



Industria y Comercio

S U P E R I N T E N D E N C I A

Delegatura de propiedad Industrial

División de Nuevas Creaciones

PCT

SOLICITUD

PATENTE DE INVENCION

21. EXPEDIENTE No. 05-101444

54. TÍTULO NUEVO PROCESO PARA LA PREPARACION DE
ANALOGOS DE CECOSPORIN NA A.

51. CLASIFICACIÓN INTERNACIONAL A61K38/13 // A61P37/02

71. SOLICITANTE ISO TECHNIKA INC.

DOMICILIO ALBERTA, CANADA.

74. APODERADO JASME EDUARDO DELEADO VILLEGAS..

22. BOGOTÁ, D. C., 06/10/2003.

SUPERINDUSTRIA Y COMERCIO
Radicación: 05101444 00000000 Folios: 246
Fecha (MM): 2005-10-06 12:15:09
Trámite: 002 PATENTES D 1 REGISTRO/ 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

FORMULARIO ÚNICO DE SOLICITUD DE
PCT

ENTRADA EN FASE NACIONAL

SUPERINDUSTRIA Y COMERCIO (COPIA)
Radicación: 05101444 00000000 Folios: 246
Fecha (MM): 2005-10-06 12:15:09
Trámite: 011 PCT-PATENT 378 FASE NACI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

1 SOLICITUD DE: ☒ Patente de Invención ☐ Patente de Modelo de Utilidad

☒ Capítulo I ☐ Capítulo II

2 SOLICITANTE (71)
Nombre: ISOTECHNIKA INC.
Dirección: 2100 College Plaza, 8215 112th Street, Edmond, Alberta T8G 2C8, Canadá
Nacionalidad o domicilio: Canadiense
Lugar de Constitución:
Teléfono: Fax:
E-mail:
IDENTIFICACIÓN
C.C. ☐ NIT ☐
C.E. ☐ Otro ☐
Cual: _____
Número: _____

3 REPRESENTANTE O APODERADO
Nombre: JAIME EDUARDO DELGADO VILLEGAS
Dirección: Avenida 22 No. 37 - 31 OF. 202 BOGOTA, COLOMBIA
Teléfono: 2444098 Fax: 2442498
E-mail: mde@mdelaw.com
IDENTIFICACIÓN
C.C. ☒ NIT ☐
C.E. ☐ Otro ☐
Cual: _____
Número: 18.270.955 T.P. 43.308 C.S.J.
Bogotá

4 INVENTOR (ES) (72)
Nombre: Jean-Michel Adam; Mark Abel y Seetharaman Jayaraman
Dirección: Ver Hoja Anexa
Nacionalidad o Domicilio: Ver Hoja Anexa

5 PCT '(86)
Solicitud Internacional No. PCT / EP2004/003804 Fecha 02 / 04 / 2004
Publicación Internacional No. WO 2004 / 089989 A2 Fecha 21 / 10 / 2004

6 Título (84) NUEVO PROCESO PARA LA PREPARACION DE ANÁLOGOS DE CICLOSPORINA A

7 Clasificación Internacional (81) C07K 5/02 A61K38/13. // A61P37/02

8 Prioridad	(33) País de Origen	(32) Fecha	(31) Número de Solicitud
SI ☒ NO ☐	EP	08/04/2003	3007921,4

9 Comprobante de pago No. Fecha:

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

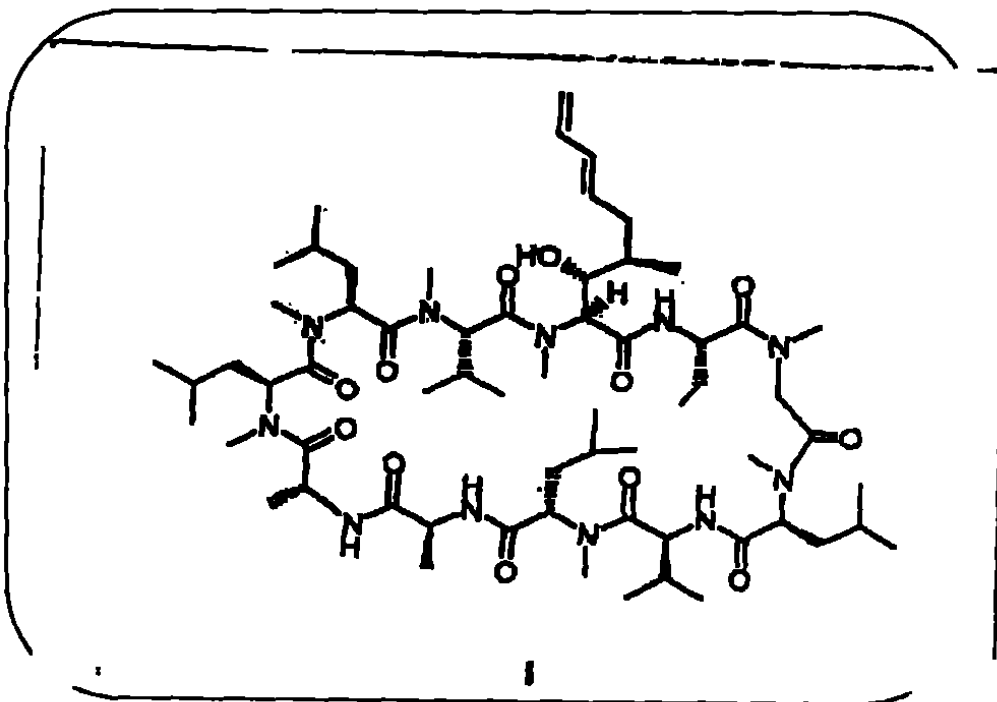
10

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud.
- ☐ Documento que acredite 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
- ☒ Copia de la solicitud internacional (Resumen, descripción, reivindicaciones, dibujos)
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicaciones, dibujos)
- ☐ Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA) y solicitud examen preliminar
- ☐ Traducción 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
- ☒ Arte final 12X12 cm.
- ☐ De ser el caso, información de otras solicitudes de patentes 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
- ☒ Otros: ver hoja anexa

11

FIGURA CARACTERÍSTICA



12

Solicito la concesión de la patente,

NOMBRE: JAIME EDUARDO DELGADO VILLEGAS

FIRMA:

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

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

Expediente No		0510444	No de Folio
Item		No de unidades	✓
1	CD		
2	DVD		
3	REVISTA		
4	LIBRO		
5	PERIODICO		
6	DISQUETES		
7	SOBRE DE MANILA		
8	SOBRE BLANCO TAMAÑO CARTA	✓	
9	SOBRE BLANCO TAMAÑO OFICIO		
10	OTROS:		

Observaciones:

contiene 1 figura

PCT/EP2004/003504

PUBLICACION No. WO 2004 / 089960 A2 Fecha 21 / 10 / 2004

**TITULO DE LA INVENCION: NUEVO PROCESO PARA LA PREPARACION
DE ANÁLOGOS DE CICLOSPORINA A**

SOLICITANTE:

NOMBRE:	ISOTECHNIKA INC.
DOMICILIO:	2100 College Plaza 8215 – 112th Street Edmonton, Alberta T6G 2C8 Canadá

INVENTORES:

NOMBRE:	Jean – Michel Adam
DOMICILIO:	Im Reinacherhof 187 CH-4153 Reinach Suiza
NACIONALIDAD:	Suizo

NOMBRE:	Mark Abel
DOMICILIO:	11530 – 9 A Avenue Edmonton, Alberta T6J 7B2 Canadá
NACIONALIDAD:	Canadiense

NOMBRE:	Seetharaman Jayaraman
DOMICILIO:	11812 – 13 Avenue Edmonton, Alberta T6J 7E9 Canadá
NACIONALIDAD:	Canadiense

A
5

PCT/EP2004/003504, Documentos adjuntos al Petitorio:

1. **Solicitud PCT/EP2004/003504 en inglés, tal como fue presentada el 2 de abril de 2004 con petitorio, descripción, reivindicaciones y resumen, con notificación del número de la solicitud internacional y fecha de la solicitud internacional de la Oficina Europea de Patentes del 11 de mayo de 2004. (66 folios).**
2. **Carta Oficina Europea de Patentes sobre recibo del poder de las empresas F. Hoffmann-La Roche AG y Isotechnika Inc. al apoderado, de fecha 15 de marzo de 2004. (2 folios)**
3. **Traducción de la solicitud PCT/EP2004/003504 presentada el 2 de abril de 2004 con descripción, reivindicaciones y resumen. (77 folios)**
4. **Formulario PCT/IB/301, notificación del recibo de copia registrada de la solicitud PCT con lista de países designados con fecha de envío PCT del 8 de junio de 2004 (1 folio).**
5. **Formulario PCT/IB/304, notificación del recibo del documento de prioridad No. EP 03007921.4 del 8 de abril de 2003, con fecha de envío del PCT del 10 de junio de 2004. (1 folio).**
6. **Formulario PCT/ISA/202, notificación del recibo de la copia de la búsqueda internacional fechado el 11 de mayo de 2004, enviado por la Oficina Europea de Patentes del 17 de junio de 2004 (1 folio).**
7. **Formulario PCT/RO/132, comunicación de la Oficina Europea de Patentes, para el caso en los cuales ningún otro formato es aplicable, de fecha 29 de junio de 2004 (2 folios)**
8. **Respuesta de la empresa F. Hoffmann – La Roche Ltd. a la comunicación para casos en los cuales ningún formato es aplicable, junto con formulario PCT/RO/101, del 8 de julio de 2004. (3 folios)**
9. **Solicitud de Cambio en el nombre y dirección del agente bajo el artículo 92 bis, del 2 de agosto de 2004 a la OMPI (2 folios).**
10. **Solicitud de cambio de la persona del solicitante bajo el artículo 92 bis, enviado el 2 de agosto de 2004 a la OMPI. (4 folios)**
11. **Formulario PCT/IB/345 comunicación para casos en los cuales ningún otro formato es aplicable, enviado por la Oficina de la OMPI el 17 de agosto de 2004 (1 folio).**

12. **Formulario PCT/ISA/206 sobre la invitación a pagar tasas adicionales junto con el resultado parcial de la búsqueda de antecedentes y opinión escrita, enviado por la Oficina Europea de Patentes del 19 de agosto de 2004. (7 folios)**
13. **Respuesta de la empresa ISOTECHNIKA INC., a la invitación a pagar tasas adicionales, con fecha 27 de agosto de 2004. (2 folios)**
14. **Respuesta de la empresa ISOTECHNIKA INC., a la comunicación para casos en los cuales ningún otro formato es aplicable, con fecha 1 de septiembre de 2004 (3 folios)**
15. **Formulario PCT/IB/306 Notificación de Registro de un Cambio de nombre de apoderado, enviado por la Oficina de la OMPI, del 22 de septiembre de 2004. (1 folio).**
16. **Formulario PCT/ISA/220 y 210, notificación envío del Reporte de la Búsqueda Internacional y Opinión Escrita del 15 de octubre de 2004 de la Oficina Europea de Patentes (25 folios)**
17. **Formulario PCT/IB/311, notificación y copia de la Publicación Internacional en la Gaceta Oficial del PCT No. WO 2004/089960 A2 del 21 de Octubre de 2004. (32 folios).**
18. **Formulario PCT/IB/308 Primera notificación informando comunicación de la Oficina del PCT sobre la solicitud internacional a los países designados, lo cual no aplica 30 meses tiempo límite bajo el Artículo 22(1), de fecha 11 de Noviembre de 2004. (3 folios).**
19. **Formulario PCT/IB/311, notificación y copia de la Solicitud Internacional tal como fue publicada por segunda vez en la Gaceta Oficial del PCT No. WO 2004/0089960 A3 del 6 de Enero de 2005. (4 folios).**
20. **Formulario PCT/IB/308 Segunda y notificación suplementaria informando al solicitante la comunicación de la Oficina del PCT sobre la solicitud internacional a los países designados, que aplican el período límite de 30 meses bajo el Artículo 22(1), del 11 de agosto de 2005. (1 folio).**
21. **Tarjeta Archivo Temático y Tarjeta Propietarios.**

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) Case 21711

Box No. I		TITLE OF INVENTION Process for preparation of cyclosporin A analog	
Box No. II		APPLICANT ☐ This person is also inventor	
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. The country of the address indicated in this Box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)		Telephone No. +41 61 688 11 11	
F. Hoffmann-La Roche AG Grenzacherstrasse 124 CH-4070 Basel, Switzerland		Facsimile No. +41 61 688 13 95	
		Teleprinter No.	
		Applicant's registration No. with the Office	
State (that is, country) of nationality: CH		State (that is, country) of residence: CH	
This person is applicant for the purposes of:		☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)			
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. The country of the address indicated in this Box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)		This person is:	
Isoteknika Inc. 2100 College Plaza 8215-112th Street Edmonton, Alberta T6G 2C8, Canada		☒ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)	
		Applicant's registration No. with the Office	
State (that is, country) of nationality: CA		State (that is, country) of residence: CA	
This person is applicant for the purposes of:		☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.			
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE			
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:		☒ 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.)		Telephone No. +41 61 688 20 74	
KJELLSAA-BERGER, Hanny Grenzacherstrasse 124 CH-4070 Basel, Switzerland		Facsimile No. +41 61 688 13 95	
		Teleprinter No.	
		Agent's registration No. with the Office	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.			

67

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
<i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)</i> ADAM, Jean-Michel Im Reinacherhof 187 CH-4153 Reinach, Switzerland	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State <i>(that is, country)</i> of nationality:	State <i>(that is, country)</i> of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)</i> ABEL, Mark 11530-9A Avenue Edmonton, Alberta T6J 7B2, Canada	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State <i>(that is, country)</i> of nationality:	State <i>(that is, country)</i> of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)</i> JAYARAMAN, Seetharaman 11812-13 Avenue Edmonton, Alberta T6J 7E9, Canada	This person is: ☐ applicant only ☐ applicant and inventor ☒ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State <i>(that is, country)</i> of nationality:	State <i>(that is, country)</i> of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)</i>	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State <i>(that is, country)</i> of nationality:	State <i>(that is, country)</i> of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

Form PCT/RO/101 (second sheet) (January 2004) See Notes to the request form

Box No. IX CHECK LIST; LANGUAGE F FILING																																																								
This international application contains: (a) In paper form, the following number of sheets: <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 80%;">request (including declaration sheets)</td> <td style="width: 20%; text-align: right;">4</td> </tr> <tr> <td>description (excluding sequence listing and/or tables related thereto)</td> <td style="text-align: right;">50</td> </tr> <tr> <td>claims</td> <td style="text-align: right;">9</td> </tr> <tr> <td>abstract</td> <td style="text-align: right;">1</td> </tr> <tr> <td>drawings</td> <td style="text-align: right;">64</td> </tr> <tr> <td colspan="2">Sub-total number of sheets</td> </tr> <tr> <td>sequence listing</td> <td style="text-align: right;">64</td> </tr> <tr> <td>tables related thereto</td> <td style="text-align: right;">64</td> </tr> <tr> <td colspan="2"><i>(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)</i></td> </tr> <tr> <td>Total number of sheets</td> <td style="text-align: right;">64</td> </tr> </table> (b) ☐ only in computer readable form (Section 801(a)(i)) (i) ☐ sequence listing (ii) ☐ tables related thereto (c) ☐ also in computer readable form (Section 801(a)(ii)) (i) ☐ sequence listing (ii) ☐ tables related thereto Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the ☐ sequence listing: ☐ tables related thereto: <i>(additional copies to be indicated under items 9(i) and/or 10(ii), in right column)</i>	request (including declaration sheets)	4	description (excluding sequence listing and/or tables related thereto)	50	claims	9	abstract	1	drawings	64	Sub-total number of sheets		sequence listing	64	tables related thereto	64	<i>(for both, actual number of sheets if filed in paper form, whether or not also filed in computer readable form; see (c) below)</i>		Total number of sheets	64	This international application is accompanied by the following item(s) (mark the applicable check-boxes below and indicate in right column the number of each item): <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 80%;">1. ☒ fee calculation sheet</td> <td style="width: 20%; text-align: right;">1</td> </tr> <tr> <td>2. ☐ original separate power of attorney</td> <td style="text-align: right;">:</td> </tr> <tr> <td>3. ☐ original general power of attorney</td> <td style="text-align: right;">:</td> </tr> <tr> <td>4. ☐ copy of general power of attorney; reference number, if any:</td> <td style="text-align: right;">:</td> </tr> <tr> <td>5. ☐ statement explaining lack of signature</td> <td style="text-align: right;">:</td> </tr> <tr> <td>6. ☐ priority document(s) identified in Box No. VI as item(s):</td> <td style="text-align: right;">:</td> </tr> <tr> <td>7. ☐ translation of international application into (language):</td> <td style="text-align: right;">:</td> </tr> <tr> <td>8. ☐ separate indications concerning deposited microorganism or other biological material</td> <td style="text-align: right;">:</td> </tr> <tr> <td>9. ☐ sequence listing in computer readable form (Indicate type and number of carriers)</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="margin-left: 20px;">(i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="margin-left: 20px;">(ii) ☐ (only where check-box (b)(i) or (c)(i) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="margin-left: 20px;">(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column</td> <td style="text-align: right;">:</td> </tr> <tr> <td>10. ☐ tables in computer readable form related to sequence listing (Indicate type and number of carriers)</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="margin-left: 20px;">(i) ☐ copy submitted for the purposes of international search under Section 802(b-quater) only (and not as part of the international application)</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="margin-left: 20px;">(ii) ☐ (only where check-box (b)(ii) or (c)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-quater)</td> <td style="text-align: right;">:</td> </tr> <tr> <td style="margin-left: 20px;">(iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column</td> <td style="text-align: right;">:</td> </tr> <tr> <td>11. ☐ other (specify):</td> <td style="text-align: right;">:</td> </tr> </table>	1. ☒ fee calculation sheet	1	2. ☐ original separate power of attorney	:	3. ☐ original general power of attorney	:	4. ☐ copy of general power of attorney; reference number, if any:	:	5. ☐ statement explaining lack of signature	:	6. ☐ priority document(s) identified in Box No. VI as item(s):	:	7. ☐ translation of international application into (language):	:	8. ☐ separate indications concerning deposited microorganism or other biological material	:	9. ☐ sequence listing in computer readable form (Indicate type and number of carriers)	:	(i) ☐ copy submitted for the purposes of international search under Rule 13ter only (and not as part of the international application)	:	(ii) ☐ (only where check-box (b)(i) or (c)(i) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Rule 13ter	:	(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column	:	10. ☐ tables in computer readable form related to sequence listing (Indicate type and number of carriers)	:	(i) ☐ copy submitted for the purposes of international search under Section 802(b-quater) only (and not as part of the international application)	:	(ii) ☐ (only where check-box (b)(ii) or (c)(ii) is marked in left column) additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-quater)	:	(iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column	:	11. ☐ other (specify):	:	Number of items
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Figure of the drawings which should accompany the abstract:	Language of filing of the international application: English																																																							
Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE <i>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).</i> Hanny Kjellaas-Berger																																																								

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1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
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FEE CALCULATION SHEET

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Case 21711

Applicant

F. Hoffmann-La Roche AG / Isotechnika Inc

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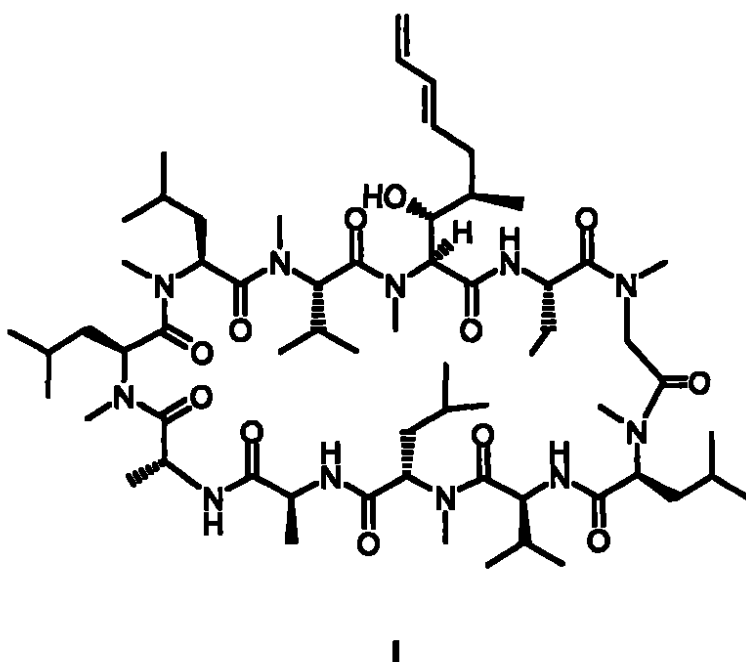
Date: March 29, 2004

Name: F. Hoffmann-La Roche AG

Signature:

Process for preparation of cyclosporin A analog

This invention relates to a new process for the preparation of cyclosporin A analog of formula I:



5

As well as intermediates for this process and processes for the preparation thereof.

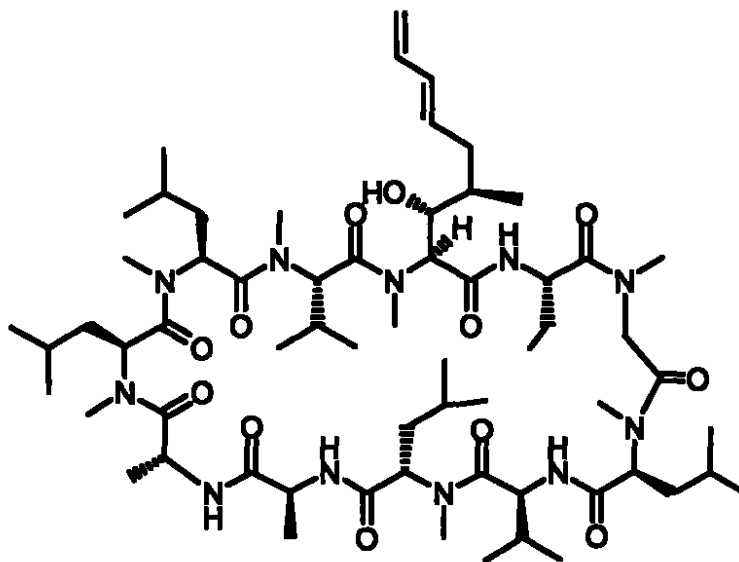
The cyclosporin A analog of formula I is structurally identical to cyclosporin A except for modification at the 1-amino acid residue. This analog is disclosed in
10 WO 99/18120 and U.S. Provisional Patent Application No. 60/346,201.
Hereinafter this analog is mentioned as (E)-ISA247.

Tetrahedron Letters, Vol.22, No.29, p2751-2752, 1981 discloses one of the intermediates of the process of this invention, namely pinacol (E)-1-trimethylsilyl-1-propene-3-boronate, and the allylation process using it.

15

Tetrahedron Letters, Vol.36, No.10, p1583, 1995 discloses allylation process using tartrate modified (E)- γ -(trimethylsilyl)allylboronate.

In a first aspect, this invention provides a process for the preparation of a cyclosporin A analog of formula I

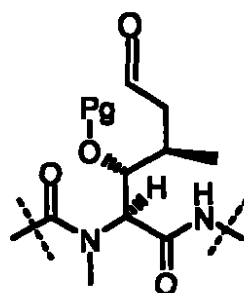


I

5

comprising

a-i) allylating a compound of formula II

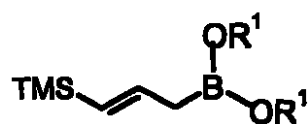


II

,wherein Pg is a protecting group and the dotted lines mean that the remainder of the compound has the same structure as that of the compound of formula I,

10

with a compound of formula III



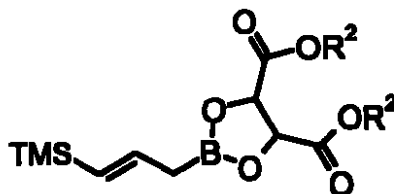
III

,wherein R¹ is hydrogen, C₁-₈ alkyl or C₃-₈ cycloalkyl and/or, when R¹ is hydrogen, a trimer thereof;

5

or

a-ii) allylating a compound of formula II with a compound of formula IV



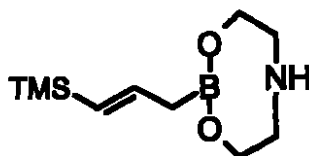
IV

10

wherein R² is C₁-₈ alkyl or C₃-₈ cycloalkyl;

or

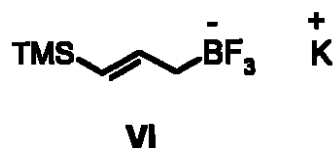
a-iii) allylating a compound of formula II with a compound of formula V



V

or

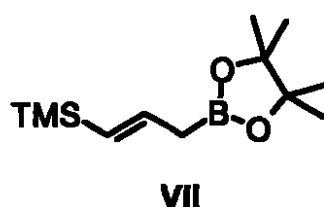
a-iv) allylating a compound of formula II with a compound of formula



VI

or

5 a-v) allylating a compound of formula II with a compound of formula VII



or

10 a-vi) allylating a compound of formula II with a reaction mixture obtained by a process comprising;

i) reacting allyltrimethylsilane with butyllithium to form trimethylsilylallyllithium;

ii) reacting trimethylsilylallyllithium with triisopropylborate or trimethylborate, and then conducting aqueous work up,

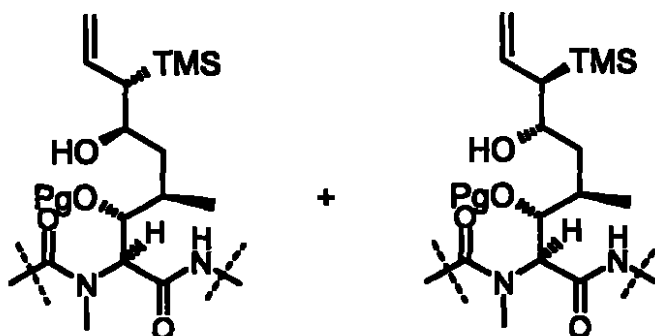
15 or

a-vii) allylating a compound of formula II with a reaction mixture obtained by reaction of the trimethylsilylallyllithium with diethylaluminum chloride,

or

a-viii) allylating a compound of formula II with a reaction mixture obtained by reaction of the trimethylsilylallyllithium with titanium tetraisopropoxide or titanium chlorotrisisopropoxide,

5 to form a compound of formula XI;



XI

wherein Pg is as defined above;

and

10 b) converting the compound of formula XI to the cyclosporin A analog of formula I.

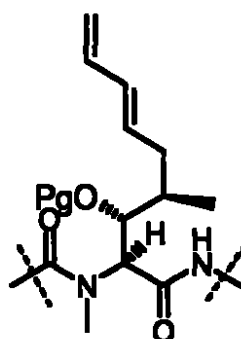
In a second aspect, this invention provides intermediates for the process mentioned above.

In a third aspect, this invention provides processes for the preparation of these intermediates.

15 Also, within the process as defined above [it will be referred to in the following under (i)], preferred are the following processes:

(ii) The process of (i), wherein step b) is conducted by

b-i) converting the compound of formula XI to a compound of formula XII



XII

wherein Pg is as defined in (i),

under acidic conditions; and

5 b-ii) converting the PgO group of the compound of formula XII to a hydroxyl group.

(iii) The process of (i) or (ii), wherein Pg is acetyl group.

(iv) The process of (iii), wherein step a-i), a-ii) or a-vi) is conducted in the presence of tartrates.

10 (v) The process of (iii), wherein step a-i), a-ii) or a-vi) is conducted in dichloromethane or toluene.

(vi) The process of (iii), wherein step a-iii) is conducted in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$, formic acid, acetic acid or tartrate esters.

(vii) The process of (vi), wherein step a-iii) is conducted in water/dichloromethane or water/toluene.

15 (viii) The process of (vi), wherein step a-iii) and b-i) are conducted in dichloromethane or tetrahydrofuran and in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

20 (ix) The process of (vi), wherein step a-iii) is conducted in acetic acid and/or formic acid; or in a mixture of acetic acid and /or formic acid and one or two cosolvents; selected from a group consisting of dichloromethane and tetrahydrofuran.

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- (x) The process of (ix), wherein step a-iii) is conducted in acetic acid and step b-i) is conducted by addition of formic acid to the reaction mixture.
- (xi) The process of (ix), wherein step a-iii) and b-i) are conducted in formic acid or acetic acid/ formic acid.
- 5 (xii) The process of (i) or (ii), wherein step a-iv) is conducted in water/dichloromethane or water/toluene.
- (xiii) The process of (iii), wherein step a-iv) and b-I) are conducted in dichlorometane, tetrahydrofuran or toluene in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- (xiv) The process of (iii), wherein step a-v) is conducted in the
10 presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- (xv) The process of (xiv), wherein step a-v) and b-i) are conducted in dichloromethane, tetrahydrofuran or toluene and in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- (xvi) The process of (iii), wherein step a-v) is conducted in the presence of formic acid or acetic acid.
- 15 (xvii) The process of (xvi), wherein step a-v) and b-i) are conducted in formic acid or acetic acid/formic acid.
- (xviii) The process of (xvii), wherein step a-v) and b-i) are conducted in a mixture of acetic acid/formic acid and co-solvent selected from dichloromethane, toluene, ethyl acetate and isopropyl acetate.
- 20 (xix) The process of (xviii), wherein co-solvent is isopropyl acetate.
- (xx) The process of (xvi), wherein step a-v) is conducted in acetic acid and step b-i) is conducted by addition of formic acid to the reaction mixture.
- (xxi) The process of (iii), wherein step a-vii) is conducted by
25 allylating the compound of formula II with a reaction mixture prepared by reaction of the trimethylsilylallyllithium with diethylaluminum chloride.
- (xxii) The process of (iii), wherein step a-viii) is conducted by allylating the compound of formula II with a reaction mixture prepared by reaction

of trimethylsilyllithium with titanium tetraisopropoxide or titanium chlorotrisisopropoxide.

The following terms used in the specification and claims have the meanings below:

5 "C_{a-b} alkyl" as used herein denotes straight chain or branched alkyl residues containing a to b carbon atoms. Therefore, for example, "C₁₋₈ alkyl" means straight chain or branched alkyl residues containing 1 to 8 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl or tert.-butyl.

10 "C₃₋₈ cycloalkyl" refers to a saturated monovalent cyclic hydrocarbon radical of three to eight ring carbons e.g., cyclopropyl, cyclobutyl, cyclohexyl.

"Protecting group" refers to a grouping of atoms that when attached to a reactive group in a molecule masks, reduces or prevents that reactivity. Examples of protecting groups can be found in T.W. Green and P.G. Futs, Protective Groups in Organic Chemistry, (Wiley, 2nd ed. 1991) and Harrison and Harrison et al., Compendium of Synthetic Organic Methods, Vols. 1-8 (John Wiley and Sons, 15 1971-1996).

In the structural formulae presented herein a broken bond (- - -) denotes that the substituent is below the plane of the paper and a wedged bond (->) denotes that the substituent is above the plane of the paper.

20 The following abbreviations used in the specification and claims, otherwise specified, have the following significances:

MTBE	methyl tert-butylether
THF	tetrahydrofuran
DCM	dichloromethane
25 DMSO	dimethylsulfoxide
HMPA	hexamethylphosphoramide
TMEDA	tetramethylethylenediamine
TMS	tetramethylsilane

	TMS-	trimethylsilyl
	Et	ethyl
	Me	methyl
	iPr	isopropyl
5	Bu	butyl
	Ac	acetyl
	RT	room temperature
	HPLC	high performance liquid chromatography
	MS	mass spectroscopy
10	TLC	thin layer chromatography
	NMR	nuclear magnetic resonance spectroscopy
	2D-COSY	2-dimensional correlated spectroscopy
	2D-TOCSY	2-dimensional total correlation spectroscopy
	HSQC	Heteronuclear Single Quantum Coherence
15	Cryst.	crystallization
	Cpd	compound
	min.	minute(s)
	h	hours

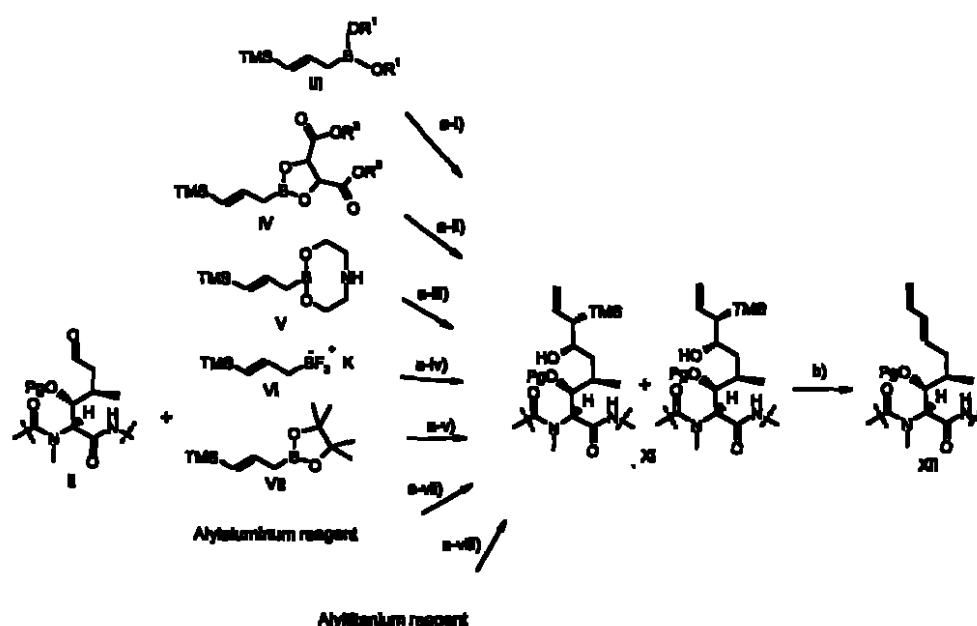
20 The starting materials and reagents used in the process of the present invention are either available from commercial suppliers such as Aldrich Chemical Co., (Milwaukee, Wisconsin, USA), Bachem (Torrance, California, USA), Emka-Chemie, or Sigma (St. Louis, Missouri, USA), Maybridge (Dist: Ryan Scientific, P.O. Box 6496, Columbia, SC 92960), Bionet Research Ltd., (Cornwall PL32 9QZ, UK), Menai Organics Ltd., (Gwynedd, N. Wales, UK), Butt Park Ltd., (Dist. 25 Interchim, Montlucon Cedex, France), Fluka (CH-9471 Buchs, CH), Acros

Organics (B-2440 Geel, BE) or are prepared by methods known to those skilled in the art following procedures set forth in references such as *Fieser and Fieser's Reagents for Organic Synthesis*, Volumes 1-17 (John Wiley and Sons, 1991), *Rodd's Chemistry of Carbon Compounds*, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989), *Organic Reactions*, Volumes 1-40 (John Wiley and Sons, 1991), *March's Advanced Organic Chemistry*, (John Wiley and Sons, 1992), and *Larock's Comprehensive Organic Transformations* (VCH Publishers Inc., 1989).

The starting materials and the intermediates of the reaction may be isolated and purified if desired using conventional techniques, including but not limited to filtration, distillation, crystallization, chromatography. Such materials may be characterized using conventional means, including physical constants and spectral data.

Compounds of formula I are prepared as illustrated in Scheme A. The allylmetal reagent also referred herein as allylating reagent is to be taken in a general sense and may comprise reagents where the metal part is based on boron although it is not per se a metal.

Scheme A:

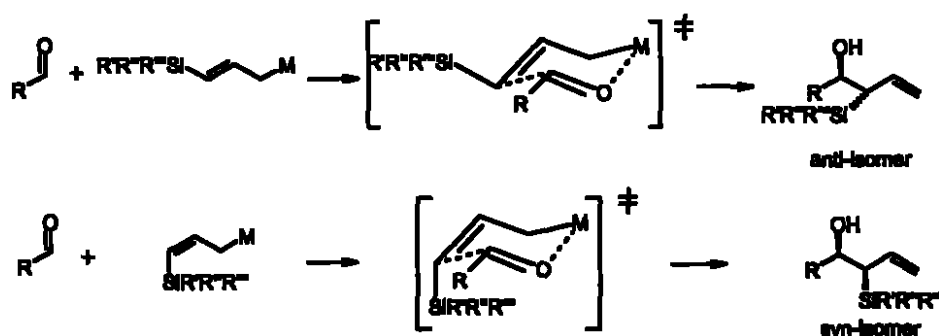


(Pg is a protecting group, the dotted lines mean that the remainder of the compound has the same structure as that of the compound of formula I, R^1 is hydrogen, C_{1-8} alkyl or C_{3-8} cycloalkyl and, when R^1 is hydrogen, a compound of formula III includes a trimer thereof, and R^2 is C_{1-8} alkyl or C_{3-8} cycloalkyl.)

In step a), protected cyclosporin A aldehyde of formula II is allylated by γ -silylated allylmetal reagent of formula III, IV, V, VI, VII, VIII etc. to form a mixture of β -silylhomomallylic alcohol diastereomers of formula XI (For a general discussion about allylmetals and allylation of aldehydes see : W. R. Roush in "Allyl Organometallics", Comprehensive Organic Synthesis, , Pergammon Press, Vol 2, pp 1-53 ; Y. Yamamoto, N. Asao in "Selective Reactions Using Allylic Metals", *Chemical Reviews* 1993, 93, p 2207-2293). The control of the relative anti or syn configuration of the β -silyl alcohol fragment will depend on the allylmetal reagent and conditions used to perform the aldehyde allylation step (For a general discussion of γ -silyl substituted allylmetal reagents see : T. H. Chan in "Silylallyl Anions in Organic Synthesis: A Study in Regio- and Stereoselectivity", *Chemical Reviews* 1995, 95, p1279-1292). This alcohol is often believed to form via a chair-like 6-membered ring transition state (also referred as Zimmerman-Traxler transition state) as shown in Scheme B.

2C
23

Scheme B:



In such a transition state, the aldehyde side chain preferably adopts a pseudo equatorial position in order to minimize 1,3-diaxial steric interactions. The relative configuration of the β -silyl alcohol fragment will therefore be determined by the configuration of C-C double bond of the allylmetal reagent.

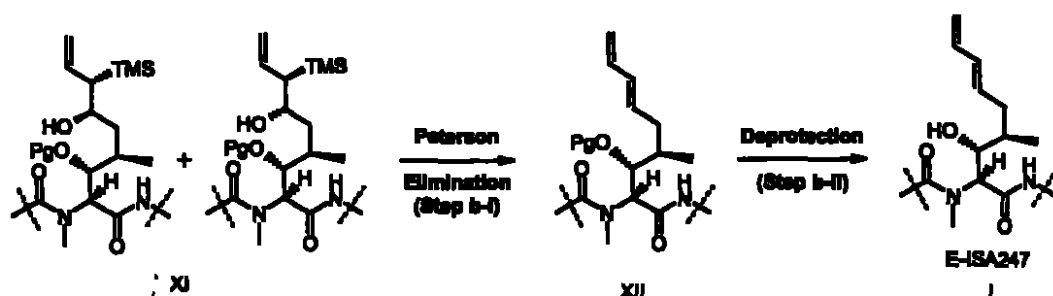
Therefore, use of trans- or cis- γ -silylated allylmetals reagents should lead predominantly to the anti- or syn- β -silyl alcohol isomer respectively. This holds in general, for example, for the allyl-boron, -titanium and -aluminum reagents.

Exception to this rule is found for example when a γ -silylated trialkylallylstannane reagent is added to aldehydes under Lewis acidic conditions, in that case the mechanism is different and the reaction provides mainly the syn β -silyl alcohol isomer.

In step b), the β -silyl alcohol of formula XI is converted to (E)-ISA247 of formula I.

Step b) can be carried out as illustrated in Scheme C.

Scheme C:



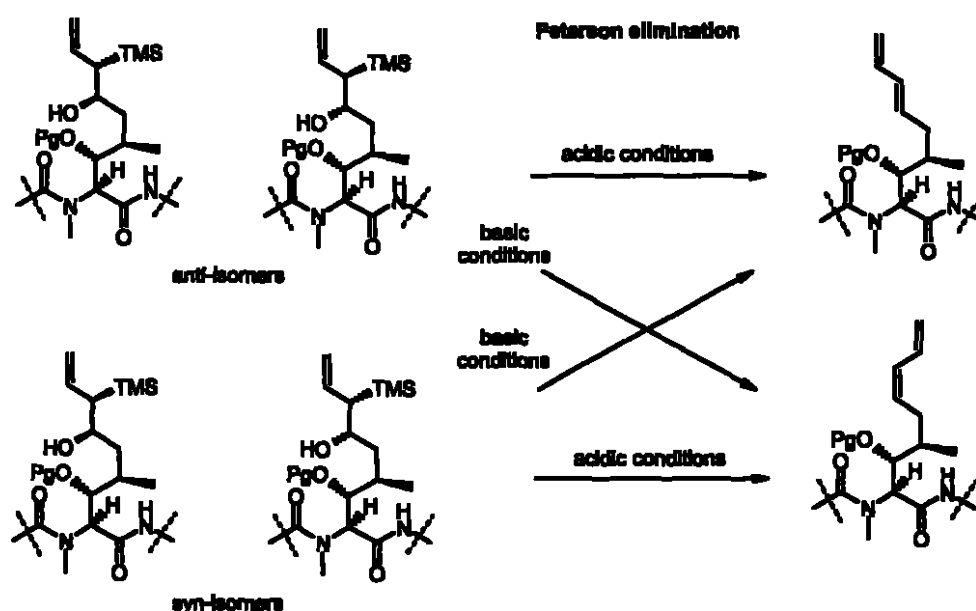
(Pg and the dotted lines have the meaning as defined above.)

In step b-i), the β -silyl alcohol of formula XI undergoes a Peterson elimination (For a general discussion about Peterson eliminations, see : D. J. Ager in "The Peterson Reaction", *Synthesis* 1984, p384-397 as well as references cited therein.) and the internal double bond is generated, i.e. the elimination of silanol from the β -silyl alcohol moiety occurs.

In view of achieving a high degree of double bond isomeric purity, the success of the allylation-Peterson elimination sequence relies on the selective introduction of a relative anti or syn configuration of the β -silyl alcohol moiety.

Indeed the Peterson elimination is known to be stereospecific. Anti isomers will provide one isomeric double bond when the syn isomers will produce the other double bond isomer under the same conditions as illustrated in Scheme D.

Scheme D:



(Pg and the dotted lines have the meaning as defined above.)

Anti isomers should give the trans double bond under acidic Peterson elimination conditions whereas syn isomers would provide the cis double bond.

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25

The reaction proceeds via a mechanism where the hydroxyl and the silyl groups are in an *anti conformation* prior to elimination.

5 The situation is opposite when the Peterson elimination is performed under basic conditions, in that case the reaction proceeds via a mechanism where the deprotonated hydroxyl and the silyl group are in a *syn conformation* prior to elimination.

Therefore, in principle, one could reach either double bond isomer by controlling the formation of either the *syn* or *anti* relative configuration of the β -silyl alcohol moiety or by using either acid or basic Peterson elimination conditions.

10 In the present invention, *trans*- γ -silylated allylmetals reagents are used for allylation of protected cyclosporin A aldehyde of formula II to form a mixture of *anti*- β -silyl alcohol diastereomers of formula XI. Therefore, a Peterson elimination is performed under acidic condition to form a *trans* double bond.

15 Typical acids for the acid-promoted reaction may include sulfuric acid, formic acid, chlorhydric acid, methanesulfonic acid tetrafluoroboric acid, perchloric acid, trifluoroacetic acid and various Lewis acids. Preferred acids are sulfuric acid, formic acid, methanesulfonic acid and $\text{BF}_3 \cdot \text{Et}_2\text{O}$, especially sulfuric acid, formic acid and $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

20 This step can be conducted at a reaction temperature from -70°C to 50°C . Preferred temperature range is 0°C to 50°C , more preferably 20°C to 40°C for formic acid. Preferred temperature range is 0°C to 40°C , more preferably 20°C to 30°C for sulfuric and methanesulfonic acid. Preferred temperature range is -80°C to 50°C , preferably -80°C to 25°C , especially -80°C to 0°C for $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

25 E-acetyl-ISA247 can be purified by crystallization in MTBE (for example via solvent exchange from dichloromethane to MTBE) or in MeOH/water mixtures.

In step b-ii), the protecting group is removed, returning the functional group on that carbon to an alcohol. The conditions and reagents to be employed depend on the protecting group used, which are known to those skilled in the art. Acyl group ($\text{R}'\text{C}(\text{O})-$; wherein R' is a linear saturated monovalent hydrocarbon radical of one to six carbon atoms or a branched saturated monovalent hydrocarbon radical of three to six carbon atoms), such as acetyl, propionyl, butyryl, isobutyryl, valeryl can preferably be used as a protecting group. When the protecting group is

an acetyl group, it can be removed, for example, by the treatment with K_2CO_3 in methanol and water. Under these conditions, the isomeric purity of the diene fragment is preserved. Therefore the double bond isomeric purity of E-ISA247 reflects the double bond isomeric purity of E-acetyl-ISA247. Bases other than
5 potassium carbonate that may be used to remove the protecting group include sodium hydroxide, sodium carbonate, sodium alkoxide and potassium alkoxide.

Synthesis of (E)-ISA247 by allylboron reagents

Allylation by γ -silylated allylboron reagent of formula III or IV (step a-i) and a-ii))

10 In general, excess of the reagent of formula III or IV is needed to complete the allylation of acetyl-cyclosporin A aldehyde (II') within an acceptable timeframe. Higher conversion and rates are achieved by using an activating agent such as a tartrate ester and/or dichloromethane as (co)-solvent. In accordance to the general behavior of boronic acids, the reagent of formula IIIa can potentially exist in the
15 form of cyclic trimer (boroxine) or oligomers (For an example of such behavior of a boronic acid, see: K. Ishihara, H. Kurihara, M. Matsumoto and H. Yamamoto in "Design of Bronsted Acid-Assisted Chiral Lewis Acid (BLA) Catalysts for Highly Enantioselective Diels-Alder Reactions", *Journal of the American Chemical Society* 1998, 120, p6920-6930.). When triisopropylborate is used for the preparation of
20 the solution of the crude reagent of formula IIIa, reagent of formula IIIa can also contain diisopropyl boronate ester ($TMS-CH=CH-CH_2-B(OiPr)_2$), from isopropanol generated from $B(OiPr)_3$ and mixed derivatives such as $TMS-CH=CH-CH_2-B(OH)(OiPr)$. This solution can be used as an allylation reagent without purification.

25 Alternatively, a solution of reagent of formula IIIa can be generated by hydrolysis of complex of formula V in organic solvent/water mixture such as a dichloromethane/water, toluene/water, ethyl acetate/water, THF/water, chloroform/water mixture, preferably a dichloromethane/water mixture, preferably in the presence of an acid such as sulfuric acid, chlorhydric acid, acetic acid,
30 preferably acetic acid. Allylation of acetyl-cyclosporin A aldehyde with a dichloromethane solution of reagent of formula IIIa prepared as just described can reach high conversions using as low as 2 equivalents of the reagent. In this case, isopropyl derivatives are of course absent.

Without tartrate activation

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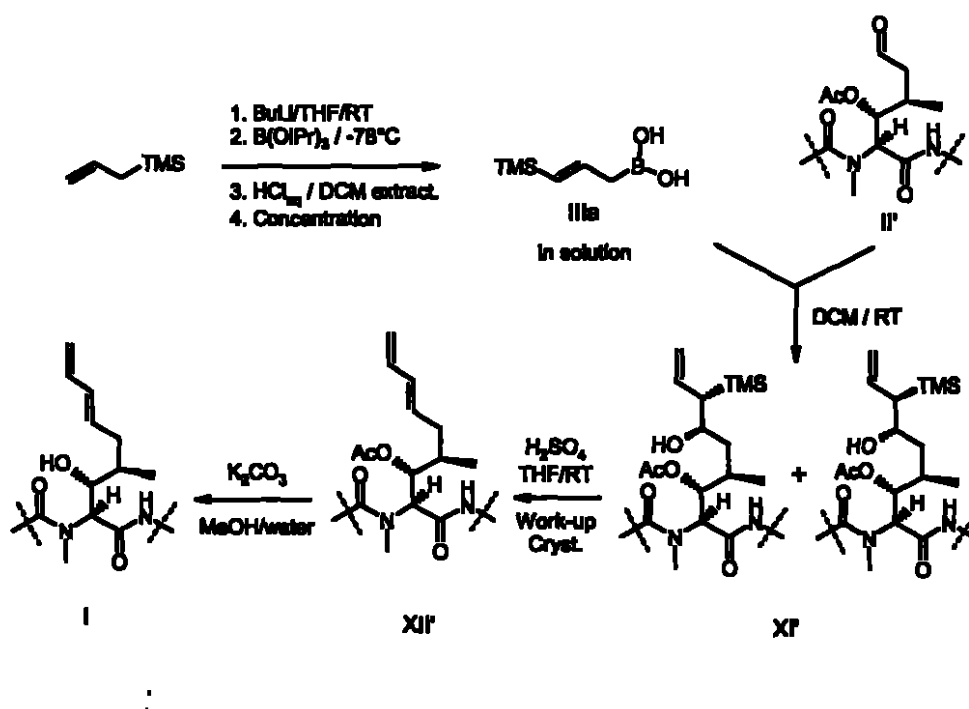
Toluene can be used as solvent for these reactions, however, marked solvent effects have been observed in these reactions. The allylation is best performed in polar non-coordinating solvents, preferably dichloromethane.

When tartrate additive is omitted, allylation is preferably performed in dichloromethane using a concentrated solution of the crude boronic acid (>10%, preferably ca 50% concentration).

Preferred reagent of formula III wherein R^1 is hydrogen, C_{1-8} alkyl or C_{3-8} cycloalkyl and/or, when R^1 is hydrogen, a trimer thereof are those wherein R^1 is hydrogen, methyl, ethyl, propyl, isopropyl, butyl or benzyl, more preferably R^1 is hydrogen, methyl, ethyl, propyl, isopropyl or butyl, further preferably hydrogen, methyl, ethyl, propyl or butyl, especially preferably hydrogen. Allylation is performed in organic solvent such as ethyl acetate, THF, toluene, chloroform or dichloromethane, preferably in ethyl acetate, toluene or dichloromethane, more preferably in toluene or dichloromethane, especially in dichloromethane.

For example, the synthesis of (E)-ISA247 by the reagent of formula III can be carried out as illustrated in Scheme E.

Scheme E:



(R^1 and the dotted lines have the meaning as defined above.)

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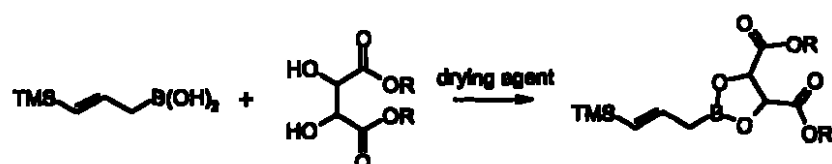
The allylation of acetyl-cylcosporine A aldehyde (II') in dichloromethane with 10 equiv. of a ca 50% solution of boronic acid reaches over 95% conversion within 60 min at RT and yields a mixture of anti β -trimethylsilyl alcohol diastereomers (XI'). The Peterson elimination can be performed after aqueous work-up on the crude allylation product at 0 °C to RT in THF with sulfuric acid. Alternatively, the Peterson elimination can take place directly on the allylation reaction mixture by addition of THF and sulfuric acid. Aqueous work-up and crystallization yields the (E)-acetyl-ISA247 (XII').

Hydrolysis of the (E)-acetyl-ISA247(XII') provides (E)-ISA247 (I).

10 With tartrate activation

As shown in Scheme F, the addition of a tartrate ester, such as, for example, L-(+)-dimethyltartrate in the presence of a drying agent, activates the boronic acid of formula IIIa by generating in-situ the corresponding boronate ester, a reagent class known in the literature to exhibit very high allylation reactivity. The reaction then proceeds partially or mainly through the generated boronate ester increasing the rate of the allylation.

Scheme F:

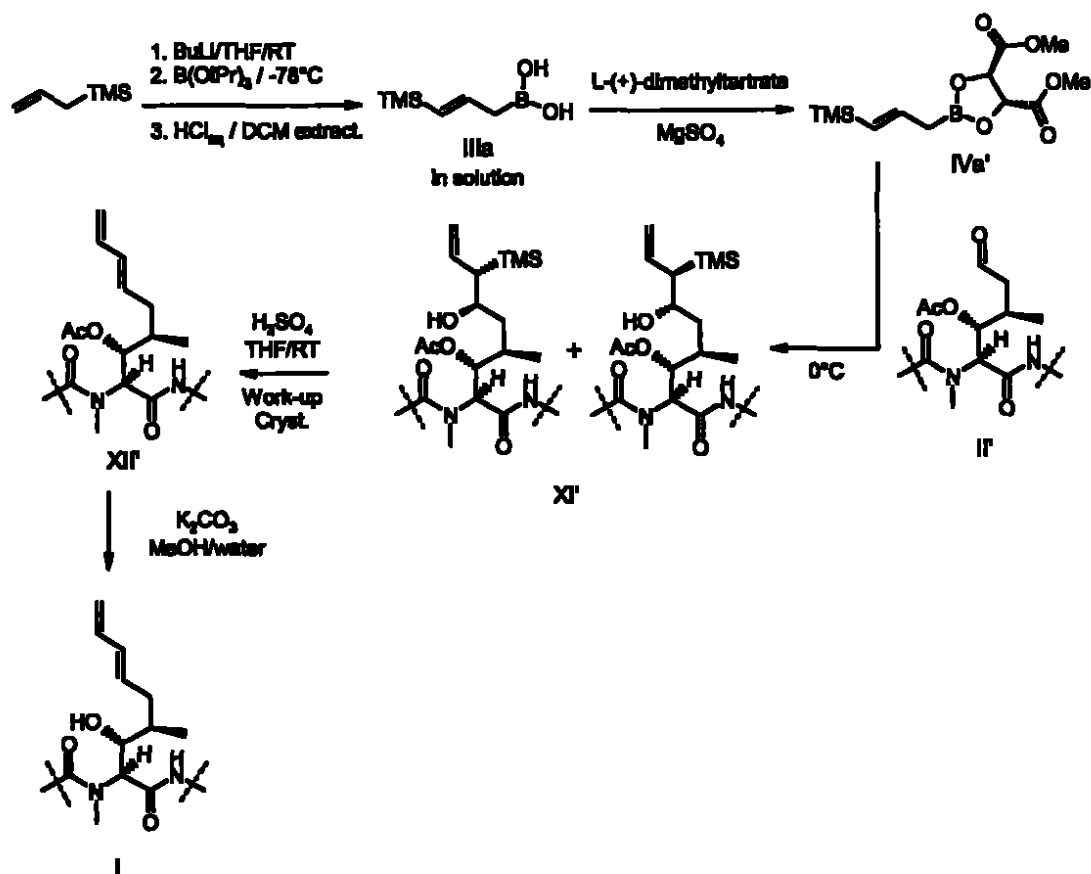


(R is C₁₋₈ alkyl, preferably C₁₋₆ alkyl, more preferably methyl, ethyl or isopropyl, especially methyl.)

Allylation with reagent of formula IV is performed in organic solvent such as ethyl acetate, THF, toluene, chloroform or dichloromethane, preferably in ethyl acetate, toluene or dichloromethane, more preferably in toluene or dichloromethane, especially in dichloromethane.

For example, the synthesis of (E)-ISA247 by the reagent of formula IV is performed as illustrated in Scheme G.

Scheme G:



(The dotted lines have the meaning as defined above.)

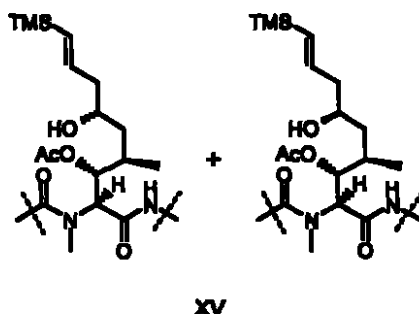
- 5 The generation of the reagent of formula IVa' by mixing a solution of boronic acid of formula IIIa with L-(+)-dimethyltartrate, in the presence of a drying agent such as molecular sieves or magnesium sulfate, preferably magnesium sulfate, is evidenced by ¹¹B and ¹H NMR analyses.

- 10 Allylation of acetyl-cyclosporin A aldehyde (II') with a boronic acid reagent in-situ activated by addition of L-(+)-dimethyltartrate at a temperature of 0 °C to RT, preferably at 0 °C, give a mixture of anti β-silyl alcohol diastereomers (XI'). Aqueous work-up followed by the Peterson elimination in THF with sulfuric acid provides, after work-up and crystallization, (E)-acetyl-ISA247 (XII'). Hydrolysis of the acetyl protecting group yields (E)-ISA247 (I).

- 15 Care should be taken that reaction involving the use of crude boronic acid solution with or without tartrate activation should be performed at neutral or acidic pH (between 3 and 7, preferably between 5 and 6). Indeed when the pH is

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over 7, substantial amount of a side-product identified as the vinylsilane of formula XV are formed. A test reaction (performed without tartrate activation) where Et₃N amine was added to reach a pH of 9-10 led to the almost exclusive formation of the vinylsilane product XV (as evidenced by MS, ¹H NMR, COSY, TOCSY and HSQC NMR experiments). Such an effect was totally unexpected.



(The dotted lines have the meaning as defined above.)

Allylation by γ -silylated allylboron reagent of formula V (step a-iii)

Without activation, the diethanolamine complex of formula V does not react at RT with acetyl-cyclosporin A aldehyde in non protic solvents like dichloromethane or THF. However, the complex of formula V represent a stable source of the corresponding boronic acid.

When treated in a water/organic solvent, (such as ethyl acetate, THF, dichloromethane or toluene, preferably ethyl acetate, dichloromethane or toluene, more preferably dichloromethane) mixture preferably in the presence of acid such as sulfuric acid, chlorhydric acid or acetic acid, preferably acetic acid, the diethanolamine complex V is hydrolyzed and liberates the reactive boronic acid as shown, for example, in Scheme H, which can then reacts with the acetyl-cyclosporin A aldehyde (II'), preferably at RT.

Scheme H:

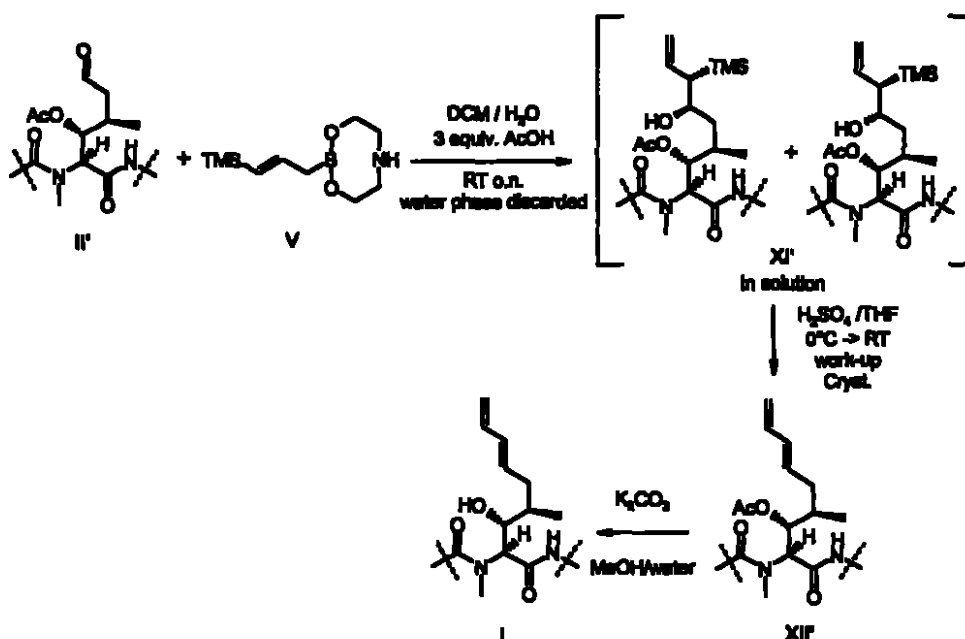


Allylation of acetyl-cyclosporin A aldehyde (II') under such conditions provides a mixture of anti β -trimethylsilyl alcohol diastereomers (XI'). After the water phase is discarded, solvent is exchanged to THF, and sulfuric acid is added to perform the Peterson elimination. Aqueous work-up and crystallization provides (E)-acetyl-ISA247 (XII'). Subsequent hydrolysis yields (E)-ISA247 (I).

Alternatively, isolation of the crude anti β -trimethylsilyl alcohol diastereomers after aqueous work-up, followed by Peterson elimination under standard conditions (concentrated sulfuric acid in THF) furnishes E-acetyl-ISA247.

For example, the synthesis of (E)-ISA247 by the complex of formula V can be performed as illustrated in Scheme I.

Scheme I:



(The dotted lines have the meaning as defined above.)

Allylmatalation of acetyl-cyclosporin A aldehyde (II') can also take place under non-aqueous conditions directly with complex of formula V. Indeed, protic solvents such as carboxylic acids are particularly effective. Solvent mixture could be acetic acid and/or formic acid or a combination of acetic acid and/or formic acid and a co-solvent such as dichloromethane and THF. The allylation is best performed in acetic acid between RT and 35 °C. This provides a mixture of anti β -silyl alcohol diastereomers (XI'). These intermediates could of course be isolated

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but the Peterson elimination can be conducted in one pot by addition to the reaction mixture of an acid such as formic acid, sulfuric acid or methanesulfonic acid, preferably formic acid. Aqueous work-up and crystallization yields (E)-acetyl-ISA247 (XII'). Subsequent hydrolysis furnishes (E)-ISA247 (I).

5 When formic acid is present in sufficient amounts in the solvent mixture used for the allylation, the Peterson elimination can take place in one-pot leading directly to (E)-acetyl-ISA247.

10 Another alternative consists in performing the addition of complex of formula V to acetyl-cyclosporin A aldehyde (II') in the presence of a Lewis acid such as BF₃.Et₂O. For example, the reaction with BF₃.Et₂O can be performed in a solvent such as dichloromethane or THF at a temperature ranging from -40 °C to RT. Under these conditions, the allylation can directly be followed by the Peterson elimination, yielding the expected (E)-acetyl-ISA247 (XII').

Allylation by γ -silylated allylboron reagent of formula VI (step a-iv))

15 Reacting the allyltrifluoroborate VI and acetyl-cyclosporin A aldehyde (II') in a biphasic water/organic solvent, preferably water/dichloromethane mixture or water/toluene mixture, more preferably water/dichloromethane mixture at RT provides a mixture of anti β -trimethylsilyl alcohol diastereomers (XI'). After the water phase is discarded, the Peterson elimination is performed by addition of THF and sulfuric acid at a temperature of 0 °C to RT providing (E)-acetyl-ISA247 (XII').
20 Peterson elimination can also be performed under standard conditions (sulfuric acid in THF) after isolation of the anti β -trimethylsilyl alcohol diastereomers to give E-acetyl-ISA247.

25 The allylation can also be promoted by a Lewis acid. In that case, the allylation and the Peterson elimination can take place in-situ. For example, addition of excess BF₃.Et₂O to a suspension of allyltrifluoroborate VI (2 equiv.) in a solution of acetyl-cyclosporin A aldehyde (XII') in dichloromethane at -70 °C provides after 60 min. reaction and aqueous work-up, (E)-acetyl-ISA247 (I). Solvents for the reaction are organic solvent such as dichloromethane, THF or
30 toluene, preferably dichloromethane.

Allylation by γ -silylated allylboron reagent of formula VII (step a-v))

Allylation of aldehydes with this reagent is known from the literature to proceed slowly (1 to several days at RT). Accordingly, allylation of acetyl-cyclosporin A aldehyde (II') with excess of reagent of formula VII (5-10 equivalents) in solvents such as THF, dichloromethane, toluene, DMF and DMSO proceeds slowly at RT.

Heating, use of large excess of reagent or high concentration could increase the rate of acetyl cyclosporin A aldehyde allylation, however, a better alternative was found by a proper choice of solvent.

Carboxylic acids such as formic acid or acetic acid were found to dramatically enhance the rate of allylation. For instance, when performed in acetic acid, allylation of acetyl cyclosporin A aldehyde (II') can reach conversion of over 95% within 5 hours at RT with 2 equivalents of reagent of formula VI, providing the β -silyl alcohols (XI'). Further addition of formic acid promotes the Peterson elimination. (E)-acetyl-ISA247 (XII') is obtained after extractive work-up ascertaining the relative anti stereochemistry of the intermediate β -silyl alcohols. Peterson elimination can also be performed under standard conditions (sulfuric acid in THF) after isolation of the anti β -trimethylsilyl alcohol diastereomers to give (E)-acetyl-ISA247.

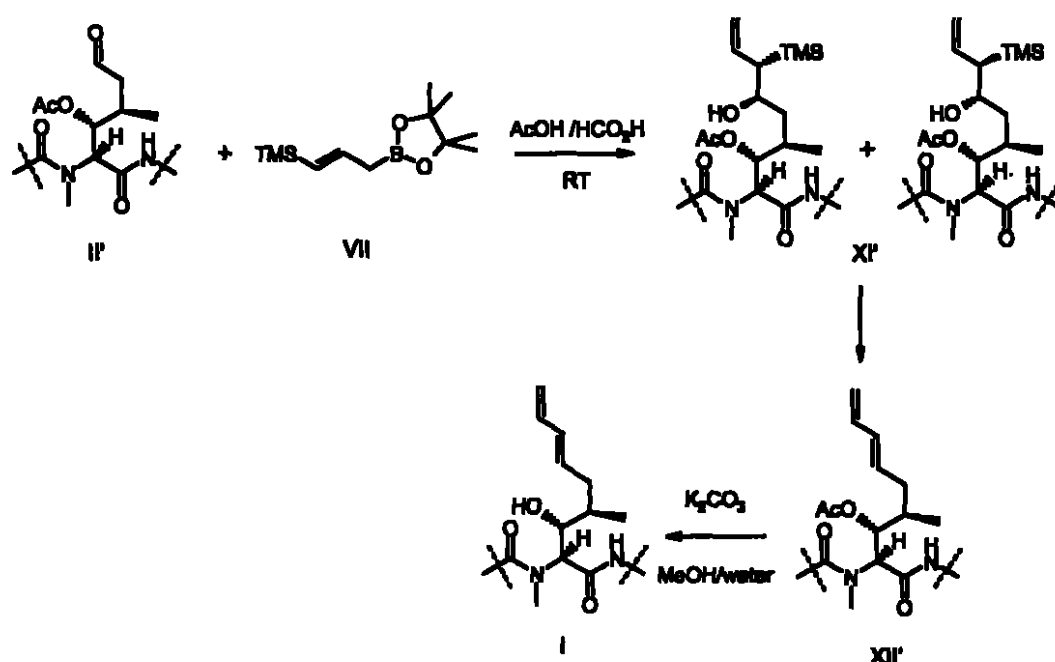
Formic acid or preferably a combination of acetic acid and formic acid (such as ca 1:1 v/v) as is also an effective solvent. In that case, the allylmetalation and the following Peterson elimination can take place in one-pot. In a combination of acetic acid and formic acid (ca 1:1 v/v) the allylation of acetyl-cyclosporin A aldehyde and the following Peterson elimination reach over 90% conversion within 60 min. at RT with 1.5 equivalent of reagent. Aqueous extractive work-up and crystallization furnishes (E)-acetyl-ISA247 (XII').

Mixture of acetic acid, formic acid and a suitable co-solvent can also be used. Dichloromethane, toluene, ethyl acetate or isopropyl acetate, preferably isopropyl acetate could be used as co-solvent. Decrease in reactivity can be observed when using a co-solvent but this could be compensated by increasing the reaction temperature.

Hydrolysis of the acetate protecting group with K_2CO_3 in aqueous methanol furnishes (E)-ISA247 (I).

For example, the synthesis of (E)-ISA247 by the reagent of formula VI can be performed as illustrated in Scheme J.

Scheme J:



The origin of the increased activity of the reagent of formula VII, when used in carboxylic acid solution like acetic acid and formic acid, could come from the high polarity and the low complexing ability of these solvents. Another effect could be found in the ability of these solvent to provide acidic catalysis of the allylation by activation of the carbonyl group of the aldehyde through protonation.

The addition of reagent of formula VII to acetyl cyclosporin A aldehyde (II') can be promoted by a Lewis acid such as BF₃·Et₂O at a temperature of -70 °C to 0 °C in toluene, THF or dichloromethane, preferably toluene or dichloromethane, preferably dichloromethane. Under the allylation reaction, the Peterson elimination also occurs and (E)-acetyl-ISA247 (XII') can be obtained after extractive aqueous work-up .

Synthesis of (E)-ISA247 by allyltitanium reagents

Reaction of acetyl-cyclosporine A aldehyde with a γ -trimethylsilylallyltitanium reagent prepared from trimethylsilylallyllithium and titanium dichlorodiisopropoxide, titanium tetraisopropoxide or titanium chlorotriisopropoxide, preferably titanium tetraisopropoxide or titanium chlorotriisopropoxide performed in THF, at a temperature of -80°C to 0 °C, preferably -80 °C to -30 °C, more preferably -80 °C to -50 °C, especially -80 °C to

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-60 °C, furnishes, after aqueous work-up, a mixture of diastreomeric anti β-silyl alcohols XI'. Peterson elimination under the standard conditions (H₂SO₄ in THF) furnishes E-acetyl-ISA247 (XII').

Synthesis of (E)-ISA247 by allyl aluminum reagents

5 Reaction of acetyl-cyclosporine A aldehyde with a γ-trimethylsilylallyl aluminum reagent prepared from trimethylsilylallyllithium and ethyl aluminum dichloride or diethyl aluminum chloride, preferably diethyl aluminum chloride, performed in THF, at a temperature of -80 °C to 0 °C, preferably -80 °C to -30 °C, more preferably -80 °C to -50 °C, especially -80 °C to -
10 60 °C furnishes after aqueous work-up a mixture of diastreomeric anti β-silyl alcohols XI'. Peterson elimination under the standard conditions (H₂SO₄ in THF) furnishes E-acetyl-ISA247 (XII').

Preparation of the γ-silylated allyl metal reagents

15 The γ-silylated allyl metal reagents required for the allyl metalation step are best generated from the corresponding allylsilanes via deprotonation, trapping with an adequate metal reagent and optionally by further complexation of the metal rest by a suitable ligand. The resulting reagents can, depending on their stability and the process, be used in situ or be isolated and stored.

20 Although other silyl-substituted allylsilanes could obviously have been used, the trimethylsilyl derivative leads to minimum amount of waste. Indeed, the silyl fragment is lost upon Peterson elimination.

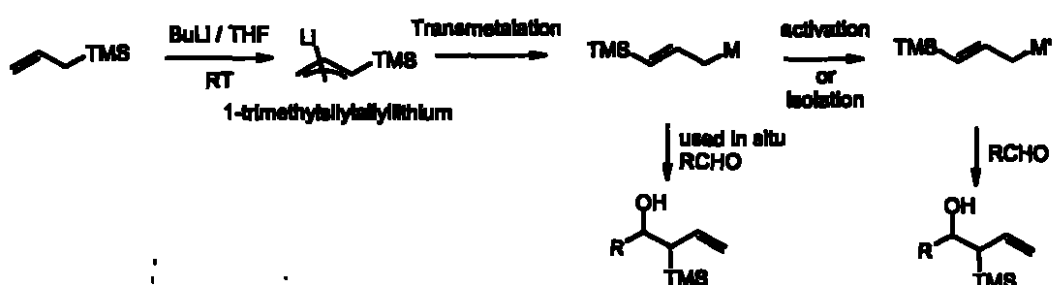
 Many conditions for the deprotonation of allylsilanes have been published in the literature and usually make use of n-butyllithium (or the sec- and tert-
25 isomers) in an organic solvent (generally THF) in combination or not with a co-solvent or co-reagent such as HMPA, TMEDA or potassium t-butoxide at temperatures ranging from -100 °C to RT. (see for example: T. H. Chan in "Silylallyl Anions in Organic Synthesis: A Study in Regio- and Stereoselectivity", *Chemical Reviews* 1995, 95, p1279-1292 ; Kohei Tamao, Eiji Nakajo, and Yoshihiko
30 Ito in "Silafunctional compounds in organic synthesis. 33. Metalated allylaminosilane: a new, practical reagent for the stereoselective .alpha.-hydroxyallylation of aldehydes to erythro-1,2-diol skeletons", *Journal of Organic*

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Chemistry 1987, 52, pp 957 – 958 ; E. Ehlinger and P. Magnus in "Silicon in Synthesis. 10. The (Trimethylsilyl)allyl Anion: A β -Acyl Anion Equivalent for the Conversion of Aldehydes and Ketones into γ -Lactones", *Journal of the American Chemical Society* 1980, 102, pp 5004-5011 ; M. Schlosser, L. Franzini in "The Regioselectivity of 1,3-Disubstituted Allylmetal Species Towards Electrophiles: 1-(Trimethylsilyl)alk-2-enylpotassium Compounds", *Synthesis* 1998, pp 707-709 ; E. Schaumann and A. Kirschning in "Ring-opening of oxiranes by silyl-substituted allyl anions. A regiochemical chameleon", *Tetrahedron Letters* 1988, 29, pp 4281-4284).

As illustrated in Scheme K, the allyltrimethylsilane is deprotonated by *n*-butyllithium in THF at a temperature ranging from 0 °C to 35 °C, preferably between 0 °C and 25 °C for 30 min. up to 3 hours. This generates a trimethylsilylallyllithium intermediate. This intermediate most probably exists in solution as a π -allyl complex of lithium in a trans configuration (T. H. Chan in "Silylallyl Anions in Organic Synthesis: A Study in Regio- and Stereoselectivity", *Chemical Reviews* 1995, 95, p1279-1292; M. Schlosser, O. Desponds, R. Lehmann, E. Moret and G. Rauschschwalbe in "Polar Allyl Type Organometallics as Key Intermediates in Regio- and Stereocontrolled Reactions : Conformational Mobilities and Preferences", *Tetrahedron* 1993, 49, p10175-10203). This anion is then trapped (transmetalated) by an electrophilic metal source usually at a temperature of –80 °C to –60 °C, generating the corresponding trans-allylmetal reagent. Depending on the metal rest, these reagents are reacted in-situ with the aldehyde or can be isolated for later use. It can also be transformed into another complex by addition of a proper reagent in order to activate the reagent for the allylation or to allow their isolation.

Scheme K:



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(M and M' are a metallic fragment comprising the metal and its ligands.)

Preparation of allylboron reagents

γ -silylated allylboron reagent of formula IIIa

5 A solution of crude boronic acid of formula IIIa is obtained after deprotonation of allyltrimethylsilane, trapping with an electrophilic boron reagent and aqueous work-up. The deprotonation of allyltrimethylsilane is performed in THF with butyllithium, between 0 °C and 35 °C, preferably between 0 °C and 25 °C for 30min. to 3 hours. The electrophilic boron reagent is a trialkylborate such as
10 triisopropyl borate or trimethyl borate, preferably triisopropyl borate. The trapping of the trimethylsilylallyllithium intermediate with triisopropyl borate is performed between -80 °C and -20 °C, preferably below -60 °C for 30 min. to 2 hours. The trapping of the trimethylsilylallyllithium intermediate with trimethyl borate is performed between -80 °C and -60 °C for 30 min. to 2 hours.

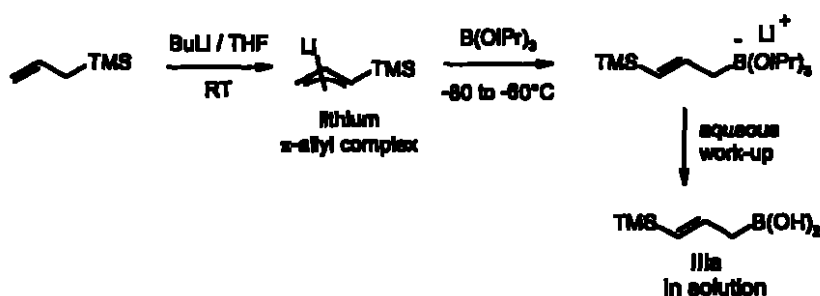
15 It is known that allylboronic acids are unstable, therefore, the crude boronic acid is kept in solution (P. G. M. Wuts and Y.-W. Jung in "The Addition of γ -(Trimethylsilyl)allylboronates to Imines", *Journal of Organic Chemistry* 1991, 56, p365-372(see comment in the example for the preparation of compound 9); W. R. Roush, K. Ando, D. B. Powers, A. D. Palkowitz and R. L. Halterman in
20 "Asymmetric Synthesis Using Diisopropyl Tartrate Modified (E)- and (Z)-Crotylboronates; Preparation of the Chiral Crotylboronates and Reactions with Achiral Aldehydes", *Journal of the American Chemical Society* 1990, 112, 6339 (see reference 17)). Concentrated solutions (>10% concentration, for example ca 50% concentration) are not stable at RT and decompose. They should be rapidly used.
25 If needed they should be stored at 5 °C maximum. In accordance to the general behaviour of boronic acids, the boronic acid of formula IIIa might in principle also exists in the form of a cyclic trimer (also called a boroxine) or oligomers. Since isopropanol coming from triisopropyl borate can also present (depending on the process), (mixed) isopropyl boron esters (TMS-CH=CH-CH₂-B(OH)(OiPr) and/or TMS-CH=CH-CH₂-B (OiPr)₂) could also be present. Indeed, ¹H NMR and
30 ¹¹B NMR analyses of concentrated solutions of boron reagent of formula IIIa (ca 50%) diluted with an equal volume of CD₂Cl₂ shows the presence of several boronate species, although reverse phase HPLC analysis shows mainly the boronic

acid (under the HPLC conditions, the oligomers, dimers and trimers should be hydrolyzed and converted to the boronic acid).

Alternatively, a solution of reagent of formula IIIa can be prepared by hydrolysis of complex of formula V in an organic solvent/water mixture such as a dichloromethane/water, toluene/water, ethyl acetate/water, THF/water, chloroform/water mixture, preferably a dichloromethane/water mixture preferably in the presence of an acid such as sulfuric acid, chlorhydric acid, acetic acid, preferably acetic acid.

Preparation of the γ -silylated allylmetal reagent of formula IIIa is, for example, performed as illustrated in Scheme L.

Scheme L:



γ -silylated allylboron reagent of formula IV

Boronate reagent of formula IV where R is C₁₋₆ alkyl, preferably C₁₋₆ alkyl, more preferably methyl, ethyl or isopropyl, especially methyl is prepared by treating boron reagent of formula IIIa with the required tartrate ester in the presence of a drying agent such molecular sieves or magnesium sulfate, preferably magnesium sulfate.

Reagent of formula IVa' is prepared by mixing a solution of boronic acid of formula IIIa with L-(+)-dimethyltartrate, in the presence of a drying agent such molecular sieves or magnesium sulfate, preferably magnesium sulfate, as evidenced by ¹¹B and ¹H NMR analyses.

γ -silylated allylboron reagent of formula III

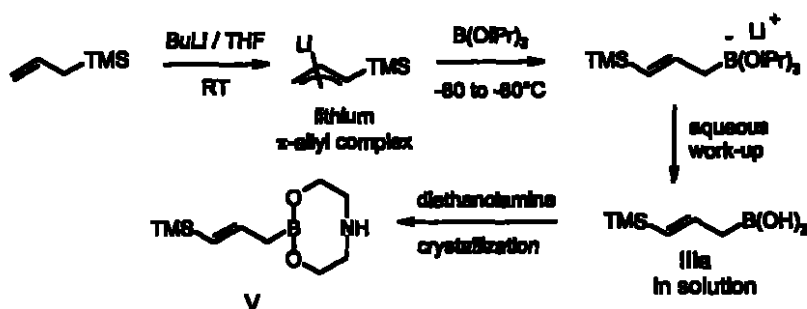
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This reagent can be prepared by treating boron reagent of formula IIIa with the required alcohol in the presence of a drying agent such molecular sieves or magnesium sulfate, preferably magnesium sulfate. Preferred boronate reagent of formula IV wherein R¹ is C₁₋₃ alkyl or C₃₋₈ cycloalkyl are those wherein R¹ is methyl, ethyl, propyl, isopropyl, butyl or benzyl, more preferably R¹ is methyl, ethyl, propyl, isopropyl or butyl, further preferably R¹ is methyl, ethyl, propyl or butyl, especially preferably R¹ is methyl.

γ-silylated allylboron reagent of formula V

To a solution of crude boronic acid of formula IIIa, preparation of which was described above, is added diethanolamine. Solvent exchange to heptane and crystallization provides the diethanolamine complex of formula V as a solid. A special feature of this reagent is the presence of an interaction between the nitrogen lone pair of the diethanolamine fragment and the boron atom (i.e. complexation of the nitrogen atom by the boron atom) as evidence by ¹¹B NMR of the complex (δ = 11ppm relative to BF₃·Et₂O, external reference).

Preparation of the γ-silylated allylmetal reagent of formula V is, for example, performed as illustrated in Scheme M:



γ-silylated allylboron reagent of formula VI

Scheme N:



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In general, allyltrifluoroborate potassium salts are prepared by treating the corresponding boronic acid with 3 equivalents of KHF_2 in a water/methanol solvent mixture (see for example : R. A. Batey in "Diastereoselective Allylation and Crotylation Reactions of Aldehydes with Potassium Allyl- and Crotyltrifluoroborates under Lewis acid Catalysis", *Synthesis* 2000, pp 990-998).
However, direct application of these procedures to the preparation of trifluoroborate of formula VI lead to the formation of substantial amounts of allyltrimethylsilane via protodeborylation due to the acidic pH of the reaction mixture.

Modification was made in order to avoid this side-reaction. Thus, as illustrated in Scheme O, a methanol solution of boronic acid is treated with 2 equivalents of KHF_2 as fluoride source, at RT. The suspension is stirred at RT for 60 min. The residual organic salts are removed by filtration. The methanolic solution of trifluoroborate salt VI is concentrated under reduced pressure and the product is crystallized at 0-5 °C. The trifluoroborate salt VI is isolated by filtration and dried under vacuum.

The required boronic acid solution is prepared by hydrolyzing the diethanolamine complex of formula V in a water/dichloromethane mixture in the presence of an acid such as acetic acid. The aqueous phase is discarded and the solvent is exchanged from dichloromethane to methanol.

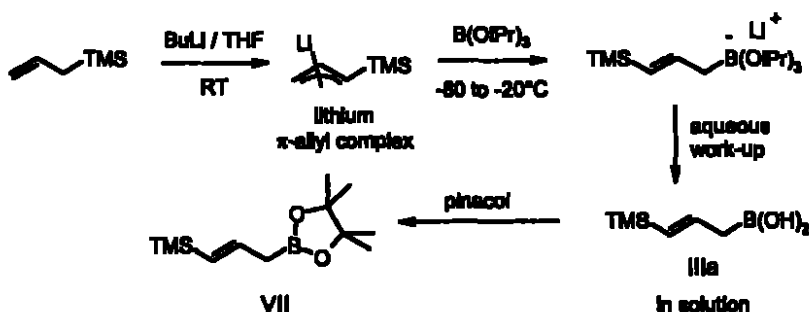
Alternatively, the diethanolamine complex V can be used directly as starting material. The trifluoroborate salts VI can be prepared, however, crystallization does not occur.

γ -silylated allylboron reagent of formula VII

To a solution of crude boronic acid of formula IIIa, preparation of which was described above, is added pinacol. The reaction mixture is stirred at RT and then concentrated under reduced pressure. The pinacol complex VII can then be distilled under low pressure or used directly in the allylmetalation step. The preparation of the pinacol complex of formula VII is, for example, performed as illustrated in Scheme O.

Scheme O: :

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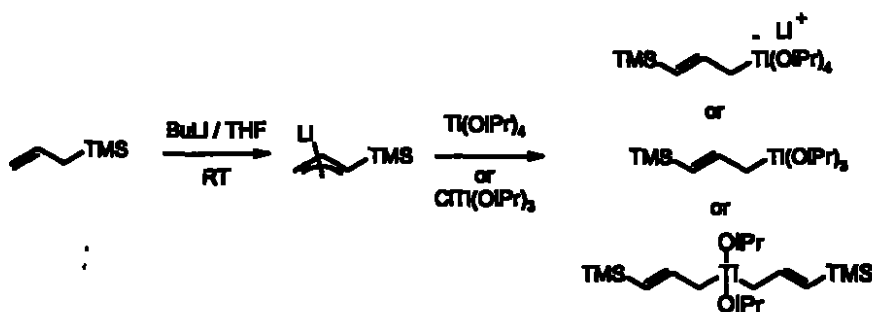


Alternatively, the trapping of the 1-trimethylsilylallyl lithium can be performed with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, leading directly after aqueous work-up to the pinacol boronate of formula VII.

Alternatively, the reagent can be prepared by deprotonation of allyltrimethylsilane at room temperature with butyllithium, quench of the allyllithium intermediate with tri-isopropylborate (between -80 °C to -20 °C, preferably between -80 °C and -30 °C), addition of pinacol and then aqueous work-up.

Preparation of the allyltitanium reagents

The (trimethylsilyl)allyltitanium reagents are prepared in-situ via deprotonation of allyltrimethylsilane to form trimethylsilylallyllithium, as described above, and reaction of this intermediate with titanium dichlorodiisopropoxide, titanium tetraisopropoxide or titanium chlorotriisopropoxide, preferably titanium tetraisopropoxide or titanium chlorotriisopropoxide at a temperature of -80 °C to 0 °C, preferably -80 °C to -30 °C, more preferably -80 °C to -50 °C, especially -80 °C to -60 °C. The resulting titanium reagents are used in situ for the allylation of protected cyclosporin A aldehydes. Putative structures for these reagents are presented below :



Preparation of the allylaluminum reagents

The (trimethylsilyl)allylaluminum reagents are prepared in-situ via deprotonation of allyltrimethylsilane to form trimethylsilylallyllithium, as described above, and reaction of this intermediate with a dialkylaluminum chloride such as diethylaluminum chloride or with an alkylaluminum dichloride such as ethylaluminum dichloride, preferably with diethylaluminum chloride, at a temperature of -80 °C to 0 °C, preferably -80 °C to -30 °C, more preferably -80 °C to -50 °C, especially -80 °C to -60 °C. The resulting aluminum reagents are used in situ for the allylation of protected cyclosporin A aldehydes.

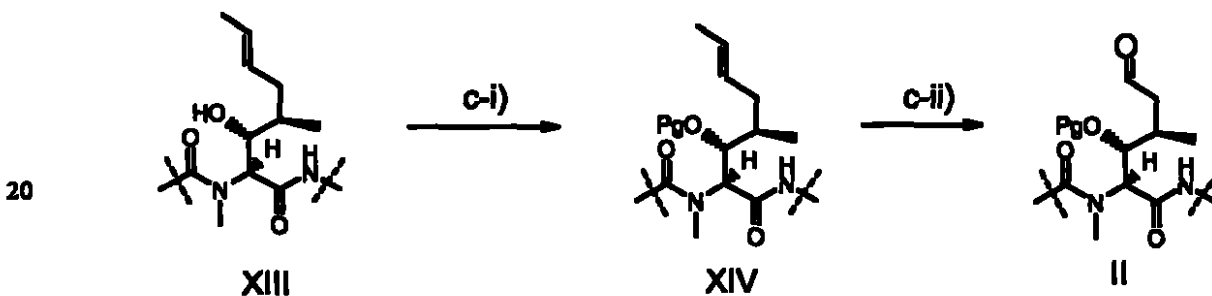
A putative structure for one of these reagents is presented below :



Preparation of protected cyclosporin A aldehyde

Protected cyclosporin A aldehyde of formula II can be prepared as illustrated in Scheme P.

Scheme P:



(The dotted lines have the meaning as defined above.)

In step c-i), a protecting group is introduced in cyclosporin A of formula XIII, to protect hydroxyl group at the β -position of the side chain of the 1-amino acid residue. Protecting groups are well known in organic synthesis, and have been

discussed by J. R. Hanson in Chapter 2, "The Protection of Alcohols," of the publication *Protecting Groups in Organic Synthesis* (Sheffield Academic Press, Sheffield, England, 1999), pp. 24-25. Hanson teaches how to protect hydroxyl groups by converting them to either esters or ethers. Acetate esters are perhaps the most frequently used type of chemistry for protecting hydroxyl groups. There are a wide range of conditions that may be used to introduce the acetate group. These reagents and solvents include acetic anhydride and pyridine; acetic anhydride, pyridine and dimethylaminopyridine (DMAP); acetic anhydride and sodium acetate; acetic anhydride and toluene-p-sulphonic acid, acetyl chloride, pyridine and DMAP; and ketene. DMAP is a useful acylation catalyst because of the formation of a highly reactive N-acylpyridium salt from the anhydride.

For example, the β -alcohol of cyclosporin A is protected as an acetate by reacting cyclosporin A (XIII) with acetyl chloride, ethyl acetate, or combinations thereof, forming the compound, acetyl cyclosporin A. In another example, the β -alcohol undergoes a nucleophilic addition to acetic anhydride, forming acetyl cyclosporin A and acetic acid. These reactions may be carried out in the presence of dimethylaminopyridine (DMAP) where an excess of acetic anhydride acts as the solvent.

Although the preparation of acetyl cyclosporin A is well established in the literature, it will be appreciated by those skilled in the art that protecting groups other than acetate esters may be used to protect the β -alcohol of the 1-amino acid residue of cyclosporin A. These protecting groups may include benzoate esters, substituted benzoate esters, ethers, and silyl ethers. Under certain reaction conditions, the acetate protecting group is prone to undesirable side reactions such as elimination and hydrolysis. Since benzoate esters, ethers and silyl ethers are often more resistant to such side reactions under those same reaction conditions, it is often advantageous to employ such protecting groups in place of acetate.

In step c-ii), the protected cyclosporin A of formula XIV is converted to a protected cyclosporin A aldehyde of formula II.

This step can be carried out, for example, by using ozone as an oxidizing agent followed by work-up with a reducing agent to form a protected cyclosporin A aldehyde (II). Ozonolysis step is conducted at a temperature range from about -80°C to 0°C . The solvent used during the ozonolysis may be a lower alcohol such as methanol. The reducing agent may be a trialkylphosphine such as

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tributylphosphine, a triarylphosphine, a trialkylamine such as triethylamine, an alkylaminosulfide, a thiosulfate or a dialkylsulfide such as dimethylsulfide. When working with tributylphosphine as the reducing agent, the person of ordinary skill in the art will know that the reaction is dose-controlled.

5 Furthermore, a protected cyclosporin A aldehyde (II) can be prepared by converting the protected cyclosporinA XIV, such as acetyl cyclosporin A, to the protected cyclosporin A epoxide with a monopersulfate, preferably oxone, in the presence of a ketone, such as acetoxycetone or diacetoxycetone. This step is performed in an organic solvent which is inert under these reaction conditions
10 such as acetonitrile and water. Ethylenediaminetetra-acetic acid disodium salt is added to capture any heavy metal ions which might be present. The epoxidation reaction is carried out preferably at a pH over 7. This epoxidation reaction is followed by oxidative cleavage of the epoxide with periodic acid or periodate salt under acidic conditions. Optionally, the oxidation and the oxidative cleavage can
15 be combined in a work-up procedure. These reactions have been discussed by Dan Yang, et al., in "A C₂ Symmetric Chiral Ketone for Catalytic Asymmetric Epoxidation of Unfunctionalized Olefins," J. Am. Chem. Soc., Vol. 118, pp. 491-492 (1996), and "Novel Cyclic Ketones for Catalytic Oxidation Reactions," J. Org. Chem., Vol. 63, pp. 9888-9894 (1998).

20 The use of ruthenium based oxidizing agents has been discussed by H. J. Carlsen et al. in "A Greatly Improved Procedure for Ruthenium Tetroxide Catalyzed Oxidations of Organic Compounds," J. Org. Chem., Vol. 46, No. 19, pp 3736-3738 (1981). Carlsen et al. teach that, historically, the expense of ruthenium metal provided an incentive for the development of catalytic procedures, the most
25 popular of which used periodate or hypochlorite as stoichiometric oxidants. These investigators found a loss of catalytic activity during the course of the reaction with the conventional use of ruthenium which they postulated to be due to the presence of carboxylic acids. The addition of nitriles to the reaction mixture, especially acetonitrile, was found to significantly enhance the rate and extent of the oxidative
30 cleavage of alkenes in a CCl₄/H₂O/IO₄⁻ system.

For example, protected cyclosporin A aldehyde (II) can be produced from protected cyclosporin A (XIV), such as acetyl cyclosporin A, by dissolving it in a mixture of acetonitrile and water, and then adding first sodium periodate and then ruthenium chloride hydrate. The aldehyde (II) may be extracted with ethyl acetate.

EXAMPLES

5 The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

10 Although most of the examples have been provided for the allylation-Peterson elimination sequence on acetyl-cyclosporin A aldehyde, other protecting group could in principle be used. Of course, this will be limited by their compatibility with the reaction conditions as well as the possibility to remove them efficiently to provide (E)-ISA247.

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Example 1

i) Preparation of a solution of crude the boronic acid of formula III : (E)-3-(trimethylsilyl)allylboronic acid

20 g (169.8 mmol, 1 equiv.) of allyltrimethylsilane (Fluka 06073) was
 5 dissolved in 140 ml of dry THF (Fluka 87368) at RT. 106.1 ml (169.8 mmol, 1
 equiv.) of a 1.6 M solution of butyllithium in hexane (Acros 181270100) was added
 in 10 min. maintaining the temperature between 20 °C and 25 °C. After 30 min.
 reaction, the resulting yellow to orange solution was cooled to -70 °C. 40.24 ml
 (169.8 mmol, 1 equiv.) triisopropylborate (Fluka 92085) is added in 10 min.,
 10 keeping the temperature below -60 °C. After 30 min. reaction at -74°C, the cold
 solution was poured onto 170 ml of a 1M aqueous HCl solution. The pH was
 adjusted to 7-8 by further addition of of 1M HCl_{aq} (in this particular case, 26 ml).
 80 ml of dichloromethane were added for extraction. The water phase was
 separated and re-extracted with 80 ml of dichloromethane. The organic phases
 15 were washed sequentially with 150 ml of a saturated aqueous NaCl solution,
 combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure to
 ca 40 ml. The weight of the solution was adjusted to 53.6 g by addition of
 dichloromethane in order to obtain a ca 50% solution of boronic acid (based on
 the starting allyltrimethylsilane).

20 ii) Allylation of acetyl-cyclosporin A aldehyde

20 g (16.23 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde were dissolved
 in 100 ml of dichloromethane at RT. 25.66 g (81.15 mmol, 5 equiv.) of the
 previously prepared boronic acid solution (ca 50% concentration) were added in
 one portion. The conversion of the reaction was monitored by HPLC. Reaction
 25 was complete within 1-3 hours at RT. A ca 85:15 mixture of β-trimethylsilyl alcohol
 diastereomers was obtained.

iii) Peterson elimination

The Peterson elimination was conducted directly on the reaction mixture.

20 ml of THF were added and the reaction mixture was cooled to 0 °C. 2.7
 30 ml (48.69 mmol, 3 equiv.) of concentrated sulfuric acid were added. The
 temperature was raised to RT. After completion of the reaction (ca 1 hour), 100 ml
 of water were added. The organic phase was separated and washed 2 times with 50

ml water. The water phases were re-extracted sequentially with 50 ml dichloromethane. The combined organic phases were dried over Na_2SO_4 , filtered and concentrated under reduced pressure at 30°C. The resulting white foam was re-dissolved in 250 ml MTBE and after a few minutes, the crystallization started. After stirring 15 min. at RT and 2 hours at 0-2 °C, the suspension was filtered. The crystals were washed with 50 ml cold MTBE (-20 °C) and dried at 40-50 °C under reduced pressure to provide 19.2 g of (E)-acetyl-ISA247 as white powder in >98% isomeric purity (400MHz ^1H NMR).

(E)-acetyl-ISA247 can be recrystallized by dissolving the solid in dichloromethane at room temperature and exchanging the solvent to MTBE (by adding MTBE, concentrating the solution to half its volume under reduced pressure at 40°C and repeating these operation 2 to three times). The solution is cooled to room temperature and the crystallization then starts within a few minutes. The suspension is stirred at room temperature for 2h and 30min at 0°C. The crystals of (E)-acetyl-ISA247 are isolated after filtration, washing with MTBE and drying under reduced pressure at 40°C.

iv) Hydrolysis

Hydrolysis of E-acetyl-ISA247 provided (E)-ISA247 in 99.5% double bond isomeric purity (by HPLC).

20 Example 2

i) Preparation of a solution of the crude boronic acid of formula IIIa IIIa: (E)-3-(trimethylsilyl)allylboronic acid

6.67 ml (40.56 mmol, 10 equiv.) of allyltrimethylsilane were dissolved in 33.3 ml of dry THF at RT. 25.35 ml (40.56 mmol, 10 equiv.) of a 1.6M solution of butyllithium in hexane were added in 5 min. maintaining the temperature between 14 °C and 16 °C. After 60 min. reaction at RT, the resulting yellow to orange solution was cooled to -70 °C. 9.614 ml (40.56 mmol, 10 equiv.) triisopropylborate were added in 10 min., keeping the temperature below -65 °C. After 60 min. reaction at -70 °C, the cold solution was poured onto 35 ml of a 1M aqueous HCl solution (pH=7-8). The reaction mixture was extracted with 30 ml of dichloromethane. The water phase was separated and re-extracted with 30 ml of dichloromethane. The organic phases were washed sequentially with 30 ml of a

saturated aqueous NaCl solution, combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure to ca 50 ml.

ii) Generation of the boron reagent of formula IVa' ((R,R)-2-[(E)-(3-trimethylsilyl-allyl)]-[1,3,2]dioxaborolane-4,5-dicarboxylic acid dimethyl ester)
5 and Allylation of acetyl-cyclosporin A aldehyde

4.88 g (40.56 mmol, 10 equiv) magnesium sulfate dihydrate were added under stirring, followed by 7.23 g (40.56 mmol, 10 equiv.) L-(+)-dimethyltartrate. After 2 hours stirring at RT, the suspension was cooled to 0 °C and 5 g (4.056 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde were added in one portion. The reaction
10 was monitored by HPLC (ca 90% conversion after 3 hours). After 17 hours stirring at 0 °C, the suspension was filtered and the filtrate was washed with 50 ml half saturated aqueous NH₄Cl solution, 50 ml half saturated aqueous NaHCO₃ solution and 50 ml half saturated aqueous NaCl solution. The aqueous phases were re-extracted with 50 ml THF and discarded. The combined organic phases were dried
15 over Na₂SO₄, filtered and concentrated at 40 °C under reduced pressure to provide 11.1 g of a ca 75:25 mixture of β-trimethylsilyl alcohol diastereomers as a light yellow oil.

iii) Peterson elimination

The crude β-trimethylsilyl alcohol diastereomers mixture (11 g, maximum
20 4.056 mmol) was dissolved in 25 ml THF. 0.679 ml (12.16 mmol, 3 equiv.) concentrated sulfuric were added dropwise maintaining the temperature between 20 °C and 25 °C. After 2 hours at RT, 50 ml half saturated aqueous NaCl solution were added. The resulting mixture was extracted twice with 50 ml MTBE. The organic phases were washed with 50ml of a half saturated aqueous NaCl solution,
25 combined, dried over Na₂SO₄ and concentrated under reduce pressure at 40°C. The resulting crude E-acetyl-ISA247 was re-dissolved in 20 ml dichloromethane and concentrated under reduced pressure. The crude product was dissolved in 60 ml MTBE. The crystallization started within 10 min. The suspension was stirred for an additional 15 min. at RT and 2 hours at -10 °C. The crystals were isolated by
30 filtration, washed with 20 ml cold MTBE (-20 °C) and dried under reduced pressure to provide 3.6 g of (E)-acetyl-ISA247 in ca 98% isomeric purity by NMR.

Example 3

i) Preparation of diethanolamine complex of formula V : 2-(E-(3-trimethylsilyl-allyl))-[1.3.6.2]dioxazaborocane

50 g (424.5 mmol, 1 equiv.) allyltrimethylsilane were charged in the reaction vessel followed by 150 ml THF. To the clear colorless solution were added dropwise over 15 min., 165.1 ml (445.7 mmol, 1.05 equiv.) of a 2.7M butyllithium solution in heptane, maintaining the temperature between 20 °C and 26 °C. After 2 hours reaction at RT, the orange solution was cooled to -78 °C. 105.7 ml (445.8 mmol, 1.05 equiv.) triisopropylborate were added dropwise over 20 min., maintaining the temperature below -60 °C. After 1 hour at -70 °C, the reaction mixture was poured onto 250 ml of a 2M aqueous chlorhydric acid solution (resulting pH : 5-6). After 10 min. stirring, the water phase was separated and discarded. 42.4 g (403.3 mmol, 0.95 equiv) diethanolamine were added to the organic phase. The solution was stirred for 60 min. at RT. 750 ml heptane were added. The biphasic emulsion was partially concentrated at 40 °C (ca 750ml solvent distilled) under reduced pressure. A white precipitate appeared and the suspension was stirred 2 hours at RT. The suspension was filtered. The white solid was washed with 125 ml heptane and dried at 40 °C under reduced pressure overnight to provide 85.7 g of the diethanolamine complex of formula V.

¹H NMR (DMSO, in ppm rel. to TMS) : 6.5 (br s, 1H), 6.15 (dt, 1H), 5.32 (d, 1H), 3.7 (m, 2H), 3.55 (m, 2H), 2.95 (m, 2H), 2.72 (m, 2H), 1.29 (d, 2H), 0 (s, 9H).

¹¹B NMR (DMSO, rel. to external ref. BF₃.Et₂O) : 11.1 br. s

Microanalysis : (contains 0.11 equiv. H₂O by Karl-Fischer titration)

Calcd : C 52.43%, H 9.71%, N 6.12%, B 4.72%, Si 12.27%

Found : C 52.04%, H 9.63%, N 6.36%, B 4.79%, Si 11.3%

ii) Allylation

7.375 g (32.46 mmol, 2 equiv.) of diethanolamine complex of formula V, 20 g (16.23 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde and 80 ml dichloromethane were charged in the reaction vessel at RT. 40 ml water and 2.79 ml (48.69 mmol, 3 equiv.) acetic acid were added under stirring. After 10 min. stirring, a clear biphasic mixture was obtained. The reaction was monitored by HPLC.

iii) Peterson elimination

After overnight reaction, the organic layer was separated and the water phase was discarded. 50 ml THF were added to the organic phase. The solution was concentrated under reduced pressure at 30 °C to half its volume. 100 ml THF were added and the solution was concentrated to 80 ml. The volume was adjusted to 100 ml with THF and the solution was cooled to 0-2 °C. 1.812 ml (32.46 mmol, 2 equiv.) concentrated sulfuric acid were added dropwise over 5 min., maintaining the temperature below 5 °C. After addition, the reaction cooling bath was removed and the temperature was raised to RT. After 4 hours reaction, 40 ml water were added followed by 20 ml MTBE. The aqueous layer was separated and discarded. The organic phase was washed with 40 ml NaHCO₃aq, 20 ml saturated NaCl_{aq}, 40 ml saturated NaCl_{aq}, dried over Na₂SO₄, filtered and concentrated at 40 °C under reduced pressure. The crude E-acetyl-ISA247 was re-dissolved in 200 ml MTBE and crystallization started within a few minutes. After 15 min. at RT and 2.5 hours at 0 °C, the suspension was filtered, the crystals were washed with 50 ml MTBE and dried at 50 °C under reduced pressure to give 18.45 g of (E)-acetyl-ISA247 as a white powder (>98% isomeric purity by NMR).

iv) Hydrolysis

This crude product was hydrolyzed to give (E)-ISA247 in 99% isomeric purity by HPLC.

20 Example 4

i) Allylation

10.02 g (44.1 mmol, 2 equiv.) diethanolamine complex of formula V obtained by the method described in Example 3, i), and 30 g (22.05 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde were charged in the reaction vessel. 36 ml acetic acid were added at RT. A clear solution was obtained after 15 min. stirring at RT. The reaction was monitored by HPLC.

ii) Peterson elimination

After ca 6 hours reaction at RT, 60 ml formic acid were added, maintaining the temperature below 30 °C. The clear light yellow solution was stirred overnight at RT. 18 ml dichloromethane and 300 ml MTBE were added followed by 180 ml of a 10% NaCl_{aq} solution. The aqueous phase was separated and discarded. The organic phase was washed with 180 ml water, 300 ml 2M aqueous NaOH and 90 ml

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water. The organic phase was concentrated at RT under reduced pressure. The crystallization started and the suspension was diluted by addition of 300 ml MTBE and concentrated to ca 330 ml. After stirring 3 hours at RT and 1 hour at 0-2 °C, the white suspension was filtered. The crystals were washed with 50 ml MTBE and dried at 50 °C under reduced pressure to give 27.4 g of (E)-acetyl-ISA247 as a white powder in >98% double bond isomeric purity by NMR.

iii) Hydrolysis

This product was hydrolyzed to give (E)-ISA247 in 99.6% double bond isomeric purity by HPLC.

10 Example 5

1 g (0.82 mmol, 1 equiv.) acetyl-cyclosporine A aldehyde were dissolved in 10 ml dichloromethane followed by 369 mg (1.62 mmol, 2 equiv.) diethanolamine complex of formula V obtained by the method described in Example 3, i). The turbid solution was cooled to -40 °C. 180 µl (369 mmol, 2 equiv.) boron trifluoride etherate were added keeping the temperature below -40 °C. After 1 hour at -40 °C, the cooling bath was removed and the reaction mixture was warmed up to RT. After 50 min. reaction at RT, 15 ml of a 5% aqueous NaHCO₃ solution were added. The aqueous phase was separated and re-extracted with 15 ml dichloromethane. The combined organic phases were dried over MgSO₄, filtered and concentrated under reduced pressure at 40 °C to give 0.99 g of (E)-acetyl-ISA247 in >95% double bond isomeric purity (NMR) as a white foam.

Example 6

25 i) Preparation of the pinacol complex of formula VII: 4,4,5,5-tetramethyl-2-(E-(3-trimethylsilyl-allyl))-[1,3,2]dioxaborolane

20 g (169.8 mmol, 1 equiv.) of allyltrimethylsilane were dissolved in 60 ml THF. 69.18 ml (186.8 mmol, 1.1 equiv.) of a 2.7M butyllithium solution in heptane were added dropwise over 10 min. maintaining the temperature between 20 °C and 26 °C. After 2 hours reaction at RT, the yellow solution was cooled to -78 °C. 42.26 ml (178.3 mmol, 1.05 equiv.) of triisopropylborate were added dropwise over 10 min., maintaining the temperature below -65 °C. After 1 hour reaction, the reaction mixture was poured onto 100 ml of a 2M aqueous chlorhydric acid

solution (resulting pH 6-7). 20 ml dichloromethane were added and the water phase was separated and discarded. The organic layer was dried over MgSO_4 , filtered and concentrated to about 100 ml. 20.48 g (169.8 mmol, 1.0 equiv.) pinacol were added and the resulting solution was stirred 18 hours at RT. The reaction mixture was concentrated at 40 °C under reduced pressure and the resulting oil was distilled at 43-50 °C under 0.2 mbar pressure to give 37.2 g of a colorless oil.

ii) One-pot allylation/Peterson elimination

20 g (15.06 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde and 5.427 g (22.59 mmol, 1.5 equiv.) pinacol complex obtained in i) and 30 ml acetic acid were charged in the reaction vessel at RT under stirring. 30 ml of formic acid were added under water bath cooling, maintaining the temperature between 20-22 °C. After 2 hours reaction at RT, 12 ml dichloromethane and 200 ml MTBE were added followed by 120 ml of a 10% aqueous NaCl solution. The water phase was separated and discarded. The organic phase was washed with 120 ml water, 204 ml 2M aqueous NaOH solution and 60 ml water. The organic phase was concentrated at 30 °C until the crystallization started. 200 ml MTBE were added and the suspension was concentrated to ca 220 ml. After stirring at RT for 2 hours and for 1 hour at 0-2 °C, the suspension was filtered. The solid was washed with 30 ml MTBE and dried at 50 °C under reduced pressure to provide 18 g of (E)-acetyl-ISA247 as a white powder in >98% double bond isomeric purity (by NMR).

iii) Hydrolysis

This product was hydrolyzed to give (E)-ISA247 in 99.7% double bond isomeric purity by HPLC.

Example 7

2 g (1.623 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde and 779.8 mg (3.246 mmol, 2 equiv.) pinacol boronate obtained by the method described in Example 6, i) were dissolved in 20 ml dichloromethane. The solution was cooled to -70 °C and 1.28 ml (10.22 mmol, 6.30 equiv.) borontrifluoride etherate were added. After 30 min. at -70 °C, the reaction mixture was slowly warmed up to 0 °C and reaction was continued for 60 min. at 0 °C. 20 ml water were added. The organic phase was separated, washed with 20 ml of a 5% aqueous NaHCO_3 solution, dried over MgSO_4 , filtered and concentrated at 40 °C under reduced pressure to give 2.1

g of (E)-acetyl-ISA247 in >95% double bond isomeric purity (NMR) as a white foam.

Example 8

5 i) Preparation of allyltrifluoroborate reagent: Potassium B-(E-(3-trimethylsilyl-allyl))-trifluoroborate

5 g (21.35 mmol, 1 equiv) of diethanolamine complex obtained by the method described in Example 3, i), 20 ml dichloromethane, 20 ml water and 2.44 ml (42.70 mmol, 2 equiv.) acetic acid were charged in the reaction vessel under stirring at RT. After 30 min. stirring, the water phase was separated and discarded.
10 20 ml methanol were added to the organic phases and the solution was concentrated at 40 °C under reduced pressure to 5-10 ml. 40 ml methanol were added followed by 3.34 g (42.70 mmol, 2 equiv.) KHF₂. After 60 min. stirring at RT, the remaining solid was filtered discarded. The filtrate was concentrated under reduced pressure at 40 °C to ca 25 ml. The solution was cooled to 0-2 °C and the a
15 white suspension was obtained. After 30 min. at 0-2 °C, the suspension was filtered and the solid was washed with cold methanol (-20 °C) and dried under reduced pressure at 40 °C to give 3.4 g of a white powder.

¹H NMR (DMSO, δ in ppm rel. to TMS) : 6.15 (1H, dt), 5.15 (1H, d), 3.5 (2H, br s water), 1.1 (2H, m), 0 (9H, s).

20 ¹¹B NMR (δ in ppm rel. to BF₃.Et₂O external ref.) : 3.8 (q)

Microanalysis : C₁₅H₁₃F₃BKSi (contains 1.08 equiv. H₂O by Karl-Fischer titration and 0.5 equiv. KP) :

Calcd : C 26.83%, H 5.65%, F 24.78%, B 4.02%, K 21.8%, Si 10.5%

Found : C 26.33%, H 5.74%, F 24.71%, B 3.89%, K 22%, Si 9.93%

25 ii) Allylation

2 g (1.623 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde were dissolved in 10 ml dichloromethane. 10 ml water were added, followed by 735 mg (3.246 mmol, 2 equiv.) of trifluoroborate obtained in i). After 2 hours stirring at RT, the organic phase was separated and the water phase discarded.

30 iii) Peterson elimination

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5 ml THF were added to the organic phase and the solution was cooled to 0-2 °C. 181 µl (3.246, 2 equiv.) concentrated sulfuric acid were added. The reaction mixture was warmed up to RT. After stirring overnight, 20 ml water were added. The aqueous layer was separated and discarded. The organic phase was washed with 20 ml of 5% aqueous NaHCO₃ solution, dried over MgSO₄, filtered and concentrated under reduced pressure at 40 °C to give 2 g of (E)-acetyl-ISA247 as a white foam in >98% double bond isomeric purity (by NMR).

Example 9

2 g (1.623 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde and 735 mg (3.246 mmol, 2 equiv.) trifluoroborate obtained by the method described in Example 8, i) and 20 ml dichloromethane were charged in the reaction vessel. The suspension was cooled to -70 °C and 1.28 ml (10.22 mmol, 6.3 equiv.) borontrifluoride etherate were added. After 60 min. at -70 °C, 20 ml water were added. The organic phase was separated, washed with 20 ml of a 5% aqueous NaHCO₃ solution, dried over MgSO₄, filtered and concentrated at 40 °C under reduced pressure to give 2.0 g of (E)-acetyl-ISA247 in >98% double bond isomeric purity (NMR) as a white foam.

Example 10

i) Allylation by an allyltitanium reagent

2.67 ml (16.23 mmol, 10 equiv.) allyltrimethylsilane were dissolved in 6 ml THF. 10.14 ml (16.23 mmol, 10 equiv.) of a 1.6M butyllithium solution in hexane were added dropwise maintaining the temperature between 14-20 °C. After 30 min. at 26 °C, the orange solution was cooled down to -75 °C. 4.8 ml (16.23 mmol, 10 equiv.) titanium tetraisopropoxide were added dropwise over 10 min., maintaining the temperature below -68 °C. After 1 hour reaction at -77 °C, 2 g (1.623 mmol, 1 equiv.) in solution in 6 ml THF were added dropwise maintaining the temperature below -72 °C. The reaction mixture was stirred for 2 hours at -76 °C. The temperature was slowly raised to -40 °C and the stirring was continued for a further 2 hours at -40 °C. The reaction mixture was poured onto a mixture consisting of 32.5 ml of a 1M aqueous HCl solution and 20 ml MTBE. 16.2 ml of a 1M aqueous HCl solution and 25 ml water were added. The aqueous layer was separated and re-extracted with 25 ml MTBE. The organic layers were washed with 30 ml of 0.5 M HCl_{aq}, combined, dried over Na₂SO₄, filtered and concentrated

under reduced pressure at 40 °C to give 2.26 g of crude mixture of diastereomeric anti β -trimethylsilyl alcohols as a white foam.

ii) Peterson elimination

The crude product was dissolved in 11.15 ml THF and 268 μ l concentrated sulfuric acid were added. The reaction mixture was heated at 33 °C for 1.5 hour and then cooled to RT. 22 ml water were added and the reaction mixture was extracted with 22 ml MTBE. The aqueous phase was re-extracted with 11 ml MTBE. The organic layer were washed with 11 ml water, combined, dried over Na_2SO_4 , filtered and concentrated at 40 °C under reduced pressure to give 1.89 g of crude (E)-acetyl-ISA247 as a beige powder. The crude product was re-dissolved in 20 ml MTBE at RT. The crystallization started within a few minutes. The suspension was stirred 30 min. at RT, 45 min. at -10 °C and was filtered. The solid was washed with cold MTBE and dried at 40 °C under reduced pressure to give 1.02 g of (E)-acetylISA247 as a white powder in ca 98% double bond isomeric purity (NMR).

Example 11

i) Allylation by an allyltitanium reagent

1.87 g (15.85 mmol, 10 equiv.) of allyltrimethylsilane were dissolved in 20 ml of THF at RT. 5.87 ml (15.85 mmol, 10 equiv.) of a 2.7 M solution of butyllithium in heptane were added dropwise over 5 min., keeping the temperature between 16 °C and 20 °C. After 1 hour stirring at RT, the yellow to orange solution was cooled to -76 °C. A solution of 4.22 g (15.85 mmol, 10 equiv.) of titanium chlorotriisopropoxide in 10 ml THF was added dropwise over 4 min., keeping the temperature below -60 °C. The resulting brown-red solution was stirred for 30 min. at -75 °C. A solution of 2 g (1.585 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde in 10 ml of THF was added dropwise over 5 min. maintaining the temperature below -60 °C. After 30 min. at -75 °C, the cooling bath was removed and the temperature was raised to -10 °C over ca 15 min. The reaction mixture was added to a biphasic mixture consisting of 40 ml MTBE and 35 ml of a 2M aqueous HCl solution. The aqueous layer was separated and discarded. The organic phase was washed with 24 ml of 1M aqueous HCl solution, 15 ml of a 10% aqueous NaCl solution, 15 ml of a half saturated aqueous NaCl solution, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to provide 2 g of the crude mixture of anti β -trimethylsilyl alcohol diastereomers as a solidifying oil.

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ii) Peterson elimination

The crude product was dissolved in 8 ml THF at RT. The solution was cooled to 0-5 °C and 200 µl of concentrated sulfuric acid were added dropwise. The temperature was raised to RT and the reaction mixture was stirred 10 hours. 40 ml MTBE and 15 ml of water were added. The water phase was separated and discarded. The organic phase was washed 15 ml of a 5% aqueous NaHCO₃ solution, 15 ml of a half saturated aqueous NaCl solution, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give 1.8 g of crude E-acetyl-ISA247. The crude diene was dissolved in 20 ml dichloromethane. 20 ml MTBE were added, and the solution was concentrated at 40 °C under reduced pressure to half its volume. The last two operations was repeated three times to in order to exchange the solvent from dichloromethane to MTBE. The solution was cooled to RT and the crystallization started within a few minutes. The suspension was stirred 2 hours at RT and 30 min. at 0 °C. The suspension was filtered. The solid was washed with 15 ml MTBE and dried under reduced pressure at 40 °C to give 1.1 g of E-acetyl-ISA247 in >95% double bond isomeric purity (NMR), as a white powder.

Example 12

i) Allylation by allylaluminum reagent :

1.87 g (15.85 mmol, 10 equiv.) of allyltrimethylsilane were dissolved in 20 ml of THF at RT. 5.87 ml (15.85 mmol, 10 equiv.) of a 2.7M solution of butyllithium in heptane were added dropwise over 5 min., keeping the temperature between 20 °C and 25 °C. After 1 hour stirring at RT, the yellow to orange solution was cooled to -75 °C. 8.6 ml (15.85 mmol, 10 equiv.) of a 25% solution of diethylaluminum chloride in toluene were added over 10 min., keeping the temperature below -55 °C. The resulting clear colorless solution was stirred for 30 min. at -75 °C. A solution of 2 g (1.585 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde in 10ml of THF was added dropwise over 5min. maintaining the temperature below -60 °C. After 30 min. at -75 °C, the cooling bath was removed and the temperature was raised to -10 °C over 15 min. The reaction mixture was slowly added (under cold water bath cooling 10 °C) to a biphasic mixture consisting of 40 ml MTBE and 35 ml of a 1M aqueous HCl solution. The aqueous layer was separated and discarded. The organic phase was washed with 35 ml of 1M aqueous HCl solution, 25 ml of water, 25 ml of a saturated aqueous NaCl

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solution, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to provide 2 g of the crude mixture of anti β -trimethylsilyl alcohol diastereomers as a solidifying oil.

ii) Peterson elimination

5 The crude product was dissolved in 10 ml THF at RT. The solution was cooled to 0-5 °C and 200 μl of concentrated sulfuric acid were added dropwise. The temperature was raised to RT and the reaction mixture was stirred overnight. 40 ml MTBE and 15 ml of water were added. The water phase was separated and discarded. The organic phase was washed with 15 ml water, 15 ml of a 5% aqueous
10 NaHCO_3 solution, 15 ml of a half saturated aqueous NaCl solution, filtered and concentrated under reduced pressure to give 1.8 g of crude E-acetyl-ISA247. The crude diene was redissolved in 35 ml of MTBE. The crystallization started within a few minutes. The suspension was stirred 2 hours at RT and 30 min. at 0 °C. The suspension was filtered. The solid was washed with 15 ml MTBE and dried under
15 reduced pressure at 40 °C to give 1 g of E-acetyl-ISA247 in >95% double bond isomeric purity (NMR), as a white powder.

Example 13

Preparation of a solution of boron reagent of formula IIIa : (E)-3-(trimethylsilyl)allylboronic acid

20 2 g (8.8 mmol, 1 equiv.) of diethanolamine complex of formula V as prepared in Example 3-i) was dissolved in 16 ml of d_2 -dichloromethane (deuterated dichloromethane). 759 μl (13.2 mmol, 1.5 equiv.) of acetic acid were added, followed by 4 ml of water. The biphasic mixture was stirred for 20 min. at RT to give a light yellow clear biphasic mixture. Stirring was stopped, the water phase
25 was separated and discarded. The organic phase (16 ml volume) consisted in a solution of boronic acid of formula IIIa as evidenced by ^{11}B NMR and ^1H NMR.

^{11}B NMR (δ in ppm relative to $\text{BF}_3 \cdot \text{Et}_2\text{O}$ as external reference) : 31.7

^1H NMR (in CD_2Cl_2 , δ in ppm relative to TMS) : 6.1 (1H, dt), 5.6 (1H, d), 1.77 (2H, d), 0 (9H, s).

30 Example 14

Preparation of a solution of boron reagent of formula IVa': (R,R)-2-[(E)-(3-trimethylsilyl-allyl)]-[1,3,2]dioxaborolane-4,5-dicarboxylic acid dimethyl ester

To 4 ml (2.2 mmol, 1 equiv.) of the solution of the boronic acid of formula IIIa, prepared as described in Example 13, were added 396 mg (2.2 mmol, 1 equiv.) of L-(+)-dimethyltartrate and 265 mg (2.2 mmol, 1 equiv.) of magnesium sulfate dihydrate. The suspension was stirred for 40 min. at RT and was filtered. The filtrate was analyzed by NMR and was shown to contain, as main product the boronate ester of formula IVa' as evidenced by the appearance of consistent ^{11}B and ^1H NMR signals.

^{11}B NMR (in CD_2Cl_2 , δ in ppm relative to $\text{BF}_3 \cdot \text{Et}_2\text{O}$ as external reference) : 34.2

^1H NMR (in CD_2Cl_2 , δ in ppm relative to TMS) : 6.07 (1H, dt), 5.64 (1H, d), 1.93 (2H, d), 0 (9H, s).

Example 15

Hydrolysis of E-acetyl-ISA247 to E-ISA247

15 g (11.94 mmol, 1 equiv.) of E-acetyl-ISA247 were dissolved at RT in 270 ml of methanol. A solution of 14.85 g (107.5 mmol, 9 equiv.) of potassium carbonate in 60 ml of water was added keeping the temperature below 27 °C. The white suspension was heated to 30 °C. The reaction was monitored by HPLC. After 22 hours of reaction, the methanol was evaporated at 40 °C under reduced pressure. The residue was taken up in 150 ml of ethylacetate. The water phase was separated and discarded. The organic phase was washed with 45 ml of a 5% aqueous solution of citric acid and 45 ml of a half saturated aqueous NaCl solution, dried over Na_2SO_4 , filtered and concentrated under reduced pressure at 40 °C to give 15.1 g of E-ISA247 (85% assay). Purification can be performed by chromatographic techniques like preparative HPLC.

Example 16

i) Preparation of the pinacol complex of formula VII : 4,4,5,5-tetramethyl-2-(E-(3-trimethylsilyl-allyl))-[1,3,2]dioxaborolane

100 g allyltrimethylsilane (1 equiv.) were charged in reactor 1 followed by 300 ml THF. The solution was cooled to 10-15 °C and 374 ml of 2.5M Butyllithium

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solution in hexane (1.1 equiv.) were added keeping the temperature below 25 °C (over ca 30 min.). After 1-2 hours at 20-25 °C, the yellow to orange solution was cooled -50 °C. 173 g Triisopropylborate (1.05 equiv.) were added dropwise keeping the temperature below -40 °C (over ca 30-45 min.). The dropping funnel
5 was washed with 25 ml THF. After 30 min. to 1 hour at -50 °C to -40 °C, a solution of 102.4 g of pinacol (1 equiv.) in 100 ml THF was added, keeping the temperature below -30 °C. The dropping funnel was washed with 25 ml THF. After 30 min. at -50 °C to -30 °C, the content of reactor 1 was poured, under stirring, onto a mixture of 61.2 g AcOH (1.2 equiv.) and 250 ml water (contained
10 in reactor 2), keeping the temperature between 0-25 °C. Reactor 1 was washed with 50 ml THF.

Stirring was discontinued in reactor 2, the aqueous phase was separated and discarded. The organic layer was washed with 250 ml water. The organic phase was concentrated to ca 500 ml (Ti = 20-40 °C, 150-200 mbar). 500 ml Toluene
15 were added and the organic phase was concentrated to 500 ml (Ti = 40-50 °C, Tj = 50 °C, 150-40 mbar). 500 ml Toluene were added and the organic phase was concentrated until constant volume (Ti = 40-50 °C, Tj = 50 °C, 150-10 mbar) to provide crude pinacol complex in >90% yield. The complex could be used directly in the allylation-Peterson elimination sequence or could be distilled under reduced
20 pressure (Ti = ca 65 °C, Tdest = ca 50 °C, P = 0.05-0.15 mbar).

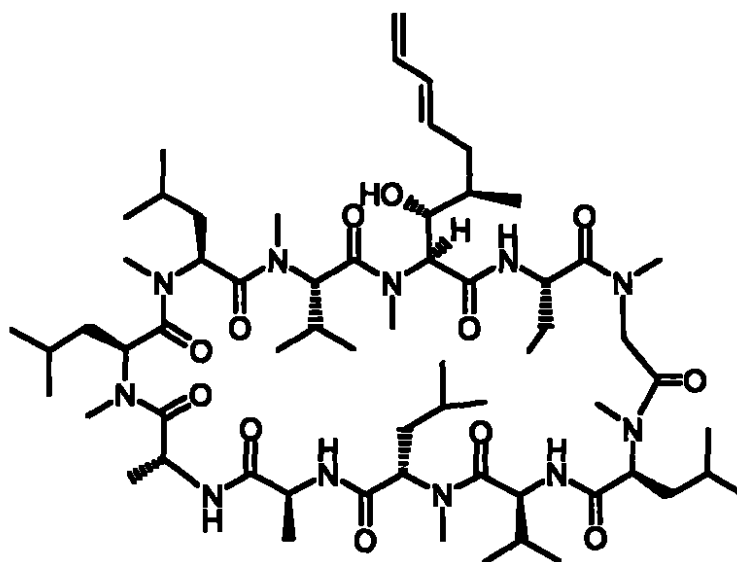
ii) One-Pot Allylation-Peterson Elimination

40 g acetyl protected CsA-Aldehyde were charged in a feed vessel followed by 80 ml isopropyl acetate. The suspension was transferred to the reactor. The feed vessel was washed with 50 ml acetic acid which was transferred to the reactor. A
25 clear solution was then obtained. Pinacol complex (1.25-1.5 equiv.) was added. The clear solution was heated to 40 °C. 50 ml formic acid were added. After completion of the allylation and Peterson elimination as evidenced by HPLC analysis (after ca 15-20 hours), 246 ml isopropyl acetate were added. The reaction mixture was washed twice with 200 ml water, 300 g of 2M aqueous KOH solution
30 (pH of the aqueous phase set between 5-8, if necessary with additional KOH solution) and 200 ml of 5% aqueous ammonium formate. The organic phase was concentrated to ca 120 ml (Ti = ca 40 °C, ca 200 mbar) and was diluted with 300 ml methanol. The organic phase was concentrated to ca 120 ml (Ti = ca 40 °C, 200 mbar) and was diluted with 240 ml methanol (Ti = ca 40 °C, 200 mbar). The
35 organic phase was concentrated to ca 280 ml. 130 ml water were added over ca 60

min. at 20-25 °C. The resulting white suspension was stirred 60 min. at room temperature. The solid was isolated by filtration, washed twice with 52 ml of a water/methanol mixture, dried under vacuum (T=50 °C) until constant weight to provide E-acetyl-ISA247 (ca 35 g).

Claims

1. A process for the preparation of a cyclosporin A analog of formula I

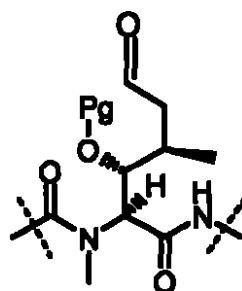


I

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comprising

- a-i) allylating a compound of formula II



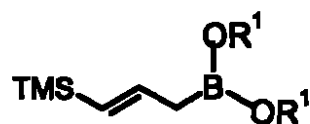
II

:

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,wherein Pg is a protecting group and the dotted lines mean that the remainder of the compound has the same structure as that of the compound of formula I,

with a compound of formula III



III

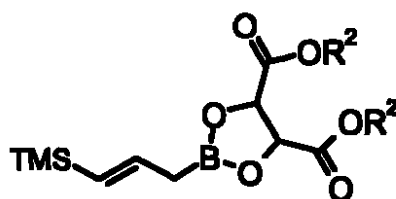
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,wherein R¹ is hydrogen, C₁-₈ alkyl or C₃-₈ cycloalkyl and/or, when R¹ is hydrogen, a trimer thereof;

or

a-ii) allylating a compound of formula II with a compound of formula IV

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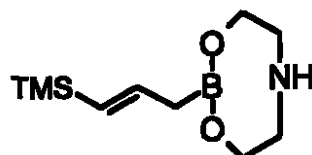
IV

wherein R² is C₁-₈ alkyl or C₃-₈ cycloalkyl;

or

a-iii) allylating a compound of formula II with a compound of formula V

15



V

or

a-iv) allylating a compound of formula II with a compound of formula



VI

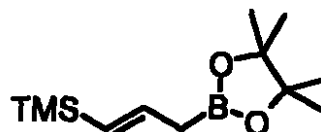
VI

5

or

a-v) allylating a compound of formula II with a compound of formula

VII



VII

10

or

a-vi) allylating a compound of formula II with a reaction mixture obtained by a process comprising;

i) reacting allyltrimethylsilane with butyllithium to form trimethylsilylallyllithium;

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ii) reacting trimethylsilylallyllithium with triisopropylborate or trimethylborate, and then conducting aqueous work up,

or

;

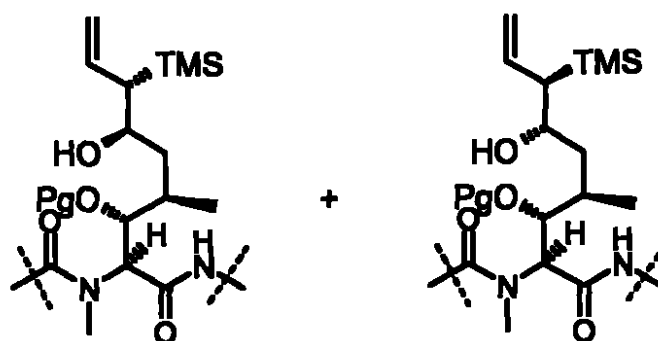
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a-vii) allylating a compound of formula II with a reaction mixture obtained by reaction of the trimethylsilylallyllithium with diethylaluminum chloride,

or

5 a-viii) allylating a compound of formula II with a reaction mixture obtained by reaction of the trimethylsilylallyllithium with titanium tetraisopropoxide or titanium chlorotrisopropoxide,

to form a compound of formula XI;



XI

10 wherein Pg is as defined above;

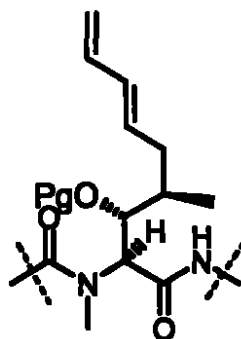
and

b) converting the compound of formula XI to the cyclosporin A analog of formula I.

2. The process according to claim 1, wherein step b) is conducted by

15 b-i) converting the compound of formula XI to a compound of formula XII

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XII

wherein Pg is as defined in claim 1,

under acidic conditions; and

- 5 b-ii) converting the PgO group of the compound of formula XII to a hydroxyl group.
3. The process according to claim 1 or 2, wherein Pg is acetyl group.
4. The process according to claim 3, wherein step a-i), a-ii) or a-vi) is conducted in the presence of tartrates.
- 10 5. The process according to claim 3, wherein step a-i), a-ii) or a-vi) is conducted in dichloromethane or toluene.
6. The process according to claim 3, wherein step a-iii) is conducted in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$, formic acid, acetic acid or tartrate esters.
7. The process according to claim 6, wherein step a-iii) is conducted in
15 water/dichloromethane or water/toluene.
8. The process according to claim 6, wherein step a-iii) and b-i) are conducted in dichloromethane or tetrahydrofuran and in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
9. The process according to claim 6, wherein step a-iii) is conducted in
20 acetic acid and/or formic acid; or in a mixture of acetic acid and /or formic

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acid and one or two cosolvents selected from a group consisting of dichloromethane and tetrahydrofuran.

- 5 10. The process according to claim 9, wherein step a-iii) is conducted in acetic acid and step b-i) is conducted by addition of formic acid to the reaction mixture.
11. The process according to claim 9, wherein step a-iii) and b-i) are conducted in formic acid or acetic acid/ formic acid.
- 10 12. The process according to claim 1 or 2, wherein step a-iv) is conducted in water/dichloromethane or water/toluene.
13. The process according to claim 3, wherein step a-iv) and b-i) are conducted in dichloromethane, tetrahydrofuran or toluene in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- 15 14. The process according to claim 3, wherein step a-v) is conducted in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
15. The process according to claim 14, wherein step a-v) and b-i) are conducted in dichloromethane, tetrahydrofuran or toluene and in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- 20 16. The process according to claim 3, wherein step a-v) is conducted in the presence of formic acid or acetic acid.
17. The process according to claim 16, wherein step a-v) and b-i) are conducted in formic acid or acetic acid/formic acid.
- 25 18. The process according to claim 17, wherein step a-v) and b-i) are conducted in a mixture of acetic acid/formic acid and co-solvent selected from dichloromethane, toluene, ethyl acetate and isopropyl acetate.
19. The process according to claim 18, wherein co-solvent is isopropyl acetate.

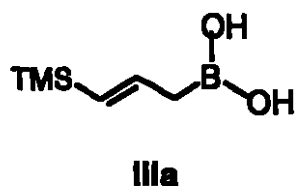
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20. The process according to claim 16, wherein step a-v) is conducted in acetic acid and step b-i) is conducted by addition of formic acid to the reaction mixture.

5 21. The process according to claim 3, wherein step a-vii) is conducted by allylating the compound of formula II with a reaction mixture prepared by reaction of the trimethylsilylallyllithium with diethylaluminum chloride.

10 22. The process according to claim 3, wherein step a-viii) is conducted by allylating the compound of formula II with a reaction mixture prepared by reaction of trimethylsilylallyllithium with titanium tetraisopropoxide or titanium chlorotrisopropoxide.

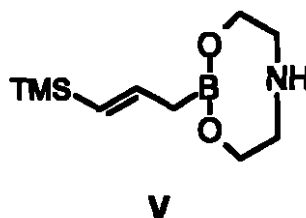
23. A process for the preparation of the compound of formula IIIa



and/or a trimer thereof,

15 comprising reacting the compound of formula V with water in dichloromethane.

24. A compound of formula V



25. A process for the preparation of a compound of formula V, comprising

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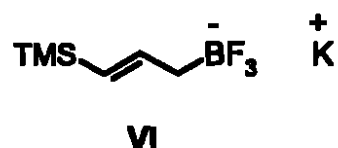
i) reacting allyltrimethylsilane with butyllithium to form trimethylsilylallyllithium;

ii) reacting trimethylsilylallyllithium with triisopropylborate or trimethylborate;

5 iii) conducting aqueous work up; and

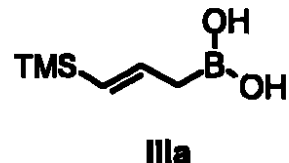
iv) reacting the compounds formed in iii) with diethanolamine to form a compound of formula V.

26. A compound of formula VI



10 27. A process for the preparation of a compound of formula VI, comprising

i) reacting a compound of formula V with water to form a compound of formula IIIa

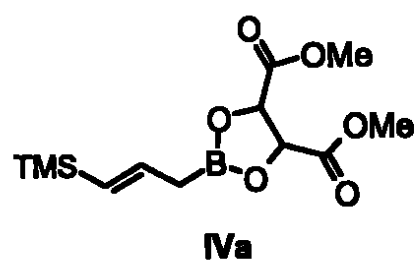


15 and

ii) exchanging the solvent of the separated organic phase of the reaction mixture obtained in step i) to methanol,

iii) reacting a solution of compound of formula IIIa obtained in step ii) with KHF_2 to form a compound of formula VI.

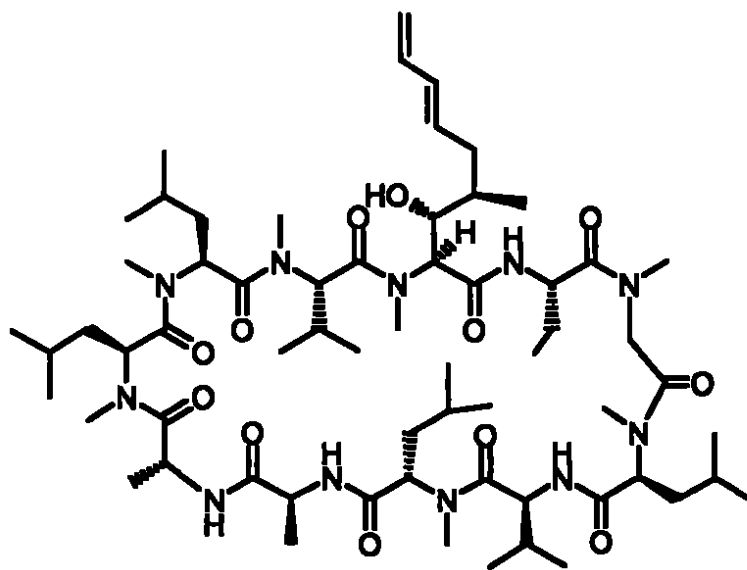
20 28. A compound of formula IVa



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Abstract

The invention is concerned with a new process for the preparation of a cyclosporin A analog of formula I



I

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comprising

a) allylating a protected cyclosporin A aldehyde with a allylmetal reagent and

b) converting the compound obtained in step a) to the cyclosporin A analog
of formula I.

10

PATENT COOPERATION TREATY

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Applicant's or agent's file reference

Case 21711

IMPORTANT NOTIFICATION

International application No.

PCT/EP2004/003504

International filing date (day/month/year)

02/04/2004

Priority date (day/month/year)

08/04/2003

Applicant

F. HOFFMANN-LA ROCHE AG

Title of the invention

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The undersigned applicant(s) (Names should be indicated as they appear in the request):

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Isotechnika Inc.
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Edmonton, Alberta T6G 2C8, Canada

hereby appoints (appoint) the following person as:

☒ agent

☐ common representative

Name and address

(Family name followed by given names; for a legal entity, full official designation. The address must include postal code and name of country.)

Witte, Hubert
Rauber, Beat
Kjellss-Berger, Hanny

} Grenzacherstrasse 124
CH-4070 Basel, Switzerland

to represent the undersigned before

☒ all the competent International Authorities

☐ the International Searching Authority

☐ the International Preliminary Examining Authority only

in connection with the international application identified below:

Title of the invention: Process for preparation of cyclosporin A analog

Applicant's or agent's file reference: Case 21711

International application number (if already available):

filed with the following Office European Patent Office, Munich, Germany
and to make or receive payments on behalf of the undersigned.

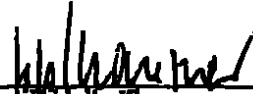
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Date: March 15, 2004

F.HOFFMANN-LA ROCHE AG


Fridolin Klausner
Holder of procuration

i.v. 
Denise Strebel
Mandatory

Date: May 18, 2004.

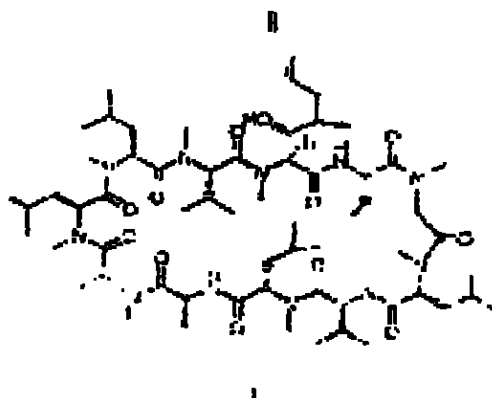
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Proceso para la preparación del análogo de ciclosporina A

El presente invento se relaciona con un nuevo proceso para la preparación de análogo de la ciclosporina A de la formula I:



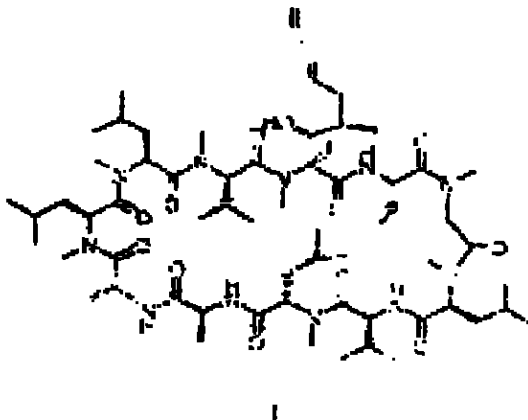
Igualmente se aplica a los intermedios para este proceso y demás procesos utilizados en su preparación.

El análogo de la ciclosporina A de la formula I es estructuralmente idéntico a la ciclosporina A excepto por las modificaciones que se encuentran en el residuo del 1-amino ácido. Este análogo se divulga en los documentos WO 99/18120 y en la Solicitud Provisional de Patente U.S. No. 60/346.201. En este documento se conocerá con el nombre de (E)-ISA247.

El documento Tetrahedron Letters, Vol.22, No. 29, p2751-2752, 1981 divulga uno de los intermedios del proceso del presente invento, principalmente el pinacol (E)-1-trimetilsilil-1-propene-3-boronato, y el proceso de alilación que lo utiliza.

El Tetrahedron Letters, Vol.36, No. 10, p1583, 1995 divulga el proceso de alilación que utiliza el tartrato modificado (E)- γ -(trimetilsilil)alilboronato.

En un primer aspecto, este invento proporciona un proceso para la preparación de un análogo de ciclosporina A de la formula I



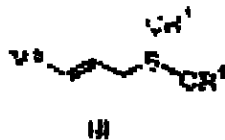
Que comprende:

a-i) alilación de un compuesto de la formula II



donde Pg es un grupo protector y las líneas punteadas significan que el resto del compuesto tiene la misma estructura que la que se encuentra en el compuesto de la formula I,

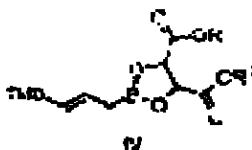
con un compuesto de la formula III



donde R¹ es hidrógeno, alquilo C₁₋₈ o cicloalquilo C₃₋₈ y/o, donde R¹ es hidrógeno, o uno de sus trimeros;

o

a-ii) alilación de un compuesto de la formula II con el compuesto de la formula IV



donde R² es alquilo C₁₋₈ o cicloalquilo C₃₋₈;

o

a-iii) alilación de un compuesto de la formula II con un compuesto de la formula V



a-iv) alilación de un compuesto de la formula II con un compuesto de la formula VI



o

a-v) alilación de un compuesto de la formula II con un compuesto de la formula VII

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o

a-vi) alilación de un compuesto de la formula II con una mezcla de reacción que se obtiene mediante un proceso que comprende;

- i) reacción del aliltrimetilsilano con butillitio para formar trimetilsililalilitio;
- ii) reacción del trimetilsililalilitio con el triisopropilborato o con el trimetilborato,

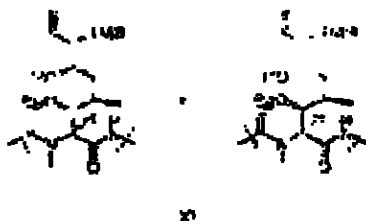
y luego la realización de la conformación acuosa,

o

a-vii) alilación de un compuesto de la formula II con una mezcla de reacción obtenida mediante la reacción del trimetilsililalilitio con el cloruro de dietilaluminio,

o

a-viii) alilación de un compuesto de la formula II con una mezcla de reacción obtenida mediante la reacción del trimetilsililalilitio con tetraisopropoxido de titanio o clorotrisisopropoxido de titanio, para formar un compuesto de la formula XI;



donde Pg corresponde a la definición dada anteriormente;

y

b) la transformación del compuesto de la formula XI en el análogo de la ciclosporina A de la formula I.

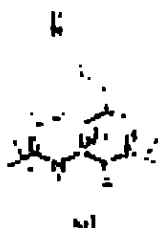
En un segundo aspecto, este invento proporciona intermedios para el proceso mencionado antes.

En un tercer aspecto, este invento proporciona procesos para la preparación de estos mismos intermedios.

Igualmente, dentro del proceso que se definió anteriormente [el cual se analizará mas adelante en el numeral (i)], se prefieren los siguientes procesos:

(ii) El proceso de (i), donde el paso b) es realizado por:

b-i) transformación del compuesto de la formula XI en un compuesto de la formula XII



donde Pg se define en (i),

bajo condiciones ácidas; y

b-ii) la transformación del grupo PgO del compuesto de la formula XII en un grupo hidróxilo.

(iii) El proceso de (i) o (ii), donde Pg es el grupo acetilo.

- (iv) El proceso de (iii), donde el paso a-1), a-ii) o a-vi) se realiza en presencia de tartratos.
- (v) El proceso de (iii), donde el paso a-i), a-ii) o a-vi) se realiza en diclorometano o tolueno.
- (vi) El proceso de (iii), donde el paso a-iii) se realiza en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$, ácido fórmico, ácido acético o esteres de tartrato.
- (vii) El proceso de (vi), donde el paso a-iii) se realiza en agua / diclorometano o agua / tolueno.
- (viii) El proceso de (vi), donde el paso a-iii) y b-i) se realizan en diclorometano o tetrahidrofurano y en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- (ix) El proceso de (vi), donde el paso a-iii) se realiza en ácido acético y/o ácido fórmico; o en una mezcla de ácido acético y/o ácido fórmico y uno o dos cosolventes seleccionados a partir de un grupo conformado por diclorometano y tetrahidrofurano.
- (x) El proceso de (ix), donde el paso a-iii) se realiza en ácido acético y paso b-i) que se realiza por adición de ácido fórmico a la mezcla de reacción.
- (xi) El proceso de (ix), donde el paso a-iii) y b-i) se realizan en ácido fórmico o ácido acético / ácido fórmico.
- (xii) El proceso de (i) o (ii), donde el paso a-iv) se realiza en agua/diclorometano o agua / tolueno.
- (xiii) El proceso de (iii), donde el paso a-iv) y b-i) se realizan en diclorometano, tetrahidrofurano o tolueno en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- (xiv) El proceso de (iii), donde el paso a-v) se realiza en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

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91

(xv) El proceso de (xiv), donde el paso a-v) y b-i) se realizan en diclorometano, tetrahydrofurano o tolueno y en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

(xvi) El proceso de (iii), donde el paso a-v) se realizan en presencia de ácido fórmico o ácido acético.

(xvii) El proceso de (xvi), donde el paso a-iv) y b-i) se realizan en el ácido fórmico o ácido acético/ácido fórmico.

(xviii) El proceso de (xvii), donde el paso a-v) y b-i) se realizan en una mezcla de ácido acético/ácido fórmico y co-solvente seleccionado a partir de diclorometano, tolueno, etil acetato e isopropil acetato.

(xix) El proceso de (xviii), donde el co-solvente es isopropil acetato.

(xx) El proceso de (xvi), donde el paso a-v) se realiza en ácido acético y el paso b-i) se lleva a cabo mediante la adición de ácido fórmico a la mezcla de reacción.

(xxi) El proceso de (iii), donde el paso a-vii) se realiza por alilación del compuesto de la formula II con una mezcla de reacción que se prepara mediante la reacción del trimetilsililalilitio con cloruro de dietilaluminio.

(xxii) El proceso de (iii), donde el paso a-viii) se realiza mediante la alilación del compuesto de la formula II con una mezcla de reacción preparada por reacción del trimetilsililalilitio con tetraisopropoxido de titanio o clorotrisopropoxido de titanio.

Los siguientes términos utilizados en la especificación y reivindicaciones tienen los significados señalados a continuación:

81
82

"alquilo C_{a-b}" tal y como se utiliza en este documento denota una cadena recta o residuos alquilo bifurcados que contienen átomos de carbono *a* a *b*. Por consiguiente, el ejemplo "alquilo C₁₋₈" significa una cadena recta o residuos de alquilo bifurcados que contienen átomos de carbono de 1 a 8, tales como metilo, etilo, propilo, isopropilo, butilo, isobutilo o tert-butilo.

"Cicloalquilo C₃₋₈" se refiere a un radical de hidrocarburo cíclico monovalente saturado de tres a ocho carbonos anulares, por ejemplo, ciclopropilo, ciclobutilo, o ciclohexilo.

"Grupo protector" se refiere a una agrupación de átomos que cuando se adhieren a un grupo reactivo en una molécula enmascara, reduce o evita tal reactividad. Entre los ejemplos de los grupos protectores se pueden citar los encontrados en la obra T.W. Green y P.G. Futs, Protective Groups in Organic Chemistry, (Wiley, 2nd ed. 1991) y Harrison y Harrison et al., Compendium of Synthetic Organic Methods, Volúmenes 1-8 (John Wiley y Sons, 1971-1996).

En las formulas estructurales presentadas en este documento el enlace discontinuo (""") denota que el sustituto se encuentra por debajo del plano del papel y el enlace acuñaado (ˆ) denota que el sustituto está por encima del plano del papel.

Las siguientes abreviaturas utilizadas en la especificación y reivindicaciones especificadas en el texto, tendrán los significados siguientes:

82
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MTBE	metil tert-butileter
THF	tetrahidrofurano
DCM	diclorometano
DMSO	dimetilsulfoxido
HMPA	hexametilfosforamida
TMEDA	tetrametiletilenodiamina
TMS	tetrametilsilano
TMS-	trimetilsililo
Et	etilo
Me	metilo
iPr	isopropilo
Bu	butilo
Ac	acetilo
RT	temperatura ambiente
HPLC	cromatografía líquida de alto desempeño
MS	espectroscopia de masa
TLC	cromatografía de capa delgada
NMR	espectroscopia de resonancia magnética nuclear
2D-COSY	espectroscopia correlacionada de 2 dimensiones
2D-TOCSY	espectroscopia de correlación total bidimensional
HSQC	Coherencia Heteronuclear de Un Solo Quantum
Cryst.	Cristalización
Cpd	compuesto
min.	minuto(s)

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84

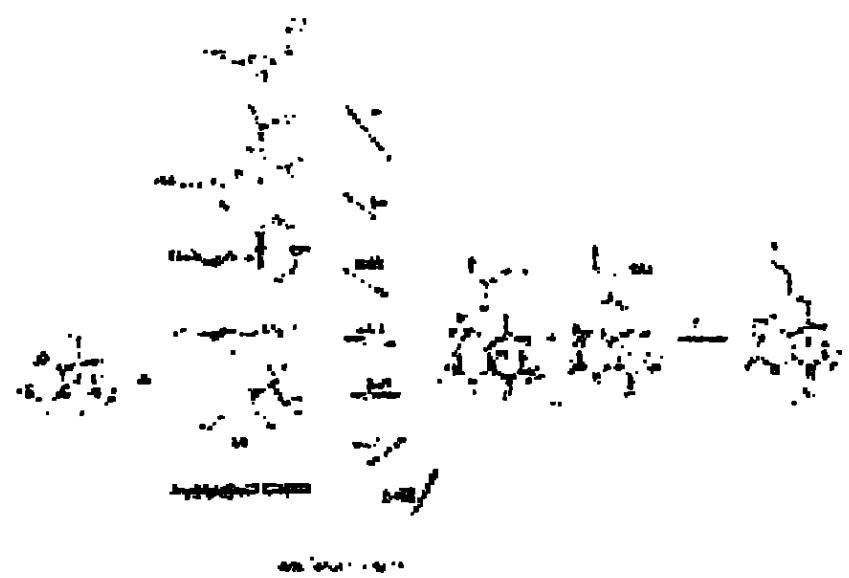
h horas

Los materiales de inicio y los reactivos utilizados en el proceso del presente invento se pueden obtener a través de proveedores comerciales tales como Aldrich Chemical Co., (Milwaukee, Wisconsin, USA), Bachem (Torrance, California, USA), Emka-Chemie, or Sigma (St. Louis, Missouri, USA), Maybridge (Dist: Ryan Scientific, P.O. Box 6496, Columbia, SC 92960), Bionet Research Ltd., (Cornwall PL32 9QZ, UK), Menai Organics Ltd., (Gwynedd, N. Wales, UK), Butt Park Ltd., (Dist. Interchim, Montlucon Cedex, France), Fluka (CH-9471 Buchs, CH), Acros Organics (B-2440 Geel, BE) o se preparan siguiendo los métodos conocidos por expertos en esta técnica, según procedimientos de la literatura como el de *Fieser and Fieser's Reagents for Organic Synthesis*, Volúmenes 1-17 (John Wiley and Sons, 1991), *Rodd's Chemistry of Carbon Compounds*, Volúmenes 1-5 y Suplementarios (Elsevier Science Publishers, 1989), *Organic Reactions*, Volúmenes 1-40 (John Wiley and Sons, 1991), *March's Advanced Organic Chemistry*, (John Wiley and Sons, 1992), y *Larock's Comprehensive Organic Transformations* (VCH Publishers Inc., 1989).

Los materiales de inicio e intermedios de la reacción pueden aislarse y purificarse si así se desea aplicando técnicas convencionales, incluyendo pero no limitándose a la filtración, destilación, cristalización, y cromatografía. Estos materiales se pueden caracterizar aplicando medios convencionales, entre los cuales están las constantes físicas y los datos espectrales.

Los compuestos de la formula I se preparan según se ilustra en el Esquema A. El reactivo alilmetal al cual se hace igualmente referencia en este documento como reactivo de alilación deberá tenerse en cuenta dentro de un sentido general y puede comprender reactivos en los cuales la parte metálica se basa en el boro aun cuando no se trate de un metal por si mismo.

Esquema A:

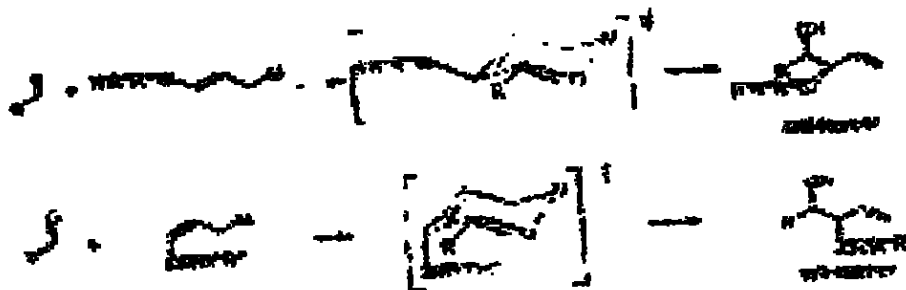


(Pg es un grupo protector, en donde las líneas punteadas significan que el resto del compuesto tiene la misma estructura que el compuesto de la formula I. R^1 es hidrógeno, alquilo C_{1-3} o cicloalquilo C_{3-6} y cuando R^1 es hidrógeno, un compuesto de la formula III incluye uno de sus trimeros, y R^2 es alquilo C_{1-3} o cicloalquilo C_{3-6}).

En el paso a), el aldehído protegido de ciclosporina A de la formula II es alilado por el reactivo alilmetálico alilado y de la formula III, IV, V, VI, VII, VIII, etc., para

formar una mezcla de diastereómeros del alcohol β -sililhomocíclico de la formula XI (El análisis general sobre los alilmetales y la alilación de los aldehídos se encuentra en: W. R. Roush en "Allyl Organometallics", *Comprehensive Organic Synthesis*, Pergammon Press, Vol. 2, pp 1-53; Y. Yamamoto, N. Asao en "Selective Reactions Using Allylic Metals", *Chemical Reviews* 1993, 93, p 2207-2293). El control de la configuración relativa anti o sin del fragmento β -sililalcohol dependerá del reactivo alilmetálico y de las condiciones utilizadas para realizar el paso de alilación del aldehído (para un análisis general de los reactivos alilmetales sustituidos del γ -sililo consultar: T: H. Chan en "Silylallyl Anions in Organic Synthesis: A Study in Regio- and Stereoselectivity", *Chemical Reviews* 1995. 95, p1279-1292). Frecuentemente se considera que este alcohol forma un estado de transición vía anillo de 6 miembros que se asemejan a una silla (conocido también como estado de transición de Zimmerman-Traxler) según se puede apreciar en el Esquema B.

Esquema B



En este estado de transición, la cadena lateral de aldehídos adopta preferiblemente una posición pseudo ecuatorial con el fin de minimizar

interacciones estéricas de 1,3-diales. La configuración relativa del fragmento β -sililalcohol se determinará por lo tanto mediante la configuración de doble enlace C-C del reactivo alilmetal.

Por consiguiente, el uso de los reactivos alilmetálicos trans - cis- γ -sililatados deberá conducir principalmente al anti o syn- β -sililalcohol isómero respectivamente. Lo anterior se aplica en términos generales para el alil-boro, titanio y reactivos de aluminio.

Una excepción a esta norma se encuentra por ejemplo en los casos en los cuales un reactivo trialilalilestanani γ -sililatado se adiciona a los aldehídos bajo condiciones de ácido Lewis, caso en el cual el mecanismo es distinto y la reacción proporciona principalmente el isómero sin β -sililalcohol.

En el paso b), el β -sililalcohol de la formula XI se transforma en (E)-ISA247 de la formula I.

Paso b) se puede realizar según se ilustra en el Esquema C.

Esquema C



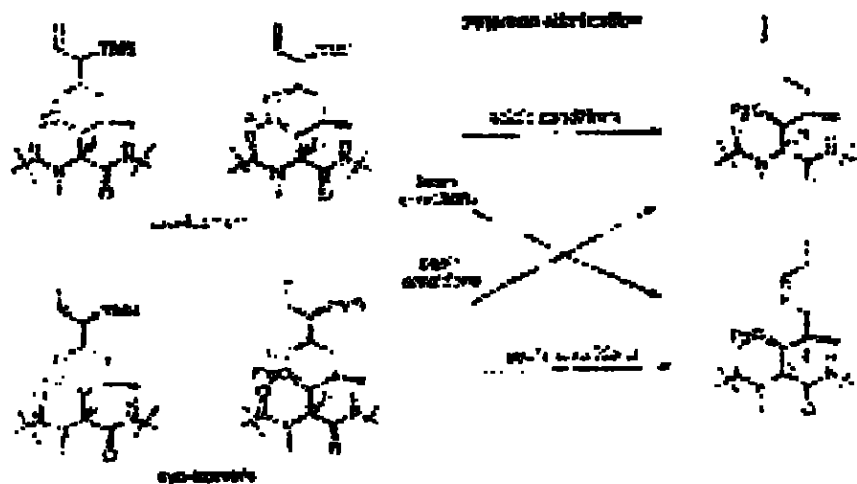
(El Pg y las líneas punteadas tienen los significados indicados anteriormente).

En el paso b-i), el β -sililalcohol de la formula XI se somete a una eliminación Peterson (para el análisis general sobre estas eliminaciones Peterson, consultar la obra: D. J. Ager in "The Peterson Reaction", *Synthesis* 1984, p384-397 al igual que las referencias allí citadas), generándose un doble enlace interno, es decir eliminación del silanol a partir de la ocurrencia del medio β -sililalcohol.

Con miras a alcanzar un alto grado de pureza isomérica de doble enlace, el éxito de la secuencia de eliminación de alilación-Peterson descansa en la introducción selectiva de una anti o syn *configuración* relativa del medio β -sililalcohol.

En realidad la eliminación de Peterson es estereoscópica según se sabe. Los anti isómeros proporcionarán un doble enlace isomérico cuando los syn isómeros produzcan los demás isómeros de doble enlace bajo las mismas condiciones según se ilustra en el Esquema D.

Esquema D



(Pg y líneas puntadas con significado según se indicó anteriormente).

Los anti isómeros deberán producir el enlace trans doble de acuerdo con las condiciones de eliminación ácida de Peterson en tanto que los syn isómeros podrían proporcionar el cis doble enlace. La reacción procede vía un mecanismo en el cual el hidróxilo y los grupos sililo se encuentran en una anti *conformación* con anterioridad a la eliminación.

La situación es opuesta cuando la eliminación Peterson se realiza bajo condiciones básicas, y en tal caso la reacción procede vía un mecanismo en el cual el hidróxilo deprotonado y el grupo sililo se encuentran en una syn *conformación* con anterioridad a la eliminación.

Por consiguiente, y en principio podría alcanzarse bien un isómero de doble enlace controlando la formación de la syn o anti configuración relativa del medio β -sililalcohol o bien utilizando condiciones de eliminación ácidas o básicas de Peterson.

De acuerdo con el presente invento, los reactivos trans- γ -sililatados de alilmetales son utilizados para la alilación de aldehídos de ciclosporina A protegidos de la formula II para formar una mezcla de diasterómeros anti- β -sililalcohol de la formula XI. Por lo tanto, se realiza una eliminación de Peterson bajo condiciones ácidas para formar un trans doble enlace.

Los ácidos típicos para la reacción promovida por el ácido pueden incluir el ácido sulfúrico, el fórmico, el clorhídrico, el metanosulfónico, tetrafluorobórico,

perclórico, trifluoroacético y diversos ácidos Lewis. Los ácidos preferidos son el sulfúrico, fórmico, metanosulfónico y $\text{BF}_3 \cdot \text{Et}_2\text{O}$, especialmente el ácido sulfúrico, el fórmico y el $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

Este paso puede realizarse a una temperatura de reacción desde -70°C hasta 50°C . El rango preferido de temperatura es de 0°C hasta 50°C , mas preferiblemente de 20°C hasta 40°C para el ácido fórmico. El rango de temperatura preferida es de 0°C hasta 40°C , mas preferiblemente de 20°C hasta 30°C para el ácido sulfúrico y metanosulfónico. El rango de temperatura preferida es de -80°C hasta 50°C , preferiblemente de -80°C hasta 25°C , y especialmente entre -80°C y 0°C para $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

El E-acetil-ISA247 se puede purificar por cristalización en MTBE (por ejemplo vía intercambio de solventes a partir del diclorometano hasta el MTBE), o en una mezcla de MeOH/agua.

En el paso b-ii), el grupo protector se retira, regresando el grupo funcional de ese carbono a un alcohol. Las condiciones y reactivos que deban utilizarse dependerán del grupo protector utilizado, que se conoce entre los expertos en esta técnica. El grupo acilo ($\text{R}'\text{C}(\text{O})-$; donde R' es un radical de hidrocarbano monovalente saturado y lineal de uno a seis átomos de carbono de un radical de hidrocarbano monovalente saturado y bifurcado de tres a seis átomos de carbono), tales como el acetilo, propionilo, butirilo, isobutirilo, valerilo, pueden

utilizarse preferiblemente como grupo protector. Cuando el grupo protector es un grupo acetilo, puede retirarse, por ejemplo mediante el tratamiento con K_2CO_3 en metanol y agua. Bajo estas condiciones, la pureza isomérica del fragmento dieno se conserva. Por lo tanto la pureza isomérica de doble enlace de E-ISA247 reflejará la pureza isomérica de doble enlace de E-acetil-ISA247. Las bases distintas del carbonato potásico que pueden utilizarse para remover el grupo protector incluyen hidróxido sódico, carbonato sódico, alcóxido sódico y alcóxido potásico.

Síntesis de (E)-ISA247 por reactivos de alilboro

Alilación por reactivo - γ -silitado alilboro de la forma III o IV (paso a-i) y a-ii)

En términos generales, el exceso de reactivos de la fórmula III o IV se necesita para completar la alilación del aldehído acetil-ciclosporina A (II'), dentro de un período de tiempo aceptable. Se logran niveles y frecuencias de conversión mas elevadas utilizando un agente de activación tal como el éster de tartrato y/o diclorometano como (co)-solvente. De acuerdo con el comportamiento generalizado de los ácidos borónicos, el reactivos de la fórmula IIIa puede existir potencialmente en forma de trimero cíclico (boroxina) u oligómeros (como ejemplo de este comportamiento de ácido borónico, consultar: K. Ishihara, H. Kurihara, M. Matsumoto y H. Yamamoto en "Design of Bronsted Acid-Assisted Chiral Lewis Acid (BLA) Catalysts for Highly Enantioselective Diels-Alder Reactions", *Journal of the American Chemical Society* 1998, 120, p6920-6930). Cuando se utiliza el

triisopropilborato para la preparación de la solución del reactivo crudo de la formula IIIa, el reactivo de la formula IIIa también puede contener éster diisopropilo boronato ($\text{TMS-CH=CH-CH}_2\text{-B (OiPr)}_2$), a partir del isopropanol generado a partir del B(OiPr)_3 y derivados mezclados tales como el $\text{TMS-CH=CH-CH}_2\text{-B(OH)(OiPr)}$. Esta solución se puede utilizar como reactivo de alilación sin purificación.

De manera alterna, se puede generar una solución del reactivo de la formula IIIa por hidrólisis del complejo de la formula V en una mezcla de solvente orgánico/agua tal como la combinación de diclorometano/agua, tolueno/agua, etil acetato/agua, THF/agua, cloroformo/agua, preferiblemente se utilizará una mezcla de diclorometano/agua, preferiblemente en presencia de un ácido que puede ser el sulfúrico, el clorhídrico, acético, prefiriéndose el ácido acético. La alilación del aldehído acetil-ciclosporina A con una solución de diclorometano del reactivo de la formula IIIa preparado según se describió anteriormente podrá alcanzar un alto nivel de conversión utilizando como mínimo hasta 2 equivalentes del reactivo. En este caso en particular, los derivados del isopropilo están por lo tanto ausentes.

Sin activación por tartrato

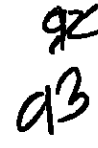
El tolueno puede utilizarse como solvente para estas reacciones, sin embargo, los efectos marcados del solvente han sido observados en estas reacciones. La alilación se realiza en mejores condiciones en solventes polares no coordinantes, preferiblemente el diclorometano.

92
d3

92
d3

92
d3

92
d3



(R¹ y las líneas puntadas tienen los significados definidos anteriormente).

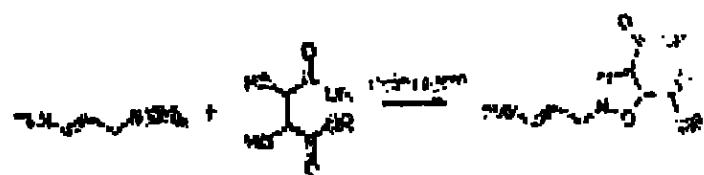
La alilación del aldehído acetil-ciclosporina A (II') en diclorometano con 10 equivalentes de una solución de aproximadamente el 50% de ácido borónico alcanza una conversión superior al 95% dentro de un tiempo de 60 minutos a temperatura ambiente (RT) y produce una mezcla de diastómeros anti β -trimetilsililalcohol (XI'). Se puede realizar la eliminación de Peterson después de la elaboración acuosa en el producto de alilación cruda a una temperatura de 0°C hasta un nivel de temperatura ambiente (RT) en THF utilizando ácido sulfúrico. De manera alterna, la eliminación Peterson puede tener lugar directamente en la mezcla de reacción de alilación mediante adición de THF y ácido sulfúrico. La elaboración acuosa y la cristalización produce el (E)-acetil-ISA247 (XII').

La hidrólisis del (E)-acetil-ISA247 (XII') proporciona (E)-ISA247 (I).

Con activación del tartrato

Como se observa en el Esquema F, la adición de un éster tartrato, tal como el L-(+)-dimetiltartrato por ejemplo en presencia de un agente para el secado, activará el ácido borónico de la fórmula IIIa generando in-situ el éster de boronato correspondiente, que es una clase de reactivo que se conoce en la literatura que muestra una reactividad de alilación muy elevada. La reacción procede luego en forma parcial o principalmente a través del éster generado de boronato incrementando la frecuencia de alilación.

Esquema F:

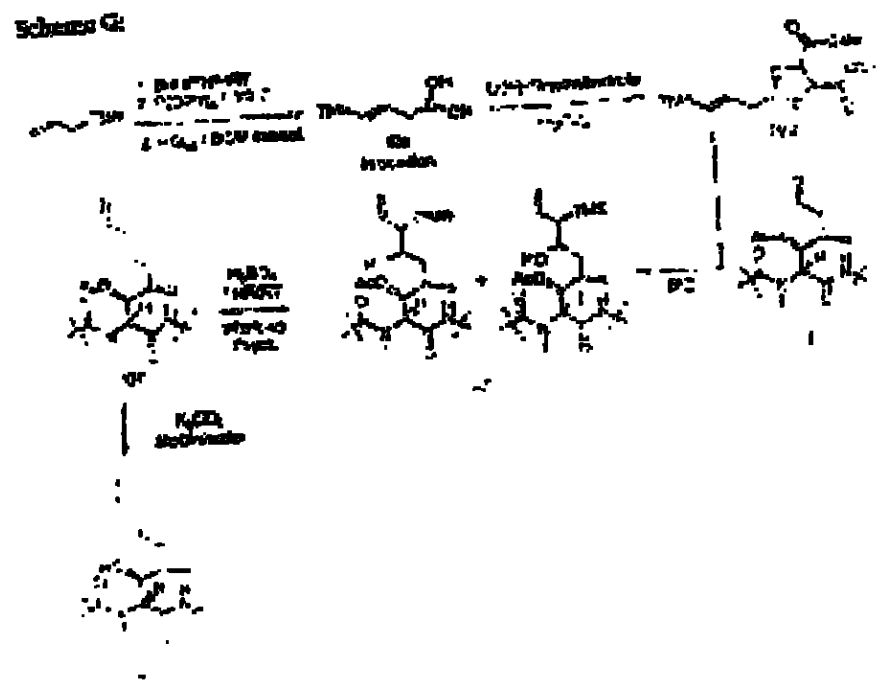


(R es alquilo C₁₋₈, preferiblemente alquilo C₁₋₆, y mas preferiblemente metilo, etilo o isopropilo, especialmente el metilo).

La alilación con la reactivo de la formula IV se realiza en un solvente orgánico que puede ser el etil acetato, THF, tolueno, cloroformo o diclorometano, preferiblemente en etil acetato, tolueno o diclorometano, y más preferiblemente en tolueno o diclorometano, especialmente en diclorometano.

Por ejemplo, la síntesis de (E)-ISA247 por el reactivo de la formula IV se cumple según se ilustra en el Esquema G.

Esquema G:

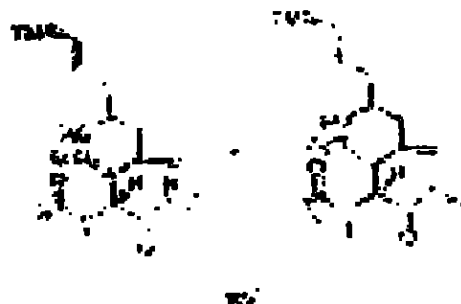


(Las líneas punteadas tienen los significados definidos anteriormente).

La generación del reactivo de la formula IVa' mezclando una solución de ácido borónico de la formula IIIa con L-(+)-dimetiltartrato en presencia de un agente para secado tal como las cribas moleculares o el sulfato de magnesio, preferiblemente sulfato de magnesio, se evidencia mediante ^{11}B y ^1H NMR en análisis.

La alilación del acetil-ciclosporina A aldehído (II') con un reactivo del ácido borónico in-situ que se activa por adición de L-(+)-dimetiltartrato a una temperatura de 0°C a temperatura ambiente, preferiblemente a 0°C, proporcionará una mezcla de diastéromeros anti β -sililalcohol (XI'). La conformación acuosa seguida por la eliminación de Peterson en THF con ácido sulfúrico proporciona después de la elaboración y cristalización, el (E)-acetil-ISA247 (XII'). La hidrólisis del grupo protector de acetilo proporciona (E)-ISA247 (I).

Debe tenerse especial cuidado en que la reacción que involucre el uso de solución de ácido borónico crudo con o sin activación de tartrato debe realizarse en neutro o con un pH ácido (entre 3 y 7, preferiblemente entre 5 y 6). En realidad cuando el pH es superior a 7, se formará una cantidad sustancial de un producto lateral identificado como el vinilsilano de la formula XV. Una reacción de prueba (realizada sin activación de tartrato) donde Et_3N amino se adicionó para alcanzar un pH de 9-10 condujo a una formación casi exclusiva del producto vinilsilano XV (según se evidencia mediante MS, ^1H NMR, COSY, TOCSY y HSQC NMR en experimentos). Un efecto de esta naturaleza fue totalmente inesperado.



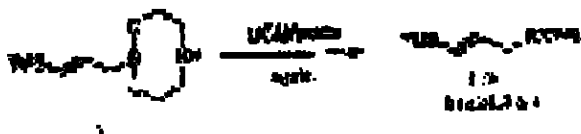
(Las líneas punteadas tienen el significado definido anteriormente).

Alilación por reactivo de alilboro γ -silitado de la formula V (paso a-iii))

Sin activación, el complejo de dietanolamina de la formula V no reacciona a temperatura ambiente con el aldehído acetil-ciclosporina A en solvente son próticos como el diclorometano o el THF. Sin embargo, el complejo de la formula V representa una fuente estable del ácido borónico correspondiente.

Quando se hace un tratamiento en un solvente de agua/orgánico (tal como el etil acetato, THF, diclorometano o tolueno, preferiblemente etil acetato, diclorometano o tolueno, más preferiblemente diclorometano) o una mezcla preferiblemente en presencia de ácidos como el ácido sulfúrico, clorhídrico o acético, preferiblemente el acético, el complejo V dietanolamina se hidroliza y libera el ácido borónico reactivo como se muestra en la ilustración, por ejemplo en el Esquema H, que puede luego reaccionar con el acetil-ciclosporina A aldehído (II'), preferiblemente a temperatura ambiente.

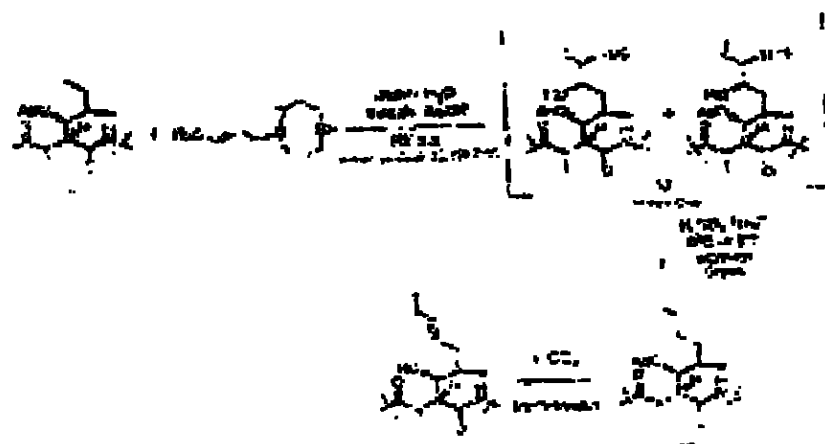
Esquema H:



La alilación del aldehído de acetil-ciclosporina A (II') bajo tales condiciones proporciona una mezcla de diasterómeros anti β -trimetilsililalcohol (XI'). Después de descartar la fase hídrica, el solvente se intercambia por THF, y se agrega ácido sulfúrico para realizar la eliminación Peterson. La conformación acuosa y la cristalización proporciona (E)-acetil-ISA247 (XII'). La hidrólisis posterior produce (E)-ISA247 (I). De manera alterna, el aislamiento de los diasterómeros crudos anti β -trimetilsililalcohol después de la conformación acuosa, seguida de la eliminación de Peterson, bajo condiciones estándar (ácido sulfúrico concentrado en THF) suministrará el E-acetil-ISA247.

Por ejemplo, la síntesis de (E)-ISA247 mediante el complejo de la formula V se puede realizar según se ilustra en el Esquema I.

Esquema I:



(Las líneas punteadas tienen los significados establecidos anteriormente).

La alilmetalización del aldehído acetil-ciclosporina A (II') también tiene lugar bajo condiciones no acuosas directamente con el complejo de la fórmula V. En realidad, los solventes próticos tales como los ácidos carboxílicos son particularmente eficaces. La mezcla de solventes podría ser ácido acético y/o ácido fórmico o una combinación del ácido acético y/o ácido fórmico y un co-solvente como el diclorometano y THF. La alilación se realiza en mejores condiciones en ácido acético entre temperatura ambiente y 35°C. Lo anterior permite lograr una mezcla de diastéromeros anti β-sililalcohol (XI'). Estos intermedios podrían obviamente aislarse, sin embargo la eliminación Peterson se puede realizar en un recipiente mediante la adición a la mezcla de reacción de un ácido como el fórmico, el sulfúrico o el metanosulfónico, preferiblemente ácido fórmico. La conformación hídrica y la cristalización producirá (E)-acetil-ISA247 (XII'). La hidrólisis posterior suministrará (E)-ISA247 (I).

Cuando el ácido fórmico se encuentra presente en cantidades suficientes en la mezcla del solvente utilizada para la alilación, la eliminación Peterson podrá tener lugar en un recipiente conduciendo directamente al (E)-acetil-ISA247.

Otra alternativa consiste en realizar la adición del complejo de la fórmula V al aldehído acetil-ciclosporina A (II') en presencia de un ácido Lewis tal como el BF₃.Et₂O. Por ejemplo, la reacción con BF₃.Et₂O se puede realizar en un solvente de tal manera que se emplee el diclorometano o THF a una temperatura en un

rango de -40°C hasta temperatura ambiente. Bajo estas condiciones, la alilación puede estar directamente seguida de la eliminación Peterson, produciendo el valor esperado de (E)-acetil-ISA247 (XII').

Alilación por reactivo alilboro γ -silitado de la formula VI (paso a-iv))

La reacción del aliltrifluoroborato VI y acetil-ciclosporina A aldehído (II') en un solvente bifásico agua/sustancia orgánica, preferiblemente una mezcla entre agua/diclorometano o mezcla de agua/tolueno, y más preferiblemente la mezcla de agua/diclorometano a temperatura ambiente proporcionará una mezcla de diastéromeros anti β -trimetilsililalcohol (XI'). Después de descartar la fase hídrica, se realiza la eliminación Peterson mediante adición de THF y ácido sulfúrico a una temperatura entre 0°C y temperatura ambiente proporcionando (E)-acetil-ISA247 (XII'). La eliminación Peterson también se puede realizar bajo condiciones estándar (ácido sulfúrico en THF) después del aislamiento de los diastéromeros anti β -trimetilsililalcohol hasta lograr E-acetil-ISA247.

La alilación se puede igualmente promover mediante un ácido Lewis. En tal caso, la alilación y la eliminación Peterson pueden tener lugar in-situ. Por ejemplo, la adición de un exceso de $\text{BF}_3 \cdot \text{Et}_2\text{O}$ a una suspensión de aliltrifluoroborato VI (2 equivalentes) en una solución de aldehído acetil-ciclosporina A (XII') en diclorometano a -70°C proporciona después de 60 minutos una reacción y una conformación acuosa (E)-acetil-ISA247 (I). Los solventes para la reacción son orgánicos como el diclorometano, THF o tolueno, preferiblemente diclorometano.

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Alilación por reactivo alilboro γ -silitado de la formula VII (paso a-v)

La alilación de los aldehídos con estos reactivos se conoce a partir de la literatura en el sentido de avanzar lentamente (1 a varios días a temperatura ambiente). En este orden de ideas, la alilación del aldehído acetil-ciclosporina A (II') con exceso de reactivo de la formula VII (5-10 equivalentes) en solventes tales como el THF, diclorometano, tolueno, DMF y DMSO procederá lentamente a temperatura ambiente.

El calentamiento, uso de grandes excesos de reactivo o altas concentraciones podrían aumentar la frecuencia de alilación del aldehído acetil-ciclosporina A, sin embargo, se encontró que una mejor alternativa sería una escogencia adecuada del solvente.

Los ácidos carboxílicos tales como el fórmico o el acético demostraron mejorar de manera sorprendente la frecuencia de alilación. Por ejemplo, cuando se realizó en ácido acético la alilación del aldehído de ciclosporina de acetilo (II') se pudo lograr una conversión de mas del 95% dentro de un término de 5 horas a temperatura ambiente con 2 equivalentes del reactivo de la formula VI, proporcionando el β -silitalcoholes (XI'). La adición del ácido fórmico promueve la eliminación Peterson. El (E)-acetil-ISA247 (XII') se obtiene después de la elaboración extractiva demostrando la relativa anti estereoquímica del β -silitalcohol intermedio. La eliminación Peterson también se puede realizar bajo condiciones estándar (ácido

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sulfúrico en THF) después del aislamiento de los diastereómeros anti β -trimetilsililalcohol para lograr el (E)-acetil-ISA247.

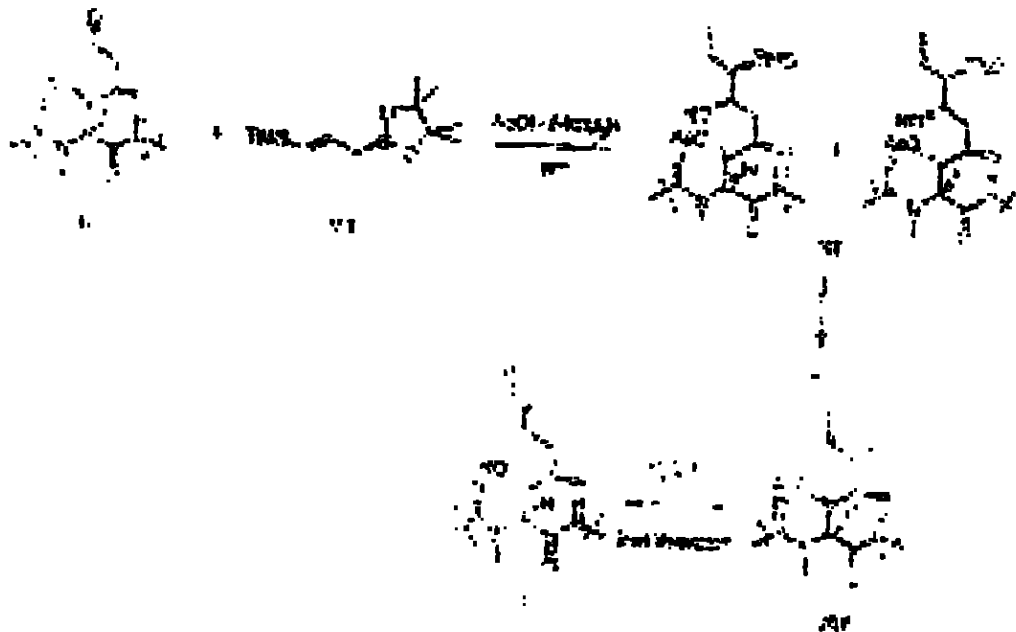
El ácido fórmico o preferiblemente una combinación de ácido acético y ácido fórmico (tal como una relación aproximada de 1:1 v/v) tal y como se proporciona es también un solvente eficaz. En tal caso, la alilmetalación y la posterior eliminación Peterson puede tener lugar en un recipiente. En una combinación de ácido acético y ácido fórmico (ca 1:1 v/v) la aligación del aldehído del acetil-ciclosporina A y la posterior eliminación de Peterson alcanzan una conversión superior al 90% dentro de un lapso de 60 minutos a temperatura ambiente con 1.5 equivalentes del reactivo. La conformación extractiva acuosa y la cristalización proporciona (E)-acetil-ISA247 (XII').

La mezcla de ácido acético, fórmico y un co-solvente adecuado pueden igualmente utilizarse. El diclorometano, tolueno, etil acetato o isopropil acetato, preferiblemente isopropil acetato podrían utilizarse como un co-solvente. La disminución en reactividad puede observarse cuando se utiliza un co-solvente, pero este hecho podría compensarse aumentando la temperatura de reacción.

La hidrólisis del grupo protector de acetato con K_2CO_3 en metanol acuoso proporciona (E)-ISA247 (I).

Por ejemplo, la síntesis de (E)-ISA247 por un reactivo de la formula VI se puede realizar según se ilustra en el Esquema J.

Esquema J:



El origen de la mayor actividad del reactivo de la formula VII, cuando se utiliza en solución de ácido carboxílico como ácido acético y ácido fórmico, podría derivarse de la alta polaridad y baja complejidad de tales solventes. Otro efecto podría encontrarse en la capacidad de dicho solvente de proporcionar catálisis ácida de la alilación por medio de la activación del grupo carbonilo del aldehído a través de la protonación.

La adición del reactivo de la formula VII al aldehído ciclosporina A acetilo (II') se puede promover mediante un ácido Lewis de tal manera que pueda emplearse $\text{BF}_3 \cdot \text{Et}_2\text{O}$ a una temperatura de -70°C hasta 0°C en tolueno, THF o diclorometano,

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preferiblemente tolueno o diclorometano, o preferiblemente diclorometano. Bajo la reacción de alilación, puede también ocurrir la eliminación Peterson pudiéndose obtener el (E)-acetil-ISA247 (XII') después de la formación extractiva acuosa.

Síntesis de (E)-ISA247 por reactivos de alilititanio

La reacción del aldehído acetil-ciclosporina A con un reactivo γ -trimetilsililalilititanio que se prepare a partir del trimetilsililalilitio y diclorodiisopropóxido de titanio, tetraisopropóxido de titanio o clorotrisopropóxido de titanio, preferiblemente tetraisopropóxido de titanio o clorotrisopropóxido de titanio en THF, a una temperatura de -80°C hasta 0°C , preferiblemente -80°C hasta -30°C , y mas preferiblemente de -80°C hasta -50°C , especialmente de -80°C hasta -60°C , proporcionará después de la formación acuosa, una mezcla de anti β -sililalcoholes XI' diastoméricos. La eliminación Peterson bajo las condiciones estándar (H_2SO_4 en THF) proporciona E-acetil-ISA247 (XII').

Síntesis de (E)-ISA247 por reactivos de alilaluminio

La reacción del acetil-ciclosporina A aldehído con un reactivo γ -trimetilsililalilaluminio que se prepare a partir de trimetilsililalilitio y dicloruro de etilaluminio o cloruro de dietilaluminio, preferiblemente cloruro de dietilaluminio, realizado en THF, a una temperatura de -80°C hasta 0°C , preferiblemente de -80°C hasta -30°C , y más preferiblemente de -80°C hasta -50°C , especialmente -80°C hasta -60°C proporcionará luego de la formación acuosa una mezcla de anti

β -sililalcoholes XI' diastomérico. La eliminación Peterson bajo las condiciones estándar (H_2SO_4 en THF) proporciona E-acetil-ISA247 (XII').

Preparación de los reactivos alilmetales γ -sililados

Los reactivos alilmetales γ -sililados requeridos para realizar el paso de alilmetalación se generan en mejores condiciones a partir de los alilsilanos correspondientes vía deprotonación, atrapado con un reactivo metálico adecuado y opcionalmente haciendo una complexación posterior del reposo del metal a través de un ligando adecuado. Los reactivos resultantes podrán de acuerdo con su estabilidad y el proceso, utilizarse in situ o aislados y luego almacenarse.

Aun cuando otro alilsilano silil-sustituidos pudieran haberse utilizando obviamente, el derivado de trimetilsililo conduce a una cantidad mínima de desechos. En realidad, el fragmento sililo se pierde mediante la eliminación Peterson.

Se han publicado muchas condiciones para la deprotonación de los alilsilanos en la literatura y usualmente se hace uso del n-butililitio (o de los sec- y tert- isómeros) en un solvente orgánico (generalmente THF) en combinación o no con un co-solvente o co-reactivo tal como HMPA, TMEDA o t-butoxido potásico a temperaturas en un rango de -100°C hasta temperatura ambiente (ver por ejemplo: T. H. Chan en "Silylallyl Anions in Organics Synthesis: A Study in Regio- and Stereoselectivity", *Chemical Reviews* 1995, 95, p1279-1292; Kohei Tamao, Eiji Nakajo, y Yoshihiko Ito en "Silafunctional compounds in organic synthesis. 33.

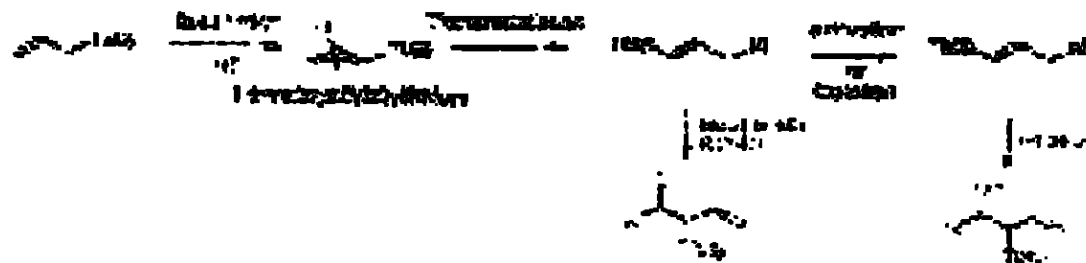
Metallated allylaminosilane: a new, practical reagent for the stereoselective α -hidroxiallylation of aldehydes to erythro-1,2-diol skeletons", *Journal of Organic Chemistry* 1987, 52, pp957-958; E. Ehlinger y P. Magnus en "Silicon in Synthesis. 10. The (Trimethylsilyl) allyl Anion: A β -Acyl Anion Equivalent for the Conversion of Aldehydes and Ketones into γ -Lactones", *Journal of the American Chemical Society* 1980, 102, pp 5004-5011; M. Schlosser, L. Franzini en "The Regioselectivity of 1,3-Disubstituted Allylmetal Species Towards Electrophiles: 1-(Trimethylsilyl)alk-2-enylpotassium Compounds", *Synthesis* 1998, pp 707-709; E. Shaumann y A. Kirschning en "Ring-opening of oxiranes by silyl-substituted allyl anions. A regiochemical chameleon", *Tetrahedron Letters* 1988, 29, pp 4281-4284).

Como se ilustra en el Esquema K, el aliltrimetilsilano es deprotonado por el *n*-butillitio en THF a una temperatura en un rango de 0°C hasta 35°C, preferiblemente entre 0°C y 25°C durante 30 minutos en un término máximo de 3 horas. Esta preparación genera un intermedio de trimetilsililalilitio. Este intermedio existe mas probablemente en soluciones tales el complejo π -alilo de litio en una trans configuración (T. H. Chan en "Silylallyl Anions in Organics Synthesis: A Study in Regio- and Stereoselectivity", *Chemical Reviews* 1995, 95, p1279-1292; M. Schlosser, O. Desponds, R. Lehmann, E. Moret y G. Rauschschwalbe en "Polar Allyl Typen Organometallics as Key Intermediates in Regio- and Stereocontrolled Reactions: Conformational Mobilities and Preferences", *Tetrahedron* 1993, 49, p10175-10203). Este anión es luego atrapado (transmetalado) por una fuente metálica electrofílica usualmente a una temperatura de -80°C hasta -60°C,

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generando el reactivo correspondiente del trans-alilmetal. De acuerdo con el reposo metálico, estos reactivos se hacen reaccionar in situ con el aldehído o bien se pueden aislar para uso posterior. Igualmente se puede hacer la transformación en otro complejo mediante la adición de un reactivo apropiado con el propósito de activar el reactivo para la alilación o permitir su aislamiento.

Esquema K:



(M y M' son fragmentos metálicos que comprenden el metal y sus ligandos).

Preparación de los reactivos del alilboro

Reactivo alilboro γ-sililado de la formula IIIa

Se obtiene una solución de ácido borónico crudo de la formula IIIa después de la deprotonación del aliltrimetilsilano, que se atrapa con un reactivo de boro electrofílico y conformación acuosa. La deprotonación del aliltrimetilsilano se realiza en THF con butillitio, entre 0°C y 35°C, preferiblemente entre 0°C y 25°C durante 30 minutos hasta un máximo de 3 horas. El reactivo de boro electrofílico es un trialquilborato tal como el borato de trisopropilo o el borato de trimetilo, preferiblemente borato de trisopropilo. El atrapamiento del intermedio de trimetilsililalilitio con borato trisopropilo se realiza entre -80°C y -20°C,

preferiblemente por debajo de -60°C durante 30 minutos hasta un máximo de 2 horas. El atrapamiento del intermedio trimetilsililalilitio con el borato de trimetilo se realiza entre -80°C y -60°C durante 30 minutos hasta un máximo de 2 horas.

Se conoce perfectamente que los ácidos alilborónicos son inestables; por lo tanto, el ácido borónico crudo se mantiene en la solución (P. G. M Wuts y Y.-W. Jung en "The Addition of γ -(Trimethylsilyl)alilboronates to Imines", *Journal of Organic Chemistry* 1991, 56, p356-372 (ver comentarios en el ejemplo para la preparación del compuesto 9); W.R. Roush, K. Ando, D. B. Powers, A.D. Palkowitz y R.L. Halterman en "Asymmetric Synthesis Using Diisopropyl Tartrate Modified (E)- and (Z)-Crotylboronates; Preparation of the Chiral Crotylboronates and Reactions with Achiral Aldehydes", *Journal of the American Chemical Society* 1990, 112, 6339 (ver referencia 17)). Las soluciones concentradas (concentración a $>10\%$, por ejemplo aproximadamente una concentración de 50%) no son estables a temperatura ambiente y se descomponen. En estas condiciones deben utilizarse rápidamente. Si fuere necesario, deberán almacenarse a una temperatura de 5°C máximo. De acuerdo con el comportamiento generalizado de los ácidos borónicos, el ácido borónico de la fórmula IIIa podría en principio también existir en la forma de un trimero cíclico (denominado también boroxina) u oligómeros. Debido a que el isopropanol que se deriva del borato triisopropilo puede estar también presente (de acuerdo con el proceso), los ésteres de boro isopropilo (mezclado) ($\text{TMS-CH=CH-CH}_2\text{-B(OH)(OiPr)}$ y/o $\text{TMS-CH=CH-CH}_2\text{-B(OiPr)}_2$) podrían también estar presentes. En realidad, ^1H NMR y ^{11}B NMR un análisis de soluciones

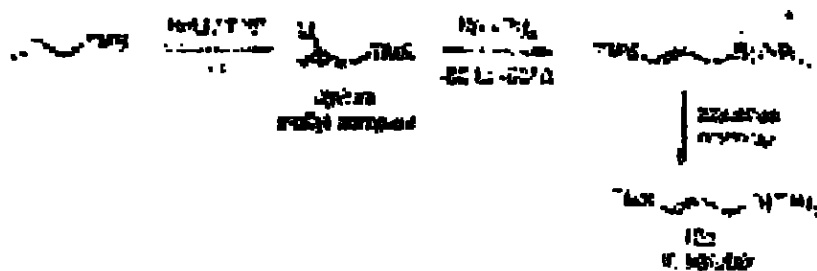
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concentradas de reactivo de boro de la formula IIIa (aproximadamente 50%) diluidos con un volumen equivalente de CD_2Cl_2 , mostrará la presencia de varias especies de boronato, aun cuando el análisis reversivo de fase HPLC demuestra principalmente el ácido borónico (bajo condiciones HPLC, oligómeros, dimeros y trimeros pueden ser hidrolizados y transformados en ácido borónico).

De manera alterna, se puede preparar una solución del reactivo de la formula IIIa mediante hidrólisis del complejo de la formula V en una mezcla de solvente orgánico/agua tal como mezclas de diclorometano/agua, tolueno/agua, etil acetato/agua, THF/agua, cloroformo/agua, preferiblemente mezcla de diclorometano/agua, preferiblemente en presencia de un ácido como el sulfúrico, clorhídrico, acético, y preferiblemente ácido acético.

La preparación del reactivo alilmetálico γ -silitado de la formula IIIa se realiza por ejemplo según se ilustra en el Esquema L.

Esquema L:



Reactivo alilboro γ -silitado de la formula IV

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El reactivo de boronato de la formula IV donde R es alquilo C₁₋₃, preferiblemente alquilo C₁₋₃, y más preferiblemente metilo, etilo o isopropilo, especialmente metilo se prepara mediante un tratamiento del reactivo de boro de la formula IIIa con el éster requerido de tartrato en presencia de un agente de secamiento como las cribas moleculares o el sulfato de magnesio, preferiblemente sulfato de magnesio.

El reactivo de la formula IVa' se prepara mediante el mezclado de una solución de ácido borónico de la formula IIIa con L-(+)-dimetiltartrato, en presencia de un agente de secado tal como cribas moleculares o sulfato de magnesio, preferiblemente este último, según se evidencia mediante los análisis ¹¹B y ¹H NMR.

Reactivo alilboro γ-sililado de la formula III

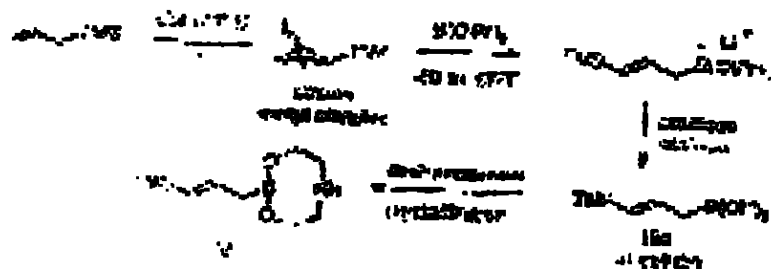
Este reactivo se puede preparar tratando el reactivo de boro de la formula IIIa con el alcohol requerido en presencia de un agente de secado como cribas moleculares o sulfato de magnesio, preferiblemente este último. El reactivo preferido de boronato de la formula IV donde R¹ es alquilo C₁₋₃ o cicloalquilo C₃₋₆ son aquellos en los cuales R¹ es metilo, etilo, propilo, isopropilo, butilo o bencilo, y más preferiblemente R¹ es metilo, etilo, propilo, isopropilo o butilo, y más preferiblemente R¹ es metilo, etilo, propilo o butilo, especialmente se prefiere R¹ como metilo.

Reactivo alilboro γ-sililado de la formula IV

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A una solución de ácido borónico crudo de la formula IIIa, cuya preparación fue descrita anteriormente, se le agrega dietanolamina. El intercambio de solvente por heptano y cristalización proporciona el complejo dietanolamina de la formula V en forma de sólido. Una característica especial de este reactivo es la presencia de una interacción entre el par nitrógeno lone del fragmento dietanolamina y el átomo de boro (es decir, complexación del átomo de nitrógeno por el átomo de boro) según se evidencia por ^{11}B NMR del complejo ($\delta = 11\text{ppm}$ en relación con $\text{BF}_3 \cdot \text{Et}_2\text{O}$, a manera de referencia externa).

La preparación del reactivo alilmetálico γ -silitado de la formula V se realiza por ejemplo según se ilustra en el Esquema M:



Reactivo aliboro γ -silitado de la formula VI

Esquema N:



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En términos generales, las sales de potasio de alitrifluoroborato se preparan mediante el tratamiento del ácido borónico correspondiente con 3 equivalentes de KHF_2 en una mezcla de solvente de agua/metanol (ver por ejemplo, R.A. Batey en "Diastereoselective Allylation and Crotylation Reactions of Aldehydes with Potassium Allyl- and Crotyltrifluoroborates under Lewis acid Catalysis", *Synthesis* 2000, pp 990-998). Sin embargo, la aplicación directa de estos procedimientos a la preparación del trifluoroborato de la fórmula VI conducen a la formación de cantidades sustanciales de aliltrimetilsilano vía protodeborilación debida al pH ácido de la mezcla de reacción.

La modificación se realizó con el fin de evitar esta reacción colateral. De esta manera, según se ilustra en el Esquema O, una solución de metanol de ácido borónico se trata con 2 equivalentes de KHF_2 en forma de fuente de fluoruro, a temperatura ambiente. La suspensión se agita a temperatura ambiente durante 60 minutos. Las sales orgánicas residuales se remueven mediante filtración. La solución metanólica de sal de trifluoroborato VI se concentra bajo presión reducida y el producto es cristalizado a 0-5°C. La sal de trifluoroborato VI se aísla mediante filtración y se seca al vacío.

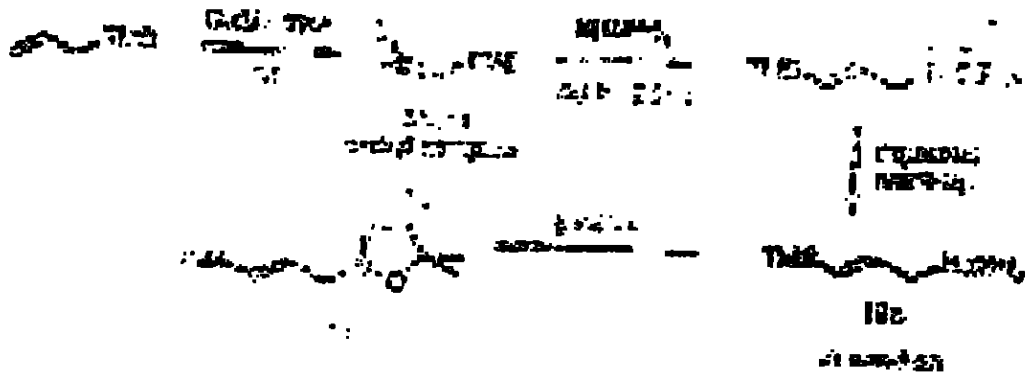
La solución de ácido borónico requerida se prepara mediante la hidrolización del complejo de dietanolamina de la fórmula V en una mezcla de agua/diclorometano en presencia de un ácido como el acético. La fase acuosa se descarga y el solvente es intercambiado a partir del diclorometano por metanol.

De manera alterna, el complejo V de dietanolamina se puede utilizar directamente como material de partida. Las sales de trifluoroborato VI pueden prepararse sin embargo no ocurre cristalización.

Reactivo alilboro γ -silylato de la formula VII

A una solución de ácido borónico crudo de la formula IIIa, cuya preparación se describió anteriormente, se le agrega pinacol. La mezcla de reacción se agita a temperatura ambiente y luego se concentra bajo presión reducida. El complejo VII de pinacol puede luego destilarse bajo presión baja o utilizarse directamente en el paso de alilmetalación. La preparación del complejo de pinacol de la formula VII se realiza por ejemplo según se ilustra en el Esquema O.

Esquema O:

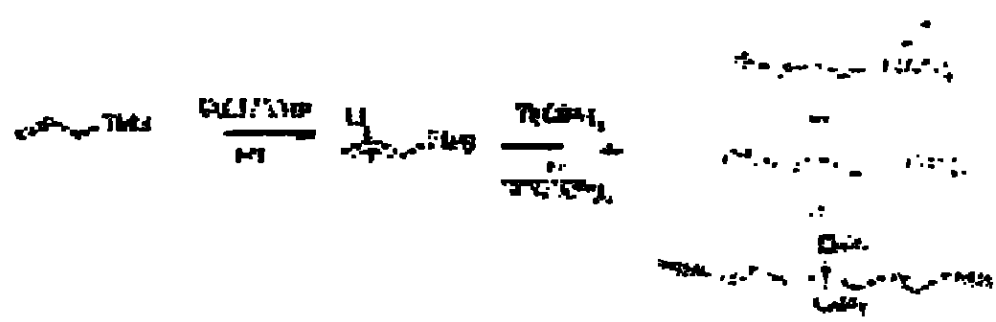


De manera alterna, el atrapamiento del 1-trimetilsililalilo litio se puede realizar con 2-isopropoxi-4,4,5,5-tetrametil-1,3,2-dioxaborolano, que conduce directamente después de la formación acuosa al boronato de pinacol de la formula VII.

En forma alterna, el reactivo se puede preparar mediante deprotonación del aliltrimetilsilano a temperatura ambiente con butyllitio neutralizando el intermedio de alillitio con tri-isopropilborato (entre -80°C hasta -20°C, preferiblemente entre -80°C y -30°C), con adición de pinacol y luego formación acuosa.

Preparación de los reactivos aliltitanio

Los reactivos aliltitanio (trimetilsililo) se preparan in situ vía desprotonación del aliltrimetilsilano para formar trimetilsililalitio, según se describió anteriormente, y la reacción de este intermedio con el diclorodiisopropóxido de titanio, tetraisopropóxido de titanio o clorotriisopropóxido de titanio, preferiblemente tetraisopropóxido de titanio o clorotriisopropóxido de titanio a una temperatura de -80°C hasta 0°C, preferiblemente -80°C hasta -30°C, más preferiblemente de -80°C hasta -50°C, especialmente -80°C a -60°C. Los reactivos resultantes de titanio se utilizan in situ para la alilación de los aldehídos ciclosporina A protegidos. Las estructuras putativas de estos reactivos se presentan a continuación:



Preparación de los reactivos alilaluminio

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Los reactivos alilaluminio (trimetilsililo) se preparan in situ vía desprotonación del aliltrimetilsilano para formar trimetilsililalio, según se describió anteriormente, y la reacción de este intermedio con un cloruro de dialquilaluminio tal como el cloruro dialquilaluminio o con un dicloruro de alquilaluminio como el dicloruro etilaluminio, preferiblemente con cloruro dietilaluminio, a una temperatura de -80°C hasta 0°C, preferiblemente -80°C hasta -30°C, y más preferiblemente de -80°C hasta -50°C, especialmente de -80°C a -60°C. Los reactivos de aluminio resultante son utilizados in situ para la alilación de los aldehídos ciclosporina A protegidos.

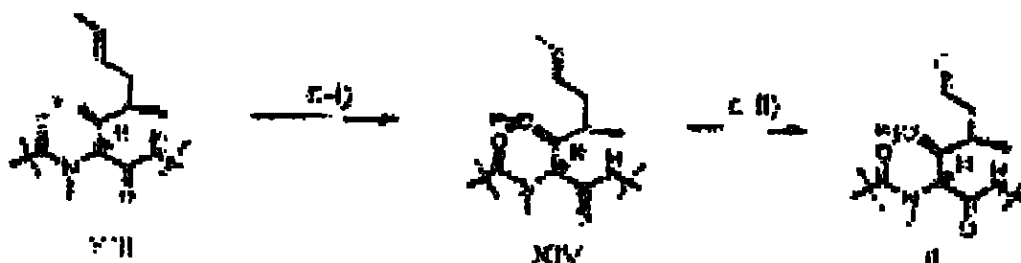
Una estructura putativa para uno de estos reactivos se presenta a continuación:



Preparación del aldehído ciclosporina A protegido

El aldehído ciclosporina A protegido de la fórmula II se puede preparar como se ilustra en el esquema P.

Esquema P:



(Las líneas punteadas tienen los significados definidos anteriormente).

En el paso c-i), se introduce un grupo protector en la ciclosporina A de la fórmula XIII, con el fin de proteger el grupo hidróxilo en la posición β de la cadena lateral del residuo del ácido 1-amino. Los grupos protectores son bien conocidos en la síntesis orgánica, y han sido analizados por J. R. Hanston en el Capítulo 2, "The Protection of Alcohols", de la publicación de Protecting Groups in Organic Synthesis (Sheffield Academic Press, Sheffield, England, 1999), pp. 24-25. Hanson enseña como proteger los grupos hidróxilos convirtiéndolos en esteres o éteres. Los esteres de acetato son posiblemente el tipo más frecuentemente utilizado de química para la protección de los grupos hidróxilos. Existe una amplia gama de condiciones que pueden emplearse para introducir el grupo acetato. Estos reactivos y solventes incluyen anhídrido acético y piridina; anhídrido acético, piridina y dimetilaminopiridina (DMAP); anhídrido acético y acetato sódico; anhídrido acético y tolueno p-ácido sulfónico, cloruro de acetilo, piridina y DMAP; y cetona. EL DMPA es un catalizador de acilación útil debido a la formación de sales altamente reactivas de N-acilpiridium a partir del anhídrido.

Por ejemplo, el β -alcohol de la ciclosporina A se protege como un acetato haciendo reaccionar la ciclosporina A (XIII) con cloruro de acetilo, etil acetato, o sus combinaciones, formando el compuesto acetil ciclosporina A. En otro ejemplo, el alcohol β se somete a una adición nucleofílica al anhídrido acético, formando acetil ciclosporina A y ácido acético. Estas reacciones se pueden realizar en presencia del dimetilaminopiridina (DMAP) en donde el exceso de anhídrido acético actúa como solvente.

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Aun cuando la preparación del acetil ciclosporina A está bien establecido en la literatura, los expertos en la materia podrán apreciar que los grupos protectores distintos de los esteres de acetato pueden ser útiles para proteger el β -alcohol del residuo 1-amino ácido de la ciclosporina A. Estos grupos protectores pueden incluir esteres de benzoato, esteres sustituido de benzoato, éteres, y silil éteres. Bajo ciertas condiciones de reacción, el grupo protector de acetato es propenso a reacciones colaterales no deseables tales como la eliminación y la hidrólisis. Debido a que los esteres de benzoato, éteres y silil éteres son frecuentemente mas resistentes a tales reacciones colaterales bajo tales condiciones de reacción iguales, resulta frecuentemente ventajoso utilizar tales grupos protectores en lugar del acetato.

En el paso c-ii), la ciclosporina A protegida de la formula XIV se transforma en un aldehído de ciclosporina A protegido de la formula II.

Este paso se puede realizar por ejemplo utilizando el ozono como agente oxidante seguido de una elaboración que se hace utilizando un agente reductor para formar un aldehído de la ciclosporina A protegida (II). El paso de ozonolisis se realiza en un rango de temperatura de -80°C hasta 0°C aproximadamente. El solvente utilizando durante la ozonolisis puede ser un alcohol inferior como metanol. El agente reductor puede ser una trialquilfosfina como tributilfosfina, triarilfosfina, trialquilamina como la trietilamina, puede ser un alquilaminosulfido, un tiosulfato o un dialquilsulfido como el dimetilsulfido. Cuando se trabaja con el tributilfosfina

como agente reductor, la persona con conocimientos básicos en la materia sabrá que la reacción es controlada por dosificación.

Por otro lado, se puede preparar un aldehído de ciclosporina A protegido (II) mediante la transformación de la ciclosporina A protegida XIV, como la acetil ciclosporina A, en el epóxido de ciclosporina A protegida con un monopersulfato, preferiblemente el oxono, en presencia de la cetona, como la acetoxiacetona o diacetoxiacetona. Este paso realiza en un solvente orgánico que es inerte bajo estas condiciones de reacción tal como el acetonitrilo y el agua. La sal disódica del ácido etilenediamintetra acético se adiciona para capturar iones de metales pesados que pudieren estar presentes. La reacción epoxidación se lleva a cabo preferiblemente a un pH por encima de 7. Esta reacción de epoxidación es seguida de una excisión oxidativa del epóxido con ácido periódico o sal de periodato bajo condiciones ácidas. Opcionalmente, la oxidación y la excisión oxidativa pueden combinarse en un procedimiento de elaboración. Estas reacciones han sido analizadas por Dan Yang, et al, en "A C_2 Symmetric Chiral Ketone for Catalytic Asymmetric Epoxidation of Unfunctionalized Olefins," J. Am. Chem. Soc., Vol. 118, pp. 491-492 (1996), y "Novel Cyclic Ketones for Catalytic Oxidation Reactions" J. Org. Chem., Vol. 63, pp. 9888-9894 (1998).

El uso de los agentes oxidantes de base de rutenio ha sido analizado por H. J. Carlsen et al. en "A Greatly Improved Procedure for Ruthenium Tetroxide Catalyzed Oxidations of Organic Compounds," J. Org. Chem., Vol. 46, No. 19, pp

3736-3738 (1981). Carlsen et al., enseña que históricamente el gasto del metal rutenio proporcionaba un incentivo para el desarrollo de los procedimientos catalíticos, y que el mas conocido utilizaba periodato o hipoclorito a manera de oxidantes estequiométricos. Estos investigadores encontraron una pérdida de la actividad catalítica durante el curso de la reacción mediante el uso convencional del rutenio que postularon se debía a la presencia de los ácidos carboxílicos. La adición de nitrilos a la mezcla de reacción, especialmente acetonitrilo, demostró mejorar significativamente la frecuencia y alcance de la excisión oxidativa de los alquenos en un sistema de $\text{CCl}_4/\text{H}_2\text{O}/\text{O}_4$.

Por ejemplo, el aldehído (II) de la ciclosporina A protegida se puede producir a partir de la ciclosporina A protegida (XIV), tal como el acetil ciclosporina A, disolviéndola en una mezcla de acetonitrilo y agua, y luego adicionando el primer periodato sódico y posteriormente el hidrato de cloruro de rutenio. El aldehído (II) se puede extraer con etil acetato.

EJEMPLOS

Las siguientes preparaciones y ejemplos se proporcionan con el fin de permitirle a las personas expertas en la materia comprender mas claramente y practicar el presente invento. Estas prácticas no deben considerarse como limitantes del alcance del invento, sino simplemente como ilustraciones y representaciones del mismo.

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Aun cuando la mayor parte de los ejemplos han sido suministrados para la secuencia de eliminación de alilación - Peterson en los aldehídos acetil-ciclosporina A, podría en principio utilizarse otro grupo protector. Obviamente, esta práctica estará limitada por su compatibilidad con las condiciones de reacción al igual que con la posibilidad de retirarlas eficientemente para lograr el (E)-ISA247.

Ejemplo 1

I) Preparación de una solución del ácido borónico crudo de la formula

III: ácido (E)-3-(trimetilsilil)alilborónico

Se disolvieron 20 g (169.8 mmol, 1 equivalente) de aliltrimetilsilano (Fluka 06073) en 140 ml de THF seco THF (Fluka 87368) a temperatura ambiente. Se adicionaron 106.1 ml (69.8 mmol, 1 Equivalente) de una solución de 1.6 M de butillitio en hexano (Acros 181270100) en un lapso de 10 minutos, conservando la temperatura entre 20°C y 25°C. Después de 30 minutos de reacción, la solución resultante de color amarillo a naranja se enfrió a -70°C. Se adicionaron 40.24 ml (169.8 mmol, 1 Equivalente) de trisopropilborato (Fluka 92085) en 10 minutos, manteniendo la temperatura por debajo de -60°C. Después de 30 minutos, de reacción a -74°C, la solución fría fue vertida en una solución de 170 ml de 1M acuoso HCl. El pH se ajustó a 7-8 mediante adición de 1M HCl_{aq} (en este caso en particular, 26 ml). Se agregaron 80 ml de diclorometano para extracción. La fase acuosa se separó y se extrajo con 80ml de diclorometano. Las fases orgánicas se lavaron secuencialmente con 150 ml de una solución saturada acuosa de NaCl, se combinaron y se secaron con Na₂SO₄, luego se filtraron y concentraron bajo

presión reducida hasta 40 ml aproximadamente. El peso de la solución se ajustó a 53.6g mediante la adición de diclorometano con el fin de obtener una solución de aproximadamente el 50% de ácido borónico (de acuerdo con el alitrimetilsilano de inicio).

II) Alilación de aldehído acetil-ciclosporina A

Se disolvieron 20g (6.23 mmol, 1 equivalente) de acetil-ciclosporina A aldehído en 100 ml de diclorometano a temperatura ambiente. Se agregaron 25.66 g (81.15 mmol, 5 equivalente) de la solución de ácido borónico previamente preparada (concentración aproximada de 50%) en una porción. La conversión de la reacción fue monitoreada por HPLC. La reacción quedó completa en un término de 1-3 horas a temperatura ambiente. Se obtuvo una mezcla de aproximadamente 85:15 de β -trimetilsililalcohol en diastómeros.

III) Eliminación Peterson

Esta eliminación se realizó directamente en la mezcla de reacción.

Se agregaron 20 ml de THF y la mezcla de reacción se enfrió a 0°C. Se agregaron 2.7ml (48.69 mmol, 3 equivalente) de ácido sulfúrico concentrado. La temperatura se elevó a temperatura ambiente. Después de terminar la reacción (aproximadamente 1 hora), se agregaron 100ml de agua. La fase orgánica se separó y se lavó dos veces con 50 ml de agua. Las fases de agua fueron re-extraídas secuencialmente con 50 ml de diclorometano. Las fases orgánicas combinadas se secaron en Na_2SO_4 , se filtraron y concentraron bajo presión

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reducida a 30°C. La espuma blanca resultante se re-disolvió en 250 ml MTBE y después de unos minutos, se inició la cristalización. Después de agitar durante 15 minutos a temperatura ambiente y 2 horas a 0-2°C, se filtró la suspensión. Los cristales se lavaron con 50ml en frío MTBE (-20°C) y se sacaron a 40-50°C bajo presión reducida hasta lograr 19.2 g de (E)-acetil-ISA247 en forma de polvo blanco en una pureza de >98% isomérica (400MHz ¹H NMR).

El (E)-acetil-ISA247 se puede recrystalizar disolviendo el sólido en diclorometano a temperatura ambiente e intercambiando el solvente por MTBE (mediante la adición de MTBE, concentrando la solución a la mitad de su volumen bajo presión reducida a 40°C y repitiendo estas operaciones de dos a tres veces). La solución se enfría a temperatura ambiente y se inicia luego la cristalización dentro de unos cuantos minutos posteriores. La suspensión se agita a temperatura ambiente durante 2 horas y 30 minutos a 0°C. Los cristales de (E)-acetil-ISA247 se aíslan después de filtración, lavándose con MTBE y secándose bajo una presión reducida a 40°C.

iv) Hidrólisis

La hidrólisis del E-acetil-ISA247 proporcionó (E)-ISA247 en una pureza de 99.5% isomérica de doble enlace (mediante HPLC).

Ejemplo 2

i) Preparación de una solución del ácido borónico crudo de la formula IIIa

IIIa: ácido (E)-3-(trimetilsilil)alilborónico

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Se disolvieron 8.67ml (40.56 mmol, 10 equivalente) de alitrimetilsilano en 33.3 ml de THF seco a temperatura ambiente. Se agregaron 25.35 ml (40.56 mmol, 10 equivalente) de una solución de 1.6 M de butilitio en hexano en 5 minutos, conservando la temperatura entre 14°C y 16°C. Después 60 minutos, de reacción a temperatura ambiente, se enfrió la solución resultante de amarillo a naranja a -70°C. Se agregaron 9.614 ml (40.56 mmol, 10 equivalente) de triisopropilborato en 10 minutos, manteniendo la temperatura de -65°C. Después de 60 minutos, la reacción a -70°C, enfrió se vertió en una solución HCl acuosa de 1M en cantidad de 35 ml (pH=7-8). La mezcla de reacción se extrajo con 30ml de diclorometano. Se separó la fase hídrica y se re-extrajo con 30ml de diclorometano. Las fases orgánicas se lavaron secuencialmente con 30ml de una solución NaCl acuosa saturada, combinada, secada en Na₂SO₄, filtrada y concentrada bajo presión reducida a 50ml aproximadamente.

ii) Generación del reactivo de boro de la formula IVa' ((R,R)-2-[(E)-(3-trimetilsilil-alil)]-[1,3,2]dioxaborolano-éster dimetil del ácido 4,5-dicarboxílico) y alilación del aldehído acetil-ciclosporina A

Se agregaron 4.88 g (40.56 mmol, 10 equivalente) de dihidrato de sulfato de magnesio agitándose, seguido de 7.23g (40.56 mmol, 10 equivalente) L-(+)-dimetiltartrato. Después de 2 horas de agitación a temperatura ambiente, la suspensión se enfrió a 0°C y 5g (4.056 mmol, 1 equivalente) de aldehído acetil-ciclosporina A se agregaron en una porción. La reacción fue monitoreada por HPLC (conversión aproximada de 90% después de 3 horas). Después de 17 horas

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de agitación a 0°C, la suspensión se filtró y el filtrado se lavó con 50ml de solución NH₄Cl acuosa medio saturada, solución NaHCO₃ acuosa medio saturada de 50 ml y solución NaCl acuosa medio saturada de 50ml. Las fases acuosas fueron extraídas con 50 ml THF y desechadas. Las fases orgánicas combinadas se secaron en Na₂SO₄ filtrado y se concentraron a 40°C bajo presión reducida hasta lograr 11.1g de una mezcla de aproximadamente 75:25 de diastereómeros β-trimetilsililalcohol en forma de aceite amarillo pálido.

iii) Eliminación de Peterson

La mezcla de diastereómeros crudos β-trimetilsililalcohol (11g, máximo 4.056 mmol) se disolvió en 25 ml THF. 0.679 ml (12.16 mmol, 3 equivalente) y se agregó sulfúrico concentrado por goteo manteniendo la temperatura entre 20°C y 25°C. Después de 2 horas a temperatura ambiente, se agregó solución NaCl acuosa medio saturada en cantidad de 50 ml. La mezcla resultante se extrajo dos veces con 50ml MTBE. Las fases orgánicas fueron lavadas con 50 ml de una solución NaCl acuosa medio saturada, combinada, secada con Na₂SO₄ y concentrada bajo presión reducida a 40°C. El E-acetil-ISA247 crudo resultante se re-disolvió en diclorometano 20ml y se concentró bajo presión reducida. El producto crudo fue disuelto en 60ml MTBE. LA cristalización se inicio dentro de los 10 minutos siguientes. Luego la suspensión fue agitada durante 15 minutos adicionales a temperatura ambiente y durante 2 horas a -10°C. Los cristales fueron aislados mediante filtración, lavados con 20ml de MTBE frío (-20°C) y se secaron bajo

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presión reducida hasta lograr 3.6g de (E)-acetil-ISA247 en una cantidad aproximada de 98% de pureza isomérica por medio del NMR.

Ejemplo 3

1) Preparación del complejo dietanolamina de la formula V: 2-(E-(3-trimetilsilil-allyl))-1,3,6,2dioxazaborocano

Se hizo una carga de 50g (424.5 mmol, 1 equivalente) de alitrimetilsilano en el recipiente de reacción seguida por 150ml THF. A la solución transparente e incolora se le agregaron por goteo durante 15 minutos, 165.1ml (445.7 mmol, 1.05 equivalente) de una solución de 2.7M butilitio en heptano, conservando la temperatura entre 20°C y 26°C. Después de 2 horas de reacción a temperatura ambiente, la solución naranja se enfrió a -78°C. Se agregaron 105.7ml (445.8mmol, 1.05 equivalente) trisopropilborato por goteo durante 20 minutos, conservando la temperatura por debajo de -60°C. Después de 1 hora a -70°C, la mezcla de reacción se virió en 250ml de una solución de 2M acuosa de ácido clorhídrico (pH resultante: 5-6). Después de 10 minutos de agitación, se separó la fase de agua y se descartó. Se agregaron 42.4 g (403.3 mmol, 0.95 equivalente) de dietanolamina a la fase orgánica. La solución se agitó durante 70 minutos a temperatura ambiente. Se agregaron 750 ml de heptano. La emulsión bifásica fue concentrada parcialmente a 40°C (aproximadamente 750ml de solvente se destilaron) bajo presión reducida. Luego apareció un precipitado blanco y la suspensión se agitó 2 horas a temperatura ambiente. La suspensión fue luego filtrada. El sólido blanco se lavó con 125ml de heptano y se secó a 40°C bajo

presión reducida durante la noche hasta lograr 85.7g del complejo dietanolamina de la formula V.

^1H NMR (DMSO, en ppm rel. hasta TMS): 6.5 (br s, 1 H), 6.15 (dt, 1H), 5.32 (d, 1H), 3.7 (m, 2H), 3.55 (m, 2H), 2.95 (m, 2H), 2.72 (m, 2H), 1.29 (d, 2H), 0 (s, 9H).

^{11}B NMR (DMSO, en relación con referencia externa $\text{BF}_3 \cdot \text{Et}_2\text{O}$): 11.1 br.s

Microanálisis: (contiene 0.11 equivalente H_2O por titulación Karl-Fischer)

Calculo: C 52.43%, H 9.71%, N 6.12%, B 4.72%, Si 12.27%

Resultado: C 52.04%, H 9.63%, N 6.36%, B 4.79%, Si 11.3%.

ii) Alilación

Se hizo una aplicación de 7.375 g (32.46 mmol, 2 equivalente) del complejo de dietanolamina de la formula V, 20g (16.23 mmol, 1 equivalente) de aldehído de acetil-ciclosporina A y 80ml diclorometano en el recipiente de reacción a temperatura ambiente. Se adicionaron 40ml de agua y 2.79 ml (48.69 mmol, 3 equivalente) de ácido acético en agitación. Después 10 minutos de agitación, se obtuvo una mezcla bifásica transparente. La reacción fue monitoreada mediante HPLC.

iii) Eliminación de Peterson

Después de la reacción durante la noche, la capa orgánica se separó y se descartó la fase hídrica. Se agregaron 50ml THF a la fase orgánica. La solución se concentró bajo presión reducida a 30°C hasta alcanzar la mitad de su volumen. Se

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agregaron 100ml THF y la solución se concentró a 80ml. El volumen se ajustó a 100ml con THF y la solución se enfrió a 0-2°C. Se agregaron 1.812 ml (32.46 mmol, 2 equivalente) de ácido sulfúrico concentrado por goteo durante 5 minutos, conservando la temperatura por debajo de 5°C. Después de la adición, se removió el baño de enfriamiento de la reacción y la temperatura se elevó a temperatura ambiente. Después de 4 horas de reacción, se agregaron 40ml de agua seguidos de 20ml de MTBE. La capa acuosa se separó y descartó. La fase orgánica fue lavada con 40ml $\text{NaHCO}_{3\text{aq}}$, 20 ml de NaCl_{aq} saturado, 40ml de NaCl_{aq} saturado, se secó en Na_2SO_4 , se filtró y concentró a 40°C bajo presión reducida. El E-acetil-ISA247 crudo se re-disolvió en 200ml MTBE y se inició la cristalización dentro de unos cuantos minutos después. Luego de transcurridos 15 minutos a temperatura ambiente y 2.5 horas a 0°C, la suspensión fue filtrada, y los cristales se lavaron con 50ml MTBE y se secaron a 50°C bajo presión reducida hasta obtener 18.45 g de (E)-acetil-ISA247 en forma de polvo blanco (>98% de pureza isomérica por NMR).

iv) Hidrólisis

Este producto crudo fue hidrolizado hasta obtener (E)-ISA247 en 99% de pureza isomérica por HPLC.

Ejemplo 4

i) Alilación

Se hizo una carga de 10.02g (44.1 mmol, 2 equivalente) del complejo de dietanolamina de la formula V obtenida por el método descrito en el Ejemplo 3, i) y

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30g (22.05 mmol, 1 equivalente) de acetil-ciclosporina A aldehído dentro del recipiente de reacción. Se agregaron 36ml de ácido acético a temperatura ambiente. Se obtuvo una solución transparente después de 15 minutos agitándose a temperatura ambiente. La reacción fue monitoreada por HPLC.

ii) Eliminación de Peterson

Después de una reacción de aproximadamente 6 horas a temperatura ambiente, se agregaron 60ml de ácido fórmico, manteniendo la temperatura por debajo de 30°C. La solución transparente amarilla pálida se agitó durante la noche a temperatura ambiente. Se agregaron 18ml de diclorometano y 300ml de MTBE seguidos de 180ml de una solución de NaCl_{aq} al 10%. La fase acuosa se separó y desechó. La fase orgánica se lavó con 180ml de agua, 300ml 2M acuosa NaOH y 90ml de agua. La fase orgánica se concentró a temperatura ambiente bajo presión reducida. La cristalización se inició y la suspensión fue diluida mediante adición de 300ml MTBE y luego se concentró a 330ml aproximadamente. Después de agitar 3 horas a temperatura ambiente y 1 hora a 0-2°C, se filtró la suspensión blanca. Los cristales fueron lavados con 50ml MTBE y secados a 50°C bajo presión reducida hasta obtener 37.4g de (E)-acetil-ISA247 en forma de polvo blanco en >98% de pureza de doble enlace isomérico mediante NMR.

iii) Hidrólisis

Este producto fue hidrolizado hasta obtener (E)-ISA247 en 99.6% de pureza isomérica de doble enlace por HPLC.

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Ejemplo 5

Se disolvieron 1g (0.82 mmol, 1 equivalente) de aldehído acetyl-ciclosporina A en 10ml de diclorometano seguidos de 369 mg (1.62 mmol, 2 equivalente) del complejo de dietanolamina de la formula V obtenida mediante el método descrito en el Ejemplo 3, i). La solución turbia fue enfriada a -40°C. Se agregaron 180 µl (369 mmol, 2 equivalente) de éterato de trifluoruro de boro manteniendo la temperatura por debajo de -40°C. Después de 1 hora a -40°C, se retiró el baño de enfriamiento y la mezcla de reacción se calentó hasta alcanzar la temperatura ambiente. Después de 50 minutos, de reacción a temperatura ambiente, se agregaron 15ml de una solución acuosa al 5% de NaHCO₃. La fase acuosa se separó y se re-extrajo con 15ml de diclorometano. Las fases orgánicas combinadas se secaron en MgSO₄, se filtraron y concentraron bajo presión reducida a 40°C hasta lograr 0.99g de (E)-acetyl-ISA247 en >95% de pureza isomérica de doble enlace (NMR) en forma de espuma blanca.

Ejemplo 6

i) Preparación del complejo pinacol de la formula VII: 4.4.5.5-tetrametil-2-(E-(3-trimetilsilil-allyl))-1.3.2]dioxaborolano

Se disolvieron 20g (169.8 mmol, 1 equivalente) de aliltrimetilsilano en 60ml THF 69.18 ml (186.8 mmol, 1.1 equivalente) de una solución de 2.7M butillitio en heptano se agregaron por goteo durante 10 minutos manteniendo la temperatura entre 20°C y 26°C. Después de 2 horas de reacción a temperatura ambiente, se enfrió la solución amarilla a -78°C. Se agregaron 42.26ml (178.3 mmol, 1.05

equivalente) de triisopropilborato por goteo durante 10 minutos, conservando la temperatura por debajo de -65°C . Después de 1 hora de reacción, la mezcla de esta reacción fue vertida en una solución de 100 ml de 2M de ácido clorhídrico acuoso (resultando pH 6-7). Se agregaron 20ml de diclorometano y la fase acuosa se separó y desechó. La capa orgánica se secó en MgSO_4 , se filtró y concentró a 100ml aproximadamente. Se agregaron 20.48g (169.8 mmol, 1.0 equivalente) de pinacol y la solución resultante se agitó durante 18 horas a temperatura ambiente. La mezcla de reacción se concentró a 40°C bajo presión reducida y luego el aceite resultante se destiló a $43-50^{\circ}\text{C}$ bajo una presión de 0.2mbar hasta obtener 37.2g de un aceite incoloro.

ii) Alilación en un recipiente / eliminación Peterson

Se hizo una carga de 20g (5.06 mmol, 1 equivalente) de aldehído acetil-ciclosporina A y de 5.427g (22.59 mmol, 1.5 equivalente) del complejo pinacol obtenido en i) y 30ml de ácido acético dentro del recipiente de reacción a temperatura ambiente bajo agitación. Se agregaron 30ml de ácido fórmico en condiciones de enfriamiento con baño de agua, conservando la temperatura entre $20-22^{\circ}\text{C}$. Después de 2 horas de reacción a temperatura ambiente, se agregaron 12ml de diclorometano y 200ml de MTBE seguidos de 120ml de una solución NaCl acuosa al 10%. La fase hídrica se separó y retiró. La fase orgánica fue lavada con 120ml de agua, 204ml de solución acuosa 2M NaOH y 60ml de agua. La fase orgánica fue concentrada a 30°C hasta cuando se inició la cristalización. Se agregaron 200ml MTBE y la suspensión fue luego concentrada a 220ml

aproximadamente. Después de agitar a temperatura ambiente durante 2 horas y durante 1 hora a 0-2°C, se filtró la suspensión. El sólido fue lavado con 30ml MTBE y secado a 50°C bajo presión reducida hasta lograr 18g de (E)-acetil-ISA247 en forma de polvo blanco en pureza >98% isomérica de doble enlace (por NMR).

iii) Hidrólisis

Este producto fue hidrolizado hasta obtener (E)-ISA247 en 99.7% de pureza isomérica de doble enlace por HPLC.

Ejemplo 7

Se disolvieron 2g (1.623 mmol, 1 equivalente) de aldehído acetil-ciclosporina A y 779.8mg (3.246 mmol, 2 equivalente) de boronato de pinacol obtenido mediante el método descrito en el Ejemplo 6, i) en 20ml de diclorometano. La solución fue enfriada a -70°C y se agregaron 1.28ml (10.22 mmol, 6.30 equivalente) de eterato de borontrifluoruro. Después de 30 minutos a -70°C, la mezcla de reacción se calentó lentamente a una temperatura de 0°C y luego la reacción se continuó durante otros 60 minutos a una temperatura de 0°C. Se agregaron 20 ml de agua. La fase orgánica se separó, se lavó con 20 ml de solución acuosa de NaHCO₃ al 5%, y se secó con MgSO₄, luego se filtró y concentró a una temperatura de 40°C bajo presión reducida hasta obtener 2.1g de (E)-acetil-ISA247 en >95% de pureza isomérica de doble enlace (NMR) en forma de espuma blanca.

Ejemplo 8

i) Preparación del reactivo alitrifluoroborato: Potasio B-(E-(3-trimetilsilil-ali))-trifluoroborato

Se hizo una carga de 5g (21.35 mmol, 1 equivalente) del complejo dietanolamina obtenido mediante el método descrito en el Ejemplo 3, i), 20 ml de diclorometano, 20ml de agua y 2.44ml (42.70 mmol, 2 equivalente) de ácido acético dentro del recipiente de reacción bajo agitación a temperatura ambiente. Después de 30 minutos de agitación, la fase hídrica se separó y se desechó. Se agregaron 20ml de metanol a las fases orgánicas y la solución se concentró a 40°C bajo presión reducida a 5-10ml. Se agregaron 40ml de metanol seguidos de 3.34g (42.70mmol, 2 equivalente) KHF_2 . Después de 60 minutos de agitación a temperatura ambiente, el sólido restante fue filtrado y descartado. El filtrado se concentró bajo presión reducida a 40°C hasta 25ml aproximadamente. La solución enfriada a 0-2°C y se obtuvo luego una suspensión blanca. Después de 30 minutos, a 0-2°C, la suspensión fue filtrada y el sólido se lavó con metanol frío (-20°C) y posteriormente se secó bajo presión reducida a 40°C hasta obtener 3.4g de un polvo blanco.

^1H NMR (DMSO, δ en ppm rel. a TMS): 6.15 (1H, dt), 5.15 (1H,d), 3.5 (2H, br s agua), 1.1 (2H, m), 0 (9H, s).

^{11}BNM (δ en ppm en relación con referencia externa $\text{BF}_3 \cdot \text{Et}_2\text{O}$): 3.8 (a)

Microanálisis: $\text{C}_{15}\text{H}_{13}\text{F}_3\text{BKSi}$ (contiene 1.08 equivalente. H_2O por titulación Karl-Fischer y 0.5 equivalente KF):

Calculo: C 28.83%, H 5.65%, F 24.78%, B 4.02%, K 21.8%, Si 10.5%

Resultado: C 26.33%, H 5.74%, F 24.71%, B 3.89%, K 22%, Si 9.93%.

ii) Alilación

Se disolvieron 2g (1.623 mmol, 1 equivalente) de aldehídos acetil-ciclosporina A en 10ml de diclorometano. Se agregaron 10ml de agua, seguidos de 735mg (3.246 mmol, 2 equivalente) de trifluoroborato obtenido en i). Después de 2 horas de agitación a temperatura ambiente, la fase orgánica se separó y se descartó la fase de agua.

iii) Eliminación de Peterson

Se agregaron 5ml THF a la fase orgánica y la solución se enfrió a 0-2°C. Se agregaron 181µl (3.246 mmol, 2 equivalente) de ácido sulfúrico concentrado. La mezcla de reacción se calentó a temperatura ambiente. Después de agitar durante la noche, se agregaron 20ml de agua. La capa acuosa se separó y se desecho. La fase orgánica se lavó con 20ml de solución acuosa de NaHCO₃ al 5%, y se secó con MgSO₄, luego se filtró y concentró bajo presión reducida a 40°C hasta lograr 2g de (E)-acetil-ISA247 en forma de espuma blanca con >98% de pureza isomérica de doble enlace (por NMR)..

Ejemplo 9

Se hizo una carga de 2g (1.623 mmol, 1 equivalente) de aldehído acetil-ciclosporina A y 735mg (3.246 mmol, 2 equivalente) de trifluoroborato obtenido mediante el método descrito en el Ejemplo 8, i) y 20ml de diclorometano dentro del

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recipiente de reacción. La suspensión se enfrió a -70°C y 1.28 ml (10.22 mmol, 6.3 equivalente) de eterato de borontrifluoruro se agregaron a esta mezcla. Después de 60 minutos a -70°C , se agregaron 20ml de agua. La fase orgánica se separó, y luego se lavó con 20ml de una solución acuosa al 5% de NaHCO_3 , y se secó en MgSO_4 , luego se filtró y concentró a una temperatura de 40°C bajo presión reducida hasta obtener 2.0g de (E)-acetil-ISA247 en $>98\%$ de pureza isomérica de doble enlace (NMR) en forma de espuma blanca.

Ejemplo 10

i) Alilación por medio del reactivo alilititanio

Se disolvieron 2.67ml (16.23 mmol, 10 equivalente) de aliltrimetilsilano en 6ml THF. Se agregaron 10.14ml (16.23 mmol, 10 equivalente) de una solución de 1.6M butillitio en hexano por goteo manteniendo la temperatura entre $14-20^{\circ}\text{C}$. Después de 30 minutos, a 26°C , la solución naranja se enfrió a -75°C . Se agregaron 4.8ml (16.23 mmol, 10 equivalente) de tetraisopropóxido de titanio por goteo durante 10 minutos, manteniendo la temperatura por debajo de -68°C . Después de 1 hora de reacción a -77°C , se agregaron 2g (1.623 mmol, 1 equivalente) en solución en 6 ml THF por goteo manteniendo la temperatura por debajo de -72°C . La mezcla de reacción se agitó durante 2 horas a -76°C . La temperatura se aumentó lentamente a -40°C y se continuó la agitación por otras 2 horas a -40°C . La mezcla de reacción se virió en una mezcla consistente en 32.5ml de una solución acuosa HCl de 1M y 20ml MTBE. Se agregaron 16.2ml de una solución acuosa HCl 1M y 25ml de agua. La capa acuosa se separó y se re-extrajo con 25ml MTBE. Las

capas orgánicas se lavaron con 30ml de 0.5M HCl_{aq}, se combinaron y se secaron en Na₂SO₄ para luego filtrarse y concentrarse en presión reducida a 40°C hasta lograr 2.26g de la mezcla cruda del anti β-trimetilsililalcohol diastereomérico en forma de espuma blanca.

ii) Eliminación de Peterson

El producto crudo se disolvió en 11.15ml THF y se agregaron 268μl de ácido sulfúrico concentrado. La mezcla de reacción se calentó a 33°C durante 1.5 horas y luego se enfrió a temperatura ambiente. Se agregaron 23ml de agua y la mezcla de reacción se extrajo con 22 ml MTBE. La fase acuosa fue re-extraída con 11ml MTBE. La capa orgánica fue lavada con 11ml de agua, combinada, secada en Na₂SO₄ filtrada y concentrada a 40°C bajo presión reducida hasta obtener 1.89g de crudo (E)-acetil-ISA247 en forma de polvo beige. El producto crudo fue redisoluelto en 20ml MTBE a temperatura ambiente. La cristalización se inicio dentro de unos cuantos minutos más. La suspensión fue agitada 30 minutos a temperatura ambiente, 45 minutos a -10°C y luego se filtró. El sólido se lavó con MTBE frío y se secó a 40°C bajo presión reducida hasta obtener 1.02g de (E)-acetil-ISA247 en forma de polvo blanco en aproximadamente el 98% de pureza isomérica de doble enlace (NMR).

Ejemplo 11

i) Alilación por reactivo de alititanio

Se disolvieron 1.87g (15.85 mmol, 10 equivalente) de aliltrimetilsilano en 20 ml de THF a temperatura ambiente. Se agregaron 5.87ml (15.85 mmol, 10 equivalente)

de una solución de 2.7M de butillitio en heptano por goteo durante 5 minutos, manteniendo la temperatura entre 16°C y 20°C. Después de 1 hora de agitación a temperatura ambiente, la solución amarilla a naranja se enfrió a -78°C. Se agregó una solución de 4.22g (15.85 mmol, 10 equivalente) de clorotrisisopropóxido de titanio en 10ml THF por goteo durante 4 minutos, manteniendo la temperatura por debajo de -60°C. La solución resultante pardo roja se agitó durante 30 minutos a -75°C. Se agregó una solución de 2g (1.585 mmol, 1 equivalente) de aldehído acetil-ciclosporina A en 10ml THF por goteo durante 5 minutos manteniendo la temperatura por debajo de -60°C. Después de 30 minutos a -75°C, se retiró el baño de enfriamiento y la temperatura se elevó a -10°C por 15 minutos aproximadamente. La mezcla de reacción se agregó a una mezcla bifásica consistente en 40ml MTBE y 35ml de una solución acuosa HCl de 2M. La capa acuosa se separó y descartó. La capa orgánica fue lavada con 24ml de solución acuosa HCl de 1M, 15 ml de una solución NaCl acuosa al 10%, 15 ml de una solución NaCl acuosa medio saturada, de cada Na_2SO_4 , filtrada y concentrada bajo presión reducida hasta lograr 2g de la mezcla cruda de los diastereómeros de anti β -trimetilsililalcohol en forma de aceite solidificante.

ii) Eliminación Peterson

El producto crudo fue disuelto en 8 ml THF a temperatura ambiente. La solución se enfrió a 0-5°C y se agregaron 200 μ l de ácido sulfúrico concentrado por goteo. La temperatura se elevó a temperatura ambiente y la mezcla de reacción se agitó durante 10 horas. Se agregaron de 40 ml MTBE y 15 ml de agua. La fase acuosa

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se separó y descartó. La fase orgánica se lavó en 15ml de una solución NaHCO_3 acuosa al 5%, 15ml de una solución NaCl acuosa medio saturada, de cada Na_2SO_4 , filtrada y concentrada a presión reducida hasta lograr 1.8g del crudo E-acetil-ISA247. El diene crudo se disolvió en 20ml de diclorometano. Se agregaron 20ml MTBE y la solución se concentró a 40°C bajo presión reducida hasta la mitad de su volumen. Las últimas dos operaciones se repitieron tres veces con el fin de intercambiar el solvente a partir del diclorometano en MTBE. La solución se enfrió a temperatura ambiente iniciándose la cristalización dentro de unos minutos. La suspensión se agitó durante 2 horas a temperatura ambiente y 30 minutos a 0°C; luego se filtró la suspensión. El sólido se lavó con 15ml MTBE y se secó a presión reducida a 40°C hasta obtener 1.1g de E-acetil-ISA247 en >95% de pureza isomérica de doble enlace (NMR), en forma de polvo blanco.

Ejemplo 12

i) Alilación por reactivo de alilaluminio

Se disolvieron 1.87g (15.85 mmol, 10 equivalente) de aliltrimetilsilano en 20ml de THF a temperatura ambiente. Se agregaron 5.87ml (15.85 mmol, 10 equivalente) de una solución de 2.7M de butilitio en heptano por goteo en 5 minutos, manteniendo la temperatura entre 20°C y 25°C. Después de 1 hora de agitación a temperatura ambiente, la solución amarilla a naranja se enfrió a -75°C. Se agregaron 8.6ml (15.85 mmol, 10 equivalente) de una solución al 25% de cloruro de dietilaluminio en tolueno durante 10 minutos, manteniendo la temperatura por debajo de -55°C. La solución resultante transparente e incolora se agito durante

30 minutos a -75°C . Se agregó una solución de 2g (1.585 mmol, 1 equivalente) de aldehído acetil-ciclosporina A en 10ml de THF por goteo durante 5 minutos manteniendo la temperatura por debajo de -60°C . Después de 30 minutos a -75°C , se retiró el baño de enfriamiento y la temperatura se elevó a -10°C durante 15 minutos. La mezcla de reacción se agregó lentamente (bajo condiciones de baño de agua fría para lograr 10°C) y obtener una mezcla bifásica en consistente en 40ml MTBE y 35ml de una solución acuosa HCl de 1M. La capa acuosa se separó y descartó. La fase orgánica se lavó con 35ml de una solución acuosa HCl de 1M, 25 ml de agua, 25ml de una solución NaCl acuosa saturada, se secó en Na_2SO_4 , se filtró y concentró bajo presión reducida hasta lograr 2g de la mezcla cruda de los diastereómeros anti β -trimetilsililalcohol en forma de aceite solidificante.

ii) Eliminación de Peterson

El producto crudo se disolvió en 10ml THF a temperatura ambiente. La solución se enfrió a $0-5^{\circ}\text{C}$ y se agregaron 200 μl de ácido sulfúrico concentrado por goteo. La temperatura se elevó a temperatura ambiente y las mezclas de reacción se agitaron durante la noche. Se agregaron 40ml MTBE y 15 ml de agua. La fase de agua se separó y descartó. La fase orgánica se lavó con 15ml agua, 15 ml de solución NaHCO_3 acuosa al 5%, 15ml de una solución NaCl acuosa medio saturada, se filtró y concentró bajo presión reducida hasta obtener 1.8g del crudo E-acetil-ISA247. El diene crudo se re-disolvió en 35ml de MTBE. La cristalización se inició dentro de unos minutos. La suspensión se agitó 2 horas a temperatura ambiente y 30 minutos a 0°C . La suspensión se filtró. El sólido se lavó con 15ml

MTBE y se secó bajo presión reducida a 40°C hasta obtener 1g de E-acetil-ISA247 en >95% de pureza isomérica de doble enlace (NMR) en forma de polvo blanco.

Ejemplo 13

Preparación de una solución de reactivo de boro de la formula IIIa: (E)-3-(trimetilsilil) ácido alilborónico

Se disolvió 2g (8.8 mmol, 1 equivalente) del complejo dietanolamina de la formula V como se preparó en el Ejemplo 3-i) en 16ml de d₂-diclorometano (diclorometano deuterado). Se agregaron 759µl (13.2 mmol, 1.5 equivalente) de ácido acético, seguidos de 4ml de agua. La mezcla bifásica se agitó durante 20 minutos a temperatura ambiente hasta obtener una mezcla bifásica transparente de color amarillo pálido. Se suspendió la agitación, y luego la fase hídrica se separó y descartó. La fase orgánica (16 ml de volumen) consistió en una solución de ácido borónico de la formula IIIa según se evidencia mediante ¹¹B NMR y ¹H NMR.

¹¹B NMR (δ en ppm en relación con BF₃.Et₂O como referencia externa): 31.7

¹H NMR (en CD₂Cl₂, δ en ppm relativo a TMS): 6.1 (1H, dt), 5.6 (1H,d), 1.77 (2H,d), 0 (9H,s)

Ejemplo 14

Preparación de una solución de reactivo de boro de la formula IVa': (R,R)-2-[(E)-(3-trimetilsilil-alil)-[1,3,2]dioxaborolano-4,5-éster dimetil del ácido dicarboxílico

A 4ml (2.2 mmol, 1 equivalente) de la solución del ácido borónico de la formula IIIa, que se preparó según descripción del Ejemplo 13, se le agregaron 396 mg

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(2.2 mmol, 1 equivalente) de L-(+)-dimetiltartrato y 265 mg (2.2 mmol, 1 equivalente) de dihidrato de sulfato de magnesio. La suspensión se agito durante 40 minutos a temperatura ambiente y luego se filtró. El filtrado se analizó mediante NMR y demostró que contenía como producto principal el éster boronato de la formula IVa'. según se evidencia por la presentación del ^{11}B y de las señales ^1H NMR consistentes.

^{11}B NMR (en CD_2Cl_2 , δ en ppm en relación con $\text{BF}_3\cdot\text{Et}_2\text{O}$ como referencia externa):

34.2

^1H NMR (en CD_2Cl_2 , δ en ppm relativo a TMS): 6.07 (1H, dt), 5.64 (1H,d), 1.93 (2H,d), 0 (9H,s).

Ejemplo 15

Hidrólisis de E-acetil-ISA247 hasta E-ISA247

Se disolvieron 15g (11.94 mmol, 1 equivalente) de E-acetil-ISA247 a temperatura ambiente en 270ml de metanol. Se agregó una solución de 14.85g (107.5 mmol, 9 equivalente) de carbonato potásico en 60 ml de agua manteniendo la temperatura por debajo de 27°C. La suspensión blanca se calentó a 30°C. La reacción fue monitoreada mediante HPLC. Después de 22 horas de reacción, el metanol se evaporó a 40°C bajo presión reducida. El residuo fue captado en 150ml de etilacetato. La fase de agua se separó y descartó. La fase orgánica se lavó con 45 ml de una solución acuosa al 5% de ácido cítrico y 45ml de una solución acuosa de NaCl medio saturada, secada en Na_2SO_4 , filtrada y concentrada bajo presión

reducida a 40°C hasta obtener 15.1g de E-ISA247 (ensayo al 85%). La purificación se puede realizar mediante técnicas de cromatografía como el HPLC preparativo.

Ejemplo 16

i) Preparación del complejo pinacol de la formula VII: 4.4.5.5-tetrametil-2-(E-(3-trimetilsilil-*alil*))-1,3,2-dioxaborolano

Se hizo una carga de 100g aliltrimetilsilano (1 equivalente) en el reactor 1 seguida de 300 ml THF. La solución se enfrió a 10-15°C y 374 ml, se agregaron 2.5M Butillitio en solución en hexano (1.1 equivalente) manteniendo la temperatura por debajo de 25°C (por espacio de 30 minutos aproximadamente). Después de 1-2 horas a 20-25°C, se enfrió la solución amarilla a naranja a -50°C. Se agregaron 173g de triisopropilborato (1.05 equivalente) por goteo manteniendo la temperatura por debajo de -40°C (durante aproximadamente 30-45 minutos). El embudo de goteo se lavó con 25ml THF. Después de 30 minutos hasta 1 hora a -50°C y hasta -40°C, se agregó una solución de 102.4g de pinacol (1 equivalente) en 100 ml THF, manteniendo la temperatura por debajo de -30°C. El embudo de goteo se lavó con 25ml THF. Después de 30 minutos a -50°C y hasta -30°C, el contenido del reactor 1 fue vertido, bajo agitación, en una mezcla de 81.2g AcOH (1.2 equivalente) y 250 ml de agua (contenida en el reactor 2), manteniendo la temperatura entre 0-25°C. El reactor 1 fue lavado con 50ml THF.

La agitación se discontinuó en el reactor 2, y la fase acuosa se separó y descartó.

La capa orgánica se lavó con 250ml de agua. La fase orgánica se concentró a 500

ml aproximadamente ($T_i = 20-40^{\circ}\text{C}$, 150-200 mbar). Se agregaron 500 ml de Tolueno y la fase orgánica se concentró a 500ml ($T_i = 40-50^{\circ}\text{C}$, $T_j = 50^{\circ}\text{C}$, 150-40 mbar). Se agregaron 500 ml de Tolueno y la fase orgánica se concentró hasta cuando se obtuvo un volumen constante ($T_i = 40-50^{\circ}\text{C}$, $T_j = 50^{\circ}\text{C}$, 150-10 mbar) con el fin de lograr el complejo crudo pinacol en >90% de producción. El complejo pudo utilizarse directamente en la secuencia alilación-eliminación Peterson o bien se pudo destilar bajo presión reducida ($T_i =$ aproximadamente 65°C , $T_{\text{dest}} =$ aproximadamente 50°C , $P = 0.05-0.15$ mbar).

ii) Alilación en un Recipiente - Eliminación de Peterson

Se hizo una carga de 40g de CsA-Aldehído protegido por acetilo en un recipiente de alimentación seguido por 80ml de acetato isopropilo. La suspensión fue transferida al reactor. El recipiente de alimentación se lavó con 50ml de ácido acético el cual se transfirió al reactor. Se obtuvo luego una solución transparente. Se agregó el complejo pinacol (1.25-1.5 equivalente). La solución transparente se calentó a 40°C . Se agregaron 50ml de ácido fórmico. Luego de terminar la alilación y la eliminación Peterson según se evidencia en el análisis HPLC (después de 15-20 horas aproximadamente), se agregaron 246 ml de acetato de isopropilo. La mezcla de reacción se lavó dos veces con 200ml de agua, 300g de solución acuosa KOH en 2M (pH de fase acuosa establecido entre 5-8, y si fuere necesario con solución adicional KOH) y 200ml de formiato de amonio acuoso al 5%. La fase orgánica se concentró a 120ml aproximadamente ($T_i =$ aproximadamente 40°C , 200 mbar aproximadamente) y luego se diluyó con metanol en cantidad de 300ml.

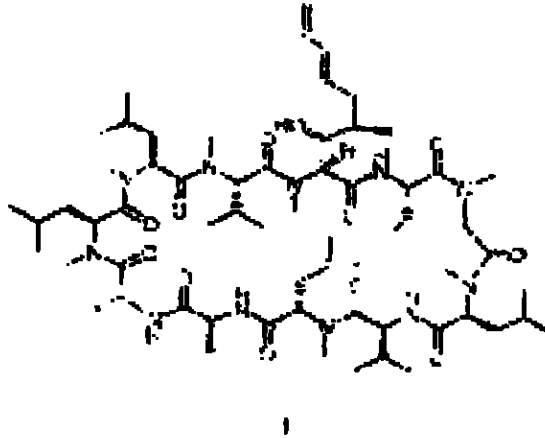
192
143

La fase orgánica se concentró a 120ml aproximadamente ($T_i = 40^{\circ}\text{C}$ aproximadamente, 200 mbar) y se diluyó con 240ml de metanol ($T_i = 40^{\circ}\text{C}$ aproximadamente, 200 mbar). La fase orgánica se concentró a 280ml aproximadamente. Se agregaron 130 ml de agua en 60 minutos aproximadamente a una temperatura de $20-25^{\circ}\text{C}$. La suspensión blanca resultante se agitó durante 60 minutos a temperatura ambiente. El sólido se aisló por filtración, se lavó dos veces con 52ml de una mezcla conformada por agua/metanol, luego se secó al vacío ($T=50^{\circ}\text{C}$) hasta cuando se obtuvo un peso constante para lograr el E-acetil-ISA247 (aproximadamente 35g).


GABRIEL RUEDA CHADID
Traductor e Interpreté Oficial
Fus. 6606 de 1982 del Min. de Justicia
Min. de Relaciones Exteriores

Reivindicaciones

1. Un proceso para la preparación de un análogo de ciclosporina A de la formula I:



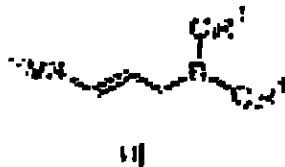
Que comprende:

a-i) alilación de un compuesto de la formula II



donde Pg es un grupo protector y las líneas punteadas significan que el resto del compuesto tiene la misma estructura que la que se encuentra en el compuesto de la formula I,

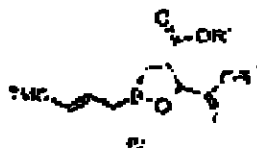
con un compuesto de la formula III



donde R¹ es hidrógeno, alquilo C₁₋₈ o cicloalquilo C₃₋₈ y/o, donde R¹ es hidrógeno.

o uno de sus trimeros; o

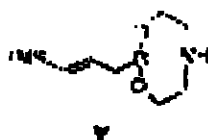
a-ii) alilación de un compuesto de la formula II con el compuesto de la formula IV



donde R² es alquilo C₁₋₈ o cicloalquilo C₃₋₈;

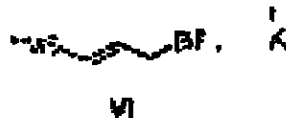
o

a-iii) alilación de un compuesto de la formula II con un compuesto de la formula V



o

a-iv) alilación de un compuesto de la formula II con un compuesto de la formula VI



o

a-v) alilación de un compuesto de la formula II con un compuesto de la formula VII



o

a-vi) alilación de un compuesto de la formula II con una mezcla de reacción que se obtiene mediante un proceso que comprende;

- i) reacción del alitrimetilsilano con butillitio para formar trimetilsililalilitio;
- ii) reacción del trimetilsililalilitio con el trisopropilborato o con el trimetilborato,

y luego la realización de la conformación acuosa,

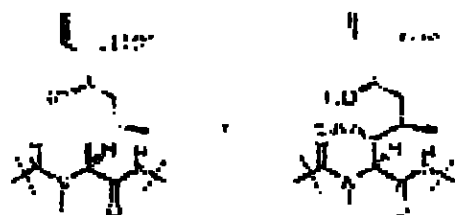
o

a-vii) alilación de un compuesto de la formula II con una mezcla de reacción obtenida mediante la reacción del trimetilsililalilitio con el cloruro de dietilaluminio,

o

a-viii) alilación de un compuesto de la formula II con una mezcla de reacción obtenida mediante la reacción del trimetilsililalilitio con tetraisopropoxido de titanio o clorotrisisopropoxido de titanio,

para formar un compuesto de la formula XI;



XI

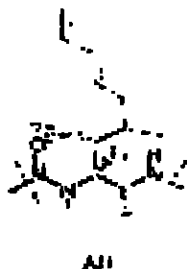
donde Pg corresponde a la definición dada anteriormente;

y

b) la transformación del compuesto de la formula XI en el análogo de la ciclosporina A de la formula I.

2. El proceso según la reivindicación 1, donde el paso b) es realizado por:

b-i) transformación del compuesto de la fórmula XI en un compuesto de la fórmula XII



donde Pg corresponde a la definición dada en la reivindicación 1,

bajo condiciones ácidas; y

b-ii) la transformación del grupo PgO del compuesto de la fórmula XII en un grupo hidróxilo.

3. El proceso según las reivindicaciones 1 o 2, en donde Pg es el grupo acetilo.

4. El proceso según la reivindicación 3, donde el paso a-1), a-ii) o a-vi) se realiza en presencia de tartratos.

5. El proceso según la reivindicación 3, donde el paso a-i), a-ii) o a-vi) se realiza en diclorometano o tolueno.

6. El proceso según la reivindicación 3, donde el paso a-iii) se realiza en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$, ácido fórmico, ácido acético o esteres de tartrato.

7. El proceso según la reivindicación 6, donde el paso a-iii) se realiza en agua / diclorometano o agua / tolueno.

8. El proceso según la reivindicación 6, donde el paso a-iii) y b-i) se realizan en diclorometano o tetrahidrofurano y en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

9. El proceso según la reivindicación 6, donde el paso a-iii) se realiza en ácido acético y/o ácido fórmico; o en una mezcla de ácido acético y/o ácido fórmico y uno o dos cosolventes seleccionados a partir de un grupo conformado por diclorometano y tetrahidrofurano.

10. El proceso según la reivindicación 9, donde el paso a-iii) se realiza en ácido acético y paso b-i) que se realiza por adición de ácido fórmico a la mezcla de reacción.

11. El proceso según la reivindicación 9, donde el paso a-iii) y b-i) se realizan en ácido fórmico o ácido acético / ácido fórmico.

12. El proceso según las reivindicaciones 1 o 2, donde el paso a-iv) se realiza en agua/diclorometano o agua / tolueno.

13. El proceso según la reivindicación 3, donde el paso a-iv) y b-i) se realizan en diclorometano, tetrahidrofurano o tolueno en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

14. El proceso según la reivindicación 3, donde el paso a-v) se realiza en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

15. El proceso según la reivindicación 14, donde el paso a-v) y b-i) se realizan en diclorometano, tetrahidrofurano o tolueno y en presencia de $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

16. El proceso según la reivindicación 3, donde el paso a-v) se realizan en presencia de ácido fórmico o ácido acético.

17. El proceso según la reivindicación 16, donde el paso a-iv) y b-i) se realizan en el ácido fórmico o ácido acético/ácido fórmico.

18. El proceso según la reivindicación 17, donde el paso a-v) y b-i) se realizan en una mezcla de ácido acético/ácido fórmico y co-solvente seleccionado a partir de diclorometano, tolueno, etil acetato e isopropil acetato.

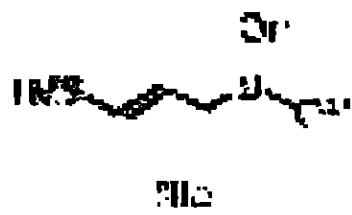
19. El proceso según la reivindicación 18, donde el co-solvente es isopropil acetato.

20. El proceso según la reivindicación 18, donde el paso a-v) se realiza en ácido acético y el paso b-i) se lleva a cabo mediante la adición de ácido fórmico a la mezcla de reacción.

21. El proceso según la reivindicación 3, donde el paso a-vii) se realiza por alilación del compuesto de la formula II con una mezcla de reacción que se prepara mediante la reacción del trimetilsililalilitio con cloruro de dietilaluminio.

22. El proceso según la reivindicación 3, donde el paso a-viii) se realiza mediante la alilación del compuesto de la formula II con una mezcla de reacción preparada por reacción del trimetilsililalilitio con tetraisopropoxido de titanio o clorotrisisopropoxido de titanio.

23. Un proceso para la preparación del un compuesto de la formula IIIa



y/o su trimero;

que comprende la reacción del compuesto de la formula V con agua en diclorometano.

24. Un compuesto de la formula V



25. Un proceso para la preparación de un compuesto de la formula V, que comprende:

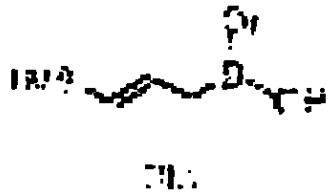
- i) reacción del alitrimetilsilano con butillitio para formar trimetilsililalilitio;
- ii) reacción del trimetilsililalilitio con triisopropilborato o trimetilborato;
- iii) realización de la formación acuosa; y
- iv) reacción del compuesto formado en iii) con dietanolamina para formar un compuesto de la formula VI.

26. Un compuesto de la formula VI



27. Un proceso para la preparación de un compuesto de la formula VI, que comprende:

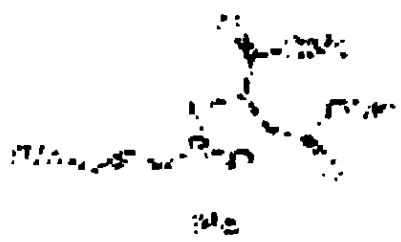
i) reacción de un compuesto de la formula V con agua para formar un compuesto de la formula IIIa



y

- ii) intercambio del solvente de la fase orgánica separada de la mezcla de reacción obtenida en el paso i) hacia metanol,
- iii) reacción de una solución de un compuesto de la formula IIIa que se obtiene en el paso ii) con KHF_2 para formar un compuesto de la formula VI.

28. Un compuesto de la formula IVa



La anterior es traducción oficial de un documento presentado originalmente en Inglés. Esta traducción fue realizada por **GABRIEL RUEDA CHADID, Traductor Oficial**. Resolución 5986 del Minjusticia, por lo cual estampo mi sello y firma.

GABRIEL RUEDA CHADID
 Traductor e Interprete Oficial
 Res. 5986 de 1982 del Min. de Justicia
 Min. de Relaciones Exteriores

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-22

Methods for the preparation of (E)-ISA 247 by means of the addition reaction between a cyclosporin-aldehyde derivative and a 1-(trimethylsilyl)-propen-3-yl organometallic compound, followed by Peterson elimination.

2. claims: 23-25 and 27

Diethanolamine 1-(trimethylsilyl)-propene-3-boronate ester, its preparation and conversion to other 1-(trimethylsilyl)-propene-3-boron compounds.

3. claim: 26

Potassium 1-(trimethylsilyl)-propene-3-boron-trifluoride.

4. claim: 28

dimethyltartrate 1-(trimethylsilyl)-propene-3-boronate ester.

INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/EP2004/003504

Patent document cited in search report	Publication date	Patent family member	Publication date
WO 03033527 A	24-04-2003	WO 03033526 A2	24-04-2003
		WO 03033527 A2	24-04-2003
		WO 03032949 A1	24-04-2003
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		HU 9500332 A3	30-10-1995
		IE 62482 B1	08-02-1995
		IL 86751 A	27-02-1994

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
21 October 2004 (21.10.2004)

PCT

(10) International Publication Number
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(51) International Patent Classification⁷: C07K 7/64.
C07F 5/02

(21) International Application Number:
PCT/EP2004/003504

(22) International Filing Date: 2 April 2004 (02.04.2004)

(25) Filing Language: English

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(30) Priority Data:
03007921.4 8 April 2003 (08.04.2003) EP

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85255 (US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): 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, GR, GU, HD, IL, IN, IS, JP, KE,
KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,
MG, MK, MN, MW, MX, MY, 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.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), Euro-
pean (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), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
ML, MR, NE, SN, TD, TG).

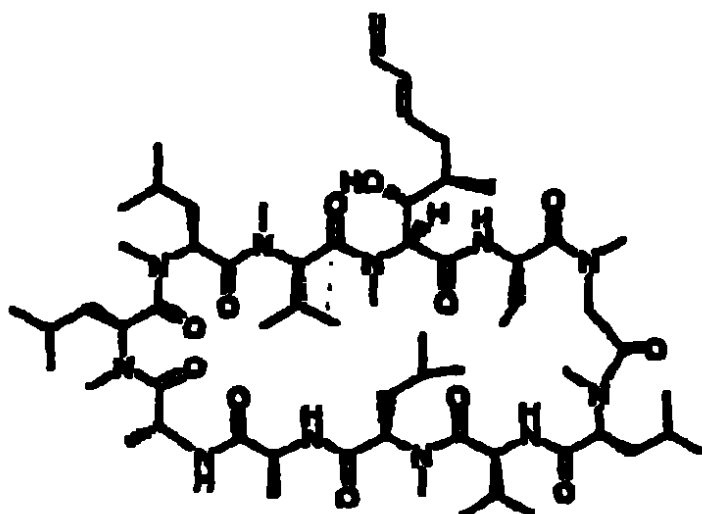
Published:

— with international search report

(88) Date of publication of the international search report:
6 January 2005

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: PROCESS FOR PREPARATION OF CYCLOSPORIN "A" ANALOGUES HAVING A TERMINAL DIENE GROUP



(57) Abstract: The invention is concerned
with a new process for the preparation of a
cyclosporin A analog of formula I comprising,
a) allylating a protected cyclosporin A aldehyde
with an allylmetal reagent and, b) converting the
compound obtained in step a) to the cyclosporin
A analog of formula I. In particular, the allyl
reagent is a trimethylsilyl-allyl borate ester or
a trimethylsilyl-allyl organometallic compound
containing aluminium or titanium.

From the INTERNATIONAL BUREAU

PCT**NOTIFICATION CONCERNING
TRANSMITTAL OF COPY OF INTERNATIONAL
APPLICATION AS PUBLISHED OR REPUBLISHED**

To:

WILKS, Susan, Sutterfield
Isotechnika Inc.
Suite 100
17301 North Perimeter Drive
Scottsdale, AZ 85255
ETATS-UNIS D'AMERIQUE

2650.PCT

Date of mailing (day/month/year)
06 January 2005 (06.01.2005)

Applicant's or agent's file reference
Case 21711

IMPORTANT NOTICE

International application No.
PCT/EP2004/003504

International filing date (day/month/year)
02 April 2004 (02.04.2004)

Priority date (day/month/year)
08 April 2003 (08.04.2003)

Applicant

ISOTECHNIKA INC. et al

The International Bureau transmits herewith the following documents:

- ☐ copy of the international application as published by the International Bureau on under No. W()
- ☒ copy of international application as republished by the International Bureau on 06 January 2005 (06.01.2005) under No. WO 2004/089960
For an explanation as to the reason for this republication of the international application, reference is made to INID codes (15), (48) or (88) (as the case may be) on the front page of the attached document.

DOCKETED**RECEIVED**
JAN 12 2005

BY:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Ellen Moyse

Facsimile No.+41 22 740 14 35

Facsimile No.+41 22 338 89 75

INTERNATIONAL SEARCH REPORT

PCT/EP2004/003504

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/EP2004/003504

C. (Classification) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BATEY R A ET AL: "Potassium Allyl- and Crotyltrifluoroborates: Stable and Efficient Agents for Allylation and Crotylation" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL. vol. 40, no. 23, 4 June 1999 (1999-06-04), pages 4289-4292, XP004164628 ISSN: 0040-4039 tables 1-2; scheme 2	26,27
Y	THADANI AVINASH N ET AL: "A mild protocol for allylation and highly diastereoselective syn or anti crotylation of aldehydes in biphasic and aqueous media utilizing potassium allyl- and crotyltrifluoroborates." ORGANIC LETTERS. 31 OCT 2002, vol. 4, no. 22, 31 October 2002 (2002-10-31), pages 3827-3830, XP002296391 ISSN: 1523-7060 tables 2 and 3	26
Y	MARRON T G ET AL: "Diastereoselective Synthesis of N-Benzoyl Mycalamine, the Fully Elaborated Trioxadecalin Nucleus of Mycalamide A. Control of the Key N-Acyl Amino Stereocenter via Carbamate Acylation" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL. vol. 36, no. 10, 6 March 1995 (1995-03-06), pages 1581-1584, XP004028537 ISSN: 0040-4039 cited in the application page 1583, the compounds 15-17 and the corresponding reaction scheme	28
Y	RATHKE, M. R.; CHAO, E.; MU, G.: "The preparation and reactions of esters of dichloromethaneboronic acid" JOURNAL OF ORGANOMETALLIC CHEMISTRY, vol. 122, no. 2, 1976, pages 145-149, XP002296392 page 146, third paragraph	25

INTERNATIONAL SEARCH REPORT

PCT/EP2004/003504

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	TSAI D J S ET AL: "A STEREO CONTROLLED SYNTHESIS OF Z AND E TERMINAL DIENES FROM PINACOL E-1 TRI METHYLSILYL-1 PROPENE-3-BORONATE" TETRAHEDRON LETTERS, vol. 22, no. 29, 1981, pages 2751-2752, XP002288404 ISSN: 0040-4039 cited in the application abstract; reaction scheme on page 2751	1-27
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A	EP 0 296 122 A (SANDOZ AG ; SANDOZ AG (CH); SANDOZ AG (DE)) 21-December 1988 (1988-12-21) page 8, line 50; examples E,F; compounds 1.24,6.3	
Y	EP 1 291 347 A (CLARIANT GMBH) 12 March 2003 (2003-03-12) page 3, the last reaction scheme; page 4, lines 52-54	23-25,27

-/-

INTERNATIONAL SEARCH REPORT

PCT/EP2004/003504

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K 004 C07F5/02

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 C07K C07F

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, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 03/033527 A (FOSTER ROBERT T ; ISOTECHNIKA INC (CA); NAICKER SELVARAJ (CA); YATSCOF) 24 Apr11 2003 (2003-04-24) page 21, line 30 to page 26, line 23; page 40, line 23 to page 41, line 4; figures 2A, 5, 6 and 12	1-22
P, X	WO 03/033526 A (JAYARAMAN SEETHARAMAN ; ABEL MARK (CA); FOSTER ROBERT T (CA); ISOTECHN) 24 Apr11 2003 (2003-04-24) page 31, second paragraph; page 52, third paragraph; figures 2A, 5, 6 and 12.	1-22
Y	WO 99/18120 A (FOSTER ROBERT T ; ISOTECHNIKA INC (CA); NAICKER'S SELVARAJ (CA); YATSC) 15 Apr11 1999 (1999-04-15) cited in the application figure 4; examples 5, 7 <i>disclosure with chemistry</i> <i>the - Patent Office</i> <i>-/-</i>	1-22

☒ 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 claims 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.

"Z" document member of the same patent family

Date of the actual completion of the international search

15 September 2004

Date of mailing of the international search report

15. 09. 04

Name and mailing address of the ISA

European Patent Office, P.O. Box 5818 Patentplan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Fausti, S

11-30-04

From the INTERNATIONAL BUREAU

PCT

FIRST NOTICE INFORMING THE APPLICANT OF
THE COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES WHICH
DO NOT APPLY THE 30 MONTH TIME LIMIT
UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

WILKS, Susan, Sutterfield
Isotechnika Inc.
Suite 100
17301 North Perimeter Drive
Scottsdale, AZ 85255
ETATS-UNIS D'AMERIQUE

2650-PCT

Date of mailing (day/month/year) 11 November 2004 (11.11.2004)		IMPORTANT NOTICE	
Applicant's or agent's file reference Case 21711			
International application No. PCT/EP2004/003504	International filing date (day/month/year) 02 April 2004 (02.04.2004)	Priority date (day/month/year) 08 April 2003 (08.04.2003)	
Applicant ISOTECHNIKA INC. et al			

1. **ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), does apply, please see Form PCT/IB/308 (Second and Supplementary Notice) (to be issued promptly after the expiration of 28 months from the priority date).

2. Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, does not apply, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
21 October 2004 (21.10.2004)

CH

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

3. The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, does not apply, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1:

FI, LU, SE, TZ, UG, ZM

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

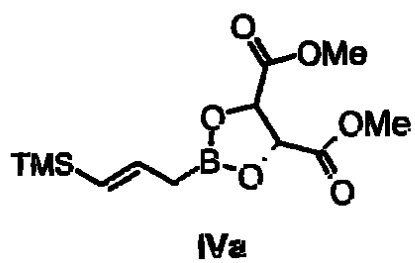
4. **TIME LIMITS for entry into the national phase**

For the designated Office(s) listed above, and unless a demand for international preliminary examination has been filed before the expiration of 19 months from the priority date (see Article 39(1)), the applicable time limit for entering the national phase will, subject to what is said in the following paragraph, be 20 MONTHS from the priority date.

In practice, time limits other than the 20-month time limit will continue to apply, for various periods of time, in respect of certain of the designated Offices listed above. For regular updates on the applicable time limits (20 or 21 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/co/index.html>.

It is the applicant's sole responsibility to monitor all these time limits.

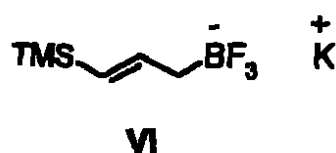
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Ellen Moyse
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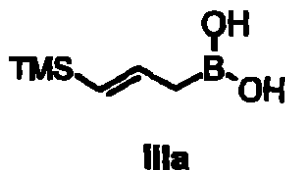
- i) reacting allyltrimethylsilane with butyllithium to form trimethylsilylallyllithium;
- ii) reacting trimethylsilylallyllithium with triisopropylborate or trimethylborate;
- 5 iii) conducting aqueous work up; and
- iv) reacting the compounds formed in iii) with diethanolamine to form a compound of formula V.

26. A compound of formula VI



10 27. A process for the preparation of a compound of formula VI, comprising

- i) reacting a compound of formula V with water to form a compound of formula IIIa



15 and

- ii) exchanging the solvent of the separated organic phase of the reaction mixture obtained in step i) to methanol,
- iii) reacting a solution of compound of formula IIIa obtained in step ii) with KHF_2 to form a compound of formula VI.

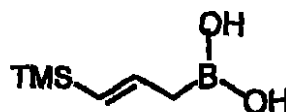
20 28. A compound of formula IVa

20. The process according to claim 16, wherein step a-v) is conducted in acetic acid and step b-i) is conducted by addition of formic acid to the reaction mixture.

5 21. The process according to claim 3, wherein step a-vii) is conducted by allylating the compound of formula II with a reaction mixture prepared by reaction of the trimethylsilylallyllithium with diethylaluminum chloride.

22. The process according to claim 3, wherein step a-viii) is conducted by allylating the compound of formula II with a reaction mixture prepared by reaction of trimethylsilylallyllithium with titanium tetraisopropoxide or
10 titanium chlorotrisopropoxide.

23. A process for the preparation of the compound of formula IIIa

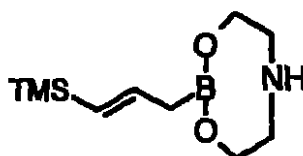


IIIa

and/or a trimer thereof,

15 comprising reacting the compound of formula V with water in dichloromethane.

24. A compound of formula V

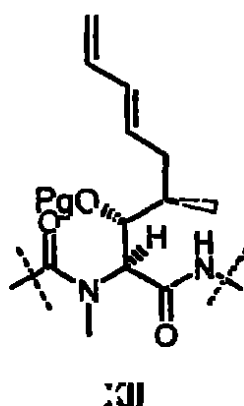


V

25. A process for the preparation of a compound of formula V, comprising

acid and one or two cosolvents selected from a group consisting of dichloromethane and tetrahydrofuran.

- 5 10. The process according to claim 9, wherein step a-iii) is conducted in acetic acid and step b-i) is conducted by addition of formic acid to the reaction mixture.
11. The process according to claim 9, wherein step a-iii) and b-i) are conducted in formic acid or acetic acid/ formic acid.
- 10 12. The process according to claim 1 or 2, wherein step a-iv) is conducted in water/dichloromethane or water/toluene.
13. The process according to claim 3, wherein step a-iv) and b-i) are conducted in dichloromethane, tetrahydrofuran or toluene in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- 15 14. The process according to claim 3, wherein step a-v) is conducted in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
15. The process according to claim 14, wherein step a-v) and b-i) are conducted in dichloromethane, tetrahydrofuran or toluene and in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- 20 16. The process according to claim 3, wherein step a-v) is conducted in the presence of formic acid or acetic acid.
17. The process according to claim 16, wherein step a-v) and b-i) are conducted in formic acid or acetic acid/formic acid.
- 25 18. The process according to claim 17, wherein step a-v) and b-i) are conducted in a mixture of acetic acid/formic acid and co-solvent selected from dichloromethane, toluene, ethyl acetate and isopropyl acetate.
19. The process according to claim 18, wherein co-solvent is isopropyl acetate.



wherein Pg is as defined in claim 1,

under acidic conditions; and

5 b-ii) converting the PgO group of the compound of formula XII to a hydroxyl group.

3. The process according to claim 1 or 2, wherein Pg is acetyl group.

4. The process according to claim 3, wherein step a-i), a-ii) or a-vi) is conducted in the presence of tartrates.

10 5. The process according to claim 3, wherein step a-i), a-ii) or a-vi) is conducted in dichloromethane or toluene.

6. The process according to claim 3, wherein step a-iii) is conducted in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$, formic acid, acetic acid or tartrate esters.

15 7. The process according to claim 6, wherein step a-iii) is conducted in water/dichloromethane or water/toluene.

8. The process according to claim 6, wherein step a-iii) and b-i) are conducted in dichloromethane or tetrahydrofuran and in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

20 9. The process according to claim 6, wherein step a-iii) is conducted in acetic acid and/or formic acid; or in a mixture of acetic acid and /or formic

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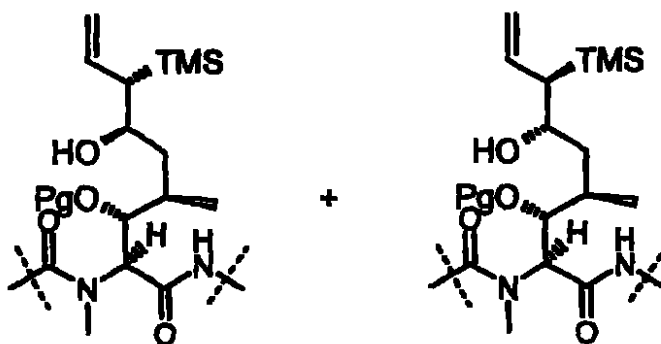
- 54 -

a-vii) allylating a compound of formula II with a reaction mixture obtained by reaction of the trimethylsilylallyllithium with diethylaluminum chloride,

or

5 a-viii) allylating a compound of formula II with a reaction mixture obtained by reaction of the trimethylsilylallyllithium with titanium tetraisopropoxide or titanium chlorotrisisopropoxide,

to form a compound of formula XI;



XI

10 wherein Pg is as defined above;

and

b) converting the compound of formula XI to the cyclosporin A analog of formula I.

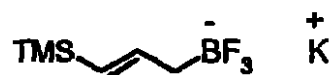
2. The process according to claim 1, wherein step b) is conducted by

15 b-i) converting the compound of formula XI to a compound of formula XII

- 53 -

or

a-iv) allylating a compound of formula II with a compound of formula



VI

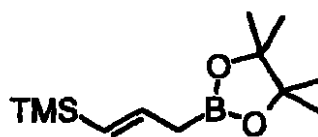
VI

5

or

a-v) allylating a compound of formula II with a compound of formula

VII



VII

or

a-vi) allylating a compound of formula II with a reaction mixture obtained by a process comprising;

i) reacting allyltrimethylsilane with butyllithium to form trimethylsilylallyllithium;

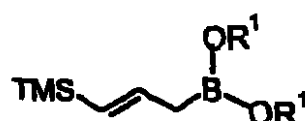
ii) reacting trimethylsilylallyllithium with triisopropylborate or trimethylborate, and then conducting aqueous work up,

or

15

,wherein Pg is a protecting group and the dotted lines mean that the remainder of the compound has the same structure as that of the compound of formula I,

with a compound of formula III

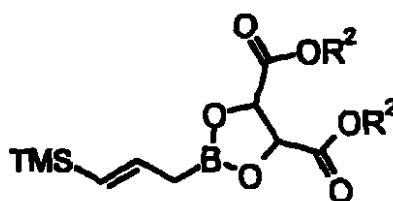


III

,wherein R¹ is hydrogen, C₁-₈ alkyl or C₃-₈ cycloalkyl and/or, when R¹ is hydrogen, a trimer thereof;

or

a-ii) allylating a compound of formula II with a compound of formula IV

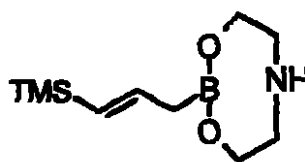


IV

wherein R² is C₁-₈ alkyl or C₃-₈ cycloalkyl;

or

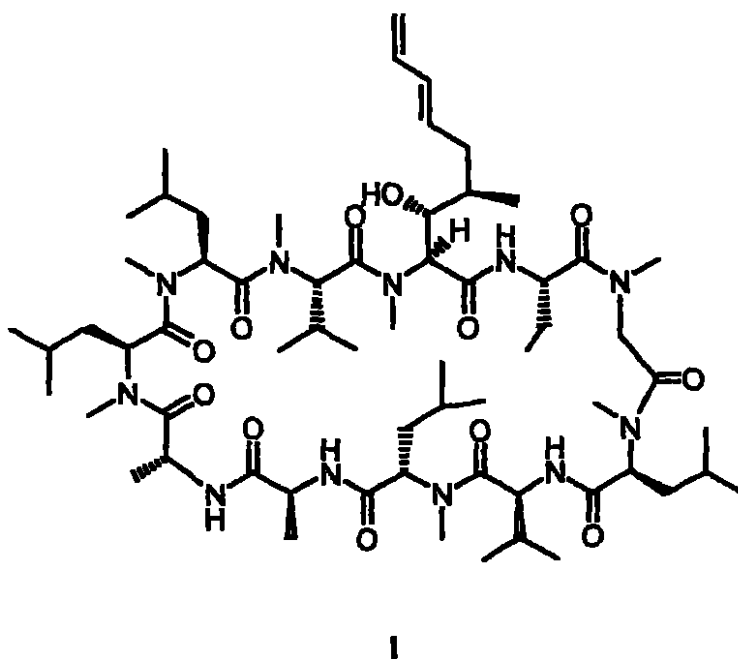
a-iii) allylating a compound of formula II with a compound of formula V



V

Claims

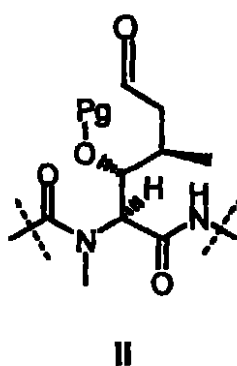
1. A process for the preparation of a cyclosporin A analog of formula I



5

comprising

- a-i) allylating a compound of formula II



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- 50 -

min. at 20-25 °C. The resulting white suspension was stirred 60 min. at room temperature. The solid was isolated by filtration, washed twice with 52 ml of a water/methanol mixture, dried under vacuum (T=50 °C) until constant weight to provide E-acetyl-ISA247 (ca 35 g).

solution in hexane (1.1 equiv.) were added keeping the temperature below 25 °C (over ca 30 min.). After 1-2 hours at 20-25 °C, the yellow to orange solution was cooled -50 °C. 173 g Triisopropylborate (1.05 equiv.) were added dropwise keeping the temperature below -40 °C (over ca 30-45 min.). The dropping funnel
5 was washed with 25 ml THF. After 30 min. to 1 hour at -50 °C to -40 °C, a solution of 102.4 g of pinacol (1 equiv.) in 100 ml THF was added, keeping the temperature below -30 °C. The dropping funnel was washed with 25 ml THF. After 30 min. at -50 °C to -30 °C, the content of reactor 1 was poured, under stirring, onto a mixture of 61.2 g AcOH (1.2 equiv.) and 250 ml water (contained
10 in reactor 2), keeping the temperature between 0-25 °C. Reactor 1 was washed with 50 ml THF.

Stirring was discontinued in reactor 2, the aqueous phase was separated and discarded. The organic layer was washed with 250 ml water. The organic phase was concentrated to ca 500 ml (Ti = 20-40 °C, 150-200 mbar). 500 ml Toluene
15 were added and the organic phase was concentrated to 500 ml (Ti = 40-50 °C, Tj = 50 °C, 150-40 mbar). 500 ml Toluene were added and the organic phase was concentrated until constant volume (Ti = 40-50 °C, Tj = 50 °C, 150-10 mbar) to provide crude pinacol complex in >90% yield. The complex could be used directly in the allylation-Peterson elimination sequence or could be distilled under reduced
20 pressure (Ti = ca 65 °C, Tdest = ca 50 °C, P = 0.05-0.15 mbar).

ii) One-Pot Allylation-Peterson Elimination

40 g acetyl protected CaA-Aldehyde were charged in a feed vessel followed by 80 ml isopropyl acetate. The suspension was transferred to the reactor. The feed vessel was washed with 50 ml acetic acid which was transferred to the reactor. A
25 clear solution was then obtained. Pinacol complex (1.25-1.5 equiv.) was added. The clear solution was heated to 40 °C. 50 ml formic acid were added. After completion of the allylation and Peterson elimination as evidenced by HPLC analysis (after ca 15-20 hours), 246 ml isopropyl acetate were added. The reaction mixture was washed twice with 200 ml water, 300 g of 2M aqueous KOH solution
30 (pH of the aqueous phase set between 5-8, if necessary with additional KOH solution) and 200 ml of 5% aqueous ammonium formate. The organic phase was concentrated to ca 120 ml (Ti = ca 40 °C, ca 200 mbar) and was diluted with 300 ml methanol. The organic phase was concentrated to ca 120 ml (Ti = ca 40 °C, 200 mbar) and was diluted with 240 ml methanol (Ti = ca 40 °C, 200 mbar). The
35 organic phase was concentrated to ca 280 ml. 130 ml water were added over ca 60

Preparation of a solution of boron reagent of formula IVa' : (R,R)-2-[(E)-(3-trimethylsilyl-allyl)]-[1,3,2]dioxaborolane-4,5-dicarboxylic acid dimethyl ester

To 4 ml (2.2 mmol, 1 equiv.) of the solution of the boronic acid of formula IIIa, prepared as described in Example 13, were added 396 mg (2.2 mmol, 1 equiv.) of L-(+)-dimethyltartrate and 265 mg (2.2 mmol, 1 equiv.) of magnesium sulfate dihydrate. The suspension was stirred for 40 min. at RT and was filtered. The filtrate was analyzed by NMR and was shown to contain, as main product the boronate ester of formula IVa' as evidenced by the appearance of consistent ^{11}B and ^1H NMR signals.

^{11}B NMR (in CD_2Cl_2 , δ in ppm relative to $\text{BF}_3 \cdot \text{Et}_2\text{O}$ as external reference) : 34.2

^1H NMR (in CD_2Cl_2 , δ in ppm relative to TMS) : 6.07 (1H, dt), 5.64 (1H, d), 1.93 (2H, d), 0 (9H, s).

Example 15

Hydrolysis of E-acetyl-ISA247 to E-ISA247

15 g (11.94 mmol, 1 equiv.) of E-acetyl-ISA247 were dissolved at RT in 270 ml of methanol. A solution of 14.85 g (107.5 mmol, 9 equiv.) of potassium carbonate in 60 ml of water was added keeping the temperature below 27 °C. The white suspension was heated to 30 °C. The reaction was monitored by HPLC. After 22 hours of reaction, the methanol was evaporated at 40 °C under reduced pressure. The residue was taken up in 150 ml of ethylacetate. The water phase was separated and discarded. The organic phase was washed with 45 ml of a 5% aqueous solution of citric acid and 45 ml of a half saturated aqueous NaCl solution, dried over Na_2SO_4 , filtered and concentrated under reduced pressure at 40 °C to give 15.1 g of E-ISA247 (85% assay). Purification can be performed by chromatographic techniques like preparative HPLC.

Example 16

i) Preparation of the pinacol complex of formula VII : 4,4,5,5-tetramethyl-2-(E-(3-trimethylsilyl-allyl))-1,3,2-dioxaborolane

100 g allyltrimethylsilane (1 equiv.) were charged in reactor 1 followed by 300 ml THF. The solution was cooled to 10-15 °C and 374 ml of 2.5M Butyllithium

solution, dried over Na_2SO_4 , filtered and concentrated under reduced pressure to provide 2 g of the crude mixture of anti β -trimethylsilyl alcohol diastereomers as a solidifying oil.

ii) Peterson elimination

5 The crude product was dissolved in 10 ml THF at RT. The solution was cooled to 0-5 °C and 200 μl of concentrated sulfuric acid were added dropwise. The temperature was raised to RT and the reaction mixture was stirred overnight. 40 ml MTBE and 15 ml of water were added. The water phase was separated and discarded. The organic phase was washed with 15 ml water, 15 ml of a 5% aqueous
10 NaHCO_3 solution, 15 ml of a half saturated aqueous NaCl solution, filtered and concentrated under reduced pressure to give 1.8 g of crude E-acetyl-ISA247. The crude diene was redissolved in 35 ml of MTBE. The crystallization started within a few minutes. The suspension was stirred 2 hours at RT and 30 min. at 0 °C. The suspension was filtered. The solid was washed with 15 ml MTBE and dried under
15 reduced pressure at 40 °C to give 1 g of E-acetyl-ISA247 in >95% double bond isomeric purity (NMR), as a white powder.

Example 13

Preparation of a solution of boron reagent of formula IIIa : (E)-3-(trimethylsilyl)allylboronic acid

20 2 g (8.8 mmol, 1 equiv.) of diethanolamine complex of formula V as prepared in Example 3-i) was dissolved in 16 ml of d_2 -dichloromethane (deuterated dichloromethane). 759 μl (13.2 mmol, 1.5 equiv.) of acetic acid were added, followed by 4 ml of water. The biphasic mixture was stirred for 20 min. at RT to give a light yellow clear biphasic mixture. Stirring was stopped, the water phase
25 was separated and discarded. The organic phase (16 ml volume) consisted in a solution of boronic acid of formula IIIa as evidenced by ^{11}B NMR and ^1H NMR.

^{11}B NMR (δ in ppm relative to $\text{BF}_3 \cdot \text{Et}_2\text{O}$ as external reference) : 31.7

^1H NMR (in CD_2Cl_2 , δ in ppm relative to TMS) : 6.1 (1H, dt), 5.6 (1H, d), 1.77 (2H, d), 0 (9H, s).

30 Example 14

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ii) Peterson elimination

The crude product was dissolved in 8 ml THF at RT. The solution was cooled to 0-5 °C and 200 µl of concentrated sulfuric acid were added dropwise. The temperature was raised to RT and the reaction mixture was stirred 10 hours.
5 40 ml MTBE and 15 ml of water were added. The water phase was separated and discarded. The organic phase was washed 15 ml of a 5% aqueous NaHCO₃ solution, 15 ml of a half saturated aqueous NaCl solution, dried over Na₂SO₄, filtered and concentrated under reduced pressure to give 1.8 g of crude E-acetyl-ISA247. The crude diene was dissolved in 20 ml dichloromethane. 20 ml MTBE
10 were added, and the solution was concentrated at 40 °C under reduced pressure to half its volume. The last two operations was repeated three times to in order to exchange the solvent from dichloromethane to MTBE. The solution was cooled to RT and the crystallization started within a few minutes. The suspension was stirred 2 hours at RT and 30 min. at 0 °C. The suspension was filtered. The solid was
15 washed with 15 ml MTBE and dried under reduced pressure at 40 °C to give 1.1 g of E-acetyl-ISA247 in >95% double bond isomeric purity (NMR), as a white powder.

Example 12

i) Allylation by allylaluminum reagent:

20 1.87 g (15.85 mmol, 10 equiv.) of allyltrimethylsilane were dissolved in 20 ml of THF at RT. 5.87 ml (15.85 mmol, 10 equiv.) of a 2.7M solution of butyllithium in heptane were added dropwise over 5 min., keeping the temperature between 20 °C and 25 °C. After 1 hour stirring at RT, the yellow to orange solution was cooled to -75 °C. 8.6 ml (15.85 mmol, 10 equiv.) of a 25% solution of
25 diethylaluminum chloride in toluene were added over 10 min., keeping the temperature below -55 °C. The resulting clear colorless solution was stirred for 30 min. at -75 °C. A solution of 2 g (1.585 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde in 10ml of THF was added dropwise over 5min. maintaining the temperature below -60 °C. After 30 min. at -75 °C, the cooling bath was removed
30 and the temperature was raised to -10 °C over 15 min. The reaction mixture was slowly added (under cold water bath cooling 10 °C) to a biphasic mixture consisting of 40 ml MTBE and 35 ml of a 1M aqueous HCl solution. The aqueous layer was separated and discarded. The organic phase was washed with 35 ml of 1M aqueous HCl solution, 25 ml of water, 25 ml of a saturated aqueous NaCl

under reduced pressure at 40 °C to give 2.26 g of crude mixture of diastereomeric anti β -trimethylsilyl alcohols as a white foam.

ii) Peterson elimination

The crude product was dissolved in 11.15 ml THF and 268 μ l concentrated sulfuric acid were added. The reaction mixture was heated at 33 °C for 1.5 hour and then cooled to RT. 22 ml water were added and the reaction mixture was extracted with 22 ml MTBE. The aqueous phase was re-extracted with 11 ml MTBE. The organic layer were washed with 11 ml water, combined, dried over Na₂SO₄, filtered and concentrated at 40 °C under reduced pressure to give 1.89 g of crude (E)-acetyl-ISA247 as a beige powder. The crude product was re-dissolved in 20 ml MTBE at RT. The crystallization started within a few minutes. The suspension was stirred 30 min. at RT, 45 min. at -10 °C and was filtered. The solid was washed with cold MTBE and dried at 40 °C under reduced pressure to give 1.02 g of (E)-acetylISA247 as a white powder in ca 98% double bond isomeric purity (NMR).

Example 11

i) Allylation by an allyltitanium reagent

1.87 g (15.85 mmol, 10 equiv.) of allyltrimethylsilane were dissolved in 20 ml of THF at RT. 5.87 ml (15.85 mmol, 10 equiv.) of a 2.7 M solution of butyllithium in heptane were added dropwise over 5 min., keeping the temperature between 16 °C and 20 °C. After 1 hour stirring at RT, the yellow to orange solution was cooled to -76 °C. A solution of 4.22 g (15.85 mmol, 10 equiv.) of titanium chlorotriisopropoxide in 10 ml THF was added dropwise over 4 min., keeping the temperature below -60 °C. The resulting brown-red solution was stirred for 30 min. at -75 °C. A solution of 2 g (1.585 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde in 10 ml of THF was added dropwise over 5 min. maintaining the temperature below -60 °C. After 30 min. at -75 °C, the cooling bath was removed and the temperature was raised to -10 °C over ca 15 min. The reaction mixture was added to a biphasic mixture consisting of 40 ml MTBE and 35 ml of a 2M aqueous HCl solution. The aqueous layer was separated and discarded. The organic phase was washed with 24 ml of 1M aqueous HCl solution, 15 ml of a 10% aqueous NaCl solution, 15 ml of a half saturated aqueous NaCl solution, dried over Na₂SO₄, filtered and concentrated under reduced pressure to provide 2 g of the crude mixture of anti β -trimethylsilyl alcohol diastereomers as a solidifying oil.

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5 ml THF were added to the organic phase and the solution was cooled to 0-2 °C. 181 µl (3.246, 2 equiv.) concentrated sulfuric acid were added. The reaction mixture was warmed up to RT. After stirring overnight, 20 ml water were added. The aqueous layer was separated and discarded. The organic phase was washed with 20 ml of 5% aqueous NaHCO₃ solution, dried over MgSO₄, filtered and concentrated under reduced pressure at 40 °C to give 2 g of (E)-acetyl-ISA247 as a white foam in >98% double bond isomeric purity (by NMR).

Example 9

2 g (1.623 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde and 735 mg (3.246 mmol, 2 equiv.) trifluoroborate obtained by the method described in Example 8, i) and 20 ml dichloromethane were charged in the reaction vessel. The suspension was cooled to -70 °C and 1.28 ml (10.22 mmol, 6.3 equiv.) borontrifluoride etherate were added. After 60 min. at -70 °C, 20 ml water were added. The organic phase was separated, washed with 20 ml of a 5% aqueous NaHCO₃ solution, dried over MgSO₄, filtered and concentrated at 40 °C under reduced pressure to give 2.0 g of (E)-acetyl-ISA247 in >98% double bond isomeric purity (NMR) as a white foam.

Example 10

i) Allylation by an allyltitanium reagent

2.67 ml (16.23 mmol, 10 equiv.) allyltrimethylsilane were dissolved in 6 ml THF. 10.14 ml (16.23 mmol, 10 equiv.) of a 1.6M butyllithium solution in hexane were added dropwise maintaining the temperature between 14-20 °C. After 30 min. at 26 °C, the orange solution was cooled down to -75 °C. 4.8 ml (16.23 mmol, 10 equiv.) titanium tetraisopropoxide were added dropwise over 10 min., maintaining the temperature below -68 °C. After 1 hour reaction at -77 °C, 2 g (1.623 mmol, 1 equiv.) in solution in 6 ml THF were added dropwise maintaining the temperature below -72 °C. The reaction mixture was stirred for 2 hours at -76 °C. The temperature was slowly raised to -40 °C and the stirring was continued for a further 2 hours at -40 °C. The reaction mixture was poured onto a mixture consisting of 32.5 ml of a 1M aqueous HCl solution and 20 ml MTBE. 16.2 ml of a 1M aqueous HCl solution and 25 ml water were added. The aqueous layer was separated and re-extracted with 25 ml MTBE. The organic layers were washed with 30 ml of 0.5 M HCl_{aq}, combined, dried over Na₂SO₄, filtered and concentrated

g of (E)-acetyl-ISA247 in >95% double bond isomeric purity (NMR) as a white foam.

Example 8

5 i) Preparation of allyltrifluoroborate reagent: Potassium B-(E-(3-trimethylsilyl-allyl))-trifluoroborate

5 g (21.35 mmol, 1 equiv) of diethanolamine complex obtained by the method described in Example 3, i), 20 ml dichloromethane, 20 ml water and 2.44 ml (42.70 mmol, 2 equiv.) acetic acid were charged in the reaction vessel under stirring at RT. After 30 min. stirring, the water phase was separated and discarded.
10 20 ml methanol were added to the organic phases and the solution was concentrated at 40 °C under reduced pressure to 5-10 ml. 40 ml methanol were added followed by 3.34 g (42.70 mmol, 2 equiv.) KHF₂. After 60 min. stirring at RT, the remaining solid was filtered discarded. The filtrate was concentrated under reduced pressure at 40 °C to ca 25 ml. The solution was cooled to 0-2 °C and the a
15 white suspension was obtained. After 30 min. at 0-2 °C, the suspension was filtered and the solid was washed with cold methanol (-20 °C) and dried under reduced pressure at 40 °C to give 3.4 g of a white powder.

¹H NMR (DMSO, δ in ppm rel. to TMS) : 6.15 (1H, dt), 5.15 (1H, d), 3.5 (2H, br s water), 1.1 (2H, m), 0 (9H, s).

20 ¹¹B NMR (δ in ppm rel. to BF₃.Et₂O external ref.) : 3.8 (q)

Microanalysis : C₁₅H₁₃F₃BKSi (contains 1.08 equiv. H₂O by Karl-Fischer titration and 0.5 equiv. KF) :

Calcd : C 26.83%, H 5.65%, F 24.78%, B 4.02%, K 21.8%, Si 10.5%

Found : C 26.33%, H 5.74%, F 24.71%, B 3.89%, K 22%, Si 9.93%

25 ii) Allylation

2 g (1.623 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde were dissolved in 10 ml dichloromethane. 10 ml water were added, followed by 735 mg (3.246 mmol, 2 equiv.) of trifluoroborate obtained in i). After 2 hours stirring at RT, the organic phase was separated and the water phase discarded.

30 iii) Peterson elimination

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solution (resulting pH 6-7). 20 ml dichloromethane were added and the water phase was separated and discarded. The organic layer was dried over MgSO_4 , filtered and concentrated to about 100 ml. 20.48 g (169.8 mmol, 1.0 equiv.) pinacol were added and the resulting solution was stirred 18 hours at RT. The reaction mixture was concentrated at 40 °C under reduced pressure and the resulting oil was distilled at 43-50 °C under 0.2 mbar pressure to give 37.2 g of a colorless oil.

ii) One-pot allylation/Peterson elimination

20 g (15.06 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde and 5.427 g (22.59 mmol, 1.5 equiv.) pinacol complex obtained in i) and 30 ml acetic acid were charged in the reaction vessel at RT under stirring. 30 ml of formic acid were added under water bath cooling, maintaining the temperature between 20-22 °C. After 2 hours reaction at RT, 12 ml dichloromethane and 200 ml MTBE were added followed by 120 ml of a 10% aqueous NaCl solution. The water phase was separated and discarded. The organic phase was washed with 120 ml water, 204 ml 2M aqueous NaOH solution and 60 ml water. The organic phase was concentrated at 30 °C until the crystallization started. 200 ml MTBE were added and the suspension was concentrated to ca 220 ml. After stirring at RT for 2 hours and for 1 hour at 0-2 °C, the suspension was filtered. The solid was washed with 30 ml MTBE and dried at 50 °C under reduced pressure to provide 18 g of (E)-acetyl-ISA247 as a white powder in >98% double bond isomeric purity (by NMR).

iii) Hydrolysis

This product was hydrolyzed to give (E)-ISA247 in 99.7% double bond isomeric purity by HPLC.

Example 7

25 2 g (1.623 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde and 779.8 mg (3.246 mmol, 2 equiv.) pinacol boronate obtained by the method described in Example 6, i) were dissolved in 20 ml dichloromethane. The solution was cooled to -70 °C and 1.28 ml (10.22 mmol, 6.30 equiv.) borontrifluoride etherate were added. After 30 min. at -70 °C, the reaction mixture was slowly warmed up to 0 °C and reaction was continued for 60 min. at 0 °C. 20 ml water were added. The organic phase was separated, washed with 20 ml of a 5% aqueous NaHCO_3 solution, dried over MgSO_4 , filtered and concentrated at 40 °C under reduced pressure to give 2.1

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water. The organic phase was concentrated at RT under reduced pressure. The crystallization started and the suspension was diluted by addition of 300 ml MTBE and concentrated to ca 330 ml. After stirring 3 hours at RT and 1 hour at 0-2 °C, the white suspension was filtered. The crystals were washed with 50 ml MTBE and dried at 50 °C under reduced pressure to give 27.4 g of (E)-acetyl-ISA247 as a white powder in >98% double bond isomeric purity by NMR.

iii) Hydrolysis

This product was hydrolyzed to give (E)-ISA247 in 99.6% double bond isomeric purity by HPLC.

Example 5

1 g (0.82 mmol, 1 equiv.) acetyl-cyclosporine A aldehyde were dissolved in 10 ml dichloromethane followed by 369 mg (1.62 mmol, 2 equiv.) diethanolamine complex of formula V obtained by the method described in Example 3, i). The turbid solution was cooled to -40 °C. 180 µl (369 mmol, 2 equiv.) boron trifluoride etherate were added keeping the temperature below -40 °C. After 1 hour at -40 °C, the cooling bath was removed and the reaction mixture was warmed up to RT. After 50 min. reaction at RT, 15 ml of a 5% aqueous NaHCO₃ solution were added. The aqueous phase was separated and re-extracted with 15 ml dichloromethane. The combined organic phases were dried over MgSO₄, filtered and concentrated under reduced pressure at 40 °C to give 0.99 g of (E)-acetyl-ISA247 in >95% double bond isomeric purity (NMR) as a white foam.

Example 6

i) Preparation of the pinacol complex of formula VII : 4,4,5,5-tetramethyl-2-(E-(3-trimethylsilyl-allyl))-1,3,2-dioxaborolane

20 g (169.8 mmol, 1 equiv.) of allyltrimethylsilane were dissolved in 60 ml THF. 69.18 ml (186.8 mmol, 1.1 equiv.) of a 2.7M butyllithium solution in heptane were added dropwise over 10 min. maintaining the temperature between 20 °C and 26 °C. After 2 hours reaction at RT, the yellow solution was cooled to -78 °C. 42.26 ml (178.3 mmol, 1.05 equiv.) of triisopropylborate were added dropwise over 10 min., maintaining the temperature below -65 °C. After 1 hour reaction, the reaction mixture was poured onto 100 ml of a 2M aqueous chlorhydric acid

After overnight reaction, the organic layer was separated and the water phase was discarded. 50 ml THF were added to the organic phase. The solution was concentrated under reduced pressure at 30 °C to half its volume. 100 ml THF were added and the solution was concentrated to 80 ml. The volume was adjusted to 100 ml with THF and the solution was cooled to 0-2 °C. 1.812 ml (32.46 mmol, 2 equiv.) concentrated sulfuric acid were added dropwise over 5 min., maintaining the temperature below 5 °C. After addition, the reaction cooling bath was removed and the temperature was raised to RT. After 4 hours reaction, 40 ml water were added followed by 20 ml MTBE. The aqueous layer was separated and discarded. The organic phase was washed with 40 ml NaHCO₃ _{aq}, 20 ml saturated NaCl_{aq}, 40 ml saturated NaCl_{aq}, dried over Na₂SO₄, filtered and concentrated at 40 °C under reduced pressure. The crude E-acetyl-ISA247 was re-dissolved in 200 ml MTBE and crystallization started within a few minutes. After 15 min. at RT and 2.5 hours at 0 °C, the suspension was filtered, the crystals were washed with 50 ml MTBE and dried at 50 °C under reduced pressure to give 18.45 g of (E)-acetyl-ISA247 as a white powder (>98% isomeric purity by NMR).

iv) Hydrolysis

This crude product was hydrolyzed to give (E)-ISA247 in 99% isomeric purity by HPLC.

Example 4

i) Allylation

10.02 g (44.1 mmol, 2 equiv.) diethanolamine complex of formula V obtained by the method described in Example 3, i), and 30 g (22.05 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde were charged in the reaction vessel. 36 ml acetic acid were added at RT. A clear solution was obtained after 15 min. stirring at RT. The reaction was monitored by HPLC.

ii) Peterson elimination

After ca 6 hours reaction at RT, 60 ml formic acid were added, maintaining the temperature below 30 °C. The clear light yellow solution was stirred overnight at RT. 18 ml dichloromethane and 300 ml MTBE were added followed by 180 ml of a 10% NaCl_{aq} solution. The aqueous phase was separated and discarded. The organic phase was washed with 180 ml water, 300 ml 2M aqueous NaOH and 90 ml

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i) Preparation of diethanolamine complex of formula V : 2-(B-(3-trimethylsilyl-allyl))-[1,3,6,2]dioxazaborocane

50 g (424.5 mmol, 1 equiv.) allyltrimethylsilane were charged in the reaction vessel followed by 150 ml THF. To the clear colorless solution were added dropwise over 15 min., 165.1 ml (445.7 mmol, 1.05 equiv.) of a 2.7M butyllithium solution in heptane, maintaining the temperature between 20 °C and 26 °C. After 2 hours reaction at RT, the orange solution was cooled to -78 °C. 105.7 ml (445.8 mmol, 1.05 equiv.) triisopropylborate were added dropwise over 20 min., maintaining the temperature below -60 °C. After 1 hour at -70 °C, the reaction mixture was poured onto 250 ml of a 2M aqueous chlorhydric acid solution (resulting pH : 5-6). After 10 min. stirring, the water phase was separated and discarded. 42.4 g (403.3 mmol, 0.95 equiv) diethanolamine were added to the organic phase. The solution was stirred for 60 min. at RT. 750 ml heptane were added. The biphasic emulsion was partially concentrated at 40 °C (ca 750ml solvent distilled) under reduced pressure. A white precipitate appeared and the suspension was stirred 2 hours at RT. The suspension was filtered. The white solid was washed with 125 ml heptane and dried at 40 °C under reduced pressure overnight to provide 85.7 g of the diethanolamine complex of formula V.

¹H NMR (DMSO, in ppm rel. to TMS) : 6.5 (br s, 1H), 6.15 (dt, 1H), 5.32 (d, 1H), 3.7 (m, 2H), 3.55 (m, 2H), 2.95 (m, 2H), 2.72 (m, 2H), 1.29 (d, 2H), 0 (s, 9H).

¹¹B NMR (DMSO, rel. to external ref. BF₃·Et₂O) : 11.1 br. s

Microanalysis : (contains 0.11 equiv. H₂O by Karl-Fischer titration)

Calcd : C 52.43%, H 9.71%, N 6.12%, B 4.72%, Si 12.27%

Found : C 52.04%, H 9.63%, N 6.36%, B 4.79%, Si 11.3%

ii) Allylation

7.375 g (32.46 mmol, 2 equiv.) of diethanolamine complex of formula V, 20 g (16.23 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde and 80 ml dichloromethane were charged in the reaction vessel at RT. 40 ml water and 2.79 ml (48.69 mmol, 3 equiv.) acetic acid were added under stirring. After 10 min. stirring, a clear biphasic mixture was obtained. The reaction was monitored by HPLC.

iii) Peterson elimination

saturated aqueous NaCl solution, combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure to ca 50 ml.

5 ii) Generation of the boron reagent of formula IVa' ((R,R)-2-[(E)-(3-trimethylsilyl-allyl)]-[1,3,2]dioxaborolane-4,5-dicarboxylic acid dimethyl ester) and Alkylation of acetyl-cyclosporin A aldehyde

4.88 g (40.56 mmol, 10 equiv) magnesium sulfate dihydrate were added under stirring, followed by 7.23 g (40.56 mmol, 10 equiv.) L-(+)-dimethyltartrate. After 2 hours stirring at RT, the suspension was cooled to 0 °C and 5 g (4.056 mmol, 1 equiv.) acetyl-cyclosporin A aldehyde were added in one portion. The reaction
10 was monitored by HPLC (ca 90% conversion after 3 hours). After 17 hours stirring at 0 °C, the suspension was filtered and the filtrate was washed with 50 ml half saturated aqueous NH₄Cl solution, 50 ml half saturated aqueous NaHCO₃ solution and 50 ml half saturated aqueous NaCl solution. The aqueous phases were re-extracted with 50 ml THF and discarded. The combined organic phases were dried
15 over Na₂SO₄, filtered and concentrated at 40 °C under reduced pressure to provide 11.1 g of a ca 75:25 mixture of β-trimethylsilyl alcohol diastereomers as a light yellow oil.

iii) Peterson elimination

The crude β-trimethylsilyl alcohol diastereomers mixture (11 g, maximum
20 4.056 mmol) was dissolved in 25 ml THF. 0.679 ml (12.16 mmol, 3 equiv.) concentrated sulfuric were added dropwise maintaining the temperature between 20 °C and 25 °C. After 2 hours at RT, 50 ml half saturated aqueous NaCl solution were added. The resulting mixture was extracted twice with 50 ml MTBE. The organic phases were washed with 50ml of a half saturated aqueous NaCl solution,
25 combined, dried over Na₂SO₄ and concentrated under reduce pressure at 40°C. The resulting crude E-acetyl-ISA247 was re-dissolved in 20 ml dichloromethane and concentrated under reduced pressure. The crude product was dissolved in 60 ml MTBE. The crystallization started within 10 min. The suspension was stirred for an additional 15 min. at RT and 2 hours at -10 °C. The crystals were isolated by
30 filtration, washed with 20 ml cold MTBE (-20 °C) and dried under reduced pressure to provide 3.6 g of (E)-acetyl-ISA247 in ca 98% isomeric purity by NMR.

Example 3

ml water. The water phases were re-extracted sequentially with 50 ml dichloromethane. The combined organic phases were dried over Na_2SO_4 , filtered and concentrated under reduced pressure at 30°C. The resulting white foam was re-dissolved in 250 ml MTBE and after a few minutes, the crystallization started.
5 After stirring 15 min. at RT and 2 hours at 0-2 °C, the suspension was filtered. The crystals were washed with 50 ml cold MTBE (-20 °C) and dried at 40-50 °C under reduced pressure to provide 19.2 g of (E)-acetyl-ISA247 as white powder in >98% isomeric purity (400MHz ^1H NMR).

(E)-acetyl-ISA247 can be recrystallized by dissolving the solid in
10 dichloromethane at room temperature and exchanging the solvent to MTBE (by adding MTBE, concentrating the solution to half its volume under reduced pressure at 40°C and repeating these operation 2 to three times). The solution is cooled to room temperature and the crystallization then starts within a few minutes. The suspension is stirred at room temperature for 2h and 30min at 0°C. The
15 crystals of (E)-acetyl-ISA247 are isolated after filtration, washing with MTBE and drying under reduced pressure at 40°C.

iv) Hydrolysis

Hydrolysis of E-acetyl-ISA247 provided (E)-ISA247 in 99.5% double bond isomeric purity (by HPLC).

20 Example 2

i) Preparation of a solution of the crude boronic acid of formula IIIa IIIa: (E)-3-(trimethylsilyl)allylboronic acid

6.67 ml (40.56 mmol, 10 equiv.) of allyltrimethylsilane were dissolved in 33.3 ml of dry THF at RT. 25.35 ml (40.56 mmol, 10 equiv.) of a 1.6M solution of
25 butyllithium in hexane were added in 5 min. maintaining the temperature between 14 °C and 16 °C. After 60 min. reaction at RT, the resulting yellow to orange solution was cooled to -70 °C. 9.614 ml (40.56 mmol, 10 equiv.) triisopropylborate were added in 10 min., keeping the temperature below -65 °C. After 60 min. reaction at -70 °C, the cold solution was poured onto 35 ml of a 1M aqueous HCl
30 solution (pH=7-8). The reaction mixture was extracted with 30 ml of dichloromethane. The water phase was separated and re-extracted with 30 ml of dichloromethane. The organic phases were washed sequentially with 30 ml of a

Example 1**i) Preparation of a solution of crude the boronic acid of formula III:**
(E)-3-(trimethylsilyl)allylboronic acid

20 g (169.8 mmol, 1 equiv.) of allyltrimethylsilane (Fluka 06073) was dissolved in 140 ml of dry THF (Fluka 87368) at RT. 106.1 ml (169.8 mmol, 1 equiv.) of a 1.6 M solution of butyllithium in hexane (Acros 181270100) was added in 10 min. maintaining the temperature between 20 °C and 25 °C. After 30 min. reaction, the resulting yellow to orange solution was cooled to -70 °C. 40.24 ml (169.8 mmol, 1 equiv.) triisopropylborate (Fluka 92085) is added in 10 min., keeping the temperature below -60 °C. After 30 min. reaction at -74 °C, the cold solution was poured onto 170 ml of a 1M aqueous HCl solution. The pH was adjusted to 7-8 by further addition of 1M HCl_{aq} (in this particular case, 26 ml). 80 ml of dichloromethane were added for extraction. The water phase was separated and re-extracted with 80 ml of dichloromethane. The organic phases were washed sequentially with 150 ml of a saturated aqueous NaCl solution, combined, dried over Na₂SO₄, filtered and concentrated under reduced pressure to ca 40 ml. The weight of the solution was adjusted to 53.6 g by addition of dichloromethane in order to obtain a ca 50% solution of boronic acid (based on the starting allyltrimethylsilane).

ii) Allylation of acetyl-cyclosporin A aldehyde

20 g (16.23 mmol, 1 equiv.) of acetyl-cyclosporin A aldehyde were dissolved in 100 ml of dichloromethane at RT. 25.66 g (81.15 mmol, 5 equiv.) of the previously prepared boronic acid solution (ca 50% concentration) were added in one portion. The conversion of the reaction was monitored by HPLC. Reaction was complete within 1-3 hours at RT. A ca 85:15 mixture of β -trimethylsilyl alcohol diastereomers was obtained.

iii) Peterson elimination

The Peterson elimination was conducted directly on the reaction mixture.

20 ml of THF were added and the reaction mixture was cooled to 0 °C. 2.7 ml (48.69 mmol, 3 equiv.) of concentrated sulfuric acid were added. The temperature was raised to RT. After completion of the reaction (ca 1 hour), 100 ml of water were added. The organic phase was separated and washed 2 times with 50

EXAMPLES

The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

Although most of the examples have been provided for the allylation-Peterson elimination sequence on acetyl-cyclosporin A aldehyde, other protecting group could in principle be used. Of course, this will be limited by their compatibility with the reaction conditions as well as the possibility to remove them efficiently to provide (E)-ISA247.

tributylphosphine, a triarylphosphine, a trialkylamine such as triethylamine, an alkylaminosulfide, a thiosulfate or a dialkylsulfide such as dimethylsulfide. When working with tributylphosphine as the reducing agent, the person of ordinary skill in the art will know that the reaction is dose-controlled.

5 Furthermore, a protected cyclosporin A aldehyde (II) can be prepared by converting the protected cyclosporin A XIV, such as acetyl cyclosporin A, to the protected cyclosporin A epoxide with a monopersulfate, preferably oxone, in the presence of a ketone, such as acetoxyacetone or diacetoxyacetone. This step is performed in an organic solvent which is inert under these reaction conditions
10 such as acetonitrile and water. Ethylenediaminetetra-acetic acid disodium salt is added to capture any heavy metal ions which might be present. The epoxidation reaction is carried out preferably at a pH over 7. This epoxidation reaction is followed by oxidative cleavage of the epoxide with periodic acid or periodate salt under acidic conditions. Optionally, the oxidation and the oxidative cleavage can be combined in a work-up procedure. These reactions have been discussed by Dan
15 Yang, et al., in "A C₂ Symmetric Chiral Ketone for Catalytic Asymmetric Epoxidation of Unfunctionalized Olefins," J. Am. Chem. Soc., Vol. 118, pp. 491-492 (1996), and "Novel Cyclic Ketones for Catalytic Oxidation Reactions," J. Org. Chem., Vol. 63, pp. 9888-9894 (1998).

20 The use of ruthenium based oxidizing agents has been discussed by H. J. Carlsen et al. in "A Greatly Improved Procedure for Ruthenium Tetroxide Catalyzed Oxidations of Organic Compounds," J. Org. Chem., Vol. 46, No. 19, pp 3736-3738 (1981). Carlsen et al. teach that, historically, the expense of ruthenium metal provided an incentive for the development of catalytic procedures, the most
25 popular of which used periodate or hypochlorite as stoichiometric oxidants. These investigators found a loss of catalytic activity during the course of the reaction with the conventional use of ruthenium which they postulated to be due to the presence of carboxylic acids. The addition of nitriles to the reaction mixture, especially acetonitrile, was found to significantly enhance the rate and extent of the oxidative
30 cleavage of alkenes in a CCl₄/H₂O/IO₄⁻ system.

For example, protected cyclosporin A aldehyde (II) can be produced from protected cyclosporin A (XIV), such as acetyl cyclosporin A, by dissolving it in a mixture of acetonitrile and water, and then adding first sodium periodate and then ruthenium chloride hydrate. The aldehyde (II) may be extracted with ethyl acetate.

discussed by J. R. Hanson in Chapter 2, "The Protection of Alcohols," of the publication *Protecting Groups in Organic Synthesis* (Sheffield Academic Press, Sheffield, England, 1999), pp. 24-25. Hanson teaches how to protect hydroxyl groups by converting them to either esters or ethers. Acetate esters are perhaps the most frequently used type of chemistry for protecting hydroxyl groups. There are a wide range of conditions that may be used to introduce the acetate group. These reagents and solvents include acetic anhydride and pyridine; acetic anhydride, pyridine and dimethylaminopyridine (DMAP); acetic anhydride and sodium acetate; acetic anhydride and toluene-p-sulphonic acid, acetyl chloride, pyridine and DMAP; and ketene. DMAP is a useful acylation catalyst because of the formation of a highly reactive N-acylpyridium salt from the anhydride.

For example, the β -alcohol of cyclosporin A is protected as an acetate by reacting cyclosporin A (XIII) with acetyl chloride, ethyl acetate, or combinations thereof, forming the compound, acetyl cyclosporin A. In another example, the β -alcohol undergoes a nucleophilic addition to acetic anhydride, forming acetyl cyclosporin A and acetic acid. These reactions may be carried out in the presence of dimethylaminopyridine (DMAP) where an excess of acetic anhydride acts as the solvent.

Although the preparation of acetyl cyclosporin A is well established in the literature, it will be appreciated by those skilled in the art that protecting groups other than acetate esters may be used to protect the β -alcohol of the 1-amino acid residue of cyclosporin A. These protecting groups may include benzoate esters, substituted benzoate esters, ethers, and silyl ethers. Under certain reaction conditions, the acetate protecting group is prone to undesirable side reactions such as elimination and hydrolysis. Since benzoate esters, ethers and silyl ethers are often more resistant to such side reactions under those same reaction conditions, it is often advantageous to employ such protecting groups in place of acetate.

In step c-ii), the protected cyclosporin A of formula XIV is converted to a protected cyclosporin A aldehyde of formula II.

This step can be carried out, for example, by using ozone as an oxidizing agent followed by work-up with a reducing agent to form a protected cyclosporin A aldehyde (II). Ozonolysis step is conducted at a temperature range from about -80°C to 0°C . The solvent used during the ozonolysis may be a lower alcohol such as methanol. The reducing agent may be a trialkylphosphine such as

Preparation of the allylaluminum reagents

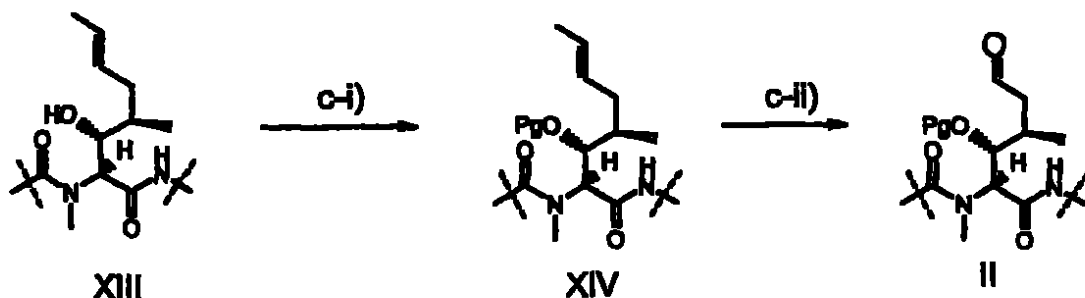
The (trimethylsilyl)allylaluminum reagents are prepared in-situ via deprotonation of allyltrimethylsilane to form trimethylsilylallyllithium, as described above, and reaction of this intermediate with a dialkylaluminum chloride such as diethylaluminum chloride or with an alkylaluminum dichloride such as ethylaluminum dichloride, preferably with diethylaluminum chloride, at a temperature of -80 °C to 0 °C, preferably -80 °C to -30 °C, more preferably -80 °C to -50 °C, especially -80 °C to -60 °C. The resulting aluminum reagents are used in situ for the allylation of protected cyclosporin A aldehydes.

A putative structure for one of these reagents is presented below :

Preparation of protected cyclosporin A aldehyde

Protected cyclosporin A aldehyde of formula II can be prepared as illustrated in Scheme P.

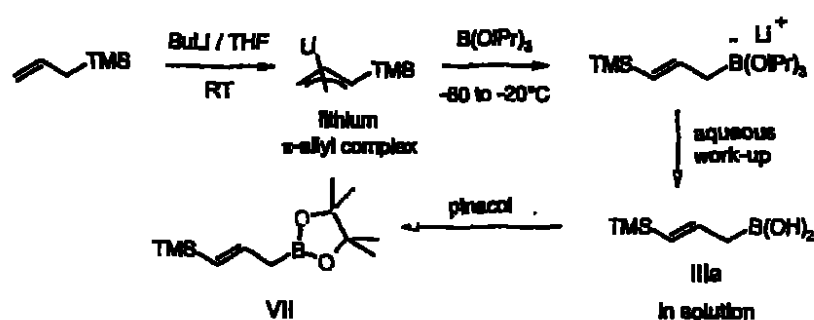
Scheme P:



(The dotted lines have the meaning as defined above.)

In step c-i), a protecting group is introduced in cyclosporin A of formula XIII, to protect hydroxyl group at the β -position of the side chain of the 1-amino acid residue. Protecting groups are well known in organic synthesis, and have been

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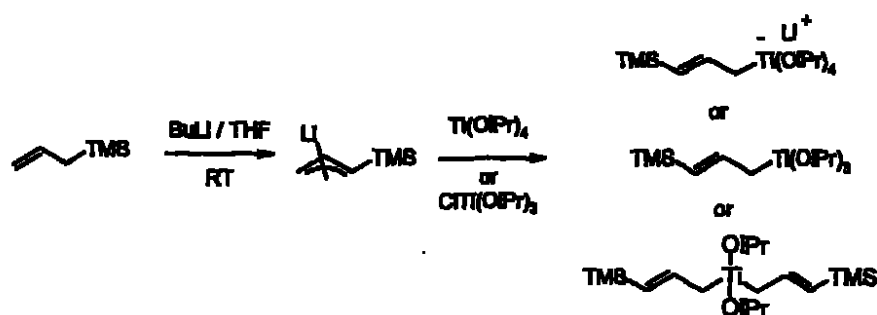


Alternatively, the trapping of the 1-trimethylallyl lithium can be performed with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, leading directly after aqueous work-up to the pinacol boronate of formula VII.

Alternatively, the reagent can be prepared by deprotonation of allyltrimethylsilane at room temperature with butyllithium, quench of the allyllithium intermediate with tri-isopropylborate (between -80 °C to -20 °C, preferably between -80 °C and -30 °C), addition of pinacol and then aqueous work-up.

Preparation of the allyltitanium reagents

The (trimethylsilyl)allyltitanium reagents are prepared in-situ via deprotonation of allyltrimethylsilane to form trimethylsilylallyllithium, as described above, and reaction of this intermediate with titanium dichlorodiisopropoxide, titanium tetraisopropoxide or titanium chlorotriisopropoxide, preferably titanium tetraisopropoxide or titanium chlorotriisopropoxide at a temperature of -80 °C to 0 °C, preferably -80 °C to -30 °C, more preferably -80 °C to -50 °C, especially -80 °C to -60 °C. The resulting titanium reagents are used in situ for the allylation of protected cyclosporin A aldehydes. Putative structures for these reagents are presented below :



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In general, allyltrifluoroborate potassium salts are prepared by treating the corresponding boronic acid with 3 equivalents of KHF_2 in a water/methanol solvent mixture (see for example : R. A. Batey in "Diastereoselective Allylation and Crotylation Reactions of Aldehydes with Potassium Allyl- and Crotyltrifluoroborates under Lewis acid Catalysis", *Synthesis* 2000, pp 990-998).
However, direct application of these procedures to the preparation of trifluoroborate of formula VI lead to the formation of substantial amounts of allyltrimethylsilane via protodeborylation due to the acidic pH of the reaction mixture.

Modification was made in order to avoid this side-reaction. Thus, as illustrated in Scheme O, a methanol solution of boronic acid is treated with 2 equivalents of KHF_2 as fluoride source, at RT. The suspension is stirred at RT for 60 min. The residual organic salts are removed by filtration. The methanolic solution of trifluoroborate salt VI is concentrated under reduced pressure and the product is crystallized at 0-5 °C. The trifluoroborate salt VI is isolated by filtration and dried under vacuum.

The required boronic acid solution is prepared by hydrolyzing the diethanolamine complex of formula V in a water/dichloromethane mixture in the presence of an acid such as acetic acid. The aqueous phase is discarded and the solvent is exchanged from dichloromethane to methanol.

Alternatively, the diethanolamine complex V can be used directly as starting material. The trifluoroborate salts VI can be prepared, however, crystallization does not occur.

γ -silylated allylboron reagent of formula VII

To a solution of crude boronic acid of formula IIIa, preparation of which was described above, is added pinacol. The reaction mixture is stirred at RT and then concentrated under reduced pressure. The pinacol complex VII can then be distilled under low pressure or used directly in the allylmethylation step. The preparation of the pinacol complex of formula VII is, for example, performed as illustrated in Scheme O.

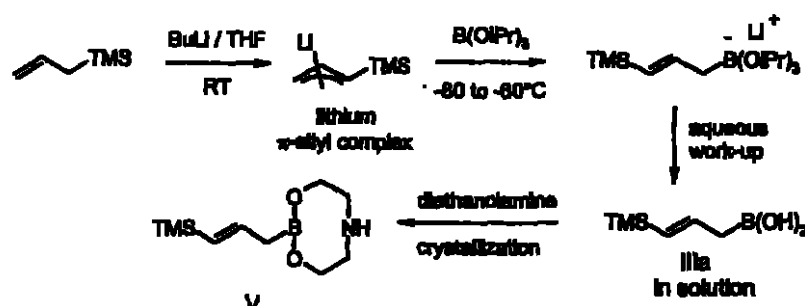
Scheme O:

This reagent can be prepared by treating boron reagent of formula IIIa with the required alcohol in the presence of a drying agent such molecular sieves or magnesium sulfate, preferably magnesium sulfate. Preferred boronate reagent of formula IV wherein R¹ is C₁₋₈ alkyl or C₃₋₈ cycloalkyl are those wherein R¹ is methyl, ethyl, propyl, isopropyl, butyl or benzyl, more preferably R¹ is methyl, ethyl, propyl, isopropyl or butyl, further preferably R¹ is methyl, ethyl, propyl or butyl, especially preferably R¹ is methyl.

γ-silylated allylboron reagent of formula V

To a solution of crude boronic acid of formula IIIa, preparation of which was described above, is added diethanolamine. Solvent exchange to heptane and crystallization provides the diethanolamine complex of formula V as a solid. A special feature of this reagent is the presence of an interaction between the nitrogen lone pair of the diethanolamine fragment and the boron atom (i.e. complexation of the nitrogen atom by the boron atom) as evidence by ¹¹B NMR of the complex (δ = 11ppm relative to BF₃·Et₂O, external reference).

Preparation of the γ-silylated allylmetal reagent of formula V is, for example, performed as illustrated in Scheme M:



γ-silylated allylboron reagent of formula VI

Scheme N:

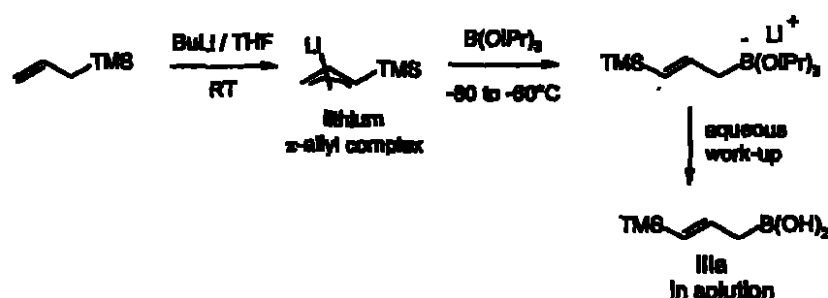


acid (under the HPLC conditions, the oligomers, dimers and trimers should be hydrolyzed and converted to the boronic acid).

Alternatively, a solution of reagent of formula IIIa can be prepared by hydrolysis of complex of formula V in an organic solvent/water mixture such as a dichloromethane/water, toluene/water, ethyl acetate/water, THF/water, chloroform/water mixture, preferably a dichloromethane/water mixture preferably in the presence of an acid such as sulfuric acid, chlorhydric acid, acetic acid, preferably acetic acid.

Preparation of the γ -silylated allylmetal reagent of formula IIIa is, for example, performed as illustrated in Scheme L.

Scheme L:



γ -silylated allylboron reagent of formula IV

Boronate reagent of formula IV where R is C_{1-8} alkyl, preferably C_{1-6} alkyl, more preferably methyl, ethyl or isopropyl, especially methyl is prepared by treating boron reagent of formula IIIa with the required tartrate ester in the presence of a drying agent such molecular sieves or magnesium sulfate, preferably magnesium sulfate.

Reagent of formula IVa' is prepared by mixing a solution of boronic acid of formula IIIa with L-(+)-dimethyltartrate, in the presence of a drying agent such molecular sieves or magnesium sulfate, preferably magnesium sulfate, as evidenced by ^{11}B and ^1H NMR analyses.

γ -silylated allylboron reagent of formula III

(M and M' are a metallic fragment comprising the metal and its ligands.)

Preparation of allylboron reagents

γ -silylated allylboron reagent of formula IIIa

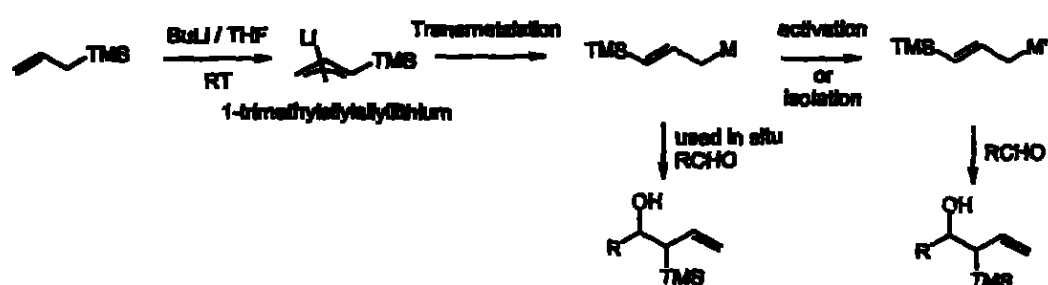
5 A solution of crude boronic acid of formula IIIa is obtained after deprotonation of allyltrimethylsilane, trapping with an electrophilic boron reagent and aqueous work-up. The deprotonation of allyltrimethylsilane is performed in THF with butyllithium, between 0 °C and 35 °C, preferably between 0 °C and 25 °C for 30min. to 3 hours. The electrophilic boron reagent is a trialkylborate such as
10 triisopropyl borate or trimethyl borate, preferably triisopropyl borate. The trapping of the trimethylsilylallyllithium intermediate with triisopropyl borate is performed between -80 °C and -20 °C, preferably below -60 °C for 30 min. to 2 hours. The trapping of the trimethylsilylallyllithium intermediate with trimethyl borate is performed between -80 °C and -60 °C for 30 min. to 2 hours.

15 It is known that allylboronic acids are unstable, therefore, the crude boronic acid is kept in solution (P. G. M. Wuts and Y.-W. Jung in "The Addition of γ -(Trimethylsilyl)allylboronates to Imines", *Journal of Organic Chemistry* 1991, 56, p365-372(see comment in the example for the preparation of compound 9); W. R. Roush, K. Ando, D. B. Powers, A. D. Palkowitz and R. L. Halterman in
20 "Asymmetric Synthesis Using Diisopropyl Tartrate Modified (E)- and (Z)-Crotylboronates; Preparation of the Chiral Crotylboronates and Reactions with Achiral Aldehydes", *Journal of the American Chemical Society* 1990, 112, 6339 (see reference 17)). Concentrated solutions (>10% concentration, for example ca 50% concentration) are not stable at RT and decompose. They should be rapidly used.
25 If needed they should be stored at 5 °C maximum. In accordance to the general behaviour of boronic acids, the boronic acid of formula IIIa might in principle also exist in the form of a cyclic trimer (also called a boroxine) or oligomers. Since isopropanol coming from triisopropyl borate can also present (depending on the process), (mixed) isopropyl boron esters (TMS-CH=CH-CH₂-B(OH)(OiPr) and/or TMS-CH=CH-CH₂-B (OiPr)₂) could also be present. Indeed, ¹H NMR and
30 ¹¹B NMR analyses of concentrated solutions of boron reagent of formula IIIa (ca 50%) diluted with an equal volume of CD₂Cl₂ shows the presence of several boronate species, although reverse phase HPLC analysis shows mainly the boronic

Chemistry 1987, 52, pp 957 – 958 ; E. Ehlinger and P. Magnus in "Silicon in
Synthesis. 10. The (Trimethylsilyl)allyl Anion: A β -Acyl Anion Equivalent for the
Conversion of Aldehydes and Ketones into γ -Lactones", *Journal of the American
Chemical Society* 1980, 102, pp 5004-5011 ; M. Schlosser, L. Franzini in "The
Regioselectivity of 1,3-Disubstituted Allylmetal Species Towards Electrophiles: 1-
(Trimethylsilyl)alk-2-enylpotassium Compounds", *Synthesis* 1998, pp 707-709 ; E.
Schaumann and A. Kirschning in "Ring-opening of oxiranes by silyl-substituted
allyl anions. A regiochemical chameleon", *Tetrahedron Letters* 1988, 29, pp 4281-
4284).

As illustrated in Scheme K, the allyltrimethylsilane is deprotonated by *n*-
butyllithium in THF at a temperature ranging from 0 °C to 35 °C, preferably
between 0 °C and 25 °C for 30 min. up to 3 hours. This generates
a trimethylsilylallyllithium intermediate. This intermediate most probably exists in
solution as a π -allyl complex of lithium in a trans configuration (T. H. Chan in
"Silylallyl Anions in Organic Synthesis: A Study in Regio- and Stereoselectivity",
Chemical Reviews 1995, 95, p1279-1292; M. Schlosser, O. Desponds, R. Lehmann,
E. Moret and G. Ransachschwalbe in "Polar Allyl Type Organometallics as Key
Intermediates in Regio- and Stereocontrolled Reactions : Conformational
Mobilities and Preferences", *Tetrahedron* 1993, 49, p10175-10203). This anion is
then trapped (transmetalated) by an electrophilic metal source usually at a
temperature of -80 °C to -60 °C, generating the corresponding trans-allylmetal
reagent. Depending on the metal rest, these reagents are reacted in-situ with the
aldehyde or can be isolated for later use. It can also be transformed into another
complex by addition of a proper reagent in order to activate the reagent for the
allylation or to allow their isolation.

Scheme K:



-60 °C, furnishes, after aqueous work-up, a mixture of diastreomeric anti β -silyl alcohols XI'. Peterson elimination under the standard conditions (H_2SO_4 in THF) furnishes E-acetyl-ISA247 (XII').

Synthesis of (E)-ISA247 by allylaluminum reagents

5 Reaction of acetyl-cyclosporine A aldehyde with a γ -trimethylsilylallylaluminum reagent prepared from trimethylsilylallyllithium and ethylaluminum dichloride or diethylaluminum chloride, preferably diethylaluminum chloride, performed in THF, at a temperature of -80 °C to 0 °C, preferably -80 °C to -30 °C, more preferably -80 °C to -50 °C, especially -80 °C to -
10 60 °C furnishes after aqueous work-up a mixture of diastreomeric anti β -silyl alcohols XI'. Peterson elimination under the standard conditions (H_2SO_4 in THF) furnishes E-acetyl-ISA247 (XII').

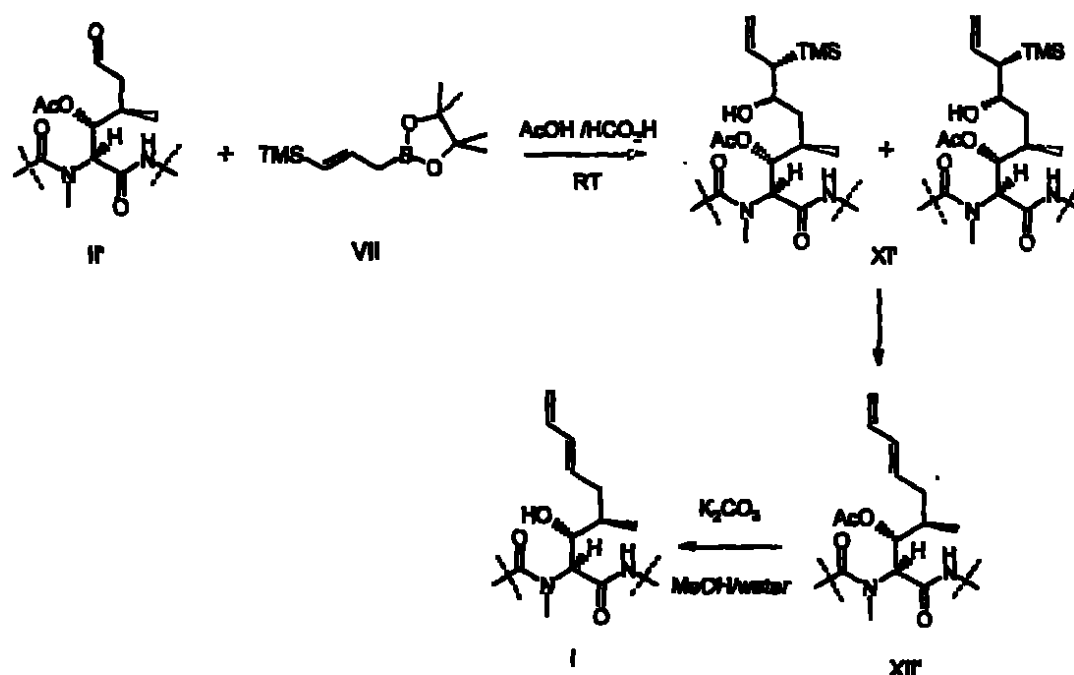
Preparation of the γ -silylated allylmetal reagents

15 The γ -silylated allylmetal reagents required for the allylmetalation step are best generated from the corresponding allylsilanes via deprotonation, trapping with an adequate metal reagent and optionally by further complexation of the metal rest by a suitable ligand. The resulting reagents can, depending on their stability and the process, be used in situ or be isolated and stored.

20 Although other silyl-substituted allylsilanes could obviously have been used, the trimethylsilyl derivative leads to minimum amount of waste. Indeed, the silyl fragment is lost upon Peterson elimination.

 Many conditions for the deprotonation of allylsilanes have been published in the literature and usually make use of n-butyllithium (or the sec- and tert-isomers) in an organic solvent (generally THF) in combination or not with a co-solvent or co-reagent such as HMPA, TMEDA or potassium t-butoxide at
25 temperatures ranging from -100 °C to RT. (see for example: T. H. Chan in "Silylallyl Anions in Organic Synthesis: A Study in Regio- and Stereoselectivity", *Chemical Reviews* 1995, 95, p1279-1292 ; Kohei Tamao, Eiji Nakajo, and Yoshihiko Ito in "Silafunctional compounds in organic synthesis. 33. Metalated
30 allylamino silane: a new, practical reagent for the stereoselective α -hydroxyallylation of aldehydes to erythro-1,2-diol skeletons", *Journal of Organic*

Scheme J:



The origin of the increased activity of the reagent of formula VII, when used in carboxylic acid solution like acetic acid and formic acid, could come from the high polarity and the low complexing ability of these solvents. Another effect could be found in the ability of these solvent to provide acidic catalysis of the allylation by activation of the carbonyl group of the aldehyde through protonation.

The addition of reagent of formula VII to acetyl cyclosporin A aldehyde (II') can be promoted by a Lewis acid such as BF₃·Et₂O at a temperature of -70 °C to 0 °C in toluene, THF or dichloromethane, preferably toluene or dichloromethane, preferably dichloromethane. Under the allylation reaction, the Peterson elimination also occurs and (E)-acetyl-ISA247 (XII') can be obtained after extractive aqueous work-up.

Synthesis of (E)-ISA247 by allyltitanium reagents

Reaction of acetyl-cyclosporine A aldehyde with a γ -trimethylsilylallyltitanium reagent prepared from trimethylsilylallyllithium and titanium dichlorodiisopropoxide, titanium tetraisopropoxide or titanium chlorotrisopropoxide, preferably titanium tetraisopropoxide or titanium chlorotrisopropoxide performed in THF, at a temperature of -80 °C to 0 °C, preferably -80 °C to -30 °C, more preferably -80 °C to -50 °C, especially -80 °C to

For example, the synthesis of (E)-ISA247 by the reagent of formula VI can be performed as illustrated in Scheme J.

Allylation of aldehydes with this reagent is known from the literature to proceed slowly (1 to several days at RT). Accordingly, allylation of acetyl-cyclosporin A aldehyde (II') with excess of reagent of formula VII (5-10 equivalents) in solvents such as THF, dichloromethane, toluene, DMF and DMSO proceeds slowly at RT.

Heating, use of large excess of reagent or high concentration could increase the rate of acetyl cyclosporin A aldehyde allylation, however, a better alternative was found by a proper choice of solvent.

Carboxylic acids such as formic acid or acetic acid were found to dramatically enhance the rate of allylation. For instance, when performed in acetic acid, allylation of acetyl cyclosporin A aldehyde (II') can reach conversion of over 95% within 5 hours at RT with 2 equivalents of reagent of formula VI, providing the β -silyl alcohols (XI'). Further addition of formic acid promotes the Peterson elimination. (E)-acetyl-ISA247 (XII') is obtained after extractive work-up ascertaining the relative anti stereochemistry of the intermediate β -silyl alcohols. Peterson elimination can also be performed under standard conditions (sulfuric acid in THF) after isolation of the anti β -trimethylsilyl alcohol diastereomers to give (E)-acetyl-ISA247.

Formic acid or preferably a combination of acetic acid and formic acid (such as ca 1:1 v/v) as is also an effective solvent. In that case, the allylmatalation and the following Peterson elimination can take place in one-pot. In a combination of acetic acid and formic acid (ca 1:1 v/v) the allylation of acetyl-cyclosporin A aldehyde and the following Peterson elimination reach over 90% conversion within 60 min. at RT with 1.5 equivalent of reagent. Aqueous extractive work-up and crystallization furnishes (E)-acetyl-ISA247 (XII').

Mixture of acetic acid, formic acid and a suitable co-solvent can also be used. Dichloromethane, toluene, ethyl acetate or isopropyl acetate, preferably isopropyl acetate could be used as co-solvent. Decrease in reactivity can be observed when using a co-solvent but this could be compensated by increasing the reaction temperature.

Hydrolysis of the acetate protecting group with K_2CO_3 in aqueous methanol furnishes (E)-ISA247 (I).

but the Peterson elimination can be conducted in one pot by addition to the reaction mixture of an acid such as formic acid, sulfuric acid or methanesulfonic acid, preferably formic acid. Aqueous work-up and crystallization yields (E)-acetyl-ISA247 (XII'). Subsequent hydrolysis furnishes (E)-ISA247 (I).

- 5 When formic acid is present in sufficient amounts in the solvent mixture used for the allylation, the Peterson elimination can take place in one-pot leading directly to (E)-acetyl-ISA247.

- 10 Another alternative consists in performing the addition of complex of formula V to acetyl-cyclosporin A aldehyde (II') in the presence of a Lewis acid such as $\text{BF}_3 \cdot \text{Et}_2\text{O}$. For example, the reaction with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ can be performed in a solvent such as dichloromethane or THF at a temperature ranging from -40°C to RT. Under these conditions, the allylation can directly be followed by the Peterson elimination, yielding the expected (E)-acetyl-ISA247 (XII').

Allylation by γ -silylated allylboron reagent of formula VI (step a-iv))

- 15 Reacting the allyltrifluoroborate VI and acetyl-cyclosporin A aldehyde (II') in a biphasic water/organic solvent, preferably water/dichloromethane mixture or water/toluene mixture, more preferably water/dichloromethane mixture at RT provides a mixture of anti β -trimethylsilyl alcohol diastereomers (XI'). After the water phase is discarded, the Peterson elimination is performed by addition of THF and sulfuric acid at a temperature of 0°C to RT providing (E)-acetyl-ISA247 (XII').
20 Peterson elimination can also be performed under standard conditions (sulfuric acid in THF) after isolation of the anti β -trimethylsilyl alcohol diastereomers to give E-acetyl-ISA247.

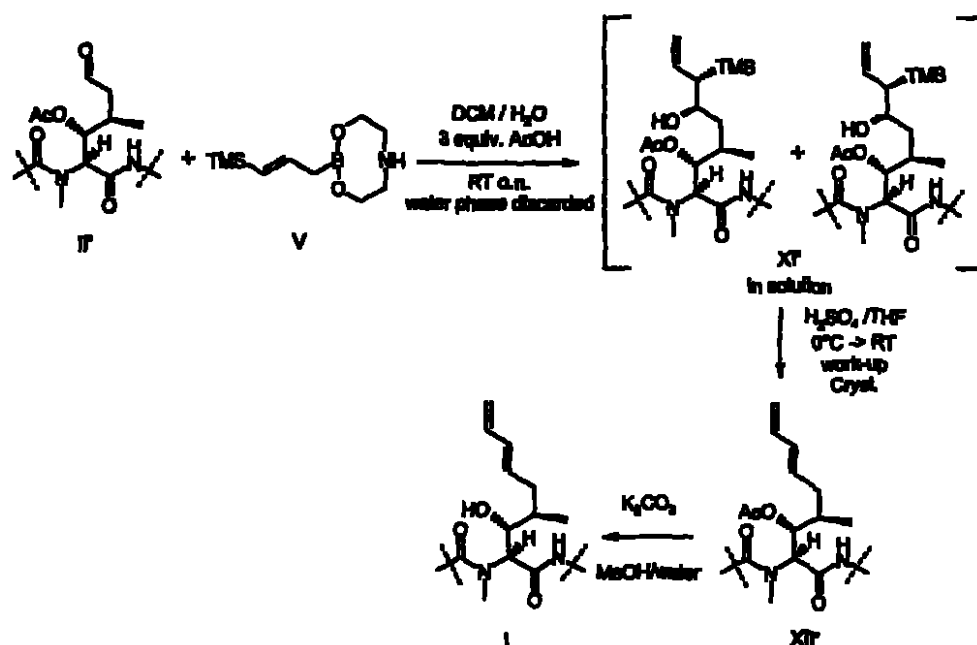
- 25 The allylation can also be promoted by a Lewis acid. In that case, the allylation and the Peterson elimination can take place in-situ. For example, addition of excess $\text{BF}_3 \cdot \text{Et}_2\text{O}$ to a suspension of allyltrifluoroborate VI (2 equiv.) in a solution of acetyl-cyclosporin A aldehyde (XII') in dichloromethane at -70°C provides after 60 min. reaction and aqueous work-up, (E)-acetyl-ISA247 (I).
30 Solvents for the reaction are organic solvent such as dichloromethane, THF or toluene, preferably dichloromethane.

Allylation by γ -silylated allylboron reagent of formula VII (step a-v))

Allylation of acetyl-cyclosporin A aldehyde (II') under such conditions provides a mixture of anti β -trimethylsilyl alcohol diastereomers (XI'). After the water phase is discarded, solvent is exchanged to THF, and sulfuric acid is added to perform the Peterson elimination. Aqueous work-up and crystallization provides (E)-acetyl-ISA247 (XII'). Subsequent hydrolysis yields (E)-ISA247 (I). Alternatively, isolation of the crude anti β -trimethylsilyl alcohol diastereomers after aqueous work-up, followed by Peterson elimination under standard conditions (concentrated sulfuric acid in THF) furnishes E-acetyl-ISA247.

For example, the synthesis of (E)-ISA247 by the complex of formula V can be performed as illustrated in Scheme I.

Scheme I:

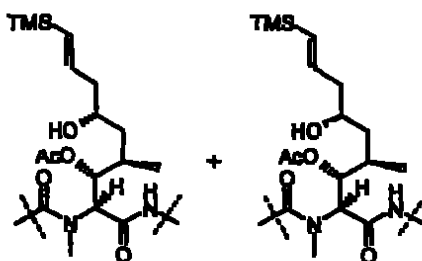


(The dotted lines have the meaning as defined above.)

Allylmatalation of acetyl-cyclosporin A aldehyde (II') can also take place under non-aqueous conditions directly with complex of formula V. Indeed, protic solvents such as carboxylic acids are particularly effective. Solvent mixture could be acetic acid and/or formic acid or a combination of acetic acid and/or formic acid and a co-solvent such as dichloromethane and THF. The allylation is best performed in acetic acid between RT and 35 °C. This provides a mixture of anti β -silyl alcohol diastereomers (XI'). These intermediates could of course be isolated

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over 7, substantial amount of a side-product identified as the vinylsilane of formula XV are formed. A test reaction (performed without tartrate activation) where Et₃N amine was added to reach a pH of 9-10 led to the almost exclusive formation of the vinylsilane product XV (as evidenced by MS, ¹H NMR, COSY, TOCSY and HSQC NMR experiments). Such an effect was totally unexpected.



XV

(The dotted lines have the meaning as defined above.)

Allylation by γ -silylated allylboron reagent of formula V (step a-iii))

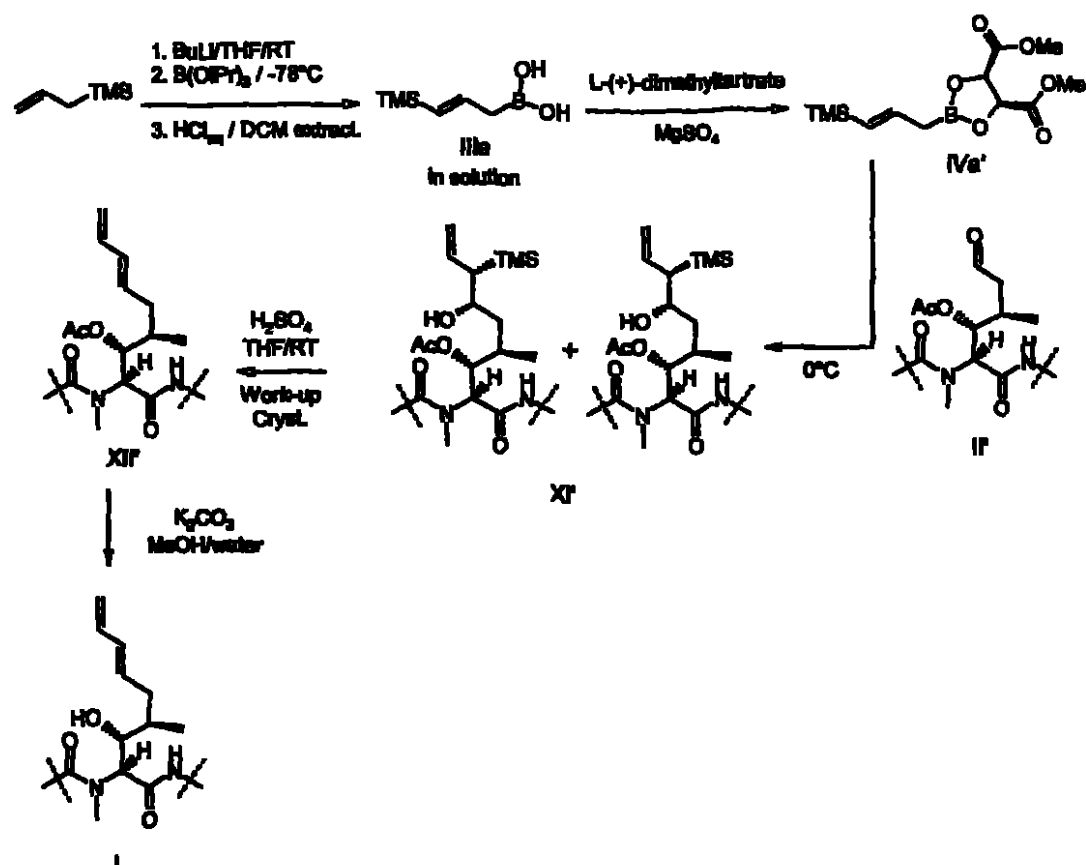
Without activation, the diethanolamine complex of formula V does not react at RT with acetyl-cyclosporin A aldehyde in non protic solvents like dichloromethane or THF. However, the complex of formula V represent a stable source of the corresponding boronic acid.

When treated in a water/organic solvent, (such as ethyl acetate, THF, dichloromethane or toluene, preferably ethyl acetate, dichloromethane or toluene, more preferably dichloromethane) mixture preferably in the presence of acid such as sulfuric acid, chlorhydric acid or acetic acid, preferably acetic acid, the diethanolamine complex V is hydrolyzed and liberates the reactive boronic acid as shown, for example, in Scheme H, which can then reacts with the acetyl-cyclosporin A aldehyde (II'), preferably at RT.

Scheme H:



Scheme G:



(The dotted lines have the meaning as defined above.)

- 5 The generation of the reagent of formula IVa' by mixing a solution of boronic acid of formula IIIa with L-(+)-dimethyltartrate, in the presence of a drying agent such as molecular sieves or magnesium sulfate, preferably magnesium sulfate, is evidenced by ¹¹B and ¹H NMR analyses.

- 10 Allylation of acetyl-cyclosporin A aldehyde (II') with a boronic acid reagent in-situ activated by addition of L-(+)-dimethyltartrate at a temperature of 0 °C to RT, preferably at 0 °C, give a mixture of anti β-silyl alcohol diastereomers (XI'). Aqueous work-up followed by the Peterson elimination in THF with sulfuric acid provides, after work-up and crystallization, (E)-acetyl-ISA247 (XII'). Hydrolysis of the acetyl protecting group yields (E)-ISA247 (I).

- 15 Care should be taken that reaction involving the use of crude boronic acid solution with or without tartrate activation should be performed at neutral or acidic pH (between 3 and 7, preferably between 5 and 6). Indeed when the pH is

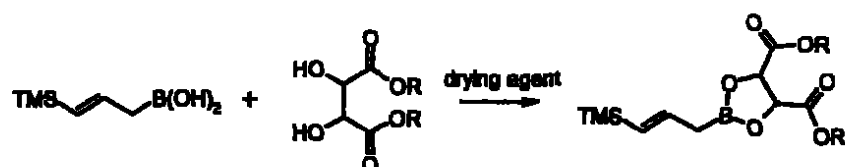
The allylation of acetyl-cyclosporine A aldehyde (II') in dichloromethane with 10 equiv. of a ca 50% solution of boronic acid reaches over 95% conversion within 60 min at RT and yields a mixture of anti β -trimethylsilyl alcohol diastereomers (XI'). The Peterson elimination can be performed after aqueous work-up on the crude allylation product at 0 °C to RT in THF with sulfuric acid. Alternatively, the Peterson elimination can take place directly on the allylation reaction mixture by addition of THF and sulfuric acid. Aqueous work-up and crystallization yields the (E)-acetyl-ISA247 (XII').

Hydrolysis of the (E)-acetyl-ISA247(XII') provides (E)-ISA247 (I).

With tartrate activation

As shown in Scheme F, the addition of a tartrate ester, such as, for example, L-(+)-dimethyltartrate in the presence of a drying agent, activates the boronic acid of formula IIIa by generating in-situ the corresponding boronate ester, a reagent class known in the literature to exhibit very high allylation reactivity. The reaction then proceeds partially or mainly through the generated boronate ester increasing the rate of the allylation.

Scheme F:



(R is C_{1-8} alkyl, preferably C_{1-6} alkyl, more preferably methyl, ethyl or isopropyl, especially methyl.)

Allylation with reagent of formula IV is performed in organic solvent such as ethyl acetate, THF, toluene, chloroform or dichloromethane, preferably in ethyl acetate, toluene or dichloromethane, more preferably in toluene or dichloromethane, especially in dichloromethane.

For example, the synthesis of (E)-ISA247 by the reagent of formula IV is performed as illustrated in Scheme G.

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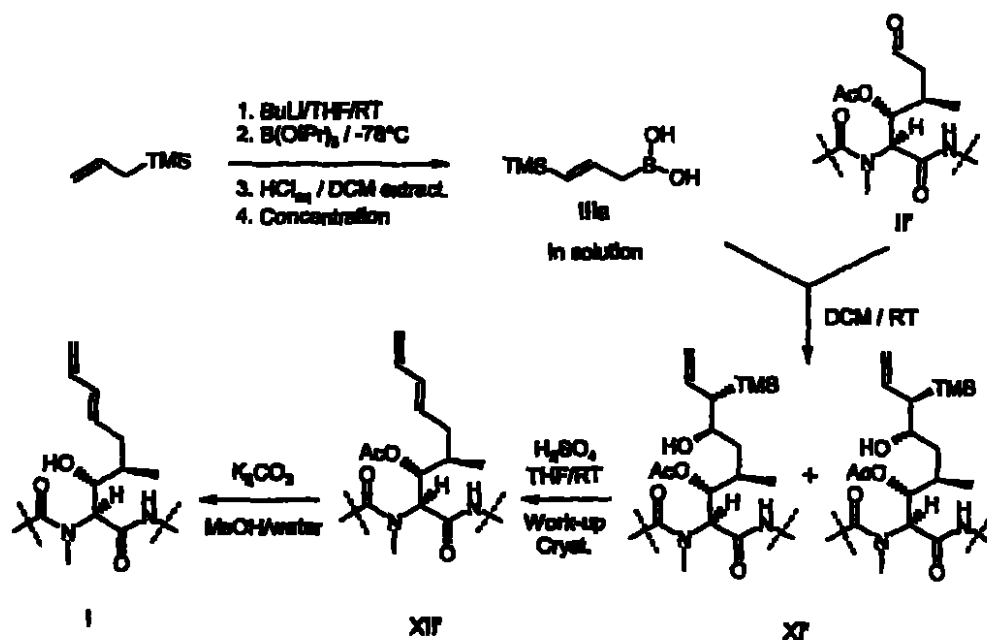
Toluene can be used as solvent for these reactions, however, marked solvent effects have been observed in these reactions. The allylation is best performed in polar non-coordinating solvents, preferably dichloromethane.

When tartrate additive is omitted, allylation is preferably performed in dichloromethane using a concentrated solution of the crude boronic acid (>10%, preferably ca 50% concentration).

Preferred reagent of formula III wherein R^1 is hydrogen, C_{1-8} alkyl or C_{3-8} cycloalkyl and/or, when R^1 is hydrogen, a trimer thereof are those wherein R^1 is hydrogen, methyl, ethyl, propyl, isopropyl, butyl or benzyl, more preferably R^1 is hydrogen, methyl, ethyl, propyl, isopropyl or butyl, further preferably hydrogen, methyl, ethyl, propyl or butyl, especially preferably hydrogen. Allylation is performed in organic solvent such as ethyl acetate, THF, toluene, chloroform or dichloromethane, preferably in ethyl acetate, toluene or dichloromethane, more preferably in toluene or dichloromethane, especially in dichloromethane.

For example, the synthesis of (E)-ISA247 by the reagent of formula III can be carried out as illustrated in Scheme E.

Scheme E:



20

(R^1 and the dotted lines have the meaning as defined above.)

an acetyl group, it can be removed, for example, by the treatment with K_2CO_3 in methanol and water. Under these conditions, the isomeric purity of the diene fragment is preserved. Therefore the double bond isomeric purity of E-ISA247 reflects the double bond isomeric purity of E-acetyl-ISA247. Bases other than potassium carbonate that may be used to remove the protecting group include sodium hydroxide, sodium carbonate, sodium alkoxide and potassium alkoxide.

Synthesis of (E)-ISA247 by allylboron reagents

Allylation by γ -silylated allylboron reagent of formula III or IV (step a-i) and a-ii))

In general, excess of the reagent of formula III or IV is needed to complete the allylation of acetyl-cyclosporin A aldehyde (II') within an acceptable timeframe. Higher conversion and rates are achieved by using an activating agent such as a tartrate ester and/or dichloromethane as (co)-solvent. In accordance to the general behavior of boronic acids, the reagent of formula IIIa can potentially exist in the form of cyclic trimer (boroxine) or oligomers (For an example of such behavior of a boronic acid, see: K. Ishihara, H. Kurihara, M. Matsumoto and H. Yamamoto in "Design of Bronsted Acid-Assisted Chiral Lewis Acid (BLA) Catalysts for Highly Enantioselective Diels-Alder Reactions", *Journal of the American Chemical Society* 1998, 120, p6920-6930.). When triisopropylborate is used for the preparation of the solution of the crude reagent of formula IIIa, reagent of formula IIIa can also contain diisopropyl boronate ester ($TMS-CH=CH-CH_2-B(OiPr)_2$), from isopropanol generated from $B(OiPr)_3$ and mixed derivatives such as $TMS-CH=CH-CH_2-B(OH)(OiPr)$. This solution can be used as an allylation reagent without purification.

Alternatively, a solution of reagent of formula IIIa can be generated by hydrolysis of complex of formula V in organic solvent/water mixture such as a dichloromethane/water, toluene/water, ethyl acetate/water, THF/water, chloroform/water mixture, preferably a dichloromethane/water mixture, preferably in the presence of an acid such as sulfuric acid, chlorhydric acid, acetic acid, preferably acetic acid. Allylation of acetyl-cyclosporin A aldehyde with a dichloromethane solution of reagent of formula IIIa prepared as just described can reach high conversions using as low as 2 equivalents of the reagent. In this case, isopropyl derivatives are of course absent.

Without tartrate activation

The reaction proceeds via a mechanism where the hydroxyl and the silyl groups are in an *anti conformation* prior to elimination.

5 The situation is opposite when the Peterson elimination is performed under basic conditions, in that case the reaction proceeds via a mechanism where the deprotonated hydroxyl and the silyl group are in a *syn conformation* prior to elimination.

Therefore, in principle, one could reach either double bond isomer by controlling the formation of either the *syn* or *anti* relative configuration of the β -silyl alcohol moiety or by using either acid or basic Peterson elimination conditions.

10 In the present invention, *trans*- γ -silylated allylmetals reagents are used for allylation of protected cyclosporin A aldehyde of formula II to form a mixture of *anti*- β -silyl alcohol diastereomers of formula XI. Therefore, a Peterson elimination is performed under acidic condition to form a *trans* double bond.

15 Typical acids for the acid-promoted reaction may include sulfuric acid, formic acid, chlorhydric acid, methanesulfonic acid tetrafluoroboric acid, perchloric acid, trifluoroacetic acid and various Lewis acids. Preferred acids are sulfuric acid, formic acid, methanesulfonic acid and $\text{BF}_3 \cdot \text{Et}_2\text{O}$, especially sulfuric acid, formic acid and $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

20 This step can be conducted at a reaction temperature from -70°C to 50°C . Preferred temperature range is 0°C to 50°C , more preferably 20°C to 40°C for formic acid. Preferred temperature range is 0°C to 40°C , more preferably 20°C to 30°C for sulfuric and methanesulfonic acid. Preferred temperature range is -80°C to 50°C , preferably -80°C to 25°C , especially -80°C to 0°C for $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

25 *E*-acetyl-ISA247 can be purified by crystallization in MTBE (for example via solvent exchange from dichloromethane to MTBE) or in MeOH/water mixtures.

30 In step b-ii), the protecting group is removed, returning the functional group on that carbon to an alcohol. The conditions and reagents to be employed depend on the protecting group used, which are known to those skilled in the art. Acyl group ($\text{R}'\text{C}(\text{O})-$; wherein R' is a linear saturated monovalent hydrocarbon radical of one to six carbon atoms or a branched saturated monovalent hydrocarbon radical of three to six carbon atoms), such as acetyl, propionyl, butyryl, isobutyryl, valeryl can preferably be used as a protecting group. When the protecting group is

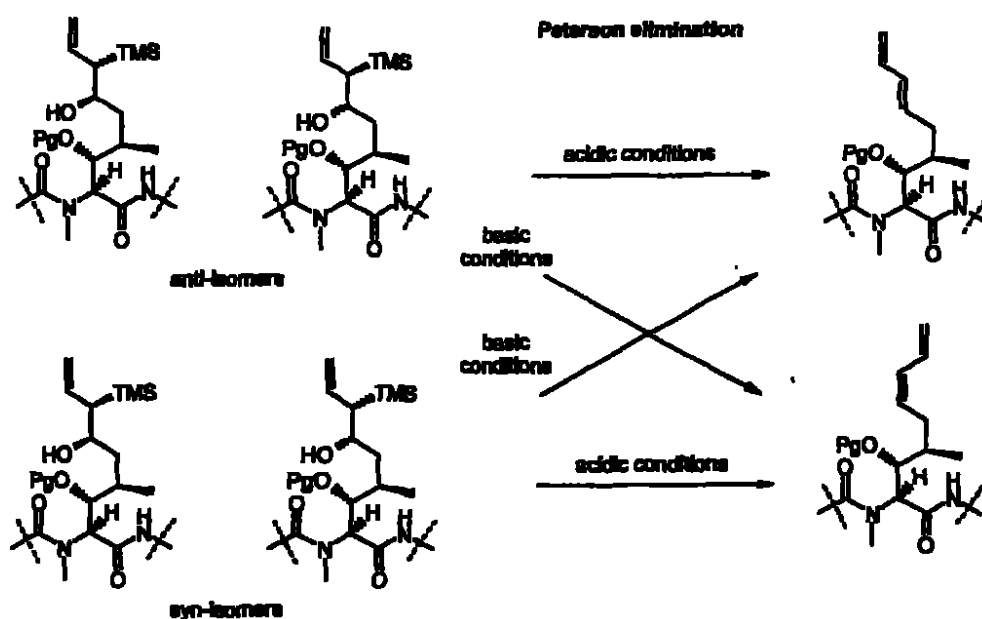
(Pg and the dotted lines have the meaning as defined above.)

In step b-i), the β -silyl alcohol of formula XI undergoes a Peterson
elimination (For a general discussion about Peterson eliminations, see : D. J. Ager
in "The Peterson Reaction", *Synthesis* 1984, p384-397 as well as references cited
therein.) and the internal double bond is generated, i.e. the elimination of silanol
from the β -silyl alcohol moiety occurs.

In view of achieving a high degree of double bond isomeric purity, the
success of the allylation-Peterson elimination sequence relies on the selective
introduction of a relative anti or syn configuration of the β -silyl alcohol moiety.

Indeed the Peterson elimination is known to be stereospecific. Anti isomers
will provide one isomeric double bond when the syn isomers will produce the other
double bond isomer under the same conditions as illustrated in Scheme D.

Scheme D:

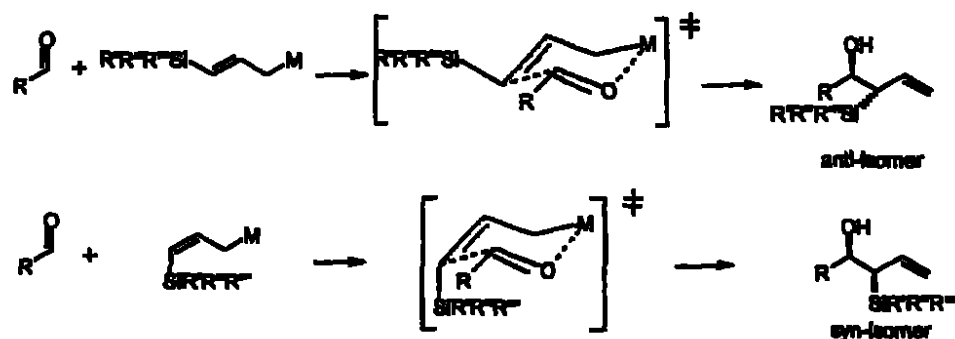


(Pg and the dotted lines have the meaning as defined above.)

Anti isomers should give the trans double bond under acidic Peterson
elimination conditions whereas syn isomers would provide the cis double bond.

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Scheme B:



5 In such a transition state, the aldehyde side chain preferably adopts a pseudo equatorial position in order to minimize 1,3-diaxial steric interactions. The relative configuration of the β -silyl alcohol fragment will therefore be determined by the configuration of C-C double bond of the allylmetal reagent.

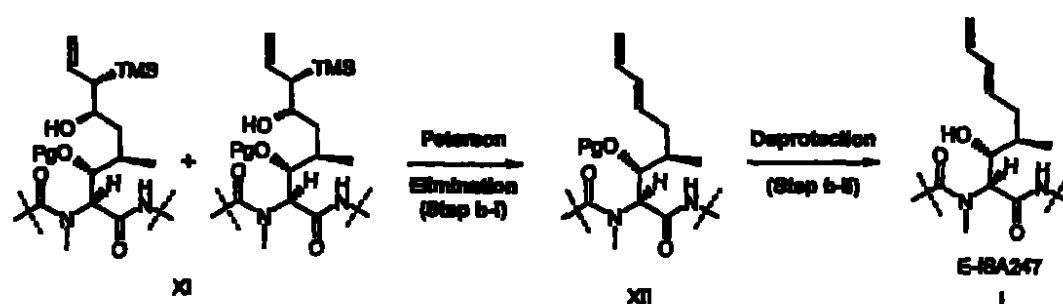
Therefore, use of *trans*- or *cis*- γ -silylated allylmetals reagents should lead predominantly to the anti- or *syn*- β -silyl alcohol isomer respectively. This holds in general, for example, for the allyl-boron, -titanium and -aluminum reagents.

10 Exception to this rule is found for example when a γ -silylated trialkylallylstannane reagent is added to aldehydes under Lewis acidic conditions, in that case the mechanism is different and the reaction provides mainly the *syn* β -silyl alcohol isomer.

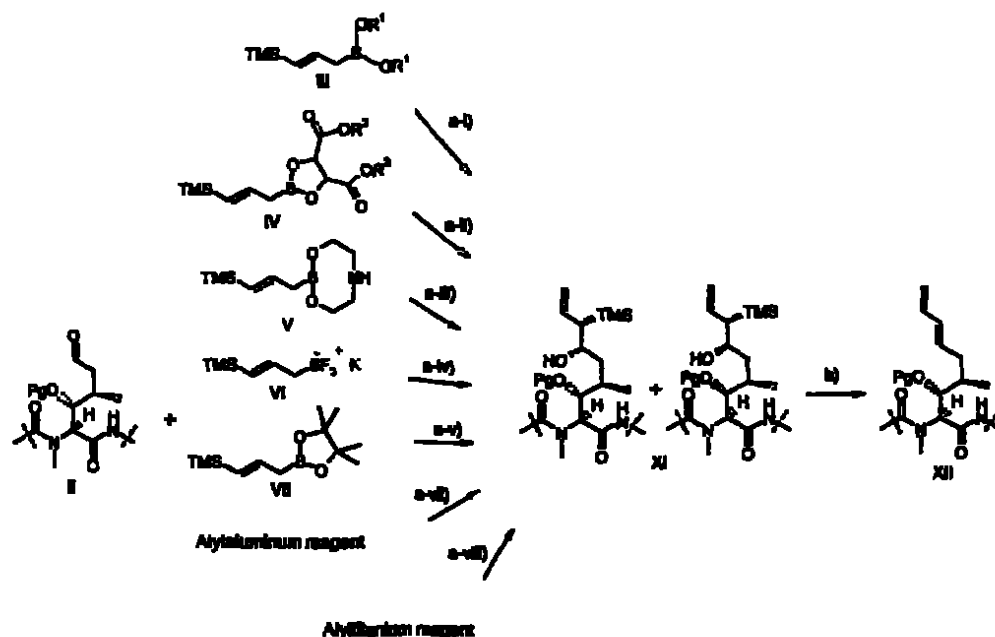
15 In step b), the β -silyl alcohol of formula XI is converted to (E)-ISA247 of formula I.

Step b) can be carried out as illustrated in Scheme C.

Scheme C:



Scheme A:



(Pg is a protecting group, the dotted lines mean that the remainder of the compound has the same structure as that of the compound of formula I, R^1 is hydrogen, C_{1-8} alkyl or C_{3-8} cycloalkyl and, when R^1 is hydrogen, a compound of formula III includes a trimer thereof, and R^2 is C_{1-8} alkyl or C_{3-8} cycloalkyl.)

In step a), protected cyclosporin A aldehyde of formula II is allylated by γ -silylated allylmetal reagent of formula III, IV, V, VI, VII, VIII etc. to form a mixture of β -silylhomomallylic alcohol diastereomers of formula XI (For a general discussion about allylmetals and allylation of aldehydes see : W. R. Roush in "Allyl Organometallics", Comprehensive Organic Synthesis, , Pergammon Press, Vol 2, pp 1-53 ; Y. Yamamoto, N. Asao in "Selective Reactions Using Allylic Metals", Chemical Reviews 1993, 93, p 2207-2293). The control of the relative anti or syn configuration of the β -silyl alcohol fragment will depend on the allylmetal reagent and conditions used to perform the aldehyde allylation step (For a general discussion of γ -silyl substituted allylmetal reagents see : T. H. Chan in "Silylallyl Anions in Organic Synthesis: A Study in Regio- and Stereoselectivity", Chemical Reviews 1995, 95, p1279-1292). This alcohol is often believed to form via a chair-like 6-membered ring transition state (also referred as Zimmerman-Traxler transition state) as shown in Scheme B.

Organics (B-2440 Geel, BE) or are prepared by methods known to those skilled in the art following procedures set forth in references such as *Fieser and Fieser's Reagents for Organic Synthesis*, Volumes 1-17 (John Wiley and Sons, 1991), *Rodd's Chemistry of Carbon Compounds*, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989), *Organic Reactions*, Volumes 1-40 (John Wiley and Sons, 1991), *March's Advanced Organic Chemistry*, (John Wiley and Sons, 1992), and *Larock's Comprehensive Organic Transformations* (VCH Publishers Inc., 1989).

The starting materials and the intermediates of the reaction may be isolated and purified if desired using conventional techniques, including but not limited to filtration, distillation, crystallization, chromatography. Such materials may be characterized using conventional means, including physical constants and spectral data.

Compounds of formula I are prepared as illustrated in Scheme A. The allylmetal reagent also referred herein as allylating reagent is to be taken in a general sense and may comprise reagents where the metal part is based on boron although it is not per se a metal.

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	TMS-	trimethylsilyl
	Et	ethyl
	Me	methyl
	iPr	isopropyl
5	Bu	butyl
	Ac	acetyl
	RT	room temperature
	HPLC	high performance liquid chromatography
	MS	mass spectroscopy
10	TLC	thin layer chromatography
	NMR	nuclear magnetic resonance spectroscopy
	2D-COSY	2-dimensional correlated spectroscopy
	2D-TOCSY	2-dimensional total correlation spectroscopy
	HSQC	Heteronuclear Single Quantum Coherence
15	Cryst.	crystallization
	Cpd	compound
	min.	minute(s)
	h	hours

The starting materials and reagents used in the process of the present
20 invention are either available from commercial suppliers such as Aldrich Chemical
Co., (Milwaukee, Wisconsin, USA), Bachem (Torrance, California, USA), Emka-
Chemie, or Sigma (St. Louis, Missouri, USA), Maybridge (Dist: Ryan Scientific,
P.O. Box 6496, Columbia, SC 92960), Bionet Research Ltd., (Cornwall PL32 9QZ,
UK), Menai Organics Ltd., (Gwynedd, N. Wales, UK), Butt Park Ltd., (Dist.
25 Interchim, Montlucon Cedex, France), Fluka (CH-9471 Buchs, CH), Acros

of trimethylsilyllithium with titanium tetraisopropoxide or titanium chlorotrisopropoxide.

The following terms used in the specification and claims have the meanings below:

5 "C_{a-b} alkyl" as used herein denotes straight chain or branched alkyl residues containing a to b carbon atoms. Therefore, for example, "C₁₋₈ alkyl" means straight chain or branched alkyl residues containing 1 to 8 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl or tert.-butyl.

10 "C₃₋₈ cycloalkyl" refers to a saturated monovalent cyclic hydrocarbon radical of three to eight ring carbons e.g., cyclopropyl, cyclobutyl, cyclohexyl.

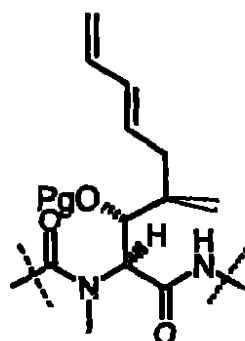
"Protecting group" refers to a grouping of atoms that when attached to a reactive group in a molecule masks, reduces or prevents that reactivity. Examples of protecting groups can be found in T.W. Green and P.G. Futs, Protective Groups in Organic Chemistry, (Wiley, 2nd ed. 1991) and Harrison and Harrison et al., Compendium of Synthetic Organic Methods, Vols. 1-8 (John Wiley and Sons, 15 1971-1996).

In the structural formulae presented herein a broken bond (- - -) denotes that the substituent is below the plane of the paper and a wedged bond (->) denotes that the substituent is above the plane of the paper.

20 The following abbreviations used in the specification and claims, otherwise specified, have the following significances:

MTBE	methyl tert-butylether
THF	tetrahydrofuran
DCM	dichloromethane
25 DMSO	dimethylsulfoxide
HMPA	hexamethylphosphoramide
TMEDA	tetramethylethylenediamine
TMS	tetramethylsilane

- (x) The process of (ix), wherein step a-iii) is conducted in acetic acid and step b-i) is conducted by addition of formic acid to the reaction mixture.
- (xi) The process of (ix), wherein step a-iii) and b-i) are conducted in formic acid or acetic acid/ formic acid.
- 5 (xii) The process of (i) or (ii), wherein step a-iv) is conducted in water/dichloromethane or water/toluene.
- (xiii) The process of (iii), wherein step a-iv) and b-I) are conducted in dichlorometane, tetrahydrofuran or toluene in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- (xiv) The process of (iii), wherein step a-v) is conducted in the
10 presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- (xv) The process of (xiv), wherein step a-v) and b-i) are conducted in dichloromethane, tetrahydrofuran or toluene and in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.
- (xvi) The process of (iii), wherein step a-v) is conducted in the presence of formic acid or acetic acid.
- 15 (xvii) The process of (xvi), wherein step a-v) and b-i) are conducted in formic acid or acetic acid/formic acid.
- (xviii) The process of (xvii), wherein step a-v) and b-i) are conducted in a mixture of acetic acid/formic acid and co-solvent selected from dichloromethane, toluene, ethyl acetate and isopropyl acetate.
- 20 (xix) The process of (xviii), wherein co-solvent is isopropyl acetate.
- (xx) The process of (xvi), wherein step a-v) is conducted in acetic acid and step b-i) is conducted by addition of formic acid to the reaction mixture.
- (xxi) The process of (iii), wherein step a-vii) is conducted by
25 allylating the compound of formula II with a reaction mixture prepared by reaction of the trimethylsilylallyllithium with diethylaluminum chloride.
- (xxii) The process of (iii), wherein step a-viii) is conducted by allylating the compound of formula II with a reaction mixture prepared by reaction



XII

wherein Pg is as defined in (i),

under acidic conditions; and

5 b-ii) converting the PgO group of the compound of formula XII to a hydroxyl group.

(iii) The process of (i) or (ii), wherein Pg is acetyl group.

(iv) The process of (iii), wherein step a-i), a-ii) or a-vi) is conducted in the presence of tartrates.

10 (v) The process of (iii), wherein step a-i), a-ii) or a-vi) is conducted in dichloromethane or toluene.

(vi) The process of (iii), wherein step a-iii) is conducted in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$, formic acid, acetic acid or tartrate esters.

(vii) The process of (vi), wherein step a-iii) is conducted in water/dichloromethane or water/toluene.

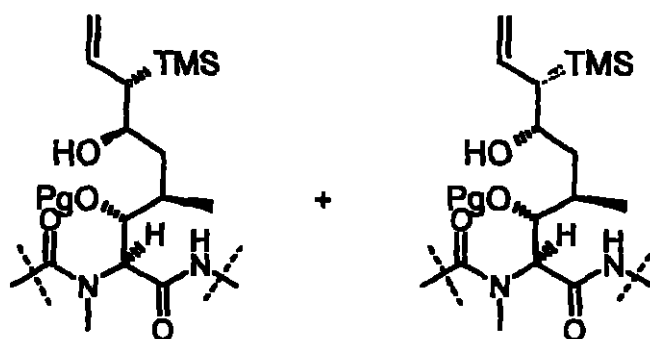
15 (viii) The process of (vi), wherein step a-iii) and b-i) are conducted in dichloromethane or tetrahydrofuran and in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$.

20 (ix) The process of (vi), wherein step a-iii) is conducted in acetic acid and/or formic acid; or in a mixture of acetic acid and /or formic acid and one or two cosolvents selected from a group consisting of dichloromethane and tetrahydrofuran.

- 5 -

a-viii) allylating a compound of formula II with a reaction mixture obtained by reaction of the trimethylsilylallyllithium with titanium tetraisopropoxide or titanium chlorotrisisopropoxide,

5 to form a compound of formula XI;



XI

wherein Pg is as defined above;

and

10 b) converting the compound of formula XI to the cyclosporin A analog of formula I.

In a second aspect, this invention provides intermediates for the process mentioned above.

In a third aspect, this invention provides processes for the preparation of these intermediates.

15 Also, within the process as defined above [it will be referred to in the following under (i)], preferred are the following processes:

(ii) The process of (i), wherein step b) is conducted by

b-i) converting the compound of formula XI to a compound of formula XII

- 4 -

a-iv) allylating a compound of formula II with a compound of formula

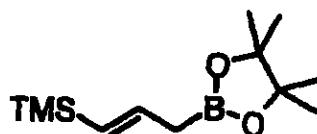


VI

VI

or

5 a-v) allylating a compound of formula II with a compound of formula VII



VII

or

10 a-vi) allylating a compound of formula II with a reaction mixture obtained by a process comprising;

- i) reacting allyltrimethylsilane with butyllithium to form trimethylsilylallyllithium;
- ii) reacting trimethylsilylallyllithium with triisopropylborate or trimethylborate, and then conducting aqueous work up,

15

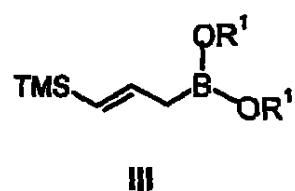
or

a-vii) allylating a compound of formula II with a reaction mixture obtained by reaction of the trimethylsilylallyllithium with diethylaluminum chloride,

or

- 3 -

with a compound of formula III

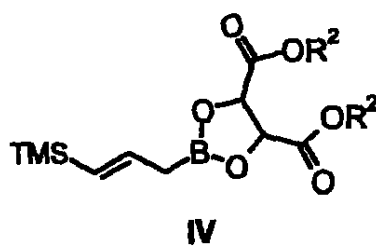


,wherein R¹ is hydrogen, C₁-₈ alkyl or C₃-₈ cycloalkyl and/or, when R¹ is hydrogen, a trimer thereof;

5

or

a-ii) allylating a compound of formula II with a compound of formula IV

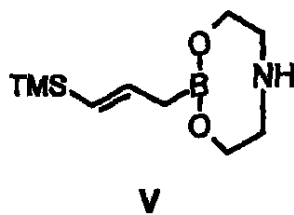


10

wherein R² is C₁-₈ alkyl or C₃-₈ cycloalkyl;

or

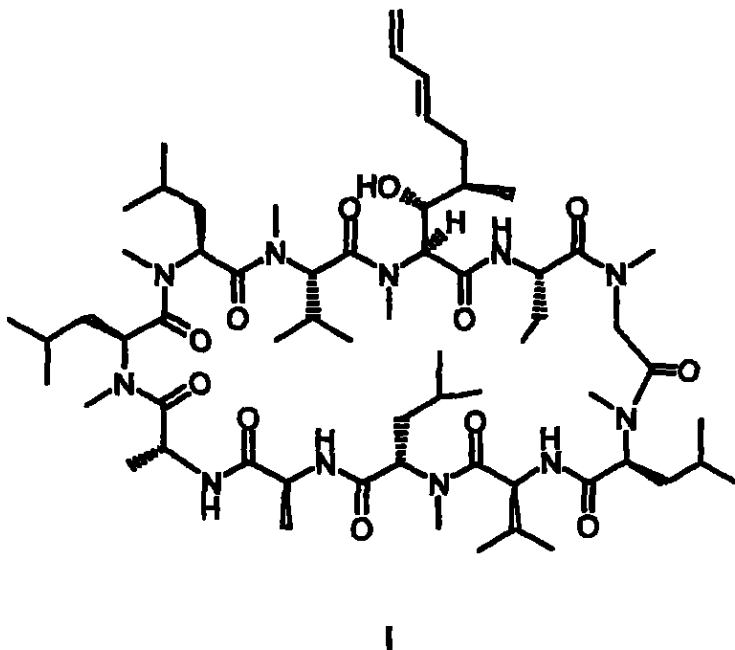
a-iii) allylating a compound of formula II with a compound of formula V



or

Tetrahedron Letters, Vol.36, No.10, p1583, 1995 discloses allylation process using tartrate modified (E)- γ -(trimethylsilyl)allylboronate.

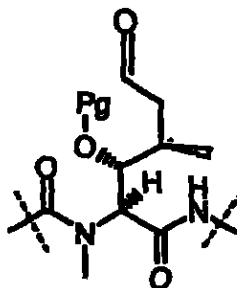
In a first aspect, this invention provides a process for the preparation of a cyclosporin A analog of formula I



5

comprising

a-i) allylating a compound of formula II

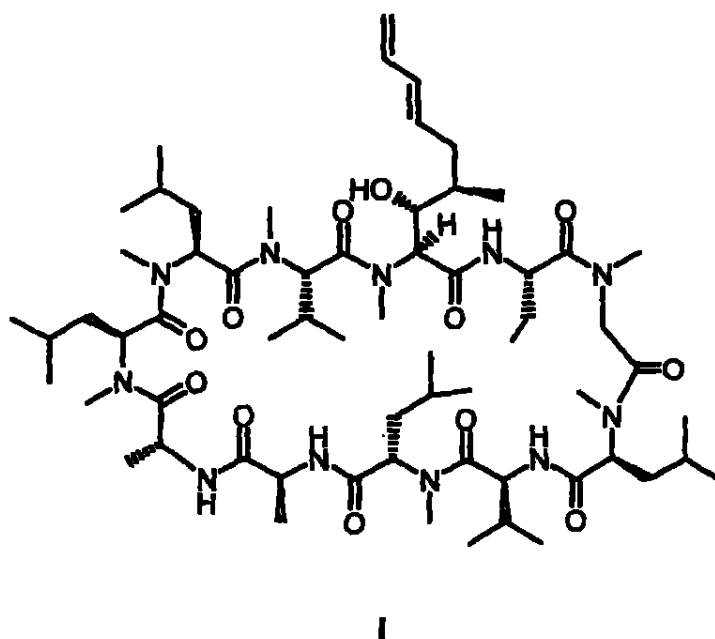


,wherein Pg is a protecting group and the dotted lines mean that the remainder of the compound has the same structure as that of the compound of formula I,

10

Process for preparation of cyclosporin A analog

This invention relates to a new process for the preparation of cyclosporin A analog of formula I:



As well as intermediates for this process and processes for the preparation thereof.

The cyclosporin A analog of formula I is structurally identical to cyclosporin A except for modification at the 1-amino acid residue. This analog is disclosed in WO 99/18120 and U.S. Provisional Patent Application No. 60/346,201. Hereinafter this analog is mentioned as (E)-ISA247.

Tetrahedron Letters, Vol.22, No.29, p2751-2752, 1981 discloses one of the intermediates of the process of this invention, namely pinacol (E)-1-trimethylsilyl-1-propene-3-boronate, and the allylation process using it.

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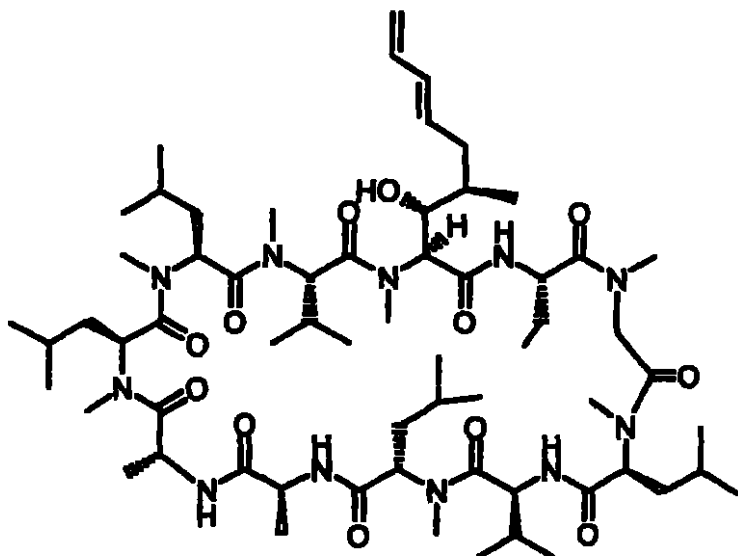
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(Continued on next page)

(54) Title: PROCESS FOR PREPARATION OF CYCLOSPORIN A ANALOG



(57) Abstract: The invention is
concerned with a new process for the
preparation of a cyclosporin A analog
of formula I comprising, a) alkylating a
protected cyclosporin A aldehyde with
an allylmetal reagent and, b) converting
the compound obtained in step a) to the
cyclosporin A analog of formula I.

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WO 2004/089960 A2



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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From the INTERNATIONAL BUREAU

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Applicant

ISOTECHNIKA INC. et al

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Re Item VI.

8. CERTAIN PUBLISHED DOCUMENTS (Rules 64.3 and 70.10 PCT).

- 8.1 D1 and D2 disclose synthetic methods, which fall within the scope of claim 1 (see point 1.1 above). These documents have been published on 24.04.2003 and relate to International Patent Applications, which have been filed on 17.10.2002 and claim the priority dates of 19.10.2001 and 05.04.2002.**

Re Item VII.

- 9.1 Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1, D2 and D5-D7 is not mentioned in the description nor are these documents identified therein.**
- 9.1^a In addition, D4 is not correctly identified in the description on page 2, line 1 (see the wrong page number for this document).**

Re Item VIII.

10. CLARITY (Art. 6 PCT).

- 10.1 It is clear from the description on page 14 (see lines 10-13) that acidic conditions for Peterson elimination (see step b) of claim 1) are essential in order to obtain the trans double bond characterizing the desired cyclosporin analogue (E)-ISA 247. The acidic conditions for the Peterson elimination are therefore among the features essential to the definition of the invention. Since independent claim 1 does not contain these features it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.**

the prior art as reactants for the addition reaction with aldehydes (see D5, point 1.4 above, and point 1.12 above).

The choice of the methyl groups for the dialkyl-tartrate moiety is therefore to be considered a selection among equivalent alternatives, i.e. lower alkyl groups, which come within the customary practice followed by the skilled person. Such a selection can only be regarded as inventive, if it presents unexpected effects or properties in relation to the other possibilities. No such effects or properties are indicated in the application, and therefore no inventive step is present in the subject-matter of claim 28.

7. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT).

7.1 Claims 1-22 relate to methods of producing a cyclosporin analogue, which can be applied in the pharmaceutical industry. Hence, the subject-matter of these claims is to be considered industrially applicable according to article 33(4) PCT.

7.2 Claims 23-28 relate to chemical compounds and methods for their preparation. Said compounds and methods can be made or used in industrial processes, e.g. for the synthesis of cyclosporin analogues, and therefore the subject-matter of these claims is to be considered industrially applicable according to article 33(4) PCT.

:

- 4.4^a (to claim 23) the hydrolysis of boronic acid esters comes within the customary practice of the skilled person;
- 4.4^b (to claim 25) the prior art discloses the esterification of boronic acids with diethanolamine (see for example point 1.13 above);
- 4.4^b (to claim 27) the skilled person would have considered allylboron-trifluoride derivatives for solving the problem posed (see paragraph 6 below).

5. INVENTIVE STEP (Art. 33(3) PCT) ** Non-Unity Subject 3 ****.**

- 5.1 Following the observation of point 4.1 above, the problem to be solved can be regarded as the provision of an alternative 1-(trimethylsilyl)-propene-3-boron organometallic compound as reagent for introducing a diene group on an aldehyde functionality.
- 5.2 The solution proposed in claim 26, i.e. boron-trifluoride, cannot be considered as involving any inventive step because the addition reaction between allylboron-trifluoride compounds and aldehydes is known (see point 1.10 above). Moreover, this reaction also occurs in the presence of substituents on the distal carbon of the allylic double bond (see the reaction involving the crothyl moiety). Hence, the skilled person would have considered to modify the 1-(trimethylsilyl)-propene-3-boronate esters of D4 and D5 into the corresponding boron-trifluoride derivatives in order to solve the problem posed, thereby obtaining an organometallic compound according to claim 26.
For this reason, the subject-matter of claim 26 does not involve any inventive step over D4 or D5 in combination with D10 and/or D11.

6. INVENTIVE STEP (Art. 33(3) PCT) ** Non-Unity Subject 4 ****.**

- 6.1 Following the observation of point 4.1 above, the problem to be solved can be regarded as the provision of an alternative 1-(trimethylsilyl)-propene-3-boronate ester as reagent for introducing a diene group on an aldehyde functionality.
- 6.2 The solution proposed consists in the dimethyl-tartrate ester or the allylboron derivative and cannot be considered as involving any inventive step because dialkyl-tartrate esters of the same organometallic reagent have been disclosed in

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4. INVENTIVE STEP (Art. 33(3) PCT) ** Non-Unity Subject 2 ****.**

- 4.1 D4 and D5 can independently be considered as representing the relevant state of the art because they disclose 1-(trimethylsilyl)-propene-3-boronate esters as reactants for introducing a diene group on an aldehyde functionality by means of: (i) the addition reaction between said aldehyde and the allylboron moiety, and (ii) the successive elimination reaction involving the trimethylsilyl group. The organometallic compound of claim 24 differs in that it corresponds to the boronate ester of diethanolamine, rather than a boronate ester of pinacol or dialkyl-tartrate.**
- 4.2 The problem to be solved can therefore be regarded as the provision of an alternative 1-(trimethylsilyl)-propene-3-boronate ester as reagent for introducing a diene group on an aldehyde functionality, eventually by converting it to an other 1-(trimethylsilyl)-propene-3-boron reactant.**
- 4.3 Having regards to the reaction scheme (see for example D4), the boron substituents other than the allyl moiety are not involved in the addition reaction with the aldehyde. These are cleaved, together with the boron atom, before the successive reaction step takes place. Accordingly, the skilled person would have considered the nature of these substituents, i.e. the structure of the alcohol moiety forming the allylboronate ester, as not essential for the reaction. The skilled person would therefore have attempted to modify the allylboronate esters of D4 and D5 by replacing the pinacol or dialkyl-tartrate moiety with any of the alcohol moieties known to form allylboronate esters in order to solve the problem posed. For example, he could have considered the diethylamino group of the allylboronate ester disclosed in D9, thereby obtaining an organometallic compound according to claim 24. Hence, the claimed subject-matter is to be considered as the result of a the selection of diethanolamine among known alcohol moieties for allylboronate esters. Such a selection can only be regarded as inventive, if it presents unexpected effects or properties. No such effects or properties are indicated in the application and therefore the subject-matter of claim 24 does not involve any inventive step over D4 or D5, eventually in combination with D9.**
- 4.4 Claims 23, 25 and 27 do not contain any features which, in combination with the features of the compound of claim 24, to which they refer, meet the requirements of the PCT in respect of inventive step, because:**

200

3. INVENTIVE STEP (Art. 33(3) PCT) ** Non-Unity Subject 1 ****.**

- 3.1** As indicated on page 1 of the present application (see lines 8-11), the cyclosporin analogue (E)-ISA 247 is known. This has been produced by means of a synthetic method, which differs from the claimed subject-matter in that it does not involve the use of any of the 1-(trimethylsilyl)-propene organometallic compounds of claim 1 (see point 1.3 above).
- 3.2** In view of that, the problem to be solved can be regarded as the provision of an alternative process for the preparation of this cyclosporin analogue.
- 3.3** The solution proposed in claim 1 of the present application cannot be considered as involving any inventive step because the use of 1-(trimethylsilyl)-propene organometallic synthetic fragments for attaching a terminal diene onto an aldehyde functionality is of customary practice in the synthesis of organic compounds (see points 1.4 and 1.6 above). Any of the allylating reagents of claim 1 is therefore to be considered a selection of a specific reagent structure among the 1-(trimethylsilyl)-propene organometallic compound disclosed in the prior art or similar equivalent synthetic fragments. Such a selection can only be regarded as inventive, if it presents unexpected effects or properties. However, no such effect or properties are indicated in the application. Hence, no inventive step is present in the subject-matter of claim 1.
- 3.3^a** Moreover, such unexpected effects or properties should be the same for all the different reagents of claim 1 in order to have a unifying technical relationship among these different reagents (Rule 13.1 and 13.2 PCT).
- 3.4** Dependent claims 2-22 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step because they relate to embodiments which comes within the customary practice of the skilled person and do not lead to any unexpected effect or property, given the disclosure of the cited prior art (see points 1.4 and 1.6 above).

:

2. NOVELTY (Art. 33(2) PCT).

****** Non-Unity Subject 1 ******

- 2.1 The subject-matter of claims 1-22 is novel because the available prior art does not disclose any process for the synthesis of the cyclosporin analogue (E)-ISA 247 by means of a 1-(trimethylsilyl)-propene organometallic compound as claimed. For example, the methods for producing cyclosporin analogues disclosed in D3 and D6 differ in that triphenylphosphonium reagents are used (see points 1.3 and 1.8 above).**

****** Non-Unity Subject 2 ******

- 2.2 The subject-matter of claim 24 is novel because none of the available prior art documents discloses the claimed compound of formula (V), namely diethylamine 1-(trimethylsilyl)-propene-3-boronate ester. The prior art only discloses similar boronate esters, which lack the trimethylsilyl substituent (see for example point 1. above).**
- 2.2^a The subject-matter of claims 23, 25 and 27 is also novel for the same reason. These claims relate to the preparation of the novel compound of formula (V) and its conversion to other 1-(trimethylsilyl)-propene-3-boron esters.**

****** Non-Unity Subject 3 ******

- 2.3 The subject-matter of claim 26 is novel because the available prior art does not disclose the claimed compound of formula (VI), namely potassium 1-(trimethylsilyl)-propene-3-boron-trifluoride. The potassium allylboron-trifluoride derivatives disclosed in D10 and D11 differ in that the trimethylsilyl substituent is missing (see point 1.10 above).**

****** Non-Unity Subject 4 ******

- 2.4 The subject-matter of claim 28 is novel over the dialkyl-tartrate 1-(trimethylsilyl)-propene-3-boronate esters, which are disclosed in the available prior art, in view of the methyl groups of the tartrate ester moiety. Indeed, the known dialkyl-tartrate 1-(trimethylsilyl)-propene-3-boronate esters are diethyl- or diisopropyl-tartrate derivatives (see points 1.5 and 1.12 above).**

- 1.4 D4 and D5 disclose pinacol and diisopropyl-tartrate 1-(trimethylsilyl)-propene-3-boronate esters as reactants for the addition reaction with aldehyde functionalities to produce substituted allyl groups, which are then converted to dienes by means of Peterson elimination (see: D4, the abstract and the reaction scheme on page 2751; D5, page 11, line 28 to page 12, line 34 and figure 5).
- 1.6 D6 and D7 disclose organometallic compounds, which are obtained by contacting the 1-(trimethylsilyl)-propene carbanion with triethyl-aluminium or tetraisopropyl orthotitanate, as aldehyde-reactive synthetic fragments for introducing allyl group in the synthesis of organic compounds. The conversion of such an allyl group to a terminal diene is also suggested (see D6, reaction equation 2 on page 4598 and equation 3 on page 4599; D7, paragraph joining pages 1449 and 1450).
- 1.8 D8 discloses the cyclosporin analogue (Z)-ISA 247 and a method for its production involving allyl-triphenylphosphonium reagents (see compound 1.24 or page 8, compound 6.3 on page 23 and example E).
- 1.9 D9 discloses tetrahydro-2-(2-propenyl)-4H-1,3,6,2-Dioxazaborocine, i.e. the ester of 1-(trimethylsilyl)-propene-3-boronate and diethanolamine, among allylborane reactants for a substitution reaction (see the last reaction scheme on page 3 and lines 52-54 on page 4).
- 1.10 D10 and D11 disclose the addition reaction of allyltrifluoroborates with aldehydes in organic and biphasic media and the preparation of the allylboron reactants from the corresponding boric acids (see: D10, tables 1-2 and scheme 2; D11, tables 2 and 3).
- 1.12 D12 discloses the diethyl-tartrate 1-(trimethylsilyl)-propene-3-boronate ester as reactants for the addition reaction with aldehydes (see compounds 15-17 of the reaction scheme on page 1583).
- 1.13 D13 discloses the preparation of boronate esters from the corresponding acid. In particular, the boronic acid ester of diethanolamine is prepared (see page 146, third paragraph).

Re Item V.

1. DOCUMENTS.

The following documents are referred to in this communication:

- D1: WO 03/033527 A;
- D2: WO 03/033526 A;
- D3: WO 99/18120 A;
- D4: Tsai D. J. S. et Matteson D. S., *Tetrahedron Lett.* (1981) vol. 22, no. 29, pages 2751-2752;
- D5: WO 98/48791 A;
- D6: Yamamoto Y. et Al., *Tetrahedron Lett.* (1982) vol. 23, no. 44, pages 4597-4600;
- D7: Reetz M. T. et Al., *Chemische Berichte* (1985) vol. 118, no. 4, pages 1441-1454;
- D8: EP0296122 A;
- D9: EP1291347 A;
- D10: Batey R. A. et Al., *Tetrahedron Lett.* (1999) vol. 40, pages 4289-4292;
- D11: Thadani A. N. et Al., *Organic Lett.* (2002) vol. 4, no. 22, pages 3827-3830;
- D12: Marron T. G. et Al., *Tetrahedron Lett.* (1995) vol. 36, pages 1581-1584;
- D13: Rathke M. W. et Al., *J.Org.Chem.* (1976) vol 122, pages 145-149.

- 1.1 D1 and D2 disclose the cyclosporin analogue (E)-ISA 247 and processes for its preparation, which involve: (i) the addition reaction between the corresponding cyclosporin aldehyde and 1-(trimethylsilyl)-propene-boron compounds, and (ii) the Peterson elimination reaction to obtain the terminal diene group (see D1: page 21, line 30 to page 26, line 23; page 40, line 23 to page 41, line 4; figures 2A,5,6 and 12 and D2: page 31, second paragraph; page 52, third paragraph; figures 2A,5,6 and 12).
In particular, the pinacol 1-(trimethylsilyl)-propene-boronate ester is used.
- 1.3 D3 discloses the deuterated cyclosporin analogue (E)-ISA 247 and a process for its preparation, which involves two successive addition reactions between a cyclosporin aldehyde derivative and a triphenylphosphonium compound (see figure 4 and examples 5 and 7).

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U.4 Accordingly, the synthetic methods of claim 1-22 and each of the different 1-(trimethylsilyl)-propene-3-boron compounds of claims 23-28 relate to separate inventions, which are not so linked as to form a single general inventive concept.

U.4^a Subject 1: claims 1-22.

Methods for the preparation of (E)-ISA 247 by means of the addition reaction between a cyclosporin-aldehyde derivative and a 1-(trimethylsilyl)-propen-3-yl organometallic compound, followed by Peterson elimination.

U.4^b Subject 2: claims 23-25 and 27.

Diethanolamine 1-(trimethylsilyl)-propene-3-boronate ester, its preparation and conversion to other 1-(trimethylsilyl)-propene-3-boron compounds.

U.4^c Subject 3: claim 26.

Potassium 1-(trimethylsilyl)-propene-3-boron-trifluoride.

U.4^d Subject 4: claim 28.

Dimethyl-tartrate 1-(trimethylsilyl)-propene-3-boronate ester.

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Re Item II.

P. PRIORITY (Rule 64(1)(b)(ii)).

- P.1** The priority document, namely the European Application EP03009721, does not disclose any of the specific embodiments relating to the choice of the solvents and solvent mixtures of present claims 16-19. Hence, the priority date of 08.04.2003 cannot be accorded to the subject-matter of these claims.
- P.2** The priority date appears to be validly claimed for the subject-matter of claims 1-15 and 20-28.

Re Item IV.

U. UNITY (Rule 13.1 PCT).

- U.1** The common concept, which would link together the subject-matter of claims 1-22 and claims 23-28, is the 1-(trimethylsilyl)-propen-3-yl structure of the organometallic compound.
- U.1^a** This is not a single inventive concept according to Rule 13.1 PCT because it is not novel in view of the 1-(trimethylsilyl)-propene-3-boronate esters and the 1-(trimethylsilyl)-propene-3-aluminum/titanium compounds disclosed in the prior art (see points 1.4 and 1.6 below). In particular, these boronate esters and organometallic compounds are disclosed as synthetic fragments that react with aldehydes and produce substituted allyl groups, which are eventually converted to the corresponding dienes.
- U.1^b** A lack of unity "a posteriori" is therefore indicated.
- U.3** The subject-matter of claims 1-22 is further characterized by the specific use of the 1-(trimethylsilyl)-propen-3-yl organometallic species for the preparation of the cyclosporin analogue (E)-ISA 247, whereas the subject-matter of claims 23-28 is further characterized by the substituents around the boron atom.
- U.3^a** In view of the prior art and the different characterizing features, there is no common technical relationship among the subject-matter of claims 1-22 and 23-28 involving one or more of the technical features, to which an inventive step could be addressed (Rule 13.2 PCT).

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

1. Statement

Novelty (N)	Yes: Claims	1-28
	No: Claims	-
Inventive step (IS)	Yes: Claims	-
	No: Claims	1-28
Industrial applicability (IA)	Yes: Claims	1-28
	No: Claims	-

2. Citations and explanations

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43bis.1 and 70.10)

and/or

2. Non-written disclosures (Rules 43bis.1 and 70.9)

see form 210

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

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Box No. II Priority

1. ☐ The following document has not been furnished:
- ☐ copy of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).
 - ☐ translation of the earlier application whose priority has been claimed (Rule 43bis.1 and 66.7(a)).
- 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 43bis.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:
- see separate sheet**

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:
- ☒ paid additional fees.
 - ☐ paid additional fees under protest.
 - ☐ not paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to require the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3:
- ☐ complied with
 - ☒ not complied with for the following reasons:
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
- ☒ all parts.
 - ☐ the parts relating to claims Nos.

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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 as filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the language of the international application, 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 as 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 to the invention 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:

To:

see form PCT/ISA/220

DOCKETED

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PCT
8.2.05 / -
WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.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.
PCT/EP2004/003504

International filing date (day/month/year)
02.04.2004

Priority date (day/month/year)
08.04.2003

International Patent Classification (IPC) or both national classification and IPC
C07K7/64, C07F5/02

Applicant
F. HOFFMANN-LA ROCHE AG

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 43bis.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 when the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 68.1bis(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:



European Patent Office
D-80299 Munich
Tel. +49 89 2369 - 0 Tx: 523656 epmu d
Fax: +49 89 2369 - 4465

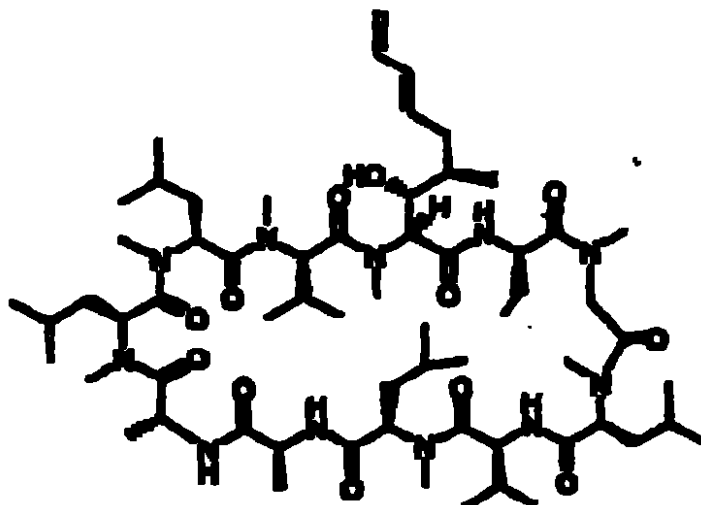
Authorized Officer

Fausti, S

Telephone No. +49 89 2369-7389

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The invention is concerned with a new process for the preparation of a cyclosporin A analog of formula I



I

comprising

a) alkylating a protected cyclosporin A aldehyde with an allylmetal reagent and

b) converting the compound obtained in step a) to the cyclosporin A analog of formula I.

In particular, the allyl reagent is a trimethylsilyl-allyl borate ester or a trimethylsilyl-allyl organometallic compound containing aluminium or titanium.

Patent document cited in search	Patent class	Publication date	Patent family number(s)	Publication date
EP 0296122	A		JP 1045396 A	17-02-1989
			JP 2116212 C	06-12-1996
			JP 8032724 B	29-03-1996
			JP 2772372 B2	02-07-1998
			JP 8048696 A	20-02-1996
			KR 9710927 B1	02-07-1997
			NZ 225041 A	26-03-1991
			PT 87745 A ,B	01-07-1988
			SK 411791 A3	11-07-1995
			US 5525590 A	11-06-1996
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			JP 2003096083 A	03-04-2003
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Patent document cited in search report		Publication date	Parent family number(s)	Publication date	187 188
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			WO 03033527 A2	24-04-2003	
			WO 03032949 A1	24-04-2003	
			CA 2460685 A1	24-04-2003	
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			EP 1436321 A2	14-07-2004	
			EP 1436322 A2	14-07-2004	
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			US 2003139326 A1	24-07-2003	
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			AU 9454298 A	27-04-1999	
			BR 9811716 A	18-07-2000	
			CA 2298572 A1	15-04-1999	
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			JP 2001519355 T	23-10-2001	
			JP 2004143168 A	20-05-2004	
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			NZ 502362 A	25-05-2001	
			US 6605593 B1	12-08-2003	
			US 2002132763 A1	19-09-2002	
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			AU 614086 B2	22-08-1991	
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			DE 3884470 D1	04-11-1993	
			DE 3884470 T2	10-03-1994	
			DK 326588 A	18-12-1988	
			EP 0296122 A2	21-12-1988	
			ES 2059558 T3	16-11-1994	
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			HU 9500332 A3	30-10-1995	
			IE 62482 B1	08-02-1995	
			IL 86751 A	27-02-1994	

Category *	Citation of document, with indication, where appropriate, of the relevant passage	Relevant to claim No.
Y	BATEY R A ET AL: "Potassium Allyl- and Crotyltrifluoroborates: Stable and Efficient Agents for Allylation and Crotylation" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 40, no. 23, 4 June 1999 (1999-06-04), pages 4289-4292, XP004164628 ISSN: 0040-4039 tables 1-2; scheme 2	26,27 187
Y	THADANI AVINASH N ET AL: "A mild protocol for allylation and highly diastereoselective syn or anti crotylation of aldehydes in biphasic and aqueous media utilizing potassium allyl- and crotyltrifluoroborates." ORGANIC LETTERS. 31 OCT 2002, vol. 4, no. 22, 31 October 2002 (2002-10-31), pages 3827-3830, XP002296391 ISSN: 1523-7060 tables 2 and 3	26
Y	MARRON T G ET AL: "Diastereoselective Synthesis of N-Benzoyl Mycalamine, the Fully Elaborated Trioxadecalin Nucleus of Mycalamide A. Control of the Key N-Acyl Amino Stereocenter via Carbamate Acylation" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 36, no. 10, 6 March 1995 (1995-03-06), pages 1581-1584, XP004028537 ISSN: 0040-4039 cited in the application page 1583, the compounds 15-17 and the corresponding reaction scheme	28
Y	RATHKE, M. R.; CHAO, E.; WU, G.: "The preparation and reactions of esters of dichloromethaneboronic acid" JOURNAL OF ORGANOMETALLIC CHEMISTRY, vol. 122, no. 2, 1976, pages 145-149, XP002296392 page 146, third paragraph	25

Category *	Citation of document, with indication, where appropriate, of the relevant passage	Relevant to claim No.
Y	TSAI D J S ET AL: "A STEREO CONTROLLED SYNTHESIS OF Z AND E TERMINAL DIENES FROM PINACOL E-1 TRI METHYLSILYL-1 PROPENE-3-BORONATE" TETRAHEDRON LETTERS, vol. 22, no. 29, 1981, pages 2751-2752, XP002288404 ISSN: 0040-4039 cited in the application abstract; reaction scheme on page 2751	1-27 186
Y	WO 98/48791 A (YANG GE ; MYLES DAVID C (US); HARRIED SCOTT S (US); UNIV CALIFORNIA (U) 5 November 1998 (1998-11-05) page 11, line 28 - page 12, line 34; figure 5	1-28
Y	YAMAMOTO, YOSHINORI ET AL: "Regioreversed reactions of the trimethylsilyl or phenylselenyl allylic carbanion with carbonyl compounds via allylic aluminum "ate" complexes" TETRAHEDRON LETTERS , 23(44), 4597-600 CODEN: TELEAY; ISSN: 0040-4039, 1982, XP002288405 reaction equation 2 on page 4598 and equation 3 on page 4599	1-22
Y	MANFRED T REETZ ET AL: "Stereoselective addition of organotitanium reagents to carbonyl compounds" CHEMISCHE BERICHTE, VERLAG CHEMIE GMBH. WEINHEIM, DE, vol. 118, no. 4, 1985, pages 1441-1454, XP002117771 ISSN: 0009-2940 paragraph joining pages 1449 and 1450	1-22
A	EP 0 296 122 A (SANDOZ AG ; SANDOZ AG (CH); SANDOZ AG (DE)) 21 December 1988 (1988-12-21) page 8, line 50; examples E,F; compounds 1.24,6.3	
Y	EP 1 291 347 A (CLARIANT GMBH) 12 March 2003 (2003-03-12) page 3, the last reaction scheme; page 4, lines 52-54	23-25,27
	-/-	

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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 C07K C07F

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, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
P,X	WO 03/033527 A (FOSTER ROBERT T ; ISOTECHNIKA INC (CA); NAICKER SELVARAJ (CA); YATSCOF) 24 Apr11 2003 (2003-04-24) page 21, line 30 to page 26, line 23; page 40, line 23 to page 41, line 4; figures 2A,5,6 and 12	1-22
P,X	WO 03/033526 A (JAYARAMAN SEETHARAMAN ; ABEL MARK (CA); FOSTER ROBERT T (CA); ISOTECHN) 24 Apr11 2003 (2003-04-24) page 31, second paragraph; page 52, third paragraph; figures 2A,5,6 and 12	1-22
Y	WO 99/18120 A (FOSTER ROBERT T ; ISOTECHNIKA INC (CA); NAICKER S SELVARAJ (CA); YATSC) 15 Apr11 1999 (1999-04-15) cited in the application figure 4; examples 5,7	1-22
-/-		

☒ 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 relating 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.
- *B* document member of the same patent family

Date of the actual completion of the international search

15 September 2004

Date of mailing of the international search report

15.10.04

Name and mailing address of the ISA

European Patent Office, P.B. 6818 Patenthaus 2
NL - 2260 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
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Authorized officer

Fausti, S

3

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows: 184

1. claims: 1-22

Methods for the preparation of (E)-ISA 247 by means of the addition reaction between a cyclosporin-aldehyde derivative and a 1-(trimethylsilyl)-propen-3-yl organometallic compound, followed by Peterson elimination.

2. claims: 23-25 and 27

Diethanolamine 1-(trimethylsilyl)-propene-3-boronate ester, its preparation and conversion to other 1-(trimethylsilyl)-propene-3-boron compounds.

3. claim: 26

Potassium 1-(trimethylsilyl)-propene-3-boron-trifluoride.

4. claim: 28

dimethyltartrate 1-(trimethylsilyl)-propene-3-boronate ester.

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

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

- 183
1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
 2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
 3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest :

- ☐ The additional search fees were accompanied by the applicant's protest
- ☒ No protest accompanied the payment of additional search fees.

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference Case 21711	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, where applicable, Item 5 below.	
International application No. PCT/EP2004/003504	International filing date (day/month/year) 02/04/2004	(Earliest) Priority Date (day/month/year) 08/04/2003
Applicant F. HOFFMANN-LA ROCHE AG		

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

This International Search Report consists of a total of 9 sheets.

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

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ The international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.

2. ☐ Certain claims were found unsearchable (See Box II).**3. ☒ Unity of invention is lacking (see Box III).****4. With regard to the title,**

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

PROCESS FOR PREPARATION OF CYCLOSPORIN "A" ANALOGUES HAVING A TERMINAL DIENE GROUP

5. With regard to the abstract,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regards to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

- b. ☐ none of the figures is to be published with the abstract.

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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 and the written opinion of the International Searching Authority, 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 (see *PCT Applicant's Guide*, Annexes B1 and B2).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no International search report would be established (see *PCT Applicant's Guide*, Volume I/A, paragraph 296).

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 29 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 48.1).

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 48.2).

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)):

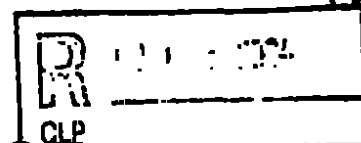
The amendments must be submitted with a letter.

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.

PATENT COOPERATION TREATY

DOCKETED



From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

KJELLSAAS-BERGER, Hanny
124 Grenzacherstrasse
CH-4070 Basle
SWITZERLAND

Frist: 19.11.04 / 2
Orientierung US
+ Überprüf. AB-IL

RECEIVED
19.11.04

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

15.12.04 / 2

(PCT Rule 44.1)

Date of mailing
(day/month/year)

15/10/2004

Applicant's or agent's file reference

Case 21711

2650.PCT

FOR FURTHER ACTION

See paragraphs 1 and 4 below

International application No.

PCT/EP2004/003504

International filing date
(day/month/year)

02/04/2004

Applicant

F. HOFFMANN-LA ROCHE AG

1. ☒ 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 48):

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, Facsimile 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. 31 851 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Bruno Danti

Form PCT/ISA/220 (January 2004)

Bitte sofort Literatur im Zedo
eintragen und Fehlendes beschaffen

(See notes on accompanying sheet)

PATENT COOPERATION TREATY

RECEIVED
19/12/04

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

To:

WILKS, Susan, Sutterfield
Isotechnika Inc.
Suite 100
17301 North Perimeter Drive
Scottsdale, AZ 85255
United States of America

Date of mailing (day/month/year) 22 September 2004 (22.09.2004)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference Case 21711	
International application No. PCT/EP2004/003504	International filing date (day/month/year) 02 April 2004 (02.04.2004)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address KJELLSAA-BERGER, Hanny Grenzacherstrasse 124 CH-4070 Basel Switzerland	State of Nationality	State of Residence
	Telephone No. (61) 688 20 74	
	Facsimile No. (61) 688 13 95	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address WILKS, Susan, Sutterfield Isotechnika Inc. Suite 100 17301 North Perimeter Drive Scottsdale, AZ 85255 United States of America	State of Nationality	State of Residence
	Telephone No. 480 505 0540 ext3213	
	Facsimile No. 480 505 0545	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office	☐ the designated Offices concerned
☒ the International Searching Authority	☐ the elected Offices concerned
☐ the International Preliminary Examining Authority	☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338.89.75	Authorized officer Rosana REYES (Fax : 338 89 75) Telephone No. (41-22) 338 8471
--	--

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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 INC.
2100 College Plaza
8215-112th Street
Edmonton, Alberta T8G 2C8
CANADA

hereby appoints (appoint) the following person as:



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.)

WILKS, Susan SUTTERFIELD, and DAY, Sharon L., of ISOTECHNIKA INC., 17301 North Perimeter Drive, Suite 100, Scottsdale, Arizona 85255, UNITED STATES OF AMERICA

to represent the undersigned before



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: PROCESS FOR PREPARATION OF CYCLOSPORIN A ANALOG

Applicant's or agent's file reference: 2850.PCT

International application number (if already available): PCT/EP2004/003504

filed with the following Office European 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 INC.



By: Joseph Kozlek
Title: Executive Vice President

Date:

3 SEPT 2004

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THE INTERNATIONAL BUREAU OF WIPO

APPLICANT : ISOTECHNIKA INC.
FOR : PROCESS FOR PREPARATION OF
CYCLOSPORIN A ANALOG
INTERNATIONAL APPLICATION NO.: PCT/EP2004/003504
INTERNATIONAL FILING DATE : 02 APRIL 2004
(02.04.2004)
INTERNATIONAL PRIORITY DATE : 08 APRIL 2003
(08.04.2003)
ATTORNEY DOCKET NO. : 2650.PCT

**RESPONSE TO COMMUNICATION IN CASES FOR WHICH
NO OTHER FORM IS APPLICABLE**

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
SWITZERLAND

Sir:

In response to the *Communication In Cases For Which No Other Form Is Applicable* dated 17 AUGUST 2004 (17.08.2004), Applicant encloses herewith a *Power Of Attorney*, which has been duly executed by Joseph Koziak, Executive Vice President of Isotechnika Inc. It is respectfully requested that the International Bureau proceed with the recordal of the requests for change of the Applicant and Agent under PCT Rule 92bis submitted to the International Bureau on August 2, 2004, and that the undersigned be notified of the change once the recordal has been effected.

Respectfully submitted,

September 7, 2004
Date

Susan S. Wilks
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

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this communication is being transmitted, via facsimile 011-41-22-338-89-75, to the International Bureau of WIPO, 34, chemin des Colombettes, 1211 Geneva 20, SWITZERLAND, on this 7th day of September, 2004.

Pamela A. Matheis
Pamela A. Matheis

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IN THE EUROPEAN PATENT OFFICE

APPLICANT : ISOTECHNIKA INC.
FOR : PROCESS FOR PREPARATION OF
CYCLOSPORIN A ANALOG
INTERNATIONAL APPLICATION NO.: PCT/EP2004/003504
INTERNATIONAL FILING DATE : 02 APRIL 2004
(02.04.2004)
INTERNATIONAL PRIORITY DATE : 08 APRIL 2003
(08.04.2003)
ATTORNEY DOCKET NO. : 2650.PCT

RESPONSE TO INVITATION TO PAY ADDITIONAL FEES

European Patent Office
Erhardtstrasse 27
D-80298 Munich
GERMANY

Sir:

In response to the *Invitation To Pay Additional Fees* dated 19 AUGUST 2004 (19.08.2004) received in connection with the above-identified International application, Applicant hereby submits the additional fee in the amount of USD \$5,720.58. Applicant further submits that the fee is being timely paid within thirty days from the mailing date of the Invitation.

Also enclosed are copies of a *Change In The Person Of The Applicant Under 92bis* together with a *Change In The Name And Address Of The Agent Under 92bis*, which were sent, via facsimile, to the International Bureau of WIPO on August 2, 2004, for recording.

A copy of this communication is being forwarded to the International Bureau of WIPO so that they may update their records accordingly.

Respectfully submitted,

August 27, 2004
Date

Susan S. Wilks
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

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CERTIFICATE OF DELIVERY BY FEDERAL EXPRESS

I hereby certify that the attached *Response To Invitation To Pay Additional Fees*, together with enclosures, is being sent via Federal Express Label No. 7913 2592 1763 this date, in an envelope addressed to Bruno Danti, European Patent Office, Erhardtstrasse 27, D-80298 Munich, Germany.

August 27, 2004
Date:

Pamela A. Matheis
Signature of Person Depositing
Federal Express Mail
Name: Pamela A. Matheis

Patent Family Annex
Information on patent family members

International Application No

PCT/EP2004/003504

7/2

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0296122	A	JP 1045396 A	17-02-1989
		JP 2116212 C	06-12-1996
		JP 8032724 B	29-03-1996
		JP 2772372 B2	02-07-1998
		JP 8048696 A	20-02-1996
		KR 9710927 B1	02-07-1997
		NZ 225041 A	26-03-1991
		PT 87745 A ,B	01-07-1988
		SK 411791 A3	11-07-1995
		US 5525590 A	11-06-1996
		ZA 8804345 A	28-02-1990

Patent Family Annex

Information on patent family members

International Application No

PCT/EP2004/003504

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03033527	A	24-04-2003	WO 03033526 A2 24-04-2003
			WO 03033527 A2 24-04-2003
			WO 03032949 A1 24-04-2003
			CA 2460685 A1 24-04-2003
			CA 2461730 A1 24-04-2003
			CA 2461740 A1 24-04-2003
			EP 1436321 A2 14-07-2004
			EP 1436322 A2 14-07-2004
			EP 1435910 A1 14-07-2004
			US 2003139326 A1 24-07-2003
			US 2003171264 A1 11-09-2003
			US 2003212249 A1 13-11-2003
WO 03033526	A	24-04-2003	WO 03033526 A2 24-04-2003
			WO 03033527 A2 24-04-2003
			WO 03032949 A1 24-04-2003
			CA 2460685 A1 24-04-2003
			CA 2461730 A1 24-04-2003
			CA 2461740 A1 24-04-2003
			EP 1436321 A2 14-07-2004
			EP 1436322 A2 14-07-2004
			EP 1435910 A1 14-07-2004
			US 2003139326 A1 24-07-2003
			US 2003171264 A1 11-09-2003
			US 2003212249 A1 13-11-2003
WO 9918120	A	15-04-1999	AU 750245 B2 11-07-2002
			AU 9454298 A 27-04-1999
			BR 9811716 A 18-07-2000
			CA 2298572 A1 15-04-1999
			CA 2372639 A1 15-04-1999
			EP 0991660 A1 12-04-2000
			WO 9918120 A1 15-04-1999
			JP 2001519355 T 23-10-2001
			JP 2004143168 A 20-05-2004
			NO 20001020 A 29-02-2000
			NZ 502362 A 25-05-2001
			US 6605593 B1 12-08-2003
			US 2002132763 A1 19-09-2002
WO 9848791	A	05-11-1998	AU 7267298 A 24-11-1998
			WO 9848791 A1 05-11-1998
EP 0296122	A	21-12-1988	AT 95193 T 15-10-1993
			AU 614086 B2 22-08-1991
			AU 1767988 A 22-12-1988
			CA 1338728 C 19-11-1996
			CZ 9104117 A3 17-02-1993
			CY 1939 A 16-05-1997
			DE 3884470 D1 04-11-1993
			DE 3884470 T2 10-03-1994
			DK 326588 A 18-12-1988
			EP 0296122 A2 21-12-1988
			ES 2059558 T3 16-11-1994
			HK 152796 A 16-08-1996
			HU 9500332 A3 30-10-1995
			IE 62482 B1 08-02-1995
			IL 86751 A 27-02-1994

INVITATION TO PAY ADDITIONAL FEES

170
International application No.

PCT/EP2004/003504 121

1-22 and 23-28 involving one or more of the technical features to which an inventive step could be addressed (Rule 13.2 PCT). Accordingly, the synthetic methods of claim 1-22, and each of the different 1-(trimethylsilyl)-propene-3-boron compounds of claims 23-28 relate to separate inventions, which are not so linked as to form a single general inventive concept.

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

1. claims: 1-22

Methods for the preparation of (E)-ISA 247 by means of the addition reaction between a cyclosporin-aldehyde derivative and a 1-(trimethylsilyl)-propen-3-yl organometallic compound, followed by Peterson elimination.

2. claims: 23-25 and 27

Diethanolamine 1-(trimethylsilyl)-propene-3-boronate ester, its preparation and conversion to other 1-(trimethylsilyl)-propene-3-boron compounds.

3. claim: 26

Potassium 1-(trimethylsilyl)-propene-3-boron-trifluoride.

4. claim: 28

dimethyltartrate 1-(trimethylsilyl)-propene-3-boronate ester.

The common concept, which would link together the subject-matter of claims 1-22 and claims 23-28, is the 1-(trimethylsilyl)-propen-3-yl structure of the organometallic compound.

This is not a single inventive concept according to Rule 13.1 PCT because it is not novel in view of the 1-(trimethylsilyl)-propene-3-boronate esters and the 1-(trimethylsilyl)-propene-3-aluminium/titanium compounds disclosed in the prior art (see for example: Tsai & Matteson (1981), compound 1; W098/48791, line 7 on page 12 and figure 5; Yamamoto et Al. (1982), reaction equation 2; Reetz et Al. (1985), paragraph joining pages 1449 and 1450). In particular, these boronate esters and organometallic compounds are disclosed as synthetic fragments that react with aldehydes and produce substituted allyl groups, which are eventually converted to the corresponding dienes.

A lack of unity "a posteriori" is therefore indicated.

The subject-matter of claims 1-22 is further characterized by the specific use of the 1-(trimethylsilyl)-propen-3-yl organometallic species for the preparation of the cyclosporine analogue (E)-ISA 247, whereas the subject-matter of claims 23-28 is further characterized by the substituents, other than 1-(trimethylsilyl)-propene, around the boron atom.

In view of the prior art and the different characterizing features, there is no common technical relationship among the subject-matter of claims

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98/48791 A (YANG GE ; MYLES DAVID C (US); HARRIED SCOTT S (US); UNIV CALIFORNIA (U) 5 November 1998 (1998-11-05) page 11, line 28 - page 12, line 34; figure 5	1-22
Y	YAMAMOTO, YOSHINORI ET AL: "Regioreversed reactions of the trimethylsilyl or phenylselenyl allylic carbanion with carbonyl compounds via allylic aluminum "ate" complexes" TETRAHEDRON LETTERS , 23(44), 4597-600 CODEN: TELEAY; ISSN: 0040-4039, 1982, XP002288405 reaction equation 2 on page 4598 and equation 3 on page 4599	1-22
Y	MANFRED T REETZ ET AL: "Stereoselective addition of organotitanium reagents to carbonyl compounds" CHEMISCHE BERICHTE, VERLAG CHEMIE GMBH. WEINHEIM, DE, vol. 118, no. 4, 1985, pages 1441-1454, XP002117771 ISSN: 0009-2940 paragraph joining pages 1449 and 1450	1-22
A	EP 0 296 122 A (SANDOZ AG ; SANDOZ AG (CH); SANDOZ AG (DE)) 21 December 1988 (1988-12-21) page 8, line 50; examples E,F; compounds 1.24,6.3	

1. The present communication is an Annex to the invitation to pay additional fees (Form PCT/ISA/206). It shows the results of the international search established on the parts of the international application which relate to the invention first mentioned in claim Nos.:
- see 'Invitation to pay additional fees'
2. This communication is not the international search report which will be established according to Article 18 and Rule 43.
3. If the applicant does not pay any additional search fees, the information appearing in this communication will be considered as the result of the international search and will be included as such in the international search report.
4. If the applicant pays additional fees, the international search report will contain both the information appearing in this communication and the results of the international search on other parts of the international application for which such fees will have been paid.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 03/033527 A (FOSTER ROBERT T ; ISOTECHNIKA INC (CA); NAICKER SELVARAJ (CA); YATSCOF) 24 Apr11 2003 (2003-04-24) page 21, line 30 to page 26, line 23; page 40, line 23 to page 41, line 4; figures 2A, 5, 6 and 12	1-22
P, X	WO 03/033526 A (JAYARAMAN SEETHARAMAN ; ABEL MARK (CA); FOSTER ROBERT T (CA); ISOTECHNI) 24 Apr11 2003 (2003-04-24) page 31, second paragraph; page 52, third paragraph; figures 2A, 5, 6 and 12	1-22
Y	WO 99/18120 A (FOSTER ROBERT T ; ISOTECHNIKA INC (CA); NAICKER S SELVARAJ (CA); YATSC) 15 Apr11 1999 (1999-04-15) cited in the application figure 4; examples 5, 7	1-22
Y	TSAI D J S ET AL: "A STEREO CONTROLLED SYNTHESIS OF Z AND E TERMINAL DIENES FROM PINACOL E-1 TRI METHYLSILYL-1 PROPENE-3-BORONATE" TETRAHEDRON LETTERS, vol. 22, no. 29, 1981, pages 2751-2752, XP002288404 ISSN: 0040-4039 cited in the application abstract; reaction scheme on page 2751	1-22
-/-		

☒ 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.

"B" document member of the same patent family

From the INTERNATIONAL SEARCHING AUTHORITY

DOCKETED PCT

To:

KJELLSA-BERGER, Hanny
124 Grenzacherstrasse
CH-4070 Basle
SWITZERLANDFrist: 23.9.04
Orientierung US
+ Überprüf. At. Nr.

INVITATION TO PAY ADDITIONAL FEES

(PCT Article 17(3)(a) and Rule 40.1)

Frist: 19.9.04 ber. uf.

Date of mailing
(day/month/year)

19/08/2004

Applicant's or agent's file reference

Case 21711

PAYMENT DUE

within 30 months/days
from the above date of mailing

International application No.

PCT/EP2004/003504

International filing date
(day/month/year)

02/04/2004

Applicant

F. HOFFMANN-LA ROCHE AG

1. This International Searching Authority

- (i) considers that there are
- 4
- (number of) inventions claimed in the international application covered by the claims indicated
- MMM
- on the extra sheet

and it considers that the international application does not comply with the requirements of unity of invention (Rules 13.1, 13.2 and 13.3) for the reasons indicated MMM on the extra sheet

- (ii)
- ☒
- has carried out a partial international search (see Annex)
- ☐
- will establish the international search report on those parts of the international application which relate to the invention first mentioned in claims Nos.:
-
- see annex

- (iii) will establish the international search report on the other parts of the international application only if, and to the extent to which, additional fees are paid

2. The applicant is hereby invited, within the time limit indicated above, to pay the amount indicated below:

EUR 1.550,00 x 3 = EUR 4.650,00
Fee per additional invention number of additional inventions total amount of additional fees

Or, _____ x _____ = _____

The applicant is informed that, according to Rule 40.2(c), the payment of any additional fee may be made under protest, i.e., a reasoned statement to the effect that the international application complies with the requirement of unity of invention or that the amount of the required additional fee is excessive.

- 3.
- ☐
- Claim(s) No. _____ have been found to be unsearchable under Article 17(2)(b) because of defects under Article 17(2)(a) and therefore have not been included with any invention.

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. 31 851 epo nl,
Fax (+31-70) 340-3018

Authorized officer

Bruno Danti

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT DOCKETED

COMMUNICATION IN CASES FOR WHICH
NO OTHER FORM IS APPLICABLE

RECEIVED
9/1/04

To:

SUTTERFIELD WILKS, Susan
Isotechnika Inc.
17301 North Perimeter Drive
Suite 100
Scottsdale, AZ 85255
UNITED STATES OF AMERICA

Date of mailing (day/month/year) 17 August 2004 (17.08.2004)	
Applicant's or agent's file reference Case 21711	REPLY DUE see paragraph 1 below
International application No. PCT/EP2004/003504	International filing date (day/month/year) 02 April 2004 (02.04.2004)
Applicant ISOTECHNIKA INC.	

1. ☒ REPLY DUE within 1 months/days from the above date of mailing
- ☐ NO REPLY DUE, however, see below
- ☐ IMPORTANT COMMUNICATION
- ☐ INFORMATION ONLY

2. COMMUNICATION:

The International Bureau acknowledges receipt of a request for change under PCT Rule 92bis for the applicant and agent.

Where the change of applicant has been duly recorded (see attached PCT Form IB/306), the agent may only be changed upon receipt of a power of attorney signed at least by the deemed common representative.

Please send the required document to the fax number below; upon its receipt, the change will be recorded promptly.

A copy of the present communication is being forwarded to the agent of record and to the receiving Office (RO/EP) for information.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Antonia MULLER (Fax : 338 89 75)
Facsimile No. (41-22) 338.89.75	Telephone No. (41-22) 338.82.43

its successors, legal representatives and assigns, but at the cost and expense of the Assignee, its successors, legal representatives and assigns;

AND the Assignors hereby request 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 use and behalf of the Assignee, its successors, legal representatives, and assigns.

F. HOFFMANN-LA ROCHE LTD

X pp Heiroth
Name: Ulrike Heiroth
Title: Holder of Procurement

X June 1, 2004
Date

X PP Jo Niizuma
Name: Yo Niizuma
Title: Holder of Procurement

X June 1, 2004
Date

X Denise Strebel
Witness Name: X Denise Strebel
Address: X Im Niederholzboden 48
X CH-4125 Riehen

X Beatrice Slevi
Witness Name: X Beatrice Slevi
Address: X Megastrasse 25
X CH-4123 Allschwil

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ASSIGNMENT

For valuable consideration and pursuant to an agreement entitled "Second Amendment to Development Collaboration and Licensing Agreement" dated April 19, 2004;

F. Hoffmann-La Roche Ltd, a Swiss Company having a place of business at Grenzacherstrasse 124, CH-4070 Basel, Switzerland (hereinafter the Assignor);

hereby assigns, sells, transfers and sets over to:

Isotechnika Inc., a corporation of Canada having a place of business at 2100 College Plaza, 8215-112th Street, Edmonton Alberta, Canada T6G 2C8, its successors, legal representatives and assigns (collectively hereinafter called "the Assignee");

Assignor's entire right, title and interest in and to the invention(s) described in European Patent Application 03007921.4 filed 8 April, 2003 entitled **PROCESS FOR THE PREPARATION OF CYCLOSPORIN A ANALOG** *this Assignment including said 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, SPCs 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 Assignors 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,

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THE INTERNATIONAL BUREAU OF WIPO

APPLICANTS : F. HOFFMANN-LA ROCHE AG and
ISOTECHNIKA INC.

FOR : PROCESS FOR PREPARATION OF
CYCLOSPORIN A ANALOG

INTERNATIONAL APPLICATION NO.: PCT/EP2004/003504

INTERNATIONAL FILING DATE : 02 APRIL 2004
(02.04.2004)

INTERNATIONAL PRIORITY DATE : 08 APRIL 2003
(08.04.2003)

ATTORNEY DOCKET NO. : 2650.PCT

CHANGE IN THE PERSON OF THE APPLICANT UNDER 92bis

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
SWITZERLAND

Sir:

Pursuant to an executed Assignment dated 01 JUNE 2004 (01.06.2004) between Applicants F. HOFFMANN-LA ROCHE LTD 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 by removing F. HOFFMANN-LA ROCHE AG as an Applicant in this application so that the application will proceed in the name of sole Applicant ISOTECHNIKA INC.

Respectfully submitted,

X Basel July 23, 04 X Hanny Kjellse-Berger
Date

Hanny Kjellse-Berger
Agent of Record
F. Hoffmann-La Roche Ltd
Grenzacherstrasse 124
CH-4070 Basel
SWITZERLAND
Telephone: (41)-61-688-2074
Fax: (41)-61-688-1395

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this communication is being transmitted, via facsimile 011-41-22-740-1435, to the International Bureau of WIPO, 34, chemin des Colombettes, 1211 Geneva 20, SWITZERLAND, on this 2nd day of August 2004.

Pamela A. Matheis

Pamela A. Matheis

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THE INTERNATIONAL BUREAU OF WIPO

APPLICANTS : F. HOFFMANN-LA ROCHE AG and
ISOTECHNIKA INC.
FOR : PROCESS FOR PREPARATION OF
CYCLOSPORIN A ANALOG
INTERNATIONAL APPLICATION NO.: PCT/EP2004/003504
INTERNATIONAL FILING DATE : 02 APRIL 2004
(02.04.2004)
INTERNATIONAL PRIORITY DATE : 08 APRIL 2003
(08.04.2003)
ATTORNEY DOCKET NO. : 2650.PCT

CHANGE IN THE NAME AND ADDRESS OF THE AGENT UNDER 92bis

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20
SWITZERLAND

Sir:

In accordance with Rule 92bis Section 422, the International Bureau is respectfully
requested to record a change in the name and address of the agent of record to the following:

WILKS, Susan Sutterfield
ISOTECHNIKA INC.
17301 North Perimeter Drive, Suite 100
Scottsdale, Arizona 85255
UNITED STATES OF AMERICA
Telephone No. (480)-505-0540 (ext. 3213)
Facsimile No. (480)-505-0545

Respectfully submitted,

July 28, 2004
Date

Susan Sutterfield Wilks
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

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CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this communication is being transmitted, via facsimile 011-41-22-740-1435, to the International Bureau of WIPO, 34, chemin des Colombettes, 1211 Geneva 20, SWITZERLAND, on this 2nd day of August, 2004.

Pamela A. Matheis
Pamela A. Matheis

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
<i>If none of the following sub-boxes is used, this sheet should not be included in the request.</i>	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)</i> ADAM, Jean-Michel Im Rehnacherhof 187 CH-4153 Reinach, Switzerland	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality: BE	State (that is, country) of residence: CH
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)</i> ABEL, Mark 11530-9A Avenue Edmonton, Alberta T6J 7B2, Canada	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality: CA	State (that is, country) of residence: CA
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)</i> JAYARAMAN, Seetharaman 11812-13 Avenue Edmonton, Alberta T6J 7E9, Canada	This person is: ☐ applicant only ☒ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality: CA	State (that is, country) of residence: CA
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box	
Name and address: <i>(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.)</i>	This person is: ☐ applicant only ☐ applicant and inventor ☐ inventor only <i>(If this check-box is marked, do not fill in below.)</i> Applicant's registration No. with the Office
State (that is, country) of nationality:	State (that is, country) of residence:
This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.	

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) Case 21711

Box No. I TITLE OF INVENTION Process for preparation of cyclosporin A analog	
Box No. II APPLICANT ☐ This person is also inventor	
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. The country of the address indicated in this Box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.) F. Hoffmann-La Roche AG Grenzacherstrasse 124 CH-4070 Basel, Switzerland	
Telephone No. +41 61 688 11 11	
Facsimile No. +41 61 688 13 95	
Teleprinter No.	
Applicant's registration No. with the Office	
State (that is, country) of nationality: CH	State (that is, country) of residence: CH
This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
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. The country of the address indicated in this Box is the applicant's State (that is, country) of residence (if no State of residence is indicated below.) Isotechnika Inc. 2100 College Plaza 8215-112th Street Edmonton, Alberta T6G 2C8, Canada	
This person is: ☒ applicant only ☐ applicant and inventor ☐ inventor only (If this check-box is marked, do not fill in below.)	
Applicant's registration No. with the Office	
State (that is, country) of nationality: CA	State (that is, country) of residence: CA
This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box	
☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.	
Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ 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.) KJELLSAA-BERGER, Hanny Grenzacherstrasse 124 CH-4070 Basel, Switzerland	
Telephone No. +41 61 688 20 74	
Facsimile No. +41 61 688 13 95	
Teleprinter No.	
Agent's registration No. with the Office	
☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.	



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Registered – Case 21711 WO
European Patent Office
Erhardtstrasse 27

D-80298 Munich

Basel, July 8, 2004

International Patent Application No. PCT/EP2004/003504 filed on April 2, 2004
Case 21711 WO

Dear Sirs,

We refer to your communication of June 29, 2004.

Attached please receive replacement sheets No. 1 and 2 of the PCT request indicating the inventor as applicants for the United States of America. At the same time the state of nationality and residence of the inventors/applicants were completed.

Yours sincerely,

F. Hoffmann-La Roche Ltd

F. Klausner


D. Strebel


enc.

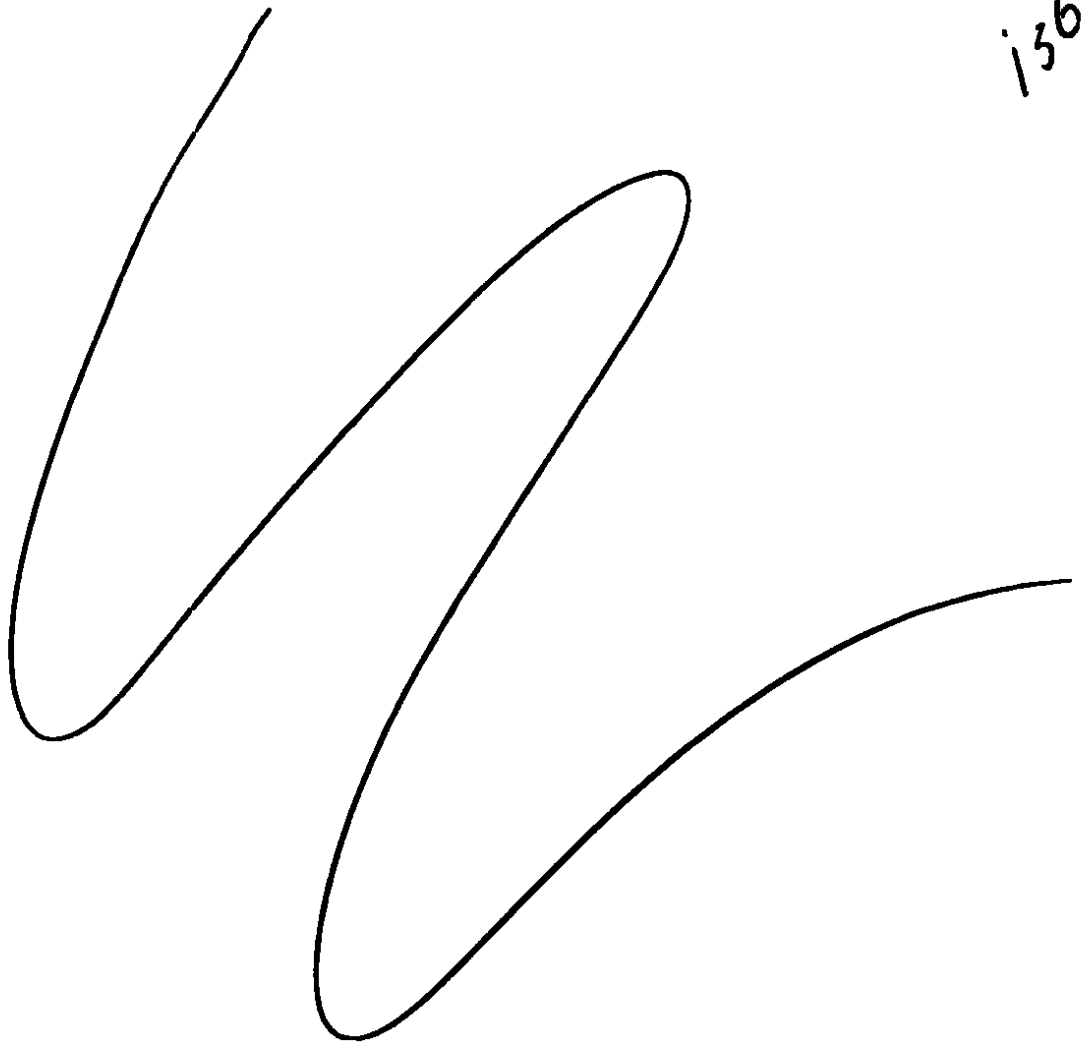
F. Hoffmann-La Roche Ltd

CH-4070 Basel

Patent Department
Bldg. 87B / 4th floor

email:
daniel.strebel@roche.com

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The applicant's attention is drawn to the fact that according to the national law of the United States of America, only the inventor is entitled to file a national application (Article 27(3)). The United States of America is designated in the international application, however,

(i) the named inventor(s) is (are) not also indicated as applicant(s) for the purposes of the designation of the United States of America, and is (are) not indicated as being deceased; or

(ii) no inventor is indicated.

If no inventor is indicated as applicant for the purposes of the designation of the United States of America, the application may be rejected by the United States Patent and Trademark Office as a designated Office."

PATENT COOPERATION TREATY

R 01. Juli 2004
CLP *rel. 7/8.7.04*

PCT

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From the RECEIVING OFFICE

To:

KJELLSA-BERGER, Hanny
Grenzacherstrasse 124
CH-4070 Basle
SUISSE

COMMUNICATION IN CASES FOR WHICH
NO OTHER FORM IS APPLICABLE

Date of mailing (day/month/year) 29.06.2004	
Applicant's or agent's file reference Case 21711	REPLY DUE See paragraph 1 below
International application No. PCT/EP2004/003504	International filing date (day/month/year) 02/04/2004
Applicant F. HOFFMANN-LA ROCHE AG	

1. ☒ REPLY DUE within 1 months/days from the above date of mailing
- ☐ NO REPLY DUE, however see below _____
- ☐ IMPORTANT COMMUNICATION
- ☐ INFORMATION ONLY

2. COMMUNICATION : see attached information

Name and mailing address of the Receiving Office
European Patent Office, P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk
Tel. (+31-70) 340-2040
Fax: (+31-70) 340-3016

Authorized officer

J. de Jong



From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF RECEIPT
OF SEARCH COPY

(PCT Rule 25.1)

To:

KJELLBAA-BERGER, Hanny
124 Grenzacherstrasse
CH-4070 Basle
SWITZERLANDDate of mailing
(day/month/year)

17/06/2004

Applicant's or agent's file reference

Case 21711

IMPORTANT NOTIFICATION

International application No.

PCT/EP2004/003504

International filing date (day/month/year)

02/04/2004

Priority date (day/month/year)

08/04/2003

Applicant

F. HOFFMANN-LA ROCHE AG

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.

11/05/2004 (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 43bis.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.

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

ISA/EP

PATENT COOPERATION TREATY

R 14. Juni 2004
 From the INTERNATIONAL BUREAU

PCT

**NOTIFICATION CONCERNING
 SUBMISSION OR TRANSMITTAL
 OF PRIORITY DOCUMENT**

(PCT Administrative Instructions, Section 411)

To:

KJELLSAA-BERGER, Hanny
 Grenzachstrasse 124
 CH-4070 Basel
 Switzerland

Date of mailing (day/month/year) 10 June 2004 (10.06.2004)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference Case 21711	
International application No. PCT/EP2004/003504	International filing date (day/month/year) 02 April 2004 (02.04.2004)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 08 April 2003 (08.04.2003)
Applicant F. HOFFMANN-LA ROCHE AG et al	

- By means of this Form, which replaces any previously issued notification concerning submission or transmittal of priority documents, the applicant is hereby notified of the date of receipt by the International Bureau of the priority document(s) relating to all earlier application(s) whose priority is claimed. Unless otherwise indicated by the letters "NR", in the right-hand column or by an asterisk appearing next to a date of receipt, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).
- (If applicable) The letters "NR" appearing in the right-hand column denote a priority document which, on the date of mailing of this Form, had not yet been received by the International Bureau under Rule 17.1(a) or (b). Where, under Rule 17.1(a), the priority document must be submitted by the applicant to the receiving Office or the International Bureau, but the applicant fails to submit the priority document within the applicable time limit under that Rule, the attention of the applicant is directed to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.
- (If applicable) An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b) (the priority document was received after the time limit prescribed in Rule 17.1(a) or the request to prepare and transmit the priority document was submitted to the receiving Office after the applicable time limit under Rule 17.1(b)). Even though the priority document was not furnished in compliance with Rule 17.1(a) or (b), the International Bureau will nevertheless transmit a copy of the document to the designated Offices, for their consideration. In case such a copy is not accepted by the designated Office as priority document, Rule 17.1(c) provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
08 April 2003 (08.04.2003)	03007921.4	EP	26 May 2004 (26.05.2004)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. (41-22) 338.89.75	Authorized officer Silvie STENDER Telephone No. (41-22) 338 9928
--	--

PATENT COOPERATION TREATY

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PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF RECEIPT OF
RECORD COPY

(PCT Rule 24.2(a))

To:

KJELLSAA-BERGER, Hanny
Grenzacherstrasse 124
CH-4070 Basel
Switzerland

Date of mailing (day/month/year) 08 June 2004 (08.06.2004)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference Case 21711	International application No. PCT/EP2004/003504

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:

F. HOFFMANN-LA ROCHE AG et al (for all designated States except US)

International filing date : 02 April 2004 (02.04.2004)

Priority date(s) claimed : 08 April 2003 (08.04.2003)

Date of receipt of the record copy
by the International Bureau : 13 May 2004 (13.05.2004)

List of designated Offices :

AP : BW, GH, GM, KE, LS, MW, MZ, 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

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:

☒ time limits for entry into the national phase - see updated important information (as of April 2002)

☒ requirements regarding priority documents (if applicable)

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 338.89.76

Authorized officer:

Mathieu BLANC (Fax 338 89 75)

Telephone No. (41-22) 338 8988

INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/EP2004/003504

Patent document cited in search report	Publication date	Patent family member	Publication date
EP 0296122 A		JP 1045396 A	17-02-1989
		JP 2116212 C	06-12-1996
		JP 8032724 B	29-03-1996
		JP 2772372 B2	02-07-1998
		JP 8048696 A	20-02-1996
		KR 9710927 B1	02-07-1997
		NZ 225041 A	26-03-1991
		PT 87745 A , B	01-07-1988
		SK 411791 A3	11-07-1995
		US 5525590 A	11-06-1996
		ZA 8804345 A	28-02-1990
EP 1291347 A	12-03-2003	DE 10143979 A1	27-03-2003
		EP 1291347 A2	12-03-2003
		JP 2003096083 A	03-04-2003
		US 2003096995 A1	22-05-2003
		US 2004039233 A1	26-02-2004

From the INTERNATIONAL BUREAU

PCT

SECOND AND SUPPLEMENTARY NOTICE
INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION (TO DESIGNATED OFFICES
WHICH APPLY THE 30 MONTH TIME
LIMIT UNDER ARTICLE 22(1))

(PCT Rule 47.1(c))

To:

WILKS, Susan, Sutterfield
Isotechnika Inc.
Suite 100
17301 North Perimeter Drive
Scottsdale, AZ 85255
ETATS-UNIS D'AMERIQUE

DOCKETED

Date of mailing (day/month/year)
11 August 2005 (11.08.2005)

Applicant's or agent's file reference

Case 21711 2650. PCT

IMPORTANT NOTICE

International application No.
PCT/EP2004/003504International filing date (day/month/year)
02 April 2004 (02.04.2004)Priority date (day/month/year)
08 April 2003 (08.04.2003)

Applicant

ISOTECHNIKA INC. et al

1. **ATTENTION:** For any designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002 (30 months from the priority date), does not apply, please see Form PCT/IB/308(First Notice) issued previously.

2. Notice is hereby given that the following designated Office(s), for which the time limit under Article 22(1), as in force from 1 April 2002, does apply, has/have requested that the communication of the international application, as provided for in Article 20, be effected under Rule 93bis.1. The International Bureau has effected that communication on the date indicated below:
21 October 2004 (21.10.2004)

AU, AZ, BY, CN, CO, DZ, EP, HU, KG, KP, KR, MD, MK, MZ, NA, RU, SY, TM, US

In accordance with Rule 47.1(c-bis)(i), those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

3. The following designated Offices, for which the time limit under Article 22(1), as in force from 1 April 2002, does apply, have not requested, as at the time of mailing of the present notice, that the communication of the international application be effected under Rule 93bis.1:

AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BW, BZ, CA, CR, CU, CZ, DE, DK, DM, EA, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, ID, IL, IN, IS, JP, KE, KZ, LC, LK, LR, LS, LT, LV, MA, MG, MN, MW, MX, NI, NO, NZ, OA, OM, PG, PH, PL, PT, RO, SC, SD, SG, SK, SL, TJ, TN, TR, TT, UA, UZ, VC, VN, YU, ZA, ZW

In accordance with Rule 47.1(c-bis)(ii), those Offices accept the present notice as conclusive evidence that the Contracting State for which that Office acts as a designated Office does not require the furnishing, under Article 22, by the applicant of a copy of the international application.

4. **TIME LIMITS** for entry into the national phase

For the designated or elected Office(s) listed above, 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.

In practice, time limits other than the 30-month time limit will continue to apply, for various periods of time, in respect of certain of the designated or elected Office(s) listed above. For regular updates on the applicable time limits (30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at <http://www.wipo.int/pct/en/index.html>.

It is the applicant's sole responsibility to monitor all these time limits.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Ellen Moyse

Facsimile No. +41 22 740 14 35

Facsimile No. +41 22 338 89 75

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de Colombia A
Expediente: _____**

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

SUPERINDUSTRIA Y COMERCIO (COPIA)
Radicacion : 05101444 00000000 Folios: 246
Fecha (AND): 2005-10-06 12:15:09
MIT : 900176089-2 Trámite : 811 PCT-PATENT 378 FASE NDCI 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 05 - 63,845
FECHA : AGOSTO 23 DE 2005

SUPERINDUSTRIA Y COMERCIO
Radicacion : 05101444 00000000 Folios: 246
Fecha (AND): 2005-10-06 12:15:09
Trámite : 002 PATENTES D I REGISTRO/ 411 PRESENTAC
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
MARIO DELGADO ECHEVERRY	CONSIGNACION	BANCO POPULAR	050-00110-6	14464947	23/08/2005	9,894,000.00

***** CONCEPTO *****

CANT. CONTABILITICO	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. _____

Retiro Folio 245 y 246
Tolosa

REQUISITOS NECESARIOS PARA PRESENTACION Y AGILIZACION DEL
TRAMITE

PATENTE DE INVENCION	MODELO DE UTILIDAD	()	()
- Indicación de que se solicita la concesión de una patente.		()	(X)
- Datos de identificación del solicitante o de la persona que se presenta la solicitud.		()	(X)
- Descripción de la invención.		()	(X)
- Dibujos de ser estos pertinentes.		()	(X)
- Comprobante de Pago de las tasas establecidas.			

COMPLETA	(X)	INCOMPLETA	()	()
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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.		(X)	()
- Representación gráfica o fotográfica del Diseño Industrial o muestra del Material que incorpora el diseño.		()	()
- Comprobante de pago de las tasas establecidas		()	()

COMPLETA	()	INCOMPLETA	()	()
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ESQUEMA DE TRAZADO	()		
- Indicación de que solicita el registro de un Esquema de trazado.		()	()
- Datos de identificación del solicitante o de la persona que presenta La solicitud.		()	()
- Representación gráfica del esquema de trazado		()	()
- Comprobante de pago de las tasas establecidas.		()	()

COMPLETA	()	INCOMPLETA	()	()
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Firma Responsable:

Fecha:

248
243

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
DELGADO VILLEGAS JAIME EDUARDO

REFERENCIA	Radicación No.	5 101444
	Solicitud internacional	EP2004/003504
	Fecha inicio fase nacional	08/11/2005
	Trámite	11
	Evento	378
	Actuación	430
	Oficio No.	3602

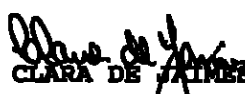
Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se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

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

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

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

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


CLARA DE J. VILLEGAS
Jefe de la División de Nuevas Creaciones (E)

El anterior requerimiento fue notificado por fijación en lista de fecha 23 DIC 2005
adpall