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Communications to the Editor

Design and Synthesis of 2′,3′-Dideoxy-2′,3′-didehydro-β-L-cytidine (β-L-d4C) and 2′,3′-Dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C), Two Exceptionally Potent Inhibitors of Human Hepatitis B Virus (HBV) and Potent Inhibitors of Human Immunodeficiency Virus (HIV) in Vitro

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It has been estimated that 300 million people worldwide are now infected with the hepatitis B virus (HBV), a causative agent of both acute and chronic forms of hepatitis.[1] Furthermore, chronic infection with HBV has been associated with a high risk for the development of primary hepatocellular carcinogen.[2] In fact, at the present time there is no clinically useful drug for the treatment of HBV infection. Considerable effort has been directed in the search for novel nucleoside structures for use as antiviral and anticancer agents. Most of these analogues are synthesized by modification of the naturally occurring nucleosides and, therefore, possess the β-D-configuration. In the past, little attention has been given to the synthesis and study of the biological activity of L-nucleosides, the enantiomers of natural D-nucleosides. Recently, a number of the unnatural L-configuration nucleoside analogues have emerged as potent antiviral agents against HBV and human immunodeficiency virus (HIV), which include β-L-[2-(hydroxymethyl)-1,3-oxathiolan-4-yl]cytosine [β-L-SddC, (−)-SddC, 3TC, Lamivudine],[3−5] β-L-[2-(hydroxymethyl)-1,3-oxathiolan-4-yl]-5-fluorocytosine [(−)-FSddC, (−)-FTC],[3,6,7] 2′,3′-dideoxy-β-L-cytidine (β-L-ddC), 2′,3′-dideoxy-β-L-5-fluorocytidine (β-L-FddC),[8−11] and 2′-fluoro-5-methyl-β-L-arabinofuranosyluracil (L-FMAU)[12] (Figure 1). Since 2′,3′-dideoxy-2′,3′-didehydro-β-D-cytidine (β-D-d4C) has proven to be about as equipotent as β-L-ddC as an anti-HIV agent,[13] it seemed logical to synthesize and evaluate the antiviral activity of its L-enantiomer, 2′,3′-dideoxy-2′,3′-didehydro-β-L-cytidine (β-L-d4C, **1**), and its 5-fluoro derivative, 2′,3′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C, **2**). In this communication, we report the synthesis and biological evaluation of β-L-d4C and β-L-Fd4C, which show exceptional potent activity against HBV and significant activity against HIV. Since patients receiving long-term, anti-HBV or -HIV nucleoside therapy have experienced delayed toxicity, which may be linked to the drugs inhibition of mitochondrial DNA synthesis,[14] the effect of β-L-d4C and β-L-Fd4C in decreasing the mitochondrial DNA content in cells upon long-term exposure to these two drugs was also studied. β-L-d4C (**1**) was also independently synthesized by Mansuri et al.;[15] however, no physical properties and spectroscopic data were reported. Furthermore, contrary to our results, they reported that this compound has no antiviral activity.

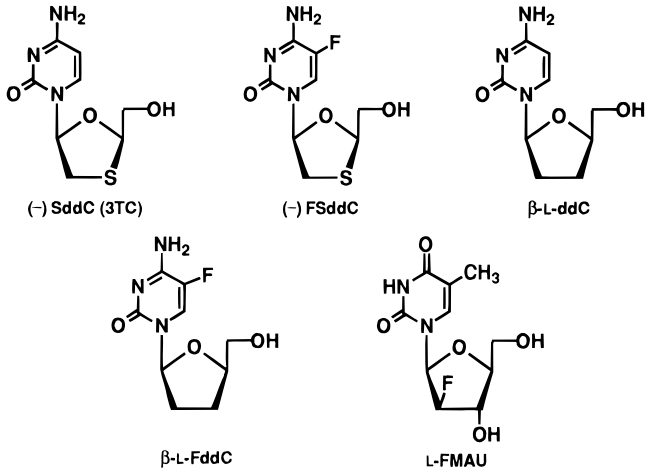

Figure 1.

Chemistry. The synthesis of 2′,3′-dideoxy-2′,3′-didehydro-β-L-cytidine (β-L-d4C, **1**) and 2′,3′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C, **2**) is depicted

† Deceased.

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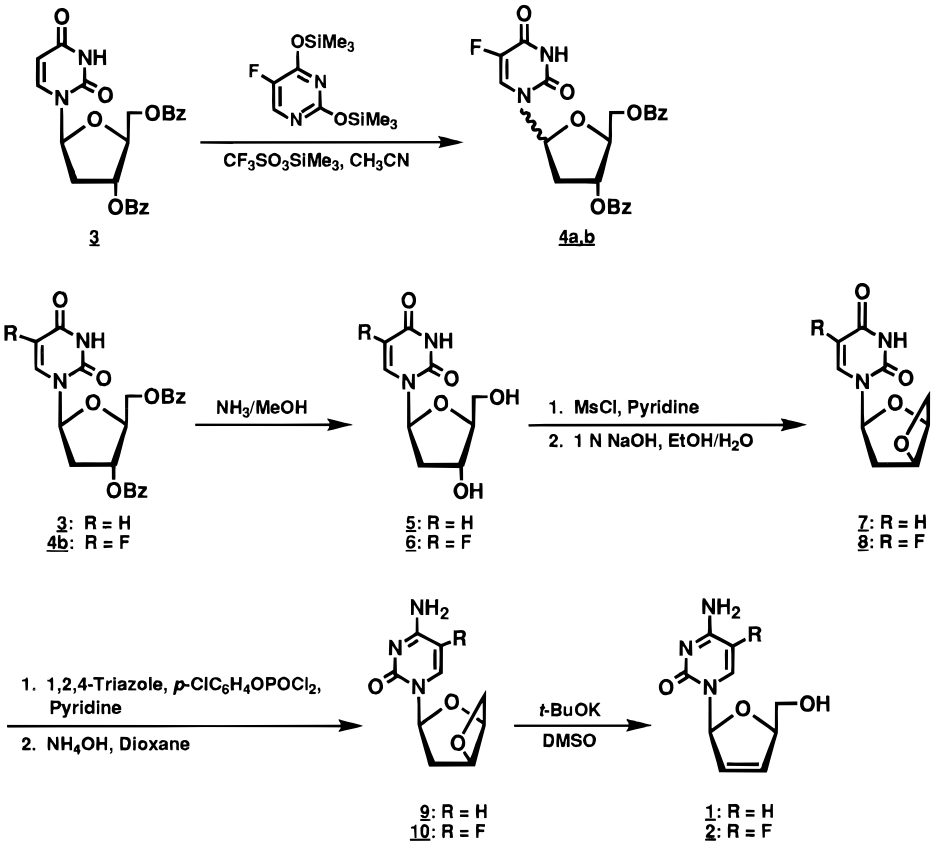
Scheme 1



in Scheme 1. The key intermediate 3′,5′-dibenzoyl-2′-deoxy-β-L-uridine (**3**) was synthesized from L-arabinose by the methodology of Holy[16] with minor modification. Transglycosylation[17] reaction of **3** with silylated 5-fluorouracil using trimethylsilyl trifluoromethanesulfonate as a catalyst afforded a mixture of the α- and β-anomers 3′,5′-dibenzoyl-2′-deoxy-α-L-5-fluorouridine (**4a**) and 3′,5′-dibenzoyl-2′-deoxy-β-L-5-fluorouridine (**4b**), which were separated by silica gel chromatography. Treatment of compounds **3** and **4b** with saturated NH_3/MeOH solution overnight produced 2′-deoxy-β-L-uridine (**5**) and 2′-deoxy-β-L-5-fluorouridine (**6**). Reaction of **5** and **6** with 2 M equiv of mesyl chloride in anhydrous pyridine followed by 1 N NaOH solution[18] resulted in the formation of the respective cyclic ethers **7** and **8**. Treatment[19] of compounds **7** and **8** with 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in anhydrous pyridine followed by a mixture of ammonium hydroxide/dioxane (2:1, v/v) afforded the corresponding cytidine derivatives **9** and **10**, which were then treated with *tert*-butoxide in DMSO to furnish the target compounds 2′,3′-dideoxy-2′,3′-didehydro-β-L-cytidine (β-L-d4C, **1**)[20] and 2′,3′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C, **2**), respectively.[21]

Biological Evaluation. The synthesized compounds β-L-d4C (**1**) and β-L-Fd4C (**2**), along with the known antiviral compounds β-D-ddC, β-D-d4C, β-L-FddC, and β-L-SddC, were tested for their antiviral activities in vitro, and the results are shown in Table 1. The procedures used were the same as described previously.[3,9] Among these nucleoside analogues, 2′,3′-dideoxy-2,3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C, **2**) was found to be most active against HBV. Compounds demonstrating significant anti-HBV activity (extracellular and intracellular, respectively) in decreasing activity were β-L-Fd4C, β-L-d4C, β-L-SddC, and β-L-FddC. To the best of our knowledge, β-L-Fd4C appears to be the most potent and selective compound against HBV reported to date. In addition, the compounds exhibiting activity against HIV in decreasing antiviral activity were β-L-Fd4C, β-L-FddC, β-D-d4C, β-L-d4C, β-D-ddC, and β-L-SddC. The compounds were also tested in vitro for their cytotoxicity against CEM cell lines. The cytotoxity of these nucleotides in decreasing order were β-L-Fd4C, β-D-ddC, β-L-d4C, β-D-d4C, β-L-SddC, and β-L-FddC.

Table 1. Evaluation of 2′,3′-Dideoxycytidine Analogue Antiviral Activity against Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV-1), Effects on Mitochondrial DNA Synthesis, and Cytotoxicity against CEM Cells in Vitro

	HBV ED_{50}[a] (nM)			cytotoxicity ED_{50}[c] (μM)	
compd	extracellular	intracellular	HIV-1[b] ED_{50}(μM)	cell growth	mt-DNA
DddC	5200	6200	1.5	10	0.022
Dd4C	3000		0.7	22	2
1 (β-L-d4C)	10	8	~1.0	20	>20
2 (β-L-Fd4C)	2	2	0.09	7	>100
β-L-FddC	55	36	0.5	67	>100
β-L-SddC (3TC)	17	30	2.0	50	>50

[a] Concentration required to inhibit 50% of extracellular circular and intracellular replicating HBV DNA using 2215 cell line.[3] [b] Concentration required to inhibit 50% of HIV activity in MT-2 cells.[9] [c] Concentration required to inhibit 50% of cell growth and to decrease 50% of mt-DNA content in CEM cells.[3,9]

Since anti-HIV, D-nucleoside analogues produce delayed toxicity in patients from long-term treatment with these drugs, and this delayed toxicity caused by these drugs may relate to their ability to inhibit mitochondrial DNA synthesis,[14] the ability of β-L-Fd4C and β-L-d4C

to decrease the mitochondrial DNA content in cells upon long-term exposure to these two drugs was also evaluated. Both compounds showed no effect on mitochondrial DNA content of CEM cells after a 6 day exposure at 10 μM, which is a much higher concentration required to inhibit HBV in culture. The spectrum of biological activity of β-L-Fd4C and β-L-d4C in culture is similar to β-L-SddC (3TC), which has just been approved by the FDA for use in combination drug therapy against HIV and HBV.

In conclusion, we present the synthesis and preliminary biological profile of 2′,3′-dideoxy-2′,3′-didehydro-β-L-cytidine (β-L-d4C, **1**) and 2′,3′-dideoxy-2′,3′-didehydro-β-L-5-fluorocytidine (β-L-Fd4C, **2**), which demonstrated exceptional potent activity against HBV and significant activity against HIV. Therefore, these two compounds merit further development as potential anti-HBV and HIV agents.

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Supporting Information Available: Experimental procedures for compounds **1**−**4a**,**b** and **7**−**10** and elemental analyses for compounds **1**, **2**, **4b**, and **7**−**10** (5 pages). Ordering information is given on any current masthead page.

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(20) Isolated as a white solid: mp 148−150 °C dec; TLC R_f 0.54 (CH_2Cl_2/MeOH, 2:1, v/v); $[\alpha]_D$ −23.2° (c = 0.11, MeOH); UV (MeOH) λ_{max} 268 nm (ϵ 5795), λ_{min} 250 nm; UV (0.01 N HCl) λ_{max} 278 nm (ϵ 9711), λ_{min} 240 nm; UV (0.01 N NaOH) λ_{max} 270 nm (ϵ 6735), λ_{min} 250 nm; ^{1}H NMR (Me_2SO-d_6) δ 3.52−3.56 (dd, 2 H, 5′-H), 4.74 (m, 1 H, 4′-H), 4.94−4.98 (t, 1 H, 5′-OH, D_2O exchangeable), 5.68 (d, 1 H, 5-H, J = 7.6 Hz), 5.86 (m, 1 H, 3′-H), 6.32 (m, 1 H, 2-H′), 6.87 (d, 1 H, 1′-H), 7.12 and 7.22 (2 s, 2 H, 4-NH_2, D_2O exchangeable), 7.67 (d, 1 H, 6-H, J = 7.6 Hz).

(21) Isolated as a white solid: mp 118 °C dec; TLC R_f 0.46 (AcOEt/EtOH, 5:1, v/v); $[\alpha]_D$ −23.4° (c = 0.11, MeOH); UV (MeOH) λ_{max} 280 nm (ϵ 5966), λ_{min} 262 nm; UV (0.01 N HCl) λ_{max} 285 nm (ϵ 7192), λ_{min} 247 nm; UV (0.01 N NaOH) λ_{max} 281 nm (ϵ 6735), λ_{min} 262 nm; ^{1}H NMR (Me_2SO-d_6) δ 3.31−3.70 (dd, 2 H, 5′-H), 4.45−4.48 (t, 1 H, 5′-OH, D_2O exchangeable), 4.77 (m, 1 H, 4′-H), 5.87 (m, 1 H, 3′-H), 6.31 (m, 1 H, 2′-H), 6.82 (d, 1 H, 1′-H), 7.55 and 7.78 (2 br s, 2 H, 4-NH_2, D_2O exchangeable), 8.03 (dd, 1 H, 6-H, J = 7.1 Hz).

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