Synthesis and Antiviral Activity of Fluoro Sugar Nucleosides 2: Synthesis and Biological Evaluations of 2',3'-Dideoxy-2'-Fluoro-3'-C-Hydroxymethyl-β-D-Arabinofuranosyl Nucleosides

Moon Woo Chun[1]*, Kyung Lee[1], Yong Suk Choi[1], Jeewoo Lee[1], Joong Hyup Kim[2], Chong Kyo Lee[3], Bo Gil Choi[4] and Yong Can Xu[5]

[1]College of Pharmacy, Seoul National University, San 56-1, Shillim-Dong, Kwanak-Gu, Seoul 151-742, Korea, [2]Korea Institute of Science and Technology, P.O. Box 130-650, Cheongryang, Seoul 130-650, Korea, [3]Korea Research Institute of Chemical Technology, P.O. Box 107, Yusong, Daejeon 305-606, Korea, [4]College of Pharmacy, Chonnam National Univerisity, Yongbong-Dong, Book-Gu, Kwangju 500-757, Korea and [5]Yanbian Medical College, Yanji City, Jilin Province, China

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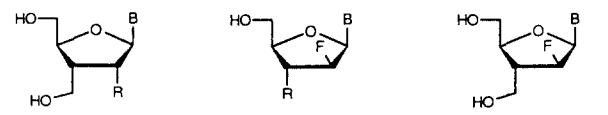
Since the oxetanocins, naturally occurring nucleosides, were reported to have a broad spectrum antiviral activity (Shimada *et al.*, 1986), a variety of nucleosides having hydroxylmethyl group on furanosyl ring were synthesized and identified as potential antiviral agents. These include a ring-enlarged oxetanocin A analogue (Tseng *et al*, 1991), 3'-deoxy (or 2',3'-dideoxy)-3'-C-hydroxymethyl nucleosides (Sterzycki *et al.*, 1991), 2',3'-deoxy-C-(hydroxymethyl) thioguanosine (Acton *et al.*, 1979), 3'-deoxy-3'-C-heteromethyl-substituted nucleosides (Lin *et al.*, 1993), isonucleoside analogues of hydroxylmethyl sugar (Tino *et al.*, 1993), carbocyclic 2',3'-dideoxy-2'-C-hydroxymethyl nucleosides (Rosenquist *et al.*, 1994) and 2',3'-dideoxy-3'-C-hydroxymethyl-4'-thionucleoside derivatives (Branalt *et al.*, 1994). Among these compounds, 2',3'-dideoxy-3'-C-(hydroxymethyl)cytidine (**1**) had a high level of anti-viral activity aginst HIV and a broad range of DNA viruses (Sterzycki *et al.*, 1991), and 3'-Deoxy-3'-C-(hydroxymethyl)thymidine (**2**) was founded to show significant anticancer activity against L1210, P388, S-180, and CCRF-CEM cells (Fig. 1) (Lin *et al.*, 1993).

On the other hand incorporation of fluorine into the sugar ring of dideoxynucleosides has been known to provide a profound effect on the chemical stability and biological potency of the resulting modified analogues (Balzalini *et al.*, 1988; Marquez *et al.*, 1990; Bamford *et al.*, 1990). For instance, 2'-fluoronucleosides such as FMAU (**3**), FIAU (**4**) (Watanabe *et al.*, 1979, 1985; Fox *et al.*, 1981), and F-DDC (**5**) (Watanabe *et al.*, 1990) were widely known to be active against various viral diseases. We therefore decided to synthesize and test 2'-fluoro-3'-hydroxymethyl nucleosides (**6**), which have both structurally requirements for biological activity for their antiviral activity and anticancer activity. In this report we want to describe the synthesis of 2',3'-dideoxy-2'-fluoro-3'-C-hydroxymethyl-β-D-arabino-furanosyl nucleosides and compare their biological activities with the other known active nucleosides.

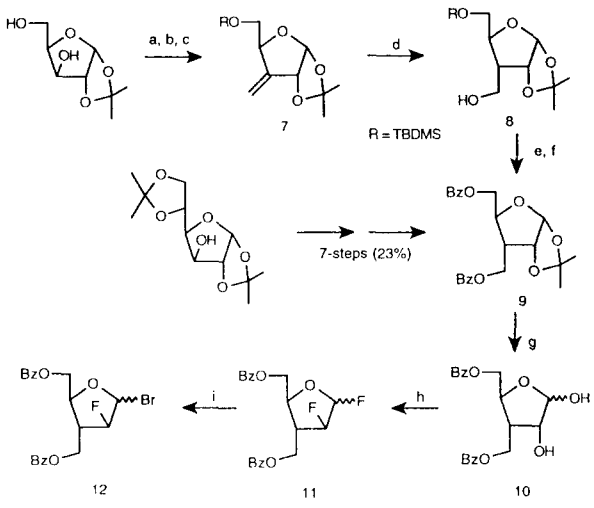
Following the published procedure, we used a suitably protected xylofuranose and glucofuranose as the starting materials (Scheme 1). Among the various protecting groups for the primary alcohol of 1,2-O-isopropylidene-α-D-xylofuranose we have tried, we obtained the best yield (52%) of **9** using tert-butyldimethylsilylchloride while obtained 11.3% yield of **9** when benzoyl chloride was used. Alternatively, it could be prepared from 1,2;5,6-O-diisopropylidene-α-D-gluco-furanose as a starting material in a yield of 23% in **7** steps. Treatment of 1,2-O-isopropylidene-α-D-xylofuranose with tert-butyldimethylsilylchloride in methylene chloride produced its 5-O-(tert-butyldimethylsilyl) derivative, whose secondary alcohol was oxidized to the corresponding ketone with chromium trioxide/pyridine/acetic anhydride complex (Garegg et al., 1978) (1 : 2 : 1, molar ratio) in methylene chloride. The resulting ketone was converted to the 3-methylene analogue **7** by a Wittig reaction with methylenetriphenylphosphrane and followed by



1. B = cytosine, R = H
2. B = thymine, R = OH
3. B = thymine, R = OH **FMAU**
4. B = 5-iodocytosine, R = OH **FIAC**
5. B = cytosine, R = H **F-DDC**
6. B = pyrimidines

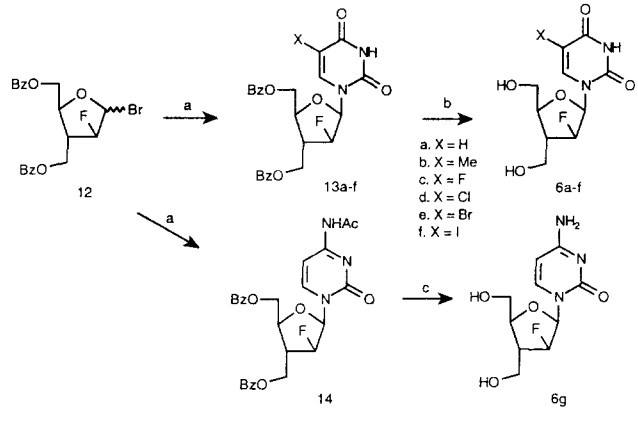
Fig. 1.

Correspondence to: Moon Woo Chun, College of Pharmacy, Seoul National University, San 56-1, Shillim-Dong, Kwanak-Gu, Seoul 151-742, Korea



Reagent: (a) TBDMSCl, imidazole, CH_2Cl_2 (b) CrO_3, pyridine, Ac_2O, CH_2Cl_2 (c) $Ph_3P^+CH_3I^-$, n-BuLi, THF (d) BH_3, THF; H_2O_2, NaOH (e) Bu_4NF, THF (f) BzCl, pyridine (g) 80% CF_3COOH (h) DAST, CH_2Cl_2, 57% from 9 (i) 30% HBr, CH_2Cl_2

Scheme 1.



Reagent: (a) i) base, HMDS ii) $CHCl_3$, reflux (b) NaOMe, MeOH (c) conc. NH_3 in MeOH

Scheme 2.

stereoselective hydroboration-oxidation to afford 3-deoxy-3-hydroxymethyl derivative **8**. The protecting silyl group must be converted to benzoyl group prior to deprotection of acetonide moiety because it was labile under such acidic condition. Treatment of compound **8** with TBAF in THF gave the corresponding diol, which was then protected with benzoyl chloride in pyridine to give a dibenzoate **9**. Acetonide group in **9** was removed under acidic condition using 80% trifluoroacetic acid followed by difluorination with diethylaminosulfurtrifluoride (DAST) (Tewson *et al.*, 1978) in methylene chloride to afford the difluoride derivative **11**. Because of a low yield on direct bases replacement on **11**, it was converted into the bromo derivative **12**, which was then condensed with uracil, thymine, N4-acetylcytosine, 5-halouracils, and to give **13a-f** and **14** (Scheme 2) (Mansuri *et al.*, 1987). In case of uracil bases, final products were prepared

Table I.

No.	Toxicity	Antiviral Activity (EC50)		Selectivity Index	
	CC50	HSV-1	HSV-2	HSV-1	HSV-2
6a	>100	>100	>100	NC	NC
6b	>100	43	80	>2.33	>1.25
6c	>100	>100	>100	NC	NC
6d	>100	>100	>100	NC	NC
6e	>100	>100	>100	NC	NC
6f	>100	61.5	>100	>1.63	NC
6g	>100	28.9	>100	3.46	NC
ACA	>250	0.14	4.69	1.786	>53
Ara-C	4.73	0.21	2.95	22.52	1.6

by deprotection of **13** with NaOMe in MeOH. We also obtained cytosine derivative by deprotection in methanolic ammonia from **14**. The structures of all prepared compounds was confirmed by spectra data.

The synthesized nucleosides **6a-g** were tested for anti-HSV-1,2 activity in a Vero (CCL81) cell and for antitumor activity *in vitro* on the replication of L1210, P388, and CCRF-CEM cells. Of these compounds, all were inactive except **6b**, **6f**, and **6g** which showed a low activity against HSV-1 and HSV-2 as shown in Table I.

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Spectral Data of Final Compounds

2',3'-dideoxy-2'-fluoro-3'-hydroxymethyl-α-D-arabino-furanosyl-5-uracil (6a) mp 194-195°C; $[\alpha]^D$+ 76.7 (c 0.15, MeOH); UV (H_2O, pH 7.2, HEPES buffer) λ_{max} 261 nm (ε 10,800), (0.1 N HCl) λ_{max} 258 nm (ε 10,100), (1 N NaOH) λ_{max} 260 nm (ε 10, 100); ^{1}H NMR (CD_3OD) δ 2.59 (dm, 1 H, H-3', $J_{3',F}$ =27.6 Hz), 3.70-3.90 (m, 4 H, H-5', 3'-CH_2-), 4.06 (q, 1 H, H-4'), 5.24 (dt, 1 H, H-2', $J_{2',F}$=53.6 Hz), 5. 73 (q, 1 H, H-5, $J_{5,6}$=7.6 Hz), 6.12 (dt, 1 H, H-1', $J_{1',F}$=16.8 Hz), 7.97 (t, 1 H, H-6, $J_{6,5}$=7.6 Hz); high-resolution FAB MS m/z 260.0778 (M+, calcd. 260. 0808).

2',3'-dideoxy-2'-fluoro-3'-hydroxymethyl-α-D-arabino-furanosyl-5-thymine (6b) mp 183-185°C; $[\alpha]^D$+ 83.2 (c 0.125, MeOH); UV (H_2O, pH 7.2, HEPES buffer) λ_{max} 265 nm (ε 11,600), (0.1 N HCl) λ_{max} 264 nm (ε 11,000), (1 N NaOH) λ_{max} 260 nm (ε 10, 100); ^{1}H NMR(CD_3OD) δ 1.94 (s, 3 H, 5-CH_3), 2.60 (dm, 1 H, H-3', $J_{3',F}$=30.4 Hz), 3.70-3.93 (m, 4 H, H-5', 3'-CH_2-), 4.02-4.05 (m, 1 H, H-4'), 5.22 (dt, 1H, H-2', $J_{2',F}$=54 Hz), 6.10 (dd, 1 H, H-1', $J_{1',F}$=16.8 Hz, $J_{1',2'}$=4 Hz), 7.83 (t, 1 H, H-6); high-resolution FAB MS m/z 274.0957 (M+, calcd. 274.0965).

2',3'-dideoxy-2'-fluroro-3'-hydroxymethyl-α-D-arabino-furanosyl-5-fluorouracil (6c) mp 158-160°C; $[\alpha]^D$+77.5 (c 0.12, MeOH); UV (H_2O, pH 7.2, HEPES buffer) λ_{max} 267 nm (ε 8,910), (0,1 N HCl) λ_{max} 258 nm (ε 10,100), (1 N NaOH) λ_{max} 260 nm (ε 10,100); ^{1}H NMR (CD_3OD) δ 2.61 (dm, 1H, H-3', $J_{3',F}$=29.6 Hz), 3.70-3.93 (m, 4 H, H-5', 3'-CH_2-), 4.03-4.07 (m, 1 H, H-4'), 5.25 (dt, 1H, H-2', $J_{2',F}$= 54.4 Hz), 6.10 (dq, 1H, H-1', $J_{1',F}$=15.6 Hz, $J_{1',5\text{-}F}$=1. 6 Hz), 8.18 (dd, 1H, H-6, $J_{6,F}$=7.2 Hz) ; high-resolution FAB MS m/z 278.0700 (M+, calcd. 278. 0714).

2',3'-dideoxy-2'-fluoro-3'-hydroxymethyl-α-D-arabino-furanosyl-5-chlorouracil (6d) mp 195-196°C; $[\alpha]^D$+87.0 (c 0.10, MeOH); UV (H_2O, pH 7.2, HEPES buffer) λ_{max} 273 nm (ε 10,800), (0.1 N HCl) λ_{max} 258 nm (ε 10,100), (1 N NaOH) λ_{max} 260 nm (ε 10,100); ^{1}H NMR (CD_3OD) δ 2.62 (dm, 1 H, H-3', $J_{3',F}$=30 Hz), 3.70-3.94 (m, 4 H, H-5', 3'-CH_2-), 4.05-4.09 (m, 1 H, H-4'), 5.25 (dt, 1 H, H-2', $J_{2',F}$= 54 Hz), 6.11 (dd, 1 H, H-1', $J_{1',F}$=15.6 Hz, $J_{1',2'}$=3.6 Hz), 8.31 (s, 1 H, H-6); high-resolution FAB MS m/ z 294.0423 (M+, calcd. 294.0419).

2',3'-dideoxy-2'-fluoro-3'-hydroxymethyl-α-D-arabino-furanosyl-5-bromouracil (6e) mp 274-275°C; $[\alpha]^D$+51.4 (c 0.105, MeOH); UV (H_2O, pH 7.2, HEPES buffer) λ_{max} 261 nm (ε 10,800). (0.1 N HCl) λ_{max} 258 nm (ε 10,100), (1 N NaOH) λ_{max} 260 nm (ε 10,100); ^{1}H NMR (CD_3OD) δ 2.60 (dm, 1 H, H-3', $J_{3',F}$=29.6 Hz), 3.73-3.93 (m, 4 H, H-5', 3-CH_2-), 4.06 (m, 1 H, H-4'), 5.25 (dm, 1 H, H-2', $J_{2',F}$=53.6 Hz), 6.11 (dd, 1H, H-1', $J_{1',F}$=16.4 Hz, $J_{1',2'}$=3.6 Hz), 8.34 (s, 1 H, H-6); high-resolution FAB MS m/z 338.4934 (M+, calcd. 338.4932)

2',3'-dideoxy-2'-fluoro-3'-hydroxymethyl-α-D-arabino-furanosyl-5-iodouracil (6f) mp 194°C ; $[\alpha]^D$+ 47.5 (c 0.10, MeOH); UV (H_2O, pH 7.2, HEPES buffer) λ_{max} 261 nm (ε 10,800), (0.1 N HCl) λ_{max} 258 nm (ε 10,100), (1 N NaOH) λ_{max} 260 nm (ε 10, 100); ^{1}H NMR (CD_3OD) δ 2.61 (dm, 1 H, H-3', $J_{3',F}$ =30.4 Hz), 3.70-3.94 (m, 4 H, H-5', 3'-CH_2-), 4.05-4.08 (m, 1 H, H-4'), 5.25 (dt, 1 H, H-2', $J_{2',F}$=54 Hz), 6.10 (dd, 1H, H-1', $J_{1',F}$=15.6 Hz, $J_{1',2'}$=4.4 Hz), 8.44 (s, 1 H, H-6); high-resolution FAB MS m/z 385.9781 (M+, calcd. 385.9775).

2',3'-dideoxy-2'-fluoro-3'-hydroxymethyl-α-D-arabino-furanosyl-5-cytosine (6g) mp 212°C; $[\alpha]^D$+76.9 (c 0.15, MeOH);, ^{1}H NMR (CD_3OD) δ 2.47 (dm, 1 H, H-3', $J_{3',F}$=27.6 Hz), 3.61-3.80 (m, 4 H, H-5', 3'-CH_2-), 3.96 (q, 1 H, H-4'), 5.15 (dm, 1 H, H-2', $J_{2',F}$ =53.6 Hz), 5.86 (q, 1 H, H-5, $J_{6,5}$=7.2 Hz), 6.03 (dd, 1H, H-1', $J_{1',F}$=18 Hz, $J_{1',2'}$=3.6 Hz), 7.86 (d, 1 H, H-5, $J_{5,6}$=7.6 Hz); high-resolution FAB MS m/z 259.1002 (M+, calcd. 259.0968).