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SYNTHESIS AND ANTI-HIV ACTIVITIES OF 2'-DEOXY-2',2''-DIFLUORO-ß-*L*-RIBOFURANOSYL-PYRIMIDINE AND -PURINE NUCLEOSIDES

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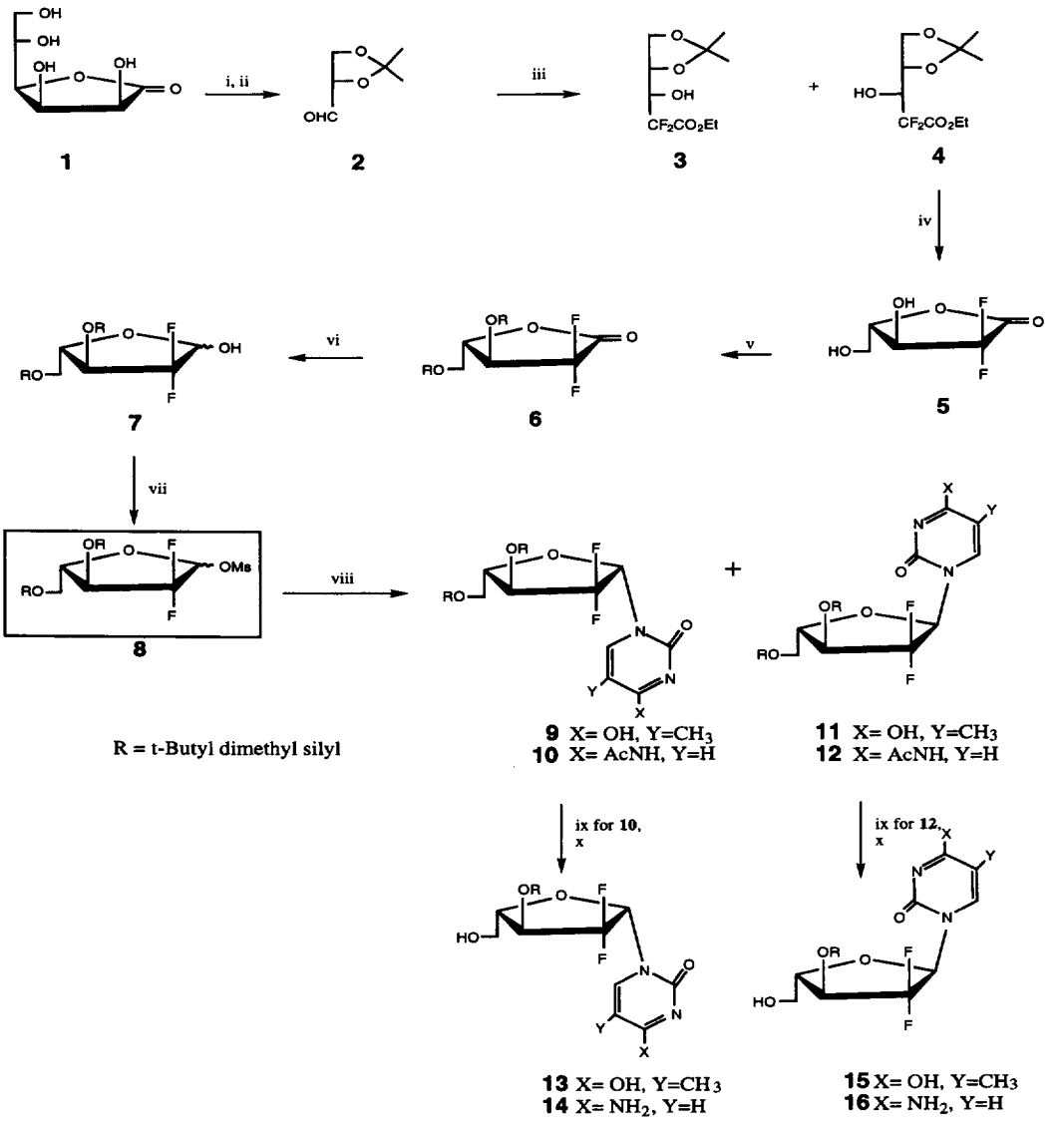
Abstract. 1- or 9-(2-Deoxy-2,2-difluoro-ß-*L*-ribofuranosyl)-thymine, -cytosine and -adenine were synthesized and their *in vitro* activitity against HIV were evaluated.

L-Nucleosides have been reported to show interesting anti-HIV and anti-HBV activities. (-)-(2R,5S)-1-[(2-Hydroxymethyl)oxathiolan-5-yl]cytosine (3TC),[1-6] (-)-ß-*L*-2',3'-dideoxy-5-fluoro-3'-thiacytidine (FTC),[7,8] 5-fluoro-2',3'-dideoxy-ß -*L*-cytidine (*L*-FddC),[9,10] and 2'-fluoro-5-methyl-ß-*L*-arabinofuranosyluracil (*L*-FMAU)[11] are currently the most promising *L*-nucleosides identified, which are undergoing preclinical and clinical evaluations as anti-HIV and anti-HBV agents. In view of the interesting biological activities exhibited by 2',2''-difluoro-substituted nucleosides with the natural *D*-configuration,[12-16] as well as the potent biological activities shown by the *L*-nucleosides described above, in this communication we report the synthesis of the corresponding *L*-isomers, 2'-deoxy-2',2''-difluoro-ß-*L*-ribofuranosyl nucleosides and their preliminary *in vitro* anti-HIV activities in acutely infected peripheral blood mononuclear(PBM) cells.

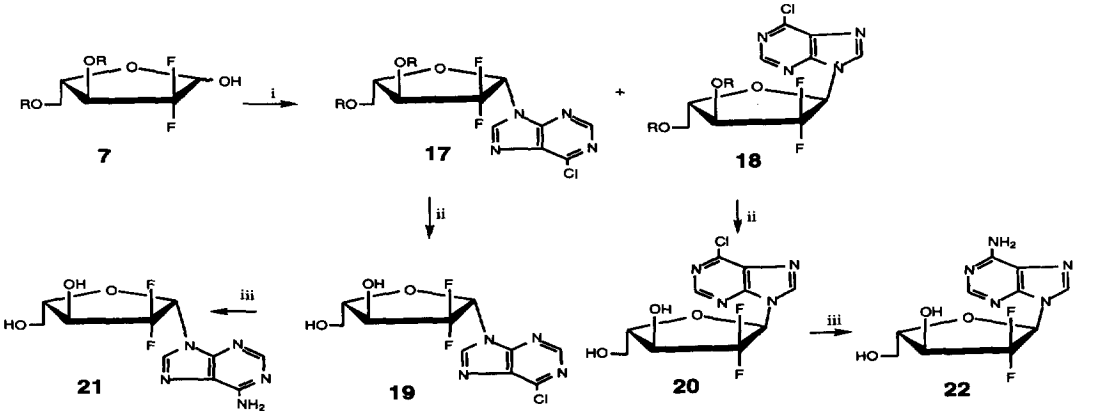
(S)-2,3-O-Isopropylideneglyceraldehyde **2** was prepared from *L*-gulonic-γ-lactone by selective isopropylidenation with 2-methoxypropene to 5,6-O-isopropylidene-*L*-gulono-γ-lactone (82% yield) followed by oxidative cleavage with sodium periodate at pH 5.5 (51% yield).[17] The coupling reaction, using the Reformatzkii conditions with activated zinc and ethyl bromodifluoroacetate in diethyl ether/THF under refluxing conditions[18], gave a mixture of diastereomers **3** and **4** in a ratio of 1:3. The mixture was separated by silica gel column chromatography using MeOH/$CHCl_3$ (1:200) as the eluent to give **3** and **4** in 24% and 72% yields, respectively. The product distribution of the above coupling reaction was similar to the corresponding *D*-isomers[18]: the addition of a nucleophile to the carbonyl compound bearing an α–asymmetric center was anti to the large group. The

Scheme 1



i. 2-Methoxy propene/TsOH/DMF; ii.$NaIO_4$/water pH 5.5; iii. $BrCF_2CO_2Et$/Zn, THF/Et_2O; iv. Dowex 50(H^+) v. *t*-Butyldimethylsilyl chloride/Imidazole/DMF; vi. DIBAL-H; vii. MsCl/Et_3N ; viii. Silylated base,NaI/CH_3CN; ix. NaOMe, MeOH, RT; x. *n*-Bu_4NF/THF.

Scheme 2



R = t-Butyldimethylsilyl

i. 6-Chloropurine/DEAD/Ph_3P/THF; ii. n-Bu_4NF/THF; iii. NH_3/MeOH

assignment of the configuration of **3** and **4** was based on the differences of chemical shifts and the coupling constants as described for the D-isomers[18], in which the hydroxyl proton at 3.08 ppm of **4** (major isomer) appeared at lower field, with a coupling constant of 4.9 Hz than that for **3** at 2.97 ppm with a coupling constant of 8.3 Hz. The major isomer **4** was subjected to the hydrolysis followed by the concomitant ring closure under the same conditions to give the lactone **5** in 93% yield. The lactone **5** was silylated with *t*-butyldimethylsilyl chloride in DMF at 40°C to give the silyl derivative **6** in 54% yield. Reduction of **6** with DIBAL-H gave the lactol **7** in 96% yield. The presence of the electron-withdrawing 2,2 -difluoro substitutent at C-2 was found to deactivate the anomeric position for the condensation as observed for the D-isomers.[18] The first attempt to condense the acetate of **7** with silylated thymine in the presence of TMSOTf in acetonitrile only resulted in the recovery of the starting material. The compound **7** was then converted to the mesylated derivative **8** with methanesulfonyl chloride in 84% yield. However, direct condensation of the mesylate compound **8** with trimethylsilylated thymine gave a low yield of the desired compound with substantial amounts of the unreacted starting material. However, addition of sodium iodide was found to catalyze the condensation reaction, in which the condensation of thymine with the intermediate **8** gave a mixture of α and ß isomers in 71% yield. The mixture of **9** and **11** was desilylated by n-Bu_4NF in THF to afford a mixure of **13** and **15** (88% yield) in a ratio of 1:4. These two isomers were separated into individual compounds by HPLC (C-18 column) using water as the eluent to give the minor ß-isomer **13**[19] and major α-isomer **15**.[20]

The cytosine analogues **14** and **16** were also obtained by the same procedure as described for the thymine derivatives. Condensation of **8** with trimethylsilylated N^4-acetylcytosine gave a mixture of N^4-acetylcytosine

nucleosides **10** and **12**, which was reacted with sodium methoxide in methanol at room temperature followed by treatment with *n*-Bu_4NF in THF to give a mixture of **14** and **16** (56 % yield). The α and ß mixture was separated by HPLC (C-18 column) using water as the eluent to give the individual compounds **14**[21] and **16**[22] in the ratio of 1:4, respectively. The purine derivatives were also synthesized: the reaction of the lactol **7** with 6-chloropurine using Mitsunobu conditions[16] (triphenyl phosphine/DEAD) yielded a ß/α-mixture (3:1) of the purine nucleosides, which was separated by a silica column to give individual isomers **17** (47%) and **18** (15%). Desilylation with *n*-Bu_4NF followed by amination with saturated methanolic ammonia afforded the free nucleosides **21**[23] and **22**.[24] The assignment of the anomeric configuration of the synthesized nucleosides was done on the basis of that followed for corresponding D-isomers by comparing the H-1' splitting pattern.[16,18]

Table 1. Median Effective (EC_{50}) and Inhibitory (IC_{50}) Concentrations for 2'-deoxy-2',2''-difluoro-*L*-ribofuranosyl nucleosides in PBM and Vero Cells.

Com-pound	Anti-HIV-1 activity in PBM cells EC_{50} (μM)	Cytotoxicity in PBM cells IC_{50} (μM)	Cytotoxicity in Vero cells IC_{50} (μM)
13	>100	>100	>100
14	>100	>100	>100
15	>100	>100	>100
16	>100	>100	>100
21	3.4	>100	>100
22	>100	>100	>100
AZT	0.004	>100	28.0 (Ref. 25)

Anti-HIV activities of the synthesized free nucleosides were evaluated against HIV-1 in human PBM cells[26] and are shown in Table 1. Among the compounds evaluated, the adenosine derivative **21**exhibited moderately potent anti-HIV activity without cytotoxicity up to 100 μM in PBM and rapidly dividing Vero cells. This encouraging antiviral activity prompts us to study comprehensive structure-activity relationships for this class of compounds, which are in progress in our laboratories.

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19. Compound**13**: $[\alpha]^{25}_D$ -44.4 (c 0.14, MeOH); UV λ_{max} (H_2O) 266.5nm (ε 9870)(pH 2), 266.0 (ε 8860) (pH 7); 266.5 (ε 7670) (pH 11); ^{1}H NMR (DMSO-d_6) δ 1.76 (s, 3H, CH_3); 3.63 (m, 1H, H-4'), 3.77 (m, 2H, H-5'), 4.02 (m, 1H, H-3'), 5.33 (t, J-5.4 Hz, 1H, 5'-OH), 6.03 (t, J = 8.0 Hz, 1H, 1'-H), 6.33 (d, J = 5.6 Hz, 1H, 3'-OH), 7.64 (s, 1H, H-6), 11.55 (s, 1H, NH). Anal. calcd for $C_{10}H_{12}F_2N_2O_5{\cdot}0.5H_2O$+0.2EtOAc: C, 42.57; H, 4.79; N, 9.19; Found: C, 42.52; H, 4.68; N, 9.13.
20. Compound **15**: $[\alpha]^{25}_D$ +32.4 (c 0.33, MeOH); UV λ_{max}(H_2O): 266.0nm (ε 10210) (pH 2); 266.5 (ε7890)(pH 7); 266.5 (ε 9870) (pH 11); ^{1}H NMR (DMSO-d_6) δ 1.80 (s, 3H, CH_3), 3.56 (m, 2H, H-5'), 4.27 (m, 1H, H-4'), 4.40 (m, 1H, H-3'), 5.09 (t, J=5.5 Hz, 5'-OH), 6.22 (dd, J = 6.5 Hz and 11.0 Hz,1H,H-1'), 6.36 (d, J = 5.8 Hz, 1H, 3'-OH), 7.45 (s, 1H, H-6), 11.54 (s,1H, NH); Anal. calcd for $C_{10}H_{12}F_2N_2O_5{\cdot}0.5H_2O$+0.2EtOAc: C, 42.57; H, 4.79; N, 9.19; Found: C, 42.37; H, 4.47; N, 8.99.
21. Compound **14**: $[\alpha]^{25}_D$ +11.0 (c 0.36, MeOH); UV λ_{max}(H_2O) 272.0 nm(ε 8140) (pH 2); 271.5 (ε 7180) (pH 7); 271.5 (ε 9830)(pH 11); ^{1}H NMR(DMSO-d_6) δ 7.69 (d, J = 7.4 Hz, 1H, H-6), 7.37 (bs, 1H, NH_2), 7.34 (bs, 1H, NH_2), 6.42 (bs, 1H, 2'-OH), 6.12 (t, J = 8.2Hz, H-1'), 5.77 (d,J = 7.4 Hz, 1H, H-5), 5.33 (bs, 1H, 5'-OH), 4.12 (m, 1H, H-3'), 3.59-3.78 (m, 3H, H-4' and H-5'); Anal. calcd for

$C_9H_{11}N_3O_4$+1.5H_2O: C, 37.84; H, 4.76; N, 14.71. Found: C, 37.86; H, 4.73; N, 14.81.

22. Compound **16:** $[\alpha]^{25}_D$ -39.4(c 0.22, MeOH); UV λ_{max}(H_2O) 271.5nm (ε 8840) (pH 2), 271.0 (ε9180) (pH 7), 271.5 (ε 7830)(pH 11); ^{1}H NMR(DMSO-d_6) δ 7.52 (d, J = 7.5 Hz, 1H, H-6), 7.36 (bs, 1H, NH_2), 7.30 (bs, 1H, NH_2), 6.51 (bs, 1H, 2'-OH), 6.26(dd, J = 6.6 and 10.6Hz,H-1'), 5.77 (d, J = 7.5 Hz, 1H, H-5), 5.17(bs, 1H, 5'-OH), 4.13(m, 1H, H-4'), 3.48-3.62(m, H,H-5'); Anal. calcd for $C_9H_{11}N_3O_4$+2.5H_2O: C, 35.07; H, 5.23; N, 13.63. Found: C, 35.04; H, 5.45; N, 13.43.
23. Compound **21:** m.p. 243-244°C; $[\alpha]^{25}_D$ +2.1 (c 0.38, MeOH); UV λ_{max}(H_2O): 258.5nm (ε13670) (pH 2), 258.5nm (ε 14460)(pH 7), 258.0nm (ε 13660)(pH 11); ^{1}H NMR(DMSO-d_6) δ 3.68-3.77 (m, 2H,H-5'), 3.93 (m, 1H, H-4'), 4.56 (m, 1H, H-3'), 5.25 (t, J=5.2 Hz, 5'-OH), 6.30 (dd, J = 5.0 Hz and 10.7 Hz, 1H, H-1'), 6.36 (d, J = 6.4 Hz, 1H, 3'-OH), 7.44 (s, 2H, NH_2), 8.16 (s, 1H, H-2), 8.35 (s, 1H, H-8); Anal. calcd for $C_{10}H_{11}F_2N_5O_5$ + 0.25H_2O: C, 41.17; H, 3.88; N, 24.00; Found: C, 41.07; H, 3.88; N, 23.96.
24. Compound **22:** m.p. 168-170°C; $[\alpha]^{25}_D$ -60.2 (c 0.26, MeOH); UV λ_{max}(H_2O) 258.5nm (ε 14760) (pH2), 258.0 (ε 15220) (pH 7), 258.5 (ε 13270) (pH 11); ^{1}H NMR(DMSO-d_6) δ 3.64 (m, 2H, H-5'), 4.39-4.46 (m, 2H, H-3' and H-4'), 5.11 (t, J = 5.2 Hz, 5'-OH), 6.45 (t, J = 8.2 Hz, 1H, H-1'), 6.55 (d, J = 5.7 Hz,1H, 3'-OH), 7.44 (s, 2H, NH_2), 8.17 (s, 1H, H-2), 8.29 (s, 1H, H-8); Anal. calcd for $C_{10}H_{11}F_2N_5O_5$+0.25H_2O: C, 41.17; H, 3.88; N, 24.00; Found: C, 41.03; H, 3.88; N, 23.62.
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