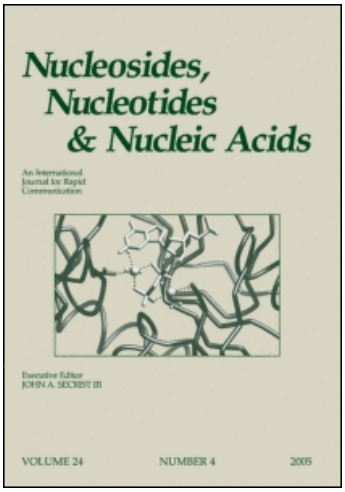


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Design and Synthesis of Novel Sate Derivatives of Acyclic Isocytosine and 9-Deazaadenine *C*-Nucleosides

Lian Jin Liu[a]; Joon Hee Hong[a]
[a] College of Pharmacy, Chosun University, Kwangju, Republic of Korea

Online publication date: 19 April 2010

To cite this Article Liu, Lian Jin and Hong, Joon Hee(2010) 'Design and Synthesis of Novel Sate Derivatives of Acyclic Isocytosine and 9-Deazaadenine *C*-Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 29: 3, 257 — 266
To link to this Article: DOI: 10.1080/15257771003745704
URL: http://dx.doi.org/10.1080/15257771003745704

Nucleosides, Nucleotides and Nucleic Acids, 29:257–266, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257771003745704

Taylor & Francis
Taylor & Francis Group

DESIGN AND SYNTHESIS OF NOVEL SATE DERIVATIVES OF ACYCLIC ISOCYTOSINE AND 9-DEAZAADENINE *C*-NUCLEOSIDES

Lian Jin Liu and Joon Hee Hong
College of Pharmacy, Chosun University, Kwangju 501-759, Republic of Korea

□ *This article describes a very simple route for synthesizing novel lipophilic phosphate bis(t-butSATE) prodrugs of acyclic cyclobutylated* C*-nucleosides such as isocytosine* ***12*** *and 9-deazaadenine* ***19****, which were prepared from 1,1-gem cyclobutyl dicarboxylate. Synthesized compounds were evaluated as potential antiviral agents against HIV virus. Some phosphate SATE prodrugs were more active against HIV than parent nucleosides.*

Keywords Acyclic nucleoside; antiviral agent; SATE prodrug

INTRODUCTION

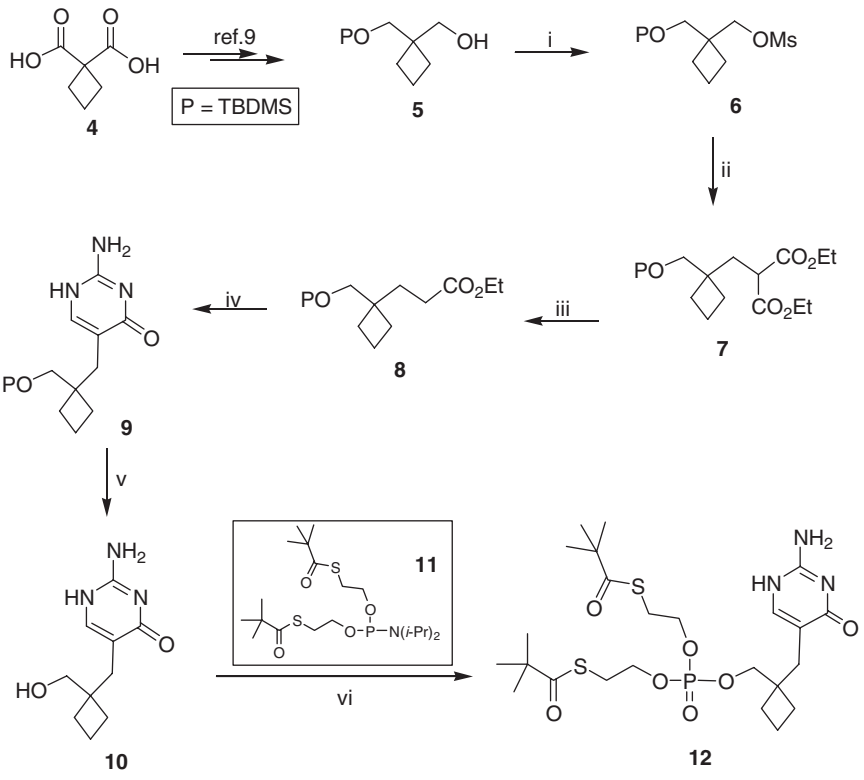
Acyclic nucleosides[1] in which the notional 5′-hydroxy group has been replaced by a phosphate or phosphonate ester can act as stable mimics of nucleoside monophosphates and undergo further phosphorylation in cells to afford species that are analogous to nucleoside triphosphates and can inhibit polymerases. These compounds circumvent the need for primary phosphorylation of the parent nucleoside, which is often the stumbling block in attaining active compounds. During the past 20 years, many new synthetic schemes for acyclic nucleoside phosphate and phosphonic acid analogues have been reported, and many of these molecules have exhibited promising antiviral activity.[2] Among them, PMEA **1**,[3] PMPA **2**[4] and HPMPC **3**[5] exhibit potent antiviral activity against human immunodeficiency virus (HIV), hepatitis B virus (HBV), and herpes simplex virus (HSV). However, the limited oral bioavailability of PMEA caused by the negatively charged phosphonate moiety at physiological pH limits the clinical usefulness of these molecules. PMEA has <1% oral bioavailability in monkeys and <11% in rats.[6] Neutral, membrane-permeable prodrugs such as bis(POM)-PMEA,[7] bis(DTE)-PMEA,[8] and bis(SATE)-PMEA,[9] can penetrate cellular membranes and deliver the parent PMEA by enzymatic activation. We, therefore, applied the

Received 20 January 2010; accepted 2 March 2010.
Address correspondence to Joon Hee Hong, College of Pharmacy, Chosun University, Kwangju 501-759, Republic of Korea. E-mail: hongjh@chosun.ac.kr

bis(SATE) approach to novel acyclic *C*-nucleosides. Here we report on the synthesis and antiviral activity of isocytosine and 9-deazaadenine prodrug analogues.

RESULTS AND DISCUSSION

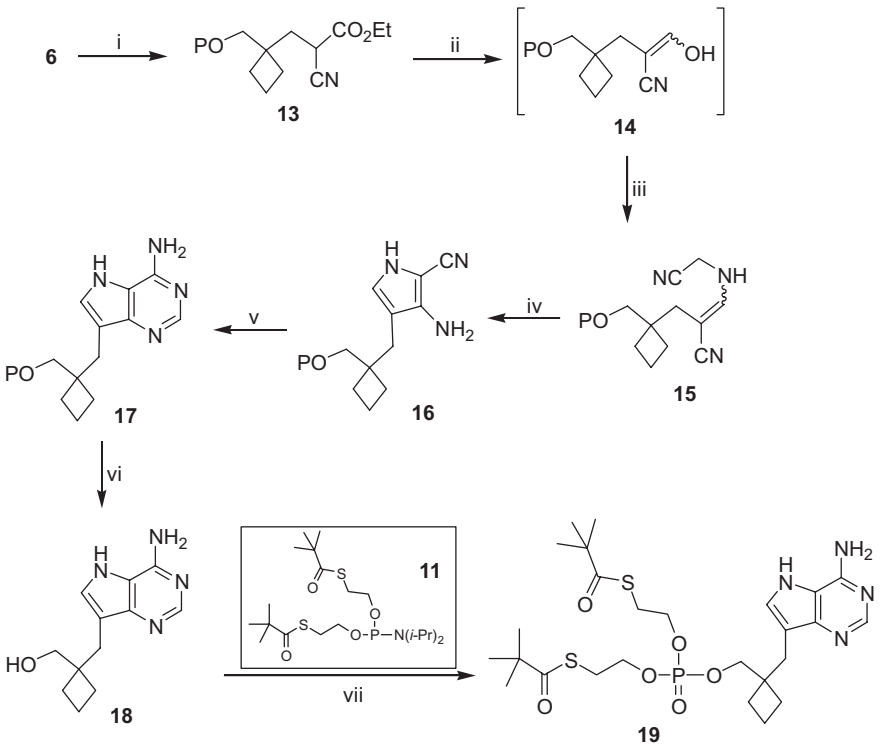
For the synthesis of cyclobutylated acyclic *C*-nucleoside phosphate, the commercially available cyclobutyl dicarboxylic acid **4** was selected as a starting material (Scheme 1). A reduction of the carboxylic functional group of **4** followed by a monosilylation of the diol afforded the alcohol derivative **5**, as described in the literature.[10] The hydroxyl group of **5** was methanesulfonylated with MsCl and triethylamine (TEA) in anhydrous CH_2Cl_2 to provide a key intermediate mesylate **6**, which was alkylated with diethyl malonate by nucleophilic S_N2 substitution conditions to give **7**. Decarboethoxylation of **7** in the presence of lithium chloride in DMSO solvent provided ester derivative **8**. Sequential treatment of **8** with lithium diisopropylamide, ethyl



SCHEME 1 Synthesis of acyclic C-isocytosine phosphate SATE prodrug analogue. Reagents: i) MsCl, TEA, CH_2Cl_2; ii) NaH, $CH_2(CO_2Et)_2$, THF; iii) LiCl, DMSO; iv) (a) LDA/THF, HCOEt; (b) $H_2NC(=NH)NH_2$·carbonate, NaOEt/EtOH; v) TBAF, THF; vi) **11**, 1*H*-tetrazole/THF, then *m*-CPBA/CH_2Cl_2.

formate, and guanidine carbonate in the presence of sodium ethoxide in ethanol gave an isocytosine analogue **9**, which was desilylated with tetrabutyl ammonium fluoride in THF, provided the acyclic *C*-isocytosine nucleoside analogue **10** (Scheme 1). Di-*t*-butyl diisopropylphosphoamidite **11** is a highly reactive phosphitylating agent, which, on activation with 1*H*-tetrazole, reacts rapidily with a simple alcohol moiety of nucleoside **11**, the reaction being complete within 15 minutes.[11] Subsequent oxidation of the corresponding phosphate triester with *m*-chloroperbenzoic acid (*m*-CPBA) proceeds rapidly and gives the desired bis(*t*-Bu-SATE) nucleoside phosphate prodrug analogue **12**.[12]

For the similar synthesis of *C*-isocytosine phosphate prodrug, the mesylate **6** was alkylated with ethylcyanoacetate by nucleophilic S_N2 substitution conditions to give **13**. Compound **13** was selectively reduced to the enol intermediate **14** by one equivalent of diisobutylaluminium hydride (DIBAL-H), which was converted to enamine **15** by treatment of aminoacetonitrile monosulfate ($H_2NCH_2CN{\cdot}H_2SO_4$) and sodium acetate three hydrate ($NaOAc{\cdot}3H_2O$) in MeOH (Scheme 2). The pyrrole



SCHEME 2 Synthesis of cyclic C-9-deazaadenine phosphate SATE prodrug analogue. Reagents: i) $NCCH_2CO_2Et$, NaH, THF; ii) DIBALH, ether; iii) $NH_2CH_2CN{\cdot}H_2SO_4$, $NaOAc{\cdot}3H_2O$, MeOH; iv) (a)ethyl chloroformate, DBU, CH_2Cl_2; (b) DBU; (c) Na_2CO_3, MeOH; V) formamidine acetate, EtOH; vi) TBAF, THF; vii) **11**, 1*H*-tetrazole/THF, then *m*-CPBA/CH_2Cl_2.

TABLE 1 Antiviral activities of the synthesized compounds

	HIV-1 EC_{50} (μM)	Cytotoxicity CC_{50} (μM)
10	>100	>100
12	61.3	89.5
18	87.3	>100
19	26.5	44.7
PMEA	>10	>10
bis(t-Bu-SATE)-PMEA	0.67	1.8

EC_{50} (μM): 50% effective concentration or concentration (in μM) required to inhibit the replication of HIV-1 by 50%.

CC_{50}(μM): 50% cytotoxic concentration (in μM) or concentration required to reduced the viability of uninfected cells by 50%.

structure **16** was successfully achieved by sequential treatment of ethyl chloroformate and 1,8-diazabicyclo[5,4,0] undec-7-ene (DBU) in CH_2Cl_2 followed by Na_2CO_3.[13] Subjection of pyrrole analogue **16** to formamidine acetate ($H_2NC{=}NH{\cdot}HOAc$) gave the protected 9-deazaadenine derivative **17**, which was desilylated with tetrabutylammonium fluoride (TBAF) to give the *C*-9-deazaadenine **18** (Scheme 2). Similar reaction conditions were used for the synthesis of the acyclic *C*-9-deazaadenine SATE prodrug **19** as described for prodrug **12**.

Both SATE prodrugs showed higher antiviral activities based along with increased cytotoxicity against human immunodeficiency virus 1 (HIV-1) in MT-4 cells than the parent nucleosides (Table 1).

In conclusion, we have synthesized and compared the antiviral activity of bis(t-Bu-SATE) prodrugs **12**, **19** and parent nucleosides **10**, **18**. Also, the synthesis of other *C*-nucleoside analogues (U,T) and the relative chemical and enzymatic stability study of the synthesized prodrugs in various media using Gosselin's method[9] are in progress and will be reported elsewhere .

EXPERIMENTAL

Melting points were determined on a Mel-tem II laboratory device and are uncorrected. The NMR spectra were recorded on a JEOL JNM-LA

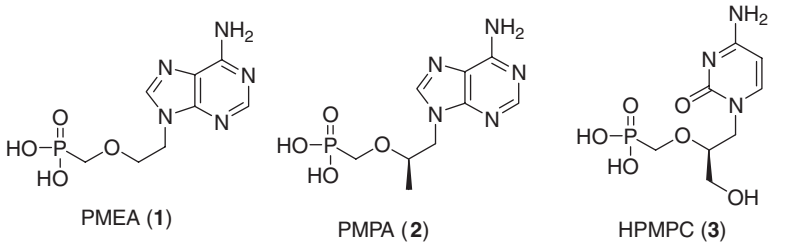


FIGURE 1 Synthesis rationale of acyclic phosphate analogues.

300 spectrometer. The chemical shifts are reported as parts per million (δ) and the signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and dd (doublet of doublets). The ultra violet (UV) spectra were obtained using 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). The thin liquid chromatography (TLC) was performed on Uniplates (silica gel) purchased from Analtech Co. (7558, Newark, DE, USA). All reactions were carried out under N_2 unless otherwise specified. Dry dichloromethane, benzene, and pyridine were obtained by distillation from CaH_2. Dry tetrahydrofuran (THF) was obtained by distillation from Na and benzophenone immediately prior to use.

Methanesulfonic acid 1-(t-butyldimethylsilanyloxymethyl) cyclobutylmethyl ester (6): To a solution of the alcohol **5** (320 mg, 1.39 mmol) in anhydrous CH_2Cl_2 (10 mL), anhydrous triethylamine (0.38 mL) and MsCl (195 mg, 1.68 mmol) was added at 0°C. The mixture was stirred at the same temperature for 5 hours, and quenched by adding a cold saturated $NaHCO_3$ solution (2.0 mL). The mixture was diluted with water (100 mL) and extracted with EtOAc (100 mL) two times. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash silica gel column chromatography (EtOAc/hexane, 1:2) to give compound **6** (373 mg, 87%) as colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.21 (s, 2H), 3.61 (s, 2H), 3.00 (s, 3H), 1.91–1.72 (m, 6H), 0.89 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 72.80, 65.42, 42.64, 36.85, 25.80, 25.33, 18.24, 15.11, −5.50.

2-[1-(t-Butyldimethylsilanyloxymethyl) cyclobutylmethyl] malonic acid diethyl ester (7): To a solution of NaH (0.45 g, 18.7 mmol) in THF (70 mL), diethyl malonate (4.5 g, 28.1 mmol) in THF (20 mL) was added under nitrogen atmosphere at room temperature. After 1 hour, compound **6** (3.42 g, 7.6 mmol) in THF (50 mL) was slowly added to the reaction mixture. The mixture was stirred overnight at room temperature and quenched by the addition of sat. ammonium chloride (10 mL) and further diluted with water (100 mL). The mixture was extracted with ethyl acetate (100 mL) twice. The combined organic layer was washed with brine, dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **7** (2.01 g, 71%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.18 (q, J = 6.9 Hz, 4H), 3.48 (s, 2H), 3.37 (t, J = 6.9 Hz, 1H), 2.10 (d, J = 6.6 Hz, 2H), 1.79–1.63 (m, 6H), 1.20 (t, J = 6.9 Hz, 6H), 0.88 (s, 9H), 0.02 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.13, 67.37, 61.21, 48.35, 42.23, 36.06, 28.43, 25.80, 18.21, 15.36, 13.98, −5.54; Anal calc for $C_{19}H_{36}O_5Si$: C, 61.25; H, 9.74. Found: C, 61.21; H, 9.70.

3-[1-(t-Butyldimethylsilanyloxymethyl) cyclobutyl] propionic acid ethyl ester (8): To a solution of **7** (1.38 g, 3.7 mmol) in DMSO (10 mL), LiCl

(470 mg, 11.1 mmol) and H_2O (2 drops) were added. The mixture was stirred overnight at 170°C. After cooling to room temperature, the mixture was diluted with H_2O (150 mL) and extracted with diethyl ether (150 mL) two times. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and filtered. The filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:12) to give **8** (767 mg, 69%) as a colorless syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.10 (q, $J = 7.2$ Hz, 2H), 3.45 (s, 2H), 2.26–2.20 (m, 2H), 1.85–1.70 (m, 6H), 1.25 (t, $J = 7.1$ Hz, 3H), 0.89 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 174.36, 67.87, 60.18, 42.48, 32.22, 29.60, 27.95, 25.85, 18.25, 15.05, 14.18, −5.50; Anal calc for $C_{16}H_{32}O_3Si$: C, 63.95; H, 10.73. Found: C, 63.89; H, 10.69.

5-[1-(t-butyldimethylsilanyloxymethyl) cyclobutylmethyl] isocytosine (9): To a solution of lithium diisopropylamide (6.0 mL, 1 M in hexane) in THF (10 mL), a solution of **8** (1.19 g, 3.97 mmol in THF 20 mL) was slowly added at −78°C under a nitrogen atmosphere. The mixture was stirred at the same temperature for 1.5 hours and ethyl formate (1.26 mL, 15.0 mmol) was added to the mixture. After stirring overnight at room temperature, saturated NH_4Cl solution (10 mL) was added to the mixture and further diluted with water (100 mL). The mixture was extracted with EtOAc (2 × 100 mL), washed with water and brine solution, dried over $MgSO_4$, and evaporated to dryness to obtain a crude residue. The residue was dissolved in absolute ethanol (10 mL). To a solution of guanidine carbonate (2.0 g, 11.25 mmol) in EtOH (30 mL), EtONa/EtOH (21% solution, 4.84 mL, 15.0 mmol) was added and stirred for 1.5 hours. To this mixture, the above-obtained residue in ethanol (10 mL) was added and refluxed overnight. The mixture was filtered, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/hexane/MeOH, 3:1:0.1) to give **9** (706 mg, 55%): m.p. 144∼146°C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.88 (br s, 1H), 7.39 (s, 1H), 6.52 (br s, 2H), 3.48 (s, 2H), 2.48 (s, 2H), 1.86–1.69 (m, 6H), 0.89 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 161.51, 154.95, 126.23, 117.66, 68.06, 43.06, 32.53, 27.76, 25.96, 18.36, 15.34, −5.34; Anal calc for $C_{16}H_{29}N_3O_2Si$: C, 59.40; H, 9.04; N, 12.99. Found: C, 59.36; H, 8.98; N, 13.04.

5-[1-(t-Hydroxymethyl) cyclobutylmethyl] isocytosine (10): To a solution of compound **9** (216 mg, 0.67 mmol) in THF/CH_3CN (1:1) (10 mL), tetrabutylammonium fluoride (TBAF, 1.0 mL, 1.0 M solution in THF) at 0°C was added. The mixture was stirred overnight at room temperature and concentrated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:5) to give compound **10** (110 mg, 79%) as a white solid: m.p. 182–184°C; UV (MeOH) λ_{max} 289.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.95 (br s, 1H), 7.44 (s, 1H), 6.40 (br s, 2H), 4.80 (t, $J = 5.0$ Hz, 1H), 3.45 (s, 2H), 2.49 (s, 2H), 1.87–1.68 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 161.56, 154.99, 127.09, 116.98, 66.17, 43.81, 32.41, 27.74, 14.46; Anal calc

for $C_{10}H_{15}N_3O_2$ (+1.0 H_2O): C, 52.85; H, 7.54; N, 18.49. Found: C, 52.89; H, 7.51; N, 18.53.

1-(Isocytosine-5-ylmethyl)-cyclobutylmethyl bis (pivaloyl-2-thioethyl) phosphate (12): 1*H*-Tetrazole (0.252 g, 3.6 mmol) was added to a stirred solution of **10** (251 mg, 1.2 mmol) and the bis(pivaloyl-2-thioethyl) phosphoramidite **11** (653 mg, 1.44 mmol) in THF (5.0 mL) at room temperature. After 30 minutes, the reaction mixture was cooled to −40°C, and a solution of 3-chloroperbenzoic acid, (*m*-CPBA) (276 mg, 1.56 mmol) in CH_2Cl_2 (5.0 mL) was added; the mixture was then allowed to warm to room temperature over 1 hour. Sodium sulfite (10% solution, 1.6 mL) was added to the mixture to destroy the excess *m*-CPBA, after which the organic layer was separated and the aqueous layer washed with dichloromethane (15 mL) two times. The combined organic layers were washed with saturated aqueous sodium hydrogen carbonate (7 mL) and then water (3 × 7 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane/MeOH, 2:1:0.1) to give compound **9** (409 mg, 59%) as a pale yellow oil: UV (EtOH) λ_{max} 290 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.91 (br s, 1H), 7.49 (s, 1H), 6.44 (br s, 2H), 4.12 (m, 4H), 3.42 (s, 2H), 3.12 (t, J = 6.6 Hz, 4H), 2.46 (s, 2H), 1.85–1.67 (m, 6H), 1.18 (s, 18H); ^{13}C NMR (DMSO-d_6) δ 202.24, 163.21, 154.83, 128.27, 117.65, 73.43, 64.44, 51.62, 43.76, 33.78, 32.12, 27.65, 25.21, 14.36; ^{31}P NMR (DMSO-d_6) δ–0.71; FABMS m/z 578 $(M + H)^+$.

3-[1-(t-Butyldimethylsilanyloxymethyl) cyclobutyl] 2-cyanopropionic acid ethyl ester (13): To a suspension of sodium hydride (111 mg, 4.625 mmol) in distilled THF (20 mL) was added dropwise ethyl cyanoacetate (525 mg, 4.625 mmol) at 0°C, and the mixture was stirred at room temperature for 1 hour. Mesylate **6** (1.426 g, 4.625 mmol) dissolved in THF (5 mL) was added to this mixture and the mixture was stirred overnight at room temperature. The reaction mixture was quenched by saturated ammonium chloride and further diluted with H_2O (50 mL). The mixture was extracted with EtOAc (50 mL × 2). The combined organic layer was washed with brine and dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:15) to give **13** (978 mg, 65%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.21 (q, J = 6.9 Hz, 2H), 3.42 (s, 2H), 3.03 (t, J = 6.8 Hz, 1H), 2.57 (d, J = 6.7 Hz, 2H), 1.80–1.65 (m, 6H), 1.28 (t, J = 6.9 Hz, 3H), 0.87 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.74, 66.29, 61.07, 48.35, 41.74, 34.67, 30.31, 28.43, 25.74, 18.42, 15.70, 13.82, −5.51; Anal calc for $C_{17}H_{31}NO_3Si$: C, 62.73; H, 9.60; N, 4.30. Found: C, 62.71; H, 9.55; N, 4.26.

2-[1-(t-Butyldimethylsilanyloxymethyl) cyclobutylmethyl] 3-hydroxyacryl onitrile (14). DIBALH (3.64 mL, 1 M in hexane) was added to a solution of **13** (592 mg, 1.82 mmol) in anhydrous ether at −78°C over 10 minutes. The resulting mixture was stirred for 20 minutes and quenched with MeOH

(4 mL). The resulting white solid was filtered, and the filtrate was concentrated in reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:10) to give enol **14** (374 mg, 73%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.59 (s, 1H), 3.50 (s, 2H), 1.94 (s, 2H), 1.81–1.66 (m, 6H), 0.88 (s, 9H), 0.01 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 156.47, 118.32, 89.71, 69.39, 40.87, 36.09, 28.48, 25.38, 18.38, 14.74, −5.53; Anal calc for $C_{15}H_{27}NO_2Si$: C, 64.01; H, 9.67; N, 4.98. Found: C, 63.97; H, 9.65; N, 4.99.

2-[1-(t-Butyldimethylsilanyloxymethyl) cyclobutylmethyl]-3-(isocyanomethylamino)-acrylonitrile (15). To a solution of **14** (955 mg, 3.24 mmol) in MeOH (30 mL), aminoacetonitrile monosulfate (2.09 g, 13.57 mmol) and sodium acetate trihydrate (1.84 g, 13.52 mmol) was added. The mixture was stirred overnight at room temperature, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (EtOAc/Hexane, 1:5) to give **15** (455 mg, 44%) as a mixture of *E/Z* diastereomers. The mixture was subjected directly to the next step.

3-Amino-4-[1-(t-butyl-dimethyl-silanyloxymethyl)-cyclobutylmethyl]-1H-pyrrole-2-carbonitrile (16): 1,8-Diazabicyclo[5,4,0] undec-7-ene (DBU, 470 mg, 3.14 mmol) and ethyl chloroformate (255 mg, 2.355 mmol) were added to a solution of **15** (503 mg, 1.575 mmol) in anhydrous CH_2Cl_2 (15 mL). The mixture was stirred at 0°C for 4 hours and the reaction temperature was elevated to room temperature. DBU (478 mg, 3.14 mmol) was added to the mixture and stirred overnight at the same temperature. After the reaction solvent was concentrated under reduced pressure and replaced with MeOH (10 mL), solid Na_2CO_3 (17 mg, 0. 158 mmol) was added to the mixture and stirred for 4 hours. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in water (60 mL) and extracted with CH_2Cl_2 two times (50 mL × 2), and the organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography (EtOAc/Hexane, 2:1) to give **16** (256 mg, 51%); UV (MeOH) λ_{max} 249.5 nm;^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.94 (br s, 1H), 6.44 (s, 1H), 3.72 (br s, 2H), 3.58 (s, 2H), 2.37 (s, 2H), 1.81–1.64 (m, 6H), 0.87 (s, 9H), 0.01 (s, 6H);^{13}C NMR ($CDCl_3$) δ 120.81, 118.32, 116.32, 113.37, 111.76, 68.88, 45.32, 35.32, 27.69, 25.54, 18.48, 14.43, −5.56; Anal calc for $C_{17}H_{29}N_3OSi$: C, 63.90; H, 9.15; N, 13.15. Found: C, 63.94; H, 9.11; N, 13.16.

7-[1-(t-Butyldimethylsilanyloxymethyl) cyclobutylmethyl] 5H-pyrrolo[3, 2-d]pyrimidin-4-ylamine (17). To a solution of **16** (715 mg, 2.24 mmol) in EtOH (25 mL), formamidine acetate (1.17 g, 11.27 mmol) was added and the reaction mixture and refluxed overnight. The solvent was concentrated under reduced pressure to give the residue. The residue was purified by silica gel column chromatography using an elution solvent system (EtOAc/Hexane, 3:1) to provide **17** (481 mg, 62%) as a white solid: m.p. 159–161°C; UV (MeOH) λ_{max} 274.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ

10.80 (br s, 1H), 8.12 (s, 1H), 7.34 (d, J = 1.9 Hz, 1H), 6.73 (br s, 2H), 3.78 (s, 2H), 2.43 (s, 2H), 1.82∼1.67 (m, 6H), 0.88 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 150.71, 149.29, 120.35, 118.32, 116.21, 113.21, 69.43, 46.75, 36.49, 27.02, 25.48, 18.52, 14.19, −5.51; Anal calc for $C_{18}H_{30}N_4OSi$: C, 62.39; H, 8.73; N, 16.17. Found: C, 62.43; H, 8.70; N, 16.18.

7-[1-(Hydroxymethyl) cyclobutylmethyl] 5H-pyrrolo[3,2-d]pyrimidin-4-ylamine (18). To a solution of compound **17** (250 mg, 0.721 mmol) in THF (8.0 mL), tetrabutylammonium fluoride (TBAF, 1.0 mL, 1.0 M solution in THF) at 0°C was added. The mixture was stirred overnight at room temperature, and concentrated. The residue was purified by silica gel column chromatography (MeOH/CH_2Cl_2, 1:5) to give compound **18** (125 mg, 75%) as a white solid: m.p. 184–185°C; UV (MeOH) λ_{max} 274.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.82 (br s, 1H), 8.13 (s, 1H), 7.32 (d, J = 2.0 Hz, 1H), 6.70 (br s, 2H), 4.82 (t, J = 4.8 Hz, 1H), 3.72 (s, 2H), 2.46 (s, 2H), 1.80∼1.63 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 150.32, 148.53, 120.17, 118.42, 116.15, 112.90, 68.57, 45.43, 37.76, 27.45, 14.64; Anal calc for $C_{12}H_{16}N_4O$ (+ 1.0 MeOH): C, 59.07; H, 7.63; N, 21.19. Found: C, 59.02; H, 7.64; N, 21.15.

1-(4-amino-5H-pyrrolo[3,2-d]pyrimidin-7-ylmethyl) cyclobutylmethyl bis (pivaloyl-2-thioethyl) phosphate (19): Compound **19** was synthesized from **18** using a similar procedure as described for **12**: yield 45%; UV (EtOH) λ_{max} 275 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.79 (br s, 1H), 8.14 (s, 1H), 7.34 (d, J = 2.0 Hz, 1H), 6.72 (br s, 2H), 4.15 (m, 4H), 3.77 (s, 2H), 3.14 (t, J = 6.6 Hz, 4H), 2.49 (s, 2H), 1.84∼1.65 (m, 6H), 1.19 (s, 18H); ^{13}C NMR (DMSO-d_6) δ 201.9, 151.57, 149.12, 119.59, 117.32, 116.27, 113.28, 73.71, 65.11, 52.01, 42.44, 34.52, 31.72, 26.55, 24.37, 14.21; ^{31}P NMR (DMSO-d_6) δ—0.73; FABMS m/z 601 $(M + H)^+$.

REFERENCES

1. a) De Clercq, E.; Holý, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P.C. A novel selective broad-spectrum anti-DNA virus agent. *Nature* **1986**, 323, 464–467; b) De Clercq, E. Codofovir in the treatment of poxvirus infections. *Antiviral Res.* **2002**, 55, 1–13; c) Robbins, B.L.; Srinivas, R.V.; Kim, C.; Bischofberger, N.; Fridland, A. Anti-human immunodeficiency virus activity and cellular metabolism of a potential prodrug of the acyclic nucleoside phosphonate 9-R-(2-phosphonomethoxypropyl)adenine (PMPA), Bis(isopropyloxymethylcarbonyl)PMPA. *Antimicrob. Agents Chemother.* **1998**, 2, 612–617.
2. a) Vandendriessche, F.; Snoeck, R.; Janssen, G.; Hoogmartens, J.; Van Aerschot, A.; De Clercq, E.; Herdewijn, P. Synthesis and antiviral activity of acyclic nucleosides with a 3(*S*),5-dihydroxypentyl or 4(*R*)-methoxy-3(*S*),5-dihydroxypentyl side chain. *J. Med. Chem.* **1992**, 35, 1458–1465; b) Liu, L.J.; Yoo, J.C.; Hong, J.H. Short synthesis and antiviral activity of acyclic phosphonic acid nucleoside analogues. *Nucleosides Nucleotides Nucleic Acids* **2009**, 28, 150–164; c) Topalis, D.; Alvarez, K.; Barral, K.; Munier-Lehmann, H.; Schneider, B.; Véron, M.; Guerreiro, C.; Mulard, L.; El-Amri, C.; Canard, B.; Deville-Bonne, D. Acyclic phosphonate nucleotides and human adenylate kinases: impact of a borano group on alpha-P position. *Nucleosides Nucleotides Nucleic Acids* **2008**, 27, 319–331.

3. a) Naesens, L.; Balzarini, J.; De Clercq, E. Therapeutic potential of PMEA as an antiviral drug. *Med. Virol.* **1994**, 4, 147–159; b) De Clercq, E. Broad-spectrum anti-DNA virus and anti-retrovirus activity of phosphonylmethoxyalkylpurines and pyrimidines. *Biochem. Pharmacol.* **1991**, 42, 963–972.
4. Pauwels, R.; Balzarini, J.; Schols, D.; Baba, M.; Desmyter, J.; Rosenberg, I.; Holy, A.; De Clercq, E. Phosphonylmethoxyethyl purine derivatives, a new class of anti-human immunodeficiency virus agents. *Antimicrob Agents Chemother.* **1988**, 32, 1025–1030.
5. De Clercq, E. Potential of acyclic nucleoside phosphonates in the treatment of DNA virus and retrovirus infections. *Expert. Rev. Anti. Infect. Ther.* **2003**, 1, 21–43.
6. Görbig, U.; Balzarini, J.; Meier, C. New cycloAmb-nucleoside phosphonate prodrugs. *Nucleosides Nucleotides Nucleic Acids* **2007**, 26, 831–834.
7. Starrett Jr., J.E.; Tortolani, D.R.; Hitchcock, M.J.M.; Martin, J.C.; Mansuri, M.M. Synthesis and in vivo evaluation of a phosphonate prodrug: bis(pivaloylosymethyl) 9-(2-phosphonylmethoxyethyl) adenine. *Antiviral Res.* **1992**, 19, 267–273.
8. Puech, F.; Gosselin, G.; Lefebvre, I.; Pompon, A.; Aubertin, A.M.; Kirn, A.; Imbach, J.L. Intracellular delivery of nucleoside monophosphates through a reductase-mediated activation process. *Antiviral Res.* **1993**, 22, 155–174.
9. Benzaria, S.; Pélicano, H.; Johnson, R.; Maury, G.; Imbach, J.L.; Aubertin, A.M.; Obert, G.; Gosselin, G. Synthesis, in vitro antiviral evaluation, and stability studies of bis(S-acyl-2-thio-ethyl) ester derivatives of 9-(2-phosphonomethoxy) ethyladenine (PMEA) as potential PMEA prodrugs with improved oral bioavailability. *J. Med. Chem.* **1996**, 39, 4958–4965.
10. a) Zelinskii, N.D.; Uyedinov, M.N. 1,1-Dimethy cyclobutane. *Ber.* **1913**, 46, 1093–1094; b) Kim, A.; Hong, J. H. Synthesis and anti-HCMV activity of novel 5′-norcarboacyclic nucleosides. *Arch. Pharm.* **2005**, 338, 522–527.
11. a) Tanaka, T.; Tamatsukuri, S.; Ikehara, M. New approach to the synthesis of deoxynucleoside phosphoramidite derivatives. *Tetrahedron Lett.* **1986**, 27, 199–202; b) Perich, J.W.; Hohn, R.B. Di-tert-butyl N,N-diethyl phosphoramidite. A new phosphitylating agent for the efficient phosphorylation of alcohol. *Synthesis* **1987**, 142–144.
12. Lefebvre, I.; Périgaud, C.; Pompon, A.; Aubertin, A.M.; Girardet, J.L.; Kirn, A.; Gosselin, G.; Imbach, J.L. Mononucleoside phosphotriester derivatives with S-acyl-2-thioethyl bioreversible phosphate-protecting groups: Intracellular delivery of 3′-azido-2′,3′-dideoxythymidine 5′-monophosphate. *J. Med. Chem.* **1995**, 38, 3941–3950.
13. Kamath, V.P.; Ananth, S.; Bantia, S.; Morris, P.E. Synthesis of a potent transit-state inhibitor of 5′-deoxy-5′-methylthioadenosine phosphorylase. *J. Med. Chem.* **2004**, 47, 1322–1324.