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Mini review

Recent advances in L-nucleosides: chemistry and biology

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1. Introduction

L-Nucleosides are the enantiomers of the natural nucleosides which have an inverted configuration at all chiral centers (Fig. 1). By analogy with the natural D-nucleosides, a heterocycle moiety is designated to be in β-orientation if it is cis to the 4′-hydroxymethyl group in the sugar moiety. The classification of L-nucleoside and related analogs is also based on the 1,3-relationship between nucleobase and the 4′-hydroxymethyl group through the first ring oxygen, which may be either transposed to a new position and/or replaced with a methylene group, sulfur or other single heteroatom in the sugar moiety.

Although the first synthesis of L-nucleoside was reported in the 1960s, little attention has been given to L-nucleosides until the emergence of 3TC. The living world is supposed to be in a completely asymmetric environment at the molecular level and most biologically active nucleosides have the natural β-D-configuration as most amino acids have the L-configurations. Enzyme reactions are normally highly stereoselective (nearly stereospecific) and act mainly on one enantiomer, such as D-nucleosides and L-amino acids. This may be the possible reason why it took so long to pay attention on the L-nucleosides. It was believed that the optically active compounds resembling to natural nucleosides would be invariably more active than the 'unnatural' L-enantiomers. Belleau et al. first described the synthesis and anti-HIV activity of an unusual nucleoside analog, ($\pm$)-2,3′-dideoxy-3′-thiacytidine (BCH-189) (Belleau et al., 1989a). Subsequently, it was found rather surprisingly that the L-isomer of BCH-189 (3TC) was more potent and less toxic than than its D-isomer (Coates et al., 1992; Schinazi et al., 1992a). Since then, a number of L-nucleoside analogs have been synthesized and biologically evaluated, and the importance of chirality and the influence on an-

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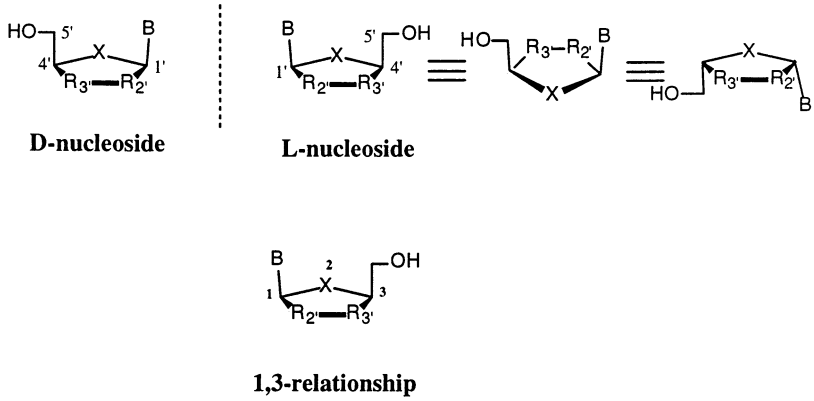


Fig. 1. D,L-Nucleoside and 1,3 relationship.

tiviral activity of the L-nucleosides has been recognized. However, the most surprising aspect in the related subject was the fact that in some instances cellular kinases are able to phosphorylate the L-nucleosides to their triphosphates, and thereby provide various biological activities.

The physical and chemical properties of L-nucleosides are identical with those of D-counterparts except optical rotation. However, their pharmacological properties in chiral environment can be different for each isomer. The origin of these differences may be (1) transportation of nucleoside into the cells, (2) anabolic and catabolic enzymes that act on these compounds, (3) interaction with the viral and/or cellular target enzymes.

The intent of this paper is to provide an overview of biologically active L-nucleosides, with emphasis on anti-HIV and anti-HBV agents.

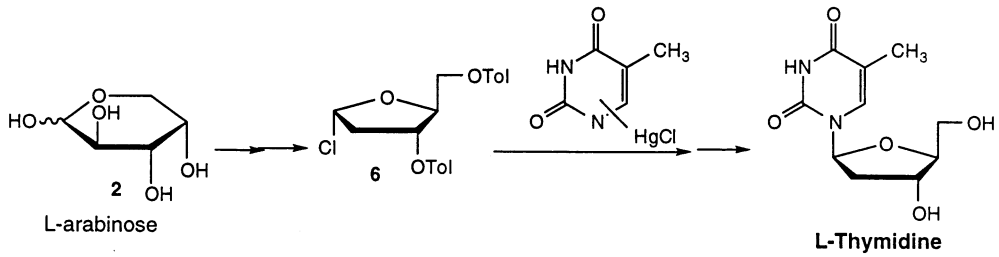
2. History of L-nucleosides

In 1964, Smejkal and Sorm reported the synthesis of the first L-nucleoside, L-thymidine, (Smejkal and Sorm, 1964) which was synthesized from 2-deoxy-L-riboside by the procedure described for the D-isomer (Scheme 1). In the same year, Acton et al. reported a synthetic method for L-adenosine from L-arabinose and L-xylose using coupling of the resultant chlorosugar with chloromercuri-6-benzamidopurine, according to the known procedure for the adenosine (Scheme 2) (Acton et al., 1964).

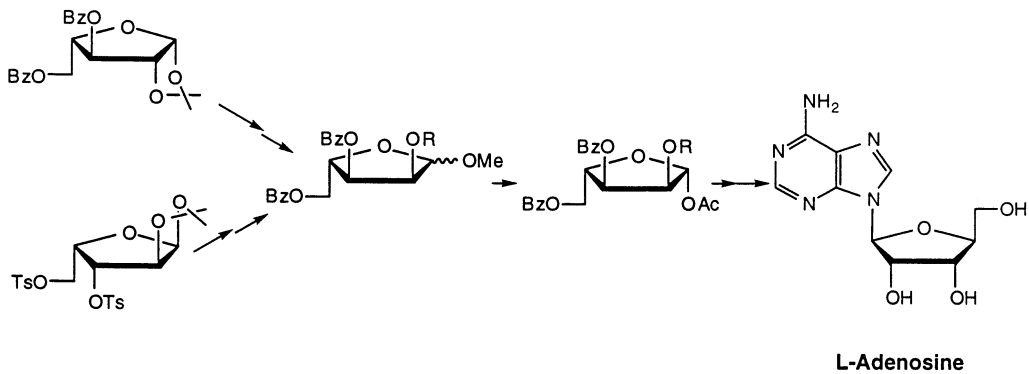
Other L-nucleosides such as L-uridine, L-cytidine, L-adenosine, L-guanosine and 5-methyl-L-uridine were synthesized by Holy et al. (Scheme 3) (Holy and Sorm, 1969). The synthesis of 2′-deoxy-L-adenosine and 2′-deoxy-L-guanosine was reported by Robins et al. using the fusion method (Robins et al., 1969). Holy also reported the synthetic method of 2′-deoxy-L-uridine, 2′-deoxy-L-thymidine and 2′-deoxy-L-cytidine using the linear base-construction method instead of the direct condensation (Holy, 1971, 1972).

During the past ten years, additional L-nucleosides have been prepared and their biological activities have been evaluated. L-AZT, the L-isomer of anti-HIV drug 3′-azido-3′-deoxy-thymidine (AZT) was synthesized by Wengel et al. using L-arabinose as a starting material (Scheme 4) (Wengel et al., 1991). The anti-HIV activity of L-AZT was reported to be about 10000 times lower than that of AZT. The general synthetic method of β-L-2′-deoxyribo-purine and pyrimidine nucleosides was also reported by condensing the corresponding chlorosugar with sodium salt of 6-chloropurine or with silylated pyrimidine bases (Fujimori et al., 1992).

Previously, only a few studies have been reported on the biological study of L-nucleosides and nucleotides. It was reported that L-nucleosides neither penetrate bacterial cell walls nor interfere with the penetration of naturally occur-



Scheme 1. Synthesis of L-thymidine

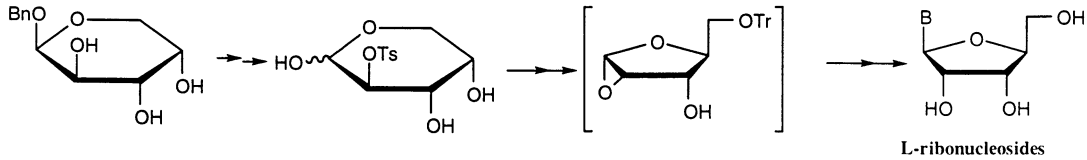


Scheme 2. Synthesis of L-adenosine.

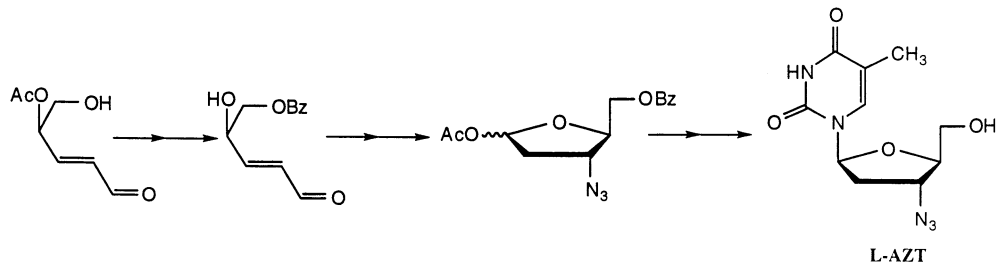
ing nucleosides through bacterial cell membrane (Votruba et al., 1971). Also, neither splitting of a nucleosidic linkage nor deamination of the heterocyclic base (with L-adenosine and L-cytidine) was observed. It was reported that the administration of L-nucleosides (L-cytidine, L-uridine, and L-thymidine) to mice resulted in distribution of these compounds into tissues and gradual excretion of the unchanged form (Jurovcik and Holy, 1976).

The incorporation of L-nucleosides into oligonucleotides to produce L-DNA (Hashimoto et al., 1993; Garbesi et al., 1993) and L-RNA (Ashley, 1992) has been reported. Two unnatural L-oligodeoxyribonucleotides, namely α- and β-L-$dT_8PO(CH_3)OH$, have been synthesized. Interestingly, these oligomers are resisitant towards nuclease degradation. They do not show any base pairing with β-D-dA_8 and poly rA (Morvan et al., 1990). Some L-nucleosides were found to have biological activity: L-adenosine diphosphate (L-ADP) interacts with bacterial polynucleotide phosphorylase (Simuth and Holy, 1975). It was also found that L-adenosine can act as a plant growth-regulating second messenger and increase Ca^{2+}, Mg^{2+} and K^+ concentration of the exudate from the stumps of excised plants (Ries et al., 1993). Spadari et al. have demonstrated that L-thymidine is not recognized by human thymidine kinase (TK), but functions as a substrate for herpes simplex 1 (HSV1) thymidine kinase in HeLa cells (Spadari et al., 1992). HSV1 thymidine kinase shows no stereospecificity and phosphorylates both D- and L-thymidine to their corresponding monophosphates with identical efficiency, and the K_i of L-thymidine (2 μM) is almost identical to the K_m for the natural substrate thymidine (2.8 μM). Also, Van Draanen et al. reported that β-L-3′-deoxythymidine 5′-triphosphate (L-ddTTP) and β-L-3′-deoxy-2′,3′-didehydrothymidine 5′-triphosphate (L-d4TTP) were substrates for human immunodeficiency virus reverse transcriptase, *Escherichia coli* DNA polymerase I, and sequenase (Van Draanen et al., 1992).

Bennett et al. showed that D- and L-carbocyclic analogs of 2″-deoxyguanosine (L-CdG) (Bennett et al., 1993) were equally effective competitive



Scheme 3. Synthesis of L-ribonucleosides.



Scheme 4. Synthesis of L-AZT.

inhibitors of the phosphorylation of thymine (dThd) by partially purified HSV-1 TK (K_i values were 2.1 and 3.4 mM, respectively) and were also equally effective as substrates (K_m values were 17 and 26 μM, respectively). In CEM cells, deoxycytidine (dCyd) kinase seemed to be the enzyme principally responsible for the phosphorylation of both enantiomers. Thus, both HSV-1 TK and cellular dCyd kinase have shown no selectivity for enantiomers of CdG.

It is reported that L-thymidine (L-dT) is selectively phosphorylated in vitro to L-dT monophosphate (L-dTMP) by HSV-1 thymidine kinase (TK) and L-dTMP is further phosphorylated to the di- and triphosphate by non-stereospecific cellular kinases (Focher et al., 1995). L-dTTP not only inhibits HSV-1 DNA polymerase in vitro, but also human DNA polymerases α, β, δ and ε, human immunodeficiency virus reverse transcriptase (HIV-1 RT). Spadari et al. also reported (Spadari et al., 1995) that deoxycytidine kinase, HSV-TK, deoxynucleoside mono- and diphosphate kinases, and cellular and viral DNA polymerases lack stereospecificity in the synthesis of nucleotides and DNA. From the chemotherapeutic point of view, the ability of certain enzymes to utilize certain unnatural L-nucleosides can be exploited for the design of selective antiviral agents.

3. Biologically active L-nucleosides

3.1. Oxathiolane nucleosides

The most exciting and surprising development in L-nucleosides was the discovery of (±)-BCH-189, a compound in which the 3′-carbon of the furanose sugar moiety is replaced by a sulfur atom (Fig. 2) (Belleau et al., 1989a). (±)-BCH-189 exhibits potent in vitro anti-HIV activity in T-cell lines as well as in primary cultures of human peripheral lymphocytes. Moreover, in cell culture assays, (±)-BCH-189 is less toxic than AZT, inhibited AZT-resistant virus and was non-toxic at 100 mg/kg given orally over 14 days in rats (Soudeyns et al., 1991). (±)-BCH-189 also shows potent anti-HBV activity in 2.2.15 cells (Doong et al., 1991).

(±)-BCH-189 was resolved first in milligram quantity by preparative HPLC and subsequently in gram quantities by enzymatic methods (Storer et al., 1993; Mahmoudian et al., 1993). Resolution of (±)-BCH-189 showed that both enantiomers had potent anti-HIV activity whereas cytotoxicity resides with the natural (+)-D-form (Coates et al., 1992).

The first synthetic approach to (±)-BCH-189 reported by Belleau et al. utilized an oxathiolane

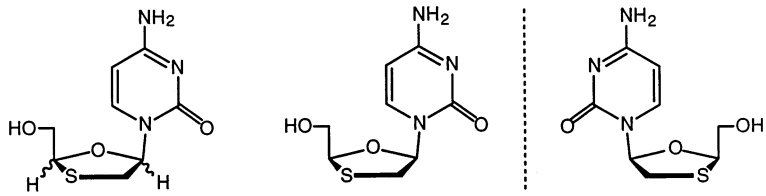


Fig. 2. BCH-189 and 3TC.

intermediate obtained as a 1:1 mixture of anomers from reaction of benzoyl oxyacetaldehyde with mercaptoacetaldehyde, was then coupled with silylated cytosine to give a 1:1 mixture of cis and trans nucleosides (Belleau et al., 1989b). After separation by chromatography followed by deprotection, (±)-BCH-189 was obtained (Scheme 5).

Various approaches for the synthesis of 3TC have been reported. Liotta et al. first reported the synthesis of 3TC using $SnCl_4$ as the catalyst afforded the β-anomer as the major product, followed by enzyme resolution (Scheme 6) (Choi et al., 1991).

Asymmetric synthesis of the enantiomeric pure isomers of BCH-189 was first reported by Chu and co-workers from D-mannose and D-galactose, (Jeong et al., 1993a) or L-gulose as starting materials (Scheme 7) (Jeong et al., 1993b; Beach et al., 1992).

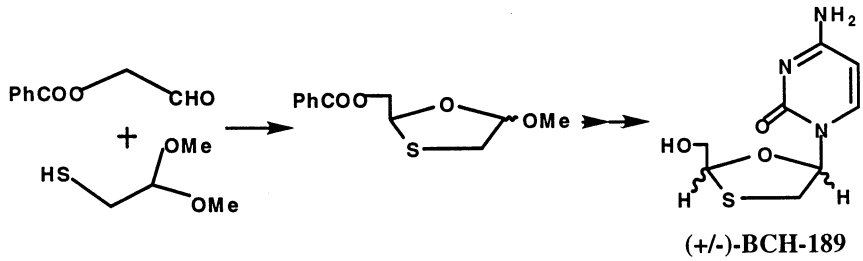
The isomer with unnatural configuration, (−)-β-L-1,3-oxathiolanyl-cytosine (now called 3TC, lamivudine, Fig. 2) showed potent anti-HIV activity and its antiviral potency is dependent on the cell type used in the assay. Comparison of the antiviral activity indicated that 3TC (EC_{50} 0.0018 μM in PBM cells) showed more potent activity than its racemate, (±)-BCH-189 (EC_{50} 0.02–0.06 μM) (Schinazi et al., 1992a) or nucleoside with the natural nucleoside configuration, (+)-β-D-1,3-oxathiolanyl-cytosine, (+)-BCH-189 (EC_{50} 0.21 μM) in PBM cells (Chu et al., 1991a) against HIV-1. Against HIV-2, 3TC and its D-enantiomer exhibit comparable antiviral activities. 3TC shows little or no cytotoxicity, whereas (+)-BCH-189 shows cytotoxicity (IC_{50} 2.7 μM) in human PBM cells (Table 1).

3TC (EC_{50} 0.01 μM) shows also anti-HBV activity, in which it is more potent than its racemate (EC_{50} 0.05 μM) or (+)-BCH-189 (EC_{50} 0.5 μM) (Jeong et al., 1993b). 3TC has no effect on mitochondrial DNA synthesis, while (+)-BCH-189 exhibits a dose-dependent effect on mitochondrial DNA synthesis (Parker and Cheng, 1994). 3TC has been approved by FDA for the treatment of AIDS and phase III clinical trials have been completed as an anti-HBV agent (Dienstag et al., 1995).

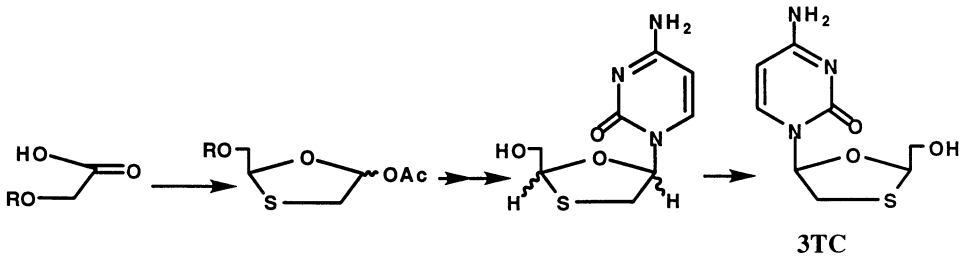
The cellular metabolism of the D- and L-isomers of BCH-189 has been reported. It was found that deoxycytidine kinase is the enzyme responsible for the monophosphorylation of 3TC (Severini et al., 1995) and that 3TC is a better substrate for this enzyme than the D-enantiomer (Shewach et al., 1993). Deoxycytidylate kinase and nucleoside diphosphate kinases are the possible enzymes that phosphorylate the monophosphate of 3TC to its di- and triphosphate, respectively (Scheme 8).

3TC is resistant to deamination by deoxycytidine deaminase, whereas the D-enantiomer is deaminated to 2′-deoxy-3′-thiauridine by the same enzyme (Chang et al., 1992). The half-life of 3TC triphosphate was 12–15.5 h in mock-infected cells and 10.5–13 h in HIV-1-infected cells compared to significantly shorter half-life for the D-enantiomer (3.5 h in mock-infected cells and 5–7 h in HIV-1-infected cells) (Cammack et al., 1992). The better substrate specificity for deoxycytidine kinase, the resistance to deamination as well as the longer half-life of the triphosphate of 3TC may explain the observation that 3TC is more potent than its D-enantiomer against HIV-1 in vitro.

3TC is active against AZT-resistant strains (Boucher et al., 1993). Furthermore, the combination of 3TC with AZT provide more thorough viral suppression and may limit the emergence of



Scheme 5. Synthesis of (±)-BCH-189.

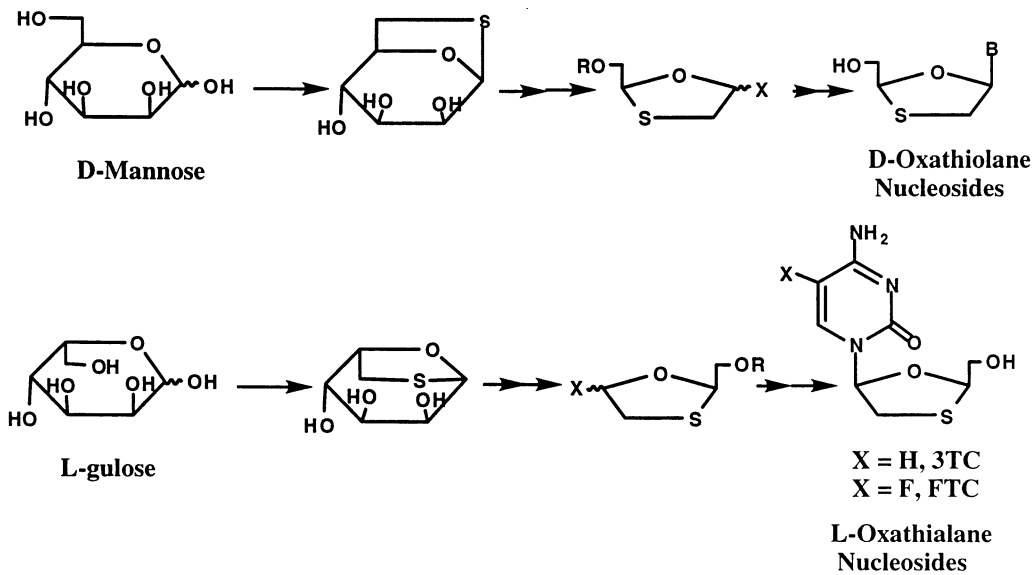


Scheme 6. Synthesis and kinetic resolution of 3TC.

drug resistance (Bartlett et al., 1996; Staszewski et al., 1996). In 3TC/AZT combination therapy, as much as a 2 log decrease in viral burden was observed, which was sustained for nearly one year. Also, combination treatment with 3TC and AZT is well tolerated and provides greater and more sustained increase in $CD4^+$ cell counts of the HIV-1-infected patients. Adverse events were no more frequent with the combination therapy than with AZT alone (Eron et al., 1995; Katlama et al., 1996). Recently, triple combination therapy against HIV has also been found to be the most promising (Gulick et al., 1997; Hammer et al., 1997). The combination of 3TC, AZT and indinavir reduces the levels of HIV-RNA to less than 500 copies/ml for HIV-infected patients for as long as one year and CD4 counts increased. To achieve the sustained potent anti-HIV effects as well as reduce the drug resistance, combination therapy will likely be routing for HIV chemotherapy.

It has been reported that the triphosphate of 3TC showed potent inhibitory activity against HBV-associated DNA polymerase and that it was a better inhibitor of HBV DNA polymerase than its D-enantiomer (Furman et al., 1995). The triphosphate of 3TC has quite high K_i values of 175 ± 31, 24.8 ± 10.9 and 43.8 ± 16.4 μM against human DNA polymerase α, β, and γ, respectively, whereas the triphosphate of the D-enantiomer has a K_i value of 0.049 ± 0.005 μM for human DNA polymerase γ. It was also reported that 3TC triphosphate is a rather weak inhibitor of DNA polymerase γ. It has been also found that 3TC triphosphates is a rather weak inhibitor of DNA polymerase γ but the triphosphates of racemic BCH-189 and D-BCH-189 are much more inhibitory (Hart et al., 1992). This may explain why 3TC was significantly less toxic than either the racemic or the D-enantiomer.

Schinazi et al. reported the anti-HIV activity of racemates and enantiomers of 5-fluoro analog of 3TC, (−)-2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC) (Fig. 3) (Schinazi et al., 1992b). FTC showed potent antiretroviral activity against HIV-1, HIV-2, simian immunodeficiency virus, and feline immunodeficiency virus in various cell cultures. Similar to 3TC, the unnatural form, (−)-β-L-FTC exhibited significant difference in antiviral activity compared to its natural D-enantiomer, (+)-FTC. The (−)-enantiomer exhibits 20-fold more potent anti-HIV-1 activity (EC_{50} 0.008 μM) than its D-counterpart (EC_{50} 0.84 μM) in PBM cell. (−)-FTC exhibits 40 times more active than



Scheme 7. Enantiomeric synthesis of 1,3-oxathiolane nucleosides.

its D-counterpart against HIV-2 in vitro. It is also significantly less toxic than its D-enantiomer to myeloid progenitor cells. (−)-FTC also exhibits anti-HBV activity (EC_{50} 0.01 μM) in hepatoma cell lines (HepG2 cells) whereas the D-enantiomer is significantly less potent (Sommadossi et al., 1992; Furman et al., 1992). Unlike 3TC and (+)-BCH-189, neither the D- nor the L-isomer of FTC showed significant cytotoxicity in human bone marrow progenitor cell assays. Both D- and L-isomers of FTC did not display any detectable hepatoxic effects at concentrations above their antiviral activities (Cui et al., 1996).

Similar to 3TC, enzymatic studies showed that the (+)-FTC was a substrate for deoxycytidine deaminase, whereas the (−)-FTC was resistant to deamination by the enzyme. (−)-FTC demonstrates an oral bioavailability of 73%, and penetrates the blood-brain barrier (Schinazi et al., 1992c). Additionally, (−)-FTC also showed strong inhibition to the replication of DHBV in vivo in chronically infected ducks (Fourel et al., 1994). Recently, it was reported that neither the D- or L-isomer nor the racemate of (±)-FTC shows any dose-dependent adverse effect on the mitochondrial function (Frick et al., 1994). (−)-FTC is currently undergoing clinical trials as an anti-HIV and anti-HBV agent.

Biological studies suggested that similar to 3TC, deoxycytidine kinase, deoxycytidylate kinase and possibly nucleoside diphosphate kinases are the enzymes that phosphorylate L-FTC to its triphosphates, which function as chain terminators of the viral DNA synthesis (Scheme 9) (Shewach et al., 1993).

The uptake and metabolism of the resolved enantiomers of FTC indicated that L-FTC entered cells readily by multiple transport mechanisms while D-FTC entered HepG2 cells by the NBMPR-susceptible equilibrative nucleoside transporter (Paff et al., 1994). Although the D- and L-enantiomers of FTC triphosphates were equipotent and alternative substrate inhibitors for HIV reverse transcriptase, L-FTC proved to be anabolized more efficiently and to be a better substrate for the deoxycytidine kinase than the corresponding D-enantiomer (Furman et al., 1992). Intracellular concentrations of the D- and L-FTC triphosphates were compared following incubation with racemic FTC and there were 30 times more L-FTC triphosphate than the corresponding D-FTC triphosphate. Therefore, potent anti-HIV activity of L-FTC and 3TC is caused by their selective phosphorylation steps together with the stereoselective deaminations of the D-enantiomers.

Table 1
Comparison of D- and L-oxathiolane-cytosine analogs

	(+)-D-BCH-189	(−)-L-3TC	(+)-D-FTC	(−)-L-FTC
Anti-HIV-1 (EC_{50}, μM)				
PBM	0.2	0.002	0.84	0.008
CEM	0.1	0.07	1.4	0.009
Anti-HBV (EC_{50}, μM)				
2.2.15	0.5	0.01	0.96	0.01
Cytotoxicity (ID_{50}, μM)				
PBM	2.7	>100	>100	>100
CEM	>100	>100	>100	>100

(Schinazi et al., 1992a; Beach et al., 1992; Schinazi et al., 1992b; Furman et al., 1992).

3.2. Dioxolane nucleosides

Dioxolane nucleosides are also 3′-heteroatom substituted analogs, where the 3′-carbon of the furanose sugar moiety is replaced by an oxygen atom. Originally, (±)-dioxolane-cytosine was reported by Belleau and co-workers (Belleau et al., 1989a) as anti-HIV agent and subsequently, (±)-dioxolane-thymine (Norbeck et al., 1989) was also reported as moderately active against HIV (EC_{50} 20 μM in ATH8 cells).

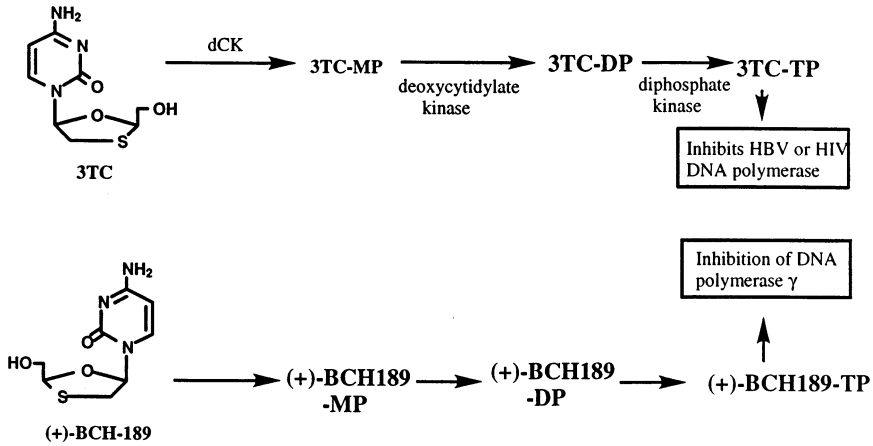
Chu and co-workers synthesized enantiomerically pure isomers of (±)-dioxolane-thymine and and (±)-dioxolane-cytosine (Chu et al., 1991b). Extensive studies of the structure–activity relationships led to the syntheses of β-L-dioxolanylcytosine (L-OddC) and its 5-fluoro derivative (L-OddFC), which exhibited potent activity against HIV-1 and HBV activities in vitro (Fig. 4) (Kim et al., 1992).

Synthesis of L-OddC and its analogs has been carried out using L-gulose as the starting material (Scheme 10) (Kim et al., 1993a).

Although L-OddC exhibits extremely potent anti-HIV activity (EC_{50} 2 nM in PBM cell) and anti-HBV activity (EC_{50} 0.5 nM in 2.2.15 cells), it is also quite toxic (IC_{50} 0.1 μM and IC_{50} 0.26 μM in Vero and CEM cells, respectively). Unlike its D-enantiomer, L-OddC is not susceptible to degradation by cytidine deaminase. L-OddC was found to be stable against deoxycytidine deaminase and metabolized in cells by deoxycytidine kinase to its monophosphate, and subsequently to the di- and triphosphate. Its triphosphate inhibits DNA polymerase α, β and γ (Scheme 11).

L-OddC may not be useful as an agent because of its cytotoxicty. The in vitro and in vivo data demonstrate that L-OddC has significant anticancer potential and has unique properties which make it an interesting candidate for further testing as an anticancer agents. Not only is it the first L-nucleoside analog ever shown to have anticancer activity, but also the first true chain-terminator, capable of inhibiting tumor growth. Although its unnatural stereochemistry does not prevent L-OddC from being activated by cellular metabolic enzymes or from being incorporated into DNA, the lack of substrate specificity for dCyd deaminase strengthens the biological activity. L-OddC is also unique in that it is active in solid tumors that are usually unresponsive to nucleoside analogs. L-OddC exhibits potent antitumor activity against various solid tumor cell lines, including prostate, renal, hepatoma, and colon (Grove et al., 1997). L-OddC is currently undergoing Phase I clinical trials as an anticancer agent (Grove et al., 1995).

The structure–activity relationships of various enantiomerically pure dioxolanyl purine nucleosides were also reported (Kim et al., 1993a,b). The enantiomers of 2,6-diaminopurine-dioxolane analogs, L-DAPD and D-DAPD, exhibit potent anti-HIV and anti-HBV activities (Fig. 4). Interestingly, L-DAPD exibits more potent anti-HIV activity (EC_{50} 0.014 μM) than D-DAPD (EC_{50} 0.7 μM) in PBM cell, while D-DAPD exhibits more



Scheme 8. Metabolism of optical isomers of BCH-189.

potent anti-HBV activity (EC_{50} 0.009 μM in 2.2.15 cells) than its L-isomer (EC_{50} 8.3 μM) with favorable toxicity profiles (Schinazi et al., 1994). Pharmacokinetic studies suggest that D-DAPD is the prodrug of the corresponding guanine derivative, dioxolane-guanine (DXG), and it is converted to DXG by adenosine deaminase (Rajagopalan et al., 1994). Currently, D-DAPD is undergoing preclinical evaluation as a potential anti-HIV and anti-HBV agent.

3.3. 2′,3′-Dideoxy-L-nucleosides

Several groups reported the synthesis and biological evaluation of various 2′,3′-dideoxy-L-nucleosides, including L-ddC and L-FddC (Fig. 5).

Okabe et al. reported (Okabe et al., 1988) that L-ddC showed no activity against HIV, however, Mansuri et al. and Van Draaken et al. reported (Mansuri et al., 1991; Van Draanen et al., 1994) that the compound exhibited some activity against the same virus. Gosselin et al. confirmed the anti-HIV and anti-HBV activity of L-ddC (Gosselin et al., 1994). Lin et al. also reported (Lin et al., 1994a) that L-ddC showed potent anti-HBV activity (EC_{50} 0.01 μM) in 2.2.15 cells and moderately potent anti-HIV activity (EC_{50} 5 μM) without significant mitochondrial DNA toxicity up to 100 μM.

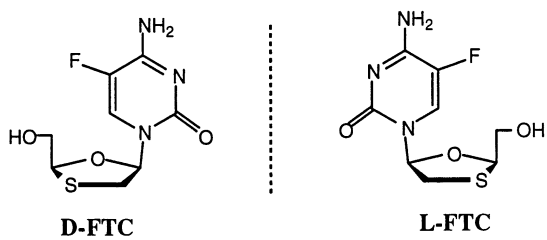
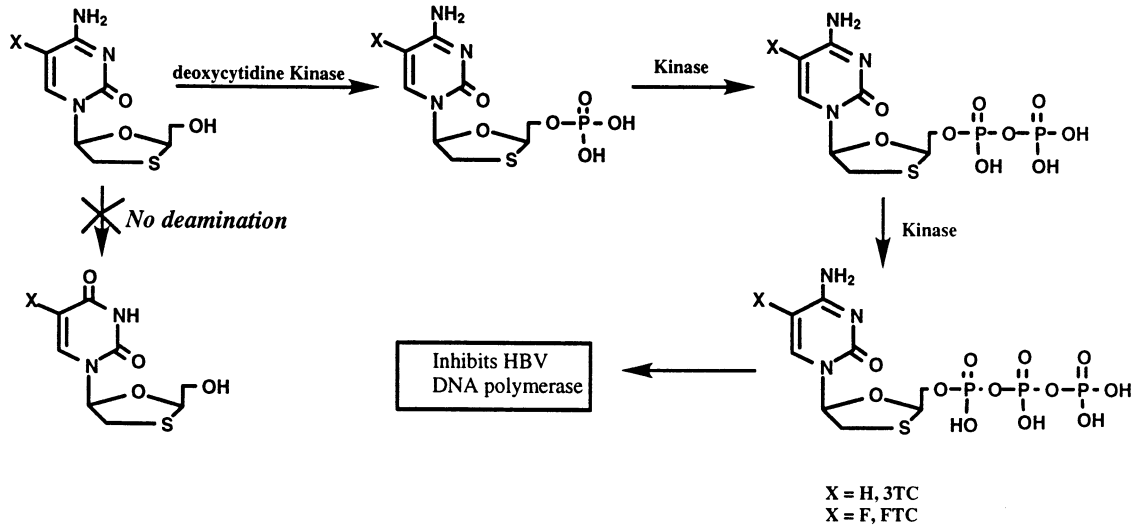


Fig. 3. FTC.

The synthesis of L-ddC was accomplished by several groups. Lin et al. reported the preparation of L-ddC from L-arabinose (Scheme 12) (Lin et al., 1994b).

Its 5-fluoro analog, L-FddC, has been reported to exhibit anti-HIV (EC_{50} 0.5 μM) and anti-HBV activity (EC_{50} 0.01 μM) without significant toxicity in vitro (Gosselin et al., 1994) (Table 2). L-FddC is approximately 3 and 4 times more potent against HIV-1 and 280 and 1000 times more potent against HBV in vitro than D-ddC and D-FddC (Fig. 5), respectively. Toxicity studies indicated that L-FddC increased lactic acid production, although it does not significantly increase the mitochondrial DNA content.

Biochemical studies of D- and L-ddC suggest that both enantiomers are activated by deoxycytidine kinase, and the L-enantiomer is a better substrate for this enzyme than the D-isomer (Van Draanen et al., 1994). However, there is little difference between the K_i values for the D- and L-ddC triphosphates (K_i 1.1 and 2.0 μM, respectively) against HIV reverse transcriptase (RT),



Scheme 9. Mechanism of 3TC and FTC.

indicating the ddC triphosphates have no stereoselectivity against the target enzyme, HIV RT (Faraj et al., 1994). L-ddC and L-FddC triphosphates did not inhibit human DNA polymerase α and β up to 100 μM, whereas D-ddC and D-FddC triphosphates inhibited human DNA polymerase β with K_i values of 0.5 and 2.5 μM, respectively.

Anti-HIV and anti-HBV activities as well as cytotoxicities of β-L-2′,3′-dideoxyadenosine (L-ddA) (Fig. 6) and its analogs have been reported (Bolon et al., 1996). L-ddA was found to have modest anti-HIV (EC_{50} 8.2 μM in PBM cells) activity, which is less potent than its D-enantiomer. L-ddA has also moderate anti-HBV activity with an EC_{50} value of 6.0 μM in 2.2.15 cells, while its D-enantiomer, D-ddA, was inactive (Mansuri et al., 1989). Recently, Imbach and coworkers reported that the prodrug of L-ddA monophosphate showed potent antiviral activity against HBV in vitro (Loi et al., 1997; Placidi et al., 1997; Imbach et al., 1998). The prodrug may effectively deliver the monophospahate to the target cells, where the phosphorylation of L-ddA is not efficient or lacking as indicated by modest antiviral potency described above.

L-ddG showed modest cytotoxicity in CEM cells with no significant antiviral properties (Lin et al., 1995a). Another L-nucleoside, 2′,3′-β-L-dideoxy-5-azacytidine (L-AzaddC) was reported to have moderately potent anti-HBV activity with an ED_{50} value of 1.5 μM, approximately the same level as that of ddC (Lin et al., 1995b).

3.4. 2′,3′-Dideoxy-2′,3′-didehydro-L-nucleosides

Two cytidine derivatives 2′,3′-dideoxy-2′,3′-didehydro-β-L-cytidine (L-d4C) and 2′,3′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (L-Fd4C) were reported to have potent anti-HIV and anti-HBV activities (Fig. 7) (Lin et al., 1996). L-d4C exhibits anti-HIV (EC_{50} 1.0 μM in CEM cells) and anti-HBV (EC_{50} 0.008 μM in 2.2.15 cells) activity. Furthermore, L-Fd4C shows more potent antiviral activity, which is 4-fold and 10-fold more potent than that of L-d4C as anti-HIV(EC_{50} 0. 09 μM in CEM cells) and anti-HBV agent (EC_{50} 0.002 μM in 2.2.15 cells), respectively. However, both compounds were potent inhibitors of cell growth at concentrations below 20 μM, although L-Fd4C does not exhibit any significant mitochondrial DNA inhibition in CEM cells at 100 μM.

Several 2′,3′-dideoxy-2′,3′-didehydro-β-L-purine nucleosides have been synthesized as potential antiviral agents (Bolon et al., 1996). Among these, L-d4A exhibited significant anti-HIV (EC_{50} 0.38

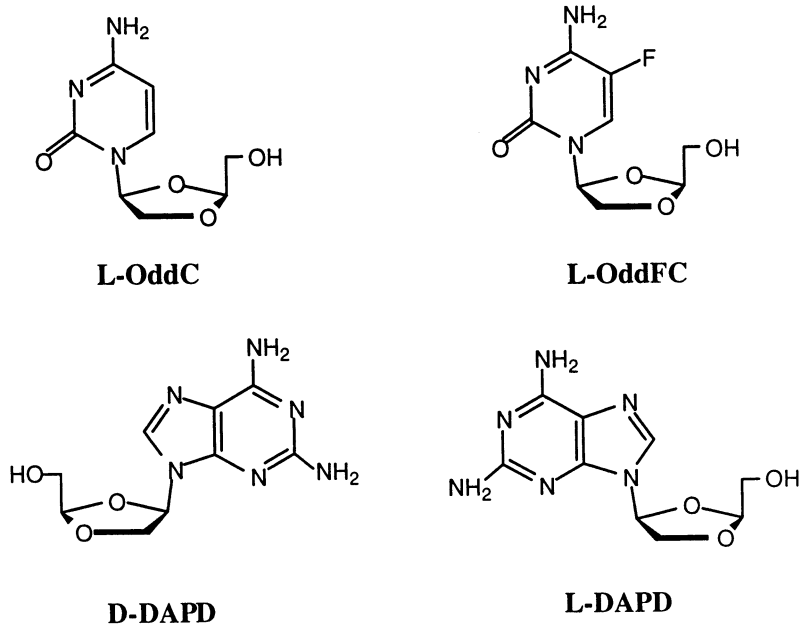


Fig. 4. Dioxolane nucleosides.

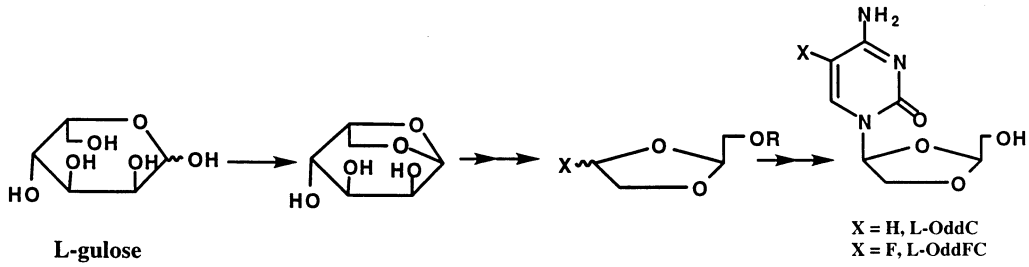
μM in PBM cells) and anti-HBV (EC_{50} 1.2 μM in 2.2.15 cells) activities, and its D-enantiomer, β-D-d4A, was also active against HIV (EC_{50} 0.76 μM in PBM cells) (Chu et al., 1988). The hypoxanthine and guanine analogs, L-d4I and L-d4G, were less potent against HIV and inactive against HBV. The adenine analog (L-d4A) exhibited a similar toxicity profile when compared to the hypoxanthine and guanine analog.

L-2′-Fluoro-2′,3′-dideoxy-2′,3′-didehydroadenosine (L-2′-Fd4A) was synthesized and it exhibited moderately potent anti-HIV activity with EC_{50} 1.5 μM in PBM cells (Choi et al., 1998).

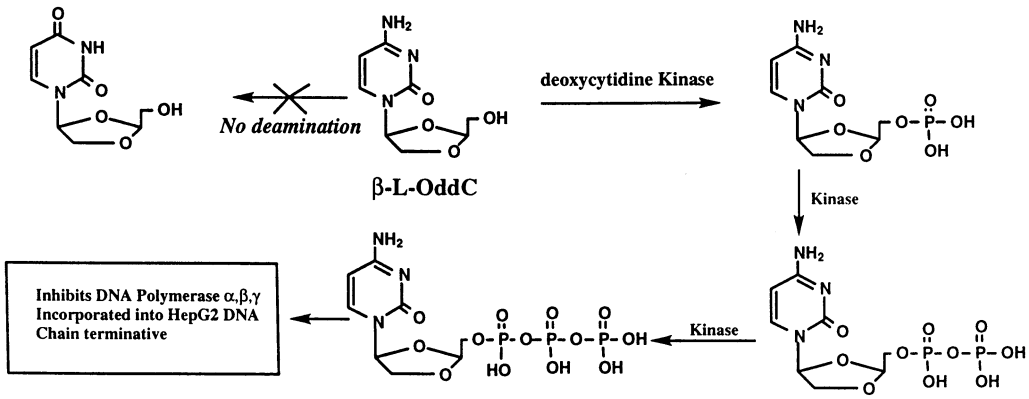
Enzymatic properties of L-d4A, L-d2A and their corresponding D-enantiomers were studied with respect to adenosine kinase (AK), deoxycytidine kinase (dCK), adenosine deaminase (ADA), and purine nucleoside phosphorylase (PNP) (Pèlicano et al., 1997). Human deoxycytidine kinase is the enzyme to phosphorylate both enantiomers of d4A and d2A. The L-enantiomers showed unexpected enantioselectivity, in which the L-isomers are better substrates than their the D-counterparts. Adenosine deaminase was strictly enantioselective, which is favored for D-d4A and D-d2A, while none of the enantiomers of d4A and d2A are substrates for either adenosine kinase or purine nucleoside phosphorylase.

3.5. 2′-Fluorinated L-nucleosides

2′-Fluoro-substituted D-arabinofuranosyl pyrimidine nucleosides were reported to have potent activity against herpes viruses (Watanabe et al., 1979, 1983). Several analogs, such as FMAU, FIAU, FIAC (Fig. 8) also inhibited woodchuck hepatitis virus replication in chronically infected woodchucks (Fourel et al., 1990; Lin et al., 1996) as well as duck hepatitis B virus in the duck models (Fourel et al., 1992). Although FIAU is a potent anti-HBV agent, it caused death of several patients due to liver failure during clinical trials (Macilwain, 1993; Touchette, 1993). It was suggested that the FIAU is incorporated into mitochondrial DNA by DNA polymerase γ resulting in the mitochondrial dysfunction, thereby causing lactic acidosis and steatosis (Parker and Cheng, 1994).



Scheme 10. Enantiomeric synthesis of L-1,3-dioxolane nucleosides.



Scheme 11. Mechanism of (−)-β-OddC.

In order to reduce the toxicity of 2′-fluorinated D-nucleosides while hopefully maintaining the antiviral activity, Chu et al. synthesized L-FMAU, which was found to be a potent antiviral agent against HBV (EC_{50} 0.1 μM in 2.2.15 cells) and Epstein–Barr virus (EC_{90} 5 μM in H1 cells) (Fig. 9) (Chu et al., 1995).

Synthetic methods for L-FMAU were developed by Chu and co-workers using L-xylose (Ma et al., 1996) and L-arabinose (Du et al., 1998) (Scheme 13).

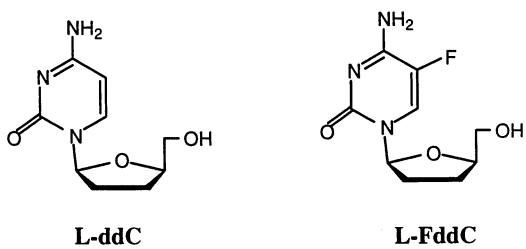
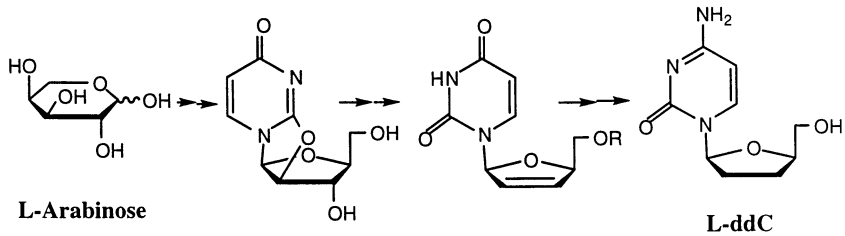


Fig. 5. Structures of L-ddC and L-FddC.

L-FMAU exhibits low cytotoxicities in a variety of cell lines, while the D-counterpart, D-FMAU, is less potent and significantly more toxic (Table 3). It did not adversely affect mitochondrial function at a concentration of 200 μM in hepatoma cell lines and no significant lactic acid production was observed. L-FMAU exhibited in vivo activity in duck hepatitis model (Stéphanie et al., 1998). Furthermore, L-FMAU demonstrated potent in vivo activity in woodchucks against chronically infected with woodchuck hepatitis virus. The most interesting aspect of in vivo studies of L-FMAU is that it demonstrated not only potent antiviral activity, but also no significant virus rebound was observed up to 18 weeks after cessation of the treatment (Tennant et al., 1997). The pharmacokinetics study indicated that L-FMAU provided respectable oral bioavailability in rats (Wright et al., 1996) (59–64%) and woodchucks (20–40%) (Witcher et al., 1997).

Preliminary biochemical studies indicated that L-FMAU is phosphorylated by the cellular



Scheme 12. Synthesis of L-ddC.

thymidine kinase and deoxycytidine kinase as well as mitochondrial pyrimidine kinase to its monophosphate (Scheme 14) (Pai et al., 1996). In order to investigate whether L-FMAU could be phosphorylated by cytosolic dCyd kinase and dThd kinase, the metabolism of L-FMAU was studied in cells that are deficient in these enzymes. L-FMAU was phosphorylated to a lesser extent but still substantially phosphorylated in both enzymes deficient cell lines. dThd and dCyd influenced the degree of L-FMAU phosphorylation in those cell lines. There was a more pronounced inhibition of L-FMAU phosphorylation in cytosolic dCyd kinase deficient cell lines by dThd. This is due to the fact that cytosolic dThd kinase is the only major enzyme responsible for its phosphorylation in these cells. Likewise, the phosphorylation of L-FMAU is much less influenced by dCyd in HeLa cells than in dThd kinase deficient HeLa (Bu) cells. This observation was well explained when the action of dCyd and/or dThd on L-FMAU metabolism was studied in HepG2 cells. Either dThd or dCyd could suppress L-FMAU phosphorylation to some extent. However, the combined effect of dThd and dCyd was the most effective (Liu et al., 1998).

Table 2
Anti-HIV and anti-HBV activities of D- and L-2′,3′-dideoxycytosine analogs

	L-ddC	D-ddC	L-FddC	D-FddC
Anti-HIV-1 (EC_{50}, μM)				
CEM	5.0	1.5	0.51	2.0
Anti-HBV (EC_{50}, μM)				
2.2.15	0.01	2.8	0.01	10.0
Cytotoxicity (IC_{50}, μM)				
CEM	70	28	67	2.0

(Lin et al., 1994a).

In addition to its potent anti-HBV activity, L-FMAU also exhibits potent anti-EBV activity. Thus far, few L-nucleoside analogs with anti-EBV activity have been reported in the literature. The EC_{90} value of L-FMAU is as potent as that of DHPG (5.0 μM in H1 cells), whereas the selectivity of L-FMAU is higher than that of DHPG (183 versus 15). The metabolic study of L-FMAU in H1 cells indicated that it is converted to its mono-, di- and tri-phosphates. Given the differences in the formation of L-FMAUMP in H1 and L5 cells, it is possible that EBV-specific thymidine kinase in H1 cells could utilize L-FMAU as a substrate and could be responsible for the quantitative difference of L-FMAU phosphates formed. Since L-FMAUMP could also be formed in L5 cells as well as in other non-EBV containing cells, human enzymes may also be capable of utilizing L-FMAU as a substrate. One interesting feature of L-FMAU metabolism is that the major metabolite is L-FMAUMP. This finding suggests that the intracellular conversion to L-FMAUDP from L-FMAUMP could be the rate-limiting step in the L-FMAU phosphorylation pathway in L5 cells (Yao et al., 1996).

The structure–activity relationships of 2′-fluoro-β-arabinofuranosyl pyrimidine and purine nucleosides as anti-HBV agents were reported (Ma et al., 1996, 1997). It was found that L-FMAU exhibits the most potent anti-HBV activity among synthesized nucleosides. Two other pyrimidine derivatives, 2′-deoxy-2-fluoro-β-arabinofuranosylcytosine (L-FAC) and its 5-iodocytosine analog (L-FIAC), exhibit good anti-HBV

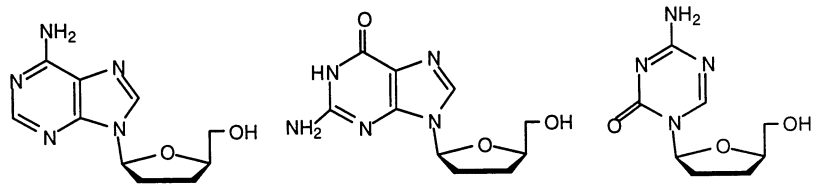


Fig. 6. Other L-dideoxynucleosides.

activity (Fig. 10). Among the purine derivatives, the adenine and hypoxanthine analogs show good anti-HBV activity (EC_{50} value of 1.5 and 8 μM, respectively) without significant toxicity in vitro.

L-2′-F-ara-ddC and L-2′-F-ara-ddA, were also synthesized and their anti-HIV and anti-HBV activities were evaluated (Xiang et al., 1995). In contrast to the D-isomers, the corresponding L-isomers exhibited only moderate or weak anti-HIV activity. The difluoro-β-L-ribofuranosyl pyrimidine and purine nucleosides were also synthesized, among which only adenosine derivative (L-difluoro-deoxyadenosine) exhibited moderately potent anti-HIV activity (EC_{50} 3.4 μM) (Kotra et al., 1997).

3.6. Carbocyclic L-nucleosides

Carbovir was the first carbocyclic nucleoside analog, which exhibited potent anti-HIV activity in vitro. It was reported that the anti-HIV activity of carbovir resides in its natural β-D enantiomer (Fig. 11) (Coates et al., 1991) with EC_{50} of 0.31 μg/ml in MT-4 cells, whereas the β-L enantiomer is weakly active against HBV (Furman et al., 1995). The results indicate that the anabolic enzymes exhibited marked selectivity toward the β-D-enantiomer of carbovir. It has also been shown that the triphosphates of β-D- and β-L-carbovir are approximately equipotent as HIV reverse transcriptase inhibitors. Therefore, it appears that the inactivity of β-L-carbovir against HIV is due to the lack of the initial phosphorylation in vivo (Miller et al., 1992).

The carbovir triphosphate mimic (Fig. 11) was also synthesized and its anti-HIV activity was evaluated. Surprisingly, the L-enantiomer showed 75 times as potent anti-HIV activity as did its D-enantiomer (Merlo et al., 1994).

Several other carbocyclic nucleosides have been reported as potential antiviral agents (Fig. 12): It has been found that L-carbocyclic-2′,3′-didehydro-2′,3′-dideoxyadenosine (L-Cd4A) exhibited moderately potent anti-HIV ($EC_{50} = 2.4$ μM) activity in human PBM cells without cytotoxicity up to 100 μM (Wang et al., 1998). (−)-Bis-(hydroxymethyl)-cyclopentenyladenine [(−)-BCA], which is like L-carbocyclic nucleoside, was reported to have potent anti-HIV activity, which was synthesized via a chemoenzymatic method using *Rhizopus delemer* lipase for asymmetric hydrolysis of the key intermediate, meso-3,5-bis-(acetoxymethyl)-cyclopentenes (Tanaka et al., 1996). L-Carbocyclic BVDU has been reported to be active against herpes simplex virus, however, it is one order of magnitude less active than its D-enantiomer, carbocyclic-BVDU (Balzarini et al., 1989).

Several L-carbocyclic nucleosides have been additionally reported: (+)-7-deaza-5′-nor-aristeromycin has been reported to possess significant anti-trypanosomal activities (Seley et al., 1997a). Another analog, (+)-5′-nor-aristeromycin, exhibited antiviral activity against hepatitis B virus (Seley et al., 1997b).

3.7. Other miscellanous L-nucleosides

L-Like reverse oxathiolane analogs, 2′-deoxy-3′-oxa-4′-thiacytidine [(+)-dOTC)] and its 5-

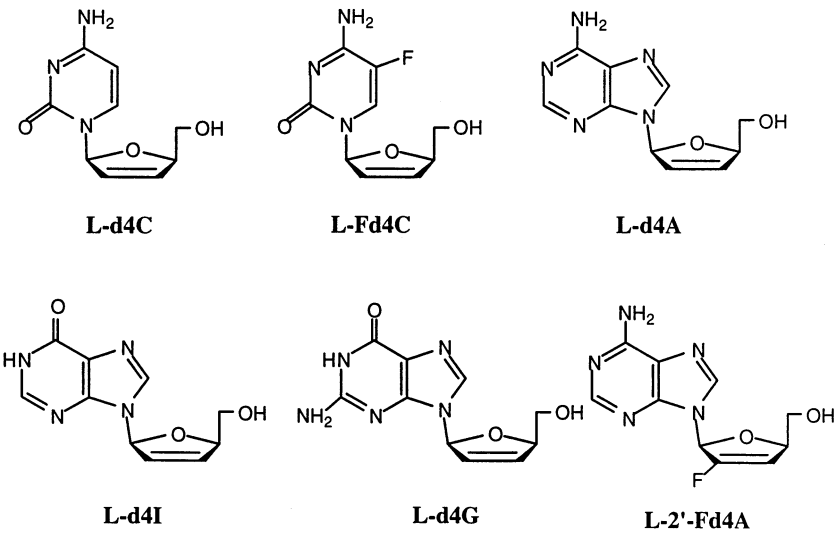


Fig. 7. L-Dideoxydidehyronucleosides.

fluoro analog [(+)-dOTFC)] as well as their D-enantiomers, have been reported to have potent anti-HIV and anti-HBV activities (Mansour et al., 1995). A series of branched-chain sugar isonucleoside, which may be viewed as L-related nucleoside analogs, were also synthesized and evaluated for antiviral activity. Among these compounds, BMS-181,164, BMS-181,165 and their adenine analog, exhibited antiviral activity against herpes simplex virus type 1 and 2 (HSV-1 and -2), varicella-zoster virus (VZV), and human cytomegalovirus (HCMV) (Fig. 13) (Tino et al., 1993).

The 4′-thio analogs of L-d4C and L-Fd4C, L-d4TC and L-d4TFC exhibited marked activiral activities against both HIV and HBV whereas no significant antiviral activity of their natural D-enantiomers was observed (Young et al., 1995).

1263W94 is a L-ribofuranosyl benzimidazole nucleoside-like compound, which possess selective and potent antiviral activity against human cytomegalovirus (Koszalka et al., 1996). The mode of reaction of this compound appears to be different from other regular nucleosides. It was reported to inhibit viral replication by interfering with CMV DNA synthesis by blocking a virus-specific process. It is more potent than ganciclovir, cidofovir, lobucavir and foscarnet.

4. Mode of action of L-nucleosides and the enantioselectivity issue

Since the emergence of HIV, a number of nucleoside analogs with anti-HIV and anti-HBV activity have been synthesized. This antiviral activty is generally associated with one of the enantiomers, and more interestingly, some unnatural L-nucleosides are more potent than their natural D-counterparts. Biochemical studies suggest that among initial enzymes involved in the phosphorylation of these nucleosides, cellular deoxycytidine kinase (dCK) lacks stereospecificity and often can phosphorylate both enantiomers, such as (±)-BCH-189, FTC and ddC (Table 4). Furthermore, cellular deoxycytidine kinase can activate not only the cytosine derivatives but also both enantiomers of adenine analogs, such as D- and L-ddA as well as D- and L-d4A. L-FMAU was found to be metabolized to its monophosphate by not only the cellular deoxycitidine kinase as a major, but also by thymidine kinase as a minor. L-FMAU is the only reported L-nucleosides to date where the cellular thymidine kinase can activate the unnatural L-nucleoside. This makes L-FMAU a unique antiviral agent among anti-HBV nucleosides for future use in combination chemotherapy. Other enzymes involved in the synthesis of nucleotide

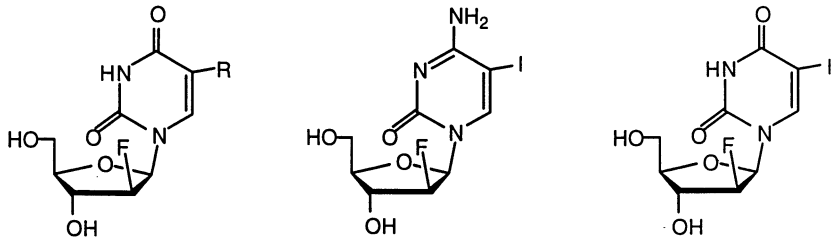


Fig. 8. D-2′-Fluoroarabinofuranosyl nucleosides.

include herpes virus thymidine kinases, deoxynucleoside mono- and diphosphate kinase, cellular and viral DNA polymerases (Spadari et al., 1995). These enzymes appear to lack stereospecificity.

The mode of action of L-nucleosides has been found to be similar as to that of D-nucleosides as a antiviral agent; that is, following intracellular phosphorylation to 5′-triphosphates, which inhibit the viral polymerase as the triphosphate and/or act as the substrates to viral polymerase resulting in chain terminations of the viral DNA (Shewach et al., 1993). However, the L-nucleosides exhibit different substrate specificity toward catabolizing enzymes, such as deoxycytidine deaminase and adenosine deaminase (Pèlicano et al., 1997). Therefore, it has been reported that unlike their D-enantiomers, 3TC, L-FTC and L-OddC are resistant to deoxycytidine deaminase (Chang et al., 1992; Grove et al., 1995). This property of L-nucleosides may be related to their enhanced potentcy as anti-HIV and/or anti-HBV agents.

As cellular deoxycytidine kinase can phosphorylate both enantiomers of some nucleosides, the enzyme has stereoselectivity to certain extent. As results, enantiomers may differ in antiviral activity and toxicity due to the rate of phosphorylation of the enantiomers. For nucleosides with anti-HIV activity, the origin of stereoselectivity appears to reside with the host cell metabolic enzymes, such as deoxycytidine kinase, rather than the target enzyme, HIV reverse transcriptase. For example, the discrepancy of anti-HIV activity of D- and L-FTC (and 3TC) appears to reside with the selective phosphorylation steps together with the stereoselective deaminations of the D-enantiomers. For anti-HBV activity, the source of stereoselectivity appears to be the results of differential metabolism as well as the differential inhibition of the target enzyme. For example, both the D- and L-enantiomers of ddC have similar anti-HIV activity, while the L-enantiomer is much more active against HBV than the D-counterpart. The difference in anti-HBV activity most likely can be accounted for the fact that L-ddC triphosphate is a better inhibitor of the HBV polymerase than the D-enantiomer (Furman et al., 1995).

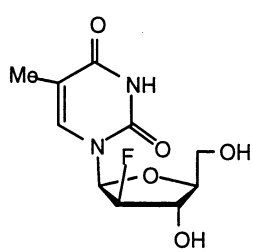
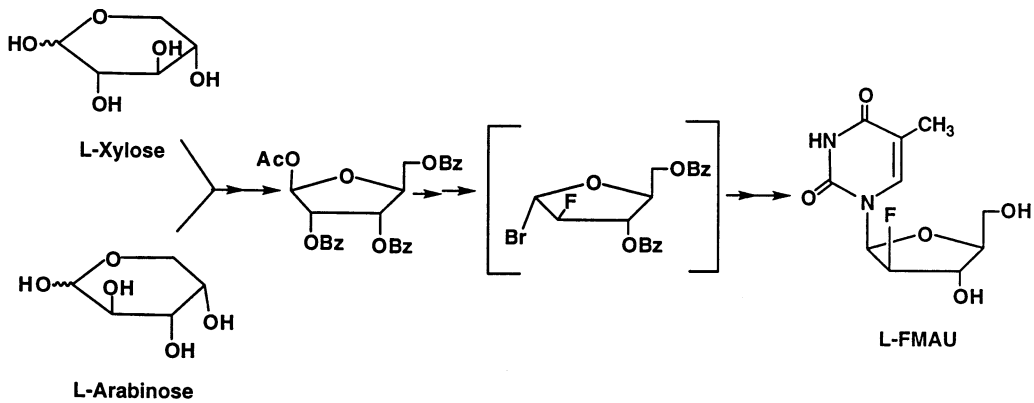


Fig. 9. Structure of L-FMAU.

Enantiomeric forms are critical for the development of life, i.e. proteins and enzymes are made up only of L-amino acids and nucleic acids are composed of D-nucleosides. Enzymes catalyze reactions in biological systems. They are in general stereospecific and/or at least stereoselective, which can be related to the three-dimensional folding of the chiral polypeptide. Therefore, only one of the enantiomer of the chiral molecule may effectively interact with the catalytic site, exhibiting biological activity. The question is: Why can some unnatural L-nucleosides be phosphorylated by



Scheme 13. Chemical synthesis of L-FMAU.

cellular enzymes and show antiviral activity like their D-enantiomers? The biological activity of a nucleoside depends on the conformation of the sugar, which dictates the orientation and conformation of 5′-OH and orientation of the base moiety at C1′ position. Therefore, the configuration and conformation of the base as well as the 5′-hydroxy group are critical to a nucleoside for biological activity. In order to understand this issue, we have to consider both the enzyme as well as the nucleosides involved.

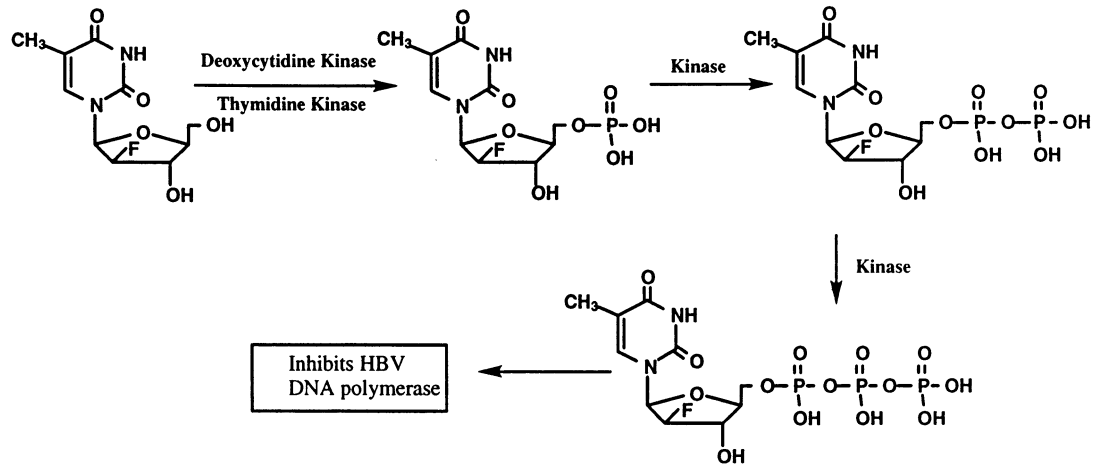
In view of the fact that three dimensional X-ray structures of associated enzymes (kinase) are not available at this time, we attempted to understand the structural characteristics of nucleosides by superimposing several enantiomeric pairs of X-ray structures, including FTC, (Van Roey et al., 1993) and ddC (Silverton et al., 1988) (Fig. 14).

When the base moiety and the 5′-hydroxymethyl group are considered as reference points, it is suprising that the D- and L-nucleosides superimpose very well. Although the furanose rings were the only area of these molecules that could not be superimposed on the atom-by-atom basis, the furanose rings with each pair of the D- and L-nucleosides were sterically similar. Due to the comformational similarity of the D- and L-enantiomers, it is understandable that these D- and L-forms of the nucleoside analogs can be recognized by the same cellular kinase which are known to be less enantioselective, such as deoxycytidine kinase.

Computer modeling was also used to superimpose the X-ray structure of L-FMAU (Ma et al., 1996) and its D-enantiomer (Fig. 15). The nucleobase moiety and the 5′-hydroxymethyl groups of the D- and L-isomers superimpose very well. The furanose rings were also sterically similar. The 2′-fluoro and 3′-hydroxy groups of FMAU can not overlap, which may create differences in antiviral activity and toxicity.

Table 3
Anti-HBV and anti-EBV activities of D- and L-FMAU analogs

	Anti-HBV activity	Anti-EBV activity	Growth inhibition (μM)				Selectivity	
	2.2.15 cells EC_{50} (μM)	H1 cells EC_{90} (μM)	MT2 ID_{50}	CEM ID_{50}	H1 ID_{50}	2.2.15 ID_{50}	2.2.15/HBV	H1/EBV
L-FMAU	0.1	5 ± 0.8	100	>100	913 ± 70	>200	>2000	9130
D-FMAU	2.0	0.1 ± 0.02	8–9	0	<10	50	25	<5



Scheme 14. Mechanism of L-FMAU.

The X-ray structure of AZT (Birnbaun et al., 1987) and its L-enantiomer were also superimposed by computer modelling (Fig. 15). The thymine moiety and hydroxymethyl group of D- and L-AZT overlap closely, while the furanose rings do not match very well but they are still sterically similar like other pairs of D- and L-nucleosides, such as ddC. However, the orientation of the azido groups of D- and L-AZT are significantly different and they can not match each other. This difference together with the steric and electronic effects of azido group of D-AZT may play an important role at the catalytic site. Such an observation leads to the conclusion that the difference between the active D-AZT and its inactive L-enantiomer is in the configuration of the azido groups. However, it is not known whether if L-AZT would be recognized by the host kinases or the triphosphate of L-AZT would inhibit reverse transcriptase.

In summary, Due to the similarity of the conformations of the D- and L-enantiomers, particularly the orientations of the nucleobases and the hydroxy group at 5′ positions, both D- and L-forms of the nucleosides can be recognized by certain cellular enzymes and exhibit biological activities (Fig. 16).

5. Conclusions

In the search for effective, selective, and nontoxic antiviral agents, a variety of strategies have been exploited to design nucleoside

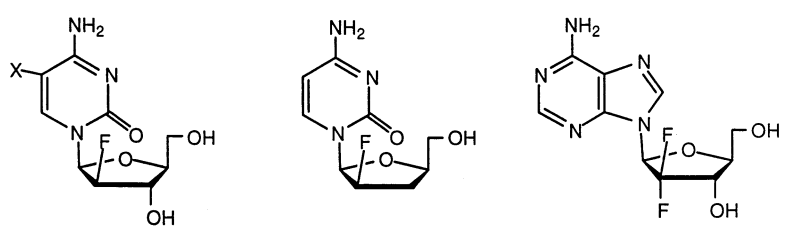
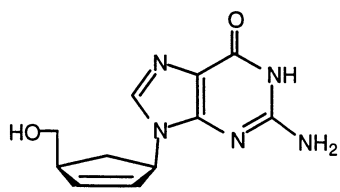
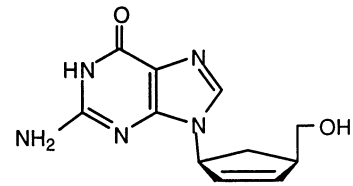


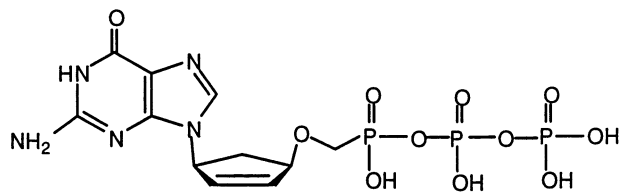
Fig. 10. 2′-Fluorinated nucleosides.



Carbovir (CBV)

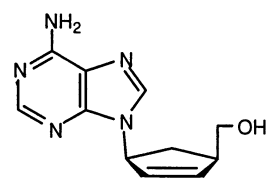


L-Carbovir (L-CBV)

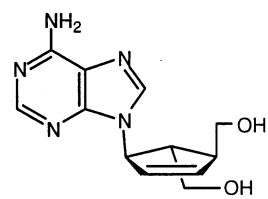


L-Carbovir TP mimic

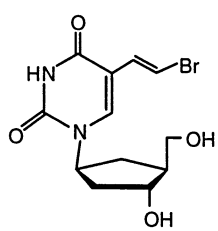
Fig. 11. Structures of carbovir and its mimic.



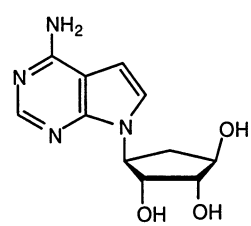
L-Cd4A



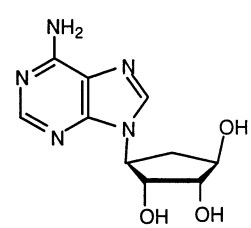
(-)-BCA



L-C-BVDU



(+)-7-Deaza-5'-aristeromycin



(+)-5'-Noraristeromycin

Fig. 12. Some potential antiviral agents.

analogs, which block viral replication without affecting host cellular processes. L-Nucleosides have drawn great attention and significant progress has been made in the past several years in the battle against HIV, hepatitis B and other viruses. 3TC has been approved for the treat-

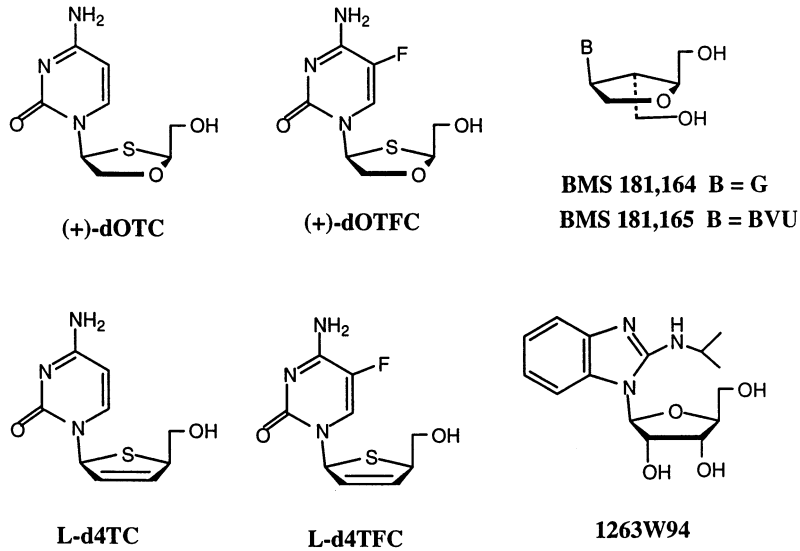


Fig. 13. Miscellaneous L-nucleosides.

ment of AIDS and is now undergoing clinical trial as an anti-HBV agent. Other L-nucleoside analogs, L-FTC and L-FMAU have shown promise in the treatment of HIV and/or HBV.

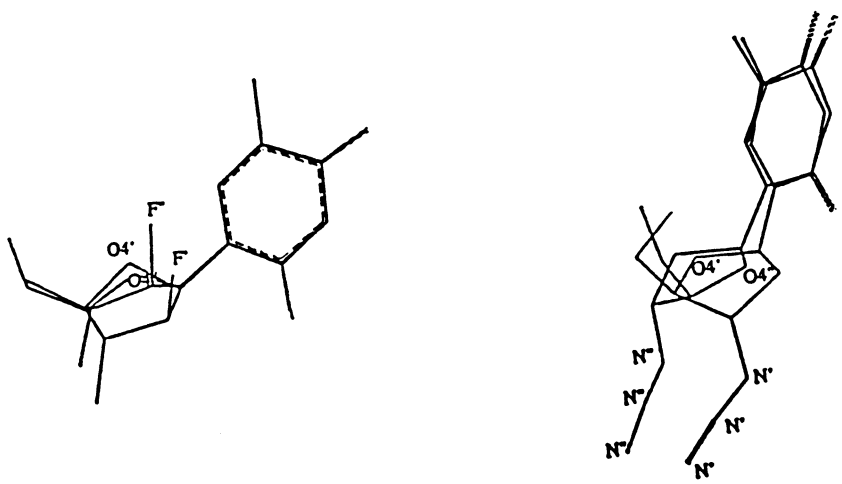
Based on the observation through modeling study that the conformation of selected L-nucleosides are similar to their natural D-enantiomers, some L-nucleoside as well as their D-enantiomers can be recognized by host enzymes, therefore exhibit biological activities. Since the monophosphorylation of a nucleoside analog is the crucial step for biological activity,

Table 4
Anti-HIV, anti-HBV activity, cytotoxicity, kinases and deaminases of selected pairs of D- and L-nucleosides

Compounds	Antiviral activity (EC_{50}, μM)		Cytotoxicity (IC_{50}, μM)	Phosphorylation enzymes	Deamination emzymes
	HIV-1	HBV			
(+)-D-BCH 189	0.21 (PBM)	0.5 (2.2.15)	2.7 (CEM)	dCK	dCDA
(−)-3TC (L-BCH 189)	0.0018 (CEM)	0.01 (2.2.15)	>100 (CEM)	dCK	NS of dCDA
(+)-D-FTC	0.008 (PBM)	0.36 (2.2.15)	>100 (CEM)	dCK	dCDA
(−)-L-FTC	0.0013 (CEM)	0.01 (2.2.15)	>100 (CEM)	dCK	NS of dCDA
(+)-D-OddC	0.016 (PBM)	0.01 (2.2.15)	12.3 (CEM)	—	—
(−)-L-OddC	0.002 (CEM)	0.0005 (2.2.15)	0.26 (CEM)	dCK	NS of dCDA
D-ddC	1.5 (CEM)	2.8 (2.2.15)	28 (CEM)	dCK	dCDA
L-ddC	5.0 (CEM)	0.01 (2.2.15)	70 (CEM)	dCK	—
D-FMAU	—	2.0 (2.2.15)	50 (2.2.15)	—	—
L-FMAU	Inactive	0.1 (2.2.15)	>200 (2.2.15)	dCK, TK	—
D-d4A	0.76 (PBM)	—	>100 (PBM)	dCK	ADA
L-d4A	0.38 (CEM)	1.2 (2.2.15)	37 (CEM)	dCK	NS of ADA
D-AZT	0.004 (PBM)	—	14.0 (CEM)	TK	—
L-AZT	>100 (MT4)	—	100 (MT4)	—	—

D-and L-FTC **D-and L-ddC**

Fig. 14. Overlap of the X-ray structures of D- and L-nucleosides. The modeling was performed on a Silicon Graphics Indy workstation using the 'Fit Atoms' module in SYBYL (Tripos Associations, St Louis, MO) based on the X-ray crystal structures of L-FTC and D-ddC. Each pair of enantiomers was fitted together by pairing C1′, C4′, C5′, O5′, N1, C2, N3, C4, C5 and C6. The oxygen atoms in sugar rings are situated on the backs of the D-nucleosides and on the fronts of the L-nucleosides as shown.



D-and L-FMAU **D-and L-AZT**

Fig. 15. Overlap of the X-ray structures of D- and L-FMAU and AZT. The modeling was performed based on the X-ray crystal structures of L-FMAU and D-AZT. Each pair of enantiomers were fit together by pairing C1′, C4′, C5′, O5′, N1, C2, N3, C4, C5 and C6. The oxygen atom in the sugar ring is situated on the left side in D-FMAU and on the right side in L-FMAU. The oxygen atom in sugar ring is situated on the left side in D-AZT and on right side on L-AZT.

the lack of enantiodifferentiation of cellular kinase, such as deoxycitidine kinase, thymidine kinase and other phosphorylating enzymes, could be utilized to exploit to design novel L-nucleosides as potential antiviral and anticancer drugs.

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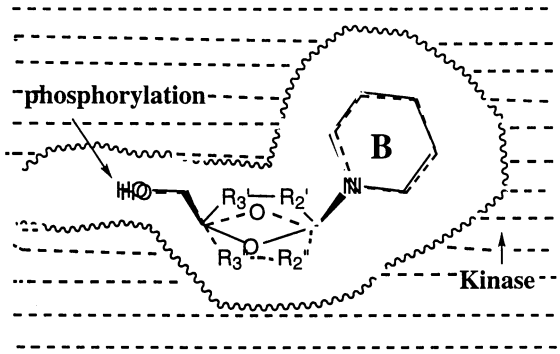

Fig. 16. Hypothetical mechanism for both L- and D-nucleoside phosphorylation by kinase. Solid line represents L-nucleosides and dash line on the structure represent D-nucleosides. **B**: base.

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