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Lamivudine, an important drug in aids treatment

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Lamivudine, an L-nucleoside, has been considered the cornerstone of antiretroviral therapy programs considering its favorable efficacy, convenient dosing, and safety profile. The low incidence of side effects in comparison with D-nucleoside analogs is due to the presence of sulfur and also to its stereochemistry difference. Considering the importance of lamivudine especially in acquired immune deficiency syndrome treatment, but also in hepatitis B infection (HBV), two critical diseases nowadays, the purpose of this article is to highlight different aspects and synthetic strategies of this important drug.

Keywords: lamivudine; AIDS; sulfur

1. Introduction

The acquired immune deficiency syndrome (AIDS) is a contagious disease that causes specific damage to the immunological system. The etiological agent of AIDS, known as human immunodeficiency virus (HIV), is a retrovirus (RNA genome), which infects the cells through enzymatic mechanisms and incorporates its genome into the host genome.

The main targets of this virus are CD4 cells, that once infected, lead to a progressive immunodeficiency. The life cycle of the AIDS virus begins with its attachment to the target cell. The structure responsible for the virus attachment is the heavily glycosylated viral envelope protein gp120 (*1*). The corresponding receptor on the target cell is the CD4 receptor protein. After binding to the surface of the host cell, the proteic membrane of the virus fuses with membrane of the cell. The genetic material of the virus, after replication by the viral enzyme reverse transcriptase (RT), is translocated into the nucleus, where it is integrated into the host genome by another viral enzyme integrase (*2*). Nowadays, AIDS is responsible for millions of deaths every year. In 2005, it is estimated that 38.6 million people were infected with HIV, and another 2.8 million people died of AIDS. This picture is particulary serious in underdeveloped countries, mainly in the African continent, where during this same year, 24.5 million people were infected with HIV, in other words, 64% of the total number of cases in the world are concentrated in this region (*3*).

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2. AIDS drugs available in the market

Depending on the mechanism of action, anti-HIV agents can be classified into six categories: the nucleoside reverse trancriptase inhibitors, which include zidovudine, abacavir, lamivudine, stavudine, didadosine, zalcitabine, entricitabine, and tenofovir; the non-nucleoside reverse trancriptase inhibitors (NNRTIs) such as nevirapine, delavirdine, and efavirenz; the protease inhibitors (PIs) which include saquinavir, ritonavir, lopinavir, indinavir, nelfinavir, amprenavir, tipranavir, fosamprenavir, darunavir, and atazanavir, a fusion inhibitor, enfuvirtide, a entry inhibitor, maraviroc, and the recently approved integrase inhibitor raltegravir (*4*).

It is important to be mentioned that among the 23 AIDS drugs in the market, 8 of them possess the element sulfur in their structures (Figure 1).

Sulfur is an important element in the Earth being essential for life, it is present in many applications such as fertilizers, insecticides, fungicides, and as a building block for vitamins,

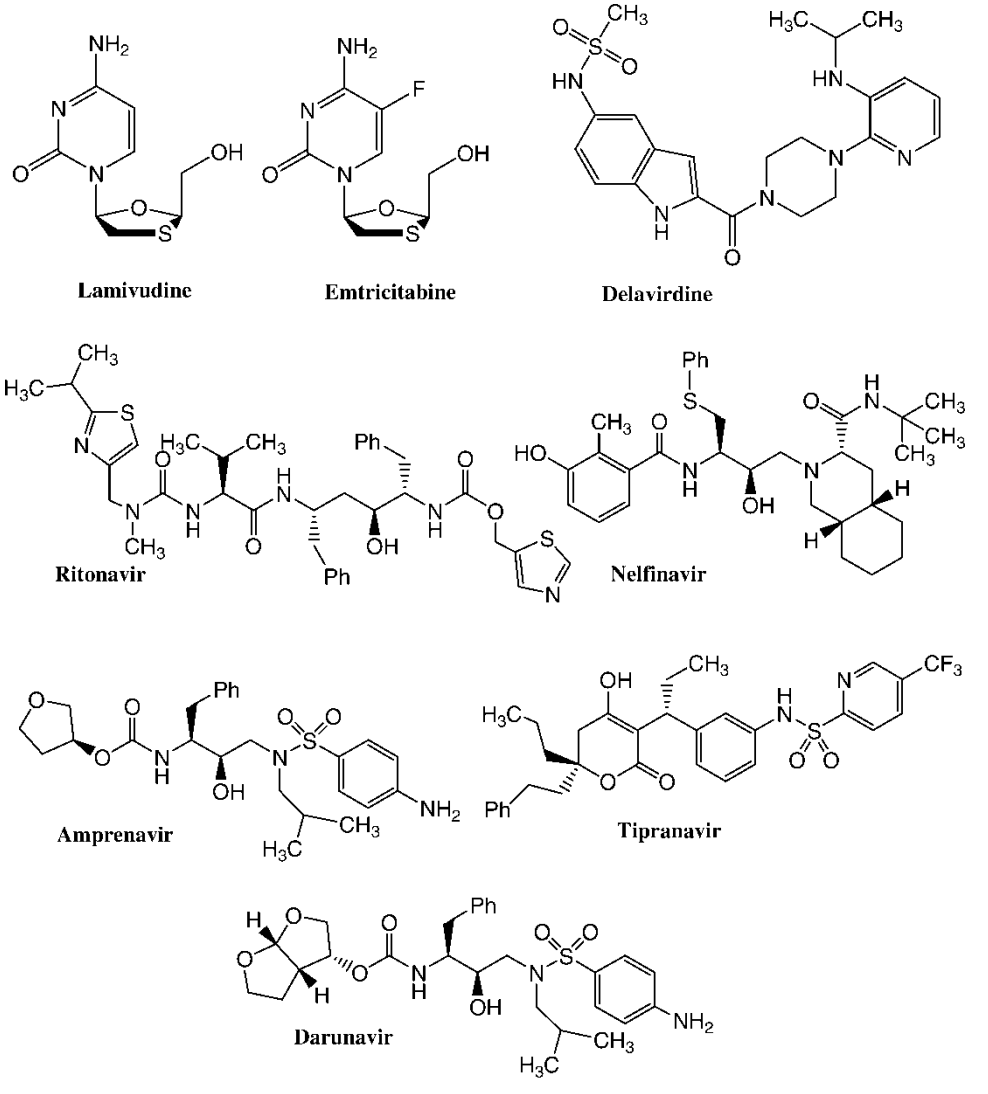


Figure 1. AIDS drugs in the market that possess sulfur in their structures.

proteins, and enzymes. Sulfur is also an essential component in different classes of drugs, one of the most prominent classes being AIDS drugs, indicating the importance of this atom (*5–8*).

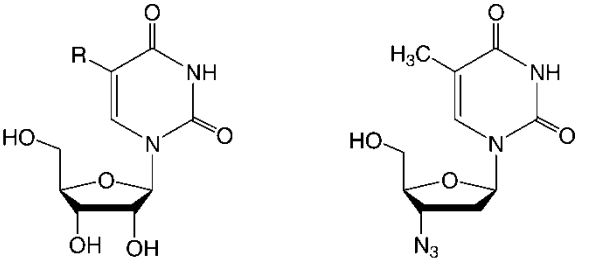
Lamivudine (3TC), an L-nucleoside, has been considered the cornerstone of antiretroviral therapy programs considering its favorable efficacy, convenient dosing, and safety profile. The low incidence of side effects in comparison with its D-nucleoside analogs is due to the presence of sulfur and also its stereochemistry difference. These structural aspects blocks its recognition by host polymerases cells (*9*).

3TC is the only one drug approved for use in the treatment of AIDS that possess the 'unnatural' L-configuration and its role has been mainly as a combination agent since a well-tolerated safety profile is essential to maintain patient compliance, drug efficacy, and also quality of life. This drug presents a potential for combination with a variety of others RT inhibitors, PIs, and NNRTI. For example, the combination between lamivudine and zidovudine which was approved by the Food and Drug Administration (FDA) on 17 November 1995. In another combination, lamivudine and abacavir was licensed in 2004 as a once daily dosed in treatment-naive individuals in combination with another class of antiretroviral. A triple formulation involving the abacavir sulfate, lamivudine, and zidovudine, marketed under the brand name of Trivizir®, was also approved for use in AIDS therapy in 2000 (*4,10*).

Due to the significant impact of lamivudine, specially on AIDS treatment, but also in hepatitis B infection (HBV), two critical diseases nowadays, the purpose of this article is to highlight different synthetic aspects of this important drug.

3. The beginning of AIDS drugs

The history of AIDS drugs has close connections with the sea. In 1955, Bergmann and Burke (*11*) isolated the marine nucleosides spongouridine and spongothymidine (Figure 2), which were prototypes for the development of a new class of drugs, nucleoside analogs, and 3′azido-2′-3′-dideoxythymidine (AZT) (Figure 2) was the first synthetic drug based on these natural products. AZT was first synthesized in 1964 by Horwitz *et al.* (*12*), and was initially evaluated for an anti-tumor agent. In 1986 AZT became the first drug to gain FDA approval for AIDS treatment, due to its inhibiting action toward the retroviral RT, an enzyme required for replication of the AIDS virus. Because of the importance of this drug in AIDS treatment, several nucleoside analogs were synthesized and evaluated, resulting in seven AIDS drugs approved by FDA, such as lamivudine and emtricitabine (Figure 1), didanosine, zalcitabine, stavudine, abacavir, and tenofovir (Figure 3).



R = H - **Spongouridine**
R = CH_3 - **Spongothimidyne**

Azidothymidine (AZT)

Figure 2. Structures of marine nucleosides spongouridine and spongothymidine and the respective synthetic analog, AZT.

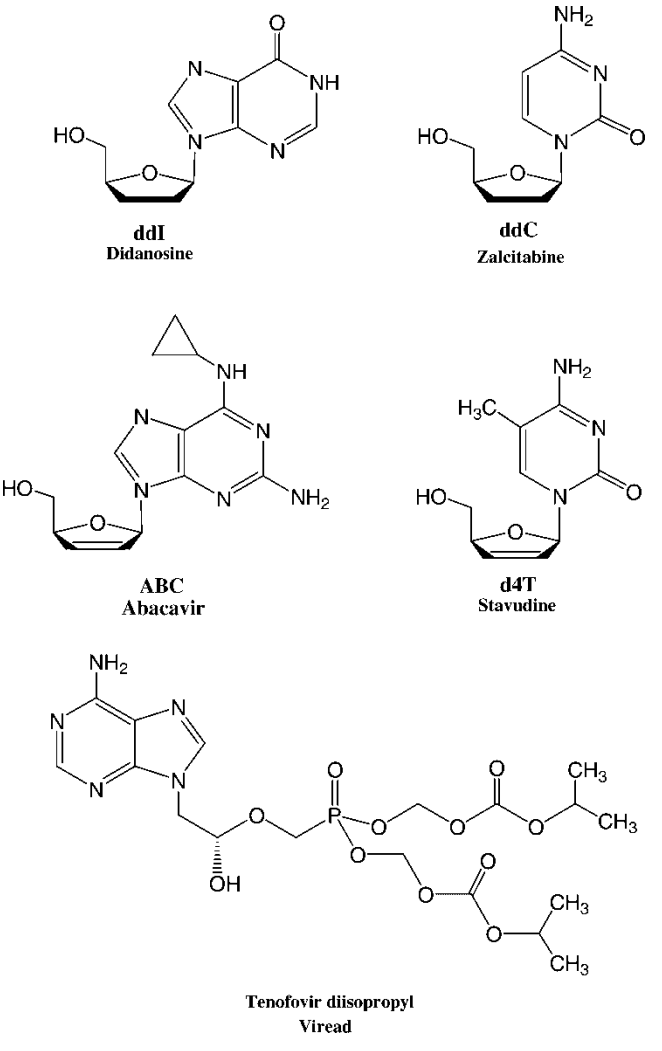


Figure 3. Structures of seven synthetic nucleosides analogs used in AIDS treatment.

4. The Canadian discovery of lamivudine

Lamivudine is a synthetic oxathiolane analog, marketed under the brand name of Epivir® (Figure 1). In 1987, a research program aiming to synthesize novel nucleosides analogs capable of inhibiting RT was started by Belleau's group from McGill University (Montreal, Quebec, Canada). They found that the activity and specificity of such compounds would be dependent on the shape of the deoxyribose ring as well as the electronic environment in the C-3′ region. Considering this, they designed novel nucleoside analogs containing the isosteric ring of deoxyribose, in which the C-3′ carbon had been replaced by a heteroatom. Belleau's group reported for the first time the synthesis of a racemic mixture called (±)-BCH-189 (Figure 4), in which C-3′ position of the ribose ring had been replaced by a sulfur atom. Evaluation of this racemic mixture *in vitro* against HIV replication in collaboration with Dr Mark Wainberg at the Lady Davis Institute of the Jewish General Hospital (Montreal, Quebec, Canada), demonstrated the promising anti-HIV activity of this mixture (*13*). Further, it was found the both enantiomers were active against HIV but only the L-like β-anomer was non cytotoxic to human cells (*14*).

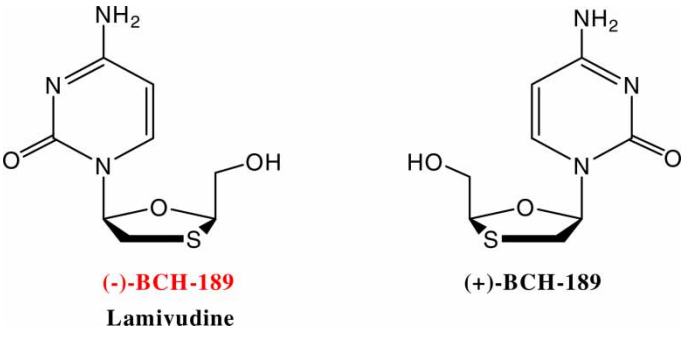


Figure 4. Structures of Lamivudine and its respective enantiomer.

5. Mechanism of action and some properties of lamivudine drug

Lamivudine is carried across the plasmatic membrane by the same transporters that carry natural nucleosides. Intracellularly, this drug is phosphorylated three times to lamivudine triphosphate (L-TP) by different enzymes. The first phosphorylation is carried out by deoxycytidine kinase that forms lamivudine monophosphate (L-MP). Then, cytidine monophosphate kinase phosphorylates L-MP to lamivudine diphosphate (L-DP).

Finally, L-DP suffers the last phosphorylation to yield L-TP, by the enzyme nucleoside diphosphate kinase (Figure 5). Although L-TP is considered to be a weak inhibitor of mammalian DNA polumerases alpha and beta, and also of the mitochondrial DNA polymerase, it inhibits HIV-1 RT via DNA chain termination after incorporation of the nucleoside analog into the viral DNa during

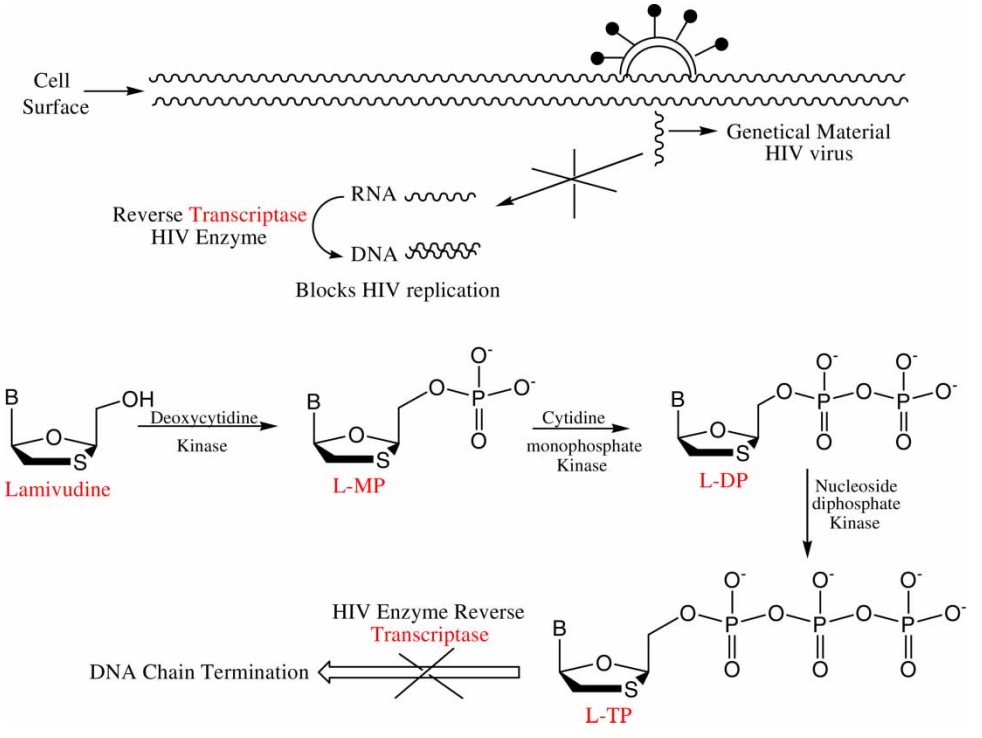


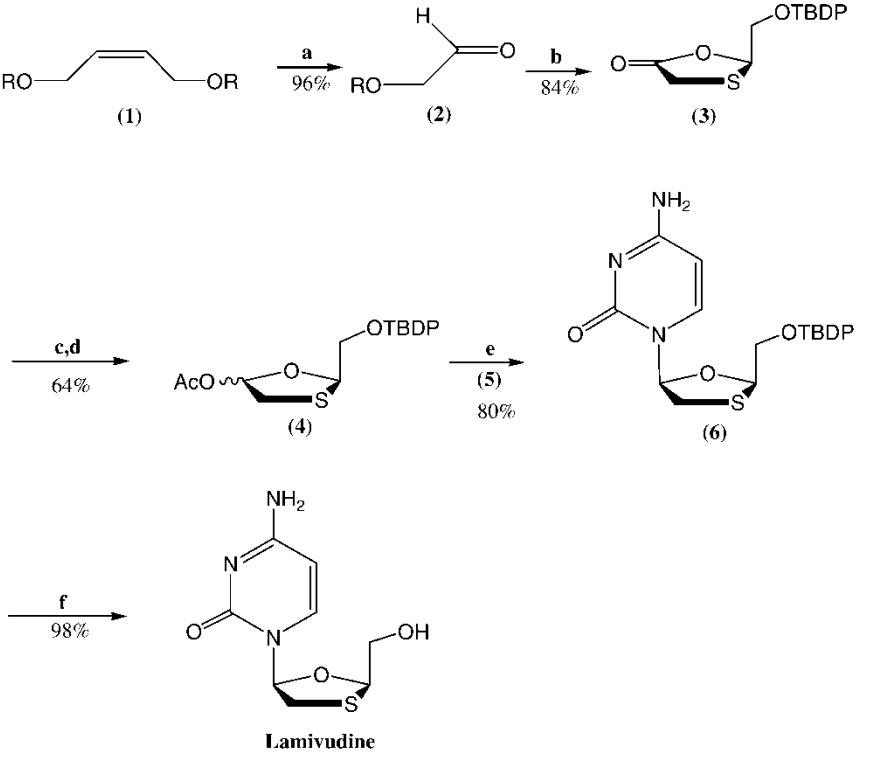
Figure 5. Mechanism of action of Lamivudine.

HIV replication (*15,16*). It means that the lamivudine is not recognized by host polymerases cells but by the viral-encoded that leads to a more specific action toward the viral cells. This fact is explained by the absolute configuration of this drug which is opposite to that of natural nucleoside analogs that compose the DNA molecule and justifies the low toxicity of this drug when compared with other structural D-analogs (*9*).

6. Synthetic methodologies developed for preparation of lamivudine and its enantiomer (+)-BCH-189

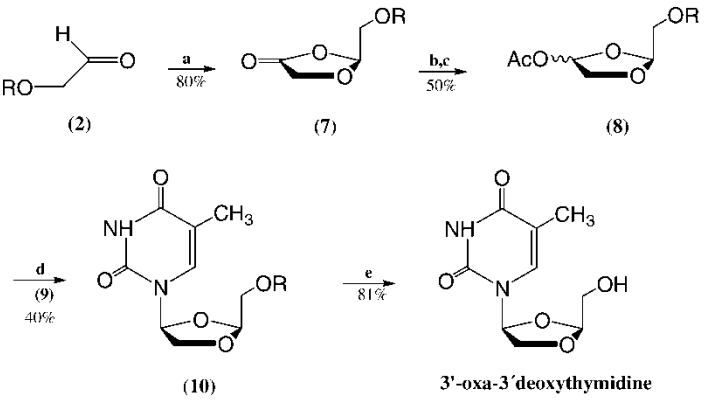
Due to the promising perspectives in AIDS treatment with (±) BCH-189, several groups have been interested in the development of practical syntheses for the preparation of the racemic mixture, including diastereo, and enantioselective syntheses. For example, Liotta and co-workers (*17*) at the department of chemistry, Emory University (Atlanta, Georgia, USA) developed the syntheses of both 3′-thia-2′,3′-di-deoxycytidine (Scheme 1) and the 3′-oxa-3′deoxythymidine (Scheme 2) using a highly stereoselective base-coupling reaction which operates via the *in situ* formation of a complex between a suitable cyclic precursor and an appropriate Lewis acid as a key step.

The synthesis of lamivudine was based on the anomeric mixture (**4**), which was synthesized from the protected glyco aldehyde (**2**). This aldehyde when refluxed with mercaptoacetic acid in toluene produced the thia lactone (**3**), which was reduced with diisobutylaluminum hydride



R= *tert*-butyldiphenylsilyl. **a:** O_3, Me_2S; **b:** $HSCH_2CO_2H$, toluene -78^{0}C; **c:** DIBAL-H; **d:** Ac_2O; **e:** TMS-cytosine **(5)**, DCM, stannic chloride; **f:** Bu_4NF.

Scheme 1. Syntheses of 3′-thia-2′,3′-di-deoxycytidine (Lamivudine) developed by Liotta and co-workers.



R= *tert*-butyldiphenylsilyl. **a:** O_3, Me_2S; **b:** $HSCH_2CO_2H$, toluene -78^{0}C; **c:** DIBAL-H; **d:** Ac_2O; **e:** TMS-cytosine (**5**), DCM, stannic chloride; **f:** Bu_4NF.

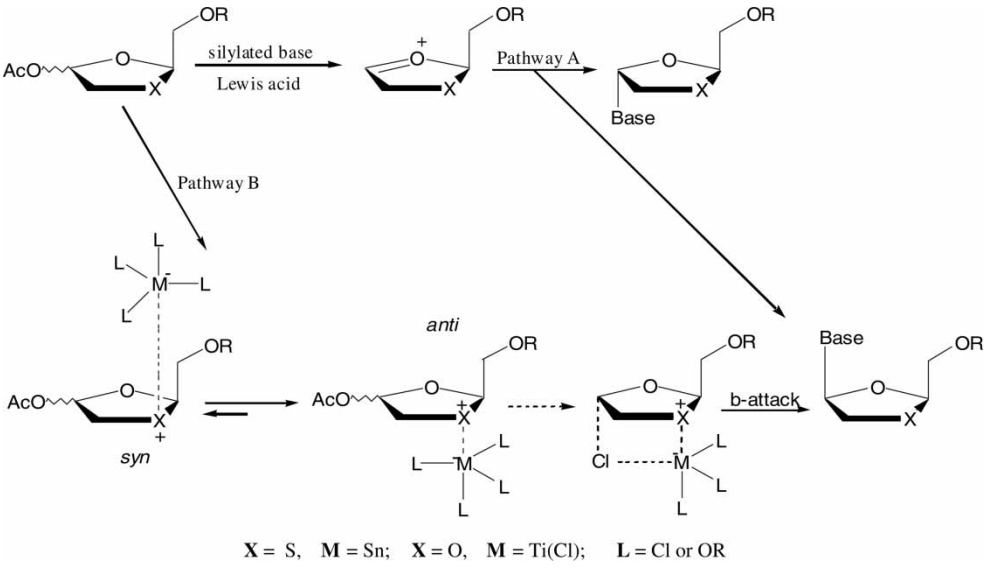
Scheme 2. Syntheses of 3′-oxa-3′-deoxythymidine developed by Liotta and co-workers.

(DIBAL-H) or lithium tri-tert-butoxyaluminum hydride (LiAlH(O*t*-Bu)$_3$) and trapped with acetic anhydride (Ac_2O) to furnish the acylated derivative (**4**). Reaction of (**4**) with sylilated cytosine (**5**) using stannic chloride as catalyst led to the exclusive formation of the β-cytosine adduct (**6**), which was further deprotected with tetrabutylammonium (Bu_4NF) to afford lamivudine in 98% yield (Scheme 1).

The 3′-oxa-3′deoxythymidine was obtained through a similar route, except that in this case titanium Lewis acid was used to provide selectivity during the introduction of the base (Scheme 2). The dioxa lactone (**7**) was synthesized in 80% yield through the reaction between (**2**) and glycolic acid in refluxing 1,2-dichloroethane. Reduction with LiAlH(O*t*-Bu)$_3$ at 0°C in tetrahydrofuran (THF), followed by acylation with acetic anhydride in the presence of 4-dimethylaminopyridine (DMAP), led to the acetyl *O*-glycoside (**8**). Reaction of this mixture with dichlorotitanium diisopropoxide and silylated thymine (**9**) in dichloromethane (DCM) at 25°C produced the analog (**10**), which after deprotection gave the 3′-oxa-3′deoxythymidine (Scheme 2).

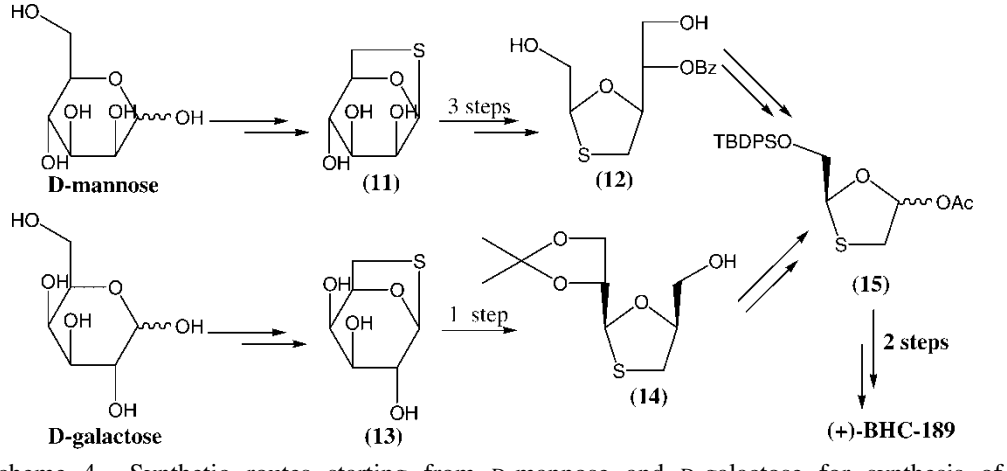
The use of stannic chloride (Scheme 1) and a titanium Lewis acid, dichlorotitanium diisopropoxide, (Scheme 2) in glycosilations steps contributed to the stereoselectivity in these reactions. On the other hand, the use of a commom Lewis acid lead, exclusively, to the formation of an oxonium ion, resulting in no stereochemistry control (pathway A, Scheme 3). However, a precomplexation *in situ* between a proper Lewis acid and a ring heteroatom is capable of blocking the α-face, selecting the silylated base's attack to the β-face. This study also suggests the possibility of an intermediate, formed by the attack of one of the metal ligands (in this specific case, chloride) to the anomeric carbon, C-1, that also contributes to the stereocontrol of these kind of reactions (pathway B, Scheme 3) (*17*).

Almost at the same time of Liotta's publication, Chu and co-workers reported the first asymmetric synthesis of enantiomerically pure (+)-BCH-189 from D-mannose, as well as its anti-HIV activity in human peripheral blood mononuclear cells (PBM) (*18,19*). Although this approach led to the desired product, the synthetic route had many steps and the key intermediate, 1,6-thioanhydro-D-mannose (**11**), was obtained with low yields. In another study, Jeong *et al.* (*20*) developed a more efficient and shorter route from 1,6-thioanhydro-D-galactose (*13*), a chiral intermediate obtained through large-scale preparation from D-galactose (*20*). Once obtained the key intermediate (**11**) or (**13**), the next steps resulted in an oxidation cleavage gave the acetate (**15**).



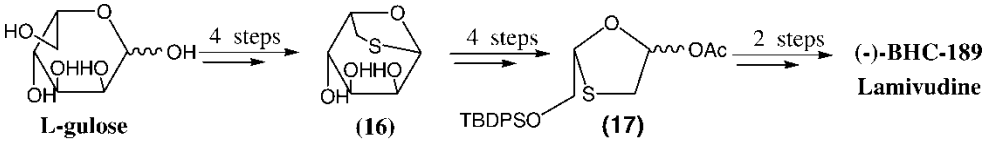
Scheme 3. Two different mechanisms of base-coupling reaction in the presence of Lewis acids.

Again, the synthetic route starting from D-galactose showed advantages over the previously described from D-mannose. In fact, the use of (**13**) as key intermediate allowed selective cleavage of the *cis*-diol and no sulfur oxidation, that also contributed to a lengthy synthetic route (Scheme 4).



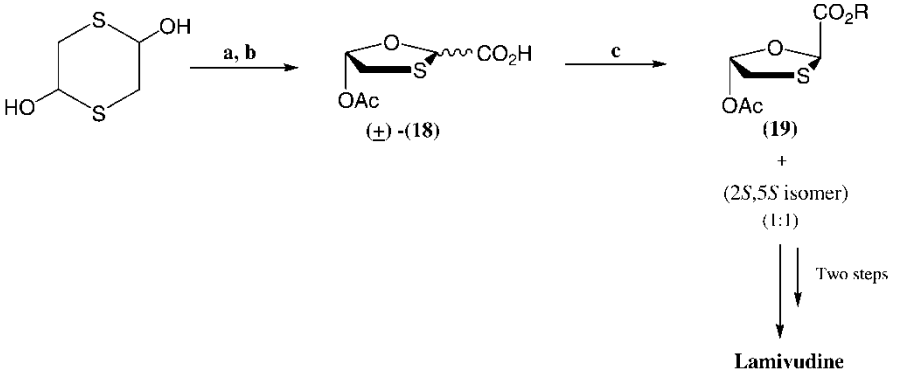
Scheme 4. Synthetic routes starting from D-mannose and D-galactose for synthesis of (+)-BCH-189.

Based on the discovery that the 'D-like' isomer was less potent than racemic BCH-189 in human PBM, Chu and co-workers (*21*) also reported the enantiomeric synthesis of (2′*R*,5′*S*)-(−)-BCH-189 (lamivudine) from L-gulose (*21*) and its anti-HIV and HBV-hepatitis. Initially, they obtained the key intermediate, 1,6-thioanhydro-L-gulose (**16**) in four steps from D-gulose. This route showed any similarity with the route for the (+)-BCH-189's synthesis from 1,6-thioanhydro-D-galactose (**13**), that was the viability of a direct cleavage of the *cis*-diol under appropriate conditions (Scheme 5).



Scheme 5. Synthetic routes starting from D-gulose for synthesis of Lamivudine.

Tse and co-workers (*22*) described the first diastereoselective synthesis of the lamivudine and its (+) enantiomer through a key intermediate (**19**), which was previously generated by chemical resolution of compound (**18**) via its menthyl ester derivatives (Scheme 6) (*22*).

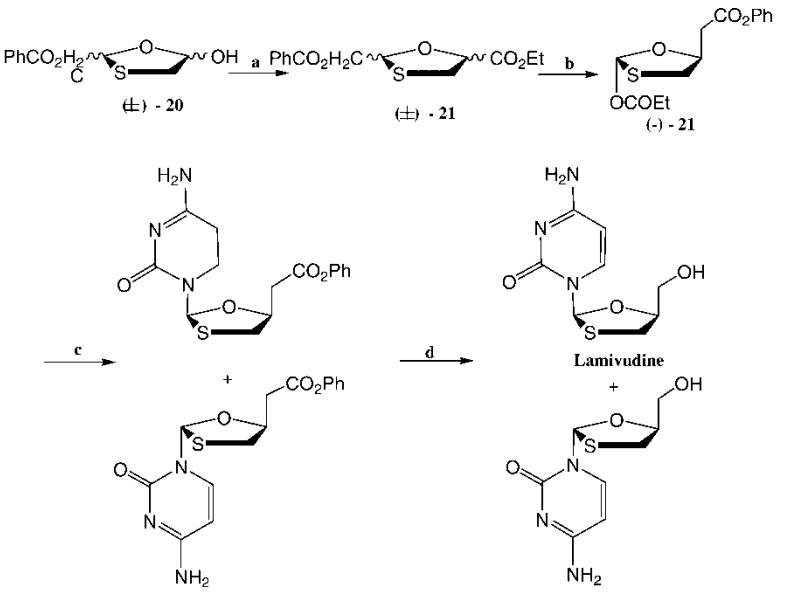


R=(-)-L-menthyl ; **a:** Glyoxylic acid hydrate, *t*-BuOMe, reflux; **b:** Ac_2O, $MeSO_3H$; **c:** (-)-L-menthol, dicyclohexylearbodiimide, DMAP, DCM.

Scheme 6. The first diastereoselective synthesis of the Lamivudine and its enantiomer developed by Tse and co-workers.

The use of enzyme is an important tool in asymmetric synthesis. In this context, an alternative to asymmetric total synthesis of lamivudine was disclosed by Cousins and co-workers (*23*), which was based on an enzymatic resolution of intermediates prior to the addition of the cytosine base (Scheme 7). Considering that, a number of commercially available lipases and proteases were screened for the ability to hydrolyze enantioselectively racemic oxathiolane (**21**) (Table 1). It was observed that enzymatic resolution of the oxathiolane propionate (**21**) with *Mucor miehei* lipase led to enantiomerically enriched substrate (−)-**21** which was converted into the nucleoside lamivudine with an induced enantiomeric excess of 70% (Scheme 7) (*23*).

Rayner and co-workers (*24,25*) also employed an enzymatic resolution of α-acetoxysulfides in an enantioselective synthesis of the lamivudine. The synthesis proceeds via a configurationally stable thioacetal (**23**) which cyclizes *in situ* to form the required oxathiolane nucleus (**24**), being converted into lamivudine in four steps. (Scheme 8). The overall enantiomeric excess (ee) obtained during the synthesis of (**24**) was significant. Initially, the enzymatic resolution of (**22**) showed dependence between the employed solvent and the ee obtained; the change from $CHCl_3$ to *t*-BuOMe resulted in a dramatic increase in ee. Soon afterwards, the conversion of (−)-(**22**) into the oxathiolane (**24**) processed with high yield and selectivity, considering the intermediate's stability (**23**) (*26*). Although the cyclization of (**23**) had been carried out in acid conditions, which could lead to racemization, the process maintained a high level of stereochemical integrity in relation to the resolved chiral center (Scheme 8).



a: EtCOCl, Pyridine, 0°C, DCM; **b:** *Mucor miehei*, Buffer, 28°C; **c:** silylated cytosine, TMSI, 1,2-dichloroethane; **d:** amberlite IRA400 (OH), IMS, reflux.

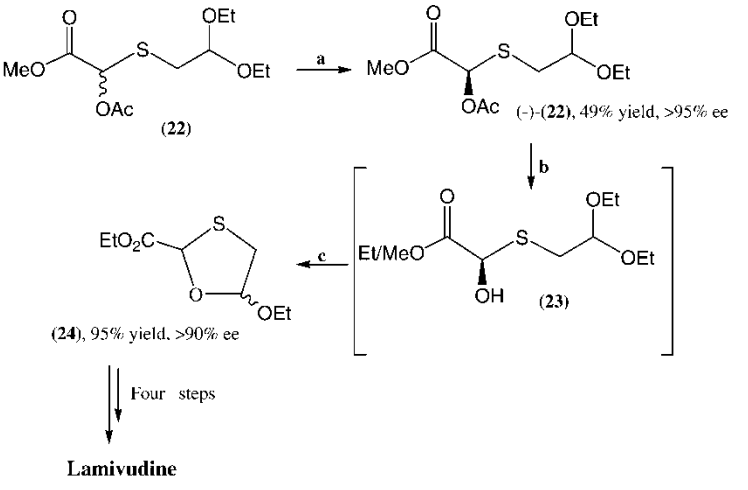
Scheme 7. An alternative asymmetric total synthesis of Lamivudine developed by Cousins and co-workers.

Table 1. Enzyme catalyzed resolution of racemic (±)-**21**.

Biocatalyst	Time (h)	Conversion (%)	Residual ester[a] (ee)	Absolute configuration[b]	E[c]
Aspergillus niger lipase[d]	1.5	67	16	(−)-2*R*	1
Candida cylindracae lipase[e]	3	89	5	(+)-2*S*	1
Chromobacterium viscosum					
Lipase[d]	15	91	10	(+)-2*S*	1
Lipase B1[f]	3	87	88	(+)-2*R*	3
Lipase F13[f]	24	67	48	(−)-2*R*	2
Lipase F14[f]	5	44	39	(−)-2*R*	4
Mucor miehei lipase[d]	2	48	65	(−)-2*R*	11
Pseudomonas fluorescens					
Lipase[d]	15	71	63	(−)-2*R*	3
Rhizopus delemar lipase[d]	22	34	36	(−)-2*R*	8
Subtilisin[e]	0.01	34	15	(+)-2*S*	2
Trypsin[e]	5	34	0	(±)	–

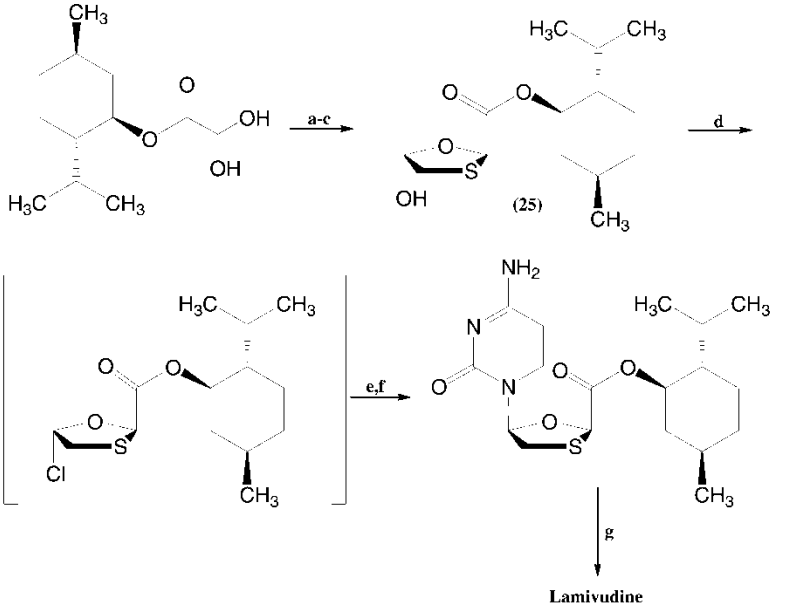
[a]ee, enantiomeric excess, by chiral hplc; [b]all compounds in trans series; [c]E, enantiomeric ratio; [d]supplied by Biocatalysts Ltd; [e]supplied by Sigma; [f]supplied by Chiroscience Ltd.

In 2005, Whitehead and co-workers (*27*) developed an enantioselective synthesis of lamivudine, which involved a dynamic kinetic resolution to obtain the 5-hydroxy-oxathiolane (**25**), followed by chlorination and coupling with pre-silylated cytosine (**5**). Further reduction of the ester group with sodium borohydride ($NaBH_4$) afforded lamivudine (Scheme 9) (*27*). Initially, the authors proposed a route based on the coupling reaction between an appropriately activated sugar moiety and pre-silylated cytosine, using trimethylsilyl iodide (TMSI) as the Lewis acid, which proved to



a: *Pseudomonas fluorescens* lipase / pH 7 phosphate buffer, 30°C, solvent; **b:** EtOH/HCl; **c:** cyclization *in situ*.

Scheme 8. Enantioselective synthesis of the Lamivudine through an enzymatic resolution of α-acetoxy sulfides.



a: Toluene, reflux; **b:** Dithiane diol; **c:** *n*-Hexane, Et_3N crystallisation (80% for 3 steps); **d:** $SOCl_2$, *N*,*N*-dimethylformamide, DCM; **e:** **(5)**, Et_3N, Toluene; **f:** Et_3N, water, n-hexane; **g:** $NaBH_4$, EtOH.

Scheme 9. Enantioselective synthesis of Lamivudine that involves, initially, a dynamic kinetic resolution to obtain the 5-hydroxy-oxathiolane (**25**).

be inefficient to achieve stereoselective and good yeilds. Mechanistic's studies suggested leaving groups change at C-5 during the process. In view of the problems encountered, it was decided to explore an alternative method which employed the synthesis of an chloride intermediate, as shown in Scheme 9. The 5-hydroxy-oxathiolane (**25**) was efficiently synthesized through a

highly effective crystallization-induced dynamic kinetic resolution employing trietylamine that was capable to convert unwanted diastereoisomers to the desired one.

7. Emtricitabine, a fluorine analog of lamivudine

Due to the success of the lamivudine in AIDS treatment and also in HBV, several analogs of this class have been synthesized and evaluated. In the begining of 1990s, a fluorine analog of lamivudine was synthesized by Liotta, Schinazi and Choi of Emory University (Atlanta, Georgia, USA) named emtricitabine (2′,3′-dideoxy-3′-thia-5-fluorocytidine, FTC) (Figure 1), which was licensed to Triangle Pharmaceuticals in 1996. This company was bought by Gilead Sciences, who finished the development of this drug with the brand name Emtriva®, being approved by FDA in 2003 (*28*). In comparison with lamivudine, emtricitabine is very similar in many aspects such as safety, activity, resistance, and convenience profile. However, one difference is that emtricitabine triphosphate possesses a longer intracellular half-life than L-TP. Usually, the choice between lamivudine and emtricitabine in patients with HIV is based on the drugs coformulated called the fixed dose combinations (FDCs).

8. Fixed dose combinations

FDCs are defined as combinations of two or more medications with the same indication in a single pill. This combination therapy is essential for the treatment of diseases in which drug resistance is a problem, such as malaria, tuberculosis, and specially HIV/AIDS. The advantages of FDCs compared with individual drugs given in combination are better prices, easier drug storage and use, which increase the patient adherence, reducing the risk of drug resistance (*29*). Currently, there are three kinds of FDCs approved by FDA using lamivudine with other AIDS drugs (Table 2). Considering one of these is in combination with zidovudine (AZT) under the brand name of Combivir®, approved by FDA in 1997. Another combination, a triple formulation involving lamivudine, abacavir sulfate, and zidovudine (Trizivir®) was approved by FDA in 2000. This regime allows a dosing schedule of one pill twice daily and also avoids side effects related to antiretroviral therapy, such as hyperlipidemia. The double combination lamivudine and abacavir sulfate (Epzicom®) was approved by FDA in 2004 as a once daily dosed in treatment-naive individuals in combination with other class of antiretroviral. The fluorine analog of lamivudine, emtricitabine has also FDCs approved by FDA, one with tenofovir (Truvada®) and other with efavirenz and tenofovir (Atripla®) (Table 2) (*30*).

Table 2. Lamivudine used in FDC in the treatment of HIV infection approved by FDA.

Chemical names	Brand name	FDA approved year
Drugs combination		
Lamivudine + zidovudine	Combivir	09/1997
Lamivudine + abacavir + zidovudine	Trizivir	11/2000
Lamivudine + abacavir	Epzicom	08/2004
emtricitabine + tenofovir	Truvada	10/2004
emtricitabine + efavirenz + tenofovir	Atripla	07/2007

9. Conclusion

Nowadays, AIDS is a very serious contagious disease, responsible for millions of deaths every year. In this context, lamivudine, the first unnatural L-nucleoside approved by the FDA for the treatment of HIV, is widely employed in combination drug therapy, therefore this drug possess some advantages in comparison with its respective D-nucleosides analogs. These include a well-tolerated safety profile and convenient dosing with maintenance of a favorable efficacy. Sulfur, an essential component of this drug also contributes to a low-host toxicity, once it blocks, as well as the drug's absolute configuration, the recognition by the host polymerases cells. Since the discovery of lamivudine for the treatment of HIV and also of HBV, a large number of analogs have been synthesized and evaluated.

References

(1) Sirois, S.; Sing, T.; Chou, K. *Curr. Protein Pept. Sci.* **2005,** *6,* 413–422.
(2) Wang, T.; Gong, Y.; Shi, P. *Front. Biotechnol. Pharm.* **2000,** *1,* 282–304.
(3) UNESCO. 2006 report on the global AIDS epidemic, 2006.
(4) www.fda.gov/oashi/aids/virals.html (accessed Nov 28, 2007)
(5) Brun, E.M.; García, M.G.; Puchades, R.; Maquieira, A. *J. Immunol. Methods* **2004,** *295,* 21–35.
(6) Curtin, D.; Beare, M.H.; McCallum, F.M. *Soil Biol. Biochem.* **2007,** *39,* 2547–2554.
(7) Cundari, S.; Dalpozzo, R.; Nino, A.; Procopio, A.; Sindona, G. *Tetrahedron* **1999,** *55,* 10155–10162.
(8) De Souza, M.V.N. *J. Sulfur Chem.* **2005,** *26,* 429–449.
(9) Chen, S. *Curr. Med. Chem.* **2002,** *9,* 899–912.
(10) Taylor, C.L.; Wilkins, E.G. *Future Virol.* **2007,** *2* (1), 11–21.
(11) Bergmann, W.; Burke, D.C. *J. Org. Chem.* **1955,** *20,* 1501–1507.
(12) Horwitz, J.P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964,** *29,* 2076–2078.
(13) Dionne, G. *McGill J. Med.* **1999,** *5,* 60–69.
(14) Coates, J.A.V.; Mutton, I.M.; Penn, C.R.; Storer, R.; Williamson, C. WO 91/17159, 1991.
(15) Ohta, Y.; Shinkai, I. *Bioorg. Med. Chem*. **1997,** *5,* 639–640.
(16) Anglada, M.P.; Felipe, A.; Casado, F.J. *Trends Pharmacol. Sci.* **1998,** *19,* 424–430.
(17) Choi, W.; Schinazi, R.; Wilson, L.J.; Yeola, S.; Liotta, D.C. *J. Am. Chem. Soc.* **1991,** *113,* 9377–9379.
(18) Jeong, L.S.; Schinazi, R.F.; Beach, J.W.; Shanmu, K.; Choi, B.G.; Comer, F.I.; Alves, A.J.; Chu, C.H. *J. Org. Chem.* 1991, *56,* 6503–6505.
(19) Jeong, L.S.; Schinazi, R.F.; Beach, J.W.; Kim, H.O.; Shanmu, K.; Nampalli, S.; Chun, M.W.; Chung, W.-K.; Choi, B.G.; Chu, C.K. *J. Med. Chem.* **1993,** *36,* 2627–2638.
(20) Jeong, L.S.; Alves, A.J.; Carrigan, S.W.; Kim, H.O.; Beach, J.W.; Chu, C.K. *Tetrahedron Lett.* **1992,** *33,* 595–598.
(21) Jeong, L.S.; Beach, J.W.; Kim, H.O.; Alves, A.J.; Pohl, D.; Chang, C.-N.; Doong, S.-L.; Schinazi, R.F.; Cheng, Y.-C.; Chu, K. *J. Org. Chem.* **1992,** *57,* 2217–2219.
(22) Jin, H.; Siddiqui, M.A.; Evans, C.A.; Allan, H.L.; Mansour, T.S. *J. Org. Chem*. **1995,** *60,* 2621–2623.
(23) Cousins, R.P.C.; Mahmoudian, M.; Youds, P.M. *Tetrahedron Asymmetry*, **1995,** *6,* 393–396.
(24) Milton, J.; Brand, S.; Jones, M.F.; Rayner, C.M. *Tetrahedron Lett.* **1995,** *36,* 6961–6964.
(25) Milton, J.; Brand, S.; Jones, M.F.; Rayner, C.M. *Tetrahedron Asymmetry*, 1995, *6,* 1903–1906.
(26) Linderman, R.J.; Tennyson, S.D.; Shultz, D.A. *Tetrahedron Lett.* **1994,** *46,* 6437–6440.
(27) Goodyear, M.D.; Hill, M.L.; West, J.P.; Whitehead, A.J. *Tetrahedron Lett.* **2005,** *46,* 9535–9538.
(28) Jian, Z.; Gregory, C.E.; Wang, H.; Laurene, H.; James, B.J.A.; Robert, B.M. *J. Clin. Pharm*. **2007,** *47,* 877–881.
(29) World Health Organisation. *Fixed-Dose Combinations for HIV/AIDS*, *Tuberculosis and Malaria*, WHO: Geneva, 2003.
(30) Food and Drug Administration. *Guidance for Industry: Fixed Dose Combinations*, *Co-Packaged Drug Products and Single-Entity Versions of Previously Approved Antiretrovirals for the Treatment of HIV*, FDA/CDER: Rockville, 2006.