



No. 11-067785- -00006-0000

Fecha: 2013-10-22 16:52:29 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 523 RESPUESTA Folios: 25

Señor Director
DIRECCIÓN DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite : 011
Evento : 379
Actuación : 523

Referencia : Expediente No. **11.067.785**
Asunto : Solicitud de patente para: **COMPUESTOS**
 CICLOUNDECADEPSIPÉPTIDOS
Solicitante : **DEBIO RECHERCHE PHARMACEUTIQUE S.A.**
Fecha de solicitud : 01 junio de 2011

1. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, me refiero al oficio No. **13476** notificado por fijación en lista el 30 de julio de 2013.

2. Mediante oficio No. **13476** se puso en conocimiento del solicitante el informe que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente. Las observaciones realizadas en el oficio se refieren a que la reivindicación 7 se refiere a un uso, a la claridad del título, a la claridad del pliego reivindicatorio y al nivel inventivo de las reivindicaciones 1-6 y 8-9 en vista de los documentos D1: WO2002092033 y D2: WO2006071619.

3. **Respuesta a las objeciones**

De este modo y con el objeto de atender las objeciones arriba mencionadas, se desea presentar las siguientes modificaciones, aclaraciones y argumentos:

"EXCELENCIA LEGAL EN UN MUNDO SIN FRONTERAS"

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3.1 Modificación de una solicitud en trámite

De acuerdo con el artículo 34 de la Decisión 486 de la Comisión de la Comunidad Andina, donde se establece que la modificación de una solicitud es posible en cualquier momento del trámite mientras no implique una ampliación de la solicitud inicial, me permito presentar un nuevo pliego reivindicatorio conformado por 3 reivindicaciones.

4.1.1 El título se ha modificado de la siguiente manera:

COMPUESTOS CICLOUNDECADEPSIPÉPTIDOS

4.1.2 Se han introducido las siguientes modificaciones al capítulo reivindicatorio:

- Se modificó la reivindicación 1 para incluir las limitaciones de las reivindicaciones 2 y 3.
- Se eliminaron las reivindicaciones 6, 7, 8 y 9.
- Se reenumeraron las reivindicaciones.

La reivindicación 1 se unió con las reivindicaciones 2 y 3 de manera tal que quedará una sola reivindicación independiente y más limitada de acuerdo con las sustituciones de las reivindicaciones 2 y 3. Se modificó la reivindicación 4 de acuerdo con las sugerencias del Examinador para restringir la invención a los tipos de compuestos que están debidamente sustentados de la descripción. La reivindicación original 5 se modificó de manera tal que se eliminó la referencia al método de tratamiento. Finalmente se modificó el título de la solicitud para eliminar la referencia y la expresión “y el uso” y se eliminaron las reivindicaciones 7, 8 y 9 que también hacían referencia a los usos de la invención.

De esta manera, con los cambios realizados al capítulo reivindicatorio las objeciones formuladas por el Examinador en cuanto a la claridad y concisión del pliego reivindicatorio se ven superadas. En consecuencia, se solicita atentamente tener en cuenta este nuevo pliego reivindicatorio para el nuevo análisis de patentabilidad.

4.2 Argumentos a favor del nivel inventivo de la presente solicitud

El artículo 18 de la Decisión 486 señala lo siguiente:

Artículo 18.- Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica.

Consecuentemente, una invención se considerará inventiva si la misma no hubiese resultado obvia a partir del arte previo, para una persona del oficio normalmente versada en la materia. Esto a su vez implica que el Examinador a cargo de esta evaluación debe colocarse en el momento exacto en el que el inventor buscaba resolver el problema técnico contemplado en la solicitud y para el cual poseía la literatura y el conocimiento disponibles en dicho momento.

Siguiendo entonces los lineamientos de la Jurisprudencia aplicable al análisis del nivel inventivo de una solicitud de patente, se considera que una anterioridad o una combinación de dos anterioridades puede afectar dicho requerimiento de patentabilidad, si una persona medianamente versada en la materia encuentra enseñanzas o sugerencias claras que, a partir de un conocimiento medio en dicho campo, le permitan llegar de manera obvia a la invención reivindicada. Adicionalmente se considera que una mejora sustancial, un efecto inesperado o una ventaja con respecto al arte previo es un indicio de nivel inventivo, por cuanto aporta un salto tecnológico en la regla técnica.

La presente solicitud si tiene nivel inventivo

El Examinador objeta las reivindicaciones 1-6 y 8-9 por falta de nivel inventivo en vista de los documentos D1 y D2. El Examinador establece que la diferencia entre la reivindicación de la presente solicitud y el documento D1 es que D1 no enseña en la posición 4 residuos de MeThr y que el documento D2 subsana dichas diferencias ya que divulga en la posición 4 un MeThr. Respetuosamente manifestamos nuestro desacuerdo con esta objeción por las razones que se presentarán a continuación.

El documento D1 divulga un agente que promueve el crecimiento del cabello que comprende un derivado de ciclosporina como ingrediente activo, que tiene una excelente habilidad para estimular el crecimiento del cabello con pocos efectos inmunosupresores. También se divulga un agente para la promoción del crecimiento del cabello que comprende un derivado de ciclosporina representado por la Fórmula Química 1. Los derivados fueron preparados por la derivación de ciclosporinas en el aminoácido del residuo 4 N-metil-L-leucina, y se examinaron los efectos que promueven el crecimiento del cabello del mismo. Dicho agente que promueve el crecimiento del cabello que comprende como ingrediente activo derivado de ciclosporina, exhibe un excelente efecto en el crecimiento del cabello, mientras que muestra una actividad inmunosupresora muy baja, comparado con la ciclosporina A sin modificar.

Particularmente, se encontró que [γ-hidroxi-N-metil-L-leucina⁴] ciclosporina A era un agente novedoso que promueve el crecimiento del cabello que tiene un efecto único para restaurar el cabello sin actividad inmunosupresora (pág. 6 de D1). El problema técnico del documento D1 era buscar derivados distintos a la ciclosporina A que tuvieran un efecto mejorado en la restauración del cabello mientras que la actividad inmunosupresora era reducida. Como uno de los resultados del documento D1 se encontró que [N-metil-D-alanina³] [γ-hidroxi-N-metil-L-leucina⁴] ciclosporina A tiene un efecto de restaurar el cabello mientras que la actividad inmunosupresora es reducida. Consecuentemente, la solución del documento D1 está dirigida a derivados de ciclosporina [γ-hidroxi-N-metil-L-leucina⁴] que tienen un efecto excelente restaurando el cabello sin actividad inmunosupresora.

Las fórmulas I, II o III del documento D1 muestran secuencias de amino ácidos muy distintas de los compuestos de acuerdo a la presente invención. La diferencia más notable es la presencia del residuo [γ-hidroxi-N-metil-L-leucina] en la posición 4 de todos los compuestos de acuerdo con el documento D1, dicho residuo es la característica clave de los compuestos del documento D1. Los compuestos preferidos del documento D1 como se muestran en el proceso de preparación, están divulgados en los ejemplos 1-4, denominados:

[N-metil-D-alanina³] **[y-hidroxi-N-metil-L-leucina⁴]** ciclosporina A;
[y-hidroxi-N-metil-L-leucina⁴] [alanina thiomida⁷, 7Ψ8 CS-NH] ciclosporina A;
[y-hidroxi-N-metil-L-leucina⁴] [y-hidroxi-N-metil-L-leucina⁹] ciclosporina A;
[L-treonin²] **[y-hidroxi-N-metil-L-leucina⁴]** [L-leucina⁵] [D-2-ácido hidroxi-isovalérico⁸] [L-leucina¹⁰] ciclosporina A;
[L-treonina²] **[y-hidroxi-N-metil-L-leucina⁴]** [L-leucina⁵] [D-2-ácido hidroxi-isovalérico⁸] [L-leucina] ciclosporina A.

Esos compuestos son utilizados en la preparación de formulaciones de tónicos para el cabello (págs. 18-20), cremas para el cabello (págs. 21-24), champús (págs. 24-27) y acondicionadores para el cabello (págs. 27-31). Sin embargo, no existe ninguna indicación en este documento de que los compuestos de D1 se puedan utilizar para prevenir o tratar infecciones por Hepatitis C o trastornos inducidos por VHC. Adicionalmente no hay ninguna sugerencia en el documento D1 de que los compuestos divulgados iban a reducir los efectos adversos potenciales relacionados con la inhibición de transportadores hepáticos o la droga potencial para interacción de drogas relacionado con la inhibición de transportadores hepáticos ni incluso ambos.

Consideramos que el documento D1 pertenece a otro campo técnico que está alejado del de la presente invención. En el documento D1 no existe ninguna indicación de que los compuestos de D1 se puedan utilizar para la prevención o el tratamiento de infecciones por Hepatitis C o trastornos inducidos por VHC. El enfoque del documento D1 es completamente diferente y no es aplicable ya que se refiere a compuestos para la preparación de tónicos para el cabello, cremas para el cabello, champús y acondicionadores. Por el contrario, los compuestos de la presente invención tienen como objetivo reducir los efectos adversos potenciales relacionados con la inhibición de transportadores hepáticos o la droga potencial para interacciones de drogas relacionado con la inhibición de transportadores hepáticos o incluso ambos.

La diferencia entre la presente invención y el documento D1 es el residuo de la posición 4. Como se estableció anteriormente, el documento D1 no debería de ser

considerado como el estado de la técnica más cercano a la invención ya que divulga derivados de ciclosporinas para el tratamiento del cabello y nunca mencionan nada sobre el uso de estos compuestos para tratar infecciones de hepatitis C o trastornos causados por VHC. Sin embargo, si se llegara a considerar que una persona versada en la materia desea partir de este documento para resolver el problema técnico de la presente solicitud también es improbable que logre la solución propuesta aunque conozca también el documento D2.

Primero que todo, partiendo de los compuestos del documento D1 la persona versada en la materia no se vería motivada a modificar el sustituyente de la posición 4, de todos los demás aminoácidos, porque no existe ninguna sugerencia en este documento que le indique a la persona versada que debe buscar otro sustituyente para específicamente esta posición y de esta manera para lograr el componente de la presente solicitud. Por ende la persona versada en la materia no sabría qué cambios hacer y consecuentemente que documentos buscar por lo que es dudoso que se encontrara con el documento D2 y mucho menos sabría que era el documento D2 con el que podía lograr la composición de la presente solicitud, teniendo en cuenta que no sabía que tenía que reemplazar el sustituyente de la posición 4. De hecho, en el documento D1 hacen énfasis y se refieren mucho a la HMeLeu en la posición 4 de su compuesto ya que es la característica principal de su componente y lo que la diferencia del arte previo porque le proporciona su efecto técnico y por eso está presente en todos los componentes ejemplos de esa solicitud y específicamente en esa posición. De esta manera, una persona versada en la materia observando los compuestos de ciclosporina del documento D1 que tienen efectos no inmunosupresores y que estos compuestos están caracterizados por tener todos en su posición 4 una HMeLeu, queriendo obtener también un efecto no inmunosupresor no hubiera pensado que el cambio debía ser en la posición 4, por el contrario el documento D1 se aleja en ese sentido de las enseñanzas de la presente solicitud.

Igualmente supongamos que la persona versada en la materia parte del documento D1 porque son compuestos de ciclosporinas que tienen actividad no inmunosupresora y decide que necesita encontrar un segundo documento que le muestre

otros compuestos de ciclosporina que se utilicen para infecciones con hepatitis C. En este punto la persona versada en la materia al observar las fórmulas de D1 y de D2 tampoco hubiera podido lograr de manera obvia la invención de la presente solicitud. El documento D2 divulga 14 distintas posibilidades para la posición 4, de las cuales solo 4 coincide con los sustituyentes de los componentes que se divulgan en la presente solicitud. De esta manera es muy poco probable que la persona versada en la materia pudiera saber que eran solo esos 4 los que tenía que elegir para poder lograr los compuestos de la presente solicitud.

Por otro lado, el documento D2 también se aleja de las enseñanzas de la presente solicitud ya que D2 divulga compuestos cicloundecapéptidos, contrario a la presente solicitud que tiene compuestos cicloundecadepsipéptidos. Los compuestos cicloundecapéptidos tienen en su posición 8 una D(Ala) en vez de un ácido hidroxiiisovarílico. Como se menciona en la descripción de la presente solicitud un cicloundecadepsipéptido difiere de un cicloundecapéptido en que los cicloundecadepsipéptidos tienen al menos un enlace éster en donde un cicloundecapéptido tiene un enlace de amida. Específicamente en la descripción de la presente solicitud se establece: "Una de las ventajas de los compuestos de acuerdo con la presente invención puede residir en el impacto de un enlace éster en el macroesqueleto cíclico en comparación con el esqueleto amida cíclica regular de los compuestos cicloundecapéptidos correspondientes descritos en el arte previo. Sin sujetarse a la teoría, se considera que la sustitución de una amida por un enlace éster entre el aminoácido AXX7 en la posición 7 y D-HIV en la posición 8 resulta en un fuerte efecto sobre las propiedades conformacionales y físico-químicas, como un aumento en la flexibilidad conformacional (en PJ Flory, Statistical Mechanics of Chain Molecules, Hanser Publishers, NY, 1988) y la lipofilicidad, así como también la ausencia de una unión donante de hidrógeno. Se cree que estos rasgos estructurales llevan a diferencias pronunciadas en las propiedades físico-químicas, farmacocinéticas y biológicas de los compuestos de la invención en comparación con la clase de análogos derivados de CsA.". De esta manera es difícil pensar que la persona versada en la materia hubiera sabido que tenía que mantener el sustituyente de la posición 8 del documento D1 pero cambiar a uno de los 14 sustituyentes de la posición 4 de los compuestos del documento D2 y además escoger

precisamente entre esas 14 posibilidades las 4 que se divulgan en la presente solicitud y que muestran los mejores resultados. Adicionalmente, es difícil pensar esto porque en el documento D1 no existe ninguna sugerencia o enseñanza de que se deba cambiar el sustituyente de la posición 4 o de que se deba mantener el sustituyente D-HIV de la posición 8.

De hecho el documento D2 divulga entre las posibilidades de la posición 4 a la HMeLeu que coincide con el aminoácido divulgado en esta posición en el documento D1, por lo que hubiera podido pensar que el cambio del aminoácido de esta posición no era la solución para que el compuesto del documento D1 tuviera su efecto técnico para la hepatitis C. Esta solución no es obvia, solamente se puede considerar obvia si se mira desde un punto de vista retrospectivo.

Lograr el compuesto de la presente solicitud no es para nada fácil si no se tiene alguna sugerencia de que aminoácidos se deben cambiar y porque razones. Se debe tener en cuenta que los documentos D1 y D2 en combinación reportan las siguientes posibles combinaciones de sustituyentes en cada posición: 1(5); 2(5); 3(25)¹; 4(14); 5(5); 6(4); 7(2); 8(2); 9(3); 10(3); 11(1). Esta cantidad da a una posibilidad de 6,300,000 combinaciones y sin ninguna guía en los documentos D1 o D2 hacia el grupo específico de componentes que se reclaman en la presente solicitud, hubiera sido imposible escoger las combinaciones exactas.

Se solicita al Examinador también tener en cuenta para este análisis las modificaciones que se realizaron al nuevo pliego reivindicatorio, ya que se limitaron los sustituyentes de cada radical y se limitaron los compuestos preferidos de la presente invención que se reclamaban en la reivindicación original 4. Los compuestos derivados de la fórmula I que se divulgan ahora en la nueva reivindicación 2 corresponden a una optimización global de los criterios más importantes de la presente invención, es decir, la

¹ No se calculó exhaustivamente todos los posibles residuos que el documento D1 indica como útiles en esta posición. Teniendo en cuenta el número de sustituciones aceptables, el número real estaría entre los cientos. Por cuestiones de simplicidad el valor 25 es usado para el presente cálculo.

mejora de la síntesis química, las propiedades farmacológicas, los perfiles farmacocinéticos y toxicológicos. En particular, como efectos técnicos de la invención estos compuestos específicamente muestran una potencia mucho menor para inhibir transportadores hepáticos como MRP2 o OATP1B1. Por lo tanto, se espera que estos compuestos reduzcan posibles efectos adversos (por ej. hiperbilirrubinemia) o interacciones farmacológicas potenciales (por ej. con inhibidores de HMG-CoA reductasa como atorvastatina) relacionadas con la inhibición de transportadores hepáticos, o incluso ambos.

Finalmente esta invención no se debe considerar como obvia teniendo en cuenta que en la química de medicamentos, especialmente la química de péptidos cíclicos como ciclosporinas que son macromoléculas flexibles, es casi imposible predecir (incluso con modelos en computador) los efectos de cambios de aminoácidos aunque estos cambios sean cambios menores. La predicción de la actividad de la estructura es casi imposible con estos compuestos de macromoléculas, especialmente esto es cierto cuando se trata de cambiar varios aminoácidos al mismo tiempo. Por ejemplo, en la patente US 5,767,069 (anexada), se observa un efecto inmunosupresor de la ciclosporina A comparado con la ausencia de dicho efecto en NIM 811, con tan solo una diferencia de los aminoácidos en la estructura. En la columna 3, líneas 10 a 14 de dicho documento de patente se establece que las ciclosporinas: "... no son del todo inmunosupresoras..." y la Tabla 1 que muestra 7 derivados de ciclosporina que son diferentes a la ciclosporina A por tan solo un aminoácido y sin embargo cada uno de estos tiene un efecto inmuno-modulador muy diferente relativo con Ciclosporina A. Los compuestos que están en la Tabla 1 de este documento denominados (b), (c) y (e), cada uno tiene menos de 1% de potencia inmunosupresora de Ciclosporina A, aunque su diferencia con Ciclosporina A es solo un aminoácido. De esta manera es claro que incluso pequeños cambios en una secuencia de ciclosporina puede tener efectos dramáticos e impredecibles en las propiedades farmacológicas. Esto demuestra que en términos del efecto, la modificación de un solo aminoácido no se puede anticipar y por ende no es posible predecir ninguna modificación en la estructura.

En conclusión por todo lo anterior, los documentos D1, D2 o su combinación, no resultan anterioridades válidas capaces de afectar el nivel inventivo de la invención de la presente solicitud. Por lo tanto, consideramos que el Examinador está excluyendo el hecho de que el inventor en el momento de solucionar el problema técnico de la solicitud tuvo que emplear habilidad inventiva, pues la presente invención no resulta de la mera extrapolación de las enseñanzas del arte previo, como se afirma. Se está desconociendo el mérito investigativo empleado y que está aportando una ventaja al arte previo.

En adición a los argumentos presentados, el mérito investigativo de la presente solicitud ha sido reconocido por otras oficinas de patentes del mundo, las cuales han otorgado y aceptado la novedad y el nivel inventivo de las patentes equivalentes, como es el ejemplo de la USPTO que otorgó la patentes equivalente US 8,501,683 (anexo la primera página de ésta).

Si bien es cierto que cada país es autónomo en conceder o negar el privilegio de patente para una solicitud, también es cierto que los requisitos de concesión de dichos privilegios son universales, esto es, novedad, nivel inventivo y aplicación industrial, como también la forma de evaluarlos. Así las cosas, el hecho de que la misma solicitud cumpla con los mencionados requisitos en diferentes países, como se mencionó anteriormente, es un claro indicio del merecimiento de la patente en Colombia.

5. Puesto que se ha dado respuesta a todas las objeciones formuladas por el Examinador, se solicita atentamente proceder al otorgamiento de la solicitud de patente. En este sentido, respetuosamente recordamos al Examinador que la patente no podrá ser negada con base en objeciones diferentes a las expuestas en la acción oficial No. 13476, toda vez que al no haber sido trasladadas a mi representada, dicha decisión violaría tanto el tenor literal del artículo 45 de la Decisión 486 como el derecho constitucional al debido proceso. En este evento, y con base en el inciso 2 del artículo 45, respetuosamente solicitamos al Examinador expedir una nueva acción oficial que permita a mi representada contra argumentar las mismas o modificar la solicitud con el fin de cumplir los requisitos de patentabilidad.

Respetuosamente manifiesto que a pesar de eliminar materia del pliego reivindicatorio originalmente presentado, mi representada no renuncia a la protección que pueda obtener sobre dicha materia y se reserva el derecho de presentar una solicitud divisional que la contenga durante el trámite de la presente solicitud, beneficiándose de la fecha de prioridad inicialmente reivindicada.

6. Anexos

- Nuevo pliego reivindicatorio conformado por 3 reivindicaciones.
- Copia de Patente US 5,767,069.
- Portada de patente US 8,501,683.

Señor Director,

Luiz Clemencia de Páez
LUZ CLEMENCIA DE PÁEZ

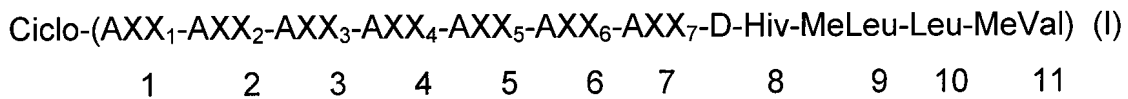
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REIVINDICACIONES

1. Un compuesto cicloundecadepsipéptido de Fórmula (I)



en el cual:

A $X\text{X}_1$ es MeBmt, o 8-hidroxi-MeBmt;

A $X\text{X}_2$ es Abu, Val, Thr, Thr(OMe), Thr(OAc), Thr(OCOCH₂CH₂CH₂OH), o 5-hidroxi-Nva;

A $X\text{X}_3$ es D-MeAla, D-3-fluoro-MeAla, D-MeSer, D-MeSer(OAc), o D-MeAsp(OMe);

A $X\text{X}_4$ es Melle, MeMet, MeMet(Ox) en el cual Ox es -SOMe, -SO₂Me, MeVal, EtVal, EtIle, o MeTyr;

A $X\text{X}_5$ es Leu, Val, o Ile

A $X\text{X}_6$ es MeAla, Sar, o MeLeu; y

A $X\text{X}_7$ es Gly, o Ala.

2. El compuesto de acuerdo con cualquiera de la reivindicación 1 que tiene las siguientes fórmulas:

ciclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal) (Ia)

ciclo-(MeBmt-Abu-D-MeAla-EtVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (Ib)

ciclo-(MeBmt-Val-D-MeAla-MeVal-Ile-MeLeu-Gly-D-Hiv-MeLeu-Leu-MeVal) (Ic)

ciclo-(MeBmt-Abu-D-MeAla-MeVal-Leu-MeLeu-Ala-D-Hiv-MeLeu-Leu-MeVal) (Id)

3. Una composición farmacéutica que comprende el compuesto de acuerdo con cualquiera de las reivindicaciones 1 a 2, junto con uno o más diluyentes o vehículos aceptables desde el punto de vista farmacéutico.



US008501683B2

(12) **United States Patent**
Wenger et al.

(10) **Patent No.:** **US 8,501,683 B2**
(45) **Date of Patent:** **Aug. 6, 2013**

(54) **CYCLOUNDECADEPSIPEPTIDE
COMPOUNDS AND USE OF SAID
COMPOUNDS AS A MEDICAMENT**

(58) **Field of Classification Search**
None
See application file for complete search history.

(75) **Inventors:** **Roland Wenger**, Riehen (CH); **Manfred Mutter**, Belmont-Sur-Lausanne (CH); **Patrick Garrouste**, Saxon (CH); **Robert Lysek**, Martigny (CH); **Olivier Turpin**, Martigny (CH); **Grégoire Vuagniaux**, Lausanne (CH); **Valérie Nicolas**, St-Prex (CH); **Laura Novaroli Zanolari**, Lausanne (CH); **Rafael Crabbé**, Bursins (CH)

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(74) *Attorney, Agent, or Firm* — Harness, Dickey & Pierce, P.L.C.

(57) **ABSTRACT**

At least one embodiment of the present invention relates to new cycloundecadepsipeptide compounds and to the use of said compounds as a medicament, in particular as antiviral agents, more particularly for preventing or treating Hepatitis C infections or HCV induced disorders.

18 Claims, 1 Drawing Sheet

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(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 159 days.

(21) **Appl. No.:** **12/998,588**

(22) **PCT Filed:** **Nov. 6, 2009**

(86) **PCT No.:** **PCT/IB2009/007361**

§ 371 (c)(1),
(2), (4) **Date:** **May 5, 2011**

(87) **PCT Pub. No.:** **WO2010/052559**

PCT Pub. Date: **May 14, 2010**

(65) **Prior Publication Data**

US 2011/0212057 A1 Sep. 1, 2011

(30) **Foreign Application Priority Data**

Nov. 6, 2008 (WO) PCT/IB2008/002982

(51) **Int. Cl.**
A61K 38/12 (2006.01)

(52) **U.S. Cl.**
USPC 514/4.3; 514/21.1



US005767069A

United States Patent [19]
Ko et al.

[11] **Patent Number:** **5,767,069**
[45] **Date of Patent:** **Jun. 16, 1998**

[54] **CYCLOSPORINS**

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[21] **Appl. No.:** **427,312**

[22] **Filed:** **Apr. 24, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 232,795, Apr. 25, 1994, abandoned,
which is a continuation of Ser. No. 57,067, May 3, 1993,
abandoned, which is a continuation of Ser. No. 785,959, Oct.
31, 1991, abandoned.

[30] **Foreign Application Priority Data**

Nov. 2, 1990	[GB]	United Kingdom	9023859
Nov. 5, 1990	[GB]	United Kingdom	9023970
Nov. 5, 1990	[GB]	United Kingdom	9023971
Nov. 5, 1990	[GB]	United Kingdom	9023972
Aug. 5, 1991	[GB]	United Kingdom	9116836

[51] **Int. Cl.⁶** **A61K 38/13; C07K 7/64**

[52] **U.S. Cl.** **514/11; 514/9; 514/2;**
530/317; 930/270

[58] **Field of Search** **514/9, 11, 2; 530/317;**
930/270

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Attorney, Agent, or Firm—Marla J. Mathias; Thomas O.
McGovern

[57] **ABSTRACT**

Nonimmunosuppressant cyclosporin derivatives having
cyclophilin-binding activity, for example, the compound,
[MeIle]⁴-ciclosporin, are useful in inhibiting HIV-1 replica-
tion in treating AIDS and AIDS related disorders.

6 Claims, 3 Drawing Sheets

FIG. 1

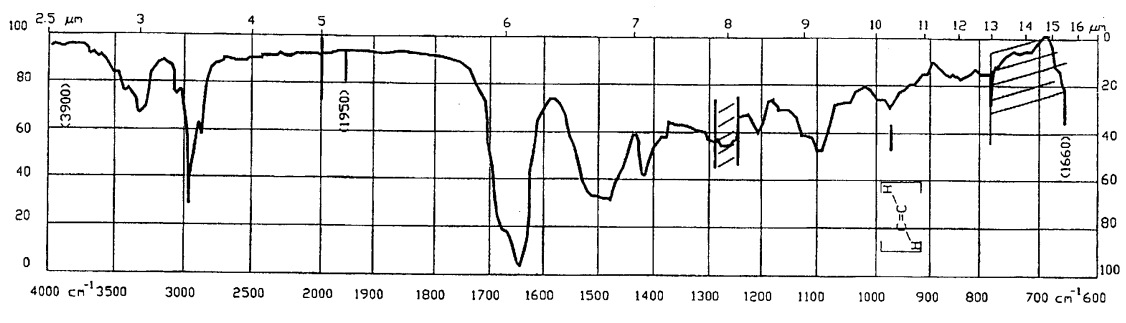


FIG. 2

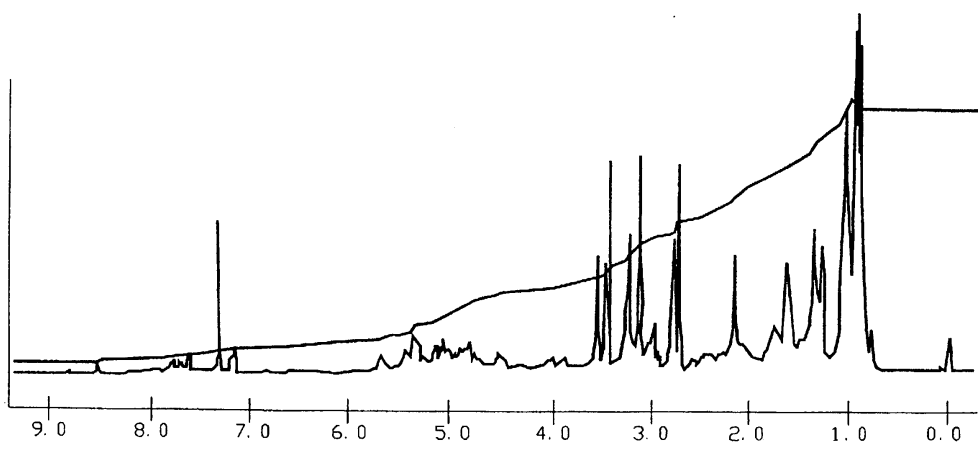
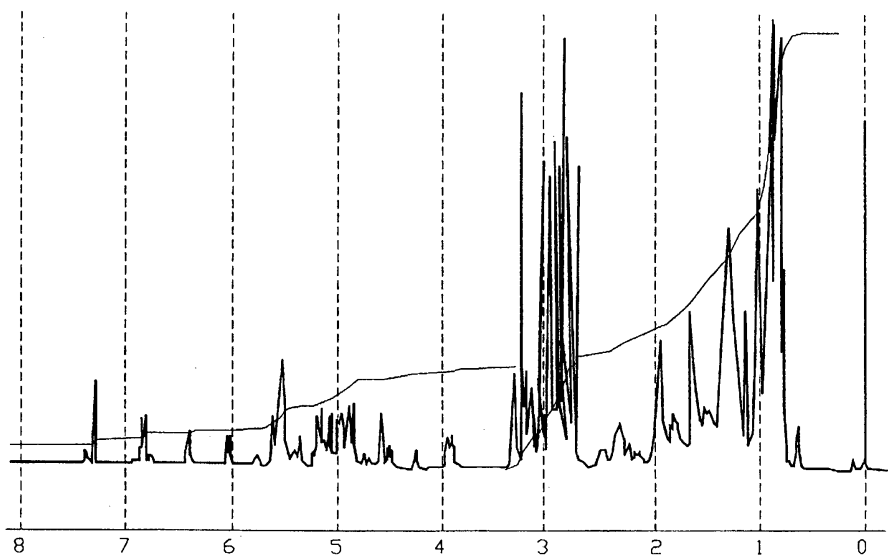


FIG. 3

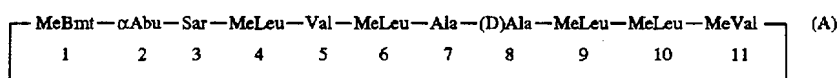


1 CYCLOSPORINS

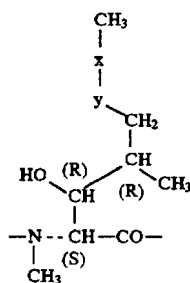
This is a continuation of application Ser. No. 08/232,795, filed Apr. 25, 1994, which in turn is a continuation of application Ser. No. 08/057,067, filed May 3, 1993, which in turn is a continuation of application Ser. No. 07/785,959, filed Oct. 31, 1991, all of which are now abandoned.

The present invention relates to novel cyclosporins, their use as pharmaceuticals and pharmaceutical compositions comprising them, as well as to processes for their production.

The cyclosporins comprise a class of structurally distinctive, cyclic, poly-N-methylated undecapeptides, commonly possessing pharmacological, in particular immunosuppressive, anti-inflammatory and/or antiparasitic activity. The first of the cyclosporins to be isolated was the naturally occurring fungal metabolite Ciclosporin or Cyclosporine, also known as cyclosporin A and commercially available under the Registered Trademark SANDIMUN® or SANDIMMUNE®. Ciclosporin is the cyclosporin of formula A.



where MeBmt represents the N-methyl-(4R)-4-but-2E-en-1-yl-4-methyl-(L)threonyl residue of formula B



in which -x-y- is —CH=CH— (trans).

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is the IR spectrum in CH₂Cl₂ of cyclosporin.

FIG. 2 is the proton NMR spectrum in CDCl₃ of cyclosporin.

FIG. 3 is a 300 MHz HNMR spectrum in CDCl₃ of the product of Example 5.

Since the original discovery of Ciclosporin, a wide variety of naturally occurring cyclosporins have been isolated and identified and many further non-natural cyclosporins have been prepared by total- or semi-synthetic means or by the application of modified culture techniques. The class comprised by the cyclosporins is thus now substantial and includes, for example, the naturally occurring cyclosporins A through Z [cf. Traber et al; 1, *Helv. Chim. Acta*, 60, 1247-1255 (1977); Traber et al; 2, *Helv. Chim. Acta*, 65, 1655-1667 (1982); Kobel et al. *Europ. J. Applied Microbiology and Biotechnology*, 14, 273-240 (1982); and von Wartburg et al, *Progress in Allergy*, 38, 28-45, (1986)], as well as various non-natural cyclosporin derivatives and artificial or synthetic cyclosporins including dihydro-cyclosporins [in which the moiety -x-y- of the MeBmt residue (formula B above) is saturated to give -x-y—CH₂—CH₂—]; derivatised cyclosporins (e.g. in which the

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3'-O-atom of the MeBmt residue is acylated or a further substituent is introduced at the α-carbon atom of the sarcosyl residue at the 3-position); cyclosporins in which the MeBmt residue is present in isomeric form (e.g. in which the configuration across positions 6' and 7' of the MeBmt residue is cis rather than trans); and cyclosporins in which variant amino acids are incorporated at specific positions within the peptide sequence, e.g. employing the total synthetic method for the production of cyclosporins developed by R. Wenger—see e.g. Traber et al. 1, Traber et al. 2 and Kobel et al., loc. cit.; U.S. Pat. Nos. 4,108,985, 4,220,641, 4,288,431, 4,554,351, 4,396,542 and 4,798,823 European Patent Publications Nos. 34,567A, 56,782A, 300,784A, 300,785A and 414,632A; International Patent Publication No WO 86/02080 and UK Patent Publications Nos. 2,206,119 and 2,207,678; Wenger 1, *Transpl. Proc.*, 15 Suppl. 1:2230 (1983); Wenger 2., *Angew. Chem. Int. Ed.* 24 77 (1985) and Wenger 3., *Progress in the Chemistry of Organic Natural Products*, 50, 123 (1986).

The class comprised by the cyclosporins is thus now very large indeed and includes, for example, [Thr]²-, [Val]²-,

[Nva]²- and [Nva]²-[Nva]⁵-Ciclosporin (also known as cyclosporins C, D, G and M respectively), [3-O-acetyl-MeBmt]¹-Ciclosporin (also known as cyclosporin A acetate), [Dihydro-MeBmt]¹-[Val]²-Ciclosporin (also known as dihydro-cyclosporin D), [(D)Ser]⁸-Ciclosporin, [MeIle]¹¹-Ciclosporin, [(D)MeVal]¹¹-Ciclosporin (also known as cyclosporin H), [MeAla]⁶-Ciclosporin, [(D)Pro]³-Ciclosporin and so on.

In accordance with conventional nomenclature for cyclosporins, these are defined throughout the present specification and claims by reference to the structure of Ciclosporin (i.e. Cyclosporin A). This is done by first indicating those residues in the molecule which differ from those present in Ciclosporin and then applying the term "Ciclosporin" to characterise the remaining residues which are identical to those present in Ciclosporin. At the same time the prefix "dihydro" is employed to designate cyclosporins wherein the MeBmt residue is hydrogenated (dihydro-MeBmt) i.e. where -x-y- in formula B is —CH₂—CH₂—. Thus [Thr]²-Ciclosporine is the cyclosporin having the sequence shown in Formula A but in which αAbu at the 2-position is replaced by Thr. and [Dihydro-MeBmt]¹-[Val]²-Ciclosporin is the cyclosporin having the sequence shown in Formula A but in which the MeBmt residue at position 1 is hydrogenated and αAbu at the 2-position is replaced by Val.

In addition, amino acid residues referred to by abbreviation, e.g. Ala, MeVal, αAbu etc. are, in accordance with conventional practice, to be understood as having the (L)-configuration unless otherwise indicated, e.g. as in the case of "(D)Ala". Residue abbreviations preceded by "Me" as in the case of "MeLeu", represent α-N-methylated residues. Individual residues of the cyclosporin molecule are numbered, as in the art, clockwise and starting with the residue MeBmt or dihydro-MeBmt in position 1. The same numerical sequence is employed throughout the present specification and claims.

It is now well established that Ciclosporin acts by interfering with the process of T cell activation by blocking transcription initiation of IL-2, although the precise mecha-

nism has not yet been elucidated. Ciclosporin has been shown to form a complex with a 17 kD cytosolic protein (cyclophilin) that occurs in many cell types and has been shown to be identical to peptidyl-prolyl cis-trans isomerase, an enzyme involved in protein folding. Up to now, however, it has not been clear whether binding to cyclophilin is directly correlated with immunosuppressive activity in ciclosporins, or indeed whether cyclophilin binding is itself a sufficient criterion for immunosuppressive activity.

It has now been found that there are ciclosporins which bind strongly to cyclophilin, but are not at all immunosuppressive. It therefore follows that binding to cyclophilin is a necessary, but not a sufficient, criterion for immunosuppressant activity.

The present invention provides ciclosporins which are active against HIV-1 replication.

Human immune deficiency virus (HIV) infects preferentially T-helper (T4) lymphocytes, although it replicates also in various other cell types, especially those of the monocytic lineage. It causes a slowly progressing disease of the immune system characterised by a gradual T4-cell destruction, named AIDS. Other immunological abnormalities of AIDS are increase of cytotoxic/suppressor (T8) lymphocytes, a defect in the antigen presentation/recognition process and polyclonal activation of B-cells. The mechanism of T4-cell destruction is still not clear. Relatively few T4-cells seem to be infected, thus, a direct cytopathic effect caused by the virus may not be the only reason for T4-cell depletion. It has been hypothesised that T4-cell destruction could be amplified by an autoimmune process triggered by HIV-producing or HIV-protein-coated T4-cells. This continuous antigenic stimulation may lead to a state of permanent activation of T4-cells which would enhance HIV-replication in these cells and expand T-cytotoxic clones. Uninfected T4-cells may be rendered antigenic by binding exogenous viral gp120 to their CD4 molecules and would thus be a target of a T-cytotoxic response.

U.S. Pat. No. 4,814,323 discloses that Ciclosporin has activity against AIDS, and that in general "ciclosporins known as immunosuppressors" may be useful in this indication. There is no suggestion that non-immunosuppressive ciclosporins might be expected to have this property.

Surprisingly, it has now been found that ciclosporins which bind to cyclophilin, but are not immunosuppressive, exhibit an inhibitory effect upon HIV-1 replication.

A ciclosporin is considered as binding to cyclophilin if it binds to human recombinant cyclophilin at least one fifth as well as does Ciclosporin in the competitive ELISA test described by Quesniaux in *Eur. J. Immunol.* 1987 17 1359-1365. In this test, the ciclosporin to be tested is added during the incubation of cyclophilin with coated BSA-Ciclosporin and the concentration required to give a 50% inhibition of the control reaction without competitor is calculated (IC_{50}). The results are expressed as the Binding Ratio (BR), which is the log to the base 10 of the ratio of the IC_{50} of the test compound and the IC_{50} in a simultaneous test of Ciclosporin itself. Thus a BR of 1.0 indicates that the test compound binds cyclophilin one factor of ten less well than does Ciclosporin, and a negative value indicates binding stronger than that of Ciclosporin.

The ciclosporins active against HIV have a BR lower than 0.7. (since $\log_{10} 5=0.7$ approx), preferably equal to or lower than zero.

A ciclosporin is considered to be non-immunosuppressive when it has an activity in the Mixed

Lymphocyte Reaction (MLR) of no more than 5%, preferably no more than 2%, that of Ciclosporin. The Mixed Lymphocyte Reaction is described by T.Meo in "Immunological Methods", L. Lefkovits and B. Peris, Eds., Academic Press, N.Y. pp. 227-239 (1979). Spleen cells (0.5×10^6) from Balb/c mice (female, 8-10 weeks) are co-incubated for 5 days with 0.5×10^6 irradiated (2000 rads) or mitomycin C treated spleen cells from CBA mice (female, 8-10 weeks). The irradiated allogeneic cells induce a proliferative response in the Balb c spleen cells which can be measured by labeled precursor incorporation into the DNA. Since the stimulator cells are irradiated (or mitomycin C treated) they do not respond to the Balb/c cells with proliferation but do retain their antigenicity. The IC_{50} found for the test compound in the MLR is compared with that found for Ciclosporin in a parallel experiment.

The activity of ciclosporins as inhibitors of HIV-1 replication may be demonstrated in the following test systems:

1. Inhibition of HIV-1-induced cytopathic effect in MT4-cells

The assay procedure described by Pauwels et al., *J. Virol. Meth.* 20/309 (1988), is used with minor modifications. The HTLV-I-transformed T4-cell line, MT4, which was previously shown to be highly permissive for HIV-infection, is used as the target cell. Inhibition of HIV-1, strain HTLV-IIIb-induced cytopathic effect is determined by measuring the viability of both HIV-infected and mock-infected cells. Viability is assessed spectrophotometrically via in situ reduction of 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT). Virus-infected and uninfected cultures without compound are included as controls as are uninfected cells treated with compound. The cell concentration is chosen so that the number of cells per ml increases by a factor of 10 during the 4 days of incubation in the mock-infected cultures. Virus inoculum is adjusted so as to cause cell death in 90% of the target cells after 4 days of incubation. The virus is adsorbed onto a 10-fold concentrated cell suspension at 37° C. for 1 hour. Then, the infected cells are diluted 1:10 and added to microtiter plates containing the test compound.

2. Cytotoxicity

To evaluate the anti-HIV potential in additional cell lines and particularly in a monocytic cell line (U937), cellular toxicity of the test compound to these cell lines is first assessed. Jurkat and U937 cell suspensions are adjusted to 1×10^5 cells/ml and incubated in the presence of various concentrations of test compound. After 48 hours the amounts of cells per ml are compared by staining with MTT. Cytotoxicity in MT4 cells may be measured in the same way.

3. Inhibition of HIV-1 replication in Jurkat and U937 cells

The T4-cell line Jurkat and the monocytic cell line U937 are infected by suspending the cells 10-fold concentrated in virus solution. Adsorption is allowed for 2 hours at 37° C. The cells are then spun down, the inoculum is removed and the cells are resuspended at their original concentration in fresh culture medium containing test compound. Thus, the substance is added post adsorption. At days 2, 5, 8, 12, 15 and 19 post-infection, aliquots of the infected cultures are removed. Cells are spun down and the supernatants collected. The concentration of viral p24 antigen is determined in the supernatants by means of a commercial ELISA kit and serves as a parameter for virus production. After each removal of aliquots, cells are counted and adjusted to 2×10^5 cells/ml by adding fresh medium containing the test compound at the particular concentration.

Of the compounds according to the invention, i.e. non-immunosuppressive, cyclophilin-binding ciclosporins

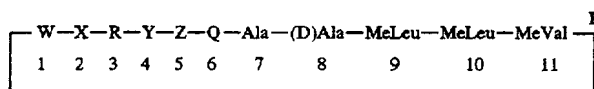
active against HIV-1 (Active Compounds), some are novel and others are known; however the anti-HIV activity of the known Active Compounds has not previously been disclosed, and in many cases the known Active Compounds have not been disclosed to have any pharmaceutical activity whatsoever.

It is found that many of the Active Compounds have structures differing from that of Ciclosporin specifically at the 4 and/or 5 positions.

One group of Active Compounds are cyclosporins in which the MeLeu group at position 4 is replaced by a different N-methylated amino acid, for example γ -hydroxy-MeLeu, MeIle, MeVal, MeThr or MeAla. In addition to MeIle and MeThr, the allo-forms MeaIle and MeaThr may also be used. In the allo-form, the stereochemistry at the β -position has the opposite configuration to that of the natural amino acid, so that the normal form and the allo-form constitute a pair of diastereoisomers.

A further group of Active Compounds is that in which Val at the 5-position is replaced by an N-alkyl-, preferably N-methyl-, amino acid. Preferably the amino acid which is N-alkylated is Val or Leu. Preferably the hydrogen of the imino group of [Val]⁵ is replaced by a non-branched C₁₋₆alkyl group, preferably methyl, ethyl or n-propyl, particularly methyl. The latter preferred group of Active Compounds are all novel.

Additionally or alternatively, certain Active Compounds may differ from Ciclosporin at the 1, 2, 3, and/or 6 positions. A preferred group of Active Compounds is constituted by the compounds of Formula I:



in which

W is MeBmt, dihydro-MeBmt or 8'-hydroxy-MeBmt;
X is α Abu, Val, Thr, Nva or O-methyl threonine (MeOThr);

R is Sar or (D)-MeAla;

Y is MeLeu, γ -hydroxy-MeLeu, MeIle, MeVal, MeThr, MeAla, MeaIle or MeaThr;

Z is Val, Leu, MeVal or MeLeu; and

Q is MeLeu, γ -hydroxy-MeLeu or MeAla;

provided that when Y is MeLeu then either Z is MeVal or MeLeu, or W is 8'-hydroxy-MeBmt.

The groups W,X,Y,Z,Q and R have, independently, the following preferred significances:

W is preferably W' where W' is MeBmt or dihydro-MeBmt;

X is preferably X' where X' is α Abu or Nva, more preferably X'' where X'' is α Abu;

R is preferably R' where R' is Sar;

Y is preferably Y' where Y' is γ -hydroxy-MeLeu, MeVal, MeThr or MeIle;

Z is preferably Z' where Z' is Val or MeVal; and

Q is preferably Q' where Q' is MeLeu;

One especially preferred group of Active Compounds are the compounds of Formula I in which W is W', X is X', Y is Y', Z is Z', Q is Q' and R is R'.

Particularly preferred compounds of Formula I are:

a) [dihydro-MeBmt]¹-[γ -hydroxy-MeLeu]⁴-Ciclosporin

b) [MeVal]⁴-Ciclosporin

c) [MeIle]⁴-Ciclosporin

d) [MeThr]⁴-Ciclosporin

e) [γ -hydroxy-MeLeu]⁴-Ciclosporin

f) [Nva]²-[γ -hydroxy-MeLeu]⁴-Ciclosporin

g) [γ -hydroxy-MeLeu]⁴-[γ -hydroxy-MeLeu]⁶-Ciclosporin

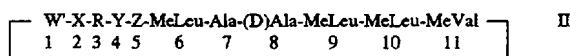
h) [MeVal]⁵-Ciclosporin

i) [MeOThr]²-[(D)MeAla]³-[MeVal]⁵-Ciclosporin

j) [8'-hydroxy-MeBmt]¹-Ciclosporin

In addition, certain compounds not within the scope of Formula I are Active Compounds, for example k) [MeAla]⁶-Ciclosporin, and l) [γ -hydroxy-MeLeu]⁹-Ciclosporin.

The invention also provides novel Active Compounds, which are compounds of Formula II:



where W', X, R, Y and Z are as defined above, provided that

1) when Y is MeLeu or MeAla then Z is MeVal or MeLeu and 2) when W' is MeBmt, R is Sar, and Y is γ -hydroxy-MeLeu, then Z is other than Val.

A preferred group of novel Active Compounds consists of the compounds of Formula II in which X is X'', Y is Y' and Z is Z', provided that when W' is MeBmt, Y' is other than γ -hydroxy-MeLeu.

Particularly preferred novel Active Compounds are the compounds a), b), c), d), f) and h) above. Certain of these compounds, for example compounds b) and c), are found to block the immunosuppressive action of Ciclosporin by blocking its binding to cyclophilin, and thus act as Ciclosporin antagonists.

The Active Compounds may be obtained in a variety of ways, which may be classified as:

- 1) Fermentation
- 2) Biotransformation
- 3) Derivatisation
- 4) Partial Synthesis
- 5) Total Synthesis.

1) Certain of the Active Compounds are produced as by-products of the fermentation of original or modified strains of Ciclosporin-producing organisms such as *Tolypocladium inflatum* Gams, as exemplified by the production of compound c), described in Example 1 below.

2) Other Active Compounds, including the known compounds j) and l) are metabolites of Ciclosporin, and can be isolated by chromatographic methods from the urine of humans or animals dosed with Ciclosporin. Furthermore, these and other metabolic transformations are possible using microorganisms, for example the production of compounds e) and g) by biotransformation of cyclosporin A, as described in Examples 2 and 3, or that of compound f) by biotransformation of cyclosporin G (Example 4). These examples demonstrate a novel process for the preparation of cyclosporins having one or more γ -hydroxy-MeLeu residues, comprising the steps of culturing a novel modified strain of *Sebekia benihana*, adding a cyclosporin having one or more MeLeu residues, and isolating the product from the fermentation broth.

3) By derivatisation is meant that natural or synthetic cyclosporins may be converted into Active Compounds by one or more chemical reactions in which one or more of the amino acids are modified without the peptide bonds being opened and reformed. For example, the class of Active Compounds in which the Val at position 5 is N-alkylated may be obtained by reacting the corresponding cyclosporin having Val at position 5 with butyllithium, followed by

reaction with an alkylating agent, as exemplified for compound h) in Example 5 and for compound i) in Example 6.

As further examples, compound a) may be prepared by hydrogenation of compound e) (Example 7), and compound j), which is also a major metabolite of Cyclosporin, may be prepared from the known compound Cyclosporin acetate as described in Example 8.

4) The term partial synthesis is used to mean a series of chemical reactions in which the ring of a natural cyclosporin is opened, one or more amino acids are removed, different amino acids are added, and the ring is closed again.

5) The total synthesis of cyclosporins may be carried out by building up a linear undecapeptide and cyclising, as described by Wenger (loc. cit.), see also U.S. Pat. Nos. 4,396,542 and 4,798,823. In principle, any cyclosporin may be prepared by the total synthesis route, although where one of the other methods is available, this may well be more convenient than total synthesis. Total synthesis may be used for the preparation of compound d) (Example 9), and for the known metabolite compound l).

Compound k) is a known substance whose properties have been described by Quesniaux et al (*Mol. Immunol.* 24 1159 1987) and which can also be prepared by total synthesis. For example, a total synthesis of this compound is described in U.S. Pat. No. 4,914,188.

EXAMPLE 1

[MeIle]⁴-Cyclosporin (Compound c)

Producing Strain

Compound c) is obtained by fermentation of the fungal strain *Tolypocladium inflatum* Cy E 4556, deposited at the Deutsche Sammlung für Mikroorganismen under the provisions of the Budapest treaty on 24 Jul. 1991 under the accession number DSM 6627. This strain is a mutant of the strain NRRL 8044 of the species *Tolypocladium inflatum* Gams, and is taxonomically identical with the parent strain, which has been fully described, e.g. in British Patent 1,491,509.

Culture 1. Agar starting culture: Agar slant cultures of the strain DSM 6627 are grown for 14 days at 27° C. on the following agar medium:

Yeast extract (Gistex)	4 g
Malt extract (Wander)	20 g
Agar	20 g
Demineralised water to	1000 ml.

The medium has a pH of 5.4–5.6 and is sterilised for 20 min. at 120° C.

2. Preculture: Spores from the mycelium of 4 starting cultures are suspended in 40 ml of a 0.9% salt solution. A series of 500 ml Erlenmeyer flasks each containing 100 ml of preculture medium are inoculated each with 20 ml of this suspension. The composition of the preculture medium is as follows:

Casein (Amber EHC)	25 g
Maltose	75 g
KH ₂ PO ₄	1 g
KCl	2.5 g
Demineralised water to	1000 ml

The medium is adjusted to pH 5.2–5.5 with HCl, then sterilised for 20 min. at 120° C. The precultures are fermented for 24 hr. at 27° C. on a rotary shaker at 200 rpm with an eccentricity of 50 mm.

3. Intermediate culture: One 25-liter steel fermenter containing 20 liters of preculture medium is inoculated with 200 ml of preculture. The intermediate culture is fermented during 5 days at 27° C., with a stirring rate of 150 rpm and a rate of air flow of 0.5 l/min. per liter medium at 0.5 bar pressure.

4. Main culture: 100 liter of preculture medium are inoculated with 10 l of intermediate culture and fermented in a 120-liter steel fermenter. To this medium is added 4 g/l D-Threonin, sterilised by filtration. Fermentation is carried out at 27° C. for 14 days, with a stirring rate of 70 rpm and a rate of air flow of 0.4 l/min per liter medium at 0.5 bar pressure being used for the 5 first days, the stirring rate then being increased to 100 rpm and the air flow rate to 0.5 l/min for the remainder of the fermentation period.

Isolation: The mycelium is separated from the culture medium and extracted in a Turrax apparatus by crushing and stirring 3 times with 10 liters of methanol/water (9:1 by vol.). The crushed mycelium is separated from the solvent by suction-filtration and the combined filtrates are concentrated by evaporation under vacuum at a temperature of 40° C. until the vapour consists mainly of water alone. The obtained mixture is extracted four times using 2 liters 1,2-dichloroethane at each extraction and the combined 1,2-dichloroethane solutions are concentrated by evaporation under vacuum at a temperature of 40° C.

The residue is subjected to silica gel column chromatography (10 kg of silica gel, granulate size 0.02–0.045 mm, "Grace") using ethyl acetate/water as eluent (fractions of 2.5 l). Fractions 20–23, containing [MeIle]⁴-Cyclosporin are pooled and then further separated by silica gel column chromatography (600 g of silica gel, granulate size 0.04–0.063 mm, "Merck") using chloroform/methanol (98:2 by vol.) as eluent (fraction size 300 ml). Further purification is achieved by silica gel column chromatography (400 g of silica gel, granulate size 0.04–0.063 mm, "Merck") using methylene chloride/methanol (98:2 by vol.) as eluent (fraction size 200 ml) yielding pure [MeIle]⁴-Cyclosporin as an amorphous white powder: m.p. 155°–158° C.; [α]_D²⁰ = –235° (c=0.68 in CHCl₃) and –193° (c=0.74 in CH₃OH).

The IR spectrum in CH₂Cl₂ is as shown in FIG. 1, and the proton NMR spectrum in CDCl₃ is as shown in FIG. 2.

EXAMPLE 2

[γ -hydroxy-MeLeu]⁴-Cyclosporin (Compound e)

Compound e) is obtained from biotransformation of Cyclosporin by the microorganism *Sebekia benihana*. The original strain used is named NRLL 11111 and belongs to the species *Sebekia benihana* (Dietz and Li: *Sebekia*, a new genus of the family Actinoplanaceae. Abstrs. 82nd Ann.Meet.Amer.Soc.Microbiol., 163, Atlanta, 1982). This strain is able to hydroxylate novobiocin. The subcultured strain used for the preparation of compound e and related compounds has been deposited at the German Collection of Microorganisms (D-3300 Braunschweig) under the number DSM 6182.

1. Agar starting culture: Agar slant cultures of the strain DSM 6182 are grown for 10 days at 27° C. on the following agar medium:

Glucose	10 g
Starch (soluble)	20 g
Yeast extract (Gistex)	5 g
Peptone (N-Z-Amine Typ A, Sheffield)	5 g
CaCO ₃	1 g

-continued

Agar (Bacto)	18 g
Demineralised water to up to 1 Liter.	

pH: neutralised at 7 with NaOH/H₂SO₄
Sterilisation: 120° C./20 min.

2. Preculture: Spores and mycelium of one starting culture are suspended in 10 ml of a 0.9% salt solution. A series of 200 ml Erlenmeyer flasks each containing 50 ml of preculture medium are inoculated each with 5 ml of this suspension. The composition of the preculture medium is as follows:

Glucose	7 g
Starch (soluble)	10 g
Yeast extract (Gistex)	4.5 g
Malt extract (liquid, Wander)	10 g
Peptone (N-Z-Amine Typ A, Sheffield)	2.5 g
CaCO ₃	1 g
Trace solution Nr 235	1 ml
Demineralised water to up to 1 Liter.	

pH neutralised to 7 with NaOH/H₂SO₄
Sterilisation: 120° C./20 min.
Trace solution Nr 235:

H ₃ BO ₃	0.1 g
FeSO ₄ ·7H ₂ O	5 g
KI	0.05 g
CoCl ₂ ·6H ₂ O	2 g
CuSO ₄ ·5H ₂ O	0.2 g
MnCl ₂ ·4H ₂ O	2 g
ZnSO ₄ ·7H ₂ O	4 g
Demineralised water up to 999 ml	
H ₂ SO ₄ (97%)	1 ml

The precultures are fermented for 4 days at 27° C. on a rotary shaker at 200 rpm with an eccentricity of 50 mm.

3. Intermediate culture: A series of 200 ml Erlenmeyer flasks each containing 50 ml of the preculture medium are inoculated each with 5 ml of preculture. The intermediate culture is fermented for 3 days at 27° C. on a rotary shaker at 200 rpm with an eccentricity of 50 mm.

4. Main culture: A series of 500 ml Erlenmeyer flasks each containing 50 ml of the main medium (total 12 liters) are inoculated each with 5 ml of intermediate culture. These cultures are fermented for 3 days at 27° C. on a rotary shaker at 200 rpm with an eccentricity of 50 mm. After 24 hours cyclosporin A (7.5 mg) solubilized in methanol is added to each main culture (=150mg/L).

The composition of the main culture medium is as follows:

Cerelease	10 g
Dextrine	10 g
Starch (soluble)	10 g
Yeast extract (Gistex)	2.5 g
Soyabean flour (Nurupan, Edelseya)	12.5 g
K ₂ HPO ₄	0.25 g
KH ₂ PO ₄	0.12 g
MgSO ₄ ·7H ₂ O	0.10 g
CaCl ₂ ·6H ₂ O	0.05 g
Trace element solution AC-1	1 ml
Demineralised water up to 1 Liter.	

pH: adjusted at 7.2–7.5 (KOH/H₂SO₄)
Sterilisation: 120° C./20 min.

Trace element solution AC-1: This solution has the same composition as the solution Nr 235 with the addition of: (NH₄)₆Mo₇O₂₄ 0.2 g.

5. Isolation: The mycelium is separated from the culture medium and the resulting culture filtrate (13 L) is extracted three times with 1,2-dichloroethane using 1.5 L at each extraction. The combined 1,2-dichloroethane solutions are evaporated under vacuum at a temperature of 40° C. The crude residue is subjected to Sephadex LH-20 gel filtration using methanol as eluent. Those fractions containing the cyclosporin compounds (525 mg) are pooled and chromatographed on silica gel (50 g, granulate size 0.04–0.063 mm, 'Merck') using chloroform/methanol as eluent. Repeated chromatography using the same system yields pure [γ-hydroxy-MeLeu]⁴-Ciclosporin as an amorphous white powder. m.p. 150°–153°, [α]_D²⁰ –225° (c=0.53 in CHCl₃), –171° (c=0.44 in CH₃OH).

EXAMPLE 3

[γ-hydroxy-MeLeu]⁴-[γ-hydroxy-MeLeu]⁶-Ciclosporin (compound g)

The more polar side fractions originating from the purification of [γ-hydroxy-MeLeu]⁴-Ciclosporin are further separated by repeated silica gel column chromatography (granulate size 0.04–0.063 mm) using acetone/hexane 2:1 as eluent followed by chromatography on silica gel using methyl t-butyl ether/methanol/water 90:9:1 as eluent. The first fractions contain [γ-hydroxy-Leu]⁴-Ciclosporin which is further purified by decolorizing with charcoal yielding the pure compound as an amorphous white powder. m.p. 162°–164°, [α]_D²⁰ –211° (c=0.50 in CHCl₃), –157° (c=0.52 in CH₃OH).

The later fractions from the above silica gel column chromatography contain [γ-hydroxy-MeLeu]⁴-[γ-hydroxy-MeLeu]⁶-Ciclosporin and are further purified by decolorizing with charcoal yielding the pure title compound as an amorphous white powder. m.p. 157°–160°, [α]_D²⁰ –217° (c=0.54 in CHCl₃), –176° (c=0.42 in CH₃OH).

EXAMPLE 4

[Nva]²-[γ-hydroxy-MeLeu]⁴-Ciclosporin (compound f)

This compound is prepared by a procedure analogous to the preparation of compound e ([γ-hydroxy-MeLeu]⁴-Ciclosporin), but using cyclosporin G ([Nva]²-Ciclosporin) as starting material. After purification by repeated silica gel column chromatography using ethyl acetate saturated with water and acetone/hexane 2:1 as eluent, respectively, the title compound is obtained as an amorphous white powder. m.p. 138°–141°, [α]_D²⁰ –213° (c=0.69 in CHCl₃), –168° (c=0.70 in CH₃OH).

EXAMPLE 5

[MeVal]⁵-ciclosporin (compound h)

Cyclosporin A (0.60 g=0.5 mmol) dissolved in tetrahydrofuran (20 ml) is incubated with 0.63 ml of a 1.6M solution of butyllithium (1.0 mmol) in hexane. The resulting solution is reacted with dimethyl sulphate (0.1 ml; 1.5 mmol) at –78° C. The reaction mixture is slowly warmed to room temperature and stirred overnight.

Isolation by flash chromatography (SiO₂, 5% methanol/ether), followed by HPLC (reverse phase) gave the title product. The compound is characterised by a 300 MHz ¹H NMR spectrum in CDCl₃ which is shown in FIG. 3.

EXAMPLE 6

[MeOThr]²-[(D)MeAla]³-[MeVal]⁵-Ciclosporin (Compound i)

A mixture of 480 ml THF (absolute) and 6.96g (49.2 mMol) diisopropyl amine is cooled to –80° C., and 33.5 ml

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of a 1.33M solution of butyllithium in hexane (=44.5 mMol) is added slowly through a syringe. The mixture is stirred for 30 min. at -80°C ., then a solution of 8 g (6.6 mMol) cyclosporin C ([Thr]²-Ciclosporin) in 120 ml absolute THF is added through a syringe over 2–3 minutes. The clear solution is stirred for a further hour at -80°C ., then 2.06 ml methyl iodide is slowly added.

The mixture is allowed to warm to room temperature over 2 hr. then 40 ml water is added and the solvents are evaporated on a rotary evaporator at $30^{\circ}\text{C}/15\text{ mm Hg}$. The residue is partitioned between water and ether, and the ether layer is washed four times with semi-saturated brine, dried over MgSO_4 and evaporated, to give a residue of 8.1 g.

The residue is chromatographed on 1200 g Kieselgel with water-saturated ethyl acetate, to give a crude product which after a further chromatography on 200 g Kieselgel with 5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$ gives the pure title product, $[\alpha]_D^{20} \approx -195^{\circ}$ ($C=1.0$ in CHCl_3).

EXAMPLE 7

[dihydro-MeBmt]¹-[γ -hydroxy-MeLeu]⁴-Ciclosporin (compound a)

To a suspension of 200 mg pre-hydrogenated 10% palladium/charcoal in 4 ml ethanol is added 1.2 g of [γ -hydroxy-MeLeu]⁴-Ciclosporin (compound e) in 10 ml ethanol and hydrogenation is carried out at room temperature until no more hydrogen is taken up. After removal of the catalyst by filtration the solution is evaporated, yielding the title compound as an amorphous white powder, m.p. $154^{\circ}\text{--}156^{\circ}$, $[\alpha]_D^{20} -225^{\circ}$ ($C=0.87$ in CHCl_3), -169° ($C=0.70$ in CH_3OH).

EXAMPLE 8

[8'-hydroxy-MeBmt]¹-Ciclosporin (compound j)

1. [0-acetyl- ω -bromo-MeBmt]¹-Ciclosporin: A mixture of 25.0 g (20 mmol) of [0-acetyl-MeBt]¹-Ciclosporin (Traber et al. *Helv.Chim. Acta* 1982, 65, 1653), 4.4 g (25 mmol) of N-bromo succinimide and 400 mg of azobisisobutyronitrile in 250 ml carbon tetrachloride is heated to reflux for 2.5 hrs. The solvent is evaporated and replaced by ether, filtered from solids, washed with water, dried over magnesium sulfate and evaporated to dryness. The residue is chromatographed over silica gel with ethyl ether/ethyl acetate (4:1) to give 10.7 g (40%) of amorphous product which is crystallized from ether/hexane to yield 8.4 g of pure substance; m.p. $207^{\circ}\text{--}209^{\circ}\text{C}$. Later fractions of the chromatogram contained an additional 11.2 g of product of slightly lesser quality.

2. [0-acetyl- ω -acetoxy-MeBmt]¹-Ciclosporin: A mixture of 4.31 g (3.3 mmol) of the product of step 1 (contaminated with an estimated 15–20% of starting material) and 2.1 g (8 mmol) of tetraethylammonium acetate tetrahydrate in 30 ml of methyl ethyl ketone, containing a catalytic amount of sodium iodide, is heated in an oil bath at 105°C . for 3 hrs. and kept at room temperature over the weekend. The solvent is diluted with methyl t-butyl ether and washed with water and brine. The organic layer is dried over MgSO_4 and evaporated to leave 4.0 g of crude product which is purified on a RP-18 reverse phase column (240 g) to yield 3.07 g of the title product; m.p. $191^{\circ}\text{--}192^{\circ}\text{C}$.

3. [ω -hydroxy-MeBmt]¹-Ciclosporin: A solution of 1.72 g (1.3 mmol) of the product of step 2 in 75 ml of methanol and a solution of 1.2 g of sodium in 50 ml of methanol are mixed and kept at r.t. for 2.5 hours. Then the solution is acidified with acetic acid. The solvent is evaporated under reduced pressure and the residue is dissolved in methyl t-butyl ether, washed sequentially with water, brine, and sodium bicar-

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bonate solution, dried over MgSO_4 and evaporated. The crude product (1.6 g) is eluted from a RP-18 column (240 g) with methanol/water 75:15 to give 1.5 g of pure product. A sample is crystallized from ether/hexane to give a crystalline product of m.p. $181^{\circ}\text{--}183^{\circ}\text{C}$.

EXAMPLE 9

[MeThr]⁴-Ciclosporin (compound d)

The total synthesis of Ciclosporin as described in U.S. Pat. Nos. 4,396,542 and 4,798,823 is carried out using MeThr in the 4-position in place of MeLeu. The product has $[\alpha]_D^{20} = -249.6^{\circ}$ ($C=1.0$ in CHCl_3).

EXAMPLE 10

[MeVal]⁴-Ciclosporin (compound b)

The total synthesis of Ciclosporin as described in U.S. Pat. Nos. 4,396,542 and 4,798,823 is carried out using MeVal in the 4-position in place of MeLeu. The product has $[\alpha]_D^{20} = -226^{\circ}$ ($C=0.358$ in CHCl_3).

EXAMPLE 11

Immunosuppression and cyclophilin-binding of Active Compounds relative to Ciclosporin

Table I gives examples of (1) the cyclophilin Binding Ratio (BR) of Active Compounds as measured in an ELISA assay and (2) the immunosuppressive activity of Active Compounds relative to Ciclosporin as measured in an MLR assay and expressed as a percentage of activity relative to Ciclosporin (Immunosuppressive Ratio or IR).

Further explanation of the significance of these values and the methods for conducting these tests are set forth supra.

TABLE I

Compound	BR(log ₁₀)	IR (%)
a) [dihydro-MeBmt] ¹ -[γ -hydroxy-MeLeu] ⁴ -Ciclosporin	0.1	<1
b) [MeVal] ⁴ -Ciclosporin	0.1	<1
c) [MeIle] ⁴ -Ciclosporin	-0.2	<1
e) [γ -hydroxy-MeLeu] ⁴ -Ciclosporin	-0.3	<1
f) [Nva] ² -[γ -hydroxy-MeLeu] ⁴ -Ciclosporin	0.4	<1
h) [MeVal] ⁵ -Ciclosporin	0.4	5.3
j) [8'-hydroxy-MeBmt] ¹ -Ciclosporin	0.35	1.8
k) [MeAla] ⁶ -Ciclosporin	-0.4	3.2
l) [γ -hydroxy-MeLeu] ⁹ -Ciclosporin	0.15	2.9

EXAMPLE 12

Anti-HIV activity and cytotoxicity of Active Compounds

Examples of the activity of Active Compounds and Ciclosporin as inhibitors of HIV-1 replication in MT4-cells and the cytotoxicity of Active Compounds and Ciclosporin in MT4-cells are set forth in Table II. The significance of these numbers and the appropriate methods are discussed supra.

TABLE II

Compound	Cytotoxicity ($\mu\text{g/ml}$)	Anti-HIV (IC ₅₀)
a) [dihydro-MeBmt] ¹ -[γ -hydroxy-MeLeu] ⁴ -Ciclosporin	16.3	0.12
b) [MeVal] ⁴ -Ciclosporin	5.4	0.064
c) [MeIle] ⁴ -Ciclosporin	4.5	0.056
e) [γ -hydroxy-MeLeu] ⁴ -Ciclosporin	15.5	0.34
f) [Nva] ² -[γ -hydroxy-MeLeu] ⁴ -Ciclosporin	14.0	0.85

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TABLE II-continued

Compound	Cytotoxicity ($\mu\text{g/ml}$)	Anti-HIV (IC_{50})
h) [MeVal] ² -Ciclosporin	4.4	0.45
i) [MeOThr] ² -(D)MeAla ³ -[MeVal] ² -Ciclosporin	>10	0.45
j) [8'-hydroxy-MeBmt] ¹ -Ciclosporin	10.6	0.56
k) [MeAla] ⁶ -Ciclosporin	5.2	1.34
l) [γ -hydroxy-MeLeu] ⁹ -Ciclosporin	>10	0.31
Ciclosporin	8.45	0.53

The Active Compounds are indicated both for the prevention of AIDS in asymptomatic HIV-positive patients and in the treatment of patients suffering from AIDS. In the patients in which AIDS has appeared the administration of the Active Compounds reverse the T4-cell depletion associated with AIDS, induce regression of AIDS-related disorders, such as Kaposi's sarcoma, and reduce the likelihood of new opportunistic infections.

Thus the invention provides a process for the treatment and the prevention of the acquired immunodeficiency syndrome and other disorders induced by the HIV-1 virus in a patient infected with said virus, comprising administering to said patient an effective amount of an Active Compound of the invention.

The Active Compound may be administered by any conventional route, in particular enterally, e.g. orally, for example in the form of solutions for drinking, tablets or capsules or parenterally, for example in the form of injectible solutions or suspensions. By the intravenous route an indicated daily dosage may be from 1 to 20 mg/kg, preferably from 3 to 10 mg/kg, and by the oral route from 1 to 50 mg/kg, preferably from 7 to 20 mg/kg.

The toxicity of the Active Compounds is believed to be similar to that of Ciclosporin. As the Active Compounds are not immunosuppressive, certain side effects of Ciclosporin related to immunosuppression are avoided. Other side effects associated with Ciclosporin, however, particularly nephrotoxicity in long term use, may also be associated with the Active Compounds.

Preferred galenic formulations for the Active Compounds include those based on microemulsions as described in British Patent Application 2 222 770A, which include topi-

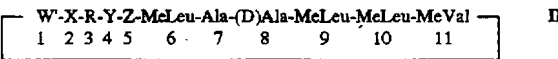
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Tetraglycol	20.0 mg
Captex 800	20.0 mg
Nikkol HCO-40	860.0 mg
Butylhydroxytoluene(BHT)	1.0 mg
Formulation Example C:	
Product of Ex. 1	25.0 mg
Glycofurol 75	100.0 mg
Miglyol 812	35.0 mg
Cremophor RH 40	90.0 mg
Butylhydroxyanisole(BHA)	0.2 mg
Formulation Example D:	
Product of Ex. 1	10.0 mg
Tetraglycol	10.0 mg
Myritol	5.0 mg
Cremophor RH 40	75.0 mg
alpha-Tocopherol	0.1 mg

The individual components of these formulations, as well as the methods for their preparation, are fully described in British Patent Application 2 222 770, the contents of which are incorporated herein by reference.

We claim:

1. A non-immunosuppressive, cyclophilin-binding compound of Formula II

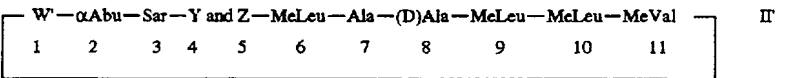


where

- W' is MeBmt or dihydro-MeBmt;
X is α Abu, Val, Thr, Nva or MeOThr;
R is Sar or (D)-MeAla;
Y is MeLeu, γ -hydroxy-MeLeu, MeIle, MeVal, MeThr, MeAla, MeIle or MeaThr; and
Z is Val, Leu, MeVal or MeLeu;

provided that 1) when Y is MeLeu or MeAla then Z is MeVal or MeLeu; and 2) when W' is MeBmt, R is Sar, and Y is γ -hydroxy-MeLeu, then Z is other than Val.

2. A compound according to claim 1 of Formula II



cal as well as oral forms; also oral and injectable forms obtained from solid solutions comprising a fatty acid saccharide monoester, e.g. saccharose monolaurate, as described in British Patent Application 2 209 671A. Suitable unit dosage forms for oral administration comprise e.g. from 25 to 200 mg Active Compound per dosage.

Formulation Example A:

Product of Ex. 1	50.0 mg
Glycofurol 75	180.0 mg
Miglyol 812	90.0 mg
Cremophor RH40	180.0 mg
alpha-Tocopherol	0.5 mg

Formulation Example B:

Product of Ex. 1	100.0 mg
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in which

- W' is MeBmt or dihydro-MeBmt;
Y is γ -hydroxy-MeLeu, MeVal, MeThr, or MeIle; and
Z is Val or MeVal;

provided that when W' is MeBmt, Y' is other than γ -hydroxy-MeLeu.

3. A non-immunosuppressive, cyclophilin-binding compound selected from the group consisting of:

- [dihydro-MeBmt]¹-[γ -hydroxy-MeLeu]⁴-Ciclosporin,
[MeVal]⁴-Ciclosporin,
[Mele]⁴-Ciclosporin,
[MeThr]⁴-Ciclosporin,
[Nva]²-[γ -hydroxy-MeLeu]⁴-Ciclosporin,
[γ -hydroxy-MeLeu]⁴-[γ -hydroxy-MeLeu]⁶-Ciclosporin,
[MeVal]⁵-Ciclosporin, and
[MeOThr]²-(D)MeAla³-[MeVal]⁵-Ciclosporin.

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5,767,069

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4. A pharmaceutical composition comprising a compound as described in claims 1, 2 or 3 together with a pharmaceutically acceptable carrier or diluent, therefor.

5. The compound of claim 3 which is [MeIle]⁴-cyclosporin.

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6. The pharmaceutical composition according to claim 4 comprising [MeIle]⁴-cyclosporin and a pharmaceutically acceptable carrier therefor.

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