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Antiviral Activity Enhancement through the SATE Prodrug of a 2'-Modified 5'-Norcarbocyclic Adenine Analogue

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We synthesized and tested the anti-HIV activity of the SATE prodrug of a 2'-methyl 5'-norcarbocyclic adenine analogue. The introduction of a methyl group in the 2'-position was performed by the addition of a carbonyl using isopropenyl magnesium bromide. The adenine base was efficiently coupled using the Mitsunobu reaction. The chemical stability study of the bis(SATE) derivative **18** was measured at neutral (pH 7.2) and slightly acid (milli-Q water, pH 5.5) pH, and compounds **16** and **18** were evaluated as potential anti-HIV-1 agents.

Key Words: Antiviral agent, Carbocyclic nucleoside, SATE prodrug, Phosphonic acid

Introduction

Emerging drug-resistant viral strains and drug toxicity are major problems in antiviral chemotherapy,[1] leading to research for structurally modified nucleosides. Although the pharmacophore of nucleoside antiviral activity is not completely defined, olefinic nucleosides such as stavudine (**1**),[2] d4AP (**2**),[3] and 2'-Fd4AP (**3**, GS-9148)[4] as potential antiviral agents have encouraged the search for novel nucleosides in this class of compounds (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.[5] However, the poor oral bioavailability of these nucleoside analogues is due to 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.[6] Since 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.[7] Usually, phosphonic acid nucleosides require an endocytosis-like process[8] or the ATP membrane receptor[9] to cross the cell membrane.

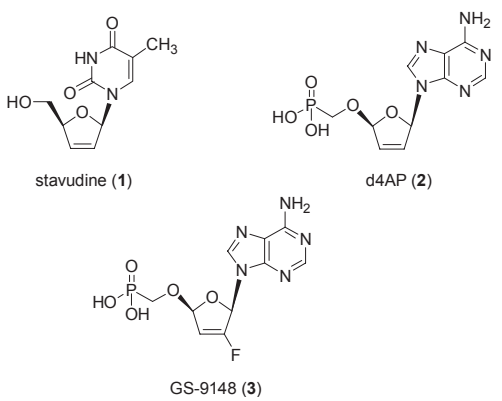


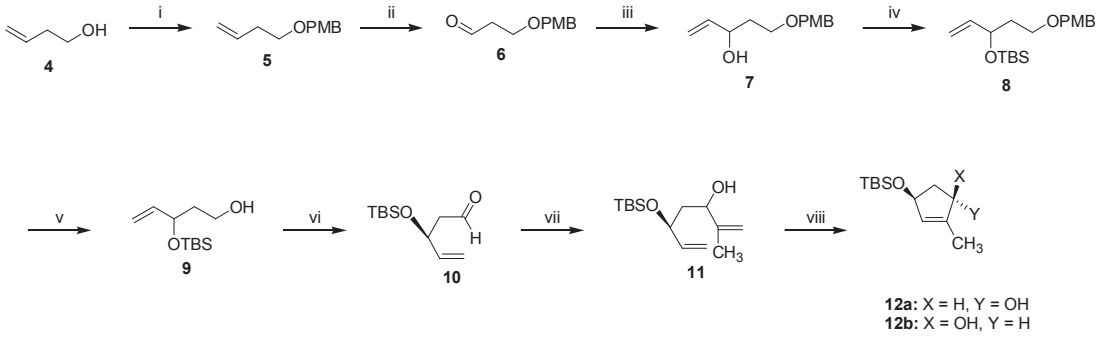
Figure 1. Rationale to the design of target 5'-norcarbocyclic nucleoside analogue

We therefore applied the bis(SATE) approach to a novel nucleoside phosphonic acid. Herein, we synthesized a novel SATE prodrug of 2'-methyl 5'-norcarbocyclic nucleoside to find new lead compounds with improved antiviral activity.

Results and Discussion

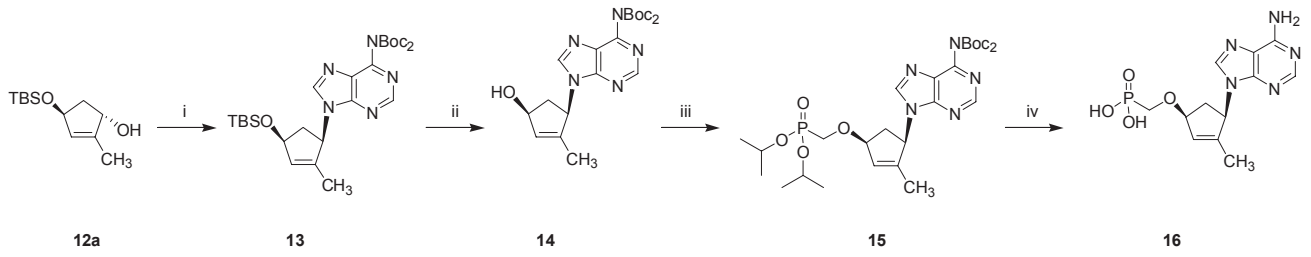
For the synthesis of phosphonate adenine nucleoside, the commercially available but-3-en-1-ol **4** was selected as a starting material. As shown in Scheme 1, the synthetic route is very simple and straightforward. The primary hydroxyl group of **4** was protected as a temporary *p*-methoxy benzyl ether (PMB) by reaction[10] with PMBCl and NaH in DMF to afford the protected olefin **5** in a yield of 97%. The olefin of **5** was treated with ozone in methylene chloride at −78 °C, followed by the decomposition of the ozonide by dimethylsulfide (DMS) to give the aldehyde **6**. Compound **6** was subjected to carbonyl addition with vinyl magnesium bromide to provide the secondary alcohol derivative **7**, this was protected with *t*-butyldimethylsilyl chloride (TBDMSCl) to give compound **8**. Oxidative deprotection of the PMB ether moiety of **8** was effected with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[11] in methylene chloride with a small amount of water to give the alcohol **9**, which was then oxidized to the aldehyde **10** using pyridinium chlorochromate (PCC), which again underwent an addition reaction with isopropenyl magnesium bromide to provide a divinyl **11**. The divinyl **11** was subjected to standard ring-closing metathesis (RCM) conditions using a 2nd generation Grubbs catalyst to provide cyclopentenol **12a** (43%) and **12b** (42%), which were readily separated by silica gel column chromatography (Scheme 1). As shown in Figure 2, the stereochemistry of **12a** and **12b** were unambiguously determined on the basis of the NOE correlations. On irradiation of C_4-H, relatively weak NOE was observed at C_1-H of **12a** (0.6%), but not at C_1-H of **12b** (1.1%).

For coupling with a nucleobase, the hydroxyl group can ac-



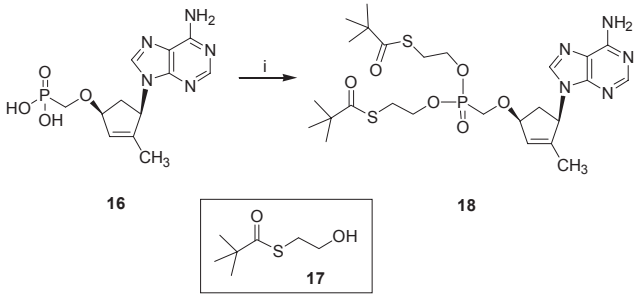
Reagents: i) PMBCl, NaH, DMF, 0 °C; ii) O_3, DMS, -78 °C; iii) vinylMgBr, THF, -78 °C; iv) TBDMSCl, imidazole, CH_2Cl_2, 0 °C; v) DDQ, CH_2Cl_2/H_2O, rt; vi) PCC, 4MS, CH_2Cl_2; vii) isopropenylMgBr, THF, -78 °C; viii) Grubbs II, benzene, 60 °C, reflux, overnight.

Scheme 1. Synthesis of cyclopentene intermediate (**12a**)



Reagents: i) PPh_3, DIAD, N^6-bis-Boc-adenine, THF, -20 °C; ii) TBAF, THF, rt; iii) Diisopropyl bromomethylphosphonate, LiO-^{t}Bu, LiI, DMF, 60 °C; iv) TMSBr, CH_3CN.

Scheme 2. Synthesis of carbocyclic adenine nucleoside (**16**)



Reagents: i) **17**, 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole, pyridine.

Scheme 3. Synthesis of final SATE prodrug of adenine analogue (**18**)

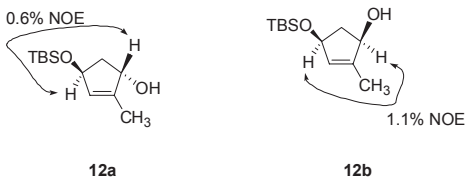


Figure 2. NOE differences of isomers **12a** & **12b**.

tivate mesylate for nucleophilic substitution. However, the yield of mesylation was very low and its product was unstable during work-up for storage. Alternatively, the alkylation of adenine was attempted under Mitsunobu conditions using diisopropyl azodicarboxylate (DIAD) and PPh_3 under a THF solvent. Unfortunately, the direct coupling of adenine with alcohol **12a** failed. A nucleobase precursor such as N^6-bis-Boc-adenine[12] was coupled with alcohol **12a** under Mitsunobu conditions[13] to give compound **13** with a chirality inversion. The silicon protection group of compound **13** was readily removed by treating it with tetrabutylammonium fluoride (TBAF) in THF to give compound **14**. For the synthesis of phosphonate nucleoside, the hydroxyl group of **14** was phosphonated by treating them with diisopropyl bromomethylphosphonate[14] in anhydrous DMF to give the phosphonate nucleoside intermediate **15** (Scheme 2). Both protecting groups (N^6-bis-BOC & di-*O*-isopropyl) of the phosphonate nucleoside were simultaneously removed using trimethylsilylbromide[15] to give nucleoside phosphonic acids **16**. To synthesize the thioester-protected analogues, phosphonic acid nucleoside was reacted with thioester[16] **17** in the presence of 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT)[17] to provide the final *t*-bu-SATE prodrug **18** (Scheme 3). The newly synthesized phosphonic nucleoside analogues **16** and **18** were assayed against HIV-1 virus. *In vitro* anti-HIV-1 drug testing involved the killing of T4-lymphocytes by HIV-1. T4-lymphocytes (MT-4 cell line) were exposed to HIV at a virus-to-cell ratio of approximately 0.05 and treated with the compounds, dissolved in dimethylformamide (DMF), at doses ranging from 10^{-8} to 10^{-4}. A complete cycle of virus reproduction is necessary to obtain the required cell killing (incubation at 37 °C in a 5% carbon dioxide atmosphere for 6 days). Uninfected cells with the compounds served as a toxicity control, whereas

Table 1. Enhanced antiviral activity of the synthesized compounds.

Compd	HIV-1 EC_{50} (μM)	cytotoxicity CC_{50} (μM)
16	85	90
18	20	24
PMEA	10.6	14.7

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

the infected and uninfected cells without the compound served as basic control. After incubation, the tetrazolium salt XTT was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Formazan production was measured spectrophotopmetically and possible protective activity was confirmed by microscopical detection of viable cells. The effect of each compound on cell growth of HIV-infected and uninfected cells was compared to that of untreated uninfected cells.

The tested prodrug **18** enhanced the *in vitro* anti-HIV activity of the parent phosphonic acid **16** (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.

In order to measure the relative chemical stability of the SATE prodrug using Gosselin's method,[7] we measured the percent of decomposition after 36 h for **18** at 37 °C: (i) in Milli-Q water (pH 5.5), 2.3% decomposition was observed, and (ii) in pH 7.2 (ammonium buffer, 0.02 M), 4.1% decomposition was observed.

In conclusion, it is interesting to note that the SATE analogue **18** exhibited 4-fold higher anti-HIV activity compared to its parent nucleoside **16**, indicating that this virus might allow the sugar moiety can serve as a template for DNA polymerase. The synthesis of other nucleoside analogues (U,T,C) and enzymatic stability data will be reported elsewhere.

Experimental Section

The melting points were determined on a Mel-temp II laboratory device and are uncorrected. NMR spectra were recorded on a JEOL 300 Fourier transform; chemical shifts are reported in parts per million (δ) and signals are quoted 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 an Elemental Analyzer System (EA1112). 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.

1-But-3-enyloxymethyl-4-methoxy-benzene (5). NaH (60% in mineral oil, 3.33 g, 83.21 mmol) was added portion wise to a cooled (0 °C) solution of but-3-en-1-ol **4** (5.0 g, 69.34 mmol) and *p*-methoxybenzyl chloride (10.34 mL, 76.27 mmol) in DMF (100 mL). The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue was quenched with H_2O followed by extraction with EtOAc two times. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give **5** (12.93 g, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (m, 2H), 6.87 (m, 2H), 5.87-5.97 (m, 1H), 5.13-5.01 (m, 2H), 4.45 (s, 2H), 3.80 (s, 3H), 3.49 (t, J = 6.7 Hz, 2H), 2.36 (q, J = 6.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.12, 135.29, 130.52, 129.24, 116.28, 113.74, 72.53, 69.28, 55.25, 34.21.

3-(4-Methoxy-benzyloxy)-propionaldehyde (6). A solution of compound **5** (3.7 g, 19.25 mmol) in anhydrous CH_2Cl_2 (50 mL) was cooled down to −78 °C, and ozone gas was then bubbled into the reaction mixture until a blue color persisted for an additional 5 minutes. The reaction mixture was degassed with nitrogen, and dimethyl sulfide (5.94 mL, 80.83 mmol) was slowly added at 78 °C. The mixture was stirred for 1 h at −78 °C under argon gas and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:12) to give compound **6** (2.99 g, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.78 (s, 1H), 7.26 (m, 2H), 6.88 (m, 2H), 4.44 (s, 2H), 3.80 (s, 3H), 3.59 (t, J = 6.1 Hz, 2H), 2.02 (q, J = 6.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.26, 159.20, 130.09, 129.34, 113.79, 72.78, 64.90, 55.25, 32.15.

(±)-5-(4-Methoxy-benzyloxy)-pent-1-en-3-ol (7). To a solution of **6** (2.4 g, 12.36 mmol) in dry THF (35 mL) was slowly added vinyl magnesium bromide (18.53 mL, 1.0 M solution in THF) at −78 °C. After 5 h, saturated NH_4Cl solution (20 mL) was added, and the reaction mixture was slowly warmed to room temperature. The mixture was diluted with water (100 mL) and extracted with EtOAc (100 mL) two times. The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:5) to give **7** (2.09 g, 76%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (d, J = 6.7 Hz, 2H), 6.88 (d, J = 6.6 Hz, 2H), 5.92-5.81 (m, 1H), 5.26 (d, J = 13.8 Hz, 1H), 5.10 (d, J = 7.2 Hz, 1H), 4.44 (s, 2H), 3.80 (s, 3H), 3.69-3.57 (m, 2H), 2.90 (s, 1H), 1.86-1.78 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.27, 140.52, 130.00, 129.33, 114.31, 113.83, 72.95, 71.97, 68.07, 55.27, 36.25; Anal. Calc. for $C_{13}H_{18}O_3$: C, 70.24; H, 8.16. Found: C, 70.26; H, 8.20.

(±)-*tert*-Butyl-{1-[2-(4-methoxybenzyloxy) ethyl] allyloxy} dimethylsilane (8). TBDMSCl (0.97 g, 6.43 mmol) was added slowly to a solution of **7** (1.3 g, 5.85 mmol) and imidazole (0.60 g, 8.77 mmol) in CH_2Cl_2 (20 mL) at 0 °C, and stirred for 5 h at the same temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in water 100 mL) and extracted with diethyl ether (100 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, 1:15) to give compound **8** (1.71 g, 87%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (d, J = 6.9 Hz, 2H), 6.84 (d, J = 6.6 Hz, 2H), 5.79-5.71 (m, 1H), 5.11 (d, J = 17.1 Hz, 1H), 4.98 (d, J = 10.5 Hz, 1H), 4.45-4.32 (m, 2H), 4.25 (q, J = 6.2 Hz, 1H),

3.77 (s, 3H), 3.55-3.41 (m, 2H), 1.73 (q, J = 13.2 Hz, 2H), 0.86 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.09, 141.59, 130.62, 129.29, 113.71, 113.65, 72.62, 70.75, 66.39, 55.24, 38.08, 25.85, 18.18, −4.67; Anal. Calc. for $C_{19}H_{32}O_3Si$: C, 67.81; H, 9.58. Found: C, 67.88; H, 9.54.

(±)-3-(*tert*-Butyldimethylsilanyloxy) pent-4-en-1-ol (9). To a solution of compound **8** (0.76 g, 2.26 mmol) in CH_2Cl_2/H_2O mixture (10 mL, 20:1 v/v) was added DDQ (0.56 g, 2.48 mmol) and the mixture was stirred for 2 h at room temperature. Saturated $NaHCO_3$ (2 mL) was added to quench the reaction and further diluted with water (20 mL). The organic layer was separated, washed with brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give compound **9** (0.43 g, 87%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.91-5.79 (m, 1H), 5.22 (d, J = 17.4 Hz, 1H), 5.07 (d, J = 10.2 Hz, 1H), 4.42 (q, J = 4.5 Hz, 1H), 3.82-3.69 (m, 2H), 2.44 (br s, 1H), 1.89-1.66 (m, 2H), 0.91 (s, 9H), 0.07 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 140.60, 114.39, 73.20, 60.10, 39.10, 25.80, 18.09, −5.07; Anal. Calc. for $C_{11}H_{24}O_2Si$: C, 61.05; H, 11.18. Found: C, 60.98; H, 11.21.

(±)-3-(*tert*-Butyldimethylsilanyloxy) pent-4-enal (10). 4 Å Molecular sieves (3.0 g) and PCC (2.99 g, 13.86 mmol) were added slowly to a solution of compound **9** (1.2 g, 5.55 mmol) in CH_2Cl_2 (15 mL) at 0 °C, and stirred overnight at room temperature. An excess of diethyl ether (20 mL) was then added to the mixture. The mixture was stirred vigorously for 2 h at the same temperature, and the resulting solid was filtered through a short silica gel column. The filtrate was concentrated under vacuum and purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give compound **10** (0.95 g, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.77 (s, 1H), 5.93-5.82 (m, 1H), 5.26 (d, J = 17.4 Hz, 1H), 5.12 (d, J = 10.2 Hz, 1H), 4.65 (q, J = 5.4 Hz, 1H), 2.65-2.48 (m, 2H), 0.88 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.59, 139.82, 114.85, 69.38, 51.19, 25.68, 18.05, −5.07; Anal. Calc. for $C_{11}H_{22}O_2Si$: C, 61.63; H, 10.34. Found: C, 61.66; H, 10.29.

(*rel*)-5-(*tert*-Butyldimethylsilanyloxy) 2-methyl-hepta-1,6-dien-3-ol (11). To a solution of compound **10** (0.25 g, 1.17 mmol) in dry THF (4 mL), isopropenyl magnesium bromide (3.50 mL, 1.0 M solution in THF) was added slowly at −78 °C. After 3 h, a saturated NH_4Cl solution (4 mL) was added, and the reaction mixture was warmed slowly to room temperature. The mixture was extracted with EtOAc/water two times. The combined organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **11** (0.24 g, 80%) as a diastereomeric mixture: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.81-5.71 (m, 1H), 5.20-4.98 (m, 4H), 4.42-4.15 (m, 2H), 1.67 (m, 5H), 0.84 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 147.28, 140.75, 114.61, 110.28, 74.65, 72.19, 43.28, 25.82, 18.37, 18.06, −4.84; Anal. Calc. for $C_{14}H_{28}O_2Si$: C, 65.57; H, 11.00. Found: C, 66.61; H, 10.97.

(*rel*)-(1*S*,4*S*)-4-(*tert*-Butyldimethylsilanyloxy) 2-methyl-cyclopent-2-enol (12a) and (*rel*)-(1*R*,4*S*)-4-(*tert*-Butyldimethylsilanyloxy) 2-methyl-cyclopent-2-enol (12b). To a solution of **10** (254 mg, 0.99 mmol) in dry benzene (3 mL) was added 2nd generation Grubbs catalyst (10 mg). The reaction mixture was refluxed overnight at 60 °C, and cooled to room temperature. The mixture was concentrated in vacuum, and residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give cyclopentenol **12a** (97 mg, 43%) and **12b** (95 mg, 42%) as colorless oils. Cyclopentenol **12a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.43 (s, 1H), 4.51 (m, 1H), 4.23 (m, 1H), 1.93 (s, 3H), 1.82-1.70 (m, 2H), 0.81 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 144.96, 130.92, 73.94, 45.13, 29.68, 25.92, 18.20, 13.52, −4.64; Anal. Calc. for $C_{12}H_{24}O_2Si$: C, 63.10; H, 10.59. Found: C, 63.02; H, 10.52. Cyclopentenol **12b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.40 (s, 1H), 4.53 (m, 1H), 4.23 (m, 1H), 1.99 (s, 3H), 1.88-1.72 (m, 2H), 0.83 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 145.02, 130.98, 74.00, 45.43, 28.54, 25.72, 18.65, 13.81, −5.31; Anal. Calc. for $C_{12}H_{24}O_2Si$: C, 63.10; H, 10.59. Found: C, 63.14; H, 10.63.

(*rel*)-(1*R*,4*S*)-9-[4-(*tert*-Butyldimethylsilanyloxy) 2-methyl-cyclopent-2-enyl]-N^6,N^6-bis-(*tert*-butoxy-carbonyl)adenine (13). To a stirred solution of triphenylphosphine (518 mg, 1.98 mmol) in THF (4 mL) at 0 °C was added dropwise the diisopropyl azodicarboxylate (DIAD, 0.38 mL, 1.98 mmol) and the yellow reaction mixture was stirred at this temperature for 30min. After that, a solution of compound **12a** (347 mg, 1.52 mmol) in THF (3.0 mL), was added and the reaction mixture was stirred at 0 °C for 10 min. Then, the cold bath was removed and the yellow solution was stirred for 30 min at room temperature. Bis-BOC adenine (662 mg, 1.98 mmol) was added and the solution became clear after 2 min. The reaction mixture was stirred overnight at room temperature. The volatiles were removed under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:3) to give **13** (522 mg, 63%) as a yellow solid: mp 134-136 ºC; UV (MeOH) λ_{max} 264.5 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.86 (s, 1H), 7.92 (s, 1H), 5.88 (s, 1H), 5.71 (s, 1H), 5.18 (s, 1H), 2.44 (t, J = 5.5 Hz, 2H), 1.63 (s, 3H), 1.46 (s, 18H), 0.91 (s, 9H), 0.10 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.28, 152.05, 150.53, 143.00, 139.65, 135.50, 83.72, 75.84, 62.38, 42.85, 27.79, 25.87, 13.94, −4.64; Anal. Calc. for $C_{27}H_{43}N_5O_5Si$: C, 59.42; H, 7.94; N, 12.83. Found: C, 59.39; H, 8.01; N, 12.85.

(*rel*)-(1*R*,4*S*)-(N^6,N^6-Bis-(*tert*-butoxycarbonyl)adenine) 3-methyl-cyclopent-2-enol (14). To a solution of **13** (132 mg, 0.24 mmol) in THF (3 mL) was added TBAF (0.51 mL, 1.0 M solution in THF) at 0 °C. The mixture was stirred overnight at room temperature and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 2:1) to give **14** (87 mg, 83%) as a white solid: mp 152-154 ºC; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.81 (s, 1H), 8.55 (s, 1H), 5.82 (s, 1H), 5.72 (s, 1H), 5.05 (s, 1H), 2.30-2.21 (m, 2H), 1.42 (s, 3H), 1.34 (s, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 149.93, 145.83, 134.89, 128.13, 83.35, 73.69, 41.00, 27.23, 21.91, 13.21; Anal. Calc. for $C_{21}H_{29}N_5O_5$: C, 58.45; H, 6.77; N, 16.23. Found: C, 58.47; H, 6.74; N, 16.27.

(*rel*)-(1*R*,4*S*)-{4-[N^6,N^6-Bis-(*tert*-butoxycarbonyl)adenine]-3-methyl-cyclopent-2-enyloxymethyl}-phosphonic acid diisopropyl ester (15). To a solution of **14** (85 g, 0.20 mmol) in DMF (2 mL), LiI (1.98 mg, 0.015 mmol) was added at 25 °C. LiO *t*-Bu (0.32 Ml, 1.0 M solution in THF) and a solution of diisopropyl bromomethylphosphonate (0.06 mL, 0.24 mmol) in DMF

(2 mL) were slowly and simultaneously added to the reaction mixture for 5 h at 60 °C under anhydrous conditions. The mixture was quenched by adding water (10 mL), and the organic solvents (THF) were removed in vacuo. The aqueous layer was extracted with EtOAc two times. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane, 2:1) to give **15** (77 mg, 64%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.74 (s, 1H), 7.81 (s, 1H), 6.00 (s, 1H), 5.82 (s, 1H), 5.73 (s, 1H), 4.83-4.73 (m, 2H), 3.24 (d, J = 9.9 Hz, 2H), 2.72-2.53 (m, 2H), 1.56 (s, 18H), 1.50 (s, 3H), 1.36 (s, 12H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.02, 144.93, 140.55, 129.74, 82.55, 80.21, 75.55, 72.23, 61.71, 39.11, 28.12, 27.77, 23.89, 19.89, 17.78; Anal. Calc. for $C_{28}H_{44}N_5O_8P$ (+ 0.5 MeOH): C, 54.71; H, 7.41; N, 11.19. Found: C, 54.68; H, 7.43; N, 11.21.

(*rel*)-(1*R*,4*S*)-[4-(6-Amino-purin-9-yl)-3-methyl-cyclopent-2-enyloxymethyl] phosphonic acid (16). To a solution of the phosphonate **15** (67 mg, 0.11 mmol) in CH_3CN (8 mL) was added trimethylsilyl bromide (168 mg, 1.11 mmol). The mixture was heated overnight at 60 °C and concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 (10 mL) and distilled H_2O (10 mL). The aqueous layer was washed with CH_2Cl_2 and then freeze-dried to give target compound **16** (23 mg, 64%) as a yellowish foamy solid. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.74 (s, 1H), 7.81 (s, 1H), 6.00 (s, 1H), 5.82 (m, 1H), 5.73 (m, 1H), 3.73 (d, J = 9.2 Hz, 2H), 2.72-2.53 (m, 2H), 1.50 (s, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.24, 149.51, 145.57, 138.54, 129.74, 79.59, 69.36, 60.65, 31.61, 17.78; Anal. Calc. for $C_{12}H_{16}N_5O_4P$ (+ 2.0 H_2O): C, 39.89; H, 5.58; N, 19.38. Found: C, 39.91; H, 5.60; N, 19.41.

(*rel*)-(1*R*,4*S*)-*t*-Butyl SATE phosphoester of 9-[1-(3,4-dimethylcyclopent-3-enylmethoxymethyl)] adenine (18). A solution of adenine phosphonic acid derivative **15** (69 mg, 0.212 mmol) and tributylamine (360 μL, 1.44 mmol) in water (2.4 mL) was mixed for 40 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 (15 mL) to which thioester **17** (324 mg, 1.98 mmol) and 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (266 mg, 0.9 mmol) were added. The mixture was stirred for 15 h at room temperature and quenched with tetrabutylammonium bicarbonate buffer (9.0 mL, 1 M solution, pH 8.0). The mixture was concentrated under reduced pressure and the residue was diluted with water (80 mL) and extracted twice with CH_2Cl_2 (60 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 (MeOH/Hexane/EtOAc, 0.03:4:1) to give **18** (42 mg, 33%) as a solid: mp 121 - 123 ºC; UV (H_2O) λ_{max} 262.0 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.24 (s, 1H), 8.12 (s, 1H), 5.89 (s, 1H), 5.76 (m, 1H), 5.32 (dd, J = 6.2, 2.4 Hz, 1H), 4.02 (m, 4H), 3.55 (d, J = 9.0 Hz, 2H), 3.16 (t, J = 6.3 Hz, 4H), 2.62-2.50 (m, 2H), 1.51 (s, 3H), 1.20 (s, 18H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.32, 154.64, 146.32, 143.51, 139.54, 128.43, 78.81, 70.21, 62.54, 59.43, 45.12, 33.45, 30.61, 18.21; Anal. Calc. for $C_{26}H_{40}N_5O_6PS_2$ (+ 1.0 MeOH): C, 50.22; H, 6.87; N, 10.84. Found: C, 50.19; H, 6.85; N, 10.88.

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