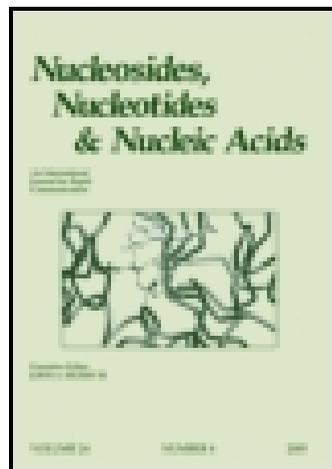


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### Synthesis and Anti-HIV Evaluation of New Acyclic Phosphonate Nucleotide Analogues and Their Bis(SATE) Derivatives

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## SYNTHESIS AND ANTI-HIV EVALUATION OF NEW ACYCLIC PHOSPHONATE NUCLEOTIDE ANALOGUES AND THEIR BIS(SATE) DERIVATIVES

Hua Li and Joon Hee Hong

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□ This article describes a very simple route for synthesizing novel lipophilic phosphonate bis(*t*-bu-SATE) prodrugs of acyclic cyclopentenylated nucleosides such as adenine 17 and cytosine 18. The key intermediate 6 was constructed via a ring-closing metathesis of compound 5, which could be readily prepared from diethylmalonate 4. The chemical stability of the bis(SATE) derivatives was tested at neutral ( $pH = 7.2$ ) and slightly acid (milli-Q water,  $pH = 5.5$ )  $pH$ . The synthesized compounds were evaluated as potential antiviral agents against HIV-1 virus.

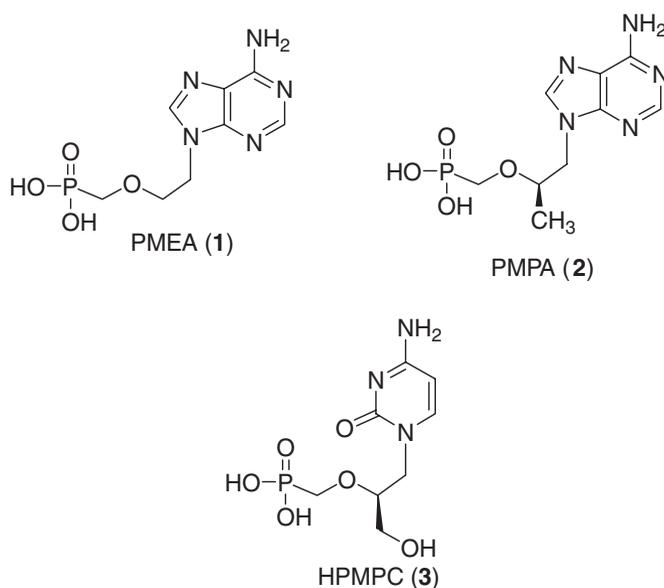
**Keywords** Antiviral agent; SATE prodrug; phosphonate nucleoside

### INTRODUCTION

Emerging drug-resistant virus strains and toxicity are major problems with antiviral chemotherapy. A number of structurally modified nucleosides have been synthesized in an attempt to overcome these drawbacks. Fundamental modifications of the classical pentofuranose moiety have been designed, and many sugar modified nucleoside analogues have shown excellent antiviral or antitumor activity. Although structure-activity relationship studies have not led to a pharmacophore model for the antiviral activities of nucleosides, some structural features have been particularly successful. Recently, a number of acyclic nucleoside,<sup>[1]</sup> analogues with phosphonic acid groups have been synthesized and evaluated for antiviral activity. Among them, PMEA 1,<sup>[2]</sup> PMPA 2,<sup>[3]</sup> and HPMPC 3<sup>[4]</sup> exhibit potent antiviral activity against HIV, HBV, and HSV (Figure 1). Unlike nucleoside agents, a phosphonate nucleoside has the advantage of skipping the requisite initial phosphorylation, which is a crucial step for the activation of nucleosides.<sup>[5]</sup> However, the poor oral bioavailability of these nucleoside analogues are

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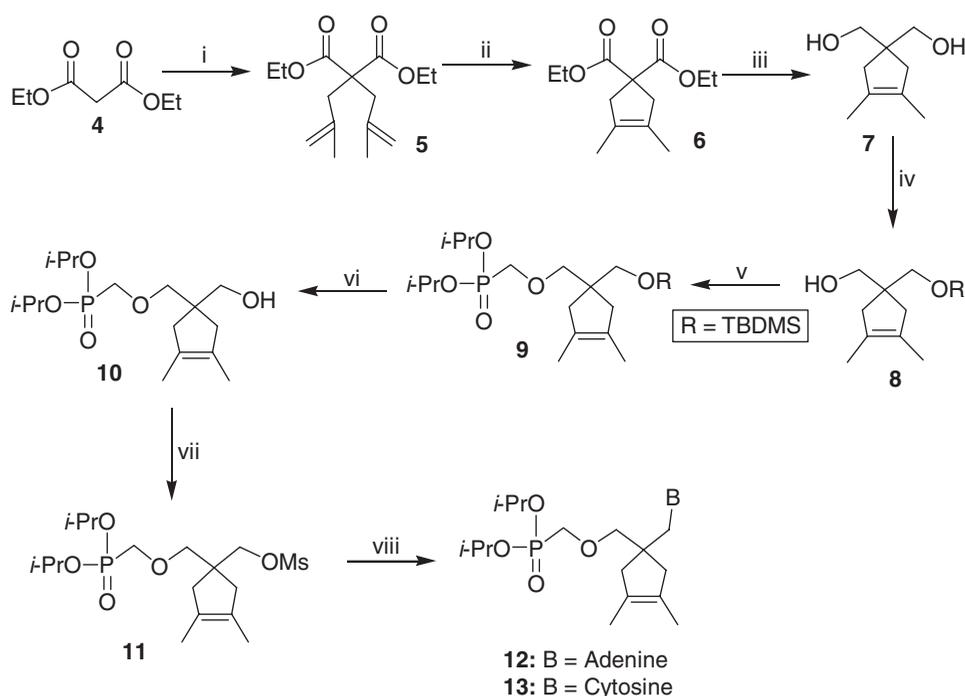


**FIGURE 1** Rationale to the design of the target nucleotide analogues.

due to the phosphonate negative charges present in nucleoside phosphonic acid at physiological pH. Therefore, the concept of temporarily masking these charges with neutral groups to form more lipophilic derivatives capable of crossing the gastrointestinal wall and reverting back to the parent nucleoside phosphonic acid was attempted.<sup>[6]</sup> Since the ionic character of a phosphonic acid would present an obstacle for cellular permeability, an S-acyl-2-thioethyl (SATE) prodrug was prepared. Esterification of a phosphonic acid with two SATE groups is a feasible strategy to deliver a phosphate or phosphonate drug into cells.<sup>[7]</sup> Usually, phosphonic acid nucleosides require an endocytosis-like process<sup>[8]</sup> or the ATP membrane receptor<sup>[9]</sup> to cross the cellular membrane. We therefore applied the bis(SATE) approach to novel acyclic nucleoside phosphonic acids. Here, we report on the synthesis, antiviral activity, and stability of the t-bu-SATE prodrugs of novel acyclic nucleoside analogues.

## RESULTS AND DISCUSSION

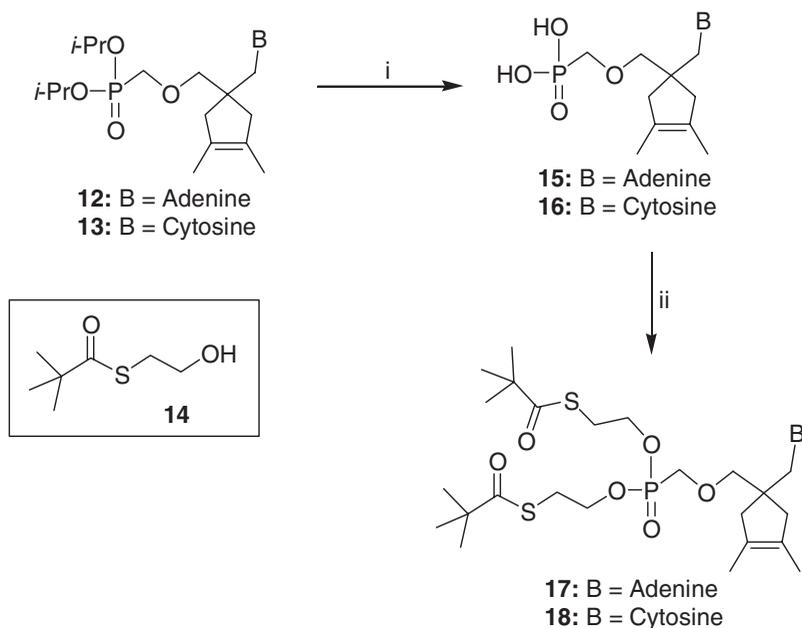
For the synthesis of carboacyclic nucleosides, the commercially available diethyl malonate **4** was selected as a starting material. As shown in Scheme 1, the synthetic route is very simple and straightforward. Double alkylation of the active methylene group of **4** and ring-closing metathesis (RCM) of corresponding methyl-allyl **5** provided dimethyl-cyclopentene derivative **6** in



**SCHEME 1** Synthesis of phosphonate nucleosides. Reagents: i) NaH, 3-chloro-2-methyl-propene, THF; ii) Grubb's catalyst (II), benzene; iii) LiAlH<sub>4</sub>, THF; iv) TBDMSCl, imidazole, CH<sub>2</sub>Cl<sub>2</sub>; v) Diisopropyl bromomethylphosphonate, LiO*t*-Bu, Lil, DMF; vi) TBAF, THF; vii) MsCl, TEA, CH<sub>2</sub>Cl<sub>2</sub>; viii) adenine, cytosine K<sub>2</sub>CO<sub>3</sub>, 18-Crown-6, DMF.

a high yield.<sup>[10]</sup> Reduction of the ester functional group of **6**, followed by a monosilylation of diol **7** provided the alcohol derivative **8**.

For the synthesis of phosphonate nucleosides, the hydroxyl group of **8** was phosphonated by treating with diisopropyl bromomethylphosphonate<sup>[11]</sup> in anhydrous DMF to give the phosphonate intermediate **9** (Scheme 1), which was readily desilylated by TBAF to provide **10**. Compound **10** was activated by methanesulfonylation with MsCl and triethylamine in anhydrous CH<sub>2</sub>Cl<sub>2</sub> to give **11**, which was coupled with natural base (adenine, cytosine) under nucleophilic S<sub>N</sub>2 substitution conditions (K<sub>2</sub>CO<sub>3</sub>, 18-Crown-6, DMF)<sup>[12]</sup> to give the dimethylcyclopentene nucleoside phosphonate **12** and **13**, respectively. The diisopropyl protecting groups of the phosphonates were readily removed using trimethylsilylbromide<sup>[13]</sup> to give nucleoside phosphonic acids **15** and **16**. To synthesize the thioester-protected analogues, phosphonic acid nucleosides were reacted with thioester **14** in the presence of 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole (MSNT)<sup>[14]</sup> to provide the final *t*-bu-SATE prodrugs **17** and **18** (Scheme 2). The newly synthesized phosphonic nucleoside analogues **15**, **16**, **17**, and **18** were assayed in the MT-4 cell line for anti-HIV activity using the previously reported assay system.<sup>[15]</sup> The



**SCHEME 2** Synthesis of SATE prodrug of phosphonic acid nucleoside. Reagents: i) TMSBr, CH<sub>3</sub>CN; ii) **14**, 1-(2-mesitylenesulfonyl)-3-nitro-1H-1,2,4-triazole, pyridine.

tested prodrugs enhanced the *in vitro* anti-HIV activity of parent phosphonic acid as well as the cytotoxicity (Table 1) via an increase in cellular uptake followed by intracellular release of parent phosphonic acids.

To measure the relative chemical stabilities of the SATE prodrugs, the percent of decomposition was briefly measured for SATE prodrugs **17**, **18** (i) at 37°C in milli-Q water (pH = 5.5) and (ii) in pH = 7.2 buffer using the Gosselin's method (Table 2).<sup>[7]</sup>

**TABLE 1** Antiviral activity of the synthesized compounds

Compd	HIV-1 EC <sub>50</sub> (μM)	Cytotoxicity CC <sub>50</sub> (μM)
<b>15</b>	97	>100
<b>16</b>	99	>100
<b>17</b>	41	47
<b>18</b>	39	44
bis(POM)PMEA	0.09	0.78

Bis(POM)PMEA: bis(pivaloyloxymethyl)9-[2-(phosphonomethoxy)ethyl]adenine.

EC<sub>50</sub>: Concentration (μM) required to inhibit the replication of HIV-1 by 50%.

CC<sub>50</sub>: Concentration (μM) required to reduce the viability of unaffected cells by 50%.

**TABLE 2** Percentage of decomposition after 24 hours of incubation at two different media

Compd	Water	pH = 7.2
<b>17</b>	1.6%	3.5%
<b>18</b>	1.5%	3.2%

Water condition: Milli-Q, pH = 5.5.

pH = 7.2 condition: Ammonium acetate buffer, 0.02 M.

## CONCLUSION

We have synthesized and tested the anti-HIV activity and chemical stability of novel SATE prodrugs of cyclopentene phosphonate nucleosides. The tested prodrugs showed increased anti-HIV activity over parent phosphonic acids. Also, the synthesized prodrugs were relatively stable in neutral and weakly acidic media.

## EXPERIMENTAL

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 ( $\delta$ ) and signals are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets). Ultra-violet (UV) spectra were obtained on a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). The elemental analyses were performed using a Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT, USA). Thin layer chromatography (TLC) was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were performed under an atmosphere of nitrogen unless specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from  $\text{CaH}_2$ . Dry THF was obtained by distillation from Na and benzophenone immediately prior to use.

**2,2-Bis-(2-methylallyl) malonic acid diethyl ester (5):** To a stirred suspension of sodium hydride (1.87 g, 0.078 mol) in tetrahydrofuran (150 mL) was slowly added diethyl malonate **4** (5 g, 0.0312 mol) at  $0^\circ\text{C}$  and stirred for 2.5 hours at room temperature. To this mixture was slowly added 3-chloro-2-methyl-propene (5.9 g, 65.5 mmol) at  $0^\circ\text{C}$  and stirred overnight at room temperature. The mixture was quenched with saturated  $\text{NaHCO}_3$  solution (10 mL) and further diluted with water (200 mL). The mixture was extracted using EtOAc (300 mL), and the organic layer was washed with brine, dried over  $\text{MgSO}_4$ , filtered, and then concentrated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **5** (7.1 g, 85%)

as a colorless oil:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  4.84 (s, 2H), 4.73 (s, 2H), 4.17 (q,  $J = 7.0$  Hz, 4H), 2.78 (s, 4H), 1.68 (s, 6H), 1.25 (t,  $J = 7.0$  Hz, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  171.5, 141.0, 115.1, 61.2, 56.7, 40.5, 23.6, 13.9; Anal. Calc. for  $\text{C}_{15}\text{H}_{24}\text{O}_4$ : C, 67.14; H, 9.01. Found: C, 67.27; H, 8.94.

**3,4-Dimethylcyclopent-3-ene-1,1-dicarboxylic acid diethyl ester (6):** To a stirred solution of **5** (2.79 g, 10.4 mmol) in anhydrous benzene (10 mL) was added Grubbs catalyst, 2nd generation (50 mg, 58.89  $\mu\text{mol}$ ) at  $0^\circ\text{C}$  and refluxed for 12 hours. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:12) to give **6** (2.25 g, 90%) as a colorless oil:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  4.19 (q,  $J = 6.9$  Hz, 4H), 2.92 (s, 4H), 1.57 (s, 6H), 1.22 (t,  $J = 6.9$  Hz, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  172.6, 128.0, 61.3, 45.8, 14.0, 13.3; Anal. Calc. for  $\text{C}_{13}\text{H}_{20}\text{O}_4$ : C, 64.98; H, 8.39. Found: C, 65.15; H, 8.28.

**(1-Hydroxymethyl-3,4-dimethylcyclopent-3-enyl) methanol (7):** To a suspension of lithium aluminum hydride (0.85 g, 22.55 mmol) in dry tetrahydrofuran (30 mL) was added a solution of diethylester **6** (2.58 g, 10.74 mmol) in dry tetrahydrofuran (15 mL) drop wise at  $0^\circ\text{C}$ . The resulting suspension was stirred overnight at  $0^\circ\text{C}$ . The suspension was quenched with water (0.86 mL), 15% sodium hydroxide (0.86 mL), and water (2.58 mL) at the same temperature. The mixture was stirred at room temperature for 2 hours. The white gel suspension was filtered through a pad of Celite and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/n-Hexane, 4:1) to give diol **7** (1.38 g, 82%) as a colorless oil:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  3.68 (s, 4H), 2.14 (s, 4H), 1.58 (s, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  128.77, 70.55, 145.41, 44.08, 13.73; Anal. Calc. for  $\text{C}_9\text{H}_{16}\text{O}_2$ : C, 69.19; H, 10.32. Found: C, 69.23; H, 10.29.

**(*t*-Butyldimethylsilyloxymethyl) 3,4-dimethyl-cyclopent-3-enyl] methanol (8):** *t*-Butyldi methylsilyl chloride (1.33 g, 8.83 mmol) was added slowly to a solution of **7** (1.38 g, 8.83 mmol) and imidazole (1.20 g, 17.67 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) at  $0^\circ\text{C}$ , and stirred for 5 hours at the same temperature. The solvent was evaporated under reduced pressure. The residue was extracted twice with EtOAc and water. The combined organic layer was dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **8** (2.27 g, 95%) as a colorless syrup:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  s (3.53, 4H), 2.13–1.95 (m, 4H), 1.50 (s, 6H), 0.83 (s, 9H), 0.01 (s, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  128.75, 71.11, 45.26, 44.09, 25.78, 18.09, 13.76, –5.76; Anal. Calc. for  $\text{C}_{15}\text{H}_{30}\text{O}_2\text{Si}$ : C, 66.61; H, 11.18. Found: C, 66.55; H, 11.08.

**[1-(*t*-Butyldimethyl-silyloxymethyl) 3,4-dimethyl-cyclopent-3-enyl methoxymethyl] phos phonic acid diisopropyl ester (9):** To a solution of the cyclopentenol **8** (2.3 g, 8.50 mmol) in 5 mL of DMF was added LiI (85.36 mg, 0.64 mmol) at  $25^\circ\text{C}$ . Both LiO*t*-Bu (13.61 mL of 1.0 M solution in THF, 13.61 mmol) and a solution of diisopropyl bromomethylphosphonate (2.74 mL, 11.52 mmol) in 5 mL of DMF were slowly added to the reaction mixture and

stirred for 2 hours at 60°C under anhydrous conditions. The mixture was quenched by adding water (50 mL), and the organic solvents were removed in vacuo. The aqueous layer was extracted with EtOAc (3 × 100 mL). The combined extracts were washed with brine, dried over MgSO<sub>4</sub>, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:2) to give **9** (2.44 g, 64%) as a colorless syrup: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 4.72 (m, 2H), 3.73 (m, 2H), 3.42 (s, 2H), 3.41 (s, 2H), 2.14–1.99 (m, 4H), 1.52 (s, 6H), 1.29 (m, 12H), 0.85 (s, 9H), 0.00 (s, 6H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 128.81, 70.87, 67.36, 66.30, 65.15, 45.78, 44.20, 25.87, 24.13, 18.25, 13.76, –5.46; Anal. Calc. for C<sub>22</sub>H<sub>45</sub>O<sub>5</sub>PSi: C, 58.90; H, 10.11, Found: C, 58.95; H, 10.08.

**(1-Hydroxymethyl-3,4-dimethyl-cyclopent-3-enylmethoxymethyl) phosphonic acid diisopropyl ester (10):** To a solution of **9** (176 mg, 0.392 mmol) in tetrahydrofuran (6 mL) was added tetrabutylammonium fluoride (TBAF) (0.60 mL, 1.0 M solution in THF) at 0°C and stirred for 5 hours at room temperature. The reaction mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography (EtOAc/hexane, 4:1) to give **10** (108 mg, 83%) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 4.68 (m, 2H), 3.69 (m, 2H), 3.54 (s, 2H), 3.47 (s, 2H), 2.12–1.99 (m, 4H), 1.50 (s, 6H), 1.29 (m, 12H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 128.68, 78.97, 71.18, 67.75, 66.79, 64.59, 45.40, 44.40, 23.98, 13.67; Anal. Calc. for C<sub>16</sub>H<sub>31</sub>O<sub>5</sub>P: C, 57.47; H, 9.34. Found: C, 57.52; H, 9.43.

**Methanesulfonic acid 1-(diisopropoxy-phosphorylmethoxymethyl) 3,4-dimethylcyclopent-3-enylmethyl ester (11):** To a solution of the alcohol **10** (789 mg, 2.36 mmol) in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (25 mL), anhydrous triethylamine (0.75 mL, 5.42 mmol) and MsCl (0.22 mL, 2.83 mmol) were added at 0°C. The mixture was stirred overnight at the same temperature, quenched by a cold saturated NaHCO<sub>3</sub> solution (1.5 mL), and further diluted with water (100 mL). The mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> (100 mL × 2) and water. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under vacuum, and the residue was purified by flash silica gel column chromatography (EtOAc/hexane, 1:1) to give **11** (749 mg, 77%) as a colorless syrup: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 4.73 (m, 2H), 4.14 (s, 2H), 3.73 (m, 2H), 3.49 (s, 2H), 3.03 (s, 3H), 2.18 (s, 4H), 1.56 (s, 6H), 1.34 (m, 12H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 128.55, 72.84, 71.03, 67.27, 65.05, 44.20, 36.88, 24.09, 13.62; Anal. Calc. for C<sub>17</sub>H<sub>33</sub>O<sub>7</sub>PS: C, 49.50, H, 8.06. Found: C, 49.56; H, 7.93.

**[1-(6-Aminopurin-9-ylmethyl) 3,4-dimethylcyclopent-3-enylmethoxymethyl] phosphonic acid diisopropyl ester (12):** A solution of the mesylate **11** (453 mg, 1.10 mmol), K<sub>2</sub>CO<sub>3</sub> (303 mg, 2.19 mmol), 18-crown-6 (441 mg, 1.67 mmol), and adenine (150 mg, 1.11 mmol) in dry DMF (15 mL) was stirred overnight at 90°C. The mixture was cooled to room temperature and concentrated under vacuum. The residue was diluted with water (100 mL)

and extracted with  $\text{CH}_2\text{Cl}_2$  (100 mL  $\times$  3). The combined organic layer was dried over anhydrous  $\text{MgSO}_4$ , filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ( $\text{MeOH}/\text{CH}_2\text{Cl}_2$ , 1:10) to give compound **12** (159 mg, 32%) as a white solid:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  8.34 (s, 1H), 8.03 (s, 1H), 4.80 (m, 2H), 4.28 (s, 2H), 3.75 (m, 2H), 3.35 (s, 2H), 2.40–2.04 (m, 4H), 1.55 (s, 6H), 1.36 (m, 12H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  155.32, 152.73, 142.24, 128.68, 119.12, 71.07, 48.09, 45.45, 24.13, 13.66; Anal. Calc. for  $\text{C}_{21}\text{H}_{34}\text{N}_5\text{O}_4\text{P}$ : C, 55.86; H, 7.59; N, 15.51. Found: C, 55.76; H, 7.44; N, 15.47.

**[1-(4-Amino-2-oxo-2H-pyrimidin-1-ylmethyl) 3,4-dimethylcyclopent-3-enylmethoxymethyl] phosphonic acid diisopropyl ester (13)**: Cytosine derivative **13** was synthesized from **11** by a similar procedure as described for **12**. Yield 37% as a white solid: m.p. 146–148°C;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 300 MHz)  $\delta$  7.33 (d,  $J = 7.0$  Hz, 1H), 5.54 (s,  $J = 7.0$  Hz, 1H), 4.75 (m, 2H), 4.31 (s, 2H), 3.74 (d,  $J = 8.0$  Hz, 2H), 3.32 (s, 2H), 2.38 (d,  $J = 10.2$  Hz, 2H), 2.08 (d,  $J = 10.0$  Hz, 2H), 1.55 (s, 6H), 1.34 (m, 12H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  165.23, 156.62, 145.70, 127.43, 98.32, 71.11, 48.56, 45.38, 25.49, 12.99; Anal. Calc. for  $\text{C}_{20}\text{H}_{34}\text{N}_3\text{O}_5\text{P}$  (+ 1.0 MeOH): C, 54.89; H, 8.33; N, 9.14. Found: C, 54.92; H, 8.28; N, 9.17.

**[1-(6-Aminopurin-9-ylmethyl) 3,4-dimethylcyclopent-3-enylmethoxy methyl] phosphonic acid (15)**: To a solution of the phosphonate **12** (92 mg, 0.204 mmol) in  $\text{CH}_3\text{CN}$  (8 mL) was added trimethylsilyl bromide (336 mg, 2.22 mmol). The mixture was heated under reflux for 15 hours and then concentrated under reduced pressure. The residue was partitioned between  $\text{CH}_2\text{Cl}_2$  (20 mL) and distilled  $\text{H}_2\text{O}$  (20 mL). The aqueous layer was washed out with  $\text{CH}_2\text{Cl}_2$  and then freeze-dried to give target compound **15** (64 mg, 86%) as a yellowish foamy solid. UV ( $\text{H}_2\text{O}$ )  $\lambda_{\text{max}}$  261.5 nm ( $\epsilon$  14 723);  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 300 MHz)  $\delta$  8.19 (s, 1H), 8.05 (s, 1H), 4.79 (m, 2H), 3.78 (d,  $J = 8.0$  Hz, 2H), 3.38 (s, 2H), 2.42–2.11 (m, 4H), 1.58 (s, 6H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  154.95, 153.0q, 143.43, 128.32, 118.96, 69.34, 66.21, 49.86, 29.87, 13.50; Anal. Calc. for  $\text{C}_{15}\text{H}_{22}\text{N}_5\text{O}_4\text{P}$  (+ 2.0  $\text{H}_2\text{O}$ ): C, 44.66; H, 6.49; N, 17.36. Found: C, 44.61; H, 6.52; N, 17.40.

**[1-(4-Amino-2-oxo-2H-pyrimidin-1-ylmethyl)-3,4-dimethylcyclopent-3-enylmethoxy methyl] phosphonic acid (16)**: Cytosine phosphonic acid derivative **16** was synthesized from **13** by the similar procedure as described for **15** as a yellowish foamy solid. Yield 80%, UV ( $\text{H}_2\text{O}$ )  $\lambda_{\text{max}}$  272.0 nm ( $\epsilon$  5 432);  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ , 300 MHz)  $\delta$  7.41 (d,  $J = 7.0$  Hz, 1H), 5.56 (s,  $J = 7.0$  Hz, 1H), 4.42 (s, 2H), 3.73 (d,  $J = 8.1$  Hz, 2H), 3.31 (s, 2H), 2.39–2.16 (m, 4H), 1.56 (s, 6H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  165.62, 155.60, 146.71, 128.56, 99.81, 69.35, 66.34, 51.56, 42.38, 30.22, 13.48; Anal. Calc. for  $\text{C}_{14}\text{H}_{22}\text{N}_3\text{O}_5\text{P}$  (+ 1.0  $\text{H}_2\text{O}$ ): C, 46.54; H, 6.69; N, 11.63. Found: C, 46.59; H, 6.72; N, 11.59.

***t*-Butyl SATE phosphoester of 9-[1-(3,4-dimethylcyclopent-3-enylmethoxymethyl)] adenine (17):** A solution of adenine phosphonic acid derivative **15** (65 mg, 0.177 mmol) and tributylamine (300  $\mu$ L, 1.2 mmol) in water (2.0 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 (15 mL) to which thioester **14** (270 mg, 1.65 mmol) and 1-mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole (222 mg, 0.75 mmol) were added. The mixture was stirred for 16 hours at room temperature and quenched with tetrabutylammonium bicarbonate buffer (7.5 mL, 1 M solution, pH = 8.0). The mixture was concentrated under reduced pressure and the residue was diluted with water (70 mL) and extracted twice with CH<sub>2</sub>Cl<sub>2</sub> (70 mL). The combined organic layer was washed with brine, dried over MgSO<sub>4</sub>, filtered, and evaporated. The residue was purified by silica gel column chromatography (MeOH/Hexane/EtOAc, 0.04:4:1) to give **17** (43 mg, 37%) as a white solid: m.p. 116–119°C; UV (MeOH)  $\lambda_{\max}$  263.0 nm ( $\epsilon$  14 811); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 300 MHz)  $\delta$  8.27 (s, 1H), 8.15 (s, 1H), 4.18 (t, *J* = 6.4 Hz, 4H), 3.89 (d, *J* = 8.2 Hz, 2H), 3.69 (s, 2H), 3.34 (s, 2H), 3.21 (t, *J* = 6.3 Hz, 4H), 2.32–2.11 (m, 4H), 1.57 (s, 6H), 1.25 (s, 18H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>)  $\delta$  202.71, 154.44, 152.18, 149.73, 144.34, 128.40, 119.73, 79.82, 69.64, 67.47, 60.19, 52.15, 43.62, 30.22, 26.16, 17.0. Analysis for C<sub>29</sub>H<sub>46</sub>N<sub>5</sub>O<sub>6</sub>PS<sub>2</sub> (+ 1.0 MeOH): C, 52.38; H, 7.33; N, 10.18; Found: C, 52.35; H, 7.36; N, 10.16.

***t*-Butyl SATE phosphoester of 1-[1-(3,4-dimethylcyclopent-3-enylmethoxymethyl)] cytosine (18):** The SATE prodrug of cytosine derivative **18** was synthesized from **16** using a similar procedure as described for **17** as a white solid: yield 31%; m.p. 107–109°C; UV (MeOH)  $\lambda_{\max}$  271.0 nm ( $\epsilon$  5 529); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$  7.46 (d, *J* = 7.1 Hz, 1H), 5.61 (s, *J* = 7.0 Hz, 1H), 4.22 (t, *J* = 6.3 Hz, 4H), 3.72 (d, *J* = 8.0 Hz, 2H), 3.30 (s, 2H), 3.10 (t, *J* = 6.4 Hz, 4H), 2.96 (s, 1H), 2.31–2.13 (m, 4H), 1.57 (s, 6H), 1.23 (s, 18H); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  202.67, 165.66, 157.76, 145.32, 129.21, 100.03, 78.31, 68.54, 67.81, 55.76, 51.21, 41.79, 29.54, 26.24, 15.48; Anal. Calc. for C<sub>28</sub>H<sub>46</sub>N<sub>3</sub>O<sub>7</sub>PS<sub>2</sub> (+ 1.0 MeOH): C, 52.27; H, 7.59; N, 6.33. Found: C, 52.29; H, 7.61; N, 6.30.

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