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SYNTHESIS AND COMPARATIVE EVALUATION OF TWO ANTIVIRAL AGENTS: β-L-Fd_4C AND β-D-Fd_4C

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Abstract: The synthesis of β-D-Fd_4C was achieved in a stereoselective fashion from D-xylose. The antiviral activity and cytotoxicity of β-D-Fd_4C was compared with that of β-L-Fd_4C and 3TC (Lamivudine). Of the three agents compared, β-L-Fd_4C was found to be the most potent antiviral agent.

Introduction: The approval of 3′-deoxy-3′-azidothymidine (AZT) as the first anti-HIV agent[1] by the FDA has spurred considerable research effort aimed at design and synthesis of other nucleoside analogs that would inhibit the replication of HIV and related viruses, such as hepatitis B virus (HBV). To date, five nucleoside reverse transcriptase inhibitors (AZT, ddI, ddC, d_4T and 3TC) and four protease inhibitors (saquinavir, ritonavir, indinavir and nelfinavir) have been approved by the FDA for use in combination therapy against HIV.[2] The most effective cocktail treatment now being administered to AIDS patients is a combination method using both reverse transcriptase and protease inhibitors. Additionally, several NNRTIs have also been approved for the HIV combination therapy. Although such combination therapy has delayed disease onset and death associated with HIV infection, the cocktail method can also fail for a number of reasons.[3] Thus, there is still an urgent need for the development of new agents possessing potent and broad antiviral activities.

Since reverse transcriptase activity is required for both HIV and HBV replication, anti-HIV reverse transcriptase inhibitors have been tested for activity against HBV replication.[4] Recent findings from different laboratories demonstrated that many reverse transcriptase inhibitors indeed exhibit potent and selective activity against HIV and HBV infections. These include β-L-Fd_4C (**1**),[5] β-D-Fd_4C (**ent-1**)[6] and 3TC (Lamivudine),[7] the first unnatural L-nucleoside approved by the FDA for the treatment of HIV. On the basis of its promising activity against HBV,[8] 3TC (**2**) will soon be used to treat HBV infection (see Figure 1 for structures). We have previously demonstrated that β-L-Fd_4C (**1**) was at least five- to tenfold more potent than 3TC (**2**) against both

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HIV and HBV.[9] More recently, Faraj et al. reported that β-D-Fd$_4$C (**ent-1**) also exhibited good activity towards both HIV and HBV.[6] Due to the therapeutic potential of these compounds the development of efficient synthetic routes for both **1** and **ent-1** from inexpensive starting materials would be a major step toward their clinical development.[11] Furthermore, we were also interested in the comparative evalaution of these agents. In this communication, we wish to report our recent progress towards these goals.

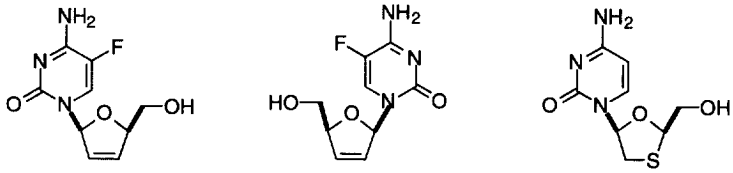


Figure 1: Structures of nucleoside analogs of interest

Synthesis: In a recent publication from our laboratory, we disclosed a highly stereocontrolled ten-step route to β-L-Fd$_4$C (**1**) starting from D-glutamic acid (**3**).[10] When N-phenylseleno-phthalimide was used as the electrophile, the conversion of 5′-silylated lactone **4** to 2′-phenylselenolactone **5** was achieved in a highly stereoselective manner. Following the known procedure thereafter, the resulting intermediacy **5** was converted to the 2′-phenylseleno-bearing nucleoside **6**, enroute to the final product β-L-Fd$_4$C **1** (Figure 2).

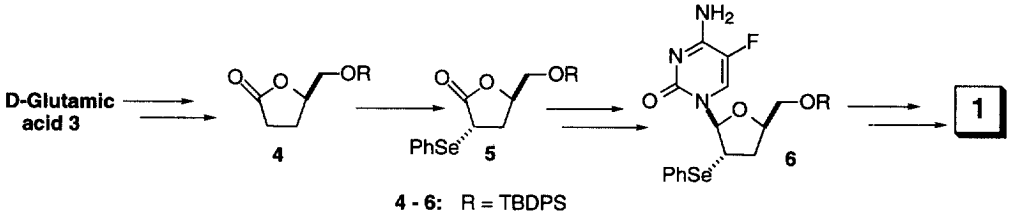
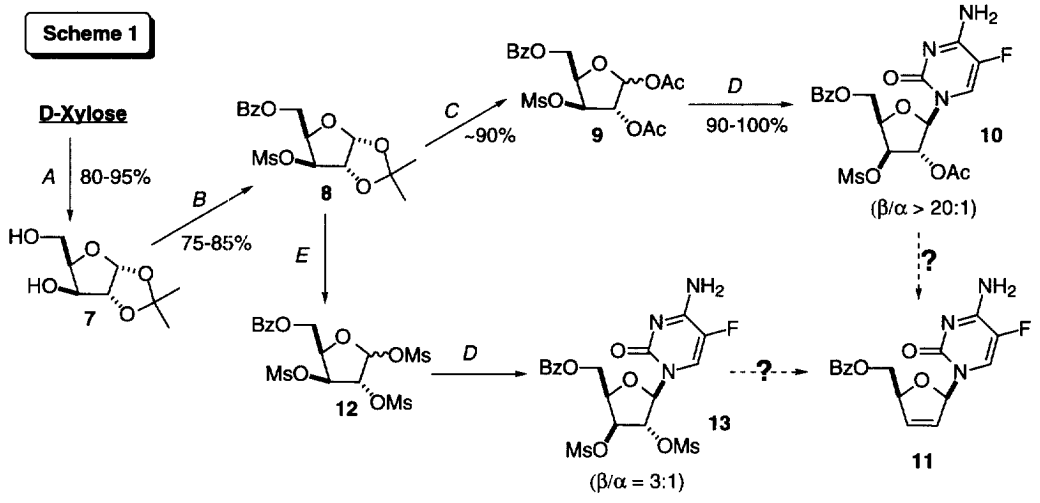


Figure 2: Synthesis of β-L-Fd$_4$C **1** via D-glutamic acid **3**

Although the highly stereoselective route outlined in Figure 2 was used successfully to prepare sufficient amounts of β-L-Fd$_4$C **1** for extensive biological evaluation and preclinical toxicology study, the relatively lengthy sequence may limit its utility for large scale manufacturing of β-L-Fd$_4$C **1**. Therefore, we decided not to use the synthetic route as shown in Figure 2 to prepare the requisite β-D-Fd$_4$C (from L-glutamic acid). In this paper, we will disclose a highly stereoselective six-step synthesis of β-D-Fd$_4$C **ent-1** starting from inexpensive D-xylose (see Schemes **1** and **2**). It is conceivable that simply switching the starting material to L-xylose, the newly devised xylose route can be used to synthesize β-L-Fd$_4$C **1**.

The starting material for the new synthesis, 1,2-*O*-isopropylidene-a-D-xylofuranose (**7**) was prepared according to Gosselin protocol[11] in high yield. As shown in Scheme 1, diol **7** was converted to the 5′-OBz-3′-OMs-xylofuranose derivative **8** via an one-pot process in 75–85% yield. Compound **8** was next converted to the 1′,2′-bisacetate **9**, thereafter the protected nucleoside analog **10** in a highly stereoselective manner with excellent overall yield. Unfortunately, further transformation of **10** to **11** failed so far. The conditions used for this reductive elimination include (I) NaI/Zn in DMF or DME;[12] (II) Li or Na/NH_3,[13] (III) Li/naphthalide/DME.[14] Alternatively, compound **8** was converted to the tri-O-mesylate **12**, which was then coupled with bis-TMS silylated 5-flurocytosine to afford the corresponding 2′,3′-bis-OMs nucleosides **13** in a 3:1 ratio favoring the desired β-isomer. Once again, attempted reductive elimination of bis-mesylate under various conditions (e.g., Li/naphthalide)[14] failed to give the desired product **11**.

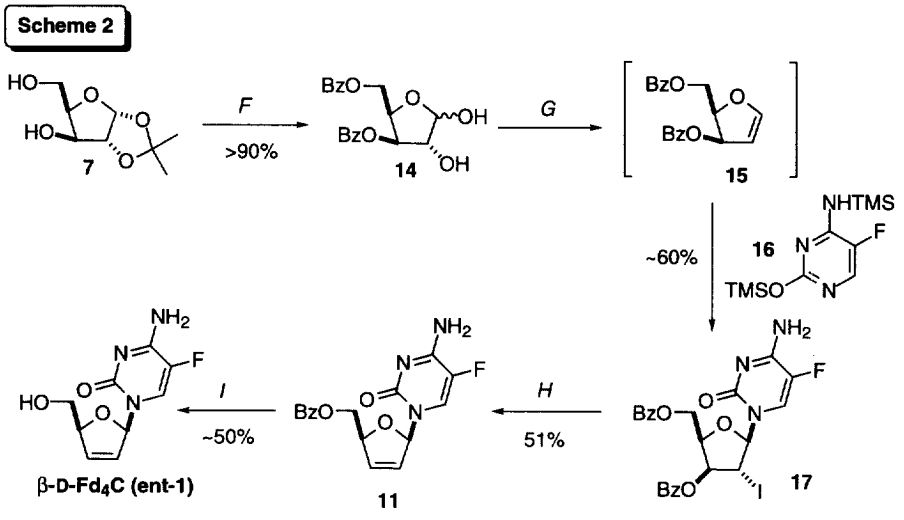
Scheme 1



Reagents and Conditions: (A) $Me_2C(OMe)_2$/acetone/cat. conc. H_2SO_4/rt, then 0.2% aq. HCl/rt; (B) BzCl/Pyridine/0 °C, then MsCl/Et_3N/CH_2Cl_2/0 °C; (C) 4M H_2SO_4/85% HOAc/60 °C or TFA/THF/rt, then Ac_2O/Et_3N/CH_2Cl_2; (D) $(TMS)_2$-5FC/TMSOTf/$ClCH_2CH_2Cl$/60 °C; (E) TFA/THF/rt, then MsCl/Et_3N/CH_2Cl_2/0 °C.

In view of the problems encountered in Scheme 1, it was decided to explore the glycal-based second approach shown in Scheme 2. Thus, diol **7** was subjected to bis-benzoylation (at C-3′ and C-5′) followed by the removal of acetonide protective group (at 1′ and 2′) to give the desired diol **14** in greater than 90% yield. Upon reaction with I_2/Ph_3P/imidazole, diol **14** was converted to the corresponding glycal **15**, which was used in the subsequent N-glycosylation reaction to provide the 2α-iodo-bearing β-nucleoside **17** in a highly stereoselective fashion with an overall yield of 60%. It should be mentioned that the similar reaction sequence (from **14** to **17**)

was used by Robles et al. for the synthesis of other nucleosides.[15] Treatment of an ethanol and EtOAc solution **17** with zinc and catalytic amount of acetic acid at 70 °C afforded the desired d_4-nucleoside **11** (51%), which was converted to the final product β-D-Fd_4C **ent-1** after standard 5′-debenzoylation.[16] The proton NMR of **ent-1** is identical to the previously synthesized β-L-Fd_4C **1**.[10]



Reagents and Conditions: (F) BzCl/Pyridine/CH_2Cl_2/0 °C; 4M H_2SO_4/85% HOAc/THF/60 °C; (G) I_2/Ph_3P/imidazole/CH_2Cl_2/rt; **16**/NIS/$ClCH_2CH_2Cl$/rt; (H) Zn/EtOH (MeOH)/EtOAc/cat. HOAc/rt; (I) n-$BuNH_2$/THF.

In vitro evaluation: β-L-Fd_4C **1** and β-D-Fd_4C **ent-1**, obtained respectively from L-glutamic acid according to Figure 2 and D-xylose via Scheme 2 were evaluated for their antiviral activity against HIV and HBV. The results of these investigations are shown in Table 1 below. When tested against HBV, β-L-Fd_4C **1** showed an EC_{50} value of 8 nM, which was at least fourfold more potent than its corresponding enantiomer β-D-Fd_4C **ent-1**. It should be mentioned that this result differs from that published by Faraj and coworkers[6] who reported that β-D-Fd_4C was threefold more potent than β-L-Fd_4C. The reason for this discrepancy is not clear at this moment. When both **1** and **ent-1** were evaluated against HIV in MT-2/IIIB cell line, they displayed identical activity with EC_{50} value of 0.2 μM (~tenfold lower than that of 3TC **2**[9]). Interestingly β-L-Fd_4C was found to be about 11-fold more toxic than its enantiomer β-D-Fd_4C in this cell line. The cytotoxicity of both **1** and **ent-1** were determined in several additional cell lines. As can be seen in Table 1, both β-L-Fd_4C and β-D-Fd_4C were not toxic in DLD-1, Hep G2 Rat-1 and B16 cell lines (IC_{50} > 200 μM). However, the L-nucleoside showed an IC_{50} value of 6.5 μM in CEM cell line,[17] which was more toxic than its corresponding D-nucleoside (IC_{50} = 100 μM). This sensitivity of CEM cells combined with preliminary results on the murine CTLL-2 T lymphoblast

cell line, which also displayed sensitivity to both **ent-1** (IC_{50} = 127 μM) and **1** (IC_{50} = 24 μM) suggests that the differential cytotoxicity of these enantiomers could be confined to T-cell lineage cell lines. This is supported by animal toxicology and efficacy studies (in mice and ducks respectively) in which β-L-Fd_4C has been administrated at high doses (Cmax in plasma >100 μM) for prolonged periods of time without evidence of drug toxicity being observed.

Table 1. Antiviral activity and cytotoxicity of **1** and **ent-1**

Compounds	*HBV (μM)*	*HIV (μM)*	*IC_{50} (μM)*					
	EC_{50}	*EC_{50}*	*MT-2/IIIB*	*CEM*	*DLD-1*	*HepG2*	*B16*	*Rat-1*
1	0.008	0.2	9	6.5	>200	>200	>200	>200
ent-1	>0.3	0.2	~100	~100	>200	>200	>200	>200

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

In summary, a highly stereoselective synthesis of β-D-Fd_4C (**ent-1**) was accomplished in six-step starting from D-xylose. In contrast to the previous syntheses completed in this institution,[10a] the introduction of the 2′,3′ double bond in **11** was achieved via reductive elimination of the 2′-iodo and the 3′-O-benzoate moieties from **17**. It is worthwhile to point out that none of the synthetic steps described in Scheme 2 requires either low temperature or selenium reagent. When compared with β-L-Fd_4C **1**, the newly synthesized β-D-Fd_4C **ent-1** showed similar anti-HIV activity yet reduced anti-HBV activity.

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