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Synthesis of SATE Prodrug of 6'-Fluoro-6'-methyl-5'-noradenosine Nucleoside Phosphonic Acid as a New Class of Anti-HIV Agent

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A very simple synthetic route of a novel SATE prodrug type of 6'-fluoro-6'-methyl-5'-noradeonosine carbocyclic nucleoside phosphonic acid is described. The key fluorinated alcohol intermediate **7** was prepared from the epoxide intermediate **6a** *via* selective ring-opening of epoxide. Coupling of **7** with N^6-bis-Boc-adenine under a Mitsunobu reaction followed by phosphonation and deprotection afforded the carbocyclic phosphonic acid. The chemical stability of the bis(SATE) derivative **13** was measured at neutral (pH 7.2) and slightly acidic (Milli-Q water, pH 5.5) pH. The antiviral activity test of the SATE prodrug **13** and its parent nucleoside phosphonic acid **11** were evaluated against HIV-1.

Key Words: Anti-HIV agent, SATE prodrug, Nucleoside phosphonic acid, Mitsunobu reaction

Introduction

Carbocyclic nucleosides[1] are a group of compounds structurally analogous to natural and synthetic nucleosides in which the furanose oxygen has been replaced by a methylene group. This replacement changes the furanose ring into a cyclopentane. The expected similarity in bond lengths and bond angles of the tetrahydrofuran and cyclopentane rings allows these analogues to behave as substrates or inhibitors of the enzymes in living cells. Therefore, the carbocyclic nucleosides possess a wide range of biological activities such as antiviral and antitumor effects.

Although the pharmacophore of nucleoside antiviral activity is not completely defined, structurally modified 5'-nornucleoside phosphonic acids such as d4AP (**1**, EC_{50} = 2.1 μM, CC_{50} = > 100 μM)[2] and 2'-Fd4AP (**2**, GS-9148, EC_{50} = 10.6 μM, CC_{50} = > 100 μM)[3] possess potent activity against viruses with a low level of toxicity (Figure 1). Unlike nucleoside agents, a phosphonic acid nucleoside can skip the requisite initial phosphorylation, which is the crucial step for the activation of nucleosides.[4] However, poor oral bioavailability of these nucleoside analogues results from the phosphonate negative charges present in nucleoside phosphonic acid at physiological pH. One strategy has been to temporarily mask these charges with neutral groups to form more lipophilic derivatives capable of crossing the gastrointestinal wall and reverting back to the parent nucleoside phosphonic acid.[5] Because the ionic character of a phosphonic acid presents an obstacle for cellular permeability, an *S*-acyl-2-thioethyl (SATE) prodrug was prepared. Esterification of a phosphonic acid with two SATE groups is a feasible strategy to deliver a phosphate or phosphonate drug into cells.[6] Usually, phosphonic acid nucleosides require an endocytosis-like process[7] or the ATP membrane receptor to cross the cell membrane.[8]

We therefore applied the bis(SATE) approach to a novel carbocyclic nucleoside phosphonic acid. Herein, we synthesized a novel SATE prodrug of 6'-fluoro-6'-methyl-5'-norcarbocyclic adenosine nucleoside to find new lead compounds with improved antiviral activity.

Results and Discussion

As depicted in Scheme 1, we used the cyclopentenone intermediate **3** as commercially available starting material. When **3** was subjected to Luche's reduction conditions ($NaBH_4/CeCl_3 \cdot 7H_2O$),[9] the allylic alcohol derivative **4** was obtained. The hydroxy functional group was protected with a benzyl group under the usual benzylation conditions (BnBr, NaH, DMF) to provide an intermediate **5**, which was epoxidized with *m*-chloroperoxybenzoic acid to give **6a** (55%) as the major isomer compared to minor isomer **6b** (22%). As shown in Figure 2, the relative stereochemistry of **6a** and **6b** were unambiguously determined on the basis of the NOE correlations. On irradiation of C_2-H, NOE was observed at C_5-H (0.72%) and C_1-CH_3 (0.82%) of **6a**, but also at C_5-H (1.14%) and C_1-CH_3 (1.34%) of **6b**. This

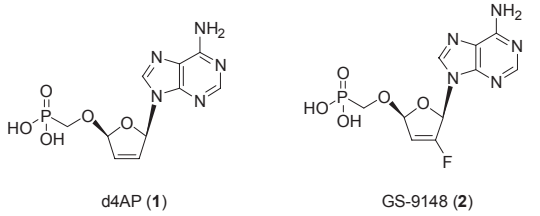


Figure 1. Structures of 5'-nornucleoside analogues as potent anti-HIV agents.

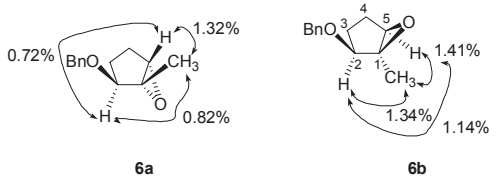
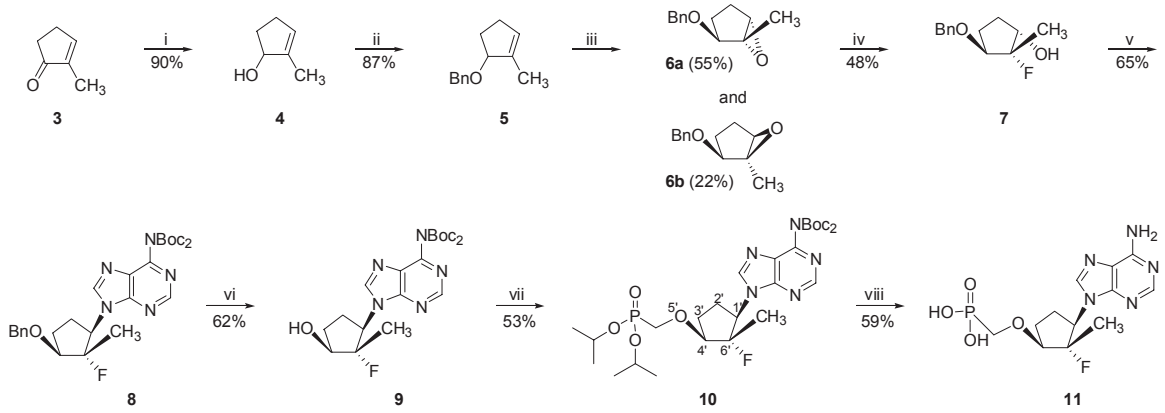


Figure 2. NOE percentage differences of isomers **6a** and **6b**.



Reagents: i) $NaBH_4$, $CeCl_3.7H_2O$, MeOH, 0 °C; ii) BnBr, NaH, DMF; iii) m-CPBA, CH_2Cl_2; iv) 47% HF, $(NH_4)_2SiF_6$, CsF; (v) PPh_3, DIAD, N^6-bis-BOC-adenine; (vi) $Pa(OH)_2$, cyclohexene, MeOH, reflux; vii) Diisopropyl bromomethylphosphonate, LiO^t-Bu, LiI, DMF, 60 °C; viii) TMSBr, CH_3CN.

Scheme 1. Synthesis of adenine phosphonic acid nucleoside

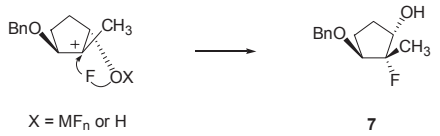
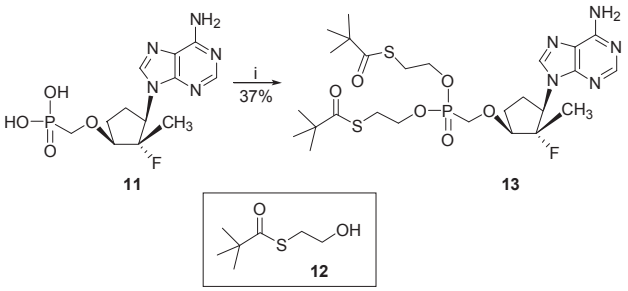


Figure 3. Possible mechanism for the formation of **7**.

stereochemical outcome suggests that a non-polar group such as benzyloxy reinforces the steric hindrance to the approach of the peroxy-acid. The epoxide **6a** was subjected to a region and stereoselective ring-opening fluorination reaction with hydrofluoric acid in the presence of silicon fluorides and additives to provide *cis*-fluorohydrin **7** in good yield. The formation of the *cis*-isomer may be due to the hydrogen bonding and/or silyl ether formation as shown in Figure 3.[10]

To synthesize the desired carbocyclic nucleoside analogues, the alcohol derivative **7** was subjected to a Mitsunobu coupling condition, which is the most useful and common method for the direct substitution of the hydroxyl group with an inverted configuration.[11] First, N^6-bis-Boc-adenine[12] was treated with the protected fluorohydrin **7** in the presence of diisopropylazodicarboxylate (DIAD) and PPh_3 to give **8** in 65% yield. Hydrogenolysis of the benzyl protecting group of **8** with palladium hydroxide and cyclohexene gave the adenine derivative **9**. For the synthesis of phosphonate nucleoside, the hydroxyl group of **9** was phosphonated with diisopropyl bromomethylphosphonate[13] in anhydrous DMF to give the phosphonate nucleoside intermediate **10**. Both protecting groups (N^6-bis-BOC & di-*O*-isopropyl) of the phosphonate nucleoside were simultaneously removed using trimethylsilylbromide[14] to give nucleoside phosphonic acids **11**. To synthesize the thioester-protected analogues, phosphonic acid nucleoside was reacted with thioester **12**[15] in the presence of 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT)[16] to provide the final *t*-Bu-SATE prodrug **13** (Scheme 2).

The newly synthesized phosphonic nucleoside analogues **11** and its prodrug **13** were assayed in MT-4 cells for anti-HIV activity. The SATE prodrug analogue **13** showed enhanced cytotoxicity-dependent anti-HIV activity compared to the parent



Reagents: i) **12**, 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole, pyridine.

Scheme 2. Synthesis of target SATE prodrug **13**

Table 1. Antiviral activity of the SATE derivative **13** and its parent nucleotide **11**

Compd	HIV-1 EC_{50} (μM)[c]	cytotoxicity CC_{50} (μM)[d]
11	62	70
13	16.7	31.4
PMEA	7.8	12.3
bis(SATE)PMEA[a]	0.69	1.9
AZT[b]	0.004	6.3

[a]bis(SATE)PMEA: bis(*t*-Bu-SATE)-9-[2-(phosphonomethoxy)ethyl]adenine.[17] [b]AZT: Azidothymidine. [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%.

phosphonic acid **11** (Table 1).

The increased anti-HIV activity for the neutral phosphodiester derivative could result from increased cellular uptake followed by intracellular release of the parent phosphonic acid. The procedures for measuring the anti-HIV activity toward HIV-1 and cytotoxicity have been reported previously.[18]

To measure the relative chemical stability of the SATE prodrug using an on-line HPLC cleaning method,[17] we measured the percentage of decomposition after 48 h for **13** at 37 °C: (i) in Milli-Q water (pH 5.5), 5.1% decomposition was observed, and (ii) in pH 7.2 (ammonium buffer, 0.02 M), 5.8% decomposition was observed.

Conclusion

We successfully synthesized novel 6'-fluoro-6'-methyl-5'-noradeonosine carbocyclic nucleoside phosphonic acid and its SATE prodrug from commercially available 2-methyl-cyclopentenone. The anti-HIV activity of the bis(*t*-Bu-SATE) prodrug **13** was higher than the parent nucleoside **11**. These molecules putatively have different (potentially better) *in vivo* bioavailability and biodistribution than the parent nucleosides. The synthesis of other nucleoside analogues (U,T,C) and enzymatic stability data will be reported elsewhere.

Experimental Section

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. NMR spectra were recorded on a JEOL JNM-AL300 Fourier transform; chemical shifts are reported in parts per million (δ) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and dd (doublet of doublets). UV spectra were obtained on a Beckman DU-7 spectrophotometer. The elemental analyses were performed using a Perkin-Elmer 2400 analyzer. TLC was performed on Uniplates (silica gel) purchased from Analtech Co. All reactions were performed under an atmosphere of nitrogen unless 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.

(±)-2-Methyl-cyclopent-2-enol (4). $CeCl_3 \cdot 7H_2O$ (3.13 g, 8.4 mmol) was added to a solution of **3** (530 mg, 5.52 mmol) in MeOH (25 mL) at 0 °C and stirred for 30 min. Then, $NaBH_4$ (416 mg, 11.0 mmol) was carefully added to the mixture and stirred for 3 h at rt. The reaction mixture was quenched by addition of acetic acid (1.0 mL) and concentrated under reduced pressure. The resulting residue was diluted with H_2O (100 mL) and extracted with EtOAc (2 × 100 mL). The organic layer was sequentially washed with sat. $NaHCO_3$ (100 mL) and brine (100 mL). The organic phase was dried over anhydrous magnesium sulfate, filtered, concentrated in vacuo, and purified by column chromatography (EtOAc/hexane, 1:5) to give compound **4** (487 mg, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.39 (dd, *J* = 5.8, 2.6 Hz, 1H), 4.07 (dd, *J* = 6.0, 2.8 Hz, 1H), 2.34-2.22 (m, 2H), 1.94 (m, 2H), 1.80 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 138.6, 123.2, 83.9, 29.6, 26.2, 16.9.

(±)-(2-Methyl-cyclopent-2-enyloxymethyl) benzene (5). To a solution of enol derivative **4** (439 mg, 4.48 mmol) in dry DMF (10 mL) was slowly added NaH (129 mg, 5.37 mmol) at 0 °C. After 30 min, benzyl bromide (840 mg, 4.92 mmol) was added, and the reaction mixture was stirred for 5 h at rt. The mixture was quenched by adding of saturated ammonium chloride (2 mL) and concentrated in a high vacuum. The residue was diluted with water (50 mL) and with EtOAc (2 × 30 mL). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$, filtered, and evaporated. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:25) to give **5** (733 mg, 87%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33-7.25 (m, 5H), 5.21 (m, 1H), 4.59 (s, 2H), 3.52 (dd, *J* = 8.4, 5.2 Hz, 1H), 2.35 (m, 2H), 2.05 (m, 2H), 1.66 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 138.3, 128.7, 127.5, 127.0, 126.3, 123.4, 84.2, 74.1, 27.8, 25.6, 15.9.

(*rel*)-(1*R*,2*S*,5*S*)-2-Benzyloxy-1-methyl-6-oxa-bicyclo [3.1.0]hexane (6a) and (*rel*)-(1*S*,2*S*,5*R*)-2-benzyloxy-1-methyl-6-oxa-bicyclo[3.1.0]hexane (6b). *m*-Chloroperbenzoic acid (1.82 g, 7.8 mmol, 77% purity) was added to a solution of **5** (1.12 g, 5.98 mmol) in anhydrous CH_2Cl_2 (40 mL) at 0 °C. The solution was stirred at 0 °C for 2 h and stirred for an additional 2 h at rt. A saturated $NaHCO_3$ solution (80 mL) was added to the reaction mixture and extracted with EtOAc (2 × 80 mL). The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, filtered, concentrated in vacuo, and purified by column chromatography (EtOAc/hexane, 1:8) to give compound **6a** (672 mg, 55%) and **6b** (269 mg, 22%) as syrup, respectively: Compound **6a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31-7.25 (m, 5H), 4.58 (s, 2H), 3.21 (dd, *J* = 6.2, 4.8 Hz, 1H), 2.58 (m, 1H), 1.71-1.62 (m, 4H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 137.9, 128.4, 127.6, 126.2, 82.9, 75.6, 70.4, 64.4, 21.1, 16.5, 14.3; Anal. Calcd. for $C_{13}H_{16}O_2$: C, 76.44; H, 7.90. Found: C, 76.41; H, 7.89; Compound **6b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35-7.29 (m, 5H), 4.60 (s, 2H), 3.27 (dd, *J* = 6.4, 4.8 Hz, 1H), 2.62 (dd, *J* = 6.8, 5.2 Hz, 1H), 1.69-1.58 (m, 4H), 1.41 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 137.4, 128.1, 127.7, 126.4, 83.5, 76.3, 71.7, 65.7, 23.5, 17.7, 14.9; Anal. Calcd. for $C_{13}H_{16}O_2$: C, 76.44; H, 7.90. Found: C, 76.48; H, 7.93.

(*rel*)-(1*S*,2*S*,3*S*)-3-Benzyloxy-2-fluoro-2-methyl-cyclopentanol (7). To a mixture of $(NH_4)_2SiF_6$ (1.068 g, 6.0 mmol), CsF (182.3 mg, 1.2 mmol) and epoxide **6a** (245 mg, 1.2 mmol) in 1,2-dichloroethane (15 mL) was added 47% hydrofluoric acid (0.152 mL, 3.6 mmol) and *i*Pr_2NEt (0.21 mL, 1.2 mmol) at 0 °C, and the mixture was stirred for 5 h at 0 °C. A saturated $NaHCO_3$ solution (15 mL) was slowly added and the mixture was extracted with diethyl ether (2 × 80 mL). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$, filtered, and evaporated. The filtrate was concentrated under vacuum and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:12) to give alcohol **7** (129 mg, 48%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33-7.25 (m, 5H), 4.60 (s, 2H), 3.73 (ddd, *J* = 2.8, 6.4, 19.8 Hz, 1H), 3.21 (dd, *J* = 5.4, 14.2 Hz, 1H), 1.70 (m, 2H), 1.62 (m, 2H), 1.30 (d, *J* = 21.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 137.8, 128.3, 127.8, 126.7, 104.2 (d, *J* = 180.8 Hz), 81.3 (d, *J* = 44.0 Hz), 76.4 (d, *J* = 19.2 Hz), 73.8, 20.3, 17.4, 13.4 (d, *J* = 22.2 Hz); Anal. Calcd. for $C_{13}H_{17}FO_2$: C, 69.62; H, 7.64. Found: C, 69.59; H, 7.61.

(*rel*)-(1*R*,2*S*,3*S*)-9-(3-Benzyloxy-2-fluoro-2-methyl-cyclopentan-1-yl) N^6-bis-Boc-adenine (8). To a stirred solution of triphenylphosphine (673 mg, 2.57 mmol) in dry THF (15 mL) at 0 °C was added dropwise the diisopropyl azodicarboxylate (DIAD) (518 mg, 2.57 mmol), and the reaction mixture was stirred at this temperature for 30 min. After that, a solution of the fluorinated alcohol **7** (287 mg, 1.28 mmol) in THF (15 mL) was added and the reaction mixture was stirred at 0 °C for 30 min. Then the cold bath was removed and the yellow solution was stirred for 30 min at room temperature. N^6-bis-Boc adenine (861 mg, 2.57 mmol) was then added and the reaction mixture was stirred overnight at room temperature. The reaction mix-

ture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 2:1) to give compound **8** (450 mg, 65%) as a white solid; mp 167 - 169 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.86 (s, 1H), 7.85 (s, 1H), 7.31-7.24 (m, 5H), 4.69 (s, 2H), 4.01 (dd, *J* = 5.6, 17.6 Hz, 1H), 3.18 (ddd, *J* = 2.2, 6.0, 15.4 Hz, 1H), 2.01 (m, 2H), 1.75 (m, 2H), 1.41 (d, *J* = 21.8 Hz, 3H), 1.35 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 155.0, 152.3, 150.5, 147.7, 143.5, 138.3, 128.7, 128.0, 127.3, 101.3 (d, *J* = 179.8 Hz), 82.9, 80.2 (d, *J* = 40.8 Hz), 74.0, 58.6 (d, *J* = 18.2 Hz), 27.7, 17.2, 15.5, 14.2 (d, *J* = 24.4 Hz); Anal. Calcd. for $C_{28}H_{36}FN_5O_5$: C, 62.09; H, 6.70; N, 12.93. Found: C, 62.12; H, 6.68; N, 12.89.

(*rel*)-(1*R*,2*S*,3*S*)-9-(3-Hydroxy-2-fluoro-2-methyl-cyclopentan-1-yl) N^6-bis-Boc-adenine (9). A solution of **8** (230 mg, 0.425 mmol) in MeOH (10 mL) was treated with palladium hydroxide (80 mg, 20% in activated charcoal) at 0 °C. Cyclohexene (5 mL) was added and the reaction mixture was refluxed overnight. The suspension was cooled to room temperature, filtered over Celite, and the filtrates were concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane/MeOH, 4:1:0.05) to give compound **9** (119 mg, 62%) as a white solid: mp 175 - 177 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.81 (s, 1H), 7.83 (s, 1H), 4.70 (s, 2H), 4.00 (dd, *J* = 5.7, 18.0 Hz, 1H), 3.18 (ddd, *J* = 2.2, 6.0, 15.4 Hz, 1H), 2.04 (m, 2H), 1.77 (m, 2H), 1.39 (d, *J* = 21.8 Hz, 3H), 1.34 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 153.7, 152.6, 150.6, 147.3, 142.4, 127.5, 102.6 (d, *J* = 176.6 Hz), 82.4, 79.9 (d, *J* = 42.4 Hz), 73.6, 57.8 (d, *J* = 17.8 Hz), 28.0, 17.7, 15.6, 14.1 (d, *J* = 22.0 Hz); Anal. Calcd. for $C_{21}H_{30}FN_5O_5$: C, 55.86; H, 6.70; N, 15.51. Found: C, 55.83; H, 6.72; N, 15.49.

(*rel*)-(1*R*,2*S*,3*S*)-[3-(N^6-Bis-Boc-adenine-9-yl)-2-fluoro-2-methyl-cyclopentyloxymethyl]-phosphonic acid diisopropyl ester (10). To a solution of the cyclopentenol **9** (1.92 g, 4.25 mmol) in 15 mL of DMF was added LiI (42.68 mg, 0.32 mmol) at rt. Both LiO-*t*Bu (6.8 mL of 1.0 M solution in THF, 6.8 mmol) and a solution of diisopropyl bromomethylphosphonate (1.38 mL, 5.76 mmol) in 10 mL of DMF were slowly added to the reaction mixture and stirred for 3 h at 60 °C under anhydrous conditions. The mixture was quenched by adding water (10 mL) and the organic solvents were removed in vacuo. The residue was diluted with water (80 mL) and was extracted with EtOAc (3 × 80 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1) to give **10** (1.42 g, 53%) as a solid: mp 162 - 164 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.87 (s, 1H), 7.86 (s, 1H), 4.71 (m, 2H), 4.03 (ddd, *J* = 1.8, 6.0, 18.8 Hz, 1H), 3.62 (d, *J* = 8.8 Hz, 2H), 3.18 (ddd, *J* = 1.8, 6.2, 14.6 Hz, 1H), 2.01 (m, 2H), 1.69 (m, 2H), 1.38 (d, *J* = 20.6 Hz, 3H), 1.36 (s, 12H), 1.34 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 153.8, 152.3, 150.9, 150.3, 142.7, 130.4, 101.4 (d, *J* = 173.2 Hz), 83.6, 82.0 (d, *J* = 43.4 Hz), 70.4, 65.4, 59.5 (d, *J* = 16.8 Hz), 28.1, 23.6, 17.2, 15.1, 14.2 (d, *J* = 20.8 Hz); Anal. Calcd. for $C_{28}H_{45}FN_5O_8P$·0.5 MeOH: C, 53.01; H, 7.33; N, 10.85. Found: C, 52.97; H, 7.35; N, 10.81.

(*rel*)-(1*R*,2*S*,3*S*)-[3-(6-Amino-purin-9-yl)-2-fluoro-2-methyl-cyclopentyloxymethyl]-phosphonic acid (11). To a solution of the phosphonate **10** (104 mg, 0.165 mmol) in CH_3CN (11 mL) was added trimethylsilyl bromide (252 mg, 1.665 mmol). The mixture was heated overnight at 60 °C and concentrated under reduced pressure. The residue was partitioned between pure CH_2Cl_2 (16 mL) and distilled H_2O (16 mL). The aqueous layer was washed out with CH_2Cl_2 and then freeze-dried to give target compound **11** (33.6 mg, 59%) as a yellowish foamy solid. UV (H_2O) λ_{max} 262.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.86 (s, 1H), 7.85 (s, 1H), 4.01 (dd, *J* = 6.2, 20.4 Hz, 1H), 3.60 (d, *J* = 8.6 Hz, 2H), 3.17 (dd, *J* = 6.1, 16.2 Hz, 1H), 2.08 (m, 2H), 1.71 (m, 2H), 1.37 (d, *J* = 20.2 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 155.4, 152.7, 150.5, 142.3, 120.4, 101.1 (d, *J* = 172.4 Hz), 82.6 (d, *J* = 40.8 Hz), 64.6, 58.9 (d, *J* = 18.3 Hz), 17.3, 15.1, 14.2 (d, *J* = 22.8 Hz); Anal. Calcd. for $C_{12}H_{17}FN_5O_4P$·2.0 H_2O: C, 37.80; H, 5.55; N, 18.37. Found: C, 37.83; H, 5.52; N, 18.35.

(*rel*)-(1*R*,2*S*,3*S*)-*t*-Butyl SATE phosphoester of 9-(2-fluoro-2-methyl-cyclopentyloxymethan-1-yl) adenine (13). A solution of adenine phosphonic acid derivative **11** (81.6 mg, 0.254 mmol) and tributylamine (432 μL, 1.728 mmol) in methanol (8.6 mL) was mixed for 30 min and concentrated under reduced pressure. The residue was thoroughly dried with anhydrous ethanol and toluene. The resulting foamy solid was dissolved in anhydrous pyridine (16 mL) to which thioester **12** (389 mg, 2.37 mmol) and 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (319 mg, 1.08 mmol) were added. The mixture was stirred for 16 h at room temperature and quenched with tetrabutylammonium bicarbonate buffer (12 mL, 1 M solution, pH 8.0). The mixture was concentrated under reduced pressure and the residue was diluted with water (100 mL) and extracted twice with CH_2Cl_2 (100 mL). The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (Hexane/EtOAc/MeOH, 4:1:0.01) to give **13** (59 mg, 37%) as a solid: mp 125 - 127 °C; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.79 (s, 1H), 7.80 (s, 1H), 4.05-4.00 (m, 5H), 3.61 (d, *J* = 8.8 Hz, 2H), 3.17-3.14 (m, 5H), 2.05 (m, 2H), 1.75 (m, 2H), 1.38 (d, *J* = 21.2 Hz, 3H), 1.20 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 205.1, 154.7, 151.9, 149.4, 143.6, 119.6, 103.2 (d, *J* = 170.8 Hz), 81.7 (d, *J* = 38.2 Hz), 64.4, 62.2, 57.8 (d, *J* = 20.4 Hz), 46.2, 30.5, 26.2, 18.1, 16.0, 14.5 (d, *J* = 20.2 Hz); Anal. Calcd. for $C_{26}H_{41}FN_5O_6PS_2$·1.0 MeOH: C, 48.71; H, 6.81; N, 10.52. Found: C, 48.68; H, 6.83; N, 10.49.

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