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Synthesis and Potent Anti-HIV Activity of L-3′-Fluoro-2′,3′-Unsaturated Cytidine

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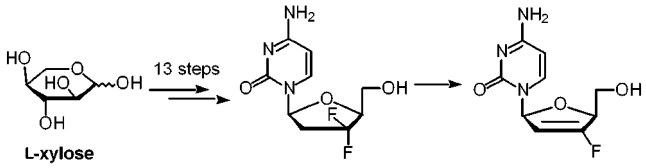
ABSTRACT



L-2′,3′-Didehydro-2′,3′-dideoxy-3′-fluorocytidine (L-3′-Fd4C), a novel potent anti-HIV agent (EC_{50} 0.03 μM in PBM cells), has been synthesized from L-xylose in 14 steps.

Nucleoside analogues have been the cornerstone of antiviral therapy over the past thirty years. In the effort to discover effective antiviral agents against AIDS and viral hepatitis, a large number of nucleoside analogues have been synthesized and evaluated. Although structure−activity relationship studies have not led to a pharmacophore model for the antiviral activities of nucleosides, some structural features have been particularly successful. For example, all six of the nucleoside reverse transcriptase inhibitors approved by the FDA for the treatment of AIDS[1] can be considered as 2′,3′-dideoxynucleosides. In addition, among ring substituents, electron-withdrawing groups such as azido[2] and fluorine[3] have often produced potent antiviral agents. Another structural feature that is often beneficial for antiviral activity is a 2′,3′-unsaturated bond. We have extensively explored these substitutions in nucleoside analogues, particularly with the synthesis and biological evaluation of D- and L-2′,3′-didehydro-2′,3′-dideoxy-2′-fluoro nucleosides (Figure 1).[3d−f]

Figure 1. Structures of D- and L-2′,3′-didehydro-2′,3′-dideoxy-2′-fluoro nucleosides.

Among them, the cytosine and 5-fluorocytosine derivatives displayed potent anti-HIV and anti-HBV activity without significant cytotoxicity. For this reason, we decided to explore the chemistry and biology of 2′,3′-didehydro-2′,3′-dideoxy-3′-fluoronucleosides. In this series, the D-cytidine and D-thymidine analogues were previously synthesized and shown to have low to moderate anti-HIV-1 activity without cytotoxicity.[4] The D-adenine derivative also showed moderate anti-HIV activity with some toxicity.[4a] In view of the fact

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(1) Zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), and abacavir (1596U89).

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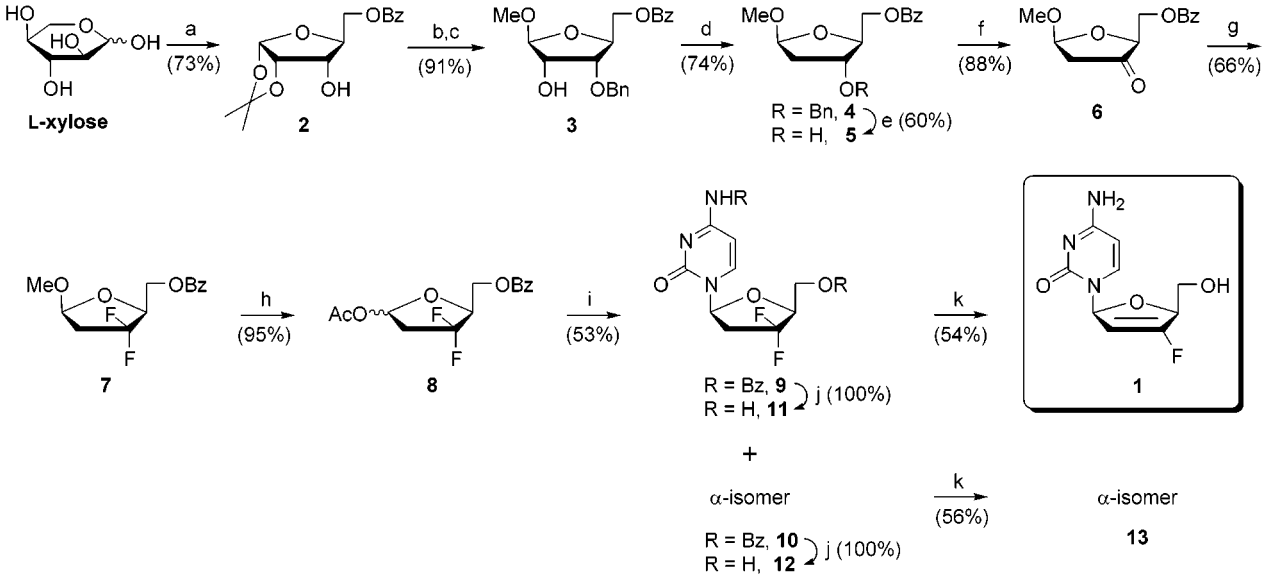
Scheme 1. Synthesis of L-2′,3′-Didehydro-2′,3′-dideoxy-3′-fluorocytidine



[a] Reagents and conditions: (a) ref 5; (b) NaH, THF, from 0 °C to room temperature, 1 h, then BnBr, TBAI, from 0 °C to room temperature, overnight; (c) 1:2 (4.0 M HCl/dioxane)/MeOH, room temp, 1.5 h; (d) (i) PhOC(S)Cl, DMAP, Tol, 90 °C, 3 h, (ii) Bu_3SnH, AIBN, reflux, 1h; (e) H_2 (55 psi), 10% Pd/C, EtOH, room temp, 72 h; (f) CrO_3, Ac_2O, Py, CH_2Cl_2, room temp, 15 min; (g) DAST, CH_2Cl_2, reflux, 36 h; (h) concentrated H_2SO_4, Ac_2O, AcOH, 0 °C, 5 min; (i) persilylated N^4-benzoylcytosine, TMSOTf, MeCN, from 0 °C to room temperature, 72 h; (j) saturated NH_3/MeOH, room temp, 4 h; (k) MeONa, DMF, room temp, overnight.

that among the 2′-fluoro derivatives L-isomers have potent antiviral activity with no toxicity or less toxicity than their D-counterparts, it was of interest to synthesize L-2′,3′-didehydro-2′,3′-dideoxy-3′-fluorocytidine (L-3′-Fd4C) **1** (Scheme 1). Our synthetic method can also provide an entry to the L-2′,3′-dideoxy-3′,3′-difluoro nucleoside **11**.

The starting material of our synthetic approach (Scheme 1) was L-xylose, which was converted to the protected L-ribose analogue **2** in four steps in 73% overall yield by a well-known procedure in our laboratory.[5] Benzylation of **2** was easily accomplished by treatment with sodium hydride, followed by benzyl bromide and catalytic tetrabutylammonium iodide. Methanolysis of the resulting benzyl ether gave the intermediate **3** as the sole isomer. ^{1}H NMR showed the signal related to the H-1 as a singlet, which indicates the β-stereochemistry.[6] Comparison of the proton spectrum with that of the known enantiomer[7] confirmed the assignment. Conversion of **3** to the phenoxythiocarbonyl derivative followed by the radical deoxygenation of the latter gave protected L-2-deoxyribose **4**. Compound **4** was rather unreactive toward catalytic hydrogenation, and its palladium-catalyzed debenzylation required treatment with hydrogen at 55 psi for 3 days. Although the yield was modest (60%), most of the unreacted starting material could be recovered and recycled. Oxidation of the debenzylated product **5** by chromic anhydride/pyridine/acetic anhydride gave ketone **6** in 88% yield. Treatment with (diethylamino)sulfur trifluoride (DAST) afforded difluorinated intermediate **7**[8] in 66% yield. Compound **7** was converted to the acetate **8** by the modification of a known literature method.[9] Condensation of the acetate **8** with persilylated N^4-benzoylcytosine was effected under Vorbrüggen conditions using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst. The epimeric products **9** and **10** were chromatographically separable, and the β-isomer was more abundant. In fact, ^{1}H NMR of the crude reaction mixture showed an epimeric ratio of 5:4. Deprotection of each isomer was accomplished by ammonolysis to give difluorinated nucleosides **11**[10] and **12**. Elimination by treatment with sodium methoxide in DMF

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(6) Binkley, R. W. *Modern Carbohydrate Chemistry*; Marcel Dekker Inc.: New York, 1988; pp 53−60.

(7) Kosma, P.; Christian, R.; Schulz, G.; Unger, F. M. *Carbohydr. Res.* **1985**, *141*, 239−253.

(8) Yellow oil: $[\alpha]^{23}_D$ 40.78° (*c* 4.28, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08 (m, 2H), 7.57 (m, 1H), 7.45 (m, 2H), 5.15 (dt, 1H, H_1, J = 5.7, 1.7 Hz), 4.63−4.41 (m, 3H, H_4, H_5), 3.40 (s, 3H), 2.67 (tdd, 1H, $H_{2\alpha}$, J = 16.6, 15.0, 5.7 Hz), 2.49 (tdd, 1H, $H_{2\beta}$, J = 15.0, 9.0, 2.0 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.05, 133.14, 129.71, 128.38, 128.32 (t, J_{C-F} = 252.8 Hz), 103.70 (dd, J_{C-F} = 7.1, 4.2 Hz), 79.46 (dd, J_{C-F} = 32.1, 24.9 Hz), 62.94 (dd, J_{C-F} = 7.5, 4.5 Hz), 55.38, 42.26 (t, J_{C-F} = 24.3 Hz); HRMS (FAB) *m/z* found 273.0950, calcd for $C_{13}H_{15}F_2O_4$ 273.0938 (MH^+). Anal. Calcd for $C_{13}H_{14}F_2O_4$: C, 57.35; H, 5.18. Found: C, 57.64; H, 5.30.

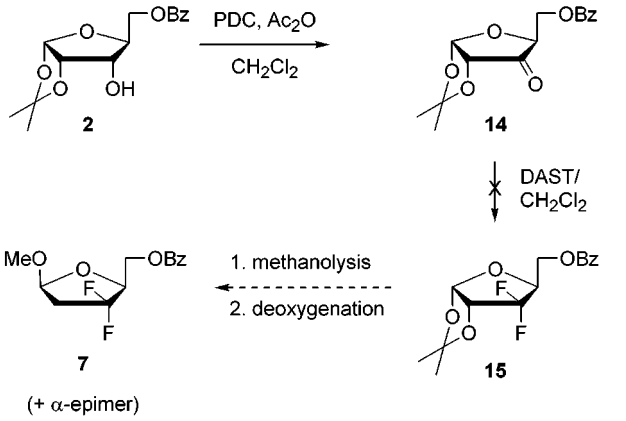
(9) Mikhailopulo, I. A.; Poopeiko, N. E.; Pricota, T. I.; Sivets, G. G.; Kvasyuk, E. I.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1991**, *34*, 2195−2202.

(10) White solid: mp 194−196 °C (dec); $[\alpha]^{24}_D$ −51.89° (*c* 1.15, MeOH); UV (MeOH) λ_{max} 276.5 (ϵ 18 160) (pH 2), 268.0 (ϵ 13 280) (pH 7), 268.5 (ϵ 13 580) (pH 11); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.97 (d, 1H, H_6, J = 7.3 Hz), 6.27 (t, 1H, $H_{1'}$, J = 6.8 Hz), 5.93 (d, 1H, H_5, J = 7.3 Hz), 4.17 (m, 1H, $H_{4'}$), 3.83 (m, 2H, $H_{5'}$), 2.90 (m, 1H, $H_{2'}$), 2.51 (m, 1H, $H_{2'}$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.19, 158.46, 142.62, 128.71 (dd, J_{C-F} = 255.1, 247.4 Hz), 97.04, 84.73 (dd, J_{C-F} = 6.7, 4.9 Hz), 83.74

afforded the target unsaturated nucleosides **1**[11] and **13** in 53 and 51% yields, respectively.

Intermediate **7** could have been obtained from **2** through a more straightforward route (Scheme 2) involving oxidation

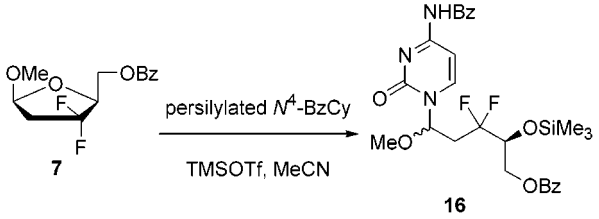
Scheme 2. Alternative Route to **7**



of **2** to the known ketone **14**, difluorination of **14** to the difluororibose derivative **15**, followed by methanolysis and deoxygenation. Since **14** can also be obtained from L-xylose in three steps,[5] this route would have saved four steps in our synthetic scheme. Unfortunately, ketone **14** proved to be unreactive in the classic fluorinating conditions. Even when the reaction was run in neat DAST, no products could be detected, and attempts to increase the reactivity by increasing the temperature only caused decomposition of **14**. It is known that the steric hindrance as well as electronic effects of the isopropylidene moiety of **14** (Scheme 2) can prevent nucleophilic attack on position 3 of the furanose ring.[12] Besides, literature examples show that high temperatures are required to difluorinate sterically hindered ketones. Furthermore, in the only example of difluorination of an α,α′-trans-disubstituted five-membered cyclic ketone found in the literature, the carbonyl group is relatively unhindered and the yield is still low (25%).[13]

Direct reaction of intermediate **7** with N^4-benzoylcytosine in the standard Vorbrüggen conditions did not give the expected protected nucleosides. Instead, the inseparable epimeric mixture **16**[14] (Scheme 3) was isolated in 60% yield.

Scheme 3. Ring-Opening of **7** in Vorbrüggen Conditions



The stereochemistry of condensation products **9** and **10** has been established by 2D NMR NOESY experiments. Thus, the NOESY spectrum of epimer **9** showed clear correlations between protons $H_{1'}$ and $H_{4'}$. Correlations between $H_{4'}$ and $H_{2'\alpha}$ and between the latter and $H_{1'}$ were also observed. In the case of **10**, the $H_{4'}$ proton strongly correlated with one of the $H_{2'}$ protons (which could thus be identified as α), while the other $H_{2'}$ (β) correlated with $H_{1'}$.

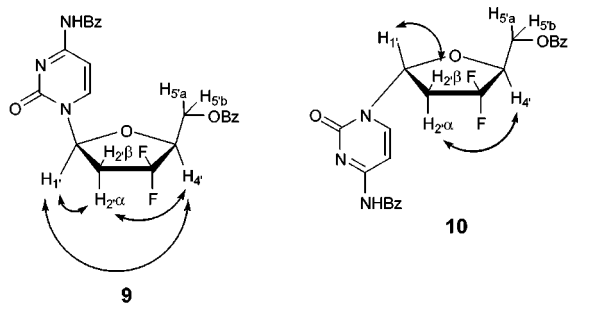


Figure 2. NOE correlations and stereochemistry of **9** and **10**.

In summary, L-2′,3′-dideoxy-3′,3′-difluoro- and L-2′,3′-didehydro-2′,3′-dideoxy-3′-fluoro nucleosides have been synthesized from L-xylose in 13 and 14 steps, respectively. The target compounds were obtained by condensation of a difluorinated intermediate **8** with a nucleobase in a process of general applicability. The key difluorination step of ketone **6** proceeded with a 66% yield. Our approach seems to be more convenient and versatile than the reported synthesis of the D-thymidine analogue.[15]

(dd, J_{C-F} = 29.5, 25.0 Hz), 60.73 (t, J_{C-F} = 4.8 Hz), 42.14 (t, J_{C-F} = 23.4 Hz), 12.29; HRMS (FAB) *m/z* found 248.0850, calcd for $C_9H_{12}F_2N_3O_3$ 248.0847 (MH^+). Anal. Calcd for $C_9H_{11}F_2N_3O_3 \cdot 0.1H_2O$: C, 43.41; H, 4.53; N, 16.88. Found: C, 43.63; H, 4.50; N, 16.48.

(11) White solid: mp 182−183 °C (dec); $[\alpha]^{22}_D$ 6.67° (*c* 0.54, MeOH); UV (MeOH) λ_{max} 276.0 (ε 11 990) (pH 2), 267.5 (ε 8010) (pH 7), 264.5 (ε 8060) (pH 11); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.14 (d, 1H, H_6, J = 7.5 Hz), 7.01 (m, 1H, $H_{1'}$), 5.90 (d, 1H, H_5 J = 7.5 Hz), 5.46 (m, 1H, $H_{2'}$), 4.76 (m, 1H, $H_{4'}$), 3.80 (m, 1H, $H_{5'}$), 3.79 (m, 1H, $H_{5'}$); ^{13}C NMR (CD_3OD, 100 MHz) δ 168.31, 163.40 (d, J_{C-F} = 284.8 Hz), 159.05, 144.05, 102.34 (d, J_{C-F} = 9.7 Hz), 95.68, 88.42 (d, J_{C-F} = 14.9 Hz), 81.96 (d, J_{C-F} = 24.7 Hz), 61.82 (d, J_{C-F} = 2.2 Hz); HRMS (FAB) *m/z* found 228.0778, calcd for $C_9H_{11}FN_3O_3$ 228.0784 (MH^+). Anal. Calcd for $C_9H_{10}FN_3O_3$: C, 47.58; H, 4.44; N, 18.50. Found: C, 47.47; H, 4.44; N, 18.17.

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(14) Yellow oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.75 (bs, 2H), 8.06−7.45 (m, 24H), 6.14−6.10 (m, 2H), 7.57 (m, 2H), 4.56 (dd, 1H, J = 11.5, 3.2 Hz), 4.51 (dd, 1H, J = 11.7, 4.2 Hz), 4.37 (m, 2H), 4.27 (m, 2H), 3.40 (s, 3H), 3.38 (s, 3H), 2.57 (m, 2H), 2.42 (m, 2H), 0.17 (s, 9H), 0.16 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.13, 166.07, 154.90, 154.81, 143.12, 133.20, 133.16, 129.60, 129.56, 128.97, 128.45, 128.41, 127.60, 121.33 (t, J_{C-F} = 248.2 Hz), 121.10 (t, J_{C-F} = 248.2 Hz), 97.69, 97.53, 83.91, (dd, J_{C-F} = 5.1, 3.1), 83.76 (dd, J_{C-F} = 7.1, 3.1), 72.13 (t, J_{C-F} = 29.3 Hz), 72.08 (t, J_{C-F} = 28.0 Hz), 64.20, 64.16, 57.08, 57.01, 37.92 (t, J_{C-F} = 23.5 Hz), 37.81 (t, J_{C-F} = 23.6 Hz), 0.11; MS (FAB) *m/z* 560 (MH^+).

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Preliminary biological evaluation of the synthesized compounds showed that L-3′-Fd4C **1** has potent anti-HIV activity (EC_{50} 0.03 μM in PBM cells) with little or no significant toxicity (IC_{50} = 86.9 μM in PBM cells and IC_{50} > 100 μM in CEM cells).[16] The difluorinated analogue **11** was inactive. These promising results prompt us to synthesize other pyrimidine and purine analogues in order to study the full structure−activity relationships, which is in progress in our laboratory.

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(16) Whereas AZT showed EC_{50} of 0.004 μM, with IC_{50} > 100 in PBM cells and IC_{50} = 14.3 in CEM cells (ref 3d).