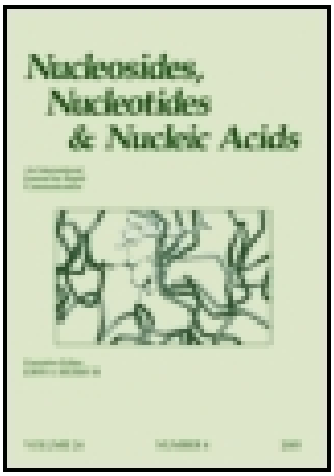

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New Unnatural L-Nucleoside Enantiomers:From Their Stereospecific Synthesis to Their Biological Activities

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NEW UNNATURAL L-NUCLEOSIDE ENANTIOMERS: FROM THEIR STEREOSPECIFIC SYNTHESIS TO THEIR BIOLOGICAL ACTIVITIES

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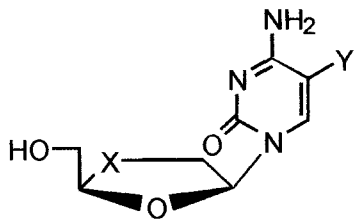
Abstract: Several purine and pyrimidine β-L-dideoxynucleosides were stereospecifically synthesized and their antiviral properties examined. Two of them, namely β-L-2',3'-dideoxyadenosine (β-L-ddA) and its 2',3'-didehydro derivative (β-L-d4A) were found to have significant anti-human immunodeficiency virus (HIV) and anti-hepatitis B virus (HBV) activities in cell culture.

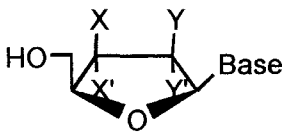
INTRODUCTION

In the search for potent and relatively non-toxic nucleoside analogues as effective drugs for the treatment of severe viral infections, β-L-enantiomers have emerged as a new class of sugar-modified derivatives with anti-HIV and anti-HBV activities.[1,2] The first such compound, β-L(-)-2',3'-dideoxy-3'-thiacytidine (3TC, Lamivudine; Fig. 1a) was recently approved as an anti-HIV agent, and its 5-fluoro derivative [(-)-FTC; Fig. 1a] has also been found to exibit antiviral activity against HIV, as well as HBV, *in vitro* and *in vivo*.[3] 2',3'-Dideoxycytidine (β-L-ddC; Fig. 1a) and its 5-fluoro derivative (β-L-FddC; Fig. 1a), which have



X = S, Y = H → 3TC
X = S, Y = F → (-)-FTC
X = CH_2, Y = H → β-L-ddC
X = CH_2, Y = F → β-L-FddC

- Figure 1a -



Base = purine-9-yl or pyrimidine-1-yl derivatives

X, X' } = didehydro, H, F, N_3, NH_2
Y, Y' }

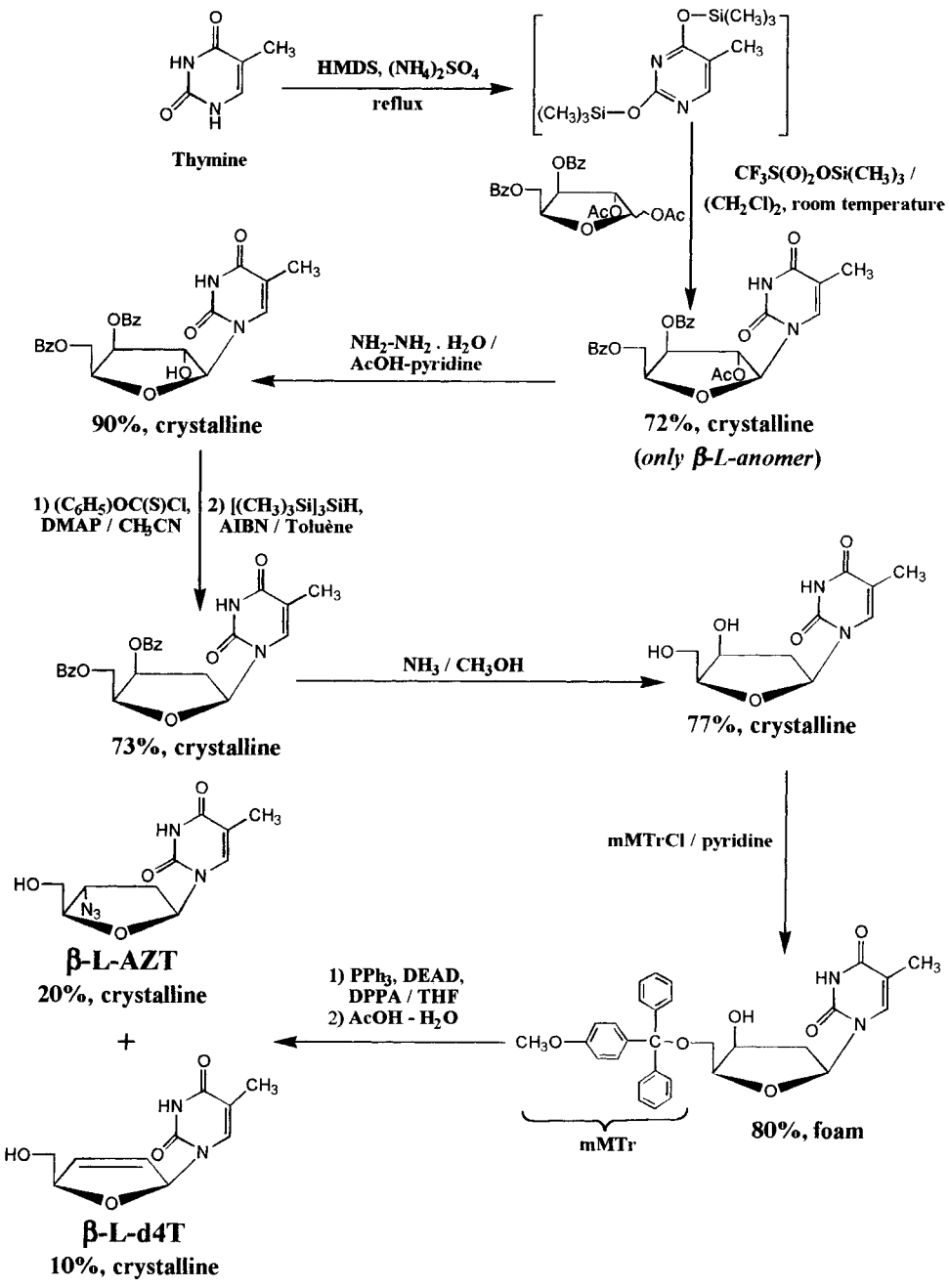
- Figure 1b -

been intensively studied by our groups[4-17] as well as in other laboratories[2, 18-32] are additional L-nucleoside analogues which should deserve preclinical development in order to determine their merit as new antiviral drugs to treat infections caused by HIV and HBV.

In continuation of our research programme on L-sugar-modified nucleoside analogues[33-40] and in view of the interesting antiviral properties of the β-L-enantiomers, we now report the stereospecific synthesis and the biological evaluations of a number of pyrimidine and purine dideoxy-β-L-nucleosides (Fig. 1b).

β-L-AZT AND β-L-d4T

From a synthetic view point (Scheme 1), the dissymmetric peracylated 1,2-di-O-acetyl-3,5-di-O-benzoyl-L-xylofuranose[38] was condensed with silylated thymine to afford exclusively (in accord with Baker's rule[41], and owing to 2-O-acyl participation during the condensation) the corresponding fully protected β-L-nucleoside anomer. This compound was selectively deacylated at its 2'-position, and then subjected to a deoxygenative hydrogenolysis. Deacylation with saturated methanolic ammonia afforded 1-(2-deoxy-β-L-*threo*-pentofuranosyl) thymine, of which the 5'-hydroxyl function was selectively monomethoxytritylated



- Scheme 1 -

Finally, reaction with diphenyl phosphorazidate (DPPA), triphenyl-phosphine and diethyl azodicarboxylate (DEAD), followed by detritylation with aqueous acetic acid, gave a mixture of β-L-AZT (20% yield) and β-L-d4T (10% yield) which could be separately isolated as pure and crystalline after silica gel column chromatography.

β-L-AZT and β-L-d4T were tested against HIV-1 in PBM cells and against HBV in Hep-G2 cells, but none of them displayed activity at the highest concentration tested (Table 1).

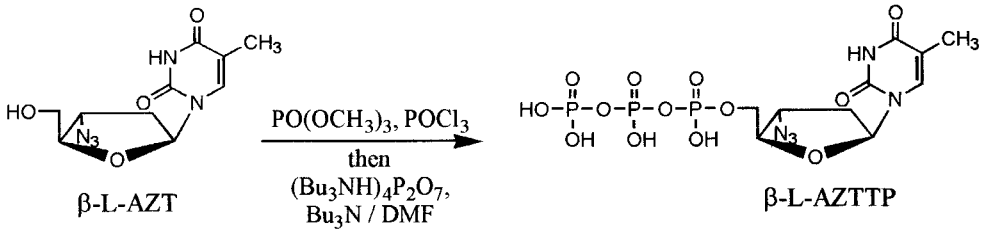
The lack of anti-HIV activity is in accordance with previously published results, since it has been reported that β-L-AZT and β-L-d4T [45-46] (synthesized following other approaches) had no biological activity. However β-L-d4T 5'-triphosphate was shown to be a potent inhibitor of HIV reverse transcriptase[46] and HBV DNA polymerase.[31] In order to determine whether β-L-AZT 5'-triphosphate could interact with viral and human DNA polymerases, we synthesized this hitherto unknown nucleotide analogue (Scheme 2) and studied its inhibitory activities on HIV reverse transcriptase, woodchuck HBV DNA polymerase and cellular DNA polymerases α and β (Table 2). Since we found that β-L-AZTTP inhibits HIV reverse transcriptase and HBV DNA polymerase at a sub-micromolar concentration, we can conclude that β-L-AZT is devoid of antiviral activity because it is not efficiently phosphorylated intracellularly in lymphocytes and liver hepatocytes.

β-L-ddA AND β-L-d4A

For the synthesis of these β-L purine nucleosides, coupling of 1,2-di-O-acetyl-3-deoxy-5-O-benzoyl-L-*erythro*-pentofuranose[14] with adenine, followed by selective deacetylation of the 2'-hydroxyl functionality provided 9-(5-O-benzoyl-3-deoxy-β-L-*erythro*-pentofuranosyl)adenine (Scheme 3). This key intermediate was converted to either β-L-ddA (*via* a radical reductive process) or β-L-d4A (*via* an elimination of the mesylate). For example, treatment of this intermediate with phenyl chlorothionoformate and dimethylaminopyridine, followed successively by

Table 1. Antiviral and cytotoxicity evaluation of β-L-AZT and β-L-d4T.

	HIV-1 / PBM cells		HBV / Hep-G2 cells	
	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
β-L-AZT	> 100	> 100	> 10	> 200
β-L-d4T	> 100	> 100	> 10	> 200



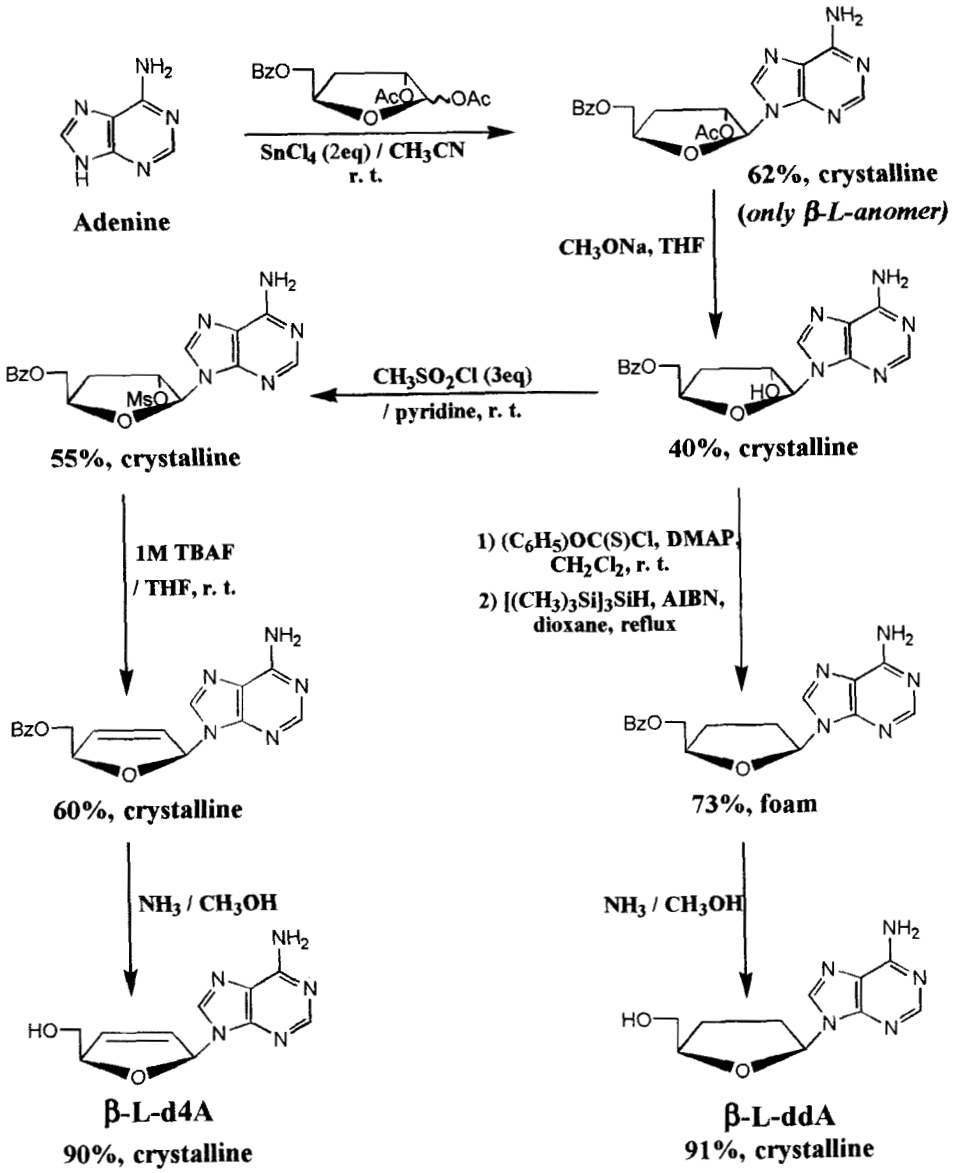
-Scheme 2-

Table 2. Inhibitory activities of β-L-AZT 5'-triphosphate on various polymerases.

	HIV reverse transcriptase	Woodchuck HBV DNA polymerase	Cellular DNA polymerases	
			DNA pol α	DNA pol β
	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
β-L-AZTTP	0.27	0.20	> 100	> 100

subsequent radical deoxygenation and debenzoylation, afforded the desired β-L-ddA.[47] On the other hand, reaction of the same intermediate with mesyl chloride and pyridine, followed successively by subsequent treatment with tetrabutylammonium fluoride in THF and debenzoylation provided β-L-d4A.[47]

Both β-L-ddA and β-L-d4A were evaluated against HIV-1 in PBM cells[47] and against HBV in Hep-G2 cells[47,48] (Table 3).



- Scheme 3 -

Table 3. Antiviral and cytotoxicity evaluation of β-L-ddA and β-L-d4A.[47,48]

	HIV-1 / PBM cells		HBV / Hep-G2 cells	
	EC_{50} (μM)	CC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
β-L-ddA	8.2	> 100	6.0 5.0	> 200 > 200
β-L-d4A	0.38	59	1.2 0.10	70 180

Both β-L-ddA and β-L-d4A inhibited HIV and HBV replication *in vitro*, β-L-d4A being the more potent but also the more cytotoxic. Regarding β-L-ddA, during the course of our work, other authors reported similar anti-HBV properties (EC_{50} = 6 μM in Hep-G2 cells), but none anti-HIV activity (EC_{50} > 100 μM in MT-2 cells).[49] Since we have also found that β-L-ddA and β-L-d4A are not substrates for adenosine deaminase (data not shown), their observed antiviral activity suggests direct intracellular phosphorylation to the respective nucleotide derivatives.

CONCLUSION

The previously published results on β-L-FddC,[4-32] as well as the data so far presented, provide a strong rationale for synthesizing and evaluating the β-L-enantiomers of other nucleoside analogues. Work is in progress in our laboratory on this topic, with the hope to identify new potent and selective antiviral agents.

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