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

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Abstract—A series of 2-amino-9-(3-acyloxymethyl-4-alkoxycarbonyloxybut-1-yl)purines (**1**–**8**) and 2-amino-9-(3-alkoxycarbonyloxymethyl-4-alkoxycarbonyloxybut-1-yl)purines (**9**–**12**) were synthesized as potential prodrugs of penciclovir. Treatment of 6-deoxypenciclovir with trimethyl orthoacetate or triethyl orthopropionate (1.2 equiv) in DMF in the presence of *p*-TsOH·H_2O (0.1 equiv) followed by quenching with excess H_2O gave the corresponding mono-*O*-acetyl or mono-*O*-propionyl compound, **17** or **18**, in excellent yields of 95 and 92%, respectively. Reactions of **17** or **18** with an appropriate alkyl (Me, Et, *n*-Pr, and *i*-Pr) 4-nitrophenyl carbonate (1.2 equiv) in pyridine in the presence of a catalytic amount of DMAP (0.1 equiv) at 80°C afforded the monoacyl, monocarbonate derivatives of 6-deoxypenciclovir, **1**–**8**, in 86–94% yields. Similar reactions of 6-deoxypenciclovir with 2.1 equiv of alkyl 4-nitrophenyl carbonate produced the dicarbonate derivatives **9**–**12** in 81–83% yields. Of the prodrugs tested in rats, 2-amino-9-(3-acetoxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (**4**) achieved the highest mean urinary recovery of penciclovir (36%), followed in order by compounds **2** (35%), **6** (35%), **7** (34%), **10** (34%), **8** (32%), **3** (32%), and famciclovir (31%). The mean urinary recovery of penciclovir and concentrations of penciclovir in the blood from **4** in mice were also slightly higher than those from famciclovir. The in vivo antiviral efficacy of **4** in HSV-1-infected normal BALB/c mice was higher than those of famciclovir and valaciclovir in terms of mortality (100, 80, and 40%) and mean survival time (>21, 13 ± 5.0 (SEM), and 13 ± 1.6 days). Compound **4** demonstrated an effective anti-hepadnaviral response with intrahepatic viral load being reduced by 90%, the viral supercoiled DNA levels reduced by 70% and Pre-S expression inhibited by 30% against duck hepatitis B virus (DHBV) in vivo, and did not cause any significant hepatotoxicity after 4 weeks of treatment.

Introduction

Penciclovir [9-(4-hydroxy-3-hydroxymethylbut-1-yl)-guanine] is a potent and selective inhibitor of members of the herpesvirus family including herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), varicella-zoster virus (VZV), and Epstein–Barr virus (EBV) both in cell cultures and in animal models,[1–4] and of hepatitis B virus (HBV) and duck hepatitis B virus (DHBV) in cell cultures.[5,6] The advantage of penciclovir over acyclovir is that its antiviral activity in cell culture is more persistent than that of acyclovir because penciclovir triphosphate has a much greater stability than acyclovir triphosphate within virus-infected cells.[2,7] However, like other acycloguanosine analogues such as acyclovir,[8] ganciclovir,[9] and buciclovir,[10] penciclovir was poorly absorbed when given orally to rodents.[11,12] Therefore, the search for a prodrug that is orally well absorbed and then readily converted to penciclovir is of high priority. Harnden et al. developed famciclovir [2-amino-9-(4-acetoxy-3-acetoxymethylbut-1-yl)purine], the diacetyl 6-deoxy analogue of penciclovir, as a prodrug of penciclovir.[11] In mice, rats, and humans, 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.[11–13] In the US, famciclovir has recently been approved by the FDA for the treatment

Key words: Antivirals; nucleosides; chemotherapy; enzyme inhibitors.
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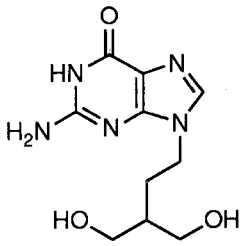
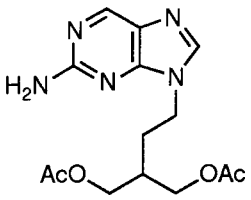
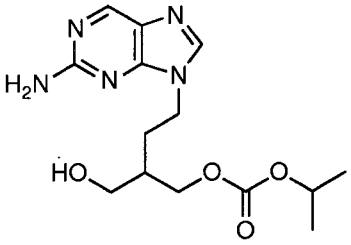


penciclovir

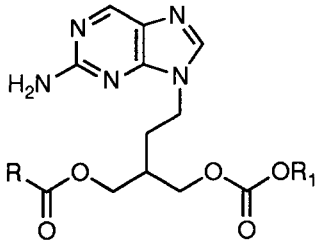


famciclovir

6-deoxypenciclovir



SK 1875



1: R = R_1 = Me
2: R = Me, R_1 = Et
3: R = Me, R_1 = *n*-Pr
4: R = Me, R_1 = *i*-Pr
5: R = Et, R_1 = Me
6: R = R_1 = Et
7: R = Et, R_1 = *n*-Pr
8: R = Et, R_1 = *i*-Pr
9: R = MeO, R_1 = Me
10: R = EtO, R_1 = Et
11: R = *n*-PrO, R_1 = *n*-Pr
12: R = *i*-PrO, R_1 = *i*-Pr

Chart 1.

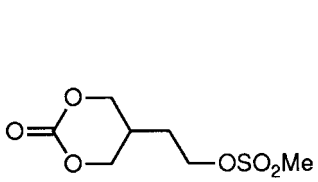
of herpes zoster (shingles) and acute recurrent genital herpes in immunocompetent individuals. Famciclovir has been shown to inhibit DHBV replication in vivo in hepatic and nonhepatic tissues of ducklings that had been infected in ovo with DHBV.[14] It also controlled HBV replication effectively in orthotopic liver transplant patients either alone or in combination with a short course of prostaglandin E[15,16] and inhibited HBV replication in a double-blind, placebo-controlled, pilot study in patients with chronic HBV infection.[17] A large, multicenter clinical trial of famciclovir against HBV infection is currently in progress. We have recently reported the synthesis of amino acid esters of penciclovir and 6-deoxypenciclovir,[18,19] esters of 2-amino-6-fluoro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purines,[20] and a series of 2-amino-9-(3-hydroxymethyl-4-alkoxycarbonyloxybut-1-yl)purines as potential prodrugs of penciclovir.[21] Amomg them, SK 1875 [2-amino-9-(3-hydroxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine] showed oral penciclovir bioavailability comparable to famciclovir in mice and rats,[21] and had desirable physical properties as a prodrug.[21]

On the basis of these results, the extensive preclinical studies on SK 1875 have been undertaken. Treatment of ducklings congenitally infected with DHBV with SK 1875 at the dose of 15 mg/kg/day given orally in two equally divided doses for 4 weeks reduced serum DHBV-DNA levels below detectable limits, intrahepatic viral load by 87%, the level of supercoiled DNA by 50%, and viral RNA expression by 31%.[22] Unfortunately, after 4 weeks of drug-free follow-up, liver histology showed some significant pathological changes such as steatosis and nucleoli.[22] This hepatotoxicity was unexpected to us since SK 1875 was readily metabolized to penciclovir and 6-deoxypenciclovir in rodents,[21] the same metabolites from famciclovir. It was of particular interest to us to determine whether hepatotoxicity of SK 1875 attributed to a carbonate group or an unprotected hydroxyl group. In addition to that, we like to examine if further modification of a hydroxyl group of SK 1875 with an acyl or a carbonate group could increase their oral bioavailability compared with SK 1875. Therefore, in this report, we synthesized the monoacyl, monocarbonate derivatives of 6-deoxypenciclovir, **1**–**8** and the dicarbonate derivatives **9**–**12**, and evaluated for their potential as prodrugs of penciclovir.

Chemistry

We have recently reported a new method for the synthesis of the C_1–C_5 alkyl monocarbonate derivatives of 6-deoxypenciclovir, where the introduction of cyclic carbonate group to the acyclic moiety at the early stage of the synthesis, the successful coupling of cyclic carbonate mesylate **13** with 2-amino-6-chloropurine, and the opening of the cyclic carbonate **14** with an appropriate alcohol as a nucleophile in the presence of activated silica gel as a mild Lewis acid under a mild

condition were the key steps.[21] Thus, it was first envisioned that all the target compounds **1**–**12** could be prepared from the alkyl monocarbonate derivatives of 6-deoxypenciclovir by acylation reactions using appropriate acylating reagents such as acid anhydrides and alkyl chloroformates, or their equivalents. Unfortunately, this approach was hampered by the fact that our new method was found not to be practical for the large scale production of SK 1875 due to the following reasons: (1) the reagent for the formation of cyclic carbonate, 1,1′-carbonyldiimidazole, is very expensive, (2) the coupling reaction of cyclic carbonate mesylate **13** with 2-amino-6-chloropurine was rather low yielding (about 30% in 56 mmol scale), and (3) the product purification was very difficult. So, it was decided to develop another practical synthetic method which can be readily scaled up for the mass production of any preclinical candidate. Our works have been mainly focused on those approaches using 6-deoxy-6-chloropenciclovir or 6-deoxypenciclovir as the starting materials, since the potentially practical methods for the preparation of them have been well documented.[23,24] Even though the direct conversion of 6-deoxypenciclovir to the monoalkyl carbonate using alkyl chloroformate has met with failure, as described in our previous work,[21] it occurred to us that the chlorine atom in the 6-deoxy-6-chloropenciclovir could make some difference, hopefully due to its electronic effect on the purine ring. Many reactions with 6-deoxy-6-chloropenciclovir and isopropyl chloroformate were performed using various solvents (DMF, CH_2Cl_2, $CHCl_3$, THF, or pyridine), and pyridine was found to be best, affording the desired monoisopropyl carbonate **15** in a reasonable yield of 60% in a small scale reaction (0.10 mmol). Unfortunately, it was observed the yields of this reaction were really variable and dropped dramatically as the reaction scale increased. As shown in Scheme 1, the large scale (2 mol) reaction was a real disaster, yielding less than 10% of the desired product **15** along with a mess of a dark yellow crystalline by-product, whereas the yields were around 40–45% in the medium size scale (0.5 mol). This unexpected by-product was assigned as a pyridium salt **16** based on the spectroscopic data (^{1}H NMR, IR, and FAB–MS) and elemental analysis, which could be formed via the displacement of chlorine atom by pyridine. Formation of the pyridium salt **16** became serious in the large scale, probably because it required prolonged time to remove a large amount of pyridine solvent during the work-up. Therefore, it was concluded that the direct conversion of the diols, 6-deoxypenciclovir or 6-deoxy-6-chloropenciclovir, to the corresponding mono-alkyl carbonate using alkyl chloroformate was not really feasible and the late introduction of carbonate group would be better.



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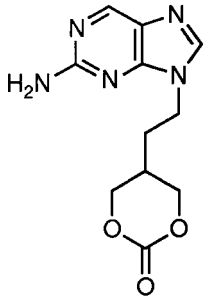
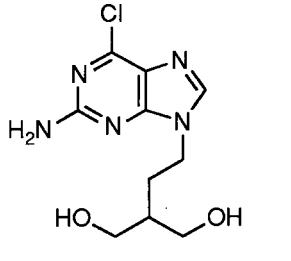


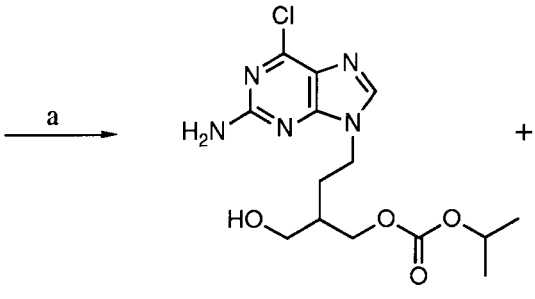
14

In the mean time, it was decided to use 6-deoxypenciclovir as a starting material and find any practical ways to differentiate the diol functionalilty prior to the introduction of carbonate group. Literature search revealed that there are several efficient ways to convert the diols into their mono-functionalized alcohols with high selectivities.[25,26] Among them, a procedure using trimethyl orthoacetate and a Lewis acid caught our attention because it was reported to be very mild and highly selective.[26] This reaction is known to proceed through the formation of cyclic orthoacetate intermediate followed by hydrolysis to yield the corresponding monoacetate derivative. Gratifyingly, this procedure tuned out to be suitable for our purpose. As shown in Scheme 2, the starting 6-deoxypenciclovir was first treated with trimethyl orthoacetate or triethyl orthopropionate (1.2 equiv) in DMF in the presence of *p*-TsOH·H_2O (0.1 equiv) at room temperature for 2 h, and then the reaction mixture was quenched with excess H_2O at ambient temperature to provide the corresponding mono-*O*-acetyl or -propionyl compound, **17** or **18**, in excellent yields of 95 and 92%, respectively. Indeed, these reactions were very mild and highly efficient.

At this stage, it is necessary to seek out an efficient way to convert the mono-*O*-acyl derivatives, **17** and **18**, to



6-deoxy-6-chloropenciclovir



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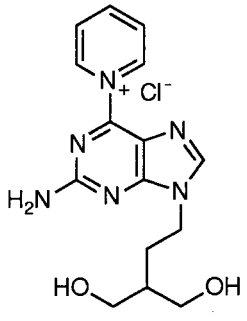
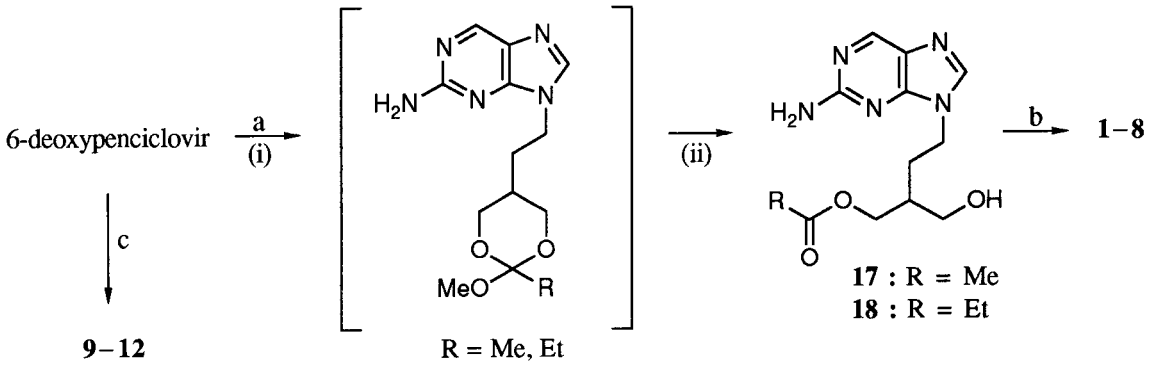
+



16

Scheme 1. (a) Isopropyl chloroformate (1.1 equiv), pyridine, 0°C to rt.



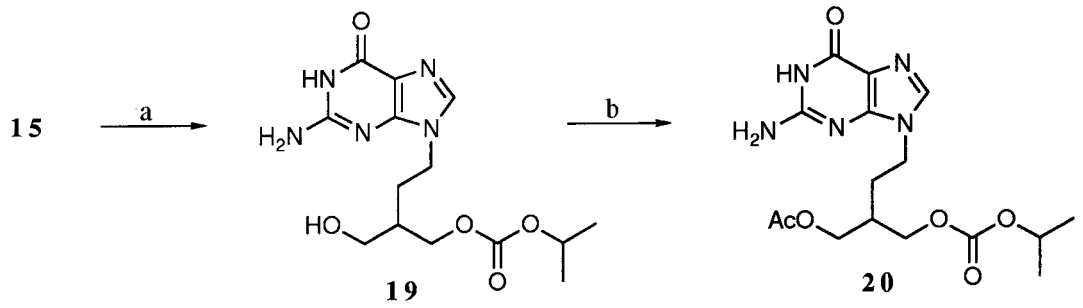
Scheme 2. (a) (i) Trimethyl orthoacetate or triethyl orthopropionate (1.2 equiv), *p*-TsOH·H_2O (0.1 equiv), DMF, rt, 2 h; (ii) H_2O, 1 h; (b) methyl, ethyl, *n*-propyl, or isopropyl 4-nitrophenyl carbonate (1.2 equiv), DMAP (0.1 equiv), pyridine, 80°C, 20 h; (c) methyl, ethyl, *n*-propyl, or isopropyl 4-nitrophenyl carbonate (2.1 equiv), DMAP (0.1 equiv), pryidine, 80°C, 24 h.

the desired target compounds, **1**–**8**. As was expected from our previous work,[21] the introducton of carbonate group was not straightforward, although one of the diol functionality was protected as an ester. A lot of reactions were carried out with the mono-*O*-acetyl compound **17** using various carbonate transfer reagents such as alkyl chloroformate, dialkyl carbonate, dialkyl dicarbonate (dialkyl pyrocarbonate), and so on. Whereas diethyl carbonate did not react at all even under very harsh conditions, isopropyl chloroformate and diisopropyl dicarbonate[27] produced rather complex mixtures under various conditions. These results indicate that alkyl chloroformate and dialkyl dicarbonate might be too much reactive to control and, on the other hand, dialkyl carbonate is not reactive enough. It is not too much surprising to observe that the reactive isopropyl chloroformate and diisopropyl dicarbonate produced rather complex mixtures, since the substrate **17** has five nitrogen atoms including an amino group which could be potentially competing reaction sites. Based on these frustrating results, it was concluded that the search for the right reagent with intermediate reactivity between alkyl chloroformate and dialkyl carbonate is a first priority. It was reasoned by us that the alkyl phenyl carbonate, where a phenyl ring is substituted with electron withdrawing groups, might be suitable since various phenols have been used in the peptide synthesis as an activated form of esters. We chose 4-nitrophenol because it is very cheap, and isopropyl 4-nitrophenyl carbonate was prepared in high yield by reacting 4-nitrophenol with isopropyl chloroformate in the presence of Et_3N in Et_2O at room temperature. We performed several reactions using isopropyl 4-nitrophenyl carbonate and **17** to find an optimal condition. Due to the low solubility of **17** in organic solvents, DMF and pyridine were tested at various temperature in the presence or absence of a catalytic amount of 4-dimethylaminopyridine (DMAP). Fortunately, the reaction worked fine in pyridine at 80°C in the presence of a catalytic amount of DMAP (0.1 equiv) and was completed in about 12 to 24 h using slight excess of isopropyl 4-nitrophenyl carbonate (1.2 equiv) to give the desired product **4** in 89%. It was really slow in the absence of DMAP at 80–100°C or at room temperature in the presence of DMAP. In contrast, it was observed that the reaction using DMF was somewhat complicated probably because DMF itself might be reacting with isopropyl 4-nitrophenyl carbonate. Other alkyl (methyl, ethyl, and *n*-propyl) 4-nitrophenyl carbonates were prepared in the same manner as described for isopropyl 4-nitrophenyl carbonate, also in high yields. Reactions of **17** and **18** with each alkyl (Me, Et, *n*-Pr and *i*-Pr) 4-nitrophenyl carbonate were carried out under the previously mentioned conditions, and the target compounds, 2-amino-9-(3-acyloxymethyl-4-alkoxycarbonyloxybut-1-yl)purines **1**–**8**, were obtained in high yields of 86–94%. Uneventfully, the di-carbonate derivatives **9**–**12** were prepared in 81–83% yields directly from 6-deoxypenciclovir by reacting with appropriate alkyl 4-nitrophenyl carbonate (2.1 equiv) in pyridine at 80°C in the presence of a catalytic amount of DMAP (0.1 equiv). It should be pointed out that it was possible to prepare a large quantity of the selected preclinical candidate by adopting this process.

To characterize the metabolites of **4** in vivo, the unknown, postulated metabolites 9-(3-hydroxymethyl-4-isopropoxycarbonyloxybut-1-yl)guanine (**19**) and 9-(3-acetoxymethyl-4-isopropoxycarbonyloxybut-1-yl)-guanine (**20**) were prepared from **15** as shown in Scheme 3 as standard compounds for HPLC analysis. First, the potential metabolite **19** was efficiently synthesized in 93% yield from **15** by enzymatic reaction using adenosine deaminase (Sigma) in phosphate buffer (pH 7.5) at room temperature. Even though this enzymatic conversion required 5 days at room temperature for completion, it was very clean, and only the desired product was isolated. Subsequent acetylation of **19** with acetic anhydride was carried out in anhydrous DMF at room temperature in the presence of a catalytic amount of DMAP to give the potential metabolite **20** in 83% yield.

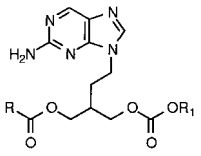
Results and Discussion

The bioavailability of penciclovir following a single oral administration (0.2 mmol/kg) of the prodrugs **1**–**12** to rats was evaluated and compared with those from penciclovir, famciclovir and SK 1875. The total amount of penciclovir recovered in the urine over a 48-h period was determined by HPLC (Table 1). The mean urinary



Scheme 3. (a) Adenosine deaminase (10% by wt), phosphate buffer (pH 7.5), rt, 5 days; (b) acetic anhydride (2 equiv), DMAP (0.1 equiv), rt, 1 h.

Table 1. Urinary recovery of penciclovir and 6-deoxypenciclovir following oral administration of 2-amino-9-(3-acyloxymethyl-4-alkoxycarbonyloxybut-1-yl)purines **1**–**8** and 2-amino-9-(3-alkoxycarbonyloxymethyl-4-alkoxycarbonyloxybut-1-yl)purines **9**–**12** to rats



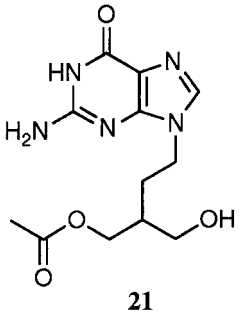
1–12

			Urinary recovery of penciclovir and 6-deoxypenciclovir[a] (% dose)[b]	
Compd	R	R_1	Penciclovir	6-Deoxypenciclovir
1	Me	Me	30	23
2	Me	Et	35	26
3	Me	*n*-Pr	32	22
4	Me	*i*-Pr	36	27
5	Et	Me	28	22
6	Et	Et	35	24
7	Et	*n*-Pr	34	24
8	Et	*i*-Pr	32	28
9	MeO	Me	30	17
10	EtO	Et	34	23
11	*n*-PrO	*n*-Pr	32	20
12	*i*-PrO	*i*-Pr	29	16
SK 1875			31	28
Famciclovir			31	27
Penciclovir			6	

[a] A single oral dose of the test compound (0.2 mmol/kg) was administered to two male Sprague–Dawley rats. The total amount of penciclovir and 6-deoxypenciclovir recovered in the urine over a 48-h period was determined by HPLC using a C_{18} reversed-phase column.
[b] Mean values of two independent experiments.

recovery of penciclovir from all of the prodrugs (28–36%) was 4.7–6.0-fold higher than that from penciclovir (6%). Compound **4** (R=Me, R_1=*i*-Pr in Table 1) achieved the highest penciclovir bioavailability (36%), followed in order by compounds **2** (35%; R=Me, R_1=Et), **6** (35%; R=R_1=Et), **7** (34%; R=Et, R_1=*n*-Pr), and **10** (34%; R=OEt, R_1=Et). The mean urinary recovery of penciclovir from compounds **8** (32%; R=Et, R_1=*i*-Pr), **3** (32%; R=Me, R_1=*n*-Pr), and **9** (30%; R=OMe, R_1=Me) was comparable to those from famciclovir (31%) and SK 1875 (31%), while compounds **12** (29%; R=*i*-PrO, R_1=*i*-Pr) and **5** (28%; R=Et, R_1=Me) were slightly less bioavailable. A substantial amount of 6-deoxypenciclovir (16–28%) was also found in the urine from all of the prodrugs (Table 1), indicating that the rate-determining step in the conversion of these prodrugs to penciclovir was oxidation at the 6-position of the purine ring.

Since compound **4** showed the highest mean urinary recovery of penciclovir and total mean urinary recovery of penciclovir and 6-deoxypenciclovir (63%) among these prodrugs, it was selected for further evaluation. Following a single oral administration (0.2 mmol/kg) of **4** to mice, concentrations of penciclovir and 6-deoxypenciclovir in the blood were determined by HPLC and compared with those obtained after oral administration of the equivalent dose of famciclovir (Table 2). The mean values (±SD) for maximum concentrations of penciclovir and 6-deoxypenciclovir in the blood were 63.5±14.9 and 29.4±9.0 μM for **4** and 62.6±2.5 and 25.9±5.7 μM for famciclovir, respectively, and these were achieved 15 min after administration. The values of concentrations of penciclovir and 6-deoxypenciclovir from **4** were similar to those from famciclovir at all time points tested with the exception of that concentration of penciclovir from **4** (54.4±7.2 μM) was much higher than that from famciclovir (41.9±8.3 μM) 30 min after administraton.



21

The parent prodrug **4** and all the other postulated metabolites SK 1875, **17**, **19**, **20**, and 9-(3-acetoxymethyl-4-hydroxybut-1-yl)guanine (**21**)[12] were also checked in the blood, but they were not detected at any time points in any mice, demonstrating very rapid hydrolysis of acyl group and carbonate group after oral administration.

The urinary recovery of penciclovir and 6-deoxypenciclovir following a single oral administration (0.2 mmol/kg) of **4** to mice was determined and compared with that from

Table 2. Concentrations of penciclovir and 6-deoxypenciclovir in the blood following oral administration of 2-amino-9-(3-acetoxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (**4**) and famciclovir to mice

	Concn (μM) in blood[a,b]			
	4		Famciclovir	
Time after dosing (h)	Penciclovir	6-Deoxypenciclovir	Penciclovir	6-Deoxypenciclovir
0.25	63.5 ± 14.9	29.4 ± 9.0	62.6 ± 2.5	25.9 ± 5.7
0.5	54.4 ± 7.2	13.2 ± 2.4	41.9 ± 8.3	12.8 ± 1.0
1	24.4 ± 5.8	3.4 ± 1.4	23.2 ± 2.8	4.0 ± 1.1
1.5	22.1 ± 6.3	2.8 ± 0.6	22.4 ± 7.0	3.4 ± 0.5
2	12.2 ± 4.7	1.0 ± 0.1[c]	6.0 ± 1.9	1.4 ± 0.7[c]
4	< 0.3[d]	< 0.8[d]	< 0.3[d]	< 0.8[d]

[a] A single oral dose of the test compound (0.2 mmol/kg) was administered to four male ICR mice. Concentrations of penciclovir and 6-deoxypenciclovir in the blood at 0.25, 0.5, 1, 1.5, 2, and 4 h after dosing were determined by HPLC using a C_{18} reversed-phase column.
[b] Mean ± SD of four animals.
[c] Mean ± SD of three animals. In the fourth animal, the value was below the limit of reliable determination.
[d] Below the limit of reliable determination in all animals (0.3 μM for penciclovir and 0.8 μM for 6-deoxypenciclovir).

the equivalent oral dose of famciclovir (Table 3). Although the mean urinary recovery of 6-deoxypenciclovir over a 24-h period from both **4** and famciclovir was the same (each 11%), **4** (53%) achieved slightly higher mean urinary recovery of penciclovir compared with that from famciclovir (49%). Again, **4** and aforementioned, all the other postulated metabolites were not detected at all in the urine. Although the total urinary recovery of penciclovir and 6-deoxypenciclovir from **4** in mice (64% over a 24-h period) was comparable to that in rats (63% over a 24-h period), the amount of penciclovir in mice (53%) is much higher than that in rats (36%), thus confirming that more extensive oxidation at the 6-position of the purine ring occurred in mice. The in vivo antiviral efficacy of **4** on HSV-1-induced mortality in BALB/c mice was evaluated and compared with those of famciclovir and valaciclovir (Table 4). The mean survival time of HSV-1-infected mice receiving no antiviral therapy was 6.5 ± 0.5 days (mean ± SEM), and no aminal survived on the last day of evaluation (day 21). Following oral administration of **4** (0.075 mmol/kg) twice daily for 5 consecutive days, it completely protected HSV-1-induced mortality. Although both famciclovir and valaciclovir caused increases in the survival rate (80 and 40%) and the mean survival time (13 ± 5.0 and 13 ± 1.6 days) as compared with those of untreated control, their in vivo antiviral efficacy was lower than that of **4**. Treatment of DHBV-infected ducklings with **4** at the dose of 30 mg/kg/day given orally in two equally divided doses for **4** weeks showed rapid decrease in serum DHBV-DNA levels below detectable limits, reduction of intrahepatic viral load and viral supercoiled DNA by 90 and 70%, respectively, and inhibition of Pre-S expression by 30%.[22] In contrast with SK 1875, after 4 weeks of drug-free follow-up, there was no difference in the histological appearance in the liver between the treated group and the control group, even though the administered dose of **4** was twofold higher than that of SK 1875.[22] These results indicate that protection of both hydroxyl groups in the acyclic moiety of the prodrug is necessary to minimize or eliminate the hepatotoxicity so that the parent prodrug and its oxidized metabolite can not be used as substrates for the host cellular DNA polymerase in the liver.

As previously shown in the amino acid ester prodrugs of acyclovir, the absolute stereochemistry of the prodrugs might affect hydrolytic cleavage and absorption.[28] However, it seems to be not the case for **4** since both protecting groups of **4** were readily cleaved and each enantiomer of **4** does not contain any distinguishable

Table 3. Urinary recovery of penciclovir and 6-deoxypenciclovir following oral administration of 2-amino-9-(3-acetoxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (**4**) and famciclovir to mice

	Urinary recovery of penciclovir and 6-deoxypenciclovir[a] (% dose)[b]	
Compd	Penciclovir	6-Deoxypenciclovir
4	53	11
Famciclovir	49	11

[a] A single oral dose of the test compound (0.2 mmol/kg) was administered to five male ICR mice. The total amount of penciclovir and 6-deoxypenciclovir recovered in the urine over a 24-h period was determined by HPLC using a C_{18} reversed-phase column.
[b] Mean values of five animals.

Table 4. Effects of **4**, famciclovir, and valaciclovir on HSV-1-induced mortality in BALB/c mice[a]

Compd	% Survival	Mean survival time (days)[b]
Untreated control	0	6.5 ± 0.5
4	100[c,d]	> 21
Famciclovir	80[c]	13 ± 5.0
Valaciclovir	40[e]	13 ± 1.6[f]

[a] Ten mice per group were infected i.p. with 3×10^4 PFU of HSV-1 (KOS strain) and treated with oral dose of 0.075 mmol/kg of the test compounds twice daily for 5 consecutive days postinfection.
[b] Each value represents the mean ± SEM.
[c] Significantly different from untreated control group ($p < 0.001$).
[d] Significantly different from valaciclovir-treated group ($p < 0.01$).
[e] Significantly different from untreated control group ($p < 0.05$).
[f] Significantly different from untreated control group ($p < 0.01$).

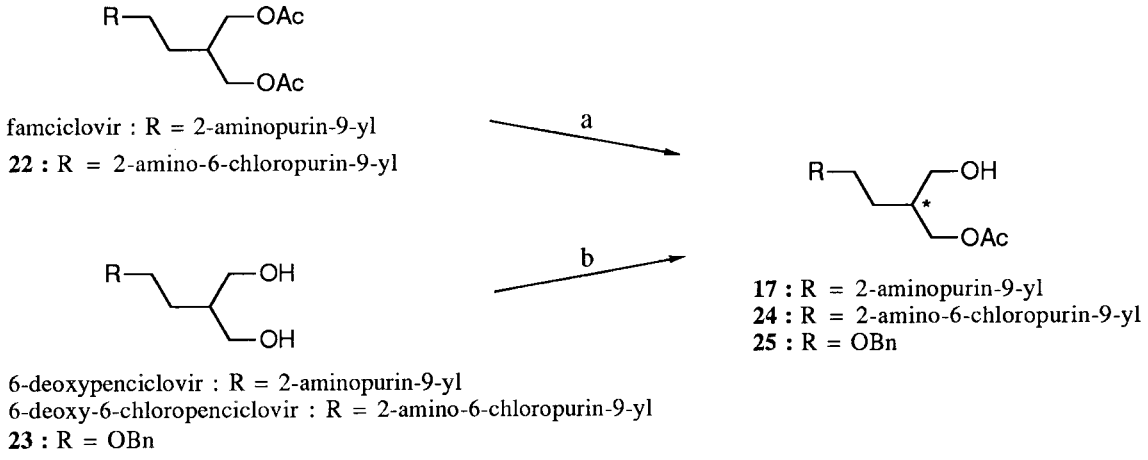
protecting groups to show different absorption rate in the gastrointestinal tract. To prove this, it seemed necessary to prepare each enantiomer of **4** and subject each enantiomer for the biological evaluation. Initial efforts for HPLC separation of the racemic **4** using chiral columns such as Chiralcel-OD (Daicel), Whelk-OI (Regis) and Cyclobondi 2000-DMP (Astec), proved unsuccessful under various chromatographic separation conditions. We then decided to resort to enzymatic reactions since there have been a lot of reports on the asymmetric enzymatic acylations of 2-substituted-1,3-propanediols or hydrosis of 1,3-diacetates of them.[29] As shown in Scheme 4, a few reactions were first tried with diols (6-deoxypenciclovir and 6-deoxy-6-chloropenciclovir) or diacetates (famciclovir and **22**), using three lipases from Pig Pancreas (PPL), *Pseudomonas fluorescens* (PFL), and *Candida Antarctica* (CAL), hoping to get the corresponding mono-acetate **17** or **24** in enantiomerically enriched form. It was disappointing to find that only the racemic mixtures of mono-acetates **17** and **24** were obtained under various conditions. Achiwa et al. have reported that the diol **23** can be converted to the mono-acetate **25** in 81% enantiomeric purity using PFL (supplied by Amano Pharmaceutical Co.).[30] Thus, we have repeated the same reaction using PFL (purchased from Aldrich) under the identical condition, but their result could not be reproduced by us. Disappointingly, only the 59:41 ratio of two enantiomers by HPLC (Chiralcel OD) was the best we could get. We tentatively attributed this discrepency to the different sources of PFL used. For now, it was concluded that either the separation or synthetic preparation of each enantiomer of **4** at this stage was too difficult. The study on the stereochemical effect of the 2-amino-9-(3-acyloxymethyl-4-alkoxycarbonyloxybut-1-yl)purine prodrugs will be undertaken when a new asymmetric synthesis of each enantiomer is developed.

In conclusion, 2-amino-9-(3-acetoxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (**4**) showed the highest oral mean urinary recovery of penciclovir in rats in a series of 2-amino-9-(3-acyloxymethyl-4-alkoxycarbonyloxybut-1-yl)purines and 2-amino-9-(3-alkoxycarbonyloxymethyl-4-alkoxycarbonyloxy-but-1-yl)purines. The mean urinary recovery of penciclovir in mice and rats, and concentrations of penciclovir in the blood in mice from **4** were slightly higher than those from famciclovir after oral administration of the equivalent dose. The in vivo antiviral efficacy of **4** in HSV-1-infected normal BALB/c mice was higher than those of famciclovir and valaciclovir in terms of mortality and mean survival time. Compound **4** demonstrated an effective anti-hepadnaviral response against DHBV in vivo and did not cause any significant hepatotoxicity after 4 weeks of treatment. On the basis of these results, the extensive preclinical studies of **4** are presently under way in our laboratory.

Experimental

Melting points were determined on a Mettler melting point apparatus and are uncorrected. Infrared spectra were recorded on a Magna 750 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 noisy-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. Fast-atom bombardment mass spectra (FAB–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.

2-Amino-6-chloro-9-(4-hydroxy-3-isopropoxycarbonyloxymethylbut-1-yl)purine (15) and 2-amino-9-(3-hydroxy-4-hydroxymethylbut-1-yl)purin-6-ylpyridinium chloride (16). To a cooled solution of 6-deoxy-6-chloropenciclovir (537 g, 1.98 mol) in anhydrous pyridine (5.7 L) in an ice bath under N_2 was added dropwise isopropyl chloroformate (267 g, 2.18 mol) as a neat solution over 1.5 h,



Scheme 4. (a) Lipase (PPL, PFL or CAL), sodium phosphate buffer (pH 7.0), rt; (b) lipase (PPL, PFL or CAL), vinyl acetate, acetone or toluene, rt.

and the mixture was stirred in an ice bath for 4 h after the addition. The reaction mixture was evaporated to dryness under the reduced pressure, and a mess of a dark yellow solid was formed during the evaporation. The resulting dark brown residue was suspended in $CHCl_3$ (2 L), and insoluble dark yellow solid was collected by filtration. Filtrate was concentrated in vacuo, and the gummy residue was partitioned between H_2O (1 L) and EtOAc (7×500 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to dryness under the reduced pressure to give a dark brown oil. The crude mixture was purified by MPLC on silica gel with CH_3CN/EtOAc (1/9) as eluent to afford 67 g (9.5%) of **15** as an off-white solid, which was crystallized from EtOAc: mp 117°C dec; IR (neat) 3347, 3209, 3104 (NH_2 and OH), 1738 (C=O); ^{1}H NMR ($CDCl_3$) δ 1.30 (d, J=6.0 Hz, 6H, $CH(CH_3)_2$), 1.85–2.09 (m, 3H, $CHCH_2CH_2$), 2.38 (br s, 1H, OH), 3.71 (t, J=4.2 Hz, 2H, CH_2OH), 4.20–4.26 (m, 4H, NCH_2 and OCH_2CH), 4.88 (septet, J=6.0 Hz, 1H, $CH(CH_3)_2$), 5.14 (br s, 2H, NH_2), 7.80 (s, 1H, H-8); MS (FAB) m/z 358 (MH^+). Anal. calcd for $C_{14}H_{20}ClN_5O_4$: C, 47.00; H, 5.63; N, 19.57. Found: C, 47.11; H, 5.67; N, 19.42. The above obtained dark yellow solid was triturated from MeOH/$CHCl_3$ to give 360 g (52%) of **16**: mp 152°C dec (MeOH/$CHCl_3$); IR (neat) 3345, 3292, 3257, 3182 (NH_2 and OH), 1659, 1636, 1574, 1562 (C=N and C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.44–1.55 (m, 1H, CH), 1.76–1.88 (m, 2H, $CHCH_2CH_2$), 3.34–3.50 (m, 4H, 2 OCH_2), 4.26 (t, J=7.4 Hz, 2H, NCH_2), 4.58 (t, J=5.0 Hz, 2H, 2 OH), 7.35 (br s, 2H, NH_2), 8.46 (dd, J=7.3 Hz, 6.2 Hz, 2H, H-3′ and H-5), 8.59 (s, 1H, H-8), 8.93 (t, J=7.3 Hz, 1H, H-4′), 10.08 (d, J=6.2 Hz, 2H, H-2′ and H-6′); MS (FAB) m/z 315 $(M-Cl)^+$. Anal. calcd for $C_{15}H_{19}ClN_6O_2$: C, 51.36; H, 5.46; N, 23.96. Found: C, 51.53; H, 5.59; N, 24.07.

General procedure for the preparation of 2-amino-9-(3-acyloxymethyl-4-hydroxybut-1-yl)purines 17 and 18. To a stirred solution of 6-deoxypenciclovir (6.00 g, 25.3 mmol) and a catalytic amount of p-TsOH·H_2O (0.48 g, 2.53 mmol) in anhydrous DMF (70 mL) was added dropwise trimethyl orthoacetate or triethyl orthopropionate (30.4 mmol) via a syringe at room temperature, and the resulting reaction mixture was stirred at room temperature for 2 h. H_2O (3 mL) was added, and the mixture was stirred for an additional 1 h. The reaction mixture was neutralized with $NaHCO_3$ (0.21 g, 2.53 mmol) in 3 mL of H_2O and was evaporated to dryness in vacuo to afford a yellowish residue. The crude product was purified by MPLC on silica gel with MeOH/$CHCl_3$ (1/9) as eluent to afford the titled compound as a white solid, which was crystallized from a suitable solvent.

2-Amino-9-(3-acetoxymethyl-4-hydroxybut-1-yl)purine (17). Yield 95%; mp 164.2–164.5°C (MeOH) (lit.[11] 166–168°C); spectroscopic data were identical to those reported.

2-Amino-9-(4-hydroxy-3-propionyloxymethylbut-1-yl)-purine (18). Yield 92%; mp 149.5–150.3°C (EtOH); IR (neat) 3367, 3326, 3191 (NH_2 and OH), 1727 (C=O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.99 (t, J=7.5 Hz, 3H, CH_2CH_3), 1.60–1.70 (m, 1H, CH), 1.75–1.90 (m, 2H, $CHCH_2CH_2$), 2.27 (q, J=7.5 Hz, 2H, CH_2CH_3), 3.42 (m, 2H, $CHCH_2OH$), 3.95–4.06 (m, 2H, OCH_2CH), 4.10 (t, J=7.2 Hz, 2H, NCH_2), 4.64 (t, J=5.1 Hz, 1H, OH), 6.47 (br s, 2H, NH_2), 8.08 (s, 1H, H-8), 8.56 (s, 1H, H-6); ^{13}C NMR (DMSO-d_6) δ 8.9, 26.7, 28.0, 37.6, 40.3, 60.5, 63.9, 126.9, 142.6, 148.9, 152.9, 160.4, 173.6; MS (FAB) m/z 294 (MH^+). Anal. calcd for $C_{13}H_{19}N_5O_3$: C, 53.23; H, 6.53; N, 23.88. Found: C, 53.11; H, 6.43; N, 23.92.

General procedure for the preparation of 2-amino-9-(3-acyloxymethyl-4-alkoxycarbonyloxybut-1-yl)purines 1–8. A mixture of **17** or **18** (2.16 mmol), alkyl 4-nitrophenyl carbonate (2.59 mmol), and a catalytic amount of DMAP (26 mg, 0.21 mmol) in anhydrous pyridine (7 mL) was heated at 80°C under N_2 for 20 h, and the resulting reaction mixture was evaporated to dryness in vacuo to afford a yellow residue. The crude product was purified by MPLC on silica gel (gradient elution: 2% MeOH in $CHCl_3$ followed by 3% MeOH in $CHCl_3$) to afford the titled compound as a white solid, which was crystallized from a suitable solvent indicated.

2-Amino-9-(3-acetoxymethyl-4-methoxycarbonyloxybut-1-yl)purine (1). Yield 86%; mp 71.0–72.5°C (EtOAc/Et_2O); IR (neat) 3338 (NH_2), 1747, 1730 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.90–2.05 (m, 3H, $CHCH_2CH_2$), 2.06 (s, 3H, $COCH_3$), 3.80 (s, 3H, OCH_3), 4.13–4.29 (m, 6H, NCH_2 and 2 OCH_2CH), 5.09 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.70 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 20.8, 28.7, 35.1, 40.7, 53.9, 63.4, 67.3, 128.3, 142.1, 149.9, 153.2, 155.6, 159.9, 170.8; MS (FAB) m/z 338 (MH^+). Anal. calcd for $C_{14}H_{19}N_5O_5$: C, 49.85; H, 5.68; N, 20.76. Found: C, 49.97; H, 5.62; N, 20.65.

2-Amino-9-(3-acetoxymethyl-4-ethoxycarbonyloxybut-1-yl)purine (2). Yield 87%; mp 92.0–93.5°C (EtOAc/Et_2O); IR (neat) 3336 (NH_2), 1746, 1735 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J=7.2 Hz, 3H, CH_2CH_3), 1.90–2.05 (m, 3H, $CHCH_2CH_2$), 2.06 (s, 3H, $COCH_3$), 4.14–4.26 (m, 8H, NCH_2 and 3 OCH_2), 5.10 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 14.2, 20.8, 28.7, 35.1, 40.8, 63.5, 65.8, 67.0, 128.3, 142.2, 149.9, 153.2, 155.0, 159.9, 170.8; MS (FAB) m/z 352 (MH^+). Anal. calcd for $C_{15}H_{21}N_5O_5$:C, 51.28; H, 6.02; N, 19.93. Found: C, 51.37; H, 6.16; N, 19.81.

2-Amino-9-(3-acetoxymethyl-4-propoxycarbonyloxybut-1-yl)purine (3). Yield 86%; mp 72.5–73.5°C (EtOAc/Et_2O/hexanes); IR (neat) 3337 (NH_2), 1750, 1735 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.96 (t, J=7.4 Hz, 3H, $CH_2CH_2CH_3$), 1.60–1.74 (m, 2H, $CH_2CH_2CH_3$), 1.90–2.05 (m, 3H, $CHCH_2CH_2$), 2.06 (s, 3H, $COCH_3$), 4.11 (t, J=6.8 Hz, 2H, OCH_2CH_2), 4.15–4.24 (m, 6H, NCH_2 and 2 OCH_2), 5.08 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.70 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 10.1, 20.8, 22.0, 28.7, 35.1, 40.8, 63.5, 67.1, 69.9, 128.3, 142.2, 149.9, 153.2, 155.2, 159.9, 170.8; MS (FAB) m/z 366 (MH^+). Anal. calcd for $C_{16}H_{23}N_5O_5$: C, 52.60; H, 6.34; N, 19.17. Found: C, 52.32; H, 6.39; N, 19.31.

2-Amino-9-(3-acetoxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (4). Yield 89%; mp 106.5–107.7°C (EtOAc/hexanes); IR (neat) 3337 (NH_2), 1744, 1736 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (d, $J=6.3$ Hz, 6H, $CH(CH_3)_2$), 1.90–2.05 (m, 3H, $CHCH_2CH_2$), 2.06 (s, 3H, $COCH_3$), 4.15–4.24 (m, 6H, NCH_2 and 2 OCH_2), 4.88 (septet, $J=6.3$ Hz, 1H, $CH(CH_3)_2$), 5.10 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 20.8, 21.7, 28.7, 35.1, 40.8, 63.5, 66.8, 72.3, 128.3, 142.1, 149.9, 153.2, 154.5, 159.9, 170.8; MS (FAB) m/z 366 (MH^+). Anal. calcd for $C_{16}H_{23}N_5O_5$: C, 52.60; H, 6.34; N, 19.17. Found: C, 52.45; H, 6.30; N, 19.19.

2-Amino-9-(4-methoxycarbonyloxy-3-propionyloxymethylbut-1-yl)purine (5). Yield 93%; mp 72.0–73.0°C (EtOAc/Et_2O); IR (neat) 3383, 3335 (NH_2), 1746, 1652 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (t, $J=7.8$ Hz, 3H, $COCH_2CH_3$), 1.95–2.08 (m, 3H, $CHCH_2CH_2$), 2.34 (q, $J=7.8$ Hz, 2H, $COCH_2CH_3$), 3.80 (s, 3H, OCH_3), 4.16-4.24 (m, 6H, NCH_2 and 2 OCH_2), 5.14 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 9.0, 27.4, 28.7, 35.1, 40.7, 54.9, 63.2, 67.3, 128.2, 142.1, 149.8, 153.2, 155.6, 159.9, 174.1; MS (FAB) m/z 352 (MH^+). Anal. calcd for $C_{15}H_{21}N_5O_5$: C, 51.28; H, 6.02; N, 19.93. Found: C, 51.35; H, 6.15; N, 20.08.

2-Amino-9-(4-ethoxycarbonyloxy-3-propionyloxymethylbut-1-yl)purine (6). Yield 93%; mp 65.0–66.2°C (EtOAc/Et_2O); IR (neat) 3387, 3336 (NH_2), 1746, 1652 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (t, $J=7.8$ Hz, 3H, $COCH_2CH_3$), 1.31 (t, $J=7.2$ Hz, 3H, OCH_2CH_3), 1.95–2.07 (m, 3H, $CHCH_2CH_2$), 2.34 (q, $J=7.8$ Hz, 2H, $COCH_2CH_3$), 4.17–4.24 (m, 8H, NCH_2 and 3 OCH_2), 5.14 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 9.0, 14.2, 27.4, 28.7, 35.1, 40.7, 63.3, 64.2, 67.0, 128.2, 142.1, 149.8, 153.2, 155.0, 159.9, 174.1; MS (FAB) m/z 366 (MH^+). Anal. calcd for $C_{16}H_{23}N_5O_5$: C, 52.60; H, 6.34; N, 19.17. Found: C, 52.83; H, 6.46; N, 19.06.

2-Amino-9-(3-propionyloxymethyl-4-propoxycarbonyloxybut-1-yl)purine (7). Yield 94%; mp 63.0–64.5°C (Et_2O/hexanes); IR (neat) 3387, 3336 (NH_2), 1747, 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (t, $J=7.5$ Hz, 3H, $OCH_2CH_2CH_3$), 1.14 (t, $J=7.8$ Hz, 3H, $COCH_2CH_3$), 1.64–1.76 (m, 2H, $OCH_2CH_2CH_3$), 1.96–2.07 (m, 3H, $CHCH_2CH_2$), 2.34 (q, $J=7.8$ Hz, 2H, $COCH_2CH_3$), 4.13 (t, $J=6.9$ Hz, 2H, $OCH_2CH_2CH_3$), 4.17–4.24 (m, 6H, NCH_2 and 2 OCH_2), 5.09 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 9.0, 10.1, 21.9, 27.4, 28.7, 35.2, 40.8, 63.3, 67.1, 69.9, 128.3, 142.2, 149.9, 153.2, 155.1, 159.9, 174.2; MS (FAB) m/z 380 (MH^+). Anal. calcd for $C_{17}H_{25}N_5O_5$: C, 53.82; H, 6.64; N, 18.46. Found: C, 53.60; H, 6.65; N, 18.59.

2-Amino-9-(4-isopropoxycarbonyloxy-3-propionyloxymethylbut-1-yl)purine (8). Yield 94%; mp 72.5–73.5°C (Et_2O/hexanes); IR (neat) 3386, 3336 (NH_2), 1742, 1651 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (t, $J=7.5$ Hz, 3H, $COCH_2CH_3$), 1.30 (d, $J=6.3$ Hz, 6H, $CH(CH_3)_2$), 1.94–2.10 (m, 3H, $CHCH_2CH_2$), 2.34 (q, $J=7.5$ Hz, 2H, $COCH_2CH_3$), 4.17–4.24 (m, 6H, NCH_2 and 2 OCH_2), 4.88 (septet, $J=6.3$ Hz, 1H, $CH(CH_3)_2$), 5.10 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 9.0, 21.7, 27.4, 28.7, 35.2, 40.8, 63.3, 66.9, 72.3, 128.2, 142.1, 149.8, 153.2, 154.5, 159.9, 174.2; MS (FAB) m/z 380 (MH^+). Anal. calcd for $C_{17}H_{25}N_5O_5$: C, 53.82; H, 6.64; N, 18.46. Found: C, 53.56; H, 6.67; N, 18.43.

General procedure for the preparation of 2-amino-9-(3-alkoxycarbonyloxymethyl-4-alkoxycarbonyloxybut-1-yl)-purines 9–12. A stirred suspension of 6-deoxypenciclovir (0.50 g, 2.11 mmol), alkyl 4-nitrophenyl carbonate (4.43 mmol) and a catalytic amount of DMAP (26 mg, 0.21 mmol) in anhydrous pyridine (8 mL) was heated at 80°C for 24 h, and the resulting homogeneous mixture was evaporated to dryness in vacuo to afford a yellow residue. The crude product was purified by MPLC on silica gel (gradient elution: 2% MeOH in $CHCl_3$ followed by 3% MeOH in $CHCl_3$) to afford the titled compound as a white solid, which was crystallized from a suitable solvent indicated.

2-Amino-9-(3-methoxycarbonyloxymethyl-4-methoxycarbonyloxybut-1-yl)purine (9). Yield 83%; mp 111.0–112.5°C (EtOAc/hexanes); IR (neat) 3460, 3309 (NH_2), 1742, 1732 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.97–2.11 (m, 3H, $CHCH_2CH_2$), 3.79 (s, 6H, 2 OCH_3), 4.20–4.24 (m, 6H, NCH_2 and 2 OCH_2CH), 5.03 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 28.6, 35.2, 40.8, 55.0, 67.0, 128.3, 142.2, 149.9, 153.2, 155.6, 159.8; MS (FAB) m/z 354 (MH^+). Anal. calcd for $C_{14}H_{19}N_5O_6$: C, 47.59; H, 5.42; N, 19.82. Found: C, 47.68; H, 5.52; N, 19.85.

2-Amino-9-(3-ethoxycarbonyloxymethyl-4-ethoxycarbonyloxybut-1-yl)purine (10). Yield 91%; mp 72.5–73.5°C (EtOAc/hexanes); IR (neat) 3383, 3334 (NH_2), 1743 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (t, $J=7.2$ Hz, 6H, 2 OCH_2CH_3), 1.95–2.15 (m, 3H, $CHCH_2CH_2$), 4.17–4.25 (m, 10H, NCH_2 and 4 OCH_2), 5.04 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 14.2, 28.5, 35.2, 40.7, 64.3, 66.7, 128.2, 142.2, 149.8, 153.2, 154.9, 159.8; MS (FAB) m/z 382 (MH^+). Anal. calcd for $C_{16}H_{23}N_5O_6$: C, 50.39; H, 6.08; N, 18.36. Found: C, 50.12; H, 6.01; N, 18.51.

2-Amino-9-(3-propoxycarbonyloxymethyl-4-propoxycarbonyloxybut-1-yl)purine (11). Yield 83%; mp 72.0–73.5°C (EtOAc/hexanes); IR (neat) 3401, 3332 (NH_2), 1746 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (t, $J=7.5$ Hz, 6H, 2 $OCH_2CH_2CH_3$), 1.65–1.75 (m, 4H, 2 $OCH_2CH_2CH_3$), 1.95–2.17 (m, 3H, $CHCH_2CH_2$), 4.10 (t, $J=6.8$ Hz, 4H, 2 $OCH_2CH_2CH_3$), 4.20–4.25 (m, 6H, NCH_2 and 2 OCH_2), 5.04 (br s, 2H, NH_2), 7.78 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 10.1, 22.0, 28.6, 35.3, 40.8, 66.8, 69.9, 128.3, 142.3, 149.8, 153.2, 155.1, 159.8; MS (FAB) m/z 410 (MH^+). Anal. calcd for $C_{18}H_{27}N_5O_6$: C, 52.80; H, 6.65; N, 17.10. Found: C, 52.47; H, 6.69; N, 17.15.

2-Amino-9-(3-isopropoxycarbonyloxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (12). Yield 85%; mp 74.5–75.0°C (EtOAc/hexanes); IR (neat) 3397, 3335 (NH_2),

1738 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (d, $J=6.3$ Hz, 12H, 2 $CH(CH_3)_2$), 1.95–2.10 (m, 3H, $CHCH_2CH_2$), 4.21–4.25 (m, 6H, NCH_2 and 2 OCH_2), 4.87 (septet, $J=6.3$ Hz, 2H, 2 $CH(CH_3)_2$), 5.12 (br s, 2H, NH_2), 7.79 (s, 1H, H-8), 8.69 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$) δ 21.7, 28.6, 35.2, 40.7, 66.6, 72.3, 128.2, 142.2, 149.8, 153.2, 154.5, 159.9; MS (FAB) *m/z* 410 (MH^+). Anal. calcd for $C_{18}H_{27}N_5O_6$: C, 52.80; H, 6.65; N, 17.10. Found: C, 52.72; H, 6.69; N, 17.17.

9-(3-Hydroxymethyl-4-isopropoxycarbonyloxybut-1-yl)-guanine (19). A mixture of **15** (420 mg, 1.17 mmol) and adenosine deaminase (42 mg; Sigma, Type VII from Calf Intestinal Mucosa) in phosphate buffer solution (80 mL, pH 7.5) was vigorously stirred at room temperature for 5 days. The reaction mixture was filtered through a Celite pad, and the filtrate was evaporated to dryness under the reduced pressure. The resulting residue was purified by MPLC on silica gel (gradient elution: 15% MeOH in $CHCl_3$ followed by 20% MeOH in $CHCl_3$) to afford 370 mg (93%) of **19** as a white solid, which was crystallized from absolute EtOH: mp 143°C dec; IR (neat) 3434, 3317, 3197 (NH_2 and OH), 1741, 1635 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.21 (d, $J=6.0$ Hz, 6H, $CH(CH_3)_2$), 1.63–1.84 (m, 3H, $CHCH_2CH_2$), 3.39 (t, $J=5.1$ Hz, 2H, CH_2OH), 3.96–4.11 (m, 4H, NCH_2 and OCH_2), 4.66 (t, $J=5.1$ Hz, 1H, CH_2OH), 4.73 (septet, $J=6.0$ Hz, 1H, $CH(CH_3)_2$), 6.42 (br s, 2H, NH_2), 7.69 (s, 1H, H-8),10.51 (br s, 1H, NH); MS (FAB) *m/z* 340 (MH^+). Anal. calcd for $C_{14}H_{21}N_5O_5$: C, 49.55; H, 6.24; N, 20.64. Found: C, 49.67; H, 6.29; N, 20.52.

9-(3-Acetoxymethyl-4-isopropoxycarbonyloxybut-1-yl)-guanine (20). To a solution of **19** (80 mg, 0.24 mmol) and a catalytic amount of DMAP (3 mg, 0.02 mmol) in anhydrous DMF (1 mL) at room temperature under N_2 was added acetic anhydride (48 mg, 45 μL, 0.47 mmol), and the mixture was stirred at room temperature for 1 h. The reaction was quenched with MeOH (1 mL), and the mixture was evaporated to dryness under the reduced pressure. The resulting residue was purified by MPLC on silica gel (gradient elution: 5% MeOH in $CHCl_3$ followed by 10% MeOH in $CHCl_3$) to afford an off-white solid, which was crystallized from absolute MeOH/Et_2O to give 75 mg (83%) of **20**: mp 186°C dec; IR (neat) 3319, 3161 (NH_2), 1741, 1691, 1651 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.21 (d, $J=6.0$ Hz, 6H, $CH(CH_3)_2$), 1.75–1.82 (m, 2H, $CHCH_2CH_2$), 1.90–1.99 (m, 1H, $CHCH_2CH_2$), 2.00 (s, 3H, $COCH_3$), 3.99–4.08 (m, 6H, NCH_2 and 2 OCH_2), 4.73 (septet, $J=6.0$ Hz, 1H, $CH(CH_3)_2$), 6.41 (br s, 2H, NH_2), 7.70 (s, 1H, H-8),10.53 (br s, 1H, NH); MS (FAB) *m/z* 382 (MH^+). Anal. calcd for $C_{16}H_{23}N_5O_6$: C, 50.39; H, 6.08; N, 18.36. Found: C, 50.47; H, 6.13; N, 18.41.

Urinary recovery in rats. The bioavailability of the test compound was estimated by determining the total amount of penciclovir and 6-deoxypenciclovir 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 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 concentrations of penciclovir and 6-deoxypenciclovir were analyzed by HPLC as follows. A C_{18} reversed-phase 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.

Pharmacokinetics in mice. Four male ICR mice (25–30 g) were each given a single dose of **4** and famciclovir (0.2 mmol/kg/10 mL) with an intragastric needle. Blood was collected at 0.25, 0.5, 1, 1.5, 2, and 4 h after dosing by cardiac puncture using heparinized syringe. An aliquot (150 μL) of each sample was immediately mixed with trichloroacetic acid (150 μL) (8% final concentration) in a separate tube and centrifuged. The supernatant (200 μL) was neutralized with 1/5 volume (40 μL) of saturated aqueous $NaHCO_3$. The concentrations of **4**, penciclovir, 6-deoxypenciclovir, and other postulated metabolites of **4**, SK 1875, **17**, **19**, **20**, and **21**, were analyzed by HPLC as follows. A C_{18} reversed-phase column equipped with a precolumn was eluted at a flow rate of 1 mL/min with the following four-step gradient: (step 1) a 5-min isocratic elution with 99% solution A (5 mM K_2HPO_4) and 1% solution B (80% MeOH in 5 mM K_2HPO_4), (step 2) a 10-min linear gradient from 99% solution A and 1% solution B to 85% solution A and 15% solution B, (step 3) a 19-min linear gradient from 85% solution A and 15% solution B to 5% solution A and 95% solution B, and (step 4) a 5-min isocratic elution with 5% solution A and 95% solution B. Before each sample injection, the column was re-equilibrated to initial conditions. The UV absorbance of the column effluent was monitored at both 256 and 300 nm with dual λ absorbance detector.

Urinary recovery in mice. Five male ICR mice (25–30 g) were each given a single dose of **4** and famciclovir (0.2 mmol/kg/10 mL) with an intragastric needle. Urine was collected for 24 h in a metabolic cage after dosing. 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. An aliquot (100 μL) of each sample was diluted with 1 mM Na_2HPO_4 (pH 7.0) (500 μL), was loaded onto the cation-exchange Sep-Pak cartridge pretreated with MeOH (1 mL) and H_2O (1 mL), and was then eluted with 100 mM Na_2HPO_4 (pH 11.0) (1 mL). The concentrations of **4**, penciclovir, 6-deoxypenciclovir, and other postulated metabolites of **4**, SK 1875, **17**, **19**, **20**, and **21** in the urine samples were analyzed by reversed-phase HPLC using a C_{18} column with a UV detector as afore-mentioned conditions in the pharmacokinetic study.

In vivo antiviral efficacy. HSV-1 (KOS strain) suspension (3×10^4 PFU/0.1 mL) was inoculated i.p. to 4.5-week-old female BALB/c mice (17–21 g). Each group consisted of 10 mice. Test compounds were dissolved in H_2O to give a dose of 0.075 mmol/kg of body weight per mouse in 10 mL. The compounds were administered by oral gavage twice daily at 8 a.m. and 6 p.m. for 5 days, commencing at 18 h postinfection. Mortality was observed for 21 days after virus inoculation. The two-tailed Fisher's exact test was used for determination of significant difference in survival rate, and the Mann–Whitney rank sum test was used for determination of significant difference in mean survival time. *P* Values of <0.05 were considered statistically significant.

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