# Synthesis and Anti-Retroviral Activity of Novel 5′-Deoxy-5′,5′-difluoro-threosyl Nucleoside Phosphonic Acid Analogs

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Novel 5′-deoxy-5′,5′-difluoro-threose purine phosphonic acid analogs were designed and synthesized from 2-propanone-1,3-diacetate. Direct displacement of the triflate intermediate **12** with diethyl (lithiodifluoromethyl) phosphonate provided the corresponding (α,α-difluoroalkyl) phosphonate **13**. Condensation successfully proceeded from a glycosyl donor **14** under Vorbrüggen conditions to provide the nucleoside phosphonate analogs **15α**, **15β, 18α,** and **18β**, respectively. Ammonolysis and hydrolysis of the phosphonates **15β** and **18β** yielded the nucleoside phosphonic acid analogs **16**, **17**, **19b**, and **20**. Also, synthesis of prodrugs of adenine derivative was performed to increase cellular uptake. The synthesized nucleoside analogs were subjected to antiviral screening against human immunodeficiency virus (HIV)-1. Bis(SATE) prodrug **22** of the adenine analog exhibited significant *in vitro* activity against HIV-1.

**Keywords:** Anti-human immunodeficiency virus agents, 5′-Deoxy-5′,5′-difluoro-threosyl phosphonic acid analog, Vorbrüggen reaction

## Introduction

The fluorine atom plays an important role in medicinal chemistry because fluorine substitution has a strong impact on the physical, chemical, and biological properties of bioactive compounds.[1] Such fluorine modifications have been extensively studied among the pharmaceutically important class of nucleoside phosphonates, which are nucleotide analogs in which the phosphate group is replaced by the enzymatically and chemically stable phosphonate moiety. The fluorinated nucleoside phosphonates abound with antiviral, antiparasitic, and anticancer properties because they are able to act as inhibitors of important enzymes of nucleoside and nucleotide metabolism.[2]

Phosphonic acids often exhibit important biological properties because of their similarity to phosphates.[3] The carbon–phoshophorus bond in phosphonates, unlike phosphates, is not susceptible to the hydrolytic action of phosphatases, thereby imparting greater stability under physiological conditions. In particular, alkyl phosphonate esters of nucleosides are generally more stable to nucleases and have greater cell permeability.[4] It has been suggested by Blackburn and Kent[5] that α-fluoro and α,α-difluoromethylphosphonates should mimic phosphate esters better than the corresponding phosphonates.[6] This assumption was based on electronic and steric considerations. Groups such as CHF and $CF_2$ could be incorporated either in place of 3′- or 5′-oxygens, or as $CFH_2$, $CF_2H$, or $CF_3$ in place of the hydroxyl on the phosphates. 9-(5,5-difluoro-5-phosphonopentyl)guanine **1** has been utilized as a multisubstrate analog inhibitor of purine nucleoside phosphorylase.[7] Furthermore, the α-monofluorinated poly(2-methoxyethyl acrylate) (PMEA) analog was prepared by Chen *et al.* in order to improve the biological properties of PMEA. Among them, the monoester derivative **2** exhibited significant activity against both human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV).[8]

Threose 5′-nor nucleoside phosphonates,[9] such as PMDTA (**3**, $EC_{50} = 4.5$ μM) and PMDTT (**4**), have been assembled from natural precursor molecules (Figure 1). Furthermore, threose nucleic acids (TNAs) form thermally stable duplexes with DNA and RNA that resemble the natural associations of nucleic acids.[10] Moreover, diphosphates in threose nucleosides are accepted as substrates by several polymerases and can be enzymatically incorporated into DNA.[11] In addition,

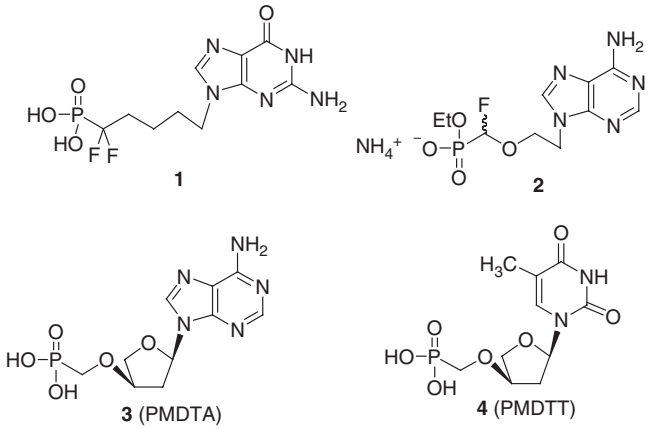



**Figure 1.** Synthesis rationale of 5′-deoxy-5′,5′-difluoro-threose nucleoside phosphonic acids as potent anti-HIV agents.

these nucleosides are accepted as substitutes for ribonucleosides at the catalytic site of the hammerhead ribozyme, although then the catalytic efficiency of ribozymes is significantly reduced.[12] PMDTA has a phosphonomethoxy group at the 3′-position of its furanose ring and no substituent at the 4′-position.[13] This absence of a 4′-hydroxymethyl group avoids problems of steric hindrance during phosphorylation reactions with kinases. Recently, we have designed and synthesized several related structures to find potent antiviral agents in this category.[14]

Stimulated by the findings that 5′-mono- and difluorinated nucleoside analogs and threose 5′-nornucleoside phosphonic acids have excellent antiviral activities, we sought to synthesize a novel class of nucleosides consisting of 5′-deoxy-5′,5′-difluoro-threose phosphonic acid analogs to find more effective therapeutics against human immunodeficiency virus (HIV).

## Experimental

**General.** Uncorrected melting points were determined using a Mel-temp II laboratory device (Brossard Quebeck CANADA J4Y2Z2). 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 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.

**(±)-Dihydro-4-(hydroxymethyl)furan-2(3*H*)-one (7):** To a solution of lactone **6** (1.14 g, 10 mmol) in 50 mL of MeOH, 0.5 g of Pd/C (5% w/w) was added under $H_2$ atmosphere; the mixture was stirred for 6 h. After filtration of the reaction mixture through a Celite pad, the filtrate was concentrated and purified using silica gel column chromatography (EtOAc/hexane, 1:15) to yield compound **7** (1.05 g, 91%). $^1$H NMR ($CDCl_3$, 300 MHz) δ 4.42 (dd, $J$ = 10.8, 4.8 Hz, 1H), 4.21 (dd, $J$ = 10.8, 6.8 Hz, 1H), 3.69 (dd, $J$ = 10.2, 6.6 Hz, 1H), 3.43 (dd, $J$ = 10.2, 8.0 Hz, 1H), 2.37 (dd, $J$ = 9.8, 6.6 Hz, 1H), 2.20–2.11 (m, 2H); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 175.4, 72.5, 69.4, 35.1, 32.6.

**(±)-4-[(Benzyloxy)methyl]-dihydrofuran-2(3*H*)-one (8):** To a stirred solution of alcohol **7** (495 mg, 4.27 mmol) and benzyl trichloroacetimidate in 33% methylene chloride/cyclohexane (25 mL) at room temperature was added trifluoromethane sulfonic acid (0.05 mL). The reaction mixture was stirred at 25 °C for 18 h, and then diluted with hexane (25 mL). The white precipitate was filtered off through a plug of Celite and washed with 5% ethyl acetate/hexane. The filtrate was concentrated and purified using silica gel column chromatography (EtOAc/hexane, 1:10) to yield compound **8** (712 mg, 81%). $^1$H NMR ($CDCl_3$, 300 MHz) δ 7.23–7.17 (m, 5H), 4.65 (s, 2H), 4.47 (dd, $J$ = 10.6, 5.0 Hz, 1H), 4.23 (dd, $J$ = 10.6, 7.0 Hz, 1H), 3.48 (dd, $J$ = 10.2, 6.8 Hz, 1H), 3.25 (dd, $J$ = 10.2, 7.8 Hz, 1H), 2.36–2.29 (m, 2H), 2.15 (dd, $J$ = 10.4, 6.6 Hz, 1H); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 175.7, 137.5, 128.5, 127.8, 127.3, 80.2, 76.4, 71.5, 36.6, 29.2.

**(*rel*)-(1*S*/1*R*,3*S*)-3-[(Benzyloxy)methyl]-tetrahydrofuran-1-ol (9):** A solution of compound **8** (451 mg, 2.19 mmol) in toluene (25 mL) was treated with 4.37 mL of 1 M DIBAL-H in hexane at −78 °C for 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 (50 mL) were added to the mixture. The resulting mixture was filtered and the filtrate was concentrated to dryness and purified using silica gel column chromatography (EtOAc/hexane, 1:7) to yield compound **9** (405 mg, 89%) as an oil. $^1$H NMR ($CDCl_3$, 300 MHz) δ 7.22–7.17 (m, 5H), 5.52–5.48 (m, 1H), 4.65 (s, 2H), 3.86 (m, 1H), 3.57–3.47 (m, 2H), 3.24 (m, 1H), 2.14–2.03 (m, 3H); Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.21; H, 7.74. Found: C, 69.36; H, 7.66; MS $m/z$ 209 $(M + H)^+$.

**(*rel*)-(1*S*/1*R*,3*S*)-3-[(Benzyloxy)methyl]-tetrahydro-1-methoxyfuran (10):** To a stirred solution of lactol **9** (2.58 g, 12.4 mmol) in methanol (30 mL) was added 1% methanolic hydrogen chloride solution (prepared by adding 85 μL acetyl chloride to 5 mL MeOH). The reaction mixture was stirred for 30 min at room temperature under argon atmosphere, then sodium bicarbonate (700 mg) added, and the stirring continued for further 10 min. The solids were filtered and the solvent removed *in vacuo* to give the residue as an oil. The residue was purified using silica gel column chromatography (EtOAc/hexane, 1:20) to yield compound **10** (2.28 g, 83%) as an oil. $^1$H NMR ($CDCl_3$, 300 MHz) δ 7.21–7.16 (m, 5H), 5.13–5.09 (m, 1H), 4.62 (s, 2H), 3.87–3.85 (m, 1H), 3.54–3.50 (m, 1H), 3.44–3.42 (m, 1H), 3.21–3.18 (m, 1H), 2.21–2.18 (m, 4H), 2.09–2.01 (m, 2H); Anal. Calcd. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.11; H, 8.27; MS $m/z$ 223 $(M + H)^+$.

**(*rel*)-(1*S*/1*R*,3*S*)-(Tetrahydro-1-methoxyfuran-3-yl) methanol (11):** Anhydrous ammonia (15 mL) was condensed into a three-necked, round-bottomed flask containing a solution of methyl glycoside **10** (222 mg, 1.0 mmol) in dry tetrahydrofuran (3 mL) at −78 °C. To this mixture was added a minimum amount of lithium (~60 mg) sufficient to maintain a blue color, and the resulting deep blue solution was stirred for 30 min at −78 °C for 2 min. Methanol was added dropwise at the same temperature until the deep blue color disappeared. The colorless solution was stirred for 30 min at −78 °C, and then solid ammonia chloride (6 g) was added. After stirring for 1 h at −78 °C, ammonia was allowed to evaporate (6 h). Ether (40 mL) was added, and the mixture was dried over

anhydrous magnesium sulfate, filtered through a pad of Celite, and washed with ether (150 mL). The filtrate and washings were concentrated *in vacuo*, and the residue was purified using silica gel column chromatography (EtOAc/hexane, 1:4) to yield compound **11** (2.28 g, 78%) as an oil. $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.09–5.05 (m, 1H), 3.83–3.79 (m, 1H), 3.61–3.55 (m, 2H), 3.39–3.34 (m, 1H), 3.26 (s, s, 3H), 2.09–1.87 (m, 3H); Anal. Calcd. for $C_6H_{12}O_3$: C, 54.53; H, 9.15. Found: C, 54.68; H, 9.06; MS *m/z* 133 $(M+H)^+$.

**(*rel*)-(1*S*/1*R*,3*R*)-(Tetrahydro-1-methoxyfuran-3-yl) methyl trifluoromethanesulfonate (12):** To a cooled solution of glycoside **11** (304 mg, 2.304 mmol) in pyridine (0.72 mL, 8.9 mmol) and $CH_2Cl_2$ (48 mL), triflic anhydride (0.456 mL, 2.76 mmol) was slowly added. After 1.5 h, the reaction mixture was poured onto a mixture of ice and sodium hydrogen carbonate. The aqueous layer was extracted with $CH_2Cl_2$ (3 × 60 mL), and the combined $CH_2Cl_2$ solution was dried and rapidly and repeatedly concentrated with toluene to remove any residual pyridine. The residue was extracted with light petroleum (3 × 60 mL), and the combined extracts were filtered and cooled. After careful evaporation of additional solvent, the crude residue **12** (605 mg, ~100%) was subjected to the next reaction without further purification.

**(1*S*/1*R*,3*S*)-(Diethyl 5,5-difluoro-4-tetrahydro-1-methoxyfuran-3-yl) ethylphosphonate (13):** To a solution of diisopropylamine (182 μL, 1.3 mmol) and HMPA (226 μL, 1.3 mmol) at −78 °C in THF (2 mL) under Ar was added *n*-butyllithium (814 μL of a 1.6 M solution in hexane, 1.3 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 a cannula, a (−78 °C) solution of diethyl (α,α-difluoromethyl) phosphonate (204 μL, 1.3 mmol) in THF (1.0 mL), and, 2 min later, a (−78 °C) solution of triflate **12** (98 mg, 0.372 mmol) in THF (2.0 mL), dropwise, via a cannula. After 10 min at −78 °C, the reaction was quenched by adding aqueous $NH_4Cl$ (6.0 mL) and $Et_2O$ (6.0 mL). The aqueous layer was further extracted with EtOAc (2 × 20 mL), and the combined organic extracts were dried, filtered, and evaporated. Silica gel flash chromatography (EtOAc/hexane, 1:1) gave **13** (78 mg, 70%). $^1$H NMR ($CDCl_3$, 300 MHz) δ 5.12–5.09 (m, 1H), 4.34–4.26 (m, 4H), 3.83–3.79 (m, 1H), 3.55–3.51 (m, 1H), 3.23 (s, s, 3H), 2.13–1.99 (m, 5H), 1.38–1.33 (m, 6H); $^{31}$P (81 MHz, $CDCl_3$) δ 6.67 (app t, $J_{P,F}$ = 108 Hz); Anal. Calcd. for $C_{11}H_{21}F_2O_5P$: C, 43.71; H, 7.00. Found: C, 43.62; H, 6.96; MS *m/z* 303 $(M+H)^+$.

**(*rel*)-Diethyl 4-[(1*S*/1*R*,3*S*)-1-acetoxy-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (14):** Glycoside **13** (725 mg, 2.4 mmol) was dissolved in EtOAc (20 mL). The solution was cooled to −15 °C and mixed with a cooled (−15 °C) solution of EtOAc (40 mL), acetic anhydride (22.0 mL), acetic acid (16.6 mL) and conc. $H_2SO_4$ (0.1 mL). The solution mixture was stirred for 18 h at 0 °C. The reaction was diluted with $CHCl_3$ (150 mL) and poured into cold 5% aqueous $NaHCO_3$ (200 mL). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 50 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, 1:2) to yield compound **14** (641 mg, 81%) as a syrup. $^1$H NMR ($CDCl_3$, 300 MHz) δ 6.23–6.19 (m, 1H), 4.32–4.24 (app quintet, *J* = 7.0 Hz, 4H), 3.86–3.81 (m, 1H), 3.61–3.56 (m, 1H), 2.24–2.19 (m, 4H), 2.04 (s, s, 3H), 1.86 (m, 1H), 1.36–1.32 (m, 6H); $^{31}$P (81 MHz, $CDCl_3$) δ 6.87 (t, $J_{P,F}$ = 98 Hz); Anal. Calcd. for $C_{12}H_{21}F_2O_6P$: C, 43.64; H, 6.41. Found: C, 43.79; H, 6.36; MS *m/z* 331 $(M+H)^+$.

**(*rel*)-Diethyl 4-[(1*S*,3*S*)-1-(6-chloro-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (15α) and (*rel*)-diethyl 4-[(1*R*,3*S*)-1-(6-chloro-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (15β):** 6-Chloropurine (260 mg, 1.68 mmol), anhydrous HMDS (12 mL), and a catalytic amount of ammonium sulfate (16.8 mg) were refluxed to a clear solution (10 h); the solvent was then distilled under anhydrous conditions. The residue obtained was dissolved in anhydrous 1,2-dichloroethane (10 mL), and to this mixture a solution of **14** (277 mg, 0.84 mmol) in dry dichloroethane (DCE) (12 mL) and TMSOTf (373 mg, 1.68 mmol) was added, and stirred for 4 h at room temperature. The reaction mixture was quenched with 6.0 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). The 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, 3:1:0.03) to yield compounds **15α** (114 mg, 32%) and **15β** (117 mg, 33%). Data for **15α**: $^1$H NMR ($CDCl_3$, 300 MHz) δ 8.71 (s, 1H), 8.29 (s, 1H), 6.03 (dd, *J* = 5.8, 4.0 Hz, 1H), 4.23–4.18 (app quintet, *J* = 7.2 Hz, 4H), 3.81 (dd, *J* = 10.6, 8.0 Hz, 1H), 3.67 (dd, *J* = 10.6, 6.4 Hz, 1H), 2.17–2.10 (m, 2H), 2.05–1.95 (m, 3H), 1.35–1.30 (app t, *J* = 7.2 Hz, 6H); $^{31}$P (81 MHz, $CDCl_3$) δ 6.89 (t, $J_{P,F}$ = 99.2 Hz); Anal. Calc. for $C_{15}H_{20}ClF_2N_4O_4P$: C, 42.41; H, 4.75; N, 13.19. Found: C, 42.38; H, 4.86; N, 13.22; MS *m/z* 425 $(M+H)^+$; Data for **15β**: $^1$H NMR ($CDCl_3$, 300 MHz) δ 8.74 (s, 1H), 8.32 (s, 1H), 5.99 (t, *J* = 6.0 Hz, 1H), 4.25–4.20 (app quintet, *J* = 7.1 Hz, 4H), 3.85 (dd, *J* = 10.2, 7.6 Hz, 1H), 3.63 (dd, *J* = 10.3, 6.6 Hz, 1H), 2.21–2.15 (m, 2H), 2.09–1.98 (m, 3H), 1.34–1.28 (app t, *J* = 7.2 Hz, 6H); $^{31}$P (81 MHz, $CDCl_3$) δ 6.85 (t, $J_{P,F}$ = 99.4 Hz); Anal. Calcd. for $C_{15}H_{20}ClF_2N_4O_4P$: C, 42.41; H, 4.75; N, 13.19. Found: C, 42.56; H, 4.61; N, 13.13; MS *m/z* 425 $(M+H)^+$.

**(*rel*)-Diethyl 4-[(1*R*,3*S*)-1-(6-amino-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-5,5-difluoroethyl phosphonate (16):** A solution of **15β** (315 mg, 0.742 mmol) in saturated methanolic ammonia (15 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/$CH_2Cl_2$, 1:10) to yield **16** (186 mg, 62%) as a white solid: UV (MeOH) $\lambda_{max}$ 262.0 nm; $^1$H NMR (DMSO-$d_6$, 300 MHz) δ 8.40 (s, 1H), 8.21 (s, 1H), 5.97 (dd, *J* = 10.8, 8.2 Hz, 1H), 4.17 (quintet, *J* = 7.0 Hz, 4H), 3.86 (dd, *J* = 10.2, 7.2 Hz, 1H), 3.58 (dd, *J* = 10.2, 6.4 Hz, 1H), 2.42 (m,

1H), 2.17–1.97 (m, 4H), 1.34 (t, $J$ = 7.0 Hz, 6H); $^{31}$P (81 MHz, DMSO-$d_6$) δ 6.79 (app t, $J_{P,F}$ = 102.4 Hz); Anal. Calcd. for $C_{15}H_{22}F_2N_5O_4P$ (+1.0 MeOH): C, 43.95; H, 5.99; N, 16.02; Found: C, 43.85; H, 6.04; N, 16.13; MS *m/z* 406 $(M + H)^+$.

**(*rel*)-4-[(1*R*,3*S*)-1-(6-Amino-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-5,5-difluoroethyl phosphonic acid (17):** To a solution of phosphonate **16** (172 mg, 0.426 mmol) in anhydrous $CH_3CN$ (8.5 mL) and 2,6-lutidine (912 mg, 8.52 mmol), trimethylsilyl bromide (652 mg, 4.26 mmol) was added. The mixture was heated overnight at 75 °C under nitrogen and then concentrated *in vacuo* to yield a brown residue, and then co-evaporated from conc. aq. $NH_4OH$ (2 × 21.3 mL). The resultant compound was purified by twice triturating the residue in acetone (8.5 mL) and removing the acetone by evaporation. The residue was then purified using preparative reverse-phase chromatography. Lyophilization of the appropriate fraction yielded the phosphonic acid salt **17** (92 mg, 59%) as a white salt (ammonium salt): UV ($H_2O$) $\lambda_{max}$ 261.0 nm; $^1$H NMR ($D_2O$, 300 MHz) δ 8.38 (s, 1H), 8.18 (s, 1H), 5.94 (dd, $J$ = 10.4, 7.2 Hz, 1H), 3.86 (dd, $J$ = 10.0, 6.2 Hz, 1H), 3.62 (dd, $J$ = 10.0, 7.8 Hz, 2H), 2.40 (m, 1H), 2.13–1.92 (m, 4H); $^{13}$C NMR ($D_2O$, 75 MHz) δ 155.6, 152.7, 150.7, 141.2, 120.6, 119.2 (dt, $J_{C,P}$ = 204.8 Hz, $J_{C,F}$ = 258.2 Hz), 90.1 72.9, 36.2, 24.2 (dt, $J_{C,P}$ = 18.6 Hz, $J_{C,F}$ = 23.8 Hz), 20.4; $^{31}$P (81 MHz, $D_2O$) δ 6.83 (app t, $J_{P,F}$ = 104.2 Hz); HPLC $t_R$ = 10.38; HRMS $[M - H]^+$ req. 348.0682, found 328.0683.

**(*rel*)-Diethyl 4-[(1*S*,3*S*)-3-(6-chloro-2-fluoro-9*H*-purin-9-yl)-tetrahydrofuran-1-yl]-5,5-difluoroethylphosphonate (18α) and (*rel*)-diethyl 4-[(1*R*,3*S*)-1-(6-chloro-2-fluoro-9*H*-purin-9-yl)-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**. Data for **18α**: yield 34%; UV (MeOH) $\lambda_{max}$ 267.5 nm; $^1$H NMR ($CDCl_3$, 300 MHz) δ 8.51 (s, 1H), 6.02 (dd, $J$ = 10.6, 6.8 Hz, 1H), 4.19 (app quintet, $J$ = 7.0 Hz, 1H), 3.83 (dd, $J$ = 9.9, 5.4 Hz, 1H), 3.59 (dd, $J$ = 10.0, 8.2 Hz, 1H), 2.39 (m, 1H), 2.16–1.96 (m, 4H), 1.32 (app t, $J$ = 7.0 Hz, 6H); $^{31}$P (81 MHz, $CDCl_3$) δ 6.87 (t, $J_{P,F}$ = 102.0 Hz); Anal. Calcd. for $C_{15}H_{19}ClF_3N_4O_4P$: C, 40.69; H, 4.33; N, 12.65; Found: C, 40.82; H, 4.41; N, 12.52; MS *m/z* 443 $(M + H)^+$. data for **18β**: yield 35%; UV (MeOH) $\lambda_{max}$ 268.0 nm; $^1$H NMR ($CDCl_3$, 300 MHz) δ 8.46 (s, 1H), 5.96 (dd, $J$ = 10.4, 8.0 Hz, 1H), 4.23 (app quintet, $J$ = 7.0 Hz, 1H), 3.85 (dd, $J$ = 9.8, 6.4 Hz, 1H), 3.53 (dd, $J$ = 9.8, 7.2 Hz, 1H), 2.35–2.32 (m, 1H), 2.11–1.94 (m, 4H), 1.35 (app t, $J$ = 7.0 Hz, 6H); $^{31}$P (81 MHz, $CDCl_3$) δ 6.86 (t, $J_{P,F}$ = 102.2 Hz); Anal. Calcd. for $C_{15}H_{19}ClF_3N_4O_4P$: C, 40.69; H, 4.33; N, 12.65; Found: C, 40.54; H, 4.20; N, 12.76; MS *m/z* 443 $(M + H)^+$.

**(*rel*)-Diethyl 4-[(1*R*,3*S*)-1-(6-amino-2-fluoro-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (19a) and (*rel*)-diethyl 4-[(1*R*,3*S*)-1-(2-amino-6-chloro-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonate (19b):** Dry ammonia gas was bubbled into a stirred solution of **18β** (310 mg, 0.7 mmol) in DME (15.0 mL) at room temperature overnight. 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 **19a** (29 mg, 11%) and **19b** (98 mg, 39%). Data for **19a**; UV (MeOH) $\lambda_{max}$ 262.5 nm; $^1$H NMR (DMSO-$d_6$, 300 MHz) δ 8.25 (s, 1H), 7.71 (br s, $NH_2$, 2H, $D_2O$ exchangeable), 5.91 (dd, $J$ = 10.4, 7.6 Hz, 1H), 4.20 (app quintete, $J$ = 7.0 Hz, 4H), 3.85 (dd, $J$ = 10.1, 6.8 Hz, 1H), 3.62 (dd, $J$ = 10.2, 7.8 Hz, 1H), 2.43–2.39 (m, 1H), 2.11–1.92 (m, 4H), 1.37 (app t, $J$ = 7.0 Hz, 1H); $^{31}$P (81 MHz, DMSO-$d_6$) δ 6.83 (t, $J_{P,F}$ = 101.8 Hz); Anal. Calcd. for $C_{15}H_{21}F_3N_5O_4P$ (+0.5 MeOH): C, 42.39; H, 5.28; N, 15.94; Found: C, 42.42; H, 5.15; N, 15.84; MS *m/z* 424 $(M + H)^+$. Data for **19b**; UV (MeOH) $\lambda_{max}$ 308.0 nm; $^1$H NMR (DMSO-$d_6$, 300 MHz) δ 10.8 (br s, 1H, $D_2O$ exchangeable), 8.35 (s, 1H), 7.67 (br s, $NH_2$, 2H, $D_2O$ exchangeable), 5.94 (dd, $J$ = 10.2, 7.4 Hz, 1H), 4.21 (app quintet, 4H), 3.85 (dd, $J$ = 10.6, 8.0 Hz, 1H), 3.62 (dd, $J$ = 10.6, 6.2 Hz, 1H), 2.84–2.78 (m, 1H), 2.12–1.94 (m, 4H), 1.36 (app t, 6H); $^{31}$P (81 MHz, DMSO-$d_6$) δ 6.81 (t, $J_{P,F}$ = 102.8 Hz); Anal. Calcd. for $C_{15}H_{21}ClF_2N_5O_4P$ (+1.0 MeOH): C, 40.78; H, 5.34; N, 14.86; Found: C, 40.83 H, 5.48; N, 14.91; MS *m/z* 440 $(M + H)^+$.

**(*rel*)-4-[(1*R*,3*S*)-1-(2-Amino-1,6-dihydro-6-oxopurin-9-yl)-tetrahydrofuran-3-yl]-5,5-difluoroethylphosphonic acid (20):** To a solution of **19b** (202 mg, 0.46 mmol) in dry $CH_3CN$ (18.4 mL), trimethylsilyl bromide (1.40 g, 9.2 mmol) was added at room temperature. The mixture was stirred for 20 h and the solvent was removed using co-evaporation with MeOH thrice. The residue was dissolved in MeOH (18.4 mL) and 2-mercaptoethanol (143 mg, 1.84 mmol), and then NaOMe (99 mg, 1.84 mmol) was added. The mixture was refluxed for 14 h under $N_2$, cooled, neutralized with glacial AcOH, and evaporated to dryness under vacuum. The residue obtained was co-evaporated from conc. $NH_4OH$ (2 × 18.4 mL) and the resultant solid was triturated with acetone (2 × 10.5 mL). After evaporating the acetone, the residue was purified using preparative column chromatography with reverse-phase C18 silica gel and elution with water. Lyophilization of the appropriate fraction yielded **20** (103 mg, 59%) as a yellowish salt (ammonium salt). UV ($H_2O$) $\lambda_{max}$ 253.0 nm; $^1$H NMR ($D_2O$, 300 MHz) δ 7.79 (s, 1H), 5.93 (dd, $J$ = 10.4, 7.2 Hz, 1H), 3.85 (dd, $J$ = 10.0, 7.6 Hz, 1H), 3.58 (dd, $J$ = 10.0, 8.8 Hz, 1H), 2.42–2.37 (m, 1H), 2.15–1.97 (m, 4H); $^{13}$C NMR ($D_2O$, 75 MHz) δ 157.5, 154.5, 152.2, 136.4, 120.2 (dt, $J_{C,P}$ = 214.0 Hz, $J_{C,F}$ = 258.4 Hz), 80.3, 73.7, 36.9, 25.5 (dt, $J_{C,P}$ = 19.1 Hz, $J_{C,F}$ = 23.6 Hz), 21.5; $^{31}$P (81 MHz, $D_2O$) δ 6.45 (app t, $J_{P,F}$ = 101.7 Hz); HPLC $t_R$ = 9.82 min; HRMS $[M - H]^+$ req. 438.0823, found 438.0824.

**(*rel*)-[(1*R*,3*S*)-Bis(SATE) ester of-[1-(6-amino-9*H*-purin-9-yl)-tetrahydrofuran-3-yl]-5,5-difluoroethyl phosphonate (22):** A solution of adenine phosphonic acid derivative **17** (236 mg, 0.676 mmol) and tri-*n*-butylamine (378 mg, 2.04 mmol) in methanol (15 mL) was mixed for 30 min and evaporated to dryness under reduced pressure. The residue was thoroughly dried with anhydrous ethanol and toluene.

The resulting foamy solid was dissolved in anhydrous pyridine (40 mL) to which thioester **21** (2.08 g, 12.8 mmol) and 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (804 mg, 2.712 mmol) were added. The mixture was stirred overnight at room temperature and quenched with tetrabutylammonium bicarbonate buffer (40.0 mL, 1 M solution, pH 8.0). The mixture was concentrated under reduced pressure, and the residue was diluted with water (200 mL) and extracted with $CHCl_3$ (200 mL) two times. The combined organic layer was washed with brine, dried over $Na_2SO_4$, filtered, and evaporated to dryness under vacuum. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.02:3:1) to give **22** (68 mg, 33%) as a yellow solid: m.p. 144–146 °C; UV (MeOH) $\lambda_{max}$ 262.0 nm; $^1$H NMR ($CDCl_3$, 300 MHz) δ 8.31 (s, 1H), 8.15 (s, 1H), 5.99 (dd, $J$ = 10.4, 7.2 Hz, 1H), 4.12–4.07 (m, 4H), 3.86 (dd, $J$ = 10.3, 6.4 Hz, 1H), 3.61 (dd, $J$ = 10.2, 7.6 Hz, 1H), 3.18–3.15 (m, 4H), 2.40 (m, 1H), 2.14–1.06 (m, 22); $^{13}$C NMR ($CDCl_3$, 75 MHz) δ 202.7, 154.6, 152.1, 147.3, 144.3, 129.7, 119.4, 118.2 (dt, $J_{C,P}$ = 211.6 Hz, $J_{C,F}$ = 256.2 Hz), 90.2, 71.7, 63.8 (d, $J$ = 6.0 Hz), 49.3, 35.8, 34.9, 27.1, 25.5 (dt, $J_{C,P}$ = 19.1 Hz, $J_{C,F}$ = 21.8 Hz), 20.2; $^{31}$P (81 MHz, $CDCl_3$) δ 6.51 (app t, $J_{P,F}$ = 100.8 Hz); Anal. Calcd. for $C_{25}H_{38}F_2N_5O_6PS_2$ (+1.0 MeOH): C, 46.66; H, 6.32; N, 10.96; Found: C, 46.78; H, 6.35; N, 10.88; MS *m/z* 638 $(M + H)^+$.

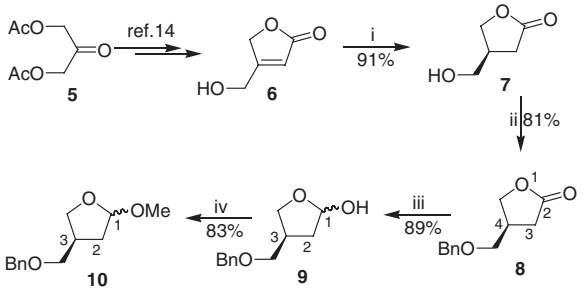
## Results and Discussion

As depicted in Scheme 1, target compound was synthesized from butenolide **6**, which was readily obtained from 2-propanone-1,3-diacetate **5**, as previously described.[15] Catalytic hydrogenation of butenolide **6** to lactone **7** was achieved with 5% Pd/C under $H_2$ treatment with a yield of 91%. Treatment of **7** with benzyl trichloroacetimidate and a catalytic amount of $CF_3SO_3H$ in cyclohexane/methylene chloride (2/1) at 25 °C furnished the desired *O*-benzyl ether **8,** which was converted to lactol **9** by DIBALH reduction in toluene at −78 °C for 1.5 h (72% yield for two steps). Protection of the anomeric position was needed prior to phosphonation. Hence, methoxylation of the anomeric position furnished glycoside **10** in 83% yield. Removal of the benzyl group of glycoside **10** under routine dissolving metal reduction for a prolonged time (~30 min) furnished alcohol **11** with 78% yield, which was converted to **13** using triflation followed by a triflate displacement according to the procedure of Berkowitz *et al.*[16] Finally, the preparation of a suitable glycosylating agent was achieved by acetolysis of **13** under mild acidic conditions ($Ac_2O$, AcOH, $H_2SO_4$, EtOAc, 0 °C) to afford an anomeric mixture of 1-*O*-acetyl-furanoside **14** in high yield (Scheme 2).[17]

The synthesis of adenine nucleoside was performed using a Vorbrüggen condensation[18] of compound **14** with silylated 6-chloropurine and trimethylsilyltriflate (TMSOTf ) as a catalyst in DCE to yield the protected 6-chloropurine derivatives **15α** and **15β**, respectively.

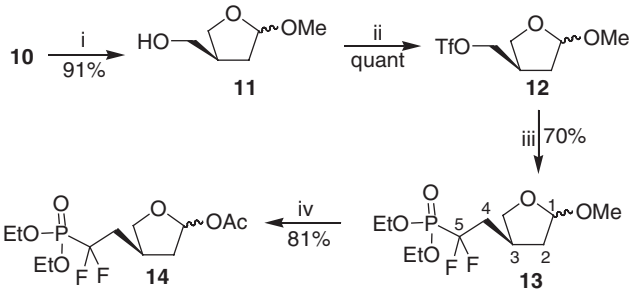
A complete nuclear Overhauser effect (NOE) study led to the unambiguous determinations of their relative stereochemistry (Figure 2). Compound **15β** showed a strong NOE (1.2%) of H-1′ ↔ H-3′, which indicated a 1′,3′-*cis* relationship. According to this result, the 3′-difluoromethyl phosphonate group and the 1′-purine base of **15β** were located on the β face. On the other hand, compound **15α** showed a weak NOE (0.5%) of H-1′ ↔ H-3′, indicating a 1′,3′-*trans* relationship.

The chlorine group from the purine analog **15β** was then converted to an amine with methanolic ammonia at 63 °C to produce the corresponding adenosine phosphonate derivative **16** with yield 62%. Hydrolysis of the diethyl phosphonate functional groups of **16** with bromotrimethylsilane treatments in $CH_3CN$ in the presence of 2,6-lutidine yielded the adenosine phosphonic acid compound **17** (Scheme 3).[19] For the




Reagents: i) $H_2$, Pd/C, EtOAc; ii) $PhCH_2OC(=NH)CCl_3$, $CF_3SO_3H$, $CH_2Cl_2$; iii) DIBALH, toluene, -78 °C; iv) MeOH, AcCl.

**Scheme 1.** Synthesis of threose methyl glycoside **10**.




Reagents: i) Li, $NH_3$, -78 °C; 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**.

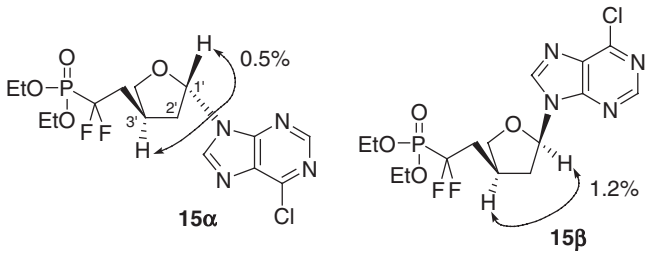


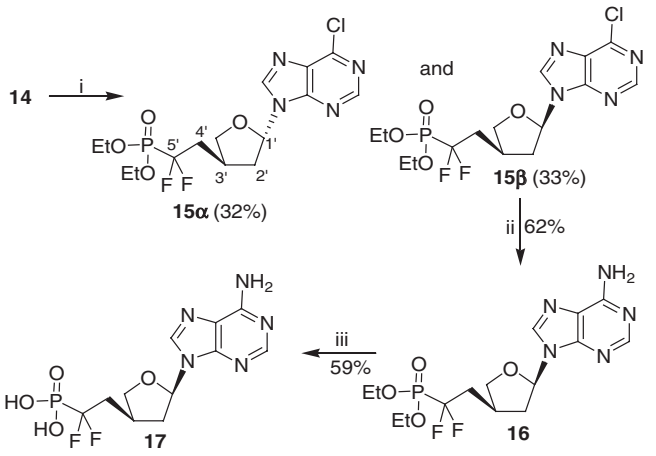

**Figure 2.** NOE differences between the proximal hydrogens of **15α** and **16β**.

structural elucidation of **17**, $^{1}H$, $^{13}C$, $^{31}P$ NMR, and high-resolution mass spectrometry (HRMS) were used.

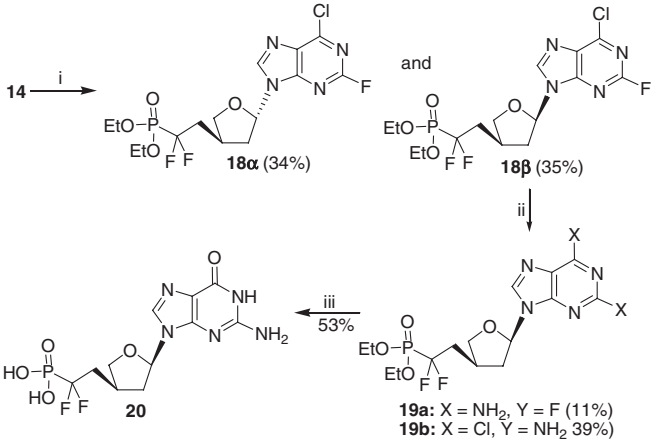
Condensation of 2-fluoro-6-chloropurine[20] with glycosyl donor **14** proceeded under conditions similar to those used for synthesis of analogs **15α** and **15β** from 6-chloropurine to yield **18α** (34%) and **18β** (35%), respectively. A complete NOE study led to the unambiguous determination of the relative stereochemistry of purine analogs **18α** and **18β**.

Bubbling ammonia into compound **18β** yielded the 2-fluoro-6-aminopurine[21] analog **19a** (11%) and the 2-amino-6-chloropurine analog **19b** (39%). The 2-amino-6-chloropurine derivative **19b** was treated with TMSBr to yield phosphonic acid and was then treated with sodium methoxide and 2-mercaptoethanol in methanol to produce guanosine phosphonic acid **20** (Scheme 4).[22]




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

**Scheme 3.** Synthesis of 5′-deoxy-5′,5′-difluoro-threosyl phosphonic acid adenosine analog.




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

**Scheme 4.** Synthesis of 5′-deoxy-5′,5′-difluoro-threosyl phosphonic acid guanosine analog.

First, the synthesized nucleoside phosphonate and phosphonic acids **16**, **17**, and **20** were evaluated for antiviral activity against HIV. The procedures for measuring the antiviral activity toward wild-type HIV and cytotoxicity have been reported previously.[23] As shown in Table 1, the adenine nucleoside analogs **16** and **17** exhibited weak anti-HIV activity. Furthermore, the guanine nucleoside analog **20** did not show anti-HIV activity or cytotoxicity at concentrations up to 100 μM. The reason for the weak antiviral activity of the final compounds might be the result of poor cell membrane diffusion of phosphonate ions *in vitro*. To increase the cellular uptake of adenine phosphonic acid analog **17**, development of bis-SATE phosphonodiester prodrugs was performed.

To synthesize the thioester prodrug of the adenine analog, the derivative **17** was reacted with thioester **21**[24] in the presence of 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT)[25] to provide the bis(SATE) derivative as a target compound **22** (Scheme 5). Much increased antiviral anti-HIV activity of bis(SATE) analog **22** was observed, which was almost equivalent to that of PMDTA.

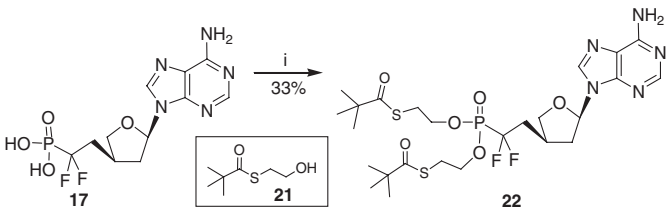
## Conclusion

Based on the potent anti-HIV activities of the 5′,5′-difluoro-nucleosides and threosyl 5′-nornucleoside phosphonic acid analogs, we designed and successfully synthesized novel 5′-deoxy-5′,5′-difluoro-threosyl nucleoside phosphonic acid

**Table 1.** Median effective ($EC_{50}$) and cytotoxic ($CC_{50}$) concentrations of the synthesized nucleoside analogs.

| Compound no. | Anti-HIV-1 $EC_{50}$ (μM)[a] | Cytotoxicity $CC_{50}$ (μM)[b] |
|---|---|---|
| 16 | 28.2 | 98 |
| 17 | 33.4 | >100 |
| 20 | 78.6 | >100 |
| 22 | 5.7 | 90 |
| AZT[a] | 0.002 | >100 |
| PMEA[b] | 1.56 | >100 |

AZT, azidothymidine; PMEA, 9-[2-(phosphonomethoxy)ethyl]adenine.
[a] $EC_{50}$ (μM): $EC_{50}$ values are for 50% inhibition of virus production as indicated by supernatant RT levels.
[b] $CC_{50}$ (μM): $CC_{50}$ values indicate 50% cytotoxic concentration.




Reagents: i) thioester, **21**, 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole, pyridine.

**Scheme 5.** Synthesis of target bis(SATE) prodrug of adenine analog **22**.

analogs from 2-propanone-1,3-diacetate. The bis(SATE) prodrug analog **22** showed significant activity in a cell-based assay compared to the phosphonic acid analogs **16**, **17**, and **20**.

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