# Enzymatic Properties of the Unnatural β-L-Enantiomers of 2′,3′-Dideoxyadenosine and 2′,3′-Didehydro-2′,3′-dideoxyadenosine

Hélène Pélicano,[†] Claire Pierra,[†] Staffan Eriksson,[‡] Gilles Gosselin,[†] Jean-Louis Imbach,[†] and Georges Maury*[,†]

*Laboratoire de Chimie Bioorganique, UMR 5626 du CNRS, Département de Chimie Organique Fine, Université Montpellier-2 des Sciences et Techniques du Languedoc, 34095 Montpellier Cedex 5, France, and Department of Veterinary Medical Chemistry, Swedish University of Agricultural Sciences, Biomedical Centre, Box 575, S-751 23 Uppsala, Sweden*

*Received March 10, 1997*[⊗]

The β-L-enantiomers of 2′,3′-dideoxyadenosine and 2′,3′-didehydro-2′,3′-dideoxyadenosine have been stereospecifically synthesized. In an attempt to explain the previously reported antiviral activities of these compounds, their enzymatic properties were studied with respect to adenosine kinase, deoxycytidine kinase, adenosine deaminase, and purine nucleoside phosphorylase. Adenosine deaminase was strictly enantioselective and favored β-D-ddA and β-D-$d_4$A, whereas adenosine kinase and purine nucleoside phosphorylase had no apparent substrate properties for the D- or L-enantiomers of β-ddA or β-$d_4$A. Human deoxycytidine kinase showed a remarkable inversion of the expected enantioselectivity, with β-L-ddA and β-L-$d_4$A having better substrate efficiencies than their corresponding β-D-enantiomers. Our results demonstrate the potential of β-L-adenosine analogues as antiviral agents and suggest that deoxycytidine kinase has a strategic importance in their cellular activation.

## Introduction

Until recently, it was generally assumed that enzymes catalyzing the transformation of nucleoside analogues are enantioselective and preferred natural D-enantiomers as substrates. Consequently, some of these enzymes have been successfully used in synthetic organic chemistry to kinetically resolve racemic mixtures of carbocyclic nucleosides or other analogues. In these cases, enzyme catalysis favors the corresponding D-enantiomers.[1−3] However, the enantioselectivity of these enzymes has recently been questioned and recognized as an important factor for the antiviral activity of several L-enantiomers of nucleoside analogues.[4] Thus, β-L-2′,3′-dideoxycytidine (L-ddC) and β-L-2′,3′-dideoxy-5-fluorocytidine (L-FddC) are potent inhibitors of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) replication, L-FddC being even more active and more selective than its corresponding D-enantiomer.[5−7] Two other cytidine analogues, (2*R*,5*S*)-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (3TC) and (2*R*,5*S*)-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine (FTC), have potent inhibition properties against both HIV and HBV replication. 3TC has the further advantage of being less cytotoxic than the corresponding D-enantiomer, whereas FTC and its D-enantiomer have similar cytotoxicities.[4] Unnatural enantiomers of uridine analogues, 5-iodo-2′-deoxyuridine and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, have been reported to be active against herpes simplex virus type 1 (HSV-1), less active but also less toxic than the D-enantiomers.[8]

To explain the observed anti-HIV activity of β-L-nucleoside analogues, the enantioselectivity of the target enzyme HIV-1 reverse transcriptase (RT) was first studied. In most examined cases, the enzyme catalyzes the incorporation of both D- and L-nucleotide analogues, although with markedly different rates.[4,9] Of particular significance was the finding that the D- and L-enantiomers of ddCTP and FddCTP[10] have similar inhibitory effects on HIV-1 RT, whereas 3TCTP is a stronger inhibitor than its D-enantiomer.[4] In contrast, the enantioselectivities of other DNA polymerases are variable depending on the nature of the substrate.[4,9,11] The enantioselectivity of the enzymes involved in the activation (mostly kinases) or deactivation (deaminases) of nucleoside analogues has been examined in order to obtain a complete assessment of their antiviral data.[4] These enzymes apparently present strict enantioselectivities[12−14] except for the cellular deoxycytidine kinase,[6,15] HSV-1 thymidine kinase,[8,16] and, to a certain extent, some cellular nucleotide kinases and nucleoside diphosphate kinases.[4]

In the present study, we have evaluated the properties of the L-enantiomers of two nucleoside analogues whose D-enantiomers are known to display some antiviral activity: β-L-2′,3′-dideoxyadenosine (**2**, β-L-ddA) and β-L-2′,3′-didehydro-2′,3′-dideoxyadenosine (**4**, β-L-$d_4$A) (Chart 1). The enantioselectivities of adenosine kinase, deoxycytidine kinase, adenosine deaminase, and purine nucleoside phosphorylase, which may be important in their metabolism and for their antiviral efficiency, were examined with respect to these nucleoside analogues, and the enzymatic properties of the compounds were compared to their antiviral activities.

## Chemistry and Biological Evaluation

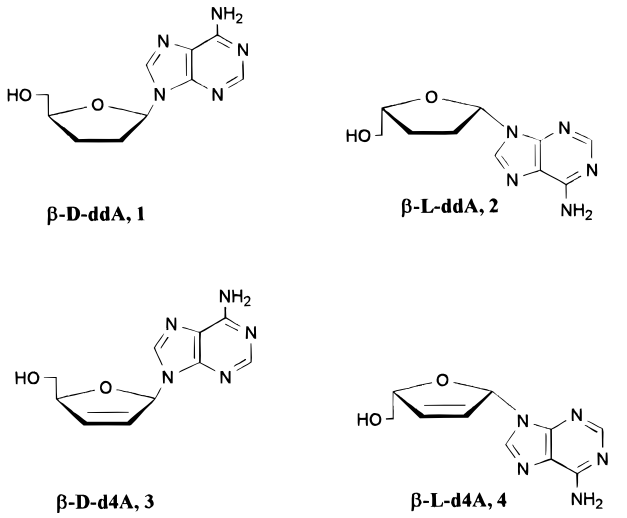
The synthetic strategy developed for the preparation of β-L-ddA and β-L-$d_4$A is similar to that previously mentioned in our preliminary communication.[17] The physicochemical properties of β-L-ddA, **2**, prepared by our procedure were in accordance with those of samples obtained following nonstereospecific routes.[18,19] Also, the physicochemical data of β-L-$d_4$A, **4**, independent of enantiomerism, were in agreement with those published for its corresponding β-D-enantiomer.[18,19]

The anti-HIV and anti-HBV activities and the cytotoxicities of the unnatural enantiomers β-L-ddA, **2**, and

* Corresponding author. Phone: 33 4 67 14 33 16. Fax: 33 4 67 04 20 29. E-mail: glmaury@univ-montp2.fr.
[†] Université Montpellier-2 des Sciences et Techniques du Languedoc.
[‡] Swedish University of Agricultural Sciences.
[⊗] Abstract published in *Advance ACS Abstracts,* November 1, 1997.

S0022-2623(97)00148-9 CCC: $14.00 

**Chart 1**




$\beta$-L-$d_4$A, **4**, as well as those of the corresponding D-enantiomers have been previously reported.[17,20] $\beta$-D-ddA was more active against HIV than its L-enantiomer at least by 1 order of magnitude in all assays. In contrast, $\beta$-D- and $\beta$-L-$d_4$A exhibited similar and significant anti-HIV activities as well as comparable toxicity profiles.[17]

In anti-HBV assays, $\beta$-D-ddA was inactive.[20] Under the same conditions, $\beta$-L-ddA, **2**, had a moderate inhibiting effect with an $EC_{50}$ close to 5.5 $\mu$M.[20] Neither $\beta$-D-ddA or $\beta$-L-ddA exhibited cytotoxicity effects against noninfected cells.[17,20] On the other hand, $\beta$-L-$d_4$A, **4**, was found to exert a more pronounced inhibition of HIV and HBV replications but with some concomitant cytotoxicity.[17,20]

## Enzymology

The antiviral activity or inactivity of L-enantiomers of nucleoside analogues depends, at least in part, on the enantioselectivity of cellular enzymes. To assess the enantioselectivity of critical enzymes related to adenosine derivatives, the substrate and inhibitory properties of the D- and L-enantiomers of ddA and $d_4$A have been studied with respect to adenosine kinase (AK), deoxycytidine kinase (dCK), adenosine deaminase (ADA), and purine nucleoside phosphorylase (PNP). Adenosine kinase was purified to homogeneity from bovine liver.[21] Human deoxycytidine kinase has been cloned, expressed in *Escherichia coli*, and purified to functional homogeneity.[22,23] The other enzymes were from commercial sources.

The results obtained in our substrate and inhibitory studies with the enzymes have been summarized in Table 1. As previously shown, $\beta$-D-ddA is an extremely poor substrate of adenosine kinase from human T lymphoid cells.[24] Using purified bovine liver adenosine kinase, no phosphorylation of either $\beta$-D- or $\beta$-L-ddA was detected even at high concentration of enzyme or substrate (up to 1 and 100 $\mu$M, respectively). Under the same conditions, the treatment of the acid-sensitive $\beta$-D- or $\beta$-L-$d_4$A led only to partial depurination as expected from previously published results,[25] without apparent formation of the corresponding monophosphates. However, the stability of $d_4$A ($T_{1/2}$ = 3.1 days) was sufficient to rule out any substrate property of this compound with respect to adenosine kinase. Finally, the 5′-phosphorylation of adenosine catalyzed by AK was not influenced by the presence of $\beta$-D-ddA, $\beta$-L-ddA, $\beta$-D-$d_4$A, and $\beta$-L-$d_4$A, suggesting that none of these compounds is an inhibitor of the enzyme under the experimental conditions.

Recent reports indicate that deoxycytidine kinase catalyzes the 5′-phosphorylation of both enantiomers of several antiviral analogues of dideoxycytidine.[4,6,15] Moreover, conflicting reports concerning the ability of this enzyme to catalyze the phosphorylation of $\beta$-D-ddA have been published.[26] We have therefore investigated the enantioselectivity of dCK, and by using pure recombinant human dCK, we have found that each enantiomer of ddA or $d_4$A is a substrate of the enzyme, albeit with marked differences in the phosphorylation rates. Under the assumption that the kinetics follow the Michaelis−Menten equation, $K_m$ and $V_m$ parameters were measured (Figure 1) in an appropriate range of substrate concentrations and in the presence of a relatively high concentration of dCK owing to the fact that these compounds are relatively inefficient substrates. The $K_m$ constants for the two enantiomers of ddA are practically equivalent, but the maximum rates $V_m$ are distinctly different giving the $\beta$-L-enantiomer a higher substrate efficiency than the $\beta$-D one [$(V_m/K_m)_L/(V_m/K_m)_D$ = 8.3] (Figure 1A and Table 1). The enantiomers of $d_4$A display poorer substrate properties with dCK than $\beta$-D- and $\beta$-L-ddA (Figure 1B and Table 1). The $K_m$ values of $\beta$-L- and $\beta$-D-$d_4$A are higher and the substrate efficiencies 2- and 5-fold lower than for the corresponding enantiomers of ddA. Human deoxycytidine kinase favors the $\beta$-L-enantiomer over the D-enantiomer in the case of $d_4$A even more than in the case of ddA since the ratio of the substrate efficiencies of the L- to D-enantiomer is 24.

$\beta$-D-2′,3′-Dideoxyadenosine has long been known as a relatively good substrate of adenosine deaminase, thus explaining the importance of the in vivo activation process of $\beta$-D-ddA to $\beta$-D-ddAMP via $\beta$-D-ddI and $\beta$-D-ddIMP.[27,28] In contrast, we have found the unnatural enantiomer $\beta$-L-ddA, **6**, to be a much poorer substrate for calf intestine ADA than the $\beta$-D-enantiomer. Under our conditions, $\beta$-D-ddA (50 $\mu$M) was entirely deami-

**Table 1.** Substrate and Inhibition Properties of the Studied Nucleoside Analogues[a]

| compd | AK | dCK | ADA | PNP |
|---|---|---|---|---|
| $\beta$-D-ddA, **1** | NS, NI/Ado | $K_m$ = 195 $\mu$M | $K_m$ = 38 $\mu$M | NS, NI/Ino |
| $\beta$-L-ddA, **2** | NS, NI/Ado | $K_m$ = 220 $\mu$M | S[b]<br>$K_i$/Ado = 120 $\mu$M | NS, NI/Ino |
| $\beta$-D−$d_4$A, **3** | NS, NI/Ado | $K_m$ = 4400 $\mu$M | $K_m$ = 30 $\mu$M[53]<br>I/Ado | NS, NI/Ino |
| $\beta$-L−$d_4$A, **4** | NS, NI/Ado | $K_m$ = 620 $\mu$M | S[b]<br>I[b]/Ado | NS, NI/Ino |

[a] S, substrate; NS, nonsubstrate; I, inhibitor; NI, noninhibitor (under the conditions reported in the Experimental Section). [b] Very weak substrate or inhibitor, precluding the determination of kinetic parameters.

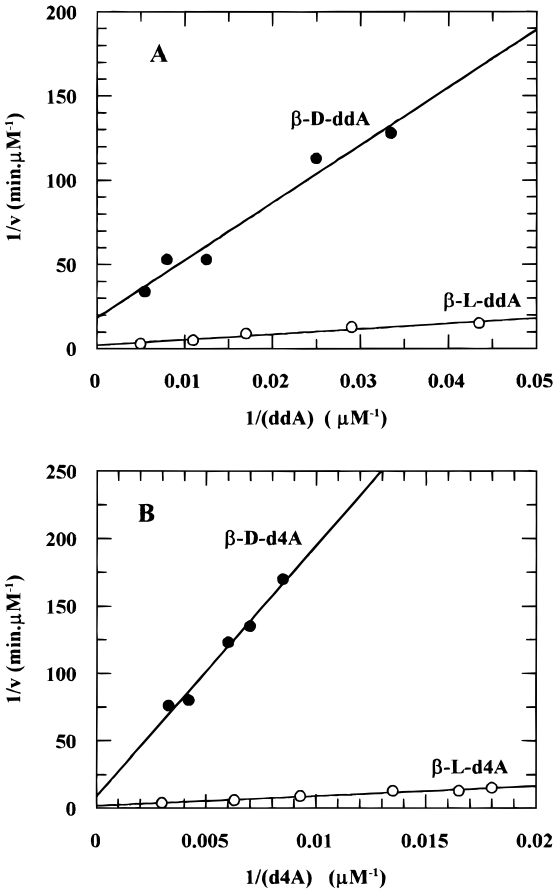



**Figure 1.** Substrate properties of β-D- and β-L-2′,3′-dideoxyadenosine (A) and β-D- and β-L-2′,3′-didehydro-2′,3′-dideoxyadenosine (B) with respect to human deoxycytidine kinase. Experiments were performed as described in the Experimental Section using a 265 $\mu g \cdot mL^{-1}$ concentration of enzyme.

nated after 5 min, whereas β-L-ddA underwent a 20% deamination only after 3 h and with an enzyme concentration increased 100-fold. Similarly, β-D-$d_4$A was a much better substrate of ADA than the L-enantiomer. Under the conditions reported, β-D-$d_4$A was almost totally converted after 1 h and β-L-$d_4$A was deaminated to the extent of only 5% with ADA concentration increased 200-fold. Inhibition studies using adenosine as the substrate showed that both β-D- and β-L-ddA, or β-D- and β-L-$d_4$A, are weak inhibitors of ADA, and a relatively high inhibition constant (120 μM) was determined for β-L-ddA from the Lineweaver−Burk equation for competitive inhibition.

Finally, the β-D- and β-L-enantiomers of ddA and $d_4$A were tested for their substrate or inhibitory properties with respect to purine nucleoside phosphorylases (PNPs) from human blood or calf spleen. Under the conditions reported in the Experimental Section, β-D- and β-L-ddA and β-D- and β-L-$d_4$A were neither substrates nor inhibitors of these PNPs.

The results presented above show that the β-L- and β-D-enantiomers of ddA are neither substrates nor inhibitors of bovine liver AK or human blood and calf spleen PNP. The very poor substrate property of β-D-ddA with regard to human AK has already been established.[24] In contrast, both β-D- and β-L-ddA are substrates for human dCK with the L-enantiomer having a markedly higher substrate efficiency than the D-enantiomer. To our knowledge, this is a first demonstration that the well-documented relaxation of the enantioselectivity of dCK toward cytidine derivatives[4] can be extended also to the adenosine derivatives. In fact, in this case, there seems to be a reversal of the "normal" or expected enantioselectivity compared to other metabolic enzymes which favor the natural β-D-enantiomers of nucleosides and their analogues. Both enantiomers of ddA are poor substrates, especially β-D-ddA with an activity 3 orders of magnitude lower than that of β-D-2′-deoxyadenosine under the same conditions, thus explaining the conflicting reports concerning the substrate properties of β-D-ddA.[26] The values of the kinetic parameters suggest that both enantiomers of ddA have about the same affinities for the enzyme and thus that the bond-forming step is more efficient in the case of the β-L-enantiomer. Adenosine deaminase discriminates β-D- and β-L-ddA, with the L-enantiomer being a very poor substrate compared to the D-enantiomer. Therefore, an activation of β-L-ddA to its monophosphate via 5′-nucleotidase phosphorylation of the corresponding inosine analogue as in the case of the D-enantiomer[27] appears unlikely. Moreover, the enzymatic properties of β-L-ddA with respect to dCK and ADA are consistent with its fairly efficient conversion to the monophosphate and its stability toward deamination in cells. Preliminary results based on fluorescence spectroscopy demonstrate that β-L-ddATP is a substrate of HIV-1 reverse transcriptase which catalyzes its incorporation into DNA, albeit less efficiently than β-D-ddATP.[29] The relaxed enantioselectivities of the viral polymerase and cellular deoxycytidine kinase with respect to β-L-ddA are in agreement with the observed anti-HIV activity of this compound in infected cells. This lack of anti-HBV activities of β-D-ddA may arise either from an inefficient metabolism of the corresponding 5′-monophosphate or from ineffective inhibition of the polymerase induced by the related 5′-triphosphate derivative.

The properties of β-D- and β-L-$d_4$A with regard to the studied enzymes follow the same patterns as those of β-D- and β-L-ddA. Both enantiomers of $d_4$A are substrates of dCK and ADA, but not substrates of AK and PNP. Our results suggest that β-L-$d_4$A phosphorylation could be catalyzed by dCK in cells. In this regard, the anti-HBV activity of β-L-$d_4$A in infected cells[17,20] is compatible with its substrate properties with regard to dCK. We observed for the two enantiomers of $d_4$A the same tendency of dCK to favor the L-enantiomer as for ddA, with an increased inversion of the expected enantioselectivity from ddA to $d_4$A. The variation of dCK enantioselectivity with respect to nucleoside analogues appears to be a general fact and is probably related to the particular 3-dimensional structure of the enzyme which is not yet known. Comparative studies of the substrate properties of the triphosphates of β-D- and β-L-$d_4$A with regard to HIV reverse transcriptase and HBV DNA polymerase are being investigated, and these investigations could allow the correlation of the enzymatic properties of β-D- and β-L-$d_4$A with their in vitro antiviral activities. The very poor substrate properties of β-L-$d_4$A with respect to ADA make the activation of this compound through deamination and subsequent phosphorylation catalyzed by 5′-nucleotidase a very unlikely process.

## Experimental Section

**Chemistry.** The chemical syntheses of β-L-ddA, **2**, and β-L-$d_4$A, **4**, have been reported previously in a preliminary form.[17,20] The compounds were characterized on the basis of their physical properties [melting point and optical rotation: for β-L-ddA, **2**, mp 185−187 °C, $[\alpha]^{20}_D$ +35.6 (*c* 0.9, DMSO); for β-L-d4A, **4**, mp 189−191 °C, $[\alpha]^{20}_D$ −0.24.0 (*c* 1.0, DMSO)] and spectroscopic properties (UV, $^1$H NMR, and FAB-MS). Their purities were ascertained by combustion analyses and high-pressure liquid chromatography.

**Biological Methods.** The data concerning the anti-HIV and anti-HBV assays on cell culture have been previously reported.[17,20]

**Enzymology. 1. Enzymes.** Bovine liver adenosine kinase was purified to homogeneity using a method described previously.[21] Human recombinant deoxycytidine kinase has been produced and purified as reported.[22,23] Other enzymes were purchased from Sigma: adenosine deaminase from calf intestinal mucosa type VIII, purine nucleoside phosphorylases from human blood and calf spleen, pyruvate kinase, and L-lactate dehydrogenase. Standard conditions of enzymatic assays were used.[21,23,30] Kinetic and stability studies were based on spectrometric or HPLC analysis, whereas inhibition studies involved radiometric and spectrometric methods. Product studies of the reaction medium with increased enzyme concentrations and reaction times were performed, especially with weak substrates.

**2. Kinetic Studies. Adenosine Kinase**: Kinetic studies were performed at 37 °C in a reaction medium containing Tris HCl (100 mM, pH 7.4), KCl (100 mM), BSA (4.8 mg·mL$^{-1}$), $MgCl_2$ (0.5 mM), ATP (1 mM), substrate, and AK (0.38−7.6 μg·mL$^{-1}$). Kinetics were followed by HPLC analysis of the reaction medium using a C-18 column. Elution was achieved in 30 min with a gradient of phosphate buffer (50 mM, pH 6) (A) and phosphate buffer (50 mM, pH 6) with 40% acetonitrile (B) (flow rate: 1 mL·min$^{-1}$). β-D-ddAMP as standard was prepared by controlled degradation of β-D-ddATP (Sigma) catalyzed by bovine alcaline phosphatase. HPLC analysis of the reaction medium was performed assuming that the differences in retention time between the nucleoside and its 5′-monophosphate were similar in the case of ddA and $d_4$A. Inhibition studies were performed in the presence of adenosine 50 μM.

**Deoxycytidine Kinase**: Kinetic studies of the phosphorylation of β-D- and β-L-ddA and β-D- and β-L-$d_4$A were performed at 37 °C using HPLC under the same conditions as with adenosine kinase. The reaction medium contained Tris HCl (50 mM, pH 7.5), DTT (1 mM), NaF (15 mM), $MgCl_2$ (5 mM), ATP (5 mM), an appropriate amount of dCK, and substrate (in 20−200 μM concentrations).

**Adenosine Deaminase**: Assays were performed at 37 °C in phosphate buffer (50 mM, pH 7.5) with substrate concentrations in the range of 20−80 μM and with 0.01−1 unit of enzyme depending on the substrate. HPLC analysis of the reaction medium in the same conditions as described for adenosine kinase was used in all cases to ensure that the sole reaction product was the deaminated substrate. HPLC was also used with poor substrates to detect minor quantities of products. In inhibition studies using UV spectrometry, adenosine concentration varied from 20 to 100 μM.

**Purine Nucleoside Phosphorylase**: Experiments were performed at 37 °C in phosphate buffer (50 mM, pH 7.5) with 0.18 unit·mL$^{-1}$ enzyme from calf spleen (or 9 units·mL$^{-1}$ human blood purine nucleoside phosphorylase) and the substrate in 50 μM concentration. Inhibition studies were carried out in the presence of inosine (40 μM) and inhibitor (100 μM). The reaction medium was analyzed by HPLC in the same conditions as described above for adenosine kinase.

Kinetic constants were measured from reciprocal plots according to Lineweaver−Burk.

**Acknowledgment.** We thank the Association pour la Recherche sur le Cancer for a research fellowship to H.P. and the Agence Nationale de Recherche sur le Sida (France) for financial support. S.E. was supported by funds from the Swedish National Research Council, the Swedish Medical Research Council, and the Swedish Board for Technical Development. We are grateful to J.-P. Sommadossi, University of Alabama at Birmingham, and Anne-Marie Aubertin, University Louis Pasteur (INSERM) at Strasbourg, for anti-HBV and anti-HIV evaluations and fruitful discussions.

## References

(1) Secrist, J. A., III; Montgomery, J. A.; Shealy, Y. F.; O. Dell, C. A.; Clayton, S. J. Resolution of racemic carboxylic analogues of purine nucleosides through the action of adenosine deaminase. antiviral activity of the carbocylic 2′-deoxyguanosine enantiomers. *J. Med. Chem.* **1987**, *30*, 746−749.

(2) Mahmoudian, M.; Baines, B. S.; Drake, C. S.; Hale, R. S.; Jones, P.; Piercey, J. E.; Montgomery, D. S.; Purvis, I. J.; Storer, R.; Dawson, M. J.; Lawrence, G. C. Enzymatic production of optically pure (2′*R*-*cis*)-2′-deoxy-3′-thiacytidine (3TC, Lamivudine): A potent anti HIV agent. *Enzyme Microb. Technol.* **1993**, *15*, 749−755.

(3) Hutchinson, D. W. New approaches to the synthesis of antiviral nucleosides. *TIBTECH* **1990**, *8*, 348−353.

(4) Furman, P. A.; Wilson, J. E.; Reardon, J. E.; Painter, G. R. The effect of absolute configuration on the anti-HIV and anti-HBV activity of nucleoside analogues. *Antiviral Chem. Chemother.* **1995**, *6*, 345−355.

(5) Gosselin, G.; Schinazi, R. F.; Sommadossi, J.-P.; Mathe, C.; Bergogne, M.-C.; Aubertin, A.-M.; Kirn, A.; Imbach, J.-L. Anti-human immunodeficiency virus activities of the β-L enantiomers of 2′,3′-dideoxycytidine and its 5-fluoro derivative in vitro. *Antimicrob. Agents Chemother.* **1994**, *38*, 1292−1297.

(6) Van Draanen, N. A.; Tisdale, M.; Parry, N. R.; Jansen, R.; Dorsife, R. E.; Tuttle, J. V.; Averett, D. R.; Koszalka, G. W. Influence of stereochemistry on antiviral activities and resistance profiles of dideoxycytidine nucleosides. *Antimicrob. Agents Chemother.* **1994**, *38*, 868−871.

(7) Schinazi, R. F.; Gosselin, G.; Faraj, A.; Korba, B. E.; Liotta, D. C.; Chu, C. K.; Mathé, C.; Imbach, J.-L.; Sommadossi, J.-P. Pure nucleoside enantiomers of β-2′,3′-dideoxycytidine analogues are selective inhibitors of hepatitis B virus in vitro. *Antimicrob. Agents Chemother.* **1994**, *38*, 2172−2174.

(8) Spadari, S.; Maga, G.; Verri, A.; Bendiscioli, A.; Tondelli, L.; Capobianco, M.; Colonna, F.; Garbesi, A.; Focher, F. Lack of stereospecificity of some cellular and viral enzymes involved in the synthesis of deoxyribonucleotides and DNA: Molecular basis for the antiviral activity of unnatural L-β-nucleosides. *Biochimie* **1995**, *77*, 861−867.

(9) Van Draanen, N. A.; Tucker, S. C.; Boyd, F. L.; Trotter, B. W.; Reardon, J. E. β-L-Thymidine 5′-triphosphate analogues as DNA polymerase substrates. *J. Biol. Chem.* **1992**, *267*, 25019−25024.

(10) Faraj, A.; Agrofoglio, L. A.; Wakefield, J. K.; McPherson, S.; Morrow, C. D.; Gosselin, G.; Mathe, C.; Imbach, J.-L.; Schinazi, R. F.; Sommadossi, J.-P. Inhibition of human immunodeficiency virus type 1 reverse transcriptase by the 5′-triphosphate β-enantiomers of cytidine analogues. *Antimicrob. Agents Chemother.* **1994**, *38*, 2300−2305.

(11) Focher, F.; Maga, G.; Bendiscioli, A.; Capobianco, M.; Colonna, F.; Garbesi, A.; Spadari, S. Stereospecificity of human DNA polymerases α,β,γ, δ and ϵ, HIV-reverse transcriptase, HSV-1 DNA polymerase, calf thymus terminal transferase and *E. coli* DNA polymerase I in recognizing D- and L-thymidine 5′-triphosphate as substrate. *Nucleic Acids Res.* **1995**, *23*, 2840−2847.

(12) Miller, W. H.; Daluge, S. M.; Garvey, E. P.; Hopkins, S.; Reardon, J. E.; Boyd, F. L.; Miller, R. L. Phosphorylation of carbovir enantiomers by cellular enzymes determines the stereoselectivity of antiviral activity. *J. Biol. Chem.* **1992**, *267*, 21220−21224.

(13) Chang, C.; Doong, S.; Zhou, J. H.; Beach, J. W.; Jeong, L. S.; Chu, C. K.; Tsai, C.; Cheng, Y. Deoxycytidine deaminase-resistant stereoisomer is the active form of (±)-dideoxy-3′-thiacytidine in the inhibition of hepatitis B virus replication. *J. Biol. Chem.* **1992**, *267*, 13938−13942.

(14) Furman, P. A.; Davis, M.; Liotta, D. C.; Paff, M.; Frick, L. W.; Nelson, D. J.; Dornsife, R. E.; Wurster, J. A.; Wilson, L. J.; Fyfe, J. A.; Tuttle, J. V.; Miller, W. H.; Condreay, L.; Averett, D. R.; Schinazi, R. F.; Painter, G. R. The anti-hepatitis B virus activities, cytotoxicities, and anabolic profiles of the (−) and (+) enantiomers of *cis*-5-fluoro-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. *Antimicrob. Agents Chemother.* **1992**, *36*, 2686−2692.

(15) Shewach, D. S.; Liotta, D. C.; Schinazi, R. F. Affinity of the antiviral enantiomers of oxathiolane cytosine nucleosides for human 2′-deoxycytidine kinase. *Biochem. Pharmacol.* **1993**, *45*, 1540−1543.

(16) Bennett, L. L.; Parker, W. B.; Allan, P. W.; Rose, L. M.; Shealy, Y. F.; Secrist, J. A., III; Montgomery, J. A.; Arnett, G.; Kirkman, R. L.; Shannon, W. M. Phosphorylation of the enantiomers of the carbocyclic analogue of 2′-deoxyguanosine in cells infected with herpes simplex virus type 1 and in uninfected cells. Lack of enantiomeric selectivity with the viral thymidine kinase. *Mol. Pharmacol.* **1993**, *44*, 1258−1266.

(17) Bolon, P. J.; Wang, P.; Chu, C. K.; Gosselin, G.; Boudou, V.; Pierra, C.; Mathé, C.; Imbach, J.-L.; Faraj, A.; El Alaoui, M. A.; Sommadossi, J.-P.; Pai, S. B.; Zhu, Y.-L.; Lin, J.-S.; Cheng, Y.-C.; Schinazi, R. F. Anti-human immunodeficiency and anti-hepatitis B virus activities of β-L-2′,3′-dideoxy purine nucleosides. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1657−1662.

(18) Lin, T.-S.; Luo, M.-Z.; Zhu, J.-L.; Liu, M.-C.; Zhu, Y.-L.; Dutschman, G. E.; Cheng, Y.-C. Synthesis of a series of purine 2′,3′-dideoxy-L-nucleoside analogues as potential antiviral agents. *Nucleosides Nucleotides* **1995**, *14*, 1759−1783.

(19) Chu, C. K.; Bhadti, V. S.; Doboszewski, B.; Gu, Z. P.; Kosugi, Y.; Pullaiah, K. C.; Van Roey, P. General Syntheses of 2′,3′-dideoxynucleosides and 2′,3′-didehydro-2′,3′-dideoxynucleosides *J. Org. Chem.* **1989**, *54*, 2217−2225.

(20) Mansuri, M. M.; Starrett, J. E.; Wos, J. A.; Tortolani, D. R.; Brodfuehrer, P. R.; Howell, H. G.; Martin, J. C. Preparation of 1-(2,3-dideoxy-β-D-glycero-pent-2-enofuranosyl)thymine ($d_4T$) and 2′,3′-dideoxyadenosine (ddA): General methods for the synthesis of 2′,3′-olefinic and 2′,3′-dideoxy nucleoside analogues active against HIV. *J. Org. Chem.* **1989**, *54*, 4780−4785.

(21) Elalaoui, A.; Divita, G.; Maury, G.; Imbach, J.-L.; Goody, R. S. Intrinsic tryptophan fluorescence of bovine liver adenosine kinase, characterization of ligand binding sites and conformational changes. *Eur. J. Biochem.* **1994**, *221*, 839−846.

(22) Chottiner, E. G.; Shewach, D. S.; Datta, N. S.; Ashcraft, E.; Gribbin, D.; Ginsburg, D.; Fox, I. H.; Mitchell, B. S. Cloning and expression of human deoxycytidine kinase cDNA. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1531−1535.

(23) Usova, E. V.; Eriksson, S. The effects of high salt concentrations on the regulation of the substrate specificity of human recombinant deoxycytidine kinase. *Eur. J. Biochem.* **1997**, in press.

(24) Johnson, M. A.; Ahluwalia, G.; Connelly, M. C.; Cooney, D. A.; Broder, S.; Johns, D. G.; Fridland, A. Metabolic pathways for the activation of the antiretroviral agent 2′,3′-dideoxyadenosine in human lymphoid cells. *J. Biol. Chem.* **1988**, *263*, 15354−15357.

(25) Balzarini, J.; Kang, G.; Dalal, M.; Herdewijn, P.; De Clercq, E.; Broder, S.; Johns, D. G. The Anti-HTLV-III (anti-HIV) and cytotoxic activity of 2′,3′-didehydro-2′,3′-dideoxyribonucleosides: A comparison with their parental 2′,3′-dideoxyribonucleosides. *Mol. Pharmacol.* **1987**, *32*, 162−167.

(26) Arner, E. S. J.; Erikson, S. Mammalian deoxyribonucleoside kinases. *Pharmacol. Ther.* **1995**, *67*, 155−186.

(27) Cooney, D. A.; Ahluwalia, G.; Mitsuya, H.; Fridland, A.; Johnson, M.; Hao, Z.; Dalal, M.; Balzarini, J.; Broder, S.; Johns, D. G. Initial studies on the cellular pharmacology of 2′,3′-dideoxyadenosine, an inhibitor of HT LV-III infectivity. *Biochem. Pharmacol.* **1987**, *36*, 1765−1768.

(28) Nair, V.; Sells, T. B. Interpretation of the roles of adenylosuccinate lyase and of AMP deaminase in the anti-HIV activity of 2′,3′-dideoxyadenosine and 2′,3′-dideoxyinosine. *Biochim. Biophys. Acta* **1992**, *1119*, 201−204.

(29) Maury, G.; Gosselin, G.; Sommadossi, J.-P.; Imbach, J.-L.; Goody, R. S. Unpublished results.

(30) Maury, G.; Daiboun, T.; Elalaoui, A.; Genu-Dellac, C.; Perigaud, C.; Bergogne, C.; Gosselin, G.; Imbach, J.-L. Inhibition and substrate specificity of adenosine deaminase. interaction with 2′-3′- and/or 5′-substituted adenine nucleoside derivatives. *Nucleosides Nucleotides* **1991**, *10*, 1677−1692.

JM9701482