US 20070203339A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2007/0203339 A1

KOPCHO et al. (43) Pub. Date: Aug. 30, 2007

(54) **PROCESS FOR PREPARATION OF CYCLIC PRODRUGS OF PMEA AND PMPA**

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(21) Appl. No.: **11/670,806**

(22) Filed: **Feb. 2, 2007**

**Related U.S. Application Data**

(63) Continuation of application No. 10/436,799, filed on May 12, 2003, now Pat. No. 7,193,081.

(60) Provisional application No. 60/380,321, filed on May 13, 2002.

**Publication Classification**

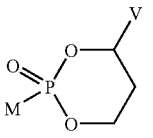
(51) **Int. Cl.**
***C07F 9/655*** (2006.01)
***C07D 263/04*** (2006.01)

(52) **U.S. Cl.** ..... **544/244**; 549/369

(57) **ABSTRACT**

The method of preparing compounds of Formula I is described:

Formula I




wherein:

M and V are cis to one another and $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine, and (R)-9-(2-phosphonylmethoxypropyl)adenine; wherein V is phenyl, optionally substituted with 1-2 substituents selected from a group consisting of fluoro, chloro, and bromo; comprising: coupling a chiral 1-phenylpropane-1,3-diol, wherein the phenyl may be optionally substituted, with $MPOCl_2$ or an N-6 substituted analogue thereof. Additionally, methods and salt forms are described that enable isolation and purification of the desired isomer.

# PROCESS FOR PREPARATION OF CYCLIC PRODRUGS OF PMEA AND PMPA

## FIELD OF INVENTION

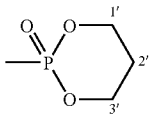
[0001] The present invention is directed towards a process of synthesis of substituted six-membered cyclic 1-aryl-1,3-propanyl esters of PMEA and PMPA. More specifically, the invention relates to the process of synthesis of halogen substituted cyclic-1-phenyl-1,3-propanyl esters of PMEA and PMPA that have cis stereochemistry.

## BACKGROUND OF THE INVENTION

[0002] The following description of the background of the invention is provided to aid in understanding the invention, but is not admitted to be, or to describe, prior art to the invention. All publications are incorporated by reference in their entirety.

[0003] 9-(2-phosphonylmethoxyethyl)adenine (PMEA), (R)-9-(2-phosphonyl-methoxypropyl)adenine (PMPA) and related analogues (U.S. Pat. No. 4,808,716; U.S. Pat. No. 5,142,051) are phosphonic acids that exhibit antiviral activity, including activity against hepatitis B and HIV (De Clercq et al., *Antiviral Res.* 8: 261-7(1987); Balzarini et al., *Biochem Biophys. Res. Commun.* 219(2): 337-41(1996)). The dipivaloyloxy methylene ester of PMEA ("BisPOM PMEA") is in clinical trials for the treatment of hepatitis B (Benhamou et al., *Lancet* 358(9283): 718-23 (2001)). In addition, some studies have shown that these compounds also show anticancer activity (Murono et al., *Cancer Res.* 61(21): 7875-7(2001)).

[0004] Compounds containing phosphonic acids and their salts are highly charged at physiological pH and therefore frequently exhibit poor oral bioavailability, poor cell penetration and limited tissue distribution (e.g., CNS). In addition, these acids are also commonly associated with several other properties that hinder their use as drugs, including short plasma half-life due to rapid renal clearance, as well as toxicities (e.g., renal, gastrointestinal, etc.) (e.g., Bijsterbosch et al., *Antimicrob Agents Chemother.* 42(5): 1146-50 (1998)). Cyclic phosphonate esters have also been described for PMEA and related analogues. The numbering for these cyclic esters is shown below:




Unsubstituted cyclic 1',3'-propanyl esters of PMEA were prepared but showed no in vivo activity. EP 0 481214 B1 discloses examples of cyclic prodrugs of PMEA wherein the 1' and 3' positions are unsubstituted. The application and a subsequent publication by the inventors (Starrett et al., *J. Med. Chem.* 37:1857-1864 (1994)) further disclose their findings with the compounds, namely that these compounds showed no oral bioavailability and no biological activity. The compounds were shown to be unstable at low pH, e.g., the cyclic 2',2'-difluoro-1',3'-propane ester is reported to be hydrolytically unstable with rapid generation of the ring-opened monoester.

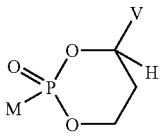
## SUMMARY OF THE INVENTION

[0005] The present invention is directed towards a novel process for the synthesis of cyclic 1-aryl-1,3 propanyl phosphonate cyclic esters of PMEA and PMPA with an enhanced d.e. for the cis isomer. In one aspect the process enhances the cis isomers via a coupling method. In another aspect this process for the cis isomers is enhanced by the temperature of the process. In an additional aspect the order of addition of the reactants enhanced the production of the cis isomer. Further aspect is additional enrichment of the desired cis isomer through the addition of an acid and the crystallization of the salt. Another aspect of the process is the enhancement of cis isomer that occurred with the crystallization solvent.

[0006] In another aspect, this invention is directed towards a method of making substantially enantiomerically pure cis cyclic esters having S stereochemistry where the V is attached.

[0007] One aspect of the invention concerns the method for the preparation of compounds of Formula I:

Formula I




[0008] wherein:

[0009] M and V are cis to one another and $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine, and (R)-9-(2-phosphonylmethoxypropyl)adenine; wherein V is phenyl, optionally substituted with 1-2 substituents selected from a group consisting of fluoro, chloro, and bromo; comprising: coupling a chiral 1-phenylpropane-1,3-diol, wherein the phenyl may be optionally substituted, with $MPOCl_2$ or an N-6 substituted analogue thereof.

[0010] Additionally, methods and salt forms are described that enable isolation and purification of the desired isomer.

### Definitions

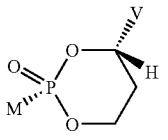
[0011] In accordance with the present invention and as used herein, the following terms are defined with the following meanings, unless explicitly stated otherwise.

[0012] The term "hexanes" refers to commercially available HPLC reagent solutions which contains approximately 95% hexane, methylcyclopropane, and methylpentane.

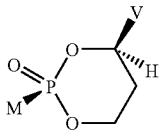
[0013] The term "dialkyl" refers to a compound containing two alkyl groups. The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched chain and cyclic groups. Suitable alkyl groups include methyl, ethyl, isopropyl, and cyclopropyl.

[0014] The term "optionally substituted" or "substituted" includes aryl groups substituted with one to two substituents, independently selected from lower alkyl lower aryl, and halogens. Preferably these substituents are selected from the group consisting of halogens.

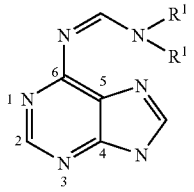
[0015] The term "Cis" stereochemistry refers to the relationship of the V group and M group positions on the six-membered ring. The formula below shows a cis stereochemistry.



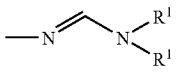
Another cis stereochemistry would have V and M pointing above the plane. The formula below shows this cis stereochemistry.



[0016] The term "N6-substituted" refers to the substitution at the amine attached at the 6-position of a purine ring system. N6- is generally substituted with a dialkylaminomethylene group wherein $R^1$ groups include but are not limited to C1-C4 acyclic alkyl, C5-C6 cyclic alkyl, benzyl, phenethyl, or $R^1$ groups together form piperdine, morpholine, and pyrrolidine.



[0017] The term "dialkylaminomethyleneimine" refers to functional group or substitution of the following structure:



[0018] wherein $R^1$ groups include but are not limited to C1-C4 acyclic alkyl, C5-C6 cyclic alkyl, benzyl, phenethyl, or $R^1$ groups together form piperdine, morpholine, and pyrrolidine.

[0019] The term "percent enantiomeric excess (% ee)" refers to optical purity. It is obtained by using the following formula:

$$\frac{[R]-[S]}{[R]+[S]}\times 100 = \%\ R - \%\ S$$

[0020] where [R] is the amount of the R isomer and [S] is the amount of the S isomer. This formula provides the % ee when R is the dominant isomer.

[0021] The term "d.e." refers to diastereomeric excess. It is obtained by using the following formula:

$$\frac{[cis]-[trans]}{[cis]+[trans]}\times 100 = \%\ [cis] - \%\ [trans]$$

[0022] The term "diastereoisomer" refers to compounds with two or more asymmetric centers having the same substituent groups and undergoing the same types of chemical reactions wherein the diastereoisomers have different physical properties, have substituent groups which occupy different relative positions in space, and may have different biological properties.

[0023] The term "racemic" refers to a compound or mixture that is composed of equal amounts of dextrorotatory and levorotatory forms of the same compound and is not optically active.

[0024] The term "enantiomer" refers to either of a pair of chemical compounds whose molecular structures have a mirror-image relationship to each other.

[0025] The term "acid dissociation constant" ($K_a$) refers to the equilibrium constant for the ionization of an acid, e.g. HA is the formula for a weak acid, then:

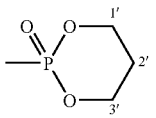
$$K_a=([H^+][A^-]/[HA])$$

[0026] The term "halogen" refers to chlorine, bromine, or fluorine.

[0027] The term "prodrug" as used herein refers to any M compound that when administered to a biological system generates a biologically active compound as a result of spontaneous chemical reaction(s), enzyme catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination of each. Standard prodrugs are formed using groups attached to functionality, e.g., HO—, HS—, HOOC—, $R_2N$—, associated with the drug, that cleave in vivo. Standard prodrugs include but are not limited to carboxylate esters where the group is alkyl, aryl, aralkyl, acyloxyalkyl, alkoxycarbonyloxyalkyl as well as esters of hydroxyl, thiol and amines where the group attached is an acyl group, an alkoxycarbonyl, aminocarbonyl, phosphate or sulfate. The groups illustrated are exemplary, not exhaustive, and one skilled in the art could prepare other known varieties of prodrugs. Such prodrugs of the compounds of Formula I fall within the scope of the present invention. Prodrugs must undergo some form of a chemical transformation to produce the compound that is biologically active or is a precursor of the biologically active compound. In some cases, the prodrug is biologically active, usually less than the drug itself, and serves to improve drug efficacy or safety through improved oral bioavailability, pharmacodynamic half-life,

etc. The biologically active compounds include, for example, anticancer agents, and antiviral agents.

[0028] The term "cyclic 1',3'-propane ester", "cyclic 1,3-propane ester", "cyclic 1',3'-propanyl ester", and "cyclic 1,3-propanyl ester" refers to the following:



[0029] The term "enhancing" refers to increasing or improving a specific property.

[0030] The term "enriching" refers to increasing the quantity of a specific isomer produced by a reaction.

[0031] The term "pharmaceutically acceptable salt" includes salts of compounds of Formula I derived from the combination of a compound of this invention and an organic or inorganic acid or base, such that they are acceptable to be safely administered to animals. Suitable acids include acetic acid, adipic acid, benzenesulfonic acid, (+)-7,7-dimethyl-2-oxobicyclo[2.2.1]heptane-1-methanesulfonic acid, citric acid, 1,2-ethanedisulfonic acid, dodecyl sulfonic acid, fumaric acid, glucoheptonic acid, gluconic acid, glucuronic acid, hippuric acid, hydrochloride hemiethanolic acid, HBr, HCl, HI, 2-hydroxyethanesulfonic acid, lactic acid, lactobionic acid, maleic acid, methanesulfonic acid, methylbromide acid, methyl sulfuric acid, 2-naphthalenesulfonic acid, nitric acid, oleic acid, 4,4'-methylenebis[3-hydroxy-2-naphthalenecarboxylic acid], phosphoric acid, polygalacturonic acid, stearic acid, succinic acid, sulfuric acid, sulfosalicylic acid, tannic acid, tartaric acid, terephthalic acid, and p-toluenesulfonic acid.

[0032] The following well known chemicals are referred to in the specification and the claims. Abbreviations, and common names are also provided.

[0033] $CH_2Cl_2$; Dichloromethane or methylene chloride

[0034] DCM; dichloromethane

[0035] (−)-DIP—Cl; (−)-β-Chlorodiisopinocampheylborane

[0036] DMAP; 4-dimethylaminopyridine

[0037] DMF; Dimethylformamide

[0038] HCl; hydrochloric acid

[0039] KI; potassium iodide

[0040] $MgSO_4$; magnesium sulfate

[0041] MTBE; t-butyl methyl ether

[0042] NaCl; sodium chloride

[0043] NaOH; sodium hydroxide

[0044] PyBOP; benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate

[0045] TEA; triethylamine

[0046] THF; tetrahydrofuran

[0047] TMSCl; chlorotrimethylsilane

[0048] bis POM PMEA; bis(pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl) adenine (Adefovir dipivoxil)

[0049] The following well known drugs are referred to in the specification and the claims. Abbreviations and common names are also provided.

[0050] PMEA; 9-(2-phosphonylmethoxyethyl)adenine (Adefovir)

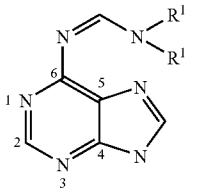
[0051] (R)—PMPA; (R)-9-(2-phosphonylmethoxypropyl)adenine (Tenofovir)

## DETAILED DESCRIPTION OF THE INVENTION

[0052] This invention is directed to the discovery that the process for the synthesis of cyclic 1-aryl-1,3-propanyl esters of PMEA and PMPA determined the stereochemistry of the resultant product. Compounds synthesized by the process of the present invention are directed towards the cis stereochemistry of the cyclic esters of PMEA and PMPA. In one aspect of this invention the stereochemistry at the methine carbon which is identified as C1' in the cyclic 1-aryl-1,3-propanyl esters was established through the synthesis of the corresponding chiral 1-aryl-1,3-propane diol e.g., via the chiral reduction of an intermediate ketoacid.

[0053] In another aspect it was found that the chirality at the phosphorus of the cyclic phosphonate ring was established during the reaction with the diol. Production of the cis diastereoisomer was dependent on the reaction temperature and the order of addition of the chiral diol and protected parent phosphonic dichloridate to the reaction mixture.

[0054] An additional aspect of the invention is the protection of the nitrogen attached to the carbon labeled 6 in the structure below.



The concentration of the desired cis isomer, wherein cis refers to the geometric relationship between the phosphorus-carbon bond and the carbon-aryl bond of the cyclic phosphonate ring, was enhanced by additional isolation via selective crystallization of the acid salt, which is a further aspect of this invention. Still further enhancement was achieved through recrystallization of the acid addition salt.

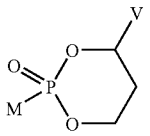
[0055] The process for the synthesis of cyclic 1-aryl-1,3-propanyl esters of PMEA or PMPA with the desired stereochemistry is via a convergent synthetic sequence starting with adenine and a halogen substituted benzoyl chloride. The final resultant compound contained two stereocenters, (1) the methine carbon which is identified as C1' in the stereoisomeric structures and (2) the phosphorus of the cyclic phosphonate ring. The stereochemistry at the carbon,

C1', resulted from the chiral reduction of an intermediate ketoacid and the phosphorus chirality was the result of the diastereoselective coupling of the parent phosphonic acid and the chiral diol. The desired cis isomer, wherein cis refers to the isomeric relationship between the phosphorus-carbon bond and the carbon-phenyl bond of the cyclic phosphonate ring, was isolated via a selective crystallization of the acid salt.

Compounds Prepared by the Invention

[0056] The compounds prepared by the invention are substituted 6-membered cyclic 1,3-propane diester prodrugs of PMEA and analogues as represented by Formula I:

Formula I



[0057] wherein:

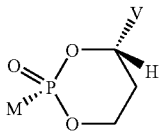
[0058] M and V are cis to one another and $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine, and (R)-9-(2-phosphonylmethoxypropyl) adenine;

[0059] V is phenyl, optionally substituted with 1-2 substituents selected from a group consisting of F, Cl, and Br;

[0060] and pharmaceutically acceptable salts thereof.

[0061] Another aspect of the invention is the preparation of the compounds of Formula II

Formula II



[0062] wherein:

[0063] $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine and (R)-9-(2-phosphonylmethoxypropyl)adenine;

[0064] V is phenyl, optionally substituted with 1-2 substituents selected from a group consisting of F, Cl, and Br;

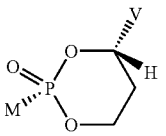
[0065] and pharmaceutically acceptable salts thereof.

[0066] Another aspect is directed to salts of such compounds formed with methanesulfonic acid or succinic acid.

[0067] Another aspect is directed to salts formed with methanesulfonic acid.

[0068] Another aspect of the invention is the preparation of compounds of Formula II

Formula II



[0069] wherein:

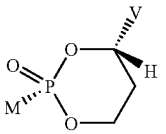
[0070] $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine and (R)-9-(2-phosphonylmethoxypropyl)adenine; V is 3-chlorophenyl;

[0071] and pharmaceutically acceptable salts thereof.

[0072] Another aspect is directed to salts formed with methanesulfonic acid of such compounds.

[0073] Another aspect of the invention are the compounds of Formula II

Formula II



[0074] wherein:

[0075] $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine and (R)-9-(2-phosphonylmethoxypropyl)adenine; V is 2-bromophenyl;

[0076] and pharmaceutically acceptable salts thereof.

[0077] Another aspect is directed to salts formed with methanesulfonic acid of such compounds.

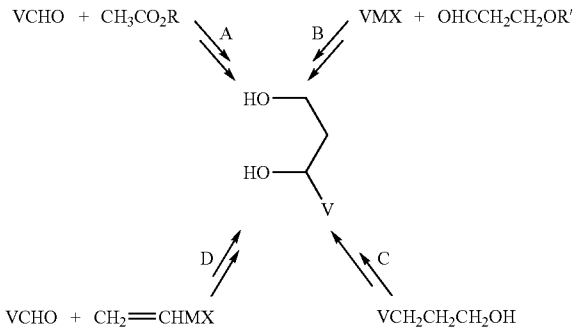
1. Synthesis of 1-(Aryl)-Propane-1,3-Diols

[0078] A variety of synthetic methods are known to prepare 1,3-diols. These suitable methods are divided into two types as following: 1) synthesis of racemic 1-(aryl)-propane-1,3-diol; 2) synthesis of chiral 1-(aryl)-propane-1,3-diol.

1.1 Synthesis of Racemic 1-(Aryl)-Propane-1,3-Diol

[0079] 1,3-Dihydroxy compounds can be synthesized by several well known methods in literature. Substituted aromatic aldehydes are utilized to synthesize racemic 1-(aryl-)propane-1,3-diol via addition of lithium enolate of alkyl acetate followed by ester reduction (path A) (Turner, *J. Org. Chem.* 55:4744 (1990)). Alternatively, aryl Grignard additions to 1-hydroxy propan-3-al also give 1-(arylsubstitued-)propane-1,3-diols (path B). This method will enable conversion of various substituted aryl halides to 1-(arylsubstituted)-1,3-propane diols (Coppi, et al., *J. Org. Chem.* 53:911 (1988)). Aryl halides can also be used to synthesize 1-substituted propane diols by Heck coupling of

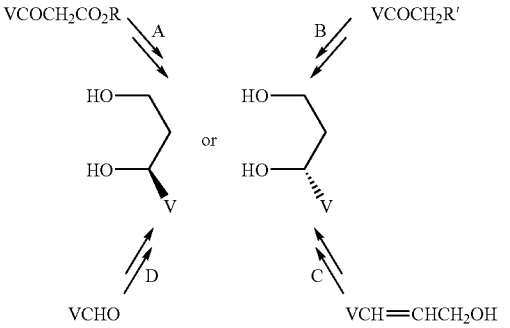
1,3-diox-4-ene followed by reduction and hydrolysis (Sakamoto, et al., *Tetrahedron Lett.* 33:6845 (1992)). Pyridyl, quinoline, isoquinoline propan-3-ol derivatives can be oxygenated to 1-substituted-1,3-diols by N-oxide formation followed by rearrangement in acetic anhydride conditions (path C) (Yamamoto, et al., *Tetrahedron* 37:1871 (1981)). A variety of aromatic aldehydes can also be converted to 1-substituted-1,3-diols by vinyl Grignard addition followed by hydroboration reaction (path D).




V = Aryl, R = Alkyl, R′ = benzyl, M = Mg or Li, X = Halide or null

1.2 Synthesis of Chiral 1-(aryl)-Propane-1,3-Diol

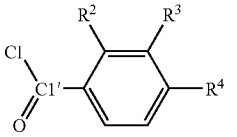
**[0080]** A variety of known methods for chiral resolution of secondary alcohols via chemical or enzymatic agents may be utilized for preparation of diol enantiomers (Harada, et al., *Tetrahedron Lett.* 28:4843 (1987)). Transition metal catalyzed hydrogenation of substituted 3-aryl-3-oxo propionic acids or esters is an efficient method to prepare R or S isomers of optically pure beta hydroxy acids or esters (*Comprehensive Asymmetric Catalysis,* Jacobsen, E. N., Pfaltz, A., Yamamoto, H. (Eds), Springer, (1999); *Asymmetric Catalysis in Organic Synthesis,* Noyori, R., John Wiley, (1994)). These beta hydroxy acid or ester products can be further reduced to give required chiral 1-(aryl)-propane-1, 3-diols. (path A). The β-keto acid or ester substrates for high pressure hydrogenation or hydrogen transfer reactions may be prepared by a variety of methods such as condensation of acetophenone with dimethylcarbonate in the presence of a base (Chu, et al, *J. Het Chem.* 22:1033 (1985)), by ester condensation (Turner, et al., *J. Org. Chem.* 54:4229 (1989)) or from aryl halides (Kobayashi, et al., *Tetrahedron Lett.* 27:4745 (1986)). Alternatively, enantiomerically pure 1,3-diols can be obtained by chiral borane reduction of β-hydroxyethyl aryl ketone derivatives or β-keto acid derivatives (path B) (Ramachandran, et al., *Tetrahedron Lett.* 38:761 (1997)). In another method, commercially available cinnamyl alcohols may be converted to epoxy alcohols under catalytic asymmetric epoxidation conditions. These epoxy alcohols are reduced by Red-Al to result in enantiomerically pure 1,3-diols (path C) (Gao, et al., *J. Org. Chem.* 53:4081 (1980)). Aldol condensation is another well described method for synthesis of the chiral 1,3-oxygenated functionality starting from aromatic aldehydes. (path D) (Mukaiyama, *Org. React.* 28:203 (1982)).




V = Aryl, R = Alkyl or H, R′ = —$CH_2OH$, $CO_2R$

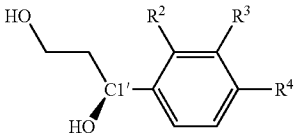
**[0081]** For the purpose of this invention the intermediate ketoacid is prepared from a halogen substituted benzoyl chloride of Formula A wherein the benzoyl chloride may be optionally substituted at any position on the phenyl ring with 1-2 halogens. In a preferred embodiment if $R^2$ is a halogen then $R^3$ must be a hydrogen and if $R^3$ is a halogen then $R^2$ must be a hydrogen. In one embodiment Formula A is 3-chlorobenzoyl chloride and in another embodiment, Formula A is 2-bromobenzoyl chloride. The C1' identifies the carbon that is the methine carbon stereocenter in the final compound prepared by this invention.

Formula A




The compound of Formula A is reacted with trimethylsilyl acetate and lithium diisopropylamide (generated in situ by reaction of diisopropylamine and n-butyllithium) to obtain the oxo-propanoic acid. The hydroxypropanoic acid is synthesized from the oxo-propanoic acid via reaction with (−)-DIP—Cl and then the hydroxypropanoic acid is reduced to the chiral 1,3-diol, shown in the following Formula B:

Formula B



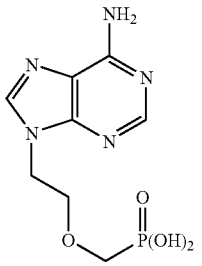

The chiral center at the carbon, C1', has been established in this process step and the ratio of enantiomers was conserved throughout the remainder of the process.

2.0 Synthesis of PMEA

**[0082]** Various preparations of PMEA and (R)-PMPA and their analogues are described in the literature (Arimilli et al., WO 99/04774; Schultze et al., *Tetrahedron Letters* 1998, 39,

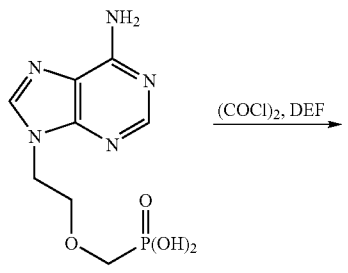
1853-1856; Bischofberger et al., U.S. Pat. No. 5,514,798, U.S. Pat. No. 5,686,629; Holy et al., U.S. Pat. No. 4,659, 825, U.S. Pat. No. 4,808,716, U.S. Pat. No. 5,130,427, U.S. Pat. No. 5,142,051) and are known to those skilled in the art. These procedures were modified for use herein and the modifications were unexpectedly found to eliminate both the time consuming isolation and purification steps given in the earlier literature. For the purpose of this invention the isolation of the diethyl ester of the phosphonic acid was not required to proceed to the next step. It was found that the ester could be deprotected without purification in this process.

[0083] In a typical method the adenine is reacted with a substituted or nonsubstituted ethylene carbonate and a base to generate 9-hydroxyethyladenine which was further alkylated with $TsOCH_2P(O)OEt_2$. The final step entailed a hydrolysis of the diethyl ester to generate PMEA, (R)-PMPA or their analogues.



3. Synthesis of N6-protected PMEA-dichloridate

[0084] In another step chlorination of PMEA is achieved using oxalyl chloride and N,N-diethylformamide to give N6 protected-PMEA-dichloridate. N,N-dialkylformamide used in the chlorination step not only forms a Vilsmeyer chlorinating agent, but also protects the $NH_2$ group at the 6 position. The protected chloridate intermediate was found to have favorable solubility properties that improved the overall yield and the diastereomeric ratio of the product. Use of other protecting groups such as acyl, alkoxycarbonyl, aryloxycarbonyl, and aralkyloxycarbonyl also enhance the solubility of the dichloridate and diastereomeric ratio of the expected product.

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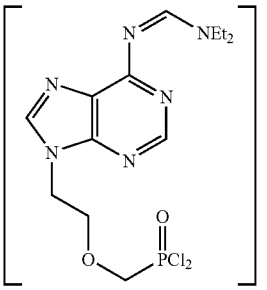


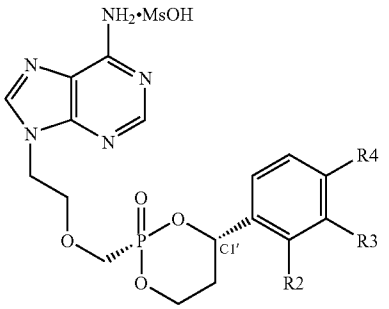
4. Coupling of Phosphonic Dichloridate and Chiral Diol

[0085] Coupling of the protected parent phosphonic dichloridate and the chiral diol in the presence of a base resulted in a protected intermediate soluble in dichloromethane at lower temperatures.

4.1 Crystallization of cis Prodrug Salt

[0086] Deprotection of the N6 position of the coupled phosphonic acid and chiral diol under mild acidic conditions and crystallization of the resultant product using methanesulfonic acid gave rise to the cis prodrug as a mesylate salt (Formula C) with 92-93% chemical purity. The trans isomer is the major impurity and a second crystallization of the final material from an alcohol such as methanol gave greater than 96% diastereomeric purity.

Formula C



[0087] The use of other acids including but not limited to such as sulfuric, nitric, hydrochloric, phosphoric, sulfonic, tartaric, citric, maleic, malic, malonic, lactic, oxalic acids and the like, may lead to better recovery and isomeric ratio of the product. The protocol as described for PMEA is also applicable to other PME or PMP derivatives.

4.2 Synthesis of 9-{2-[2,4-cis-(S)-(+)-4-(halophenyl)-2-oxo-1,3,2-dioxa-phosphorinan-2-yl] methoxyethyl}adenine mesylate

[0088] The 9-{2-[2,4-cis-(S)-(+)-4-(halophenyl)-2-oxo-1, 3,2-dioxaphosphorinan-2-yl]methoxyethyl}adenine mesylate (Formula C) was prepared via an eight step convergent synthetic sequence starting with adenine and halobenzoyl

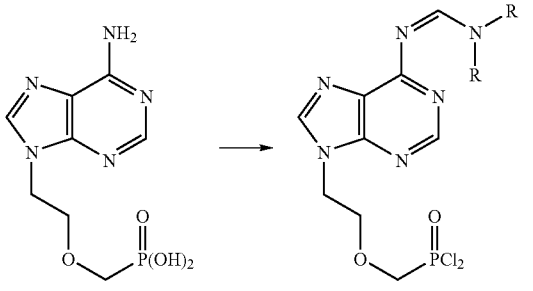
chloride. The final resultant compound (Formula C) contained two stereocenters: (1) the methine carbon (C1'); and (2) the phosphorus of the cyclic phosphonate ring. The stereochemistry at the carbon (C1') resulted from the chiral reduction of the intermediate ketoacid and the phosphorus chirality was the result of the diastereoselective coupling of the parent phosphonic acid and the chiral diol. The desired cis isomer, wherein cis refers to the isomeric relationship between the phosphorus-carbon bond and the carbon-phenyl bond of the cyclic phosphonate ring, was isolated via a selective crystallization of the methanesulfonic acid salt.

[0089] The starting materials of the chiral diol and the parent phosphonic acid were synthesized using modified procedures. The chiral diol was synthesized from 3-chlorobenzoyl chloride via a three step sequence and the parent phosphonic acid was synthesized from adenine via a four step sequence.

[0090] The final desired (cis) stereoisomer product is obtained with high purity via a novel coupling step wherein the parent phosphonic acid and the chiral diol were coupled to produce the final stereoisomer product.

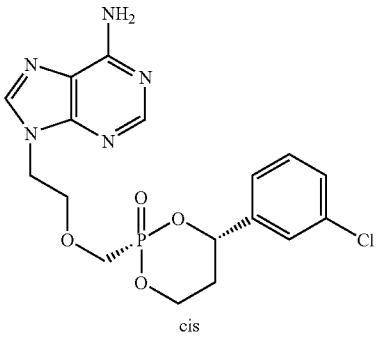
[0091] Previous coupling efforts wherein PMEA was reacted with racemic diol using dehydrating agents such as, N,N'-dicyclohexylcarbodiimide and PyBOP in DMF/pyridine solvent systems, were found to require elevated temperatures (at least 100° C.) to achieve complete coupling. These reactions proceeded with a relatively minor diastereomeric excess (5-10% of the desired cis isomer). Unexpectedly and surprisingly, improved d.e.'s were noted when the reaction temperature was lowered. This aspect of the invention led to an effort to activate PMEA as the dichloridate, a more reactive chemical species. It was the inventors' desire to react the dichloridate of PMEA with the diol at lower temperatures. The dichloridate of PMEA is readily prepared using standard chlorination conditions. The coupling reaction with the dichloridate at low temperature was complicated by the poor solubility of the dichloridate. Accordingly, the inventors sought protecting groups of N6 that would aid in the solubilization of the dichloridate. One preferred protecting group was the N-(dialkylaminomethyleneimine).

[0092] Formation of the N-(dialkylaminomethyleneimine)-protected PMEA dichloridate was achieved by treatment of PMEA in the presence of a dialkylformamide, such as, dimethylformamide, diethylformamide, dibutylformamide, N-formylpiperidine, or N-formylmorpholine, with oxalyl chloride in refluxing dichloromethane.

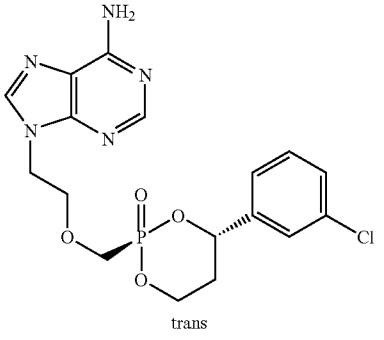



5.0 Effect of Dichloridate Addition Order and Temperature

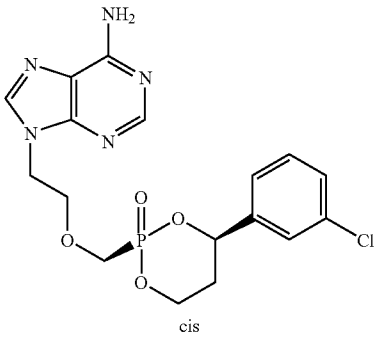
[0093] The higher order formamides (diethyl and higher) gave a more lipophilic nature to the dichloridate. This lipophilic nature was found to make the dichloridate more soluble in dichloromethane (DCM). The addition of the racemic diol to the dichloridate intermediate, in the presence of an excess of triethylamine (TEA), gave complete reaction but the reaction was found to have only a modest d.e. In a preferred embodiment, when the reagents were added in reverse order (i.e. the dichloridate was added to the diol/base mixture), an improved d.e. was obtained (cis:trans=71:29). Surprisingly the inventors found that the order of the addition and a low temperature produced a method for enriching the d.e. in favor of the cis isomer. The results are given in Table 1 (see entries 1-3).




cis




trans




cis

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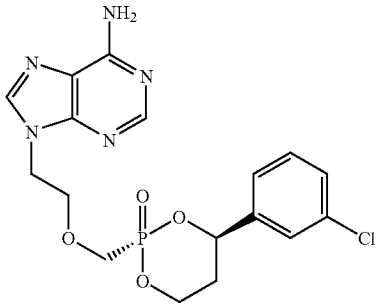

trans

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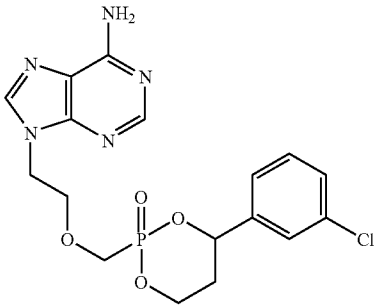


(cis:trans = 75:25)

TABLE 1

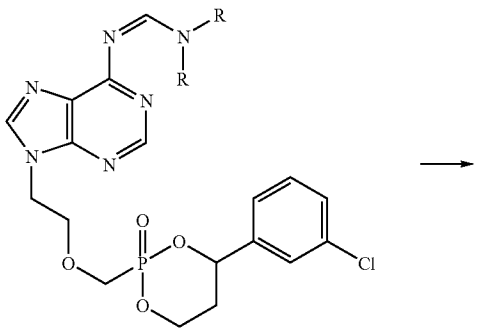
EFFECT OF TEMPERATURE AND ADDITION ORDER

| Entry | R Group | Solvent | Temp (° C.) | Addition | cis:trans |
|---|---|---|---|---|---|
| 1 | Methyl | DCM | −70 | Dichloridate to diol | 75:25 |
| 2 | Methyl | DCM | −50 | Dichloridate to diol | 71:29 |
| 3 | Methyl | DCM | 0 | Dichloridate to diol | 63:36 |
| 4 | Methyl | DCM | −50 | Diol to dichloridate | 57:42 |
| 5 | Ethyl | DCM | −50 | Base to mixture | 66:34 |

[0094] Table 1 also shows that superior cis-trans ratios are achieved by lowering the temperature of the coupling reaction. See entries (1-3).

[0095] With the unexpected advantage of dichloridate being added to the diol, it was preferable for the dichloridate to remain in solution for transferring. When R of the protecting group was methyl, the resulting dichloridate remained a slurry. Surprisingly, it was found that with an addition of a slight excess of pyridine (1.1 equivalents), the dichloridate slurry dissolved. This may be due to the neutralization of one equivalent of HCl and the resulting greater solubility of the free base dichloridate versus the dichloridate hydrochloride.

[0096] The resulting crude reaction mixture was subjected to a water/dichloromethane partition work-up and the isolated coupling mixture was treated with refluxing acetic acid in ethanol to effect nitrogen deprotection.



(cis:trans = 75:25)

5.1 cis Isomer Salt Formation and Solvents

[0097] When the coupling/deprotection sequence was performed with the chiral diol (S or R), the same d.e. was observed (50%), and it was discovered that the cis enantiomer did not crystallize from the reaction solution as did the cis racemate. Surprisingly, formation of certain salts of the 75:25 cis:trans mixture led to a crystallization of the desired cis diastereomer. A list of some salts that were used and the d.e.'s that were found of the solid and filtrates are listed in Table 2.

TABLE 2

cis ISOMER SALT AND CRYSTALLIZATION SOLVENTS

| SALT | SOLVENT | SOLID (cis:trans) | FILTRATE (cis:trans) |
|---|---|---|---|
| Free base | | 75:25 | |
| Succinic acid | Ethanol | 82:18 | 70:30 |
| L-Tartaric acid | Ethanol | 70:30 | 85:15 |
| D-Tartaric acid | Ethanol | 76:24 | 77:23 |
| Maleic acid | Ethanol | 66:31 | 88:12 |
| Methanesulfonic acid | Ethanol:acetone | 93:7 | 57:43 |
| L-Malic acid | Ethanol:acetone | 56:43 | 93:6 |
| D-Malic acid | Ethanol:acetone | 56:43 | 95:2 |

[0098] It was found that the methanesulfonic acid salt of the 75:25 cis:trans mixture gave the highest enrichment of the desired cis diastereomer (93:7). Deprotection was conducted by refluxing with a weak acid, such as acetic acid in an alcoholic solvent, e.g., ethanol. Methanesulfonic acid was then added to the reaction solution after deprotection was complete. At this stage it was found that the methanesulfonic acid selectively crystallized the desired cis diastereomer. The crude mesylate salt typically contained only 5-7% of the trans isomer, and a final recrystallization was developed to further decrease the trans levels to 1-3%. Table 3 lists some of the recrystallization solvent systems tried.

[0099] Using a sample containing 4% trans isomer dissolved in the solvents listed below, the cis isomer was enriched.

TABLE 3

FINAL RECRYSTALLIZATION SOLVENTS

| Solvent | *Volume (mL)/g | % Recovery | A % Trans |
|---|---|---|---|
| Methanol | 2.5 | 71.8 | 1.0 |
| Ethanol | 10.5 | 86.4 | 1.6 |
| Isopropanol | 48 | 88.4 | 2.2 |
| Methanol/Toluene (1/1) | 5.0 | 58.8 | 1.0 |
| Methanol/Isopropanol (1/1) | 4.0 | 85.0 | 2.0 |
| Methanol/Isopropanol (2/5) | 7.0 | 89.2 | 2.5 |
| Methanol/Ethanol (2/5) | 7.0 | 83.4 | 2.7 |
| Methanol/Acetone (1/1) | 4.0 | 73.2 | 1.2 |

*Volume used is per gram of sample.

[0100] The compounds used in this invention and their preparation can be understood further by the examples which illustrate some of the processes by which these compounds are prepared. These examples should not however be construed as specifically limiting the invention and variations of the compounds, now known or later developed, are considered to fall within the scope of the present invention as hereinafter claimed.

## EXAMPLES

### Example 1

### Preparation of 3-(3-Chlorophenyl)-3-oxo-propanoic acid (1)

[0101] A 12 L, 3-neck round bottom flask was equipped with a mechanical stirrer and addition funnel (2 L). The flask was flushed with nitrogen and charged with diisopropylamine (636 mL) and THF (1.80 L). The stirred contents were cooled to −20° C. n-Butyllithium (1.81 L of a 2.5 M solution in hexanes) was added slowly with stirring, and the temperature was maintained between −20 and −28° C. After the addition was complete (30 min), the addition funnel was rinsed with hexanes (30 mL) and the stirred solution was then cooled to −62° C. Trimethylsilyl acetate (300 g) was added slowly with stirring, maintaining the temperature at <−60° C. After the addition was complete (about 30 minutes), the solution was stirred at −60° C. for 15 minutes. 3-Chlorobenzoyl chloride (295 mL) was added slowly with stirring, maintaining the temperature at <−60° C. After the addition was complete (about 65 minutes), the cooling bath was removed and the reaction solution was stirred for approximately 1.25 hours, with gradual warming to 0° C. The reaction flask was cooled with an ice bath, then water (1.8 L) was added to the stirred solution. The reaction mixture was stirred for 10 minutes, and then diluted with t-butyl methyl ether (MTBE) (1.0 L). The lower aqueous phase was separated and transferred to a round bottom flask equipped with a mechanical stirrer. MTBE was added (1.8 L) and the stirred mixture was cooled to <10° C. in an ice bath. Concentrated HCl solution (300 mL of 12 M solution) was added and the mixture was vigorously stirred. The layers were separated and aqueous phase was further acidified with concentrated HCl (30 mL) and extracted again with MTBE (1.0 L). The combined MTBE extracts were washed with approximately 10% NaCl solution (1 L), dried ($MgSO_4$, 70 g), filtered and concentrated under reduced pressure to give 827 g of a yellow solid. The crude solid was slurried in hexanes (2.2 L) and transferred to a round bottom flask equipped with a mechanical stirrer. The mixture was stirred at <10° C. for 1 hour, then filtered, washed with hexanes (4×100 mL) and dried to constant weight (−30 in. Hg, ambient temperature, 14 hours). The $^1$H-NMR analysis for this example and all following examples were performed on a VARIAN GEMNI-200 MHz Spectrometer. The samples were dissolved in the indicated solvent and the chemical shifts are referenced to the residual solvent.

[0102] Recovery=309 g

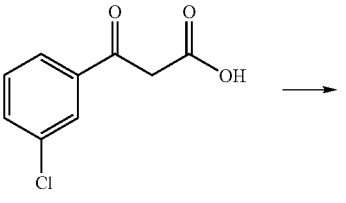
[0103] Pale yellow powder 1 (68.6%).

[0104] $^1$H-NMR (acetone-$d_6$): δ=4.1 (s, 2H), 7.5-8.1 (m, 4H)

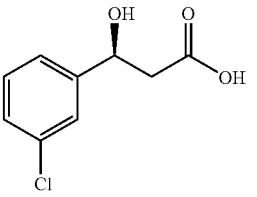
### Example 2

### Preparation of (S)-3-(3-Chlorophenyl)-3-hydroxypropanoic acid (2)

[0105]




1




2

[0106] A 12 L, 3-neck round bottom flask was equipped with a mechanical stirrer and addition funnel (1 L). The flask was flushed with nitrogen and charged with 3-(3-chlorophenyl)-3-oxo-propanoic acid (275.5 g) 1 and dichloromethane (2.2 L). A thermocouple probe was immersed in the reaction slurry and the stirred contents were cooled to −20° C. Triethylamine (211 mL) was added over 5 minutes to the stirred slurry and all solids dissolved. A dichloromethane solution of (−)-B-chlorodiisopinocampheylborane (1.60 M, 1.04 L) was charged to the addition funnel, and then added slowly with stirring while maintaining the temperature between −20 and −25° C. After the addition was complete (approximately 35 min), the solution was warmed to ice bath temperature (2-3° C.) and stirred. After approximately 4 hours of stirring an in-process NMR analysis indicated the starting material 1 was <4%.

[0107] The residual starting material 1 was measured by proton NMR as follows: removing a 0.5 mL sample of the reaction mixture and quenching with water (0.5 mL) and 3 M NaOH solution (0.5 mL). The quenched mixture was stirred and the layers separated. The aqueous phase was acidified with 2 M HCl (1 mL) and extracted with ethyl acetate (1 mL). The organic phase was separated, filtered

through a plug of $MgSO_4$ and concentrated with a stream of nitrogen. The residue was dissolved in $CH_2Cl_2$ and the solvent was evaporated with a stream of nitrogen. This residue was dissolved in acetone-$d_6$ and an analysis was done by $^1$H-NMR(acetone-$d_6$).

[0108] Water (1.2 L) was added to the cloudy orange reaction mixture, followed by 3 M NaOH solution (1.44 L). The mixture was vigorously stirred for 5 minutes and then transferred to a separatory funnel. The layers were separated and the basic aqueous phase was washed with ethyl acetate (1 L). The aqueous phase was acidified with concentrated HCl (300 mL) and extracted with ethyl acetate (2 times with 1.3 L each). The two acidic ethyl acetate extracts were combined, washed with approximately 10% NaCl solution (600 mL), dried with $MgSO_4$ (130 g), filtered and concentrated under reduced pressure to provide 328 g of a yellow oil. The oil crystallized upon standing. The resulting solid was slurried in ethyl acetate (180 mL) and transferred to a 2 L, 3-neck round bottom flask, equipped with a mechanical stirrer. The stirred ethyl acetate mixture was cooled to <10° C. (ice bath), then diluted with hexanes (800 mL). The resulting mixture was stirred at ice bath temperature for 4 h, and then filtered. The collected solid was washed with 4:1 hexanes:ethyl acetate (3×50 mL) and dried to constant weight (−30 inches of Hg, ambient temperature, 12 h).

[0109] Recovery=207.5 g

[0110] White powder 2 (74.5%)

[0111] $^1$H-NMR(acetone-$d_6$): δ=2.7 (d, J=6 Hz, 2H), 4.7 (d, J=4 Hz, 1H), 5.1-5.2 (m, 1H), 7.2-7.5 (m, 4H).

Example 3

Preparation of (S)-(−)-1-(3-Chlorophenyl)-1,3-propanediol (3)

[0112] A 12 L, 3-neck round bottom flask was equipped with a mechanical stirrer, addition funnel (2 L) and thermometer. The flask was flushed with nitrogen and charged with (S)-3-(3-chlorophenyl)-3-hydroxypropanoic acid 2 (206.7 g) and THF (850 mL), and the stirred solution was cooled to 5° C. (ice bath). A 1 M borane in THF solution (2.14 L) was charged to the addition funnel, and then added slowly with stirring maintaining the temperature at <10° C. After the addition was complete (approximately 1 hour), the cooling bath was removed and the solution was stirred at ambient temperature for 1 hour. The reaction solution was slowly and cautiously quenched with water (600 mL), followed by 3 M NaOH solution (850 mL). The mixture was stirred for 10 minutes with an observed temperature increase to approximately 40° C., and then the mixture was transferred to a separatory funnel. The layers were separated and the aqueous phase was extracted again with ethyl acetate (600 mL). The combined organic phase was washed with approximately 10% NaCl solution (500 mL), dried ($MgSO_4$, 322 g), filtered and concentrated under reduced pressure to provide 189.0 g of a pale yellow oil (101%). Preliminary analysis of the oil was by $^1$H-NMR ($CDCl_3$).

[0113] The oil was purified by vacuum distillation and the fraction at 125-155° C./0.15 mmHg was collected.

[0114] Recovery=180.9 g

[0115] Colorless oil 3 (94.0%).

[0116] $^1$H-NMR ($CDCl_3$): δ=2.9-3.1 (m, 2H), 2.5 (bs, 2H), 3.9 (t, J=5 Hz, 2H), 4.9(dd, J=7.4, 4.8 Hz, 1H), 7.2-7.4 (m, 4H).

Procedure for ee Determination

[0117] For the chiral HPLC analysis the diol 3 was derivatized to the diacetate as follows:

[0118] The resultant diol 3 (5.0 mg, 0.026 mmol) was dissolved in dichloromethane (2.0 mL). Acetic anhydride (15 μL, 0.15 mmol) and 4-(dimethylamino)pyridine (13 mg, 0.10 mmol) were added and the solution was stirred at ambient temperature for 15 minutes. The reaction solution was quenched with 1 M HCl solution (3 mL) and the lower organic phase was separated, passed through a plug of $MgSO_4$, and concentrated with a stream of nitrogen. The residue was dissolved in methanol (1 mL) and analyzed by chiral HPLC. Surprisingly, the ee for the diol 3 was determined to be >98%.

[0119] HPLC conditions:

[0120] Column: Pirkle covalent (S,S) Whelk-O 10/100 krom FEC, 250×4.6 mm; mobile phase=70:30, methanol:water, isocratic; flow rate=1.5 mL/min; injection volume=10 μL UV detection at 220 nm.

[0121] Retention times: S-diol (diacetate)=12.1 min, R-diol (diacetate)=8.6 min.

Example 4

Preparation of Diethyl p-toluenesulfonyloxymethylphosphonate (4)

[0122] A 12 L, 3-neck round bottom flask was equipped with a mechanical stirrer, condenser, thermometer and heating mantle. The flask was flushed with nitrogen and charged with diethyl phosphite (554 g), paraformaldehyde (142 g), toluene (2 L) and triethylamine (53 mL). The mixture was stirred at 85-90° C. for 2 hours, and then refluxed for 1 hour. The resulting yellow solution was cooled to 4° C. in an ice bath and p-toluenesulfonyl chloride (718 g) was added. The condenser was replaced with an addition funnel and triethylamine (750 mL) was added slowly with stirring, maintaining the temperature at <10° C. After the addition was complete (45 minutes), the resulting mixture was stirred at ambient temperature for 14 hours. The mixture was filtered and the filtercake was washed with toluene (2×250 mL). The combined filtrate and washings were washed with water (2×1 L), dried ($MgSO_4$, 200 g), filtered through diatomaceous earth (Celite 521, CAS 61790-53-2), and concentrated under reduced pressure.

[0123] Recovery=1004 g.

[0124] Cloudy yellow oil 4 (77.6%).

[0125] $^1$H-NMR ($CDCl_3$). Δ=1.3 (t, J=8H, m, 3H), 2.4(s, 3H), 4.0-4.2 (m, 4H), 7.2 (d, J=8 Hz, 2H), 7.8 (d, J=8 Hz, 2H).

Example 5

Preparation of 9-(2-Hydroxyethyl)adenine (5)

[0126] A 12 L, 3-necked round bottom flask was equipped with a mechanical stirrer, condenser, thermometer and heating mantle. The flask was flushed with nitrogen and charged

with adenine (504 g), ethylene carbonate (343 g), DMF (3.7 L) and sodium hydroxide (7.80 g). The stirred mixture was heated to reflux (approximately 80 minutes to reach reflux, pot temperature=145° C.), and then refluxed for 2 hours. The heating mantle was removed and the yellow solution was cooled to below 100° C. The resulting mixture was then cooled to 5° C. in an ice bath and diluted with toluene (3.8 L). The resulting mixture was stirred at <10° C. for 2 hours and then filtered. The collected solid was washed with toluene (2×0.5) and cold ethanol (1.5 L), then dried to constant weight (−30 in. Hg, 50° C., 14 h).

[0127] The solid 5 was analyzed by HPLC and $^1$H-NMR (DMSO-$d_6$).

[0128] HPLC conditions:

[0129] Silica column (particle size,10 microns) (Phenomenex Bondclone) 10 C18 column, 300×3.9 mm; Mobile phase: Solvent A=20 mM potassium phosphate, pH 6.2; Solvent B=acetonitrile; Gradient: 0-60% B/15 min.,60-0% B/2 min., 0% B/3 min.; UV detection at 270 nm.

[0130] Retention times: Product=6.5 min., 3-regioisomer (tentative)=5.6 min.

[0131] Recovery=624 g.

[0132] Pale yellow solid 5 (93.3%).

[0133] $^1$H-NMR (DMSO-$d_6$): δ=3.6-3.8 (m, 2H), 4.1 (t, J=6 Hz, 2H), 5.0 (bs, 1H), 7.2(bs, 2H), 8.05(s, 1H), 8.10(s, 1H).

Example 6

Preparation of
9-(2-Diethylphosphonylmethoxyethyl)adenine (6)

[0134] A 5 L, 3-neck round bottom flask was equipped with a mechanical stirrer and thermometer. The flask was flushed with nitrogen and charged with 9-(2-hydroxethyl)adenine 5 (464 g) and DMF (1.40 L). The stirred slurry was cooled to 10° C. in an ice bath and sodium tert-butoxide (436 g) was added in one portion with a corresponding increase in temperature to 29° C. The ice bath was removed and the mixture was stirred at ambient temperature for 1 hour yielding a slightly cloudy solution. The reaction flask was equipped with an addition funnel (2 L) and the stirred contents were cooled to 5° C. (ice bath). Diethyl p-toluenesulfonyloxymethylphosphonate (1130 g), as a solution in DMF (700 mL), was added slowly with stirring, maintaining the temperature at <10° C. After the addition was complete (2 hours), the cooling bath was removed and the mixture was stirred at ambient temperature for 1 hour. HPLC was used to determine completeness of the reaction. The mixture was sampled by removing 0.05 mL of the reaction mixture and dissolving the material in 10 mL of 20 mM potassium phosphate buffer, pH 6.2.

[0135] HPLC conditions:

[0136] Silica column (particle size, 10 microns) (Phenomenex Bondclone) 10 C18 column, 300×3.9 mm; Mobile phase: Solvent A=20 mM potassium phosphate (pH 6.2, Solvent B=acetonitrile; Gradient: 0-60% B/15 min., 60-0% B/2 min., 0% B/3 min.; UV detection at 270 nm; Injection volume=10 uL.

[0137] Retention times: Product 6=9.2 minutes, Starting material 5=6.5 minutes.

[0138] The stirred mixture was cooled to 10° C. and 80% acetic acid (250 mL) was slowly added. After the addition was complete (approximately 15 minutes), the mixture was stirred at ambient temperature for 30 minutes and the temperature gradually increased to 30° C. The solvent was evaporated under reduced pressure (R-152 rotary evaporator, 5 mm Hg) to provide 2115 g of an orange sludge. The material was used without purification for the next step.

Example 7

Preparation of
9-(2-Phosphonylmethoxyetnyl)adenine (7)

[0139] A 12 L, 3-neck round bottom flask was equipped with a mechanical stirrer. The flask was charged with the crude 9-(2-diethylphosphonylmethyoxyethyl)adenine 6, as a slurry in acetonitrile (4.0 L). The mixture was stirred at ambient temperature for 30 minutes and then filtered. The filter cake was washed with acetonitrile (2×0.5 L) and the combined filtrate and washings were used directly as follows.

[0140] A 22 L, 3-neck round bottom flask was equipped with a mechanical stirrer, thermometer, condenser and heating mantle. The flask was flushed with nitrogen and charged with the 9-(2-diethylphosphonylmethoxyethyl)adenine 6 solution (2.59 mol), chlorotrimethylsilane (1.315 L) and potassium iodide (1.719 kg). There was a gradual increase in temperature after the addition of KI to 35° C. The stirred mixture was then heated to 55° C. and stirred at 50-55° C. for 1 hour. The mixture was stirred for an additional 3 hours with gradual cooling to 38° C. HPLC was used to determine completeness of the reaction.

[0141] HPLC conditions:

[0142] Silica column (particle size,10 microns) (Phenomenex Bondclone) 10 C18, 300×3.9 mm column; mobile phase: Solvent A=20 mM potassium phosphate, pH 6.2, Solvent B=acetonitrile; Gradient: 0-60% B/15 min., 60-0% B/2 min., 0% B/3 min.; UV detection at 270 nm.

[0143] Retention times: Product 7=5.2 min., starting material 6=9.2 min.

[0144] The reaction flask was equipped with an addition funnel (2 L) and 3.5 M NaOH solution (4 L) was slowly added with a temperature increase from 32 to 44° C. The two liquid phase system was transferred to a 5 gal. stationary separatory funnel and the layers were separated. The basic aqueous phase was extracted with ethyl acetate (2 L) and then transferred to a 12 L, 3-neck flask, equipped with a mechanical stirrer and an addition funnel (1 L). Concentrated HCl was added slowly with stirring until the pH was 3.0 as determined by standard laboratory pH meter. The resulting yellow solution was stirred at ambient temperature for 12 hours. A precipitate formed. The stirred mixture was cooled to 7° C. in an ice bath and the pH was readjusted to 3.0 with concentrated HCl. The mixture was stirred at ice bath temperature for 5 hours and then filtered. Filtration took approximately 4 hours. The collected solid was washed with acetone and air dried on the filter funnel.

[0145] A 5 L, 3-neck round bottom flask was equipped with a mechanical stirrer and a 250 mL addition funnel. The

flask was charged with the crude solid and 1 M sodium hydroxide solution (1.25 L). The mixture was stirred until all solids were dissolved (15 minutes). Concentrated HCl solution was added slowly to the stirred solution until the pH was 3.0. The resulting mixture was stirred at ambient temperature for 4 hours and then filtered. The collected solid was washed with water (2×250 mL) and acetone (200 mL), and dried to constant weight (−30 in. Hg, 60° C., 14 hours).

[0146] Recovery=292 g

[0147] Off-white solid (41.3%).

[0148] $^1$H-NMR ($D_2O$); δ=3.25 (d, J=8 Hz, 2H), 3.70 (t, J=4 Hz, 2H), 4.10 (t, J=4 Hz, 2H), 4.60 (s, 4H), 7.80 (s, 1H), 7.90 (s, 1H).

Example 8

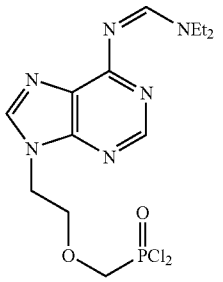
Preparation 9-{2-[2,4- cis(S)-(+)-4-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl] methoxyethyl}adenine methanesulfonate (9)

Example 8.1

Formation of Dichloridate (8)

[0149]

8



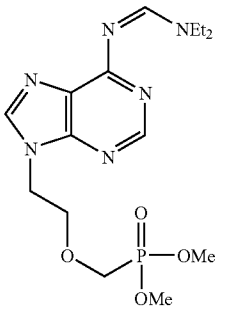
[0150] A 2 L, 3-neck round bottom flask was equipped with a mechanical stirrer, condenser, addition funnel (125 mL) and heating mantle. The flask was flushed with nitrogen and charged with PMEA 7 (50.0 g), dichloromethane (650 mL) and N,N-diethylformamide (22.5 mL). Oxalyl chloride (58.0 mL) was charged to the addition funnel, and added slowly to the stirred reaction mixture. Vigorous gas evolution occurred and the nitrogen inlet was removed to facilitate the gas to escape. After the addition was complete (15 minutes), the addition funnel was removed and the vigorously stirred mixture was heated at reflux for 2 hours. The solution remained a slurry during this process. The reaction mixture was cooled slightly, and additional oxalyl chloride (1.0 mL) and N,N-diethylformamide (0.4 ml) were added. The addition of N,N-diethylformamide produced vigorous gas evolution. The resulting mixture was heated at reflux until all solids were dissolved (additional 2.5 hours, total reaction time was approximately 4.5 hours). HPLC analysis of the reaction solution indicated the product 8 at 83 Area %. The reaction was monitored for formation of the dichloridate. A sample of the reaction mixture (approximately 50 μL) was removed and quenched in anhydrous methanol (1 mL) containing 1 drop of triethylamine. The resulting methyl phosphonate(s) were analyzed by HPLC.

[0151] HPLC conditions:

[0152] YMC-Pack R & D, R-33-5 S-5 120A, 250×4.6 mm; mobile phase: Solvent A=20 mM potassium phosphate, pH 6.2; Solvent B=acetonitrile; gradient: 10-60% B/15 min., 60-10% B/2 min., 10% B/3 min.; 1.4 mL/min.; inj. vol.=10 μL; UV detection at 270 nm.

[0153] Retention times: Dimethylphosphonate 11=8.5 min., monomethyl phosphonate 12=5.8 min.

11



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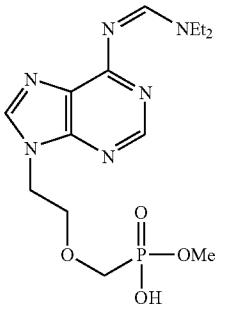

[0154] The reaction solution was cooled slightly and the condenser was replaced with a distillation head with thermometer, condenser and collection flask (250 mL). The reaction solution was heated to reflux and 250 mL of distillate was collected. The pot solution was diluted with dichloromethane (250 mL) and an additional 250 mL of distillate was collected. The distillation head was removed and the reaction flask was placed under nitrogen. The solution was diluted with dichloromethane (100 mL) and cooled to ice bath temperature. HPLC analysis of the reaction solution indicated the product at 89 Area %.

[0155] HPLC conditions:

[0156] YMC-Pack R & D, R-33-5 S-5 120A, 250×4.6 mm; mobile phase: Solvent A=20 mM potassium phosphate, pH 6.2; Solvent B=acetonitrile; gradient: 10-60% B/15 min., 60-10% B/2 min., 10% B/3 min.; 1.4 mL/min.; inj. Vol=10 μL; UV detection at 270 nm.

[0157] Retention times: Product 8=8.5 min., starting material 7=5.9 min

[0158] Pyridine (18 mL) was added slowly to the stirred solution. After the addition was complete (5 minutes), the resulting pale orange solution was stored at ice bath temperature until used (30 minutes).

Example 8.2

Coupling Reaction

[0159] A 2 L, 3-neck round bottom flask was equipped with a mechanical stirrer, and addition funnel (1 L). The flask was flushed with nitrogen and charged with (S)-(−)-(3-chlorophenyl)-1,3-propanediol 3 (34.1 g), as a solution in dichloromethane (500 mL) and triethylamine (125 ml). A thermocouple probe was immersed in the reaction solution and the stirred contents were cooled to −71° C. (dry ice/isopropanol). The dichloridate solution 8 was charged to the addition funnel, then added slowly with stirring, maintaining the temperature <−67° C. After the addition was complete (1.25 h), the cooling bath was removed and the stirred mixture was warmed to 0° C. over 30 min. The reaction mixture was washed with water (550 mL) and the layers were separated. The dichloromethane phase was diluted with ethyl acetate (500 mL) and washed with 5% NaCl solution (600 mL). The organic phase was dried ($MgSO_4$, 50 g), filtered through diatomaceous earth (Celite 521), and concentrated under reduced pressure to provide 108 g of a dark red sludge. The sample was dissolved in methanol.

[0160] HPLC conditions:

[0161] YMC-Pack R & D, R-33-5 S-5 120A, 250×4.6 mm; mobile phase: Solvent A=20 mM potassium phosphate, pH 6.2; Solvent B=acetonitrile; gradient: 10-60% B/15 min., 60-10% B/2 min., 10% B/3 min.; 1.4 mL/min.; inj. vol.=10 μL; UV detection at 270 nm.

[0162] Retention times: cis 13=12.5 min., trans 14=13.0 min.

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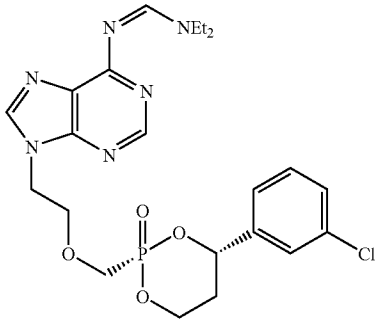

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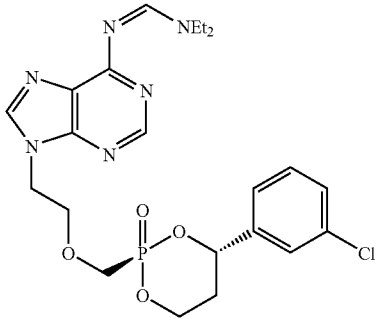


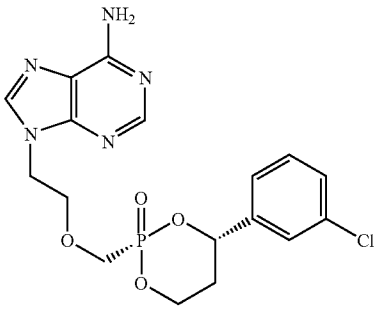
[0163] The material was dissolved in ethanol (500 mL) and transferred to a 2 L round bottom flask equipped with magnetic stirring, condenser and heating mantle. Acetic acid (55 mL) was added and the red solution was heated at reflux for 8 hours. HPLC indicated the reaction was complete. The sample was dissolved in methanol.

[0164] HPLC conditions:

[0165] YMC-Pack R & D, R-33-5 S-5 120A, 250×4.6 mm; mobile phase: Solvent A=20 mM potassium phosphate, pH 6.2; Solvent B=acetonitrile; gradient: 10-60% B/15 min., 60-10% B/2 min., 10% B/3 min.; 1.4 mL/min.; inj. vol.=10 μL; UV detection at 270 nm. 6.

[0166] Retention times: cis 15=9.5 min., trans 16=9.8 min.

15



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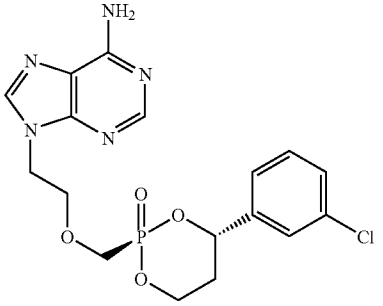


Example 8.3

Crystallization of 9-{2-[2,4-cis(S)-(+)-4-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl] methoxyethyl}adenine methanesulfonate (9)

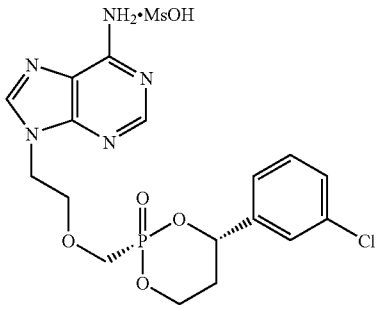
[0167] Methanesulfonic acid (21.5 mL) was added and a precipitate formed after 15 min. The mixture was diluted with ethanol (400 mL) and heated until all solids dissolved (pot temperature=70° C.). The solution was cooled with stirring and a precipitate formed at 46° C. The resulting mixture was stirred for 2 h, with cooling to ambient temperature, then at ice bath temperature for 2.5 h. The mixture was filtered and the collected solid was washed with ethanol (2×15 mL) and dried to constant weight (−30 in. Hg, 55° C., 14 h). Recovery=49.4 g of a white powder 9 (51.9%). The solid contained 6.5 Area % of the trans diastereomer.

[0168] Chiral HPLC: Pirkle covalent (S,S) Whelk-O 1 10/100 krom FEC 250×4.6 mm; mobile phase=55:45,

methanol: 0.1% HOAc in water; isocratic; 1.0 mL/min.; inj. Vol.=10 μL; UV detection at 260 nm; sample preparation= 2.0 mg/mL in water. Retention times: cis-(R) 5=24.6 min., trans-(R) 6=27.5 min., cis-(S) 7=18.0 min.

[0169] $^1$H-NMR ($D_2O$) was used to confirm structure of components.

9




Example 8.4

Recrystallization of 9-{2-[2,4-cis-(S)-(+)-4-(3-Chlorophenyl)-2-oxo-1,3,2-dioxaphosphorinan-2-yl] methoxyethyl}adenine methanesulfonate (9)

[0170] A 3 L, 3-neck round bottom flask was equipped with a mechanical stirrer, condenser, heating mantle and thermometer. The flask was charged with 2 batches of crude mesylate salt 9 and ethanol (1.4 L). The stirred mixture was heated at reflux (pot temperature was 78° C.) until all solids dissolved (approximately 10 minutes). The stirred mixture was gradually cooled to ambient temperature over 1.5 hours (a precipitate formed at 56° C). The mixture was stirred at ambient temperature for an additional 2 hrs., then filtered. The collected solid was washed with ethanol (2×15 mL) and dried to constant weight (−30 in Hg, 65° C., 60 hrs.).

[0171] Color: off white granular solid

[0172] Purity=97% (HPLC)

[0173] Optical purity (Chiral HPLC) >99.5%.

[0174] M.P.(° C.): 186.5-188

[0175] Specific Rotation (MeOH, 25° C., 589 nm): +16.429

[0176] Composition: C, 41.58; H, 4.56; N, 13.37 [Theoretical: C, 41.50; H, 4.53; N, 13.35]

[0177] $^1$H NMR ($D_2O$): δ=1.30-1.60 (m, 1H), 1.80-1.95 (m, 1H), 2.60 (s, 3H), 3.70-3.90 (m, 4H),4.10-4.50 (m, 2H), 4.60 (s, 3H), 5.15-5.40 (m, 1H), 6.70-6.80 (m, 2H), 7.00-7.10 (m, 2H), 8.00 (s, 1H), 8.10 (s, 1H).

Example 9

Formation of Hydrochloride Salt via Salt Exchange

[0178] In this instant invention the oxalate salt of the phosphonic acid based prodrugs was also formed. This salt form of the prodrug could be exchanged for other salts that are pharmaceutically acceptable. The oxalate salt is dissolved in a solution containing an acid with a higher $pK_a$, the acid dissociation constant.

[0179] A 3-neck round bottom flask is equipped with a mechanical stirrer, condenser, heating mantle and thermometer. The flask is charged with crude oxalate salt and ethanol (5-10% solution by weight). The stirred mixture is heated at reflux (pot temperature is 78° C.) until all solids dissolve. The solution is acidified with HCl and the stirred mixture is gradually cooled to ambient temperature (a precipitate forms as the temperature cools). The mixture is stirred at ambient temperature with filtration following. The collected solid consisting of the hydrochloride salt is washed with ethanol and is dried in a vacuum oven to constant weight (oven temperature=65° C.).

Example 10

Formation of the Sulfate Salt via Salt Exchange

[0180] A 3-neck round bottom flask is equipped with a mechanical stirrer, condenser, heating mantle and thermometer. The flask is charged with crude mesylate salt 9 and ethanol (5-10% solution by weight). The stirred mixture is heated at reflux (pot temperature is 78° C.) until all solids dissolve. The solution is acidified with sulfuric acid and the stirred mixture is gradually cooled to ambient temperature (a precipitate forms as the temperature decreases). The mixture is stirred at ambient temperature and filtration of desired product follows. The collected solid consisting of the sulfate salt is washed with ethanol and is dried in a vacuum oven to constant weight (oven temperature=65° C.).

Example 11

Formation of the Sulfate Salt via Free Base Reaction

[0181] A 3-neck round bottom flask is equipped with a mechanical stirrer, condenser, heating mantle and thermometer. The flask is charged with crude mesylate salt 9 and $NaHCO_3$ solution. The stirred mixture is heated until all solids dissolve. The solution is acidified with sulfuric acid and the stirred mixture is gradually cooled to ambient temperature (a precipitate forms as the temperature decreases). The mixture is stirred at ambient temperature followed by filtration. The collected solid consisting of the sulfate salt is washed with ethanol and is dried in a vacuum oven to constant weight (oven temperature=65° C.).

Example 12

Formation of the Maleate Salt via Anionic Resin Reaction

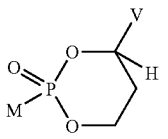
[0182] A 3-neck round bottom flask is equipped with a mechanical stirrer, condenser, heating mantle and thermometer. The flask is charged with crude mesylate salt 9. The stirred mixture is heated until all solids dissolve. The mixture containing the mesylate salt 9 is run through an anionic resin. The resultant solution containing the free base of the compound of Formula 1 is acidified with maleic acid and the stirred mixture is gradually cooled to ambient temperature (a precipitate forms as the temperature decreases). The mixture is stirred at ambient temperature followed by filtration. The

collected solid consisting of the maleate salt is washed with ethanol and is dried in a vacuum oven to constant weight (oven temperature=65° C.).

We claim:

**1**. A method for the preparation of compounds of Formula I:

Formula I



wherein:

M and V are cis to one another and $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine and (R)-9-(2-phosphonylmethoxypropyl)adenine; wherein V is phenyl, optionally substituted with 1-2 substituents selected from a group consisting of fluoro, chloro, and bromo; comprising:

(a) coupling a chiral 1-arylpropane-1,3-diol, wherein the aryl is a phenyl optionally substituted with 1-2 substituents selected from the group consisting of fluoro, chloro, and bromo, with $MPOCl_2$ or an N-6 substituted analogue thereof.

**2**. The method of claim 1 wherein the cis isomer has a diastereomeric excess of at least 50%.

**3**. The method of claim 2 further comprising adding acid to form an acid addition salt of compounds of Formula I.

**4**. The method of claim 3 wherein said acid is selected from the group consisting of HCl, HBr, acetic acid, citric acid, maleic acid, methanesulfonic acid, nitric acid, phosphoric acid, succinic acid, sulfuric acid, and tartaric acid.

**5**. The method of claim 3 wherein said acid is selected from a group consisting of methanesulfonic acid, succinic acid, citric acid, and oxalic acid.

**6**. The method of claim 5 wherein said acid is methanesulfonic acid.

**7**. The method of claim 3 further comprising crystallizing said acid addition salt.

**8**. The method of claim 7 wherein the solvent for crystallizing said acid addition salt is selected from a group consisting of methanol, ethanol, isopropanol, acetone, toluene, and mixtures thereof.

**9**. The method of claim 3 further comprising:

(a) reacting a first acid addition salt compound of Formula I with a second acid that has a higher acid dissociation constant than the first acid, and

(b) crystallizing the desired second acid addition salt compound of Formula I.

**10**. The method of claim 3 further comprising:

(a) neutralizing a first acid addition salt compound of Formula I,

(b) obtaining the free base of the compound of Formula I,

(c) adding a pharmaceutically acceptable acid, and

(d) crystallizing the desired second acid addition salt compound of Formula I.

**11**. The method of claim 3 further comprising:

(a) utilizing an anionic resin to obtain free base of a first acid addition salt compound of Formula I,

(b) adding a pharmaceutically acceptable acid, and

(c) crystallizing the desired second acid addition salt compound of Formula I.

**12**. The method of claim 1 wherein the reaction solution for the coupling step is at or below −50° C.

**13**. The method in claim 12 wherein said reaction solution is at or below −70° C.

**14**. The method as recited in claim 1 wherein said $MPOCl_2$ is added to said chiral 1-phenylpropane-1,3-diol.

**15**. The method of claim 1 wherein said $MPOCl_2$ is added to said chiral 1-phenylpropane-1,3-diol at a reaction solution temperature at or below −50° C.

**16**. The method of claim 1 further comprising addition of a base to the reaction solution.

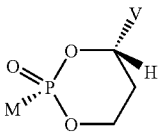
**17**. The method of claim 1 wherein the $MPOCl_2$ has an N-6 substituent to form a dialkylaminomethyleneimine.

**18**. The method of claim 17 wherein said N-6 substituent is produced as part of the reaction to form the dichloridate.

**19**. The method of claim 17 wherein said dialkylaminomethyleneimine is selected from the group consisting of dimethylaminomethyleneimine, diethylaminomethylene, dipropylaminomethyleneimine, dibutylaminomethyleneimine, N-piperidinomethyleneimine, and N-morpholinomethyleneimine.

**20**. The method of claim 1 wherein the compounds of Formula I have the stereochemistry of Formula II:

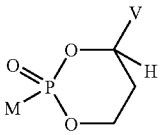
Formula II



**21**. The method of claim 20 wherein the temperature for the coupling step is at or below −50° C.

**22**. A method for the preparation of compounds of Formula I:

Formula I



wherein:

M and V are cis to one another and $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine and (R)-9-(2-phosphonylmethoxypropyl)adenine;

wherein V is 3-chlorophenyl; comprising:

(a) coupling a chiral 1-(3-chlorophenyl)propane-1,3-diol with $MPOCl_2$ or an N-6 substituted analogue thereof.

**23**. The method of claim 22 wherein the cis isomer has a diastereomeric excess of at least 50%.

**24**. The method of claim 23 further comprising adding acid to form an acid addition salt of Formula I.

**25**. The method of claim 24 wherein said acid is selected from the group consisting of methanesulfonic acid, succinic acid, citric acid, and oxalic acid.

**26**. The method of claim 25 wherein said acid is methanesulfonic acid.

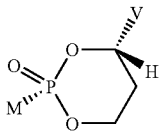
**27**. The method of claim 22 wherein the reaction solution for the coupling step is at or below −50° C.

**28**. The method of claim 27 wherein said reaction solution is at or below −70° C.

**29**. The method of claim 22 wherein said $MPOCl_2$ is added to said chiral 1-(3-chlorophenyl)propane-1,3-diol.

**30**. The method of claim 22 wherein the compounds of Formula I have the stereochemistry of Formula II:

Formula II



**31**. The method of claim 30 wherein the temperature for the coupling step is at or below −50° C.

**32**. The method of claim 30 further comprising adding acid to form an acid addition salt of Formula II.

**33**. The method of claim 32 wherein said acid is selected from the group consisting of methanesulfonic acid, succinic acid, citric acid, and oxalic acid.

**34**. The method of claim 33 wherein said acid is methanesulfonic acid.

**35**. The method of claim 24 further comprising crystallizing said acid addition salt from a solvent selected from the group consisting of methanol, ethanol, isopropanol, toluene, acetone, and mixtures thereof.

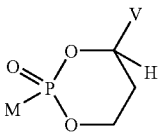
**36**. The method as recited in claim 22 wherein the $MPOCl_2$ has an N-6 substituent to form a dialkylaminomethyleneimine.

**37**. The method as recited in claim 36 wherein the N-6 substituent is produced as part of the reaction to form the dichloridate.

**38**. The method as recited in claim 36 wherein the dialkylaminomethyleneimine is selected from a group consisting of dimethylaminomethyleneimine, diethylaminomethylene, dipropylaminomethyleneimine, dibutylaminomethyleneimine, N-piperidinomethyleneimine, and N-morpholinomethyleneimine.

**39**. A method for the preparation of compounds of Formula I:

Formula I



wherein:

M and V are cis to one another and $MPO_3H_2$ is phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine, and (R)-9-(2-phosphonylmethoxypropyl)adenine;

wherein V is 2-bromophenyl; comprising:

(a) coupling a chiral 1-(2-bromophenyl)propane-1,3-diol with $MPOCl_2$ or an N-6 substituted analogue thereof.

**40**. The method of claim 39 wherein the cis isomer has a diastereomeric excess of at least 50%.

**41**. The method of claim 40 further comprising adding acid to form an acid addition salt of Formula I.

**42**. The method of claim 41 wherein said acid is selected from the group consisting of methanesulfonic acid, succinic acid, citric acid, and oxalic acid.

**43**. The method of claim 42 wherein said acid is methanesulfonic acid.

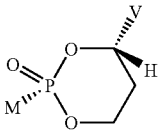
**44**. The method of claim 39 wherein the reaction solution for the coupling step is at or below −50° C.

**45**. The method of claim 44 wherein said reaction solution is at or below −70° C.

**46**. The method of claim 39 wherein said $MPOCl_2$ is added to said chiral 1-(2-bromophenyl)propane-1,3-diol.

**47**. The method of claim 39 wherein the compounds of Formula I have the stereochemistry of Formula II:

Formula II



**48**. The method of claim 40 wherein the temperature for the coupling step is at or below −50° C.

**49**. The method of claim 47 further comprising adding acid to form an acid addition salt of Formula II.

**50**. The method of claim 49 wherein said acid is selected from the group consisting of methanesulfonic acid, succinic acid, citric acid, and oxalic acid.

**51**. The method of claim 50 wherein said acid is methanesulfonic acid.

**52**. The method of claim 41 further comprising crystallization of said acid addition salt from a solvent selected from the group consisting of methanol, ethanol, isopropanol, acetone, toluene, and mixtures thereof.

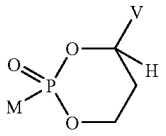
**53**. The method of claim 39 wherein the $MPOCl_2$ has an N-6 substituent to form a dialkylaminomethyleneimine.

**54**. The method of claim 53 wherein the N-6 substituent is produced as part of the reaction to form the dichloridate.

**55**. The method of claim 53 wherein the dialkylaminomethyleneimine is selected from the group consisting of dimethylaminomethyleneimine, diethylaminomethylene, dipropylaminomethyleneimine, dibutylaminomethyleneimine, N-piperidinomethyleneimine, and N-morpholinomethyleneimine.

**56**. A method for the conversion of acid addition salt compounds of Formula I:

Formula I




wherein:

M and V are cis to one another and $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine, and (R)-9-(2-phosphonylmethoxypropyl)adenine; wherein V is phenyl, optionally substituted with 1-2 substituents selected from a group consisting of fluoro, chloro, and bromo; comprising:

(a) reacting said first acid addition salt compound of Formula I with a second acid that has a higher acid dissociation constant than the first acid, and

(b) crystallizing desired second acid salt compound.

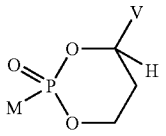
**57**. The method of claim 56 wherein said second acid is selected from the group consisting of HCl, HBr, acetic acid, citric acid, maleic acid, methanesulfonic acid, nitric acid, phosphoric acid, succinic acid, sulfuric acid, and tartaric acid.

**58**. The method of claim 57 wherein said second acid is selected from the group consisting of methanesulfonic acid, succinic acid, citric acid, and oxalic acid.

**59**. The method of claim 58 wherein said second acid is methanesulfonic acid.

**60**. A method for the conversion of first acid addition salt compounds of Formula I:

Formula I




wherein:

M and V are cis to one another and $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine, and (R)-9-(2-phosphonylmethoxypropyl)adenine; wherein V is phenyl, optionally substituted with 1-2 substituents selected from a group consisting of fluoro, chloro, and bromo; comprising:

(a) neutralizing said first acid addition salt compound of Formula I,

(b) obtaining the free base of the compound of Formula I,

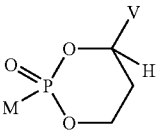
(c) adding pharmaceutically acceptable acid, and

(d) crystallizing desired second acid salt compound.

**61**. The method of claim 60 wherein the acid is selected from the group consisting of said pharmaceutically acceptable HCl, HBr, acetic acid, citric acid, maleic acid, methane sulfonic acid, nitric acid, phosphoric acid, succinic acid, sulfuric acid, and tartaric acid.

**62**. A method for the conversion of a first acid addition salt compounds of Formula I:

Formula I




wherein:

M and V are cis to one another and $MPO_3H_2$ is a phosphonic acid selected from the group consisting of 9-(2-phosphonylmethoxyethyl)adenine, and (R)-9-(2-phosphonylmethoxypropyl)adenine; wherein V is phenyl, optionally substituted with 1-2 substituents selected from a group consisting of fluoro, chloro, and bromo; comprising:

(a) utilizing an anionic resin to obtain the free base of the compound of Formula I,

(b) adding a pharmaceutically acceptable acid, and

(c) crystallizing the desired second acid addition salt compound of Formula I.

**63**. The method of claim 62 wherein said pharmaceutically acceptable acid is selected from the consisting of HCl, HBr, acetic acid, citric acid, maleic acid, methanesulfonic acid, nitric acid, phosphoric acid, succinic acid, sulfuric acid, and tartaric acid.

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