Brief Articles

Synthesis and Evaluation of 2-Amino-9-(1,3-dihydroxy-2-propoxymethyl)-6-fluoropurine Mono- and Diesters as Potential Prodrugs of Ganciclovir

Dae-Kee Kim,* Kieyoung Chang, Guang-Jin Im, Hun-Taek Kim, Namkyu Lee, and Key H. Kim

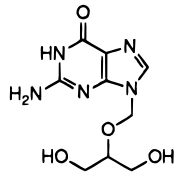
Life Science Research Center, SK Chemicals, 600 Jungja-Dong, Changan-Ku, Suwon-Si, Kyungki-Do 440-745, Korea

Received May 22, 1998

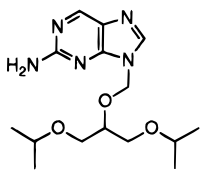
A series of 2-amino-9-(1,3-dihydroxy-2-propoxymethyl)-6-fluoropurine mono- and diesters, **6a**−**h**, were synthesized as potential prodrugs of ganciclovir and evaluated for their oral ganciclovir bioavailability in rats. Treatment of 2-amino-6-chloro-9-(1,3-dihydroxy-2-propoxymethyl)purine (**4**) with Me_3N in DMF/THF (1/4) followed by the reaction of the resulting trimethylammonium chloride salt **5** with KF in DMF gave 2-amino-9-(1,3-dihydroxy-2-propoxymethyl)-6-fluoropurine (**2**) in 83% yield. Esterification of **2** with an appropriate acid anhydride (Ac_2O, $(EtCO)_2O$, (n-$PrCO)_2O$, or (i-$PrCO)_2O$) (6 equiv for **6a**−**d** or 1 equiv for **6e**−**h**) in DMF in the presence of a catalytic amount of DMAP produced the diesters **6a**−**d** in 92−98% yields and the monoesters **6e**−**h** in 37−44% yields. Of the prodrugs tested in rats, the monoisobutyrate **6h** achieved the highest ganciclovir bioavailability (45%) that is 15-fold higher than that from ganciclovir (3%), followed in order by the diisobutyrate **6d** (42%), the diacetate **6a** (41%), the monobutyrate **6g** (41%), the monopropionate **6f** (39%), the dipropionate **6b** (35%), the dibutyrate **6c** (35%), and the monoacetate **6e** (29%). The prodrugs **6e**−**h** were found to be quite stable at pH 6.0 ($t_{1/2}$ = >29 days), 7.4 ($t_{1/2}$ = >7 days), and 8.0 ($t_{1/2}$ = >2 days) but had relatively short half-lives at pH 1.2 ($t_{1/2}$ = 60−83 min).

Introduction

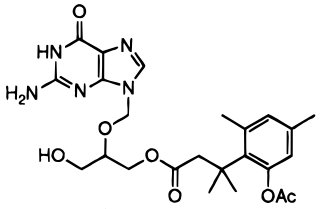
Human cytomegalovirus (HCMV) is a herpesvirus that causes serious infections in immunologically immature or compromised hosts such as neonates, organ transplant recipients, cancer patients, and AIDS patients.[1] CMV retinitis develops in an estimated 20%−30% of AIDS patients; if untreated, it invariably results in retinal necrosis with complete blindness.[2] So far, 9-(1,3-dihydroxy-2-propoxymethyl)guanine (ganciclovir) is the drug of choice for treatment and maintenance therapy of CMV retinitis.[3−6] Recently, the FDA has approved oral ganciclovir as an alternative to iv infusion ganciclovir for maintenance therapy of CMV retinitis in immunocompromised patients, whose retinitis is stable after iv ganciclovir induction therapy.[7,8] The oral formulation is more convenient than the iv formulation, and it avoids catheter-related infections and sepsis. However, the bioavailability of orally administered ganciclovir in humans ranged from 2.6% to 7.3%,[9] although giving the drug with food increases absorption.[10] Thus, the search for a prodrug that is orally well-absorbed and then readily converted to ganciclovir is of high priority. Winkler et al. recently reported that the bioavailability of ganciclovir after oral administration of 2-amino-9-(1,3-diisopropoxy-2-propoxymethyl)purine (HOE 602) was approximately 4-fold higher than that from oral ganciclovir in monkeys.[11] More recently, Dillon et al. reported that the mono-3-(2′-acetoxy-4′,6′-dimethylphenyl)-3,3-dimethylpropanoic ester of ganciclovir, **1**, showed a 4-fold increase in oral bioavailability over the parent drug in rats.[12]



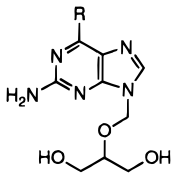
ganciclovir



HOE 602



1



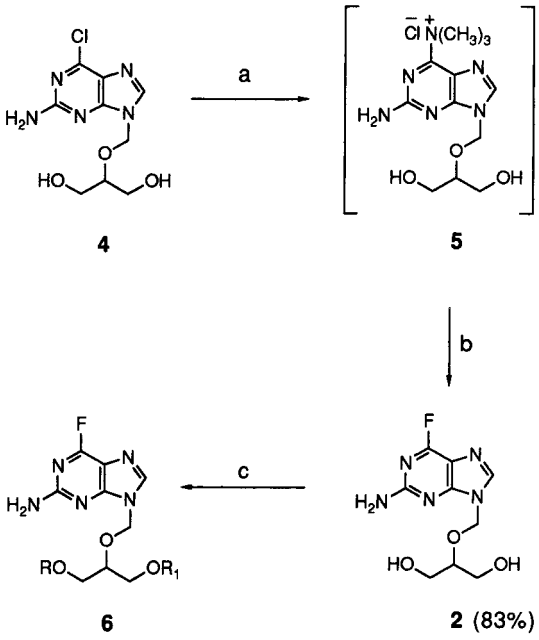
2 : R = F
3 : R = NH_2
4 : R = Cl

In a previous communication, we prepared a 6-fluoropurine acyclonucleoside, 2-amino-9-(1,3-dihydroxy-2-propoxymethyl)-6-fluoropurine (**2**), as a potential prodrug of ganciclovir and demonstrated that **2** was readily converted to ganciclovir in the presence of calf intestinal mucosal adenosine deaminase in phosphate buffer solution.[13] From enzyme kinetic studies, it was found that

* To whom correspondence should be addressed.

10.1021/jm980321+ CCC: $18.00
Published on Web 01/05/1999

Scheme 1[a]



4 **5** **6** **2** (83%)

a : R = R_1 = $COCH_3$ (95%)
b : R = R_1 = COC_2H_5 (93%)
c : R = R_1 = $CO(CH_2)_2CH_3$ (92%)
d : R = R_1 = $COCH(CH_3)_2$ (98%)
e : R = H, R_1 = $COCH_3$ (41%)
f : R = H, R_1 = COC_2H_5 (37%)
g : R = H, R_1 = $CO(CH_2)_2CH_3$ (44%)
h : R = H, R_1 = $COCH(CH_3)_2$ (42%)

[a] (a) $N(CH_3)_3$ (10 equiv), DMF/THF (1/4), −78 °C to rt, 5 days; (b) KF (10 equiv), DMF, 80 °C, 3 h; (c) $(RCO)_2O$ (6 equiv for **6a**−**d** or 1 equiv for **6e**−**h**), DMAP (0.1 equiv), DMF, rt, 1 h.

2 was 7.6 and 12.7 times a more efficient substrate for adenosine deaminase in terms of V_{max}/K_m than the corresponding 6-aminopurine acyclonucleoside **3** and 6-chloropurine acyclonucleoside **4**, respectively.[13] Therefore, in this study, we prepared the mono- and diacyl esters of **2** to maximize its oral bioavailability and evaluated them for their potential as prodrugs of ganciclovir.

Chemistry

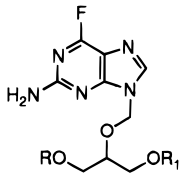
Target compounds, 6-deoxy-6-fluoroganciclovir mono- and diesters **6a**−**h**, were synthesized as shown in Scheme 1, starting from 6-deoxy-6-chloroganciclovir (**4**)[14] which was prepared by a known method. It has been known for years that the 6-chloro atom at the C-6 position of a purine ring could be converted to a fluoro atom by activating the chloro atom to the trimethylammonium salt followed by replacing it with an appropoate fluoride nucleophile.[15] In our earlier work, **4** was treated with excess anhydrous Me_3N in DMF at room temperature, and the isolated trimethylammonium salt **5** was further reacted with excess anhydrous KF in DMF to afford the desired 6-deoxy-6-fluoroganciclovir (**2**) in a somewhat low yield (39%).[13] This rather inefficient conversion attributed to the formation of a major byproduct, 6-deoxy-6-(dimethylamino)ganciclovir, which was mainly produced from the trimethylammonium chloride salt-forming step probably via intramolecular S_N2 displacement by chloride. The trimethylammonium chloride salt **5** was found to be indefinitely stable at room temperature under N_2 atmosphere once it is isolated as a white solid. Thus, it was reasoned that the intramolecular S_N2 side reacton might be facile because the salt **5** is fairly soluble in DMF. Based on this reasoning, it occurred to us that this side reaction might be suppressed if the trimethylammonium chloride salt could be precipitated out completely from the reaction medium by using less polar solvents than DMF. A mixed solvent system, a 4:1 mixture of THF and DMF, served this purpose well, showing only a small amount of the 6-dimethylamino byproduct by TLC. After the complete salt formation, the solvents were decanted, and the residual solvents were removed thoroughly in vacuo. Resulting crude salts were subsequently treated with excess anhydrous KF in DMF at 80 °C to afford **2** in an improved yield of 83%. Reactions of **2** with an appropriate acid anhydride (Ac_2O, $(EtCO)_2O$, $(n\text{-}PrCO)_2O$, or $(i\text{-}PrCO)_2O$) (6 equiv for **6a**−**d** or 1 equiv for **6e**−**h**) in DMF in the presence of a catalytic amount of DMAP at room temperature produced the diesters **6a**−**d** in 92−98% yields and the monoesters **6e**−**h** in 37−44% yields.

Results and Discussion

The in vitro antiviral activity and cytotoxicity of the prodrugs **2** and **6a**−**h** along with ganciclovir and HOE 602 against HCMV (AD-169) in human embryonic lung fibroblast (HEL) 299 cells were evaluated (Table 1). As was expected, all the prodrugs showed no significant antiviral activity at concentrations up to 100 μM, while ganciclovir was active against HCMV replication with an EC_{50} value of 0.63 μM. None of the prodrugs showed cytotoxicity to HEL 299 cells at the maximum concentration of 100 μM. We evaluated the bioavailability of ganciclovir after a single oral administration (0.1 mmol/kg) of the prodrugs **2** and **6a**−**h** in rats and compared them with those from ganciclovir and HOE 602. The total amount of ganciclovir recovered in the urine over a 48-h period was determined by reversed-phase HPLC. The mean urinary recovery of ganciclovir from **2** (8%) was approximately 3-fold higher than that from ganciclovir (3%) but was approximately one-half that from HOE 602 (14%). However, its mono- and diesters **6a**−**h** showed significantly higher bioavailability as compared with ganciclovir and HOE 602. The monoisobutyrate **6h** achieved the highest ganciclovir bioavailability (45%): 15- and 3-fold higher than those from ganciclovir and HOE 602, respectively. The diisobutyrate **6d** (42%), the diacetate **6a** (41%), the monobutyrate **6g** (41%), the monopropionate **6f** (39%), the dipropionate **6b** (35%), the dibutyrate **6c** (35%), and the monoacetate **6e** (29%) also showed 10−14-fold higher mean urinary recovery of ganciclovir compared with that from ganciclovir.

Among the ester derivatives **6a**−**h**, **6e** was the most soluble in H_2O (44.4 mg/mL) at 25 °C, showing a remarkable increase in aqueous solubility compared with those of the parent compound **2** (11.3 mg/mL) and ganciclovir (4.3 mg/mL). **6f** (19.0 mg/mL) and **6g** (19.4 mg/mL) also showed fair increases in water solubility. Although the water solubility of **6h** (5.7 mg/mL) and **6a** (4.2 mg/mL) was comparable to that of ganciclovir, the other diester derivatives **6b**−**d** were quite less soluble in H_2O (<1.0 mg/mL). The aqueous stability of **2** and **6a**−**h** was examined at pH 1.2, 6.0, 7.4, and 8.0 at 37 °C, and the calculated half-lives ($t_{1/2}$) are shown in Table 1. The prodrugs **6a**−**h** were found to be quite stable at

Table 1. Solubility and Stability in Aqueous Solution, in Vitro Anti-HCMV Activity in HEL Cells, and Oral Bioavailability in Rats of 2-Amino-9-(1,3-dihydroxy-2-propoxymethyl)-6-fluoropurine Mono- and Diesters **6a**−**h**



6a−**h**

compd	R	R_1	solubility in H_2O[a] (mg/mL, 25 °C)	half-life[b] (37 °C) pH 1.2[c]	pH 6.0[d]	pH 7.4[d]	pH 8.0[d]	EC_{50}[e] (μM) AD-169	CC_{50}[f] (μM) HEL 299	urinary recovery of ganciclovir (% dose)[g]
6a	$COCH_3$	$COCH_3$	4.2	62 min	30 days	8 days	3 days	>100.0[h]	>100.0[h]	41[i]
6b	COC_2H_5	COC_2H_5	0.7	83 min	35 days	7 days	2 days	>100.0	>100.0	35
6c	$CO(CH_2)_2CH_3$	$CO(CH_2)_2CH_3$	0.6	65 min	39 days	11 days	4 days	>100.0	>100.0	35
6d	$COCH(CH_3)_2$	$COCH(CH_3)_2$	0.4	74 min	29 days	7 days	5 days	>100.0	>100.0	42
6e	H	$COCH_3$	44.4	61 min	56 days	14 days	7 days	>100.0	>100.0	29
6f	H	COC_2H_5	19.0	60 min	37 days	17 days	7 days	>100.0	>100.0	39
6g	H	$CO(CH_2)_2CH_3$	19.4	69 min	51 days	21 days	12 days	>100.0	>100.0	41
6h	H	$COCH(CH_3)_2$	5.7	65 min	56 days	32 days	14 days	>100.0	>100.0	45
2			11.3	62 min	107 days	69 days	30 days	>100.0	>100.0	8
ganciclovir			4.3					0.63	>100.0	3
HOE 602										14

[a] Determined by the comparison of the UV absorbance of the saturated solution of each compound at 245 nm with that of the standard curve. [b] Determined by HPLC using a C_{18} reversed-phase column. [c] HCl/NaCl buffer. [d] Sodium phosphate buffer. [e] Concentration required to reduce the number of plaques by 50% of the virus-infected control as determined by plaque-reduction assay. [f] Concentration required to reduce the OD value by 50% of the cell control as determined by MTT assay. [g] A single oral dose of the test compound (0.1 mmol/kg) was administered to two male SD rats. The total amount of ganciclovir recovered in the urine over a 48-h period was determined by HPLC using a C_{18} reversed-phase column. [h] Values are the mean of at least two independent experiments run in quadruplicate. [i] Values are the mean of two independent experiments.

pH 6.0 ($t_{1/2}$ = >29 days), 7.4 ($t_{1/2}$ = >7 days), and 8.0 ($t_{1/2}$ = >2 days), although the stability of the prodrugs decreased with increasing pH. Among **6a**−**h**, 6h was the most stable, and the parent compound **2** was more stable at these buffer solutions than the ester derivatives **6a**−**h**. However, the prodrugs **6a**−**h** were relatively unstable at pH 1.2, showing $t_{1/2}$ values of 60−83 min. These values were similar to that for **2** ($t_{1/2}$ = 62 min), indicating that the fluoro atom at the C-6 of the purine ring is relatively sensitive to undergo hydrolysis in the acid buffer solution.

In conclusion, 2-amino-6-fluoro-9-(1-isobutyryloxy-3-hydroxy-2-propoxymethyl)purine (**6h**) showed the highest oral ganciclovir bioavailability in rats in a series of 2-amino-9-(1,3-dihydroxy-2-propoxymethyl)-6-fluoropurine mono- and diesters that was 15-fold higher than that from ganciclovir. On the basis of these results, extensive studies on pharmacokinetics, enteric-coating formulation, and toxicology of **6h** are presently underway in our laboratory.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. ^{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 $CDCl_3$ or DMSO-d_6. Electron impact mass spectra (EI-MS) were obtained on a VG Quattro mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60F-254 glass plates. Medium-pressure chromatography (MPLC) was performed using Merck silica gel 60 (230−400 mesh) with a VSP-2200 ceramic pump (Eyela). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer. Where indicated by the symbols of the elements, analyses were within ±0.4% of theoretical values.

2-Amino-9-(1,3-dihydroxy-2-propoxymethyl)-6-fluoropurine (2). To a stirred solution of 2-amino-6-chloro-9-(1,3-dihydroxy-2-propoxymethyl)purine (**4**) (12.3 g, 45 mmol) in a mixture of anhydrous DMF (100 mL) and anhydrous THF (400 mL) at −78 °C was added anhydrous Me_3N (26.2 g, 444 mmol, 40 mL) precooled to −78 °C via a cannula. The mixture was allowed to warm to room temperature and stirred for 5 days. The organic solvent was decanted off, and the white precipitate and the residual solvent were evaporated to dryness in vacuo to give the crude trimethylammonium chloride salt **5**. A stirred suspension of **5** and anhydrous KF (26.1 g, 449 mmol) in anhydrous DMF (400 mL) was heated at 80 °C for 3 h under an aspirator pressure (~30 mmHg) to release the resulting Me_3N. The reaction mixture was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in hot MeOH and stored in a refrigerator to afford 7.90 g (68%) of **2** as white crystals. The filtrate was concentrated, and the residue was purified by MPLC over SiO_2 with 15% MeOH/$CHCl_3$ as eluent to afford an additional 1.69 g (15%) of **2**: physical and spectral data were identical with those reported previously.[13]

General Procedure for the Preparation of 2-Amino-9-(1,3-dihydroxy-2-propoxymethyl)-6-fluoropurine Mono- and Diesters. To a stirred solution of **2** (1.03 g, 4.0 mmol) in anhydrous DMF (40 mL) were added DMAP (49 mg, 0.4 mmol) and an appropriate acid anhydride (6 equiv for **6a**−**d** or 1 equiv for **6e**−**h**), and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated to dryness in vacuo, and the residue was purified by MPLC over SiO_2. Acid anhydride used, eluent for MPLC, yield, physical appearance, and spectral data are given below.

2-Amino-9-(1,3-diacetoxy-2-propoxymethyl)-6-fluoropurine (6a): Ac_2O; 10% MeOH/$CHCl_3$; 95%; mp 113.2−113.9 °C (EtOAc−hexane); IR (KBr) 3470, 3303, 3201, 3124, 1753, 1734 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 6 H, 2 CH_3), 4.08 (m, 3 H, CH and CH_2OAc), 4.19 (m, 2 H, CH_2OAc), 5.17 (s, 2 H, NH_2), 5.29 (s, 2 H, NCH_2O), 7.88 (s, 1 H, H-8); EI-MS *m*/*z* 341 (M^+). Anal. ($C_{13}H_{16}FN_5O_5$) C, H, N.

2-Amino-9-(1,3-dipropionyloxy-2-propoxymethyl)-6-fluoropurine (6b): $(EtCO)_2O$; 10% MeOH/$CHCl_3$; 93%; mp 101.5−102.6 °C (EtOAc−hexane); IR (KBr) 3415, 3334, 3228, 1748 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.10 (t, J = 7.5 Hz, 6 H, 2 CH_2-CH_3), 2.25 (q, J = 7.5 Hz, 4 H, 2 CH_2CH$_3$), 4.08 (m, 3 H, CH and CH_2OCO), 4.21 (m, 2 H, CH_2OCO), 5.18 (s, 2 H, NH_2),

5.59 (s, 2 H, NCH_2O), 7.88 (s, 1 H, H-8); EI-MS *m*/*z* 369 (M^+). Anal. ($C_{15}H_{20}FN_5O_5$) C, H, N.

2-Amino-9-(1,3-dibutyryloxy-2-propoxymethyl)-6-fluoropurine (6c): (n-PrCO)$_2$O; 10% MeOH/$CHCl_3$; 92%; mp 106.2−108.0 °C (EtOAc−hexane); IR (KBr) 3419, 3329, 3213, 1734 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (t, J = 7.5 Hz, 6 H, 2 $COCH_2CH_2CH_3$), 1.60 (qt, J = 7.5 Hz, J = 7.5 Hz, 4 H, $COCH_2CH_2CH_3$), 2.21 (t, J = 7.5 Hz, 4 H, 2 $COCH_2$), 4.08 (m, 3 H, CH and CH_2OCO), 4.21 (m, 2 H, CH_2OCO), 5.18 (s, 2 H, NH_2), 5.59 (s, 2 H, NCH_2O), 7.87 (s, 1 H, H-8); EI-MS *m*/*z* 397 (M^+). Anal. ($C_{17}H_{24}FN_5O_5$) C, H, N.

2-Amino-9-(1,3-diisobutyryloxy-2-propoxymethyl)-6-fluoropurine (6d): (i-PrCO)$_2$O; 10% MeOH/$CHCl_3$; 98%; mp 107.5−107.9 °C (EtOAc−hexane); IR (KBr) 3500, 3331, 3210, 1732 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.11 (d, J = 6.9 Hz, 6 H, 2 $CHCH_3$), 1.13 (d, J = 6.9 Hz, 6 H, 2 $CHCH_3$), 2.47 (septet, J = 6.9 Hz, 2 H, 2 $CH(CH_3)_2$), 4.08 (m, 3 H, CH and CH_2OCO), 4.23 (m, 2 H, CH_2OCO), 5.16 (s, 2 H, NH_2), 5.59 (s, 2 H, NCH_2O), 7.88 (s, 1 H, H-8); EI-MS *m*/*z* 397 (M^+). Anal. ($C_{17}H_{24}FN_5O_5$) C, H, N.

2-Amino-9-(1-acetoxy-3-hydroxy-2-propoxymethyl)-6-fluoropurine (6e): Ac_2O; 10% MeOH/$CHCl_3$, then 20% MeOH/$CHCl_3$; 41%; mp 115.0−116.5 °C (EtOAc−hexane); IR (KBr) 3419, 3316, 3201, 1740 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.80 (s, 3 H, CH_3), 3.41 (m, 2 H, CH_2OH), 3.82 (m, 1 H, CH), 3.87 (dd, J = 11.1 Hz, J = 6.9 Hz, 1 H, $CHOAc$), 4.06 (dd, J = 11.1 Hz, J = 2.4 Hz, 1 H, $CHOAc$), 4.84 (t, J = 5.6 Hz, 1 H, OH), 5.53 (d, J = 11.4 Hz, 1 H, NCHO), 5.55 (d, J = 11.4 Hz, 1 H, NCHO) 6.96 (s, 2 H, NH_2), 8.22 (s, 1 H, H-8); EI-MS *m*/*z* 299 (M^+). Anal. ($C_{11}H_{14}FN_5O_4$) C, H, N.

2-Amino-6-fluoro-9-(1-propionyloxy-3-hydroxy-2-propoxymethyl)purine (6f): ($EtCO)_2O$; 10% MeOH/$CHCl_3$, then 20% MeOH/$CHCl_3$; 37%; mp 120.5−122.5 °C (EtOAc−hexane); IR (KBr) 3483, 3331, 3213, 1737 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.89 (t, J = 7.5 Hz, 3 H, CH_2CH_3), 2.06 (q, J = 7.5 Hz, 2 H, CH_2CH_3), 3.42 (m, 2 H, CH_2OH), 3.82 (m, 1 H, CH), 3.88 (dd, J = 11.1 Hz, J = 6.9 Hz, 1 H, CHOCO), 4.08 (dd, J = 11.1 Hz, J = 2.4 Hz, 1 H, CHOCO), 4.84 (t, J = 5.6 Hz, 1 H, OH), 5.53 (d, J = 11.4 Hz, 1 H, NCHO), 5.55 (d, J = 11.4 Hz, 1 H, NCHO) 6.96 (s, 2 H, NH_2), 8.22 (s, 1 H, H-8); EI-MS *m*/*z* 313 (M^+). Anal. ($C_{12}H_{16}FN_5O_4$) C, H, N.

2-Amino-9-(1-butyryloxy-3-hydroxy-2-propoxymethyl)-6-fluoropurine (6g): (n-PrCO)$_2$O; 10% MeOH/$CHCl_3$, then 20% MeOH/$CHCl_3$; 44%; mp 120.2−120.6 °C (EtOAc−hexane); IR (KBr) 3316, 3252, 3201, 1747, 1728 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.79 (t, J = 7.4 Hz, 3 H, $COCH_2CH_2CH_3$), 1.36 (qt, J = 7.4 Hz, J = 7.3 Hz, 2 H, $COCH_2CH_2CH_3$), 1.99 (t, J = 7.3 Hz, 2 H, $COCH_2$), 3.42 (m, 2 H, CH_2OH), 3.82 (m, 1 H, CH), 3.87 (dd, J = 11.1 Hz, J = 6.9 Hz, 1 H, CHOCO), 4.07 (dd, J = 11.1 Hz, J = 2.4 Hz, 1 H, CHOCO), 4.85 (t, J = 5.6 Hz, 1 H, OH), 5.53 (d, J = 11.4 Hz, 1 H, NCHO), 5.55 (d, J = 11.4 Hz, 1 H, NCHO) 6.96 (s, 2 H, NH_2), 8.22 (s, 1 H, H-8); EI-MS *m*/*z* 327 (M^+). Anal. ($C_{13}H_{18}FN_5O_4$) C, H, N.

2-Amino-6-fluoro-9-(1-isobutyryloxy-3-hydroxy-2-propoxymethyl)purine (6h): (i-PrCO)$_2$O; 10% MeOH/$CHCl_3$, then 20% MeOH/$CHCl_3$; 42%; mp 125.4−125.8 °C (EtOAc−hexane); IR (KBr) 3483, 3329, 3201, 1728 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.92 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 0.93 (d, J = 6.9 Hz, 3 H, $CHCH_3$), 2.28 (septet, J = 6.9 Hz, 1 H, $CH(CH_3)_2$), 3.43 (m, 2 H, CH_2OH), 3.82 (m, 1 H, CH), 3.88 (dd, J = 11.1 Hz, J = 6.9 Hz, 1 H, CHOCO), 4.08 (d, J = 11.1 Hz, 1 H, CHOCO), 4.85 (t, J = 5.5 Hz, 1 H, OH), 5.54 (s, 2 H, NCH_2O), 6.95 (s, 2 H, NH_2), 8.23 (s, 1 H, H-8); EI-MS *m*/*z* 327 (M^+). Anal. ($C_{13}H_{18}FN_5O_4$) C, H, N.

Water Solubility. The aqueous solubility of the test compound was measured as described previously.[16]

Aqueous Stability. The analytical HPLC samples of the test compound were prepared as described previously[16] and eluted at a flow rate of 1 mL/min with the following three-step gradient: (step 1) a 10-min isocratic elution with 100% buffer A (0.1% phosphoric acid), (step 2) a 25-min linear gradient from 100% buffer A to 55% buffer A and 45% buffer B (80% MeCN in 0.1% phosphoric acid), and (step 3) a 4-min isocratic elution with 55% buffer A and 45% buffer B. A C_{18} reversed-phase column was equilibrated with 100% buffer A for 10 min before each sample injection. The UV absorbance of the column effluent was monitored at 245 nm. The half-life of the compound was calculated from the concentration of the parent compound.

In Vitro Cytotoxicity. HEL 299 cells obtained from American Type Culture Collection (ATCC, Manassas, VA) were seeded at 1×10^4 cells/well into 96-well microtiter plates and allowed to proliferate for 24 h in Eagle's minimum essential medium (EMEM; Gibco, Gaithersburg, MD). The test compound at various concentrations was added to each well. After the plates were incubated at 37 °C in a humidified incubator containing 5% CO_2 for 4 days, 20 μL of MTT dissolved in phosphate-buffered saline (PBS) (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 h. Thereafter, the incubation plates were centrifuged at 1000 rpm for 10 min in a plate holder, and then the majority of the media was aspirated, leaving 60 μL of media in each well. To solubilize the formazan crystals that formed, 240 μL of DMSO was added to each well, and the plates were placed on a plate shaker for 5 min. The optical density (OD) was measured immediately at 540 nm using an ELISA reader (Dynatech, MR 5000). Cytotoxicity was expressed as a 50% cytotoxic concentration (CC_{50}), the concentration required to reduce the OD by 50% of cell control, which was calculated from a dose−response curve plotted with probit analysis. Each experiment was performed in quadruplicate and repeated twice.

In Vitro Antiviral Activity. The in vitro antiviral activity was tested by plaque-reduction assay. Confluent cells in 24-well multidish plates were infected with about 50 plaque forming units (PFU) of HCMV (AD-169) in culture medium per well. After a 1-h adsorption period at 37 °C in a highly humidified incubator containing 5% CO_2, the medium containing residual virus was removed, and 1 mL of EMEM containing 2% fetal bovine serum (FBS), 1% methyl cellulose, and the test compound at various concentrations was added to each well. After a 10-day incubation at 37 °C in a highly humidified incubator containing 5% CO_2, plaques were fixed with methanol, stained with crystal violet, and counted. Antiviral activity was expressed as a 50% effective concentration (EC_{50}), the concentration required to reduce the number of plaques by 50% of the virus-infected control, which was calculated from a dose−response curve plotted with probit analysis. Each experiment was performed in quadruplicate and repeated twice.

Oral Bioavailability. The bioavailability of the test compound was estimated by determining the total amount of ganciclovir in the urine using HPLC. Urine was collected for 48 h in a metabolic cage after oral administration of a single 0.1 mmol/kg dose of the test compound to two male Sprague−Dawley rats (200−250 g). A 5% solution of sodium azide (0.4 mL per estimated 100 mL of urine) was added to each urine receptacle before collection to prevent bacterial growth. The collected urine was filtered (0.45 μm), and the ganciclovir concentration was analyzed by HPLC. A C_{18} reversed-phase column equipped with a compatible guard column was eluted at a flow rate of 1 mL/min with the same three-step gradient as used for aqueous stability.

References

(1) Alford, C. A.; Britt, W. J. Cytomegalovirus. In *Fields Virology*, 2nd ed.; Fields, B. N., Knipe, D. M., Eds.; Raven Press: New York, 1990; pp 1981−2010.

(2) Luckie, A.; Ai, E. Diagnosis and Management of Cytomegalovirus Retinitis in AIDS. *Curr. Opin. Ophthalmol.* **1993**, *4*, 81−89.

(3) Laskin, O. L.; Cederberg, D. M.; Mills, J.; Eron, L. J.; Mildvan, D.; Spector, S. S. Ganciclovir for the Treatment and Suppression of Serious Infections Caused by Cytomegaloviruses. *Am. J. Med.* **1987**, *83*, 201−207.

(4) Buhles, W. C.; Mastre, B. J., Jr.; Tinker, A. J. Ganciclovir Tratment of Life- or Sight-threatening Cytomegalovirus Infection: Experience in 314 Immunocompromised Patients. *Rev. Infect. Dis.* **1988**, *10* (Suppl. 3), S495−506.

(5) Markham, A.; Faulds, D. Ganciclovir. An Update of its Therapeutic Use in Cytomegalovirus Infection. *Drugs* **1994**, *48*, 455−484.

(6) Hall, A. J.; Jennens, I. D.; Lucas, C. R.; MacLean, H.; Sandland, A. M. Low Frequency Maintenance Ganciclovir for Cytomegalovirus Retinitis. *Scand. J. Infect. Dis.* **1991**, *23*, 43−46.
(7) Lalczari, J. P. Oral vs Intravenous Ganciclovir (GCV) as Maintenance Treatment of Newly Diagnosed Cytomegalovirus Retinitis (CMVR) in AIDS. *Antiviral Res.* **1994**, *23* (Suppl. 1), 45.
(8) Drew, W. L.; Ives, D.; Lalezari, J. P.; Crumpacker, C.; Follansbee, S. E.; Spector, S. A.; Benson, C. A.; Friedberg, D. N.; Hubbard, L. Oral Ganciclovir as Maintenance Treatment for Cytomegalovirus Retinitis in Patients with AIDS. *N. Engl. J. Med.* **1995**, *333*, 615−620.
(9) Spector, S. A.; Busch, D. F.; Follansbee, S.; Squires, K.; Lalezari, J. P.; Jacobson, M. A.; Connor, J. D.; Jung, D.; Shadman, A.; Mastre, B.; Buhles, W.; Drew, W. L. Pharmacokinetics, Safety, and Antiviral Profiles of Oral Ganciclovir in Persons Infected with Human Immunodeficiency Virus: A Phase I−II Study. *J. Infect. Dis.* **1995**, *171*, 1431−1437.
(10) Byrne, A. K.; Lavelle, J.; Follansbee, S.; Trapnell, C. B.; Jung, D.; Fratis, A.; Scofield, C.; Buhles, W. Multiple Dose Crossover Pharmacokinetic Study to Evaluate the Effect of Food on the Relative Bioavailability of Oral Ganciclovir. Eighth International Conference on AIDS and the Third STD World Congress, Amsterdam, The Netherlands, July 19−24, 1992; Abstract PuB 7077.
(11) Winkler, I.; Winkelmann, E.; Scholl, T.; Rosner, M.; Jahne, G.; Helsberg, M. Antiviral Activity and Pharmacokinetics of HOE 602, an Acyclic Nucleoside, in Animal Models. *Antiviral Res.* **1990**, *14*, 61−74.
(12) Dillon, M. P.; Cai, H.; Maag, H. Application of the "Trimethyl Lock" to Ganciclovir, a Pro-Prodrug with Increased Oral Bioavailability. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1653−1656.
(13) Kim, D.-K.; Kim, H.-K.; Chae, Y.-B. Design and Synthesis of 6-Fluoropurine Acyclonucleosides: Potential Prodrugs of Acyclovir and Ganciclovir. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1309−1312.
(14) Ogilvie, K. K.; Nguyen-ba, N.; Gillen, M. F.; Radatus, B. K.; Cheriyan, U. O. Synthesis of a Purine Acyclonucleoside Series Having Pronounced Antiviral Activity. The Glyceropurines. *Can. J. Chem.* **1984**, *62*, 241−252.
(15) Robins, M. J.; Basom, G. L. Nucleic Acid Related Compounds. 8. Direct Conversion of 2′-Deoxyinosine to 6-Chloropurine 2′-Deoxyriboside and Selected 6-Substituted Deoxynucleosides and Their Evaluation as Substrates of Adenosine Deaminase. *Can. J. Chem.* **1973**, *51*, 3161−3169.
(16) Kim, D.-K.; Lee, N.; Kim, Y.-W.; Chang, K.; Kim, J.-S.; Im, G.-J.; Choi, W.-S.; Jung, I.; Kim, T.-S.; Hwang, Y.-Y.; Min, D.-S.; Um, K. A.; Cho, Y.-B.; Kim, K. H. Synthesis and Evaluation of 2-Amino-9-(3-hydroxymethyl-4-alkoxycarbonyloxybut-1-yl)purines as Potential Prodrugs of Penciclovir. *J. Med. Chem.* **1998**, *41*, 3435−3441.

JM980321+