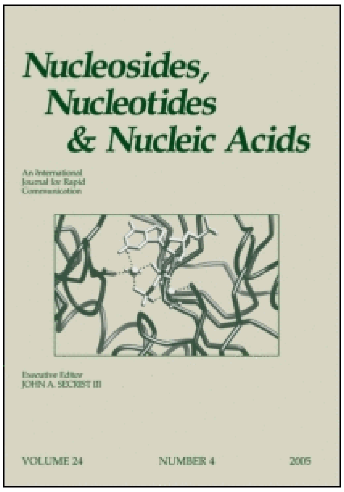

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Design, Synthesis and Anti-HIV Activity of Homologous PMEA Derivatives

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DESIGN, SYNTHESIS, AND ANTI-HIV ACTIVITY OF HOMOLOGOUS PMEA DERIVATIVES

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□ *This article describes an efficient route for synthesizing novel cyclopropyl homologous PMEA analogues. The condensation of the bromide* ***8*** *with nucleosidic bases (A, U, T, C, 5-FU, G) under standard nucleophilic substitution and deprotection conditions, afforded the target phosphonic acid analogues* ***14∼18*** *and* ***21****. These compounds were evaluated for their potential antiviral properties against various viruses. Guanine derivative* ***21*** *showed significant antiviral activity.*

Keywords Homologous PMEA; acyclic nucleoside; antiviral agent

INTRODUCTION

Acyclic nucleosides,[1] in which the 5′-hydroxy group has been replaced by either a phosphonate group or phosphonate ester, can act as stable mimics of nucleoside monophosphates and undergo further phosphorylation in cells to produce species that are analogous to nucleoside triphosphates and can inhibit polymerases. The advantage of these compounds is that primary phosphorylation of the parent nucleoside is unnecessary, which is often a stumbling block in attaining active compounds. Over the past several years, many new synthetic schemes for various acyclic nucleoside phosphonate and phosphonic acid analogues have been reported. Many of these molecules have shown promising antiviral activity.[2] Among them, PMEA **1**,[3] and PMCG **2**[4] exhibit potent antiviral activity against the HIV and HBV. The recent Food and Drug Administration (FDA)-approval of bis(POC)PMPA **3**[5] as an anti-HIV agent highlights the need for further research into acyclic phsophonate analogues as potential chemotherapeutic agents.

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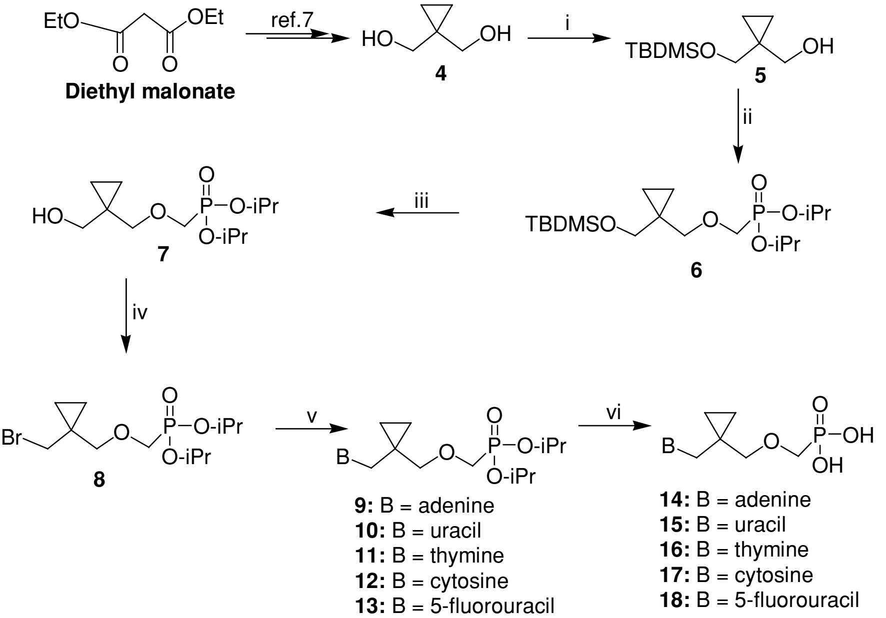
The efficacy of these drugs is limited by their toxicity and side effects, as well as the emergence of many drug resistant viral strains.[6] Therefore, there is a need for less toxic and more effective antiviral agents that do not exhibit any cross-resistance with existing drugs. As a part of our ongoing research into drug discovery and in view of the stimulating results reported for acyclic nucleoside phosphonate analogues, we designed and synthesized one-carbon elongated homologous PMEA analogues and examined their potential antiviral activity in this study.

RESULTS AND DISCUSSION

First, the commercially available diethyl malonate was selected as a starting material for the synthesis of target nucleoside phosphonic acids (Scheme 1). The double alkylation of diethyl malonate using 1,2-dibromoethane and reduction with $LiAlH_4$ using the reported procedure[7] provided the diol derivative **4**. Selective monosilylation of the diol **4** afforded the alcohol derivative **5**. The hydroxy group of compound **5** was phosphonylated by treating it with diisopropyl bromomethylphosphonate in the presence of anhydrous DMF to give compound **6**. A silyl protection group was removed by a treatment with tetrabutylammonium fluoride (TBAF) to produce compound **7**, which was then brominated by the sequential addition of NBS to a solution of the alcohol and triphenylphosphine to give key intermediate **8**.[8] The direct coupling of the bromide **9** with the bases (A, U, T, C, 5-FU) in DMF containing cesium carbonate (Cs_2CO_3) as a catalyst provided the desired and N^9-alkylated purine derivative **9** in the case of adenine[9] and N^1-alkylated derivatives (**10–13**) in the case of pyrimidine bases.[10] The UV data was in good agreement with those of the appropriate model compounds. The removal of the isopropyl groups of compounds **9**~**13** was accomplished using trimethylsilyl bromide[11] to afford the desired nucleoside phosphonic acids **14**~**18**.

For the synthesis of the target guanine derivative **21**, the bromide **8** was coupled with the sodium salt of 2-amino-6-chloropurine, which was prepared by adding sodium hydride to 2-amino-6-chloropurine in DMF. The isopropylidene groups of compound **19** was removed by a similar treatment of $(CH_3)_3SiBr$ to that used for compound **9** to produce compound **20**, which was subjected to hydrolysis conditions in a 2N HCl solution to give the desired guanine phosphonic acid nucleoside **21** (Scheme 2).

All the synthesized compounds were tested against several viruses such as HIV-1 (MT-4 cells), HSV-1 and HSV-2 (CCL 18 cells), and HCMV (AD-169).[12] As shown in Table 1, some of the compounds showed good antiviral activity. Especially, the guanine nucleotide analogue **21**, exhibited significant anti-HIV activity in the MT-4 cells (EC_{50} = 8.5 μmol). It is believed that the arrangement between the guanine and the phosphorus atom may



Reagents: i) TBDMSCl, imidazole, CH_2Cl_2; ii) Diisopropyl bromomethylphosphonate, LiO*t*-Bu, LiI, DMF; iii) TBAF, THF; vi) PPh_3, NBS, CH_2Cl_2; v) Bases, Cs_2CO_3, DMF, rt; vi) $(CH_3)_3SiBr$, CH_2Cl_2.

SCHEME 1 Synthesis of 1,1′-cyclopropyl phosphonic acid nucleosides. Reagents: i) K_2CO_3, CH_2Br_2, DMF; ii) $LiAlH_4$, THF; iii) TBDMSCl, imidazole, CH_2C_{l2}; iv) Diisopropyl bromomethylphosphonate, LiO*t*-Bu, LiI, DMF; v) TBAF, THF; vi) PPh_3, NBS, CH_2Cl_2; vii) Bases, Cs_2CO_3, DMF, rt; viii) $(CH_3)3SiBr$, CH_2C_{l2}.

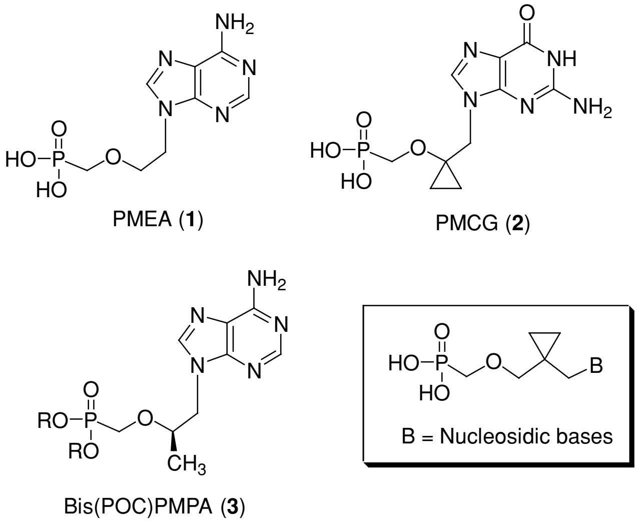
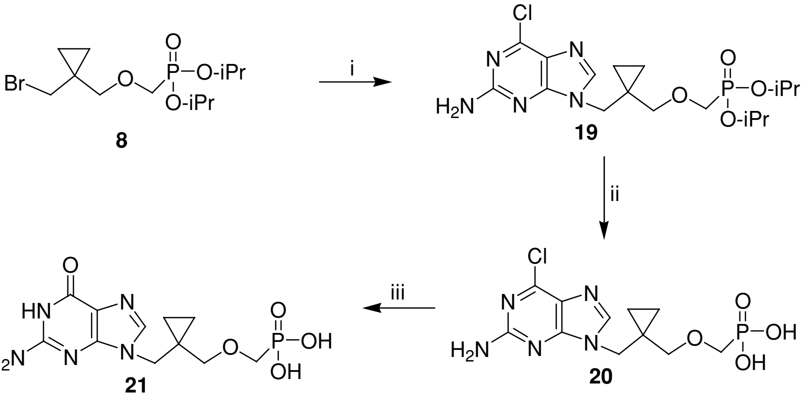


FIGURE 1 Design of the target homo-PMEA analogues.



Reagents: i) 2-amino-6-chloropurine, NaH, DMF, rt; ii) $(CH_3)_3SiBr$, CH_2Cl_2;
iii) 2 N HCl, reflux.

SCHEME 2 Synthesis of guanosine phosphonic acid derivative. Reagents: i) 2-amino-6-chloropurine, NaH, DMF, rt; ii) $(CH_3)_3SiBr$, CH_2Cl_2; iii) 2N HCl, reflux.

be conformationally similar to the natural nucleosides containing ribose. Hence, this arrangement would enhance the level of phosphorylation by kinase to produce the active diphosphate form. The findings of some antiviral activity in this series will allow this class of nucleoside to be a new template for the development of new antiviral agents.

In summary, a simple synthetic method for synthesizing novel acyclic homo-PMEA analogues from diethyl malonate was developed. When the synthesized compounds were tested against several viruses, the guanine derivative **21** exhibited significant anti-HIV-1 activity.

TABLE 1 The antiviral activity of the synthesized compounds

	HIV-1 EC_{50} (μM)	HSV-1 EC_{50} (μM)	HSV-2 EC_{50} (μM)	HCMV EC_{50} (μM)	cytotoxicity CC_{50} (μM)
14	81.2	77.3	>100	>100	99
15	28.3	>100	90.5	95.1	98
16	>100	>100	>100	61.2	>100
17	41.6	65.8	>100	>100	>100
18	27.8	>100	73.2	83.7	98
21	**8.5**	>100	89.0	16.7	99
AZT	0.007	ND	ND	ND	1.12
GCV	ND	ND	ND	0.6	>10
ACV	ND	0.1	ND	ND	>100

AZT: azidothymidine; GCV: ganciclovir; ACV: acyclovir.

ND: not determined.

EC_{50} (μM): concentration required to inhibit 50% of the virus induced cytopathicity.

CC_{50} (μM): concentration required to reduce the cell viability by 50%.

EXPERIMENTAL

The melting points were determined on a Mel-tem II laboratory device and were uncorrected. The NMR spectra were recorded on a JEOL JNM-LA 300 spectrometer (JEOL, Tokyo, Japan). The chemical shifts are reported in 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 UV spectra were obtained using a Beckman DU-7 spectrophotometer (Beckman, South Pasadena, CA, USA). Elemental analysis was carried out using an Elemental Analyzer System (Profile HV-3). The mass spectra were obtained on a Finnigan MAT SSQ 7000 spectrometer (Thermo Electron Corp., Bremen, Germany). The TLC was performed on Uniplates (silica gel) purchased from Analtech Co. All the reactions were carried out under N_2 unless otherwise specified. Dry dichloromethane, benzene and pyridine were obtained by distillation from CaH_2. The dry THF was obtained by distillation from Na and benzophenone immediately before use.

[1-(tert-Butyldimethylsilyloxymethyl)cyclopropyl]methanol (5): *t*-Butyldimethylsilyl chloride (743 mg, 4.93 mmol) was added to a stirred solution of compound **4** (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 room temperature 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 **5** (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 76.57, 66.10, 25.84, 24.70, 18.21, 13.74, −5.47.

[1-(tert-Butyldimethylsilyloxymethyl) cyclopropyl] methyl phosphonic acid diisopropyl ester (6): LiI (18 mg, 0.135 mmol) was added to a solution of compound **5** (384 mg, 1.775 mmol) in 5 mL of DMF at 25°C. LiO*t*-Bu (2.5 mL of 1.0 M solution in THF) and a solution of diisopropyl bromomethylphosphonate (625 mg, 2.4 mmol) in 3 mL of DMF were slowly added simultaneously to the reaction mixture over a 6 hours period at 65°C under anhydrous conditions. The mixture was quenched by adding water (10 mL), and the organic solvents (THF) were removed under reduced pressure. The aqueous layer was extracted three times with EtOAc and the combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane, 1:2) to give compound **6** (413 mg, 59%) as a syrup: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.72 (m, 2H), 3.73 (d, $J = 11.2$ Hz, 1H), 3.60 (d, $J = 11.4$ Hz, 1H), 3.51 (d, $J = 15.9$ Hz, 2H), 3.24 (d, $J = 9.3$ Hz, 2H), 1.82-1.60 (m, 6H), 1.35 (m 12H), 0.85 (s, 9H), 0.45 (dd, $J = 13.2$, 3.3 Hz, 4H), 0.15 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 72.16, 70.87, 69.54, 64.71, 25.85, 24.16, 23.92, 21.49, 18.28, 7.77, −5.42; Anal. Calcd for $C_{18}H_{39}O_5PSi$: C, 54.79; H, 9.96. Found: C, 54.63; H, 10.12; MS (EI) for $C_{18}H_{39}O_5PSi$: m/z 395 $(M)^+$.

[1-(Hydroxymethyl) cyclopropyl] methyl phosphonic acid diisopropyl ester (7): Tetrabutylammonium fluoride (2.85 mL, 1.0 M solution in THF) was added to a solution of compound **6** (749 mg, 1.9 mmol) in tetrahydrofuran (9 mL) at 0°C, and stirred for 4 hours at room temperature. The reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 4:1) to give compound **7** (592 mg, 79%) as a colorless syrup: ^1H NMR ($CDCl_3$, 300 MHz) δ 4.77 (m, 2H), 3.75 (d, J = 11.1 Hz, 2H), 3.59 (s, 2H), 3.52 (s, 2H), 1.35 (m 12H), 0.59-0.46 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 71.25, 67.72, 66.68, 64.48, 24.05, 13.55, 8.63; Anal. Calcd for $C_{12}H_{25}O_5P$: C, 51.42; H, 8.99. Found: C, 51.27; H, 9.19; MS (EI) for $C_{12}H_{25}O_5P$: m/z 280 $(M)^+$.

(1-Bromomethylcyclopropylmethoxymethyl)phosphonic acid diisopropyl ester (8): *N*-bromosuccinimide (2.465 g, 6.93 mmol) was added slowly to a solution of compound **7** (971 mg, 3.465 mmol) and triphenylphosphine (1.81 g, 6.93 mmol) in CH_2Cl_2 (20 mL) at 0°C, stirred overnight at room temperature (rt), and diluted with CH_2Cl_2 (30 mL). The organic layer was washed with water and brine, dried over anhydrous magnesium sulfate and filtered through a Celite pad. The filtrate was concentrated under vacuum and the residue was purified by flash silica gel column chromatography (EtOAc/n-hexane, 1:8) to give the allylic bromide **8** (939 mg, 79%) as a yellow oil: ^1H NMR ($CDCl_3$, 300 MHz) δ 4.83-4.69 (m, 2H), 3.78 (d, J = 8.4 Hz, 2H), 3.54 (s, 2H), 3.47 (s, 2H), 1.35 (dd, J = 6.3, 1.2 Hz, 12H), 0.78 (dd, J = 6.6, 4.8 Hz, 2H), 0.67 (dd, J = 6.6, 4.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 70.99, 66.84, 64.63, 40.95, 24.15, 24.00, 13.46; Anal. Calcd for $C_{12}H_{24}BrO_4P$: C, 42.00; H, 7.05. Found: C, 41.87; H, 6.92; MS (EI) for $C_{12}H_{24}BrO_4P$: m/z 343 $(M)^+$.

9-[1-(Diisopropoxyphosphorylmethoxymethyl)cyclopropylmethyl]adenine (9): A solution of the bromide **8** (458 mg, 1.335 mmol), adenine (272 mg, 2.01 mmol) and cesium carbonate (655 mg, 2.01 mmol) in anhydrous DMF (8 mL) was stirred overnight at rt. The mixture was quenched by the addition of water and diluted with ethyl acetate. The organic layer was separated and washed with brine, dried over anhydrous magnesium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography (EtOAc/n-hexane/MeOH, 4:1:0.1) to give compound **9** (302 mg, 57%) as a solid: ^1H NMR ($CDCl_3$, 300 MHz) δ 8.35 (s, 1H), 8.06 (s, 1H), 5.88 (br s, 2H), 4.84-4.71 (m, 2H), 4.22 (s, 2H), 3.71 (d, J = 8.4 Hz, 2H), 3.31 (s, 2H), 1.37 (dd, J = 6.2, 1.4 Hz, 12H), 0.90 (t, J = 6.0 Hz, 2H), 0.57 (t, J = 6.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 155.37, 152.76, 150.40, 141.59, 119.37, 71.21, 66.62, 64.39, 47.51, 24.10, 20.94, 10.01; Anal. Calcd for $C_{17}H_{28}N_5O_4P$: C, 51.38; H, 7.10; N, 17.62. Found: C, 51.25; H, 7.21; N, 17.75; MS (EI) for $C_{17}H_{28}N_5O_4P$: m/z 397 $(M)^+$.

Compounds **10**, **11**, **12**, **13** were synthesized from the compound **8** using the procedures described for synthesizing adenine derivative **9**.

1-[1-(Diisopropoxyphosphorylmethoxymethyl)cyclopropylmethyl] uracil (10): yield 34%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.33 (br s, 1H), 7.43 (d, J = 7.8 Hz, 1H), 5.66 (d, J = 7.8 Hz, 1H), 4.75 (m, 2H), 3.76 (s, 2H), 3.69 (d, J = 8.7 Hz, 2H), 3.35 (s, 2H), 1.35 (d, J = 6.4 Hz, 12H), 0.78 (t, J = 4.0 Hz, 2H), 0.53 (t, J = 3.9 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 163.74, 151.70, 143.30, 101.37, 71.19, 66.84, 64.13, 52.51, 24.04, 19.10, 9.73; Anal. Calcd for $C_{16}H_{27}N_2O_6P$: C, 51.33; H, 7.27; N, 7.48. Found: C, 51.22; H, 7.08; N, 7.30; MS (EI) for $C_{16}H_{27}N_2O_6P$: m/z 374 $(M)^+$.

1-[1-(Diisopropoxyphosphorylmethoxymethyl)cyclopropylmethyl] thymine (11): yield 30%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.35 (br s, 1H), 7.38 (s, 1H), 4.76 (m, 2H), 3.75 (s, 2H), 3.67 (d, J = 8.6 Hz, 2H), 3.35 (s, 2H), 1.78 (s, 3H), 1.36 (dd, J = 6.8, 1.6 Hz, 12H), 0.79 (t, J = 5.1 Hz, 2H), 0.53 (t, J = 5.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 164.12, 151.29, 141.84, 109.99, 71.19, 70.53, 60.41, 51.75, 24.09, 20.28, 12.97, 9.60; Anal. Calcd for $C_{17}H_{29}N_2O_6P$: C, 52.57; H, 7.53; N, 7.21. Found: C, 52.75; H, 7.38; N, 7.04; MS (EI) for $C_{17}H_{29}N_2O_6P$: m/z 389 $(M+1)^+$.

1-[1-(Diisopropoxyphosphorylmethoxymethyl)cyclopropylmethyl] cytosine (12): yield 39%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (d, J = 7.2 Hz, 1H), 5.84 (d, J = 7.2 Hz, 1H), 4.96 (m, 2H), 3.82 (s, 2H), 3.73 (d, J = 8.7 Hz, 2H), 3.64 (s, 2H), 1.36 (dd, J = 6.8, 1.6 Hz, 12H), 0.83 (t, J = 5.0 Hz, 2H), 0.48 (t, J = 5.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 164.93, 156.30, 141.14, 94.32, 69.56, 68.31, 58.95, 47.31, 24.09, 19.75, 9.87; Anal. Calc for $C_{16}H_{28}N_3O_5P$: C, 51.47; H, 7.56; N, 11.25. Found: C, 51.35; H, 7.41; N, 7.72; MS (EI) for $C_{16}H_{28}N_3O_5P$: m/z 373 $(M)^+$.

1-[1-(Diisopropoxyphosphorylmethoxymethyl)cyclopropylmethyl] 5-fluorouracil (13): yield 29%; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.55 (br s, 1H), 7.89 (d, J = 6.8 Hz, 1H), 4.88 (m, 2H), 3.79 (s, 2H), 3.69 (d, J = 8.8 Hz, 2H), 3.57 (s, 2H), 1.34 (d, J = 6.6 Hz, 12H), 0.80 (t, J = 5.2 Hz, 2H), 0.46 (t, J = 5.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 157.65, 157.31, 150.52, 140.78, 137.74, 131.32, 130.88, 70.78, 70.35, 61.21, 49.57, 24.45, 20.51, 9.62; Anal. Calcd for $C_{16}H_{26}FN_2O_6P$: C, 48.98; H, 6.68; N, 7.14. Found: C, 49.15; H, 6.57; N, 7.03; MS (EI) for $C_{16}H_{26}FN_2O_6P$: m/z 392 $(M)^+$.

9-[1-(Hydromethyl)cyclopropylmethylphosphonic acid]adenine (14): $(CH_3)_3SiBr$ (0.472 g, 3.12 mmol) was added to a solution of the phosphonate **9** (113 mg, 0.286 mmol) in 10 mL of anhydrous methylene chloride. The mixture was heated overnight under reflux and concentrated under reduced pressure. The residue was dissolved in distilled water and washed out with CH_2Cl_2 twice. The aqueous layer was lyophilized by freeze dryer to give compound **14** (62 mg, 70%): UV (H_2O) λ_{max} 262.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.42 (s, 1H), 8.12 (s, 1H), 4.12 (s, 2H), 3.68 (d, J = 8.2 Hz, 2H), 3.42 (s, 2H), 0.91 (dd, J = 6.4, 1.2 Hz, 2H), 0.50 (dd, J = 6.6, 1.2 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 154.89, 152.21, 150.02, 142.11, 118.21, 67.56, 65.43, 47.72, 19.54, 9.21; Anal. Calcd for $C_{11}H_{16}N_5O_4P\cdot1.0H_2O$: C, 39.88; H, 5.48; N, 21.14. Found: C, 39.76; H, 5.41; N, 21.30; MS (EI) for $C_{11}H_{16}N_5O_4P$: m/z 313 (M)$^+$.

Compounds **15**, **16**, **17**, **18** were synthesized from the corresponding starting material using the procedure described for the adenine phsophonic acid derivative **14**.

1-[1-(Hydroxymethyl)cyclopropylmethylphosphonic acid] uracil (15): yield 75%; UV (H_2O) λ_{max} 263.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.37 (d, J = 7.6 Hz, 1H), 5.52 (d, J = 7.6 Hz, 1H), 3.78 (s, 2H), 3.67 (d, J = 8.6 Hz, 2H), 3.36 (s, 2H), 0.81 (d, J = 5.0 Hz, 2H), 0.51 (d, J = 5.0 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 164.21, 151.43, 142.78, 102.10, 66.91, 65.28, 50.25, 20.31, 10.22; Anal. Calcd for $C_{10}H_{15}N_2O_6P\cdot1.0H_2O$: C, 38.97; H, 5.56; N, 9.09. Found: C, 39.12; H, 5.63; N, 9.02; MS (EI) for $C_{10}H_{15}N_2O_6P$: m/z 291 (M+1)$^+$.

1-[1-(Hydroxymethyl)cyclopropylmethylphosphonic acid] thymine (16): yield 69%; m.p. 102–105°C; UV (H_2O) λ_{max} 267.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.40 (s, 1H), 3.78 (s, 2H), 3.69 (d, J = 8.8 Hz, 2H), 3.39 (s, 2H), 1.81 (s, 3H), 0.79 (d, J = 6.1 Hz, 2H), 0.57 (t, J = 6.1 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 164.78, 151.56, 142.38, 110.02, 69.53, 62.67, 52.28, 20.09, 12.96, 9.46; Anal. Calcd for $C_{11}H_{17}N_2O_6P\cdot1.0H_2O$: C, 40.99; H, 5.94; N, 8.69. Found: C, 40.82; H, 6.01; N, 8.56; MS (EI) for $C_{11}H_{17}N_2O_6P$: m/z 304 (M+1)$^+$.

1-[1-(Hydroxymethyl)cyclopropylmethylphosphonic acid] cytosine (17): yield 65%; as a form; UV (H_2O) λ_{max} 271.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.29 (d, J = 7.4 Hz, 1H), 5.78 (d, J = 7.2 Hz, 1H), 3.80 (s, 2H), 3.71 (d, J = 8.2 Hz, 2H), 3.57 (s, 2H), 0.88 (dd, J = 6.0, 1.2 Hz, 2H), 0.49 (d, J = 6.0 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 164.72, 155.27, 142.42, 95.61, 69.31, 59.56, 49.63, 19.70, 9.80; Anal. Calcd for $C_{10}H_{16}N_3O_5P\cdot1.0H_2O$: C, 39.09; H, 5.90; N, 13.67. Found: C, 39.15; H, 5.97; N, 13.54; MS (EI) for $C_{10}H_{16}N_3O_5P$: m/z 288 (M)$^+$.

1-[1-(Hydroxymethyl)cyclopropylmethylphophonic acid] 5-fluorouracil (18): yield 77%; m.p. 99–101°C; UV (H_2O) λ_{max} 270.0 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.82 (d, J = 6.8 Hz, 1H), 3.81 (s, 2H), 3.70 (d, J = 8.6 Hz, 2H), 3.56 (s, 2H), 0.82 (t, J = 5.0 Hz, 2H), 0.49 (t, J = 5.0 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 158.42, 158.06, 150.99, 141.21, 138.34, 131.73, 131.30, 69.55, 63.71, 50.45, 20.81, 9.85; Anal. Calcd for $C_{10}H_{14}FN_2O_6P\cdot1.0H_2O$: C, 36.82; H, 4.94; N, 8.59. Found: C, 36.92; H, 5.07; N, 8.41; MS (EI) for $C_{10}H_{14}FN_2O_6P$: m/z 308 (M+1)$^+$.

9-[1-(Diisopropoxyphosphorylmethoxymethyl)cyclopropylmethyl] 2-amino-6-chloropurine (19): A solution of the 2-amino-6-chloropurine (248 mg, 1.464 mmol) and sodium hydride (41 mg, 1.7 mmol) in anhydrous DMF (10 mL) was stirred for 1 hour at rt. A solution of the bromide **8** (251 mg, 0.732 mmol) in DMF (5 mL) was then added to the mixture and stirred 4 hours at 80°C. The mixture was quenched by the addition of a saturated ammonium chloride solution (5 mL) and concentrated under reduced pressure. The residue was dissolved in water and extracted three times with CH_2Cl_2. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel column chromatography (EtOAc/Hexane, 4:1) to give compound **19** (123 mg, 39%) as a solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.93 (s, 1H), 5.35 (br s, 2H), 4.85–4.70 (m, 2H), 3.92 (s, 2H), 3.73 (dd, $J = 8.7$, 1.5 Hz, 2H), 3.25 (s, 2H), 1.35 (dd, $J = 6.4$, 1.6 Hz, 12H), 0.82 (s, 2H), 0.58 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 159.19, 154.22, 151.07, 143.15, 124.99, 71.21, 66.76, 64.52, 47.49, 24.04, 20.98, 9.94; Anal. Calcd for $C_{17}H_{27}ClN_5O_4P$: C, 47.28; H, 6.30; N, 16.22. Found: C, 47.12; H, 6.21; N, 16.36; MS (EI) for $C_{17}H_{27}ClN_5O_4P$: m/z 432 $(M)^+$.

9-[1-(Hydroxymethyl)cyclopropylmethylphophonic acid] 2-amino-6-chloropurine (20): The removal of the diisopropylidene groups of compound **19** was performed using a similar procedure described for removing these groups from compound **9**: yield 85%; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.73 (s, 1H), 3.82 (s, 2H), 3.70 (d, $J = 8.5$ Hz, 2H), 3.33 (s, 2H), 0.90 (s, 2H), 0.47 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 159.54, 154.79, 151.41, 143.48, 125.06, 68.43, 64.62, 47.72, 20.44, 9.93; Anal. Calcd for $C_{11}H_{15}ClN_5O_4P \cdot 1.5H_2O$: C, 35.26; H, 4.84; N, 18.69. Found: C, 35.14; H, 5.76; N, 18.73; MS (EI) for $C_{11}H_{15}ClN_5O_4P$: m/z 349 $(M+1)^+$.

9-[1-(Hydroxymethyl)cyclopropylmethylphophonic acid] guanine (21): Compound **20** (121 mg, 0.348 mmol) was dissolved in 2 N HCl (7 mL) and heated under reflux for 7 hours. The mixture was washed out by CH_2Cl_2 twice. The aqueous layer was dried by freeze dryer to give compound **14** (86 mg, 75%): UV (H_2O) λ_{max} 253.5 nm; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.09 (s, 1H), 3.88 (s, 2H), 3.74 (d, $J = 8.4$ Hz, 2H), 3.42 (s, 2H), 0.89 (s, 2H), 0.51 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 157.88, 154.43, 152.43, 137.31, 118.54, 68.71, 64.82, 46.82, 20.28, 10.21; Anal. Calcd for $C_{11}H_{16}N_5O_5P \cdot 1.0H_2O$: C, 38.04; H, 5.22; N, 20.17. Found: C, 37.92; H, 5.31; N, 20.21; MS (EI) for $C_{11}H_{16}N_5O_5P$: m/z 330 $(M+1)^+$.

REFERENCES

1. a) Chu, C.K.; Cutler, S.J. Chemistry and antiviral activities of acyclonucleosides. *J. Heterocycl. Chem.* **1986**, 23, 289–319; b) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P.C. A novel selective broad-spectrum anti-DNA virus agent. *Nature* **1986**, 323, 464–467.
2. a) Prisbe, E.J.; Martin, J.C.; McGee, D.P.; Barker, M.F.; Smee, D.F.; Duke, A.E.; Mattews, T.R.; Verheyden, J.P. Synthesis and antiherpes virus activity of phosphate and phosphonate derivatives of 9-[(1,3-dihydroxy-2-propoxy)methyl] guanine. *J. Med. Chem.* **1986**, 29, 671–675; b) De Clercq, E.; Holy, A. Efficacy of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine in various models of herpes simplex virus infection in mice. *Antimicrob. Agents Chemother.* **1991**, 37, 701–706; c) De Clercq, E. Therapeutic potential of phosphonylmethoxyalkylpurines and-pyrimidines as antiviral agents. *Drugs Exp. Clin. Res.* **1990**, 16, 319–326; d) De Clercq, E. Broad-spectrum anti-DNA virus and anti-retrovirus

activity of phosphonylmethoxyalkylpurines and –pyrimidines. *Biochem. Pharmacol.* **1991**, 42, 963–972.

3. Balzarini, J.; Aquaro, S.; Perno, C.F.; Witvrouw, M.; Holy, A.; De Clercq, E. Activity of the (*R*)-enantiomers of 9-(2-phosphonylmethoxypropyl)-adenine and 9-(2-phosphonylmethoxy propyl)-2,6-diaminopurine against HIV in different human cell systems. *Biochem. Biophys. Res. Commun.* **1996**, 219, 337–341.
4. Choi, J.R.; Cho, D.G.; Roh, K.Y.; Hwang, J.T.; Ahn, S.; Jang, H.S.; Cho, W.Y.; Kim, K.W.; Cho, Y.G.; Kim, J.; Kim, Y.Z. A novel class of phosphonate nucleosides. 9-[1-Phosphonomethoxycyclopropyl]methyl]guanine as a potent and selective anti-HBV agent. *J. Med. Chem.* **2004**, 47, 2864–2869.
5. Robbins, B.L.; Srinivas, R.V.; 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**, 42, 612–617.
6. Chatis, P.A.; Crumpacker, C.S. Resistance of herpesviruses to antiviral drugs. *Antimicrob. Agents Chemother.* **1992**, 36, 1589–1595.
7. a) House, H.O.; Lord, R.C.; Rao, H.S.J. The synthesis of spiropentane-d_8. *J. Org. Chem.* **1956**, 21, 1487–1491; b) Gosselck, J.; Schmidt, G. A new route to 1,1-disubstituted cyclopropane. *Angew. Chem. Int. Ed.* **1968**, 7, 456–457.
8. Borcherding, D.R.; Narayanan, S.; Hasobe, M.; McKee, J.G.; Keller, B.T.; Borchardt, R.T. Potential inhibitors of S-adenosylmethionine-dependent methyltransferases. 11. Molecular dissections of neplanocin A as potential inhibitors of S-adenosylhomocysteine hydrolase. *J. Med. Chem.* **1988**, 31, 1729–1738.
9. Bronson, J.J.; Ghazzouli, I.; Hichcock, M.J.M.; Webb, R.R.; Martin, J.C. Synthesis and antiviral activity of the nucleotide analogue (S)-1-[3-hydroxy-2-(phosphonyl-methoxy)propyl]cytosine. *J. Med. Chem.* **1989**, 32, 1457–1463.
10. Cook, A.F.; Holman, M.J.; Kramer, M.J. Fluorinated pyrimidine nucleosides. 4. Synthesis and antitumor testing of a series of 2′,5′-dideoxy- and 2′,3′,5′-trideoxynucleosides of 5-fluorouracil. *J. Med. Chem.* **1980**, 23, 852–857.
11. El Subbagh, H.I.; Racha, S.; Abushanab, E.; Panzica, R.P. Synthesis of phosphonate isosteres of 2′-deoxy-1′,2′-seco-nucleosides. *J. Org. Chem.* **1996**, 61, 890–894.
12. Jeong, L.S.; Kim, H.O.; Moon, H.R.; Hong, J.H.; Yoo, S.J.; Choi, W.J.; Chun, M.W.; Lee, C.K. Synthesis and structure-activity relationships of novel apio- and thio-apiodideoxydidehydro nucleosides as anti-HCMV agents. *J. Med. Chem.* **2001**, 44, 806–813.