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(71) Applicant *(for all designated States except US)*: **LUPIN LIMITED** [IN/IN]; 159 CST Road, Kalina, Santacruz (East), State of Maharashtra, 400 098 Mumbai (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ROY, Bhairab, Nath** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Maharashtra, 411 042 Pune (IN). **SINGH, Girij, Pal** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Maharashtra, 411 042 Pune (IN). **SHRIVASTAVA, Dhananjai** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Maharashtra, 411 042 Pune (IN). **JADHAV, Harishchandra, Sambhaji** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Maharashtra, 411 042 Pune (IN). **AHER, Umesh, Parashram** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Maharashtra, 411 042 Pune (IN). **DEOKAR, Sharad, Chandrabhan** [IN/IN]; Lupin Limited (Research Park), 46A/47A, Village Nande, Taluka Mulshi, Maharashtra, 411 042 Pune (IN).

(74) Agents: **MAJUMDAR, Subhatosh** et al.; S. Majumdar & Co., 5, Harish Mukherjee Road, 700 025 Kolkata (IN).

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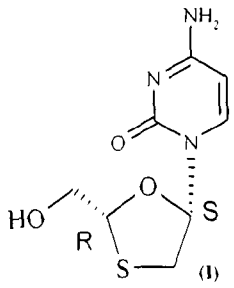
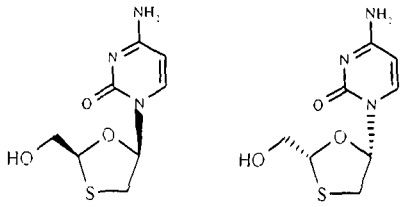
[Continued on next page]

(54) Title: AN IMPROVED PROCESS FOR THE MANUFACTURE OF LAMIVUDINE

(57) Abstract: The present invention relates to an improved process for the Manufacture of Lamivudine. A process for the preparation of essentially enantiomerically pure (-)-[2R, 5S]-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one of formula (I), from L-menthyl glyoxylate is described. Also provided is a process for preparation of (+)-1- (2R/S-Cis)-4-amino-1-[(2-hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one of formula (XII), from L-menthyl glyoxylate.



(I)



XII

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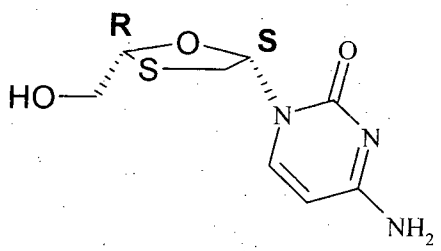
AN IMPROVED PROCESS FOR THE MANUFACTURE OF LAMIVUDINE

Field of invention

The present invention relates to an improved process for the Manufacture of Lamivudine.

Background of the invention

Lamivudine (I) (CAS No. 134678-17-4) is chemically known as (−)-[2R,5S]-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one.



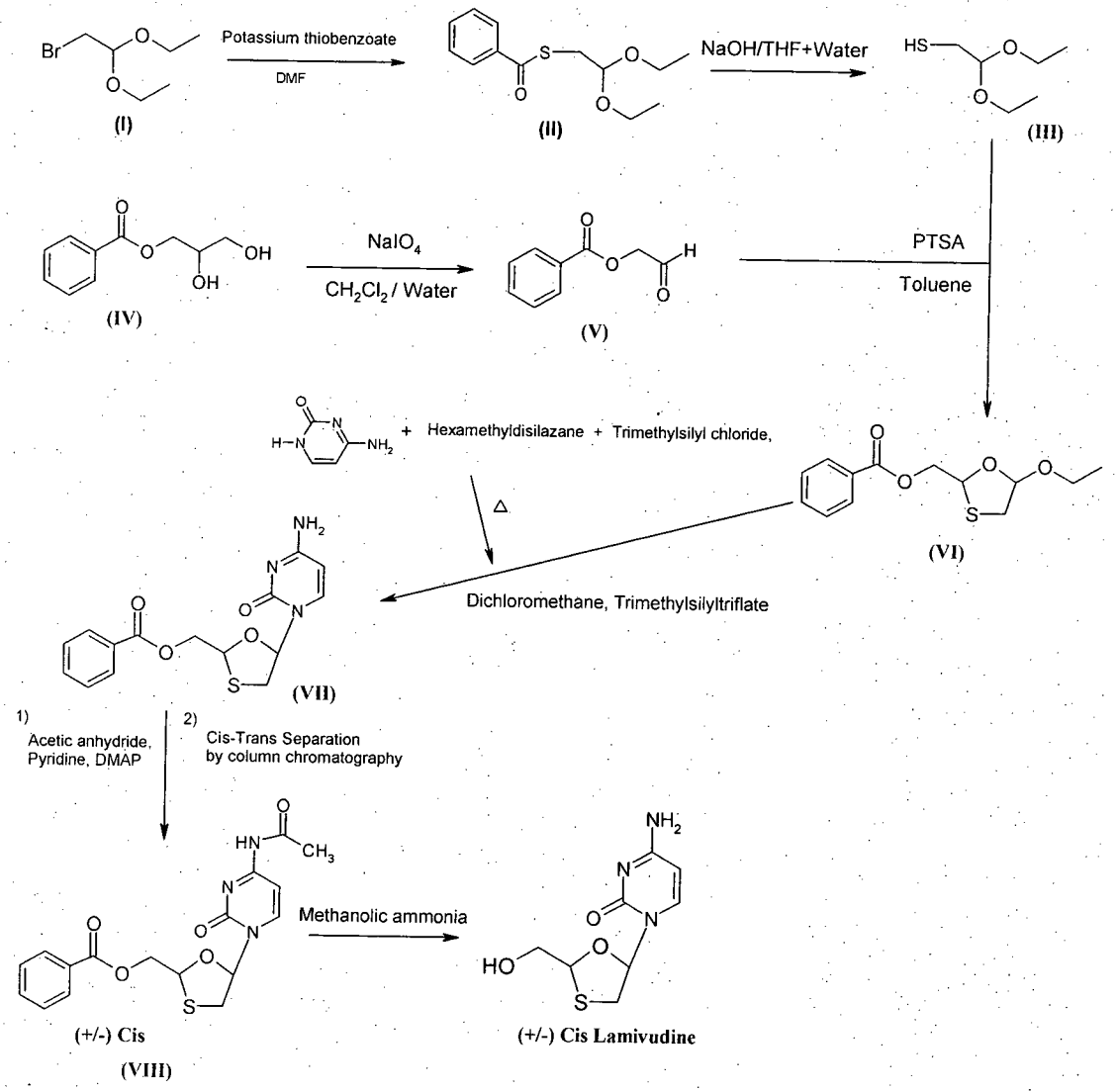
Formula (I)

Lamivudine is a reverse transcriptase inhibitor used alone or in combination with other classes of Anti-HIV drugs in the treatment of HIV infection. It is available commercially as a pharmaceutical composition under the brand name EPIVIR®, marketed by GlaxoSmithKline, and is covered under **US 5,047,407**.

This molecule has two stereo-centres, thus giving rise to four stereoisomers: (±)-Cis Lamivudine and (±)-Trans Lamivudine. The pharmaceutically active isomer however is the (−)-Cis isomer which has the absolute configuration [2R,5S] as show in Formula (I).

US 5,047,407 discloses the 1,3-oxathiolane derivatives; their geometric (cis/trans) and optical isomers. This patent describes the preparation of Lamivudine as a mixture of cis and trans isomers (shown in scheme I). The diastereomers obtained are converted into N-acetyl derivatives before separation by column chromatography using ethylacetate and methanol (99:1); however, this patent remains silent about further resolution of the cis isomer to the

desired (−)-[2R,5S]-Cis-Lamivudine. Secondly, as the ethoxy group is a poor leaving group, the condensation of cytosine with compound VI gives a poor yield, i.e. 30 – 40%, of compound VII. Thirdly, chromatographic separation that has been achieved only after acetylation requires a further step of de-acetylation of the cis-(±)-isomer. Also, separation of large volumes of a compound by column chromatography makes the process undesirable on a commercial scale.

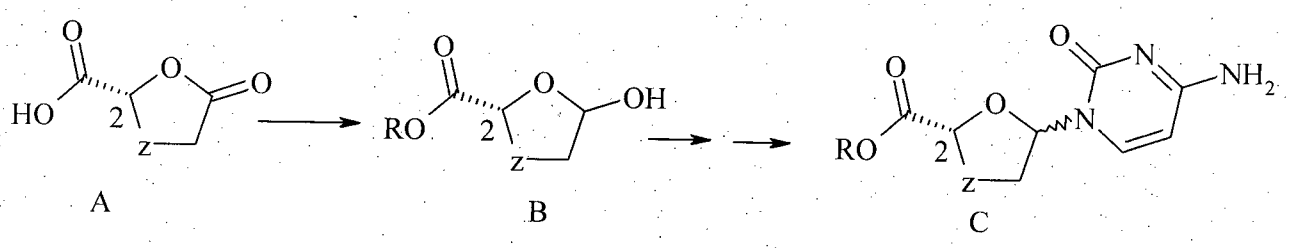


Scheme - I

Efforts have been made in the past to overcome the shortcomings of low yield and enantiomeric enrichment. In general, there have been two approaches to synthesize (–)-[2R,5S]-Cis-Lamivudine. One approach involves stereoselective synthesis, some examples of which are discussed below.

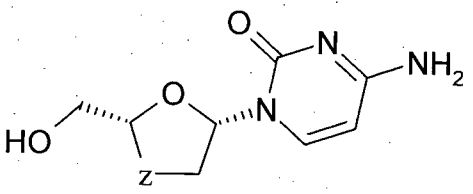
US **5,248,776** describes an asymmetric process for the synthesis of enantiomerically pure β-L-(–)-1,3-oxathiolone-nucleosides starting from optically pure 1,6-thioanhydro-L-gulose, which in turn can be easily prepared from L-Gulose. The condensation of the 1,3-oxathiolane derivative with the heterocyclic base is carried out in the presence of a Lewis acid, most preferably $SnCl_4$ to give the [2R,5R] and [2R,5S] diastereomers that are then separated chromatographically.

US 5,756,706 relates a process where compound A is esterified and reduced to compound B. The hydroxy group is then converted to a leaving group (like acetyl) and the cis- and trans-2R-tetrahydrofuran derivatives are treated with a pyrimidine base, like N-acetylcytosine, in the presence trimethylsilyl triflate to give compound C in the diastereomeric ratio 4:1 of cis and trans isomers.



Z = S, CH

Dissolving compound C in a mixture of 3:7 ethyl acetate-hexane separates the cis isomer. The product containing predominantly the cis-2R,5S isomer and some trans-2R,5R compound is reduced with $NaBH_4$ and subjected to column chromatography (30% MeOH-EtOAc) to yield the below compound.

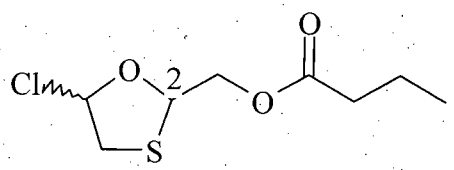


D

I (z = S)

US 6,175,008 describes the preparation of Lamivudine by reacting mercaptoacetaldehyde dimer with glyoxylate and further with silylated pyrimidine base to give mainly the cis-isomer by using an appropriate Lewis acid, like TMS-I, TMS-Tf, $TiCl_4$ *et cetera*. However the stereoselectivity is not absolute and although the cis isomer is obtained in excess, this process still requires its separation from the trans isomer. The separation of the diastereomers is done by acetylation and chromatographic separation followed by deacetylation. Further separation of the enantiomers of the cis-isomer is not mentioned.

US 6,939,965 discloses the glycosylation of 5-fluoro-cytosine with compound E (configuration: 2R and 2S)



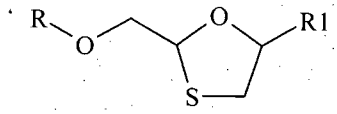
(E)

The glycosylation is carried out in the presence of $TiCl_3(OiPr)$ which is stereoselective and the cis-2R,5S-isomer is obtained in excess over the trans-2S,5S-isomer. These diastereomers are then separated by fractional crystallization.

US 6,600,044 relates a method for converting the undesired trans-1,3-oxathiolane nucleoside to the desired cis isomer by a method of anomerization or transglycosylation and the

separation of the hydroxy-protected form of cis-, trans-(−)-nucleosides by fractional crystallization of their hydrochloride, hydrobromide, methanesulfonate salts. However, these cis-trans isomers already bear the [R] configuration at C2 and only differ in their configuration at C5; i.e. the isomers are [2R,5R] and [2R,5S]. Hence diastereomeric separation directly yields the desired [2R, 5S] enantiomer of Lamivudine.

In the second approach to prepare enantiomerically pure Lamivudine the resolution of racemic mixtures of nucleosides is carried out. **US 5,728,575** provides one such method by using enzyme-mediated enantioselective hydrolysis of esters of the formula



(F)

wherein, 'R' is an acyl group and 'R1' represents the purine or pyrimidine base. 'R' may be alkyl carboxylic, substituted alkyl carboxylic and preferably an acyl group that is significantly electron-withdrawing, eg. α-haloesters. After selective hydrolysis, the process involves further separation of the unhydrolyzed ester from the enantiomerically pure 1,3-oxathiolane-nucleoside. Three methods are suggested in this patent, which are:

1. Separation of the more lipophilic unhydrolyzed ester by solvent extraction with one of a wide variety of nonpolar organic solvents.
2. Lyophilization followed by extraction into MeOH or EtOH.
3. Using an HPLC column designed for chiral separations.

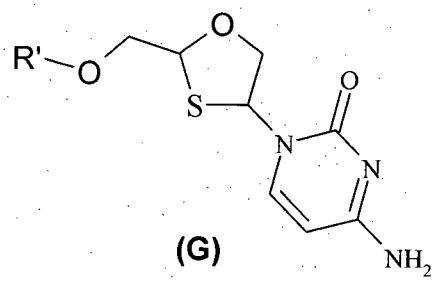
In another of its aspects, this patent also refers to the use of the enzyme cytidine-deoxycytidine deaminase, which is enantiomer-specific, to catalyze the deamination of the cytosine moiety and thereby converting it to uridine. Thus, the enantiomer that remains unreacted is still basic and can be extracted by using an acidic solution.

However, the above methods suffer from the following drawbacks:

(a) Enzymatic hydrolysis sets down limitations on choice of solvents: alcohol solvents cannot be used as they denature enzymes.

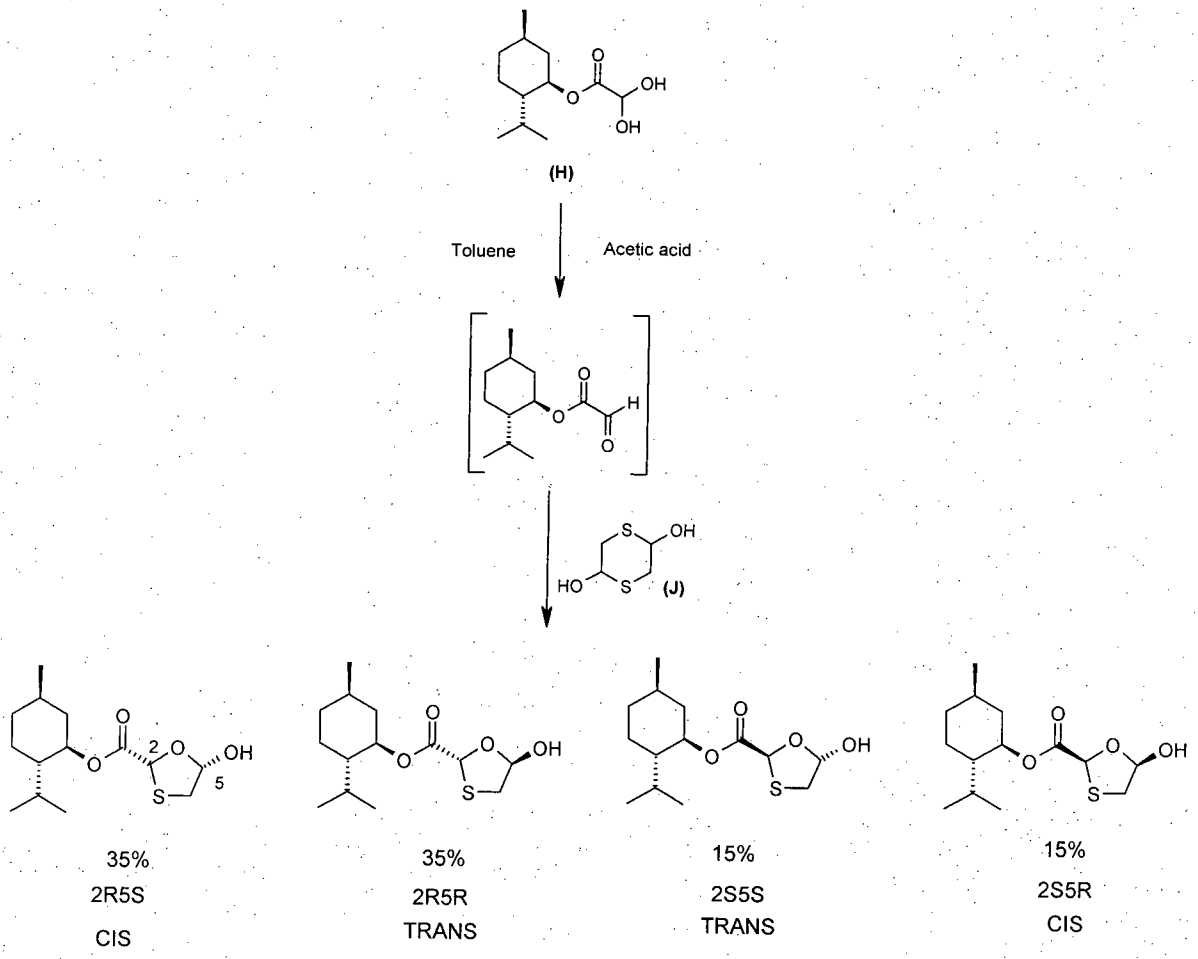
(b) Lyophilization on an industrial scale is tedious. (c) Chiral column chromatographic separations are expensive.

WO 2006/096954 describes the separation of protected or unprotected enantiomers of the cis nucleosides of below formula by using a chiral acid to form diastereomeric salts that are isolated by filtration. Some of the acids used are R-(−)-Camphorsulfonic acid, L-(−)-Tartaric acid, L-(−)-Malic acid, *et cetera*. However, the configuration of these CIS-nucleosides are [2R,4R] and [2S,4S] as the heterocyclic base is attached at the 4 position of the oxathiolane ring and the overall stereo-structure of the molecule changes from that of the 2,5-substituted oxathiolane ring.



(G)

Goodyear *et al.*, ['Practical enantioselective synthesis of lamivudine (3TC™) via a dyanamic kinetic resolution', *Tetrahedron Letter*, 46 (2005) 8535 – 8538.] describes synthesis of lamivudine from L-L-menthyl glyoxylate. Wherein, L-menthyl glyoxylate (H) is condensed with dithiane 2,5-diol (J) (*i.e.* dimer of mercaptoacetaldehyde) to obtain 5-hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester (K), the distribution of various isomers of compound K is as shown in scheme – II below:



On treatment of the whole residue with triethylamine 2R5R isomer could be enriched, however, once hydroxy is converted to leaving group such as chloro, which is supposed to be a leaving group of choice, position 5 gets racemized and further glycosylation lead to mixture of isomers, as per the literature cited hereinbefore, it is very difficult to separate cis (-) isomer from cis (+) isomer.

Thus various methods are described for the preparation of Lamivudine in the literature. However there is no mention in the prior art about the separation of an enantiomeric pair, either cis-(±) or trans-(±), from a mixture containing cis-[2R,5S], [2S,5R] and trans-[2R,5R], [2S,5S] isomers. Further, there also is a need to provide resolution of the cis-(±) isomers to yield the desired enantiomer in high optical purity.

CN 1223262 (Deng *et al*) teaches the resolution of a certain class of compounds called *Prazoles* by using chiral host compounds such as dinaphthalenephenols (BINOL), diphenanthrenols or tartaric acid derivatives. The method consists of the formation of a 1:1 complex between the chiral host (BINOL) and one of the enantiomers, the guest molecule. The other enantiomer remains in solution. (S)-Omeprazole, which is pharmaceutically active as a highly potent inhibitor of gastric acid secretion, has been isolated from its racemic mixture in this manner by using S-BINOL.

BINOL is a versatile chiral ligand that has found its uses in various reactions involving asymmetric synthesis (Noyori, R. *Asymmetric Catalysis in Organic Synthesis)* and optical resolution (Cram, D. J. et al *J. Org. Chem.* **1977,** *42,* 4173-4184). Some of these reactions include BINOL-mediated oxidation and reduction reactions, C-C bond formation reactions such as Aldol reaction, Michael addition, Mannich reaction *et cetera* (Brunel *Chem. Rev.* **2005** *105,* 857-897) and kinetic resolution, resolution by inclusion complexation *et cetera.*

BINOL, or 1,1′-bi-2-Naphthol, being an atropoisomer possesses the property of chiral recognition towards appropriate compounds. One of the uses of BINOL in resolution that is known in literature is in Host-Guest complexation. In one such example, 1,1-binaphthyl derivatives have been successfully incorporated into optically active crown ethers for the enantioselective complexation of amino acid esters and chiral primary ammonium ions (Cram, D. J. *Acc. Chem. Res.* **1978,** *11,* 8-14). The chiral 'host' is thus able to discriminate between enantiomeric compounds by the formation of hydrogen bonds between the ether oxygen and the enantiomers. The complex formed with one of the isomers, the 'guest', will be less stable on steric grounds and this forms the basis for its separation.

It is evident from the literature cited that there exists a need to (a) synthesize Lamivudine by a process requiring less expensive, less hazardous and easily available reagents, and (b) achieve good yields with superior quality of product without resorting to column

chromatography as a means of separation, thereby making the process of Lamivudine manufacture more acceptable industrially.

Our efforts on commercialization of Lamivudine process lead to the process described in WO2008053496, to further improve the process claimed in the '496 application with respect to the cost, ease in the operation and minimization in the unit process operations we surprisingly found that if one react L-menthyl glyoxylate with mercaptoacetaldehyde dimer under certain optimized conditions it lead to formation of cis (+/-) isomer of 5-hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester predominantly. Further, when cis (+/-)5-hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester which was then further converted to cis racemate Lamivudine with high yield; however, in the process described in the '496 application lead to formation of cis(+/-)/trans (+/-) *i.e.* four isomers. Further cis (+/-) lamivudine was further resolved by formation of co-crystal with S-BINOL.

Object of the invention

Thus, one object of the present invention is to provide a process for the synthesis of Lamivudine which is cost effective, uses less hazardous and easily available reagents, yet achieves good yields with superior quality of product without resorting to column chromatography.

A further object of the present invention is to provide an improved process for the synthesis of Lamivudine, by separating the mixture of diastereomers: Cis-[2R,5S], [2S,5R] from Trans-[2R,5R], [2S,5S] and then resolving the Cis isomers using BINOL to obtain (–)-[2R,5S]-Cis-Lamivudine with at least 99% ee.

Summary of the invention

Thus, according to one aspect of the present invention there is provided a process to predominantly form cis (+/-) lamivudine and resolve the product by forming co-crystals with S-BINOL, the process comprising the following steps:

a. dehydration of L-menthyl glyoxylate hydrate.

b. condensation of dehydrated L-menthyl glyoxylate with 1,4-dithiane-2,5-diol to form 5-hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester.

c. Conversion of 5-hydroxy[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester to 5-chloro[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester by treating 5-hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester with thionyl chloride in dichloromethane in presence of N,N-dimethyl formamide.

d. Condensation of 5-chloro[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester with silylated cytosine to obtain racemic 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester.

e. Reduction of 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester using sodiumborohydride in organic solvent like ethanol and further crystallization in methanol to obtain cis (±)-Lamivudine.

f. reacting Cis (±)-Lamivudine with a chiral host in an organic solvent,

g. selectively crystallizing out the adduct formed by Cis (-)-Lamivudine and the chiral host,

h. optionally treating the adduct with an acid and then neutralizing it to get (–)-[2R,5S]-Cis-Lamivudine,

i. isolating the product using suitable method such as partitioning in the biphasic system or crystallizing out from the solution,

j. optionally purifying it by crystallization from a suitable organic solvent, thereby obtaining the (−)-[2R,5S]-Cis-Lamivudine in a substantially optically pure or optically enriched form.

Thus, the yield loss problems associated with distereomeric separation of cis(+/-)/trans(+/-) into cis (+/-) and trans (+/-) isomers was successfully eliminated and further resolution was also improved by removing tedious operations like chromatographic separations.

Detailed description of the invention:

The process of the present invention for the manufacture of Lamivudine is as presented in Scheme 2, and comprises Following chemistry

L-Menthyl Glyoxylate, toluene and catalytic quantity of acetic acid were heated to reflux and formed water was removed azeotropically, then the reaction mixture was cooled to room temperature. Then 1,4-Dithiane 2,5-Diol was added to the reaction mixture and the reaction mixture was heated to reflux and maintained for four to five hours or till completion of reaction. After completion of the reaction the mixture was cooled to 50 to 55°C and the solvent was distilled out at this temperature under vacuum to obtain sticky mass. Analysis of this mass revealed composition of isomers as (+/-) cis around 95% and (+/-) trans around 5%.

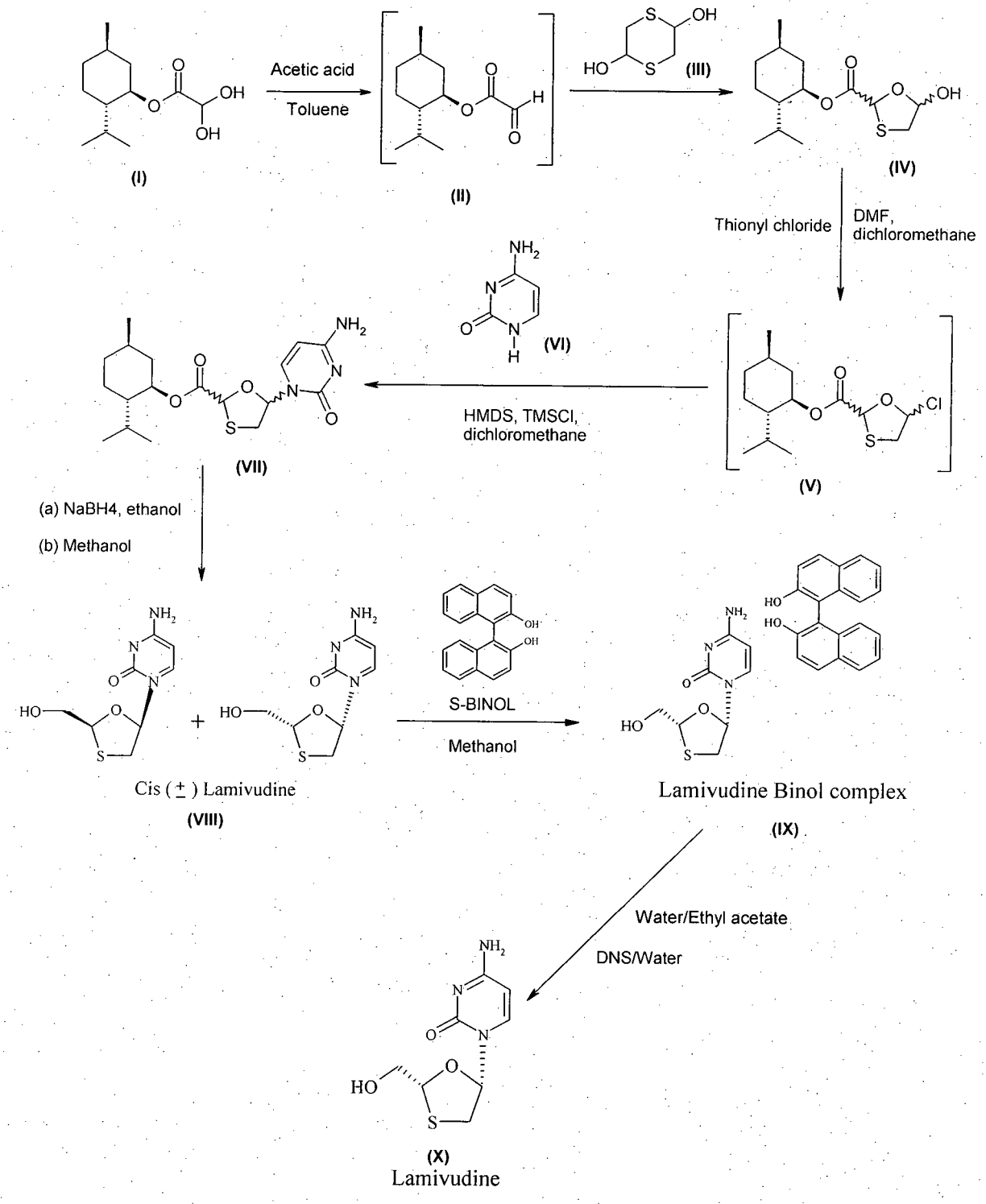
Dichloromethane was added to the reaction mass obtained in step 1, to this mixture catalytic N,N-dimethylformamide was added and then the mixture was cooled to 5 to 10°C. Thionyl chloride was added slowly to the mixture keeping temperature between 5 to 10°C. The mixture was stirred for half an hour maintaining the temperature of reaction between 5 to 10°C. Further the temperature was raised to 25 to 30°C and maintained at this temperature till completion of reaction, which was monitored by TLC. Solvents were distilled completely initially at atmospheric pressure and at the end under vacuum to assure complete removal. Fresh dichloromethane was added to the residue to obtain a clear solution (solution 1). In a separate vessel was added cytosine, HMDS and TMSCl, and the reaction mass was heated to

115 to 125° and maintained till clear solution was observed. Then the reaction mass was cooled and excess HMDS and TMSCl was distilled out under vacuum. Obtained residue was cooled to room temperature and to it was added dichloromethane, triethylamine and solution 1 obtained above. The reaction mass was then heated to 35 to 40 °C and maintained the temperature for 8 to 10 hours or till completion of the reaction, which was monitored by TLC. The reaction mass was cooled to 25 to 30°C. Further, the reaction mixture was quenched into a mixture of sodiumbicarbonate, triethylamine, dichloromethane and water. Organic layer was further washed with water and concentrated completely under vacuum. n-heptane and water were added to the residue and the mixture was stirred for 2-4 hrs at room temperature. The solidified material was filtered, washed with water, slurry washed with isopropyl acetate at room temperature and the isolated product was dried under vacuum.

A mixture of dipotassium hydrogen phosphate, water, ethanol and 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester was prepared at 25 to 30°C and cooled the reaction mass to 10 to 15°C to which was added a solution of sodium borohydride (prepared by dissolving sodium borohydride in aqueous sodium hydroxide solution) at 10 to 15°C slowly. The reaction mass was maintained for 4 to 5 hours at 10 to 15°C or till completion of reaction, which was monitored by TLC. After completion of the reaction the layers were separated and the pH of the organic layer was adjusted to 4 to 4.5 using conc. hydrochloric acid. Then the pH was adjusted to 6.8 to 7.2 using 2M sodium hydroxide solution. The reaction mixture was then stirred for 10 to 15 minutes at 25 to 30°C. Further, the reaction mass was concentrated under vacuum. Water and charcoal was then added to the residue and the mixture was subjected to heating to 70 to 75°C and maintained for 30 to 60 minutes. The reaction mass was then cooled to 30 to 35°C and the charcoal was filtered out using celite cake. The clear filtrate was then concentrated under vacuum at 40 to 45°C. The mixture was then cooled and the aqueous solution so obtained was washed with Toluene and was concentrated under vacuum, to ensure removal of water the residue was co-distilled with methanol. The residue so obtained was dissolved in methanol and the mass was heated to 60 to 65°C and stirred the solution for

half an hour and then the solution was filtered out. The filtrate was then subjected to concentration. The concentrated solution was then cooled to 25 to 30 and stirred for an hour to obtain solid product. The product was then filtered and dried.

cis (+/-) lamivudine was treated with (-) Binol in methanol to obtain Binol addition complex of cis (-) Lamivudine as a crystalline material. This complex was further treated with water at 35 to 40°C, which was further washed with ethyl acetate and the product was isolated from the aqueous layer


Acetic acid
Toluene
(I)
(II)
(III)
(IV)
Thionyl chloride
DMF, dichloromethane
(V)
(VI)
HMDS, TMSCl, dichloromethane
(VII)
(a) NaBH4, ethanol
(b) Methanol
Cis (±) Lamivudine
(VIII)
S-BINOL
Methanol
Lamivudine Binol complex
(IX)
Water/Ethyl acetate
DNS/Water
(X)
Lamivudine

SCHEME 2

Hence, the aspect of the present invention is to improve the yield of cis (±)-Lamivudine by utilizing the chemistry provided below:

a. dehydration of L-menthyl glyoxylate hydrate.
b. condensation of dehydrated L-menthyl glyoxylate with 1,4-dithiane-2,5-diol to form 5-Hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester.
c. Conversion of 5-hydroxy[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester to 5-chloro[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester by treating 5-Hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester with thionyl chloride in dichloromethane in presence of N,N-dimethyl formamide.
d. Condensation of 5-chloro[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester with silylated cytosine to obtain racemic 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester.
e. Reduction of 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester using sodiumborohydride in organic solvent like ethanol and further crystallization in methanol to obtain cis (±)-Lamivudine.

Another aspect of the present invention provides a method for the separation of (−)-[2R,5S]-Cis-Lamivudine from its (+)-enantiomer using optically pure (S)-2,2'-dihydroxy-1,1'-binaphthyl [(S)-BINOL].

The process is operationally simple and comprises the following steps:

a) treating the racemates with (S)-BINOL in the presence of a hydroxylic solvent like methanol,
b) isolating the adduct formed by the enantiomer and the chiral host by filtration,
c) if desired, crystallizing the adduct,

d) optionally treating the adduct with an acid and then neutralizing it to get (–)-[2R,5S]-Cis-Lamivudine,

e) isolating the product using suitable method such as partitioning in the biphasic system or crystallizing out from the solution,

f) optionally purifying it by crystallization from a suitable organic solvent, thereby obtaining the (–)-[2R,5S]-Cis-Lamivudine in a substantially optically pure or optically enriched form.

Thereby yielding the desired (–)-[2R,5S] -Lamivudine with an enantiomeric excess greater than 99% .

Thus, reacting the racemates with (S)-BINOL results in the formation of a clathrate of (–)-[2R,5S]-Cis-Lamivudine by Host-Guest complexation. On filtration it affords a clean separation between the enantiomers. In the final step, the (–)-[2R,5S]-Cis-Lamivudine-(S)-BINOL complex is broken in an acidic medium and then neutralized to obtain the desired product: (–)-[2R,5S]-Lamivudine, in high optical purity of greater than 97.5% before purification. Re-crystallization with isopropanol raises the enantiomeric excess to 99.5%. The IR spectra of racemic Lamivudine, S-BINOL and the Lamivudine – S-BINOL complex are provided in Figures 1, 2, and 3 respectively. It is to be emphasized that when the same separation was attempted using R-BINOL, no resolution of enantiomers was achieved.

Resolution of the Cis enantiomers of Lamivudine by the formation of diastereomeric salts of cis-(±) lamivudine with acids like malic acid, mandelic acid, dibenzoyl tartaric acid, 3-bromocamphor-8-sulfonic acid, 10-camphorsulfonic acid, and di-p-toluoyltartaric acid have been attempted before by Liotta *et al*, one of the inventors named in US. 5,204,466. However, these attempts were unsuccessful as revealed by a declaration attached with the prosecution history file of US 6,703,396.

Interestingly, the inventors have found that when the salts Cis-(±)-Lamivudine were prepared with R-(–)-CSA or with D-(–)-tartaric acid, no separation of enantiomers was achieved.

The following examples illustrate the practice of the invention without being limiting in any way.

Example 1: Preparation of 5-Hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester

To a 1.0-lit 4-necked RB flask, charged 500 ml of Toluene, L-Menthyl Glyoxylate (100 gm) followed by acetic acid (10 ml). Heated the reaction mixture upto 110-115°C, to remove water by azeotrope. After removal of water cooled the R.M., recovered the R.M. under vacuum up to 300 ml solvents remains in the flak Cooled the R.M. Charged 1,4-Dithiane 2,5-Diol (33.1 gm) and heated the reaction mixture. Maintained the R.M. by TLC monitoring. After completion of the reaction cooled the mass and concentrate the mass at 50-55°C and degassed it to get Hydroxy compound Lami-1 (120-125 gm).

Results:

Out put (gm)	% Yield in Range	Chiral purity by HPLC %
120-125 gm	96-100 %	41.90: 53.84:2.92:1.34

Example 2: Preparation of racemic 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester.

Charged dichloromethane (500 ml) in 1.0 lit 4-neck RBF and 5-Hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester (125 gm) followed by DMF (6.4 gm). Cooled the reaction mixture upto 5-10°C and thionyl chloride (61.2 gm) was added by dropping funnel. Stirred the mass for 30 min and raise the temp upto 25-30°C. Monitor the progress of reaction by TLC. After consumption of starting materials distill out solvent atmospherically. To remove the traces of thionyl apply vacuum. Charged fresh DCM (125 ml) into concentrated residue.

In another 2.0 lit round bottom flask charged cytosine (48.22 gm), HMDS (54.12 gm) and TMSCl (22.76 gm) and heated reaction mass upto 110-120°C, till clear solution appears. Cooled the reaction mixture upto 90-100°C, distill out excess HMDS and TMSCl and cooled the reaction mass. Charged DCM (250 ml), TEA (43.91 gm) in above residue. Charged slowly DCM solution of Chloro compound into the above reaction mass Heated reaction mass upto 30-35°C, maintain the temperature with stirring for 8-10 hrs Monitor progress of reaction by TLC after completion of the reaction cooled reaction mass. In another flask, charged $NaHCO_3$ (125 gm) TEA (12.5 ml) and DCM (750 ml) and Water (1250 ml). Charged the reaction mass into the above mixture. Stirred the reaction mass, settled the mixture and separate the DCM layer. Washed the organic layer with 250 ml water and concentrate the organic layer completely under vacuum. Charged water (400 ml) in above residue followed by n-Heptane (400 ml) and stir the mixture for 2-4 hrs at RT. Filter the solid and suck dry under vacuum washed wet cake with 200 ml of water and suck dry. Pour the above wet cake in Isopropyl acetate (400 ml) Stir for 15-30 min at RT Filter the solid, wash wet cake with with Isopropyl acetate (200 ml) and suck dried Dry the solid under vacuum for 6-8 hrs to afford condensed compound (Lami-2)

Result:

Chiral Purity

Out put (gm)	Yield Range (w/w)	% Yield in Range	Chiral purity by HPLC %
			46.98:53.02 (Crude Concentrated Mass)
62.5-87.5 gm	0.6-0.7	45-53 %	50-72:49.28 (Isolated solid)

Example 3: Preparation of Cis (±) Lamivudine

Charged Di-potassium hydrogen phosphate (137 gm) in DM water (150 ml) followed by Lami-02 (50 gm) at RT. Charged ethanol (375 ml) in above flask. In another flask prepared the $NaBH_4$ solution using the NaOH (0.125 gm) in Dm water (100 ml) at 10-15°C and added the slowly 20 gm $NaBH_4$ Cooled the reaction mass upto 10-15°C Added the sodium

borohydride solution into the above solution at 10-15°C. Maintain the reaction mass at 10-15°C and monitor by TLC/HPLC After completion of the reaction separate the layer adjusted the pH of organic layer to 4 to 4.5 using the con. HCl (11 ml), adjusted the pH to 6.8 –7.2 using 2M NaOH solution 40 ml (3.2 gm NaOH in 40 ml Water) stirred the R.M Concentrate the mass under vacuum charged water (45 ml) followed by act. Carbon (5 gm) in above mass Heat the mass upto 70-75°C, maintain the temperature cool the mass filter activated carbon through celite. Recover the solvent under vacuum upto 325 ml solvents remains in the flask Cool the mass and wash above aqueous layer with Toluene (200 ml X 3 times). Concentrate the aqueous layer under vacuum Co distill traces of water with methanol (50 ml X 2 times). Dissolve the above residue in methanol (600 ml) Heated the mass upto 60-65°C, stirred the solution and filter the mass Recover the solvent under vacuum upto 2 volume. Stir the mass and filter the solid, wash wet cake with methanol (50 ml), suck the solid and dry under vacuum to afford racemic Lamivudine.

Result of Crude racemic Lamivudine

Out put (gm)	Yield Range (w/w)	Chiral purity by HPLC %
30 gm	0.55-0.65	Cis (+): 50.67 % Cis (-): 49.33 % Trans (+): 0.18 % Trans (-): 0.04 %

Result of Purified racemic Lamivudine

Out put (gm)	Yield Range (w/w)	Chiral purity by HPLC
15-20	0.3-0.4	Cis (+): 49.66 % Cis ((-): 50.13 % Trans (+): 0.18 % Trans (-): 0.04 %

Example 4: Preparation of (-)-1-(2R-Cis)-4-amino-1- [(2-hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one- (*S*)-Binol Co-Crystal.

Charged S (-) Binol (50 g) followed by methanol (200 ml) in a RBF. Stirred to get clear solution at 30-35°C and charged racemic Lamivudine (25gm) and heated the mass upto 60-65°C to get clear solution. Cooled the mass at ambient temperature at 10 °C/ hr. Stirred the mixture at RT for 2-4.5 hrs, filtered the solid. Slurry washed the solid with methanol (25ml) in the flask. Suck dry the solid, dried the solid under vacuum to get the S-BINOL Cocrystal.

Results:

Out put	Yield Range (w/w)	% Yield in Range	Chiral purity by HPLC
17.5-22.5g	0.70-0.90	63—82 %	Cis (-): 99.76 % Cis (+): 0.24%

Example 5: Preparation of 4-amino-1- [(2*R*, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one; (Lamivudine).

Charged (S) BINOL Cocrystal (20g) in ethyl acetate (100ml) and D.M. water (200ml) at RT. Heat the solution upto 35-40°C Maintain the temperature Cool the solution upto RT and settle the layer, separate the layer. Charged ethyl acetate (100 ml) into the aqueous layer Stirred the solution settle the layer Separate the layers. Combine both the ethyl acetate layer and charge water (100 ml). Stirred the solution Settle the layer Separate the layers. Combine both the aq. layer and charge activated carbon (2.0 gm) Heated the reaction mixture upto 45-50°C and stirred the reaction mixture. Filtered the reaction mixture through celite bed. Wash celite bed with 20 ml chloride free water. Recovered the solvent under vacuum at 45-50°C till 1.2 volumes left in the flask. Charged DNS (8 ml) to the reaction mixture and stirred the reaction mixture Filter the solution through 0.5 μ cooled the solution and seeded with 0.025 gm of Lamivudine form-1 at 30-32°C. Further cooled the mixture upto 8-10°C and stirred the solid for 1 hrs. Filtered the solid and washed the wet cake with 4 ml of precooled DM

Water and DNS mixture (3:1) Sucked Dry the solid, dry the solid under vacuum to afford Lamivudine form –1.

Results:

Out put (Range)	Yield (w/w) (Range)	% Yield in Range	HPLC purity (RS)	Chiral purity by HPLC
7-8 gm	0.30-0.40	68-90%	Cis (-): 99.93 % Individual Impurity: 0.03 %	Cis (-): 99.94% Cis (+): 0.06 %

Example 6: Preparation of 4-amino-1- [(2*R*, 5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-1,2-dihydropyrimidin-2-one; (Lamivudine).

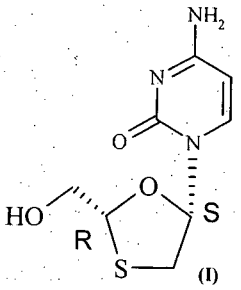
Charged (S) BINOL Cocrystal (20g) in ethyl acetate (100ml) and D.M. water (100ml) at RT. Conc. Hydrochloric acid (4 to 5 ml) was added to the mixture gradually and stirred (pH of the mixture was between about 3 to 4) for an hour and allowed the layers to settle, separated the layers. Aqueous layer was washed with fresh ethyl acetate to remove S(-) BINOL completely. pH of the solution was adjusted to about 7 using 10% sodium hydroxide solution. The solution was then passed though activated resin 225-H column. The column was then washed with purified water, thereafter with 15% aqueous ammonia solution. Combined solutions were subjected to evaporation till 20 to 50 ml. To the mass was then added 8 ml of DNS and stirred to homogenize. The solution was then passed through 0.5 micron filter and then the solution was seeded with Lamivudine form I. Further, the mixture was cooled to around 8 – 10°C and stirred for an hour maintaining the same temperature. The crystalline product was then filtered and washed with precooled water and DNS mixture. The product was then dried under vacuum to afford Lamivudine form I.

Results:

Out put (Range)	Yield (w/w) (Range)	% Yield in Range	HPLC purity (RS)	Chiral purity by HPLC
6.5-8 gm	0.30-0.40	68-90%	Cis (-): 99.94 % Individual Impurity: 0.03 %	Cis (-): 99.95% Cis (+): 0.06 %

CLAIMS

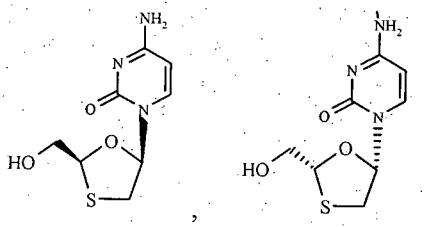
1) A process for the preparation of essentially enantiomerically pure (-)-[2R, 5S]-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one of formula (I),



(I)

from L-menthyl glyoxylate comprising the steps of:

i. preparation of (±)-1-(2R/S-Cis)-4-amino-1-[(2-hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one of formula (XII),



XII

from L-menthyl glyoxylate comprising the steps of:

a. dehydration of L-menthyl glyoxylate hydrate.

b. condensation of dehydrated L-menthyl glyoxylate with 1,4-dithiane-2,5-diol to form 5-Hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester.

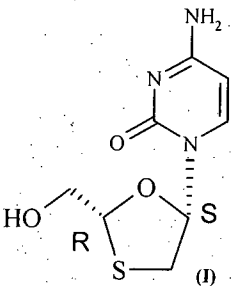
c. Conversion of 5-hydroxy[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester to 5-chloro[1,3]oxathiolane-2-carboxylic

acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester by treating 5-Hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester with thionyl chloride in dichloromethane in presence of N,N-dimethyl formamide.

d. Condensation of 5-chloro[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester with silylated cytosine to obtain racemic 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester.

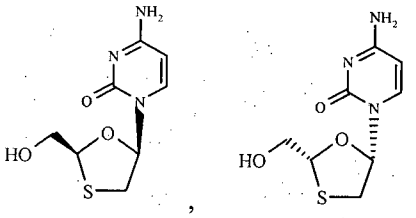
e. Reduction of 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester using sodiumborohydride in organic solvent like ethanol and further crystallization in methanol to obtain cis (±)-Lamivudine.;

ii. preparation of essentially enantiomerically pure (-)-[2R, 5S]-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one of formula (I),



(I)

from a mixture of racemic cis-(±)-Lamivudine of formula (XII),



XII

comprising the steps of:

a treating (±)-1-(2R/S-Cis)-4-amino-1-[(2-hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one of formula (XII) with S-Binol in an organic solvent such as C1 to C8 alcohol;

b isolating the adduct formed by the cis (-) enantiomer and S-Binol;

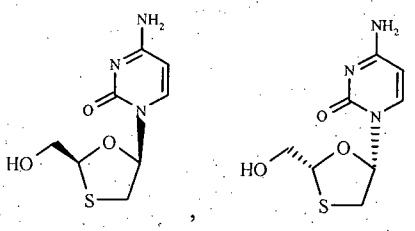
c optionally purifying the adduct by crystallizing,

d optionally treating the adduct with an acid and then neutralizing it.,

e isolating the product using suitable method such as partitioning in the biphasic system or crystallizing out from the solution,

f optionally purifying it by crystallization from a suitable organic solvent, thereby obtaining the (−)-[2R,5S]-Cis-Lamivudine in a substantially optically pure or optically enriched form.

2. A process for preparation of (±)-1-(2R/S-Cis)-4-amino-1-[(2-hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidin-2-one of formula (XII),



XII

from L-menthyl glyoxylate comprising the steps of:

a. dehydration of L-menthyl glyoxylate hydrate.

b. condensation of dehydrated L-menthyl glyoxylate with 1,4-dithiane-2,5-diol to form 5-Hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester.

c. Conversion of 5-hydroxy[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester to 5-chloro[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester by treating 5-Hydroxy [1,3] oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester

with thionyl chloride in dichloromethane in presence of N,N-dimethyl formamide.

d. Condensation of 5-chloro[1,3]oxathiolane-2-carboxylic acid 2S-isopropyl-5R-methyl-1R-cyclohexyl ester with silylated cytosine to obtain racemic 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester.

e. Reduction of 5-(4-Amino-2-oxo-2H-pyrimidin-1-yl)-[1,3]oxathiolane-2-carboxylic acid (1R,2S,5R)-2-isopropyl-5-methyl-cyclohexyl ester using sodiumborohydride in organic solvent like ethanol and further optional crystallization in methanol to obtain cis (±)-Lamivudine.

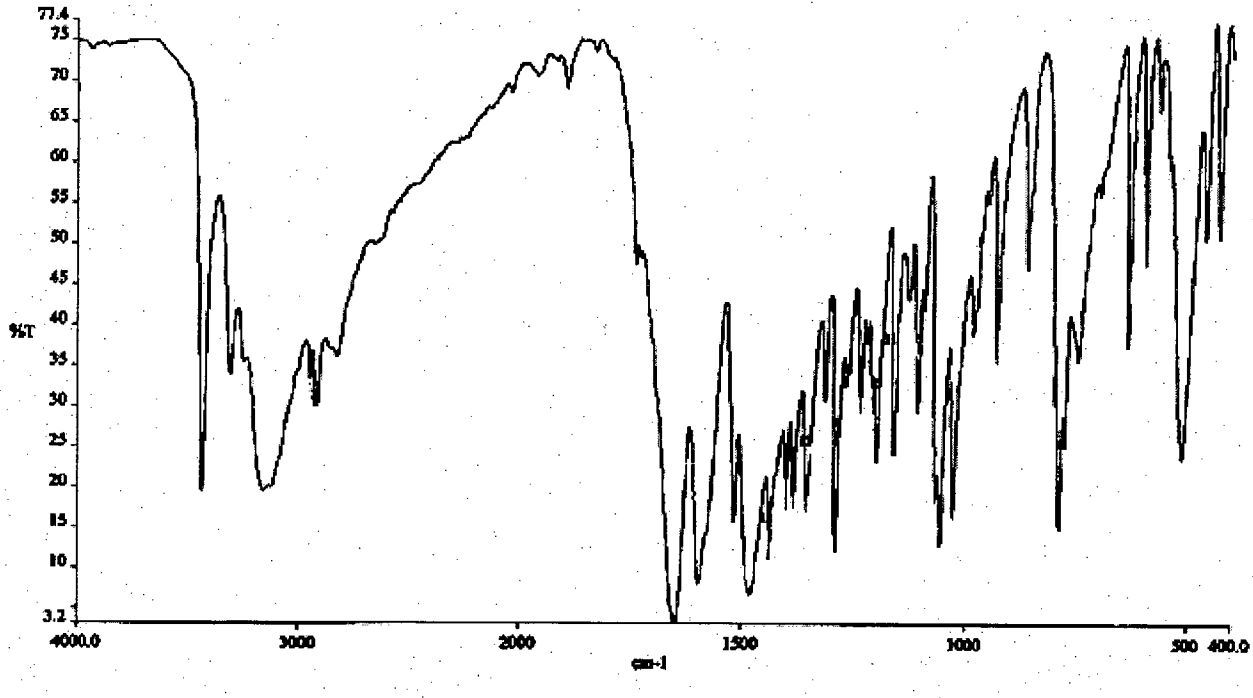

77.4
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Figure 1

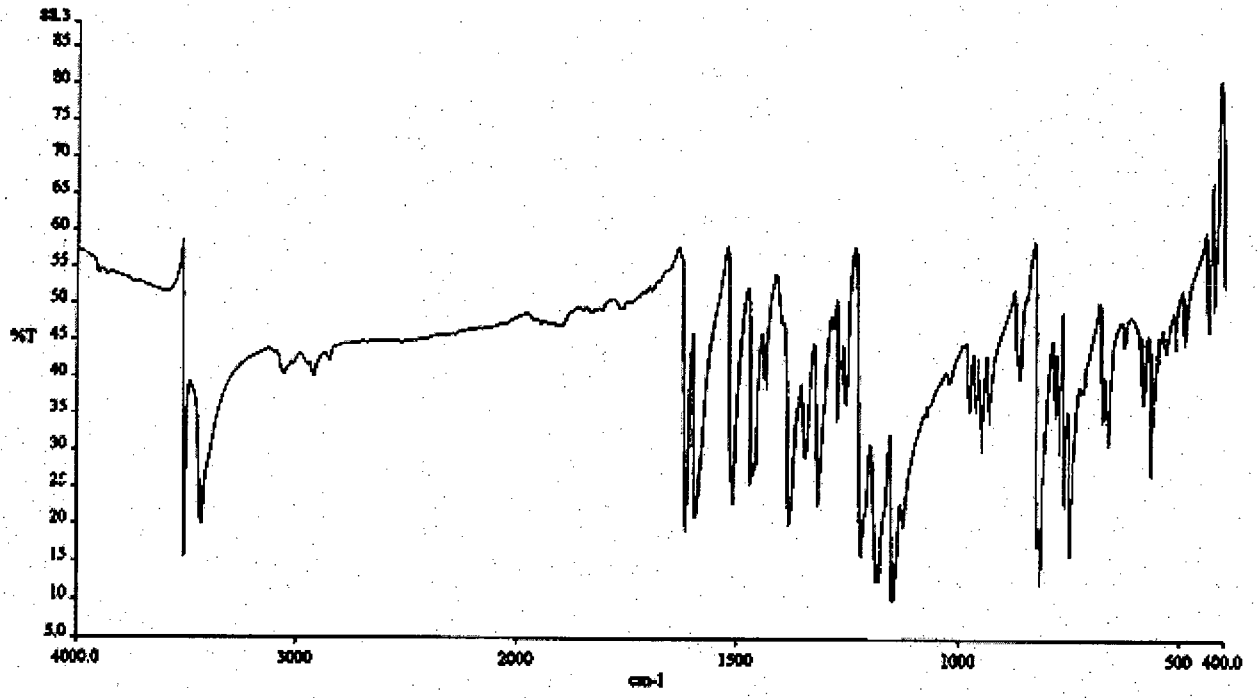

Figure 2

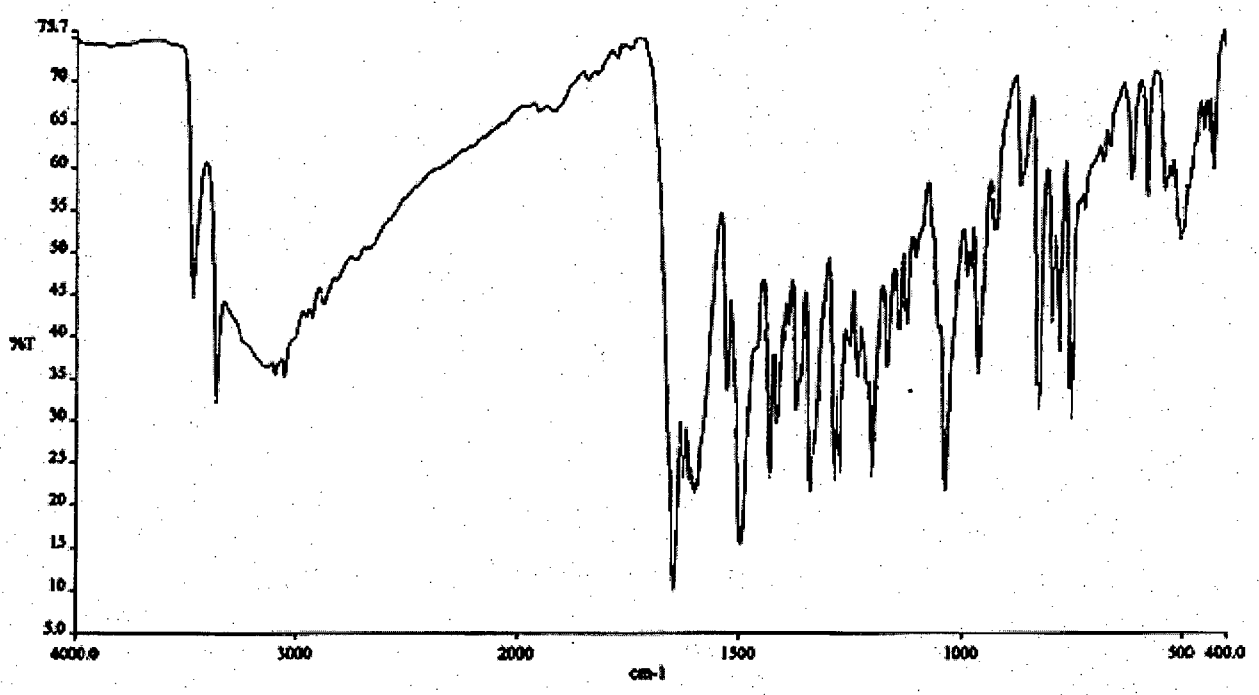

Figure 3