Synthesis of 9-[(2-Hydroxy-1-phosphonylethoxy)ethyl]guanine, 1-[(2-Hydroxy-1-phosphonylethoxy)ethyl]cytosine and 9-[(2-Hydroxy-1-phosphonylethoxy)ethyl]adenine

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The acyclic nucleoside phosphonate analogues, 9-[(2-hydroxy-1-phosphonylethoxy)ethyl]guanine **6**, 1-[(2-hydroxy-1-phosphonylethoxy)ethyl]cytosine **7** and 9-[(2-hydroxy-1-phosphonylethoxy)ethyl]adenine **8**, have been prepared by the coupling of a tosylate of the phosphonate side chain **12** with a purine or pyrimidine base followed by deprotection of the blocking groups.

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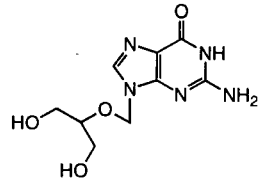
Human cytomegalovirus (HCMV) is one of the most important pathogens in immunologically immature or compromised hosts such as neonates, organ transplant recipients, cancer patients and AIDS patients [1]. So far, only 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG, ganciclovir, **1**) and the trisodium salt of phosphonoformic acid (PFA, foscarnet, **2**) have been approved for the treatment of HCMV infections [2]. 9-(1,3-Dihydroxy-2-propoxymethyl)guanine has been found to be one of the most potent inhibitors of HCMV [2]. However, prolonged use of this drug is often associated with serious side effects such as anemia and neutropenia [3]. Moreover, virus resistance to 9-(1,3-dihydroxy-2-propoxymethyl)guanine often develops during treatment [4]. It seems, therefore, still imperative to find novel chemotherapeutic agents active against HCMV, preferably, through a different mechanism of action.

Recently, metabolically and chemically stable acyclic nucleoside phosphonate analogues, (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (**3**) [5] and 9-[2-(phosphonomethoxy)ethyl]guanine (**4**) [6] have been reported as potent and selective inhibitors against various DNA virus infections including HCMV. Since these phosphonate analogues are structural mimics of acyclic nucleoside monophosphates, they can by-pass the initial enzymatic phosphorylation. Consequently, these compounds are highly effective against HCMV which does not encode a thymidine kinase and thymidine kinase-deficient strains of herpes simplex virus and varicella zoster virus [5]. While (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-cytosine elicits a long-lasting antiviral effect against cytomegalovirus *in vitro* [7] and *in vivo* [8], it has been observed that systemic administration of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine was toxic to guinea pigs [9] and humans [10]. Although 9-[2-(phosphonomethoxy)ethyl]guanine exhibits excellent antiviral activity against cytomegalovirus, it also has high cellular toxicity. Thus, overall therapeutic index of 9-[2-(phosphonomethoxy)ethyl]guanine did not exceed that of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine [9]. We have recently reported an isostere of 9-[2-(phosphono-



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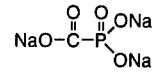

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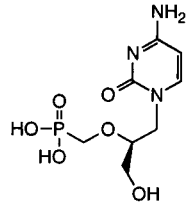

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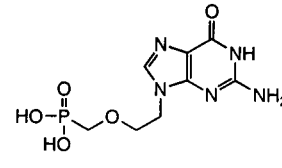

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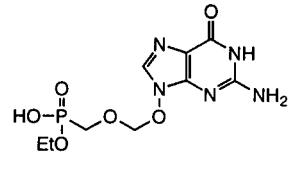

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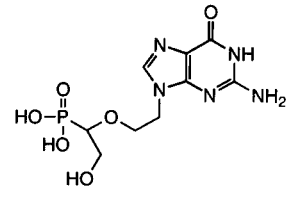

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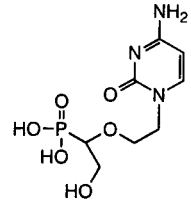

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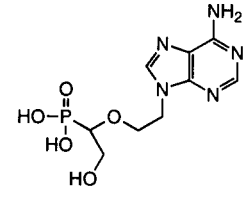

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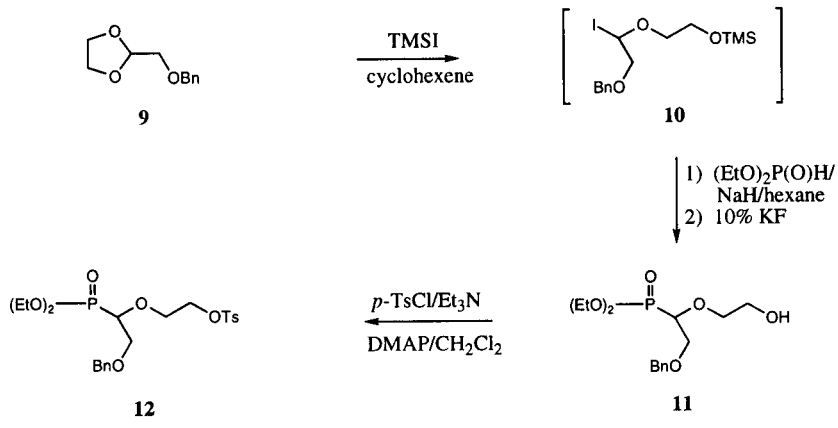
Scheme 1

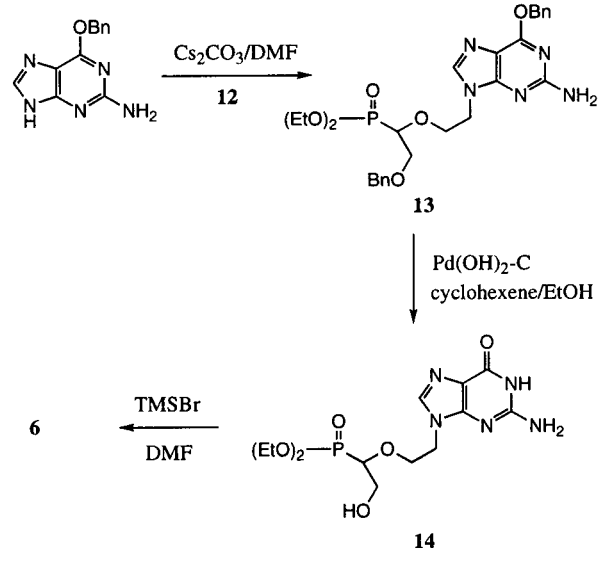


methoxy)ethyl]guanine monoethyl ester, 9-[[(ethoxyhydroxyphosphinyl)methoxy]methoxy]guanine **5**, to examine the role of an additional oxygen atom adjacent to the guanine base in anti-HCMV activity [11]. The phosphonate **5** showed comparable anti-HCMV activity to 9-(1,3-dihydroxy-2-propoxymethyl)guanine in tissue culture [11,12].

In our continued efforts to study the structure-activity relationships of acyclic nucleoside phosphonate analogues, we have introduced a hydroxymethyl group at the 4'-position of acyclic (phosphonomethoxy)ethyl moiety. 9-[(2-Hydroxy-1-phosphonylethoxy)ethyl]guanine **6** is structurally more closely related to 9-(1,3-dihydroxy-2-propoxymethyl)guanine monophosphate than 9-[2-(phosphonomethoxy)ethyl]guanine, and 1-[(2-hydroxy-1-phosphonylethoxy)ethyl]cytosine **7** is a positional isomer of (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine. This report describes the synthesis and *in vitro* anti-HCMV activity of **6**, **7** and 9-[(2-hydroxy-1-phosphonylethoxy)ethyl]adenine **8**.

The general strategy employed for the synthesis of 4'-hydroxymethyl(phosphonomethoxy)ethyl analogues involves coupling of a suitable tosylate of the phosphonate side chain with a purine or pyrimidine base. The synthesis of the required tosylate side chain **12** is depicted in Scheme 1. Treatment of 2-(benzyloxymethyl)-1,3-dioxolane **9** [13] with iodotrimethylsilane in cyclohexene at -78° according to a published procedure [14] generated the reactive iodomethyl ether **10** *in situ*. The subsequent reaction of **10** with the sodium salt of diethyl phosphite in hexane followed by desilylation with 10% aqueous potassium fluoride solution afforded alcohol **11** in 86% yield. Conversion of **11** to the tosylate derivative **12** was accomplished by addition of *p*-toluenesulfonyl chloride, triethylamine and 4-dimethylaminopyridine to a cooled (0°) solution of the alcohol in dichloromethane in 86% yield. Coupling of 2-amino-6-(benzyloxy)purine [15] with tosylate **12** in the presence of cesium carbonate in *N,N*-dimethylformamide at 90° produced the N-9 isomer **13** in 35% yield. Removal of the benzyl protecting group was achieved by catalytic transfer hydrogenation with 20% palladium hydroxide on carbon in a mixture of cyclohexene and ethanol at reflux temperature to provide the phosphonate diethyl ester **14** in 55% yield. Ester cleavage of **14** was effected by treatment with excess bromotrimethylsilane in *N,N*-dimethylformamide at room temperature to give guanine derivative **6** in 62% yield (Scheme 2). Reaction of cytosine and **12** in the presence of cesium carbonate in *N,N*-dimethylformamide under the same reaction

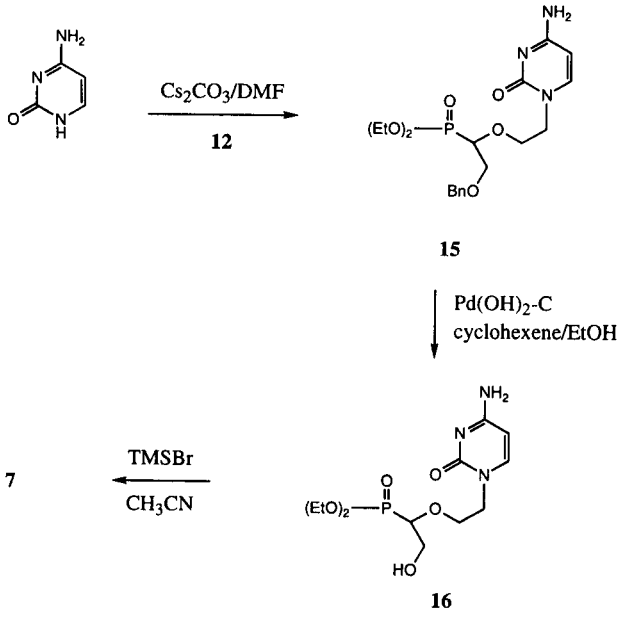
Scheme 2



condition as for **13** afforded the *N*-alkylated product **15** in 63% yield. Cleavage of the benzyl protecting group of **15** by catalytic transfer hydrogenation and hydrolysis of the resulting phosphonate diethyl ester **16** with bromotrimethylsilane in acetonitrile gave cytosine derivative **7** in a

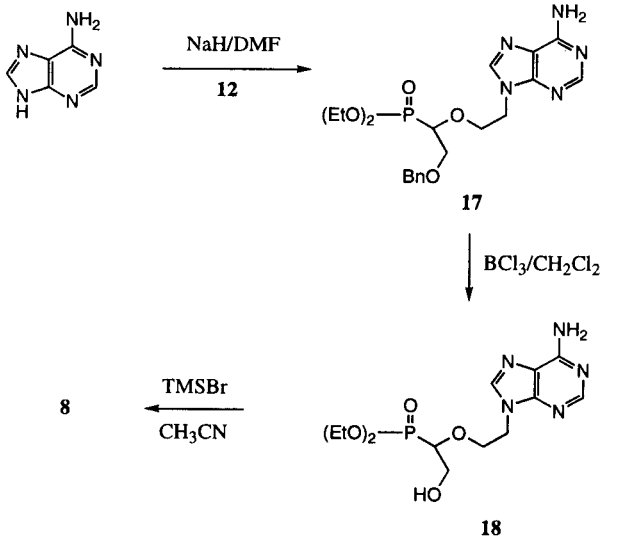
two-step yield of 49% (Scheme 3). For the synthesis of adenine derivative, the sodium salt of adenine was first

Scheme 3



generated by treatment with sodium hydride in *N,N*-dimethylformamide at 80°, and then the resulting slurry was reacted with a solution of **12** in *N,N*-dimethylformamide at 65° to give the N-9 isomer **17** in 59% yield. The benzyl protecting group of **17** was removed by treatment with boron trichloride in dichloromethane to produce **18** in 94% yield. Hydrolysis of the phosphonate diethyl ester **18** with bromotrimethylsilane in acetonitrile at room temperature afforded **8** in 76% yield (Scheme 4).

Scheme 4



The antiviral activity of the phosphonates **6-8** has been evaluated against two strains of HCMV, AD-169 and Davis, by cytopathic effect inhibition assay. Surprisingly, these compounds were found to be completely inactive at concentrations up to 100 μg/ml, suggesting that introduction of a hydroxymethyl group at the 4'-position of acyclic (phosphonomethoxy)ethyl moiety may inhibit their enzymatic phosphorylation by steric hindrance.

EXPERIMENTAL

Melting points were determined on either an Electrothermal F500MA digital or a Mettler FP62 melting point apparatus and are uncorrected. The ir spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. The ^{1}H nmr spectra were recorded on a Varian Unity 300 spectrometer. The chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane in deuteriochloroform or deuteriodimethyl sulfoxide and to sodium 2,2-dimethyl-2-silapentane-5-sulfonate in deuterium oxide. The ^{13}C nmr spectra were recorded on a Varian Unity 300 spectrometer at 75.4 MHz. When deuteriochloroform or deuteriodimethyl sulfoxide was used as solvent, it served as the internal standard at δ 77.0 or 39.5, respectively. When deuterium oxide was used, sodium 2,2-dimethyl-2-silapentane-5-sulfonate (-1.6 ppm) was added as the internal standard. The tlc analysis was performed on Merck silica gel 60F-254 glass plates. Flash chromatography was performed using Merck silica gel 60 (230-400 mesh). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

1-[2-(Benzyloxy)-1-(diethylphosphonyl)ethoxy]-2-hydroxyethane (**11**).

Iodotrimethylsilane (34.00 g, 170 mmoles, 24.2 ml) in cyclohexene (80 ml) was added to 2-(benzyloxymethyl)-1,3-dioxolane **9** (30.00 g, 154 mmoles) in cyclohexene (80 ml) dropwise at -78° under a nitrogen atmosphere, and then the mixture was allowed to warm to 0° over 15 minutes to generate 1-[2-(benzyloxy)-1-iodoethoxy]-2-(trimethylsilyloxy)ethane **10**. To a suspension of 60% sodium hydride in mineral oil (6.80 g, 170 mmoles) in hexane (200 ml) at 0° was added dropwise diethyl phosphite (23.48 g, 170 mmoles, 21.9 ml). After the mixture was stirred for 1 hour at 0°, and into this, the above *in situ* generated **10** was added. The mixture was allowed to warm to room temperature over 1 hour and then concentrated under reduced pressure. The residue was dissolved in dichloromethane (200 ml), washed with water (100 ml), and evaporated to dryness. The oily residue was dissolved in ethanol (200 ml) and treated with 10% aqueous potassium fluoride solution (100 ml), and the mixture was stirred at room temperature for 24 hours. The reaction mixture was concentrated to 100 ml under reduced pressure and was extracted with dichloromethane (2 x 100 ml). The dichloromethane solution was dried over anhydrous magnesium sulfate and evaporated to dryness to give an oily residue, which was purified by flash column chromatography on silica gel with a mixture of methanol and chloroform (3:97, v/v) as the eluent to give **11** (44.04 g, 86%) as a colorless oil; ir (neat): 3423, 1240, 1047, 1024, 972 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.32 (t, J = 7.2 Hz, 3H, $POCH_2CH_3$), 1.33 (t, J = 7.2 Hz, 3H, $POCH_2CH_3$), 3.64-3.90 (m, 7H, CH_2CH_2OH and CH_2OBn), 3.98 (m, 1H, OCHP), 4.15 (quintet, J = 7.2 Hz, 2H, $POCH_2$), 4.19 (quintet, J = 7.2 Hz, 2H, $POCH_2$), 4.58 (d, J = 12.0 Hz, 1H,

benzylic), 4.59 (d, J = 12.0 Hz, 1H, benzylic), 7.26-7.42 (m, 5H, ArH); ^{13}C nmr (deuteriochloroform): δ 16.5 (d, J = 5.0 Hz, $POCH_2CH_3$), 61.8 (CH_2OH), 62.6 (d, J = 7.0 Hz, $POCH_2$), 63.1 (d, J = 6.5 Hz, $POCH_2$), 70.1 (d, J = 12.1 Hz, CH_2OBn), 73.6 (OCH_2Ph), 75.2 (d, J = 4.5 Hz, CH_2CH_2OH), 76.7 (d, J = 163.2 Hz, OCHP), 127.7, 127.9, 128.5, 137.5.

Anal. Calcd. for $C_{15}H_{25}O_6P$: C, 54.21; H, 7.58. Found: C, 54.07; H, 7.62.

1-[2-(Benzyloxy)-1-(diethylphosphonyl)ethoxy]-2-(*p*-toluenesulfonyloxy)ethane (**12**).

To a stirred solution of **11** (14.10 g, 42.4 mmoles) in anhydrous dichloromethane (200 ml) at 0° were added *p*-toluenesulfonyl chloride (9.71 g, 50.9 mmoles), triethylamine (10.30 g, 101.8 mmoles, 14.2 ml), and 4-dimethylaminopyridine (0.26 g, 2.1 mmoles). The mixture was stirred at room temperature for 16 hours, diluted with dichloromethane (200 ml), washed with water (50 ml), and evaporated to dryness. The residue was purified by flash column chromatography on silica gel with a mixture of ethyl acetate and hexane (3:1, v/v) as the eluent to give **12** (17.78 g, 86%) as a colorless oil; ir (neat): 1360, 1250, 1190, 1178, 1097, 1049, 1024, 970, 925, 753 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.29 (t, J = 7.2 Hz, 3H, $POCH_2CH_3$), 1.30 (t, J = 7.2 Hz, 3H, $POCH_2CH_3$), 2.42 (s, 3H, CH_3), 3.63-4.03 (m, 3H, $CHCH_2OBn$), 3.95 (m, 2H, CH_2CH_2OTs), 4.05-4.23 (m, 6H, 2 $POCH_2$ and CH_2OTs), 4.53 (s, 2H, benzylic), 7.21-7.42 (m, 7H, ArH), 7.77 (d, J = 8.4 Hz, 2H, ArH); ^{13}C nmr (deuteriochloroform): δ 16.4 (d, J = 5.5 Hz, $POCH_2CH_3$), 21.6 (Ar-CH_3), 62.7 (d, J = 7.1 Hz, $POCH_2$), 62.8 (d, J = 7.4 Hz, $POCH_2$), 68.9 (CH_2OTs), 70.0 (d, J = 10.9 Hz, CH_2OBn), 70.1 (d, J = 6.8 Hz, CH_2CH_2OTs), 73.5 (OCH_2Ph), 77.1 (d, J = 162.2 Hz, OCHP), 127.6, 127.7, 127.9, 128.4, 129.7, 133.0, 137.8, 144.7.

Anal. Calcd. for $C_{22}H_{31}O_8PS$: C, 54.31; H, 6.42. Found: C, 54.02; H, 6.61.

2-Amino-6-(benzyloxy)-9-[[2-(benzyloxy)-1-(diethylphosphonyl)ethoxy]ethyl]purine (**13**).

A mixture of **12** (6.39 g, 13.1 mmoles), 2-amino-6-(benzyloxy)purine [15] (3.80 g, 15.8 mmoles), and cesium carbonate (17.46 g, 53.6 mmoles) in anhydrous *N,N*-dimethylformamide (25 ml) was heated at 90° for 3 hours under a nitrogen atmosphere. The reaction mixture was filtered, and the filtrate was evaporated *in vacuo* to dryness. The oily residue was dissolved in dichloromethane (200 ml) and washed with water (50 ml). The dichloromethane solution was evaporated to dryness, and the residue was purified by flash column chromatography on silica gel with a mixture of methanol and ethyl acetate (5:95, v/v) as the eluent to give **13** (2.55 g, 35%) as a yellow oil; ir (neat): 3332, 1613, 1581, 1456, 1411, 1247, 1047, 1028, 970, 735 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.24 (t, J = 6.9 Hz, 3H, $POCH_2CH_3$), 1.26 (t, J = 6.9 Hz, 3H, $POCH_2CH_3$), 3.60-3.91 (m, 3H, $CHCH_2OBn$), 3.96-4.16 (m, 6H, 2 $POCH_2$ and CH_2N), 4.24 (t, J = 4.5 Hz, 2H, OCH_2CH_2N), 4.47 (s, 2H, benzylic), 4.82 (s, 2H, benzylic), 5.56 (s, 2H, NH_2), 7.20-7.55 (m, 10H, ArH), 7.79 (s, 1H, H-8); ^{13}C nmr (deuteriochloroform): δ 16.4 (d, J = 5.5 Hz, $POCH_2CH_3$), 43.4 (CH_2N), 62.6 (d, J = 6.7 Hz, $POCH_2$), 62.7 (d, J = 6.1 Hz, $POCH_2$), 68.0 (OCH_2Ph), 70.0 (d, J = 10.3 Hz, CH_2OBn), 70.9 (d, J = 6.1 Hz, OCH_2CH_2N), 73.5 (OCH_2Ph), 76.9 (d, J = 162.6 Hz, OCHP), 115.4 (C-5), 127.6, 127.7, 127.9, 128.2, 128.3, 128.4, 136.6, 137.6, 140.6 (C-8), 154.1 (C-4), 159.0 (C-2), 161.0 (C-6).

Anal. Calcd. for $C_{27}H_{34}N_5O_6P$: C, 58.37; H, 6.17; N, 12.61. Found: C, 58.16; H, 6.30; N, 12.48.

9-[[2-Hydroxy-1-(diethylphosphonyl)ethoxy]ethyl]guanine (**14**).

To a stirred solution of **13** (2.40 g, 4.3 mmoles) in a mixture of ethanol (25 ml) and cyclohexene (25 ml) was added palladium hydroxide on carbon (2.40 g, 20%), and this mixture was heated at reflux for 16 hours. The hot reaction mixture was filtered through a pad of Celite, and the pad was washed well with hot ethanol. The combined filtrate and washings were evaporated to dryness, dissolved in methanol, adsorbed on silica gel, and loaded on the top of a silica gel column. Elution with a mixture of methanol and chloroform (2:8, v/v) yielded **14** (0.89 g, 55%) as a white solid, mp 142°; ir (potassium bromide): 3392, 3348, 3132, 1692, 1607, 1541, 1369, 1234, 1111, 1048, 1019, 964, 780 cm^{-1}; ^{1}H nmr (deuteriodimethyl sulfoxide): δ 1.17 (t, J = 7.2 Hz, 6H, 2 $POCH_2CH_3$), 3.45-3.82 (m, 3H, $CHCH_2OH$), 3.88-4.03 (m, 6H, 2 $POCH_2$ and CH_2N), 4.11 (t, J = 4.5 Hz, 2H, OCH_2CH_2N), 4.98 (t, J = 5.4 Hz, 1H, OH), 6.42 (s, 2H, NH_2), 7.69 (s, 1H, H-8), 10.56 (s, 1H, NH) ; ^{13}C nmr (deuteriodimethyl sulfoxide): δ 16.4 (d, J = 5.0 Hz, $POCH_2CH_3$), 16.5 (d, J = 5.0 Hz, $POCH_2CH_3$), 43.1 (CH_2N), 60.8 (d, J = 9.0 Hz, CH_2OH), 62.1 (d, J = 6.5 Hz, $POCH_2$), 62.3 (d, J = 6.5 Hz, $POCH_2$), 70.4 (d, J = 5.0 Hz, $OCHCH_2N$), 77.9 (d, J = 156.8 Hz, OCHP), 116.4 (C-5), 138.3 (C-8), 151.4 (C-4), 153.5 (C-2), 157.1 (C-6).

Anal. Calcd. for $C_{13}H_{22}N_5O_6P$: C, 41.60; H, 5.90; N, 18.66. Found: C, 41.25; H, 6.13; N, 18.48.

9-[(2-Hydroxy-1-phosphonylethoxy)ethyl]guanine (**6**).

To a stirred solution of **14** (0.83 g, 2.2 mmoles) in anhydrous *N,N*-dimethylformamide (25 ml) was added bromotrimethylsilane (3 ml) dropwise under a nitrogen atmosphere. The mixture was stirred at room temperature for 16 hours, and then evaporated to dryness *in vacuo*. The residue was basified to *p*H 11 by addition of 2*N* sodium hydroxide solution and loaded on the top of a column packed with Amberlite IRA-400(OH) ion-exchange resin. The column was washed with water and then eluted with 20% aqueous acetic acid solution with monitoring the peak at 254 nm. The combined acetic acid solution was evaporated to dryness, and the residue was co-evaporated with toluene twice. The residue was treated with water (15 ml) and ethanol (30 ml), and the white precipitate was filtered, washed with ethanol, and dried thoroughly *in vacuo* to give **6** (0.44 g, 62%), mp 217-220° dec; ir (potassium bromide): 3335, 3148, 1703, 1644, 1606, 1164, 1117, 1064, 931 cm^{-1}; ^{1}H nmr (deuterium oxide): δ 3.67 (m, 2H, CH_2OH), 3.90 (m, 1H, OCHP), 4.08 (dt, J = 11.1, 4.5 Hz, 1H, CH_2N), 4.16 (dt, J = 11.1, 4.5 Hz, 1H, CH_2N), 4.42 (t, J = 4.5 Hz, 2H, OCH_2CH_2N), 8.91 (s, 1H, H-8).

Anal. Calcd. for $C_9H_{14}N_5O_6P$: C, 33.86; H, 4.42; N, 21.94. Found: C, 33.61; H, 4.53; N, 21.80.

1-[[2-(Benzyloxy)-1-(diethylphosphonyl)ethoxy]ethyl]cytosine (**15**).

A mixture of **12** (11.68 g, 24.0 mmoles), cytosine (3.25 g, 29.3 mmoles) and cesium carbonate (15.97 g, 49.0 mmoles) in anhydrous *N,N*-dimethylformamide (50 ml) was heated at 90° for 2 hours under a nitrogen atmosphere. The reaction mixture was filtered, and the filtrate was evaporated *in vacuo* to dryness. The oily residue was dissolved in dichloromethane (200 ml) and washed with water (50 ml). The dichloromethane solution was evaporated to dryness, and the residue was purified by flash col-

umn chromatography on silica gel with a mixture of methanol and chloroform (7:93, v/v) as the eluent to give **15** (6.46 g, 63%), mp 121-123°; ir (potassium bromide): 3324, 1649, 1499, 1385, 1243, 1213, 1121, 1024, 973 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.29 (t, J = 7.2 Hz, 6H, 2 $POCH_2CH_3$), 3.64-3.88 (m, 3H, $CHCH_2OBn$), 3.92-4.18 (m, 8H, 2 $POCH_2$ and OCH_2CH_2N), 4.51 (s, 2H, benzylic), 5.52 (d, J = 7.2 Hz, 1H, H-5), 5.88 (br s, 2H, NH_2), 7.24-7.40 (m, 5H, Ar H), 7.47 (d, J = 7.2 Hz, 1H, H-6); ^{13}C nmr (deuteriochloroform): δ 16.5 (d, J = 5.0 Hz, $POCH_2CH_3$), 49.7 (CH_2N), 62.6 (d, J = 6.5 Hz, $POCH_2$), 69.8 (d, J = 10.1 Hz, CH_2OBn), 70.9 (d, J = 6.0 Hz, OCH_2CH_2N), 73.5 (OCH_2Ph), 76.7 (d, J = 162.2 Hz, OCHP), 93.0 (C-5), 127.7, 127.8, 128.4, 137.7, 147.5 (C-6), 156.3 (C-2), 165.6 (C-4).

Anal. Calcd. for $C_{19}H_{28}N_3O_6P$: C, 53.64; H, 6.63; N, 9.88. Found: C, 53.42; H, 6.85; N, 9.55.

1-[[2-Hydroxy-1-(diethylphosphonyl)ethoxy]ethyl]cytosine (**16**).

To a stirred solution of **15** (6.46 g, 15.2 mmoles) in a mixture of ethanol (40 ml) and cyclohexene (40 ml) was added palladium hydroxide on carbon (6.46 g, 20%), and this mixture was heated at reflux. The tlc indicated a constant ratio of starting material/product (~1:2) after 6 hours, so the hot reaction mixture was filtered through a pad of Celite, and the pad was washed with hot ethanol. The combined filtrate and washings were evaporated to dryness, and the residue was dissolved in a mixture of ethanol (40 ml) and cyclohexene (40 ml). Palladium hydroxide on carbon (6.46 g, 20%) was added, and the mixture was heated at reflux for 8 hours. The hot reaction mixture was filtered through a pad of Celite, and the pad was washed with hot ethanol. The combined filtrate and washings were evaporated to dryness, and the residue was purified by flash column chromatography on silica gel with a mixture of methanol and chloroform (2:8, v/v) as the eluent to give **16** (3.47 g, 68%) as an oil which was solidified on standing, mp 137-138.5°; ir (potassium bromide): 3364, 1655, 1611, 1491, 1391, 1230, 1104, 1020, 966, 790 cm^{-1}; ^1H nmr (deuteriodimethyl sulfoxide): δ 1.19 (t, J = 7.2 Hz, 6H, 2 $POCH_2CH_3$), 3.40-3.90 (m, 7H, OCH_2CH_2N and $CHCH_2OH$), 3.98 (quintet, J = 7.2 Hz, 4H, 2 $POCH_2$), 5.06 (t, J = 4.5 Hz, 1H, OH), 5.64 (d, J = 7.2 Hz, 1H, H-5), 6.89 (br s, 1H, NH), 7.11 (br s, 1H, NH), 7.56 (d, J = 7.2 Hz, 1H, H-6); ^{13}C nmr (deuteriodimethyl sulfoxide): δ 16.2 (d, J = 5.0 Hz, $POCH_2CH_3$), 16.3 (d, J = 5.0 Hz $POCH_2CH_3$), 48.6 (CH_2N), 60.7 (d, J = 9.2 Hz, CH_2OH), 61.6 (d, J = 6.5 Hz, $POCH_2$), 61.7 (d, J = 6.5 Hz, $POCH_2$), 70.0 (d, J = 5.5 Hz, OCH_2CH_2N), 77.9 (d, J = 155.6 Hz, OCHP), 92.6 (C-5), 146.6 (C-6), 155.7 (C-2), 165.9 (C-4).

Anal. Calcd. for $C_{12}H_{22}N_3O_6P$: C, 42.99; H, 6.61; N, 12.53. Found: C, 43.22; H, 6.50; N, 12.48.

1-[(2-Hydroxy-1-phosphonylethoxy)ethyl]cytosine (**7**).

To a stirred suspension of **16** (2.18 g, 6.5 mmoles) in anhydrous acetonitrile (60 ml) was added bromotrimethylsilane (9 ml) dropwise under a nitrogen atmosphere. The resulting bright red solution was stirred at room temperature for 15 hours, and then the reaction mixture was evaporated to dryness *in vacuo*. The residue was basified to *p*H 11 by addition of 2*N* sodium hydroxide solution and loaded on the top of a column packed with Amberlite IRA-400(OH) ion-exchange resin. The column was washed with water, and then eluted with 20% aqueous acetic acid solution with monitoring the peak 274 nm. The combined acetic acid solution was evaporated to dryness, and the residue was dissolved in a small volume of water and freeze-dried to give **7** (1.31 g, 72%), mp 128-130° dec; ir (potassium bromide): 3325, 3201, 3116, 1716, 1674, 1119, 1054, 915 cm^{-1}; ^1H nmr (deuterium oxide): δ 3.62 (m, 2H, CH_2OH), 3.84-3.98 (m, 2H, CHN and OCHP), 3.99-4.14 (m, 3H, OCH_2CHN), 6.12 (d, J = 7.8 Hz, 1H, H-5), 7.92 (d, J = 7.8 Hz, 1H, H-6); ^{13}C nmr (deuterium oxide): δ 50.8 (CH_2N), 62.9 (d, J = 10.4 Hz, CH_2OH), 70.1 (d, J = 3.8 Hz, OCH_2CH_2N), 80.7 (d, J = 151.3 Hz, OCHP), 95.0 (C-5), 150.3 (C-2), 151.8 (C-6), 160.6 (C-4).

Anal. Calcd. for $C_8H_{14}N_3O_6P$: C, 34.42; H, 5.05; N, 15.05. Found: C, 34.18; H, 5.15; N, 14.92.

9-[[2-(Benzyloxy)-1-(diethylphosphonyl)ethoxy]ethyl]adenine (**17**).

To a suspension of 60% sodium hydride in mineral oil (0.45 g, 11.3 mmoles) in anhydrous *N,N*-dimethylformamide (50 ml) was added adenine (1.53 g, 11.3 mmoles) under a nitrogen atmosphere, and the mixture was heated at 80° for 1 hour. To the resulting slurry was added dropwise a solution of **12** (5.00 g, 10.3 mmoles) in anhydrous *N,N*-dimethylformamide (10 ml) at room temperature. The mixture was heated at 65° for 16 hours, and then evaporated to dryness *in vacuo*. The residue was dissolved in chloroform (100 ml) and washed with water (50 ml). The chloroform solution was evaporated to dryness, and the residue was purified by flash column chromatography on silica gel with a mixture of methanol-chloroform (7:93, v/v) as the eluent to give **17** (2.72 g, 59%) as a colorless oil; ir (neat): 3331, 3187, 1652, 1601, 1243, 1046, 1023, 970, 753 cm^{-1}; ^1H nmr (deuteriochloroform): δ 1.25 (t, J = 6.9 Hz, 3H, $POCH_2CH_3$), 1.26 (t, J = 6.9 Hz, 3H, $POCH_2CH_3$), 3.63-3.92 (m, 3H, $CHCH_2OBn$), 3.97-4.21 (m, 6H, 2 $POCH_2$ and CH_2N), 4.39 (t, J = 4.5 Hz, 2H, OCH_2CH_2N), 4.47 (s, 2H, benzylic), 5.71 (br s, 2H, NH_2), 7.20-7.40 (m, 5H, ArH), 8.03 (s, 1H, H-2), 8.34 (s, 1H, H-8); ^{13}C nmr (deuteriochloroform): δ 16.4 (d, J = 5.5 Hz, $POCH_2CH_3$), 43.7 (CH_2N), 62.6 (d, J = 6.8 Hz, $POCH_2$), 62.6 (d, J = 7.3 Hz, $POCH_2$), 69.9 (d, J = 10.3 Hz, CH_2OBn), 70.9 (d, J = 6.1 Hz, OCH_2CH_2N), 73.5 (OCH_2Ph), 76.9 (d, J = 162.7 Hz, OCHP), 119.4 (C-5), 127.6, 127.8, 128.4, 137.6, 141.8 (C-8), 150.0 (C-4), 152.8 (C-2), 155.3 (C-6).

Anal. Calcd. for $C_{20}H_{28}N_5O_5P$: C, 53.45; H, 6.28; N, 15.58. Found: C, 53.40; H, 6.35; N, 15.48.

9-[[2-Hydroxy-1-(diethylphosphonyl)ethoxy]ethyl]adenine (**18**).

To a stirred solution of **17** (1.17 g, 2.6 mmoles) in anhydrous dichloromethane (25 ml) at -78° was added 1*M* boron trichloride solution in dichloromethane (13.0 ml) dropwise under a nitrogen atmosphere. The mixture was stirred at -78° for 1 hour and then at -20° for 1 hour. To the reaction mixture was added a mixture of methanol and dichloromethane (25 ml, 1:1, v/v), and the mixture was stirred at room temperature for an additional 1 hour. After cooling to 0°, the mixture was neutralized with 28% ammonium hydroxide (3.6 ml), and the resulting solid was removed by filtration. The filtrate was evaporated *in vacuo* to dryness, dissolved in methanol, adsorbed on silica gel, and loaded on the top of a silica gel column. Elution with a mixture of methanol and dichloromethane (1:9, v/v) yielded **18** (0.88 g, 94%) as a white solid, mp 155-156°; ir (potassium bromide): 3350, 3298, 3125, 1674, 1605, 1311, 1236, 1226, 1205, 1123, 1059, 1013, 976 cm^{-1}; ^1H nmr (deuteriodimethyl sulfoxide): δ

1.13 (t, J = 6.9 Hz, 3H, $POCH_2CH_3$), 1.14 (t, J = 6.9 Hz, 3H, $POCH_2CH_3$), 3.46-3.60 (m, 1H, CH_2OH), 3.62-3.74 (m, 1H, CH_2OH), 3.78 (m, 1H, OCHP), 3.91 (quintet, J = 6.9 Hz, 4H, 2 $POCH_2$), 4.01 (m, 2H, CH_2N), 4.31 (m, 2H, OCH_2CH_2N), 5.10 (t, J = 5.7 Hz, 1H, OH), 7.15 (br s, 2H, NH_2), 8.231 (s, 1H, H-2), 8.234 (s, 1H, H-8); ^{13}C nmr (deuteriodimethyl sulfoxide): δ 16.0 (d, J = 5.0 Hz, $POCH_2CH_3$), 16.1 (d, J = 5.0 Hz, $POCH_2CH_3$), 43.1 (CH_2N), 60.4 (d, J = 9.5 Hz, CH_2OH), 61.8 (d, J = 6.5 Hz, $POCH_2$), 62.0 (d, J = 7.1 Hz, $POCH_2$), 70.1 (d, J = 5.0 Hz, OCH_2CH_2N), 77.6 (d, J = 156.8 Hz, OCHP), 118.3 (C-5), 141.4 (C-8), 149.3 (C-4), 152.2 (C-2), 155.6 (C-6).

Anal. Calcd. for $C_{13}H_{22}N_5O_5P$: C, 43.46; H, 6.17; N, 19.49. Found: C, 43.28; H, 6.13; N, 19.36.

9-[(2-Hydroxy-1-phosphonylethoxy)ethyl]adenine (**8**).

To a stirred suspension of **18** (0.50 g, 1.4 mmoles) in anhydrous acetonitrile (10 ml) was added bromotrimethylsilane (2 ml) dropwise under a nitrogen atmosphere. The resulting bright red solution was stirred at room temperature for 15 hours, and then the reaction mixture was evaporated to dryness *in vacuo*. The residual oil was placed under high vacuum (0.1 mm Hg) for 2 hours and then was treated with water (2 ml) and ethanol (7.5 ml). The resulting solution was allowed to stand at 0° overnight, and then the white precipitate was filtered to give **8** (0.32 g, 76%), mp 229-231° dec; ir (potassium bromide): 3323, 3105, 1693, 1607, 1414, 1221, 1119, 1061, 930 cm^{-1}; ^1H nmr (deuterium oxide): δ 3.65 (m, 2H, CH_2OH), 3.87 (m, 1H, OCHP), 4.10 (dt, J = 11.1, 4.5 Hz, 1H, CH_2N), 4.20 (dt, J = 11.1, 4.5 Hz, 1H, CH_2N), 4.53 (t, J = 4.5 Hz, 2H, OCH_2CH_2N), 8.40 (s, 1H, H-2), 8.47 (s, 1H, H-8); ^{13}C nmr (deuterium oxide): δ 45.8 (CH_2N), 62.8 (d, J = 10.6 Hz, CH_2OH), 70.9 (d, J = 3.5 Hz, OCH_2CH_2N), 80.7 (d, J = 151.2 Hz, OCHP), 118.9 (C-5), 145.7 (C-8), 146.5 (C-4), 149.6 (C-2), 151.1 (C-6).

Anal. Calcd. for $C_9H_{14}N_5O_5P$: C, 35.65; H, 4.65; N, 23.10. Found: C, 35.70; H, 4.72; N, 22.92.

Cytopathic Effect Inhibition Assay.

HEL 299 cells (ATCC CCL 137) in stationary phase were infected with virus at an M.O.I. (multiplicity of infection) of 2-4 $CCID_{50}$ (50% cell culture inhibitory dose) per well of 96-well plates. After a 2 hour adsorption period at 37° in 5% carbon dioxide incubator, the liquid was aspirated off, and 100 µl of Dulbecco's modified Eagle medium (DMEM) (GIBCO)/2% fetal bovine serum (FBS) (GIBCO) containing a compound was applied to each well in triplicate for each concentration. After 7 days of incubation at 37° in 5% carbon dioxide incubator, Giemsa staining was performed. The antiviral activity was measured by microscopic observation of cytopathic effect and expressed as EC_{50}, a concentration of the compound required to inhibit virus-induced cytopathic effect by 50%.

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