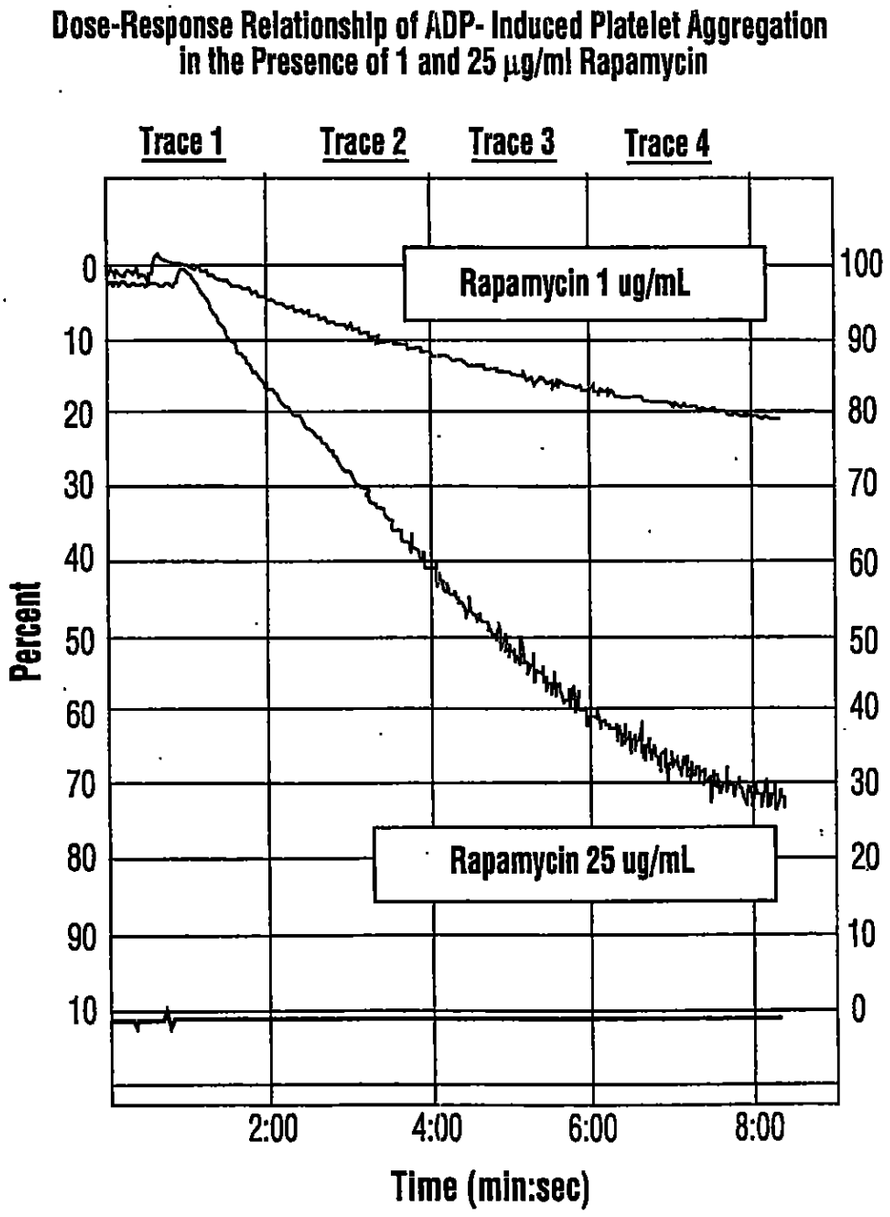

Dose-Response Relationship of ADP- Induced Platelet Aggregation
in the Presence of 1 and 25 µg/ml Rapamycin
Trace 1
Trace 2
Trace 3
Trace 4
Rapamycin 1 ug/mL
Rapamycin 25 ug/mL
Percent
0
10
20
30
40
50
60
70
80
90
10
100
90
80
70
60
50
40
30
20
10
0
2:00
4:00
6:00
8:00
Time (min:sec)

FIG. 10

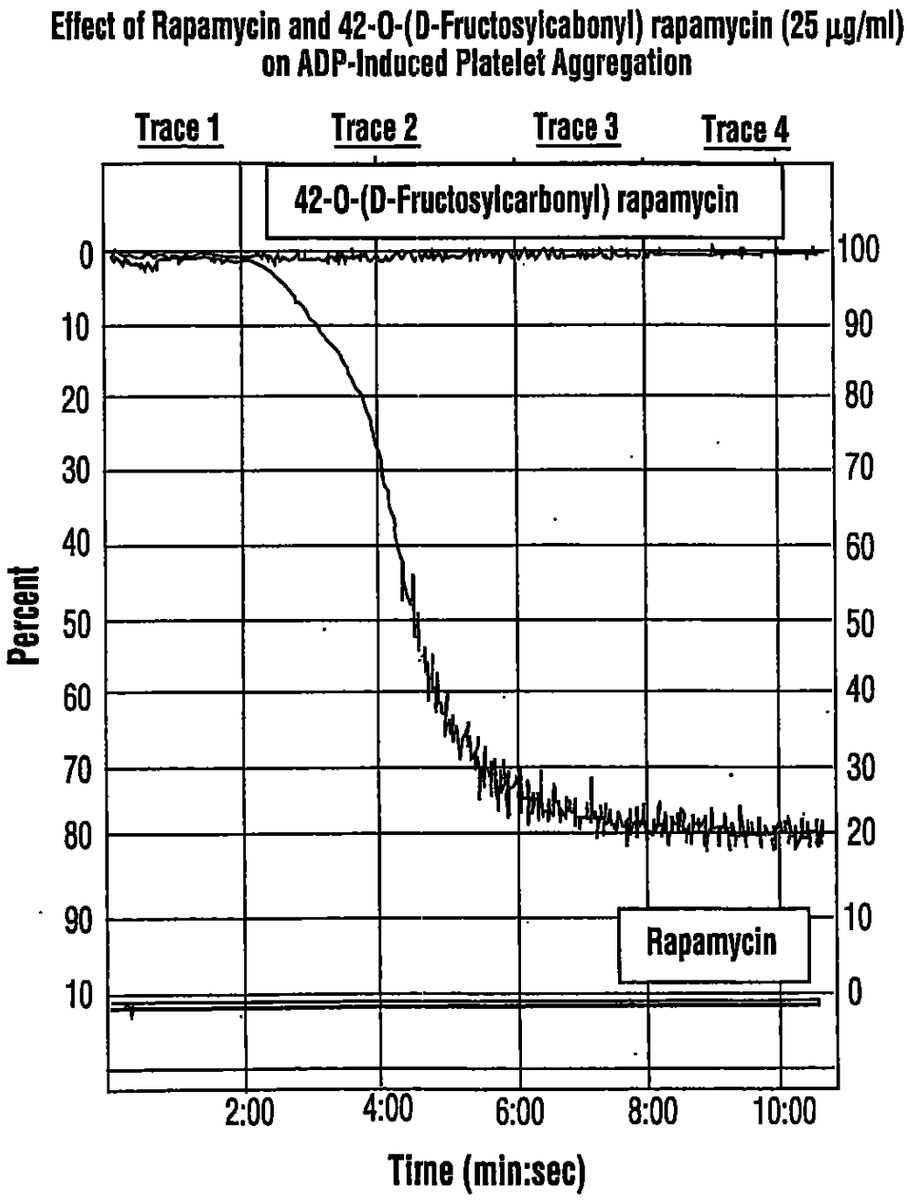
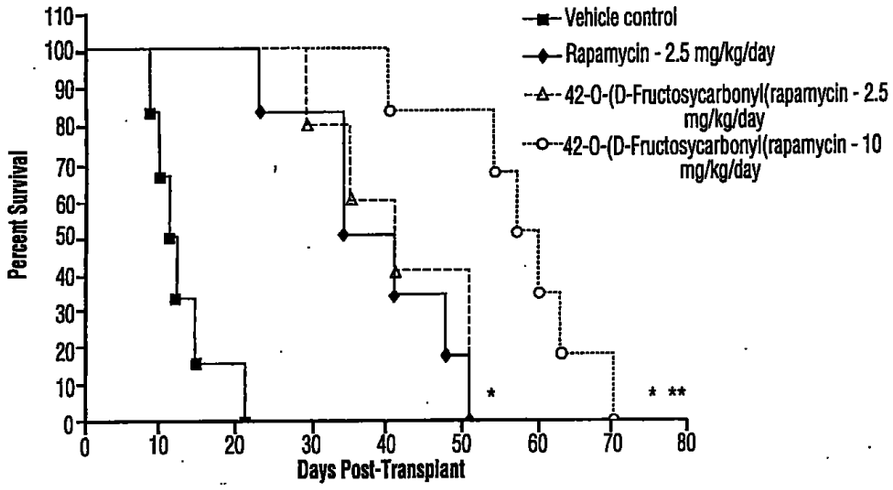

Effect of Rapamycin and 42-O-(D-Fructosylcabonyl) rapamycin (25 µg/ml) on ADP-Induced Platelet Aggregation
Trace 1
Trace 2
Trace 3
Trace 4
42-O-(D-Fructosylcarbonyl) rapamycin
Rapamycin
Percent
0
10
20
30
40
50
60
70
80
90
10
100
90
80
70
60
50
40
30
20
10
0
2:00
4:00
6:00
8:00
10:00
Time (min:sec)

FIG. 11

Graft Survival Curves of Heterotopic Heart Allografts in Rats Receiving Oral 42-O-(D-Fructosylcarbonyl)rapamycin (2.5 and 10 mg/kg/day), Rapamycin (2.5 mg/kg/day),or Vehicle



* Different from Vehicle, $p < 0.005$
** Different from Rapamycin, $p < 0.05$
Wilcoxon Rank Sum

FIG. 12

DOCKETED

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
TORYS LLP
Maritime Life Tower
Suite 3000
79 Wellington St. W.
Box 270, TD Centre
Toronto, Ontario
M5K 1N2 Canada

PCT

INVITATION TO CORRECT DEFECTS IN THE INTERNATIONAL APPLICATION

(PCT Articles 3(4)(i) and 14(1) and Rule 26)

Date of mailing (day/month/year): 04 June 2004 (04-06-2004)

Applicant's or agent's file reference	REPLY DUE
31284-2007	within ONE months/days from the above date of mailing
International application No. PCT/CA2004/000724	International filing date (day/month/year): 14 May 2004 (14-05-2004)

Applicant: ISOTECHNIKA INTERNATIONAL INC. ET AL

1. [X] The applicant is hereby invited, within the time limit indicated above, to correct, in the international application as filed, the defects specified on the attached:

   [X] Annex A
   [ ] Annex B1 (*text matter of the international application as filed*)
   [X] Annex C1 (*drawings of the international application as filed*)

2. [ ] The applicant is hereby invited, within the time limit indicated above, to correct, in the translation of the international application furnished under Rule 12.3 or 12.4, the defects specified on the attached:

   [ ] Annex A
   [ ] Annex B2 (*text matter of the translation of the international application*)
   [ ] Annex C2 (*drawings of the translation of the international application*)

DUE DATE PROCESSED
DUE JUL 4/04

Additional observations (if necessary):

HOW TO CORRECT THE DEFECTS?

Correction must be submitted by filing a replacement sheet embodying the correction and a letter accompanying the replacement sheet, which shall draw attention to the difference between the replaced sheet and the replacement sheet. A correction may be stated in a letter only if it is of such a nature that it can be transferred from the letter to the record copy without adversely affecting the clarity and direct reproducibility of the sheet onto which the correction is to be transferred (*Rule 26.4*).

ATTENTION

Failure to correct the defects will result in the international application being considered withdrawn by this receiving Office (see Rule 26.5 for further details).

A copy of this invitation and any attachment has been sent to the International Bureau
[X] and the International Searching Authority.

Name and mailing address of the Receiving Office	Authorized Officer
Commissioner of Patents Canadian Receiving Office Box PCT, Ottawa/Hull K1A 0C9 Facsimile No. (819) 953-9538	Jean-Luc Robert (819) 994-6587

Form PCT/RO/106 (January 2004)

ANNEX A TO FORM PCT/RO/106

International application No.
PCT/CA2004/000724

The receiving Office has found the following defects in the international application as filed:

1. As to signature of the international application (Rules 4.15, 26.2*bis* and and 90.4), the request:

a. [ ] is not signed* by the applicant or, if there is more than one applicant, by at least one of them

b. [ ] is not accompanied by the statement referred to in the check list in Box No. IX of the request explaining the lack of the signature of an applicant for the designation of the United States of America

c. [X] is signed by what appears to be an agent/common representative but:

[X] the international application is not accompanied by a power of attorney appointing him.

[ ] the power of attorney accompanying the international application was not signed by all the applicants

d. [X] other (*specify*):PLEASE NOTE THE CANADIAN RECEIVING OFFICE IS AN EXCEPTION TO RULE 26.2bis(A)

* Although Rule 4.15 requires that all applicants must sign the request (e.g. including all inventors/applicants for the designation of the United States of America), for the purposes of Article 14(1)(a)(i), if there is more than one applicant, it shall be sufficient that the request be signed by one of them (Rule 26.2*bis*(a)).

However, the applicant's attention is drawn to the fact that the national law applied by each designated Office may require, in connection with the processing of the international application in the national phase, that the applicant furnish the confirmation of the international application by the signature of any applicant for the designated State who has not signed the request (Rule 51*bis*.1(a)(vi)).

2. As to indications concerning the applicant* who is entitled, according to Rule 19.1, to file the international application with the receiving Office, the request (Rules 4.4, 4.5 and 26.2*bis*(b)):

a. [ ] does not properly indicate the applicant's name (*specify*):

b. [ ] does not indicates the applicant's address
c. [ ] does not properly indicate the applicant's address (*specify*):

d. [ ] does not indicate the applicant's *nationality*
e. [ ] does not indicate the applicant's residence

[ ] Further observations about *indications concerning* other applicants (if applicable):

* Although Rules 4.4 and 4.5 require indications concerning the applicant, or if there are several applicants, of each of them, for the purposes of Article 14(1)(a)(ii), if there is more than one applicant, it shall be sufficient that the indications required under Rule 4.5(a)(ii) and (iii) be provided in respect of one of the m who is entitled according to Rule 19.1 to file the international application with the receiving Office (Rule 26.2*bis*(b)).

However, the applicant's attention is drawn to the fact that the national law applied by each designated Office may require, in connection with the processing of the international application in the national phase, that the applicant furnish any missing indication required under Rule 4.5(a)(ii) and (iii) in respect of any applicant for the designated State (Rule 51*bis*.1(a)(vii)).

3. As to the language of certain elements of the international application, other than the description and claims (Rules 12.1(c) and 26.3*ter*(a) and (c)):

a. [ ] the request is not in a language of publication accepted by this receiving Office; (the) language(s) accepted by this receiving Office is/are: English or French

b. [ ] the text matter of the drawings is not in the language in which the international application is to be published, which is: English or French

c. [ ] the abstract is not in the language in which the international application is to be published, which is: English or French

4. The title of the invention:

a. [ ] is not indicated in Box No. *I* of the request (*Rule* 4.1(a))

b. [ ] is not indicated at the top of the first sheet of the description (Rule 5.1(a))

c. [ ] as appearing in Box No. *I* of the request is *not* identical with the title heading the description (Rule 5.1(a)).

5. As to the abstract (Rule 8):

[ok] the international application does not contain an abstract (RECEIVED)

other (*specify*): PLEASE REMOVE THE TITLE ON THE ABSTRACT SHEETS

ANNEX C1 TO FORM PCT/RO/106

International application No.
PCT/CA2004/000724

This receiving Office has found that, with regard to the presentation of the drawings of the international application as filed, the physical requirement are not complied with to the extent that compliance therewith is necessary for:

1. [X] reasonably uniform international publication (Rules 11 and 26.3(a)(i)) (*defects to be specified*):

Sheets containing drawings:

a [ ] the sheets do not admit of direct reproduction
b [ ] the sheets are not free from creases, cracks, folds
c [ ] one side of the sheets is not left unused
d [ ] the paper of the sheets is not flexible/strong/white/smooth/non-shiny/durable
e [ ] the drawings do not commence on a new sheet
f [ ] the sheets are not connected as prescribed (Rule 114(b))
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Date of mailing (day/month/year) 04 June 2004 (04-06-2004)

Applicant's or agent's file reference	REPLY DUE
31284-2007	See paragraph 1 below
International Application No. PCT/CA2004/000724	International filing date (day/month/year) 14 May 2004 (14-05-2004)
Applicant ISOTECHNIKA INTERNATIONAL INC. ET AL	

1. [ ] REPLY DUE within ____ months/days from the above date of mailing

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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.
31284-2007		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA2004/000724	14/05/2004	16/05/2003
Applicant		
ISOTECHNIKA INTERNATIONAL INC.		

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.

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[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.
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   b. [ ] none of the figures is to be published with the abstract.

ERNATIONAL SEARCH REPORT

.rnational Application No
PCT/CA2004/000724

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H15/04 C07D498/18 A61F2/02 A61K31/436

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 C07H C07D A61F A61K

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, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 342 507 B1 (FOSTER ROBERT T ET AL) 29 January 2002 (2002-01-29) figure 5	1-24.
A	WO 92/05179 A (AMERICAN HOME PROD) 2 April 1992 (1992-04-02) cited in the application	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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Name and mailing address of the ISA	Authorized officer
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TERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA2004/000724

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 6342507 B1	29-01-2002	US	2003139440 A1	24-07-2003
		US	2003130206 A1	10-07-2003
		US	2002028827 A1	07-03-2002
WO 9205179 A	02-04-1992	AU	653175 B2	22-09-1994
		AU	8659991 A	15-04-1992
		CA	2051781 A1	20-03-1992
		EP	0549727 A1	07-07-1993
		FI	931203 A	18-03-1993
		HU	65763 A2	28-07-1994
		IE	913302 A1	25-02-1992
		JP	6501012 T	27-01-1994
		MX	9101139 A1	04-05-1992
		NZ	239852 A	26-03-1993
		PT	98990 A	31-08-1992
		US	5358944 A	25-10-1994
		US	5378696 A	03-01-1995
		WO	9205179 A1	02-04-1992
		US	5221670 A	22-06-1993
		CA	2051782 A1	29-03-1992
		US	5130307 A	14-07-1992

US006342507B1

(12) **United States Patent**
Naicker et al.

(10) **Patent No.: US 6,342,507 B1**
(45) **Date of Patent: Jan. 29, 2002**

(54) **DEUTERATED RAPAMYCIN COMPOUNDS, METHOD AND USES THEREOF**

(75) Inventors: **Selvaraj Naicker; Randall W. Yatscoff; Robert T. Foster, all of Edmonton (CA)**

(73) Assignee: **Isotechnika, Inc., Edmonton (CA)**

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/348,015**

(22) Filed: **Jul. 6, 1999**

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/148,623, filed on Sep. 4, 1998, now abandoned.
(60) Provisional application No. 60/057,632, filed on Sep. 5, 1997.

(51) **Int. Cl.**[7] **C07D 491/16; A61K 31/445**
(52) **U.S. Cl.** **514/291; 540/456**
(58) **Field of Search** **540/456; 514/291**

(56) **References Cited**

PUBLICATIONS

Curran et al. (Tetrahedron Letters, vol. 33, No. 17, pp. 2295–2298, 1992).*
Park et al. (Journal of Biological Chemistry, vol. 267, No. 5 (15), pp 3316–3324, 1992).*
Connelly et al. (Biochemistry 1993, 32, 5583–5590).*

Dennis P. Curran, et al, Intramolecular Hydrogen Transfer Reactions of o-(Bromophenyl)dialkylsilyl Ethers. Preparationof Rapamycin-d_3, Tetrahedron Letters, vol. 33, No. 17, 1992, pp. 2295–2298 (Particular page of interest, p. 2297).

Don Stricker, Senior Technical Information Specialist of Chemical Abstract Service, C.A.S., Commercial Database Search of Deuterated Rapamycin, search conducted Mar. 9, 2000.

* cited by examiner

Primary Examiner—Bruck Kifle
(74) *Attorney, Agent, or Firm*—Burns, Doane, Swecker & Mathis, L.L.P.

(57) **ABSTRACT**

The synthesis of deuterated analogues of rapamycin is disclosed together with a method for use for inducing immunosupression and in the treatment of transplantation rejection, graft vs host disease, autoimmune diseases, diseases of inflammation leukemia/lymphoma, solid tumors, fungal infections, hyperproliferative vascular disorders. Also described is a method for the synthesis of water soluble deuteratred rapamycin compounds and their use as described above.

6 Claims, 2 Drawing Sheets

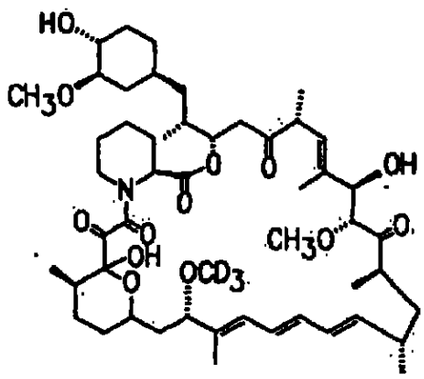

FIG. 1

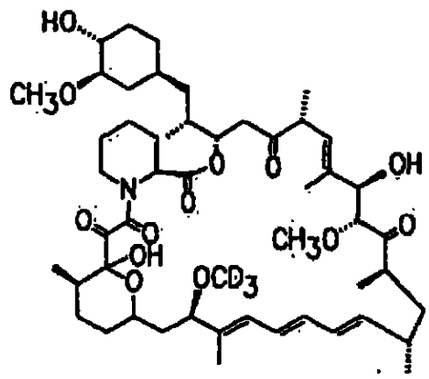

FIG. 2

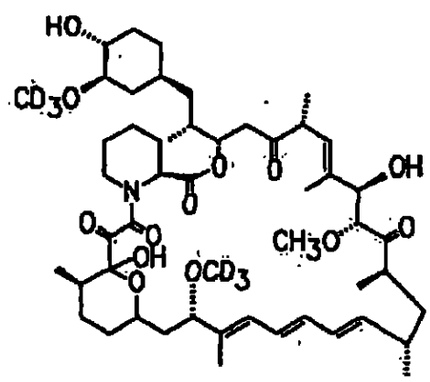

FIG. 3

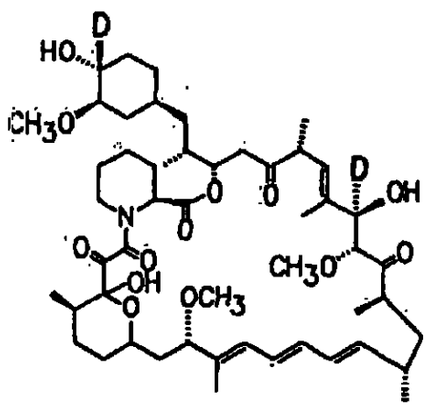

FIG. 4

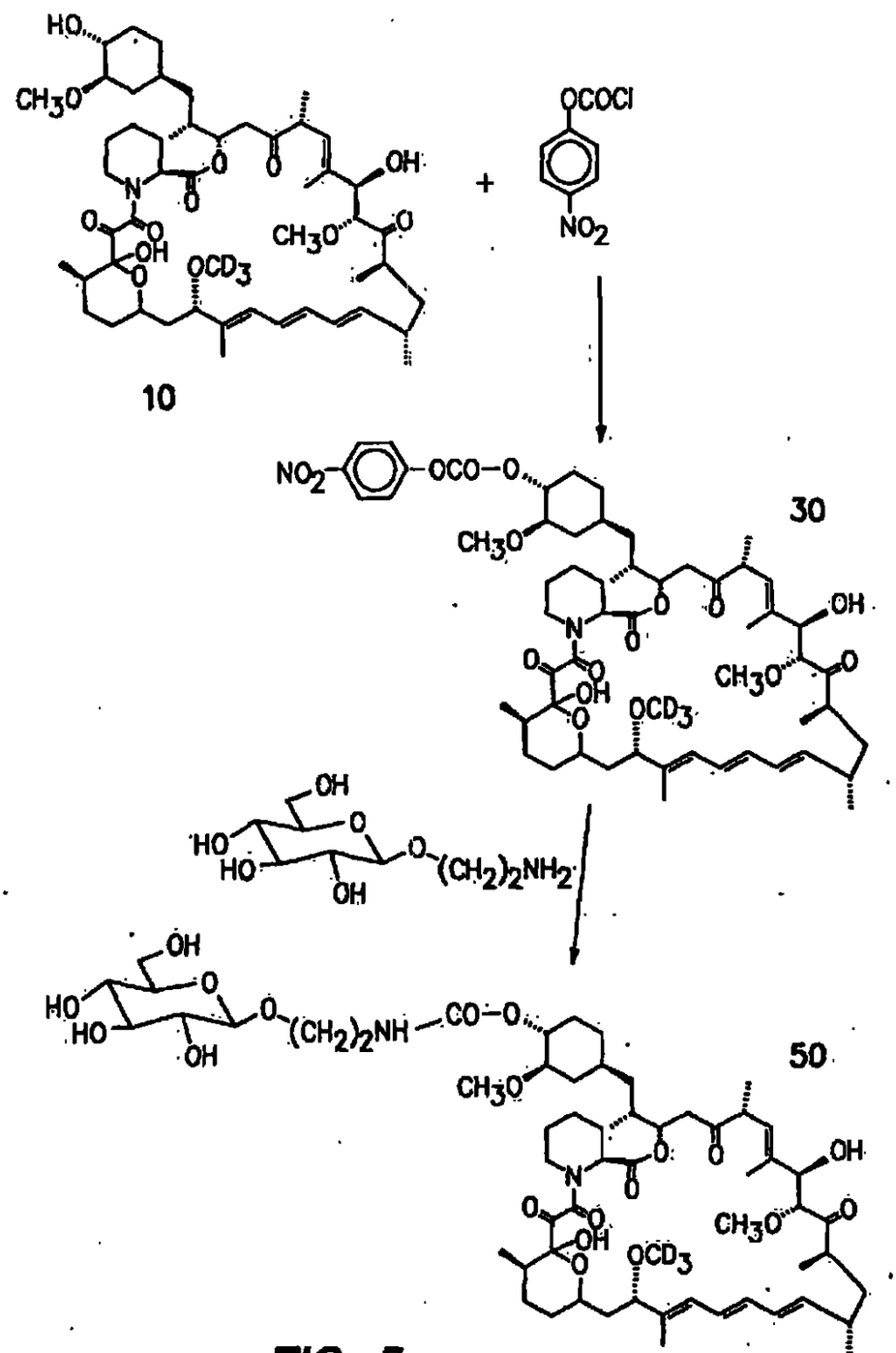

FIG. 5

DEUTERATED RAPAMYCIN COMPOUNDS, METHOD AND USES THEREOF

REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. patent application Ser. No. 09/148,623 filed on Sep. 4, 1998 now abandoned, which is based on provisional patent application No. 60/057,632 filed on Sep. 5, 1997, both of which are relied on and incorporated herein by reference.

BACKGROUND OF THE INVENTION

This invention relates to deuterated derivatives of rapamycin and a method for using them in the treatment of transplantation rejection, host vs. graft disease, graft vs. host disease, leukemia/lymphoma, hyperproliferative vascular disorders, autoimmune diseases, diseases of inflammation, solid tumors, and fungal infections.

Rapamycin, known as sirolimus, is a 31-membered macrolide lactone, $C_{51}H_{79}NO_{13}$, with a molecular mass of 913.6 Da. In solution, sirolimus forms two conformational trans-, cis-isomers with a ratio of 4:1 (chloroform) due to hindered rotation around the pipecolic acid amide bond. It is sparingly soluble in water, aliphatic hydrocarbons and diethyl ether, whereas it is soluble in alcohols, halogenated hydrocarbons and dimethyl sulfoxide. Rapamycin is unstable in solution and degrades in plasma and low-, and neuteral -pH buffers at 37° C. with half-life of <10 h. the structures of the degradation products have recently been characterized. Rapamycin is a macrocyclic trieno antibiotic produced by *Streptomyces hygroscopicus*, which was found to have antifungal activity, particularly against *Candida albicans*, both in vitro and in vivo [C. Vezina et al., J. Antibiot. 28, 721 (1975); S. N. Sehgal et al., J. Antibiot. 28, 727 (1975); H. A. Baker et al., J. Antibiot. 31, 539 (1978); U.S. Pat. Nos. 3,929,992; and 3,993,749].

Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has been shown to have antitumor activity. R. Martel et al. [Can. J. Physiol. Pharmacol. 55, 48 (1977)] disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Cyclosporin A and FK-506, other macrocyclic molecules, also have been shown to be effective as immunosuppressive agents, therefore useful in preventing transplant rejection [FASEB 3, 3411 (1989); FASEB 3, 5256 (1989); and R. Y. Calne et al., Lancet 1183 (1978)]. Although it shares structural homology with the immunosuppressant tacrolimus and binds to the same intracellular binding protein in lymphocytes, rapamycin inhibits S6p70-kinase and therefore has a mechanism of immunosuppressive action distinct from that of tacrolimus. Rapamycin was found to prolong graft survival of different transplants in several species alone or in combination with other immunosupressants. In animal models its spectrum of toxic effects is different from that of cyclosporin or FK-506., comprising impairment of glucose homeostasis, stomach, ulceration, weight loss and thrombocytopenia, although no nephrotoxicity has been detected.

Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat. No. 4,316,885) and used to make water soluble prodrugs of rapamycin (U.S. Pat. No. 4,650,803). Recently, the numbering convention for rapamycin has been changed; therefore according to Chemical Abstracts nomenclature, the esters described above would be at the 31- and 42-positions. Carboxylic acid esters (PCT application No. WO 92/05179), carbamates (U.S. Pat. No. 5,118,678), amide esters (U.S. Pat. No. 5,118,678), (U.S. Pat. No. 5,118,678) fluorinated esters (U.S. Pat. No. 5,100, 883), acetals (U.S. Pat. No. 5,151,413), silyl ethers (U.S. Pat. No. 5,120,842), bicyclic derivatives (U.S. Pat. No. 5,120,725), rapamycin dimers (U.S. Pat. No. 5,120,727) and O-aryl, O-alkyl, O-alkyenyl and O-alkynyl derivatives (U.S. Pat. No. 5,258,389) have been described.

Rapamycin is metabolized by cytochrome P-450 3A to at least six metabolites. During incubation with human liver and small intestinal microsomes, sirolimus was hydroxylated and demethylated and the structure of 39-O-demethyl sirolimus was identified. In bile of sirolimus-treated rats >16 hydroxylated and demethylated metabolites were detected.

In rapamycin, demethylation of methoxy group at C-7 Carbon will lead to the change in the conformation of the Rapamycin due to the interaction of the released C-7 hydroxyl group with the neighbouring pyran ring system which is in equilibrium with the open form of the ring system. The C-7 hydroxyl group will also interact with the triene system and possibly alter the immunosuppressive activity of rapamycin. This accounts for the degradation of rapamycin molecule and its altered activity.

Stable isotopes (e.g., deuterium, ^{13}C, ^{15}N, ^{18}O) are non-radioactive isotopes which contain one additional neutron than the normally abundant isotope of the atom in question. Deuterated compounds have been used in pharmaceutical research to investigate the in vivo metabolic fate of the compounds by evaluation of the mechanism of action and metabolic pathway of the non deuterated parent compound. (Blake et al. J. Pharm. Sci. 64, 3, 367–391,1975). Such metabolic studies are important in the design of safe, effective therapeutic drugs, either because the in vivo active compound administered to the patient or because the metabolites produced from the parent compound prove to be toxic or carcinogenic (Foster et al., Advances in drug Research Vol. 14, pp. 2–36, Academic press, London, 1985).

Incorporation of a heavy atom particularly substitution of deuterium for hydrogen, can give rise to an isotope effect that can alter the pharmacokinetics of the drug. This effect is usually insignificant if the label is placed in a molecule at the metabolically inert position of the molecule.

Stable isotope labeling of a drug can alter its physicochemical properties such as pKa and lipid solubility. These changes may influence the fate of the drug at different steps along its passage through the body. Absorption, distribution, metabolism or excretion can be changed. Absorption and distribution are processes that depend primarily on the molecular size and the lipophilicity of the substance.

Drug metabolism can give rise to large isotopic effect if the breaking of a chemical bond to a deuterium atom is the rate limiting step in the process. While some of the physical properties of a stable isotope-labeled molecule are different from those of the unlabeled one, the chemical and biological properties are the same, with one important exception: because of the increased mass of the heavy isotope, any bond involving the heavy isotope and another atom will be stronger than the same bond between the light isotope and that atom. In any reaction in which the breaking of this bond is the rate limiting step, the reaction will proceed slower for the molecule with the heavy isotope due to kinetic isotope effect. A reaction involving breaking a C—D bond can be up to 700 percent slower than a similar reaction involving breaking a C—H bond.

More caution has to be observed when using deuterium labeled drugs. If the C—D bond is not involved in any of the steps leading to the metabolite, there may not be any effect to alter the behavior of the drug. If a deuterium is placed at a site involved in the metabolism of a drug, an isotope effect will be observed only if breaking of the C—D bond is the rate limiting step. There are evidences to suggest that whenever cleavage of an aliphatic C—H bond occurs, usually by oxidation catalyzed by a mixed-function oxidase, replacement of the hydrogen by deuterium will lead to observable isotope effect. It is also important to understand that the incorporation of deuterium at the site of metabolism slows its rate to the point where another metabolite produced by attack at a carbon atom not substituted by deuterium becomes the major pathway by a process called "metabolic switching".

It is also observed that one of the most important metabolic pathways of compounds containing aromatic systems is hydroxylation leading to a phenolic group in the 3 or 4 position to carbon substituents. Although this pathway involves cleavage of the C—H bond, it is often not accompanied by an isotope effect, because the cleavage of this bond is mostly not involved in the rate-limiting step. The substitution of hydrogen by deuterium at the stereo center will induce a greater effect on the activity of the drug.

Clinically relevant questions include the toxicity of the drug and its metabolite derivatives, the changes in distribution or elimination (enzyme induction), lipophilicity which will have an effect on absorption of the drug. Replacement of hydrogen by deuterium at the site involving the metabolic reaction will lead to increased toxicity of the drug. Replacement of hydrogen by deuterium at the aliphatic carbons will have an isotopic effect to a larger extent. Deuterium placed at an aromatic carbon atom, which will be the site of hydroxylation, may lead to an observable isotope effect, although this is less often the case than with aliphatic carbons. But in few cases such as in penicillin, the substitution on the aromatic ring will induce the restriction of rotation of the ring around the C—C bond leading to a favorable stereo-specific situation to enhance the activity of the drug.

Approaching half a century of stable-isotope usage in human metabolic studies has been without documented significant adverse effect. Side-effects with acute D dosing are transitory with no demonstrated evidence of permanent deleterious action. The threshold of D toxicity has been defined in animals and is far in excess of concentrations conceivably used in human studies (Jones P. J, Leatherdale S T Clin Sci (Colch) 1991 Apr;80(4):277–280). The possibility that D may have additional beneficial pharmacological applications cannot be excluded. For isotopes other than D, evidence of observed toxicity remains to be produced even at dosages far in excess of the range used in metabolic studies. Absence of adverse effect may be attributable to small mass differences and the similar properties of tracer and predominantly abundant isotopes. The precision of extrapolating toxicity thresholds from animal studies remains unknown. However, should perturbation of the delicate homoeostatic characteristic of living organisms occur with use of stable isotopes, it is almost undoubtedly at some level of administration greatly in excess of those administered currently in biomedical research.

In the prior art, no details are described regarding deuterated derivatives to improve the stability of rapamycin molecule and also about glycosylated deuterated rapamycin to improve the stability and also the solubility of the molecule in order to increase the bio-availability of the drug.

We therefore defined the global objective of preparing a rapamycin derivative which is more stable, less prone to degradation, and more water soluble to improve the bio-availability.

SUMMARY OF THE INVENTION

Deuteration of the rapamycin molecule results in altered physicochemical and pharmacokinetic properties which enhance its usefulness in the treatment of transplantation rejection, host vs. graft disease, graft vs. host disease, leukemia/lymphoma, hyperproliferative vascular disorders, autoimmune diseases, diseases of inflammation, solid tumors, and fungal infections.

Deuterium isotope is selected based on the fact that if ^{13}C, ^{15}N or another heavy isotope differing from the light one by less than 10% in mass is incorporated at the site of metabolism, there may be a small isotope effect. In addition to this, there are secondary isotope effects away from the site of isotope substitution due to changes in electronic environment.

Substitution of deuterium in methyl groups of rapamycin will result in a slower rate of oxidation of the C—D bond relative to the rate of oxidation of a non deuterium substituted C—H bond. The isotopic effect acts to reduce formation of demethylated metabolites and thereby alters the pharmacokinetic parameters of the drug. Lower rates of oxidation, metabolism and clearance result in greater and more sustained biological activity. Deuteration is targeted at various sites of the rapamycin molecule to increase the potency of drug, reduce toxicity of the drug, reduce the clearance of the pharmacologically active moiety and improve the stability of the molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is the chemical structure of 7-deuteromethyl rapamycin showing sites of deuteration.

FIG. 2 is the chemical structure of epi-7 deuteromethyl rapamycin showing sites of deuteration.

FIG. 3 is the chemical structure of 7,43-d_6-rapamycin showing sites of deuteration.

FIG. 4 is the chemical structure of 31,42-d_2 showing sites of deuteration.

FIG. 5 illustrates the preparation of glycosylated deuteriorapamycin.

DETAILED DESCRIPTION OF THE INVENTION

Substitution of deuterium for ordinary hydrogen and deuterated substrates for protio metabolites can produce profound changes in biosystems. Isotopically altered drugs have shown widely divergent pharmacological effects. Patterson et al., found increased anti-cancer affect with deuterated 5,6-benzylidene-dl-L-ascorbic acid (Zilascorb) [Anticancer Res. 12, 33 (1992)].

Substitution of deuterium in methyl groups of rapamycin will result in a slower rate of oxidation of the C—D bond relative to the rate of oxidation of a non deuterium substituted C—H bond. The isotopic effect acts to reduce formation of demethylated metabolites and thereby alters the pharmacokinetic parameters of the drug. Lower rates of oxidation, metabolism and clearance result in greater and more sustained biological activity. Deuteration is targeted at various sites of the rapamycin molecule to increase the potency of drug, reduce toxicity of the drug, reduce the clearance of the pharmacologically active moiety and improve the stability of the molecule.

Determination of the physicochemical, toxicological and pharmacokinetic properties can be made using standard chemical and biological assays and through the use of mathematical modeling techniques which are known in the chemical and pharmacological/toxicological arts. The therapeutic utility and dosing regimen can be extrapolated from the results of such techniques and through the use of appropriate pharmacokinetic and/or pharmacodynamic models.

The compounds of this invention may be administered neat or with a pharmaceutical carrier to an animal, such as a warm blooded mammal, and especially humans, in need thereof. The pharmaceutically effective carrier may be solid or liquid.

A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets may contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose derivatives, possibly sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellent.

Liquid pharmaceutical compositions which are sterile solutions or suspensions can be utilized by, for example, intramuscular, intraperitoneal or subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form.

The pharmaceutical composition can be in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form. The dosage to be used in the treatment must be subjectively determined by the attending physician.

In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles administered to a fungally affected area.

EXAMPLES

FIGS. 1–4 show examples of sites for deuteration of the rapamycin molecule. Nonlimiting examples of deuterated rapamycin molecules include the compounds; 7-deuteromethyl rapamycin (FIG. 1), epi-7-deuteromethyl rapamycin (FIG. 2), 7,43-d_6-rapamycin (FIG. 3) and 31,42-d_2-rapamycin (FIG. 4) including the cis and trans isomers of the compounds shown in FIGS. 1–4. FIG. 5 shows the preparation and structure of the compound glycosylated deuterorapamycin.

Example 1

Preparation of 7-Deuteromethyl Rapamycin (FIG. 1)

5 mg of Rapamycin was dissolved in 2.5 ml of dichloromethane. 40 mg of deuterated methanol was added. 10 beads of NAFION® catalyst were added to the above solution. The contents were stirred under nitrogen at room temperature for 14 hours. The reaction was monitored by mass spectrum. The solution was filtered and concentrated The residue was dissolved in dry benzene and freeze dried. The white solid obtained was homogenous by mass spectrum analysis and characterized by LC/MS.

Example 2

Preparation of 31, 42 d_2-7-deuterated Rapamycin (FIG.3)

Rapamycin (11 mM) was dissolved in a mixture of cyclohexane and dichloromethane (1:1) 10 ml. The contents were cooled in ice bath and poly(vinylpyridinium)dichromate 0.5 grams was added. The reaction mixture was stirred overnight and the reaction was followed by mass spectrum. The reaction mixture was filtered, washed with water and dried using anhydrous magnesium sulphate. The organic solution was filtered and concentrated. The crude product was subjected to purification by silica column using chloroform-methanol (20:10) mixture. The pure fractions were collected and concentrated. The residue was dissolved in benzene and freeze dried. The product was characterized by LC/MS. M+(Na) 932. This material was dissolved in dry ether (10 ml). 10 equivalents of lithium aluminum deuteride was added. The reaction mixture was stirred for 24 hours. After the completion of the reaction, the excess of $LiAlD_4$ was decomposed by the addition of acetone. The complex was decomposed by adding ice cooled acetic acid. The mixture is filtered. The filtrate was diluted with ether and washed with water, dried, and concentrated. The crude mixture was subjected to column chromatography and the required material was eluted using chloroform-methanol solvent system. The pure fractions were collected and concentrated. The compound was tested by mass spectrum. M–(Na) 940. This compound was converted to the desired final compound (2) by following the procedure as described in Example 1.

Example 3

Preparation of Glycosylated deuteroRapamycin (FIG. 5)

Referring to FIG. 5, compound 10 prepared by example 1 (20 mg) was dissolved in 5 ml of dichloromethane. Dim-

ethylaminopyridine (2.2 mg) was added to the above solution. The contents were cooled to −70 C. 4-Nitrophenylchloroformate in dichloromethane was added to the reaction mixture. The solution was stirred under nitrogen at room temperature for 14 hours. The reaction was followed by mass spectrum. After the completion of the reaction, the reaction mixture was diluted with dichloromethane and the organic solution was washed with water, 0.2M ice cold HCl solution. The organic layer was dried over anhydrous magnesium sulphate. After filtration, the organic solution was filtered and concentrated. The crude product was purified by LC/MS to provide the pure compound 30 (Yield 10 mg.) Compound 30 (0.9 m.mol)was dissolved in dry DMF(0.5 ml) To this mixture, a solution of 2-aminoethyl-a-D-glucopyranoside (7.2 m.mol) was added. The reaction mixture was stirred for 14 hours at room temperature. After the completion of the reaction, the mixture was diluted with dichloromethane. The organic solution was concentrated in vacuum. The residue was extracted with water and the aqueous solution was subjected to biogel column to get the required pure compound 50. This material was characterized by LC/MS. M+(Na)1185.

Further variations and modifications of the present invention will be apparent to those skilled in the art from the foregoing and are intended to be encompassed by the claims appended hereto.

We claim:

1. The deuterated rapamycin compound which is epi-7-deuteromethyl rapamycin.

2. The deuterated rapamycin compound which is 7,43-d_6-rapamycin.

3. A pharmaceutical composition comprising deuterated rapamycin or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier, wherein the deuterated rapamycin is epi-7-deuteromethyl rapamycin and isomers thereof.

4. The pharmaceutical composition of claim 3 that is in tablet form.

5. A pharmaceutical composition comprising deuterated rapamycin or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier, wherein the deuterated rapamycin is 7,43-d_6-rapamycin and isomers thereof.

6. The pharmaceutical composition of claim 5 that is in tablet form.

* * * * *

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PATENT COOPERATION TREATY

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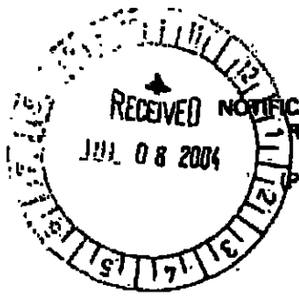

From the INTERNATIONAL BUREAU

To:

TORYS LLP
Maritime Life Tower
Suite 3000
79 Wellington St. W.
Box 270, TD Centre
Toronto, Ontario M5K 1N2
Canada

NOTIFICATION OF RECEIPT OF RECORD COPY (PCT Rule 24.2(a))

DOCKETED
DUE _____

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
29 June 2004 (29.06.2004)	
Applicant's or agent's file reference	**International application No.**
31284-2007	PCT/CA2004/000724

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

ISOTECHNIKA INTERNATIONAL INC. (for all designated States except US)
ABEL, Mark et al (for US)

International filing date	:	14 May 2004 (14.05.2004)
Priority date(s) claimed	:	16 May 2003 (16.05.2003) 20 February 2004 (20.02.2004) 16 April 2004 (16.04.2004)
Date of receipt of the record copy by the International Bureau	:	09 June 2004 (09.06.2004)
List of designated Offices	:	

AP :BW,GH,GM,KE,LS,MW,MZ,NA,SD,SL,SZ,TZ,UG,ZM,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,BG,CH,CY,CZ,DE,DK,EE,ES,FI,FR,GB,GR,HU,IE,IT,LU,MC,NL,PL,PT,RO,SE,SI,SK,TR
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GQ,GW,ML,MR,NE,SN,TD,TG
National :AE,AG,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BW,BY,BZ,CA,CH,CN,CO,CR,CU,CZ,DE,DK,DM,DZ,EC,EE,EG,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,MZ,NA,NI,NO,NZ,OM,PG,PH,PL,PT,RO,RU,SC,SD,SE,SG,SK,SL,SY,TJ,TM,TN,TR,TT,TZ,UA,UG,US,UZ,VC,VN,YU,ZA,ZM,ZW

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.89.85

Authorized officer:
Sophie Charnot (Fax 338-8995)

Telephone No. (41-22) 338 8081

Continuation of Form PCT/IB/301

NOTIFICATION OF RECEIPT OF RECORD COPY

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
29 June 2004 (29.06.2004)	
Applicant's or agent's file reference	International application No.
31284-2007	PCT/CA2004/000724

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

[X] time limits for entry into the national phase - see updated important information (as of April 2002)

[X] requirements regarding priority documents (if applicable)

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

ANNEX TO FORM PCT/IB/301

International application No.
PCT/CA2004/000724

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated on the cover sheet of this Notification by paying national fees and furnishing translations, as prescribed by Articles 22 and 39 and the applicable national laws. In addition, the applicant may also have to comply with other special requirements applicable in certain Offices. It is the applicant's responsibility to ensure the necessary steps to enter the national phase are taken in a timely fashion. Most Offices do not issue reminders to applicants in connection with the entry into the national phase.

The applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be **30 MONTHS** from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of 19 months from the priority date (see Article 39(1)), but also in respect of any designated Office, in the absence of filing of such demand, where Article22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see PCT Gazette No. 44/2001 of 1 November 2001, pages 19928, 19932 and 19934, as well as the PCT Newsletter, October and November 2001 and February 2002 issues.

In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For regular updates on the applicable time limits (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the PCT Gazette ("Section IV" part published on a weekly basis), to the PCT Newsletter (on a monthly basis) and to the relevant National Chapters in Volume II of the PCT Applicant's Guide (the paper version of which is updated usually twice a year and the Internet version of which is updated usually on a weekly basis). Finally, a cumulative table of all applicable time limits for entering the national phase is available from WIPO's Internet site, via links from various pages of the site including those of the Gazette, Newsletter and Guide, at http://www.wipo.int/pct/en/index.html.

Information about the requirements for filing a demand for international preliminary examination is set out in the PCT Applicant's Guide, Volume I/A, Chapter IX. Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided 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 the time limit which is reasonable under the circumstances (Rule 17.1(c)).

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit (and all other PCT time limits) is the filing date of the earliest application whose priority is claimed (Article 2(xi)(b)).

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PATENT COOPERATION TREATY

DOCKETED

From the INTERNATIONAL SEARCHING AUTHORITY

To:
TORYS LLP
Maritime Life Tower
Suite 3000, 79 Wellington St. W.
Box 270, TD Centre
Toronto, Ontario M5K 1N2
CANADA

Date of mailing (day/month/year): 01/07/2004

Applicant's or agent's file reference: 31284-2007

IMPORTANT NOTIFICATION

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/CA2004/000724	14/05/2004	16/05/2003

Applicant: ISOTECHNIKA INTERNATIONAL INC.

1. Where the International Searching Authority and the receiving Office are not the same office:

The applicant is hereby notified that the search copy of the international application was received by this International Searching Authority on the date indicated below.

Where the International Searching Authority and the receiving Office are the same office:

The applicant is hereby notified that the search copy of the international application was received on the date indicated below.

09/06/2004 *(date of receipt).*

2. ☐ The search copy was accompanied by a nucleotide and/or amino acid sequence listing or tables related thereto in computer readable form.

3. Time limit for establishment of international search report and written opinion of the International Searching Authority

The applicant is informed that the time limit for establishing the international search report and the written opinion of the International Searching Authority is three months from the date of receipt indicated above or nine months from the priority date, whichever time limit expires later (Rules 42.1 and 43*bis*.1(a))

4. A copy of this notification has been sent to the International Bureau and, where the first sentence of paragraph 1 applies, to the receiving Office.

DUE DATE
PROCESSED
DUE

Name and mailing address of the International Searching Authority	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	ISA/EP

Form PCT/ISA/202 (January 2004)

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RECEIVED 31241047

PATENT COOPERATION TREATY

RECEIVED PCT 2004

From the INTERNATIONAL SEARCHING AUTHORITY

To:

TORYS LLP
Maritime Life Tower
Suite 3000, 79 Wellington St. W.
Box 270, TD Centre
Toronto, Ontario M5K 1N2
CANADA

COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE

	Date of mailing (day/month/year) 01/07/2004
Applicant's or agent's file reference 31284-2007	REPLY DUE See paragraph 1 below
International application No. PCT/CA2004/000724	International filing date (day/month/year) 14/05/2004

Applicant

ISOTECHNIKA INTERNATIONAL INC.

DUE DATE
PROCESSED
DUE

1. [ ] REPLY DUE within ~~3 months~~ days from the above date of mailing

   [X] NO REPLY DUE

2. COMMUNICATION:

The applicant is informed that establishment of the international search report (ISR) for non first-filings may be delayed due to a current search backlog.

Although the time limit for entering the national phase before designated offices under Article 22(1) PCT and elected offices under Article 39(1) PCT has, with effect from 1 April 2002 (see PCT Gazette 44/2001 Section IV) been set at 30 months from the priority date (before the EPO the time limit is 31 months from the priority date - see Rule 107 EPC as amended with effect from 2 January 2002 - OJ EPO 8-9/2001, 373) not all PCT contracting states have yet made the necessary changes to their national laws and will for the time being continue to require entry to the national phase at 20/21 months from the priority date if a demand has not been filed before the end of 19 months from the priority date - see PCT Gazette/PCT Newsletter available on the WIPO internet site at http://www.wipo.int/pct/en/index.html for an up to date list of the applicable time limits.

In these circumstances, the EPO acting as IPEA will accept, without any late payment fee under Rule 58bis PCT, the handling fee and the preliminary examination fee due in respect of the demand relating to the present application, even if they are not paid within the time limit prescribed in Rules 57.3 and 58.1(b) PCT, provided that they are paid within one month from the date of transmittal of the ISR; i.e., the EPO will only send an invitation pursuant to Rule 58bis.1(a) PCT after expiry of this one-month period. In all cases where the EPO has sent an invitation to pay and the applicant has not paid in full the amount due, the demand shall be considered as if it had not been submitted (Rule 58bis.1(b)-(d) PCT). A loss of rights may well be the consequence in designated states where the time limit for entry into the national phase under Article 22 PCT has already expired (see also Article 37(4) PCT).

Note that if the competent IPEA chosen by the applicant is not the EPO and if the fees mentioned above are not paid within the time limit prescribed in Rules 57.3 and 58.1(b) PCT, the competent IPEA is entitled to apply Rule 58bis.1(a) PCT immediately thereafter.

If your application is affected, we apologise for any inconvenience caused.

Finally, applicants are reminded that as of 3 January 2002 a rationalised PCT II procedure may apply, see OJ EPO 11/2001, 539 and that the EPO as ISA will not carry out international search on an application which relates to no more than a method of doing business, see OJ EPO 10/2001, 462. Applicants should also bear in mind the restriction of the EPO's competence as ISA and IPEA in certain technical fields in respect of certain international applications, see OJ EPO 1/2002, 52 and PCT Newsletter 1/2002 for further details.

Name and mailing address of the International Searching Authority	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	ISA/EP

Form PCT/ISA/224 (January 1994)

LB3

PATENT COOPERATION T. ATY

DOCKETED

From the
INTERNATIONAL SEARCHING AUTHORITY

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/CA2004/000724	14.05.2004	16.05.2003

International Patent Classification (IPC) or both national classification and IPC
C07H15/04, C07D498/18, A61F2/02, A61K31/436

Applicant
ISOTECHNIKA INTERNATIONAL INC.

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	Klein, D Telephone No. +49 89 2399-7896

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

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CA2004/000724

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 field, 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:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/CA2004/000724

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the *assumption* that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

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	1-24
	No:	Claims	---
Inventive step (IS)	Yes:	Claims	2-8,10-16
	No:	Claims	1,9,17-24
Industrial applicability (IA)	Yes:	Claims	1-17,20-23
	No:	Claims	18,19,24

2. *Citations and explanations*

   see separate sheet

WRITTEN OPINION OF THE INTERNATI NAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.

PCT/CA2004/000724

Re Item V

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

Reference is made to the following document:
D1: US-B-6 342 5071 (FOSTER ROBERT T ET AL) 29 January 2002 (2002-01-29)

Novelty (Art. 33(2) PCT) :

The subject-matter of the present application concerns glycosylated derivatives of Rapamycin and uses thereof.

Since none of the available discloses the subject-matter of claims 1-24, novelty of said claims is acknowledged.

Inventive step (Art. 33(3) PCT) :

a) D1 discloses on figure 5 a deuterated rapamycin derivative bearing on position 42 a linker coupled to glucopyranose unit from which the subject-matter of present claim 1 differs in that the claimed compounds are not deuterated.

Since the deuteration of the compound is D1 has been performed in order to enhance its usefulness in transplantation processes (see page 4 "summary of the invention" of D1), a non-deuterated compound is also expected to have a biological activity.

Thus, the subject-matter of claim 1 is merely considered as an adaptation of D1 and is therefore not considered inventive.

As a consequence claims 9,17,20-23 are not considered inventive either.

b) Claims 10-16, dependant on claim 9 further specify the linkers and/or the nature of the sugar.
Since none of the available prior art discloses the above-mentioned linkers coupled to different sugars, the subject-matter of claims 10-16 is considered inventive.

c) Since none of the available prior art suggest the presence of a linker coupled to a sugar unit on position 31 of the macrocycle, the subject-matter of claims 2-8 is considered inventive.

Industrial application (Art. 33(4) PCT) :

For the assessment of the present claims 18-19,24 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

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Marks & Clerk
Patent and Trademark Agents

P.O. Box 957, Station B, Ottawa, Ontario K1P 5S7
CANADA

Street address: Suite 1800, 250 Slater Street
Ottawa, Ontario K1P 1C2

Tel: (613) 236-9561
Fax: (613) 230-8821
e-mail: rmitchell@marksclerk.com

Please reply to: Richard J. Mitchell

July 21, 2004

Our Ref: 16902-PCT
Your Ref: ------

Commissioner of Patents
Canadian Receiving Office
Box PCT
Ottawa-Gatineau
K1A 0C9

Dear Sir:

Re: International Patent Application No. PCT/CA04/00724
Applicant: Mark Abel et al.
Title: Rapamycin Carbohydrate Derivatives

Pursuant to the Invitation to Correct Defects dated June 4, 2004, we are submitting herewith the three priority documents, namely:

United States Provisional Patent Application No. 60/562,840, filed April 16, 2004;
United States Provisional Patent Application No. 60/546,240, filed February 20, 2004; &
United States Provisional Patent Application No. 60/471,367, filed May 16, 2003.

Please forward these documents to the International Authorities in Geneva.

Yours truly,

Richard J. Mitchell
RJM:ci
Enc.

PA 1189814

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

July 01, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *60/471,367*
FILING DATE: *May 16, 2003*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. SWAIN
Certifying Officer

PA 1189814

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

July 01, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *60/546,240*
FILING DATE: *February 20, 2004*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. SWAIN
Certifying Officer

PA 1169814

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

July 01, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: *60/562,840*
FILING DATE: *April 16, 2004*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. SWAIN
Certifying Officer

192

PATENT CO PERATION TREATY

From the RECEIVING OFFICE

To:

The International Bureau of WIPO
34, chemin des Colombettes
1211, Geneva 20
Switzerland

PCT

REQUEST FOR THE RECORDING OF A CHANGE

(PCT Rule 92*bis*.1)

Date of mailing (*day/month/year*): 13 August 2004 (13-08-2004)

International application No.: PCT/CA2004/000724

International filing date (*day/month/year*): 14 May 2004 (14-05-2004)

1. The following indications appear on record concerning:

[ ] the applicant [ ] the inventor [x] the agent [ ] the common representative

Name and address:

TORYS LLP
Maritime Life Tower
3000-79 Wellington St. W.
Box 270, TD Centre
Toronto, Ontario
M5K 1N2 Canada

State of Nationality*:
State of Residence*:
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. This receiving Office hereby requests the International Bureau to record the following change in:

[x] the person [x] the name [x] the address [ ] the nationality* [ ] the residence*

Name and address:

Marks & Clerk
280 Slater Street Suite 1800
Ottawa, Ontario
K1P 5S7 Canada
Attn: Richard J. Mitchell

State of Nationality*:
State of Residence*:
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

* To be indicated only for a change concerning the applicant.

Name and mailing address of the Receiving Office:
Commissioner of Patents
Canadian Receiving Office
Box PCT, Ottawa/Gatineau K1A 0C9
Facsimile No. (819) 953-9538

Authorized Officer:
Diane Leclaire (819) 997-7623

Form PCT/RO/113 (July 1992; reprint January 2004)

Marks & Clerk
Patent and Trade Mark Agents
P.O.Box 957, Station B, Ottawa, Canada K1P 5S7

Street address: Suite 1800, 280 Slater Street,
OTTAWA, Ontario K2K 1C2

Tel:(613) 236-9561
Fax:(613) 230-8821
e-mail: rmitchell@marksclerk.com

Please reply to: Richard J. Mitchell

Our Ref: 16902-PCT
Your Ref: --------

August 3, 2004

Commissioner of Patents
Canadian Receiving Office
Box PCT
Ottawa Hull
K1A 0C1

Dear Sir:

Re: International Patent Application. No. PCT/CA2004/000724
Applicant: ISOTECHNIKA INTERNATIONAL, INC. et al
Filed: 14-May-2004

Pursuant to the Communication Regarding Extension of Time, we are now attaching the required Powers of Attorney (3), one executed by an officer of Isotechnika International Inc., one executed by inventor Randall W. Yatscoff and another executed by the other four inventors.

We are also attaching herewith the newly-prepared formal drawings.

I trust this now completes the formalities.

Yours very truly,

RJM/pm

Richard J. Mitchell

PCT

POWER OF ATTORNEY

(for an international application filed under the Patent Cooperation Treaty)

(PCT Rule 90.4)

The undersigned applicant(s) *(Names should be indicated as they appear in the request)*:

ISOTECHNIKA INTERNATIONAL INC.
2nd Floor, Musson Building
Hincks Street
Bridgetown
BARBADOS

hereby appoints (appoint) the following person as: [X] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Richard J. MITCHELL, George M. MacGREGOR, S. Mark BUDD, and Ian D. CLARK, c/o Marks & Clerk, P.O. Box 957, Station B, Ottawa, Ontario Canada K1P 5S7

to represent the undersigned before

[X] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with the international application identified below:

Title of the invention: RAPAMYCIN CARBOHYDRATE DERIVATIVES

Applicant's or agent's file reference: 16902-PCT

International application number (if already available): PCT/CA2004/000724

filed with the following Office Canadian Patent Office as receiving Office and to make or receive payments on behalf of the undersigned.

Signature of the applicant(s) *(where there are several applicants, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading the request or this power)*:

ISOTECHNIKA INTERNATIONAL INC.

By: Joseph Kozlak
Title: Executive Vice President

Date: JUNE 14, 2004

Form PCT/Model of power of attorney (for a given international application) (July 1992)

PCT

POWER OF ATTORNEY

(for an international application filed under the Patent Cooperation Treaty)

(PCT Rule 90.4)

The undersigned applicant(s) *(Names should be indicated as they appear in the request)*:

ABEL, Mark
11530-9A Avenue
Edmonton, Alberta T6J 7B2
CANADA

SZWEDA, Roman
#705, 9916 113th Street
Edmonton, Alberta T5K 2N3
CANADA

TREPANIER, Daniel
1417 Haswell Place
Edmonton, Alberta T6R 3E1
CANADA

YATSCOFF, Randall W.
1596 Hector Road
Edmonton, Alberta T6R 2Z5

FOSTER, Robert T.
34 Westbrook Drive
Edmonton, Alberta T6J 2C9

hereby appoints (appoint) the following person as: [X] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Richard J. MITCHELL, George M. MacGREGOR, S. Mark BUDD, and Ian D. CLARK, c/o Marks & Clerk, P.O. Box 957, Station B, Ottawa, Ontario Canada K1P 5S7

to represent the undersigned before

[X] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with the international application identified below:

Title of the invention: RAPAMYCIN CARBOHYDRATE DERIVATIVES

Applicant's or agent's file reference: 16902-PCT

International application number (if already available): PCT/CA2004/000724

filed with the following Office Canadian Patent Office as receiving Office and to make or receive payments on behalf of the undersigned.

Signature of the applicant(s) *(where there are several applicants, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading the request or this power)*:

Mark Abel: _____ Date: June 17/04

Roman Szweda: _____ Date: June 17, 2004

Daniel Trepanier: _____ Date: June 16/04

Randall W. Yatscoff: X _____ Date: X

Robert T. Foster: _____ Date: June 16/04

Form PCT/Model of power of attorney (for a given international application) (July 1992)

PCT

POWER OF ATTORNEY

(for an international application filed under the Patent Cooperation Treaty)

(PCT Rule 90.4)

The undersigned applicant(s) *(Names should be indicated as they appear in the request)*:

ABEL, Mark
11530-9A Avenue
Edmonton, Alberta T6J 7B2
CANADA

SZWEDA, Roman
#705, 9816 113th Street
Edmonton, Alberta T5K 2N3
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CANADA

YATSCOFF, Randall W.
1596 Hector Road
Edmonton, Alberta T6R 2Z5

FOSTER, Robert T.
34 Westbrook Drive
Edmonton, Alberta T6J 2C9

hereby appoints (appoint) the following person as: [X] agent [ ] common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

Richard J. MITCHELL, George M. MacGREGOR, S. Mark BUDD, and Ian D. CLARK, c/o Marks & Clerk, P.O. Box 957, Station B, Ottawa, Ontario Canada K1P 5S7

to represent the undersigned before

[X] all the competent International Authorities
[ ] the International Searching Authority only
[ ] the International Preliminary Examining Authority only

in connection with the international application identified below:

Title of the invention: RAPAMYCIN CARBOHYDRATE DERIVATIVES

Applicant's or agent's file reference: 16902-PCT

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Signature of the applicant(s) *(where there are several applicants, each of them must sign; refer to each signature, indicate the name of the person signing and the capacity in which that person signs, if such capacity is not obvious from reading the request or this power)*

Mark Abel: X Date: X

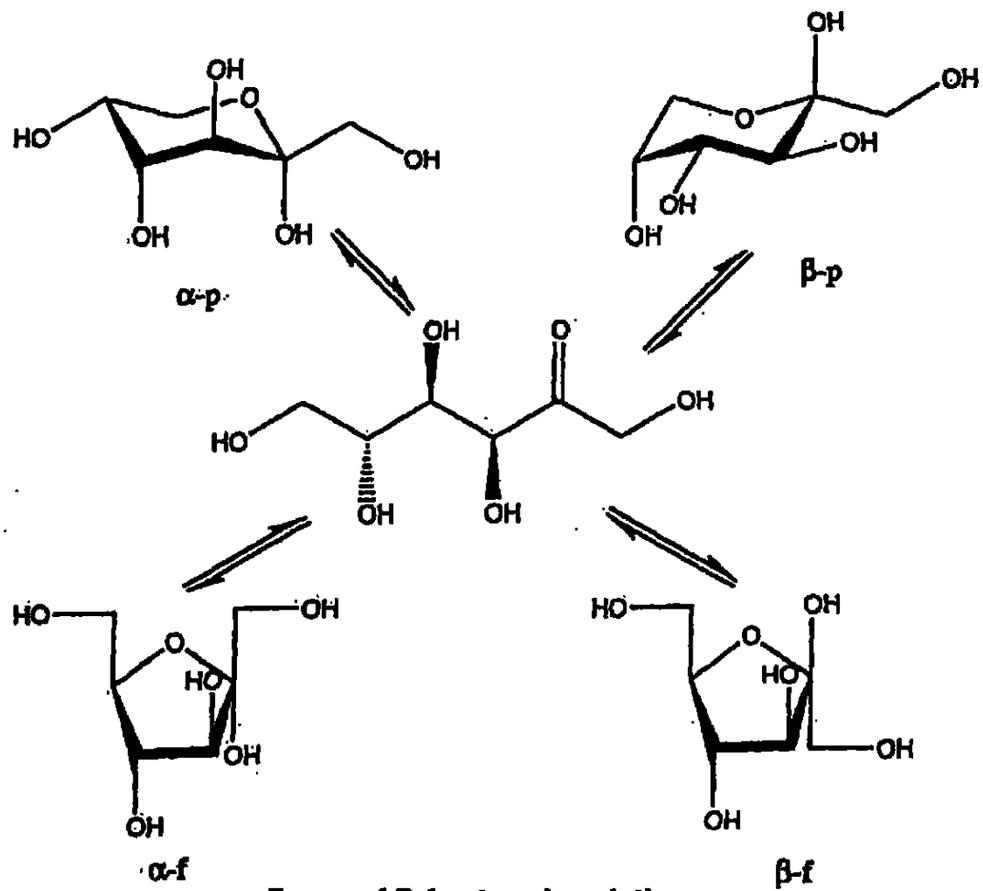
Roman Szweda: X Date: X

Daniel Trepanier: X Date: X

Randall W. Yatscoff: X Date: X June 15/04

Robert T. Foster: X Date: X

Form PCT/Model of power of attorney (for a given international application) (July 1992)



Forms of D-fructose in solution

FIG. 1

General Route of Synthesis for Rapamycin Carbohydrate Derivatives

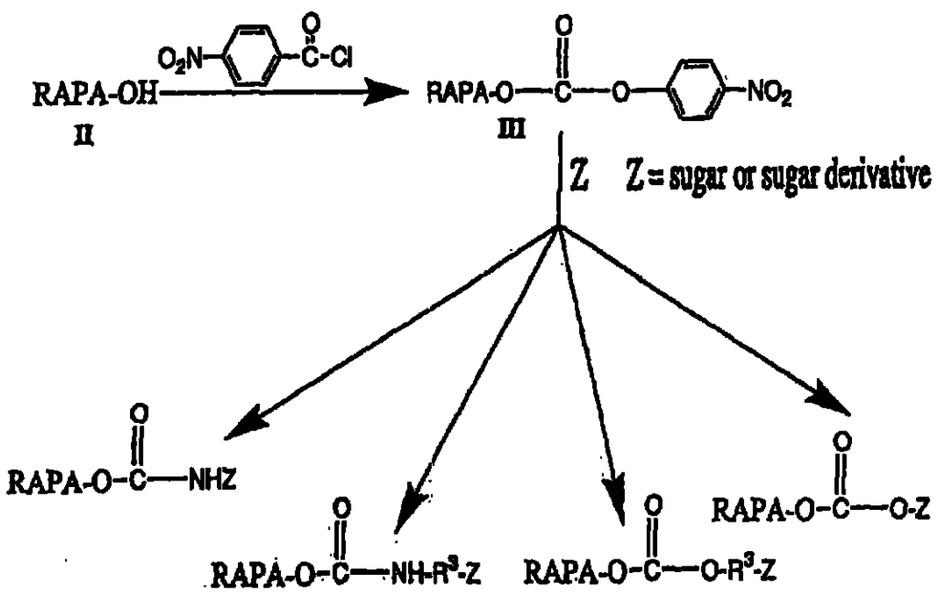
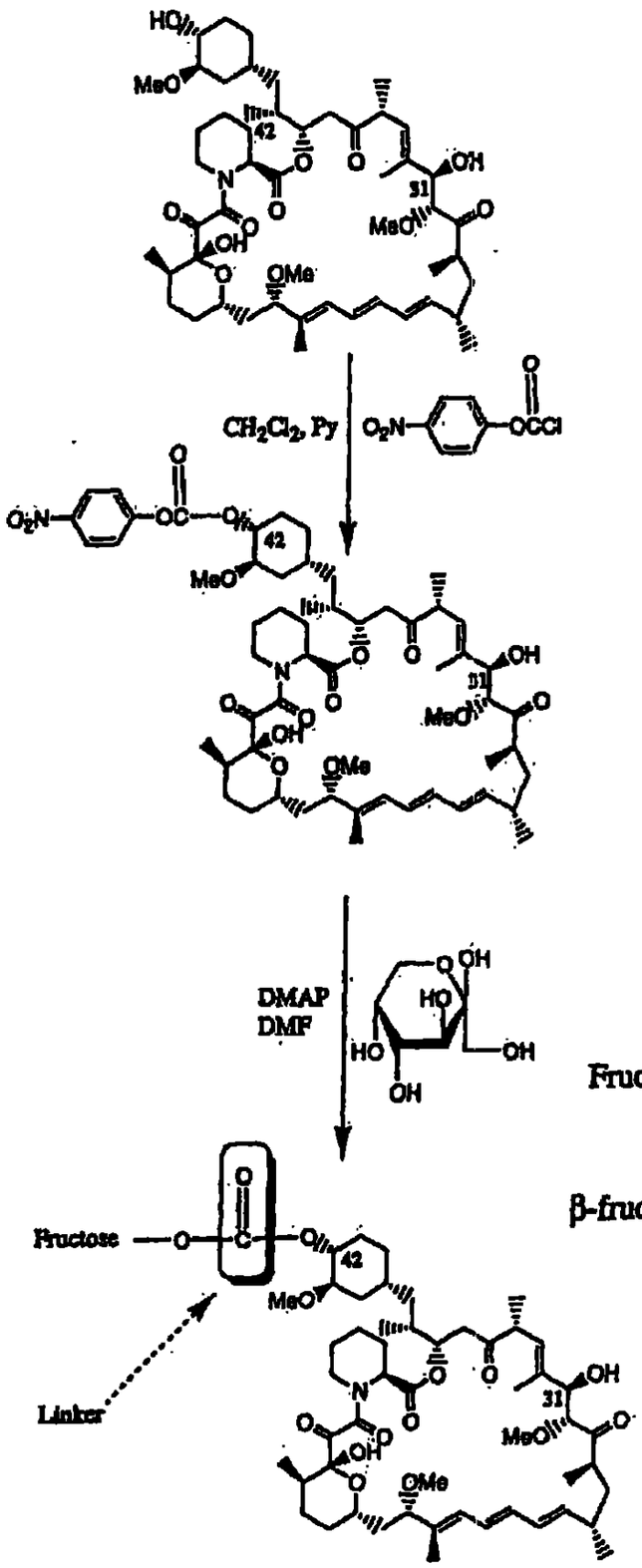


FIG. 2



Fructose = α-fructopyranose or
β-fructopyranose or
α-fructofuranose or
β-fructofuranose attached through
the 1 or 6 position.

FIG. 3

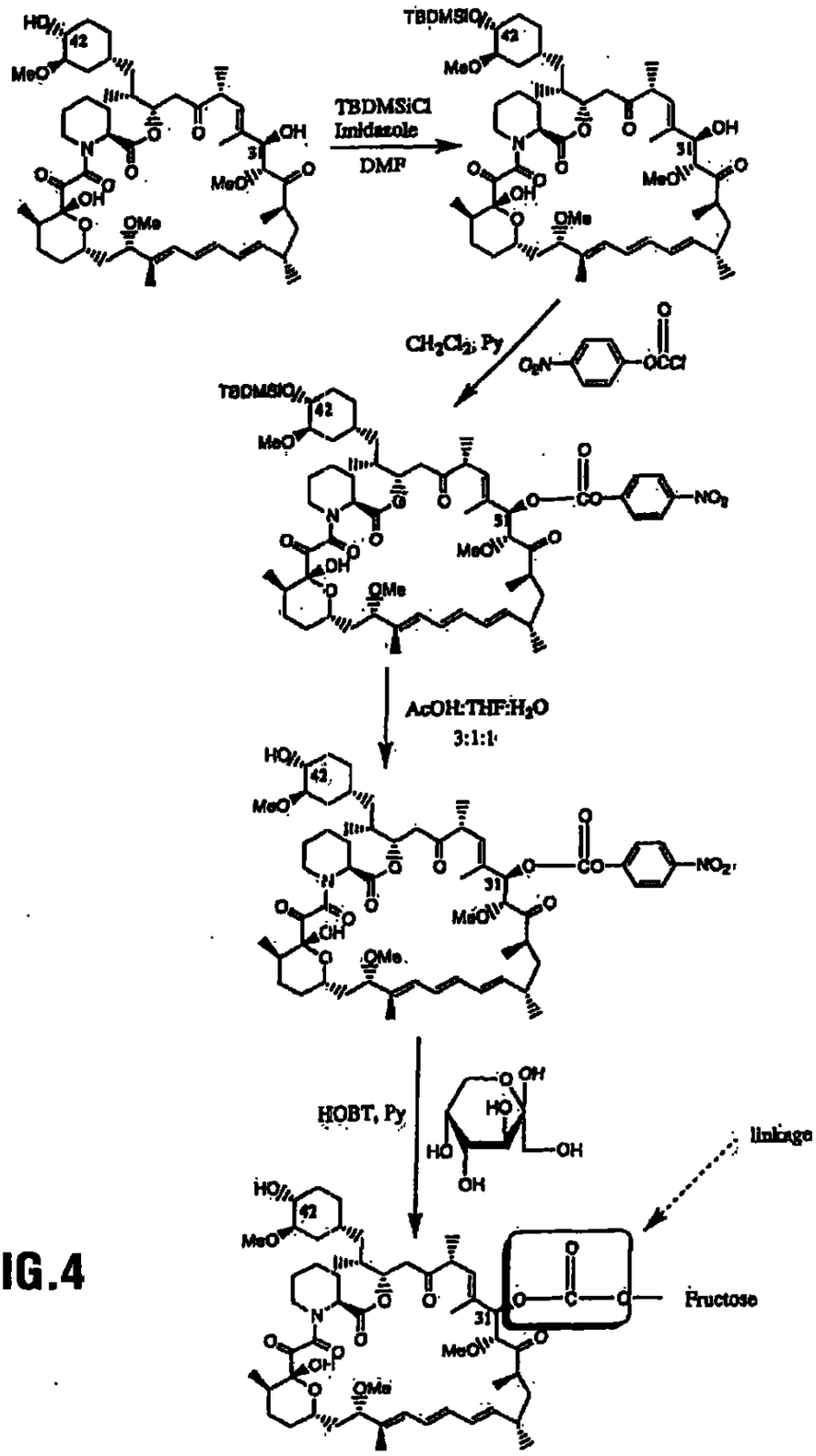

TBDMSiCl
Imidazole
DMF
CH_2Cl_2, Py
AcOH:THF:H_2O
3:1:1
HOBT, Py
linkage
Fructose

FIG.4

***In vitro* Immunosuppressive Activity of Rapamycin 42-O-(D-Fructosylcarbonyl)rapamycin, and a Carbamate-linked Analog in a Cell Proliferation assay.**

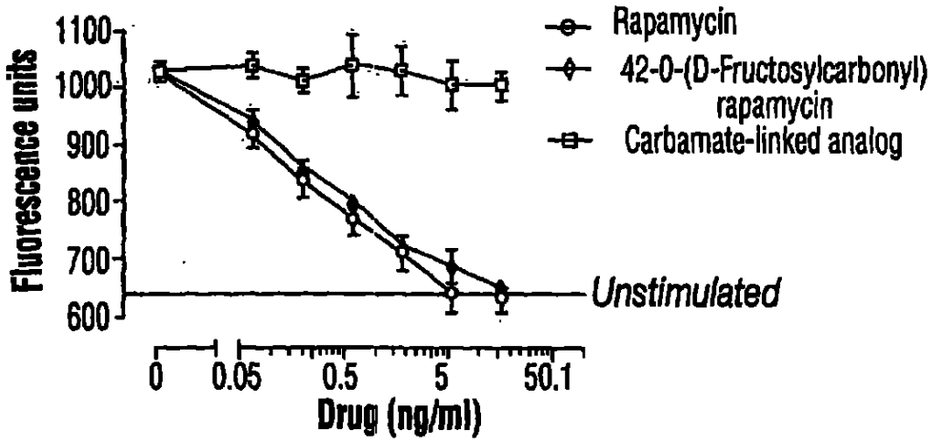


FIG. 5

Pharmacokinetic Profiles In the Rat of Rapamycin-42-O-Carbohydrate Derivatives

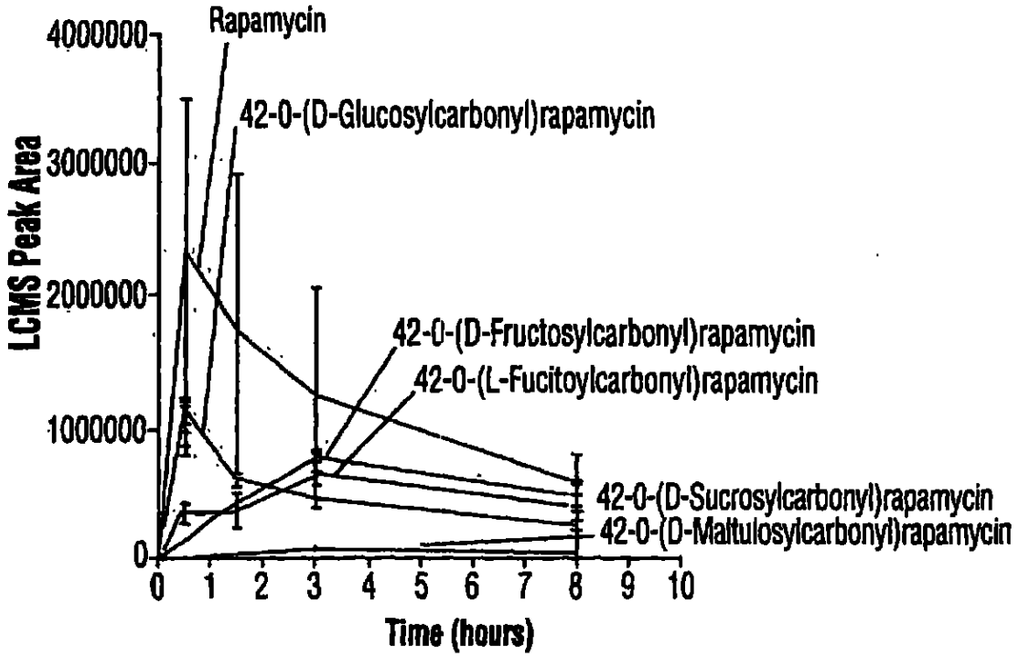


FIG. 6

Pharmacokinetic Profiles In the Rat of Rapamycin-31-O-Carbohydrate Derivitives

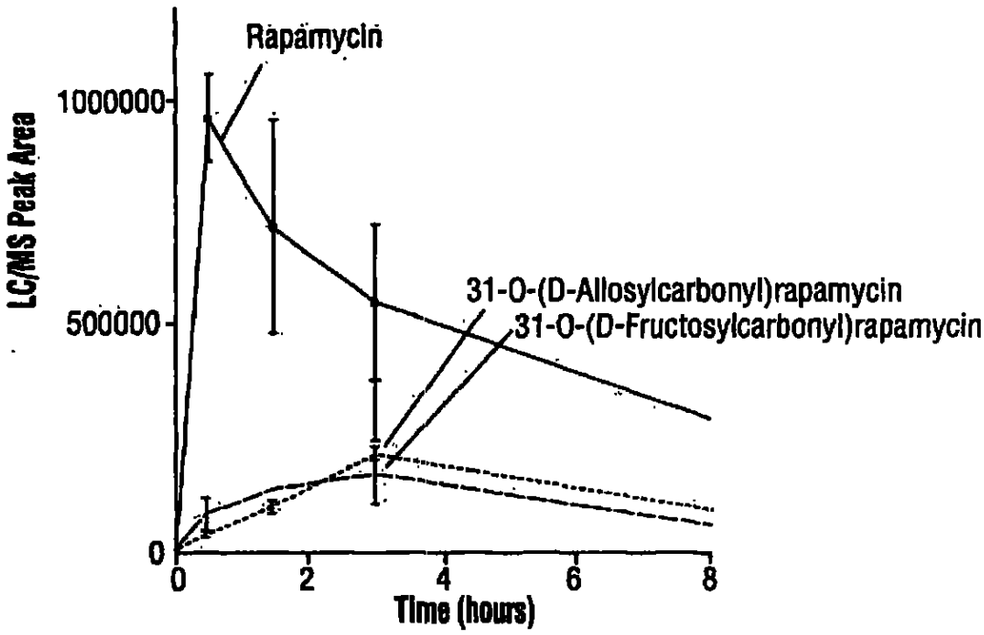


FIG. 7

Pharmacokinetic Profiles in the Rat of Rapamycin-42-O-Carbohydrate Derivitives

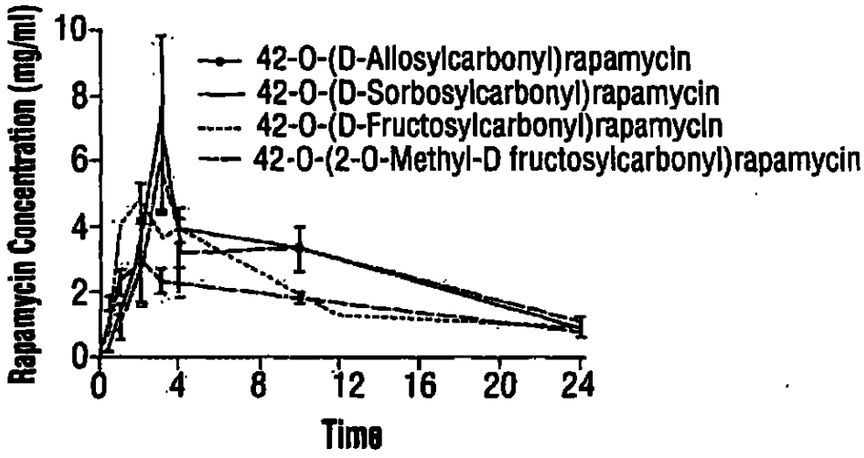
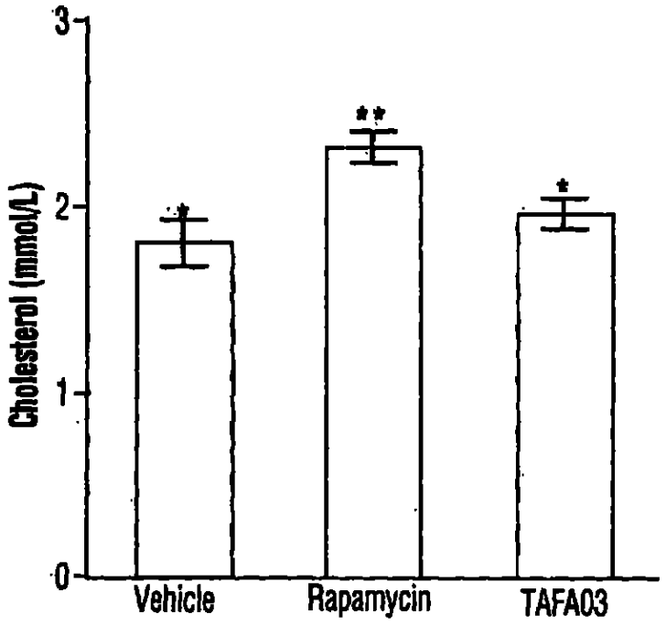


FIG. 8

Serum Cholesterol Levels Following 12-Day Dosing of 42-O-(D-Fructosylcarbonyl)rapamycin and Rapamycin in Sprague-Dawley Rats



* Significantly Lower than Rapamycin $p < 0.01$

** Significantly higher than Vehicle, $p < 0.01$

ANOVA, Dunnell's Post Hoc Test

FIG. 9

Dose-Response Relationship of ADP- Induced Platelet Aggregation
In the Presence of 1 and 25 µg/ml Rapamycin

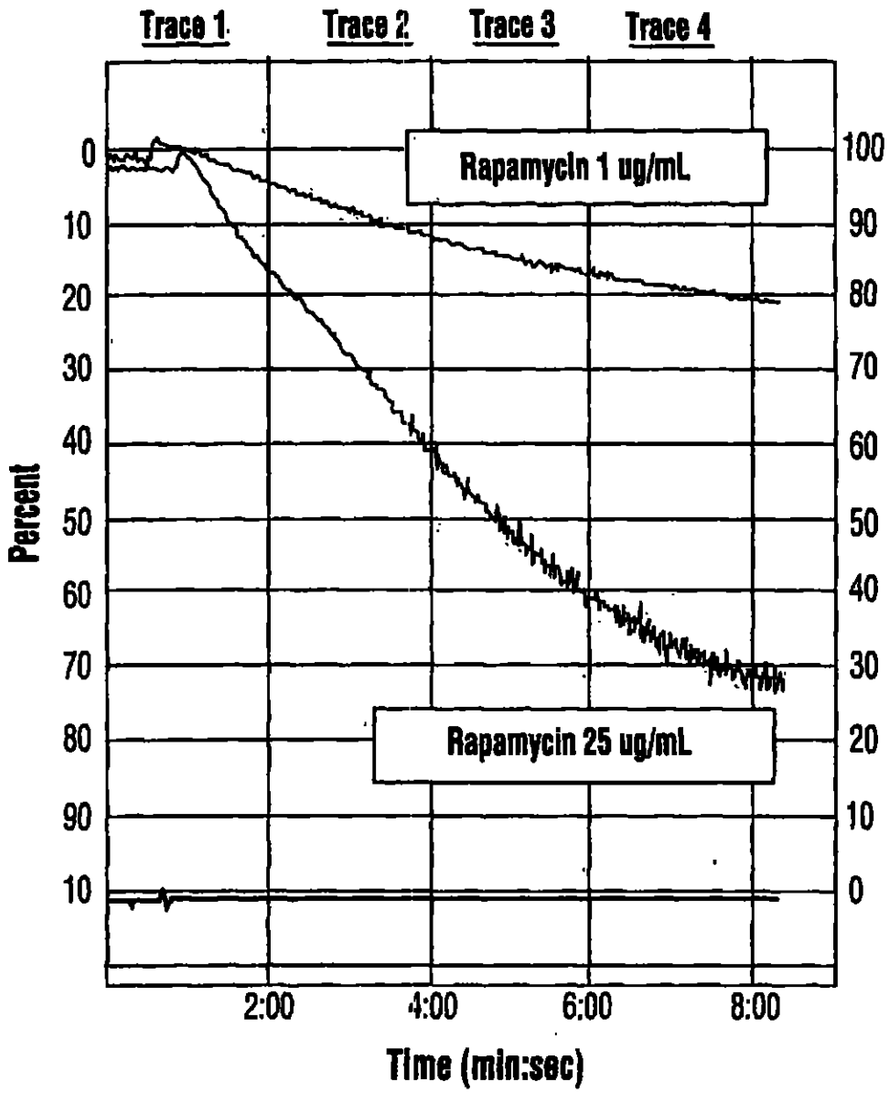


FIG. 10

Effect of Rapamycin and 42-O-(D-Fructosylcabonyl) rapamycin (25 µg/ml) on ADP-Induced Platelet Aggregation

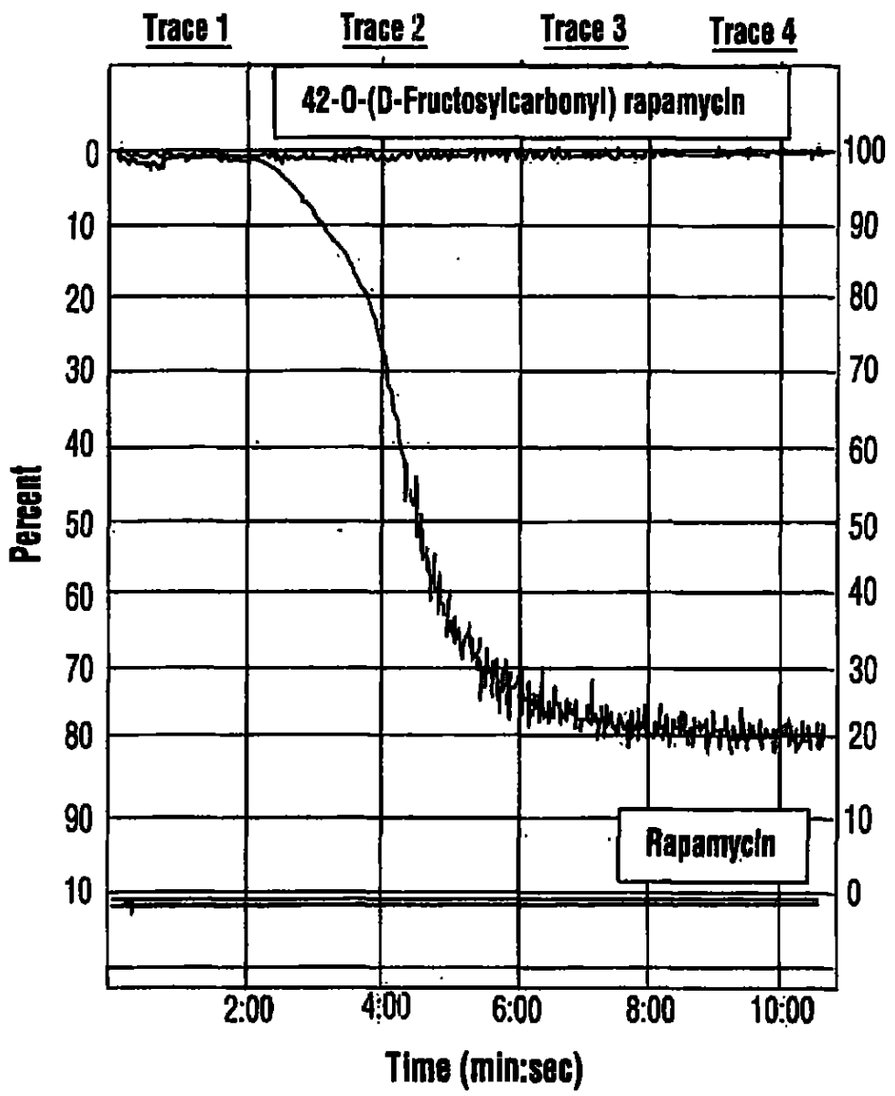
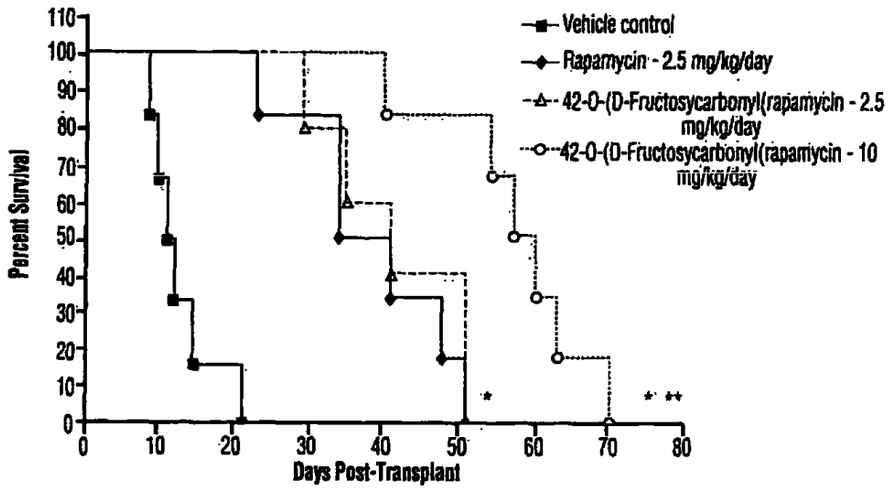


FIG. 11

Graft Survival Curves of Heterotopic Heart Allografts in Rats Receiving Oral 42-O-(D-Fructosylcarbonyl)rapamycin (2.5 and 10 mg/kg/day), Rapamycin (2.5 mg/kg/day),or Vehicle



* Different from Vehicle, $p < 0.005$
** Different from Rapamycin, $p < 0.05$
Wilcoxon Rank Sum

FIG. 12

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PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
Switzerland

PCT

NOTIFICATION OF DATE OF RECEIPT OF PRIORITY DOCUMENT OR OF PRIORITY APPLICATION NUMBER

(PCT Administrative Instructions, Section 323(a), (b) and (c))

Applicant's or agent's file reference 31284-2007	Date of mailing *(day/month/year)* 13 August 2004 (13-08-2004)
International application No. PCT/CA2004/000724	International filing date *(day/month/year)* 14 May 2004 (14-05-2004)
Applicant ISOTECHNIKA INTERNATIONAL INC. ET AL	

1. [X] This receiving Office hereby gives notice of the receipt of the priority document(s) identified below on:

22 July 2004 (22-07-2004)

2. [ ] This receiving Office hereby gives notice of the receipt of a request (made under Rule 17.1(b)) to prepare and transmit to the International Bureau the priority document(s) identified below on:

Identification of the priority document(s):

Priority date	Priority application No.	Country or regional Office or PCT receiving Office
16 May 2003 (16-05-2003)	60/471,367	US
20 February 2004 (20-02-2004)	60/546,240	US
16 April 2004 (16-04-2004)	60/562,840	US

Name and mailing address of the receiving Office Commissioner of Patents Canadian Receiving Office Box PCT, Ottawa/Gatineau K1A 0C9 Facsimile No. (819) 953-9338	Authorized Officer Diane Leclaire (819) 997-7623

Form PCT/RO/135 (July 1998; reprint January 2004)

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PATENT COOPERATION TREATY

To the RECEIVING OFFICE

To:
EUROPEAN PATENT OFFICE
SEARCH ADMINISTRATION
PATENTLAAN 2
NL-2280 HV RIJSWIJK (ZH)
NETHERLANDS

PCT

NOTIFICATION CONCERNING REPRESENTATION

(PCT Rule 90 and Administrative Instructions, Section 328)

Applicant's or agent's file reference 31284-2007	Date of Mailing (day/month/year) 13 August 2004 (13-08-2004)
International application No. PCT/CA2004/000724	International filing date (day/month/year) 14 May 2004 (14-05-2004)
Applicant ISOTECHNIKA INTERNATIONAL INC. ET AL	

1. This receiving Office hereby gives notice of the receipt of a document containing:

   [X] a power of attorney ISOTECHNIKA INTERNATIONAL INC. ET AL

   [ ] a revocation of power of attorney

   [ ] a renunciation of appointment

2. This notification, together with a copy of the document indicated above, is sent to the addressee in its capacity as:

   [ ] the International Searching Authority.

   [ ] the International Bureau, which is requested to record a change in the person of the agent or common representative under Rule 92*bis*.1(a)(ii).

Name and mailing address of the Receiving Office Commissioner of Patents Canadian Receiving Office Box PCT, Ottawa/Gatineau K1A 0C9 Facsimile No. (819) 953-9538	Authorized Officer Diane Leclaire (819) 997-7623

Form PCT/RO/123 (July 1992)

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PATENT COOPERATION TREATY

DOCKETED

From the INTERNATIONAL SEARCHING AUTHORITY

To:
TORYS LLP
Maritime Life Tower
Suite 3000, 79 Wellington St. W.
Box 270, TD Centre
Toronto, Ontario M5K 1N2
CANADA

PCT RECEIVED

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing *(day/month/year)* 23/08/2004
Applicant's or agent's file reference 31284-2007	FOR FURTHER ACTION See paragraphs 1 and 4 below
International application No. PCT/CA2004/000724	International filing date *(day/month/year)* 14/05/2004
Applicant ISOTECHNIKA INTERNATIONAL INC.	

1. [x] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 740.14.35

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules *90bis.1* and *90bis.3*, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the International Searching Authority 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	Authorized officer Dominique Hundt

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220 (continued)

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international application is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

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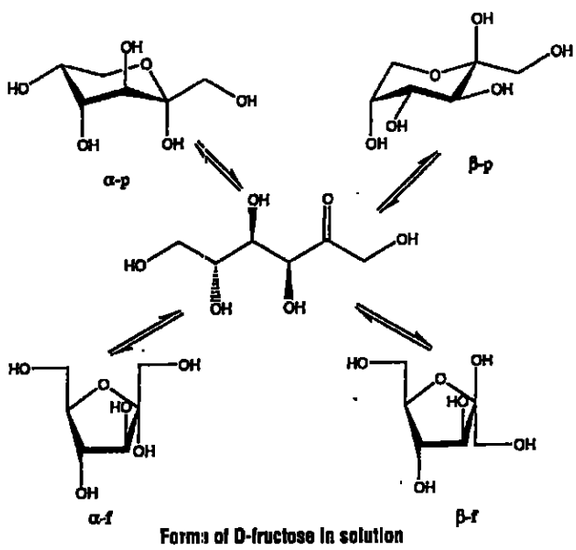

Forms of D-fructose in solution

(57) Abstract: This invention provides modified rapamycins that have specific monosaccharide(s), oligosaccharide(s), pseudosugar(s) or derivatives thereof attached through a linker to create rapamycin carbohydrate derivatives having enhanced pharmacokinetic and/or pharmacodynamic profiles. For example, administration of the rapamycin carbohydrate derivative results in altered pharmacokinetic profiles and reduced toxicities. Thus, the present invention provides compounds with characteristics that are distinct from other drugs in its class such as rapamycin.

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RAPAMYCIN CARBOHYDRATE DERIVATIVES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Application Serial Nos. 60/471,367 filed May 16, 2003, 60/546,240 filed February 20, 2004 and 60/562,840 filed April 16, 2004.

FIELD OF THE INVENTION

[0002] This application relates to carbohydrate derivatives of rapamycin, a potent immunosuppressant, with structurally defined carbohydrate moieties attached to the rapamycin structure via a connective moiety. The rapamycin carbohydrate derivatives may act as prodrugs. That is, they may be substantially without immunosuppressive activity themselves, but *in vivo* be converted to rapamycin which then exhibits an immunosuppressive effect.

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[0003] The following references are related hereto or referred to herein by patent or application number or by author and year at the relevant portions of this specification.

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[0017] U.S. Patent No. 3,929,992.

[0018] U.S. Patent No. 3,993,749.

[0019] U.S. Patent No. 4,885,171.

[0020] U.S. Patent No. 4,401,653.

[0021] U.S. Patent No. 4,316,885.

[0022] U.S. Patent No. 4,650,803.

[0023] PCT application No.WO 92/05179.

[0024] U.S. Patent No. 5,118,678.

[0025] U.S. Patent No. 5,260,300.

[0026] U.S. Patent No. 5,118,678.

[0027] U.S. Patent No. 5,118,678.

[0028] U.S. Patent No. 5,100,883.

[0029] U.S. Patent No. 5,151,413.

[0030] U.S. Patent No. 5,120,842.

[0031] U.S. Patent No. 5,120,725.

[0032] U.S. Patent No. 5,120,727.

[0033] U.S. Patent No. 5,258,389.

[0034] U.S. Patent No. 5,672,605.

[0035] U.S. Patent No. 5,583,139.

[0036] U.S. Patent No. 5,527,907.

[0037] U.S. Patent No. 5,457,111.

[0038] U.S. Patent No. 5,955,100.

[0039] U.S. Patent No. 6,146,658.

[0040] U.S. Patent No. 5,935,995.

[0041] U.S. Patent No. 5,665,728.

[0042] U.S. Patent No. 6,146,658.

BACKGROUND OF THE INVENTION

[0043] Rapamycin, also known as sirolimus, is a 31-membered macrolide lactone, $C_{51}H_{79}NO_{13}$, with a molecular mass of 913.6 Da. In solution, rapamycin forms conformational trans- and cis-isomers with a ratio of 4:1 (in chloroform solution) due to hindered rotation around the pipecolic acid amide bond. It is sparingly soluble in water, aliphatic hydrocarbons and diethyl ether, whereas it is soluble in alcohols, halogenated hydrocarbons and dimethyl sulfoxide. Rapamycin is unstable in solution; it degrades in plasma and in low and neutral pH buffers at 37°C with a half-life of less than 10 hours.

[0044] Produced by *Streptomyces hygroscopicus*, rapamycin has been shown to possess a number of valuable pharmacological attributes. The compound is a macrocyclic triene antibiotic that possesses antifungal activity, particularly against *Candida albicans*, both *in vitro* and *in vivo*. See, C. Vezina et al., *J. Antibiot.* 28, 721 (1975), S. N. Sehgal et al., *J. Antibiot.* 28, 727 (1975), H. A. Baker et al., *J. Antibiot.* 31, 539 (1978), and U.S. Patent Numbers 3,929,992; and 3,993,749. Rapamycin alone (U.S. Pat. No. 4,885,171) or in combination with picibanil (U.S. Pat. No. 4,401,653) has also been shown to have antitumor activity. Futhermore, R. Martel et al. *Can. J. Physiol. Pharmacol.* 55, 48 (1977) disclosed that rapamycin is effective in the experimental allergic encephalomyelitis model, a model for

multiple sclerosis; in the adjuvant arthritis model, a model for rheumatoid arthritis; and effectively inhibited the formation of IgE-like antibodies.

[0045] The immunosuppressive effects of rapamycin have been disclosed in FASEB 3, 3411 (1989). Rapamycin, Cyclosporin A, FK-506 (also known as tacrolimus), and other macrocyclic molecules, have been shown to be effective immunosuppressive agents and therefore are useful in preventing transplant rejection. See, FASEB 3, 3411 (1989), FASEB 3, 5256 (1989), and R. Y. Calne et al., *Lancet* 1183 (1978). Although rapamycin shares structural homology with the immunosuppressant tacrolimus (FK506) and binds to the same intracellular binding protein in lymphocytes, rapamycin and tacrolimus have been shown to have different mechanisms of immunospressive action. Rapamycin inhibits S6p70-kinase whereas Tacrolimus inhibits calcineurin. Rapamycin was found to prolong graft survival of different transplants in several species alone or in combination with other immunosuppressants. See, S.N. Sehgal et al., *Medicinal Research Reviews* 14, 1 (1994).

[0046] Rapamycin is also known as an mTOR inhibitor. These inhibitors are a class of immunosuppressive drugs that inhibit T cell activation at a later stage in the immune response than other types of inhibitors like calcineurin inhibitors and DNA synthesis inhibitors. In transplantation, mTOR inhibitors are typically used in combination with calcineurin inhibitors.

[0047] Unfortunately, the side effects (e.g., gastrointestinal effects, hyperlipidemia) of mTOR inhibitors currently limit their broader use in transplantation and the treatment of autoimmune diseases. And, while not having been shown to induce nephrotoxicity, rapamycin has been shown to induce a number of toxic side effects in animal model. Such toxic effects include, for example, impairment of glucose homeostasis, gastrointestinal tract ulceration, weight loss, diarrhea and thrombocytopenia.

[0048] Numerous rapamycin derivatives have been synthesized in the hopes of alleviating and improving some drawbacks that rapamycin retains, which include low and/or variable bioavailability and solubility, and high toxicity. Mono- and diacylated derivatives of rapamycin (esterified at the 28 and 43 positions) have been shown to be useful as antifungal agents (U.S. Pat. No. 4,316,885) and used to make water soluble prodrugs (U.S. Pat. No.

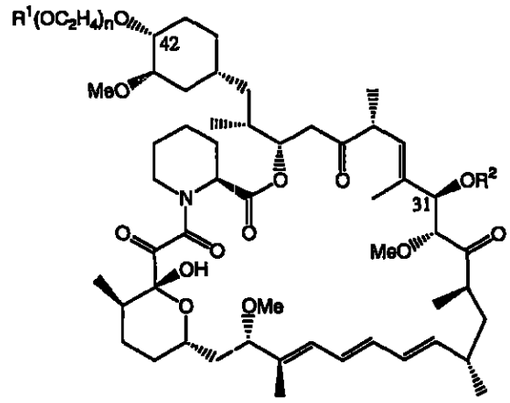
4,650,803). Other derivatives include, carboxylic acid esters (PCT Publication No.WO 92/05179), carbamates (U.S. Pat. No. 5,118,678), carbonates (U.S. Pat. No. 5,260,300), amide esters (U.S. Pat. No. 5,118,678), fluorinated esters (U.S. Pat. No. 5,100,883), acetals (U.S. Pat. No. 5,151,413), silyl ethers (U.S. Pat. No. 5,120,842), bicyclic derivatives (U.S. Pat. No. 5,120,725), rapamycin dimers (U.S. Pat. No. 5,120,727) and O-aryl, O-alkyl, O-alkenyl and O-alkynyl derivatives (U.S. Pat. No. 5,258,389). Various rapamycin prodrugs have also been developed (U.S. Pat. Nos. 5,672,605, 5,583,139, 5,527,907, 5,457,111, 5,955,100, 6,146,658, and 5,935,995).

[0049] As rapamycin has been shown to possess excellent immunosuppressant, antifungal, antitumor, and other important biological activities, a need still exists for improved derivatives that increase solubility and improve the pharmakokinetic profile while decreasing its toxicity. The present invention addresses these needs.

SUMMARY OF THE INVENTION

[0050] The present invention is based in part on the recognition that carbohydrate derivatives of rapamycin in which the rapamycin molecule is modified at the 31- and/or 42- position, as defined by the current Chemical Abstracts nomenclature, by attaching monosaccharide(s), oligosaccharide(s) or pseudosugars, have similar or enhanced pharmacokinetic and/or pharmacodynamic profiles compared to rapamycin. In addition, administration of the rapamycin carbohydrate derivatives may result in reduced toxicity with retention of the desired pharmacological effect. In addition, the rapamycin carbohydrate derivatives may render rapamycin more water soluble allowing for simplified drug formulation. Thus, the present invention provides compounds with characteristics that are distinct from other drugs in its class such as rapamycin.

[0051] In one aspect, the invention is directed to a rapamycin carbohydrate derivative having the structure of formula (I):



(I)

wherein

n = 0 or 1, R^1 and R^2 are independently hydrogen or-X-Z, wherein each X is a linker, and each Z is a carbohydrate moiety independently selected from the group consisting of a monosaccharide, an oligosaccharide and a pseudosugar wherein Z is attached to X through a hydroxyl oxygen atom of Z with the proviso that R^1 and R^2 are not both hydrogen. In an embodiment, R^1 can be hydrogen and R^2 can be X-Z or in another embodiment, R^2 is hydrogen and R^1 is X-Z. In a further embodiment, X can be selected from the group consisting of (i) $-R^3C(O)-$; (ii) $-C(O)R^3-$; iii) $-R^3S(O)_2-$; and (iv) $-S(O)_2R^3-$; wherein R^3 is selected from the group consisting of: (a) $-(CH_2)_p-$ where p is an integer from 1 to 18; (b) $-(CH_2)_n-O-(CH_2)_m-$ where n and m are each independently an integer from 2 to 6; and(c) a bond. In another embodiment, X can be selected from the group consisting of -C(O)- and $-SO_2-$. In an additional embodiment, X can be a single functional group. In another aspect, Z can be selected from the group consisting of fructose, fucitol and allose. In yet a further aspect, Z can be a monosaccharide derivative wherein at least one of the hydroxyl groups of the monosaccharide is replaced with a hydrogen, an alkoxy, an alkanoate or a halogen group.

[0052] Rapamycin carbohydrate derivatives having the structure of formula I that are within the scope of this invention include, for example, those set forth below (including pharmaceutically acceptable salts thereof):

42-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-[2-(Methyl-D-glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-D-fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-L-fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-D-fructosylcarbonyl)rapamycin;

31-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-L-fructosylcarbonyl)rapamycin;

42-O-(D-Allosylcarbonyl)rapamycin;

42-O-[2-(D-Allosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Allosylcarbonyl)rapamycin;

42-O-[2-(L-Allosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-allosylcarbonyl)rapamycin;

31-O-(L-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-allosylcarbonyl)rapamycin;

42-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-[2-(D-Fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-[2-(L-Fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fructoslylcarbonyl)rapamycin;

31-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fructoslylcarbonyl)rapamycin;

42-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-[2-(D-Fucitolylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-[2-(L-Fucitolylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fucitolylcarbonyl)rapamycin;

31-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fucitolylcarbonyl)rapamycin;

42-O-(D-Glucalylcarbonyl)rapamycin;

42-O-[2-(D-Glucalylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Glucosylcarbonyl)rapamycin;

42-O-[2-(D-Glucosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Glucosylcarbonyl)rapamycin;

42-O-[2-(L-Glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Glucalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucalylcarbonyl)rapamycin;

31-O-(D-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucosylcarbonyl)rapamycin;

31-O-(L-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-glucosylcarbonyl)rapamycin;

42-O-(L-Sorbosylcarbonyl)rapamycin;

42-O-(D-Sorbosylcarbonyl)rapamycin;

31-O-(L-Sorbosylcarbonyl)rapamycin;

31-O-(D-Sorbosylcarbonyl)rapamycin;

42-O-[2-(L-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-[2-(D-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sorbosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-sorbosylcarbonyl)rapamycin;

42-O-(D-Lactalylcarbonyl)rapamycin;

42-O-[2-(D-Lactalylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Lactalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-lactalylcarbonyl)rapamycin;

42-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-[2-(D-Sucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sucrosylcarbonyl)rapamycin;

42-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-[2-(D-Gentobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(D-gentobiosylcarbonyl)rapamycin

42-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-[2-(D-Cellobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellobiosylcarbonyl)rapamycin;

42-O-(D-Turanosylcarbonyl)rapamycin;

42-O-[2-(D-Turanosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Turanosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-turanosylcarbonyl)rapamycin;

42-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-[2-(D-Palatinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-palatinosylcarbonyl)rapamycin;

42-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltosylcarbonyl)rapamycin;

42-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-[2-(D-Maltulosylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Maltosylcarbonyl)rapamycin;

42-O-[2-(D-Maltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltulosylcarbonyl)rapamycin;

31-O-(D-Maltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltosylcarbonyl)rapamycin;

42-O-(D-Lactosylcarbonyl)rapamycin;

42-O-[2-(D-Lactosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-lactosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-lactosylcarbonyl)rapamycin;

42-O-(D-Melibiosylcarbonyl)rapamycin;

31-O-(D-Melibiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-melibiosylcarbonyl)rapamycin;

42-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-[2-(D-Leucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-leucrosylcarbonyl)rapamycin;

42-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-[2-(D-Rafinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-rafinosylcarbonyl)rapamycin;

42-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltotriosylcarbonyl)rapamycin;

42-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-[2-(D-Cellotetraosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellotetraosylcarbonyl)rapamycin;

42-O-(Valiolylcarbonyl)rapamycin

42-O-[2-(D-Valiolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolylcarbonyl)rapamycin

42-O-(Valiolonylcarbonyl)rapamycin

42-O-[2-(D-Valiolonylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolonylcarbonyl)rapamycin
42-O-(2-Hydroxyethyl)-31-O-(valiolonylcarbonyl)rapamycin
42-O-(Valienolylcarbonyl)rapamycin
42-O-[2-(D-Valienolylcarbonyloxy)ethyl]rapamycin;
31-O-(Valienolylcarbonyl)rapamycin
42-O-(2-Hydroxyethyl)-31-O-(valienolylcarbonyl)rapamycin
42-O-(Valienoneylcarbonyl)rapamycin
42-O-[2-(D-Valienoneylcarbonyloxy)ethyl]rapamycin;
31-O-(Valienoneylcarbonyl)rapamycin
42-O-(2-Hydroxyethyl)- 31-O-(valienoneylcarbonyl)rapamycin

[0053] In another aspect, the invention is directed to a pharmaceutical composition comprising the aforementioned rapamycin carbohydrate derivative, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. In addition, an embodiment of the invention is a method for treating a disease treatable by rapamycin by administering a therapeutically effective amount of the rapamycin carbohydrate derivative to a subject in need thereof.

[0054] In yet another aspect of the current invention, a method is presented for treating a condition such as transplantation rejection, host vs. graft disease, graft vs. host disease, leukemia, lymphoma, hyperproliferative vascular disorders, autoimmune disease, diseases of inflammation, solid tumors, and fungal infections in a patient, said method comprising administering a therapeutically effective amount of the aforementioned pharmaceutical composition to a patient in need thereof.

[0055] Still further, the invention provides a medical device where the medical device comprises a rapamycin carbohydrate derivative or a pharmaceutically acceptable salt thereof. In an embodiment, the device is coated with the rapamycin carbohydrate derivative.

[0056] And, the invention provides a method for treating a disease treatable by rapamycin, comprising coadministering a therapeutically effective amount of a rapamycin carbohydrate derivative of the present invention to a subject in need thereof with a pharmaceutical

composition selected from the group consisting of a cyclosporine or cyclosporine derivative, a steroid, or an immunomodulatory compound.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] Figure 1 depicts fructose as it exists in several configurations in solution

[0058] Figure 2 depicts a general approach to the preparation of rapamycin carbohydrate derivatives of the present invention. "RAPA-OH" stands for rapamycin, wherein the hydroxyl group (-OH) may be any of the hydroxyl groups of rapamycin.

[0059] Figure 3 depicts the reaction pathway for the synthesis of 42-O-(D-fructosylcarbonyl)rapamycin.

[0060] Figure 4 depicts the reaction pathway for the synthesis of 31-O-(D-fructosylcarbonyl)rapamycin.

[0061] Figure 5 depicts the *in vitro* immunosuppressive activity of rapamycin, 42-O-(D-fructosylcarbonyl)rapamycin and a carbamate-linked analog of 42-O-(D-fructosylcarbonyl)rapamycin.

[0062] Figure 6, 7 and 8 depict pharmacokinetic profiles of selected rapamycin carbohydrate derivatives in rats.

[0063] Figure 9 depicts serum cholesterol levels in rats following treatment with 42-O-(D-fructosylcarbonyl)rapamycin and rapamycin.

[0064] Figure 10 shows the effect of two different doses of rapamycin on platelet aggregation.

[0065] Figure 11 compares the effect of 42-O-(D-fructosylcarbonyl)rapamycin and rapamycin on platelet aggregation.

[0066] Figure 12 illustrates the effect of 42-O-(D-fructosylcarbonyl)rapamycin and rapamycin on survival rates in a rat heart transplant model.

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DETAILED DESCRIPTION OF THE INVENTION

[0067] This invention relates to the unexpected discovery that the claimed rapamycin carbohydrate derivatives have improved pharmacological properties as compared to underivatized rapamycin.

10 **[0068]** It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural references unless context clearly dictates otherwise. Thus, for example, a reference to "a rapamycin carbohydrate derivative" includes a plurality of such derivatives; a reference to a "pharmaceutically acceptable carrier" is a reference to one or more carriers and to equivalents thereof known to those skilled in the art, and so forth.

15 **[0069]** Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described.

20 **[0070]** All publications cited herein are incorporated herein by reference in their entirety for the purpose of describing and disclosing the methodologies, reagents, and tools reported in the publications that might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

25 **[0071]** The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, cell biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. (See, e.g., Gennaro, A.R., ed. (1990) Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co.; Colowick, S. et al., eds., Short Protocols in
30 Molecular Biology, 4th edition, John Wiley & Sons; Ream et al., eds. (1998) Molecular Biology Techniques: An Intensive Laboratory Course, Academic Press).

Definitions

[0072] When discussing rapamycin carbohydrate derivatives, compositions, or methods, the following terms have the following meanings unless otherwise indicated. Undefined terms have their art-recognized meanings.

[0073] The term "alkyl" refers to alkyl groups having from 1 to 10 carbon atoms, for example 1 to 6 carbon atoms, and includes both straight chain and branched chain alkyl groups. This term is exemplified by groups such as methyl, t-butyl, n-heptyl, octyl and the like.

[0074] The term "alkene" refers to an unsaturated alkyl group having at least one point of alkene unsaturation (i.e. -C=C-) and further having from 1 to 10 carbon atoms, for example from 1 to 6 carbon atoms, and includes both straight chain and branched chain alkyl groups.

[0075] The term "alkyne" refers to an unsaturated alkyl group having at least one point of alkyne unsaturation (i.e. –C≡C-) and further having from 1 to 10 carbon atoms, for example from 1 to 6 carbon atoms, and includes both straight and branched chain alkyl groups.

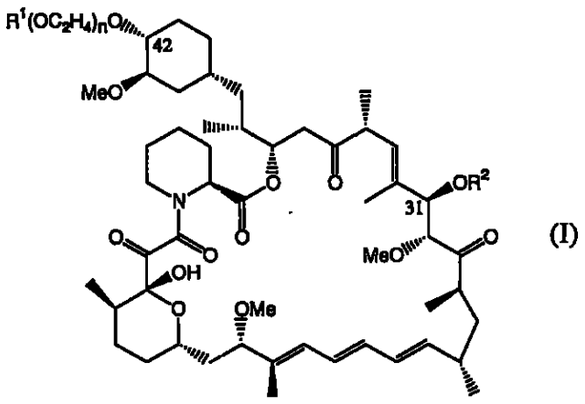
[0076] The term "aromatic" group refers to an aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring (e.g., phenyl) or multiple condensed rings (e.g., naphthyl or anthryl) which condensed rings may or may not be aromatic (e.g., 2-benzoxazolinone, 2H-1,4-benzoxazin-3(4H)-one-7-yl, and the like).

[0077] The term "protecting group" or "blocking group" refers to any group which, when bound to one or more hydroxyl group(s) of rapamycin or a sugar moiety, prevents reactions from occurring at these hydroxyl group(s) and which protecting groups can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl group(s). The particular removable protecting group employed is determined by the nature of the compounds and chemical processes being utilized. Removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidene, phenacyl, t-butyldimethylsilyl and trialkylsilyls such as triethylsilyl, triisopropylsilyl, trimethylsily, tributylsilyl and the like, and any other group that can be introduced chemically

onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

The Rapamycin Carbohydrate Derivatives

[0078] As discussed above, the rapamycin carbohydrate derivatives of the invention are compounds having the structure of formula (I):



(I)

wherein

n=0 or 1, R^1 and R^2 are independently hydrogen or-X-Z, wherein each X is a linker, and each Z is a carbohydrate moiety independently selected from the group consisting of a monosaccharide, oligosaccharide and pseudosugar, with the proviso that R^1 and R^2 are not both hydrogen.

[0079] As will be evident, the rapamycin derivatives can have sugar derivative moieties attached at the 42-position, the 31-position, or both the 42- and the 31-positions. In one embodiment, the sugar derivative is attached at the 42-position alone and in another embodiment the sugar derivative is attached at the 31-position alone.

[0080] It is important to note that in naturally-occurring rapamycin, the 41-methoxy and 42-hydroxy substituents exist in a *trans* configuration relative to each other. The 42-O-

(glycosylcarbonyl) rapamycin compounds of the present invention are prepared in such a way as to retain the *trans* configuration of the 41- and 42-substituents. Consequently, upon hydrolysis of the carbonate linkage, the rapamycin will be released in its naturally-occurring configuration. Similarly, the 31-O-(glycosylcarbonyl)rapamycin compounds of the present invention are prepared in such a way as to retain the naturally-occurring stereochemistry of the 31-hydroxyl substitutent of rapamycin.

The Carbohydrate Moiety

[0081] The carbohydrate (or sugar) moiety, represented by the identifier "Z" in formula (I), is formed from a monosaccharide, oligosaccharide, pseudosugar or derivative thereof having a reactive functional group that can be coupled (i) directly to the hydroxyl group(s) at either or both of the 31- and 42- positions of rapamycin or (ii) to a reactive functional group(s) on an activated rapamycin to yield a rapamycin carbohydrate derivative of the present invention. The functional group is optionally attached to a linker that in turn is attached to the sugar, typically but not necessarily at the anomeric center. Such optional linker groups are discussed further below.

[0082] The sugars that comprise the sugar derivative can differ from each other in a multitude of ways. For example, they can exist in the pyranose or furanose forms to differing degrees. Certain sugars such as fucitol exist exclusively in the open chain form whereas many others (e.g. glucose, ribose, allose) exist predominantly in the cyclic form. Physicochemical properties of the sugar such as the geometry, composition, size, flexibility or rigidity, and the relative hydrophilicity can all affect the chemical characteristics of the rapamycin carbohydrate derivative.

[0083] In solution fructose, for example, can exist mostly in a 6-membered pyranose form and/or its 5-membered furanose form. (See Figure 1). And, each of these forms can exist in alpha or beta configurations. In addition, fructose can exist in an open-chain form. Therefore, fructose can exist in its α-fructopyranose, β-fructopyranose, α-fructofuranose or , β-fructofuranose or open chain forms as shown in Fig. 6. And, fructose can be attached to a

drug in any of these configurations. In its 6-membered pyranose form, fructose is more likely to form a bond, to a drug, for example, through the single primary alcohol that is present at the 1 position in the pyranose form. In its 5-membered furanose form, fructose has two primary alcohols which are at the 1 and 6 position. In the furanose form, bonds are likely through either of these primary alcohols at the 1 or the 6 position.

[0084] The manner in which the body absorbs and processes the specific sugars varies. Many people have difficulty processing lactose, for instance. Such lactose intolerance could make the incorporation of lactose into the rapamycin carbohydrate derivative unsuitable. Further, oligosaccharides are not absorbed intact from the digestive tract and must first be digested to their monosaccharide constituents. Monosaccharides, however, are absorbed by transporter systems located in the brush border membrane of enterocytes. See, S. Miura, Journal of Gastroenterology 37, 491 (2002). For example, glucose and galactose are absorbed by the SGLT1 transporter system. Another transporter, GLUT2, facilitates mainly glucose transport, and yet another transporter known as GLUT5 transports fructose. The presence in the gastrointestinal tract of different monosaccharide transporter proteins with differing selectivities could lead to varied absorption of various rapamycin carbohydrate derivatives. That is, certain derivatives may be more readily able to take advantage of an available transporter in order to facilitate the passage of the rapamycin carbohydrate derivative out of the gastrointestinal tract and deliver it to the blood stream where the carbohydrate moiety can be cleaved off, thereby releasing rapamycin. It is believed that such a facilitated process may result in lowered gastrointestinal toxicity associated with localized exposure to rapamycin.

[0085] In view of the above, it is apparent that the appropriate selection of the carbohydrate moiety (monosaccharide, oligosaccharide, or pseudosugar) incorporated into the rapamycin carbohydrate derivative can have a major influence on the derivative's pharmacokinetic and/or pharmacodynamic properties. Accordingly, the carbohydrate moiety can be carefully chosen to optimize the pharmacokinetic and/or pharmacodynamic properties of the rapamycin carbohydrate derivative.

[0086] Suitable monosaccharides include, but are not limited to, any of several simple open or closed chain sugars (in the L or D configuration), typically having 5 or 6 carbons (a

pentose monosaccharide or a hexose monosaccharide), as well as 7 carbons (heptose monosaccharide). Included are sugar derivatives in which the ring oxygen atom has been replaced by carbon, nitrogen or sulfur, amino sugars in which a hydroxyl substituent on the simple sugar is replaced with an amino group or sugars having a double bond between two adjacent carbon atoms, (e.g. glucosamine, 5-thio-D-glucose, nojirimycin, deoxynojirimycin, 1,5-anhydro-D-sorbitol, 2,5-anhydro-D-mannitol, 2-deoxy-D-galactose, 2-deoxy-D-glucose, 3-deoxy-D-glucose, allose, arabinose, arabinitol, fucitol, fucose, galactitol, glucitol, iditol, lyxose, mannitol, levo-rhamnitol, 2-deoxy-D-ribose, ribose, ribitol, ribulose, rhamnose, xylose, xylulose, allose, altrose, fructose, galactose, glucose, gulose, idose, levulose, mannose, psicose, sorbose, tagatose, talose, galactal, glucal, fucal, rhamnal, arabinal, xylal, valienamine, validamine, valiolamine, valiol, valiolon, valienol, valienone, glucuronic acid, galacturonic acid, N-acetylneuraminic acid, gluconic acid D-lactone, galactonic acid γ-lactone, galactonic acid δ-lactone, mannonic acid γ-lactone, D-*altro*-heptulose, D-*manno*-heptulose, D-*glycero*-D-*manno*-heptose, D-*glycero*-D-*gluco*-heptose, D-*allo*-heptulose, D-*altro*-3-heptulose, D-*glycero*-D-*manno*-heptitol, D-*glycero*-D-*altro*-heptitol and the like), The hydroxyl groups on monosaccharides may optionally be replaced with hydrogen, alkoxy (e.g. 2-O-methyl-D-fructose), alkanoate or halogen groups. Included are sulfate and/or phosphate derivatives of monosaccharides as defined herein.

[0087] Suitable oligosaccharides include, but are not limited to, carbohydrates having from 2 to 10 or more monosaccharides linked together. The constituent monosaccharide unit may be, for example, a pentose monosaccharide, a hexose monosaccharide, or a pseudosugar (including a pseudoaminosugar). Oligosaccharides do not include bicyclic groups that are formed by fusing a monosaccharide to a benzene ring, a cyclohexane ring, or a heterocyclic ring.

[0088] Pseudosugars that may be used in the invention are members of the class of compounds wherein the ring oxygen atom of the cyclic monosaccharide is replaced by a methylene group. Pseudosugars are also known as "carba-sugars."

The Linker

[0089] As discussed above, the carbohydrate moiety is covalently attached to the rapamycin via a linker, indicated as "X" in formula I. In it's simplest form, the linker is a chemical functional group that is formed when the sugar derivative is covalently attached to rapamycin, but is itself part of neither rapamycin nor the sugar molecule. In one embodiment, the nature of the linker is determined by the chemistry employed to covalently attach the sugar or sugar derivative to rapamycin. For example, if 42-O-(4-nitrophenyloxycarbonyl) rapamycin (an activated rapamycin) is reacted with a sugar the result is a rapamycin carbohydrate derivative wherein the 42-hydroxyl oxygen of rapamycin is covalently bonded to a carbonyl group which in turn is covalently bonded to a hydroxyl oxygen on the sugar. In this example, the linker is the carbonyl group (C=O). An example of a linker as defined herein is depicted in Figure 3. Examples of linkers include moieties such as carbonyl (C=O) and sulfonyl (O=S=O). Such a carbonyl or sulfonyl linker is recognized as a single functional group linker.

[0090] The linker together with the functionality through which the sugar and rapamycin are attached to that linker form a "linkage." For example, when a carbonyl linker is attached to a sugar via one of its hydroxyl oxygen atoms and then to a rapamycin hydroxyl oxygen, the resulting "linkage" is a carbonate (i.e. –OC(O)O-). An example of a linkage as defined herein in depicted in Figure 4. Examples of linkages include esters, ethers, carbonates, carbamates, sulfates and urethanes.

[0091] The linker and associated linkage are selected to provide a biocompatible, substantially non-immunogenic rapamycin carbohydrate derivative. While the present invention is based on the recognition that the presence of the sugar(s) will improve the effectiveness of rapamycin, its pharmacokinetic and/or pharmacodynamic properties may also be enhanced by the geometry, composition, size, flexibility or rigidity, the relative hydrophobicity or hydrophilicity, and similar properties of the linker and/or linkage. Accordingly, the linker or linkage can be chosen to optimize the pharmacokinetic and/or pharmacodynamic properties of the rapamycin carbohydrate derivative. For example, the rates of acid-catalyzed or enzymatic hydrolysis of the derivatives resulting in the release of

free rapamycin will vary depending on which linkage is created. The linker or linkage may be biologically "neutral," i.e., not itself contribute any additional biological activity to the carbohydrate rapamycin derivative, or it may be chosen to further enhance the biological activity of the compound.

[0092] The reaction chemistries resulting in linkers and linkages employ conventional techniques. These techniques generally involve the use of complimentary reactive functional groups located on rapamycin or activated rapamycin and the sugar or sugar derivative. Examples of complimentary functional groups and the resulting linkages are found in Table 1.

TABLE 1. LINKAGES RESULTING FROM COMPLIMENTARY FUNCTIONALITIES

FIRST REACTIVE GROUP	SECOND REACTIVE GROUP	LINKAGE
hydroxyl	isocyanate	urethane
amine	epoxide	ß-aminohydroxy
sulfonyl halide	amine	sulfonamide
carboxyl acid	amine	amide
hydroxyl	alkyl/aryl halide	ether
aldehyde	amine/$NaCNBH_3$	amine
ketone	amine/$NaCNBH_3$	amine
amine	isocyanate	carbamate
carboxyl acid	hydroxyl	ester
chloroformate	hydroxyl	carbonate

[0093] If desired, the linker may contain more than a single functional group. Complex linkers may be used to provide different chemical properties into the rapamycin carbohydrate derivative. For example, different hydrophobic/hydrophilic characteristics may be imparted to the rapamycin carbohydrate derivative by manipulation of the linker. Similarly, charged moieties may also be introduced. Techniques for modification of the linker will be readily understood by those of skill in the art. For example, the hydrophobic nature of a linker

derived from hexamethylene diamine or a related polyamine can be modifed to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group.

[0094] For example, glycosyl-Y[-C(=Y)-X-]p-W(R)n-X-C(=Y)-drug where W is an aromatic or heteroaromatic or aliphatic group with conjugated double bonds or an amino-acid derivative radical which cyclizes after elimination of the glycosyl radical was disclosed in U.S. Pat. 6,146,658. These complex linkers are designed to self-eliminate via cyclization subsequent to enzymatic removal of the glycosyl moiety.

[0095] Wide varieties of linkers are suitable for use in the invention. Ordinarily skilled artisans will recognize that a carbonyl (C=O) or a sulfonyl (O=S=O) linker, or a carbonyl or sulfonyl linker combined with a simple alkyl chain or a polyether chain (e.g. a small number of repeating ethylene oxide units) will have properties that are different from those of the more complex linkers described above. For example, it is contemplated that the linkers of the present invention will not undergo cyclization to self-eliminate. Further, the susceptibility of the linker/drug compound to enzymatic or hydrolytic cleavage will be highly dependent on the nature of the spacer or linker moiety. In addition, it is contemplated that compounds with complicated linkers may be more difficult and expensive to produce than compounds with only single functional group linkers, such as those of the present invention.

[0096] Wide varieties of linkers are commercially available (e.g., Chem Sources USA and Chem Sources International; the ACD electronic database; and Chemical Abstracts). Many of the linkers that are suitable for use in this invention fall into this category. Others can be readily synthesized by methods known in the art, and as described below. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples of commercially available linkers are peptides or polyamides, hydrocarbons, aromatics, heterocyclics, ethers, lipids, cationic or anionic groups, or a combination thereof.

[0097] It is contemplated that the properties of the linker of this invention can be modified by the addition or insertion of ancillary groups, for example, to change the solubility of the multibinding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity,

hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more polyethylene glycol (PEG) groups onto the linker enhances the hydrophilicity and water solubility of the rapamycin carbohydrate derivative, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further, PEG may decrease antigenicity and potentially enhances the overall rigidity of the linker.

[0098] Ancillary groups that enhance the water solubility/hydrophilicity of the linker, and accordingly, the resulting compounds, are useful in practicing this invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, small repeating units of ethylene glycols, alcohols, polyols, (e.g., glycerin, glycerol propoxylate, etc.) carboxylates (e.g., small repeating units of glutamic acid, acrylic acid, etc.), amines (e.g., tetraethylenepentamine), and the like to enhance the water solubility and/or hydrophilicity of the carbohydrate rapamycin derivatives of this invention. For example, the ancillary group used to improve water solubility/hydrophilicity may be a polyether containing a small number of repeating ethylene oxide (-CH_2CH_2O-) units.

[0099] The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the rapamycin carbohydrate derivatives is also within the scope of this invention. Lipophilic groups useful with the linkers of this invention include, but are not limited to, lower alkyl, aromatic groups, and polycyclic aromatic groups. The aromatic groups may be either unsubstituted or substituted with other groups, but are at least substituted with a group that allows their covalent attachment to the linker. As used herein the term "aromatic groups" incorporates both aromatic hydrocarbons and heterocyclic aromatics. Other lipophilic groups useful with the linker of this invention include fatty acid derivatives that may or may not form micelles in aqueous medium and other specific lipophilic groups that modulate interactions between the carbohydrate rapamycin derivative and biological membranes.

[00100] The flexibility of the linker can be manipulated by the inclusion of ancillary groups that are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker, or bonds between the linker and the ancillary group(s), or

bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational freedom is restrained by the presence of rings and/or bonds, for example, aryl, heteroaryl and heterocyclic groups. Other groups that can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

[00101] Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the linker into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other, which is inversely related to the square of the distance between the groups, will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further, ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker in a conformation that allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, protected groups that bear a latent charge which is unmasked, following addition to the linker, by deprotection, a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art, is within the scope of this invention.

[00102] Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups, and structures incorporating one or more carbon-carbon bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers that are branched- or straight-chain species. Branched-species are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

[00103] In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art.

[00104] Linkers can be attached to rapamycin or a sugar by employing reactive functional groups. The reactive functional groups are selected relative to the functional

groups available on rapamycin or the sugar for coupling, or which are introduced onto rapamycin or the sugar for this purpose. For example, reaction between a carboxylic acid of the linker and a primary or secondary amine of the sugar in the presence of suitable activating agents results in formation of an amide moiety covalently linking the sugar to the linker. Reaction between an amine group of the linker and a sulfonyl halide of the sugar results in formation of a sulfonamide moiety covalently linking the sugar to the linker. Reaction between an alkyl or aryl halide of the linker and an alcohol of the sugar results in formation of an ether moiety covalently linking the sugar to the linker.

[00105] Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as Advanced Organic Chemistry, Jerry March, John Wiley &Sons (5th Ed., 2000). The term linker embraces everything that is not considered to be part of the sugar or rapamycin. Linkers can be derived from linear compounds having reactive functional groups at the end of the linker.

[00106] Suitable divalent linkers include, by way of example, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates, diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the sugar and rapamycin to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the above-discussed Table 1.

[00107] In embodiments of the invention, the linker (X) is selected from the group consisting of: (i) $-R^3C(O)$; (ii) $-C(O)R^3$, (iii) $-R^3S(O)_2$; or (iv) $-S(O)_2R^3$ where R^3 is selected from the group consisting of: (i) $-(CH_2)_p-$ where p is an integer from 1 to 18, (ii) $-(CH_2)_n-O-(CH_2)_m-$ where n and m are each independently an integer from 2 to 6, or (iii) a bond. It is to be understood that the linker (X) may be the same or different at each occurrence, i.e., at the 31 and 42 positions. In additional embodiments of the invention, the linker (X) is carbonyl (C=O), sulfonyl (O=S=O), or a single functional group. Carbonyl linkers may be in the 31 position, the 42 position, or both. In other embodiments, R^1 is –C(O)-Z and R^2 is H or R^2 is –C(O)-Z and R^1 is H.

[00108] Accordingly, rapamycin carbohydrate derivatives having the structure of formula I that are within the scope of this invention include, for example, those set forth below (including pharmaceutically acceptable salts thereof):

42-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-[2-(Methyl-D-glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-D-fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-L-fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-D-fructosylcarbonyl)rapamycin;

31-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-L-fructosylcarbonyl)rapamycin;

42-O-(D-Allosylcarbonyl)rapamycin;

42-O-[2-(D-Allosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Allosylcarbonyl)rapamycin;

42-O-[2-(L-Allosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-allosylcarbonyl)rapamycin;

31-O-(L-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-allosylcarbonyl)rapamycin;

42-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-[2-(D-Fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-[2-(L-Fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fructoslylcarbonyl)rapamycin;

31-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fructoslylcarbonyl)rapamycin;

42-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-[2-(D-Fucitolylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-[2-(L-Fucitolylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fucitolylcarbonyl)rapamycin;

31-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fucitolylcarbonyl)rapamycin;

42-O-(D-Glucalylcarbonyl)rapamycin;

42-O-[2-(D-Glucalylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Glucosylcarbonyl)rapamycin;

42-O-[2-(D-Glucosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Glucosylcarbonyl)rapamycin;

42-O-[2-(L-Glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Glucalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucalylcarbonyl)rapamycin;

31-O-(D-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucosylcarbonyl)rapamycin;

31-O-(L-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-glucosylcarbonyl)rapamycin;

42-O-(D-Lactalylcarbonyl)rapamycin;

42-O-[2-(D-Lactalylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Lactalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-lactalylcarbonyl)rapamycin;

42-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-[2-(D-Sucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sucrosylcarbonyl)rapamycin;

42-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-[2-(D-Gentobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(D-gentobiosylcarbonyl)rapamycin
42-O-(D-Cellobiosylcarbonyl)rapamycin;
42-O-[2-(D-Cellobiosylcarbonyloxy)ethyl]rapamycin;
31-O-(D-Cellobiosylcarbonyl)rapamycin;
42-O-(2-Hydroxyethyl)-31-O-(D-cellobiosylcarbonyl)rapamycin;
42-O-(D-Turanosylcarbonyl)rapamycin;
42-O-[2-(D-Turanosylcarbonyloxy)ethyl]rapamycin;
31-O-(D-Turanosylcarbonyl)rapamycin;
42-O-(2-Hydroxyethyl)-31-O-(D-turanosylcarbonyl)rapamycin;
42-O-(D-Palatinosylcarbonyl)rapamycin;
42-O-[2-(D-Palatinosylcarbonyloxy)ethyl]rapamycin;
31-O-(D-Palatinosylcarbonyl)rapamycin;
42-O-(2-Hydroxyethyl)-31-O-(D-palatinosylcarbonyl)rapamycin;
42-O-(D-Isomaltosylcarbonyl)rapamycin;
42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;
31-O-(D-Isomaltosylcarbonyl)rapamycin;
42-O-(2-Hydroxyethyl)-31-O-(D-isomaltosylcarbonyl)rapamycin;
42-O-(D-Maltulosylcarbonyl)rapamycin;
42-O-[2-(D-Maltulosylcarbonyloxy)ethyl]rapamycin;
42-O-(D-Maltosylcarbonyl)rapamycin;
42-O-[2-(D-Maltosylcarbonyloxy)ethyl]rapamycin;
31-O-(D-Maltulosylcarbonyl)rapamycin;
42-O-(2-Hydroxyethyl)-31-O-(D-maltulosylcarbonyl)rapamycin;
31-O-(D-Maltosylcarbonyl)rapamycin;
42-O-(2-Hydroxyethyl)-31-O-(D-maltosylcarbonyl)rapamycin;\
42-O-(L-Sorbosylcarbonyl)rapamycin;
42-O-(D-Sorbosylcarbonyl)rapamycin;
31-O-(L-Sorbosylcarbonyl)rapamycin;
31-O-(D-Sorbosylcarbonyl)rapamycin;
42-O-[2-(L-Sorbosylcarbonyloxy)ethyl]rapamycin;
42-O-[2-(D-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sorbosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-sorbosylcarbonyl)rapamycin;

42-O-(D-Lactosylcarbonyl)rapamycin;

42-O-[2-(D-Lactosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-lactosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-lactosylcarbonyl)rapamycin;

42-O-(D-Melibiosylcarbonyl)rapamycin;

31-O-(D-Melibiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-melibiosylcarbonyl)rapamycin;

42-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-[2-(D-Leucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-leucrosylcarbonyl)rapamycin;

42-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-[2-(D-Rafinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-rafinosylcarbonyl)rapamycin;

42-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltotriosylcarbonyl)rapamycin;

42-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-[2-(D-Cellotetraosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellotetraosylcarbonyl)rapamycin;

42-O-(Valiolylcarbonyl)rapamycin

42-O-[2-(D-Valiolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolylcarbonyl)rapamycin

42-O-(Valiolonylcarbonyl)rapamycin

42-O-[2-(D-Valiolonylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolonylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolonylcarbonyl)rapamycin

42-O-(Valienolylcarbonyl)rapamycin

42-O-[2-(D-Valienolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valienolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valienolylcarbonyl)rapamycin

42-O-(Valienoneylcarbonyl)rapamycin

42-O-[2-(D-Valienoneylcarbonyloxy)ethyl]rapamycin;

31-O-(Valienoneylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)- 31-O-(valienoneylcarbonyl)rapamycin

[00109] In addition, the invention is directed to a rapamycin derivative having the structure of formula I wherein n=1, R^1 is H and R^2 is X-Z, wherein X is a linker, and Z is a carbohydrate moiety independently selected from the group consisting of a monosaccharide, an oligosaccharide and a pseudosugar.

[00110] The invention is also directed to a pharmaceutical composition comprising the aforementioned rapamycin carbohydrate derivative, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

Preparation of the Rapamycin Carbohydrate Derivatives

[00111] A general procedure for synthesizing rapamycin carbohydrate derivatives of the present invention involves the coupling of a monosaccharide, oligosaccharide, pseudosugar or sugar derivative to the 31- and/or 42- positions of activated rapamycin.

[00112] For example, as depicted in Figure 2, rapamycin (II) can be reacted with p-nitrophenyl chloroformate to yield an activated rapamycin (III). Under carefully controlled conditions, the reaction will preferentially take place at the 42-hydroxyl position. By altering the reaction conditions, both the 31- and 42-hydroxyl groups can be similarly activated. Selective activation of the 31-hydroxyl group can be accomplished by first protecting the 42-hydroxyl moiety with, for example an alkylsilyl group such as triethylsilyl, triisopropylsilyl or *tert*-butyldimethylsilyl, and then reacting with p-nitrophenyl chloroformate or other

chloroformates. Removal of the protecting group then affords rapamycin activated at the 31-hydroxyl position. Thus, it is possible to prepare a rapamycin derivative that is selectively activated at the 31-position, the 42-position, or both.

[00113] Thereafter, in the second step, a sugar moiety or sugar derivative is reacted with an activated rapamycin (III) to give a rapamycin carbohydrate derivative. When the reactive functional group on the sugar moiety or sugar derivative is a hydroxyl group, the reaction with activated rapamycin may take place on a primary hydroxyl group to form a carbonate linkage to rapamycin. The resulting linker is a carbonyl group. When the reactive functional group on the sugar or sugar derivative is an amino group (which can be situated at any position of the sugar), a carbamate linkage to rapamycin results and the resulting linker is a carbonyl group. Amino substituted sugar derivatives, e.g, sugar-X-NH_2 or sugar-NH_2, can be prepared from precursors by conventional methods. For example, reduction of sugar-X-N_3 or de-phthaloylation of sugar-X-NPhth, where Phth is phthalyl, yields the amino substituted sugar derivative. These precursors can be synthesized by glycosidation of linker moieties with appropriately activated glycosyl donors.

[00114] Thus, using the general approach described herein is it possible to prepare a wide range of rapamycin carbohydrate derivatives in which the 31- and/or 42-hydroxyl groups are modified with a wide range of sugars or sugar derivatives.

[00115] It is contemplated that hydroxyl groups of rapamycin or a rapamycin metabolite, including those that are located in positions other than 31- and 42-, can also be glycosylated as described herein, and the resultant rapamycin carbohydrate derivatives will also exhibit higher water-solubility and/or enhanced pharmacokinetic and/or pharmacodynamic properties compared to their unglycosylated counterparts.

[00116] Rapamycin metabolites are known in the art. For example, Streit *et. al.* structurally identified several rapamycin metabolites from human liver microsomes. See, F. Streit et al., Drug Metabol. Disp., 24, 1272 (1996). These include 41-demethyl rapamycin, 7-demethyl rapamycin, 11-hydroxy rapamycin, and a 24-hydroxy ester hydrolysis degradation product of rapamycin. It has also been shown that the metabolites of rapamycin can undergo this ester hydrolysis. Streit also partially identified di, tri, and tetra hydroxylated rapamycin

metabolites. Wang *et. al.* found 16 hydroxylated and/or demethylated metabolites in the bile of rapamycin treated rats. See, C.K. Wang et al., Proceedings of the 41st ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, 545 (1993). Nickmilder *et. al.* identified a 3,4 and 5,6 dihydrodiol rapamycin metabolite in rat liver microsomes. See, M.J.M. Nickmilder et al., Xenobiotica, 27, 869 (1997). In trough whole blood, Streit *et. al.* have identified 41-demethyl, hydroxy, dihydroxy, and didemethyl rapamycin metabolites. See, F. Streit et al., Clin. Chem., 42, 1417 (1996). These metabolites accounted for 56% of total rapamycin derivatives measured. Finally, Leung *et. al.* looked at the disposition of [^{14}C]-rapamycin in healthy male volunteers. They found that rapamycin represented approximately 35% of the total radioactivity in blood and that 41-demethyl, 7-demethyl, and several hydroxy, hydroxydemethyl, and didemethyl rapamycin metabolites individually represented between 1 and 12% of the total radioactivity. Rapamycin metabolites can be isolated from a number of various sources, including but not limited to blood, urine or feces samples, from liver microsomes or from microorganism cultures.

[00117] Accordingly, in addition to 31- and 42-, the hydroxyl groups of particular interest include those at the 27-, 41-, 3-, 4-, 5-, 6-, 7-, 11- and 24-positions of rapamycin and rapamycin metabolites.

Pharmaceutical Compositions and Utility

[00118] The compounds of this invention may be administered neat or with a pharmaceutical carrier to an animal, such as a warm blooded mammal, and especially humans, in need thereof. The pharmaceutical compositions may also contain other drugs, particularly drugs known to have different mechanisms of action. Thus, the pharmaceutical compositions may contain, in addition to the compounds of the present invention, at least one drug of another class, for example,a calcineurin inhibitor, a steroid or other immunomodulatory compounds which may interfere with DNA synthesis or intra- or inter-cellular signaling or other cell processes. Examples of calcineurin inhibitors include. Cyclosporin A (available from Novartis as Sandimmune® and Neoral® and FK506 (also known as tacrolimus or Prograf® available from Fujisawa). Examples of cyclosporine

derivatives are those disclosed in W099/18120. Examples of steroids include predisone, prednisolone or methylprednisolone. Examples of these immunomodulatory compounds include azothioprine, mycophenolic acid (mycophenolate mofitil or Cellcept® available from Roche), leflunomide available from Aventis, Brequinar, Mizoribine, antibodies including α-LFA-1, and α-ICAM-1, thimoglobuline, IL2R antagonists including basiliximab (Stimulect®), and daclizumab (Zenapax®), alemtuzumab (Campath 1H®, a humanized monoclonal antibody recognizing CD52), Orthoclone OKT3® or muromonab CD3, Atgam(R) lymphocyte immune globulin, ATG (antithymocyte globulin), and other compounds. The individual drugs, and the rapamycin carbohydrate derivative, may be formulated separately as distinct components of the pharmaceutical composition, and administered together or separately.

[00119] The pharmaceutically effective carrier may be solid or liquid. A solid carrier can include one or more substances which may also act as flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid that is in admixture with the finely divided active ingredient. In tablets, the active ingredient is mixed with a carrier having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets may contain up to 99% of the active ingredient. Suitable solid carriers include, for example, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose, polyvinylpyrrolidine, low melting waxes and ion exchange resins.

[00120] Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs, and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, surfactants, buffers, preservatives, sweeteners, flavoring agents, suspending agents, thickening agents, colors, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water (partially containing additives as above, e.g. cellulose

derivatives, possibly sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the carrier can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid carriers are useful in sterile liquid form compositions for parenteral administration. The liquid carrier for pressurized compositions can be halogenated hydrocarbon or other pharmaceutically acceptable propellant.

[00121] Liquid pharmaceutical compositions that are sterile solutions or suspensions are suitable for intramuscular, intraperitoneal, and subcutaneous injection. Sterile solutions can also be administered intravenously. The compound can also be administered orally either in liquid or solid composition form. Pulmonary administration is also contemplated.

[00122] The pharmaceutical composition can be in unit dosage form, e.g. as tablets or capsules. In such form, the composition is sub-divided in unit dose containing appropriate quantities of the active ingredient; the unit dosage forms can be packaged compositions, for example, packeted powders, vials, ampoules, prefilled syringes or sachets containing liquids. The unit dosage form can be, for example, a capsule or tablet itself, or it can be the appropriate number of any such compositions in package form. The dosage to be used in the treatment must be subjectively determined by the attending physician.

[00123] In addition, the compounds of this invention may be employed as a solution, cream, or lotion by formulation with pharmaceutically acceptable vehicles administered to an affected area.

[00124] The compounds of this invention can also be used in conjunction with a medical device. For example, a rapamycin carbohydrate derivative as a component of a drug-coated or impregnated intravascular stent may be used to inhibit neointimal tissue proliferation and thereby prevent restenosis (See, for example U.S. Pat. No. 5,665,728). Rapamycin carbohydrate derivatives can also be used as a component of other drug-coated or impregnated medical devices such as catheters, pumps, or drug-delivery medical devices such as beads or discs containing drugs. The presence of rapamycin, a potent immunosuppressant,

may decrease inflammation, rejection, or other immune responses to the presence of these implantable medical devices in the body.

[00125] The following examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of the present invention.

EXAMPLES

[00126] In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

g	=	gram
mg	=	milligram
kg	=	kilogram
mmol	=	millimole
M	=	molar
N	=	Normal
mL	=	milliliter
min	=	minute
BzCl	=	benzoyl chloride
DMAP	=	4-dimethylaminopyridine
DMS	=	dimethyl sulfate
Py	=	Pyridine
DMF	=	N,N-dimethylformamide
Me	=	methyl
HOAt	=	1-hydroxy-7-azabenzotriazole
HOBT	=	1-hydroxybenzotriazole hydrate
TBDMSiCl	=	tert-butyldimethylsilyl chloride
AcOH	=	acetic acid
THF	=	tetrahydrofuran
LC/MS or LCMS	=	liquid chromatography/mass spectroscopy
HPLC	=	high performance liquid chromatography
conc.	=	concentrated
eq.	=	equivalents

[00127] In the following examples and procedures, the starting materials are available commercially from Aldrich Chemical Company, Inc., Milwaukee, WI 53233 USA; Lancaster Synthesis, Inc., NH 03087 USA; Sigma, St. Louis MO 63178 USA; Maybridge Chemical Co. Trevillett, Tintagel, Cornwall PL34 OHW United Kingdom; TCI America, Portland OR 97203; Frontier Scientific, Utah, USA; and Bachem, Torrance, California, USA.

EXAMPLE 1

Synthesis of 42-O-(D-Fructosylcarbonyl)rapamycin

[00128] Figure 3 depicts the method of synthesis for 42-O-(D-Fructosylcarbonyl)rapamycin. The detailed procedure is described below:

42-O-(4-nitrophenyloxycarbonyl)rapamycin

[00129] A solution of 10.0 g of rapamycin in 50mL of dichloromethane and 10 mL of dry pyridine was cooled to -78°C under a nitrogen atmosphere. To this solution 3.31g of 4-nitrophenyl chloroformate was added and the reaction mixture was stirred for 1 hour at -78°C, and then brought directly to room temperature. Reaction was complete after 2 hours. The mixture was diluted with water and extracted with dichloromethane. The organic phase was washed with water, dried over Na_2SO_4 and evaporated. Chromatography on a silica gel column (solvent: hexanes – ethyl acetate, 2:1) gave 9.66g of 42-O-(4-nitrophenyloxycarbonyl)rapamycin as a yellowish solid (freeze dried from benzene). $C_{58}H_{82}N_2O_{17}$, M=1078.6; MS(ES+): m/z=1101.7 $(M+Na)^+$

42-O-(D-Fructosylcarbonyl)rapamycin

[00130] To a solution of D-fructose (2.025g, 11.24mmol) and DMAP (250mg) in *N,N*-dimethylformamide (25mL) 42-O-(4-nitrophenyloxycarbonyl)rapamycin (4.05g, 3.757mmol)

was added and the reaction mixture was stirred at room temperature for 24 hours. The solvent was evaporated under high vacuum and the residue was flash chromatographed on a silica gel column using dichloromethane – methanol (9:1) as eluent. Obtained product was re-purified on a preparative HPLC column (80% methanol – 20% water, flow 10mL/min.), yielding 1.461g of 42-O-(D-fructosylcarbonyl)rapamycin as white solid (freeze dried from benzene). $C_{58}H_{89}NO_{20}$, M=1119.6; MS(ES+): m/z=1142.7 $(M+Na)^+$ In the alternative HOBT (or HOAT) may be employed in place of DMAP as disclosed in example 3.

EXAMPLE 2

[00131] Following procedures analogous to that outlined in Example 1, and employing the appropriate sugars or sugar derivatives, the following compounds have been obtained:

42-O-(D-Glucosylcarbonyl)rapamycin
42-O-(Methyl-D-glucosylcarbonyl)rapamycin
42-O-(D-Allosylcarbonyl)rapamycin
42-O-(D-Fructosylcarbonyl)rapamycin
42-O-(L-Fructosylcarbonyl)rapamycin
42-O-(D-Fucitolylcarbonyl)rapamycin
42-O-(L-Fucitolylcarbonyl)rapamycin
42-O-(D-Glucalylcarbonyl)rapamycin
42-O-(L-Sorbosylcarbonyl)rapamycin
42-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin
42-O-(D-Lactalylcarbonyl)rapamycin
42-O-(D-Sucrosylcarbonyl)rapamycin
42-O-(D-Gentobiosylcarbonyl)rapamycin
42-O-(D-Cellobiosylcarbonyl)rapamycin
42-O-(D-Turanosylcarbonyl)rapamycin
42-O-(D-Palatinosylcarbonyl)rapamycin
42-O-(D-Isomaltosylcarbonyl)rapamycin
42-O-(D-Maltulosylcarbonyl)rapamycin

42-O-(D-Maltosylcarbonyl)rapamycin

42-O-(D-Lactosylcarbonyl)rapamycin

42-O-(Methyl-D-lactosylcarbonyl)rapamycin

42-O-(D-Melibiosylcarbonyl)rapamycin

42-O-(D-Leucrosylcarbonyl)rapamycin

42-O-(D-Rafinosylcarbonyl)rapamycin

42-O-(D-Isomaltotriosylcarbonyl)rapamycin

42-O-(D-Cellotetraosylcarbonyl)rapamycin

EXAMPLE 3

Synthesis of 31-O-(D-Fructosylcarbonyl)rapamycin

[00132] Figure 4 depicts the method of synthesis for 31-O-(D-Fructosylcarbonyl)rapamycin. The detailed procedure is described below:

42-O-(tert-Butyldimethylsilyl)rapamycin

[00133] To a solution of rapamycin (10g) and imidazole (2.2g) in *N,N*-dimethylformamide (40mL) *tert*-butyldimethylsilyl chloride (1.76g) was added, and the reaction mixture was stirred at room temperature under nitrogen for 5 days. The solvent was evaporated under high vacuum and the residue was chromatographed on a silica gel column (solvent: hexanes – ethyl acetate, 3:2) yielding 5.84g of 42-O-(tert-butyldimethylsilyl)rapamycin as off-white foam. $C_{57}H_{93}NO_{13}Si$, M=1027.6; MS(ES+): m/z=1050.7 $(M+Na)^+$

42-O-(tert-Butyldimethylsilyl)-31-O-(4-nitrophenyloxycarbonyl)rapamycin

[00134] 42-O-(*tert*-Butyldimethylsilyl)rapamycin (5.84g) was dissolved in dichloromethane (30mL) and pyridine (6mL), 4-nitrophenyl chloroformate (2.582g) was added and the reaction mixture was stirred under nitrogen at room temperature for 2 hours. Solvents were evaporated and the residue purified on a silica gel column. Elution with hexanes – ethyl acetate (3:1) gave title compound as yellowish foam (5.4g). $C_{64}H_{96}N_2O_{17}Si$, M=1192.6; MS(ES+): m/z=1215.6 $(M+Na)^+$

31-O-(4-nitrophenyloxycarbonyl)rapamycin

[00135] 42-O-(*tert*-Butyldimethylsilyl)-31-O-(4-nitrophenyloxycarbonyl) rapamycin (5.4g) was dissolved in a mixture of acetic acid (30mL), tetrahydrofuran (10mL) and water (10mL). It was stirred at room temperature for 20 hours. The reaction mixture was diluted with water and extracted with ethyl acetate (3 x 200mL).The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated. Silica gel column chromatography (solvent: hexanes – ethyl acetate, 3:2) gave 2.1g of 31-O-(4-nitrophenyloxycarbonyl) rapamycin as yellowish solid (freeze dried from benzene). $C_{58}H_{82}N_2O_{17}$, M=1078.6; MS(ES+): m/z=1101.6 $(M+Na)^+$

31-O-(D-Fructosylcarbonyl)rapamycin

[00136] A mixture of 31-O-(4-nitrophenyloxycarbonyl)rapamycin (1.3g), D-fructose (0.434g), and 1-hydroxybenzotriazole (HOBT) (0.325g) in dry pyridine (20mL) was stirred at room temperature for 3 days. Then, the solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel column. Elution with dichloromethane – methanol (10:1) provided 31-O-(D-fructosylcarbonyl)rapamycin (0.625g, 46%) as white solid (freeze dried from benzene). $C_{58}H_{89}NO_{20}$, M=1119.6; MS(ES+): m/z=1142.7 $(M+Na)^+$

[00137] In an analogous manner, 31-O-(D-allosylcarbonyl)rapamycin was also prepared. In addition, in the alternative, DMAP may be employed in place of HOBT as disclosed in example 1.

Example 4

Synthesis of 42-O-(2-O-methyl-β-D-fructosylcarbonyl)rapamycin

1,3,4,5-Tetra-O-benzoyl-β-D-fructopyranose

[00138] A mixture of anhydrous pyridine (52 mL), benzoyl chloride (51.5 mL, 0.444 mmol) and anhydrous dichloromethane (125 mL) was cooled to -10°C. Finely powdered fructose (20 g, 0.111 mmol) was added portion wise and the reaction mixture was stirred at -10°C for 18 hours. The reaction mixture was quenched with ice water, diluted with dichloromethane and transferred to separatory funnel. Organic layer was separated and washed with 5 % citric acid, saturated sodium bicarbonate, water, and dried over sodium sulfate. It was filtered and the solvent evaporated. The residue was dissolved in diethyl ether (75 ml) then added slowly to hexanes (300 ml) to give a white solid which upon drying afforded 58.9 g (89 %) of 1,3,4,5-tetra-O-benzoyl-β-D-fructopyranose

1,3,4,5-Tetra-O-benzoyl-2-O-methyl-β-D-fructopyranose

[00139] To a solution of 1,3,4,5-tetra-O-benzoyl-β-D-fructopyranose (10.00g, 16.8mmol) in acetone (40 mL) dimethyl sulfate (2.4ml, 25.16 mmoles, 1.5 eq.) was added followed by potassium carbonate (3.48g, 25.16 mmoles) and the reaction mixture was stirred at 50°C under nitrogen for 18 hours. Solvents were removed under vacuo and the resulting residue was dissolved in ethyl acetate (150ml), washed with 5% citric acid, water, brine, dried over sodium sulfate, filtered and concentrated to an oily solid. Purification on a silica gel column (hexane: ethyl acetate, 4:1) afforded 10.0g (98%) of title compound as white foam.

2-O-methyl-β-D-fructopyranose

[00140] A solution of sodium methoxide (0.5 M in methanol, 10.0 ml) was added to a vigorously stirred solution of 1,3,4,5-tetra-O-benzoyl-2-O-methyl-β-D-fructopyranose (10.0g, 16.4 mmol) in anhydrous methanol. After stirring for 1.5 hrs at room temperature the reaction was complete. The pH was adjusted to 7.0 with Amberlite IRC-50 (~4.0g). The solids were removed by filtration and the filtrate was concentrated in vacuo. Purification on a silica gel column (methanol: dichloromethane, 4:1 and 3:1) provided the product as white foam 2.70 g (81%).

42-O-(2-O-methyl-β-D-fructosylcarbonyl)rapamycin

[00141] A mixture of 42-O-(4-nitrophenyloxycarbonyl)rapamycin (10.0 g, 9.3 mmol), 2-O-methyl-β-D-fructopyranose (6.2g, 23 mmol, 3 eq.), and 1-hydroxy-7-azabenzotriazole (HOAt) (2.5 g, 2 eq.) in dry pyridine (60mL) was stirred at room temperature for 4 days. Then, the solvent was evaporated under reduced pressure and the residue was re-dissolved in ethyl acetate, washed with water. Organic layer was evaporated and the residue chromatographed on a silica gel column. Elution with dichloromethane – methanol (100:5) gave 42-O-(2-O-methyl-β-D-fructosylcarbonyl)rapamycin (4.7g,) as white solid (freeze dried from benzene). $C_{59}H_{91}NO_{20}$, M=1133.7; MS (ES+): m/z=1156.7 $(M+Na)^+$

EXAMPLE 5

Stability of Rapamycin Carbohydrate Derivatives Toward Hydrolysis in Acidic Media

[00142] The stability toward hydrolysis of various rapamycin carbohydrate derivatives in acidic media was investigated by dissolving the compounds in 70/30 methanol/0.1N HCl (pH 1.5) and then measuring by HPLC the amount of free rapamycin in the samples at 1 hour to determine the extent of hydrolysis. The results are summarized in Table 2.

TABLE 2. ACID CATALYZED HYDROLYSIS OF RAPAMYCIN CARBOHYDRATE DERIVATIVES

Compound	Extent of Hydrolysis (%)
42-O-(D-Fructosylcarbonyl)rapamycin	<1
42-O-(D-Glucosylcarbonyl)rapamycin	<1
42-O-(D-Maltulosylcarbonyl)rapamycin	<5
42-O-(L-Fucitolylcarbonyl)rapamycin	<1
42-O-(D-Lactalylcarbonyl)rapamycin	<1
31-O-(D-Fructosylcarbonyl)rapamycin	<1
42-O-(D-Allosylcarbonyl)rapamycin	<1

[00143] As can be seen in Table 2, all compounds exhibited good stability in acidic media with little or no observable hydrolysis.

EXAMPLE 6

Hydrolysis of Rapamycin Carbohydrate Derivatives in Human Whole Blood

[00144] The ability of various rapamycin carbohydrate derivatives to release free rapamycin via hydrolysis in whole blood was investigated by spiking the compounds in human whole blood and then measuring by HPLC the amount of free rapamycin in the samples at 1 hour to determine the extent of hydrolysis. The results are summarized in Table 3.

TABLE 3. HYDROLYSIS OF RAPAMYCIN CARBOHYDRATE DERIVATIVES IN WHOLE BLOOD

Compound	Extent of Hydrolysis (%)
42-O-(D-Fructosylcarbonyl)rapamycin	28±2
42-O-(D-Glucosylcarbonyl)rapamycin	5±1
42-O-(D-Maltulosylcarbonyl)rapamycin	13±4
42-O-(L-Fucitolylcarbonyl)rapamycin	31±4
42-O-(D-Lactalylcarbonyl)rapamycin	2±1
31-O-(D-Fructosylcarbonyl)rapamycin	23±0.5
42-O-(D-Allosylcarbonyl)rapamycin	39±4
31-O-(D-Allosylcarbonyl)rapamycin	34±2

[00145] As shown in Table 3, the extent of hydrolysis in human whole blood varied greatly depending on the nature of the carbohydrate moiety. The incorporation via carbonate linkages of sugars such as D-fructose, L-fucitol or D-allose generally led to a higher degree of hydrolysis than was observed when D-glucose, D-maltulose, and D-lactal were employed. The results also show that when a suitable sugar is chosen, both 31-O- and 42-O-rapamycin carbohydrate derivatives are effective at releasing free rapamycin in whole blood. Additionally, previous, similar experiments that investigated the hydrolysis in whole blood of rapamycin carbohydrate derivatives with carbamate linkages showed little or no hydrolysis in contrast to many of the compounds with carbonate linkages in Table 3.

Example 7

Comparison of the *In Vitro* Immunosuppressive Activity of Rapamycin Carbohydrate Derivatives with Carbonate and Carbamate Linkages

[00146] The immunosuppressive activity of rapamycin, 42-O-(D-Fructosylcarbonyl)rapamycin and a carbamate-linked analog of 42-O-(D-Fructosylcarbonyl)rapamycin (a non-hydrolyzable form of 42-O-(D-Fructosylcarbonyl)rapamycin) was assessed in primary blood lymphocyte cultures (PBMC) using alamar blue as detection of cell proliferation. The carbamate analog of 42-O-(D-Fructosylcarbonyl)rapamycin is formed from an amino sugar wherein the carbohydrate moiety is attached to the carbonyl linker through the amino nitrogen atom of the amino sugar, thereby forming a carbamate linkage. Figure 5 illustrates that 42-O-(D-Fructosylcarbonyl)rapamycin shows cell proliferation inhibition equivalent to rapamycin, while the non-hydrolyzable carbamate-linked analog does not possess any intrinsic immunosuppressive activity. This data indicates that the active species is rapamycin resulting from the hydrolysis of 42-O-(D-Fructosylcarbonyl)rapamycin over the course of the 3-day culturing, and not non-hydrolysed 42-O-(D-Fructosylcarbonyl)rapamycin. In other words, the prodrug does not appear to possess any intrinsic immunosuppressive activity and must be

hydrolyzed to rapamycin in order to exhibit the desired pharmacological effect. This experiment also demonstrates the importance of the selection of the linkage between the carbohydrate moiety and rapamycin as, in this example, a carbonate linkage allows for the desired hydrolysis to occur whereas the carbamate linkage remains intact and little or no rapamycin is released.

EXAMPLE 8

Comparison of Pharmacokinetic Profiles of Rapamycin and Rapamycin Carbohydrate Derivatives in Rats

[00147] The pharmacokinetic profiles in rats of selected rapamycin carbohydrate derivatives were determined to investigate the ability of the derivatives to deliver free rapamycin into the bloodstream *in vivo*. Briefly, Sprague Dawley rats were orally dosed with rapamycin and derivatives at 2.5 or 10 mg/kg. Whole blood was extracted via jugular bleed over 24 hours and frozen at -20 °C until analysis. Whole blood was analyzed by Liquid Chromatography Mass Spectrometry for the presence of rapamycin. The results are summarized in Figures 6,7 and 8.

[00148] As can be seen in Figures 6 and 7, upon oral administration of rapamycin a rapid rise in rapamycin concentration in the blood was observed with a maximum concentration reached at approximately 30 minutes. The level of rapamycin then dropped quite rapidly over the next several hours. A similar profile was observed for 42-O-(D-glucosylcarbonyl)rapamycin. Surprisingly, however, when 42-O-(D-fructosylcarbonyl)rapamycin or 42-O-(L-fucitolylcarbonyl)rapamycin were administered orally the level of rapamycin in the blood rose gradually to reach a maximum concentration at approximately 3 hours before gradually decreasing over time (Figure 6). A similar profile was observed for both 31-O-(D-fructosylcarbonyl)rapamycin and 31-O-(D-allosylcarbonyl)rapamycin (Figure 7) as well as 42-O-(D-allosylcarbonyl)rapamycin, 42-O-(D-sorbosylcarbonyl)rapamycin and 42-O-(2-O-methyl-D-fructosylcarbonyl)rapamycin (Figure 8),. The delayed kinetics observed for selected compounds may offer the advantage

of allowing for less frequent dosing than that typical for rapamycin. Additionally, the gradual rise in rapamycin concentration associated with selected rapamycin carbohydrate derivatives may ameliorate toxic effects associated with the rapid rise in drug concentration when rapamycin itself is orally administered.

[00149] A second observation from Figures 6, 7 and 8 is that the variability in the concentration of rapamycin, as demonstrated by the standard deviations shown on the graphs, was considerably lower for the rapamycin carbohydrate derivatives than for rapamycin itself. Thus, the compounds of the present invention may also have the advantage of reduced inter-individual variability that could allow for more consistent, predictable dosing.

[00150] These experiments demonstrated that careful selection of the glycosyl substituent has a profound impact on the pharmacokinetic profiles of the rapamycin carbohydrate derivatives and that the compounds of the present invention may possess considerable advantage, including pharmacokinetic advantages, over rapamycin itself.

EXAMPLE 9

Comparison of GI Tract Toxicity of Rapamycin and 42-O-(D-Fructosylcarbonyl)rapamycin in a Canine Model

[00151] Beagle dogs are considered to be a hypersensitive model of gastrointestinal tract toxicity associated with rapamycin. See, S.N. Sehgal et al., Medicinal Research Reviews 14, 1 (1994). Even short exposures of low dose rapamycin given orally to dogs is known to result in rapid weight loss due to ulceration occurring from the mouth to the colon secondary to necrotizing fibrinoid vasculitis. As summarized in Table 4, when 2 beagle dogs were given a single oral dose of rapamycin at 10 mg/kg both dogs became lethargic and lost over 30% of their body weight in less than 1 week resulting from reduced food intake. The animals did not recover. Another beagle dog given the same dose of 42-O-(D-fructosylcarbonyl)rapamycin showed no overt physical changes and maintained a normal food intake. All three dogs had similar blood levels of rapamycin as measured by LCMS. In a fourth dog a dose of 1 mg/kg 42-O-(D-fructosylcarbonyl)rapamycin resulted in slight lethargy but the dog quickly

recovered. A subsequent dose of rapamycin (1mg/kg) one week later resulted in severe lethargy and weight loss, from which the animal did not recover.

TABLE 4. GASTROINTESTINAL TOLERABILITY IN BEAGLE DOGS

Dog #	Drug Treatment	Dose (mg/kg)	Observations
1	Rapamycin	10	Lethargic 30 % weight loss No recovery
2	Rapamycin	10	Lethargic 30 % weight loss No recovery
3	42-O-(D-Fructosylcarbonyl) rapamycin	10	Normal
4	42-O-(D-Fructosylcarbonyl) rapamycin	1	Lethargic Recovered
4	Rapamycin	1	30 % weight loss No recovery

[00152] This experiment clearly demonstrates that oral administration of 42-O-(D-fructosylcarbonyl)rapamycin resulted in little or no overt sign of gastrointestinal toxicity in a hypersensitive dog model as compared to rapamycin which led to signs of severe toxicity. This result further demonstrates the potential of the rapamycin carbohydrate derivatives of the present invention to improve the pharmacodynamic profile of rapamycin.

Example 10

Comparison of Serum Cholesterol Levels in Rats Treated with Rapamycin and 42-O-(D-Fructosylcarbonyl)rapamycin

[00153] 42-O-(D-fructosylcarbonyl)rapamycin and rapamaycin were tested head-to-head in Sprague-Dawley rats for changes in cholesterol level. Rats (n = 12) were dosed daily for 12 days with equivalent doses (2.5 mg/kg/day) of either rapamycin or 42-O-(D-fructosylcarbonyl)rapamycin. Cholesterol levels were measured at the 24-hour trough level on day 11. Results (Figure 9) indicate that rats treated with rapamycin displayed significantly increased levels of cholesterol relative to a vehicle control group, while cholesterol levels in the 42-O-(D-fructosylcarbonyl)rapamycin group were significantly lower ($p < 0.01$) than in the rapamycin group and not significantly different than the vehicle. It is important to note that both compounds showed equivalent efficacy in a heterotopic rat heart transplant model at the same 2.5 mg/kg/day dose used in this study (Example 12). This experiment demonstrates the ability of the rapamycin carbohydrate derivatives to improve the side effect profile of rapamycin while maintaining efficacy.

EXAMPLE 11

Comparison of Platelet Aggregation due to Rapamycin and 42-O-(D-Fructosylcarbonyl)rapamycin in a Washed Human Platelet Aggregation Assay

[00154] Platelet aggregation is suspected to be a side effect of rapamycin and has been implicated in increasing chronic rejection and other long term side effects of the use of rapamycin in transplantation. (Ann Babinska et. al., Enhancement of Human Platelet Aggregation and Secretion Induced by Rapamycin. (1998) Nephrology Dialysis Transplantation Vol. 13 pp. 3153-3159. These experiments were conducted using fresh washed human platelets stimulated with 2 μM ADP and spiked with either Rapamycin or 42-O-(D-Fructosylcarbonyl)rapamycin at time zero. The treated platelet aggregation was continuously read over a period of 8 to 10 minutes in a ChronoLog™ Platelet Aggregometer.

[00155] Figure 10 shows a plot of percent platelet aggregation versus time for two concentrations of rapamycin, 1 μg/ml and 25 μg/ml. Figure 10 illustrates that rapamycin

induces platelet aggregation in a dose dependent manner. Rapamycin at the 1 μg/ml dose induced platelet aggregation by approximately 20% after 8 minutes. Rapamycin at the 25 μg/ml dose induced platelet aggregation by approximately 70% after 8 minutes. Figure 11 shows a plot of percent platelet aggregation versus time for washed human platelets, stimulated with 2 μM ADP dosed with 25 μg/ml rapamycin or 25 μg/ml 42-O-(D-Fructosylcarbonyl)rapamycin. Fig. 11 shows that while rapamycin induces nearly 80% platelet aggregation after 8 minutes, 42-O-(D-Fructosylcarbonyl)rapamycin does not exhibit a measurable effect on platelet aggregation in the same time.

EXAMPLE 12

Graft Survival of Heterotopic Heart Allografts in Rats Receiving Oral 42-O-(D-Fructosylcarbonyl)rapamycin (2.5 and 10 mg/kg/day), Rapamycin (2.5 mg/kg/day), or Vehicle

[00156] Heterotopic transplants were administered to the abdominal aorta and inferior vena cava of a genetically unmatched (allogenic) heart from Wistar Furth rats to Lewis rats. The controls (vehicle and Rapamycin at 2.5 mg/kg/day) or 42-O-(D-Fructosylcarbonyl)rapamycin, at 2.5 and 10 mg/kg/day were administered once daily by oral gavage to the transplant recipients (6 rats per group) starting 3 days prior to transplantation and continuing for 30 days post-transplantation. If graft dysfunction was noted during the 30-day post-transplantation period, the animal was sacrificed. If the animal survived longer than 30 days post-transplantation, the test and control articles were discontinued and the animal was allowed to continue until graft dysfunction or up to 100 days post-transplantation. The average survival rates for each group of recipient animals are summarized in Table 5 and in Figure 12. As shown in Table 5, 42-O-(D-Fructosylcarbonyl)rapamycin prolonged survival of the graft at the 2.5 and 10 mg/kg/day dose level by 214 and 341% over the vehicle control. This was similar to the prolonged survival exhibited with rapamycin at the 2.5 mg/kg/day dose. Figure 12 illustrates that 42-O-(D-Fructosylcarbonyl)rapamycin prolonged survival of the graft over the vehicle as did rapamycin at the 2.5 mg/kg/day dose level. These data

demonstrate the immunosuppressive activity of 42-O-(D-Fructosylcarbonyl)rapamycin in preventing graft rejection.

TABLE 5. AVERAGE SURVIVAL RATES AFTER HETEROTOPIC RAT HEART TRANSPLANTS (N=6)

Dose (mg/kg/day)	Average Survival Time (days post-transplant) Mean ± SEM		
	Vehicle Control	Rapamycin	42-O-(D-Fructosylcarbonyl)rapamycin
0	13 ± 2		
2.5		39 ± 4	41 ± 4*
10			57 ± 4

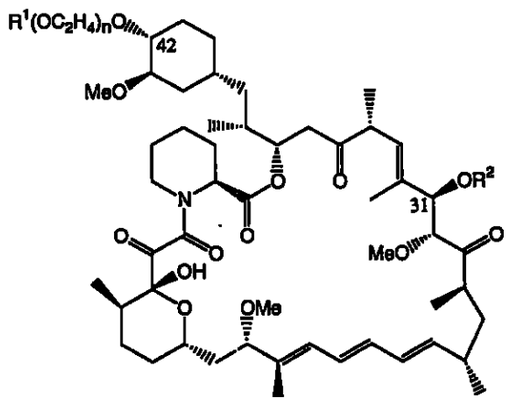
* n=5

[00157] Although only some embodiments of the invention are specifically disclosed and described above, it will be appreciated that many modifications and variations of the present invention are possible in light of the above teachings and within the purview of the appended claims without departing from the spirit and intended scope of the invention.

[00158] All of the above publications as well as any other references mentioned hereafter are herein incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated as incorporated herein by reference in its entirety.

We Claim:

1. A rapamycin carbohydrate derivative having the structure of formula (I):



(I)

wherein

R^1 and R^2 are independently hydrogen or-X-Z,

wherein n= 0 or 1; and

wherein each X is a linker, and each Z is a carbohydrate moiety independently selected from the group consisting of a monosaccharide, oligosaccharide and pseudosugar, wherein Z is attached to X through a hydroxyl oxygen atom of Z with the proviso that R^1 and R^2 are not both hydrogen.

2. The rapamycin carbohydrate derivative of claim 1, wherein R^1 is hydrogen and R^2 is –X-Z.

3. The rapamycin carbohydrate derivative of claim 2, wherein X is selected from the group consisting of:

(i) $-R^3C(O)-$;

(ii) $-C(O)R^3-$;

(iii) $-R^3S(O)_2-$; and

(iv) $-S(O)_2R^3-$;

wherein R^3 is selected from the group consisting of:

(a) $-(CH_2)_p-$ where p is an integer from 1 to 18;

(b) $-(CH_2)_n-O-(CH_2)_m-$ where n and m are each independently an integer from 2 to 6; and

(c) a bond.

4. The rapamycin carbohydrate derivative of claim 3, wherein X is selected from the group consisting of -C(O)- and $-SO_2-$.

5. The rapamycin carbohydrate derivative of claim 2, wherein X is a single functional group.

6. The rapamycin carbohydrate derivative of claim 2, wherein Z is selected from the group consisting of fructose, fucitol and allose.

7. The rapamycin carbohydrate derivative of claim 6, wherein Z is D-fructose.

8. The rapamycin carbohydrate derivative of claim 3 wherein Z is a monosaccharide derivative wherein at least one of the hydroxyl groups of the monosaccharide is replaced with a hydrogen, an alkoxy, an alkanoate or a halogen group.

9. The rapamycin carbohydrate derivative of claim 1, wherein R^1 is –X-Z and R^2 is hydrogen.

10. The rapamycin carbohydrate derivative of claim 9, wherein X is selected from the group consisting of:

(i) $-R^3C(O)-$;

(ii) $-C(O)R^3-$;

(iii) $-R^3S(O)_2-$; and

(iv) $-S(O)_2R^3-$;

wherein R^3 is selected from the group consisting of:

(a) $-(CH_2)_p-$ where p is an integer from 1 to 18;

(b) $-(CH_2)_n-O-(CH_2)_m-$ where n and m are each independently an integer from 2 to 6; and

(c) a bond.

11. The rapamycin carbohydrate derivative of claim 10, wherein X is selected from the group consisting of -C(O)- and $-SO_2-$.

12. The rapamycin carbohydrate derivative of claim 9, wherein X is a single functional group.

13. The rapamycin carbohydrate derivative of claim 9, wherein Z is selected from the group consisting of fructose, fucitol and allose.

14. The rapamycin carbohydrate derivative of claim 13, wherein Z is D-fructose.

15. The rapamycin carbohydrate derivative of claim 9 wherein Z is a monosaccharide derivative wherein at least one of the hydroxyl groups of the monosaccharide is replaced with a hydrogen, an alkoxy, an alkanoate or a halogen group.

16. A rapamycin carbohydrate derivative selected from the group consisting of:

42-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-[2-(Methyl-D-glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-glucosylcarbonyl)rapamycin;

42-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-D-fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-[2-(2-O-Methyl-L-fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(2-O-Methyl-D-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-D-fructosylcarbonyl)rapamycin;

31-O-(2-O-Methyl-L-fructosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(2-O-methyl-L-fructosylcarbonyl)rapamycin;

42-O-(D-Allosylcarbonyl)rapamycin;

42-O-[2-(D-Allosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Allosylcarbonyl)rapamycin;

42-O-[2-(L-Allosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-allosylcarbonyl)rapamycin;

31-O-(L-Allosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-allosylcarbonyl)rapamycin;

42-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-[2-(D-Fructosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-[2-(L-Fructosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fructoslylcarbonyl)rapamycin;

31-O-(L-Fructoslylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fructoslylcarbonyl)rapamycin;

42-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-[2-(D-Fucitolylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-[2-(L-Fucitolylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-fucitolylcarbonyl)rapamycin;

31-O-(L-Fucitolylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-fucitolylcarbonyl)rapamycin;

42-O-(D-Glucalylcarbonyl)rapamycin;

42-O-[2-(D-Glucalylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Glucosylcarbonyl)rapamycin;

42-O-[2-(D-Glucosylcarbonyloxy)ethyl]rapamycin;

42-O-(L-Glucosylcarbonyl)rapamycin;

42-O-[2-(L-Glucosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Glucalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucalylcarbonyl)rapamycin;

31-O-(D-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-glucosylcarbonyl)rapamycin;

31-O-(L-Glucosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-glucosylcarbonyl)rapamycin;

42-O-(L-Sorbosylcarbonyl)rapamycin;

42-O-(D-Sorbosylcarbonyl)rapamycin;

31-O-(L-Sorbosylcarbonyl)rapamycin;

31-O-(D-Sorbosylcarbonyl)rapamycin;

42-O-[2-(L-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-[2-(D-Sorbosylcarbonyloxy)ethyl]rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sorbosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(L-sorbosylcarbonyl)rapamycin;

42-O-(D-Lactalylcarbonyl)rapamycin;

42-O-[2-(D-Lactalylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Lactalylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-lactalylcarbonyl)rapamycin;

42-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-[2-(D-Sucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Sucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-sucrosylcarbonyl)rapamycin;

42-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-[2-(D-Gentobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Gentobiosylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(D-gentobiosylcarbonyl)rapamycin

42-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-[2-(D-Cellobiosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellobiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellobiosylcarbonyl)rapamycin;

42-O-(D-Turanosylcarbonyl)rapamycin;

42-O-[2-(D-Turanosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Turanosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-turanosylcarbonyl)rapamycin;

42-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-[2-(D-Palatinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Palatinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-palatinosylcarbonyl)rapamycin;

42-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltosylcarbonyl)rapamycin;

42-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-[2-(D-Maltulosylcarbonyloxy)ethyl]rapamycin;

42-O-(D-Maltosylcarbonyl)rapamycin;

42-O-[2-(D-Maltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Maltulosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltulosylcarbonyl)rapamycin;

31-O-(D-Maltosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-maltosylcarbonyl)rapamycin;

42-O-(D-Lactosylcarbonyl)rapamycin;

42-O-[2-(D-Lactosylcarbonyloxy)ethyl]rapamycin;

31-O-(Methyl-D-lactosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(methyl-D-lactosylcarbonyl)rapamycin;

42-O-(D-Melibiosylcarbonyl)rapamycin;

31-O-(D-Melibiosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-melibiosylcarbonyl)rapamycin;

42-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-[2-(D-Leucrosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Leucrosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-leucrosylcarbonyl)rapamycin;

42-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-[2-(D-Rafinosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Rafinosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-rafinosylcarbonyl)rapamycin;

42-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-[2-(D-Isomaltosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Isomaltotriosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-isomaltotriosylcarbonyl)rapamycin;

42-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-[2-(D-Cellotetraosylcarbonyloxy)ethyl]rapamycin;

31-O-(D-Cellotetraosylcarbonyl)rapamycin;

42-O-(2-Hydroxyethyl)-31-O-(D-cellotetraosylcarbonyl)rapamycin;

42-O-(Valiolylcarbonyl)rapamycin

42-O-[2-(D-Valiolylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolylcarbonyl)rapamycin

42-O-(Valiolonylcarbonyl)rapamycin

42-O-[2-(D-Valiolonylcarbonyloxy)ethyl]rapamycin;

31-O-(Valiolonylcarbonyl)rapamycin

42-O-(2-Hydroxyethyl)-31-O-(valiolonylcarbonyl)rapamycin

42-O-(Valienolylcarbonyl)rapamycin
42-O-[2-(D-Valienolylcarbonyloxy)ethyl]rapamycin;
31-O-(Valienolylcarbonyl)rapamycin
42-O-(2-Hydroxyethyl)-31-O-(valienolylcarbonyl)rapamycin
42-O-(Valienoneylcarbonyl)rapamycin
42-O-[2-(D-Valienoneylcarbonyloxy)ethyl]rapamycin;
31-O-(Valienoneylcarbonyl)rapamycin
42-O-(2-Hydroxyethyl)- 31-O-(valienoneylcarbonyl)rapamycin

17. A pharmaceutical composition comprising the rapamycin carbohydrate derivative of claim 1 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

18. A method for treating a disease treatable by rapamycin, comprising administering a therapeutically effective amount of a pharmaceutical composition of claim 17 to a subject in need thereof.

19. The method of claim 18, wherein the disease is selected from the group consisting of transplantation rejection, host vs. graft disease, graft vs. host disease, leukemia, lymphoma, hyperproliferative vascular disorders, autoimmune disease, diseases of inflammation, solid tumors, and fungal infections.

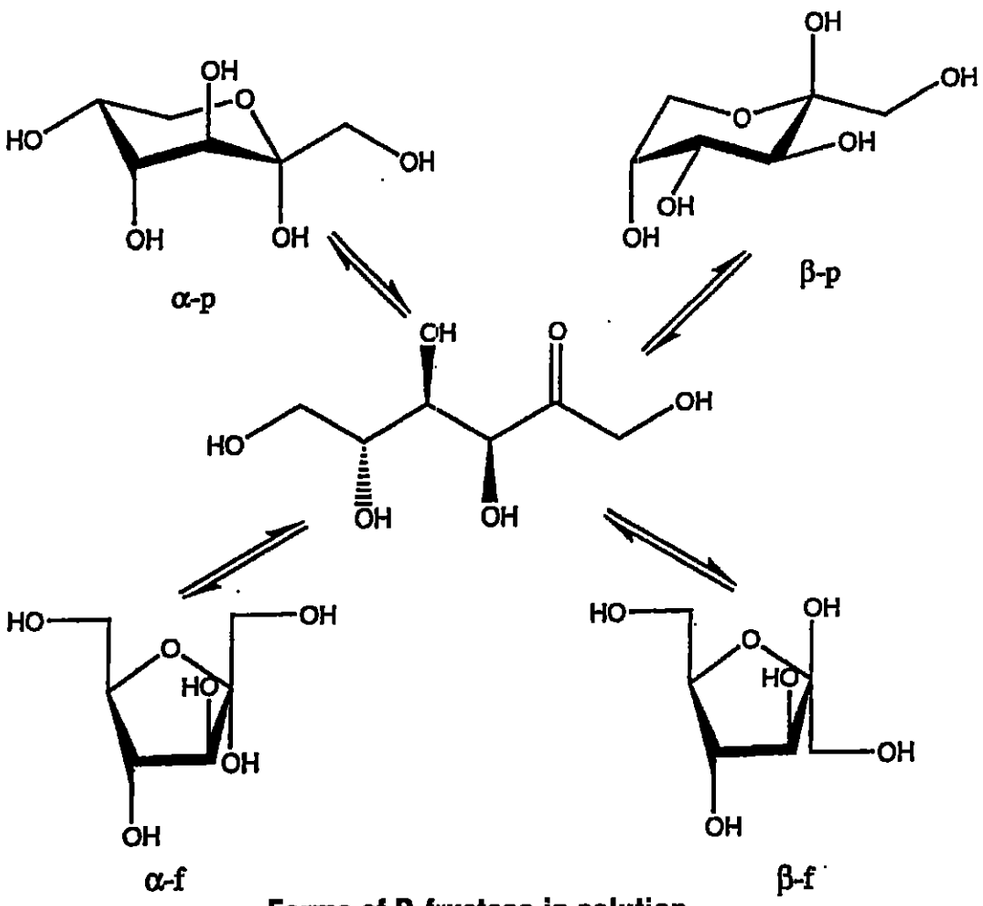
20. A medical device comprising a rapamycin carbohydrate derivative of claim 1 or a pharmaceutically acceptable salt thereof.

21. A medical device wherein said medical device is coated with a rapamycin carbohydrate derivative of claim 1.

22. A medical device of claim 21, wherein the medical device is selected from the group consisting of stents, grafts, and implants

23. The medical device of claim 20, wherein the medical device is selected from the group consisting of stents, grafts, and implants.

24. A method for treating a disease treatable by rapamycin, comprising coadministering a therapeutically effective amount of a pharmaceutical composition of claim 17 to a subject in need thereof with a pharmaceutical composition comprising a compound selected from the group consisting of a cyclosporine or cyclosporine derivative, a steroid, or an immunomodulatory compound.



Forms of D-fructose in solution

FIG. 1

General Route of Synthesis for Rapamycin Carbohydrate Derivatives

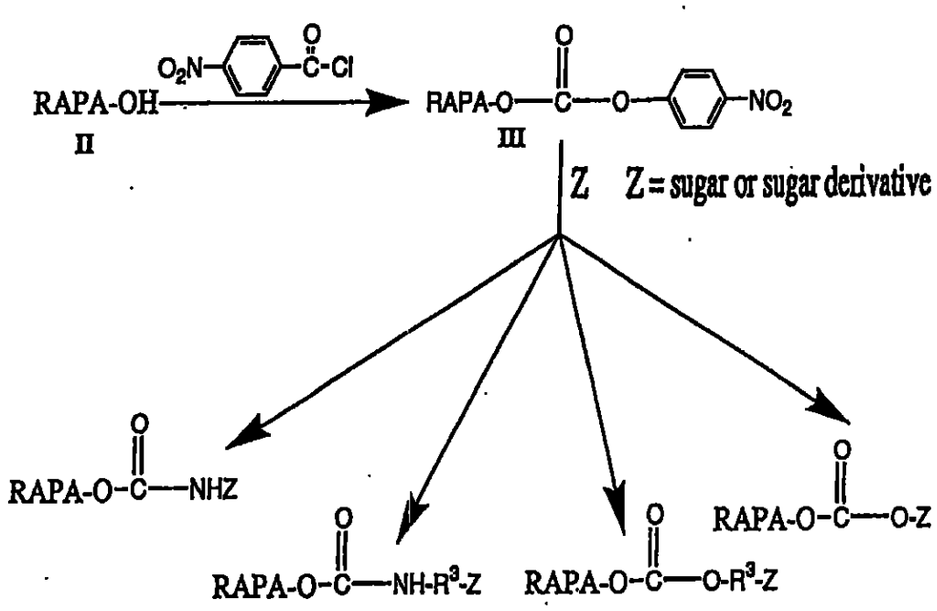
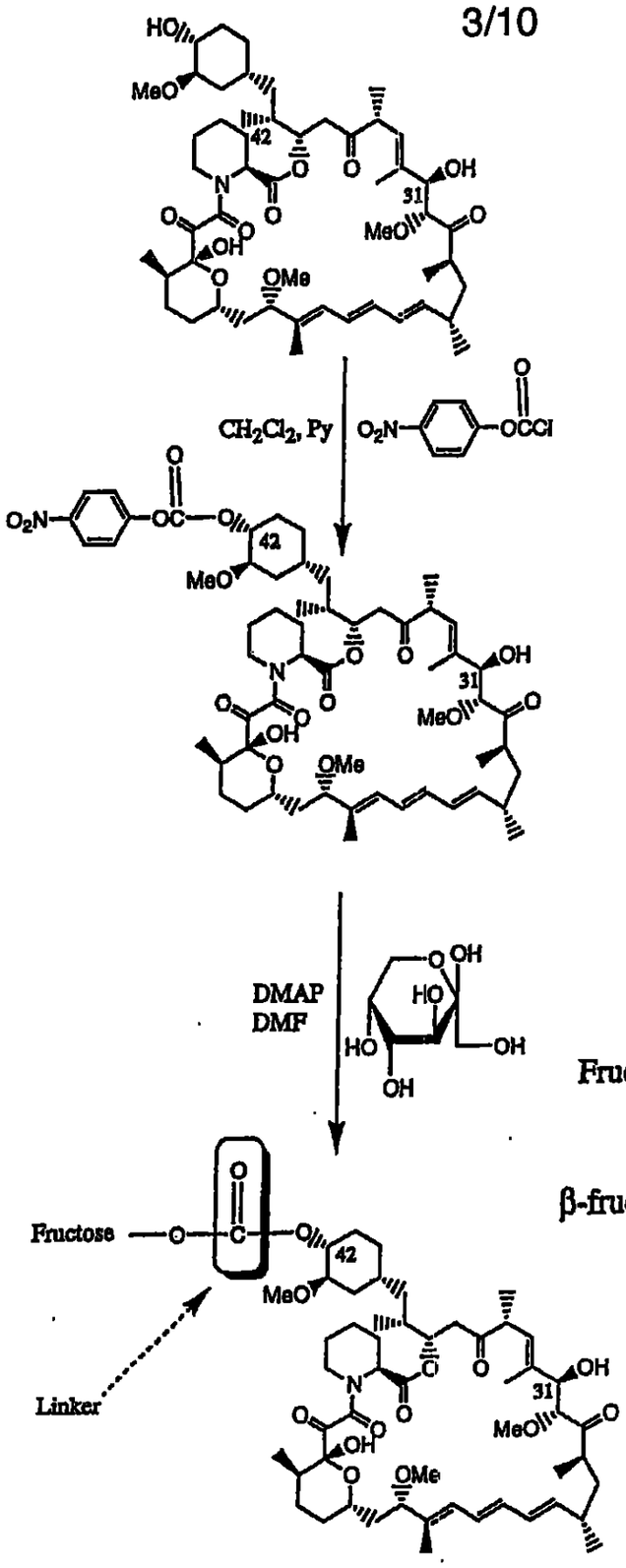


FIG. 2



Fructose = α-fructopyranose or β-fructopyranose or α-fructofuranose or β-fructofuranose attached through the 1 or 6 position.

FIG. 3

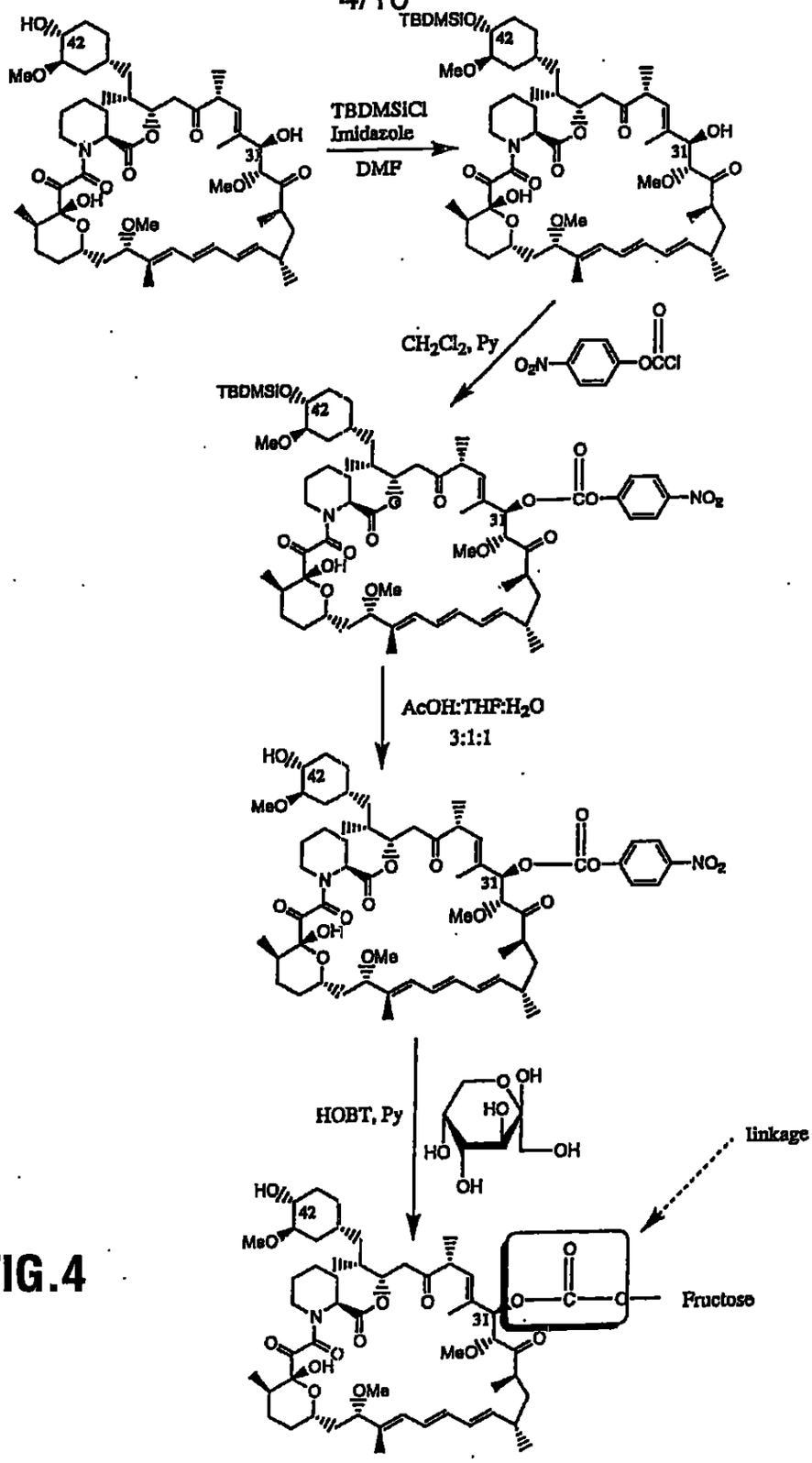

TBDMSiCl
Imidazole
DMF
CH2Cl2, Py
AcOH:THF:H2O
3:1:1
HOBT, Py
linkage
Fructose

FIG.4

***In vitro* Immunosuppressive Activity of Rapamycin 42-O-(D-Fructosylcarbonyl)rapamycin, and a Carbamate-linked Analog in a Cell Proliferation assay.**

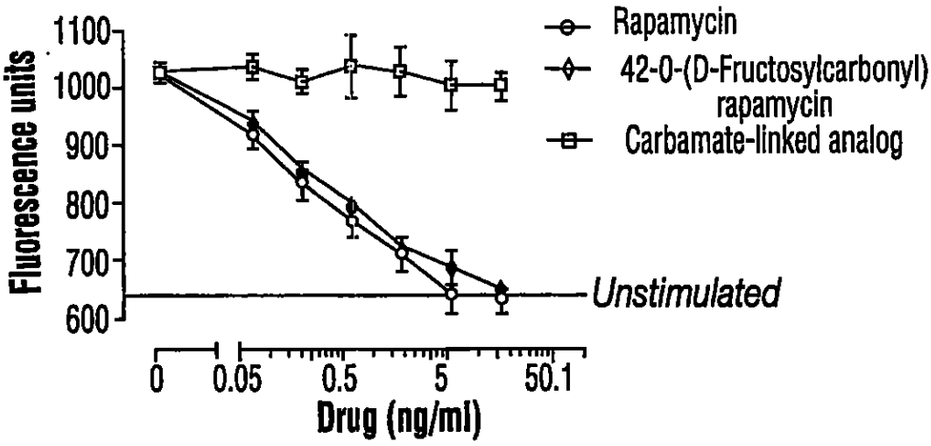


FIG. 5

Pharmacokinetic Profiles in the Rat of Rapamycin-42-O-Carbohydrate Derivatives

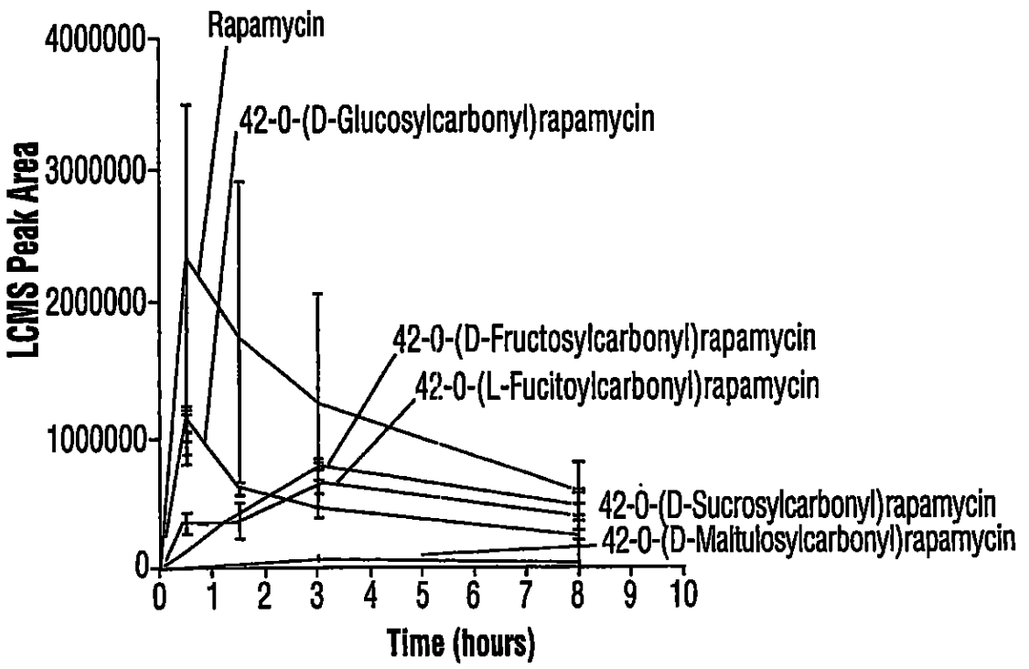


FIG. 6

Pharmacokinetic Profiles in the Rat of Rapamycin-31-O-Carbohydrate Derivitives

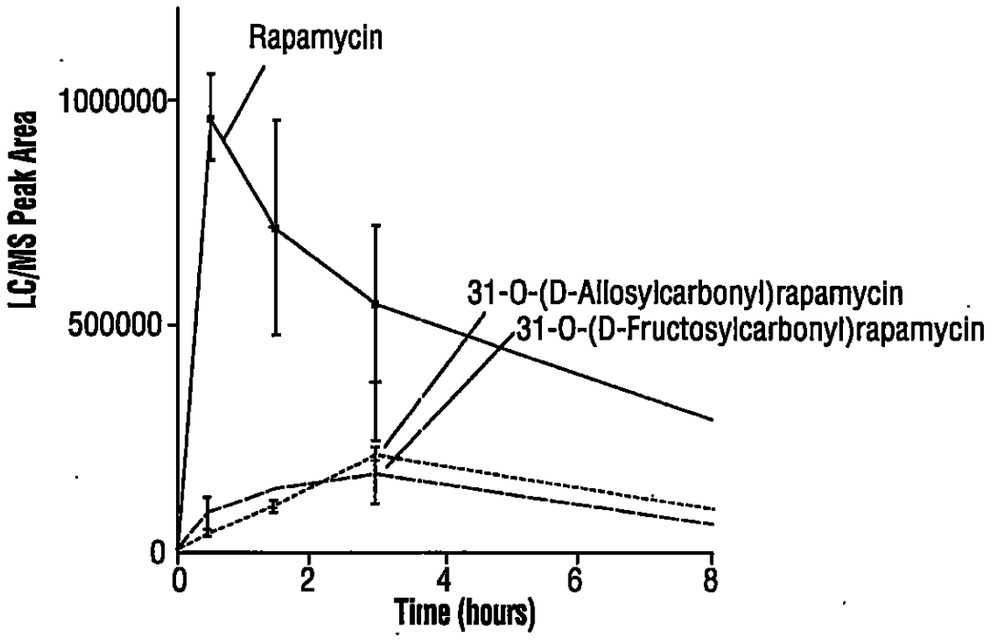


FIG. 7

Pharmacokinetic Profiles in the Rat of Rapamycin-42-O-Carbohydrate Derivitives

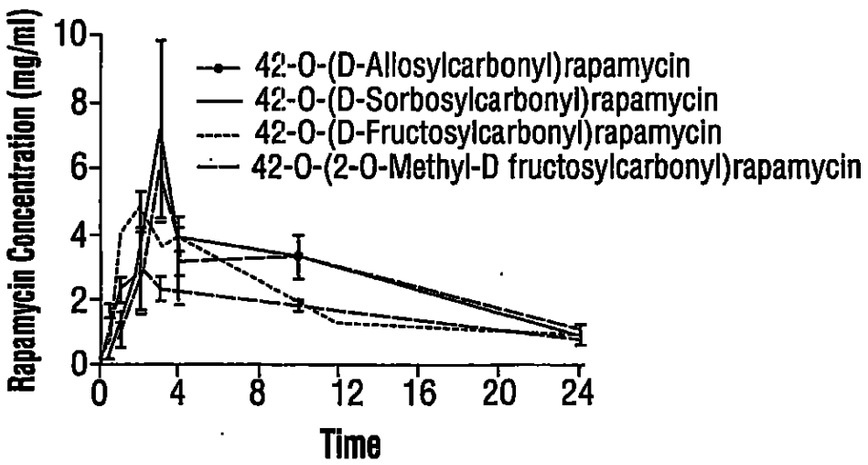
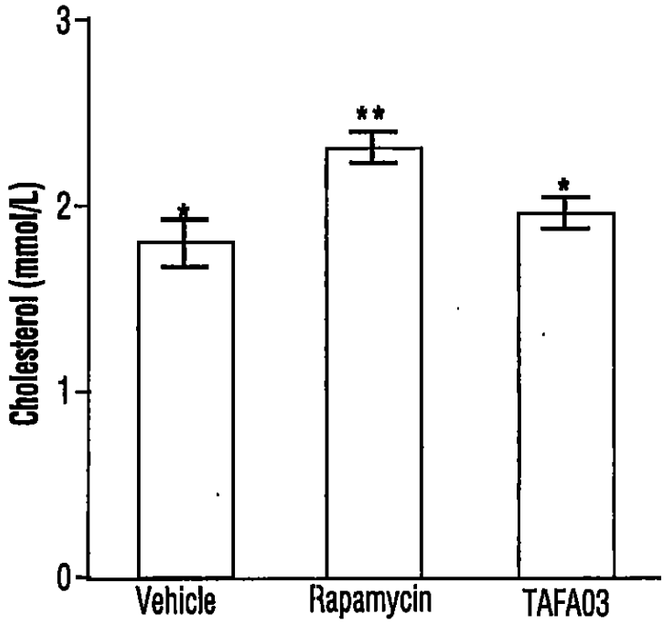


FIG. 8

Serum Cholesterol Levels Following 12-Day Dosing of 42-O-(D-Fructosylcarbonyl)rapamycin and Rapamycin in Sprague-Dawley Rats



*** Significantly Lower than Rapamycin p < 0.01**

****Significantly higher than Vehicle, p < 0.01**

ANOVA, Dunnell's Post Hoc Test

FIG. 9

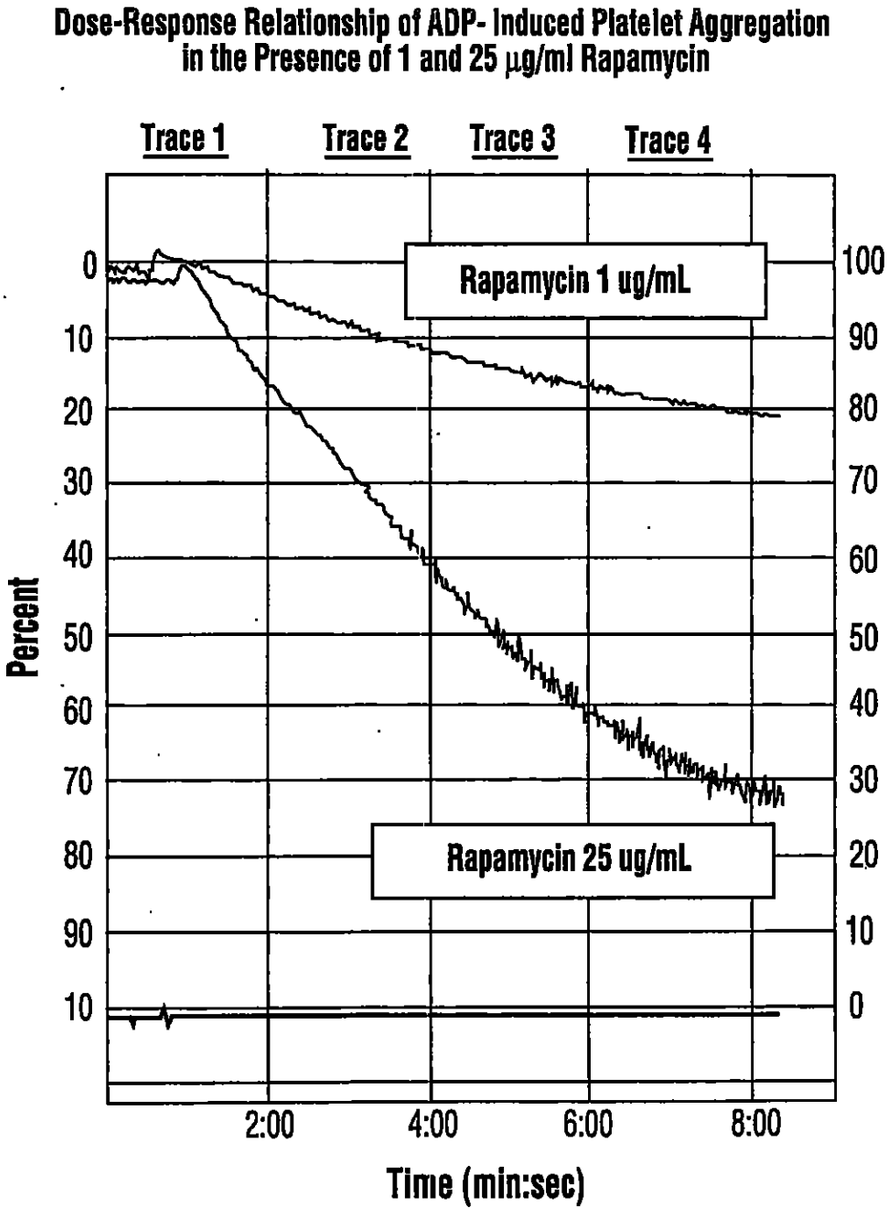

Dose-Response Relationship of ADP- Induced Platelet Aggregation in the Presence of 1 and 25 µg/ml Rapamycin
Trace 1
Trace 2
Trace 3
Trace 4
Rapamycin 1 ug/mL
Rapamycin 25 ug/mL
Percent
0
10
20
30
40
50
60
70
80
90
10
100
90
80
70
60
50
40
30
20
10
0
2:00
4:00
6:00
8:00
Time (min:sec)

FIG. 10

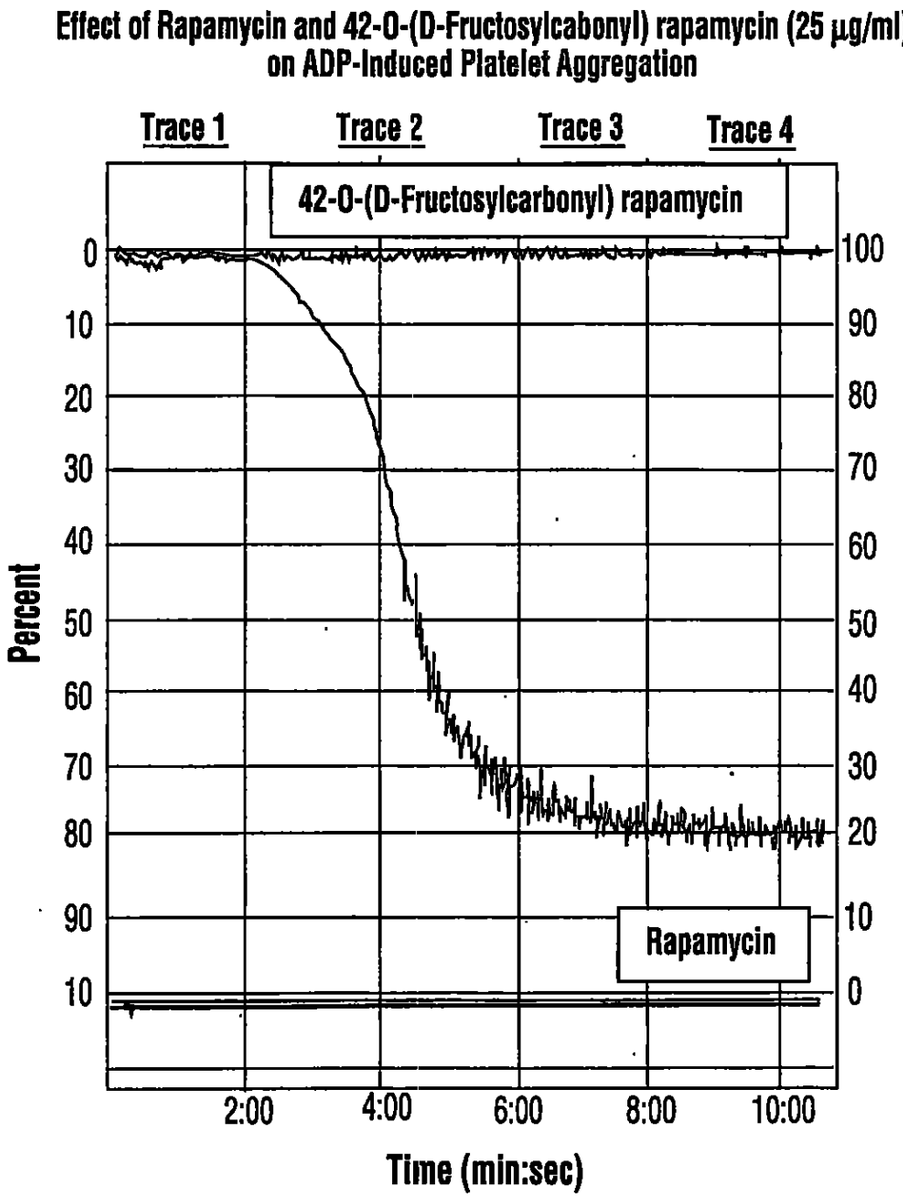
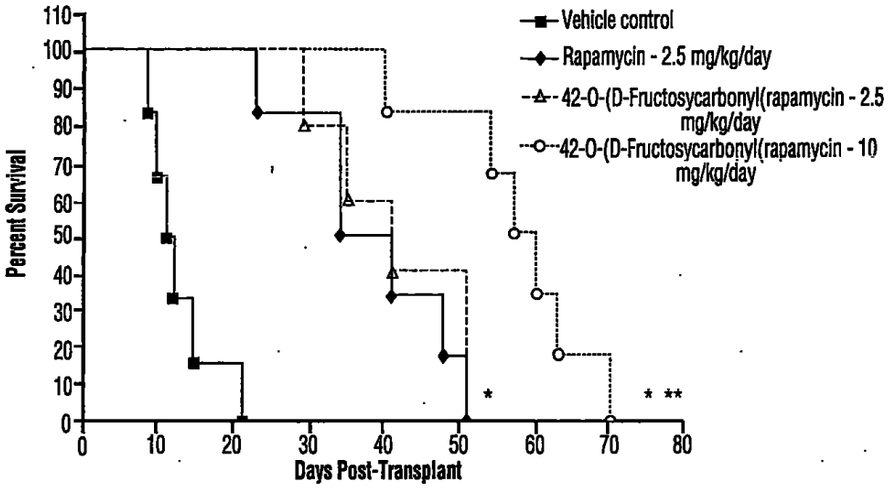

Effect of Rapamycin and 42-O-(D-Fructosylcabonyl) rapamycin (25 µg/ml) on ADP-Induced Platelet Aggregation
Trace 1
Trace 2
Trace 3
Trace 4
42-O-(D-Fructosylcarbonyl) rapamycin
Rapamycin
Percent
0
10
20
30
40
50
60
70
80
90
10
100
90
80
70
60
50
40
30
20
10
0
2:00
4:00
6:00
8:00
10:00
Time (min:sec)

FIG. 11

Graft Survival Curves of Heterotopic Heart Allografts in Rats Receiving Oral 42-O-(D-Fructosylcarbonyl)rapamycin (2.5 and 10 mg/kg/day), Rapamycin (2.5 mg/kg/day),or Vehicle



* Different from Vehicle, $p < 0.005$
** Different from Rapamycin, $p < 0.05$
Wilcoxon Rank Sum

FIG. 12

INTERNATIONAL SEARCH REPORT

[redacted]ational Application No
PCT/CA2004/000724

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07H15/04 C07D498/18 A61F2/02 A61K31/436

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 C07H C07D A61F A61K

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, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6 342 507 B1 (FOSTER ROBERT T ET AL) 29 January 2002 (2002-01-29) figure 5	1-24
A	WO 92/05179 A (AMERICAN HOME PROD) 2 April 1992 (1992-04-02) cited in the application	

☐ Further documents are listed in the continuation of box C.

☒ 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
11 August 2004	23/08/2004

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	Klein, D

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA2004/000724

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6342507 B1	29-01-2002	US 2003139440 A1	24-07-2003
		US 2003130206 A1	10-07-2003
		US 2002028827 A1	07-03-2002
WO 9205179 A	02-04-1992	AU 653175 B2	22-09-1994
		AU 8659991 A	15-04-1992
		CA 2051781 A1	20-03-1992
		EP 0549727 A1	07-07-1993
		FI 931203 A	18-03-1993
		HU 65763 A2	28-07-1994
		IE 913302 A1	25-02-1992
		JP 6501012 T	27-01-1994
		MX 9101139 A1	04-05-1992
		NZ 239852 A	26-03-1993
		PT 98990 A	31-08-1992
		US 5358944 A	25-10-1994
		US 5378696 A	03-01-1995
		WO 9205179 A1	02-04-1992
		US 5221670 A	22-06-1993
		CA 2051782 A1	29-03-1992
		US 5130307 A	14-07-1992

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this communication is being transmitted, via facsimile 011-41-22-733-5428, to the World Intellectual Property Organization, 34, chemin des Colombettes, P.O. Box 18, CH-1211 Geneva 20, SWITZERLAND, on this 31st day of October, 2005.

Pamela A. Matheis

Assignment

Pursuant to the Purchase Agreement dated September 13, 2005,

ISOTECHNIKA INTERNATIONAL INC., a corporation duly organized and existing pursuant to the laws of BARBADOS, having its principal place of business at Suite No. 1, Evergreen House, 3rd Avenue, Belleville, St. Michael, BARBADOS (hereinafter referred to as the "Assignor");

hereby sells, assigns and transfers to:

ISOTECHNIKA INC., a corporation duly organized under and existing pursuant to the laws of CANADA, having its principal place of business at 2100 College Plaza, 8215-112th Street, Edmonton, Alberta T6G 2C8, CANADA (hereinafter referred to as the "Assignee");

Assignor's entire right, title and interest in and to the invention(s) described in U.S. Patent Application No. 10/845,747, filed on May 13, 2004, entitled "RAPAMYCIN CARBOHYDRATE DERIVATIVES". This Assignment including said applications, provisional applications, any and all United States and international patents, utility models and design registrations, including any divisionals, continuations, continued prosecution applications and continuations-in-part thereof, confirmations, and reexamination certificates, renewals, reissue patents, patent extensions, SPC's and patent term restorations throughout the world, the right to file applications on said inventions, and Assignor's entire right, title and interest in and to any applications for Letters Patent of the United States or other countries claiming priority to said applications, the same to be held and enjoyed by the Assignee, for its own use and behalf and the use and behalf of its successors, legal representatives and assigns, to the full end of the term or terms for which Letters Patent or Patents may be granted as fully and entirely as the same would have been held and enjoyed by the Assignor had this sale and assignment not been made;

AND for the same consideration, Assignor hereby covenants and agrees to and with the Assignee, its successors, legal representatives, and assigns that the Assignor and its employees will, whenever counsel for, or an authorized representative of the Assignee, or the counsel or representative of its successors, legal representatives, and assigns, shall advise that any proceeding in connection with said inventions or said applications for Letters Patent or Patents, or any proceeding in connection with Letters Patent or Patents for said inventions in any country, including Interference proceedings, is lawful and desirable, or that any application claiming priority to said application, division, continuation, or continuation-in-part of any applications for Letters Patent or Patents, or any reissue or extension of any Letters Patent or Patents to be obtained thereon, is lawful and desirable, sign all papers and documents, take all lawful oaths, and do all acts necessary or required to be done for the procurement, maintenance, enforcement,

and defense of Letters Patent or Patents for said inventions, without charge to the Assignee, its successors, legal representatives and assigns, but at the cost and expense of the Assignee, its successors, legal representatives and assigns;

AND the Assignor hereby requests the Commissioner of Patents to issue any and all said Letters Patent or Patents to the Assignee as the Assignee of said inventions, the Letters Patent to be issued for the sole user and on behalf of the Assignee, its successors, legal representatives, and assigns.

ISOTECHNIKA INTERNATIONAL INC.

Date: October 30, 2005

By: _____
Name: Joseph Koziak
Title: Executive Vice President

STATE OF ARIZONA)
):SS
COUNTY OF MARICOPA)

Personally appeared before me this day, the above-named Joseph Koziak, to me personally known, who acknowledged that he signed the foregoing instrument for the uses and purposes therein mentioned, and that the same is his free act and deed.

In testimony whereof, I have hereunto set my hand and affixed my seal, this 30th day of October, 2005.

Notary Public State of Arizona
Maricopa County
Pamela A Mathis
Expires October 31 2005

_____
Notary Public

(Notarial Seal)

THE WORLD INTELLECTUAL PROPERTY ORGANIZATION

APPLICANT	:	ISOTECHNIKA INTERNATIONAL INC.
FOR	:	RAPAMYCIN CARBOHYDRATE DERIVATIVES
INTERNATIONAL APPLICATION NO.:		PCT/CA2004/000724
INTERNATIONAL FILING DATE	:	14 MAY 2004 (14.05.2004)
INTERNATIONAL PRIORITY DATE	:	16 MAY2003 (16.05.2003)
ATTORNEY DOCKET NO.	:	550.PCT

CHANGE IN THE PERSON OF THE APPLICANT UNDER 92bis

World Intellectual Property Organization
34, chemin des Colombettes
P.O. Box 18
CH-1211 Geneva 20
SWITZERLAND

Sir:

Pursuant to an executed Assignment dated 31 OCT 2005 (31.10.2005) between Applicant ISOTECHNIKA INTERNATIONAL INC., and ISOTECHNIKA INC., the person of the Applicant has been changed for the above-referenced International application. A copy of the executed Assignment is attached herewith.

In accordance with Rule 92bis Section 422, the International Bureau is respectfully requested to record a change in the person of the Applicant to ISOTECHNIKA INC., as follows:

NAME:	ISOTECHNIKA INC.
ADDRESS:	2100 College Plaza 8215 112th Street Edmonton, Alberta T6G 2C8
COUNTRY:	CANADA
COUNTRY OF NATIONALITY:	CANADA
TELEPHONE NO.:	(780)-487-1600
FACSIMILE NO.:	(780)-484-4105

Respectfully submitted,

October 31, 2005
Date

Susan Sutterfield Wilks, Reg. No. 48,192
Associate General Counsel
Isotechnika Inc.
17301 North Perimeter Drive, Suite 100
Scottsdale, AZ 85255
Telephone: (480)-505-0540 (ext. 3213)
Fax: (480)-505-0545

Estado de publicación
Folio 288

Fols 289 y 290 tarjeta.
Folio 291 hoja
en blanco

Documentos anexos a la solicitud ***PCT/CA2004/000724***

1. *Solicitud* ***PCT/CA2004/00724*** *tal como fue presentada el 14 de mayo de 2004 con descripción, dibujos, reivindicaciones y resumen. (64 folios) con notificación del recibo de la solicitud (1 folio)*

2. *Traducción de la solicitud* ***PCT/CA2004/000724*** *descripción, figuras (10 folios), reivindicaciones y resumen. (84 folios)*

3. *Formulario PCT/RO/106 Invitación a corregir defectos en la solicitud internacional del 4 de junio de 2004 (3 folios)*

4. *Formulario PCT/RO/132 Comunicación para casos en los cuales ningún otro formulario es aplicable del 4 de junio de 2004. (1folio)*

5. *Formulario PCT/ISA/210, notificación del Reporte de la Búsqueda Internacional del 16 de mayo de 2004, (3 folios) junto con documentos del arte anterior:*

    a) *Patente US No.6.342.507 del 29 de enero de 2002 (7 folios).*

6. *Formulario PCT/IB/301, Notificación de recibo de la copia de la solicitud en la oficina del PCT para los países designados, del 29 de junio de 2004. (3 folios)*

7. *Formulario PCT/ISA/202 Notificación de recibo de la copia de la búsqueda del 1 de julio de 2004 (1 folio)*

8. *Formulario PCT/ISA/224 Comunicación para casos en los cuales ningún otro formulario es aplicable del 1 de julio de 2004. (1folio)*
9. *Formulario PCT/ISA/237 Opinión escrita de la Autoridad Internacional de búsqueda (5 folios)*

10. *Respuesta a la carta de invitación para corregir defectos acompañada de tres certificados de documentos de prioridad del 21 de julio de 2004 (4 folios)*

11. *Formulario PCT/RO/113, solicitud de registro de un cambio de apoderado del 13 de agosto de 2004. (1 folio).*

12. *Carta a la Oficina Receptora acompañada de poderes y dibujos formales del 3 de agosto de 2004 (14 folios)*

13. *Formulario PCT/RO/135, notificación de la fecha de recibo del documento de prioridad o del número de solicitud prioritaria del 13 de agosto de 2004 (1 folio)*

14. *Formulario PCT/RO/123, notificación ccerniente a la representación del 13 de agosto de 2004 (1 folio)*

15. *Formulario PCT/ISA/220, notificación de la transmisión de la búsqueda internacional y opinión escrita del 23 de agosto de 2004 (3 folios)*

16. *Copia de la Publicación Internacional en la Gaceta Oficial del PCT No. WO 2004/101583 A1 del 25 de noviembre de 2004. (70 folios).*

17. *Copia del Cambio en la persona del solicitante del 31 de octubre de 2005 (4 folios)*

18. *Extracto para la publicación con Arte Final en 12 cm x 12 cm.*
19. *Tarjeta Archivo Temático y Tarjeta Propietarios.*

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REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL TRAMITE

	USUARIO	RADICADOR
PATENTE DE INVENCIÓN ✓ MODELO DE UTILIDAD	( )	(✓)
- Indicación de que se solicita la concesión de una patente.	( )	(✓)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.	( )	(✓)
- Descripción de la invención.	( )	( )
- Dibujos de ser estos pertinentes.	( )	(✓)
- Comprobante de Pago de las tasas establecidas.		
COMPLETA (✓) 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 o 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 solicita el registro de un Esquema de trazado.	( )	( )
- Datos de Identificación del solcitante o de la persona que presenta La solicitud.	( )	( )
Representación gráfica del esquema de trazado	( )	( )
Comprobante de pago de las tasas establecidas.	( )	( )
COMPLETA ( ) INCOMPLETA	( )	( )

Firma Responsable:

Fecha:

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REPUBLICA DE COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
DELGADO VILLEGAS JAIME EDUARDO

REFERENCIA	Radicación No.	5 116201
	Solicitud internacional	CA2004/000724
	Fecha inicio fase nacional	16/12/2005
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	3137

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.

3. Debe aclarar el nombre del solicitante, por cuanto la publicación internacional difiere del petitorio presentado en fase nacional.

4. Debe aclarar la fecha de la prioridad 60/471.367, puesto que difieren la citada en la publicación internacional y en el memorial petitorio .

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.

Continua en la página siguiente...

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REPUBLICA DE COLOMBIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCI

Radicación No.:5 116201

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

El anterior requerimiento fue notificado por fijación en lista de fecha 16 MAR. 2006
adpal