

AO 120 (Rev. 08/10)

TO: Mail Stop 8 Director of the U.S. Patent and Trademark Office P.O. Box 1450 Alexandria, VA 22313-1450	REPORT ON THE FILING OR DETERMINATION OF AN ACTION REGARDING A PATENT OR TRADEMARK
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In Compliance with 35 U.S.C. § 290 and/or 15 U.S.C. § 1116 you are hereby advised that a court action has been
filed in the U.S. District Court Western District of Texas, Waco Division on the following

☐ Trademarks or ☒ Patents. (☐ the patent action involves 35 U.S.C. § 292.):

DOCKET NO. 6:22-cv-00975	DATE FILED 9/16/2022	U.S. DISTRICT COURT Western District of Texas, Waco Division
PLAINTIFF ALD SOCIAL, LLC		DEFENDANT VERKADA, INC.
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1 US 9,198,054	11/24/2015	ALD SOCIAL, LLC
2 US 9,402,158	7/26/2016	ALD SOCIAL, LLC
3		
4		
5		

In the above—entitled case, the following patent(s)/ trademark(s) have been included:

DATE INCLUDED	INCLUDED BY ☐ Amendment ☐ Answer ☐ Cross Bill ☐ Other Pleading	
PATENT OR TRADEMARK NO.	DATE OF PATENT OR TRADEMARK	HOLDER OF PATENT OR TRADEMARK
1		
2		
3		
4		
5		

In the above—entitled case, the following decision has been rendered or judgement issued:

DECISION/JUDGEMENT

CLERK	(BY) DEPUTY CLERK	DATE
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Copy 1—Upon initiation of action, mail this copy to Director Copy 3—Upon termination of action, mail this copy to Director
Copy 2—Upon filing document adding patent(s), mail this copy to Director Copy 4—Case file copy

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 10,849,919 B2
APPLICATION NO. : 16/199119
DATED : December 1, 2020
INVENTOR(S) : Fernando Dangond

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification

Column 1, Line 9:

“Nov. 24, 2018,”

Should read:

--Nov. 24, 2017,--; and

In the Claims

Column 65, Line 5, Claim 6:

“according to claim 4”

Should read:

--according to claim 5--.

Signed and Sealed this
Twenty-fifth Day of October, 2022


Katherine Kelly Vidal
Director of the United States Patent and Trademark Office



ELECTRONIC PAYMENT RECEIPT

APPLICATION #
16/199,119

RECEIPT DATE / TIME
09/21/2022 04:59:53 PM ET

ATTORNEY DOCKET #
000696US

Title of Invention

Cladribine regimen for treating progressive forms of Multiple Sclerosis

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # 10849919

CONFIRMATION # 8647

FILED BY Ashley Connelly

PATENT CENTER # 60990286

AUTHORIZED BY Eric Myers

CUSTOMER # 151167

FILING DATE 11/23/2018

CORRESPONDENCE
ADDRESS -

FIRST NAMED
INVENTOR Fernando Dangond

Payment Information

PAYMENT METHOD
CARD / 6425

PAYMENT TRANSACTION ID
E20229KH00511159

PAYMENT AUTHORIZED BY
Ashley Connelly

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
1811	CERTIFICATE OF CORRECTION	160.00	1	160.00
TOTAL AMOUNT:				\$160.00

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

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APPLICATION #
16/199,119

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FILING DATE 11/23/2018

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FIRST NAMED
INVENTOR Fernando Dangond

CORRESPONDENCE
ADDRESS -

AUTHORIZED BY Eric Myers

Documents

TOTAL DOCUMENTS: 2

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2022-09-21-Request-for-COC-as-filed.pdf	4	-	576 KB
2022-09-21-Request-for-COC-as-filed-TRAN.LET.pdf	(1-2) 2	Transmittal Letter	282 KB
2022-09-21-Request-for-COC-as-filed-COCIN.pdf	(3-4) 2	Request for Certificate of Correction	282 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
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2022-09-21-Request-for-COC-as-filed.pdf	542F21D2139C082B920EC04AC244C9CDB5BBFA6FD94FCB0B3C241F319490153BA04294A05295B8B79A1E4A085110AF4B804C0A00E0DE77BD2D38880F43EFD0B2
2022-09-21-Request-for-COC-as-filed-TRAN.LET.pdf	D4EBD3BA9E1767D919F6B0895767A09821A881A20DA1428DA8F564AE94119597841C687F40DCC292AC711890C34775F102ECA90F84E1E7ACE031CFFC63B6DAEE
2022-09-21-Request-for-COC-as-filed-COCIN.pdf	B059221BAB996653F3B91295C27879A1B866CFB2AF05229F85703C395834EA9C6A7C7A091963851235F983B5750B74F460C9661CDAA24424E2D7234F7FDE3683

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Docket No. 000696US

GRÜNEBERG
MYERS
P L L C

ATTORNEYS AT LAW

Application of Fernando Dangond

Serial No.: 16/199,119

Filed on: November 23, 2018

Examiner: ULM, JOHN D.

Group Art Unit: 1649

For: Cladribine regimen for treating progressive forms of Multiple Sclerosis

Commissioner for Patents
Alexandria, Virginia 22313

Commissioner:

The following papers are attached herewith:

**FEE TRANSMITTAL;
REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 CFR § 1.322;
CERTIFICATE OF CORRECTION (FORM PTO/SB/44)**

Please charge additional fee(s) or underpayment of fee(s) to Deposit Account No. 601920 under 37 CFR 1.16 and 1.17, and please credit any overpayment of fee(s) to Deposit Account No. 601920. If these papers are not considered timely, then Applicants hereby petition under 37 CFR 1.136 for any necessary extension of time, further authorizing any necessary extension of time fees to be charged to Deposit Account No. 601920.

Respectfully Submitted,
GRÜNEBERG AND MYERS PLLC

/Kirsten Grueneberg/

Customer Number

151167

Phone: (571) 458-7790

Fax: (571) 458-7789

Dr. Kirsten Grueneberg
Attorney of Record
Registration No. 47,297

Eric Myers
Registration No. 68,546

FEE TRANSMITTAL	Application Number	16/199,119
	Filing Date	November 23, 2018
	First Named Inventor	Fernando Dangond
	Art Unit	1649
	Examiner Name	ULM, JOHN D.
	Attorney Docket No.	000696US
☐ No fee required	Title	Cladribine regimen for treating progressive forms of Multiple Sclerosis
☒ Total payment \$160.00		

- ☐ Applicant asserts small entity status, 37 CFR 1.27
- ☐ Applicant asserts micro entity status, 37 CFR 1.29 (Form PTO/SB/15 or equivalent enclosed or already submitted)
- ☐ Track 1 Prioritized Examination

Claims Fees:

Total: (- 20) × \$100 \$

Independent: (- 3) × \$480 \$

☐ Multiple dependency \$860 \$

☐ Late filing declaration \$160 \$

☐ Non-electronic filing fee \$400 \$

☐ Non-English translation \$140 \$

☐ Terminal Disclaimer \$ 170 \$

☐ RCE – 1st Request \$1360 \$

☐ RCE – 2nd or Subseq. \$2000 \$

☐ Notice of Appeal \$ 840 \$

☐ Appl'n Size (pp.-100)/50 ×\$ 420 \$

Extension Fees under 37 CFR 1.136(a) and 1.17(a), see petition filed herewith, if applicable:

☐ Within first month \$ 220 \$

☐ Within second month \$ 640 \$

☐ Within third month \$1480 \$

☐ Within fourth month \$2320 \$

☐ Within fifth month \$3160 \$

Other:

☒ Request for Certificate of Correction \$160.00

Payment in the amount of **\$160.00** paid by:

- ☒ Credit Card (online if electronically filed, or attached if paper filed)
- ☐ Deposit Account No. **601920**

- ☒ Please charge additional fee(s) or underpayment of fee(s) to Deposit Account No. **601920** under 37 CFR 1.16 and 1.17, and please credit any overpayment of fee(s) to Deposit Account No. **601920**.

- ☒ If these papers are not considered timely, then Applicants hereby petition under 37 CFR 1.136 for any necessary extension of time, further authorizing any necessary extension of time fees to be charged to Deposit Account No. **601920**.

Respectfully Submitted,
GRÜNEBERG AND MYERS PLLC

/Kirsten Grueneberg/

Customer Number

151167

Phone: (571) 458-7790

Fax: (571) 458-7789

Dr. Kirsten Grueneberg
Registration No. 47,297

Eric Myers
Registration No. 68,546

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	16/199,119
Filing Date	November 23, 2018
First Named Inventor	Fernando Dangond
Art Unit	1649
Examiner Name	ULM, JOHN D.
Attorney Docket No.	000696US
Title	Cladribine regimen for treating progressive forms of Multiple Sclerosis

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. § 1.322

ATTN: Certificate of Corrections Branch
Commissioner For Patents
P.O. Box 1450
Alexandria, VA 22313

Commissioner:

The undersigned requests the issuance of a Certificate of Correction in the above-identified patent to correct typographical errors in the Related U.S. Application Data and in Claim 6. Accompanying this request is a completed Certificate of Correction form (PTO/SB/44).

Applicant submits that the request for this Certificate of Correction does not raise issues of new matter.

Applicant submits that the typographical error in the Related U.S. Application Data is clearly disclosed in the records of the Office, notably the Application Data Sheet filed November 23, 2018.

Applicant submits that the support for the correction to Claim 6 is found in the claims and specification as originally filed.

The required fee of \$160.00 is enclosed.

Grüneberg and Myers, PLLC

/Kirsten Grueneberg/

Customer No. 151167
Telephone No. 571 458 7790
Facsimile No. 571 458 7789

Name	Dr. Kirsten Grueneberg
Reg. No.	47,297
Name	Eric Myers
Reg. No.	68,546

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.
(Also Form PTO-1050)**UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION**Page 1 of 1

PATENT NO. : 10,849,919
 APPLICATION NO.: 16/199,119
 ISSUE DATE : December 1, 2020
 INVENTOR(S) : Fernando Dangond

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1, Line 9, "Nov. 24, 2018,"

should read

--Nov. 24, 2017,--; and

Column 65, Line 5, Claim 6., "according to claim 4"

should read

--according to claim 5--.

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):

1775 Tysons Blvd, 5th Floor
 Tysons, VA 22102

This collection of information is required by 37 CFR 1.322, 1.323, and 1.324. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1.0 hour to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Attention Certificate of Corrections Branch, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/199,119	12/01/2020	10849919	000696US	8647

151167 759 11/11/2020
Gruneberg and Myers PLLC
1775 Tysons Blvd
5th Floor
Tysons, VA 22102

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 0 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Fernando Dangond, Weston, MA;
Merck Patent GmbH, Darmstadt, GERMANY;
Matthias Dotzauer, Erzhausen, GERMANY;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the **ISSUE FEE** and **PUBLICATION FEE** (if required). Blocks I through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block I, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block I for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

151167 7590 07/23/2020
Gruneberg and Myers PLLC
1775 Tysons Blvd
5th Floor
Tysons, VA 22102

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/199,119	11/23/2018	Fernando Dangond	000696US	8647

TITLE OF INVENTION: Cladribine regimen for treating progressive forms of Multiple Sclerosis

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	10/23/2020

EXAMINER	ART UNIT	CLASS-SUBCLASS
ULM, JOHN D	1649	424-085600

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

Grüneberg and Myers PLLC

1 _____

2 _____

3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Merck Patent GmbH

Darmstadt, GERMANY

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☒ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☒ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. **601920**

5. **Change in Entity Status** (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature /Eric Myers/

Date October 23, 2020

Typed or printed name Eric Myers

Registration No. 68,546



ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
16/199,119

RECEIPT DATE / TIME
10/23/2020 07:29:06 AM ET

ATTORNEY DOCKET #
000696US

Title of Invention

Cladribine regimen for treating progressive forms of Multiple Sclerosis

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under 35 USC 111(a)

PATENT # -

CONFIRMATION # 8647

FILED BY Malika Ash Shakur

PATENT CENTER # 60099402

FILING DATE 11/23/2018

CUSTOMER # 151167

FIRST NAMED
INVENTOR Fernando Dangond

CORRESPONDENCE
ADDRESS -

AUTHORIZED BY Eric Myers

Documents

TOTAL DOCUMENTS: 1

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
2020-10-23-Issue-Fee-as-filed.pdf	1	Issue Fee Payment (PTO-85B)	126 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
2020-10-23-Issue-Fee-as-filed.pdf	85A058100FB319223BE0C07245D97BEFE4C558DAA9FC28314 8C8EA0E3DD031A3140F3892D636AF5506EA72879825D6BFE2 821EAB04CFC4D91A9BFC1F4C7E6DBC

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ELECTRONIC PAYMENT RECEIPT

APPLICATION #
16/199,119

RECEIPT DATE / TIME
10/23/2020 07:29:06 AM ET

ATTORNEY DOCKET #
000696US

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FILED BY Malika Ash Shakur

PATENT CENTER # 60099402

AUTHORIZED BY Eric Myers

CUSTOMER # 151167

FILING DATE 11/23/2018

CORRESPONDENCE
ADDRESS -

FIRST NAMED
INVENTOR Fernando Dangond

Payment Information

PAYMENT METHOD
DA / 601920

PAYMENT TRANSACTION ID
E20200M830044227

PAYMENT AUTHORIZED BY
Malika Ash Shakur

FEE CODE	DESCRIPTION	ITEM PRICE(\$)	QUANTITY	ITEM TOTAL(\$)
1501	UTILITY ISSUE FEE	1000.00	1	1000.00
TOTAL AMOUNT:				\$1,000.00

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UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

151167 759 07/23/2020
Gruneberg and Myers PLLC
1775 Tysons Blvd
5th Floor
Tysons, VA 22102

EXAMINER

ULM, JOHN D

ART UNIT

PAPER NUMBER

1649

DATE MAILED: 07/23/2020

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/199,119	11/23/2018	Fernando Dangond	000696US	8647

TITLE OF INVENTION: Cladribine regimen for treating progressive forms of Multiple Sclerosis

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	10/23/2020

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

151167 759 07/23/2020
Gruneberg and Myers PLLC
1775 Tysons Blvd
5th Floor
Tysons, VA 22102

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/199,119	11/23/2018	Fernando Dangond	000696US	8647

TITLE OF INVENTION: Cladribine regimen for treating progressive forms of Multiple Sclerosis

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	10/23/2020

EXAMINER	ART UNIT	CLASS-SUBCLASS
ULM, JOHN D	1649	424-085600

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. Use of a Customer Number is required.

2. For printing on the patent front page, list

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,

(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☐ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29

☐ Applicant asserting small entity status. See 37 CFR 1.27

☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/199,119	11/23/2018	Fernando Dangond	000696US	8647
151167 7590 07/23/2020 Gruneberg and Myers PLLC 1775 Tysons Blvd 5th Floor Tysons, VA 22102			EXAMINER ULM, JOHN D	
			ART UNIT 1649	PAPER NUMBER
DATE MAILED: 07/23/2020				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	Application No. 16/199,119	Applicant(s) Dangond et al.	
	Examiner JOHN D ULM	Art Unit 1649	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to Applicant's amendment filed 07 July, 2020.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are See Continuation Sheet. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov**.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☐ All b) ☐ Some *c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. ____.

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: ____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date ____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)	5. ☒ Examiner's Amendment/Comment
2. ☒ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date <u>07/07/2020</u> .	6. ☐ Examiner's Statement of Reasons for Allowance
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material ____.	7. ☐ Other ____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. ____.	

/JOHN D ULM/ Primary Examiner, Art Unit 1649	
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Continuation of 3. The allowed claim(s) is/are: 1,3-4,16,19,21-22,26-27,29-31,33-37,40-42,48-50,52-54,56-59,64-65 and 67-68

DETAILED ACTION

1) Claims 1, 3, 4, 16, 19, 21, 22, 26, 27, 29 to 31, 33 to 37, 40 to 42, 48 to 50, 52 to 54, 56 to 59, 64, 65, 67 and 68 are pending in the instant application. Claims 1, 34, 49, 56 and 65 have been amended as requested by Applicant in the correspondence filed 07 July of 2020.

2) Any objection or rejection of record that is not expressly repeated in this action has been overcome by Applicant's response and withdrawn.

3) The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Notice of Pre-AIA or AIA Status

4) The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

Information Disclosure Statement

5) The information disclosure statement (IDS) submitted on 07 July, 2020 is in compliance with the provisions of 37 CFR 1.97 and has been considered by the examiner.

Allowable Subject Matter

6) Claims 1, 3, 4, 16, 19, 21, 22, 26, 27, 29 to 31, 33 to 37, 40 to 42, 48 to 50, 52 to 54, 56 to 59, 64, 65, 67 and 68 are allowed and have been renumbered 1 to 34, respectively.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to JOHN D ULM whose telephone number is (571)272-0880. The examiner can normally be reached on 8:00 to 4:30, Monday through Friday.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffery Stucker can be reached on (571) 272-0911. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Application/Control Number: 16/199,119
Art Unit: 1649

Page 4

/JOHN D ULM/
Primary Examiner, Art Unit 1649

Issue Classification 	Application/Control No. 16/199,119	Applicant(s)/Patent Under Reexamination Dangond et al.
	Examiner JOHN D ULM	Art Unit 1649

CPC						
Symbol					Type	Version
A61K	/	31	/	7076	F	2013-01-01
A61K	/	9	/	205	I	2013-01-01
A61K	/	9	/	20	I	2013-01-01
A61P	/	37	/	02	I	2018-01-01
A61P	/	25	/	00	I	2018-01-01
A61K	/	9	/	2013	I	2013-01-01
A61K	/	9	/	2018	I	2013-01-01
A61K	/	38	/	215	I	2013-01-01

CPC Combination Sets				
Symbol	Type	Set	Ranking	Version

NONE (Assistant Examiner) (Date)		Total Claims Allowed: 34	
/JOHN D ULM/ Primary Examiner, Art Unit 1649 (Primary Examiner) (Date)		17 July 2020 (Date)	O.G. Print Claim(s) 1
			O.G. Print Figure None

Issue Classification 	Application/Control No. 16/199,119	Applicant(s)/Patent Under Reexamination Dangond et al.
	Examiner JOHN D ULM	Art Unit 1649

INTERNATIONAL CLASSIFICATION			
CLAIMED			
A61P	/	37	/ 02
NON-CLAIMED			
/		/	

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS
424	85.6

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					

NONE (Assistant Examiner) (Date)		Total Claims Allowed: 34	
/JOHN D ULM/ Primary Examiner, Art Unit 1649 (Primary Examiner) (Date)		17 July 2020 (Date)	O.G. Print Claim(s) 1
			O.G. Print Figure None

Issue Classification 	Application/Control No. 16/199,119	Applicant(s)/Patent Under Reexamination Dangond et al.
	Examiner JOHN D ULM	Art Unit 1649

☒ Claims renumbered in the same order as presented by applicant	☐ CPA	☐ T.D.	☐ R.1.47
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CLAIMS															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE (Assistant Examiner) _____ (Date) _____		Total Claims Allowed: 34	
/JOHN D ULM/ Primary Examiner, Art Unit 1649 (Primary Examiner) _____ (Date) 17 July 2020		O.G. Print Claim(s) 1	O.G. Print Figure None

<i>Search Notes</i> 	Application/Control No. 16/199,119	Applicant(s)/Patent Under Reexamination Dangond et al.
	Examiner JOHN D ULM	Art Unit 1649

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Searched Inventor Names in PALM & NPL; Searched GOOGLE, Medline & WEST for: "cladribine", "mavenclad", "leustatin", "progressive multiple sclerosis", "interferon beta", peginterferon beta"	04/09/2020	JDU
Updated, Searched GOOGLE & WEST for: "Cladribine", "sclerosis", "ESPMS"	07/17/2020	JDU

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
	WEST Searches Included Issued Patents & Published Applications	07/17/2020	JDU

/JOHN D ULM/ Primary Examiner, Art Unit 1649	
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L1: Records 1 through 1 of 1- Filtered by Family ID (1 Families)

Sorted by: Rec #

Sort is done only on each individual page

<< First	< Prev		Go	Next >	Last >>	Select All	Generate Collection
Generate L#	Download Selected	Default Sort	Settings	Help	Close Window		

	Rec #	Sel	U	Doc ID	DBNM	Pub Date	Title
	1	☐	☒	US 20190167707 A1	PGPB	20190606	Cladribine regimen for treating progress Sclerosis

<< First	< Prev		Go	Next >	Last >>	Select All	Generate Collection
Generate L#	Download Selected	Default Sort	Settings	Help	Close Window		

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		US Appln. No.	16/199,119		
		Filing Date	November 23, 2018		
		First Named Inventor	Fernando Dangond		
		Art Unit	1649		
		Examiner Name	ULM, JOHN D.		
		FOR: Cladribine regimen for treating progressive forms of Multiple Sclerosis			
US Patent Documents					
Examiner Initials	Cite No.	Document Number	Date	Name	
/J.D.U/	A1	5,208,327	May 4, 1993	Robert H. K. Chen	
/J.D.U/	A2	5,506,214	April 9, 1996	Ernest Beutler	
/J.D.U/	A3	6,194,395	February 27, 2001	Schultz et al.	
Foreign Patent Documents					
Examiner Initials	Cite No.	Document Number	Date	Country	Translation
/J.D.U/	A4	0 173 059	March 5, 1986	Europe	
/J.D.U/	A5	0 626 853	April 26, 2000	Europe	
/J.D.U/	A6	96/19229	June 27, 1996	WIPO	
/J.D.U/	A7	96/19230	June 27, 1996	WIPO	
/J.D.U/	A8	00/64918	November 2, 2000	WIPO	
/J.D.U/	A9	2004/028462	April 8, 2004	WIPO	
/J.D.U/	A10	2004/087100	October 14, 2004	WIPO	
/J.D.U/	A11	2004/087101	October 14, 2004	WIPO	
/J.D.U/	A12	2006/067141	June 29, 2006	WIPO	
Non-Patent Literature (including Author, Title, Date, etc.)					
Examiner Initials	Cite No.	Document			
/J.D.U/	A13	Beutler et al., Acta Haematol; 1994, 91:10-15			
/J.D.U/	A14	Beutler et al., Proc. Natl. Acad. Sci.; 1996, 93:1716-1720			
/J.D.U/	A15	Beutler et al., Seminars in Hematology; 1996, 33(1):45-52			
/J.D.U/	A16	Evans et al., Annals of Neurology, 1997; 41(1):125-132			
/J.D.U/	A17	Giovannoni et al., The New England Journal of Medicine; 2010, 362(5):416-426			
/J.D.U/	A18	Grieb et al., Archivum Immunologiae et Therapiae Experimentalis; 1995, 43:323-327			
/J.D.U/	A19	Janiec et al., Clinical Research, Med Sci Monit; 2001, 7(1):93-98			
/J.D.U/	A20	Kazimierzczuk et al., J. Am. Chem. Soc.; 1984, 106:6379-6382			
/J.D.U/	A21	Kurtzke, John F., Neurology; 1983, 33:1444-1452			
/J.D.U/	A22	Lassmann et al., Trends in Molecular Medicine; 2001, 7(3):115-121			
/J.D.U/	A23	Lublin et al., Neurology; 1996, 46:907-911			
/J.D.U/	A24	Lucchinetti et al., Current Opinion in Neurology; 2001, 14:259-269			
/J.D.U/	A25	Mattson, David H., Expert Rev. Neurotherapeutics; 2002, 2(3):319-327			
Examiner	/JOHN D ULM/	Date Considered	07/19/2020		
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.					

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)		US Appln. No.	16/199,119
		Filing Date	November 23, 2018
		First Named Inventor	Fernando Dangond
		Art Unit	1649
		Examiner Name	ULM, JOHN D.
		FOR: Cladribine regimen for treating progressive forms of Multiple Sclerosis	
Non-Patent Literature (including Author, Title, Date, etc.)			
Examiner Initials	Cite No.	Document	
/J.D.U/	A26	McDonald et al., Ann Neurol; 2001, 50:121-127	
/J.D.U/	A27	Miller et al., Neurology; 1996, 47(4):S217	
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Examiner	/JOHN D ULM/	Date Considered	07/19/2020
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Prior Art Searches

DB=PGPB,USPT; PLUR=YES; OP=ADJ

Date	<u>L1</u>	Cladribine same ESPMS	1	<u>L1</u>	<u>L1</u>	<u>L1</u>
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END OF SEARCH HISTORY

Bibliographic Data

Application No: 16/199,119

Foreign Priority claimed: ☐ Yes ☒ No

35 USC 119 (a-d) conditions met: ☐ Yes ☐ No ☐ Met After Allowance

Verified and Acknowledged:

/JOHN D ULM/

Examiner's Signature

Initials

Title:

Cladribine regimen for treating progressive forms of Multiple Sclerosis

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/23/2018	424	1649	000696US
RULE			

APPLICANTS

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Matthias Dotzauer, Erzhausen, GERMANY

CONTINUING DATA

This application has PRO of 62590442 11/24/2017

FOREIGN APPLICATIONS

IF REQUIRED, FOREIGN LICENSE GRANTED**

12/17/2018

STATE OR COUNTRY

UNITED STATES

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UNITED STATES

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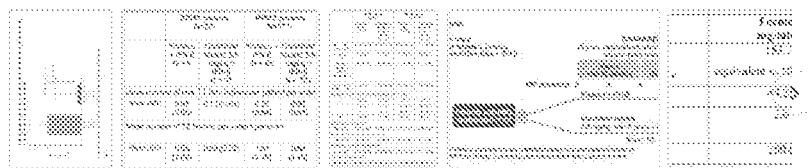
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		Filing Date	November 23, 2018		
		First Named Inventor	Fernando Dangond		
		Art Unit	1649		
		Examiner Name	ULM, JOHN D.		
		FOR: Cladribine regimen for treating progressive forms of Multiple Sclerosis			
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Title of Invention

Cladribine regimen for treating progressive forms of Multiple Sclerosis

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

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FILED BY W. Michael Buck

PATENT CENTER # 60049787

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CUSTOMER # 151167

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INVENTOR Fernando Dangond

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AUTHORIZED BY Eric Myers

Documents

TOTAL DOCUMENTS: 41

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US99/09158 (22) International Filing Date: 28 April 1999 (28.04.99) (71) Applicant (for all designated States except US): STERRENBELD BIOTECHNOLOGIE NORTH AMERICA, INC. [US/US]; 1209 Orange Street, Wilmington, DE 19801 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): GERSZBERG, Szepsel [AR/AR]; Apartment 5-9, R. Scalabrini Ortiz 254, Buenos Aires 1414 (AR). ALONSO, Daniel [AR/AR]; Gualeguaychu 1443, Buenos Aires 1407 (AR). (74) Agents: GOLDSTEIN, Jorge, A. et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Suite 600, 1100 New York Avenue, N.W., Washington, DC 20005-3934 (US).		(81) Designated States: BR, CA, US. Published <i>With international search report.</i> <i>With a statement concerning non-prejudicial disclosure or exception to lack of novelty.</i>
(54) Title: METHOD FOR THE PRODUCTION OF 2-CHLORO-2'-DEOXYADENOSINE (CLADRIBINE) AND ITS 3,5-DI-O-p-TOLUOYL DERIVATIVE (57) Abstract A process for the production of cladribine, 2-chloro-2'-deoxyadenosine, is provided which involves the direct glycosylation of 2-chloro-6-aminopurine with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-erythropentofuranose. The process is carried out by first forming the sodium salt of 2-chloro-6-aminopurine and allowing the sodium salt to react with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-erythropentofuranose in the presence of a moderately polar solvent such as acetone. The final product, cladribine, is produced by removal of the p-toluoyl groups by the action of methanolic ammonia or methanolic sodium methoxide.		

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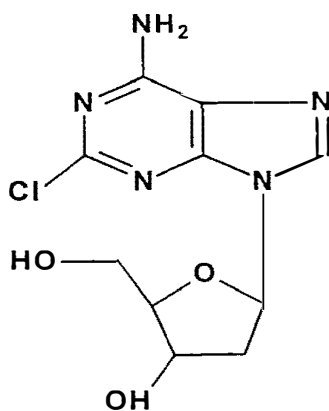
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Method for the Production of 2-Chloro-2'-Deoxyadenosine (Cladribine) and its 3,5-di-O-*p*-Toluoyl Derivative

Background of the Invention

Technical Field

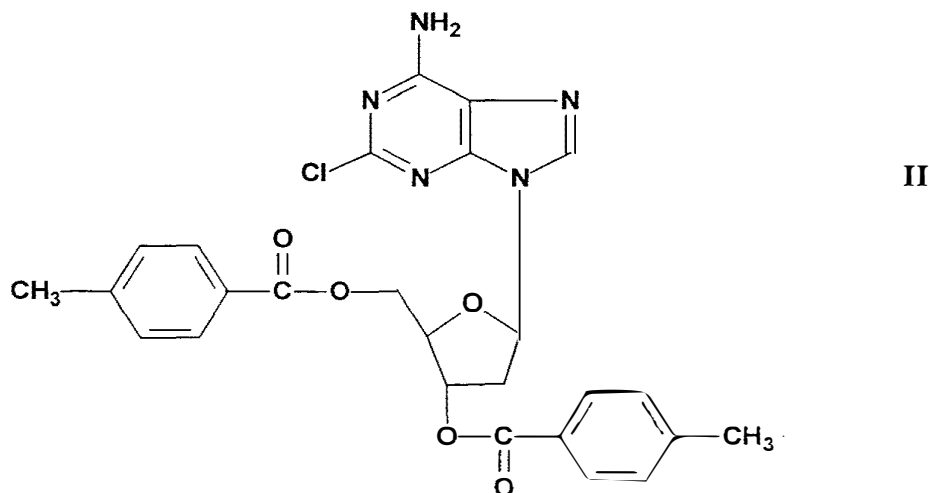
The present invention relates generally to a method of producing 2-chloro-2'-deoxyadenosine (2-CdA; cladribine, I).



I

More particularly, the invention relates to a method of producing 2-CdA by the glycosylation of the sodium salt of 2-chloro-6-aminopurine with 1-chloro-2-deoxy-3,5-di-O-*p*-toluoyl- α -D-erythropentofuranose (II), followed by isolation of the 3,5-di-O-*p*-toluoyl derivative and removal of the *p*-toluoyl protecting groups.

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Related Art

Cladribine is useful as an antileukemic agent, i.e., in the treatment of leukemia, such as hairy cell leukemia. 2-CdA is also known to have immunosuppressive activity. Many processes for preparing 2-CdA and similar compounds are known which involve the direct condensation or coupling of a heterocycle (e.g., purine, pyrimidine, imidazole) with an activated, conveniently protected sugar at C-1.

The first synthesis of 2-CdA was described by Ikehara and Tada as an intermediate in the preparation of 2'-deoxydenosine. (Ikehara, M. *et al*, *J. Am. Chem. Soc.* 12(17):2344-2345 (1963); *Id.* 87(3):606-610 (1965)). The starting material (2,8-dichloroadenine), the use of the chloromercury salt of the purine base in the condensation step, the sugar employed (2-*O*-acetyl-3-*O*-tosyl-5-*O*-methoxycarbonyl-D-xylofuranosylchloride), as well as the deoxygenation process at C-2' of this method, are different from all of the methods hereinafter described.

A second synthesis was published by Christensen *et al.* based on the fusion coupling of 2,6-dichloropurine with 1,3,5-tri-*O*-acetyl-2-deoxy-D-erythropentofuranose resulting in an anomeric mixture of the acetyl-protected deoxynucleosides. (Christensen, L.F. *et al.*, *J. Med. Chem.* 15(7):735-739 (1972). When the resulting mixture of this coupling was treated with liquid ammonia, the

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clean ammonolysis of the chlorine atom at C-6 and deacetylation took place and 2-chloro-2'-deoxy-9- α -adenosine and 2-chloro-9- β -adenosine were obtained. This mixture was then reacylated with *p*-toluoyl chloride and the new blocked mixture was finally resolved by chromatography. Removal of the *p*-toluoyl groups with methanolic sodium methoxide at room temperature resulted in a mixture of 2-chloro-2'-deoxyadenosine and its α -anomer with a total yield of 16% and 9%, respectively. Fusion of 2,6-dichloropurine with methyl 3,5-di-*O-p*-toluoyl-2-deoxy-D-erythropentofuranoside gave a mixture of blocked deoxyribonucleosides which was separated by silica gel chromatography. Treatment of each anomer with liquid ammonia was used as an alternative route and deprotection of the toluoyl groups and simultaneous substitution at C-6 took place resulting in 2-chloro-2'-deoxyadenosine (I) with a total yield of 8%, while the yield for the α -anomer was 13%.

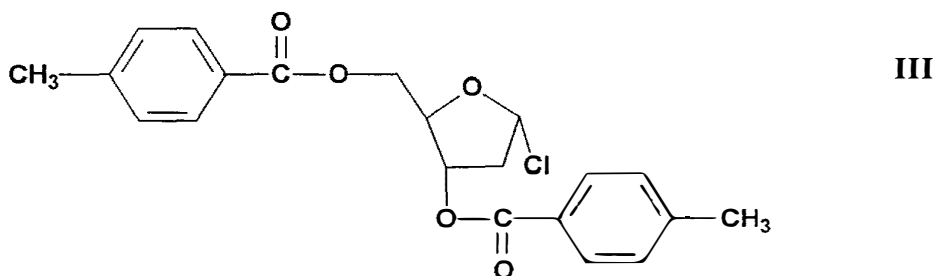
The synthesis of biologically active 9- β -purine-2-deoxyribonucleosides commonly involves direct glycosylation of the heterocycle with an activated 2-deoxyribose derivative. Glycosylation procedures introducing the 2-deoxy- β -D-ribofuranosyl moiety into an aglycon invariably provide anomeric mixtures as well as positional isomers. Not only is the yield of the desired product low in such reactions, but purification is often difficult due to the similar mobility of isomeric products during chromatographic separation. Such a process is both time consuming and costly.

The third synthesis of 2-CdA was postulated by Kazimierczuk *et al.* and Robbins *et al.* and minimizes the intrinsic difficulties caused by methods involving direct glycosylation. (Kazimierczuk, Z. *et al.*, *J. Am. Chem. Soc.* 106(21):6379-6382 (1984); Robbins, R.K. *et al.*, Eur. Patent No. 173059 (1985)). This coupling method comprises the use of chloroheterocyclic derivatives and a protected 1- α -chloro-2-deoxyribose. This method provides a clear improvement in reaction selectivity.

The respective sodium salts of the bases to be glycosylated were prepared *in situ* using sodium hydride in acetonitrile. This solution was made to react in

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the same medium and at room temperature with 1-chloro-2-deoxy-3,5-di-*p*-*O*-toluoyl- α -D-erythropentofuranose (III), having the formula



The α -configuration of the starting sugar in the solid state facilitates the formation of the blocked 2-deoxy- β -nucleosides during the anionic attack of the heterocyclic nitrogen at the C-1 site. The formation of the β -anomer is attributed to a direct Walden inversion (S_N2). This sodium salt glycosylation procedure is considerably superior to those reported, including silyl derivatives, molecular sieves and phase transfer catalysis.

In contrast to ribofuranose synthons which possess an acyloxy substituent at C-2 that directs base attack on the β face of the sugar, glycosylation with 2-deoxyribofuranoses may result in the formation of α and β anomeric products, due to the diminished steric control exhibited by deoxyribofuranoses. In fact, the non-desired N-7 coupling product evidentiates the loss of regioselectivity resulting from the ambidentate character of the anion. Hildebrand *et al.* described for this particular case the formation of 9- β - (50 %), 7- β - (15%) and 9- α - (1.5%) nucleosides, while Kazimierczuk *et al.* reported a 59% and 13% yield for the first two nucleosides (9- β - and 7- β -), respectively.

Purification by column chromatography is often difficult due to the quite similar mobility of these products which lowers the yield and makes the isolation of pure fractions difficult. Pure 2,6-dichloro-9-(2'-deoxy-3,5-di-*O*-*p*-toluoyl- β -D-erythropentafuranosyl) purine was ammonolysed with methanolic ammonia at 100 °C giving 2-chloro-2'-deoxyadenosine (I) which must be purified by a silica gel column. The total yield of isomer 9- β was 42%.

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A fourth process for preparing 2-CdA was described by Chen. (Chen, R.H.K., U.S. Patent No. 5,208,327). This process differs from the previous ones by employing guanosine, whose β -glycoside bond is between C-1 of the ribose and N-9 of the guanine moiety, as a starting material. The main disadvantages of this method are the low total yield (2.75 %) and the complexity of the method which involves eight steps with four chromatographic purification steps of certain intermediates and an additional chromatographic step to obtain the final product, cladribine.

A fifth preparation method is an enzymatic synthesis achieved by direct transfer of the 2-deoxyribofuranose moiety from 2'-deoxythymidine to 2-chloroadenine catalyzed by purified trans-N-deoxyribosylase obtained from α *E. Coli* BMT-1D/1A strain. (Mikhailopulo, I.A. *et al.*, *Nucleosides & Nucleotides* 12(3-4):417-422 (1993)). This method is highly stereospecific, but the reaction scale is limited to the production of less than 1 millimole of 2-CdA.

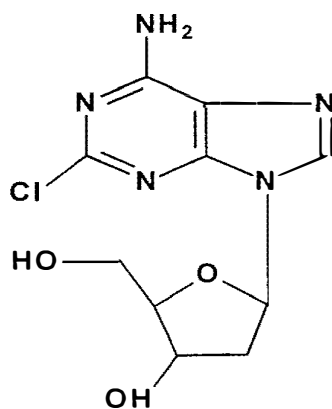
Accordingly, the above-discussed prior art describes the preparation of 2-chloro-2'-deoxyadenosine based on the direct glycosylation of 2,6 dichloropurines and further dehalogenation with liquid ammonia or methanolic ammonia. Because of the above-described disadvantages of this method, there is a need for the synthesis of 2-chloro-2'-deoxyadenosine by means of direct glycosylation of 2-substituted adenines.

Summary of the Invention

The present invention relates to the synthesis of 2-chloro-2'-deoxyadenosine by direct glycosylation of 2-chloro-6-aminopurine. The principal advantage of this method is that the intermediate and final products obtained do not require column chromatography purification, facilitating the synthesis of pure cladribine. Another of the advantages of this method is the surprisingly high yield of pure cladribine obtained.

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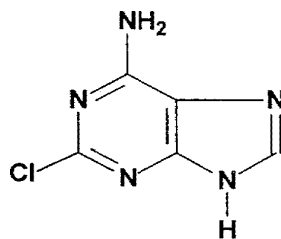
Accordingly, it is an aspect of the present invention to provide a novel procedure for the preparation 2-chloro-2'-deoxyadenosine having the formula



I

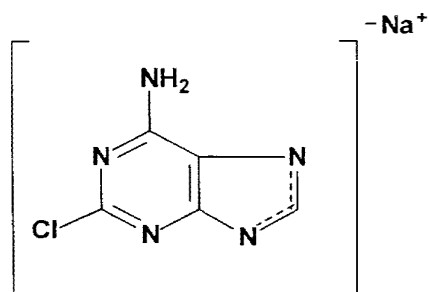
comprising:

- (a) glycosylating 2-chloro-6-aminopurine having the formula



IV ;

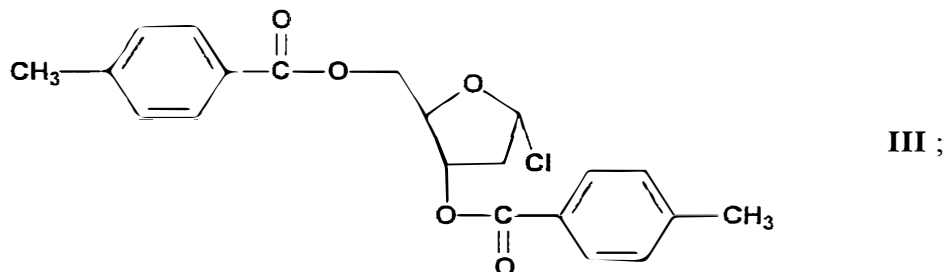
as its sodium salt, having the formula



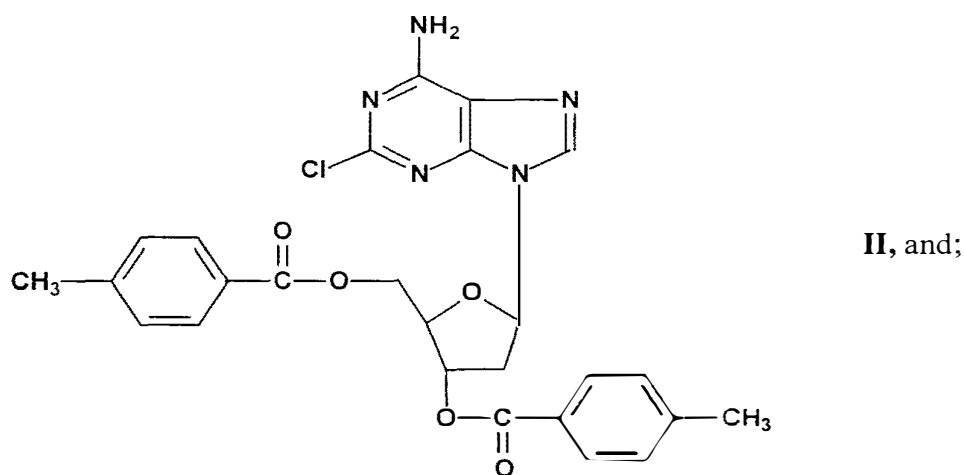
IV-a ;

with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl- α -D-erythropentofuranose having the formula

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(b) isolating the resulting glycoside having the formula



(c) removing the *p*-toluoyl groups to obtain 2-chloro-2'-deoxyadenosine (I).

Detailed Description of the Preferred Embodiments

The starting material, 2-chloro-6-aminopurine (IV). is prepared from 2,6-dichloropurine according to Brown *et al.* (Brown, G.B. *et al.*, *J. Org. Chem.* 23:125-126 (1958)).

Since the H-9 proton of adenine is known to be sufficiently acidic (pKa=9,80) the sodium salt (IVa) is prepared by reacting (IV) with one equivalent of methanolic sodium methoxide, instead of sodium hydride in acetonitrile.

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Excess sodium methoxide must be avoided because it is harmful to the coupling reaction with the protected sugar III.

It is known to those of skill in the art that solvent effects can provide good anomeric specificities in moderately polar solvents. As used herein, the term "moderately polar solvent" refers to a solvent with a dielectric constant at 20-25 °C between about 6 and about 25. A preferred solvent is anhydrous acetone. Accordingly, after removal of the solvent used to produce the sodium salt (IVa), methanol, the sodium salt of 2-chloro-6-aminopurine (IVa) was obtained and used directly for the coupling reaction with the 1-chlorosugar (III) by suspending it in anhydrous acetone.

A prerequisite for the successful formation of high yields of β -2-deoxynucleosides, assuming that a bimolecular nucleophilic substitution (S_N2) reaction mechanism can be invoked, is the prevention of anomerization of the α -chlorosugar III. Hubbard *et al.* studied the stability of this α -chlorosugar in solvents having a range of dielectric constants. (Hubbard, A.J. *et al.*, *Nucl. Acids Res.* 12(7):6827-6837 (1984)). As expected, much more β -chlorosugar was formed in polar solvents like acetonitrile (70% in 2 hours) than in chloroform (20% in 2 hours). In benzene, no anomerization was detectable. Chloroform and benzene are inadequate solvents for nucleoside synthesis by this route, due to the insolubility of sodium salts in non-polar solvents. In addition, it is known that these solvents do not promote nucleophilic substitution.

The protected 1-chloro-2-deoxyribose (III) has the α -configuration in the solid state, but in solution undergoes an equilibrium process which results in a mixture composed mostly of the α -anomer with small quantities of the β -anomer. Accordingly, reaction conditions which prevent the α -anomer from having enough time to undergo such anomerization should be chosen.

Preferential formation of β -anomers in reactions with 1- α -chlorosugars may result if the rate of glycosylation is much faster than the sugar anomerization or if the 1- β -chloro sugar is unreactive. The use of acetone as solvent favors the preferential formation of the 9- β -nucleoside.

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In these sodium salt glycosylation procedures, the attack of the ambidentate purine anion on the 1- α -chlorosugar induces 7- α and 7- β nucleoside formation. A systematic study of 2-deoxyribonucleoside isomer distribution from this procedure of substituted purines at C-6 (H, Cl, Br, SCH₃) was studied by Hildebrand *et al.*; in each case, 9- β -nucleoside always prevails, but 9- α , 7- α and 7- β isomer ratio vary with the C-6 substituent (Hildebrand, C. *et al.*, *J. Org. Chem.* 57(6):1808-1813 (1992)).

In this invention the use of 2-chloro-6-amino-purine in a sodium salt glycosylation reaction is described for the first time.

A similar glycosylation of the sodium salt of 2,6-dichloropurine results in a mixture of 9- β and the corresponding 7- β and 9- α isomers, which are difficult to separate on a silica gel column. In our case, the main product of the reaction is accompanied only by traces of other compounds of similar mobility and by unreacted chlorosugar starting material and degradation products of the chlorosugar.

Therefore, the presence of an amino group at C-6 directs a more selective attack by the N-9 anion on the β -face of the α -chlorosugar, forming mainly the 9- β -nucleoside. This high regioselectivity and anomeric specificity is achieved in a moderately polar solvent such as acetone.

It should be recalled that the 1-chlorosugar is easily anomerized in polar solvents and on the other hand, the purine sodium salt is insoluble in non-polar organic solvents. Therefore, in a moderately polar solvent such as acetone a good anomeric specificity is achieved though its yield is not very high, probably due to the low solubility of the sodium salt and/or sugar degradation.

The coupling reaction preferably takes place in anhydrous acetone at room temperature under a nitrogen atmosphere.

The best results (yield 60%) were obtained when the reaction was carried out at approximately a 2:1 molar ratio of sodium salt of 2-chloroadenine (IVa) to 1 α -chlorosugar (III). Under these conditions, the reaction proceeds to completion in about 2 hours.

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The solution is evaporated to dryness and the residue obtained is substantially pure as demonstrated by thin layer chromatography. The product, 2-chloro-6-amino-9-(3,5-di-O-*p*-toluoyl-2-deoxy- β -D-erythropentofuranosyl) purine (II), is crystallized from ethanol. The melting point of the intermediate product (II) agrees with that described for the intermediate product synthesized by another pathway.

Ammonolysis of both *p*-toluoyl groups may be conducted at room temperature, either by the action of methanolic ammonia or methanolic sodium methoxide. In the event methanolic ammonia is used, the work up consists of solvent removal by evaporation and then treatment of the resulting residue with n-hexane to remove methyl *p*-toluate and *p*-toluamide. When methanolic sodium methoxide is employed, the solution must be neutralized, preferably with an ion exchange resin (cationic form), and then treated with n-hexane. The yield of this step, including ammonolysis of the protecting groups and purification, is 70% and the total yield starting from 2-chloro-6-aminopurine is 42%. This method is advantageous in that it does not need additional purification of the intermediate product II or the final product I through chromatographic columns.

The residue obtained is 2-chloro-2'-deoxyadenosine, impurified with traces of other products. By recrystallization from an ethanol-water mixture, a chromatographically pure product is obtained having a melting point, specific optical rotation, ^1H NMR, ^{13}C NMR, mass and UV spectra identical to those reported in the literature for cladribine.

In Vitro Activity of Cladribine

The *in vitro* effect of cladribine (2-CdA) has been studied on a number of cell lines, showing different degrees of sensitivity, represented by the IC_{50} value (concentration required to inhibit 50% of growth) on a case-by-case basis.

Cladribine shows *in vitro* cytotoxicity against cell lines derived from human cells like T lymphocytes, B lymphocytes, non-T/non-B lymphocytes and myeloblastoid lines (Avery, T.L. *et al.*, *Cancer Res.* 49:4972-4978 (1989);

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Carson, D.A. *et al.*, *Proc. Nat'l Acad. Sci. (USA)* 77:6865-6869 (1980); Wataya, Y. *et al.*, *Adv. Exp. Med. Biol.* 253(B):227-234 (1989)). IC₅₀ values for cladribine range between 3-338 nmol/L for T cells, between 6-67 nmol/L for B cells, and between 5-70 nmol/L for myeloid cells (Avery, T.L. *et al.*, *Cancer Res.* 49:4972-4978 (1989)). Concentration of cladribine necessary to obtain an inhibitory effect depends on the cell type and cell pathogenic condition. The 5 nmol/L concentration inhibits the thymidine uptake in lymphoblastoid leukemia cells and has no effect on the granulocyte and macrophage colony formation in normal bone marrow. (Carson, D.A. *et al.*, *Blood* 62(4):737-743 (1983)).

Cladribine has low activity on multiple myeloma cells (Nagourney, R.A. *et al.*, *Br. J. Cancer* 67:10-14 (1993)) and cell lines of bone tumors (Carson, D.A. *et al.*, *Blood* 62(4):737-743 (1983)). Activity reported for some human melanoma cell lines was IC₃₇ at 12-22 nmol/L (Parson, D.G. *et al.*, *Biochem. Pharm.* 35:4025-4029 (1986)) and for a neuroblastoma cell line, IC₅₀ at 60 nmol/L (Avery, T.L. *et al.*, *Cancer Res.* 49:4972-4978 (1989)).

Petzer *et al.* investigated the effects of cladribine on clonal growth of hematopoietic progenitor cells. (Petzer, A.L. *et al.*, *Blood* 78:2583-2587 (1991)). Cladribine inhibits both myeloid progenitor cell growth and T lymphocyte colony forming cells on a dose-dependent basis. Erythroid progenitor cells show a dose-dependent sensitivity to cladribine, and this sensitivity decreases as the maturation state of said progenitors rises. Cladribine has higher effect on progenitor cells in the early maturation state; IC₅₀ values for more immature progenitors (granulocyte-macrophage colony forming units and erythrocyte burst forming units) are of approximately 16 to 20 nmoles/L. Mature erythroid colony forming units (mBFU-E) show an IC₅₀ value of 38 nM. More differentiated erythroid colony forming cells (CFU-E), show an IC₅₀ value of 56 nM. A higher concentration is needed for a complete inhibition of lymphoid colony formation (1280 nmol/L). Cladribine is active both in dividing and non-dividing cells (Carson, D.A. *et al.*, *Proc. Nat'l Acad. Sci. (USA)* 79:3848 (1982); Carson D.A. *et al.*, *Blood* 62(4):737-743 (1983)). This activity distinguishes this drug from

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other agents that affect purine and pyrimidine metabolism (Carson D.A. *et al.*, *Blood* 62(4):737-743 (1983); Carson, D.A. *et al.*, *Adv. Exp. Med. And Biol.* 165(B):351-356 (1984).

Cladribine activity against T lymphocytes that are not in cell division process depends on the duration of exposure to drug. Peripheral lymphocytes incubated with cladribine 100 nmol/l during 24 hours, followed by washing, retain viability after washing, while 60% of cells die after seven-day incubation with cladribine 8 nmol/L. (Carson, D.A. *et al.*, *Proc. Nat'l Acad. Sci. (USA)* 79:3848 (1982); Carson D.A. *et al.*, *Blood* 62(4):737-743 (1983)).

The following synthetic examples describe the invention in greater extent particularly and are intended to be a way of illustrating but not limiting the invention.

Table Number 1. Summary of *in vitro* Sensitivity to Cladribine of Different Cell Lines and Types.

CELL LINE	IC ₅₀ (nmol/L)**
Acute lymphoblastic leukemia*	34
Acute myelogenous leukemia*	20
Chronic lymphocytic leukemia*	39
Hairy cell leukemia*	31
Multiple myeloma*	1750
Non-Hodgkin's lymphoma*	87
T cells#	3-338
B cells#	6-67
Normal bone marrow °	15
Epstein Barr B lymphoblastoid cell lines	200
DAUDI cell line &	210

** IC₅₀ Drug concentration required to inhibit 50% of growth.

* Cladribine (Bryson, H.M. *et al.*, *Drugs* 46(5):872-894 (1993))

Avery, T.L. *et al.*, *Cancer Res.* 49:4972-4978 (1989)

° Carson D.A. *et al.*, *Blood* 62(4):737-743 (1983)

& Data Bio Sidus

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Examples

General Methods

Melting points were taken on a Mel-Temp II apparatus and are uncorrected.

NMR spectra were recorded on a Bruker MSL 300 spectrometer at ^1H (300.1 MHz) and ^{13}C (75.47 MHz). Chemical shifts are reported in parts per million (δ) relative to internal tetramethylsilane and deuterated dimethylsulfoxide as the solvent.

Mass spectra were recorded on a Quatro II-Micromass instrument using positive ion electrospray.

Infrared spectra were recorded as KBr pellets on a Nicolet-Magna 560 FT-IR spectrophotometer.

Ultraviolet spectra were recorded using a Shimadzu UV 1603 spectrophotometer.

Optical rotations were determined using a Perkin-Elmer Model 343 polarimeter with a 1 cm³, 1 dm cell.

Evaporations were carried out with a Büchi Rotavapor R-114.

TLC was performed using aluminium-backed sheets of silica gel 60 F₂₅₄. Spots were visualized under 254 nm UV light.

HPLC assays were carried out with a Waters 600 E high-pressure liquid chromatographer with 214 nm detection (Waters 991); a Merck-Lichrocart column in the reverse phase mode (Cartridge lichrospher 100 RP 18 (4 x 25 mm) and a linear gradient mobile phase of 0.1 M NH₄CH₃CO₂ (pH 6.55) and CH₃CN, and a flow rate of 1.2 min.

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Example 1 Preparation of 2-Chloro-6-amino-9-(3,5-di-O-p-toluoyl-2-β-D-erythropentofuranosyl)-purine (II)

A solution containing 7.2 g (58.9 mmole) of sodium methoxide in 300 ml of methanol was added to a suspension of 22.5 g (133 mmole) of 2-chloro-6-aminopurine in 260 ml of methanol. The mixture was magnetically stirred for 20 minutes at room temperature.

The solvent was then removed from the reaction mixture under reduced pressure in a rotavapor not exceeding the bath temperature 55 °C.

Then 350 ml of anhydrous acetone was added and removed as indicated.

The resulting residue of 2-chloro-6-aminopurine sodium salt, was suspended in 1,300 ml of anhydrous acetone and afterwards 22.9 g (58.9 mmole) of 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-α-D-erythropentofuranose was added.

The mixture was stirred at room temperature under a nitrogen atmosphere for 2 hours. The solution was then filtered and the filtrate evaporated to dryness. The residue was then dissolved in boiling ethanol (1,100 ml) and decolorized with activated carbon (2.2 g).

The clear solution was cooled overnight, filtered, washed with cold ethanol (3 x 20 ml) and dried in a vacuum oven at 40 °C, to give the title compound (18.5g, 60.2%), mp 190-192 °C with softening at 170 °C. (Lit.: Christensen *et al.*, 192-194 °C, (s) 170-180 °C); TLC (CHCl₃-Acetone 4:3): R_f 0.37.

Example 2 Preparation of 2-chloro-2'-deoxyadenosine. (Cladribine) (I)

A suspension of 2-chloro-6-amino-9-(3,5-di-O-p-toluoyl-2-β-D-erythropentofuranosyl)-purine (II) (5.6 g, 10.7 mmole) in methanolic ammonia (saturated at 10 °C, 285 ml) was stirred at room temperature for approximately 20 h, and the reaction was monitored by TLC (CHCl₃:MeOH 9:1, R_f 0.68) until disappearance of the starting material.

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The clear yellowish solution was evaporated to dryness and the resulting pasty residue was triturated with n-hexane (3 x 25 ml), heated to reflux in a mixture of ethanol-water (9.5:1; 42 ml) and decolorized with activated carbon (0.28 g).

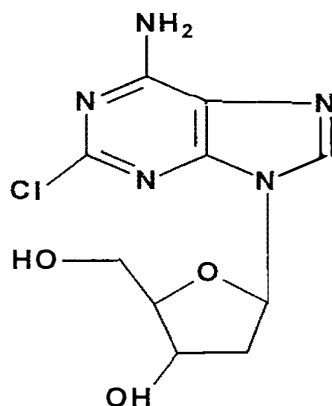
The solution was cooled overnight, filtered, washed with cold ethanol (1 x 2 ml) and dried in a vacuum oven at 40 °C to give analytically pure colorless cladribine (2.15 g, 70.2 %); mp 210-214 °C (softens) and then solidifies and turns brown, does not melt below 300 °C; TLC (CHCl₃:MeOH 7:3) R_f 0.60; UV (95 % EtOH): λ max. 265 nm (E 15,600); IR (Fig. 1);

¹H NMR (d₆-DMSO): δ 2.27 (m, 1H, H-2 α), 2.63 (m, 1H, H-2 β), 3.84 (m, 1H, H-4), 4.37 (m, 1H, H-3), 4.97 (t, 1H, J = 5.6 Hz, OH-5), 5.32 (d, 1H, J = 4.2 Hz, OH-3), 6.24 (t, 1H, J_{1-2 α} = J_{1-2 β} = 6.8 Hz, H-1), 7.81 (s, 2H, NH₂), 8.34 (s, 1H, H-8); ¹³C NMR (d₆-DMSO): δ 39.7 (C-2), 61.9 (C-5), 71.0 (C-3), 84.0 (C-1), 88.2 (C-4), 118.4 (C-5), 140.3 (C-8), 150.3 (C-4), 153.3 (C-2), 157.0 (C-6); Mass spectrum (electrospray): m/z (Ir) 288 ([MH+2]⁺, 33 %), 286 ([MH]⁺, 95 %), 172 ([MH+2-sugar]⁺, 34%), 170 ([MH-sugar]⁺, 100 %): HPLC: 16.32 min; [α]_D²⁶ -19.2 (C1, DMF).

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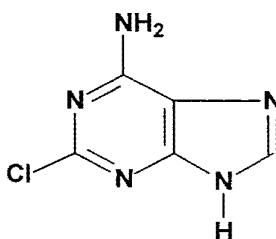
What Is Claimed Is:

1. A process for forming 2-chloro-2'-deoxyadenosine having formula I

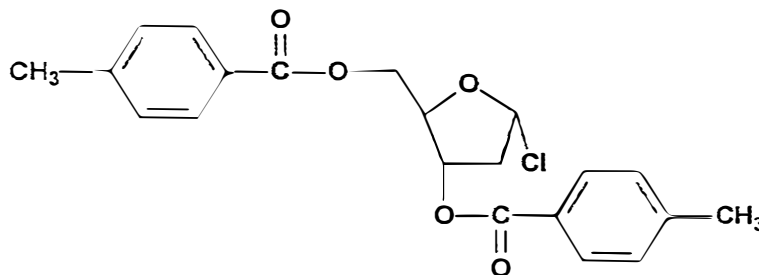
**I ,**

comprising:

- (a) glycosylating 2-chloro-6-aminopurine having formula IV

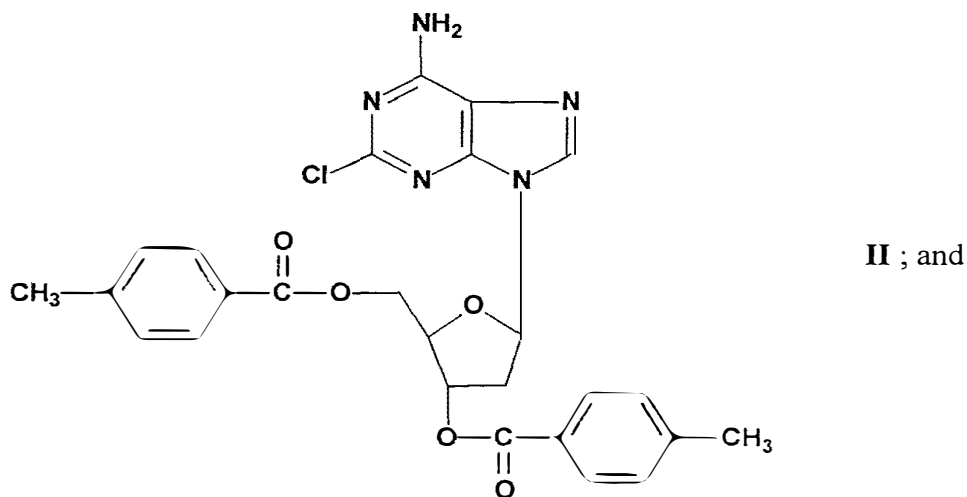
**IV**

as its sodium salt, with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl- α -D-erythropentofuranose having formula III

**III ;**

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- (b) isolating the resulting compound, 2-chloro-6-amino-9-(3,5-di-O-p-toluoyl-2'-deoxy- β -D-erythropentofuranosyl) purine, having formula II



- (c) removing the p-toluoyl groups from the compound of formula II, to produce 2-chloro-2'-deoxyadenosine having formula I.

2. A process according to claim 1, where a moderately polar anhydrous solvent is employed as a solvent in step (a).
3. A process according to claim 2 wherein said moderately polar solvent is acetone.
4. A process according to claim 1 where the removal of p-toluoyl groups in step (c) is carried out by the action of methanolic ammonia or methanolic sodium methoxide.
5. A process according to claim 1 where said sodium salt is formed by reaction of 2-chloro-6-aminopurine with anhydrous methanolic sodium methoxide at room temperature.

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6. A process according to claim 1, wherein said sodium salt is present in approximately a 2:1 molar ratio to said 1-chloro-2-deoxy-3,5-di-*O-p*-toluoyl- α -D-erythropentofuranose.
7. 2-Chloro-6-amino-9-(3,5-di-*O-p*-toluoyl-2-deoxy- β -D-erythropentofuranosyl) purine, produced by the process of claim 1.
8. Cladribine, produced by the process of claim 1.

Statement Concerning Non-Prejudicial Disclosure or Exception to Lack of Novelty

Due to disclosures on April 29, 1998, the applicant respectfully requests that the subject International application be granted the respective provisions under national laws concerning Exceptions to Lack of Novelty in each of the designated countries. This is not an admission that the subject invention is not novel. Exception to Lack of Novelty is hereby requested for purposes of disclosure and precautionary measures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/09158

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C07H 19/167

US CL : 536/27.7

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)

U.S. : 536/27.7; 536/26.26, 26.8

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

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE
USPATFULL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,760,137 A (ROBINS et al.) 26 June 1988, columns 4, 5 and 6.	1-8
Y	US 4,997,926 A (HAERTLE et al) 05 March 1991, columns 16-20.	1-8
Y	US 5,208,327 A (CHEN) 04 May 1993, columns 3-7.	1-8
Y	US 5,654,286 A (HOSTETLER) 05 August 1997, columns 25-26.	1-8



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	*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
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Date of the actual completion of the international search

13 AUGUST 1999

Date of mailing of the international search report

02 SEP 1999

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US95/16238 (22) International Filing Date: 15 December 1995 (15.12.95) (30) Priority Data: 08/362,106 22 December 1994 (22.12.94) US (71) Applicant: ORTHO PHARMACEUTICAL CORPORATION [US/US]; U.S. Route #202, P.O. Box 300, Raritan, NJ 08869-0602 (US). (72) Inventors: BORNSTEIN, Michael; 90 Barchester Way, Westfield, NJ 07090 (US). ROZMAN, Rosemary; 514 Village Commons, Flemington, NJ 08822 (US). LONG, Kevin, Francis; 17 Dogwood Circle, Flemington, NJ 08822 (US). GUH, Hsiao, Yung; 845 Mara Drive, Blue Bell, PA 19422 (US). (74) Agents: CIAMPORCERO, Audley, A., Jr. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933 (US).		(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: STABLE SOLUTIONS OF 2-CHLORO-2'-DEOXYADENOSINE FORMULATIONS (57) Abstract Shelf stable formulations of 2-CdA in water are disclosed which contain benzyl alcohol, m-cresol, a buffer and sodium chloride or a solubilizing agent such as propylene glycol or polyethylene glycol.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

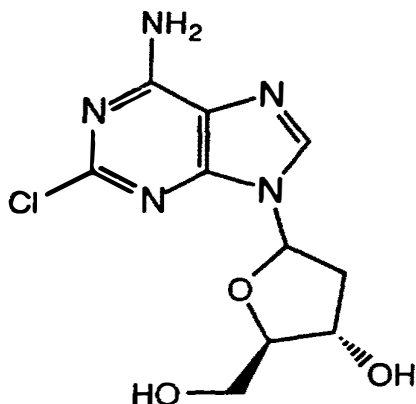
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STABLE SOLUTIONS OF 2-CHLORO-2'-DEOXYADENOSINE FORMULATIONS

This invention relates to pharmaceutically useful and stable formulations of 2-chloro-2'-deoxyadenosine (2-CdA) in water. More particularly, this invention relates to stable formulations of 2-CdA in water with certain preservatives, buffers and solubilizers which are injectable in humans and have a improved shelf-life.

BACKGROUND OF THE INVENTION

The compound 2-CdA has the following formula:



2-CdA is known as an antileukemic agent, i.e., in treating leukemias, such as, hairy cell leukemia and L 1210 leukemia, and as an immunosuppressive agent (D. A. Carson, D. Bruce Wasson, and Ernst Beutler, Proc. Soc. Acad. Sci. USA, Vol. 81, pp 2232- 2236, 1984). More recently, 2-CdA has been disclosed as effective in the treatment of rheumatoid arthritis and multiple sclerosis, U.S. Pat. No. 5,310,732.

To date, 2-CdA has been administered by intravenous injection of saline solutions presenting two problems for subcutaneous or intramuscular injection. First, 2-CdA is slightly soluble in water which requires a large volume of material to be injected subcutaneously or intramuscularly to achieve the required dose. Secondly, 2-CdA has limited stability in simple saline solutions. Longer shelf-life is beneficial for extended storage at refrigerated or room temperature conditions.

U.S. Pat. No. 5,310,732, col. 8, teaches a 0.1 mg/mL isotonic saline solution of 2-CdA. There has been marketed a non-buffered solution containing 1.0 mg/mL of 2-CdA in 9.0 mg/mL sodium chloride injection, USP.

5 SUMMARY OF THE INVENTION

There is provided by the present invention a first improved shelf stable solution of 2-CdA in water comprising:

- 10 a) from about 1 to about 8 mg/mL 2-CdA;
- b) from about 50 to about 400 mg/mL of solubilizing agent selected from the group consisting of propylene glycol and polyethylene glycol;
- 15 c) from about 5 to about 50 mg/mL of benzyl alcohol;
- d) from 0 to about 3 mg/mL of m-cresol; and
- e) an effective amount of a pharmaceutically acceptable buffer to
20 stabilize the pH at between about 5.5 and about 8.5.

There is provided by the present invention a second improved shelf stable solution of 2-CdA in water comprising:

- 25 a) from about 1 to about 5 mg/mL 2-CdA;
- b) from about 2 to about 10 mg/mL of sodium chloride;
- c) from about 5 to about 50 mg/mL of benzyl alcohol;
- 30 d) from about 0 to about 3 mg/mL of m-cresol; and
- e) an effective amount of a pharmaceutically acceptable buffer to
35 stabilize the pH at between about 5.5 and about 8.5.

DETAILED DESCRIPTION OF THE INVENTION

Processes for preparing 2-CdA are known. European Patent Application No. 173,059 A2 and Robins et al., J. Am. Chem. Soc., 106, 6379 (1984) disclose the preparation of 2-CdA. The preparation consists of the glycosilation of 2,6-dichloropurine with 1-chloro-2'-deoxy-3',5'-di-O-p-toluoyl-b-D-erythropentofuranose to yield the N-9 glycosilated purine, 2,6-dichloro-9-(2-deoxy-3,5-di-O-p-toluoyl-b-D-erythropentofuranosyl)-purine, which is subsequently reacted with ammonia to yield 2-CdA. An alternative method to manufacture 2-CdA is taught in U.S. Pat. No. 5,208,327 by Robert H. K. Chen.

Preferably, the first shelf stable solution contains from about 3 to about 7 mg/mL and most preferably from about 4 to about 6 mg/mL of 2-CdA. The second shelf stable solution preferably contains from about 2 to about 4 mg/mL of 2-CdA.

Propylene glycol and polyethylene glycol are known solubilizing agents for a variety of pharmaceuticals. Suitable polyethylene glycols are exemplified by polyethylene glycol 300 or polyethylene glycol 400. Preferably in the first shelf stable solution there is from about 75 to about 200 mg/mL of propylene glycol or polyethylene glycol and most preferably from about 100 to about 150 mg/mL.

The second shelf stable solution is isotonic and should contain sufficient sodium chloride to render it so. Preferably this solution should contain from about 3 to about 8 mg/mL sodium chloride and most preferably 3 to 6 mg/mL.

Benzyl alcohol is known generally as a preservative in pharmaceutical formulations based on its antibacterial action and as a solubilizing agent for certain pharmaceutical compounds. Preferably, in both the first and second shelf stable solutions there is present from about 8 to about 20 mg/mL benzyl alcohol and most preferably from about 10 to about 15 mg/mL.

The m-cresol is known generally as a preservative in pharmaceutical formulations based on its antibacterial action. Preferably, in both the first and second shelf stable solutions there is present from about 1.5 to about 2.5 mg/mL of m-cresol and most preferably about 2 mg/mL. Use of m-cresol may be eliminated provided that sufficient other preservative is used to render a suitably preserved formulation.

Suitable buffers are any of those available for pharmaceutical application and which are capable of stabilizing the pH between 5.5 and 8.5. Such buffers include but are not limited to phosphate, citrate, acetate, borate and tris. The preferred buffer for use herein is a sodium phosphate buffer system. A preferred pH range for the shelf stable solutions herein is between about 6.0 and about 7.0. The ratio of the sodium phosphate monobasic, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and the sodium phosphate dibasic, $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, are adjusted to achieve the pH desired. This buffer is generally useful to achieve a pH in the range of from 4.5 to 8.5. Of course, sufficient buffer should be employed not only to obtain the desired pH but to stabilize the pH at that value. For the first shelf stable solution, there should be employed about a 2 to 1 weight ratio of the sodium phosphate monobasic to the sodium phosphate dibasic. For the second shelf stable solution, there should be employed about a 1 to 1 weight ratio of the sodium phosphate monobasic to the sodium phosphate dibasic.

2-CdA may be administered to a patient in need of the same in a daily dose of 0.05 to 0.15 mg/Kg. A more desirable daily dose would be from 0.07 to 0.1 mg/Kg.

The invention is illustrated, but in no way limited, by the following examples.

30

EXAMPLE

The following formulations were prepared. Solutions containing a phosphate buffer were adjusted to pH 6.5. All figures shown are in mg/mL. The following abbreviations were employed: polyethylene glycol, NF, (PEG), propylene glycol, USP, (PG), benzyl alcohol, NF, (BA), m-cresol, (MC), sodium phosphate monobasic, monohydrate, USP, (SPMM) and sodium

35

phosphate dibasic, heptahydrate, USP, (SPDH). Water for injection, USP, was added to make the final volume of 1.0 mL.

5

TABLE 1.

<u>Formulation</u>		<u>1</u>	<u>2</u>	<u>3</u>	<u>Control</u>
10	2-CdA	5.0	5.0	3.0	1.0
	NaCl			4.0	9.0
	PEG 300		100.0		
	PG	100.0			
	BA	10.0	10.0	10.0	
15	MC	2.0	2.0	2.0	
	SPMM	1.816	1.816	1.555	
	SPDH	0.941	0.941	1.446	

The formulations of Table 1 were tested for stability after being stored for various times at various temperatures. The formulations were tested for 2-CdA content, 2-CAD content (hydrolysis product of 2-CdA), benzyl alcohol content and m-cresol content. The reported figure is in % of initial with the % of 2-CAD expressed in % initial of 2-CdA.

20

TABLE 2

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<u>Formulation</u>		<u>1</u>	<u>2</u>	<u>3</u>	<u>Control</u>
Initial, % label					
30	2-CdA	98.8	99.1	104.9	102.3
	2-CAD	ND	ND	ND	ND
	BA	109	108.7	111.4	N/A
	MC	99.6	99.9	101.5	N/A

TABLE 2 - Cont'd.

	Formulation	1	2	3	Control
5	50°C/4 wk				
	2-CdA	99.4	99.1	93.2	82.9
	2-CAD	2.8	2.7	3.6	6.0
	BA	95.1	95.3	91.9	N/A
10	MC	98.8	99.2	94.6	N/A
	50°C/8 wk				
	2-CdA	96.6	95.8	89.8	81.2
15	2-CAD	3.7	3.6	3.0	5.7
	BA	90.9	90.2	86.8	N/A
	MC	98.2	96.3	92.6	N/A
	40°C/4 wk				
20	2-CdA	101.8	101.8	96.3	96.2
	2-CAD	0.7	0.7	0.9	1.3
	BA	95.4	95.8	92.4	N/A
	MC	99.5	100.0	95.9	N/A
25	40°C/8 wk				
	2-CdA	102.3	100.2	96.3	94.8
	2-CAD	1.0	0.9	1.2	2.9
30	BA	90.8	90.5	87.1	N/A
	MC	96.5	98.0	94.4	N/A
	25°C/12 wk				
35	2-CdA	103.7		97.6	98.3
	2-CAD	0.21		0.25	0.36
	BA	92.8		88.1	N/A
	MC	99.6		96.4	N/A

TABLE 2 - Cont'd.

<u>Formulation</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>Control</u>
5 5°C/8 wk				
2-CdA	103.4	104.5	97.9	100.7
2-CAD	0.0	0.0	0.1	0.1
BA	92.5	93.4	89.9	N/A
10 MC	99.0	100.2	97.0	N/A

It is evident from the data above, that the formulations of the present invention are exceptionally shelf stable as compared to the control, which is the presently marketed formulation. Nowhere are such stable formulations of 2-CdA taught or suggested in the prior art. In addition, the data show that significantly less 2-CAD hydrolysis degradation product is formed in formulations 1, 2 and 3 compared to the control.

WHAT IS CLAIMED IS:

1. A solution of 2-CdA in water comprising:
 - 5 a) from about 1 to about 8 mg/mL 2-CdA;
 - b) from about 50 to about 400 mg/mL of solubilizing agent selected from the group consisting of propylene glycol and polyethylene glycol;
 - 10 c) from about 5 to about 50 mg/mL of benzyl alcohol;
 - d) from 0 to about 3 mg/mL of m-cresol; and
 - 15 e) an effective amount of a pharmaceutically acceptable buffer to stabilize the pH at between about 5.5 and about 8.5.
2. The solution of claim 1 comprising from about 3 to about 7 mg/mL of 2-CdA, from 75 to about 200 mg/mL of solubilizing agent, from about 8 to about 20 mg/mL of benzyl alcohol and from about 1.5 to about 2.5
20 mg/mL of m-cresol.
3. The solution of claim 1 wherein the buffer is selected from the group consisting of phosphate, citrate, acetate, borate and tris buffers.
- 25 4. The solution of claim 1 wherein the buffer is an effective amount of phosphate buffer comprising sodium phosphate monobasic, monohydrate, and sodium phosphate dibasic, heptahydrate, to stabilize the pH at between about 6.0 and about 7.0.
- 30 5. The solution of claim 1 comprising:
 - a) from about 4 to about 6 mg/mL 2-CdA;
 - b) from about 100 to about 150 mg/mL of solubilizing agent selected
35 from the group consisting of propylene glycol and polyethylene glycol;
 - c) from about 10 to about 15 mg/mL of benzyl alcohol;

d) about 2 mg/mL of m-cresol; and

e) about 1.816 mg/mL sodium phosphate monobasic, monohydrate,
and about 0.941 mg/mL sodium phosphate dibasic, heptahydrate.

5

6. The solution of claim 1 wherein the solubilizing agent is propylene glycol.

7. A solution of 2-CdA in water comprising:

10

a) from about 1 to about 5 mg/mL 2-CdA;

b) from about 2 to about 10 mg/mL of sodium chloride;

c) from about 5 to about 50 mg/mL of benzyl alcohol;

15

d) from about 0 to about 3 mg/mL of m-cresol; and

e) an effective amount of a pharmaceutically acceptable buffer to
stabilize the pH at between about 5.5 and about 8.5.

20

8. The solution of claim 7 comprising from about 2 to about 4 mg/mL of 2-CdA, from 3 to about 8 mg/mL of sodium chloride, from about 8 to about 20 mg/mL of benzyl alcohol and from about 1.5 to about 2.5 mg/mL of m-cresol.

25

9. The solution of claim 7 wherein the buffer is selected from the group consisting of phosphate, citrate, acetate, borate and tris buffers.

30

10. The solution of claim 7 wherein the buffer is an effective amount of phosphate buffer comprising sodium phosphate monobasic, monohydrate, and sodium phosphate dibasic, heptahydrate, to stabilize the pH at between about 6.0 and about 7.0.

11. The solution of claim 7 comprising:

- a) about 3 mg/mL 2-CdA;
- 5 b) about 4 mg/mL of sodium chloride;
- c) about 10 to about 15 mg/mL of benzyl alcohol;
- d) about 2 mg/mL of m-cresol; and
- 10 e) about 1.555 mg/mL sodium phosphate monobasic, monohydrate,
and about 1.446 mg/mL sodium phosphate dibasic, heptahydrate.

INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/US 95/16238

A. CLASSIFICATION OF SUBJECT MATTER
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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 6 A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,93 23058 (THE SCRIPPS RESEARCH INSTITUTE,U.S.A.) 25 November 1993 see claims see page 14, line 1 - line 4 see example 9 ---	1-11
A	US,A,5 310 732 (D.A.CARSON ET AL.) 10 May 1994 cited in the application see claims see column 13, line 48 - line 54 see example 18 ---	1-11
A	US,A,4 719 295 (P.D.COOK ET AL.) 12 January 1988 see column 6, line 20 - line 64 see example 3 -----	1-11

☐ 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
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- *&* document member of the same patent family

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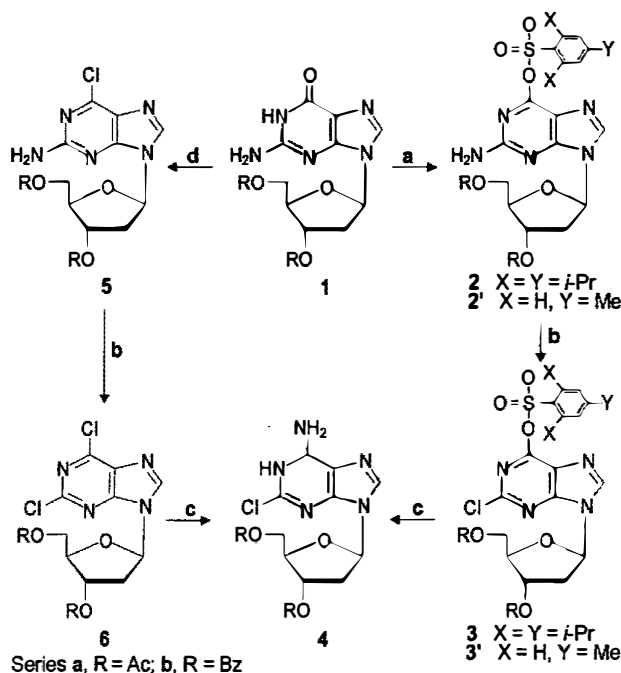
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[Continued on next page]

(54) Title: METHOD FOR THE PREPARATION OF 2-HALO-2'-DEOXYADENOSINE COMPOUNDS FROM 2'-DEOXYGUANOSINE



(57) Abstract: The present invention is a method for preparing 2-halo-6-aminopurines, and more specifically for preparing the clinical agent cladribine (2-chloro-2'-deoxyadenosine, C1dAdo, 4), a drug of choice against hairy-cell leukemia and other neoplasms, from 2-amino-6-oxopurines, which are readily obtained from the naturally occurring compound 2'-deoxyguanosine. According to the methods of the present invention, the 6-oxo group of a protected 2'-deoxyguanosine (1) is converted to a 6-(substituted oxy) leaving group, or alternatively to a 6-chloro leaving group, the 2-amino group is replaced with a 2-chloro group, the 6-(substituted oxy) leaving group, or alternatively the 6-chloro leaving group, is replaced with a 6-amino group or, alternatively, a 2,6-dichloro substituted compound is selectively replaced group, and the protecting groups are removed.

^a (a) TlPBS-Cl/Et₃N/DMAP/CH₂Cl₂. (b) AcCl/BTEA-NO₂/CH₂Cl₂/-5 to 0 °C. (c) NH₃/MeOH/CH₂Cl₂/Δ. (d) POCl₃/BTEA-Cl/N,N-dimethylaniline/McCN/Δ.



European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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TITLE OF THE INVENTION

Method for the Preparation of 2-Halo-2'-Deoxyadenosine Compounds from 2'-Deoxyguanosine.

FIELD OF THE INVENTION

5 The present invention is directed to processes for preparing 2-halo-6-aminopurines, and more particularly to a process for preparing 2-chloro-2'-deoxyadenosine.

BACKGROUND OF THE INVENTION

10 The lymphoselective toxicity of 2-chloro-2'-deoxyadenosine (CldAdo, cladribine) and its potential as a chemotherapeutic agent against lymphoid neoplasms were reported by Carson et al.¹ This potent, deaminase-resistant analogue of 2'-deoxyadenosine (dAdo) is currently the drug of choice for hairy-cell leukemia.^{2,3} It also has significant activity against chronic lymphocytic leukemia,^{4,5} indolent non-Hodgkin's lymphoma,⁶ and Waldenström's macroglobulinemia.⁷ Investigations with cladribine treatment of multiple sclerosis,⁸ systemic lupus erythematosus-associated glomerulonephritis,⁹ and other rheumatoid and immune disorders are in progress. Cladribine is a nucleoside prodrug, which is phosphorylated by deoxycytidine kinase to CldAMP, and then sequentially to CldADP and the active CldATP.^{1a,10a} Cladribine also is a good substrate for mitochondrial 2'-deoxyguanosine (dGuo) kinase,¹⁰ and induction of programmed cell death by direct effects on mitochondria has been implicated in its potent activity against indolent lymphoid malignancies (via apoptosis) as well as in proliferating cells.^{11,12}

15 Various methodologies have been published for the production of Cladribine. Venner reported Fischer-Helferich syntheses of naturally occurring 2'-deoxynucleosides in 1960,¹³ and employed 2-chloro-2'-deoxyadenosine as an intermediate for 2'-deoxy(guanosine and inosine). Ikehara and Tada also synthesized dAdo with CldAdo as an intermediate [obtained by desulfurization of 8,2'-anhydro-9-(β-D-arabinofuranosyl)-2-chloro-8-thioadenine].¹⁴

20

Syntheses of CldAdo as a target compound have exploited the greater reactivity of leaving groups at C6 relative to those at C2 of the purine ring in S_NAr displacement reactions. Robins and Robins¹⁵ employed fusion coupling of 2,6-dichloropurine with 1,3,5-tri-*O*-acetyl-2-deoxy- α -D-ribofuranose. The 9-(3,5-di-*O*-acetyl-2-deoxy- α -D-*erythro*-pentofuranosyl)-
5 2,6-dichloropurine anomer was obtained by fractional crystallization. Selective ammonolysis at C6 and accompanying deprotection gave 6-amino-2-chloro-9-(2-deoxy- α -D-*erythro*-pentofuranosyl)purine. The pharmacologically active β -anomer (cladribine) was prepared by an analogous coupling, chromatographic separation of anomers, and ammonolysis.¹⁶

Stereoselective glycosylation of sodium salts of halopurines and analogues with 2-
10 deoxy-3,5-di-*O*-*p*-toluoyl- α -D-*erythro*-pentofuranosyl chloride gave β -nucleoside anomers via predominant Walden inversion,^{17,18} and ammonolysis/deprotection gave CldAdo.¹⁹ Although the sodium salt glycosylation usually gave good anomeric stereoselectivity, minor quantities of α anomers and >10% of N7 regioisomers were usually formed.^{20,21} This requires separations and results in diminished yields of the desired N9 product. Carson et al.¹
15 had reported an enzymatic transfer of the 2-deoxy sugar from thymidine to 2-chloroadenine (ClAde). Holy and coworkers noted that cells of a strain of *Escherichia coli* performed glycosyl transfer from 2'-deoxyuridine to 2-chloro-6-[(dimethylaminomethylene)amino]purine to give a derivative of CldAdo.²² Very recently Barai, Mikhailopulo, and coworkers²³ described an *E. coli*-mediated glycosyl transfer
20 synthesis of 2,6-diamino-9-(3-deoxy- β -D-*erythro*-pentofuranosyl)purine,²⁴ and its enzymatic deamination to 3'-deoxyguanosine.²⁴ They reported glycosyl transfer from 2'-deoxyguanosine to ClAde, and claimed a yield of 81% of CldAdo (based on ClAde).²³ However, a 3:1 ratio of dGuo/ClAde was employed, so the yield of CldAdo was <27% based on dGuo.

Sampath et al. have recently shown (U.S. Patent No. 6,596,858 B2) a method for making 2-chloro-2'-deoxyadenosine compounds, using 2-amino-2'-deoxyadenosine as a starting compound, but beginning with an initial diazotization/chloro-dediazoni-
ation reaction on the unprotected nucleoside to replace the 2-amino group with a 6-chloro group. This
5 method, however, creates various impurities, which requires extensive purification procedures, and results in an overall yield of only 27%.

Accordingly, there is a significant need to produce CldAdo using methods that result in a higher yield, are more cost effective, and result in a more purified form.

SUMMARY OF THE INVENTION

10 The present invention is a method for producing 2-chloro-2'-deoxyadenosine (CldAdo) comprising the steps of: (a) converting the 6-oxo group of a compound having the formula (1) wherein R is a protecting group, into a 6-leaving group having sufficient reactivity for an S_NAr displacement reaction; (b) replacing the 2-amino group with a 2-chloro group in a diazotization/chloro-dediazoni-
ation reaction; (c) replacing the 6-leaving group
15 with a 6-amino group; and (d) removing the R protecting groups, to produce 2-chloro-2'-deoxyadenosine.

DESCRIPTION OF THE FIGURES

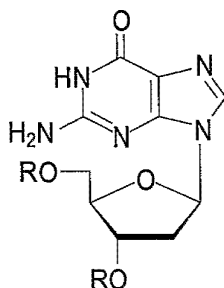
Figure 1 shows the chemical synthesis of 2-chloro-2'-deoxyadenosine from protected forms of naturally occurring 2'-deoxyguanosine.

20 Figure 2 shows the diazotization/halo-dediazoni-
ation conversion reaction from a 2-aminopurine nucleoside to a 2-halopurine nucleoside.

DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

In accordance with the present invention, synthesis of regio- and stereochemically pure 2-chloro-2'-deoxyadenosine (Cladrabine, or CldAdo), which avoids separation of
25 mixtures with fusion and sodium salt glycosylation procedures, is accomplished by

transformation of the naturally occurring nucleoside 2'-deoxyguanosine (dGuo) as the starting compound.²⁴ Methods for producing CldAdo from 2'-deoxyguanosine (dGuo) according to the present invention begin with protected forms of dGuo including, but not restricted to, acyl, silyl, amide, and other derivatives useful in the field of nucleoside/nucleotide/nucleic acid chemistry and protection strategies. Methods for obtaining protected forms of dGuo are well-known in the art.^{25,26,27,28,29,30} The preferred starting products for efficient synthesis of CldAdo are defined by the following chemical structure:



where R is any suitable protecting group, and preferably R is Ac or Bz.

In order to obtain the desired CldAdo compounds, protected dGuo derivatives are treated with combinations of chemicals that effect functionalization at the C6 position to give groups that can be replaced, followed by transformation of the 2-amino function to a 2-chloro group, followed by replacement of the 6-functional group to give a 6-amino group (or a 6-substituent that can be converted into a 6-amino group, followed by conversion to the 6-amino group), and concomitant or subsequent deprotection of the resulting 6-amino-2-chloropurine derivative to give CldAdo. For example, from dGuo, CldAdo (4) (Figure 1) is synthesized by converting the 6-oxo function into appropriate 6-(substituted oxy) leaving groups that can be replaced without protection of the 2-amino moiety, transformation of the

6-chloro derivatives **5a** (90%) and **5b** (85%) were obtained in high yields under carefully controlled conditions.

Following the step of converting the 6-oxo group to a 6-(substituted oxy) leaving group, the 2-amino group is replaced with a 2-chloro group by a diazotization/halo-dediazoni-
5 dediazoni-
ation reaction. Improved methods are disclosed for replacement of an amino group on purine nucleoside derivatives with chlorine, bromine, or iodine under non-aqueous conditions by diazotization/halo-dediazoni-
ation methods.²⁷ These mild diazotization/halo-dediazoni-
ation methods are applicable at C6 of dAdo derivatives as well as at C2 of 2-amino-6-chloropurine nucleosides. In accordance with the present invention, CldAdo is produced
10 from dGuo by treatment of protected forms of dGuo that contain a respective 6-*O*-(alkyl, cycloalkyl, or aryl)sulfonyl group with reagents that effect diazotization/chloro-dediazoni-
ation at C2 to give a 2-chloro group. CldAdo is also produced by treating protected 2-amino-6-chloropurine derivatives with reagents that effect diazotization/chloro-dediazoni-
ation at C2 to give respective 2,6-dichloropurine derivatives.

15 Reagents that effect diazotization/chloro-dediazoni-
ation at C2 to give a 2-chloro group include a halide source (such as metal chlorides, metal chloride salts, acyl chlorides, sulfonyl chlorides, and silyl chlorides, alkyl and aryl substituted ammonium chloride salts, including but not limited to tetraalkyl and aryl ammonium chloride salts) and a nitrite source (such as metal nitrites, metal nitrite salts, organic nitrites, such as tert-butyl nitrite, pentyl
20 nitrite, and isoamyl nitrite, and complex quaternary ammonium nitrites, such as benzyltriethylammonium nitrite).

In preferred embodiments of the present invention, CldAdo is synthesized by employing acetyl chloride and benzyltriethylammonium nitrite (BTEA-NO₂)-mediated diazotization/chloro-dediazoni-
ation of 6-*O*-(2,4,6-triisopropylbenzenesulfonyl) (TiPBS) or 6-
25 chloro derivatives that are readily obtained from dGuo. Non-aqueous diazotization/chloro-

dediazonation (acetyl chloride/benzyltriethylammonium nitrite) of **2**, **2'b**, or **5** gave the 2-chloropurine derivatives **3**, **3'b**, or **6**, respectively. This new procedure for non-aqueous diazotization/chloro-dediazonation²⁷ (AcCl/BTEA-NO₂/CH₂Cl₂/-5 to 0 °C) worked well for replacement of the 2-amino group of **2**, **2'b**, and **5** with chlorine to give **3a** (89%), **3b** (90%),
5 **3'b** (87%), **6a** (95%), and **6b** (91%).

Efficient diazotization/chloro-dediazonation of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2-amino-6-chloropurine^{26,33} (**7**) (Figure 2) was effected with TMS-Cl (9 equivalents) and benzyltriethylammonium nitrite (BTEA-NO₂) (3 equivalents) in CH₂Cl₂ at ambient temperature. The process was rapid (<30 min), and the desired 9-(2,3,5-tri-*O*-acetyl-
10 β-D-ribofuranosyl)-2,6-dichloropurine^{27,37} (**8**) (83%, without chromatography) was obtained as a white crystalline solid. Comparable yields were obtained at 0° C. TMS-Cl (3.5 equivalents) and BTEA-NO₂ (1.5 equivalents) with powdered NaNO₂ (5 equivalents) gave **8** (86%) within 1 hr. By contrast, an alternative method for non-aqueous diazotization/chloro-dediazonation of **7** employed Cl₂/TBN/CuCl in a strongly exothermic reaction, and removal
15 of colloidal material by filtration was required prior to crystallization of **7**.³⁵

Compound **7** underwent efficient diazotization/bromo-dediazonation with TMS-Br and tert-butyl nitrite (TBN). Competing redox interactions between nitrite anion and TMS-Br precluded the use of NaNO₂. The 2-bromo-6-chloropurine nucleoside **3**^{27,37} (85%, without chromatography) was obtained as a crystalline solid with TMS-Br (9 equivalents)/TBN (20
20 equivalents)/CH₂Br₂/ambient temperature within 1 h.

NOCl/CH₂Cl₂ or NOBr/CH₂Br₂ is presumed to be generated from (Me₃SiX or AcCl) and (TBN or BTEA-NO₂). These procedures provide efficient diazotization/halo-dediazonation of protected (2 or 6)-aminopurine nucleosides as well as the acid-sensitive 2'-deoxynucleosides. The reactions are cost-effective and proceed at or below ambient
25 temperature with convenient reagents and standard laboratory equipment and conditions.

Following replacement of the 2-amino group with a 2-chloro group, the protected forms of dGuo that contain a respective 6-*O*-(alkyl, cycloalkyl, or aryl)sulfonyl or phosphoryl group and a 2-chloro group is reacted with chemicals that cause replacement of the 6-*O*-(alkyl, cycloalkyl, or aryl) sulfonyl or phosphoryl group to give a 6-amino group (or a
5 substituent that can be converted into a 6-amino group, resulting in overall conversion of the substituent into a 6-amino group) of a resulting 6-amino-2-chloropurine derivative. In preferred embodiments, the 6-leaving group is replaced with a 6-amino group by reacting the product of step (b) with a nitrogen source capable of being converted to an amino group in a solvent compatible with the nitrogen source to replace the 6-leaving group with a 6-amino
10 group by selective ammonolysis of the 6-leaving group. The nitrogen source is selected from the group consisting of ammonia, azides, hydrazines, benzylic amines, or compatible ammonium salts. The solvent may be any solvent that is compatible with the nitrogen source, such as methanol, ethanol, higher alcohols, or aprotic solvents, such as 1,2-dimethoxyethane, tetrahydrofuran, or DMF.

15 In other preferred embodiments, the respective 6-*O*-(alkyl, cycloalkyl, or aryl)sulfonyl leaving group is reacted with ammonia in a compatible aprotic solvent to give a 6-amino-2-chloropurine derivative, followed by deprotection (if necessary) to give CldAdo. In a more preferred embodiment, a 9-(3,5-di-*O*-acyl- β -D-*erythro*-pentofuranosyl-6-*O*-(alkyl, cycloalkyl, or aryl)sulfonyl-2-chloropurine is treated with ammonia in methanol or other
20 compatible solvent to give CldAdo.

Further, protected derivatives of 2,6-dichloropurine are treated with reagents that cause selective replacement of the 6-chloro group to give a 6-amino group (or a substituent that can be converted into a 6-amino group, followed by conversion of the substituent into a 6-amino group) of a resulting 6-amino-2-chloropurine derivative. In a more preferred

embodiment, 9-(3,5-di-*O*-acyl- β -D-*erythro*-pentofuranosyl)-2,6-dichloropurine is treated with ammonia in methanol or other compatible solvent to give CldAdo.

Selective ammonolysis at C6 (arylsulfonate with **3** or chloride with **6**) and accompanying deprotection of the sugar moiety gave CldAdo (**4**) (64–75% overall from **1**).

5 Specifically, displacements of the hindered arylsulfonate (from **3**) or chloride (from **6**) at C6 with accompanying cleavage of the sugar esters were effected at 80 °C with NH₃/MeOH/CH₂Cl₂. Cladribine (**4**) was obtained in high yields from **3a** (81%), **3b** (83%), **6a** (87%), and **6b** (94%), but only in moderate yield from the 6-*O*-tosyl derivative **3'b** (43%).

The R protecting groups are removed by deacylation using a basic reagent well known in the art in a solvent compatible with the basic reagent, to remove the R protecting groups and produce 2-chloro-2'-deoxyadenosine. The basic reagent is selected from the group consisting of ammonia, metal alkoxides, metal hydroxides, and metal carbonates. The solvent may be any solvent that is compatible with the basic reagent, such as methanol, ethanol, 1,2-dimethoxyethane/H₂O, or tetrahydrofuran/H₂O.

15 It is to be noted that removal of the R protecting groups may occur concomitantly with or subsequent to the replacement of the 6-(substituted oxy) leaving group with a 6-amino group by ammonolysis. Both steps of replacement of the 6-(substituted oxy) leaving group with a 6-amino group and removal of the R protecting groups is accomplished by use of a nitrogen source in a solvent compatible with the nitrogen source. Where the nitrogen source is ammonia in a protic solvent, such as methanol or ethanol, both steps proceed simultaneously. However, where the nitrogen source is anhydrous ammonia in a dry solvent, such as 1,2-dimethoxyethane, or tetrahydrofuran, only the step of replacement of the 6-(substituted oxy) leaving group with a 6-amino group proceeds. In this case, it is necessary and sometimes desirable to remove the R protecting groups in a separate step.

(nucleoside/NH₃/MeOH/ Δ) for **3a** $\rightarrow$ **4**. Analogous reactions with equivalent molar proportions of other nucleosides gave the indicated products and quantities.

Example 1

Preparation of 9-(3,5-Di-*O*-acetyl-2-deoxy- β -D-*erythro*-pentofuranosyl)-2-amino-6-*O*-(2,4,6-triisopropylbenzenesulfonyl)purine (2a**).** Method 1. Et₃N (1.25 mL, 910 mg, 9.0 mmol) was added to a stirred solution of **1a** (1.67 g, 4.8 mmol), TiPBS-Cl (2.73 g, 9.0 mmol), and DMAP (72 mg, 0.6 mmol) in dried CHCl₃ (70 mL) under N₂. Stirring was continued for 24 h, and volatiles were evaporated. The orange residue was chromatographed (CH₂Cl₂/MeOH) give **2a**³² (2.67 g, 91%) as a slightly yellow foam with UV max 238, 291 nm, min 264 nm; ¹H NMR (500 MHz) δ 1.26–1.32 (m, 18H), 2.08 (s, 3H), 2.14 (s, 3H), 2.54 (ddd, J = 4.7, 9.0, 14.0 Hz, 1H), 2.91–2.99 (m, 2H), 4.22–4.37 (m, 3H), 4.43–4.47 (m, 2H), 4.97 (br s, 2H), 5.41–5.42 ("d", 1H), 6.26–6.29 (m, 1H), 7.21 (s, 2H), 7.84 (s, 1H); LRMS m/z 618 (MH⁺ [C₂₉H₄₀N₅O₈S] = 618); HRMS m/z 640.2413 (MNa⁺ [C₂₉H₃₉N₅O₈SNa] = 640.2417).

Example 2

Preparation of 2-Amino-9-(3,5-di-*O*-benzoyl-2-deoxy- β -D-*erythro*-pentofuranosyl)-6-*O*-(2,4,6-triisopropylbenzenesulfonyl) purine (2b**).** Treatment of **1b** (950 mg, 2.0 mmol) by method 1 gave **2b** (1.27 g, 86%) as a white solid foam with UV max 289 nm, min 264 nm; ¹H NMR δ 1.29–1.32 (m, 18H), 2.76 (ddd, J = 2.1, 6.0, 14.3 Hz, 1H), 2.96 ("quint", J = 6.8 Hz, 1H), 3.15–3.25 (m, 1H), 4.34 ("quint", J = 6.8 Hz, 2H), 4.65–4.74 (m, 2H), 4.85–4.90 (m, 1 H), 5.00 (br s, 2H), 5.84–5.86 ("d", 1H), 6.38–6.43 (m, 1H), 7.30 (s, 2H), 7.44–7.55 (m, 4H), 7.58–7.69 (m, 2H), 7.85 (s, 1H), 8.04 (d, J = 7.1 Hz, 2H), 8.11 (d, J = 7.1 Hz, 2H); LRMS m/z 742 (MH⁺ [C₃₉H₄₄N₅O₈S] = 742), 764 (MNa⁺ [C₃₉H₄₃N₅O₈SNa] = 764); HRMS m/z 764.2730 (MNa⁺ [C₃₉H₄₃N₅O₈SNa] = 764.2730).

Example 3

Preparation of 2-Amino-9-(3,5-di-*O*-benzoyl-2-deoxy- β -D-erythro-pentofuranosyl)-6-*O*-(4-methylbenzenesulfonyl)purine (**2'b**). Et₃N (700 μ L, 506 mg, 5.0 mmol) was added to a stirred solution of **1b** (1.43 g, 3.0 mmol), TsCl (858 mg, 4.5 mmol),
5 and DMAP (36 mg, 0.3 mmol) in dried CHCl₃ (45 mL) under N₂. Stirring was continued for 15 h, and volatiles were evaporated. The slightly yellow residue was chromatographed (CH₂Cl₂/MeOH) to give **2'b** (1.68 g, 89%) as a white solid foam with UV max 300 nm; ¹H NMR (DMSO-*d*₆) δ 2.43 (s, 3H), 2.73 (ddd, *J* = 2.1, 8.4, 14.4 Hz, 1H), 3.17–3.27 ("quint", *J* = 7.2 Hz, 1H), 4.52–4.65 (m, 3H), 5.76–5.78 ("d", 1H), 6.37–6.41 (m, 1H), 6.95 (br s, 2H),
10 7.47–7.73 (m, 8H), 7.95 (d, *J* = 7.8 Hz, 2H), 8.03–8.11 (m, 4H), 8.30 (s, 1H); LRMS *m/z* 630 (MH⁺ [C₃₁H₂₈N₅O₈S] = 630), *m/z* 652 (MNa⁺ [C₃₁H₂₇N₅O₈SNa] = 652); HRMS *m/z* 652.1467 (MNa⁺ [C₃₁H₂₇N₅O₈SNa] = 652.1478).

Example 4

Preparation of 9-(3,5-Di-*O*-acetyl-2-deoxy- β -D-erythro-pentofuranosyl)-2-chloro-
15 6-*O*-(2,4,6-triisopropylbenzenesulfonyl)purine (**3a**). Method 2. A solution of AcCl (200 μ L, 220 mg, 2.8 mmol) in dried CH₂Cl₂ (12 mL) under N₂ was chilled in a NaCl/ice/H₂O bath (–5 to 0 °C) for 15 min. BTEA-NO₂ (520 mg, 2.2 mmol) was dissolved in dried CH₂Cl₂ (8 mL) and this solution was immediately added dropwise to the cold, stirred solution of AcCl/CH₂Cl₂. A solution of **2a** (288 mg, 0.5 mmol) in dried CH₂Cl₂ (5 mL) was then added
20 dropwise to the cold solution, and stirring was continued for 5 min (TLC, CH₂Cl₂/MeOH, 95:5, showed complete conversion of **2a** into a single product). The reaction mixture was added dropwise at a rapid rate to a cold (ice/H₂O bath), vigorously stirred mixture of saturated NaHCO₃/H₂O (100 mL)//CH₂Cl₂ (100 mL). The layers were separated, and the organic phase was washed with cold (0 °C) H₂O (2 X 100 mL) and dried (MgSO₄) for 1 h.
25 Volatiles were evaporated, and the residue was chromatographed (CH₂Cl₂/MeOH) to give **3a**

(267 mg, 89%) as a white solid foam with UV max 240, 266 nm, min 255 nm; ^1H NMR (500 MHz) δ 1.24–1.31 (m, 18H), 2.08 (s, 3H), 2.13 (s, 3H), 2.67 (ddd, $J = 2.5, 7.0, 15.5$ Hz, 1H), 2.76–2.81 (m, 1H), 2.90–2.96 ("quint", $J = 7.0$ Hz, 1H), 4.28–4.33 ("quint", $J = 7.0$ Hz, 2H), 4.35–4.39 (m, 3H), 5.38–5.39 ("d", 1H), 6.42–6.45 (m, 1H), 7.22 (s, 2H), 8.23 (s, 1H); LRMS m/z 637 (MH^+ [$\text{C}_{29}\text{H}_{38}\text{ClN}_4\text{O}_8\text{S}$] = 637); HRMS m/z 659.1902 (MNa^+ [$\text{C}_{29}\text{H}_{37}\text{ClN}_4\text{O}_8\text{SNa}$] = 659.1918).

Example 5

Preparation of 9-(3,5-Di-*O*-benzoyl-2-deoxy- β -D-erythro-pentofuranosyl)-2-chloro-6-*O*-(2,4,6-triisopropylbenzenesulfonyl)purine (3b). Treatment **2b** (1.20 g, 1.6 mmol) by method 2 gave **3b** (1.10 g, 90%) as a yellow solid foam with UV max 230, 266 nm, min 255 nm; ^1H NMR δ 1.22–1.34 (m, 18H), 2.92–3.00 (m, 3H), 4.30–4.34 (m, 2H), 4.68–4.77 (m, 3H), 5.80–5.82 ("d", 1H), 6.54–6.57 (m, 1H), 7.42–7.64 (m, 8H), 8.00 (d, $J = 8.4$ Hz, 2H), 8.10 (d, $J = 8.4$ Hz, 2H), 8.26 (s, 1H); HRMS m/z 783.2224 (MNa^+ [$\text{C}_{39}\text{H}_{41}\text{ClN}_4\text{O}_8\text{SNa}$] = 783.2231).

Example 6

Preparation of 9-(3,5-Di-*O*-benzoyl-2-deoxy- β -D-erythro-pentofuranosyl)-2-chloro-6-*O*-(4-methylbenzenesulfonyl)purine (3'b). Treatment of **2'b** (1.45 g, 2.3 mmol) by method 2 gave **3'b** (1.30 g, 87%) as a slightly yellow foam with UV max 267 nm, min 255 nm; ^1H NMR ($\text{DMSO}-d_6$) δ 2.45 (s, 3H), 2.86 (ddd, $J = 3.4, 6.2, 14.0$ Hz, 1H), 3.21–3.30 ("quint", $J = 7.0$ Hz, 1H), 4.55–4.67 (m, 3H), 5.82–5.84 (m, 1H), 6.56–6.61 (m, 1H), 7.42–7.72 (m, 8H), 7.88 (d, $J = 7.8$ Hz, 2H), 8.04–8.07 (m, 4H), 8.88 (s, 1H); HRMS m/z 671.0983 (MNa^+ [$\text{C}_{31}\text{H}_{25}\text{ClN}_4\text{O}_8\text{SNa}$] = 671.0979).

Example 7

Preparation of 9-(3,5-Di-*O*-acetyl-2-deoxy- β -D-erythro-pentofuranosyl)-2-amino-6-chloropurine (5a). **Method 3.** A mixture of **1a** (540 mg, 1.54 mmol), BTEA-Cl (710 mg,

3.1 mmol), *N,N*-dimethylaniline (215 μ L, 206 mg, 1.7 mmol), and POCl₃ (720 μ L, 1.2 g, 7.7 mmol) in MeCN (6 mL) was stirred in a preheated oil bath (85 °C) for 10 min. Volatiles were flash evaporated immediately (in vacuo), and the yellow foam was dissolved (CHCl₃, 15 mL) and stirred vigorously with crushed ice for 15 min. The layers were separated, and the aqueous phase was extracted (CHCl₃, 3 X 5 mL). Crushed ice was frequently added to the combined organic phase, which was washed [(ice-H₂O (3 X 5 mL), 5% NaHCO₃/H₂O (to pH ~7)] and dried (MgSO₄). Volatiles were evaporated, and the residue was chromatographed (CH₂Cl₂/MeOH) to give **5a**^{36,38} (517 mg, 90%) as a white solid foam with UV max 248, 310 nm, min 268 nm; ¹H NMR (500 MHz, DMSO-*d*₆) δ 2.02 (s, 3H), 2.08 (s, 3H), 2.49–2.52 (m, 1H), 3.04–3.06 (m, 1H), 4.20–4.29 (m, 3H), 5.32–5.34 ("d", 1H), 6.23–6.26 (m, 1H); 7.03 (br s, 2H), 8.35 (s, 1H); ¹H NMR δ 2.03 (s, 3H), 2.08 (s, 3H), 2.51 (ddd, *J* = 2.7, 5.9, 14.2 Hz, 1H), 2.85–2.94 ("quint", *J* = 7.1 Hz, 1H), 4.28–4.42 (m, 3H), 5.35–5.37 (m, 1H), 6.21–6.26 (m, 1H), 7.89 (s, 1H); HRMS (EI) *m/z* 369.0844 (*M*⁺ [C₁₄H₁₆ClN₅O₅] = 369.0840).

Example 8

Preparation of 2-Amino-9-(3,5-di-*O*-benzoyl-2-deoxy- β -D-erythro-pentofuranosyl)-6-chloropurine (5b**).** Treatment of **1b** (2.38 g, 5 mmol) by method 3 gave **5b** (2.10 g, 85%) as a slightly yellow solid foam with UV max 310 nm; ¹H NMR (DMSO-*d*₆) δ 2.69–2.78 ("ddd", 1H), 3.20–3.24 ("quint", *J* = 7.2 Hz, 1H), 4.56–4.67 (m, 3H), 5.77–5.79 ("d", 1H), 6.39–6.44 (m, 1H), 7.02 (br s, 2H), 7.48–7.71 (m, 6H), 7.96 (d, *J* = 8.4 Hz, 2H), 8.06 (d, *J* = 8.7 Hz, 2H), 8.37 (s, 1H); LRMS *m/z* 494 (*MH*⁺ [C₂₄H₂₁ClN₅O₅] = 494); HRMS *m/z* 516.1042 (*MNa*⁺ [C₂₄H₂₀ClN₅O₅Na] = 516.1051).

Example 9

Preparation of 9-(3,5-Di-*O*-acetyl-2-deoxy- β -D-erythro-pentofuranosyl)-2,6-dichloropurine (6a**).** Treatment of **5a** (265 mg, 0.7 mmol) by method 2 gave **6a**³⁹ (266 mg,

Method C. A solution of AcCl/CH₂Cl₂ (1 M, 2.5mL, 2.5 mmol) was added to dried CH₂Cl₂ (10 mL) under N₂, and the solution was cooled for 15 min. BTEA-NO₂ (535.5 mg, 2.25 mmol) in CH₂Cl₂ (5 mL) was then added dropwise (1 drop/2 sec) to the cold, stirred solution of AcCl/CH₂Cl₂. A cold solution of **7** (214 mg., 0.5 mmol) in dried CH₂Cl₂ (5 mL) was then added dropwise to the cooled, stirred AcCl/BETA-NO₂Cl₂ solution (TLC, hexanes/EtOAc, 3:7). Immediate workup and recrystallization (as in method B) gave **8** (187 mg, 84%) as pale-yellow crystals.

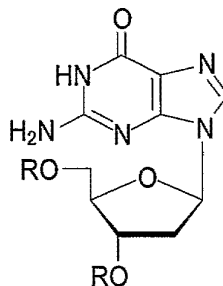
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CLAIMS

We claim:

1. A method for producing 2-chloro-2'-deoxyadenosine comprising the steps of:

(a) converting the 6-oxo group of a compound having the formula



wherein R is a protecting group, into a 6-(substituted oxy) group having sufficient reactivity in an S_NAr displacement reaction;

(b) replacing the 2-amino group with a 2-chloro group by a diazotization/chloro-dediazoniatio reaction;

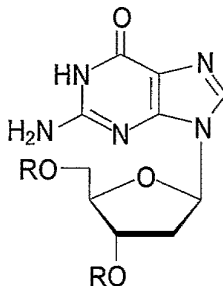
(c) replacing the 6-(substituted oxy) leaving group with a 6-amino group;

and

(d) removing the R protecting groups, to produce 2-chloro-2'-deoxyadenosine.

5. A method for producing 2-chloro-2'-deoxyadenosine comprising the steps of:

(a) converting the 6-oxo group of a compound having the formula



wherein R is a protecting group selected from the group consisting of acetyl, benzoyl, into a 6-leaving group having greater reactivity than that of the 2-chloro group in an S_NAr displacement reaction;

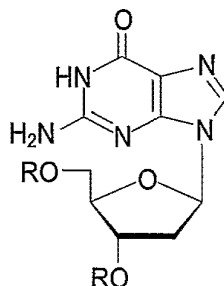
(b) replacing the 2-amino group with a 2-chloro group by diazotization/chloro-dediazoniation of the 2-amino group;

(c) replacing the 6-leaving group with a 6-amino group by selective ammonolysis of the 6-leaving group; and

(d) removing the R protecting groups by deacylation, to produce 2-chloro-2'-deoxyadenosine.

6. A method for producing 2-chloro-2'-deoxyadenosine comprising the steps of:

(a) reacting the 6-oxo group of a compound having the formula

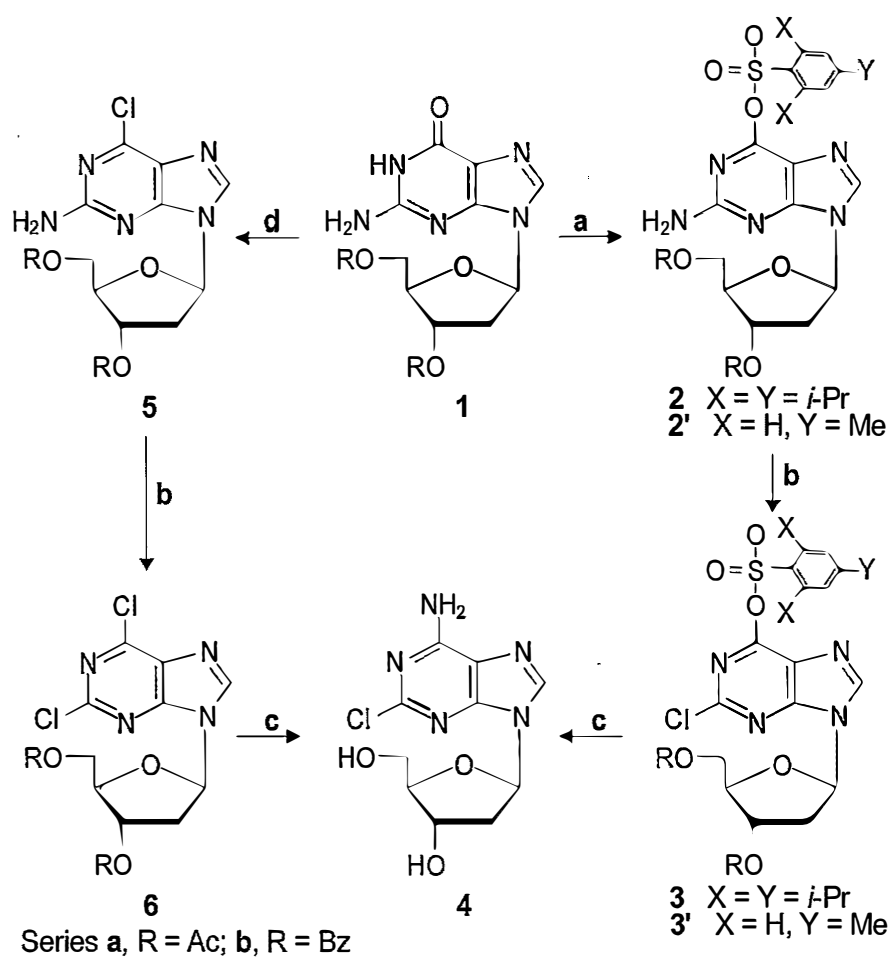


wherein R is a protecting group selected from the group consisting of acyl and silyl, with an (alkyl or any substituted alkyl or cycloalkyl) sulfonyl or phosphoryl reagent or (aryl or any substituted aryl) sulfonyl or phosphoryl reagent to convert the 6-oxo group to a 6-O-(alkyl, cycloalkyl, or aryl) sulfonyl or phosphoryl group;

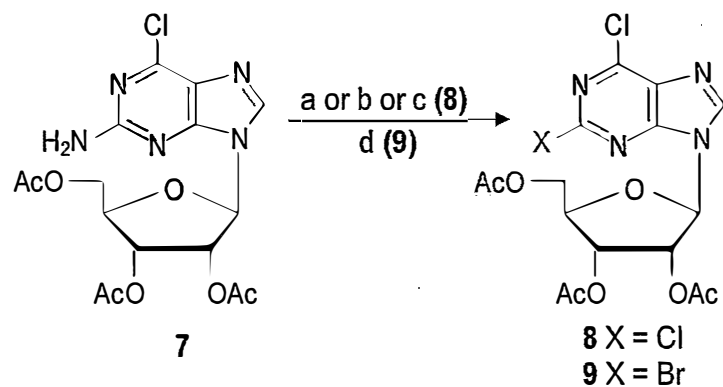
(b) reacting the product of step (a) with a halide and an organic nitrite in a solvent compatible with the halide to replace the 2-amino group with a 2-chloro group by diazotization/chloro-dediazoniation of the 2-amino group;

(c) reacting the product of step (b) with ammonia in a solvent or with a nitrogen source capable of being converted to an amino group in a solvent compatible with the nitrogen source to replace the 6-leaving group with a 6-amino group by selective ammonolysis of the 6-leaving group; and

(d) reacting the product of step (c) with a basic reagent in a compatible solvent to remove the R protecting groups by deacylation, to produce 2-chloro-2'-deoxyadenosine.

**Figure 1**

^a(a) TiPBS-Cl/Et₃N/DMAP/CH₂Cl₂. (b) AcCl/BTEA-NO₂/CH₂Cl₂/-5 to 0 °C. (c) NH₃/MeOH/CH₂Cl₂/Δ. (d) POCl₃/BTEA-Cl/*N,N*-dimethylaniline/MeCN/Δ.

**Figure 2**

(a) TMS-Cl/BTEA-NO₂/CH₂Cl₂ (83%); (b) TMS-Cl/BTEA-NO₂/NaNO₂/CH₂Cl₂ (86%); (c) AcCl/BTEA-NO₂/CH₂Cl₂/0-5 C° (84%); (d) TMS-Br/TBN/CH₂Br₂ (85%)

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(54) Title: CLADRIBINE REGIMEN FOR TREATING MULTIPLE SCLEROSIS

(57) Abstract: The present invention is related to the use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis, wherein the preparation is to be orally administered and wherein re-treatments are possible.

WO 2006/067141 A1

Cladribine regimen for treating Multiple Sclerosis

Field of the Invention

5 The present invention relates to the use of multiple doses of Cladribine for the treatment of multiple sclerosis, especially relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis.

Background of the Invention

10 Multiple sclerosis (MS) is the most known chronic inflammatory demyelinating disease of the central nervous system in humans. The onset of the disease typically occurs during ages 20 to 40. Women are affected approximately twice as often as men.

Over time, MS may result in the accumulation of various neurological disabilities. Clinical
15 disability in MS is presumed to be a result of repeated inflammatory injury with subsequent loss of myelin and axons, leading to tissue atrophy.

MS is manifested in physical symptoms (relapses and disability progression), Central Nervous System (CNS) inflammation, brain atrophy and cognitive impairment. Presenting
20 symptoms include focal sensory deficits, focal weakness, visual problems, imbalance and fatigue. Sexual impairment and sphincter dysfunction may occur. Approximately half of the patients with MS may experience cognitive impairment or depression.

MS is now considered to be a multi-phasic disease and periods of clinical quiescence
25 (remissions) occur between exacerbations. Remissions vary in length and may last several years but are infrequently permanent.

Four courses of the disease are individualized: relapsing-remitting (RR), secondary progressive (SP), primary progressive (PP) and progressive relapsing (PR) multiple sclerosis.

More than 80% of patients with MS will initially display a RR course with clinical exacerbation of neurological symptoms, followed by a recovery that may or may not be complete (*Lublin and Reingold, Neurology, 1996, 46:907-911*).

5 During RRMS, accumulation of disability results from incomplete recovery from relapses. Approximately, half of the patients with RRMS switch to a progressive course, called SPMS, 10 years after the diseased onset. During the SP phase, worsening of disability results from the accumulation of residual symptoms after exarcerbation but also from insidious progression between exacerbations (*Lublin and Reingold above*). 10% of MS
10 patients have PPMS which is characterized by insidious progression of the symptoms from the disease onset. Less than 5 % of patients have PRMS and are often considered to have the same prognosis as PPMS. It is suggested that distinct pathogenic mechanisms may be involved in different patient sub-groups and have wide-ranging implications for disease classification (*Lassmann et al., 2001, Trends Mol. Med., 7, 115-121; Lucchinetti et al.,
15 Curr. Opin. Neurol., 2001, 14, 259-269*).

MS onset is defined by the occurrence of the first neurological symptoms of CNS dysfunction. Advances in cerebrospinal fluid (CSF) analysis and magnetic resonance imaging (MRI) have simplified the diagnostic process and facilitated early diagnostic
20 (*Noseworthy et al., The New England Journal of Medicine, 2000, 343, 13, 938-952*). The International Panel on the Diagnosis of MS issued revised criteria facilitating the diagnosis of MS and including MRI together with clinical and para-clinical diagnostic methods (*Mc Donald et al., 2001, Ann. Neurol., 50:121-127*).

25 Current medications for MS which are disease modifying treatments, i.e. modifying the course of MS, modulate or suppress the immune system. There are four FDA approved immunomodulating agents for RRMS: three beta interferons (Betaseron®, Berlex; Avonex®, Biogen; Rebif®, Serono) and Glatimarer Acetate (Copaxone®, Amgen). There is also one FDA approved immunosuppressing drug for worsening MS, Mitoxantrone

(Novantrone®, Amgen). Several other immunosuppressive agents are used, although not FDA approved.

Among them, Cladribine, a chlorinated purine analogue 2-chloro-2'deoxyadenosine analogue (2-CdA), has been suggested to be useful in the treatment of MS (*EP 626853B1* and *US 5,506,214*).

Several clinical studies with Cladribine in patients with multiple sclerosis have investigated the use of i.v. and s.c. Cladribine in MS.

Two double-blind, placebo controlled Phase II studies were conducted respectively in the treatment of Chronic Progressive MS (*Selby et al., 1998, Can. J. Neurol. Sci., 25:295-299*) and Relapsing-Remitting MS respectively (*Romine et al., 1999, Proceedings of the Association of American Physicians, 111, 1, 35-44*).

In the first trial, the Cladribine dose used was 0.1 mg/kg/day for 7 days by continuous i.v. infusion. The treatment was repeated for 4 consecutive months.

In the second clinical trial, the Cladribine dose used was 0.07mg/kg/day for 5 days by subcutaneous injection. The treatment was repeated for 6 consecutive months.

In addition, placebo controlled Phase III study was conducted in patients with primary progressive (PP) or secondary progressive (SP) multiple sclerosis (*Rice et al., 2000, Neurology, 54, 5, 1145-1155*). In this study, both patient groups received Cladribine by subcutaneous injection at a dose of 0.07 mg/kg/day. The treatment was repeated for either 2 months or 6 months.

The Phase II clinical studies provided evidence for the positive effects of Cladribine in patients with MS in terms of Kutzke Extended Disability Status Scale (EDSS), Scripps Neurologic rating Scale (SNRS) scores and Magnetic Resonance Imaging (MRI) findings (*Beutler et al., 1996, Proc. Nat. Acad. Sci. USA, 93, 1716-1720; Romine et al., 1999 above*). Phase III study results, were positive on the significant reduction of MRI-measured brain lesions (*Rice et al., 2000, above*).

Some adverse effects (AEs), such as increased incidence of infections related to compromised immune function or myelosuppression, were observed with the highest doses (Selby et al., 1998, above; Beutler et al., 1994, *Acta hematol.*, 91:10-15). Due to the narrow margin of safety between the efficacy dose and the dose of occurrence of AEs, to date, all clinical trials for Cladribine in multiple sclerosis have been conducted using either i.v. or s.c. administration. As a result, Beutler et al. (Beutler et al., 1996, *Seminars in Hematology*, 33, 1(S1), 45-52) excluded the oral route for the treatment of multiple sclerosis with Cladribine.

Grieb et al. reported a small trial in 11 patients with relapsing-remitting multiple sclerosis (Grieb et al., 1995, *Archivum Immunologiae et Therapiae Experimentalis*, 43 (5-6), 323-327) wherein Cladribine has been orally administered during 6 monthly courses of 5 days at a total dose of about 4-5.7 mg/kg (patients of about 52 and about 75 kilos, respectively) i.e. a total effective dose of 2-2.85 mg/kg. For some patients, a single re-treatment of 5 days was performed at a cumulative dose of 0.4-0.66 mg/kg after a cladribine free-period of 3 or 6 months. The side effects observed with the regimen above were said to be less severe than the ones observed in the study on patients suffering from chronic progressive multiple sclerosis treated by i.v. infusion of Cladribine (Sipe et al., 1994, *Lancet*, 344, 9-13) but were still present. In addition, the therapeutic efficacy of the oral regimen above versus the i.v. infusion therapy was questioned (Grieb et al., 1995, above) and a group of "non-responders" has been identified (Stelmasiak et al., 1998, *Laboratory Investigations*, 4(1), 4-8).

Therefore, it would be desirable to have a method for treating multiple sclerosis comprising the oral administration of Cladribine that would permit the same or improved effect on MS lesions while decreasing the occurrence and/or severity adverse events. In addition, as MS is a chronic disease, it would be desirable to decrease the occurrence and/or severity adverse events in such a way that re-treatments are possible. A sustained benefit of Cladribine treatment between the treatment periods is also desirable.

Summary of the Invention

The present invention is directed towards a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis, wherein the preparation is to be the orally administered. Particularly, the invention is directed towards a use of Cladribine for the preparation of a medicament for the treatment of relapsing-remitting multiple sclerosis or early secondary progressive multiple sclerosis and wherein re-treatments are possible.

An embodiment of the invention provides an improved dosing regimen for Cladribine in the treatment of multiple sclerosis.

An additional embodiment of the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein adverse effects are reduced, allowing further use of Cladribine.

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation wherein the formulation is to be orally administered following the sequential steps below:

- (i) An induction period wherein the Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;
- (ii) A Cladribine-free period wherein no Cladribine is administered;
- (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
- (iv) A Cladribine-free period wherein no Cladribine is administered.

In another embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a formulation thereof in a patient in need thereof comprising the following steps:

- (i) An induction treatment wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;
- (ii) A Cladribine-free period wherein no Cladribine is administered;
- (iii) A maintenance treatment wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
- (iv) A Cladribine-free period wherein no Cladribine is administered.

Detailed Description of the invention

Definitions

The “total dose” or “cumulative dose” refers to the total dose of Cladribine administered during the treatment, i.e. the dose reached at the end of the treatment that is calculated by adding the daily doses. For example, the total dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days is 3.5 mg/kg or the total dose of Cladribine corresponding to a treatment of 0.35 mg/kg Cladribine per day during 5 days is 1.7 mg/kg.

“The total effective dose” or “cumulative effective dose” refers to the bioavailable dose of Cladribine after a given administration period, i.e. the bioavailable dose reached at the end of the treatment that is calculated by adding the daily doses reduced by the bioavailability coefficient. For example, the total effective dose of Cladribine corresponding to a treatment of 0.7 mg/kg Cladribine per day during 5 days wherein the bioavailability of Cladribine is of about 40% is 1.4 mg/kg or the total effective dose of Cladribine corresponding to a treatment of 0.35 mg/kg Cladribine per day during 5 days wherein the bioavailability of Cladribine is of about 40% is 0.7 mg/kg.

Typically, the bioavailability of Cladribine or of a Cladribine formulation used in the context of this invention is from about 30% to about 90%, preferably from about 40% to about 60%, such as about 50%.

5 “A week” refers to a period of time of or about 5, about 6 or about 7 days.

“A month” refers to a period of time of or about 28, about 29, about 30 or about 31 days.

10 “Treatment” comprises the sequential succession of an “induction treatment” and at least a “maintenance treatment”. Typically, a treatment according to the invention comprises an “induction treatment” and about one or about two or about three maintenance treatments. Typically, a treatment according to the invention is of about 2 years (about 24 months) or about 3 years (about 36 months) or about 4 years (about 48 months).

15 An “Induction Treatment” consists in the sequential succession of (i) an induction period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention is orally administered and (ii) a Cladribine-free period. An induction period lasts up to about 4 months or up to about 3 month or up to about 2 months. For example, an induction period lasts for about 2 to about 4 months. An induction period consists in the oral administration
20 of Cladribine or a pharmaceutical preparation thereof during about 1 to about 7 days each month.

A “Cladribine-free period” is a period wherein no Cladribine is administered to the patient. During a Cladribine-free period, the patient can be free of any administration or be dosed with a placebo-pill or another drug except. A Cladribine-free period lasts up to about 10
25 months or up to 9 months or up to about 8 months. For example, a Cladribine-free period lasts from about 8 to about 10 months, typically at least of about 8 months.

A “Maintenance Treatment” consists in the sequential succession of (i) a maintenance period wherein the Cladribine or the Cladribine pharmaceutical preparation of the invention
30 is orally administered at a lower dose than the Cladribine dose orally administered during

the induction treatment and (ii) a Cladribine-free period. A maintenance period lasts for up to about 4 months, or up to about 3 months, or up to about 2 months, preferably up to about 2 months. For example, a maintenance period lasts for about 2 to about 4 months, preferably for about 2 months. A maintenance period consists in the oral administration of Cladribine or of a pharmaceutical preparation thereof during about 1 to about 7 days each month.

Within the context of this invention, the beneficial effect, including but not limited to an attenuation, reduction, decrease or diminishing of the pathological development after onset of the disease, may be seen after one or more a “treatments”, after an “induction treatment”, after a “maintenance treatment” or during a Cladribine-free period.

“Daily dose” refers to the total dose of Cladribine orally administered to the patient each day of administration. The daily dose can be reached through a single or several administrations per day, such as for example once a day, twice a day or three times a day.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factors, including pharmacokinetic properties, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired.

Patients suffering from MS can be defined for example as having clinically definite or laboratory-definite MS according to Schumacher or Poser criteria (*Schumacher et al.*, 1965, *Ann. NY Acad. Sci.* 1965; 122:552-568; *Poser et al.*, 1983, *Ann. Neurol.* 13(3): 227-31).

“Relapses” involve neurologic problems that occur over a short period, typically days but sometimes as short as hours or even minutes. These attacks most often involve motor, sensory, visual or coordination problems early in the disease. Later, bladder, bowel, sexual and cognitive problems may be shown. Sometimes the attack onset occurs over several weeks. Typical MS relapse involves a period of worsening, with development of neurological deficits, then a plateau, in which the patient is not getting any better but also

not getting any worse followed by a recovery period. Recovery usually begins within a few weeks.

“Efficacy” of a treatment according to the invention can be measured based on changes in the course of disease in response to a use according to the invention. For example, treatment of MS efficacy can be measured by the frequency of relapses in RRMS and the presence or absence of new lesions in the CNS as detected using methods such as MRI technique (*Miller et al., 1996, Neurology, 47(Suppl 4): S217; Evans et al., 1997, Ann. Neurology, 41:125-132*).

The observation of the reduction and/or suppression of MRI T₁ gadolinium-enhanced lesions (thought to represent areas of active inflammation) gives a primary efficacy variable.

Secondary efficacy variables include MRI T₁ enhanced brain lesion volume, MRI T₁ enhanced lesion number, MRI T₂ lesion volume (thought to represent total disease burden, i.e. demyelination, gliosis, inflammation and axon loss), MRI T₁ enhanced hypointense lesion volume (thought to represent primarily demyelination and axon loss), time-to-progression of MS, frequency and severity of exacerbations and time-to-exacerbation, Expanded Disability Status Scale score and Scripps Neurologic Rating Scale (SNRS) score (*Sipe et al., 1984, Neurology, 34, 1368-1372*). Methods of early and accurate diagnosis of multiple sclerosis and of following the disease progression are described in *Mattson, 2002, Expert Rev. Neurotherapeutics, 319-328*.

Degree of disability of MS patients can be for example measured by Kurtzke Expanded Disability Status Scale (EDSS) score (*Kurtzke, 1983, Neurology, 33, 1444-1452*). Typically a decrease in EDSS score corresponds to an improvement in the disease and conversely, an increase in EDSS score corresponds to a worsening of the disease.

Cladribine (2-CdA)

2-CdA and its pharmacologically acceptable salts may be used in the practice of this invention.

Cladribine can be formulated in any pharmaceutical preparation suitable for oral administration. Representative oral formulations of 2-CdA are described in (*WO 96/19230; WO 96/19229; US 6,194,395; US 5,506,214; WO 2004/087100; WO 2004/087101*), the contents of which are incorporated herein by reference. Examples of ingredients for oral formulations are given below.

Processes for preparing 2-CdA are well known in the art. For example, the preparation of 2-CdA is described in (*EP 173,059; WO 04/028462; WO 04/028462; US 5,208,327; WO 00/64918*) and *Robins et al., J. Am. Chem. Soc., 1984, 106: 6379*. Alternatively, pharmaceutical preparations of 2-CdA may be purchased from Bedford Laboratories, Bedford, Ohio.

Oral administration of Cladribine may be in capsule, tablet, oral suspension, or syrup form. The tablet or capsules may contain from about 3 to 500 mg of Cladribine. Preferably they may contain about 3 to about 10 mg of Cladribine, more preferably about 3, about 5 or about 10 mg of Cladribine. The capsules may be gelatin capsules and may contain, in addition to Cladribine in the quantity indicated above, a small quantity, for example less than 5% by weight, magnesium stearate or other excipient. Tablets may contain the foregoing amount of the compound and a binder, which may be a gelatin solution, a starch paste in water, polyvinyl polyvinyl alcohol in water, etc. with a typical sugar coating.

Compositions

Compositions of this invention may further comprise one or more pharmaceutically acceptable additional ingredient(s) such as alum, stabilizers, antimicrobial agents, buffers, coloring agents, flavoring agents, adjuvants, and the like.

Compositions of this invention may be in the form of tablets or lozenges formulated in a conventional manner. For example, tablets and capsules for oral administration may contain conventional excipients including, but not limited to, binding agents, fillers, lubricants,

disintegrants and wetting agents. Binding agents include, but are not limited to, syrup, accacia, gelatin, sorbitol, tragacanth, mucilage of starch and polyvinylpyrrolidone. Fillers include, but are not limited to, lactose, sugar, microcrystalline cellulose, maizestarch, calcium phosphate, and sorbitol. Lubricants include, but are not limited to, magnesium stearate, stearic acid, talc, polyethylene glycol, and silica. Disintegrants include, but are not limited to, potato starch and sodium starch glycollate. Wetting agents include, but are not limited to, sodium lauryl sulfate). Tablets may be coated according to methods well known in the art.

Compositions of this invention may also be liquid formulations including, but not limited to, aqueous or oily suspensions, solutions, emulsions, syrups, and elixirs. The compositions may also be formulated as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain additives including, but not limited to, suspending agents, emulsifying agents, nonaqueous vehicles and preservatives. Suspending agent include, but are not limited to, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxyethylcellulose, carboxymethyl cellulose, aluminum stearate gel, and hydrogenated edible fats. Emulsifying agents include, but are not limited to, lecithin, sorbitan monooleate, and acacia. Nonaqueous vehicles include, but are not limited to, edible oils, almond oil, fractionated coconut oil, oily esters, propylene glycol, and ethyl alcohol. Preservatives include, but are not limited to, methyl or propyl p-hydroxybenzoate and sorbic acid.

Combination

According to the invention, Cladribine can be administered alone or in combination with IFN-beta, prophylactically or therapeutically to an individual prior to, simultaneously or sequentially with other therapeutic regimens or agents (e.g. multiple drug regimens), in a therapeutically effective amount, especially therapeutic agents for the treatment of multiple sclerosis. Active agents that are administered simultaneously with other therapeutic agents can be administered in the same or different compositions and in the same or different routes of administration.

In one embodiment, when Cladribine is administered in combination with IFN-beta, IFN-beta is administered during the Cladribine-free period.

In another embodiment, when Cladribine is administered in combination with IFN-beta,
5 IFN-beta is administered after the "treatment" according to the invention.

The term "interferon-beta (IFN- β)", as used herein, is intended to include fibroblast interferon in particular of human origin, as obtained by isolation from biological fluids or as obtained by DNA recombinant techniques from prokaryotic or eukaryotic host cells, as well as its
10 salts, functional derivatives, variants, analogs and active fragments.

IFN- β suitable in accordance with the present invention is commercially available e.g. as Rebif® (Serono), Avonex® (Biogen) or Betaferon® (Schering). The use of interferons of human origin is also preferred in accordance with the present invention. The term interferon, as used herein, is intended to encompass salts, functional derivatives, variants, analogs and
15 active fragments thereof.

Rebif® (recombinant human interferon- β) is the latest development in interferon therapy for multiple sclerosis (MS) and represents a significant advance in treatment. Rebif® is interferon (IFN)-beta 1a, produced from mammalian cell lines. It was established that interferon beta-1a given subcutaneously three times per week is efficacious in the treatment
20 of Relapsing-Remitting Multiple Sclerosis (RRMS). Interferon beta-1a can have a positive effect on the long-term course of MS by reducing number and severity of relapses and reducing the burden of the disease and disease activity as measured by MRI.

The dosing of IFN- β in the treatment of relapsing-remitting MS according to the invention
25 depends on the type of IFN- β used.

In accordance with the present invention, where IFN is recombinant IFN- β 1b produced in E. Coli, commercially available under the trademark Betaseron®, it may preferably be

administered sub-cutaneously every second day at a dosage of about of 250 to 300 µg or 8 MIU to 9.6 MIU per person.

In accordance with the present invention, where IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Avonex®, it may preferably be administered intra-muscularly once a week at a dosage of about of 30µg to 33 µg or 6 MIU to 6.6 MIU per person.

In accordance with the present invention, when IFN is recombinant IFN-β1a, produced in Chinese Hamster Ovary cells (CHO cells), commercially available under the trademark Rebif®, it may preferably be administered sub-cutaneously three times a week (TIW) at a dosage of 22 to 44 µg or 6 MIU to 12 MIU per person.

Patients

Patients according to the invention are patients suffering from multiple sclerosis, preferably RRMS or early SPMS.

In an embodiment of the invention, patients are selected from human males or females between 18 and 55 years age.

In another embodiment of the invention, patients had at least one relapse within the prior 12 months of the treatment.

Use according to the invention

In one embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

- (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;

- (ii) A Cladribine-free period wherein no Cladribine is administered;
- (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
- (iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months or up to about 3 months or up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 2 months.

In a further embodiment, the invention provides a use according to the invention wherein the induction period lasts up to about 4 months.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

In a further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months or up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (ii) period lasts up to about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (ii) period lasts at least about 8 months.

In another further embodiment, the invention provides a use according to the invention
5 wherein the Cladribine-free period (ii) lasts up to about 10 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (iv) period lasts up to about 10 months.

10 In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free (iv) period lasts at least about 8 months.

In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free periods (ii) and/or (iv) last between about 8 and about 10
15 months.

In another further embodiment, the invention provides a use according to the invention wherein a placebo-pill is administered during the Cladribine-free period.

20 In another further embodiment, the invention provides a use according to the invention wherein the Cladribine-free period is free of any administration.

In another further embodiment, the invention provides a use according to the invention wherein the maintenance period lasts up to about 4 months, or up to about 3 months, or up
25 to about 2 months, preferably up to about 2 months.

In another further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the maintenance period (iii) is about 1.7 mg/kg.

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In another further embodiment, the invention provides a use according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

In a preferred embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

- (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;
 - (ii) A Cladribine-free period wherein no Cladribine is administered;
 - (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i)
 - (iv) A Cladribine-free period wherein no Cladribine is administered;
- wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

In another embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

- (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;
- (ii) A Cladribine-free period wherein no Cladribine is administered;

- (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period (iii) is lower than the total effective dose of Cladribine reached at the end of the induction period (i);
- 5 (iv) A Cladribine-free period wherein no Cladribine is administered.

In a further embodiment, the invention provides a use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:

- 10 (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;
- (ii) A Cladribine-free period wherein no Cladribine is administered;
- (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);
- 15 (iv) A Cladribine-free period wherein no Cladribine is administered;

wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months; the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

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In a preferred embodiment, the invention provides Cladribine for use as a medicament for the treatment of multiple sclerosis wherein the medicament is to be orally administered following the sequential steps below:

- (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;
- (ii) A Cladribine-free period wherein no Cladribine is administered;
- 5 (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
- (iv) A Cladribine-free period wherein no Cladribine is administered;
- 10 wherein the induction period last up to about 4 months, or up to about 3 months, or up to about 2 months; the Cladribine-free period (ii) lasts up to about 10 months, or up to about 9 months, or up to about 8 months; the maintenance period (iii) lasts up to about 2 months; the Cladribine-free period (iv) lasts up to about 10 months; the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg and steps (iii) to (iv) are
- 15 repeated performed one, two or three times.

In another embodiment, the invention provides a a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine about 3 to 30 mg Cladribine, preferably 5 to 20 mg Cladribine, most
20 preferably 10 mg Cladribine.

In another further embodiment, the invention provides a use according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of Cladribine reached at the end of the maintenance period is
25 about 1.7 mg/kg.

In another further embodiment, the invention provides a use according to the invention wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg and the total effective dose of Cladribine reached at the end of the
30 maintenance period is about 0.7 mg/kg.

In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered once a day during the induction period.

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In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered several times a day administered once a day during the induction period, preferably twice or three times a day, more preferably twice a day.

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In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 1 to about 7 days per month, preferably from about 5 to about 7 days per month during the induction period.

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In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 0.02 days/kg to about 0.08 days/kg per month during the induction period.

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In another embodiment, the invention provides a use of Cladribine according to the invention whereby the pharmaceutical formulation is orally administered about 0.02 days/kg to about 0.08 days/kg per month during the maintenance period.

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In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 2 each month during the induction period.

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In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily

dose of about 10 mg Cladribine from day 1 to about day 3 each month during the induction period.

5 In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 4 each month during the induction period.

10 In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 5 each month during the induction period.

15 In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 6 each month during the induction period.

20 In another embodiment, the invention provides a use of Cladribine according to the invention wherein the pharmaceutical formulation is to be orally administered at a daily dose of about 10 mg Cladribine from day 1 to about day 4 each month during the induction period and wherein the pharmaceutical formulation is a pharmaceutical formulation described in WO 2004/087101 or in WO 2004/087100.

25 In another embodiment, the invention provides a use of Cladribine according to any of the preceding claims wherein the pharmaceutical formulation is to be administered in combination with interferon-beta.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical formulation thereof in a patient in need thereof comprising the following steps:

- 5 (i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the end of the induction period is from about 1.5 mg/kg to about 3.5 mg/kg;
- (ii) A Cladribine-free period wherein no Cladribine is administered;
- (iii) A maintenance period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total dose of Cladribine reached at the
10 end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
- (iv) A Cladribine-free period wherein no Cladribine is administered.

In a preferred embodiment, the invention provides a method for the treatment of multiple sclerosis, comprising the oral administration of Cladribine or of a pharmaceutical
15 formulation thereof in a patient in need thereof comprising the following steps:

- (i) An induction period wherein Cladribine or a pharmaceutical formulation thereof is administered and wherein the total effective dose of Cladribine reached at the end of the induction period is from about 0.7 mg/kg to about 1.4 mg/kg;
- 20 (ii) A Cladribine-free period wherein no Cladribine is administered;
- (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total effective dose of Cladribine reached at the end of the maintenance period is lower than the total effective dose of Cladribine reached at the end of the induction period (i);
- 25 (iv) A Cladribine-free period wherein no Cladribine is administered.

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) to (iv) are repeated at least one or two times.

In a preferred embodiment, the invention provides a method of treating multiple sclerosis with Cladribine, wherein Cladribine is orally administered following the sequential steps below:

- 5 (i) Administering Cladribine, such that the total dose of Cladribine reached at the end of the induction period is from about 1.7 mg/kg to about 3.5 mg/kg;
- (ii) Administering no Cladribine during a Cladribine free period;
- (iii) Administering Cladribine such that the total dose of Cladribine reached at the end of a maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
- 10 (iv) And optionally, a Cladribine-free period wherein no Cladribine is administered.

15 In a further preferred embodiment, the invention provides a method wherein the induction period lasts up to about 4 months, or up to about 3 months, or up to about 2 months.

In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the induction period is about 1.7 mg/kg.

20 In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg.

In a further preferred embodiment, the invention provides a method wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg.

25 In a further preferred embodiment, the invention provides a method wherein the Cladribine-free period lasts up to about 10 months, or up to about 9 months, or up to about 8 months.

In a further preferred embodiment, the invention provides a method wherein the maintenance period lasts up to about 4 months, or up to about 3 months or up to about 2
30 months.

In a further preferred embodiment, the invention provides a method wherein the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

- 5 In a further preferred embodiment, the invention provides a method wherein the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg.

- 10 In a further preferred embodiment, the invention provides a method wherein the maintenance period is followed by a Cladribine-free period.

- 15 In another further embodiment, the invention provides a method according to the invention wherein the total dose of Cladribine reached at the end of the induction period is about 3.5 mg/kg and the total dose of Cladribine reached at the end of the maintenance period is about 1.7 mg/kg.

- 20 In another further embodiment, the invention provides a method according to the invention wherein the total effective dose of Cladribine reached at the end of the induction period is about 1.4 mg/kg and the total effective dose of Cladribine reached at the end of the maintenance period is about 0.7 mg/kg.

In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be orally administered at a daily dose of about 3 to about 30 mg.

- 25 In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be orally administered at a daily dose of about 10 mg.

- 30 In another further embodiment, the invention provides a method according to the invention wherein Cladribine is orally administered about 1 to about 7 days per month during the induction period.

In another further embodiment, the invention provides a method according to the invention wherein the steps (iii) are repeated at least one or two times.

- 5 In another further embodiment, the invention provides a method according to the invention wherein Cladribine is to be administered in combination with interferon-beta.

Examples

The following abbreviations refer respectively to the definitions below:

- 10 **kg** (kilogram), **μg** (microgram), **mg** (milligram), **AEs** (Adverse effects), **CNS** (Central nervous system), **CSF** (Cerebrospinal fluid), **EDSS** (Expanded Disability Status Scale, **SNRS** (Scripps Neurologic Rating Scale), **IFN** (interferon), **i.v.** (intra-veinous), **MIU** (Million International units), **MS** (multiple sclerosis), **MRI** (Magnetic resonance imaging), **p.o.** (per os), **PPMS** (Primary progressive multiple sclerosis), **PRMS** (Progressive relapsing multiple sclerosis), **RRMS** (Relapsing-remitting multiple sclerosis), **SPMS** (Secondary progressive multiple sclerosis), **s.c.** (subcutaneous), **TIW** (Three times a week),
15 **2-CdA** (2-chloro-2'deoxyadenosine or Cladribine), **UI** (International unit).

- The efficacy and safety of oral Cladribine administration, eventually multi-dose
20 administration, according to the invention can be assessed for example following the protocol below:

Example 1: Oral cladribine in the treatment of relapsing forms of MS

- A study of sixty patients with relapsing forms of clinically definite multiple sclerosis is
25 undertaken. Each patient is first examined for normal hepatic, renal, and bone marrow functioning to establish baseline values.

Patients are selected from Male or Female, between 18 and 55 years of age who had one or more relapses within the prior 12 months. Female patients are non-pregnant female.

Patients are randomly assigned to one of the treatment groups listed in Table 1 below:

Table 1:

Group	2-CdA
1	-
2	1.75 mg/kg
3	3.5 mg/kg

Each of the patients in Groups 2 and 3 receives 3 mg or 10 mg 2-CdA (1, 2 or 3 administration(s) a day depending on the patient's weight) combined in cyclodextrin formulation as described in WO 2004/087101, Example 3. The Compositions of the Cladribine formulations in 3 mg or 10 mg 2-CdA tablets containing hydroxypropyl-beta-cyclodextrin are listed in Table 2 below:

Table 2:

Name of ingredients	Formula mg/tablet	Formula mg/tablet
Cladribine-2-hydroxypropyl- β -cyclodextrin- complex*	153.75 equivalent to 10 mg 2-CdA	30.60 equivalent to 3 mg 2-CdA
Sorbitol powder	44.25	68.4
Magnesium Stearate (vegetable grade)	2.0	1.00
Total	200.0	100

* Cladribine is complexed and lyophilised with 2-hydroxypropyl- β -cyclodextrin as a separate process as described in WO 2004/087101.

Examples of administration schemes for the induction period depending on the patient's weight are given below in Tables 3 and 4 for the target doses of 1.75 mg/kg and 3.5 mg/kg respectively. For the maintenance period, the example of administration scheme of Table 3 is applicable.

Table 3:

Patient weight ranges (kg)			Total target dose (kg) equivalent to 1.75 mg/kg		Number of pills (10 mg)/induction period		
Min	Mid range	Max	Min	Max	Month 1	Month 2	Total
40	42.5	44.9	28	31.4	4	3	7
45	47.5	49.9	31.5	34.9	4	4	8
50	52.5	54.9	35	38.4	5	4	9
55	57.5	59.9	38.5	41.9	5	5	10
60	62.5	64.9	42	45.4	5	5	10
65	67.5	69.9	45.5	48.9	6	5	11
70	72.5	74.9	49	52.4	6	6	12
75	77.5	79.9	52.5	55.9	7	6	13
80	82.5	84.9	56	59.4	7	6	13
85	87.5	89.9	59.5	62.9	7	7	14
90	92.5	94.9	63	66.4	8	7	15
95	97.5	99.9	66.5	69.9	8	8	16
100	102.5	104.9	70	73.4	9	8	17
105	107.5	109.9	73.5	76.9	9	9	18
110	112.5	114.9	77	80.4	9	9	18
115	117.5	119.9	80.5	83.9	10	9	19

Table 4:

Patient weight ranges (kg)			Total target dose (kg) equivalent to 3.5 mg/kg		Number of pills (10 mg)/induction period				
Min	Mid range	Max	Min	Max	Month 1	Month 2	Month 3	Month 4	Total
40	42.5	44.9	56	62.9	4	4	3	3	14
45	47.5	49.9	63	69.9	4	4	4	4	16
50	52.5	54.9	70	76.9	5	4	4	4	17
55	57.5	59.9	77	83.9	5	5	5	4	19
60	62.5	64.9	84	90.9	6	5	5	5	21
65	67.5	69.9	91	97.9	6	6	5	5	22
70	72.5	74.9	98	104.9	6	6	6	6	24

Patient weight ranges (kg)			Total target dose (kg) equivalent to 3.5 mg/kg		Number of pills (10 mg)/induction period				
Min	Mid range	Max	Min	Max	Month 1	Month 2	Month 3	Month 4	Total
75	77.5	79.9	105	111.9	7	7	6	6	26
80	82.5	84.9	112	118.9	7	7	7	6	27
85	87.5	89.9	119	125.9	7	7	7	7	28
90	92.5	94.9	126	132.9	8	8	7	7	30
95	97.5	99.9	133	139.9	8	8	8	8	32
100	102.5	104.9	140	146.9	9	8	8	8	33
105	107.5	109.9	147	153.9	9	9	9	8	35
110	112.5	114.9	154	160.9	10	9	9	9	37
115	117.5	119.9	161	167.9	10	10	9	9	38

In Group 1 patients receive a placebo (saline) for 4 months followed by 8 months of no treatment.

- 5 In Group 2 patients receive a daily oral administration of Cladribine for about 5 days a month during 2 months (induction period) of 2-CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg (total dose of about 1.75 mg/kg for a bioavailability of about 40%); followed by administration of placebo for 2 months; followed by 8 months of no treatment.

10

In Group 3 patients receive a daily oral administration of Cladribine for about 5 days a month during 4 months (induction period) of 2-CdA cyclodextrin formulation such as the total effective dose administered at the end of the first 4 months approximates about 1.4 mg/kg (total dose of about 3.5 mg/kg for a bioavailability of about 40%); followed by 8 months of no treatment.

15

Beginning at month 13, all 3 patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with

the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 months of no treatment.

5 Finally, beginning at month 25, all patient groups receive re-treatment with Cladribine cyclodextrin formulation for about 5 days a month for 2 months (maintenance period) with the lower dose (such as the total effective dose administered at the end of the first 2 months approximates about 0.7 mg/kg) followed by 10 more months of no treatment.

10 Patients are monitored to determine whether there is any progression or improvement of brain lesions associated with progression of MS through MRI scans and neurological examination as described in *Miller et al., 1996, above; Evans et al., 1997, above; Sipe et al., 1984, above; and Mattson, 2002, above*. All patients have a baseline and MRI study (brain or spinal cord, according to localization of the lesions) at month 12.

15 The patient's disability progression and the time for having a first relapse are monitored as well as the proportion of relapse-free patients at 24 months.

Lymphocyte markers and monocyte counts are monitored in the patients.

Patients in Groups 2 and 3 have a decrease in brain lesions.

20 The data show that the 2-CdA regimen consisting in the succession of an induction treatment and maintenance treatments is efficient in decreasing brain lesions and no severe adverse effect is observed.

Claims:

1. Use of Cladribine for the preparation of a pharmaceutical formulation for the treatment of multiple sclerosis wherein the formulation is to be orally administered following the sequential steps below:
 - (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from 1.7 mg/kg to 3.5 mg/kg;
 - (ii) A Cladribine-free period wherein no Cladribine is administered;
 - (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
 - (iv) A Cladribine-free period wherein no Cladribine is administered.
2. Use according to claim 1 wherein the induction period lasts up to 4 months, or up to 3 months, or up to 2 months.
3. Use according to claims 1 or 2 wherein the induction period lasts up to 2 months.
4. Use according to any of the preceding claims wherein the induction period lasts up to 4 months.
5. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the induction period is 1.7 mg/kg.
6. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the induction period is 3.5 mg/kg.

7. Use according to any of the preceding claims wherein the Cladribine-free period lasts up to 10 months, or up to 9 months, or up to 8 months.
8. Use according to any of the preceding claims wherein the Cladribine-free (iv) period lasts up to 10 months.
9. Use according to any of the preceding claims wherein the maintenance period lasts up to 4 months, or up to 3 months or up to 2 months.
10. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the maintenance period is 1.7 mg/kg.
11. Use according to claim 1 wherein the formulation is to be orally administered following the sequential steps below:
- (i) An induction period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the induction period is from 1.7 mg/kg to 3.5 mg/kg;
 - (ii) A Cladribine-free period wherein no Cladribine is administered;
 - (iii) A maintenance period wherein Cladribine pharmaceutical formulation is administered and wherein the total dose of Cladribine reached at the end of the maintenance period is lower than the total dose of Cladribine reached at the end of the induction period (i);
 - (iv) A Cladribine-free period wherein no Cladribine is administered;
- wherein the induction period lasts up to 4 months, or up to 3 months or up to 2 months; the Cladribine-free period (ii) lasts up to 10 months, or up to 8 months or up to 10 months; the maintenance period (iii) lasts up to 2 months; the Cladribine-free period (iv) lasts up to 10 months; the total dose of Cladribine reached at the end of the maintenance period is 1.7 mg/kg and steps (iii) to (iv) are repeated performed one, two or three times.

12. Use according to any of the preceding claims wherein the total dose of Cladribine reached at the end of the induction period is 3.5 mg/kg and the total dose of Cladribine reached at the end of the maintenance period is 1.7 mg/kg.
- 5 13. Use according to any of the preceding claims wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine 3 to 30 mg Cladribine.
- 10 14. Use according to any of the preceding claims wherein the pharmaceutical formulation is to be orally administered at a daily dose of Cladribine 10 mg Cladribine.
- 15 15. Use according to any of the preceding claims wherein the pharmaceutical formulation is orally administered 1 to 7 days per month during the induction period.
16. Use according to any of the preceding claims wherein the steps (iii) to (iv) are repeated at least one or two times.
- 20 17. Use according to any of the preceding claims wherein the pharmaceutical formulation is to be administered in combination with interferon-beta.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/7076 A61K38/21 A61P25/00

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)
A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, EMBASE, SCISEARCH, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRIEB PAWEL ET AL: "Effect of repeated treatments with cladribine (2-chlorodeoxyadenosine) on blood counts in multiple sclerosis patients" ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS, vol. 43, no. 5-6, 1995, pages 323-327, XP008047072 ISSN: 0004-069X cited in the application abstract page 324, column 1, paragraph 3 page 326, column 2, paragraph 3 ----- -/--	1,7-9, 13-16

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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- * & * document member of the same patent family

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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.
X	STELMASIAK Z ET AL: "A pilot trial of cladribine (2-chlorodeoxyadenosine) in remitting- relapsing multiple sclerosis" MEDICAL SCIENCE MONITOR 1998 POLAND, vol. 4, no. 1, 1998, pages 4-8, XP008047060 ISSN: 1234-1010 cited in the application abstract page 5, column 1, paragraph 2 - column 2, paragraph 1 page 7, column 1, paragraph 3	1,7-9, 13-16
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2005/056954

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2005/056954

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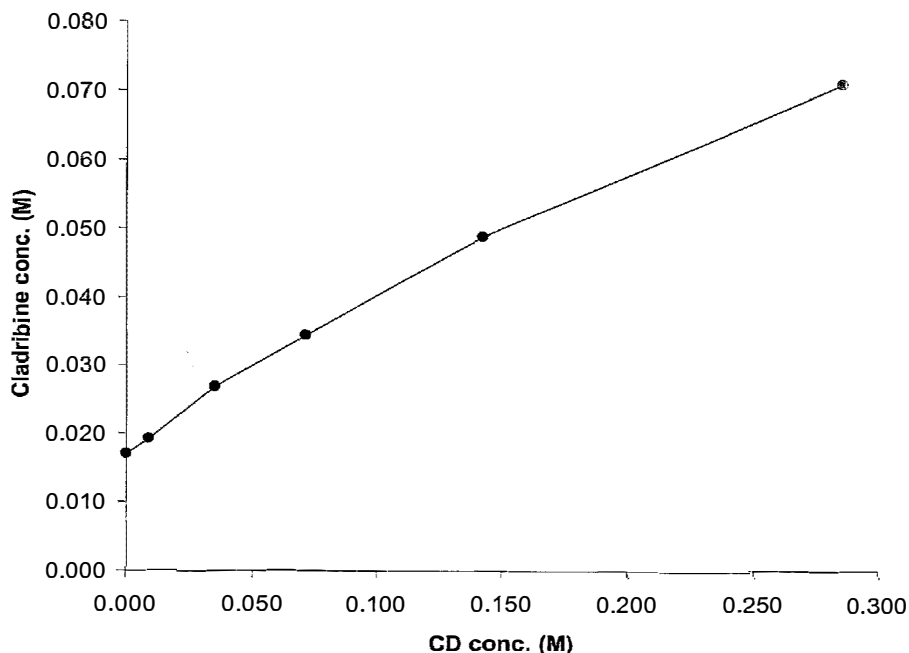
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- (71) Applicant (for all designated States except US): **IVAX CORPORATION** [US/US]; 4400 Biscayne Boulevard, Miami, Florida 33137 (US).
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- (74) Agents: **STEPNO, Norman, H.** et al.; BURNS, DOANE, SWECKER & MATHIS, LLP, PO BOX 1404, Alexandria, VA 22313-01404 (US).
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[Continued on next page]

(54) Title: ORAL FORMULATIONS OF CLADRIBINE



(57) Abstract: ABSTRACT OF THE DISCLOSURE Provided are compositions of cladribine and cyclodextrin which are especially suited for the oral administration of cladribine.

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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.

-1-

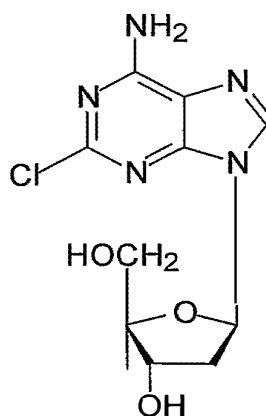
ORAL FORMULATIONS OF CLADRIBINE

FIELD OF THE INVENTION

5 The invention relates to a composition comprising a complex
cladribine-cyclodextrin complex formulated into a solid oral dosage form and
to a method for enhancing the oral bioavailability of cladribine.

BACKGROUND OF THE INVENTION

10 Cladribine, which is an acid-labile drug, has the chemical structure as
set forth below:



It is also known as 2-chloro-2'-deoxyadenosine or 2-CdA. Cladribine exists
as a white, nonhygroscopic, crystalline powder, consisting of individual
crystals and of crystalline aggregates.

15 Cladribine is an antimetabolite which has use in the treatment of
lymphoproliferative disorders. It has been used to treat experimental
leukemias such as L1210 and clinically for hairy cell leukemia and chronic
lymphocytic leukemia as well as Waldenstrom's macroglobulinaemia. It has

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also been used as an immunosuppressive agent and as a modality for the treatment of a variety of autoimmune conditions including rheumatoid arthritis, inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis) and multiple sclerosis (see e.g., J. Liliemark, *Clin. Pharmacokinet*, 5 32(2): 120-131, 1997). It has also been investigated, either experimentally or clinically in, for example, lymphomas, Langerhan's cell histiocytosis, lupus erythematosus, chronic plaque psoriasis, Sezary syndrome, Bing-Neel syndrome, recurrent glioma, and solid tumors.

Oral delivery of drugs is often preferred to parenteral delivery for a variety of reasons, foremost patient compliance, or for cost or therapeutic considerations. Patient compliance is enhanced insofar as oral dosage forms alleviate repeated health care provider visits, or the discomfort of injections or prolonged infusion times associated with some active drugs. At a time of escalating health care costs, the reduced costs associated with oral administration versus parenteral administration costs gain importance. The cost of parenteral administration is much higher due to the requirement that a health care professional administer the cladribine in the health care provider setting, which also includes all attendant costs associated with such administration. Furthermore, in certain instances, therapeutic considerations 15 such as the need for a slow release of cladribine over a prolonged period of time may be practically met only by oral or transmucosal delivery.

However, to date the oral delivery of cladribine has been plagued by low bioavailability (see, e.g., J. Liliemark *et al.*, *J. Clin. Oncol.*, 10(10): 1514-1518, 1992), and suboptimal interpatient variation (see, e.g., J. Liliemark, 25 *Clin. Pharmacokinet*, 32 (2): 120-131, 1997). See also, A. Tarasuik, *et al.* reporting poor absorption and pH dependent lability (*Arch. Immunol. et Therapiae Exper.*, 42: 13-15, 1994).

Cyclodextrins are cyclic oligosaccharides composed of cyclic α -(1 $\rightarrow$ 4) linked D-glucopyranose units. Cyclodextrins with six to eight units have

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been named α -, β - and γ -cyclodextrin, respectively. The number of units determines the size of the cone-shaped cavity which characterizes cyclodextrins and into which drugs may be included to form stable complexes. A number of derivatives of α -, β - and γ -cyclodextrin are known in which one or more hydroxyl groups is/are replaced with ether groups or other radicals. These compounds are thus known complexing agents and have been previously used in the pharmaceutical field to form inclusion complexes with water-insoluble drugs and to thus solubilize them in aqueous media.

Recently, Schultz *et al.*, in U.S. Patent No. 6,194,395 B1, have described complexing and solubilizing cladribine with cyclodextrin. The Schultz *et al.* patent primarily addresses the problems inherent in previously described aqueous formulations of cladribine, particularly for subcutaneous and intramuscular injection. Schultz *et al.* have found that cladribine is not only significantly more soluble in aqueous media when formulated with cyclodextrin, but also is more stable against acid-catalyzed hydrolysis when combined with cyclodextrin. The latter finding is taught to be of particular benefit in the formulation of solid oral dosage forms, where the compound would normally undergo hydrolysis in the acid pH of the stomach contents. Schultz *et al.* do not appear to have described any actual work in connection with solid oral dosage forms. In fact, they describe only one method of preparing the solid dosage form, which is a melt extrusion process, in which the cladribine and cyclodextrin are mixed with other optional additives and then heated until melting occurs. Furthermore, the broad dosage ranges of 1 mg to 15 mg of cladribine and 100 mg to 500 mg of cyclodextrin listed in the patent suggest no criticality to the particular amount of cyclodextrin to be present with a given amount of cladribine in a solid oral dosage form. Indeed, these dosage ranges include many combinations which may be suitable as mixtures but not for complex formation. For example, a ratio of 1 mg of cladribine to 500 mg of cyclodextrin contains too much cyclodextrin, so

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that the drug would not readily leave the complex and achieve its therapeutic function. On the other hand, 15 mg of cladribine and only 100 mg of cyclodextrin would not be enough to complex that amount of cladribine.

5 The Schultz *et al.* patent does suggest improving the stability of cladribine in oral dosage forms by combining/complexing it with cyclodextrin, but does not suggest improving the drug's oral bioavailability by such means; in fact, the patent does not describe or suggest a method for enhancing or maximizing the bioavailability of cladribine from a solid oral dosage form of cladribine and cyclodextrin, or a composition specially designed to do so.

10 Many workers have studied the solubility of specific drugs in water containing various concentrations of selected cyclodextrins in order to demonstrate that increasing concentrations of cyclodextrins increase the solubility of the drugs at selected temperatures and pH levels, as for example reported in the Schultz *et al.* patent. Phase solubility studies have
15 also been performed by various workers in order to elucidate the nature of the complex formation, for example, whether the cyclodextrin and drug form a 1:1 complex or a 1:2 complex; see, for example, Harada *et al.* U.S. Patent No. 4,497,803, relating to inclusion complexes of lankacidin-group antibiotics with cyclodextrin, and Shinoda *et al.* U.S. Patent No. 4,478,995, relating to a
20 complex of an acid addition salt of (2'-benzyloxycarbonyl)phenyl trans-4-guanidinomethylcyclohexanecarboxylate with a cyclodextrin.

 While Schultz *et al.* teach that a cladribine-cyclodextrin complex improves the water solubility and acid stability of cladribine, the art does not suggest how to maximize or enhance the benefits of the complexation in
25 terms of bioavailability and interpatient variation when the complex is to be administered in a solid oral dosage form.

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SUMMARY OF THE INVENTION

It has now been found that amorphous cyclodextrins can be combined with cladribine to form a particularly advantageous product which can be incorporated into a solid oral dosage form. This product is a complex
5 cladribine-cyclodextrin complex, and the solid oral dosage form containing it improves oral bioavailability and/or achieves lower interpatient and/or inpatient variation of the drug.

The present invention provides a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous
10 inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, and a pharmaceutical composition comprising said complex, formulated into a solid oral dosage form. Thus, the cyclodextrin itself is amorphous, the inclusion complex with cladribine is amorphous (and
15 is preferably saturated with cladribine) and the free cladribine which forms the non-inclusion complex is amorphous.

The invention also provides a method for increasing or enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof, a pharmaceutical composition comprising a complex
20 cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form which maximizes the amount of cladribine in the inclusion and non-
25 inclusion complexes.

The invention further provides for treatment of conditions responsive to administration of cladribine in mammals by administering thereto the composition of the invention. Use of cladribine in the preparation of the pharmaceutical compositions of the invention for administration to treat

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cladribine-responsive conditions and for enhancing the oral bioavailability of cladribine is also provided.

Still further, the invention provides a process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

5 (i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature; and

(iii) lyophilizing the cooled solution to afford an amorphous product.

10 In yet a further aspect the invention provides a pharmaceutical composition obtainable by a process comprising the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

15 (ii) cooling the resultant aqueous solution to room temperature;

(iii) lyophilizing the cooled solution to afford an amorphous product;

and

(iv) formulating the amorphous product into a solid oral dosage form.

20 BRIEF DESCRIPTION OF THE DRAWING

A more complete appreciation of the invention and its many attendant advantages will be readily understood by reference to the following detailed description and the accompanying drawing, wherein the sole Figure is a graphical representation of the results of a phase solubility study where
25 various molar concentrations of hydroxypropyl- β -cyclodextrin (HP β CD) are plotted against various cladribine molar concentrations, with (●) representing

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the data points obtained for complexation under conditions specified in
EXAMPLE 2 below.

DETAILED DESCRIPTION OF THE INVENTION

5 Throughout the instant specification and claims, the following
definitions and general statements are applicable.

 The patents, published applications, and scientific literature referred
to herein establish the knowledge of those with skill in the art and are hereby
incorporated by reference in their entirety to the same extent as if each was
10 specifically and individually indicated to be incorporated by reference. Any
conflict between any reference cited herein and the specific teachings of this
specification shall be resolved in favor of the latter. Likewise, any conflict
between an art-understood definition of a word or phrase and a definition of
the word or phrase as specifically taught in this specification shall be
15 resolved in favor of the latter.

 The term "inclusion complex" as used herein refers to a complex of
cladribine with the selected cyclodextrin wherein the hydrophobic portion of
the cladribine molecule (the nitrogen-containing ring system) is inserted into
the hydrophobic cavity of the cyclodextrin molecule. This is often referred to
20 simply as a cyclodextrin complex of the drug.

 The term "non-inclusion complex" refers to a complex which is not an
inclusion complex; rather than the hydrophobic portion of cladribine being
inserted in the cyclodextrin cavity, the non-inclusion complex is formed
primarily by hydrogen-bonding of the hydroxyls and amino group on "free"
25 cladribine, (*i.e.* cladribine not in the inclusion complex) to the hydroxyls on
the exterior of the cyclodextrin torus (*e.g.* in the case of hydroxypropyl- β -
cyclodextrin, hydroxypropyl and hydroxyl groups on the glucose rings). This
is a more loosely-held association than an inclusion complex.

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As used herein, whether in a transitional phrase or in the body of a claim, the terms "comprise(s)" and "comprising" are to be interpreted as having an open-ended meaning. That is, the terms are to be interpreted synonymously with the phrases "having at least" or "including at least".

5 When used in the context of a process, the term "comprising" means that the process includes at least the recited steps, but may include additional steps. When used in the context of a composition, the term "comprising" means that the composition includes at least the recited features or components, but may also include additional features or components.

10 The terms "consists essentially of" or "consisting essentially of" have a partially closed meaning, that is, they do not permit inclusion of steps or features or components which would substantially change the essential characteristics of a process or composition; for example, steps or features or components which would significantly interfere with the desired properties of

15 the compositions described herein, *i.e.*, the process or composition is limited to the specified steps or materials and those which do not materially affect the basic and novel characteristics of the invention. The basic and novel features herein are the provision of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous

20 inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form, so as to provide improved bioavailability and/or lower interpatient and/or inpatient variation following administration. Essential to the invention is the combination of the

25 amorphous nature of the starting cyclodextrin, and the level of water solubility exhibited by cladribine (about 5 mg/ml at room temperature), and consequently its capability for hydrogen bonding, which can be taken advantage of under particular conditions described hereinafter, and which afford a special amorphous mixture uniquely well-suited for optimizing the

30 oral bioavailability of cladribine.

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The terms "consists of" and "consists" are closed terminology and allow only for the inclusion of the recited steps or features or components.

As used herein, the singular forms "a," "an" and "the" specifically also encompass the plural forms of the terms to which they refer, unless the
5 content clearly dictates otherwise.

The term "about" is used herein to means approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" or
10 "approximately" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

The term "amorphous" is used herein to refer to a noncrystalline solid. The cyclodextrins encompassed herein themselves are amorphous because they are each composed of a multitude of individual isomers, and their
15 complexes with cladribine are also amorphous. Further, conditions for complexation can be selected (elevated temperature and prolonged complexation times, as described hereinafter) so that a supersaturated cladribine solution will be formed. When cooled, because of the amorphous nature of the complex and the cyclodextrin, some excess free cladribine
20 does not precipitate but rather is trapped in amorphous form in intimate admixture with the (preferably saturated) amorphous cladribine-cyclodextrin inclusion complex. This excess cladribine forms a loosely-held association, or non-inclusion complex, with the cyclodextrin through hydrogen bonding. This, then, further increases the amount of cladribine in the product; this
25 additional cladribine, because it is amorphous and also because it is in intimate admixture with the amorphous inclusion complex, is expected to be somewhat protected from degradation by stomach acid (although it may not be as protected as the cladribine which is in the form of the inclusion complex).

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The term "saturated" when used in conjunction with a complex of cladribine in amorphous cyclodextrin means that the complex is saturated with cladribine, that is, the complex contains the maximum amount of cladribine which can be complexed (by means of both inclusion and non-inclusion complexes) with a given amount of cyclodextrin under the conditions of complexation used. A phase solubility study can be used to provide this information, as described in more detail hereinafter. (Conditions for the complexation are also described in more detail below.) Alternatively, a saturated complex may be arrived at empirically by simply adding cladribine to an aqueous solution of the selected cyclodextrin until no more cladribine goes into solution; ultimately, excess cladribine, if any, is removed (by filtration or centrifugation) and the solution lyophilized to provide the dry saturated complex.

The expression "substantially", as in "substantially free" means within 20% of the exact calculated amount, preferably within 10%, most preferably within 5%.

The term "interpatient variability" refers to variation among patients to which a drug is administered. The term "intrapatient variability" refers to variation experienced by a single patient when dosed at different times.

As used herein, the recitation of a numerical range for a variable is intended to convey that the invention may be practiced with the variable equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable can be equal to any integer value of the numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable can be equal to any real value of the numerical range, including the end-points of the range. As an example, a variable which is described as having values between 0 and 2, can be 0, 1 or 2 for variables which are inherently discrete, and can be 0.0,

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0.1, 0.01, 0.001, or any other real value for variables which are inherently continuous.

In the specification and claims, the singular forms include plural referents unless the context clearly dictates otherwise. As used herein,
5 unless specifically indicated otherwise, the word "or" is used in the "inclusive" sense of "and/or" and not the "exclusive" sense of "either/or."

Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present invention pertains, unless otherwise defined. Reference is made herein to various
10 methodologies and materials known to those of skill in the art. Standard reference works setting forth the general principles of pharmacology include Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 10th Ed., McGraw Hill Companies Inc., New York (2001).

Reference is made hereinafter in detail to specific embodiments of the
15 invention. While the invention will be described in conjunction with these specific embodiments, it will be understood that it is not intended to limit the invention to such specific embodiments. On the contrary, it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims. In
20 the following description, numerous specific details are set forth in order to provided a thorough understanding of the present invention. The present invention may be practiced without some or all of these specific details. In other instances, well-known process operations have not been described in detail, in order not to unnecessarily obscure the present invention.

25 There is provided by the present invention compositions, as well as methods of making and of using pharmaceutical compositions, useful to achieve desirable pharmacokinetic properties. Such compositions stem from the discovery that solutions of cyclodextrin and cladribine in which cladribine is in a high thermodynamic state, when presented to the gastric mucosa

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through which they are absorbed are associated with improved cladribine absorption, as reflected by higher bioavailability and/or lower interpatient variation.

It is postulated, without wishing to so limit the invention, that upon
5 dissolution (e.g., by contact with a fluid, such as a bodily fluid), dry compositions according to the invention form a locally saturated cladribine solution in which cladribine is in the state of highest thermodynamic activity (HTA), thus favoring absorption. Cladribine has a fairly low, although not insignificant, intrinsic aqueous solubility; it is in fact somewhat water soluble.

10 The free cladribine formed from dissociation of the inclusion and non-inclusion complexes in a saturated aqueous solution seeks a more stable activity level by being absorbed through the gastric mucosa.

In view of the foregoing, it is apparent that to produce optimal pharmaceutical compositions, in a solid oral dosage form, these dosage
15 forms should be formulated to release a localized saturated cladribine solution, upon contact of the solid dosage forms with body fluid at the mucosa, in which cladribine is in its HTA state. To provide such a localized saturated solution *in vivo*, it is important to first identify the optimal ratio of cladribine to amorphous cyclodextrin, which ratio is referred to herein as the
20 HTA ratio, to be used in the solid dosage form.

The HTA ratio is empirically determined and is identified as the ratio of cladribine to amorphous cyclodextrin which corresponds to the maximum amount of cladribine that can be complexed with a given amount of the cyclodextrin. The HTA ratio may be determined using an empirical method
25 such as a phase solubility study to determine the saturation concentration of cladribine that can be solubilized with different concentrations of amorphous cyclodextrin solutions. Hence, the method identifies the concentrations at which a saturated cladribine-cyclodextrin complex is formed. It is noted that the molar ratio represented by a point on the phase solubility graph shows

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how many moles of amorphous cyclodextrin are the minimum needed to maintain the drug in the complex, under given conditions; this may then be converted to a weight ratio. For example, if a phase solubility diagram shows that 9 moles of a given cyclodextrin are needed to maintain the cladribine in a saturated complex, then multiplying the number of moles of cladribine by its molecular weight and multiplying the number of moles of the selected cyclodextrin by its molecular weight, one can arrive at the ratio of the products as an appropriate optimized weight ratio. A phase solubility study also provides information about the nature of the cladribine-cyclodextrin inclusion complex formed, for example whether the inclusion complex is a 1:1 complex (1 molecule of drug complexed with 1 molecule of cyclodextrin) or a 1:2 complex (1 molecule of drug complexed with 2 molecules of cyclodextrin).

In accordance with the present invention, one can start using either the selected amorphous cyclodextrin, such as hydroxypropyl- β -cyclodextrin (HP β CD) or hydroxypropyl- γ -cyclodextrin, or cladribine as the fixed variable to which an excess of the other is added to identify various solubility data points (indicating saturated cladribine-cyclodextrin complexes) and draw the resultant line. Typically, cladribine is added to an aqueous solution having a known concentration of amorphous cyclodextrin under conditions empirically found to promote complex formation. Generally, the complexation is conducted with heating, for example at about 45 to about 60°C for a significant period of time, *e.g.*, at least 6-9 hours; it is believed that even better results can be obtained by heating at up to about 80°C for up to 24 hours. Excess precipitated cladribine is then removed and the cladribine concentration is subsequently measured. This concentration represents the amount of cladribine solubilized for a given amorphous cyclodextrin concentration. This process is repeated for a different known concentration of cyclodextrin until several data points are obtained. Each data point represents the concentration of the cladribine dissolved in a known

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concentration of the selected amorphous cyclodextrin. The data points are then plotted to show the concentration of cladribine against the various cyclodextrin concentrations used. The graph is a phase solubility diagram which can be used to determine the amount of cladribine for any specific concentration of cyclodextrin used to form the solution under a given set of complexation conditions. It will be appreciated that the aqueous solubility of cladribine is about 5 mg/ml at room temperature and would be higher at elevated temperature. Consequently, the data points correspond to the amount of cladribine dissolved in aqueous HP β CD or other amorphous cyclodextrin under the selected conditions; when later lyophilized, the solution yields a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex. If equilibrium conditions are reached during the complexation, the amorphous cladribine-cyclodextrin complex will be saturated with cladribine.

One of skill in the art will appreciate that concentrations at which saturated complexes of cladribine with amorphous cyclodextrins are formed (and thus HTA ratios as well) may be identified by a variety of alternative methodologies. Accordingly, any method known in the field suitable to identify these concentrations is within the scope of the invention.

It has been discovered that desirable pharmacological properties (improved bioavailability and/or coefficient of variation as compared to traditional approaches) are associated with mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin.

Using intrinsically amorphous cyclodextrins, for example hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, randomly methylated cyclodextrins, and the like, with cladribine, which is a somewhat water soluble compound (capable of H-bonding through its free hydroxyl and

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amino groups), the cladribine provides increased solubility in solutions of these cyclodextrins. Not only is there increased water solubility but also H-bonded association of the cladribine with the cyclodextrin, separately from the actual inclusion complexed material.

5 One of skill in the art will appreciate that the phase solubility diagram for each given starting concentration ratio represents the starting point of one's investigation on the basis of which variables (reactants' concentrations, temperature and time) may be altered to promote inclusion complex and non-inclusion complex associations favoring a higher or lower
10 proportion of either type of association in the final product. Departure from the ratio of cladribine to cyclodextrin, the temperature and/or the dilution empirically found to promote equilibrium towards complex formation is then analyzed to promote the formation of mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin in various proportions
15 according to the invention.

 Thus, for example, by starting with more diluted cyclodextrin (*i.e.*, larger water volumes than that used for solubility plot analysis) logically will accommodate more cladribine in solution sequestering more of the same from complex formation. Upon evaporation, some of the solubilized
20 cladribine will tend to associate with cyclodextrin in a non-inclusion complex fashion. By altering the initial dilution, one may shift equilibrium towards inclusion complex or non-inclusion complex formation. Similarly, by increasing complexation temperature, the water solubility of cladribine may be increased while decreasing the stability of inclusion complexes, thus
25 promoting non-inclusion complexes. Thus, by altering complexation temperature, one may shift equilibrium towards inclusion complex or non-inclusion complex formation. Finally, complexation time may be altered to favor the formation of mixtures of inclusion complexes and non-inclusion complexes of cladribine and cyclodextrin according to the invention.

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As exemplified hereinafter, it is possible to maximize the cladribine in solid amorphous mixtures, by forcing additional cladribine into solution (using more dilute solutions of cyclodextrin, higher temperatures and longer complexation times, as indicated above). When the solution is cooled off, the extensively amorphous nature of these cyclodextrins does not allow crystallization of an excess amount of cladribine beyond that which forms an inclusion complex with the cyclodextrin; and upon freeze-drying/lyophilization, one obtains an amorphous mixture of cladribine-cyclodextrin inclusion complex (which is amorphous) and amorphous free cladribine, loosely associated with uncomplexed cyclodextrin (and even with complexed cyclodextrin) by hydrogen-bonding, that is, the non-inclusion complex.

As shown in the EXAMPLES, this may be done by maximizing solubilization by elevating the temperature (for example, to about 50° to 80°C), and stirring for many hours (up to 24 hours) before freeze-drying. The weight/weight ratios obtained were about 1:14 and 1:11. The apparent optimum weight/weight ratio under these exemplified conditions is the higher of these, or about 1:14 of cladribine: cyclodextrin. If too much excess cladribine is added to the complexation medium, then crystallization of some of the cladribine takes place, which would in turn result in some crystalline cladribine in the product; this undesired excess cladribine is not in solution and is not H-bonded to the amorphous cyclodextrin and lowers the weight ratio. Therefore, it is desirable to carefully control the amount of excess cladribine beyond that which will form the inclusion complex to only the amount which will dissolve in the solution. The desired amorphous mixture of amorphous inclusion complex and amorphous free cladribine can be termed a "complex cladribine-cyclodextrin complex," which includes both inclusion and non-inclusion/H-bonded complexes. The inclusion complex is a complex of cladribine inserted into the hydrophobic cavity of the selected amorphous cyclodextrin, while the non-inclusion/H-bonded complex is

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amorphous free cladribine loosely hydrogen-bonded to the cyclodextrin. It is estimated that about two-thirds (60 to 70%) of the cladribine will be in the non-inclusion complex, with the remaining one third (30 to 40%) being in the inclusion complex when the product is obtained as exemplified hereinbelow (17% HP β CD solution, 45 to 50°C complexation temperature for about 9 hours); by increasing the percentage of cyclodextrin used and/or manipulating the temperature, products can be readily obtained in which a much greater proportion of the amorphous mixture is in the form of the inclusion complex. In the case of a representative amorphous cyclodextrin, hydroxypropyl- β -cyclodextrin (HP β CD) a cladribine:cyclodextrin weight ratio of from about 1:10 to about 1:16 is appropriate for the exemplified conditions; the ratio is expected to be the same for hydroxypropyl- γ -cyclodextrin under those conditions. The material obtained is characterized by rapid dissolution of the cladribine in aqueous media.

Freeze-drying, also known as lyophilization, comprises three basic stages: first a freezing stage, then a primary drying stage and finally a secondary drying stage. EXAMPLE 2 below provides details of lyophilization as conducted on the batches described therein. This procedure can be further optimized by following the principles described by Xiaolin (Charlie) Tang and Michael J. Pikal in *Pharmaceutical Research*, Vol. 21, No. 2, February 2004, 191-200, incorporated by reference herein in its entirety and relied upon.

The above-described method requires amorphous cyclodextrins rather than originally crystalline cyclodextrins which have relatively low water solubilities, such as α -, β - or γ -cyclodextrin, 2,6-dimethyl- β -cyclodextrin and the like, because these cyclodextrins would allow crystallization of cladribine in excess of that forming an inclusion complex and therefore would not afford the desired amorphous mixture. The method also would not be useful if cladribine were highly hydrophobic/lipophilic, because in such a situation the drug would not have intrinsic aqueous solubility/H-bonding capability and

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could not provide the unique mixture obtained herein. However, in point of fact, cladribine has an aqueous solubility of 5 mg/ml at room temperature, thus a significant amount of the drug will be simply soluble in the water phase especially at higher than room temperature; also, as in the case of HP β CD, for example, some of the cladribine will be associated by hydrogen-bonding to the 2-hydroxypropyl and free glucose-OH groups in the cyclodextrin via the two hydroxy functions found in the deoxyadenosine moiety of the cladribine.

The cyclodextrins within the scope of this invention are amorphous derivatives of the natural cyclodextrins α -, β - or γ -cyclodextrin wherein one or more of the hydroxy groups are substituted, for example, by alkyl, hydroxyalkyl, carboxyalkyl, alkylcarbonyl, carboxyalkoxyalkyl, alkylcarbonyloxyalkyl, alkoxycarbonylalkyl or hydroxy-(mono or polyalkoxy)alkyl groups; and wherein each alkyl or alkylene moiety preferably contains up to six carbons. Although commonly referred to as a single entity, an amorphous cyclodextrin is actually a mixture of many different entities, since the substituent groups can be located on various hydroxyls of the basic cyclodextrin structure. This in turn results in the amorphous nature of these cyclodextrins, which is indeed well-known. Moreover, these cyclodextrins can be obtained in varying degrees of substitution, for example from 1 to 14, preferably from 4 to 7; the degree of substitution is the approximate average number of substituent groups on the cyclodextrin molecule, for example, the approximate number of hydroxypropyl groups in the case of the hydroxypropyl- β -cyclodextrin molecule, and all such variations are within the ambit of this invention. Substituted amorphous cyclodextrins which can be used in the invention include polyethers, for example, as described in U.S. Patent No. 3,459,731. Further examples of substituted cyclodextrins include ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C₁₋₆alkyl, hydroxy-C₁₋₆alkyl, carboxy-C₁₋₆alkyl or C₁₋₆alkyloxycarbonyl-

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C₁₋₆alkyl groups or mixed ethers thereof. In particular, such substituted cyclodextrins are ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C₁₋₃alkyl, hydroxy-C₂₋₄alkyl or carboxy-C₁₋₂alkyl or more particularly by methyl, ethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, carboxymethyl or carboxyethyl. The term "C₁₋₆alkyl" is meant to include straight and branched saturated hydrocarbon radicals, having from 1 to 6 carbon atoms such as methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, butyl, pentyl, hexyl and the like. Other cyclodextrins contemplated for use herein included glucosyl- β -cyclodextrin and maltosyl- β -cyclodextrin. Of particular utility in the present invention are randomly methylated β -cyclodextrin and polyethers such as hydroxypropyl- β -cyclodextrin, hydroxyethyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, and hydroxyethyl- γ -cyclodextrin, as well as sulfobutyl ethers, especially β -cyclodextrin sulfobutyl ether. In addition to simple cyclodextrins, branched cyclodextrins and cyclodextrin polymers may also be used. Other cyclodextrins are described, for example, in *Chemical and Pharmaceutical Bulletin* 28: 1552-1558 (1980); Yakugyo Jiho No. 6452 (28 March 1983); *Angew. Chem. Int. Ed. Engl.* 19: 344-362 (1980); U.S. Patent Nos. 3,459,731 and 4,535,152; European Patent Nos. EP 0 149 197A and EP 0 197 571A; PCT International Patent Publication No. WO90/12035; and UK Patent Publication GB 2,189,245.

References describing cyclodextrins for use in the compositions according to the present invention, and/or which provide a guide for the preparation, purification and analysis of cyclodextrins include the following:

Cyclodextrin Technology by Jozsef Szejtli, Kluwer Academic Publishers (1988) in the chapter Cyclodextrins in Pharmaceuticals; *Cyclodextrin Chemistry* by M. L. Bender *et al.*, Springer-Verlag, Berlin (1978); *Advances in Carbohydrate Chemistry*, Vol. 12, Ed. By M. L. Wolfrom, Academic Press, New York in the chapter "The Schardinger Dextrins" by Dexter French, pp. 189-260; *Cyclodextrins and their Inclusion Complexes* by J. Szejtli,

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Adakemiai Kiado, Budapest, Hungary (1982); I. Tabushi, *Acc. Chem. Research*, 1982, 15, pp. 66-72; W. Sanger, *Angewandte Chemie*, 92, p. 343-361 (1981); A. P. Croft *et al.*, *Tetrahedron*, 39, pp. 1417-1474 (1983); Irie *et al. Pharmaceutical Research*, 5, pp. 713-716 (1988); Pitha *et al.*, *Int. J. Pharm.* 29, 73 (1986); U.S. Patent Nos. 4,659,696 and 4,383,992; German Patent Nos. DE 3,118,218 and DE-3,317,064; and European Patent No. EP 0 094 157A. Patents describing hydroxyalkylated derivative of β - and γ -cyclodextrin include Pitha U.S. Patent Nos. 4,596,795 and 4,727,064, Müller U.S. Patent Nos. 4,764,604 and 4,870,060 and Müller *et al.* U.S. Patent No. 6,407,079.

Amorphous cyclodextrins of particular interest for complexation with cladribine include: hydroxyalkyl, *e.g.* hydroxyethyl or hydroxypropyl, derivatives of β - and γ -cyclodextrin; carboxyalkyl, *e.g.* carboxymethyl or carboxyethyl, derivatives of β - or γ -cyclodextrin; β -cyclodextrin sulfobutyl ether; and randomly methylated β -cyclodextrin. 2-Hydroxypropyl- β -cyclodextrin (HP β CD), 2-hydroxypropyl- γ -cyclodextrin (HP γ CD), randomly methylated β -cyclodextrin, β -cyclodextrin sulfobutyl ether, carboxymethyl- β -cyclodextrin (CM β CD) and carboxymethyl- γ -cyclodextrin (CM γ CD) are of special interest, especially hydroxypropyl- β -cyclodextrin and hydroxypropyl- γ -cyclodextrin.

Compositions of an amorphous mixture of amorphous free cladribine and an amorphous, preferably saturated, cladribine-cyclodextrin inclusion complex for use in the present invention can be prepared under conditions favoring complex formation in a liquid environment as described and as exemplified herein. The resultant liquid preparations can be subsequently converted to a dry form suitable for administration as a solid oral or transmucosal dosage form.

One of skill will appreciate that a variety of approaches are available in the field to prepare compositions as described herein. One available

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method exemplified herein includes the steps of mixing the cladribine in an aqueous solution of an amorphous cyclodextrin, separating un-dissolved cladribine (e.g., by filtering or centrifugation), and lyophilizing or freeze-drying the saturated solution to form a solid amorphous mixture.

5 Pharmaceutical compositions according to the invention may optionally include one or more excipients or other pharmaceutically inert components. One of the advantages of the invention, however, is that cladribine drug forms as described herein can be prepared with the minimal
10 amount of excipients necessary for shaping and producing the particular form, such as a tablet or patch. Excipients may be chosen from those that do not interfere with cladribine, with cyclodextrin or with complex formation.

 Dosage forms are optionally formulated in a pharmaceutically acceptable vehicle with any of the well-known pharmaceutically acceptable carriers, diluents, binders, lubricants, disintegrants, scavengers, flavoring
15 agents, coloring agents, and excipients (see *Handbook of Pharmaceutical Excipients*, Marcel Dekker Inc., New York and Basel (1998); Lachman *et al.* Eds., *The Theory and Practice of Industrial Pharmacy*, 3rd Ed., (1986); Lieberman *et al.*, Eds. *Pharmaceutical Dosage Forms*, Marcel Dekker Inc., New York and Basel (1989); and *The Handbook of Pharmaceutical*
20 *Excipients*, 3rd Ed., American Pharmaceutical Association and Pharmaceutical Press, 2000); see also *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, Mack Publishing Co., Easton, PA (1990) and *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams & Wilkins, (1995)). A simple solid oral dosage form consists of the amorphous
25 mixture of amorphous free cladribine and amorphous cladribine-cyclodextrin complex (preferably saturated) as described above, *i.e.* the complex cladribine-cyclodextrin complex, compressed with a small amount (e.g. about 1% by weight) of a suitable binder or lubricant such as magnesium stearate.

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In certain instances, oral absorption may be further facilitated by the addition of various excipients and additives to increase solubility or to enhance penetration, such as by the modification of the microenvironment.

5 The methods and pharmaceutical compositions described herein offer novel therapeutic modalities for the treatment of patients in need of treatment with cladribine. As shown herein, the invention addresses the problems of poor bioavailability traditionally associated with oral cladribine.

10 The compositions of the invention are particularly suitable as modalities for the treatment of any cladribine-responsive disease. Several disease states responsive to cladribine are well-documented in the literature (see *infra*). For any target disease state, an effective amount of the complex cladribine-cyclodextrin complex, *i.e.* the amorphous mixture of the optimized amorphous saturated cladribine-amorphous cyclodextrin complex with amorphous free cladribine as described above is used (*e.g.*, an amount
15 affective for the treatment of multiple sclerosis, rheumatoid arthritis, or leukemia).

The term "therapeutically effective amount" or "effective amount" is used to denote treatments at dosages effective to achieve the therapeutic result sought. Therapeutically effective dosages described in the literature
20 include those for hairy cell leukemia (0.09 mg/kg/day for 7 days), for multiple sclerosis (from about 0.04 to about 1.0 mg/kg/day (see U.S. Patent No. 5,506,214)); for other diseases, see also U.S. Patent Nos. 5,106,837 (autohemolytic anemia); 5,310,732 (inflammatory bowel disease); 5,401,724 (rheumatoid arthritis); 5,424,296 (malignant astrocytoma); 5,510,336
25 (histiocytosis); 5,401,724 (chronic myelogenous leukemia); and 6,239,118 (atherosclerosis).

Further, various dosage amounts and dosing regimens have been reported in the literature for use in the treatment of multiple sclerosis; see, for example: Romine et al., *Proceedings of the Association of American*

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Physicians, Vol. 111, No. 1, 35-44 (1999); Selby et al., *The Canadian Journal of Neurological Sciences*, 25, 295-299 (1998); Tortorella et al., *Current Opinion in Investigational Drugs*, 2 (12), 1751-1756 (2001); Rice et al., *Neurology*, 54, 1145-1155 (2000); and Karlsson et al., *British Journal of Haematology*, 116, 538-548 (2002); all of which are incorporated by
5 reference herein in their entireties and relied upon.

Moreover, the route of administration for which the therapeutically effective dosages are taught in the literature should be taken into consideration. While the instant compositions optimize the bioavailability of
10 cladribine following oral administration, it will be appreciated that even optimal bioavailability from oral dosage forms is not expected to approach bioavailability obtain after intravenous administration, particularly at early time points. Thus, it is often appropriate to increase a dosage suggested for intravenous administration to arrive at a suitable dosage for incorporation
15 into a solid oral dosage form. At the present time, it is envisioned that, for the treatment of multiple sclerosis, 10 mg of cladribine in the instant complex cladribine-cyclodextrin complex in the instant solid dosage form would be administered once per day for a period of five to seven days in the first month, repeated for another period of five to seven days in the second
20 month, followed by ten months of no treatment. Alternatively the patient would be treated with 10 mg of cladribine in the instant complex cladribine-cyclodextrin complex in the instant dosage form once per day for a period of five to seven days per month for a total of six months, followed by eighteen months of no treatment. For further dosing information, see also U.S.
25 Provisional Patent Application No. _____ [IVAX0021-P-USA/Attorney Docket No. 033935-011], and U.S. Provisional Patent Application No. _____ [IVAX0022-P-USA/Attorney Docket No. 033935-012], both entitled "Cladribine Regimen for Treating Multiple Sclerosis", both filed on March 25, 2004 and incorporated by reference herein in their entireties.

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Furthermore, one of skill will appreciate that the therapeutically effective amount of cladribine administered herein may be lowered or increased by fine tuning and/or by administering cladribine according to the invention with another active ingredient. The invention therefore provides a method to tailor the administration/treatment to the particular exigencies specific to a given mammal. Therapeutically effective amounts may be easily determined, for example, empirically by starting at relatively low amounts and by step-wise increments with concurrent evaluation of beneficial effect.

As noted in the preceding paragraph, administration of cladribine in accord with this invention may be accompanied by administration of one or more additional active ingredients for treating the cladribine-responsive condition. The additional active ingredient will be administered by a route of administration and in dosing amounts and frequencies appropriate for each additional active ingredient and the condition being treated. For example, in the treatment of multiple sclerosis, other useful drugs include interferon beta (Rebif[®], Betaseron[®]/Betaferon[®], Avonex[®]), identical to the naturally occurring protein found in the human body; glatiramer acetate (Copaxone[®]), a random chain (polymer) of the amino acids glutamic acid, lysine, alanine and tyrosine; natalizumab (Antegren[®]), a monoclonal antibody; alemtuzumab (Campath-1H[®]), a humanized anti-CD52 monoclonal antibody; 4-aminopyridine (also known as 4-AP and Fampridine), a drug that blocks the potassium channels in neurons; and amantadine, an anti-viral agent which improves muscle control and reduces muscle stiffness and is used to alleviate the symptoms of fatigue in multiple sclerosis, a purpose for which pemoline (Cylert[®]) and L-Carnitine (a herbal product) may also be useful. In the treatment of hairy cell leukemia, additional active ingredients may include interferon alpha, pentostatin, fludarabine, rituximab (an anti-CD 20 monoclonal antibody) and the anti-CD22 recombinant immunotoxin BL 22; other additional active ingredients may be appropriate in other types of

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leukemias. In the treatment of rheumatoid arthritis, there are many other active ingredients which may be selected. These include NSAIDS (non-steroidal anti-inflammatory drugs), which are of three types: salicylates such as aspirin, traditional NSAIDS such as ibuprofen and indomethacin, and COX-2 inhibitors such as celecoxib (Celebrex[®]), rofecoxib (Vioxx[®]), meloxicam (Mobic[®]), valdecoxib (Bextra[®]), lumiracoxib (Prexige[®]) and etoricoxib (Arcoxia[®]). Other drugs useful in treating rheumatoid arthritis which may be used in conjunction with the present invention include DMARDS, glucocorticoids, biological response modifiers and non-NSAID analgesics. DMARDS are disease-modifying anti-rheumatic drugs which include methotrexate, plaquenil, leflunomide (Arava[®]), sulfasalazine, gold, penicillamide, cyclosporine, methyl cyclophosphamide and azathioprine. Glucocorticoids include dexamethasone, prednisolone, triamcinolone and many others. Biological response modifiers (which restore the disease-fighting ability of the immune system), include etanercept (Enrel[®]), a tumor-necrosis factor inhibitor, infliximab (Remicade[®]), which is also an anti-TNF drug, anakinra (Kineret[®]), a selective IL-1 blocker, and Humira[®], a human monoclonal antibody which is another anti-TNF drug. The non-NSAID analgesics include acetaminophen as well as narcotic analgesics such as hydrocodone, oxycodone and propoxyphene. Generally speaking, those drugs which work by a mechanism different from that of cladribine are particularly useful for concomitant therapy with the cladribine composition described herein. Those drugs which are effective by the oral route of administration and which are compatible with the instant cladribine complexes in a single dosage form may be incorporated into the instant dosage forms; otherwise, they should of course be separately administered in amounts, frequencies and via administration routes suitable to them.

As used herein, "treating" means reducing, preventing, hindering the development of, controlling, alleviating and/or reversing the symptoms in the individual to which a compound of the invention has been administered, as

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compared to the symptoms of an individual not being treated according to the invention. A practitioner will appreciate that the complexes, compositions, dosage forms and methods described herein are to be used in concomitance with continuous clinical evaluations by a skilled practitioner (physician or veterinarian) to determine subsequent therapy. Such evaluation will aid and inform in evaluating whether to increase, reduce or continue a particular treatment dose, and/or to alter the mode of administration.

The methods of the present invention are intended for use with any subject/patient that may experience the benefits of the methods of the invention. Thus, in accordance with the invention, the terms "subjects" as well as "patients" include humans as well as non-human subjects, particularly domesticated animals.

Any suitable materials and/or methods known to those of skill can be utilized in carrying out the present invention. However, preferred materials and methods are described. Materials, reagents and the like to which reference are made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein.

25

EXAMPLES

EXAMPLE 1

PHASE SOLUBILITY STUDY

A phase solubility study can be carried out as follows. Excess cladribine is added to cyclodextrin solutions of various concentrations of

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hydroxypropyl- β -cyclodextrin (HP β CD) and allowed to complex as described in EXAMPLE 2 below. The excess, undissolved cladribine is removed by filtration. The amount of cladribine in the complexation solution is measured to obtain a data point. This process is repeated with different known concentrations of HP β CD until several data points are obtained. These data points are then plotted graphically, each data point representing the amount of cladribine that can be dissolved in water with a specific concentration of cyclodextrin. Points on the line generated by the data points represent ratios for the product. One of skill in the art will realize the same results will be generated if excess cyclodextrin is added to cladribine solutions of known concentration.

The molar concentrations of cladribine to cyclodextrin obtained are plotted and presented graphically. A representative phase solubility diagram is shown in the Figure. The plotted lines for cladribine-HP β CD represent cladribine solubilization for the conditions tested, that is, the ratio of the concentration of cladribine to the concentration of cyclodextrin. The area above each of the plotted lines represents conditions where excess insoluble cladribine is present. The area below each of the plotted lines represents the conditions where cyclodextrin is in excess.

The plot for cladribine-HP β CD shown in the Figure is approximately linear; this is indicative of a 1:1 complex, in which one molecule of the drug is complexed with one molecule of cyclodextrin. The Figure also shows that additional cyclodextrin is needed to maintain the cladribine in the complex. For example, about 0.14 mole of HP β CD is needed to maintain about 0.049 mole of cladribine dissolved under the selected conditions, which will ultimately provide the amorphous mixture of the amorphous, preferably saturated, cladribine-HP β CD inclusion complex and amorphous free cladribine (as a non-inclusion complex). Under the conditions of EXAMPLE 2 below, a significant portion of the cladribine in the product can be expected to be not in the inclusion complex but rather in amorphous form loosely held

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in intimate admixture therewith by hydrogen bonding as a non-inclusion complex.

EXAMPLE 2

5 PREPARATION OF CLADRIBINE-CYCLODEXTRIN COMPLEX FOR HUMAN TRIALS

Cladribine is complexed with HP β CD by the following method.

10 In 825 mL of distilled water, 172.5 g of hydroxypropyl- β -cyclodextrin are dissolved (forming an approximately 17% solution), then cladribine is added and the mixture is stirred at about 45 to about 50°C for about nine hours. Stirring is continued for an additional 6 to 9 hours at room temperature. Any undissolved cladribine is removed by filtration and the solution is cooled to room temperature. To form the amorphous mixture of amorphous cladribine-cyclodextrin complex and amorphous free cladribine, the aqueous cladribine-cyclodextrin solution is dried by lyophilization prior to
15 incorporation into solid oral tablets. The lyophilization procedure comprises a freezing stage of rapidly bringing the complexation solution to about -40°C to about -80°C (e.g., about -45°C) for approximately 2 to 4 hours (preferably about 3 to 4 hours), followed by a primary drying stage at about -25°C for approximately 80-90 hours, typically under low pressure, and a second
20 drying stage at about 30°C for about 15-20 hours.

Product made by the foregoing general procedure can be analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase, with UV detection at 264 nm) to find the weight ratio of cladribine to cyclodextrin in the final product. Final product preparations can
25 be further characterized by methods known in the art, including, for example by inspecting appearance, ascertaining the overall impurity content by HPLC, ascertaining the water content using a Karl Fischer titrator, determining the dissolution profile by a standard method, for example using USP<711>Apparatus II equipment and UV detection at 264 nm, inspecting

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the content uniformity and performing quantitative assay by HPLC analysis of the active ingredient.

Two batches of cladribine/cyclodextrin product, FD04 and FD05, were made by the foregoing general procedure as follows:

5 Purified water (825 mL) was pre-heated at 48°C (target range 45°C to 50°C) in a 1-liter glass vessel by immersion in a water bath. The heated water was stirred to achieve a controlled central vortex. 2-hydroxypropyl- β -cyclodextrin (172.50 g) was weighed and slowly added to the heated water over a period of 40 minutes. The resulting solution was
10 stirred for a further 10 minutes to ensure complete dissolution of the cyclodextrin. Cladribine (12.00 g for FD04 and 18.75 g for FD05) was weighed and added to the stirred cyclodextrin solution, which turned cloudy before becoming clear. The resulting clear solution was maintained at 48°C and continually stirred for 9 hours. Stirring continued for a further 7 hours
15 while the solution cooled to room temperature.

 Use of a larger amount of cladribine in the preparation of FD05 was part of an attempt to optimize the procedure; however, it was found that the initial amount of cladribine in that case was too great and precipitation was observed at the end of the cooling step for batch FD05. The solution was
20 filtered to remove the precipitate. Analysis of the resultant product revealed (assay value = 87.2%) that 16.35 g of cladribine had been incorporated into the cyclodextrin complex in the case of FD05. No filtration was required for batch FD04, indicating that the amounts used in the preparation of FD04 were more appropriate and that the FD05 procedure could be optimized by
25 beginning with a smaller amount of cladribine (16.35 g rather than 18.75 g), thus avoiding the filtration step.

 After cooling to room temperature and, in the case of FD05, filtering, the solutions were filled into 100 mL lyophilization vials (20 mL solutions per vial), the filled vials were partially stoppered and lyophilized. The

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lyophilization included freezing at -45°C for about 200 minutes, a primary drying phase at -25°C under a pressure of 100 mTorr for about 5,200 minutes and a secondary drying phase at 30°C for about 1,080 minutes as set forth below:

5

TABLE I

Step	Process	Temperature	Pressure (mTorr)	Time (min)
1	Load	4°C		
2	Load Hold	4°C	n/a	120
3	Ramp	-45°C	n/a	120
4	Freezing	-45°C	n/a	200
5	Ramp	-25°C	100	120
6	Primary drying	-25°C	100	5200
7	Ramp	30°C	50	240
8	Secondary drying	30°C	50	1080
9	Finish	30°C	Vials closed under vacuum	

The FD04 and FD05 batches of cladribine/cyclodextrin product made by the foregoing procedure were analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase with UV detection at 264 nm) and empirically found to have the following characteristics:

10

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TABLE II

Lot No.	Cladribine: HP β CD w/w	Cladribine: HP β CD Weight Ratio
FD04	12.00g:172.50g	1:14.38
FD05	16.35g:172.50g	1:10.55

The products were analyzed by DSC thermograms and X-ray diffraction methods to determine any free crystalline cladribine in the lyophilized material. Importantly, the samples exhibited no transitions in the region of 210°C to 230°C, which is associated with the melting of crystalline cladribine. In both cases, no significant thermal activity was recorded in the range of 210°C to 230°C, suggesting that the complexes obtained at the end of the lyophilization do not have any significant amount of free crystalline cladribine, considering the sensitivity of the analytical method (up to 3% w/w). This conclusion was supported by the absence of peaks for crystalline cladribine from X-ray diffraction traces for both complexes FD04 and FD05.

The products are amorphous mixtures of amorphous cladribine-HP β CD inclusion complex and amorphous free cladribine hydrogen-bonded to the cyclodextrin as a non-inclusion complex. The cladribine:HP β CD weight ratios obtained were about 1:14 and 1:11.

Generally speaking, amorphous mixtures within the scope of the present invention have cladribine:HP β CD weight ratios of from about 1:10 to 1:16.

EXAMPLE 3

PREPARATION OF ORAL TABLETS

Tablets were manufactured using batches of amorphous mixtures FD04 and FD05 described in EXAMPLE 2 for use in a clinical study.

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Batch N0120 was manufactured using cladribine-2-HP β CD complex mixture DF05 to a batch size of 3,000 tablets and batch N0126 was manufactured using cladribine-HP β CD complex mixture FD04 to a batch size of 800 tablets. The master formulations for the two batches are shown in TABLE III. Batch N0120 represented 3.0 g tablets and Batch N0126 represented 10 mg tablets for clinical study.

TABLE III

Constituent	Lot Number	mg/tablet	mg/tablet
		3.0 mg Batch N0120	10.0 mg Batch N0126
Cladribine-HP β CD complex mix	FD05	30.60*	
Cladribine-HP β CD complex mix	FD04		153.75**
Sorbitol powder NF	1007403	68.4	44.25
Magnesium stearate NF	1006280	1.00	2.00
Total		100.00	200.00

*Equivalent to 3.0 mg cladribine per tablet.

**Equivalent to 10.0 mg cladribine per tablet.

The following table sets forth the method of manufacture of the Batch N0120 and N0126 tablets.

TABLE IV

1.	Pre-mix the magnesium stearate with an approximately equal quantity of sorbitol powder.
2.	Pass the cladribine-HP β CD complex and the remainder of the sorbitol powder into a one-liter glass jar via a 40-mesh screen.
3.	Blend the contents for 10 minutes at 12 rpm.
4.	Pass the magnesium stearate/sorbitol powder pre-mix into the glass jar via the 40-mesh screen.
5.	Blend the final mixture for 5 minutes at 12 rpm.
6.	Compress into 3.0 mg and 10.0 mg tablets at a target compression weight of 100 mg and 200 mg, respectively.

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Both the Batch N0120 3.0 mg tablets and the Batch N0126 10.0 mg tablets were round, with one side flat-beveled edged and the other side shallow convex. The Batch N0120 3.0 mg tablets had an average weight of 100 mg, a thickness of 2.7 mm, a friability of 0.2%, a hardness of 4 Kp and a disintegration time of 3 minutes. The Batch N0126 10.0 mg tablets had an average weight of 198 mg, a thickness of 4.2 mm, a friability of 1%, a hardness of 2.8 Kp and a disintegration time of 5 minutes 42 seconds.

The Batch N0120 3.0 mg and N0126 10.0 mg tablets were used in the clinical study summarized in EXAMPLE 5 below.

10

EXAMPLE 4

CLINICAL STUDY: RELATIVE BIOAVAILABILITY

The objective of this study was to assess the relative bioavailability of three oral cladribine formulations: (1) a cyclodextrin-based formulation according to the instant invention (Tablet 1: complex FD05, i.e. Batch No. N0120 tablets described above); (2) a mucoadhesive formulation (Tablet 2: containing 3.0 mg cladribine, 10 mg of Carbopol 71G NF, 22.2 mg of dicalcium phosphate, 64.3 mg of lactose and 0.5 mg of magnesium stearate, Batch No. N0121); and (3) a hard-gel capsule (Capsule containing 3.0 mg cladribine, 5.0 mg Carbopol 974P, 91.3 mg Avicel PH101, 100.0 mg Avicel PH102, 0.2 mg colloidal silicon dioxide and 0.5 mg magnesium stearate, Batch No. RD03030) in comparison with one fixed subcutaneous cladribine administration (reference formulation) in patients with MS (multiple sclerosis).

This study was a 2 center, open-label, randomized, 4-way crossover single dose study using twelve patients with MS. Patients received randomly three different fixed oral doses (3.0 mg) and a fixed subcutaneous dose of 3.0 mg. The four treatment days were separated by a drug-free interval of at

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least 5 days. In each treatment period, blood samples were collected over a 24-hour period for evaluation of plasma cladribine.

The plasma concentration of cladribine was measured by a HPLC/MS/MS method. Using this method, the relationship between concentration versus peak area ratio was found to be linear within the range of 100 pg/ml to 50,000 pg/ml for cladribine. The limit of quantification was 100 pg/ml. Analysis of samples was carried out in 16 runs. No calibrator had to be excluded from fitting of the calibration curve and accuracy of each quality control sample met the GLP requirements.

576 clinical plasma samples were analyzed and concentration values of cladribine were determined. The results were compiled and are summarized in the tables below (Tables V and VI). In these tables, the following definitions are applicable: $T_{\max}$ is the time to reach maximum concentration in the plasma; $T_{1/2}$ is the half-life of cladribine in the plasma; $C_{\max}$ is the maximum concentration of cladribine in the plasma; $AUC_{\inf}$ is the area under the curve for the measured data from zero extrapolated to infinity; AUC_t is the area under the curve for the measured data (from zero to the last time point); Geom Mean is the geometric mean; CV is the coefficient of variation (relative standard deviation); LL is the lower limit; UL is the upper limit.

TABLE V
Summary Statistics for Pharmacokinetic Parameters for Cladribine Study
Obtained via Non-Compartmental Analyses. (n=12).

Pharmacokinetic Parameter	3.0 mg subcutaneous			3mg Tablet 1			3mg Tablet 2			3 mg Capsule		
	Geom Mean	Mean $\pm$ SD	CV** (%)	Geom Mean	Mean $\pm$ SD	CV** (%)	Geom Mean	Mean $\pm$ SD	CV** (%)	Geom Mean	Mean $\pm$ SD	CV** (%)
$T_{max}(hr)$	N/A	.313 $\pm$.113	36.2	N/A	.521 $\pm$.167	32.1	N/A	1.25 $\pm$.839	67.1	N/A	2.25 $\pm$.622	27.7
$T_{1/2}(hr)$	N/A	6.69 $\pm$ 2.01	30.1	N/A	7.55 $\pm$ 2.50	33.1	N/A	6.73 $\pm$ 2.82	41.9	N/A	6.27 $\pm$ 2.31	36.9
$C_{max}(pg/ml)$	23186	N/A	40.1	6597	N/A	24.7	5041	N/A	52.6	3818	N/A	36.8
$AUC_{inf}(hr \cdot pg/ml)$	57254	N/A	44.4	24936	N/A	28.8	21676	N/A	42.7	22604	N/A	39.5
$AUC_t(hr \cdot pg/ml)$	54725	N/A	43.8	23182	N/A	28.0	20063	N/A	42.1	20951	N/A	42.0

5 **CV=SD/mean for T_{max} and $T_{1/2}$ and CV% geometric mean for C_{max} , AUC_{inf} and AUC_t .

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TABLE VI

Ratios of Oral to Subcutaneous Pharmacokinetic Parameters and Corresponding Two-Sided 90% Confidence Intervals for Cladribine Study (n=12).

5

Pharmacokinetic Parameter	3 mg Tablet 1		3mg Tablet 2		3mg Capsule	
	Ratio*	LL, UL	Ratio*	LL, UL	Ratio*	LL, UL
AUC _{inf}	43.1	35.7, 52.1	38.4	31.8, 46.4	38.9	32.1, 47.0
AUC _t	41.9	34.6, 50.8	37.2	30.7, 45.0	37.6	31.0, 45.5

*Ratios (dose normalized) and Corresponding 95% LL obtained via inverse transformation of log-transformed data.

EXAMPLE 5

10

CLINICAL STUDY: DOSE RESPONSE AND ABSOLUTE BIOAVAILABILITY

The objective of this study was to assess the systemic availability of cladribine after oral administration in two different fixed oral doses, in comparison with one fixed intravenous administration (reference formulation) in patients with MS (multiple sclerosis), and to evaluate the safety and tolerability of cladribine in this population.

15

This study was a 3 center, open-label, randomized, 3-way crossover single dose study using twenty-six patients with MS. Patients received randomly two different fixed oral doses (3.0 mg and 10.0 mg) and a fixed intravenous dose of 3.0 mg (administered as a 1 hour infusion). The three treatment days were separated by a drug-free interval of at least 5 days. In

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each treatment period blood samples were collected over a 24-hour period for evaluation of plasma cladribine.

The plasma concentrations of cladribine were measured by a HPLC/MS/MS method. Using this method the relationship between concentrations versus peak area ratios was found to be linear within the range of 100 pg/ml to 50,000 pg/ml for cladribine. The limit of quantification was 100 pg/ml. Analysis of samples was carried out in 16 runs. Except the first run (which had to be rejected because of equipment failure), all other runs could be accepted. No calibrator had to be excluded from fitting of the calibration curve and accuracy of each quality control sample met the GLP requirements.

858 clinical plasma samples were analyzed and concentration values of cladribine were determined. The results were compiled and are summarized in the tables below [TABLES VII through X]. In these tables, the following definitions are applicable: $T_{\max}$ is the time to reach maximum concentration in the plasma; $T_{1/2}$ is the half-life of cladribine in the plasma; $C_{\max}$ is the maximum concentration of cladribine in the plasma; AUC_{inf} is the area under the curve for the measured data from zero extrapolated to infinity; AUC_t is the area under the curve for the measured data (from zero to the last time point); Geom Mean is the geometric mean; CV is the coefficient of variation (relative standard deviation); LL is the lower limit; UL is the upper limit; σ^2 is the mean variance; σ_B^2 is the mean variance between subjects; σ_W^2 is the mean variance within subjects; CV_T is the total coefficient of variation; and CV_W is the coefficient of variation within subjects.

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TABLE VII

Summary Statistics for Pharmacokinetic Parameters for Cladribine Study
Obtained via Non-Compartmental Analysis (n=26)

Pharmaco-kinetic Parameter	3.0 mg IV infusion			Oral Administration					
				3.0 mg			10.0 mg		
	Geom Mean	Mean $\pm$ SD	CV** (%)	Geom Mean	Mean $\pm$ SD	CV** (%)	Geom Mean	Mean $\pm$ SD	CV** (%)
T _{max} (hr)	N/A	.817 $\pm$.397	48.6	N/A	.548 $\pm$.300	54.8	N/A	.558 $\pm$.204	36.5
T _{1/2} (hr)	N/A	6.50 $\pm$ 1.27	19.5	N/A	5.85 $\pm$ 1.18	20.2	N/A	5.60 $\pm$ 0.75	13.3
C _{max} (pg/ml)	21425	N/A	27.6	5608	N/A	49.5	21242	N/A	50.5
AUC _{inf} (hr·pg/ml)	58528	N/A	24.0	20159	N/A	35.0	76690	N/A	30.3
AUC _t (hr·pg/ml)	56396	N/A	24.0	19166	N/A	36.9	74532	N/A	30.3

**CV=SD/mean for T_{max} and T_{1/2} and CV% geometric mean for C_{max}, AUC_{inf} and AUC_t.

TABLE VIII

Ratios of Oral to I.V. Pharmacokinetic Parameters and Corresponding Lower Limit (LL) for the one-sided 95% Confidence Interval for Cladribine Study (n=26)

Pharmacokinetic Parameter	Oral Administration			
	3.0 mg		10.0 mg	
	Ratio*	LL	Ratio*	LL
AUC _{inf}	34.5	31.7	39.1	35.9
AUC _t	34.0	31.2	39.4	36.1

*Ratios (dose normalized) and Corresponding 95% LL obtained via inverse transformation of log-transformed data.

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TABLE IX

Ratios and Corresponding two-sided 90% Confidence Intervals for Cladribine Study (n=26)

5

Pharmacokinetic Parameter	10.0 mg/3.0 mg		
	Ratio*	LL	UL
C _{max}	112.6	95.1	133.3
AUC _{inf}	113.3	104.2	123.3
AUC _t	115.8	106.1	126.5

*Ratios (dose normalized) and Corresponding 90% CI obtained via inverse transformation of log-transformed data.

TABLE X

10

Variance components for Cladribine Study (n=26)

Source of variation	C _{max}	AUC _{inf}	AUC _t
Between (σ_B^2)	.0380	.0487	.0492
With (σ_W^2)	.1315	.0330	.0357
TOTAL ($\sigma_B^2 + \sigma_W^2$)	.1695	.0816	.0849
CV _T (%)	43.0	29.2	29.8
CV _W (%)	37.5	18.3	19.1

Where PK parameters are dose-adjusted and $CV = \sqrt{\exp(\sigma^2) - 1}$

15

The foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents thereof may be resorted to, falling within the scope of the invention claimed.

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WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.
2. The pharmaceutical composition according to Claim 1, wherein the complex is saturated with cladribine.
3. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, randomly methylated β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.
4. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.
5. The composition according to Claim 1 or 2, wherein the amorphous cyclodextrin is hydroxypropyl- γ -cyclodextrin.
6. The composition according to any one of Claims 1 to 3, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.
7. The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.

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8. The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:14.

5 9. The composition according to Claim 7, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:11.

10. The composition according to Claim 6, wherein the amorphous cyclodextrin is hydroxypropyl- γ -cyclodextrin.

10 11. The composition according to any one of Claims 1 to 10, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

15 12. The composition according to any one of Claims 1 to 11, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

20 13. A method for enhancing the oral bioavailability of cladribine comprising orally administering to a subject in need thereof a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine
25 associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

14. The method according to Claim 13, wherein the complex is saturated with cladribine.

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15. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, randomly methylated β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

5

16. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.

10

17. The method according to Claim 13 or 14, wherein the amorphous cyclodextrin is hydroxypropyl- γ -cyclodextrin.

15

18. The method according to any one of Claims 13 to 15, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

19. The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.

20

20. The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:14.

21. The method according to Claim 19, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:11.

25

22. The method according to Claim 18, wherein the amorphous cyclodextrin is hydroxypropyl- γ -cyclodextrin.

23. The method according to any one of Claims 13 to 22, wherein the approximate molar ratio of cladribine to amorphous cyclodextrin

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corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

24. The method according to any one of Claims 13 to 23, wherein
5 from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

25. A method for the treatment of symptoms of a
10 cladribine-responsive condition in a subject suffering from said symptoms comprising orally administering to said subject a pharmaceutical composition comprising a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine
15 associated with amorphous cyclodextrin as a non-inclusion complex, formulated into a solid oral dosage form.

26. The method according to Claim 25, wherein the complex is
20 saturated with cladribine.

27. The method according to Claim 25 or 26, wherein the
cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

28. The method according to Claim 27, wherein the
25 cladribine-responsive condition is multiple sclerosis.

29. The method according to Claim 25, 26, 27 or 28, wherein the
amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin,

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hydroxypropyl- γ -cyclodextrin, randomly methylated β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

5 30. The method according to any one of Claims 25 to 29, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

10 31. The method according to any one of Claims 25 to 30, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.

 32. The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:14.

15 33. The method according to Claim 31, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:11.

 34. The method according to Claim 25, 26, 27 or 28, wherein the amorphous cyclodextrin is hydropropyl- γ -cyclodextrin.

20 35. The method according to any one of Claims 25 to 34, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

25 36. Use of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, in the formulation of a solid oral dosage form, for administration in the treatment of
30 symptoms of a cladribine-responsive condition.

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37. Use according to Claim 36, wherein the complex is saturated with cladribine.

5 38. Use according to Claim 36 or 37, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

10 39. Use according to Claim 38, wherein the cladribine-responsive condition is multiple sclerosis.

15 40. Use according to Claim 36, 37, 38 or 39, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, randomly methylated β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

20 41. Use according to any one of Claims 36 to 40, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

 42. Use according to any one of Claims 36 to 41, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.

25 43. Use according to Claim 42, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:14.

 44. Use according to Claim 42, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:11.

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45. Use according to any one of Claims 36 to 41, wherein the amorphous cyclodextrin is hydroxypropyl- γ -cyclodextrin.

5 46. Use according to any one of Claims 36 to 45, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

10 47. Use of a complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex, in the formulation of a solid oral dosage form, for enhancing the oral bioavailability of cladribine.

15

48. Use according to Claim 47, wherein the complex is saturated with cladribine.

20 49. Use according to Claim 47 or 48, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, randomly methylated β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

25 50. Use according to any one of Claims 47 to 49, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

30 51. Use according to any one of Claims 47 to 50, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.

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52. Use according to Claim 51, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:14.

5 53. Use according to Claim 51, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:11.

54. Use according to any one of Claims 47 to 50, wherein the amorphous cyclodextrin is hydroxypropyl- γ -cyclodextrin.

10 55. Use according to any one of Claims 47 to 54, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

15 56. A complex cladribine-cyclodextrin complex which is an intimate amorphous admixture of (a) an amorphous inclusion complex of cladribine with an amorphous cyclodextrin and (b) amorphous free cladribine associated with amorphous cyclodextrin as a non-inclusion complex.

20 57. The complex according to Claim 56, saturated with cladribine.

58. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, randomly methylated β -cyclodextrin,
25 carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

59. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.

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60. The complex according to Claim 56 or 57, wherein the amorphous cyclodextrin is hydroxypropyl- γ -cyclodextrin.

5 61. The complex according to any one of Claims 56 to 58, wherein the weight ratio of cladribine to amorphous cyclodextrin is from about 1:10 to about 1:16.

10 62. The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl- β -cyclodextrin.

63. The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:14.

15 64. The complex according to Claim 62, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:11.

65. The complex according to Claim 61, wherein the amorphous cyclodextrin is hydroxypropyl- γ -cyclodextrin.

20 66. The complex according to any one of Claims 56 to 65, wherein from about 30 to about 40 percent by weight of the cladribine is in the inclusion complex (a) and from about 70 to about 60 percent by weight of the cladribine is in the non-inclusion complex (b).

25 67. A process for the preparation of a complex cladribine-cyclodextrin complex which comprises the steps of:

(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

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- (ii) cooling the resultant aqueous solution to room temperature;
and
- (iii) lyophilizing the cooled solution to afford an amorphous product.

5 68. A process according to Claim 67, further comprising a filtration step following step (ii).

10 69. A process according to Claim 67 or 68, wherein step (i) is performed at a temperature of from about 45 to about 60°C.

 70. A process according to any one of Claims 67 to 69, wherein step (i) is performed at a temperature of from about 45 to about 50°C.

15 71. A process according to Claim 69 or 70, wherein step (i) is performed with stirring.

 72. A process according to Claim 71, wherein step (i) is performed for a period of from about 6 to about 9 hours.

20 73. A process according to any one of Claims 67 to 72, wherein step (ii) is performed for a period of from about 6 to about 9 hours.

25 74. A process according to any one of Claims 67 to 73, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80° C, and held at said temperature for a period of from about 2 to about 4 hours.

30 75. A process according to Claim 74, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

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76. A process according to any one of Claims 67 to 75, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl- β -cyclodextrin are introduced in step (i).

5 77. A process according to any one of Claims 67 to 75, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of hydroxypropyl- β -cyclodextrin are introduced in step (i).

10 78. A process according to Claim 76 or 77, wherein 825 parts by volume of water are introduced in step (i).

79. A process according to any one of Claims 67 to 78, wherein the lyophilization step (iii) comprises:

15 (a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;
(b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and
(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

20

80. A process according to Claim 79, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

25 81. A process according to Claim 79 or 80, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

82. A pharmaceutical composition obtainable by a process comprising the steps of:

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(i) combining cladribine and an amorphous cyclodextrin in water at a temperature of from about 40 to about 80°C and maintaining said temperature for a period of from about 6 to about 24 hours;

(ii) cooling the resultant aqueous solution to room temperature;

5 (iii) lyophilizing the cooled solution to afford an amorphous product; and

(iv) formulating the amorphous product into a solid oral dosage form.

10 83. A pharmaceutical composition according to Claim 82, wherein the process further comprises a filtration step following step (i) or (ii).

84. A pharmaceutical composition according to Claim 82 or 83, wherein step (i) of the process is performed at a temperature of from about
15 45 to about 60°C.

85. A pharmaceutical composition according to any one of Claims 82 to 84, wherein step (i) of the process is performed at a temperature of from about 45 to about 50°C.

20

86. A pharmaceutical composition according to Claim 84 or 85, wherein step (i) of the process is performed with stirring.

87. A pharmaceutical composition according to Claim 86, wherein
25 step (i) of the process is performed for a period of from about 6 to about 9 hours.

88. A pharmaceutical composition according to any one of Claims 82 to 87, wherein step (ii) of the process is performed for a period of from
30 about 6 to about 9 hours.

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89. A pharmaceutical composition according to any one of Claims 82 to 88, wherein step (iii) comprises an initial freezing stage in which the solution is cooled to from about -40 to about -80°C, and held at said
5 temperature for a period of from about 2 to about 4 hours.

90. A pharmaceutical composition according to Claim 89, wherein, in the initial freezing stage of step (iii), the solution is cooled to about -45°C.

10 91. A pharmaceutical composition according to any one of Claims 82 to 90, wherein 12.00 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

15 92. A pharmaceutical composition according to any one of Claims 82 to 90, wherein 16.35 parts by weight of cladribine and 172.50 parts by weight of the hydroxypropyl-β-cyclodextrin are introduced in step (i) of the process.

20 93. A pharmaceutical composition according to Claim 91 or 92, wherein 825 parts by volume of water are introduced in step (i) of the process.

25 94. A pharmaceutical composition according to any one of Claims 82 to 93, wherein the lyophilization step (iii) of the process comprises:

- (a) an initial freezing stage in which the complexation solution is brought to from about -40°C to about -80°C for approximately 2 to 4 hours;
- (b) a primary drying stage at about -25°C for approximately 80 to 90 hours; and

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(c) a secondary drying stage at about 30°C for approximately 15 to 20 hours.

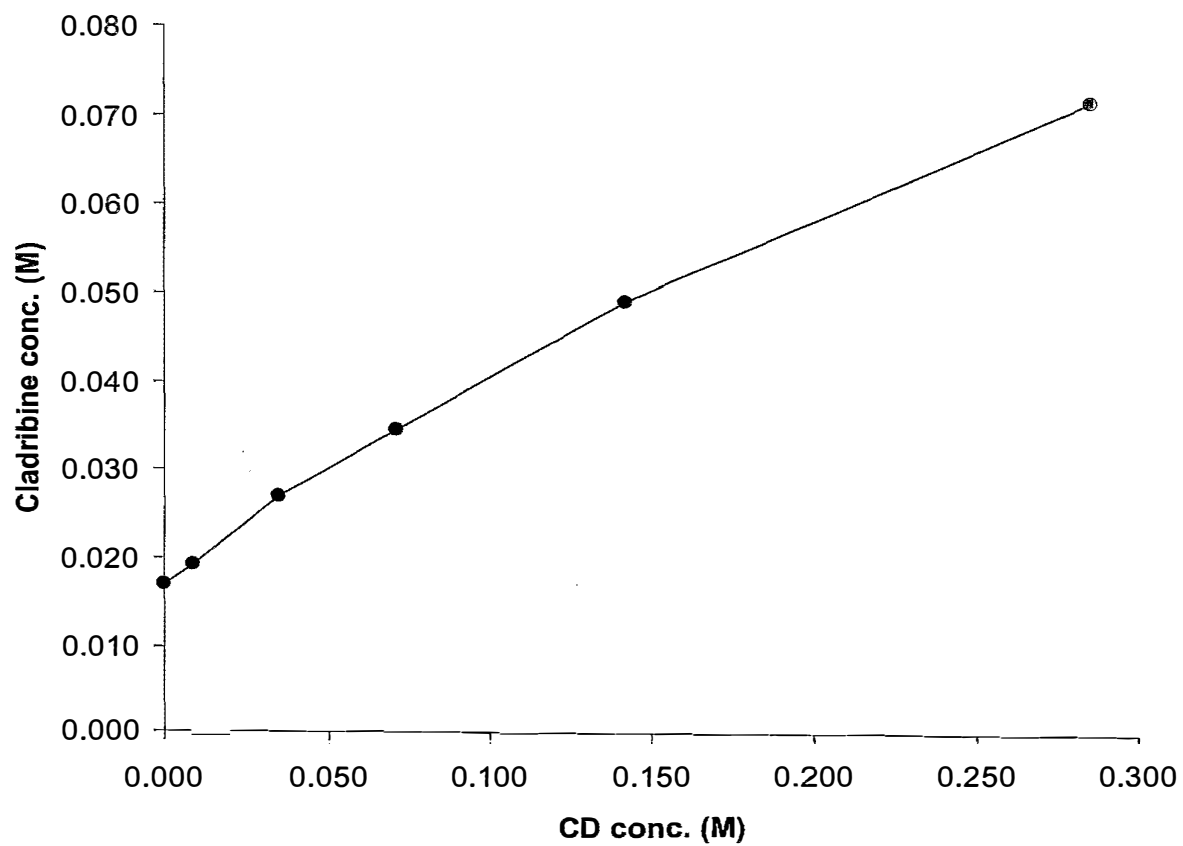
5 95. A pharmaceutical composition according to Claim 94, wherein stage (a) of the lyophilization is conducted at about -45°C for approximately 3 to 4 hours.

10 96. A pharmaceutical composition according to Claim 94 or 95, wherein stage (b) of the lyophilization is conducted under a pressure of about 100 mTorr.

15 97. A pharmaceutical composition according to any one of Claims 82 to 96, wherein the formulation step (iv) of the process comprises blending the complex with magnesium stearate and compressing into tablets.

 98. A pharmaceutical composition according to Claim 97, wherein magnesium stearate is pre-mixed with sorbitol powder before blending with the complex.

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(21) International Application Number: PCT/US95/16418 (22) International Filing Date: 15 December 1995 (15.12.95) (30) Priority Data: 08/362,083 22 December 1994 (22.12.94) US (71) Applicant: ORTHO PHARMACEUTICAL CORPORATION [US/US]; U.S. Route #202, P.O. Box 300, Raritan, NJ 08869-0602 (US). (72) Inventors: BORNSTEIN, Michael; 90 Barchester Way, West- field, NJ 07090 (US). ROZMAN, Rosemary; 514 Village Commons, Flemington, NJ 08822 (US). LONG, Kevin, Francis; 17 Dogwood Circle, Flemington, NJ 08822 (US). WONG, George, Kaon; 672 Marshall Road #G, Somerville, NJ 08876 (US). (74) Agents: CIAMPORCERO, Audley, A., Jr. et al.; Johnson and Johnson, One Johnson and Johnson Plaza, New Brunswick, NJ 08933 (US).		(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: SOLUBLE 2-CHLORO-2'-DEOXYADENOSINE FORMULATIONS (57) Abstract Shelf stable formulations of 2-CdA in water are disclosed which contain benzyl alcohol and/or propylene glycol and a buffer system.		

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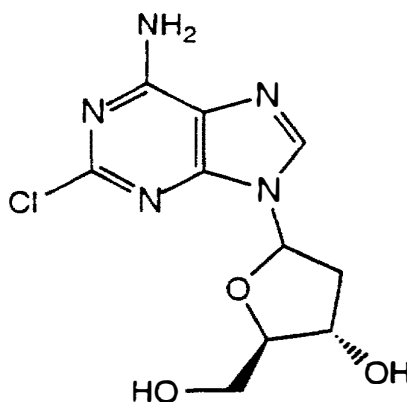
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SOLUBLE 2-CHLORO-2'-DEOXYADENOSINE FORMULATIONS

This invention relates to pharmaceutically useful and soluble formulations of 2-chloro-2'-deoxyadenosine (2-CdA) in water. More particularly, this invention relates to soluble formulations of 2-CdA in water with certain solubilizers and optionally certain preservatives and buffers which are injectable in humans and have a improved shelf-life.

BACKGROUND OF THE INVENTION

The compound 2-CdA has the following formula:



2-CdA is known as an antileukemic agent, i.e., in treating leukemias, such as, hairy cell leukemia and L 1210 leukemia, and as an immunosuppressive agent (D. A. Carson, D. Bruce Wasson, and Ernst Beutler, Proc. Soc. Acad. Sci. USA, Vol. 81, pp 2232- 2236, 1984). More recently, 2-CdA has been disclosed as effective in the treatment of rheumatoid arthritis and multiple sclerosis, U.S. Pat. No. 5,310,732.

To date, 2-CdA has been administered by intravenous injection of saline solutions presenting two problems for subcutaneous or intramuscular injection. First, 2-CdA is slightly soluble in water which requires a large volume of material to be injected subcutaneously or intramuscularly to achieve the required dose. Dilute solutions are acceptable for intravenous injection, but may create pain or inflammatory difficulties for subcutaneous or intramuscular injection. Secondly, 2-CdA has limited stability in simple saline solutions. Longer shelf-life is beneficial for extended storage at refrigerated or room temperature conditions.

U.S. Pat. No. 5,310,732, col. 8, teaches a 0.1 mg/mL isotonic saline solution of 2-CdA. There has been marketed a non-buffered solution containing 1.0 mg/mL of 2-CdA in 9.0 mg/mL Sodium Chloride Injection, USP.

5

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the concentration of 2-CdA versus time in water and with 1% benzyl alcohol as a cosolvent.

10 Figure 2 is the concentration of 2-CdA versus time with 10% propylene glycol as a cosolvent.

Figure 3 is the concentration of 2-CdA versus time with various levels of benzyl alcohol and a single combination of benzyl alcohol and propylene glycol as shown.

15

SUMMARY OF THE INVENTION

There is provided by the present invention a solution of 2-CdA in water comprising:

20

a) from about 1 to about 8 mg/mL of 2-CdA; and

b) a solubilizing agent selected from the group consisting of from about 5 to about 30 mg/mL of benzyl alcohol and from about 50 to about 400 mg/mL of propylene glycol.

25

DETAILED DESCRIPTION OF THE INVENTION

Processes for preparing 2-CdA are known. European Patent Application No. 173,059 A2 and Robins et al., J. Am. Chem. Soc., 106, 6379 (1984) disclose the preparation of 2-CdA. The preparation consists of the glycosilation of 2,6-dichloropurine with 1-chloro-2'-deoxy-3',5'-di-O-p-toluoyl-b-D-erythropentofuranose to yield the N-9 glycosilated purine, 2,6-dichloro-9-(2-deoxy-3,5-di-O-p-toluoyl-b-D-erythropentofuranosyl)-purine, which is subsequently reacted with ammonia to yield 2-CdA. An alternative method to manufacture 2-CdA is taught in U.S. Pat. No. 5,208,327 by Robert H. K. Chen.

30

35

Preferably, the solution contains from about 2 to about 7 mg/mL of 2-CdA. Most preferably the solution contains from about 3 to about 5 mg/mL of 2-CdA.

5 Benzyl alcohol is known generally as a preservative in pharmaceutical formulations based on its antibacterial action and as a solubilizing agent for certain pharmaceutical compounds. Preferably, in the solutions of the present invention there is present from about 8 to about 20 mg/mL benzyl alcohol and most preferably from about 10 to about 15 mg/mL.

10

Propylene glycol is a known solubilizing agent for a variety of pharmaceuticals. Preferably in the solution of the present invention there is from about 75 to about 200 mg/mL of propylene glycol and most preferably from about 100 to about 150 mg/mL.

15

It is preferred that both benzyl alcohol and propylene glycol be present within the prescribed ranges to obtain optimum solubility of 2-CdA in water. Of course, where one of either benzyl alcohol or propylene glycol it is contemplated within the present invention that the other may be present in less than the prescribed minimum amount.

20

To provide for useful solutions of 2-CdA which may be injected to achieve the desired pharmaceutical effect, it may be necessary or desirable to include further compounds in the formulation.

25

In the absence of propylene glycol, sodium chloride might be added to render the solution isotonic. Where employed, it might constitute from about 2 to about 6 mg/mL of the solution.

30

The m-cresol is known generally as a preservative in pharmaceutical formulations based on its antibacterial action. In preferred solutions herein there might be added from about 1.5 to about 2.5 mg/mL of m-cresol and most preferably about 2 mg/mL.

35

Suitable buffers are any of those available for pharmaceutical application and which are capable of stabilizing pH for the instant solutions between 5.5 and 8.5. Such buffers include but are not limited to phosphate, citrate, acetate, borate and tris. The preferred buffer for use herein is sodium

phosphate buffer. A preferred pH range for the shelf stable solutions herein is between about 6.0 and about 7.0. The ratio of the sodium phosphate monobasic, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, and the sodium phosphate dibasic, $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, are adjusted to achieve the pH desired. This buffer is generally useful to achieve a pH in the range of from 4.5 to 8.5. Of course, sufficient buffer should be employed not only to obtain the desired pH but to stabilize the pH at that value. For solutions herein there might be employed a weight ratio from about 2 to 1 to about 1 to 1 of the sodium phosphate monobasic to the sodium phosphate dibasic.

The formulation of Table 1 was prepared and found suitable for use as an injectable and pharmaceutically useful solution. This solution is pH 6.5. All figures shown are in mg/mL. The following abbreviations are employed in Table 1 and elsewhere herein: propylene glycol, USP, (PG), benzyl alcohol, NF, (BA), m-cresol, (MC), sodium phosphate monobasic, monohydrate, USP, (SPMM) and sodium phosphate dibasic, heptahydrate, USP, (SPDH). Water for injection, USP (WFI), was added to 1.0 mL.

TABLE 1.

Formulation		<u>1</u>
2-CdA		5.0
PG		100.0
BA		10.0
MC		2.0
SPMM		1.816
SPDH		0.941

2-CdA may be administered to a patient in need of the same in a daily dose of 0.05 to about 0.15 mg/Kg. A more desirable daily dose would be from 0.07 to about 0.1 mg/Kg.

The invention is illustrated, but in no way limited, by the following examples.

5

EXAMPLE

The following solutions were prepared with water for injection to make a total volume of 1.0 mL. All figures are in mg/mL.

5

TABLE 2

	<u>Formulation</u>	<u>2-CdA</u>	<u>BA</u>	<u>PG</u>
10	Control	10.0		
	1	10.0		100.0
	2	10.0	5.0	
	3	10.0	10.0	
	4	10.0	15.0	
15	5	10.0	20.0	
	6	10.0	25.0	
	7	10.0	30.0	
	8	10.0	10.0	100.0

20

The formulations of Table 2 were stored in a darkened container at room temperature and tested periodically for the amount of 2-CdA in solution. The reported figure is 2-CdA in solution in mg/mL.

TABLE 3

25

	<u>Days</u>	<u>Formulation</u>								<u>Control</u>
		<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	
	0 (target)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
30	3			10.1						7.65
	4		8.29		9.97	9.80	10.1	9.99	9.67	
	6	4.71								
	10			9.54						7.30
	17			4.66						2.21
35	22	2.94								
	24			4.25						2.26
	32		2.61		4.42	6.25	6.85	7.92	4.71	
	38			3.64						2.09

6

59		3.63	2.15
74	2.66		
102	2.85		
111		3.7	2.24

5

It is evident from the data above, that the solubility of 2-CdA in water is greatly increased by the addition of either propylene glycol or benzyl alcohol. Nowhere are co-solvents having such a marked effect on the solubility of 2-CdA taught or suggested in the prior art.

WHAT IS CLAIMED IS:

1. A solution of 2-CdA in water comprising:
 - 5 a) from about 1 to about 8 mg/mL 2-CdA; and
 - b) a solubilizing agent selected from the group consisting of from about 5 to about 30 mg/mL of benzyl alcohol and from about 50 to about 400 mg/mL of propylene glycol.
- 10 2. The solution of claim 1 comprising from about 2 to about 7 mg/mL of 2-CdA.
- 15 3. The solution of claim 1 comprising from about 3 to about 5 mg/mL of 2-CdA.
4. The solution of claim 1 wherein the solubilizing agent is propylene glycol.
5. The solution of claim 1 wherein the solubilizing agent is benzyl alcohol.
- 20 6. The solution of claim 1 wherein the solubilizing agent selected from the group consisting of from about 8 to about 20 mg/mL of benzyl alcohol and from about 75 to about 200 mg/mL of propylene glycol.
- 25 7. The solution of claim 1 wherein the solubilizing agent selected from the group consisting of from about 10 to about 15 mg/mL of benzyl alcohol and from about 100 to about 150 nmg/mL of propylene glycol.

Figure 1

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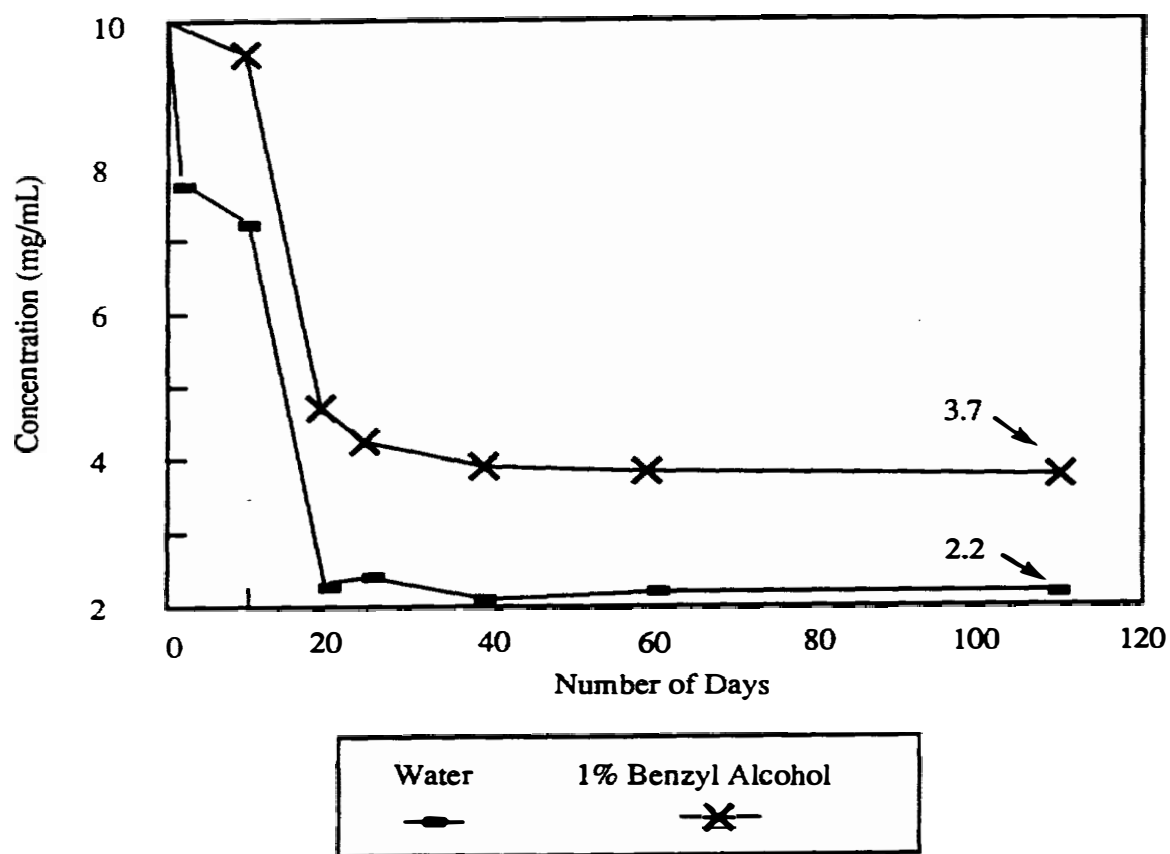


Figure 2

2/3

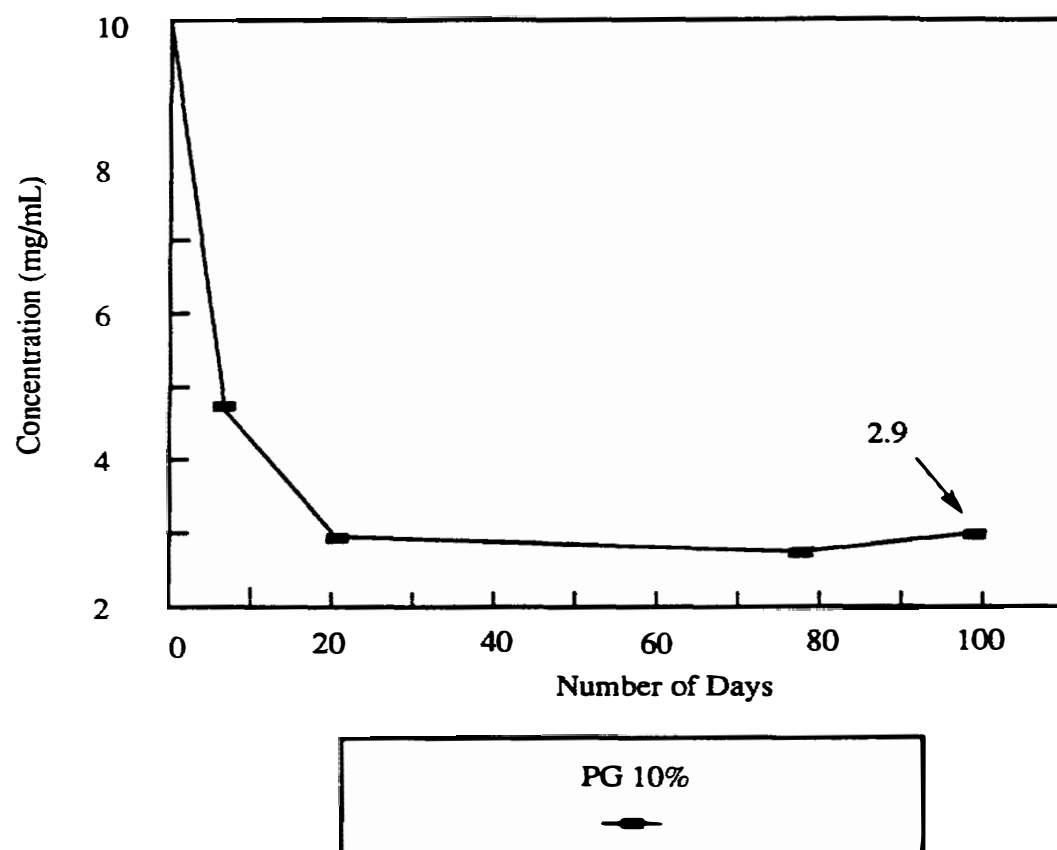
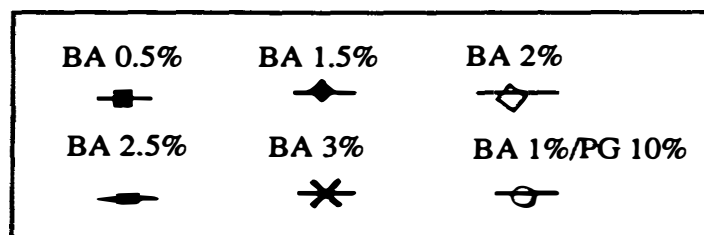
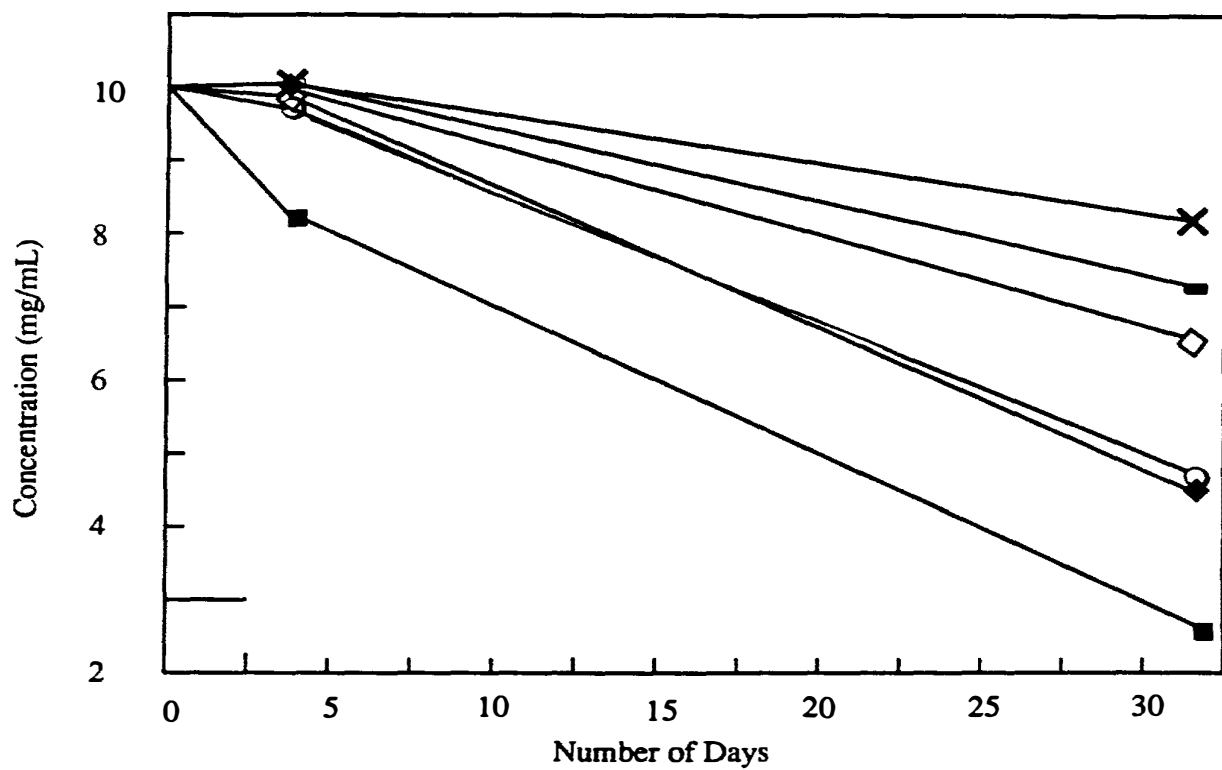


Figure 3

3/3



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/16418

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/70 A61K47/10

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 6 A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,5 310 732 (D.A.CARSON ET AL.) 10 May 1994 cited in the application see claims see example 18 ---	1-3,5-7
X	WO,A,93 23058 (THE SCRIPPS RESEARCH INSTITUTE.U.S.A.) 25 November 1993 see example 9 ---	1-3,5-7
A	EP,A,0 190 726 (WARNER-LAMBERT COMPANY,U.S.A.) 13 August 1986 see the whole document -----	1-7

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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- *E* earlier document but published on or after the international filing date
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- *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
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- *&* document member of the same patent family

Date of the actual completion of the international search

29 March 1996

Date of mailing of the international search report

10. 04. 96

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Authorized officer

Scarponi, U

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/16418

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(11)

EP 0 626 853 B1

(12)

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PCT/US93/01467

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WO 93/16706 (02.09.1993 Gazette 1993/21)

(54) **USE OF SUBSTITUTED ADENINE DERIVATIVES FOR TREATING MULTIPLE SCLEROSIS**

Verwendung von substituierten Adeninderivaten zur Behandlung von MultipleSklerose

UTILISATION DE DERIVES D'ADENINE SUBSTITUEE POUR LE TRAITEMENT DE LA SCLEROSE
EN PLAQUES

(84) Designated Contracting States:
**AT BE DE DK ES FR GB GR IE IT LU MC NL PT
SE**

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- Acta Neurol. Scand., vol. 75, 1987, p.352-355
- J. Exp. Med., vol. 160, 1984, p.310-316
- Nervenarzt, vol. 66, 1995, p.299-303

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 0 626 853 B1

DescriptionTechnical Field

- 5 **[0001]** This invention relates to the use of substituted adenine derivatives for the manufacture of a medicament for treating multiple sclerosis.

Background of the invention

- 10 **[0002]** Multiple sclerosis (MS) is the result of demyelination in the brain and spinal cord (central nervous system). Symptoms resulting from this demyelination include weakness, visual impairment, incoordination, and paresthesia (abnormal tingling). The course of the disease is largely unpredictable, but often progresses through a cycle of exacerbation of symptoms followed by remission.

- [0003]** Conventional treatments presently employ therapy with ACTH or corticosteroids such as prednisone. Controlled studies suggest that such treatments induce more rapid clearing of acute symptoms and signs but leave the long-term outcome of the disease unaffected. Long-term maintenance therapy with ACTH or corticosteroids is contraindicated. Evidence indicates that immunosuppressant agents have no long-term benefit. (Cecil, Textbook of Medicine, Beeson et al., eds., 15th ed., W.B. Saunders Company, Philadelphia, (1979) page 847)

- [0004]** The etiology of multiple sclerosis is unknown but is linked to a variety of genetic and environmental factors. Both cell-mediated and humoral immune responses, triggered by extraneous or autoantigens may contribute to the pathogenesis of multiple sclerosis. Certain immune response genes may be associated with an increased susceptibility to the disease. The disease may be mediated by T cells that recognize an as yet unidentified autoantigen. For example, experimental allergic encephalomyelitis (EAE), an animal model of demyelinating diseases such as multiple sclerosis, can be induced by immunizing mice with whole myelin or specific myelin components such as myelin basic protein.

- 25 **[0005]** In humans with multiple sclerosis, exacerbations are correlated with high levels of neopterin in blood and cerebrospinal fluid. Neopterin is a factor released from monocytes and macrophages in the presence of activated T-cells, thereby implicating these cells as being involved in multiple sclerosis exacerbations. (Fredrickson et al. (1987), *Acta Neurol. Scand.*, 75:352-355; Huber et al. (1984), *J. Exp. Med.*, 160:310-316). At the microscopic level, monocytes, microglial cells (macrophages of the central nervous system) and activated T-cells are found within the demyelinated regions of the nerve cells during multiple sclerosis exacerbations. (Cecil, Textbook of Medicine (1979), Beeson et al. (eds.), W.B. Saunders Co., Philadelphia, PA).

[0006] Various conventional treatment methodologies have been employed to ameliorate the symptoms of multiple sclerosis. Many of these are directed to use of palliative, anti-inflammatory agents. No treatment to date has had any consistent positive effect on the course of the disease.

- 35 **[0007]** Recently, the art has described the use of specific deoxyribosides as anti-inflammatory agents. For instance, U.S. Patent No. 4,481,197 (Rideout et al.) relates to the use of unsubstituted 3-deaza-2'-deoxyadenosine derivatives in the treatment of inflammation. U.S. Patent No. 4,381,344 (Rideout et al.) relates to a process for the synthesis of deoxyribosides that utilizes a bacterial phosphorylase.

- [0008]** A deoxyriboside derivative, 2-chloro-2'-deoxyadenosine (CdA), has been found to be an effective agent for the treatment of chronic lymphocytic leukemia and some T cell malignancies. (Carson et al. (1984) *Proc. Natl. Acad. Sci. U.S.A.*, 81:2232-2236; Piro et al. (1988), *Blood* 72:1069-1073) The pharmacokinetics of orally and subcutaneously administered 2-chloro-2'-deoxyadenosine in the treatment of chronic lymphocytic leukemia have been described and compared. (Liliemark et al. (1992) *Journal of Clinical Oncology*, 10, (10): 1514-1518; Juliusson et al. (1992) *Blood*, 80 (Suppl. 1): 1427) Chronic lymphocytic leukemia is a malignancy of B lymphocytes that bear the Leu-I surface antigen.

- 45 **[0009]** The Leu-I B cells represent a minor proportion of the normal pool of B lymphocytes, usually less than 20 percent. The Leu-I B cells express surface markers that are typically found on monocytes (Mac-I antigen) and T-lymphocytes (Leu-I antigen). Approximately 10 percent of patients with chronic lymphocytic leukemia exhibit accompanying autoimmunity, and recently, Leu-I B cells have been implicated in the pathogenesis of autoimmune diseases.

- [0010]** Phase I clinical trials on human patients with chronic lymphocytic leukemia indicate that infusion of increasing doses of 2-chloro-2'-deoxyadenosine [0.1-0.5 milligrams per kilogram of body weight per day (mg/kg/day)] yielded increasing plasma concentrations of the drug [10-50 nanomolar (nM)]. Those infusions indicated that the drug was well tolerated and did not induce nausea, vomiting or fever. The dose-limiting toxicity was bone marrow suppression, which usually occurred at doses greater than about 0.2 mg/kg/day or at plasma levels of greater than about 20 nM.

- [0011]** Other studies, Montgomery et al. (1959) *J. Am. Chem. Soc.*, 82:463-468, indicated that 2-fluoroadenosine exhibits a relatively high degree of cytotoxicity. Those workers reported that C57 black mice implanted with Adenocarcinoma 755 (Ad755) could tolerate only about 1 milligram per kilogram of body weight. 2-Fluoroadenosine was found to be inactive at that level against Ad755 as well as leukemia L1210 and the Erlich ascites tumor.

- [0012]** U.S. Patent No. 4,751,221 and its division No. 4,918,179 to Watanabe et al. describe the synthesis and use

of several 2-substituted-2'-deoxy-2'-fluoroarabino-furanosyl nucleosides including adenine derivatives. Those compounds were said to have anti-tumor and antitrypanosomal biological activities. Cytotoxicity data showing anti-tumor activity of 2-amino-6-thiopurine, guanine and thiopurine derivatives against murine and human cell lines were reported.

[0013] U.S. Patent No. 5,034,518 to Montgomery et al. teaches the synthesis of 2-substituted-2'-deoxy-2'-fluoro-araadenosines. Those compounds were said to have anticancer activity, and data for prolongation of life of mice transplanted with P388 leukemia cells were provided.

[0014] The biochemical activity of 2-CdA in cells has been reviewed by Ernest Beutler. (The Lancet (1992), 340: 952-956 - incorporated herein by reference)

[0015] The 2',3'-dideoxynucleosides are phosphorylated at the 5'-position in T cells to form the 5'-nucleotide triphosphate derivatives. Those derivatives are well known to be substrates for reverse transcriptase molecules. (Ono et al. (1986) Biochem. Biophys. Res. Comm., 2:498-507)

[0016] Those 2',3'-dideoxynucleoside 5'-triphosphates are also utilized by mammalian DNA polymerases beta and gamma. (Waquar et al. (1984) J. Cell. Physiol., 121:402-408) They are, however, poor substrates for DNA polymerase-alpha, the main enzyme responsible for both repair and replicative DNA synthesis in human lymphocytes. In part, these properties may explain the selective anti-HIV activity of the 2',3'-dideoxynucleosides.

[0017] Chan et al. (1982) J. Cell Physiol., 111:28-32 studied the pathways of pyrimidine nucleotide metabolism in murine peritoneal macrophages and monocytes, and reported undetectable levels of deoxycytidine kinase or thymidine kinase in these cells. High levels of adenosine kinase were found, however.

[0018] Similar high levels of adenosine kinase have been found in human monocytes and human monocyte-derived macrophages (MDM). MDM were found to exhibit about one-tenth to about one-fourth the nucleoside kinase activity of GEM T lymphoblasts (e.g. ATCC CCL 119) toward uridine, deoxycytidine and thymidine, and about two-thirds the adenosine kinase activity of GEM cells. In addition, that adenosine kinase activity of MDM cells was at least about 10-fold higher than any of the other kinase activities. Those studies also indicated relatively low levels of nucleoside phosphorylation using AZT, dideoxycytidine (ddC) and 2',3'-dideoxyadenosine (ddA) in intact GEM T lymphoblasts and still lower levels with the MDM.

[0019] Several 2-substituted adenosine derivatives have been reported not to be deaminated by adenosine deaminase. For example, Coddington (1965) Biochim. Biophys. Acta, 99:442-451 reported that deoxyadenosine-1-N-oxide, as well as 2-hydroxy-, 2-methyl-, 2-chloro-, 2-acetamido-, and 2-methylthio-adenosines were neither substrates nor inhibitors for adenosine deaminase. Montgomery, in Nucleosides, Nucleotides, and Their Biological Applications, Rideout et al. eds., Academic Press, New York, page 19 (1983) provides a table of comparative K_m and V_{max} data for the deamination of adenosine, 2-halo-adenosines 2-halo-deoxyadenosines and 2-fluoro-arabinoadenosine that also indicates that those 2-halo adenine derivatives are poor substrates for the enzyme relative to adenine itself. Stoeckler et al. (1982) Biochem. Pharm., 31:1723-1728 reported that the 2'-deoxy-2'-azidoribosyl and 2'-deoxy-2'-azidoarabinosyl-adenine derivatives were substrates for human erythrocytic adenosine deaminase, whereas work of others indicated 2-fluoroadenosine to have negligible activity with adenosine deaminase.

[0020] 2-Chloro-2'-deoxyadenosine is phosphorylated by non-dividing (normal) human peripheral blood lymphocytes and is converted to the 5'-triphosphate. This adenine derivative is not catabolized significantly by intact human cells or cell extracts, and is phosphorylated efficiently by T lymphocytes. (Carson et al. (1980) Proc. Natl. Acad. Sci. USA, 77:6865-6869)

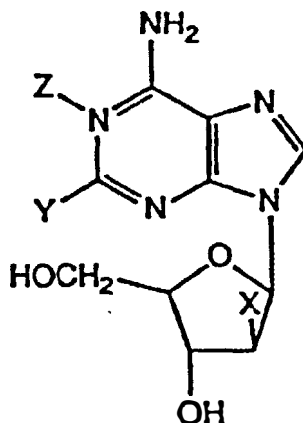
[0021] As discussed before, high levels of adenosine kinase have been found in murine peritoneal macrophages and in human monocytes. Adenosine kinase can phosphorylate 2'-deoxyadenosine derivatives, but does so less efficiently than deoxycytidine kinase. (Hershfield et al. (1982) J. Biol. Chem., 257:6380-6386)

[0022] Chemotherapeutic agents are described hereinafter that may be employed as therapeutic agents in the treatment of multiple sclerosis.

Summary of the Invention

[0023] The present invention relates the use of an substituted derivative for the preparation of a medicament for treating multiple sclerosis. The medicament has a pharmacologically acceptable carrier and a substituted adenine derivative dissolved or dispersed therein. The substituted adenine derivative is present in the pharmacologically acceptable carrier in an amount sufficient to provide a therapeutically effective dose over the course of treatment.

[0024] The substituted adenine derivatives useful for treating multiple sclerosis may be represented by Formula I having a structural formula corresponding to:



wherein Z is O^- or absent,

Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, provides a soluble adenine derivative and whose presence on the adenine moiety inhibits deamination of the adenine derivative by adenosine deaminase; and

X is hydrogen or fluoro, with the proviso that when Z is absent, Y is not hydrogen.

[0025] Particularly preferred compounds of Formula I are free of the Z group; i.e. Z is absent, and contain a halo group at the 2-position. The most preferred compounds are 2-chloro-2'-deoxyadenosine and 2-chloro-2'-deoxy-2'-arafluoroadenosine.

[0026] Methods for synthesizing all of the above compounds are indicated in U.S. Patent 5,106,837 (Carson et al., April 21, 1992, incorporated herein by reference).

[0027] The invention teaches that the disease condition of a patient having multiple sclerosis may be ameliorated by administration of an amount of the above-described composition having a sufficient quantity of the compound of Formula I to provide a therapeutically effective dose. Exemplary dosages range from about 0.04 to about 1.0 mg/kg/day, with dosages of about 0.04 to about 0.2 mg/kg/day being more preferred. Typically, the amount is sufficient to provide a concentration in the patient's plasma of about 0.5 nanomolar (nM) to about 50 nM, more preferably of about 1 nM to about 10 nM.

[0028] Preferably, the agent contemplated for use in the present invention is a 2-halo-2'-deoxyadenosine (2-halo-2'-deoxy-9,1'-beta-ribofuranosyladenine) or a 2-halo-2'-deoxy-2'-arafluoroadenosine, and most preferably the halo group is chloro.

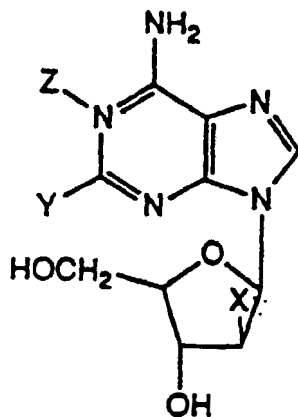
[0029] A further aspect contemplated by the present invention comprises the use of subcutaneous injection for administering an effective amount of the active ingredient (agent) of the invention for treating multiple sclerosis.

[0030] An alternative aspect contemplated by the present invention comprises the peroral administration of an effective amount of the active ingredient (agent) of the invention in a method of treating disease. Preferred compounds of Formula I for oral administration include compounds in which X is fluoro.

[0031] In each of the before-described methods, the substituted 2'-deoxyadenosine derivative is administered in a therapeutically effective amount. The effect of a compound of Formula I is dependent upon the route of administration and upon the time and dosage. As a consequence, one can tailor the dosage and duration for which a particular compound is administered to the stage of the disease and the condition of the patient being treated. Where the stage of multiple sclerosis is advanced or life-threatening, treatment may be more aggressive, and a therapeutically effective amount is an amount that is sufficient to kill at least 50 percent of the monocytes present but is less than that which substantially impairs bone marrow function as determined by usual procedures when administration is *in vivo*. The monocyte killing amount of a compound of Formula I is another measure of a therapeutically effective dose and monocyte death is measured at a time seven days after the initial administration.

Detailed Description of the InventionA. Compounds

5 **[0032]** The present invention contemplates the use of substituted adenine derivatives, i.e. substituted-2'-deoxy-arabinofuranosyladenine, for treating multiple sclerosis. Preferred substituted adenine derivatives have a structure represented by the following formula, viz. Formula I:



30

wherein Z is an oxide radical (O⁻) or is absent;

Y is hydrogen or a radical containing one to about twenty atoms that is free from net ionic charge at physiological pH values, provides a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of the adenine derivative by adenosine deaminase; and

35 X is hydrogen or fluorine, with the proviso that Y is hydrogen only when Z is present.

[0033] Preferably, Y is chloro. Other Y substituents may be selected from the group consisting of lower alkyl, lower alkanoylamido, lower alkylthio and hydroxyl radicals. In particularly preferred embodiments, when Y is chloro, X is fluo-
40 rine.

[0034] The preferred compound included in Formula I is 2-chloro-9,1'-beta-D-2'-deoxyriboseyladenine, otherwise known as 2-chlorodeoxyadenosine or CdA.

[0035] Of the compounds of Formula I, those where X is fluoro are among the preferred compounds for use by oral administration.

45 **[0036]** Other illustrative compounds included in Formula I are:

- 2-bromo-9,1'-beta-D-2'-deoxyriboseyladenine;
 2-methyl-9,1'-beta-D-2'-deoxyriboseyladenine;
 2-fluoro-9,1'-beta-D-2'-deoxyriboseyladenine;
 50 2-acetoamido-9,1'-beta-D-2'-deoxyriboseyladenine;
 2-methylthio-9,1'-beta-D-2'-deoxyriboseyladenine;
 2-chloro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
 2-bromo-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
 2-(N-acetamido)-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine;
 55 2-methylthio-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine.

[0037] Further illustrative of compounds of Formula I include the following arabinofuranosyl derivatives of adenine:

2-methyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
 2-isopropyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
 2-hydroxy-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyl-adenine;
 2-chloro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
 5 2-fluoro-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
 2-bromo-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
 2-methyl-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
 2-(N-acetamido)-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
 2-hydroxy-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
 10 2-(2-methylbutyl)-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine-1-N-oxide;
 2-fluoro-9,1'-beta-D-2'-deoxyadenosine-1-oxide;
 and
 2-chloro-9,1'-beta-D-2'-deoxyadenosine-1-oxide.

15 **[0038]** It is noted that when X is hydrogen the sugar ring can be named as a 2'-deoxyribosyl or 2'-deoxyarabino-furanosyl radical. Both nomenclatures are utilized herein. When the class of compounds embraced by Formula I is discussed, all of the compounds are considered herein as derivatives of arabinose. However, when specific compounds of the subclass where X = H are discussed, the more familiar deoxyribose nomenclature is used, such as in deoxyadenosine. These compounds are also referred to herein more simply as adenine derivatives.

20 **[0039]** In the above formulas, and in all other formulas shown herein, hydrogen atoms on the purine and furanosidyl rings that are not needed to show conformation about a particular bond are not shown. Thus, the 8-position adenine hydrogen is not shown.

[0040] It is also to be understood that the D isomers of compounds of the formulas are the isomers contemplated. It is further to be noted that the designation "halo" used herein is meant to include fluorine, chlorine and bromine derivatives, and to exclude iodine derivatives, which are unstable and decompose, and astatine derivatives that are radioactive. Where specific halogen derivatives are intended, those compounds are named specifically.

25 **[0041]** As used herein, "a substituent free from net ionic charge" includes both charged and uncharged radicals, wherein when the substituent radical is charged, an internal zwitterionic charge pair is present that results in the absence of a net ionic charge for the molecule at physiologic pH values. N-oxide compounds are exemplary of such substituents.

[0042] As used herein, a "soluble adenine derivative" is an adenine derivative which is able to dissolve and remain soluble in a body fluid such as blood at a therapeutically effective dose as is discussed hereinafter.

30 **[0043]** As used herein, a "substituent whose presence on the adenine moiety inhibits deamination of an adenine derivative by adenosine deaminase" is one that, when 100 microliters of a 1 millimolar solution of the substituted adenine derivative is incubated for three hours at room temperature with 25 units of calf spleen adenosine deaminase (1 unit catalyzes the deamination of 1 micromole of adenosine per minute), produces a single UV-absorbing spot upon cellulose-thin layer chromatography of the reaction mixture whose R_f value is the same as that of the substituted adenine derivative used.

40 **[0044]** The metabolism of a compound by adenosine deaminase can be investigated by the following procedure. The individual nucleosides, at concentrations from 5-200 μ M in 10 mM sodium phosphate, pH 7.5, are incubated at 18-20 degrees C with 0.01 EU/ml calf intestinal adenosine deaminase. The change in the optical density at 265 nm and 250 nm is monitored spectrophotometrically. The K_m and V_{max} values are determined by the Lineweaver-Burke method, utilizing the ΔE_{265}^M between adenosine and inosine.

45 **[0045]** The ratio V_{max}/K_m also provides a measure of relative efficiency of deamination by the enzyme. A substituent that provides a V_{max}/K_m ratio that is about 1 percent or less than that for the ratio obtained using 2'-deoxyadenosine is also a "substituent whose presence on the adenine moiety inhibits deamination of an adenine derivative by adenosine deaminase."

50 **[0046]** As used herein, lower alkyl radicals include C_1 - C_6 straight chain, branched and cyclic alkyl groups, for example, methyl, ethyl, n-butyl, t-butyl, n-hexyl, 1-ethylbutyl, cyclopentyl, cyclohexyl and the like. Lower alkanoylamido radicals include C_1 - C_6 radicals, for example, formamido, acetylamido, propionamido, hexamoylamido and the like. Lower alkylthio radicals include C_1 - C_6 straight chain, branched and cyclic alkyl groups as discussed above linked to a thio radical.

55 **[0047]** The pharmacologically acceptable salts of a compound of the above Formula are also utilized. The phrase "pharmacologically acceptable salts," as used herein, refers to non-toxic acid addition salts that are generally prepared by reacting a compound with a suitable organic or inorganic acid. Representative salts include the hydrochloride, hydrobromide, sulfate, phosphate, citrate, acetate, maleate and the like.

B. Compositions

[0048] A compound of Formula I dissolved or dispersed in or together with a pharmacologically acceptable carrier constitutes a composition of this invention.

5 **[0049]** A compound of Formula I and its pharmacologically acceptable salts are useful in both short and long term treatment. For instance, a 2-substituted-9,1'-beta-2'-deoxy-2'-fluoro-D-arabinofuranosyladenine is administered to the patient internally, e.g., subcutaneously by injection, parenterally, orally, or rectally as a suppository, in an effective amount.

10 **[0050]** Although a compound of Formula I and its pharmacologically acceptable salts can be administered as the pure chemical, it is preferred that it be administered as a pharmaceutical composition. In either event, it is administered in an amount sufficient to provide a therapeutically effective dose as is discussed hereinafter.

[0051] Accordingly, the present invention utilizes a pharmaceutical composition comprising a therapeutically effective dose of a compound of Formula I or a pharmacologically acceptable salt thereof, hereinafter referred to as the "active ingredient" or "agent," dissolved or dispersed in a pharmacologically acceptable carrier or diluent.

15 **[0052]** A pharmaceutical composition is prepared by any of the methods well known in the art of pharmacy all of which involve bringing into association the active compound and the carrier therefor. For therapeutic use, a compound utilized in the present invention can be administered in the form of conventional pharmaceutical compositions. Such compositions can be formulated so as to be suitable for oral, subcutaneous, or parenteral administration, or as suppositories. In these compositions, the agent is typically dissolved or dispersed in a physiologically tolerable carrier.

20 **[0053]** A carrier or diluent is a material useful for administering the active compound and must be "pharmacologically acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. Thus, as used herein, the phrases "physiologically tolerable" and "pharmacologically acceptable" are used interchangeably and refer to molecular entities and compositions that do not produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a mammal. The physiologically tolerable carrier can take a wide variety of forms depending upon the preparation desired for administration and the intended route of administration.

25 **[0054]** As an example of a useful composition, a compound of Formula I can be utilized in liquid compositions such as sterile suspensions or solutions, or as isotonic preparations containing suitable preservatives. Particularly well-suited for the present purposes are injectable media constituted by aqueous injectable isotonic and sterile saline or glucose solutions. Additional liquid forms in which these compounds can be incorporated for administration include flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, peanut oil, and the like, as well as elixirs and similar pharmaceutical vehicles.

30 **[0055]** The agents can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain stabilizers, preservatives, excipients, and the like in addition to the agent. The preferred lipids are the phospholipids and the phosphatidylcholines (lecithins), both natural and synthetic.

40 **[0056]** Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 et seq.

[0057] An agent of Formula I can also be used in compositions such as tablets or pills, preferably containing a unit dose of the compound. To this end, the agent (active ingredient) is mixed with conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate, gums, or similar materials as non-toxic, physiologically tolerable carriers. The tablets or pills can be laminated or otherwise compounded to provide unit dosage forms affording prolonged or delayed action.

45 **[0058]** It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical formulation described herein can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, flavoring agents, binders, surface active agents, thickeners, lubricants, preservatives (including antioxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient.

50 **[0059]** The tablets or pills can also be provided with an enteric layer in the form of an envelope that serves to resist disintegration in the stomach and permits the active ingredient to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, including polymeric acids or mixtures of such acids with such materials as shellac, shellac and cetyl alcohol, cellulose acetate phthalate, and the like. A particularly suitable enteric coating comprises a styrene-maleic acid copolymer together with known materials that contribute to the enteric properties of the coating. Methods for producing enteric coated tablets are described in U.S. Patent 4,079,125 to Sipos, which is herein incorporated by reference.

[0060] The term "unit dose", as used herein, refers to physically discrete units suitable as unitary dosages for

administration to patients, each such unit containing a predetermined quantity of the agent calculated to produce the desired therapeutic effect in association with the pharmaceutically acceptable diluent. Examples of suitable unit dosage forms in accord with this invention are tablets, capsules, pills, powder packets, granules, wafers, cachets, teaspoonfuls, dropperfuls, ampules, vials, segregated multiples of any of the foregoing, and the like.

[0061] Administration of the compound by subcutaneous injection is a particularly attractive mode of administration due to the favorable pharmacokinetics of this mode of administration.

[0062] Oral administration of the compound is also an attractive mode of administration. One drawback usually associated with oral administrations of bioactive nucleoside compounds, however, is their potential decomposition in the acidic conditions of the stomach. That is, the glycosidic bond tends to hydrolyze under acid conditions.

[0063] However, where oral administration is desired, substitutions on the 2-position of the adenine ring of the compound of Formula I are utilized along with a 2'-fluoro-substituted arabinofuranosidyl ring.

[0064] Marquez et al. (1987) *Biochem. Pharm.*, **36**:2719-2722 reported preparation of 2'-fluoro-2',3'-dideoxyribose and 2'-fluoro-2',3'-dideoxyarabinose derivatives of adenine. Their findings stated that both derivatives were stable at a pH value of 1 at 37 degrees C, whereas dideoxyadenosine had a half-time of 35 seconds under those conditions.

[0065] The ability of an adenine derivative to be or not to be a substrate for adenosine deaminase is more a function of the 2-substituent or lack thereof on the adenine portion of the molecule than a function of substituents on the linked sugar ring portion, at least as far as the substituents on both rings herein are concerned.

C. Methods

[0066] As noted earlier, a method of treating multiple sclerosis is contemplated herein. Broadly in that method, a patient having multiple sclerosis is treated with a composition containing a pharmacologically acceptable carrier having dissolved or dispersed therein, as an active ingredient, a substituted adenine derivative (substituted 2'-deoxy-adenosine) whose structure corresponds to that of previously discussed Formula I. The substituted adenine derivative is present in the composition in an amount sufficient to provide a therapeutically effective dose over the period of contacting. The above treatment is typically repeated periodically such as weekly or monthly over a time period of several months to about one year.

[0067] The amount of a compound of Formula I present in a composition and used in a method as described above is a function of several variables. Among those variables is the method of administration. Exemplary concentrations for various modes of administration are illustrated hereinafter.

[0068] When the administration is in vivo, the amount administered is less than that which substantially impairs bone marrow functions as determined by usual procedures. An amount sufficient to kill at least about 50 percent of the monocytes originally present while not substantially impairing bone marrow function over the course of the administration of the agent is one way of defining a therapeutic dose.

[0069] The above amount of a 2'-deoxyadenine derivative of Formula I or its pharmacologically acceptable salt present in the composition is also an amount sufficient to provide about 0.04 to about 1.0 mg/kg of body weight of the treated host mammal per day, more preferably about 0.04 to about 0.20 mg/kg/day, more preferably still at about 0.05 to about 0.15 mg/kg/day and most preferably about 0.1 mg/kg/day, when given in vivo. This amount is another way of defining a therapeutically effective dose that is particularly useful when a compound of Formula I is administered by infusion.

[0070] The molar plasma concentration of the compound of Formula I or the pharmacologically acceptable salts thereof during treatment is preferably in the range of about 1 nanomolar (nM) to about 100 nM, particularly about 5 nM to about 50 nM, and more preferably about 10 nM to about 20 nM. Molarity of the 2'-deoxyadenine derivative in plasma of the treated (administered to) patient thus provides still another measure of a therapeutically effective dose from which the amount in a composition can be calculated.

[0071] It is to be understood that the above therapeutically effective dosages need not be the result of a single administration, and are usually the result of the administration of a plurality of unit doses. Those unit doses can in turn comprise portions of a daily or weekly dosage, and thus, the therapeutically effective dose is determined over the period of treatment (contacting).

[0072] Oral administration and subcutaneous injection are preferred modes of administration, as already noted. To achieve the desired plasma concentration of the agent, a range of doses can be employed depending upon the specific mode of administration, objective of the particular treatment, the particular compound being used, and like considerations.

[0073] For example, for oral administration, the daily dose can be about 0.04 to about 1.0 mg/kg of body weight, more preferably about 0.04 to about 0.20 mg/kg/day, more preferably still at about 0.05 to about 0.15 mg/kg/day, and most preferably about 0.1 mg/kg body weight. In general, the amount of active substituted adenine derivative administered can vary over a relatively wide range to achieve, and preferably maintain, the desired plasma concentration.

[0074] Unit dosage forms of the adenine derivative can contain about 0.1 milligrams to about 15 milligrams thereof.

A preferred unit dosage form contains about 0.1 to about 1 milligram of agent and can be administered 2 to 5 times per day. However, it should be noted that continuous infusion at a rate designed to maintain the above described plasma concentration is also contemplated.

[0075] Duration of a particular treatment can also vary, depending on severity of the disease, whether the treatment is intended for an acute manifestation or for prophylactic purposes, and like considerations. Typical administration lasts for a time period of about 5 to about 14 days, with a 7-day time course being usual. Courses (cycles) of administration can also be repeated at monthly intervals, or parenteral unit dosages can be delivered at weekly intervals. Oral unit dosages can be administered at intervals of one to several days to provide the determined therapeutically effective dose. Thus, *in vivo* administration of a before-discussed dosage over a time period of about 5 to about 14 days or at weekly or daily intervals provides an amount sufficient to kill at least about 50 percent of the originally present monocytes.

[0076] This method of treatment produces a decrease in the level of monocytes in the blood due to the toxicity of the utilized compounds of Formula I toward monocytes. This method can be used to reduce the number of monocytes circulating in a treated mammal's blood stream by about 90 percent of the number present prior to treatment over a seven day treatment period with the level of circulating monocytes returning to pretreatment levels about two weeks after the treatment stopped. This exemplary study is illustrated hereinafter.

[0077] A less aggressive treatment regimen is also therefore contemplated. Here, a before-described dosage, e.g., plasma concentration, is again utilized, but for a shorter contact time course so that monocyte function is impaired, but the monocytes are not substantially killed as is the result of the before-discussed treatment regimen. Impairment of monocyte function is herein defined as a reduction of at least about 25 percent in the spontaneous secretion of interleukin-6 (IL-6) by monocytes cultured in the presence of a compound of Formula I for a time period of 72 hours. A useful assay for monocyte impairment is discussed hereinafter.

[0078] In an exemplary treatment regimen, a compound of Formula I is administered in an amount of about 0.04 to about 1.0 mg/kg/day, more preferably about 0.04 to 0.20 mg/kg/day, more preferably still about 0.05 to about 0.15 mg/kg/day, and most preferably about 0.1 mg/kg/day. Such treatments typically provide a plasma concentration of about 0.5 nM to about 50 μ M, and more preferably about 10 nM to about 10 μ M. That single administration is repeated periodically such as weekly over a time period of several months, e.g. about three to about nine months. In usual practice, treatments are administered over a period of about five to seven days and are repeated at about three to about four week intervals for several months, e.g. about three to about nine months.

[0079] Such an administration can be carried out on an out-patient basis for humans using an intravenous infusion lasting about 2 to about 4 hours in a doctor's office. As such, the treatment is far less invasive than is a continuous infusion over a period of several days that usually requires a hospital stay for the host mammal; i.e., human patient. A less invasive continuous infusion method that employs a pump linked to a catheter that automatically infuses a predetermined dosage permits the patient to be ambulatory during the infusion.

[0080] Any of the before-discussed methods can be carried out while the patient is continuing therapy with a previous drug or drugs, or after cessation of such prior treatment. When a patient is removed from a prior even partially effective treatment, a flare-up (exacerbation) of symptoms sometimes occurs that typically abates after several months. In addition, where a prior treatment regimen is halted while an above method is practiced, that prior treatment can be continued after cessation of an above method, often with quite positive results.

[0081] Dosage schedules and protocols for administering 2-chlorodeoxyadenosine to treat patients having disease conditions other than multiple sclerosis have been reviewed in the literature. (Ernest Beutler (1992), *The Lancet*, 340: 952-956) To a first approximation, the pharmacokinetics of 2-chlorodeoxyadenosine and its effect upon monocyte levels are independent of the disease condition being treated.

D. Compound Synthesis

[0082] A compound useful herein where Z is absent can be prepared by condensing an appropriately substituted adenine directly with an appropriately substituted sugar ring as by the techniques described in Montgomery et al., (1986) *J. Med. Chem.*, 29:2389-2392, by the method taught in U.S. Patent No. 4,082,911, or as described in the citations of Herdewijn et al. (1987) *J. Med. Chem.*, 30:2131-2137, which disclosures are incorporated herein by reference. An appropriately substituted adenine can be prepared by following reported literature syntheses or analogous syntheses. Still further, Wright et al. (1987) *J. Org. Chem.*, 35:4617-4618 recently prepared 2-chloro- and 2-bromo-2'-deoxyadenosines by direct reaction of the appropriate 2,6-dihalo purine with a 3',5'-protected-alpha-l-chlororibose using sodium hydride in acetonitrile, followed by treatment with methanolic ammonia at 60 degrees C to deprotect the resulting 3',5'-hydroxyls and form the 6-amino group of the finally produced adenosine. Fukukawa et al. (1983) *Chem. Pharm. Bull.*, 31(5):1582-1592 also report syntheses of 2'-deoxy-2'-arabalo-substituted adenosine derivatives.

[0083] The 2'-deoxy-2'-fluoroarabinofuranosyladenine compounds of the present invention are produced as described hereinafter in the Examples. The synthesis is similar to that taught in Marquez et al. (1987) *Biochem. Pharmacol.*, 36:2719-2722, herein incorporated by reference, in which 6-chloropurine is condensed with 3-O-acetyl-5-O-

benzoyl-2-deoxy-2-fluoro-D-arabinofuranosyl bromide. The functionalized halosugar is produced according to the method reported by Reichman et al. (1975) J. Carbohydr. Res., 42:233 and the 2'-deoxy-2'-fluoro-arabinofuranosyladenine compound is obtained by ammonolysis with concentrated methanolic ammonia which removes the protective groups. Syntheses of 2-substituted-2'-deoxy-2'-arafluoroadenosines are also described in U.S. Patents No. 4,918,179 and No. 5,034,518, whose disclosures are incorporated by reference.

[0084] The adenosine-1-N-oxide group of compounds, i.e., where Z is present, is of particular interest since those materials, per se, are most likely not incorporated into a growing polynucleotide chain because the presence of the N-oxide group probably interferes with hydrogen bonding during that synthesis. Rather, it is believed that the N-oxide compounds are reduced by an endogenous reductase prior to their incorporation into and termination of the growing chain.

[0085] Nevertheless, being free from a net ionic charge, but possessing an internal zwitterionic charge pair, the N-oxide compounds can penetrate cell membranes. Those compounds are also somewhat more water-soluble than are the corresponding un-oxidized compounds.

[0086] Without wishing to be bound by theory, it is nevertheless believed that the N-oxide compounds enter the cell and are phosphorylated, in keeping with the report of such phosphorylation in Lindberg et al. (1967) J. Biol. Chem., 242:350-356. A pool of such derivatives is maintained intracellularly until such time as the N-oxide function is reduced and the nucleotide is incorporated to terminate the appropriate, growing polynucleotide chain.

[0087] The 1-N-oxide compounds are readily prepared by the method of Klenow et al. (1961) Biochim. Biophys. Acta, 52:386-389, with slight modification, as discussed hereinafter.

[0088] The present invention is further illustrated by the following examples which are not intended to limit the scope of the invention in any way.

Example 1

Treatment of Multiple Sclerosis with CdA

[0089] A study of four patients with chronic multiple sclerosis was undertaken. Each patient was first examined for normal hepatic, renal, and bone marrow functioning to establish baseline values. Each of the patients was then treated with CdA dissolved in sterile preservative-free isotonic saline. The CdA was administered intravenously at a dosage of 0.1 mg/kg each day for a total of seven days. Each patient received six courses of intravenous therapy, once monthly for a total of six months. Patients were examined on a daily basis while hospitalized. During that time, daily blood counts and twice weekly blood chemistries were performed on each patient. CdA levels were also measured in blood and spinal fluid.

[0090] The neurologic function of each of these patients was measured using the expanded Krutzke disability status scale (EDSS), and the Scripps neurologic rating scale (SNRS).

[0091] There was no evidence of any significant toxic side effects. None of the four patients exhibited any nausea, vomiting, skin rash, or hepatic or renal dysfunction. Each of the patients developed lymphopenia (reduction in the level of lymphocytes in the blood), with absolute lymphocyte counts being suppressed 0.5 to about 10 percent for more than one year.

[0092] Monocyte levels dropped after each treatment. For example, in one patient, monocytes dropped 40 percent after the first treatment, and were substantially absent after each of the remaining five treatments. For another patient, monocytes were substantially absent after two treatments, and depleted by about 85, 50, 40 and 73 percents after the other four treatments.

[0093] In some cases, there was leukopenia (reduction in the level of total white blood cells). There was also a modest macrocytosis in all patients lasting for six to eight months after cessation of treatment. However, the platelet counts of all four patients remained within the normal range. In essence, there was no evidence of toxicity in these four patients with normal marrow, hepatic and renal functions. Likewise, the side effects of CdA were imperceptible in these four patients.

[0094] Measurement of neurologic function using the EDSS and SNRS scales provided evidence of improvement in all four patients during treatment with CdA. Cerebrospinal fluid studies (CSF) showed a marked drop in lymphocyte counts and, quite remarkably, complete disappearance of IgG oligoclonal bands in all cases. There was no significant change in total CFS IgG.

[0095] In particular, the SNRS data demonstrated between 5 and 50 percent improvement from baseline pre-treatment values in all patients. One of the four patients was completely bed-ridden at the beginning of the treatment, and this patient was able to walk with the aid of a walker by the end of the treatment. All patients reported subjective feelings of improved energy and stamina.

Example 2Treatment of Multiple Sclerosis with CdA

- 5 **[0096]** The study indicated above in Example 1 involving four patients was then enlarged. A double-blind placebo study involving 50 patients was performed to further demonstrate the effectiveness of 2-CdA for treating multiple sclerosis. The dosage schedules and protocols for this second study were similar or substantially the same as the dosage schedules and protocols employed in Example 1. The same two neurologic rating scales were employed, i.e. the SNRS scale and the EDSS scale. 28 patients were tested with the SNRS scale; 23 patients were tested with the EDSS scale.
- 10 The SNRS scale is substantially more sensitive than the EDSS scale. The inventor's most recent data indicate that a highly significant improvement ($p=0.0004$) was observed in patients treated with 2-CdA as compared with placebo in the 28 patients tested for changes in the SNRS scale.

Table I
Changes in SNRS

	<u>Absolute</u>	<u>Relative</u>
20 CdA	4.83 $\pm$ 5.71	0.076 $\pm$ 0.089 (N=14)
Placebo	-4.40 $\pm$ 5.14	-0.062 $\pm$ 0.071 (N=14)
25	p=0.0004	p=0.0005

Changes in EDSS

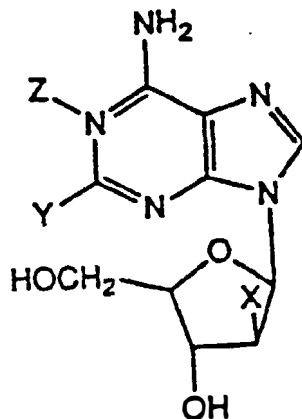
	<u>Absolute</u>	<u>Relative</u>
30 CdA	-0.018 $\pm$ 0.222	-0.011 $\pm$ 0.081 (N=12)
35 Placebo	0.038 $\pm$ 0.9233	0.039 $\pm$ 0.240 (N=11)
	p=0.84	p=0.50

40	SNRS	0	100
	EDSS	10	1
		Worst	Best

Claims

- 50 1. Use of a substituted adenine derivative having a structure represented by the formula:

55



or a pharmacologically acceptable salt thereof
where:

Z is O⁻ or absent;

Y is hydrogen or a substituent containing one to about 20 atoms that is free from net ionic charge at physiological pH values, produces a soluble adenine derivative, and whose presence on the adenine moiety inhibits deamination of said adenine derivative by adenosine deaminase; and

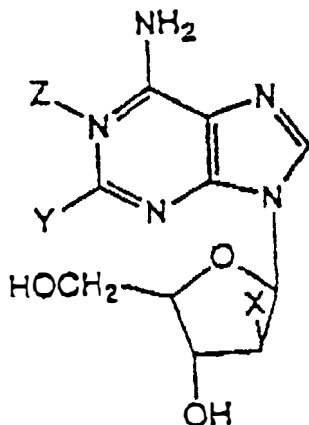
X is hydrogen or fluoro,

with the proviso that when Z is absent, Y is not hydrogen for the preparation of a medicament for treating multiple sclerosis.

2. The use of claim 1, wherein Z is absent and Y is halo group.
3. The use of claim 1 wherein said substituted adenine is 2-chloro-2'-deoxyadenosine.
4. The use of any one of claims 1 to 3, wherein the treatment is by application of the adenine derivative to the patient's serum.
5. The use of any one of claims 1 to 4, wherein the medicament is in the form of a subcutaneous injectable medium.
6. The use of any one of claims 1 to 4, wherein the medicament is in the form of an intravenous infusion.
7. The use of any one of claims 1 to 4, wherein the medicament is in the form of capsules, powders or granules, in the form of tablets or pills that are optionally provided with an enteric layer, in the form of a suppository, in the form of a solution, suspension or elixir or in the form of liposomes.

Patentansprüche

1. Verwendung eines substituierten Adeninderivats mit einer Struktur der Formel:



oder eines pharmakologisch annehmbaren Salzes davon,
worin:

Z O⁻ ist oder nicht vorliegt;

Y Wasserstoff oder ein Substituent ist, welcher 1 bis etwa 20 Atome aufweist, der frei ist von einer Netto-Ionenladung bei physiologischen pH-Werten, ein lösliches Adeninderivat erzeugt und dessen Anwesenheit auf dem Adeninrest eine Desaminierung des Adeninderivats durch Adenosindesaminase inhibiert; und

X Wasserstoff oder Fluor ist,

mit der Maßgabe, daß, wenn Z nicht vorliegt, Y nicht Wasserstoff ist, für die Herstellung eines Medikaments zur Behandlung von Multiple Sklerose.

2. Verwendung gemäß Anspruch 1, worin Z nicht vorliegt und Y eine Halogengruppe ist.

3. Verwendung gemäß Anspruch 1, worin das substituierte Adenin 2-Chlor-2'-desoxyadenosin ist.

4. Verwendung gemäß einem der Ansprüche 1 bis 3, worin die Behandlung durch die Anwendung des Adeninderivats auf das Serum des Patienten geschieht.

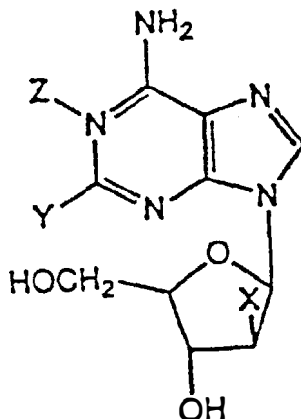
5. Verwendung gemäß einem der Ansprüche 1 bis 4, wobei das Medikament in Form eines subkutanen injizierbaren Mediums vorliegt.

6. Verwendung gemäß einem der Ansprüche 1 bis 4, wobei das Medikament in Form einer intravenösen Infusion vorliegt.

7. Verwendung gemäß einem der Ansprüche 1 bis 4, wobei das Medikament in Form von Kapseln, Pulvern oder Granulaten, in der Form von Tabletten oder Pillen, welche vorzugsweise mit einer enterischen Schicht versehen sind, in der Form ein Zäpfchens, in der Form einer Lösung, einer Suspension oder eines Elixiers oder in der Form von Liposomen vorliegt.

Revendications

1. Utilisation d'un dérivé d'adénine substituée ayant la structure représentée par la formule :



ou d'un sel pharmacologiquement acceptable d'un tel dérivé

formule dans laquelle :

Z représente O^- ou est absent ;

Y représente l'hydrogène ou un substituant contenant de 1 à environ 20 atomes qui est dépourvu de charge ionique nette aux valeurs de pH physiologiques, produit un dérivé soluble de l'adénine et dont la présence sur le motif adénine inhibe la désamination dudit dérivé de l'adénine par l'adénosine-désaminase ; et

X représente l'hydrogène ou fluoro, étant entendu que lorsque Z est absent, Y ne représente pas l'hydrogène, pour la préparation d'un médicament pour le traitement de la sclérose en plaques.

2. Utilisation selon la revendication 1, dans laquelle Z est absent et Y est un groupe halogéno.
3. Utilisation selon la revendication 1, dans laquelle ladite adénine substituée est la 2-chloro-2'-déoxyadénosine.
4. Utilisation selon l'une quelconque des revendications 1 à 3, dans laquelle le traitement se fait par application du dérivé de l'adénine au sérum du patient.
5. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle le médicament est sous la forme d'un milieu injectable par voie sous-cutanée.
6. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle le médicament est sous la forme d'une perfusion intraveineuse.
7. Utilisation selon l'une quelconque des revendications 1 à 4, dans laquelle le médicament est sous la forme de capsules, poudres ou granules, sous la forme de comprimés ou de pilules qui sont éventuellement pourvus d'une couche entérique, sous la forme d'un suppositoire, sous la forme d'une solution, d'une suspension ou d'un élixir ou sous la forme de liposomes.

(19) World Intellectual Property
Organization
International Bureau



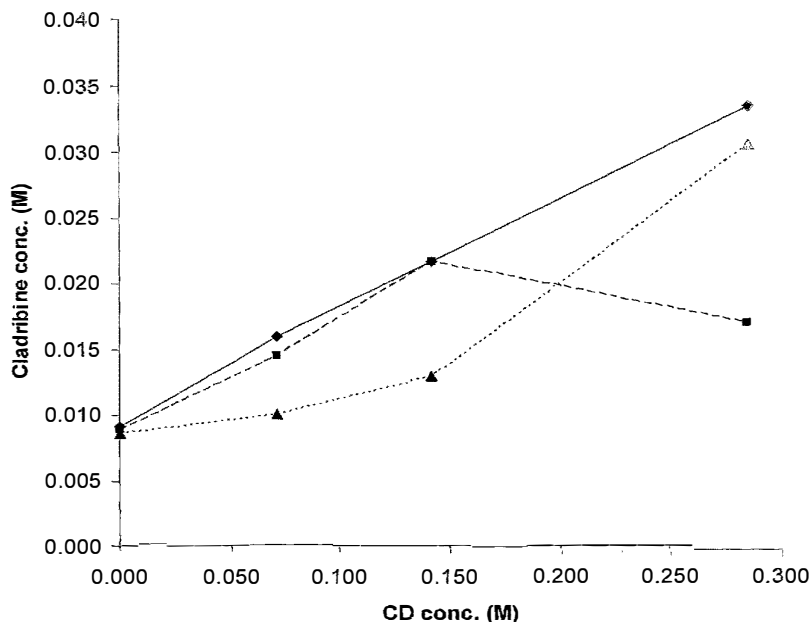
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(54) Title: CLADRIBINE FORMULATIONS FOR IMPROVED ORAL AND TRANSMUCOSAL DELIVERY



(57) Abstract: Provided are compositions of cladribine and cyclodextrin which are especially suited for the oral and buccal administration of cladribine.

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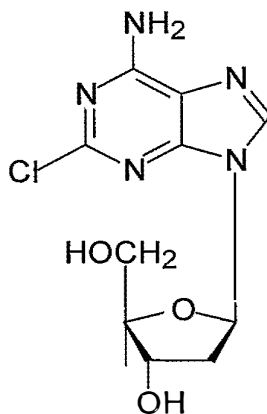
CLADRIBINE FORMULATIONS FOR IMPROVED ORAL AND TRANSMUCOSAL DELIVERY

FIELD OF THE INVENTION

5 The invention relates to a composition comprising a cladribine-cyclodextrin complex formulated into a solid oral dosage form or a transmucosal dosage form and to a method for enhancing the oral and transmucosal bioavailability of cladribine.

10 BACKGROUND OF THE INVENTION

 Cladribine, which is an acid-labile drug, has the chemical structure as set forth below:



 It is also known as 2-chloro-2'-deoxyadenosine or 2-CdA.

15 Cladribine is an antimetabolite which has use in the treatment of lymphoproliferative disorders. It has been used to treat experimental leukemias such as L1210 and clinically for hairy cell leukemia and chronic lymphocytic leukemia as well as Waldenstrom's macroglobulinaemia. It has also been used as an immunosuppressive agent and as a modality for the
20 treatment of a variety of autoimmune conditions including rheumatoid arthritis, inflammatory bowel disease (e.g., Crohn's disease, ulcerative

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colitis) and multiple sclerosis (see e.g., J. Liliemark, *Clin. Pharmacokinet*, 32(2): 120-131, 1997). It has also been investigated, either experimentally or clinically in, for example, lymphomas, Langerhan's cell histiocytosis, lupus erythematosus, chronic plaque psoriasis, Sezary syndrome, Bing-Neel syndrome, recurrent glioma, and solid tumors.

Oral delivery of drugs is often preferred to parenteral delivery for a variety of reasons, foremost patient compliance, or for cost or therapeutic considerations. Patient compliance is enhanced insofar as oral dosage forms alleviate repeated health care provider visits, or the discomfort of injections or prolonged infusion times associated with some active drugs. At a time of escalating health care costs, the reduced costs associated with oral or transmucosal administration versus parenteral administration costs gain importance. The cost of parenteral administration is much higher due to the requirement that a health care professional administer the cladribine in the health care provider setting, which also includes all attendant costs associated with such administration. Furthermore, in certain instances, therapeutic considerations such as the need for a slow release of cladribine over a prolonged period of time may be practically met only by oral or transmucosal delivery.

However, to date the oral and transmucosal delivery of cladribine has been plagued by low bioavailability (see, e.g., J. Liliemark *et al.*, *J. Clin. Oncol.*, 10(10): 1514-1518, 1992), and suboptimal interpatient variation (see, e.g., J. Liliemark, *Clin. Pharmacokinet*, 32 (2): 120-131, 1997). See also, A. Tarasuik, *et al.* reporting poor absorption and pH dependent lability (*Arch. Immunol. et Therapiae Exper.*, 42: 13-15, 1994).

Cyclodextrins are cyclic oligosaccharides composed of cyclic α -(1 $\rightarrow$ 4) linked D-glucopyranose units. Cyclodextrins with six to eight units have been named α -, β - and γ -cyclodextrin, respectively. The number of units determines the size of the cone-shaped cavity which characterizes cyclodextrins and into which drugs may include to form stable complexes. A

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number of derivatives of α -, β - and γ -cyclodextrin are known in which one or more hydroxyl groups is/are replaced with ether groups or other radicals. These compounds are thus known complexing agents and have been previously used in the pharmaceutical field to form inclusion complexes with water-insoluble drugs and to thus solubilize them in aqueous media.

Recently, Schultz *et al.*, in U.S. Patent No. 6,194,395 B1, have described complexing and solubilizing cladribine with cyclodextrin. The Schultz *et al.* patent primarily addresses the problems inherent in previously described aqueous formulations of cladribine, particularly for subcutaneous and intramuscular injection. Schultz *et al.* have found that cladribine is not only significantly more soluble in aqueous media when formulated with cyclodextrin, but also is more stable against acid-catalyzed hydrolysis when combined with cyclodextrin. The latter finding is taught to be of particular benefit in the formulation of solid oral dosage forms, where the compound would normally undergo hydrolysis in the acid pH of the stomach contents. Schultz *et al.* do not appear to have described any actual work in connection with solid oral dosage forms. In fact, they describe only one method of preparing the solid dosage form, which is a melt extrusion process, in which the cladribine and cyclodextrin are mixed with other optional additives and then heated until melting occurs. Furthermore, the broad dosage ranges of 1 mg to 15 mg of cladribine and 100 mg to 500 mg of cyclodextrin listed in the patent suggest no criticality to the particular amount of cyclodextrin to be present with a given amount of cladribine in a solid oral dosage form. Indeed, these dosage ranges include many combinations which may be suitable as mixtures but not for complex formation. For example, a ratio of 1 mg of cladribine to 500 mg of cyclodextrin contains too much cyclodextrin, so that the drug would not readily leave the complex and achieve its therapeutic function. On the other hand, 15 mg of cladribine and only 100 mg of cyclodextrin would not be enough to complex that amount of cladribine.

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The Schultz *et al.* patent does suggest improving the stability of cladribine in oral dosage forms by combining/complexing it with cyclodextrin, but does not suggest improving the drug's oral bioavailability by such means; in fact, the patent does not describe or suggest a method for enhancing or maximizing the bioavailability of cladribine from a solid oral dosage form of cladribine and cyclodextrin, or a composition specially designed to do so. Further, Schultz *et al.* do not suggest cladribine/cyclodextrin combinations for transmucosal administration, that is, in a form intended for administration through the mucosa lining the nasal, oral, vaginal or rectal cavities rather than via the orogastric route, much less enhancing the bioavailability of the drug when administered via such a dosage form.

Many workers have studied the solubility of specific drugs in water containing various concentrations of selected cyclodextrins in order to demonstrate that increasing concentrations of cyclodextrins increase the solubility of the drugs at selected temperatures and pH levels, as for example reported in the Schultz *et al.* patent. Phase solubility studies have also been performed by various workers in order to elucidate the nature of the complex formation, for example, whether the cyclodextrin and drug form a 1:1 complex or a 1:2 complex; see, for example, Harada *et al.* U.S. Patent No. 4,497,803, relating to inclusion complexes of lankacidin-group antibiotics with cyclodextrin, and Shinoda *et al.* U.S. Patent No. 4,478,995, relating to a complex of an acid addition salt of (2'-benzyloxycarbonyl)phenyl trans-4-guanidinomethylcyclohexanecarboxylate with a cyclodextrin.

It has been a common practice in the pharmaceutical arts to use a surplus of cyclodextrin in solid dosage forms in which the cyclodextrin is used to improve drug solubilization, unless the solubility is such that excess cyclodextrin would result in too large a dosage form. Conventional wisdom would dictate that for a solid oral dosage form, especially of an acid-labile drug such as cladribine, excess cyclodextrin would be expected to protect the drug in the acid environment of the stomach and, ideally, deliver it

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through the gut wall/gastric mucosa still protected as a complex with the cyclodextrin. In the bloodstream, away from the deleterious influence of stomach acid, the drug would then be expected to dissociate from the complex and perform its therapeutic function.

5 While Schultz *et al.* teach that a cladribine-cyclodextrin complex improves the water solubility and acid stability of cladribine, the art does not suggest how to maximize or enhance the benefits of the complexation in terms of bioavailability and interpatient variation when the complex is to be administered in a solid oral dosage form or a transmucosal dosage form.

10

SUMMARY OF THE INVENTION

 It has now been found that excess cyclodextrin inhibits the absorption of cladribine from a solid oral dosage form or a transmucosal dosage form comprising a cladribine-cyclodextrin complex, and that a solid oral or a
15 transmucosal dosage form of a saturated cladribine-cyclodextrin complex improves oral and/or transmucosal bioavailability and/or achieves lower interpatient and/or inpatient variation of the drug.

 The present invention provides a pharmaceutical composition comprising a saturated cladribine-cyclodextrin complex formulated into a
20 solid oral dosage form or a transmucosal dosage form which is substantially free of cyclodextrin in excess of the minimum amount needed to maximize the amount of cladribine in the complex. In a particular aspect of the invention, the pharmaceutical composition comprises a saturated cladribine-cyclodextrin complex formulated into a solid oral dosage form or a
25 transmucosal dosage form which is substantially free of cyclodextrin in excess of the minimum amount needed to maintain substantially all of the cladribine in the complex. This composition provides cladribine in its highest thermodynamic activity state at the time it contacts the gastric mucosa (in the case of an oral dosage form) or the rectal, vaginal, buccal or nasal
30 mucosa (in the case of transmucosal dosage forms).

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The invention also provides a method for increasing the oral or transmucosal bioavailability of cladribine comprising administering to a subject in need thereof, a pharmaceutical composition comprising a saturated cladribine-cyclodextrin complex formulated into a solid oral dosage form or a transmucosal dosage form which is substantially free of cyclodextrin in excess of the minimum amount needed to maximize the amount of the cladribine in the complex. In a particular aspect of the method, the composition administered comprises a saturated cladribine-cyclodextrin complex formulated into a solid oral dosage form or a transmucosal dosage form which is substantially free of cyclodextrin in excess of the minimum amount needed to maintain substantially all of the cladribine in the complex.

The invention further provides a method for enhancing the bioavailability of cladribine from a solid oral dosage form or a transmucosal dosage form in a mammal in need of treatment with cladribine, the method comprising: (a) determining the minimum amount of cyclodextrin required to complex with a selected amount of cladribine and to maintain said selected amount of cladribine in the complex; (b) combining an amount of cladribine in excess of said selected amount with said minimum amount of cyclodextrin in an aqueous medium; (c) removing uncomplexed cladribine from the complexation medium; (d) removing water from the resultant solution to afford the dry saturated cladribine-cyclodextrin complex; (e) formulating said dry saturated cladribine-cyclodextrin complex into a solid oral dosage form or a transmucosal dosage form substantially free of cyclodextrin in excess of the minimum amount required to maximize the amount of cladribine in the complex; and (f) administering the dosage form orally or transmucosally to the mammal. In a particular aspect of this method, step (e) comprises formulating said dry saturated cladribine-cyclodextrin complex into a solid oral dosage form or a transmucosal dosage form substantially free of

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cyclodextrin in excess of the minimum amount required to maintain substantially all of the cladribine in the complex.

The invention further provides for treatment of conditions responsive to administration of cladribine in mammals by administering thereto the composition of the invention. Use of cladribine in the preparation of the pharmaceutical compositions of the invention for administration to treat symptoms of cladribine-responsive conditions and for enhancing the oral or transmucosal bioavailability of cladribine is also provided.

In one particular embodiment, the invention provides a novel 1:2 complex of cladribine: γ -cyclodextrin which is particularly advantageous. In a related embodiment, there is provided a mixture of a 1:1 cladribine: γ -cyclodextrin complex and a 1:2 cladribine: γ -cyclodextrin complex, wherein the 1:2 complex is predominant.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and its many attendant advantages will be readily understood by reference to the following detailed description and the accompanying drawings, wherein:

Fig. 1 is a graphical representation of the results of phase solubility studies, where various cyclodextrin (CD) molar concentrations are plotted against various cladribine molar concentrations, with (◆) representing hydroxypropyl- β -cyclodextrin, (■) representing hydroxypropyl- β -cyclodextrin with added hydroxypropyl methylcellulose, and (▲) representing γ -cyclodextrin.

Fig. 2 shows plasma profiles for cladribine in dogs after administration of 5 mg single doses of cladribine with data showing the average concentration of cladribine in the plasma, in pg/ml, $\pm$ SD for 5-6 animals per group, plotted against time in hours, following administration of the following cladribine formulations: (◆) intravenous (i.v.) bolus; (⊖) saturated buccal cladribine γ -cyclodextrin complex; (x) saturated buccal cladribine-

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hydroxypropyl- β -cyclodextrin complex; (●) saturated oral cladribine- γ -cyclodextrin complex; (○) oral capsule of physical mixture of cladribine with ten times excess γ -cyclodextrin; (□) oral capsule of cladribine complex with ten times excess γ -cyclodextrin; (▲) saturated oral cladribine-hydroxypropyl- β -cyclodextrin complex; and (Δ) oral capsule of physical mixture of cladribine with ten times excess hydroxypropyl- β -cyclodextrin.

Fig. 3 represents a comparison of plasma profiles for cladribine in dogs after administration of 5 mg single doses of cladribine, with data showing the average concentration, in pg/mL, $\pm$ SD for 5-6 animals per group, plotted against time in hours, following administration of the following cladribine formulations: (◆) intravenous (i.v.) bolus, (●) saturated oral cladribine- γ -cyclodextrin complex, and (▲) saturated oral cladribine-hydroxypropyl- β -cyclodextrin complex.

Fig. 4 represents a comparison of plasma profiles for cladribine in dogs after oral administration of 5 mg single doses of cladribine, with data showing the average concentration, in pg/mL, $\pm$ SD for 5-6 animals per group, plotted against time in hours, following administration of the following cladribine formulations: (●) saturated oral cladribine- γ -cyclodextrin complex; (○) oral capsule of physical mixture of cladribine with ten times excess γ -cyclodextrin; and (□) oral capsule of cladribine complex with ten times excess γ -cyclodextrin.

Fig. 5 represents a comparison of plasma profiles for cladribine in dogs after oral administration of 5 mg single doses of cladribine, with data showing the average concentration, in pg/mL, $\pm$ SD for 5-6 animals per group, plotted against time in hours, following administration of the following cladribine formulations: (▲) saturated oral cladribine-hydroxypropyl- β -cyclodextrin complex; and (Δ) oral capsule of physical mixture of cladribine with ten times excess hydroxypropyl- β -cyclodextrin.

Fig. 6 illustrates the average cumulative area under the curve (AUC), in pg x h/mL, for cladribine in groups of 5-6 dogs, plotted against time in

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hours, after administration of each of the formulations described with reference to Fig. 2, where the symbols are as indicated in that paragraph.

DETAILED DESCRIPTION OF THE INVENTION

5 Throughout the instant specification and claims, the following definitions and general statements are applicable.

 The patents, published applications, and scientific literature referred to herein establish the knowledge of those with skill in the art and are hereby incorporated by reference in their entirety to the same extent as if each was
10 specifically and individually indicated to be incorporated by reference. Any conflict between any reference cited herein and the specific teachings of this specification shall be resolved in favor of the latter. Likewise, any conflict between an art-understood definition of a word or phrase and a definition of the word or phrase as specifically taught in this specification shall be
15 resolved in favor of the latter.

 The term "complex" as used herein means an inclusion complex, in which the hydrophobic portion of the cladribine molecule (the nitrogen-containing ring system) is inserted into the hydrophobic cavity of the cyclodextrin molecule.

20 As used herein, whether in a transitional phrase or in the body of a claim, the terms "comprise(s)" and "comprising" are to be interpreted as having an open-ended meaning. That is, the terms are to be interpreted synonymously with the phrases "having at least" or "including at least". When used in the context of a process, the term "comprising" means that the
25 process includes at least the recited steps, but may include additional steps. When used in the context of a composition, the term "comprising" means that the composition includes at least the recited features or components, but may also include additional features or components.

 The terms "consists essentially of" or "consisting essentially of" have
30 a partially closed meaning, that is, they do not permit inclusion of steps or

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features or components which would substantially change the essential characteristics of a process or composition; for example, steps or features or components which would significantly interfere with the desired properties of the compositions described herein, *i.e.*, the process or composition is limited to the specified steps or materials and those which do not materially affect the basic and novel characteristics of the invention. The basic and novel features herein are the provision of a saturated cladribine-cyclodextrin complex in a solid oral dosage form or a transmucosal dosage form which is substantially free of cyclodextrin in excess of the minimum amount required to maximize the amount of cladribine in the complex, so as to provide improved bioavailability and/or lower interpatient variation following administration. In a particular embodiment of the invention, the basic and novel features herein are the provision of a saturated cladribine-cyclodextrin complex in a solid oral dosage form or a transmucosal dosage form which is substantially free of cyclodextrin in excess of the minimum amount required to maintain substantially all of the cladribine in the complex, providing particularly enhanced bioavailability and/or low interpatient and/or low inpatient variability following administration.

The terms "consists of" and "consists" are closed terminology and allow only for the inclusion of the recited steps or features or components.

As used herein, the singular forms "a," "an" and "the" specifically also encompass the plural forms of the terms to which they refer, unless the content clearly dictates otherwise.

The term "about" is used herein to mean approximately, in the region of, roughly, or around. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" or "approximately" is used herein to modify a numerical value above and below the stated value by a variance of 20%.

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The term "saturated" when used in conjunction with a complex of cladribine in cyclodextrin means that the complex is saturated with cladribine, that is, the complex contains the maximum amount of cladribine which can be complexed with a given amount of cyclodextrin under the conditions of complexation used. A phase solubility study can be used to provide this information, as described in more detail hereinafter. (Conditions for the complexation are also described in more detail below.) Alternatively, a saturated complex may be arrived at empirically by simply adding cladribine to an aqueous solution of the selected cyclodextrin until a precipitate (of uncomplexed cladribine) forms; ultimately, the precipitate is removed and the solution lyophilized to provide the dry saturated complex.

The expression "substantially", as in "substantially free" or "substantially all", means within 20% of the exact calculated amount. In the case of the expression "substantially free of cyclodextrin in excess of the minimum amount needed to maintain substantially all of the cladribine in the complex," the minimum amount of cyclodextrin needed to maintain the cladribine in the complex can be obtained from phase solubility studies as explained in more detail below. The actual amount of cyclodextrin should be within 20% of that minimum, plus or minus, preferably within 10% of that minimum, plus or minus, even more preferably within 5% of that minimum, plus or minus, and should maintain at least 90% or more, preferably at least 95% or more, of the drug in the complex. On the other hand, when the expression "substantially free of cyclodextrin in excess of the minimum amount needed to maximize the amount of cladribine in the complex" is used, less than the aforementioned amount of cyclodextrin may be utilized and a larger amount of cladribine may be present in the dosage form in uncomplexed form as a result. This may occur by using a less concentrated cyclodextrin solution for the complexation reaction and/or by conducting the complexation at the upper end of the temperature range suggested below. It is considered particularly advantageous, however, to use enough

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cyclodextrin to maintain substantially all of the cladribine in the complex, and to thus minimize the amount of uncomplexed cladribine in the dosage form.

The term "interpatient variability" refers to variation among patients to which a drug is administered. The term "intrapatient variability" refers to
5 variation experienced by a single patient when dosed at different times.

As used herein, the recitation of a numerical range for a variable is intended to convey that the invention may be practiced with the variable equal to any of the values within that range. Thus, for a variable which is inherently discrete, the variable can be equal to any integer value of the
10 numerical range, including the end-points of the range. Similarly, for a variable which is inherently continuous, the variable can be equal to any real value of the numerical range, including the end-points of the range. As an example, a variable which is described as having values between 0 and 2, can be 0, 1 or 2 for variables which are inherently discrete, and can be 0.0,
15 0.1, 0.01, 0.001, or any other real value for variables which are inherently continuous.

In the specification and claims, the singular forms include plural referents unless the context clearly dictates otherwise. As used herein, unless specifically indicated otherwise, the word "or" is used in the
20 "inclusive" sense of "and/or" and not the "exclusive" sense of "either/or."

Technical and scientific terms used herein have the meaning commonly understood by one of skill in the art to which the present invention pertains, unless otherwise defined. Reference is made herein to various methodologies and materials known to those of skill in the art. Standard
25 reference works setting forth the general principles of pharmacology include Goodman and Gilman's *The Pharmacological Basis of Therapeutics*, 10th Ed., McGraw Hill Companies Inc., New York (2001).

Reference is made hereinafter in detail to specific embodiments of the invention. While the invention will be described in conjunction with these
30 specific embodiments, it will be understood that it is not intended to limit the

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invention to such specific embodiments. On the contrary, it is intended to cover alternatives, modifications, and equivalents as may be included within the spirit and scope of the invention as defined by the appended claims. In the following description, numerous specific details are set forth in order to provide a thorough understanding of the present invention. The present invention may be practiced without some or all of these specific details. In other instances, well-known process operations have not been described in detail, in order not to unnecessarily obscure the present invention.

There is provided by the present invention compositions, as well as methods of making and of using pharmaceutical compositions, useful to achieve desirable pharmacokinetic properties. Such compositions stem from the discovery that solutions of cyclodextrin and cladribine in which cladribine is in its highest thermodynamic state, when presented to the mucosa through which they are absorbed (gastric, nasal, rectal, buccal, sublingual or vaginal) are associated with improved cladribine absorption, as reflected by higher bioavailability and/or lower interpatient variation.

It is postulated, without wishing to so limit the invention, that upon dissolution (*e.g.*, by contact with a fluid, such as a bodily fluid), dry compositions of a saturated cladribine-cyclodextrin complex not containing excess cyclodextrin form a locally saturated cladribine solution in which cladribine is in the state of highest thermodynamic activity (HTA), thus favoring absorption. Cladribine has a fairly low, although not insignificant, intrinsic aqueous solubility. The free cladribine formed from dissociation of the complex in a saturated aqueous solution seeks a more stable activity level, and if excess cyclodextrin were present, the cladribine would seek greater stability by re-complexing with the cyclodextrin. By controlling the amount of cyclodextrin so that the dosage form is substantially free of cyclodextrin in excess of the amount needed to keep the cladribine in the complex, it will not be easy for the cladribine in the locally saturated solution to recombine with cyclodextrin. Therefore, this cladribine will seek a state of

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lower thermodynamic activity/greater stability by being absorbed through the gastric mucosa (in the case of a solid oral dosage form) or through the nasal, buccal, vaginal or rectal mucosa (in the case of a transmucosal dosage form). This approach is shown hereinafter *inter alia* to increase

5 bioavailability, likely by avoiding or minimizing the inhibition of cladribine absorption which would result from the presence of excess cyclodextrin. In the presence of a large amount of excess cyclodextrin, the cladribine in solution would be expected to recombine with cyclodextrin. This will not

10 achieve optimum bioavailability, because it is essential that the cladribine move out of the complex in which it is encapsulated if the drug is to accomplish its therapeutic function.

In view of the foregoing, it is apparent that to produce optimal pharmaceutical compositions, in a solid oral or a transmucosal dosage form, these dosage forms should be formulated to release a localized saturated

15 cladribine solution, upon contact of the solid dosage forms with body fluid at the mucosa, in which cladribine is in its HTA state. To provide such a localized saturated solution *in vivo*, it is important to first identify the optimal ratio of cladribine to cyclodextrin, which ratio is referred to herein as the HTA ratio, to be used in the solid dosage form. In the case of a buccal dosage

20 form, a highly concentrated solution made by dissolving the saturated complex in a minimal amount of water and placing this solution in the buccal cavity can accomplish the same effect.

The HTA ratio is empirically determined and is identified as the ratio of cladribine to a specific cyclodextrin which corresponds to the maximum

25 amount of cladribine that can be complexed with a given amount of cyclodextrin. The HTA ratio may be determined using an empirical method such as a phase solubility study to determine the saturation concentration of cladribine that can be solubilized with different concentrations of cyclodextrin solutions. Hence, the method identifies the concentrations at which a

30 saturated cladribine-cyclodextrin complex is formed. It is noted that the

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molar ratio represented by a point on the phase solubility graph shows how many moles of cyclodextrin are the minimum needed to maintain the drug in the complex, under given conditions; this may then be converted to a weight ratio. For example, if a phase solubility diagram shows that 9 moles of a given cyclodextrin are needed to maintain substantially all of the cladribine in a saturated complex, then multiplying the number of moles of cladribine by its molecular weight and multiplying the number of moles the cyclodextrin by its molecular weight, one can arrive at the ratio of the products as an appropriate optimized weight ratio. A phase solubility study also provides information about the nature of the cladribine-cyclodextrin complex formed, for example whether the complex is a 1:1 complex (1 molecule of drug complexed with 1 molecule of cyclodextrin) or a 1:2 complex (1 molecule of drug complexed with 2 molecules of cyclodextrin).

In accordance with the present invention, one can start using either cyclodextrin or cladribine as the fixed variable to which an excess of the other is added to identify various HTA data points (indicating saturated cladribine-cyclodextrin complexes) and draw the resultant HTA line. Typically, cladribine is added to an aqueous solution having a known concentration of cyclodextrin under conditions empirically found to promote complex formation. A concentrated solution, for example, of approximately 27% for γ -cyclodextrin and approximately 40% for hydroxypropyl- β -cyclodextrin, is in one embodiment particularly advantageous. Generally, the complexation is conducted at room temperature or with slight heating (up to about 50°C or even up to 60°C). Excess cladribine, if any, is then removed and the cladribine concentration in the complex is subsequently measured. The concentration measured represents the cladribine saturation concentration for the given cyclodextrin concentration. This process is repeated for a different known concentration of cyclodextrin until several data points are obtained. Each data point represents the saturated concentration of the cladribine dissolved in a known concentration of

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cyclodextrin. The data points are then plotted to show the saturated concentration of cladribine against the various cyclodextrin concentrations used. The graph is a phase solubility diagram which can be used to determine the saturation amount of cladribine for any specific concentration of cyclodextrin used to form a saturated cladribine-cyclodextrin complex under a given set of complexation conditions.

One of skill in the art will appreciate that concentrations at which saturated cladribine-cyclodextrin complexes are formed (and thus HTA ratios as well) may be identified by a variety of alternative methodologies. Accordingly, any method known in the field suitable to identify these concentrations is within the scope of the invention.

It has been discovered that desirable pharmacological properties (improved bioavailability and/or lower interpatient and/or intrapatient variation) are associated with the inclusion complexes of this invention.

The cyclodextrins within the scope of this invention include the natural cyclodextrins α -, β , and γ -cyclodextrin, and derivatives thereof, in particular, derivatives wherein one or more of the hydroxy groups are substituted, for example, by alkyl, hydroxyalkyl, carboxyalkyl, alkylcarbonyl, carboxyalkoxyalkyl, alkylcarbonyloxyalkyl, alkoxyalkyl or hydroxy-(mono or polyalkoxy)alkyl groups; and wherein each alkyl or alkylene moiety preferably contains up to six carbons. Substituted cyclodextrins can generally be obtained in varying degrees of substitution, for example, from 1 to 14, preferably from 4 to 7; the degree of substitution is the approximate average number of substituent groups on the cyclodextrin molecule, for example, the approximate number of hydroxypropyl groups in the case of the hydroxypropyl- β -cyclodextrin molecule, and all such variations are within the ambit of this invention. Substituted cyclodextrins which can be used in the invention include polyethers, for example, as described in U.S. Patent No. 3,459,731. Further examples of substituted cyclodextrins include ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is

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replaced by C₁₋₆ alkyl, hydroxy-C₁₋₆ alkyl, carboxy-C₁₋₆ alkyl or C₁₋₆ alkyloxycarbonyl-C₁₋₆ alkyl groups or mixed ethers thereof. In particular, such substituted cyclodextrins are ethers wherein the hydrogen of one or more cyclodextrin hydroxy groups is replaced by C₁₋₃ alkyl, hydroxy-C₂₋₄ alkyl or carboxy-C₁₋₂ alkyl or more particularly by methyl, ethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, carboxymethyl or carboxyethyl. The term "C₁₋₆ alkyl" is meant to include straight and branched saturated hydrocarbon radicals, having from 1 to 6 carbon atoms such as methyl, ethyl, 1-methylethyl, 1,1-dimethylethyl, propyl, 2-methylpropyl, butyl, pentyl, hexyl and the like. Other cyclodextrins contemplated for use herein include glucosyl- β -cyclodextrin and maltosyl- β -cyclodextrin. Of particular utility in the present invention are the β -cyclodextrin ethers such as dimethyl- β -cyclodextrin as described in *Cyclodextrins of the Future*, Vol. 9, No. 8, p. 577-578 by M. Nogradi (1984), randomly methylated β -cyclodextrin and polyethers such as hydroxypropyl- β -cyclodextrin, hydroxyethyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, and hydroxyethyl- γ -cyclodextrin, as well as sulfobutyl ethers, especially β -cyclodextrin sulfobutyl ether. In addition to simple cyclodextrins, branched cyclodextrins and cyclodextrin polymers may also be used. Other cyclodextrins are described, for example, in *Chemical and Pharmaceutical Bulletin* 28: 1552-1558 (1980); Yakugyo Jiho No. 6452 (28 March 1983); *Angew. Chem. Int. Ed. Engl.* 19: 344-362 (1980); U.S. Patent. Nos. 3,459,731 and 4,535,152; European Patent Nos. EP 0 149 197A and EP 0 197 571A; PCT International Patent Publication No. WO90/12035; and UK Patent Publication GB 2,189,245. Other references describing cyclodextrins for use in the compositions according to the present invention, and which provide a guide for the preparation, purification and analysis of cyclodextrins include the following: *Cyclodextrin Technology* by Jozsef Szejtli, Kluwer Academic Publishers (1988) in the chapter Cyclodextrins in Pharmaceuticals; *Cyclodextrin Chemistry* by M. L. Bender *et. al.*, Springer-Verlag, Berlin (1978); *Advances in Carbohydrate*

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Chemistry, Vol. 12, Ed. by M. L. Wolfrom, Academic Press, New York in the chapter "The Schardinger Dextrins" by Dexter French, pp. 189-260;

Cyclodextrins and their Inclusion Complexes by J. Szejtli, Akademiai Kiado, Budapest, Hungary (1982); I. Tabushi, *Acc. Chem. Research*, 1982, 15, pp.

5 66-72; W. Sanger, *Angewandte Chemie*, 92, p. 343-361 (1981); A. P. Croft *et. al.*, *Tetrahedron*, 39, pp. 1417-1474 (1983); Irie *et. al.*, *Pharmaceutical Research*, 5, pp. 713-716 (1988); Pitha *et. al.*, *Int. J. Pharm.* 29, 73 (1986); U.S. Patent Nos. 4,659,696 and 4,383,992; German Patent Nos. DE 3,118,218 and DE-3,317,064; and European Patent No. EP 0 094 157A.

10 Patents describing hydroxyalkylated derivative of β - and γ -cyclodextrin include Pitha U.S. Patent Nos. 4,596,795 and 4,727,064, Müller U.S. Patent Nos. 4,764,604 4,870,060 and Müller *et al.* U.S. Patent No. 6,407,079.

Cyclodextrins of particular interest for complexation with cladribine include: γ -cyclodextrin; hydroxyalkyl, e.g. hydroxyethyl or hydroxypropyl, derivatives of β - and γ -cyclodextrin; carboxyalkyl, e.g. carboxymethyl or carboxyethyl, derivatives of β - or γ -cyclodextrin; β -cyclodextrin sulfobutyl ether; dimethyl- β -cyclodextrin; and randomly methylated β -cyclodextrin. 2-Hydroxypropyl- β -cyclodextrin (HP β CD), 2-hydroxypropyl- γ -cyclodextrin (HP γ CD), randomly methylated β -cyclodextrin, dimethyl- β -cyclodextrin, 15 β -cyclodextrin sulfobutyl ether, carboxymethyl- β -cyclodextrin (CM β CD), carboxymethyl- γ -cyclodextrin (CM γ CD) and γ -cyclodextrin (γ CD) itself are of special interest, especially γ -cyclodextrin and hydroxypropyl- β -cyclodextrin, most especially γ -cyclodextrin.

25 Compositions of a saturated cladribine-cyclodextrin complex for use in the present invention can be prepared under conditions favoring complex formation in a liquid environment as described and as exemplified herein. The resultant liquid preparations can be subsequently converted to a dry form suitable for administration as a solid oral or transmucosal dosage form.

30 One of skill will appreciate that a variety of approaches are available in the field to prepare compositions as described herein. One available

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method exemplified herein includes the steps of mixing the cladribine in an aqueous cyclodextrin solution, maintaining the complexation medium at room temperature, with stirring, for from about 6 to about 24 hours, that is, for a sufficient time to achieve equilibrium, separating un-complexed
5 cladribine, if any (e.g., by filtering or centrifugation), and lyophilizing or freeze-drying the saturated solution to form a solid saturated cladribine-cyclodextrin complex mixture.

Freeze-drying, also known as lyophilization, consists of three basic stages: first a freezing stage, then a primary drying stage and finally a
10 secondary drying stage. EXAMPLE 2 below provides details of lyophilization as conducted on the batches described therein. This procedure can be further optimized by following the principles described by Xiaolin (Charlie) Tang and Michael J. Pikal in *Pharmaceutical Research*, Vol. 21, No. 2, February 2004, 191-200, incorporated by reference herein in its entirety and
15 relied upon.

Pharmaceutical compositions according to the invention may optionally include one or more excipients or other pharmaceutically inert components. One of the advantages of the invention, however, is that cladribine drug forms as described herein can be prepared with the minimal
20 amount of excipients necessary for shaping and producing the particular form, such as a tablet or patch. Excipients may be chosen from those that do not interfere with cladribine, with cyclodextrin or with complex formation.

Dosage forms are optionally formulated in a pharmaceutically acceptable vehicle with any of the well-known pharmaceutically acceptable
25 carriers, diluents, binders, lubricants, disintegrants, scavengers, flavoring agents, coloring agents, and excipients (see *Handbook of Pharmaceutical Excipients*, Marcel Dekker Inc., New York and Basel (1998); Lachman *et al.* Eds., *The Theory and Practice of Industrial Pharmacy*, 3rd Ed., (1986); Lieberman *et al.*, Eds. *Pharmaceutical Dosage Forms*, Marcel Dekker Inc.,
30 New York and Basel (1989); and *The Handbook of Pharmaceutical*

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Excipients, 3rd Ed., American Pharmaceutical Association and Pharmaceutical Press, 2000); see also *Remington's Pharmaceutical Sciences*, 18th Ed., Gennaro, Mack Publishing Co., Easton, PA (1990) and *Remington: The Science and Practice of Pharmacy*, Lippincott, Williams & Wilkins, (1995)). A simple solid oral or transmucosal dosage form consists of the saturated cladribine-cyclodextrin complex compressed with a small amount (e.g. about 1% by weight) of a suitable binder or lubricant such as magnesium stearate.

In particular embodiments, the saturated cladribine-cyclodextrin complex is used for the transmucosal or the oral administration of cladribine.

As used herein, "mucosa" means the epithelial membranes lining the nasal, oral, vaginal and rectal cavities, as well as those lining the stomach (the gastric mucosa). As used herein, mucosal and transmucosal are used interchangeably. Transmucosal delivery methods and forms are well-known in the art. These include buccal and sublingual tablets, lozenges, adhesive patches, gels, solutions or sprays (powder, liquid or aerosol), and suppositories or foams (for rectal or vaginal administration). Transmucosal delivery methods and forms do not include the methods and forms for oral use, which are intended to be swallowed and are simply called oral dosage forms herein, despite the fact that they ultimately deliver the drug through the gastric mucosa. When the transmucosal form is a liquid, it can be obtained by dissolving the saturated complex in a minimum amount of water, for example 500 mg of the saturated complex with HP β CD in 0.5 ml water (50% w/w solution), or 500 mg of the saturated γ CD complex in 1.0 ml of water. A few drops of such a solution can be inserted into the buccal cavity and retained there for about 2 minutes to allow for absorption through the buccal mucosa. Nevertheless, solid transmucosal dosage forms are generally preferred over liquid forms.

In certain instances, oral or mucosal absorption may be further facilitated by the addition of various excipients and additives to increase

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solubility or to enhance penetration, such as by the modification of the microenvironment, or by the addition of mucoadhesive excipients to improve contact between the delivery system and the mucosal tissue.

5 Buccal drug delivery can be effected by placing the buccal dosage unit between the lower gum and the oral mucosa opposite thereto of the individual undergoing drug therapy. Excipients or vehicles suitable for buccal drug administration can be used, and include any such materials known in the art, *e.g.*, any liquid, gel, solvent, liquid diluent, solubilizer, or the like, which is nontoxic and does not interact with other components of the composition in a deleterious manner. A solid dosage unit is fabricated so as to dissolve gradually over a predetermined time period, to produce a substantially saturated drug solution in the saliva of the buccal cavity, allowing absorption of cladribine through the mucosa, wherein drug delivery is provided essentially throughout the time period. The buccal dosage unit 10 may further comprise a lubricant to facilitate manufacture, *e.g.*, magnesium stearate or the like. Additional components that may be included in the buccal dosage unit include but are not limited to flavorings, permeation enhancers, diluents, binders, and the like. The remainder of the buccal dosage unit may comprise a bioerodible polymeric carrier, and any 15 excipients that may be desired, *e.g.*, binders, disintegrants, lubricants, diluents, flavorings, colorings, and the like, and/or additional active agents. 20

The buccal carrier can comprise a polymer having sufficient tack to ensure that the dosage unit adheres to the buccal mucosa for the necessary time period, *i.e.*, the time period during which the cladribine is to be delivered 25 to the buccal mucosa. Additionally, the polymeric carrier is gradually "bioerodible", *i.e.*, the polymer hydrolyzes at a predetermined rate upon contact with moisture. Any polymeric carriers can be used that are pharmaceutically acceptable, provide both a suitable degree of adhesion and the desired drug release profile, and are compatible with the cladribine 30 to be administered and any other components that may be present in the

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buccal dosage unit. Generally, the polymeric carriers comprise hydrophilic (water-soluble and water-swellaable) polymers that adhere to the wet surface of the buccal mucosa. Examples of polymeric carriers useful herein include acrylic acid polymers and copolymers, *e.g.*, those known as "carbomers" for example, Carbopol[®]. Other suitable polymers include, but are not limited to, hydrolyzed polyvinyl alcohol, polyethylene oxides (*e.g.*, Sentry Polyox[®]), polyacrylates (*e.g.*, Gantrez[®]), vinyl polymers and copolymers, polyvinylpyrrolidone, dextran, guar gum, pectins, starches, and cellulosic polymers such as hydroxypropyl methylcellulose (*e.g.*, Methocel[®]), hydroxypropyl cellulose (*e.g.*, Klucel[®]), hydroxypropyl cellulose ethers, hydroxyethyl cellulose, sodium carboxymethyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetate phthalate, cellulose acetate butyrate, and the like. The dosage unit need contain only the saturated cladribine-cyclodextrin complex. However, it may be desirable in some cases to include one or more of the aforementioned carriers and/or one or more additional components. For example, a lubricant may be included to facilitate the process of manufacturing the dosage units; lubricants may also optimize erosion rate and drug flux. If a lubricant is present, it will represent on the order of 0.01 wt.% to about 2 wt.%, preferably about 0.01 wt.% to 1.0 wt.%, of the dosage unit. Suitable lubricants include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, sodium stearyl fumarate, talc, hydrogenated vegetable oils and polyethylene glycol.

The saturated cladribine-cyclodextrin complex may also be administered in accord with this invention in the form of suppositories or foams for vaginal or rectal administration. These compositions can be prepared by well-known methods, for example, in the case of suppositories, by mixing the saturated complex with a suitable non-irritating excipient or binder which is solid at ordinary temperatures but liquid at the vaginal or rectal temperature and will, therefore, melt in the vagina or rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

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Traditional binders and carriers include, for example, polyalkylene glycols or triglycerides [e.g., PEG 1000 (96%) and PEG 4000 (4%)]. Such suppositories may be formed from mixtures containing active ingredients in the range of from about 0.5 wt/wt% to about 10 wt/wt%; preferably from about 1 wt/wt% to about 2 wt/wt%.

For intranasal use, a powder spray, gel or ointment may be utilized, preferably a powder form of the saturated complex.

Moreover, for use in humans, a buccal dosage form, especially a buccal tablet or wafer or disk, advantageously having a disintegration time of about 15-30 minutes, or a buccal patch (in which the drug is released only from the side which adheres to the buccal mucosa while the other side is nonpermeable), is of interest. Buccal administration may make use of the inventions of Nagai *et al.* described in U.S. Patent Nos. 4,226,848 and 4,250,163, both of which are incorporated by reference herein in their entireties and relied upon. Thus, a buccal mucosa-adhesive tablet may be formulated for use herein comprising: (a) a water-swelling and mucosa-adhesive polymeric matrix comprising about 50% to about 95% by weight of a cellulose ether and about 50% to about 95% by weight of a homo- or copolymer of acrylic acid or a pharmaceutically acceptable salt thereof, and (b) dispersed therein, an appropriate quantity of cladribine, as a saturated complex with 2-hydroxypropyl- β -cyclodextrin or γ -cyclodextrin. Ideally, for storage stability, the tablet is anhydrous.

The methods and pharmaceutical compositions described herein offer novel therapeutic modalities for the treatment of patients in need of treatment with cladribine. As shown herein, the invention addresses the problems of poor bioavailability traditionally associated with oral cladribine. Alternatively, the orogastric route may be avoided entirely by administering a transmucosal delivery form.

The compositions of the invention are particularly suitable as modalities for the treatment of any cladribine-responsive disease. Several

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disease states responsive to cladribine are well-documented in the literature (see *infra*). For any target disease state, an effective amount of the optimized cladribine-cyclodextrin complex is used (e.g., an amount effective for the treatment of multiple sclerosis, rheumatoid arthritis, or leukemia).

5 The term "therapeutically effective amount" or "effective amount" is used to denote treatments at dosages effective to achieve the therapeutic result sought. Therapeutically effective dosages described in the literature include those for hairy cell leukemia (0.09 mg/kg/day for 7 days), for multiple sclerosis (from about 0.04 to about 1.0 mg/kg/day (see U.S. Patent No.
10 5,506,214)); for other diseases, see also U.S. Patent Nos. 5,106,837 (autohemolytic anemia); 5,310,732 (inflammatory bowel disease); 5,401,724 (rheumatoid arthritis); 5,424,296 (malignant astrocytoma); 5,510,336 (histiocytosis); 5,401,724 (chronic myelogenous leukemia); and 6,239,118 (atherosclerosis).

15 Further, various dosage amounts and dosing regimens have been reported in the literature for use in the treatment of multiple sclerosis; see, for example: Romine et al., *Proceedings of the Association of American Physicians*, Vol. 111, No. 1, 35-44 (1999); Selby et al., *The Canadian Journal of Neurological Sciences*, 25, 295-299 (1998); Tortorella et al.,
20 *Current Opinion in Investigational Drugs*, 2 (12), 1751-1756 (2001); Rice et al., *Neurology*, 54, 1145-1155 (2000); and Karlsson et al., *British Journal of Haematology*, 116, 538-548 (2002); all of which are incorporated by reference herein in their entireties and relied upon.

25 Moreover, the route of administration for which the therapeutically effective dosages are taught in the literature should be taken into consideration. While the instant compositions optimize the bioavailability of cladribine following oral or transmucosal administration, it will be appreciated that even optimal bioavailability from oral or transmucosal dosage forms is not expected to approach bioavailability obtained after intravenous
30 administration, particularly at early time points; see, for example, Fig. 3

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hereinafter. Thus, it is often appropriate to increase a dosage suggested for intravenous administration to arrive at a suitable dosage for incorporation into a solid oral dosage form or a transmucosal dosage form. At the present time, it is envisioned that, for the treatment of multiple sclerosis, 10 mg of

5 cladribine in the instant solid dosage form as the saturated cladribine-cyclodextrin complex would be administered once per day for a period of five to seven days in the first month, repeated for another period of five to seven days in the second month, followed by ten months of no treatment.

Alternatively, the patient would be administered the 10 mg dose once per

10 day for a period of five to seven days per month for six months, followed by eighteen months of no treatment. For further dosing information, see also U.S. Provisional Patent Application No. _____ [IVAX0021-P-USA/Attorney Docket No. 033935-011], and U.S. Provisional Patent Application No. _____ [IVAX0022-P-USA/Attorney Docket No. 033935-012], both entitled

15 "Cladribine Regimen for Treating Multiple Sclerosis", both filed on March 25, 2004 and incorporated by reference herein in their entireties.

Furthermore, one of skill will appreciate that the therapeutically effective amount of cladribine administered herein may be lowered or increased by fine tuning and/or by administering cladribine according to the

20 invention with another active ingredient. The invention therefore provides a method to tailor the administration/treatment to the particular exigencies specific to a given mammal. Therapeutically effective amounts may be easily determined, for example, empirically by starting at relatively low amounts and by step-wise increments with concurrent evaluation of

25 beneficial effect.

As noted in the preceding paragraph, administration of cladribine in accord with this invention may be accompanied by administration of one or more additional active ingredients for treating the cladribine-responsive condition. The additional active ingredient will be administered by a route of

30 administration and in dosing amounts and frequencies appropriate for each

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additional active ingredient and the condition being treated. For example, in the treatment of multiple sclerosis, other useful drugs include interferon beta (Rebif[®], Betaseron[®]/Betaferon[®], Avonex[®]), identical to the naturally occurring protein found in the human body; glatiramer acetate (Copaxone[®]),
5 a random chain (polymer) of the amino acids glutamic acid, lysine, alanine and tyrosine; natalizumab (Antegren[®]), a monoclonal antibody; alemtuzumab (Campath-1H[®]), a humanized anti-CD52 monoclonal antibody; 4-aminopyridine (also known as 4-AP and Fampridine), a drug that blocks the potassium channels in neurons; and amantadine, an anti-viral agent which
10 improves muscle control and reduces muscle stiffness and is used to alleviate the symptoms of fatigue in multiple sclerosis, a purpose for which pemoline (Cylert[®]) and L-Carnitine (a herbal product) may also be useful. In the treatment of hairy cell leukemia, additional active ingredients may include interferon alpha, pentostatin, fludarabine, rituximab (an anti-CD 20
15 monoclonal antibody) and the anti-CD22 recombinant immunotoxin BL 22; other additional active ingredients may be appropriate in other types of leukemias. In the treatment of rheumatoid arthritis, there are many other active ingredients which may be selected. These include NSAIDS (non-steroidal anti-inflammatory drugs), which are of three types: salicylates such
20 as aspirin, traditional NSAIDS such as ibuprofen and indomethacin, and COX-2 inhibitors such as celecoxib (Celebrex[®]), rofecoxib (Vioxx[®]), meloxicam (Mobic[®]), valdecoxib (Bextra[®]), lumiracoxib (Prexige[®]) and etoricoxib (Arcoxia[®]). Other drugs useful in treating rheumatoid arthritis which may be used in conjunction with the present invention include
25 DMARDS, glucocorticoids, biological response modifiers and non-NSAID analgesics. DMARDS are disease-modifying anti-rheumatic drugs which include methotrexate, plaquenil, leflunomide (Arava[®]), sulfasalazine, gold, penicillamide, cyclosporine, methyl cyclophosphamide and azathioprine. Glucocorticoids include dexamethasone, prednisolone, triamcinolone and
30 many others. Biological response modifiers (which restore the disease-

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fighting ability of the immune system), include etanercept (Enrel[®]), a tumor-necrosis factor inhibitor, infliximab (Remicade[®]), which is also an anti-TNF drug, anakinra (Kineret[®]), a selective IL-1 blocker, and Humira[®], a human monoclonal antibody which is another anti-TNF drug. The non-NSAID

5 analgesics include acetaminophen as well as narcotic analgesics such as hydrocodone, oxycodone and propoxyphene. Generally speaking, those drugs which work by a mechanism different from that of cladribine are particularly useful for concomitant therapy with the cladribine composition described herein. Those drugs which are effective by the oral or
10 transmucosal route of administration and which are compatible with the instant cladribine complexes in a single dosage form may be incorporated into the instant dosage forms; otherwise, they should of course be separately administered in amounts, frequencies and via administration routes suitable to them.

15 As used herein, "treating" means reducing, preventing, hindering the development of, controlling, alleviating and/or reversing the symptoms in the individual to which a compound of the invention has been administered, as compared to the symptoms of an individual not being treated according to the invention. A practitioner will appreciate that the complexes,
20 compositions, dosage forms and methods described herein are to be used in concomitance with continuous clinical evaluations by a skilled practitioner (physician or veterinarian) to determine subsequent therapy. Such evaluation will aid and inform in evaluating whether to increase, reduce or continue a particular treatment dose, and/or to alter the mode of
25 administration.

The methods of the present invention are intended for use with any subject/patient that may experience the benefits of the methods of the invention. Thus, in accordance with the invention, the terms "subjects" as well as "patients" include humans as well as non-human subjects,
30 particularly domesticated animals.

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Any suitable materials and/or methods known to those of skill can be utilized in carrying out the present invention. However, preferred materials and methods are described. Materials, reagents and the like to which reference are made in the following description and examples are obtainable from commercial sources, unless otherwise noted.

The following examples are intended to further illustrate certain preferred embodiments of the invention and are not limiting in nature. Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein.

EXAMPLES

EXAMPLE 1

PHASE SOLUBILITY STUDY

A phase solubility study was carried out as follows. Excess cladribine was added to cyclodextrin solutions of various concentrations of γ -cyclodextrin (γ CD) or hydroxypropyl- β -cyclodextrin (HP β CD) and allowed to complex as described in Example 2 below. In addition, in one set of experiments, the effect of hydroxypropylmethyl cellulose (HPMC) on complexation was investigated. The excess, undissolved cladribine was removed by filtration. The amount of cladribine in the complexation solution was measured to obtain a data point. This process was repeated with different known concentrations of cyclodextrin until several data points were obtained. These data points were then plotted graphically, each data point representing the maximum amount of cladribine that can be complexed with a specific concentration of cyclodextrin, *i.e.* each point represents a saturated cladribine-cyclodextrin complex. Points on the line generated by the data points represent HTA ratios. Any point on the line represents a specific, unique saturated cladribine-cyclodextrin complex. One of skill in the

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art will realize the same results will be generated if excess cyclodextrin is added to cladribine solutions of known concentration.

As an example, cyclodextrin solutions of varying concentrations were prepared and saturated with cladribine by providing cladribine in excess.

5 Saturated cyclodextrin solutions for a given cyclodextrin at a given cyclodextrin concentration are exemplified in Table I below.

Table I

Cyclo- dex- trin Molar Conc.	Cladribine-HP β CD (Trial A)			Cladribine-HP β CD and HPMC (0.1%) (Trial B)			Cladribine- γ CD (Trial C)		
	Absorb- ance	mg/ ml	Molar conc.	Absorb- ance	mg/ ml	Molar conc.	Absorb- ance	mg/ ml	Molar conc.
0.00	0.140	2.610	0.0091	0.137	2.550	0.0089	0.132	2.459	0.0086
0.018	0.169	3.139	0.011	0.146	2.711	0.0095	0.1352	2.519	0.0088
0.035	0.191	3.554	0.0124	0.175	3.262	0.0114	0.1531	2.852	0.0100
0.071	0.245	4.570	0.016	0.223	4.149	0.0145	0.1542	2.873	0.0101
0.142	0.333	6.211	0.0217	0.332	6.185	0.0216	0.1965	3.661	0.0128
0.285	0.514	9.581	0.0335	0.259	4.831	0.0169	0.4688	8.733	0.0306

10 The molar concentrations of cladribine to cyclodextrin in Table I are plotted and presented graphically as Fig. 1. The plotted lines for cladribine-HP β CD, cladribine-HP β CD and 0.1% HPMC, and cladribine- γ CD represent maximal cladribine solubilization for the conditions tested, that is, the HTA ratio of the concentration of cladribine to the concentration of cyclodextrin.

15 The area above each of the plotted lines represents conditions where excess insoluble cladribine is present. The area below each of the plotted lines represents the conditions where cyclodextrin is in excess of the amount needed to maintain the complex in solution. Clearly, the data in Table I and in Fig. 1 shows that HPMC, a known complexation facilitator, has no effect at

20 lower concentrations and has a negative effect at higher concentrations.

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The HTA plot for cladribine-HP β CD shown in Fig. 1 is approximately linear; this is indicative of a 1:1 complex, in which one molecule of the drug is complexed with one molecule of cyclodextrin. Fig. 1 also shows that additional cyclodextrin is needed to maintain the cladribine in the complex.

5 For example, in the case of γ -cyclodextrin, about 0.10 mole of γ CD is needed to maintain about 0.01 mole of cladribine in its saturated complex; in the case of HP β CD, about 0.10 mole of cyclodextrin is needed to maintain about 0.017 mole of cladribine in its saturated complex. However, in the case of γ CD, drug solubility significantly increases at higher concentrations
10 of the cyclodextrin; at a molar concentration of γ CD of about 0.15, the slope of the line changes, indicating formation of a 1:2 complex of cladribine to cyclodextrin, that is, one molecule of cladribine is complexed with 2 molecules of γ -CD, which essentially surround and protect the cladribine molecule.

15 The two molecules of γ -CD are believed to hydrogen-bond to each other at high cyclodextrin concentration and incorporate in the cavity between them the cladribine molecule. This is thought to be a stepwise process, in which the 1:1 complex first forms, then a second γ -CD molecule H-bonds with the γ -CD in the 1:1 complex, forming the 1:2 complex. Of
20 course, frequently a mixture of 1:1 and 1:2 complexes will be obtained, but a predominance of the 1:2 complex is advantageous. Thus, in the case of γ -CD, a molar concentration of about 0.20 of the cyclodextrin maintains about 0.017 mole of cladribine in its saturated complex. At higher cyclodextrin and drug concentrations, then, there is less difference between γ CD and HP β CD
25 in the amount of cyclodextrin needed for a given amount of cladribine, and γ -CD dissolves proportionately more cladribine than does HP β CD. Since the 1:2 complex formed at higher concentrations of γ CD is a stronger complex than a 1:1 complex, the cladribine in the saturated solution formed when such a 1:2 complex releases the drug in the body fluid at the mucosa is even
30 more unstable, *i.e.* has even higher thermodynamic activity, than the

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cladribine released from a 1:1 complex, favoring even greater movement of the drug through the mucosa. The complex with γ CD is also advantageous because γ CD is a natural cyclodextrin, thus presents fewer issues vis-à-vis toxicity. Further, in the case of solid oral dosage forms, it is believed that the 1:2 complex with γ -CD will better protect the cladribine from attack by stomach acid because it is able to essentially surround the drug molecule with cyclodextrin and thus is uniquely well-suited for the purposes of this invention.

10

EXAMPLE 2

PREPARATION OF CLADRIBINE-CYCLODEXTRIN COMPLEX
PART A:

Cladribine is complexed with either HP β CD or γ CD by the following general method.

15

An aqueous suspension of cladribine, in excess, and a concentrated solution (approximately 27% for γ -cyclodextrin and approximately 40% for HP β CD) of cyclodextrin are mixed with stirring at room temperature for about nine hours. This achieves equilibration. Excess, non-complexed cladribine, if any, is removed by filtration. To form the solid saturated cladribine-cyclodextrin complex, the aqueous cladribine-cyclodextrin solutions are dried by lyophilization prior to incorporation into solid buccal or oral tablets. The lyophilization procedure comprises a freezing stage of rapidly bringing the complexation solution to a temperature of from about -40°C to about -80°C for a period of from about 2 to 4 hours, preferably from about 3 to 4 hours, for example a temperature of about -45°C for approximately 200 minutes, followed by a primary drying stage at about -25°C for approximately 80-90 hours, typically under low pressure, and then a secondary drying stage at about 30°C for about 15-20 hours.

25

30

Product made by the foregoing general procedure can be analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based

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mobile phase, with UV detection at 264 nm) to find the weight ratio of cladribine to cyclodextrin in the final product. Final product preparations can be further characterized by methods known in the art, including, for example by inspecting appearance, ascertaining the overall impurity content by

5 HPLC, ascertaining the water content using a Karl Fischer titrator, determining the dissolution profile by a standard method, for example using USP<711>Apparatus II equipment and UV detection at 264 nm, inspecting the content uniformity and performing quantitative assay by HPLC analysis of the active ingredient.

10 PART B:

Two batches of cladribine/cyclodextrin product, FD02, in which γ -CD was used, and FD03 in which HP β CD was used, were prepared by the foregoing general procedure as follows:

Purified water (585 ml for FD02 and 575 ml for FD03) was dispensed

15 into a 1 liter glass vessel for each batch. The γ -cyclodextrin (116 g) and 2-hydroxypropyl- β -cyclodextrin (115 g) were weighed and slowly added to the stirred water over a period of 30 minutes. Cladribine (2.53 g for FD02 and 2.76 g for FD03) was weighed and added to the respective stirred cyclodextrin solutions. The solutions were sonicated for 20 minutes. The

20 resulting clear solution was stirred at room temperature for 9 hours. The solutions were then filled into 100 ml lyophilization vials (20 ml solution per vial) and the filled vials were partially stoppered. The lyophilization included freezing at -45°C for about 3.3 hours, a primary drying phase at -25°C under a pressure of 100 mTorr for about 85.8 hours, and a secondary drying phase

25 at 30°C for about 17.5 hours as set forth below:

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LYOPHILIZATION CYCLE

Step	Process	Temperature	Pressure (mTorr)	Time (hrs)
1	Load	4°C		
2	Load Hold	4°C	n/a	2.0
3	Ramp	-45°C	n/a	2.0
4	Freezing	-45°C	n/a	3.3
5	Ramp	-25°C	100	2.2
6	Primary drying	-25°C	100	85.8
7	Ramp	30°C	50	4.0
8	Secondary drying	30°C	50	17.5
9	Finish	30°C	Vials closed under vacuum	

- 5 The FD02 and FD03 batches of cladribine/cyclodextrin made by the foregoing procedure were analyzed by HPLC (utilizing a Hypersil ODS 3 micron column and an acetonitrile based mobile phase with UV detection at 264 nm) and empirically found to have the following characteristics:

Complex	Weight:Weight	Weight Ratio
Cladribine: γ -CD	2.53 g:116.0 g	1:46
Cladribine: HP β CD	2.76 g:115.0 g	1:42

- 10 The products were analyzed by DSR and X-ray diffraction methods to determine any free cladribine in the lyophilized material. Importantly, the samples exhibited no transitions in the region of 210°C to 230°C, which is associated with the melting of crystalline cladribine. In both cases, no significant thermal activity was recorded in the range of 210°C to 230°C,

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suggesting that the complexes obtained at the end of the lyophilization do not have any significant amount of free crystalline cladribine, considering the sensitivity of the analytical method (up to 3% w/w). This conclusion was supported by the absence of peaks for crystalline cladribine from X-ray diffraction traces for both complexes FD02 and FD03.

As noted above, these cladribine:cyclodextrin complexes have cladribine:cyclodextrin weight ratios of about 1:46 for cladribine: γ -cyclodextrin and about 1:42 for cladribine:hydroxypropyl- β -cyclodextrin. Cladribine:cyclodextrin weight ratios close to these, for example from about 1:35 to about 1:50, are most desirable. These ratios can vary depending upon the particular cyclodextrin used and the amount of cyclodextrin in the complexation solution, as well as the complexation temperature.

EXAMPLE 3

PHARMACOKINETIC STUDIES

The bioavailability of cladribine when complexed with γ CD or HP β CD was evaluated in a beagle dog model. The data obtained from this model are expected to be representative for the human experience.

The saturated cladribine-cyclodextrin complex as prepared in EXAMPLE 2, Part B, FD02 and FD03, were used to prepare oral and buccal tablets. The complex materials were passed through a #18 mesh (0.9 mm) screen with magnesium stearate, blended for five minutes and compressed using 10 mm punches. The 10 mm tablets had upper shallow convex tooling and lower flat beveled edge tooling. The formulations for the manufacture were as follows:

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TABLE II, PART A

Batch No.		RDT-0418/C	RDT-0418/D
Ingredient	Lot Number	mg/tablet	mg/tablet
Cladribine/ γ -CD complex	FD 02	232.65*	
Cladribine/2-HP β CD complex	FD 03		212.85*
Magnesium stearate		2.35	2.15
Total		235.00	215.00

*This amount of complex contains approximately 5 mg of cladribine/tablet.

5

TABLE II, PART B

Property of Finished Tablet	Cladribine: γ -Cyclodextrin Tablet RDT-0418/C	Cladribine:HP- β -Cyclodextrin Tablet RDT-0418/D
Average Weight	237.0 mg	217 mg
Hardness	4.0 Kp	3.72 Kp
Friability	0.5%	0.4%
Thickness	3.8 mm	3.3 mm
Disintegration	8 minutes	8 minutes

Bioavailability and pharmacokinetic studies were conducted in a beagle dog model as follows.

10

Outbred male beagle dogs (identified as PM01-PM06) obtained from IDRI (Dunakeszi, Hungary) were allowed laboratory diet and water *ad libitum*. The same dogs were used throughout the study to minimize inter- and intrasubject variability. The bioavailability and pharmacokinetic studies were conducted as follows.

15

In the first test period, 5 mg cladribine (0.25 mg/ml in isotonic saline) was administered intravenously to test subjects. Blood samples were collected at various time intervals over 48 hours. In the second test period,

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half of the subjects received buccally a tablet as described above containing a saturated cladribine- γ CD or -HP β CD complex. Serial blood samples were collected over 48 hours. The third test period repeated the second test period with the exception that the subjects previously receiving the γ -cyclodextrin buccal tablet were now given hydroxypropyl- β -cyclodextrin buccal tablets, with hydroxypropyl- β -cyclodextrin tablet recipients from the second period receiving γ -cyclodextrin buccal tablets. The fourth and fifth test periods repeated test periods two and three with the exception that the tablets were given orally.

Cladribine levels in the blood were measured by HPLC and an LC/MS/MS method. The TopFit 2.0 Pharmacokinetic and Pharmacodynamic Data Analysis System was used for the pharmacokinetic analysis of the data. The results of the bioavailability study for control (intravenous) and cladribine-cyclodextrin complexes are presented in Tables III to VII and summarized in Table VIII.

The headings of the columns in Table III are defined as follows:

C_{initial} is the extrapolated value at the end of the bolus;

C_{first} is the first measured concentration at 5 minutes after the dose is administered;

$t_{1/2}^{\text{terminal}}$ is the terminal elimination half-life;

AUD is the area under the measured data, integrated with the linear trapezoidal rule;

AUD_{ext} is the extrapolated area from the last measured time-point to infinity;

AUC is AUD extrapolated to infinity;

Cl_{tot} is total clearance (dose/AUC); and

MRT_{tot} is the mean residence time.

The headings of the columns in Tables IV to VII are defined as follows:

C_{max} is the peak concentration measured;

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$T_{\max}$ is the time to $C_{\max}$;

$t_{1/2}$ terminal is the terminal elimination half-life;

AUD is the area under the measured data, integrated with the linear trapezoidal rule;

5 AUD_{ext} is the extrapolated area from the last measured time-point to infinity;

AUC is AUD extrapolated to infinity;

MRT_{tot} is the mean residence time; and

F is the bioavailability expressed in %.

10 The peak areas, calibration curves, accuracy, precision values and the concentrations were determined using Analyst Software 1.1 (PE SCIEX, Foster City, US). For calculation of mean and standard deviation, Excel 5.0 software was used. The calibration curve was fitted using the ratio of concentrations of analyte and internal standard versus the ratio of the peak
15 areas of them. A straight line was fitted on the experimental points by weighted least squares linear regression analysis. The weighting scheme used was 1/concentration squared as pg/ml plasma.

20 At early time-points after intravenous administration, the plasma concentrations exceeded the upper limit of the calibration curve. Thus a lower amount of the sample was re-injected to prove the linear detector response. Since the standard/internal standard ratio remained the same at the appropriate detector response, concentrations higher than 100 ng/ml could be accepted. For calculation of mean, standard deviation and CV%, Excel 5.0 software was used. Lower levels than the limit of quantification
25 were not accepted. Mean and S.D. values were calculated only for the concentrations measured after intravenous administration.

Table III

Dog	Intravenous bolus, 5 mg cladribine/animal											
	C_{initial}	C_{first}	$t_{1/2}$	AUD	AUD _{ext}	AUC	Cl _{tot}	MRT _{tot}	Body Weight	Dose	AUC/Dose	Cl _{tot} /kg
	(ng/ml)	(ng/ml)	(h)	(ng·h/ml)	(%)	(ng·h/ml)	(ml/min)	(h)	(kg)	(mg/kg)		(ml/min/kg)
PM01	655	560	10.4	426	1.4	432	193	1.3	10.98	0.46	949	17.6
PM02	525	446	8.5	337	1.4	342	244	1.2	13.86	0.36	948	17.6
PM03	726	605	10.7	426	1.5	433	192	1.3	12.44	0.40	1077	15.4
PM04	569	477	11.0	379	1.4	383	217	1.4	11.98	0.42	918	18.1
PM05	335	306	11.3	334	1.0	338	247	1.5	14.28	0.35	965	17.3
PM06	567	478	9.9	359	1.9	366	228	1.3	12.94	0.39	947	17.6
Mean	563	479	10.3	377	1.4	382	220	1.3	12.75	0.40	967	17.3
S.D.	133	103	1.0	41	0.3	42	24	0.1	1.22	0.04	56	0.9
CV%	24	22	9.8	11	20	11	11	9	10	10	6	5

Table IV

Dog	Buccal administration 5 mg cladribine/animal										
	Tablet-1: γ -cyclodextrin complex (RDT-0418/C)										
	$C_{\max}$	$T_{\max}$	$t_{1/2}$ terminal	AUD	AUD _{ext}	AUC	MRT _{tot}	Body Weight	Dose	AUC/Dose	F
	(ng/ml)	(h)	(h)	(ng·h/ml)	(%)	(ng·h/ml)	(h)	(kg)	(mg/kg)		(%)
PM01	31.1	4.0	15.1	115	1.8	117	6.7	12.16	0.41	285	30.0
PM02	78.8	2.0	16.9	159	1.2	161	4.2	14.72	0.34	474	50.0
PM03	107.0	2.0	21.5	214	1.6	218	4.6	12.50	0.40	545	50.6
PM04	37.3	1.5	13.3	96	1.0	97	4.2	13.02	0.38	253	27.5
PM05	30.7	3.0	9.2	92	0.4	93	4.9	15.52	0.32	289	29.9
PM06	65.4	3.0	13.2	108	0.7	109	4.5	14.40	0.35	314	33.1
Mean	58.4	2.6	14.9	131	1.1	133	4.8	13.72	0.37	360	36.9
S.D.	30.9	0.9	4.1	47	0.5	48	1.0	1.35	0.04	120	10.6
CV%	53	36	28	36	48	37	20	10	10	33	29

Table V

Dog	Buccal administration; 5 mg cladribine/animal										
	Tablet-2: hydroxypropyl- β -cyclodextrin complex (RDT-0418/D)										
	C _{max}	T _{max}	t _{1/2} terminal	AUD	AUD _{ext}	AUC	MRT _{tot}	Body Weight	Dose	AUC/Dose	F
	(ng/ml)	(h)	(h)	(ng·h/ml)	(%)	(ng·h/ml)	(h)	(kg)	(mg/kg)		(%)
PM01	78.0	2.0	10.9	128	0.5	129	3.7	12.32	0.41	318	33.5
PM02	95.6	0.8	13.8	102	1.3	103	3.7	14.96	0.33	308	32.5
PM03	54.0	2.0	9.1	106	0.6	107	4.7	12.90	0.39	276	25.6
PM04	51.5	1.0	15.5	120	0.8	121	3.3	12.74	0.39	308	33.6
PM05	50.9*	0.75*	13.9	83*	0.6*	84*	2.88*	15.08	0.33	253*	29.6*
PM06	33.4	2.0	13.1	84	1.4	85	5.3	13.68	0.37	233	24.6
Mean	62.5	1.6	12.7	108	0.9	109	4.1	13.61	0.37	289	30.0
S.D.	24.4	0.6	2.3	17	0.4	17	0.8	1.18	0.03	35	4.5
CV%	39	40	18	16	44	16	20	9	8	12	15

*: excluded from mean (because the subject swallowed the drug form)

Table VI

Dog	Oral administration; 5 mg cladribine/animal Tablet-1: γ -cyclodextrin complex (RDT-0418/C)										
	C _{max}	T _{max}	t _{1/2} terminal	AUD	AUD _{ext}	AUC	MRT _{tot}	Body Weight	Dose	AUC/Dose	F
	(ng/ml)	(h)	(h)	(ng·h/ml)	(%)	(ng·h/ml)	(h)	(kg)	(mg/kg)		(%)
PM01	218.9	0.8	11.0	193	0.4	194	2.6	12.70	0.39	493	51.9
PM02	141.2	0.5	10.2	156	0.3	156	2.3	15.08	0.33	470	49.6
PM03	234.9	0.3	13.3	214	0.4	215	1.9	13.18	0.38	567	52.6
PM04	99.6	2.0	16.2	172	0.8	174	3.5	13.18	0.38	459	50.0
PM05	17.6*	1.5*	10.2*	52*	2*	53*	3.29*	15.52	0.32	165*	17.0*
PM06	161.8	0.3	11.1	148	0.4	148	2.1	14.52	0.34	430	45.4
Mean	171.3	0.8	12.4	177	0.5	177	2.5	14.03	0.36	484	49.9
S.D.	55.8	0.7	2.4	27	0.2	27	0.6	1.16	0.03	52	2.8
CV%	33	97	20	15	42	15	25	8	8	11	6

*: excluded from mean (because the values obtained were highly anomalous compared to those for other subjects)

Table VII

Dog	Oral administration; 5 mg cladribine/animal Tablet-2: hydroxypropyl- β -cyclodextrin complex (RDT-0418/D)										
	$C_{\max}$	$T_{\max}$	$t_{1/2}$ terminal	AUD	AUD _{ext}	AUC	MRT _{tot}	Body Weight	Dose	AUC/Dose	F
	(ng/ml)	(h)	(h)	(ng·h/ml)	(%)	(ng·h/ml)	(h)	(kg)	(mg/kg)		(%)
PM01	143.3	0.5	12.8	174	0.5	175	2.3	12.72	0.39	445	46.9
PM02	86.9	1.5	10.6	121	0.5	122	3.4	15.00	0.33	366	38.6
PM03	231.5	0.3	12.6	199	0.4	200	2.0	13.24	0.38	530	49.2
PM04	114.3	1.5	15.0	171	0.8	172	3.7	13.16	0.38	453	49.3
PM05	147.5	1.0	14.2	149	0.7	150	3.2	15.32	0.33	460	47.6
PM06	68.5	0.5	16.9	121	0.9	123	2.8	14.32	0.35	352	37.2
Mean	132.0	0.9	13.7	156	0.6	157	2.9	13.96	0.36	434	44.8
S.D.	57.7	0.5	2.2	31	0.2	31	0.6	1.07	0.03	66	5.4
CV%	44	62	16	20	31	20	22	8	8	15	12

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Table VIII

Dog	Bioavailability of cladribine in dogs (dose given in mg cladribine/animal)					
	γ -Cyclodextrin Complex			HP- β -Cyclodextrin Complex		
	Buccal (5 mg)	Oral (5 mg)	Oral ^a (5 mg)	Buccal (5 mg)	Oral (5 mg)	Oral ^a (5 mg)
PM01	30.0	51.9	35.3	33.5	46.9	17.0
PM02	50.0	49.6	39.5	32.5	38.6	19.0
PM03	50.6	52.6	27.4	25.6	49.2	25.1
PM04	27.5	50.0	24.9	33.6	49.3	26.5
PM05	29.9	17.0*	36.1	29.6**	47.6	27.1
PM06	33.1	45.4	42.3	24.6	37.2	31.7
Mean	36.9	49.9	34.2	30.0	44.8	24.5
S.D.	10.6	2.8	6.8	4.5	5.4	5.3
CV%	29	6	20	15	12	21

*: excluded from mean (because the values obtained were highly anomalous compared to those for the other subjects)

**: excluded from mean (because the subject swallowed the drug form)

a: excess cyclodextrin, $\approx 10\times$ that for the saturated complex. The total amount of cyclodextrin in these studies is ~ 2.5 gm with 5 mg cladribine.

Pharmacokinetic analysis was performed on the basis of individual plasma concentration versus time curves. The mean and S.D. of parameters obtained from individual data were calculated. The linear-trapezoidal rule was used for calculation of the area under the plasma concentration time curve from 0 until the last measured concentration (AUD). Using the terminal regression line the area extrapolated to infinity ($AUC_{t-\infty}$) was calculated in the following way: $AUC_{t-\infty} = C_{calc}/\lambda_z$

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where: C_{calc} represents the estimated plasma concentration by the regression line at the last sampling time point with measured concentration above the limit of quantification, and λ_z represents the rate constant calculated from the regression line.

5

The points determining the regression line, *i.e.* the terminal phase, were selected by visual determination of the linear segment of the semilogarithmic curve. The total area under the curve (AUC) was calculated by adding together the partial areas: $AUC = AUD + AUC_{t-\infty}$. The AUC values were normalized by the actual dose given in the particular period. For calculation of the bioavailability, the buccal/oral AUC/dose value was divided by the intravenous AUC/dose value.

10

The individual plasma level-time curves were obtained. Small inter-individual variability was found after intravenous administration. After a very rapid initial decrease, the terminal elimination half-life of cladribine was about 10 hours. The mean total clearance proved to be 17 ml/min/kg. During buccal administration of the two formulations, the dissolution period was longer for the cladribine: γ -cyclodextrin complex compared to the cladribine:HP- β -cyclodextrin complex. Although the peak concentrations and the absorption profiles showed high inter-individual variability after both buccal and oral administrations, the total exposures (AUC) showed much lower variability. The oral bioavailability proved to be good: $50 \pm 3 \%$ and $45 \pm 5 \%$ for γ -cyclodextrin and hydroxypropyl- β -cyclodextrin complexes, respectively. The buccal bioavailability values were lower: $37 \pm 10 \%$ for the γ -cyclodextrin complex and $30 \pm 4.5 \%$ for the hydroxypropyl- β -cyclodextrin complex.

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The results from further comparative dog pharmacokinetic studies of the γ -cyclodextrin complex and mixture are set forth in Table IX below. The "Oral Complex" column describes the results of absolute bioavailability of 5 mg cladribine in 2.5 g of γ -cyclodextrin; this is approximately 10 times the

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amount of cyclodextrin in a saturated complex with 5 mg cladribine. This preparation was the same as in the "Oral Mix" column, with the exception that the complex was preformed as opposed to only mixing the components. The results for the "Oral Mix" and "Oral Complex" are comparable, indicating that with such a large excess of cyclodextrin, a complex forms during dissolution, interfering with dissolution and exerting a negative impact on bioavailability. The same dogs and the same experimental method were used as before. These results show that the use of excess cyclodextrin is counter-productive, *i.e.*, the saturated cladribine-cyclodextrin complex substantially free of cyclodextrin in excess of the minimum amount required to maintain substantially all of the cladribine in the complex provided both enhanced bioavailability and decreased interpatient variability.

Table IX

Dog	Bioavailability of 5 mg cladribine in 2.5 g γ -Cyclodextrin Complex dose: mg cladribine/animal			
	Buccal Tablet	Oral Tablet	Oral Mix 10x in Capsule ^a	Oral Complex 10x in Capsule ^a
PM01	30.0	51.9	35.3	39.5
PM02	50.0	49.6	29.5	27.5
PM03	50.6	52.6	27.4	32.6
PM04	27.5	50.0	24.9	34.7
PM05	29.9	17.0*	36.1	12
PM06	33.1	45.4	42.3	32.7
Mean	36.9	49.9	34.2	33.4
S.D.	10.6	2.8	6.8	4.3
CV%	29	6	20	1.3

*:excluded from mean

a: excess cyclodextrin, $\approx$ 10x that for the saturated complex. The total amount of cyclodextrin in these studies is $\sim$ 2.5 gm with 5 mg cladribine.

The results of the foregoing pharmacokinetic studies are graphically represented in Figs. 2-6.

Fig. 2 shows the plasma profile for cladribine in dogs after administration of 5 mg single doses in the various formulations described

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above, where the data are the average $\pm$ SD for 5-6 animals per group. The average drug concentration in pg/ml of plasma is plotted against time in hours. Although each test was conducted for a 48-hour period, only the first 6 hours were presented in the graphs; after 6 hours, most concentrations had returned to or near baseline and therefore are not shown in the graphs. Intravenous values ($\diamond$) are considered to give 100% bioavailability, and plasma levels for oral and buccal forms were compared thereto. The meanings of the symbols are given in the BRIEF DESCRIPTION OF THE DRAWINGS hereinabove. The buccal formulations of the saturated complexes with γ -cyclodextrin (*) and hydroxypropyl- β -cyclodextrin (x) were found to be less effective than the oral formulations in these tests, and this is readily seen in Fig. 2. The results for the five different oral formulations can be more readily seen by reference to Figs. 3-5.

Fig. 3 provides a comparison of the plasma profiles for the intravenous formulation ($\diamond$), the oral saturated cladribine- γ -cyclodextrin complex formulation ($\bullet$) and the oral saturated cladribine-hydroxypropyl- β -cyclodextrin complex formulation ($\blacktriangle$) shown in Fig. 2. Both of these oral formulations afforded desirable profiles.

Fig. 4 provides a comparison of the plasma profiles for the oral saturated cladribine- γ -cyclodextrin complex formulation ($\bullet$), the oral capsule of a physical mixture of cladribine with ten-times excess γ -cyclodextrin ($\circ$) and the oral capsule of the cladribine- γ CD complex with ten times excess γ -cyclodextrin ($\square$) shown in Fig. 2. Here it is apparent that excess cyclodextrin decreases the amount of cladribine in the plasma, particularly in the first hour after administration.

Fig. 5 provides a comparison of the plasma profiles for the oral saturated hydroxypropyl- β -cyclodextrin complex formulation ($\blacktriangle$) and the oral capsule of a physical mixture of cladribine with ten-times excess hydroxypropyl- β -cyclodextrin (Δ) shown in Fig. 2. Here it is again readily apparent that excess cyclodextrin decreases the amount of cladribine in the

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complex. In this case, the decrease is seen in the first two hours after administration.

Fig. 6 depicts the cumulative areas under the curves (AUCs) in pg xh/ml for the eight formulations shown in Fig. 2. Again, data are the average for 5-6 animals per group.

The figures thus graphically illustrate what is shown by the data in Tables III through IX, that the saturated cladribine-cyclodextrin complex, formulated as a solid oral or a transmucosal dosage form substantially free of cyclodextrin in excess of the minimum amount required to maintain substantially all of the cladribine in the complex, provides enhanced bioavailability with acceptable interpatient variability.

The foregoing is considered as illustrative only of the principles of the invention. Further, since numerous modifications and changes will readily occur to those skilled in the art, it is not desired to limit the invention to the exact construction and operation shown and described, and accordingly, all suitable modifications and equivalents thereof may be resorted to, falling within the scope of the invention claimed.

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WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising a saturated
cladribine-cyclodextrin complex formulated into a solid oral dosage form or a
5 transmucosal dosage form, said composition being substantially free of
cyclodextrin in excess of the minimum amount required to maximize the
amount of cladribine in the complex.
2. A pharmaceutical composition comprising a saturated
10 cladribine-cyclodextrin complex formulated into a solid oral dosage form or a
transmucosal dosage form, said composition being substantially free of
cyclodextrin in excess of the minimum amount required to maintain
substantially all of the cladribine in the complex.
3. The composition according to Claim 1 or 2, wherein the
15 saturated cladribine-cyclodextrin complex is formulated into a solid oral
dosage form.
4. The composition according to Claim 1, 2 or 3, wherein the
20 cyclodextrin is γ -cyclodextrin, hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -
cyclodextrin, dimethyl- β -cyclodextrin, randomly methylated β -cyclodextrin,
carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.
5. The composition according to Claim 1, 2 or 3, wherein the
25 cyclodextrin is γ -cyclodextrin.
6. The composition according to Claim 1, 2 or 3, wherein the
cyclodextrin is hydroxypropyl- β -cyclodextrin.

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7. The composition according to Claim 5, wherein the complex comprises a 1:2 cladribine:γ-cyclodextrin complex.

5 8. The composition according to Claim 4, 5 or 6, wherein the weight ratio of cladribine to cyclodextrin is from about 1:35 to about 1:50.

9. The composition according to Claim 5, wherein the weight ratio of cladribine to γ-cyclodextrin is about 1:46.

10 10. The composition according to Claim 6, wherein the weight ratio of cladribine to hydroxypropyl-β-cyclodextrin is about 1:42.

11. The composition according to any one of Claims 2 to 6, wherein the approximate molar ratio of cladribine to cyclodextrin
15 corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

12. The composition according to Claim 11, wherein the cyclodextrin is γ-cyclodextrin and the point is taken from the portion of the
20 phase solubility diagram indicative of formation of a 1:2 complex of cladribine:γ-cyclodextrin.

13. A method for enhancing the oral or transmucosal bioavailability of cladribine comprising administering to a subject in need thereof a
25 pharmaceutical composition comprising a saturated cladribine-cyclodextrin complex formulated into a solid oral dosage form or a transmucosal dosage form, said composition being substantially free of cyclodextrin in excess of the minimum amount required to maximize the amount of cladribine in the complex.

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14. A method for enhancing the oral or transmucosal bioavailability of cladribine comprising administering to a subject in need thereof a pharmaceutical composition comprising a saturated cladribine-cyclodextrin complex formulated into a solid oral dosage form or a transmucosal dosage form, said composition being substantially free of cyclodextrin in excess of the minimum amount required to maintain substantially all of the cladribine in the complex.

15. The method according to Claim 13 or 14, wherein the saturated cladribine-cyclodextrin complex is formulated into a solid oral dosage form.

16. The method according to Claim 13, 14 or 15, wherein the cyclodextrin is γ -cyclodextrin, hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, dimethyl- β -cyclodextrin, randomly methylated β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

17. The method according to Claim 13, 14 or 15, wherein the cyclodextrin is γ -cyclodextrin.

18. The method according to Claim 13, 14 or 15, wherein the cyclodextrin is hydroxypropyl- β -cyclodextrin.

19. The method according to Claim 17, wherein the complex comprises a 1:2 cladribine: γ -cyclodextrin complex.

20. The method according to Claim 16, 17 or 18, wherein the weight ratio of cladribine to cyclodextrin is from about 1:35 to about 1:50.

21. The method according to Claim 17, wherein the weight ratio of cladribine to γ -cyclodextrin is about 1:46.

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22. The method according to Claim 18, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:42.

5 23. The method according to any one of Claims 14 to 18, wherein the approximate molar ratio of cladribine to cyclodextrin corresponds to a point located on a phase solubility diagram for saturated complexes of cladribine in varying concentrations of the cyclodextrin.

10 24. The method according to Claim 23, wherein the cyclodextrin is γ -cyclodextrin and the point is taken from the portion of the phase solubility diagram indicative of formation of a 1:2 complex of cladribine: γ -cyclodextrin.

15 25. A method for the treatment of symptoms of a cladribine-responsive condition in a subject suffering from said symptoms comprising administering to said subject a pharmaceutical composition comprising a saturated cladribine-cyclodextrin complex formulated into a solid oral dosage form, said composition being substantially free of cyclodextrin in excess of the minimum amount required to maximize the amount of cladribine in the
20 complex.

25 26. A method for the treatment of symptoms of a cladribine-responsive condition in a subject suffering from said symptoms comprising administering to said subject a pharmaceutical composition comprising a saturated cladribine-cyclodextrin complex formulated into a solid oral dosage form, said composition being substantially free of cyclodextrin in excess of the minimum amount required to maintain substantially all of the cladribine in the complex.

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27. The method according to Claim 25 or 26, wherein the cladribine-responsive condition is selected from the group consisting of multiple sclerosis, rheumatoid arthritis and leukemia.

5 28. The method according to Claim 27, wherein the cladribine-responsive condition is multiple sclerosis.

29. The method according to Claim 25, 26, 27 or 28, wherein the saturated cladribine-cyclodextrin complex is formulated into a solid oral
10 dosage form.

30. The method according to any one of Claims 25 to 29, wherein the cyclodextrin is γ -cyclodextrin, hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, dimethyl- β -cyclodextrin, randomly methylated
15 β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

31. The method according to any one of Claims 25 to 29, wherein the cyclodextrin is γ -cyclodextrin.

20 32. The method according to any one of Claim 25 to 29, wherein the cyclodextrin is hydroxypropyl- β -cyclodextrin.

33. The method according to Claim 30, 31 or 32, wherein the weight ratio of cladribine to cyclodextrin is from about 1:35 to about 1:50.
25

34. The method according to Claim 31, wherein the weight ratio of cladribine to γ -cyclodextrin is about 1:46.

35. The method according to Claim 32, wherein the weight ratio of
30 cladribine to hydroxypropyl- β -cyclodextrin is about 1:42.

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36. The method according to Claim 31, wherein the complex comprises a 1:2 cladribine:γ-cyclodextrin complex.

5 37. A method for enhancing the bioavailability of cladribine from a solid oral or transmucosal dosage form administered to a mammal in need of treatment with cladribine, said method comprising:

 (a) determining the minimum amount of cyclodextrin required to complex with a selected amount of cladribine and to maintain said selected
10 amount of cladribine in the complex;

 (b) combining an amount of cladribine in excess of said selected amount with said minimum amount of cyclodextrin in an aqueous medium;

 (c) removing uncomplexed cladribine from the aqueous complexation medium;

15 (d) removing water from the aqueous complexation medium to afford the dry saturated cladribine-cyclodextrin complex;

 (e) formulating said dry saturated cladribine-cyclodextrin complex into a solid oral dosage form or a transmucosal dosage form substantially free of cyclodextrin in excess of the minimum amount required to maintain
20 substantially all of the cladribine in the complex; and

 (f) administering said dosage form orally or transmucosally to said mammal.

 38. Use of a saturated cladribine-cyclodextrin complex in the
25 formulation of a solid oral dosage form or a transmucosal dosage form substantially free of cyclodextrin in excess of the minimum amount required to maximize the amount of cladribine in the complex, for administration in the treatment of symptoms of a cladribine-responsive condition.

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39. Use of a saturated cladribine-cyclodextrin complex in the formulation of a solid oral dosage form or a transmucosal dosage form substantially free of cyclodextrin in excess of the minimum amount required to maintain substantially all of the cladribine in the complex, for
5 administration in the treatment of symptoms of a cladribine-responsive condition.

40. Use according to Claim 38 or 39, wherein the cladribine-responsive condition is selected from the group consisting of multiple
10 sclerosis, rheumatoid arthritis and leukemia.

41. Use according to Claim 40, wherein the cladribine-responsive condition is multiple sclerosis.

42. Use according to Claim 38, 39, 40 or 41, wherein the saturated
15 cladribine-cyclodextrin complex is formulated into a solid oral dosage form.

43. Use according to any one of Claims 38 to 42, wherein the cyclodextrin is γ -cyclodextrin, hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -
20 cyclodextrin, dimethyl- β -cyclodextrin, randomly methylated β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

44. Use according to any one of Claims 38 to 42, wherein the cyclodextrin is γ -cyclodextrin.
25

45. Use according to any one of Claims 38 to 42, wherein the cyclodextrin is hydroxypropyl- β -cyclodextrin.

46. Use according to Claim 43, 44 or 45, wherein the weight ratio
30 of cladribine to cyclodextrin is from about 1:35 to about 1:50.

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47. Use according to Claim 44, wherein the weight ratio of cladribine to γ -cyclodextrin is about 1:46.

5 48. Use according to Claim 45, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:42.

49. Use according to Claim 44, wherein the complex comprises a 1:2 cladribine: γ -cyclodextrin complex.

10

50. Use of a saturated cladribine-cyclodextrin complex in the formulation of a solid oral dosage form or a transmucosal dosage form substantially free of cyclodextrin in excess of the minimum amount required to maximize the amount of cladribine in the complex, for enhancing the oral or transmucosal bioavailability of cladribine.

15

51. Use of a saturated cladribine-cyclodextrin complex in the formulation of a solid oral dosage form or a transmucosal dosage form substantially free of cyclodextrin in excess of the minimum amount required to maintain substantially all of the cladribine in the complex, for enhancing the oral or transmucosal bioavailability of cladribine.

20

52. Use according to Claim 50 or 51, wherein the saturated cladribine-cyclodextrin complex is formulated into a solid oral dosage form.

25

53. Use according to Claim 50, 51 or 52, wherein the cyclodextrin is γ -cyclodextrin, hydroxypropyl- β -cyclodextrin, hydroxypropyl- γ -cyclodextrin, dimethyl- β -cyclodextrin, randomly methylated β -cyclodextrin, carboxymethyl- β -cyclodextrin or sulfobutyl- β -cyclodextrin.

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54. Use according to Claim 50, 51 or 52, wherein the cyclodextrin is γ -cyclodextrin.

5 55. Use according to Claim 50, 51 or 52, wherein the cyclodextrin is hydroxypropyl- β -cyclodextrin.

56. Use according to Claim 53, 54 or 55, wherein the weight ratio of cladribine to cyclodextrin is from about 1:35 to about 1:50.

10

57. Use according to Claim 54, wherein the weight ratio of cladribine to γ -cyclodextrin is about 1:46.

15 58. Use according to Claim 55, wherein the weight ratio of cladribine to hydroxypropyl- β -cyclodextrin is about 1:42.

59. Use according to Claim 54, wherein the complex comprises a 1:2 cladribine: γ -cyclodextrin complex.

20 60. A 1:2 cladribine: γ -cyclodextrin complex.

61. A mixture of a 1:1 cladribine: γ -cyclodextrin complex and a 1:2 cladribine: γ -cyclodextrin complex, wherein the 1:2 complex is predominant.

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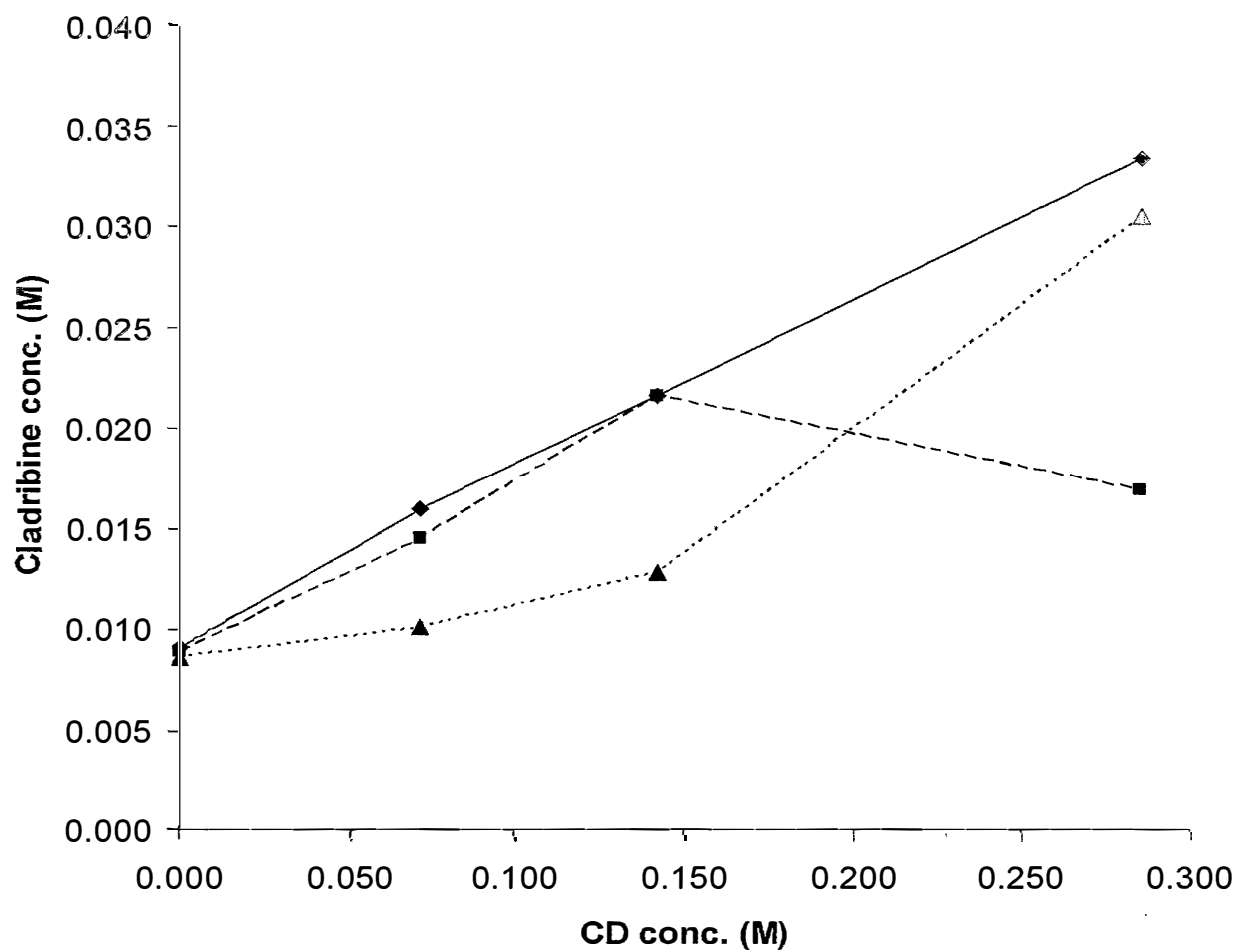


Fig. 1

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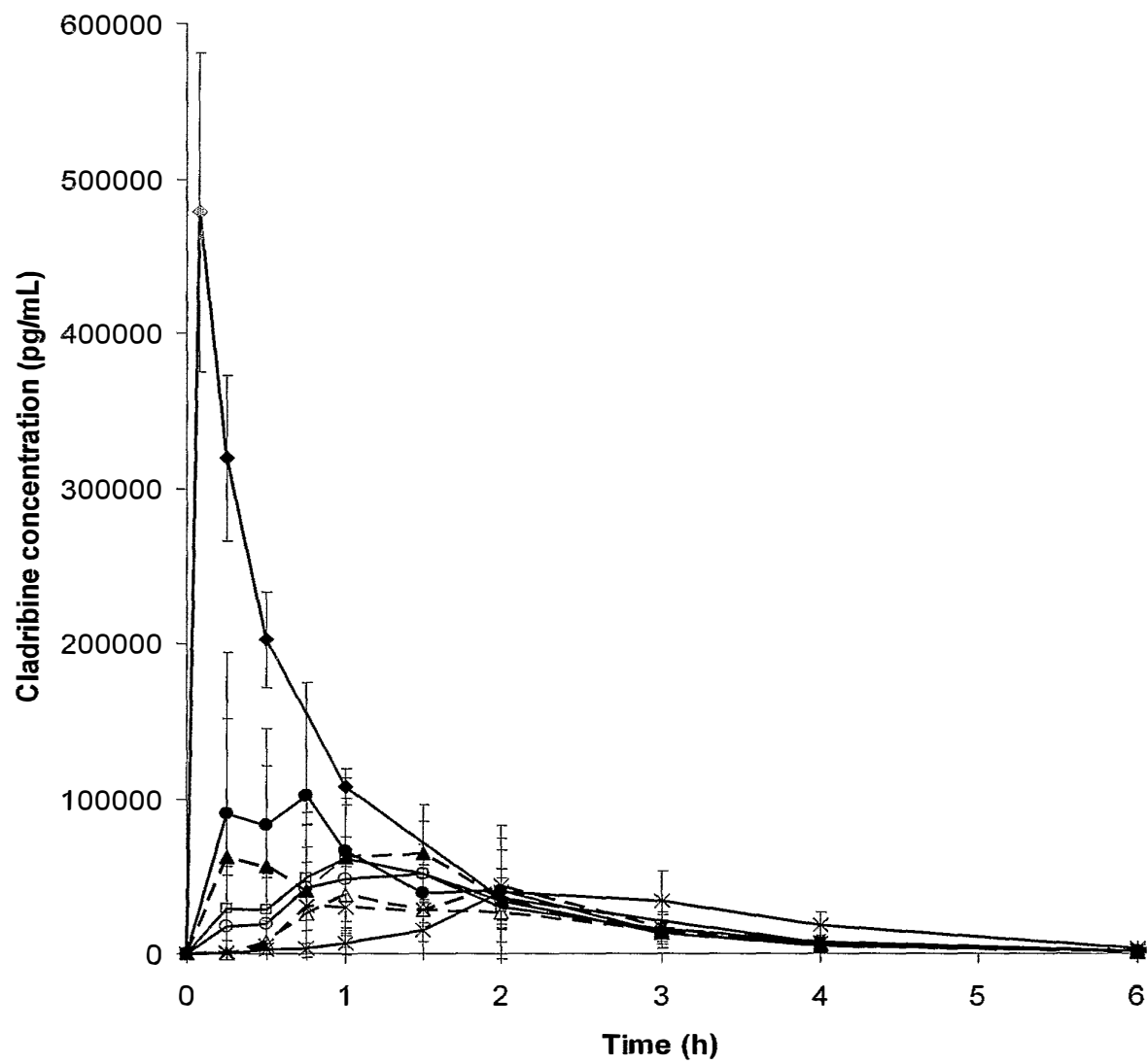


Fig. 2

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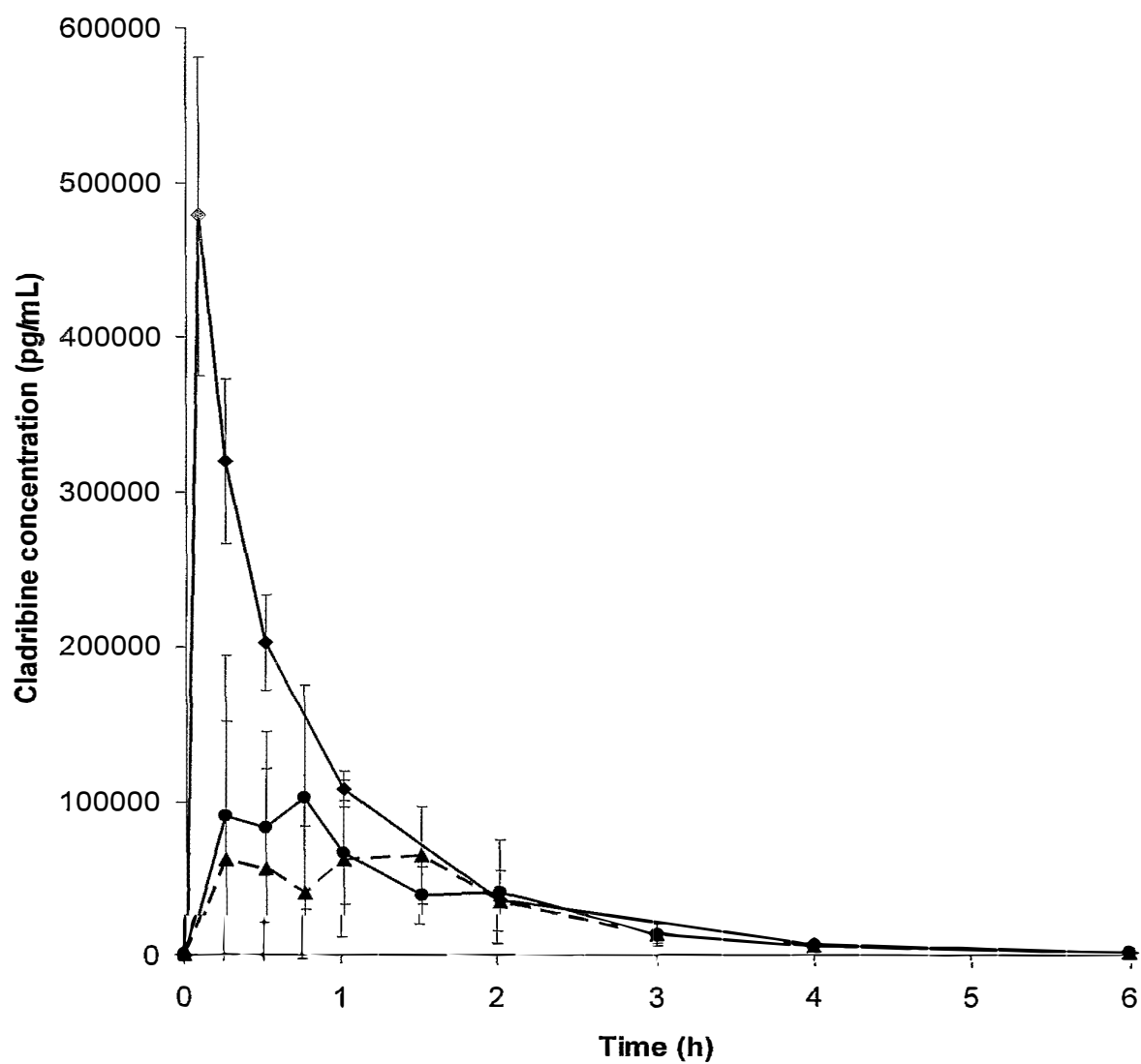


Fig. 3

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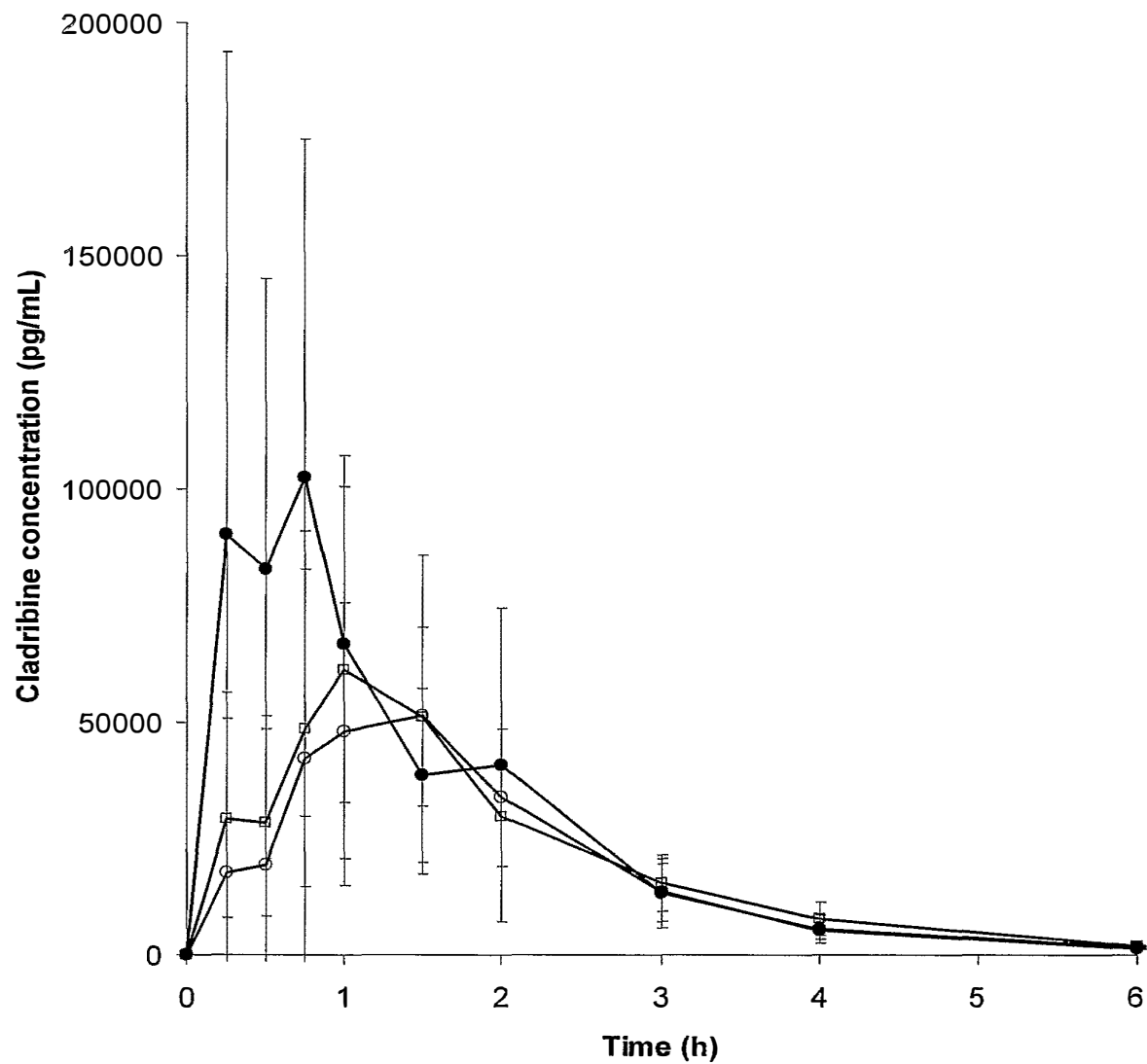


Fig. 4

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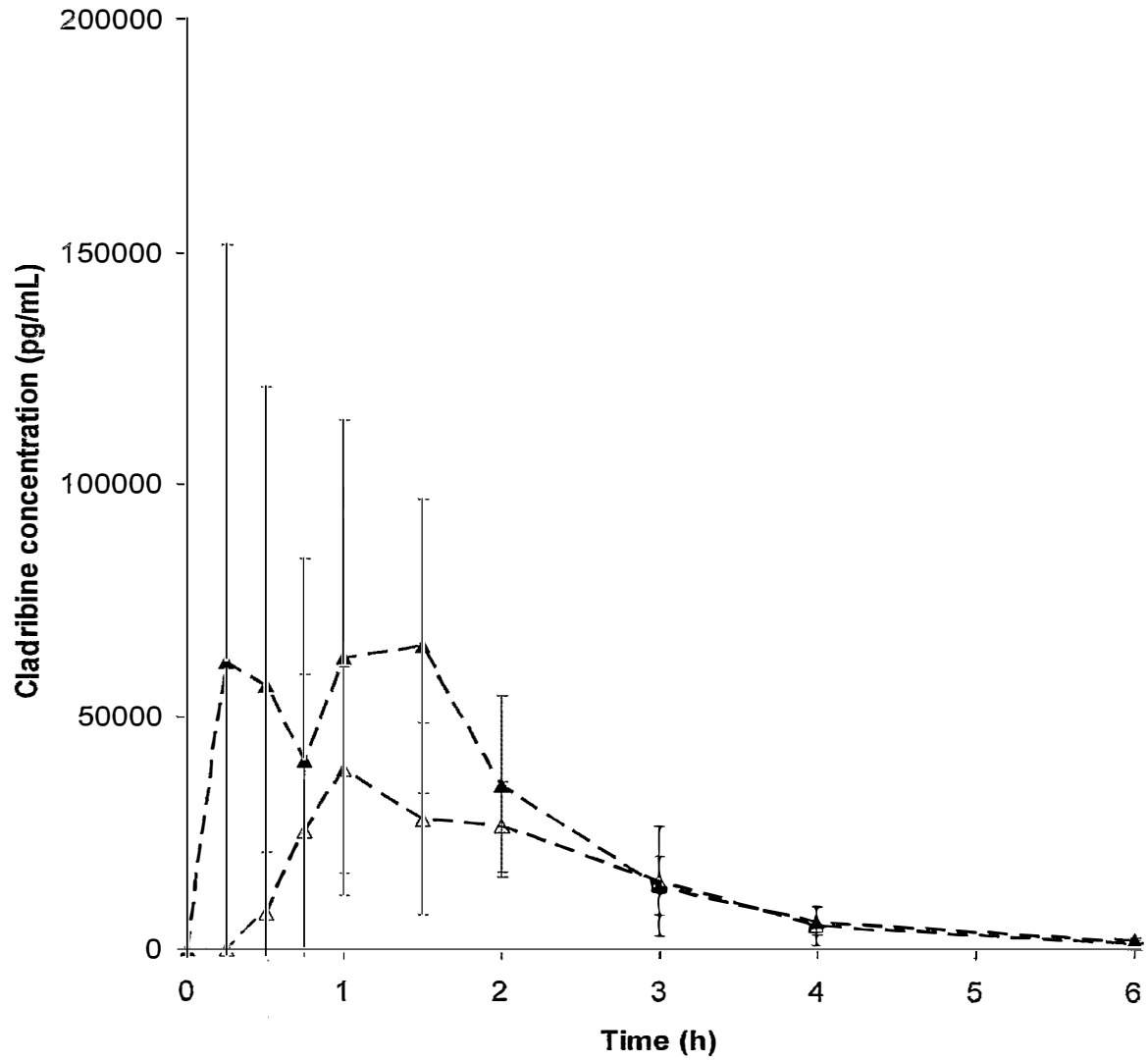


Fig. 5

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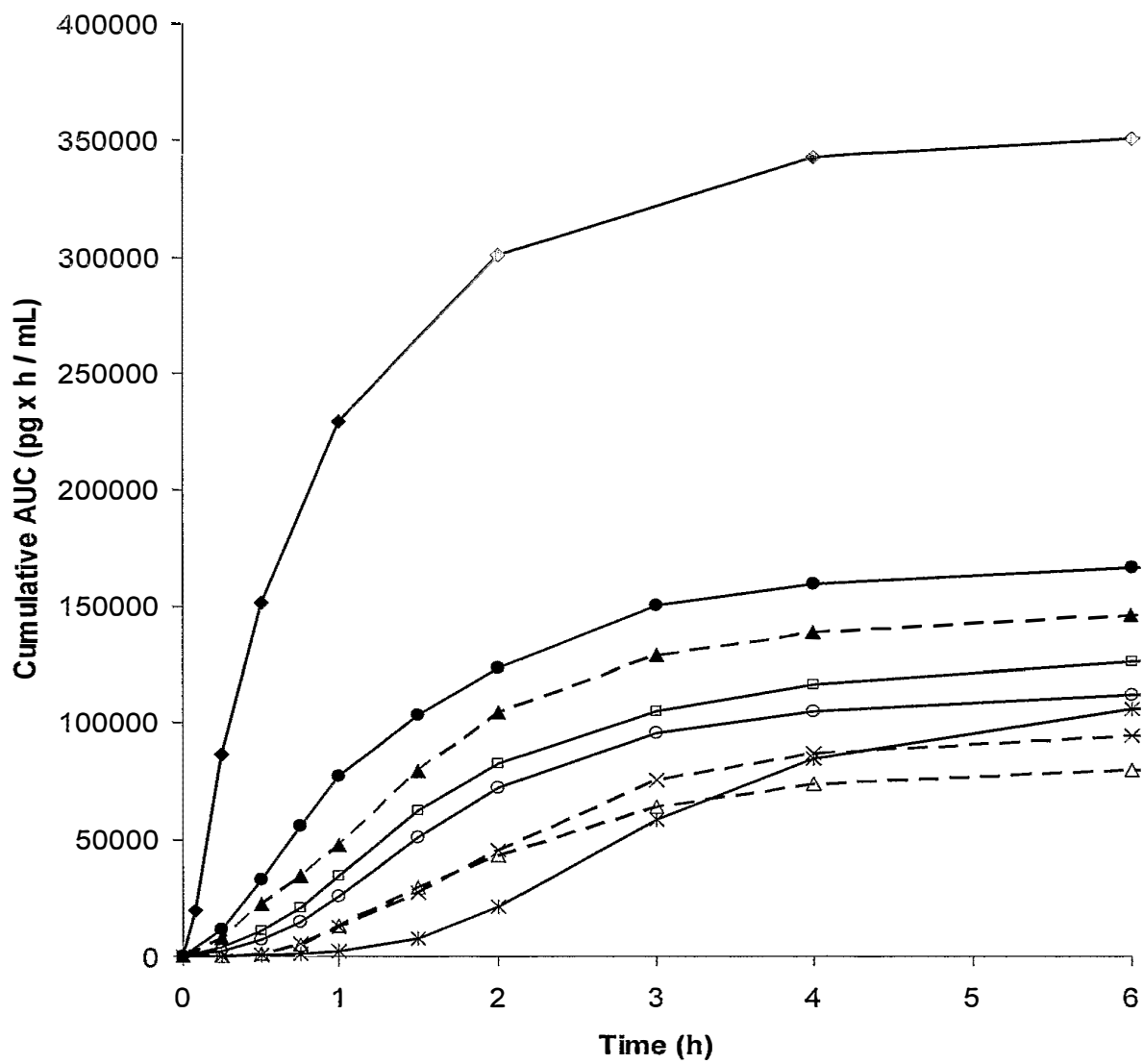


Fig. 6

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54

Method for the production of 2'-deoxyadenosine compounds.

57

A method that is direct and stereospecific is provided for the production of 2'-deoxyadenosine derivatives and related analogs. The method comprises glycosylation of the sodium salt of 2,6-dichloropurine or 6-chloropurine and ammonolysis of the glycosylate to obtain the corresponding 2-chloro-2'-deoxyadenosine or 2'-deoxyadenosine.

EP 0 173 059 A2

Description

Method for the Production of
2'-Deoxyadenosine Compounds

Technical Field

5 This invention relates to a method for the production of 2'-deoxyadenosine compounds and related analogs, comprising a direct stereospecific glycosylation of a purine aglycon.

Background of the Invention

10 Prior glycosylation procedures in which the 2-deoxy- β -D-ribofuranosyl (2-deoxy- β -D-erythro-pentofuranosyl moiety is introduced into an aglycon invariably provide anomeric mixtures as well as positional isomers which result in very low yields of the desired 2'-deoxy-

15 nucleoside. In view of these difficulties, a four-step deoxygenation procedure using phenoxythiocarbonylation (J.A.C.S. 1983, 105, 4059) or imidazolylthiocarbonylation (J. Org. Chem. 1982, 47, 485; Chem. Pharm. Bull. 1983, 31, 1842) of the 2'-

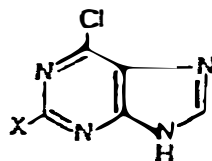
20 hydroxy group of the corresponding 3',5'-protected β -D-ribonucleoside has been developed to provide the requisite 2'-deoxynucleoside. What the art lacks, however, are improved procedures that do not require the

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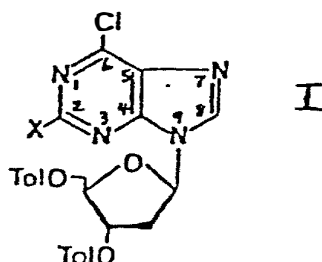
availability of the preformed ribonucleoside and also are applicable in the presence of haloheterocyclic derivatives, which are the most useful for further nucleophilic displacement.

5 Summary and Detailed Description

The present invention, which overcomes the difficulties and shortcomings of the prior art, concerns a method of producing 2'-deoxyadenosine compounds, comprising glycosylating a sodium salt of a purine compound having the formula

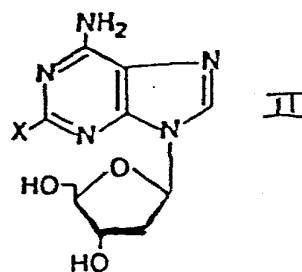


with 1-chloro-2-deoxy-3,5-di-O-p-toluoyl- α -D-erythro-pentofuranose, isolating the resulting 9-(2-deoxy-3,5-di-O-p-toluoyl- β -D-erythro-pentofuranosyl) purine having the formula I



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and subjecting the same to ammonolysis under deblocking conditions to obtain the corresponding 2'-deoxyadenosine having the formula II



where X is hydrogen or Cl.

The glycosylation step of the method is carried out in a polar solvent, preferably acetonitrile, at ambient temperature. The sodium salt of the purine compound preferably is formed in situ by reaction of the purine compound and sodium hydride. The reaction is usually complete within 0.5 hour. The glycosylation is carried out at ambient temperature until complete, usually within about 15 hours. The specificity of the glycosylation step and the high yield produced of the desired 6-chloro-9-glycosylated product were both unexpected. Although a polar solvent was used, which would tend to favor sugar anomerization and thus resulting in approximately equal amounts of the alpha and beta nucleosidic products, the desired β -glycosylation was rapid and preferentially took place without appreciable anomerization. Since the starting 1-chloro-2-deoxy-3,5-di-O-p-toluoyl-D-erythro-