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Synthesis of Novel 2′(β)-Methyl-5′-deoxyapiose Nucleoside Phosphonic Acid Analogues as Antiviral Agents

Joon Hee Hong†

Abstract

Novel 2'(β)-methyl-5'-deoxyapiose purine phosphonic acid analogues were designed and synthesized from 2-propanone-1,3-diacetate. Condensation successfully proceeded from a glycosyl donor **9** under Vorbrüggen conditions. Condensation of aldehyde **14** with Wittig reagent [(diethoxyphosphinyl)methylidene] triphenylphosphorane gave the desired nucleoside phosphonate analogues **15**. Ammonolysis and hydrolysis of phosphonates gave the nucleoside phosphonic acid analogue **17** and **19**. The synthesized nucleoside analogues were subjected to antiviral screening against HIV-1. The adenine analogue **19** exhibited weak *in vitro* activities against HIV-1.

Key word: Anti-HIV Agents, 2'-Methyl-5'-deoxyphosphonic Acid Analogue, Vorbrüggen Reaction

1. Introduction

Nonclassical nucleosides continue to be a promising challenge for the development of new antiviral agents since the discovery of lamivudine as anti-human immunodeficiency virus (HIV) and anti-hepatitis B virus (HBV) agent[1]. The apio dideoxynucleoside also belong to the class of nonclassical nucleoside in that 4-hydroxymethyl of the 2.3-dideoxyribose moves to the *C*3 position. This class of nucleosides like (**1**) (Figure 1) showed not only the antiviral activity, but also metabolic advantage such as resistance to adenosine deaminase and glycosyl bond hydrolysis, when compared to the classical 2,3-dideoxynucleosides[2].

Apiose 5'-nornucleoside phosphonates[3], such as, PMDTA (**2**) and PMDTT (**3**), have been assembled from natural precursor molecules. Furthermore, it has been demonstrated apiose nucleic acids (TNA) form thermal stable duplexes with DNA and RNA that are reminiscent of the natural associations of nucleic acids[4]. Moreover, diphosphates of apiose nucleosides are accepted as substrates by several polymerases, and can be enzymatically incorporated into DNA[5]. In addition, these nucleosides are also accepted as substitutes for ribonucleosides at the catalytic site of hammerhead ribozyme, although the catalytic efficiency of ribozyme is then significantly reduced[6]. PMDTA has a phosphonomethoxy group at the 3'-position of its furanose ring and no substituent at the 4'-position. This absence of a 4'-hydroxymethyl group avoids problems of steric hindrance during phosphorylation reactions with kinases.

Recently, 2'(β)-azido-apiose 5'-nornucleoside phosphonic acid (**4**) was synthesized from 2,3-dihydroxy-dihydrofuran-1-one[7]. To study the influences of

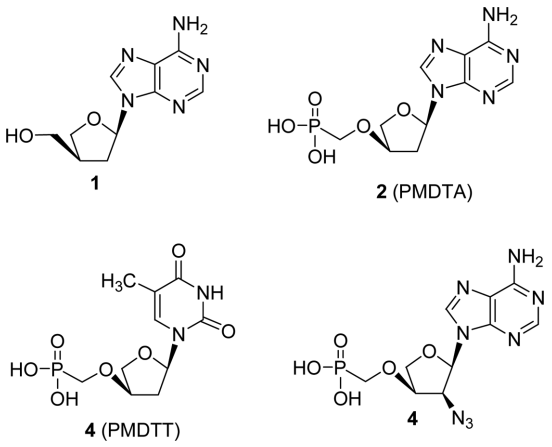

Fig. 1. Synthesis rationale of 2'(β)-methyl-5'-deoxyapiose nucleoside phosphonic acids as potent antiviral agents.

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

†Corresponding author : hongjh@chosun.ac.kr
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different substituents on anti-HIV activity further, we undertook to synthesize 2'(β)-fluorinated analogues of PMDTA.

Stimulated by the findings that apiosyl nucleoside analogues and 5'-nornucleoside phosphonic acids have excellent antiviral activities, we sought to synthesize a novel class of nucleosides consisting of 2'(β)-methyl-apiose nucleoside and its 5'-deoxyphosphonic acid analogues to find therapeutics that are more effective against HIV.

2. Experimental Section

Uncorrected melting points were determined on a Mel-temp II laboratory device. NMR spectra were recorded on 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). UV spectra were obtained on a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). MS spectra were collected in electrospray ionization (ESI) mode. Elemental analyses were performed using a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA). TLC was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were carried out in a nitrogen atmosphere unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

3-Methyl-4-(*t*-butyldimethylsilyloxymethyl)furan-2(5*H*)-one (7): TBDMSCl (2.25 g, 14.99 mmol) was added slowly at 0°C to a solution of **6** (1.74 g, 13.63 mmol) and imidazole (1.86 g, 27.26 mmol) in CH_2Cl_2 (60 mL), and stirred for 4 h at room temperature. The solvent was evaporated under a reduced pressure. The residue was diluted with H_2O (100 mL) and extracted twice with EtOAc (100 mL x2). The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under a reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:8) to give compound **7** (2.77 g, 84%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.90-4.87 (m, 2H), 4.63 (s, 2H), 1.85 (s, 3H), 0.88 (m, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 175.4, 158.6, 123.8, 71.2, 58.9, 25.3, 18.5, 8.7, -4.7.

(*rel*)-(3*S*,4*R*)-4-(*t*-Butyldimethylsilyloxymethyl)-dihydro-3-methylfuran-2(3*H*)-one (8): To a solution of lactone **7** (4.92 g, 20 mmol) in 100 mL of EtOAc was added 1.0 g of Pd/C (5% w/w) under H_2 atmosphere, and the mixture was stirred for 6 h. After filtration of the reaction mixture through a celite pad, the filtrate was concentrated and purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give compound **8** (4.46 g, 88%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.41 (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, 8.4 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.

(*rel*)-(1*R*/*S*,2*S*,3*R*)-3-Methyl-tetrahydro-4-(*t*-butyldimethylsilyloxymethyl)furan-2-yl acetate (9): A solution of compound **8** (1.28 g, 5.25 mmol) in toluene (60 mL) was treated with 10.5 mL of 1 M DIBAL-H in hexane at -78°C for 30 min. The reaction was quenched with 3 mL of MeOH and warmed to room temperature for 1 h and aq $NaHCO_3$ (5 mL) and EtOAc (60 mL) were added to the mixture. The resulting mixture was filtered, and the filtrate was concentrated to dryness. A solution of the crude lactol in CH_2Cl_2 (60 mL) was treated with Ac_2O (1.48 mL, 15.84 mmol), TEA (2.2 mL, 15.84 mmol) and a catalytic amount of 4-DMAP (15 mg) at room temperature for 6 h. The resulting mixture was concentrated and purified by silica gel column chromatography (EtOAc/hexane, 1:20) to give compound **9** (1.03 g, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.36, 6.30 (d and d, *J* = 5.8 and 5.2 Hz, 1H), 3.87 (m, 2H), 3.62 (m, 2H), 2.63-2.59 (m, 1H), 1.94 (m, 1H), 1.07, 1.05 (d, d, *J* = 4.4 Hz, 3H), 0.89 (m, 9H), 0.03 (s, 6H).

(*rel*)-(1′*S*,2′*S*,3′*R*)-9-(3′-*t*-Butyldimethylsilanyloxymethyl-2′-methyl-tetrahydrofuran-1′-yl) 6-chloropurine (10α) and (*rel*)-(1′*R*,2′*S*,3′*R*)-9-(3′-*t*-butyldimethylsilanyloxymethyl-2′-methyl-tetrahydrofuran-1′-yl) 6-chloropurine (10β): 6-Chloropurine (235 mg, 1.52 mmol), anhydrous HMDS (12 mL), and a catalytic amount of ammonium sulfate (15.2 mg) were refluxed

to a clear solution, and the solvent was then distilled off under anhydrous conditions. The residue obtained was dissolved in anhydrous 1,2-dichloroethane (12 mL), and to this mixture, a solution of **9** (290 mg, 0.76 mmol) in dry DCE (12 mL) and TMSOTf (338 mg, 1.52 mmol) was added, and stirred for 6 h at rt. The reaction mixture was quenched with 10.0 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 (100 mL). Combined organic layers were dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAc/hexane/3:1) to give compounds **10α** (101 mg, 35%) and **10β** (96 mg, 33%). Data for **10α**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.70 (s, 1H), 8.31 (s, 1H), 5.97 (d, J = 4.2 Hz, 1H), 3.87-3.85 (m, 2H), 3.62-3.58 (m, 2H), 2.04-1.98 (m, 2H), 1.09 (d, J = 3.8 Hz, 3H), 0.89 (m, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.7, 151.5, 151.2, 144.8, 132.5, 90.6, 67.4, 62.7, 41.2, 33.0, 25.7, 18.4, 10.5, -4.8; Data for **10β**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (s, 1H), 8.30 (s, 1H), 6.02 (d, J = 5.4 Hz, 1H), 3.86 (m, 2H), 3.65-3.62 (m, 2H), 2.09-2.05 (m, 1H), 1.96 (m, 1H), 1.10 (d, J = 4.8 Hz, 3H), 0.88 (m, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.4, 151.0, 150.8, 142.8, 132.7, 92.4, 68.5, 63.8, 42.4, 33.5, 25.4, 18.4, 10.7, -4.8.

(*rel*)-(1′*R*,2′*S*,3′*R*)-9-(3′-*t*-Butyldimethylsilanyloxymethyl-2′-methyl-tetrahydrofuran-1′-yl) adenine (11): A solution of **10β** (244 mg, 0.64 mmol) in saturated methanolic ammonia (10 mL) was stirred overnight at 62°C in a steel bomb, and volatiles were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:10) to give **11** (141 mg, 61%) as a white solid: UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.39 (s, 1H), 8.21 (s, 1H), 5.98 (d, J = 4.2 Hz, 1H), 3.88-3.85 (m, 2H), 3.65-3.62 (m, 2H), 2.04-1.97 (m, 2H), 1.08 (d, J = 4.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.6, 152.1, 147.5, 141.3, 119.3, 93.5, 67.5, 62.5, 41.7, 32.8, 25.4, 18.3, 12.2, -5.1.

(*rel*)-(1′*R*,2′*S*,3′*S*)-9-(3′-Hydroxymethyl-2′-methyl-tetrahydrofuran-1′-yl) adenine (12): To a solution of **11** (675 mg, 1.86 mmol) in THF (8 mL), TBAF (2.8 mL, 1.0 M solution in THF) was added at 0°C. The mixture was stirred overnight at rt and concentrated *in vacuum*. The residue was purified by silica gel column chromatography (Hexane/EtOAc/MeOH, 3:1:0.02) to give **12** (412 mg, 89%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (s, 1H), 8.35 (s, 1H), 6.18 (dd, J = 19.3, 6.2 Hz, 1H), 3.84-3.78 (m, 2H), 3.63-3.59 (m, 2H), 3.38-3.34 (m, 1H), 2.29-2.25 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.3, 152.7, 151.2, 142.5, 118.7, 92.5, 67.7, 62.5, 41.3, 32.7, 12.3; Anal. Calc. for $C_{11}H_{15}N_5O_2$: C, 53.00; H, 6.07; N, 28.10. Found: C, 53.12; H, 6.21; N, 28.17; MS m/z 250 $(M+H)^+$.

(*rel*)-(1′*R*,2′*S*,3′*S*)-9-(3′-Hydroxymethyl-2′-methyl-tetrahydrofuran-1′-yl) 6-chloropurine (13): Compound **13** was synthesized from **10β** by the similar procedure described for **12**: yield 90%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.70 (s, 1H), 8.32 (s, 1H), 5.98 (d, J = 4.4 Hz, 1H), 3.85 (dd, J = 10.2, 6.8 Hz, 1H), 3.64-3.60 (m, 2H), 3.39-3.35 (dd, J = 10.4, 8.2 Hz, 1H), 2.05-1.95 (m, 2H), 1.09 (d, J = 4.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.8, 151.4, 151.0, 142.5, 118.7, 91.2, 66.5, 60.9, 40.7, 32.4, 12.3.

(*rel*)-(1′*R*,2′*S*,3′*R*)-9-(3′-Carbaldehyde-2′-methyl-tetrahydrofuran-1′-yl) 6-chloropurine (14): To a mixture of CrO_3 (1.58 g, 15.93 mmol) in CH_2Cl_2 (25 mL) and DMF (6 mL) was added pyridine (2.61 mL, 32.4 mmol) and stirred for 20 min at rt. To the mixture, nucleoside analogue **13** (1.05 g, 3.97 mmol) was added as a solution in CH_2Cl_2 (12 mL) and DMF (3.0 mL), followed by addition Ac_2O (1.62 g, 15.93 mmol) and stirred for 10 min at rt. The reaction was quenched by addition of ethanol (4.32 mL), further diluted with ethyl acetate (250 mL). The resulting mixture was filtered through a silica gel column topped with a layer of anhydrous Na_2SO_4, and filtrate was concentrated in vacuo to give the crude aldehyde **14**. The crude product was subjected to next reaction without further purification.

(*rel*)-(1′*R*,2′*S*,3′*S*)-Diethyl {9-(3′-vinyl-2′-fluoro-tetrahydrofuran-1′-yl) 6-chloropurine} phosphonate (15): Immediately, after its preparation, a solution of the unpurified previous aldehyde **14** in anhydrous DMSO (28 mL) was treated with Wittig reagent (1.22 g, 3.97 mmol) and the resulting solution was stirred for 20 hr at rt. After the reaction was diluted with distilled water (28 mL) and stirred for 1 h, the mixture was extracted

with CH_2Cl_2 (200 mL) three times. The combined extract was washed with water and then saturated brine. The organic layer was dried over anhydrous Na_2SO_4, concentrated in vacuo, and the residue was purified by column chromatography using a gradient solvent system (1 to 4% CH_3OH in CH_2Cl_2) to give vinylphosphonate **15** (921 mg, 58% in two step yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.69 (s, 1H), 7.24 (s, 1H), 6.66-6.61 (m, 1H), 6.15 (dd, J = 20.6, 17.3 Hz, 1H), 5.92 (d, J = 4.4 Hz, 1H), 4.12-4.08 (m, 4H), 3.85 (dd, J = 9.8, 6.4 Hz, 1H), 3.64 (dd, J = 9.9, 7.6 Hz, 1H), 2.58 (m, 1H), 1.98-1.95 (m, 1H), 1.12-1.08 (m, 9H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 151.6, 151.4, 151.2, 149.7, 145.2, 132.8, 116.2, 91.6, 73.7, 62.3, 61.7, 51.3, 35.6, 15.7, 12.2.

(*rel*)-(1′*R*,2′*S*,3′*S*)-Diethyl {9-(3′-vinyl-2′-methyltetrahydrofuran-1′-yl) adenine} phosphonate (16): A solution of **15** (184 mg, 0.46 mmol) in saturated methanolic ammonia (10 mL) was stirred overnight at 63°C in a steel bomb, and volatiles were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:10) to give **16** (110 mg, 63%) as a white solid: UV (MeOH) λ_{max} 262.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.42 (s, 1H), 8.20 (s, 1H), 6.74-6.69 (m, 1H), 6.17 (dd, J = 19.2, 17.8 Hz, 1H), 5.92 (d, J = 4.6 Hz, 1H), 4.09-4.02 (m, 4H), 3.85 (dd, J = 10.2, 6.4 Hz, 1H), 3.67 (dd, J = 10.2, 8.0 Hz, 1H), 2.57-2.52 (m, 1H), 2.05-1.99 (m, 1H), 1.23-1.18 (m, 9H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.4, 152.6, 150.5, 149.3, 120.6, 118.5, 93.1, 72.8, 61.5, 61.1, 50.3, 35.5, 15.2, 13.5.

(*rel*)-(1′*R*,2′*S*,3′*S*)-9-[(3′-Vinyl-2′-methyl-tetrahydrofuran-1′-yl) adenine]phosphonic acid (17): To a solution of the phosphonate **16** (183 mg, 0.48 mmol) in anhydrous CH_3CN (10 mL) and 2,6-lutidine (1.03 g, 9.6 mmol) was added trimethylsilyl bromide (734 mg, 4.8 mmol). The mixture was heated overnight at 78°C under nitrogen and then concentrated *in vacuo* to give a brown residue, and then co-evaporated from conc-aqueous NH_4OH (2×24 mL). The resultant purified by twice triturating the residue in acetone (10 mL) and removing the acetone by evaporation. The residue so obtained was then purified by preparative reverse-phase chromatography. Lyophilization of the appropriate fraction provided the phosphonic acid salt **17** (95 mg, 58%) as a white salt (ammonium salt): mp 175-177°C; UV (H_2O) λ_{max} 261.0 nm; ^{1}H NMR (D_2O, 300 MHz) δ 8.41 (s, 1H), 8.22 (s, 1H), 6.35 (dd, J = 20.4, 18.0 Hz, 1H), 6.11 (dd, J = 18.0, 12.5 Hz, 1H), 3.85 (dd, J = 10.4, 7.6 Hz, 1H), 3.64 (dd, J = 10.4, 6.2 Hz, 1H), 2.54 (m, 1H), 2.01 (m, 1H), 1.08 (d, J = 3.8 Hz, 3H); ^{13}C NMR (D_2O, 75 MHz) δ 155.4, 152.5, 150.4, 146.3, 142.8, 120.2, 114.7, 92.3, 73.7, 52.0, 37.3, 11.2; HPLC t_R = 10.37; HRMS [M-H]$^+$ req. 324.0647, found 324.0648.

(*rel*)-(1′*R*,2′*S*,3′*S*)-Diethyl {9-(3′-ethyl-2′-methyltetrahydrofuran-1′-yl) adenine} phosphonate (18): A solution of vinyl phosphonate nucleoside analogue **16** (323 mg, 0.85 mmol) in methanol (12 mL) was added 10% Pd/C (24 mg) and cyclohexene (6 mL) under argon. The reaction mixture was refluxed for 24 h. The reaction mixture was filtered through a pad of Celite, evaporated, and purified by silica gel column chromatography using methanol and methylene chloride (10:1) to give ethyl phosphonate analogue **18** (217 mg, 67%) as a white solid: mp 171-173°C; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.39 (s, 1H), 8.19 (s, 1H), 5.98 (d, J = 4.2 Hz, 1H), 4.10-4.06 (m, 4H), 3.85 (dd, J = 9.8, 6.2 Hz, 1H), 3.61-3.57 (m, 1H), 2.13-1.88 (m, 6H), 1.21-1.18 (m, 9H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.6, 153.7, 152.3, 140.4, 117.9, 91.2, 72.2, 62.1, 61.6, 44.7, 36.2, 28.6, 19.5, 14.5, 11.4.

(*rel*)-(1′*R*,2′*S*,3′*S*)-{9-(3′-Ethyl-2′-methyl-tetrahydrofuran-1′-yl) adenine} phosphonic acid (19): Phosphonic acid **19** was synthesized from **18** using hydrolysis conditions identical to that for **17**: yield 58%, mp 173-175°C; UV (H_2O) λ_{max} 262.0 nm; ^{1}H NMR (D_2O, 300 MHz) δ 8.38 (s, 1H), 8.17 (s, 1H), 5.97 (d, J = 4.2 Hz, 1H), 3.81 (dd, J = 10.0, 6.8 Hz, 1H), 3.56 (dd, J = 10.0, 8.2 Hz, 1H), 2.21-2.16 (m, 2H), 2.05-1.95 (m, 4H), 1.09 (d, J = 4.2 Hz, 3H); ^{13}C NMR (D_2O, 75 MHz) δ 155.3, 152.5, 150.7, 142.5, 119.3, 91.7, 71.6, 44.3, 36.2, 28.2, 19.2, 11.7; HPLC t_R = 10.54; HRMS [M-H]$^+$ req. 326.0658, found 326.0659.

Anti-HIV assay

Flat bottom, 96-well plastic microtiter trays were filled with 100 μL of complete medium using a Titertek Multidrop dispenser. This eight-channel dispenser could fill a microtiter tray in less than 10 s. subsequently,

stock solutions (10 x final test concentration) of compounds were added in 25-μL volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on HIV–and mock–infected cells. Serial five-fold dilutions were made directly in the microtiter trays using an eight-channel Titertek^R pipette. Untreated control HIV–and mock–infected cell samples were included for each compound. Exponentially growing MT-4 cells were centrifuged for 10 min at 140 × g and the supernatants were discarded. The pellet was either infected with 100 $CCID_{50}$ or mock-infected. The MT-4 cells were resuspended at 4×10^5 cells/mL in two flasks which were connected with an autoclavable dispensing cassette of a Titertek^R Mul-tidrop dispenser. Under slight magnetic stirring 100-μL volumes were then transferred to the microtiter tray wells. The outer row wells were filled with 100 μL of medium. The cell cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. The cells remained in contact with the test compounds during the whole incubation period. Five days after infection the viability of mock– and HIV–infected cells was examined either microscopically in a hemacytometer by the trypan blue exclusion method or spectrophotometrically by the MTT method.

MTT Assay

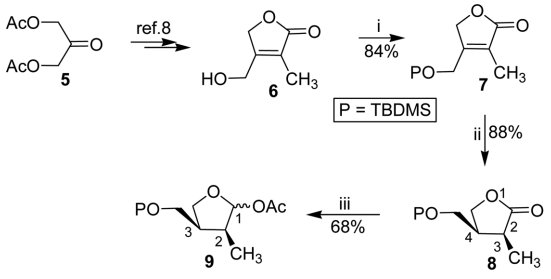
The MTT assay is based on the reduction of the yellow coloured 3-(4,5-dime-thylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenases of metabolically active cells to a blue formasan which can be measured spectrophotometrically. Therefore, to each well of the microtiter trays 20 μL of a solution of MTT (7.5 mg/mL) in phosphate-buffered saline was added using the Titertek^R Multidrop. The trays were further incubated at 37°C in a CO_2 incubator for the indicated times. A fixed volume of medium (150 μL) was then removed from each cup using an eight-channel pipette without disturbing the MT-4 cell clusters containing the formazan crystals. If necessary, this step was preceded by centrifugation of the microtiter trays (450×g, 5 min) in a plate holder (8 trays per run). Solubilization of the formazan crystals was achieved by adding 100 μL 10% (v/v) Triton X-100 in acidified isopropanol (2 mL concentrated HCl per 500 mL solvent) using the Titertek^R Multidrop. Complete dissolution of the formazan crystals could be obtained after the trays had been placed on a plate snaker for 10 min. Finally, the absorbances were read in an eight-channel computer-controlled photometer (Multiskan MCC, Flow Laboratories) at two wavelengths (540 and 690 nm). The absorbance measured at 690 nm was automatically subtracted from the absorbance at 540 nm, so as to eliminate the effects of non-specific absorption. Blanking was carried out directly on the microtiter trays with the first column wells which contained all reagents except for the MT-4 cells. All data (CD_{50}) was defined as the concentration of compound that reduced the absorbance (OD_{540}) of the mock-infected control sample by 50%. The percent protection achieved by the compounds in HIV-infected cells was calculated by the following formula:

$$\frac{(OD_T)_{HIV}-(OD_C)_{HIV}}{(OD_C)_{mock}-(OD_C)_{HIV}} \text{ expressed in \%}$$

whereby $(OD_T)_{HIV}$ is the optical density measured with a given concentration of the test compound in HIV-infected cells; $(OD_C)_{mock}$ is the optical density measured for the control untreated mock-infected cells; all OD values determined at 540 nm. The dose achieving 50% protection according to the above formula was defined as the 50% effective dose (ED_{50}).

3. Results and Discussion

As depicted in Scheme 1, target compounds were prepared from the 2-methylbutenolide **6**, which was readily prepared from 2-propanone-1,3-diacetate **5**, as previously described[8]. 2-Methylbutenolide 7 was hydrogenated to 2-methyllactone **8** by treatment with 5% Pd/C under H_2 in a 90% yield. DIBAL reduction of the 2-



Reagents: i) TBDMS, imidazole, CH_2Cl_2; ii) H_2, Pd/C, EtOAc; iii) (a) DIBALH, toluene; (b) Ac_2O, pyridine.

Scheme 1. Synthesis of methylated apiose glycosyl donor intermediate 9.

methyllactone **8** followed by acetylation using Ac_2O and triethylamine in CH_2Cl_2 gave the key intermediate **9** (Scheme 1). The synthesis of adenine nucleoside was carried out by Vorbrüggen condensation[9] of compound **9** with silylated 6-chloropurine using TMSOTf as a catalyst in DCE to give the protected 6-chloropurine derivatives **10a** and **10b**, respectively. A complete NOE study allowed the unambiguous determinations of their relative stereochemistries (Figure 2). For compound **10b**, strong NOE (1.5%) of H-1′↔CH-3′, which showed a 1′,3′-*cis* relationship, was observed. According to this result, the 3′-hydroxymethyl group and the 1′-purine base of **10b** were located on the β face. On the other hand, for **10a** compound, weak NOE (0.7%) of H-1′↔CH-3′, demonstrating a 1′,3′-*trans* relationship. Transformation of chlorine group of the purine to amine and sequential desilyaltion of corresponding nucleoside gave apiosyl adenine nucleoside **12** (Scheme 2).

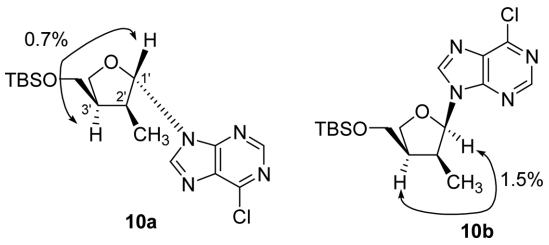
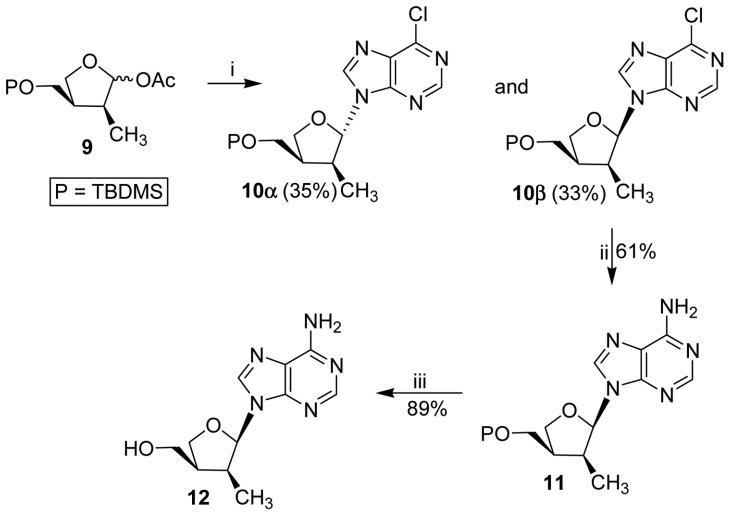


Fig. 2. NOE differences between the proximal hydrogens of **10a** and **10b**.

Moffat oxidation[10] of the alcohol of **13** gave the aldehyde **14**, which was subjected to Wittig condensation[11] using [(diethoxyphosphinyl)methylidene] triphenylphosphorane to give phosphonate nucleoside analogue **15** at a yield of 58%. The chlorine group of the purine phosphonate analogue **15** was then converted to amine with methanolic ammonia at 63°C to give the corresponding adenosine phosphonate derivative **16** at a yield of 59%. Hydrolysis of the diethyl phosphonate functional groups of **16** by treatment with bromotrimethylsilane in CH_3CN in the presence of 2,6-lutidine then gave the adenosine phosphonic acid derivative **17**[12]. When the vinylidene phosphonate **16** was saturated under transfer catalytic hydrogenation conditions[13], it produced the ethyl phosphonate nucleoside analogue **18** at a yield of 67%. The adenosine phosphonic acid analogue **19** was prepared using conditions similar to the ammonolysis and hydrolysis described to produce **17** (Scheme 3).

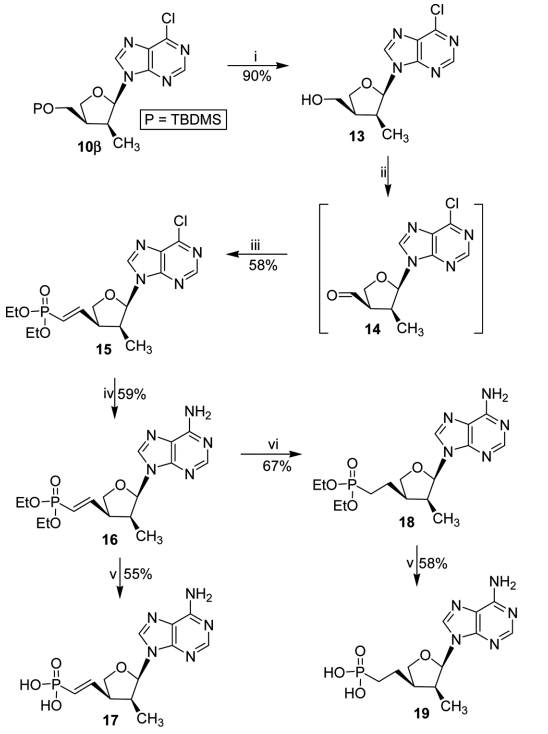
Anti-HIV Activity

A rapid, sensitive and automated assay procedure was developed for the in vitro evaluation of anti-HIV agents[14]. The synthesized nucleoside, its phosphonic acids **12**, **17** and **19** were then evaluated for antiviral activity against human immunodeficiency virus[15]. As



Reagents: i) Silylated 6-chloropurine, TMSOTf, DCE; ii) NH_3/MeOH; iii) TBAF, THF.

Scheme 2. Synthesis of 2′(β)-methyl-apiose adenosine analogue.



Reagents: i) TBAF, THF; ii) CrO_3, pyridine, DMF, CH_2Cl_2; iii) $(EtO)_2P(O)CH=PPh_3$; iv) NH_3/MeOH; v) TMSBr, 2,6-lutidine, CH_3CN; vi) Pd/C, cyclohexene, MeOH.

Scheme 3. Synthesis of 2'(β)-methyl-5'-deoxyapiose phosphonic acid adenosine analogues.

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

Compound No.	Anti-HIV-1 EC50 (μM)[c]	Cytotoxicity CC50 (μM)[d]
12	84.2	>100
17	77.1	>100
19	**36.7**	**95**
AZT[a]	0.003	>100
PMEA[b]	0.32	>100

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

shown in Table 1, adenine nucleoside phosphonic acid **19** exhibits weak anti-HIV activity. However, nucleoside analogues **12** and **17** did not show anti-HIV activity or cytotoxicity at concentrations up to 100 μM.

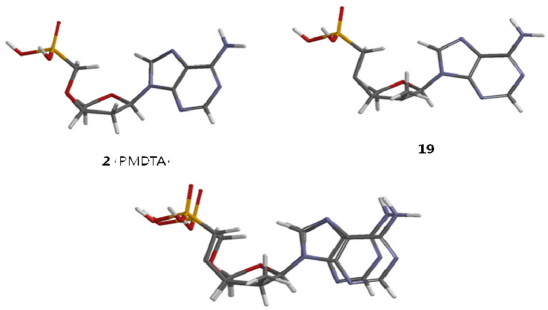


Fig. 3. Superimpose of PMDTA(**2**) and **19**.

Conclusion

Based on the potent anti-HIV activity of 5′-norapiosenucleoside phosphonic acid analogues, we have designed and successfully synthesized novel 5′-norcarbocyclic nucleoside analogues starting from 2-propanone-1,3-diacetate **5**. Although the adenine 5′-norapiose nucleoside phosphonic acid analogue **2** inhibits *in vitro* anti-HIV activity comparable to that of PMEA, the synthesized 2′-branched-5′-deoxyversions did not show anti-HIV activity. Only adenine analogue **19** showed weak anti-HIV activity. Since apiofurans are not perfect mimics for ribofuranose moiety, the mechanisms of virus inhibition, that is, either phosphorylation or inhibition of RNA synthesis, might be impaired in these compounds. Methylation of 2′-position is another possible reason for the apparent lack of activity. Figure 3 shows the superposition of the calculated low energy conformers of **2** and **19**, highlighting closer position parts such as phosphonic acid parts and purine bases moieties[16].

Acknowledgment

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[16] All geometries were optimized with the framework of the density functional theory (DFT), with Spartan modeling software. The B3LYP functional with 6-31G* basis set was employed.