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Synthesis and Anti-HCV Activity of 3',5'-cyclic SATE Phosphonodiester Nucleoside as a Novel Prodrug

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A novel 2',4'-dimethyl carbocyclic adenosine 5'-phosphonic acid analogue (**20**) was prepared using acyclic stereoselective route from commercially available 4-hydroxybutan-2-one (**4**). To improve cellular permeability and enhance the anti-HCV activity of this phosphonic acid, a 3',5'-cyclic SATE phosphonodiester nucleoside prodrug (**22**) was prepared. The synthesized phosphonic nucleoside analogues, (**20**) and (**22**), were assayed for their ability to inhibit HCV RNA replication in a subgenomic replicon Huh7 cell line.

Key Words: Anti-HCV agent, Prodrug, Nucleoside phosphonate

Introduction

Hepatitis C virus (HCV) is the major causative agent for non-A, non-B virally induced hepatitis.[1] HCV infections often lead to reduced liver function, cirrhosis and hepatocellular carcinoma and eventually liver transplantation. The current approved therapy based on pegylated interferon alone or in combination with ribavirin is effective in only approximately half of the genotype 1 population.[2] Moreover, this limited efficacy is often associated with significant side effects, leading to discontinuation of treatment.[3] Therefore, there is a need for the development of more effective therapeutic agents for the treatment of HCV infection.[4] HCV RNA-dependent RNA polymerase (NS5B) and NS3/4A protease are currently the most promising targets for the development of novel treatments. The activities of these virally encoded enzymes are essential for HCV replication, and antiviral agents targeting these enzymes are in both preclinical and clinical development. To date, most of the reported nucleoside analogues[5] that inhibit HCV polymerase have modifications at the 2' or 4' position of the sugar.[6]

For example, 2'-methyl ribonucleosides yield compounds with excellent chain-terminating properties. Among them, 2'-*C*-methylcytidine[7] (**1**) and 2'-*C*-methyladenosine[8] (**2**) were discovered as potent anti-HCV agents and 2'-*C*-methylcytidine is in phase II clinical trials. Furthermore, 4'-bis-SATE (bis-*S*-acyl-2-thioethyl) prodrug of adenine analogue[9] (**3**) showed excellent anti-HCV activity in the genotype 1b subgenomic replicon system (Figure 1).

Actually, these antiviral nucleoside analogues do not directly exert antiviral activity, but rather are prodrugs of active phosphorylated metabolites that are formed by the actions of various kinases in cells. Ultimately, the triphosphates of these drugs are responsible for their actual antiviral activity by acting as inhibitors of HCV polymerase.[10]

A nucleoside 5'-phosphonate is essentially a nucleoside monophosphate analogue. However, a phosphonate has certain advantages over its phosphate counterpart as it is metabolically stable because its phosphorus-carbon bond is not susceptible

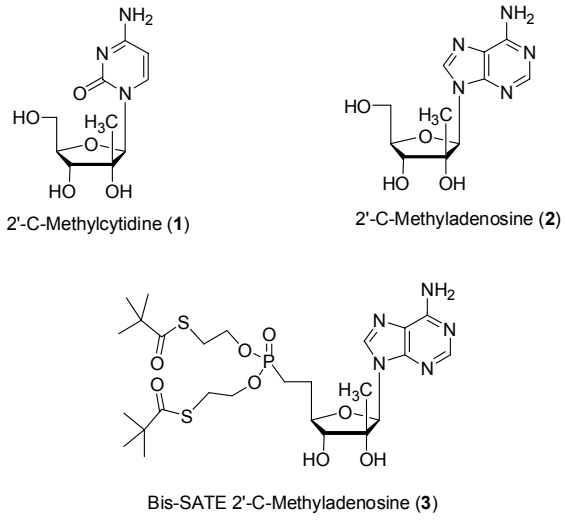
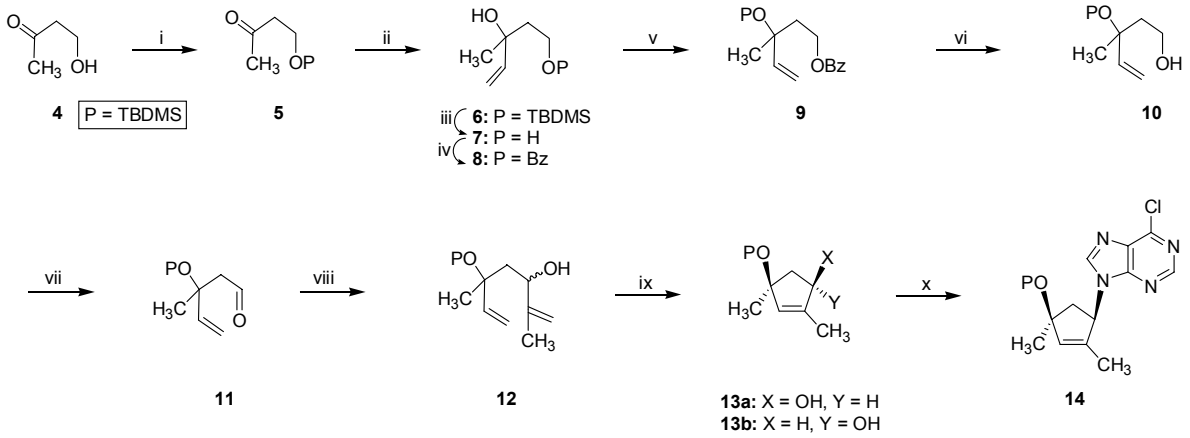


Figure 1. Structure of potent anti-HCV agents.

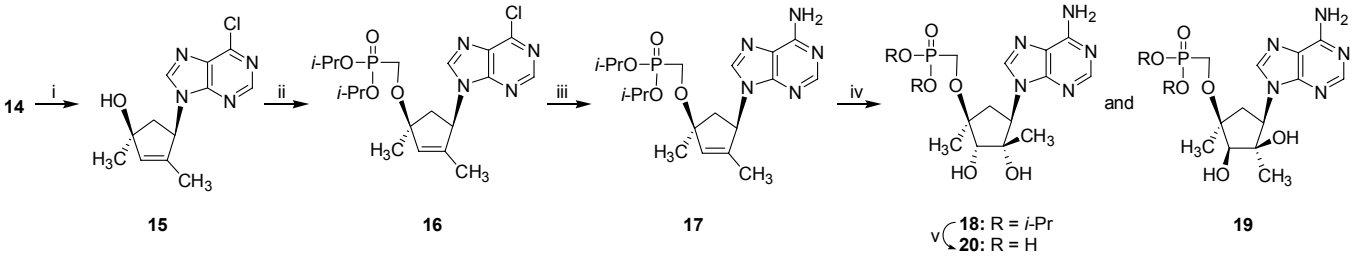
to hydrolytic cleavage.[11] Moreover, a phosphonate nucleoside can skip the requisite first phosphorylation, which is a crucial step for the activation of nucleosides. Though triphosphates of several of these nucleoside analogues exhibit excellent RdRp inhibitory potency, only a few nucleoside derivatives have exhibited biological activity in cell culture assays. This might be due to poor cellular penetration coupled with insufficient metabolism of these nucleoside derivatives to 5'-triphosphates.

Stimulated by the finding that branched nucleoside analogues exhibit excellent antiviral activities, we sought to synthesize novel classes of nucleotides comprising 2',4'-*C*-dimethyl carbocyclic 5'-phosphonic acid (**20**) and its cyclic SATE phosphonodiester prodrug (**22**) rather than its bis-SATE counterpart which has a bigger molecular size.

The target compounds were prepared from the commercially available starting material (**4**) as shown in Scheme 1. The alcohol functional group of (**4**) was temporary masked with *t*-butyldimethylchlorosilane to give ketone derivative (**5**), which was



Scheme 1. Synthesis of 6-chloropurine nucleoside intermediate. Reagents: i) TBDMSCl, imidazole, CH_2Cl_2; ii) VinylMgBr, THF; iii) TBAF, THF/CH_3CN; iv) BzCl, DMAP, pyridine; v) TBDMSOTf, DMAP, TEA, CH_2Cl_2; vi) NaOMe, MeOH; vii) $(COCl)_2$, DMSO, TEA, CH_2Cl_2; viii) IsopropenylMgBr, THF; ix) 2nd-Grubbs cat. benzene, reflux; x) 6-chloropurine, DIAD, PPh_3, 1,4-dioxane



Scheme 2. Synthesis of carbocyclic adenine phosphonic acid (20). Reagents: i) TBAF, THF; ii) $(i\text{-PrO})_2POCH_2Br$, LiO-*t*-Bu, THF; iii) NH_3, MeOH, steel bomb; iv) OsO_4, NMO, acetone/*t*-BuOH/H_2O; v) TMSBr, CH_2Cl_2

subject to carbonyl addition using vinylmagnesium bromide to yield tertiary alcohol derivative (**6**). In order to differentiate between the two hydroxyl groups, the silicon protection group of the primary hydroxyl was replaced with a benzoyl group by sequential desilylation and benzoylation to provide (**8**). Silylation of the tertiary hydroxyl group of (**8**) was successfully accomplished using trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give fully protected compound (**9**). The benzoyl protection group of the primary hydroxyl was removed by sodium methoxide (NaOMe) to provide the alcohol derivative (**10**), which was oxidized to the aldehyde (**11**) using Swern oxidation conditions (DMSO, oxalyl chloride, TEA). The aldehyde (**11**) was subjected to nucleophilic Grignard conditions by isopropenylmagnesium bromide [$CH_2 = C(CH_3)MgBr$] to yield divinyl (**12**).

Without separation of diastereomeric mixture, divinyl (**12**) was subjected to ring-closing metathesis (RCM) condition[12] using 2nd generation Grubbs catalyst to provide cyclopentenol (**13α**) (38%) and (**13β**) (39%), which were readily separated by silica gel column chromatography. The relative stereochemical assignments of the two isomers were made readily based on NOE comparisons. Upon irradiation of C_1-H, weak NOE patterns were observed at the proximal hydrogens of compound (**13b**) [C_4-CH_3 (0.14%)] versus those of compound (**13a**) [C_4-CH_3 (0.42%)] (Figure 2).

To synthesize the desired carbocyclic nucleoside analogues,

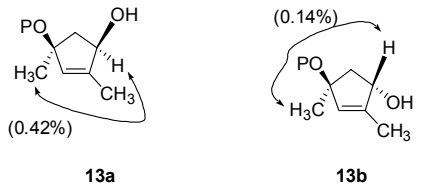
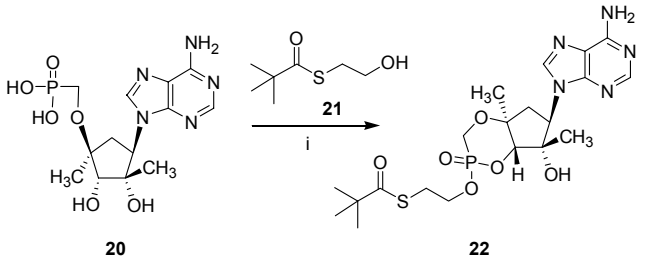


Figure 2. NOE between the proximal hydrogens of **13a** and **13b**.

6-chloropurine was treated with the protected cyclopentenol (**13b**) in the presence of diisopropylazodicarboxylate (DIAD) and PPh_3 to give (**14**) with a correct configuration. For the synthesis of nucleoside phosphonate by alkylation of the oxygen at C_4 of (**14**), the silyl protection was removed. Further treatment of the nucleoside (**15**) with diisopropylphosphonomethyl bromide yielded the desired compound (**16**); the chlorine group of (**16**) was then transformed to amine with methanolic ammonia in a steel bomb to give adenine derivative (**17**). The resultant nucleoside phosphonate mimics the overall shape and geometry of a nucleoside monophosphate. Bishydroxylation[13] of the double bond in (**17**) was accomplished with a catalytic amount of osmium tetraoxide (OsO_4) and 4-methyl-morpholine *N*-oxide (NMO) as the oxidant to give the dihydroxylated (**18**) as a major reaction product compared to minor isomer (**19**) (Scheme 2). Their stereochemistries were also readily determined by NOE experiment. These stereochemical outcomes suggest that a bul-



Scheme 3. Synthesis of 3',5'-cyclic-SATE prodrug of adenine analogue Reagents: i) thioester **21**, 1-mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole, pyridine

Table 1. Anti-HCV activity of synthesized compounds

Compound No.	Anti-HCV EC_{50} (μg/mL)	Cytotoxicity CC_{50} (μg/mL)
20	76.9	> 100
22	27.6	> 100
2'-*C*-Me-A	1.9	> 100

2'-*C*-Me-A: 2'-*C*-Methyladenosine. EC_{50} (μg/mL): concentration needed to reduce cell replication by 50%. CC_{50} (μg/mL): concentration needed to reduce cell viability by 50%

ky group such as the diisopropyl phosphonate group reinforce the steric hindrance of the β-faces.[14] Hydrolysis of (**18**) by treatment with bromotrimethylsilane afforded the adenine phosphonic acid (**20**). To synthesize the thioester-protected analogue, compound (**20**) was reacted with thioester (**21**) in the presence of 1-mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole (MSNT)[15] to provide the final prodrug (**22**) (Scheme 3).

The newly synthesized nucleoside analogues (**20**) and (**22**) were assayed for anti-HCV activity using an *in vitro* assay system that is suitable for monitoring anti-HCV activities of compounds.[16] This system is composed of a human hepatocarcinoma cell line (Huh-7) supporting multiplication of a HCV replication, and the results are summarized in Table 1. These cells contain a HCV subgenomic replicon RNA encoding a luciferase reporter gene as a marker. The antiviral potency of the nucleoside analogues against the HCV replicon is expressed as EC_{50}, which was quantified by a luciferase assay after a two-days incubation period with the tested compound. To confirm the anti-HCV potency of compounds, subgenomic replicon RNA levels were quantified by real-time RT-PCR analysis. In addition, the associated cytotoxicity (expressed as CC_{50} in Table 1) was evaluated in a tetrazolium (XTT)-based assay. 2'-*C*-methyladenosine was selected as the reference standard due to its structure similarity to the newly synthesized compounds.

The synthesized nucleoside prodrug (**22**) exhibited encouraging improvement in cell-based activity compared with phosphonic acid (**20**). A significant step forward in terms of activity could then be made with the introduction of 3',5'-cyclic-SATE protecting group as a prodrug scaffold.

Experimental Section

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. 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) and dd (doublet of doublets). UV spectra were obtained on a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). MS spectra were run in electrospray ionization (ESI) mode. The 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 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.

4-(*t*-Butyldimethylsilanyloxy) butan-2-one (5). To a solution of 4-hydroxy-butan-2-one **4** (10.0 g, 113.5 mmol) and imidazole (11.59 g, 170.24 mmol) in CH_2Cl_2 (250 mL), TBDMSCl (18.82 g, 124.85 mmol) was added slowly at 0 °C and stirred overnight at rt. The reaction solvent was evaporated under reduced pressure. The residue was poured into water (200 mL) and extracted with ethyl acetate (200 mL) two times. The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:25) to give compound **5** (21.36 g, 93%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.91 (t, J = 7.0 Hz, 2H), 2.72 (t, J = 7.0 Hz, 2H), 2.17 (s, 3H), 0.81 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 208.6, 59.3, 46.2, 25.5, 18.7, -5.5.

(±)-5-(*t*-Butyldimethylsilanyloxy)-3-methylpent-1-en-3-ol (6). To a solution of **5** (5.4 g, 26.68 mmol) in dry THF (50 mL) was slowly added vinylMgBr (32.79 mL, 1.0 M solution in THF) at −78 °C. After 5 h, saturated NH_4Cl solution (32 mL) and water (100 mL) were sequentially added to the mixture, and the reaction mixture was slowly warmed to rt. The mixture was extracted with EtOAc (100 mL) 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 **6** (4.92 g, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.78 (dd, J = 17.4, 10.8 Hz, 1H), 5.28 (d, J = 17.3 Hz, 1H), 5.04 (d, J = 10.8 Hz, 1H), 3.81-3.69 (m, 2H), 1.85-1.76 (m, 1H), 1.59-1.53 (m, 1H), 1.18 (s, 3H), 0.83 (s, 9H), 0.02 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 144.3, 112.2, 73.75, 61.0, 41.6, 28.6, 25.7, 18.2, -5.5; Analysis for $C_{12}H_{26}O_2Si$, Calcd.: C, 62.55; H, 11.37; Found: C, 62.47; H, 11.31.

(±)-3-Methylpent-4-ene-1,3-diol (7). TBAF (7.8 mL, 1.0 M solution in THF) was added to a solution of **6** (1.5 g, 6.51 mmol) in cosolvent (10 mL, THF/CH_3CN 1:1 v/v) at 0 °C. The mixture was stirred overnight at room temperature and concentrated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:7) to give **7** (635 mg, 84%): ^{1}H NMR (DMSO-d_6, 300 MHz) δ 5.92 (dd, J = 17.4, 11.1 Hz, 1H), 5.34 (d, J = 17.3 Hz, 1H), 5.14 (d, J = 11.2 Hz, 1H), 5.09 (s, 1H), 4.88 (t, J = 4.8 Hz, 1H), 3.84 (br s, 2H), 1.88 (m, 1H), 1.71 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 144.3, 112.3, 74.4, 60.1, 42.1, 28.8; Analysis for $C_6H_{12}O_2$, Calcd.: C, 62.04; H, 10.41; Found: C, 61.96; H, 10.44.

(±)-Benzoic acid 3-hydroxy-3-methylpent-4-enyl ester (8). To a solution of **7** (2.5 g, 21.52 mmol) in anhydrous pyridine

(25 mL) was added benzoyl chloride (3.66 g, 23.67 mmol) and dimethylamino pyridine (DMAP) (525 mg, 4.3 mmol) at 0 °C. The reaction mixture was stirred overnight at rt. The reaction mixture was quenched with saturated $NaHCO_3$ solution (10 mL), stirred for 20 minutes and concentrated under reduced pressure. The residue was poured into water (100 mL) and extracted with EtOAc (100 mL) twice. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give **8** (3.46 g, 73%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.03 (m, 2H), 7.57 (m, 1H), 7.45 (m, 2H), 5.95 (dd, *J* = 17.4, 10.8 Hz, 1H), 5.25 (d, *J* = 17.4 Hz, 1H), 5.07 (d, *J* = 10.8 Hz, 1H), 4.50-4.40 (m, 2H), 2.10-1.99 (m, 2H), 1.37 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 166.5, 144.2, 132.9, 130.1, 129.5, 128.3, 112.3, 72.3, 61.6, 40.3, 28.5; Analysis for $C_{13}H_{16}O_3$, Calcd.: C, 70.89; H, 7.32; Found: C, 70.92; H, 7.36.

(±)-Benzoic acid 3-(*t*-butyldimethylsilanyloxy)-3-methylpent-4-enyl ester (9). To a solution of **8** (2.2 g, 9.99 mmol) in anhydrous CH_2Cl_2 (20 mL) was added TEA (2.02 g, 19.98 mmol) and DMAP (262 mg, 2.15 mmol) at 0 °C. *t*-Butyldimethylsilyl trifluomethanesulfonate (TBDMSOTf) (2.9 g, 10.99 mmol) was added to this mixture and the reaction mixture was stirred overnight at rt and quenched with cold H_2O (10 mL). The mixture was diluted with water (100 mL) and extracted with EtOAc (80 mL) two times. Combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:25) to give **9** (2.7 g, 81%) as colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.93 (m, 2H), 7.45 (m, 1H), 7.32 (m, 2H), 5.81 (dd, *J* = 17.3, 10.8 Hz, 1H), 5.14 (d, *J* = 17.2 Hz, 1H), 4.93 (d, *J* = 10.8 Hz, 1H), 4.36-4.30 (m, 2H), 1.95-1.87 (m, 2H), 1.31 (s, 3H), 0.81 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 166.5, 144.8, 132.7, 130.4, 129.5, 128.2, 112.2, 74.4, 61.8, 42.1, 27.8, 25.8, 18.2, -5.56; Analysis for $C_{19}H_{30}O_3Si$, Calcd.: C, 68.22; H, 9.04; Found: C, 68.27; H, 8.97.

(±)-3-(*t*-Butyldimethylsilanyloxy)-3-methylpent-4-en-1-ol (10). To a solution of **9** (1.6 g, 4.78 mmol) in MeOH (12 mL) was added NaOMe (1.43 mL, 1.0 M in MeOH) at 0 °C. The reaction mixture was stirred overnight at rt and concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give **10** (991 mg, 90%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.91 (dd, *J* = 17.3, 10.7 Hz, 1H), 5.20 (d, *J* = 17.3 Hz, 1H), 5.04 (d, *J* = 10.8 Hz, 1H), 4.41-4.36 (m, 2H), 2.08-1.99 (m, 2H), 1.32 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 144.4, 112.3, 71.7, 61.3, 42.5, 28.9, 25.6, 18.4, -5.6; Analysis for $C_{12}H_{26}O_2Si$, Calcd.: C, 62.55; H, 11.37; Found: C, 62.47; H, 11.31.

3-(*t*-Butyldimethylsilanyloxy)-3-methylpent-4-enal (11). To a stirred solution of oxalyl chloride (0.072 mL, 0.816 mmol) in CH_2Cl_2 (8 mL) was added a solution of DMSO (0.072 mL, 1.08 mmol) in CH_2Cl_2 (0.8 mL) dropwise at −78 °C. The resulting solution was stirred at −78 °C for 5 min, and a solution of alcohol **10** (197 mg, 0.454 mmol) in CH_2Cl_2 (3 mL) was added dropwise. The mixture was stirred at −78 °C for 20 min and TEA (0.32 mL, 2.26 mmol) was added. The resulting mixture was warmed to 0 °C and stirred for 30 min. H_2O (6 mL) was added, and the solution was stirred at room temperature for 20 min. The mixture was diluted with water (50 mL) and then extracted with EtOAc (40 mL) two times. The combined organic layer was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:30) to give aldehyde compound **11** (193 mg, 99%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.94 (s, 1H), 5.92 (dd, *J* = 17.3, 10.7 Hz, 1H), 5.13 (d, *J* = 17.2 Hz, 1H), 5.00 (d, *J* = 10.7 Hz, 1H), 2.63-2.57 (m, 2H), 1.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 201.3, 144.8, 112.6, 61.9, 58.6, 28.8, 25.7, 18.6, -5.4.

(*rel*)-(3*R* and *3S*,5*S*)-5-(*t*-Butyldimethylsilanyloxy)-2,5-dimethylhepta-1,6-dien-3-ol (12). To a solution of **11** (2.5 g, 10.95 mmol) in dry THF (20 mL) was slowly added isopropenylMgBr (13.13 mL, 1.0 M solution in THF) at −20 °C and stirred 5 h at the same temperature. Saturated NH_4Cl solution (13 mL) and water (70 mL) were sequentially added to the mixture, which was slowly warmed to rt and extracted with EtOAc (70 mL) 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, 20) to give **12** (2.39 g, 81%) as diastereomeric mixture: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.01 (dd, *J* = 17.4, 10.8 Hz, 1H), 5.13 (d, *J* = 17.2 Hz, 1H), 5.26-5.00 (m, 4H), 3.82-3.87 (m, 1H), 1.87-1.55 (m, 2H), 1.45 (s, 3H), 1.34 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 147.2, 145.9, 143.4, 113.3, 112.3, 110.0, 109.9, 78.0, 72.5, 72.4, 48.6, 48.3, 29.0, 25.8, 18.1, −5.5.

(*rel*)-(1*S* and 4*S*)-4-(*t*-Butyldimethylsilanyloxy)-2,4-dimethylcyclopent-2-enol (13a) and (*rel*)-(1*R* and 4*S*)-4-(*t*-butyldimethylsilanyloxy)-2,4-dimethylcyclopent-2-enol (13b). To a solution of **12** (1.16 g, 4.3 mmol) in dry benzene (6 mL) was added 2nd generation Grubbs catalyst (63.5 mg 0.075 mmol). The reaction mixture was refluxed overnight and cooled to room temperature. The mixture was concentrated in vacuum, and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give cyclopentenol **13a** (354 mg, 34%) and **13b** (344 mg, 33%) as colorless oils, respectively. Cyclopentenol **13a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.40 (s, 1H), 4.21 (dd, *J* = 6.6, 1.8 Hz, 1H), 2.17 (dd, *J* = 12.6, 8.8 Hz, 1H), 1.99 (dd, *J* = 12.6, 6.6 Hz, 1H), 1.72 (s, 3H), 1.51 (s, 3H), 0.82 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 139.6, 125.8, 73.2, 70.1, 45.7, 29.3, 25.6, 18.7, 16.7, -5.5; Analysis for $C_{13}H_{26}O_2Si$, Calcd.: C, 64.41; H, 10.81; Found: C, 64.36; H, 10.80; Cyclopentenol **13b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.41 (s, 1H), 4.19 (d, *J* = 7.8 Hz, 1H), 2.19 (dd, *J* = 12.2, 8.4 Hz, 1H), 2.04 (dd, *J* = 12.3, 7.8 Hz, 1H), 1.74 (s, 3H), 1.50 (s, 3H), 0.83 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 138.9, 124.3, 73.8, 69.9, 44.2, 29.6, 25.5, 18.6, 15.8, −5.6; Analysis for $C_{13}H_{26}O_2Si$, Calcd.: C, 64.41; H, 10.81; Found: C, 64.43; H, 10.85.

(*rel*)-(1'*R*,4'*S*)-9-[4-(*t*-Butyldimethylsilyloxy)-2,4-dimethylcyclopent-2-en-1-yl] 6-chloropurine (14). To a solution containing compound **13b** (263 mg, 1.085 mmol), triphenylphosphine (570 mg, 2.17 mmol) and 6-chloropurine (335 mg, 2.17 mmol) in anhydrous 1,4-dioxane (10 mL), diisopropyl azodicarboxylate (DIAD) (438 mg, 2.17 mmol) was added dropwise at −20 °C for 30 min. under nitrogen. The reaction mixture was stirred for 5 h at 0 °C under nitrogen. The solvent was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 2:1) to give compound **14** (189 mg, 46%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71

(s, 1H), 8.52 (s, 1H), 5.40 (s, 1H), 4.52 (dd, J = 7.8, 1.4 Hz, 1H), 2.54 (dd, J = 12.8, 8.8 Hz, 1H), 2.10 (dd, J = 12.9, 7.8 Hz, 1H), 1.72 (s, 3H), 1.53 (s, 3H), 0.82 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 152.0, 151.4, 150.9, 146.0, 139.4, 134.1, 131.1, 71.3, 58.5, 41.6, 28.9, 25.5, 18.8, 17.8, −5.4; Analysis for $C_{18}H_{27}ClN_4OSi$, Calcd.: C, 57.05; H, 7.18; N, 14.78; Found: C, 56.96; H, 7.11; N, 14.81.

(*rel*)-(1'*R*,4'*S*)-9-[4-Hydroxy-2,4-dimethylcyclopent-2-en-1-yl] 6-chloropurine (15). TBAF (0.444 mL, 1.0 M solution in THF) was added to a solution of **14** (140 mg, 0.37 mmol) in THF (6.0 mL) at 0 °C. The mixture was stirred overnight at room temperature and concentrated. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.1:3:1) to give **15** (85 mg, 87%) as a white solid: mp 160 - 162 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.72 (s, 1H), 8.53 (s, 1H), 5.42 (s, 1H), 4.50 (dd, J = 7.6, 1.6 Hz, 1H), 2.59 (dd, J = 12.7, 8.8 Hz, 1H), 2.12 (dd, J = 12.8, 7.6 Hz, 1H), 1.73 (s, 3H), 1.49 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 152.3, 151.3, 150.5, 146.2, 138.2, 133.6, 131.4, 71.8, 58.2, 40.9, 29.0, 17.9; Analysis for $C_{12}H_{13}ClN_4O$, Calcd.: C, 54.45; H, 4.95; N, 21.17; Found: C, 54.38; H, 5.00; N, 21.13.

(*rel*)-(1'*R*,4'*S*)-Diisopropyl [9-(4-hydroxy-2,4-dimethylcyclopent-2-en-1-yl)] 6-chloropurine] methylphosphonate (16). Both Li*Ot*-Bu (7.6 mL of 1.0 M solution in THF, 7.6 mmol) and a solution of diisopropyl bromomethylphosphonate (1.665 g, 6.42 mmol) in 5 mL of THF were slowly added to a solution of the cyclopentene nucleoside analogue **15** (1.25 g, 4.74 mmol) in 5 mL of THF at 0 °C and stirred for 3 h at rt under anhydrous conditions. The mixture was quenched by adding water (10 mL) and further diluted with additional H_2O (60 mL). The aqueous layer was extracted with EtOAc (3 × 70 mL). The combined organic layer were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.1:4:1) to give **16** (1.34 g, 64%) as a foamy syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (s, 1H), 8.52 (s, 1H), 5.45 (s, 1H), 4.71 (m, 2H), 4.47 (d, J = 7.8 Hz, 1H), 3.72 (d, J = 8.0 Hz, 2H), 2.57 (dd, J = 12.8, 8.8 Hz, 1H), 2.16 (dd, J = 12.8, 7.4 Hz, 1H), 1.75 (s, 3H), 1.50 (s, 3H), 1.34 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 152.4, 151.3, 150.4, 146.6, 138.6, 133.2, 131.1, 72.2, 70.7, 65.6, 58.2, 41.3, 28.9, 23.7, 17.5; Analysis for $C_{19}H_{28}ClN_4O_4P$, Calcd.: C, 51.53; H, 6.37; N, 12.65; Found: C, 51.47; H, 6.40; N, 12.71.

(*rel*)-(1'*R*,4'*S*)-Diisopropyl [9-(4-hydroxy-2,4-dimethylcyclopent-2-en-1-yl)] adenine] methylphosphonate (17). A solution of **16** (150 mg, 0.34 mmol) in saturated methanolic ammonia (7 mL) was stirred on a steel bomb at 80 °C for 12 h, and the volatile components were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:7) to give **17** (86 mg, 60%) as a solid: mp 134 - 136 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.26 (s, 1H), 8.15 (s, 1H), 5.46 (s, 1H), 4.71 (m, 2H), 4.48 (dd, J = 7.6, 1.4 Hz, 1H), 3.71 (d, J = 8.0 Hz, 2H), 2.55 (dd, J = 12.6, 8.7 Hz, 1H), 2.17 (dd, J = 12.6, 7.5 Hz, 1H), 1.79 (s, 3H), 1.51 (s, 3H), 1.35 (m, 12H); ^{13}C NMR (DMSO-d_6) δ 155.4, 152.4, 150.7, 146.6, 138.1, 133.7, 119.4, 72.6, 70.2, 65.4, 59.6, 40.8, 29.1, 23.8, 17.3; Analysis for $C_{19}H_{30}N_5O_4P$, Calcd.: C, 53.89; H, 7.14; N, 16.54; Found: C, 53.85; H, 7.16; N, 16.47.

(*rel*)-(1'*R*, 2'*S*,3'*S*,4'*S*)-Diisopropyl [9-(2,3,4-trihydroxy-2,4-dimethylcyclopentyl)] adenine] methylphosphonate (18) and (*rel*)-(1'*R*,2'*R*,3'*R*,4'*S*)-diisopropyl [9-(2,3,4-trihydroxy-2,4-dimethylcyclopentyl)] adenine] methylphosphonate (19). Compound **17** (338 mg, 0.8 mmol) was dissolved in a mixture of acetone (12 mL), *t*-BuOH (2 mL) and H_2O (2 mL) along with 4-methylmorpholine *N*-oxide (140 mg, 1.2 mmol). Subsequently, OsO_4 (0.254 mL, 0.04 mmol, 4% wt % in H_2O) was added. The mixture was stirred overnight at rt and quenched with saturated Na_2SO_3 solution (4 mL). The resulting solid was removed by filtration through a pad of Celite, and filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:5) to give **18** (139 mg, 38%) and **19** (51 mg, 14%): Spectroscopical data for **18:** mp 121-123 °C; UV (H_2O) λ_{max} 260.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.30 (s, 1H), 8.14 (s, 1H), 7.17 (br s, 2H), 5.11 (s, 1H), 4.83 (d, J = 4.6 Hz, 1H), 4.70 (m, 2H), 3.86 (dd, J = 7.6, 1.6 Hz, 1H), 3.71 (d, J = 8.1 Hz, 2H), 3.61 (s, 1H), 2.16 (dd, J = 12.8, 8.8 Hz, 1H), 1.97 (dd, J = 12.7, 7.6 Hz, 1H), 1.36 (m, 15H), 1.31 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 155.6, 152.3, 150.1, 145.9, 120.3, 89.8, 73.0, 70.5, 69.1, 65.7, 54.2, 29.4, 23.3, 19.3, 16.2; Analysis for $C_{19}H_{32}N_5O_6P$, Calcd.: C, 49.88; H, 7.05; N, 15.31; Found: C, 49.92; H, 6.98; N, 15.27; Spectroscopical data for **19:** mp 117-119 °C; UV (H_2O) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.33 (s, 1H), 8.16 (s, 1H), 7.18 (br s, 2H), 5.13 (s, 1H), 4.85 (br s, 1H), 4.71 (m, 2H), 3.86 (dd, J = 7.7, 4.2 Hz, 1H), 3.72 (d, J = 8.0 Hz, 2H), 3.60 (s, 1H), 2.15 (dd, J = 12.7, 8.8 Hz, 1H), 1.99 (dd, J = 12.8, 7.4 Hz, 1H), 1.35-1.32 (m, 15H), 1.30 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 155.2, 152.7, 150.6, 145.2, 119.8, 90.0, 72.7, 70.9, 68.8, 65.3, 55.3, 30.1, 23.8, 19.5, 16.8; Analysis for $C_{19}H_{32}N_5O_6P$, Calcd.: C, 49.88; H, 7.05; N, 15.31; Found: C, 49.84; H, 7.09; N, 15.37.

(*rel*)-(1'*R*,2'*S*,3'*S*,4'*S*)-[9-(2,3,4-Trihydroxy-2,4-dimethylcyclopentyl)] adenine] methylphosphonic acid (20). To a solution of the phosphonate **18** (186 mg, 0.408 mmol) in CH_2Cl_2 (14 mL) was added trimethylsilyl bromide (672 mg, 4.44 mmol). The mixture was heated under reflux for 20 h and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 (20 mL) and distilled H_2O (20 mL). The aqueous layer was washed out with CH_2Cl_2 and then freeze-dried to give target compound **20** (125 mg, 82%) as a yellowish foamy solid. mp 106 - 108 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.26 (s, 1H), 8.12 (s, 1H), 7.13 (br s, 2H), 5.13 (s, 1H), 4.85 (br s, 1H), 3.82 (d, J = 7.8 Hz, 1H), 3.72 (d, J = 8.1 Hz, 2H), 3.63 (s, 1H), 2.14 (dd, J = 12.7, 8.6 Hz, 1H), 1.96 (dd, J = 12.8, 7.7 Hz, 1H), 1.33 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 154.9, 152.0, 150.2, 145.5, 119.7, 88.9, 72.6, 69.6, 65.7, 54.0, 28.8, 20.1, 16.1; Analysis for $C_{13}H_{20}N_5O_6P$ (+2 H_2O), Calcd.: C, 38.14; H, 5.91; N, 17.11; Found: C, 38.07; H, 5.99; N, 17.08.

(*rel*)-(1'*R*,2'*S*,3'*S*,4'*S*)-Cyclic SATE phosphoester of [9-(4-methyloxyphosphonate-2,3,4-trihydroxy-2,4-dimethylcyclopentyl)] adenine (22). A solution of adenine phosphonic acid derivative **20** (44 mg, 0.118 mmol) and tributylamine (200 μL, 0.80 mmol) in water (1.5 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 (10 mL) to which thioester **21** (180 mg, 1.1 mmol) and 1-mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole (148 mg, 0.5 mmol) were added. The mix-

ture was stirred for 16 h at room temperature and quenched with tetrabutylammonium bicarbonate buffer (5 mL, 1 M solution, pH 8.0). The mixture was concentrated under reduced pressure and the residue was diluted with water (50 mL) and extracted twice with CH_2Cl_2 (50 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, 0.05:3:1) to give **22** (16 mg, 28%) as a solid: mp 113 - 115 °C; UV (H_2O) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.25 (s, 1H), 8.12 (s, 1H), 4.19 (t, J = 6.4 Hz, 2H), 3.70 (d, J = 8.2 Hz, 2H), 3.32 (s, 1H), 3.24 (t, J = 6.3 Hz, 2H), 2.10 (dd, J = 12.6, 8.6 Hz, 1H), 1.91 (dd, J = 12.6, 7.2 Hz, 1H), 1.29 (s, 3H), 1.21 (s, 9H), 1.01 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 202.4, 154.7, 151.8, 150.5, 145.7, 119.5, 94.4, 70.6, 69.2, 67.4, 65.2, 54.3, 51.3, 33.2, 28.3, 24.2, 19.3, 15.3. Analysis for $C_{20}H_{30}N_5O_6PS$ (+0.5 MeOH): C, 47.76; H, 6.26; N, 13.58; Found: C, 47.83; H, 6.19; N, 13.50.

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