Efficient Synthesis of Novel 4'-Trifluoromethyl-5'-norcarbocyclic Purine Phosphonic Acid Analogs by Using the Ruppert-Prakash Reaction

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Novel 4'-trifluoromethyl-5'-norcarbocyclic purine phosphonic acid analogs were efficiently synthesized from commercially available 1,3-dihydroxy cyclopentane (5). Trifluoromethylation was successfully performed by using the Ruppert-Prakash reaction. The purine nucleosidic bases were efficiently coupled by using the Mitsunobu reaction. The synthesized adenosine phosphonic acids analogs 13 and 16 were screened for antiviral activity against human immunodeficiency virus-1 (HIV-1). Adenine derivative 13 exhibited significant anti-HIV-1 activity.

**Key Words**: Anti-HIV agents, 4'- Branched carbocyclic nucleoside, Phosphonic acid nucleosides

**Introduction**

4'-Branched-5'-norcarbocyclic phosphonic acid analogs, such as 4'-vinyl-cpAP (1) and 4'-ethynyl-cpAP (2), have encouraged the search for novel nucleosides as potential anti-human immunodeficiency virus (HIV) agents among this class of compounds. Molecular modeling studies demonstrated the presence of a hydrophobic 4'-pocket that could accommodate these substitutions and enhance anti-HIV activity.

Although monofluorinated and gem-difluorinated nucleosides have been widely studied, only a few trifluoromethylated nucleosides have been reported, which is probably because of the limitations of existing synthesis methods. The presence of a CF$_3$ group on the sugar moiety of nucleosides could confer many advantages including increased lipophilicity and improved chemical and/or enzymatic stability. In addition, the trifluoromethyl group can enhance the therapeutic properties of bioactive compounds. There has been increased interest in introducing a trifluoromethyl group into nucleosides in order to discover new nucleoside derivatives with high antiviral activities. Li et al. (2001) reported the first synthesis of 2'-C-β-trifluoromethyl pyrimidine ribonucleoside (3) with the Ruppert-Prakash reagent. Johnson and Kozak successfully synthesized a 4'-trifluoromethylated nucleoside analog (4) by introducing a CF$_3$ group into the C-4' position of ribose derivatives (Figure 1).

Phosphonate has certain advantages over its phosphate counterpart, as it is metabolically stable because of its phosphorus-carbon bond, which is not susceptible to hydrolytic cleavage. The spatial location of the oxygen atom, the β-position from the phosphorus atom in the nucleoside analog, is critical for antiviral activity. The increased antiviral activity conferred by the oxygen atom may be attributed to the increased binding capacity of the phosphonate analog to target enzymes. Moreover, a phosphonate nucleoside does not require the first phosphorylation, which is a crucial step for the activation of nucleosides. This is frequently a limiting step in the phosphorylation sequence, which ultimately leads to triphosphates.

Given that 4'-branched nucleoside analogs and 5'-norcarbocyclic nucleoside phosphonate have excellent biological activities, we aimed to synthesize a novel class of nucleosides, including 4'-trifluoromethyl-5'-norcarbocyclic phosphonic acid analogs, in order to identify more effective therapeutics against HIV and to provide analogs for probing the conformational preferences of enzymes associated with the nucleoside kinases of nucleosides and nucleotides.

As depicted in Scheme 1, the target compounds were readily prepared from 1,3-dihydroxy cyclopentane (5). Selective monosilylation of diol 5 produced alcohol derivative 6, which was oxidized to the ketone 7 by using Dess-Martin conditions. Lavaire et al. (1996) reported fluoride-induced trifluoromethylation conditions by using the Ruppert-Prakash reagent, in which the tert-butyldimethylsilyl (TBDMS) protective group is retained. The ketone 7 was subjected to nucleophilic addition conditions with CF$_3$SiMe$_2$/t-butyl-
ammonium fluoride (TBAF) followed by treatment with NaOMe in MeOH, and to give the cyclopentanols $8a$ (34%) and $8b$ (35%). The stereochemical assignment of $8a$ and $8b$ as $\alpha$ and $\beta$ anomers, respectively, was determined by the $^{19}$F-$^1$H nuclear Overhauser effects (NOE) experiments. For $8b$, we observed strong NOE signals for $^1$H when the $^{19}$F nuclei were irradiated (Figure 2).

The hydroxyl functional group of $8b$ was treated with diethylphosphonomethyl triflate $17$ by using lithium $\beta$-butoxide to yield the phosphonate analog $9$ (Scheme 2). Removal of the silyl protective group of $9$ by using TBAF produced the secondary alcohol $10$. To synthesize the desired 5'-norcarbocyclic adenosine nucleoside analogs, the cyclopentanol $10$ was treated with 6-chloropurine under Mitsunobu conditions $18$ [diethyl azodicarboxylate (DEAD) and PPh$_3$]. Slow addition of DEAD to a mixture of cyclopentanol $10$, triphenylphosphine, and the 6-chloropurine in anhydrous tetrahydrofuran (THF) solvent produced a yellow solution, which was stirred for 2 h at $-40 \, ^\circ$C and further stirred overnight at room temperature to produce the protected 6-chloropurine analog $11$ as a single $N^9$-regioisomer (UV [MeOH] $\lambda_{\text{max}}$ 264.0 nm). The chlorine group of $11$ was then converted to an amine group with methanolic ammonia at 72 $^\circ$C to produce the corresponding adenine phosphonate derivative $12$. Hydrolysis of $12$ by treatment with bromotrimethylsilane (TMSBr) in CH$_3$CN in the presence of 2,6-lutidine produced an adenine phosphonic acid derivative $13$ (Scheme 2).

For the synthesis of guanine analogs, 2-fluoro-6-chloropurine $21$ was condensed with alcohol derivative $10$ under similar coupling conditions as those used for the condensation of 6-chloropurine to produce the 2-fluoro-6-chloropurine analog $14$ (61% yield). Bubbling ammonia into compound $14$ produced the 2-fluoro-6-aminopurine analog $22$ $15a$ and 2-amino-6-chloropurine analog $15b$ with 12% and 42% yields, respectively. The 2-amino-6-chloropurine derivative $15b$ was treated with TMSBr and 2,6-lutidine to produce the corresponding phosphonic acid, which was successively treated with sodium methoxide and 2-mercaptoethanol in MeOH resulting in the desired guanine phosphonic acid $16$ (Scheme 3).

The synthesized nucleoside phosphonic acid analogs $13$ and $16$ were then evaluated for antiviral activity against...
The antiviral activity of phosphonate nucleosides is due to their intracellular metabolism to diphosphates followed by incorporation into the viral genome and chain termination. Anti-HIV activity was determined in human peripheral blood mononuclear (PBMC) cells infected with HIV-1 strain LAI. PBMC cells (1 × 10⁶ cell/mL) were infected with HIV-1 at a multiplicity of infection of 0.02 and cultured in the presence of different concentrations of the test compounds. After 4 days of incubation at 37 °C, numbers of viable cells were determined by using the 3-(4,5-di-methylthiazole-2-yl)-2,5-diphenyltetrazolium bromide method. The cytoxicity of the compounds was evaluated in parallel with their antiviral activity, based on the viability of mock-infected cells. In particular, the adenine analog 13 showed significant anti-HIV-1 activity (Table 1), indicating diphosphorylation of the sugar moiety of the analog or some affinity of viral polymerases for its diphosphate. However, guanine nucleoside analog 16 showed weak anti-HIV activity at concentrations of up to 100 μM.

In summary, we have designed and successfully synthesized novel 4'-trifluoromethyl-5'-norcarbocyclic phosphonic acid nucleoside analogs starting from 1,3-dihydroxy cyclopentane (5). The adenine analog 13 exhibited significant antiviral activity against HIV-1 (EC₅₀ = 8.4 μM).

### Experimental Section

Melting points (mp) were determined by using a Meltemp II laboratory device and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL 300 Fourier transform spectrometer (JEOL, Tokyo, Japan). Chemical shifts are reported in parts per million (d) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doubles). Ultraviolet (UV) spectra were obtained on a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). Mass spectrometry (MS) spectra were collected in the electrospray ionization mode. The elemental analyses were performed by using a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA). Thin layer chromatography was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were carried out under a nitrogen atmosphere unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH₂. Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

### Table 1. Anti-HIV activity of synthesized compounds

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The solvent was concentrated under high vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:8) to produce 8a (695 mg, 34%) and 8b (760 mg, 35%) as oils. Compound 8a: 'H NMR (CDCl3, 300 MHz) δ 3.25-3.23 (m, 1H), 1.90-1.56 (m, 6H), 0.90 (s, 9H), 0.02 (s, 6H); 13C NMR (CDCl3, 75 MHz) δ 140.4 (q, J = 283.7 Hz), 80.2 (q, J = 30.4 Hz), 68.5, 35.2, 29.8, 25.5, 19.3, 18.4, -5. Analysis. Calcd. for C11H16O: C, 65.17; H, 8.21; MS m/z 285 (M+H)+.

Compound 8b: 'H NMR (CDCl3, 300 MHz) δ 3.37-3.34 (m, 1H), 1.91-1.55 (m, 6H), 0.89 (s, 1H), 0.02 (s, 6H); 13C NMR (CDCl3, 75 MHz) δ 139.7 (q, J = 282.8 Hz), 79.5 (q, J = 31.8 Hz), 66.8, 36.2, 30.2, 25.7, 19.5, 18.1, -4.4. Analysis. Calcd. for C11H16F3O5Si: C, 50.68; H, 8.15; Found: C, 50.77; H, 8.21; MS m/z 285 (M+H)+.

(ref)-(1R,4R)-Diethyl 1-(β-Butyldimethylsilanyloxy)-4-[(trifluoromethyl) cyclopentanoyloxy] methylphosphonate (9). Both LiOt-Bu (3.172 mL of 0.5 M solution in THF, 1.586 mmol) and a solution of diethyl phosphonomethyl-triflate (475 mg, 1.586 mmol) in 12.0 mL of THF were slowly added to a solution of the 8b analog (225 mg, 0.793 mmol) in 6.0 mL of THF at 0 °C and further diluted with additional H2O (100 mL). The aquaeous layer was extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with saturated NH4Cl solution (5 mL) and 2,6-lutidine (1.09 mL, 9.38 mmol). The mixture was quenched by adding saturated NH4Cl solution (5 mL) and 2,6-lutidine (1.09 mL, 9.38 mmol). The residue was purified by silica gel column chromatography (EtOAc/hexane, 3:1) to produce compound 11 (226 mg, 69%): mp 176-178 °C; UV (MeOH) λmax 263.0 nm; 'H NMR (DMSO-d6, 300 MHz) δ 8.80 (s, 1H), 8.33 (s, 1H), 4.35 (m, 1H), 3.84 (d, J = 8.2 Hz, 2H), 3.75 (m, 1H), 2.21-1.52 (m, 6H), 1.12 (m, 6H); 13C NMR (DMSO-d6, 75 MHz) δ 151.7, 151.6, 151.4, 145.2, 137.6 (q, J = 282.8 Hz), 132.5, 85.3 (q, J = 28.6 Hz), 63.0, 62.5, 61.9, 52.5, 26.7, 23.5, 17.5, 15.2. Analysis. Calcd. for C16H25ClF5N2O5P (+1.0 MeOH): C, 41.83; H, 5.16; N, 11.48; Found: C, 41.74; H, 5.25; N, 11.53; MS m/z 457 (M+H)+.

(ref)-(1'S,4'R)-Diethyl 9-[4-(trifluoromethyl)cyclopentanoyloxy]-1'-yl) adenine methylphosphonate (12). A solution of 11 (220 mg, 0.482 mmol) in saturated methanolic ammonia (12 mL) was stirred overnight at 72 °C in a steel bomb and the volatiles were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH2Cl2, 1:8) to produce 12 (140 mg, 62%) as a white solid: mp 163-165 °C; UV (MeOH) λmax 261.0 nm; 'H NMR (DMSO-d6, 300 MHz) δ 8.28 (s, 1H), 8.13 (s, 1H), 4.37-4.34 (m, 1H), 3.87 (d, J = 8.1 Hz, 2H), 3.72 (m, 1H), 2.19-1.49 (m, 6H), 1.11 (m, 6H); 13C NMR (DMSO-d6, 75 MHz) δ 155.5, 152.7, 150.5, 141.4, 137.3 (q, J = 283.2 Hz), 120.1, 86.1 (q, J = 30.2 Hz), 62.6, 61.8, 52.2, 26.7, 22.5, 17.5, 13.9. Analysis. Calcd. for C16H25F3N2O5P (+1.0 MeOH): C, 43.52; H, 5.80; N, 14.93; Found: C, 43.65; H, 5.73; N, 14.85; MS m/z 438 (M+H)+.

(ref)-(1'S,4'R)-Diethyl 9-[4-(4'-Trifluoromethylcyclopentanoyloxy)-1'-yl)-adenine] 4'-Methylphosphonic Acid (13). TMSBr (0.619 mL, 4.69 mmol) was added to a solution of phosphonate 12 (205 mg, 0.469 mmol) in anhydrous CH2Cl2 (10 mL) and 2,6-lutidine (1.09 mL, 9.38 mmol). The mixture was heated overnight at 72 °C under nitrogen and then concentrated under vacuum. The residue was co-evaporated from concentrated aqueous ammonium hydroxide (NH4OH; 2 × 20 mL) and purified by triturating with acetone (10 mL) twice and removing the acetone by evaporation. The residue was then purified by preparative reverse-phase column chromatography using C18 silica gel. Lyophilization of the appropriate fraction produced phosphonic acid salt 13 (128 mg, 69% yield) as a white salt (ammonium salt): UV (H2O) λmax 262.0 nm; 'H NMR (D2O, 300 MHz) δ 8.38 (s, 1H), 8.19 (s, 1H), 3.81 (d, J = 8.2 Hz, 2H), 3.72 (m, 1H), 2.15-1.51 (m, 6H); 13C NMR (D2O, 75 MHz) 155.7, 152.9, 150.8, 141.6, 138.5 (q, J = 281.8 Hz), 120.5, 85.9 (q, J = 29.4 Hz), 63.2, 52.2, 26.7, 22.5, 18.0; High-performance liquid chromatography (HPLC), tR = 10.26 min; High-resolution mass spectrometry (HRMS) [M − H]+ calcd. 380.0692, found 380.0693.

(ref)-(1'S,4'R)-Diethyl 9-[4'-Trifluoromethylcyclopentanoyloxy]-1'-yl)-2-fluoro-6-chloropurine] Methylphosphonate (14). Coupling of 10 with 2-fluoro-6-chloropurine was accomplished by using a similar Mitsunobu reaction as described for the synthesis of 11: yield 61%; mp 176-178 °C; UV (MeOH) λmax 270.5 nm; 'H NMR (DMSO-d6, 300 MHz) δ 8.45 (s, 1H), 4.21-4.15 (m, 4H), 3.81 (d, J = 10.5 Hz)
4'-Trifluoromethyl-5'-norcarbocyclic Purine Analogs


Hz, 2H), 3.69 (m, 1H), 2.15-1.50 (m, 6H), 1.14 (m, 6H); 13C NMR (DMSO- d6, 75 MHz) δ 158.4, 145.2, 137.3 (q, J = 30.4 Hz), 62.7, 62.4, 61.5, 52.2, 26.7, 23.1, 18.2, 13.5. Anal. Calcd. for C13H12F2NO3P (+0.5 MeOH): C, 41.91; H, 5.38; N, 14.37; Found: C, 41.88; H, 5.28.

Data for 15b: Dry ammonia gas was bubbled into a solution of 14 (310 mg, 0.65 mmol) in methanol (15 mL) while stirring at room temperature overnight. The salts were removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/CH2Cl2, 1:10) to produce 15a (35 mg, 12%) and 15b (128 mg, 42%).

References


