Synthesis and Evaluation of Amino Acid Esters of 6-Deoxypenciclovir as Potential Prodrugs of Penciclovir

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Abstract—The amino acid ester derivatives of 6-deoxypenciclovir, **11–20**, were synthesized as potential prodrugs of penciclovir, and were evaluated for their oral penciclovir bioavailability in mice and rats. Esterification of 6-deoxypenciclovir with *N*-carbobenzyl-oxyglycine, -L-alanine, -L-valine, -L-leucine, or -L-isoleucine (3.75 equiv.) using conventional coupling method (DCC/DMAP) afforded the mono-*O*-ester derivatives **1–5** in 47–55% yields as a mixture of two diastereomers along with the di-*O*-ester derivatives **6–10** in 20–29% yields. Reductive cleavage of carbobenzyloxy (Cbz) group (10% Pd/C, 1 atmosphere of H₂, room temperature in methanol) followed by subsequent treatment of the resulting free amine with methanolic HCl solution provided the mono-*O*-ester derivatives **11–15** as di-HCl salt in 51–98% yields and the di-*O*-ester derivatives **16–20** as tri-HCl salt in 65–98% yields. Of the prodrugs tested in mice and rats, 6-deoxypenciclovir *O*-L-valinate (**13**), *O*-L-isoleucinate (**15**), and *O,O*-di-glycinate (**16**) showed significantly higher urinary recovery of penciclovir compared with that of penciclovir, but those are somewhat lower than that of famciclovir. © 1999 Elsevier Science Ltd. All rights reserved.

Introduction

An acyclonucleoside 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (penciclovir) is a potent and highly 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) in cell cultures and in animal models.[1,2] The antiviral spectrum of penciclovir against human herpesviruses is similar to that of 9-(2-hydroxy-ethoxymethyl)guanine (acyclovir), and both compounds have highly selective activity against these viruses because they are phosphorylated only in herpesvirus infected cells.[3] The advantage of penciclovir over acyclovir is that its antiviral activity in cell culture is more persistent than that of acyclovir since penciclovir triphosphate has a much greater stability than acyclovir triphosphate within virus-infected cells.[2,4] However, like other acycloguanosine analogues such as acyclovir,[5] ganciclovir,[6] and buciclovir,[7] penciclovir was poorly absorbed when given orally to rodents.[8,9] In order 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.[8] 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,[8] rats,[9] and humans.[10] Famciclovir has recently been approved by FDA for the treatment of herpes zoster (shingles).

Recently, Beauchamp et al. prepared a series of amino acid ester derivatives of acyclovir to increase its oral bioavailability.[11] Among them, the L-valyl ester of acyclovir (valaciclovir), recently approved by FDA, is rapidly absorbed and extensively converted to acyclovir after oral administration; the resulting plasma levels of acyclovir in rats and humans are 3 to 5 times higher than those attainable with oral acyclovir itself.[11,12] We also prepared the amino acid esters of penciclovir and a series of 2-amino-9-(3-hydroxymethyl-4-alkoxy-carbonyloxybut-1-yl)purines as potential prodrugs of penciclovir, and found that the oral penciclovir bioavailabilities of *O*-acetyl-*O*-L-valylpenciclovir (SK 1826) and 2-amino-9-(3-hydroxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine (SK 1875) in mice and/or rats were comparable to that of famciclovir.[13,14] On the basis of these findings, it was of our particular interest to determine whether amino acid ester derivatives of 6-deoxy-penciclovir could increase its oral penciclovir bioavailability. Therefore, in this report, we prepared the amino acid esters of 6-deoxypenciclovir, **11–20**, and evaluated for their oral penciclovir bioavailability in mice and rats.

Chemistry

Target compounds, the *O*-amino acid esters of 6-deoxy-penciclovir, **11–20**, were synthesized as shown in Scheme 1. Starting 6-deoxypenciclovir was prepared according to the known procedure[1] and was esterified with *N*-carbobenzyloxyglycine, -L-alanine, -L-valine, -L-leucine or -L-isoleucine (3.75 equiv.) using conventional coupling method (DCC/DMAP) under a dilute condition (0.04 M in DMF) to obtain a mixture of mono- and di-*N*-carbobenzyloxy-amino acid derivatives, **1–10**. Chromatographic purification of the crude mixtures on
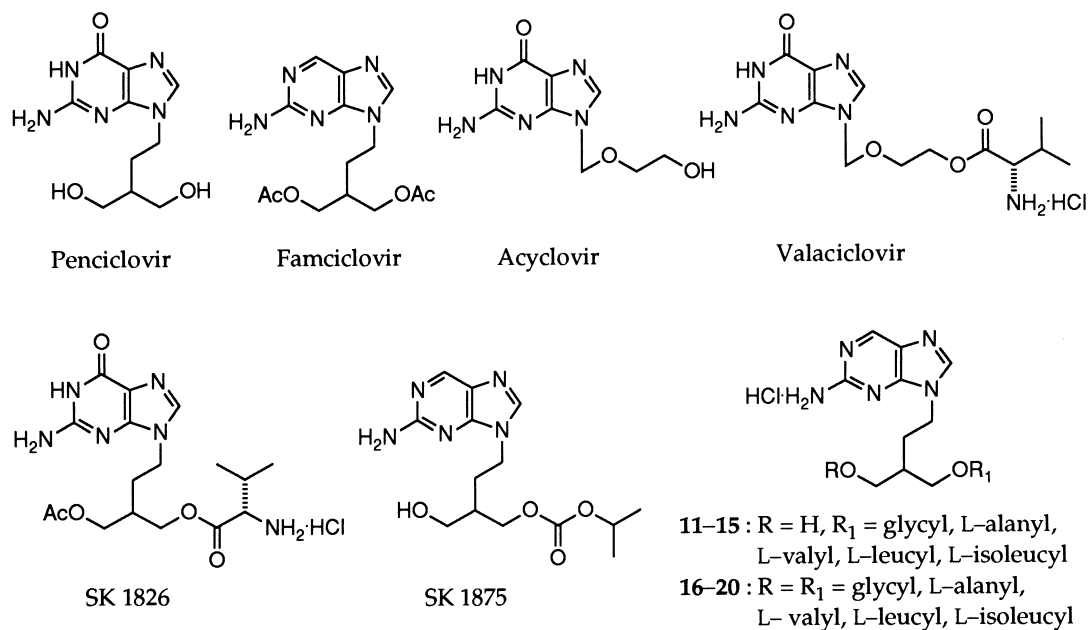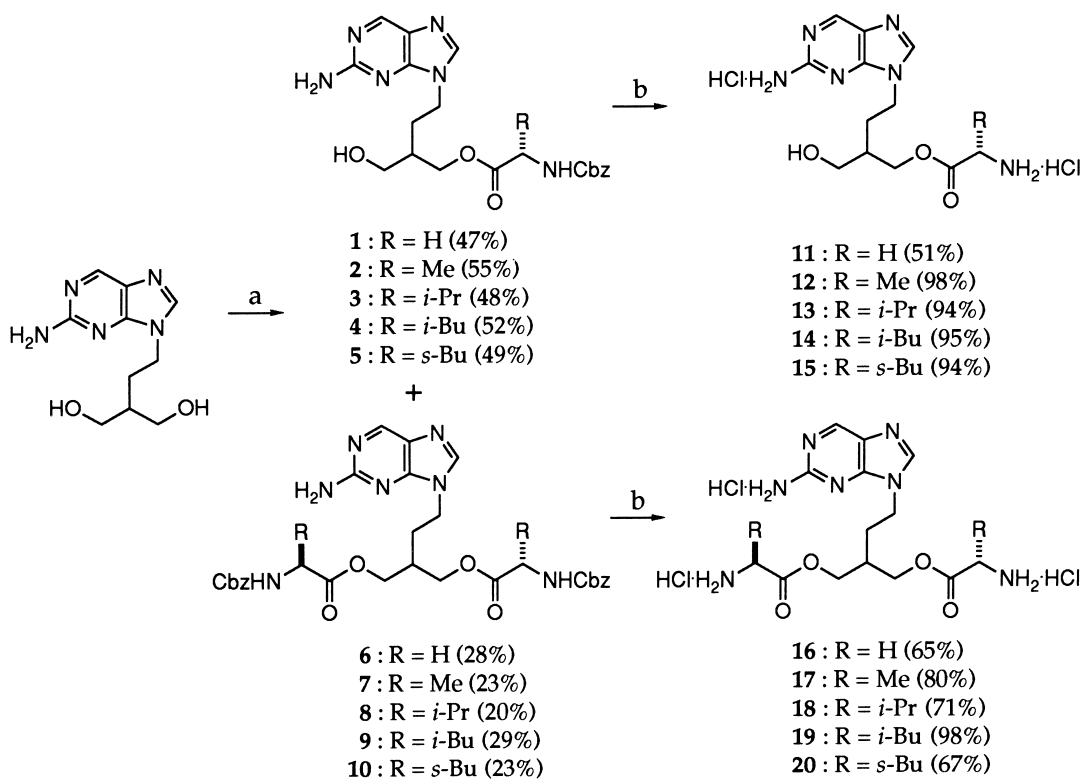

Penciclovir   Famciclovir   Acyclovir   Valaciclovir

SK 1826   SK 1875

11–15 : R = H, R$_1$ = glycyl, L–alanyl, L–valyl, L–leucyl, L–isoleucyl
16–20 : R = R$_1$ = glycyl, L–alanyl, L–valyl, L–leucyl, L–isoleucyl

Chart 1.

1 : R = H (47%)
2 : R = Me (55%)
3 : R = *i*-Pr (48%)
4 : R = *i*-Bu (52%)
5 : R = *s*-Bu (49%)

11 : R = H (51%)
12 : R = Me (98%)
13 : R = *i*-Pr (94%)
14 : R = *i*-Bu (95%)
15 : R = *s*-Bu (94%)

+

6 : R = H (28%)
7 : R = Me (23%)
8 : R = *i*-Pr (20%)
9 : R = *i*-Bu (29%)
10 : R = *s*-Bu (23%)

16 : R = H (65%)
17 : R = Me (80%)
18 : R = *i*-Pr (71%)
19 : R = *i*-Bu (98%)
20 : R = *s*-Bu (67%)

Scheme 1. (a) (i) *N*-Cbz-glycine, -L-alanine, -L-valine, -L-leucine or -L-isoleucine (1.25 equiv.), DCC (1.5 equiv.), DMAP (0.15 equiv.), DMF, rt, 16 h; (ii) repeat process (i) twice; (b) (i) H$_2$ (1 atm), 10% Pd/C (20 wt% for **11–15** or 40 wt% for **16–20**), MeOH, rt, 3 h, (ii) SiO$_2$ flash chromatography, (ii) 2*N* HCl in MeOH (3.0 equiv. for **11–15** or 5.0 equiv. for **16–20**), MeOH.

silica gel afforded the mono-*O*-ester derivatives **1–5** in 47–55% yields as a mixture of two diastereomers along with the di-*O*-ester derivatives **6–10** in 20–29% yields. Reductive cleavage of carbobenzyloxy (Cbz) group and formation of HCl salts from **11–20** were performed in a stepwise manner because an impurity problem was observed with one-pot procedure in our previous work of *O*-acyl-*O*-amino acid ester prodrugs of penciclovir.[13] Hydrogenolysis of Cbz group (10% Pd/C, 1 atmosphere of H_2, room temperature in methanol) in the absence of aqueous HCl solution, and purification by flash chromatography on silica gel followed by subsequent treatment of the resulting free amine with 2.0 N methanolic HCl solution (3.0 equiv. for **11–15** or 5.0 equiv. for **16–20**) provided the mono-*O*-ester derivatives **11–15** as di-HCl salt in 51–98% yields and the di-*O*-ester derivatives **16–20** as tri-HCl salt in 65–98% yields.

As pointed out in our earlier work of *O*-acyl-*O*-amino acid ester prodrugs of penciclovir,[13] it was again apparent from their ^{1}H and ^{13}C NMR spectra that the mono-*O*-ester compounds **12–15** consisted of two diastereomers in equal amount. Two asymmetric carbon centers and two carbons bridging them were showed up as pairs in their ^{13}C NMR spectra. For example, the ^{13}C NMR spectrum of **13** in DMSO-d_6 showed pairs of two asymmetric carbons at 37.3, 37.4, 57.4, and 57.5 ppm, a pair of methylene carbon at 65.3 and 65.4 ppm, and a pair of carbonyl carbon at 168.55 and 168.64 ppm, respectively. As previously shown in the amino acid ester prodrugs of acyclovir,[11] the stereochemistry of the prodrugs might affect hydrolytic cleavage and absorption. Thus, it seems necessary to separate aforementioned diastereomers before biological evaluation. However, since the separation of those isomers was practically difficult even by using HPLC, they were tested as a mixture of diastereomers without further separation.

Results and Discussion

The bioavailability of penciclovir after a single oral administration (0.2 mmol/kg) of the amino acid derivatives **11–20** to mice and rats was estimated by determining the total amount of penciclovir recovered in the urine over a 48 h period and was compared with those of penciclovir and famciclovir (Table 1). Among the five mono-amino acid ester derivatives **11–15**, the L-valine ester derivative **13** (32% in mice and 30% in rats) and the L-isoleucine ester derivative **15** (36% in mice and 29% in rats) showed high urinary recovery of penciclovir that was comparatively lower than that of famciclovir (39% in mice and 38% in rats), but significantly higher than that of penciclovir (9% in mice and 3% in rats). This result is consistent with an early observation of Beauchamp et al. that the esters of L-valine and L-isoleucine of acyclovir were much more bioavailable than the other amino acid ester derivatives.[11] In the di-amino acid ester derivatives **16–20**, the glycine ester derivative **16** achieved high urinary recovery of penciclovir in both mice (31%) and rats (30%), but the L-valine ester derivative **18** showed good urinary recovery of penciclovir in mice only (34%). In conclusion,

Table 1 Oral bioavailability of 6-deoxypenciclovir amino acid ester prodrugs of penciclovir, **11–20**, in mice and rats

Compd	R	Urinary recovery of penciclovir[a] (% dose)[b]	
		Mouse	Rat
11	H	18	16
12	Me	14	11
13	*i*-Pr	32	30
14	*i*-Bu	14	10
15	*s*-Bu	36	29
16	H	31	30
17	Me	10	9
18	*i*-Pr	34	14
19	*i*-Bu	16	10
20	*s*-Bu	25	15
Penciclovir		9	3
Famciclovir		39	38

[a] A single oral dose of test compound (0.2 mmol/kg) was administered to six male ICR mice or four 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.
[b] Mean values.

6-deoxypenciclovir *O*-L-valinate (**13**), *O*-L-isoleucinate (**15**), and *O*,*O*-di-glycinate (**16**) showed significantly higher oral penciclovir bioavailability compared with that of penciclovir in mice and rats, but their oral bioavailability is somewhat lower than that of famciclovir.

Experimental

Infrared spectra were recorded on a Perkin–Elmer 1600 FTIR spectrophotometer and UV spectra on a Hewlett-Packard 8452A spectrometer. ^{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 DMSO-d_6. ^{1}H noise-decoupled ^{13}C NMR spectra were recorded on a Varian Unity 300 spectrometer at 75.4 MHz. DMSO-d_6 was used as solvent, and it served as the internal standard at δ 39.5. 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. Flash column chromatography was performed using Merck silica gel 60 (230-400 mesh). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

Synthesis of *N*-Cbz amino acid ester derivatives (1-10): general procedure. A mixture of 6-deoxypenciclovir (1.50 g, 6.32 mmol), DCC (1.96 g, 9.48 mmol), a catalytic amount of DMAP (116 mg, 0.95 mmol) and an appropriate *N*-Cbz protected amino acid (*N*-carbobenzyloxy-glycine, -L-alanine, -L-valine, -L-leucine or -L-isoleucine) (1.25 equiv.) in anhydrous DMF (150 mL) was stirred at

room temperature under nitrogen atmosphere for 16 h. The reaction was replenished with the same amounts of amino acid, DCC and DMAP twice, and was stirred for an additional 11 h after each replenishment. The reaction mixture was filtered, and the filtrate was evaporated to dryness in vacuo. The resulting residue was purified by column chromatography on silica gel (gradient elution: 2% MeOH in CHCl$_3$, 5% MeOH in CHCl$_3$, and 10% MeOH in CHCl$_3$) to afford the corresponding mono-*O*-ester (**1–5**) and di-*O*-ester (**6–10**) compounds as a white foamy solid.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl *N*-carbobenzyloxyglycinate (1). Yield 47%; UV (MeOH) λ_{max} 224 (ε 22489), 246 (4301), and 310 (6015) nm; IR (KBr) 3330, 3217, 1718, 1614 cm^{-1}; ^{1}H NMR δ 1.64–1.98 (m, 3H, CH$_2$CH), 3.35–3.43 (m, 2H, C*H*$_2$OH), 3.78 (d, $J = 6.0$ Hz, 2H, αCH$_2$), 4.02–4.17 (m, 4H, OCH$_2$ and NCH$_2$), 4.66 (t, $J = 5.1$ Hz, 1H, OH), 5.03 (s, 2H, PhC*H*$_2$O), 6.48 (br s, 2H, NH$_2$), 7.25–7.40 (m, 5H, ArH), 7.70 (t, $J = 6.0$ Hz, 1H, NH), 8.07 (s, 1H, H-8), 8.56 (s, 1H, H-6); FAB-MS m/z 429 (MH$^+$). Anal. calcd for C$_{20}$H$_{24}$N$_6$O$_5$: C, 56.07; H, 5.65; N, 19.62. Found: C, 56.17; H, 5.74; N, 19.75.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl *N*-carbobenzyloxy-L-alaninate (2). Yield 55%; UV (MeOH) λ_{max} 224 (ε 19348), 246 (3474), and 310 (5185) nm; IR (KBr) 3331, 3215, 1716, 1614 cm^{-1}; ^{1}H NMR δ 1.25 (d, $J = 7.2$ Hz, 1.5H, CH$_3$), 1.27 (d, $J = 7.2$ Hz, 1.5H, CH$_3$), 1.67–1.98 (m, 3H, CH$_2$CH), 3.37–3.42 (m, 2H, C*H*$_2$OH), 3.99–4.19 (m, 5H, OCH$_2$, NCH$_2$ and αCH), 4.66 (t, $J = 5.4$ Hz, 1H, OH), 4.98 (ABq, $J = 14.4$ Hz, 1H, PhC*H*$_2$O), 5.02 (ABq, $J = 14.4$ Hz, 1H, PhC*H*$_2$O), 6.48 (br s, 2H, NH$_2$), 7.25–7.40 (m, 5H, ArH), 7.75 (t, $J = 7.5$ Hz, 1H, NH), 8.07 (s, 1H, H-8), 8.56 (s, 1H, H-6); FAB-MS m/z 443 (MH$^+$). Anal. calcd for C$_{21}$H$_{26}$N$_6$O$_5$: C, 57.01; H, 5.92; N, 18.99. Found: C, 56.92; H, 6.05; N, 18.86.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl *N*-carbobenzyloxy-L-valinate (3). Yield 48%; UV (MeOH) λ_{max} 224 (ε 22895), 246 (4057), and 310 (6192) nm; IR (KBr) 3333, 3214, 1716, 1612 cm^{-1}; ^{1}H NMR δ 0.84–0.88 (m, 6H, CH(C*H*$_3$)$_2$), 1.70–1.90 (m, 3H, CH$_2$CH), 1.92–2.10 (m, 1H, C*H*(CH$_3$)$_2$), 3.42 (br s, 2H, C*H*$_2$OH), 3.94 (dd, $J = 7.5$ Hz, $J = 6.9$ Hz, 1H, αCH), 4.04–4.15 (m, 4H, OCH$_2$ and NCH$_2$), 4.65 (t, $J = 5.1$ Hz, 1H, OH), 5.02 (s, 2H, PhC*H*$_2$O), 6.47 (br s, 2H, NH$_2$), 7.20–7.34 (m, 5H, ArH), 7.66 (t, $J = 6.9$ Hz, 1H, NH), 8.06 (s, 1H, H-8), 8.57 (s, 1H, H-6); FAB-MS m/z 471 (MH$^+$). Anal. calcd for C$_{23}$H$_{30}$N$_6$O$_5$: C, 58.71; H, 6.43; N, 17.86. Found: C, 58.65; H, 6.49; N, 17.54.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl *N*-carbobenzyloxy-L-leucinate (4). Yield 52%; UV (MeOH) λ_{max} 224 (ε 24004), 246 (4349), and 310 (6486) nm; IR (KBr) 3333, 3214, 1718, 1614 cm^{-1}; ^{1}H NMR δ 0.83 (d, $J = 6.6$ Hz, 3H, CH(C*H*$_3$)$_2$), 0.86 (d, $J = 6.9$ Hz, 3H, CH(C*H*$_3$)$_2$), 1.37–1.97 (m, 6H, CH$_2$CH and C*H*$_2$C*H*(CH$_3$)$_2$), 3.40 (br s, 2H, C*H*$_2$OH), 4.00–4.22 (m, 5H, OCH$_2$, NCH$_2$ and αCH), 4.65 (br s, 1H, OH), 5.01 (s, 2H, PhC*H*$_2$O), 6.63 (br s, 2H, NH$_2$), 7.25–7.40 (m, 5H,

ArH), 7.72 (t, $J = 7.5$ Hz, 1H, NH), 8.12 (s, 1H, H-8), 8.60 (s, 1H, H-6); FAB-MS m/z 485 (MH$^+$). Anal. calcd for C$_{24}$H$_{32}$N$_6$O$_5$: C, 59.49; H, 6.66; N, 17.34. Found: C, 59.54; H, 6.78; N, 17.48.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl *N*-carbobenzyloxy-L-isoleucinate (5). Yield 49%; UV (MeOH) λ_{max} 224 (ε 24343), 246 (4308), and 310 (6636) nm; IR (KBr) 3336, 3214, 1717, 1614 cm^{-1}; ^{1}H NMR δ 0.76–0.84 (m, 6H, 2 CH$_3$), 1.15–1.23 (m, 2H, CHC*H*$_2$CH$_3$), 1.65–1.93 (m, 4H, CH$_2$CH and CH), 3.42 (br s, 2H, C*H*$_2$OH), 3.99 (dd, $J = 7.5$ Hz, $J = 7.2$ Hz, 1H, αCH), 4.06–4.15 (m, 4H, OCH$_2$ and NCH$_2$), 4.65 (t, $J = 5.1$ Hz, 1H, OH), 5.02 (s, 2H, PhC*H*$_2$O), 6.47 (br s, 2H, NH$_2$), 7.29–7.34 (m, 5H, ArH), 7.67 (t, $J = 8.1$ Hz, 1H, NH), 8.06 (s, 1H, H-8), 8.57 (s, 1H, H-6); FAB-MS m/z 485 (MH$^+$). Anal. calcd for C$_{24}$H$_{32}$N$_6$O$_5$: C, 59.49; H, 6.66; N, 17.34. Found: C, 59.32; H, 6.81; N, 17.39.

[4-(2-Aminopurin-9-yl)-2-(*N*-carbobenzyloxyglycyloxy)methyl]butyl *N*-carbobenzyloxyglycinate (6). Yield 28%; UV (MeOH) λ_{max} 218 (ε 23528), 246 (4272), and 310 (6256) nm; IR (KBr) 3355, 3216, 1724, 1614 cm^{-1}; ^{1}H NMR δ 1.78–2.02 (m, 3H, CH$_2$CH), 3.75–3.82 (m, 4H, 2 αCH$_2$), 4.05–4.15 (m, 6H, 2 OCH$_2$ and NCH$_2$), 5.03 (s, 2H, PhC*H*$_2$O), 6.48 (br s, 2H, NH$_2$), 7.27–7.39 (m, 10H, ArH), 7.71 (t, $J = 6.3$ Hz, 1H, NH), 8.08 (s, 1H, H-8), 8.57 (s, 1H, H-6); FAB-MS m/z 620 (MH$^+$). Anal. calcd for C$_{30}$H$_{33}$N$_7$O$_8$: C, 58.15; H, 5.37; N, 15.82. Found: C, 58.24; H, 5.55; N, 15.66.

[4-(2-Aminopurin-9-yl)-2-(*N*-carbobenzyloxy-L-alanyloxy)methyl]butyl *N*-carbobenzyloxy-L-alaninate (7). Yield 23%; UV (MeOH) λ_{max} 212 (ε 21288), 246 (3623), and 310 (5334) nm; IR (KBr) 3328, 3222, 1718, 1625, 1613 cm^{-1}; ^{1}H NMR δ 1.25 (d, $J = 5.4$ Hz, 3H, CH$_3$), 1.27 (d, $J = 7.2$ Hz, 3H, CH$_3$), 1.60–1.98 (m, 3H, CH$_2$CH), 4.00–4.20 (m, 8H, 2 OCH$_2$, NCH$_2$ and 2 αCH), 4.98 (ABq, $J = 15.0$ Hz, 2H, PhC*H*$_2$O), 5.02 (ABq, $J = 15.0$ Hz, 2H, PhC*H*$_2$O), 6.47 (br s, 2H, NH$_2$), 7.20–7.38 (m, 10H, ArH), 7.78 (d, $J = 6.0$ Hz, 2H, 2 NH), 8.07 (s, 1H, H-8), 8.57 (s, 1H, H-6); FAB-MS m/z 648 (MH$^+$). Anal. calcd for C$_{32}$H$_{37}$N$_7$O$_8$: C, 59.34; H, 5.76; N, 15.14. Found: C, 59.55; H, 5.87; N, 14.99.

[4-(2-Aminopurin-9-yl)-2-(*N*-carbobenzyloxy-L-valyloxy)methyl]butyl *N*-carbobenzyloxy-L-valinate (8). Yield 20%; UV (MeOH) λ_{max} 218 (ε 22813), 246 (4052), and 310 (5893) nm; IR (KBr) 3356, 3214, 1719, 1613 cm^{-1}; ^{1}H NMR δ 0.80–0.87 (m, 12H, 2 CH(C*H*$_3$)$_2$), 1.90–2.10 (m, 5H, CH$_2$CH and 2 C*H*(CH$_3$)$_2$), 3.95 (dd, $J = 7.5$ Hz, $J = 6.9$ Hz, 2H, 2 αCH), 4.12 (br s, 6H, 2 OCH$_2$ and NCH$_2$), 5.02 (s, 4H, 2 PhC*H*$_2$O), 6.46 (br s, 2H, NH$_2$), 7.15–7.34 (m, 10H, ArH), 7.69 (t, $J = 7.8$ Hz, 2H, 2 NH), 8.06 (s, 1H, H-8), 8.57 (s, 1H, H-6); FAB-MS m/z 704 (MH$^+$). Anal. calcd for C$_{36}$H$_{45}$N$_7$O$_8$: C, 61.44; H, 6.44; N, 13.93. Found: C, 61.32; H, 6.41; N, 13.88.

[4-(2-Aminopurin-9-yl)-2-(*N*-carbobenzyloxy-L-leucyloxy)methyl]butyl *N*-carbobenzyloxy-L-leucinate (9). Yield 29%; UV (MeOH) λ_{max} 218 (ε 23796), 246 (4183), and

310 (6209) nm; IR (KBr) 3337, 3220, 1720, 1613 cm^{-1}; ^{1}H NMR δ 0.83 (d, $J = 8.4$ Hz, 6H, CH(CH_3)$_2$), 0.85 (d, $J = 8.1$ Hz, 6H, CH(CH_3)$_2$), 1.38–1.70 (m, 6H, 2 CH_2CH(CH$_3$)$_2$), 1.85–2.10 (m, 3H, CH$_2$CH), 3.50 (br s, 2H, 2 αCH), 3.95–4.15 (m, 6H, 2 OCH$_2$ and NCH$_2$), 5.01 (s, 4H, 2 PhCH_2O), 6.75 (br s, 2H, NH$_2$), 7.25–7.40 (m, 10H, ArH), 7.75 (d, $J = 7.8$ Hz, 2H, 2 NH), 8.17 (s, 1H, H-8), 8.65 (s, 1H, H-6); FAB-MS m/z 732 (MH$^+$). Anal. calcd for C$_{38}$H$_{49}$N$_7$O$_8$: C, 62.37; H, 6.75; N, 13.40. Found: C, 62.26; H, 6.79; N, 13.55.

[4-(2-Aminopurin-9-yl)-2-(*N*-carbobenzyloxy-L-isoleucyl-oxy)methyl]butyl *N*-carbobenzyloxy-L-leucinate (10). Yield 23%; UV (MeOH) λ_{max} 218 (ε 24838), 246 (4333), and 310 (6522) nm; IR (KBr) 3358, 3214, 1718, 1612 cm^{-1}; ^{1}H NMR δ 0.76–0.83 (m, 12H, 4 CH$_3$), 1.10–1.45 (m, 4H, 2 CHCH_2CH$_3$), 1.70–2.05 (m, 5H, CH$_2$CH and 2 CH), 4.01 (dd, $J = 7.2$ Hz, $J = 6.9$ Hz, 2H, 2 αCH), 4.12 (m, 6H, 2 OCH$_2$ and NCH$_2$), 5.01 (s, 4H, 2 PhCH_2O), 6.46 (br s, 2H, NH$_2$), 7.25–7.40 (m, 10H, ArH), 7.70 (t, $J = 7.8$ Hz, 2H, 2 NH), 8.06 (s, 1H, H-8), 8.57 (s, 1H, H-6); FAB-MS m/z 732 (MH$^+$). Anal. calcd for C$_{38}$H$_{49}$N$_7$O$_8$: C, 62.37; H, 6.75; N, 13.40. Found: C, 62.48; H, 6.89; N, 13.49.

Synthesis of 11–20: general procedure. A mixture of *N*-Cbz amino ester compound and 10% Pd/C (20 wt% for mono-*O*-esters **1–5** and 40 wt% for di-*O*-ester **6–10**) in methanol was purged with H$_2$ three times, and was vigorously stirred under H$_2$ atmosphere (1 atm; a balloon) at room temperature for 3 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated under reduced pressure to give an oily residue. The crude material was purified by column chromatography on silica gel (20% MeOH in CHCl$_3$) to afford the corresponding free amine compound as a colorless oil. Each purified free amine compound dissolved in anhydrous MeOH (20 mL) was treated with 2.0 *N* methanolic HCl solution (3.0 equiv. for mono-*O*-ester **11–15** and 5.0 equiv. for di-*O*-ester **16–20**), evaporated to dryness in vacuo, and the resulting HCl salt was triturated from dry acetone to obtain the target products **11–20** as an off-white solid or an yellowish solid.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl glycinate di-hydrochloride (11). Yield 51%; UV (H$_2$O) λ_{max} 222 (ε 16974), 248 (3215), and 272 (3345) nm; IR (KBr) 3280, 3103, 1746, 1666, 1573 cm^{-1}; ^{1}H NMR δ 1.85 (br s, 3H, CH$_2$CH), 3.45 (br s, 2H, CH_2OH), 3.83 (br s, 2H, αCH$_2$), 4.10–4.35 (m, 4H, OCH$_2$ and NCH$_2$), 8.02 (br s, 3H, 2-NH$_3^+$), 8.56 (br s, 3H, αNH$_3^+$), 8.72 (s, 1H, H-8), 9.01 (s, 1H, H-6); FAB-MS m/z 295 (MH$^+$). Anal. calcd for C$_{12}$H$_{18}$N$_6$O$_3$·2 HCl·1.7 CH$_3$OH: C, 39.02; H, 6.41; N, 19.93. Found: C, 38.73; H, 6.49; N, 19.81.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl L-alaninate di-hydrochloride (12). Yield 98%; UV (H$_2$O) λ_{max} 226 (ε 13196) and 266 (4662) nm; IR (KBr) 3292, 3066, 1745, 1702, 1667, 1585 cm^{-1}; ^{1}H NMR δ 1.44 (d, $J = 7.2$ Hz, 3H, CH$_3$), 1.86 (br s, 3H, CH$_2$CH), 3.46 (br s, 2H, CH_2OH), 4.00–4.35 (m, 5H, OCH$_2$, NCH$_2$ and αCH), 8.00 (br s, 3H, 2-NH$_3^+$), 8.68 (br s, 3H, αNH$_3^+$), 9.00 (s, 1H, H-8), 9.17 (s, 1H, H-6); FAB-MS

m/z 309 (MH$^+$). Anal. calcd for C$_{13}$H$_{20}$N$_6$O$_3$·2 HCl·CH$_3$OH·CH$_3$COCH$_3$: C, 43.32; H, 6.84; N, 17.83. Found: C, 43.47; H, 6.98; N, 17.95.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl L-valinate di-hydrochloride (13). Yield 94%; UV (H$_2$O) λ_{max} 222 (ε 22893), 244 (3891), and 306 (5787) nm; IR (KBr) 3302, 3029, 1742, 1648, 1523, 1424 cm^{-1}; ^{1}H NMR δ 0.92 (d, $J = 6.9$ Hz, 1.5H, CH(CH_3)$_2$), 0.93 (d, $J = 7.2$ Hz, 1.5H, CH(CH_3)$_2$), 0.96 (d, $J = 7.2$ Hz, 1.5H, CH(CH_3)$_2$), 0.97 (d, $J = 6.9$ Hz, 1.5H, CH(CH_3)$_2$), 1.75–1.95 (m, 3H, CH$_2$CH), 2.15–2.25 (m, 1H, CH(CH$_3$)$_2$), 3.45 (d, $J = 4.5$ Hz, 2H, CH_2OH), 3.79 (br s, 1H, αCH), 4.11–4.23 (m, 4H, COOCH$_2$ and NCH$_2$), 8.25 (br s, 3H, 2-NH$_3^+$), 8.76 (br s, 3H, αNH$_3^+$), 8.78 (s, 1H, H-8), 9.03 (s, 1H, H-6); ^{13}C NMR δ 17.6 (CH(CH$_3$)$_2$), 18.5 (CH(CH$_3$)$_2$), 27.5 (NCH$_2$CH$_2$), 29.3 (CH(CH$_3$)$_2$), 37.3 and 37.4 (CH), 41.3 (NCH$_2$), 57.4 and 57.5 (αC), 60.1 and 60.2 (CH$_2$OH), 65.3 and 65.4 (CH$_2$OCOCH), 125.9 (C-5), 138.0 (C-8), 149.9 (C-6), 154.12 (C-4), 156.92 and 156.93 (C-2), 168.55 and 168.64 (CO); FAB-MS m/z 337 (MH$^+$). Anal. calcd for C$_{15}$H$_{24}$N$_6$O$_3$·2 HCl·H$_2$O: C, 42.16; H, 6.60; N, 19.67. Found: C, 42.28; H, 6.87; N, 19.45.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl L-leucin-ate di-hydrochloride (14). Yield 95%; UV (H$_2$O) λ_{max} 228 (ε 12811) and 266 (4644) nm; IR (KBr) 3339, 3113, 1742, 1706, 1673, 1584 cm^{-1}; ^{1}H NMR δ 0.89 (d, $J = 5.4$ Hz, 6H, CH(CH_3)$_2$), 1.65–1.86 (m, 7H, CH$_2$CH, CH_2CH(CH$_3$)$_2$, and CH(CH$_3$)$_2$), 3.46 (br s, 2H, CH_2OH), 3.93 (br s, 1H, αCH), 4.10–4.18 (m, 4H, COOCH$_2$ and NCH$_2$), 7.99 (br s, 3H, 2-NH$_3^+$), 8.72 (br s, 3H, αNH$_3^+$), 8.96 (s, 1H, H-8), 9.18 (s, 1H, H-6); FAB-MS m/z 351 (MH$^+$). Anal. calcd for C$_{16}$H$_{26}$N$_6$O$_3$·2 HCl·1.2 CH$_3$OH: C, 44.74; H, 7.16; N, 18.20. Found: C, 44.86; H, 7.24; N, 18.27.

[4-(2-Aminopurin-9-yl)-2-hydroxymethyl]butyl L-isoleuc-inate di-hydrochloride (15). Yield 94%; UV (H$_2$O) λ_{max} 222 (ε 22795), 244 (4047), and 306 (5914) nm; IR (KBr) 3307, 3029, 1744, 1651, 1523, 1424 cm^{-1}; ^{1}H NMR δ 0.83–0.90 (m, 6H, 2 CH$_3$), 1.20–1.47 (m, 2H, CHCH_2CH$_3$), 1.72–2.02 (m, 4H, CH$_2$CH and CHCH$_3$), 3.45 (dd, $J = 5.0$ Hz, 2.3 Hz, 2H, CH_2OH), 3.85 (br s, 1H, αCH), 4.14–4.23 (m, 4H, COOCH$_2$ and NCH$_2$), 8.25 (br s, 3H, 2-NH$_3^+$), 8.76 (br s, 3H, αNH$_3^+$), 8.78 (s, 1H, H-8), 9.03 (s, 1H, H-6); ^{13}C NMR δ 11.4 (CH$_2$CH$_3$), 14.3 (CHCH$_3$), 25.4 (CH$_2$CH$_3$), 27.5 (NCH$_2$CH$_2$), 35.8 (CH$_2$CHCH$_3$), 37.3 and 37.4 (CH), 41.2 (NCH$_2$), 56.06 and 56.10 (αC), 60.1 and 60.2 (CH$_2$OH), 65.3 and 65.4 (CH$_2$OCOCH), 125.9 (C-5), 138.1 (C-8), 149.8 and 149.9 (C-6), 154.1 (C-4), 156.9 (C-2), 168.4 and 168.5 (CO); FAB-MS m/z 351 (MH$^+$). Anal. calcd for C$_{16}$H$_{26}$N$_6$O$_3$·2 HCl·0.5H$_2$O·CH$_3$OH: C, 43.97; H, 7.16; N, 18.10. Found: C, 44.09; H, 7.27 N, 18.24.

[4-(2-Aminopurin-9-yl)-2-glycyloxymethyl]butyl glycinate tri-hydrochloride (16). Yield 65%; UV (H$_2$O) λ_{max} 222 (ε 23231), 244 (4341), and 306 (6300) nm; IR (KBr) 3309, 1747, 1659, 1646, 1425 cm^{-1}; ^{1}H NMR δ 1.89–2.14 (m, 3H, CH$_2$CH), 3.81 (br s, 4H, 2 αCH$_2$), 4.11–4.36 (m, 6H, 2 OCH$_2$ and NCH$_2$), 8.09 (br s, 3H, 2-NH$_3^+$), 8.65 (br s, 6H, 2 αNH$_3^+$), 8.73 (s, 1H, H-8), 9.00 (s, 1H,

H-6); ^{13}C NMR δ 27.6 (NCH$_2$CH$_2$), 34.3 (CH), 40.8 (NCH$_2$), 64.5 (αC), 65.4 (CH$_2$OCOCH$_2$), 126.0 (C-5), 139.1 (C-8), 149.2 (C-6), 154.7 (C-4), 156.6 (C-2), 167.4 (CO); FAB-MS *m/z* 352 (MH$^+$). Anal. calcd for C$_{14}$H$_{21}$N$_7$O$_4$·3 HCl·0.7 H$_2$O·1.5 CH$_3$OH: C, 35.70; H, 6.07; N, 18.80. Found: C, 35.81; H, 6.20; N, 18.82.

[4-(2-Aminopurin-9-yl)-2-(L-alanyloxy)methyl]butyl L-alaninate tri-hydrochloride (17). Yield 80%; UV (H$_2$O) λ$_{max}$ 224 (ε 13504) and 268 (4473) nm; IR (KBr) 3364, 3029, 1743, 1698, 1576, 1458 cm^{-1}; ^{1}H NMR δ 1.45 (d, *J* = 6.9 Hz, 6H, 2 CH$_3$), 1.93–2.26 (m, 3H, CH$_2$CH), 4.11 (br s, 2H, 2 αCH), 4.20–4.35 (m, 6H, 2 OCH$_2$, and NCH$_2$), 7.95 (br s, 3H, 2-NH$_3$$^+$), 8.74 (br s, 6H, αNH$_3$$^+$), 8.94 (s, 1H, H-8), 9.13 (s, 1H, H-6); FAB-MS *m/z* 380 (MH$^+$). Anal. calcd for C$_{16}$H$_{25}$N$_7$O$_4$·3 HCl·H$_2$O·0.4 CH$_3$OH: C, 37.91; H, 6.13; N, 18.87. Found: C, 38.01; H, 6.29; N, 18.78.

[4-(2-Aminopurin-9-yl)-2-(L-valyloxy)methyl]butyl L-valinate tri-hydrochloride (18). Yield 71%; UV (H$_2$O) λ$_{max}$ 222 (ε 23103), 244 (4377), and 306 (6209) nm; IR (KBr) 3309, 1746, 1661, 1649, 1427 cm^{-1}; ^{1}H NMR δ 0.91–0.97 (m, 12H, 4 CH$_3$), 1.95–2.15 (m, 3H, CH$_2$CH), 2.15–2.30 (m, 2H, 2 CH(CH$_3$)$_2$), 3.79 (br s, 2H, 2 αCH), 4.23 (br s, 6H, 2 OCH$_2$ and NCH$_2$), 8.25 (br s, 3H, 2-NH$_3$$^+$), 8.80 (br s, 6H, 2 αNH$_3$$^+$), 8.85 (s, 1H, H-8), 9.04 (s, 1H, H-6); ^{13}C NMR δ 17.6 (CH(CH$_3$)$_2$), 18.6 (CH(CH$_3$)$_2$), 27.0 (NCH$_2$CH$_2$), 29.2 (CH(CH$_3$)$_2$), 34.1 (CH), 40.8 (NCH$_2$), 57.45 and 57.53 (αC), 64.37 and 64.43 (CH$_2$OCOCH), 125.9 (C-5), 138.1 (C-8), 149.8 (C-6), 154.2 (C-4), 156.9 (C-2), 168.25 and 168.34 (CO); FAB-MS *m/z* 436 (MH$^+$). Anal. calcd for C$_{20}$H$_{33}$N$_7$O$_4$·3 HCl·H$_2$O·0.8 CH$_3$COCH$_3$: C, 44.15; H, 7.08; N, 16.09. Found: C, 44.27; H, 7.21; N, 16.24.

[4-(2-Aminopurin-9-yl)-2-(L-leucyloxy)methyl]butyl L-leucinate tri-hydrochloride (19). Yield 98%; UV (H$_2$O) λ$_{max}$ 222 (ε 12565), 246 (3795), and 298 (3828) nm; IR (KBr) 3339, 1747, 1663, 1647, 1428 cm^{-1}; ^{1}H NMR δ 0.87 (d, *J* = 5.4 Hz, 6H, CH(CH$_3$)$_2$), 0.88 (d, *J* = 5.4 Hz, 6H, CH(CH$_3$)$_2$), 1.60–1.82 (m, 6H, 2 CH$_2$CH(CH$_3$)$_2$), 1.95–2.30 (m, 3H, CH$_2$CH), 3.90 (br s, 2H, 2 αCH), 4.15–4.40 (m, 6H, 2 OCH$_2$ and NCH$_2$), 8.10 (br s, 3H, 2-NH$_3$$^+$), 8.50 (br s, 6H, 2 αNH$_3$$^+$), 9.04 (s, 1H, H-8), 9.17 (s, 1H, H-6); FAB-MS *m/z* 464 (MH$^+$). Anal. calcd for C$_{22}$H$_{37}$N$_7$O$_4$·3 HCl·2 CH$_3$OH·0.5 CH$_3$COCH$_3$: C, 45.98; H, 7.72; N, 14.72. Found: C, 45.77; H, 7.85; N, 14.79.

[4-(2-Aminopurin-9-yl)-2-(L-isoleucyloxy)methyl]butyl L-isoleucinate tri-hydrochloride (20). Yield 67%; UV (H$_2$O) λ$_{max}$ 222 (ε 23867), 244 (4288), and 306 (6446) nm; IR (KBr) 3307, 1746, 1660, 1648, 1426 cm^{-1}; ^{1}H NMR δ 0.82–0.90 (m, 12H, 4 CH$_3$), 1.15–1.50 (m, 4H, 2 CH CH$_2$CH$_3$), 1.90–2.15 (m, 5H, CH$_2$CH and 2 CHCH$_3$), 3.86 (br s, 2H, 2 αCH), 4.15–4.32 (m, 6H, 2 OCH$_2$ and NCH$_2$), 8.20 (br s, 3H, 2-NH$_3$$^+$), 8.84 (br s, 7H, H-8 and 2 αNH$_3$$^+$), 9.03 (s, 1H, H-6); ^{13}C NMR δ 11.4 (CH$_2$CH$_3$), 14.3 (CHCH$_3$), 25.4 (CH$_2$CH$_3$), 27.0 (NCH$_2$CH$_2$), 34.1 (CHCH$_3$), 35.7 (CH), 40.8 (NCH$_2$), 56.06 and 56.14 (αC), 64.3 and 64.4 (CH$_2$OCOCH), 125.9 (C-5), 138.3 (C-8), 149.7 (C-6), 154.3 (C-4), 156.8 (C-2),

168.1 and 168.2 (CO); FAB-MS *m/z* 464 (MH$^+$). Anal. calcd for C$_{22}$H$_{37}$N$_7$O$_4$·3 HCl·CH$_3$COCH$_3$: C, 47.58; H, 7.35; N, 15.54. Found: C, 47.69; H, 7.57; N, 15.36.

Oral bioavailability. The bioavailability of 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^{-1} dose of test compound to six male ICR mice (25–30 g) or four 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^{-1} 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), (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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