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SYNTHESIS AND BIOLOGICAL EVALUATION OF A SERIES OF 2′-FLUORINATED-2′,3′-DIDEOXY-2′,3′-DIDEHYDRO-(L)-NUCLEOSIDES

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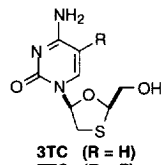
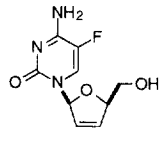
Introduction

L-Nucleoside analogs, the enantiomers of the natural D-nucleosides, have emerged as potent antiviral agents against HIV, HBV, and other related viruses. It is generally believed that L-nucleosides are endowed with minimal host toxicity while maintaining good antiviral/antibacterial activity. This is because L-nucleosides are usually not recognized by normal mammalian enzymes but by virus-encoded or bacterial enzymes.[1] For example, 3TC (Lamivudine)[2] is the first L-nucleoside derivative that has been approved by the FDA for use in combination therapy against HIV and HBV. (–)FTC,[3] a 5-fluorinated analog of 3TC, has also demonstrated potent antiviral activity against HIV and HBV. More recently, β-L-FddC[4] and β-L-FD_4C[5] have been identified, on the basis of their excellent antiviral activity, as new leads. In particular, β-L-FD_4C has shown exceptional activity against HBV, which is 10- to 20-fold greater than 3TC (Lamivudine). Careful examination of the chemical structures of these four L-nucleosides reveals that three of them contain a fluorine atom at the 5-position on the base (see Figure 1).

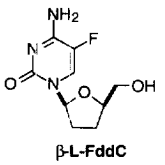
Figure 1: Structures of Nucleoside Analogs



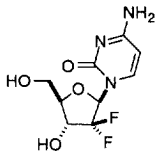
3TC (R = H)
FTC (R = F)



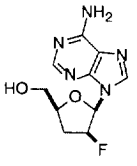
β-L-FD4C (1)



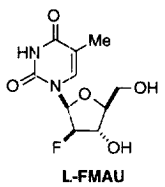
β-L-FddC



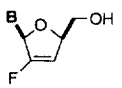
Gemcitabine



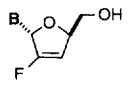
F-ddA



L-FMAU



2a B = 5-Fluorocytosine
3a B = Thymine
4a B = 5-Iodouracil
5a B = Adenine



2b B = 5-Fluorocytosine
3b B = Thymine
4b B = 5-Iodouracil
5b B = Adenine

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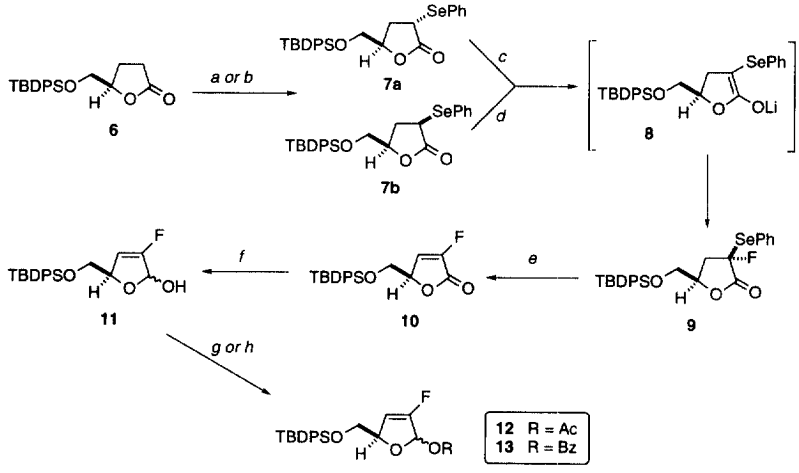
Similarly, fluorine substitution at the 2′-position often results in nucleoside analogs that exhibit favorable acid stability and antiviral/antitumor activity. Examples of this type (shown in Figure 1) include Gemcitabine,[6] a newly approved anticancer agent; F-ddA,[7] a drug currently in clinical trials against drug-resistant HIV isolates; L-FMAU,[8] a potent anti-HBV, and anti-Epstein-Barr virus (EBV) L-nucleoside with a favorable toxicity profile, which is being evaluated in preclinical toxicology studies (Figure 1).

Taking these observations together as a whole, it appears reasonable to expect that incorporation of a fluorine atom at either the 5-position on the base or the 2′-position on the sugar ring leads to nucleoside analogs possessing enhanced biological activity acid stability.[9] Guided by this generalization, we became interested in the synthesis and evaluation of a series of L-nucleoside analogs that contain a 2′-fluorine atom such as 2′-vinylfluoro bearing nucleosides. A careful substructure search on this subject reveals that only a few 2′-vinylfluorine containing nucleosides in the D-series (e.g., 2′-FD$_4$T, 2′-FD$_4$C, 2′-FD$_4$U) were synthesized and reported to be much less potent than AZT.[10] So far, no 2′-vinylfluoro-bearing L-nucleosides have been reported. In this report, we wish to disclose the synthesis and evaluation of a series of 2′-fluorinated nucleosides **2–5** shown in Figure 1.

Synthesis

The synthesis of two 2′-fluorinated acetals **12** and **13**, the key intermediates employed for the synthesis of our target nucleosides (**2–5**) is shown in Scheme 1. Stereospecific introduction of the 2′-phenylseleno moiety in **6** afforded the 2′α-phenylselenolactone **7a** (75%).[11] Treatment of **7a** with LiHMDS in THF followed by F-N(SO$_2$Ph)$_2$ provided the 2′a-fluorinated lactone **9** in 86–95% yield. As expected, fluorination of the 2′β-phenylselenolactone **7b**, obtained from nonspecific 2′-phenylselenation,[12] under identical conditions provided the same product **9** (87%), suggesting that the lithium enolate **8** was the common intermediate in this fluorination reaction.[13] Oxidative elimination of the 2′-phenylseleno moiety in **9** afforded the 2′-fluoro-enonelactone **10** (90%), which was reduced with DIBAL-H to the corresponding lactol **11** in 93% yield. Acylation of **11** with acetic anhydride or benzoyl chloride afforded compounds **12** (96%) and **13** (87%), respectively.

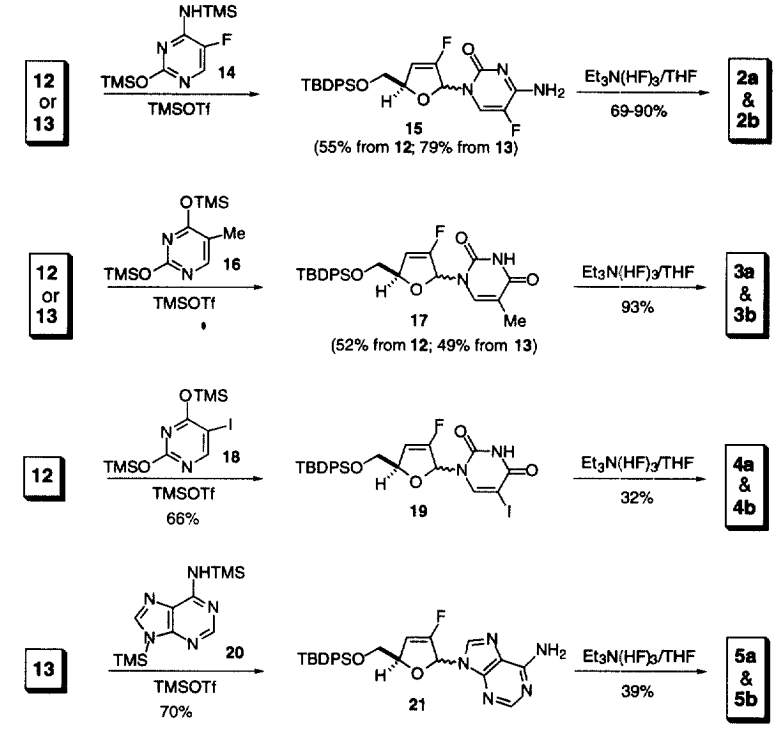
Scheme 1



Reagents and Conditions: (a) LiHMDS/THF/-78 °C, PhSeBr, 60% **7a** + 24% **7b**; (b) LiHMDS/THF/-78 °C, N-(phenylseleno) phthalimide, 75% **7a** + <2% **7b**; (c) & (d) LiHMDS/THF/-78 °C, F-N(SO$_2$Ph)$_2$, 86-95% from **7a**; 87% from **7b**; (e) 30% H$_2$O$_2$/pyridine/rt, 90%; (f) DIBAL-H/Toluene/-78 °C, 93%; (g) Ac$_2$O/TEA, 96%; (h) BzCl/pyridine, 87%.

With **12** and **13** in hand, we attempted the N-glycosylation reactions shown in Scheme 2. Stirring a 1,2-dichloroethane solution containing **12** (1 equiv) together with 3 equiv of bisTMS-5-fluorocytosine **14** and 1.2 equiv of TMSOTf overnight at room temperature provided the desired product **15** (55%) as an anomeric mixture in a ratio of ~1.5:1 favoring the *trans*-isomer. The yield of this coupling reaction was later improved to 79% by employing the C-1 benzoate **13** as the acceptor for this N-glycosylation reaction. When this base coupling reaction was performed at 0 °C, no coupled product **15** was formed. These findings appear to indicate that the formation of the C-1 oxonium ion derived from either **12** or **13** was rather difficult. This observation correlates well with the excellent acid stability associated with these 2′-fluorinated nucleosides, because acid promoted decomposition of these nucleosides must proceed through the same C-1 oxonium intermediated mentioned above (see Acid Stability Section for detail). Finally, triethylamine-trihydrofluoride[14] was used to remove the 5′-silyl protecting group in **15** to give an anomeric mixture **2a** and **2b** in 69–90% yield.

Scheme 2



Following the same sequence, bis(TMS)thymine **16** was coupled successfully with **12** and **13** to afford the intermediate **17**, as an anomeric mixture with a ratio of ~1.5:1 favoring the *trans*-nucleoside, in 52% and 49% yield, respectively. Upon desilylation, **17** was converted to the desired nucleosides **3a** and **3b** in 93% overall yield. Likewise, bis(TMS)-5-iodouracil **18** and bis(TMS)adenine **20** were reacted with **12** or **13** under the identical N-glycosylation conditions to give the desired products **19** (66%) and **21** (70%). After standard desilylation, these intermediates were converted to the target products **4a/4b** and **5a/5b** with a ratio of ~1.5:1 again in favor of the corresponding *trans*-isomers, albeit in 30–40% yield.

Pure *cis*-nucleosides, **2a**, **3a**, and **5a** were separated from their corresponding *trans*-counterparts (**2b**, **3b**, and **5b**) via reverse-phase HPLC.[15] 5-Iodouracil-bearing nucleoside **4a** was separated from **4b** via repeated silica gel chromatography.[15]

Structure Assignment

The assignment of the anomeric configuration of the 2′-fluorinated nucleosides (**2–5**) was made on the basis of the characteristic proton NMR spectra. This method was used previously by Okabe et al.[16] for D-nucleosides and by Lin et al.[4,17] for L-nucleosides. The H-4′ protons of the α-anomer appear at a lower field than those of the β-anomers. Conversely, the H-5′ protons of the α-anomers appear at a higher field than those of β-anomers. It is also worthwhile to mentioning that all *cis*-nucleosides (β-anomers) were less polar than their corresponding *trans*-nucleosides (α-anomers). The detailed proton chemical shifts for H-4′ and H-5′ protons of our 2′-fluorinated L-nucleosides are listed in Table 1 below.

Table 1. Proton NMR chemical shifts (PPM)

Compd	H-4′	H-5′
2a (*cis*)	4.79	3.59
2b (*trans*)	4.92	3.50–3.40
3a (*cis*)	4.80	3.59
3b (*trans*)	5.03	3.52–3.45
4a (*cis*)	4.95	3.82
4b (*trans*)	5.20	3.75–3.62
5a (*cis*)	4.90	3.65–3.60
5b (*trans*)	4.96	3.60–3.50

Acid Stability Assessment

The aqueous stability of **2a** and **2b** was investigated at pH 2.0, 4.3, 7.1, and 9.1. The results showed that both 2′-fluorinated nucleosides were very stable at pH 4.3, 7.1, and 9.1. No change in HPLC peak area was detected during the one week study period. At pH 2.0, the degradation of **2a** and **2b** followed first order kinetics with the half-lives of approximately 21 and 22 days, respectively. When β-L-FD_4C was studied in a separate experiment at pH 2.0, the half-life was less than 30 min. Obviously, incorporation of a fluorine atom at the 2′-position can greatly enhance the acid stability of the resulting 2′-vinylfluoronucleosides.

Biological Activity Assessment

All new 2′-fluorinated L-nucleosides (**2–5**) were evaluated in a semiquantitative assay for their cytotoxicity and anti-HIV activity in CEM-SS cells. β-L-FD_4C and AZT were also included as positive controls. With the exception of β-L-FD_4C and **2b**, all other nucleosides were found to be nontoxic at 25 μM. When tested in HIV-infected CEM-SS cells, only three nucleoside analogs (β-L-FD_4C, AZT and **2a**) were found to be active. The antiviral activity of these nucleosides decreases in the following order: β-L-FD_4C > AZT = 2′F-L-FD_4C (**2a**).

Encouraged by its favorable biological profile, 2′F-β-L-FD_4C (**2a**) was further evaluated for its activity against HBV[18] and HIV[19] as well as its in vitro cytotoxicity in CEM cell lines.[20] β-L-FD_4C, β-L-FddC and 3TC (Lamivudine) were included as positive control. The results from these experiments are listed in Table 2. When evaluated in the HBV assay, 2′-fluorinated β-L-FD_4C **2a** was the least potent nucleoside, about 40-fold less potent than β-L-FD_4C. The anti-HIV activity (in MT-2/IIIB cells) of four L-nucleosides tested decreases in the following order: β-L-FD_4C > β-L-FddC >> 3TC > **2a**. Although β-L-FD_4C displayed best antiviral activity, this analog was also the most cytotoxic one. The newly synthesized analog **2a** was at least sevenfold less toxic than β-L-FD_4C.

Table 2. Antiviral and cytotoxicity of a series of L-nucleosides

Compounds	HBV (μM) EC_{50}	HIV (μM) EC_{50}	(MT-2/IIIB) ID_{50}	CEM (μM) ID_{50}
β-L-FD_4C	0.008	0.1–0.2	9	7
β-L-FddC	0.036	0.3–0.5	>100	67
3TC	0.030	2.0–3.0	>100	50
2′F-β-L-FD_4C	>0.03	3.6	>100	>50

EC_{50}: Drug concentration required to inhibit the viral cell proliferation by 50%.
ID_{50}: Drug concentration required to inhibit cell growth by 50%.

In summary, we have described herein a general method for the synthesis of both the *cis*- and *trans*-2′-vinylfluoro containing nucleosides. Of the eight nucleosides prepared (**2–5**), the 5-fluorocytosine bearing nucleoside **2a** displayed the best biological activity. It is also interesting to note that incorporation of a fluorine atom at the 2′-position of β-L-FD_4C indeed improved its cytotoxicity profile, yet failed to further enhance its antiviral activity.

Acknowledgments

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15. Proton NMR data for final products (**2–5**):

^{1}H NMR of **2a** (300 MHz, DMSO-d_6): δ 8.27 (d, J = 5.2 Hz, 1H), 7.98 (bs, 1H), 7.73 (bs, 1H), 6.80 (m, 1H), 5.92 (t, J = 1.6 Hz, 1H), 5.25 (bs, 1H), 4.79 (m, 1H), 3.59 (m, 2H).

^{1}H NMR of **2b** (300 MHz, DMSO-d_6): d 7.98 (bs, 1H), 7.74 (d, J = 6.6 Hz, 1H), 7.73 (bs, 1H), 5.92 (m, 1H), 5.03 (m, 1H), 4.92 (m, 1H), 3.50–3.40 (m, 2H).

HRMS (FAB) calcd for **2b** $C_9H_{10}F_2N_3O_3$ (MH^+): 246.0690, found: 246.0690.

^{1}H NMR of **3a** (300 MHz, DMSO-d_6): δ 7.83 (s, 1H), 6.76 (m, 1H), 5.98 (s, 1H), 5.20 (bs, 1H), 4.80 (m, 1H), 3.59 (m, 2H), 1.73 (d, J = 0.8 Hz, 3H).

^{1}H NMR of **3b** (300 MHz, DMSO-d_6): δ 8.31 (s, 1H), 7.34 (d, J = 1.2 Hz, 1H), 6.77 (m, 1H), 5.99 (t, J = 1.5 Hz, 1H), 5.03 (m, 2H), 3.52–3.45 (m, 2H), 1.79 (d, J = 1.1 Hz, 3H).

^{1}H NMR of **4a** (300 MHz, acetone-d_6): δ 8.75 (d, J = 0.5 Hz, 1H), 6.83 (m, 1H), 5.94 (t, J = 1.7 Hz, 1H), 4.95 (m, 1H), 3.82 (bs, 2H).

^{1}H NMR of **4b** (300 MHz, acetone-d_6): δ 7.89 (s, 1H), 6.86 (m, 1H), 5.96 (t, J = 1.5 Hz, 1H), 5.20 (m, 1H), 3.75–3.62 (m, 2H).

^{1}H NMR of **5a** (300 MHz, DMSO-d_6): δ 8.38 (s, 1H), 8.15 (s, 1H), 7.39 (bs, 2H), 6.88 (m, 1H), 6.06 (t, J = 1.6 Hz, 1H), 5.20 (bs, 1H), 4.90 (m, 1H), 3.65–3.60 (m, 2H).

^{1}H NMR of **5b** (300 MHz, DMSO-d_6): δ 8.30 (s, 1H), 8.15 (s, 1H), 7.35 (bs, 2H), 6.87 (m, 1H), 6.04 (t, J = 1.6 Hz, 1H), 5.14 (m, 1H), 4.96 (bs, 1H), 3.60–3.50 (m, 2H).

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20. Protocol for determining CEM cytotoxicity: (i) seed CEM at 2.5 x 10^4 per mL in RPMI plus 20% dialyzed FBS; (ii) when cells double, usually after 24 h, seed onto 24-well dishes at 5 × 10^4 per mL per well; (iii) add drugs and incubate at 37 °C, 5% CO_2 for three days; (iv) determine cell numbers using the coulter counter.