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SYNTHESIS AND ANTI-HIV ACTIVITY OF L-2'-FLUORO-2',3'-UNSATURATED PURINE NUCLEOSIDES

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Abstract: The synthesis of 9-(2,3-dideoxy-2-fluoro-L-glycero-pent-2-eno-furanosyl)adenine and -hypoxanthine has been accomplished by direct condensation of silylated 6-chloropurine with key intermediates 8, which were prepared starting from 2,3-O-isopropylidene-L-glyceraldehyde. The synthesized nucleosides were evaluated against HIV-1 in vitro in primary human lymphocytes (PMB cells). It was found that β -L-Fd4A 12 exhibited moderately potent anti-HIV activity (EC₅₀ 1.5 μ M). © 1998 Elsevier Science Ltd. All rights reserved.

The intense efforts in the search for more effective and less toxic antiviral agents against the human immunodeficiency virus (HIV) and hepatitis B virus (HBV) have led to the discovery of 2',3'-dideoxy nucleoside analogs, such as AZT,¹ ddC,² ddI,³ and d4T⁴ and unnatural L-nucleoside analogs, such as 3TC,⁵ FTC,⁶ and L-FMAU⁷. Recently, we have reported the synthesis and antiviral activity of β -L-2',3'-dideoxy (β -L-d2N)- and 2',3'-dideoxy (β -L-d4N)-purine nucleosides, among which β -L-d4A exhibited the most potent antiviral activity against HIV.⁸ However, it has been well known that d2- and d4-purine nucleosides are unstable in acidic media, resulting in glycosyl bond cleavage, thus limiting their use as orally bioavailable drugs.⁹ Introduction of a fluorine atom at the 2'-position in those dideoxypurine nucleosides is known to stabilize the glycosyl bond^{10, 11} and, moreover, a variety of fluorinated compounds have been shown to exhibit broad biological activities.¹¹ Therefore, it was of interest to sythesize d4N with a fluorine atom at the 2'-position, which could result in significant biological activity and the stabilization of glycosyl bond. Herein, we describe the synthesis of purine nucleosides containing vinylic fluorine and their anti-HIV activity.

Previously, the synthesis of 2',3'-unsaturated D-nucleosides was achieved via elimination reactions starting from readily available nucleoside analogs. This involved a lengthy modification for individual nucleosides. Several groups reported the preparation of D-2'-fluoro-2',3'-unsaturated pyrimidine nucleosides by the elimination of suitable 2'-fluorinated nucleoside analogs.¹² This strategy for the synthesis of L-Fd4N, however, is accompanied by additional difficulties in the use of L-nucleosides as the starting material. There are few examples for the synthesis of 2',3'-unsaturated purine nucleosides by the direct condensation due to the lability of the 2,3unsaturated sugar moiety under the coupling conditions in the presence of Lewis acid, except one case for the pyrimidine analogs using a thiophenyl intermediate.¹³ In contrast to the 2,3-unsaturated sugar moiety, the 2fluoro-2,3-unsaturated sugar bears enhanced stability of the glycosyl bond during its condensation with a heterocycle and was expected to be more suitable for the direct coupling reaction. Thus, (*R*)-2-fluorobutenolide **6**, as a precusor for the key intermediates **8**, was chosen and prepared from 2,3-O-isopropylidene-Lglyceraldehyde (**Scheme 1**).

Starting from 2,3-O-isopropylidene-L-glyceraldehyde (1), a mixture of (E)-2/(Z)-2 (9:1 by ¹H NMR) was



Reagents: (i) $(EtO)_2P(O)CHFCO_2Et$, $[(CH_3)_3Si]_2NNa$, THF, -78 °C (ii) HCl/EtOH (iii) TBDMSCl, imidazole, CH_2Cl_2 (iv) 1 M DIBAL-H in CH_2Cl_2 , CH_2Cl_2 , -78 °C (v) Ac_2O , pyr., CH_2Cl_2 (vi) silylated 6-Cl-purine, TMSOTf, DCE (vii) TBAF, CH_3CN (viii) NH₃/MeOH, 90 °C (ix) HS(CH₂)₂OH, NaOMe/MeOH, reflux

Table 1. Median I Hypoxant	Effective (EC ₅₀) and hine against HIV-1 in	Inhibitory (IC ₅₀) Con human PBM cells	centration of L-2'-Fl	uoro-d4Adenii	ie and
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Compound No.	EC ₅₀ (μM) (PBM Cells)	EC ₉₀ (μM) (PBM Cells)	Cytotoxicity			
			PBM Cells	Vero Cells	CEM Cells	
			IC ₅₀ (μM)	IC ₅₀ (μM)	IC ₅₀ (μM)	
12	1.5	15.1	> 100	> 100	> 100	
13	47.6	332	> 100	> 100	> 100	
14	> 100	> 100	> 100	> 100	> 100	
15	> 100	> 100	> 100	> 100	> 100	
AZT	0.004	0.04	> 100	29.0	14.3	

Scheme 1. Synthesis of L-2'-Fluoro-d4Adenine and -Hypoxanthine by Direct Condensation

obtained via the Horner-Emmons reaction in the presence of triethyl α -fluorophosphonoacetate and sodium bis(trimethylsilyl) amide in THF.¹⁴ Due to the difficulties in separating the (*E*)-2/(*Z*)-2 isomers, the mixture was used in the following cyclization reaction under acidic condition to give the desired lactone 3 and uncyclized diol 4. The resulting mixture was converted to the corresponding silyl derivatives, which were readily separated to give 5 and 6 (70.2% yield from compound 1). The silylated lactone 6 was subjected to reduction with DIBAL-H in CH₂Cl₂ at -78 °C to give a mixture of lactols 7. The lactols 7 were treated with acetic anhydride to yield the key intermediates 8, which were condensed with silylated 6-chloropurine under Vorbrüggen conditions to afford anomeric isomers 9. Treatment of 9 with TBAF in THF gave free nucleosides 10 and 11, which were readily separated by silica gel column chromatography. Adenine analogs 12¹⁵ and 13¹⁶ were obtained by the treatment of compound 10 and 11 in methanolic ammonia in a steel bomb at 90 °C, respectively. Treatment of compound 10 and 11 with mercaptoethanol and NaOMe afforded the inosine analogs 14¹⁷ and 15,¹⁸ respectively. The stereochemical assignment of these compounds was based on the NOESY spectroscopy (cross peak between H-1' and H-4' in β -isomer 12).

In conclusion, we accomplished the direct condensation of a 2,3-unsaturated sugar moiety with a silylated purine base in an efficient manner. The synthesized nucleoside 12 exhibited moderately potent anti-HIV activity (**Table 1**). The synthesis of other purine and pyrimidine nucleosides is in progress and will be reported shortly.

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- 15. 9-(2,3-Dideoxy-2-fluoro-β-L-glycero-pent-2-enofuranosyl)adenine (12): mp 188-190 °C; [α] 26 _D = -54.9 (c 0.17, MeOH); UV (H₂O) λ_{max} 258 nm (ε 18,800) (pH 2), 258.5 nm (ε 18,800) (pH 7), 258.5 nm (ε 19,100) (pH 11); ¹H NMR (DMSO-d) δ 3.63 (s, H-5'), 4.91 (s, H-4'), 6.08 (s, H-3'), 6.90 (s, H-1'), 8.17 (s, H-2), 8.40 (s, H-8); ¹³C NMR (DMSO-d) δ 65.06, 83.74, 85.62, 109.28, 121.17, 141.65, 151.79, 152.76, 155.40, 158.57; Anal. Calcd for C₁₀H₁₀FN₅O₂·0.2H₂O: C, 47.13; H, 4.11; N, 27.48. Found: C, 47.02; H, 4.13; N, 27.26.
- 16. 9-(2,3-Dideoxy-2-fluoro-α-L-glycero-pent-2-enofuranosyl)adenine (13): mp 168-171 °C; [α]²⁶_D = +160.6 (c 0.19, MeOH); UV (H₂O) λ_{max} 258 nm (ε 21,100) (pH 2), 259 nm (ε 21,500) (pH 7), 259 nm (ε 22,600) (pH 11); ¹H NMR (DMSO-*d*) δ 3.57 (m, H-5'), 5.14 (ps t, J = 3.9, 4.3 Hz, H-4'), 6.06 (s, H-3'), 6.89 (ps t, J = 3.9, 4.1 Hz, H-1'), 8.17 (s, H-2), 8.31 (s, H-8); ¹³C NMR (DMSO-*d*) δ 62.92, 81.88, 83.44, 106.99, 118.89, 139.03, 149.12, 150.27, 152.99, 155.95; Anal. Calcd for C₁₀H₁₀FN₅O₂·0.3MeOH: C, 47.33; H, 4.33; N, 26.85. Found: C, 47.42; H, 4.23; N, 26.91.
- 17. **9-(2,3-Dideoxy-2-fluoro-β-L-glycero-pent-2-enofuranosyl)hypoxanthine** (14): mp 128-130 'C; $[\alpha]^{24}_{D}$ = -50.2 (c 0.2, MeOH); UV (H₂O) λ_{max} 247 nm (ε 12,400) (pH 2), 247.5 nm (ε 13,000) (pH 7), 253 nm (ε 13,100) (pH 11); ¹H NMR (DMSO-*d*) δ 3.67 (s, H-5'), 4.98 (s, H-4'), 6.15 (ps t, J = 1.6 Hz, H-3'), 6.94 (m, H-1'), 8.17 (s, H-2), 8.43 (s, H-8); ¹³C NMR (DMSO-*d*) δ 65.97, 84.87, 86.94, 110.62, 127.64, 142.00, 149.93, 151.74, 153.56, 159.97; Anal. Calcd for C₁₀H₉FN₄O₃·0.2MeOH: C, 47.37; H, 3.82; N, 21.66. Found: C, 47.11; H, 3.77; N, 21.50.
- 18. 9-(2,3-Dideoxy-2-fluoro-α-L-glycero-pent-2-enofuranosyl)hypoxanthine (15): mp 200 °C (dec.); [α]²⁶_D = +157.3 (c 0.22, MeOH); UV (H₂O) λ_{max} 247.5 nm (ε 12,700) (pH 2), 247.5 nm (ε 13,700) (pH 7), 252.5 nm (ε 13,100) (pH 11); ¹H NMR (DMSO-d) δ 3.56 (m, H-5'), 5.13 (ps t, J = 3.6, 3.7 Hz, H-4'), 6.06 (s, H-3'), 6.87 (ps t, J = 2.7, 5.3 Hz, H-1'), 8.09 (s, H-2), 8.26 (s, H-8); ¹³C NMR (DMSO-d) δ 63.39, 82.88, 84.24, 107.84, 125.13, 139.20, 146.84, 148.59, 150.75, 156.92; Anal. Calcd for C₁₀H₉FN₄O₃·0.3H₂O: C, 46.62; H, 3.76; N, 21.75. Found: C, 46.86; H, 3.80; N, 21.56.