# A Novel Class of Phosphonate Nucleosides. 9-[(1-Phosphonomethoxycyclopropyl)methyl]guanine as a Potent and Selective Anti-HBV Agent

Jong-Ryoo Choi,* Dong-Gyu Cho, Kee Y. Roh, Jae-Taeg Hwang, Sinbyoung Ahn, Hyun S. Jang, Woo-Young Cho, Kyong W. Kim, Young-Gyo Cho, Jeongmin Kim, and Yong-Zu Kim

*LG Life Sciences Ltd., R & D Park, 104-1 Moongi-dong, Yusung-gu, Daejeon 305-380, Korea*

*Received October 16, 2003*

9-[1-(Phosphonomethoxycyclopropyl)methyl]guanine (PMCG, **1**), representative of a novel class of phosphonate nucleosides, blocks HBV replication with excellent potency ($EC_{50}$ = 0.5 $\mu$M) in a primary culture of HepG2 2.2.15 cells. It exhibits no significant cytotoxicity in several human cell lines up to 1.0 mM. It does not inhibit replication of human immunodeficiency virus (HIV-1) or herpes simplex virus (HSV-1) at 30 $\mu$M. Many purine base analogues of **1** also exhibit inhibitory activity against HBV, but at 30 $\mu$M, pyrimidine analogues do not. **1** is 4 times more potent than 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA), which was used as a positive control ($EC_{50}$ = 2.0 $\mu$M). The characteristic cyclopropyl moiety at the 2′-position of **1** was prepared by titanium-mediated Kulinkovich cyclopropanation. **1** was modified to give the orally available drug candidate, PMCDG Dipivoxil (**2**). Compound **2** exhibited excellent efficacy when administered at 5 mg per kg per day in a study with woodchucks infected with woodchuck hepatitis B virus (WHBV). Drug candidate **2** has successfully completed phase I clinical trials and is currently undergoing phase II clinical studies for evaluation of efficacy.

## Introduction

Despite extensive vaccinations, the World Health Organization (WHO) estimates that over 350 million people are chronically infected by hepatitis B virus (HBV) throughout the world.[1] Although interferon-α and lamivudine (3-TC) have been used for treatment of HBV infection, they have a low cure rate and drug resistance develops, respectively.[2,3a,b] Several other nucleoside analogues including phosphonate nucleosides have been undergoing clinical studies.[4a,b] For HBV therapy, the FDA recently approved Adefovir Dipivoxil, which is a prodrug of the phosphonate nucleoside, 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA).[5a,b] PMEA shows a broad spectrum of antiviral activity and is effective against HBV, human immunodeficiency virus (HIV), and also the herpes simplex virus (HSV).[6a,b] PMEA has been reported to show kidney toxicity with longer treatment at higher doses during clinical studies for HIV therapy.[7] Unlike nucleoside agents, a phosphonate nucleoside has the advantage of skipping the requisite first phosphorylation step to reach its active metabolic form.[4a] Many PMEA analogues, particularly 9-[2-(phosphonomethoxy)ethyl]guanine (PMEG) and 9-[2-(phosphonomethoxy)ethyl]-2,6-diaminopurine (PME-DAP), have a broad spectrum as antiviral agents, and some of them also exhibit severe cytotoxicity.[8a,b]

In this article, we describe a novel phosphonate nucleoside, 9-[1-(phosphonomethoxycyclopropyl)methyl]-guanine (PMCG, **1**), which exhibits a highly potent and selective anti-HBV activity ($EC_{50}$ = 0.5 $\mu$M). Its orally available drug candidate, 9-[1-(phosphonomethoxycyclopropyl)methyl]-6-deoxyguanine Dipivoxil (PMCDG Dipivoxil, **2**) (Figure 1) is also described.

* To whom correspondence should be addressed. Phone: 82-42-866-2252. Fax: 82-42-866-5754. E-mail: jrchoi@lgls.co.kr.

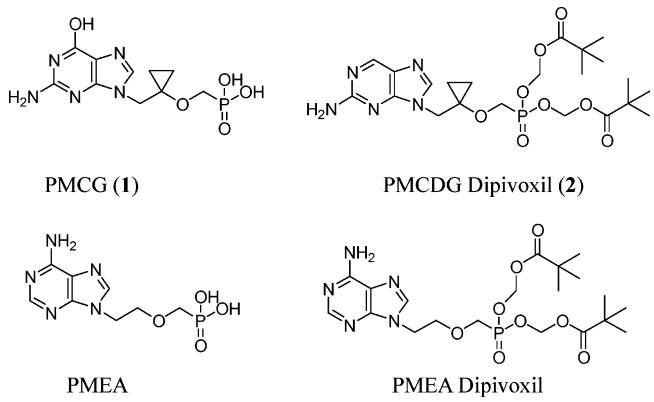


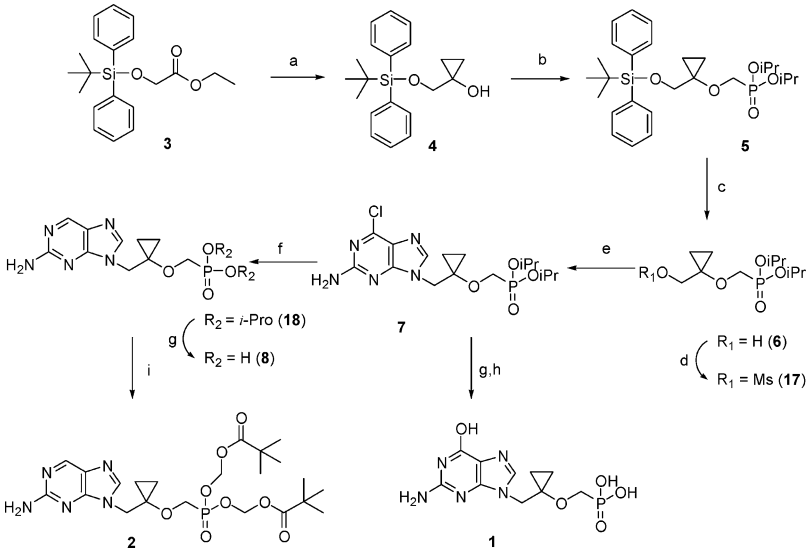

**Figure 1.** Structures of PMCG (**1**), PMCDG Dipivoxil (**2**), and PMEA.

## Chemistry

PMCG (**1**) was synthesized in seven steps from *O*-silylprotected ethyl glycolate in 17−20% overall yield as shown in Scheme 1. The key intermediate cyclopropanol **4** was prepared by titanium-mediated Kulinkovich cyclopropanation of ester **3** in over 80% yield as white solids.[9a,b] Etherification of **4** with diisopropyl bromomethylphosphonate in the presence of LiO*t*-Bu in THF and DMF at 50−60 °C gave the phosphonate **5** in over 70% yield. This etherification was highly dependent on base and reaction temperature. Desilylation of the phosphonate **5** with ammonium fluoride, followed by mesylation and subsequent coupling reaction with 6-chloroguanine, gave 9-substituted isomer **7** in 27% overall yield; 7% of the corresponding 7-isomer **9** and 1−2% of cyclobutane derivative **10** were also obtained. Compound **10** may form through a rearrangement in which **17** solvolyzes to generate cyclopropylmethyl

10.1021/jm0305265 CCC: $27.50 
Published on Web 04/16/2004

**Scheme 1**[a] Synthesis of PMCG (**1**), PMCDG (**8**), and PMCDG Dipivoxil (**2**)




[a] Reagents and conditions: (a) $CH_3CH_2MgBr$, Ti(O*i*-Pr)$_4$ (0.25 equiv), THF, 0 °C to 25 °C, 10 h; (b) $BrCH_2P(O)(O$*i*-Pr$)_2$, LiO*t*-Bu, LiI (cat.), DMF, THF, 60 °C, 4 h; (c) $NH_4F$, MeOH, reflux, 10 h; (d) MsCl, TEA, MDC, 0 °C to 25 °C; (e) 6-chloroguanine, NaH, DMF, 80 °C, 4 h; (f) $H_2$, 5% Pd on C, THF, 1 atm, 18 h; (g) TMSBr, MDC, reflux, 18 h; (h) 2 N HCl, reflux, 6 h; (i) chloromethyl pivalate, TEA, 1-methyl-2-pyrrolidinone, 25 °C, 48 h.

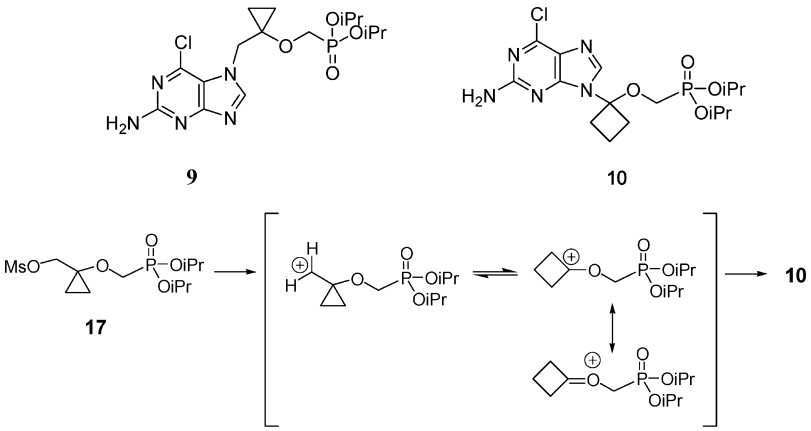



**Figure 2.** Structures of **9** and **10** and formation of **10**.

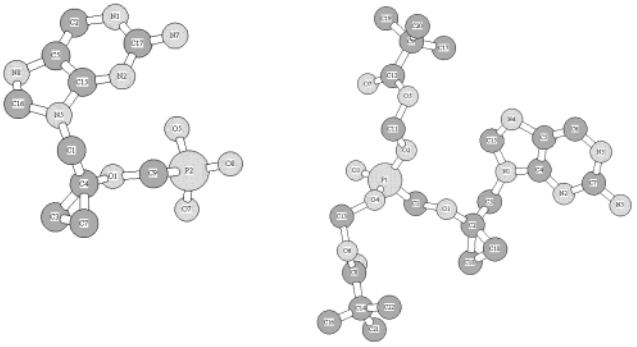
cation. Subsequent rearrangement affords the cyclobutane cation, which is stabilized by resonance, and the cyclobutane cation is captured by 6-chloroguanine anion to produce compound **10** (Figure 2).[10a,b]

Compound **7** was hydrolyzed with trimethylsilyl bromide, followed by treatment with 2 N HCl, to afford **1** in quantitative yield.[11] 9-[1-(Phosphonomethoxycyclopropyl)methyl]-6-deoxyguanine (PMCDG, **8**) was obtained from **7** in 94% overall yield by hydrogenation in the presence of Pd and subsequent hydrolysis with trimethylsilyl bromide. **8** was then converted to an orally available drug candidate PMCDG Dipivoxil (**2**) by etherification with chloromethyl pivalate in the presence of triethylamine in 1-methyl-2-pyrrolidinone in 39% yield.[12a,b] Other derivatives including thymine derivative **11** were also synthesized by the same procedure as outlined in Scheme 1.

## Result and Discussion

Evaluation of anti-HBV activity of these synthetic compounds was conducted with HepG2 2.2.15 cells transfected with the HBV genome.[13] As shown in Table 1, **1** and **16** are the most potent ($EC_{50}$ = 0.5 $\mu$M). The other modified guanine base analogues also exhibit moderate to high anti-HBV activity ($EC_{50}$ = 1.5−8.0 $\mu$M), while the thymine base analogue **11** and 6-ethylaminoguanine analogue **14** do not show anti-HBV activity at 30 $\mu$M. Among the modified guanine base analogues, the anti-HBV activity of **8**, **15**, and **16** are comparable to that of **1**. Compounds **8**, **15**, and **16** would most likely be converted to **1** by oxidation or hydrolysis inside the cell.[14a,b] All novel phosphonate nucleoside analogues show an excellent cellular toxicity profile with $CC_{50}$ > 1.0 mM and do not inhibit replication of HIV-1 and HSV-1 at 30 $\mu$M.[15,16]

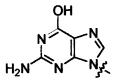
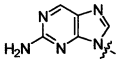
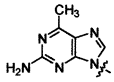
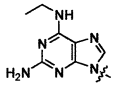
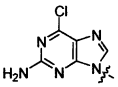
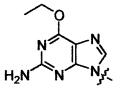
The potent anti-HBV activity and therapeutic selectivity of these novel phosphonate nucleosides may be attributable to their conformation. The presence of the cyclopropyl moiety would play an important role in controlling the global conformation of the phosphonate nucleoside. Compared to PMEA or the monophosphate of acyclic nucleoside agents, **8** containing a cyclopropyl moiety at the 2′-position strongly favors the cis arrangement between the guanine and the phosphorus atom as shown in the X-ray structure (Figure 3).



PMCDG (8) PMCDG Dipivoxil (2)

**Figure 3.** Molecular structures of PMCDG (**8**) and PMCDG Dipivoxil (**2**) from X-ray diffraction study (hydrogen atoms are omitted for clarity).

**Table 1.** Anti-HBV Activity and Cytotoxicity of PMCG Analogues

| Compound | Q | $EC_{50}$/ $EC_{80}$ | $CC_{50}$ |
|---|---|---|---|
| **1** |  | 0.5 / 2.3 | >1000 |
| **8** |  | 4.0 / - | >1000 |
| **11** |  | > 30 /- | >1000 |
| **12** |  | 8.0 / - | >1000 |
| **13** |  | 8.0 / - | >1000 |
| **14** |  | >30 / - | >1000 |
| **15** |  | 1.5 / 7.5 | >1000 |
| **16** |  | 0.5 / 3.0 | >1000 |
| | **PMEA** | 2.0 / 44.0 | |

Although an X-ray crystallographic analysis of **1** is not available, its structure is expected to resemble the global conformation of **8**. The cis arrangement between the guanine and the phosphorus atom may be conformationally similar to the 1′-base and 5′-OH arrangement of natural nucleosides having a ribose ring. The natural nucleosides have their 1′-base and 5′-OH on the same side of the ribose ring plane. This cis arrangement would enhance phosphorylation of PMCG by human kinase to produce the active form PMCG-diphosphate, which then may be properly incorporated into DNA during viral replication, resulting in termination of the polymerization.[17]

Although **8** is less potent than **1** in vitro, it exhibits better pharmacokinetic profiles and much better efficacy in vivo.[18] It may be that **8** easily permeates the cell membrane and is intracellularly metabolized to yield **1** in the liver. **8** has poor oral bioavailability (>5% in rats) as expected due to the highly polar nature of the phosphonic acid. Masking the phosphonic acid functionality with Dipivoxil increased its oral bioavailbility to 25% in rats and 60% in dogs.

In conclusion, the introduction of a cyclopropyl moiety at the 2′-position to restrict conformational mobility of an acyclic phosphonate nucleoside was shown to result in highly potent, specific, and selective anti-HBV activity. The orally available drug candidate PMCDG Dipivoxil (**2**) is a double prodrug of PMCG (**1**), which drastically reduced DNA titers of woodchuck hepatitis B virus (WHBV) in an in vivo study with woodchucks at 5 mg/kg per day.[19] The drug candidate **2** has successfully completed phase I and is currently undergoing phase II clinical study for evaluation of efficacy in human.

## Experimental Section

Tetrahydrofuran (THF) and methylene dichloride (MDC) were dried by distillation from sodium/benzophenone and caclium hydride, respectively. Other commercially available chemicals and solvents were used without further purification. Merck silica gel 60 was used for column chromatography. NMR spectra were recorded on a Bruker ARX-300, Bruker DRX-400, or JEOL GSX-500. Preparative HPLC for purifications was performed with Waters Delta Prep-400 using $\mu$-Bondapak $C_{18}$ column. HRMS data were obtained with a Q-Tof2 mass spectrometer (Micromass, Manchester, UK). Elemental analysis for carbon, hydrogen, and nitrogen was determined on a CE EA-1110 Elemental Analyzer (Italy).

**1-({[*tert*-Butyl(diphenyl)silyl]oxy}methyl)cyclopropanol (4).** To a solution of ethyl 2-{[*tert*-butyl(diphenyl)silyl]-oxy}acetate (**3**) (150 g, 0.46 mole) in 3 L of THF was added $Ti(O\textit{i}\text{-}Pr)_4$ (30 mL, 0.10 mole) at −10 °C. A solution of ethylmagnesium bromide (365 mL of 3.0 M in THF, 1.09 mole) was added to the above mixture dropwise for 4 h below 5 °C under nitrogen. The mixture was allowed to warm to 25 °C and stirred for 1 h more. The reaction mixture was quenched by adding water (400 mL) dropwise for 1 h below 10 °C. After removal of THF, the aqueous layer was extracted with *n*-hexane (3 × 500 mL), and the combined extracts were washed with brine (300 mL) and water (300 mL). The organic layer was concentrated in vacuo, and the residue was diluted with *n*-heptane (150 mL). The solution was slowly stirred to generate white solids at −5 °C, and the solids were collected to afford 84 g of cyclopropanol **4**. The second crop (30 g) of **4** was obtained from the mother liquor. Total 114 g of the cyclopropanol **4** (80% yield) was obtained. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.43 (dd, $J$ = 6.8, 5.6 Hz, 2H), 0.77 (dd, $J$ = 6.8, 5.6 Hz, 2H), 1.01 (s, 9H), 2.69 (s, 1H), 3.68 (s, 2H), 7.43 (m, 6H), 7.69 (m, 4H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 11.8 (2C), 19.8, 27.3 (3C), 56.8, 69.7, 128.2 (4C), 130.2 (2C), 133.9 (2C), 136 (4C). HRMS $(M + Na)^+$: 349.1600 calcd for $C_{20}H_{26}O_2Si$, found 349.1583. Anal. ($C_{20}H_{26}O_2Si$) C, H.

**Diisopropyl {[1-({[*tert*-Butyl(diphenyl)silyl]oxy}methyl)cyclopropyl]oxy}methylphosphonate (5).** To a solution of the cyclopropanol **4** (6.5 g, 20 mmol) in 10 mL of DMF was added LiI (200 mg, 1.49 mmol) at 25 °C. Li*Ot*-Bu (32.0 mL of 1.0 M solution in THF, 32.0 mmol) and a solution of diisopropyl bromomethylphosphonate (7.0 g, 27 mmol) in 10 mL of DMF were slowly and simultaneously added to the above mixture for 1−2 h at 50−60 °C under nitrogen. The reaction mixture was quenched by adding water (100 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 (100 mL), dried over $MgSO_4$, and

concentrated in vacuo. The residue was purified by a silica gel column chromatography using EtOAc/*n*-hexane (1/1) to afford 7.1 g of the phosphonate **5** (70% yield) as a pale yellow liquid. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.54 (dd, $J$ = 7.1, 4.2 Hz, 2H), 0.88 (dd, $J$ = 7.1, 4.3 Hz, 2H), 1.05 (s, 9H), 1.29 (d, $J$ = 6.6 Hz, 6H), 1.33 (d, $J$ = 6.6 Hz, 6H), 3.79 (s, 2H), 3.97 (d, $J$ = 9.3 Hz, 2H), 4.71 (m, 2H), 7.43 (m, 6H), 7.67 (m, 4H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 10.8 (2C), 19.1, 23.8 (d, $J$ = 4.6 Hz, 2C), 24.0 (d, $J$ = 3.8 Hz, 2C), 26.7 (3C), 63.6 (d, $J$ = 168 Hz), 64.4 (d, $J$ = 15.0 Hz), 67.1, 70.6 (d, $J$ = 7.5 Hz, 2C), 127.6 (4C), 129.6 (2C), 133.1 (2C), 135.5 (4C).

**Diisopropyl {[1-(Hydroxymethyl)cyclopropyl]oxy}-methylphosphonate (6).** To a solution of the phosphonate **5** (8.3 g, 16.5 mmol) in 100 mL of MeOH was added ammonium fluoride (3.1 g, 83.4 mmol). The mixture was heated under reflux for 4 h. After removal of MeOH, the residue was directly purified by a silica gel column chromatography using MeOH/MDC (1/20) to afford 3.6 g of the phosphonate **6** (82% yield) as a colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.62 (t, $J$ = 5.6 Hz, 2H), 0.89 (d, $J$ = 5.6 Hz, 2H), 1.35 (d, $J$ = 6.4 Hz, 12H), 3.66 (s, 2H), 3.86 (d, $J$ = 8.0 Hz, 2H), 4.78 (m, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 11.2 (2C), 23.8 (d, $J$ = 4.5 Hz, 2C), 23.9 (d, $J$ = 3.8 Hz, 2C), 62.3 (d, $J$ = 172 Hz), 65.9, 71.3 (d, $J$ = 6.8, 2C). HRMS ($M^+$): 267.1361 calcd for $C_{11}H_{23}O_5P$, found 267.1368. Anal. ($C_{11}H_{23}O_5P$) C, H.

**{1-[(Diisopropylphosphoryl)methoxy]cyclopropyl}-methyl Methanesulfonate (17).** To a solution of the phosphonate **6** (1.5 g, 5.6 mmol) in 50 mL of MDC were added methansulfonyl chloride (0.84 g, 7.3 mmol) and triethylamine (0.85 g, 8.4 mmol) at 0 °C. The mixture was stirred for 30 min at 25 °C and then quenched with saturated $NH_4Cl$. The organic layer was washed with brine (20 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by a flash silica gel column chromatography using EtOAc/n-hexane (1/1) to afford 1.64 g of the methansulfonate **17** (85% yield). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.77 (t, $J$ = 6.0 Hz, 2H), 1.10 (d, $J$ = 6.0 Hz, 2H), 1.31 (t, $J$ = 5.0 Hz, 12H), 3.10 (s, 3H), 3.86 (d, $J$ = 10.0 Hz, 2H), 4.33 (s, 2H), 4.70 (m, 2H). MS (ESI): 421 $(MH)^+$.

**Diisopropyl ({1-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]cyclopropyl}oxy)methylphosphonate (7).** To a suspension of 2-amino-6-chloro-9*H*-purine (773 mg, 4.6 mmol) in 10 mL of DMF was added NaH (219 mg, 5.47 mmol) at 25 °C and stirred for 30 min. A solution of the methansulfonate **17** (1.63 g, 4.7 mmol) in 10 mL of DMF at 25 °C was added to the above mixture and stirred at 80 °C for 4 h. The reaction mixture was quenched with saturated $NH_4Cl$ at 25 °C. After removal of DMF in vacuo, the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by a flash silica gel column chromatography using MeOH/MDC (1/20) to afford 765 mg of **7** (39% yield), 130 mg of **9** (7.0%), and 20 mg of **10** (1.0%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.86 (t, $J$ = 6.8 Hz, 2H), 1.03 (t, $J$ = 6.8 Hz, 2H), 1.26 (d, $J$ = 6.0 Hz, 6H), 1.31 (d, $J$ = 6.0 Hz, 6H), 3.84 (d, $J$ = 8.0 Hz, 2H), 4.23 (s, 2H), 4.71 (m, 2H), 5.20 (s, 2H), 8.17 (s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$11.2 (2C), 23.8 (d, $J$ = 4.5 Hz, 2C), 23.9 (d, $J$ = 3.8 Hz, 2C), 62.3 (d, $J$ = 172 Hz), 65.9, 71.3 (d, $J$ = 6.8 Hz, 2C). HRMS ($M^+$): 418.1411 calcd for $C_{11}H_{23}O_5P$, found 418.1412. Anal.($C_{16}H_{25}N_5O_4P$) C, H, N.

**Diisopropyl ({1-[(2-Amino-6-chloro-7*H*-purin-7-yl)methyl]cyclopropyl}oxy)methylphosphonate (9).** $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.85 (br t, 2H), 1.11 (br t, 2H), 1.29 (d, $J$ = 8.0 Hz, 6H), 1.32 (d, $J$ = 8.0 Hz, 6H), 3.76 (d, $J$ = 12.0 Hz, 2H), 4.55 (s, 2H), 4.73 (m, 2H), 5.01 (s, 2H), 8.50 (s, 1H). MS *m/e* ($M^+$): 418.

**Diisopropyl {[1-(2-Amino-6-chloro-9*H*-purin-9-yl)cyclobutyl]oxy}methylphosphonate (10).** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.27 (d, $J$ = 5.7 Hz, 6H), 1.30 (d, $J$ = 5.7 Hz, 6H), 1.95 (m, 1H), 2.10 (m, 1H), 2.78 (m, 2H), 2.95 (m, 2H), 3.52 (d, $J$ = 11.1 Hz, 2H), 4.55 (s, 2H), 4.73 (m, 2H), 5.01 (s, 2H), 8.50 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 13.8, 23.8 (4C), 32.8 (2C), 58.7 (d, $J$ = 200 Hz), 71.4 (2C), 90.0, 125.8, 140.5, 151.6, 154.0, 159.0. MS *m/e* ($M^+$): 418.

**({1-[(2-Amino-6-chloro-9*H*-purin-9-yl)methyl]cyclopropyl}oxy)methylphosphonic Acid (15).** To a solution of the phosphonate **7** (765 mg, 1.8 mmol) in 85 mL of MDC was added trimethylsilyl bromide (5.6 g, 36.6 mmol). The mixture was heated under reflux for 18 h and then concentrated in vacuo. The residue was partitioned between MDC and water. The aqueous layer was freeze-dried to afford 600 mg of the phosphonic acid **15** (100% yield) as pale yellowish solids. $^1$H NMR (500 MHz, MeOH-$d_4$): $\delta$ 0.99 (br s, 2H), 1.03 (br s, 2H), 3.92 (d, $J$ = 10.0 Hz, 2H), 4.50 (s, 2H), 9.29 (s, 1H). Purity: HPLC analysis.

**({1-[(2-Amino-6-hydroxy-9*H*-purin-9-yl)methyl]-cyclopropyl}oxy)methyl phosphonic Acid (PMCG, 1).** A solution of the phosphonic acid **15** (820 mg, 2.4 mmol) in 20.0 mL of 2 N HCl was heated under reflux for 6 h and then was freeze-dried. The residue was recrystallized from MeOH to afford 740 mg of PMCG-monohydrogen bromide as white solids. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 0.92 (br q, 4H), 3.74 (d, $J$ = 10.0 Hz, 2H), 4.30 (s, 2H), 6.98 (br s, 2H), 7.0−8.5 (br, 2H), 8.98 (s, 1H), 11.33 (s, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 11.2 (2C), 46.7, 62.70 (d, $J$ = 6.0 Hz), 63.0 (d, $J$ = 159.0 Hz), 109.4, 137.7, 150.2, 154.2, 155.0. HRMS ($M^+$): 316.0811 calcd for $C_{10}H_{14}N_5O_5P$, found 316.0810. Anal. Calcd for ($C_{10}H_{14}N_5O_5P$·HBr): C, 30.32; H, 3.82; N, 17.68. found: C, 30.20; H, 4.13; N, 17.16.

**Diisopropyl ({1-[(2-Amino-9*H*-purin-9-yl)methyl]cyclopropyl}oxy)methylphosphonate (18).** To a solution of the phosphonate **7** (150 mg, 0.36 mmol) in 15.0 mL of THF was added 5 wt % palladium on carbon (15 mg). The mixture was hydrogenated under $H_2$ atmosphere for 18 h. The Pd on C was filtered out through a Celite, and the filtrate was concentrated in vacuo, which was purified by a silica gel column chromatography to afford 130 mg of **18** (94% yield) as white solids. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.86 (t, $J$ = 6.5 Hz, 2H), 1.03 (t, $J$ = 6.5 Hz, 2H), 1.23 (d, $J$ = 6.5 Hz, 6H), 1.30 (d, $J$ = 6.5 Hz, 6H), 3.83 (d, $J$ = 8.0 Hz, 2H), 4.23 (s, 2H), 4.71 (m, 2H), 5.20 (s, 2H), 8.17 (s, 1H).

**({1-[(2-Amino-9*H*-purin-9-yl)methyl]cyclopropyl}oxy)-methylphosphonic Acid (PMCDG, 8).** To a solution of the phosphonate **18** (130 mg, 0.34 mmol) in 10 mL of MDC was added trimethylsilyl bromide (560 mg, 3.7 mmol). The mixture was heated under reflux for 18 h and then concentrated in vacuo. The residue was partitioned between MDC and water. The aqueous layer was freeze-dried to afford 91 mg of **8** (89.5% yield) as yellowish solids. The compound was recrystallized from water for X-ray crystallography. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 0.92 (br q, 4H), 3.76 (d, $J$ = 12.0 Hz, 2H), 4.33 (s, 2H), 8.0 (br s, 2H), 8.74 (s, 1H), 9.00 (s, 1H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 11.6 (2C), 45.9, 62.9 (d, $J$ = 15.0 Hz), 63.0 (d, $J$ = 161 Hz), 125.6, 139.1, 149.8, 154.2, 157.1. HRMS ($MH^+$): 300.0862 calcd for $C_{10}H_{14}N_5O_4P$, found 300.0872. Anal. ($C_{10}H_{14}N_5O_4P$·$H_2O$) C, H, N.

**({1-[(2-Amino-9*H*-purin-9-yl)methyl]cyclopropyl}oxy)-methylphosphonic Acid Dipivoxyl (PMCDG Dipivoxyl, 2).** To a solution of **8** (42.8 g, 143 mmol) in 257 mL of 1-methyl-2-pyrrolidinone was added TEA (43.4 g, 430 mmol) at 60 °C and stirred for 30 min at the same temperature. Chloromethyl pivaloate (64.6 g, 429 mmol) was added the above mixture for 20 min and stirred for 48 h at 25 °C. The reaction mixture was diluted with EtOAc (640 mL) and water (1000 mL). After separation of the organic layer, the aqueous layer was back extracted with EtOAc (640 mL). The combined organic layers were washed water (3 × 600 mL) and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was diluted with diethyl ether (210 mL) and stored for 12 h at −4 °C to afford 29 g of **2** (38.5% yield) as white solids. mp: 92 °C. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 0.89 (br t, 2H), 1.06 (br t, 2H), 1.21 (s, 18H), 3.97 (d, $J$ = 10.0 Hz, 2H), 4.23 (s, 2H), 5.0 (br s, 2H), 5.62 (m, 2H), 8.01 (s, 1H), 8.68 (s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 12.3 (2C), 26.7 (6C), 38.6 (2C), 46.0, 62.1 (d, $J$ = 170 Hz), 64.1 (d, $J$ = 15.0 Hz), 81.6 (d, $J$ = 6.0 Hz, 2C), 127.6, 143.0, 149.4, 153.4, 158.9, 176.6 (2C). HRMS ($MH^+$): 528.2223 calcd for $C_{10}H_{14}N_5O_4P$, found 528.2233. Anal. ($C_{22}H_{34}N_5O_8P$) C, H, N.

**[(1-[{[5-Methyl-2,4-dioxo-3,4-dihydro-1(2*H*)-pyrimidinyl]-methyl}cyclopropyl]oxy) methylphosphonic Acid (11).** $^1$H NMR (500 MHz, MeOH-$d_4$): $\delta$ 0.82 (br s, 2H), 0.96 (br s, 2H), 1.87 (s, 3H), 3.81 (d, $J$ = 10.5 Hz, 2H), 3.96 (s, 2H), 7.57 (s, 1H). HRMS (MH$^+$): 291.0743 calcd for $C_{10}H_{15}N_2O_6P$, found 291.0746. Purity: HPLC analysis.

**({1-[(6-Amino-9*H*-purin-9-yl)methyl]cyclopropyl}oxy)-methylphosphonic Acid (PMCA, 12).** $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 0.92 (br s, 4H), 3.76 (d, $J$ = 8.0 Hz, 2H), 4.47 (s, 2H), 8.49 (s, 1H), 8.63 (s, 1H), 8.86 (br s, 2H), 9.50 (br s, 2 H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 11.2 (2C), 45.8, 62.7 (d, $J$ = 14.0 Hz), 62.4 (d, $J$ = 161 Hz), 117.2 143.9, 144.5, 148.2, 149.6. HRMS (MH$^+$): 300.0867 calcd for $C_{10}H_{14}N_5O_4P$, found 300.0862. Purity: HPLC analysis.

**({1-[(2-Amino-6-methyl-9*H*-purin-9-yl)methyl]-cyclopropyl}oxy)methylphosphonic Acid (13).** To a suspension of $ZnBr_2$ (53 mg, 0.24 mmol) in 2 mL of THF was added methylmagnesium bromide (0.08 mL of 3.0 M solution in diethyl ether, 0.24 mmol) at −78 °C. The mixture was warmed to 25 °C and stirred for 1 h. Pd(PPh$_3$)$_4$ (14 mg, 0.012 mmol) and a solution of the compound **7** (50 mg, 0.12 mmol) in 1 mL of THF were sequentially added to the above mixture. The resulting mixture was heated under reflux for 1 h. After removal of the solvent, the residue was partitioned between EtOAc and water. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by a flash silica gel column chromatography using MeOH/MDC (1/20) to afford 20 mg (42% yield) of diisopropyl ({1-[(2-amino-6-methyl-9*H*-purin-9-yl)methyl]cyclopropyl}oxy)methylphosphonate. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.95 (br s, 2H), 0.98 (br s, 2H), 1.17 (d, $J$ = 6.0 Hz, 6H), 1.23 (d, $J$ = 6.0 Hz, 6H), 2.59 (s, 3H), 4.02 (d, $J$ = 10.6 Hz, 2H), 4.32 (s, 2H), 4.59 (m, 2H), 8.12 (s, 1H). MS (ES): $m/z$ 400 (MH$^+$).

To a solution of the above phosphonate (20 mg, 0.05 mmol) in 10 mL of MDC was added trimethylsilyl bromide (0.2 mL, 1.5 mmol) and heated under reflux for 12 h. The reaction mixture was partitioned between MDC and water. The aqueous layer was freeze-dried, and the residue was purified by HPLC to afford 8.5 mg of the compound **13** (53% yield). $^1$H NMR (500 MHz, MeOH-$d_4$ + $D_2O$): $\delta$ 0.87 (br s, 2H), 1.02 (br s, 2H), 2.72 (s, 3H), 3.80 (br d, 2H), 4.53 (s, 2H), 8.12 (s, 1H). HRMS (MH$^+$): 314.1018 calcd for $C_{11}H_{16}N_5O_4P$, found 314.1014. Purity: HPLC analysis.

**({1-[(2-Amino-6-ethylamino-9*H*-purin-9-yl)methyl]-cyclopropyl}oxy)methylphosphonic Acid (14).** $^1$H NMR (500 MHz, MeOH-$d_4$): $\delta$ 0.89 (br s, 2H), 1.04 (br s, 2H), 1.32 (t, $J$ = 7.5 Hz, 3H), 3.59 (br s, 2H), 3.89 (d, $J$ = 10.5 Hz, 2H), 4.35 (br s, 2H), 7.95 (s, 1H). HRMS (MH$^+$): 343.1284 calcd for $C_{12}H_{19}N_6O_4P$, found 343.1281. Purity: HPLC analysis.

**({1-[(2-Amino-6-ethoxy-9*H*-purin-9-yl)methyl]-cyclopropyl}oxy)methylphosphonic Acid (16).** To a solution of the compound **7** (700 mg, 1.68 mmol) in 10 mL of ethanol were added triethylamine (203.4 mg, 2.1 mmol) and NaOEt (312 mg, 5.0 mmol). The resulting mixture was heated under reflux for 4 h. After removal of the solvent, the residue was partitioned between water and MDC. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by a flash silica gel column chromatography using MeOH/MDC (1/33) to afford 705 mg (93% yield) of diisopropyl ({1-[(2-amino-6-ethoxy-9*H*-purin-9-yl)methyl]cyclopropyl}oxy)methylphosphonate (93% yield) as a yellowish solid. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 0.84 (t, $J$ = 8.0 Hz, 2H), 1.02 (t, $J$ = 8.0 Hz, 2H), 1.25 (d, $J$ = 6.0 Hz, 6H), 1.30 (d, $J$ = 6.0 Hz, 6H), 1.46 (t, $J$ = 7.0 Hz, 3H), 3.82 (d, $J$ = 10.0 Hz, 2H), 4.20 (s, 2H), 4.56 (q, $J$ = 7.0 Hz, 2H), 4.59 (m, 2H), 4.77 (br s, 2H), 7.91 (s, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 12 (2C), 14.3, 23.6 (d, $J$ = 5.0 Hz, 2C), 23.7 (d, $J$ = 3.0 Hz, 2C), 45.8, 62.3, 62.4 (d, $J$ = 170 Hz), 63.5 (d, $J$ = 15 Hz), 70.9, 71.1, 114.8, 142.8, 150.7, 154.0, 159.0, 161.0. MS (ES): $m/z$ 428 (MH$^+$). To a solution of the above phosphonate (100 mg, 0.23 mmol) in 8.0 mL of MDC was added trimethylsilyl bromide (358 mg, 2.34 mmol). The mixture was heated under reflux for 4 h and then concentrated in vacuo. The residue was purified by HPLC to afford 30 mg of **16** (39% yield) and 22 mg of **1** (30% yield). $^1$H NMR (500 MHz, MeOH-d$_4$): $\delta$ 0.88 (br s, 2H), 1.02 (br s, 2H), 1.45 (t, $J$ = 7.0 Hz, 3H), 3.81 (d, $J$ = 10.0 Hz, 2H), 4.39 (s, 2H), 4.60 (d, $J$ = 7.0 Hz, 2H), 8.33 (s, 1H). $^{13}$C NMR (150 MHz, MeOH-$d_4$): $\delta$ 6.0 (2C), 8.9, 23.6, 58.9, 59.5 (d, $J$ = 12.0 Hz), 58.6 (d, $J$ = 150 Hz), 107.3, 135.9, 145.7, 154.8, 156.8. MS (ES): $m/z$ 344 (MH$^+$). Purity: HPLC analysis.

**Procedure To Evaluate Anti-HBV Activity and Cytotoxicity. Anti-HBV Activity**. The compounds listed above were evaluated for anti-HBV activity, referring to the article.[13] The HepG2 2.2.15 cells, hepatitis B virus (HBV) producing cell line, were cultured at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM; GIBCOBRL) supplemented with 10% fetal bovine serum (FBS, Life Technologies), 1% Antibiotic-Antimycotics (ABAM, GIBCOBRL), and 400 $\mu$g/mL Geneticin (GIBCOBRL) in the presence of 5% $CO_2$. The HepG2 2.2.15 cells were plated at a density of $1.5 \times 10^4$ cells/well on 96-well plates and incubated for 2 days. When confluence was about 80−90%, the media were replaced with fresh DMEM containing 2% FBS, 1% ABAM, and 400 $\mu$g/mL G 418. The synthetic compounds with concentrations of 100, 20, 4, 0.8, 0.16, and 0 $\mu$M were treated every other day for 9 days. On day 10 the culture media were collected, boiled, and diluted serially. The samples were analyzed by real-time PCR using a Rotor-Gene 2000 (Corbett Research, Austraslia). Amplification primers were HBV2005F (5′-TCA GCT CTG TAT CGG GAA GCC TTA G-3′) and HBV2122R (5′-CAC CCA CCC AGG TAG CTA GAG TCA-3′), and TaqMan probe was 5′-6-FAM-CCT CAC CAT ACT GCA CTC AGG CAA-BHQ-1−3′. The dual-labeled fluorescent probe was purchased from Biosearch Technologies, Inc. PCR samples were denatured for 10 min at 94 °C, followed by 40 cycles of 94° C for 30 s, 55 °C for 30 s, and 86 °C for 30 s. Emitting fluorescence was detected at 86 °C and the data analyzed statistically using PRISM (GraphPad Software, Inc.).

**Cytotoxicity**. The 50% cytotoxic concentration of each compound was determined in HepG2 2.2.15 cell line. After compounds treatments for the evaluation of anti-HBV activity, each well of the 96-well plate was treated with 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazdium bromide (MTT) and incubated for 2 h at 37 °C. At the end of the incubation period, the medium was removed, and the converted dye was solubilized with acidic isopropyl alcohol (0.05% HCl in 95% isopropyl alcohol). The metabolized formazan reduction product was colorimetrically measured at 490 nm.

**Acknowledgment.** We thank Dr. T.-S. Choi, Ms. C.-M. Kim, and Mr. M.-J. Han for in vivo evaluation with HBV transfected transgenic mice; Dr. B. C. Tennant, Marmotech Inc., for in vivo evaluation with woodchucks; Dr. S. H. Lee, Ms. K. H. Chung, Ms. O. H. Choi, and Dr. In C. Kim for pharmacokinetics; Dr. S. K. Lee, Mr. S. H. Kim, and Ms. E. Choi for evaluation of animal toxicities; Ms. Y. H. Im and Ms. H. S. Cho for mass spectral data. We acknowledge professors Marc M. Greenberg, David Crich, and Jin K. Cha, and Mr. Fred E. Dutton, for proofreading the manuscript.

## References

(1) Marques, A. R.; Straus, S. E. Advances in the Treatment of Chronic Hepatitis B Virus Infection *Rev. Med. Virol.* **1998**, *8*, 223−234 and references therein.

(2) Fattovich, G.; Brollo, L.; Alberti, A.; Pontisso, P.; Giustina, G.; Realdi, G. Long-term follow-up of HBe-positive chronic active hepatitis B. *Hepatology* **1988**, *8*, 1651−1654.

(3) (a) Lai, C. L.; Chien, R. N.; Leung, N. W. Y.; Chang, T. T.; Guan, R.; Tai, D. I.; Ng, K. Y.; Wu, P. C.; Dent, J. C.; Barber, J.; Stephenson, S. L.; Gray, D. F. A One-Year Trial of Lamivudine for Chronic Hepatitis B. *N. Engl. J. Med.* **1998**, *339*, 61−68. (b) Dienstag, J. L.; Schiff. E. R.; Wright, T. L.; Perrillo, R. P.; Hann, H. L.; Goodman, Z.; Crowther, L. D.; Woessner, M.; Rubin, M.; Brown, N. A. Lamivudine as initial treatment for chronic hepatitis B in the United State. *N. Engl. J. Med.* **1999**, *341*, 1256−1263.

(4) (a) De Clercq, E. Perspectives for the treatment of hepatitis B virus infections. *Int. J. Antimicrob. Agents* **1999**, *12*, 81−95 and references therein. (b) Gumina, G.; Song, G.-Y.; Chu, C. K. Advances in antiviral agents for hepatitis B virus. *Antiviral Chem., Chemother.* **2001**, *12 (Suppl. 1)*, 93−117.

(5) (a) Marcellin, P.; Chang, T.-T.; Lim, S. G.; Tong, M. J.; Sievert, W.; Shiffman, M. L.; Jeffers, L.; Goodman, Z.; Wulfsohn, M. S.; Xiong, S.; Fry, J.; Brosgart, C. L. Adefovir Dipivoxil for the Treatment of Hepatitis B e Antigen-Positive Chronic Hepatitis B. *N. Engl. J. Med*. **2003**, *348*, *808*−*16*; (b) Hadziyannis, S. J.; Tassopoulos, N. C.; Heathcote, E. J.; Chang, T.-T.; Kitis, G.; Rizzetto, M.; Marcellin, P.; Lim, S. G.; Fry, J.; Brosgart, C. L. Adefovir Dipivoxil for the Treatment of Hepatitis B e Antigen-Negative Chronic Hepatitis B. *N. Engl. J. Med*. **2003**, *348*, 800−807.

(6) (a) Balzarini, J.; Naesens, L.; Herdewijn, P.; Rosenberg, I.; Holy, A.; Pauwels, R.; Baba, M.; Johns, D. G.; De Clercq, E. Marked in vivo antiretrovirus activity of 9-(2-phosphonomethoxyethyl)-adenine, a selective anti-human immunodeficiency virus agent. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 332−336. (b) De Clercq, E.; Sakuma, T.; Sakuma, T.; Baba, M.; Pauwels, R.; Balzarini, J.; Rosenberg, I.; Holy, A. Antiviral activity of phosphonylmethoxyalkyl derivatives of purine and pyrimidines *Antiviral Res.* **1987**, *8*, 262−272.

(7) Noble, S.; Goa, K. L. Adefovir Dipivoxil. *Drugs* **1999**, *58* (3), 479−487.

(8) (a) Yokota, T.; Konno, N.; Holy, A.; Balzarini, J.; De Clercq, E. Inhibitory effects of acyclic nucleoside phosphonate analogues on hepatitis B virus DNA synthesis in HB611 cells *Antiviral Chem. Chemother.* **1994**, *5*, 57−63; (b) Holy, A.; Gunter, J.; Dvorakova, H.; Masojidkova, M.; Andrei, G.; Snoeck, R.; Balzarini, J.; De Clercq, E. Structure-Antiviral Activity Relationship in the Series of Pyrimidine and Purine N-[2-(2-Phosphonomethoxy)ethyl] Nucleotide Analogues. 1. Derivatives Substituted at the Carbon Atom of the Base. *J. Med. Chem*. **1999**, *42*, 2064−2086.

(9) (a) Raiman, M. V.; II'ina, N. A.; Kulinkovich, O. G. A Convenient Method for Preparation of *N*-Substituted 1-Acetonylaminocyclopropanes from Acetoacetic Ester Ethylene Acetal. *Synlett*. **1999**, *07*, 1053−1054. (b) Lee, J. C.; Sung, M. J.; Cha, J. K. Evaluation of titanium alkoxides and aryloxides in the Kulinkovich cyclopropanation of carboxylic esters. *Tetrahedron Lett*. **2001**, *42*, 2059−2061 and references therein.

(10) (a) Roberts, J. D.; Mazur, R. H. Small-Ring Compounds. IV. Interconversion Reaction of Cyclobutyl, Cyclopropylcarbinyl and Allylcarbinyl Derivatives *J. Am. Chem. Soc.* **1951**, *73*, 2509−2518. (b) Roberts, D. D.; Snyder, R. C., Jr. Cyclopropylcarbinyl Pentamethylbenzenesulfinate Solvolysis. A Study of Response to Solvent Ionizing Strength. *J. Org. Chem*. **1979**, *44*, 2860−2863 and references therein.

(11) Kim, C. U.; Misco, P. F.; Luh, B. Y.; Hitchcock, M. J. M.; Ghazzouli, I.; Martin, J. C. A New Class of Acyclic Phosphonate Nucleotide Analogues: Phosphonate Isosteres of Acyclovir and Ganciclovir Monophosphates as Antiviral Agents. *J. Med. Chem*. **1991**, *34*, 2286−2294.

(12) (a) Starrett, J. E., Jr.; Tortolani, D. R.; Russell, J.; Hitchcock, M. J. M.; Whiterock, V.; Martin, J. C.; Mansuri, M. M. Synthesis, Oral Bioavailability Determination, and in Vitro Evaluation of Prodrugs of the Antiviral Agent 9-[2-(Phosphonomethoxy)ethyl]-adenine (PMEA). *J. Med. Chem*. **1994**, *37*, 1857−1864. (b) Bischofberger, N.; Jones, R. J. Minireview: nucleotide prodrugs. *Antiviral Res.* **1995**, *27*, 1−17.

(13) Sells, M. A.; Chen, M.-L.; Acs, G. Production of hepatitis B virus particles in HepG2 cells transfected with cloned hepatitis B virus DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1005−1009.

(14) (a) Rashidi, M. R.; Smith, J. A.; Clarke, S. E.; Beedcham, C. *In votro* Oxidation of Famciclovir and 6-Deoxypenciclovir by Aldehyde Oxidase From Human, Guinea Pig, Rabbit, and Rat Liver. *Drug Metab. Dispos.* **1997**, *25* (7), 805−813. (b) Faletto, M. B.; Miller, W. H.; Garvey, E. P.; St. Clair, M. H.; Daluge, S. M.; Good, S. S. Unique Intracellular Activation of the Potent Anti-Human Immunodeficiency Virus Agent 1592U89. *Antimicrob. Agents Chemother.* **1997**, *41* (5), 1099−1107.

(15) Moore, S. M.; Mckeating, J. A.; Weiss, R. A.; Satterntau, Q. J. Dissociation of gp120 from HIV-1 Virions Induced by Soluble CD4. *Science* **1990**, *250*, 1139−1141.

(16) Mahy, B. W. *Virology a Practical Approach*; IRL Press: Washington D. C., **1985**, 207−236 and references therein.

(17) Balzarini, J.; Hao, Z.; Herdewijin, P.; Hohns, D.; De Clercq, E. Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1499−1503.

(18) In vivo efficacy evaluation of PMCG (**1**) and PMCDG (**8**) was conducted with HBV transfected transgenic mice by subcutaneous administration, and the results will be described in detail soon.

(19) In vivo efficacy evaluation of PMCDG Dipivoxil (**2**) was conducted with WHBV-infected woodchucks by oral administration at 5 mg and 15 mg/kg/day. WHBV DNA titers of the woodchucks after 1 month treatment were reduced by $10^8$ at both of two doses, and the details will be published soon.

JM0305265