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SYNTHESIS OF NOVEL 6′-SPIROCYCLOPROPYL-5′-NORCARBOCYCLIC ADENOSINE PHOSPHONIC ACID ANALOGUES AS POTENT ANTI-HIV AGENTS

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□ *Novel 5′-norcarbocyclic adenosine phosphonic acid analogues with 6′-electropositive moiety such as spirocyclopropane were designed and synthesized from the commercially available diethylmalonate* ***5****. Regioselective Mitsunobu reaction proceeded in the presence of an allylic functional group at a low reaction temperature in polar cosolvent [dimethylformamide (DMF)/1,4-dioxane] to give purine analogue* ***15****. To improve cellular permeability and enhance the anti-human immunodeficiency virus (HIV) activity of this phosphonic acid, a SATE phosphonodiester nucleoside prodrug* ***23*** *was prepared. The synthesized adenosine phosphonic acids analogues* ***18–21*** *and* ***23*** *were subjected to antiviral screening against HIV-1.*

Keywords Anti-HIV agents; 6′-spirocyclopropyl nucleosides; bis-SATE prodrug; mitsunobu reaction

INTRODUCTION

Much attention has been paid to unusual nucleosides since 6′-modified nucleosides were reported to be promising anti-human immunodeficiency virus (anti-HIV) and anti-hepatitis B virus (anti-HBV) agents. Among these compounds, entecavir[1] (**1**) is being clinically used in anti-HBV drugs (Figure 1). Carbovir analogue[2] **2** also exhibited potent anti-HIV activity, but its cytotoxicity hindered it from being further developed as an antiviral agent. Recently, an isonucleoside with an exomethylene[3] **3** or with a spirocyclopane[4] **4** moiety in place of a carbon atom of a furanose ring was reported to show antiviral activity, especially anti-HIV activity. Molecular modeling studies demonstrated the presence of an electropositive moiety at the 6′-position that could accommodate these substitutions and contribute to the observed enhancement in potency in anti-HIV activity.[4] The spatial location of the oxygen atom, namely the β-position from the phosphorus atom in the nucleoside analogue, plays a critical role in antiviral activity.

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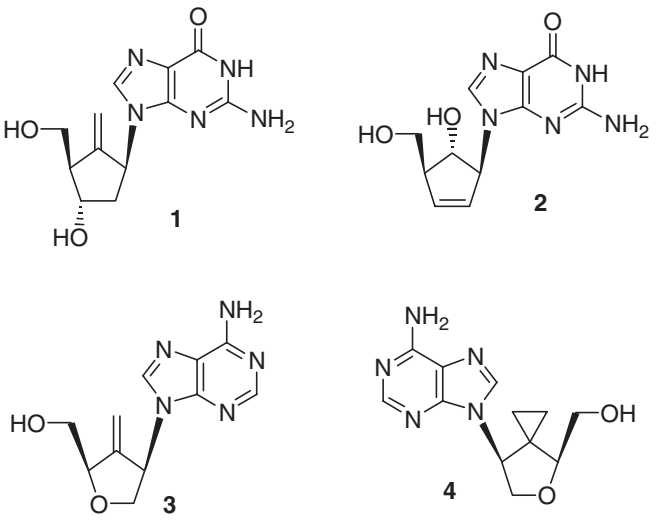


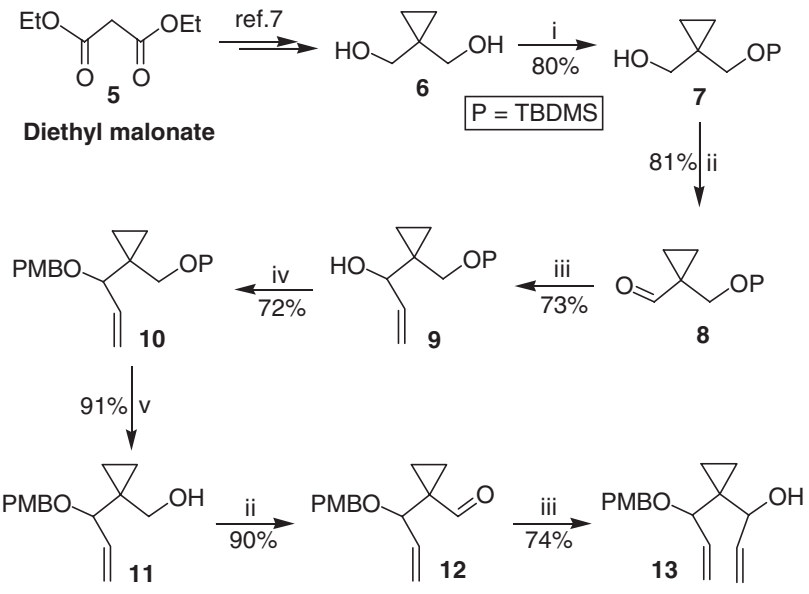
FIGURE 1 Structures of nucleoside analogues as potent antiviral agents.

This increased antiviral activity due to this oxygen atom may be attributed to the increased binding capacity of the phosphonate analogues to target enzymes.[5] Moreover, a phosphonate nucleoside analogue can skip the requisite first phosphorylation, which is a crucial step for the activation of nucleosides. This is frequently a limiting step in the phosphorylation sequence, which ultimately leads to triphosphates.[6] Though triphosphates of several of the nucleoside analogues exhibit excellent RT 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 these findings that 6′-electropositive nucleoside analogues and 5′-norcarbocyclic nucleoside phosphonate have excellent biological activities, we sought to synthesize a novel class of nucleosides comprising 6′-spirocyclopropyl-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 diethylmalonate **5**.[7] Monosilylation of diol **6** and subsequent oxidation of corresponding alcohol **7** gave aldehyde **8**.

The aldehyde functional group of **8** was subjected to a carbonyl addition reaction by vinylmagnesium bromide to furnish the secondary alcohol **9**, which was successfully protected using *p*-methoxybenzyl chloride (PMBCl)[8] to provide compound **10**. Removal of the silyl protecting group of **10** using *t*-butylammonium fluoride (TBAF) gave the primary alcohol **11**, which was oxidized to the aldehyde **12** using Swern oxidation conditions[9] [dimethyl



Reagents: i) TBDMSCl, imidazole, DMF; ii) $(COCl)_2$, DMSO, TEA; iii) vinylMgBr, THF; iv) PMBCl, NaH, DMF; v) TBAF, THF.

SCHEME 1 Synthesis of cyclopropyl divinyl intermediate **13**.

sulfoxide (DMSO), oxalyl chloride, TEA]. The aldehyde **12** was subjected to nucleophilic Grignard conditions[10] with vinylmagnesium bromide to give divinyl **13**, which was subjected to ring-closing metathesis (RCM) conditions using a 2nd generation Grubbs catalyst ($C_{46}H_{65}Cl_2N_2PRu$)[11] to provide spirocyclopropyl cyclopentenol **14a** (40%) and **14b** (39%), which were readily separated by silica gel column chromatography. The nuclear Overhauser enhancement (NOE) experiments with cyclopentenols **14a** and **14b** confirmed these assignments. As expected, NOEs were found between the cis-oriented hydrogens. Upon irradiation of C_4-*H*, weak NOE patterns were observed at the proximal hydrogens of compound **14b** [C_7-C*H*- (1.5%)] versus those of compound **14a** [C_7-C*H*- (2.1%)] (Figure 2).

To synthesize the desired 5′-norcarbocyclic adenosine nucleoside analogues, the protected cyclopentenol **14b** was treated with 6-chloropurine

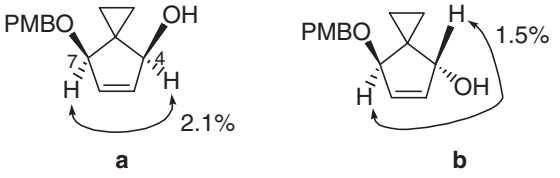


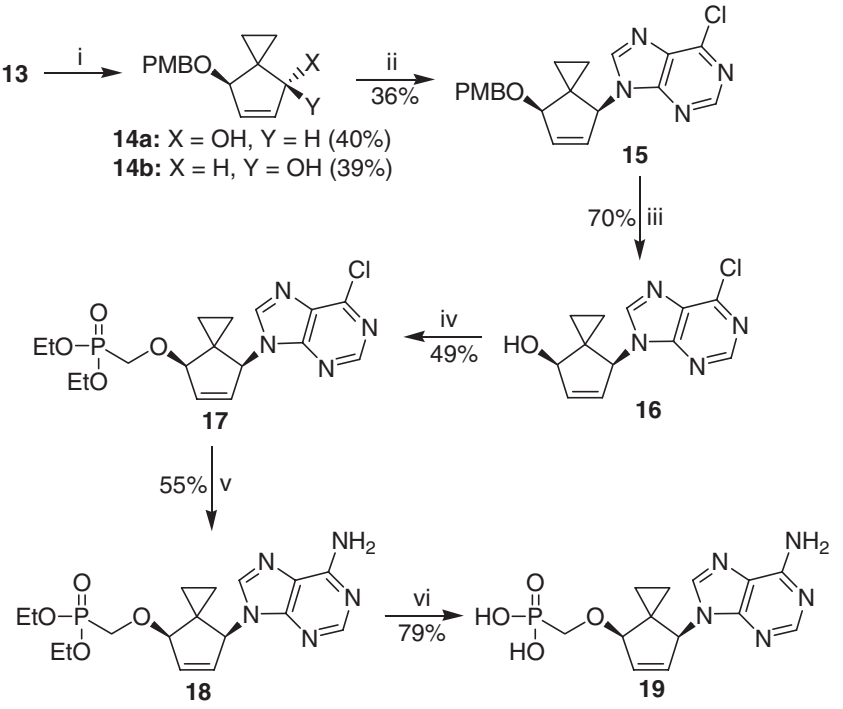
FIGURE 2 NOE differences between the proximal hydrogens.

under Mitsunobu conditions[12] [diethyl azodicarboxylate (DEAD) and PPh_3]. An appropriate choice of the solvent system, temperature, and procedure is essential for the regioselectivity as well as for the yield. In purine synthesis, a mixture of dioxane and DMF was used as the solvent for the coupling of the cyclopentenol **14b** with 6-chloropurine, instead of tetrahydrofuran (THF). The heterocyclic bases had better solubility in the dioxane-DMF mixture, resulting in better yields. The slow addition of diethyl azodicarboxylate (DEAD) to a mixture of cyclopentenol **14b**, triphenylphosphine, and the 6-chloropurine in anhydrous cosolvent (dioxane-DMF) gave a yellow solution, which was stirred for 1.5 hours at −40°C and further stirred overnight at room temperature (rt) to give the protected 6-chloropurine analogue **15** as N^9-regioisomer [UV (MeOH) λ_{max} 265.0 nm].[13] The PMB protection group was removed, along with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ),[14] to produce the 5′-nornucleoside analogue **16**, which was treated with diethylphosphonomethyl triflate[15] using lithium *t*-butoxide to yield the nucleoside phosphonate analogue **17** (Scheme 2). The chlorine group of **17** was then converted to amine with methanolic ammonia at 70°C to give the corresponding adenine phosphonate derivative **18**. Hydrolysis of **18** by treatment with bromotrimethylsilane in CH_3CN in the presence of 2,6-lutidine gave an adenine phosphonic acid derivative **19** (Scheme 2).[16]

In order to synthesize the 2′,3′-dihydroxy nucleoside analogues, the protected nucleoside **18** was subjected to vicinal hydroxylation conditions[17] using a catalytic amount of OsO_4 and NMO to give **20** (33%) and **20′** (31%), respectively.[18] As shown in Figure 3, the stereochemistry was readily determined by the NOE experiment. Upon irradiation of C_4-*H*, a relatively strong NOE was observed at C_5-*H* and C_6-*H* of **20′**, which showed 1′,2′,3′-*cis* relationships. But a relatively weak NOE was observed at C_5-*H* and C_6-*H* of **20**, which indicates 1′,2′- and 1′,3′-*trans* relationships. The adenosine phosphonic acid **21** was synthesized from **20** by similar procedures described for **19** (Scheme 3).

To synthesize the thioester prodrug analogue, compound **19** was reacted with thioester **22**[19] in the presence of 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT)[20] to provide the bis(SATE) derivative as a target compound **23** (Scheme 4).

The synthesized nucleoside phosphonate and phosphonic acid analogues **18–21** and **23** were then evaluated for antiviral activity against HIV. The procedures for measuring the antiviral activity toward wild-type HIV and cytotoxicity have been reported previously.[21] As shown in Table 1, nucleoside phosphonic acid **23** exhibited increased anti-HIV activity compared with its parent nucleoside phosphonic acid **19**. However, nucleoside analogues **18**, **20**, and **21** did not show anti-HIV activity or cytotoxicity at concentrations up to 100 μM.



Reagents: i) Grubbs (II), CH_2Cl_2; ii) 6-chloropurine, DEAD, PPh_3, 1,4-dioxane/DMF; iii) DDQ, CH_2Cl_2/H_2O (10:1); iv) $(EtO)_2POCH_2OTf$, LiO-*t*-Bu, THF; v) NH_3/MeOH, 70 °C; vi) TMSBr, 2,6-lutidine, CH_3CN;

SCHEME 2 Synthesis of 6′-spirocyclopropyl cyclopentenyl adenine phosphonic acid.

In summary, on the basis of the potent anti-HIV activity of 6′-electropositive nucleosides and 5′-norcarbocyclic nucleoside analogues, we have designed and successfully synthesized novel 6′-spirocyclopropyl-5′-norcarbocyclic nucleoside analogues starting from the commercially available diethylmalonate **5**.

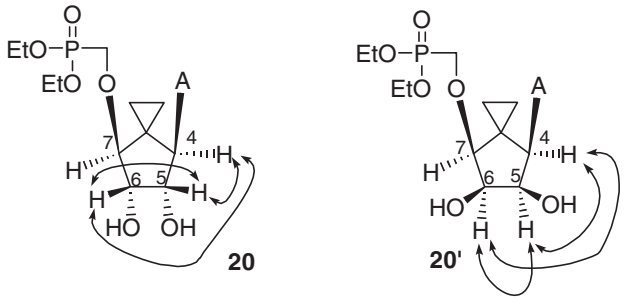
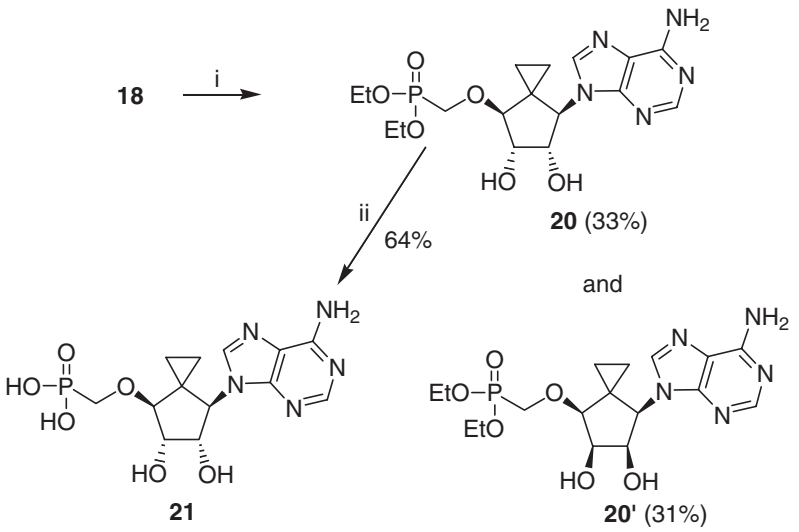


FIGURE 3 NOE relationships between the proximal hydrogens.



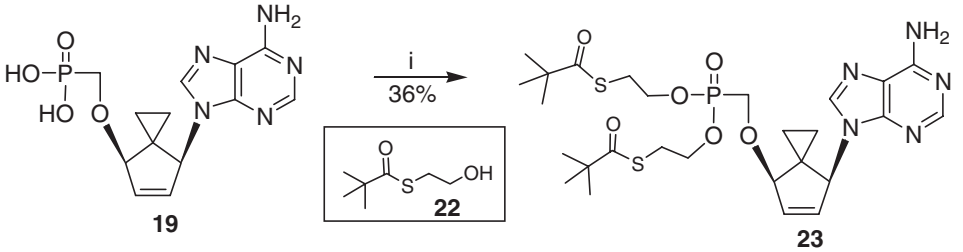
Reagents: i) OsO_4, NMO, acetone/*t*-BuOH/H_2O (6:1:1);
ii) TMSBr, 2,6-lutidine, CH_3CN;

SCHEME 3 Synthesis of 6′-cyclopropyl-2′,3′-dihydroxy-cyclopentanyl adenine phosphonic acid.

The synthesized nucleoside prodrug **23** exhibited slight improvement in cell-based activity compared with phosphonic acid **19**. Although the SATE protecting group as a prodrug scaffold was introduced, the antiviral activity was slightly increased.

EXPERIMENTAL

Melting points were determined on a Mel-temp II laboratory device and are uncorrected. NMR spectra were recorded on a JEOL 300 Fourier



Reagents: i) thioester, **22**, 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole, pyridine.

SCHEME 4 Synthesis of the target bis(SATE) prodrug of adenine analogue **23**.

TABLE 1 Anti-HIV activity of synthesized compounds

Compound no.	Anti-HIV EC_{50} (μM)[c]	Cytotoxicity CC_{50} (μM)[d]
18	80.0	98
19	19.1	80
20	88.9	100
21	90	100
23	**12.3**	50
AZT[a]	0.01	100
PMEA[b]	0.51	10

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

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. 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 in 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.

[1-(t-Butyldimethylsilyloxymethyl) cyclopropyl]methanol (7). *t*-Butyldimethylsilyl chloride (743 mg, 4.93 mmol) was added to a stirred solution of compound **6** (480 mg, 4.7 mmol) and imidazole (477 mg, 7.02 mmol) in CH_2Cl_2 (40 mL) at 0°C. The mixture was stirred at rt for 3 hours, and quenched by adding a $NaHCO_3$ solution (5 mL). The mixture was extracted using an EtOAc/water system, dried over $MgSO_4$, filtered, and then concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:5) to give compound **7** (813 mg, 80%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.54 (s, 2H), 3.49 (s, 2H), 0.84 (s, 9H), 0.44 (d, $J = 3.9$ Hz, 2H), 0.39 (d, $J = 3.9$ Hz, 2H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 76.6, 66.1, 25.8, 24.7, 18.2, 13.7, −5.5; MS m/z 217 $(M+H)^+$.

1-(t-Butyldimethylsilanyloxymethyl) cyclopropanecarbaldehyde (8). 4Å molecular sieves (1.9 g) and PCC (1.95 g, 9.06 mmol) were added slowly to a solution of compound **7** (779 mg, 3.6 mmol) in CH_2Cl_2 (40 mL) at 0°C, and the mixture was stirred at rt for 10 hours. An excess of diethyl ether (120 mL) was then added to the mixture. The mixture was stirred vigorously at rt for 2 hours, 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, 1:35) to give compound **8** (617 mg, 81%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.58 (s, 1H), 4.06 (s, 2H), 0.82 (s, 9H), 0.75 (d, $J = 3.8$ Hz, 2H), 0.58 (d, $J = 3.8$ Hz, 2H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.2, 71.5, 40.6, 25.3, 18.5, 13.5, −5.3; MS m/z 215 $(M+H)^+$.

(±)-1-[1-(t-Butyldimethylsilanyloxymethyl)cyclopropyl]prop-2-en-1-ol (9). To a solution of **8** (1.64 g, 7.66 mmol) in dry THF (40 mL), vinylmagnesium bromide (9.2 mL, 1.0 M solution in THF) was slowly added at −20°C and stirred at 0°C for 4 hours. A saturated NH_4Cl solution (10 mL) was added to the mixture, which was slowly warmed to rt. The mixture was diluted with water (80 mL) and extracted with EtOAc (2 × 80 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, 1:12) to give **9** (1.35 g, 73%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.90 (m, 1H), 5.27–5.18 (m, 2H), 3.89 (d, $J = 4.2$ Hz, 1H), 3.73 (m, 2H), 0.82 (s, 9H), 0.72–0.67 (m, 2H), 0.44–0.36 (m, 2H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 141.8, 114.5, 82.7, 73.4, 30.3, 25.4, 9.2, –5.5; MS m/z 243 $(M+H)^+$.

(±)-t-Butyl-{1-[1-(4-methoxybenzyloxy)allyl]cyclopropylmethoxy} dimethylsilane (10). NaH (60% in mineral oil, 264 mg, 6.64 mmol) was added portion-wise to a cooled (0°C) solution of secondary alcohol **9** (1.34 g, 5.54 mmol) and *p*-methoxybenzyl chloride (0.82 mL, 6.09 mmol) in anhydrous DMF (20 mL). The reaction mixture was stirred overnight at rt. The solvent was removed in vacuo and the residue was diluted with H_2O (100 mL), followed by extraction with diethyl ether (2 × 80 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 **10** (1.47 g, 72%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29–7.23 (m, 2H), 6.92–6.86 (m, 2H), 5.88 (m, 1H), 5.29–5.23 (m, 2H), 4.50 (s, 2H), 3.79 (s, 3H), 3.64–3.52 (m, 3H), 0.83 (s, 9H), 0.74-0.69 (m, 2H), 0.48–0.40 (m, 2H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.6, 143.4, 131.7, 128.7, 117.5, 114.5, 86.7, 77.4, 75.2, 55.9, 25.6, 24.2, 18.5, 10.5, 9.9, –5.3; MS m/z 363 $(M+H)^+$.

(±)—1-[1-(4-Methoxybenzyloxy)allyl]cyclopropyl˝methanol (11). To a solution of **10** (1.2 g, 3.31 mmol) in THF (15 mL), TBAF (4.96 mL, 1.0 M solution in THF) was added at 0°C. The mixture was stirred overnight at rt and concentrated in vacuo. The residue was purified by silica gel column chromatography (Hexane/EtOAc, 7:1) to give **11** (747.9 mg, 91%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33–7.28 (m, 2H), 6.96–6.87 (m, 2H), 5.91 (m, 1H), 5.28–5.21 (m, 2H), 4.61 (s, 2H), 3.76 (s, 3H), 3.55–3.47 (m, 3H), 0.70-0.64 (m, 2H), 0.49–0.41 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.5, 143.0, 131.5, 129.4, 118.6, 114.3, 87.1, 74.8, 70.8, 57.1, 25.5, 10.0, 9.4; MS m/z 249 $(M+H)^+$.

(±)-1-[1-(4-Methoxybenzyloxy)allyl]cyclopropanecarbaldehyde (12). To a stirred solution of oxalyl chloride (258 mg, 2.04 mmol) in CH_2Cl_2 (17 mL) was added a solution of DMSO (318 mg, 4.08 mmol) in CH_2Cl_2 (6.0 mL) dropwise at –78°C. The resulting solution was stirred at –78°C for 30 minutes, and a solution of alcohol **11** (253 mg, 1.02 mmol) in CH_2Cl_2 (12 mL) was added dropwise. The mixture was stirred at –78°C for 30 min and TEA (1.14 mL, 8.16 mmol) was added. The resulting mixture was warmed to 0°C and stirred for 30 minutes. H_2O (15 mL) was added, and the solution was stirred for 30 min at rt. The mixture was diluted with water (150 mL) and then extracted with EtOAc (2 × 150 mL). The combined organic layer was washed with brine, 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, 1:20) to give aldehyde compound **12** (226 mg, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.68 (s, 1H), 7.29–7.22 (m, 2H), 6.91–6.86 (m, 2H), 5.89 (m, 1H), 5.26-5.19 (m, 2H), 4.69 (s, 2H), 3.75 (s, 3H), 3.71 (d, J = 4.0 Hz, 1H), 0.73–0.67 (m, 2H), 0.49-0.41 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.1, 159.6, 143.5, 132.0, 128.2, 117.8, 114.5, 83.2, 74.7, 56.9, 39.3, 10.7, 10.2; MS m/z 247 $(M+H)^+$.

(*rel*)-(3*R* and *3S*,5*S*)- 5-(4-Methoxybenzyloxy)-4-cyclopropyl-hepta-1,6-dien-3-ol (13). Divinyl analogue **13** was synthesized as a diastereomeric mixture from aldehyde **12** by a procedure similar to that described for **8** as a diastereomeric mixture: yield 74%; ^{1}H NMR ($CDCl_3$, 300MHz) δ 7.32–7.24 (m, 2H), 6.92–6.83 (m, 2H), 5.93–5.85 (m, 2H), 5.23–5.15 (m, 4H), 4.65 (s, 2H), 3.89 (d, J = 4.1 Hz, 1H), 3.76–3.68 (m, 4H), 0.75–0.65 (m, 2H), 0.48–0.37 (m, 2H).

(*rel*)-(4*S*,7*S*)-7-(4-Methoxybenzyloxy)-spiro[2.4]hept-5-en-4-ol (14a) and (*rel*)-(4*R*,7*S*)-7-(4-methoxybenzyloxy)-spiro[2.4]hept-5-en-4-ol (14b). To a solution of **13** (296 mg, 1.08 mmol) in dry methylene chloride (7 mL) was added a 2nd generation Grubbs catalyst (40.0 mg, 0.0471 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 **14a** (106 mg, 40%) and **14b** (103 mg, 39%). Data for **14a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31–7.26 (m, 2H), 6.89–6.83 (m, 2H), 5.66 (dd, J = 2.8, 5.3 Hz, 1H), 5.38 (m, 1H), 4.65 (s, 2H), 4.02 (d, J = 2.9 Hz, 1H), 3.77 (s, 3H), 3.62 (d, J = 3.1 Hz, 1H), 2.20 (dd, J = 13.6. 8.2 Hz, 1H), 0.77–0.71 (m, 2H), 0.51–0.47 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.6, 138.1, 137.0, 132.2, 127.6, 118.4, 87.4, 82.6, 74.5, 57.7, 24.7, 11.5, 9.9; MS m/z 247 $(M+H)^+$.

Data for **14b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29–7.21 (m, 2H), 6.88–6.83 (m, 2H), 5.66 (dd, J = 2.8, 5.4 Hz, 1H), 5.34 (m, 1H), 4.68 (s, 2H), 4.00 (dd, J = 4.2, 2.6 Hz, 1H), 3.73-3.65 (m, 4H), 0.78–0.73 (m, 2H), 0.49–0.44 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.8, 138.7, 136.1, 134.5, 129.8, 118.4, 88.2, 73.8, 27.5, 8.8, 7.7; MS m/z 247 $(M+H)^+$.

(*rel*)-(4′*R*,7′*S*)-9-[7-(4-Methoxybenzyloxy)-spiro[2.4]hept-5-en-4-yl] 6-chloropurine (15). To a solution containing compound **14b** (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), DEAD (0.271 mL, 1.488 mmol) was added dropwise at –40°C for 10 minutes under nitrogen. The reaction mixture was stirred at the same temperature for 1.5 hours 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 **15** (102 mg, 36%): mp 162–164°C; UV (MeOH) λ_{max} 265.0 nm: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (s, 1H), 8.36 (s, 1H), 7.20–7.24 (m, 2H), 6.93–6.86 (m, 2H), 5.67 (d, J = 5.4 Hz, 1H), 5.34 (dd, J = 3.2, 5.5 Hz, 1H), 4.66 (s, 2H), 4.45 (d, J = 3.3 Hz, 1H), 3.74 (s, 3H), 0.78–0.72 (m, 2H), 0.48–0.43 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.7, 151.4, 149.9, 143.8, 137.5, 135.7, 132.3, 131.5, 128.5, 118.4, 89.5, 75.1, 67.7, 56.3, 21.5, 8.6, 8.0; MS m/z 383 $(M+H)^+$.

(*rel*)-(4′*R*,7′*S*)-[9-(7-Hydroxy)-spiro[2.4]hept-5-en-4-yl] 6-chloropurine (16). To a solution of compound **15** (324 mg, 0.846 mmol) in CH_2Cl_2/H_2O (10 mL, 10:1 v/v) was added DDQ (286 mg, 1.264 mmol), and the mixture was stirred overnight at room temperature. Saturated $NaHCO_3$ (1.7 mL) was added to quench the reaction, which was then stirred at rt for 2 hours. The mixture was diluted with water (150 mL) and extracted with CH_2Cl_2 (3 × 150 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, 3:1:0.05) to give compound **16** (155 mg, 70%): mp 179–181°C; UV (MeOH) λ_{max} 264.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.67 (s, 1H), 8.29 (s, 1H), 5.62 (d, J = 5.5 Hz, 1H), 5.34 (dd, J = 5.6, 3.9 Hz, 1H), 4.50 (d, J = 5.4 Hz, 1H), 3.98 (d, J = 5.2 Hz, 1H), 0.39–0.29 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.0, 151.5, 149.9, 145.4, 137.5, 135.2, 132.4, 83.8, 67.4, 21.9, 8.5, 7.8; MS m/z 263 $(M+H)^+$.

(*rel*)-(4′*R*,7′*S*)-Diethyl [9-(7-hydroxymethyl)-spiro[2.4]hept-5-en-4-yl) 6-chloropurine] phosphonate (17). Both LiO*t*-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 6-chloropurine analogue **16** (183 mg, 0.696 mmol) in 9.0 mL of THF at –25°C and stirred overnight at rt under nitrogen. The mixture was quenched by adding a 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, 0.04:3:1) to give **17** (140 mg, 49%) as a foam: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.78 (s, 1H), 8.49 (s, 1H), 5.63 (d, J = 5.4 Hz, 1H), 5.38 (dd, J = 5.3, 4.2 Hz, 1H), 4.48 (d, J = 4.2 Hz, 1H),

4.32 (m, 4H), 3.94 (d, J = 8.1 Hz, 2H), 3.67 (d, J = 5.1 Hz, 1H), 1.38 (m 6H), 0.41–0.32 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 151.7, 151.0, 150.2, 146.5, 137.8, 134.2, 132.2, 90.4, 69.1, 65.4, 64.6, 59.2, 22.1, 14.7, 9.1, 8.3; MS m/z 413 $(M+H)^+$.

(*rel*)-(4′*R*,7′*S*)-Diethyl [9-(7-hydroxymethyl)spiro[2.4]hept-5-en-4-yl)-adenine]-phosphonate (18). A solution of **17** (180 mg, 0.436 mmol) in saturated methanolic ammonia (10 mL) was stirred overnight at 65°C in a steel bomb, and the volatiles were evaporated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:7) to give **18** (94 mg, 55%) as a white solid: mp 144–146°C; UV (MeOH) λ_{max} 261.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.28 (s, 1H), 8.13 (s, 1H), 5.66 (d, J = 5.4 Hz, 1H), 5.37 (m, 1H), 4.43 (d, J = 5.2 Hz, 1H), 4.35–4.30 (m, 4H), 4.00 (d, J = 8.2 Hz, 2H), 3.59 (d, J = 5.0 Hz, 1H), 1.41–1.37 (m 6H), 0.41–0.36 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.6, 152.7, 150.1, 142.2, 137.3, 132.9, 119.4, 90.5, 67.7, 64.6, 63.7, 57.7, 21.5, 15.2, 8.0, 7.2; Anal. Calc. for $C_{17}H_{24}N_5O_4P$ (+0.5 MeOH): C, 51.39; H, 6.40; N, 17.11; Found: C, 51.44; H, 6.42; N, 17.09; MS m/z 394 $(M+H)^+$.

(*rel*)-(4′*R*,7′*S*)-[9-(7-Hydroxymethyl)-spiro[2.4]hept-5-en-4-yl)-adenine]-7-phosphonic acid (19). To a solution of the phosphonate **18** (82.5 mg, 0.21 mmol) in anhydrous CH_3CN (7 mL) and 2,6-lutidine (0.489 mL, 4.2 mmol) was added trimethylsilyl bromide (0.321 mg, 2.1 mmol). The mixture was heated overnight at 60°C under nitrogen gas and then concentrated in vacuo. The residue was partitioned between CH_2Cl_2 (50 mL) and distilled purified water (50 mL). The aqueous layer was washed with CH_2Cl_2 (2 × 30 mL) and then freeze-dried to give phosphonic acid **19** (56 mg, 79%) as a yellowish foam: UV (H_2O) λ_{max} 260.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.29 (s, 1H), 8.10 (s, 1H), 5.67 (d, J = 5.3 Hz, 1H), 5.35 (dd, J = 5.4, 4.3 Hz, 1H), 4.49 (d, J = 4.5 Hz, 1H), 4.15 (d, J = 8.2 Hz, 2H), 0.39–0.32 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.7, 152.1, 150.2, 141.7, 136.9, 133.6, 120.0, 90.2, 67.3, 57.8, 21.4, 8.7, 7.6; Anal. Calc. for $C_{13}H_{16}N_5O_4P$ (+3.0 H_2O): C, 39.92; H, 5.67; N, 18.76; Found: C, 39.89; H, 5.66; N, 18.78; MS m/z 338 $(M+H)^+$.

(*rel*)-(4′*R*,5′*R*,6′*S*,7′*S*)-Diethyl [9-(7-hydroxymethyl)-5,6-dihydroxy-spiro [2.4]hept-5-en-4-yl)-adenine] phosphonate (20) and (*rel*)-(4′*R*,5′*S*,6′*R*,7′*S*)-diethyl [9-(7-hydroxymethyl)-5,6-dihydroxy-spiro[2.4]hept-5-en-4-yl)-adenine] phosphonate (20′). Compound **18** (205 mg, 0.522 mmol) was dissolved in a cosolvent system (10 mL) (acetone: *t*-BuOH:H_2O = 6:1:1) along with 4-methylmorpholine *N*-oxide (121 mg, 1.044 mmol). Subsequently, OsO_4 (0.261 mL, 4% wt. % in H_2O) was added. The mixture was stirred overnight at rt and quenched with a saturated Na_2SO_3 solution (5 mL). The resulting solid was removed by filtration through a pad of Celite, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:4) to give **20** (73 mg, 33%) and **20'** (66 mg, 31%) as a solid: compound **20:** mp 147–149°C;

UV (H_2O) λ_{max} 260.0 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.30 (s, 1H), 8.19 (s, 1H), 4.24–4.17 (m, 4H), 4.09 (d, $J = 8.1$ Hz, 2H), 3.79 (d, $J = 5.0$ Hz, 1H), 3.67 (m, 1H), 3.30 (dd, $J = 6.8$, 4.6 Hz, 1H), 2.96 (d, $J = 6.1$ Hz, 1H), 1.36–1.30 (m 6H), 0.38-0.31 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.8, 152.2, 149.5, 142.7, 119.8, 84.6, 67.6, 66.2, 64.2, 63.7, 59.3, 20.4, 9.2, 8.5; Anal. Calc. for $C_{17}H_{26}N_5O_6P$ (+0.5 MeOH): C, 47.42; H, 6.37; N, 15.80; Found: C, 47.39; H, 6.39; N, 15.78; MS m/z 428 $(M+H)^+$.

Compound **20′**: mp 153–154°C; UV (H_2O) λ_{max} 260.5 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.29 (s, 1H), 8.15 (s, 1H), 4.35-4.28 (m, 4H), 4.15 (d, $J = 8.0$ Hz, 2H), 3.76 (br s, 1H), 3.59–3.46 (m, 2H), 1.38–1.34 (m 6H), 0.41-0.37 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.6, 152.5, 150.7, 143.2, 120.2, 85.2, 68.1, 65.3, 64.7, 60.8, 19.9, 8.0, 7.3; Anal. Calc. for $C_{17}H_{26}N_5O_6P$ (+1.0 MeOH): C, 47.08; H, 6.58; N, 15.25; Found: C, 47.11; H, 6.60; N, 15.27; MS m/z 428 $(M+H)^+$.

(*rel*)-(4′*R*,5′*R*,6′*S*,7′*S*)-[9-(7-Hydroxymethyl)-5,6-dihydroxy-spiro[2.4] hept-5-en-4-yl)-adenine] phosphonic acid (21). The final adenosine phosphonic acid **21** was synthesized from **20** using a similar procedure described for **19** as a light yellow foamy solid: yield 64%; UV (H_2O) λ_{max} 261.5 nm; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.29 (s, 1H), 8.17 (s, 1H), 4.14 (d, $J = 8.2$ Hz, 2H), 3.80 (d, $J = 5.5$ Hz, 1H), 3.66 (dd, $J = 5.8$, 4.8 Hz, 1H), 3.39 (dd, $J = 5.4$, 4.7 Hz, 1H), 0.41–0.34 (m, 4H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 154.5, 152.6, 151.7, 142.5, 120.0, 84.2, 67.4, 64.4, 63.7, 59.8, 19.6, 8.4, 7.3; Anal. Calc. for $C_{13}H_{18}N_5O_6P$ (+2.0 H_2O): C, 38.35; H, 5.44; N, 17.20; Found: C, 38.32; H, 5.46; N, 17.17; MS m/z 372 $(M+H)^+$.

(*rel*)-(4′*R*,7′*S*)-Bis(SATE) phosphoester of [9-(7-methyloxyphosphonate-spiro[2.4]hept-5-en-4-yl)]-adenine (23). A solution of adenine phosphonic acid derivative **19** (57 mg, 0.169 mmol) and tri-*n*-butylamine (94 mg, 0.510 mmol) in methanol (3.9 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 **22** (519 mg, 3.2 mmol) and 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole (201 mg, 0.678 mmol) were added. The mixture was stirred overnight at rt and quenched with tetrabutylammonium bicarbonate buffer (10.0 mL, 1 M solution, pH 8.0). The mixture was concentrated under reduced pressure and the residue was diluted with water (60 mL) and extracted with $CHCl_3$ (70 mL) two times. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.05:4:1) to give **23** (38 mg, 36%) as a white solid: mp 130–133°C; UV (MeOH) λ_{max} 260.5 nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.30 (s, 1H), 8.12 (s, 1H), 5.54 (d, $J = 5.7$ Hz, 1H), 5.31 (dd, $J = 5.6$, 4.4 Hz, 1H), 4.49 (m, 1H), 4.22 (d, $J = 8.0$ Hz, 2H), 3.93-3.90 (m, 4H), 3.17–3.14 (m, 4H), 1.22–1.18 (m, 18), 0.88 (m, 1H), 0.39-0.34 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 203.8, 154.7, 152.3, 148.5, 143.8, 127.6, 123.7,

119.8, 84.7, 66.8, 64.5, 62.8, 58.5, 48.3, 35.8, 26.2, 19.7, 8.8, 7.5; Anal. Calc. for $C_{27}H_{40}N_5O_6PS_2$ (+0.5 MeOH): C, 51.46; H, 6.59; N, 10.91; Found: C, 51.49; H, 6.61; N, 10.89; MS m/z 626 $(M+H)^+$.

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