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Synthesis and Antiviral Evaluation of 1'-Branched-5'-Norcarbocyclic Adenosine Phosphonic Acid Analogues

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Novel 1'-methyl-5'-norcarbocyclic adenosine phosphonic acid analogues were synthesized using an acyclic stereoselective route from commercially available 3,3-diethoxy-propan-1-ol **4**. The synthesized nucleoside phosphonate **19** and phosphonic acid **21** were subjected to antiviral screening against various viruses.

Key Words: Antiviral agent, 1'-Branched nucleoside, 5'-Nornucleoside, Phosphonic acid nucleoside

Introduction

Nucleosides play a major role in combating tumors and viruses, with modifications of natural nucleosides leading to novel antitumor and/or antiviral agents, including branched nucleosides such as DMDC,[1] CNDAC,[2] ECyd,[3] 4'α-*C*-ethenylthymidine,[4] and 4'α-*C*-ethynylthymidine[5] as potent antiviral or antitumor agents.

The 1'-substituted nucleosides such as angustmycin **1**[6] and hydantocidin **2**[7] are herbicidal and affect plant growth (Figure 1). However, only a few examples of 1'-substituted nucleosides of defined absolute stereochemistry are reported.[8] The scarcity of examples of 1'-substituted nucleosides may be due to the synthetic difficulties for elaborating a necessary tertiary carbon center. We therefore developed efficient methodology to synthesize furanosyl or cyclopentane rings containing stereochemically defined tertiary carbons.

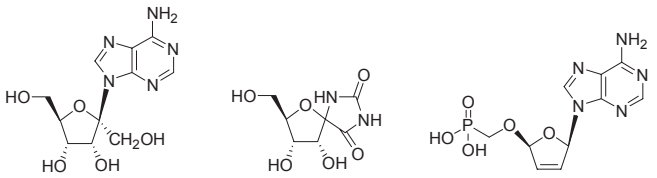


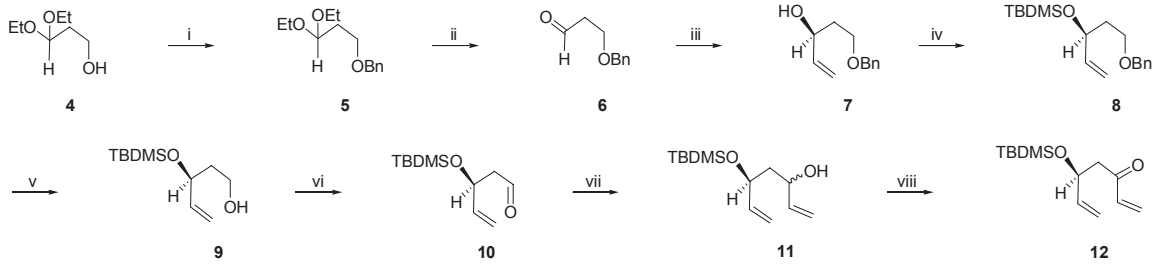
Figure 1. Structures of potent 1'-branched or 5'-nornucleoside analogues.

5'-Nornucleoside phosphonic acid analogues such as d4AP **3**[9] as potential anti-HIV agents have encouraged the search for novel nucleosides in this class of compounds. A nucleoside 5'-nornucleoside phosphonate analogue is essentially a nucleoside monophosphate analogue.[10] The phosphonate has certain advantages over its phosphate counterpart as it is metabolically stable because its phosphorus-carbon bond is not susceptible to hydrolytic cleavage.[11] Moreover, a phosphonate nucleoside can skip the requisite first phosphorylation, which is a crucial step for the activation of nucleosides. This is frequently a limiting event in the phosphorylation sequence, which ultimately leads to triphosphates.

Because 1'-branched nucleoside analogues and 5'-nornucleoside phosphate have excellent biological activities, we sought to synthesize and evaluate a novel class of nucleosides comprising 1'-methyl branched carbocyclic-5'-norcarbocyclic phosphonic acid.

Results and Discussion

The target compounds were prepared from commercially available starting material **4** as shown in Scheme 1. The alcohol functional group of **4** was protected with benzyl bromide to give the acetal derivative **5**, which was subject to the acidic hydrolysis conditions using *p*-toluene sulfonic acid (PTSA) to give the aldehyde derivative **6**. Aldehyde functional **6** was sub-



Reagents: i) BnBr, NaH, THF; ii) PTSA, H_2O/acetone; iii) vinylMgBr, THF; iv) TBDMSOTf, 2,6-lutidine, CH_2Cl_2; v) Li, NH_3, THF; vi) $(COCl)_2$, DMSO, TEA, CH_2Cl_2; vii) vinylMgBr, THF; viii) MnO_2, CCl_4.

Scheme 1. Synthesis of cyclopentenone intermediate **12**

ject to carbonyl addition using vinylmagnesium bromide to provide the tertiary alcohol derivative **7** as a racemic mixture. The corresponding tertiary hydroxyl group of **7** was successfully silylated using *t*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) with 2,6-lutidine[12] to give the fully protected compound **9**. Removal of the benzyl protecting group of **8** under dissolving metal reduction furnished desired alcohol **9**, which was oxidized to the aldehyde **10** using Swern oxidation conditions (DMSO, oxalyl chloride, TEA). The aldehyde **10** was again subjected to nucleophilic Grignard conditions with vinylmagnesium bromide to give divinyl **11**, which was subjected to allylic oxidation conditions (MnO_2, CCl_4) to provide **12**. Dienone **12** was subjected to addition by methyllithium in dry diethyl ether to give **13** as an inseparable isomer, which was subjected to ring-closing metathesis (RCM) conditions using 2nd generation Grubbs catalyst[13] to provide cyclopentenol **14a** (41%) and **14b** (40%), which were readily separated by silica gel column chromatography. The NOE experiments with cyclopentenols **14a** and **14b** confirmed these assignments. As expected, NOE enhancements were found between the *cis*-oriented hydrogens. Upon irradiation of C_1-CH_3, weak NOE patterns were observed at the proximal hydrogens of compound **14b** [C_4-H (0.03%)] *versus* those of compound **14a** [C_4-H (0.06%)] (Figure 2).

To synthesize the desired 5'-norcarbocyclic adenine nucleoside analogue, the protected cyclopentenol **14b** was treated with 6-chloropurine in the presence of diisopropyl azodicarboxylate (DIAD) and PPh_3 to give **15** with a correct configuration. The choice of solvent system, temperature, and procedure are critical in the selective nucleobase coupling using Mitsunobu conditions (Scheme 2). Slow addition of diisopropyl azodicarboxylate (DIAD) to a mixture of cyclopentenol **14b**, triphenylphosphine, and the 6-chloropurine in anhydrous dioxane-DMF mixture solvent gave a yellow solution that was stirred for 2 h at −20 °C to give protected 6-chloropurine analogue **15**. The silyl protection group was removed with tetrabutylammonium fluoride (TBAF) to give **16**. Treatment of the nucleoside **16** with diethylphosphonomethyl triflate prepared from diethyl hydroxymethylphosphonate yielded the nucleoside phosphonate **17**.[14] The chlorine group of **17** was then transformed to amine with methanolic ammonia at 70 °C to give a corresponding adenine phosphonate derivative **18**.[15]

The resultant nucleoside phosphonate mimics the overall shape and geometry of a nucleoside monophosphate. Bishydroxylation[16] of the double bond in **18** 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 isomer **19** (37%) as a major reaction product compared to minor isomer **20** (17%) (Scheme 3). Their stereochemistries were also readily determined by NOE. These stereochemical outcomes suggest that two bulky groups such as the diethyl phosphonate and purine nucleobase reinforce the steric hindrance of the β-faces.[17] Hydrolysis of **19** by treatment with bromotrimethylsilane in CH_3CN in the presence of 2,6-lutidine gave an adenine phosphonic acid derivative **21**.[18]

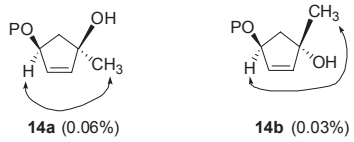
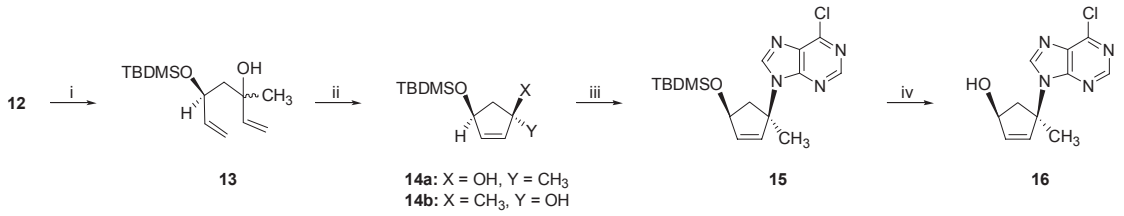


Figure 2. NOE difference between the hydrogens of **14a** and **14b**.

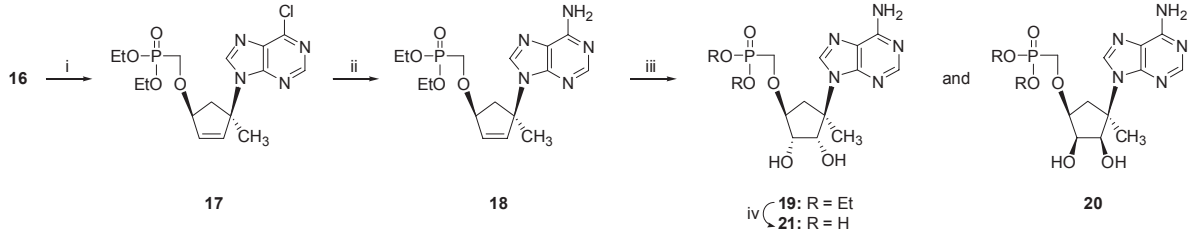
Conclusions

As shown in Table 1, the synthesized nucleoside phosphonate **19** and phosphonic acid **21** were subjected to antiviral screening against herpes simplex virus, coxsakie B virus, poliovirus, and human immunodeficiency virus according to the assay procedure.[19] They only showed weak antiviral activity (**19**, EC_{50} = 62.7 μM) and (**21**, EC_{50} = 41.2 μM) against herpes simples virus type 1 without cytotoxicity.



Reagents: i) MeLi, diethylether; ii) Grubbs (II) catalyst, CH_2Cl_2; iii) 6-chloropurine, DIAD, dioxane/DMF; iv) TBAF, THF.

Scheme 2. Synthesis of 5'-norcarbocyclic 6-chloropurine **16**



Reagents: i) $(EtO)_2POCH_2OTf$, LiO-*t*-Bu, THF; ii) NH_3/MeOH, 70 °C; iii) OsO_4, NMO, acetone/*t*-BuOH/H_2O; iv) TMSBr, 2,6-lutidine, CH_3CN.

Scheme 3. Synthesis of target 5'-norcarbocyclic phosphonic acid analogues

Table 1. Antiviral activity of synthesized compounds

Compound No.	Virus	Activity EC_{50} (μM)	Cytotoxicity IC_{50} (μM)
19	HSV-1	**62.7**	> 100
	coxsakie B	> 100	> 100
	Polio	> 100	> 100
	HIV-1	> 100	> 100
21	HSV-1	**41.2**	> 100
	coxsakie B	> 100	> 100
	Polio	> 100	> 100
	HIV-1	90	98

On the basis of potent antiviral and antitumor activity of 1'-branched nucleoside analogues, we have accomplished the stereoselective synthesis of its carbocyclic version starting from 3,3-diethoxy-propan-1-ol. The synthesis of other nucleoside analogues with different nucleobases (T, C, U), their SATE-prodrugs and stability study 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 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 collected 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 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.

(3,3-Diethoxy-propoxymethyl)-benzene (5). To a stirred suspension of NaH (0.7 g, 29.16 mmol) in THF (75 mL), a solution of **4** (3.13 g, 21.15 mmol) in dry THF (30 mL) was added slowly, and stirred for 1 h at rt. To this mixture, benzyl bromide (4.27 g, 25 mmol) was added, and stirred overnight at rt. The mixture was quenched using a saturated ammonium chloride solution (10 mL) and further diluted with water (100 mL). The mixture was extracted with EtOAc (100 mL) twice. The combined organic layer was 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 **4** (4.33g, 86%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27-7.20 (m, 5H), 4.68 (t, J = 6.0 Hz, 1H), 4.57 (s, 2H), 3.71-3.60 (m, 6H), 1.98 (m, 2H), 1.35 (t, J = 6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 137.8, 128.4, 127.4, 104.6, 75.4, 62.2, 60.6, 38.2, 16.2.

3-Benzyloxy-propionaldehyde (6). The mixture of acetal **5** (216.9 mg, 0.91 mmol) and *p*-toluenesulfonic acid (PTSA, 17.4 mg, 0.092 mmol) in 10% aqueous acetone (10 mL) was heated overnight at 35 - 40 °C, and then quenched by addition of triethylamine and concentrated in vacuo. The residue was extracted with ethylacetate, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:5) to give compound **6** (118 mg, 79%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.87 (s, 1H), 7.25-7.19 (m, 5H), 4.67 (s, 2H), 3.69 (t, J = 7.0 Hz, 2H), 2.58 (t, J = 7.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 200.8, 138.1, 128.8, 127.5, 74.6, 63.7, 46.4.

(±)-5-Benzyloxy-pent-1-en-3-ol (7). To a solution of **6** (1.55 g, 9.44 mmol) in dry THF (20 mL) was slowly added vinylMgBr (11.32 mL, 1.0 M solution in THF) at −20 °C and stirred for 3 h at the same temperature. Saturated NH_4Cl solution (10 mL) was added to the mixture, which was slowly warmed to rt. The mixture was further 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 under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **7** (1.50 g, 83%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28-7.23 (m, 5H), 5.91-5.83 (m, 1H), 5.25 (d, J = 12.2 Hz, 1H), 5.12 (d, J = 7.0 Hz, 1H), 4.64 (s, 2H), 3.90 (m, 1H), 3.39 (t, J = 6.8 Hz, 2H), 1.67 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 138.5, 137.9, 128.4, 127.3, 115.3, 74.9, 72.0, 64.2, 37.8.

(±)-[1-(2-Benzyloxyethyl)-allyloxy]-*t*-butyldimethylsilane (8). To a stirred solution of tertiary alcohol **7** (138.4 mg, 0.72 mmol) and 2,6-lutidine (0.6 mL, 6.14 mmol) in dry methylene chloride (10 mL) was added *t*-butyldimethylsilyltrifluoromethane sulfonate (TBDMSOTf, 0.9 mL, 3.07 mmol) at −10 °C. The reaction mixture was warmed to room temperature, and stirred for 3 h at the same temperature. The mixture was quenched by saturated sodium bicarbonate (5 mL) and water (60 mL) was added. The mixture was extracted with ethyl acetate (70 mL) twice. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:35) to give **8** (185 mg, 84%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28-7.22 (m, 5H), 5.90 (m, 1H), 5.22 (dd, J = 2.4, 12.4 Hz, 1H), 5.15 (d, J = 6.9 Hz, 1H), 4.65 (s, 2H), 3.92 (m, 1H), 3.40 (t, J = 7.0 Hz, 2H), 1.68 (m, 2H), 0.81 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 138.8, 138.1, 129.2, 127.7, 116.4, 75.3, 71.8, 63.2, 38.2, 25.6, 18.6, −5.5.

(±)-3-(*t*-Butyldimethylsilanyloxy)-pent-4-en-1-ol (9). Ammonia (approximately 10 mL) was condensed into a flask containing a solution of benzyl ether **8** (167 mg, 0.546 mmol) in dry tetrahydrofuran (4 mL) at −78 °C. To this mixture was added a minimum amount of metallic lithium sufficient to maintain a blue color, and the resulting deep blue solution was stirred at −78 °C for 4 min. Methanol was added dropwise at the same temperature until the blue color disappeared. The colorless solution was stirred for 30 min at −78 °C, and then solid ammonium chloride (*ca*. 4.0 g) was added. After stirring for 1 h at −78 °C, ammonia was allowed to evaporate. Diethyl ether (40 mL) was added, and the mixture was dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **9** (101 mg, 86%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300

MHz) δ 5.89 (m, 1H), 5.27 (d, J = 12.4 Hz, 1H), 5.14 (d, J = 7.0 Hz, 1H), 3.91 (m, 1H), 3.56 (m, 2H), 1.66 (m, 2H), 0.82 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 138.5, 115.7, 70.8, 59.2, 42.5, 25.7, 18.4, −5.6.

(±)-3-(*t*-Butyldimethylsilanyloxy)-pent-4-enal (10). To a stirred solution of oxalyl chloride (132 mg, 1.04 mmol) in CH_2Cl_2 (8 mL) was added a solution of DMSO (122 mg, 1.56 mmol) in CH_2Cl_2 (2.5 mL) dropwise at –78 °C. The resulting solution was stirred at –78 °C for 10 min, and a solution of alcohol **9** (114.6 mg, 0.53 mmol) in CH_2Cl_2 (5 mL) was added dropwise. The mixture was stirred at –78 °C for 20 min and TEA (316 mg, 3.12 mmol) was added. The resulting mixture was warmed to 0 °C and stirred for 30 min. H_2O (10 mL) was added, and the solution was stirred at room temperature for 20 min. The mixture was diluted with water (60 mL) and then extracted with EtOAc (70 mL) twice. The combined organic layer was 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, 1:20) to give aldehyde compound **10** (101 mg, 89%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.90 (s, 1H), 5.91 (m, 1H), 5.29 (dd, J = 2.6, 12.2 Hz, 1H), 5.17 (dd, J = 2.7, 7.0 Hz, 1H), 4.09 (m, 1H), 2.61 (m, 2H), 0.82 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 200.3, 137.8, 116.1, 68.4, 53.2, 25.5, 18.6, −5.3.

(*rel*)-(3*R* and 3*S*,5*S*)-5-(*t*-Butyldimethylsilanyloxy)-hepta-1,6-dien-3-ol (11). Divinyl derivative **11** was synthesized from aldehyde **10** using a similar procedure as described for **7** as a diastereomeric mixture: yield 81%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.82-5.77 (m, 2H), 5.31-5.20 (m, 4H), 3.93-3.85 (m, 2H), 1.69-1.61 (m, 2H), 0.82 (m, 9H), 0.02 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 138.2, 137.6, 118.5, 117.6, 69.1, 68.3, 46.7, 25.7, 18.3, −5.5.

(±)-5-(*t*-Butyldimethylsilanyloxy)-hepta-1,6-dien-3-one (12). To a mixture of allylic alcohol **11** (1.2 g, 4.97 mmol), manganese (IV) dioxide (1.29 g, 14.8 mmol) and CCl_4 (15 mL) were stirred at room temperature. Additional manganese (IV) dioxide (216 mg, 2.47 mmol) was added at 1 h. The progress of the reaction was monitored by TLC. The resulting mixture was filtered through a pad of Celite and washed with ethyl acetate. The filtrate and washings were condensed in vacuo and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:20) to give dieneone **12** (896 mg, 75%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.40-6.23 (m, 4H), 5.23 (m, 2H), 3.16 (dd, J = 12.4, 6.6 Hz, 1H), 0.83 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 197.5, 139.1, 138.5, 128.3, 116.2, 68.7, 50.5, 25.4, 18.7, −5.3.

(*rel*)-(1*R* and 1*S*,4*S*)-4-(*t*-Butyldimethylsilanyloxy)-3-methyl-hepta-1,6-dien-3-ol (13). To a solution of compound **12** (2.8 g, 11.66 mmol) in dry diethyl ether (60 mL), methyllithium (8.7 mL, 1.6 M solution in diethyl ether) was added slowly at −78 °C. After 1 h, a saturated NH_4Cl solution (15 mL) was added, and the reaction mixture was warmed slowly to room temperature. The mixture was diluted with water (100 mL) and extracted with EtOAc (100 mL) twice. The combined organic layer was washed with water, dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **13** (2.1 g, 71%) as a diastereomeric mixture: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.92-2.86 (m, 2H), 5.31-5.20 (m, 4H), 3.88 (m, 1H), 1.65-1.60 (m, 2H), 1.42 (s, 3H), 0.81 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 143.1, 140.8, 115.5, 113.2, 67.3, 65.2, 52.7, 25.7, 18.3, −5.4.

(*rel*)-(1*S*,4*S*)-4-(*t*-Butyldimethylsilanyloxy)-1-methyl-cyclopent-2-enol (14a) and (*rel*)-(1*R*,4*S*)-4-(*t*-butyldimethylsilanyloxy)-1-methyl-cyclopent-2-enol (14b). To a solution of **13** (456 mg, 1.78 mmol) in dry methylene chloride (7 mL) was added 2nd generation Grubbs catalyst (36.0 mg 0.0424 mmol). The reaction mixture was refluxed overnight and cooled to room temperature. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give cyclopentenol **14a** (166 mg, 41%) and **14b** (162 mg, 40%). Data for **14a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.64-5.55 (m, 2H), 4.06 (m, 1H), 2.17 (dd, J = 12.2, 6.8 Hz, 1H), 2.08 (dd, J = 12.1, 8.6 Hz, 1H), 1.45 (s, 3H), 0.81 (m, 9H), 0.02 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 135.1, 130.6, 71.4, 67.4, 47.7, 26.7, 25.4, 18.3, −5.5; Anal. Calc. for $C_{12}H_{24}O_2Si$: C, 63.10; H, 10.59; Found: C, 63.04; H, 10.56; Data for **14b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.62-5.53 (m, 2H), 4.10 (m, 1H), 2.18 (dd, J = 12.2, 6.6 Hz, 1H), 2.07 (dd, J = 12.2, 8.8 Hz, 1H), 1.49 (s, 3H), 0.82 (m, 9H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 136.3, 131.4, 70.9, 66.8, 48.9, 26.2, 25.7, 18.6, −5.3; Anal. Calc. for $C_{12}H_{24}O_2Si$: C, 63.10; H, 10.59; Found: C, 63.15; H, 10.66.

(*rel*)-(1'*R*,4'*S*)-9-[4-(*t*-Butyldimethylsilanyloxy)-1-methyl-cyclopent-2-enyl]-6-chloropurine (15). To a solution containing compound **14b** (66.46 mg, 0.291 mmol), triphenylphosphine (305 mg, 1.164 mmol) and 6-chloropurine (90 mg, 0.582 mmol) in anhydrous co-solvent (dioxane/DMF:3/1, 7.0 mL), diisopropyl azodicarboxylate (DIAD) (117 mg, 0.582 mmol) was added dropwise at –78 °C for 30 min under nitrogen. The reaction mixture was stirred for 2 h at –20 °C under nitrogen and further stirred for 2 h at rt. The solvent was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 2:1) to give compound **15** (46 mg, 44%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.77 (s, 1H), 8.54 (s, 1H), 5.62-5.55 (m, 2H), 4.09 (m, 1H), 2.17 (dd, J = 12.3, 6.8 Hz, 1H), 2.07 (dd, J = 12.2, 8.8 Hz, 1H), 1.89 (s, 3H), 0.81 (m, 9H), 0.01 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 154.7, 152.3, 147.6, 145.7, 134.3, 129.4, 123.5, 70.5, 57.4, 46.7, 25.4, 23.8, 18.5, −5.3; Anal. Calc. for $C_{17}H_{25}ClN_4OSi$·1.0 MeOH: C, 54.46; H, 7.36; N, 14.11; Found: C, 54.49; H, 7.40; N, 14.08.

(*rel*)-(1'*R*,4'*S*)-9-[4-Hydroxy-1-methyl-cyclopent-2-en-1-yl] 6-chloropurine (16). To a solution of **15** (126 mg, 0.345 mmol) in THF (6.0 mL), TBAF (0.449 mL, 1.0 M solution in THF) was added at 0 °C. The mixture was stirred overnight at room temperature and concentrated in vacuo. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.05:4:1) to give **16** (94 mg, 84%) as a white solid: mp 156 - 158 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.79 (s, 1H), 8.55 (s, 1H), 5.56-5.47 (m, 2H), 5.17 (s, 1H, D_2O exchangeable), 4.12 (m, 1H), 2.56 (dd, J = 12.3, 6.8 Hz, 1H), 2.12 (dd, J = 12.4, 6.8 Hz, 1H), 1.85 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 155.0, 153.5, 147.3, 135.2, 128.6, 127.2, 123.2, 72.1, 58.5, 44.8, 23.6; Anal. Calc. for $C_{11}H_{11}ClN_4O$: C, 52.70; H, 4.42; N, 22.35; Found: C, 52.67; H, 4.39; N, 22.33.

(*rel*)-(1'*R*,4'*S*)-Diethyl [9-(4-hydroxy-1-methyl-cyclopent-2-en-1-yl)] 6-chloropurine] methylphosphonate (17). Both Li*O*t-Bu (3.8 mL of 0.5 M solution in THF, 1.9 mmol) and a

solution of diethyl phosphonomethyltriflate (480 mg, 1.6 mmol) in 5.0 mL of THF were slowly added to a solution of the purine analogue **16** (296 mg, 1.18 mmol) in 5.0 mL of THF at 0 °C and stirred for 4 h at rt under anhydrous conditions. The mixture was quenched by adding water (5 mL) and further diluted with additional H_2O (50 mL). The aqueous layer was extracted with EtOAc (3 × 50 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 (MeOH/Hexane/EtOAc, 0.05:3:1) to give **17** (274 mg, 58%) as a foamy solid: mp 109-111 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.78 (s, 1H), 8.54 (s, 1H), 5.56-5.46 (m, 2H), 4.29-4.15 (m, 6H), 3.68 (d, *J* = 8.0 Hz, 2H), 2.54 (dd, *J* = 12.3, 6.5 Hz, 1H), 2.08 (dd, *J* = 12.4, 8.7 Hz, 1H), 1.84 (s, 3H), 1.39 (m, 6H); ^{13}C NMR ($CDCl_3$)) δ 154.8, 152.8, 146.5, 133.2, 129.5, 127.4, 119.4, 71.4, 67.4, 65.3, 63.2, 57.6, 46.5, 22.9, 16.2; Anal. Calc. for $C_{16}H_{22}ClN_4O_4P$·1.0 MeOH: C, 47.17; H, 6.05; N, 12.94; Found: C, 47.21; H, 6.01; N, 12.97.

(*rel*)-(1'*R*,4'*S*)-Diethyl [9-(4-hydroxy-1-methyl-cyclopent-2-en-1-yl)] adenine] methylphosphonate (18). A solution of **17** (120 mg, 0.299 mmol) in saturated methanolic ammonia (5 mL) was stirred at 70 °C in a steel bomb, and the volatile components were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:10) to give **18** (77 mg, 68%) as a solid: mp 130 - 132 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.28 (s, 1H), 8.10 (s, 1H), 5.57-5.48 (m, 2H), 4.30-4.17 (m, 5H), 3.68 (d, *J* = 8.2 Hz, 2H), 2.33 (dd, *J* = 12.4, 6.7 Hz, 1H), 2.05 (dd, *J* = 12.3, 8.8 Hz, 1H), 1.82 (s, 3H), 1.37 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 155.1, 153.4, 147.8, 134.6, 128.6, 126.7, 118.4, 70.5, 67.8, 65.3, 63.7, 58.7, 48.1, 23.4, 16.8; Anal. Calc. for $C_{16}H_{24}N_5O_4P$·1.0 MeOH: C, 49.39; H, 6.83; N, 16.94; Found: C, 49.43; H, 6.80; N, 16.91.

(*rel*)-(1'*R*, 2'*S*,3'*S*,4'*S*)-Diethyl [9-(2,3-dihydroxy-1-methyl-cyclopent-1-yl)] adenine] 4-methylphosphonate (19) and (*rel*)-(1'*R*,2'*R*,3'*R*,4'*S*)-diethyl [9-(2,3-dihydroxy-1-methyl-cyclopent-1-yl)] adenine] 4-methylphosphonate (20). Compound **18** (152 mg, 0.4 mmol) was dissolved in a mixture of acetone (6 mL), *t*-BuOH (1 mL), and H_2O (1 mL) along with 4-methylmorpholine *N*-oxide (70 mg, 0.6 mmol). Subsequently, OsO_4 (0.127 mL, 0.02 mmol, 4% wt % in H_2O) was added. The mixture was stirred overnight at rt and quenched with saturated Na_2SO_3 solution (2 mL). The resulting solid was removed by filtration through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:5) to give **19** (61 mg, 37%) and **20** (28 mg, 17%):

Spectroscopical data for 19: mp 148 - 150 °C; UV (H_2O) λ_{max} 262.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.28 (s, 1H), 8.15 (s, 1H), 7.15 (br s, 2H), 4.99 (d, *J* = 4.0 Hz, 1H), 4.21 (m, 4H), 3.71-3.63 (m, 3H), 3.31 (d, *J* = 6.8 Hz, 1H), 3.02 (m, 1H), 2.12 (dd, *J* = 12.4, 6.8 Hz, 1H), 1.93 (dd, *J* = 12.4, 8.6 Hz, 1H), 1.72 (s, 3H), 1.37 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 154.9, 152.4, 149.3, 144.6, 119.8, 80.2, 71.3, 67.8, 65.9, 64.6, 63.2, 53.2, 31.6, 18.0, 16.2; Anal. Calc. for $C_{16}H_{26}N_5O_6P$·1.0 MeOH: C, 45.63; H, 6.76; N, 15.65; Found: C, 45.59; H, 6.79; N, 15.68.

Spectroscopical data for 20: mp 156 - 158 °C; UV (H_2O) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.30 (s, 1H), 8.17 (s, 1H), 7.13 (br s, 2H), 4.89 (d, *J* = 4.2 Hz, 1H), 4.22-4.26 (m, 4H), 3.70-3.62 (m, 3H), 3.36 (d, *J* = 6.7 Hz, 1H), 3.07 (m, 1H), 2.12 (dd, *J* = 12.5, 6.8 Hz, 1H), 1.94 (dd, *J* = 12.4, 8.8 Hz, 1H), 1.77 (s, 3H), 1.38 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 155.1, 152.8, 148.6, 143.8, 120.3, 79.8, 71.5, 68.2, 65.2, 62.4, 54.6, 33.7, 17.6, 15.9; Anal. Calc. for $C_{16}H_{26}N_5O_6P$·1.5 MeOH: C, 45.35; H, 6.96; N, 15.11; Found: C, 45.31; H, 7.00; N, 15.08.

(*rel*)-(1'*R*,2'*S*,3'*S*,4'*S*)-[9-(2,3-dihydroxy-1-methyl-cyclopent-1-yl)] adenine] 4-methylphosphonic acid (21): To a solution of the phosphonate **19** (87 mg, 0.211 mmol) in anhydrous CH_3CN (7 mL) and 2,6-lutidine (0.5 mL) was added trimethylsilyl bromide (350 mg, 2.3 mmol). The mixture was heated under reflux for 6 h and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 (50 mL) and distilled clean water (50 mL). The aqueous layer was washed out with CH_2Cl_2 two times and then freeze-dried to give target compound **21** (47 mg, 83%) as a yellowish solid. mp 121 - 123 °C; UV (H_2O) λ_{max} 263.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.29 (s, 1H), 8.14 (s, 1H), 5.01 (br s, 1H), 3.72-3.66 (m, 3H), 3.41 (d, *J* = 6.6 Hz, 1H), 3.09 (m, 1H), 2.21 (dd, *J* = 12.4, 6.7 Hz, 1H), 1.98 (dd, *J* = 12.5, 8.9 Hz, 1H), 1.69 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 155.1, 153.7, 148.3, 145.8, 121.0, 80.1, 78.6, 73.2, 67.5, 64.2, 53.1, 31.4; Anal. Calc. for $C_{12}H_{18}N_5O_6P$·3.0 H_2O: C, 34.87; H, 5.85; N, 16.94; Found: C, 34.92; H, 5.82; N, 16.95.

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