Synthesis and Evaluation of 2-Amino-9-(3-hydroxymethyl-4-alkoxycarbonyloxybut-1-yl)purines as Potential Prodrugs of Penciclovir

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A series of 2-amino-9-(3-hydroxymethyl-4-alkoxycarbonyloxybut-1-yl)purines (**4**−**10**) and 2-amino-9-(2-(2-oxo-1,3-dioxan-5-yl)ethyl)purine (**1**) were synthesized as potential prodrugs of penciclovir and evaluated for their oral penciclovir bioavailability in mice and rats. Treatment of 2-(2-benzyloxyethyl)propane-1,3-diol (**11**) with 1,1′-carbonyldiimidazole in THF followed by hydrogenolytic removal of the benzyl group of the resulting cyclic carbonate **12** gave 5-(2-hydroxyethyl)-1,3-dioxan-2-one (**13**). Mesylation of the alcohol **13** and then a coupling reaction of the resulting mesylate **14** with 2-amino-6-chloropurine using anhydrous Cs_2CO_3 in DMF afforded 2-amino-6-chloro-9-(2-(2-oxo-1,3-dioxan-5-yl)ethyl)purine (**16**) after purification by flash column chromatography on silica gel using EtOAc/MeCN/Et_3N as eluent. Hydrogenation of the 6-chloro cyclic carbonate **16** followed by a ring-opening reaction of the 6-deoxy cyclic carbonate **1** in a mixture of an appropriate alcohol and $CHCl_3$ using activated SiO_2 as a Lewis acid afforded the corresponding alkyl monocarbonate derivatives **3**−**10** in fair to good yields. Of the prodrugs tested in mice, the isopropyl monocarbonate **6** achieved the highest mean urinary recovery of penciclovir (53%), followed in order by the propyl monocarbonate **5** (51%), the isopentyl monocarbonate **10** (51%), the ethyl monocarbonate **4** (50%), and famciclovir (48%). In rats, the methyl monocarbonate **3**, **4**, **6**, the *n*-butyl monocarbonate **7**, and **10** (39−41%) showed levels of mean urinary recovery of penciclovir similar to that from famciclovir (40%). The alkyl monocarbonates **4**−**10** were found to be quite stable in the aqueous buffer solutions, and among them, **6** was the most stable with the half-lives ($t_{1/2}$) of 88, >200, 61, and 26 days at pH 1.2, 6.0, 7.4, and 8.0, respectively. In addition, **6** was highly soluble in H_2O (138.8 mg/mL, 20 °C).

Introduction

An acyclonucleoside 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (penciclovir) is a potent and highly selective inhibitor of the replication of herpesviruses including herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV) in cell cultures and in animals,[1−4] and of hepatitis B virus (HBV) and duck hepatitis B virus (DHBV) in cell cultures.[5,6] The antiviral spectrum of penciclovir against human herpesviruses is similar to that of 9-(2-hydroxyethoxymethyl)guanine (acyclovir), and both compounds have comparable activity against these viruses.[7] The advantage of penciclovir over acyclovir is that its antiviral activity in cell culture is more pesistent than that of acyclovir since penciclovir triphosphate is much more stable than acyclovir triphosphate within infected cells.[2,8]

However, like other acyclic nucleoside analogues such as acyclovir,[9] ganciclovir,[10] and buciclovir,[11] penciclovir has poor oral bioavailability in mice and rats.[12,13] To overcome this inadequate oral bioavailability, 2-amino-9-(4-acetoxy-3-acetoxymethylbut-1-yl)purine (famciclovir), the diacetyl 6-deoxy analogue of penciclovir, has been developed as a prodrug of penciclovir.[12] Famciclovir is orally well absorbed and then extensively converted to penciclovir by the enzymatic removal of two *O*-acetyl groups, followed by oxidation at the 6-position of the purine ring by xanthine oxidase in mice,[12] rats,[13] and humans.[14] Famciclovir has recently been approved by FDA for the treatment of herpes zoster (shingles) and acute recurrent genital herpes. Famciclovir has been reported to inhibit DHBV replication in chronically infected ducks,[15] to control HBV replication effectively in liver transplant patients,[16,17] and to inhibit HBV replication in a double-blind, placebo-controlled, single-center clinical trial in patients with chronic HBV infection.[18] A large, multicenter trial of famciclovir against chronic HBV infection is currently in progress.

In a previous report, Harnden et al. attempted to synthesize the cyclic carbonate **1** as a potential prodrug of penciclovir.[12] However, treatment of 6-deoxypenciclovir **2** with tetramethyl orthocarbonate gave the methyl monocarbonate 3 in a low yield (22%) instead of the desired cyclic carbonate **1**.[12] When **3** was evaluated for its bioavailability in mice, it achieved maximum concentration of penciclovir in the blood that was comparatively lower (73%) than that from famciclovir, but 11 times higher than that from penciclovir when assayed after administration of the equivalent oral doses of each compound.[12] On the basis of these findings, it was of particular interest to us to determine whether introduction of a higher alkyl carbonate group rather

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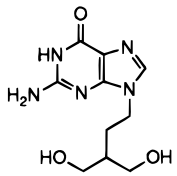
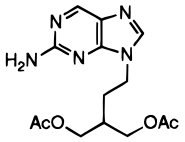
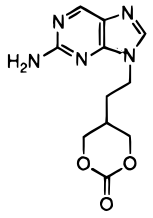


penciclovir



famciclovir



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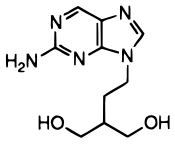

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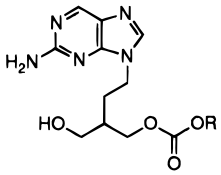


3 : R = Me **7** : R = *n*-Bu
4 : R = Et **8** : R = *i*-Bu
5 : R = *n*-Pr **9** : R = *n*-pentyl
6 : R = *i*-Pr **10** : R = isopentyl

than that of a methyl carbonate group into one of the two hydroxyl groups of **2** could increase its oral bioavailability. These alkyl monocarbonates are expected to be more efficiently converted to penciclovir as compared with famciclovir after gastrointestinal absorption since they have only one carbonate group to be hydrolyzed in an acyclic moiety of the molecule.

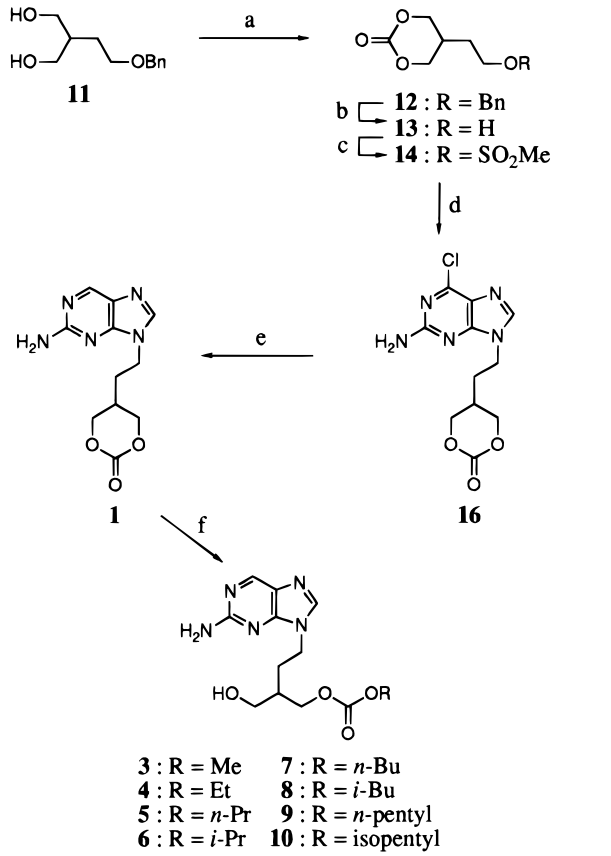
In this report, we describe the synthesis of the cyclic carbonate **1** and the C_2-C_5 alkyl monocarbonates **4**−**10** as potential prodrugs of penciclovir and their physical properties and oral bioavailability in mice and rats.

Chemistry

First, we have investigated the possibility of converting the diol **2** into the desired monocarbonate derivatives, which seemed to be the most obvious approach since the starting 2 can be readily prepared by using well-documented procedures.[1,12] Several reactions of **2** with ethyl chloroformate (1 equiv) were carried out by using an amine base (Et_3N or 2,6-lutidine) in DMF solvent or using pyridine as a base and solvent. These reactions were rather complex and unfruitful. Thus it was conceived that the carbonate group must be introduced to the acyclic portion prior to being coupled with the purine moiety. Cyclic carbonate was chosen because it can act as a protecting group for the diol functionality, and it can also undergo ring-opening reactions to afford the target monocarbonate compounds.

As shown in Scheme 1, the synthesis of the target carbonate compounds **1** and **4**−**10** began with the known diol **11**[19] which was prepared from diethyl malonate and *O*-benzyl-2-bromoethanol using a known procedure. It has been reported that the formation of cyclic carbonate from a diol can be easily accomplished by using 1,1′-carbonyldiimidazole or triphosgene under

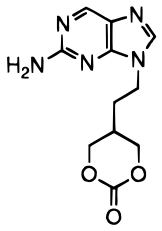
Scheme 1[a]



[a] (a) 1,1′-carbonyldiimidazole, THF, reflux, 8 h; (b) H_2 (1 atm), 10% Pd/C, THF, room temperature, 3 h; (c) MsCl, Et_3N, CH_2Cl_2, 0 °C, 1 h; (d) 2-amino-6-chloropurine, Cs_2CO_3, DMF, room temperature, 16 h; (e) H_2 (1 atm), 10% Pd/C, Et_3N, MeCN/DMF, room temperature, 3 h; (f) ROH, SiO_2, $CHCl_3$, 70 °C, 4 h (for **3**−**5** and **7**−**10**) or 24 h (for **6**).

various reaction conditions. We performed several reactions on a small scale using 1,1′-carbonyldiimidazole or triphosgene with 2,6-lutidine as a base in THF or benzene as solvents. Both reagents (1,1′-carbonyldiimidazole and triphosgene) showed comparable results; fairly good yields of 80% and 85%, respectively, were obtained in THF, and lower yields of 64% and 59%, respectively, were obtained in benzene. However, the large-scale reaction with 1,1′-carbonyldiimidazole was more reproducible in yields (79−80%) than that with triphosgene (61%). Therefore, 1,1′-carbonyldiimidazole was selected as the choice of reagent for the carbonylation of the diol **11**, and the cyclic carbonate **12** was prepared in 79% yield. Removal of the benzyl group in the cyclic carbonate **12** proceeded smoothly under hydrogenolytic condition (1 atm of H_2, THF, room temperature) to give the corresponding alcohol **13** in almost quantitative yield. Treatment of the alcohol **13** with methanesulfonyl chloride (MsCl) using Et_3N as a base in CH_2Cl_2 at 0 °C afforded the mesylate **14** in a good yield of 84%. With the mesylate **14** in hand, the coupling reaction of 2-amino-6-chloropurine with the mesylate **14** using anhydrous cesium carbonate (Cs_2CO_3) in DMF was first carried out to see if the cyclic carbonate group would survive under the basic reaction conditions. Gratifyingly, this reaction worked well (TLC analysis), and the methyl monocarbonate **15**, instead of the desired cyclic carbonate **16**, was isolated in 50%

Table 1. Solubility and Stability in Aqueous Solution, In Vitro Anti-HSV-1 Activity, and Oral Bioavailability of 2-Amino-9-(2-(2-oxo-1,3-dioxan-5-yl)ethyl)purine (**1**) and 2-Amino-9-(3-hydroxymethyl-4-alkoxycarbonyloxybut-1-yl)purines **3**−**10**



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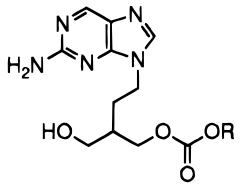
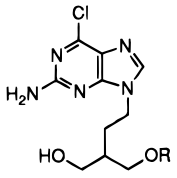


3 – 10

compd	R	solubility[a] (mg/mL, 20 °C)	half-life (day, 37 °C)[b] pH 1.2[c]	pH 6.0[d]	pH 7.4[d]	pH 8.0[d]	EC_{50} (μM)[e] HSV-1 (KOS)	CC_{50} (μM)[f] Vero	urinary recovery of penciclovir[g] (% dose)[h] mouse	rat
1		9.9	0	2.6	0.12	0.07	>125[i]	>500[i]	27	15
4	Et	20.0	35	112	7	3	>125	>500	50	40
5	*n*-Pr	68.0	29	89	10	4	>125	>500	51	34
6	*i*-Pr	138.8	88	>200	61	26	>125	>500	53	40
7	*n*-Bu	15.0	29	74	11	5	>125	>500	38	41
8	*i*-Bu	1.5	28	87	17	8	>125	>500	38	37
9	*n*-pentyl	4.2	25	17	7	5	>125	>500	47	38
10	isopentyl	5.6	24	65	11	6	>125	357	51	40
3	Me	14.1	23	18	1	0.5	>125	>500	45	39
famciclovir							>125	>500	48	40
penciclovir							8.65	>500	3	9

[a] Determined by the comparison of the UV absorbance of the saturated solution of each compound at 290 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 OD value by 50% of the virus-infected control. [f] Concentration required to reduce the OD value by 50% of the control. [g] A single oral dose of the test compound (0.2 mmol/kg) was administered to six male ICR mice or to six male Sprague-Dawley rats. The total amount of penciclovir recovered in the urine over a 48-h period was determined by HPLC using a C_{18} reversed-phase column. [h] Mean values of six animals. [i] Mean values of two independent experiments run in quadruplicate.

yield after flash column chromatography on silica gel using 10% MeOH in $CHCl_3$. This unexpected product could have resulted from the ring opening of the desired cyclic carbonate product **16** by MeOH of the eluent. This accidental opening of the cyclic carbonate **16** could be avoided by using a different solvent system as eluent. Thus, the alkylation of 2-amino-6-chloropurine with mesylate **14** in DMF at room temperature using anhydrous Cs_2CO_3 and purification by flash column chromatography on silica gel using EtOAc/MeCN/Et_3N as eluent afforded the desired cyclic carbonate product **16** in 48% yield. It occurred to us that the cyclic carbonate group in **16** might be opened up to produce a variety of monocarbonate derivatives directly by using an appropriate alcohol and activated silica gel as a mild Lewis acid since the 6-chloro cyclic carbonate **16** was quantitatively converted to the methyl monocarbonate **15**



15 : R = CO_2Me
17 : R = H

while it was being purified by flash column chromatography on silica gel using 10% MeOH in $CHCl_3$. This approach was tested with the 6-chloro cyclic carbonate **16** on a small scale using activated silica gel in a 2:1 mixture of an alcohol (MeOH, EtOH, *i*-PrOH, and isopentanol) and $CHCl_3$ (a spectroscopic grade free from EtOH stabilizer), and TLC analysis revealed that the reaction proceeded fairly well even with *i*-PrOH at 70 °C although a small amount of 6-chloropenciclovir **17** was also formed. On the basis of this promising result, we felt confident it would be feasible to prepare all the target monocarbonate compounds in a single step by opening a common intermediate, 6-deoxy cyclic carbonate **1** with an appropriate alcohol under the previously mentioned reaction condition. As a first step, the chloro atom in the compound **16** was removed by hydrogenation (1 atm of H_2, Et_3N, MeCN/DMF, room temperature) to afford the 6-deoxy product **1** in 90% yield. Ring-opening reactions of the 6-deoxy cyclic carbonate **1** were performed in a 2:1 mixture of an appropriate alcohol (MeOH, EtOH, *n*-PrOH, *i*-PrOH, *n*-BuOH, *i*-BuOH, *n*-pentanol, and isopentanol) and $CHCl_3$ (free from EtOH stabilizer) at 70 °C using activated silica gel (10-fold excess by weight, activated by heating overnight at 80 °C under vacuum), and the corresponding monocarbonate derivatives **3**−**10** were obtained as racemates in fair to good yields (64−89%). It should be pointed out that the activated silica gel is essential for the ring opening since even the reaction with MeOH under otherwise identical conditions did not proceed at all without activated silica gel.

Results and Discussion

The in vitro antiviral activity and cytotoxicity of the prodrugs **1** and **4**−**10** along with the methyl monocarbonate **3**, famciclovir, and penciclovir against HSV-1 (KOS strain) in Vero cells were evaluated (Table 1). As was expected, all the prodrugs showed no significant antiviral activity at concentrations up to 125 μM, while penciclovir was active against HSV-1 replication with an EC_{50} value of 8.65 μM. With the exception of the

isopentyl monocarbonate **10** (CC_{50} = 357 μM), none of the prodrugs showed cytotoxicity to Vero cells even at the maximum concentration of 500 μM. We evaluated the bioavailability of penciclovir after a single oral administration (0.2 mmol/kg) of the prodrugs in mice and rats and compared them with that from famciclovir. The total amount of penciclovir recovered in the urine over a 48-h period was determined by HPLC. Of the prodrugs tested in mice, the isopropyl monocarbonate **6** achieved the highest mean urinary recovery of penciclovir (53%), followed in order by the propyl monocarbonate **5** (51%), the isopentyl monocarbonate **10** (51%), the ethyl monocarbonate **4** (50%), famciclovir (48%), the *n*-pentyl monocarbonate **9** (47%), and the methyl monocarbonate **3** (45%). The mean urinary recoveries of penciclovir from the *n*-butyl monocarbonate **7** (38%), the isobutyl monocarbonate **8** (38%), and the cyclic carbonate **1** (27%) were comparatively lower than that from famciclovir but significantly higher than that from penciclovir (3%). In rats, compounds **3**, **4**, **6**, **7**, and **10** (39−41%) showed levels of mean urinary recovery of penciclovir similar to that from famciclovir (40%), while compounds **5** (34%), **8** (37%), and **9** (38%) were slightly less bioavailable. Once again, **1** (15%) showed the lowest mean urinary recovery of penciclovir in this series of compounds, which was consistent with the result in mice. The low oral bioavailability of **1** could be due to its extreme instability in acidic aqueous solution since **1** was immediately converted to the diol **2** in the artificial gastric juice (HCl/NaCl buffer, pH 1.2). However, the alkyl monocarbonates **4**−**10** were found to be quite stable in the examined aqueous buffer solutions (pH 1.2, 6.0, 7.4, and 8.0), and they were much more stable at pH 1.2 and 6.0 than at pH 7.4 and 8.0. The isopropyl monocarbonate **6** was the most stable with the half-lives ($t_{1/2}$) of 88, >200, 61, and 26 days at pH 1.2, 6.0, 7.4, and 8.0, respectively, but the methyl monocarbonate **3**, having the smallest alkyl group in this series, had relatively short half-lives at pH 7.0 (24 h) and 8.4 (12 h). In addition, compounds **5** (68.0 mg/mL) and **6** (138.8 mg/mL) were highly soluble in H_2O at 20 °C, showing a remarkable increase in aqueous solubility compared with that of penciclovir (3.2 mg/mL).

As previously shown in the amino acid ester prodrugs of acyclovir, the absolute stereochemistry of the prodrugs might affect hydrolytic cleavage and absorption.[20] Thus, it seemed necessary to separate the enantiomers of **6** since it showed the best bioavailability and physical properties among the alkyl monocarbonates **3**−**10** tested and to subject each enantiomer to biological evaluation to investigate the effect of the stereochemistry. Initially, HPLC separation of the racemic alcohol **6** using chiral columns, Chiralcel-OD (Daicel) and Whelk-OI (Regis), was attempted. Unfortunately, all of the efforts with various eluents proved unsuccessful. We then prepared Mosher's ester of the racemic alcohol **6** by coupling it with (S)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid using DCC, hoping to obtain one pure diastereomer by conventional purification methods. Unfortunately, various attempts to purify Mosher's ester of racemic **6** either by repeated recrystallization or by HPLC were unfruitful. We concluded that the separation of enantiomers at this stage was too difficult. The study of the stereochemical effect of the monocarbonate prodrugs will be undertaken when a new asymmetric synthesis of each enantiomer is developed.

In conclusion, 2-amino-9-(3-hydroxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (**6**) showed the highest oral mean urinary recovery of penciclovir in rodents in a series of 2-amino-9-(3-hydroxymethyl-4-alkoxycarbonyloxybut-1-yl)purines that was comparable to or slightly higher than that from famciclovir, and it was found to be quite stable and soluble in the aqueous solution. On the basis of these results, the extensive studies on pharmacokinetics, metabolism, disposition, and toxicology of the racemic **6** are presently under way in our laboratory.

Experimental Section

Melting points were determined on a Thomas-Hoover 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. ^{1}H noise-decoupled ^{13}C NMR spectra were recorded on a Varian Unity 300 spectrometer at 75.4 MHz. When $CDCl_3$ or DMSO-d_6 was used as solvent, it served as the internal standard at δ 77.0 or 39.5, respectively. 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. Flash column chromatography was performed using Merck silica gel 60 (230−400 mesh). 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.

5-(2-Benzyloxyethyl)-1,3-dioxan-2-one (12). To a stirred solution of 2-(2-benzyloxyethyl)propane-1,3-diol (**11**) (15.30 g, 72.9 mmol) in anhydrous THF (1 L) was added 1,1′-carbonyldiimidazole (14.18 g, 87.4 mmol) in one portion, and the mixture was refluxed for 4 h. A second portion of 1,1′-carbonyldiimidazole (3.54 g, 21.9 mmol) was added to the reaction mixture, and it was refluxed for an additional 2 h. Another 0.3 equiv of 1,1′-carbonyldiimidazole (3.54 g, 21.9 mmol) was added, and refluxing was continued for 2 h to complete the reaction. The solvent was evaporated to dryness, and the oily residue was dissolved in EtOAc (600 mL). The EtOAc solution was washed with 1 N aqueous HCl solution (600 mL). The separated aqueous phase was saturated with NaCl and extracted with EtOAc (600 mL × 3). The combined EtOAc solution was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. The oily residue was purified by MPLC over SiO_2 with a mixture of EtOAc/hexane (2:1, v/v) as eluent to give 13.50 g (79%) of **12** as a white solid, which was crystallized from Et_2O: mp 38.7−39.5 °C; IR (KBr) 1746 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.60−1.75 (m, 2 H, CHCH_2CH$_2$), 2.36−2.48 (m, 1 H, CH), 3.54 (t, J = 5.7 Hz, 2 H, CHCH$_2$CH_2), 4.12 (dd, J = 11.1 Hz, J = 9.3 Hz, 2 H, (C=O)OCH$_{ax}$), 4.41 (dd, J = 11.1 Hz, J = 4.8 Hz, 2 H, (C=O)OCH$_{eq}$), 4.49 (s, 2 H, OCH_2Ph), 7.28−7.35 (m, 5 H, Ar H); ^{13}C NMR ($CDCl_3$) δ 27.57, 29.41, 66.96, 71.93, 73.16, 127.60, 127.82, 128.45, 137.71, 148.44; MS (EI) m/z 236 (M^+). Anal. ($C_{13}H_{16}O_4$) C, H.

5-(2-Hydroxyethyl)-1,3-dioxan-2-one (13). A stirred solution of **12** (12.50 g, 53.0 mmol) in anhydrous THF (300 mL) was treated with 10% Pd/C (1.00 g) and purged with H_2 gas three times. The mixture was stirred under H_2 atmosphere at room temperature for 3 h with a hydrogen-filled balloon and filtered. The organic solvent was evaporated to dryness under reduced pressure to give the almost pure (>95% by ^{1}H NMR analysis) 7.73 g (100%) of **13** as a pale yellow oil, which was used in the next step without further purification. An aliquot was purified for analysis by MPLC over SiO_2 with a mixture of MeOH/$CHCl_3$ (1:9, v/v) as eluent: IR (neat) 3433 (OH), 1730 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.62 (m, 2 H,

$CHCH_2CH_2$), 2.40−2.55 (m, 1 H, CH), 2.66 (br s, 1 H, OH), 3.74 (t, J = 5.7 Hz, 2 H, CH_2OH), 4.19 (dd, J = 10.8 Hz, J = 9.0 Hz, 2 H, (C=O)OCH_{ax}), 4.51 (dd, J = 10.8 Hz, J = 4.8 Hz, 2 H, (C=O)OCH_{eq}); ^{13}C NMR ($CDCl_3$) δ 28.94, 29.80, 59.45, 72.18, 149.03; MS (EI) m/z 147 $(M + H)^+$. Anal. ($C_6H_{10}O_4$) C, H.

5-(2-Methanesulfonyloxyethyl)-1,3-dioxan-2-one (14). To a stirred solution of **13** (4.50 g, 30.8 mmol) in anhydrous CH_2Cl_2 (40 mL) cooled at 0 °C under N_2 atmosphere was slowly added Et_3N (9.36 g, 92.5 mmol) followed by dropwise addition of MsCl (3.88 g, 33.9 mmol) via a syringe, and the mixture was stirred at 0 °C for 30 min. Additional MsCl (1.77 g, 15.4 mmol) was added dropwise to the reaction mixture at 0 °C, and it was stirred for another 30 min. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by MPLC over SiO_2 with a mixture of EtOAc/hexane (9:1, v/v) followed by EtOAc as eluent to give 5.77 g (84%) of **14** as a white solid that should be used as soon as possible: mp 71.8−74.1 °C; IR (KBr) 1751 (C=O), 1358, 1182 (OSO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.91 (m, 2 H, $CHCH_2CH_2$), 2.40−2.51 (m, 1 H, CH), 3.06 (s, 3 H, CH_3), 4.21 (dd, J = 11.1 Hz, J = 7.8 Hz, 2 H, (C=O)OCH_{ax}), 4.34 (t, J = 6.3 Hz, 2 H, $CHCH_2CH_2$), 4.52 (dd, J = 11.1 Hz, J = 3.9 Hz, 2 H, (C=O)OCH_{eq}); ^{13}C NMR ($CDCl_3$) δ 27.35, 28.37, 37.62, 66.22, 71.15, 147.89. Anal. ($C_7H_{12}O_6S$) C, H.

2-Amino-6-chloro-9-(3-hydroxymethyl-4-methoxycarbonyloxybut-1-yl)purine (15). A mixture of 2-amino-6-chloropurine (9.49 g, 56.0 mmol), **14** (12.54 g, 56.0 mmol), and Cs_2CO_3 (22.8 g, 70.0 mmol) in anhydrous DMF (150 mL) was stirred at room temperature for 16 h under N_2 atmosphere. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. The residue adsorbed to SiO_2 using DMF as a solvent was placed on the top of the SiO_2 column and purified by MPLC with a mixture of MeOH/$CHCl_3$ (1:9, v/v) as eluent to give 9.25 g (50%) of **15** as a white solid, which was crystallized from EtOH: mp 140.2−141.2 °C dec; IR (KBr) 3379, 3310, 3212 (NH_2 and OH), 1733 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.66−1.92 (m, 3 H, $CHCH_2CH_2$), 3.41 (t, J = 5.0 Hz, 2 H, $CHCH_2OH$), 3.68 (s, 3 H, OCH_3), 4.06−4.18 (m, 4 H, NCH_2 and OCH_2CH), 4.67 (t, J = 5.3 Hz, 1 H, OH), 6.88 (br s, 2 H, NH_2), 8.14 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) δ 27.73, 37.57, 41.05, 54.51, 60.20, 67.55, 123.34, 143.10, 149.27, 154.01, 155.10, 159.69. Anal. ($C_{12}H_{16}ClN_5O_4$) C, H, N.

2-Amino-6-chloro-9-(2-(2-oxo-1,3-dioxan-5-yl)ethyl)purine (16). A mixture of 2-amino-6-chloropurine (9.49 g, 56.0 mmol), **14** (12.54 g, 56.0 mmol), and Cs_2CO_3 (22.8 g, 70.0 mmol) in anhydrous DMF (150 mL) was stirred at room temperature for 16 h under N_2 atmosphere. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. The residue adsorbed to SiO_2 using DMF as a solvent was placed on the top of the SiO_2 column and purified by MPLC with a mixture of EtOAc/MeCN/Et_3N (49:49:2, v/v/v) as eluent to give 8.02 g (48%) of **16** as a white solid, which was crystallized from a mixture of MeCN/THF/hexane (1:4:10, v/v/v): mp 185.2−186.0 °C dec; IR (KBr) 3347, 3322, 3199 (NH_2), 1733 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.86 (m, 2 H, $CHCH_2CH_2$), 2.07−2.19 (m, 1 H, CH), 4.13 (t, J = 7.2 Hz, 2 H, NCH_2), 4.21 (dd, J = 10.5 Hz, J = 7.8 Hz, 2 H, (C=O)OCH_{ax}), 4.46 (dd, J = 10.5 Hz, J = 4.2 Hz, 2 H, (C=O)OCH_{eq}), 6.90 (br s, 2 H, NH_2), 8.17 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) δ 26.78, 27.97, 40.27, 71.01, 123.30, 143.04, 147.83, 149.35, 154.06, 159.72; MS (EI) m/z 297 (M^+). Anal. ($C_{11}H_{12}ClN_5O_3$) C, H, N.

2-Amino-9-(2-(2-oxo-1,3-dioxan-5-yl)ethyl)purine (1). A stirred solution of **16** (534 mg, 1.79 mmol) in a mixture of anhydrous MeCN (25 mL) and anhydrous DMF (10 mL) was treated with Et_3N (543 mg, 5.37 mmol) and 10% Pd/C (60 mg) and purged with H_2 gas three times. The mixture was stirred under H_2 atmosphere at room temperature for 3 h with a hydrogen-filled balloon and filtered. The filtrate was evaporated to dryness under reduced pressure, and the residue was triturated with a small volume of absolute EtOH (2 mL) to give 427 mg (90%) of **1** as a white solid, which was crystallized from a mixture of MeCN/THF/hexane (1:4:10, v/v/v): mp 183.0−183.5 °C (dec, brown from 175.0 °C); IR (KBr) 3365, 3326, 3189 (NH_2), 1749 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.84 (m, 2 H, $CHCH_2CH_2$), 2.07−2.17 (m, 1H, CH), 4.14 (t, J = 7.1 Hz, 2 H, NCH_2), 4.22 (dd, J = 10.8 Hz, J = 8.1 Hz, 2 H, (C=O)OCH_{ax}), 4.46 (dd, J = 10.8 Hz, J = 4.5 Hz, 2 H, (C=O)OCH_{eq}), 6.51 (br s, 2 H, NH_2), 8.11 (s, 1 H, H-8), 8.57 (s, 1 H, H-6); ^{13}C NMR (DMSO-d_6) δ 26.90, 28.11, 40.05, 71.03, 126.79, 142.57, 147.83, 148.95, 152.98, 160.42; MS (EI) m/z 263 (M^+). Anal. ($C_{11}H_{13}N_5O_3$) C, H, N.

General Procedure for the Preparation of 2-Amino-9-(3-hydroxymethyl-4-alkoxycarbonyloxybut-1-yl)-purines 3−10. A mixture of **1** (395 mg, 1.50 mmol) and dried SiO_2 (4.0 g) in $CHCl_3$ (20 mL) was treated with an appropriate alcohol (40 mL) and heated at 70 °C for 4 h (for **3**−**5** and **7**−**10**) or 24 h (for **6**) under N_2 atmosphere. After being cooled, the reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by MPLC over SiO_2 using a mixture of MeOH/$CHCl_3$ (1:9, v/v) as eluent to give the titled compound, which was crystallized from a suitable solvent.

2-Amino-9-(3-hydroxymethyl-4-methoxycarbonyloxybut-1-yl)purine (3): yield 91%; mp 129.0−132.0 ° (lit.[12] 129−132 °C); spectral data were identical with those reported.[12]

2-Amino-9-(3-hydroxymethyl-4-ethoxycarbonyloxybut-1-yl)purine (4): yield 87%; mp 107.5−109.5 °C (EtOAc/hexane); IR (KBr) 3487, 3307, 3187 (NH_2 and OH), 1731 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.85−2.02 (m, 2 H, $CHCH_2CH_2$), 1.98−2.06 (m, 1 H, CH), 3.64−3.76 (m, 2 H, $CHCH_2OH$), 4.12−4.28 (m, 6 H, NCH_2 and 2 OCH_2), 5.53 (br s, 2 H, NH_2), 7.79 (s, 1 H, H-8), 8.64 (s, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 14.15, 28.65, 37.87, 41.09, 61.64, 64.23, 67.66, 127.87, 142.39, 149.70, 152.98, 155.26, 159.79; MS (EI) m/z 309 (M^+). Anal. ($C_{13}H_{19}N_5O_4$) C, H, N.

2-Amino-9-(3-hydroxymethyl-4-propoxycarbonyloxybut-1-yl)purine (5): yield 89%; mp 67.0−68.7 °C (EtOAc/hexane); IR (KBr) 3350, 3221 (NH_2 and OH), 1750 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, J = 7.5 Hz, 3 H, CH_2CH_3), 1.62−1.76 (m, 2 H, CH_2CH_3), 1.84−2.01 (m, 2 H, $CHCH_2CH_2$), 1.97−2.06 (m, 1 H, CH), 3.72 (m, 2 H, $CHCH_2OH$), 4.13 (t, J = 6.9 Hz, 2 H, OCH_2CH_2), 4.18−4.30 (m, 4 H, NCH_2 and OCH_2CH), 5.18 (br s, 2 H, NH_2), 7.81 (s, 1 H, H-8), 8.69 (s, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 10.07, 21.90, 28.66, 37.88, 41.09, 61.66, 67.66, 69.83, 127.89, 142.38, 149.72, 152.98, 155.41, 159.80; MS (EI) m/z 323 (M^+). Anal. ($C_{14}H_{21}N_5O_4$) C, H, N.

2-Amino-9-(3-hydroxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (6): yield 64%; mp 75.0−78.0 °C (EtOAc); IR (KBr) 3350, 3221 (NH_2 and OH), 1747 (C=O); ^{1}H NMR ($CDCl_3$) δ 1.29 (d, J = 6.3 Hz, 6 H, $CH(CH_3)_2$), 1.85−2.01 (m, 2 H, $CHCH_2CH_2$), 1.98−2.10 (m, 1 H, CH) 3.71 (m, 2 H, $CHCH_2OH$), 4.15−4.28 (m, 4 H, NCH_2 and OCH_2CH), 4.87 (septet, J = 6.3 Hz, 1 H, $CH(CH_3)_2$), 5.47 (br s, 2 H, NH_2), 7.79 (s, 1 H, H-8), 8.66 (s, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 21.67, 28.68, 37.89, 41.09, 61.71, 67.44, 72.30, 127.92, 142.38, 149.73, 152.99, 154.81, 159.78; MS (EI) m/z 323 (M^+). Anal. ($C_{14}H_{21}N_5O_4$) C, H, N.

2-Amino-9-(3-hydroxymethyl-4-butoxycarbonyloxybut-1-yl)purine (7): yield 69%; mp 76.9−78.1 °C (EtOAc/hexane); IR (KBr) 3346, 3219 (NH_2 and OH), 1746 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, J = 7.4 Hz, 3 H, CH_2CH_3), 1.40 (m, 2 H, CH_2CH_3), 1.66 (m, 2 H, $CH_2CH_2CH_3$), 1.84−2.02 (m, 2 H, $CHCH_2CH_2$), 1.99−2.06 (m, 1 H, CH), 3.72 (m, 2 H, $CHCH_2OH$), 4.15 (t, J = 6.8 Hz, 2 H, $OCH_2CH_2CH_2$), 4.18−4.34 (m, 4 H, NCH_2 and OCH_2CH), 5.19 (br s, 2 H, NH_2), 7.78 (s, 1 H, H-8), 8.69 (s, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 13.55, 18.81, 28.66, 30.54, 37.88, 41.09, 61.65, 67.66, 68.14, 127.88, 142.38, 149.71, 152.97, 155.41, 159.80; MS (EI) m/z 337 (M^+). Anal. ($C_{15}H_{23}N_5O_4$) C, H, N.

2-Amino-9-(3-hydroxymethyl-4-isobutoxycarbonyloxybut-1-yl)purine (8): yield 87%; mp 117.5−118.7 °C (EtOAc); IR (KBr) 3411, 3312, 3175 (NH_2 and OH), 1735 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (d, J = 6.6 Hz, 6 H, $CH(CH_3)_2$), 1.85−2.12 (m, 4 H, $CHCH_2CH_2$ and $CH(CH_3)_2$), 3.73 (m, 2 H, $CHCH_2OH$), 3.92 (d, J = 6.9 Hz, 2 H, OCH_2CH), 4.18−4.29 (m, 4 H,

NCH_2 and OCH_2), 5.25 (br s, 2 H, NH_2), 7.78 (s, 1 H, H-8), 8.69 (s, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 18.85, 27.74, 28.74, 37.87, 41.04, 62.00, 67.62, 74.34, 128.17, 142.34, 149.86, 153.12, 155.54, 159.78; MS (EI) *m/z* 337 (M^+). Anal. ($C_{15}H_{23}N_5O_4$) C, H, N.

2-Amino-9-(3-hydroxymethyl-4-pentoxycarbonyloxybut-1-yl)purine (9): yield 74%; mp 66.5−68.5 °C; IR (KBr) 3374, 3341, 3211 (NH_2 and OH), 1747 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (t, J = 7.1 Hz, 3 H, CH_2CH_3), 1.34 (m, 4 H, CH_2CH_2-CH_3), 1.67 (m, 2 H, $CH_2CH_2CH_2CH_3$), 1.85−2.10 (m, 3 H, $CHCH_2CH_2$), 3.72 (m, 2 H, $CHCH_2OH$), 4.13 (t, J = 6.9 Hz, 2 H, OCH_2CH_2), 4.18−4.31 (m, 4 H, NCH_2 and OCH_2), 5.15 (br s, 2 H, NH_2), 7.78 (s, 1 H, H-8), 8.69 (s, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 13.88, 22.24, 27.77, 28.30, 28.74, 37.85, 41.01, 62.14, 67.57, 68.52, 128.29, 142.32, 149.97, 153.13, 155.53, 159.74; MS (EI) *m/z* 351 (M^+). Anal. ($C_{16}H_{25}N_5O_4$) C, H, N.

2-Amino-9-(3-hydroxymethyl-4-isopentoxycarbonyloxybut-1-yl)purine (10): yield 84%; mp 81.3−83.1 °C (EtOAc/hexane); IR (KBr) 3377, 3340, 3211 (NH_2 and OH), 1750 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (d, J = 6.9 Hz, 6 H, $CH(CH_3)_2$), 1.56 (m, 2 H, $CHCH_2CH_2$), 1.71 (septet, J = 6.9 Hz, 1 H, $CH(CH_3)_2$), 1.88−2.11 (m, 3 H, $CHCH_2CH_2$), 3.73 (m, 2 H, $CHCH_2OH$), 4.17 (t, J = 6.9 Hz, 2 H, OCH_2CH_2), 4.18−4.28 (m, 4 H, NCH_2 and OCH_2), 5.28 (br s, 2 H, NH_2), 7.78 (s, 1 H, H-8), 8.69 (s, 1 H, H-6); ^{13}C NMR ($CDCl_3$) δ 22.36, 24.79, 28.74, 37.25, 37.87, 41.04, 62.02, 67.00, 67.60, 128.17, 142.32, 149.88, 153.10, 155.47, 159.78; MS (EI) *m/z* 351 (M^+). Anal. ($C_{16}H_{25}N_5O_4$) C, H, N.

Water Solubility. A standard solution was prepared by dissolving 1 mg of the test compound in 10 mL of H_2O at 20 °C. The standard solution was diluted with H_2O as necessary, and the diluted standard solutions were scanned by UV at 290 nm to obtain a standard curve. A saturated solution was prepared by vortex-mixing in H_2O for 1 min, ultrasonification for 1 min, vortex-mixing for 3 min, ultrasonification for 1 min, and finally vortex-mixing for 5 min in the presence of excess compound. After the saturated solution was filtered to remove excess compound using 0.45-μm Millipore filters, the solution was diluted with H_2O and then scanned by UV at 290 nm. Total solubility was then determined by the comparison of the absorbance of the saturated solution with that of the standard curve.

Aqueous Stability. Fifty microliters of 2.5 mM stock solution of the test compound was added to sodium phosphate buffer (pH 6.0, pH 7.4, or pH 8.0) or HCl/NaCl buffer (pH 1.2) to give a final concentration of 125 μM. Immediately after the compound was mixed with the buffer, incubation of the solution was initiated at 37 °C. At various intervals, 100 μL of the sample was taken throughout the indicated incubation times and immediately mixed with 900 μL of 0.1 M phosphate buffer (pH 7.0). The sample was then isocratically eluted at a flow rate of 1 mL/min with buffer A (0.1% phosphoric acid) and buffer B (80% MeCN in 0.1% phosphoric acid) with various ratios using a C_{18} symmetry column. The half-life of the compound was calculated from the concentration of the parent compound.

In Vitro Cytotoxicity. African green monkey kidney (Vero) cells obtained from American Type Culture Collection (ATCC, Manassas, VA) were seeded at 5×10^3 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 3 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). Each experiment was performed in quadruplicate and repeated twice.

In Vitro Antiviral Activity. The in vitro antiviral activity was tested by dye-uptake assay.[21] Vero cells were grown in EMEM containing 5% fetal bovine serum (FBS) (Gibco). Herpes simplex virus type 1 (HSV-1) (KOS strain) was obtained from ATCC, and stocks were prepared in Vero cell suspensions and stored at −70 °C. One hundred microliters of Vero cell suspensions (10^5 cells/mL) was plated in 96-well plates. After a 1-h plating period at 37 °C in an incubator containing 5% CO_2, 50 μL of 100 PFU/well of virus in EMEM with 5% FBS was added into each well. One hundred microliters of the test compound (serial 3-fold dilutions in EMEM from maximum concentration of 125 μM) was added into 96-well plates in quadruplicate following a 1-h virus adsorption period. After 48 h of incubation at 37 °C in an incubator containing 5% CO_2, 100 μL of neutral red dye (0.15% in PBS, pH 5.5) was placed into each well. The plates were then incubated for an additional 30 min at 37 °C, and then the medium and residual stain were removed, and the wells were rinsed twice with PBS (pH 6.5). Thereafter, 200 μL of phosphate ethanol buffer (PBS/EtOH = 1:1, v/v, pH 4.2) was distributed into each well for elution of the dye incorporated by supposedly viable cells. The OD of the solutions was read at 550 nm using an ELISA reader. The drug concentration exhibiting a dye uptake of 50% (EC_{50}) in comparison to that of the virus control (= 0%) and that of the cell control (= 100%) was calculated by quantal probit analysis of pharmacologic calculations with a computer program.[22]

Oral Bioavailability. The bioavilability of the test compound was estimated by determining the total amount of penciclovir in the urine using HPLC. Urine was collected for 48 h in a metabolic cage after oral administration of a single 0.2 mmol/kg dose of the test compound to six male ICR mice (25−30 g) or six 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 penciclovir concentration was analyzed by HPLC as follows. A C_{18} symmetry column equipped with a compatible guard column was 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. The column was equilibrated with 100% buffer A for 10 min before each sample injection. The UV absorbance of the column effluent was monitored at 248 nm.

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