J. Chosun Natural Sci.
Vol. 8, No. 3 (2015) pp. 153 – 163
http://dx.doi.org/10.13160/ricns.2015.8.3.153

Synthesis and Antiviral Activity Evaluation of 5′,5′-Difluoro-2′-methyl-apiosyl Nucleoside Phosphonic Acid Analogs

Joon Hee Hong†

Abstract

Racemic synthesis of novel 5′,5′-difluoro-2′-methyl-apiose nucleoside phosphonic acid analogs was achieved as potent antiviral agents. Phosphonation was performed by direct displacement of triflate intermediate with diethyl (lithiodifluoromethyl) phosphonate to give the corresponding (α,α-difluoroalkyl) phosphonate. Condensation successfully proceeded from a glycosyl donor with persilylated bases to yield the nucleoside phosphonate analogs. Deprotection of diethyl phosphonates provided the target nucleoside analogs. An antiviral evaluation of the synthesized compounds against various viruses such as HIV, HSV-1, HSV-2 and HCMV revealed that the pyrimidine analogs (cytosine, uracil, and thymine) have weak anti-HIV or HCMV activity.

Keywords: Antiviral Agents; 5′,5′-Difluoro-2′-methyl-apiose Nucleoside Phosphonic Acid Analogues; Vorbrüggen Reaction

1. Introduction

The modification of the nucleosides and/or sugar moiety of a natural nucleoside is an obvious choice for developing new antiviral compounds, and apiose-based nucleoside could serve this purpose.

Recently, apiose 5′-nor nucleoside phosphonate[1], such as, PMDTA (**1**), has been synthesized and has shown promising anti-HIV properties. The 4′-*C*-ethynyl substitution of natural nucleoside has a beneficial effect on anti-HIV activity[2]. Herdewijn *et al*. reported the synthetic procedure of 3′-*C*-ethynyl analog of PMTA (**2**)[3]. This absence of a 4′-hydroxymethyl group avoids problems of steric hindrance during phosphorylation reactions with kinases.

Phosphonates and structurally modified phosphonates isosters can mimic phosphates in biological system[4]. The resistance of the phosphorus-carbon phosphonate linkage to hydrolysis by chemical agents or esterases is one of the features responsible for their increasing popularity. Fluoro-substitution at the α-carbon of phosphonates may increase the effectiveness of these phosphate mimetics as a result both geometric and electronic factors[5]. The replacement of phosphonates by fluorophosphonates has provided a number of analogs showing significant biological activity[6].

9-(5,5-Difluoro-5-phosphonopentyl)guanine (**3**) has been utilized as a substrate analog inhibitor of purine nucleoside phosphorylase[7]. 2-Chloro-2′,5′-dideoxy-5′-difluoromethylphosphinyl adenosine (2CDPA, **4**), the nonhydrolyzable analog of 2-chlorodeoxyadenosine monophosphate was prepared for the treatment of refractory chronic leukemia and hairy cell leukemia to overcome the undesired metabolic pathway of 2CDA[8]. However, biological testing performed on various T cells showed that 2CDPA does not exhibit expected cytotoxic effect. The lack of cytotoxicity is probably caused by an insufficient level of phosphorylation inside T cells.

On the basis of the above encouraging results, we undertook the synthesis of isosteric and isopolar 5′,5′-difluoromethyl phosphonate derivatives of apiosyl nucleoside to find more effective antiviral agents.

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

BK-21 Project Team, College of Pharmacy, Chosun University, Kwangju 501-759, Korea

†Corresponding author : hongjh@chosun.ac.kr
(Received: July 10, 2015, Revised: September 17, 2015, Accepted: September 25, 2015)

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). Ultraviolet (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*)-(3S,4S)-Dihydro-4-(hydroxymethyl)-3-methylfuran-2(3*H*)-one (**7**)

To a solution of lactone **6** (2.51 g, 19.6 mmol) in 98 mL of EtOAc, 0.98 g of Pd/C (5% w/w) was added under H_2 atmosphere; the mixture was stirred for 10 h. After filtration of the reaction mixture through a celite pad, the filtrate was concentrated and purified using silica gel column chromatography (EtOAc/hexane, 1:4) to yield compound **7** (2.34 g, 92%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.44 (dd, J = 9.8, 6.2 Hz, 1H), 4.15 (dd, J = 9.8, 7.8 Hz, 1H), 3.61 (dd, J = 10.0, 6.4 Hz, 1H), 3.34 (dd, J = 10.0, 8.0 Hz, 1H), 2.49 (m, 1H), 2.18 (m, 1H), 1.25 (d, J = 4.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.1, 69.4, 67.5, 40.7, 36.3, 14.6; MS *m/z* 131 $(M + H)^+$.

2.2. (*rel*)-(3*S*,4*S*)-Dihydro-4-(t-butyldimethylsilyloxymethyl)-3-methylfuran-2(3*H*)-one (**8**)

t-Butyldimethylsilyl chloride (TBDMSCl) (1.60 g, 10.64 mmol) was added slowly at 0°C to a solution of **7** (1.26 g, 9.68 mmol) and imidazole (659 mg, 17.24 mmol) in CH_2Cl_2 (30 mL), and stirred for 8 h at rt. The solvent was evaporated under reduced pressure. The residue was diluted with H_2O (100 mL) and extracted twice with ethyl acetate (EtOAc) (100 mL×2). The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (EtOAc/hexane, 1:3) to yield compound **8** (2.15 g, 91%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.43 (dd, J = 10.4, 8.4 Hz, 1H), 4.15 (dd, J = 10.4, 6.6 Hz, 1H), 3.87 (dd, J = 10.8, 6.2 Hz, 1H), 3.63 (dd, J = 10.8, 8.0 Hz, 1H), 2.45 (m, 1H), 2.20 (m, 1H), 1.25 (d, J = 4.2 Hz, 3H), 0.87 (m, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 176.2, 69.7, 67.5, 40.3, 37.4, 25.3, 18.6, 14.2, -5.1.

2.3. (*rel*)-(4*R*,3*S*,2*R*/*S*)-Tetrahydro-4-(*t*-butyldimethylsilyloxymethyl)-3-methylfuran-2-ol (**9**)

A solution of compound **8** (1.68 g, 6.88 mmol) in toluene (60 mL) was treated with 13.76 mL of 1 M DIBAL-H in hexane at -78°C for 1 h. The reaction was quenched with 4 mL of methanol and warmed to room temperature for 1 h before aqueous $NaHCO_3$ (6 mL) and EtOAc (60 mL) were added to the mixture. The resulting mixture was filtered and the filtrate was concentrated to dryness. The residue was purified using silica gel column chromatography (EtOAc/hexane, 1:10) to yield compound **9** (1.44 g, 85%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.48, 5.39 (d and d, J = 6.8 and 7.2 Hz, 1H), 3.86-3.82 (m, 2H), 3.65-3.59 (m, 2H), 2.11-1.96 (m, 2H), 0.88 (m, 9H), 0.02 (m, 6H); Anal. Calcd. for $C_{13}H_{28}O_3Si$: C, 59.95; H, 10.84; found: C, 60.10; H, 10.86.

2.4. (*rel*)-(3*R*,4*S*,5*R*/*S*)-[(4-Methyl-tetrahydro-5-methoxyfuran-3-yl)methoxy](*t*-butyl)dimethylsilane (**10**)

Lactol **9** (1.59 g, 6.48 mmol) was dissolved in anhydrous diethyl ether (20 mL), and powdered anhydrous molecular sieves (4 Å, 0.16 g) were added. With stirring, trimethyl orthoformate (1.42 mL, 12.96 mmol) and $BF_3{\cdot}OEt_2$ (194 μL) were added, and stirred for 50 min. The reaction mixture was quenched with Et_3N and brine until neutral. The mixture was extracted with diethyl ether, dried over anhydrous $MgSO_4$, and concentrated to give a residue. The residue was purified by using silica gel column chromatography (EtOAc/hexane, 1:15) to yield compound **10** (1.39 g, 83%) as diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.06, 4.97 (d and d, J = 7.2 and 6.6 Hz, 1H), 3.90-3.81 (m, 2H), 3.64-3.57 (m, 2H), 3.31 (d, J = 5.2 Hz, 3H), 2.32-2.28 (m, 1H), 1.99-1.94 (m, 1H), 1.09 (d, J = 6.0 Hz, 3H), 0.87 (m, 9H), 0.01 (m, 6H); Anal. Calcd. for $C_{13}H_{28}O_3Si$: C, 59.95; H, 10.84. Found: C, 59.84; H, 10.77; MS *m/z* 261 $(M + H)^+$.

2.5. (*rel*)-(3*S*,4*S*,5*R*/*S*)-(4-Methyl-tetrahydro-5-methoxyfuran-3-yl)methanol (**11**)

To a solution of compound **10** (2.17 g, 8.35 mmol) in THF (20 mL), TBAF (12.52 mL, 1.0 M solution in THF) at 0°C was added. The mixture was stirred for 5 h at rt, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:4) to give compound **11** (1.07 g, 88%) as diastereomeric mixture. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.03 (d, J = 6.8 Hz, 0.5H), 4.96 (d, J = 6.2 Hz, 0.5H), 3.85-3.80 (m, 1H), 3.65-3.49 (m, 2H), 3.40-3.35 (m, 1H), 3.26, 3. 25 (s, s, 3H), 2.35-2.29 (m, 1H), 1.94 (m, 1H); Anal. Calcd. for $C_7H_{14}O_3$: C, 57.51; H, 9.65. Found: C, 57.42; H, 9.55; MS m/z 147 $(M + H)^+$.

2.6. (*rel*)-(3*R*,4*S*,5*R*/*S*)-(4-Methyl-tetrahydro-5-methoxyfuran-3-yl)methyl trifluoromethanesulfonate (**12**)

To a cooled solution (-78°C) of glycoside **11** (315 mg, 2.16 mmol) in pyridine (0.873 mL, 10.8 mmol) and CH_2Cl_2 (25 mL), triflic anhydride (730 mg, 2.59 mmol) was slowly added. After 3.5 h, the reaction mixture was poured into a mixture of ice and sodium hydrogen carbonate. The aqueous layer was extracted with CH_2Cl_2 (3×50 mL), and the combined CH_2Cl_2 solution were dried, and rapidly and repeatedly concentrated with toluene to remove any residual pyridine. The residue was extracted with light petroleum (3×50 mL), and the combined extracted were filtered and cooled. After careful evaporation of additional solvent, the crude residue **12** (594 mg, ~99%) was subjected to next reaction without further purification.

2.7. (*rel*)-Diethyl 1,1-difluoro-2-[(3*S*,4*S*,5*R*/*S*)-4-methyl-tetrahydro-5-methoxyfuran-3-yl] ethylphosphonate (**13**)

To a solution of diisopropylamine (521 μL, 3.72 mmol) and HMPA (647 μL, 3.72 mmol) at -78°C in THF (10 mL) under Ar was added *n*-butyllithium (2.32 mL of a 1.6 M solution in hexane, 3.72 mmol). The resulting solution was allowed to stir for 30 min at 0°C and then cooled to -78°C. To this solution of LDA at -78°C were added *via* cannula, a (-78°C) solution of diethyl (α,α-difluoromethyl) phosphonate (699 mg, 3.72 mmol) in THF (2.8 mL), and, 3 min later, a (-78°C) solution of triflate **12** (517 mg, 1.86 mmol) in THF (4.0 mL), dropwise, *via* cannula. After 10 min at -78°C, the reaction was quenched by adding aqueous NH_4Cl (18.6 mL) and Et_2O (18.6 mL). The aqueous layer was further extracted with EtOAc (2×74 mL), and the combined organic extracts were dried, filtered, and evaporated. Silica gel flash chromatography (EtOAc/hexane, 1:2) gave **13** (352 mg, 60%) as a form. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.06 (d, J = 7.0 Hz, 0.5H), 5.01 (d, J = 6.4 Hz, 0.5H), 4.29-4.24 (m, 4H), 3.92 (m, 1H), 3.65 (m, 1H), 3.23, 3.21 (s, s, 3H), 2.35-2.29 (m, 1H), 2.04-1.94 (m, 3H), 1.13-1.07 (m, 9H); Anal. Calcd. for $C_{12}H_{23}F_2O_5P$: C, 45.57; H, 7.33. Found: C, 45.65; H, 7.39; MS m/z 317 $(M + H)^+$.

2.8. (*rel*)-Diethyl 2-[(3*S*,4*S*,5*R*/*S*)-5-acetoxy-4-methyl-tetrahydrofuran-3-yl]-1,1-difluoroethylphosphonate (**14**)

Glycoside **13** (493 mg, 1.56 mmol)was dissolved in EtOAc (13 mL), mixed with a solution of EtOAc (26 mL), acetic anhydride (14.3 mL), acetic acid (10.8 mL) and conc H_2SO_4 (0.065 mL) at -15°C, and stirred for 20 h at 0°C. The reaction was diluted with $CHCl_3$ (97 mL) and poured into cold 5% aqueous $NaHCO_3$ (130 mL). The organic layer was separated and the aqueous layer extracted with $CHCl_3$ (3×35 mL). The combined organic layers were washed with brine, dried and evaporated to dryness. The residue was purified using silica gel column chromatography (EtOAc/hexane, 1:3) to yield compound **14** (381 mg, 71%) as a form. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.24 (d, J = 7.4 Hz, 0.5H), 6.17 (d, J = 8.0 Hz, 0.5H), 4.29-4.24 (m, 4H), 3.83-3.80 (m, 1H), 3.62-3.58 (m, 1H), 2.65-2.62 (m, 1H), 2.08-1.83 (m, 3H), 2.03, 2.01 (s, s, 3H), 1.22-1.18 (m, 6H); Anal. Calcd. for $C_{13}H_{23}F_2O_6P$: C, 45.35; H, 6.73. Found: C, 45.48; H, 6.86; MS m/z 345 $(M + H)^+$.

2.9. (*rel*)-Diethyl 4-[(1*S*,2*S*,3*S*)-1-(6-chloro-9*H*-purin-9-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**15**α) and (*rel*)-diethyl 4-[(1*R*,2*S*,3*S*)-1-(6-chloro-9*H*-purin-9-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**15**β)

6-Chloropurine (280 mg, 1.81 mmol), anhydrous HMDS (15 mL), and a catalytic amount of ammonium sulfate (15 mg) were refluxed for 16 h 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 **14** (309 mg, 0.90 mmol) in dry DCE

(12 mL) and TMSOTf (0.327 mL, 1.81 mmol) was added, and stirred for 8 h at rt. The reaction mixture was quenched with 12.0 mL of saturated $NaHCO_3$, stirred for 2 h, filtered through a Celite pad, and the filtrate obtained was then extracted twice with CH_2Cl_2 (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, 3:1:0.02) to yield compounds **15α** (134 mg, 34%) and **15β** (130 mg, 33%), respectively. Data for **15α**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71 (s, 1H), 8.18 (s, 1H), 5.98 (d, J = 6.8 Hz, 1H), 4.25-4.21 (m, 4H), 3.88 (dd, J = 10.2, 6.4 Hz, 1H), 3.60 (dd, J = 10.2, 7.6Hz, 1H), 2.06 (m, 1H), 1.86 (m, 1H), 1.73-1.61 (m, 2H), 1.09-1.03 (m, 9H); ^{31}P (121.5 MHz, $CDCl_3$) δ 7.66 (t, $J_{P,F}$ = 107.2 Hz); Anal. Calc. for $C_{16}H_{22}ClF_2N_4O_4P$: C, 43.80; H, 5.05; N, 12.77. Found: C, 43.92; H, 5.16; N, 12.83; MS *m/z* 439 $(M + H)^+$; Data for **15β**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.75 (s, 1H), 8.21 (s, 1H), 6.01 (d, J = 7.2 Hz, 1H), 4.28-4.25 (m, 4H), 3.85 (dd, J = 9.8, 7.4 Hz, 1H), 3.62 (dd, J = 9.8, 7.6Hz, 1H), 2.09 (m, 1H), 1.84 (m, 1H), 1.70-1.55 (m, 2H), 1.10-1.05 (m, 9H); ^{31}P (121.5 MHz, $CDCl_3$) δ 7.58 (t, $J_{P,F}$ = 106.4 Hz); Anal. Calc. for $C_{16}H_{22}ClF_2N_4O_4P$ (+1.0MeOH): C, 43.43; H, 5.57; N, 11.91. Found: C, 43.51; H, 4.54; N, 11.85; MS *m/z* 439 $(M + H)^+$.

2.10. (*rel*)-Diethyl 4-[(1*R*,2*S*,3*S*)-1-(6-amino-9*H*-purin-9-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethyl phosphonate (**16**)

A solution of **15β** (280 mg, 0.64 mmol) in saturated methanolic ammonia (10 mL) was stirred overnight at 66°C in a steel bomb and the volatiles were evaporated. The residue was purified using silica gel column chromatography (MeOH/CH_2Cl_2, 1:14) to yield **16** (163 mg, 61%) as a white solid: UV (MeOH) λ_{max} 260.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.39 (s, 1H), 8.11 (s, 1H), 7.44 (br s, 2H, D_2O exchangeable), 5.97 (d, J = 7.4 Hz, 1H), 4.28-4.25 (m, 4H), 3.82 (dd, J = 10.0, 7.2 Hz, 1H), 3.62 (dd, J = 10.0, 6.8 Hz, 1H), 2.04 (m, 1H), 1.84 (m, 1H), 1.66-1.50 (m, 2H), 1.15 (m, 6H), 1.01 (d, J = 5.8 Hz, 3H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.66 (dd, $J_{P,F}$ = 104.2, 98.8 Hz); Anal. Calc. for $C_{16}H_{24}F_2N_5O_4P$ (+0.5 MeOH): C, 45.54; H, 6.02; N, 16.09; Found: C, 45.63; H, 5.97; N, 16.15; MS *m/z* 420 $(M + H)^+$.

2.11. (*rel*)-4-[(1*R*,2*S*,3*S*)-1-(6-Amino-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-2-methyl-5,5-difluoroethyl-phosphonic acid sodium salt (**17**)

To a solution of compound **16** (159 mg, 0.38 mmol) and 2,6-lutidine (2.65 mL, 22.8 mmol) in 25 mL of dry CH_3CN was added bromotrimethylsilane (1.16 g, 7.6 mmol) at room temperature under nitrogen. The reaction mixture was continuously refluxed for 22 h. The reaction mixture was concentrated under high vacuum at room temperature, and the residue was coevaporated with MeOH and 0.5 M TEAB solution. Purification by HPLC using reverse phase C_{18} and ion exchange with Dowex-Na^+ resin offered **17** (64 mg, 44%) as a colourless solid (sodium salt) after lyophilization. ^{1}H NMR (D_2O, 300 MHz) δ 8.35 (s, 1H), 8.12 (s, 1H), 5.97 (d, J = 7.6 Hz, 1H), 3.82 (dd, J = 10.2, 7.2 Hz, 1H), 3.58 (dd, J = 10.2, 6.6 Hz, 1H), 2.11 (m, 1H), 1.84 (m, 1H), 1.72-1.58 (m, 2H), 1.09 (d, J = 6.0 Hz, 3H); ^{13}C NMR (D_2O, 75 MHz) δ 156.2, 153.3, 149.1, 141.5, 125.7 (dt, J = 224.6, 268.4 Hz), 119.3, 89.5, 71.4, 35.2, 30.7, 22.5 (dd, J = 26.2, 20.4 Hz), 9.6; ^{31}P (121.5 MHz, D_2O) δ 5.92 (dd, $J_{P,F}$ = 103.6, 88.4 Hz); HPLC t_R = 10.67; HRMS $[M-H]^+$ req. 362.0656, found 362.0654.

2.12. (*rel*)-Diethyl 4-[(1*S*,2*S*,3*S*)-1-(2-fluoro-6-chloro-9*H*-purin-9-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**18α**) and (*rel*)-diethyl 4-[(1*R*,2*S*,3*S*)-1-(2-methyl-6-chloro-9*H*-purin-9-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**18β**)

Condensation of **14** with 2-fluoro-6-chloropurine under Vorbrüggen condensation conditions similar to those described for **15α** and **15β** yielded **18α** and **18β**, respectively. Data for **18α**: yield 36%; UV (MeOH) λ_{max} 268.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.31 (s, 1H), 6.02 (d, J = 7.4 Hz, 1H), 4.31-4.28 (m, 4H), 3.82 (dd, J = 9.8, 6.8 Hz, 1H), 3.60 (d, J = 9.9, 7.8 Hz, 1H), 2.09 (m, 1H), 1.84-1.80 (m, 1H), 1.64-1.53 (m, 2H), 1.12-1.08 (m, 9H); ^{31}P (121.5 MHz, $CDCl_3$) δ 7.57 (t, $J_{P,F}$ = 111.0 Hz); Anal. Calc. for $C_{16}H_{21}ClF_3N_4O_4P$ (+1.0MeOH): C, 41.83; H, 5.16; N, 11.47; Found: C, 42.98; H, 5.24; N, 11.49; MS *m/z* 457 $(M + H)^+$; data for **18β**: yield 36%; UV (MeOH) λ_{max} 267.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.28 (s, 1H), 5.97 (d, J = 6.8 Hz, 1H), 4.30-4.26 (m, 4H), 3.85 (dd, J = 10.4, 7.2 Hz, 1H), 3.66 (d, J = 10.4, 8.2 Hz, 1H), 2.03 (m, 1H), 1.81-1.77 (m, 1H), 1.65-1.56 (m, 2H), 1.10-1.06 (m,

9H); ^{31}P (121.5 MHz, $CDCl_3$) δ 7.58 (t, $J_{P,F}$ = 109.2 Hz); Anal. Calc. for $C_{16}H_{21}ClF_3N_4O_4P$: C, 42.07; H, 4.63; N, 12.27; Found: C, 41.97; H, 4.56; N, 12.35; MS *m/z* 457 $(M + H)^+$.

2.13. (*rel*)-Diethyl 4-[(1*R*,2*S*,3*S*)-1-(2-fluoro-6-amino-9*H*-purin-9-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**19**) and (*rel*)-diethyl 4-[(1*R*,2*S*,3*S*)-1-(2-amino-6-chloro-9*H*-purin-9-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**20**)

Dry ammonia gas was bubbled into a stirred solution of **18β** (522 mg, 1.14 mmol) in DME (15.0 mL) overnight at rt. Salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue obtained was purified using silica gel column chromatography (MeOH/CH_2Cl_2, 1:10) to produce **19** (49 mg, 10%) and **20** (216 mg, 42%). Data for **19**; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.34 (s, 1H), 7.72 (br s, NH_2, 2H, D_2O exchangeable), 5.93 (d, *J* = 7.2 Hz, 1H), 4.31-4.28 (m, 4H), 3.85 (dd, *J* = 10.2, 7.4 Hz, 1H), 3.61 (dd, *J* = 10.2, 6.8 Hz, 1H), 2.07 (m, 1H), 1.82 (m, 1H), 1.67-1.58 (m, 2H), 1.21 (m, 6H), 1.03 (d, *J* = 6.0 Hz, 3H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.21(t, $J_{P,F}$ = 105.8 Hz); Anal. Calc. for $C_{16}H_{23}F_3N_5O_4P$ (+0.5MeOH): C, 43.73; H, 5.56; N, 15.45; Found: C, 43.87; H, 5.53; N, 15.36; MS *m/z* 438 $(M + H)^+$. Data for **20**; UV (MeOH) λ_{max} 308.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.16 (s, 1H), 7.69 (br s, NH_2, 2H, D_2O exchangeable), 5.97 (d, *J* =7.6 Hz, 1H), 4.30-4.27 (m, 4H), 3.80 (dd, *J* = 10.1, 7.8 Hz, 1H), 3.65 (dd, *J* = 10.0, 7.0 Hz, 1H), 2.03 (m, 1H), 1.81 (m, 1H), 1.62-1.54 (m, 2H), 1.21-1.18 (m, 6H), 1.09 (d, *J* = 6.0 Hz, 3H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.19 (t, $J_{P,F}$ = 106.6 Hz); Anal. Calc. for $C_{16}H_{23}ClF_2N_5O_4P$ (+1.0MeOH): C, 42.08; H, 5.61; N, 14.43; Found: C, 42.15; H, 5.52; N, 14.59; MS *m/z* 454 $(M + H)^+$.

2.14. (*rel*)-4-[(1*R*,2*S*,3*S*)-1-(2-Amino-6-oxo-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-2-methyl-5,5-difluoroethyl-phosphonic acid sodium salt (**21**)

To a solution of **20** (308 mg, 0.68 mmol) and 2,6-lutidine (4.75 mL, 40.8 mmol) in dry CH_3CN (27.2 mL), trimethylsilyl bromide (2.08 g, 13.6 mmol) was added at rt. The mixture was stirred for 30 h and the solvent was removed using evaporation with MeOH three times. The residue was dissolved in MeOH (27.2 mL) and 2-mercaptoethanol (0.19 mL, 2.72 mmol), and then NaOMe (147 mg, 2.72 mmol) was added. The mixture was refluxed for 16 h under N_2, cooled, neutralized with glacial AcOH, and evaporated to dryness under vacuum. The residue obtained was evaporated with methanol. Purification by HPLC using reverse phase C_{18} and ion exchange with Dowex-Na^+ resin yielded **21** (128 mg, 47%) as a colourless solid (sodium salt) after lyophilization. ^{1}H NMR (D_2O, 300 MHz) δ 7.87 (s, 1H), 5.92 (d, *J* = 7.2 Hz, 1H), 3.80 (dd, *J* = 9.8, 7.4 Hz, 1H), 3.58 (d, *J* = 9.8, 7.8 Hz, 1H), 2.06-2.00 (m, 1H), 1.80-1.67 (m, 3H), 1.11 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (D_2O, 75 MHz) δ 157.5, 154.3, 152.1, 136.2, 126.2 (δ, *J* = 216.2, 266.8, Hz), 117.4, 82.4, 71.6, 36.2, 30.5, 24.7 (dd, *J* = 21.8, 26.6 Hz), 10.2; ^{31}P (121.5 MHz, D_2O) δ 5.74 (t, $J_{P,F}$ = 101.2 Hz); HPLC t_R = 9.86 min; HRMS $[M-H]^+$ req. 378.0573, found 378.0571.

2.15. (*rel*)-Diethyl 4-[(1*S*,2*S*,3*S*)-1-(N_4-benzoylamino-2-oxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**22α**) and (*rel*)-diethyl 4-[(1*R*,2*S*,3*S*)-1-(N_4-benzoylamino-2-oxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**22β**)

Condensation of **14** with N_4-benzoyl cytosine under Vorbrüggen condensation conditions similar to those described for **15α** and **15β** yielded **22α** and **22β** as solids. Data for **22α**: yield 31%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.20 (d, *J* = 6.8 Hz, 1H), 8.01-7.97 (m, 2H), 7.64-7.53 (m, 4H), 5.91 (d, *J* = 7.2 Hz, 1H), 4.30-4.27 (m, 4H), 3.86 (dd, *J* = 10.2, 7.2 Hz, 1H), 3.56 (dd, *J* = 10.2, 6.4 Hz, 1H), 2.57 (m, 1H), 1.85 (m, 1H), 1.78-1.65 (m, 2H), 1.18 (m, 6H), 1.05 (d, *J* = 6.0 Hz, 3H); ^{31}P (121.5 MHz, $CDCl_3$) δ 7.39 (t, $J_{P,F}$ = 108.2 Hz); Anal. Calc. for $C_{22}H_{28}F_2N_3O_6P$ (+0.5MeOH): C, 52.45; H, 5.87; N, 8.15; Found: C, 52.60; H, 5.77; N, 8.28; MS *m/z* 500 $(M + H)^+$. data for **22β**: yield 32%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.17 (d, *J* = 6.9 Hz, 1H), 8.00-7.95 (m, 2H), 7.62-7.51 (m, 4H), 5.89 (d, *J* = 7.0 Hz, 1H), 4.28-4.25 (m, 4H), 3.87 (dd, *J* = 10.0, 7.8 Hz, 1H), 3.52 (dd, *J* = 10.0, 6.0 Hz, 1H), 2.49-2.44 (m, 1H), 1.81 (m, 1H), 1.72-1.60 (m, 2H), 1.21 (m, 6H), 1.01 (d, *J* = 5.8 Hz, 3H); ^{31}P (121.5 MHz, $CDCl_3$) δ 7.45 (t, $J_{P,F}$ = 110.0 Hz); Anal. Calc. for $C_{22}H_{28}F_2N_3O_6P$ (+1.0MeOH): C, 52.00; H, 6.07; N, 7.91; Found: C, 51.98; H, 6.15; N, 7.87; MS *m/z* 500 $(M + H)^+$.

2.16. (*rel*)-Diethyl 4-[(1*R*,2*S*,3*S*)-1-(4-amino-2-oxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**23**)

Compound **22β** (509 mg, 1.02 mmol) was treated with saturated methanolic ammonia (17 mL) overnight at rt. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$/1:10) to give compound **23** (322 mg, 80%): UV (MeOH) λ_{max} 272.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.82 (d, J = 7.0 Hz, 1H), 7.21 (br d, 2H, D_2O exchangeable), 5.90 (d, J = 7.4 Hz, 1H), 5.73 (d, J = 7.0 Hz, 1H), 4.29-4.25 (m, 4.5H), 3.82 (dd, J = 10.1, 7.2 Hz, 1H), 3.58 (dd, J = 10.2, 8.2 Hz, 1H), 2.61-2.57 (m, 1H), 1.90-1.87 (m, 1H), 1.75-1.60 (m, 2H), 1.23 (m, 6H), 1.02 (d, J = 6.4 Hz, 3H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.21 (t, $J_{P,F}$ = 112.2 Hz); Anal. Calc. for $C_{15}H_{24}F_2N_3O_5P$ (+1.0MeOH): C, 44.98; H, 6.60; N, 9.83; Found: C, 44.89; H, 6.57; N, 9.76; MS *m/z* 396 $(M + H)^+$.

2.17. (*rel*)-4-[(1*R*,2*S*,3*S*)-1-(4-Amino-2-oxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3-yl]-2-methyl-5,5-difluoroethyl-phosphonic acid sodium salt (**24**)

Final cytosine analogue **24** was synthesized from **23** by the similar deprotection procedure as described for **17**: Yield 51%; UV (H_2O) λ_{max} 271.5 nm; ^{1}H NMR (D_2O, 300 MHz) δ 7.51 (d, J = 7.0 Hz, 1H), 5.91 (d, J = 7.8 Hz, 1H), 5.53 (d, J = 7.0 Hz, 1H), 3.84 (dd, J = 9.8, 6.8 Hz, 1H), 3.56 (dd, J = 9.8, 7.6 Hz, 1H), 2.54-2.50 (m, 1H), 1.88-1.84 (m, 1H), 1.68-1.54 (m, 2H); ^{13}C NMR (D_2O, 75 MHz) δ 165.6, 155.8, 141.6, 126.1 (dt, J = 222.7, 267.4 Hz), 98.7, 71.2, 35.5, 31.6, 22.4 (dd, J = 21.2, 26.5 Hz), 9.8; ^{31}P (121.5 MHz, D_2O) δ 5.87 (dd, $J_{P,F}$ = 109.4, 94.6 Hz); HPLC t_R = 9.78 min; HRMS $[M-H]^+$ req. 338.0758, found 338.0759.

2.18. (*rel*)-Diethyl 4-[(1*S*,2*S*,3*S*)-1-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**25**α) and (*rel*)-diethyl 4-[(1*R*,2*S*,3*S*)-1-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**25**β)

Uracil analogues were synthesized using the similar Vorbrüggen condensation conditions as described for the synthesis of 6-chloropurine analogues **15**α and **15**β. Data for **25**α: yield 33%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.16 (br s, 1H, D_2O exchangeable), 7.75 (d, J = 7.8 Hz, 1H), 5.87 (d, J = 7.2 Hz, 1H), 5.62 (d, J = 7.8 Hz, 1H), 4.28-4.25 (m, 4H), 3.81 (dd, J = 10.0, 7.8 Hz, 1H), 3.57 (dd, J = 10.1, 6.8 Hz, 1H), 2.54-2.49 (m, 1H), 1.90-1.86 (m, 1H), 1.68-1.54 (m, 2H), 1.26 (m, 6H), 1.11 (d, J = 6.2 Hz, 3H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.15 (t, $J_{P,F}$ = 107.6 Hz); Anal. Calc. for $C_{15}H_{23}F_2N_2O_6P$ (+0.5MeOH): C, 45.17; H, 6.11; N, 6.79; Found: C, 45.22; H, 6.18; N, 6.83; MS *m/z* 397 $(M + H)^+$. Data for **25**β: yield 34%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.21 (br s, 1H, D_2O exchangeable), 7.78 (d, J = 7.8 Hz, 1H), 5.84 (d, J = 7.0 Hz, 1H), 5.62 (d, J = 7.8 Hz, 1H), 4.31-4.27 (m, 4H), 3.79 (dd, J = 10.2, 7.6 Hz, 1H), 3.57 (dd, J = 10.2, 6.6 Hz, 1H), 2.61 (m, 1H), 1.81 (m, 1H), 1.72-1.60 (m, 2H), 1.27-25 (m, 6H), 1.04 (d, J = 6.0 Hz, 3H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.20 (t, $J_{P,F}$ = 108.8 Hz); Anal. Calc. for $C_{15}H_{23}F_2N_2O_6P$ (+1.0MeOH): C, 44.88; H, 6.35; N, 6.54; Found: C, 44.75; H, 6.22; N, 6.47; MS *m/z* 397 $(M + H)^+$.

2.19. (*rel*)-Diethyl 4-[(1*S*,2*S*,3*S*)-1-(2,4-dioxo-5-methyl-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**26**α) and (*rel*)-diethyl 4-[(1*R*,2*S*,3*S*)-1-(2,4-dioxo-5-methyl-3,4-dihydropyrimidin-1(2*H*)-yl)-2-methyl-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (**26**β)

Thymine analogs were synthesized using the similar Vorbrüggen condensation conditions as described for the synthesis of 6-chloropurine analogues **15**α and **15**β. Data for **26**α: yield 35%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.25 (br s, 1H, D_2O exchangeable), 7.70 (s, 1H), 5.88 (d, J = 7.8 Hz, 1H), 4.27-4.23 (m, 4H), 3.83 (dd, J = 10.2, 7.8 Hz, 1H), 3.60 (dd, J = 10.2, 6.6 Hz, 1H), 2.56-2.53 (m, 1H), 1.82-1.76 (s, 4H), 1.64-1.53 (m, 2H), 1.29 (m, 6H), 1.02 (d, J = 6.2 Hz, 3H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.28 (t, $J_{P,F}$ = 109.2 Hz); Anal. Calc. for $C_{16}H_{25}F_2N_2O_6P$ (+1.0MeOH): C, 46.17; H, 6.61; N, 6.66; Found: C, 46.26; H, 6.68; N, 6.70; MS *m/z* 411 $(M + H)^+$. Data for **27**β: yield 34%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.19 (br s, 1H, D_2O exchangeable), 7.73 (s, 1H), 5.85 (d, J = 7.6 Hz, 1H), 4.29-4.25 (m, 4H), 3.78 (dd, J = 10.0, 7.6 Hz, 1H), 3.58 (dd, J = 10.1, 6.2 Hz, 1H), 2.61 (m, 1H), 1.80-1.72 (s, 4H), 1.61-1.50 (m, 2H), 1.23 (m, 6H), 1.07 (d, J = 6.4 Hz, 3H); ^{31}P (121.5 MHz, DMSO-d_6) δ 7.36 (t, $J_{P,F}$ = 108.8 Hz);

Anal. Calc. for $C_{16}H_{25}F_2N_2O_6P$ (+1.0MeOH): C, 46.17; H, 6.61; N, 6.66; Found: C, 46.08; H, 6.72; N, 6.53; MS *m/z* 411 $(M + H)^+$.

2.20. (*rel*)-4-[(1*R*,2*S*,3*S*)-1-(2,4-Dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3-yl]-2-methyl-5,5-difluoroethyl-phosphonic acid sodium salt (**27**)

Uracil phosphonic acid analog **27** was synthesized from **25β** using the similar hydrolysis conditions as described for **18**: Yield 45%; UV (H_2O) λ_{max} 260.5 nm; ^{1}H NMR (D_2O, 300 MHz) δ 7.76 (d, *J* = 7.2 Hz, 1H), 5.92 (d, *J* = 7.2 Hz, 1H), 5.73 (d, *J* = 7.2 Hz, 1H), 3.83 (dd, *J* = 10.2, 7.4 Hz, 1H), 3.58 (dd, *J* = 10.2, 6.6 Hz, 1H), 2.58-2.48 (m, 1H), 1.86 (m, 1H), 1.59-1.48 (m, 2H), ; ^{13}C NMR (D_2O, 75 MHz) δ 166.7, 152.1, 142.5, 124.4 (dt, *J* = 212.2, 264.8 Hz), 101.3, 72.4, 35.3, 29.7, 23.2 (dd, *J* = 20.8, 26.6 Hz), 10.9; ^{31}P (121.5 MHz, D_2O) δ 5.68 (dd, $J_{P,F}$ = 106.4, 91.2 Hz); HPLC t_R = 10.54 min; HRMS $[M-H]^+$ req. 339.0645, found 339.0643.

2.21. (*rel*)-4-[(1*R*,2*S*,3*S*)-1-(2,4-Dioxo-5-methyl-3,4-dihydropyrimidin-1(2*H*)-yl)-tetrahydrofuran-3-yl]-2-methyl-5,5-difluoroethyl-phosphonic acid sodium salt (**28**)

Thymine analog **28** was synthesized from **26β** using the similar hydrolysis conditions as described for **18**: Yield 43%; UV (H_2O) λ_{max} 267.0 nm; ^{1}H NMR (D_2O, 300 MHz) δ 7.71 (s, 1H), 5.90 (d, *J* = 7.6 Hz, 1H), 3.80 (dd, *J* = 9.9, 7.6 Hz, 1H), 3.57 (dd, *J* = 9.8, 6.2 Hz), 2.53 (m, 1H), 1.80-1.71 (m, 4H), 1.65-1.53 (m, 2H), 1.08; ^{13}C NMR (D_2O, 75 MHz) δ 163.8, 150.6, 136.2, 125.1 (dt, *J* = 208.4, 265.6 Hz), 109.6, 99.6, 71.4, 35.5, 30.3, 22.7 (dd, *J* = 20.4, 26.2 Hz), 12.5, 9.9; ^{31}P (121.5 MHz, D_2O) δ 5.78 (t, $J_{P,F}$ = 107.4 Hz); HPLC t_R = 10.62 min; HRMS $[M-H]^+$ req. 353.0567, found 353.0565.

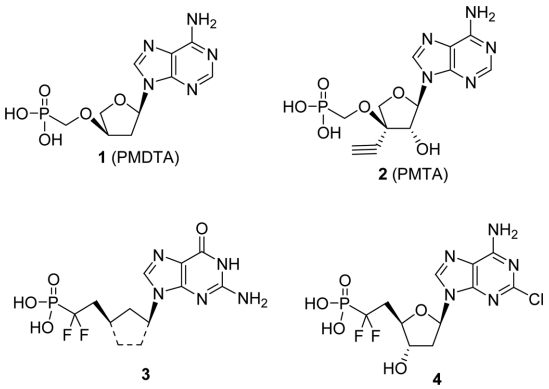
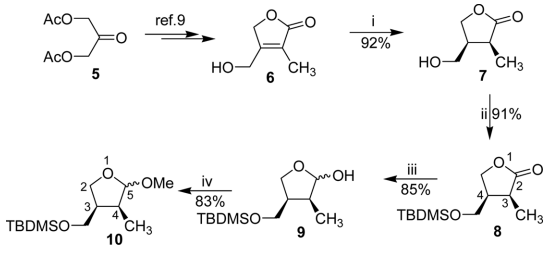


Fig. 1. Synthesis rationale of 5',5'-difluoro and apiose nucleoside phosphonic acids showing potent biological activity.

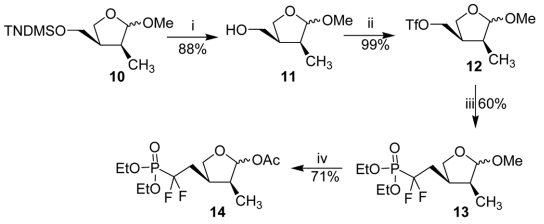
3. Results and Discussion

Target compounds were synthesized from lactone derivative **6**, which was readily obtained from 1,3-dihydroxyacetone, as previously described (Scheme 1)[9]. Hydrogenation of 2-methyl-butenolide **6** was achieved with 5% Pd/C under H_2 treatment with a yield of 92% to give lactone **7**. Protection[10] of **7** with TBDMSCl in methylene chloride at 25°C furnished the desired *O*-silyl ether **8**, which was converted to lactol **9** by DIBALH reduction in toluene at -78°C for 1.0 h in 77% two step yield. Protection of anomeric position was needed prior to phosphonation. Hence, methoxylation of anomeric position furnished glycoside **10** in a 83% yield using the conditions [$CH(OMe)_3$, BF_3/Et_2O] even in the presence of acid labile silyl protection group[11]. Removal of the TBDMS group of glycoside **10** by TBAF furnished alcohol **11** with a 88% yield which was converted to difluorophosphonate derivative **13** using triflation followed by a triflate displacement according to the procedure of Berkowitz *et al*.[12] The preparation of a suitable glycosylating agent **14** was attempted by direct acetolysis of **13** under acidic conditions (Ac_2O, AcOH, H_2SO_4, EtOAc, 0°C)[13] to afford an anomeric mixture of 1-*O*-acetyl-furanoside **14** in a 71% yield (Scheme 2). The synthesis of adenine nucleoside was performed using a Vorbrüggen condensation[14] of compound **14** with silylated 6-chloropurine and trimethylsilyltriflate



Reagents: i) H_2, Pd/C, EtOAc; ii) TBDMS, imidazole, CH_2Cl_2; iii) iii) DIBALH, toluene; iv) $CH(OMe)_3$, BF_3/Et_2O, 4 A MS, Et_2O.

Scheme 1. Synthesis of fluorinated apiose glycosyl donor intermediate **10**.



Reagents: i) TBAF, THF; ii) Tf_2O, pyridine, CH_2Cl_2; iii) $LiCF_2P(O)(OEt)_2$, HMPA, THF; iv) Ac_2O, AcOH, H_2SO_4, EtOAc.

Scheme 2. Synthesis of fluorinated apiose glycosyl donor intermediate **14**.

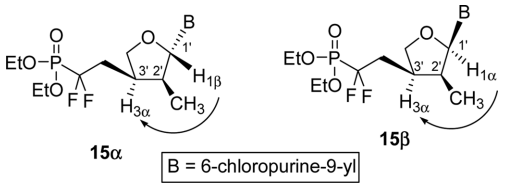
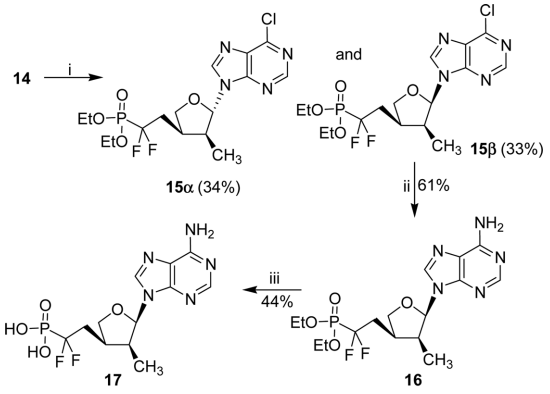


Fig. 2. NOE differences between the proximal hydrogens of **15α** and **15β**.

(TMSOTf) as a catalyst in dichloroethane (DCE) to yield the protected 6-chloropurine derivatives, **15α** and **15β**, respectively. A complete nuclear overhauser effect (NOE) study between proximal hydrogens verified their relative stereochemistry (Figure 2). NOE experiments of both products showed that glycosylation in α-direction is isomer **15α** (NOE: $H_{1\beta}/H_{3\alpha}$, 0.7%), and glycosylation of β-direction is isomer **15β** (NOE: $H_{1\alpha}/H_{3\alpha}$, 1.5%). The chlorine group from purine analog **15β** was then converted to an amine with methanolic ammonia at 66°C to produce the adenosine phosphonate derivative **16** in 61% yield. Hydrolysis of the diethyl phosphonate functional groups of **16** with bromotrimethylsilane treatments in CH_3CN in the presence of 2,6-lutidine yielded adenosine phosphonic acid derivative **17** (Scheme 3)[15].

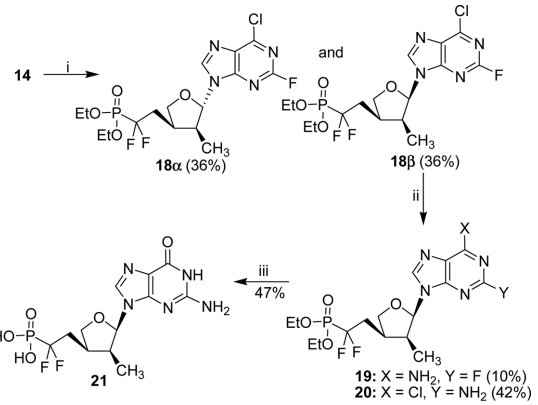
Condensation of 2-fluoro-6-chloropurine[16] with glycosyl donor **14** proceeded under conditions similar to those used for synthesis of analogues **15α** and **15β** to yield **18α** (36%) and **18β** (36%), respectively. The relative stereochemistries of purine analogs **18α** and **18β** were also determined by the study of NOE experiments between proximal hydrogens.

Mild bubbling ammonia into compound **18β** in DME yielded 2-fluoro-6-aminopurine[17] analogue **19** (10%) and 2-amino-6-chloropurine analogue **20** (42%), respectively. The 2-amino-6-chloropurine derivative **20** was treated with TMSBr and 2,6-lutidine to yield phosphonic acid and was then treated with sodium methoxide and 2-mercaptoethanol in methanol to produce guanosine phosphonic acid **21** (Scheme 4)[18].



Reagents: i) Silylated 6-chloropurine, TMSOTf, DCE; ii) NH_3/MeOH, 66 °C; iii) TMSBr, 2,6-lutidine, CH_3CN.

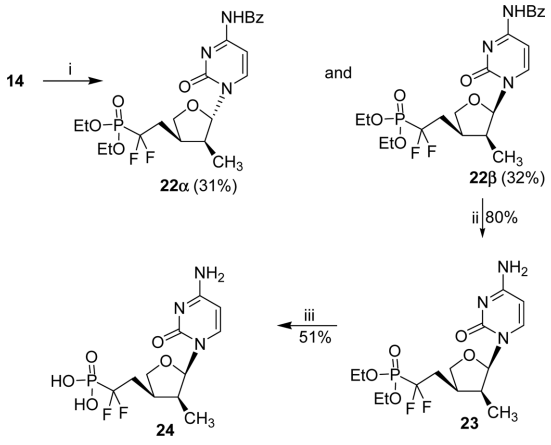
Scheme 3. Synthesis of 5',5'-difluoro-2'-methyl-apiosyl adenosine phosphonic acid analogues.



Reagents: i) Silylated 2-fluoro-6-chloropurine, TMSOTf, DCE; ii) NH_3, DME; iii) (a) TMSBr, 2,6-lutidine, CH_3CN; (b) NaOMe, $HSCH_2CH_2OH$, MeOH.

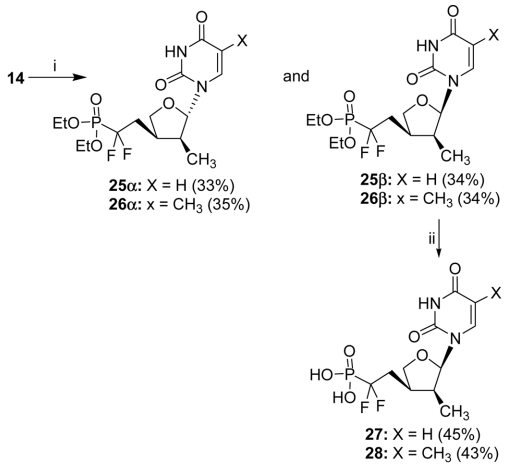
Scheme 4. Synthesis of 5',5'-difluoro-2'-methyl-apiosyl guanosine phosphonic acid analogues.

Condensation of N_4-benzoyl cytosine with glycosyl donor **14** proceeded under conditions similar to those used for the synthesis of adenine analogs to yield **22α** (31%) and **22β** (32%), respectively. Ammonolysis of **22β** followed by deprotection of diethyl phosphonate furnished the target cytosine phosphonic acid **24** (Scheme 5). Also, uracil and thymine nucleoside ana-



Reagents: i) N_4-Benzoyl cytosine, TMSOTf, DCE; ii) NH_3/MeOH, rt; iii) TMSBr, 2,6-lutidine, CH_3CN.

Scheme 5. Synthesis of 5',5'-difluoro-2'-methyl-apiosyl cytosine phosphonic acid analogues.



Reagents: i) silylated uracil and silylated thymine, TMSOTf, DCE; ii) TMSBr, 2,6-lutidine, CH_3CN.

Scheme 6. Synthesis of 5',5'-difluoro-2'-methyl-apiosyl uracil and thymine phosphonic acid analogues.

logs **27** and **28** were also prepared from **14** *via* condensation and deprotection procedures (Scheme 6).

3.1. Biological Activity Evaluation

The antiviral activity of nucleoside phosphonic acid is mostly attributable to their intracellular conversion to the diphosphate form, which is incorporated into the viral genome, causing chain termination[19]. The antiviral assay against several viruses such as the human immunodeficiency virus 1 (HIV-1), herpes simplex virus-1,2 (HSV-1,2) and human cytomegalovirus (HCMV) was performed. As shown in Table 1, compound cytosine analog exhibited weak antiviral activity against HIV without any cytotoxicity up to 100 μM.[20] Also, uracil and thymine analogs showed weak anti-HCMV activity in the Davis cell line. It is impossible that the sugar moiety of the purine analogs (adenine and guanine) either inhibited diphosphorylation or binding to viral polymerases.

4. Conclusion

Based on the potent biological activities of the fluorinated phosphonate nucleosides and apiosyl nucleoside phosphonic acid analogs, we designed and successfully synthesized novel 5′,5′-difluoro-2′-methyl apiosyl nucl-

Table 1. The antiviral activity of the synthesized compounds

	HIV-1 EC_{50}(μM)	HSV-1 EC_{50}(μM)	HSV-2 EC_{50}(μM)	HCMV EC_{50}(μM)	cytotoxicity CC_{50}(μM)
17	>100	>100	>100	>100	>100
21	>100	>100	>100	>100	>100
24	54.2	>100	>100	>100	98
27	>100	>100	>100	57	>100
28	>100	>100	>100	39.6	>100
AZT	0.012	ND	ND	ND	2.58
GCV	ND	ND	ND	0.42	>10
ACV	ND	0.38	ND	ND	>100

AZT: Azidothymidine; GCV: Ganciclovir; ACV: Acyclovir, ND: Not Determined
EC_{50}(μM): Concentration required to inhibit 50% of the virus induced cytopathicity
CC_{50}(μM): Concentration required to reduce the cell viability by 50%

eoside phosphonic acid analogs from 1,3-dihydroxyacetone. Among them, pyrimidine analogs **24**, **27** and **28** showed weak anti-HIV or HCMV activity.

Acknowledgment

This study was supported by research fund from Chosun University, 2014.

References

[1] D. Vina, T. Wu, M. Renders, G. Laflamme, and P. Herdewijn, "Synthesis of 3′-*O*-phosphonomethyl nucleosides with an adenine base moiety", Tetrahedron, Vol. 63, pp. 2634-2646, 2007.

[2] E. I. Kodama, S. Kohgo, K. Kitano, H. Machida, H. Gatanaga, S. Shigeta, M. Matsuoka, H. Ohrui, and H. Mitsuya, "4′-Ethynyl nucleoside analogs: potent inhibitors of multidrug-resistant human immunodeficiency virus variants in vitro", Antimicrob. Agents Chemother., Vol. 45, pp. 1539-1546, 2001.

[3] S. G. Dumbre, M.-Y. Jang, and P. Herdewijn, "Synthesis of α-L-threose nucleoside phosphonates via regioselective sugar protection", J. Org. Chem., Vol. 78, pp. 7137-7144, 2013.

[4] (a) U. Pradere, E. C. Garnier-Amblard, S. J. Coats, F. Amblard, and R. F. Schinazi, "Synthesis of nucleoside phosphate and phosphonate prodrugs", Chem. Rev., Vol. 114, pp. 9154-9218, 2014; (b) R. Engel, "Phosphonates as analogues of natural phosphates", Chem. Rev., Vol. 77, pp. 349-367, 1977; (c) O. Baszczyňski, and Z. Janeba, "Medicinal chemistry of fluorinated cyclic and acyclic nucleoside phosphonates", Med. Chem. Rev., Vol. 33, pp. 1304-1344, 2013; (d) E. De Clercq, "The holy trinity: the acyclic nucleoside phosphonates", Adv. Pharmacol., Vol. 67, pp. 293-316, 2013.

[5] (a) G. M. Blackburn, and D. E. Kent, "Synthesis of α- and γ-fluoroalkylphosphonates", J. Chem. Soc., Perkin Trans. 1, pp. 913-917, 1986; (b) G. R. J. Thatcher, and A. S. Campbell, "Phosphonates as mimics of phosphate biomolecules: ab initio calculations on tetrahedral ground states and pentacoordinate intermediates for phosphoryl transfer", J. Org. Chem., Vol. 58, pp. 2272-2281, 1993; (c) T. R Jr. Burke, M. S. Smyth, A. Otaka, M. Nomizu, M.; P. P. Roller, G. Wolf, R. Case, and S. E. Shoelson, "Nonhydrolyzable phosphotyrosyl mimetics for the preparation of phosphatase-resistant SH2 domain inhibitors", Biochemistry, Vol. 33, pp. 6490-6494, 1994.

[6] (a) R. D. Chambers, R. Jaouhari, and D. O'Hagan, "The preparation of difluoromethylenephosphonate analogues of glycolytic phosphates. Approaching an isosteric and isoelectronic phosphate mimic", Tetrahedron, Vol. 45, pp. 5101-5108, 1989; (b) D. B. Berkowitz, H. J. Eggen, Q. Shen, and R. K. Shoemaker, "Ready access to fluorinated phosphonate mimics of secondary phosphates. Synthesis of the (α,α-difluoroalkyl) phosphonate analogues of L-phosphoserine, L-phosphoallothreonine, and L-phosphothreonine", J. Org. Chem., Vol. 91, pp. 4666-4675, 1996; (c) D. J. Burton, Z.-Y. Yang, and W. Qiu, "Fluorinated ylides and related compounds", Chem. Rev., Vol. 96, pp. 1641-1715, 1996.

[7] S. Halazy, A. Ehrhard, and C. Danzin, "(9-(Difluorophosphonoalkyl)guanines as a new class of multisubstrate analogue inhibitors of purine nucleoside phosphorylase", J. Am. Chem. Soc., Vol. 113, pp. 315-317, 1991.

[8] S. G. Levy, D. B. Wasson, D. A. Carson, and H. B Cottam, "Synthesis of 2-chloro-2′,5′-dideoxy-5′-(phosphinyldifluoromethyl)adenosine: A nonhydrolyzable isosteric, isopolar analog of 2-chlorodeoxyadenosine monophosphate", Synthesis, Vol. 7, pp. 843-846, 1996.

[9] D. A. Fort, T. J. Woltering, A. M. Alker, and T. Bach, "Photochemical reactions of prop-2-enyl and prop-2-ynyl substituted 4-aminomethyl- and 4-oxymethyl-2(*5H*)-furanones", Heterocycles, Vol. 88, pp. 1079-1100, 2004.

[10] E. J. Corey, and A.Venkateswarlu, "Protection of hydroxyl groups as *t*-butyldimethylsilyl derivatives", J. Am. Chem. Soc., Vol. 94, pp. 6190-6172, 1972.

[11] G. Rassu, F. Zanardi, L. Battistini, E. Caetani, and G. Casirsghi, "Expeditious synthesis of sugar-modified nucleosides and collections thereof exploiting furan-, pyrrole-, and thiophen-based dienes", J. Med. Chem., Vol. 40, pp. 168-180, 1997.

[12] D. B. Berkowitz, M. Eggen, Q. Shen, and D. G. Sloss, "Synthesis of (α,α,-difluoroalkyl) phosphonates by displacement of primary triflates", J. Org. chem., Vol. 58, pp. 6174-6176, 1993.

[13] K. Walczak, J. Lau, and E. B. Pedesen, "2,3,6-Trideoxy-5-*O*-(4-nitrobenzoyl)-3-trifluoroacetamido-L-*ribo*-hexofuranosyl bromide - A suitable furanoid ristosamine glycosylation reagent", Synthesis, pp. 790-792, 1993.

[14] H. Vorbrüggen, "Nucleoside analogs, Chemistry", Biology and Medical Applications, NATO ASI

Series A 26; Plenum Press: New Tork, pp. 35, 1980.
[15] (a) D. Hocková, A. Holý, M. Masojídková, D. T. Keough, J. De Jersey, and L. W. Guddat, "Synthesis of branched 9-[2-(2-phosphonoethoxy)ethyl]purines as a new class of acyclic nucleoside phosphonates which inhibit Plasmodium falciparum hypoxanthine-guanine-xanthine phosphoribosyltransferase", Bioorg. Med. Chem., Vol. 17, pp. 6218-6232, 2009; (b) J. Matulic-Adamic, P. Haeberli, and N. Usman, "Synthesis of 5′-deoxy-difluoromethyl phosphonate nucleoside analogs", J. Org. Chem. Vol. 60, pp. 2563-2569, 1995.
[16] M. J. Robins, and B. Uznanski, "Non-aqueous diazotization with *t*-butyl nitrite. Introduction of fluorine, chlorine, and bromine at *C*-2 of purine nucleoside", Can. J. Chem. Vol. 59, pp. 2608-2611, 1981.
[17] J. Montgomery, and K. Hewson, "Nucleosides of 2-fluoroadenine", J. Med. Chem., Vol. 12, pp. 498-504, 1969.
[18] G. L. Tong, K. J. Ryan, W. W. Lee, E. M. Acton, and L. Goodman, "Nucleosides of thioguanine and other 2-amino-6-substituted purines from 2-acetamido-5-chloropurine", J. Org. Chem., Vol. 32, pp. 859-862, 1967.
[19] A. Holy, I. Votruba, A. Merta, J. Cerny, J. Vesely, J. Vlach, K. Sediva, I. Rosenberg, M. Otmar, H. Hrebabecky, M. Travniekb, V. Vonkac, R. Snoeck, and E. De Clercq, "Acyclic nucleotide analogues: synthesis, antiviral activity and inhibitory effects on some cellular and virus-encoded enzymes in vitro", Antiviral Res., Vol. 13, pp. 295-311, 1990.
[20] O. H. Ko, and J. H. Hong, "Synthesis and biological evaluation of novel 2′,3′-4′-triply branched carbocyclic nucleosides as potential antiviral agents", Arch. Pharm. Pharm. Med. Chem., Vol. 337, pp. 579-586, 2004.