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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**)[1] and 4'-ethynyl-cpAP (**2**),[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.[1]

Although monofluorinated[3] and *gem*-difluorinated[4] nucleosides have been widely studied, only a few trifluoromethylated[5] 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[6] and improved chemical and/or enzymatic stability.[7] In addition, the trifluoromethyl group can enhance the therapeutic properties of bioactive compounds.[8] 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.[9] 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).[10]

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.[11] 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.[12] 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.[13]

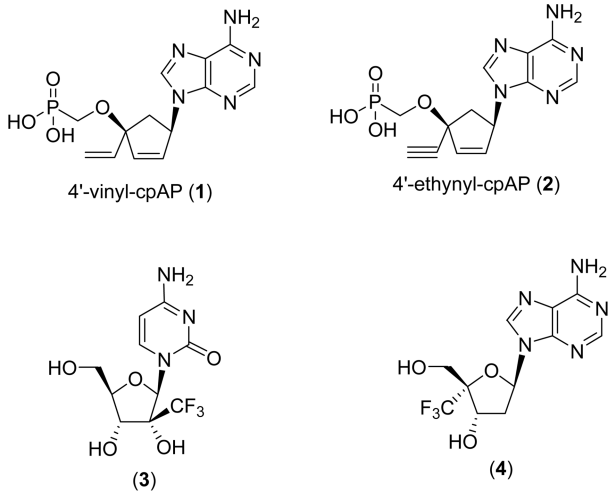
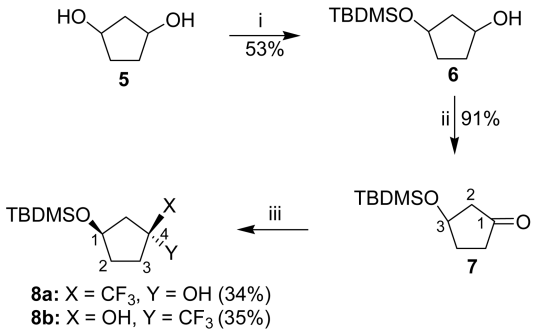


Figure 1. Design rationale of 4'-trifluoromethyl-5'-norcarbocyclic phosphonic acid nucleoside analogs.

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.[14] 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.[15] The ketone **7** was subjected to nucleophilic addition conditions[16] with CF_3SiMe_3/*t*-butyl-



Reagents: i) TBDMSCl, imidazole, CH_2Cl_2, -10 °C; ii) Dess-Martin periodinane, CH_2Cl_2; iii) (a) CF_3SiMe_3, TBAF (cat), THF; (b) Na (cat), MeOH.

Scheme 1. Synthesis of trifluoromethylated cyclopentanol intermediate.

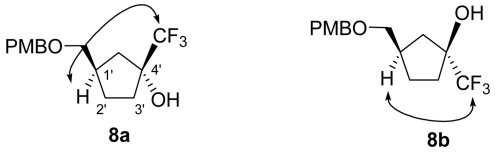
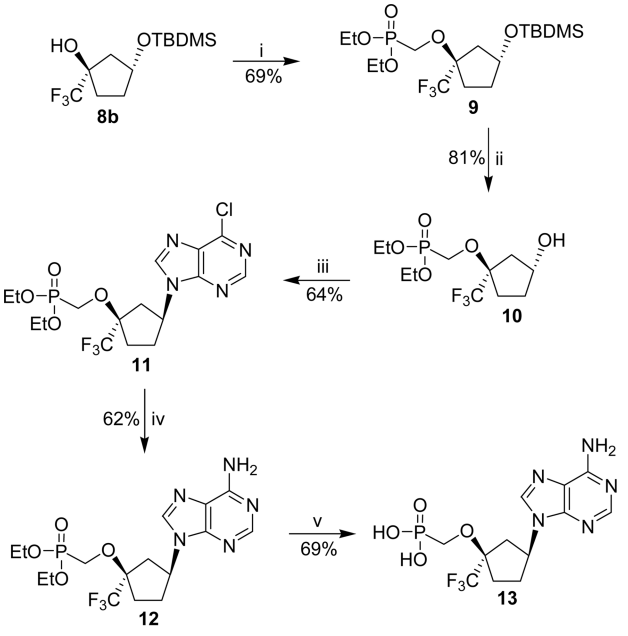


Figure 2. ^{19}F-1H NOE differences between the proximal hydrogens of **8a** and **8b**.

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 α and β 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).

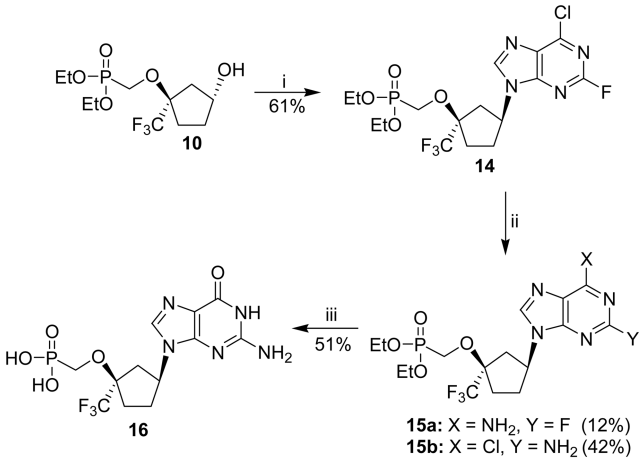
The hydroxyl functional group of **8b** was treated with diethylphosphonomethyl triflate[17] by using lithium *t*-butoxide to yield the phosphonate analog **9** (Scheme 2). Removal of the silyl protective group of **9** by using TBAF produced the secondary alcohol **10**. To synthesize the desired 5'-norcarbocyclic 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 °C and further stirred overnight at room temperature to produce the protected 6-chloropurine analog **11** as a single N^9-regioisomer (UV [MeOH] λ_{max} 264.0 nm).[19] The chlorine group of **11** was then converted to an amine group with methanolic ammonia at 72 °C to produce the corresponding adenine phosphonate derivative **12**. Hydrolysis of **12** by treatment with bromotrimethylsilane (TMSBr) in CH_3CN in the presence of 2,6-lutidine produced an adenine phosphonic acid derivative **13** (Scheme 2).[20]

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



Reagents: i) $(EtO)_2POCH_2OTf$, LiO-*t*-Bu, THF; ii) TBAF, THF; iii) 6-chloropurine, DEAD, PPh_3, THF; iv) NH_3/MeOH, 70 °C; v) TMSBr, 2,6-lutidine, CH_3CN.

Scheme 2. Synthesis of 5'-norcarbocyclic adenine phosphonic acid.



Reagents: i) 2-fluoro-6-chloropurine, DEAD, PPh_3, THF; ii) iv) NH_3/DME, 70 °C; iii) (a) TMSBr, 2,6-lutidine, CH_3CN; (b) NaOMe, $HSCH_2CH_2OH$, MeOH.

Scheme 3. Synthesis of 5'-norcarbocyclic guanine phosphonic acid.

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).[23]

The synthesized nucleoside phosphonic acid analogs **13** and **16** were then evaluated for antiviral activity against

Table 1. Anti-HIV activity of synthesized compounds

Compound No.	anti-HIV EC_{50} (μM)[c]	Cytotoxicity CC_{50} (μM)[d]
13	8.4	90
16	39.2	98
AZT[a]	0.009	100
PMEA[b]	0.54	10

[a]**AZT**: azidothymidine. [b]**PMEA**: 9-(2-[phosphonomethoxy]ethyl)adenine. [c]EC_{50} (μM): Concentration (μM) required to inhibit the replication of HIV-1 by 50%. [d]CC_{50} (μM): Concentration (μM) required to reduce the viability of unaffected cells by 50%

HIV-1. The antiviral activity of phosphonate nucleosides is due to their intracellular metabolism to diphosphates followed by incorporation into the viral genome and chain termination.[24] Anti-HIV activity was determined in human peripheral blood mononuclear (PBM) cells infected with HIV-1 strain *LAI*. PBM cells (1×10^5 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 cytotoxicity of the compounds was evaluated in parallel with their antiviral activity, based on the viability of mock-infected cells.[25] 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 (EC_{50} = 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 doublets). 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 CaH_2. Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

(*rel*)-(1*S* and 1*R*,3*S*)-3-(*t*-Butyldimethylsilanyloxy) cyclopentanol (6). TBDMSCl (2.29 g, 15.25 mmol) was added slowly to a solution of **5** (1.41 g, 13.87 mmol) and imidazole (1.41 g, 20.80 mmol) in CH_2Cl_2 (100 mL) at −10 °C and stirred for 7 h at the same temperature. Saturated $NaHCO_3$ solution (10 mL) was poured into the mixture and stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in water (200 mL) and extracted with diethyl ether (200 mL). The organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to produce compound **6** (1.58 g, 53%) as an isomeric mixture: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.27-3.20 (m, 2H), 1.98-1.52 (m, 6H), 0.89 (m, 9H), 0.02 (m, 6H).

(±)-3-(*t*-Butyldimethylsilanyloxy) Cyclopentanone (7). Compound **6** (2.43 g, 11.25 mmol) was added to a solution of Dess-Martin periodinane (10.38 g, 24.5 mmol) in CH_2Cl_2 (100 mL) at 0 °C, and stirred for 24 h at room temperature under argon gas. The solvent was removed and the residue was triturated with diethyl ether (150 mL). Following filtration through a pad of silica gel, the organic solution was washed with a solution of sodium thiosulfate pentahydrate (13 g) in water (100 mL), ice-cold saturated $NaHCO_3$ (80 mL), and brine (80 mL) and dried over $MgSO_4$. The solvent was filtered, concentrated under vacuum, and purified by silica gel column chromatography (EtOAc/hexane, 1:10) to produce compound **7** (2.19 g, 91%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.76-3.74 (m, 1H), 2.34-2.02 (m, 6H), 0.89 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 215.6, 63.5, 52.6, 37.1, 31.5, 25.7, 18.4, −4.7. Anal. Calcd. for $C_{11}H_{22}O_2Si$: C, 61.63; H, 10.34; Found: C, 61.72; H, 10.26; MS *m/z* 215 $(M+H)^+$.

(*rel*)-(1*R*,4*S*)-1-(*t*-Butyldimethylsilanyloxy)-4-(trifluoromethyl)cyclopentanyl-4-ol (8a) and (*rel*)-(1*R*,4*R*)-1-(*t*-butyldimethylsilanyloxy)-4-(trifluoromethyl)cyclopentanyl-4-ol (8b). A catalytic amount of TBAF (0.37 mL, 1 M solution in THF) was added into mixture **7** (1.54 g, 7.2 mmol) with trifluoromethylsilane (25 mL, 12.5 mmol, 0.5 M solution in THF) in dry THF (10 mL) at −78 °C under argon gas. The reaction mixture was allowed to warm to room temperature. The solution became yellow immediately upon the addition of TBAF and eventually became dark brown. After the reaction mixture was stirred for 15 h at room temperature, it was washed with saturated NH_4Cl solution. The aqueous layer was extracted with Et_2O (2 × 20 mL) and the combined organic layer was dried with magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography. Without further purification, the crude residue was dissolved in methanol (10 mL) and a catalytic amount (0.1 eq) of metallic Na (17 mg) was added at 0 °C. The reaction was completed within 1 h, the reaction mixture was treated with a saturated NH_4Cl solution, and the aqueous layer was extracted with Et_2O. The combined organic layer was dried over Na_2SO_4 and filtered.

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**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.25-3.23 (m, 1H), 1.90-1.56 (m, 6H), 0.90 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 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. Anal. Calcd. for $C_{12}H_{23}F_3O_2Si$: C, 50.68; H, 8.15; Found: C, 50.77; H, 8.21; MS *m/z* 285 $(M+H)^+$.

Compound 8b: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.37-3.34 (m, 1H), 1.91-1.55 (m, 6H), 0.89 (s, 1H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 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. Anal. Calcd. for $C_{12}H_{23}F_3O_2Si$: C, 50.68; H, 8.15; Found: C, 50.56; H, 8.08; MS *m/z* 285 $(M+H)^+$.

(*rel*)-(1*R*,4*R*)-Diethyl 1-(*t*-butyldimethylsilanyloxy) 4-[(trifluoromethyl) cyclopentanyloxy] methylphosphonate (9). Both Li*O*t-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 stirred overnight at room temperature under anhydrous conditions. The mixture was quenched by adding saturated NH_4Cl solution (5 mL) and further diluted with additional H_2O (100 mL). The aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Hexane/EtOAc, 4:1) to produce **9** (237 mg, 69%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.38 (m, 4H), 3.94 (d, J = 8.0 Hz, 2H), 3.26 (m, 1H), 1.89-1.49 (m, 6H), 1.13 (m, 6H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 138.4 (q, J = 281.4 Hz), 85.3 (q, J = 28.2 Hz), 70.1, 62.8, 62.3, 32.1, 30.5, 25.3, 18.6, 17.4, 13.7, −4.6. Anal. Calcd. for $C_{17}H_{34}F_3O_5PSi$: C, 46.99; H, 7.89; Found: C, 47.11; H, 7.95; MS *m/z* 435 $(M+H)^+$.

(*rel*)-(1*R*,4*R*)-Diethyl [4-(trifluoromethyl) cyclopentanyloxy] methylphosphonate (10). TBAF (0.60 mL, 1.0 M solution in THF) was added to a solution of **9** (235 mg, 0.54 mmol) in THF (8 mL) at 0 °C. The mixture was stirred overnight at room temperature and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane/MeOH, 1:4:0.02) to produce **10** (140 mg, 81%) as an oil: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 4.41-4.38 (m, 4H), 3.87 (d, J = 8.1 Hz, 2H), 3.31 (m, 1H), 1.94-1.52 (m, 6H), 1.11 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 137.6 (q, J = 281.8 Hz), 84.3 (q, J = 29.7 Hz), 70.9, 62.4, 61.8, 31.3, 29.5, 17.3, 13.2. Anal. Calcd. for $C_{11}H_{20}F_3O_5P$: C, 41.26; H, 6.29; Found: C, 41.19; H, 6.34; MS *m/z* 321 $(M+H)^+$.

(*rel*)-(1'*S*,4'*R*)-Diethyl [9-(4'-trifluoromethylcyclopentanyloxy-1'-yl)-6-chloropurine] Methylphosphonate (11). DEAD (245 mg, 1.412 mmol) was added dropwise at −40 °C to a solution containing compound **10** (226 mg, 0.706 mmol), triphenylphosphine (555 mg, 2.118 mmol), and 6-chloropurine (218 mg, 1.412 mmol) in anhydrous THF (15.0 mL) for 10 min under nitrogen. The reaction mixture was stirred for 4 h at the same temperature under argon and further stirred overnight at room temperature. The solvent was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/hexane, 3:1) to produce compound **11** (206 mg, 64%): mp 171-173 °C; UV (MeOH) λ_{max} 263.0 nm; ^{1}H NMR (DMSO-d_6, 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); ^{13}C NMR (DMSO-d_6, 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. Anal. Calcd. for $C_{16}H_{21}ClF_3N_4O_4P$ (+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 $(M+H)^+$.

(*rel*)-(1'*S*,4'*R*)-Diethyl [9-(4'-trifluoromethylcyclopentanyloxy-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 ºC in a steel bomb and the volatiles were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:8) to produce **12** (130 mg, 62%) as a white solid: mp 163-165 ºC; UV (MeOH) λ_{max} 261.0 nm; ^{1}H (DMSO-d_6, 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); ^{13}C NMR (DMSO-d_6, 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. Anal. Calcd. for $C_{16}H_{23}F_3N_5O_4P$ (+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 $(M+H)^+$.

(*rel*)-(1'*S*,4'*R*)-[9-(4'-Trifluoromethylcyclopentanyloxy-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 CH_3CN (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 (NH_4OH; 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 (H_2O) λ_{max} 262.0 nm; ^{1}H NMR (D_2O, 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); ^{13}C NMR (D_2O, 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-performace liquid chromatography (HPLC), t_R = 10.26 min; High-resolution mass spectrometry (HRMS) $[M - H]^+$ calcd. 380.0692, found 380.0693.

(*rel*)-(1'*S*,4'*R*)-Diethyl [9-(4'-Trifluoromethylcyclopentanyloxy-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) λ_{max} 270.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.45 (s, 1H), 4.21-4.15 (m, 4H), 3.81 (d, J = 10.5

Hz, 2H), 3.69 (m, 1H), 2.15-1.50 (m, 6H), 1.14 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ157.6 (d, J = 220.5 Hz), 154.1, 145.2, 137.3 (q, J = 281.5 Hz), 136.5, 121.3, 86.7 (q, J = 30.4 Hz), 62.7, 62.4, 61.5, 52.2, 26.7, 23.1, 18.2, 13.5. Anal. Calcd. for $C_{16}H_{20}ClF_4N_4O_4P$ (+0.5 MeOH): C, 40.43; H, 4.52; N, 11.43; Found: C, 40.50; H, 4.48; N, 11.49; MS m/z 475 $(M+H)^+$.

(*rel*)-(1'*S*,4'*R*)-Diethyl [9-(4'-Trifluoromethylcyclopentanyloxy-1'-yl) 2-Fluoro-6-aminopurine] Methylphosphonate (15a) and (*rel*)-(1'*S*,4'*R*)-Diethyl [9-(4'-Trifluoromethylcyclopentanyloxy-1'-yl) 2-Amino-6-chloropurine] Methylphosphonate (15b). Dry ammonia gas was bubbled into a solution of **14** (310 mg, 0.65 mmol) in dimethoxyethane (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/CH_2Cl_2, 1:10) to produce **15a** (35 mg, 12%) and **15b** (128 mg, 42%).

Data for 15a: UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.21 (s, 1H), 4.15-4.09 (m, 4H), 3.84 (d, J = 10.2 Hz, 2H), 3.70 (m, 1H), 2.18-1.56 (m, 6H), 1.25-1.20 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 160.9 (d, J = 254.3 Hz), 155.2, 152.8, 142.0, 138.4 (q, J = 281.4 Hz), 120.2, 85.4 (q, J = 31.8 Hz), 63.2, 62.7, 61.7, 52.7, 26.8, 24.5, 19.2, 13.7. Anal. Calcd. for $C_{16}H_{22}F_4N_5O_4P$ (+1.0 MeOH): C, 41.91; H, 5.38; N, 14.37; Found: C, 41.88; H, 5.43; N, 14.42; MS m/z 456 $(M+H)^+$.

Data for 15b: UV (MeOH) λ_{max} 309.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.13 (s, 1H), 4.17-4.14 (m, 4H), 3.84 (d, J = 8.0 Hz, 2H), 3.73 (m, 1H), 2.17-1.57 (m, 6H), 1.24-1.20 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 158.4, 154.1, 150.7, 142.7, 136.9 (q, J = 281.3 Hz), 125.2, 86.3 (q, J = 29.5 Hz), 62.6, 62.1, 61.5, 53.7, 27.6, 23.2, 13.7. Anal. Calcd. for $C_{16}H_{22}ClF_3N_5O_4P$ (+ 1.0 MeOH): C, 40.58; H, 5.21; N, 13.92; Found: C, 40.62; H, 5.28; N, 13.87; MS m/z 472 $(M+H)^+$.

(*rel*)-(1'*S*,4'*R*)-[9-(4'-Trifluoromethylcyclopentanyloxy-1'-yl) Guanine] 4'-Methylphosphonic Acid (16). Dry acetonitrile (15 mL), 2,6-lutidine (1.01 mL, 8.72 mmol), and TMSBr (0.57 mL, 4.36 mmol) were added to a solution of **15b** (205 mg, 0.436 mmol) at room temperature. After stirring this mixture for 32 h, the solvent was removed and co-evaporaed three times with MeOH. The residue was dissolved in MeOH (16.0 mL), and 2-mercaptoethanol (170 mg, 2.18 mmol) and sodium methoxide (117 mg, 2.18 mmol) were added to the mixture. The mixture was refluxed for 24 h under nitrogen, cooled, neutralized with glacial acetic acid, and evaporated to dryness under vacuum. The obtained residue was co-evaporated from concentrated NH_4OH (2 × 16 mL) and the resultant solid was triturated with acetone (2 × 16 mL). After evaporating the acetone, the residue was purified by preparative column chromatography by using reverse-phase C18 silica gel and eluting with water. Lyophilization of the appropriate fraction produced compound **16** (92 mg, 51%) in the form of a yellowish salt (ammonium salt): mp 180-182 °C; UV (H_2O) λ_{max} 255.5 nm; ^{1}H NMR (D_2O, 300 MHz) δ 7.84 (s, 1H), 3.86 (d, J = 8.3 Hz, 1H), 3.69 (m, 1H), 2.19-1.60 (m, 6H); ^{13}C NMR (D_2O, 75 MHz) δ 157.5, 154.4, 152.1, 137.2, 136.4 (q, J = 280.8 Hz), 119.1, 86.3 (q, J = 30.4 Hz), 63.4, 44.6, 24.1, 18.4; HPLC t_R = 9.37 min; HRMS $[M-H]^+$ req. 396.0754, found 396.0755.

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