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Synthesis and Evaluation of 2-Amino-6-fluoro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine Mono- and Diesters as Potential Prodrugs of Penciclovir

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Abstract—2-Amino-6-fluoro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine (**7**), and its mono- and diesters **8**–**15** were prepared and evaluated for their potential as prodrugs of penciclovir. Treatment of 2-amino-6-chloro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine (**5**) with trimethylamine in THF followed by a reaction of the resulting trimethylammonium chloride salt **6** with KF in DMF afforded 2-amino-6-fluoro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine (**7**) in 80% yield. Esterification of **7** with an appropriate acid anhydride [Ac_2O, $(EtCO)_2O$, $(n\text{-}PrCO)_2O$, or $(i\text{-}PrCO)_2O$] in DMF in the presence of a catalytic amount of DMAP produced the mono-esters **8**–**11** in 42–45% yields and diesters **12**–**15** in 87–99% yields. Of the prodrugs tested in rats, the mono-isobutyrate **11** was the most efficiently absorbed and metabolized to **7**, showing the mean maximum total concentration of penciclovir (5.5 μg/mL) and **7** (10.8 μg/mL) in the blood was much higher than the mean maximum concentration of penciclovir (11.5 μg/mL) from famciclovir. However, the mean concentrations of penciclovir from **11** were lower than those from famciclovir because of the limited conversion of a major metabolite **7** to penciclovir by adenosine deaminase.

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.[1,2] The advantage of penciclovir over acyclovir [9-(2-hydroxyethoxymethyl) guanine] 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.[2,3] However, like other acycloguanosine analogues such as acyclovir,[4] ganciclovir,[5] and buciclovir,[6] penciclovir was poorly absorbed when given orally to rodents.[7,8] 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.[7] Famciclovir is orally well absorbed, and then extensively converted to penciclovir by the enzymatic removal of two *O*-acetyl groups and followed by oxidation at the 6-position of the purine ring by xanthine oxidase in mice,[7] rats,[8] and humans.[9] We also prepared the amino acid esters of penciclovir and 6-deoxy-penciclovir,[10,11] and a series of 2-amino-9-(3-hydroxymethyl-4-alkoxycarbonyloxybut-1-yl)purines as potential prodrugs of penciclovir.[12] Amomg them, SK 1875 [2-amino-9-(3-hydroxymethyl-4-isopropoxycarbonyloxybut-1-yl)purine] showed the comparable oral penciclovir bioavailability to famciclovir in mice and rats.[12] In a previous communication, we designed the 2-amino-6-fluoropurine acyclonucleosides **1** and **2** as potential prodrugs of acyclovir and ganciclovir, respectively, and demonstrated that **1** and **2** were readily converted to acyclovir and ganciclovir in the presence of calf intestinal mucosal adenosine deaminase in phosphate buffer solution.[13] From enzyme kinetic studies, it was found that **1** and **2** were 11.6 and 7.6 times more efficient substrates for adenosine deaminase in terms of V_{max}/K_m than the corresponding 2,6-diaminopurine acyclonucleosides **3** and **4**, respectively.[13] In order to maximize their oral acyclovir bioavailability or ganciclovir bioavailability, the acyl esters of **1** and **2** have been prepared.[14,15] Among them, SK 1809 [2-amino-6-fluoro-9-(2-isobutyryloxyethoxymethyl)purine] achieved the comparable oral acyclovir bioavailability to valaciclovir, the L-valyl ester of acyclovir, in rats,[14] and SK 1818 [2-amino-6-fluoro-9-(1-isobutyryloxy-3-hydroxy-2-propoxymethyl)purine] showed 15-fold higher oral ganciclovir bioavailability compared with that from ganciclovir itself in rats.[15] On the basis of these results, we prepared

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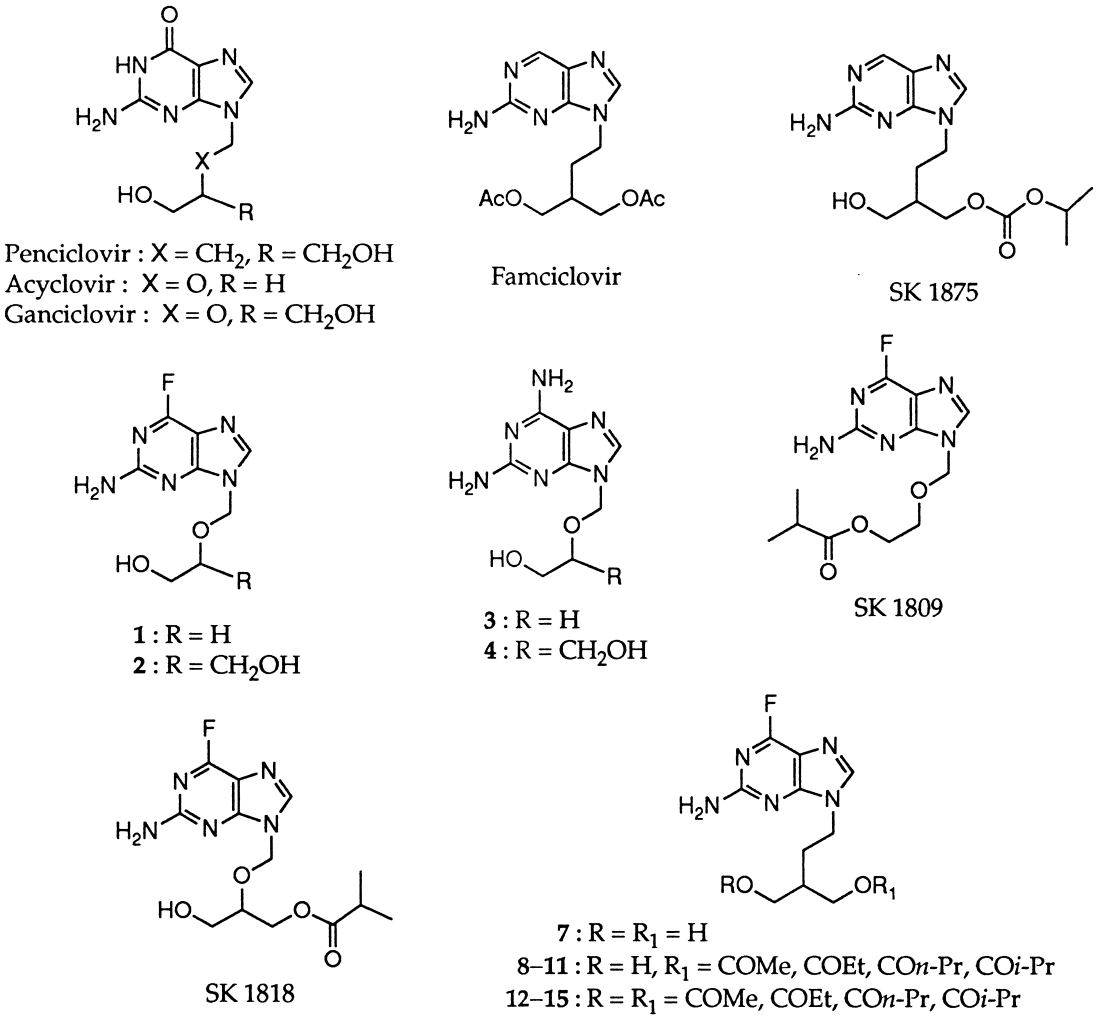


Chart 1.

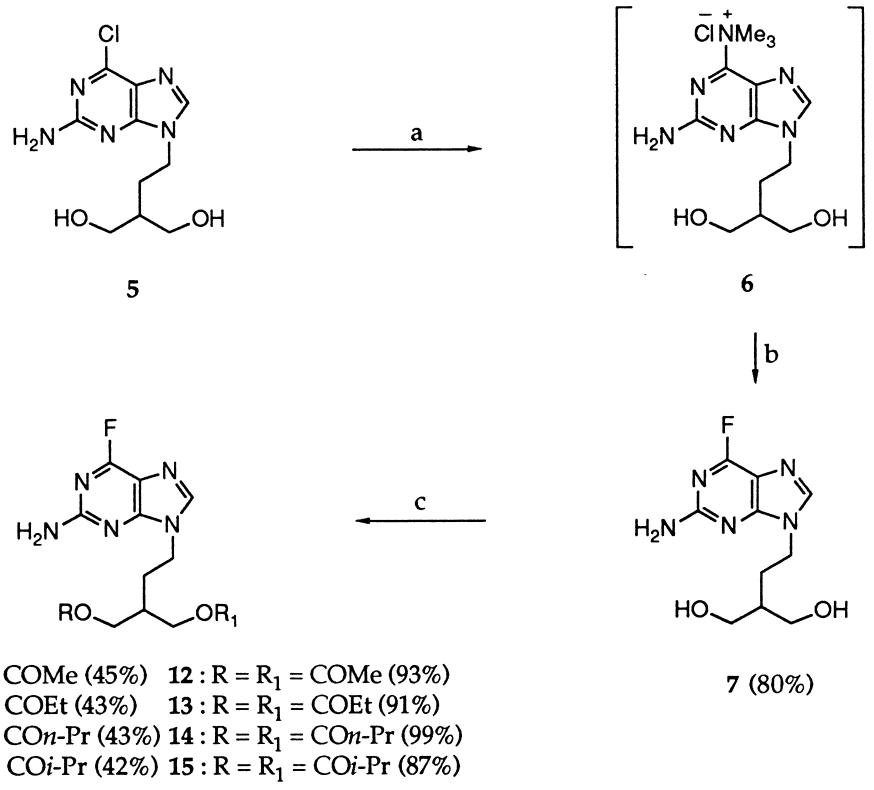
2-amino-6-fluoro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine (**7**) and its mono- and diesters **8**–**15**, and evaluated for their potential as prodrugs of penciclovir (Chart 1).

Chemistry

Target compounds **7**–**15** were synthesized from 2-amino-6-chloro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)-purine (**5**)[1] as shown in Scheme 1. The fluorination of **5** was performed by activating the chloro atom to the trimethylammonium chloride salt followed by replacing it with KF. As pointed out in our related works,[14,15] it is important to use a right solvent in which the trimethylammonium chloride salt **6** is sufficiently insoluble, in order to suppress formation of a by-product, 6-deoxy-6-dimethylaminopenciclovir. A reaction in DMF alone produced the by-product predominantly, and a 4/1 mixture of THF and DMF was not promising either, showing a substantial amount of the by-product formation on TLC. Fortunately, the use of THF alone was found to be most satisfactory, even though the formation of the trimethylammonium chloride salt **6** was rather slow. The compound **5** was treated with excess anhydrous trimethylamine in anhydrous THF at room temperature for 5 days. After the complete salt formation, the solvent was decanted, and the residual solvent was removed thoroughly in vacuo. Resulting crude trimethylammonium chloride salt **6** was subsequently treated with excess anhydrous KF in DMF at 80 °C to afford **7** in 80% yield. Reactions of **7** with an appropriate acid anhydride [Ac_2O, $(EtCO)_2O$, $(n\text{-}PrCO)_2O$, or $(i\text{-}PrCO)_2O$] (1 equiv. for **8**–**11** or 6 equiv. for **12**–**15**) in dry DMF in the presence of a catalytic amount of DMAP at room temperature produced the mono-esters **8**–**11** in 42–45% yields and diesters **12**–**15** in 87–99% yields.

Results and Discussion

Compound **7** and its ester derivatives **8**–**15** along with penciclovir and famciclovir were administered orally to rats (0.2 mmol/kg), and concentrations of penciclovir in the blood were determined by using HPLC (Table 1). The time to maximum concentrations of penciclovir in the blood from the ester derivatives **8**–**15** was about 0.25 to 0.5 h and that from **7** was about 1 h, showing that **8**–**15** were much more rapidly absorbed than **7**. The mean maximum concentration of penciclovir from **7** (0.8 μg/mL)



8 : R = H, R_1 = COMe (45%) **12** : R = R_1 = COMe (93%)
9 : R = H, R_1 = COEt (43%) **13** : R = R_1 = COEt (91%)
10 : R = H, R_1 = CO*n*-Pr (43%) **14** : R = R_1 = CO*n*-Pr (99%)
11 : R = H, R_1 = CO*i*-Pr (42%) **15** : R = R_1 = CO*i*-Pr (87%)

Scheme 1. (a) NMe_3 (10 equiv.), anh. THF, −78 °C to rt, 5 days; (b) KF (10 equiv.), DMF, 80 °C, 3 h; (c) $(RCO)_2O$ (1 equiv. for **8**–**11** or 6 equiv. for **12**–**15**), DMAP (0.1 equiv.), DMF, rt, 1 h.

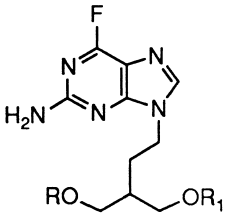
was 2-fold higher than that from penciclovir (0.4 μg/mL). All of the ester derivatives **8**–**15** showed much higher mean concentrations of penciclovir in the blood compared with those from **7**, and among these, the mono-isobutyrate **11** achieved the highest concentrations in the blood. However, since the mean maximum concentration of penciclovir from **11** (5.5 μg/mL) was approximately one-half of that from famciclovir (11.5 μg/mL), we decided to determine concentrations of parent compounds and a major metabolite **7** in the blood from the prodrugs **7**–**15**, in order to see whether the lower concentrations of penciclovir from these prodrugs attributed to poor gastrointestinal absorption or lower conversion to penciclovir by esterases or adenosine deaminase after absorption. Although the parent compounds were not detected in the blood, approximately 3-fold and 1–2-fold higher concentrations of **7** than concentrations of penciclovir from **7** and **8**–**15**, respectively, were found in the blood. Again, the prodrug **11** achieved the highest mean maximum concentration of **7** (10.8 μg/mL), thus, the mean maximum total concentration of penciclovir and **7** from **11** in the blood was much higher than the mean maximum concentration of penciclovir achieved from the equivalent dose of famciclovir. This result indicates that some of the ester prodrugs such as **11** and the mono-butyrate **10** appear to be well absorbed from gastrointestinal tract and hydrolysed to **7** by esterases, but not efficiently converted to the antiviral penciclovir by adenosine deaminase. The low substrate efficiency of **7** for adenosine deaminase seems to be due to the lack of an oxygen atom at the 2′-position in the acyclic moiety, since the 6-deoxy-6-fluoroacyclovir **1** and the 6-deoxy-6-fluoroganciclovir **2** were readily converted to acyclovir and ganciclovir, respectively, by adenosine deaminase in the enzyme kinetic study,[13] and their ester derivatives showed very high acyclovir or ganciclovir bioavailability in rats.[14,15] This assumption could be supported by an early observation of Bloch et al. that replacement of the bridge oxygen atom in the ribose ring of adenosine with a sulfur atom reduced the rate of deamination by approximately one-half that of adenosine.[16]

In conclusion, among the prodrugs **8**–**15**, the mono-isobutyrate **11** was the most efficiently absorbed and metabolized to **7** in rats, showing the mean maximum total concentration of penciclovir and **7** in the blood was much higher than the mean maximum concentration of penciclovir from famciclovir. But, the mean concentrations of penciclovir from **11** were lower than those from famciclovir because of the limited conversion of a major metabolite **7** to penciclovir by adenosine deaminase. However, since the extent of the deaminaton between in humans and in rats could be different, further evaluation of **11** is warranted.

Experimental

Melting points were determined on a Mettler (FP 62) melting point apparatus and are uncorrected. 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

Table 1. Concentrations of penciclovir and 2-amino-6-fluoro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine (**7**) in the blood following oral administration of **7** and its ester derivatives **8**–**15** to rats[a]



7–15

			Concn (μg/mL) in blood at time (h) after dosing[b]							
			Penciclovir				**7**			
Compd	R	R_1	0.25	0.5	1	2	0.25	0.5	1	2
7	H	H	0.8	1.0	1.2	0.9	2.3	2.8	2.9	2.3
8	H	COMe	2.8	2.4	1.8	0.7	5.0	4.0	2.5	1.1
9	H	COEt	1.4	1.0	1.3	0.6	1.7	1.5	1.2	0.9
10	H	CO*n*-Pr	3.5	2.7	1.6	0.8	6.6	4.3	2.9	1.2
11	H	CO*i*-Pr	5.5	5.0	3.8	2.2	10.8	8.1	4.4	1.9
12	COMe	COMe	2.9	2.5	1.8	0.6	5.9	5.9	4.1	0.7
13	COEt	COEt	1.1	1.3	1.2	0.7	2.1	1.5	1.1	0.9
14	CO*n*-Pr	CO*n*-Pr	3.3	2.7	1.7	1.0	5.2	3.8	1.9	1.0
15	CO*i*-Pr	CO*i*-Pr	2.7	4.6	1.9	1.0	3.9	3.9	2.5	0.6
Penciclovir			0.4	0.3	0.2	0.2				
Famciclovir			11.5	12.0	7.8	3.0				

[a]Test compound was administered as a single dose of 0.2 mmol/kg in 1% (carboxymethyl)cellulose in a volume of 10 mL/kg by oral gavage to four Sprague–Dawley rats weighing 360–400 g, from which food had been withheld for 16 h. Blood was collected at 0.25, 0.5, 1 and 2 h after dosing by cardiac puncture.
[b]Mean values from four animals.

a Varian Unity 300 spectrometer. The chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane in $CDCl_3$ or DMSO-d_6. Electron impact mass spectra (EI-MS) were obtained on a VG Quattro mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60F-254 glass plates. 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 2-amino-6-fluoro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine (7). Anhydrous trimethylamine (about 35 mL) was condensed at −78 °C and was added dropwise to a cooled suspension of 2-amino-6-chloro-9-(4-hydroxy-3-hydroxymethylbut-1-yl)purine (**5**) (18.99 g, 0.07 mol) in anhydrous THF (900 mL) at −78 °C under nitrogen atmosphere via a cannula. The resulting suspension was warmed to room temperature immediately after the addition of trimethylamine and was stirred under nitrogen atmosphere at room temperature for five days. After the reaction mixture was allowed to be settled down, the supernatant solvent was decanted from the white precipitates, and the residual volatiles were removed completely in vacuo to afford the corresponding trimethylammonium chloride salt **6** as a white solid. The resulting trimethylammonium chloride salt was treated with anhydrous KF (40.67 g, 0.70 mol) in anhydrous DMF (900 mL) at 80 °C for 3 h while the gaseous trimethylamine by-product was being removed from the reaction medium under reduced pressure using an aspirator (about 35 mmHg). The reaction mixture was cooled to room temperature, diluted with $CHCl_3$ (900 mL), and filtered through a glass filter. The filtrate was evaporated to dryness in vacuo to give an off-white solid and was purified by column chromatography on silica gel (gradient elution: 10% MeOH in $CHCl_3$ and 20% MeOH in $CHCl_3$). The slightly impure product was triturated once from cold MeOH to afford pure **7** (14.21 g, 80%) as a white powder: mp 180.2 °C (dec) (THF); UV (H_2O) λ_{max} 218 (ε 24002), 246 (6496), and 290 (6440) nm; IR (KBr) 3322, 3208, 1657, 1572, 1485 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.42–1.50 (m, 1H, CH_2CH), 1.74–1.81 (m, 2H, CH_2CH), 3.30–3.48 (m, 4H, 2 OCH_2), 4.13 (t, J = 7.4 Hz, 2H, NCH_2), 4.30 (t, J = 5.1 Hz, 2H, 2 OH), 6.87 (br s, 2H, NH_2), 8.11 (s, 1H, H-8); EI-MS m/z 255 (M^+). Anal. calcd for $C_{10}H_{14}FN_5O_2$: C, 47.06; H, 5.53; N, 27.44. Found: C, 47.15; H, 5.64; N, 27.31.

Synthesis of 8–11: General procedure. To a solution of **7** (1.02 g, 4.00 mmol) and a catalytic amount of DMAP (48 mg, 0.40 mmol) in anhydrous DMF (80 mL) was added dropwise an appropriate acid anhydride [Ac_2O, $(EtCO)_2O$, $(n\text{-}PrCO)_2O$, or $(i\text{-}PrCO)_2O$] (4.00 mmol) over 20 min at 0 °C under nitrogen atmosphere, and the mixture was stirred for 30 min at 0 °C. The reaction mixture was warmed gradually to room temperature and was stirred at room temperature for 1 h. MeOH (5 mL) was added, and the reaction mixture was evaporated to

dryness under reduced pressure to give a yellow solid. The crude product was purified by column chromatography on silica gel (gradient elution: 5% MeOH in $CHCl_3$, 10% MeOH in $CHCl_3$, and 20% MeOH in $CHCl_3$) to afford the corresponding mono-ester as a white solid. Analytically pure material was obtained by crystallization from the solvents indicated below.

2-Amino-6-fluoro-9-(3-acetoxymethyl-4-hydroxybut-1-yl)-purine (8). mp 159.4 °C (dec) (MeOH/EtOAc/hexanes); UV (H_2O) λ_{max} 218 (ε 24261), 246 (6766), and 290 (6523) nm; IR (KBr) 3337, 3209, 1725, 1657, 1632, 1572, 1486 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.62–1.93 (m, 3H, CH_2CH), 1.98 (s, 3H, $COCH_3$), 3.41 (t, $J=5.1$ Hz, 2H, CH_2OH), 3.93–4.01 (m, 2H, CH_2OAc), 4.14 (t, $J=7.2$ Hz, 2H, NCH_2), 4.63 (t, $J=5.1$ Hz, 1H, OH), 6.87 (br s, 2H, NH_2), 8.12 (s, 1H, H-8); EI-MS m/z 297 (M^+). Anal. calcd for $C_{12}H_{16}FN_5O_3$: C, 48.48; H, 5.42; N, 23.56. Found: C, 48.41; H, 5.54; N, 23.68.

2-Amino-6-fluoro-9-(4-hydroxy-3-propionyloxymethylbut-1-yl)purine (9). mp 171.8 °C (dec) (DMF/EtOAc/hexanes); UV (H_2O) λ_{max} 218 (ε 23842), 246 (6620), and 290 (6470) nm; IR (KBr) 3366, 3317, 3213, 1726, 1648, 1576, 1488 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.00 (t, $J=7.5$ Hz, 3H, CH_2CH_3), 1.75–1.90 (m, 3H, CH_2CH), 2.28 (q, $J=7.5$ Hz, 2H, CH_2CH_3), 3.41 (t, $J=5.1$ Hz, 2H, CH_2OH), 3.92–4.11 (m, 2H, CH_2OCO), 4.14 (t, $J=7.2$ Hz, 2H, NCH_2), 4.63 (t, $J=5.1$ Hz, 1H, OH), 6.87 (br s, 2H, NH_2), 8.12 (s, 1H, H-8); EI-MS m/z 311 (M^+). Anal. calcd for $C_{13}H_{18}FN_5O_3$: C, 50.16; H, 5.83; N, 22.50. Found: C, 50.25; H, 5.96; N, 22.37.

2-Amino-6-fluoro-9-(3-butyryloxymethyl-4-hydroxybut-1-yl)purine (10). mp 140.2 °C (dec) (EtOAc/hexanes); UV (H_2O) λ_{max} 218 (ε 24463), 246 (6769), and 290 (6610) nm; IR (KBr) 3385, 3312, 3219, 1727, 1646, 1576, 1483 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.85 (t, $J=7.2$ Hz, 3H, $CH_2CH_2CH_3$), 1.47–1.57 (m, 2H, $CH_2CH_2CH_3$), 1.63–1.90 (m, 3H, CH_2CH), 2.23 (t, $J=7.2$ Hz, 2H, $CH_2CH_2CH_3$), 3.42 (t, $J=5.1$ Hz, 2H, CH_2OH), 3.95–4.09 (m, 2H, CH_2OCO), 4.14 (t, $J=7.2$ Hz, 2H, NCH_2), 4.63 (t, $J=5.1$ Hz, 1H, OH), 6.87 (br s, 2H, NH_2), 8.12 (s, 1H, H-8); EI-MS m/z 325 (M^+). Anal. calcd for $C_{14}H_{20}FN_5O_3$: C, 51.69; H, 6.20; N, 21.53. Found: C, 51.78; H, 6.37; N, 21.65.

2-Amino-6-fluoro-9-(4-hydroxy-3-isobutyryloxymethylbut-1-yl)purine (11). mp 171.8 °C (dec) (EtOAc/hexanes); UV (H_2O) λ_{max} 218 (ε 24111), 246 (6731), and 290 (6602) nm; IR (KBr) 3369, 3202, 1720, 1647, 1574, 1484 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.04 (d, $J=6.9$ Hz, 3H, $CH(CH_3)_2$), 1.05 (d, $J=6.9$ Hz, 3H, $CH(CH_3)_2$), 1.74–1.89 (m, 3H, CH_2CH), 2.49 (septet, $J=6.9$ Hz, 1H, $CH(CH_3)_2$), 3.42 (t, $J=5.1$ Hz, 2H, CH_2OH), 3.95–4.08 (m, 2H, CH_2OCO), 4.14 (t, $J=6.9$ Hz, 2H, NCH_2), 4.64 (t, $J=5.1$ Hz, 1H, OH), 6.86 (br s, 2H, NH_2), 8.12 (s, 1H, H-8); EI-MS m/z 325 (M^+). Anal. calcd for $C_{14}H_{20}FN_5O_3$: C, 51.69; H, 6.20; N, 21.53. Found: C, 51.62; H, 6.29; N, 21.57.

Synthesis of 12–15: General procedure. To a solution of **7** (0.51 g, 2.00 mmol) and a catalytic amount of DMAP (24 mg, 0.20 mmol) in anhydrous DMF (10 mL) was added an appropriate acid anhydride [Ac_2O, $(EtCO)_2O$, $(n\text{-}PrCO)_2O$, or $(i\text{-}PrCO)_2O$] (12.00 mmol) at room temperature under nitrogen atmosphere, and the mixture was stirred for 30 min at room temperature. MeOH (5 mL) was added, and the reaction mixture was evaporated to dryness under reduced pressure. The residue was partitioned between $CHCl_3$ (2×50 mL) and saturated aqueous $NaHCO_3$ solution, and the organic layer was dried ($MgSO_4$), filtered, and evaporated to dryness under reduced pressure to give a yellow solid. The crude product was purified by column chromatography on silica gel (gradient elution: 2/1 EtOAc/hexanes and 4/1 EtOAc/hexanes) to afford the corresponding diester as a white solid. Analytically pure material was obtained by crystallization from the solvents indicated below.

2-Amino-6-fluoro-9-(4-acetoxy-3-acetoxymethylbut-1-yl)-purine (12). mp 128.3–128.9 °C (EtOAc/hexanes); UV (H_2O) λ_{max} 218 (ε 22790), 246 (6473), and 290 (6212) nm; IR (KBr) 3396, 3345, 3235, 1731, 1656, 1643, 1568, 1482 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.91–2.01 (m, 3H, CH_2CH), 2.07 (s, 6H, 2 $COCH_3$), 4.15 (d, $J=5.4$ Hz, 4H, 2 OCH_2), 4.21 (t, $J=7.1$ Hz, 2H, NCH_2), 5.13 (br s, 2H, NH_2), 7.76 (s, 1H, H-8); EI-MS m/z 339 (M^+). Anal. calcd for $C_{14}H_{18}FN_5O_4$: C, 49.56; H, 5.35; N, 20.64. Found: C, 49.67; H, 5.57; N, 20.48.

2-Amino-6-fluoro-9-(4-propionyloxy-3-propionyloxymethylbut-1-yl)purine (13). mp 112.9–113.4 °C (EtOAc/hexanes); UV (H_2O) λ_{max} 218 (ε 24269), 246 (6933), and 290 (6628) nm; IR (KBr) 3475, 3345, 3213, 1748, 1732, 1639, 1620, 1568, 1478 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (t, $J=7.5$ Hz, 6H, 2 CH_2CH_3), 1.91–2.04 (m, 3H, CH_2CH), 2.34 (q, $J=7.5$ Hz, 4H, 2 CH_2CH_3), 4.15 (d, $J=5.1$ Hz, 4H, 2 OCH_2), 4.21 (t, $J=7.2$ Hz, 2H, NCH_2), 5.09 (br s, 2H, NH_2), 7.75 (s, 1H, H-8); EI-MS m/z 367 (M^+). Anal. calcd for $C_{16}H_{22}FN_5O_4$: C, 52.31; H, 6.04; N, 19.06. Found: C,52.42; H, 6.17; N, 19.24.

2-Amino-6-fluoro-9-(4-butyryloxy-3-butyryloxymethylbut-1-yl)purine (14). mp 99.4–100.2 °C (EtOAc/hexanes); UV (H_2O) λ_{max} 218 (ε 24309), 246 (6982), and 290 (6741) nm; IR (KBr) 3383, 3333, 3206, 1735, 1735, 1656, 1631, 1571, 1479 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (t, $J=7.2$ Hz, 6H, 2 $CH_2CH_2CH_3$), 1.59–1.71 (m, 4H, 2 $CH_2CH_2CH_3$), 1.90–1.99 (m, 3H, CH_2CH), 2.30 (t, $J=7.2$ Hz, 4H, 2 $CH_2CH_2CH_3$), 4.15 (d, $J=5.4$ Hz, 4H, 2 OCH_2), 4.21 (t, $J=7.2$ Hz, 2H, NCH_2), 5.10 (br s, 2H, NH_2), 7.75 (s, 1H, H-8); EI-MS m/z 395 (M^+). Anal. calcd for $C_{18}H_{26}FN_5O_4$: C, 54.67; H, 6.63; N, 17.71. Found: C, 54.76; H, 6.78; N, 17.52.

2-Amino-6-fluoro-9-(4-isobutyryloxy-3-isobutyryloxymethylbut-1-yl)purine (15). mp 114.4–115.2 °C (EtOAc/hexanes); UV (H_2O) λ_{max} 218 (ε 22470), 246 (6353), and 290 (6120) nm; IR (KBr) 3382, 3333, 3206, 1732, 1656, 1631, 1571, 1479 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (d, $J=6.9$ Hz, 12H, 2 $CH(CH_3)_2$), 1.92–2.02 (m, 3H, CH_2CH), 2.56 (septet, $J=6.9$ Hz, 2H, 2 $CH(CH_3)_2$), 4.15 (d, $J=5.7$ Hz, 4H, 2 OCH_2), 4.22 (t, $J=7.2$ Hz, 2H, NCH_2), 5.09 (br s, 2H, NH_2), 7.75 (s, 1H, H-8); EI-MS

m/z 395 (M^+). Anal. calcd for $C_{18}H_{26}FN_5O_4$: C, 54.67; H, 6.63; N, 17.71. Found: C, 54.81; H, 6.68; N, 17.83.

Oral bioavailability. Test compound was administered as a single dose of 0.2 mmol/kg in 1% (carboxymethyl) cellulose in a volume of 10 mL/kg by oral gavage to four Sprague–Dawley rats weighing 360–400 g, from which food had been withheld for 16 h. Blood was collected at 0.25, 0.5, 1, and 2 h after dosing by cardiac puncture. An aliquot of each blood sample was immediately mixed with trichloroacetic acid (8% final concentration) in a separate tube and centrifuged, and the supernatant was neutralized with 1/5 volume of saturated aqueous $NaHCO_3$ solution. The plasma samples were then analyzed by reversed-phase HPLC (Waters Associates, Inc., Milford, USA) using a C_{18}-symmetry column equipped with a precolumn. The column was eluted at a flow rate of 1 mL/min with the following three-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 20-min linear gradient from 99% solution A and 1% solution B to 5% solution A and 95% solution B, and (step 3) a 10-min isocratic elution with 5% solution A and 95% solution B. The column was equilibrated with 99% solution A and 1% solution B for 10 min before each sample injection. The UV absorbance of the column effluent was monitored at 287 nm.

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