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Design and Synthesis of Novel 2'(β)-Fluoro-3'(α)-hydroxy-threose Nucleosides: Iso-FMAU Analogues as Potent Antiviral Agents

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Abstract

Novel 2'(β)-fluoro-3'(α)-hydroxy-threose nucleosides (iso-FMAU) as antiviral agents were designed and racemically synthesized from Solketal. Condensation successfully proceeded from a glycosyl donor **9** under Vorbrüggen conditions yielded the nucleoside analogues. Ammonolysis and hydrolysis of isopropylidene protection group gave the desired nucleoside analogues **12**, **15, 18,** and **19**. The antiviral activities of the synthesized compounds were evaluated against the HIV-1, HSV-1, HSV-2 and HCMV. Compound **12** displayed some anti-HCMV activity (EC_{50}=24.7 μg/ml) without exhibiting any cytotoxicity up to 100 μM.

Keywords: Antiviral Agents, 2'(β)-Fluoro-3'(α)-hydroxy-threose Nucleoside Analogue, Iso-FMAU, Vorbrüggen Reaction

1. Introduction

Fluorinated nucleosides (containing fluorine atom(s) in the sugar moiety) have drawn increasing attention owing to their improved biological activity and chemical stability of the corresponding compounds[1]. Especially, introduction of a fluorine atom at the 2'-β-position of nucleoside analogues has produced a variety of interesting antiviral agents. A series of pyrimidine nucleosides, including FMAU and FIAU, were prepared in a large scale for biological evaluation[2]. Unfortunately, the phase 1 trials of FMAU as an antileukemic agent were terminated due to neurological toxicity[3]. FIAU (**1**) also exhibited delayed toxicities due to the interference of mitochondrial function resulting in lactic acidosis and hepatic failure (Figure 1)[4].

Because of the broad spectrum of biological activities of 2'-F-arabinofuranosyl nucleoside, a number of structural modifications of these analogues have been carried out. Chu and coworkers have demonstrated that the enantiomer of FMAU, L-FMAU (**2**) is a promising agent against HBV[5]. It showed low toxicity in rates and woodchucks, potent in vivo antiviral activity against chronically infected woodchucks (WHV), respectable bioavailability and showed no significant virus rebound up to 36 weeks after cessation of the drug treatment. L-FMAU was approved as anti-HBV drug by the Korea FDA in 2006 and is under phase III clinical trials in the USA and Europe. Another interesting congener is clofarabine (**3**) which was approved by FDA for the treatment of acute lymphoblastic leukemia (ALL)[6]. Gemcitabine[7] (2'-deoxy-2',2'-difluorocytidine, **4**) is a clinically effective anti-cancer agent for the treatment of pancreatic cancer. It has also shown anti-

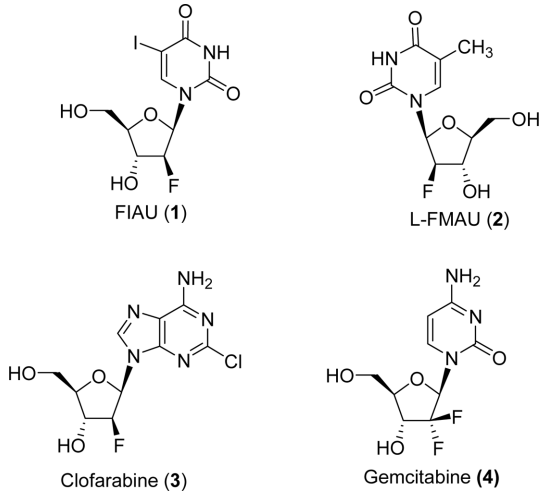

Fig. 1. Design rationale of 2'(β)-fluoro-threose nucleosides

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tumor activity against a wide spectrum of human solid tumors[8].

Nonclassical nucleosides continue to be a promising challenge for the development of new antiviral agents since the discovery of lamivudine as an anti-human immunodeficiency virus (HIV) and anti-hepatitis B virus (HBV) agent[9]. Threose dideoxynucleoside also belongs to the class of nonclassical nucleosides in that the 4-hydroxymethyl group of 2,3-dideoxyribose moves to the C-3 position. This class of nucleosides exhibited not only antiviral activity, but also metabolic advantages such as resistance to adenosine deaminase and glycosyl bond hydrolysis, compared to classical 2,3-dideoxynucleosides[10]. Furthermore, this absence of a 4'-hydroxymethyl group avoids problems of steric hindrance during phosphorylation reactions with kinases[11].

Stimulated by the findings that 2'-electropositive nucleoside analogues have excellent anti-viral activity and threosyl nucleosides exhibited potent antiviral activity, we sought to synthesize a novel hybrid class of nucleosides, consisting of 2'(β)-fluoro-3'(α)-hydroxy-threose nucleoside analogues (iso-FMAU), to find more effective therapeutics.

2. Experimental Section

Uncorrected melting points were determined using a Mel-temp II laboratory device. Nuclear magnetic resonance (NMR) spectra were recorded using a JEOL 300 Fourier transform spectrometer (JEOL, Tokyo, Japan); chemical shifts are reported in parts per million (δ) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or dd (doublet of doublets). Ultra violet (UV) spectra were obtained using a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). Mass spectra (MS) were collected in electrospray ionization (ESI) mode. Elemental analyses were performed using a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA). Thin layer chromatography (TLC) was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were performed in a nitrogen atmosphere unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry tetrahydrofuran (THF) was obtained by distillation from Na and benzophenone immediately prior to use.

2.1. (*rel*)-(4*S*,5*R*)-7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-one (**7**) and (*rel*)-(4*R*,5*R*)-7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-one (**8**)

N-Fluorodibenzenesulfonimide (NFSI) (1.272 g, 4.056 mmol) in 15 mL of anhydrous THF was added to a solution of lactone derivative **6** (698 mg, 4.056 mmol) and cooled to -78°C. LiHMDS in THF (4.89 mL, 1.0 M) was then added dropwise over 1 h, and the solution was stirred at -78°C for an additional 4.0 h then warmed to room temperature and stirred for 2.0 h. The reaction was then quenched with 1.5 mL of saturated ammonium chloride (NH_4Cl), diluted with diethyl ether (100 mL), and poured into an equal volume of saturated sodium bicarbonate ($NaHCO_3$). The organic layer was washed twice with saturated $NaHCO_3$ and once with brine, dried over magnesium sulfate ($MgSO_4$), filtered, and concentrated. The residue was purified by silica gel column chromatography (Hexane/EtOAc, 20:1) to give compounds **7** (246 mg, 32%) and **8** (231 mg, 30%). Data for compound **7**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.53 (d, J = 7.6 Hz, 1H), 4.45 (d, J = 16.8 Hz, 1H), 4.28 (d, J = 7.6 Hz, 1H), 3.95 (d, J = 8.2 Hz, 1H), 3.67 (d, J = 8.2 Hz, 1H), 1.47 (s, 3H), 1.46 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.6 (d, J = 19.2 Hz), 111.5 (d, J = 201.4 Hz), 109.2, 74.7 (d, J = 20.3 Hz), 70.4, 67.7, 26.8, 26.4; Anal. Calcd. for $C_8H_{11}FO_4$: C, 50.53; H, 5.83; found: C, 50.42; H, 5.89; MS *m/z* 191 $(M+H)^+$. Data for compound **8**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.49 (d, J = 8.2 Hz, 1H), 4.39 (d, J = 14.2 Hz, 1H), 4.17 (d, J = 8.2 Hz, 1H), 3.91 (d, J = 8.4 Hz, 1H), 3.74 (d, J = 8.3 Hz, 1H), 1.45 (s, 3H), 1.44 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.1 (d, J = 20.7 Hz), 112.3 (d, J = 209.6 Hz), 108.2, 75.8 (d, J = 19.5 Hz), 69.9, 66.4, 26.4, 25.9; Anal. Calcd. for $C_8H_{11}FO_4$: C, 50.53; H, 5.83; found: C, 50.64; H, 5.74; MS *m/z* 191 $(M+H)^+$.

2.2. (*rel*)-(3*S*/3*R*,4*S*,5*R*)-(7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) acetate (**9**)

A solution of compound **7** (500 mg, 2.63 mmol) in toluene (20 mL) was treated with 5.24 mL of 1 M DIBAL-H in hexane at -78°Cfor 1 h. The reaction was quenched with 1 mL of methanol (MeOH) and warmed to room temperature for 1 h before aqueous (aq) $NaHCO_3$ (2 mL) and EtOAc (20 mL) were added to the

mixture. The resulting mixture was filtered and the filtrate was concentrated to dryness. A solution of crude lactol in CH_2Cl_2 (30 mL) was treated with acetic anhydride (Ac_2O; 0.75 mL, 7.92 mmol), triethylamine (TEA; 1.1 mL, 7.92 mmol), and a catalytic amount of 4-dimethylaminopyridine (DMAP; 10 mg) at room temperature for 7 h. The resulting mixture was concentrated and purified using silica gel column chromatography (EtOAc/hexane, 1:20) to yield compound **9** (486 mg, 79%) as diastereomeric mixtures. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.48-6.45 (m, 1H), 4.62-4.51 (m, 1H), 3.97-3.90 (m, 2H), 3.73-3.64 (m, 2H), 2.03, 2.01 (s, s, 3H), 1.41 (d, *J* = 6.8 Hz, 6H); Anal. Calcd. for $C_{10}H_{15}FO_5$: C, 51.28; H, 6.46; found: C, 51.42; H, 6.54; MS *m/z* 235 $(M+H)^+$.

2.3. (*rel*)-(3*S*,4*S*,5*R*)-9-(7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) 6-chloropurine(10α) and (*rel*)-(3*R*,4*S*,5*R*)-9-(7,7-dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) 6-chloropurine (**10β**)

6-Chloropurine (266 mg, 1.72 mmol), anhydrous HMDS (13 mL), and a catalytic amount of ammonium sulfate (17.2 mg) were refluxed to a clear solution; the solvent was then distilled under anhydrous conditions. The residue obtained was dissolved in anhydrous 1,2-dichloroethane (12 mL), and to this mixture, a solution of **9** (282 mg, 0.86 mmol) in dry DCE (13 mL) and TMSOTf (382 mg, 1.72 mmol) was added, and stirred for 6 h at room temperature. The reaction mixture was quenched with 7.5 mL of saturated $NaHCO_3$, stirred for 1 h, filtered through a Celite pad, and the filtrate obtained was then extracted twice with CH_2Cl_2 (100 mL). Combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified using silica gel column chromatography (EtOAc/hexane/MeOH, 4:1:0.02) to yield compounds **10α** (90 mg, 32%) and **10β** (93 mg, 33%). Data for **10α**: UV (MeOH) λ_{max} 263.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.67 (s, 1H), 8.17 (s, 1H), 6.14 (dd, *J* = 14.6, 5.8 Hz, 1H), 4.09 (dd, *J* = 14.6, 5.8 Hz, 1H), 3.94 (d, *J* = 7.3 Hz, 1H), 3.88 (d, *J* = 8.0 Hz, 1H), 3.71 (dd, *J* = 7.8, 7.0 Hz, 2H), 1.42 (s, 3H), 1.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.6, 151.5, 151.3, 145.0, 132.3, 110.9, 99.5 (d, *J* = 206.4 Hz), 85.6 (d, *J* = 20.5 Hz), 79.6 (d. *J* = 19.2 Hz), 74.3, 63.8, 26.4, 26.0; Anal. Calc. for $C_{13}H_{14}ClFN_4O_3$: C, 47.50; H, 4.29; N, 17.04. Found: C, 47.64; H, 4.36; N, 17.12; MS *m/z* 329 $(M + H)^+$. Data for **10β**: UV (MeOH) λ_{max} 263.0 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71 (s, 1H), 8.19 (s, 1H), 6.19 (dd, *J* = 15.2, 6.6 Hz, 1H), 4.04-3.97 (m, 2H), 3.89 (d, *J* = 7.6 Hz, 1H), 3.82 (d, *J* = 8.1 Hz, 1H), 3.73 (d, *J* = 8.0 Hz, 1H), 1.40 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.9, 151.6, 151.5, 145.1, 132.6, 111.7, 101.2 (d, *J* = 208.8 Hz), 87.4 (d, *J* = 19.8 Hz), 77.7 (d. *J* = 18.8 Hz), 73.5, 62.4, 27.1, 26.6; Anal. Calc. for $C_{13}H_{14}ClFN_4O_3$: C, 47.50; H, 4.29; N, 17.04. Found: C, 47.37; H, 4.17; N, 16.96; MS *m/z* 329 $(M + H)^+$.

2.4. (*rel*)-(3*R*,4*S*,5*R*)-9-(7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) adenine (**11**)

A solution of **10β** (250 mg, 0.762 mmol) in saturated methanolic ammonia (10 mL) was stirred overnight at 100°C in a steel bomb and the volatiles were evaporated. The residue was purified using silica gel column chromatography (MeOH/CH_2Cl_2, 1:10) to yield **11** (207 mg, 88%) as a white solid: UV (MeOH) λ_{max} 262.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.31 (s, 1H), 8.14 (s, 1H), 7.26 (br s, 2H, D_2O exchangeable), 6.11 (dd, *J* = 14.6, 6.2 Hz, 1H), 4.02-3.91 (m, 3H), 3.70 (dd, *J* = 10.4, 6.8 Hz, 2H), 1.42 (s, 3H), 1.41 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.5, 152.4, 150.2, 141.4, 119.1, 112.7, 98.8 (d, *J* = 210.2 Hz), 84.1 (d, *J* = 20.6 Hz), 80.4 (d, *J* = 19.2 Hz), 74.5, 64.5, 26.2, 25.8; Anal. Calc. for $C_{13}H_{16}FN_5O_3$: C, 50.48; H, 5.21; N, 22.64; Found: C, 50.34; H, 5.15; N, 22.72; MS *m/z* 310 $(M + H)^+$.

2.5. (*rel*)-(1*R*,2*S*,3*R*)-9-[2-Fluoro-3-(hydroxymethyl)-3-hydroxyfuran-1-yl] adenine (**12**)

To the solution of **11** (286 mg, 0.925 mmol) in MeOH (60 mL), Dowex 50×8 resin (10 g) was added and the mixture was stirred for 10 h at 40~45°C. After removing the solvent, the residue was loaded onto a Dowex H^+ resin column and the product eluted with 14% NH_4OH. The fraction containing product were combined and evaporated under reduced pressure. The residue was purified *via* flash column chromatography (EtOAc/MeOH), 20:4) to give **12** (154 mg, 62%) as a white solid. mp 188-190°C; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.30 (s, 1H), 8.15 (s, 1H), 7.34 (br s, D_2O exchangeable), 6.15 (dd, *J* = 14.0, 6.2 Hz, 1H), 5.19 (s, 1H, D_2O exchangeable), 4.96 (t, *J* = 4.6 Hz, 1H, D_2O exchangeable), 3.99 (d, *J* = 8.2

Hz, 1H), 3.86-3.72 (m, 3H), 3.59 (d, J = 7.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.4, 152.3, 149.2, 141.9, 120.1, 101.5 (d, J = 204.6 Hz), 83.6 (d, J = 19.5 Hz), 79.2 (d, J = 18.8 Hz), 75.3, 62.2; Anal. Calc. for $C_{10}H_{12}FN_5O_3$ (+0.5 MeOH): C, 44.23; H, 4.95; N, 24.56; Found: C, 44.38; H, 4.81; N, 24.62; MS m/z 270 $(M + H)^+$.

2.6. (*rel*)-(3*S*,4*S*,5*R*)-*N*$_4$-Benzoyl-1-(7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) cytosine (13α) and (*rel*)-(3*R*,4*S*,5*R*)-*N*$_4$-benzoyl-1-(7,7-dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) cytosine (**13**β)

Condensation of **9** with N_4-benzoyl cytosine under Vorbrüggen condensation conditions similar to those described for **10**α and **10**β yielded **13**α and **13**β. Data for **13**α: yield 35%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.20 (d, J = 7.1 Hz, 1H), 7.97-7.75 (m, 2H), 7.67-7.51 (m, 4H), 6.06 (dd, J = 13.6, 6.8 Hz, 1H), 4.56 (dd, J = 15.2, 6.2 Hz, 1H), 3.94 (dd, J = 8.0, 6.6 Hz, 2H), 3.69 (dd, J = 9.2, 8.0 Hz, 2H), 1.40 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 168.7, 163.3, 156.8, 142.3, 135.8, 128.2, 127.7, 127.3, 112.0, 99.5 (d, J = 211.4 Hz), 81.9 (d, J = 19.1 Hz), 78.6 (d, J = 18.4 Hz), 74.2, 64.4, 26.4, 25.8; Anal. Calc. for $C_{19}H_{20}FN_3O_5$: C, 58.61; H, 5.18; N, 10.79; Found: C, 58.53; H, 5.22; N, 10.84; MS m/z 390 $(M + H)^+$. Data for **13**β: yield 33%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.17 (d, J = 7.2 Hz, 1H), 7.67-7.50 (m, 6H), 6.11 (dd, J = 14.2, 7.0 Hz, 1H), 4.48 (dd, J = 14.6, 7.4 Hz, 1H), 3.96 (d, J = 7.8 Hz, 1H), 3.86 (d, J = 8.0 Hz, 1H), 3.76 (d, J = 7.8 Hz, 1H), 3.68 (d, J = 8.0 Hz, 1H), 1.41 (s, 3H), 1.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 167.8, 163.6, 157.1, 142.5, 136.2, 128.7, 127.4, 110.8, 98.8 (d, J = 203.6 Hz), 82.3 (d, J = 18.8 Hz), 79.5 (d, J = 18.3 Hz), 73.7, 63.6, 27.1, 26.8; Anal. Calc. for $C_{19}H_{20}FN_3O_5$: C, 58.61; H, 5.18; N, 10.79; Found: C, 58.74; H, 5.12; N, 10.65; MS m/z 390 $(M + H)^+$.

2.7. (*rel*)-(3*R*,4*S*,5*R*)-1-(7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) cytosine (**14**)

Compound **13**β (218 mg, 0.54 mmol) was treated with saturated methanolic ammonia (10 mL) overnight at rt. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2/EtOAc, 1:15:2) to give compound **14** (145 mg, 83%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.48 (d, J = 7.0 Hz, 1H), 7.34 (br d, 2H, D_2O exchangeable), 6.08 (dd, J = 13.6, 7.2 Hz, 1H), 5.56 (d, J = 7.0 Hz, 1H), 4.58 (dd, J = 14.2, 6.8 Hz, 1H), 3.93 (dd, J = 8.8, 6.6 Hz, 2H), 3.78 (d, J = 7.4 Hz, 1H), 3.65 (d, J = 7.8 Hz, 1H), 1.42 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.4, 156.6, 143.7, 110.6, 99.3 (d, J = 202 Hz), 95.6, 81 (d, J = 18.7 Hz), 77.5 (d, J = 18.8 Hz), 73.7, 64.7, 26.5, 26.1; Anal calc for $C_{12}H_{16}FN_3O_4$: C, 50.52; H, 5.65; N, 14.73. Found: C, 50.67; H, 5.43; N, 14.68; MS m/z 286 $(M + H)^+$.

2.8 (*rel*)-(1*R*,2*S*,3*R*)-1-[2-Fluoro-3-(hydroxymethyl)-3-hydroxyfuran-1-yl] cytosine (**15**)

Cytosine analogue **15** was prepared by deprotection of **14** by the similar hydrolytic procedure described for **12**. yield 67%; mp 189-192ºC; UV (MeOH) λ_{max} 271.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.45 (d, J = 7.0 Hz, 1H), 7.36 (br d, 2H, D_2O exchangeable), 6.10 (dd, J = 13.0, 7.2 Hz, 1H), 6.09 (dd, J = 13.4, 6.8 Hz, 1H), 5.57 (d, J = 7.0 Hz, 1H), 5.19 (s, 1H, D_2O exchangeable), 4.98 (t, J = 5.2 Hz, 1H, D_2O exchangeable), 4.41 (dd, J = 12.8, 7.0 Hz, 1H), 3.97 (d, J = 7.8 Hz, 1H), 3.75 (dd, J = 8.4, 7.2 Hz, 2H), 3.67 (d, J = 7.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 167.3, 156.8, 143.2, 101.4 (d, J = 204.6 Hz), 95.4, 81.6 (d, J = 18.4 Hz), 78.5 (d, J = 19.0 Hz), 76.2, 61.3; Anal calc for $C_9H_{12}FN_3O_4$ (+1.0 MeOH): C, 43.34; H, 5.82; N, 15.16. Found: C, 43.27; H, 5.89; N, 15.23; MS m/z 246 $(M + H)^+$.

2.9 (*rel*)-(3*S*,4*S*,5*R*)-1-(7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) uracil (**16**α) and (*rel*)-(3*R*,4*S*,5*R*)-1-(7,7-dimethyl-4-fluoro-2,6,8,-trioxaspiro [4.4]nonan-3-yl) uracil (**16**β)

Uracil analogues were synthesized using the similar Vorbrüggen condensation conditions as described for the synthesis of cytosine analogues **13**α and **13**β. Data for **16**α: yield 36%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.22 (br s, 1H, D_2O exchangeable), 7.36 (d, J = 8.2 Hz, 1H), 6.09 (dd, J = 13.2, 7.0 Hz, 1H), 5.57 (d, J = 8.2 Hz, 1H), 4.58 (dd, J = 15.2, 6.2 Hz, 1H), 3.93 (dd, J = 8.8, 6.2 Hz, 2H), 3.73-3.68 (dd, J = 9.0, 8.2 Hz, 2H), 1.42 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 165.6, 153.5, 140.2, 110.8, 98.6 (d, J = 205.3 Hz), 99.1, 80.5 (d, J = 18.7 Hz), 78.6 (d, J = 18.8 Hz), 73.4, 64.7, 26.3, 25.7; Anal. Calc. for $C_{12}H_{15}FN_2O_5$: C, 50.35; H, 5.28; N, 9.79; Found: C, 50.23; H, 5.33; N, 9.86; MS m/z 287 $(M + H)^+$. Data for **16**β: yield 37%;

^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.17 (br s, 1H, D_2O exchangeable), 7.29 (d, *J* = 8.2 Hz, 1H), 6.11 (dd, *J* = 13.6, 7.2 Hz, 1H), 5.54 (d, *J* = 8.2 Hz, 1H), 4.51 (dd, *J* = 14.6, 6.4 Hz, 1H), 3.95 (d, *J* = 8.8 Hz, 1H), 3.83 (d, *J* = 7.6 Hz, H), 3.75 (d, *J* = 8.6 Hz, 1H), 3.67 (d, *J* = 7.6 Hz, 1H), 1.40 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 163.9, 151.6, 146.2, 109.8, 101.3, 99.7 (d, *J* = 208.6 Hz), 80.8 (d, *J* = 18.2 Hz), 79.4 (d, *J* = 18.6 Hz), 72.4, 62.7, 26.7, 26.4; Anal. Calc. for $C_{12}H_{15}FN_2O_5$: C, 50.35; H, 5.28; N, 9.79; Found: C, 50.43; H, 5.18; N, 9.69; MS *m/z* 287 $(M + H)^+$.

2.10. (*rel*)-(3*S*,4*S*,5*R*)-1-(7,7-Dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) thymine (**17**α) and (*rel*)-(3*R*,4*S*,5*R*)-1-(7,7-dimethyl-4-fluoro-2,6,8,-trioxaspiro[4.4]nonan-3-yl) thymine (**17**β)

Thymine analogues were synthesized using the similar Vorbrüggen condensation conditions as described for the synthesis of cytosine analogues **13**α and **13**β. Data for **17**α: yield 34%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.47 (br s, 1H), 7.16 (s, 1H), 6.04 (dd, *J* = 13.4, 7.2 Hz, 1H), 4.62 (dd, *J* = 14.6, 6.8 Hz, 1H), 3.95 (d, *J* = 8.8 Hz, 1H), 3.84 (d, *J* = 8.0 Hz, 1H), 3.77 (d, *J* = 8.7 Hz, 1H), 3.64 (d, *J* = 8.0 Hz, 1H), 1.56 (s, 3H), 1.40 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.1, 151.6, 142.2, 111.5, 109.5, 100.3 (d, *J* = 211.6 Hz), 81.3 (d, *J* = 18.2 Hz), 78.9 (d, *J* = 18.0 Hz), 72.7, 63.7, 26.3, 25.7, 14.9; Anal. Calc. for $C_{13}H_{17}FN_2O_5$: C, 52.00; H, 5.71; N, 9.33; Found: C, 52.17; H, 5.76; N, 9.46; MS *m/z* 301 $(M + H)^+$. Data for **17**β: yield 33%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.44 (br s, 1H), 7.21 (s, 1H), 6.08 (dd, *J* = 13.2, 7.4 Hz, 1H), 4.56 (dd, *J* = 14.0, 7.2 Hz, 1H), 3.91-3.85 (dd, *J* = 8.6, 7.0 Hz, 2H), 3.75 (d, *J* = 8.4 Hz, 1H), 3.66 (d, *J* = 8.2 Hz, 1H), 1.52 (s, 3H), 1.41 (s, 3H), 1.40 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 164.4, 151.8, 142.5, 110.8, 108.3, 99.6 (d, *J* = 207.8 Hz), 82.2 (d, *J* = 19.4 Hz), 79.4 (d, *J* = 18.6 Hz), 71.4, 61.3, 26.6, 24.2, 15.8; Anal. Calc. for $C_{13}H_{17}FN_2O_5$: C, 52.00; H, 5.71; N, 9.33; Found: C, 51.93; H, 5.64; N, 9.21; MS *m/z* 301 $(M + H)^+$.

2.11 (*rel*)-(1*R*,2*S*,3*R*)-1-[2-Fluoro-3-(hydroxymethyl)-3-hydroxyfuran-1-yl] uracil (**18**)

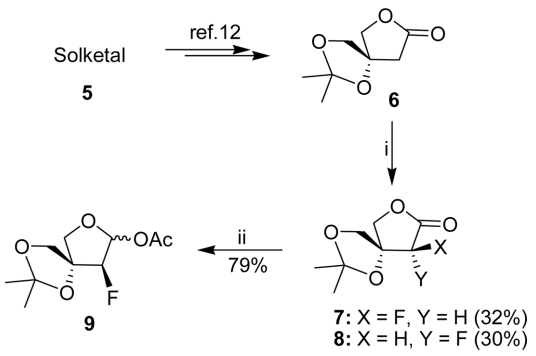
Removal of isopropylidene protection group of **16**β was performed by the similar hydrolytic conditions used for **15**. yield 65%; mp 184-186°C; UV (MeOH) λ_{max} 262.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.21 (br s, 1H, D_2O exchangeable), 7.57 (d, *J* = 8.0 Hz, 1H), 6.08 (dd, *J* = 13.8, 6.8 Hz, 1H), 5.56 (d, *J* = 8.0 Hz, 1H), 5.13 (s, 1H, D_2O exchangeable), 5.04 (t, *J* = 5.2 Hz, 1H, D_2O exchangeable), 4.42 (dd, *J* = 12.8, 6.2 Hz, 1H), 3.91 (d, *J* = 8.2 Hz, 1H), 3.84 (d, *J* = 7.8 Hz, 1H), 3.73-3.67 (m, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 164.3, 151.7, 146.2, 100.8 (d, *J* = 202.5 Hz), 99.3, 80.2 (d, *J* = 19.1 Hz), 77.6 (d, *J* = 18.2 Hz), 75.4, 61.5; Anal. Calc. for $C_9H_{11}FN_2O_5$ (+0.5 MeOH): C, 43.53; H, 4.99; N, 10.68. Found: C, 43.46; H, 4.83; N, 10.56; MS *m/z* 247 $(M + H)^+$.

2.12. (*rel*)-(1*R*,2*S*,3*R*)-1-[2-Fluoro-3-(hydroxymethyl)-3-hydroxyfuran-1-yl] thymine (**19**)

Deprotection of isopropylidene group of **17**β was performed by the similar conditions used for **15**. yield 67%; mp 189-191°C; UV (MeOH) λ_{max} 267.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.17 (br s, 1H, D_2O exchangeable), 7.28 (s, 1H), 6.12 (dd, *J* = 13.3, 7.4 Hz, 1H), 5.18 (s, 1H, D_2O exchangeable), 5.03 (t, *J* = 5.0 Hz, 1H, D_2O exchangeable), 4.47 (dd, *J* = 14.0, 8.2 Hz, 1H), 3.96 (d, *J* = 8.3 Hz, 1H), 3.84-3.78 (dd, *J* = 7.8, 6.8 Hz, 2H), 3.65 (d, *J* = 7.8 Hz, 1H), 1.62 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 164.6, 151.8, 142.7, 109.3, 99.3 (d, *J* = 198.8 Hz), 81.4 (d, *J* = 19.6 Hz), 78.4 (d, *J* = 18.4 Hz), 74.6, 62.2, 15.7; Anal. Calc. for $C_{10}H_{13}FN_2O_5$ (+1.0 MeOH): C, 45.22; H, 5.86; N, 9.59. Found: C, 45.35; H, 5.76; N, 9.62; MS *m/z* 261 $(M + H)^+$.

3. Results and Discussion

As depicted in Scheme 1, target compounds were racemically synthesized from spirolactone **6**, which was readily obtained from Solketal **5**, as previously described.[12] First, an attempt was made to fluorinate the lactone derivative **6** using a typical electrophilic fluorination[13] procedure (LiHMDS/NFSI). For fluorination, the order of addition of reagents is important. Lactone and *N*-fluorodibenzenesulfonimide (NFSI) were dissolved together in tetrahydrofuran (THF) and cooled to -78°C.[14] The slow addition of lithium hexamethyldisilazane (LiHMDS) produced compounds **7** and **8** at yields of 32% and 30%, respectively. NOE experiments of both products showed that fluorination in β-direction is isomer **7** (NOE: $H_{2\alpha}/H_{4\beta}$, 0.9%, $H_{2\alpha}/H_{4\alpha}$, 0.7%), and fluorination of α-direction is isomer **8**



Scheme 1. Synthesis of fluorinated threose glycosly donor **9**.

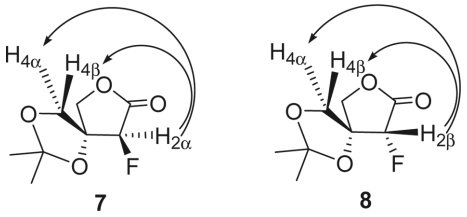


Fig. 2. NOE differences between the proximal diastereotropic hydrogens of **7** and **8**.

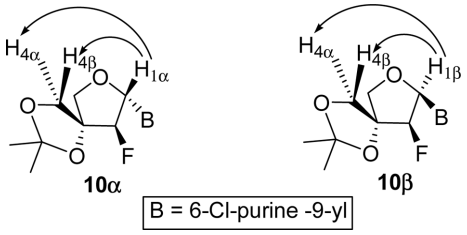
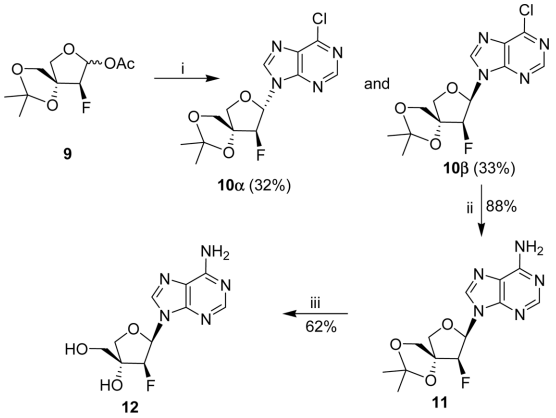
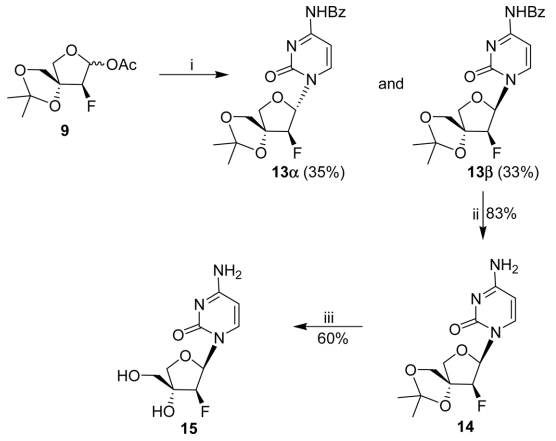


Fig. 3. NOE differences between the proximal diastereotropic hydrogens of **10α** and **10β**.



Scheme 2. Synthesis of fluorinated thresosyl adenine analogue.



Scheme 3. Synthesis of fluorinated threosyl cytosine analogue **15**.

(NOE: $H_{2\beta}/H_{4\beta}$, 1.6%, $H_{2\beta}/H_{4\alpha}$, 1.5%) (Figure 2). Diisobutylaluminium hydride (DIBAL-H) reduction of fluorospirolactone **7**, followed by acetylation using Ac_2O and triethylamine in CH_2Cl_2, yielded the key intermediate **9**. The synthesis of adenine nucleoside was performed using a Vorbrüggen condensation[15] of compound **9** with silylated 6-chloropurine and trimethylsilyltriflate (TMSOTf) as a catalyst in dichloroethane (DCE) to yield the protected 6-chloropurine derivatives, **10α** and **10β**. NOE experiments verified the unambiguous determinations of their relative stereochemistry (Figure 3).

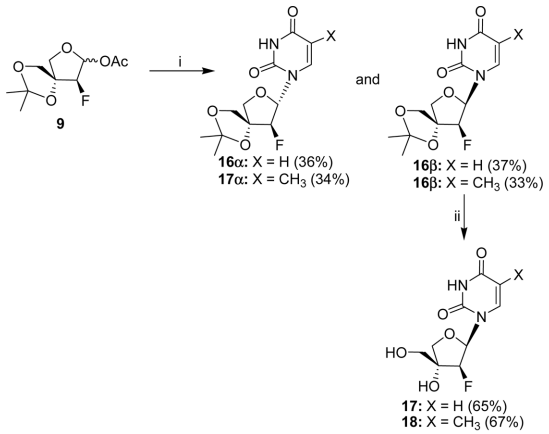
The chlorine group from purine analogue **10β** was then converted to an amine with methanolic ammonia at 100°C to produce the corresponding adenosine nucleoside derivative **11** at a yield of 88%. Hydrolysis of isoproplyidene protection groups of **12** with Dowex H^+ provided desired adenosine derivative **12** (Scheme 2).

Condensation of N_4-benzoyl cytosine with glycosyl donor **9** proceeded under conditions similar to those used for synthesis of analogues **10α** and **10β** to yield **13α** (35%) and **13β** (33%), respectively. NOE study

Table 1. Antiviral activity of the synthesized compounds

	HIV-1 EC_{50} (μg/ml)	HSV-1 EC_{50} (μg/ml)	HSV-2 EC_{50} (μg/ml)	HCMV EC_{50} (μg/ml)	Cytotoxicity CC_{50} (μg/ml)
12	>100	>100	>100	24.7	>100
15	>100	>100	>100	68.4	>100
18	>100	>100	>100	>100	>100
19	>100	>100	>100	>100	>100
AZT	0.009	ND	ND	ND	1.5
GCV	ND	ND	ND	1.4	>10
ACV	ND	0.15	ND	ND	>100

AZT: azidothymidine; GCV: Ganciclovir; ACV: Acyclovir
ND: Not Determined
EC_{50} (μg/ml): Concentration required to inhibit 50% of virus induced cytopathicity
CC_{50} (μg/ml): Concentration required to reduce cell viability by 50%



Scheme 4. Synthesis of fluorinated threosyl uracil and thymine analogues.

verified the relative stereochemistry of pyrimidine analogues **13α** and **13β**. Ammonolysis of **13β** followed by deprotection with Dowex H^+ furnished the target cytosine analogue **15** (Scheme 3). Also, uracil and thymine nucleoside analogues **18** and **19** were prepared from **9** *via* condensation and deprotection (Scheme 4).

Antiviral evaluation on the synthesized compounds was performed against several viruses such as the HIV-1 (MT-4 cells), HSV-1 (CCL81 cells), HSV-2 (CCL-81 cells), and HCMV (AD-169). Among compounds tested, adenine derivative **12** was found to be potent (EC_{50}=24.7 μg/mL) against HCMV without cytotoxicity up to 100 μg/mL when compared to positive control, Ganciclovir (EC_{50}=1.4 μg/mL, in AD-169) (Table 1). Since transposition of hydroxymethyl group from 4' to 3' position and 2'-fluorine group of threose nucleoside derivatives are not perfect mimics of the ribofuranose moiety, mechanisms of virus inhibition, including phosphorylation or the inhibition of DNA or RNA synthesis, might be impaired for these compounds.

4. Conclusion

Based on the potent antiviral activities of the 2¢-electropositive FMAU analogues and threose nucleoside analogues, we designed and successfully synthesized novel 2'(β)-fluoro-3'(α)-hydroxy-threose nucleoside analogues from Solketal. When the synthesized compounds were tested against several viruses such as the HIV-1, HSV-1, HSV-2 and HCMV, the adenine analogue **12** exhibit some antiviral activity against the HCMV. These results suggested that the 3',3'-double branched threose moiety can serve as a novel template for the development of new anti-HCMV agents.

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