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Racemic Synthesis of Novel 6'-Methylene-5'-norcarbocyclic Purine Phosphonic Acid Analogues *via* Mitsunobu Reaction

Eunae Kim, Lian Jin Liu, and Joon Hee Hong*

*BK-21 Project Team, College of Pharmacy, Chosun University, Kwangju 501-759, Korea. *E-mail: hongjh@chosun.ac.kr*
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Novel 5'-norcarbocyclic adenine and guanine phosphonic acid analogues with 6'-electronegative moiety such as unsaturated *C-C* bond were designed and synthesized from commercially available 2-methylene-propane-1,3-diol (**4**). Regioselective Mitsunobu reaction successfully proceeded from an allylic functional group (±)-**12b** at low reaction temperature in polar cosolvent (DMF/1,4-dioxane) to give purine phosphonate analogues (±)-**13** and (±)-**20**. The purine nucleoside phosphonate and phosphonic acid analogues were subjected to antiviral screening against HIV-1. Guanine analogue (±)-**23** shows significant *anti*-HIV activity in PBM cell lines (EC_{50} = 8.1 μM).

Key Words : *anti*-HIV agents, 6'-Methylene-5'-norcarbocyclic nucleoside, Mitsunobu reaction

Introduction

Several phosphonate and phosphonic acid derivatives of purines and pyrimidines have emerged as potent antiviral agents.[1] The spacial location of the oxygen atom, namely the β-position from the phosphorus atom in the nucleoside analogue, plays a critical role in the antiviral activity. This increased antiviral activity with this oxygen atom may be attributed to the increased binding capacity of the phosphonate analogues to target enzymes.[2] Moreover, a phosphonate nucleoside analogue can skip the requisite first phosphorylation, which is a crucial step for the activation of nucleosides.[3]

As mimics of nucleoside monophosphates, these nucleotide analogues exert their antiviral effect following sequential activation by cellular kinases to their diphosphate derivatives (nucleoside triphosphate analogues) which act as potent inhibitors of viral DNA polymerases.[4] A selective inhibition for these enzymes as opposed to host cell DNA polymerases is critical for the potential use of such compounds as drugs. Various attempts to improve the selectivity index have led to rigid nucleoside analogues modified in their cyclopentane ring system by introduction of unsaturations, sometimes with an exomethylene moiety in 6'-position.

Among these compounds, entecavir[5] **1** is being clinically used as *anti*-HBV drugs (Figure 1). Pyrimidine isonucleoside **2** with a methylene[6] moiety in place of carbon atom of the furanose ring was reported to show antiviral activity, especially *anti*-HIV activity. The phosphonate analogue **3** was successfully prepared and evaluated for its inhibitory effect on the replication of retroviruses, including Rauscher-murine Leukemia virus (R-MuLV) and HIV-1. Furthermore, compound **3** was superior to d4T in inhibiting R-MuLV at three orders magnitude low concentration, indicating that the murine model might be useful to evaluate **3** for its *in vivo* efficacy against the retrovirus.[7] Molecular modeling studies demonstrated the presence of an electronegative moiety at the 6'-position that could accommodate these substitutions and contribute to the observed enhancement in potency in *anti*-HIV activity.[8]

Stimulated by these findings that 6'-electronegative nucleoside analogues and 5'-norcarbocyclic nucleoside phosphonates have excellent biological activities, we sought to synthesize a novel class of nucleosides comprising 6'-methylene-5'-norcarbocyclic phosphonic acid analogues in order to search for more effective therapeutics against HIV and to provide analogues for probing the conformational preferences of enzymes associated with the nucleoside kinases of nucleosides and nucleotides.

As depicted in Scheme 1, the target compounds were prepared from 2-methylene-propane-1,3-diol (**4**). Monosilylation of diol **4** and subsequent oxidation of corresponding allylic alcohol **5** gave α,β-unsaturated aldehyde **6**. The aldehyde functional group of **6** was subjected to carbonyl addition reaction by vinylmagensium bromide[9] to furnish the secondary alcohol (±)-**7**, which was successfully protected using *p*-methoxybenzyl chloride (PMBCl)[10] to provide compound (±)-**8**. Removal of the silyl protecting group of (±)-**8** using tetra *n*-butylammonium fluoride (TBAF) gave

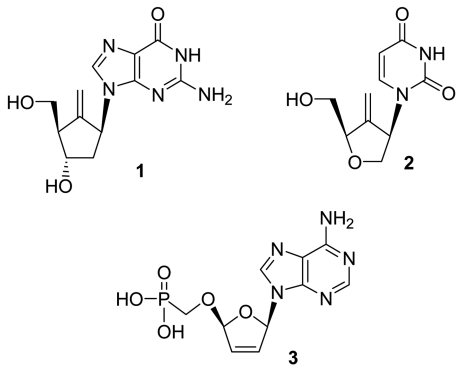
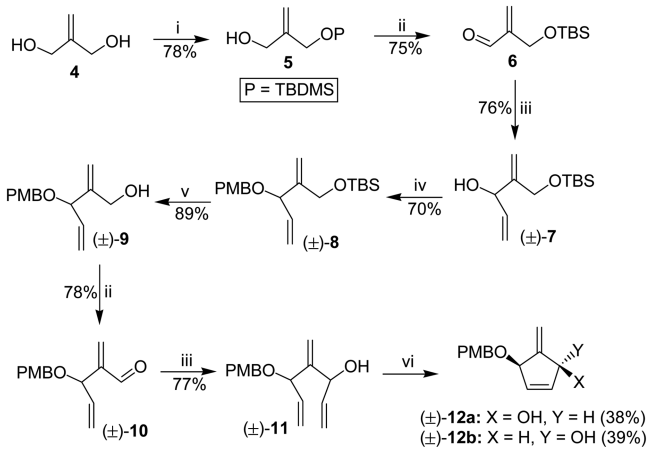
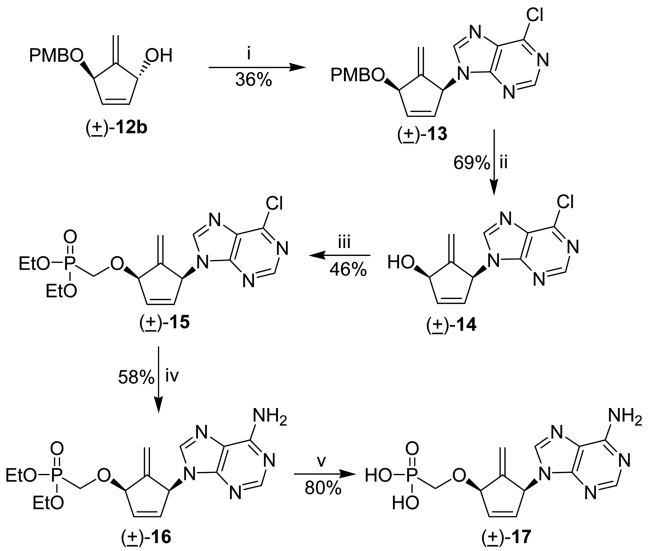


Figure 1. Structures of nucleoside analogues as potent antiviral agents.



Reagents: i) TBDMSCl, imidazole, DMF; ii) MnO_2, CCl_4; iii) vinylMgBr, THF; iv) PMBCl, NaH, DMF; v) TBAF, THF; vi) Grubbs (II), CH_2Cl_2.

Scheme 1. Synthesis of 6'-methylene cyclopentene intermediate (±)-**12b**.



Reagents: i) 6-chloropurine, DEAD, PPh_3, 1,4-dioxane/DMF; ii) DDQ, CH_2Cl_2/H_2O (10:1); iii) $(EtO)_2POCH_2OTf$, LiO-*t*-Bu, THF; iv) NH_3/MeOH, 65 °C; v) TMSBr, 2,6-lutidine, CH_3CN.

Scheme 2. Synthesis of 6'-methylene cyclopentenyl adenine phosphonic acid (±)-**17**.

the primary alcohol (±)-**9**, which was oxidized to the aldehyde (±)-**10** using same oxidation conditions as described for **6**. The aldehyde (±)-**10** was subjected to nucleophilic Grignard conditions with vinylmagnesium bromide to give divinyl (±)-**11**, which was subjected to ring-closing metathesis (RCM) conditions using 2nd generation Grubbs catalyst ($C_{46}H_{65}Cl_2N_2PRu$)[11] to provide 6'-cyclopentenol (±)-**12a** (38%) and (±)-**12b** (39%), which were readily separated by silica gel column chromatography. The Nuclear Overhauser Enhancement (NOE) experiments with cyclopentenols (±)-**12a** and (±)-**12b** confirmed these assignments. As expected, NOE enhancements were found between the *cis*-oriented hydrogens. Upon irradiation of C_1-*H*, weak NOE patterns were observed at the proximal hydrogens of compound (±)-**12b** [C_4-C*H*- (1.9%)] versus those of compound (±)-**12a** [C_4-C*H*- (3.0%)] (Figure 2).

To synthesize the desired 5'-norcarbocyclic adenosine nucleoside analogues, the protected cyclopentenol (±)-**12b** was treated with 6-chloropurine under Mitsunobu conditions[12] (DEAD and PPh_3). The appropriate choice of solvent system, temperature and procedure are essential for the regioselectivity as well as for the yield. In purine synthesis, a mixture of dioxane and DMF were used as the solvent for the coupling of the cyclopentenol (±)-**12b** with 6-chloropurine instead of THF. The heterocyclic bases had a better solubility in the dioxane-DMF mixture resulting in better yields. Slow addition of diethyl azodicarboxylate (DEAD) to a mixture of cyclopentenol (±)-**12b**, triphenylphosphine and the 6-chloropurine in anhydrous cosolvent (dioxane-DMF) gave a yellow solution, which was stirred for 2.0 h at −40 °C and further stirred overnight at rt to give the protected 6-chloropurine analogue (±)-**13** as an only N^9-regioisomer [UV (MeOH) λ_{max} 264.5 nm].[13] The PMB protection group was removed with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ)[14] to produce the 5'-nornucleoside analogue (±)-**14**, which was treated with diethylphosphonomethyl triflate[15] using lithium *t*-butoxide to yield the nucleoside phosphonate analogue (±)-**15** (Scheme 2). The chlorine group of (±)-**15** was then converted to amine with methanolic ammonia at 65 °C to give the corresponding adenine phosphonate derivative (±)-**16**. Hydrolysis of (±)-**16** by treatment with bromotrimethylsilane in CH_3CN in the presence of 2,6-lutidine gave an adenine phosphonic acid derivative (±)-**17** (Scheme 2).[16]

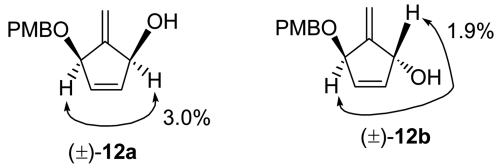
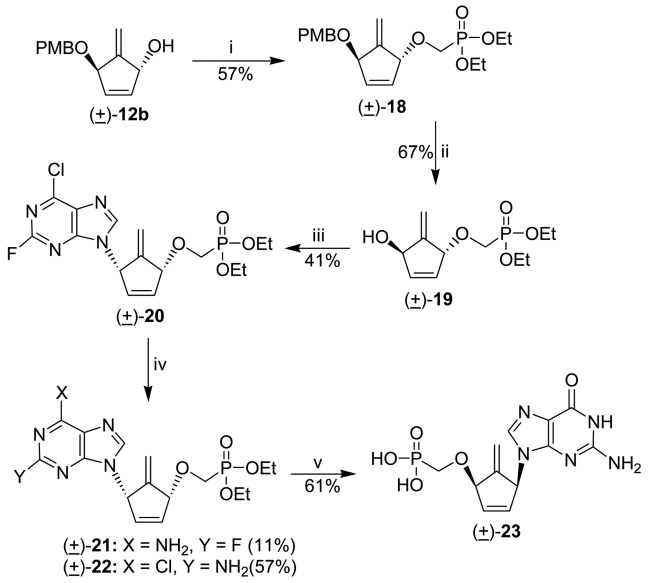


Figure 2. NOE differences between the proximal hydrogens of (±)-**12a** and (±)-**12b**.

The cyclopentenol intermediate (±)-**19** was also used for the synthesis of 2,6-disubstituted purine analogues such as guanine derivative (±)-**23**. Regioselective coupling of the enol (±)-**19** with 2-fluoro-6-chloropurine[17] under the similar conditions for 6-chloropurine gives analogue (±)-**20**. Bubbling ammonia into the compound (±)-**20** gave separable 2-fluoro-6-aminopurine[18] analogue (±)-**21** (11%) and 2-amino-6-chloropurine analogue (±)-**22** (57%), respectively. 2-Amino-6-chloropurine derivative (±)-**22** was treated with TMSBr to provide phosphonic acid and sequentially treated sodium methoxide and 2-mercaptoethanol in methanol to give desired guanine phosphonic acid (±)-**23** (Scheme 3).[19]

The synthesized nucleoside phosphonate and phosphonic acid analogues (±)-**16**, (±)-**17**, (±)-**21**, (±)-**22** and (±)-**23** were then evaluated for antiviral activity against human immunodeficiency virus. The procedures for measuring the antiviral activity toward wild-type HIV and cytotoxicity have been reported previously.[20] As shown in Table 1,



Reagents: i) $(EtO)_2POCH_2OTf$, LiO-*t*-Bu, THF; ii) DDQ, CH_2Cl_2/H_2O (10:1); iii) 2-fluoro-6-chloropurine, DEAD, PPh_3, 1,4-dioxane/DMF; iv) NH_3/DME, rt; v) (a) TMSBr, 2,6-lutidine, CH_3CN; (b) NaOMe, $HSCH_2CH_2OH$, MeOH.

Scheme 3. Synthesis of 6'-methylene cyclopentenyl guanine phosphonic acid (±)-**23**.

Table 1. Median effective (EC_{50}) and inhibitory (IC_{50}) concentration of synthesized nucleoside analogues in PBM and Vero cells

Compound No.	*Anti*-HIV-1 in PBM cells EC_{50} (μM)[c]	Cytotoxicity in PMB cells IC_{50} (μM)[d]	Cytotoxicity in Vero cells IC_{50} (μM)[d]
16	56.4	> 100	98
17	26	> 100	95
21	80	> 100	> 100
22	60	> 100	98
23	**8.1**	> 100	80
AZT[a]	0.004	> 100	> 100
PMEA[b]	0.51	> 100	> 100

[a]**AZT:** azidothymidine. [b]**PMEA**: 9-[2-(phosphonomethoxy)ethyl]adenine. [c]EC_{50}(M): EC_{50} values are for 50% inhibition of virus production as indicated by supernatant RT levels. [d]IC_{50}(M): IC_{50} values indicates 50% inhibition of cell growth.

guanine nucleoside phosphonic acid (±)-**23** exhibits significant *anti*-HIV activity. However, nucleoside analogues (±)-**16**, (±)-**17**, (±)-**21**, and (±)-**22** showed weak *anti*-HIV activity or cytotoxicity at concentrations up to 100 μM.

In summary, based on the potent *anti*-HIV activity of 6'-electronegative nucleosides and 5'-norcarbocyclic nucleoside analogues, we have designed and successfully synthesized novel 6'-methylene-5'-norcarbocyclic nucleoside analogues starting from 2-methylene-propane-1,3-diol (**4**). The synthesized nucleotide guanine (±)-**23** exhibited improvement in cell-based activity compared with adenine phosphonic acid (±)-**17**. Although the nucleotide analogue **3** inhibits in vitro *anti*-HIV activity, the carbocyclic versions shows weak *anti*-HIV activity except guanine analogue (±)-**23**. Since rigid cyclopentene carbocycles are not perfect

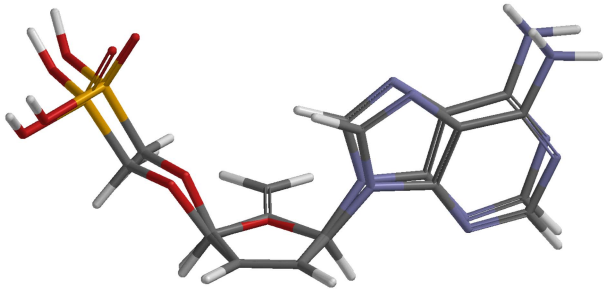


Figure 3. Superimposed conformations of nucleoside analogue **3**, as *anti*-HIV agent and an adenosine phosphonic acid derivative **17**. The lowest energy conformation for each molecules was calculated with the modeling package Spartan 02 and energy minimization with semi-empirical force field (PM3).

mimics for ribofuranose moiety, the mechanisms of virus inhibition, that is, either phosphorylation or inhibition of RNA synthesis, might be impaired in these compounds. Homologation of 6'-position is another possible reason for the apparent lack of activity. Figure 3 shows the superposition of the calculated low energy conformers of **3** and **17**, highlighting the two difference parts such as purine bases and phosphonic acid functional moieties.

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.

2-(*t*-Butyldimethylsilanyloxymethyl)prop-2-en-1-ol (5). To a stirred solution of compound **4** (3.5 g, 39.72 mmol) and imidazole (4.05 g, 59.58 mmol) in CH_2Cl_2 (150 mL), *t*-butyldimethylsilyl chloride (6.28 g, 41.7 mmol) was added at 0 °C. The mixture was stirred at the same temperature for 4 h, and quenched by adding a $NaHCO_3$ aqueous solution (10 mL). The mixture was extracted using EtOAc (200 mL), dried over $MgSO_4$, filtered and then concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:7) to give **5** (6.27 g, 78%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.99 (m, 2H), 4.15 (s, 2H), 4.07 (s, 2H), 0.83 (s, 9H), 0.03 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 147.43, 111.04, 65.05, 64.59, 25.83, 18.26, −5.46.

2-(*t*-Butyldimethylsilanyloxymethyl)propenal (6). To a mixture of allylic alcohol **5** (838 mg, 4.14 mmol), manganese (IV) dioxide (1.08 g, 12.4 mmol) and CCl_4 (12 mL) was added and refluxed overnight. Additional manganese (IV) dioxide (180 mg, 2.06 mmol) was added and refluxed for an additional 12 h. The progress of the reaction was monitored by TLC. The resulting mixture was filtered through a pad of celite, washed with ethyl acetate. The filtrate and washings were condensed *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give α,β-unsaturated aldehyde **6** (622 mg, 75%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.74 (s, 1H), 6.42-6.26 (m, 2H), 4.51 (m, 2H), 0.83 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.3, 154.9, 128.7, 65.5, 25.4, 18.6, –5.6.

(±)-2-(*t*-Butyldimethylsilanyloxymethyl)penta-1,4-dien-3-ol (7). To a solution of **6** (1.55 g, 7.73 mmol) in dry THF (20 mL), vinylmagnesium bromide (9.3 mL, 1.0 M solution in THF) was slowly added at –20 ºC and stirred 5 h at 0 ºC. Saturated NH_4Cl solution (10 mL) was added to the mixture, which was slowly warmed to rt. The mixture was diluted with water (100 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layer was washed with brine, 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 **7** (1.34 g, 76%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.91 (m, 1H), 5.31-5.22 (m, 4H), 4.61 (m, 2H), 4.42 (m, 2H), 0.81 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.2, 137.5, 115.8, 108.4, 76.6, 68.1, 25.5, –5.5; MS *m/z* 229 $(M+H)^+$.

(±)-*t*-Butyl-[3-(4-methoxybenzyloxy)-2-methylenepent-4-enyloxy]dimethylsilane (8). NaH (60% in mineral oil, 131 mg, 3.32 mmol) was added portion-wise to a cooled (0 °C) solution of secondary alcohol **7** (632 mg, 2.77 mmol) and *p*-methoxybenzyl chloride (0.41 mL, 3.04 mmol) in anhydrous DMF (10 mL). The reaction mixture was stirred overnight at rt. The solvent was removed *in vacuo* and the residue was diluted with H_2O (70 mL) followed by extraction with diethyl ether (2 × 70 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **8** (675 mg, 70%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29-7.23 (m, 2H), 6.93-6.87 (m, 2H), 5.89 (m, 1H), 5.28-5.22 (m, 4H), 4.64 (s, 2H), 4.49 (s, 2H), 4.20 (m, 1H), 3.75 (s, 3H), 0.82 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.5, 151.2, 143.4, 133.7, 128.7, 115.5, 108.5, 79.6, 74.1, 68.0, 56.3, 25.7, 18.4, –5.5; MS *m/z* 349 $(M+H)^+$.

(±)-3-(4-Methoxybenzyloxy)-2-methylenepent-4-en-1-ol (9). To a solution of **8** (1.35 g, 3.87 mmol) in THF (16 mL), TBAF (5.8 mL, 1.0 M solution in THF) was added at 0 ºC. The mixture was stirred overnight at rt and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (Hexane/EtOAc, 5:1) to give **9** (807 mg, 89%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30-7.22 (m, 2H), 6.92-6.86 (m, 2H), 5.87 (m, 1H), 5.30-5.23 (m, 4H), 4.65 (s, 2H), 4.48 (s, 2H), 4.22 (m, 1H), 3.74 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.7, 152.0, 142.7, 132.5, 127.8, 116.3, 107.6, 78.7, 73.6, 67.6, 57.5; MS *m/z* 235 $(M+H)^+$.

(±)-3-(4-Methoxybenzyloxy)-2-methylenepent-4-enal (10). Aldehyde derivative **10** was synthesized from **9** by the similar procedure as described for **6**: yield 78%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.69 (s, 1H), 7.28-7.21 (m, 2H), 6.89-6.82 (m, 2H), 6.42 (d, J = 1.6 Hz, 1H), 6.19 (d, J = 1.5 Hz, 1H), 5.88 (m, 1H), 5.25-5.20 (m, 2H), 4.65 (s, 2H), 4.23 (m, 1H), 3.74 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 191.5, 159.5, 154.7, 142.8, 132.0, 129.5, 116.1, 76.1, 73.5, 56.2; MS *m/z* 233 $(M+H)^+$.

(*rel*)-(3*R* and *3S*,5*S*)-5-(4-Methoxybenzyloxy)-4-methylenehepta-1,6-dien-3-ol (11). Divinyl analogue **11** was synthesized as a diastereomeric mixture from aldehyde **10** by a procedure similar to that described for **9** as diastereomeric mixture: yield 77%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31-7.23 (m, 2H), 6.91-6.83 (m, 2H), 5.91-5.86 (m, 2H), 5.25-5.18 (m, 6H), 4.63-4.58 (m, 3H), 4.21 (m, 1H), 3.75 (s, 3H).

(*rel*)-(1*R*,4*S*)-4-(4-Methoxybenzyloxy)-5-methylenecyclopent-2-enol (12a) and (*rel*)-(1*S*,4*S*)-4-(4-Methoxybenzyloxy)-5-methylenecyclopent-2-enol (12b). To a solution of **11** (140.5 mg, 0.54 mmol) in dry methylene chloride (5 mL) was added 2nd generation Grubbs catalyst (20.0 mg, 0.0235 mmol). The reaction mixture was refluxed overnight and cooled to rt. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give cyclopentenol **12a** (47 mg, 38%) and **12b** (49 mg, 39%). Data for **12a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.30-7.24 (m, 2H), 6.90-6.84 (m, 2H), 5.64 (dd, J = 5.4, 2.4 Hz, 1H), 5.36 (dd, J = 5.5, 3.0 Hz, 1H), 5.21-5.18 (m, 2H), 4.64-4.58 (m, 3H), 4.19 (d, J = 3.1 Hz, 1H), 3.75 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.7, 151.6, 138.4, 137.1, 132.2, 129.3, 126.9, 108.5, 80.5, 77.0, 74.2, 56.4; MS *m/z* 233 $(M+H)^+$.

Data for **12b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31-7.23 (m, 2H), 6.89-6.82 (m, 2H), 5.65 (dd, J = 5.5, 2.6 Hz, 1H), 5.40 (dd, J = 5.6, 3.2 Hz, 1H), 5.20 (m, 2H), 4.63-4.58 (m, 3H), 4.21 (d, J = 3.2 Hz, 1H), 3.73 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.5, 152.0, 138.7, 137.3, 133.7, 128.8, 127.5, 107.9, 81.1, 76.7, 73.5, 57.2; MS *m/z* 233 $(M+H)^+$.

(*rel*)-(1*R*,4*S*)-9-[4-(4-Methoxybenzyloxy)-5-methylenecyclopent-2-en-1-yl] 6-chloropurine (13). To a solution containing compound **12b** (183 mg, 0.744 mmol), triphenylphosphine (528 mg, 2.016 mmol) and 6-chloropurine (229 mg, 1.488 mmol) in anhydrous cosolvent (1,4-dioxane, 8.0 mL and DMF, 6.0 mL), diethyl azodicarboxylate (DEAD) (0.271 mL, 1.488 mmol) was added dropwise at –40 ºC for 10 min under nitrogen. The reaction mixture was stirred for 2 h at the same temperature under nitrogen and further stirred overnight at rt. The solvent was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/hexane, 3:1) to give compound **13** (102 mg, 36%): mp 162-164 ºC; UV (MeOH) λ_{max} 264.5 nm: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71 (s, 1H), 8.37 (s, 1H), 7.25-7.19 (m, 2H), 6.91-6.84 (m, 2H), 5.62 (dd, J = 5.5, 2.8

Hz, 1H), 5.38 (dd, *J* = 5.6, 3.3 Hz, 1H), 5.18-5.15 (m, 2H), 5.08 (d, *J* = 2.9 Hz, 1H), 4.64 (s, 2H), 4.22 (d, *J* = 3.2 Hz, 1H), 3.73 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.5, 149.9, 147.4, 141.3, 136.6, 131.2, 124.7, 108.5, 81.8, 74.6, 62.3, 57.0; MS *m/z* 369 $(M+H)^+$.

(*rel*)-(1*R*,4*S*)-9-(4-Hydroxy-5-methylene-cyclopent-2-en-1-yl)-6-chloropurine (14). To a solution of compound **13** (156 mg, 0.423 mmol) in CH_2Cl_2/H_2O (8 mL, 10:1 v/v) was added DDQ (143 mg, 0.623 mmol), and the mixture was stirred overnight at room temperature. Saturated $NaHCO_3$ (0.8 mL) was added to quench the reaction, which was then stirred for 2 h at rt. The mixture was diluted with water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated *in vacuo* and the residue was purified by silica gel column chromatography (EtOAc/hexane/MeOH, 4:1:0.05) to give compound **14** (72 mg, 69%): mp 166-168 ºC; UV (MeOH) λ_{max} 264.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.68 (s, 1H), 8.28 (s, 1H), 5.74 (dd, *J* = 5.4, 2.8 Hz, 1H), 5.68 (dd, *J* = 5.5, 3.4 Hz, 1H), 5.15 (dd, *J* = 4.2, 1.4 Hz, 2H), 5.03 (d, 3.0 Hz, 1H), 4.94 (d, *J* = 4.0 Hz, 1H), 4.55 (d, *J* = 3.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.4, 151.3, 147.1, 143.8, 138.6, 135.2, 125.7, 108.1, 78.2, 61.5; MS *m/z* 249 $(M+H)^+$.

(*rel*)-(1*R*,4*S*)-Diethyl [9-(4-hydroxy-5-methylene-cyclopent-2-en-1-yl)-6-chloropurine] phosphonate (15). Both Li*Ot*-Bu (2.98 mL of 0.5 M solution in THF, 1.488 mmol) and a solution of diethyl phosphonomethyltriflate (417 mg, 1.392 mmol) in 11.0 mL of THF were slowly added to a solution of the 6-chloropurine nucleoside analogue **14** (173 mg, 0.696 mmol) in 10.0 mL of THF at –20 °C and stirred overnight at rt under nitrogen. The mixture was quenched by adding saturated NH_4Cl solution (7 mL) and further diluted with additional H_2O (150 mL). The aqueous layer was extracted with EtOAc (3 × 150 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.05:4:1) to give **15** (127 mg, 46%) as a foam: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.77 (s, 1H), 8.46 (s, 1H), 5.77 (dd, *J* = 5.5, 2.8 Hz, 1H), 5.52 (dd, *J* = 5.6, 4.0 Hz, 1H), 5.17-5.15 (m, 2H), 5.01 (d, *J* = 3.2 Hz, 1H), 4.33-4.30 (m, 4H), 4.20 (d, *J* = 3.3 Hz, 1H), 3.96 (d, *J* = 8.0 Hz, 2H), 1.37 (m 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 151.8, 151.3, 150.4, 147.5, 145.2, 132.2, 130.2, 125.2, 108.2, 84.2, 65.5, 64.8, 62.3, 60.6, 14.5; Anal. Calc. for $C_{16}H_{20}ClN_4O_4P$: C, 48.19; H, 5.06; N, 14.05; Found: C, 48.21; H, 5.09; N, 14.03; MS *m/z* 399 $(M+H)^+$.

(*rel*)-(1*R*,4*S*)-Diethyl [9-(4-hydroxy-5-methylene-cyclopent-2-en-1-yl)adenine] phosphonate (16). A solution of **15** (170 mg, 0.426 mmol) in saturated methanolic ammonia (10 mL) was stirred overnight at 65 ºC in a steel bomb, and the volatiles were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:8) to give **16** (93 mg, 58%) as a white solid: mp 148-150 ºC; UV (MeOH) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.27 (s, 1H), 8.12 (s, 1H), 7.81 (br s, NH_2, 2H, D_2O exchangeable), 5.75 (dd, *J* = 5.6, 2.8 Hz, 1H), 5.55 (dd, *J* = 5.7, 3.4 Hz, 1H), 5.16 (m, 2H), 5.04 (d, *J* = 3.3 Hz, 1H), 4.35-4.31 (m, 4H), 4.21 (d, *J* = 2.9 Hz, 1H), 3.98 (d, *J* = 8.1 Hz, 2H), 1.38-1.36 (m 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.7, 152.3, 150.3, 147.3, 145.3, 132.3, 130.6, 126.0, 107.6, 83.7, 64.2, 62.6, 61.2, 14.6; Anal. Calc. for $C_{16}H_{22}N_5O_4P$ (+1.0 MeOH): C, 49.63; H, 6.37; N, 17.02; Found: C, 49.60; H, 6.39; N, 17.05; MS *m/z* 380 $(M+H)^+$.

(*rel*)-(1'*R*,4'*S*)-[9-(4-hydroxy-5-methylene-cyclopent-2-en-1-yl) adenine]-4-phosphonic Acid (17). To a solution of the phosphonate **16** (159 mg, 0.42 mmol) in anhydrous CH_3CN (13 mL) and 2,6-lutidine (0.978 mL, 8.4 mmol) was added trimethylsilyl bromide (0.642 mg, 4.2 mmol). The mixture was heated overnight at 65 ºC under nitrogen gas and then concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 (110 mL) and distilled water (110 mL). The aqueous layer was washed with CH_2Cl_2 (2 × 60 mL) and then freeze-dried to give phosphonic acid **17** (108 mg, 80%) as a yellowish foam: UV (H_2O) λ_{max} 261.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.29 (s, 1H), 8.12 (s, 1H), 5.78 (dd, *J* = 5.7, 2.9 Hz, 1H), 5.58 (dd, *J* = 5.6, 3.0 Hz, 1H), 5.17 (m, 2H), 5.04 (d, *J* = 3.0 Hz, 1H), 4.22-4.17 (m, 3H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.4, 152.3, 150.5, 147.2, 141.5, 130.3, 124.3, 119.5, 108.1, 84.1, 67.3, 61.6; Anal. Calc. for $C_{12}H_{14}N_5O_4P$ (+3.0 H_2O): C, 38.20; H, 5.34; N, 18.56; Found: C, 38.19; H, 5.36; N, 18.52; MS *m/z* 324 $(M+H)^+$.

(*rel*)-(1'*S*,4'*S*)-[4-(4-Methoxybenzyloxy)-5-methylene-cyclopent-2-enyloxymethyl]phosphonic Acid Diethyl Ester (18). Diethylphosphonate analogue **18** was synthesized from **12b** by the similar procedure used for **15**: yield 57%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27-7.21 (m, 2H), 6.90-6.82 (m, 2H), 5.75 (dd, *J* = 5.6, 3.2 Hz, 1H), 5.58 (dd, *J* = 5.6, 3.0 Hz, 1H), 5.21 (m, 2H), 4.63 (s, 2H), 4.29-4.17 (m, 6H), 4.02 (d, *J* = 8.0 Hz, 2H), 3.74 (s, 3H), 1.37 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.2. 152.0, 130.6, 129.3, 128.7, 116.5, 107.2, 83.0, 81.2, 72.8, 66.2, 64.4, 56.3, 16.7; MS *m/z* 383 $(M+H)^+$.

(*rel*)-(1'*S*,4'*S*)-[4-(4-Hydroxy-5-methylene-cyclopent-2-enyloxymethyl)]phosphonic Acid Diethyl Ester (19). Deprotection of **18** was performed under the similar procedure as described for **14**: yield 67%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.78 (dd, *J* = 5.7, 3.4 Hz, 1H), 5.58 (dd, *J* = 5.6, 3.1 Hz, 1H), 5.22-5.20 (m, 2H), 4.60 (d, *J* = 3.0 Hz, 1H), 4.23-4.18 (m, 5H), 4.05 (d, *J* = 8.2 Hz, 2H), 1.38 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 151.7, 132.2, 129.3, 108.1, 83.2, 77.3, 65.2, 64.8, 17.1; MS *m/z* 263 $(M+H)^+$.

(*rel*)-(1'*S*,4'*R*)-Diethyl [9-(4-hydroxy-5-methylene-cyclopent-2-en-1-yl)-2-fluoro-6-chloropurine] phosphonate (20). Mitsunobu coupling of **19** with 2-fluoro-6-chloropurine under the similar reaction condition as described for **13**: yield 41%; UV (MeOH) λ_{max} 269.0 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.43 (s, 1H), 5.76 (dd, *J* = 5.5, 3.3 Hz, 1H), 5.56 (dd, *J* = 5.6, 3.0 Hz, 1H), 5.18 (dd, *J* = 4.4, 1.2 Hz, 2H), 5.02 (d, *J* = 3.1 Hz, 1H), 4.21-4.18 (m, 5H), 4.01 (d, *J* = 8.1 Hz, 2H), 1.38 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 154.3, 152.3, 151.8, 148.1, 146.3, 144.6, 130.6, 128.5, 125.1, 108.1, 83.9, 64.5, 62.1, 60.7, 17.4; Anal. Calc. for $C_{16}H_{19}ClFN_4O_4P$: C, 46.11; H, 4.60; N, 13.44; Found: C, 46.08; H, 4.63; N, 13.41; MS *m/z* 417 $(M+H)^+$.

(*rel*)-(1'*S*,4'*R*)-Diethyl [9-(4-hydroxy-5-methylene-cyclopent-2-en-1-yl)-2-fluoro-6-aminopurine]phosphonate (21) and (*rel*)-(1'*S*,4'*R*)-diethyl [9-(4-hydroxy-5-methylene-cyclopent-2-en-1-yl)-2-amino-6-chloropurine]phosphonate (22). Dry ammonia gas was bubbled into a stirred solution of **20** (850 mg, 2.40 mmol) in DME (45 mL) at room temperature overnight. The salts were removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($MeOH/CH_2Cl_2$, 1:10) to give **21** (104 mg, 11%) and **22** (566 mg, 57%), respectively: Data for **21**; UV (MeOH) λ_{max} 268.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.21 (s, 1H), 7.88 (br s, NH_2, 2H, D_2O exchangeable), 5.73 (dd, J = 5.6, 3.0 Hz, 1H), 5.58 (dd, J = 5.6, 3.2 Hz, 1H), 5.15-5.13 (m, 2H), 5.02 (d, J = 3.1 Hz, 1H), 4.21-4.17 (m, 5H), 4.01 (d, J = 8.0 Hz, 2H), 1.37 (m 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 155.0, 152.3, 151.8, 147.2, 146.6, 144.5, 130.2, 128.3, 125.2, 107.9, 84.4, 65.7, 64.8, 62.7, 17.8; Anal. Calc. for $C_{16}H_{21}FN_5O_4P$ (+1.0 MeOH): C, 47.55; H, 5.87; N, 16.31; Found: C, 47.59; H, 5.85; N, 16.28; MS *m/z* 398 $(M+H)^+$. Data for **22**; UV (MeOH) λ_{max} 309.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.11 (s, 1H), 7.97 (br s, NH_2, 2H, D_2O exchangeable), 5.72 (dd, J = 5.7, 3.2 Hz, 1H), 5.54 (dd, J = 5.6, 3.0 Hz, 1H), 5.16 (dd, J = 4.8, 2.0 Hz, 2H), 5.00 (d, J = 3.2 Hz, 1H), 4.26-4.20 (m, 5H), 4.06 (d, J = 8.0 Hz, 2H), 1.38-1.36 (m 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.7, 151.9, 151.2, 147.6, 145.8, 143.1, 129.7, 124.6, 108.4, 83.5, 64.5, 63.9, 62.2, 16.9; Anal. Calc. for $C_{16}H_{21}ClN_5O_4P$ (+1.0 MeOH): C, 45.79; H, 5.65; N, 15.70; Found: C, 45.84; H, 5.63; N, 15.69; MS *m/z* 414 $(M+H)^+$.

(*rel*)-(1'*S*,4'*R*)-9-(4-Hydroxy-5-methylene-cyclopent-2-en-1-yl)guanine]phosphonic Acid (23). To a solution of **22** (13.6 mg, 0.033 mmol) dry DMF (4 mL) was added trimethylsilyl bromide (75.9 mL, 0.575 mmol) at room temperature. After this mixture was stirred for 2 days, the solvent was removed, coevaporating three times with methanol. The residue was dissolved in MeOH (1.0 mL) and 2-mercaptoethanol (9 mL, 0.132 mmol) and NaOMe (7 mg, 0.132 mmol) was added to the mixture. The mixture was refluxed for 4 h under N_2, cooled, neutralized with glacial AcOH, and evaporated to dryness under vacuum. The residue was purified by chromatography on a column of reversed-phase C18 silica gel eluting water to give **23** (6.8 mg, 61%) as a white solid. UV (H_2O) λ_{max} 253.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.5 (br s, NH, H, D_2O exchangeable), 8.03 (s, 1H), 6.98 (br s, NH_2, 2H, D_2O exchangeable), 5.77 (dd, J = 5.6, 3.3 Hz, 1H), 5.58 (dd, J = 5.5, 3.2 Hz, 1H), 5.14 (dd, J = 4.8, 2.2 Hz, 2H), 5.01 (d, J = 3.3 Hz, 1H), 4.20 (d, J = 3.0 Hz, 1H), 4.02 (d, J = 8.1 Hz, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 157.5, 154.2, 152.7, 147.2, 136.3, 125.0, 117.6, 108.9, 84.3, 64.7, 64.1; Anal. Calc. for $C_{12}H_{14}N_5O_5P$ (+2.0 H_2O): C, 38.40; H, 4.83; N, 18.66; Found: C, 38.36; H, 4.80; N, 18.61; MS *m/z* 340 $(M+H)^+$.

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