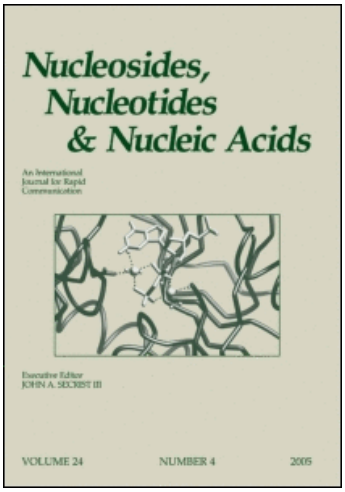

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UNNATURAL β-L-ENANTIOMERS OF NUCLEOSIDE ANALOGUES AS POTENT ANTI-HEPATITIS B VIRUS AGENTS

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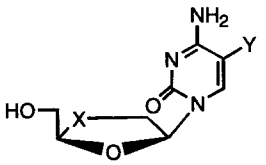
Abstract: Several 2'- or 3'- substituted 2',3'-dideoxy-β-L-nucleosides bearing adenine as the base were stereospecifically synthesized and their antiviral properties examined. Two of them, namely 2'-azido- and 3'-azido-2',3'-dideoxy-β-L-adenosine (2'-N_3-β-L-ddA and 3'-N_3-β-L-ddA) were found to have some anti-hepatitis B virus (HBV) activity in cell culture.

INTRODUCTION

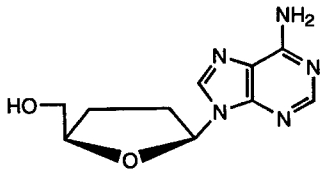
During the last decade there has been some interest in the synthesis and biological evaluation of L-nucleoside analogues, although the activities of most nucleoside analogues had been already associated with the natural D-enantiomers. However, more recently several 2',3'-dideoxy pyrimidine-β-L-nucleoside analogues have been synthesized. Among them, those bearing a cytosine base (3TC, β-L-5FddC, Fig. 1a) have been found to possess potent activity against both human immunodeficiency virus (HIV) and hepatitis B virus (HBV).[1,2]

As a part of our ongoing research programme on β-L-sugar-modified nucleoside analogues,[3-10] we have reported previously the stereospecific

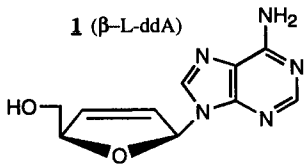


X = S, Y = H → 3TC
X = S, Y = F → (-)-FTC
X = CH_2, Y = H → β-L-ddC
X = CH_2, Y = F → β-L-FddC

- Figure 1a -



1 (β-L-ddA)



2 (β-L-d4A)

- Figure 1b -

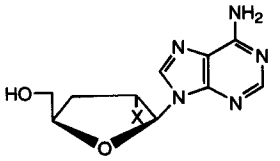
synthesis and the anti-HBV activity of 2',3'-dideoxy-β-L-adenosine (β-L-ddA, **1**) and its 2',3'-didehydro derivative (β-L-d4A, **2**)[10-12] (Fig. 1b).

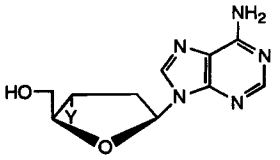
Here we described the synthesis of the 2'-fluoro (2'-F-β-L-ddA, **3**), 2'-azido (2'-N_3-β-L-ddA, **4**), 2'-amino (2'-NH_2-β-L-ddA, **5**), 3'-fluoro (3'-F-β-L-ddA, **6**), 3'-azido (3'-N_3-β-L-ddA, **7**), and 3'-amino (3'-NH_2-β-L-ddA, **8**) derivatives (Fig. 2) of β-L-ddA in order to evaluate their antiviral properties in cell cultures.

2'-Substituted β-L-ddA derivatives

From a synthetic view point, coupling of 1,2-di-*O*-acetyl-3-deoxy-5-*O*-benzoyl-L-*erythro*-pentofuranose[9] with adenine, followed by deacetylation provided 9-(3-deoxy-β-L-*erythro*-pentofuranosyl)adenine (Scheme 1). Selective bis 5'-*O* and ^{6}*N*-tritylation followed by Mitsunobu[13] inversion of the 2'-hydroxyl function and debenzoylation gave 9-(5-*O*-trityl-3-deoxy-β-L-*threo*-pentofuranosyl)^6*N*-trityladenine. Fluoration of this key intermediate was effected using (diethylamino)sulfur trifluoride (DAST) in methylene chloride. Detritylation in acidic conditions afforded the desired 2'-F-β-L-ddA **3** (Scheme 1).

Azidation of 9-(5-*O*-trityl-3-deoxy-β-L-*threo*-pentofuranosyl)^6*N*-trityl adenine was carried out following a modified Mitsunobu procedure[13, 14] and





3, X = F (2'-F-β-L-ddA)

4, X = N_3 (2'-N_3-β-L-ddA)

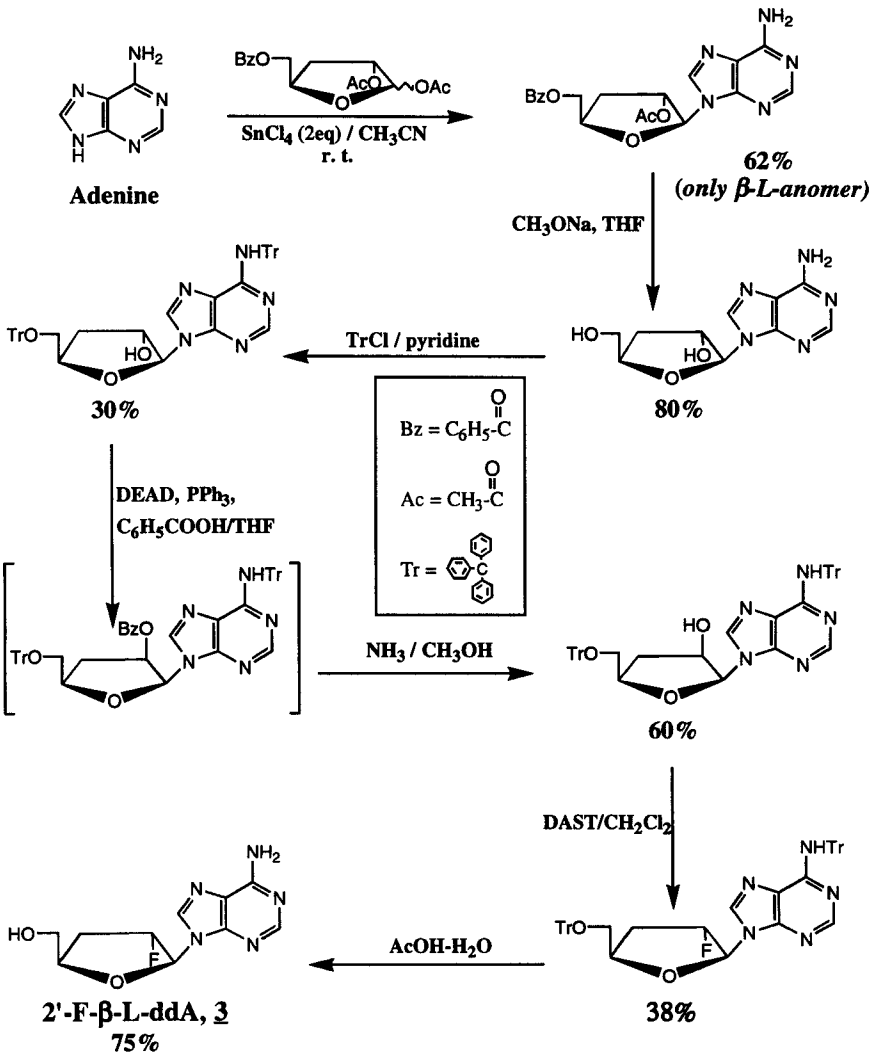
5, X = NH_2 (2'-NH_2-β-L-ddA)

6, Y = F (3'-F-β-L-ddA)

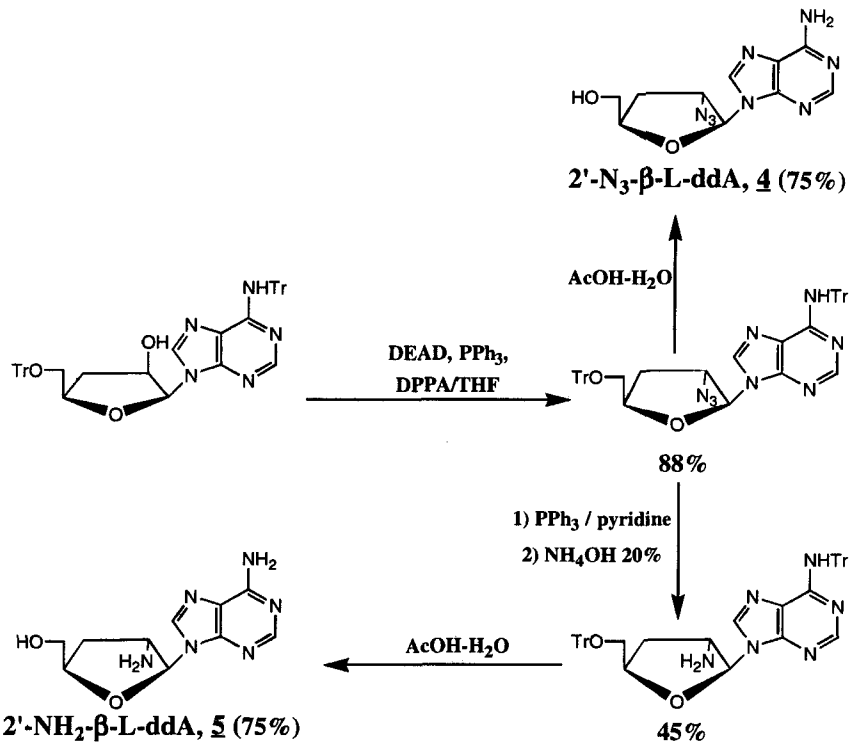
7, Y = N_3 (3'-N_3-β-L-ddA)

8, Y = NH_2 (3'-NH_2-β-L-ddA)

- Figure 2 -



- Scheme 1 -



- Scheme 2 -

afforded, after detritylation, 2'-N_3-β-L-ddA **4** (Scheme 2). The tritylated intermediate azido nucleoside was converted to the corresponding amino derivative 2'-NH_2-β-L-ddA **5** by treatment with triphenylphosphine in pyridine, followed by hydrolysis with concentrated ammonium hydroxyde and detritylation, as reported previously in other series.[15]

3'-Substituted β-L-ddA derivatives

For the synthesis of these nucleoside analogues, the dissymmetric peracylated 1,2-di-*O*-acetyl-3,5-di-*O*-benzoyl-L-xylofuranose[3] was condensed with adenine to afford exclusively (in accord with Baker's rule[16] owing to 2-*O*-acyl

participation during the condensation) the corresponding fully protected β-L-nucleoside anomer (Scheme 3).

This compound was selectively deacylated at its 2'-position, and then subjected to a deoxygenative hydrogenolysis. Successive ^{6}*N*-monomethoxytritylation, full debenzoylation and selective 5'-*O*-monomethoxytritylation afforded 9-(2-deoxy-β-L-*threo*-pentofuranosyl)^{6}*N*-monomethoxytrityladenine. This intermediate was converted to 3'-fluoro-β-L-ddA **6**, 3'-azido-β-L-ddA **7** and 3'-amino-β-L-ddA **8** following similar procedures as used during the synthesis of the corresponding 2'-substituted derivatives of β-L-ddA.

Antiviral activities

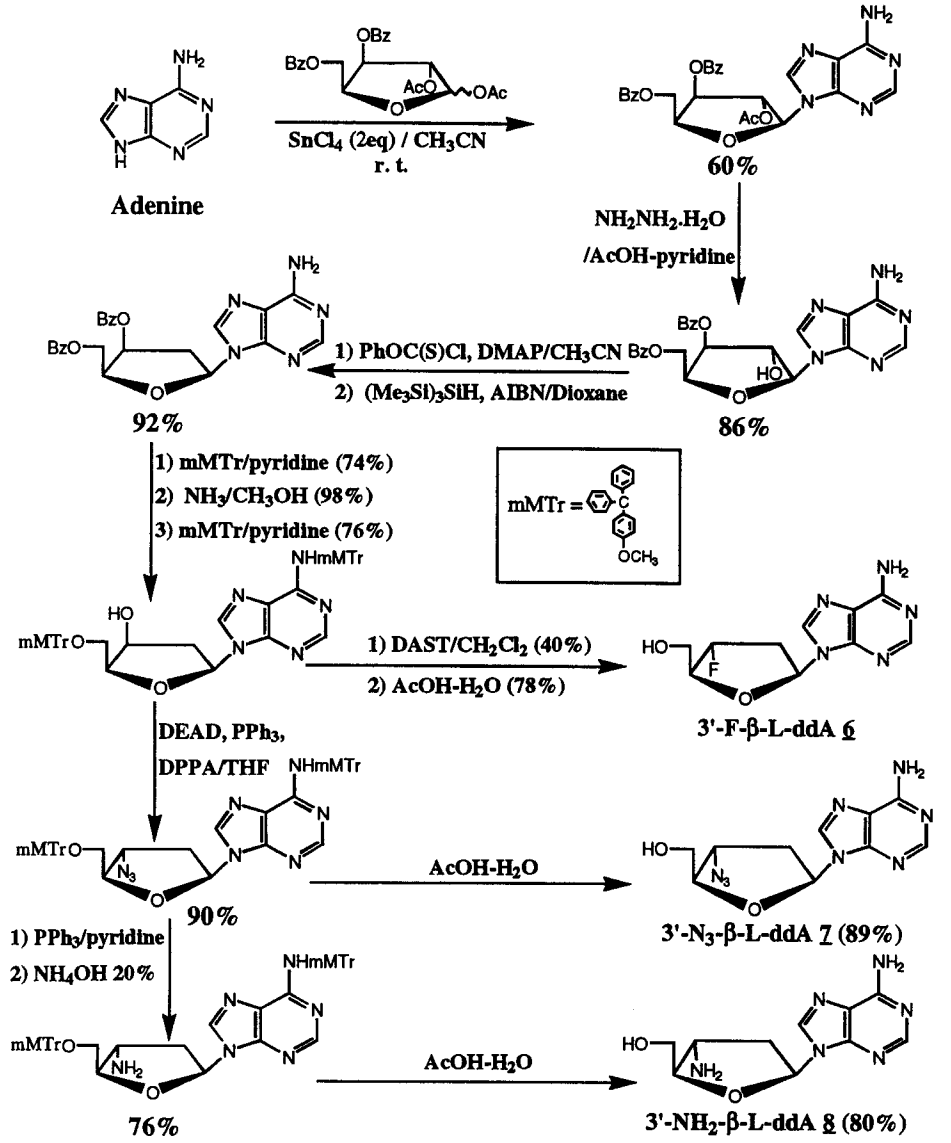
All the 2'- and 3'- substituted derivatives **3-8** of β-L-ddA **1** were evaluated against HIV in MT-4 cells and against HBV in 2.2.15 transfected Hep-G2 cells (Table).

Thus it appears that none of the studied compounds, including the parent nucleoside β-L-ddA **1**, displayed anti-HIV activity at the highest concentration tested (100 μM). Although we have recently reported that β-L-ddA **1** inhibited HIV replication in PBM cells (EC_{50} = 8.2 μM, CC_{50} > 100 μM),[10,11] the lack of anti-HIV activity of this β-L-dideoxynucleoside in MT-4 cells is in accordance with other previously published results (EC_{50} > 100 μM in MT-2 cells).[17]

On the other hand, both β-L-ddA **1** and its 2'- and 3'-azido derivatives (**4** and **7**, respectively) inhibited HBV replication at concentrations ranging between 2.2 and 5.0 μM. It is noteworthy that none of these compounds showed cytotoxicity at 200 μM.

CONCLUSION

Several 2'- and 3'-substituted derivatives (**3-8**) of 2',3'-dideoxy-β-L-adenosine **1** were stereospecifically and conveniently synthesized following a multi-step reaction. They were characterized on the basis of their physical



- Scheme 3 -

Compound	Anti-HIV evaluation [a] MT-4 cells		Anti-HBV evaluation [a]	
	HIV-1 IIIB infected cells	non infected cells	transfected 2.2.15 cells	normal Hep-G2 cells
	EC_{50} [b] (μM)	CC_{50} [c] (μM)	EC_{50} [b] (μM) R.I.[d]	CC_{50} [c] (μM)
β-L-ddA, **1**	> 100	> 100	**5.0**	> 200
2'-F-β-L-ddA, **3**	> 100	> 100	> 10	> 200
2'-N_3-β-L-ddA, **4**	> 100	> 100	**2.2**	> 200
2'-NH_2-β-L-ddA, **5**	> 100	> 100	> 10	> 200
3'-F-β-L-ddA, **6**	> 100	> 100	> 10	> 200
3'-N_3-β-L-ddA, **7**	> 100	> 100	**5.0**	> 200
3'-NH_2-β-L-ddA, **8**	> 100	> 100	> 10	> 200

[a] All data represent average values from at least three separate experiments. [b] EC_{50} values represent the drug concentration (μM) requires to inhibit the replication of HIV-1 or HBV by 50%. [c] CC_{50} represent the drug concentration (μM) required to reduce the viability of non infected cell growth by 50%. [d] R.I. : Replicative intermediate (intracellular) HBV DNA.

(melting points, optical rotations) and spectroscopic properties (UV, ^{1}H-NMR, FAB mass spectra) ; their purities were ascertained by combustion analysis and HPLC. When evaluated against HBV in cell cultures, it appeared that the 2'- and 3'-azido derivatives (**4** and **7**, respectively), as well as β-L-ddA **1** showed some anti-HBV activity without cytotoxic effect.

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