200053263

The Controller of Patents
The Patent Office
Mumbai

14021
E-105/68/2015

June 29, 2015

Dear Sir,

Re: **Opposition under Section 25(1) against-**
Patent Application No: 568/MUMNP/2011 dated July 20, 2001
Entitled: "Prodrugs of phosphonate nucleotide analogues and methods for selecting and making same"
Applicant: GILEAD SCIENCES, INC
Opponent: Amrita Majumdar
Our Ref: PII-0577

In connection with the aforesaid Patent Application I submit herewith the following documents:

1. Representation for Opposition to Grant Of Patent under section 25 (1) on Form-7A (in duplicate);
2. Annexure A, Annexure B, Annexure C, Annexure D, Annexure E, Annexure F, Annexure G, Annexure H, Annexure I, Annexure J, Annexure K, Exhibit 1, Exhibit 2, Exhibit 3, Exhibit 4, Exhibit 5, Exhibit 6, Exhibit 7, Exhibit 8, Exhibit 9, Exhibit 10, Exhibit 11. (in duplicate);

Please grant a hearing in due course.

I request you to kindly take the opposition on record under intimation to us.

Yours faithfully,

Amrita Majumdar

Amrita Majumdar

Encl.: a/a.

भारत सरकार, पेटेन्ट कार्यालय मुंबई
30 JUN 2015
GOVT. OF INDIA, PATENT OFFICE MUMBAI

IPO MUMBAI 30-06-2015 17:44

Index

Sr No.	Particulars	Exhibit/ Annexure	Page no.
	VOLUME 1		
1	Form 7A	-----	-----
2	Representation under Section 25(1)	-----	1-35
3	As filed complete specification and claims of 568/MUMNP/2011	Annexure A	36-95
4	Amended claims of 568/MUMNP/2011	Annexure B	96-97
5	Granted status of 9/MUMNP/2003	Annexure C	98
6	As filed claims of 9/MUMNP/2003	Annexure D	99-105
7	Granted claims of 9/MUMNP/2003	Annexure E	106-107
8	Refusal of patent application no:530/MUMNP/2006	Annexure F	108-144
9	First Examination Report of 568/MUMNP/2011	Annexure G	145-147
10	Applicant's response dated October 17, 2014 to First Examination Report of 568/MUMNP/2011	Annexure H	148-150
11	First Examination Report of 9/MUMNP/2003	Annexure I	151-154
12	Applicant's response First Examination Report of 9/MUMNP/2003	Annexure J	155-158
13	As filed claims of 530/MUMNP/2006	Annexure K	159
14	WO9804569 entitled "Nucleotide analogs" published on February 5, 1998	Exhibit 1/D1	160-233
	VOLUME 2		
15	WO9904774 entitled "Nucleotide analog compositions" published on February 4, 1999	Exhibit 2/D2	234-327
16	US5476938 entitled "Process for the preparation of nucleotides" published on December 19, 1995	Exhibit 3/D3	328-337
17	"Practical Synthesis of the anti-HIV drug, PMPA" by Lisa M. Schultze, published in Tetrahedron Letters 39 (1998) 1853-1856	Exhibit 4/D4	338-341
18	"Process Optimization in the synthesis of 9-[2-Diethyl phosphnomethoxy)ethyl]adenine: Replacement of Sodium Hydride with Sodium Tert-butoxide as the base for Oxygen alkylation" by Richard H. Yu, published in Organic Process Research & Development 1999, 3, 53-55	Exhibit 5/D5	342-344
19	"Standard Molar Enthalpies of formation of Mg and Ca Alkoxides" by Teresa Barreira and Joao Paulo published in Eur. J. Inorg. Chem. 2000, 987-991	Exhibit 6/D6	345-349
20	US5739314 entitled "Method of synthesis of 2'-O-Substituted Pyrimidine Nucleosides and Oligonucleotides" published on April 14, 1998	Exhibit 7/D7	350-353

21	"Ether synthesis using trifluoromethanesulfonic anhydride or triflates under mild reaction conditions" by Tomihiro Nishiyama, Hideaki Kame yama, Hideki Maekawa and Kouhei Watanuki published in Can. J. Chem. 77: 258-262 (1999).	Exhibit 8/D8	354-358
22	"Analytical Chiral Separation Methods" published in Pure & Appl. Chem. Vol. 69, No. 7, pp. 1469-1474, 1997	Exhibit 9/D9	359-364
23	"Chiral Separations Introduction 1.1 Importance" By Mey Cabusas	Exhibit10/D10	365-372
24	"Comparison of Batch elution and continuous simulated moving bed Chromatography" by Jochen Strube, Sonja Haumreisser, Henner Schimdt-Traub, Michael Schulte and Reinhard Ditz in Org. Proc. Res. Dev., 1998, 2(5), pp 305-319 dated July 18, 1998	Exhibit11/D11	373-387

Representation under Section 25(1) to Application no: 568/MUMNP/2011

VOLUME 1

Pages 1-233

FORM – 7A

THE PATENTS ACT, 1970

(39 OF 1970)

&

THE PATENTS RULES, 2003

REPRESENTATION FOR OPPOSITION TO GRANT OF PATENT

(See section 25 (1) and rule 55)

I, Amrita Majumdar based at 202, Elecon Chambers, Behind Saki Naka Tel. Ex., Off Kurla-Andheri Road, Saki Naka, Mumbai – 400 072, India hereby give representation by way of opposition to the grant of patent in respect of Patent Application No. **568/MUMNP/2011** dated July 20, 2001 (Divisional filed on March 23, 2011) by GILEAD SCIENCES, INC., of 333 Lakeside Drive, Foster City, CA 94404, United States of America. It is published under section 11A in the Special Issue of the Official Journal of Indian Patent Office dated 06/06/2014 and the publication date therein is mentioned as 02/12/2011.

The impugned Patent Application is opposed on the following grounds:-

a. **U/S 25 (1) (e):** that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published as mentioned in section 25 (1) (b) or having regard to what was used in India before the priority date of the applicant's claim;

b. **U/S 25 (1) (f):** that the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act;

My address for service in India is

202, Elecon Chambers, Behind Saki Naka Tel. Ex., Off Kurla-Andheri Road, Saki Naka, Mumbai – 400 072, India, Phone: 0-22-28522901; Fax: 0-22-28522903.

Email: amrita.majumdar@majumdarip.com.

Dated this the 29th day of June, 2015

Amrita Majumdar

To

The Controller of Patents
The Patent Office,
At Mumbai

BEFORE THE CONTROLLER OF PATENTS,

Mumbai

In the matter of section 25(1) of The Patents Act, 1970 *as amended by* The Patents (Amendment) Act 2005;

And

In the matter of The Patents (Amendment) Rules, 2006;

And

IN THE MATTER of Patents Application 568/MUMNP/2011 dated July 20, 2001 made by GILEAD SCIENCES, INC

...Applicant

And

IN THE MATTER of opposition of the grant of a patent thereto by Amrita Majumdar;

.....Opponent

REPRESENTATION UNDER SECTION 25(1)

I, Amrita Majumdar, C/o S. MAJUMDAR & CO., 202 Elecon Chambers, Behind Saki Naka Tel. Ex., Off Kurla-Andheri Road, Saki Naka, Mumbai – 400 072, India, (hereinafter called 'Opponent') make the following representation under Section 25(1) of the Act in opposing the grant of patent on the application indicated in the cause title.

1. **LOCUS STANDI**

Locus standi is not a condition precedent for an opposition under Section 25(1).

2. **GROUNDS OF OPPOSITION**

2.1 The application is opposed on the following grounds:

Section 25(1) (e)-Obviousness/lack of inventive step

that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published as mentioned in Section 25(1) (b) or having regard to what was used in India before the priority date of the applicant's claim;

Section 25(1) (f)-Not an invention

that the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act;

2.2 The opponent craves leave to alter, modify, add or delete the grounds in the course of the present proceedings.

3. **PRELIMINARY ANALYSIS AND SUBMISSIONS**

3.1 Patent Application No: 568/MUMNP/2011 entitled "Prodrugs of phosphonate nucleotide analogues and methods for selecting and making same" dated March 23, 2011. The IPAIRS site indicates that it has been published under section 11A in the Official Journal of Indian Patent Office dated December 2, 2011.

3.2 The impugned application claims an earliest priority of US dated 21 July, 2000. The impugned application earlier contained a set of 20 claims. The Claims of the impugned application have been amended on October 17, 2014. However, at

present there are only 7 claims which relate to method of making PMPA/PMEA and process for obtaining GS-7340. The as filed claims along with complete specification and the amended claims are attached herewith as Annexure A and Annexure B.

3.3 The impugned application is divisional to application no. 530/MUMNP/2006 dated May 9, 2006 which is in turn is divisional to application no: 9/MUMNP/2003 dated January 2, 2003 which has been granted as IN208435 and notified in the Official Journal of Indian Patent Office dated August 31, 2007. IN208435 claims a screening method for identifying a methoxy phosphonate nucleotide analogue prodrug conferring enhanced activity in a target tissue with antiviral or antitumour activity. The granted status, as filed claims and granted claims of IN208435 as downloaded from the IPAIRS site are attached herewith as Annexure C, Annexure D and Annexure E. 3 divisional applications have been filed for the parent application 9/MUMNP/2003 namely 529/MUMNP/2006 entitled "Methoxy Phosphonate Nucleotide Prodrug" which has been granted as IN241597, 530/MUMNP/2006 which has been refused grant of patent vide Ld Controller's order dated February 3, 2014 (copy of which is annexed herewith as Annexure F) and 532/MUMNP/2006 (refused under Section 21(1) by the Mumbai Patent Office vide letter no: 7782 dated January 31, 2011).

3.4 The First Examination Report, copy of which is annexed herewith as Annexure G for the impugned application was issued on March 10, 2014. Further continuation of First Examination Report was issued by the Ld Controller vide communication dated May 19, 2014. In view of the First Examination Report, the Applicant has amended the claims on October 17, 2014 while filing the response to the First Examination report, copy of which is annexed herewith as Annexure H and has specifically stated:

To meet the objection of paragraph 6, the claims 1 to 12 and 20 have been deleted.

3.5 Admittedly, the as filed claims 1 to 12 and 20 are in conflict with the claims of parent (main) applications and have been consequently deleted. While filing response to FER on October 17, 2014, the Applicant has provided substantive

arguments specifically with reference to the amended claims. The Opponent seeks leave to rely on additional documents and make additional submissions in case the claims are further amended with an intention to broaden the scope of the amended claims.

Preliminary Submissions

3.6 In the First Examination Report dated May 19, 2014 issued with regards to the impugned application, the Ld. Controller has stated that the claims of the impugned application are not allowable under Sec 16(1) of the Act wherein the Ld Controller has raised the following objections in point no: 5:

> 5. -----. *The further application can be filed at any time before the grant of the patent to the original or first-mentioned application but there is no provision to file the further application to already filed further application. Hence the instant application does not qualify as a further application as per sec 16(1) of the Patents Act, 1970. Further the particulars for filing divisional application provided at paragraph 7 on Form-1 is not correct as the application no: 530/MUMNP/2006 is not the original or first application. In view of the above, further examination of the application is deferred and divisional status of the instant application no. 568/MUMNP/2011 is not allowable.*

The Applicant's in its response dated October 17, 2014 submitted that:

> *Accordingly, under the law, apart from the same date of filing and priority date, the divisional is treated as a separate application---.*
> *Since, a divisional application is a separate independent application, it is not necessary for the grandparent application, i.e. the parent application of the first divisional application, to be still pending. The status of the grandparent application, e.g. the grant or refusal of the grandparent application, should have no influence on the second divisional application. As the second divisional application is divided out from the first divisional application, it is the first divisional application and not the grandparent application which represents the "parent application" of the second divisional application.*
> *Therefore, a further or second divisional application out of a divisional application is a valid divisional application.*

3.7 The Opponent states that divisional of divisional application is not allowed by the Patents Act, 1970 for the below reasons.

Section 16 (3) of the Patents Act states:

The Controller may require such amendment of the complete specification field in pursuance of either the original or the further application as may be necessary to ensure that neither of the said complete specifications includes a claim for any matter claimed in the other.

The Opponent states that the explanation portion specifically indicates that for examination purposes, divisional application should be considered as substantive application for the simple reason that the scope of the inventions claimed therein in both the applications being different, determining patentability of each of the invention would require substantive usage of Patent Office resources. The Opponent further states that if the Applicant's response as provided in the communication dated October 17, 2014 is considered, based on which the Ld Controller just evaluates the claims of the impugned patent application vis-à-vis the claims of the parent application and does not evaluate it against the claims of the grandparent application, than it is likely that same/similar claims as have been rejected/granted in the grandparent application would get granted. In the process the Ld Controller would have to re-evaluate the claims of the grandparent application which may have been objected/refused/granted therein again while evaluating the grandchild (divisional of divisional) application leading to substantial loss of energy/time of the Ld Controller as also leading to situations wherein the same invention is granted in two applications (grandchild application and grandparent application) as also leading to grant of a claim which has been refused in the grandparent application.

The Opponent therefore states that filing of Divisional of Divisional Applications should not be allowed.

3.8 It is imperative to note that the Ld Controller had never raised the plurality of invention objections with regards to the process claims for any of the parent/divisional applications.

3.9 The opponent states that even it is understood that Section 16 (1) provides applicant to file a divisional application if he desires, the proviso therein indicates that such a divisional may be filed only to overcome the objection that the claims of the complete specification relate to more than one invention. It is implied that the Applicant while filing divisional should take utmost care to

identify the different set of inventions claimed by them in the first application and accordingly file multiple divisional applications. Thus the Applicant cannot take the ground at a later date that they were not able to identify the different set of inventions within their own inventions which were claimed in the divisional application and hence sought to file another divisional to an existing divisional application. Thus the Applicant's stand at this juncture points to the ignorance of their own application/ invention claimed in their own application.

3.10 In the instant case, the grandparent application (i.e. 9/MUMNP/2003) was notified as granted in the Patent Office Journal dated August 31, 2007 whereas the impugned patent application was filed on March 23, 2011 i.e. almost about 3 and half years after the date of grant of parent application. Since Section 16(1) specifically states that **a divisional application has to be filed before the grant of the parent application**, it is imperative that if the Applicant had filed a divisional after August 2007 starting from application no: 9/MUMNP/2003, than the same would have been barred at its threshold. So the Applicant has sought to file the impugned application in disguise as a divisional of an already filed divisional application.

3.11 In this context further reference is made to the as filed claims of the grandparent application i.e. 9/MUMNP/2003 annexed herewith as Annexure D to check whether the Applicant ever intended to claim any method for manufacture of 9-[2-(phosphonomethoxy) propyl] adenine (PMPA) or 9-[2-(phosphonomethoxy) ethyl] adenine (PMEA) or to claim a process for obtaining GS-7340. The Opponent states that the as filed claims of 9/MUMNP/2003 also do not explicitly claim method for manufacture of 9-[2-(phosphonomethoxy) propyl] adenine (PMPA) or 9-[2-(phosphonomethoxy) ethyl] adenine (PMEA) neither does it claims a process for obtaining GS-7340. Thus the invention claimed in the impugned application never formed a part of the invention to be claimed by the applicant in the first application.

3.12 Even if reference is made to the as filed claim 29 of 9/MUMNP/2003, it is stated that as filed claim 29 refers to a method for use of Magnesium alkoxide, but does not refer to the method of manufacture/process for preparation of PMPA/PMEA

of GS-7340. Further from the FER dated June 16, 2005 and August 12, 2005 for application no: 9/MUMNP/2003 annexed herewith as Annexure I, it may be noted that the Ld Controller had raised an objection that claims 19-26 do not fall within the scope of claim 1, but no such objection has been raised with regards to claim 29-33. Rather the Ld Controller had raised an objection in point no.4 that claims 1-33 lack in novelty and inventive step, subsequent to which the applicant deleted the claims 29-33 and stated in the response dated February 24, 2006 /August 2006 annexed herewith as Annexure J that:

The claims have been thoroughly revised to meet the second to sixth objections.

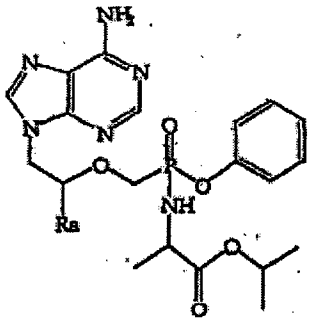
The Opponent therefore states that the grandparent application (9/MUMNP/2003) did not explicitly claim method for manufacture/process for preparation of PMPA/PMEA/GS-7340. Even if Claim 29 is considered to be an implicit reference to method for manufacture/process for preparation of PMPA/PMEA, no plurality objection was raised therein with regard to the said claims (i.e. claims 29-33 of 9/MUMNP/2003). Rather the applicant voluntarily sought to delete the said claims to overcome the novelty/inventive step objections. It is therefore stated that the Applicant has through the impugned application sought to reclaim the claims 29-33 of the grandparent application i.e. 9/MUMNP/2003 in the disguise of divisional of divisional application. Thus the impugned application is liable to be refused for not being a divisional application and hence is not liable to claim the priority of the grandparent application i.e. 9/MUMNP/2003.

3.13 The Opponent further states even though the applicant's arguments that divisional of divisional application is allowed by the Patents Act, 1970 and hence the claims of the impugned patent application ought to be allowed, it is respectfully stated that the claims of the impugned patent application still fail to satisfy the criteria for divisional application as set out in Section 16(1) of the Patents Act, 1970. Section 16(1) is reproduced below for ready reference:

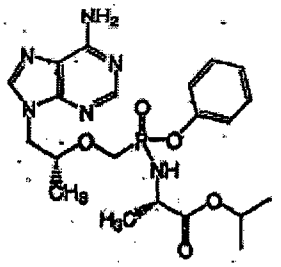
Section 16(1): *A person who has made an application for a patent under this Act may, at any time [before the grant of the patent], if he so desires, or with a view to remedy the objection raised by the Controller on the ground that*

the claims of the complete specification relate to more than one invention, *file a further application in respect of an invention disclosed in the provisional or complete specification already filed in respect of the first-mentioned application.*

The Opponent states that as per section 16 (1) of the Patents Act, it is imperative that the invention which the applicant sought to claim through the divisional application should be essentially claimed in the parent application which as per the applicant's Form 1 and as per the response dated October 17, 2014 is Application no. 530/MUMNP/2006. From the as filed claims of 530/MUMNP/2006 annexed herewith as Annexure K, it is noted that the Applicant in Application no: 530/MUMNP/2006 did not claim any method for manufacture of 9-[2-(phosphonomethoxy) propyl] adenine (PMPA) or 9-[2-(phosphonomethoxy) ethyl] adenine (PMEA) neither did the Applicant claim a process for obtaining GS-7340, rather the as filed claims indicate that the Applicant claimed a compound of structure (1) and structure (2) which is as provided below:



(1)



(2)

The Opponent states that if application no: 530/MUMNP/2006 is to be taken as Parent Application, than the Applicant is barred from claiming any invention which was not originally claimed therein and hence the Applicant is barred from claiming the method/process for preparation of any compound in a further divisional application. The Opponent therefore states that since the impugned patent application specifically claims such method/process claims which were never a part of the claims of the parent application (530/MUMNP/2006), the impugned patent application cannot be termed as a divisional application to

application no: 530/MUMNP/2006 under Section 16 (1) of the Patents Act and hence should be refused.

3.14 In view of the above submissions, the Opponent states that the impugned patent application should be refused at its threshold for failing to satisfy the criteria of divisional application as stated in Section 16 of the Patents Act.

3.15 It is further stated that three divisional applications for a parent application, followed by a further divisional application to one of the divisional application clearly indicates the malafide intention of the applicant to enlarge the scope of the claims by introducing new claim/new subject matter into the claims based on the complete specification. It is stated that the applicant has tried to misguide the Ld. Controller and the public at large. In all likelihood the wrongful act may not have come to the notice of the Ld. Controller at all in the absence of the present opposition. It is stated that this is an act of malice by the applicant. Such an action besides being wholly contrary to law is an act of fraud commissioned against the Government of India and the public of India as well with the wrongful intention of creating a monopoly for the alleged invention for which no patent can be granted in India since it is obvious and lacks inventive merit and falls under the mischief of section 3(d) of the Patents Act. Therefore it is stated that the impugned application merits refusal in limine without any further consideration of the grounds of opposition on which the present opposition is based.

3.16 The opponent further states that the process for obtaining GS-7340 by using Chiralpak AS and through batch elution as is claimed in the impugned application imply that the applicant has just separated the diastereomers via the usual chromatographic techniques.

4 ANALYSIS OF THE APPLICANT'S SPECIFICATION

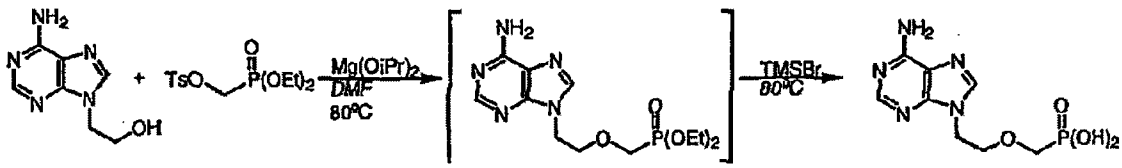
4.1 Page 2 of the specification discusses about the prodrugs of methoxyphosphonate nucleotide and its use in general and specifically states that the objective of the invention is to provide a screening method for identifying a methoxy phosphonate nucleotide analogue prodrug. Page 6 states that the preferred

embodiment of this invention is the compound of structure (6) which has been designated as GS-7340.

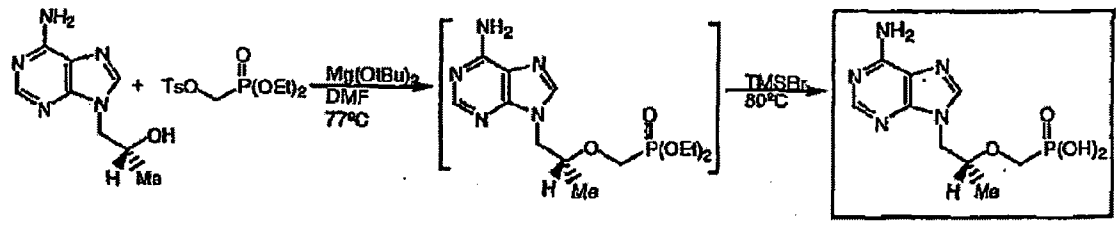
4.2 Page 7 states that a further embodiment relates to facile manufacture of PMPA/PMEA using Magnesium alkoxide.

4.3 Page 15 provides for the preparation of starting materials or intermediates. Line 35 **further states that the alkyl group of the magnesium alkoxide is not critical** and can be any C1-C6 branched or normal alkyl, but is preferably t-butyl (for PMPA) or isopropyl (for PMEA). Page 16 further indicates that **the reaction conditions are not critical,** but preferably comprise heating the reaction mixture at about 70-75°C **with stirring or other moderate agitation.**

4.4 Page 23 provides for preparation of PMEA in the presence of DMF at about 80°C and further discloses essential parameters for the preparation of PMEA and is represented as below:



4.5 Page 24-25 provides for preparation of PMPA in the presence of DMF at about 80°C and further discloses essential parameters for the preparation of PMPA and is represented as below:



4.6 Pages 26-33 provides for the preparation of GS7171 and the diastereomer separation of GS7340 from GS7171 by Batch elution chromatography, SMB chromatography, C18 RP-HPLC and by Crystallization, thereby indicating that various techniques are available for separation of diastereomers and the Applicant has routinely employed those techniques to identify which of those techniques would provide the best results. The Opponent states that identifying

of such techniques through routine experimentation is obvious to a skilled person and do not involve any technical ingenuity.

5. **Analysis of Amended claims**

The Opponent states that there are 4 independent claims and 3 dependent claims.

- Amended claim 1 refers to method for manufacture of PMPA and PMEA by reacting HPA/HEA, magnesium alkoxide and protected p-toluene sulfonylmethylphosphonate.
- Amended claim 2 is dependent on claim 1 and indicates that p-toluene sulfonylmethylphosphonate is protected by ethyl ester;
- Amended claim 3 is dependent on claim 1 and indicates the alkoxide to be selected from C1-C6 alkoxide.
- Amended claim 4 is dependent on claim 3 and states that the alkoxide is t-butyl or isopropyl oxide.
- Amended claim 5 refers to process for obtaining GS-7340 through batch elution chromatography.
- Amended claim 6 refers to process for obtaining GS-7340 by contacting with Chiralpak AS.
- Amended claim 7 refers to separation of diastereomers GS-7171 by batch elution chromatography.

6. **PRIOR ART RELIED ON IN THE PRESENT OPPOSITION**

- **Exhibit 1: WO9804569** entitled **"Nucleotide analogs"** published on February 5, 1998 (Indian equivalent is 2076/DEL/1997 which has been refused u/s 25(1)).
- **Exhibit 2: WO9904774** entitled "Nucleotide analog compositions" published on February 4, 1999.
- **Exhibit 3: US5476938** entitled "Process for the preparation of nucleotides" published on December 19, 1995.
- **Exhibit 4:** "Practical Synthesis of the anti-HIV drug, PMPA" by Lisa M. Schultze, published in Tetrahedron Letters 39 (1998) 1853-1856.
- **Exhibit 5:** "Process Optimization in the synthesis of 9-[2-Diethyl phosphnomethoxy)ethyl]adenine: Replacement of Sodium Hydride with

Sodium Tert-butoxide as the base for Oxygen alkylation" by Richard H. Yu, published in Organic Process Research & Development 1999, 3, 53-55.

- **Exhibit 6:** "Standard Molar Enthalpies of formation of Mg and Ca Alkoxides" by Teresa Barreira and Joao Paulo published in Eur. J. Inorg. Chem. 2000, 987-991.
- **Exhibit 7: US5739314** entitled "Method of synthesis of 2'-O-Substituted Pyrimidine Nucleosides and Oligonucleotides" published on April 14, 1998.
- **Exhibit 8:** "Ether synthesis using trifluoromethanesulfonic anhydride or triflates under mild reaction conditions" by Tomihiro Nishiyama, Hideaki Kame yama, Hideki Maekawa and Kouhei Watanuki published in Can. J. Chem. 77: 258-262 (1999).
- **Exhibit 9:** "Analytical Chiral Separation Methods" published in Pure & Appl. Chem. Vol. 69, No. 7, pp. 1469-1474, 1997.
- **Exhibit 10:**"Chiral Separations Introduction 1.1 Importance." By Mey Cabusas
- **Exhibit 11:** "Comparison of Batch elution and continuous simulated moving bed Chromatography" by Jochen Strube, Sonja Haumreisser, Henner Schimdt-Traub, Michael Schulte and Reinhard Ditz in Org. Proc. Res. Dev., 1998, 2(5), pp 305-319 dated July 18, 1998.

7. OBVIOUSNESS AND LACK OF INVENTIVE STEP [Section 25(1)(e)]:

7.1 The opponent states the process claimed in the impugned application is not anticipated as a whole in any document published prior to the priority date but is however obvious and devoid of inventive merit for the reasons provided hereinafter.

7.2 Under section 2(1) (ja) of the Patents Act,

> *"inventive step" means a feature of an invention that involves technical advances compared to the existing knowledge or having economic significance or both and that makes the invention not obvious to a person skilled in the art.*

7.3 The opponent therefore states that to satisfy the criteria of inventive step, it is necessary that the invention should provide technical advancement over the known prior art processes. The opponent states that mere change of intermediates to known reaction steps so as to get a molecule wherein though the intermediates are different, the actual mode of reaction remains the same and hence does not involve any inventive ingenuity.

7.4 The alleged inventive step of amended claim 1 as per the Applicant is preparation of PMPA/PMEA is as below:

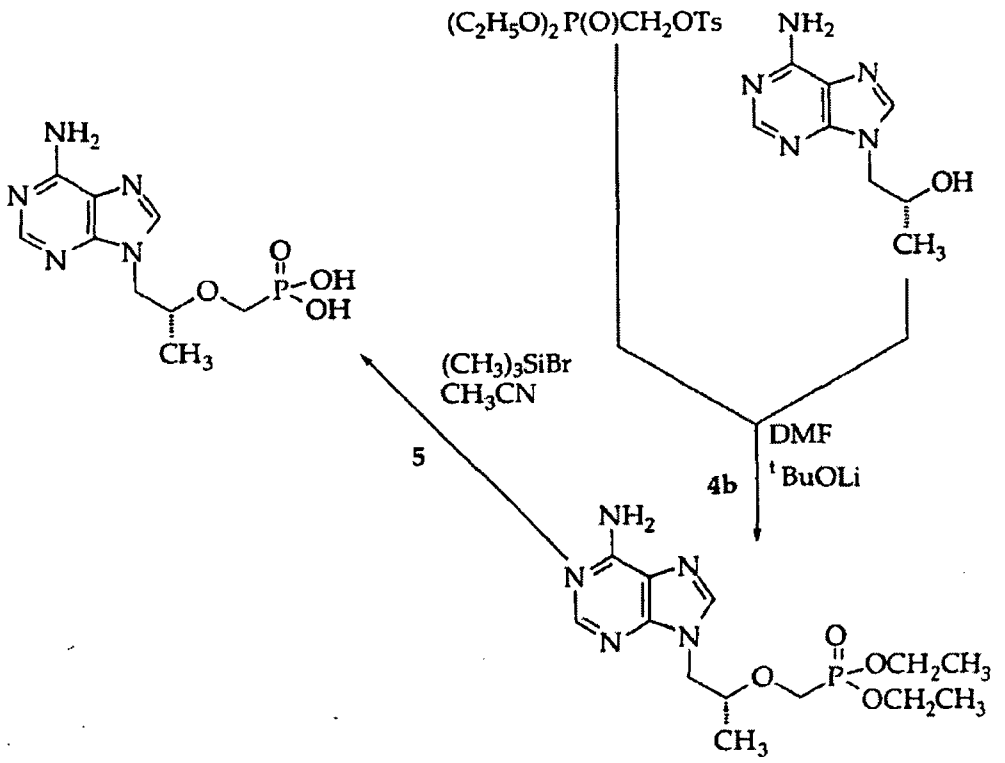
- Reacting HPA/HEA,
- With magnesium alkoxide and
- protected p-toluene sulfonylmethylphosphonate.

From the prior art cited hereinafter it may be noted that the usage of HPA/HEA and protected p-toluene sulfonylmethylphosphonate in the preparation of PMPA/PMEA is well known in prior art. So the alleged inventive step probably lies in the usage of magnesium alkoxide.

7.5 **WO9804569** entitled "Antiviral phosphonomethoxy nucleotide analogs having increased oral bioavailability" is published on February 5, 1998 and annexed hereto as Exhibit 1 and hereinafter referred to as D1. The said application claims the priority of US dated July 26, 1996.

7.6 D1 discloses compounds comprising esters of antiviral phosphonomethoxy nucleotide analogs with carbonates and/or carbamates and which are useful as intermediates for the preparation of antiviral compounds or oligonucleotides, or are useful for administration directly to patients for antiviral therapy or prophylaxis.

7.7 Example 1 of D1 discloses a process for the synthesis of PMPA which is schematically represented as below:



Page 41 of D1 provides the process summary which is as reproduced below:

Process Summary

PMPA is prepared as follows: ------. The carbonate is reacted with adenine and catalytic amounts of a base such as sodium hydroxide to give ***(R)-9-[2-(diethylphosphonomethoxy)propyl]adenine*** *which, without isolation, is* ***reacted with lithium alkoxide (alkyl containing 1, 2, 3, 4, 5 or 6 carbon atoms, e.g., n-hexoxide, n-pentoxide, n-butoxide, i-butoxide, t-butoxide, n-propoxide, i-propoxide, ethoxide, methoxide)*** *and* ***diethyl p-toluenesulfonyloxymethylphosphonate*** *(prepared by reacting diethyl phosphite and paraformaldehyde, and tosylating the product in situ). The resulting* ***(R)-9-[2-diethylphosphonomethoxypropyl]adenine is deesterified with bromotrimethylsilane to give crude PMPA****, which is then purified by precipitation from water with pH adjustment.*

------. The process uses lithium alkoxide at step 4b, which is mildly exothermic on addition to the reaction mixture. The use of a highly reactive base such as NaH, results in an exothermic reaction that generates hydrogen gas in a reaction is difficult to control. The use of NaH thus requires more labor and care to use than lithium alkoxide. Lithium alkoxide bases also give a product that has an improved by-product profile compared to that obtained using NaH, e.g., lower amounts of starting

material or overalkylated products usually result from the use of lithium alkoxide.

The scale of the following method is proportionately reduced or increased if desired. The scheme and process steps depict synthesis of (R)-PMPA.

Step 4 and 5 in pages 44-46 state:

Step 4. (R)-9-[2-(Diethylphosphonomethoxy)propyl]adenine

----. The resulting mixture is cooled to about 25°C, typically about 20-30°C, and contains the stage I intermediate, ***(R)-9-(2-hydroxypropyl)adenine,*** *which may precipitate out at this point. After cooling,* ***lithium t-butoxide*** *(3.62 kg), 2.0 M in tetrahydrofuran is added to the stage I intermediate, to produce the lithium salt of (R)-9-(2-hydroxypropyl)adenine in a mildly exothermic addition. The slurry is treated with* ***diethyl p-toluenesulfonyloxymethylphosphonate*** *(1.19 kg) and the mixture is heated to a temperature of about 32°C, typically about 30-45°C, and is stirred for at least about 2 hours (typically about 2-3 hours) during which time the mixture becomes homogeneous. More diethyl p-toluenesulfonyloxymethylphosphonate (1.43 kg) is added and the mixture is stirred at a temperature of about 32 °C (typically about 30-45°C) for at least about 2 hours (typically about 2-3 hours). Additional lithium t-butoxide (0.66 kg), 2.0 M in tetrahydrofuran and diethyl p-toluenesulfonyloxymethylphosphonate (0.48 kg) are added twice more, each time followed by stirring the mixture, which is at a temperature of about 32°C for at least about 2 hours. Reaction completion is optionally monitored by area normalized HPLC showing no more than about 10% of stage I intermediate remaining. If the reaction is incomplete, additional lithium t-butoxide (0.33 kg), 2.0 M in tetrahydrofuran and diethyl p-toluenesulfonyloxymethylphosphonate (0.24 kg) are added and the reaction mixture is maintained at a temperature of about 32°C for at least about 2 hours to achieve reaction completion. The mixture is then cooled to about 25°C (typically about 20-40°C) and glacial acetic acid (0.5 kg) is then added. The resulting mixture is concentrated in vacuo at a final maximum mixture temperature of about 80°C under about 29 in Hg vacuum. The residue is cooled to about 50°C (typically about 40-60°C) and water (1.8 kg) is added and the reaction is rinsed forward with additional water (1.8 kg). The solution is continuously extracted with dichloromethane (about 35 kg) for 12-48 hours with periodic additions of glacial acetic acid (0.2 kg) to the aqueous phase after about 5 hours and after about 10 hours of continuous extraction time. Extraction completion is optionally conformed by area normalized HPLC as shown by no more than about 7% of (R)-9-[2-(diethylphosphonomethoxy)propyl]adenine remaining in the aqueous*

phase. The combined dichloromethane extracts are concentrated initially at atmospheric pressure then in vacuo at an extract temperature of no more than about 80°C to give the title compound as a viscous orange oil. The title compound yield is about 40-45% by weight normalized HPLC and its purity is typically 60-65% by area normalized HPLC. The actual weight of the title compound after concentration is approximately 1.6 times the theoretical weight (or 3.8 times the expected yield). The additional observed weight is attributed to impurities and/or solvents remaining after the continuous extraction and concentration.

Step 5. (R)-9-[2-(Phosphonomethoxy)propyl]adenine, Crude

Bromotrimethylsilane *(1.56 kg) is added to a reactor containing a mixture of crude (R)-9-[2-(diethylphosphonomethoxy)propyl]adenine (1.0 kg calculated based on adenine input from step 4 above) and acetonitrile (0.9 kg) with cooling to maintain a temperature no higher than about 50°C. The lines are rinsed forward with acetonitrile (0.3 kg) and the mixture is refluxed at about 60-75°C for about 2-4 hours with moderate agitation to avoid splashing the reactor contents. Reaction completion is monitored by area normalized HPLC showing no more than about 3% total of monoethyl PMPA and diethyl PMPA remaining. If the reaction is incomplete, additional bromotrimethylsilane (0.04 kg) is charged into the reactor and the reaction is refluxed for at least about 1 hour with moderate agitation. The volatiles are removed by distillation at no higher than about 70°C initially at atmospheric pressure and then in vacuo (about 24-27 in Hg) at no higher than about 70°C The reactor is then cooled to about 20°C (typically about 15-25°C) and water (1.9 kg) is added (exothermic addition) to the residue with the temperature maintained at no higher than about 50°C. The mixture is cooled to 20°C and washed with dichloromethane (1.7 kg) by agitating for about 30 minutes. The isolated aqueous phase is then filtered through a 1 .mu.m cartridge filter, diluted with water (3.2 kg), heated to about 40°C (typically about 35-50°C) and adjusted to pH about 1.1 (typically about 0.9-1.3) with aqueous sodium hydroxide about 0.15 kg NaOH as a 50% solution) while the temperature is maintained at about 45°C. PMPA seed crystals are added to the mixture and the pH is adjusted to about 2.8 (typically about 2.6-3.0) with a 50% aqueous sodium hydroxide solution (about 0.15 kg NaOH required) while the temperature is maintained at about 45°C (typically about 35-50°C). The solution is cooled to about 22°C (typically about 15-25°C) over about 3-20 hours with slow to moderate agitation that avoids splashing the contents, during which time the product should precipitate, beginning at about 35°C The pH of the slurry is adjusted to about 3.2 (typically about*

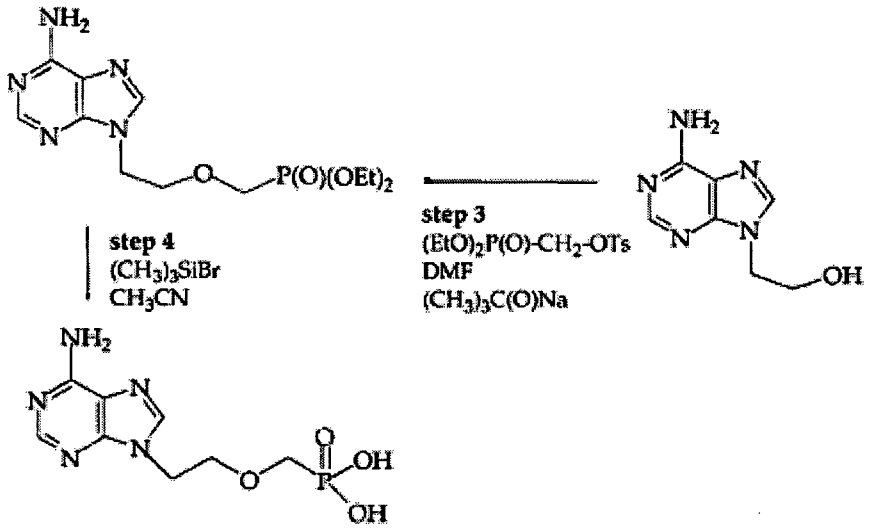
3.1-3.3), usually using 50% aqueous sodium hydroxide or concentrated hydrochloric acid, if necessary. The slurry is cooled to approximately 5°C, typically about 0-10°C, and slowly agitated for at least about 3 hours in that temperature range. The solids are collected by filtration, washed sequentially with cold water (0.35 kg) and acetone (0.3 kg) giving ***crude PMPA as a damp solid typically of about 97% purity.*** *The product is heated to about 50°C and dried in vacuo to a water content of less than 10%. The quantity of diethyl PMPA is calculated from the quantity of adenine used in the preceding step of the synthesis (assuming 100% yield) and not from the net weight of the crude diethyl PMPA, which may contain other compounds.*

The Opponent therefore states that D1 discloses in detail complete process steps inclusive of reaction parameters and using the same reactants for the preparation of PMPA as claimed in claim of the impugned patent application i.e. HPA/protected p-toluenesulfonylmoxymethylphosphonate (protected group being diethyl) and bromotrimethylsilane for the preparation of PMPA, the only exception being the usage of Lithium alkoxide instead of Magnesium alkoxide.

7.8 **WO9904774** entitled "Nucleotide analog compositions" is published on February 4, 1999 and annexed hereto as Exhibit 2 and hereinafter referred to as D2. The said application claims the priority of US dated July 25, 1997.

7.9 D2 relates to the nucleotide analog 9-[2[[bis [(pivaloyloxy)-methoxy] phosphinylmethoxyjethyl] adenine ("adefovir dipivoxil" or "AD") and to its use. D2 further discloses methods to synthesize AD and herby discloses method for the preparation of PMEA.

7.10 Page 19 Diagram A of D2 provides schematic representation for preparation of PMEA and the same is reproduced below:



Page 23 discloses in detail process for preparation of PMEA which is as reproduced below:

Methods for 9-[2-(Diethylphosphonomethoxy)ethyl]adenine Synthesis

This compound is prepared using a composition ***comprising sodium alkoxide (C1.6 alkyl) and 9-(2-hydroxyethyl)adenine****. One contacts* ***sodium alkoxide****, typically* ***sodium t-butoxide or sodium i-propoxide****, with* ***9-(2-hydroxyethyl)adenine in a solvent such as DMF****, at a temperature of about 20-30° over about 1-4 hours. The synthesis typically gives good results with 1 molar equivalent of 9-(2-hydroxyethyl)adenine and about 1.2-2.2 molar equivalents of sodium alkoxide.*

In an embodiment, ***synthesis of 9-[2-(diethylphosphonomethoxy)ethyl] adenine, shown in Diagram A, Step 3****, is described as follows. In a reactor having an inert atmosphere, e.g., nitrogen, a slurry of* ***9-(2hydroxyethyl)adenine*** *(1.0 kg) and* ***DMF*** *(4.79 kg) is warmed to about 130 (125-135°) for 30-60 minutes. The reactor contents are rapidly cooled with vigorous agitation to about 25 (20-30°) and* ***sodium tert-butoxide*** *(0.939 kg) is added in portions over about 1-3 hours while maintaining vigorous agitation and the contents temperature at about 25 (20-30°). The agitation and temperature is maintained for about 15-45 minutes after all sodium tert-butoxide has been added. Then the reactor contents are cooled to about -10 (-13 to 0°) and a solution of* ***diethyl ptoluenesulfonyloxymethyl-phosphonate*** *(2.25 kg on a pure basis) in DMF (1.22 kg) is added over about 5-10 hours. The mixture is kept at about -5° (-10 to 0°) until the reaction is complete, which is typically about*

0.5-2 hours after the final portion of diethyl p-toluenesulfonyloxymethylphosphonate has been added. Reaction completion is monitored by HPLC (not more than 3% 9-(2-hydroxyethyl)adenine remaining). Glacial acetic acid (0.67 kg) is added, with the pot temperature controlled to no more than 20°. The mixture at about 22° (15-25°) is agitated for about 15-45 minutes. The quenched mixture is concentrated in vacuo until distillation stops and the contents are then cooled to below 40°.

Dichloromethane (16.0 kg) is added and the contents at 20° (15-25°) are agitated for at least 1 hour. If the DMF content versus total solids (NaOTs (sodium tosylate), NaOAc, Et2PMEA) is greater than 20% (by 1H NMR) the mixture is concentrated in vacuo until distillation stops, the contents are cooled to below 40°C, dichloromethane (16 kg) is added and the reactor contents at about 20° (15-25°) are agitated for at least 1 hour.

Diatomaceous earth (0.5 kg) is added and the contents, which are at about 20 (15-25°), are agitated for at least 1 hour. The solids are removed by filtration and rinsed 3 times with CH2Cl2 (about 1 kg each). The filtrate and rinses at no more than 80° are concentrated in vacuo until distillation stops, the reactor contents are cooled to below 40°, dichloromethane (5.0 kg) is added to the residue and the contents at about 25° (20-40°) are agitated to dissolve the solids. The resulting solution at no more than 80° is concentrated in vacuo until distillation stops. Dichloromethane (7.0 kg) is added and the contents at about 25° (20-40°) are agitated to dissolve the solids. If the DMF content compared to diethyl PMEA is greater than 12%, the mixture at no more than 80° is concentrated in vacuo, the contents are cooled to below 40°, dichloromethane (7.0 kg) is added and the contents at about 25 (20-40°) are agitated to dissolve the solids. The mixture is washed with water (0.8 kg) at about 25° (22-30°) by agitating for about 15-45 minutes. The phases are allowed to separate for 4 hours and the phases are then separated. The aqueous phase is back-extracted twice with dichloromethane (1.5 kg per wash) by agitation for about 15-45 minutes with the solution maintained at about 25° (22-30°), followed by allowing the phases to separate for at least 2 hours. The combined organics at no more than 80° are then concentrated in vacuo until distillation stops.

Toluene (3.0 kg) is added, agitated at about 25° (22-30°) for about 15-45 minutes and the resulting mixture at no more than 80° is azeotroped in vacuo. Toluene (3.0 kg) is added and the mixture is heated to about 80° (75-85°), agitated for about 15-45 minutes, cooled to below 30° over about 60-90 minutes and then cooled to about 0° (-3 to 6°). After at least 12 hours at about 0° with slow agitation, the resulting slurry is filtered and

the filter cake is rinsed three times with cold (about 0-6°) toluene (about 0.2 kg per rinse). The wet cake is dried in vacuo at about 50° (35 to 65°) and the dried product is milled. Product drying is monitored for water removal (no more than 0.3% water detected by KF titration). The inert atmosphere is maintained throughout step 3.

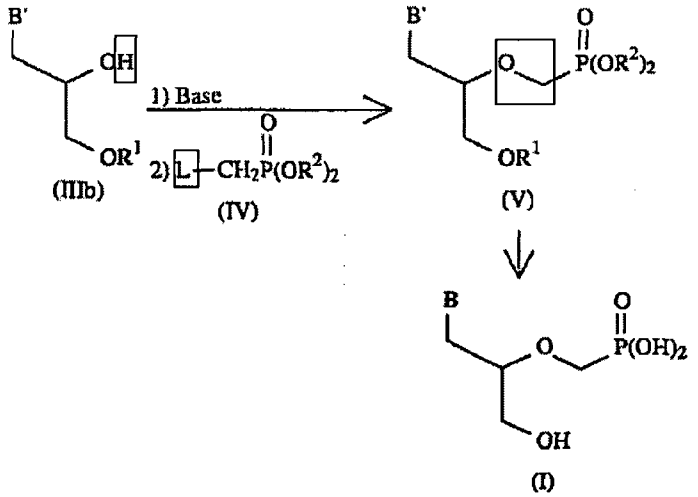
Methods for PMEA Synthesis

In an embodiment, synthesis of PMEA, shown in Diagram A, Step 4, *is described as follows. In a reactor having an inert atmosphere, e.g., nitrogen,* ***a mixture of diethyl PMEA (1.00 kg), acetonitrile (2.00 kg), and bromotrimethylsilane*** *(1.63 kg) is heated to and maintained at reflux for about 1-3 hours with agitation, until the reaction is complete. Reaction completion is monitored by 31p NMR or HPLC (no diethyl PMEA and no more than 2% monoethyl PMEA detected). The solution at ≤ 80°C is distilled in vacuo to a semi-solid, which is taken up in water (2.00 kg) and warmed to about 55°C (52-58°C) for about 30-60 minutes with agitation to dissolve all solids. The resulting mixture is cooled to about 22° C (19-25°C), adjusted to pH 3.2 with aqueous sodium hydroxide, the contents are heated to about 75°C (72-78°C) until the consistency thins (about 15-120 minutes), cooled to about 3°C (0-6°C), and stirred for at least 3 hours (3-6 hours). The slurry is filtered and the filter cake is rinsed with water (1.00 kg). The wet cake is suspended in water (3.75 kg) and the suspension is heated to about 75°C (72-78°C) with vigorous stirring. After stirring for about 2 hours, the slurry is cooled to about 3°C(0-6°C) and stirred for at least another 2 hours. The slurry is filtered and the filter cake is rinsed sequentially with two portions of water (0.50 kg per rinse) and two portions of acetone (1.00 kg per rinse). The isolated solid is dried in vacuo at no more than about 90°C to a low water content (no more than 0.5% water detected by KF titration),* ***to provide PMEA as white crystals.*** *The product is milled to a fine particle size.*

The Opponent therefore states that D2 discloses in detail complete process steps inclusive of reaction parameters and using the same reactants for the preparation of PMEA as claimed in claim of the impugned patent application i.e. HEA in DMF/protected p-toluenesulfonylmoxymethylphosphonate (protected group being diethyl) and bromotrimethylsilane for the preparation of PMEA, the only exception being the usage of Sodium alkoxide instead of Magnesium alkoxide.

7.11 In this context, the Opponent further relies on **US5476938** entitled "Process for the preparation of nucleotides" is published on December 19, 1995 and annexed hereto as Exhibit 3 and hereinafter referred to as D3.

7.12 D3 relates to a novel process for the preparation of hydroxyphosphonylmethoxypropyl nucleosides, and novel intermediates produced therein. Scheme 1 schematically represents O-alkylation reaction between compound of formula III(b) and protected phosphonate moiety in the presence of a base to generate compound of formula V which is than deprotected by treatment with trialkylsilylhalide. Schematic representation of the reaction is reproduced below:



Thus, a compound of formula (IIIb) is first treated with a base to generate the corresponding alkoxide anion. The base may be a metal hydride, for example sodium hydride, potassium hydride or lithium hydride; and metal alkoxides, for example, potassium t-butoxide or sodium methoxide and the like. The reaction mixture containing the alkoxide anion is then treated with the methanephosphonate LCH2 P(O)(OR2)2 (IV) wherein L is a leaving group and R2 is an alkyl group containing 1-5 carbon atoms as previously defined to provide the protected HPMP nucleoside of formula (V). L is preferably selected from the group consisting of p-toluenesulfonate (tosylate), methanesulfonate (mesylate), and trifluoromethanesulfonate (triflate); and R2 is preferably an alkyl group having from 1-3 carbon atoms, e.g., methyl, ethyl, n-propyl, and isopropyl.

The third step of the process involves the removal of the phosphonic protecting group, i.e. R2 the hydroxy protecting group, and if present, any protecting groups on the purine or pyrimidine base. The phosphonate may be converted to the parent acid by treatment with a trialkylsilyl

halide such as trimethylsilyl bromide or trimethylsilyl iodide, and optionally followed by the addition of water.

The Opponent states that though D3 discusses the usage of Sodium hydride as a preferred base, it also provides motivation to skilled person to use other bases such as metal alkoxides for carrying out the O-alkylation reaction between compound of formula III(b) and protected phosphonate moiety.

7.13 An article entitled "Practical Synthesis of the anti-HIV drug, PMPA" by Lisa M. Schultze, published in Tetrahedron Letters 39 (1998) 1853-1856 annexed hereto as Exhibit 4 and hereinafter referred to as D4. Abstract of D4 states:

The anti-HIV nucleotide analogue PMPA can be prepared on a kilogram-scale by a three step sequence:

i) condensation of adenine with (R)-propylene carbonate,

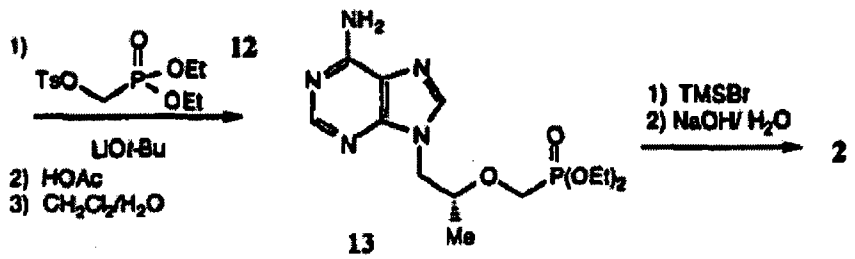
*ii) alkylation of the resulting **(R)-9-(2-hydroxypropy1)adenine** with **diethyl p-toluenesulfonyloxymethanephosphonate** using lithium tert-butoxide and*

iii) cleavage of the phosphonate ester functionalities with bromotrimethylsilane.

Page 1853 states:

The acyclic derivatives of adenosine monophosphate, 9-[2-phosphonomethoxy) ethyl] adenine (PMEA, 1) and (R)-9-[2-(phosphonomethoxy) propyl]adenine (PMPA, 2) have shown potent and selective activity against human immunodeficiency virus (HN) and other retroviruses.

Scheme 3 from D4 is reproduced below for ready reference:



Scheme 3

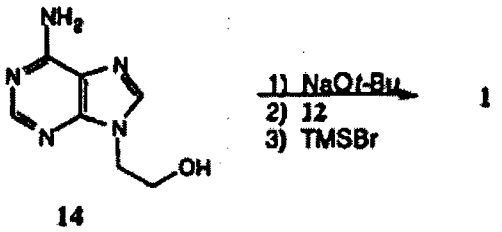
Treatment of a 1 M DMF solution of 8a with lithium tert-butoxide (1.1 eq., 2 M in THF) afforded a thick suspension of lithium salts. Addition of diethyl p-toluenesulfonyloxymethanephosphonate 12 (0.5 eq.) at 30-35°C afforded a homogenous solution (ca 1 h). This was followed by charging additional lithium tertbutoxide (0.4 eq) and 12 (1.0 eq.) to achieve complete reaction. The reaction was quenched with acetic acid and water, and the diethylphosphonate ester 13 was extracted into methylene chloride.

Yields of crude 13 were typically 55-65 % from adenine (by external standard HPLC).

To complete the synthesis of 2, the crude diethyl ester 13 was subjected to excess bromotrimethylsilane (3.5 eq.) in refluxing acetonihile.6.7 After disappearance of mono and diethyl esters by HPLC, the reaction was concentrated, diluted with water and extracted with dichloromethane to remove silylated byproducts. The phosphonic acid product 2 was precipitated by addition of 50% NaOH bringing the aqueous solution to pH 3. The product precipitated slowly over several hours under these dilute conditions (ca 0.57 M) and resulted in enrichment of the chiral purity of the product from 86% ee to > 98% ee.

An alkoxide-alkylation process has also been used to prepare PMEA 1 from HEA 14 (Scheme 4). Optimal results in this case were obtained with sodium tert-butoxide (1.75 eq.) which afforded a homogeneous solution of sodium salts upon mixing with a suspension of 14 in DMF (5 L/kg). Alkylation with tosylate 12 and TMSBr cleavage afforded PMEA 1 in 35-45% overall yield from adenine.

Scheme 4 from D4 is reproduced below for ready reference:



Scheme 4

In summary, an efficient process has been developed to prepare the anti-HIV agent, PMPA. Notable features include a practical preparation of (R)-propylene carbonate, a novel key akylation using lithium tertbutoxide, a simple crystallization process for chiral enrichment and no chromatography. Multi-kilogram batches of PMPA have been prepared by this process in standard pilot plant equipment.

The Opponent states that D4 discloses the preparation of PMPA as well as PMEA by alkylating HPA/HEA with diethyl p-toluenesulfonyloxymethane phosphonate in the presence of alkali alkoxide such as Lithium tert-butoxide and sodium tert-butoxide. Further from the abstract of D4, it may be noted that the steps (ii) and (iii) for preparation of PMPA as disclosed therein is similar to

that claimed in claim 1 of the impugned application except for the use of lithium tert-butoxide.

7.14 An article entitled "Process Optimization in the synthesis of 9-[2-Diethylphosphnomethoxy)ethyl]adenine: Replacement of Sodium Hydride with Sodium Tert-butoxide as the base for Oxygen alkylation" by Richard H. Yu, published in Organic Process Research & Development 1999, 3, 53-55 annexed hereto as Exhibit 5 and hereinafter referred to as D5. Abstract of D5 states:

> *9-12-(Diethylphosphonomethoxy)ethylladenine (diethyl-PMEA), a key intermediate in the production of the antiviral drug adefovir dipivoxil, was originally produced via a process utilizing sodium hydride (NaH) to couple hydroxyethyl adenine with diethyl p-toluenesulfonyloxymethanephosphonate. The use of NaH presented safety and consistency problems. It was found that sodium tert-butoxide (NaO'Bu) was a suitable replacement for NaH as the base to effect the coupling reaction. Optimization of reagent stoichiometry and introduction of a simplified filtration workup procedure led to a robust process affording diethyl-PMEA in consistent yields and purities.*

Page 53 Column 2 and page 54 column 1 disuses the problems associated with the use of sodium hydride for alkylating hydroxyethyl adenine with diethyl p-toluenesulfonyloxymethanephosphonate. Further on page 4 it is stated:

> *The modification of the diethyl-PMEA process **focused on replacing NaH with alternative bases while maintaining a reaction profile similar to that of the NaH process.** Lithium tertbutoxide (LiO'Bu), which previously was found to be effective for the synthesis of the related compound, PMPA, afforded a thick suspension of the lithium salt of 3 in DMF which hindered agitation and reaction progress. In contrast, the related base, sodium tert-butoxide (NaO'Bu), afforded a homogeneous mixture on reaction with 3 in DMF. In some instances, the sodium salt of 3 precipitated out of solution as a viscous resin as the reaction progressed. Counterintuitively, the viscous resin did not dissolve as more solvent was added. It was discovered that, at high concentration (1.5 M), the sodium salt of 3 was soluble, affording a homogeneous solution throughout the coupling reaction, whereas at lower concentration (0.8-1.0 M), the mixture became heterogeneous as the sodium salt of 3 precipitated. Aside from this concentration issue, it was observed in some runs that large clumps of starting 3 did not react with NaO'Bu and thus remained as floating particulates in the reaction mixture. To circumvent this issue, an initial digestion at 125-135°C in DMF, followed by fast*

cooling, fully dissolved and reprecipitated 3 as a fine solid. Once performed, addition of NaO'Bu reliably gave a homogeneous solution.

Table 2. **Comparison of large scale results using NaH and NaO'Bu**

reaction			results			
			purity (HPLC area %)		yield (% theory)	
base used	no. of batches	scale (kg of HEA, 3)	av	range	av	range
NaH	10	5–249	92.6	82.7–96.1	29.0	17.5–43.7
NaO'Bu	4	200	95.0	94.6–95.3	41.0	39.4–43.7

From Table 2 provided on page 5, D5 indicates that the usage of alkali alkoxide as a base leads to substantial increase in yield and purity of the required product.

7.15 The opponent states that from D1 to D5, it is amply clear that a skilled person involved in the preparation of PMPA/PMEA will react HPA/HEA with protected (diethyl) p-toluenesulfonyloxymethanephosphonate in the presence of an alkali alkoxide as a base followed by reacting with TMSBr to obtain PMPA/PMEA with better yield and purity. Thus the only difference in the present invention is the usage of alkaline alkoxide (Mg alkoxide) instead of Alkali alkoxide (Li/Na alkoxide).

7.16 The opponent further states that a skilled person was continuously evaluating the use of different metal alkoxides so as to proceed the said reaction in an efficient and affordable manner. This may be noted form the below fact:

- D3 published in 1995 refers to the preferable use of Sodium hydride for carrying out O-alkylation reaction between an adenine compound bearing a hydroxyl substituent and methane phosphonate moiety bearing a leaving group. It further generically discloses the use of metal alkoxides for carrying out such reactions.
- D4 which was published in 1998, discusses the usage of Lithium tert-butoxide during the reaction of HPA/HEA with protected (diethyl) p-toluenesulfonyloxymethanephosphonate;
- D5 which was published in 1999 discusses the usage of Sodium tert-butoxide during the reaction of HPA/HEA with protected (diethyl) p-

Group 2 metals. In this context, the skilled person being aware of the fact that alkali metal ions and alkaline earth metal ions have similar characteristic would be motivated to check out whether alkaline earth metal alkoxides also exhibits similar properties to those exhibited by alkali metal alkoxides .

7.20 An article entitled "Standard Molar Enthalpies of formation of Mg and Ca Alkoxides" by Teresa Barreira and Joao Paulo published in Eur. J. Inorg. Chem. 2000, 987-991 annexed hereto as Exhibit 6 and hereinafter referred to as D6 in the introduction portion states:

> *Alkaline and Alkaline-earth metal alkoxide compounds are of major importance in synthetic chemistry. However thermochemical data for these substances is rather scarce. Previous results obtained for sodium and lithium alkoxides show that there exists a linear relation between the formation of alkoxides and those of the corresponding alcohols for short-chain unbranched alkoxides. A model based on the alkoxide energy calculations and a simple electrostatic model was also developed, which enabled us to predict unknown metal alkoxide enthalpies of formation.*
> *In this paper, we extend the previously developed model to alkaline-earth alkoxides.*

Further Table 5 provides the below data

Table 5. Thermochemical radii[a] calculated or estimated[b] for the alkoxide ions in alkali and alkaline-earth metal alkoxides[c]

RO^-	$Be(OR)_2$	$LiOR^{[c]}$	$Mg(OR)_2$	$NaOR^{[f]}$	$Ca(OR)_2$	$KOR^{[g]}$	$Sr(OR)_2$	$RbOR^{[g]}$	$Ba(OR)_2$	$CsOR^{[g]}$
HO^-	120.2±1.1[d]	119.8±1.3	130.5±1.2	127.4±1.5	132.2±1.3	121±3	130.4±1.3[d]	118±3	129.7±1.4[d]	116±3
MeO^-	(130±2)	130.1±1.5	**139.8±1.2**	141.8±1.9	**140.6±1.5**	138±4	(140±6)	131±4	(140±6)	131±4
EtO^-	(132±2)	132.4±1.6	**143.4±1.3**	145.2±2.0	**147.5±1.5**	134±4	(136±6)	127±3	(138±6)	129±4
$nBuO^-$	(135±4)	135.0±3.6	(145±5)	144.9±4.8	**151.3±3.4**	144±10			(148±12)	139±10
$tBuO^-$	(146±3)	146.1±2.1	(154±3)	154.2±3.0	(167±8)	160±6			(170±8)	160±6

Thus form table 5, it is understood that the thermo chemical radii for Magnesium tert-butoxide and Sodium tert-butoxide is similar i.e. 154±3. Thus a skilled person in the process of identifying a different metal alkoxide for carrying out the reaction between HPA/HEA and phosphonate moiety will be motivated to carry out routine trials with Magnesium Alkoxide specifically Magnesium tert-butoxide. The Opponent further states that the applicant has also indicated that the preferred Magnesium alkoxide is Magnesium tert-butoxide. The Opponent therefore states that the Applicant in the preparation of PMPA/PMEA has by keeping the other reactants similar to those mentioned in the prior art, carried out routine experimentation with different metal alkoxides and arrived at an

alternative for sodium/lithium alkoxide i.e. Magnesium alkoxide. The Opponent states that such routine experimentation to arrive at the known product does not provide any technical ingenuity to the claimed process, the same being obvious to a skilled person.

7.21 Abstract of **US5739314** entitled "Method of synthesis of 2'-O-Substituted Pyrimidine Nucleosides and Oligonucleotides" published on April 14, 1998 annexed hereto as Exhibit 7 and hereinafter referred to as D7 states:

> *The present invention provides an improved method of synthesizing 2'-O-R substituted pyrimidine mononucleosides.* ***The method comprises reacting an anhydropyrimidine with magnesium alkoxide in the corresponding alcohol at elevated temperatures to directly produce the 2'-O-R substituted pyrimidine nucleoside product.*** *The method advantageously eliminates several steps from prior art methods, thereby reducing the time and cost of synthesis and increasing the yield of final product.*
>
> Column 2 further states:
>
> *The present invention discloses improved methods that provide for faster and more productive synthesis of 2'-O-R substituted pyrimidine nucleosides (wherein R is allyl or C,- C3 alkyl (i. e., methyl, ethyl, or propyl)), the method comprising reacting anhydrouridine or anhydrocytidine* ***with Mg (OR)*** *2 in corresponding alcohols (R-OH) to produce 2'-O-R substituted uridine or 2'-O-R substituted cytidine, respectively.*
>
> ---
>
> *A further advantage of the present method is the shorter time to completion of alkylation reactions. Although methods such as those reported by McGee and Zhai took 4 hours at 1 gram scale, an increase in scale to 10 grams required 3 times longer. In the present invention, the reaction was completed in 5 hours at a scale of from 5 grams to 75 grams.*

The Opponent therefore states that D7 describes the efficient usage of magnesium alkoxide in O-alkylation reaction for better yields and improved productivity.

7.22 An article entitled "Ether synthesis using trifluoromethanesulfonic anhydride or triflates under mild reaction conditions" by Tomihiro Nishiyama, Hideaki Kame yama, Hideki Maekawa and Kouhei Watanuki published in Can. J. Chem. 77: 258-262 (1999) annexed hereto as Exhibit 8 and hereinafter referred to as D8. D8 teaches the advantage of magnesium alkoxide usage for O-alkylation reactions

(like in tenofovir process). Column 2 on page 258 under the introduction portion states:

> *In this paper, we report a simple procedure for the preparation of symmetrical and unsymmetrical ethers by the reaction of magnesium alkoxides with trifluoromethanesulfonic anhydride (Tf2O) or triflates (Scheme 1).*

Further under the heading "Results and discussion" it is stated:

> *The reaction of Tf2O in dry ether with in situ generated magnesium di(benzyl alcholate) proceeded smoothly to yield dibenzyl ether 2a. The reaction was completed n 30 min at 5°C, and an 80% isolate yield of dibenzyl ether could be obtained. ----- With other magnesium alkoxides such as 2-phenylethanol **1b** and hexanol **1c**, the corresponding sym- metrical ethers, diphenethyl ether **2b** and dihexyl ether **2c**, were obtained in high yields. The reactions of magnesium alkoxides with Tf2O were usually carried out at 5°C for 30 min, although in one case, a higher temperature provided better yields. When a mixture of magnesium di(hexanolate) and Tf2O was stirred at 40°C for 1h, 2c was obtained in 90% yield. Similarly, a branched primary magnesium alkoxide gave the symmetrical ether **2d** as a mixture of diastereomers (ratio 1:1) in 60% yield (entry 7).*

The Opponent therefore states that D8 describes the efficient usage of magnesium alkoxide in O-alkylation reaction leading to preparation of ethers with better yields and improved productivity.

7.23 The Opponent states that from D6, D7 and D8 a skilled person will note that magnesium (alkaline earth metal) alkoxides can also be efficiently replaced with sodium (alkaline metal alkoxides) to carry out O-alkylation reaction so as to give better yield. The Opponent therefore states that the prior art cited hereinbefore provide sufficient motivation towards the usage of magnesium alkoxide for the reactions claimed in claim 1.

7.24 The invention claimed in claim 1 for the preparation of PMPA/PMEA differs from the cited prior art (D1 to D5) only with regards to the usage of magnesium alkoxide and D6, D7 and D8 indicates that reactions involved in claim 1 can be effectively carried out in the presence of magnesium alkoxide the Opponent states that the invention claimed in the impugned application is obvious to a skilled person.

7.25 The Opponent states that the impugned patent application fails to identify the problem associated with sodium alkoxide for carrying out the process claimed in claim 1 of the impugned application. The Opponent further states that since the preparation of PMPA/PMEA by reacting HPA/HEA with protected (diethyl) p-toluenesulfonyloxymethanephosphonate was well known in prior art, it was imperative upon the applicant to provide data to show the technical advancement achieved by the applicant by replacing sodium/lithium alkoxide with magnesium alkoxide. Since no such data is provided in the impugned patent application, no technical advancement can be attributed to the claimed invention.

7.26 Therefore, the process for the preparation of PMPA/PMEA as alleged by the applicant in its specification is a known process with the use of a new reagent (base) the usage of which is also obvious from the cited prior art and therefore does not involve any inventive step. Thus, the amended claims 1-4 of the impugned application are bad in law and should be rejected.

7.27 Amended claims 5-7 relate to process for obtaining GS-7340 by separating diastereomers of GS-7171 by subjecting it to batch elution chromatography/ by contacting it with Chiralpak AS. The Opponent states that the prior art cited hereinbefore especially D1, D2, D4 and D5 refer to the separation of stereoisomers through column chromatography.

7.28 Further an article entitled "Analytical Chiral Separation Methods" published in Pure & Appl. Chem. Vol. 69, No. 7, pp. 1469-1474, 1997 annexed hereto as Exhibit 9 and hereinafter referred to as D9 refers to the continuous usage of chromatography for separation of enantiomers/diastereomers as also refers to the common terms used in the said field. Abstract on page 1740 states:

> *In recent years there has been considerable interest in the synthesis and separation of enantiomers of organic compounds especially because of their importance in the biochemistry and pharmaceutical industry. Frequently the* ***methods used for the separations****, for monitoring the progress of an asymmetric synthesis or optical purity of the products* ***are chromatographic with either liquids, gases, or supercritical fluids as the mobile phase.*** *More recently capillary electrophoresis has been added as an analytical chiral separation method.*

These applications have lead to a number of terms and expressions in addition to those commonly used or recently recommended for the chemistry and physical properties of chiral compounds.

Page 1470 further states:

One approach to separate enantiomers, sometimes referred to as indirect enantiomeric resolution, involves the coupling of the enantiomers with an auxiliary chiral reagent to convert them into diastereomers. ***The diastereomers can then be separated by any achiral separation technique.***

Page 1471 further states:

Interaction of the chiral selector of the system with the enantiomers of the solute results in the formation of two labile diastereomers. *These differ in their thermodynamic stability, provided that at least three active points of the selector participate in the interaction with corresponding sites of the solute molecule. This three-point interaction rule is generally valid for enantioselective chromatography, with the extension to the rule, stating that one of the required interactions may be mediated by the adsorption of the two components of the interacting pair onto the sorbent surface.*

Page 1473 further refers to the terminology used for the diastereomers after separation.

*3.1 * Diastereoisomer excess/Diastereoisomeric excess*

This is defined by analogy with enantiomer excess, as $D_1 - D_2$, [and the percent diastereoisomer excess as 100 (D_1-D_2)], w here the mole fractions of the two diastereoisomers in a mixture or the fractional yields of two diastereoisomers formed in a reaction are D_1 and D_2, $(D_1 + D_2 = 1)$. The term is not applicable if more than two diastereoisomers are present. Frequently this term is abbreviated to d.e.

7.29 An article entitled "Chiral Separations Introduction 1.1 Importance." By Mey Cabusas 55 annexed hereto as Exhibit 10 and hereinafter referred to as D10 and downloaded from the link

CHIRAL SEPARATIONS INTRODUCTION 1.1. Importance ...
scholar.lib.vt.edu/theses/available/etd-32298-223814/.../ch_01.pdf
by MEY Cabusas - 1998 - Cited by 6 - Related articles
The **separation** of **chiral** compounds has been of great interest because the majority of .... form **diastereomers** and then **separated** using a **chiral** column. 40-56.

This article refers to the usage of Chiralpak column for separation of the isomers.

7.30 An article entitled: "Comparison of Batch elution and continuous simulated moving bed Chromatography" by Jochen Strube, Sonja Haumreisser, Henner Schimdt-Traub, Michael Schulte and Reinhard Ditz in Org. Proc. Res. Dev.,

1998, 2(5), pp 305-319 dated July 18, 1998 annexed hereto as Exhibit 11 and hereinafter referred to as D11.

The said article provides a comparative analysis of the chromatography technique prevalent in 1998 i.e. Batch elution chromatography and Continuous Simulated Moving bed Chromatography.

Column 1 on page 307 states:

> *At first, chromatography was only used for analytical purposes.* ***Elution chromatography is likewise applied for separations on the production scale. Because of the discontinuous operation batch chromatography has a demand on a high degree of automation*** *to save personal costs and to run the equipment in a safe and reliable way. In contrast to batch chromatography,* ***the automation of SMB chromatography is far more complex.***

The Opponent states that from D11 a skilled person will be motivated to check out the simpler method of chromatographic separation i.e. batch elution chromatography. Based on the results available, the skilled person will further try out different chromatographic techniques and determine which of the techniques provides the best results.

7.31 The Opponent states that from the teachings of D9 to D11, a skilled person will be able to identify that separation of diastereomers needs to be carried out through chromatography. Further the usage of Chiralpak AS column and Batch elution chromatography can be arrived at by a skilled person through routine experimentation as the said techniques are well known from D10 and D11.

7.32 The Opponent further states that in the impugned application also the applicant has also referred to different techniques for diastereomeric separation namely Batch elution Chromatography (Example 3 A), SMB Chromatography (Example 3 B), C18 RP-HPLC (Example 3 C) and Crystallization (Example 3 D) thereby indicating that the applicant has also carried out routine analysis to arrive at the technique to be used for chromatographic separation as also to arrive at the column to be used for such separation.

7.33 The Opponent therefore states that the process for obtaining GS-7340 through diastereomeric separation of GS-7171 is from the cited prior art and therefore

does not involve any inventive step. Thus, the amended claims 5-7 of the impugned application are bad in law and should be rejected.

7.34 It is therefore stated the subject matter covered in the impugned application being obvious and devoid of inventive merit over multiple documents, indicates that the alleged invention is a mere extension of prior art and is arrived at by trial and error techniques and hence is within the purview of a person skilled in the art and therefore ought to be rejected.

7.35 The Opponent therefore states that the invention claimed by the applicant is devoid of an inventive step having regard to the aforesaid publication viz. Exhibit 1 to Exhibit 11 which clearly indicates and makes it obvious to any person skilled in the art to arrive at the allegedly claimed invention and this knowledge can be arrived at through routine experimentation on the priority date of the application under opposition. Therefore, the opponent states that the entire claimed scope of the alleged invention now amended is incurably obvious in view of exhibits 1 to 11.

8. <u>NOT AN INVENTION / NOT PATENTABLE[Section 25(1)(f)]:</u>

8.1 **<u>Claims not an invention as per Section 2(1)(ja)</u>**

The opponent states that the claimed invention falls under the mischief of Section 2(1)(ja) being devoid of inventive step. The opponent states that Applicant's invention is neither a technical advancement nor it is giving any economic significance on the face of what is already known in the prior art and cited herein above and for the sake of brevity rely upon the arguments under paragraph 7. It is stated that to claim an invention, an inventor has to show a positive advancement of the relevant art and in the present case no technical advancement flows out of the various facets of the alleged invention claimed by the applicant.

8.2 **<u>Claims an invention not patentable as per Section 3(d)</u>**

The opponent states that the claimed invention is not patentable as it falls within the mischief of Section 3 (d) of the Indian Patent Act. The section 3(d) is stated below:

3(d) The mere discovery of a new form of a known substance which does not result in the enhancement of the known efficacy of that substance or the mere discovery of any new property or new use for a known substance or of the mere use of a known process, machine or apparatus unless such known process results in a new product or employs at least one new reactant.

Explanation- For the purposes of this clause, salts, esters, ethers, polymorphs, metabolites, pure form, particle size, isomers, mixtures of isomers, complexes, combinations and other derivatives of known substance shall be considered to be the same substance, unless they differ significantly in properties with regard to efficacy

The Manual of Indian Patent Act further states that:

Mere use of a known process is not patentable unless such known process results in a new product or employs at least one new reactant.

The opponent states that the mere use of a known process to prepare PMPA/PMEA by just interchanging one metal alkoxide base (sodium/lithium alkoxide) with another metal alkoxide base (Magnesium alkoxide) keeping the other reactants same as is mentioned in the prior art is not patentable. The process also has not resulted in any new product but the same product PMPA/PMEA which has been cited in many of the priorly known documents. The Applicant has also failed to show any improved efficacy in terms of improved yield/purity with regards to the use of Magnesium alkoxide. The amended claims of the opposed application is liable to be refused under this ground alone.

9 **RELIEF SOUGHT**

The opponent states that it has established and made out a case on each of the aforesaid grounds of opposition and pray to the Ld. Controller for the following relief(s):

1) Take on record the present representation;

2) Leave to file evidence;

3) Forward copy of reply of applicant and evidence if any and any amendments filed;

4) Leave to file a replication to the reply of the applicant and evidence;

5) Grant of hearing;

6) Refusal of the application *in toto*;

7) Such other relief or reliefs as the Controller may deem appropriate.

Dated this the 29th day of June 2015.

Amrita Majumdar

Amrita Majumdar

The Controller of Patents
The Patent Office Branch
Mumbai.

Enclosures:

- Annexure A;
- Annexure B;
- Annexure C;
- Annexure D;
- Annexure E;
- Annexure F;
- Annexure G;
- Annexure H;
- Annexure I;
- Annexure J;
- Annexure K;
- Exhibit 1;
- Exhibit 2;
- Exhibit 3;
- Exhibit 4;
- Exhibit 5;
- Exhibit 6;
- Exhibit 7;
- Exhibit 8;
- Exhibit 9;
- Exhibit 10;
- Exhibit 11.

Annexure A

36

FORM 2

THE PATENTS ACT, 1970
(39 of 1970)
&
The Patent Rules, 2003

COMPLETE SPECIFICATION
(See section 10 and rule 13)

ORIGINAL

TITLE OF THE INVENTION

"PRODRUGS OF PHOSPHONATE NUCLEOTIDE ANALOGUES AND METHODS FOR SELECTING AND MAKING SAME"

We, **GILEAD SCIENCES, INC.**, of 333 Lakeside Drive, Foster City, CA 94404, United States of America,

The following specification particularly describes the nature of the invention and the manner in which it is performed:

Prodrugs of Phosphonate Nucleotide Analogues and Methods for Selecting and Making Same

This application relates to prodrugs of methoxyphosphonate nucleotide analogues. In particular it relates to improved methods for making and identifying such prodrugs.

Many methoxyphosphonate nucleotide analogues are known. In general, such compounds have the structure A-$OCH_2P(O)(OR)_2$ where A is the residue of a nucleoside analogue and R independently is hydrogen or various protecting or prodrug functionalities. See U.S. Patent Nos. 5,663,159, 5,977,061 and 5,798,340, Oliyai et al, "Pharmaceutical Research" 16(11):1687-1693 (1999), Stella et al., "J. Med. Chem." 23(12):1275-1282 (1980), Aarons, L., Boddy, A. and Petrak, K. (1989) *Novel Drug Delivery and Its Therapeutic Application* (Prescott, L. F. and Nimmo, W. S., ed.), pp. 121–126; Bundgaard, H. (1985) *Design of Prodrugs* (Bundgaard, H., ed.) pp. 70–74 and 79-92; Banerjee, P. K. and Amidon, G. L. (1985) *Design of Prodrugs* (Bundgaard, H., ed.) pp. 118-121; Notari, R. E. (1985) *Design of Prodrugs* (Bundgaard, H., ed.) pp. 135-156; Stella, V. J. and Himmelstein, K. J. (1985) *Design of Prodrugs* (Bundgaard, H., ed.) pp. 177-198; Jones, G. (1985) *Design of Prodrugs* (Bundgaard, H., ed.) pp. 199-241; Connors, T. A. (1985) *Design of Prodrugs* (Bundgaard, H., ed.) pp. 291–316. All literature and patent citations herein are expressly incorporated by reference.

Summary of the Invention

Prodrugs of methoxyphosphonate nucleotide analogues intended for antiviral or antitumor therapy, while known, traditionally have been selected for their systemic effect. For example, such prodrugs have been selected for enhanced bioavailability, i.e., ability to be absorbed from the gastrointestinal tract and converted rapidly to parent drug to ensure that the parent drug is available to all tissues. However, applicants now have found that it is possible to select prodrugs that become enriched at therapeutic sites, as illustrated by the studies described herein where the analogues are enriched at localized focal sites of HIV infection. The objective of this invention is, among other advantages, to produce less toxicity to bystander tissues and greater potency of the parental drug in tissues which are the targets of therapy with the parent methoxyphosphonate nucleotide analogue.

Accordingly, pursuant to these observations, a screening method is provided for identifying a methoxyphosphonate nucleotide analogue prodrug conferring enhanced activity in a target tissue comprising:

(a) providing at least one of said prodrugs;

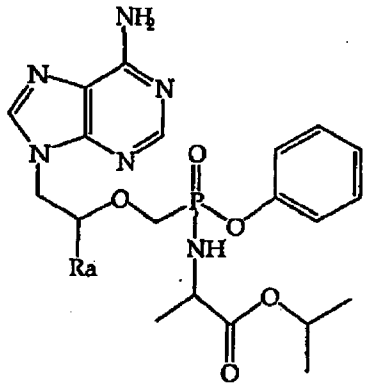
(b) selecting at least one therapeutic target tissue and at least one non-target tissue;

(c) administering the prodrug to the target tissue and to said at least one non-target tissue; and

(d) determining the relative antiviral activity conferred by the prodrug in the tissues in step (c).

In preferred embodiments, the target tissue are sites where HIV is actively replicated and/or which serve as an HIV reservoir, and the non-target tissue is an intact animal. Unexpectedly, we found that selecting lymphoid tissue as the target tissue for the practice of this method for HIV led to identification of prodrugs that enhance the delivery of active drug to such tissues.

A preferred compound of this invention, which has been identified by this method has the structure (1),

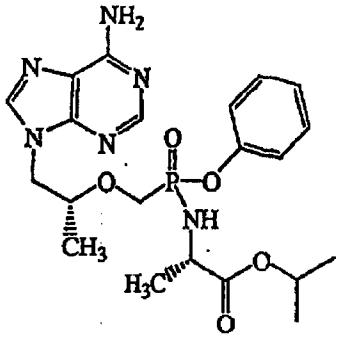


(1)

where Ra is H or methyl,

and chirally enriched compositions thereof, salts, their free base and solvates thereof.

A preferred compound of this invention has the structure (2)

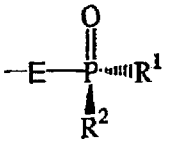


(2)

and its enriched diasteromers, salts, free base and solvates.

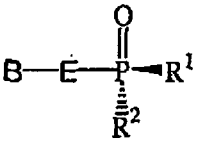
In addition, we unexpectedly found that the chirality of substituents on the phosphorous atom and/or the amidate substituent are influential in the enrichment observed in the practice of this invention. Thus, in another embodiment of this

invention, we provide diastereomerically enriched compounds of this invention *having the structure (3)*



(3)

which are substantially free of the diastereomer (4)



(4)

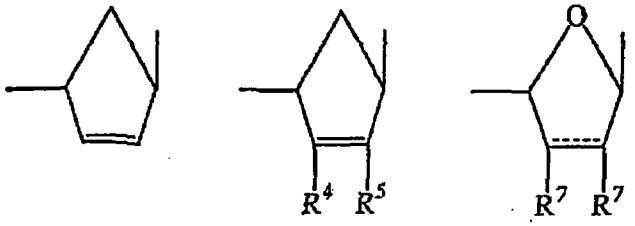
wherein

R^1 is an oxyester which is hydrolyzable *in vivo*, or hydroxyl;

B is a heterocyclic base;

R^2 is hydroxyl, or the residue of an amino acid bonded to the P atom through an amino group of the amino acid and having each carboxy substituent of the amino acid optionally esterified, but not both of R^1 and R^2 are hydroxyl;

E is $-(CH_2)2-$, $-CH(CH_3)CH_2-$, $-CH(CH_2F)CH_2-$, $-CH(CH_2OH)CH_2-$, $-CH(CH=CH_2)CH_2-$, $-CH(C\equiv CH)CH_2-$, $-CH(CH_2N_3)CH_2-$,



$-CH(R^6)OCH(R^{6'})-$, $-CH(R^7)CH_2O-$ or $-CH(R^8)O-$, wherein the right hand bond is linked to the heterocyclic base;

the broken line represents an optional double bond;

R^4 and R^5 are independently hydrogen, hydroxy, halo, amino or a substituent having 1-5 carbon atoms selected from acyloxy, alkyoxy, alkylthio, alkylamino and dialkylamino;

R^6 and $R^{6'}$ are independently H, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl, or C_2-C_7 alkanoyl;

R^7 is independently H, C_1-C_6 alkyl, or are taken together to form -O- or $-CH_2-$;

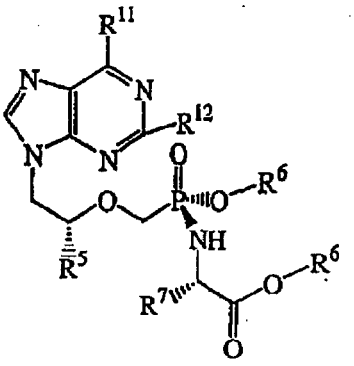
R^8 is H, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl or C_1-C_6 haloalkyl; and

R^9 is H, hydroxymethyl or acyloxymethyl;

and their salts, free base, and solvates.

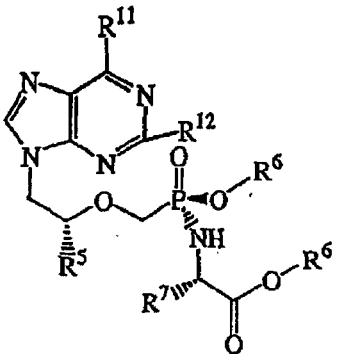
The diastereomers of structure (3) are designated the *(S)* isomers at the phosphorus chiral center.

Preferred embodiments of this invention are the diastereomerically enriched compounds having the structure (5a)



(5a)

which is substantially free of diastereomer (5b)



(5b)

wherein

R^5 is methyl or hydrogen;

R^6 independently is H, alkyl, alkenyl, alkynyl, aryl or arylalkyl, or R^6 independently is alkyl, alkenyl, alkynyl, aryl or arylalkyl which is substituted with from 1 to 3 substituents selected from alkylamino, alkylaminoalkyl, dialkylaminoalkyl, dialkylamino, hydroxyl, oxo, halo, amino, alkylthio, alkoxy, alkoxyalkyl, aryloxy, aryloxyalkyl, arylalkoxy, arylalkoxyalkyl, haloalkyl, nitro, nitroalkyl, azido, azidoalkyl, alkylacyl, alkylacylalkyl, carboxyl, or alkylacylamino;

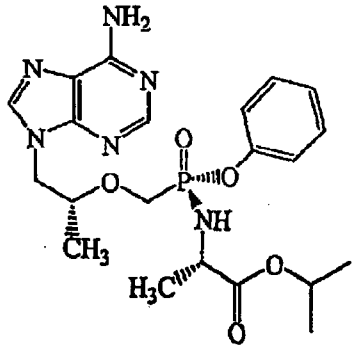
R^7 is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and which, if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group;

R^{11} is amino, alkylamino, oxo, or dialkylamino; and

R^{12} is amino or H;

and its salts, tautomers, free base and solvates.

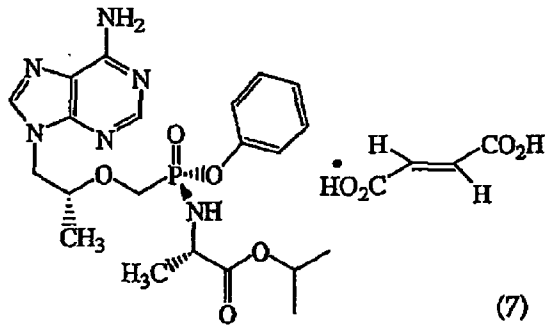
A preferred embodiment of this invention is the compound of structure (6), 9-[(*R*)-2-[[(*S*)-[[(*S*)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine, also designated herein GS-7340



(6)

Another preferred embodiment of this invention is the fumarate salt of structure (5) (structure (7)), 9-[(*R*)-2-[[(*S*)-[[(*S*)-1-

(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl]adenine fumarate (1:1), also designated herein GS-7340-2



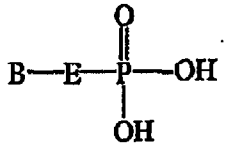
(7)

The compounds of structures (1)-(7) optionally are formulated into compositions containing pharmaceutically acceptable excipients. Such compositions are used in effective doses in the therapy or prophylaxis of viral (particularly HIV or hepadnaviral) infections.

In a further embodiment, a method is provided for the facile manufacture of 9-[2-(phosphonomethoxy)propyl]adenine (hereinafter "PMPA" or 9-[2-(phosphonomethoxy)ethyl] adenine (hereinafter "PMEA") using magnesium alkoxide, which comprises combining 9-(2-hydroxypropyl)adenine or 9-(2-hydroxyethyl)adenine, protected *p*-toluenesulfonyloxymethylphosphonate and magnesium alkoxide, and recovering PMPA or PMEA, respectively.

Detailed Description of the Invention

The methoxyphosphonate nucleotide analogue parent drugs for use in this screening method are compounds having the structure A-$OH_2P(O)(OH)_2$ wherein A is the residue of a nucleoside analogue. These compounds are known per se and are not part of this invention. More particularly, the parent compounds comprise a heterocyclic base B and an aglycon E, in general having the structure



wherein the group B is defined below and group E is defined above. Examples are described in U.S. Patent Nos. 4,659,825, 4,808,716, 4,724,233, 5,142,051, 5,130,427, 5,650,510, 5,663,159, 5,302,585, 5,476,938, 5,696,263, 5,744,600, 5,688,778, 5,386,030, 5,733,896, 5,352,786, and 5,798,340, and EP 821,690 and 654,037.

The prodrugs for use in the screening method of this invention are covalently modified analogues of the parent methoxyphosphonate nucleotide analogues described in the preceding paragraph. In general, the phosphorus atom of the parent drug is the preferred site for prodrug modification, but other sites are found on the heterocyclic base B or the aglycon E. Many such prodrugs are already known. Primarily, they are esters or amidates of the phosphorus atom, but also include substitutions on the base and aglycon. None of these modifications per se is part of this invention and none are to be considered limiting on the scope of the invention herein.

The phosphorus atom of the methoxyphosphonate nucleotide analogues contains two valences for covalent modification such as amidation or esterification (unless one phosphoryl hydroxyl is esterified to an aglycon E hydroxyl substituent, whereupon only one phosphorus valence is free for substitution). The esters typically are aryloxy. The amidates ordinarily are naturally occurring monoamino acids having free carboxyl group(s) esterified with an alkyl or aryl group, usually phenyl, cycloalkyl, or t-, n- or s- alkyl groups. Suitable prodrugs for use in the screening method of this invention are disclosed for example in U.S. Patent No. 5,798,340. However, any prodrug which is potentially believed to be capable of being converted *in vivo* within target tissue cells to the free methoxyphosphonate nucleotide analogue parent drug, e.g., whether by hydrolysis, oxidation, or other covalent transformation resulting from exposure to biological tissues, is suitable for use in the method of this invention. Such prodrugs may not be known at this time but are identified in the future and thus become suitable candidates available for testing in the method of this invention. Since the prodrugs are simply candidates for screening in the methods their structures are not relevant to practicing or enabling the screening method, although of course their structures ultimately are dispositive of whether or not a prodrug will be shown to be selective in the assay.

The pro-moieties bound to the parent drug may be the same or different. However, each prodrug to be used in the screening assay will differ structurally from the other prodrugs to be tested. Distinct, i.e. structurally different, prodrugs generally are selected on the basis of either their stereochemistry or their covalent structure, or these features are varied in combination. Each prodrug tested, however, desirably is structurally and stereochemically substantially pure, else the output of the screening assay will be less useful. It is of course within the scope of this invention to test only a single prodrug in an individual embodiment of the method of this invention, although typically then one would compare the results with prior studies with other prodrugs.

We have found that the stereochemistry of the prodrugs is capable of influencing the enrichment in target tissues. Chiral sites are at the phosphorus atom and are also found in its substituents. For example, amino acid used in preparing amidates may be D or L forms, and the phosphonate esters or the amino acid esters can contain chiral centers as well. Chiral sites also are found on the nucleoside analogue portion of the molecules, but these typically are already dictated by the stereochemistry of the parent drug and will not be varied as part of the screen. For example the R isomer of PMPA is preferred as it is more active than the corresponding S isomer. Typically these diasteromers or enantiomers will be chirally enriched if not pure at each site so that the results of the screen will be more meaningful. As noted, distinctiveness of stereoisomers is conferred by enriching or purifying the stereoisomer (typically this will be a diastereomer rather than an enantiomer in the case of most methoxyphosphonate nucleotide analogues) free of other stereoisomers at the chiral center in question, so that each test compound is substantially homogeneous. By substantially homogeneous or chirally enriched, we mean that the desired stereoisomer constitutes greater than about 60% by weight of the compound, ordinarily greater than about 80% and preferably greater than about 95%.

Novel Screening Method

Once at least one candidate prodrug has been selected, the remaining steps of the screening method of this invention are used to identify a prodrug possessing the required selectivity for the target tissue. Most conveniently the prodrugs are labeled with a detectable group, e.g. radiolabeled, in order to facilitate detection later in tissues or cells. However, a label is not required since other suitable assays for the prodrug or its metabolites (including the parent drug) can also be employed. These assays could include mass spectrometry, HPLC, bioassays or immunoassays for instance. The assay may detect the prodrug and any one or more of its metabolites, but preferably the assay is conducted to detect only the generation of the parent drug. This is based on the assumption (which may not be warranted in all cases) that the degree and rate of conversion of prodrug to antivirally active parent diphosphate is the same across all tissues tested. Otherwise, one can test for the diphosphate.

The target tissue preferably will be lymphoid tissue when screening for prodrugs useful in the treatment of HIV infection. Lymphoid tissue will be known to the artisan and includes CD4 cells, lymphocytes, lymph nodes, macrophages and macrophage-like cells including monocytes such as peripheral blood monocytic cells (PBMCs) and glial cells. Lymphoid tissue also includes non-lymphoid tissues that are enriched in lymphoid tissues or cells, e.g. lung, skin and spleen. Other targets for other antiviral drugs of course will be the primary sites of replication or latency for the particular virus concerned, e.g., liver for hepatitis and peripheral nerves for HSV. Similarly, target tissues for tumors will in fact be the tumors themselves. These tissues are all well-known to the artisan and would not require undue experimentation to select. When screening for antiviral compounds, target tissue can be infected by the virus.

Non-target tissues or cells also are screened as part of the method herein. Any number or identity of such tissues or cells can be employed in this regard. In general, tissues for which the parent drug is expected to be toxic will be used as non-target tissues. The selection of a non-target tissue is entirely dependent upon

the nature of the prodrug and the activity of the parent. For example, non-hepatic tissues would be selected for prodrugs against hepatitis, and untransformed cells of the same tissue as the tumor will suffice for the antitumor-selective prodrug screen.

It should be noted that the method of this invention is distinct from studies typically undertaken to determine oral bioavailability of prodrugs. In oral bioavailability studies, the objective is to identify a prodrug which passes into the systemic circulation substantially converted to parent drug. In the present invention, the objective is to find prodrugs that are not metabolized in the gastrointestinal tract or circulation. Thus, target tissues to be evaluated in the method of this invention generally do not include the small intestines or, if the intestines are included, then the tissues also include additional tissues other than the small intestines.

The target and non-target tissues used in the screening method of this invention typically will be in an intact living animal. Prodrugs containing esters are more desirably tested in dogs, monkeys or other animals than rodents; mice and rat plasma contains high circulating levels of esterases that may produce a misleading result if the desired therapeutic subject is a human or higher mammal.

It is not necessary to practice this method with intact animals. It also is within the scope of this invention to employ perfused organs, *in vitro* culture of organs (e.g. skin grafts) or cell lines maintained in various forms of cell culture, e.g. roller bottles or zero gravity suspension systems. For example, MT-2 cells can be used as a target tissue for selecting HIV prodrugs. Thus, the term "tissue" shall not be construed to require organized cellular structures, or the structures of tissues as they may be found in nature, although such would be preferred. Rather, the term "tissue" shall be construed to be synonymous with cells of a particular source, origin or differentiation stage.

The target and non-target tissue may in fact be the same tissue, but the tissues will be in different biological status. For example, the method herein could be used to select for prodrugs that confer activity in virally-infected tissue (target tissue) but which remain substantially inactive in virally-uninfected cells (corresponding non-target tissue). The same strategy would be employed to select

prophylactic prodrugs, i.e., prodrugs metabolized to antivirally active forms incidental to viral infection but which remain substantially unmetabolized in uninfected cells. Similarly, prodrugs could be screened in transformed cells and the untransformed counterpart tissue. This would be particularly useful in comparative testing to select prodrugs for the treatment of hematological malignancies, e.g. leukemias.

Without being limited by any particular theory of operation, tissue selective prodrugs are thought to be selectively taken up by target cells and/or selectively metabolized within the cell, as compared to other tissues or cells. The unique advantage of the methoxyphosphonate prodrugs herein is that their metabolism to the dianion at physiological pH ensures that they will be unable to diffuse back out of the cell. They therefore remain effective for lengthy periods of time and are maintained at elevated intracellular concentrations, thereby exhibiting increased potency. The mechanisms for enhanced activity in the target tissue are believed to include enhanced uptake by the target cells, enhanced intracellular retention, or both mechanisms working together. However, the manner in which selectivity or enhanced delivery occurs in the target tissue is not important. It also is not important that all of the metabolic conversion of the prodrug to the parent compound occurs within the target tissue. Only the final drug activity-conferring conversion need occur in the target tissue; metabolism in other tissues may provide intermediates finally converted to antiviral forms in the target tissue.

The degree of selectivity or enhanced delivery that is desired will vary with the parent compound and the manner in which it is measured (% dose distribution or parent drug concentration). In general, if the parent drug already possess a generous therapeutic window, a low degree of selectivity may be sufficient for the desired prodrug. On the other hand, toxic compounds may require more extensive screening to identify selective prodrugs. The relative expense of the method of this invention can be reduced by screening only in the target tissue and tissues against which the parent compound is known to be relatively toxic, e.g. for PMEA, which is nephrotoxic at higher doses, the primary focus will be on kidney and lymphoid tissues.

The step of determining the relative antiviral activity of a prodrug in the selected tissues ordinarily is accomplished by assaying target and non-target tissues for the relative presence or activity of a metabolite of the prodrug, which metabolite is known to have, or is converted to, a metabolite having antiviral or antitumor activity. Thus, typically one would determine the relative amount of the parent drug in the tissues over substantially the same time course in order to identify prodrugs that are preferentially metabolized in the target tissue to an antivirally or antitumor active metabolite or precursor thereof which in the target tissue ultimately produces the active metabolite. In the case of antiviral compounds, the active metabolite is the diphosphate of the phosphonate parent compounds. It is this metabolite that is incorporated into the viral nucleic acid, thereby truncating the elongating nucleic acid strand and halting viral replication. Metabolites of the prodrug can be anabolic metabolites, catabolic metabolites, or the product of anabolism and catabolism together. The manner in which the metabolite is produced is not important in the practice of the method of this invention.

The method of this invention is not limited to assaying a metabolite which per se possesses antiviral or antitumor activity. Instead, one can assay inactive precursors of the active metabolites. Precursors of the antivirally active diphosphate metabolite include the monophosphate of the parent drug, monophosphates of other metabolites of the parent drug (e.g., an intermediate modification of a substituent on the heterocyclic base), the parent itself and metabolites generated by the cell in converting the prodrug to the parent prior to phosphorylation. The precursor structures may vary considerably as they are the result of cellular metabolism. However, this information is already known or could be readily determined by one skilled in the art.

If the prodrug being assayed does not exhibit antitumor or antiviral activity per se then adjustments to the raw assay results may be required. For example, if the intracellular processing of the inactive metabolite to an active metabolite occurs at different rates among the tissues being tested, the raw assay results with the inactive metabolite would need to be adjusted to take account of the differences

among the cell types because the relevant parameter is the generation of activity in the target tissue, not accumulation of inactive metabolites. However, determining the proper adjustments would be within the ordinary skill. Thus, when step (d) of the method herein calls for determining the activity, activity can be either measured directly or extrapolated. It does not mean that the method herein is limited to only assaying intermediates that are active per se. For instance, the absence or decline of the prodrug in the test tissues also could be assayed. Step (d) only requires assessment of the activity conferred by the prodrug as it interacts with the tissue concerned, and this may be based on extrapolation or other indirect measurement.

Step (d) of the method of this invention calls for determining the "relative" activity of the prodrug. It will be understood that this does not require that each and every assay or series of assays necessarily must also contain runs with the selected non-target tissue. On the contrary, it is within the scope of this invention to employ historical controls of the non-target tissue or tissues, or algorithms representing results to be expected from such non-target tissues, in order to provide the benchmark non-target activity.

The results obtained in step (d) are then used optimally to select or identify a prodrug which produces greater antiviral activity in the target tissue than in the non-target tissue. It is this prodrug that is selected for further development.

It will be appreciated that some preassessment of prodrug candidates can be undertaken before the practice of the method of this invention. For example, the prodrug will need to be capable of passing largely unmetabolized through the gastrointestinal tract, it will need to be substantially stable in blood, and it should be able to permeate cells at least to some degree. In most cases it also will need to complete a first pass of the hepatic circulation without substantial metabolism. Such prestudies are optional, and are well-known to those skilled in the art.

The same reasoning as is described above for antiviral activity is applicable to antitumor prodrugs of methoxyphosphonate nucleotide analogues as well. These include, for example, prodrugs of PMEG, the guanyl analogue of PMEA. In

this case, cytotoxic phosphonates such as PMEG are worthwhile candidates to pursue as their cytotoxicity in fact confers their antitumor activity.

A compound identified by this novel screening method then can be entered into a traditional preclinical or clinical program to confirm that the desired objectives have been met. Typically, a prodrug is considered to be selective if the activity or concentration of parent drug in the target tissue (% dose distribution) is greater than 2x, and preferably 5x, that of the parent compound in non-target tissue. Alternatively, a prodrug candidate can be compared against a benchmark prodrug. In this case, selectivity is relative rather than absolute. Selective prodrugs will be those resulting in greater than about 10x concentration or activity in the target tissue as compared with the prototype, although the degree of selectivity is a matter of discretion.

Novel Method for Preparation of Starting Materials or Intermediates

Also included herein is an improved method for manufacture of preferred starting materials (parent drugs) of this invention, PMEA and (*R*)-PMPA. Typically, this method comprises reacting 9-(2-hydroxypropyl)adenine (HPA) or 9-(2-hydroxyethyl)adenine (HEA) with a magnesium alkoxide, thereafter adding the protected aglycon synthon *p*-toluene-sulfonyloxymethylphosphonate (tosylate) to the reaction mixture, and recovering PMPA or PMEA, respectively.

Preferably, HPA is the enriched or isolated R enantiomer. If a chiral HPA mixture is used, R-PMPA can be isolated from the chiral PMPA mixture after the synthesis is completed.

Typically the tosylate is protected by lower alkyl groups, but other suitable groups will be apparent to the artisan. It may be convenient to employ the tosylate presubstituted with the prodrug phosphonate substituents which are capable of acting as protecting groups in the tosylation reaction, thereby allowing one to bypass the deprotection step and directly recover prodrug or an intermediate therefore.

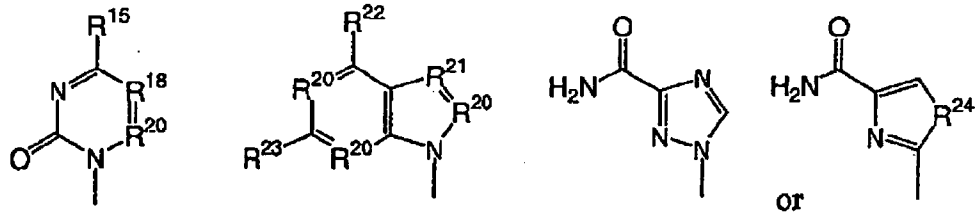
The alkyl group of the magnesium alkoxide is not critical and can be any C_1-C_6 branched or normal alkyl, but is preferably t-butyl (for PMPA) or isopropyl (for

PMEA). The reaction conditions also are not critical, but preferably comprise heating the reaction mixture at about 70-75°C with stirring or other moderate agitation.

If there is no interest in retaining the phosphonate substituents, the product is deprotected (usually with bromotrimethylsilane where the tosylate protecting group is alkyl), and the product then recovered by crystallization or other conventional method as will be apparent to the artisan.

Heterocyclic Base

In the compounds of this invention depicted in structures (3) and (4), the heterocyclic base B is selected from the structures



or

wherein

R^{15} is H, OH, F, Cl, Br, I, OR^{16}, SH, SR^{16}, NH_2, or NHR^{17};

R^{16} is C_1-C_6 alkyl or C_2-C_6 alkenyl including CH_3, CH_2CH_3, CH_2CCH, CH_2CHCH_2 and C_3H_7;

R^{17} is C_1-C_6 alkyl or C_2-C_6 alkenyl including CH_3, CH_2CH_3, CH_2CCH, CH_2CHCH_2, and C_3H_7;

R^{18} is N, CF, CCl, CBr, CI, CR^{19}, CSR^{19}, or COR^{19};

R^{19} is H, C_1-C_9 alkyl, C_2-C_9 alkenyl, C_2 - C_9 alkynyl, C_1-C_9 alkyl-C_1-C_9 alkoxy, or C_7-C_9 aryl-alkyl unsubstituted or substituted by OH, F, Cl, Br or I, R^{19} therefore including -CH_3, -CH_2CH_3, -$CHCH_2$, -CHCHBr, -CH_2CH_2Cl, -CH_2CH_2F, -CH_2CCH, -CH_2CHCH_2, -C_3H_7, -CH_2OH, -CH_2OCH_3, -$CH_2OC_2H_5$, -CH_2OCCH, -$CH_2OCH_2CHCH_2$, -$CH_2C_3H_7$, -CH_2CH_2OH, -$CH_2CH_2OCH_3$,

$-CH_2CH_2OC_2H_5$, $-CH_2CH_2OCCH$, $-CH_2CH_2OCH_2CHCH_2$, and $-CH_2CH_2OC_3H_7$;

R^{20} is N or CH;

R^{21} is N, CH, CCN, CCF_3, $CC{\equiv}CH$ or $CC(O)NH_2$;

R^{22} is H, OH, NH_2, SH, SCH_3, SCH_2CH_3, SCH_2CCH, SCH_2CHCH_2, SC_3H_7, $NH(CH_3)$, $N(CH_3)_2$, $NH(CH_2CH_3)$, $N(CH_2CH_3)_2$, $NH(CH_2CCH)$, $NH(CH_2CHCH_2)$, $NH(C_3H_7)$, halogen (F, Cl, Br or I) or X wherein X is $(CH_2)_m(O)_n(CH_2)_mN(R^{10})_2$ wherein each m is independently 0-2, n is 0-1, and

R^{10} independently is

H,

C_1-C_{15} alkyl, C_2-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_6-C_{15} arylalkynyl, C_2-C_{15} alkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroaralkyl, C_5-C_6 aryl, C_2-C_6 heterocycloalkyl,

C_2-C_{15} alkyl, C_3-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_3-C_{15} alkynyl, C_7-C_{15} arylalkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroalkyl or C_3-C_6 heterocycloalkyl wherein methylene in the alkyl moiety not adjacent to N^6 has been replaced by –O-,

optionally both R^{10} are joined together with N to form a saturated or unsaturated C_2-C_5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom,

or one of the foregoing R^{10} groups which is substituted with 1 to 3 halo, CN or N_3; but optionally at least one R^{10} group is not H;

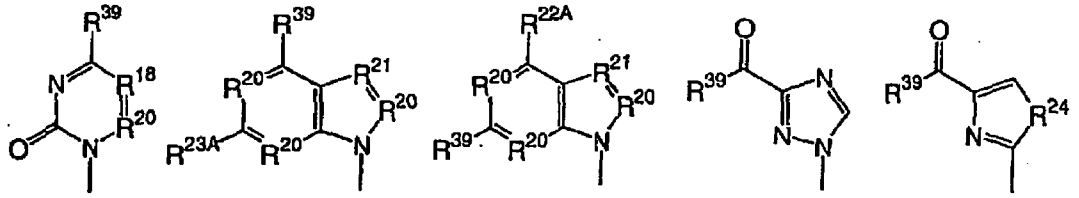
R^{23} is H, OH, F, Cl, Br, I, SCH_3, SCH_2CH_3, SCH_2CCH, SCH_2CHCH_2, SC_3H_7, OR^{16}, NH_2, NHR^{17} or R^{22}; and

R^{24} is O, S or Se.

B also includes both protected and unprotected heterocyclic bases, particularly purine and pyrimidine bases. Protecting groups for exocyclic amines and other labile groups are known (Greene et al. "Protective Groups in Organic Synthesis") and include N-benzoyl, isobutyryl, 4,4'-dimethoxytrityl (DMT) and the

like. The selection of protecting group will be apparent to the ordinary artisan and will depend upon the nature of the labile group and the chemistry which the protecting group is expected to encounter, e.g. acidic, basic, oxidative, reductive or other conditions. Exemplary protected species are N^4-benzoylcytosine, N^6-benzoyladenine, N^2-isobutyrylguanine and the like.

Protected bases have the formulas Xa.1, XIa.1, XIb.1, XIIa.1 or XIIIa.1



(Xa.1) (XIa.1) (XIb.1) (XIIa.1) (XIIIa.1)

wherein R^{18}, R^{20}, R^{21}, R^{24} have the meanings previously defined; R^{22A} is R^{39} or R^{22} provided that R^{22} is not NH_2; R^{23A} is R^{39} or R^{23} provided that R^{23} is not NH_2; R^{39} is NHR^{40}, $NHC(O)R^{36}$ or $CR^{41}N(R^{38})_2$ wherein R^{36} is C_1-C_{19} alkyl, C_1-C_{19} alkenyl, C_3-C_{10} aryl, adamantoyl, alkylanyl, or C_3-C_{10} aryl substituted with 1 or 2 atoms or groups selected from halogen, methyl, ethyl, methoxy, ethoxy, hydroxy and cyano; R^{38} is C_1-C_{10} alkyl, or both R^{38} together are 1-morpholino, 1-piperidine or 1-pyrrolidine; R^{40} is C_1-C_{16} alkyl, including methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, hexyl, octyl and decanyl; and R^{41} is hydrogen or CH_3.

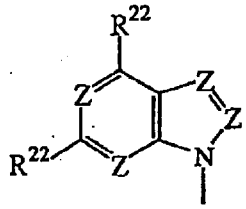
For bases of structures XIa.1 and XIb.1, if R^{39} is present at R^{22A} or R^{23A}, both R^{39} groups on the same base will generally be the same. Exemplary R^{36} are phenyl, phenyl substituted with one of the foregoing R^{36} aryl substituents, $-C_{10}H_{15}$ (where $C_{10}H_{15}$ is 2-adamantoyl), $-CH_2-C_6H_5$, $-C_6H_5$, $-CH(CH_3)_2$, $-CH_2CH_3$, methyl, butyl, t-butyl, heptanyl, nonanyl, undecanyl, or undecenyl.

Specific bases include hypoxanthine, guanine, adenine, cytosine, inosine, thymine, uracil, xanthine, 8-aza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 7-deaza-8-aza

derivatives of adenine, guanine, 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 1-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 7-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 3-deaza derivatives of 2-aminopurine, 2,6-diaminopurine, 2-amino-6-chloropurine, hypoxanthine, inosine and xanthine; 6-azacytosine; 5-fluorocytosine; 5-chlorocytosine; 5-iodocytosine; 5-bromocytosine; 5-methylcytosine; 5-bromovinyluracil; 5-fluorouracil; 5-chlorouracil; 5-iodouracil; 5-bromouracil; 5-trifluoromethyluracil; 5-methoxymethyluracil; 5-ethynyluracil and 5-propynyluracil.

Preferably, B is a 9-purinyl residue selected from guanyl, 3-deazaguanyl, 1-deazaguanyl, 8-azaguanyl, 7-deazaguanyl, adenyl, 3-deazaadenyl, 1-dezazadenyl, 8-azaadenyl, 7-deazaadenyl, 2,6-diaminopurinyl, 2-aminopurinyl, 6-chloro-2-aminopurinyl and 6-thio-2-aminopurinyl, or a B' is a 1-pyrimidinyl residue selected from cytosinyl, 5-halocytosinyl, and 5-(C_1-C_3-alkyl)cytosinyl.

Preferred B groups have the formula



wherein

R^{22} independently is halo, oxygen, NH_2, X or H, but optionally at least one R^{22} is X;

X is $-(CH_2)_m(O)_n(CH_2)_mN(R^{10})_2$ wherein m is 0-2, n is 0-1, and

R^{10} independently is

H,

C_1-C_{15} alkyl, C_2-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_6-C_{15} arylalkynyl, C_2-C_{15} alkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroaralkyl, C_5-C_6 aryl, C_2-C_6 heterocycloalkyl,

C_2-C_{15} alkyl, C_3-C_{15} alkenyl, C_6-C_{15} arylalkenyl, C_3-C_{15} alkynyl, C_7-C_{15} arylalkynyl, C_1-C_6-alkylamino-C_1-C_6 alkyl, C_5-C_{15} aralkyl, C_6-C_{15} heteroalkyl or C_3-C_6 heterocycloalkyl wherein methylene in the alkyl moiety not adjacent to N^6 has been replaced by -O-,

optionally both R^{10} are joined together with N to form a saturated or unsaturated C_2-C_5 heterocycle containing one or two N heteroatoms and optionally an additional O or S heteroatom,

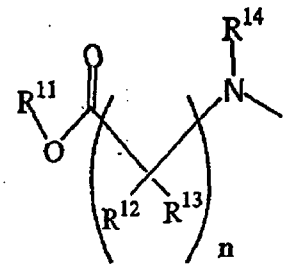
or one of the foregoing R^{10} groups is substituted with 1 to 3 halo, CN or N3; but optionally at least one R^{10} group is not H; and

Z is N or CH, provided that the heterocyclic nucleus varies from purine by no more than one Z.

E groups represent the aglycons employed in the methoxyphosphonate nucleotide analogues. Preferably, the E group is $-CH(CH_3)CH_2-$ or $-CH_2CH_2-$. Also, it is preferred that the side groups at chiral centers in the aglycon be substantially solely in the (*R*) configuration (except for hydroxymethyl, which is the enriched (*S*) enantiomer).

R^1 is an *in vivo* hydrolyzable oxyester having the structure $-OR^{35}$ or $-OR^6$ wherein R^{35} is defined in column 64, line 49 of U.S. Patent No. 5,798,340, herein incorporated by reference, and R^6 is defined above. Preferably R^1 is aryloxy, ordinarily unsubstituted or para-substituted (as defined in R^6) phenoxy.

R^2 is an amino acid residue, optionally provided that any carboxy group linked by less than about 5 atoms to the amidate N is esterified. R^2 typically has the structure



(8)

wherein

n is 1 or 2;

R^{11} is R^6 or H; preferably R^6 = C_3-C_9 alkyl; C_3-C_9 alkyl substituted independently with OH, halogen, O or N; C_3-C_6 aryl; C_3-C_6 aryl which is independently substituted with OH, halogen, O or N; or C_3-C_6arylalkyl which is independently substituted with OH, halogen, O or N;

R^{12} independently is H or C_1-C_9 alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, $COOR^{11}$ and halogen; C_3-C_6aryl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, $COOR^{11}$ and halogen; or C_3-C_9 aryl-alkyl which is unsubstituted or substituted by substituents independently selected from the group consisting of OH, O, N, $COOR^{11}$ and halogen;

R^{13} independently is C(O)-OR^{11}; amino; amide; guanidinyl; imidazolyl; indolyl; sulfoxide; phosphoryl; C_1-C_3 alkylamino; C_1-C_3 alkyldiamino; C_1-C_6 alkenylamino; hydroxy; thiol; C_1-C_3 alkoxy; C_1-C_3 alkthiol; $(CH_2)_nCOOR^{11}$; C_1-C_6 alkyl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_{10} alkoxyphenyl; C_2-C_6 alkenyl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_{10} alkoxyphenyl; and C_6-C_{12} aryl which is unsubstituted or substituted with OH, halogen, SH, NH_2, phenyl, hydroxyphenyl or C_7-C_{10} alkoxyphenyl; and

R^{14} is H or C_1-C_9 alkyl or C_1-C_3 alkyl independently substituted with OH, halogen, $COOR^{11}$, O or N; C_3-C_6 aryl; C_3-C_6 aryl which is independently substituted with OH, halogen, $COOR^{11}$, O or N; or C_3-C_6 arylalkyl which is independently substituted with OH, halogen, $COOR^{11}$, O or N.

Preferably, R^{11} is C_1-C_6 alkyl, most preferably isopropyl, R^{13} is the side chain of a naturally occurring amino acid, n = 1, R^{12} is H and R^{14} is H. In the compound of structure (2), the invention includes metabolites in which the phenoxy and isopropyl esters have been hydrolyzed to -OH. Similarly, the de-esterified enriched phosphonoamidate metabolites of compounds (5a), 5(b) and (6) are included within the scope of this invention.

Aryl and "O" or "N" substitution are defined in column 16, lines 42-58, of

United States Patent No. 5,798,340.

Typically, the amino acids are in the natural or *l* amino acids. Suitable specific examples are set forth in U. S. Patent No. 5,798,340, for instance Table 4 and col. 8-10 therein.

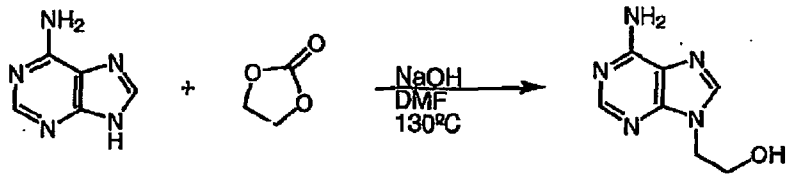
Alkyl as used herein, unless stated to the contrary, is a normal, secondary, tertiary or cyclic hydrocarbon. Unless stated to the contrary alkyl is C_1-C_{12}. Examples are -CH_3, -CH_2CH_3, -$CH_2CH_2CH_3$, -$CH(CH_3)_2$, -$CH_2CH_2CH_2CH_3$), -$CH_2CH(CH_3)_2$, -$CH(CH_3)CH_2CH_3$, -$C(CH_3)_3$, -$CH_2CH_2CH_2CH_2CH_3$, -$CH(CH_3)CH_2CH_2CH_3$, -$CH(CH_2CH_3)_2$, -$C(CH_3)_2CH_2CH_3$), -$CH(CH_3)CH(CH_3)_2$, -$CH_2CH_2CH(CH_3)_2$), -$CH_2CH(CH_3)CH_2CH_3$, -$CH_2CH_2CH_2CH_2CH_2CH_3$, -$CH(CH_3)CH_2CH_2CH_2CH_3$, -$CH(CH_2CH_3)(CH_2CH_2CH_3)$, -$C(CH_3)_2CH_2CH_2CH_3$, -$CH(CH_3)CH(CH_3)CH_2CH_3$, -$CH(CH_3)CH_2CH(CH_3)_2$, -$C(CH_3)(CH_2CH_3)_2$, -$CH(CH_2CH_3)CH(CH_3)_2$, -$C(CH_3)_2CH(CH_3)_2$, and -$CH(CH_3)C(CH_3)_3$. Alkenyl and alkynyl are defined in the same fashion, but contain at least one double or triple bond, respectively.

Where enol or keto groups are disclosed, the corresponding tautomers are to be construed as taught as well.

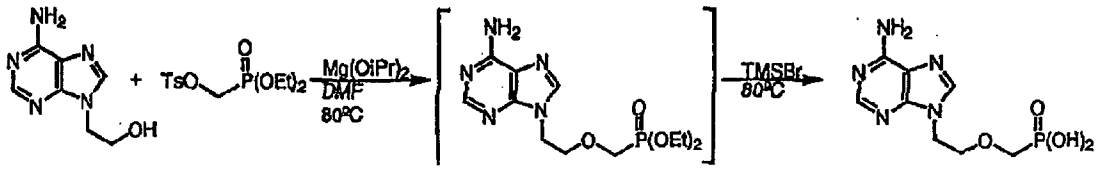
The prodrug compounds of this invention are provided in the form of free base or the various salts enumerated in U. S. Patent No. 5,798,340, and are formulated with pharmaceutically acceptable excipients or solvating diluents for use as pharmaceutical products also as set forth in U. S. Patent No. 5,798,340. These prodrugs have the antiviral and utilities already established for the parent drugs (see U. S. Patent 5,798,340 and other citations relating to the methoxyphosphonate nucleotide analogues). It will be understood that the diastereomer of structure (4) at least is useful as an intermediate in the chemical production of the parent drug by hydrolysis *in vitro*, regardless of its relatively unselective character as revealed in the studies herein.

The invention will be more fully understood by reference to the following examples:

Example 1a



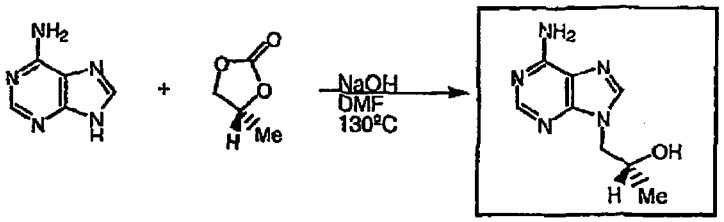
Adenine to PMEA using Magnesium Isopropoxide. To a suspension of adenine (16.8g, 0.124 mol) in DMF (41.9 ml) was added ethylene carbonate (12.1g, 0.137 mol) and sodium hydroxide (.100g, 0.0025 mol). The mixture was heated at 130°C overnight. The reaction was cooled to below 50°C and toluene (62.1 ml) was added. The slurry was further cooled to 5°C for 2 hours, filtered, and rinsed with toluene (2x). The wet solid was dried *in vacuo* at 65°C to yield 20.0g (90%) of 9-(2-hydroxyethyl)adenine as an off-white solid. Mp: 238-240°C.



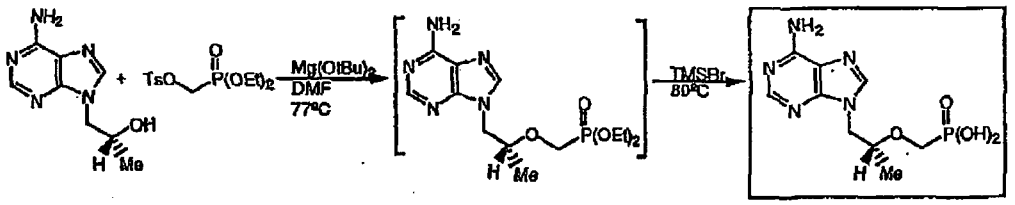
9-(2-Hydroxyethyl)adenine (HEA) (20.0g, 0.112 mol) was suspended in DMF (125 ml) and heated to 80°C. Magnesium isopropoxide (11.2g, 0.0784 mol) , or alternatively magnesium t-butoxide, was added to the mixture followed by diethyl *p*-toluenesulfonyloxymethylphosphonate (66.0g, 0.162 mol) over one hour. The mixture was stirred at 80°C for 7 hours. 30 ml of volatiles were removed via vacuum distillation and the reaction was recharged with 30 ml of fresh DMF. After cooling to room temperature, bromotrimethylsilane (69.6g, 0.450 mol) was added and the mixture heated to 80°C for 6 hours. The reaction was concentrated to yield a thick gum. The gum was dissolved into 360 ml water, extracted with 120 ml dichloromethane, adjusted to pH 3.2 with sodium hydroxide, and the resulting slurry stirred at room temperature overnight. The slurry was cooled to 4°C for one hour. The solids were isolated by filtration, washed with water (2x), and dried *in*

vacuo at 56°C to yield 20g (65.4%) of 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) as a white solid. Mp: > 200°C dec. ^{1}H NMR (D_2O) • 3.49 (t, 2H); 3.94 (t, 2H); 4.39 (t, 2H); 8.13 (*s*, 1H); 8.22 (*s*, 1H).

Example 1b



Adenine to PMPA using Magnesium t-Butoxide. To a suspension of adenine (40g, 0.296 mol) in DMF (41.9 ml) was added (*R*)-propylene carbonate (34.5g, 0.338 mol) and sodium hydroxide (.480g, 0.012 mol). The mixture was heated at 130°C overnight. The reaction was cooled to 100°C and toluene (138 ml) was added followed by methanesulfonic acid (4.7g, 0.049 mol) while maintaining the reaction temperature between 100-110°C. Additional toluene (114 ml) was added to create a homogeneous solution. The solution was cooled to 3°C over 7 hours and then held at 3°C for one hour. The resulting solid was isolated by filtration and rinsed with acetone (2x). The wet solid was dried *in vacuo* at 80°C to yield 42.6g (75%) of (*R*)-9-[2-(hydroxy)propyl]adenine (HPA) as an off-white solid. Mp: 188-190°C.



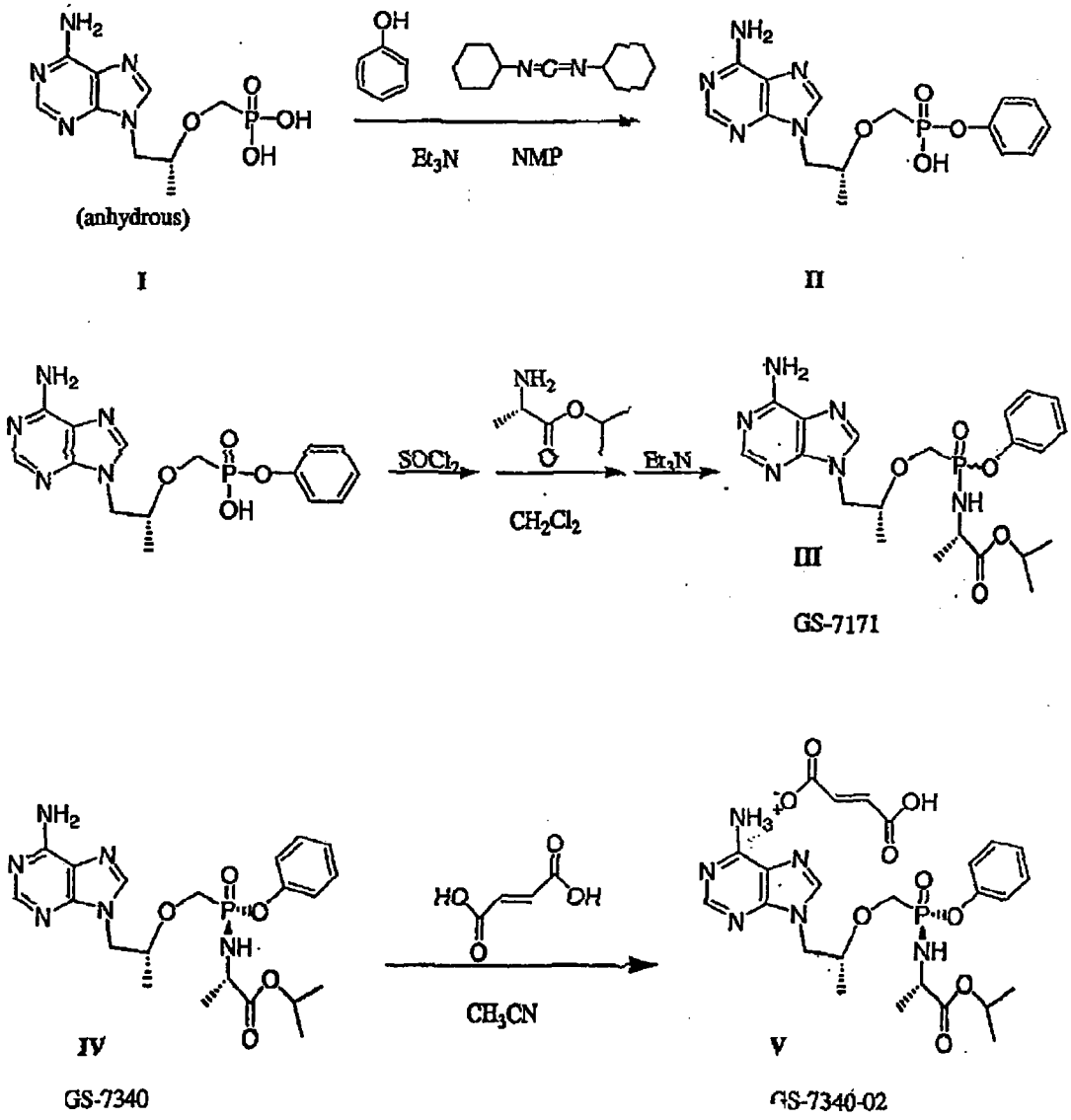
(*R*)-9-[2-(hydroxy)propyl]adenine (HPA) (20.0g, 0.104 mol) was suspended in DMF (44.5 ml) and heated to 65°C. Magnesium t-butoxide (14.2g, 0.083 mol), or alternatively magnesium isopropoxide, was added to the mixture over one hour followed by diethyl *p*-toluenesulfonyloxymethylphosphonate (66.0g, 0.205 mol)

over two hours while the temperature was kept at 78°C. The mixture was stirred at 75°C for 4 hours. After cooling to below 50°C, bromotrimethylsilane (73.9g, 0.478 mol) was added and the mixture heated to 77°C for 3 hours. When complete, the reaction was heated to 80°C and volatiles were removed via atmospheric distillation. The residue was dissolved into water (120 ml) at 50°C and then extracted with ethyl acetate (101 ml). The pH of the aqueous phase was adjusted to pH 1.1 with sodium hydroxide, seeded with authentic *(R)*-PMPA, and the pH of the aqueous layer was readjusted to pH 2.1 with sodium hydroxide. The resulting slurry was stirred at room temperature overnight. The slurry was cooled to 4°C for three hours. The solid was isolated by filtration, washed with water (60 ml), and dried *in vacuo* at 50°C to yield 18.9g (63.5%) of crude*(R)*-9-[2-(phosphonomethoxy)propyl]adenine (PMPA) as an off-white solid.

The crude*(R)*-9-[2-(phosphonomethoxy)propyl]adenine was heated at reflux in water (255 ml) until all solids dissolved. The solution was cooled to room temperature over 4 hours. The resulting slurry was cooled at 4°C for three hours. The solid was isolated by filtration, washed with water (56 ml) and acetone (56 ml), and dried *in vacuo* at 50°C to yield 15.0g (50.4%) of *(R)*-9-[2-(phosphonomethoxy)propyl]adenine (PMPA) as a white solid. Mp: 278-280°C.

Example 2
Preparation of GS-7171 (III)

Scheme 1



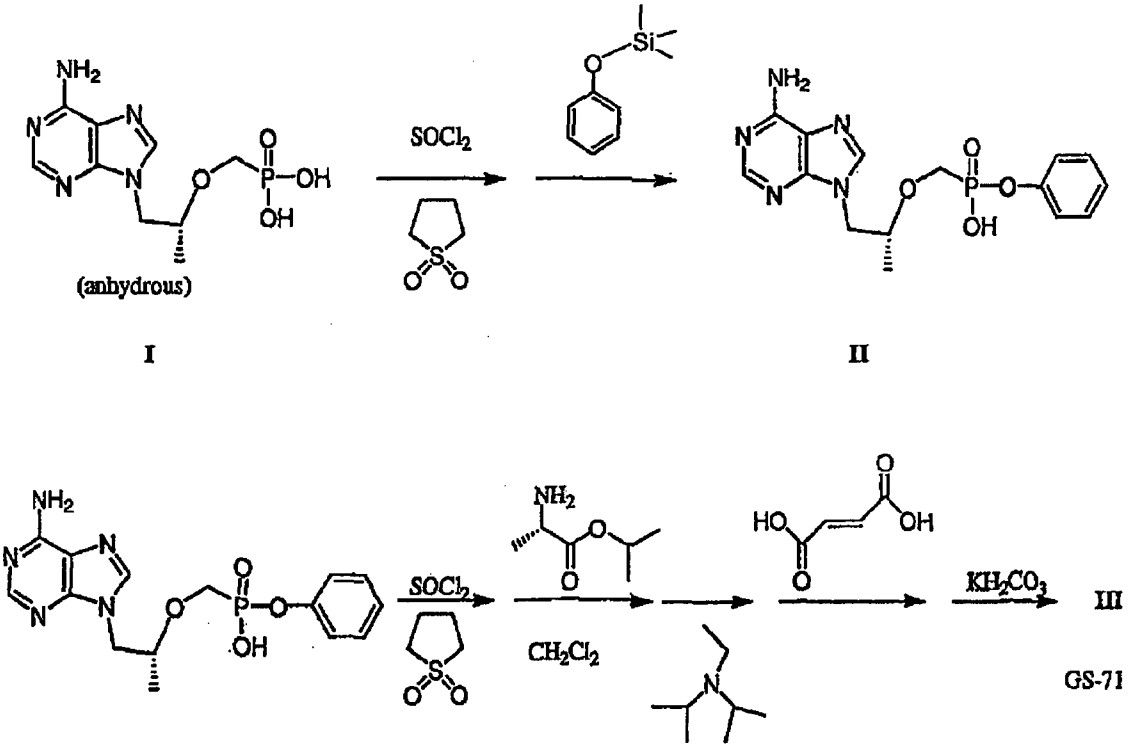
A glass-lined reactor was charged with anhydrous PMPA, (I) (14.6 kg, 50.8 mol), phenol (9.6 kg, 102 mol), and 1-methyl-2-pyrrolidinone (39 kg). The mixture was heated to 85°C and triethylamine (6.3 kg, 62.3 mol) added. A solution of 1,3-dicyclohexylcarbodiimide (17.1 kg, 82.9 mol) in 1-methyl-2-pyrrolidinone (1.6 kg) was then added over 6 hours at 100°C. Heating was continued for 16 hours. The reaction was cooled to 45°C, water (29 kg) added, and cooled to 25°C. Solids were removed from the reaction by filtration and rinsed with water (15.3 kg). The combined filtrate and rinse was concentrated to a tan slurry under reduced pressure, water (24.6 kg) added, and adjusted to pH = 11 with NaOH (25% in water). Fines were removed by filtration through diatomaceous earth (2 kg) followed by a water (4.4 kg) rinse. The combined filtrate and rinse was extracted with ethyl acetate (28 kg). The aqueous solution was adjusted to pH = 3.1 with HCl (37% in water) (4 kg). Crude II was isolated by filtration and washed with methanol (12.7 kg). The crude II wet cake was slurried in methanol (58 kg). Solids were isolated by filtration, washed with methanol (8.5 kg), and dried under reduced pressure to yield 9.33 kg II as a white powder: ^{1}H NMR (D_2O) δ 1.2 (d, 3H), 3.45 (q, 2H), 3.7 (q, 2H), 4 (m, 2H), 4.2 (q, 2H), 4.35 (dd, 2H), 6.6 (d, 2H), 7 (t, 1H), 7.15 (t, 2H), 8.15 (s, 1H), 8.2 (s, 1H); ^{31}P NMR (D_2O) δ 15.0 (decoupled).

GS-7171 (III). (Scheme 1) A glass-lined reactor was charged with monophenyl PMPA, (II), (9.12 kg, 25.1 mol) and acetonitrile (30.7 kg). Thionyl chloride (6.57 kg, 56.7 mol) was added below 50°C. The mixture was heated at 75°C until solids dissolved. Reaction temperature was increased to 80°C and volatiles (11.4 kg) collected by atmospheric distillation under nitrogen. The pot residue was cooled to 25°C, dichloromethane (41 kg) added, and cooled to -29°C. A solution of (L)-alanine isopropyl ester (7.1 kg, 54.4 mol) in dichloromethane (36 kg) was added over 60 minutes at -18°C followed by triethylamine (7.66 kg, 75.7 mol) over 30 minutes at -18 to -11°C. The reaction mixture was warmed to room temperature and washed five times with sodium dihydrogenphosphate solution (10% in water, 15.7 kg each wash). The organic solution was dried with anhydrous sodium sulfate (18.2 kg), filtered, rinsed with dichloromethane (28 kg), and concentrated to an oil

under reduced pressure. Acetone (20 kg) was charged to the oil and the mixture concentrated under reduced pressure. Acetone (18.8 kg) was charged to the resulting oil. Half the product solution was purified by chromatography over a 38 x 38 cm bed of 22 kg silica gel 60, 230 to 400 mesh. The column was eluted with 480 kg acetone. The purification was repeated on the second half of the oil using fresh silica gel and acetone. Clean product bearing fractions were concentrated under reduced pressure to an oil. Acetonitrile (19.6 kg) was charged to the oil and the mixture concentrated under reduced pressure. Acetonitrile (66.4 kg) was charged and the solution chilled to 0 to -5°C for 16 hours. Solids were removed by filtration and the filtrate concentrated under reduced pressure to 5.6 kg III as a dark oil: ^{1}H NMR ($CDCl_3$) δ 1.1 (m 12H), 3.7 (m, 1H), 4.0 (m, 5H), 4.2 (m, 1H), 5.0 (m, 1H), 6.2 (s, 2H), 7.05 (m, 5H), 8.0 (s, 1H), 8.25 (d, 1H); ^{31}P NMR ($CDCl_3$) δ 21.0, 22.5 (decoupled).

Alternate Method for GS-7171(III)

Scheme 2



I II III

GS-71

Monophenyl PMPA (II). A round-bottom flask with reflux condenser and nitrogen inlet was placed in a 70°C oil bath. The flask was charged with anhydrous PMPA (I) (19.2 g, 67 mmol), *N,N*-dimethylformamide (0.29 g, 3.3 mmol), and tetramethylene sulfone (40 mL). Thionyl chloride (14.2 g, 119 mmol) was added over 4 hours. Heating was increased to 100°C over the same time. A homogeneous solution resulted. Phenoxytrimethylsilane (11.7 g, 70 mmol) was added to the solution over 5 minutes. Heating in the 100°C oil bath continued for two hours more. The reaction was poured into rapidly stirring acetone (400 mL) with cooling at 0°C. Solids were isolated by filtration, dried under reduced pressure, and dissolved in methanol (75 mL). The solution pH was adjusted to 3.0 with potassium hydroxide solution (45% aq.) with cooling in ice/water. The resulting solids were isolated by filtration, rinsed with methanol, and dried under reduced pressure to 20.4 g II (Scheme 2) as a white powder.

GS-7171 (III). Monophenyl PMPA (II) (3 g, 8.3 mmol), tetramethylene sulfone (5 mL), and *N,N*-dimethylformamide (1 drop) were combined in a round bottom flask in a 40°C oil bath. Thionyl chloride (1.96 g, 16.5 mmol) was added. After 20 minutes the clear solution was removed from heat, diluted with dichloromethane (10 ml), and added to a solution of (L)-alanine isopropyl ester (5g, 33 mmol) and diisopropylethylamine (5.33 g, 41 mmol) in dichloromethane (20 mL) at -10°C. The reaction mixture was warmed to room temperature and washed three times with sodium dihydrogenphosphate solution (10% aq., 10 mL each wash). The organic solution was dried over anhydrous sodium sulfate and concentrated under reduced pressure to a oil. The oil was combined with fumaric acid (0.77g, 6.6 mmol) and acetonitrile (40 mL) and heated to reflux to give a homogeneous solution. The solution was cooled in an ice bath and solids isolated by filtration. The solid GS-7171 fumarate salt was dried under reduced pressure to 3.7 g. The salt (3.16 g, 5.3 mmol) was suspended in dichloromethane (30 mL) and stirred with potassium carbonate solution (5 mL, 2.5 M in water) until the solid dissolved. The organic layer was isolated, then washed with water (5 mL), dried over anhydrous

sodium sulfate, and concentrated under reduced pressure to afford 2.4 g III as a tan foam.

Example 3

A. *Diastereomer Separation* by Batch Elution Chromatography

The diastereomers of GS-7171 (III) were resolved by batch elution chromatography using a commercially available Chiralpak AS, 20 µm, 21 x 250 mm semi-preparative HPLC column with a Chiralpak AS, 20 µm, 21 x 50 mm guard column. Chiralpak® AS is a proprietary packing material manufactured by Diacel and sold in North America by Chiral Technologies, Inc. (U. S. Patent Nos. 5,202,433, RE 35,919, 5,434,298, 5,434,299 and 5,498,752). Chiralpak AS is a chiral stationary phase (CSP) comprised of amylosetris[(*S*)-α-methylbenzyl carbamate] coated onto a silica gel support.

The GS-7171 diastereomeric mixture was dissolved in mobile phase, and approximately 1 g aliquots of GS-7171 were pumped onto the chromatographic system. The undesired diastereomer, designated GS-7339, was the first major broad (approx. 15 min. duration) peak to elute from the column. When the GS-7339 peak had finished eluting, the mobile phase was immediately switched to 100% methyl alcohol, which caused the desired diastereomer, designated GS-7340 (IV), to elute as a sharp peak from the column with the methyl alcohol solvent front. The *methyl alcohol was used to reduce the over-all cycle time. After the first couple of* injections, both diastereomers were collected as a single large fractions containing one of the purified diastereomers (>99.0% single diastereomer). The mobile phase solvents were removed *in vacuo* to yield the purified diastereomer as a friable foam.

About 95% of the starting GS-7171 mass was recovered in the two diastereomer fractions. The GS-7340 fraction comprised about 50% of the total recovered mass.

The chromatographic conditions were as follows:

Mobile Phase(Initial)	:	GS-7171 – Acetonitrile : Isopropyl Alcohol (90:10)
(Final)	:	100% Methyl Alcohol
Flow	:	10 mL/minute
Run Time	:	About 45 minute
Detection	:	UV at 275 nm
Temperature	:	Ambient
Elution Profile	:	GS-7339 (diastereomer B)
	:	GS-7340 (diastereomer A; (IV))

B. Diastereomer Separation of GS-7171 by SMB Chromatography

For a general description of simulated moving bed (SMB) chromatography, see Strube et al., "Organic Process Research and Development" 2:305-319 (1998).

GS-7340 (IV). GS-7171 (III), 2.8 kg, was purified by simulated moving bed chromatography over 10 cm by 5 cm beds of packing (Chiral Technologies Inc., 20 micron Chiralpak AS coated on silica gel) (1.2 kg). The columns were eluted with 30% methanol in acetonitrile. Product bearing fractions were concentrated to a solution of IV in acetonitrile (2.48 kg). The solution solidified to a crystalline mass wet with acetonitrile on standing. The crystalline mass was dried under reduced pressure to a tan crystalline powder, 1.301 kg IV, 98.7% diastereomeric purity: mp 117 – 120°C; ^{1}H NMR ($CDCl_3$) δ 1.15 (m 12H), 3.7 (t, 1H), 4.0 (m, 5H), 4.2 (dd, 1H), 5.0 (m, 1H), 6.05 (s, 2H), 7.1 (m, 5H), 8.0 (s, 1H), 8.2 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 21.0 (decoupled).

C. Diastereomer Separation by C18 RP-HPLC

GS-7171 (III) was chromatographed by reverse phase HPLC to separate the diastereomers using the following summary protocol.

Chromatographic column:	Phenomenex Luna™ C18(2), 5 µm, 100 Å pore size, (Phenomenex, Torrance, CA), or equivalent
Guard column:	Pellicular C18 (Alltech, Deerfield, IL), or equivalent
Mobile Phase:	A — 0.02% (85%) H_3PO_4 in water : acetonitrile (95:5) B — 0.02% (85%) H_3PO_4 in water : acetonitrile (50:50)

Mobile Phase Gradient:

Time	% Mobile Phase A	% Mobile Phase B
0	100	0
5	100	0
7	70	30
32	70	30
40	0	100
50	0	100

Run Time:	50 minutes
Equilibration Delay:	10 min at 100% mobile phase A
Flow Rate:	1.2 mL/min
Temperature:	Ambient
Detection:	UV at 260 nm
Sample Solution:	20 mM sodium phosphate buffer, pH 6
Retention Times:	GS-7339, about 25 minutes
	GS-7340, about 27 minutes

D. Diastereomer Separation by Crystallization

GS-7340 (IV). A solution of GS-7171 (III) in acetonitrile was concentrated to an amber foam (14.9g) under reduced pressure. The foam was dissolved in acetonitrile (20 mL) and seeded with a crystal of IV. The mixture was stirred overnight, cooled to 5°C, and solids isolated by filtration. The solids were dried to 2.3 g IV as white crystals, 98% diastereomeric purity (^{31}P NMR): ^1H NMR ($CDCl_3$) δ 1.15 (m 12H), 3.7 (t, 1H), 3.95 (m, 2H), 4.05 (m, 2H), 4.2 (m, 2H), 5.0 (m, 1H), 6.4 (s, 2H), 7.1 (m, 5H), 8.0 (s, 1H), 8.2 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 19.5 (decoupled). X-ray crystal analysis of a single crystal selected from this product yielded the following data:

Crystal Color, Habit	colorless, column
Crystal Diminsions	0.25 X 0.12 X 0.08 mm
Crystal System	orthorhombic
Lattice Type	Primitive
Lattice Parameters	a = 8.352(1) Å b = 15.574(2) Å c = 18.253(2) Å V = 2374.2(5) Å^3
Space Group	$P2_12_12_1$ (#19)
Z value	4
D_{calc}	1.333 g/cm^3
F_{000}	1008.00
μ(MoKα)	1.60 cm^{-1}

Example 4

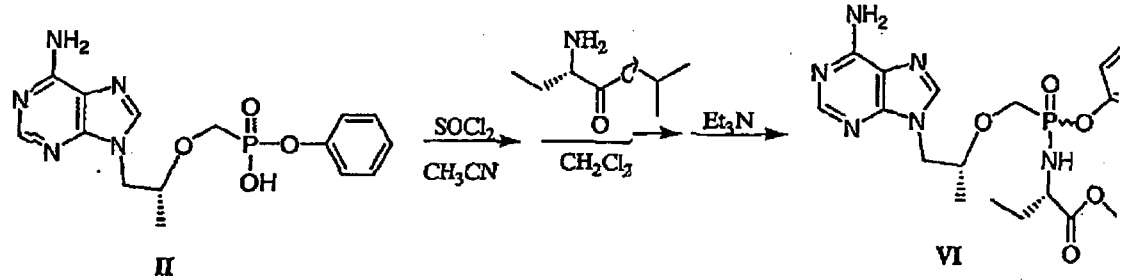
Preparation of Fumarate Salt of GS-7340

GS-7340-02 (V). (Scheme 1) A glass-lined reactor was charged with GS-7340 (IV), (1.294 kg, 2.71 mol), fumaric acid (284 g, 2.44 mol), and acetonitrile (24.6 kg). The mixture was heated to reflux to dissolve the solids, filtered while hot and cooled to 5°C for 16 hours. The product was isolated by filtration, rinsed with acetonitrile. (9.2 kg), and dried to 1329 g (V) as a white powder: mp 119.7 - 121.1°C; $[\alpha]_D^{20}$ –41.7° (*c* 1.0, acetic acid).

Example 5

Preparation of GS-7120 (VI)

Scheme 3



II VI

GS-7120

A 5 L round bottom flask was charged with monophenyl PMPA, (II), (200 g, 0.55 mol) and acetonitrile (0.629 kg). Thionyl chloride (0.144 kg, 1.21 mol) was added below 27°C. The mixture was heated at 70°C until solids dissolved. Volatiles (0.45 L) were removed by atmospheric distillation under nitrogen. The pot residue was cooled to 25°C, dichloromethane (1.6 kg) was added and the mixture was cooled to –20°C. A solution of (L)-α aminobutyric acid ethyl ester (0.144 kg, 1.1 mol) in dichloromethane (1.33 kg) was added over 18 minutes at –20 to -10°C followed by triethylamine (0.17 kg, 1.65 mol) over 15 minutes at –8 to –15°C. The reaction mixture was warmed to room temperature and washed four times with sodium dihydrogenphosphate solution (10% aq., 0.3 L each wash). The organic solution was dried with anhydrous sodium sulfate (0.5 kg) and filtered. The solids were rinsed with dichloromethane (0.6 kg) and the combined filtrate and rinse was concentrated to an oil under reduced pressure. The oil was purified by chromatography over a 15 x 13 cm bed of 1.2 kg silica gel 60, 230 to 400 mesh. The column was eluted with a gradient of dichloromethane and methanol. Product bearing fractions were concentrated under reduced pressure to afford 211 g VI (Scheme 3) as a tan foam.

Example 5a

Diastereomer Separation of GS-7120 by Batch Elution Chromatography

The diastereomeric mixture was purified using the conditions described for GS-7171 in Example 3A except for the following:

Mobile Phase	(Initial)	: GS-7120 – Acetonitrile : Isopropyl Alcohol (98:2)
	(Final)	: 100% Methyl Alcohol
Elution Profile		: GS-7341 (diastereomer B)
		: GS-7342 (diastereomer A)

Example 6

Diastereomer Separation of GS-7120 by Crystallization

A 1 L round bottom flask was charged with monophenyl PMPA, (II), (50 g, 0.137 mol) and acetonitrile (0.2 L). Thionyl chloride (0.036 kg, 0.303 mol) was added with a 10°C exotherm. The mixture was heated to reflux until solids dissolved. Volatiles (0.1 L) were removed by atmospheric distillation under nitrogen. The pot residue was cooled to 25°C, dichloromethane (0.2 kg) was added, and the mixture was cooled to –20°C. A solution of (L)-α aminobutyric acid ethyl ester (0.036 kg, 0.275 mol) in dichloromethane (0.67 kg) was added over 30 minutes at –20 to -8°C followed by triethylamine (0.042 kg, 0.41 mol) over 10 minutes at up to –6°C. The reaction mixture was warmed to room temperature and washed four times with sodium dihydrogenphosphate solution (10% aq., 0.075 L each wash). The organic solution was dried with anhydrous sodium sulfate (0.1 kg) and filtered. The solids were rinsed with ethyl acetate (0.25 L, and the combined filtrate and rinse was concentrated to an oil under reduced pressure. The oil was diluted with ethyl acetate (0.25 L), seeded, stirred overnight, and chilled to – 15°C. The solids were isolated by filtration and dried under reduced pressure to afford 17.7 g of GS-7342 (Table 5) as a tan powder: ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H), 1.3 (m, 6H), 1.7, (m, 2H),

3.7 (m, 2H), 4.1(m, 6H), 4.4 (dd, 1H), 5.8 (s, 2H), 7.1 (m, 5H), 8.0 (s, 1H), 8.4 (s, 1H); ^{31}P NMR ($CDCl_3$) δ 21 (decoupled).

Example 7

Diastereomer Separation of GS-7097

The diastereomeric mixture was purified using the conditions described for GS-7171 (Example 3A) except for the following:

Mobile Phase	(Initial)	: GS-7120 – Acetonitrile : Isopropyl Alcohol (95:5)
	(Final)	: 100% Methyl Alcohol
Elution Profile		: GS-7115 (diastereomer B)
		: GS-7114 (diastereomer A)

Example 8

Alternative Procedure for Preparation of GS-7097

GS-7097: Phenyl PMPA, Ethyl L-Alanyl Amidate. Phenyl PMPA (15.0 g, 41.3 mmol), L-alanine ethyl ester hydrochloride (12.6 g, 83 mmol) and triethylamine (11.5 mL, 83 mmol) were slurried together in 500 mL pyridine under dry N_2. This suspension was combined with a solution of triphenylphosphine (37.9 g, 145 mmol), Aldrithiol 2 (2,2'-dipyridyl disulfide) (31.8 g, 145 mmol), and 120 mL pyridine. The mixture was heated at an internal temperature of 57°C for 15 hours. The complete reaction was concentrated under vacuum to a yellow paste, 100 g. The paste was purified by column chromatography over a 25 x 11 cm bed of 1.1 kg silica gel 60, 230 to 400 mesh. The column was eluted with 8 liters of 2% methanol in dichloromethane followed by a linear gradient over a course of 26 liters eluent up to a final composition of 13% methanol. Clean product bearing fractions were concentrated to yield 12.4 g crude (5), 65% theory. This material was contaminated with about 15% (weight) triethylamine hydrochloride by ^1H NMR. The contamination was removed by dissolving the product in 350 mL ethyl acetate, extracting with 20 mL water, drying the organic solution over anhydrous sodium

sulfate, and concentrating to yield 11.1 g pure GS-7097 as a white solid, 58% yield. The process also is employed to synthesize the diastereomeric mixture of GS-7003a and GS-7003b (the phenylalanyl amidate) and the mixture GS-7119 and GS-7335 (the glycyl amidate). These diastereomers are separated using a batch elution procedure such as shown in Example 3A, 6 and 7.

Example 9

In Vitro Studies of Prodrug Diastereomers

The *in vitro* anti-HIV-1 activity and cytotoxicity in MT-2 cells and stability in human plasma and MT-2 cell extracts of GS-7340 (freebase) and tenofovir disoproxil fumarate (TDF), are shown in Table 1. GS-7340 shows a 10-fold increase in antiviral activity relative to TDF and a 200-fold increase in plasma stability. This greater plasma stability is expected to result in higher circulating levels of GS-7340 than TDF after oral administration.

Table 1. *In Vitro* Activity and Stability

	HIV-1 Activity	Cytotoxicity	Stability T 1/2 (min)		
	$IC_{50\mu M}$	$CC_{50\mu M}$	Human Plasma	MT-2 Cell Extract	(P/MT-2)
GS 7340	0.005	>40	90.0	28.3	3.2
TDF	0.05	70	0.41	70.7	0.006
Tenofovir	5	6000	--	--	--

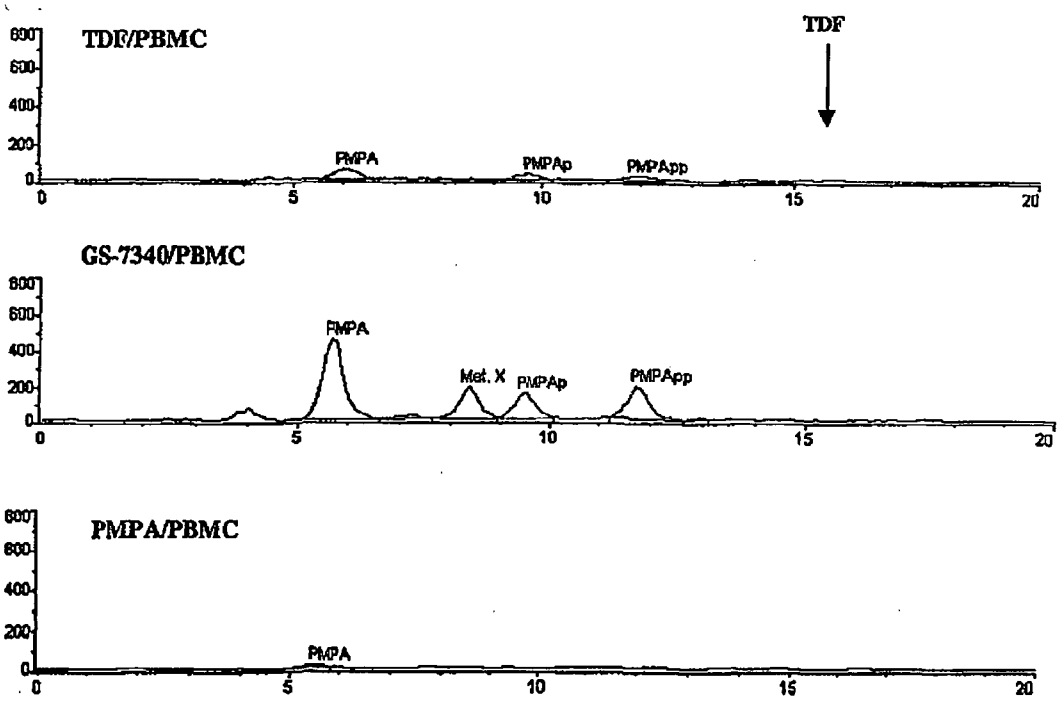
In order to estimate the relative intracellular PMPA resulting from the intracellular metabolism of TDF as compared to that from GS-7340, both prodrugs and PMPA were radiolabeled and spiked into intact human whole blood at equimolar concentrations. After 1 hour, plasma, red blood cells (RBCs) and peripheral blood mononuclear cells (PBMCs) were isolated and analyzed by HPLC with radiometric detection. The results are shown in Table 2.

After 1 hour, GS-7340 results in 10x and 30x the total intracellular concentration of PMPA species in PBMCs as compared to TDF and PMPA, respectively. In plasma after 1 hour, 84% of the radioactivity is due to intact GS-7340, whereas no TDF is detected at 1 hour. Since no intact TDF is detected in plasma, the 10x difference at 1 hour between TDF and GS-7340 is the minimum difference expected *in vivo*. The HPLC chromatogram for all three compounds in PBMCs is shown in Figure 1.

Table 2. PMPA Metabolites in Plasma, PBMCs and RBCs After 1 h Incubation of PMPA Prodrugs or PMPA in Human Blood.

Compound	Matrix	Total C-14 Recovered, µg-eq	Metabolites (% of Total Peak Area)					
			PMPA %	PMPAp, %	PMPApp, %	Met. X, %	Met. Y, %	GS 7340, %
GS-7340 (60 µg-eq)	Plasma/FP	43.0	1	-	-	2	13	84
	PBMC	1.25	45	16	21	18	-	-
	RBC/FP	12.6	8	-	-	24	11	57
			PMPA	PMPAp	PMPApp	Mono-POC	GS-4331	
GS-4331 (TDF) (60 µg-eq)	Plasma/FP	48.1	11	-	-	89	-	
	PBMC	0.133	50	25	18	7	-	
	RBC/FP	10.5	93	7.0	-	-	-	
			PMPA	PMPAp	PMPApp			
PMPA (60 µg-eq)	Plasma/FP	55.7	100	-	-			
	PBMC	0.033	86	14	-			
	RBC/FP	3.72	74	10	16			

Figure 1. HPLC/C-14 Traces of PBMC Extracts from Human Blood Incubated for 1 h at 37°C with TDF, GS-7340 or PMPA.



Met. X and Met Y (metabolites X and Y) are shown in Table 5. Lower case "p" designates phosphorylation. These results were obtained after 1 hour in human blood. With increasing time, the *in vitro* differences are expected to increase, since 84% of GS-7340 is still intact in plasma after one hour. Because intact GS-7340 is present in plasma after oral administration, the relative clinical efficacy should be related to the IC_{50} values seen *in vitro*.

In Table 3 below, IC_{50} values of tenofovir, TDF, GS-7340, several nucleosides and the protease inhibitor nelfinivir are listed. As shown, nelfinavir and GS-7340 are 2-3 orders of magnitude more potent than all other nucleotides or nucleosides.

5

Table 3. *In Vitro* Anti-HIV-1 Activities of Antiretroviral Compounds

Compound	IC_{50} (μM)
Adefovir (PMEA)	13.4 ± 4.2^1
Tenofovir (PMPA)	6.3 ± 3.3^1
AZT	0.17 ± 0.08^1
3TC	1.8 ± 0.25^1
d4T	8 ± 2.5^1
Nelfinavir	0.006 ± 0.002^1
TDF	0.05
GS 7340	0.005

1. A. S. Mulato and J. M. Cherrington, *Antiviral Research* 36, 91 (1997)

10 Additional studies of the *in vitro* cell culture anti-HIV-1 activity and CC_{50} of separated diastereomers of this invention were conducted and the results tabulated below.

15

Table 4. Effect of Diastereomer

Compound	Diastereomer	IC_{50} (μM)	Fold change	A/B activity	CC_{50} (μM)
PMPA	-	5	1x	-	6000
Ala-methylester	Mixture 1:1	0.025	200x	20x	80
GS-6957a	A	0.0075	670x		
GS-6957b		0.15	33x		
Phe-methylester	Mixture 1:1	0.03	170x	10x	60
GS-7003a	A	0.01	500x		
GS-7003b	B	0.1	50x		
Gly-ethylester	Mixture 1:1	0.5	10x	20x	
GS-7119	A	0.05	100x		>100
GS-7335	B	1.0	5x		
Ala-isopropyl	Mixture 1:1	0.01	500x	12x	
GS-7340	A	0.005	1,000x		40
GS-7339	B	0.06	83x		>100
ABA-ethyl	Mixture 1:1	0.008	625x	7.5x	>100
GS-7342	A	0.004	1,250x		
GS-7341	B	0.03	170x		
Ala-ethyl	Mixture 1:1	0.02	250x	10x	60
GS-7114	A	0.005	1,000x		
GS-7115	B	0.05	100x		

Assay reference: Arimilli, MN, et al., (1997) Synthesis, in vitro biological evaluation and oral bioavailability of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) prodrugs. Antiviral Chemistry and Chemotherapy 8(6):557-564.

"Phe-methylester" is the methylphenylalaninyl monoamidate, phenyl monoester of tenofovir; "gly-methylester" is the methylglycyl monoamidate, phenyl monoester of tenofovir.

In each instance above, isomer A is believed to have the same absolute stereochemistry as GS-7340 (*S*), and isomer B is believed to have the same absolute stereochemistry that of GS-7339.

The *in vitro* metabolism and stability of separated diastereomers were determined in PLCE, MT-2 extract and human plasma. A biological sample listed below, 80 µL, was transferred into a screw-capped centrifuge tube and incubated at 37°C for 5 min. A solution containing 0.2 mg/mL of the test compound in a suitable buffer, 20 µL, was added to the biological sample and mixed. The reaction mixture, 20 µL, was immediately sampled and mixed with 60 µL of methanol containing 0.015 mg/mL of 2-hydroxymethylnaphthalene as an internal standard for HPLC analysis. The sample was taken as the time-zero sample. Then, at specific time points, the reaction mixture, 20 µL, was sampled and mixed with 60 µL of methanol containing the internal standard. The mixture thus obtained was centrifuged at 15,000 G for 5 min and the supernatant was analyzed with HPLC under the conditions described below.

The biological samples evaluated are as follows.

(1) PLCE (porcine liver carboxyesterase from Sigma, 160 u/mg protein, 21 mg protein/mL) diluted 20 fold with PBS (phosphated-buffered saline).

(2) MT-2 cell extract was prepared from MT-2 cells according to the published procedure [A. Pompon, I. Lefebvre, J.-L. Imbach, S. Kahn, and D. Farquhar, "Antiviral Chemistry & Chemotherapy", 5:91–98 (1994)] except for using HEPES buffer described below as the medium.

(3) Human plasma (pooled normal human plasma from George King Biomedical Systems, Inc.)

The buffer systems used in the studies are as follows.

In the study for PLCE, the test compound was dissolved in PBS. PBS (phosphate buffered saline, Sigma) contains 0.01 M phosphate, 0.0027 M potassium chloride, and 0.137 M sodium chloride. pH 7.4 at 37°C.

In the study for MT-2 cell extracts, the test compound was dissolved in HEPES buffer. HEPES buffer contains 0.010 M HEPES, 0.05 M potassium chloride, 0.005 M magnesium chloride, and 0.005 M *dl*-dithiothreitol. pH 7.4 at 37°C.

In the study for human plasma, the test compound was dissolved in TBS. TBS (tris-buffered saline, Sigma) contains 0.05 M Tris, 0.0027 M potassium chloride, and 0.138 M sodium chloride. pH 7.5 at 37°C.

The HPLC analysis was carried out under the following conditions.

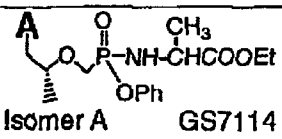
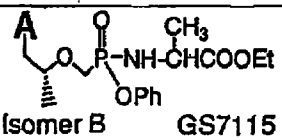
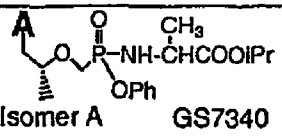
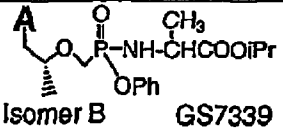
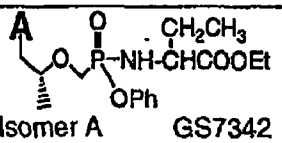
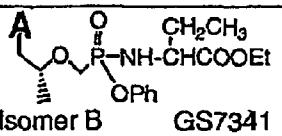
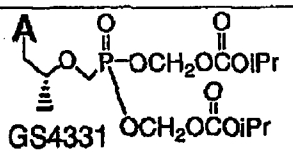
Column:	Zorbax R_X-C8, 4.6 x 250 mm, 5 µ (MAC-MOD Analytical, Inc. Chadds Ford, PA)
Detection:	UV at 260 nm
Flow Rate:	1.0 mL/min
Run Time:	30 min
Injection Volume:	20 µL
Column Temperature:	Ambient temperature

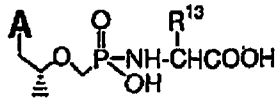
Mobile Phase A:	50 mM potassium phosphate (pH 6.0)/CH_3CN = 95/5 (v/v)
Mobile Phase B:	50 mM Potassium phosphate (pH 6.0)/CH_3CN = 50/50 (v/v)

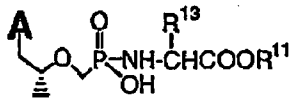
Gradient Run:	0 min	100% Mobile Phase A
	25 min	100% Mobile Phase B
	30 min	100% Mobile Phase B

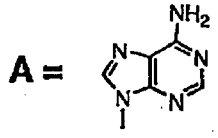
The results are shown below in Table 5 (also including selected IC_{50} data from Table 4).

Table 5. In Vitro Metabolism of Isomers A and B of PMPA monoamidate at 37°C

No.	PMPA monoamidate structure	HIV IC_{50} (μM)	PLCE hydrolysis rate and product	MT-2 extract hydrolysis rate and product	Human Plasma Stability (HP)
1	 Isomer A GS7114	0.005	$t_{1/2}$ = 2.9 min Met. X & PMPA	$t_{1/2}$ = 2.9 min Met. X & PMPA	$t_{1/2}$ = 148 min Met. Y
2	 Isomer B GS7115	0.05	$t_{1/2}$ = 8.0 min Met. X & PMPA	$t_{1/2}$ = 150.6 min Met. X & PMPA	$t_{1/2}$ = 495 min Met. Y
3	 Isomer A GS7340	0.005	$t_{1/2}$ = 3.3 min Met. X & PMPA	$t_{1/2}$ = 28.3 min Met. X & PMPA	$t_{1/2}$ = 90.0 min Met. Y
4	 Isomer B GS7339	0.06	$t_{1/2}$ = 10.1 min Met. X & PMPA	$t_{1/2}$ > 1000 min	$t_{1/2}$ = 231 min Met. Y
5	 Isomer A GS7342	0.004	$t_{1/2}$ = 3.9 min Met. X	$t_{1/2}$ = 49.2 min Met. X & PMPA	$t_{1/2}$ = 103 min Met. Y
6	 Isomer B GS7341	0.03	$t_{1/2}$ = 11.3 min Met. X	$t_{1/2}$ > 1000 min	$t_{1/2}$ = 257 min Met. Y
7	 GS4331	0.05	$t_{1/2}$ < 0.14 min MonoPOC PMPA	$t_{1/2}$ = 70.7 min monoPOC PMPA	$t_{1/2}$ = 0.41 min monoPOC PMPA

Met. X: 

Met. Y: 



Example 10

Plasma and PBMC Exposures Following Oral Administration Of Prodrug Diastereomers to Beagle Dogs

The pharmacokinetics of GS 7340 were studied in dogs after oral administration of a 10 mg-eq/kg dose.

Formulations. The prodrugs were formulated as solutions in 50 mM citric acid within 0.5 hour prior to dose. All compounds used in the studies were synthesized by Gilead Sciences. The following lots were used:

GSI	Amidate Amino acid	AA Ester	Diastereoisomer	Lot Number
GS-7340-2	Alanine	i-Propyl	Isomer A	1504-187-19
GS-7339	Alanine	i-Propyl	Isomer B	1509-185-31
GS7114	Alanine	Ethyl	Isomer A	1509-181-26
GS7115	Alanine	Ethyl	Isomer B	1509-181-22
GS7119	Glycine	Ethyl	Isomer A	1428-163-28
GS7342	α-Aminobutyric Acid	Ethyl	Isomer A	1509-191-12
GS7341	α-Aminobutyric Acid	Ethyl	Isomer B	1509-191-7

Dose Administration and Sample Collection. The in-life phase of this study was conducted in accordance with the recommendations of the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication 86-23) and was approved by an Institutional Animal Care and Use Committee. Fasted male beagle dogs (10 ± 2 kg) were used for the studies. Each drug was administered as a single dose by oral gavage (1.5–2 ml/kg). The dose was 10 mg-equivalent of PMPA/kg. For PBMCs, blood samples were collected at 0 (pre-dose), 2, 8, and 24 h post-dose. For plasma, blood samples were collected at 0 (pre-dose), 5, 15, and 30 min, and 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose. Blood (1.0 ml) was processed immediately for plasma by centrifugation at 2,000 rpm for 10 min. Plasma samples were frozen and maintained at 70°C until analyzed.

Peripheral Blood Mononuclear Cell (PBMC) preparation. Whole blood (8 ml) drawn at specified time points was mixed in equal proportion with phosphate buffered saline (PBS), layered onto 15 ml of Ficoll-Paque solution (Pharmacia Biotech,) and centrifuged at 400 x g for 40 min. PBMC layer was removed and

washed once with PBS. Formed PMBC pellet was reconstituted in 0.5 ml of PBS, cells were resuspended, counted using hemocytometer and maintained at 70°C unti analyzed. The number of cells multiplied by the mean single-cell volume was used in calculation of intracellular concentrations. A reported value of 200 femtoliters/cell was used as the resting PBMC volume (B. L. Robins, R.V. Srinivas, C. Kim, N. Bischofberger, and A. Fridland, Antimicrob. Agents Chemother. 42, 612 (1998).

Determination of PMPA and Prodrugs in plasma and PBMCs. The concentration of PMPA in dog plasma samples was determined by derivatizing PMPA with chloroacetaldehyde to yield a highly fluorescent N^1, N^6-ethenoadenine derivative (L. Naesens, J. Balzarini, and E. De Clercq, Clin. Chem. 38, 480 (1992). Briefly, plasma (100 µl) was mixed with 200 µl acetonitrile to precipitate protein. Samples were then evaporated to dryness under reduced pressure at room temperature. Dried samples were reconstituted in 200 µl derivatization cocktail (0.34% chloroacetaldehyde in 100 mM sodium acetate, pH 4.5), vortexed, and centrifuged. Supernatant was then transferred to a clean screw-cap tube and incubated at 95°C for 40 min. Derivatized samples were then evaporated to dryness and reconstituted in 100 µl of water for HPLC analysis.

Before intracellular PMPA could be determined by HPLC, the large amounts of adenine related ribonucleotides present in the PBMC extracts had to be removed by selective oxidation. We used a modified procedure of Tanaka et al (K. Tanaka, A. Yoshioka, S. Tanaka, and Y. Wataya, Anal. Biochem., 139, 35 (1984). Briefly, PBMC samples were mixed 1:2 with methanol and evaporated to dryness under reduced pressure. The dried samples were derivatized as described in the plasma assay. The derivatized samples were mixed with 20 µL of 1M rhamnose and 30 µL of 0.1M sodium periodate and incubated at 37°C for 5 min. Following incubation, 40 µL of 4M methylamine and 20 µL of 0.5M inosine were added. After incubation at 37°C for 30 min, samples were evaporated to dryness under reduced pressure and reconstituted in water for HPLC analysis.

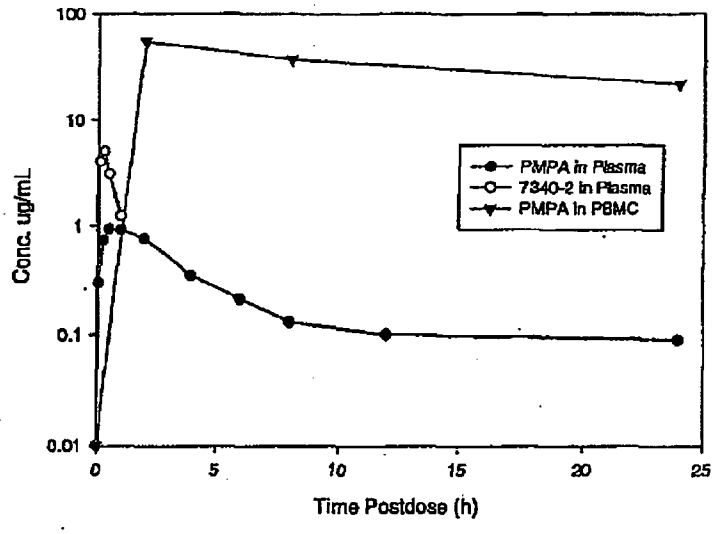
No intact prodrug was detected in any PBMC samples. For plasma samples potentially containing intact prodrugs, experiments were performed to verify that no further conversion to PMPA occurred during derivatization. Prodrug standards were added to drug-free plasma and derivatized as described. There were no detectable levels of PMPA present in any of the plasma samples, and the projected % of conversion was less than 1%.

The HPLC system was comprised of a P4000 solvent delivery system with AS3000 autoinjector and F2000 fluorescence detector (Thermo Separation, San Jose, CA). The column was an Inertsil ODS-2 column (4.6 x 150 mm). The mobile phases used were: A, 5% acetonitrile in 25 mM potassium phosphate buffer with 5 mM tetrabutyl ammonium bromide (TBABr), pH 6.0; B, 60% acetonitrile in 25 mM potassium phosphate buffer with 5 mM TBABr, pH 6.0. The flow rate was 2 ml/min and the column temperature was maintained at 35°C by a column oven. The gradient profile was 90% A/10% B for 10 min for PMPA and 65%A/35%B for 10 min for the prodrug. Detection was by fluorescence with excitation at 236 nm and emission at 420 nm, and the injection volume was 10 µl. Data was acquired and stored by a laboratory data acquisition system (PeakPro, Beckman, Allendale, NJ).

Pharmacokinetic Calculations. PMPA and prodrug exposures were expressed as areas under concentration curves in plasma or PBMC from zero to 24 hours (AUC). The AUC values were calculated using the trapezoidal rule.

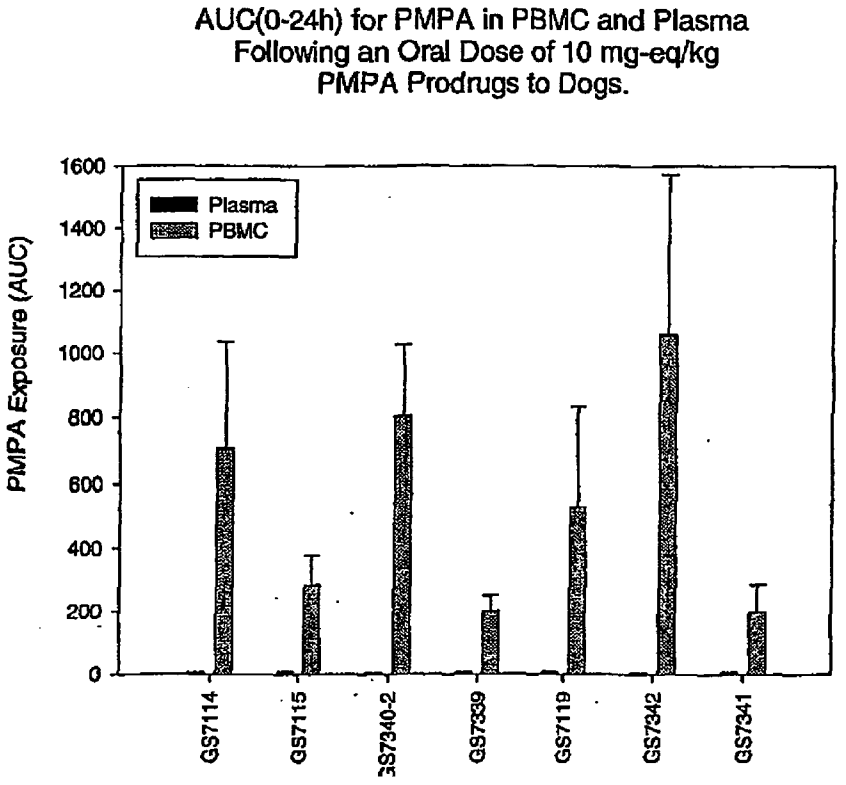
Plasma and PBMC Concentrations. The results of this study is shown in Figures 2 and 3. Figure 2 shows the time course of GS 7340-2 metabolism summary of plasma and PBMC exposures following oral administration of pure diastereoisomers of the PMPA prodrugs.

Figure 2. PMPA and Prodrug Concentration in Plasma and PBMCs Following Oral Administration of GS 7340-2 to Dogs at 10 mg-eq/kg.



The bar graph in Figure 2 shows the AUC (0-24h) for tenofovir in dog PBMCs and plasma after administration of PMPA s.c., TDF and amidate ester prodrugs. All of the amidate prodrugs exhibited increases in PBMC exposure. For example, GS 7340 results in a ~21-fold increase in PBMC exposure as compared to PMPA s.c. and TDF; and a 6.25-fold and 1.29-fold decrease in plasma exposure, respectively.

Figure 3. Depicts Tenofovir Exposure in PBMCs and Plasma Upon Administration of 10 mg-eq/kg in dogs



These data establish *in vivo* that GS 7340 can be delivered orally, minimizes systemic exposure to PMPA and greatly enhances the intracellular concentration of PMPA in the cells primarily responsible for HIV replication.

Table 6

PMPA Exposure in PBMC and Plasma from Oral Prodru

GS#	Moiety	PMPA AUC in Plasma			PMPA AUC in PBMC			Prodrug In Plasma	PBMC/Plasma Exposure Ratio
		Mean	StDev	N	Mean	StDev	N		
GS-7114	Mono-Ala-Et-A	5.8	0.9	2	706	331	5	YES	122
GS-7115	Mono-Ala-Et-B	6.6	1.5	2	284	94	5	YES	43
GS-7340-2	Mono-Ala-iPr-A	5.0	1.1	5	805	222	5	YES	161
GS-7339	Mono-Ala-iPr-A	6.4	1.3	2	200	57	5	YES	31
GS-7119	Mono-Gly-Et-A	6.11	1.86	2	530	304	5	YES	87
GS-7342	Mono-ABA-Et-A	4.6	1.2	2	1060	511	5	YES	230
GS7341	Mono-ABA-Et-B	5.8	1.4	2	199	86	5	YES	34

Example 11

Biodistribution of GS-7340

As part of the preclinical characterization of GS-7340, its biodistribution in dogs was determined. The tissue distribution of GS-7340 (isopropyl alaninyl monoamidate, phenyl monoester of tenofovir) was examined following oral administration to beagle dogs. Two male animals were dosed orally with ^{14}C=GS-7340 (8.85 mg-equiv. of PMPA/kg, 33.2 μCi/kg; the 8-carbon of adenine is labeled) in an aqueous solution (50 mM citric acid, pH 2.2). Plasma and peripheral blood mononuclear cells (PBMCs) were obtained over the 24-hr period. Urine and feces were cage collected over 24 hr. At 24 h after the dose, the animals were sacrificed and tissues removed for analysis. Total radioactivity in tissues was determined by oxidation and liquid scintillation counting.

The biodistribution of PMPA after 24 hours after a single oral dose of radiolabelled GS 7340 is shown in Table 4 along with the data from a previous study with TDF (GS-4331). In the case of TDF, the prodrug concentration in the plasma is below the level of assay detection, and the main species observed in plasma is the parent drug. Levels of PMPA in the lymphatic tissues, bone marrow, and skeletal muscle are increased 10-fold after administration of GS-7340.

Accumulation in lymphatic tissues is consistent with the data observed from the PBMC analyses, since these tissues are composed primarily of lymphocytes. Likewise, accumulation in bone marrow is probably due to the high percentage of lymphocytes (70%) in this tissue.

Table 7. Excretion and Tissue Distribution of Radiolabelled GS-7340 in Dogs (Mean, N=2) Following an Oral Dose at 10 mg-eq. PMPA/kg.

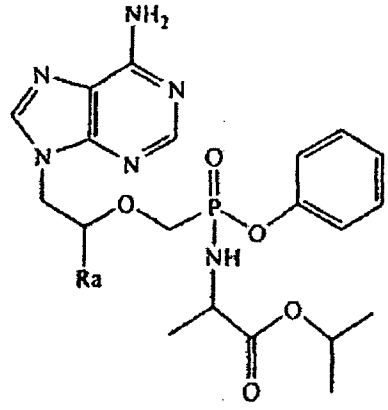
Tissue/Fluid	GS-4331		GS-7340		Tissue Conc. Ratio of GS 7340 to GS-4331
	% Dose	Conc. (ug-eq/g)	% Dose	Conc. (ug-eq/g)	
Liver	12.40	38.30	16.45	52.94	1.4
Kidney	4.58	87.90	3.78	80.21	0.9
Lungs	0.03	0.53	0.34	4.33	8.2
Iliac Lymph Nodes	0.00	0.51	0.01	5.42	10.6
Axillary Lymph Nodes	0.00	0.37	0.01	5.54	14.8
Inguinal Lymph Nodes	0.00	0.28	0.00	4.12	15.0
Mesenteric Lymph Nodes	0.00	1.20	0.04	6.88	5.7
Thyroid Gland	0.00	0.30	0.00	4.78	15.8
Pituitary Gland	0.00	0.23	0.00	1.80	7.8
Salivary Gland (L+R)	0.00	0.45	0.03	5.54	12.3
Adrenal Gland	0.00	1.90	0.00	3.47	1.8
Spleen	0.00	0.63	0.17	8.13	12.8
Pancreas	0.00	0.57	0.01	3.51	6.2
Prostate	0.00	0.23	0.00	2.14	9.1
Testes (L+R)	0.02	1.95	0.02	2.01	1.0
Skeletal Muscle	0.00	0.11	0.01	1.12	10.1
Heart	0.03	0.46	0.15	1.97	4.3
Femoral Bone	0.00	0.08	0.00	0.28	3.5
Bone Marrow	0.00	0.20	0.00	2.05	10.2
Skin	0.00	0.13	0.00	0.95	7.2
Abdominal fat	0.00	0.16	0.00	0.90	5.8
Eye (L+R)	0.00	0.06	0.00	0.23	3.7
Brain	0.00	<LOD	0.00	<LOD	n.d.
Cerebrospinal Fluid	0.00	<LOD	0.00	0.00	n.d.
Spinal Cord	0.00	<LOD	0.00	0.04	n.d.
Stomach	0.11	1.92	0.26	2.68	1.4
Jejunum	1.34	3.01	0.79	4.16	1.4
Duodenum	0.49	4.96	0.44	8.77	1.8
Ileum	0.01	0.50	0.16	4.61	9.2
Large Intestine	1.63	5.97	2.65	47.20	7.9
Gall bladder	0.00	3.58	0.04	25.02	7.0
Bile	0.00	9.63	0.22	40.48	4.2
Feces	40.96	n.d.	0.19	n.d.	n.a.
Total GI Tract Contents	5.61	n.d.	21.64	n.d.	n.a.
Urine	23.72	n.d.	14.73	n.d.	n.a.
Plasma at 24 h	0.00	0.20	0.00	0.20	1.0
Plasma at 0.25 h	n.a.	3.68	n.a.	3.48	0.9
PBMC*	0.00	n.d.	0.00	63.20	n.d.
Whole Blood	0.00	0.85	0.16	0.20	0.2
Total Recovery	81.10		68.96		

* Calculated using typical recovery of 15 x 10^6 cells total, and mean PBMC volume of 0.2 picoliters/cell

n.s. = no sample, n.a. = not applicable, n.d. = not determined.

We Claim:

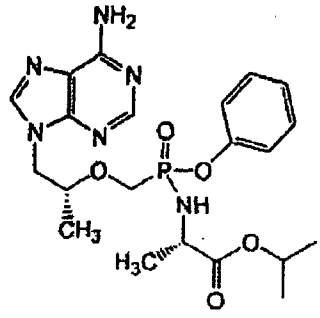
1. A compound having the structure (1)



(1)

where Ra is H or methyl, and chirally enriched compositions thereof, salts, their free base and solvates thereof.

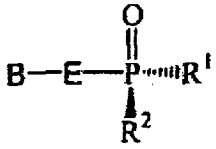
2. A compound having the structure (2)



(2)

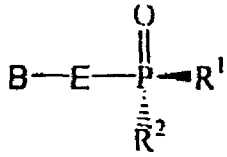
and its enriched diasteromers, salts, free base and solvates.

3. A diastereomerically enriched compound having the structure (3)



(3)

which comprises less than 40% by weight of the diastereomer (4)



(4)

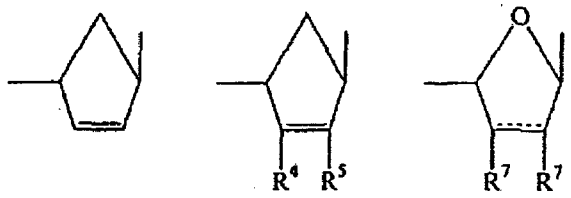
wherein

R is an oxyester which is hydrolyzable in vivo, or hydroxyl;

B is a heterocyclic base;

R^2 is hydroxyl, or the residue of an amino acid bonded to the P atom through an amino group of the amino acid and having each carboxy substituent of the amino acid optionally esterified, but not both of R^1 and R^2 are hydroxyl;

E is $-(CH_2)_2-$, $-CH(CH_3)CH_2-$,$-CH(CH_2F)CH_2-$,$-CH(CH_2OH)CH_2-$, $-CH(CH=CH_2)CH_2-$,$-CH(C\equiv CH)CH_2-$,$-CH(CH_2N_3)CH_2-$,



$-CH(R^5)OCH(R^{6'})-$,$-CH(R^9)CH_2O-$ or $-CH(R^8)O-$, wherein the right hand bond is linked to the heterocyclic base; the broken line represents an optional double bond;

R^4 and R^5 are independently hydrogen, hydroxy, halo, amino or a substituent having 1-5 carbon atoms selected from acyloxy, alkoxy, alkylthio, alkylamino and dialkylamino;

R^6 and $R^{6'}$ are independently H, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl, or C_2-C_7 alkanol;

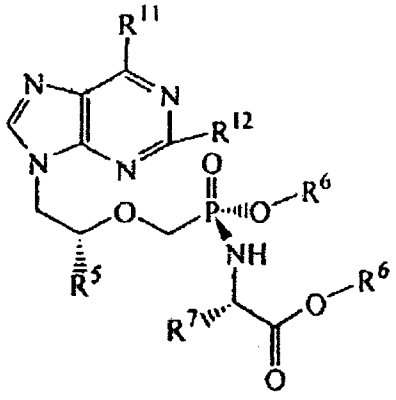
R^7 is independently H, C_1-C_6 alkyl, or are taken together to form -O- or $-CH_2-$,

R^8 is H, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl or C_1-C_6 haloalkyl; and

R^9 is H, hydroxymethyl or acyloxymethyl;

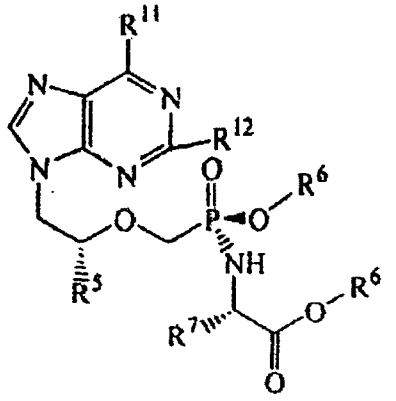
and their salts, free base, and solvates.

4. A diastereomerically enriched compound having the structure (5a)



(5a)

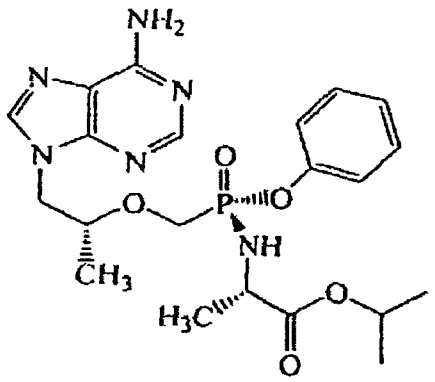
which comprises less than 40% by weight of diastereomer (5b)



(5b)

wherein R^5 is methyl or hydrogen; R^6 independently is H, alkyl, alkenyl, alkynyl, aryl or arylalkyl, or R^6 independently is alkyl, alkenyl, alkynyl, aryl or arylalkyl which is substituted *with from 1 to 3 substituents selected from alkylamino, alkylaminoalkyl, dialkylaminoalkyl,* dialkylamino, hydroxyl, oxo, halo, amino, alkylthio, alkoxy, alkoxyalkyl, aryloxy, aryloxyalkyl, arylalkoxy, arylalkoxyalkyl, haloalkyl, nitro, nitroalkyl, azido, azidoalkyl, alkylacyl, alkylacylalkyl, carboxyl, or alkylacylamino; R^7 is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and which, if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group; R^{11} is amino, alkylamino, oxo, or dialkylamino; and R^{12} is amino or H; and it salts, tautomers, free base and solvates.

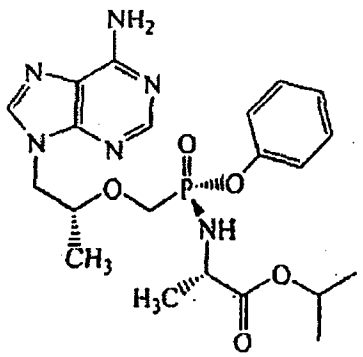
5. A compound of structure (6)



(6)

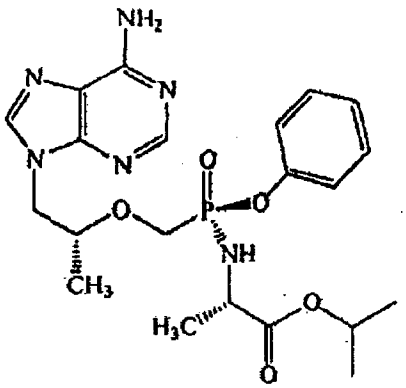
and its salts and solvates.

6. A diastereomerically enriched compound of structure 6(a)



(6a)

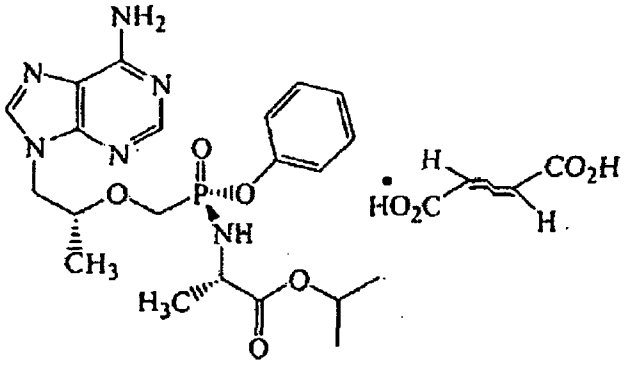
which comprises less than 40% by weight of diasteromer (6b)



(6b)

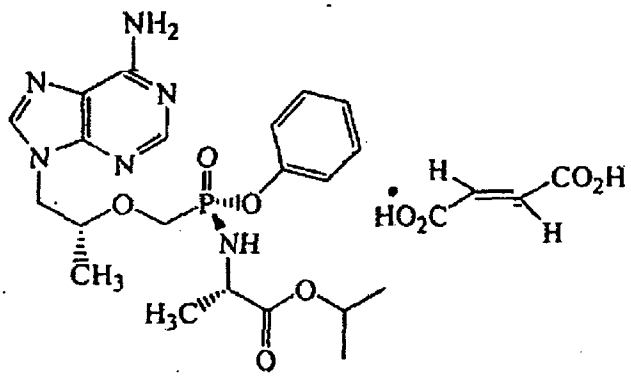
and its salts and solvates.

7. A compound of structure (7)



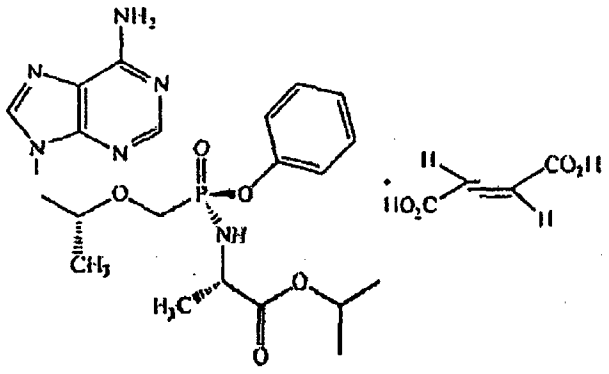
(7)

8. A diastereomerically enriched compound of structure 7(a)



(7a)

which comprises less than 40% by weight of diasteromer 7(b)



(7b)

and its salts and solvates.

9. A compound as claimed in claims 1 or 2, wherein the chirally or diasteromerically enriched compound constitutes greater than 60%, preferably greater than 80% and in particular greater than 95% by weight.

10. A diastereomerically enriched compound as claimed in any of claims 3, 4, 6 or 7, having structure (3), (5a), (6a) and (7a) respectively, and comprising less than 20% weight, preferably less than 5% by weight of corresponding diastereomer (4), 5(b), 6(b) and 7(b) respectively.

11. A composition comprising a compound as claimed in any of claims 1 - 10 and a pharmaceutically effective excipient, wherein said excipient is preferably a gel.

12. The composition as claimed in claim 11 which is suitable for topical administration.

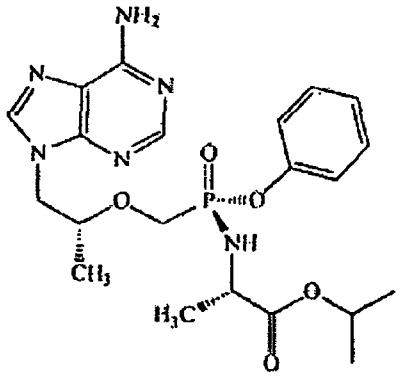
13. A method for the manufacture of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) or 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) comprising reacting 9-(2-hydroxypropyl) adenine (HPA) or 9-(2-hydroxyethyl)adenine (HEA), magnesium alkoxide, and protected *p*-toluenesulfonyloxymethylphosphonate, and if desired, recovering PMPA or PMEA, respectively.

14. The method as claimed in claim 13 wherein the phosphonate of the ptoluenesulfonyloxymethylphosphonate is protected by ethyl ester.

15. The method as claimed in claim 13 wherein the alkoxide is a C1-C6 alkoxide.

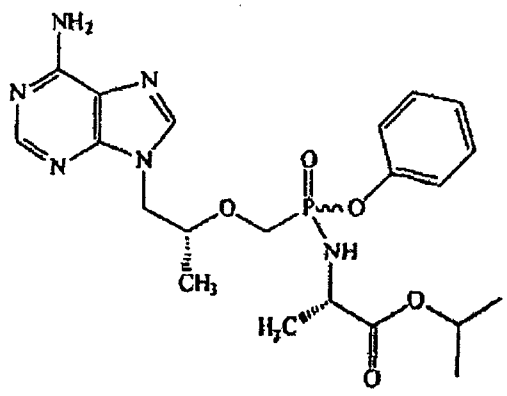
16. The method as claimed in claim 15, wherein the alkoxide is t-butyl or isopropyl oxide.

17. A process for obtaining GS-7340 of the formula



(6)

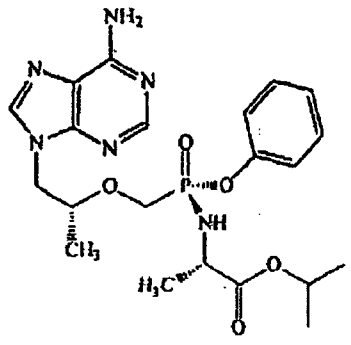
which comprises separating the diastereomers of GS-7171 of the formula



GS-7171

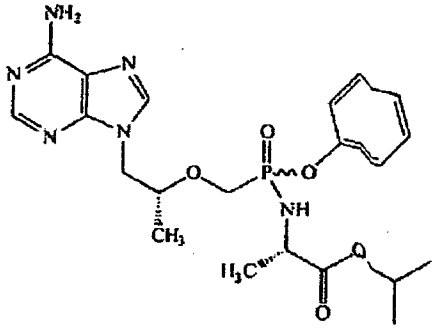
by subjecting them to batch elution chromatography and recovering GS-7340.

18. A process for obtaining GS-7340 of the formula



(6)

which comprises separating the diastereomers of FS-7171 of the formula

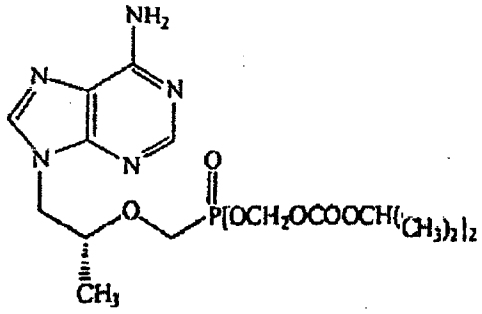


GS-7171

by contacting with Chiralpak AS and recovering GS-7340.

19. The process according as claimed in claim 18, wherein the separation of the diastereomers of GS-7171 is made using batch elution chromatography.

20. The compound of the formula



Dated this the 22nd day of March 2011.

Annexure B E91/5111/2014

We claim:

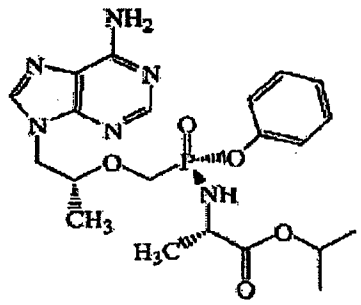
1. A method for the manufacture of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) or 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) comprising reacting 9-(2-hydroxypropyl) adenine (HPA) or 9-(2-hydroxyethyl)adenine (HEA), magnesium alkoxide, and protected p-toluenesulfonyloxymethylphosphonate, and if desired, recovering PMPA or PMEA, respectively.

2. The method as claimed in claim 1, wherein the phosphonate of the ptoluenesulfonyloxymethylphosphonate is protected by ethyl ester.

3. The method as claimed in claim 1, wherein the alkoxide is a C_1-C_6 alkoxide.

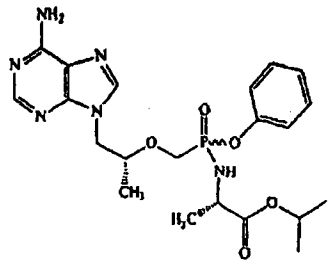
4. The method as claimed in claim 3, wherein the alkoxide is t-butyl or isopropyl oxide.

5. A process for obtaining GS-7340 of the formula



(6)

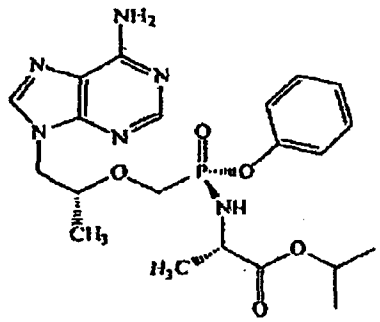
which comprises separating the diastereomers of GS-7171 of the formula



GS-7171

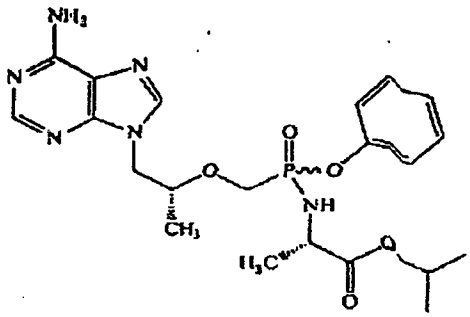
by subjecting them to batch elution chromatography and recovering GS-7340.

6. A process for obtaining GS-7340 of the formula



(6)

which comprises separating the diastereomers of GS-7171 of the formula



GS-7171

by contacting with Chiralpak AS and recovering GS-7340.

7. The process according as claimed in claim 6, wherein the separation of the diastereomers of GS-7171 is made using batch elution chromatography.

Dated this 22nd day of March 2011

(DIVYA KAPOOR)
of SUBRAMANIAM & ASSOCIATES
ATTORNEYS FOR THE APPLICANTS

Detail Annexure C

APPLICATION NUMBER	9/MUMNP/2003
APPLICANT NAME	**GILEAD SCIENCES, INC.**
DATE OF FILING	02/01/2003
DATE OF COMPLETE SPECIFICATION	02/01/2003
PCT INTERNATIONAL FILING DATE	20/07/2001
PRIORITY DATE	21/07/2000
TITLE OF INVENTION	PRODRUGS OF PHOSPHONATE NUCLEOTIDE ANALOGUES AND METHODS FOR SELECTING AND MAKING SAME
PUBLICATION DATE (U/S 11A)	04/02/2005
POST GRANT JOURNAL DATE (U/S 43(2))	31/08/2007

Application Status

Date Of Certificate Issue	08/08/2007
Status	**Granted Application, Patent Number : 208435**

Annexure D

CLAIMS:

1. A screening method for identifying a methoxyphosphonate nucleotide analogue prodrug conferring enhanced activity in a target tissue comprising:

(a) providing at least one of said prodrugs;

(b) selecting at least one therapeutic target tissue and at least one non-target tissue;

(c) administering the prodrug to the target tissue and to said at least one non-target tissue; and

(d) determining the relative activity conferred by the prodrug in the tissues in step (c).

2. The method of claim 1 wherein the activity is antiviral activity or antitumor activity.

3. The method of claim 2 wherein the activity is antiviral activity.

4. The method of claim 3 wherein the activity is anti-HIV or anti-HBV activity.

5. The method of claim 1 wherein the prodrug is a prodrug of PMPA or PMEA.

6. The method of claim 5 wherein the prodrug is a phosphonoamidate, phosphonoester or mixed phosphonoamidate/phosphonoester.

7. The method of claim 6 wherein the amidate is an amino acid amidate.

8. The method of claim 6 wherein the ester is an aryl ester.

9. The method of claim 1 further comprising selecting a prodrug having a relative activity in the target tissue that is greater than 10 times that of the non-target tissue.

10. The method of claim 1 wherein the target and non-target tissue are in an animal, the prodrug is administered to the animal and the relative activity is determined by analysis of the animal tissues after administration of the prodrug.

11. The method of claim 1 wherein activity in the target and non-target tissues is determined by assaying the amount of at least one metabolite of the prodrug in the tissues.

12. The method of claim 12 wherein the metabolite is the parental drug.

13. The method of claim 12 wherein the metabolite is the diphosphate of the parental drug.

14. The method of claim 1 wherein the target tissue is virally infected tissue and the non-target tissue is the same tissue which is not virally infected.

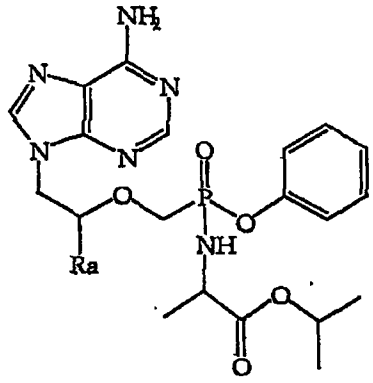
15. The method of claim 1 wherein the target tissue is lymphoid tissue and the activity is anti-HIV activity.

16. The method of claim 1 wherein the target tissue is liver and the activity is anti-HBV activity.

17. The method of claim 1 wherein the target tissue is hematological and the activity is antitumor activity.

18. The method of claim 1 wherein the target tissue is malignant and the non-target tissue is the same tissue but non-malignant.

19. A compound having the structure (1)

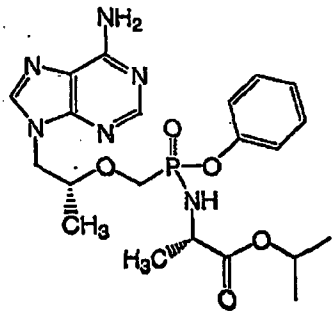


(1)

where Ra is H or methyl,

and chirally enriched compositions thereof, salts, their free base and solvates thereof.

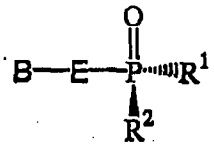
20. A compound having the structure (2)



(2)

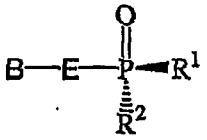
and its enriched diasteromers, salts, free base and solvates.

21. A diastereomerically enriched compound having the structure (3)



(3)

which is substantially free of the diastereomer (4).



(4)

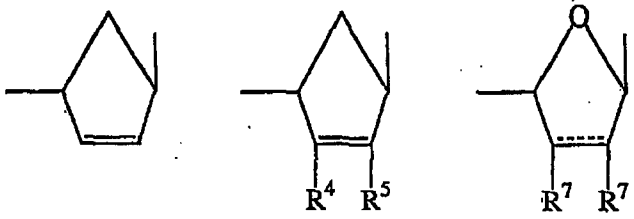
wherein

R^1 is an oxyester which is hydrolyzable *in vivo*, or hydroxyl;

B is a heterocyclic base;

R^2 is hydroxyl, or the residue of an amino acid bonded to the P atom through an amino group of the amino acid and having each carboxy substituent of the amino acid optionally esterified, but not both of R^1 and R^2 are hydroxyl;

E is $-(CH_2)_2-$, $-CH(CH_3)CH_2-$, $-CH(CH_2F)CH_2-$, $-CH(CH_2OH)CH_2-$, $-CH(CH{=}CH_2)CH_2-$, $-CH(C{\equiv}CH)CH_2-$, $-CH(CH_2N_3)CH_2-$,



$-CH(R^6)OCH(R^{6'})-$, $-CH(R^9)CH_2O-$ or $-CH(R^8)O-$, wherein the right hand bond is linked to the heterocyclic base;

the broken line represents an optional double bond;

R^4 and R^5 are independently hydrogen, hydroxy, halo, amino or a substituent having 1-5 carbon atoms selected from acyloxy, alkyoxy, alkylthio, alkylamino and dialkylamino;

R^6 and $R^{6'}$ are independently H, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl, or C_2-C_7 alkanoyl;

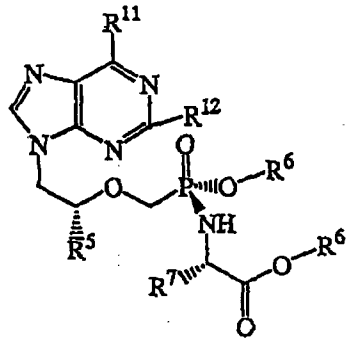
R^7 is independently H, C_1-C_6 alkyl, or are taken together to form -O- or $-CH_2-$;

R^8 is H, C_1-C_6 alkyl, C_1-C_6 hydroxyalkyl or C_1-C_6 haloalkyl; and

R^9 is H, hydroxymethyl or acyloxymethyl;

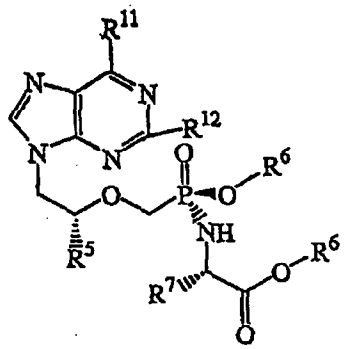
and their salts, free base, and solvates.

22. A diastereomerically enriched compound having the structure (5a)



(5a)

which is substantially free of diastereomer (5b)



(5b)

wherein

R^5 is methyl or hydrogen;

R^6 independently is H, alkyl, alkenyl, alkynyl, aryl or arylalkyl, or R^6 independently is alkyl, alkenyl, alkynyl, aryl or arylalkyl which is substituted with from 1 to 3 substituents selected from alkylamino, alkylaminoalkyl, dialkylaminoalkyl, dialkylamino, hydroxyl, oxo, halo, amino, alkylthio, alkoxy,

alkoxyalkyl, aryloxy, aryloxyalkyl, arylalkoxy, arylalkoxyalkyl, haloalkyl, nitro, nitroalkyl, azido, azidoalkyl, alkylacyl, alkylacylalkyl, carboxyl, or alkylacylamino;

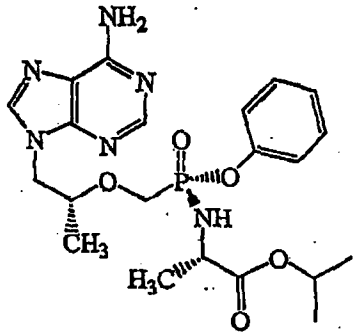
R^7 is the side chain of any naturally-occurring or pharmaceutically acceptable amino acid and which, if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group;

R^{11} is amino, alkylamino, oxo, or dialkylamino; and

R^{12} is amino or H;

and it salts, tautomers, free base and solvates.

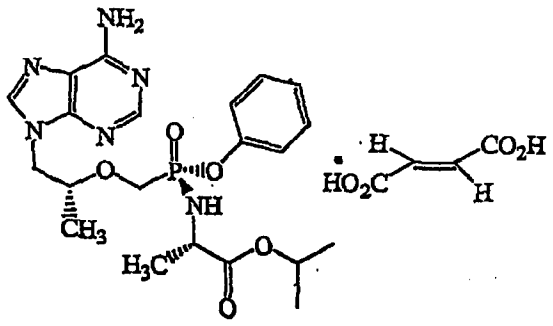
23. A compound of structure (6)



(6)

and its salts and solvates.

24. A compound of structure (7)



(7)

25. A composition comprising a compound of any of claims 19-24 and a pharmaceutically effective excipient.

26. The composition of claim 25 wherein the excipient is a gel.

27. The composition of claim 25 which is suitable for topical administration.

28. A method for antiviral therapy or prophylaxis comprising administering a compound of any of claims 19-24 in a therapeutically or prophylactically effective amount to a subject in need of such therapy or prophylaxis.

29. A method for use of magnesium alkoxide comprising reacting 9-(2-hydroxypropyl)adenine (HPA) or 9-(2-hydroxyethyl)adenine (HEA), magnesium alkoxide, and protected *p*-toluenesulfonyloxymethylphosphonate.

30. The method of claim 29 further comprising recovering PMPA or PMEA, respectively.

31. The method of claim 29 wherein the phosphonate of the *p*-toluenesulfonyloxymethylphosphonate is protected by ethyl ester.

32. The method of claim 29 wherein the alkoxide is a C_1-C_6 alkoxide.

33. The method of claim 32 wherein the alkoxide is t-butyl or isopropyl oxide.

We claim :

1. A screening method for identifying a methoxyphosphonate nucleotide analogue prodrug conferring enhanced activity in a target tissue comprising:

   (a) providing at least one of said prodrugs;
   (b) selecting at least one therapeutic target tissue and at least one non-target tissue;
   (c) administering the prodrug to the target tissue and to said at least one non-target tissue; and
   (d) determining the relative activity conferred by the prodrug in the tissue in step (c).

2. The method as claimed in claim 1 wherein the activity is antiviral activity or antitumor activity.

3. The method as claimed in claim 2 wherein the activity is anti-HIV or anti-HBV activity.

4. The method as claimed in any preceding claim wherein the prodrug is a prodrug of PMPA or PMEA.

5. The method as claimed in any preceding claim wherein the prodrug is a phosphonoamidate, phosphonoester or mixed phosphonoamidate/phosphonoester

6. The method as claimed in claim 5 wherein the amidate is an amino acid amidate.

7. The method as claimed in claim 6 wherein the ester is an aryl ester.

8. The method as claimed in any preceding claim wherein said prodrug has relative activity in the target tissue that is greater than 10 times that of the non-target tissue.

9. The method as claimed in any preceding claim wherein the target and non-target tissue are in an animal, the prodrug is administered to the animal and the relative activity is determined by analysis of the animal tissues after administration of the prodrug.

10. The method as claimed in any preceding claim wherein activity in the target and non-target tissues is determined by assaying the amount of at least one metabolite of the prodrug in the tissues.

11. The method as claimed in claim 10 wherein the metabolite is the parental drug.

12. The method as claimed in claim 11 wherein the metabolite is the diphosphate of the parental drug.

13. The method as claimed in any preceding claim wherein the target tissue is virally infected tissue and the non-target tissue is the same tissue which is not virally infected.

14. The method as claimed in any one of claims 1 to 12 wherein the target tissue is lymphoid tissue and the activity is anti-HIV activity.

15. The method as claimed in any one of claims 1 to 12 wherein the target tissue is liver and the activity is anti-HBV activity.

16. The method as claimed in any one of claims 1 to 12 wherein the target tissue is hematological and the activity is antitumor activity.

17. The method as claimed in any one of claims 1 to 12 wherein the target tissue is malignant and the non-target tissue is the same tissue but non-malignant.

Dated this the 2nd day of January 2003.

(H. SUBRAMANIAM]
of SUBRAMANIAM, NATARAJ & ASSOCIATES
ATTORNEYS FOR THE APPLICANTS

Annexure F

BEFORE CONTROLLER OF PATENTS, THE PATENTS OPFFICE, MUMBAI.

THE PATENTS ACT 1970
(Section 15)

In the matter of Patent Act, 1970 and as amended Patent (Amendment) Act, 2005

In the matter of Patents Rules, 2003 and as amended (Amendment) Rule 2006,

In the matter of Patent Application No. 530/MUMNP/2006 dated.09.05.2006, a divisional Patent Application out of 9/MUMNP/2003.

Hearing held on 12.12.2013

M/s. Gilead Sciences, INC	:	Applicant
Presence	:	Applicant not attended hearing
M/s. Cipla Limited	:	Opponent
Presence	:	S.Majumdar, An authorized Patent Agent, on behalf of the Opponent, & Mrs. Mythili Venkatesh, Patent Agent

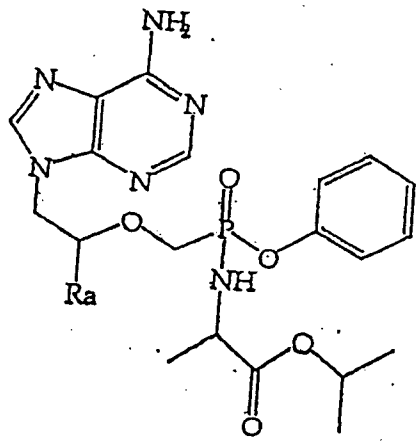
and

The Pre-Grant Opposition by way of representation, under section **25(1)** entered by the Opponent M/s. Cipla Limited, against to the grant of subject Patent Application.

ORDER

1. M/s. Gilead Sciences, INC, filed National Phase Patent Application through their Patent Attorney. SUBRAMANIAM, NATARAJ & ASSOCIATES PATENT & TRADEMARK ATTORNEYS E-556, GREATER KAILASH-II NEW DELHI-110048, bearing application no. 530/MUMNP/2006 dated. 09.05.2006, which is a divisional application of 9/MUMNP/2003, claiming the priority of parent application for their invention titled "PRODRUGS OF PHOSPHONATE NUCLEOTIDE ANALOGUES AND METHODS FOR SELECTING AND MAKING SAME" containing the following claims, which are been amended at the later stage

1 A compound having the structure (1)

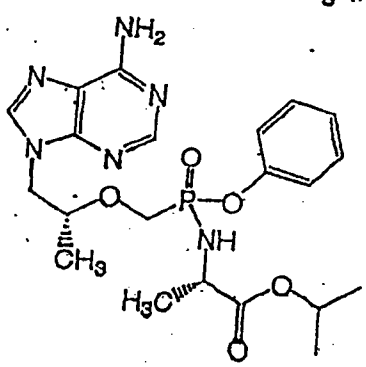


(1)

where Ra is H or methyl,

and chirally enriched compositions thereof, salts, their free base and solvates thereof

2 A compound as claimed in claim 1 having the structure (2)



(2)

and its enriched diasteromers, salts, free base and solvates

1

2. M/s. Cipla Limited, an Indian company, (herein after referred as opponent) has filed a pre-grant opposition by way of representation under section 25 (1) of Patents Act,

1970 (as amended) on 06th June, 2012 opposing grant of Patent to subject application. The pre-grant representation was officially served on the applicant on 13th June, 2013 as per Rule 55(3) and the applicant has failed to failed to file any reply under Rule 55(4) within stipulated time period. The applicant has vide letter dated.11.12.2013 acknowledged that no reply statement has been filed under Rule 55(4) within the stipulated time period. The applicant also did not attend the hearing and therefore failed to make any oral submissions on the date of the hearing i.e on 12.12.2013. it was categorically submitted that opponent's opposition based on the ground of opposition available under the Act and taken by the opponent are maintained and warrant rejection of the impugned application in its entirety

3. The first examination report (hereinafter referred as FER) issued in the subject patent application on 26.10.2007, wherein the following objections have been communicated to the applicant's agent,

1. The claims filed here in have the same inventive concept as file din parent application no.9/MUMNP/2003 according to the Sec.10(5) of the Act. As such division of the invention form the invention claimed in mail application is not clear or misleading and hence division of application is not valid. (further read sec.16 with Sec.10(50 and 7(10of th4e Act). without prejudiced to the above other requirement are;
2. Invention claimed in claims 1-2 falls within the scope of sub clause of sec 3(d) of the patents act 1970 as amended by 2005. Hence not allowed
3. Name of the compound should be given.
4. Form -3 and form -5 should be corrected with application number therein.
5. Details regarding the search and/or examination report including claims of the application allowed, as referred to in Rule 12(3) of the Patent Rule, 2003, in respect of same or substantially the same invention filed in all the major Patent office's such as USPTO,EPO and JPO etc., along with appropriate translation where applicable, should be submitted within a period of Six months from the date of receipt of this communication as provided under section 8(2) of the Indian Patents Act.
6. Kindly not that it may not be possible to repeatedly examine your amended specification therefore you should submit the documents only after fully complying with the above requirements.
7. In case, the applicant desires to hear in respect of any of the objections, may request for a hearing as provided U/s.80 of the Patents Act 1970(as amended). However, provision to the said section shall be strictly adhered to.
8. If you desire to discuss the matter, you can visit this office with prior appointment.

4. The Agent of the Applicant responded to the first examination report on 14/07/2008, wherein they have provided following explanation

With regard to objection.1

With due respects, we humbly disagree with the contents of the first paragraph of the official letter. Under Section 16 of the Patent Act, a divisional can be filed either to meet the Learned Examiner's objections or voluntarily by the Applicant. It is humbly submitted that the parent application of this divisional application was filed on 02 January 2003 with both the process and the product claims. Since prior to 2005, pharmaceutical products *per se* were not patentable under Indian Patent Law, a divisional was filed on the parent application on 09 May 2006 covering the product claims. Even under the law which was in force in 2005, section 5 permitted filing of product claims in one application and process claims in another. The only requirement which has to be met is that the divisional and the parent application should not have same claims.

Without prejudice to the submissions made above, it is also respectfully submitted that the Learned Examiner has himself held, in the First Examination Report of 9/MUMNP/2003, that claims 19 and 20 are distinct and therefore, do not fall within the scope of claim 1. This objection by the Learned Examiner itself makes clear that this divisional is valid. Accordingly, the reconsideration and withdrawal of this objection is respectfully requested.

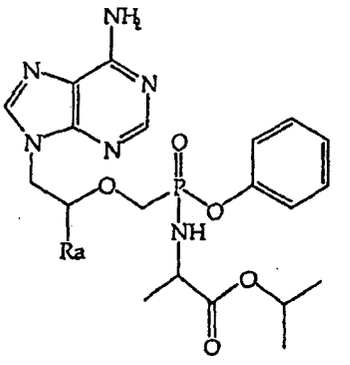
With regard to objection.1

With reference to second paragraph, we respectfully submit that section 3(d) of Patent Act applies where the subject matter of the claims is a derivative of a known compound having identical efficacy. It is respectfully submitted that there is sufficient difference in the efficacy of the present compound as compared to the parent compound which is a basic nucleotide analogue. Examples 9 to 11 of the specification clearly demonstrates the technical efficacy of the compounds claimed in the present invention. The basic nucleotide analogue, the parent compound, does not have the desired bioavailability and does not reach the affected cells easily. This basic nucleotide analogue is not the part of the present invention and what are claimed herein are pro-drugs of the parent compounds (specification pages 7 and 8). These pro-drugs are not the derivatives but bio-intermediates of the parent compound with increased bio-distribution and increased bio-availability in the blood plasma. The increase in bio-availability is about 10 times (Example 11 of specification, page 50, last line). It is respectfully submitted that 10 times increase in the bio-availability results in a significant increase in the efficacy in terms of therapeutic value. We, therefore, respectfully submit that the present invention falls outside the purview of section 3(d).

With regard to the third official requirement, the preamble of the claim 1 has been amended,

With above observation and explanations the agent on behalf of the applicant submitted the new set of amended claims in line with the objection, which are reproduced below,

1. A methoxyphosphonate nucleotide analogue having the structure (1)

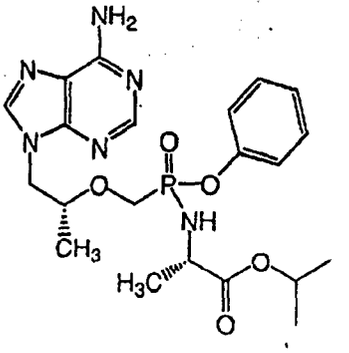


(1)

where Ra is H or methyl,

and chirally enriched compositions thereof, salts, their free base and solvates thereof.

2. A methoxyphosphonate nucleotide analogue having the structure (2)



(2)

and its enriched diasteromers, salts, free base and solvates.

5. A Pre-grant Opposition by way of representation made under Section 25(1) of the Patents Act(as amended), by M/s. Cipla limited, in respect of alleged patent application on the following, amongst other grounds listed below along with suitable analysis in each ground,

3. GROUNDS OF OPPOSITION

3.1 The application is opposed on the following grounds:

Section 25(1) (e)-Obviousness/lack of inventive step

that the invention so far as claimed in any claim of the complete specification is obvious and clearly does not involve any inventive step, having regard to the matter published as mentioned in Section 25(1) (b) or having regard to what was used in India before the priority date of the applicant's claim;

Section 25(1) (f)-Not an invention

that the subject of any claim of the complete specification is not an invention within the meaning of this Act, or is not patentable under this Act;

Section 25(1) (g)-Insufficiency

that the complete specification does not sufficiently and clearly describe the invention or the method by which it is to be performed;

Section 25(1)(h)-Failure to disclose information or furnishing false information relating to foreign filing

that the applicant has failed to disclose to the Controller the information required by Section 8 or has furnished the information which in any material particular was false to his knowledge.

3.2 The opponent craves leave to alter, modify, add or delete the grounds in the course of the present proceedings.

4. ANALYSIS OF CLAIMS OF THE IMPUGNED APPLICATION

4.1 Patent Application No: 530/MUMNP/2006 entitled "Prodrugs of phosphonate nucleotide analogues and methods for selecting and making same" dated May 9. 2006. It is published under section 11A in the Official Journal of Indian Patent Office dated August 17, 2007. The impugned application claims an earliest priority of US dated 21 July, 2000. The Claims of the impugned application have been amended on September 13, 2011. The as filed claims and the amended claims are attached herewith as Annexure A and Annexure B.

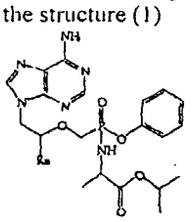
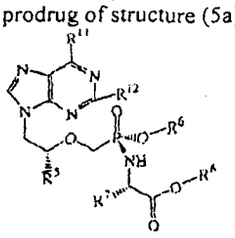
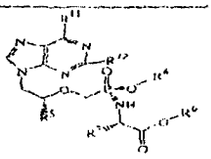
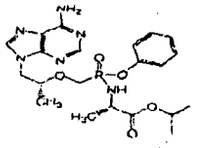
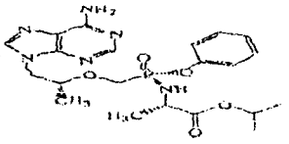
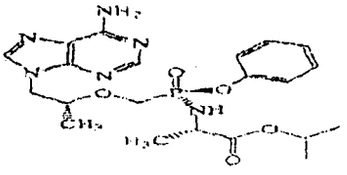
4.2 The impugned application is divisional to application no. 9/MUMNP/2003 dated January 2, 2003 which has been granted as IN208435. IN208435 claims a screening method for identifying a methoxy phosphonate nucleotide analogue prodrug conferring enhanced activity in a target tissue with antiviral or antitumour activity. 3 divisional applications have been filed for the parent application 9/MUMNP/2003

4.3 In the First Examination Report with regards to the impugned application, the Ld. Controller has stated that the claims of the impugned application are not allowable under Sec (16) read with Sec 10 (5) and Sec 7 (10) of the Act. The Applicant's response dated July 14, 2008 states that:

"Under section 16 of the Patent Act, a divisional can be filed either to meet the learned Examiner's objections or voluntarily by the applicant. It is humbly submitted that the parent application of this divisional application was filed on 02 January 2003 with both the process and the product claims. Since prior to 2005, pharmaceutical products per se were not patentable under the Indian Patent Law, a divisional was filed on the parent application on May 9, 2006 covering the product claims. Even under the law which was in force in 2005, section 5 permitted filing of the product claims in one application and process claims in another."

4.4 The opponent further submits that the claims of the impugned application completely overlaps with claim 2 and claim 3 of IN241597 as may be noted from the below table.

Table 1

Sr No	Amended Claims of impugned application	Claim 2 and claim 3 of IN241597	Comments
1	A methoxyphosphonate nucleotide analogue having the structure (1)  (1) where Ra is H or methyl, and chirally enriched	A methoxyphosphonate nucleotide prodrug of structure (5a)  (5a) which is substantially free of diastereomer (5b)	**Same** If the specific substituents are inserted in structure (5a) of IN241597, the structure of formula (1) of the impugned application is obtained. Further claim 1 of the impugned application claims chirally enriched form of structure (1) whereas claim 2 states that (5a) is substantially free of diastereomer (5b) which impliedly means that structure (5a) is chirally enriched.
	compositions thereof, salts, their free base and solvates thereof.	 (5b) wherein R5 is methyl or hydrogen, R6 independently is H, alkyl, alkenyl, aryl or arylalkyl -------; R7 is the side chain of any naturally occurring or pharmaceutically acceptable amino acid and which, if the side chain comprises carboxyl, the carboxyl group is optionally esterified with an alkyl or aryl group; R11 is amino, alkylamino, oxo, or dialkylamino and R12 is amino or H; and its salts, tautomers, free base and solvates.	
2	A methoxyphosphonate nucleotide analogue having the structure (2)  (2) and its enriched diastereomers, salts, free base and solvates.	A methoxyphosphonate nucleotide prodrug of structure (6a)  (6a) which is substantially free of diastereomer (6b)  and its salts and solvates.	**Same** If the specific substituents are inserted in structure (6a) of IN241597, the structure of formula (2) of the impugned application is obtained. Further claim 2 of the impugned application claims enriched form of structure (2) whereas claim 3 of IN241597 states that (6a) is substantially free of diastereomer (6b) which impliedly means that structure (6a) is enriched.

screening purpose is of structure (1). Page 4 states that the diastereomerically enriched compounds of this invention have the structure (3) and are designated the (S) isomers at the phosphorus chiral center and they are substantially free of the diastereomer (4).

5.2 Page 6 states that the preferred embodiment of this invention is the compound of structure (6) which has been designated as GS-7340. The specification further provides methods for the manufacture of PMPA and PMEA.

5.3 Page 8 further states:

"The prodrugs for use in the screening method of this invention are covalently modified analogues of the parent methoxyphosphonate nucleotide analogues described in the preceding paragraph. In general, the phosphorus atom of the parent drug is the preferred site for prodrug modification, but other sites are found on the heterocyclic base B or the aglycon E. Many such prodrugs are already known. Primarily, they are esters or amidates of the phosphorus atom, but also include substitutions on the base and aglycon. None of these modifications per se is part of this invention and none are to be considered limiting on the scope of the invention herein."

The opponent submits that the above paragraph clearly implies that the prodrugs of methoxyphosphonate nucleotide namely amidates are well known in the art and none of these modifications form a part of the invention, yet the applicant in the impugned application intends to claim an amidate prodrug of methoxyphosphonate nucleotide.

5.4 Page 9 of the specification further states that the R isomer of PMPA is preferred as it is more active than the corresponding S isomer which is in contradiction with the statement made on page (5) that structure (3) which is the preferred diastereomer is designated as (S) isomer at the phosphorus chiral center.

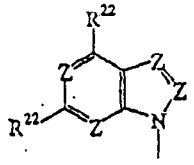
5.5 Page 11 of the specification states that the objective of the invention claimed in the impugned application is to find prodrugs that are not metabolized in the gastrointestinal tract which is different from the oral bioavailability studies undertaken to determine bioavailability of prodrugs wherein the objective is to identify a prodrug which passes into the systemic circulation substantially

converted to parent drug. Thus the screening method as provided in the impugned application specially provides for prodrug which is capable of passing largely unmetabolized through the gastrointestinal tract, is substantially stable in blood and is able to permeate cells at least to some degree.

5.6 Page 15 states that a prodrug is considered to be selective if the activity or concentration of the parent drug in the target tissue (% dose distribution) is greater than 2x, preferably 5x, that of the parent compound in non-target tissue. Alternatively a prodrug candidate can be compared against a benchmark prodrug.

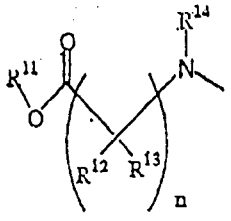
5.7 Page 15 provides for the preparation of starting materials or intermediates. Line 35 further states that the alkyl group of the magnesium alkoxide is not critical and can be any C1-C6 branched or normal alkyl, but is preferably t-butyl (for PMPA) or isopropyl (for PMEA).

5.8 Page 16 discloses the heterocyclic bases B and page 19 states that the preferred B groups have the formula



Page 20 states that R1 is an *invivo* hydrolysable oxyester having the structure –OR35 or –OR6 and preferably R1 is aryloxy, ordinarily unsubstituted or para-substituted phenoxy.

R2 is an amino acid residue, optionally provided that any carboxy group linked by less than about 5 atoms to the amidate N is esterified and R2 typically has the structure



5.9 The applicant has admitted on Page 22 of the specification that the prodrug compounds of this invention are provided in the form of free base or the various salts enumerated in US5798340.

5.10 Pages 26-33 provides for the preparation of GS7171 and the diastereomer separation of GS7340 by batch elution chromatography.

5.11 Example 9 provides the in vitro activity and stability of prodrug Diastereomers GS7340 vs Tenofovir and TDF.

6. PRIOR ART RELIED ON IN THE PRESENT OPPOSITION

- **Exhibit 1: US5798340** entitled **"Nucleotide analogs"** published on August 25, 1998.
- **Exhibit 2:** "Minireview: nucleotide prodrugs" by Robert J. Jones and Norbert Bischofberger published in Antiviral Research 27 (1995) 1-17.
- **Exhibit 3: WO9804569** entitled **"Nucleotide analogs"** published on February 5, 1998 (Indian equivalent is 2076/DEL/1997 which has been refused u/s 25(1)).
- **Exhibit 4 :** "Probing the Mechanism of Action and Decomposition of Amino Acid Phosphomonoester Amidates of Antiviral Nucleoside Prodrugs" by Edward J. McIntee, Rory P. Remmel, Raymond F. Schinazi, Timothy W. Abraham, and Carston R. Wagner published in *J. Med. Chem.* 1997, *40*, 3323-3331.

7. OBVIOUSNESS AND LACK OF INVENTIVE STEP [Section 25(1)(e)]:

7.1 **US5798340** entitled "**Nucleotide analogs**" is published on August 25, 1998 and annexed hereto as Exhibit 1 and hereinafter referred to as D1. The said application claims the priority of US dated September 17, 1993.

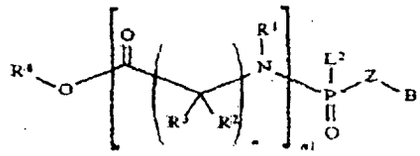
7.2 Exhibit 1 discloses Nucleotide analogs characterized by the presence of an amidate linked amino acid or an ester linked group which is bonded to the phosphorus atom of phosphonate nucleotide analogs are disclosed. The analogs comprise a phosphoamidate or ester bond that is hydrolysed in vivo to yield a corresponding phosphonate nucleotide analog. Methods and intermediates for the synthesis and use are described.

7.3 Column 2 line 20-32 discloses under the title "Summary of the invention":

"In a principal embodiment, the objects of this invention are accomplished by a nucleotide analog amidate comprising a phosphonate radical wherein the improvement comprises an amino acid residue or polypeptide radical in which an amino group of the amino acid or polypeptide is bonded to the phosphorus atom of the nucleotide analog by an amidate bond, a carboxyl group of the amino acid residue or polypeptide radical is positioned such that it is capable as the free acid of hydrolyzing the phosphoroamidate bond, and the carboxyl group is blocked (such as by moieties including esters or amides). The nucleotide analog amidates of this invention are hydrolyzed in vivo to the corresponding nucleotide analog and are thus precursors of the corresponding nucleotide analog.

7.4 Bridging paragraphs of Column 3 and column 4 states:

In a further embodiment the objects are accomplished by compounds of the formula II, Ia, IIb and IIc. The formula (II) has the structure:



wherein L^2 is OR, SR -----;

n is an integer having a value from 1 to 5 ---;

n1 is an integer;

substituents linked to the carbon atom designated # are in the R, S or RS configuration;

R is H, C1 -C20 alkyl which is ---,C3 -C20 aryl which is unsubstituted or substituted by substituents ----;

R1 is H or C1 -C9 alkyl which is unsubstituted or substituted by substituents ---;

R2 is H or C1 -C9 alkyl which is unsubstituted or substituted by substituents---;

R3 is ------, C1 -C6 alkyl which is unsubstituted or substituted ---; and

R4 is ---- is C3 -C9 alkyl which is substituted by substituents independently selected from the group consisting of OH, O, N and halogen, C3 -C6 aryl which is substituted by substituents independently selected from the group consisting of OH,

O, N and halogen or C3-C9 aryl-alkyl which is substituted by substituents independently selected from the group consisting of OH, O, N and halogen.

7.5 Column 7 under the title "Detailed description of the invention" it is stated:

When groups L1 or L2 comprise an amino acid residue they comprise any naturally-occurring or synthetic amino acid residue, i.e., any moiety comprising at least one carboxyl and at least one amino residue directly linked by at least one carbon atom, typically a single (a) carbon atom. The nature and identity of the intervening structure located between the carboxyl and amino (amidate) groups can have a variety of structures including those described herein. All that is necessary is that the group have sufficient conformation and length to be capable of acid catalysis of the phosphoroamidate bond and release of the phosphonate when the free carboxyl is generated in vivo, e.g. by deesterification, deamidation or peptidolytic cleavage of the precursor. In general, the amino acids corresponding to the residues employed in the compounds of this invention are naturally occurring and have no pharmacological activity per se. However, optimal pharmacokinetic activity (substantially complete autocatalytic hydrolysis upon hydrolysis of the distal amide or ester bond) may be achieved by using non-naturally occurring amino acid residues. The intervening structure may be as simple as methylene (when the residue is glycyl) or substituted methylene (other a amino acids). The structure ordinarily contains up to about 5 carbon or hetero atoms in the direct linkage between the carboxyl carbon and the amidate nitrogen, as for example in the case of intervening ethylene, propylene, butylene, or pentylene groups or their substituted analogs, such as for example oxyesters in which O replaces carbon and, as appropriate, hydrogen. An example of such an intervening structure would be --CH--O--CH(R3)(R2)--. In general, fewer intervening atoms are employed when more rapid hydrolysis is desired, although it will be understood that larger structures are suitable if they possess sufficient flexibility or have conformations in which the carboxyl group is positioned in proximity to the amidate bond.

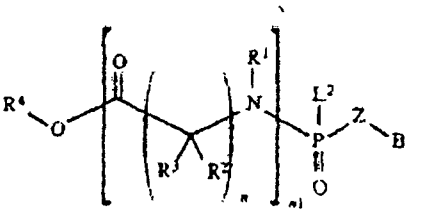
Column 20 lines 37-40 state:

The invention compounds are optionally esterified at the amino acid carboxyl moiety by the R4 group defined above. Exemplary R4 groups include H, methyl, ethyl, propyl, isopropyl, butyl, t-butyl (C(CH3)3), phenyl (-C6H5), benzyl (-CH2 -C6 H5), ---------.

Last paragraph of Column 16 states:

Nucleoside Phosphonates: Table 1 lists a group of exemplary nucleotide analogs of formula 1 having the structure (L1)(L2)P(O)--Z--B. These compounds generally have L1 and L2 groups that, when amino acids, are identical, although one of the amino acid groups can be different or replaced by another hydrolyzable group such as --O--CH2 --O--C(O)--C(CH3)3 or --O--C6 H5 as listed below.

7.6 Substitution of Specific substituents in the compound of formula (II)



provides a prodrug compound which is similar to that is intended to be claimed by the applicant in the impugned application.

7.7 The opponent submits that the applicant has also admitted the said fact on page 22 of the impugned application wherein it is stated that the prodrug compounds of this invention are provided in the form of free base or the various salts enumerated in US5798340.

7.8 Column 48 of D1 further states:

Identification of Active Precursors. It is desirable to select the amino acid residue or sequence of the invention compounds having one or more peptide bonds, such as formula VII compounds, based on the substrate specificity of esterases and/or carboxypeptidases expected to be found within cells where precursor hydrolysis is desired. To the extent that the specificity of these enzymes is unknown, one will screen a plurality of nucleotide analogs or esters until the desired substrate specificity is found. This will be apparent from assay either of the generation of free phosphonate or of antimicrobial activity. One selects compounds that are (i) not hydrolyzed or hydrolyzed comparatively slowly in the upper gut, (ii) gut and cell permeable and (iii) hydrolyzed in the cell cytoplasm and/or systemic circulation. Screens with cells from particular tissues are used to identify precursors that are released in organs susceptible to a target viral or microbial infection, e.g. in the case of liver, precursor drugs capable of hydrolysis in the liver. Other infections, e.g. CMV or HIV, are treated with a precursor that is hydrolyzed at substantially the same rate and to substantially the same degree in all tissues, with no one tissue preferentially hydrolyzing the precursor nucleosides.

Column 55 further states:

The hydrolysis products of interest are the phosphonates resulting from the hydrolysis of the amidate or ester bond(s) of the precursor compounds of this invention, for example HPMPC, 6-aza-HPMPC, cyclic HPMPC, PMEA, PMEG, PMPDAP, PMPA, D4TMPI, D4AMPI, cyclic HPMPA, FPMPA, PMEDAP, PMEMAP, 7-deaza-8-aza-FPMPA, 7-deaza-8-aza-HPMPA, cyclic 7-deaza-8-aza-HPMPA, 7-deaza-8-aza-PMPA, 8-aza-FPMPA, 8-aza-HPMPA, cyclic 8-aza-HPMPA, 8-aza-PMPA, PMPG, PMPMAP, 1-deaza-HPMPA, cyclic 1-deaza-HPMPA, 1-deaza-PMPA, 1-deaza-PMPG, 1-deaza-PMPMAP, 1-deaza-PMPDAP, 3-deaza-HPMPA, cyclic 3-deaza-HPMPA or 3-deaza-PMPA. Thus, the antibodies of this invention will be capable of binding to the precursors without binding to the hydrolysis products, will be capable of binding to the hydrolysis products without binding to the precursors, or will be capable of binding specifically to both. The antibodies will not cross-react with naturally-occurring nucleotides or nucleosides.

Further Column 57 and 58 disclose the synthesis of bis-phosphoroamidate nucleotide analogs of Formula Id.

Column 59 further discloses synthesis of mixed amidate-ester nucleotide analog amidates of Formula Id where L^1 is an amino acid ester and L^2 is a group of the formula OR, SR or OR^{31} is accomplished by conversion of a nucleotide analog (such as PMEA, HPMPC, HPMPA, PMEG, FPMPA, PMPDAP, D4AMPI, D4TMPI and the like) di- or bis-ester to a corresponding mixed ester-phosphoroamidate compound. A bis ester is converted to a mono ester by treatment with a base such as ammonia to remove one ester group. The resulting mono ester is then converted to a mixed amidate-ester as described for synthesis of bis amidate compounds.

9 The opponent submits that D1 discloses nucleotide analogs characterized by the presence of an amidate linked amino acid or an ester linked group which is bonded to the phosphorus atom of phosphonate nucleotide analogs and also discloses method of preparation of the same.

10 D2 states that

Nucleotides have shown interesting biological activities in a wide variety of antiviral, antiproliferative, immunomodulatory and other biological assays. Because of their negative charge (s) nucleotides suffer from disadvantages which can be successfully overcome by the utilization of nucleotide prodrugs. Nucleotide prodrugs were successfully used to increase oral absorption of nucleotides in

vivo. .... Nucleotides prodrugs have been also been utilized for tissue specific delivery of the nucleotides in vivo resulting in altered selectivity and reduced toxicity.

7.11 D2 further states that promised candidates were pre selected utilizing a number of assays, including chemical stability and stability in biological media.

7.12 Page 4 point 3 of D2 further discloses oral bioavailability, intracellular delivery of nucleotide prodrugs. Page 7 Fig. 5 discloses decomposition pathways of acyloxybenzyl esters A and amino acid ester amidates C. The said figure is reproduced below for ready reference:

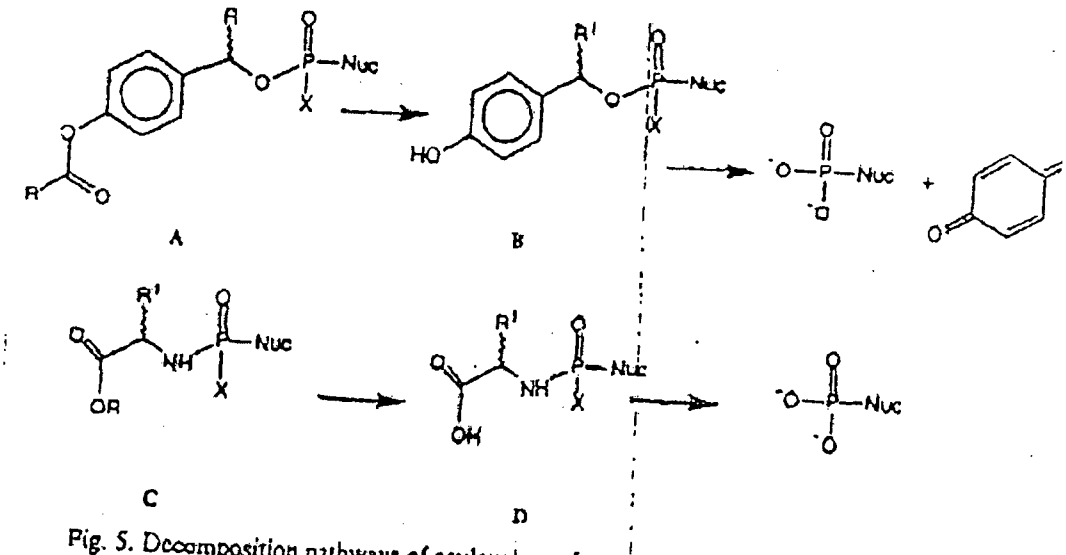


Fig. 5. Decomposition pathways of acyloxybenzyl esters A and amino acid ester amidates C.

7.13 Page 7 further states:

Another class of nucleotide prodrugs are phosphoranidates which have one or two nitrogens attached to the phosphorus. The chemical hydrolysis of phosphoramidates proceeds rapidly at lower pH and therefore the compounds may utilize an intracellular pH trigger to target endosomes, lyzosomes, or some tumour cells where the pH is lower than the normal physiological pH (Ross, 1961). Amino acid ester phosphoramidates C (Fig. 5) can also be hydrolyzed by esterases to form the free amino acid phosphoramidate D which is unstable and undergoes hydrolysis to yield the free nucleotide (Judoka and Smrt, 1974). A wide variety of amines and amino acids have been utilized in prodrug moieties (McGuigan et al, 1990 a, 1991, 1992; Gabrielsen et al, 1994). The phosphoroamidate formed between AZTp and

alanine methyl ester (13) (fig.6) was found to be active against HIV in a cell line restrictive to the activity of AZT due to cytosol.

7.14 The opponent therefore submits that from D1 and D2, it is amply clear that as on the date of priority of the impugned application, the use of amidate prodrugs for specific tissue delivery was well known in prior art.

7.15 The opponent states that though D1 and D2 does not specifically disclose PMPA, it nonetheless provides more than sufficient motivation and expectation of success to apply its teachings regarding the formation of a phosphonate nucleotide amidate ester prodrug to produce a compound with superior physical properties over a free base phosphonate nucleotide ester prodrug form. It is therefore stated that the alleged invention claimed in the instant impugned application is obvious over the teachings of D1 and D2.

7.16 WO9804569 which is the PCT equivalent of Indian application 2076/DEL/1997 hereinafter is referred to as D3 discloses Novel compounds that comprise esters of antiviral phosphonomethoxy nucleotide analogs with carbonates and/or carbamates having the structure $-OC(R^2)^2OC(O)X(R)a$, wherein R^2 independently is H, C_1-C_{12} alkyl, aryl, alkenyl, alkynyl, alkyenylaryl, alkynylaryl, alkaryl, arylalkynyl, arylalkenyl or arylalkyl which is unsubstituted or is substituted with halo, azido, nitro or OR^3 in which R^3 is C_1-C_{12} alkyl; X is N or O; R is independently H, C_1-C_{12} alkyl, aryl, alkenyl, alkynyl, alkyenylaryl, alkynylaryl, alkaryl, arylalkynyl, arylalkenyl or arylalkyl which is unsubstituted or is substituted with halo, azido, nitro, -O-, -N=, $-NR^4$-, $-N(R^4)^2$- or OR^3, R^4 independently is -H or C_1-C_8 alkyl, provided that at least one R is not H; and a is 1 or 2, with the proviso that when a is 2 and X is N, (a) two R groups can be taken together to form a carbocycle or oxygen-containing heterocycle, or (b) one R additionally can be OR^3. The compounds are useful as intermediates for the preparation of antiviral compounds or oligonucleotides, or are useful for administration directly to patients for antiviral therapy or prophylaxis. Embodiments are particularly useful when administered orally.

7.17 D3 further specifically provides carbonate and carbamate prodrugs of PMPA and also discloses methods for the preparation of the same. D3 also discloses the

preparation of fumarate salts of Tenofovir. Further pages 64-66 of D3 discloses the activity of various prodrugs of PMPA including Bis (POC) PMPA.

7.18 Table 2 further discloses antiretroviral activity of PMPA and PMPA prodrugs against HIV-1 wherein compound 2 is PMPA and compound 5a-5g are the different prodrugs of Tenofovir.

Table 2. Antiretroviral activity of PMPA and PMPA prodrugs against HIV-1.

compound	IC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
2	0.5	250	500
5a	0.002	40	20000
5c	< 0.001	30	30000
5d	0.2	10	50
5e	< 0.001	3	3000
5f	0.003	50	16600
5g	< 0.001	40	40000

7.19 The opponent therefore submits that as on the date of filing of the impugned application, it was well known to a person skilled in the art that PMEA when administered in the form of a carbonate/carbamate prodrug provides improved antiretroviral activity as may be noted from table 2 of D3.

7.20 The opponent states that in the event of the availability of a compound with similar therapeutic effect to the compound allegedly sought to be prepared by the applicant, it is evident that a person skilled in the art would first try out all known options before proceeding to look for other alternatives. The opponent therefore states that a person skilled in the art in view of the disclosures provided in D1 and D2 in combination with D3 would be motivate to try out the different prodrugs of PMPA/PMEA and would arrive at the amidate prodrugs of PMEA a so as to identify the best prodrug.

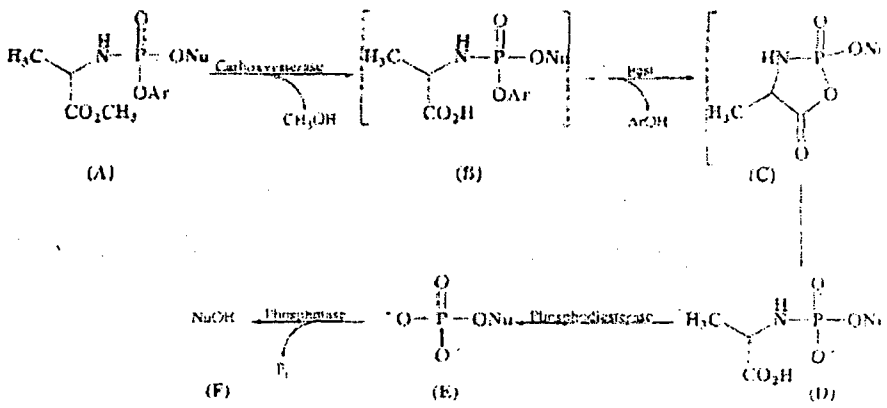
7.21 D4 probes the mechanism of action and decomposition of amino acid phosphomonoester amidates of antiviral nucleoside prodrugs.

Column 1 last paragraph states:

Of the various prodrug approaches, amino acid phosphoramidate derivatives have shown promise as potent antiviral agents, since in some cases they have exhibited enhanced antiviral activity and reduced cytotoxicity when compared to the parent nucleoside.

Scheme 1 discloses the proposed carboxyesterase – mediated breakdown of Aryl Phosphodiester amidates

Scheme 1. Proposed Carboxyesterase-Mediated Breakdown of Aryl Phosphodiester Amidates[a]



[a] Nu: either isoddA[11] or d4T.[17] Ar: either C_6H_5 or p-$NO_2C_6H_4$.

Column 1 on page 3324 further states;

Our laboratory has recently reported the biological activity of a series of aromatic amino acid carbomethoxy ester phosphomono- and -diester amidates of AZT (Table 1, 1-6).19 In contrast to amino acid carbomethoxy ester aryl phosphodiester amidates, the phosphomonoester amidates, 3 and 4, were shown to have significant antiviral activity and reduced cytotoxicity. For example, phosphoramidate 4 was found to be 8-fold more active at inhibiting the replication of HIV-1 in human peripheral blood mononuclear cells (PBMCs) than the parent nucleoside and was at least 10-fold less cytotoxic. The monoester derivatives (3 and 4) were shown to be stable in heat-inactivated fetal bovine serum and human serum (data not shown) at pH 7.2, 37 °C, for greater than 6 days. Although 3 and 4 were shown not to be substrates for phosphodiesterase I or alkaline phosphatase, they were shown to be substrates for acid phosphatase. Preliminary mechanistic studies demonstrated that 3 and 4 were internalized by PBMCs to the same extent as AZT. However, in contrast to PBMCs incubated with AZT, little or no free nucleoside and nearly 4-

fold more total phosphorylated AZT (i.e., AZT- 5'-mono-, di-, -triphosphate and phosphoramidate) were observed in cells incubated with either 3 or 4. Taken together, these results for the AZT phosphomonoester amidates were unexpected, since the decomposition of amino acid carbomethoxy ester phosphomonoester amidates of isoddA and d4T to their corresponding biologically active nucleoside monophosphates was not observed.

7.22 The opponent therefore states that the above abstracts from D4 indicate the bioavailability achieved by aminoacid phosphoramidate derivatives.

7.23 The opponent therefore states that a person skilled in the at equipped with the teachings of D4 would obviously be motivated to prepare the phosphoramidate prodrug of the parental compounds disclosed in D3 i.e. PMPA/PMEA withb a expectation of better activity.

7.24 It is therefore stated the subject matter covered in the impugned application being obvious and devoid of inventive merit over multiple documents, indicates that the alleged invention is a mere extension of prior art and is arrived at by trial and error techniques and hence is within the purview of a person skilled in the art and therefore ought to be rejected.

8. **NOT AN INVENTION / NOT PATENTABLE[Section 25(1)(f)]:**

8.1 **Claims not an invention as per Section 2(1)(ja)**

The opponent states that the claimed invention falls under the mischief of Section 2(1)(ja) being devoid of inventive step. The opponent states that Applicant's invention is neither a technical advancement nor it is giving any economic significance on the face of what is already known in the prior art and cited herein above and for the sake of brevity rely upon the arguments under paragraph 7. It is stated that to claim an invention, an inventor has to show a positive advancement of the relevant art and in the present case no technical advancement flows out of the various facets of the alleged invention claimed by the applicant.

8.2 **Claims not an invention as per Section 2(1)(j)**

The opponent states that the claimed invention falls under the mischief of Section 2(1) (j) devoid of being an "invention". The opponent states that the impugned application warrants rejection on this ground alone.

8.3 **Section 2 (1) (ta)**

The opponent further states that the alleged invention under the impugned patent is also in serious breach of Section 2(1) (ta) because the substance claimed is not a new entity involving one or more inventive steps. It is therefore specific requirement that in respect of a pharmaceutical product there has to be a new entity and over and above that there should be at least one inventive step. The case made out by the opponent clearly goes to show that there is no new entity involved in the alleged invention and neither there is any inventive step. For the purpose of the Act when the product in question is a pharmaceutical product the definition of invention has to be read harmoniously with Section 2(1) (ta) and such interpretation clearly makes the alleged invention devoid of patentability.

8.4 **Claims an invention not patentable as per Section 3(d)**

8.4.1 The term prodrug refers to a pharmacologically inactive compound that is converted to an active drug by a metabolic biotransformation. The biotransformation or activation of a prodrug may occur prior, during, and after absorption, or at specific target sites within the body.

8.4.2 The opponent states that the amidate prodrug of PMEA/PMPA claimed in claim 1 contravenes the provisions of Section 3(d) of the Patents Act, 1970. The clarification provided in Section 3(d) states that derivatives of known compounds are deemed to be the same substance unless the compounds differ significantly in efficacy in comparison with the compounds known from the prior art. The opponent submits that prodrugs being pharmaceutically inactive do not have any therapeutic efficacy. Even after biotransformation, it is the drug which shows the therapeutic effect and not the prodrug.

8.4.3 It is therefore stated that the applicant has failed to show as to how the allegedly claimed product has enhanced activity compared to the known prodrugs of PMEA/PMPA namely the prodrug of PMPA which is well documented in prior art namely D3.

8.4.4 It is further stated that it is a known fact that a prodrug will have higher bioavailability as compared to its parent drug and prodrugs of a single compound will have varying bioavailability (more or less) amongst themselves.

8.4.5 The opponent further submits that enhanced bioavailability only amounts to increased concentration of the compound at that specific site though it does not bring out any enhancement in the therapeutic activity of the parent drug. The opponent therefore submits that enhanced bioavailability of the drug due to the administration of the prodrug cannot be compared to enhanced therapeutic efficacy of the parent drug.

8.4.6 The opponent states that from Table 5 as provided in the specification it is noted that there are many other drugs such as GS7114 and GS73421 which have similar or better activity as compared to the compound intended to be claimed in the impugned application. Further from figure 3, it is noted that the AUC (0-24h) for PMPA in PBMC and plasma following an oral dose of 10 mg-eq/kg PMPA prodrugs is almost similar for GS7114, GS7340 and GS7342.It is further stated that demonstration of superior chemical stability is not in any way indicative of the activity of the said prodrug. Thus it is stated that, the applicant has failed to demonstrate that the claimed amidate prodrug of PMPA/PMEA significantly differs in its activity over the other known prodrugs of PMPA/PMEA known in the art.

8.4.7 The opponent states that the legality of this provision was challenged in the Hon'ble Madras High Court and such challenge was set aside. While setting aside the petition, the Ld. Judges were pleased to make *inter alia* the following observations:

> *The position therefore is, if the discovery of a new form of a known substance must be treated as an invention, then the patent applicant should show that the substance so discovered has a better therapeutic affect. Darland's Medical Dictionary defines the expression "efficacy" in the field of pharmacology as*

"the ability of a drug to produce the desired therapeutic affect" and efficacy is independent of the potency of the drug. The dictionary meaning of the term "therapeutic" is healing of a disease – having a good effect on the body. Going by the meaning of the terms "efficacy" and "therapeutic" extracted above, what the patent applicant is expected to show is, how effective the new discovery made would be in healing a disease or having a good effect on the body? In other words, the patent applicant is definitely aware as to what is the "therapeutic effect" of the drug for which he had already got a patent and what is the difference between the therapeutic effect of the patented drug and the drug in respect of which patent is asked for. Therefore, it is a simple exercise of, though preceded by research, we state, for any patent applicant to place on record what is the therapeutic effect/efficacy of a known substance and what is the enhancement in that known efficacy. The amended section not only covers the field of pharmacology but also the other fields. As we could see from the amended section, it is made applicable to even machine, apparatus or known process with a rider that mere use of a known process is not an invention unless such a known process results in a new product or employs atleast one new reactant. Therefore the amended Section is a comprehensive provision covering all fields of technology, including the field of pharmacology. In our opinion, the explanation would come in aid only to understand what is meant by the expression "resulting in the enhancement of a known efficacy" in the amended section and therefore we have no doubt at all that the Explanation would operate only when discovery is made in the pharmacology field"

"In our respectful opinion, when the validity of an Act is challenged on the touchstone of Article 14 of the Constitution of India, the decision has to depend upon the provisions of the concerned Statute itself, which are in challenge. Of course, law is well settled that when there is vagueness in any provision of law leading to arbitrary exercise of power / uncanalised powers, the Act should be struck down."

"We have borne in mind the object which the Amending Act wanted to achieve namely, to prevent evergreening; to provide easy access to the citizens of this country to life saving drugs and to discharge their Constitutional obligation of providing good health care to its citizens."

In the present case the applicant has at best discovered that the amidate ester prodrug of methoxyphosphonate nucleotide analogue (namely PMPA/PMEA) has enhanced bioavailability over the parental compound. Even such discovery has no merit on the face of the prior art which clearly taught that prodrug have better bioavailability over the parental compound and thereof disclosed the various prodrugs to be prepared for enhanced bioavailability. The opponent states that the alleged invention claimed in the instant impugned application under opposition is liable to be rejected on this ground alone.

9. **INSUFFICIENCY[Section 25(1)(g)]:**

9.1 The opponent states that the impugned application is liable to be rejected on the ground of insufficiency as well in that it does not describe with sufficient clarity to a general specialist in the art as to how the claimed invention may be put to practice.

9.2 Page 3 of the specification mentions compounds of formula (1) to be part of chirally enriched compositions. The opponent submits that the term chirally enriched compositions lacks clarity as to which composition is referred to therein. Further the term chirally enriched is also not clearly defined in the specification as to what is the specific amount of chiral enrichment is required. Claim 1 of the impugned application also mentions the term "chirally enriched compositions" and hence claim 1 also lacks clarity.

9.3 Page 3 of the specification further refers to enriched diastereomers of formula (2), though no specific amount of enrichment is disclosed in the specification. Claim 2 of the impugned application also mentions the term "its enriched diastereomers" and hence claim 2 also provides insufficient information.

9.4 The opponent states that page 3 of the specification states that the salts, free bases and solvates of formula (1) and formula (2) also form a part of alleged invention to be claimed in the impugned application. The opponent submits that the applicant ahs failed to provide any data with regards to the specific salts/solvates, the applicant intends to claim.

9.5 The opposed application is therefore liable to be rejected on the ground of insufficiency as well.

10. **BREACH OF SECTION 8 [Section 25(1) (h)]**

10.1 The applicant is required to provide all the information regarding the prosecution his equivalent applications till the grant of his Indian application to the Controller writing from time to time and also within the prescribed time which the applica has failed to do.

10.2 The opponent states that the applicant has not discharged its burden under Section since it failed to comply with the provisions of Section 8(1) and (2). The detai with respect to, *inter alia*, the following foreign applications in respect of tl same/substantially same invention were not disclosed completely in accordan with the requirement of the Act.

6. The pre-grant representation containing pages 192 was officially served on the Applicant on 13th June, 2013 as per Rule 55(3) and the applicant failed to file any reply under Rule 55(4) within stipulated time period. The applicants acknowledged vide letter dated.11.12.2013 that no reply statement has been filed under Rule 55(4) within the stipulated time period.

7. After expiry of time period for filing reply as per Rule 55(3) of the Patens Rules, the matter was proceeded further to next stage of prosecution i.e the hearing was offered inviting both the parties to discuss the grounds raised in pre-grant opposition vide office letter dated 26.11.2013, by fixing a hearing date on 12.12.2013.

8. The Applicant did not attend the hearing and therefore failed to make any oral submissions on the date of the hearing i.e on 12.12.2013. the applicant had written a letter on 11.12.2013 wherein it is indicated that according to Patent Rules it is not mandatory for the applicant to file a reply to the opponent's representation, hence, non – filing of the reply statement U/r. 55(4) does not amount to an implied admission of the grounds and submission raised by the opponent in the representation.

9. An authorized Patent Agent Mr.S. Majumdar, along with Mrs. Mythili Venkatesh, on behalf of the Opponent appeared for the hearing on the scheduled date and they have argued the matter on each ground maintained in the pre-grant representation. Upon hearing opponent was sought "7" days' time to submit their written submission, accordingly, an opponent has submitted their written submission etc., within the stipulated period of time.

Findings

10. Upon arguments made by opponent during the course of hearing and written submission made thereof, the following finding are derived into arrive a decision in the instant patent application by considering all the grounds maintained in the pre-grant representation.

a) Analysis of the claims of the impugned application.

The impugned application is a divisional to application 9/MUMNP/2003 dt.02.01.2003 which has been granted with a patent no.208435. The claim of the granted patent pertains to "a method for identifying a methoxy phosphate nucleotide analogue pro-drug conferring enhanced activity in a target tissue with antiviral or antitumor activity.

In this aspect the objection pertaining to allowability of divisional status has been raised in the examination report, wherein it was stated that the claims of the impugned application claims are not allowable under sect(16) read with Sec.10 (5) and Sec (7) (10) of the Act.

The applicants response dated 14th July, 2008 is as follow,

Under section 16 of the Patent Act, a divisional can be filed either to meet the learned Examiners objection or voluntarily by the applicant. It is submitted that the parent application of this divisional application was file don 02.01.2003 with both t process and the product claims,. Since prior art 2005, pharmaceutical product perse were not patentable under the Indian Patent Law, a divisional was file on the parent application 9th may, 2006 covering the products claims. Even under the law which was in force in 2005 section 5 permitted filing of the product claims in on application and process claims another.

It is further argued during the course of hearing by the opponent that the object of the invention is that about the prodrug of methoxyphosphonate mecleotide and its use in general and specifically states that invention is provide a screening method for identifying a methoxy phophonate nucleotide analogue prodrug. The compound which has been identified for the screening purpose is of structure (1). Page 4 states that the diastereomerically enriched compounds of this invention have the structure (3) and are designated the (S) isomers at the phosphorus chiral center and they are substantially free of the diastereomer (4). Further the page 8 of the specification states the preferred embodiment of this invention is the compound of structure (6) which has been designated as GS-7340. As the specification pertains to the prodrug for use in the screening method of this invention are covalently modified analogues of the parent methoxyphosphonate nucleotide analogues described. The phosphorus atom of

parent drug is the preferred site for prodrug modification , but other sites are found on the heterocyclic base B or the aglycon E. many such prodrug are already known as they are esters or amidates of the phosphorus atom but also include substitutions on the base and aglycon. Non e of these modifications per se is part of this invention and none are to be considered limiting on the scope of the invention here along with above observation, the opponent had submitted that the prodrug of methoxyphosphonate nucleotide namely amidates are well known in the aret and none of these modifications form a part of the invention, as the applicant in the impugned application intends to claim an amidate prodrug of methoxyphosphonate nucleotide.

With respect to divisional status the opponent had relied upon the hon,ble High Court Order (Glaxo Smith kline Plc and ors. Vs Controller of Patents and Designs). the opponents submitted that the case referred to by the applicant was with respet to EMR provisions while the present case relates to an application made as an ordinary application and is not comparable or equitable with the as cited above. It was submitted that the product claims of the parent application were not allowable /not patentable as on the date of the filing of the parent application.

The opponent further relied upon the IPAB order No. 243/2012 , in which it stated that the – those applications might have consisted of plurality of inventions and one of the multiple invention in the said application may have related to a product which was not patentable under the earlier Act. These can now be processed as a divisional application subject to satisfying all the criteria for grant of patent and also the criterion for treating it as a divisional application. It cannot be construed to mean the re-submission of the same claims that were made in the patent application disguising it as a divisional application will be entertained; it still has to satisfy the requirement of S.16. In view of the observations I hereby inclined with the arguments made during the course of hearing and observations provided in the written submissions.

b) Section 25(1) (e) – obviousness/lack of inventive steps.

The Opponent has analyzed all the cited documents (D1-D4) with alleged claims in the representation followed by further discussion had during the course of hearing and written submissions thereof, with respect to inventive step U/s, 25(1) (e) of the alleged claims.

They have stated that

D1 US 5798340 discloses Nucleotide analogs characterized but presence of an amidate linked amino acid or an ester linked group which is bonded to the phosphorus atom of phosphonate nucleotide analogs. The analogs comprise phosphoamidate or ester bond that is hydrolyzed in vivo to yield a corresponding phosphonate nucleotide analog. Method and intermediates for the

synthesis and use are described. The analysis of D1 under summery of the invention, in principal embodiment the objects of this invention are accomplished by a nucleotide analog comprising a phosphonate radical wherein the improvement comprises an amino acid residue or polypeptide radical I which an amino group of the amino acid or polypeptide is bonded to the phoporus ato(D1-D4) of the nucleotide analog by an amidate bond, a carboxyl group of the amino acid residue or polypeptide radical is positioned such that is tis capable as the free acid of hydrolyzing th ephophoroamide bond etc. As the same analysis in full is being provided in the representation is not repeated for sake of brevity.

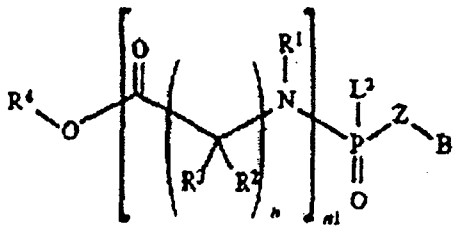
Further it is observed from the opponent's written submission that the alleged specification teaches at page 2 wherein under heading "Summery of the Invention". prodrug of methoxyphosphonate nucleotide analogues intended for antiviral or antitumor therapy / while known, traditionally have been selected for their systemic effect such prodrugs have been selected for enhanced bioavailability.

The methoxyphosponate nucleotide analogues in the present application relate to the Tenofovir drug molecule which is also known as PMPA. It addressed that the applicant has admittedly acknowledged in the specification that traditionally methoxyphosphonate nucleotide analogues are used in its prodrug form and are well known to have enhanced bioavailability. It is also clear form the specification at page no. 2 indicates and intends to provide a screening method for identifying a methoxyphosphonate nucleotide analogue prodrug which confers enhanced activity in a target tissue. Page no. further directs that the prodrugs for use I n the screening method for this invention are covalently modified analogues of the parent methoxyphosphonate nucleotide analogues. Impugned specification addressed certain facts with regards to methoxyphosphonate nucleotide which are the phosphorus atom of the parent drug is the preferred site for prodrug modification (page 8, line 11-12)

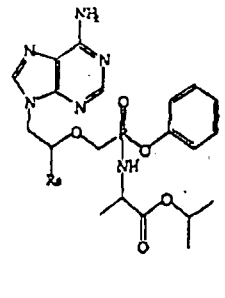
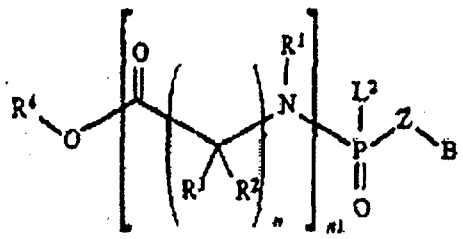
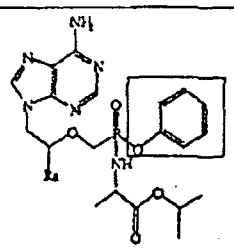
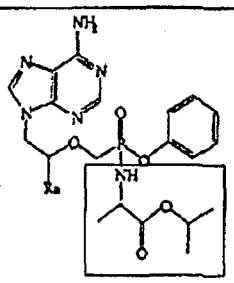
The known prodrug they are esters or amidates of the phosphorus atom (page 8, line 14-15). The use of prodrugs for use in the screening method of this invention are disclosed in D1 at page 8, line 24-26. It also reiterated the pro – moieties bound to the parent drug may be the same or different, each prodrug to be used in the screening assay will differ structurally from the other prodrugs to be tested with a varying features of combination based on the stereochemistry or their covalent structure.

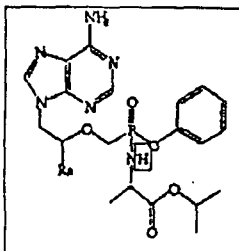
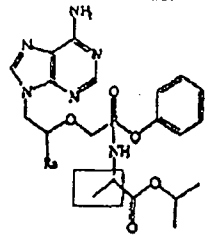
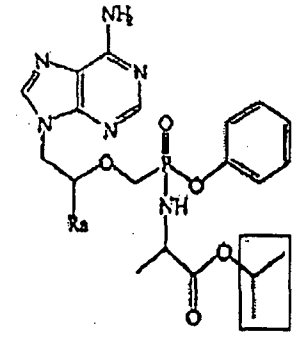
Therefore, the agent submitted that the preparation of different prodrugs of a single drug compound is well known in the prior art especially D1 and these prodrug are primarily ester prodrugs and amidate prodrugs. The opponent has further characterized presence of an amidate linked amino acid or an ester linked group which is bonded to the phosphorus atom of phosphonate nucleotide analogs, the analogs hydrolyzed in vivo to yield a corresponding phosphonate nucleotide analog. The characterization with D1 is as follow,

D1 (US5798340) discloses a compound of formula II



Specific substitutions as mentioned in the D1 specification would provide a compound similar to the structure as claimed in claim 1 and 2 of the impugned application as may be noted from the below table

530/MUMNP/2006	D1
Claim 1  (I)	Structure II of D1 
	wherein L^2 is OR, SR -----; R is H, C1 -C20 alkyl which is ---,C3 -C20 aryl which is unsubstituted or substituted by substituents ----; n is an integer having a value from 1 to 5 ---;
	

 (I)	**R1 is H** or C1-C9 alkyl which is unsubstituted or substituted by substituents ---;
 (I)	**R2** is **H** or C1 -C9 alkyl which is unsubstituted or substituted by substituents---; **R3** is ------, **C1 -C6 alkyl** which is unsubstituted or substituted ---; and
 (I)	**R4** is ---- is **C3-C9 alkyl** which is substituted by substituents independently selected from the group consisting of OH, O, N and halogen, C3 -C6 aryl which is substituted by substituents independently selected from the group consisting of OH, O, N and halogen or C3-C9 aryl-alkyl which is substituted by substituents independently selected from the group consisting of OH, O, N and halogen.

As these compounds generally have L1 and L2 groups that, when amino acids are identical, although one of the amino acid groups can be different or replaced by another hydrolysable group such as -- or –o-C6H5, thus when L1/L2 2. –NH-CH (CH_3)-C (o)-OR_4; and Z is 3. – CH2-O-CH (CH_3)-CH_2-B

And B is Adenin -0-yl wherein R4 includes H, propyl, isopropyl, t-butyl--- then the compound is as mentioned on column 25 of D1i.e 38.2.3.1 which is similar to that claim din claim 2 of the impugned application. Thus the D1 provides a list of nucleotide analogs characterized by the presence of an amidate linked amino acid or an ester linked group which is bonded to the phosphorus atom of phosphonate nucleotide analogs.

It is also noted that the D1 discloses the therapeutic indications of the compounds disclosed in the present application, wherein D1 indicated the same problems with nucleotide analogs as have been stated by the applicant in the impugned application and has also provided compounds to overcome those problems. Thus a person skilled in the art is aware of the manner in which the said problem can be overcome by the skilled person.

The Opponent had submitted the D1 addressed same problems with nucleotide analogs as have been stated by the applicant in the impugned application and has also provided compounds to overcome those problems. Thus a person skilled in the art is aware of the manner in which the said problem can be overcome. The nucleotide analogs which have been disclosed provides the various prodrugs forms the nucleotide analogs and also indicate that the same can be used for the same therapeutic activity as has been indicated in the impugned application. The opponent has been established that the D1 is the closest prior art in terms of structural and similarity of technical problem, as the impugned application is a mere extension of D1 having failed to contribute technical advancement.

D2 which is an article cited in the representation " Minireview " nucleotide prodrug by RobertJ.Jones and Norbert Bischofberger published in Antiviral Research states the disadvantages associated with nucleotides and the manner in which it is overcome by the preparation of nucleotide prodrugs. The D2 which teaches of utilizing a number of assays including chemical stability and stability in biological media, wherein at page 4 of the D2 states that the Nucleotide, however , have some disadvantages over nucleoside, because of their charge, they enter cells very poorly an show generally low or no activity in vitro, similarly radiolabelled uptake experiments showed that only 2% of extracellularly applied HPMC permeated into the cell. The charges are also responsible for their low oral bioavailability. The oral bioavailability of both PMEA and HPMPC in rats was less than 5%. Another disadvantage which pertains specifically to nucleoside phosphate analogues is their low stability in biological media, due to rapid dephosphorylation by phosphatases.

The nucleotide prodrugs can potentially overcome these difficulties, masking the negative charges on the phosphorus by suitable functionalities, which can be converted chemically or biologically to the parent nucleotide, can make nucleotides orally bioavailable, increase intracellular delivery, and alter pharmacokinetics distribution/metabolism resulting in improved efficacy and target organ specificity.

D2 gain describes for decomposition pathways of amino acid ester amidates to the nucleotides in the following manner,

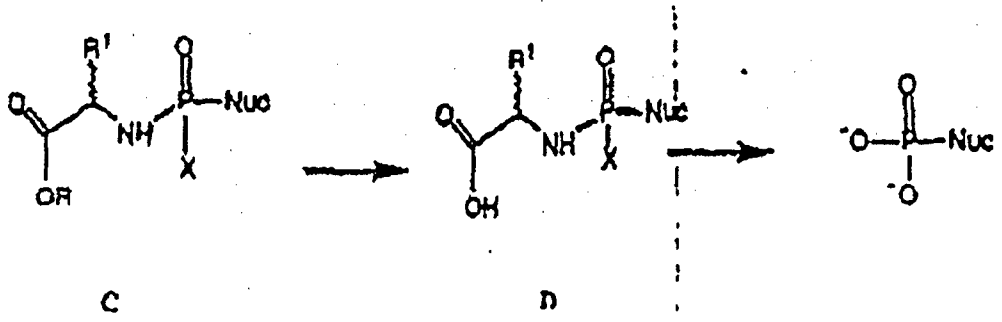
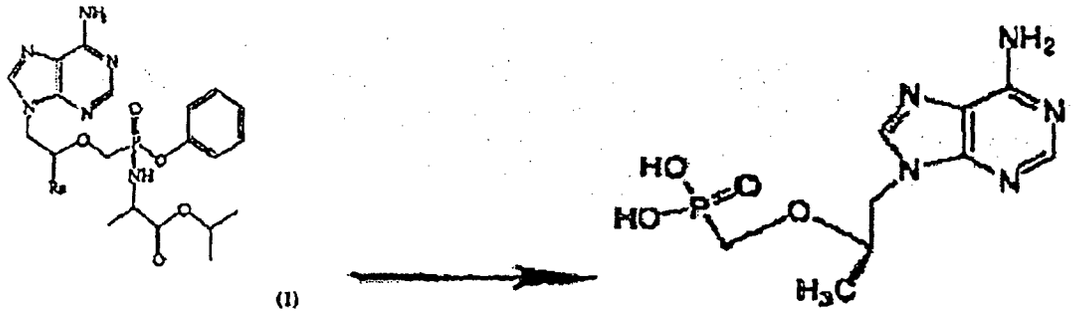


Fig. 5. Decomposition pathways of acyloxybenzyl esters A and amino acid ester amidates C.

The above chemical reaction illustrates how amino ester amidates is hydrolysed in vivo to yield a corresponding phosphonate nucleotide analog, it is observed that the skilled person will visualize that the compound of formula (I) will decompose in a similar pathway to give the compound Tenofovir.



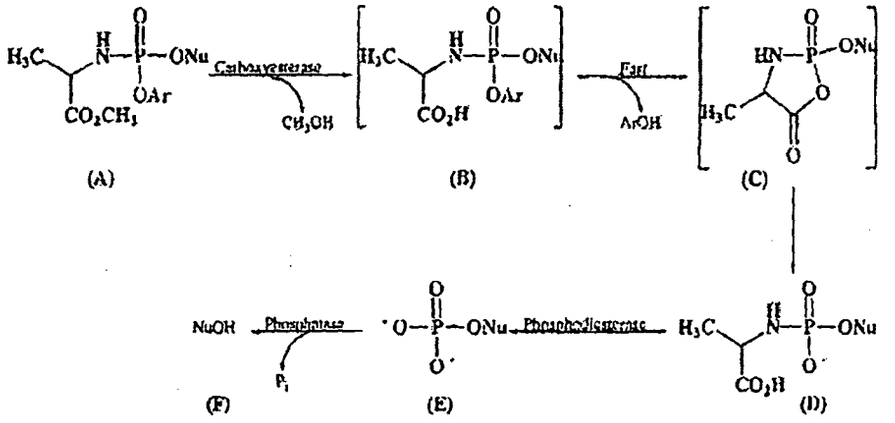
(I)

As the skilled person who is aware of D2 could able to indentify the different prodrugs and the manner in which they are likely to undergo degradation / hydrolysis so as to make the active drug available at the specific site. It's apparently clear from the reading of the D2 will provide motivation to prepare different prodrugs of Tenofovir inclusive of the amidate prodrugs as has been claimed in the alleged claims.

D3 (Wo 9804569 , which is PCT equivalent of indian patent application bearing no. 207/DEL/1997, which teaches of Novel compounds comprises esters of antiviral phosphonomethoxy nucleotide analogs with carbonates and/or carbamates. D3 specifically provides carbonate and carbamate prodrugs of PMPA and also discloses methods for the preparation of the same. Which also discloses the preparation of fumarate salts of Tenofovir, at pages 64 - 66 discloses the activity of various prodrugs of PMPA including Bis (POC) PMPA indicating that the prodrugs have a better activity as compared to Tenofovir. Therefore, D3 which indicates the activity of prodrug is better than the main nucleotide, as the D3 hhas enhanced properties as against the basic drug i.e Tenofovir, and therefore, provides motivation to a skilled person to look out for the various prodrugs of Tenofovir.

D4 an article "Probing the Mechanism of Action and Decomposition of Amino Acid Phosphomonoester Amidates of Antiviral Nucleoside prodrugs" published in J.Med . Chem, 1997 , 40 3323-3331 , which is cited at the time of making pre-grant representation, which teaches of mechanism of action and decomposition of amino acid phosphomonoester amidates of antiviral nucleodise prodrugs, D4 further highlighted the scheme I discloses the proposed carboxyesterase mediated breakdown of Aryl Phosphodiester amidartes.

Scheme 1. Proposed Carboxyesterase-Mediated Breakdown of Aryl Phosphodiester Amidates[a]



[a] Nu: either isoddA[11] or d4T.[17] Ar: either C_6H_5 or p-$NO_2C_6H_4$.

The D4 indicates that the bioavailability achieved by aminoacid phosphomonoester amidates, opponents have submitted that the skilled person were of the teaching of D4 will be inclined to think that amidate prodrugs of methoxyphosphonate nucleotide can be made so as to provide prodrugs with enhanced properties.

In this context the opponents have relied upon various decision that of European Board of Appeal decision T_1101/98 which relates a sililase case of obviousness in prodrug strategy of anticonvulsant sulfamates , the opponent further referred a decision of In Pfizer V. Apotex (U.S court of Appeal, 20061261) observed that for the test of obviousness only reasonable expectation of success and not guarantee is needed.

In Aventis V. Lupin (U.S Court of Appeal, 20061530) wherein the court held that the "where the prior art gives the reason or motivation to t make the claimed compositions, creates a prima facie case of obviousness.

The opponent again relied upon decision in Astrazenneca UK Limited V. GM Pharma Ltd, wherein the requirement of comparative tests vis-à-vis the closest prior art was again stated by the Ld. Tribunal, as follow,

The opponent relied on the European Board of Appeal decision T 181/82 which held that "an effect which may be said to be unexpected can be regarded as an indication of inventive step; where comparative tests are submitted as evidence of this, there must be the closest possible structural approximation – in a comparable type of use – to the subject –matter of the invention. In view of above skilled person would have been motivated to prepare mono L-valine ester of Tenofovir from the teachings of the '329, '924 and the Beauchamp articles. Therefore, claim 1 and dependent claims are not inventive. I agree with the analysis made out with cited prior arts, D1, D2, D3 and D4, wherein in the D1 discloses Nucleotide analogs characterized by the presence of an amidate linked amino acid which is bonded to the phosphorus atom of phosphonate nucleotide analogs, D2 discloses the manner in which amino acid ester amidate prodrugs of nucleotide will decompose to release the drug in the body at the specific site, D3 discloses that Tenofovir (PMPA) compound cannot be orally administered by administering it in the form of its prodrug which then decomposes at the specific site in the body to give

Tenofovir, thus D3 teaches that bioavailability of tenofovir is increased when administered in the form of a prodrug. D4 discusses about the bioavailability of the amino acid phosphomonoester amidates of antiviral Nucleoside prodrugs.

It observed that the person skilled in the field, could motivated to prepare the amidate prodrugs as is claimed in the present application by plain reading of D1 or in combination of the teachings of D1 to D4 with a reasonable expectation that the said prodrug will have better activity. Hence, the original claims of alleged patent application and as amended claims (in reply to First Examination Report) lacks inventive steps, and the ground of opposition under section 25(1)(e) has been established by the opponent.

Not an invention / not patentable (Section 25(1) (f)

It is apparently clear from previous discussions that invention is not patentable under section 25(1)(j) as it doesn't involve any inventive step.

Not patentable under section 3(d)

The applicant has submitted on 14/07/2008 with regard to section 3(d) against to the office objection raised under section 3(d) in the first examination report. It was stated, the basic nucleotide analogue, the parent compound, does not have the desired bioavailability and does not reach the affected cells easily. This basic nucleotide analogue is not the part of the present invention and what are claimed herein are pro-drugs of the parent compound's (specification pages 7 and 8). These pro-drugs are not the derivative but bio-intermediates of the parent compound with increased bio-distribution and increased bio – availability in the blood plasma. The increase in bio-availability is about 10 times (example's 11 of specification, page 50, last line).

The opponent stated that when the substance is known to have enhanced bioavailability over the parent drug compound as has been acknowledged by the applicant in specification, then the same factor i.e. enhanced bioavailability the prodrug compound cannot be a factor to determine the enhanced efficacy of the prodrug compound so as to satisfy the criteria of sec 3(d). It is stated that the experimental results increased 10 times as stipulated in the example no. 11 in this regard the opponent has pointed out the data related to GS -7340 i.e the diastereomer compound of formula (2) which is claimed in claim 2 of the impugned application. No data is provided in relation with regard to formula (2) of the impugned patent application. Thus the applicant has failed to provide any data let alone relevant data to show the enhanced properties achieved by the compounds to be claimed in the impugned patent application. Further it was sated that the if the data for the diastereomeric compounds is extrapolated to those compounds which the applicant has allegedly claimed in the impugned patent still the data provided relates only to bioavailability which demonstrated by the prodrugs whereas the data that is required is enhanced efficacy, for which the opponent had relied upon decision of Hon'ble High Court of Madras in the decision of Novartis Vs. UOI, has defined the therapeutic efficacy. In the same

context the opponent further relied upon Hon'ble Supreme Court in civil Appeal nos.2706-2716 of 2013 judgment, and the relevant paragraph 179.

The impugned patent application relates to prodrugs of PMPA (Tenofovir) in which the applicant has relied on enhanced bioavailability of the prodrug to show the enhanced effect over the previous prodrugs as well as PMPA. Different methods preparing different prodrugs of a known drug are well known as may be drowned from the cited references in the representation. It has been observed that when a comparison is made among the different prodrugs for the same compound, one prodrug is bound to have better effect than the other prodrug the better effect should be supported with data relating to its effect. As the present application and the parent drug is made available at the specific site without any degradation. Therefore applying known methods to arrive at the prodrug in the claimed invention without any therapeutic advantage but only advantage in terms of mode of safe delivery by masking of the parent nucleotide analogs of the alleged invention, and advantage only in terms of mode delivery of the nucleotide analogs which attracts section 3(d) of the Patents Act. Hence the ground of opposition under section 3(d) is validly established

11. In view of above findings of the case, the grounds in the statements and discussions as above and after considering written submission made by the opponent after hearing, all documents submitted during the course of hearing, under the circumstance of the case I conclude that the claims as amended of the instant patent application are not allowable as being obvious to a person skilled in the art and lacking an inventive step. Hence, the alleged claims do not constitute an invention U/s. 2(1) (j) of the Patents (Amended) Act, 2005. The claims also fall under section 3(d) of Patents (Amended) Act, 2005.

12. In view of above findings and observation provided by the opponent, I hereby order to refuse the grant of the patent on this patent application no.530/MUMNP/2006.

Dated 3rd Day of February, 2014

(N.Ramchander)
Asstt. Controller of Patents & Designs

Copy to:-

1. Subramaniam & Associates, Central Square, suite -328, Plaza III, 20 Manoharlal Khurana Marg, Bara Hindu Rao (off Rani Jhansi Road), Delhi -110006.

2. Mrs.Mythili Venkatesh, of S.Majumdar & Co., 202 Elecon Chambers, Behind Saki Naka, off Kurla – Andheri Road, Saki Naka, Mumbai – 400 072.

Annexure G

(6) 145

सत्यमेव जयते

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Date : 10/03/2014

To,
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SUB : First Examination Report

APPLICATION NUMBER : 568/MUMNP/2011
DATE OF FILING : 23/03/2011
DATE OF REQUEST FOR EXAMINATION : 24/08/2011
DATE OF PUBLICATION : 02/12/2011

a) With reference to the RQ No. 2971/RQ-MUM/2011 Dated 24/08/2011 in the above mentioned application for Grant of Patent , Examination has been conducted under Section 12 and 13 of the Patents Act 1970 , The following objections are hereby communicated.

b) Objections :

1 Subejct-matter claimed in claims 1-8, 20 is not patentable u/s 3 (d) of Patents Act;

2 The instant application claims priority from the International application No. PCT/US01/23104 which has number of claims 33, whereas the instant application at the National Phase entry has number of claims 20 for which fees is paid, there is difference of number of claims at the National phase and PCT application even after amendments before the IB, therefore in order to treat the instant application as National phase entry according to section 134 of Patents Act application should pay remaining fees for the number of claims as before the IB;

3 Details regarding application for Patents which may be filed outside India from time to time for the same or substantially the same invention should be furnished within Six months from the date of filing of the said application under clause(b) of sub section(1) of secton 8 and rule 12(1) of Indian Patent Act.

4 Details regarding the search and/or examination report including claims of the application allowed, as referred to in Rule 12(3) of the Patent Rule, 2003, in respect of same or substantially the same invention filed in all the major Patent offices along with appropriate translation where applicable, should be submitted within a period of Six months from the date of receipt of this communication as provided under section 8(2) of the Indian Patents Act.

c) You are requested to comply with the objections by filing your reply by way of explanation and/or amendments within 12 months from the date of issue of FER failing which your application will be treated as "Deemed to have been abandoned" under section 21(1) of the Act. The last Date is 10/03/2015.

d) You are advised to file your reply at the earliest so that the office can further proceed with application and complete the process within the prescribed period.

(Bharat N S) 10/3/2014
Asst. Controller of Patents & Designs

NOTE : All Communications to be sent to the Controller of Patents at INTELLECTUAL PROPERTY BUILDING S. M. Road, Antop Hill Mumbai-400 037.

146

(7)

बौध्दिक सम्पदा भारत
एकस्व / अभिकल्प / व्यापार चिन्ह / भौगोलिक संकेत
INTELLECTUAL PROPERTY **INDIA**
PATENTS / DESIGNS / TRADEMARKS / GEOGRAPHICAL INDICATIONS

सत्यमेव जयते
भारत सरकार
पेटेंट कार्यालय - बौद्धिक सम्पदा भवन
एस.एम. रोड, नजदीक अन्टॉप हिल डाकघर, अन्टॉप हिल, मुंबई - 400037
Government of India
Patent Office - Boudhik Sampada Bhawan
S.M. Road, Near Antop Hill Post Office, Antop Hill, Mumbai-400037

दूरभाष Tel: 022-24130747
फॅक्स Fax: 022-24130387
Email: mumbai-patent@nic.in
patmum@vsnl.net
Website: www. ipindia.nic.in

संख्या /No: Chem/162 दिनांक /Date: **19. 05. 2014.**

BY REGISTERED AD

To,

SUBRAMANIAM, NATARAJ & ASSOCIATES
ATTORNEYS-AT-LAW
PATENT & TRADEMARK ATTORNEYS
E-556,GREATER KAILASH-II
NEW DELHI 110 048.

In continuation of First Examination Report (FER) chem. /9030 dated 10/03/2014 the following objections are not communicated inadvertently and which are outstanding;

5. The instant application 568/MUMNP/20011 is filed as a divisional application (further application) out of 530/MUMNP/2006 and which in turn divisional application (further application) out of 9/MUMNP/2003 (original or first application). The provision of section 16 of Patents Act, 1970 in this regard is as follows:

> ***16. Power of Controller to make orders respecting division of application***
> ***(1) A person who has made an application for a patent under this Act may, at any time before the grant of the patent, if he so desires, or with a view to remedy the objection raised by the Controller on the ground that the claims of the complete specification relate to more than one invention, file a further application in respect of an invention disclosed in the provisional or complete specification already filed in respect of the first-mentioned application.***
> *(2) The further application under sub-section (1) shall be accompanied by a complete specification but such complete specification shall not include any matter not in substance disclosed in the complete specification filed in pursuance of the first-mentioned application.*

(3) The Controller may require such amendment of the complete specification filed in pursuance of either the original or the further application as may be necessary to ensure that neither of the said complete specification includes a claim for any matter claimed in the other.

[Explanation: For the purposes of this Act, the further application and the complete specification accompanying it shall be deemed to have been filed on the date on which the first mentioned application had been filed, and the further application shall be proceeded with as a substantive application and be examined when the request for examination is filed within the prescribed period.]

The further application can be filed at any time before the grant of the patent to the original or first-mentioned application but there is no provision to file the further application to already filed further application. Hence the instant application does not qualify as a further application as per sec 16(1) of the Patents Act, 1970. Further, the particulars for filing divisional application provided at paragraph 7 on Form-1 is not correct as the application no. 530/MUMNP/2006 is not the original or first application. In view of the above, further examination of the application is deferred and divisional status of the instant application no.568/MUMNP/2011 is not allowable.

6. Claims of the instant application (568/MUMNP/2011) are conflicting with the claims of parent (main) applications.

19/5/2014

(Bharat N. S.)
Asst. Controller of Patents & Designs

SNA Subramaniam & Associates

Attorneys-at-Law • Patent and Trademark Agents

2187 148

Annexure H 91/5109/2014

Our Ref: DV/PA/SNP 6811

15 October 2014

The Controller of Patents
The Patent Office
Baudhik Sampada Bhawan
SM Road, Antop Hill
Mumbai -400 037

Acceptance Due Date: 10.03.2015
Kind Attn.: Mr. Bharat N.S.
Asst. Controller of Patents & Designs

Dear Sirs,

RE: GILEAD SCIENCES, INC.
Indian Patent Application No.: 568/MUMNP/2011
Filed on : 23 March 2011
Divisional Out of Indian Application No.: 530/MUMNP/2006
Filed on : 09 May 2006 which is a
Divisional Out of Indian Application No.: 9/MUMNP/2003
Filed on : 02 January 2003
Corres to : PCT/US2001/23104
Filed on : 20 July 2001
Priority : 21 July 2000 [US: 60/220,021]

Reply to FER

17 OCT 2014

This is with reference to the Examination Reports dated 10 March 2014 and 19 May 2014. Our detailed comments with respect to the various objections raised therein are as under:

To address the objection in paragraph 1 of the First Examination Report claims 1 to 8 and 20 have been deleted. Therefore, this objection is rendered moot and the Learned Controller is respectfully requested to waive this objection.

The applicants respectfully resist the objection in paragraph 2 and submit that the present application is divisional out of 530/MUMNP/2006 and is required to be filed with the claims distinct from the parent application and not on the basis of claims as published with the PCT application. Therefore, this objection is rendered moot and the Learned Controller is respectfully requested to waive this objection.

The requirement of paragraphs 3 and 4 of the First Examination Report has been addressed in our letter of 02 June 2014 and 08 September 2014. Copy of our letters is attached herewith. Accordingly, the Learned Controller is respectfully requested to waive this requirement.

The objection in paragraph 5 is respectfully resisted and it is submitted that under Indian law a divisional application once filed is considered as an independent application. In this context, we respectfully invite the Learned Controller's attention to the *Explanation* appended with Section 16 which states that *"For the purpose of this Act, the further application and the complete specification accompanying it shall be deemed to have been filed, and the further application shall be proceeded with as a substantive application and be examined when the request for examination is filed within the prescribed period"*

Central Square, Suite-328, Plaza III, 20 Manoharlal Khurana Marg, Bara Hindu Rao (off Rani Jhansi Road), Delhi-110006
Tel: (+91 11) 30165700 • Fax: (+91 11) 30165798 / 30165799 • E-mail: sna@sna-ip.com

17 OCT 2014

Accordingly, under the law, apart from the same date of filing and priority date, the divisional is treated as a separate application because of the following reasons:

A divisional application:

1. is accorded a separate application number;
2. requires separate fees;
3. requires a separate request for examination to be filed;
4. is prosecuted separately; and
5. results in an independent patent.

Since, a divisional application is a separate independent application, it is not necessary for the grandparent application, i.e. the parent application of the first divisional application, to be still pending. The status of the grandparent application, e.g. the grant or refusal of the grandparent application, should have no influence on the second divisional application. As the second divisional application is divided out from the first divisional application, it is the first divisional application and not the grandparent application which represents the ***"parent application"*** of the second divisional application.

Therefore, a further or second divisional application out of a divisional application is a valid divisional application.

In this context, we respectfully invite the Learned Controller's attention to the European Law which also permit filing of the divisional application till the parent application is pending. However, a (first) divisional application may form the basis of a (second) divisional application; it is sufficient that the first divisional application is still pending at the filing date of the second divisional application. In this context, we attach herewith a copy of the Guidelines for Examination in the EPO, Part A, Chapter IV-1.1.1 (attached as Annexure-A). Similar stand is taken in other countries also i.e. a divisional out of divisional may be filed.

It is further submitted that it is the general principle of law that *"everything is permitted, which is not forbidden by law"*. The Patents Act does not prohibit the filing of a divisional out of divisional application. Moreover, it particularly specifies that a divisional application once filed is a separate application and therefore, it will form a valid basis for filing another divisional application. Further, since the date of filing of the divisional remains the same and does not extend the life of a patent, the filing of a divisional application out of divisional is not prejudicial and should be allowed. However, refusal of a valid divisional is prejudicial to the interest of the applicants.

Accordingly, in view of the above submissions the Learned Controller is respectfully requested to waive this objection.

To meet the objection of paragraph 6, the claims 1 to 12 and 20 have been deleted. The subject matter of claims does not conflict with the parent application.

The Learned Controller is respectfully requested to take the above submissions and enclosed documents on record.

All objections raised in first examination report have been duly complied and the amendments and submissions in preceding paragraphs are believed to place the application in condition of grant.

17 OCT 2014

Acceptance of this application within the **last date** expiring on **10 March, 2015** is respectfully requested.

The Learned Controller is respectfully requested to not to take any adverse decision on this application without giving the applicants an opportunity to be heard in this matter.

Yours sincerely,

Divya Kapoor
[Agents for Applicants]

Enclosures:

Revised claims_clean copy (in duplicate);
Revised claims_marked copy (in duplicate);
Copy of our letter dated 02 June 2014
Copy of our letter dated 08 September 2014
ANNEXURE A (in duplicate)

Annexure I

151 (11)

INTELLECTUAL PROPERTY INDIA
बौद्धिक सम्पदा भारत
एकस्व /अभिकल्प /व्यापार चिन्ह /
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भारत सरकार /Government of India
पेटेंट कार्यालय/ The Patent Office
तोडी इस्टेट्स,३ री मंजिल,सन मिल कंपाउंड, लोअर परेल ,मुंबई - 13
Todi Estate, 3rd Floor, Sun mill compound
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दूरभाष Tel 022-2492 4058
022-2492 5092
022-2496 1370
022-24949845
022-24922710
फॅक्स Fax 022-2495 0622
022-24903852
Email patmum@vsnl.net
Website www. ipindia.nic.in

संख्या /Letter No

दिनांक /Date: 12 AUG 2005

To,

Subramaniam, Nataraj & Associates
Patent & Trade Mark Attorneys,
E-556 ,Greater Kailash-II,
New Delhi-110048.

3725/12/05

Subject: First Examination Report
Reference: Patent Application No: **9/MUMNP/2003.**
Applicant: Gilead Sciences, Inc.

With reference to the request number**253/RQ/2005**, made on **1/3/2005** by **you** for the examination, the above quoted application has been examined under section 12 of The Patents Act, 1970 as amended and the First *Examination Report containing a statement of objections is forwarded herewith for compliance thereof.*

The documents enclosed shall be resubmitted **within 06(six) months from the date of issue of the said report** together with your observation if any, in connection with the compliance of the requirements of the First Examination Report. It may be noted that extension of time for a period of **not exceeding three** months can be obtained by making a request on Form 4 with prescribed fee, in exceptional circumstances beyond control of the applicant. Such extension can be availed only once and before the expiry of the said period of six months.

The application referred to will be deemed to have been abandoned under section 21(1) unless all the requirements imposed by the said Act and the rules there under are **complied with within the above said prescribed period.**

The pages of the complete specification should be freshly typed wherever corrections or interpolation are made. The typed pages in duplicate should be on white pages in order that clear photocopies of the specification can be prepared. The original pages in that case should be returned to this office duly cancelled

It is in the interest of the applicant to comply with the requirements at the earliest.

12-8-2005

(Bharat N.S.)
Examiner of Patents & Designs
For Asst. Controller of Patents & Designs

Encl: 1) Application on Form-1A
2) ~~Prov./~~Compl. Specification
~~3) Prov./Compl.Drawings.~~
~~4) Form-3~~ 5 Form-5

Note: All communications to be addressed to the Controller of Patents at the above address

(P.T.O.)

OIC RP

EXAMINATION REPORT

1. Invention claimed in claims 19-24 are not allowable u/sec 5(1)(a) of the prevailing patents act 1970,at the time of filing of application which is before 1.1.2005.For drug products claims there was a provision of WTO application & this application has not been filed as WTO application.
2. Invention claimed in claims 25-27 falls within the scope of sub clause of sec 3(e) of the patents act 1970 as amended by 2005. Hence not allowed.
3. Invention claimed in claims 28 falls within the scope of sub clause of sec 3(i) of the patents act 1970 as amended by 2005. Hence not allowed.
4. Invention claimed in claims 1-33 lacking in novelty & inventive step.
5. Claims 19-26 do not falls within the scope of claim 1.
6. Name of the compounds should be given.
7. Power of attorney in your favour should be filed.
8. Title is inconsistent with the claims.
9. Title from page 1 of the complete specification should be deleted.
10. Complete specification should be dated & signed.
11. Form 3 should be corrected according to the patents act 1970 (amended 2002), further details if any regarding foreign filing u/sec 8(1) should be furnished along with the necessary petition if & as applicable.
12. Form 5 should be corrected according to the patents act 1970(amended 2002).
13. All the irrevelent matters from complete specification should be deleted.
14. Preamble to the claims should be added i.e. we claim.
15. All the pages should be renumbered writing the page numbers at the bottom.
16. Details regarding the search and /or examination report including claims of the application allowed, as referred to the rule 12 (3) of the patents rules 2005,in respect of same or substantially the same invention filed in any one of the major patent offices, such as USPTO, EPO& JPO etc, along with appropriate translation where applicable, should be submitted within a period of 30 days from date of receipt of this communication as provided u/sec8 (2) of the Patents Act 1970 (Amended 2005).
17. Kindly note that it may not be possible to repeatedly examine your amended specification, therefore you should submit the documents only after fully complying with above requirements.
18. If you desire to discuss the matter, you can visit this office with prior appointments.

153
(5)

INTELLECTUAL PROPERTY INDIA
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PATENTS /DESIGNS/
TRADEMARKS/
GEOGRAPHICAL INDICATIONS

सत्यमेव जयते
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पेटेंट कार्यालय/ The Patent Office
तोडी इस्टेट्स,३ री मंजिल,सन मिल कंपाउंड, लोअर परेल ,मुंबई - 13
Todi Estate, 3rd Floor, Sun mill compound
Lower Parel (West), Mumbai – 400 013

दूरभाष Tel 022-2492 4058
022-2492 5092
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022-24949845
022-24922710
फॅक्स Fax 022-2495 0622
022-24903852
Email patmum@vsnl.net
Website www. ipindia.nic.in

संख्या /Letter No:

दिनांक /Date 6 JUN 2005

2014/6/6/05

To,
REMFRY &SAGAR
Attorneys-at- Law
Gresham Assurance House
1 Sir P.M. Road
MUMBAI 400001

Sub: First Examination Report
Ref: Patent Application No: 9/MUMNP/2003.
Name of the Applicant: Gilead Sciences, Inc.

With reference to the request number **253/RQ/2005**,made on **01/03/05**by you for the examination the above quoted application has been examined under section 12 of The Patents Act, 1970 as amended and the First Examination Report containing a statement of objections is forwarded herewith for compliance thereof.

The documents enclosed shall be resubmitted **within 06(six) months from the date of issue of the said report** together with your observation if any, in connection with the compliance of the requirements of the First Examination Report. It may be noted that extension of time for a period of **not exceeding three** months can be obtained by making a request on Form 4 with prescribed fee, in exceptional circumstances beyond control of the applicant. Such extension can be availed only once and before the expiry of the said period of six months.

The application referred to will be deemed to have been abandoned under section 21(1) unless All the requirements imposed by the said act and the rules thee under are complied with within the above said prescribed period.

The pages of the complete specification should be freshly typed wherever corrections or interpolation are made. The typed pages in duplicate should be on white pages in order that clear photocopies of the specification can be prepared. The original pages in that case should be returned to this office duly cancelled

It is in the interest of the applicant to comply with the requirements at the earliest.

6-6-2005

Encl: 1) Application Form
~~2) Provisional specification~~
3) Complete specification
~~4) Drawing sheets~~
5) Form 3/Form 5/~~Form 6/Form 8/Form 9/Form 13/Form 18~~
~~6) Power of Authority~~

(Bharat N.S.) 3/6/6
Examiner of Patents & Designs
For Asst. Controller of Patents & Designs

(P.T.O.)

Note: All communications to be sent to controller of patents at the above address

OLC 2

3/6

1) Invention claimed in claims19 –24 are not allowable u/sec 5(1)(a) of the prevailing patents act 1970, at the time of filing of a application which is before 1.1.2005.For drug products claims there was a provision of WTO application & this application has not been filed as WTO application.

2) Invention claimed in claims 25-27 falls within the scope of sub clause of sec3 (e) of the patents act 1970 as amended by the act 2005.

3) Invention claimed in claims 1-33 lacking in novelty/inventive step.

4) Invention claimed in claims 28 falls within the scope of sub clause of sec3 (i) of the patents act 1970 as amended by the act 2005.

5) Claims 19-26 do not falls within the scope of claim 1.

6) Name of the compounds should be given.

7) Power of attorney in your favour should be filed.

8) Title is inconsistent with the claims

9) Title from page 1 of the complete specification should be deleted.

10) Complete specification should be dated & signed.

11) Form 3 should corrected according to the patents act 1970(amended 2002), further details if any regarding foreign filing under sec 8(1) should be furnished along with necessary petition if & as applicable.

12) Form 5 should be corrected according to the patents act 1970(amended 2002).

13) All the irrevelent matters from complete specification should be deleted.

14) Preamble to the claims should be added i.e. we claim.

15) All the pages should be renumbered writing the page no, s at the bottom.

16) "Details regarding the search and /or examination report including claims of the application allowed, as referred to the rule 12(3) of the patents rule2003, in respect of same or substantially the same invention filed in any one of the major patent offices such as USPTO, EPO& JPO etc., along with appropriate translation where applicable, should be submitted within a period of 30 days from date of receipt of this communication as providedu/sec8 (2) of the Indian patents (Amendment) Act2003."

155
(13)

SUBRAMANIAM, NATARAJ & ASSOCIATES
ATTORNEYS-AT-LAW
PATENT AND TRADEMARK AGENTS

E-556, GREATER KAILASH - II
NEW DELHI-110 048

Tel: (011) 29210792, 29215603, 29216025 e-mail:sna@vsnl.com, Fax: (+91 11) 2922 6005, 2922 6012

Annexure J

HSM/SJ/RST0945

24 February 2006

The Controller of Patents
The Patent Office
Todi Estates, 3rd Floor
Lower Parel (W)
Mumbai-400 013

Kind Attn.: Mr. Bharat N.S.
Asst. Controller of Patents & Design

Dear Sirs,

Re: **GILEAD SCIENCES, INC.**
Indian Patent Application **No. 9/MUMNP/2003**
Filed on : 02 January 2003
Corres. to : PCT/US01/23104
Filed on : 20 July 2001
Priority : 21 July 2000 (US: 60/220,021)

We refer to the official letter dated 12 August and resubmit herewith the under-mentioned documents and present the following reply.

In connection with the first objection, it is respectfully submitted that Section 5(2) as it existed that time merely stated that when a claim is made for a pharmaceutical product..... it shall be dealt with according to chapter IV. There was no provision prescribed by the Act or Rules on how the claims should be made or in what form the claims should be made. All that was require was if a claim was made ..... it shall be dealt with in accordance with chapter IV. From the records, it is clear that a claim was indeed made for a pharmaceutical product per se. Therefore, the requirement of Section 5(2) was complied with. Contrary to what the Learned Examiner has said there was no provision of "WTO applications" under the Act. In fact, the Patents Rules, 1970 does not employ this terminology. This was merely an administrative convenience followed by the Patent Office which however can never overrule any legal provisions of the Patents Act, 1970.

Besides, Section 5 no longer exists. Under the recent order of the High Court of Calcutta this provision cannot be invoked any more. Nevertheless, the product claims have been deleted but they have been covered by a separate divisional application.

The claims have been thoroughly revised to meet the second to sixth objections.

It is respectfully submitted that no claim is taught by any prior art known to the applicant. With due respects it is submitted that even the ISR, a copy of which was filed at the Patent Office on 16 September 2005 holds all the claims to be novel and inventive. Reconsideration and withdrawal of the objection is respectfully requested.

A further statement and undertaking on Form 3 is submitted herewith. A Petition under Rule 137 and 138 has been filed under the cover of a separate letter. A copy of the petitions are submitted herewith. It is respectfully submitted that both form 4 and 5 originally filed still confirm to the basic requirement of the law giving the necessary information and therefore we respectfully request the Learned Examiner to withdraw the objection.

With regard to paragraph 16, it is respectfully submitted that the IPEA has found all the claims to be novel and inventive. Patents have been granted in New Zealand, South Africa and Eurasia. Notice of allowance has been issued in U.S.A. Every single jurisdiction has found every single claim to be novel and inventive. Copies of these documents are submitted herewith.

Contd....2

6-3-2006

The amendments carried out to the specification have necessitated retyping claim pages. The retyped pages are submitted herewith in triplicate alongwith original pages duly cancelled.

Acceptance of this application within the last date expiring on 12 May 2006 is respectfully requested.

Yours sincerely,

H. Subramaniam

Enclosures:
Application forms
Complete Specification
Form 3
Copy of Petition u/r 137
Copy of Petition u/r 138
Copy of notice of allowance in U.S.A.
Copy of Eurasian Patent
Copy of South African Patent
Copy of IPER
Retyped claim pages
Original pages (duly cancelled)

157

SUBRAMANIAM, NATARAJ & ASSOCIATES
ATTORNEYS-AT-LAW
PATENT AND TRADEMARK AGENTS

E-556, GREATER KAILASH - II
NEW DELHI-110 048

Tel: (011) 29210792, 29215603, 29216025 e-mail:sna@vsnl.com, Fax: (+91 11) 2922 6005, 2922 6012

HSM/SJ/RST0945

24 February 2006

Mr. Patne (Controller of Patents) 9/8/06

The Controller of Patents
The Patent Office
Todi Estates, 5rd Floor
Lower Parel (W)
Mumbai-400 013

Kind Attn.: Mr. Bharat N.S.
Asst. Controller of Patents & Design

Dear Sirs,

पेटेन्ट कार्यालय मुंबई, भारत सरकार
14 AUG 2006
PATENT OFFICE MUMBAI, GOVT. OF INDIA

Re: **GILEAD SCIENCES, INC.**
Indian Patent Application **No. 9/MUMNP/2003**
Filed on : 02 January 2003
Corres. to : PCT/US01/23104
Filed on : 20 July 2001
Priority : 21 July 2000 (US: 60/220,021)

We refer to the official letter dated 12 August and resubmit herewith the under-mentioned documents and present the following reply.

SNB 14/08/06

In connection with the first objection, it is respectfully submitted that Section 5(2) as it existed that time merely stated that when a claim is made for a pharmaceutical product..... it shall be dealt with according to chapter IV. There was no provision prescribed by the Act or Rules on how the claims should be made or in what form the claims should be made. All that was require was if a claim was made ..... it shall be dealt with in accordance with chapter IV. From the records, it is clear that a claim was indeed made for a pharmaceutical product per se. Therefore, the requirement of Section 5(2) was complied with. Contrary to what the Learned Examiner has said there was no provision of "WTO applications" under the Act. In fact, the Patents Rules, 1970 does not employ this terminology. This was merely an administrative convenience followed by the Patent Office which however can never overrule any legal provisions of the Patents Act, 1970.

Besides, Section 5 no longer exists. Under the recent order of the High Court of Calcutta this provision cannot be invoked any more. Nevertheless, the product claims have been deleted but they have been covered by a separate divisional application.

The claims have been thoroughly revised to meet the second to sixth objections.

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With regard to paragraph 16, it is respectfully submitted that the IPEA has found all the claims to be novel and inventive. Patents have been granted in New Zealand, South Africa and Eurasia. Notice of allowance has been issued in U.S.A. Every single jurisdiction has found every single claim to be novel and inventive. Copies of these documents are submitted herewith.

Contd....2

14-8-2006

The amendments carried out to the specification have necessitated retyping claim pages. The retyped pages are submitted herewith in triplicate alongwith original pages duly cancelled.

Acceptance of this application within the last date expiring on 12 May 2006 is respectfully requested.

Yours sincerely,

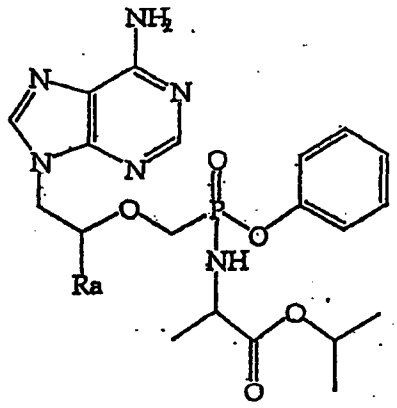
H. Subramaniam

Enclosures:
Application forms
Complete Specification
Form 3
Copy of Petition u/r 137
Copy of Petition u/r 138
Copy of notice of allowance in U.S.A.
Copy of Eurasian Patent
Copy of South African Patent
Copy of IPER
Retyped claim pages
Original pages (duly cancelled)

Annexure K

We claim

1 A compound having the structure (1)

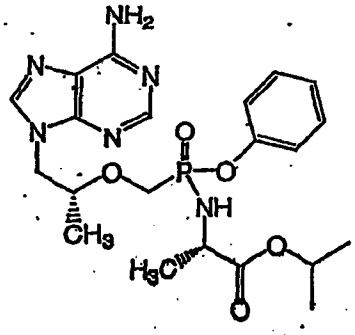


(1)

where Ra is H or methyl.

and chirally enriched compositions thereof, salts, their free base and solvates thereof

2 A compound as claimed in claim 1 having the structure (2)



(2)

and its enriched diasteromers, salts, free base and solvates

Dated this 1st day of May 2006

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Exhibit 1

160

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(57) Abstract

Novel compounds are provided that comprise esters of antiviral phosphonomethoxy nucleotide analogs with carbonates and/or carbamates having the structure $-OC(R^2)_2OC(O)X(R)_a$, wherein R^2 independently is H, C_1-C_{12} alkyl, aryl, alkenyl, alkynyl, alkyenylaryl, alkynylaryl, alkaryl, arylalkynyl, arylalkenyl or arylalkyl which is unsubstituted or is substituted with halo, azido, nitro or OR^3 in which R^3 is C_1-C_{12} alkyl; X is N or O; R is independently H, C_1-C_{12} alkyl, aryl, alkenyl, alkynyl, alkyenylaryl, alkynylaryl, alkaryl, arylalkynyl, arylalkenyl or arylalkyl which is unsubstituted or is substituted with halo, azido, nitro, -O-, -N=, $-NR^4$-, $-N(R^4)_2$- or OR^3, R^4 independently is -H or C_1-C_8 alkyl, provided that at least one R is not H; and a is 1 or 2, with the proviso that when a is 2 and X is N, (a) two R groups can be taken together to form a carbocycle or oxygen-containing heterocycle, or (b) one R additionally can be OR^3. The compounds are useful as intermediates for the preparation of antiviral compounds or oligonucleotides, or are useful for administration directly to patients for antiviral therapy or prophylaxis. Embodiments are particularly useful when administered orally.

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NUCLEOTIDE ANALOGS

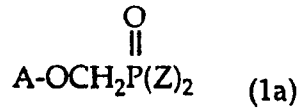
BACKGROUND OF THE INVENTION

The present invention relates to intermediates for phosphonomethoxy nucleotide analogs, in particular intermediates suitable for use in the efficient oral delivery of such analogs.

Such analogs per se and various technologies for oral delivery of these and other therapeutic compounds are known. See WO 91/19721, WO 94/03467, WO 94/03466, WO 92/13869, U.S. 5,208,221, 5,124,051, DE 41 38 584 A1, WO 94/10539, WO 94/10467, WO 96/18605, WO 95/07920, WO 95 79/07919, WO 92/09611, WO 92/01698, WO 91/19721, WO 88/05438, EP 0 632 048, EP 0 481 214, EP 0 369 409, EP 0 269 947, U.S. Patent Nos. 3,524,846 and 5,386,030, Engel *Chem. Rev.* 77:349-367 1977, Farquhar et al., J. *Pharm. Sci.* 72:324-325 1983, Starrett et al., *Antiviral Res.* 19:267-273 1992, Safadi et al., *Pharmaceutical Research* 10(9):1350-1355 1993, Sakamoto et al., *Chem. Pharm. Bull.* 32(6):2241-2248 1984, and Davidsen et al., *J. Med. Chem.* 37(26):4423-4429 1994.

SUMMARY OF THE INVENTION

In accordance with this invention, compounds are provided having formula (1a)



(1a)

wherein Z is independently $-OC(R^2)_2OC(O)X(R)_a$, an ester, an amidate or -H, but at least one Z is $-OC(R^2)_2OC(O)X(R)_a$;

A is the residue of an antiviral phosphonomethoxy nucleotide analog;

X is N or O;

R^2 independently is -H, C_1-C_{12} alkyl, C_5-C_{12} aryl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_7-C_{12} alkenylaryl, C_7-C_{12} alkynylaryl, or C_6-C_{12} alkaryl, any one of which is unsubstituted or is substituted with 1 or 2 halo, cyano, azido, nitro

or -OR^3 in which R^3 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl or C_5-C_{12} aryl;

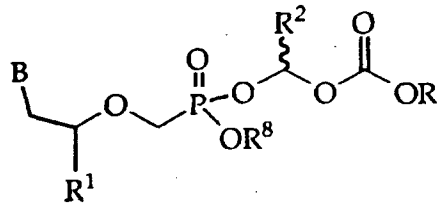
R independently is -H, C_1-C_{12} alkyl, C_5-C_{12} aryl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_7-C_{12} alkyenylaryl, C_7-C_{12} alkynylaryl, or C_6-C_{12} alkaryl, any one of which is unsubstituted or is substituted with 1 or 2 halo, cyano, azido, nitro, -$N(R^4)_2$ or -OR^3, where R^4 independently is -H or C_1-C_8 alkyl, provided that at least one R is not H; and

a is 1 when X is O, or 1 or 2 when X is N;

with the proviso that when a is 2 and X is N, (a) two N-linked R groups can be taken together to form a carbocycle or oxygen-containing heterocycle, (b) one N-linked R additionally can be -OR^3 or (c) both N-linked R groups can be -H;

and the salts, hydrates, tautomers and solvates thereof.

Further embodiments of the compounds of this invention are compounds of formula (1)



(1)

wherein B is guanin-9-yl, adenin-9-yl, 2,6-diaminopurin-9-yl, 2-aminopurin-9-yl or their 1-deaza, 3-deaza, or 8-aza analogs, or B is cytosin-1-yl;

R is independently -H, C_1-C_{12} alkyl, C_5-C_{12} aryl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_7-C_{12} alkenylaryl, C_7-C_{12} alkynylaryl, or C_6-C_{12} alkaryl, any one of which is unsubstituted or is substituted with 1 or 2 halo, cyano, azido, nitro or -OR^3 in which R^3 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl or C_5-C_{12} aryl;

R^1 is hydrogen, -CH_3, -CH_2OH, -CH_2F, -$CH{=}CH_2$, or -CH_2N_3, or R^1 and R^8 are joined to form -CH_2-;

R^2 independently is hydrogen or C_1-C_6 alkyl; and

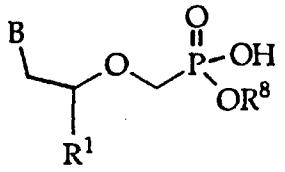
R^8 is hydrogen or -CHR^2-O-C(O)-OR, or R^8 is joined with R^1 to form -CH_2-;

and the salts, hydrates, tautomers and solvates thereof.

Other embodiments comprise orally administering to a patient infected with virus or at risk for viral infection a therapeutically effective amount of a compound of formulas (1a) or (1).

Other embodiments of this invention include a method for preparing a compound of formula (1a) which comprises reacting the diacid of a phosphonomethoxy nucleotide analog with $LC(R^2)_2OC(O)X(R)_a$ wherein L is a leaving group.

In particular embodiments of this invention, a method for preparing a compound of formula (1) is provided which comprises reacting a compound of formula (4)



(4)

with $LC(R^2)_2OC(O)X(R)_a$.

DETAILED DESCRIPTION OF THE INVENTION

The abbreviations NMP, DMF and DMPU mean, respectively, N-methylpyrrolidinone, dimethylformamide and N,N'-dimethylpropyleneurea.

Heterocycle means aromatic and nonaromatic ringed moieties. Heterocyclic moieties typically comprise one ring or two fused rings, where the ring(s) is 5- or 6-membered and typically contains 1 or 2 noncarbon atoms such as oxygen, nitrogen or sulfur, usually oxygen or nitrogen.

"Alkyl" as used herein, unless stated to the contrary, is C_1-C_{12} hydrocarbon containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 carbon atoms in the form of normal, secondary, tertiary or cyclic structures. Examples are $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH_2CH_2CH_3$, $-CH_2CH(CH_3)_2$, $-CH(CH_3)CH_2CH_3$, $-C(CH_3)_3$, $-CH_2CH_2CH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_2CH_3$, $-CH(CH_2CH_3)_2$, $-C(CH_3)_2CH_2CH_3$, $-CH(CH_3)CH(CH_3)_2$, $-CH_2CH_2CH(CH_3)_2$, $-CH_2CH(CH_3)CH_2CH_3$, $-CH_2C(CH_3)_3$, $-CH_2CH_2CH_2CH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_2CH_2CH_3$, $-CH(CH_2CH_3)(CH_2CH_2CH_3)$, $-C(CH_3)_2CH_2CH_2CH_3$, $-CH(CH_3)CH(CH_3)CH_2CH_3$, $-CH(CH_3)CH_2CH(CH_3)_2$, $-C(CH_3)(CH_2CH_3)_2$, $-CH(CH_2CH_3)CH(CH_3)_2$, $-C(CH_3)_2CH(CH_3)_2$, $-CH(CH_3)C(CH_3)_3$, cyclopropyl, cyclobutyl, cyclopropylmethyl, cyclopentyl, cyclobutylmethyl, 1-cyclopropyl-1-ethyl, 2-cyclopropyl-1-ethyl, cyclohexyl, cyclopentylmethyl, 1-cyclobutyl-1-ethyl, 2-cyclobutyl-1-ethyl, 1-cyclopropyl-1-propyl, 2-cyclopropyl-1-propyl, 3-cyclopropyl-1-propyl, 2-cyclopropyl-2-propyl, and 1-cyclopropyl-2-propyl.

"Alkenyl" as used herein, unless stated to the contrary, is C_1-C_{12} hydrocarbon containing normal, secondary, tertiary or cyclic structures.

Examples are -CH=CH_2, -CH=$CHCH_3$, -CH_2CH=CH_2, -C(=CH_2)(CH_3), -CH=$CHCH_2CH_3$, -CH_2CH=$CHCH_3$, -CH_2CH_2CH=CH_2, -CH=C(CH_3)2, -CH_2C(=CH_2)(CH_3), -C(=CH_2)CH_2CH_3, -C(CH_3)=$CHCH_3$, -CH(CH_3)CH=CH_2, -C=$CHCH_2CH_2CH_3$, -CHCH=$CHCH_2CH_3$, -$CHCH_2$CH=$CHCH_3$, -$CHCH_2CH_2$CH=CH_2, -C(=CH_2)$CH_2CH_2CH_3$, -C(CH_3)=$CH_2CH_2CH_3$, -CH(CH_3)CH=$CHCH_3$, -CH(CH_3)CH_2CH=CH_2, -CH_2CH=C(CH_3)2, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, and 1-cyclohex-3-enyl.

"Alkynyl" as used herein, unless stated to the contrary, is C_1-C_{12} hydrocarbon containing normal, secondary, tertiary or cyclic structures. Examples are -CCH, -$CCCH_3$, -CH_2CCH, -$CCCH_2CH_3$, -CH_2CCCH_3, -CH_2CH_2CCH, CH(CH_3)CCH, -$CCCH_2CH_2CH_3$, -$CH_2CCCH_2CH_3$, -$CH_2CH_2CCCH_3$ and -$CH_2CH_2CH_2$CCH.

Salt(s) include those derived by combination of appropriate anions such as inorganic or organic acids. Suitable acids include those having sufficient acidity to form a stable salt, preferably acids of low toxicity. For example, one may form invention salts from acid addition of certain organic and inorganic acids, e.g., HF, HCl, HBr, HI, H_2SO_4, H_3PO_4, or from organic sulfonic acids, organic carboxylic acids to basic centers, typically amines. Exemplary organic sulfonic acids include C_{6-16} aryl sulfonic acids, C_{6-16} heteroaryl sulfonic acids and C_{1-16} alkyl sulfonic acids such as phenyl, a-naphthyl, b-naphthyl, (*S*)-camphor, methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, *i*-butyl, *t*-butyl, pentyl and hexyl sulfonic acids. Exemplary organic carboxylic acids include C_{1-16} alkyl, C_{6-16} aryl carboxylic acids and C_{4-16} heteroaryl carboxylic acids such as acetic, glycolic, lactic, pyruvic, malonic, glutaric, tartaric, citric, fumaric, succinic, malic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, salicylic and 2-phenoxybenzoic. Salts also include the invention compound salts with one or more amino acids. Many amino acids are suitable, especially the naturally-occurring amino acids found as protein components, although the amino acid typically is one bearing a side chain with a basic or acidic group, e.g., lysine, arginine or glutamic acid, or a neutral group such as glycine, serine, threonine, alanine, isoleucine, or leucine. Salts are usually biologically compatible or pharmaceutically acceptable or non-toxic, particularly for mammalian cells. Salts that are biologically toxic are generally used with synthetic intermediates of invention compounds. The salts of invention compounds may be crystalline or noncrystalline.

A is the residue of a phosphonomethoxy nucleotide analog. The parental compounds have the structure $AOCH_2P(O)(OH)_2$. They are well known and have demonstrated antiviral activity. Per se, they are not part of this invention. In general, A has the structure BQ wherein B is a purine or pyrimidine base or the aza and/or deaza analogs thereof and Q is a cyclic or acyclic aglycon. B is linked to Q through the purine 9 or pyrimidine 1 positions. Examples of these analogs can be found in U.S. Patents 4,659,825, 4,724,233, 5,142,051 and 5,130,427, EP 369,409, EP 398,231, EP 494,370, EP 454,427, EP 270,885, EP 269,947, EP 452,935, WO 93/07157, WO 94/03467, and WO96/23801. Typically, A will have the structure $BCH_2CH(CH_3)$- or BCH_2CH_2-.

The designation "a" is an integer of 1 or 2. If X is N then a is 2 and one R is usually H and the other is not H. If X is O then a is 1.

B generally is guanin-9-yl, adenin-9-yl, 2,6-diaminopurin-9-yl, 2-aminopurin-9-yl or their 1-deaza, 3-deaza, or 8-aza analogs, or B is cytosin-1-yl. Ordinarily, B is adenin-9-yl or 2,6-diaminopurin-9-yl. In formula (1a) compounds, one Z optionally comprises an ester or an amidate. Suitable esters or amidates have been described, e.g., WO 95/07920. Exemplary esters are phenyl, benzyl, *o*-ethoxyphenyl, *p*-ethoxyphenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, N-ethylmorpholino, C_1-C_8 O-alkyl and C_1-C_8 NH-alkyl. However, every compound of the invention will contain at least one $-C(R^2)_2OC(O)X(R)_a$ moiety.

R^2 independently is -H, C_1-C_{12} alkyl, C_5-C_{12} aryl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_7-C_{12} alkenylaryl, C_7-C_{12} alkynylaryl, or C_6-C_{12} alkaryl, any one of which is unsubstituted or is substituted with 1 or 2 halo, cyano, azido, nitro or $-OR^3$ in which R^3 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl or C_5-C_{12} aryl. R^2 is usually H or C_1-C_6 alkyl, and typically only one R^2 is other than H. In most embodiments R^2 is H in both instances. The carbon atom to which R^2 is bonded is capable of chiral substitution, in which case R^2 is in the (R), (S) or racemic configuration. In most embodiments, if R^2 is other than H the compounds of this invention are chirally enriched or pure at this site. In general, however, manufacturing is somewhat less expensive if chirality at the R^2 carbon can be avoided. Thus, R^2 is H when it is desired to help minimize the cost of synthesis.

X is O or N, typically O. The carbamates (where X=N) tend to be more stable in biological environments than the carbonates. When X is O then a is 1.

R independently is -H, C_1-C_{12} alkyl, C_5-C_{12} aryl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_7-C_{12} alkyenylaryl, C_7-C_{12} alkynylaryl, or C_6-C_{12} alkaryl, any one of which is unsubstituted or is substituted with 1 or 2 halo, cyano, azido, nitro, $-N(R^4)_2$ or $-OR^3$, where R^4 independently is -H or C_1-C_8 alkyl, provided that at least one R is not H. In general, R is C_1-C_6 secondary or normal alkyl which is unsubstituted or substituted with OR^3. When X is N then a is 2. In the latter case one R is usually other than H. Alternatively, two N-linked R groups are joined to form a carbocycle or O-containing heterocycle, typically containing 3 to 5 carbon atoms in the ring. When R is unsaturated, but not aryl, the site of unsaturation is not critical and is in the Z or E configuration. The alkenyl chains of naturally occurring unsaturated fatty acids would be suitable as R groups, for example. R also includes cycloalkenyl or cycloalkynyl containing 1 or 2 unsaturated bonds, typically 1 unsaturated bond. When R is unsaturated, usually it is alkenyl or alkynyl without aryl substitution.

If R is substituted with halo, cyano, azido, nitro or OR^3, typically R will contain 1 of these substituents. If substituted with 2 of these substituents, they are same or different. Generally, substituents found on R are OR^3. An exemplary R group containing an OR^3 substituent is $-CH_2C(CH_2OCH_3)(CH_3)_2$.

When R contains an aryl group, the aryl group generally is bonded directly to X or is linked to X by methylene or ethylene. The aryl group may contain -N= or -O- as a ring atom. In general, the aryl group contains 5 or 6 carbons. If substituted, the aryl moiety is substituted with halo or OR^3 in the ortho, meta or para positions, with R^3 in this instance being typically C_1-C_3. Aryl groups containing 5 carbons are typically 2-, 3- or 4-pyridyl. In general, only one substituent group will be found on the aryl moiety if it is substituted at all. Exemplary aromatic and nonaromatic heterocyclic groups as used herein includes by way of example and not limitation the heterocycles described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and "J. Am. Chem. Soc.", 82:5566 (1960).

Examples of heterocycles include by way of example and not limitation pyridyl, thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl,

imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathiinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-indazoly, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, b-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl.

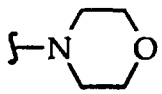
By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or b-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

R includes the structure -C_2-$C_6R^5C_2$-C_6 where each C_2-C_6 independently is a 2, 3, 4, 5 or 6 carbon linear, branched or cyclic alkyl moiety, e.g., ethylene, ethyl, propylene, propyl, isopropylene, isopropyl, cyclohexyl, etc., and R^5 is -O- or -NR^6- where R^6 is linear, branched or cyclic alkyl having 1, 2, 3, 4, 5 or 6 carbon atoms.

Embodiments include compounds where R^4 is -H or -CH_3.

R includes the structure -C_2-$C_{12}R^9$, where each C_2-C_{12} independently is a 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 carbon linear, branched or cyclic alkyl moiety, and R^9 is



N-morpholino ( ), N-piperidino, 2-pyridyl, 3-pyridyl or 4-pyridyl.

R also includes -$C(CH_2(X)_{0-1}R^7)_3$, -$CH[C(CH_2(X)_{0-1}R^7)_3]_2$ and -$CH_2(C(X)_{0-1}R^7)_3$, where R^7 is 1, 2, 3, 4, 5 or 6 carbon linear, branched or cyclic alkyl or R^7 is 5 or 6 carbon aryl. In these embodiments, one or two X are typically present, usually 1, X is usually oxygen and R^7 is typically methyl, ethyl, isopropyl, propyl or butyl, usually methyl.

R usually is phenyl, methyl, ethyl, 1-propyl, 2-propyl, *n*-butyl, *i*-butyl, *t*-butyl, pentyl or 3-pentyl.

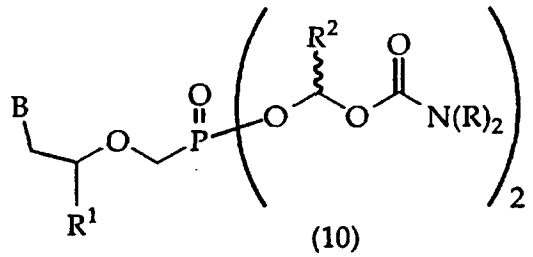
R^1 is a substituent found in prior art phosphonomethoxy nucleotide analogs. R^1 typically is any of hydrogen, -CH_3, -CH_2OH, -CH_2F, -$CH{=}CH_2$, -CH_2N_3 or R^1 and R^8 are joined to form -CH_2-. R^1 is usually H or methyl. If R^1 and R^8 are joined to form methylene, B typically is cytosin-1-yl.

R^3 is C_1-C_{12} alkyl, but typically is C_1-C_6 alkyl.

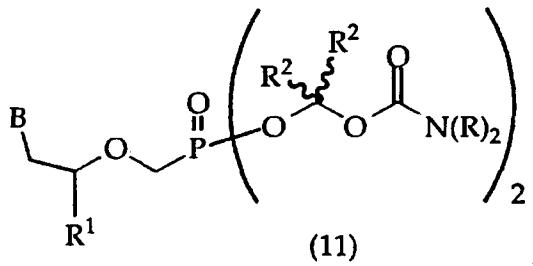
Compounds of structure (1) typically are those in which B is adenin-9-yl, R^1 is methyl or H, R^8 is -CHR^2-O-C(O)-OR and R, R^2 and R^3 are as set forth above.

The compounds of this invention are optionally enriched or resolved at the carbon atom chiral center linked to R^1 in accordance with prior findings associating optimal antiviral activity with the configuration at this site. Thus, where R^1 is methyl the compounds will be in (*R*) configuration at this center and will be substantially free of the (*S*) enantiomer.

Other embodiments include structure (10) and (11) compounds where R and each R^2 are independently chosen and R^2 is C_1-C_6 alkyl

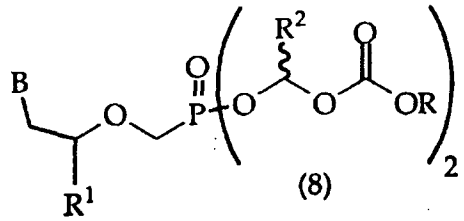


(10)



(11)

Exemplary embodiments include the compounds named in Table B. Each compound in Table B is depicted as a compound having the formula (8)



(8)

Compounds named in Table B are designated by numbers assigned to B, R, R^1 and R^2 according to the following convention, B.R.R^1.R^2, using the numbered structures depicted in Table A. Thus, the compound named 1.2.3.4 specifies adenin-9-yl at B, $-CH_2CH_3$ at both R groups, $-CH_2OH$ at R^1 and $-(CH_2)_2CH_3$ at both R^2 groups.

TABLE A

B	R^1
1 adenin-9-yl	1 $-CH_3$
2 guanin-9-yl	2 -H
3 2,6-diaminopurin-9-yl	3 $-CH_2OH$
4 2-aminopurin-9-yl	4 $-CH_2F$
5 cytosin-1-yl	5 $-CH=CH_2$
	6 $-CH_2N_3$

R	R^2
1 $-CH_3$	1 -H
2 $-C_2H_5$	2 $-CH_3$
3 $-(CH_2)_2CH_3$	3 $-C_2H_5$
4 $-CH(CH_3)_2$	4 $-(CH_2)_2CH_3$
5 $-(CH_2)_3CH_3$	5 $-CH(CH_3)_2$
6 $-CH_2CH(CH_3)_2$	6 $-(CH_2)_3CH_3$
7 $-CH(CH_3)CH_2CH_3$	7 $-CH_2CH(CH_3)_2$
8 $-C(CH_3)_3$	8 $-C(CH_3)_3$
9 $-(CH_2)_4CH_3$	9 $-(CH_2)_4CH_3$
10 $-CH(CH_3)CH_2CH_2CH_3$	10 $-(CH_2)_5CH_3$
11 $-CH(CH_2CH_3)_2$	
12 $-C(CH_3)_2CH_2CH_3$	
13 $-CH(CH_3)CH(CH_3)_2$	
14 $-CH_2CH_2CH(CH_3)_2$	
15 $-CH_2CH(CH_3)CH_2CH_3$	
16 $-CH_2CH_2CH_2CH_2CH_2CH_3$	
17 $-CH(CH_3)CH_2CH_2CH_2CH_3$	
18 $-CH(CH_2CH_3)CH_2CH_2CH_3$	
19 $-C(CH_3)_2CH_2CH_2CH_3$	
20 $-CH(CH_3)CH(CH_3)CH_2CH_3$	
21 $-CH(CH_3)CH_2CH(CH_3)_2$	
22 $-C(CH_3)(CH_2CH_3)_2$	
23 $-CH(CH_2CH_3)CH(CH_3)_2$	
24 $-CH_2C_6H_5$	
25 $-C_6H_5$	

TABLE B

1.1.1.1 1.1.1.2 1.1.1.3 1.1.1.4 1.1.1.5 1.1.1.6 1.1.1.7 1.1.1.8 1.1.1.9 1.1.1.10 1.1.2.1 1.1.2.2 1.1.2.3
1.1.2.4 1.1.2.5 1.1.2.6 1.1.2.7 1.1.2.8 1.1.2.9 1.1.2.10 1.1.3.1 1.1.3.2 1.1.3.3 1.1.3.4 1.1.3.5 1.1.3.6
1.1.3.7 1.1.3.8 1.1.3.9 1.1.3.10 1.1.4.1 1.1.4.2 1.1.4.3 1.1.4.4 1.1.4.5 1.1.4.6 1.1.4.7 1.1.4.8 1.1.4.9
1.1.4.10 1.1.5.1 1.1.5.2 1.1.5.3 1.1.5.4 1.1.5.5 1.1.5.6 1.1.5.7 1.1.5.8 1.1.5.9 1.1.5.10 1.1.6.1 1.1.6.2
1.1.6.3 1.1.6.4 1.1.6.5 1.1.6.6 1.1.6.7 1.1.6.8 1.1.6.9 1.1.6.10 1.2.1.1 1.2.1.2 1.2.1.3 1.2.1.4 1.2.1.5
1.2.1.6 1.2.1.7 1.2.1.8 1.2.1.9 1.2.1.10 1.2.2.1 1.2.2.2 1.2.2.3 1.2.2.4 1.2.2.5 1.2.2.6 1.2.2.7 1.2.2.8
1.2.2.9 1.2.2.10 1.2.3.1 1.2.3.2 1.2.3.3 1.2.3.4 1.2.3.5 1.2.3.6 1.2.3.7 1.2.3.8 1.2.3.9 1.2.3.10 1.2.4.1
1.2.4.2 1.2.4.3 1.2.4.4 1.2.4.5 1.2.4.6 1.2.4.7 1.2.4.8 1.2.4.9 1.2.4.10 1.2.5.1 1.2.5.2 1.2.5.3 1.2.5.4
1.2.5.5 1.2.5.6 1.2.5.7 1.2.5.8 1.2.5.9 1.2.5.10 1.2.6.1 1.2.6.2 1.2.6.3 1.2.6.4 1.2.6.5 1.2.6.6 1.2.6.7
1.2.6.8 1.2.6.9 1.2.6.10 1.3.1.1 1.3.1.2 1.3.1.3 1.3.1.4 1.3.1.5 1.3.1.6 1.3.1.7 1.3.1.8 1.3.1.9 1.3.1.10
1.3.2.1 1.3.2.2 1.3.2.3 1.3.2.4 1.3.2.5 1.3.2.6 1.3.2.7 1.3.2.8 1.3.2.9 1.3.2.10 1.3.3.1 1.3.3.2 1.3.3.3

1.3.3.4 1.3.3.5 1.3.3.6 1.3.3.7 1.3.3.8 1.3.3.9 1.3.3.10 1.3.4.1 1.3.4.2 1.3.4.3 1.3.4.4 1.3.4.5 1.3.4.6
1.3.4.7 1.3.4.8 1.3.4.9 1.3.4.10 1.3.5.1 1.3.5.2 1.3.5.3 1.3.5.4 1.3.5.5 1.3.5.6 1.3.5.7 1.3.5.8 1.3.5.9
1.3.5.10 1.3.6.1 1.3.6.2 1.3.6.3 1.3.6.4 1.3.6.5 1.3.6.6 1.3.6.7 1.3.6.8 1.3.6.9 1.3.6.10 1.4.1.1 1.4.1.2
1.4.1.3 1.4.1.4 1.4.1.5 1.4.1.6 1.4.1.7 1.4.1.8 1.4.1.9 1.4.1.10 1.4.2.1 1.4.2.2 1.4.2.3 1.4.2.4 1.4.2.5
1.4.2.6 1.4.2.7 1.4.2.8 1.4.2.9 1.4.2.10 1.4.3.1 1.4.3.2 1.4.3.3 1.4.3.4 1.4.3.5 1.4.3.6 1.4.3.7 1.4.3.8
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1.6.3.6 1.6.3.7 1.6.3.8 1.6.3.9 1.6.3.10 1.6.4.1 1.6.4.2 1.6.4.3 1.6.4.4 1.6.4.5 1.6.4.6 1.6.4.7 1.6.4.8
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1.11.3.3 1.11.3.4 1.11.3.5 1.11.3.6 1.11.3.7 1.11.3.8 1.11.3.9 1.11.3.10 1.11.4.1 1.11.4.2 1.11.4.3
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1.11.6.6 1.11.6.7 1.11.6.8 1.11.6.9 1.11.6.10 1.12.1.1 1.12.1.2 1.12.1.3 1.12.1.4 1.12.1.5 1.12.1.6
1.12.1.7 1.12.1.8 1.12.1.9 1.12.1.10 1.12.2.1 1.12.2.2 1.12.2.3 1.12.2.4 1.12.2.5 1.12.2.6 1.12.2.7
1.12.2.8 1.12.2.9 1.12.2.10 1.12.3.1 1.12.3.2 1.12.3.3 1.12.3.4 1.12.3.5 1.12.3.6 1.12.3.7 1.12.3.8
1.12.3.9 1.12.3.10 1.12.4.1 1.12.4.2 1.12.4.3 1.12.4.4 1.12.4.5 1.12.4.6 1.12.4.7 1.12.4.8 1.12.4.9
1.12.4.10 1.12.5.1 1.12.5.2 1.12.5.3 1.12.5.4 1.12.5.5 1.12.5.6 1.12.5.7 1.12.5.8 1.12.5.9 1.12.5.10
1.12.6.1 1.12.6.2 1.12.6.3 1.12.6.4 1.12.6.5 1.12.6.6 1.12.6.7 1.12.6.8 1.12.6.9 1.12.6.10 1.13.1.1
1.13.1.2 1.13.1.3 1.13.1.4 1.13.1.5 1.13.1.6 1.13.1.7 1.13.1.8 1.13.1.9 1.13.1.10 1.13.2.1 1.13.2.2
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5.24.4.1 5.24.4.2 5.24.4.3 5.24.4.4 5.24.4.5 5.24.4.6 5.24.4.7 5.24.4.8 5.24.4.9 5.24.4.10 5.24.5.1
5.24.5.2 5.24.5.3 5.24.5.4 5.24.5.5 5.24.5.6 5.24.5.7 5.24.5.8 5.24.5.9 5.24.5.10 5.24.6.1 5.24.6.2

5.24.6.3 5.24.6.4 5.24.6.5 5.24.6.6 5.24.6.7 5.24.6.8 5.24.6.9 5.24.6.10 5.25.1.1 5.25.1.2 5.25.1.3
5.25.1.4 5.25.1.5 5.25.1.6 5.25.1.7 5.25.1.8 5.25.1.9 5.25.1.10 5.25.2.1 5.25.2.2 5.25.2.3 5.25.2.4
5.25.2.5 5.25.2.6 5.25.2.7 5.25.2.8 5.25.2.9 5.25.2.10 5.25.3.1 5.25.3.2 5.25.3.3 5.25.3.4 5.25.3.5
5.25.3.6 5.25.3.7 5.25.3.8 5.25.3.9 5.25.3.10 5.25.4.1 5.25.4.2 5.25.4.3 5.25.4.4 5.25.4.5 5.25.4.6
5.25.4.7 5.25.4.8 5.25.4.9 5.25.4.10 5.25.5.1 5.25.5.2 5.25.5.3 5.25.5.4 5.25.5.5 5.25.5.6 5.25.5.7
5.25.5.8 5.25.5.9 5.25.5.10 5.25.6.1 5.25.6.2 5.25.6.3 5.25.6.4 5.25.6.5 5.25.6.6 5.25.6.7 5.25.6.8
5.25.6.9 5.25.6.10

Exemplary embodiments include the following numbered groups of compounds.

1 Each compound named in Table B having only one carbonate moiety and a hydroxyl group linked to the phosphorus atom in place of the second carbonate moiety, i.e., B-CH_2-CHR^1-O-CH_2-P(O)(OH)-O-CHR^2-O-C(O)-OR. Thus, the group 1 compound named 1.4.1.1 in Table B has the structure: adenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(OH)-O-CH_2-O-C(O)-$OCH(CH_3)_2$.

2 Compounds named in Table B having only one carbonate moiety and having only the R^1 moiety, #3 (-CH_2OH), which is modified such that R^8 of formula (1) compounds is joined with R^1 to form -CH_2-. Thus, the group 2 compound named 1.4.3.1 in Table B has the structure: adenin-9-yl-CH_2-$CH(CH_2$-◊)-O-CH_2-P(O)(O-◊)-O-CH_2-O-C(O)-$OCH(CH_3)_2$, where the symbols ◊ indicate a covalent bond that links the oxygen and carbon atoms together.

3 Compounds named in Table B and compounds named by compound groups 1 and 2 where each purine base listed in Table A is the 3-deaza analog, e.g., 3-deazaadenin-9-yl. Thus, the group 3 compound defined in Table A and named 1.4.1.1 in Table B has the structure: 3-deazaadenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(-O-CH_2-O-C(O)-$OCH(CH_3)_2)_2$. The group 3 compound defined in Table A and named 1.4.1.1 in compound group 1 has the structure: 3-deazaadenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(OH)-O-CH_2-O-C(O)-$OCH(CH_3)_2$.

4 Compounds named in Table B and compounds named by compound groups 1 and 2 where each purine base listed in Table A is the 1-deaza analog, e.g., 1-deazaadenin-9-yl. Thus, the group 4 compound defined in Table A and named 1.4.1.1 in Table B has the structure: 1-deazaadenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(-O-CH_2-O-C(O)-$OCH(CH_3)_2)_2$. The group 3 compound defined in Table A and named 1.4.1.1 in compound group 1 has the structure: 1-deazaadenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(OH)-O-CH_2-O-C(O)-$OCH(CH_3)_2$.

5 Compounds named in Table B and compounds named by compound groups 1 and 2 where each purine base listed in Table A is the 8-aza analog, e.g., 8-azaadenin-9-yl.

6 Compounds named in Table B and compounds named by compound groups 1-5 where R moieties 1-25 listed in Table A are replaced with the following groups:

1 -cyclopropyl (cyclopropyl replaces -CH_3, which is R moiety 1 in Table A)
2 -CH_2-cyclopropyl
3 -$(CH_2)_2$-cyclopropyl
4 -$(CH_2)_3$-cyclopropyl
5 -$(CH_2)_4$-cyclopropyl
6 -cyclobutyl
7 -CH_2-cyclobutyl
8 -$(CH_2)_2$-cyclobutyl
9 -$(CH_2)_3$-cyclobutyl
10 -$(CH_2)_4$-cyclobutyl
11 -cyclopentyl
12 -CH_2-cyclopentyl
13 -$(CH_2)_2$-cyclopentyl
14 -$(CH_2)_3$-cyclopentyl
15 -$(CH_2)_4$-cyclopentyl
16 -cyclohexyl
17 -CH_2-cyclohexyl
18 -$(CH_2)_2$-cyclohexyl
19 -$(CH_2)_3$-cyclohexyl
20 -$(CH_2)_4$-cyclohexyl
21 -$CH(CH_3)CH_2$-cyclopropyl
22 -$CH(CH_3)CH_2$-cyclobutyl
23 -$CH(CH_3)CH_2$-cyclopentyl
24 -$CH(CH_3)CH_2$-cyclohexyl
25 -$(CH_2)_{0\text{-}4}$-cyclooctyl.

Thus, the group 6 compound defined in Table A and named 1.16.1.1 in Table B has the structure:

adenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(-O-CH_2-O-C(O)-O-cyclohexyl)$_2$. The group 6 compound defined in Table A and named 1.16.1.1 in compound group 1 has the structure:

adenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(OH)-O-CH_2-O-C(O)-O-cyclohexyl. The group 6 compound defined in Table A and named 1.16.1.1 in compound group 3 has the structure:

3-deazaadenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(-O-CH_2-O-C(O)-O-cyclohexyl)$_2$.

7 Compounds named in Table B and compounds named by compound groups 1-5 where R moieties 1-25 listed in Table A are replaced with the following groups:

1 7 carbon alkyl*
2 8 carbon alkyl
3 9 carbon alkyl
4 10 carbon alkyl
5 11 carbon alkyl
6 12 carbon alkyl
7 -$(CH_2)_2C_6H_5$
8 -$(CH_2)_3C_6H_5$
9 -$(CH_2)_4C_6H_5$
10 -$C(CH_3)_2CH(CH_3)_2$
11 -$CH(CH_3)C(CH_3)_3$

12 -$(CH_2)_2CH(C_2H_5)CH_2CH_3$
13 -$(CH_2)_2CH(C_2H_5)(CH_2)_2CH_3$
14 -$(CH_2)_2CH(C_2H_5)(CH_2)_3CH_3$
15 -$(CH_2)_3CH(C_2H_5)CH_3$
16 -$(CH_2)_3CH(C_2H_5)CH_2CH_3$
17 -$(CH_2)_3CH(C_2H_5)(CH_2)_2CH_3$
18 -$CH_2CH(C_2H_5)CH_2CH_3$
19 -$CH_2CH(C_2H_5)(CH_2)_2CH_3$
20 -$CH_2CH(C_2H_5)(CH_2)_3CH_3$
21 -$(CH_2)_2CH(C_3H_7)CH_2CH_3$
22 -$(CH_2)_2CH(C_3H_7)(CH_2)_2CH_3$
23 -$(CH_2)_2CH(C_3H_7)(CH_2)_3CH_3$
24 -$CH_2CH=CH_2$
25 -$CH=CHCH_3$.

* Alkyl groups are linear, branched, cyclic or monounsaturated (-C=C-).

8 Compounds named in Table B and compounds named by compound groups 1-5 where R moieties 1-25 listed in Table A are replaced with the following groups:

1 -$(CH_2)_2OCH_3$
2 -$(CH_2)_3OCH_3$
3 -$(CH_2)_4OCH_3$
4 -$(CH_2)_5OCH_3$
5 -$(CH_2)_6OCH_3$
6 -$(CH_2)_2OCH_2CH_3$
7 -$(CH_2)_3OCH_2CH_3$
8 -$(CH_2)_4O(CH_2)_2CH_3$
9 -$(CH_2)_5O(CH_2)_2CH_3$
10 -$(CH_2)_6O(CH_2)_2CH_3$
11 -$(CH_2)_2O(CH_2)_2CH_3$
12 -$(CH_2)_3O(CH_2)_2CH_3$
13 -$(CH_2)_4O(CH_2)_2CH_3$
14 -$(CH_2)_5O(CH_2)_2CH_3$
15 -$(CH_2)_6O(CH_2)_2CH_3$
16 -$(CH_2)_2OCH(CH_3)_2$
17 -$(CH_2)_3OCH(CH_3)_2$
18 -$(CH_2)_4OCH(CH_3)_2$
19 -$(CH_2)_5OCH(CH_3)_2$
20 -$(CH_2)_6OCH(CH_3)_2$
21 -$(CH_2)_2O(CH_2)_3CH_3$
22 -$(CH_2)_2OCH_2CH(CH_3)_2$
23 -$(CH_2)_2OC(CH_3)_3$
24 -$(CH_2)_2OC_5H_{11}$
25 -$(CH_2)_2OC_6H_{13}$.

9 Compounds named in Table B and compounds named by compound groups 1-5 where R moieties 1-25 listed in Table A are replaced with the following groups:

1 -$CH(CH_3)CH_2OCH_3$
2 -$CH(CH_3)(CH_2)_2OCH_3$
3 -$CH(CH_3)(CH_2)_3OCH_3$
4 -$CH(CH_3)(CH_2)_4OCH_3$
5 -$CH(CH_3)CH_2OCH_2CH_3$
6 -$CH(CH_3)(CH_2)_2OCH_2CH_3$
7 -$CH(CH_3)(CH_2)_3OCH_2CH_3$
8 -$CH(CH_3)(CH_2)_4OCH_2CH_3$
9 -$CH(CH_3)CH_2O(CH_2)_2CH_3$
10 -$CH(CH_3)(CH_2)_2O(CH_2)_2CH_3$
11 -$CH(CH_3)(CH_2)_3O(CH_2)_2CH_3$
12 -$CH(CH_3)(CH_2)_4O(CH_2)_2CH_3$
13 -$CH(CH_3)CH_2OCH(CH_3)_2$

14 -$CH(CH_3)(CH_2)_2OCH(CH_3)_2$
15 -$CH(CH_3)(CH_2)_3OCH(CH_3)_2$
16 -$CH(CH_3)(CH_2)_4OCH(CH_3)_2$
17 -$CH(CH_3)CH_2OC_4H_9$
18 -$CH(CH_3)(CH_2)_2OC_4H_9$
19 -$CH(CH_3)(CH_2)_3OC_4H_9$
20 -$CH(CH_3)(CH_2)_4OC_4H_9$
21 -$CH(CH_3)CH_2OC_5H_{11}$
22 -$CH(CH_3)(CH_2)_2OC_5H_{11}$
23 -$CH(CH_3)(CH_2)_3OC_5H_{11}$
24 -$CH(CH_3)(CH_2)_4OC_5H_{11}$
25 -$CH(CH_3)CH_2OC_6H_{13}$.

10 Compounds named in Table B and compounds named by compound groups **1-5** where R moieties 1-25 listed in Table A are replaced with the following groups:
1 -$CH(CH_3)(CH_2)_2OC_6H_{13}$
2 -$CH(CH_3)(CH_2)_3OC_6H_{13}$
3 -$CH(CH_3)(CH_2)_4OC_6H_{13}$
4 -$CH_2CH(CH_3)OCH_3$
5 -$(CH_2)_2CH(CH_3)OCH_3$
6 -$(CH_2)_3CH(CH_3)OCH_3$
7 -$(CH_2)_4CH(CH_3)OCH_3$
8 -$CH_2CH(CH_3)OCH_2CH_3$
9 -$(CH_2)_2CH(CH_3)OCH_2CH_3$
10 -$(CH_2)_3CH(CH_3)OCH_2CH_3$
11 -$(CH_2)_4CH(CH_3)OCH_2CH_3$
12 -$CH_2CH(CH_3)OCH_2CH_3$
13 -$(CH_2)_2CH(CH_3)O(CH_2)_2CH_3$
14 -$(CH_2)_3CH(CH_3)O(CH_2)_3CH_3$
15 -$(CH_2)_4CH(CH_3)O(CH_2)_4CH_3$
16 -$CH_2CH(CH_3)OCH(CH_3)_2$
17 -$(CH_2)_2CH(CH_3)OCH(CH_3)_2$
18 -$(CH_2)_3CH(CH_3)OCH(CH_3)_2$
19 -$(CH_2)_4CH(CH_3)OCH(CH_3)_2$
20 -$CH_2CH(CH_3)OC_4H_9$
21 -$(CH_2)_2CH(CH_3)OC_4H_9$
22 -$(CH_2)_3CH(CH_3)OC_4H_9$
23 -$(CH_2)_4CH(CH_3)OC_4H_9$
24 -$CH_2CH(CH_3)OC_5H_{11}$
25 -$(CH_2)_2CH(CH_3)OC_5H_{11}$

11 Compounds named in Table B and compounds named by compound groups **1-5** where R moieties 1-25 listed in Table A are replaced with the following groups:
1 -$(CH_2)_3CH(CH_3)OC_5H_{11}$
2 -$(CH_2)_4CH(CH_3)OC_5H_{11}$
3 -$CH_2CH(CH_3)OC_6H_{13}$
4 -$(CH_2)_2CH(CH_3)OC_6H_{13}$

5 $-(CH_2)_3CH(CH_3)OC_6H_{13}$
6 $-(CH_2)_4CH(CH_3)OC_6H_{13}$
7 $-(CH_2)_2OCH(CH_3)C_2H_5$
8 $-(CH_2)_2OCH(CH_3)(CH_2)_2CH_3$
9 $-(CH_2)_2OCH(CH_3)CH(CH_3)_2$
10 $-(CH_2)_2OCH(CH_3)(CH_2)_3CH_3$
11 $-(CH_2)_2OCH(CH_3)C(CH_3)_3$
12 $-(CH_2)_2OCH(CH_3)CH(CH_3)CH_2CH_3$
13 $-(CH_2)_2OCH(CH_3)CH_2CH(CH_3)_2$
14 $-(CH_2)_3OCH(CH_3)C_2H_5$
15 $-(CH_2)_3OCH(CH_3)(CH_2)_2CH_3$
16 $-(CH_2)_3OCH(CH_3)CH(CH_3)_2$
17 $-(CH_2)_3OCH(CH_3)(CH_2)_3CH_3$
18 $-(CH_2)_3OCH(CH_3)C(CH_3)_3$
19 $-(CH_2)_3OCH(CH_3)CH(CH_3)CH_2CH_3$
20 $-(CH_2)_3OCH(CH_3)CH_2CH(CH_3)_2$
21 $-(CH_2)_4OCH(CH_3)C_2H_5$
22 $-(CH_2)_4OCH(CH_3)(CH_2)_2CH_3$
23 $-(CH_2)_4OCH(CH_3)CH(CH_3)_2$
24 $-(CH_2)_4OCH(CH_3)(CH_2)_3CH_3$
25 $-(CH_2)_4OCH(CH_3)C(CH_3)_3$

12 Compounds named in Table B and compounds named by compound groups **1-5** where R moieties 1-25 listed in Table A are replaced with the following groups:

1 $-(CH_2)_2O(CH_2)_3CH_3$
2 $-(CH_2)_2O(CH_2)_4CH_3$
3 $-(CH_2)_2O(CH_2)_5CH_3$
4 $-(CH_2)_3O(CH_2)_3CH_3$
5 $-(CH_2)_3O(CH_2)_4CH_3$
6 $-(CH_2)_3O(CH_2)_5CH_3$
7 $-(CH_2)_2OC_6H_5$
8 $-(CH_2)_2OC_6H_5$
9 $-(CH_2)_2OC_6H_5$
10 $-CH(C_2H_5)CH_2OCH_3$
11 $-CH(C_2H_5)CH_2OC_2H_5$
12 $-CH(C_2H_5)CH_2O(CH_2)_2CH_3$
13 $-CH(C_2H_5)CH_2OCH_2(CH_3)_2$
14 $-CH(C_2H_5)CH_2O(CH_2)_3CH_3$
15 $-CH(C_2H_5)CH_2OCH(CH_3)C_2H_5$
16 $-CH(C_2H_5)CH_2OCH_2CH(CH_3)_2$
17 $-CH(C_2H_5)CH_2OC(CH_3)_3$
18 $-CH(C_2H_5)CH_2O(CH_2)_4CH_3$
19 $-CH(C_2H_5)CH_2O(CH_2)_2CH(CH_3)_2$
20 $-CH(C_2H_5)CH_2O(CH_2)_5CH_3$
21 $-CH(C_2H_5)CH_2O(CH_2)_3CH(CH_3)_2$
22 $-CH_2CH(C_2H_5)OCH_3$
23 $-CH_2CH(C_2H_5)OC_2H_5$
24 $-CH_2CH(C_2H_5)OC_3H_7$

25 $-CH_2CH(C_2H_5)OC_4H_9$.

13 Compounds named in Table B and compounds named by compound groups 1-5 where R moieties 1-25 listed in Table A are replaced with the following groups:

1 $-(CH_2)_2O$-cyclopropyl
2 $-(CH_2)_2O$-cyclobutyl
3 $-(CH_2)_2O$-cyclopentyl
4 $-(CH_2)_2O$-cyclohexyl
5 $-(CH_2)_2OCH_2$-cyclopropyl
6 $-(CH_2)_2OCH_2$-cyclobutyl
7 $-(CH_2)_2OCH_2$-cyclopentyl
8 $-(CH_2)_2OCH_2$-cyclohexyl
9 $-(CH_2)_2O-(CH_2)_2$cyclopropyl
10 $-(CH_2)_2O-(CH_2)_2$cyclobutyl
11 $-(CH_2)_2O-(CH_2)_2$cyclopentyl
12 $-(CH_2)_2O-(CH_2)_2$cyclohexyl
13 $-(CH_2)_3O$-cyclopropyl
14 $-(CH_2)_3O$-cyclobutyl
15 $-(CH_2)_3O$-cyclopentyl
16 $-(CH_2)_3O$-cyclohexyl
17 $-(CH_2)_3OCH_2$-cyclopropyl
18 $-(CH_2)_3OCH_2$-cyclobutyl
19 $-(CH_2)_3OCH_2$-cyclopentyl
20 $-(CH_2)_3OCH_2$-cyclohexyl
21 $-CH(CH_3)CH_2O$-cyclopropyl
22 $-CH(CH_3)CH_2O$-cyclobutyl
23 $-CH(CH_3)CH_2O$-cyclopentyl
24 $-CH(CH_3)CH_2O$-cyclohexyl
25 $-CH(CH_3)CH_2OCH_2$-cyclohexyl.

14 Compounds named in Table B and compounds named by compound groups 1-5 where R moieties 1-25 listed in Table A are replaced with the following groups:

1 $-C(CH_2OCH_3)_3$
2 $-C(C_2H_5)_2(CH_2OCH_3)$
3 $-CH(C_2H_5)(CH_2OCH_3)$
4 $-CH_2(CH_2OCH_3)$
5 $-C(CH_3)_2(CH_2OCH_3)$
6 $-CH(CH_3)(CH_2OCH_3)$
7 $-C(CH_2OC_2H_5)_3$
8 $-C(C_2H_5)_2(CH_2OC_2H_5)$
9 $-CH(C_2H_5)(CH_2OC_2H_5)$
10 $-CH(C_4H_9)(CH_2OCH_3)$
11 $-CH_2C(CH_2OCH_3)_3$
12 $-CH_2C(C_2H_5)_2(CH_2OCH_3)$
13 $-CH_2CH(C_2H_5)(CH_2OCH_3)$
14 $-CH(CH_2OCH_3)_2$
15 $-CH_2C(CH_2OCH_3)_3$

16 $-CH_2CH(CH_2OCH_3)_2$
17 $-C(CH_2OC_2H_5)_3$
18 $-CH(CH_2OC_2H_5)_2$
19 $-CH_2C(CH_2OC_2H_5)_3$
20 $-CH_2CH(CH_2OC_2H_5)_2$
21 $-C(C_2H_5)_2(CH_2OC_3H_7)$
22 $-CH(C_3H_7)(CH_2OCH_3)$
23 $-C(C_3H_7)_2(CH_2OCH_3)$
24 $-CH(C_3H_7)(CH_2OC_2H_5)$
25 $-C(C_3H_7)_2(CH_2OC_2H_5)$

15 The following groups of compounds **A-J**.

A Compounds named in groups **8-14** where the oxygen atom (-O-) in the R moiety is replaced with -NH-.

B Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with $-N(CH_3)-$.

C Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with $-N(C_2H_5)-$.

D Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with $-N(CH_2CH_2CH_3)-$.

E Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with $-N(CH(CH_3)_2-$.

F Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with *n*-butyl substituted nitrogen ($-N(CH_2)_3CH_3)-$).

G Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with *i*-butyl substituted nitrogen.

H Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with *t*-butyl substituted nitrogen.

I Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with linear, branched or cyclic 5 carbon alkyl substituted nitrogen.

J Compounds named in groups **8-14** where the oxygen atom in the R moiety is replaced with linear, branched or cyclic 6 carbon alkyl substituted nitrogen.

Thus, the group **15B** compound defined in Table A and named 1.1.1.1 in compound group **8** has the structure:
adenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(-O-CH_2-O-C(O)-O-$(CH_2)_2N(CH_3)_2)_2$.
The group **15B** compound defined in Table A and named 1.1.1.1 in compound group **1**, as named under group **8**, has the structure:

adenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(OH)-O-CH_2-O-C(O)-O-$(CH_2)_2N(CH_3)_2$.
The group **15B** compound defined in Table A and named 1.16.1.1 in compound group 3, as named under group 8, has the structure:
3-deazaadenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(-O-CH_2-O-C(O)-O-$(CH_2)_2N(CH_3)_2)_2$.

16 Compounds named in Table B and compounds named by compound groups **1-5** where R moieties 1-25 listed in Table A are replaced with the following groups:

1 -$(CH_2)_2R^9$
2 -$(CH_2)_3R^9$
3 -$(CH_2)_4R^9$
4 -$(CH_2)_5R^9$
5 -$(CH_2)_6R^9$
6 -$(CH_2)_7R^9$
7 -$(CH_2)_8R^9$
8 -$CH(CH_3)CH_2R^9$
9 -$CH(CH_3)(CH_2)_2R^9$
10 -$CH(CH_3)(CH_2)_3R^9$
11 -$(CH_2)_2R^9$
12 -$(CH_2)_3R^9$
13 -$(CH_2)_4R^9$
14 -$(CH_2)_5R^9$
15 -$(CH_2)_6R^9$
16 -$(CH_2)_7R^9$
17 -$(CH_2)_8R^9$
18 -$CH(CH_3)CH_2R^9$
19 -$CH(CH_3)(CH_2)_2R^9$
20 -$CH(CH_3)(CH_2)_3R^9$
21 -$(CH_2)_2R^9$
22 -$(CH_2)_3R^9$
23 -$(CH_2)_4R^9$
24 -$(CH_2)_5R^9$
25 -$(CH_2)_6R^9$

In moieties 1-10, R^9 is N-morpholino, in moieties 11-20, R^9 is 2-pyridyl and in moieties 21-25, R^9 is 3-pyridyl.

17 Compounds named in Table B and compounds named by compound groups **1-5** where R moieties 1-25 listed in Table A are replaced with the following groups:

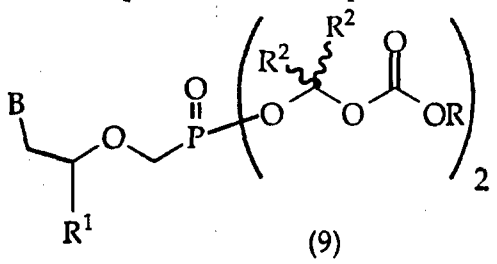
1 -$(CH_2)_2R^9$
2 -$(CH_2)_3R^9$
3 -$(CH_2)_4R^9$
4 -$(CH_2)_5R^9$
5 -$(CH_2)_6R^9$

6 $-(CH_2)_2CH(CH_3)R^9$
7 $-(CH_2)_3CH(CH_3)R^9$
8 $-(CH_2)_4CH(CH_3)R^9$
9 $-(CH_2)_2R^9$
9 $-(CH_2)_3R^9$
11 $-(CH_2)_4R^9$
12 $-(CH_2)_5R^9$
13 $-(CH_2)_6R^9$
14 $-(CH_2)_6CH_3$
15 $-(CH_2)_7CH_3$
16 $-(CH_2)_8CH_3$
17 $-(CH_2)_9CH_3$
18 $-(CH_2)_{10}CH_3$
19 $-(CH_2)_{11}CH_3$
20 $-(CH_2)_4CH(CH_3)_2$
21 $-(CH_2)_5CH(CH_3)_2$
22 $-(CH_2)_6CH(CH_3)_2$
23 $-(CH_2)_7CH(CH_3)_2$
24 $-(CH_2)_8CH(CH_3)_2$
25 $-(CH_2)_9CH(CH_3)_2$.

In moieties 1-5, R^9 is 4-pyridyl, in moieties 6-9 R^9 is N-morpholino and in moieties 9-13, R^9 is N-piperidyl.

18 The following groups of compounds **A-J**.

A Compounds named in Table B and compounds named by groups **1-17** where compound (8) is replaced with compound (9)



(9)

where one R^2 is as specified in Table A and the other R^2 is $-CH_3$.

B Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-CH_2CH_3$.

C Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-(CH_2)_2CH_3$.

D Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-CH(CH_3)_2$.

E Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-(CH_2)_3CH_3$.

F Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-(CH_2)_4CH_3$.

G Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-CH_2CH(CH_3)_2$.

H Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-C(CH_3)_3$.

I Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-C_5H_{11}$.

J Compounds named in Table B and compounds named by compound groups **1-17** where compound (8) is replaced with compound (9) where one R^2 is as specified in Table A and the other R^2 is $-C_6H_{13}$.

Thus, the group **18A** compound defined in Table A and named 1.4.2.3 in Table B has the structure:

adenin-9-yl-CH_2-CH_2-O-$CH(CH_3)$-P(O)(-O-$C(C_2H_5)(CH_3)$-O-C(O)-O-$CH(CH_3)_2)_2$.

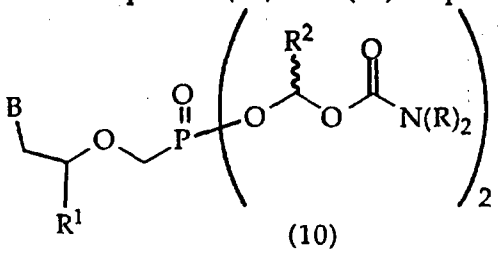
The group **18A** compound defined in Table A and named 1.4.1.1 in compound group **1** has the structure:

adenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(OH)-O-$CH(CH_3)$-O-C(O)-O-$CH(CH_3)_2$.

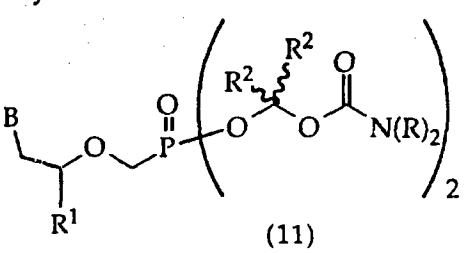
The group **18A** compound defined in Table A and named 1.1.1.1 in compound group **3**, as named under compound group 8, has the structure:

3-deazaadenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(-O-$CH(CH_3)$-O-C(O)-O-$(CH_2)_2OCH_3)_2$.

19 Compounds named in Table B and compounds named by compound groups **1-18** where compound (8) and compound (9) are replaced with compound (10) and (11) respectively



(10)



(11)

where both R moieties are the same. Thus, the group **19** compound defined in Table A and named 1.4.1.1 in Table B has the structure:

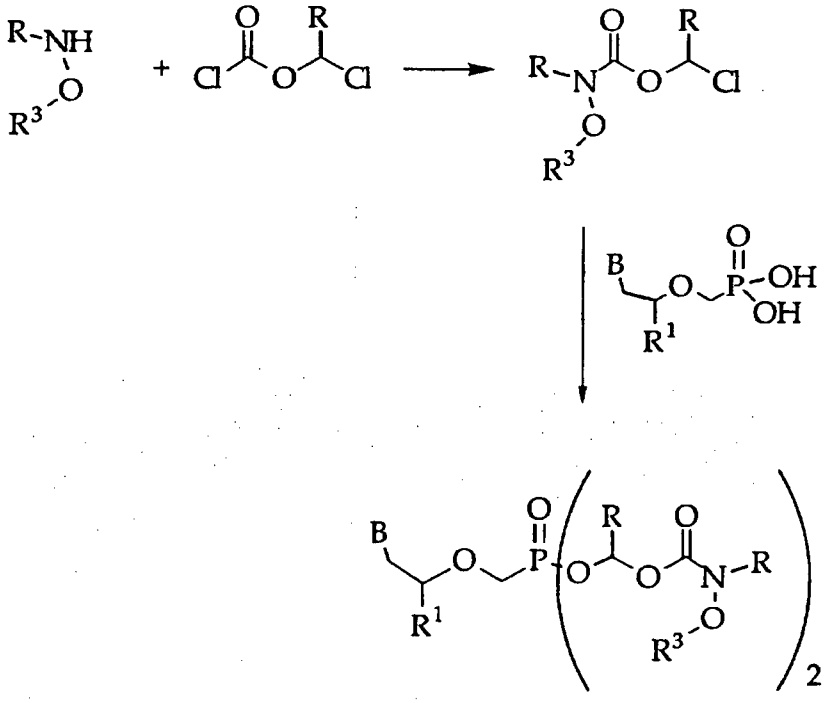
adenin-9-yl-CH_2-$CH(CH_3)$-O-$CH(CH_3)$-P(O)(-O-CH_2-O-C(O)-N-$CH(CH_3)_2)_2$. The group 19 compound defined in Table A and named 1.4.1.1 in compound group 1 has the structure: adenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(OH)-O-CH_2-O-C(O)-N-$CH(CH_3)_2$. The group 19 compound defined in Table A and named 1.1.1.1 in compound group 3, as named under compound group 8, has the structure: 3-deazaadenin-9-yl-CH_2-$CH(CH_3)$-O-CH_2-P(O)(-O-CH_2-O-C(O)-N-$(CH_2)_2OCH_3)_2$.

The compounds of this invention are, to varying degrees, chemically stable. It is preferable that the compounds be chemically stable in order to ensure an adequate shelf-life and proper biodistribution upon oral administration. In general, embodiments are selected that have a t 1/2 at pH 7.4 of greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours and preferably in addition possess a t 1/2 at pH 2.0 of greater than 1, 10 or 100 hours. For example, the t-butyl carbonate found in Table 1 has a t 1/2 that is less stable than these parameters and therefor is not preferred. In addition, the optimal compounds of this invention should have bioavailability in beagle dogs (as set forth in more detail below) that exceeds about 20%, preferably, about 30%.

Synthetic Methods

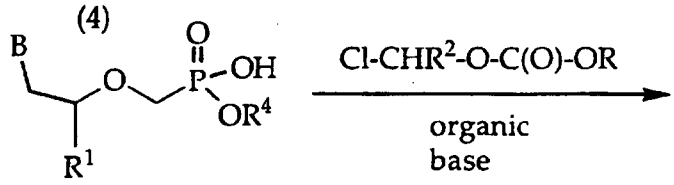
The carbamates and carbonates of this invention are prepared from the diacids of the phosphonomethoxy nucleotide analogs and the synthon $LCH(R^2)OC(O)X(R)_a$. L is a leaving group such as Cl, although it will be appreciated that any of the conventional leaving groups used in organic chemistry in nucleophilic substitution reactions can be successfully employed in place of chloro. In particular, leaving groups include halides, such as Cl, Br and I, and sulfonic acid esters such as methane, benzene or toluene sulfonic acid esters. The synthon is prepared by reacting $LCH(R^2)OC(O)L$ with HOR for preparation of the carbonate synthon or HNR_2 for the preparation of the carbamate synthon. The synthon is then reacted with the nucleotide analog of choice, typically PMPA, to form the desired carbamate or carbonate adducts. The carbamates are prepared by reacting the synthon with the nucleotide analog under typical conditions of nucleophilic attack, for example in Et_3N/DMF at room temperature. The carbonates are formed by reacting the appropriate synthon with the nucleotide analog in the presence of an organic base, typically an amine base. In addition, masked leaving groups such as thioethers, which may be activated by, for example, oxidation, and coupled directly to the phosphonic acid moiety may be used. Intermediates may be

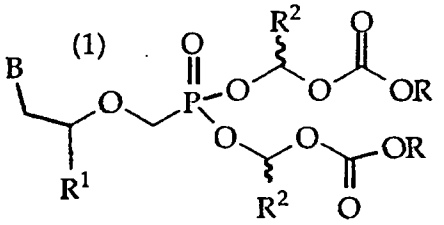
made with other leaving groups in this way, for example diphenylphosphinic acids, and others known in the chemistry of formacetals and glycosylation.



Compounds where X=N and R=OR^3 may be prepared by alkylation with the appropriate haloalkyl, O-alkyl carbamate. N, O-dialkylhydroxylamines are known in the literature, and may be prepared by alkylation of hydroxylamine, or by reductive amination of aldehydes or ketones with alkyl hydroxylamines. The dialkylhydroxylamines may be acylated with the appropriately substituted haloalkyl chloroformate under conditions analagous to those used to prepare the unsubstituted chloromethyl carbamates. Phosphonates may then be alkylated with the haloalkyl, O-alkyl carbamates to give the prodrugs under conditions used for the carbonates and carbamates. Leaving groups other than chloride may of course be used throughout.

In a typical method, the carbonate compounds of this invention are prepared by reacting L-CHR^2-O-C(O)-OR with (4) to yield a compound of formula (1).





The reaction typically proceeds in two concurrent steps in which the monoester forms first, and then the diester as the reaction proceeds longer. In this situation monoester is not typically isolated as an intermediate.

In order to make a diester that contains different carbonate or carbamate functionalities the monoester intermediate is recovered from the early reaction and the reaction is then completed with for example a second L-CHR^2-O-C(O)-OR reagent, thereby resulting in substitution with a second ester different from the first.

One optionally conducts the carbonate synthesis reactions using at least about 1.0 and typically 2 equivalents of L-CHR^2-O-C(O)-OR. The reaction is conducted in the presence of an organic base in an organic solvent at a reaction temperature of about 4-100° for about 4-72 hours. Exemplary suitable organic bases include triethylamine or Hunig's base. Exemplary suitable organic solvents include DMF, DMPU, or NMP.

The monoester or diester products are purified by standard methods including flash column chromatography or salting out. Suitable salts for purification and/or formulation will final product include the sulfuric acid, phosphoric acid, lactic acid, fumaric or citric acid salts or complexes of the diester or monoester compounds of structures (1) or (1a).

Utilities

The compounds of this invention are useful in the treatment or prophylaxis of one or more viral infections in man or animals, including infections caused by DNA viruses, RNA viruses, herpesviruses (CMV, HSV 1, HSV 2, VZV, and the like), retroviruses, hepadnaviruses, (e.g. HBV), papillomavirus, hantavirus, adenoviruses and HIV. Other infections to be treated with the compounds herein include MSV, RSV, SIV, FIV, MuLV, and other retroviral infections of rodents and other animals. The prior art describes the antiviral specificity of the nucleotide analogs, and the parental drug specificity is shared by the compounds of this invention. Dosages, viral targets, and suitable administration routes to best attack the site of infection are well known in the art for the parental drugs. Determination of proper doses is a straightforward matter for the clinician, taking into account the molecular weight of the compounds of this invention and, when adminstering them orally, their bioavailability in animals or as deduced in clinical trials with humans. Oral dosages of the compounds of this invention in humans for antiviral therapy will range about from 0.5 to 60 mg/Kg/day, usually about from 1 to 30 mg/Kg/day and typically about from 1.5 to 10 mg/Kg/day.

The compounds of this invention also are useful as intermediates in the preparation of detectable labels for oligonucleotide probes. The compounds are hydrolyzed to yield the diacid, diphosphorylated and incorporated into an oligonucleotide by conventional enzymatic or chemical means. The incorporated base from the compound of the invention will be capable of participating in base pairing and thus will not interfere substantially with the binding of the oligonucleotide to its complementary sequence (E. De Clercq *Rev. Med. Virol.* 3:85-96 1993). However, if it does interfere with binding of the oligonucleotide containing the analog to the complementary sequence, the compound of the invention optionally is incorporated into the oligonucleotide as the 3' terminal base, an innocuous position and a conventional site for oligonucleotide labeling. The aglycon donated by the nucleotide analog that is incorporated into the oligonucleotide is detected by any means, such as NMR or by binding to antibodies specific for the nucleotide analog.

Pharmaceutical Formulations

Compounds of the invention and their pharmaceutically, i.e. physiologically, acceptable salts (hereafter collectively referred to as the active

ingredients), are administered by any route appropriate to the condition to be treated, suitable routes including oral, rectal, nasal, topical (including ocular, buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). Generally, the compounds of this invention are administered orally, but if an embodiment is not sufficiently orally bioavailable it can be administered by any of the other routes noted above.

While it is possible for the active ingredients to be administered as pure compounds it is preferable to present them as pharmaceutical formulations. The formulations of the present invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the patient.

The formulations include those suitable for topical or systemic administration, including oral, rectal, nasal, buccal, sublingual, vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations are in unit dosage form and are prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may

optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein.

For infections of the eye or other external tissues, e.g. mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.01 to 10% w/w (including active ingredient(s) in a range between 0.1% and 5% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc), preferably 0.2 to 3% w/w and most preferably 0.5 to 2% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the emulsifying wax, and the wax together with the oil and fat make up the emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween® 60, Span® 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. Thus the cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene

glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is suitably present in such formulations in a concentration of 0.01 to 20%, in some embodiments 0.1 to 10%, and in others about 1.0% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for nasal or inhalational administration wherein the carrier is a solid include a powder having a particle size for example in the range 1 to 500 microns (including particle sizes in a range between 20 and 500 microns in increments of 5 microns such as 30 microns, 35 microns, etc). Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient. Formulations suitable for aerosol administration may be prepared according to conventional methods and may be delivered with other therapeutic agents. Inhalational therapy is readily administered by metered dose inhalers.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration are sterile and include aqueous and non-aqueous injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-

aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials with elastomeric stoppers, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as recited above, or an appropriate fraction thereof, of an active ingredient.

In addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

The present invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefor.

Veterinary carriers are materials useful for the purpose of administering the composition to cats, dogs, horses, rabbits and other animals and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered orally, parenterally or by any other desired route.

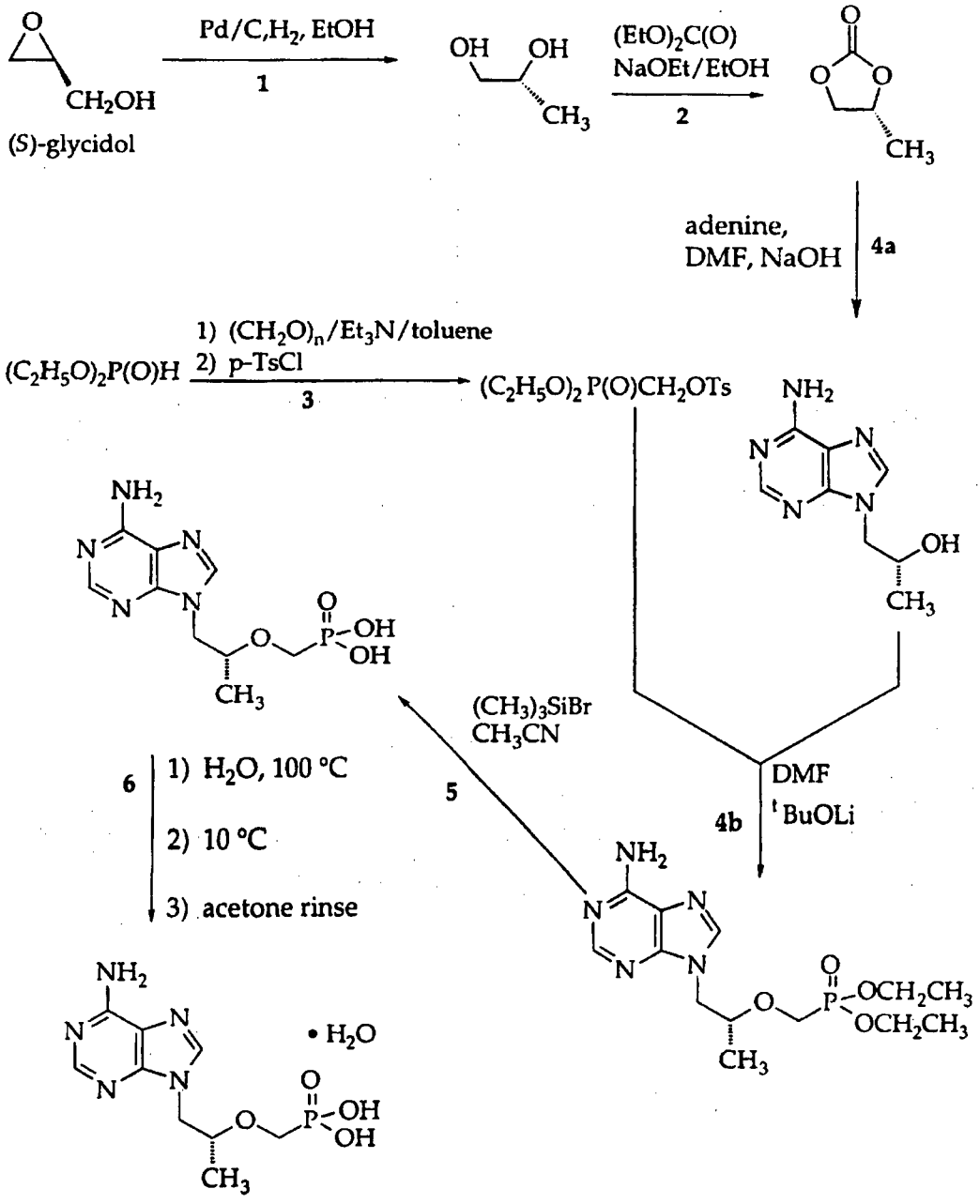
Compounds of the invention can be used to provide controlled release pharmaceutical formulations containing a matrix or absorbent material and as active ingredient one or more compounds of the invention in which the release of the active ingredient can be controlled and regulated to allow less frequent dosing or to improve the pharmacokinetic or toxicity profile of the compound. Controlled release formulations adapted for oral administration in which discrete units comprising one or more compounds of the invention can be prepared according to conventional methods.

All citations found herein are incorporated by reference.

The following examples further illustrate the invention but are not to be construed as limiting the invention.

EXAMPLES

Example 1
PMPA Synthesis



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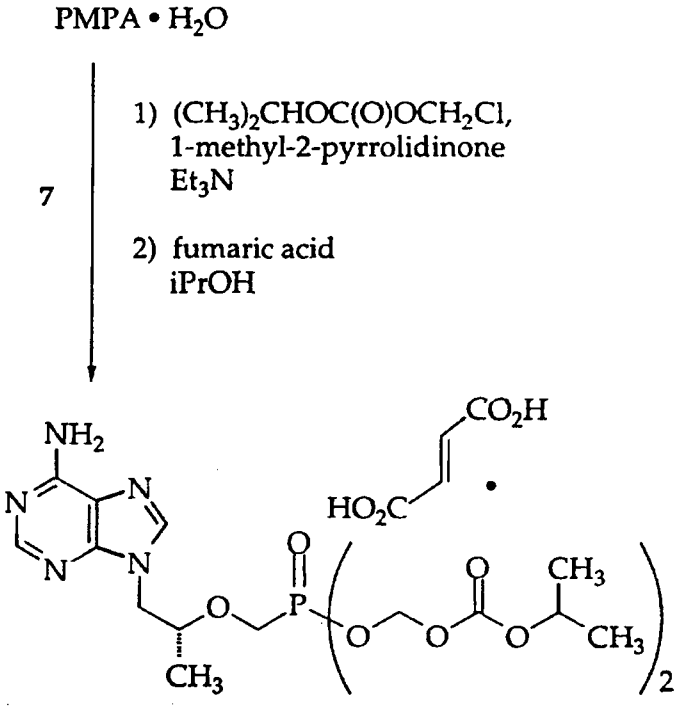


Process Summary

PMPA is prepared as follows: (*S*)-Glycidol is reduced to (*R*)-1,2-propanediol by catalytic hydrogenation, which is then reacted with diethyl carbonate to afford (*R*)-1,2-propylene carbonate. The carbonate is reacted with adenine and catalytic amounts of a base such as sodium hydroxide to give (*R*)-9-[2-(diethylphosphonomethoxy)propyl]adenine which, without isolation, is reacted with lithium alkoxide (alkyl containing 1, 2, 3, 4, 5 or 6 carbon atoms, e.g., *n*-hexoxide, *n*-pentoxide, *n*-butoxide, *i*-butoxide, *t*-butoxide, *n*-propoxide, *i*-propoxide, ethoxide, methoxide) and diethyl *p*-toluenesulfonyl-oxymethylphosphonate (prepared by reacting diethyl phosphite and paraformaldehyde, and tosylating the product *in situ*). The resulting (*R*)-9-[2-diethylphosphonomethoxypropyl]adenine is deesterified with bromotrimethylsilane to give crude PMPA, which is then purified by precipitation from water with pH adjustment. The product is further purified by recrystallization with water to afford PMPA monohydrate.

The process uses a small amount of a base such as NaOH at step 1, which increases the reaction rate about 10-fold compared to the same reaction lacking the base. Step 1 also uses hydrogen gas instead of using a reagent such as HCO_2NH_4 to generate hydrogen *in situ*. The process uses lithium alkoxide at step 4b, which is mildly exothermic on addition to the reaction mixture. The use of a highly reactive base such as NaH, results in an exothermic

reaction that generates hydrogen gas in a reaction is difficult to control. The use of NaH thus requires more labor and care to use than lithium alkoxide. Lithium alkoxide bases also give a product that has an improved by-product profile compared to that obtained using NaH, e.g., lower amounts of starting material or overalkylated products usually result from the use of lithium alkoxide.

The scale of the following method is proportionately reduced or increased if desired. The scheme and process steps depict synthesis of (*R*)-PMPA. One can practice the method using chirally impure starting materials such as (*R,S*)-glycidol to obtain a chiral mixture of intermediates or of the final product.

One can increase or decrease the scale of the process steps described below if desired. The scheme and process steps depict synthesis of (*R*)-PMPA and (*R*)-bis(POC)PMPA. One can practice the method using chirally impure starting materials such as (*R,S*)-glycidol to obtain a chiral mixture of intermediates, e.g., a chiral mixture of 1,2-propylene carbonate, PMPA or bis(POC)PMPA.

Step 1. (*R*)-1,2-Propanediol: (*S*)-Glycidol (1.0 kg, 13.5 moles) is added to a reactor containing (i) an inert, e.g., nitrogen, atmosphere and (ii) a stirred suspension of 5% palladium on activated carbon (50% wet) catalyst (100 g) in denatured ethyl alcohol containing 2 mole% sodium hydroxide (7.85 kg EtOH and 54 g of 16.7% NaOH solution). The contents of the inerted reactor containing catalyst and the ethanol solution is usually cooled to about 0°C (usually about -5 to 5°C) before the (*S*)-glycidol is added. Hydrogen gas at no more than 20 psi is then introduced to the inerted reaction vessel containing reactants at a temperature of no more than 25°C. The mixture is agitated for approximately 4–5 hours, until hydrogen consumption stops. Reaction completion is monitored by TLC (trace or no (*S*)-glycidol remaining). The mixture is then filtered e.g., diatomaceous earth (about 150 g), to remove solids and the filtrate is concentrated *in vacuo* at no more than 50°C, until volatile collection stops or is very slow, to obtain an oil containing the crude product. The crude product is used directly in the next step. Title compound yield is about 100%.

Step 2. (*R*)-1,2-Propylene carbonate: Diethyl carbonate (1.78 kg, 15.1 moles) and sodium ethoxide in denatured ethyl alcohol (210 g of 21% w/w sodium ethoxide in ethanol) are added to (*R*)-1,2-propanediol (1.0 kg

theoretical based on the quantity of (*S*)-glycidol used in step 1 above), and the solution is heated to 80 to 150°C to distill off the ethanol. If necessary to achieve reaction completion, additional diethyl carbonate (0.16 kg) is added to the reaction mixture, followed by distillation to remove ethanol. Reaction completion is monitored by TLC showing a trace or no detectable (*R*)-1,2-propanediol. The residue is fractionally distilled at 120°C and 10–17 mm Hg, to yield the title compound as a colorless liquid. The product purity is typically 96% or greater purity by GC analysis.

Step 3. Diethyl p-toluenesulfonyloxymethylphosphonate: In a reactor containing an inert atmosphere, e.g., nitrogen, a mixture of diethyl phosphite (0.80 kg), paraformaldehyde (0.22 kg), and triethylamine (0.06 kg) in toluene (0.11 kg) is heated at 87°C for about 2 hours, then refluxed for about 1 hour, until the reaction is complete as monitored by TLC showing a trace or no detectable diethyl phosphite. During the reaction, the inert atmosphere is maintained. Toluene is necessary to moderate the reaction, which may otherwise explode. Reaction completion is optionally confirmed by ^{1}H NMR (diethyl phosphite peak at δ 8.4–8.6 ppm no longer detected). The solution is cooled to about 1°C (typically about -2 to 4°C) and *p*-toluenesulfonyl chloride (1.0 kg) is added and then triethylamine (0.82 kg) at about 5°C is slowly added (exothermic addition) while maintaining the temperature at no more than about 10°C (typically 0 to 10°C). The resulting mixture is warmed to 22°C and stirred for at least about 5 hours (typically about 4.5 to 6.0 hours), until the reaction is complete as shown by TLC (trace or no *p*-toluenesulfonyl chloride detectable) and optionally confirmed by ^{1}H NMR (*p*-toluenesulfonyl chloride doublet at δ 7.9 ppm no longer detected). The solids are removed by filtration and washed with toluene (0.34 kg). The combined washings and filtrate are washed either twice with water (1.15 kg per wash), or optionally with a sequence of water (1.15 kg), 5% aqueous sodium carbonate (3.38 kg), and then twice with water (1.15 kg). After each wash, the reactor contents are briefly agitated, allowed to settle and the lower aqueous layer is then discarded. If the reaction results in an emulsion, brine (0.23 kg of water containing 0.08 kg NaCl) may be added to the first organic/water mixture, followed by agitating the reactor contents, allowing the solids to settle, discarding the lower aqueous layer, adding 1.15 kg water, agitating, allowing solids to settle and again discarding the lower aqueous layer. The organic phase is distilled *in vacuo* at no more than 50°C (to LOD at 110°C of no more than 10% and water

content, by KF titration, no more than 0.3%), affording a yield of about 60-70% of the title compound as an oil of about 85–95% purity, exclusive of toluene.

Step 4. (*R*)-9-[2-(Diethylphosphonomethoxy)propyl]adenine: In a reactor containing an inert atmosphere, e.g., nitrogen, a mixture of adenine (1.0 kg), sodium hydroxide (11.8 g), (*R*)-1,2-propylene carbonate (0.83 kg), and N,N-dimethylformamide (6.5 kg) is heated to about 130°C (typically about 125-138°C) for about 18-30 hours until the reaction is complete as optionally monitored by area normalized HPLC showing no more than about 0.5% adenine remaining. The resulting mixture is cooled to about 25°C, typically about 20-30°C, and contains the stage I intermediate, (*R*)-9-(2-hydroxypropyl)adenine, which may precipitate out at this point. After cooling, lithium *t*-butoxide (3.62 kg), 2.0 M in tetrahydrofuran is added to the stage I intermediate, to produce the lithium salt of (*R*)-9-(2-hydroxypropyl)adenine in a mildly exothermic addition. The slurry is treated with diethyl *p*-toluenesulfonyloxymethylphosphonate (1.19 kg) and the mixture is heated to a temperature of about 32°C, typically about 30-45°C, and is stirred for at least about 2 hours (typically about 2-3 hours) during which time the mixture becomes homogeneous. More diethyl *p*-toluenesulfonyloxymethylphosphonate (1.43 kg) is added and the mixture is stirred at a temperature of about 32°C (typically about 30-45°C) for at least about 2 hours (typically about 2-3 hours). Additional lithium *t*-butoxide (0.66 kg), 2.0 M in tetrahydrofuran and diethyl *p*-toluenesulfonyloxymethylphosphonate (0.48 kg) are added twice more, each time followed by stirring the mixture, which is at a temperature of about 32°C for at least about 2 hours. Reaction completion is optionally monitored by area normalized HPLC showing no more than about 10% of stage I intermediate remaining. If the reaction is incomplete, additional lithium *t*-butoxide (0.33 kg), 2.0 M in tetrahydrofuran and diethyl *p*-toluenesulfonyloxymethylphosphonate (0.24 kg) are added and the reaction mixture is maintained at a temperature of about 32°C for at least about 2 hours to achieve reaction completion. The mixture is then cooled to about 25°C (typically about 20-40°C) and glacial acetic acid (0.5 kg) is then added. The resulting mixture is concentrated *in vacuo* at a final maximum mixture temperature of about 80°C under about 29 in Hg vacuum. The residue is cooled to about 50°C (typically about 40-60°C) and water (1.8 kg) is added and the reaction is rinsed forward with additional water (1.8 kg). The solution is continuously extracted with dichloromethane (about 35 kg) for 12–48 hours

with periodic additions of glacial acetic acid (0.2 kg) to the aqueous phase after about 5 hours and after about 10 hours of continuous extraction time. Extraction completion is optionally confirmed by area normalized HPLC as shown by no more than about 7% of (*R*)-9-[2-(diethylphosphonomethoxy)propyl]adenine remaining in the aqueous phase. The combined dichloromethane extracts are concentrated initially at atmospheric pressure then *in vacuo* at an extract temperature of no more than about 80°C to give the title compound as a viscous orange oil. The title compound yield is about 40-45% by weight normalized HPLC and its purity is typically 60-65% by area normalized HPLC. The actual weight of the title compound after concentration is approximately 1.6 times the theoretical weight (or 3.8 times the expected yield). The additional observed weight is attributed to impurities and/or solvents remaining after the continuous extraction and concentration.

Step 5. (*R*)-9-[2-(Phosphonomethoxy)propyl]adenine, crude:
Bromotrimethylsilane (1.56 kg) is added to a reactor containing a mixture of crude (*R*)-9-[2-(diethylphosphonomethoxy)propyl]adenine (1.0 kg calculated based on adenine input from step 4 above) and acetonitrile (0.9 kg) with cooling to maintain a temperature no higher than about 50°C. The lines are rinsed forward with acetonitrile (0.3 kg) and the mixture is refluxed at about 60-75°C for about 2–4 hours with moderate agitation to avoid splashing the reactor contents. Reaction completion is monitored by area normalized HPLC showing no more than about 3% total of monoethyl PMPA and diethyl PMPA remaining. If the reaction is incomplete, additional bromotrimethylsilane (0.04 kg) is charged into the reactor and the reaction is refluxed for at least about 1 hour with moderate agitation. The volatiles are removed by distillation at no higher than about 70°C initially at atmospheric pressure and then *in vacuo* (about 24-27 in Hg) at no higher than about 70°C. The reactor is then cooled to about 20°C (typically about 15-25°C) and water (1.9 kg) is added (exothermic addition) to the residue with the temperature maintained at no higher than about 50°C. The mixture is cooled to 20°C and washed with dichloromethane (1.7 kg) by agitating for about 30 minutes. The isolated aqueous phase is then filtered through a 1 μm cartridge filter, diluted with water (3.2 kg), heated to about 40°C (typically about 35-50°C) and adjusted to pH about 1.1 (typically about 0.9-1.3) with aqueous sodium hydroxide (about 0.15 kg NaOH as a 50% solution) while the temperature is maintained at about 45°C. PMPA seed crystals are added to the mixture and the pH is

adjusted to about 2.8 (typically about 2.6-3.0) with a 50% aqueous sodium hydroxide solution (about 0.15 kg NaOH required) while the temperature is maintained at about 45°C (typically about 35-50° C). The solution is cooled to about 22°C (typically about 15-25°C) over about 3–20 hours with slow to moderate agitation that avoids splashing the contents, during which time the product should precipitate, beginning at about 35°C. The pH of the slurry is adjusted to about 3.2 (typically about 3.1-3.3), usually using 50% aqueous sodium hydroxide or concentrated hydrochloric acid, if necessary. The slurry is cooled to approximately 5°C, typically about 0-10°C, and slowly agitated for at least about 3 hours in that temperature range. The solids are collected by filtration, washed sequentially with cold water (0.35 kg) and acetone (0.3 kg) giving crude PMPA as a damp solid typically of about 97% purity. The product is heated to about 50°C and dried *in vacuo* to a water content of less than 10%. The quantity of diethyl PMPA is calculated from the quantity of adenine used in the preceding step of the synthesis (assuming 100% yield) and not from the net weight of the crude diethyl PMPA, which may contain other compounds.

Step 6. (*R*)-9-[2-(Phosphonomethoxy)propyl]adenine, pure: A suspension of the crude PMPA (1.00 kg corrected for water content) (Step 5 product) in water is heated to about 100°C (typically about 95-110°C) with moderate to high agitation until all solids dissolve, and the resulting solution is clarified by filtration while hot, rinsing forward using additional hot water (1 kg, about 95-110°C). The filtrate is heated to 100°C prior to cooling, first to about 30°C (typically about 20-25°C) over about 3–5 hours with slow agitation, then cooling is continued to about 10°C (typically about 5-15°C). After holding at about 10°C for at least about 3 hours, the solids are collected by filtration and washed sequentially with cold water (1.5 kg, about 0-10°C) and then acetone (1 kg). The wet cake is dried *in vacuo* at about 50 °C (typically about 40-60°C) to a water content of about 5.9% (typically about 3.9-7.9%), affording pure PMPA monohydrate. The product purity is typically 98% or greater by both area normalized and weight normalized HPLC. If the chemical purity is unsatisfactory, the product may be repurified by a repeat of this step.

Optional recrystallization: 0.75 g of PMPA (preparation A) was recrystallized from H_2O (11.3 mL, 15:1 wt. ratio) by heating the suspension to 95-100°C. Upon cooling to room temperature, the crystallized PMPA was

chilled in a freezer. After 3 h the crystals were filtered on a coarse frit fit with Tyvek™, the filter cake rinsed with ice-cold H_2O and acetone, and air dried to constant weight to give a fluffy white solid (Preparation B). Recovery was 0.64 g (85.3%). HPLC showed 98.5-98.9% pure PMPA. No 14.7 min impurity was observed. Recrystallized liquors (1039-91-23) showed 71.4% pure PMPA with a major impurity at 4.8 min (26.9%), possibly solvent. 14.7 min impurity = 0.05%.

Preparation B PMPA was recrystallized again from 9.6 mL (15:1 wt. ratio) H_2O heated to 95-100°C. Upon cooling to room temperature, the crystallized PMPA was chilled in a freezer overnight. The PMPA was filtered through a coarse frit fit with Tyvek™ and the filter cake was rinsed with ice-cold H_2O and acetone, then sucked dry to constant weight to afford a fluffy, white solid (Preparation C). Recovery was 0.52 g (81.3%). HPLC (JH52807, JH52810) showed 99.3-99.5% pure PMPA. The largest impurity at 19 min = 0.22%. Recrystallized liquors showed 64.9% pure PMPA with 0.01% 14.7 min impurity and 0.09% 19 min impurity.

Preparation C PMPA (0.50 g) was recrystallized from approximately 7.5 mL boiling H_2O (15:1 wt. ratio). Upon cooling to room temperature, the PMPA was filtered on a coarse frit fit with Tyvek™. The filter cake was rinsed with ice-cold H_2O and acetone then sucked to dryness to afford a fluffy white solid (Preparation D). The filtrate was also concentrated to afford a white solid (Preparation E).

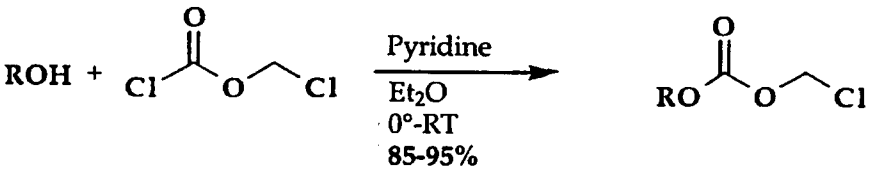
Recovery: Filter cake: 0.41 g (82%), Filtrate: 0.08 g = 0.49 g combined (98%). HPLC analysis showed the filtrate (Preparation E) was 99.9% pure. PMPA prepared in this fashion is used to manufacture the compounds of this invention.

Step 7. Bis(POC)PMPA fumarate: In a reactor with an inert atmosphere, e.g., nitrogen, a mixture of 1-methyl-2-pyrrolidinone (4.12 kg), PMPA monohydrate (1.00 kg), triethylamine (0.996 kg), are agitated for about 15-45 min., typically about 30 min, and then chloromethyl-2-propyl carbonate (2.50 kg) is added and the mixture is heated to about 55-65°C, typically about 60°C and agitated without splashing the contents for about 3–6 hours, typically about 4 hours, until the reaction is complete, as optionally indicated by HPLC (no more than 15% mono(POC)PMPA present). The mixture is diluted with isopropyl acetate (10.72 kg), cooled to about 15-30°C, typically about 25°C, as rapidly as possible, and while holding the reactor contents at a of about 15-30°C, typically at about 25°C, the mixture is agitated for about 20-60 minutes,

typically about 30 minutes. The solids are removed by filtration and washed with isopropyl acetate (4.44 kg). The combined organic phases at about 15-30°C, typically about 25°C, are extracted twice with water (3.28 kg) using moderate agitation for about 1-10 min. to avoid forming an emulsion followed by allowing the phases to separate. The combined aqueous phases are back-extracted twice with isopropyl acetate (3.56 kg) (about 15-30°C, typically about 25°C). All organic phases are combined and washed with water (2.20 kg) (about 15-30°C, typically about 25°C) using moderate agitation for about 1-10 min. to avoid forming an emulsion, then the combined organic phases, which are at about 25-43°C, but at no more than 45°C, are concentrated *in vacuo* (about 26.5-28" Hg) to approximately 30% of the original volume (about 10-12 L/kg PMPA monohydrate). After a polishing filtration using an in-line 1 µm filter, the concentration of the organic phase is resumed at about 20-38°C, but no higher than 40°C under a vacuum (about 28" Hg) until a pale yellow oil remains. The oil is dissolved in a warmed solution (about 45-55° C, typically about 50°C) of fumaric acid (0.38 kg) in 2-propanol (6.24 kg) with vigorous agitation until solids dissolve, about 0.5-2.0 hours. The warm solution is then optionally filtered using an in-line 1 µm filter while minimizing cooling of the solution. The filtrate at about 34-50°C, typically at about 40°C, is agitated using the minimum agitation needed to obtain a homogenous solution. The resulting solution is cooled to about 30-33°C, typically about 32°C, over about 30 minutes using minimal agitation, optionally seeded with a small quantity of bis(POC)PMPA fumarate (about 100 mg), and cooled to about 12-18°C, typically about 15°C, over about 1-2 hours, typically over about 1 hour. Seed crystals may not be needed if crystal formation begins before seed crystals are added. Crystals may form over a range of about 12-33°C as the solution is cooled. Crystallization will occur at lower temperatures if the solution is further chilled, e.g., to about -10° to about 11°C. Agitation is discontinued when crystal formation begins. The mixture is allowed to stand at about 15°C for at least about 12 hours, typically about 12-30 hours. The resulting slurry is filtered (Tyvek) and the filter cake is washed with a premixed solution of isopropyl acetate (0.70 kg) in butyl ether (2.44 kg) (1: 4 v/v). The filter cake, which is at no more than 40°C, is dried *in vacuo* for about 1 to 3 days and the dried product is optionally milled (Fitzmill M5A fitted with a 0.050" screen), affording bis(POC)PMPA fumarate as white, fine, powder-like crystals of about 97.0 to 99.5% purity.

Example 2

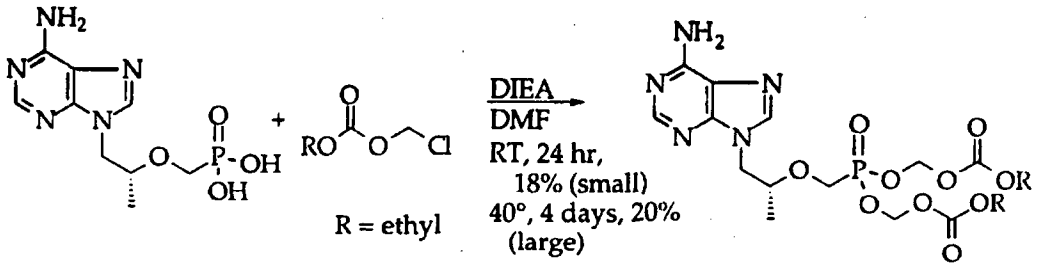
Preparation of Alkyl Chloromethylcarbonates



A solution of the alcohol (73 mmol) and chloromethyl chloroformate (Fluka, 6.23 mL, 70 mmol) in diethyl ether was cooled to 0°C under argon. Pyridine (5.7 mL, 70 mmol) was added dropwise with stirring over 10 minutes. The solution was stirred at 0°C for one hour, then allowed to warm to room temperature and stirred for three additional hours. The ether solution was filtered, washed with 1 M HCl, dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator. Brief application of 0.1 torr vacuum gave the alkyl chloromethyl carbonates in 85-95% yields. Ethyl chloromethyl carbonate is somewhat volatile, and cannot be left on the rotovap too long, or the yield suffers (87-35%).

Example 3

Preparation of the Bis-ethyl Oxymethyl Carbonate of PMPA

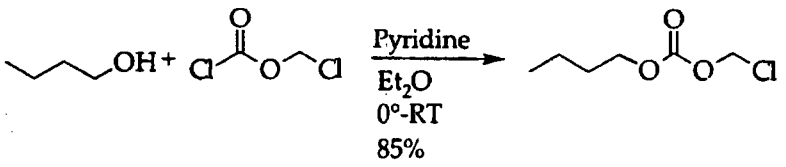


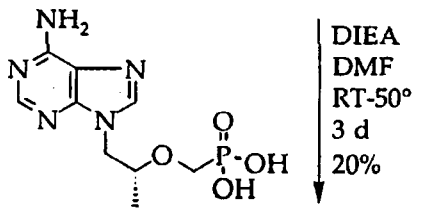
R= Et. Anhydrous PMPA (5 g, 16 mmol) and DIEA (Hunig's base) (11.5 mL, 66 mmol) were placed in anhydrous DMF (50 mL). The chloromethyl carbonate (49 mmol) was then added and the suspension heated to 50°C under argon with rapid mechanical stirring. After 1 hr the reaction was clear and the temperature was lowered to 35°C and the reaction stirred for 48 hr. The DMF was removed on a rotary evaporator, and the reaction partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was dried over magnesium sulfate, filtered, and concentrated on a rotary evaporator. The residue was taken up in methylene chloride and applied to a silica gel column (150 g SiO_2). It was eluted with 500 mL each 0,3,6,9,12,15,18% (v/v) isopropanol in

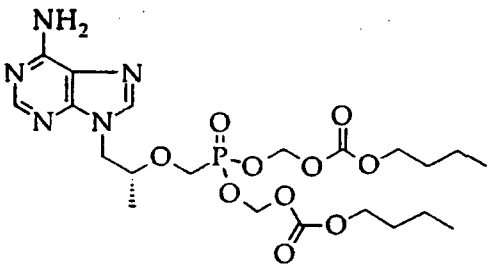
methylene chloride, and then with 2000 mL 21%. Appropriate fractions were pooled and evaporated to give the desired product.

Example 4

Preparation of the Bis-n-butyl Oxymethyl Carbonate of PMPA







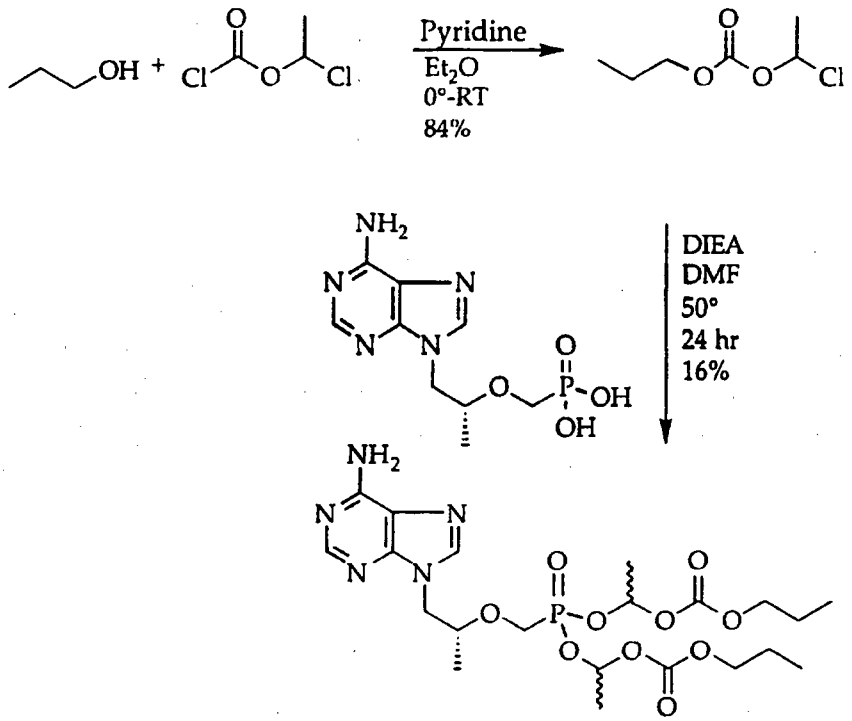
Butyl chloromethyl carbonate. A solution of butyl alcohol (50 mmol) and chloromethyl chloroformate (4.5 mL, 50 mmol) in diethyl ether (200 mL) was cooled to 0°C under argon. Pyridine (5.7 mL, 50 mmol) was added dropwise with stirring over 5 min. The solution was stirred at 0°C for 15 min, then allowed to warm to room temperature and stirred for three additional hours. The ether solution was filtered, washed with 1 M HCl, and then twice with water, dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator to give butyl chloromethyl carbonate (7 g, 85%).

Dibutyl PMPA carbonate. Anhydrous PMPA (4 g, 13 mmol) and DIEA (10.5 mL, 60 mmol) were placed in anhydrous DMF (40 mL). Butyl chloromethyl carbonate (40 mmol) was then added and the suspension stirred at room temperature for 48 hr. The reaction was then heated to 50°C for 18hr. The DMF was removed on a rotary evaporator, and the reaction partitioned between CH_2Cl_2 (250 mL) and water (250 mL). The CH_2Cl_2 layer was washed

once with saturated aqueous $NaHCO_3$, dried over magnesium sulfate, filtered, and concentrated on a rotary evaporator. The residue was taken up in methylene chloride and applied to a silica gel column (150 g SiO_2). It was eluted with 1000 mL CH_2Cl_2, 500 mL each 0,3,6,9,12,15,18% (v/v) isopropanol in methylene chloride, and then with 2000 mL 21% isopropanol in methylene chloride. Appropriate fractions were pooled and evaporated to give the desired product.

Example 5

Synthesis of Bis-n-propyl Oxyethyl Carbonate of PMPA



Preparation of propyl-1-chloroethyl carbonate. A solution of propyl alcohol (70 mmol) and 1-chloroethyl chloroformate (7.6ml, 70 mmol) in diethyl ether (200 mL) was cooled to 0°C under argon. Pyridine (70 mmol) was added dropwise with stirring over 5 min. The solution was stirred at 0°C for 15 min, then allowed to warm to room temperature and stirred for 4.5 additional hours. The ether solution was filtered, washed with 1 M HCl, and then twice with water, dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator to give propyl-1-chloroethyl carbonate (9.8 g, 84%). Anhydrous PMPA (0.3 g, 1 mmol) and DIEA (0.7 mL, 4 mmol) were placed in anhydrous DMF (2 mL) under argon. Propyl-1-chloroethyl carbonate (3 mmol) was then

added and the suspension stirred at 50°C for 20 hr. The DMF was removed on a rotary evaporator, and the reaction partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was dried over magnesium sulfate, filtered, and concentrated on a rotary evaporator. The residue was taken up in methylene chloride and applied to a silica gel column (25 g SiO_2). It was eluted with 100 mL CH_2Cl_2, 50 mL each 3,6,9,12,15,18% (v/v) isopropanol in methylene chloride, and then with 200 mL 21% isopropanol in methylene chloride. Appropriate fractions were pooled and evaporated to give the desired product.

Example 6

Synthesis of Chloromethyl Isopropyl Carbonate

To a cold solution (approximately 10°C) of chloromethylchloroformate (65 mL) in diethyl ether (1.4L) was added isopropanol (56 mL) followed by a dropwise addition of pyridine (60 mL). After the addition the cold bath was removed and the reaction mixture was stirred for 18 h. The reaction mixture was poured into a separation funnel containing cold water (100 mL). The ether layer was separated and washed with water (100 mL x 2) and then dried over Na_2SO_4. Evaporation of the solvent furnished the chloromethyl isopropyl carbonate (107 g, 95%). Chloromethyl isobutyl carbonate, chloromethyl neopentyl carbonate, chloromethyl tert butyl carbonate and chloromethyl 3-pentyl carbonate are synthesized in a similar manner.

Example 7

Synthesis of Bis Isopropyl Oxymethyl Carbonate of PMPA

To a stirred suspension of PMPA (7.26 g, 0.026 mmol) in DMF (100 mL) at 50°C was added Et_3N (10.8 mL, 0.0778 mmol). The reaction mixture became homogeneous and chloromethyl isopropyl carbonate (12.1 g, 0.0778 mol) was added to the reaction mixture and stirring continued at 50°C (oil bath temperature) for 20 h. The solvents were removed under reduced pressure and the crude was chromatographed on a silica gel column. Elution with 10% isopropanol in CH_2Cl_2 removed all the non polar impurities. Further elution with the same solvent mixture furnished the prodrug, 1.3 g (approximately 10%).

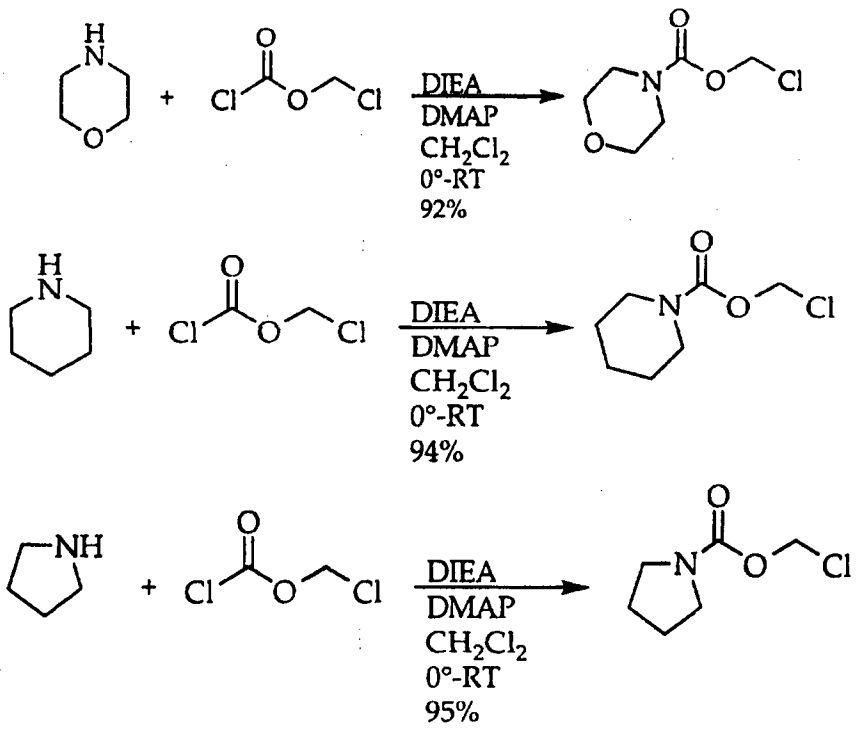
Example 8

Synthesis of Bis Isopropyl Oxymethyl Carbonate of PMPA

To a stirred suspension of PMPA (1 g, 3.57 mmol) in DMF (5 mL) were added Et_3N (1.5 mL, 10.71 mmol) and chloromethyl isopropyl carbonate (1.67 g, 10.71 mmol). The reaction mixture was then diluted with ethyl acetate (100 mL) and filtered. The filtrate was washed with water (2 x 50 mL) and finally with brine (10 mL). The crude obtained after removal of the solvent was dried under vacuum. The resulting oil was dissolved in isopropanol (7 mL) and citric acid (260 mg) was added. The mixture was stirred for 16 h at room temperature and then cooled to 0°C. The product was crystallized and crystals were filtered and dried. Mp 76-81°C.

Example 9

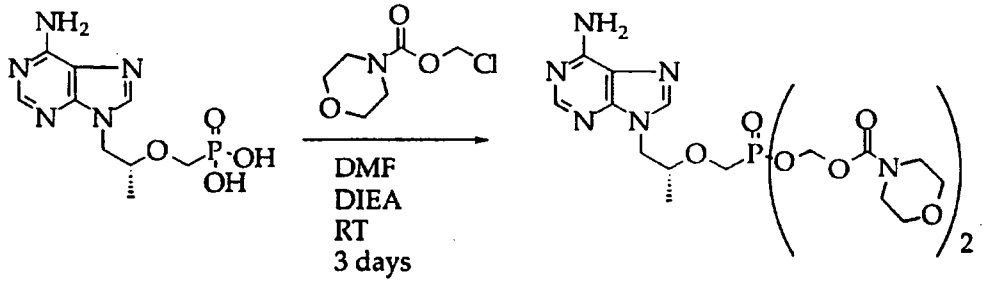
Preparation of chloromethylcarbamates



A solution of the amine (24 mmol), DIEA (30 mmol), and DMAP (.5 mmol) in methylene chloride (5 mL) was added dropwise to a cold (0°C) solution of chloromethyl chloroformate (25 mmol) in methylene chloride (45 mL) over 5 min. The solution was allowed to warm to room temperature over 1.5 hr. The solution was diluted into ethyl acetate (100 mL), and washed with saturated sodium bicarbonate, 1 M HCl, and saturated sodium chloride. It was then dried over magnesium sulfate, filtered, and evaporated to give the desired chloromethyl carbamate.

Example 10

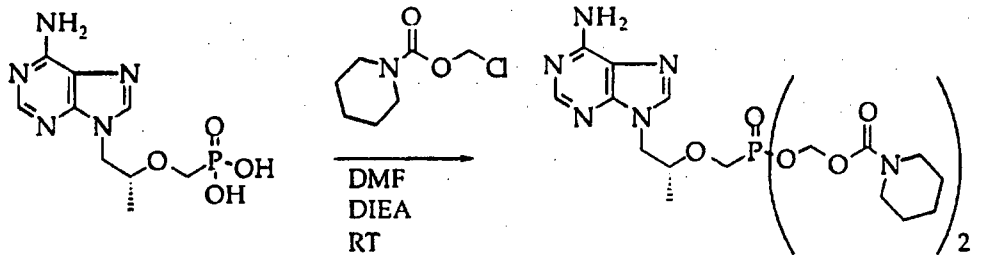
Synthesis of Bis Morpholino Oxymethyl Carbamate of PMPA



Anhydrous PMPA (0.3 g, 1 mmol) and DIEA (1 mL, 6 mmol) were placed in anhydrous DMF (2 mL). Chloromethyl morpholino carbamate (3 mmol) was then added and the suspension stirred at room temperature for 3 days. The reaction was partitioned between CH_2Cl_2/isopropanol and 0.1 M citrate buffer (pH 6). The CH_2Cl_2 layer was washed with water, dried over magnesium sulfate, filtered, and concentrated on a rotary evaporator. The residue was taken up in methylene chloride and applied to a silica gel column (5 g SiO_2). It was eluted with 25 mL each 0,3,6,9,12,15,18% (v/v) isopropanol in methylene chloride, and then with 100 mL 21% isopropanol in methylene chloride. Appropriate fractions were pooled and evaporated to give the desired product.

Example 11

Synthesis of Bis Piperidino Oxymethyl Carbamate of PMPA



Anhydrous PMPA (0.3 g, 1 mmol) and DIEA (0.7 mL, 4 mmol) were placed in anhydrous DMF (2 mL). Chloromethyl piperidino carbamate (3 mmol) was then added and the suspension stirred at room temperature for 3 days. More DIEA (4 mmol) and chloromethyl piperidino carbamate (100 µl) were added, and the reaction stirred for 27 hr. The reaction was partitioned between CH_2Cl_2/isopropanol and 0.1 M citrate buffer (pH 6). The CH_2Cl_2

layer was dried over magnesium sulfate, filtered, and concentrated on a rotary evaporator. The residue was taken up in methylene chloride and applied to a silica gel column (5 g SiO_2). It was eluted with 25 mL each 0,3,6,9,12,15,18% (v/v) isopropanol in methylene chloride, and then with 100 mL 21% isopropanol in methylene chloride. Appropriate fractions were pooled and evaporated to give the desired product.

Example 12

Other Carbamate Intermediates

To a solution of chloromethylchloroformate (4.16 mL) in CH_2Cl_2 (30 mL) were added tert butyl amine (4.9 mL) and proton sponge (10 g). The reaction mixture was stirred for 18 h and then it was poured into a separation funnel containing cold 0.5N HCl (100 mL). The CH_2Cl_2 layer was separated and washed with water (100 mL x 2) and then dried over Na_2SO_4. Evaporation of the solvent furnished the chloromethyl tert butyl carbamate (8 g). Chloromethyl n-butyl carbamate (R =*n*-butyl) and chloromethyl dimethyl carbamate (R = Me) were prepared in the same fashion.

Example 13

Other Oxymethyl Alkyl Carbamate Prodrugs of PMPA

To a stirred suspension of PMPA (4.51 g, 0.016 mmol) in DMF (50 mL) was added Et_3N (6.7 mL, 0.048 mmol). The reaction mixture became homogeneous and chloromethyl tert butyl carbamate (8 g, 0.048 mol) was added to the reaction mixture and stirring continued at room temperature for 3 days. The solvents were removed under reduced pressure and the crude was chromatographed on a silica gel column. Elution with 10% isopropanol in CH_2Cl_2 removed all the less polar impurities. Further elution with the same solvent mixture furnished the prodrug (1.25g). The n-butyl and methyl carbamates were prepared in the same fashion from the intermediates of the preceding example.

Example 14

Chemical Stability of PMPA Carbonates

The solution stability of PMPA carbonates was studied at pH 7.4 at 37°C in 10 mM buffer (NaH_2PO_4 and Na_2HPO_4) with the total ionic strength

adjusted to 0.15 M with KCl. The assays were performed by adding 200 µL of a PMPA carbonate stock solution (about 1 mg/mL in DMSO) to 10 mL of pre-equilibrated buffer at 37°C. Samples were removed at specific times points and analyzed by HPLC. The chemical t 1/2 is expressed in terms of the number of hours required to hydrolyze 50% of the carbonate at the specified pH.

Example 15

Oral Bioavailability of PMPA and PMPA Carbonates in Beagle Dogs

PMPA (9-[(R)-2-(phosphonomethoxy)propyl]adenine) and PMPA carbonates were examined to determine the effect of dose on the pharmacokinetics of PMPA in beagle dogs, in particular the bioavailability of PMPA following oral administration to beagle dogs.

PMPA monohydrate was synthesized by Gilead Sciences. Tetrabutylammonium hydrogen phosphate (TBAHP) was obtained from Fluka (Ronkonkoma, NY). Acetonitrile was obtained from Baxter (Muskegon, MI). Dibasic potassium phosphate, monobasic potassium phosphate, and sodium acetate trihydrate were obtained from Mallinckrodt (Paris, KY). Chloroacetaldehyde and trifluoroacetic acid (TFA) were from Aldrich (Milwaukee, WI).

The intravenous formulations used as standards were isotonic aqueous solutions containing 50 mg/mL PMPA. Compound was added to 10 mL of WFI (water for injection from Abbott Laboratory) and 1N NaOH was added to adjust the pH to 7.4. The solutions were diluted to 15 mL with WFI and sterile filtered with a 0.2 µm filter. The PMPA dose was 10 mg/kg (0.2 mL/kg).

The intravenous formulation for a 1 mg/kg dose was prepared as described above except only 75 mg of PMPA was added to WFI and the final concentration was 5 mg/mL. The dose was 1 mg/kg (0.2 mL/kg). Oral formulation of carbonates were prepared in 20-40% PEG 400/50 mM citric acid and were adjusted to pH 2.2. Doses ranged from 6.2-10 mg eq of PMPA/kg and are shown in Table 1.

Two groups of five adult male beagle dogs were used. The mean body weight at the time of the first dose was 9.6 ± 0.4 Kg (range 9.2-10.2). The dogs were fasted 12-18 hours prior to dosing and until 6 hours post-dose. For pentagastrin pretreatment, dogs were given a single intramuscular injection of pentagastrin (Peptavlon 0.25 mg/mL, Ayerst Laboratories, Inc.,

Philadelphia, PA) at a dose of 6 μg/kg, 20 minutes prior to dosing. Water was provided *ad lib.*

Each formulation was administered as a single dose to five male beagle dogs. Individual vials of each formulation were provided for each animal. The intravenous formulation was administrated via a cephalic vein. The oral suspension was administered by gavage, followed by two 10 mL water washes. At least one week washout period was allowed between administrations.

Blood samples (4.0 mL) were collected by direct jugular access from each animal into heparinized tubes. Animals remained conscious throughout the sample collection period. Blood was processed immediately for plasma by centrifugation at 2000 rpm for 10 minutes. Plasma samples were frozen and maintained at ≤ -20°C until analyzed.

Urine samples were collected over 0-24 and 24-48 hours time periods. Urine samples from 0-24 and 24-48 hours were divided into aliquots and mixed based on the volume collected and analyzed to determine amount of PMPA recovered from urine during 0-48 hours.

PMPA in Plasma and Urine was determined as follows. PMEA (9-(2-phosphono-methoxyethyl)adenine; adefovir) was used as the internal standard for both analyses. The total concentration of PMPA in dog plasma or urine samples was determined by derivatizing PMPA and PMEA with chloroacetaldehyde to yield a highly fluorescent N^1, N^6-ethenoadenine derivative as described (Russell, J. et al. (1991) Determination of 9-[(2-Phosphonylmethoxy)-ethyl]Adenine in Rat Urine by High-Performance Liquid Chromatography with Fluorescence Detection. ***J. Chromatogr. (Netherlands), 572, 321-326.).***

Sample Extraction for PMPA in plasma and urine was performed as follows. Plasma (200 μL) and internal standard (20 μL of 10 μg/mL PMEA providing a final PMEA concentration of 1 μg/mL) were mixed with 400 μL of acetonitrile containing 0.1 % TFA to precipitate protein. Samples were then evaporated to dryness under reduced pressure at room temperature (Savant SpeedVac). Urine samples (20 μL) and internal standard (30 μL of 10 μg/mL PMEA providing a final PMEA concentration of 1.5 μg/mL) were used directly for derivatization without drying.

The samples were derivatized for analysis as follows. Dried plasma samples or urine samples were reconstituted or mixed in 200 μL derivatization cocktail (0.34% chloroacetaldehyde in 100 mM sodium acetate, pH 4.5), vortexed, and centrifuged for 10 minutes at 14,000 rpm in an

Eppendorf Centrifuge 5402. Supernatant was then transferred to a clean screw capped tube and incubated at 95°C for 40 minutes. Derivatized samples were quenched on ice and evaporated to dryness under reduced pressure at room temperature. Dried samples were reconstituted in 200 µL Mobile Phase A (see below), vortexed and centrifuged for 10 minutes at 14,000 rpm in an Eppendorf Centrifuge 5402. The supernatant was then transferred to autoinjector vials for HPLC analysis.

The plasma and urine samples were analyzed by HPLC with Fluorescence Detection as follows. The HPLC system comprised a Model P4000 solvent delivery system with a Model AS3000 autoinjector and a Model F2000 Fluorescence detector (Thermo Separation, San Jose, CA). The column was a Zorbax RX-C18 (5 µm, 150 x 4.6 mm) (MAC-MOD, Chadds Ford, NY) equipped with a Brownlee RP-18 Newguard guard column (7 µm, 15 x 3.2 mm) (Alltech, Deerfield, IL). The mobile phases used were: A, 2% acetonitrile in 25 mM potassium phosphate buffer with 5 mM TBAHP, pH 6.0; B, 65% acetonitrile in 25 mM potassium phosphate buffer with 5 mM TBAHP, pH 6.0. The flow rate was 1.5 mL/min and the column temperature was maintained at 35°C by a column oven. The gradient profile was 100% A until 2.0 min, then a linear gradient to 100% B by 13.0 minutes, returning immediately to 100% A. Detection was by fluorescence with excitation at 236 nm and emission at 420 nm, and the injection volume was 50 µL. Total cycle time between injections was 25 min. Data was acquired and stored by a Peak Pro data acquisition system (Beckman, Palo Alto, CA).

The pharmacokinetic parameters for intravenous and oral formulations of PMPA and PMPA carbonates were assessed using non-compartmental methods. Intravenous data were analyzed using PCNONLIN Model 201 (5); oral data were analyzed using Model 200. Additional pharmacokinetic parameters were calculated as follows:

CL = Dose/AUC (0-∞) ; where CL is the total plasma clearance.

Vss = CL x MRT ; where Vss is the apparent volume of distribution at steady state. MRT is the mean residence time.

The initial plasma concentration (Co) was determined by extrapolation of log transformed data to zero time. Bioavailability was expressed as

$$\text{Bioavailability (\%)} = \frac{\text{AUC}(0\text{-}\infty)\ \text{oral or prodrug}}{\text{AUC}(0\text{-}\infty)\ \text{intravenous}} \times 100$$

Urinary recovery was expressed as

$$\text{Urinary Recovery (\%)} = \frac{\text{amount (mg) of PMPA in urine (0-48 hr)}}{\text{amount (mg) of PMPA dosed}} \times 100$$

Oral bioavailability of t-Bu, 3-pentyl, isopropyl, Et carbonate parameters were compared by unpaired t-tests (StatView® Version 4.0, Software for the Statistical Analysis. Abacus Concepts, Inc., Berkeley, CA). A P value of ≤ 0.05 was considered significant.

Biological t1/2: Dog liver was obtained fresh from Pharmakon USA (Waverly, PA). Liver homogenate was prepared following a standard protocol. Dog liver was rinsed three times with ice-cold 50 mM sodium/potassium phosphate buffer and homogenized with a Tekmar Tissumizer homogenizer (VWR 33995-060). The homogenate was centrifuged at 9000 g (11,000 rpm for Eppendorf Centrifuge 5402; Brinkmann Instruments, Westbury, NY) at 4°C for 20 minutes. The supernatant was designated as the S9 fraction. The concentration of protein in the S9 fraction was determined using a Bio-Rad Protein Assay Kit II, with bovine serum albumin as standard. Esterase activity was determined using *o*-nitrophenyl butyrate as substrate and activity was calculated based on the increase in absorbance at 420 nm after a 1 min incubation. The homogenates were stored as 1.0 mL aliquots at -70°C.

Intestinal Homogenate: Dog intestinal segments (jejunum/ileum) were obtained fresh from Pharmakon USA (Waverly, PA) and intestinal homogenate was prepared as described for liver. The intestinal homogenates were stored as 1.0 mL aliquots at -70°C.

Human intestinal homogenate (S9) was obtained from Keystone Skin Bank (Exton, PA) at concentration of 20 mg protein/mL.

Study Design: Enzymatic stability studies involving plasma and intestinal homogenate were performed with 90% biological fluids.

Stability Measurement: One blank (drug free) incubation was performed for each biological fluid. All biological fluid tubes (open) were preincubated without PMPA prodrugs in a shaker bath at 37°C and 100 oscillation/min for 5 minutes. PMPA prodrugs was added to the test incubations (final concentration: 20 µg/mL), mixed and maintained at 37°C and 100 oscillations/min. Samples (50 µL) were withdrawn at 0, 30, and 60 minutes

and the reaction was quenched with 100 µL of 0.1% trifluoroacetic acid (TFA) in acetonitrile. Quenched samples were centrifuged for 5 minutes at 14,000 rpm in an Eppendorf Centrifuge 5402, and the supernatant was used for HPLC analysis.

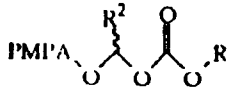
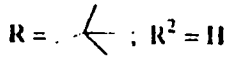
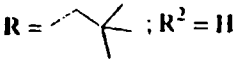
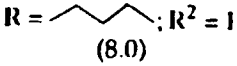
Calculations: For each incubation, the observed rate constant for degradation was calculated by plotting the log of the peak area of PMPA prodrugs versus time of incubation (min). The slope was the observed rate constant (k_{obs}). The half life was calculated according to the following equation:

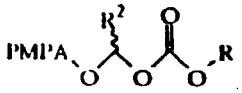
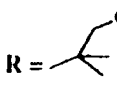
$$t1/2\ (min) = \frac{0.693}{k_{obs}}$$

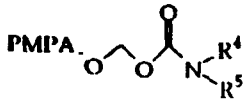
If the observed rate constant for degradation was less than 0.01 min^{-1}, then t1/2 was expressed as stable.

The results of the beagle study are shown below in Table 1.

TABLE 1
PMPA Prodrug Summary

PRODRUGS CARBONATES  (Dose mg e.g. PMPA/Kg)	Chemical t1/2 (hr)		Log PC	Biological t1/2 (min) (Dog) *(Human)*			% of PMPA IV (1 mg/kg) AUC				Urinary Recovery (% as PMPA)
	pH 7.4	pH 2.0	pH 7.4	Intestine	Plasma	Liver	PMPA	Monoester	Prodrug	other	
Bis-t-Bu COM PMPA  (6.45)	0.4	0.4	1.93	26.6 *(<5)*	21.2	14.9	36.2 ± 6.76	---	---	---	34.9 ± 14.0
Bis-IBu COM PMPA  (7.2)	9	>150	2.0	15 *(<5)*	<5	<5	24.5 ± 8.82	---	---	---	TBD
Bis-neoPentylCOM PMPA  (7.7)	6	>150	3.2	<5 *(<5)*	<5	<5	18.9 ± 6.57	---	---	---	TBD
Bis-nBuCOM PMPA  (8.0)	6	>150	2.7	<5 *(<5)*	<5	<5	17.3 ± 2.57	---	---	---	TBD
Bis-3-PentylCOM PMPA  (6.2)	8	>150	3.2	30 *(<5)*	15	<5	33.9 ± 9.02	---	---	---	TBD

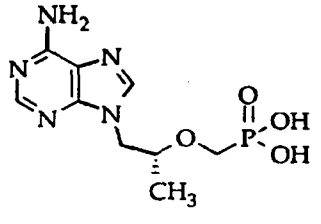
PRODRUGS CARBONATES 	Chemical t1/2 (hr)		Log PC	Biological t1/2 (min) (Dog) *(Human)*			% of PMPA IV (1 mg/kg) AUC				Urinary Recovery (% as PMPA)
	pH 7.4	pH 2.0	pH 7.4	Intestine	Plasma	Liver	PMPA	Monoester	Prodrug	other	
Bis-EtCOM PMPA R =  ; R^2 = H (10)	7		0.6	23.3 *(<5)*	16.6	<5	29.3 ± 3.4	~2	0	0	TBD
Bis-EtCOE PMPA R =  ; R^2 = Me	4			62.4	42.6	<5	NA	NA	NA	NA	NA
Bis-Methoxy diMeCOM PMPA R =  ; R^2 = H	9		1.0	Stable *(30)*	77.6	100.8	NA	NA	NA	NA	NA
Bis-isopropylCOM PMPA R =  ; R^2 = H (9)	9		1.25	52.6 *(<5)*	20.5	<5	35.8 ± 14.7	3.1± 0.67	0	0	TBD

PRODRUGS CARBAMATES 	Chemical t1/2 (hr)		Log PC	Biological t1/2 (min) (Dog) *(Human)*			% of PMPA IV (1 mg/kg) AUC				*Urinary Recovery* (% as PMPA)
	pH 7.4	pH 2.0	pH 7.4	Intestine	Plasma	Liver	PMPA	Monoester	Prodrug	other	
Bis-tBuNCOM PMPA $R^4 = H, R^5 =$ (6.4)	0.4			107.5	99.5	166.5	8.86 ± 2.38	---	9.97 ± 2.52	---	
Bis-di-n-proNCOM PMPA $R^4 = R^5 =$	13			Stable	Stable	76	NA	NA	NA	NA	NA

Example 16

Antiviral Activity of PMPA and PMPA Carbonates in Tissue Culture

PMPA (9-[(R)-2-(phosphonomethoxy)propyl]adenine) and PMPA carbonates were examined to determine their activity against HIV-1. The antiviral activity of the carbonates **5a, 5c-g** against HIV-1(IIIB) was determined in MT-2 cells and the IC_{50} (50% inhibitory concentration) and CC_{50} (concentration to kill 50% of the cells) values were measured. The carbonate prodrugs exhibited increased potency (about 2.5-500 fold) compared to PMPA (Table 2). Although cytotoxicity of the prodrugs also increased, the selectivity indices were improved compared to PMPA. The increased activity can be attributed to increased cellular uptake of the prodrugs followed by effective intracellular conversion to PMPA, which undergoes subsequent phosphorylation to the antivirally active diphosphate metabolite. The t-butyl carbonate **5d** exhibited only 2.5 fold increased activity over PMPA with reduced selectivity possibly due to chemical instability. The antiviral activity data indicate good permeability of alkyl methyl carbonate prodrugs into cells, possibly due to their increased lipophilicity. The partition coefficient values support this hypothesis, with all prodrugs being more lipophilic (logP = 0.6-3.2) compared to PMPA (logP = -2.5).



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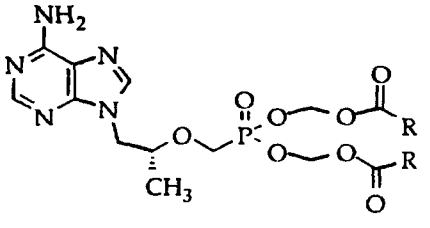


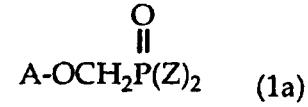
5

a. R = -OEt
c. R = -OBu^{iso}
d. R = -OBu^{t}
e. R = -OPen^{neo}
f. R = -OPro^{iso}
g. R = 3-OPen

CLAIMS

We claim:

1. A compound having formula (1a)



(1a)

wherein Z is independently $-OC(R^2)_2OC(O)X(R)_a$, an ester, an amidate or -H, but at least one Z is $-OC(R^2)_2OC(O)X(R)_a$;

A is the residue of an antiviral phosphonomethoxy nucleotide analog;

X is N or O;

R^2 independently is -H, C_1-C_{12} alkyl, C_5-C_{12} aryl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_7-C_{12} alkenylaryl, C_7-C_{12} alkynylaryl, or C_6-C_{12} alkaryl, any one of which is unsubstituted or is substituted with 1 or 2 halo, cyano, azido, nitro or $-OR^3$ in which R^3 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl or C_5-C_{12} aryl;

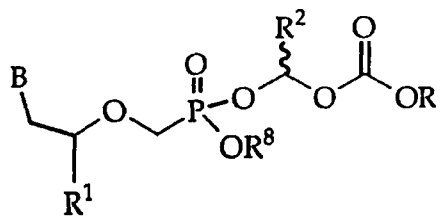
R is independently -H, C_1-C_{12} alkyl, C_5-C_{12} aryl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_7-C_{12} alkyenylaryl, C_7-C_{12} alkynylaryl, or C_6-C_{12} alkaryl, any one of which is unsubstituted or is substituted with 1 or 2 halo, cyano, azido, nitro, $-N(R^4)_2$ or $-OR^3$, where R^4 independently is -H or C_1-C_8 alkyl, provided that at least one R is not H; and

a is 1 when X is O, or 1 or 2 when X is N;

with the proviso that when a is 2 and X is N, (a) two N-linked R groups can be taken together to form a carbocycle or oxygen-containing heterocycle, (b) one N-linked R additionally can be $-OR^3$ or (c) both N-linked R groups can be -H;

and the salts, hydrates, tautomers and solvates thereof.

2. The compound of claim 1 having formula (1)



(1)

wherein B is guanin-9-yl, adenin-9-yl, 2,6-diaminopurin-9-yl, 2-aminopurin-9-yl or their 1-deaza, 3-deaza, or 8-aza analogs, or B is cytosin-1-yl;

R is independently -H, C_1-C_{12} alkyl, C_5-C_{12} aryl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_7-C_{12} alkenylaryl, C_7-C_{12} alkynylaryl, or C_6-C_{12} alkaryl, any one of which is unsubstituted or is substituted with 1 or 2 halo, cyano, azido, nitro or $-OR^3$ in which R^3 is C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl or C_5-C_{12} aryl;

R^1 is hydrogen, $-CH_3$, $-CH_2OH$, $-CH_2F$, $-CH=CH_2$, or $-CH_2N_3$, or R^1 and R^8 are joined to form $-CH_2-$;

R^2 independently is hydrogen or C_1-C_6 alkyl; and

R^8 is hydrogen or $-CHR^2-O-C(O)-OR$, or R^8 is joined with R^1 to form $-CH_2-$;

and the salts, hydrates, tautomers and solvates thereof.

3. The compound of claim 2 wherein R^2 is -H.

4. The compound of claim 3 wherein R^1 is $-CH_3$.

5. The compound of claim 1 wherein R^2 is -H.

6. The compound of claim 1 wherein one R^2 is $-CH_3$ and the other R^2 is H.

7. The compound of claim 1 wherein R^3 is C_1-C_6 alkyl or phenyl.

8. The compound of claim 1 wherein R^3 is $-CH_3$ or $-C_2H_5$.

9. The compound of claim 1 wherein X is O.

10. The compound of claim 1 wherein X is N and one R^3 is H.

11. The compound of claim 4 wherein the compound is enriched or resolved at the carbon atom chiral center linked to R^1.

12. The compound of claim 4 wherein at least about 90% of the compound is in the (*R*) configuration at the R^1 site.

13. The compound of claim 12 wherein B is adenin-9-yl.

14. The compound of claim 13 wherein each R is ethyl.

15. The compound of claim 13 wherein each R is isopropyl.

16. The compound of claim 13 wherein each R is 3-pentyl or neopentyl.

17. The compound of claim 13 wherein each R is *t*-butyl or isobutyl.

18. The compound of claim 4 wherein B is 2,6-diaminopurin-9-yl.

19. The compound of claim 3 wherein R^1 is H.

20. The compound of claim 19 wherein B is adenin-9-yl.

21. The compound of claim 4 wherein R is C_1-C_{12} alkyl.

22. The compound of claim 3 wherein R^1 is -CH_2OH.

23. The compound of claim 22 wherein B is cytosin-1-yl.

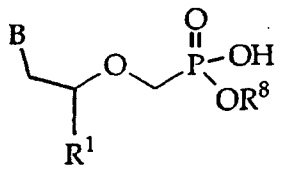
24. The compound of claim 1 named in Table B and compound groups **1-19**.

25. The compound of claim 22 wherein at least about 90% of the compound is in the (*S*) configuration at the R^1 site.

26. A method comprising orally administering to a patient infected with virus or at risk to viral infection a therapeutically effective amount of a compound of claim 1.

27. A method for preparing a compound of formula (1a) comprising reacting the diacid of a phosphonomethoxy nucleotide analog with L-$CH(R^2)OC(O)X(R)_n$ wherein L is a leaving group.

28. A method for preparing a compound of formula (1) comprising reacting a compound of formula (6)



(6)

with L-CHR^2-O-C(O)-OR and recovering a compound of formula (1), wherein B is guanin-9-yl, adenin-9-yl, 2,6-diaminopurin-9-yl, 2-aminopurin-9-yl or their 1-deaza, 3-deaza, or 8-aza analogs, or B is cytosin-1-yl;

R^1 is hydrogen, -CH_3, -CH_2OH, -CH_2F, -$CH{=}CH_2$, -CH_2N_3 or R^1 and R^8 are joined to form -CH_2-; and

R^8 is hydrogen, -CHR^2-O-C(O)-OR or R^8 is joined with R^1 to form -CH_2-; and

R^2 is H, C_1-C_{12} alkyl, aryl, alkenyl, alkynyl, alkyenylaryl, alkynylaryl, alkaryl, arylalkynyl, arylalkenyl or arylalkyl which is unsubstituted or is substituted with halo, azido, nitro or OR^3 in which R^3 is C_1-C12 alkyl;

R is independently H, C_1-C_{12} alkyl, aryl, alkenyl, alkynyl, alkyenylaryl, alkynylaryl, alkaryl, arylalkynyl, arylalkenyl or arylalkyl which is unsubstituted or is substituted with halo, azido, nitro or OR^3, provided that at least one R is not H; and

L is a leaving group.

29. The method of claim 30 comprising conducting the reaction using at least about 1.0 equivalent of L-CHR^2-O-C(O)-OR.

30. The method of claim 31 comprising conducting the reaction in the presence of an organic base in an organic solvent at a reaction temperature of about 4-100°C for about 4-72 hours.

31. The method of claim 28 wherein the compound of formula (1) is recovered by forming a salt, precipitating the salt and recovering the precipitated salt.

32. The method of claim 31 wherein the salt is formed from sulfuric acid, phosphoric acid, lactic acid, or citric acid.

231

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 97/13244

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07F9/6561 A61K31/675 C07F9/6512

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 C07F A61K

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

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LINDAHL A ET AL: "Synthesis of an acryloxymethyl prodrug of the inositol phosphate.alpha.-trinositol" J. CARBOHYDR. CHEM. (JCACDM,07328303);96; VOL.15 (5); PP.549-554, PERSTORP PHARMA;PERSTORP; S-284 80; SWED. (SE), XP002043027 see the whole document ---	1-32
Y	WO 95 07920 A (GILEAD SCIENCES INC ;BISCHOFBERGER NORBERT W (US); JONES ROBERT J) 23 March 1995 cited in the application see the whole document --- -/--	1-32

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

[X] Patent family members are listed in annex.

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1

Date of the actual completion of the international search	Date of mailing of the international search report
9 October 1997	20 -10- 1997

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/13244

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 92 09611 A (BEECHAM GROUP PLC) 11 June 1992 cited in the application see the whole document ---	1-32
Y	EP 0 481 214 A (SQUIBB BRISTOL MYERS CO) 22 April 1992 cited in the application see the whole document -----	1-32

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/13244

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 7875294 A	03-04-95
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		BR 9407510 A	07-01-97
		EP 0719273 A	03-07-96
		EP 0719274 A	03-07-96
		WO 9507919 A	23-03-95
		US 5591851 A	07-01-97
WO 9209611 A	11-06-92	AU 9044091 A	25-06-92
EP 0481214 A	22-04-92	CA 2051239 A	15-03-92
		JP 4230694 A	19-08-92
		US 5663159 A	02-09-97

Representation under Section 25(1) to Application no: 568/MUMNP/2011

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(54) Title: NUCLEOTIDE ANALOG COMPOSITIONS

(57) Abstract

The invention provides crystalline forms of adefovir dipivoxil and methods to prepare the crystals. The compositions and methods of the present invention have desirable properties for large scale synthesis of crystalline adefovir dipivoxil or for its formulation into therapeutic dosages. Invention compositions include an anhydrous crystal form of adefovir dipivoxil.

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NUCLEOTIDE ANALOG COMPOSITIONS

BACKGROUND OF THE INVENTION

The invention relates to the nucleotide analog 9-[2-[[bis[(pivaloyloxy)-methoxy]phosphinyl]methoxy]ethyl]adenine ("adefovir dipivoxil" or "AD") and to its use. The present invention also relates to methods to synthesize AD.

AD is the bis-pivaloyloxymethyl ester of the parent compound 9-[2-(phosphonomethoxy)ethyl]adenine ("PMEA"), which has antiviral activity in animals and in humans. AD and PMEA have been described, e.g., U.S. Patent Numbers 4,724,233 and 4,808,716, EP 481 214, Benzaria et al., *Nucleosides and Nucleotides* (1995) 14(3-5):563-565, Holy et al., *Collect. Czech. Chem. Commun.* (1989) 54:2190-2201, Holy et al., *Collect. Czech. Chem. Commun.* (1987) 52:2801-2809, Rosenberg et al., *Collect. Czech. Chem. Commun.* (1988) 53:2753-2777, Starrett et al., *Antiviral Res.* (1992) 19:267-273; Starrett et al., *J. Med. Chem.* (1994) 37:1857-1864. Heretofore, AD has been provided only as a noncrystalline or amorphous form. It has not been reported to have been prepared as a crystalline material.

Methods for crystallizing organic compounds *per se* are described in J.A. Landgrebe, *Theory and Practice in the Organic Laboratory*, 2nd edition, 1977, D.C. Heath and Co., Lexington, MA, p. 43-51; A.S. Myerson, *Handbook of Industrial Crystallization*, 1993, Butterworth-Heinemann, Stoneham, MA, p. 1-101).

OBJECTS OF THE INVENTION

The invention provides one or more compositions or methods that meet one or more of the following objects.

A principal object of the invention is to provide compositions comprising novel AD forms having desirable properties for large scale synthesis or for formulation into therapeutic dosages.

Another object is to provide AD having good melting point, and/or flow or bulk density properties, which facilitates manufacturing and formulation of compositions containing AD.

Another object is to provide storage-stable forms of AD.

Another object is to provide AD which can be readily filtered and easily dried.

Another object is to provide highly purified AD having at least about 97% (w/w) purity and preferably at least about 98%.

Another object is to eliminate or minimize by-products made during AD synthesis.

Another object is to provide a method for purifying AD that avoids expensive and time-consuming column chromatography.

SUMMARY OF THE INVENTION

The invention accomplishes its primary objects by providing crystalline AD, in particular, an anhydrous crystalline form (hereafter "Form 1"), a hydrated form, $C_{20}H_{32}N_5O_8P_1 \cdot 2H_2O$, (hereafter "Form 2"), a methanol solvate form, $C_{20}H_{32}N_5O_8P_1 \cdot CH_3OH$, (hereafter "Form 3"), a fumaric acid salt or complex, $C_{20}H_{32}N_5O_8P_1 \cdot C_4H_4O_4$ (hereafter "Form 4"), a hemisulfate salt or complex, a hydrobromide salt or complex, a hydrochloride salt or complex, a nitrate salt or complex, a mesylate (CH_3SO_3H) salt or complex, an ethyl sulfonate salt ($C_2H_5SO_3H$) or complex, a β-naphthylene sulfonic acid salt or complex, an α-naphthylene sulfonic acid salt or complex, an (S)-camphor sulfonic acid salt or complex, a succinic acid salt or complex, a maleic acid salt or complex, an ascorbic acid salt or complex and a nicotinic acid salt or complex.

Invention embodiments include (1) crystalline Form 1 AD essentially having an X-ray powder diffraction ("XRD") spectrum using Cu-Kα radiation, expressed in degrees 2θ at any one or more (in any combination) of about 6.9, about 11.8, about 12.7, about 15.7, about 17.2, about 20.7, about 21.5, about 22.5, and about 23.3; (2) crystalline Form 2 AD essentially having an XRD spectrum using Cu-Kα radiation, expressed in degrees 2θ at any one or more (in any combination) of about 8.7-8.9, about 9.6, about 16.3, about 18.3, about 18.9, about 19.7, about 21.0, about 21.4, about 22.0, about 24.3, about 27.9, about, 30.8, and about 32.8; (3) crystalline Form 3 AD essentially having an XRD spectrum using Cu-Kα radiation, expressed in degrees 2θ at any one or more (in any combination) of about 8.1, about 8.7, about 14.1, about 16.5, about 17.0, about 19.4, about 21.1, about 22.6, about 23.4, about 24.2, about 25.4, and about 30.9; and crystalline Form 4 AD essentially having an XRD spectrum using Cu-Kα radiation, expressed in degrees 2θ at any one or more (in any combination) of about

9.8, about 15.2, about 15.7, about 18.1, about 18.3, about 21.0, about 26.3 and about 31.7.

Invention embodiments include AD crystals having the crystal morphologies shown in any one or more of figures 4-10.

In other embodiments, the invention provides methods to produce AD crystals by allowing crystals to form from a crystallization solution comprising about 6-45% AD and about 55-94% crystallization solvent wherein the crystallization solvent is selected from the group consisting of (1) a mixture between about 1:10 v/v to about 1:3 v/v of acetone:di-n-butyl ether, (2) a mixture between about 1:10 v/v to about 1:3 v/v of ethyl acetate:di-n-propyl ether, (3) a mixture between about 1:10 v/v to about 10:1 v/v of t-butanol:di-n-butyl ether, (4) a mixture between about 1:10 v/v to about 1:3 v/v of methylene chloride:di-n-butyl ether, (5) a mixture between about 1:10 v/v to about 10:1 v/v of diethyl ether:di-n-propyl ether, (6) a mixture between about 1:10 v/v to about 1:3 v/v of tetrahydrofuran:di-n-butyl ether, (7) a mixture between about 1:10 v/v to about 1:3 v/v of ethyl acetate:di-n-butyl ether, (8) a mixture between about 1:10 v/v to about 1:3 v/v of tetrahydropyran:di-n-butyl ether, (9) a mixture between about 1:10 v/v to about 1:3 v/v of ethyl acetate:diethyl ether, (10) t-butyl-methyl ether, (11) diethyl ether, (12) di-n-butyl ether, (13) t-butanol, (14) toluene, (15) isopropyl acetate, (16) ethyl acetate, (17) a mixture consisting essentially of (A) a first crystallization solvent consisting of a first dialkyl ether of the formula R^1-O-R^2 wherein R^1 is an alkyl group having 1, 2, 3, 4, 5 or 6 carbon atoms, R^2 is an alkyl group having 2, 3, 4, 5 or 6 carbon atoms or both R^1 and R^2 are linked together to form a 5-, 6-, 7-, or 8-membered ring, provided that the dialkyl ether is not methyl-ethyl ether, and (B) a second crystallization solvent selected from the group consisting of (a) a second dialkyl ether of the formula R^1-O-R^2, wherein the second dialkyl ether is different from the first dialkyl ether, but is not methyl ethyl ether, (b) toluene, (c) tetrahydrofuran, (d) t-butanol, (e) ethyl acetate, (f) methylene chloride, (g) propyl acetate and (h) isopropanol.

Invention embodiments include purified crystalline AD (e.g., form 1 and/or form 2). Invention embodiments also include compositions comprising crystalline AD (e.g., form 1 and/or form 2) and one or more compounds, such as pharmaceutical excipients or compounds present in reaction mixtures that contain the crystalline AD.

Invention embodiments include a method to produce AD crystals comprising dissolving AD in methanol and allowing crystals to form.

Another embodiment is crystalline AD suitable for pharmaceutical compositions or uses comprising, e.g., one or more of Form 1, Form 2, Form 3 and/or Form 4 AD and a pharmaceutically acceptable carrier(s) for treating viral conditions for which PMEA is known to be active, such as a retroviral infection (HIV, SIV, FIV) or hepatitis B virus or other hepadnavirus infections, or DNA virus infection (human cytomegalovirus or herpesvirus, e.g., HSV1 or HSV2) in humans or animals.

The invention provides a method to produce crystalline Form 2 AD comprising forming AD crystals in the presence of water.

In another embodiment, a method for preparing AD comprises contacting PMEA with chloromethyl pivalate in N-methylpyrrolidinone (NMP, 1-methyl-2-pyrrolidinone) and a trialkylamine such as triethylamine (TEA) and recovering AD.

In a further embodiment, a PMEA composition containing less than about 2% salt is provided, which may be used in a method comprising contacting PMEA containing less than about 2% salt.

In a further embodiment, an AD product is obtained by a process comprising preparing wet granules from a mixture comprising a liquid, Form 1 adefovir dipivoxil and an acceptable excipient and, optionally drying the wet granules.

Brief Description of the Figures

Figure 1 shows a Form 1 crystal XRD pattern. Figure 2 shows a thermogram obtained by differential scanning calorimetry of Form 1 crystals. Figure 3 shows a Fourier transform infrared absorption spectrum of Form 1 crystals. Figures 4-10 are pictures of a photograph showing embodiments of Form 1 crystals at 100X magnification. Figures 4-10 are copies of the photographs made at a 128% enlargement. Figure 11 shows an XRD pattern of Form 2 crystals. Figure 12 shows a thermogram obtained by differential scanning calorimetry of Form 2 crystals. Figure 13 shows a Fourier transform infrared absorption spectrum of Form 2 crystals. Figure 14 shows an XRD pattern of Form 3 crystals. Figure 15 shows a thermogram obtained by differential scanning calorimetry of Form 3 crystals. Figure 16 shows a Form 4 crystal XRD pattern. Figure 17

shows a thermogram obtained by differential scanning calorimetry of Form 4 crystals. Figure 18 shows an AD hemisulfate salt crystal XRD pattern. Figure 19 shows an AD hydrobromide salt crystal XRD pattern. Figure 20 shows an AD nitrate salt crystal XRD pattern. Figure 21 shows an AD mesylate salt crystal XRD pattern. Figure 22 shows an AD ethyl sulfonate salt crystal XRD pattern. Figure 23 shows an AD β-naphthylene sulfonate salt crystal XRD pattern. Figure 24 shows an AD α-naphthylene sulfonate salt crystal XRD pattern. Figure 25 shows an AD (*S*)-camphor sulfonate salt crystal XRD pattern. Figure 26 shows an AD succinic acid salt crystal XRD pattern.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise indicated, temperatures are in degrees Celsius (°). Room temperature means about 18-23°.

As used herein, alkyl means linear, branched and cyclic saturated hydrocarbons. "Alkyl" or "alkyl moiety" as used herein, unless stated to the contrary, is a hydrocarbon containing 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 normal, secondary, tertiary or cyclic structures. The term $C_{1\text{-}10}$ alkyl means alkyl groups having 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 carbon atoms. Examples are $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH_2CH_2CH_3$, $-CH_2CH(CH_3)_2$, $-CH(CH_3)CH_2CH_3$, $-C(CH_3)_3$, $-CH_2CH_2CH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_2CH_3$, $-CH(CH_2CH_3)_2$, $-C(CH_3)_2CH_2CH_3$, $-CH(CH_3)CH(CH_3)_2$, $-CH_2CH_2CH(CH_3)_2$, $-CH_2CH(CH_3)CH_2CH_3$, $-CH_2C(CH_3)_3$, $-CH_2CH_2CH_2CH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_2CH_2CH_3$, $-CH(CH_2CH_3)(CH_2CH_2CH_3)$, $-C(CH_3)_2CH_2CH_2CH_3$, $-CH(CH_3)CH(CH_3)CH_2CH_3$, $-CH(CH_3)CH_2CH(CH_3)_2$, $-C(CH_3)(CH_2CH_3)_2$, $-CH(CH_2CH_3)CH(CH_3)_2$, $-C(CH_3)_2CH(CH_3)_2$, $-CH(CH_3)C(CH_3)_3$, cyclopropyl, cyclobutyl, cyclopropylmethyl, cyclopentyl, cyclobutylmethyl, 1-cyclopropyl-1-ethyl, 2-cyclopropyl-1-ethyl, cyclohexyl, cyclopentylmethyl, 1-cyclobutyl-1-ethyl, 2-cyclobutyl-1-ethyl, 1-cyclopropyl-1-propyl, 2-cyclopropyl-1-propyl, 3-cyclopropyl-1-propyl, 2-cyclopropyl-2-propyl, and 1-cyclopropyl-2-propyl.

"Alkoxide" as used herein, unless stated to the contrary, is a hydrocarbon containing 1, 2, 3, 4, 5 or 6 carbon atoms, as defined herein for alkyl, linked to an oxygen atom. Examples are $-OCH_3$, $-OCH_2CH_3$,

$-OCH_2CH_2CH_3$, $-OCH(CH_3)_2$, $-OCH_2CH_2CH_2CH_3$, $-OCH_2CH(CH_3)_2$, $-OCH(CH_3)CH_2CH_3$, $-OC(CH_3)_3$, $-OCH_2CH_2CH_2CH_2CH_3$, $-OCH(CH_3)CH_2CH_2CH_3$, $-OCH(CH_2CH_3)_2$, $-OC(CH_3)_2CH_2CH_3$, $-OCH(CH_3)CH(CH_3)_2$, $-OCH_2CH_2CH(CH_3)_2$, $-OCH_2CH(CH_3)CH_2CH_3$, $-OCH_2C(CH_3)_3$, $-OCH(CH_3)(CH_2)_3CH_3$, $-OC(CH_3)_2(CH_2)_2CH_3$, $-OCH(C_2H_5)(CH_2)_2CH_3$, $-O(CH_2)_3CH(CH_3)_2$, $-O(CH_2)_2C(CH_3)_3$, $-OCH_2CH(CH_3)(CH_2)_2CH_3$, and $-OCH_2CH_2CH_2CH_2CH_2CH_3$.

"Trialkylamine" means an nitrogen atom substituted with three C_{1-6} alkyl moieties, which are independently chosen. Examples are nitrogen substituted with 1, 2 or 3 $-CH_3$, $-CH_2CH_3$, $-CH_2CH_2CH_3$, $-CH(CH_3)_2$, $-CH_2CH_2CH_2CH_3$, $-CH_2CH(CH_3)_2$, $-CH(CH_3)CH_2CH_3$, $-C(CH_3)_3$, $-CH_2CH_2CH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_2CH_3$, $-CH(CH_2CH_3)_2$, $-C(CH_3)_2CH_2CH_3$, $-CH(CH_3)CH(CH_3)_2$, $-CH_2CH_2CH(CH_3)_2$, $-CH_2CH(CH_3)CH_2CH_3$, $-CH_2C(CH_3)_3$, $-CH_2CH_2CH_2CH_2CH_2CH_3$, $-CH(CH_3)CH_2CH_2CH_2CH_3$, $-CH(CH_2CH_3)(CH_2CH_2CH_3)$, $-C(CH_3)_2CH_2CH_2CH_3$, $-CH(CH_3)CH(CH_3)CH_2CH_3$, $-CH(CH_3)CH_2CH(CH_3)_2$, $-C(CH_3)(CH_2CH_3)_2$, $-CH(CH_2CH_3)CH(CH_3)_2$, $-C(CH_3)_2CH(CH_3)_2$ or $-CH(CH_3)C(CH_3)_3$ moieties.

"Heteroaryl" as used herein includes by way of example and not limitation these heterocycles described in Paquette, Leo A.; *Principles of Modern Heterocyclic Chemistry* (W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; *The Chemistry of Heterocyclic Compounds, A series of Monographs* (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.*, (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, 1H-

indazoly, purinyl, 4H-quinolizinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, b-carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolinyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolinyl, isoindolinyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolinyl, and isatinoyl.

By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β-carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

As used herein, AD that is a "crystalline material", "crystalline" or "crystal" means a solid AD having an ordered arrangement of substantially all of the constituent molecule(s) in a definite three-dimensional spatial pattern or lattice. Crystalline or crystal AD may comprise one or more than one type of composition, e.g., AD•fumaric acid or AD•2H_2O. A crystalline material or crystal may occur in one or more than one crystal habits, e.g., tablets, rods, plates or needles.

Unless specified otherwise explicitly or by context, we express percentage amounts as % by weight (w/w). Thus, a solution containing at least about 40% AD is a solution containing at least about 40% w/w AD. Solid AD containing 0.1% water means 0.1% w/w water is associated with the solid.

Crystalline AD substantially free of noncrystalline AD means a solid composition in which more than about 60% of the AD is present in the composition as crystalline material. Such compositions typically contain at least about 80%, usually at least about 90%, of one or more AD crystal forms, with the remaining AD being present as noncrystalline AD.

Invention compositions optionally comprise salts of the compounds herein, including pharmaceutically acceptable salts comprising, for example, an uncharged moiety or a monovalent anion. Salt(s) include those derived by combination of appropriate anions such as inorganic or organic acids. Suitable acids include those having sufficient acidity to form a stable salt, preferably acids of low toxicity. For example, one may form invention salts from acid addition of certain organic and inorganic acids, e.g., HF, HCl, HBr, HI, H_2SO_4, H_3PO_4, or from organic sulfonic acids, organic carboxylic acids to basic centers, typically amines. Exemplary organic sulfonic acids include C_{6-16} aryl sulfonic acids, C_{6-16} heteroaryl sulfonic acids and C_{1-16} alkyl sulfonic acids such as phenyl, α-naphthyl, β-naphthyl, (*S*)-camphor, methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *s*-butyl, *i*-butyl, *t*-butyl, pentyl and hexyl sulfonic acids. Exemplary organic carboxylic acids include C_{1-16} alkyl, C_{6-16} aryl carboxylic acids and C_{4-16} heteroaryl carboxylic acids such as acetic, glycolic, lactic, pyruvic, malonic, glutaric, tartaric, citric, fumaric, succinic, malic, maleic, hydroxymaleic, benzoic, hydroxybenzoic, phenylacetic, cinnamic, salicylic and 2-phenoxybenzoic. Salts also include the invention compound salts with one or more amino acids. Many amino acids are suitable, especially the naturally-occurring amino acids found as protein components, although the amino acid typically is one bearing a side chain with a basic or acidic group, e.g., lysine, arginine or glutamic acid, or a neutral group such as glycine, serine, threonine, alanine, isoleucine, or leucine. Salts are usually biologically compatible or pharmaceutically acceptable or non-toxic, particularly for mammalian cells. Salts that are biologically toxic are generally used with synthetic intermediates of invention compounds. The salts of AD are typically crystalline, such as Form 4 described herein.

Embodiments include compositions that transiently occur when a method step or operation is performed. For example, when a sodium alkoxide is brought into contact with a 9-(2-hydroxyethyl)adenine solution, the composition at the initiation of mixing will contain negligible amounts of the sodium alkoxide. This composition will be generally be present as a non-homogenous mixture prior to sufficient agitation to mix the solution. Such a composition usually comprises negligible reaction products and comprises mostly reactants. Similarly, as a reaction proceeds, the proportions of reactants, products and by-products will change relative to each other. These transient compositions are intermediates that arise when a process step is performed and they are expressly included as invention embodiments.

The invention includes compositions comprising mixtures of two or more different crystal types or forms, e.g., Form 1 and Form 2 crystals, Form 1, Form 2 and Form 4 crystals, or Form 2 and Form 4 crystals. Mixtures of Form 1 and Form 2 AD crystals may be present in pharmaceutical formulations or their manufacture, and typically such mixtures comprise at least about 70% Form 1, usually at least about 90%, but in some instances up to about 70% of such a mixture may comprise Form 2 and/or amorphous AD.

Crystalline forms of AD

AD prepared and recovered as described (Starrett et al., *J. Med. Chem.* (1994) 19:1857-1864) and as recovered from a silica gel column in a solution of methanol (about 4%) and methylene chloride (about 96%) by rotary evaporation under reduced pressure at about 35° precipitates as a noncrystalline or an amorphous solid. We now have discovered that AD can be prepared in crystalline form.

We have identified several different crystalline AD forms. We have characterized them by several methods, usually by XRD and DSC thermogram. Workers commonly use XRD to characterize or identify crystal compositions (see, e.g., U.S. Pharmacopoeia, volume 23, 1995, method 941, p 1843-1845, U.S.P. Pharmacopeial Convention, Inc., Rockville, MD; Stout et al, *X-Ray Structure Determination; A Practical Guide*, MacMillan Co., New York, N.Y. 1968). The diffraction pattern obtained from a crystalline compound is often diagnostic for a given crystal form, although weak or very weak diffraction peaks may not always

appear in replicate diffraction patterns obtained from successive batches of crystals. This is particularly the case if other crystal forms are present in the sample in appreciable amounts, e.g., where Form 1 crystals have become partially hydrated to Form 2 crystals. The relative intensities of bands, particularly at low angle X-ray incidence values (low 2θ), may vary due to preferred orientation effects arising from differences in, e.g., crystal habit, particle size and other conditions of measurement. Thus, the relative intensities of the diffraction peaks are not conclusively diagnostic of the crystal form in question. Instead, one should look to the relative positioning of the peaks rather than their amplitude to determine if an AD crystal is one of the forms described herein. Individual XRD peaks in different samples are generally located within about 0.3-1 2θ degree for broad peaks. Broad XRD peaks may consist of two or more individual peaks located closely together. For sharp isolated peaks, the peak is usually found within about 0.1 2θ degrees on successive XRD analyses. Assuming one uses the same instrument to measure a compound's XRD spectrum on successive XRD analyses, the differences in XRD peak locations are due primarily to differences in sample preparation or the purity of the sample itself. When we identify a sharp isolated XRD peak at a given position as being located at, e.g., about 6.9, this means that the peak is at 6.9±0.1. When we identify a broad XRD peak at a given position as being located at about a given 2θ value, this means that the peak is at that 2θ value ±0.3.

Note that it is not necessary to rely on all bands that one observes in the highly purified AD reference samples herein; even a single band may be diagnostic of a given crystal form of AD, e.g., 6.9 for Form 1. Identification should focus on band position and general pattern, particularly the selection of bands unique to the various crystal forms.

Additional diagnostic techniques that one can optionally use to identify crystalline AD include differential scanning calorimetry (DSC), melting point measurements and infrared absorption spectroscopy (IR). DSC measures thermal transition temperatures at which a crystal absorbs or releases heat when its crystal structure changes or it melts. Thermal transition temperatures and melting points are typically within about 2° C on successive analyses, usually within about 1 degree. When we state that a compound has a DSC peak or a melting point at a given value, it means that the DSC peak or a melting point is within ±2° C. DSC provides an alternate means for one to distinguish between different AD crystal forms.

Different crystal forms may be identified, at least in part, based on their different transition temperature profiles. IR measures absorption of infrared light caused by the presence of particular chemical bonds associated with groups in a molecule that vibrate in response to the light. DSC and/or IR can thus provide physicochemical information one can use to describe AD crystals.

Form 1

Single crystal X-ray crystallography was used to characterize Form 1 AD. Cell constants and an orientation matrix obtained from a least squares refinement using the measured positions of 3242 reflections with $I > 10\sigma$ in the range $3.00 < 2\theta < 45.00°$ corresponded to a C-centered monoclinic cell specified as follows: a = 12.85 Å, b = 24.50 Å, c = 8.28 Å, ß = 100.2°, Z = 4, space group Cc.

The Form 1 XRD pattern usually shows a peak(s) at about 6.9, typically at about 6.9 and about 20.7, or more typically at about 6.9, about 15.7 and about 20.7 and ordinarily at least at about 6.9, about 11.8, about 15.7 and about 20.7. Typically the XRD peak at about 6.9, or usually either (1) this peak plus one or two peaks additional peaks or (2) the peak at about 6.9 plus one or two other peaks coupled with differential scanning calorimetry data or melting point data, is sufficient to distinguish Form 1 crystals from other forms or to identify Form 1 itself. The Form 1 spectrum commonly has peaks at about 6.9, about 11.8, about 12.7, about 15.7, about 17.2, about 20.7, about 21.5, about 22.5 and about 23.3. The Form 1 XRD pattern usually shows a peak(s) at any one (or combination) of about 6.9 and/or 11.8 and/or 15.7 and/or 17.2 and/or 20.7 and/or 23.3. Figure 1 shows a typical Form 1 crystal X-ray diffraction pattern. It should be understood, however, that figures 1-26 are only exemplary and that diagnostic representations of other crystalline AD preparations may depart from these depictions.

Form 1 AD is anhydrous, containing little or no detectable water. In general, Form 1 crystals ordinarily will contain less than about 1%, typically less than about 0.5%, and usually less than about 0.2% of water. Moreover, Form 1 crystals ordinarily will contain less than about 20%, typically will contain less than about 10%, often less than about 1%, and usually less than about 0.1% noncrystalline AD. Often, Form 1 crystals will contain no noncrystalline AD that is detectable by DSC, XRD or polarized light microscopy at 100X magnification. Form 1 AD is typically

substantially free of crystallization solvent, i.e., typically less than about 1%, usually less than about 0.6%, if adequately recovered from the crystallization bath, and it does not contain lattice-entrained solvent molecules.

Form 1 crystals generally have a median size by light scattering of about 25-150 μm, usually about 30-80 μm. Individual Form 1 preparations usually contain crystals that have a length range of about 1-200 μm and have a typical maximum dimension for individual crystals in a preparation of about 60-200 μm. In some Form 1 preparations, about 1-10% of the crystals in a preparation will have a maximum dimension of greater than 250 μm. The Form 1 crystals shown in figures 4-10 typically have tablet, plate, needle and/or irregular habits. Aggregates of Form 1 crystals also occur with a typical diameter range of about 25-150 μm.

Form 1 crystals exhibit a DSC endothermic transition at about 102°C (see figure 2) and an IR spectrum essentially as depicted in figure 3. Different Form 1 crystal preparations have a bulk density of about 0.15-0.60 g/mL, usually about 0.25-0.50 g/mL, with a surface area of about 0.10-2.20 m^2/g, usually about 0.20-0.60 m^2/g. Form 1 AD is thus characterized by an XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at any one (or combination) of about 6.9 and/or 11.8 and/or 15.7 and/or 20.7 and an endothermic transition as measured by differential scanning calorimetry at about 102°. Form 1 AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 6.9±0.1, 11.8±0.1, 15.7±0.1, 17.2±0.1, 20.7±0.1 and an endothermic transition peak as measured by differential scanning calorimetry at 102.0±2° and/or an endothermic onset at 99.8±2°.

Form 2

The Form 2 XRD pattern, an example of which is depicted in figure 11, usually shows a peak(s) at about 22.0, typically at about 18.3 and about 22.0, or more typically at about 9.6, about 18.3 and about 22.0 and ordinarily at least at about 9.6, about 18.3, about 22.0 and about 32.8. Typically any three or four of these four characteristic XRD peaks, or usually either (1) four peaks or (2) two or three of these peaks coupled with differential scanning calorimetry data or melting point data, is sufficient to distinguish Form 2 crystals from other forms or to identify Form 2 itself. The Form 2 XRD pattern usually shows a peak(s) at any one (or combination) of about

8.7-8.9, about 9.6, about 16.3, about 18.3, about 18.9, about 19.7, about 21.0-21.3, about 21.4, about 22.0, about 24.3, about 27.9, about 30.8 and about 32.8.

Form 2 crystals are AD dihydrate, and they usually contain essentially no detectable crystallization solvent, other than water. Form 2 crystals ordinarily will contain less than about 30%, typically less than about 10%, often less than about 1%, usually less than about 0.1% of noncrystalline AD. Generally, the crystals will contain no noncrystalline AD that is detectable by DSC, XRD or polarized light microscopy at 100X magnification. Form 2 crystals typically have a median size of about 15-85 μm by light scattering, ordinarily about 25-80 μm. Individual Form 2 preparations usually contain crystals that have a length range of about 1-300 μm. Form 2 crystals have a DSC endothermic transition at about 73°C (see figure 12) and an IR spectrum substantially as shown in figure 13. Form 2 AD is thus characterized by an XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at any one (or combination) of about 9.6 and/or about 18.3 and/or about 22.0 and/or about 32.8 and an endothermic transition as measured by differential scanning calorimetry at about 73°. Form 2 AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 9.6±0.1, 18.3±0.1, 22.0±0.1, 24.3±0.1 and 32.8±0.1 and an endothermic transition peak as measured by differential scanning calorimetry at 72.7±2° and/or an endothermic onset at 69.5±2°.

Form 3

A Form 3 XRD pattern such as that shown in figure 14 usually shows a peak(s) at about 8.1, typically at about 8.1 and about 25.4, or more typically at about 8.1, about 19.4 and about 25.4. Typically any one or two of these three characteristic XRD peaks, or usually either (1) three or four of these peaks or (2) two or three of these peaks coupled with differential scanning calorimetry data or melting point data, is sufficient to distinguish Form 3 crystals from other forms or to identify Form 3 itself. Form 3 AD has an endothermic transition at about 85° as measured by differential scanning calorimetry (figure 15). The Form 3 spectrum commonly has peaks at any one (or combination) of about 8.1, about 8.7, about 14.1, about 16.5, about 17.0, about 19.4, about 21.1, about 22.6, about 23.4, about 24.2, about 25.4 and about 30.9.

Unlike Forms 1 and 2, Form 3 crystals contain about one equivalent of methanol in the crystal lattice. The methanol typically is donated by

crystallization solvent. However, Form 3 contains essentially no other detectable solvent or water. Form 3 crystals ordinarily will contain less than about 20%, typically less than about 10%, often less than about 1%, usually less than about 0.1% of noncrystalline AD. The crystals will contain no noncrystalline AD that is detectable by DSC, XRD or polarized light microscopy at 100X magnification. Form 3 crystals typically have a median size of about 20-150 μm by light scattering, ordinarily about 30-120 μm. Individual Form 3 preparations usually contain crystals that have a length range of about 1-300 μm.

Form 4

A Form 4 XRD pattern such as that shown in figure 16 usually shows a peak(s) at about 26.3, typically at about 26.3 and about 31.7, or typically at about 26.3, about 31.7 and about 15.2, or usually at about 26.3, about 31.7, about 15.2 and about 21.0. Typically these four characteristic XRD peaks, or usually either (1) three of these peaks or (2) two or three of these peaks coupled with differential scanning calorimetry data or melting point data, is sufficient to distinguish Form 4 crystals from other forms or to identify Form 4 itself. Form 4 AD has endothermic transitions at about 121°C and about 148°C as measured by differential scanning calorimetry (figure 17). The Form 4 spectrum commonly has peaks at any one (or combination) of about 9.8, about 15.2, about 15.7, about 18.1, about 18.3, about 21.0, about 26.3, and about 31.7. Form 4 AD is thus characterized by an XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at any one (or combination) of about 15.2 and/or about 21.0 and/or about 26.3 and/or about 31.7 and an endothermic transition as measured by differential scanning calorimetry at about 121.3° and about 148.4°. Form 4 AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 9.8±0.1, 18.1±0.1, 21.0±0.1, 26.3±0.1 and 31.7±0.1 and an endothermic transition peaks as measured by differential scanning calorimetry at 121.3±2° and 148.4±2°.

Crystalline Salts of Organic and Inorganic Acids

Figures 18-26 show XRD spectra obtained from crystalline salts or, alternatively, complexes of AD and organic and inorganic acids. These salts are a hemisulfate salt or complex (fig 18), a hydrobromide salt or complex (fig 19), a nitrate salt or complex (fig 20), a mesylate (CH_3SO_3H)

salt or complex (fig 21), an ethyl sulfonate salt ($C_2H_5SO_3H$) or complex (fig 22), a β-naphthylene sulfonic acid salt or complex (fig 23), an α-naphthylene sulfonic acid salt or complex (fig 24), an (*S*)-camphor sulfonic acid salt or complex (fig 25) and a succinic acid salt or complex (fig 26). These XRD spectra show a number of peaks that characterize the compounds and allow one to identify each compound from other crystalline forms.

Figure 18 shows that the hemisulfate salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 8.0, about 9.5, about 12.0, about 14.6, about 16.4, about 17.0, about 17.5-17.7, about 18.3, about 19.0, about 20.2, about 22.7, about 24.1 and about 28.2. The salt or complex has a melting point of about 131-134°C. It is thus characterized as having four of these distinctive XRD peaks at about 12.0, about 14.6, about 16.4 and about 17.5-17.7. One may further characterize the compound as having three or four of these XRD peaks and having a melting point of about 131-134°C. The hemisulfate of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 8.0±0.1, 12.0±0.1, 14.6±0.1, 16.4±0.1 and 17.5-17.7±0.3 and a melting point of 131-134±2°C.

Figure 19 shows that the hydrobromide salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 13.2, about 14.3, about 15.9, about 17.8, about 20.7, about 21.8, about 27.2 and about 28.1. The salt or complex decomposes on heating at about 196-199°C. It is thus characterized as having four distinctive XRD peaks at about 13.2, about 14.3, about 17.8 and about 28.1. One may further characterize the compound as having three or four of these XRD peaks and decomposing on heating to about 196-199°C. The hydrobromide of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 13.2±0.1, 14.3±0.1, 17.8±0.1, 20.7±0.1 and 27.2±0.1 and a decomposition point of 196-199±2.0°.

Figure 20 shows that the nitrate salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 8.0, about 9.7, about 14.1, about 15.2, about 16.7, about 17.1, about 18.3, about 18.9, about 19.4, about 20.0, about 21.2, about 22.3, about 23.2, about 24.9, about 27.6, about 28.2, about 29.4 and about 32.6. The salt or complex decomposes on heating at about 135-136°C. It is thus characterized as having four distinctive XRD peaks at about 14.1, about 23.2, about 29.4 and about 32.6.

One may further characterize the compound as having three or four of these XRD peaks and having a melting point of about 131-134°C. The nitrate of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 8.0±0.1, 14.1±0.1, 23.2±0.1, 29.4±0.1 and 32.6±0.1 and a decomposition point of 135-136±2°.

Figure 21 shows that the mesylate salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 4.8, about 15.5, about 16.2, about 17.5, about 18.5, about 20.2, about 24.8, about 25.4 and about 29.5. The salt or complex has a melting point of about 138-139°C. It is thus characterized as having four distinctive XRD peaks at about 4.8, about 15.5, about 20.2 and about 24.8. One may further characterize the compound as having three or four of these XRD peaks and having a melting point of about 138-139°C. The mesylate of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 4.8±0.1, 15.5±0.1, 16.2±0.1, 20.2±0.1 and 24.8±0.1 and a melting point of 138-139±2°.

Figure 22 shows that the ethyl sulfonate salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 4.4, about 8.8, about 18.8, about 23.0-23.3 and about 27.3. The salt or complex has a melting point of about 132-133°C. It is thus characterized as having four distinctive XRD peaks at about 4.4, about 8.8, about 18.8 and about 27.3. One may further characterize the compound as having three or four of these XRD peaks and having a melting point of about 132-133°C. The ethyl sulfonate of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 4.4±0.1, 8.8±0.1, 18.8±0.1, 23.0-23.3±0.3 and 27.3±0.1 and a melting point of 132-133±2°.

Figure 23 shows that the β-naphthylene sulfonic acid salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 9.8, about 13.1, about 16.3, about 17.4, about 19.6, about 21.6-22.3, about 23.4, about 24.1-24.5 and about 26.6. The salt or complex has a melting point of about 156-157°C. It is thus characterized as having four distinctive XRD peaks at about 13.1, about 17.4, about 23.4 and about 26.2. One may further characterize the compound as having three or four of these XRD peaks and having a melting point of about 156-157°C. The β-naphthylene sulfonate of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees

2θ at 9.8±0.1, 13.1±0.1, 17.4±0.1, 23.4±0.1 and 26.2±0.1 and a melting point of 156-157±2°.

Figure 24 shows that the α-naphthylene sulfonic acid salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 8.3, about 9.8, about 11.5, about 15.6, about 16.3, about 16.7-17.4, about 19.6, about 21.0, about 22.9, about 23.7, about 25.0 and about 26.1. The salt or complex has a melting point of about 122-128°C. It is thus characterized as having four distinctive XRD peaks at about 9.8, about 15.6, about 19.6 and about 26.1. One may further characterize the compound as having three or four of these XRD peaks and having a melting point of about 122-128°C. The α-naphthylene sulfonate of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 9.8±0.1, 15.6±0.1, 19.6±0.1, 21.0±0.1 and 26.1±0.1 and a melting point of 122-128±2°.

Figure 25 shows that the (*S*)-camphor sulfonic acid salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 5.4, about 6.5, about 13.7, about 15.5, about 16.8-17.2, about 19.6, about 20.4-20.7, about 21.2, about 23.1, about 26.1, about 27.5, about 28.4, about 31.3 and about 32.2. The salt or complex has a melting point of about 160-161°C. It is thus characterized as having four distinctive XRD peaks at about 5.4, about 6.5, about 13.7 and about 16.8-17.2. One may further characterize the compound as having three or four of these XRD peaks and having a melting point of about 160-161°C. The (*S*)-camphor sulfonate of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at 5.4±0.1, 6.5±0.1, 13.7±0.1, 16.8-17.2±0.3 and 19.6±0.1 and a melting point of 160-161±2°.

Figure 26 shows that the succinic acid salt or complex has distinctive XRD peaks in degrees 2θ at any one (or combination) of about 4.7, about 9.5, about 10.6, about 14.9, about 16.3, about 17.4, about 17.9, about 19.9, about 20.8, about 22.1, about 23.9-24.2, about 26.5, about 27.6 and about 28.2. The salt or complex has a melting point of about 122-124°C. It is thus characterized as having four distinctive XRD peaks at about 4.7, about 9.5 about 14.9 and about 17.4. One may further characterize the compound as having three or four of these XRD peaks and having a melting point of about 122-124°C. The succinate of AD is alternatively characterized by an obvious XRD spectrum peak using Cu-Kα radiation, expressed in degrees

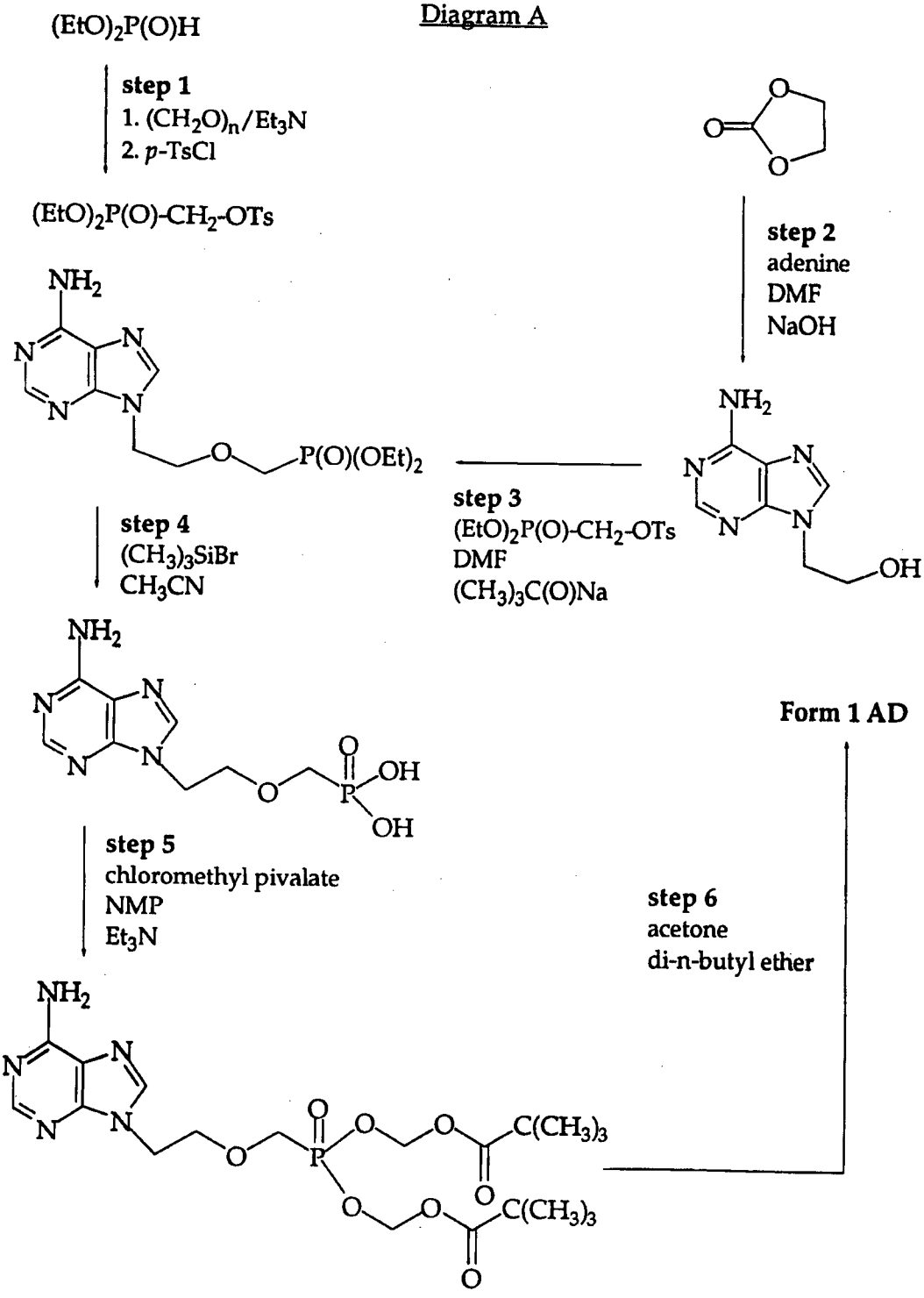
2θ at 9.5±0.1, 14.9±0.1, 16.3±0.1, 17.4±0.1 and 23.9-24.2±0.3 and a melting point of 122-124±2°.

Invention embodiments include compositions comprising a crystalline salt, e.g., a salt as characterized above, of adefovir dipivoxil and a pharmaceutically acceptable excipient(s). Other embodiments include a process to prepare a pharmaceutically acceptable composition by contacting a crystalline salt, e.g., a salt as characterized above, of adefovir dipivoxil and a pharmaceutically acceptable excipient(s). Other embodiments include the product produced by the process of contacting a crystalline salt, e.g., a salt as characterized above, of adefovir dipivoxil and a pharmaceutically acceptable excipient(s).

Methods for AD Synthesis

Diagram A below shows a representative process flow diagram for making AD and Form 1 AD crystals.

Diagram A



One can increase or decrease the scale of the process steps shown in Diagram A and described below if desired.

Methods for Diethyl *p*-toluenesulfonyloxymethylphosphonate Synthesis

In an embodiment, synthesis of diethyl *p*-toluenesulfonyloxymethyl-phosphonate, shown in Diagram A, Step 1, is described as follows. In a reactor having an inert atmosphere, e.g., nitrogen, a mixture of diethylphosphite (0.8 kg), paraformaldehyde (0.22 kg), and triethylamine (0.06 kg) in toluene (2.69 kg) is heated to 87°C (84 to 110°C) for 2 hours with agitation, then heated to reflux and maintained for at reflux for 1 hour, until the reaction is complete. Reaction completion is monitored by TLC (trace or no diethyl phosphite detectable) and confirmed by ^{1}H NMR showing no more than 1% of the diethyl phosphite peak at δ 8.4–8.6 ppm. The solution is cooled to about 1°C (-2 to 4°C) and *p*-toluenesulfonyl chloride (1.0 kg) is added and then triethylamine (0.82 kg) at no more than 10°C is slowly added (over about 3-6 hours in an exothermic reaction). The resulting mixture is warmed to 22°C (19-25°C) and stirred for at least 5 hours (typically about 16-24 hours), until the reaction is complete. Reaction completion is monitored by TLC (trace or no *p*-toluenesulfonyl chloride detectable) and confirmed by ^{1}H NMR (*p*-toluenesulfonyl chloride doublet at δ 7.9 ppm no longer detected). The solids are removed by filtration and rinsed with toluene (0.34 kg). The combined washings and filtrate are washed either twice with water (1.15 kg each), or optionally with a sequence of water (1.15 kg), 5% aqueous sodium carbonate (3.38 kg), and twice with water (1.15 kg each). In the event emulsion occurs, brine may be added to the first organic/water mixture. The organic phase, which is at no more than 50°C, is distilled *in vacuo* (to LOD no more than 10% and water content, by KF (Karl Fischer) titration, no more than 0.5%), affording the title compound as an oil of about 85–95% purity, exclusive of toluene. The oil may become viscous on cooling.

Methods for 9-(2-Hydroxyethyl)adenine Synthesis

In an embodiment, synthesis of 9-(2-hydroxyethyl)adenine, shown in Diagram A, Step 2, is described as follows. In a reactor having an inert atmosphere, e.g., nitrogen, sodium hydroxide (6 g) is added to a slurry of adenine (1.0 kg) and molten ethylene carbonate (0.72 kg, m.p. 37-39°C), in DMF (2.5 kg) and the mixture is heated to 125°C (95°C to reflux) with

agitation until the reaction is complete (about 3-9 hours if the mixture temperature is at 110°C to reflux or about 15–48 hours if at 95 to 110°C). Reaction completion is monitored by HPLC (no more than 0.5% adenine remaining). The mixture is cooled to below 50°C and diluted with toluene (3.2 kg). The resulting slurry is cooled to 3°C (0-6°C) and agitated for at least 2 hours. The slurry is filtered and the filter cake is washed twice with cold (0-5°C) toluene (0.6 kg each). The filter cake is dried *in vacuo* at 35 to 70°C (no more than 2% toluene, by ^{1}H NMR or LOD) and optionally milled, affording the title compound as a white to off-white powdery solid.

Methods for 9-[2-(Diethylphosphonomethoxy)ethyl]adenine Synthesis

This compound is prepared using a composition comprising sodium alkoxide (C_{1-6} alkyl) and 9-(2-hydroxyethyl)adenine. One contacts sodium alkoxide, typically sodium *t*-butoxide or sodium *i*-propoxide, with 9-(2-hydroxyethyl)adenine in a solvent such as DMF, at a temperature of about 20-30° over about 1-4 hours. The synthesis typically gives good results with 1 molar equivalent of 9-(2-hydroxyethyl)adenine and about 1.2-2.2 molar equivalents of sodium alkoxide.

In an embodiment, synthesis of 9-[2-(diethylphosphonomethoxy)-ethyl]adenine, shown in Diagram A, Step 3, is described as follows. In a reactor having an inert atmosphere, e.g., nitrogen, a slurry of 9-(2-hydroxyethyl)adenine (1.0 kg) and DMF (4.79 kg) is warmed to about 130° (125-135°) for 30-60 minutes. The reactor contents are rapidly cooled with vigorous agitation to about 25° (20-30°) and sodium *tert*-butoxide (0.939 kg) is added in portions over about 1-3 hours while maintaining vigorous agitation and the contents temperature at about 25° (20-30°). The agitation and temperature is maintained for about 15-45 minutes after all sodium tert-butoxide has been added. Then the reactor contents are cooled to about -10° (-13 to 0°) and a solution of diethyl *p*-toluenesulfonyloxymethyl-phosphonate (2.25 kg on a pure basis) in DMF (1.22 kg) is added over about 5-10 hours. The mixture is kept at about -5° (-10 to 0°) until the reaction is complete, which is typically about 0.5-2 hours after the final portion of diethyl *p*-toluenesulfonyloxymethyl-phosphonate has been added. Reaction completion is monitored by HPLC (not more than 3% 9-(2-hydroxyethyl)adenine remaining). Glacial acetic acid (0.67 kg) is added, with the pot temperature controlled to no more

than 20°. The mixture at about 22° (15-25°) is agitated for about 15-45 minutes. The quenched mixture is concentrated *in vacuo* until distillation stops and the contents are then cooled to below 40°. Dichloromethane (16.0 kg) is added and the contents at 20° (15-25°) are agitated for at least 1 hour. If the DMF content versus total solids (NaOTs (sodium tosylate), NaOAc, Et_2PMEA) is greater than 20% (by ^1H NMR) the mixture is concentrated *in vacuo* until distillation stops, the contents are cooled to below 40°C, dichloromethane (16 kg) is added and the reactor contents at about 20° (15-25°) are agitated for at least 1 hour. Diatomaceous earth (0.5 kg) is added and the contents, which are at about 20° (15-25°), are agitated for at least 1 hour. The solids are removed by filtration and rinsed 3 times with CH_2Cl_2 (about 1 kg each). The filtrate and rinses at no more than 80° are concentrated *in vacuo* until distillation stops, the reactor contents are cooled to below 40°, dichloromethane (5.0 kg) is added to the residue and the contents at about 25° (20-40°) are agitated to dissolve the solids. The resulting solution at no more than 80° is concentrated *in vacuo* until distillation stops. Dichloromethane (7.0 kg) is added and the contents at about 25° (20-40°) are agitated to dissolve the solids. If the DMF content compared to diethyl PMEA is greater than 12%, the mixture at no more than 80° is concentrated *in vacuo*, the contents are cooled to below 40°, dichloromethane (7.0 kg) is added and the contents at about 25° (20-40°) are agitated to dissolve the solids. The mixture is washed with water (0.8 kg) at about 25° (22-30°) by agitating for about 15-45 minutes. The phases are allowed to separate for 4 hours and the phases are then separated. The aqueous phase is back-extracted twice with dichloromethane (1.5 kg per wash) by agitation for about 15-45 minutes with the solution maintained at about 25° (22-30°), followed by allowing the phases to separate for at least 2 hours. The combined organics at no more than 80° are then concentrated *in vacuo* until distillation stops. Toluene (3.0 kg) is added, agitated at about 25° (22-30°) for about 15-45 minutes and the resulting mixture at no more than 80° is azeotroped *in vacuo*. Toluene (3.0 kg) is added and the mixture is heated to about 80° (75-85°), agitated for about 15-45 minutes, cooled to below 30° over about 60-90 minutes and then cooled to about 0° (-3 to 6°). After at least 12 hours at about 0° with slow agitation, the resulting slurry is filtered and the filter cake is rinsed three times with cold (about 0-6°) toluene (about 0.2 kg per rinse). The wet cake is dried *in vacuo* at about 50° (35 to 65°) and the dried

product is milled. Product drying is monitored for water removal (no more than 0.3% water detected by KF titration). The inert atmosphere is maintained throughout step 3.

Methods for PMEA Synthesis

In an embodiment, synthesis of PMEA, shown in Diagram A, Step 4, is described as follows. In a reactor having an inert atmosphere, e.g., nitrogen, a mixture of diethyl PMEA (1.00 kg), acetonitrile (2.00 kg), and bromotrimethylsilane (1.63 kg) is heated to and maintained at reflux for about 1-3 hours with agitation, until the reaction is complete. Reaction completion is monitored by ^{31}P NMR or HPLC (no diethyl PMEA and no more than 2% monoethyl PMEA detected). The solution at ≤ 80°C is distilled *in vacuo* to a semi-solid, which is taken up in water (2.00 kg) and warmed to about 55°C (52-58°C) for about 30-60 minutes with agitation to dissolve all solids. The resulting mixture is cooled to about 22°C (19-25°C), adjusted to pH 3.2 with aqueous sodium hydroxide, the contents are heated to about 75°C (72-78°C) until the consistency thins (about 15-120 minutes), cooled to about 3°C (0-6 °C), and stirred for at least 3 hours (3-6 hours). The slurry is filtered and the filter cake is rinsed with water (1.00 kg). The wet cake is suspended in water (3.75 kg) and the suspension is heated to about 75°C (72-78°C) with vigorous stirring. After stirring for about 2 hours, the slurry is cooled to about 3°C (0-6 °C) and stirred for at least another 2 hours. The slurry is filtered and the filter cake is rinsed sequentially with two portions of water (0.50 kg per rinse) and two portions of acetone (1.00 kg per rinse). The isolated solid is dried *in vacuo* at no more than about 90°C to a low water content (no more than 0.5% water detected by KF titration), to provide PMEA as white crystals. The product is milled to a fine particle size.

Methods for AD Synthesis

An exemplary method to prepare AD comprises suspending 1 molar equivalent of PMEA in a volume of about 5.68-56.8 equivalents of NMP/equivalent PMEA and, after one suspends the PMEA, adding about 2-5 molar equivalents, often about 2.5-3.5, usually about 3 molar equivalents, of triethylamine ("TEA") to the solution using mild to moderate agitation. One then adds about 3-6 molar equivalents, often about 4.5-5.5 molar equivalents, usually about 5 equivalents, of

chloromethyl pivalate to obtain a reaction mixture. We usually prepare the reaction mixture at room temperature. One heats the reaction mixture to maintain a temperature of less than 66°, typically about 28-65°, usually between about 55-65° for about 2-4 hours to conduct the reaction. The time needed to heat the reaction mixture to about 28-65° is not critical and can vary depending on the reaction mixture volume and the capacity of the apparatus used to heat the mixture. Mild or moderate agitation maintains solids in suspension during the reaction and this minimizes extensive splashing of the reactants in the reaction vessel. This method results in a product comprising AD produced by the process of reacting the listed reactants, typically under the given conditions.

In an embodiment, conversion of PMEA to AD, shown in Diagram A, Step 5, is described as follows. In a reactor having an inert atmosphere, e.g., nitrogen, a mixture of 1-methyl-2-pyrrolidinone (3.15 kg), PMEA (1.00 kg), triethylamine (1.11 kg), and chloromethyl pivalate (2.76 kg) is heated to about 60 ± 3°C (no more than 66°C) and stirred using moderate agitation for ≤ 4 hours (1–4 hours) until the reaction is complete, as indicated by ^{31}P NMR or HPLC (no more than 15% mono(POM)PMEA). The mixture is diluted with isopropyl acetate (12.00 kg), cooled to 25 ± 3°C, and agitated for about 30 minutes. The solids are removed by filtration and washed with isopropyl acetate (5.0 kg). The combined organics are washed twice with water (3.70 kg per wash) by moderately agitating the mixture at a mixture temperature of 25 ± 3°C for about 15-45 minutes. The combined aqueous washes are back-extracted twice with isopropyl acetate (4.00 kg per extraction) at a mixture temperature of 25 ± 3°C by agitation for 15-45 minutes. The combined organics at 25 ± 3°C are washed with water (1.80 kg) by agitation for 15-45 minutes and then the organics at about 35 ± 5°C (no more than 40°C) are concentrated *in vacuo* to approximately 40% of the original volume. After a polishing filtration (1 μm filter), and a rinse forward with 1.5 kg of isopropyl acetate, the concentration of the organics *in vacuo* is resumed until a pale oil remains the organics at about 35 ± 5°C (no more than 50°C). The oil typically comprises about 6-45% AD, usually about 30-42%.

Methods for AD Crystallization

AD Crystallization from the organic oil is usually accomplished by (1) using a relatively low volume of NMP in the AD synthesis reaction as compared to the amount of PMEA present as a reactant , i.e., less than about 10 mL NMP per gram PMEA, and/or (2) by minimizing the amount of isopropyl acetate that remains entrained in the organic oil after vacuum distillation by allowing sufficient time for vacuum distillation, i.e., usually at least about 4-6 hours. The aggregate of reaction starting materials, e.g., NMP or PMEA, in the oil can account for about 2-20% of the crystallization solution, but generally less than about 1-2%. When crystals are prepared from organic oil, about 20-45%, often about 30-42%, and usually about 35-42% of AD is present in the oil before adding crystallization solvents.

One optionally crystallizes AD optionally from a supersaturated solution. Nucleation occurs in such supersaturated solutions, and readily leads to crystal formation. Nucleation rates typically increase when the degree of supersaturation and the temperature increases. Supersaturated solutions typically are prepared by changing the temperature (usually decreasing it), solvent evaporation or altering solvent composition, e.g., by adding a miscible nonsolvent or poor solvent. Combinations of these methods also generate supersaturated AD solutions, e.g., using evaporation under reduced pressure to both cool the solution while increasing the solute concentration.

Crystalline AD is prepared by allowing crystal formation in an AD composition, usually from a solution of AD in a crystallization mixture containing at least about 6%, typically at least about 30%, usually at least about 35%, of AD. One would ordinarily conduct crystallizations by preparing an AD solution comprising about 6-45% AD and about 55-94% crystallization solvent. The upper limit of solubility of AD is about 10-41% for most crystallization solvents at room temperature. AD is not freely soluble in some crystallization solvents, e.g., AD solubility in di-n-butyl ether is less than about 0.3 mg/mL, and adding these solvents to an AD solution increases the degree of saturation or supersaturation of the solution. One usually uses organic solutions containing an amount of AD that is near the upper solubility limit in the crystallization solvent(s). The lower amount, about 6%, is the minimum amount of AD needed in a solution to consistently yield crystals. Certain solvents, e.g., methanol or CH_2Cl_2, can contain more than about 50% AD.

The temperature at which crystallization is conducted is not critical and can vary, as the crystallization process usually proceeds spontaneously over a range of temperatures. Crystallization at temperatures above about 35°, especially about 45-50° may result in reduced yield and/or in an increase in impurities associated with the crystals. Crystallizations are generally conducted at temperature ranges of about -5° to about 50°, often about 0-35°, usually about 4-23°. One can optionally use crystallization temperatures below about -5° to increase the crystal yield or to enhance the crystal formation rate, but a low temperature process may result in increased by-products. Thus it is generally more convenient and economic to use solvents either at approximately ambient temperatures (about 15-23°) or at the typical cooling temperatures that most cooling apparatus or methods can easily reach (about 0-4°). When a solution contains relatively low concentrations of AD, i.e., about 10-20%, crystallization at a relatively low temperature, i.e., about 0-15° will often enhance crystal yields.

Heating the solution containing AD and crystallization solvent(s) to a point above room temperature, preferably to about 35°, appears to facilitate crystallization, presumably by increasing the nucleation rate. The time to heat the crystallization mixture to about 35° is not critical and can vary according to the capacity of the apparatus used, generally over a period of about 20-45 minutes. Heating is then discontinued and the temperature is reduced by cooling or by allowing the temperature to fall for about 10-120 minutes. During this time, crystals form and continue to form over a period of at least about 4-36 hours. Crystallization usually begins immediately or shortly after the crystallization mixture has reached 35°. We usually conduct crystallizations by allowing the temperature to fall to about 0-23°C after the solution reaches 35°. Crystallizations conducted with or without mild to moderate agitation, typically with mild agitation, routinely give good results.

Appreciable crystallization usually occurs over a period of about 5 minutes to about 72 hours and about 10-16 hours routinely give good results regardless of the solvents used. The time of crystallization is not critical and can vary, although relatively short crystallization times (about 30-90 minutes) may result in reduced AD recovery. When one adds crystallization solvents to reaction mixtures containing other organic

solvents, e.g., NMP, crystallization usually begins immediately once the temperature has reached about 35°or less and the solution becomes hazy.

Crystallizations are conducted in common laboratory or manufacturing plant apparatus, e.g., round bottom flasks, Erlenmeyer flasks, stainless steel reactors or glass lined reactors. One will usually conduct the crystallizations using standard laboratory scale or commercial scale manufacturing apparatus for mechanical agitation and temperature control.

When using crystallization systems containing two different solvents, one generally adds the most polar solvent to the AD first, followed by addition of the least polar solvent. One optionally removes undissolved components, if any, from the solution after one has added the first crystallization solvent, e.g., by filtration or centrifugation. For example, when one uses acetone and di-n-butyl ether to prepare Form 1 crystals from an organic solution containing AD and components from the AD synthesis reaction, one usually adds acetone first. Similarly, one would add n-butanol before adding di-n-butyl ether or one would add ethyl acetate before di-n-propyl ether. A solution containing the first polar solvent may become hazy due to precipitation of mono(POM) PMEA which may be present. The mono(POM) PMEA can then be removed from the solution by standard physical methods, e.g., filtration or centrifugation, followed by adding the second solvent, e.g., di-n-butyl ether.

Crystallization solvents we use to prepare Form 1 crystals generally contain less than about 0.2% of water. When a significant amount of water is present in the crystallization solvent, i.e., about 1-2%, the crystallization process yields varying amounts of Form 2 crystals, that are also recovered together with Form 1 crystals. The amount of water that is present in a crystallization reaction is optionally reduced by conventional means, including using anhydrous reagents or by drying solvents using molecular sieves or other known drying agents. One optionally reduces the amount of water that might be present in organic solutions containing AD, e.g., from AD synthesis reactions containing by-products and solvents such as the organic oil described above, by using an azeotroping co-solvent such as isopropyl acetate to reduce water prior to adding crystallization solvents.

In an embodiment, crystallization of Form 1 AD, shown in Diagram A, Step 6, is described as follows. The pale oil containing AD described

above is dissolved in acetone (1.0 kg), heated to 35 ± 3°C , and diluted with di-*n*-butyl ether (5.00 kg) in about 4 portions while maintaining a temperature of about 32-38°C and moderate agitation. The clear solution is cooled to about 25-30°C over about 30-60 minutes (no more than 90 minutes), seeded with a small quantity of Form 1 AD crystals (about 5 g), and the contents are then cooled to 22 ± 3°C over about 30-60 minutes (no more than 90 minutes) while maintaining moderate agitation. Moderate agitation of the mixture is continued at 22 ± 3°C for a minimum of about 15 hours. The resulting slurry is filtered and the filter cake is washed with a premixed solution of acetone (0.27 kg) in di-*n*-butyl ether (2.4 kg) (1:9 v/v). The wet solids are optionally further purified by adding premixed acetone (0.57 kg) and di-*n*-butyl ether (4.92 kg), maintaining the temperature of the contents at 22 ± 3°C for about 15-24 hours with agitation. The solids are then filtered, and the filter cake is washed with premixed acetone (0.27 kg) and di-*n*-butyl ether (2.4 kg). The filter cake maintained at ≤ 35°C (about 25-35°C) is dried *in vacuo* for about 1–3 days (LOD no more than 0.5%), affording Form 1 AD as a white to off-white powdery solid. The dried product is milled.

The invention includes methods to prepare Form 2 crystals. Form 2 crystals are conveniently prepared by hydrating Form 1 crystals, although the hydrate can be obtained by crystallizing AD from crystallization solvents containing an amount of water which does not interfere with crystallization, but which provides the requisite water of hydration. The water may be present as ice, liquid water or water vapor. Typically in placed into physical contact with Form 1 crystals under conditions for formation of Form 2 crystals. Form 1 crystals are optionally contacted with water vapor in a gas such as air, carbon dioxide or nitrogen, at a relative humidity of at least about 75% to obtain complete conversion of Form 1 to Form 2 crystals. Form 1 crystals are usually contacted with air at at least about 75% relative humidity for about 1-10 days at about 18-30° or typically at room temperature to obtain complete conversion to Form 2. However, Form 1 crystals are essentially non-hygroscopic at 54% relative humidity in air at room temperature, with no increase in water content after 13 days exposure.

The process of hydrating Form 1 to Form 2 crystals generates compositions comprising a mixture of Form 1 and Form 2 AD crystals where the proportion of Form 1 AD crystals varies from about 100% to 0%,

with the balance of the AD being Form 2. Thus, the proportion of Form 2 crystals increases from 0% to 100% during the conversion process. These compositions may comprise formulations such as tablets.

As noted above Form 2 crystals are also prepared by conducting AD crystallization in the presence of water, e.g., where about 2-5% water is present in the crystallization solvent(s) otherwise used to make Form 1 AD. Crystallization occurs essentially as described above for Form 1 crystals, e.g., over about 4-36 hours at about 0-23°. Such preparations can contain some Form 1 crystals, but any residual Form 1 crystals optionally are converted to Form 2 crystals by exposure to water vapor as described above, or by adding sufficient additional water to the crystallization solvent.

One usually prepares Form 3 crystals by allowing crystals to grow in an anhydrous methanol solution of AD. One obtains AD in methanol by mixing sufficient noncrystalline or crystalline AD in methanol for about 10-15 minutes at room temperature or as needed to dissolve the solid AD to obtain a solution having at least about 100-150 mg AD/mL methanol. AD solubility in methanol at room temperature is greater than 600 mg/mL. Crystallization then proceeds for about 4 to about 48 hours at a temperature of about -5° to about 25°, usually at about 0-23°.

Crystals obtained using isopropyl acetate as the sole crystallization solvent typically are primarily rods which can be relatively long, i.e., measuring up to about 500 µm in length, with a few needles also present. Figure 8 shows rod-shaped crystals about 20-500 µm in length obtained by crystallization in isopropyl acetate at temperatures above about 15°.

Crystallization from supersaturated and from saturated or some unsaturated AD solutions is optionally facilitated or enhanced by adding seed crystals of AD to the solution, but seed crystals are not mandatory. For example, Form 1 AD is obtained by adding a small amount of crystalline Form 1 AD to an organic solution as described above, e.g., organic oil to which crystallization solvent has been added, but without heating to 35°. The seeded crystals facilitate formation of Form 1 crystals. Form 2 and Form 3 crystals can similarly be obtained by seeding suitable solutions with the respective crystal form, e.g., an organic solution containing ethyl acetate and about 2% water for Form 2 crystals or a saturated solution of AD in anhydrous methanol for Form 3 crystals. The amount of crystals used for seeding are optionally varied to obtain optimal

results. Generally about 0.1-01.0 g of crystals per L of AD recrystallization solution is sufficient.

One can optionally recrystallize crystalline AD as desired, e.g., to increase the purity of the crystals.

For example, one recrystallizes Form 1 AD by essentially the same methods used to prepare Form 1 crystals described above. For example, recrystallization using acetone and di-n-butyl ether is accomplished by dissolving crystalline AD in acetone, about 0.2-0.4 g/mL, at about 20-35°, followed by optionally removing undissolved components, e.g., by filtering or centrifuging the solution, which is usually hazy. An undissolved component is typically mono(POM) PMEA. One then warms the solution to about 35-40° and adds about 5.2-6.2 mL (usually about 5.7 mL) of warmed (about 35-40°) di-n-butyl ether per 0.2-0.4 g of crystals that were initially used in the recrystallization. The recrystallization mixture is then allowed to cool to room temperature over about 4-4.5 hours. The recrystallization mixture will cool to room temperature more rapidly if relatively small volumes, e.g., about 1-3 L, are used. The time to cool the mixture is not critical and can vary.

Recrystallization generally begins shortly after completion of adding and mixing the di-n-butyl ether and one then allows recrystallization to proceed for about 4-36 hours, usually about 6-24 hours. Additional yield of crystals from recrystallization at room temperature for about 4-36 hours is usually obtained by cooling the recrystallization mixture to about 4-10° and allowing the mixture to stand about 1-6 hours at the reduced temperature. Usually, the amount of AD one uses in a recrystallization will be sufficient to form a saturated or nearly saturated solution, i.e., about 0.4 g/mL using acetone. Dissolution of AD in acetone is complete in about 2-8 minutes using moderate agitation. Material remaining undissolved after this initial mixing period is removed and discarded, followed by adding the second less polar solvent of the solvent pair to the mixture containing the first crystallization solvent.

One optionally recrystallizes Form 1 crystals using a single solvent such as acetone. In this embodiment, one dissolves sufficient crystals in the solvent at room temperature, to afford a saturated or nearly saturated solution followed by removal of undissolved components. One then warms the mixture to 35° and allows it to cool as described for recrystallization using the acetone and di-n-butyl ether solvent pair.

Recrystallization of Form 2 crystals will proceed as described for recrystallizing Form 1 crystals but will use Form 2 crystals dissolved in the recrystallization solvents. The Form 1 crystals that are obtained from recrystallization are optionally converted to Form 2 crystals as described herein for conversion of Form 1 to Form 2 crystals. Recrystallization of Form 2 to Form 1 crystals may also be accomplished. In this case, molecular sieves or other solvent drying means can optionally be used to limit the amount of water that is present after the Form 2 crystals are dissolved in the first solvent and during the recrystallization process. One can also recrystallize Form 2 crystals using solvents containing about 1-2% water to directly obtain Form 2 crystals.

One conducts a Form 3 recrystallization in methanol in the same manner as described herein for preparation of Form 3 crystals. A saturated or nearly saturated methanol solution is used to prepare the crystals, i.e., at least about 0.6 g/mL AD.

One optionally prepares salts from acid addition of certain organic and inorganic acids with the basic center in adenosine of AD. One generally prepares acid salts by standard methods, including dissolving AD free base in an aqueous, aqueous-alcohol or aqueous-organic solution containing the selected acid or counterion of the acid, optionally allowing crystallization and optionally accompanied by evaporating, agitating or cooling the solution. One will usually react the free base in an organic solution containing the acid or counterion, in which case the salt usually separates directly or one can seed the solution with crystals or concentrate the solution to facilitate salt precipitation. Embodiments include solutions comprising AD, a solvent, usually a crystallization solvent, and a sulfonic acid such as a C_{6-16} aryl sulfonic acid, a C_{4-16} heteroaryl sulfonic acid or a C_{1-16} alkyl sulfonic acid. Embodiments also include solutions comprising AD, a solvent, usually a crystallization solvent, and a carboxylic acid, such as a tricarboxylic acid, a dicarboxylic acid or a monocarboxylic acid, any of which carboxylic acids comprise about 1-12 carbon atoms.

Pharmaceutical Formulations and Routes of Administration

Invention compositions that comprise crystalline AD, typically Form 1, (hereafter referred to as the active ingredients), are administered by any route appropriate to the condition to be treated, suitable routes including oral, rectal, nasal, topical (including ocular, buccal and

sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural). Generally, the invention compositions are administered orally, but compositions containing crystalline AD can be administered by any of the other routes noted above.

While it is possible for AD to be administered as a pure compound it is preferable to present it as a pharmaceutical formulation. The formulations of the present invention comprise AD, together with one or more pharmaceutically acceptable excipients or carriers ("acceptable excipients") and optionally other therapeutic ingredients. The excipient(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the patient.

The formulations include those suitable for topical or systemic administration, including oral, rectal, nasal, buccal, sublingual, vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations are in unit dosage form and are prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier or excipient which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with either liquid carriers or finely divided solid carriers or both, and then, if necessary, drying or shaping the product.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as sachets, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

Invention formulations include compositions comprising AD and an acceptable excipient. Such excipients include binders, diluents, disintegrants, preservatives, dispersants, glidants (antiadherents) and lubricants. Such compositions optionally comprise unit dosages, including tablets and capsules. Such compositions optionally comprise tablets containing about 5-250 mg AD, usually about 5-150 mg, including tablets comprising about 60 mg or 120 mg per tablet. Such tablets

optionally comprise about 1-10% binder, about 0.5-10% disintegrant, about 50-60% diluent or about 0.25-5% lubricant. Such compositions also comprise wet granules containing liquid, e.g., water, AD and one or more acceptable excipients selected from the group consisting of binders, diluents, dispersants and disintegrants.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients or excipients. Tablets will typically comprise about 5-250 mg of crystalline AD per tablet, usually about 30-120 mg and usually predominantly Form 1 AD, e.g., about 60 mg per tablet or about 120 mg per tablet of Form 1 AD, where only limited amounts, usually less than about 20%, of Form 2 crystals, other crystal types or noncrystalline AD are present. Compressed tablets may be prepared by compressing on a suitable machine, AD in a free-flowing form such as a powder or granules, optionally mixed with a binder, disintegrant, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound usually moistened with a liquid diluent. The tablets may optionally be coated and printed, embossed, or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein. Embodiments include a product made by the process of compressing a mixture containing crystalline AD, typically Form 1 or Form 2, and an acceptable excipient, e.g., dried wet granules containing, e.g., lactose, pregelatinized starch, croscarmellose sodium, talc and magnesium stearate.

Formulations containing crystalline AD and an excipient(s) may also contain L-carnitine or salts of L-carnitine, e.g., L-carnitine-L-tartrate (2:1). Release of pivalic acid from the pivaloyloxymethyl moiety of AD *in vivo* appears to lower the levels of L-carnitine in patients. Tablets containing L-carnitine-L-tartrate and AD may decrease the effect of pivalic acid on L-carnitine depletion in patients taking AD. The amount of L-carnitine to be included will be apparent to the clinician in view of the extent of depletion in patients.

Typical formulation ingredients for tablets or related dosage forms include one or more binders, diluents, disintegrants or lubricants. These excipients increase formulation stability, facilitate tablet compression during manufacture or formulation disintegration after ingestion. The tablets are typically made by wet granulation of one or more excipients

with AD in a mixture, followed by wet milling the granules and drying to a loss on drying of about 3% or less. A binder such as pregelatinized starch or povidone, which enhances processing, is optionally present at a level of about 1-10%. A disintegrant such as microcrystalline cellulose or a cross-linked cellulose such as coscarmellose sodium is optionally present at a level of about 0.5-5% to facilitate tablet dissolution. A diluent such as a monosaccharide or disaccharide is optionally present at a level of about 40-60% to mask the physical properties of AD or to facilitate tablet dissolution. A lubricant such as magnesium stearate, talc or silicon dioxide is optionally present at a level of about 0.25-10% to facilitate tablet ejection during manufacture. The tablets may optionally contain scavengers such as lysine or gelatin to trap formaldehyde that may be released on storage of AD. Excipients have been described, e.g., Monograph for "Pregelatinized Starch", Handbook of Pharmaceutical Excipients, Second Edition, American Pharmaceutical Association, 1994, pp: 491-493; Monograph for "Croscarmellose Sodium", Handbook of Pharmaceutical Excipients, Second Edition, American Pharmaceutical Association, 1994, pp: 141-142; Monograph for "Lactose Monohydrate", Handbook of Pharmaceutical Excipients, Second Edition, American Pharmaceutical Association, 1994, pp: 252-261; Monograph for "Talc", Handbook of Pharmaceutical Excipients, Second Edition, American Pharmaceutical Association, 1994, pp: 519-521; Monograph for "Magnesium Stearate", Handbook of Pharmaceutical Excipients, Second Edition, American Pharmaceutical Association, 1994, pp: 280-282.

Typical containers for storage of Form 1 AD formulations will limit the amount of water that is present in the container. Typically unit formulations or dosages are packaged with a desiccant such as silica gel or activated carbon, or both. The containers are typically induction sealed. Silica gel packaging alone is a sufficient desiccant for storage of tablets containing AD at ambient temperature. AD contains two pivaloyloxymethyl moieties per molecule. Silica gel is thus suitable as a single desiccant for compounds such as therapeutic agents that contain one or more pivaloyloxymethyl moieties. Water permeation characteristics of containers have been described, e.g., Containers--Permeation, Chapter, USP 23, United States Pharmacopeial Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD 20852, pp: 1787 (1995).

For infections of the eye or other external tissues, e.g. mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.01 to 10% w/w (including active ingredient(s) in a range between 0.1% and 5% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc), preferably 0.2 to 3% w/w and most preferably 0.5 to 2% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier (otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the emulsifying wax, and the wax together with the oil and fat make up the emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the present invention include Tween® 60, Span® 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. Thus the cream should preferably be a non-greasy, non-staining and washable product with

suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils can be used.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is suitably present in such formulations in a concentration of 0.01 to 20%, in some embodiments 0.1 to 10%, and in others about 1.0% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for nasal or inhalational administration, wherein the carrier is a solid, include a powder having a particle size for example in the range 1 to 500 microns (including particle sizes in a range between 20 and 500 microns in increments of 5 microns such as 30 microns, 35 microns, etc). Suitable formulations wherein the carrier is a liquid, for administration as for example a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient. Formulations suitable for aerosol administration may be prepared according to conventional methods and may be delivered with other therapeutic agents. Inhalational therapy is readily administered by metered dose inhalers.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations

containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration are sterile and include aqueous and non-aqueous injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials with elastomeric stoppers, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as recited above, or an appropriate fraction thereof, of an active ingredient.

In addition to the ingredients particularly mentioned above the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

The present invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefor.

Veterinary carriers are materials useful for the purpose of administering the composition to cats, dogs, horses, rabbits and other animals and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered orally, parenterally or by any other desired route.

Compounds of the invention can be used to provide controlled release pharmaceutical formulations containing a matrix or absorbent material and as active ingredient one or more compounds of the invention in which the release of the active ingredient can be controlled and regulated to allow less frequent dosing or to improve the pharmacokinetic or toxicity profile of the compound. Controlled release formulations adapted for oral administration in which discrete units

comprising one or more compounds of the invention can be prepared according to conventional methods.

All references cited herein are expressly incorporated by reference with specificity.

Examples

The following examples further exemplify and do not to limit the invention.

Example 1. Preparation of Form 1 crystals. To a 500 mL single-neck round bottom flask equipped with a magnetic stirring bar was added PMEA (27.3 g, 100 mmol). To this was added, under nitrogen, N-methylpyrrolidinone (109.3 mL) and triethylamine (50.6 g, 69.8 mL, 500 mmol), and the resulting suspension stirred vigorously. Chloromethyl pivalate (75.2 g, 72.0 mL, 500 mmol) was added and the stirring suspension was placed in a 45° oil bath for 18.5 hours. The resulting thick, light yellow suspension was diluted with isopropyl acetate (1.0 L) and stirred for 1 hour. The solid was removed by filtration (a Kimax glass funnel with a "C" glass frit) and washed with more isopropyl acetate (250 mL). The wash was combined with the filtrate and this organic phase extracted with water (200 mL x 2). The aqueous extracts were combined and back-extracted with isopropyl acetate (250 mL x 2). All organic phases were combined, and measured 1975 mL. Isopropyl acetate was added to bring the total volume of the organic phase up to 2.0 L. For the purpose of an internal control on this experiment, the organic phase was divided into two equal, 1.0 L portions. One portion was worked-up using a brine wash and sodium sulfate treatment while the other portion was processed without these steps (see below).

The 1.0 L organic phase sample for this new procedure was concentrated to an oil directly using a standard (Büchi) rotary evaporator employing a bath temperature of 45° and a vacuum of 50-70 mm throughout the procedure. The weight of the oil was 32.4 g, and it appeared perfectly clear, with no visible salts present. The oil was diluted with acetone (25 mL) and again a perfectly clear solution resulted with no visible precipitated salts present. After standing at room temperature for about 3 hours, the solution still remained perfectly clear. This solution was placed in an oil bath set at 45°C and di-n-butyl ether (140 mL) was

added slowly, keeping the internal temperature near 40°C. The flask was then removed from the oil bath and allowed to cool to room temperature and stirred at room temperature for about 16 hours resulting in the precipitation of Form 1 AD. The solid product was collected by filtration (a Kimax glass funnel with a "M" glass frit). The solid was washed with a 10% acetone in 90% di-n-butyl ether solution (v/v) (40 mL) and dried in a vacuum oven for 12 hours (ambient temperature, nitrogen bleed, 28" vacuum). This yielded 12.2 g (48.8% theoretical yield, based on a 50 mmol reaction scale) of a white solid, identified (HPLC) as AD of 99.8% purity versus external standard.

The remaining 1.0 L of organic phase was used as control for the above results, and was worked-up as follows. This organic phase was washed with brine (25 mL), dried over sodium sulfate (25 g, 12 hours drying time), and concentrated as described above. This afforded 27.4 g of an oil, which was crystallized as described above from acetone (25 mL) and butyl ether (135 mL). The solid was collected by filtration and dried as described above, affording 12.3 g (48.9% theoretical yield) of a white solid, identified (HPLC) as AD of 98.7% purity versus external standard.

Example 2. Preparation of Form 1 crystals. 9.7 kg of NMP at room temperature was added to 3 kg of PMEA in a 30 gallon glass-lined steel reactor vessel (Pfaudler, Rochester, NY, model No. P20-30-150-115) and the mixture was moderately agitated after NMP was added. The moderate agitation used was sufficient to maintain solid PMEA in suspension and prevent splashing of reactor contents on the walls. 5.6 kg of TEA was then added, followed by addition of 8.3 kg of chloromethyl pivalate. An additional 2.7 kg of NMP was then added to wash residual materials from the transfer lines used to feed the reactor. The temperature was adjusted to about 48° and the temperature was maintained between 38-48° for 18 hours with moderate agitation. After the reaction was complete, 48 kg of isopropyl acetate at room temperature was added to the reactor and the resulting mixture, under moderate agitation, was maintained for 1 hour at 43-48°, before filtration to remove the solids (Tyvek™ filter, 15.5" diameter, Kavon Filter Products, Wall, NJ, model No. 1058-D). The 30 gallon vessel was washed forward through the filter with 12 kg of additional isopropyl acetate. The filtrate was transferred to a 50 gallon glass-lined steel reactor vessel (Pfaudler, model No. P24-50-150-105) while

maintaining the temperature at 43-48°. The temperature was allowed to drop to ambient during subsequent steps.

The mixture was then washed with 22 kg of water by vigorous agitation for about 1.5-2 minutes. Agitation was discontinued and the phases were allowed to completely separate (about 10 min). The lower aqueous phase (about 26 L) was transferred to the 30 gallon glass-lined steel reactor vessel. Another 22 kg of water was added to the organic phase left in the 50 gallon reactor and the phases were vigorously agitated for about 1.5-2 minutes. Agitation was discontinued and the phases were allowed to completely separate (about 1 hour 40 min). The lower aqueous phase was transferred to the 30 gallon glass-lined steel reactor vessel which now contained both aqueous washes. 24 kg of isopropyl acetate was added to the aqueous washes in the 30 gallon reactor and the phases were vigorously agitated for about 1.5-2 minutes, followed by discontinued agitation for sufficient time to obtain complete phase separation (about 10 min). The upper organic phase was retained and mixed with the organic phase previously retained in the 50 gallon reactor. 24 kg of isopropyl acetate was added to the aqueous washes in the 30 gallon reactor and the phases were vigorously agitated for about 1.5-2 minutes, followed by discontinued agitation for sufficient time to obtain complete phase separation (about 20 min). The upper organic phase was retained and mixed with the organic phase previously retained in the 50 gallon reactor. The combined organic phases were then washed with a brine solution (7 kg water, 3.9 kg NaCl) by vigorous agitation for about 1.5-2 minutes followed by discontinued agitation to allow the phases to completely separate (about 5 min). The brine phase was discarded. 18 kg of sodium sulfate was added to the reactor and the mixture was agitated vigorously for about 1.5-2 minutes and then allowed to stand for 1 hour. The organic phase weighed 98.5 kg at this point.

The reactor contents were then gently agitated and filtered through a bag filter (American Felt and Filter Co, model No. RM C S/S 122). The organic solution containing AD was transferred to a clean 50 gallon reactor and the volatile organics were removed by vacuum distillation at 33°-41°C at a vacuum of 26-30" Hg until 50-55 L of condensate had collected. The organic phase was transferred from the 50 gallon reactor to a clean 30 gallon reactor via vacuum filtration through a cartridge filter (Memtec America, Corp., model No. 910044) containing a cotton spun wound

cartridge and washed forward with 8.6 kg of isopropyl acetate. The solution was held overnight at 5° then concentrated under a vacuum at 26° - 41° for 3 hours to obtain about 7-9 L of oil. 5.4 kg of acetone was added to the oil which yielded a clear solution. The solution was then agitated and warmed to 43°C and 27 kg of room temperature di-n-butyl ether was added over a period of about 4 minutes followed by warming to return the temperature to 43°C. An additional 15 kg of di-n-butyl ether was added over about 4 minutes and the temperature was returned to 43°-44°C at which time the temperature was allowed to drop to 20°C over about 7 hours 15 minutes. During this time AD crystals formed in the reactor. The crystals were recovered by filteration (Nutche filter) and dried. 2.40 kg of AD was obtained (45.1%).

Example 3. Preparation of Form 1 crystals. A 3 neck, 12 L, round bottom flask was charged with 546.3 g PMEA (2 mole), followed by 2.18 L of NMP at room temperature. Slow mechanical agitation was initiated (sufficient to keep solid PMEA suspended but without splashing flask contents) to suspend the PMEA and 1.39 L of TEA was then charged to the flask, followed by addition of 1.44 L of pivaloyloxymethyl chloride. The flask was then purged with nitrogen and the reaction was heated to 60°C over 30-45 minutes. Gentle agitation was maintained for 2-2.5 hours with the reaction at 60°. Completion of the reaction was determined by HPLC. The reaction was terminated by charging the flask with 7.48 L of cold (0-3°) isopropyl acetate when the yield of AD reached 65-68% by area normalization. The agitation was increased to moderate agitation (moderate vortex but no splashing of contents) and the mixture remained at room temperature for 30 minutes under moderate agitation while solids (e.g., TEA•HCl, mono(POM) PMEA) precipitated from the solution.

The reaction mixture was then filtered using a glass-sinter funnel (40-60 μm) and the filter cake was washed with 2.51 L of isopropyl acetate at room temperature.

The filtrate was then extracted twice with 2.0 L of potable water at room temperature. The combined aqueous phases were back extracted twice with 2.51 L of isopropyl acetate (room temperature). All organic phases were combined and extracted once with 985 mL of potable water.

The organic phase was isolated and concentrated in vacuo for about 1-2 hours at a temperature of 35-39° at a vacuum of about 30 mm Hg to obtain 1.24 kg of yellow oil.

The oil was transferred to a 3 neck, 12 L flask and cooled to room temperature over about 30 minutes. The flask was charged with 628 mL of room temperature acetone and then with 3.14 L of di-n-butyl ether. Slow agitation was initiated and the solution was heated to 35° over about 5-20 minutes. When the temperature reached 35°, heating was discontinued and no further temperature increase occurred. The solution was cooled to below 30° (20-29°) over about 30 minutes. During the cooling period Form 1 crystals formed in the crystallization mixture while slow agitation was maintained, followed by continued slow agitation for 14-20 hours at room temperature. The crystals were then filtered (Tyvek™ filter) and the filter cake was washed with 2 L of a 10% acetone, 90% di-n-butyl ether (v/v) solution. The cake was dried at room temperature in a drying oven with a nitrogen bleed until a constant weight was achieved (about 2 days).

The yield of Form 1 AD obtained was 50-54% of the theoretical yield from PMEA and the purity was 97-98.5% by HPLC by area of normalization.

Example 4. Preparation of Form 1 crystals. A 3 neck, 3 L, round bottom flask was charged with 273.14 g PMEA (1 mole), followed by 1.09 L of NMP at room temperature. Slow mechanical agitation was initiated (sufficient to keep solid PMEA suspended but without splashing flask contents) to suspend the PMEA and 0.418 L of TEA (3 equivalents) was then charged to the flask, followed by addition of 0.72 L of pivaloyloxymethyl chloride (5 equivalents). The flask was then purged with nitrogen and the reaction was heated to 60°C over 30-45 minutes. Gentle agitation was maintained for 2-2.5 hours with the reaction at 60°. Completion of the reaction was determined by HPLC. The reaction was terminated by charging the flask with 3.74 L of cold (0-3°) isopropyl acetate when the yield of AD reached 68-70% by area normalization. The agitation was increased to moderate agitation (moderate vortex but no splashing of contents) and the mixture was allowed to stand at room temperature for 30 minutes with the moderate agitation while solids (e.g., TEA•HCl, mono(POM)PMEA) precipitated from the solution. The reaction mixture

was filtered using a glass-sinter funnel (40-60 μm) and the filter cake was washed with 1.26 L of isopropyl acetate (room temperature). The filtrate was then extracted twice with 1.01 L of potable water at room temperature for each extraction. The combined aqueous phases were back extracted twice with 1.26 L of isopropyl acetate (room temperature). All organic phases were combined and extracted once with 492 mL of potable water. The organic phase was isolated and concentrated in vacuo for about 1-2 hours at a temperature of 35-39° at a vacuum of about 30 mm Hg to obtain 0.6 kg of yellow oil. The oil was transferred to a 3 neck, 3 L flask and cooled to room temperature by allowing the temperature to fall over about 30 minutes. Then the flask was charged with 314 mL of acetone (room temperature) and then charged with 1.57 L of di-n-butyl ether. Slow agitation was initiated and the solution was heated to 35° over about 5-20 minutes. When the temperature reached 35°, heating was discontinued and no further temperature increase occurred. The solution was cooled to below 30° (20-29°) over about 30 minutes. During the cooling period Form 1 crystals formed in the crystallization mixture while slow agitation was maintained. An additional volume of 1.15 L of room temperature di-n-butyl ether was added to the crystallization mixture. Moderate agitation was continued at room temperature for about 16 hours. The crystals were then filtered (Tyvek™ filter) and the cake was washed with 1 L of a 10% acetone, 90% di-n-butyl ether (v/v) solution and this solution was then removed by filtering. The cake was dried at room temperature in a drying oven with a nitrogen bleed until a constant weight was achieved (about 2 days).

The yield of Form 1 AD obtained was 55-58% of the theoretical yield from PMEA and the purity was 99-100% by HPLC by area of normalization.

Example 5. Preparation of AD crystals using isopropyl acetate as the crystallization solvent. 43.7 mL of NMP at room temperature was added to PMEA (10.93 g) under nitrogen in a 500 mL 3 neck flask fitted with a stirring apparatus. The mixture was stirred to suspend the PMEA. TEA (27.9 mL) at room temperature was then added, followed by addition of pivaloyloxymethyl chloride (28.9 mL) at room temperature. The temperature was increased to 45° and the suspension was stirred for 12 hours at 45°. The resulting thick, yellow suspension was diluted with isopropyl acetate (150 mL) at room temperature and stirred vigorously for 75 minutes at room temperature. The solids were removed by filtration with a "C" sintered glass frit and the solids were washed with 50 mL isopropyl acetate at room temperature. The filtrates were combined and washed twice with deionized water using 40 mL per wash. The combined water washes were back-extracted twice with 40 mL isopropyl acetate per extraction. All organic phases were combined, washed once with 20 mL deionized water and the aqueous and organic phases were allowed to separate and remain in contact for 2 hours at 17°. During this time long rod-like crystals were observed to form at the aqueous-organic interface. The crystals were collected by filtration using an "M" glass sintered frit and dried, affording 512 mg of long rod-shaped crystals.

Example 6. Analysis of AD by HPLC. Crystalline Form 1 AD was analyzed by HPLC to assess purity, to isolate or identify by-products and to exemplify the use of by-products as reference standards for AD. Levels of compounds present were analyzed by the area normalization method. HPLC analyses were performed within 12 hours of standard or sample preparation.

A liquid chromatograph equipped with a fixed volume sample injector, variable wavelength absorbance detector and an electronic integrator was used with a column (Alltech Mixed Mode Anion Exchange™ C8, 7µm, 100Å pore size, 250 mm x 4.6 mm (i.d.), Alltech, Deerfield, IL) and guard column (20 mm x 4.6 mm (i.d.), dry packed with Pellicular C8 particles, Alltech, Deerfield, IL). Chromatographic quality water was used. Chemicals used were chromatographic grade acetonitrile (Burdick & Jackson, Muskegon, MI), anhydrous analytical grade potassium phosphate monobasic (KH_2PO_4, Mallinckrodt, Paris, KY), anhydrous analytical grade potassium phosphate dibasic (K_2HPO_4, Mallinckrodt, Paris,

KY) and A.C.S. reagent grade phosphoric acid (Mallinckrodt, Paris, KY). Aqueous potassium phosphate solutions were filtered (0.45 μm Nylon 66 membrane filter, Rainin, Woburn, MA) and degassed prior to use. Equivalents of these components and compounds can also be used. Equivalent apparatus and/or reagents can also be used to obtain similar results.

Mobile phase A, which consisted of potassium phosphate buffer, pH 6.0:acetonitrile 70:30 v/v, was prepared by mixing 1400 mL of 200 mM potassium phosphate buffer, pH 6.0 with 600 mL acetonitrile. Mobile phase B, which consisted of potassium phosphate buffer, pH 6.0:acetonitrile 50:50 v/v, was prepared by mixing 1000 mL of 200 mM potassium phosphate buffer, pH 6.0 with 1000 mL acetonitrile.

Prior to sample analysis, the HPLC column was equilibrated with mobile phase A at 1.2 mL per minute for 1 hour at room temperature. A 5 μL sample of AD (about 1 mg/mL solution) containing by-products was analyzed in a 25 minute run at room temperature and at a flow rate of 1.2 mL per minute using 100% mobile phase A for 1 minute, followed by a 19-minute linear gradient to 100% mobile phase B. The column was then held at 100% mobile phase B for 5 minutes.

The sample containing AD was prepared by accurately weighing about 25 mg of an AD sample or preparation and dissolving the AD in a final volume of 25.0 mL of sample solvent. Sample solvent was prepared by mixing 200 mL of potassium phosphate buffer (3.40 g of potassium phosphate monobasic per 1 L water, adjusted to pH 3.0 with phosphoric acid) with 800 mL of acetonitrile and equilibrating to room temperature. Compounds are identified on the basis of their elution times and/or their retention times. AD usually elutes from such a gradient at about 9.8 minutes, mono(POM) PMEA elutes at about 6.7 minutes and PMEA elutes at about 3.5 minutes.

Example 7. Physical characterization of Form 1 crystals. Form 1 crystals were analyzed by XRD by loading about 100 to 150 mg of crystals into an aluminum holder which was mounted into a diffractometer (GE model XRD-5 automated with a Nicolet automation package). Form 1 crystals were scanned between 4 and 35 degrees 2θ at a scan speed of 0.05° per 1.5 seconds by exposure to an X-ray generator operated at 40 KV and at -20 mA using a standard focus copper X-ray tube (Varican CA-8) with a

graphite monochromator (ES Industries) and a scintillation detector. The weighted mean value of X-ray wavelengths used for the calculations was CuKα 1.541838 Å. Form 1 AD crystals exhibit characteristic XRD peaks expressed in degrees 2θ at about 6.9, 11.8, 12.7, 15.7, 17.2, 20.7, 21.5, 22.5 and 23.3. An exemplary XRD pattern for Form 1 is shown in figure 1.

Form 1 crystals were also analyzed by differential scanning calorimetry and exhibited a thermogram as shown in figure 2 with a characteristic endothermic transition at approximately 102.0°, having an onset at approximately 99.8°. The thermogram was obtained using a scan rate of 10° per minute under a nitrogen atmosphere. The sample was not sealed in a container in the DSC apparatus and instead was analyzed at ambient pressure in the DSC apparatus. The calorimetry scan was obtained using a differential scanning calorimeter (TA Instruments, model DSC 2910 with a model 2200 controller). Approximately 5 mg of AD was used to obtain the thermogram. Differential scanning calorimetry has been described (see, e.g., U.S. Pharmacopoeia, vol. 23, 1995, method 891, U.S.P. Pharmacopeial Convention, Inc, Rockville, MD).

The melting point of Form 1 crystals was determined by conventional melting point analysis. The analysis was conducted using a Mettler model FP 90 Central Processor equipped with a model FP 81 measuring cell according to the manufacturer's instructions. The sample was equilibrated for 30 seconds at an initial temperature of 63° followed by a temperature increase of 1.0°/minute. Form 1 crystals melted over a range of 99.1° to 100.7°.

The infrared absorption (IR) spectrum of Form 1 crystals was obtained using a Perkin-Elmer Model 1650 FT-IR spectrophotometer according to the manufacturer's instructions. A translucent pellet containing about 10% by weight (5 mg) of Form 1 crystals and about 90% by weight (50 mg) of dried (60°C under vacuum overnight) potassium bromide (Aldrich, IR grade) was prepared by grinding the two powders together to obtain a fine powder. IR spectroscopy has been described (see, e.g., U.S. Pharmacopoeia, vol. 23, 1995 method 197, U.S.P. Pharmacopeial Convention, Inc, Rockville, MD; Morrison, R.T. et al, *Organic Chemistry*, 3rd ed., Allyn and Bacon, Inc., Boston, p 405-412, 1973). The spectrophotometer sample chamber was purged for at least 5 minutes with high purity nitrogen gas at about 6 p.s.i. to reduce carbon dioxide absorbance interference to ≤ 3% in a background scan prior to scanning

with the sample. Form 1 crystals exhibited an infrared absorption spectrum in potassium bromide with characteristic bands expressed in reciprocal centimeters at approximately 3325-3275, 3050, 2800-1750, 1700, 1625, 1575-1525, 1200-1150, 1075 and 875. An exemplary infrared absorption spectrum for Form 1 is shown in figure 3.

Form 1 crystals usually appear as an opaque white or off-white powder when dry. The crystals obtained from a given preparation are usually polydisperse and have a range of crystal habits including tablets, needles, plates and aggregates of tablets, needles and plates. Form 1 crystals typically range in size from about 1 μm to about 300 μm in length and are irregular tablet shaped with fractured or angular edges. Form 1 crystals obtained at low temperature, usually about 2-4°, from preparations using acetone and di-n-butyl ether as crystallization solvents are typically aggregates that comprise mostly needles and some plates. Figures 4-7 are photographs showing Form 1 crystals obtained from crystallization in acetone and di-n-butyl ether at temperatures above 15°. These photographs show tablet or plate-shaped and needle-shaped crystals that range in size from about 10 μm to about 250 μm in length. Figure 9 shows Form 1 crystals obtained from crystallization in acetone and di-n-butyl ether at temperatures between about 2-4°. The photograph shows plate-shaped and needle-shaped crystal aggregates that range in diameter from about 30 μm to about 120 μm. The individual crystals in the aggregates have angular edges.

Form 1 crystals were found to have a water content of less than 1% by Karl Fischer titration. We performed the water content analysis essentially as described (see, e.g., U.S. Pharmacopoeia, 1990, pages 1619-1621, U.S. Pharmacopoeial Convention).

Example 8. Preparation of Form 2 crystals. Form 1 crystals were converted to the Form 2 dihydrate by incubation in air at 94% relative humidity for 3 days at room temperature. During conversion of Form 1 to Form 2, a mixture of Form 1 and Form 2 crystals was obtained which increased over time from no detectable Form 2 in the initial Form 1 preparation. At the end of three days incubation, the final Form 2 preparation contained no detectable Form 1 crystals.

Example 9. Physical characterization of Form 2 crystals. Form 2 crystals were analyzed by XRD by the same method that was used for Form 1. Form 2 AD crystals had characteristic XRD peaks expressed in degrees 2θ at approximately 8.7-8.9, 9.6, 16.3, 18.3, 18.9, 19.7, 21.0, 21.4, 22.0, 24.3, 27.9, 30.8 and 32.8. An exemplary XRD pattern for Form 2 is shown in figure 11.

Form 2 crystals were also analyzed by differential scanning calorimetry by the same method used to analyze Form 1 crystals and exhibited a thermogram as shown in figure 12 with a characteristic endothermic transition at about 72.7°, having an onset at about 69.5°.

The melting point of Form 2 crystals was determined by conventional melting point analysis. The analysis was conducted using the same method as described for Form 1. Form 2 crystals melted over a range of 70.9° to 71.8°.

The IR spectrum of Form 2 crystals was obtained using the same method as that described for Form 1 crystals. The IR spectrum is shown in figure 13 and exhibits the following characteristic absorption bands, expressed in reciprocal centimeters at approximately 3300-3350, 3050, 2800-1750, 1700, 1625, 1575-1525, 1200-1150, 1075 and 875. These bands are similar to those associated with Form 1 crystals, but Form 2 shows an additional O-H bond stretch band associated with water at approximately 3500.

Form 2 crystals were found to have a water content of 6.7% by Karl Fischer titration. We performed the water content analysis essentially as described (see, e.g., U.S. Pharmacopoeia, 1990, pages 1619-1621, U.S. Pharmacopoeial Convention).

Example 10. Preparation of Form 3 crystals. Sufficient Form 1 crystals (about 250 mg) were dissolved in anhydrous methanol (about 2 mL) at room temperature to obtain a solution. The solution was obtained by mixing for about 10-15 minutes until the crystals were dissolved. The solution was allowed to stand without mixing for 10-48 hours and Form 3 crystals were then recovered from the solution.

Example 11. Physical characterization of Form 3 crystals. Form 3 crystals were analyzed by XRD by the same method that was used for Form 1. Crystalline Form 3 AD crystals were characterized as essentially having XRD peaks expressed in degrees 2θ at approximately 8.1, 8.7, 14.1, 16.5, 17.0,

19.4, 21.1, 22.6, 23.4, 24.2, 25.4 and 30.9. An exemplary XRD pattern for Form 3 is shown in figure 14.

Example 12. Synthesis and purification of PMEA. PMEA used for AD synthesis and crystallization was purified to increase product yield and purity. A 12 L 3 neck round bottom flask containing 548.8 g of diethyl PMEA was charged with 637.5 mL of acetonitrile at room temperature. The diethyl PMEA was dissolved by moderate agitation (moderate vortex with little or no splashing of the flask contents). The flask was purged with nitrogen and 803.8 g of bromotrimethylsilane was slowly added (about 2-5 minutes). The flask contents were heated to reflux (65°) for 2 hours until ≤ 1% monoethyl PMEA remained by HPLC area of normalization analysis. Volatiles were distilled off at ≤ 80° and ~ 20 mm Hg. The flask was then charged with 1500 mL of room temperature water. The pH of the solution in the flask was then adjusted to 3.2 with 25% w/v NaOH. The flask contents were then heated to 75° for 2 hours and the contents were then cooled to 3-4° over 15-20 minutes and held at 3-4° for 3-3.5 hours. The flask contents were then filtered with a glass frit filter and the cake was washed with 150 mL of cold (3-4°) water. The washed cake was transferred to a clean 12 L 3 neck flask and the flask was charged with 2025 mL of water and the flask was heated to 75° and held at that temperature for 2 hours. Heating was discontinued and the flask was cooled and held at 3-4° for 3-3.5 hours. The flask contents were then filtered with a glass frit filter and the cake was washed with 150 mL of cold (3-4°) water and then washed with 1050 mL of room temperature acetone. The cake was dried to constant weight by heating at 65-70° at ~ 20 mm Hg. PMEA yield was 85.4% with 99% purity by either area of normalization or external standard HPLC analysis.

Example 13. Single crystal X-ray crystallography of Form 1.

About 200 mg of lot 840-D-1 AD drug substance was dissolved in 200 mg of acetone. The solution was heated to about 60°C. Di-*n*-butyl ether, at ambient temperature, was slowly added to the solution at 60°C until the appearance of the first trace of precipitate. The mixture was then shaken and re-heated to about 60°C to form a clear and homogeneous solution. The solution was allowed to cool to ambient temperature overnight and was held at ambient temperature for about 2 days. The resulting crystals

were highly polydisperse with some having long dimensions of up to 1 mm. The supernatant was decanted and the remaining crystals were washed with a total of about 1 mL of di-*n*-butyl ether over four cycles to remove the residual supernatant. A crystal having approximate dimensions of 150 x 200 x 320 μm was subjected to analysis using single crystal X-ray diffraction.

All measurements were made on a Siemens SMART diffractometer (Siemens Industrial Automation, Inc., Madison, WI) with graphite monochromated Mo-Kα radiation (λ = 0.71069 Å). The crystal was mounted on a glass fiber using Paratone N™ hydrocarbon oil. Data acquisition was carried out at -135±1°C. Frames for an arbitrary hemisphere of reciprocal space were collected using w scans of 0.3° per frame counted for 10 seconds per frame.

5967 integrated reflections, measured out to a maximum 2θ of 51.6°, were averaged to yield 3205 Friedel unique reflections (R_{int} = 0.044). The structure was solved with the non-hydrogen atoms refined anisotropically. The hydrogen atoms were introduced in idealized positions. The final cycle of full matrix least squares refinement, based on 2438 observed reflections having I > 3σ and 306 variable parameters, converged at R = 0.048 (R_w = 0.054).

Cell constants and an orientation matrix obtained from a least squares refinement using the measured positions of 3242 reflections with I > 10σ in the range 3.00 < 2θ < 45.00° corresponded to a C-centered monoclinic cell specified as follows: a = 12.85 Å, b = 24.50 Å, c = 8.28 Å, ß = 100.2°, Z = 4, space group Cc.

The following tables show data obtained from the study. Diagrams of AD are shown in Figures 27 and 28.

286

Fractional atomic coordinates for Form 1 AD.[a]

Atom	x	y	z
P1	1.0808	0.22760(05)	0.6554
O1	0.8826(03)	0.23934(12)	0.6880(04)
O2	1.1005(04)	0.26242(16)	0.5213(05)
O3	1.0440(03)	0.16716(14)	0.6037(05)
O4	1.0034(04)	0.12075(16)	0.3651(05)
O5	0.9271(05)	0.16940(19)	0.1501(06)
O6	1.1768(03)	0.21530(12)	0.7951(04)
O7	1.3179(03)	0.17817(13)	0.6942(04)
O8	1.3518(04)	0.13595(19)	0.9392(06)
N1	0.6976(04)	0.09182(15)	0.7806(05)
N2	0.6997(04)	0.06321(14)	0.3428(05)
N3	0.6929(04)	0.15993(15)	0.3987(05)
N4	0.6929(04)	0.17777(13)	0.6860(05)
N5	0.7041(04)	-0.00364(15)	0.5388(05)
C1	0.6935(05)	0.14417(19)	0.8165(06)
C2	0.7000(04)	0.09175(17)	0.6147(06)
C3	0.7008(04)	0.04924(19)	0.4999(06)
C4	0.6945(05)	0.11621(19)	0.3029(06)
C5	0.6962(04)	0.14452(17)	0.5538(05)
C6	0.6968(05)	0.23782(18)	0.6890(06)
C7	0.8026(04)	0.25795(18)	0.7733(06)
C8	0.9855(05)	0.25344(20)	0.7701(07)
C9	0.9597(06)	0.1557(03)	0.4715(08)

a Numbers in parentheses denote standard deviation in the last significant figures

286A

Fractional atomic coordinates for Form 1 AD.[a] *(con't)*

Atom	x	y	z
C10	0.9798(05)	0.1318(02)	0.2018(07)
C11	1.0283(04)	0.08975(19)	0.1036(06)
C12	1.1460(06)	0.1018(03)	0.1244(10)
C13	1.0105(06)	0.0329(02)	0.1618(08)
C14	0.9783(07)	0.0959(03)	-0.0773(08)
C15	1.2825(05)	0.22414(20)	0.7731(06)
C16	1.3473(05)	0.1340(02)	0.7942(09)
C17	1.3650(05)	0.0841(02)	0.6937(09)
C18	1.4337(07)	0.0440(03)	0.8045(12)
C19	1.4160(05)	0.1000(02)	0.5486(09)
C20	1.2561(06)	0.0599(03)	0.6340(11)
H1	0.6911	0.1572	0.9239
H2	0.6915	0.1239	0.1897
H3	0.7060	-0.0145	0.6494
H4	0.7044	-0.0304	0.4560
H5	0.6836	0.2511	0.5796
H6	0.6439	0.2511	0.7458
H7	0.8166	0.2445	0.8826
H8	0.8025	0.2967	0.7751

a Numbers in parentheses denote standard deviation in the last significant figures

Fractional atomic coordinates for Form 1 AD.[a] *(con't)*

Atom	x	y	z
H9	0.9977	0.2379	0.8768
H10	0.9916	0.2920	0.7786
H11	0.9032	0.1380	0.5107
H12	0.9346	0.1884	0.4165
H13	1.1770	0.0992	0.2371
H14	1.1785	0.0762	0.0630
H15	1.1561	0.1377	0.0861
H16	0.9367	0.0263	0.1513
H17	1.0404	0.0072	0.0974
H18	1.0430	0.0293	0.2736
H19	0.9919	0.1315	-0.1138
H20	1.0079	0.0696	-0.1405
H21	0.9041	0.0903	-0.0902
H22	1.2855	0.2557	0.7074
H23	1.3266	0.2293	0.8768
H24	1.3999	0.0345	0.8938
H25	1.4441	0.0122	0.7442
H26	1.5002	0.0604	0.8454
H27	1.4811	0.1181	0.5869
H28	1.4288	0.0681	0.4897
H29	1.3701	0.1237	0.4784
H30	1.2125	0.0863	0.5708
H31	1.2623	0.0287	0.5684
H32	1.2254	0.0497	0.7257

a Numbers in parentheses denote standard deviation in the last significant figures

Figure 29 shows powder X-ray diffraction patterns for Form 1 AD: (a) Observed and (b) Calculated.

Example 14. Preparation of form 4 crystals. Form 1 AD (10.05 g) was dissolved in isopropanol (50 mL) with warming (about 35°C) and then filtered through a glass frit (M frit, ASTM 10-15 μm). The filtrate was added to a stirred solution of isopropanol (49 mL) at about 35°C containing dissolved fumaric acid (2.33 g) and the mixture was allowed to passively cool to room temperature. Form 4 crystals, AD•fumaric acid (1:1) spontaneously formed in the mixture shortly after the AD solution was added to the isopropanol solution. The crystals were allowed to form for 2 days at room temperature, recovered by filtration and dried *in vacuo* under nitrogen at room temperature.

Example 15. Preparation of form 4 crystals. Form 1 AD (1005.1 g) was dissolved in warm (about 45°C) isopropanol (3.0 L). The warm AD solution was added over about 20 minutes with moderate agitation to a stirred solution of isopropanol (6.0 L) at about 45°C in a 12 L flask containing dissolved fumaric acid (233.0 g). The mixture temperature was maintained at 40-45°C for 10 minutes and warming was discontinued when thick precipitate formed. Several minutes after the all of the AD solution was added, the mixture became hazy and then a few minutes later the precipitate became thick, at which point agitation was discontinued (mixture temperature 42°C). Precipitate was allowed to form for an hour. Slow agitation was started and continued for about 2 hours, followed by immersing the 12 L flask in room temperature water with slow stirring continued overnight to facilitate mixture cooling. The precipitate was recovered by a first filtration (Tyvek™ filter) and a second filtration (M glass frit) and dried *in vacuo* at room temperature under nitrogen.

Example 16. Preparation of crystalline AD salts from organic and inorganic acids. Form 1 AD (500 mg, 1.0 mmol) was dissolved in isopropyl alcohol (5 mL) with warming (< 40°C). Acid (1.0 mmol) dissolved in 2 mL of isopropyl alcohol, or a larger volume as needed to dissolve the acid, was added to the AD solution. The solution was stored in a tightly capped scintillation vial at room temperature. In some cases, precipitated salts were observed shortly after the solution was capped (about 1 minute). For other salts, precipitate began to form at times up to

several months after the solution was capped. Melting points for all 13 salts is shown below. XRD data (degrees 2θ) for nine salts is also shown below. The XRD data shows most of the highest intensity peaks for these salts.

Acid	melting point (° C)	XRD spectrum peaks
hemisulfate	131-134	8.0, 9.5, 12.0, 14.6, 16.4, 17.0, 17.5-17.7*, 18.3, 19.0, 20.2, 22.7, 24.1, 28.2
HBr	196-199 (decomp.)	13.2, 14.3, 15.9, 17.8, 20.7, 21.8, 27.2, 28.1
HCl	204-205 (decomp.)	ND***
HNO_3	135-136 (decomp.)	8.0, 9.7, 14.1, 15.2, 16.7, 17.1, 18.3, 18.9, 19.4, 20.0, 21.2, 22.3, 23.2, 24.9, 27.6, 28.2, 29.4, 32.6
CH_3SO_3H	138-139	4.8, 15.5, 16.2, 17.5, 18.5, 20.2, 24.8, 25.4, 29.5
$C_2H_5SO_3H$	132-133	4.4, 8.8, 18.8, 23.0-23.3*, 27.3
β-naphthylene sulfonic acid	156-157	9.8, 13.1, 16.3, 17.4, 19.6, 21.6-22.3*, 23.4, 24.1-24.5**, 26.6
α-naphthylene sulfonic acid	122-128	8.3, 9.8, 11.5, 15.6, 16.3, 16.7-17.4**, 19.6, 21.0, 22.9, 23.7, 25.0, 26.1
(*S*)-camphor sulfonic acid	160-161	5.4, 6.5, 13.7, 15.5, 16.8-17.2*, 19.6, 20.4-20.7*, 21.2, 23.1, 26.1, 27.5, 28.4, 31.3, 32.2
fumaric acid	144-145	ND
succinic acid	122-124	4.7, 9.5, 10.6, 14.9, 16.3, 17.4, 17.9, 19.9, 20.8, 22.1, 23.9-24.2*, 26.5, 27.6, 28.2
maleic acid	72-75	ND
ascorbic acid	210-212	ND
nicotinic acid	192-193	ND

* present as two peaks or as a peak with shoulder
** 3-4 peaks present in broad peak
*** ND = XRD analysis not done

Example 17. AD Formulation. Form 1 AD was formulated with several excipients in tablets containing 30, 60 or 120 mg AD per tablet as follows.

Component	30 mg Tablet		60 mg Tablet		120 mg Tablet	
	% w/w	mg/tab.	% w/w	mg/tab.	% w/w	mg/tab.
Adefovir dipivoxil	7.5	30.0	15.0	60.0	30.0	120.0
Pregelatinized Starch, NF	5.0	20.0	5.0	20.0	5.0	20.0
Croscarmellose Sodium, NF[1]	6.0	24.0	6.0	24.0	6.0	24.0
Lactose Monohydrate, NF[1]	74.5	298.0	67.0	268.0	52.0	208.0
Purified Water, USP[2]	--		--		--	
Talc, USP	6.0	24.0	6.0	24.0	6.0	24.0
Magnesium Stearate, NF	1.0	4.0	1.0	4.0	1.0	4.0
Total	100.0	400.0	100.0	400.0	100.0	400.0

[1]To be incorporated into the dosage form in two portions (intragranular and extragranular) during the manufacturing process.

[2] The quantity of water added is sufficient to produce a suitable wet granulation. Water was removed to a level of not more than 3% loss on drying (LOD).

Tablets containing Form 1 AD were made by blending croscarmellose sodium, pregelatinized starch and lactose monohydrate in a granulator. Water was added and the contents were mixed in a granulator until a suitable wet granulation formed. The wet granulation was milled, dried in a dryer to a moisture content of not more than 3% loss on drying and the dried granules were passed through a mill. The milled granules were combined with extragranular excipients, lactose monohydrate, croscarmellose sodium and talc, and blended in a blender to obtain a powder blend. Magnesium stearate was added, blended in a blender, and compressed into tablets. The tablets were filled into high density polyethylene or glass bottles along with polyester fiber packing material and optionally with a silica gel desiccant.

Example 18. AD Formulation. Form 1 AD was formulated with several excipients in tablets weighing 100 mg each and containing either 25 or 50 mg AD per tablet as follows. The tablets were prepared by wet granulation in a manner similar to that described above.

	per unit content	
Component	% w/w	%w/w
Form 1 AD	25.0	50.0
Lactose Monohydrate, NF	40.5	26.5
Microcrystalline Cellulose, NF	31.0	20.0
Croscarmellose Sodium, NF	2.0	2.0
Silicon Dioxide, NF	0.5	0.5
Magnesium Stearate, NF	1.0	1.0

CLAIMS

We claim:

1. A composition comprising crystalline adefovir dipivoxil.

2. The composition of claim 1 wherein the crystalline adefovir dipivoxil is Form 1 adefovir dipivoxil.

3. The composition of claim 2 comprising a C-centered monoclinic cell specified substantially as follows: a = 12.85 Å, b = 24.50 Å, c = 8.28 Å, ß = 100.2°, Z = 4, space group Cc.

4. The composition of claim 2 having an X-ray powder diffraction spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at about 6.9.

5. The composition of claim 4 having a DSC endothermic transition at about 102°C.

6. The composition of claim 1 wherein the crystalline adefovir dipivoxil is Form 2 adefovir dipivoxil.

7. The composition of claim 6 having an X-ray powder diffraction spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at about 9.6, about 18.3, about 22.0 and about 32.8.

8. The composition of claim 7 having a DSC endothermic transition at about 73°C.

9. The composition of claim 1 wherein the crystalline adefovir dipivoxil is Form 3 adefovir dipivoxil.

10. The composition of claim 9 having an X-ray powder diffraction spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at about 8.1, about 19.4, about 25.4 and about 30.9.

11. The composition of claim 10 having a DSC endothermic transition at about 85°C.

12. The composition of claim 1 wherein the crystalline adefovir dipivoxil is Form 4 adefovir dipivoxil.

13. The composition of claim 12 having an X-ray powder diffraction spectrum peak using Cu-Kα radiation, expressed in degrees 2θ at about 9.8, about 15.2, about 26.3 and about 31.7.

14. The composition of claim 4 having a DSC endothermic transition at about 148°C.

15. The composition of claim 1 comprising a crystalline salt of adefovir dipivoxil.

16. The crystalline salt of claim 15 wherein the crystalline salt is a salt of an organic acid.

17. The crystalline salt of claim 15 wherein the crystalline salt is a salt of an inorganic acid.

18. The composition of claim 1 wherein the crystalline adefovir dipivoxil is a crystalline salt of adefovir dipivoxil selected from the group consisting of hemisulfate, hydrobromide, hydrochloride, nitrate, mesylate, ethane sulfonate, β-naphthylene sulfonate, α-naphthylene sulfonate, (*S*)-camphor sulfonate, succinic acid, maleic acid, ascorbic acid or nicotinic acid.

19. The composition of claim 1 comprising a pharmaceutically acceptable excipient.

20. A method comprising administering to a subject an antivirally effective amount of the composition of claim 19.

21. A method comprising contacting a crystallization solvent and adefovir dipivoxil.

22. The method of claim 21 wherein the adefovir dipivoxil is in a a solution.

23. The method of claim 22 wherein the crystallization solvent is mixed with the solution to obtain a second solution, which is allowed to form crystals.

24. A method comprising crystallizing adefovir dipivoxil from a solution comprising about 6-45% adefovir dipivoxil and about 55-94% crystallization solvent wherein the crystallization solvent is selected from the group consisting of (1) a mixture between about 1:10 v/v to about 1:3 v/v of acetone:di-n-butyl ether, (2) a mixture between about 1:10 v/v to about 1:3 v/v of ethyl acetate:di-n-propyl ether, (3) a mixture between about 1:10 v/v to about 10:1 v/v of t-butanol:di-n-butyl ether, (4) a mixture between about 1:10 v/v to about 1:3 v/v of methylene chloride:di-n-butyl ether, (5) a mixture between about 1:10 v/v to about 10:1 v/v of diethyl ether:di-n-propyl ether, (6) a mixture between about 1:10 v/v to about 1:3 v/v of tetrahydrofuran:di-n-butyl ether, (7) a mixture between about 1:10 v/v to about 1:3 v/v of ethyl acetate:di-n-butyl ether, (8) a mixture between about 1:10 v/v to about 1:3 v/v of tetrahydropyran:di-n-butyl ether, (9) a mixture between about 1:10 v/v to about 1:3 v/v of ethyl acetate:diethyl ether, (10) t-butyl-methyl ether, (11) diethyl ether, (12) di-n-butyl ether, (13) t-butanol, (14) toluene, (15) isopropyl acetate, (16) ethyl acetate, and (17) a mixture consisting essentially of (A) a first crystallization solvent consisting of a first dialkyl ether of the formula R^1-O-R^2 wherein R^1 is an alkyl group having 1, 2, 3, 4, 5 or 6 carbon atoms, R^2 is an alkyl group having 2, 3, 4, 5 or 6 carbon atoms wherein R^1 and R^2 are the same or different, or both R^1 and R^2 are linked together to form a 5-, 6-, 7-, or 8-membered ring, provided that the dialkyl ether is not methyl-ethyl ether, and (B) a second crystallization solvent selected from the group consisting of (a) a second dialkyl ether of the formula R^1-O-R^2, wherein the second dialkyl ether is different from the first dialkyl ether, (b) toluene, (c) tetrahydrofuran, (d) t-butanol, (e) ethyl acetate, (f) methylene chloride, (g) propyl acetate and (h) isopropanol.

296

25. A method for preparing Form 2 adefovir dipivoxil comprising forming adefovir dipivoxil crystals in the presence of water.

26. The method of claim 25 wherein the Form 2 adefovir dipivoxil is produced by (1) hydrating Form 1 adefovir dipivoxil crystals, and/or (2) crystallizing adefovir dipivoxil in the presence of water.

27. A method comprising contacting adefovir dipivoxil with methanol.

28. A method for preparing Form 4 adefovir dipivoxil comprising forming crystals comprising adefovir dipivoxil in the presence of fumaric acid.

29. A method for preparing adefovir dipivoxil comprising contacting 9-[2-(phosphonomethoxy)ethyl]adenine with chloromethyl pivalate in 1-methyl-2-pyrrolidinone and a trialkylamine and recovering adefovir dipivoxil.

30. The method of claim 29 wherein the trialkylamine is triethylamine.

31. The method of claim 30 comprising contacting 1 molar equivalent of 9-[2-(phosphonomethoxy)ethyl]adenine and about 5.6-56.8 molar equivalents of 1-methyl-2-pyrrolidinone.

32. The method of claim 29 comprising contacting 1 molar equivalent of 9-[2-(phosphonomethoxy)ethyl]adenine and about 2-5 molar equivalents of triethylamine.

33. A method comprising contacting 9-[2-(phosphonomethoxy)-ethyl]adenine containing less than about 2% salt with chloromethyl pivalate.

34. The method of claim 33 wherein the salt is NaBr or KBr.

35. A product produced by the process of compressing a mixture comprising Form 1 adefovir dipivoxil and a pharmaceutically acceptable excipient.

36. The product of claim 35 wherein the compression results in a tablet.

37. A product produced by the process of preparing wet granules from a mixture comprising a liquid, Form 1 adefovir dipivoxil and a pharmaceutically acceptable excipient.

38. The product of claim 37 wherein the liquid is water.

39. The product of claim 37 wherein the process further comprises drying the wet granules.

40. A composition comprising a tablet containing adefovir dipivoxil, 20 mg pregelatinized starch, 24 mg croscarmellose sodium, lactose monohydrate, 24 mg talc and 4 mg magnesium stearate, wherein the adefovir dipivoxil comprises at least about 70% form 1 adefovir dipivoxil.

41. The composition of claim 40 wherein the tablet contains 60 mg adefovir dipivoxil and 268 mg lactose monohydrate.

42. The composition of claim 41 wherein the tablet weighs about 400 mg.

43. The composition of claim 41 wherein the adefovir dipivoxil comprises at least about 80% form 1 adefovir dipivoxil.

44. The composition of claim 40 wherein the tablet contains 120 mg adefovir dipivoxil and 208 mg lactose monohydrate.

45. The composition of claim 44 wherein the tablet weighs about 400 mg.

46. The composition of claim 44 wherein the adefovir dipivoxil comprises at least about 80% form 1 adefovir dipivoxil.

47. A method for preparing 9-[2-(diethylphosphonomethoxy)ethyl]-adenine comprising contacting sodium alkoxide and 9-(2-hydroxyethyl)-adenine.

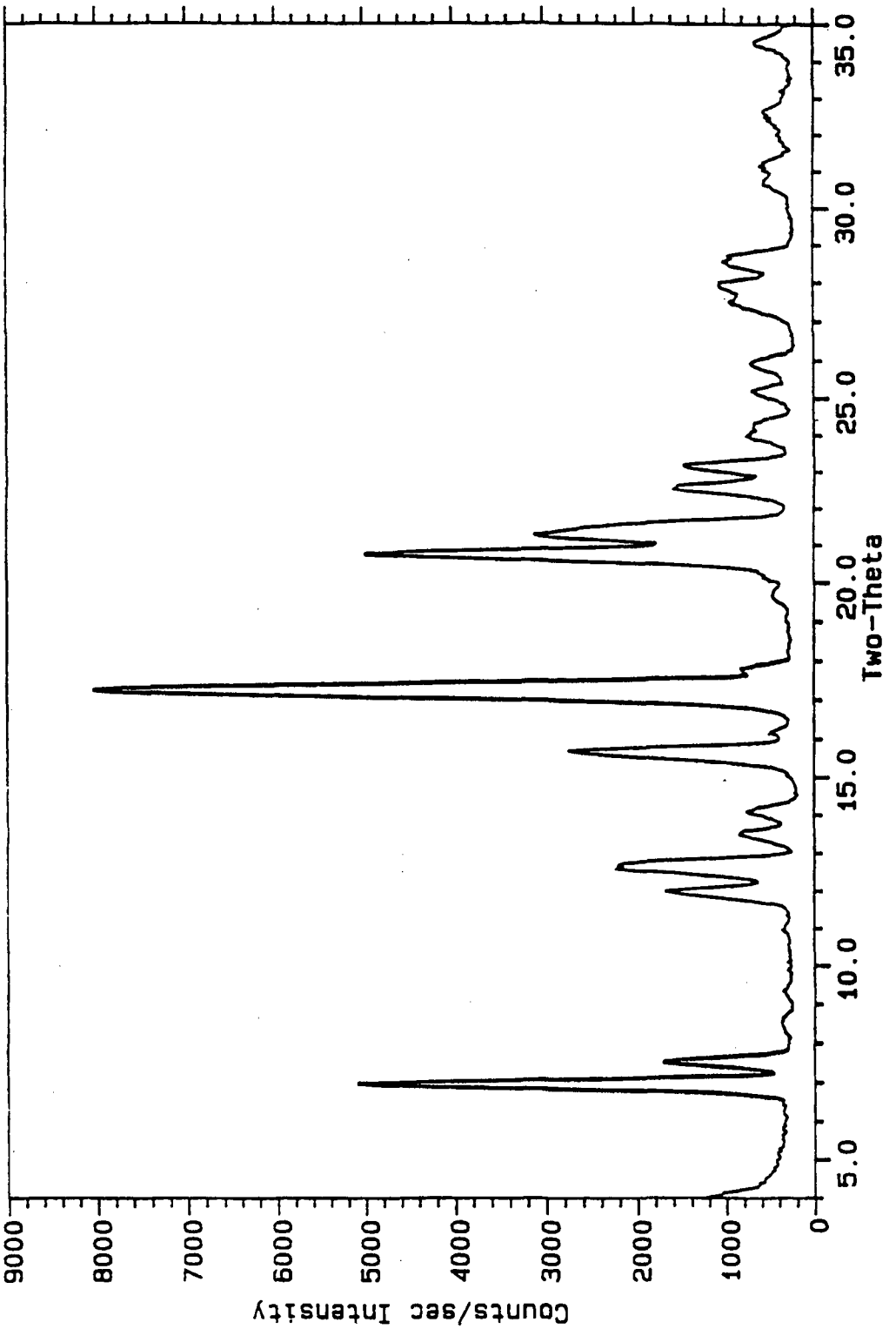


Figure 1

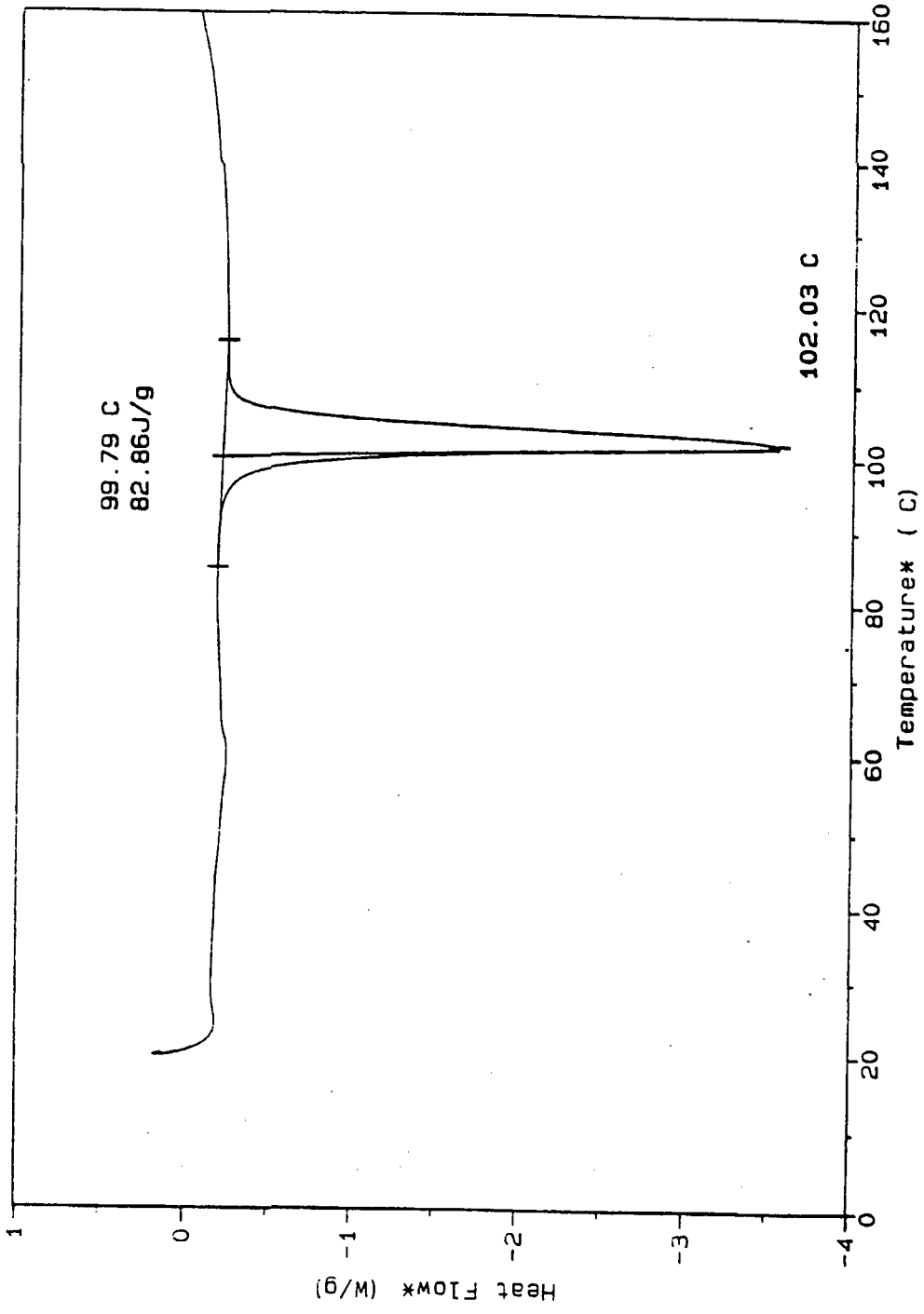


Figure 2

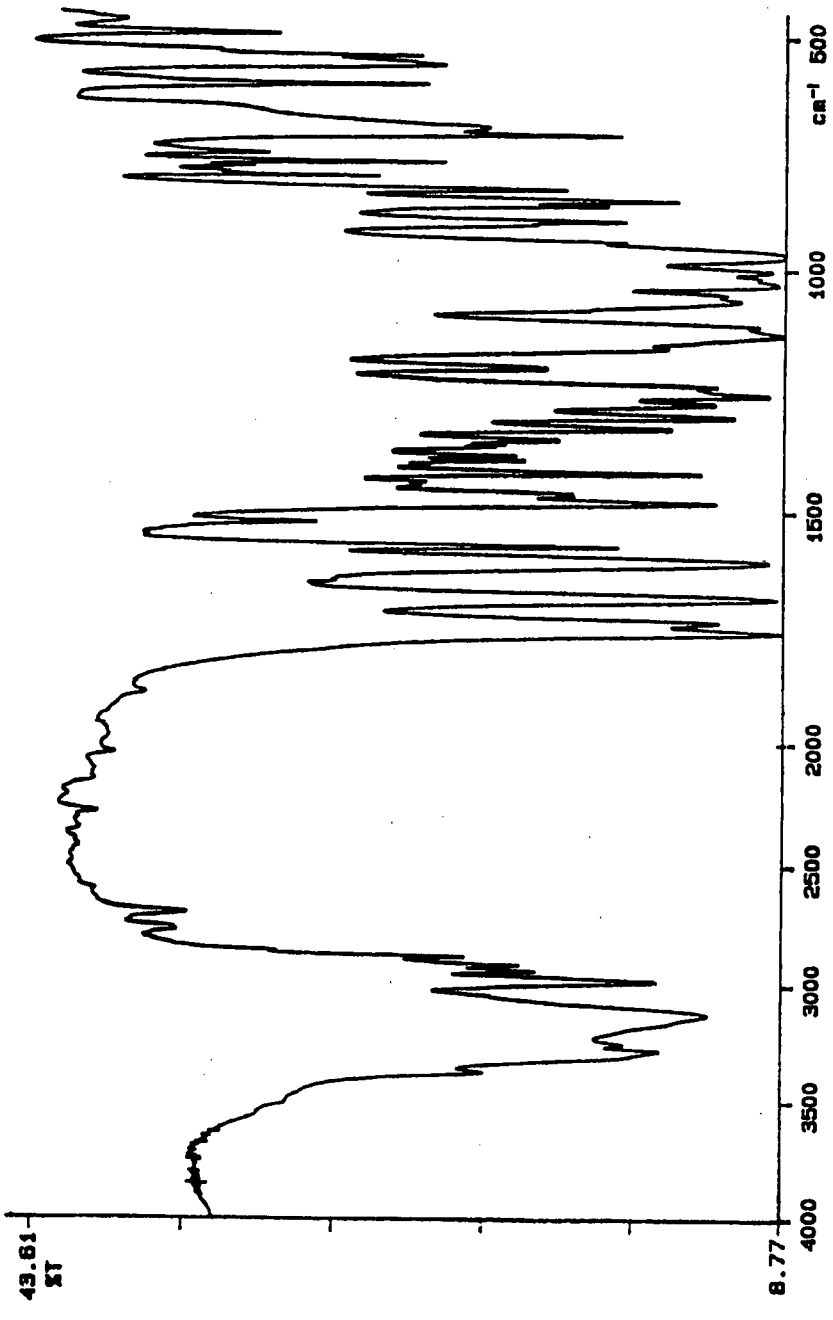


Figure 3

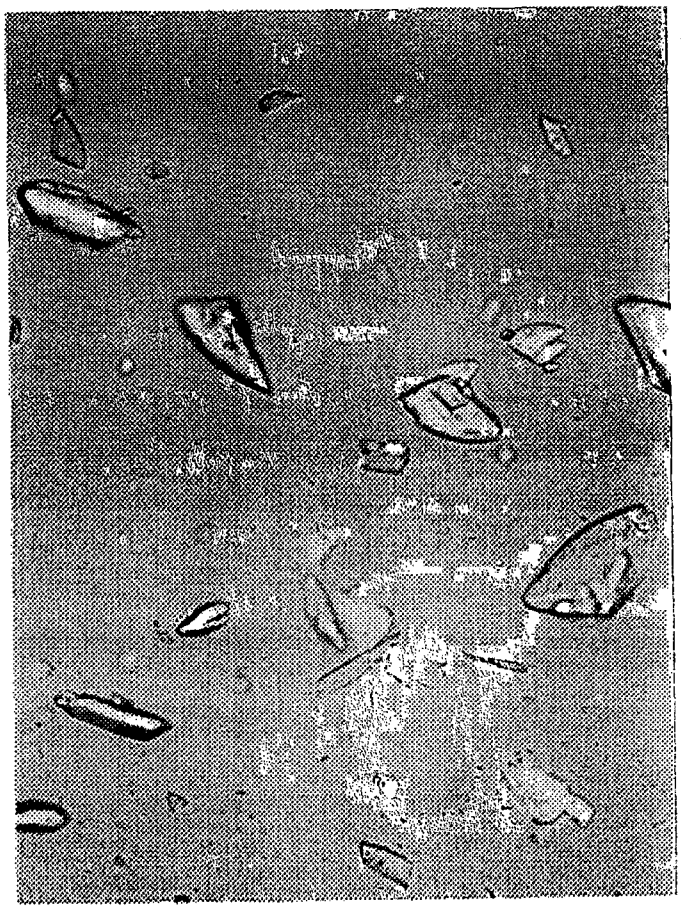


Figure 4

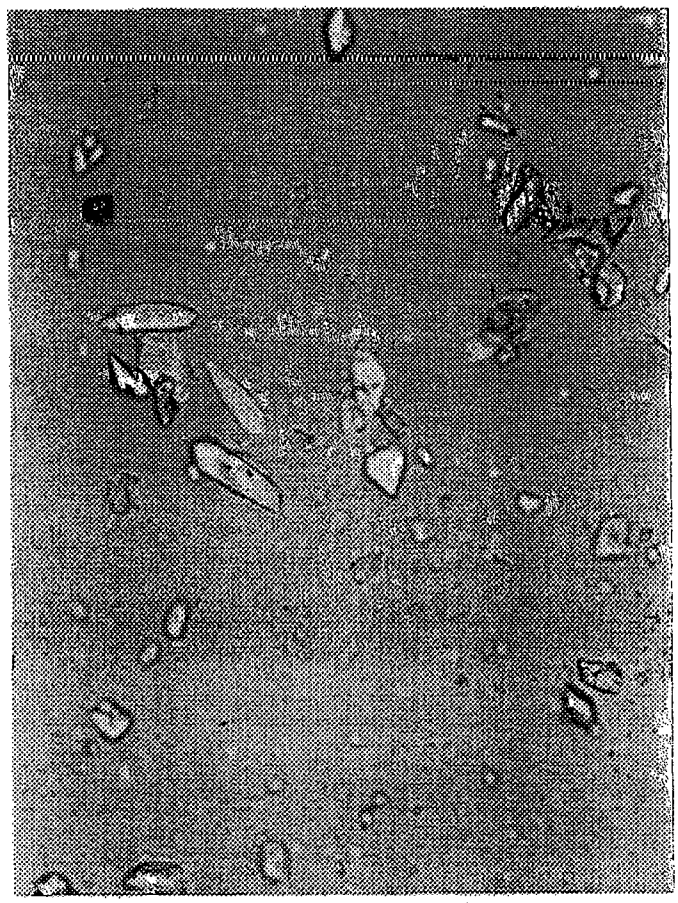


Figure 5

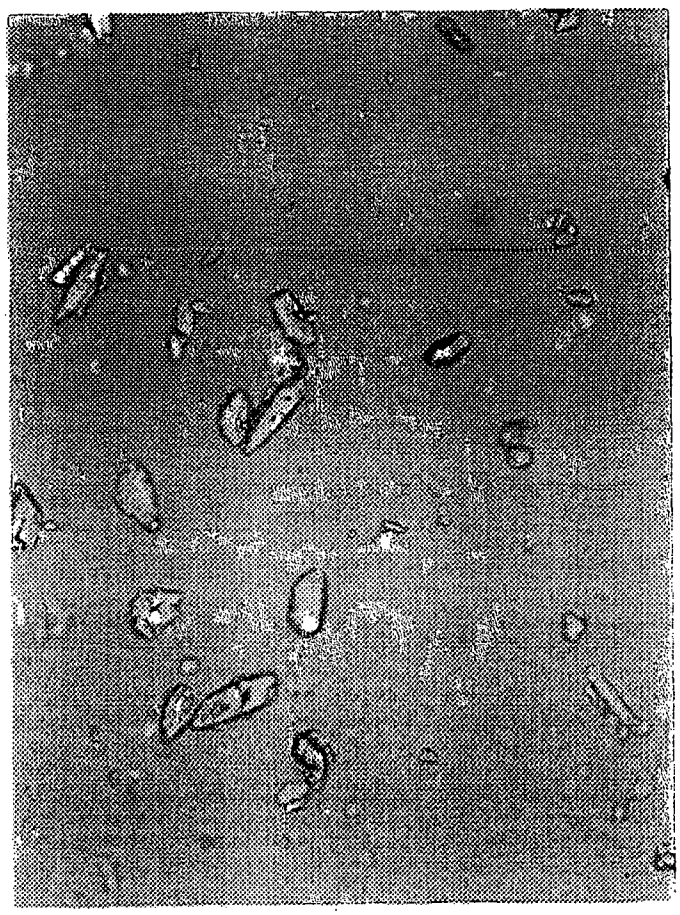


Figure 6

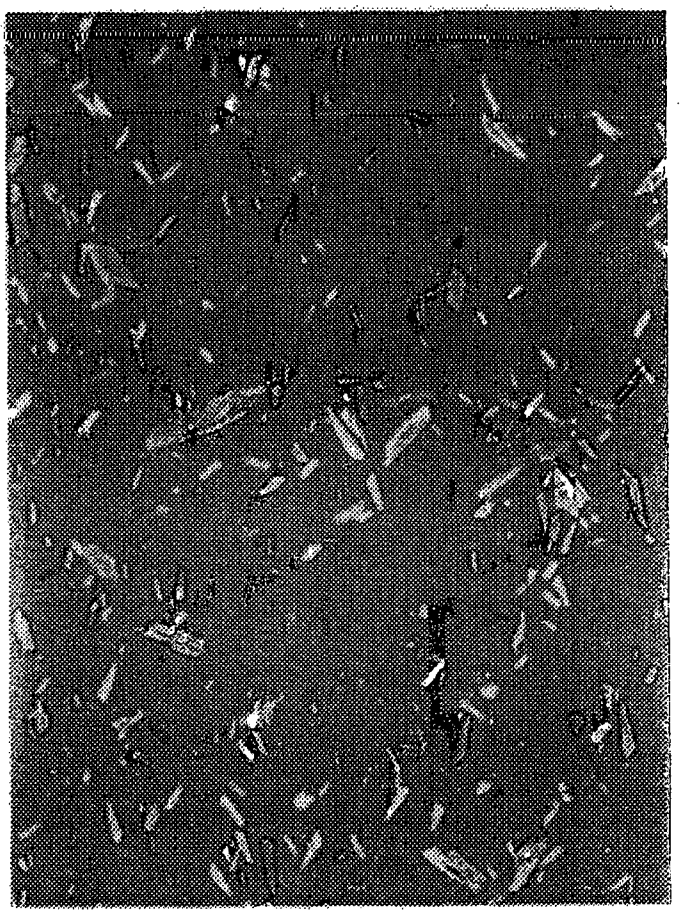


Figure 7

Figure 8

Figure 9

Figure 10

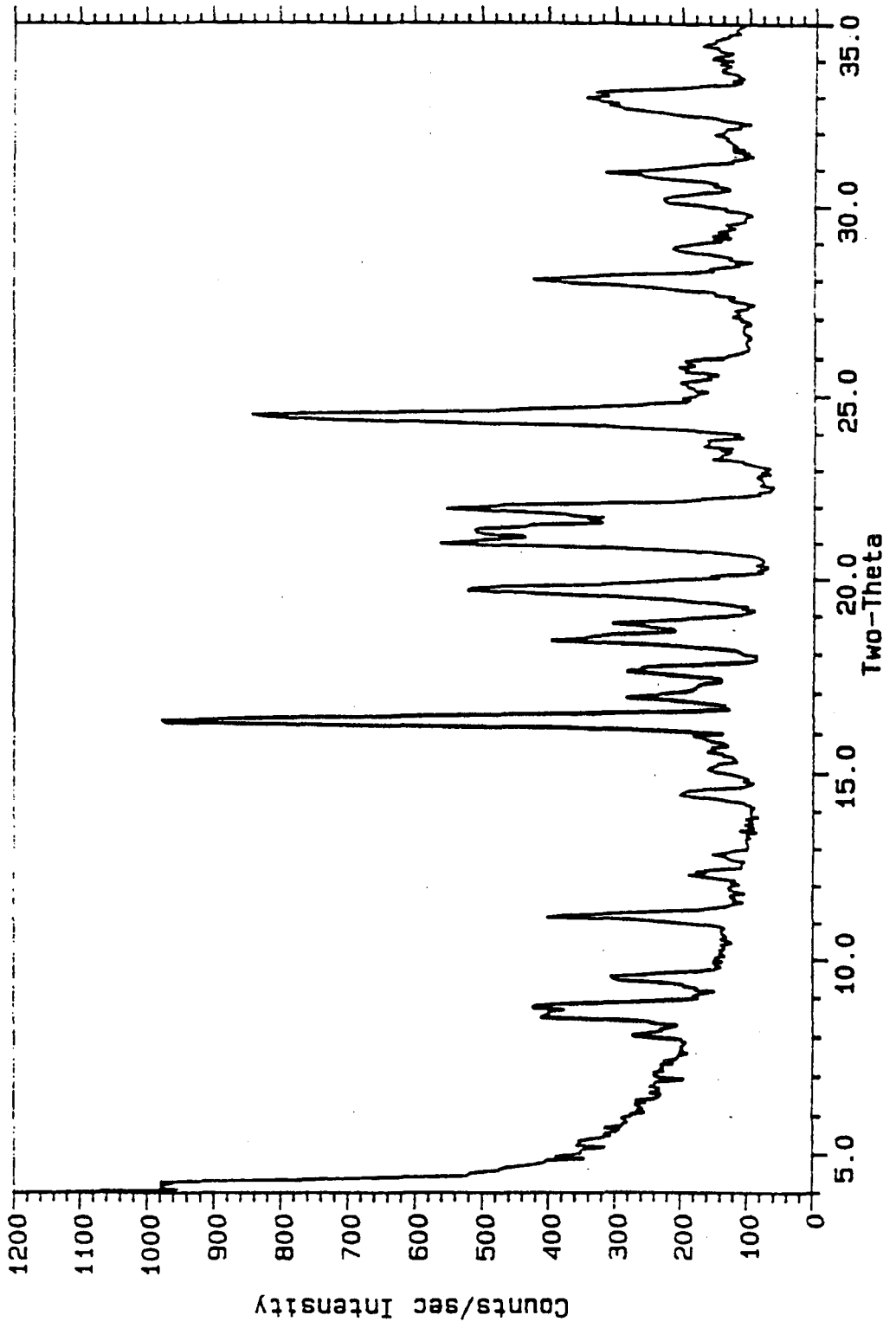
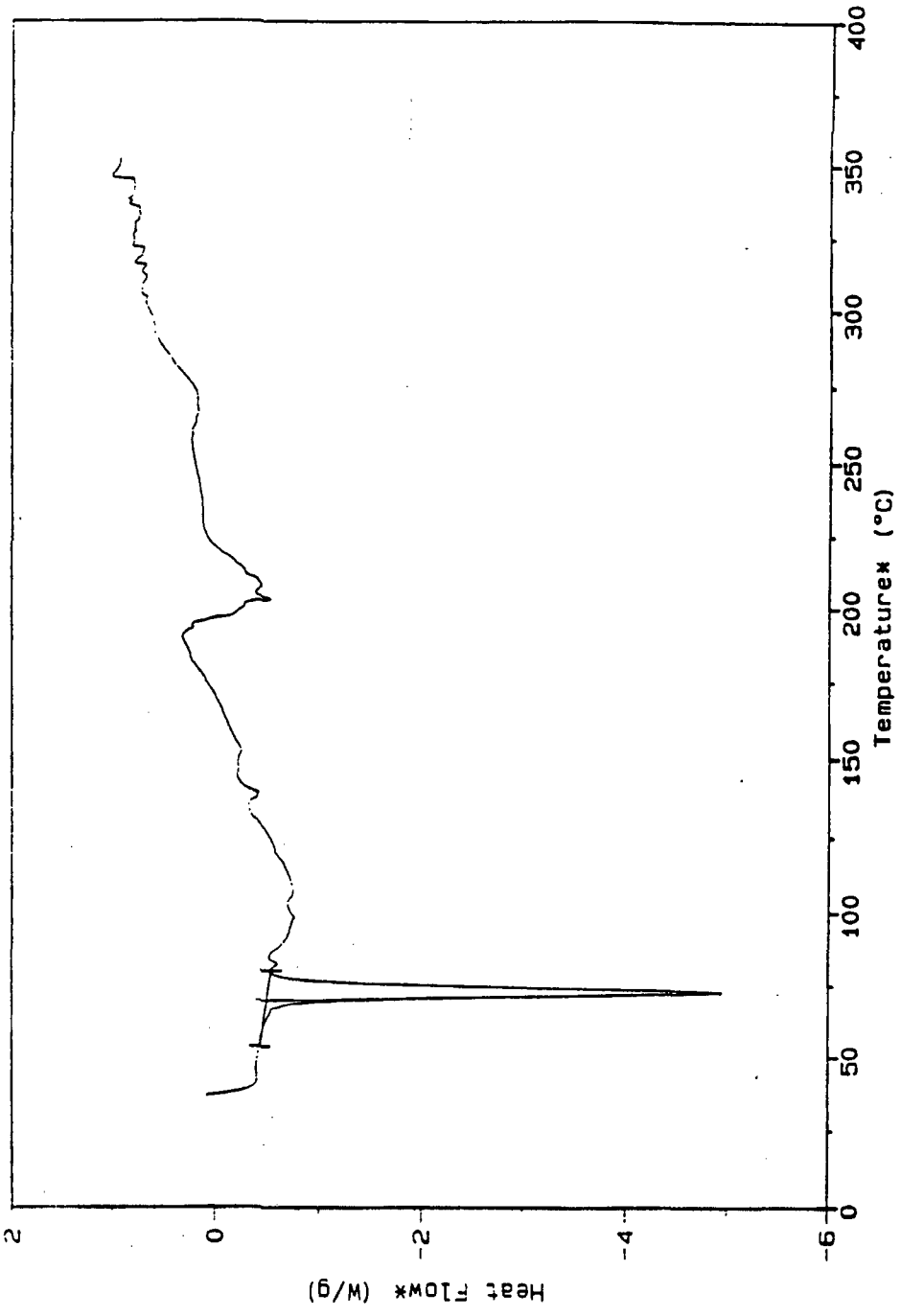


Figure 11



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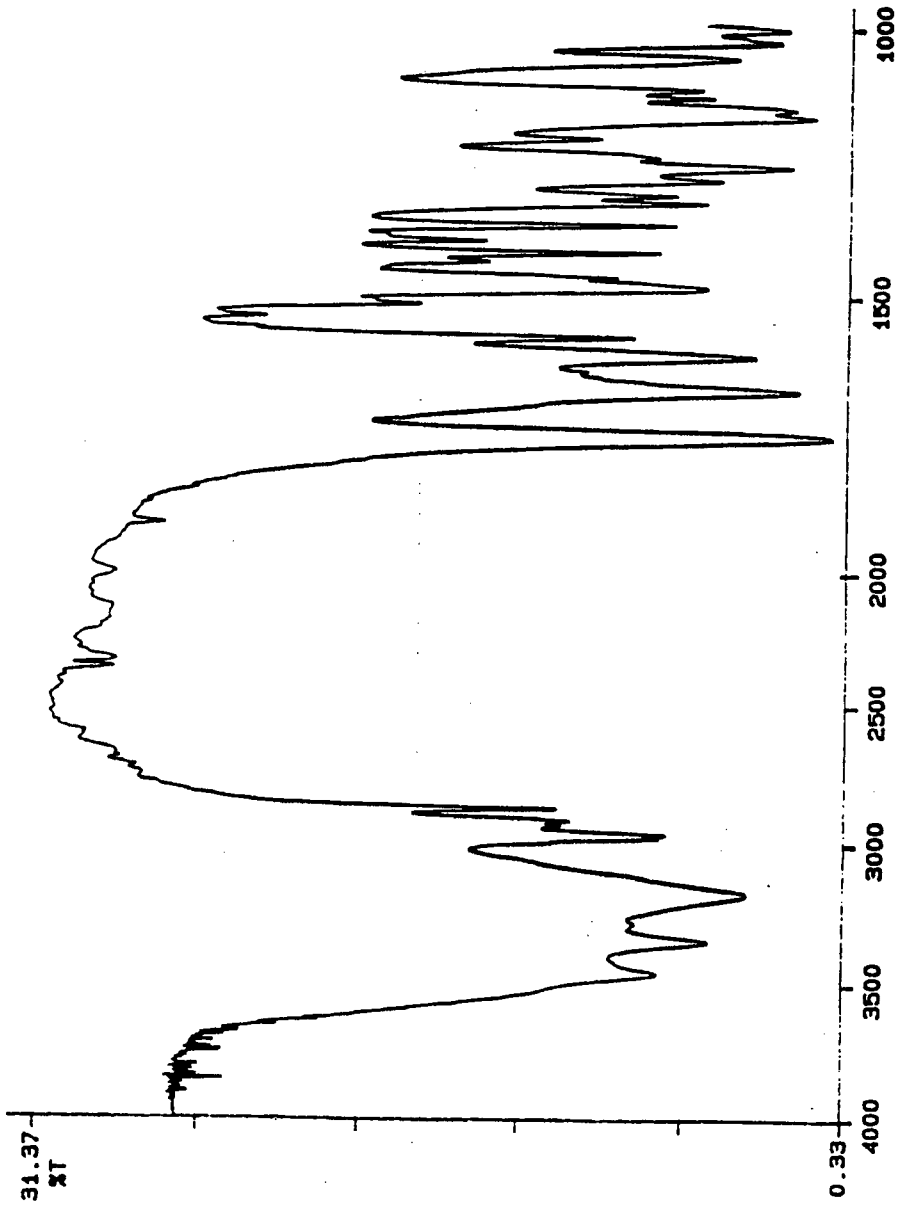
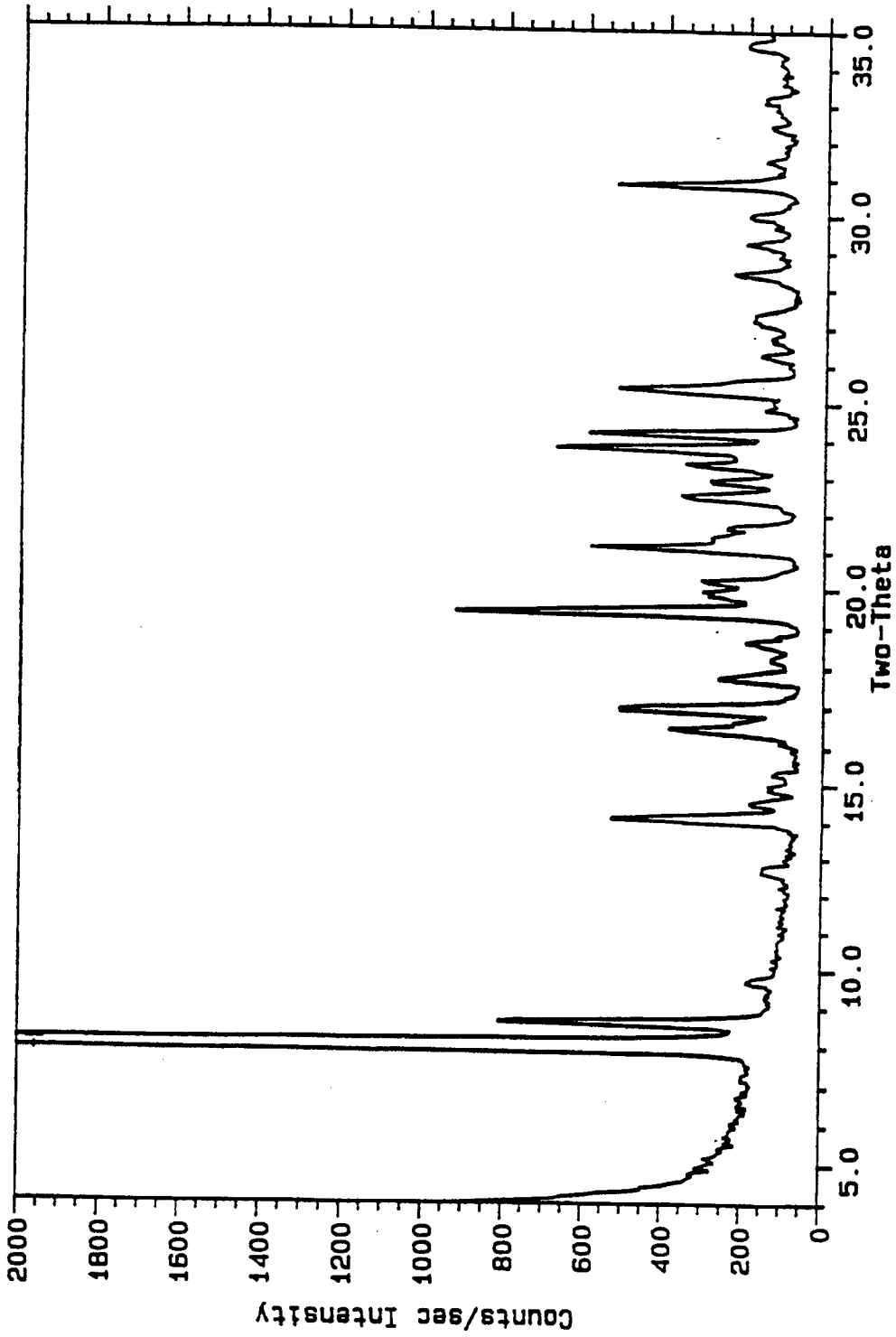


Figure 13



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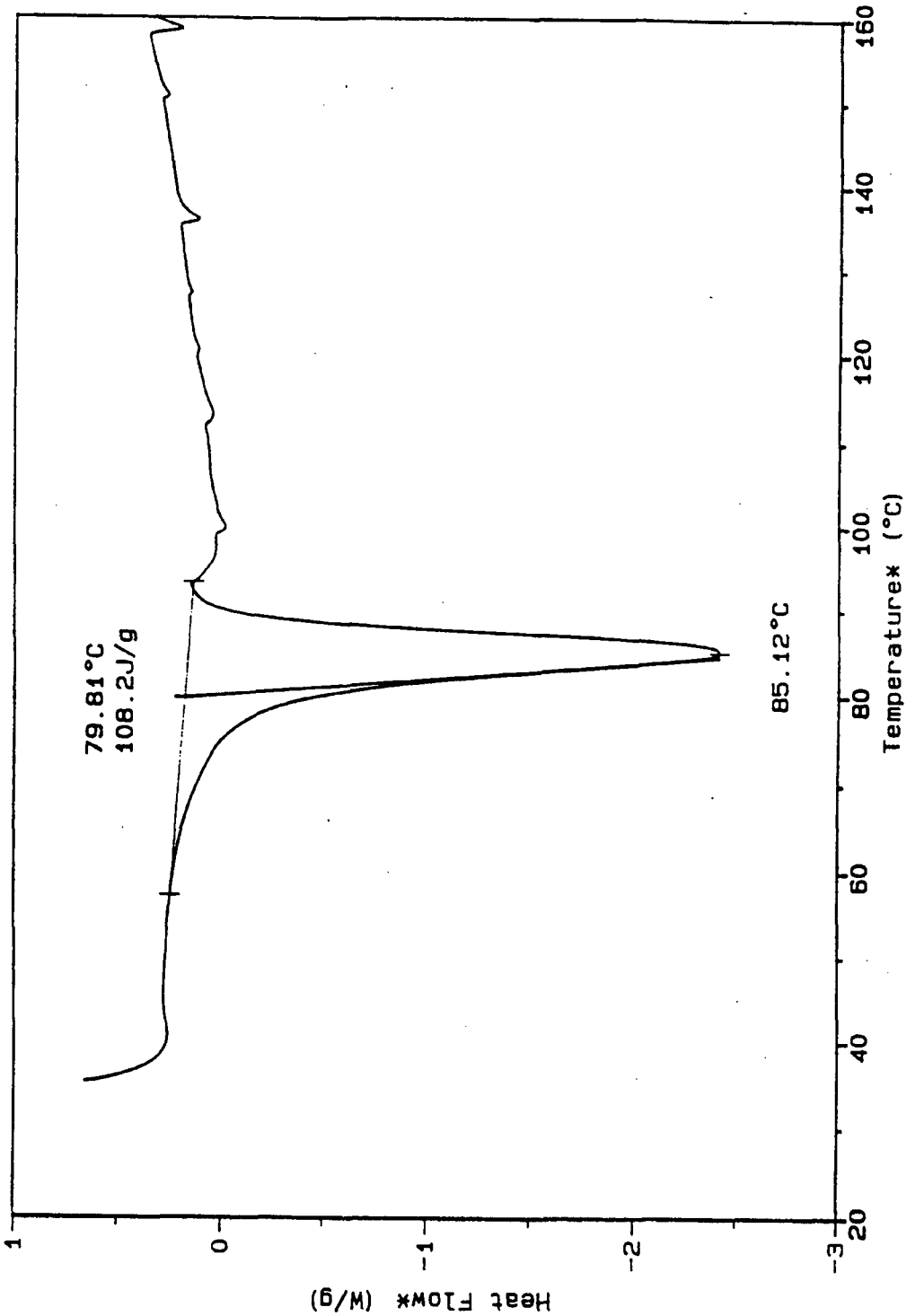

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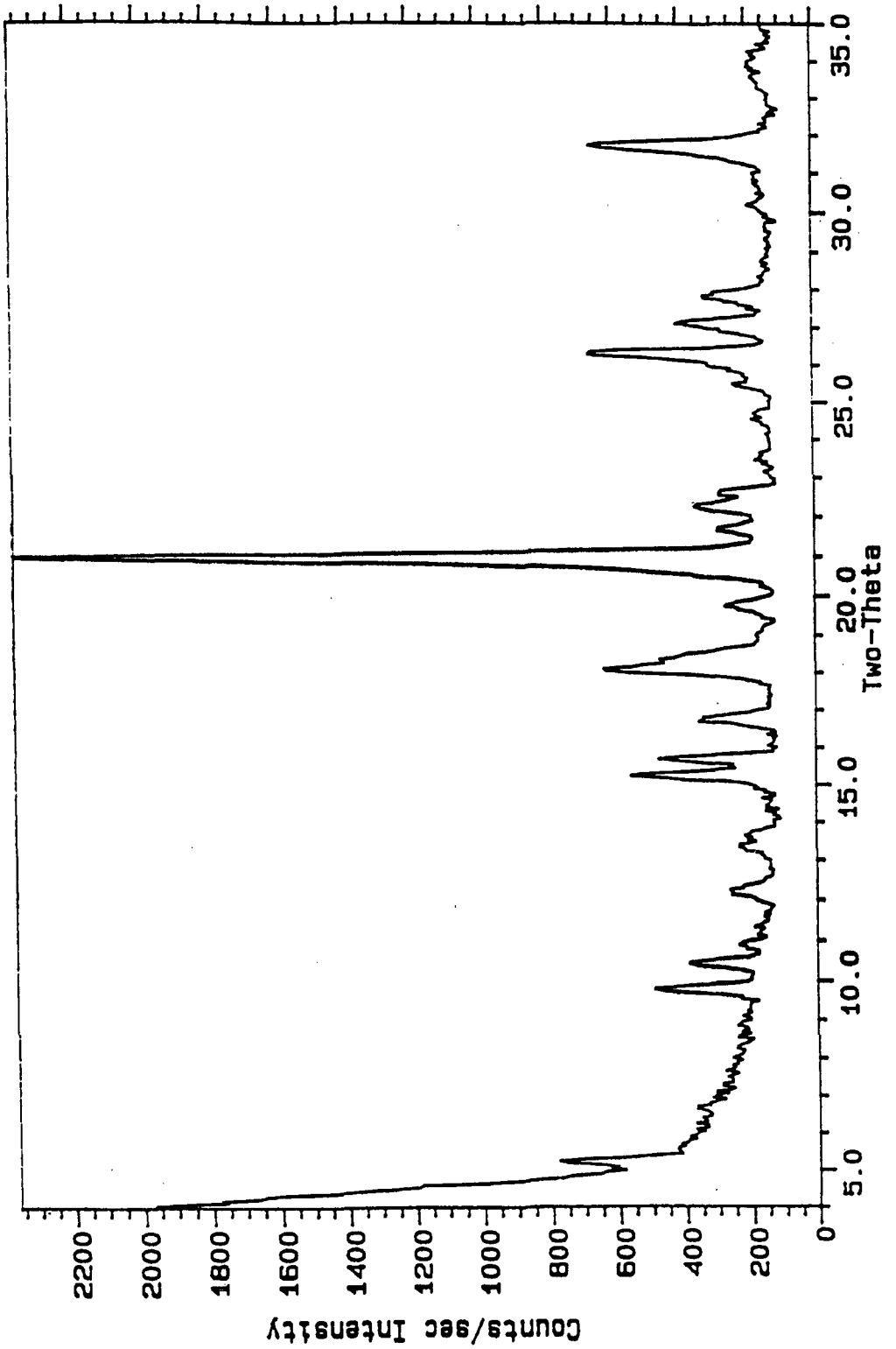

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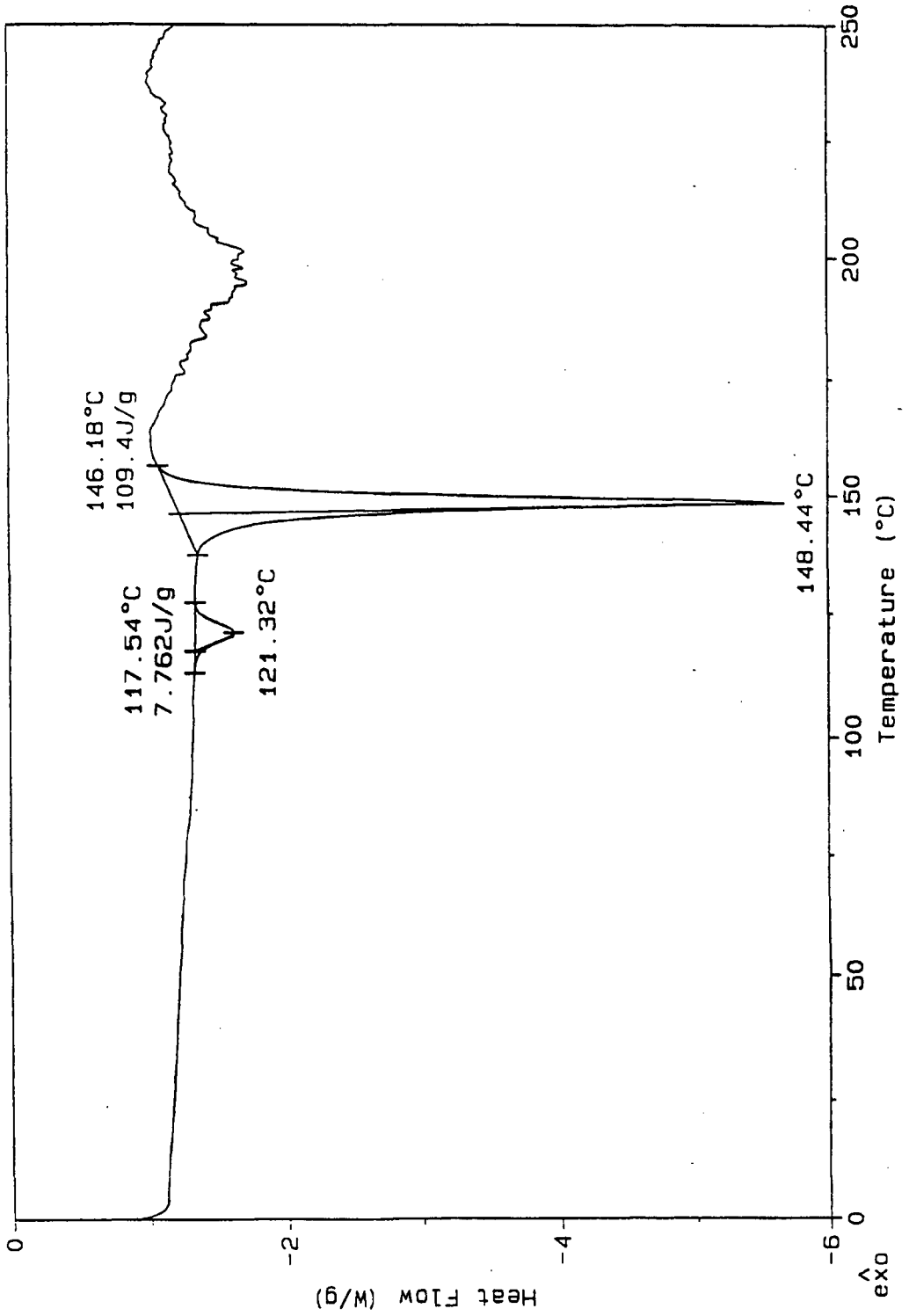
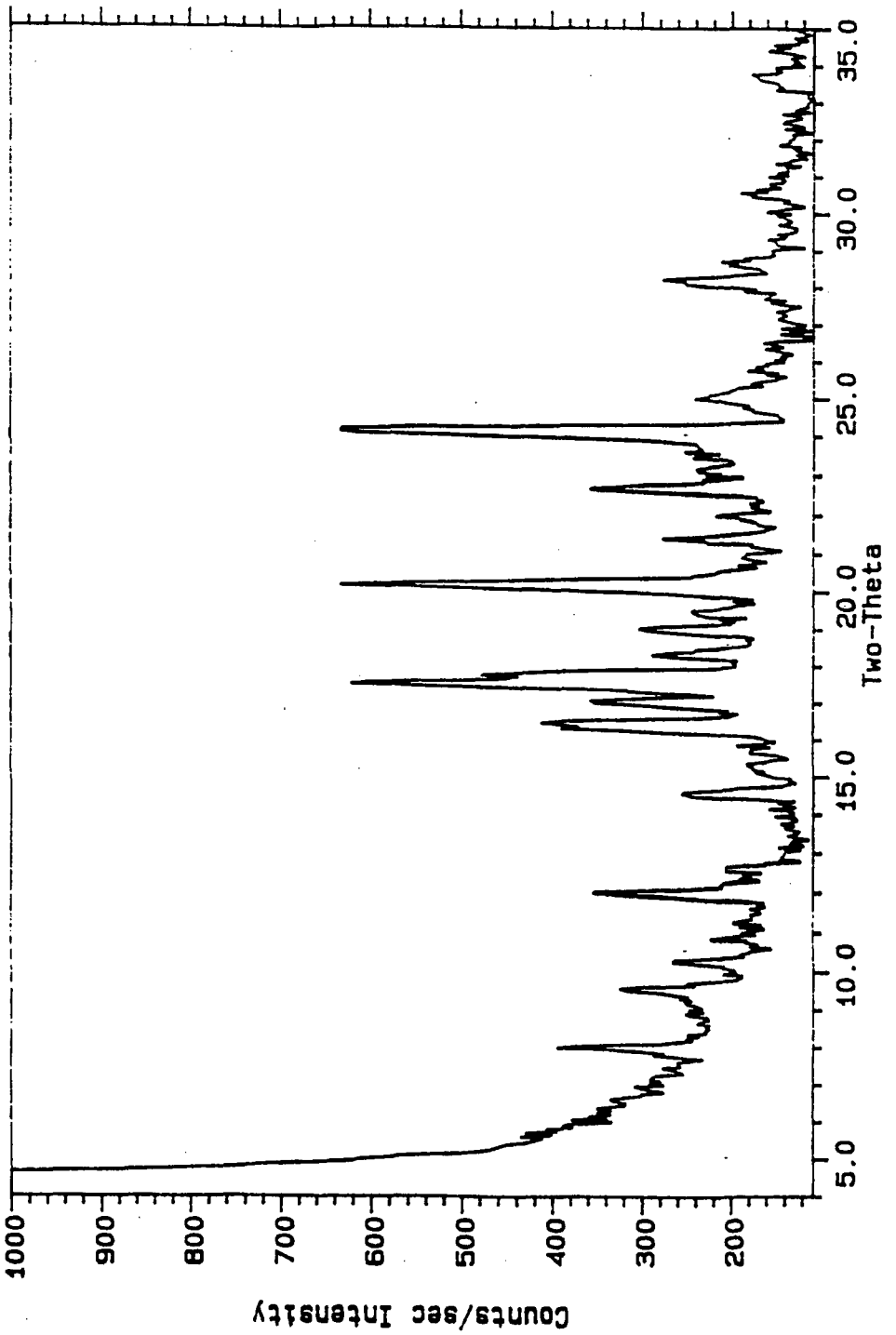


Figure 17



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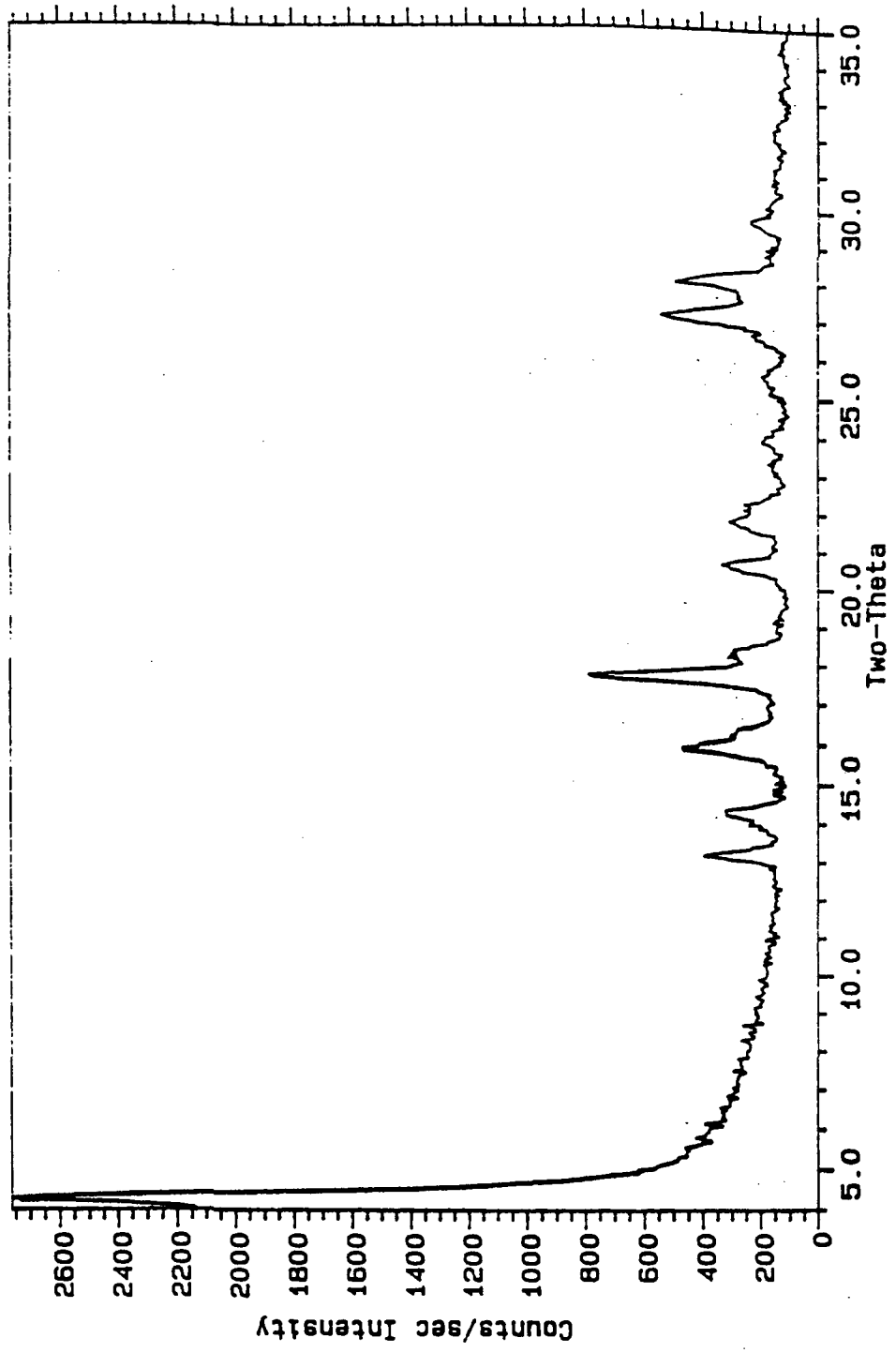

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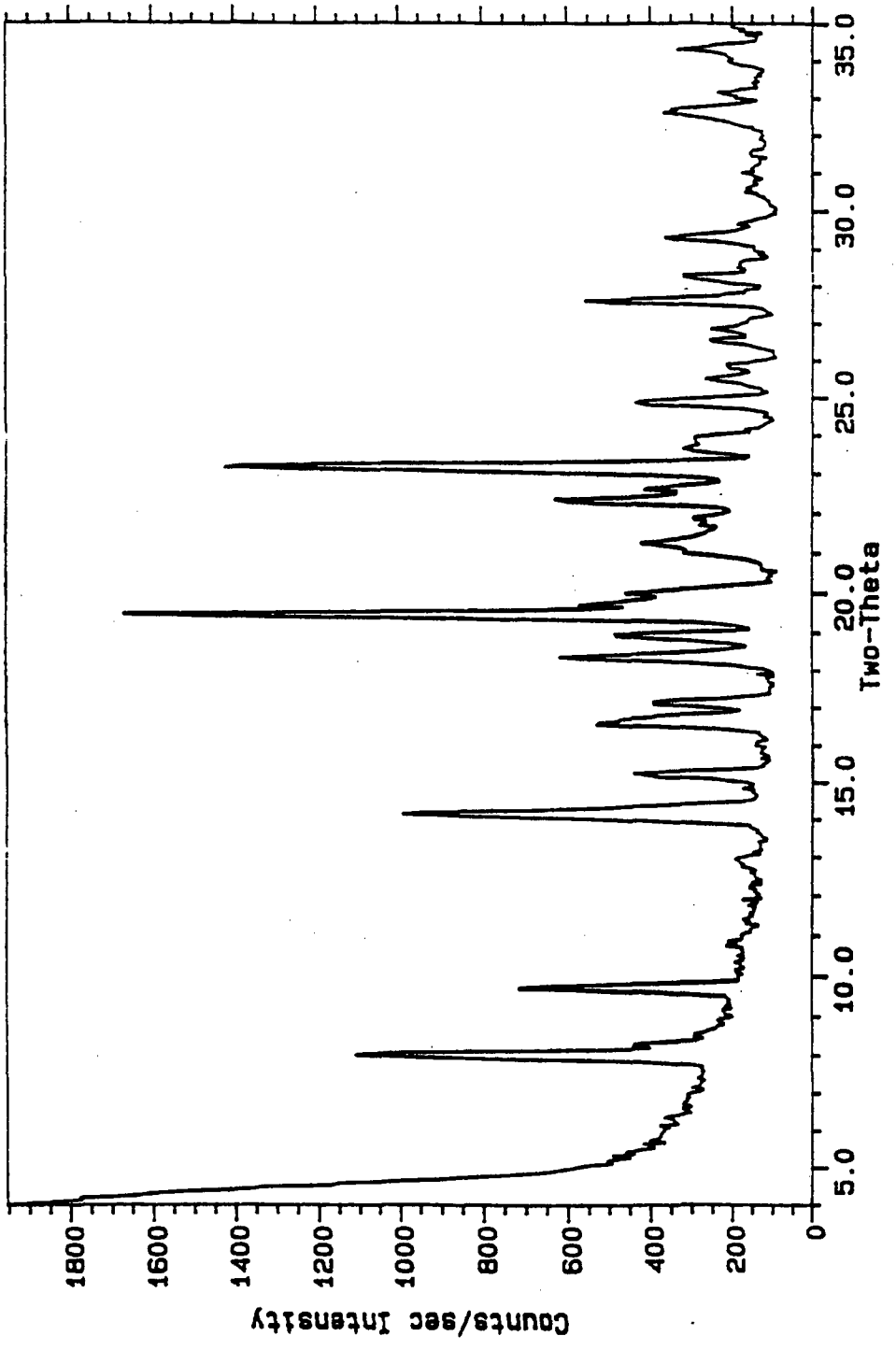

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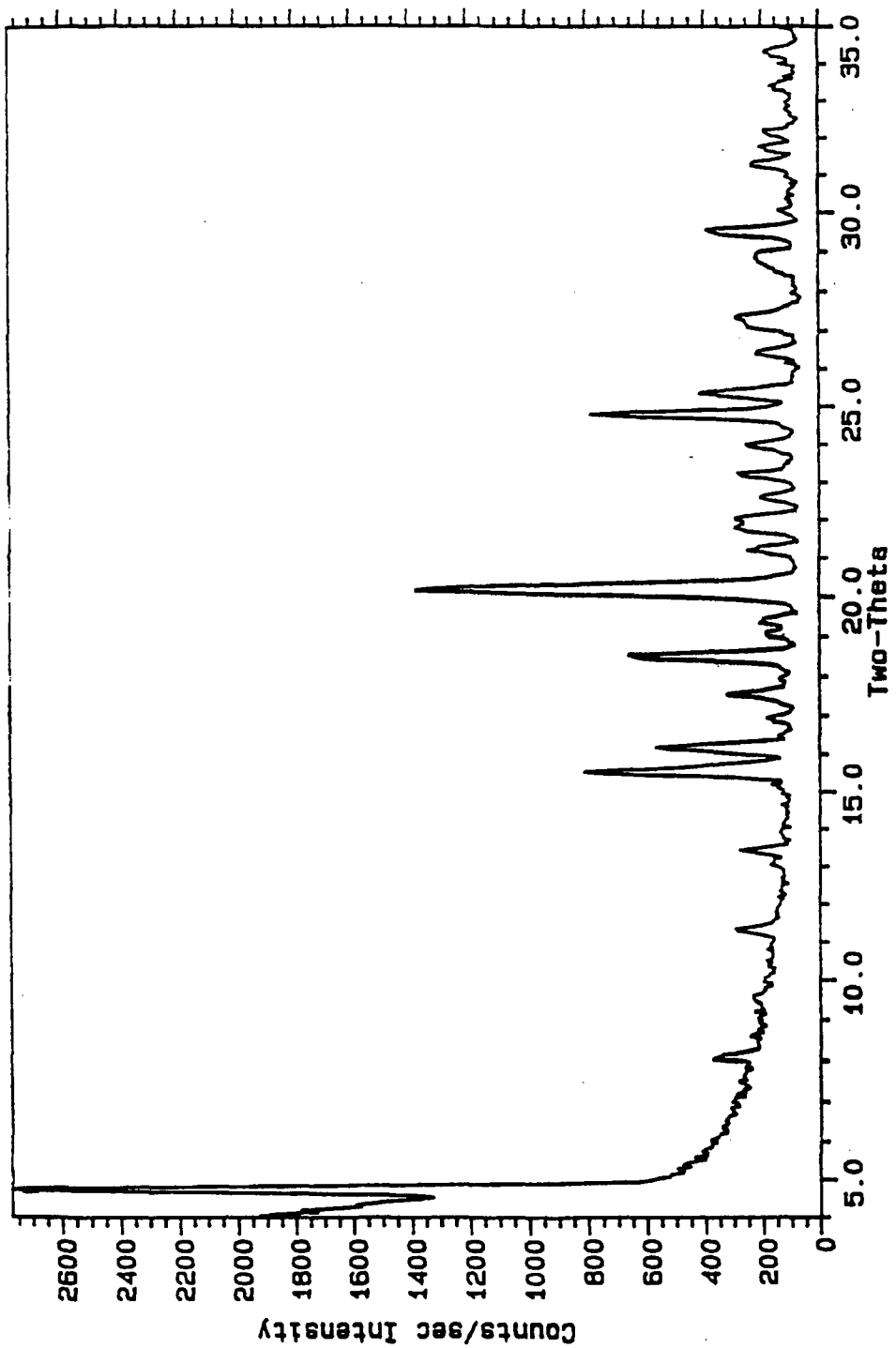

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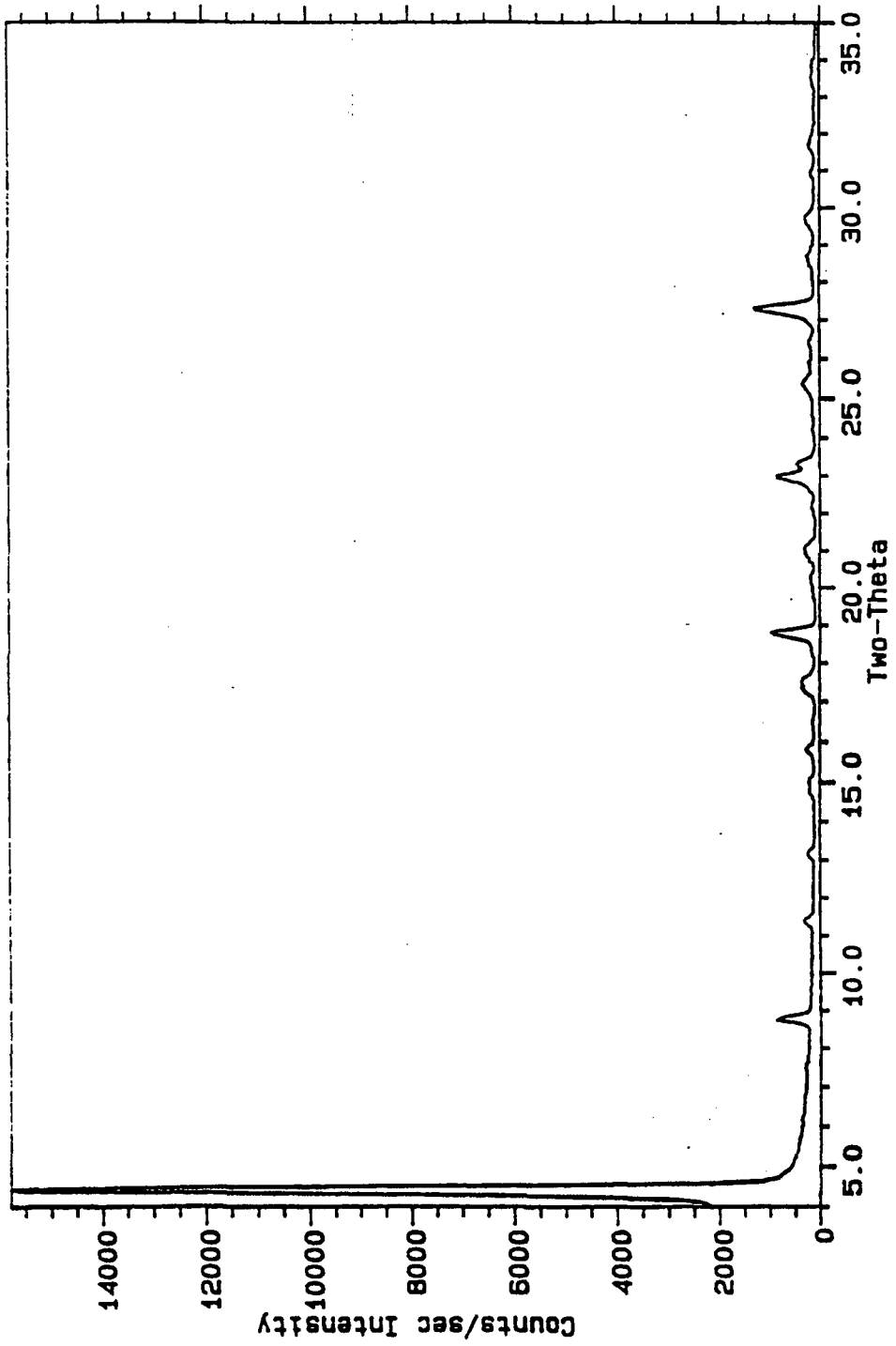
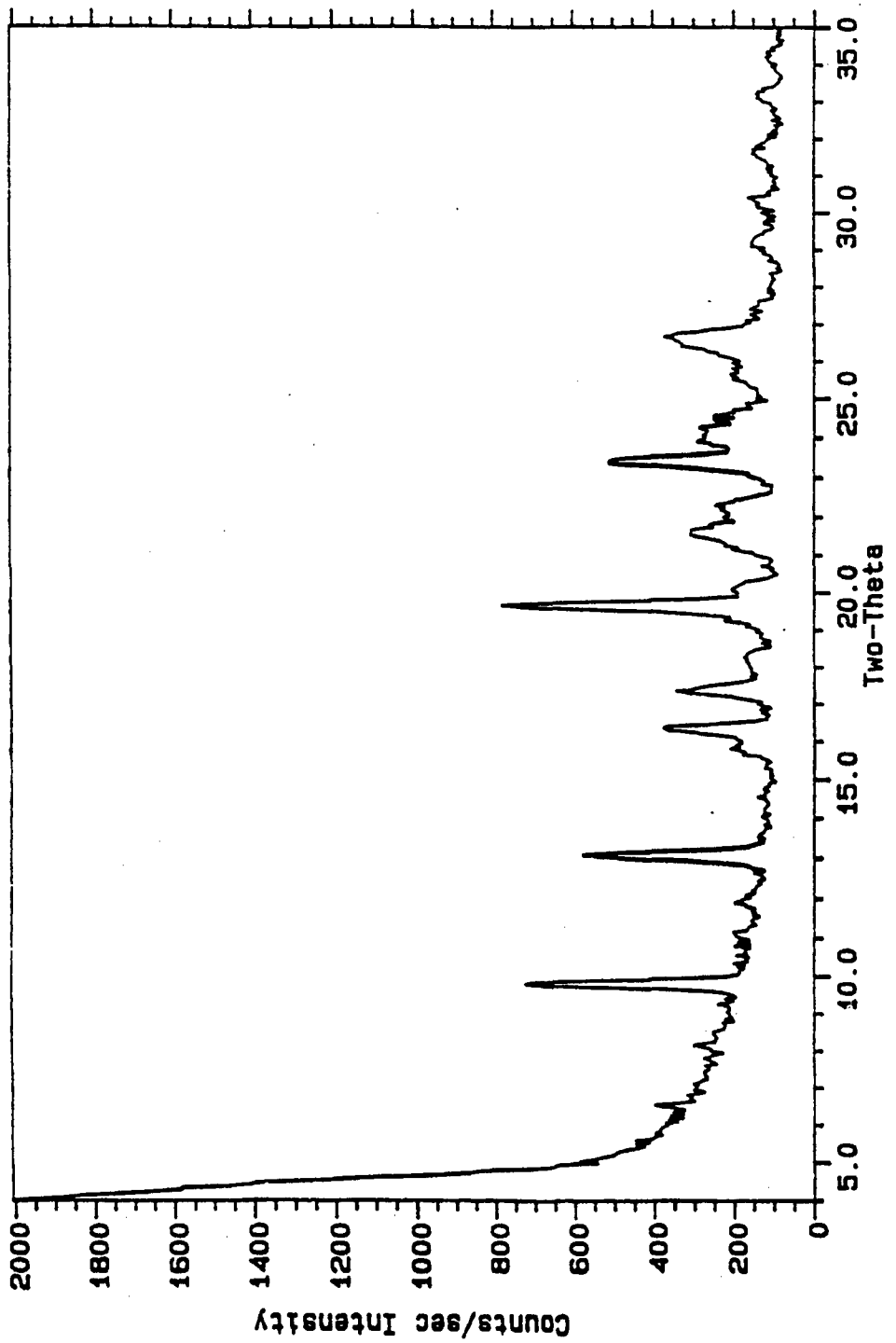


Figure 22



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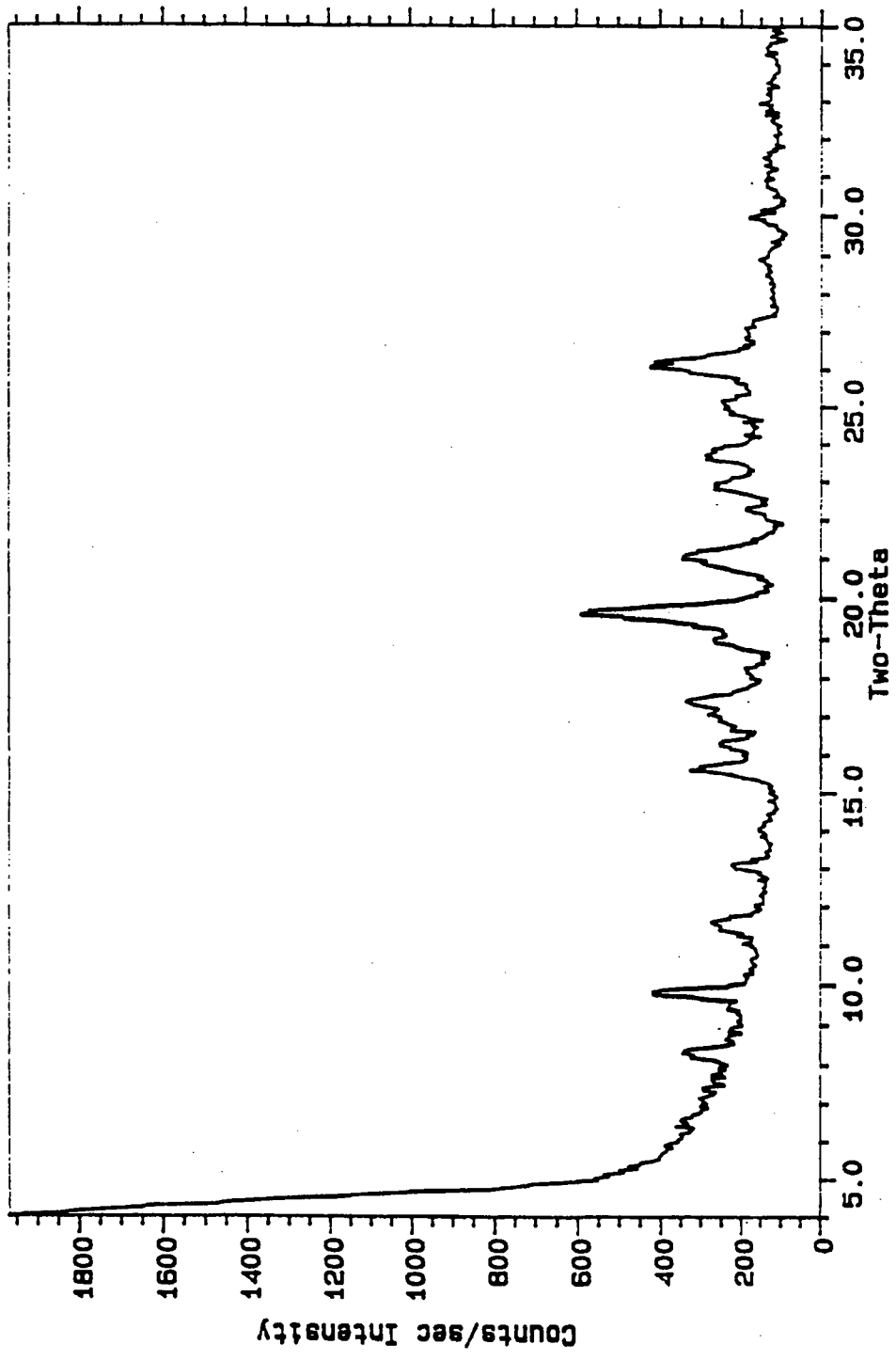


Figure 24

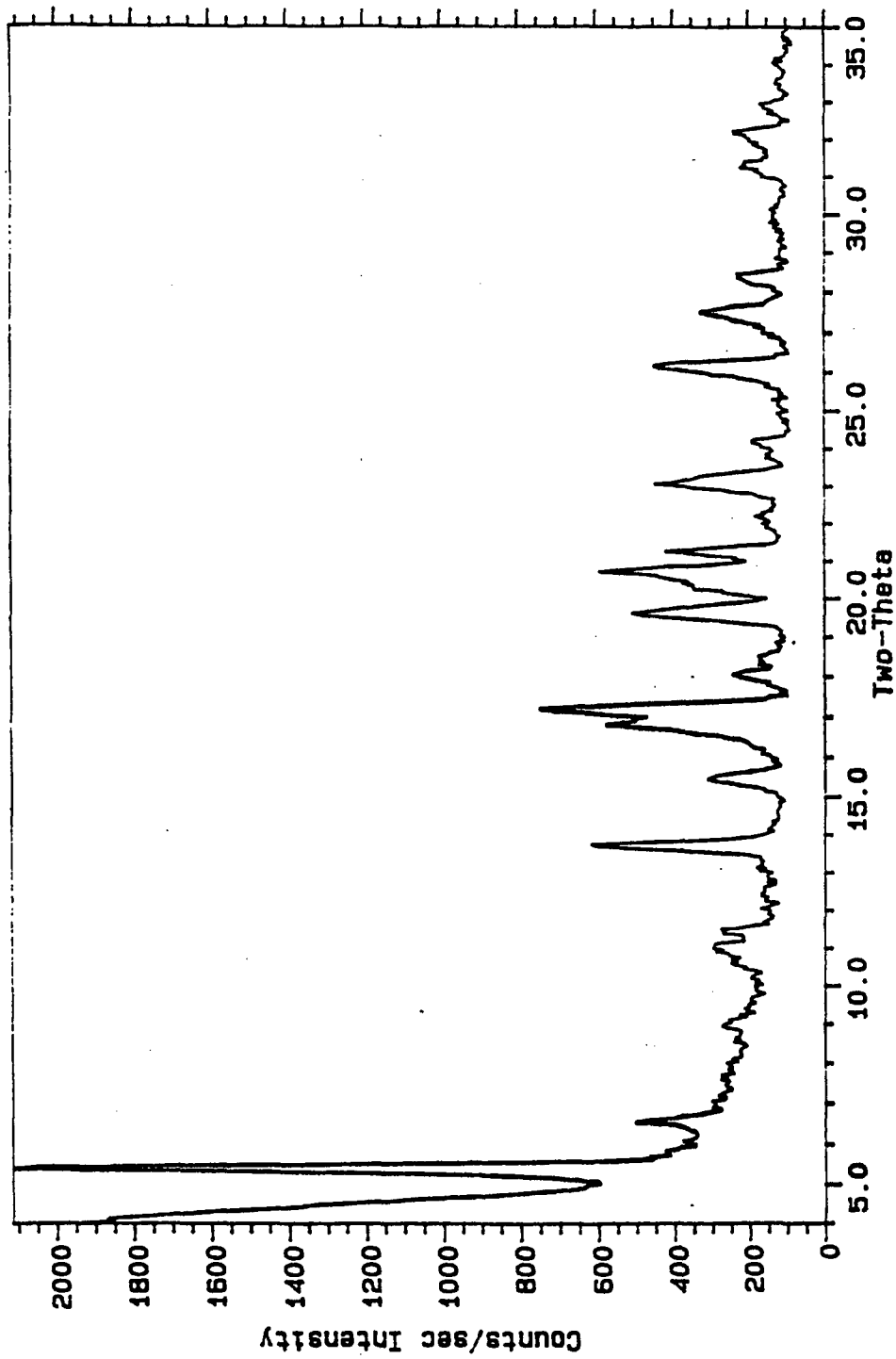


Figure 25

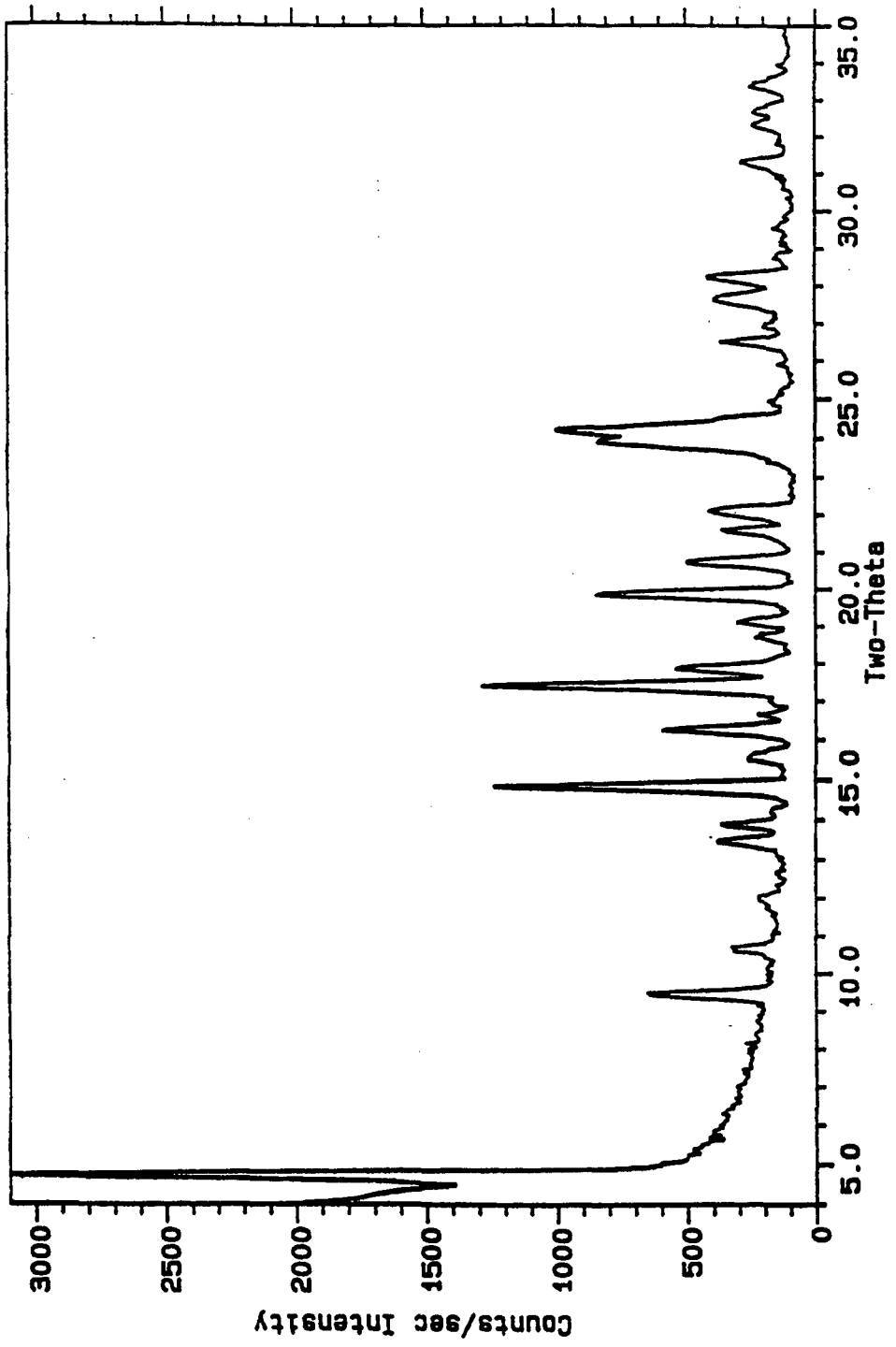


Figure 26

Packing diagram showing unit cell of Form 1 AD.

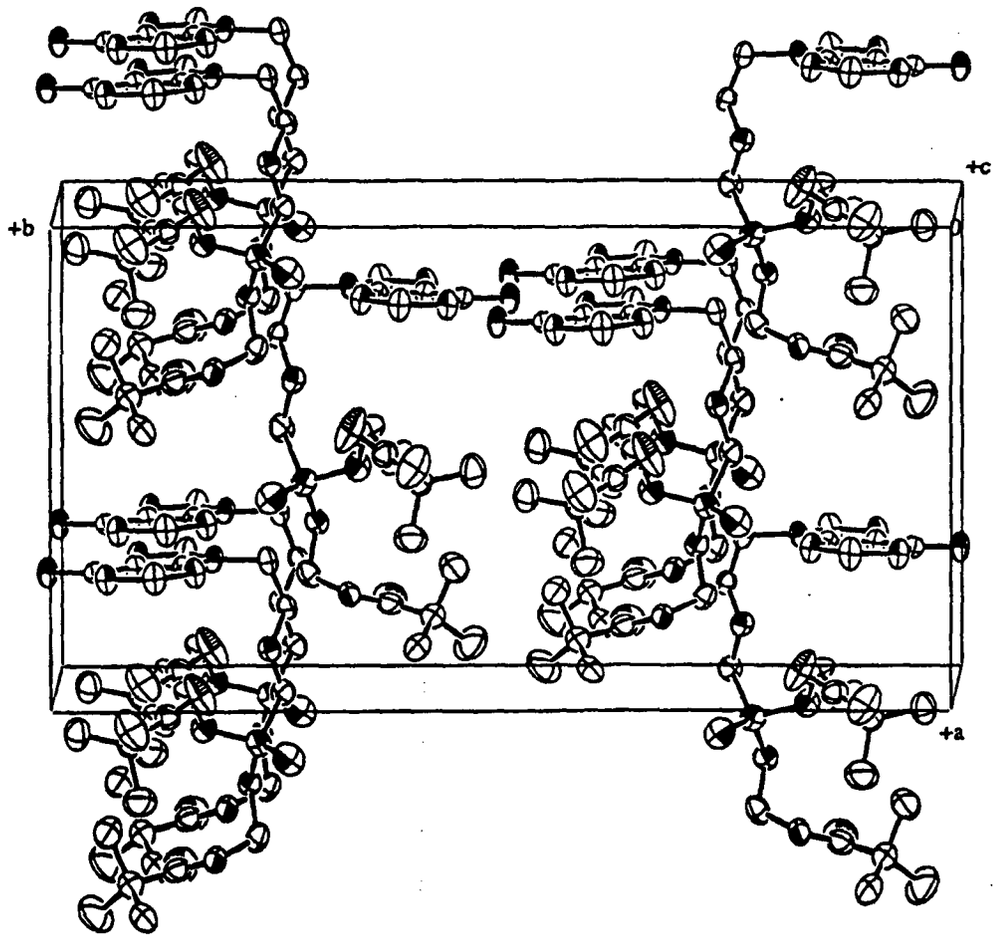


Figure 27

Atomic numbering scheme for Form 1 AD.

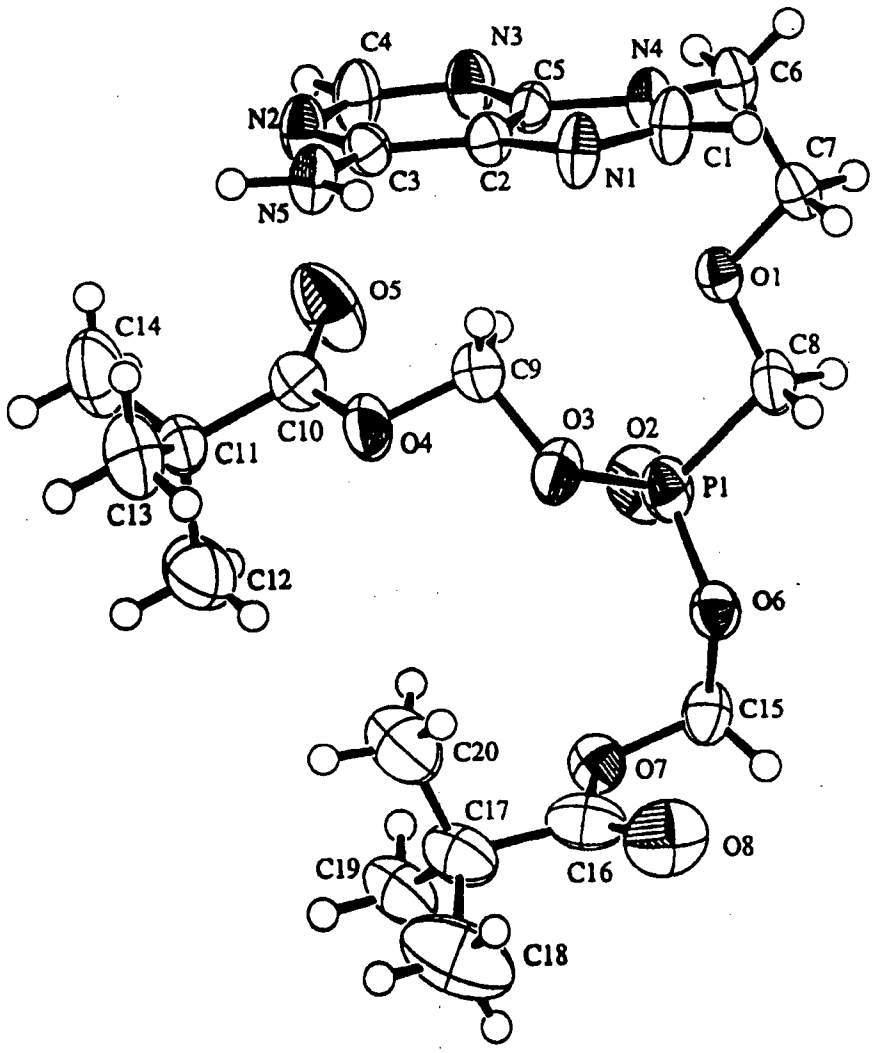
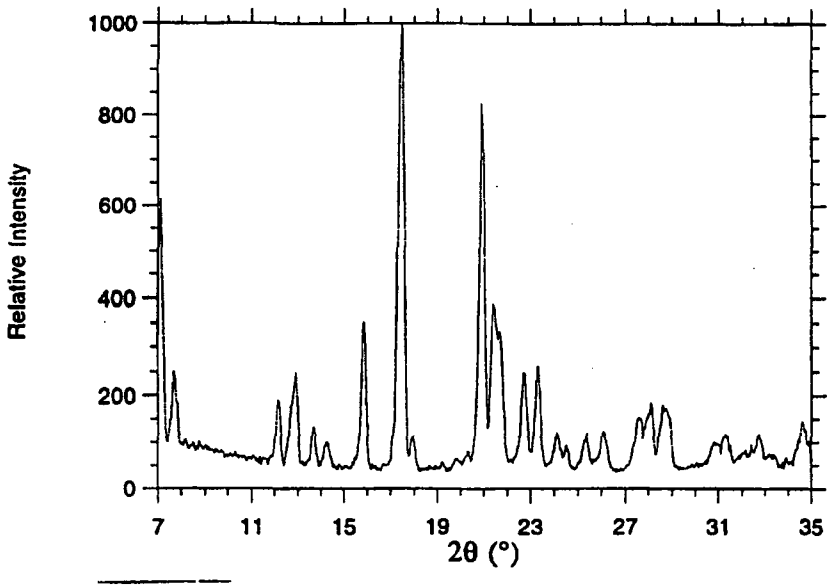


Figure 28

(a) Observed:



(b) Calculated:

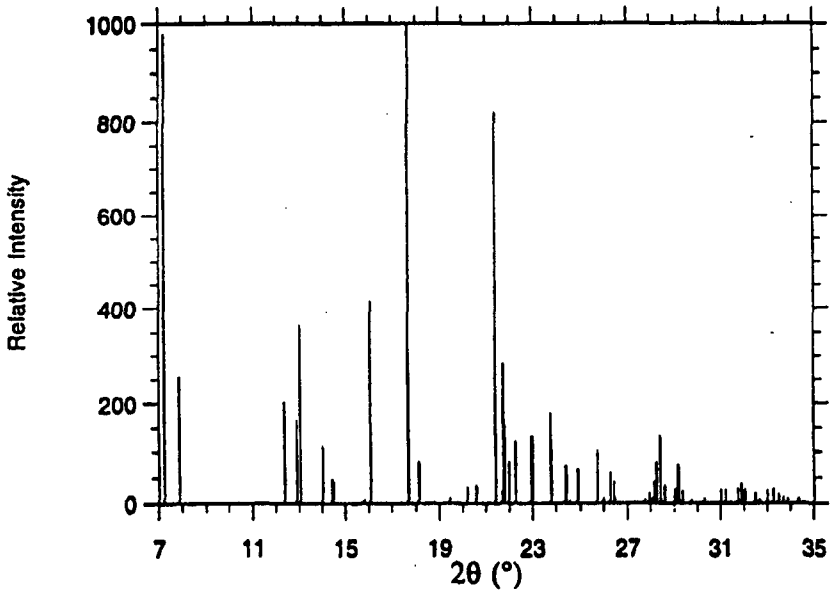


Figure 29

Exhibit 3

US005476938A

United States Patent [19]

Vemishetti et al.

[11] Patent Number: 5,476,938

[45] Date of Patent: Dec. 19, 1995

[54] **PROCESS FOR THE PREPARATION OF NUCLEOTIDES**

[75] Inventors: **Purushortham Vemishetti; Paul R. Brodfuehrer; Henry G. Howell; Chester Sapino, Jr.**, all of Onondaga County, N.Y.

[73] Assignees: **Institute of Organic Chemistry and Biochemistry of the Academy of Sciences of the Czech Republic**, Czech Rep.; **Rega Stichting v.z.w.**, Belgium

[21] Appl. No.: **822,271**

[22] Filed: **Jan. 21, 1992**

Related U.S. Application Data

[63] Continuation of Ser. No. 566,200, Aug. 10, 1990, abandoned.

[51] **Int. Cl.**[6] **C07F 9/38**; C07F 9/40; C07B 43/00

[52] **U.S. Cl.** **544/243**; 544/244

[58] **Field of Search** 544/243, 244

[56] **References Cited**

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Robert M. Hanson, "The Synthetic Methodology of Nonracemic Glycidol and Related 2,3–Epoxy Alcohols", *Chemical Reviews*, vol. 91, No. 4, (Jun. 1991) pp. 437–475.

Primary Examiner—Mark L. Berch
Attorney, Agent, or Firm—Max D. Hensley

[57] **ABSTRACT**

The present invention relates to a novel and economical process for the synthesis of HPMP-substituted nucleotide antiviral compounds. Also disclosed are novel intermediates produced in the process for the preparation of HPMPC.

12 Claims, No Drawings

PROCESS FOR THE PREPARATION OF NUCLEOTIDES

This application is a continuation of Ser. No. 07/566,200, filed Aug. 10, 1990, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a novel process for the preparation of hydroxyphosphonylmethoxypropyl nucleosides, and novel intermediates produced therein.

2. Background Art

Nucleoside analogs possessing a 3-hydroxy-2-(phosphonylmethoxy)propyl (HPMP) side chain have been reported as potent antiviral compounds having a broad spectrum of activity. Examples of compounds belonging to this class include HPMP-adenine (HPMPA), HPMP-guanine (HPMPG) and HPMP-cytosine (HPMPC). HPMP-substituted nucleosides contain a chiral center and it has been postulated that the biological activity may reside in one enantiomer and not the other. It is therefore desirable to develop a synthetic method which will preferentially yield the active enantiomer using readily available and inexpensive starting materials.

Bronson et al (*J. Med. Chem.*, 1989, 32:1457) reported the synthesis of (S)-HPMPC which involves the coupling of cytosine with 3-O-benzyl-2-O-[(diethylphosphonyl)methyl] -3-O-(methylsulfonyl)glycerol, followed by subsequent deprotection to afford the product. The glycerol starting material is derived from chiral (R)-glycerol acetonide.

Holy et al (*Coll. Czech. Chem..Comm.*, 1989, 54:2470) reported the synthesis of (S)-HPMPC by reacting (R)-glycerol acetonide tosylate with 4-methoxy-2-pyrimidinone, the resultant product is then converted to 1-[(2,3-dihydroxy)propyl]cytosine. The latter compound is reacted with chloromethylphosphonyl dichloride, and the product is converted to (S)-HPMPC by base catalyzed rearrangement.

Glycerol acetonide was also used in the synthesis of (S)-HPMPA (Webb, Nucleosides and Nucleotides, 1989, 8:619) and HPMPG (Terry et al, *Antiviral Res.*, 1988, 10:235). These procedures all require the use of the expensive chiral glycerol acetonide as starting material, and involve multi-step process requiring chromatographic purifications of intermediate compounds.

The reaction of glycidol with adenine, cytosine or uracil to form the 2,3-dihyroxypropyl substituted nucleosides was reported by Ueda et al, *J. Heterocyclic Chem.*, 1971, 8:827. The reaction of (±)- glycidol with thymine or 5-fluorouracil was reported by Seiter et al, *Bull. Chem. Soc. Jpn.*, 1973, 46:1572. The prior art does not disclose or suggest the process of the present invention for the preparation of HPMP-nucleotides which offers marked improvement over previously known methods.

SUMMARY OF THE INVENTION

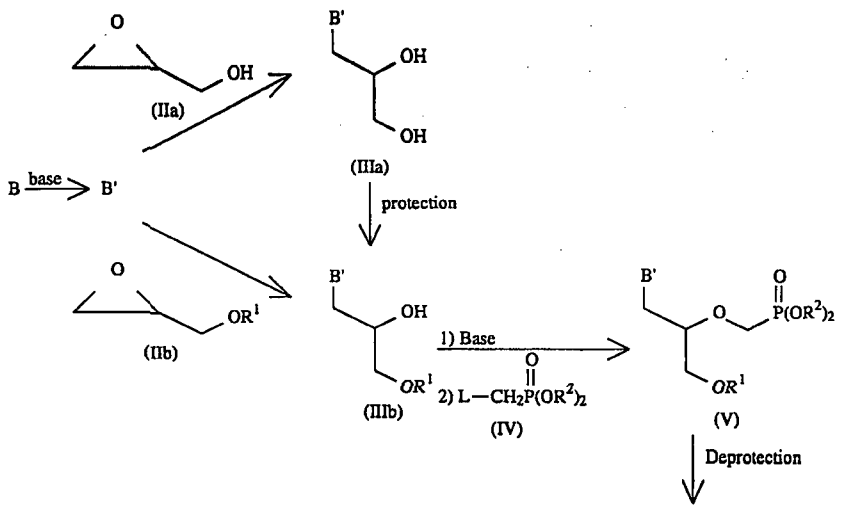
The present invention provides a novel and improved process for the preparation of hydroxyphosphonomethoxypropyl (HPMP) nucleoside antiviral compounds. The process of the instant invention comprises the steps of reacting an optionally substituted purine or pyrimidine base with an optionally substituted glycidol; if glycidol is used in the previous step, protecting the primary hydroxy group of the intermediate thus formed; reacting this product with a methanephosphonate derivative; and removing the various protecting groups to afford the final product.

The instant process starts with readily available purine and pyrimidine bases, and glycidol. The process offers advantages in economies of both material and labor costs by virtue of eliminating the need for isomer separations and subsequent chromatographic purifications; and unlike prior art processes, the instant process is suitable for large scale synthesis of the final products. Furthermore, the process is stereospecific and, starting with a chiral glycidol, produces the products without racemization.

DETAILED DESCRIPTION OF THE INVENTION

The present process for the preparation of HPMP-type nucleoside antiviral compounds is shown in Scheme I.

Scheme I



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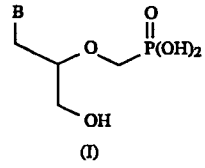
Scheme I



(I)

In Scheme I, B is a purine or a pyrimidine base; B' is a purine or pyrimidine base or a protected purine or pyrimidine base; L is a conventional leaving group; R^1 is a hydroxy protecting group; and R^2 is an alkyl group having 1–5 carbon atoms.

"Purine or pyrimidine base" includes, but is not limited to, adenine, guanine, thymine, uracil, cytosine, xanthine, hypoxanthine, 8-bromoguanine, 8-chloroguanine, 8-aminoguanine, 8-hydrazinoguanine, 8-hydroxyguanine, 8-methylguanine, 8-thioguanine, 2-aminopurine, 2,6-diaminopurine, 5-ethylcytosine, 5-methylcytosine, 5-bromouracil, 5-iodouracil, 5-ethyluracil, 5-propyluracil, 5-vinyluracil, and 5-bromovinyluracil.

"Protected purine or pyrimidine" refers to a purine or pyrimidine base in which functional groups that may interfere with the desired reaction have been blocked by a group stable under basic conditions. For example, the 4-amino group of cytosine may be blocked by the benzoyl group.

"Leaving group" includes, but is not limited to, halides such as chloride, bromide, and iodide; mesylate; and tosylate. "Alkyl" includes both straight and branched carbon chains. "Hydroxy protecting group" includes, for example, trityl, allyl, and benzyl groups.

In Scheme I, the first step involves the preparation of a compound of formula (IIIb). A purine or a pyrimidine base B' is first treated with a base in order to generate the corresponding anion. The base is not particularly restricted and may be selected from metal hydrides such as sodium and potassium hydrides, metal carbonates such as sodium and potassium carbonates, and metal alkoxides such as potassium t-butoxide; preferably the base is used in a catalytic amount.

Where the purine or pyrimidine base contains 1 or more functional groups that may be reactive to form undesired products under the reaction conditions of the present process, for example, the 4-amino group of cytosine and adenine and the 2-amino and 4-oxo groups of guanine, such functional groups may be blocked using the protecting group commonly employed in nucleoside chemistry. For example, the 4-amino group of adenine and cytosine may be protected. by benzoyl; the 4-oxo and 2-amino groups of guanine may be protected by the triphenylmethyl group. The selection of methods for introducing and subsequent removal of such protecting groups are well known to one of ordinary skill in the pertinent art.

The anion B'– generated in situ is reacted with glycidol (IIa) to generate the 2,3-dihydroxy nucleoside of formula (IIIa). The primary alcohol of the compound of formula (IIIa) is blocked prior to the addition of the phosphonate group. For the present process, however, it is preferred that the glycidol reactant is one in which the primary alcohol is protected, i.e. a compound of formula (IIb). The reaction of a protected glycidol with B' consistently gives the corresponding product of formula (IIIb) in higher yields than reactions in which unprotected glycidol is used. The hydroxy protecting group may be, for example, triphenylmethyl-type where the phenyl groups are unsubstituted or 1 or more of the phenyl groups are substituted, for example with methoxy; or allyl, benzyl, and the like. Preferably, the hydroxy protecting group is one selected from the group of triphenylmethyl type compounds.

The reaction is carried out in an inert dipolar aprotic organic solvent such as dimethylformamide, N-methyl-2-pyrrolidinone (NMPO), dimethyl sulfoxide, and hexamethyl phosphoramide at a temperature that favors the formation of the desired products; generally, the reaction temperature is elevated and may be from about 50° C. to about 150° C. Preferably, the reaction is carried out at about 100° C. to about 120° C. The starting materials B' and the glycidol are used in molar equivalent or one or the other reactant may be used in a slight excess, e.g., up to about 2 equivalents relative to the other. Preferably, B' is employed in excess in an amount up to about 1.3 equivalent of the glycidol.

The second step of the present process involves the introduction of the methanephosphonate moiety to the secondary hydroxy group of a compound of formula (IIIb). Prior to carrying out this step, if B' contains an unprotected functional group, this may be optionally protected. For example, the 4-amino group of cytosine may be converted to the corresponding dimethylformamidino derivative upon treatment with N,N-dimethylformamide or an acetal thereof.

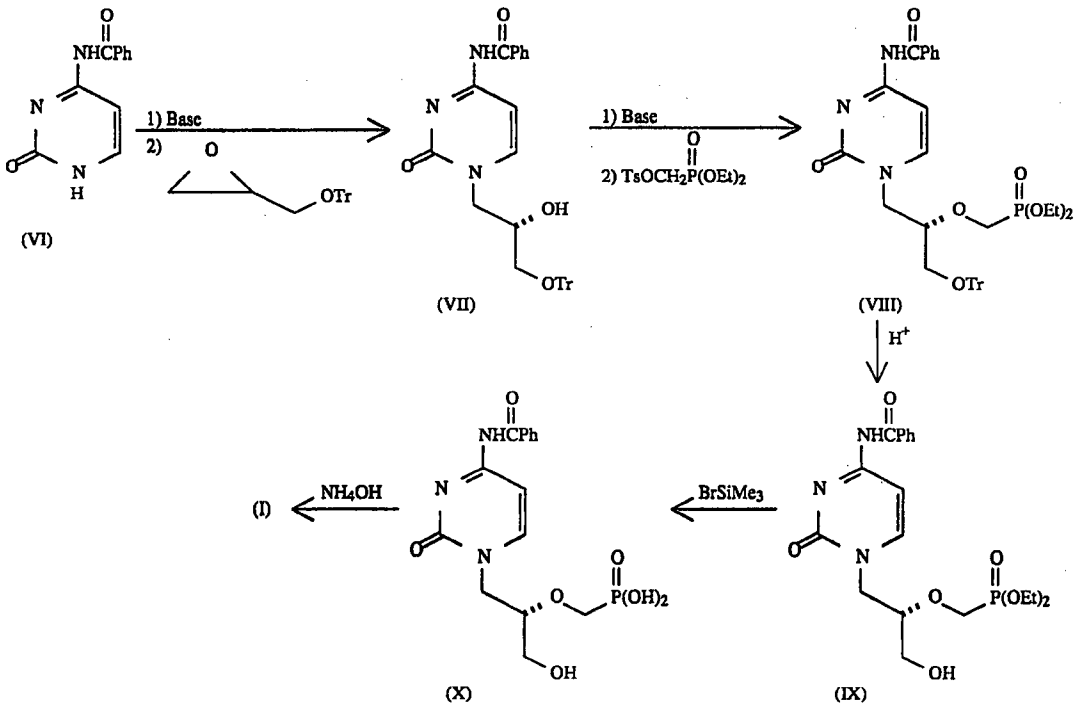
Thus, a compound of formula (IIIb) is first treated with a base to generate the corresponding alkoxide anion. The base may be a metal hydride, for example sodium hydride, potassium hydride or lithium hydride; and metal alkoxides, for example, potassium t-butoxide or sodium methoxide and the like. The reaction mixture containing the alkoxide anion is then treated with the methanephosphonate $LCH_2P(O)(OR^2)_2$ (IV) wherein L is a leaving group and R^2 is an alkyl group containing 1–5 carbon atoms as previously defined to provide the protected HPMP nucleoside of formula (V). L is preferably selected from the group consisting of p-toluenesulfonate (tosylate), methanesulfonate (mesylate), and trifluoromethanesulfonate (triflate); and R^2 is preferably an alkyl group having from 1–3 carbon atoms, e.g., methyl, ethyl, n-propyl, and isopropyl.

The third step of the process involves the removal of the phosphonic protecting group, i e R^2 the hydroxy protecting group, and if present, any protecting groups on the purine or pyrimidine base. The phosphonate may be converted to the parent acid by treatment with a trialkylsilyl halide such as trimethylsilyl bromide or trimethylsilyl iodide, and optionally followed by the addition of water. Methods to be employed for the removal of the hydroxy protecting group, and if present, protecting groups on the purine or pyrimidine base will of course depend on the nature of the protecting group; examples of typical deprotecting techniques include acid or base catalyzed hydrolysis, hydrogenation, or metal mediated deprotection.

In a preferred embodiment of the present process, the reaction sequence is conveniently carried out from the starting material to the end product without isolating and purifying the intermediate compounds formed. The elimination of the need for costly and labor intensive isolation and purification of intermediates represent a marked improvement over prior processes. Another advantage of the present invention is that the stereochemistry of the glycidol reactant is maintained throughout the process such that end product having the desired stereo configuration is obtained without racemization.

The process of the present invention, while adaptable to the synthesis of a wide variety of HPMP substituted purine and pyrimidine bases, is especially applicable to the synthesis of hydroxyphosphonomethoxypropyl cytosine (HPMPC); particularly (S)-HPMPC. A preferred embodiment of the present process suitable for the preparation of (S)-HPMPC is illustrated in Scheme II.

Scheme II



In Scheme II, Tr is triphenylmethyl and Ts is tosyl. N^4-Benzoylcytosine (VI) is converted to its anionic form by treatment with a base in an aprotic polar organic solvent at elevated temperature; suitable bases are for example sodium hydride, potassium t-butoxide, potassium or sodium carbonate, and the like; suitable solvents are for example dimethylformamide, N-methyl-2-pyrrolidinone, dimethyl sulfoxide, hexamethylphosphoramide, and the like; and typical reaction temperature ranges from about 70° C. to about 150° C. Subsequently, (S)-[(triphenylmethoxy)methyl]oxirane is added to the above reaction solution and the solution maintained at the elevated temperature to effect the formation of (S)-N^4-benzoyl-N^1-[(2-hydroxy-3-triphenylmethyl)propyl]-cytosine (VII).

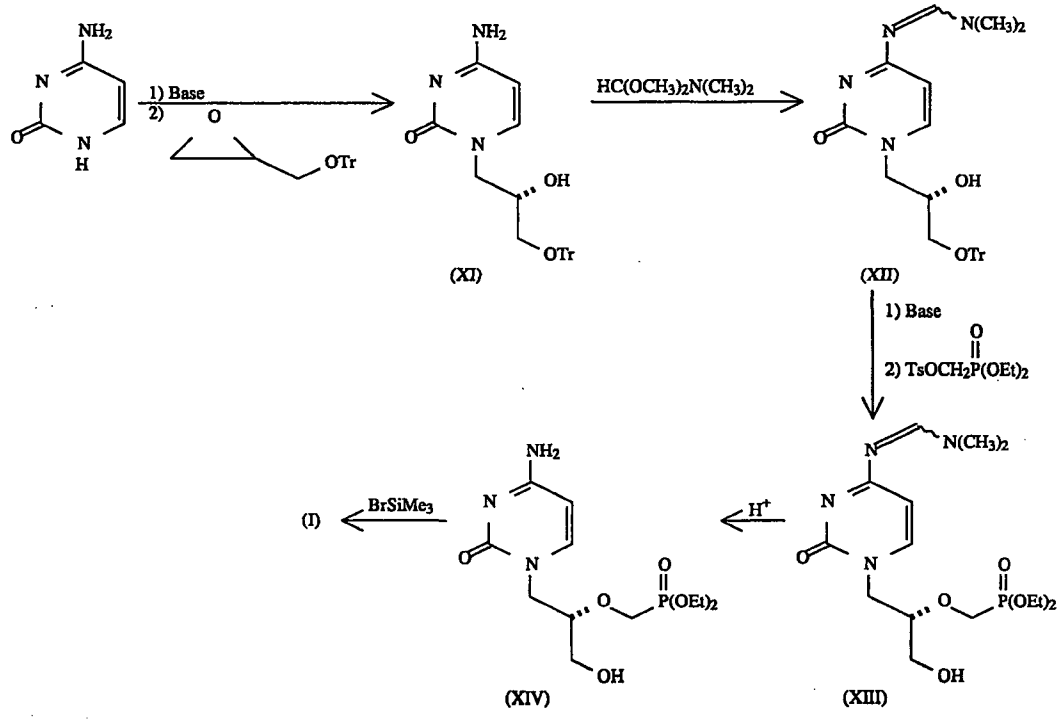
The above obtained diprotected (2,3-dihydroxy)propyl cytosine is treated with a metal hydride, e.g. sodium hydride, at ice bath temperature, and then treated with diethyl tosyloxymethylphosphonate to provide the compound (S)-N^4-benzoyl-N^1-[[(2-diethylphosphonylmethoxy)-3-triphenylmethyl]propyl]cytosine (VIII).

Next, the trityl protecting group is removed to provide the compound of formula (IX) by treating the above obtained compound (VIII) with an acidic medium, for example with hydrochloric acid at about 0°–5° C. A wide range of other acids may be employed to accomplish this step, and examples include, acetic acid, formic acid, trifluoroacetic acid, zinc bromide, acidic ion exchange resins, to name but a few. Suitable reaction temperature, and time may be readily ascertained by a person skilled in the art.

Following detritylation, the resulting compound (X) is treated with a trialkylsilyl halide such as trimethylsilyl bromide at room temperature to convert the diethyl phosphonate to the phosphonic acid. This latter compound is then treated with a base such as ammonium hydroxide to remove the benzoyl protecting group to afford the desired end product (S)-HPMPC.

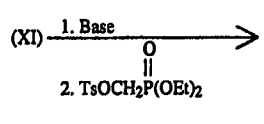
Another preferred process for the preparation of (S)-HPMPC is illustrated in Scheme III.

Scheme III



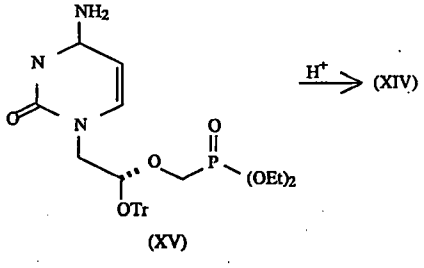
In Scheme III, cytosine is coupled with (S)-(triphenylmethoxy)methyl]oxirane in the presence of a base such as one previously enumerated to give cytosine derivative of formula (XI). The 4-amino group of compound (XI) is then converted to the corresponding dimethyl formamidine derivative (XII) upon treatment with dimethylformamide or an acetal thereof. Compound (XII) is subjected to base promoted alkylation with diethyl tosyloxymethylphosphonate as previously described to provide compound of formula (XIII). Compound (XIII) is deprotected in a acidic medium and the product thereof is treated with e.g. trimethylsilyl bromide to afford (S)-HPMPC. In another preferred process for the preparation of (S)-HPMPC, as illustrated in Scheme IV, cytosine derivative of formula (XI) is treated with a base, followed by diethyl tosyloxymethylphosphonete to afford the compound of formula (XV). The latter compound is treated with an acid to remove the trityl protecting group and affords the compound of formula (XIV) which is converted to (S)-HPMPC as previously described.

Scheme IV



-continued

Scheme IV



Another aspect of the present invention concerns novel intermediates in the synthesis of (S)-HPMPC. These include compounds of formulas (VII), (VIII), (IX), (X), (XII), (XIII), and (XV).

The process of this invention is illustrated in greater detail by the following examples which are not to be construed to limit the scope of the invention in any manner.

Preparation I. (±)-Triphenylmethoxymethyloxirane

Trityl chloride (18.816 g, 0.067 mol) was added to a stirred solution of (±)-glycidol (5 g, 0.067 mol) and triethylamine (13.84 g, 0.137 mol) in anhydrous methylene chloride (54 ml). After 15 hours of stirring at room temperature, the reaction solution was washed with water (2×10 ml) and brine (20 ml). The organic phase was evaporated after drying over anhydrous Na_2SO_4 to give a yellow foam which was purified on silica gel (5% EtOAc in hexane) to afford the title compound (17.64 g, 82.6%) as a solid. ^1H NMR ($CDCl_3$): 2.62 (dd, J=2.4 and 5.2 Hz, 1H), 2.77 (t, J=4.5 Hz, 1H), 3.08–3.18 (m, 2H), 3.29–3.80 (m, 1H), 7.20–7.38 (m, 3H), 7.45–7.52 (m, 2H).

Preparation II. (S)-Triphenylmethoxymethyloxirane

A 5 L 3-neck round bottom flask was charged with trityl chloride (133.8 g, 0.48 mol) and methylene chloride (400 ml). It was cooled to 0° C. under N_2 and treated with triethylamine (70.7 g, 0.70 mol). After an hour of stirring at 0° C., a solution of (R)-glycidol (88% ee, 37.03 g, 0.5 mol) in methylene chloride (100 ml) was added over 0.75 hour. The resulting solution was allowed to warm to ambient temperature and was stirred for 3 hours. It was then filtered, and the filtrate was washed with water (2×500 ml) and brine (2×500 ml). The organic phase was dried over $MgSO_4$ and concentrated to a foam, which on crystallization from isopropyl alcohol gave the title compound (116.2 g, 76.5%) as an off-white powder.

$[\alpha]_D$=–6.01 (C=1, MeOH).

EXAMPLE 1

Preparation of (±)-N^1-[(2,3-dihydroxy)propyl]cytosine

Cytosine (0.55 g, 4.95 mmol), (±)-glycidol (0.404 g, 5.45 mmol), and anhydrous potassium carbonate (5 mg, 0.04 mmol) in dry DMF (6 ml) were stirred at 71° C. for 3 hours. Glycidol (4) was totally reacted according to TLC of the reaction mixture. The DMF was distilled off under high vacuum, and the resulting yellowish thick liquid was absorbed on silica gel (3 g). This was placed on top of a silica gel column, which was eluted with 20% MeOH in ethyl acetate to give a mixture (0.540 g) of the title compound and a polymer derived from glycidol. Crystallization from ethanol afforded the title compound (0.44 g, 52.3%) as a solid. MP: 169°—71° C. UV: λ_{max}274 nm (ϵ=8,083). ^{1}H NMR (DMSO-d_6): 3.11–3.47 (m, 3H), 3.55–3.75 (m, 1H), 3.88 (dd, J=3.3 and 13.3 Hz, 1H), 4.71 (t, J=5.8 Hz, 1H), 4.95 (d, J=5.3 Hz, 1H), 5.61 (d, J=7.1 Hz, 1H), 7.00 (bd, J=23.7 Hz, 2H) , 7.44 (d, J=7.1 Hz, 1H) .

Analysis calcd. for $C_7H_{11}N_3O_3 \cdot 0.5H_2O$: C, 43.30; H, 6.23; N, 21.63 Found: C, 43.33; H, 5.92; N, 21.38

EXAMPLE 2

Preparation of (S)-N1-[(2,3-dihydroxy)propyl]cytosine

Reaction of cytosine (2.2 g, 19.8 mmol) with (R)-glycidol (88% ee, 1.51 ml, 22.8 mmol) in the presence of anhydrous potassium carbonate (40 mg, 0.289 mmol) in dry DMF (20 ml) at 72° C. for 5 hours, as described in Example 1, furnished the title compound (88% ee) in 43.1% yield.

EXAMPLE 3

Preparation of (±)-N^1-[(2-hydroxy-3-triphenylmethoxy)propyl]cytosine by tritylation of (±)-N^1-[(2,3-dihydroxy)propyl]cytosine

(a) using 1.1 eq. of glycidol

A mixture of cytosine (0.55 g, 4.95 mmol), (±)-glycidol (0.362 ml, 5.46 mol), and anhydrous potassium carbonate (5 mg) in dry DMF (5 ml) was stirred at 71° C. for 3 hours. It was cooled to room temperature and treated with DMAP (0,031 g, 0.25 mmol), dry pyridine (0.783 g, 9.9 mmol), and trityl chloride (1.48 g, 5.2 mmol). The resulting reaction mixture was stirred at 80° C. for 3 hours and at room temperature for 17 hours. It was diluted with ethyl acetate (60 ml), washed with saturated sodium bicarbonate (2×15 ml), water (15 ml), and brine (15 ml), and dried over $MgSO_4$. The ethyl acetate was evaporated to give a crispy foam (1.98 g), and purification by chromatography over silica gel (10–15% methanol in ethyl acetate) furnished the title compound as a crystalline solid (0.74 g, 35%). MP: 227°–228° C. UV: λ_{max}274 nm (ϵ=7,149). ^{1}H NMR (DMSO-d_6): 2.81–2.98 (m, 2H), 3.26–3.42 (m, 1H), 3.85–3.97 (m, 1H), 4.02 (dd, J=4.7 and 14.2 Hz, 1H), 5.23 (d, J=5.8 Hz, 1H), 5.54 (d, J=7.1 Hz, 1H), 7.93 (bd, 2H), 7.1–7.29 (m, 16H).

(b) using 1.5 e.g. of glycidol

Cytosine (0.275 g, 2.48 mmol), (±)-glycidol (0,281 g, 3.7 mmol), and anhydrous potassium carbonate (2.5 mg, 0,018 mmol) in dry DMF (2.5 ml) were stirred at 70° C. for 1.5 hours. The DMF was distilled under reduced pressure. PMR of the resulting solid showed that it contained (±)-N^1-[(2,3-dihydroxy)propyl]cytosine and cytosine in 89:11 ratio.

The above solid was dissolved in dry pyridine (4 ml) and trityl chloride (0.602 g, 2.14 mmol) and DMAP (13 mg) were added successively at room temperature. After 3 hours of stirring at 85° C. followed by work-up as described in Example 3 (a), supra, a foamy solid (0.88 g) was obtained. Crystallization from methylene chloride and toluene gave the title compound (0.360 g 34%). The mother liquor was concentrated and purified by silica gel chromatography (10% methanol in ethyl acetate) to give the title compound (60 mg, 5.7%) and (±)-N^1-[[2-[(2-hydroxy-3-triphenylmethoxy)propyloxy]-3-triphenylmethoxy]propyl]cytosine (hereinafter referred to as the dimer) (10 mg, 0.8%).

(c) using 2 eq. of glycidol

The above experiment was repeated using 2 eq. of (±)-glycidol to provide the title compound in 39.6% yield and the dimer in 1.6% yield.

EXAMPLE 4

Preparation of (±)-N^1-[(2-hydroxy-3-triphenylmethoxy)propyl]cytosine by reaction of cytosine and (±)-triphenylmethoxymethyloxirane

(a) using a catalytic amount of NaH

Cytosine (80 mg, 0.72 mmol) was added to a stirred suspension of 80% NaH (4 mg, 0.13 mmol) in dry DMF (3 ml). After an hour at room temperature, (±)-trityloxymethyloxirane (0.19 g, 0.6 mmol) was added to the reaction mixture, and stirring continued at 106° C. for 5 hours. The reaction was completed as indicated by TLC of the reaction mixture. It was cooled, and the DMF was distilled off under vacuum. The resulting solid was partitioned between ethyl acetate (20 ml) and water (2 ml). The organic phase was separated, washed once again with water (5 ml), and dried over Na_2SO_4. Evaporation of the ethyl acetate gave the brown solid (0.28 g) which was crystallized from methylene chloride-toluene (2 ml and 30 ml) to furnish the title compound (0.21 g) in 81.7% yield.

(b) using one equivalent of NaH

Carrying out the above reaction using (±)-trityloxymethyloxirane (0.190 g, 0.6 mmol), cytosine (67 mg, 0 6 mmol), and sodium hydride (80% 18 mg 0 6 mmol) in anhydrous DMF (4 ml) furnished the title compound (60 mg) in 23.3% yield.

c. using K_2CO_3 instead of NaH

Following the procedure described above, the title compound was obtained in 82% yield from (±)-trityloxymethyloxirane (0.19 g, 0.6 mmol) and cytosine (0.08 g, 0.72 mmol) in the presence of potassium carbonate (10 mg, 0.072 mmol) in anhydrous DMF (3 ml).

EXAMPLE 5

Preparation of (±)-N^4-benzoyl-N^1-[(2-hydroxy-3-triphenylmethoxy)propyl]cytosine

(a) using 1 equivalent of N^4-benzoylcytosine

Treatment of N^4-benzoylcytosine (0.388 g, 1.803 mmol) with (±)-trityloxymethyloxirane (0.571 g, 1.805 mmol) in

the presence of 80% sodium hydride (12 mg, 0.4 mmol), according to the procedure of Example 4 (a), gave the title compound as a crystalline solid in 72.9% yield after chromatography over silica gel using hexane-EtOAc (1:3). MP: 105°–7° C. UV: λ_{max}259 nm (ε=23,500), 306 nm (ε=10, 380). ^{1}H NMR ($CDCl_3$): 3.05–3.18 (m, 1H), 3.21–3.33 (m, 1H), 3.66–3.90 (m, 1H), 4.2 (bS, 1H), 4.35 (d, J=13.6 Hz, 1H), 7.13–7.72 (m, 15H), 7.88 (d, J=7.5 Hz, 1H), 8.73 (bS, 1H).

Analysis calcd. for $C_{33}H_{29}N_3O_4$: C, 74.56; H, 5.50; N, 7.90 Found: C, 74.02; H, 5.67; N, 7.63

(b) using 1.2 equivalents of N^4-benzoylcytosine

(±)-Trityloxymethyloxirane (0.762 g, 2.41 mmol) was reacted with N^4-benzoylcytosine (0.621 g, 2.89 mmol) in the presence of 80% sodium hydride (16 mg, 0.53 mmol) in dry DMF, as described above, to obtain the title compound in 85% yield.

EXAMPLE 6

Preparation of (S)-N^4-benzoyl-N^1-[(3-allyloxy-2-hydroxy)propyl]cytosine

To sodium hydride (80%, 12 mg, 0.4 mmol) stirring in anhydrous DMF (4.5 ml) at room temperature was added N^4-benzoylcytosine (0.466 g, 2.17 mmol). The reaction mixture was stirred for an hour and treated with (S)-allyloxymethyloxirane (0.206 g, 1.8 mmol). It was then heated at 105° C. for 6 hours, cooled, and concentrated in vacuo. The resulting orange red gummy material was treated with water (5 ml) and ethyl acetate (20 ml). It was stirred for 5 minutes, and the insoluble solid (0.145 g, 31.1% of recovery) was collected by filtration and identified as N^4-benzoylcytosine. The filtrate was transferred into a separatory funnel, and the ethyl acetate layer was separated. It was washed with water (3×5 ml), dried over Na_2SO_4, and evaporated to obtain 0.423 g of pale-yellow solid. Slurrying of this material in diethyl ether gave the title compound (0.331 g) in 55.7% yield. The ether filtrate was evaporated, and the resulting light greenish gummy material was purified by flash chromatography on silica gel (0–5% MeOH in EtOAc) to furnish the title compound (20 mg) in 3.6% yield. MP: 139°–41° C. $[\alpha]_D$=–55.06 (C=1.155, MeOH). UV: λ_{max}259 nm (ε=21,500), 305 nm (ε=10,120). ^{1}H NMR ($CDCl_3$): 3.4–3.56 (m, 2H), 3.77 (dd, J=7.6 and 13.5 Hz, 1H), 3.98 (d, J=5.7 Hz, 3H), 4.16–4.25 (m, 1H), 4.28 (dd, J=2.7 and 13.5 Hz, 1H), 5.10–5.25 (m, 2H), 5.79–5.92 (m, 1H), 7.39–7.62 (m, 4H).

Analysis calcd. for $C_{17}H_{19}N_3O_4$: C, 61.97; H, 5.85; N, 12.79 Found: C, 61.82; H, 6.05; N, 12.77

EXAMPLE 7

Preparation of (±)-N^4-benzoyl-N^1-[(3-allyloxy-2-hydroxy)propyl]cytosine

The title compound was prepared in 39.5% yield, following the procedure of Example 6, from (±)-allyloxymethyloxirane (9.02 g, 0.079 mol), N^4-benzoylcytosine (20.40 g, 0.095 mol), and 80% sodium hydride (0.526 g, 0.018 mol) in dry DMF (241 ml).

EXAMPLE 8

Preparation of (S)-N^4-benzoyl-N^1-[(3-benzyloxy-2-hydroxy)propyl]cytosine

(R)-benzyloxymethyloxirane (0.296 g, 1.8 mmol) in dry DMF (0.5 ml) was added to sodium salt of N^4-benzoylcytosine, prepared from N^4-benzoylcytosine (0.388 g, 1.8 mmol) and 80% sodium hydride (0.012 g, 0.4 mmol) in dry DMF (4 ml) at room temperature for an hour, and stirred at 110° C. for 6 hours. The reaction was complete as confirmed by HPLC of the reaction. Most of the DMF was distilled off under reduced pressure. The resulting gummy product was partitioned between ethyl acetate (20 ml) and water (5 ml). The ethyl acetate layer was separated, washed with water (3×10 ml), dried over Na_2SO_4, and evaporated to give a yellow product (0.595 g). Trituration with ethyl acetate furnished the title compound (0.392 g) in 57.3% yield. The mother liquor was concentrated and chromatographed on silica gel (ethyl acetate) to give the title compound (50 mg, 7.3%). MP: 138° C. $[\alpha]_D$=–49.19 (C=1.425, MeOH). UV: λ_{max} 259 nm (ε=22,440), 306 nm (ε=10,220). ^{1}H NMR ($CDCl_3$): 3.45–3.58 (m, 3H), 3.79 (dd, J=7.1 and 13.3 Hz, 2H), 4.16–4.33 (m, 2H), 4.51 (s, 2H), 7.2–7.6 (m, 8H), 7.69 (d, J=7.2 Hz, 1H), 7.89 (d, J=7.4 Hz, 2H), 8.91 (bS, 1H).

Analysis calcd. for $C_{21}H_{21}N^3O_4.0.9H_2O$: C, 63.76; H, 5.81; N, 10.62 Found: C, 63.97; H, 5.50; N, 10.63

Example 9

Preparation of (±)-N^4-benzoyl-N^1-[(3-benzyloxy-2-hydroxy)propyl]cytosine

Treatment of (±)-benzyloxymethyloxirane (6.0 g, 0.0365 mol) with N^4-benzoylcytosine (9. 446 g, 0.0439 mol) in the presence of sodium hydride (80% pure, 0.263 g, 8.1 mmol) in dry DMF (85 ml), according to the procedure of Example 8, afforded the title compound (8.2 g) in 59.2% yield. MP: 144°–6° C.

EXAMPLE 10.

Preparation of (±)-N^1-[(2-diethylphosphonylmethoxy-2-triphenylmethoxy)propyl]cytosine

One Pot Synthesis: A mixture of cytosine (0,134 g, 1.21 mmol) and 80% sodium hydride (8 mg, 0.27 mmol) in anhydrous DMF (3 ml) was stirred at room temperature. After 1 hour, (±)-trityloxymethyloxirane (0.38 g, 1.2 mmol) was added in 1 portion, and stirring was continued for 5 hours at 105° C. The formation of (±)-N^1-[(2-hydroxy-3-trityloxy)propyl] cytosine was noted by its HPLC.

The above homogenous reaction solution was cooled in an ice bath and successively treated with 80% sodium hydride (0.100 g, 3.3 mmol) and diethyl tosyloxymethylphosphonate (85% pure, 0.682 g, 1.8 mmol). After being stirred at 0° C. for 0.5 hour and at room temperature for 15 hours, a few drops of ethanol were added to quench excess sodium hydride. The solvent was removed under reduced pressure, and the resulting orange residue was partitioned between ethyl acetate (30 ml) and water (5 ml). The organic phase was separated and washed with saturated sodium bicarbonate (10 ml) and brine (10 ml). After drying over Na_2SO_4, the ethyl acetate was evaporated to give the orange-colored product (0.550 g), which was purified by chromatography on silica gel (10–15% MeOH in CH_2Cl_2) to furnish the title compound (0.26 g, 37.4%) as a foamy solid. ^{1}H NMR ($CDCl_3$): 1.25 (t, J=7 Hz, 6H), 2.97–3.12 (m, 1H), 3.33 (dd, J=3 and 10.5 Hz, 1H), 3.55–3.68 (m, 2H), 3.84–3.96 (m, 2H), 3.96–4.24 (m, 5H), 5.63 (d, J=6.9 Hz, 1H), 7.0–7.6 (m, 18H).

EXAMPLE 11

Preparation of (±)-N^1-[(2-dimethylphosphonylmethoxy-3-triphenylmethoxy)propyl]cytosine

Repeating the experiment of Example 10, using dimethyl tosyloxymethylphosphonate instead of diethylphosphonate afforded the title compound in 15.6% yield. ^{1}H NMR

($CDCl_3$): 3.0–3.1 (m, 1H), 3.27 (dd, J=2.8 and 10.5 Hz, 1H), 3.51–4.26 (m, 10H), 4.17 (dd, J=3.0 and 13.6 Hz, 1H), 5.69 (d, J=6.9 Hz, 1H), 7.0–7.66 (m, 18H).

EXAMPLE 12

Preparation of (±)-N^1-[(2-diethylphosphonylmethoxy- 3-hydroxy)propyl]cytosine

Treatment of cytosine (0.249 g, 2.24 mmol) with (±)-trityloxymethyloxirane (0.590 g, 1.87 mmol) in the presence of a catalytic amount of 80% sodium hydride (13 mg, 0.44 mmol) followed by in situ alkylation of the intermediate with diethyl tosyloxymethylphosphonate (85.1% pure, 1.06 g, 2.80 mmol) in the presence of 80% sodium hydride (0.099 g, 3.3 mmol), according to the procedure of Example 10, gave the crude (±)-N^1-[(2-diethylphosphonylmethoxy-3-trityloxy)propyl] cytosine (1.267 g).

To the above nucleotide, 80% acetic acid (20 ml) was added and stirred at 95° C. for 3 hours. Water (20 ml) was added to the reaction which was then cooled to –0° C. The precipitated trityl alcohol was collected by filtration. The filtrate was evaporated, and the resulting thick product was co-distilled with water (3×30 ml) and with toluene (3×30 ml) to remove acetic acid. It was then applied on a silica gel column which, on elution with 15% MeOH in CH_2Cl_2, afforded the title compound (0.147 g, 23.5%) as a gummy material. Further elution of the column with 20% MeOH in CH_2Cl_2 gave (±)-N^1-[(2,3-dihydroxy)propyl]cytosine (20.2 mg, 6%). ^1H NMR (MeOH-d_4): 1.30 (t, J=7.1 Hz, 3H), 1.31 (t, J=7.1 Hz, 3H), 3.5–3.63 (m, 1H), 3.67–3.92 (m, 4H), 3.97–4.22 (m, 6H), 5.85 (d, J=7.2 Hz, 1H), 7.53 (d, J=7.2 Hz, 1H).

EXAMPLE 13

Preparation of (±)-N^1-[(2-diethylphosphonylmethoxy- 3-hydroxy)propyl]cytosine via formamidine method

A mixture of the sodium salt of cytosine, obtained from cytosine (0.134 g, 1.21 mol) and 80% sodium hydride (8 mg, 0.27 mmol) in dry DMF (3 ml) at room temperature for 1 hour, and (±)-trityloxymethyloxirane (0.38 g, 1.2 mmol) was stirred at 110° C. for 5 hours. The resulting solution of 12 was cooled to room temperature, and DMF dimethyl acetal (0.286 g, 2.4 mmol) was added in 1 portion. It was then stirred at 85° C. for 1.5 hours and concentrated under reduced pressure to ca. 1 ml of the crude dimethylformamidine derivative of 11. This and diethyl tosyloxymethylphosphonate (0.909 g, 2.4 mmol) in anhydrous DMF (3 ml) was cooled to 0° C. and treated with 80% sodium hydride (64 mg, 2.13 mmol). The resulting yellow reaction mixture was stirred at 0° C. for 1.5 hours and at room temperature for 14 hours. The crude product obtained after work-up is a mixture of (±)-N'-[(2-diethylphosphonylmethoxy-3-trityloxy)propy] cytosine and its N^4-dimethylformamidine derivative. This mixture was dissolved in 80% acetic acid (11 ml) and refluxed for 3 hours. After work-up, the yellowish gummy product (0.693 g) was obtained which, on purification by chromatography on silica gel (15% MeOH in EtOAc), afforded the title compound (0.175 g) in 43.6% yield.

EXAMPLE 14

Preparation of (±)-N^1-[(2-ethylhydrogenphosphonylmethoxy- 3-hydroxy)propyl]cytosine

2N Sodium hydroxide solution (4.5 ml) was added to (±)-N^1-[(2-diethylphosphonylmethoxy-3-hydroxy)propyl] cytosine (0.230 g, 0.69 mmol). TLC of the reaction after 1.25 hours at room temperature showed that starting material was completely consumed. The reaction was acidified with Dowex 50×8 (H^+) and filtered. The resin was washed with 20 ml of water. The combined filtrate was evaporated to provide the title compound (0.163 g) in 77.3% yield. ^1H NMR (D_2O): 1.26 (t, J=7.1 Hz, 3H) , 2.52–2.68 (m, 2H), 3.75–4.0 (m, 6H), 4.21 (dd, J=2.8 and 14.1 Hz, 1H), 6.19 (d, J=7.6 Hz, 1H), 7.88 (d, J=7.6 Hz, 1H). MS: molecular ion (m/e) for $C_{10}H_{18}N_3O_6P$,308.1011: Found: 308. 1009

EXAMPLE 15

Preparation of (S)-N^4-benzoyl-N^1-[(2-hydroxy-3-triphenylmethoxy)propyl] cytosine

(a) To N^4-benzoylcytosine (100.1 g, 0.47 mol) in dry DMF (1,000 ml) at 100° C. under N_2 was added 80% sodium hydride (3.0 g, 0.10 mol) in 1 portion, and the slurry was stirred for 0.25 hour. (S)-trityloxymethyloxirane (88% ee, 125.1 g, 0.40 mol) was added and further stirred at 110° C. for 4 hours. The reaction was completed as confirmed by its HPLC. The reaction mixture was filtered and used in the subsequent reaction without further purification. The filtrate contained a 90% in solution yield of the title compound based on HPLC.

(b) In a separate experiment, the crude product obtained from the above reaction was purified by chromatography on silica gel (3–5% MeOH in CH_2Cl_2) and provided analytically pure title compound. MP: 105°–7° C. UV: λ_{max} 259 nm (ε=23,500), 306 nm (ε=10,380). ^1H NMR ($CDCl_3$): 3.05–3.18 (m, 1H), 3.21–3.33 (m, 1H), 3.66–3.90 (m, 1H), 4.2 (bS, 1H), 4.35 (d, J=13.6 Hz, 1H), 7.13–7.72 (m, 15H), 7.88 (d, J=7.5 Hz, 1H), 8.73 (bS, 1H).

Analysis calcd. for $C_{33}H_{29}N_3O_4$: C, 74.56; H, 5.50; N, 7.90 Found: C, 74.02; H, 5.67; N, 7.63

(c) The reaction described in (a) supra was also repeated using solvents and conditions to afford the title compound.

(1) NaH, NMPO, 70°–80° C. for 2 hours followed by 100°–104° for 3.5 hours.

(2) NaH, 18-crown-6, DMF, 103° C., 5 hours.

(3) NaH, benzyltriethylammonium chloride, DMF, 70° C. for 6 hours, 105° C. for 4 hours.

(4) $KOC(CH_3)_3$, DMF, 70° C. for 16 hours followed by 105° C. for 8 hours.

EXAMPLE 16

Preparation of (S)-N^4-benzoyl-[(diethylposphonylmethoxy-3-triphenylmethoxy)propyl]-cytosine

(a) A solution of the crude (S)-N^4-benzoyl-N^1-[(2-hydroxy- 3-triphenylmethoxy)propyl]cytosine in DMF, obtained in Example 15 (a), was placed in a 5 L 3-neck round bottom flask and cooled to 0° C. 80% sodium hydride (32.4 g, 1.06 mol) was added in 2 portions, and an exotherm of 8° C. was noted. Immediately, diethyl tosyloxymethylphosphonate (80%. pure, 215.6 g, 0.54 mol) was added, and the reaction was completed after 6 hours of stirring. The reaction was diluted with ethyl acetate (2 L), quenched with water, washed with water (2×1 L) and saturated $NaHCO_3$ (1 L), dried over $MgSO_4$, and concentrated to afford crude title compound (230.1 g) with 2% of (S)-trityloxymethyloxirane, as indicated by its proton NMR spectrum. This crude product was used in the next procedure without further purification.

(b) In a separate experiment, a small amount of the crude product was purified by column chromatography on silica gel (1–3% MeOH in CH_2Cl_2) to provide an analytical sample of the title compound. ^1H NMR (DMSO-d_6): 1.14 (t,

J=7 Hz, 3H), 1.16 (t, J=7 Hz, 3H), 2.94–2.98 (m, 1H), 3.24–3.31 (m, 1H), 3.58–4.09 (m, 9H), 7.23–7.63 (m, 19H), 7.98 (d, J=7 Hz, 3H), 11.19 (bS, 1H).

Analysis calcd. for $C_{38}H_{40}N^3O_7P.0.5H_2O$: C, 66.07; H, 5.98; N, 6.08 Found: C, 65.96; H, 5.84; N, 6.09

EXAMPLE 17

Preparation of (S)-N^4-benzoyl-N^1-[(2-diethylphosphonylmethoxy- 3-hydroxy)propyl]cytosine

(a) Hydrogen chloride gas was bubbled into a solution of the crude (S)-N^4-benzoyl-N^1-[(2-diethylphosphonyl-3-triphenylmethoxy)propyl] cytosine (230.1 g, obtained from Example 16) in methylene chloride (1.2 L) at 0°–5° C. until starting material was consumed as determined by HPLC (ca 10 minutes). Water (500 ml) was added, and the resulting 2-phase mixture stirred rigorously for 5 minutes. The organic phase was separated and extracted with 10% hydrochloric acid (2×250 ml). The combined aqueous solution was cooled to 0°–5° C., adjusted to pH=8 with 40% sodium hydroxide solution, and then extracted with CH_2Cl_2 (2×500 ml). The combined CH_2CL_2 solution was dried over $MgSO_4$ and concentrated in vacuo to give crude title compound (96.2 g), as a viscous oil, in 55% yield from (S)-trityloxymethoxyoxirane after 3 steps. ^1H NMR (DMSO-d_6): 1.16 (t, J=7 Hz, 3H), 1,18 (t, J=Hz, 3H), 3.44–3.57 (m, 2H), 3.68–3.80 (m, 3H), 3.88–4.01 (m, 5H), 4.13 (dd, J=8 and 17 Hz, 1H), 4.88 (t, J=6 Hz, 1H), 7.27 (br d, J=7 Hz, 1H), 7.49 (t, J=7 Hz, 2H), 7.60 (t, J=7 Hz, 1H), 7.98 (d, J=7 Hz, 3H), 11.18 (br s, 1H).

Analysis calcd. for $C_{19}H_{26}N^3O_7P.0.5H_2O$: C, 50.89; H, 6.07; N, 9.37 Found: C, 50.99; H, 6.03; N, 9.32

(b) Detritylation was carried out with the following reagents and conditions to afford the title compound in moderate to excellent yields:

(1) 80% acetic acid, 75° C., 45 minutes.

(2) 80% acetic acid, 100° C., 30 minutes.

(3) 80% acetic acid, 60° C., 3 hours.

(4) 80% formic acid, 0°–5° C., 30 minutes.

(5) 95–97% formic acid, room temperature, 5 minutes.

(6) Trifluoroacetic acid, n-butanol or isopropyl alcohol, or CH_2Cl_2, 22 hours.

(7) $ZnBr_2$, CH_2Cl_2, room temperature, 10 minutes-3 hours.

(8) Amberlyst 15 (H^+), MeOH, 50° C., 24 minutes.

(9) Amberlyst 15 (H^+) activated by HCl/MeOH wash, 50° C., 6.5 hours.

(10) Dowex 50×8 (H^+) activated by HCl/MeOH.

EXAMPLE 18

Preparation of (S)-N^4-benzoyl-N^1-[(3-hydroxy-2-phosphonylmethoxy)propy] cytosine

A solution of (S)-N^1-[(2-diethylphosphonylmethoxy-3-hydroxy)propyl-N^4]-benzoylcytosine (188 g, 0.428 mol) in methylene chloride (1.2 L) at room temperature under argon was treated with bromotrimethylsilane (200 ml, 1.52 mol), and the resulting mixture was stirred for 18 hours. It was then concentrated in vacuo to a residue which was redissolved in methylene chloride (500 ml) and reconcentrated to furnish the crude persilylated title compound (289 g) as a tan foam. This material was used in the next step without further purification. An analytical sample of the title compound was prepared by treating the crude foam with water from which the desired title compound crystallized. ^1H NMR (DMSO-d_6): 3.45–3.81 (m, 6H), 4.11 (dd, J=4 and 13 Hz, 1H), 7.26 (d, J=7 Hz, 1H), 7.49 (t, J=7 Hz, 2H), 7.61 (t, J=7 Hz, 1H), 7.98 (d, J=7 Hz, 2H), 8.04 (d, J=7 Hz, 1H).

Analysis calcd. for $C_{15}H_{18}N_3O_7P.0.5H_2O$: C, 45.93; H, 4.84; N, 10.71 Found: C, 46.04; H, 4.67; N, 10.71

EXAMPLE 19

Preparation of (S)-N^1-[(3-hydroxy-2-phosphonylmethoxy] propyl] cytosine

The crude persilyated (S)-N^4-benzoyl-N^1-[(3-hydroxy-2-phosphonylmethoxy)propyl] cytosine (289 g), obtained from the previous example, was dissolved on conc NH_4OH (850 ml) and stirred at room temperature for 4 hours. The aqueous reaction mixture was extracted with CH_2Cl_2 (2×600 ml) to remove most of the benzamide and then filtered and concentrated in vacuo until the pH of the aqueous solution was neutral. The concentrated solution was diluted with water to a volume of 800 ml, and ethanol (600 ml) was added. The product was precipitated by adjusting the pH to 3.0 with careful addition of conc HCl (65 ml). The resulting thick slurry was stirred at room temperature for 1 hour and then stored at 0°–5° C. for 16 hours. The solid product was collected by filtration, washed with water ethanol (2:1, 2×150 ml), and dried to constant weight in vacuo at 40° C. to give (S)-HPMPC (105 g) in 78% yield from 22S after 2 steps. This material contained 5% of the undesired (R)-isomer as determined by chiral HPLC. Two crystallizations of the crude product by adjusting an aqueous slurry to pH=6 with 40% NaOH solution, followed by reprecipitation with conc HCl to pH=3, reduced the level of the undesired (R)-isomer to 2.4, a 90% weight recovery. MP: 260° C. (decomp). $[\alpha]_D$=–86.65 (C=0.40, H_2O). ^1H NMR (D_2O): d 3.59–3.67 (m, 2H), 3.79–3.94 (m, 4H), 4.20 (dd, J=3 and 14 Hz, 1H), 6.17 (d, J=8 Hz, 1H), 7.90 (d, J=8 Hz, 1H).

Analysis calcd. for $C_8H_{14}N_3O_6.2H_2O$: C, 30.48; H, 5.75; N, 13.33 Found: C, 30.30; H, 5.70; N, 13.25

EXAMPLE 20

Preparation of (±)-N^1-[(3-hydroxy-2-phosphonylmethoxy] propyl]cytosine

Synthesis of (±)-HPMPC was achieved in 42.4% yield after 5 steps (Examples 15, 16, 17, 18, and 19) starting from (±)-trityloxymethyloxirane and N^4-benzoylcytosine.

EXAMPLE 21

Preparation of (R)-N^1-[(3-hydroxy-2-phosphonylmethoxy)propyl] cytosine

The title compound (R)-HPMPC was prepared from (S)-glycidol (88% ee) and N^4-benzoylcytosine, following the method described for (S)-HPMPC.

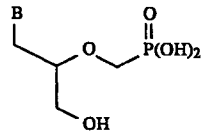
EXAMPLE 22

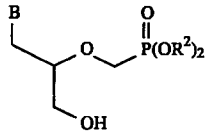
Preparation of (±)-[(3-hydroxy-2-phosphonylomethoxy)propyl] uracil

A solution of (±)-N^1-[(2-diethylphosphonyl-3-hydroxy)propyl] cytosine (0.228 g, 0.68 mol) in 2N sodium hydroxide (4.5 ml) was heated at 82° C. for 60 hours. The reaction was complete as indicated by its HPLC. It was acidified with Dowex 50×8 (H^+) form at room temperature and filtered, and the resin was washed with water (30 ml). Evaporation of the filtrate afforded the title compound (0.157 g, 82.4%) as a solid. ^1H NMR (D_2O): 3.16–3.29 (m, 1H), 2.55–4.17 (m, 9H), 5.87 (d, J=7.9 Hz, 1H), 7.72 (d, J=7.9 Hz, 1H).

What is claimed is:

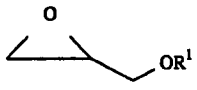
1. A process for preparing a compound of formula (Ia) or (Ib)

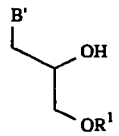

(Ia)


(Ib)

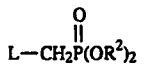
wherein B is a purine or pyrimidine base, which comprises the steps of

(a) reacting, in a reaction mixture, B' in the presence of a base with a compound of formula (IIb) to form the intermediate (IIIb)

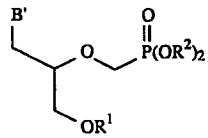

(IIb)


(IIIb)

and, without separating intermediate (IIIb) from the reaction mixture, reacting intermediate (IIIb) with a phosphonate of formula (IV)



to form an intermediate of formula (V)


(V)

where B' is a purine or pyrimidine base or a suitably protected purine or pyrimidine base, R^1 is a hydroxy protecting group, L is a leaving group, and R^2 is a phosphonic protecting group selected from alkyl having 1–5 carbon atoms; and

(b) replacing the R^1 hydroxy protecting group, and optionally, the R^2 or B' protecting groups, with hydrogen to form the product of formula (Ia) or (Ib).

2. The process of claim **1** wherein R^1 is replaced with hydrogen by treatment with an acidic medium.

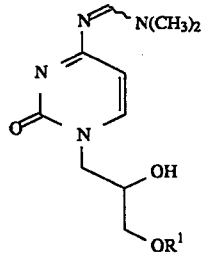
3. The process of claim **1** wherein R^1 of a compound of formula (IIIb) is triphenylmethyl and R^2 is ethyl or isopropyl.

4. The process of claim **1** wherein B' is cytosine or N^4-protected cytosine.

5. The process of claim **1** wherein the process is carried out in an inert polar aprotic solvent.

6. The process of claim **1** wherein B' is N^4-benzoylcytosine and R^1 is triphenylmethyl.

7. The process of claim **1** which further comprises reacting a compound of formula (IIIb) wherein B' is cytosine with dimethylformamide dimethyl acetal to form a compound of formula (XVI)


(XVI)

wherein R^1 is as defined in claim **1**.

8. The process of claim **1** wherein compound (IIb) is (S)-triphenylmethoxymethyloxirane.

9. The process of claim **1** wherein the product is (S)-N^1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine.

10. The process of claim **1** wherein the base is a metal hydride or metal alkoxide.

11. The process of claim **10** wherein the base is sodium hydride.

12. The process of claim **5** wherein the solvent is dimethylformamide.

* * * * *

338

Exhibit 4

Pergamon

Tetrahedron Letters 39 (1998) 1853–1856

TETRAHEDRON LETTERS

Practical Synthesis of the anti-HIV Drug, PMPA

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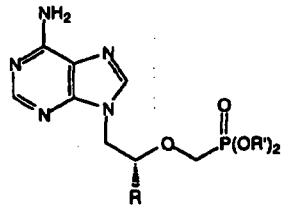
Key words: HIV, PMPA, PMEA, nucleotide, phosphonate.

Abstract: The anti-HIV nucleotide analogue PMPA can be prepared on a kilogram-scale by a three step sequence: i) condensation of adenine with (*R*)-propylene carbonate, ii) alkylation of the resulting (*R*)-9-(2-hydroxypropyl)adenine with diethyl *p*-toluenesulfonyloxymethanephosphonate using lithium *tert*-butoxide and iii) cleavage of the phosphonate ester functionalities with bromotrimethylsilane.

The acyclic derivatives of adenosine monophosphate, 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA, **1**) and (*R*)-9-[2-(phosphonomethoxy)propyl]adenine (PMPA, **2**) have shown potent and selective activity against human immunodeficiency virus (HIV) and other retroviruses.[1,2] The diester prodrugs, bis(POM)PMEA **3** and bis(POC)PMPA **4**, are currently being tested in clinical trials as oral therapies for AIDS and/or hepatitis B infection.[3,4] In order to supply the ongoing toxicological and clinical studies, a practical kilogram-scale preparation of **2** was needed.

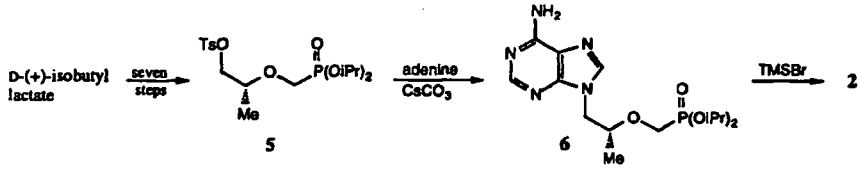


1 R = H, R' = H
2 R = Me, R' = H
3 R = H, R' = $CH_2OC(O)$*t*-Bu
4 R = Me, R' = $CH_2OC(O)OCH(CH_3)_2$

Figure 1.

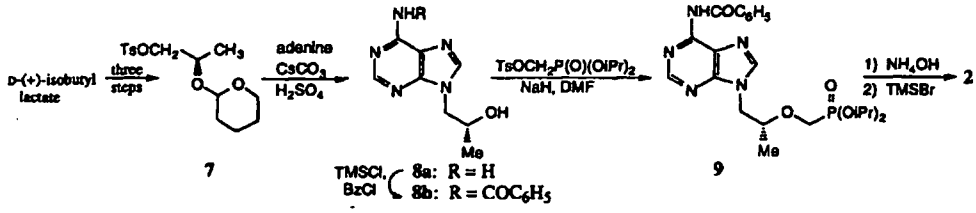
0040-4039/98/$19.00
PII: S0040-4039(98)00131-2

Two syntheses of **2** have been previously reported by Holy, both of which employed the readily available reagents, dialkyl *p*-toluenesulfonyloxymethanephosphonates.[5] The first approach (Scheme 1) was based on alkylation of adenine with the side-chain **5** already functionalized with the protected phosphonate.[6]



Scheme 1

The second approach (Scheme 2) built up the side chain stepwise by treatment of adenine with a protected tosyl alcohol **7** to afford, after deprotection, (*R*)-9-(2-hydroxypropyl)adenine (HPA, **8a**).[7] After protection of N6 as the benzoyl derivative **8b**, methylene phosphonate **9** was obtained by sodium hydride-mediated alkylation with diisopropyl *p*-toluenesulfonyloxymethanephosphonate.



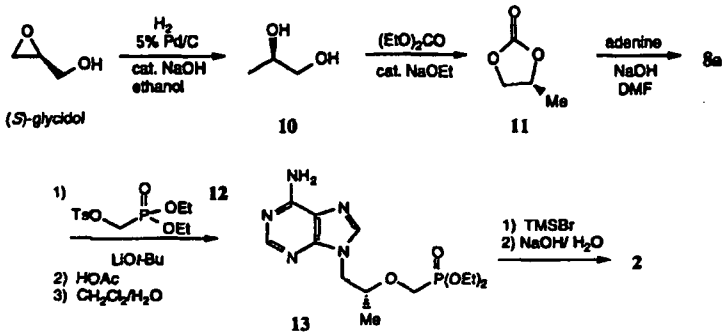
Scheme 2

The chiral synthon for both routes to **2** was derived from D-(+)-isobutyl lactate, which required aluminum hydride reagents for the ester reduction and transient protection of the lactate hydroxyl as a THP- or benzyl-ether. Both routes also required multiple chromatographic purifications. The alkylation of **8a,b** with sodium hydride (3 eq.) scaled-up poorly due to reaction heterogeneity, delayed-onset exotherms, and cross reactions between the tosylate reagent and sodium hydride. Careful quenching of excess sodium hydride was needed and the copious off-gassing of hydrogen necessitated nitrogen dilution. An alternative synthetic route was developed which was more amenable to scale-up while minimizing the use of protecting groups.

The related phosphonate **1** had been prepared via base catalyzed condensation of adenine with ethylene carbonate to generate 9-(2-hydroxyethyl)adenine (HEA).[8] Based on this precedent, it was proposed that **8a** could be prepared using (*R*)-propylene carbonate **11** (Scheme 3).

The preparation of (*R*)-propylene carbonate commenced with commercially available (*S*)-glycidol (86% ee) which was hydrogenated[9] to afford (*R*)-1,2-propanediol **10** using 5% palladium on carbon[10] (5% wt.) in ethanol (1 M) at ca. 25 psi hydrogen (6 h) with the addition of a catalytic amount of sodium hydroxide (0.05 eq.). Addition of the sodium hydroxide was found to be critical to avoid stalling on scale-up. After filtration and concentration, crude **10** was condensed with diethyl carbonate (1.2 eq.) in the presence of catalytic (0.04

eq.) sodium ethoxide.[11] The reaction was driven to completion by distillation of ethanol, followed by isolation of **11** by vacuum distillation (100 °C, 3 mm). The overall yield of **11** from (*S*)-glycidol was 75-80%. Chiral gas chromatographic analysis indicated no change in optical purity (86% ee) for the two-step sequence.[12]



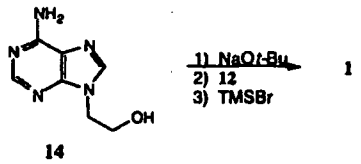
Scheme 3

Coupling of adenine with **11** (1.1 eq.) was accomplished in dimethylformamide (DMF) at 140 °C for 20 hours in the presence sodium hydroxide (0.02 eq.). Only two minor byproducts were formed at approximately 3% and 6% levels.[13] The pure alcohol **8a** could be isolated by crystallization directly from the reaction mixture by addition of toluene or, more conveniently, the crude **8a** DMF solution could be used directly in the next step.

Treatment of a 1 M DMF solution of **8a** with lithium *tert*-butoxide (1.1 eq., 2 M in THF) afforded a thick suspension of lithium salts. Addition of diethyl *p*-toluenesulfonyloxymethanephosphonate **12** (0.5 eq.) at 30-35 °C afforded a homogenous solution (ca. 1 h). This was followed by charging additional lithium *tert*-butoxide (0.4 eq) and **12** (1.0 eq.) to achieve complete reaction. The reaction was quenched with acetic acid and water, and the diethylphosphonate ester **13** was extracted into methylene chloride. Yields of crude **13** were typically 55-65% from adenine (by external standard HPLC).[13]

To complete the synthesis of **2**, the crude diethyl ester **13** was subjected to excess bromotrimethylsilane (3.5 eq.) in refluxing acetonitrile.[6,7] After disappearance of mono- and di-ethyl esters by HPLC, the reaction was concentrated, diluted with water and extracted with dichloromethane to remove silylated byproducts. The phosphonic acid product **2** was precipitated by addition of 50% NaOH bringing the aqueous solution to pH 3. The product precipitated slowly over several hours under these dilute conditions (ca. 0.57 M) and resulted in enrichment of the chiral purity of the product from 86% ee to > 98% ee.[14] A final recrystallization from boiling water (14 L/kg) afforded **2** as a stable monohydrate in 30-35% overall yield from adenine.

An alkoxide-alkylation process has also been used to prepare PMEA **1** from HEA **14** (Scheme 4). Optimal results in this case were obtained with sodium *tert*-butoxide (1.75 eq.) which afforded a *homogeneous* solution of sodium salts upon mixing with a suspension of **14** in DMF (5 L/kg). Alkylation with tosylate **12** and TMSBr cleavage afforded PMEA **1** in 35-45% overall yield from adenine.



14

Scheme 4

In summary, an efficient process has been developed to prepare the anti-HIV agent, PMPA. Notable features include a practical preparation of (*R*)-propylene carbonate, a novel key alkylation using lithium *tert*-butoxide, a simple crystallization process for chiral enrichment and no chromatography. Multi-kilogram batches of PMPA have been prepared by this process in standard pilot plant equipment.

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9. a) Park, G. J.; Fuchs, R. *J. Org. Chem.* **1957**, *22*, 93. b) Kotz, A.; Richter, K. *J. Prakt. Chem.* **1925**, *2*, 373.
10. Best results were obtained with catalyst type E101 NO/W 5% (53.75% water) available from Degussa Corp., Chemical Catalyst Div., 5150 Gilbertsville Hwy., Calvert City, KY 42029.
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12. The chiral purity of 1,2-propylene carbonate **11** was determined using a proprietary gas chromatography method (Chiraldex G-PN) available from Advanced Separation Technologies, Inc., 37 Leslie Court, P.O. Box 297, Whippany, NJ, 07981.
13. HPLC method: Mobile Phase A = 10 mM NH_4OAc in 5% MeOH in water, Mobile Phase B = MeOH; linear ramp over 20 min. from 0% B to 60% B. Column conditions: Hypersil ODS (C18), 5 µm, 4.6 x 150 mm, (Alltech Assoc., 2051 Waukegan Road, Deerfield, IL, 60015); 10 µL injection; flow rate 1 mL/min.; detection @ 262 nm by absorbance; ambient temperature.
14. The chiral purity of PMPA **2** was determined by HPLC analysis of a ternary complex of **2** with Cu(II) and L-phenylalanine in the mobile phase (0.7 mg/mL **2**, 3.8 mM L-Phe; 1.9 mM $CuSO_4$; 19.2 mM $NH_4H_2PO_4$ in a 3.8% [v/v] solution of acetonitrile in water). Column conditions: Hypersil ODS (C18), 5 µm, 4.6 x 250 mm, (Alltech Assoc., 2051 Waukegan Road, Deerfield, IL, 60015); 10 µL injection; flow rate 0.6 mL/min. (isocratic); detection @ 274 nm by absorbance; ambient temperature; RT(*ent*-**2**) = 10.5 to 12.5 min; RT(*R*-**2**) = 12.5 to 14.5 min.

Exhibit 5

342

Organic Process Research & Development 1999, 3, 53–55

Process Optimization in the Synthesis of 9-[2-(Diethylphosphonomethoxy)ethyl]adenine: Replacement of Sodium Hydride with Sodium *tert*-Butoxide as the Base for Oxygen Alkylation

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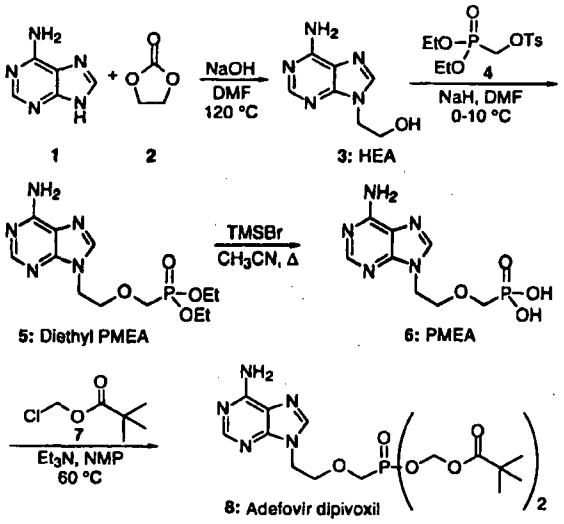
Abstract:

9-[2-(Diethylphosphonomethoxy)ethyl]adenine (diethyl-PMEA), a key intermediate in the production of the antiviral drug adefovir dipivoxil, was originally produced via a process utilizing sodium hydride (NaH) to couple hydroxyethyl adenine with diethyl *p*-toluenesulfonyloxymethanephosphonate. The use of NaH presented safety and consistency problems. It was found that sodium *tert*-butoxide (NaO^{t}Bu) was a suitable replacement for NaH as the base to effect the coupling reaction. Optimization of reagent stoichiometry and introduction of a simplified filtration workup procedure led to a robust process affording diethyl-PMEA in consistent yields and purities. The modifications and process improvements were scaled-up successfully to batch sizes of >100 kg.

Introduction

Adefovir dipivoxil (9-[2-bis(pivaloyloxymethyl)phosphonomethoxyethyl]adenine (**8**), Scheme 1)[1,2a] is an orally bioavailable prodrug of 9-[2-(phosphonomethoxy)ethyl]adenine [PMEA (**6**)], a nucleotide analogue with activity against the human immunodeficiency virus, hepatitis B virus, herpes simplex virus, cytomegalovirus, Epstein–Barr virus, and other DNA viruses.[1b,2–4] Adefovir dipivoxil is currently in late-phase clinical trials[5] for the treatment of HIV and in early-phase clinical trials for the treatment of hepatitis B virus.

Scheme 1. **Synthesis of adefovir dipivoxil from adenine**



The supply of adefovir dipivoxil for these programs depends on a four-step synthetic process (Scheme 1). In the first step, adenine (**1**) is condensed with ethylene carbonate (**2**) in hot DMF to afford the intermediate 9-(2-hydroxyethyl)-adenine [HEA (**3**)] in 83–95% yield after crystallization from toluene. This step scaled-up well and was reproducible at the 100–200-kg scale. Similarly, the third step, phosphonate ester cleavage with bromotrimethylsilane, worked well at production scale to afford **6**, as did the final esterification of the phosphonate to append the pivaloyloxymethyl groups.

The second step, the synthesis of 9-[2-(diethylphosphonomethoxy)ethyl]adenine [diethyl-PMEA (**5**)] (**3** → **5**), which at the outset of this investigation was derived from the laboratory procedure initially described by Holý and Rosenberg,[6] was problematic on large scale. In this step, the alkylation of **3** was performed using diethyl *p*-toluenesulfo-

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nyloxymethanephosphonate (4) and 3.0 equiv of sodium hydride in DMF. The heterogeneity of the reaction mixture, in which both NaH and 3 were virtually insoluble, coupled with a strong, delayed onset exotherm led to large variations in product purities (83–96%) and yields (17–44%) at production scale (Table 2). Furthermore, the hydrogen evolution during the reaction and during the acetic acid quench of the excess NaH at the end of the reaction caused safety and operational difficulties. At 200-kg scale (1116 mol of 3), the NaH process generated 7.51×10^4 L of hydrogen gas. Dilution of this hydrogen to below the 4% hydrogen flammability limit required 1.80×10^6 L of nitrogen (2.25 metric tons).[7] Additionally, concentrated mixtures of NaH in DMF can also result in runaway decomposition.[8]

A continuous extraction procedure using methylene chloride to isolate the partially water soluble diethyl-PMEA (5) worked well in the pilot plant facility; however, further scale-up proved arduous, necessitating replacement with a tedious batch extraction procedure. In addition, the removal of the sodium tosylate byproduct was variable using this extractive workup method. Finally, the mineral oil in which the NaH was dispersed required an additional hexane extraction step for its removal. Because of these process weaknesses and concerns with the use of NaH, an effort was initiated to improve the chemistry associated with this step.

Results and Discussion

Reaction Modifications and Optimization. The modification of the diethyl-PMEA process focused on replacing NaH with alternative bases while maintaining a reaction profile similar to that of the NaH process. Lithium *tert*-butoxide (LiO^{t}Bu), which previously was found to be effective for the synthesis of the related compound, PMPA,[9] afforded a thick suspension of the lithium salt of 3 in DMF which hindered agitation and reaction progress. In contrast, the related base, sodium *tert*-butoxide (NaO^{t}Bu), afforded a homogeneous mixture on reaction with 3 in DMF. In some instances, the sodium salt of 3 precipitated out of solution as a viscous resin as the reaction progressed. Counterintuitively, the viscous resin did not dissolve as more solvent was added. It was discovered that, at high concentration (1.5 M), the sodium salt of 3 was soluble, affording a homogeneous solution throughout the coupling reaction, whereas at lower concentration (0.8–1.0 M), the mixture became heterogeneous as the sodium salt of 3 precipitated. Aside from this concentration issue, it was observed in some runs that large clumps of starting 3 did not react with NaO^{t}Bu and thus remained as floating particulates in the reaction mixture. To circumvent this issue, an initial digestion at 125–135 °C in DMF, followed by fast cooling, fully dissolved and reprecipitated 3 as a fine solid. Once performed, addition of NaO^{t}Bu reliably gave a homogeneous solution.

Table 1. Effects of stoichiometry on product purity and reaction efficiency

		reaction			HPLC assay	
entry	scale (g)	NaO^{t}Bu (equiv)	tosylate (equiv)	time (h)	HEA + major impurities (area %)	product (area %)
1	35.8	1.10	1.20	26.0	21.7	46.6
2	35.8	1.30	1.20	23.0	17.5	58.7
3	50.0	1.50	1.40	20.0	18.5	56.8
4	100.0	1.50	1.40	20.0	17.8	57.6
5	35.8	1.50	1.20	11.0	14.7	56.0
6	35.8	1.75	1.25	7.5	13.4	63.6
7	239.0	1.75	1.25	7.5	12.9	55.1
8	35.8	2.00	1.25	10.0	11.8	60.4
9	35.8	3.00	1.25	30.0	>27.0	na

Previously, NaH (3.0 equiv) was added portion-wise and concurrently with a solution of tosylate 4 in DMF at less than 10 °C to minimize the side reaction of NaH with 4. Sodium *tert*-butoxide proved more selective and milder, requiring only 1.5 equiv to effect the coupling reaction. Furthermore, the entire amount of base could be charged in one portion without degradation of 4.

Ranging studies (Table 1) established that the best stoichiometry was 1.50–2.00 equiv of NaO^{t}Bu and 1.20–1.25 equiv of 4. A final adjustment to 1.75 equiv of NaO^{t}Bu and 1.25 equiv of 4 afforded a completed reaction (<3% HEA) in under 8 h with yields and purities superior to those of the NaH process. The use of NaO^{t}Bu in the optimized stoichiometry eliminated the flammability hazards and the mineral oil associated with the use of NaH, offered a milder quench with acetic acid, and reduced the amount of NaOTs and NaOAc generated, simplifying the purification procedures.

Workup Optimization. The workup optimization centered on the removal of the salt byproducts in the product mixture. In the NaH process workup procedure, the salt byproducts were removed by dissolving the concentrated reaction mixture in water, extracting with hexanes to remove the mineral oil from NaH, followed by numerous batch extractions (>12) with methylene chloride to recover the product from the aqueous phase.

In the new workup, the use of NaO^{t}Bu eliminated the need for a hexane extraction and an extensive aqueous workup. After the initial concentration of the reaction mixture, the semisolid product mixture was diluted with methylene chloride to precipitate the majority of the sodium tosylate and sodium acetate salts. Filtration of this mixture efficiently removed the salts, provided that the DMF level after distillation was <20 wt %[10] to limit the solubility of the salts. Above this level, the salts became soluble, and the filtration was slowed considerably. A final 9:1 (CH_2Cl_2/H_2O, w/w) partition of the reconcentrated filtrate was incorporated to entirely remove trace amounts of dissolved salts and residual DMF. This ratio of solvents afforded the best separation with the minimum amount of product retained in the water.

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(10) A representative sample was obtained after addition of methylene chloride. The level of DMF was determined by ^{1}H NMR comparing by wt % of DMF versus NaOTs, NaOAc, and diethyl-PMEA.

Table 2. **Comparison of large scale results using NaH and NaO^{t}Bu**

reaction			results			
			purity (HPLC area %)		yield (% theory)	
base used	no. of batches	scale (kg of HEA, 3)	av	range	av	range
NaH	10	5–249	92.6	82.7–96.1	29.0	17.5–43.7
NaO^{t}Bu	4	200	95.0	94.6–95.3	41.0	39.4–43.7

The isolation of **3** involved concentration of the methylene chloride layer, a coevaporation with toluene to remove water, and crystallization from toluene. The isolated laboratory yields of the NaO^{t}Bu procedure were 35–45%, with purities of 88–95%. On production scale, the yields and purities (Table 2) were impressively consistent compared to the NaH process.

Experimental Section

Starting material **3** was prepared according to the literature procedure.[11] Compound **4** was prepared using a method similar to the literature description[12] and was used as a ~90% concentrated toluene solution adjusted for purity. NaO^{t}Bu and acetic acid were commercially available and were used as received. All reactions were performed under a dry nitrogen atmosphere. Proton nuclear magnetic resonance spectra were obtained on a Varian spectrometer (300 MHz). The ^{1}H NMR chemical shifts are expressed in parts per million (δ) relative to the protio form of the solvent used. Cooling baths (for laboratory scale) used are as follows: −10 °C (ice/methanol), 0 °C (ice/water/salt). HPLC analyses were performed on a Rainin Dynamax HPLC system using an Alltech Alltima 5 μm, reversed-phase column with 5% MeOH in water as eluent.

9-[2-(Diethylphosphonomethoxy)ethyl]adenine (5). A suspension of **3** (200.2 kg, 1117 mol) in *N,N*-dimethylformamide (DMF) (960 kg, 1.10 M) was heated at 125–135 °C for 30 min. Upon reaching 130 °C, solution was achieved. The contents were rapidly cooled to 20–30 °C to reprecipitate **3** as a fine suspension in DMF. NaO^{t}Bu (188.0 kg, 1956 mol) was charged slowly to the suspension with cooling so as to maintain the content temperature at 20–30 °C. The resulting solution was then held at 20–30 °C for 30 min before being cooled to −10 °C. A solution of **4** (508.4 kg, 1577 mol) in DMF (241 kg) was slowly added to the reaction mixture with cooling so as to maintain the content temperature at −10 to 0 °C. After completion of addition, the mixture was stirred for an additional 1.5 h.

Acetic acid (134.5 kg, 2240 mol) was charged to the reaction mixture while the temperature was kept <20 °C. After the mixture was stirred for 15 min, the contents were concentrated in vacuo (maximum temperature of 80 °C with minimum vacuum of 25 mmHg) to remove DMF and residual solvents until the distillation stopped at a maximum pot temperature of 80 °C. The concentrate was cooled to 40 °C, diluted with methylene chloride (3201 kg), and charged with Celite (100.3 kg) before filtration. The solids containing NaOTs and NaOAc salts were removed by filtration and rinsed with methylene chloride (3 × 80 kg). The combined filtrate was concentrated in vacuo until distillation halted at a maximum pot temperature of 80 °C. The concentrate was cooled to 40 °C, diluted with methylene chloride (1000 kg), and reconcentrated in vacuo until distillation halted at a maximum pot temperature of 80 °C. The concentrate was cooled to 40 °C, diluted again with methylene chloride (1388 kg) to dissolve all solids, and washed once with water (163.3 kg). The aqueous washing was back-extracted twice with methylene chloride (300 kg each back-extraction). The methylene chloride phases were combined and concentrated in vacuo until distillation halted at a maximum pot temperature of 80 °C. The resulting oil was diluted with toluene (600 kg) and reconcentrated in vacuo until distillation halted at a maximum pot temperature of 80 °C. The concentrate was again diluted with toluene (600 kg) and then warmed to 80 °C for 30 min with moderate agitation. The solution was cooled slowly to 30 °C over 90 min and then to 0 °C, where it was maintained for 12 h with slow agitation. The white solid product was isolated by filtration, washed with cold toluene (3 × 35 kg), and dried in vacuo at 50 °C, yielding 151.6 kg (41.52%) of **5**. Purity by HPLC assay was 93–96% by area. Characterization and ^{1}H NMR data were consistent with literature values.[6]

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Exhibit 6

345

FULL PAPER

Standard Molar Enthalpies of Formation of Mg and Ca Alkoxides

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Keywords: Thermochemistry / Alkaline-Earth Alkoxides / Enthalpy of Formation / Calcium / Magnesium

The enthalpies of formation of some alkaline-earth alkoxides were determined by reaction-solution calorimetry ($\Delta_fH°[Mg(OMe)_2]$ = −792.6±1.2 kJ/mol; $\Delta_fH°[Mg(OEt)_2]$ = −847.9±2.7 kJ/mol; $\Delta_fH°[Ca(OMe)_2]$ = −890.8±6.4 kJ/mol; $\Delta_fH°[Ca(OEt)_2]$ = −924.5±2.6 kJ/mol; $\Delta_fH°[Ca(OBu)_2]$ = −989.8±5.4 kJ/mol) and allowed us to extend a previously developed model for predicting the enthalpies of formation of Be, Sr and Ba alkoxides.

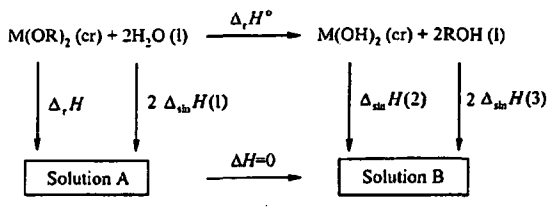
Introduction

Alkaline and alkaline-earth metal alkoxide compounds are of major importance in synthetic chemistry.[1] However, thermochemical data for these substances is rather scarce. Previous results obtained for sodium and lithium alkoxides[2,3] show that there exists a linear relation between the enthalpies of formation of the alkoxides and those of the corresponding alcohols for short-chain unbranched alkoxides. A model based on the alkoxide energy calculations and a simple electrostatic model was also developed, which enabled us to predict unknown alkali metal alkoxide enthalpies of formation.

In this paper we extend the previously developed model to alkaline-earth alkoxides and use it for the prediction of some unmeasured Be, Sr and Ba alkoxides.

Results and Discussion

The enthalpies of formation of magnesium and calcium alkoxides were calculated according to Scheme 1 (reaction with water, used for all the compounds studied in this paper) or Scheme 2 [reaction with 0.1 M HCl, used for Ca(OEt)$_2$]. In these schemes, $\Delta_rH°$ represents the reaction enthalpy with all the compounds in their standard state, Δ_rH the experimentally measured reaction enthalpy, $\Delta_{sln}H(2)$ the dissolution enthalpy for stoichiometric amounts of $M(OH)_2$ in H_2O, $\Delta_{sln}H(3)$ the dissolution enthalpy of the alcohol in H_2O + $M(OH)_2$, $\Delta_{sln}H(5)$ the dissolution of $CaCl_2$ in 0.1 M HCl, $\Delta_{sln}H(6)$ the dissolution of ethanol in a 0.1 M HCl + $CaCl_2$ solution, and $\Delta_{sln}H(7)$ the dissolution of water in 0.1 M HCl solution. $\Delta_{sln}H(1)$ (dissolution of water in water) and $\Delta_{sln}H(4)$ (dissolution of 0.1 M HCl in 0.1 M HCl) are obviously zero.



Solution A = Solution B = $M(OH)_2$ (sln) +2ROH (sln)

Scheme 1. Reaction with water

As the concentrations of $M(OH)_2$ in solution were always very small [about 1 mol $M(OH)_2$ in 4 × 10^5 mol water] an infinite dilution can be assumed and $\Delta_{sln}H(2)$ can be calculated as −2.30 kJ/mol for magnesium hydroxide and −16.73 kJ/mol for the calcium analogue using the data in Table 1.[4,5] In the cases of ethanol and methanol, $\Delta_{sln}H(3)$ values were calculated from the differences between the enthalpies of formation of the pure alcohols and the respective enthalpies of formation at infinite dilution in water (Table 2 and Equation 1 and 2). $\Delta_{sln}H(3)$ for butanol was taken as −7.39 ± 0.59 kJ/mol.[3]

$$\Delta_{sln}H(3)\ (MeOH) = \Delta_fH°\ (CH_3OH,\infty\ H_2O) - \Delta_fH°\ (CH_3OH,l) = -7.3 \pm 0.4\ kJ/mol \quad (1)$$

$$\Delta_{sln}H(3)\ (EtOH) = \Delta_fH°\ (C_2H_5OH,\infty\ H_2O) - \Delta_fH°\ (C_2H_5OH,\ l) = -10.7 \pm 0.6\ kJ/mol \quad (2)$$

The fact that the above $\Delta_{sln}H(3)$ values were obtained in water and not in H_2O + $M(OH)_2$ solutions should not be relevant due to the low concentrations of $M(OH)_2$ in solution. This is in keeping with the values in a previous publication[3] where the dissolution of butanol in water, H_2O + LiOH and H_2O + NaOH was measured, and showed no difference within experimental error.

The enthalpies of formation of aqueous 0.1 M HCl and crystalline calcium chloride are known (Table 1). The dissolution of calcium chloride in 0.1 M HCl and of ethanol in $CaCl_2$ + 0.1 M HCl solution was measured (Table 2). The dissolution of water in the solvent is negligible (smaller than the detection limit of the apparatus) and even when multiplied by 1104 yields a minor contribution to the final $\Delta_rH°$ value (smaller than the experimental error). Therefore, $\Delta_{sln}H(7)$ was neglected.

Schemes 1 and 2 lead to Equations 3 and 4, that allow the calculation of the enthalpies of formation of the metal alkoxides shown in Table 3.

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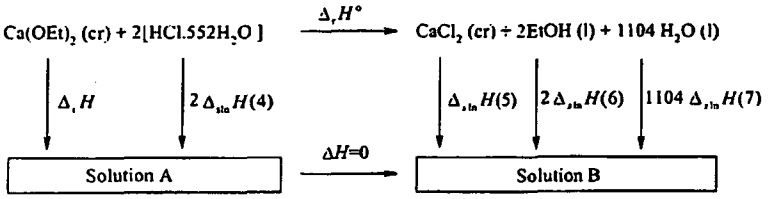


Solution A ≡ Solution B ≡ $CaCl_2$ (sln) +2EtOH (sln) + n (HCl .552H_2O)

Scheme 2. Reaction with 0.1 M HCl

Table 1. Auxiliary data (T = 298.15 K)

Compound	$\Delta_f H°$ (kJ/mol)	Ref.
H_2O, l	-285.830	4
CH_3OH, l	-239.1±0.3	5
$CH_3OH·\infty H_2O$	-246.4±0.3	5
C_2H_5OH, l	-277.5±0.4	5
$C_2H_5OH·\infty H_2O$	-288.2±0.4	5
C_4H_9OH, l	-327.3±0.4	5
$Mg(OH)_2$, cr	-924.54	4
$Mg(OH)_2$, ai	-926.84	4
Mg, g	147.70	4
$Ca(OH)_2$, cr	-986.09	4
$Ca(OH)_2$, ai	-1002.82	4
Ca, g	178.2	4
$CaCl_2$, cr	-795.8	4
HCl·552 H_2O	-166.596	4

Table 2. Dissolution enthalpies (T = 298.15 K)

Compound	$\Delta_{sln}H(3)$ (kJ/mol)[a]	$\Delta_{sln}H(6)$ (kJ/mol)[b]	$\Delta_{sln}H(5)$ (kJ/mol)[c]
CH_3OH	-7.3±0.4[d]	-	-
C_2H_5OH	-10.7±0.6[d]	-9.4±1.6	-
C_4H_9OH	-7.39±0.59[e]	-	-
$CaCl_2$	-	-	-81.8±1.1

[a] Dissolution in water. - [b] Dissolution in 0.1 M HCl + $CaCl_2$. - [c] Dissolution in 0.1 M HCl. - [d] Calculated from data in Table 1. - [e] Measured in ref.[3]

Table 3. Reaction enthalpies, and standard enthalpies of formation of magnesium and calcium alkoxides (T = 298.15 K).

Alkoxide	$\Delta_r H$[a] (kJ/mol)	$\Delta_f H°[M(OR)_2,cr]$ (kJ/mol)	$\Delta_f H°$[a] (kJ/mol)
$Mg(OMe)_2$	-55.4±0.7	-792.6±1.2	-38.5±1.1
$Mg(OEt)_2$	-83.7±2.3	-847.9±2.7	-60.0±2.6
$Ca(OMe)_2$	-33.2±6.3	-890.8±6.4	-1.9±6.4
$Ca(OEt)_2$	-83.1±2.2	-924.5±2.6	-45.0±2.5
-	-192.1±9.1[b]	-926.1±9.7	-
$Ca(OBu)_2$	-110.7±5.2	-989.8±5.4	-79.2±5.3

[a] Reaction with water unless stated otherwise. - [b] Reaction with 0.1 M HCl.

$$\Delta_f H°[M(OR)_2] = \Delta_f H°[M(OH)_2,cr] + 2\Delta_f H°[ROH, l] - 2\Delta_f H°[H_2O, l] - \Delta_r H + \Delta_{sln}H(2) + 2\Delta_{sln}H(3) \quad (3)$$

$$\Delta_f H°[Ca(OEt)_2, cr] = \Delta_f H°[CaCl_2,cr] + 2\Delta_f H°[EtOH, l] - 2\Delta_f H°[HCl·552H_2O] - \Delta_r H + \Delta_{sln}H(5) + 2\Delta_{sln}H(6) + 1104\ \Delta_{sln}H(7) \quad (4)$$

As a first comment we note the good agreement between the values for the enthalpy of formation of $Ca(OEt)_2$ obtained by the two different methods referred to above. Since the reaction with HCl was studied mainly to test the consistency of the results, in all subsequent calculations the values derived from the reactions with water will be used.

A plot of the enthalpies of formation of the crystalline alkoxides against the enthalpies of formation of the corresponding alcohols in their standard reference states is shown in Figure 1. This type of plot has been used before for many compound families,[6] including sodium[2] and lithium alkoxides,[3] and it is useful to assess the reliability of the data. For calcium alkoxides, and using the three points for R = Me, Et and Bu, a fairly linear correlation (r = 0.9922; uncertainty intervals are standard deviations) (Equation 5) is obtained. The slope defined by the two points for Mg alkoxides (R = Me and Et) is 1.44.

$$\Delta_f H°(Ca(OR)_2) = (1.13 \pm 0.14)\ \Delta_f H°(ROH, l) - (617 \pm 39) \quad (5)$$

To understand the plot of Figure 1 (or Equation 5) we can use Equation 6, where $\Delta_r H°$ is simply defined in terms of the standard enthalpies of formation of reactants and products. If a linear correlation between $\Delta_f H°[M(OR)_2,cr]$ and $\Delta_f H°(ROH,l)$ is observed, this means that $\Delta_r H°$ is constant for several R. On the other hand, as evidenced by Equation 6, the slope of the line should be close to 2 (the number of alkoxide ligands bound to M). However, this is not observed either for Mg or Ca compounds, nor is $\Delta_r H°$ constant (Table 3) in Equation 5. Similar behaviour had been noticed for some K, Rb and Cs alkoxides.[7] This type of plot should work well only if the crystal structure has little effect on the energetics. Since none of the crystal structures are known, and since changing the anions and/or the cations is very likely to change the crystal structure, Figure 1 should be used mainly as a visual aid to find trends in the values.

$$\Delta_f H°[M(OR)_2, cr] = 2\Delta_f H°[ROH, l] + \Delta_f H°[M(OH)_2, cr] - 2\Delta_f H°[H_2O, l] - \Delta_r H° \quad (6)$$

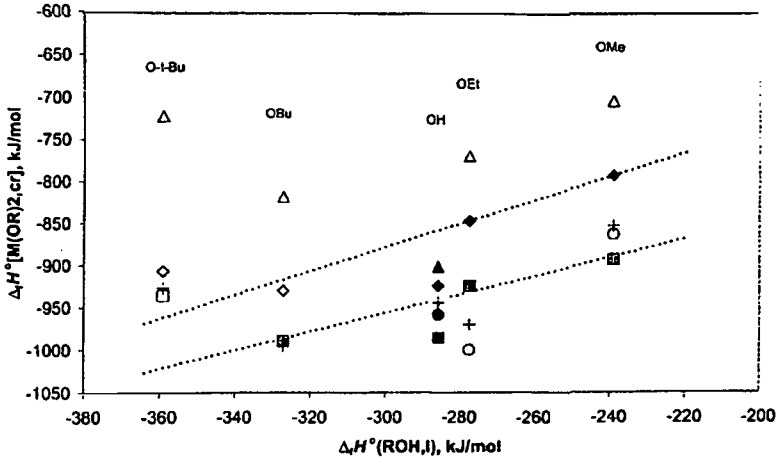
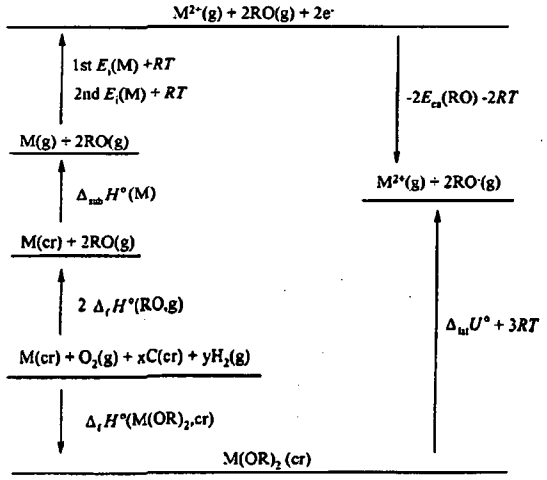


Figure 1. Enthalpies of formation of alkaline-earth metal alkoxides versus the enthalpies of formation of the corresponding alcohols (experimental data: filled symbols; estimated data: empty symbols; Be: triangle; Mg: diamond; Ca: square; Sr: circle; Ba: cross); the lower line is a linear regression using the three experimental values for Ca, and the upper line is simply a line passing through the two experimental Mg values, which are included mainly as a visual guide

Table 4. Lattice energies of magnesium and calcium alkoxides

Alkoxide $M(OR)_2$[a]	$\Delta_f H°(RO,g)$ (kJ/mol)[c]	EA(OR) (kJ/mol)[d]	$\Delta_{lat}U°[M(OR)_2]$ (kJ/mol)
$Mg(OH)_2$[b]	39±4	176.4±1.0	2990.8±8.2
$Mg(OMe)_2$	18±4	151.4±2.1	2866.9±9.1
$Mg(OEt)_2$	−17±4	166.5±3.2	2822±10
$Ca(OH)_2$[b]	39±4	176.4±1.0	2629.8±8.2
$Ca(OMe)_2$	18±4	151.4±2.1	2542±11
$Ca(OEt)_2$	−17±4	166.5±3.2	2476±10
$Ca(OBu)_2$	−63±4	171±14	2440±30

[a] $\Delta_{sub}H°(Mg)$ = 147.70 kJ/mol,[4] $\Delta_{sub}H°(Ca)$ = 178.2 kJ/mol,[4] 1st E_i (Mg) = 743.935,[4] 2nd E_i (Mg) = 1456.869,[4] 1st E_i (Ca) = 596.05,[4] 2nd E_i (Ca) = 1151.65.[4] – [b] $\Delta_f H°[M(OH)_2]$ taken from Table 1. – [c] Taken from D. F. McMillen, D. M. Golden, *Ann. Rev. Phys. Chem.* **1982**, *33*, 493. – [d] Taken from S. G. Lias, J. E. Bartmess, J. F. Liebman, J. L. Holmes, R. D. Levin, W. G. Mallard, *J. Phys. Chem.*, **1988**, *17*, Suppl. no. 1.



Scheme 3

Another way of discussing the data in Table 4 involves the calculation of lattice energies ($\Delta_{lat}U°$) of magnesium and calcium alkoxides.

These values, displayed in Table 4, were obtained from the standard enthalpies of formation of the alkoxides and from literature data, according to Scheme 3. The high ionic character of the alkoxides is indicated by their lattice energies, which are under 200 kJ/mol lower than the lattice energies of the corresponding hydroxides.

Let us assume a simple ionic model and the Kapustinskii approximation represented by Equation 7.[8] In this expression, ν represents the number of ions in the molecule, Z_+ and Z_- the charges of the cation and anion and r_+ and r_- the respective radii in pm. As the ionic radii of Mg^{2+} and Ca^{2+} are known,[9] the ionic radii of the alkoxide anions can be derived from the data in Table 4. The results are shown in Table 5, which also includes early data for alkaline-earth and alkali metal alkoxides [2,3,7] and estimates of the radii of the alkoxide ions in some alkaline-earth metal alkoxides not studied in this work.

$$\Delta_{lat}U° = \frac{1.079\times10^3\,\nu Z_+Z_-}{r_+ + r_-} \qquad (7)$$

The radii calculated by the above procedure are called "thermochemical radii" and have no special physical meaning, apart from reproducing lattice energies when introduced in Equation 7. It is to be noted that the values of the *n*-butoxy anion always have a greater error than the others. This is mainly due to the huge error in the electroaffinity of the butoxy ion.

Using these estimated radii and Scheme 3, it is possible to calculate the enthalpies of formation for some unknown alkaline-earth metal alkoxides (Table 6). The error bars are

Table 5. Thermochemical radii[a] calculated or estimated[b] for the alkoxide ions in alkali and alkaline-earth metal alkoxides[c]

RO^-	$Be(OR)_2$	LiOR[c]	$Mg(OR)_2$	NaOR[f]	$Ca(OR)_2$	KOR[g]	$Sr(OR)_2$	RbOR[g]	$Ba(OR)_2$	CsOR[g]
HO^-	120.2±1.1[d]	119.8±1.3	130.5±1.2	127.4±1.5	132.2±1.3	121±3	130.4±1.3[d]	118±3	129.7±1.4[d]	116±3
MeO^-	(130±2)	130.1±1.5	**139.8±1.2**	141.8±1.9	**140.6±1.5**	138±4	(140±6)	131±4	(140±6)	131±4
EtO^-	(132±2)	132.4±1.6	**143.4±1.3**	145.2±2.0	**147.5±1.5**	134±4	(136±6)	127±3	(138±6)	129±4
$nBuO^-$	(135±4)	135.0±3.6	(145±5)	144.9±4.8	**151.3±3.4**	144±10			(148±12)	139±10
$tBuO^-$	(146±3)	146.1±2.1	(154±3)	154.2±3.0	(167±8)	160±6			(170±8)	160±6

[a] Thermochemical radii studied in the present paper are in bold. – [b] Estimated thermochemical radii in parentheses[c] $r_+(Li^+) = 90 \pm 1$ pm, $r_+(Na^+) = 116 \pm 1$ pm, $r_+(K^+) = 152 \pm 1$ pm, $r_+(Rb^+) = 166 \pm 1$ pm, $r_+(Cs^+) = 181 \pm 1$ pm, $r_+(Be^{2+}) = 59 \pm 1$ pm, $r_+(Mg^{2+}) = 86 \pm 1$ pm, $r_+(Ca^{2+}) = 114 \pm 1$ pm, $r_+(Sr^{2+}) = 132 \pm 1$ pm, $r_+(Ba^{2+}) = 149 \pm 1$ pm.[9] – [d] $\Delta_f H°[Be(OH)_2,cr] = -902.5$ kJ/mol, $\Delta_f H°[Sr(OH)_2,cr] = -959.0$ kJ/mol, $\Delta_f H°[Ba(OH)_2,cr] = -944.7$ kJ/mol, $\Delta_f H°(Be^{2+},g) = 2993.23$ kJ/mol, $\Delta_f H°(Sr^{2+},g) = 1790.54$ kJ/mol, $\Delta_f H°(Ba^{2+},g) = 1660.38$ kJ/mol.[4] – [e] Calculated from data in ref.[3] – [f] Calculated from data in ref.[2] – [g] From ref.[7]

large (50 kJ/mol excluding the butoxides and 90 kJ/mol for the butoxides) when compared with those for the alkoxides measured on this paper (2–6 kJ/mol). The main reason is that a small error in the radii will be amplified in the lattice energy values (since $\Delta_{lat}U°$ is a big value) and therefore in the values of the enthalpy of formation.

Table 6. Lattice energies and enthalpies of formation of alkaline-earth alkoxides; estimated values in parentheses

Alkoxide	$\Delta_{lat}U°$ kJ/mol	$\Delta_f H°[M(OR)_2]$ kJ/mol
$Be(OMe)_2$	(3425±40)	(–706±41)
$Be(OEt)_2$	(3390±40)	(–771±41)
$Be(OBu)_2$	(3337±71)	(–819±77)
$Be(OtBu)_2$[a]	(3158±49)	(–723±51)
$Mg(OMe)_2$	2866.9±9.1	–792.6±1.2
$Mg(OEt)_2$	2822±10	–847.9±2.7
$Mg(OBu)_2$	(2803±62)	(–930±68)
$Mg(OtBu)_2$[a]	(2698±36)	(–907±39)
$Ca(OMe)_2$	2542±11	–890.8±6.4
$Ca(OEt)_2$	2476±10	–924.5±2.6
$Ca(OBu)_2$	2440±30	–989.8±5.4
$Ca(OtBu)_2$[a]	(2304±66)	(–936±68)
$Sr(OMe)_2$	(2380±53)	(–864±54)
$Sr(OEt)_2$	(2416±55)	(–1000±56)
$Sr(OBu)_2$	–	–
$Sr(OtBu)_2$[a]	–	–
$Ba(OMe)_2$	(2240±47)	(–854±48)
$Ba(OEt)_2$	(2256±48)	(–970±49)
$Ba(OBu)_2$	(2180±88)	(–995±93)
$Ba(OtBu)_2$[a]	(2029±51)	(–927±53)

[a] $\Delta_f H°(OtBu) = -91 \pm 5$ kJ/mol from D. F. McMillen, D. M. Golden, *Ann. Rev. Phys. Chem.* **1982**, *33*, 493; $E_{ea}(OtBu) = 184.4 \pm 5.2$ from S. G. Lias, J. E. Bartmess, J. F. Liebman, J. L. Holmes, R. D. Levin, W. G. Mallard, *J. Phys. Chem.* **1988**, *17*, Suppl. no. 1.

Nevertheless, if no other values exist, those can be quite useful. In the future we intend to extend this study to other alkaline-earth metal alkoxides to achieve better data for these compounds.

Knowing the structures would give deep insight into in these kinds of compounds. Despite all efforts, it has not yet been possible to obtain single crystals, and powder studies have yet to be undertaken.

Experimental Section

Materials: Methanol (Merck, 99.8%) and ethanol (Merck, 99.8%) were pre-dried over calcium sulfate, refluxed over activated magnesium and iodine, and finally distilled. Butanol (Sigma, 99.0%) was dried over calcium hydride and distilled. Magnesium (turnings, Sigma) and calcium (granules, Sigma) were used as supplied and stored in an oxygen- and water-free glovebox. Iodine was sublimed twice before use. Pentane was distilled over P_2O_5 and stored in the glovebox over 4 Å molecular sieves.

Physical Measurements: Infrared spectra were determined using a Perkin–Elmer 577 spectrophotometer with samples mounted as Nujol mulls between KBr plates. Elemental analyses were performed on a Perkin–Elmer 240 C (C, H) automatic analyzer or by gravimetric analyses (Mg as magnesium oxide,[10] Ca as calcium oxalate[11]). Iodine was qualitatively analyzed by an energy-dispersive X-ray spectrometer with a primary photon beam produced by a high power rhodium anode X-ray tube (Kevex Delta XRF Analyst System).

Alkoxide Syntheses: The studied alkoxides are moisture sensitive. Therefore, all syntheses were performed inside an oxygen- and water-free (<5 ppm) glovebox. Alcohols were degassed before use. Magnesium alkoxides were prepared by addition of the metal to an excess of alcohol (methanol or ethanol) containing a small amount of sublimed iodine (acting as a catalyst) and stirred for several days inside the glovebox at room temperature. The solution was taken to dryness, washed twice with pentane and dried under high vacuum (10^{-4}–10^{-5} Torr). The calcium alkoxides (methoxy, ethoxy and butoxy) were synthesized in a similar way without adding the catalyst. One of the problems that may occur is the formation of metal hydroxides due to the presence of water, and which are virtually impossible to separate from the alkoxides. Another problem is ensuring that the alkoxides are completely dry, i.e. all the excess alcohol must be removed. IR spectra showed that all the alkoxides used in

Table 7. Elemental analysis of magnesium and calcium alkoxides

Alkoxide	Found %C	%H	%M	Theoretical %C	%H	%M
$Mg(OMe)_2$	27.95	6.77	–	27.90	7.03	–
$Mg(OEt)_2$	39.83	8.51	21.1	41.99	8.81	21.24
$Ca(OMe)_2$	23.21	5.97	–	23.52	5.92	–
$Ca(OEt)_2$	38.23	7.82	31.0	36.90	7.74	30.78
$Ca(OBu)_2$	51.03	9.65	–	51.57	9.74	–

this work were alcohol- and hydroxide-free. Elemental analysis of the alkoxides (C, H, metal) was also performed (metal analysis on only some of them) to assess the purity (Table 7). For the magnesium compounds energy-dispersive X-ray spectrometry was conducted to check if any iodine was incorporated, the result always being negative.

Reaction-Solution Calorimetry: The calorimeter used was specially built for the study of oxygen- and water-sensitive compounds, and the experimental procedure was described in a previous paper.[2] All measurements were made near 298.15 K, and the results are averages of at least four runs. The errors presented are twice the standard deviation of the mean in each case. To determine the enthalpies of formation of the alkoxides, their reactions with water were studied in the calorimeter. As a test for consistency, the reaction of $Ca(OEt)_2$ with a 0.1 M aqueous solution of HCl was also studied.

Acknowledgments

The authors thank Dr. Fátima Araújo (ITN) for energy-dispersive X-ray spectrometry analysis. Financial support from FCT (PRAXIS/2/2.1/QUI/51/94) is gratefully acknowledged.

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350

Exhibit 7

US005739314A

United States Patent [19]

Roy et al.

[11] Patent Number: 5,739,314

[45] Date of Patent: Apr. 14, 1998

[54] **METHOD FOR SYNTHESIZING 2'-O-SUBSTITUTED PYRIMIDINE NUCLEOSIDES**

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[73] Assignee: **Hybridon, Inc.**, Cambridge, Mass.

[21] Appl. No.: **846,124**

[22] Filed: **Apr. 25, 1997**

[51] **Int. Cl.**^{6} **C07H 1/00**; C07H 1/02; C07H 11/067

[52] **U.S. Cl.** **536/55.3**; 536/25.3

[58] **Field of Search** 536/55.3, 25.3

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[57] **ABSTRACT**

The present invention provides an improved method of synthesizing 2'-O—R substituted pyrimidine mononucleosides. The method comprises reacting an anhydropyrimidine with magnesium alkoxide in the corresponding alcohol at elevated temperatures to directly produce the 2'-O—R substituted pyrimidine nucleoside product. The method advantageously eliminates several steps from prior art methods, thereby reducing the time and cost of synthesis and increasing the yield of final product.

6 Claims, No Drawings

METHOD FOR SYNTHESIZING 2'-O-SUBSTITUTED PYRIMIDINE NUCLEOSIDES

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to improved methods of synthesizing protected 2'-O-substituted pyrimidine nucleosides.

2. Description of the Related Art

Novel oligonucleotide analogues containing modified bases have proven to be valuable antisense probes. The presence of 2'-O-alkyl ribonucleosides in oligonucleotides has been shown to decrease both chemical and enzymatic degradation of oligonucleotides while retaining or even increasing the recognition of complementary target sequences. (Cummins et al., *Nucleic Acids Research* 23:2019, 1995; Lesnick et al., *Biochemistry* 32:7832, 1993; Kawai, et al., *Biochemistry* 31:1040, 1992). These properties of 2'-O-alkyl oligonucleotides make them extremely useful in ribozymes and antisense oligonucleotides. See, e.g., *Antisense Research and Applications* (Crooke and Lebleu, Eds., CRC Press, Boca Raton, Fla., 1993).

2'-O-methyl ribonucleosides have been prepared by partial methylation of a nucleoside with diazomethane and also by partial tritylation of uridine, methylation of the products and removal of N-alkylated isomers. (Haines, *Tetrahedron* 29:2807–2810, 1973).

2'-O-methyl nucleosides have also been prepared by using trimethylsulphonium hydroxide (Me_3SOH) and trimethylsulphonium iodide (Me_3SI) at elevated temperature, yielding 2'- and 3'-O-alkylated nucleoside. (Yamauchi, *Perkin I* 2787–2792, 1980).

A variety of N^3-protected ribonucleosides have been subject to alkylation of the ribose hydroxyls (wherein the order of reactivity is 2'-OH>3'-OH>5'-OH). E.g., Gin and Dekker, *Biochemistry* 7:1413 1968. Similar problems are inherent in base-promoted alkylation of cytidine. (Wagner, et al., *Nucleic Acids Res.* 19: 5965, 1991). Methyl iodide and silver oxide reaction on N^3-protected 3',5'-O-bis silylated nucleoside gave the product but included four extra steps, thereby decreasing the overall yield. (Suresh and Saroj, U.S. Pat. No. 5,214,135).

Tin-catalyzed reactions of unprotected uridine with either alkyl halides (Wagner et al., *J. Org. Chem.* 39:24, 1974) or diazomethane (Robbins et al., *J. Org. Chem.* 39: 1891, 1974) directly produced both 2' and 3'-O-alkyl nucleosides in high yield.

These and a variety of other methods are not selective for 2'-O-alkylation and require chromatographic or other methods of separation to isolate the desired 2'-O-alkyl nucleosides, thereby increasing the time and expense of the synthesis while greatly decreasing its efficiency and overall yield. See, e.g., Wagner, et al., *J. Org. Chem.* 39:24, 1974; Robbins et al., *J. Org. Chem.* 39:1891–1899, 1974; and Pathak and Chattopadadhyaya, *Chemica Scripta* 26:135–139, 1986.

Sproat and Lamond, in *Oligonucleotides and Analogues: A Practical Approach* (F. Eckstein, Ed., Oxford University Press 1991) describes a multi-step synthesis of 2'-O-methylribonucleosides employing a silyl blocking group at the 3' and 5' positions. Sproat, in *Methods in Molecular Biology: Protocols for Oligonucleotides and Analogs* (Vol. 20) (S. Agrawal, Ed., Humana Press, Totowa, N.J., 1993) describe a similar multi-step synthesis of 2'-allyl ribonucleotides.

Recently, McGee and Zhai, *Nucleosides & Nucleotides* 15:1797–1803, 1996, reported selective alkylation of 5'-O-dimethoxytrityl (DMT) anhydrouridine with either magnesium or calcium alkoxide in DMF at 100° C. Although initial yields of the desired product were high, subsequent steps to convert the protected 2'-O-alkyluridine to the cytidine analog resulted in a significant drop in yield. Furthermore, attempts to react the unprotected anhydrouridine with magnesium methoxide under the same conditions to produce 2'-O-methyluridine failed.

Because of the ever increasing interest in using 2'-O-substituted nucleosides in antisense oligonucleotides and elsewhere, there remains a desire for faster and more efficient methods of synthesizing 2'-O-methylated nucleosides.

SUMMARY OF THE INVENTION

The present invention discloses improved methods that provide for faster and more productive synthesis of 2'-O—R substituted pyrimidine nucleosides (wherein R is allyl or C_1–C_3 alkyl (i.e., methyl, ethyl, or propyl)), the method comprising reacting anhydrouridine or anhydrocytidine with $Mg(OR)_2$ in corresponding alcohols (R—OH) to produce 2'-O—R substituted uridine or 2'-O—R substituted cytidine, respectively.

The present method provides an unexpected improvement over prior art methods such as disclosed by McGee and Zhai, supra. The advantages of the present method arise from several factors, including the elimination of the necessity to block the 3'- and/or 5'-hydroxyl of the pyrimidine mononucleoside (e.g., with bis-silylating reagent or DMT) before the alkylation reaction as well as the ability to conduct the reaction in corresponding alcohols. Without the need to block the N^3, 3'-, and/or 5'-hydroxyls two steps are eliminated. McGee and Zhai observed no reaction of the unprotected pyrimidine nucleoside.

The ability to conduct the reaction in alcohol also eases synthesis because divalent alkoxides are synthesized in alcohols. The need to isolate $Mg(OR)_2$ is thereby eliminated. Thus, the present method comprises a one-pot synthesis. The method of McGee and Zhai, for example, entails synthesis of magnesium alkoxide in corresponding alcohols and its subsequent isolation and transfer to a DMF-containing reaction vessel for synthesis of the 2'-O-substituted pyrimidine nucleoside.

A further advantage of the present method is the shorter time to completion of alkylation reactions. Although methods such as those reported by McGee and Zhai took 4 hours at 1 gram scale, an increase in scale to 10 grams required 3 times longer. In the present invention, the reaction was completed in 5 hours at a scale of from 5 grams to 75 grams.

The efficiency of production of 2'-O-alkylcytidine is increased dramatically by the present method because it is direct from anhydrocytidine rather than from 2'-O-alkyluridine, which McGee and Zhai reported resulted in only 50% yield.

The foregoing merely summarizes certain aspects of the present invention and is not intended, nor should it be construed, as limiting the invention in any manner. All patents and other publications are hereby incorporated by reference in their entirety.

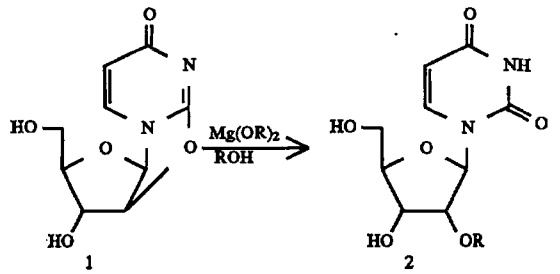
DETAILED DESCRIPTION OF THE INVENTION

The inclusion of 2'-O—R substituted nucleosides in ribozymes and antisense oligonucleotides (particularly wherein R is methyl) has been shown to greatly enhance their stability and, hence, their utility. Methods for the synthesis of oligonucleotides containing 2'-O-substituted nucleosides have suffered from the difficulties inherent in the synthesis of individual 2'-O-substituted nucleosides

themselves. These difficulties include low yields of the desired product due to non-selective methylation and/or multi-step syntheses, and high costs and/or lengthy synthesis times inherent in prior art methods.

It has been discovered that high yields of 2'-O—R substituted uridine and 2'-O—R substituted cytidine (wherein R is C_1–C_3 alkyl or allyl) can be produced when unprotected anhydrouridine or anhydrocytidine, respectively, are reacted in the presence of $Mg(OR)_2$ in the corresponding alcohol, ROH. This discovery is unexpected because previous studies suggested that reactions of unprotected anhydrouridine with magnesium methoxide at 100° C. gave no product. (McGee and Zhai, supra).

Thus, in the first aspect, the inventive method comprises the following reaction:



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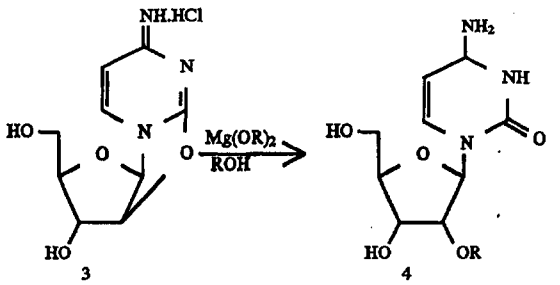
where R is a C_1–C_3 alkyl or allyl. Preferably R is methyl. The reaction is preferably conducted at about 150° C. Lower temperatures can be used, but the reaction proceeds more slowly. Product has been observed from reaction at 120° C., for example. Higher temperatures can also be employed, but significantly higher temperatures might require larger condensers or cooling equipment.

In a second aspect of the present invention, 2'-O—R substituted cytidine is produced by the same synthetic route:



3 4

The definition of R and the reaction conditions are the same as for the production of 2'-O—R substituted uridine describe above. In a preferred embodiment, R is methyl.

The synthesis of anhydrouridine (1) from uridine can be conducted according to the method described in Verheyden et al., *J. Org. Chem.* 36:25–254, 1971. Briefly, uridine is reacted with diphenyl carbonate, sodium bicarbonate and DMF at an elevated temperature, preferably 90° C. Uridine (and cytidine) are commercially available from, for example, SIGMA Chemical Co. (St. Louis, Mo.). Diphenyl carbonate, sodium bicarbonate and DMF are all commercially available as well (e.g., ALDRICH, Milwaukee, Wis.).

The synthesis of anhydrocytidine is conducted as described in Kikugawa and Kiyomi, *J. Org. Chem.* 37:284–288, 1972, where cytidine is reacted with a mixture of phosphorous oxychloride and DMF at room temperature.

The utility of 2'-substituted pyrimidine ribonucleosides for incorporation in antisense oligonucleotides and ribozymes to enhance target binding and nuclease resistance is well known to those skilled in the art and has been widely published. See, e.g., Uhlmann and Peymann, *Chem. Rev.* 90: 543, 1990; and *Antisense Research and Applications, supra.* Methods for incorporating 2'-substituted pyrimidine ribonucleosides into oligonucleotides are well known as well. E.g., Sproat and Lamond, supra.

The methods of the present invention are an improvement over prior art methods because they involve fewer steps, higher overall yield, reduced synthesis time, and reduced cost. In particular, the present methods are an improvement over that of McGee and Zhai, supra, because they eliminate the need for ribose protecting groups and utilize the corresponding alcohol as the solvent. As noted previously, McGee and Zhai did not observe any 2'-O-methyluridine product with unprotected anhydrouridine.

Furthermore, synthesis of $Mg(OR)_2$ is conducted in the corresponding alcohol, ROH. Thus, the present methods can be conducted in the same reaction vessel as that in which $Mg(OR)_2$ was synthesized. This simplifies the process. Using the method of McGee and Zhai, it is necessary to isolate $Mg(OR)_2$ from the alcohol-based reaction solution before its addition to the DMF-containing reaction vessel used by McGee and Zhai for alkylation of 5'-protected anhydrouridine.

The improved methods of synthesizing 2'-O-substituted uridine and cytidine ribonucleosides also leads to improved methods of synthesizing oligonucleotides containing them. Thus, in a third aspect, the invention provides an improved method of synthesizing oligonucleotides containing one or more 2'-O-substituted uridine or cytidine residues, the method comprising synthesizing 2'-O-substituted uridine or cytidine according to either of the first two aspects of the invention and then employing the 2'-O-substituted uridine or cytidine so-produced in the synthesis of an oligonucleotide containing one or more of such residues. Oligonucleotide synthesis can be conducted by any suitable means, a number of which are standard and well known to those skilled in the art. E.g., *Methods in Molecular Biology v.* 20: *Protocols for Oligonucleotides and Analogs* (S. Agrawal, Ed., Humana Press, Totowa, N.J. 1993).

One skilled in the art will recognize that modifications may be made in the present invention without deviating from the spirit or scope of the invention. The invention is illustrated further by the following examples, which are not be construed as limiting the invention or scope of the specific procedures described herein.

EXAMPLES

Example 1

Synthesis of 2'-O-methyl-Uridine

2,2'-anhydro uridine (1, 75 g, 331.56 mmol) was added to the freshly prepared solution of 15% magnesium methoxide (1218.75 ml, 1.66 mol) in methanol. The mixture was refluxed at 150° C. for five hours. The mixture was cooled down to room temperature and then to 5° C. on ice bath. The pH was adjusted to 7 by using glacial acetic acid. The solution was roto-evaporated to a foam. This solid was refluxed with ethyl alcohol (1500 ml) for two hours. The solid was filtered and filtrate was roto-evaporated to a solid mass. The isolated yield of the compound was 78.75 g (92%). The product was >95% pure by HPLC. The yield after quantitation against uridine was 89%. The structure of the compound was confirmed by ^{1}H-NMR and elemental analysis. Table 1 shows the PMR results of the compounds.

Example 2

Synthesis of 2'-O-methyl-Cytidine

2,2'-anhydro Cytidine HCl (3, 1 g, 3.82 mmol) was added to the freshly prepared solution of 15% magnesium methoxide (16.25 ml, 22.17 mmol) in methanol. The mixture was refluxed at 150°–160° C. for five hours. The mixture was cooled down to room temperature and then to 5° C. on ice bath. The pH was adjusted to 7 by using glacial acetic acid. The solution was roto-evaporated to a gummy mass. This material was refluxed with ethyl alcohol (25 ml) for two hours. The solid was filtered and filtrate was roto-evaporated to a solid mass. The isolated yield after quantitation against cytidine was 752 mg (76%). The product was >93% pure by HPLC. The structure of the compound was confirmed by ^{1}H-NMR and elemental analysis. Table 1 shows the PMR results of the compounds.

TABLE 1

Proton NMR (300 MHz) of 2'-O-methyl nucleosides

Cmpd	H-1'	H-2'	H-3'	H-4'	H-5' H-5"	H-5	H-6	—CH_3
2	5.90 (d, 3.36)	3.97 (m)	4.27 (m)	4.02 (m)	3.76–3.84 (m)	5.82 (d, 7.93)	7.85 (d, 8.09)	3.45 (s)
4	5.91 (d, 3.33)	3.95 (m)	4.24 (m)	4.04 (m)	3.76–3.85 (m)	6.01 (d, 7.33)	7.82 (d, 7.48)	3.47 (s)

From the foregoing, it will appreciated that although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit or scope of the invention.

What is claimed is:

1. A method of synthesizing 2'-O—R substituted uridine comprising reacting 2,2'-anhydrouridine with $Mg(OR)_2$ in ROH, wherein R is C_1–C_3 alkyl or allyl.

2. The method according to claim 1, wherein R is methyl.

3. A method of synthesizing 2'-O—R substituted cytidine comprising reacting 2,2'-anhydrocytidine with $Mg(OR)_2$ in ROH, wherein R is C_1–C_3 alkyl or allyl.

4. The method according to claim 3, wherein R is methyl.

5. A method for synthesizing oligonucleotides containing one or more 2'-O-substituted uridine or cytidine ribonucleotides wherein the improvement is preparing the 2'-O-substituted uridine or cytidine by 1) reacting 2,2'-anhydrouridine or 2,2'-anhydrocytidine with Mg(OR)2 in ROH to produce 2'-O—R substituted uridine or cytidine, respectively, wherein R is C1–C3 alkyl or allyl, 2) isolating the 2'-substituted uridine or cytidine, and 3) using said 2'-O-substituted uridine or 2'-O-substituted cytidine in said synthesis of said oligonucleotides.

6. The method according to claim 5, wherein R is methyl.

* * * * *

Ether synthesis using trifluoromethanesulfonic anhydride or triflates under mild reaction conditions

Tomihiro Nishiyama, Hideaki Kameyama, Hideki Maekawa, and Kouhei Watanuki

Abstract: Reactions of magnesium alkoxides with trifluoromethanesulfonic anhydride or triflates gave corresponding symmetrical or unsymmetrical ethers in moderate to good yields under mild reaction conditions. The scope and limitations of the reaction are discussed.

Key words: ethers, trifluoromethanesulfonic anhydride, triflates, magnesium alkoxides.

Résumé : Les réactions des alcoolates de magnésium avec l'anhydride trifluorométhanesulfonique ou avec les triflates dans des conditions douces conduit aux éthers symétriques ou non symétriques avec des rendements qui vont de modérés à bons. On discute de la portée et des limitations de la réaction.

Mots clés : éthers, anhydride trifluorométhanesulfonique, triflates, alcoolates de magnésium.

[Traduit par la Rédaction]

Introduction

Conversion of an alcohol to the corresponding ether is a widely used functional transformation in organic synthesis, and several methods are available to accomplish an *O*-alkylation reaction under a variety of conditions. For example, the Williamson ether synthesis, with its modifications (1, 2), is one of the most useful synthetic methods for the formation of a carbon–oxygen bond. The attack of alkoxides on alkyl halides, however, is synthetically useful only when the alkyl halide is primary (3). Moreover, the standard Williamson ether synthesis requires rigorous reaction conditions. The condensation of alcohols and their salts with aldehydes (4), olefins (5), *p*-toluene sulfonic acid (6), nitro compounds (7), and dialkyl phosphates (8) are also reported to be useful methods for the *O*-alkylation reaction.

Other approaches have been demonstrated by either the trimethylsilyl triflate- or trimethylsilyl iodide-catalyzed reductive coupling of carbonyl compounds with trialkylsilanes (9). Noyori and co-workers (10) have shown that acetals can be conveniently reduced to ether with trimethylsilane catalyzed by trimethylsilyl trifluoromethanesulfonate. This method is extremely mild and provides nearly equal quantitative yields of symmetrical and unsymmetrical ethers from the corresponding acetals.

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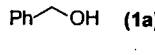
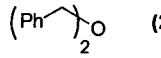
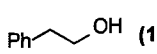
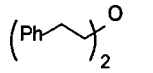
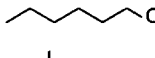
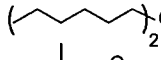
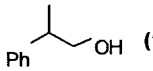
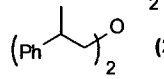
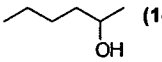
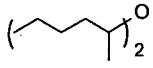
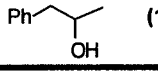
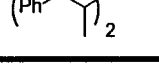
In this paper, we report a simple procedure for the preparation of symmetrical and unsymmetrical ethers by the reaction of magnesium alkoxides with trifluoromethanesulfonic anhydride (Tf_2O) or triflates (Scheme 1). The scope and limitations of this procedure are also discussed.

Results and discussion

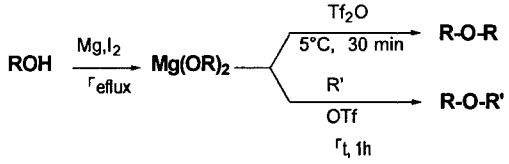
Table 1 summarizes the products and yields obtained from the reaction of magnesium alkoxides with Tf_2O, and scope of the present method. The reaction of Tf_2O in dry ether with in situ generated magnesium di(benzyl alcoholate) proceeded smoothly to yield dibenzyl ether **2a**. The reaction was completed in 30 min at 5°C, and an 80% isolated yield of dibenzyl ether could be obtained. In this reaction, a stoichiometric amount of Tf_2O was necessary for the reaction. After treatment of magnesium di(benzyl alcoholate) with 0.5 equiv. of Tf_2O, the isolated yield of symmetrical ether decreased to 35% (entry 2). The trifluoromethanesulfonic acid (TfOH) and toluene-4-sulfonic anhydride (TS_2O) were less effective (entries 3 and 4), and the reaction required longer reaction times and a higher temperature to attain a comparable yield (entry 4). With other magnesium alkoxides such as 2-phenylethanol **1b** and hexanol **1c**, the corresponding symmetrical ethers, diphenethyl ether **2b** and dihexyl ether **2c**, were obtained in high yields. The reactions of magnesium alkoxides with Tf_2O were usually carried out at 5°C for 30 min, although in one case, a higher temperature provided better yields. When a mixture of magnesium di(hexanolate) and Tf_2O was stirred at 40°C for 1h, **2c** was obtained in 90% yield. Similarly, a branched primary magnesium alkoxide gave the symmetrical ether **2d** as a mixture of diastereomers (ratio 1:1) in 60% yield (entry 7). In contrast to results obtained with primary magnesium alkoxides, the reactions with secondary magnesium alkoxides such as 2-hexanol **1e** and

Table 1. Synthesis of symmerical ethers.[a]

Entry	Alcohol	Additive	Product	Yield (%)[b]
1	 (1a)	Tf_2O	 (2a)	85 (80)
2		Tf_2O		39 (35)[c]
3		TfOH		23 (19)
4		TS_2O		86 (80)[d]
5	 (1b)	Tf_2O	 (2b)	86 (72)
6	 (1c)	Tf_2O	 (2c)	98 (90)[e]
7	 (1d)	Tf_2O	 (2d)	71 (60)[f]
8	 (1e)	Tf_2O	 (2e)	30 (25)[f]
9	 (1f)	Tf_2O	 (2f)	45 (37)[f]

[a]Unless otherwise indicated, all reactions were carried out at 5°C for 30 min.
[b]GLC yield based on Tf_2O. Value in parentheses indicates yield after purification.
[c]The amount of Tf_2O was 0.5 equiv. Yield based on Mg.
[d]The reaction was carried out at 40°C for 15 h.
[e]The reaction was carried out at 40°C for 1 h.
[f]1:1 diastereomeric ratio. The ratios of two diastereomers were estimated by GLC.

Scheme 1.



3-phenyl-2-propanol **1f** gave bis(2-hexyl) ether **2e** and bis(3-phenyl-2-propyl) ether **2f** as a mixture of diastereomers (ratio 1:1) in low yields. However, tertiary alcohol and phenol failed to give the corresponding ethers. No product was obtained upon heating magnesium di(1,1-dimethyl-2-phenylethanolate) and Tf_2O, even after several hours.

We next turned our attention to the synthesis of unsymmetrical ethers. An unsymmetrical ether can be obtained in moderate yields when an alkyl triflate is used in place of Tf_2O in the reaction. These results are summarized in Table 2. The addition of 1 equiv. of pentyl triflate in dry ether to magnesium di(benzyl alcoholate) in benzyl alcohol **1a** solution at room temperature for 1 h gave benzyl pentyl ether **3a** in 87% yield. The reaction is much less successful with mesylate or tosylate than with triflate. In the case of pentyl mesylate, the reaction required longer reaction times and a higher temperature to obtain an unsymmetrical ether (entry 2). Pentyl tosylate did not give an unsymmetrical ether even at 60°C for 17 h (entry 3). The treatment of 2 equiv. of magnesium di(2-phenylethanolate) with ditriflate at room temperature for 6 h gave symmetrical bis(phenethoxy)butane **3c** in 60% yield. When branched magnesium di(2-phenyl-1-propanolate) was used, the corresponding pentyl 2-phenyl-1-propyl ether **3d** was obtained in 88% yield. For the reactions of secondary magnesium alkoxides with triflate, the corresponding ethers **3e** and **3f** were isolated in 85 and 76% yields, respectively (entries 7 and 8). With tertiary magnesium alkoxide such as 1,1-dimethyl-2-phenylethanol **1h**, the yield was not satisfactory (entry 9). The results in Table 2 indicate that this reaction sequence is applicable to primary and secondary magnesium alkoxides in moderate yields.

In conclusion, we report a simple procedure for the preparation of symmetrical and unsymmetrical ethers by the reaction of magnesium alkoxides with Tf_2O or triflates. This method has the advantage that the reaction rapidly and cleanly takes place under mild reaction conditions.

Experimental

General methods

NMR spectra were recorded in $CDCl_3$ on a JEOL GSX-400 spectrometer operating at 400 and 100 MHz for ^1H and ^{13}C, respectively. Chemical shifts for NMR spectra were referenced to internal $(CH_3)_4Si$. Mass spectra were measured

Table 2. Synthesis of unsymmetrical ethers.[a]

Entry	Alcohol	Triflate	Product	Yield (%)[b]
1	**1a**	OTf	Ph-O- **(3a)**	98 (87)
2		OMs		52 (42)[c]
3		OTs		N.R[c]
4	**1b**	OTf	Ph-O- **(3b)**	99 (94)
5		TfO-OTf	Ph-O-O-Ph **(3c)**	66 (60)[d]
6	**1d**	OTf	Ph(Me)-O- **(3d)**	92 (88)
7	**1g**[e]		Ph(Me)-O- **(3e)**	96 (85)
8	**1f**		Ph(Et)-O- **(3f)**	86 (76)
9	**1h**[f]		Ph(Me)(Me)-O- **(3g)**	52 (40)

[a]Unless otherwise indicated, all reactions were carried out at room temperature for 1 h.
[b]GLC yield based on triflate. Value in parentheses indicates yield after purification.
[c]The reaction was carried out at 60°C for 17 h.
[d]The reaction was carried out at room temperature for 6 h.
[e]1-Phenylethanol.
[f]1,1-Dimethyl-2-phenylethanol.

with a Perkin–Elmer model 910 spectrometer operating in the electron impact mode (70 eV). IR spectra were recorded on a Perkin–Elmer model 1600 spectrophotometer. Gas chromatography was carried out on a 20 m × 0.22 mm capillary column packed with methyl silicone.

Materials

Triflates, mesylates, and tosylates were prepared in the same manner as described in the literature (11, 12). All reagents were reagent grade materials purchases obtained from Tokyo Kasei Kogyo Co. Ltd. (Tokyo) and used without further purification.

General procedure for the synthesis of symmetrical ethers

The following experimental procedure is representative. The precursor, the magnesium alkoxides, were prepared by using a slight modification of the procedure described in the literature (13). A mixture of benzyl alcohol **1a** (2.16 g, 0.02 mol), magnesium (0.24 g, 0.01 mol), and iodine (50 mg, 1.2 mmol) was stirred at 60°C for 1 h. To this solution were added **1a** (2.16 g, 0.02 mol), and the reaction mixture was refluxed until the magnesium was digested. After the reaction mixture cooled to ice-water temperature, a solution of Tf_2O (2.8 g, 0.01 mol) in dry ether (10 mL) was added, and the mixture was then stirred continuously for 30 min at 5°C. The reaction was quenched by the addition of water and extracted with ether. The extract was washed with water, and concentrated in vacuo. The residue was chromatographed on silica gel using hexane/ethyl acetate (80/20) to give dibenzyl ether **2a** in 80% yield as a colorless oil.

Dibenzyl ether (2a) (14)
^{1}H NMR δ: 4.54 (s, 4H), 7.24–7.37 (m, 10H); ^{13}C NMR δ: 72.1,127.6, 127.8, 128.4, 138.3; MS *m/z* (rel. intensity): 107(19), 92(100), 91(98), 79(29), 77(28), 65(31), 51(12).

Diphenethyl ether (2b) (15)
^{1}H NMR δ: 2.87 (t, J = 7.3 Hz, 4H), 3.64 (t, J = 7.3 Hz, 4H), 7.18–7.28 (m, 10H); ^{13}C NMR δ: 36.4, 71.9, 126.2, 128.3, 128.9, 139.1; MS *m/z* (rel. intensity): 226 (M^+, 1), 135(11), 134(11), 106(10), 105(100), 104(12), 103(13), 91(25), 77(19).

Dihexyl ether (2c) (14, 15)
^{1}H NMR δ: 0.89 (t, J = 7.0 Hz, 6H), 1.28–1.36 (m, 12H), 3.39 (t, J = 7.0 Hz, 4H); ^{13}C NMR δ: 14.1, 22.7, 25.9, 29.8, 31.8, 71.0; MS *m/z* (rel. intensity): 85(84), 84(21), 83(22), 69(15), 59(21), 56(43), 55(23), 43(100).

Bis(2-phenyl-1-propyl) ether (2d) (16*a*)
^{1}H NMR δ: 1.09 (d, J = 6.6 Hz, 3H), 1.23 (d, J = 6.6 Hz, 3H), 2.58 (dd, J = 6.6, 13.5 Hz, 1H), 2.62 (dd, J = 6.6, 13.5 Hz, 1H), 2.83 (dd, J = 6.6 Hz, 1H), 2.86 (dd, J = 6.6, 13.5 Hz, 1H), 3.53–3.63 (m, 2H), 7.09–7.29 (m, 10H); ^{13}C NMR δ: 18.3, 18.3, 19.4, 19.5, 40.3, 40.4, 43.1, 43.2, 74.7, 74.8, 76.9, 77.1, 125.9, 126.0, 126.2, 127.3, 127.4, 128.1,

128.2, 128.3, 129.4, 129.5, 139.1, 144.6, 144.7; MS *m/z* (rel. intensity): 163(12), 119(42), 105(13), 91(100), 77(10).

Bis(2-hexyl) ether (2e) (16*b*)
^{1}H NMR δ: 0.89 (t, *J* = 7.0 Hz, 3H), 1.10 (t, *J* = 6.2 Hz, 3H), 1.09 (d, *J* = 5.9 Hz, 3H), 1.11 (d, *J* = 6.2 Hz, 3H), 1.21–1.38 (m, 10H), 1.45–1.49 (m, 2H), 3.39–3.42 (m, 2H); ^{13}C NMR δ: 14.1, 20.5, 21.1, 22.8, 22.9, 27.9, 28.1, 36.9, 37.3, 72.9, 73.4; MS *m/z* (rel. intensity): 152(10), 137(20), 129(17), 85(100), 69(11), 57(17).

Bis(3-phenyl-2-propyl) ether (2f)
IR (neat, cm^{-1}): 2960, 2920, 1599, 1495, 1450, 1370, 1122, 749, 709; ^{1}H NMR δ: 0.98 (d, *J* = 5.9 Hz, 3H), 1.10 (d, *J* = 5.9 Hz, 3H), 2.50 (dd, *J* = 6.6, 13.2 Hz, 1H), 2.60 (dd, *J* = 6.6, 13.2 Hz, 1H), 2.74 (dd, *J* = 6.2, 13.2 Hz, 1H), 2.84 (dd, *J* = 6.2, 13.2 Hz, 1H), 3.54–3.62 (m, 2H), 7.08–7.27 (m, 10H); ^{13}C NMR δ: 20.2, 20.7, 43.8, 43.9, 74.9, 75.4, 126.0, 128.1, 128.2, 129.5, 129.6, 139.3; MS *m/z* (rel. intensity): 163(24), 119(51), 92(9), 91(100), 65(9). Anal. calcd. for $C_{18}H_{22}O$: C 84.99, H 8.72; found: C 84.87, H 8.97.

General procedure for the synthesis of unsymmetrical ethers

Unsymmetrical ethers were prepared using triflates in place of Tf_2O. To a solution of magnesium di(benzyl alcoholate), generated in situ from **1a** (2.16 g, 0.02 mol), magnesium (0.24 g, 0.01 mol), and iodine (50 mg, 0.2 mmol) in benzyl alcohol (2.16 g, 0.02 mol) was added dropwise a solution of pentyl triflate (2.2 g, 0.01 mol) in 5 mL of dry ether. The mixture was stirred at room temperature for 1 h, the reaction was quenched by the addition of water. After the usual work-up, chromatography of the residue with hexane/ethyl acetate (80/20) afforded benzyl pentyl ether **3a** (1.55 g, 87%).

Benzyl pentyl ether (3a) (17)
^{1}H NMR δ: 0.89 (t, *J* = 7.0 Hz, 3H), 1.29–1.35 (m, 4H), 1.57–1.64 (m, 2H), 3.46 (t, *J* = 6.6 Hz, 2H), 4.50 (s, 2H), 7.25–7.34 (m, 5H); ^{13}C NMR δ: 14.1, 22.5, 28.4, 29.5, 36.4, 71.1, 71.8, 126.1, 128.3, 128.9, 139.1; MS *m/z* (rel. intensity): 92(61), 91(100), 77(9), 65(15).

Pentyl phenetyl ether (3b)
IR (neat, cm^{-1}): 2910, 2840, 1598, 1492, 1449, 1360, 1110, 743, 695; ^{1}H NMR δ: 0.89 (t, *J* = 7.3 Hz, 3H), 1.28–1.32 (m, 4H), 1.55–1.59 (m, 2H), 2.87 (t, *J* = 6.9 Hz, 2H), 3.42 (t, *J* = 6.9 Hz, 2H), 3.63 (t, *J* = 6.9 Hz, 2H), 7.17–7.29 (m, 5H); ^{13}C NMR δ: 14.1, 22.5, 28.4, 29.5, 36.4, 71.1, 71.9, 126.1, 128.3, 128.9, 139.1; MS *m/z* (rel. intensity): 192 (M$^+$, 19), 174(8), 106(38), 105(44), 104(17), 103(18), 101(11), 92(24), 91(54), 79(21), 78(16), 77(28), 71(100), 69(18), 65(17), 55(9), 51(9), 43(63). Anal. calcd for $C_{13}H_{20}O$: C 81.19, H 10.48; found: C 81.01, H 10. 35.

Bis(phenethoxy) butane (3c)
IR (neat, cm^{-1}): 2920, 2845, 1732, 1598, 1595, 1450, 1364, 1109, 749, 699; ^{1}H NMR δ: 1.60–1.62 (m, 4H), 2.87 (t, *J* = 7.0 Hz, 4H), 3.46 (t, *J* = 7.0 Hz, 4H), 3.61 (t, *J* = 7.0 Hz, 4H); ^{13}C NMR δ: 26.5, 36.4, 70.7, 71.8, 126.1, 128.3, 128.9, 139.1; MS *m/z* (rel. intensity): 207(8), 107(25), 92(12), 91(100), 73(17), 65(17). Anal. calcd. for $C_{20}H_{26}0_2$: C 85.06, H 9.28; found; C 85.11, H 9.19.

Pentyl 2-phenyl-1-propyl ether (3d)
IR (neat, cm^{-1}): 2945, 2850, 1721, 1595, 1491, 1445, 1388, 1105, 755, 696; ^{1}H NMR δ: 0.88 (t, *J* = 7.3 Hz, 3H), 1.29 (d, *J* = 6.9 Hz, 3H), 1.25–1.30 (m, 4H), 1.53–1.55 (m, 2H), 2.98–3.02 (m, 1H), 3.38–3.45 (m, 3H), 3.53 (dd, *J* = 9.3, 6.1Hz, 1H), 7.18–7.31 (m, 5H); ^{13}C NMR δ: 14.1, 18.4, 22.5, 28.4, 29.4, 71.2, 126.3, 127.4, 128.3, 144.7; MS *m/z* (rel. intensity): 206 (M$^+$, 8), 120(17), 106(12), 105(100), 104(36), 91(50), 79(16), 78(12), 77(23), 71(80), 69(13), 43(32). Anal. calcd. for $C_{14}H_{22}O$: C 81.49, H 10.75; found: C 81.20, H 10.45.

Pentyl 1-phenylethyl ether (3e)
IR (neat, cm^{-1}): 2910, 2850, 1600, 1492, 1451, 1370, 1105, 760, 703; ^{1}H NMR δ: 0.88 (t, *J* = 7.3 Hz, 3H), 1.28–1.34 (m, 4H), 1.42 (d, *J* = 6.6 Hz, 3H), 1.55–1.56 (m, 2H), 4.37 (q, *J* = 6.6 Hz, 1H), 3.28 (t, *J* = 6.6 Hz, 2H), 7.23–7.35 (m, 5H); ^{13}C NMR δ: 14.0, 22.6, 24.3, 28.4, 29.5, 29.7, 68.8, 126.1, 127.3; MS *m/z* (rel. intensity): 121(13), 104(24), 105(100), 91(10), 79(18), 77(34). Anal. calcd. for $C_{13}H_{20}O$: C 81.19, H 10.48; found: C 81.13, H 10.26.

1-(3-Phenyl-2-propoxy) pentane (3f)
IR (neat, cm^{-1}): 2920, 2810, 1719, 1590, 1485, 1442, 1365, 1101, 735, 693; ^{1}H NMR δ: 0.88 (t, *J* = 7.3 Hz, 3H), 1.27–1.31 (m, 7H), 1.53–1.55 (m, 2H), 2.98–3.01 (m, 1H), 3.38–3.44 (m, 3H), 3.52–3.55 (m, 1H), 7.18–7.31 (m, 5H); ^{13}C NMR δ: 14.1, 19.6, 22.5, 28.4, 29.8, 43.2, 68.8, 76.7, 126.0, 128.2, 129.5, 139.3; MS *m/z* (rel. intensity): 206 (M$^+$, 4), 115(95), 97(14), 92(28), 91(100), 77(10), 72(18), 71(98), 65(21), 55(16), 45(93), 43(99). Anal. calcd. for $C_{14}H_{22}O$: C 81.49, H 10.75; found: C 81.12, H 11.01.

1-(2-Methyl-3-phenyl-2-propoxy) pentane (3g)
IR (neat, cm^{-1}): 2906, 2850, 1595, 1490, 1448, 1375, 1359, 1222, 1145, 1122, 1080, 725, 698; ^{1}H NMR δ: 0.91 (t, *J* = 6.9 Hz, 3H), 1.12 (s, 6H), 1.32–1.35 (m, 6H), 2.77 (s, 2H), 3.39 (t, *J* = 6.6 Hz, 2H), 7.19–7.25 (m, 5H); ^{13}C NMR δ: 14.1, 25.2, 28.6, 30.3, 47.1, 61.4, 71.0, 74.9, 125.9, 127.8, 130.6, 138.7; MS *m/z* (rel. intensity): 135(7), 130(8), 129(82), 117(8), 92(11), 91(53), 71(14), 65(10), 60(10), 59(100), 55(10), 43(34). Anal. calcd. for $C_{15}H_{24}O$: C 81.76, H 10.98; found: C 82.02, H 11.01.

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Exhibit 9

INTERNATIONAL UNION OF PURE
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COMMISSION ON SEPARATION METHODS IN ANALYTICAL CHEMISTRY (V3)*

ANALYTICAL CHIRAL SEPARATION METHODS

(IUPAC Recommendations 1997)

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Analytical chiral separation methods (IUPAC Recommendations 1997)

Abstract: In recent years there has been considerable interest in the synthesis and separation of enantiomers of organic compounds especially because of their importance in the biochemistry and pharmaceutical industry. Frequently the methods used for the separations, for monitoring the progress of an asymmetric synthesis or optical purity of the products are chromatographic with either liquids, gases, or supercritical fluids as the mobile phase. More recently capillary electrophoresis has been added as an analytical chiral separation method.

These applications have lead to a number of terms and expressions in addition to those commonly used or recently recommended for the chemistry and physical properties of chiral compounds. This Nomenclature provides the descriptions and definitions for additional terms particularly related to analytical separation methods, and to the formation and enantiomeric purity of chiral products.

INTRODUCTION

Enantiomers are two chemically identical molecular species which differ from each other as non-superposable mirror images. The most simple and vivid model for enantiomeric structures is the two hands, left and right. Enantiomers, in addition to diastereomers and *cis-trans*-isomers, are thus a special case of stereoisomers.

The chirality (handedness) of enantiomeric molecules is caused by the presence of one or more chirality elements (chirality axis, chirality plane, or chirality centre, e.g., asymmetric carbon atom) in their structure. The chirality sense and optical activity of the enantiomers are determined by their absolute configuration, i.e., the spatial arrangement of the atoms in the molecule. In contrast to their conformation, the configuration of enantiomers cannot be changed without a change in the connectivity of constituent atoms. Designation of the configuration of enantiomers should be made in accordance with the Cahn-Ingold-Prelog *R, S*-system. The Delta-Lambda designations for enantiomers of octahedral complexes and the D, L Fischer-Rosanoff designations for amino acids and sugars are also in use.

Conventional chemical synthesis, in contrast to asymmetric synthesis, deals mostly with the transformations of achiral compounds. If these reactions result in the formation of a chirality element in the molecule, the reaction product appears to be an equivalent mixture of a pair of enantiomers, a racemate, which is optically inactive. Racemates are also formed through racemisation of chiral compounds. Racemates crystallise in the form of a racemic compound or, less frequently, as a conglomerate.

Separation of the enantiomers comprising the racemate, i.e., the resolution of the racemate, is a common problem in stereochemical research as well as in the preparation of biologically active compounds, in particular, drugs. The problem is that in contrast to diastereomers and all other types of isomeric species, enantiomers, in an achiral environment, display identical physical and chemical properties. (Energetic inequivalence of enantiomeric species, which can arise from the violation of parity by the weak interactions [1], is negligibly small - of the order of 10^{-14} J mol^{-1}).

One approach to separate enantiomers, sometimes referred to as indirect enantiomeric resolution, involves the coupling of the enantiomers with an auxiliary chiral reagent to convert them into diastereomers. The diastereomers can then be separated by any achiral separation technique.

Nowadays, direct separation methods are commonly used in which the enantiomers are placed in an chiral environment. As a matter of principle, only chiral selectors or chiral irradiation (e.g., a polarised light beam which consists of two chiral circular-polarised components) can distinguish between two enantiomers. Chiral selectors can be an appropriate chiral molecule or a chiral surface (e.g., a chiral seed crystal). Due to the enantioselectivity (a special case of stereoselectivity) of the interaction with the two enantiomers, the chiral selector either transforms the enantiomers at a different rate into new chemical entities (kinetic enantioselectivity) or forms labile molecular adducts of differing stability with the enantiomers (thermodynamic enantioselectivity). Enzymic selective transformation of L-enantiomers of racemic D, L-amino acids is a typical example of a kinetically enantioselective process (kinetic resolution). Enantioselective (chiral) chromatography does not modify the enantiomeric species to be separated and thus represents an example of a thermodynamically enantioselective process.

Direct enantiomeric resolutions are only feasible in chromatographic systems which contain an appropriate chiral selector. The latter can be incorporated into the stationary phase (chiral stationary phase) or be permanently bonded to or coated onto the surface of the column packing material (chiral bonded and chiral coated stationary phases). In all these cases it is appropriate to refer to the chromatographic column as an enantioselective (chiral) column. Enantioselective chromatography can also be performed on achiral chromatographic columns using the required chiral selector as a chiral mobile phase or a chiral mobile phase additive. Combinations of several chiral selectors in the mobile phase [2] as well as mobile and stationary phases [3] are also feasible.

In the case of chiral stationary phases, the enantiomer that forms the more stable association with the chiral selector will be the more strongly retained species of the racemate. The enantioselectivity of the chiral chromatographic system is then expressed as the ratio of the retention factors of the two enantiomers. This ratio may approach the value of the thermodynamic enantioselectivity of the association of the chiral selector with the enantiomers. This situation occurs when the association with the chiral selector governs the retention of the enantiomers in the chromatographic system and other, non-selective types of solute-sorbent interactions are negligible. On the other hand, a chiral mobile phase reduces the retention of the solute enantiomer which forms a stronger association with the chiral selector. Here again, the limit for the enantioselectivity of the chiral chromatographic system is set by the enantioselectivity of the selector-solute association (in the mobile phase). However, in the majority of chiral mobile phase systems, the chiral selector as well as its associates with the solute enantiomers are distributed between the mobile and stationary phases. The effective enantioselectivity of the chromatographic system will therefore be proportional to the ratio of the enantioselectivities of the association processes in the stationary and mobile phases [4].

Interaction of the chiral selector of the system with the enantiomers of the solute results in the formation of two labile diastereomers. These differ in their thermodynamic stability, provided that at least three active points of the selector participate in the interaction with corresponding sites of the solute molecule. This three-point interaction rule is generally valid for enantioselective chromatography, with the extension to the rule, stating that one of the required interactions may be mediated by the adsorption of the two components of the interacting pair onto the sorbent surface [5].

Because of the multiplicity and complexity of the interactions of the enantiomers to be separated with the chiral selector, sorbent surface and other components of the chromatographic system, the total enantioselectivity can depend strongly on the composition, pH and temperature of the mobile phase. Therefore, in papers on enantioselective chromatography, it is important to define these parameters.

Enantioselective chromatography and capillary electrophoresis are extensively employed in the analysis of the enantiomeric composition (enantiomeric excess, optical purity) of chiral compounds. Liquid and supercritical fluid chromatography are also used for the isolation of chiral compounds from racemic mixtures on a preparative scale.

Enantioselective separations have been realised in all possible separation techniques, including gas chromatography, column liquid chromatography, thin-layer chromatography, supercritical fluid chromatography, as well as electromigration methods, counter current liquid chromatography and liquid-liquid extractions. Numerous review papers and special monographs [6-15] describe the technical details as well as the achievements and potential of these important modern separation techniques.

In the following glossary of definitions and terms related to the chromatographic and capillary-electrophoretic separation of chiral compounds some terms (those marked with asterisks) were defined in the Basic Terminology of Stereochemistry, recently published by the IUPAC Joint Working Party on Stereochemical Terminology [16]. Some of these definitions contain further cross references which are to be found in the original paper.

TERMS AND DEFINITIONS

1. General terms related to chirality

*1.1 *Chirality*

The geometric property of a rigid object (or spatial arrangement of points or atoms) of being non-superposable on its mirror image; such an object has no symmetry elements of the second kind (a mirror plane, $\sigma = S_1$, a centre of inversion, $i = S_2$, a rotation-reflection axis, S_{2n}). If the object is superposable on its mirror image the object is described as being achiral. See also handedness

*1.2 *Diastereoisomers (Diastereomers) see diastereoisomerism*

1.3 Diastereoisomerism

Stereoisomerism other than enantiomerism and *cis-trans* isomerism. Diastereoisomers (or diastereomers) are stereoisomers not related as mirror images. Diastereoisomers are characterised by differences in physical properties, and by differences in chemical behaviour towards achiral as well as chiral reagents.

*1.4 *Enantiomer*

One of a pair of molecular entities which are mirror images of each other and non-superposable. See also *enantiomorph.*

*1.5 *Stereoisomers*

Isomers that possess identical constitution but which differ in the arrangement of their atoms in space. See *enantiomer, diastereomer, cis-trans-isomers.*

2. Terms related to the separation process

2.1 Chiral additive

The *chiral selector* which has been added as a component of a mobile phase or electrophoretic medium.

2.2 Chiral mobile phase

A mobile phase containing a *chiral selector.*

2.3 Chiral selector

The *chiral* component of the separation system capable of interacting *enantioselectively* with the *enantiomers* to be separated.

2.4 Chiral stationary phase

A stationary phase which incorporates a *chiral selector.* If not a constituent of the stationary phase as a whole, the chiral selector can be chemically bonded to (*chiral bonded stationary phase*) or immobilised onto the surface of a solid support or column wall (*chiral coated stationary phase*), or simply dissolved in the liquid stationary phase.

2.5 Enantioselective chromatography (electrophoresis)

The separation of *enantiomeric* species due to the *enantioselectivity* of their interaction with the *chiral selector*(s) of a chromatographic (electrophoretic) system. Also called *Chiral chromatography (electrophoresis).*

2.6 Enantioselective column

A chromatographic column containing a *chiral stationary phase.* Also called a *chiral column.*

2.7 Enantioselectivity (in chiral separations)

The preferential interaction with the chiral selector of one enantiomer over the other.

2.8 Enantioselectivity of a chromatographic (electrophoretic) system

The ratio of the retention factors of two solute enantiomers in a chiral chromatographic (electrophoretic) system.

3. Terms related to the chiral purity of the sample

3.1 * Diastereoisomer excess/Diastereoisomeric excess

This is defined by analogy with *enantiomer excess*, as $D_1 - D_2$ [and the percent diastereoisomer excess as 100 $(D_1 - D_2)$], where the mole fractions of the two diastereoisomers in a mixture or the fractional yields of two diastereoisomers formed in a reaction are D_1 and D_2 ($D_1 + D_2 = 1$). The term is not applicable if more than two diastereoisomers are present. Frequently this term is abbreviated to d.e. See *stereoselectivity; diastereoisomerism.*

3.2 *Enantiomer excess/Enantiomeric excess

For a mixture of (+) and (–) enantiomers, with composition given as the mole or weight fractions $F_{(+)}$ and $F_{(-)}$ (where $F_{(+)} + F_{(-)} = 1$) the enantiomeric excess is defined as $|F_{(+)} - F_{(-)}|$ (and the percent enantiomer excess by $100|F_{(+)} - F_{(-)}|$). Frequently this term is abbreviated as e.e. See *optical purity.*

3.3 *Enantiomeric purity see Enantiomer excess.

3.4 *Optical purity

The ratio of the observed optical rotation of a sample consisting of a mixture of enantiomers to the optical rotation of one pure enantiomer. See *enantiomeric excess.*

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Exhibit 10

CHIRAL SEPARATIONS

INTRODUCTION

1.1. Importance of Chiral Separation

The separation of chiral compounds has been of great interest because the majority of bioorganic molecules are chiral.[1] Living organisms, for example, are composed of chiral biomolecules such as amino acids, sugars, proteins and nucleic acids. In nature these biomolecules exist in only one of the two possible enantiomeric forms, e.g., amino acids in the L-form and sugars in the D-form. Because of chirality, living organisms show different biological responses to one of a pair of enantiomers in drugs, pesticides, or waste compounds, etc.[2]

Chirality is a major concern in the modern pharmaceutical industry.[3-4] This interest can be attributed largely to a heightened awareness that enantiomers of a racemic drug may have different pharmacological activities, as well as different pharmacokinetic and pharmacodynamic effects.[5-6] The body being amazingly chiral selective, will interact with each racemic drug differently and metabolize each enantiomer by a separate pathway to produce different pharmacological activity. Thus, one isomer may produce the desired therapeutic activities, while the other may be inactive or, in worst cases, produce unwanted effects. Consider the tragic case of the racemic drug of n-phthalyl-glutamic acid imide that was marketed in the 1960's as the sedative Thalidomide. Its therapeutic acitivity resided exclusively in the R-(+)-enantiomer. It was discovered only after several hundred births of malformed infants that the S-(+)-enantiomer was teratogenic. [7]

The U.S. Food and Drug Administration, in 1992, issued a guideline that for chiral drugs only its therapeutically active isomer be brought to market, and that each enantiomer of the drug should be studied separately for its pharmacological and metabolic pathways.[8] In addition, a rigorous justification is required for market approval of a racemate of chiral drugs. Presently, a majority of commercially available drugs are both synthetic and chiral. However, a large number of chiral drugs are still marketed as

racemic mixtures[9-10]. Nevertheless, to avoid the possible undesirable effects of a chiral drug, it is imperative that only the pure, therapeutically active form be prepared and marketed. Hence there is a great need to develop the technology for analysis and separation of racemic drugs.

Chiral compounds are also utilized for asymmetric synthesis[11], i.e., for the preparation of pure optically active compounds. They are also used in studies for determining reaction mechanisms, as well as reaction pathways. Chiral compounds are also important in the agrochemical industries[12-13].

Current methods of enantiomeric analysis include such non-chromatographic techniques as polarimetry, nuclear magnetic resonance, isotopic dilution, calorimetry, and enzyme techniques. The disadvantages of these techniques are the need for pure samples, and no separation of enantiomers are involved. Quantitation, which does not require pure samples, and separation of enantiomers can be done simultaneously by either gas chromatography (GC) or high performance liquid chromatography (HPLC).[14]

Chiral HPLC has proven to be one of the best methods for the direct separation and analysis of enatiomers[15-16]. It is more versatile than chiral GC because it can separate a wide variety of nonvolatile compounds. It provides fast and accurate methods for chiral separation, and allows on-line detection and quantitation of both mass and optical rotation of enantiomers if appropriate detection devices are used[17-19]. Current chiral HPLC methods are either direct, which utilizes chiral stationary phases (CSPs) and chiral additives in the mobile phase, or indirect, which involves derivatization of samples.[20-22] Direct chiral separations using CSPs are more widely used and are more predictable, in mechanistic terms, than those using chiral additives in the mobile phase[23].

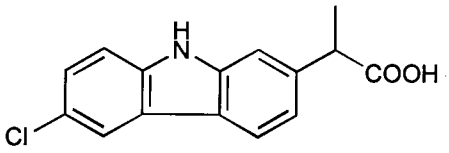
To date nearly a hundred HPLC CSPs have been developed and are commercially available.[21] However, there is no single CSP that can be considered universal, i.e., has the ability to separate all classes of racemic compounds. Choosing the right CSP for the enantioseparation of a chiral compound is difficult. The decision relies mostly on empirical data[22-23]. Most chiral separations achieved on CSPs, however, were obtained based upon the accumulated trial-and-error knowledge of the analyst, intuition, and often simply by chance. An alternative way of choosing a CSP is by using predictive empirical

rules that have been developed based on empirical structures [24-26]. Neither scheme of choosing a right CSP offers a guarantee for a successful enantiomeric separation. Although enantioseparation is hoped to be achieved by knowing the chemistry of the racemic analytes and the CSP sometimes, however, it does not work because the interactions of the mobile phase with both the racemic analyte and CSP have to be considered. All three components, analyte, CSP, and mobile phase, must be taken into consideration when developing a chiral separation method. The key, therefore, to a successful enantioseparation of a particular class of racemates on a given CSP is the understanding of the possible chiral recognition mechanisms[27-28].

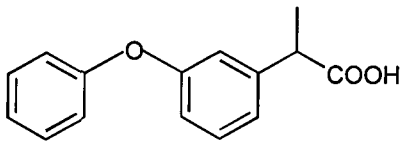
1.2 Nonsteroidal Anti-inflammatory Drugs of 2-Methylarylpropionic Acids (Profens)

A variety of 2-arylmethylpropionic acids (profens) (Fig. 1) have been widely used as nonsteroidal anti-inflammatory drugs for the relief of acute and chronic rheumatoid arthritis and osteoarthritis, as well as for other connective tissue disorders and pains[29-30]. Examples are fenoprofen, ibuprofen, ketoprofen, flurbiprofen, and naproxen. Another profen (carprofen) has been studied here but is not yet commercially available. All are chiral and, except for naproxen, are marketed in racemic form. The chirality of these molecules arise from the sp^3 - alpha carbon. Direct enantioseparations of profens have been of considerable interest because their anti-inflammatory and analgesic effects have been attributed almost exclusively to their S-enantiomers.[31] To avoid the unwanted effects of the R-enantiomer, the use of pure S-enantiomer of the profens is desirable. Hence development of a preparative scale separation direct enantiomeric analysis is important. Furthermore, some of the profens generally undergo a unidirectional in vivo chiral inversion[32-36] from the inactive R-enantiomer to the active S-form, as well as bi-directional chiral inversion[37-39]. The metabolic and pharmacokinetic studies of both isomers require direct enantioseparations.

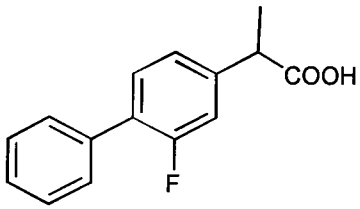
Enantioseparation of profens by HPLC has been studied extensively, using both indirect and indirect methods. In the indirect method, a racemic profen is derivatized to form diastereomers and then separated using a chiral column[40-56]. Whereas in the direct



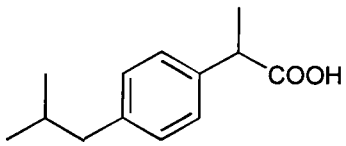
carprofen



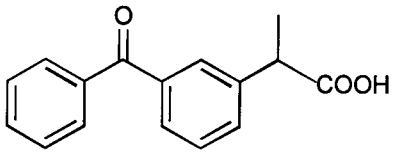
fenoprofen



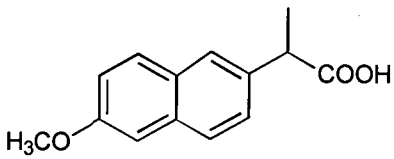
flurbiprofen



ibuprofen



ketoprofen



naproxen

Figure 1. Structures of racemic racemic 2-arylmethyl propionic acids (profens) analyzed.

method, there is no derivatization of profens, and CSPs are used to separate the isomers. At present numerous direct chiral separations of profens on HPLC chiral stationary phases have been reported. To mention some of the CSPs used for the enantioseparation of profens with aqueous buffer solutions as eluents are the α_1-acid glycoprotein[57-61], ovomucoid[62-65], bovine serum albumin[66], cyclodextrin[67-68], and serum albumin[69], and avidine[70-71]. Examples of the CSPs used for the separations of profens with normal phase eluents are the derivatized polysaccharides[72-81] adsorbed on macroporous silica gel such as Chiralcel OD, Chiralcel OJ, and Chiralpak AD.

The enantiomeric separation of profens using tris(3,5-dimethylphenylcarbamate)s of amylose and cellulose coated on macroporous silica (ADMPC and CDMPC, respectively, which was later commercialized as Chiralpak AD and Chiralcel OD) (Fig. 2) was first studied by Okamoto *et al.*[82] Enantioseparations of ibuprofen, ketoprofen, flurbiprofen, and tiaprofenic acid were performed at varying compositions of hexane/2-propanol, but with 1% trifluoroacetic acid (TFA) of the total volume of mobile phase. In their work, all racemic profens were incompletely separated on CDMPC. On ADMPC, only flurbiprofen and tiaprofenic acids were completely enantioseparated. Ibuprofen was also completely enantioseparated on ADMPC, but only in its derivatized form. Chiral separations were completed in more than 15 minutes. Based on the racemic profens separated, ADMPC appears to have a superior enantioseparating ability.

Wainer *et al.*[83] studied on the enantioseparation of 2-alkylarylpropionic acids on Chiralpak AD, including the 2-arylmethylpropionic acids. They investigated the chiral recognition mechanism for the enantiomeric separations of these acids using quantitative structure-enantioselective retention relationship (QSERR). The retention data collected from the enantioseparation of racemic acids, with 95/5/1% hexane/2-propanol/TFA as eluents, were correlated to a series of molecular descriptors including the hydrogen bonding ability and aromaticity of the analytes. In this QSERR analysis, the influence of the nature of mobile phase on the structure of CSP and analyte is not considered. Based on their results the chiral recognition mechanism on Chiralpak AD mainly involves attractive interactions, primarily hydrogen bonding, and is conformationally driven.

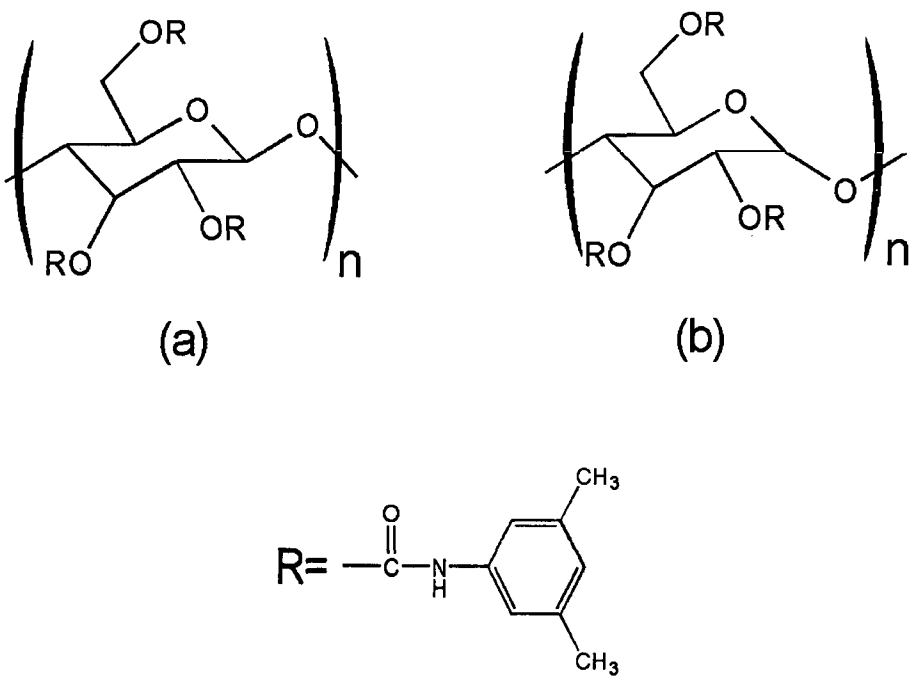


Figure 2. Structure of derivatized polysaccharide CSPs: (a) cellulose tris(3,5-dimethylphenylcarbamate) and (b) amylose tris(3,5-dimethylphenylcarbamate).

1.3 Research Objectives

There were several interrelated objectives in this dissertation. The first objective was to develop a systematic method for optimized separation of racemic profens geared for the analysis of both enantiomers. The chiral separation method should take only a short time and preferably use only inexpensive solvents. This study explored the enantioseparation of profens on Chiralpak AD and Chiralcel OD using a normal phase eluent of hexane, as the apolar solvent, and alcohol as the polar modifier. Carboxylic acids were studied as the acidic mobile phase modifiers. Variation of column temperatures are also investigated for optimization of enantioseparation of profens on both CSPs.

Chiralpak AD and Chiralcel OD were the CSPs chosen in the study because, by virtue of the tris(3,5-dimethylphenylcarbamate)-D-glucose units as the chiral adsorbing sites, they are capable of interacting with the small racemic profens leading to chiral separation. In addition, since Chiralpak AD and Chiralcel OD have the same chiral adsorbing sites but are of different structures their enantioseparating abilities for profens are expected to be different, but complementary. That is, a profen that could be only partially enantioseparated in one CSP, hopefully could be well resolved in the other. Lastly, Chiralpak AD and Chiralcel OD both require a normal phase eluent that is well suited for the analysis of the pure S-enantiomer of profens.

The second objective of this study was to investigate the influence of temperature on retention and enantioselectivity of profens on Chiralpak AD and Chiralcel OD. It is well known that a change in temperature alters retention and enantioselectivity, and thus enantiomer resolution[84-86]. From the retention behavior and the corresponding enantioselectivity, inferences can be drawn for the possible chiral recognition mechanism of profens on both CSPs. To achieve this result, the thermodynamic parameters - differences in enthalpy, entropy, and Gibbs free energies for the association of enantiomers and CSP were measured.

The third goal of this research was to explore the influence of mobile phase, both acidic and alcoholic modifiers, on the enantioseparation of profens and profen methyl esters on Chiralpak AD and Chiralcel OD. The general mechanisms for chiral recognition

on both CSPs are attractive interactions and inclusion or steric fit of analytes to the chiral cavities[87-90]. Nevertheless, it is obvious that any competing interaction involving the mobile phase may alter enantioselectivity and, thus, enantiomeric resolution. In addition, this study aimed to obtain a glimpse of the mechanism involved for chiral discrimination of profens on both CSPs.

Lastly, this study aimed to elucidate the chiral recognition of mechanisms for the separation of racemic profens on Chiralpak AD and Chiralcel OD. The retention behavior and the corresponding enantioselectivity from the optimization studies and the studies of the influence of temperature and acidic and alcoholic mobile phase modifiers were used in depth to evaluate the mechanism for chiral discrimination of profens on both CSPs. This involves structural correlation of analyte and CSP to the observed retention behavior and corresponding enantioselectivity from the different studies. In this study, methyl esters of fenoprofen, ibuprofen, and ketoprofen, and the aromatic hydrocarbons of benzene, naphthalene, and anthracene, were included as probe analytes aside from the six profen molecules (Fig. 1).

As noted in Section 1.2, Wainer *et al.* investigated the chiral recognition for the enantioseparation of 2-arylmethyl propionic acids on Chiralpak AD by QSERR analysis. The study of the chiral recognition of profens on Chiralpak AD, in this dissertation, was already completed before the work of Wainer *et al.* was reported.

373

Exhibit 11

Organic Process Research & Development 1998, 2, 305–319

Comparison of Batch Elution and Continuous Simulated Moving Bed Chromatography

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Abstract:
The production of chirality with maximum economy is one of the most challenging tasks of today's pharmaceutical industry. Apart from the use of inherent chirality (starting material from the chiral pool, e.g., amino acid derivatives, carbohydrates), the creation of chiral centers via biocatalysis or asymmetric synthesis is commonly used. Another way to obtain pure enantiomers is the separation of racemates via kinetic resolution through preferred crystallization or preparative chromatography on chiral stationary phases. This paper emphasizes this last method, explains the possibilities of this technique, especially in its application form as simulated moving bed (SMB) chromatography, and shows its benefits and limitations. Therefore, comparisons to classical batch elution chromatographic processes as well as other unit operations (such as crystallization, etc.) must take cost calculations into account. In this paper, a theoretical comparison of optimized SMB and batch elution processes by simulation studies based on rigorous process models is presented for the separation of two different binary mixtures. These examples are chosen to demonstrate the different effects which dominate the applications in large-scale isomer separations and production-scale enantiomer separation. The first example is a fructose/glucose separation with linear isotherms. The model parameters are measured by Nicoud. The second characteristic example is an enantioseparation. The corresponding isotherms are of the modified Langmuir type. The performance of each separation process is quantified by three characteristic objective functions: productivity, dilution, and solvent requirement. Last, the specific separation costs or the total costs of separation are calculated as an objective function to lay emphasis on the economy of the separation, including product recovery and solvent recycling. The comparison of these objective functions, which are determined for batch and SMB processes, leads finally to certain rules of consideration to decide what kind of process (either batch elution or SMB) is preferable as a function of the physical properties of the given binary mixture and the separation task.

1. Introduction

The basic separation principles of batch elution and the port movement of the SMB process are shown in Figure 1.

Countercurrent chromatography and especially simulated moving bed (SMB) chromatography have been known for a long time in the petrochemical industry for the separation of C_8 hydrocarbons.[1] The biggest systems produce up to 500 000 tons/year of *p*-xylene. There was never any doubt that SMB chromatography with zeolites or ion exchangers in this scale is the cheapest way to produce these pure compounds. However, there were numerous obstacles to overcome during the adoption of those big production systems to systems of a smaller dimension which would be suitable for the pharmaceutical and fine chemical industry with their typical production range of 1−50 tons/year at high purities (up to 99.9%). During the last five years, a lot of work was done to scale down the systems and optimize them for the production of pharmaceuticals.[2−5] In 1997 the first SMB unit with a production capacity of 40 tons of enantiomer/year was brought to operation.[6]

The principle of SMB chromatography is the simulation of the countercurrent movement of a stationary phase (silica, chiral modified silica, or ion-exchange resins) and a mobile phase. Due to enormous technical problems when continuously moving a solid, the setup of a true moving bed (TMB) is not feasible. Therefore, the chromatographic bed was divided into single columns, connected in series, and closed in a circle (Figure 1). Between each column, four connecting lines are present to allow the withdraw of two product lines (called extract [more retained] and raffinate [less retained]), the input of new feed solution (the educt, to be separated), and the input of fresh mobile phase to desorb the more strongly adsorbed products. All four flows are continuous with previously determined flow rates. After a given time, all four flows are shifted one position into the direction of the mobile phase movement. The feed line is now injecting into the zone of not separated products. After a series of shifts at the front and the tail of the internal concentration profile, pure products can be withdrawn from the system. It has to be pointed out that SMB systems in their normal configuration are systems to separate a feed into two product streams but not necessarily into just two products: the extract or raffinate stream can still consist of several impurities,

† University of Dortmund.
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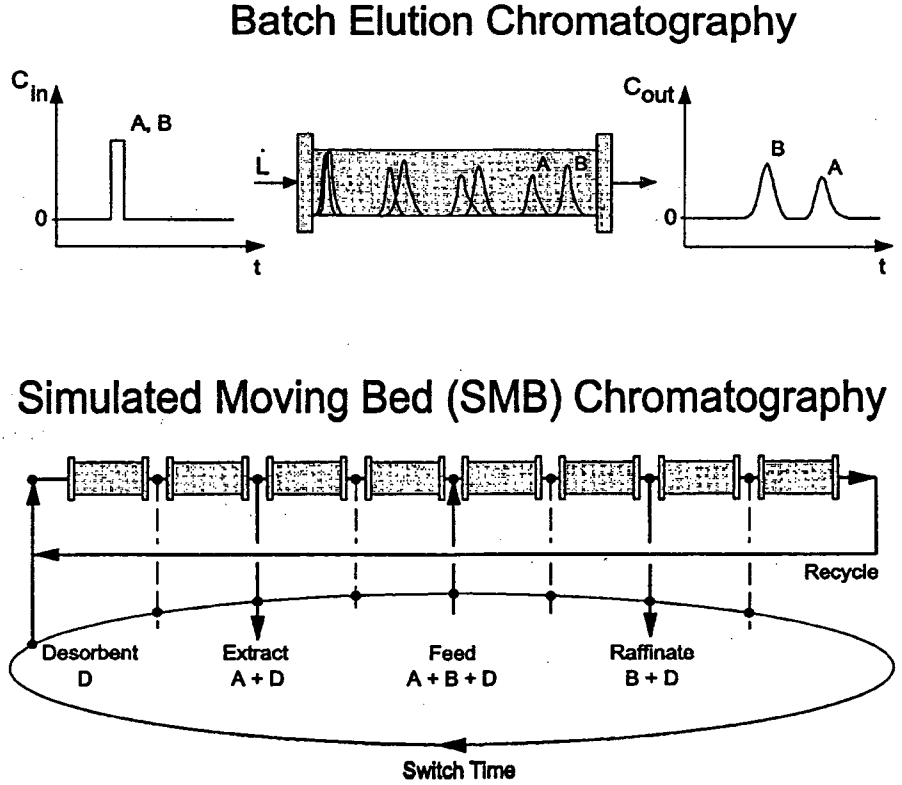

Figure 1. **Funtional scheme of batch elution and simulated moving bed (SMB) chromatography.**

separated from the value product. Through combination of different chromatographic setups, even the isolation of natural products from a big number of byproducts is possible, as it was demonstrated for the separation of cyclosporin A from a fermentation broth.[7]

Since the setup of five different flow rates (mobile phase flow rate, four input/output-line flow rates) and the line-shift timing comprises a highly complex system, it requires rigorous simulation systems which take into account the conditions of technical chromatographic separations. These are nonlinear adsorption isotherms, axial dispersion, and mass transfer resistances as well as the modeling of all parts of the plant. In addition, a high modeling accuracy requires the determination of empirical parameters by characteristic and consistent experiments for the relevant separation problem.

In this paper, a comparison by simulation studies of optimized SMB and batch elution chromatographic processes is presented for the separation of two different binary mixtures. The model parameters are summarized in Table 1. These examples are chosen to demonstrate the different effects which dominate the applications in large-scale isomer separations and production-scale enantiomer separation.

The first example is a fructose/glucose separation with linear isotherms. The model parameters are measured by Nicoud.[2] The second characteristic example is an enantioseparation (abbreviated in the following as EMD53986).[8-10] The corresponding isotherms are of the modified Langmuir type (eq 8).

Table 1. **Model parameters for the fructose/glucose and the EMD 53986 enantioseparation**

model parameters	unit	Nicoud	EN2_1
mass transfer coeff. *A*	[cm/s]	4.50E-04	3.00E-04
mass transfer coeff. *B*	[cm/s]	2.50E-04	2.50E-04
fluid density	[cm³/s]	1	0.88
dynamic fluid viscosity	[g/cm s]	8.00E-03	4.38E-03
eluent		H_2O	ETAc/ETOH (95/5)
Henry coeff. P_a	[−]		3
Henry coeff. P_b	[−]		1.5
Henry coeff. K_{Fa}	[−]	0.45	8.33
Henry coeff. K_{Fb}	[−]	0.25	2.62
Langmuir coeff. *A*	[cm³/g]		830
Langmuir coeff. *B*	[cm³/g]		260
interference coeff. *A*	[cm³/g]		260
interference coeff. *B*	[cm³/g]		830
α ($c = c_{Feed}$)	[−]	1.80	1.90229062
$q_{s,maxA}$ ($c = c_{Feed}$)	[g/cm³]	0.09	0.02557
$q_{s,maxB}$ ($c = c_{Feed}$)	[g/cm³]	0.05	0.01154813
axial dispersion *A*	[cm²/s]	4.59E-02	4.50E-04
axial dispersion *B*	[cm²/s]	4.59E-02	4.50E-04
NLFA	[−]	1	2.214

(7) German Pat., DE 196 11 094 A1; Voigt, U.; Hempel, R.; Kinkel, J. *Chromatographisches Verfahren zur Gewinnung von hochgereinigtem Cyclosporin A und verwandten Cyclosporinen*, C 07 K 1/16, 1997.

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The performance of each separation process is quantified by three characteristic objective functions: productivity, dilution, and solvent requirement.

In addition, the specific separation costs or the total costs of separation are calculated as an objective function to lay emphasis on the economy of the separation, including product recovery and solvent recycling.

The comparison of these objective functions, which are determined for batch and SMB processes, leads finally to certain rules of consideration to decide what kind of process (either batch elution or SMB) is preferable as a function of the physical properties of the given binary mixture and the separation task.

Detailed studies of binary mixtures combined with impurities have been done by the authors but are not the topic of this paper. The conclusion is that impurities influence the performance of batch elution and SMB chromatography to the same extent. The difference in process performance is still in the same magnitude.

Of course, these theoretical simulation studies have to be proven by experiments. But, there is no doubt in the literature that the rigoros process modeling approach is more exact than models with simplifying assumptions. Moreover, realistic experimental model parameters are chosen. Therefore, process simulations may function as a standardized reference for theoretical studies. The aim of this paper is to point out advantages/disadvantages and preferable ranges of application of batch elution and SMB chromatography by theoretical studies to show the benefits of both processes due to a standardized methodology. Experimental comparisons have been previously presented in the literature.

2. State of the Art: Previous Comparisons of Batch and SMB Processes

At first, chromatography was only used for analytical purposes. Elution chromatography is likewise applied for separations on the production scale. Because of the discontinuous operation batch chromatography has a demand on a high degree of automation to save personal costs and to run the equipment in a safe and reliable way. In contrast to batch chromatography, the automation of SMB chromatography is far more complex.

Feed and desorbent are injected at different inlets of the column configuration in the same manner that extract and raffinate are taken from different outlets. After a certain period of time ("switch time"), inlets and outlets are switched to the next port (all in the same direction of the fluid flow) while the columns with stationary phase remain fixed. The switching is done by a sequence of valves. The result is a simulated countercurrent flow of solid and liquid.

The driving force of the classic batch chromatographic separation is the different affinities of each adsorptive component in the fluid mixture to the stationary phase.

In the case of SMB processes, the driving force is additionally increased by the basic principle of the (simulated) countercurrent flow. Due to this principle, separations by SMB processes should lead to productivity levels which are much higher than those resulting from batch separations. In addition to this, SMB products should be achieved which are less diluted and with less solvent requirements because the solvent is recycled in the SMB process. Batch elution chromatography is an the one end of productivity range, and a countercurrent process such as SMB chromatography is on the other end. Between these methods exist modifications of batch chromatography such as recycling or peak shaving chromatography and the annular rotationg disk chromatograph, which is a cross-flow process. In this study, we compare the extremes, batch elution and SMB chromatography, to point out the different ranges of application.

Several comparisons of the chromatographic processes are documented in literature[3-5,11-13] which confirm the results discussed above by experimental comparisons. In practical applications, the SMB process is found to achieve productivity levels up to 4 times higher than those achieved by the batch process.[3,13] As a result, the factor of the resulting dilution is about 2−5 times less.

As far as Schulte's investigations[5] are concerned, the solvent requirement of the SMB process can be up to 90% less than that of the corresponding batch separation.

One problem of these current comparisons is shown by Nagamatsu.[13]

In this study the batch process is, in comparison to the SMB, a 4 times less productive separation technique which additionally has a high desorbent requirement, which leads to a solvent requirement 8 times higher. On the other hand, two completely different separations are compared and assumed as equal. However, batch and SMB separations of binary mixtures are compared in this practical study where the feed concentrations differ. This results in a different throughput at different column loadability and in a different yield. The column loadability for example is 2 times higher for the SMB than for the corresponding batch stationary phase. Furthermore, the concentration of the feed in SMB chromatography is 6 times higher than that for the analogous batch process.

Seidel-Morgenstern[12] optimizes batch elution and recycle chromatography in comparison to annular rotating disk and SMB chromatgraphy due to either productivity or product dilution. Therefore, this case study for a single separation example is limited to these boundary cases.

The study of Bauer[11] presents realistic industrial cost relations for SMB chromatography. Shortcut design rules are derived that are in agreement with these theoretical studies as shown later.

Schulte[5] describes the main problem of recent comparisons between batch and SMB technology.

Therein, Schulte reports that the application of SMB technology is connected to the use of simulative process optimization. Only during process development does the SMB process need to be optimized for the special separation problem. In this way, it is taken for granted that the plant

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Table 2. **Process parameters/characteristic numbers**

	fructose/glucose		enantiomers	
	SMB	batch	SMB	batch
	Process Parameters			
concentration feed [g/cm³]	0.2	0.2	0.00524	0.00254
u_int [cm/s]	0.78	0.78	0.25	0.25
column diameter [cm]	2.54	2.17	2.6	2.24
column length [cm]	94	1800	5.3	100
column number [−]	24	8	1	
segmentation [−]	6:6:6:6	2:2:2:2		
particle diameter [cm]	0.032	0.032	0.001	0.001
void fraction [−]	0.39	0.39	0.4	0.4
column total volume V_kol [cm³]	11431	6657	225	394
feed [cm³/s]	0.231		0.08307	
desorbent [cm³/s]	0.361		0.352	
extract [cm³/s]	0.277		0.364	
raffinate [cm³/s]	0.315		0.703	
switch time [s]	207		232.5	
solid flow [cm³/s]	33.69		0.58093927	
volume flow, batch [cm³/s]		1.125		0.39
injection time [s]		330		650.00
cycle time [s]		1600		3700.00
pressure drop [bar]	9.9	7.9	31.376	74.00
	Characteristic Numbers			
productivity A [g/g/h]	0.0181	0.0301	0.007452	0.00514
productivity B [g/g/h]	0.0182	0.0302	0.007488	0.0051
dilution A [−]	1.205	2.778	4.40336134	4.45
dilution B [−]	1.361	2.273	0.84516129	0.93
solvent requirement A [g of solv/g of prod]	7.9	19.4	712.2	786.1
solvent requirement B [g of solv/g of prod]	7.8	19.4	710.2	792.4
Ca_Extr. [g/cm³]	0.166	0.072	0.00119	0.0012
Cb_Raf. [g/cm³]	0.147	0.088	0.0062	0.0056
yield A [%]	99.3	99.2	99.8	100
yield B [%]	100	99.3	100	99.5
purity A [%]	100	99.2	100	99.5
purity B [%]	100	99.3	100	100

is used efficiently. Batch columns are widely used as "multipurpose" columns that are not optimized to a single application at all. As a result, these batch columns are operated in production with less overload than possible if the process would be optimized as carefully as the SMB process.

The low productivity of batch columns may be just a problem due to the extent the two different technologies are applied and operated correctly. The often extremly unfavourable results of batch chromatography are due to the fact that a fully optimized SMB process is compared with a batch process that is not so carefully optimized.

3. Systematic Comparison by Simulation Studies with Rigorous Process Models

3.1. Basic Assumptions of the Methodology. *A. Comparison of Optimized Processes.* The different applications of the two chromatographic processes and the resulting afore-mentioned comparisons lead to the demand that both batch and SMB processes need to be optimized. Therefore, in this study, as a first step, this process optimization has been done. In the case of SMB processes, it is performed only with regard to maximum feed throughput, minimum solvent requirement, pressure drop, and careful consideration about the breakthrough of the axial profile in the edge sections of the columns. Apart from this, yield and purity are required up to 99%. The optimization strategies for both processes consider that the optimal experimental conditions to reach maximum productivity *and* minimal product dilution between batch elution and SMB processes may strongly differ.

B. Optimized SMB Processes Determine the Conditions for Optimized Batch Processes. On the basis of the optimum SMB process parameters determined by Strube[10,14] (see also Table 2), the batch optimization is done as follows:

First, assumptions must be made as to which process parameters have to remain constant for the sake of comparability.

1. It is obvious that feed concentrations and column loadability have to be the same for the batch and SMB processes. The first rule is to maximize the feed concentrations up to the solubility range (in practice 10% less because of the chromatographic enrichment) in order to get maximal loadibility at minimal flow rates. The next step is to maximize the throughput by increasing the flow rates to their limits.

(14) Strube, J.; Altenhöner, U.; Meurer, M.; Schmidt-Traub, H. *Chem. Eng. Technol.* **1997**, *69*, 328−331.

To minimize the simulation studies to a realistic range and to achieve significant results, the following assumptions are necessary:

2. Yield and purity have to be larger than 99%. Total separation is assumed to define plain optimal operating points of the processes which are definitely comparable. The operating optima should not be affected by the effect of the costs of product losses, because otherwise differing product values as a function of market price, etc., must be taken into account. Moreover, the advantage of chromatography against other unit operations to be operated with minimal product losses should be emphasized.

3. The interstitial velocity u_{int} in the batch column shall be as high as u_{int} in zone I with the maximum liquid flow in the SMB process. The definition of u_{int} is given in eq 1.

$$u_{int} = \frac{4\dot{V}}{\pi\epsilon_0 D^2} \tag{1}$$

The reason for this assumption is that the maximal interstitial velocity of SMB expresses the real limitation of the fluid flow due to pressure drop and mass transfer resistances. This limit is valid for batch chromatography as well. Further optimization in batch chromatography may be possible, but it is guaranteed that the order of magnitude of this extremely complex parameter (which is dominated by mass transfer and fluid dynamic effects) is correct.

4. In addition to this, the feed throughput shall be the same for the batch and SMB processes as shown in the mass balance for the demand of equal throughput (eq 2).

$$\dot{V}_{SMB}\Delta t_{cyc} c_{F,i} = \dot{V}_{Batch}\Delta t_{inj} c_{F,i} \tag{2}$$

5. For optimization of batch processes, a further assumption is necessary to give maximum productivity: The peaks of each component of the binary mixture must touch each other as shown in Figures 5 and 7. This is called the "touching band assumption".

6. The underlying model assumptions which determine that the accuracy of the simulations and

7. the degree of optimization are for both processes the same.

The optimization strategies are described elsewhere in detail.[14-18]

3.2. Systematic Procedures. *1. Parameter Studies.* Starting from this methodology, the batch process is optimized by varying all process parameters such as column diameter, column length, cycle time, and injection time. In addition, the pressure drop must be taken into account, as well as energy costs, constructive requirements, and leakage risks.

2. Rigourous Process Modeling. The optimization of both SMB and batch processes are performed by process modeling using the dynamic process simulation package SPEEDUP (AspenTech., Cambridge, MA) as a modeling tool and numerical solver. To take into account such real effects as axial dispersion and finite mass transfer, rigorous dynamic models are used[14-18] which are characterized by eqs 3 and 4 (fluid and mass balance for a small volume of the column). The process models have been written during the last 6 years at the University of Dortmund.[19]

$$\frac{\partial c_i}{\partial t} = D_i\frac{\partial^2 c_i}{\partial x^2} - u_{int}\frac{\partial c_i}{\partial x} - k_{eff}\frac{6}{d_p}(c_i - c_{p,i})\frac{(1-\epsilon)}{\epsilon} \tag{3}$$

$$\frac{\partial q_i}{\partial t} = k_{eff,i}\frac{6}{d_p}(c_i - c_{p,i}) \tag{4}$$

combined with any multicomponent equilibrium phase isotherm equation.

The progress of the optimization is quantified by three main objective functions which take into account the requirements of solvent, of stationary phase, and of further preparation. These objective functions are productivity (Pro_i), dilution (Di_i), and solvent requirement ($SR)_i$ of a component under the use of the following definitions:

$$Pro_i = \frac{\dot{m}_i Y_i}{m_{ads}}[\text{g of Product/g of Adsorbent/hour}] \tag{5}$$

$$Di_i = \frac{c_{Feed,i}}{c_{ex,i}} \tag{6}$$

$$SR_i = \frac{m_{Solvent}}{m_i} \tag{7}$$

4. Separation Examples

In this paper, the comparison between batch and SMB processes is demonstrated on behalf of two different binary mixtures.

4.1. Example: Large-Scale Separation of Fructose/Glucose. The first separation example is an aqueous fructose/glucose system with linear adsorption behavior on resins. The isotherms are presented in Figure 2, the model parameters are in Table 1. The separation factor has the constant value of 1.8. Table 2 summarizes the optimized process parameters for batch and SMB chromatography.

It is striking that the columns for each process are extremely long. Due to the low separation factor, long columns are needed. Additionally, a relatively high interstitial velocity could be used. Because of good mass transfer in the resins, large particle diameters could be taken. This results in a low pressure drop per meter and in a relatively large HETP per meter.

4.2. Example: Production-Scale Separation of Enantiomers. The second binary mixture, EMD53986, is a racemic mixture with ethyl acetate/ethanol (95/5) as solvent. The liquid–solid equilibrium is described by eq 8.

$$q_i = P_i c_{pi} + \frac{K_{2i}c_{pi}}{1 + K_{3i}c_{pi} + K_{4j}c_{pj}} \tag{8}$$

(15) Strube, J.; Michel, S.; Paul, H.-I.; Schmidt-Traub, H. *Chem. Eng. Tech.* **1995**, *67*, 323–326.
(16) Strube, J.; Schmidt-Traub, H. *Comput. Chem. Eng. Suppl.* **1996**, *20*, S641–646.
(17) Strube, J.; Schmidt-Traub, H. *Comput. Chem. Eng.* In press.
(18) Strube, J.; Brozio, J.; Schmidt-Traub, H. Submitted for publication.
(19) Strube, J. Ph.D. Dissertation, University of Dortmund, Germany, 1994.

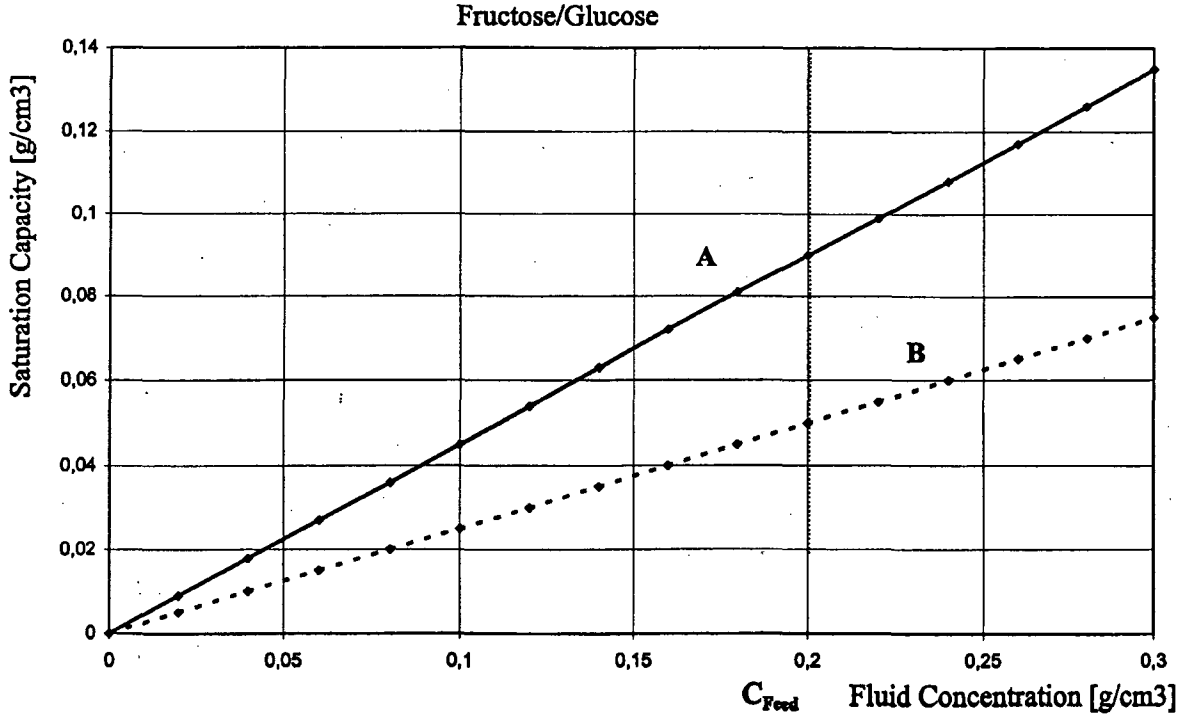

Figure 2. **Isotherms of fructose/glucose separation. Details are given in ref 1. Fructose (A); glucose (B).**

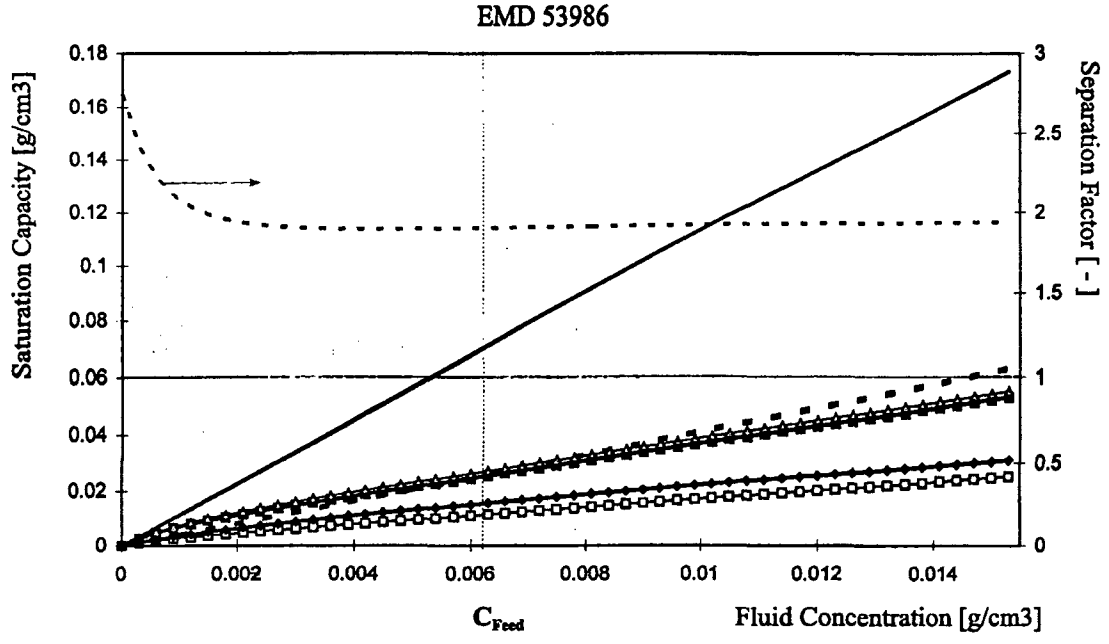

Figure 3. **Isotherms and separation factor of the enantioseparation. Details are given in refs 2 and 3. Henry isotherm (+), (−); Henry isotherm (−) pure isotherm (+) (▲); pure isotherm (−) (◆); interference isotherm (+) (■); interference isotherm (−) with $c_{(+)} = c_{(-)}$ (□); separation factor (thick dashed line).**

It is equivalent to a Langmuir isotherm with a linear prefactor due to nonselective adsorption sites of the different chiral stationary phase (CSP) compounds.

The model parameters are listed in Table 1. The separation factor and column loadability is shown in Figure 3.

4.3. Comparison of Physical Component Properties. In the area of *Henry behaviour*, the separation factor of the enantiomers is much higher (2.75) than that of the sugar separation. Despite that, it diminishes to a value of less than 2 at high liquid concentrations. Due to this fact, combined with a relatively low interstitial velocity, major deviations

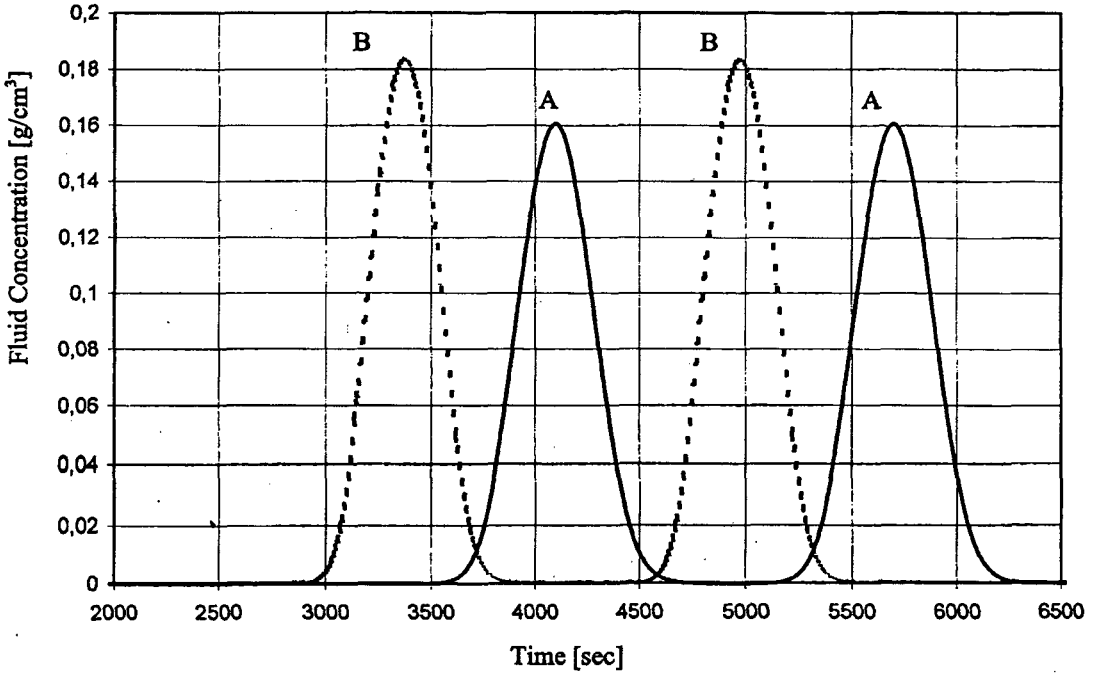

Figure 4. **Simulated chromatogram of the optimized batch elution to separate fructose/glucose. Fructose (A); glucode (B).**

of the separation tasks result. The low interstitial velocity is caused by *kinetic mass transfer resistances* which lead to low particle diameters, which in turn cause a high-pressure drop.

The *column loadabilities* of the two components are approximately 4–5 times lower than those for the sugar separation. *Mass transfer coefficients, separation factors at feed concentrations, and axial dispersion coefficients* are approximately of the same magnitude for each binary system.

The most striking difference is the *nonlinearity of the isotherm*, which is defined as the deviation from the ideal Henry isotherm at feed concentrations. In the case of EMD53986, the factor of nonlinearity is 2.2. Compared to other nonlinear systems, this is a relatively high value. The most significant difference between the two binary mixtures discussed here lies in this factor, which is a result of the wholly different equilibrium phase behaviour of these two examples.

Another striking fact concerning these binary mixtures is seen during the optimization of the separation process.

In Figures 4 and 6, the optimized concentrations which breakthrough at the column outlet are shown as a function of time, and in Figures 5 and 7, the optimized axial concentration profiles of the optimized SMB processes are plotted for the sugar separation (Figure 5) as well as for the enantioseparation (Figure 7).

Because of the *displacement* of the weaker adsorbable component B by the stronger adsorbable component A, local increase of liquid concentration of the weaker adsorbable component B over the feed concentration takes place by enantioseparation. Figures 6 and 7 demonstrate that a concentration of component B occurs which is significantly higher than the feed concentration. This displacement phenomena occurs equally in batch and SMB processes. As a result, component B can be yielded at a lower dilution than it is fed into the column.

5. Results of the Process Optimizations

The optimized parameters achieved from the simulative optimization for batch and SMB chromatography are summarized in Table 2. In addition, the optimum characteristic numbers and objective functions are listed.

For the sugar separation, one interesting fact can be noticed: the productivity of the optimum batch column is 1.7 times higher than that of the corresponding SMB column. This differs from the practical results mentioned above, where the SMB process is always much more productive than batch elution. Additional studies with other binary mixtures with linear isotherms confirm the results presented here. The reason of the differing results is the difference in the operating modes of batch chromatography: either optimized due to touching bands or not.

On the other hand, product dilution and solvent requirement of the sugar separation (and other linear systems) are much higher for the batch process: Batch chromatography has up to 2.3 times higher product dilutions and up to 2.5 times higher solvent requirements.

The latter result fits in the experimental investigations described above, although in this study this difference is not as high as described in previous literature. For example, Nicoud stated[2] that the only advantage of SMB chromatography for linear systems such as fructose/glucose separations lies in the product dilution.

For the second mixture, EMD53986, the following relations result:

(a) SMB productivity is 1.5 times higher;

(b) components are up to 1.1 times less diluted;

(c) solvent requirement is up to 1.1 times less.

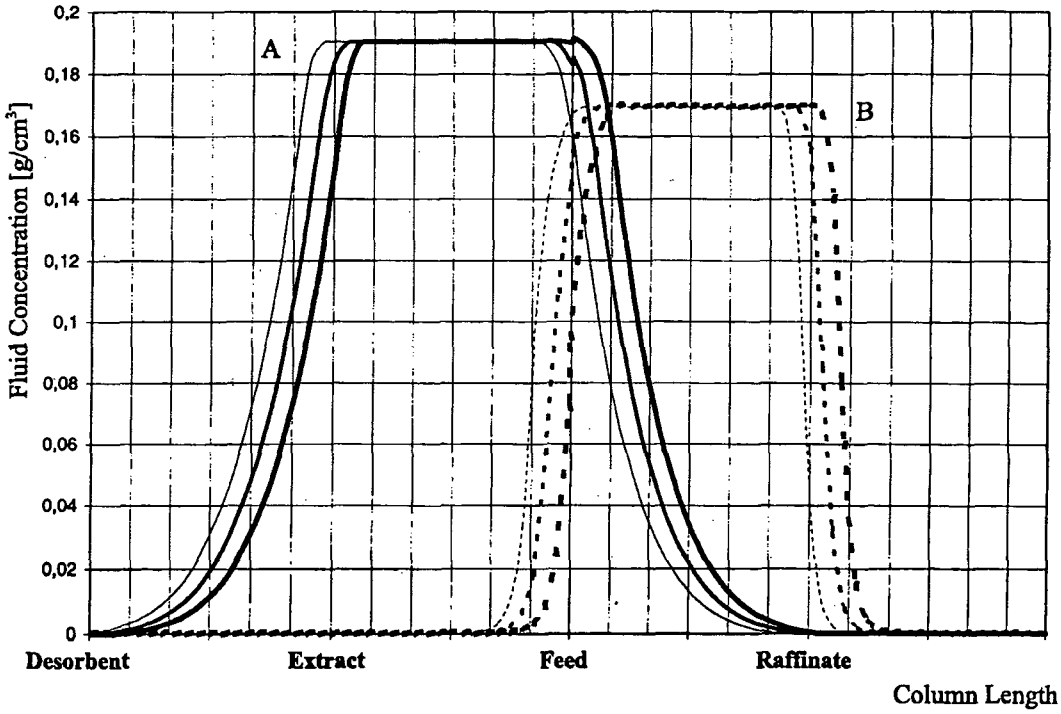

Figure 5. **Simulated axial concentration profile of the optimized SMB process to separate fructose/glucose, periodic quasi-steady-state is reached. Fructose (A); glucose (B) (with each 3 curves in fluid flow direction, beginning, middle, and end of the shown switch period).**

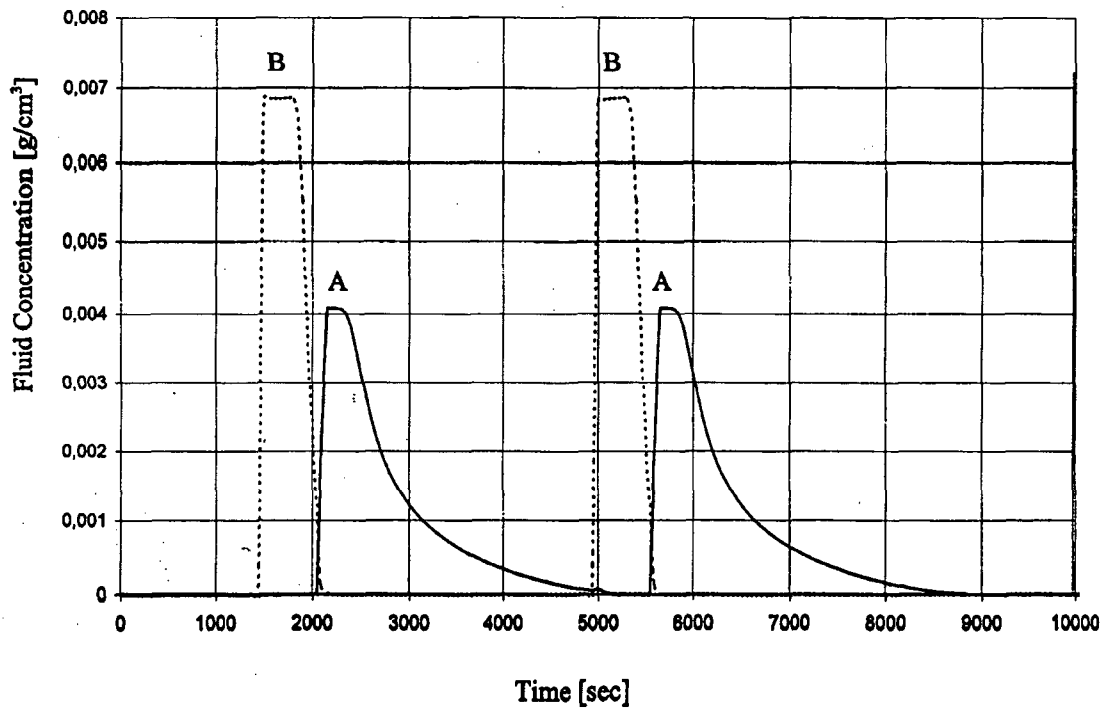

Figure 6. **Simulated chromatogram of the optimized batch elution to separate the enantiomers: (A) (+); (B) (−).**

This enantioseparation is a typical example for the class of enantioseparations in pharmaceuticals or fine chemicals, which are now scaled up into production by SMB chromatography. The separation factor is about 2, and the efficiency of CSP's is not extremely high, with relative low HETP's and extreme peak tailing due to mass transfer resistances (compare Figures 4 and 6). Therefore, small particles of about 10−40 μm diameter are chosen.

Five additional binary mixtures with nonlinear isotherms (Langmuir, modified Langmuir, and bi-Langmuir) where analyzed by process simulations in the theoretical study by the authors. The result is that there is no way to predict

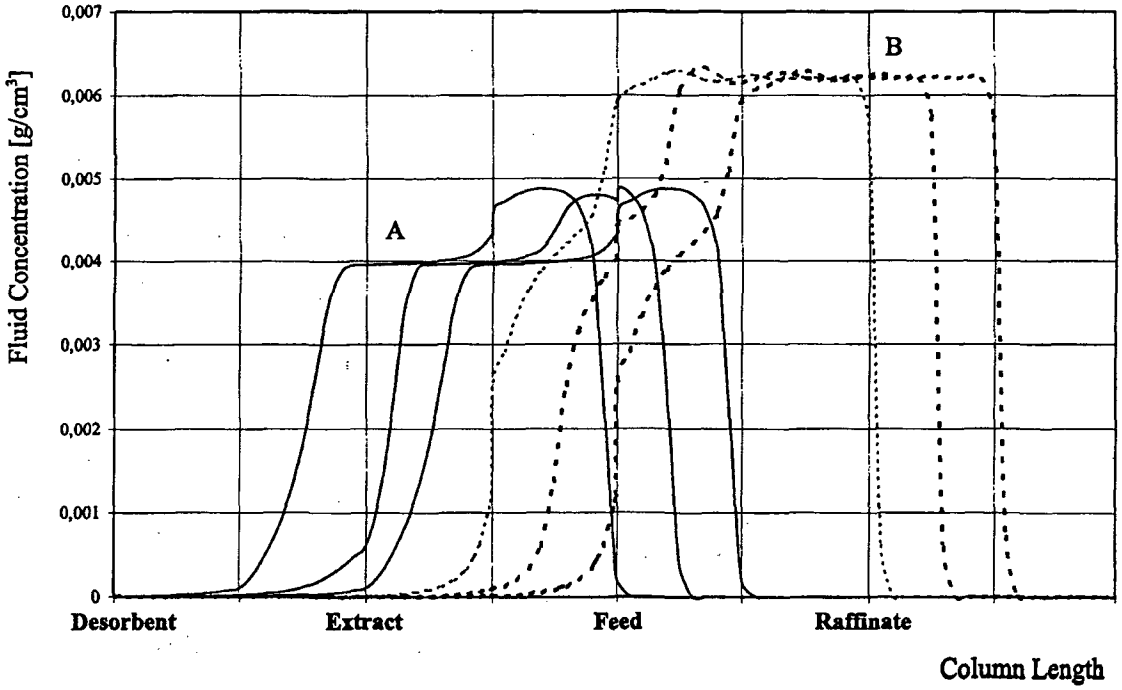

Figure 7. **Simulated axial concentration profile of the optimized SMB process to separate the enantiomers, periodic quasi-steady-state is reached. Fructose (A); glucose (B) (with each 3 curves in fluid flow direction, beginning, middle, and end of the shown switch period).**

which kind of process is the more productive one.

Dilution and solvent requirement of batch chromatography where indeed higher in each case, but the difference of both processes can be extremely small, as demonstrated for EMD53986. The reason for the generally higher solvent requirement of batch chromatography is the demand of equal throughput for batch and SMB (see eq 2). For equal concentration of the feed eq 2 simplifies:

$$\dot{V}_{SMB}\Delta t_{cyc} = \dot{V}_{Batch}\Delta t_{inj} \qquad (9)$$

The cycle time must be significantly higher than the injection time otherwise the mixture cannot be separated.

Furthermore, the results demonstrate that the assumption of the touching bands for the batch process leads to yields and purities up to 99%, even 100%, if the batch process is optimized properly. On the other hand, there is a certain level of automation required for fraction collection at the optimum time cuts. The personal costs for an automated batch column is as high as for a-SMB column to operate the equipment safe, reliable and 24 h per day. Due to that, the criteria of working costs is not a real advantage of the simulated moving bed technology. The same personnel are needed. This assumption is conservative in advantage of batch chromatography.[11]

6. Comparison of the Process Behavior by Cost Calculations

"It is well-known that there are no technical optima in industry, only economic optima," states Georges Guiochon.[20,21]

A comparison of batch elution and SMB chromatography just by a consideration of the three objective functions does not lead to a consistent answer of the question, "what kind of process should be preferred for a given separation task?"

In the case of *linear isotherms*, the productivities of batch chromatography are much higher than of the corresponding SMB processes, but product dilution and solvent requirements are also much higher.

The case of *nonlinear isotherms* is far more difficult because there is no homogeneous result for the process productivity at all. Even product dilution and solvent requirements of batch and SMB may not differ in a significant degree.

Due to that, at least a fourth objective function is calculated unifying productivity and solvent requirement—the separation costs. In this way, the economical relevance of each influencing parameter can be analyzed in a realistic way.

The third objective function, the product dilution, is taken into account. It includes the costs for further upgrading and, therefore, the energy necessary to separate different amounts of solvent from the product. Because batch chromatography generally provides the more diluted fractions, this objective function influences the total separation costs and also the decision about the more economic kind of process.

But in the forehand of the cost calculation, it is necessary to note that for the large scale fructose/glucose separation the separation costs of the SMB process are significantly lower than those of the corresponding batch process. As

(20) Guiochon, G.; Felinger, A. *J. Chromatogr. A* **1996**, *752*, 31–40.

(21) Guiochon, G.; Shirazi, S. G.; Katti, A. M. *Fundamentals of preparative and nonlinear chromatography*; Academic Press: New York, 1994.

far as the enantioseparation is concerned, the dilution of each process differs up to 10%. The financial expense is roughly the same for batch and SMB, therefore, an additional consideration about the dilution is not necessary.

7. The Proceeding Methodology

The basis of the following calculations is a Merck KGaA inhouse cost calculation program written by Dr. M. Schulte in Microsoft Excel which was extended for the presented process comparison. The cost calulations take into account that the total separation costs (TSC) consist of (1) costs for stationary phase (SP), (2) costs for mobile phase (MP), (3) personnel costs (PC), (4) plant costs, and (5) product losses.

(1) The costs for the stationary phase are a result of the column volume and therefore related to productivity (see eq 5). For the sugar separation, the stationary phase is a resin, and for EMD53986, a chiral stationary phase is applied. As a result, the price per kilogram of stationary phase differs from about \$0.5 to \$20 000 in the examples. The operation time is assumed to be 2−3 years.

(2) The costs for mobile phase contain feed as well as desorbent flow, desorbent flow for the plant startup, solvent recycling, loss of desorbent, and removal. This factor is related to solvent requirement. Solvent loss is proved to be about 1−3%.

(3) Personnel costs contain the mechanical work to prepare the equipment of the plant and labor costs for one person for each batch and SMB chromatography plant in preparative-scale and nine people for large-scale production. As discussed above, it is reasonable to assume that both processes are totally automated. A fully automated production plant can run unattended but tasks such as solvent handling, product recovery, and quality control must be done manually. The different product batches have to be prepared in order to be separated. The separation cut pints and operating parameters have to be adjusted for each batch. Moreover, the process control equipment must be watched.

(4) The plant costs include investment costs, running expenses, and maintenance. The investment costs are calculated from existing prices by using a degression coefficient for either batch or SMB chromatography. Plant costs are a function of the throughput and therefore increse with column diameter (D^2).

(5) Both processes are optimized due to total separation to avoid the need to take into account different product values and keep the comparison independent of that effect. If products are extremely expensive, it is the main argument to choose chromatography in favor of other process alternatives to gain total product yield and recovery and to achieve total separation.

The separation costs are calculated for typical production quantities. For the sugar separation, these quantities range from 10 000 to 200 000 tons per year. In the case of enantioseparations, the production scale is assumed to lie between 1 and 50 tons per year. Furthermore, the cut point of equal prices as a function of production is calculated to provide additional information for the decision about the best chromatographic process.

The main difference between the SMB and the corresponding batch column, resulting from the previous optimization, is the difference in column length and diameter. The optimized laboratory-scale columns are scaled up in relation to throughput, which increases in proportion to D^2. Due to this, the differences between batch and SMB column dimensions still remain. The column length has to be kept constant for different feed throughputs because a variation influences the separation behavior.

It is important to point out that cost distributions never can be represented in a gerneral form, because the cost structure of companies can be strongly different. Therefore, a case study is presented here with the same assumptions for both processes.

8. The Results

8.1. Large-Scale Sugar Separation. Figures 8 and 9 visualize the cost distribution for the fructose/glucose separation.

As discussed above, the optimum batch *productivity* is 1.7 times higher than the optimized SMB productivity.

The optimized *solvent requirement* is twice as high. This difference can also be noticed in the separation costs as a function of the annual production.

Stationary and mobile phase feed throughput increases linearly. The *operating costs* are assumed to remain constant and are negligible in relation to the other factors.

The *plant costs* rise with a degression exponent; near 1 for batch and about 0.5 for SMB. The reason for this sharp difference can be explained as follows: The investment costs for SMB chromatography are—compared to batch—extremely high because of the large number of equipment and high degree of automation. By scaling up the processes, the automation level remains almost the same. In the case of batch chromatography, the automation level rises faster with rising plant capacity because the demand to the single units (e.g., pumps) is much higher. The reason is that a single volume flow is set through, instead of the 4 fluid flows in the case of SMB columns. The differences in total equipment cost between batch elution chromatography and SMB are due to the fact that to operate batch chromatography units 24 h a day basis, larger storage tanks for solvent and recovered fractions as well as larger equipment for product recovery and solved recycling are required.

As a result, one interesting influence becomes visible by these cost calculations: While SMB columns of laboratory scale are significantly more expensive, this relation is reversed in production scale due to of the different degression coefficients.

The difference between batch and SMB plant costs can easily be seen in the Figures 8 and 9.

The most important factor is the cost of the mobile phase, and therefore, the different solvent requirements for batch and SMB chromatography turn the scale between the two processes. The effects on cost of alternative techniques (for example, membrane technology, crystallization, precipitation, evaporation, centrifugation, freeze-drying) to recover the isolated products and the solvent are considered in a different

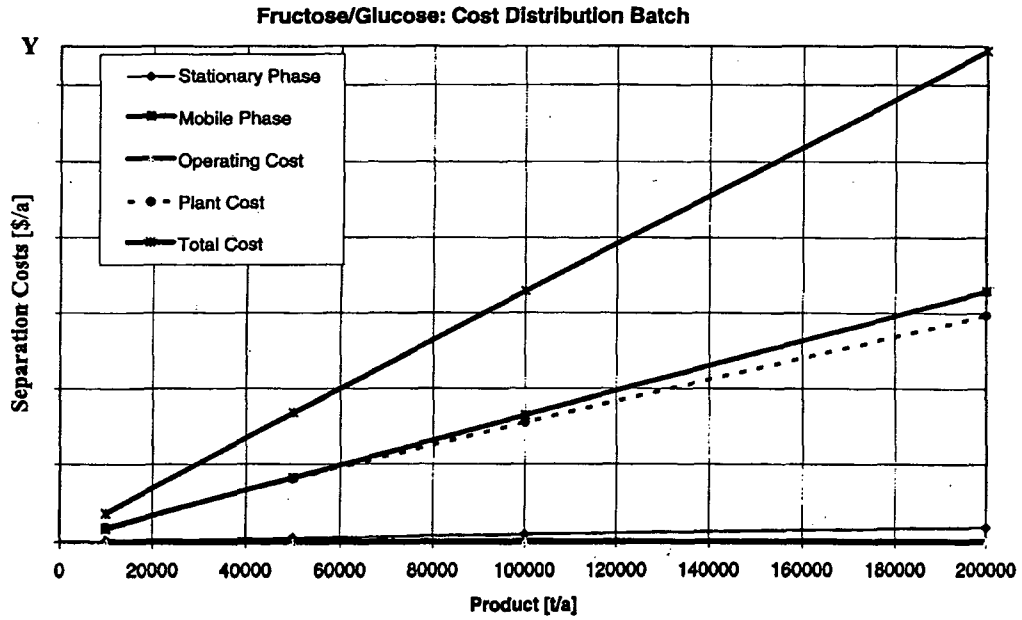

Figure 8. **Cost distribution of the optimized batch elution chromatography, fructose/glucose separation, separation costs [dollar/a] vs production in tons per year.**

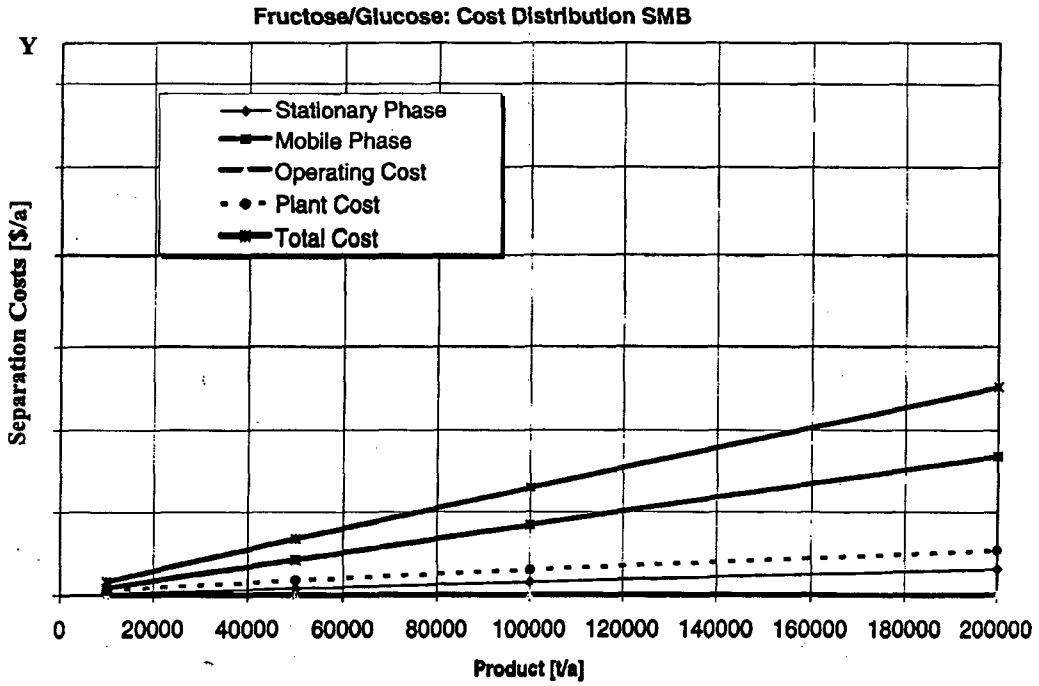

Figure 9. **Cost distribution of the optimized SMB chromatography, fructose/glucose separation, separation costs [dollar/a] vs production in tons per year.**

study at the moment. The higher productivity of the batch columns, economically characterized by the costs of the stationary phase, is not of any consequence. The reason is the relatively low price of the resins.

Finally, the specific separation costs for fructose/glucose, shown in Figure 10, are much lower for the SMB process than by applying the batchwise process.

Additionally, the annual production that provides equal separation costs by the two processes was calculated. Without taking into account different product dilutions, which determines the costs of further upgrading, this cut point is reached at 350 tons of product per year and is far below any economic production scale.

8.2. Production-Scale Enantioseparation. Figures 11 and 12 demonstrate the cost distribution for the enantioseparation of EMD53986.

An important difference to the previous sugar separation example is the much lower range of annual production (from

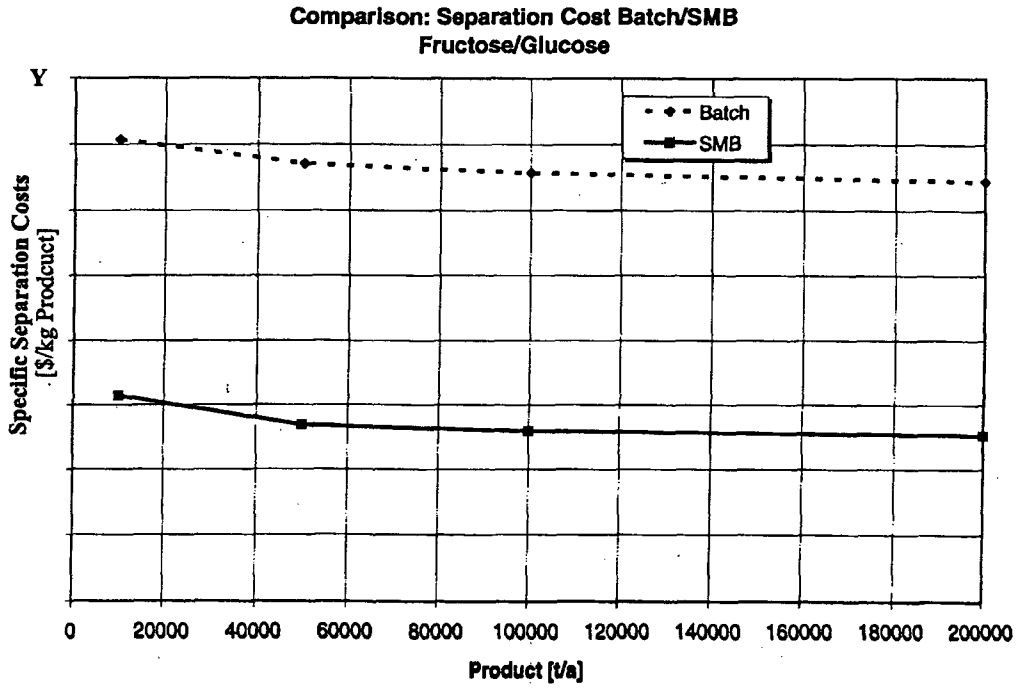

Figure 10. **Comparison of the total separation costs for the optimized batch elution and SMB chromatography, fructose/glucose separation, specific total separation costs [dollar/kg of product] vs production in tons per year.**

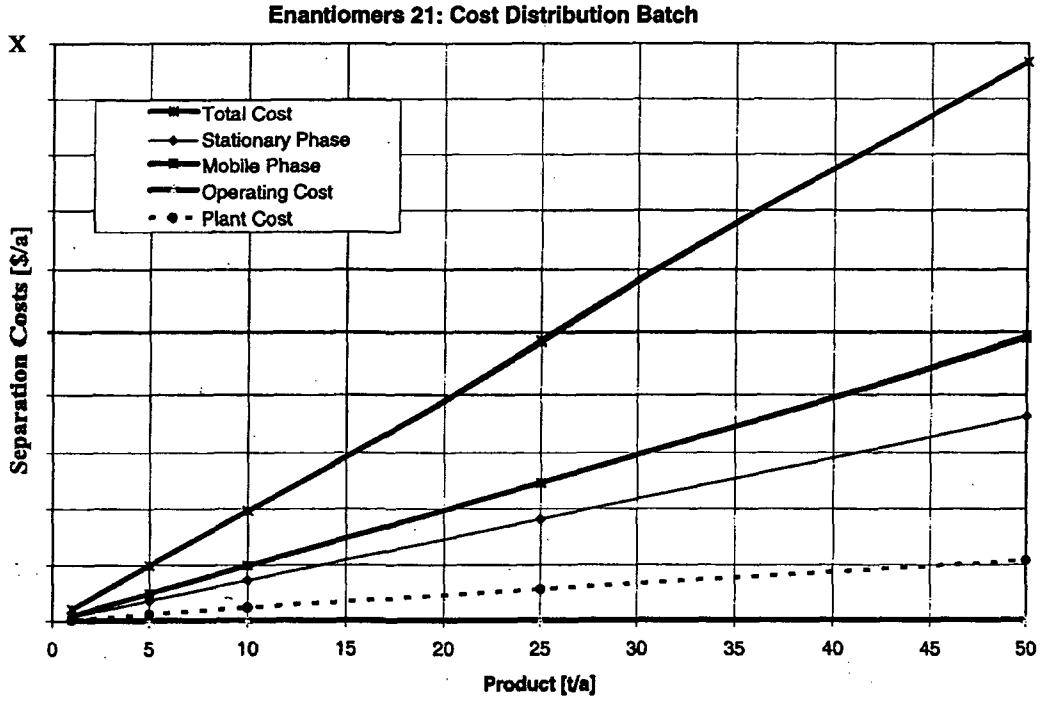

Figure 11. **Cost distribution of the optimized batch elution chromatography, enantioseparation, separation costs [dollar/a] vs production in tons per year.**

1 to 50 tons). As summarized in Table 2, the batch productivity is 1.5 times lower than for SMB, while the dilution and solvent requirement for each component differ only up to 10%—in favor of the SMB process.

In this range of annual production, the influence of plant costs is much smaller. Still, the mobile phase takes the greatest part of the total costs of the separation. But the second largest influence is the stationary phase, due to the high expenses for chiral phases. Figure 13 compares the specific separation costs of the batch and SMB process. The cut point where the costs are equal for both processes is at an annual production of 1.35 tons (see Figure 14).

As a result, the separation costs of enantiomers mostly depend on the costs of stationary phase and mobile phase.

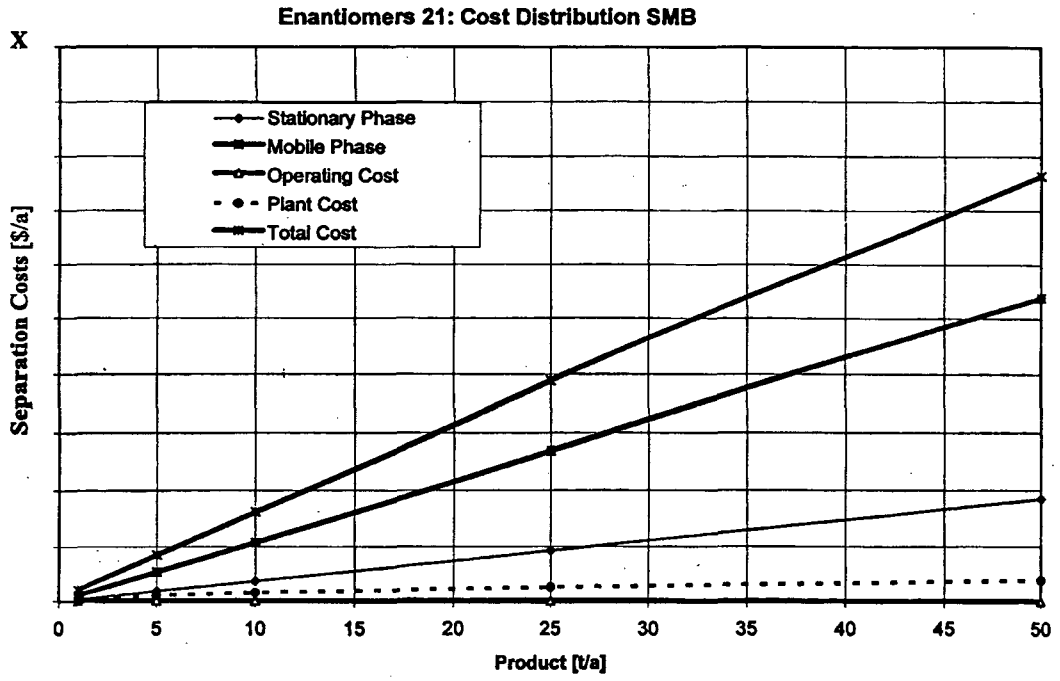

Figure 12. **Cost distribution of the optimized SMB chromatography, enantioseparation, separation costs [dollar/a] vs production in tons per year.**

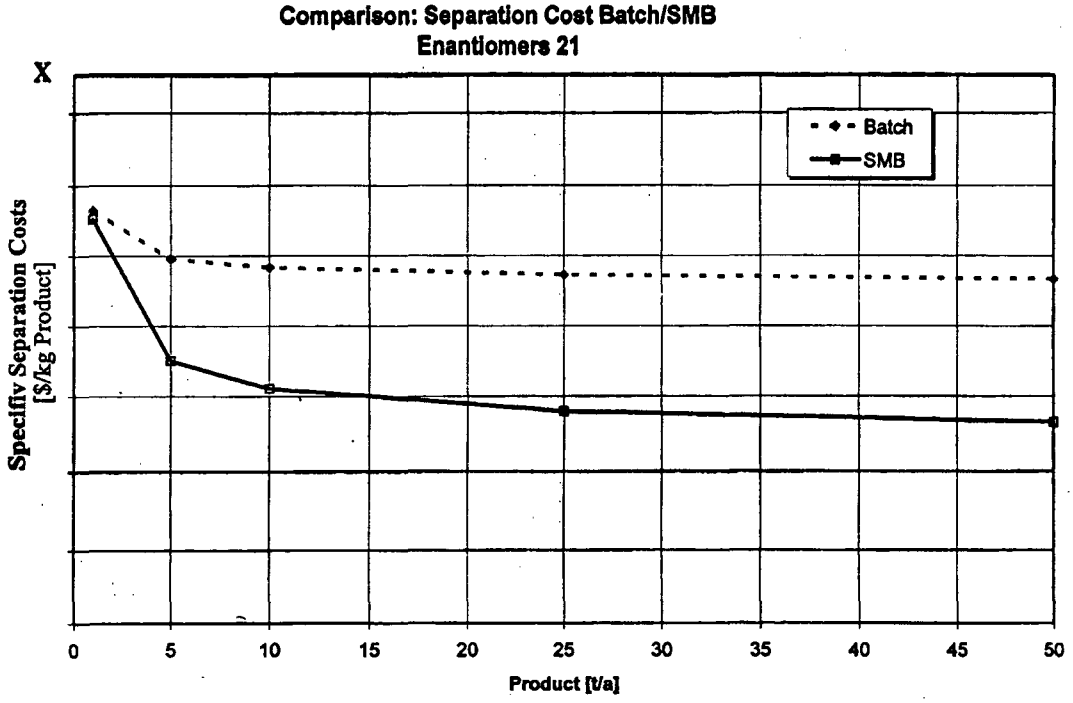

Figure 13. **Comparison of the total separation costs for the optimized batch elution and SMB chromatography, enantioseparation, specific total separation costs [dollar/kg of product] vs production in tons per year.**

But as mentioned above, these factors can be influenced strongly by optimization.

As previously noted, it is not possible to generally predict which kind of process will provide the greater productivity. If the productivity of the batch process is higher than that for the continuous one, batch chromatography may be the more economic route. On the other hand, the costs of mobile phase and their influence on the total separation costs depend, last but not least, on the specific desorbent price. The price for water is relatively low in comparison with hexane, for example, but the necessary energy for evaporation is high in product upgrading and solvent recovery.

9. Conclusions

9.1. Rules of Decision: Which Chromatographic Process Should Be Applied? Finally, the decision about

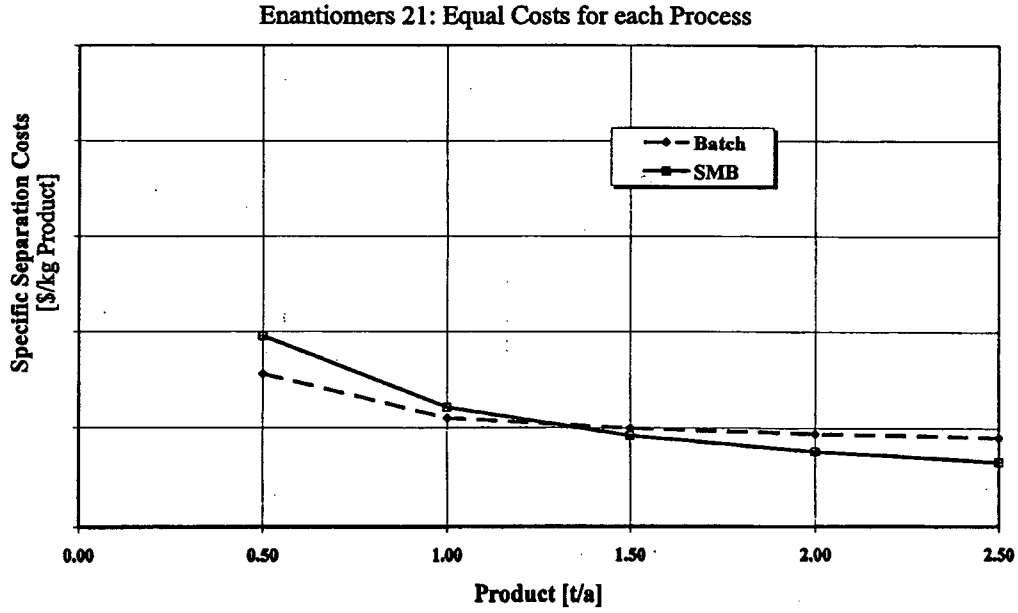

Figure 14. **Equal costs for the optimized batch elution and SMB chromatography, enantioseparation, specific total separation costs [dollar/kg of product] vs production in tons per year.**

the more economic chromatographic process is at first a question of the adsorbent used for separation:

For resins, zeolithes, and similar low-priced adsorbents, the influence of the stationary phase on the total separation costs can be neglected. Furthermore, the influence of the productivity is not remarkable as demonstrated in the fructose/glucose separation. In this case, the SMB process should be preferred—as it is done in the sugar industry since 1960s.

For expensive adsorbents, the specific separation costs depend on many details which must be considered carefully: optimum solvent requirement, optimum productivity of each process, and, of course, the relation of these objective functions.

Additionally, the prices of stationary and mobile phase should be taken into account because these prices concisely determine the degree of influence on each objective function of the different processes.

For enantioseparations in pharmaceutical or fine chemical product development or production, SMB chromatography has major advantages over batch elution such as higher productivity, lower product dilution, and lower separation costs. Both technologies have their advantages and limitations. This strongly depends on the type of problem to be handled. Often forgotten is the factor solubility. In batch chromatography, it is for example possible to inject the sample in a completely different solvent as the eluent composition, which is not so easy to perform in an SMB process.

If impurities in the binary separation cut are taken into account, as they occur in reality, the relations between the two chromatographic processes remain at the same magnitudes.

In detail, the more economic process can only be found by detailed process optimization, done by process simulations. Rigorous models are necessary because real effects for peak tailing have to be considered to optimize chromatographic processes.

The feasibility of SMB technology is demonstrated, and the process is established in product research and development and accepted by the involved scientists. Now, chemical engineers are called to develop and prove the profits of SMB chromatography in production and to identify separation task of interest. Therefore, the authors intend to give arguments for the process decision with the presented comparisons of batch elution and SMB chromatography by cost calculations. The design and optimization methodologies are described in detail in refs 14–18 and 22.

Looking at a complex production scheme, more questions must be answered. First of all, the whole process has to be taken into account. This would lead us to a very broad approach of general optimization of the chromatographic step within a production route. Some of the necessary considerations are the following:

(a) The best step for a separation has to be chosen. This depends on the selectivity and the solubility of the educts.

(b) Saving of reagents in following synthetic steps have to be achieved.

(c) An increase in yield in the following steps due to higher purity of educts should be gained.

(d) Should the mobile phase be removed or is the concentration/enrichment of the product sufficient?

(e) The costs for connecting the separation unit with the previous or next steps have to be considered: whether it is possible to take the same solvent or whether the product has to be crystallized and resoluted before the next step.

(f) The chosen separation has to be optimization as described before because process optima have to be compared.

Chemical engineers should accept chromatography as an efficient and economic unit operation which has many advantages:

1. Most pharmaceutical and fine chemical products are analyzed by chromatography in the early stage of product development. Therefore, stationary and mobile phases are chosen and experiments are done. Optimized analytical parameters are not (always) the best conditions for an optimal preparative process. Few experiments must be done on an analytical HPLC column to optimize stationary and mobile phases for the production scale and to determine the equilibrium phase isotherms. It should be kept in mind that analytical method development has the goal to achieve a total baseline separation of all components of the mixture. As a result, complex eluent mixtures may be needed in most cases. But in production, only one main or at least two main products are needed out of the feedstock. Therefore, first, the separation task is quite different and, second, taking the costs of product recovery and eluent recycling into account, pure eluents or simple mixtures have to be chosen.

2. It is not possible to derive general rules in detail. But, for single applications, the design and optimization methodology is developed and can easily be applied to industrial separation tasks as described above.

3. In product development, this methodology is a reliable method to evaluate the separation task by chromatography, to benchmark different products. Detailed optimization and consideration of unit operation alternatives could be done if the production decision is made.

4. SMB technology is accepted in laboratory scale for the production of value product. The feasibility is proven.[3,4,8,23]

5. The authors have proven[10,14,18,19] that detailed optimization of SMB chromatography by rigorous simulation studies, in contrast to an empirical trial and error approach, optimizes the operation conditions to about double feed throughput and half desorbent requirements. In production, these efforts for process optimization are of great benefit.[11]

These theoretical simulation studies need to be proven by experimentation. However, process simulations based on rigorous models function as a standardized reference for theoretical studies. Due to a standardized methodology, the advantages and limitations as well as the preferable ranges of application of batch elution and SMB chromatography are demonstrated by theoretical studies to show the benefits of both processes.

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Appendix 1: Symbols and Abbreviations Used

c	[g/cm³]	fluid concentration
c_P	[g/cm³]	fluid concentration in the adsorbent pores
D	[cm]	column diameter
D_L	[cm²/s]	axial dispersion coefficient
d_P	[cm]	particle diameter
Δt_{cyc}	[s]	cycle time
Δt_{inj}	[s]	injection time
E	[cm³/s]	extract volume flow
F	[cm³/s]	feed volume flow
H_i	[−]	Henry coefficient of component i
P	[−]	coefficient of the modified Langmuir isotherm
k_{eff}	[cm/s]	effective, overal mass transfer coefficient
k_{1-4}	[cm³/g]	Langmuir coefficient of component i
L	[cm³/s]	liquid fluid flow
m_{ads}	[g]	mass of adsorbent
$\dot{m}_j$	[−]	relative mass flow in section j
$\dot{m}_i$	[g/s]	mass flow component i
P_i	[−]	adsorbility of component i (Bi−Langmuir)
Pr	[1/s]	productivity [g of produkt/g of adsorbent/h]
q_i	[g/cm³]	solid load of componet i
R	[cm³/s]	raffinate fluid flow
r	[−]	point in the operating diagram
Rec	[cm³/s]	recycle fluid flow
t	[s]	time domain
t^*	[s]	switch time
u_{int}	[cm/s]	interstitial fluid velocity
$\dot{V}$	[cm³/s]	recycle fluid flow
V	[cm³]	column volume
w	[−]	point in the operating diagram
w_c	[cm/s]	velocity of the movement of a concentration front
x	[cm]	space domain
Y	[%]	yield [g of product/g of feed]
ϵ_0	[−]	voidage
π	[−]	circle constant
A		stronger adsorbable component
B		weeker adsorbable component
Batch		batch elution chromatography
D		desorbent
E		extract
F		feed
i		component number
j		section number
R		raffinate
Rec		recycle
SMB		simulated moving bed chromatography
CSP		chiral stationary phases
D		dilution
HETP		height equivalent to theoretical plate
Pr		productivity
Pu		purity
SR		solvent requirement
Y		yield

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