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Synthesis of 2-modified aristeromycins and their analogs as potent inhibitors against *Plasmodium falciparum* *S*-adenosyl-L-homocysteine hydrolase

Takayuki Ando,[a,b] Masafumi Iwata,[b] Fazila Zulfiqar,[b] Tatsuya Miyamoto,[b] Masayuki Nakanishi[b] and Yukio Kitade[a,b,c,d,*]

[a]*Center for Emerging Infectious Diseases, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*
[b]*Department of Biomolecular Science, Faculty of Engineering, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*
[c]*Center for Advanced Drug Research, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*
[d]*United Graduate School of Drug Discovery and Medical Information Sciences, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*

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Abstract—2-Modified aristeromycin derivatives and their related analogs were synthesized to investigate their inhibitory activity against human and *Plasmodium falciparum* *S*-adenosyl-L-homocysteine hydrolase (PfSAHH). 2-Fluoroaristeromycin showed a strong inhibitory activity against PfSAHH selectively and complete resistance to adenosine deaminase.

1. Introduction

S-Adenosyl-L-homocysteine (SAH) is formed by the donation of the methyl group of *S*-adenosyl-L-methionine (SAM) to a methyl acceptor, and is physiologically hydrolyzed to adenosine and homocysteine by SAH hydrolase (SAHH). Therefore, the inhibition of SAHH results in cellular accumulation of SAH, which is a potent feedback inhibition of SAM-dependent biological methylation such as that at the cap structure at 5′-end of eucaryotic *m*-RNA.[1–4] The eucaryotic *m*-RNA must possess a methylated 5′-cap structure for stability against phosphatases and ribonuclease, proper binding to ribosomes and the promotion of splicing. Therefore, an uncapped *m*-RNA is much less likely to be translated into its respective protein.[1–6] Hence, SAHH has emerged as a target enzyme for the molecular design of the above-mentioned chemotherapeutic agents.

Various carbocyclic adenine nucleosides (**1**–**4**, **7**) as SAHH inhibitors have been synthesized (Fig. 1).[7] It is known that aristeromycin (**4**) is a naturally occurring product possessing inhibitory activity against SAHH.[1] However, aristeromycin is deaminated by adenosine deaminase to yield chemotherapeutically inactive inosine congeners, reducing its inhibitory potency. It has been recognized that the introduction of a halogen atom at the 2-position of an adenosine nucleoside enhances its resistance to the adenosine deaminase.[8,9] Actually, 2-fluoroneplanocin A (**1**) exhibited complete resistance to this enzyme.[10]

Furthermore, we have reported that 2-fluoronoraristeromycin (**2**) selectively inhibits *Plasmodium falciparum* SAHH (PfSAHH) which has additional space near 2-position of the adenine-ring, in the substrate binding pocket, in comparison with human SAHH (HsSAHH).[11,12] Mutagenic analysis of the amino acid residue forming the additional space confirmed that the inhibition selectivity is due to the difference of only one amino acid residue.[13]

On the basis of these observations, we designed and synthesized 2-fluoroaristeromycin (**5**) and its analogs (**6**, **8**, and **9** in Figure 1; **10** and **11** in Scheme 2; **12** in Scheme 3) to investigate not only the inhibitory activities against HsSAHH and PfSAHH but also the resistance to adenosine deaminase for the development of a potent antimalarial agent.

Keywords: 2-Fluoroaristeromycin; *S*-Adenosyl-L-homocysteine hydrolase; Carbocyclic nucleoside; Enzyme inhibitor.
* Corresponding author. Tel./fax: +81 58 2932640; e-mail addresses: ykkitade@gifu-u.ac.jp; kitade@biomol.gifu-u.ac.jp

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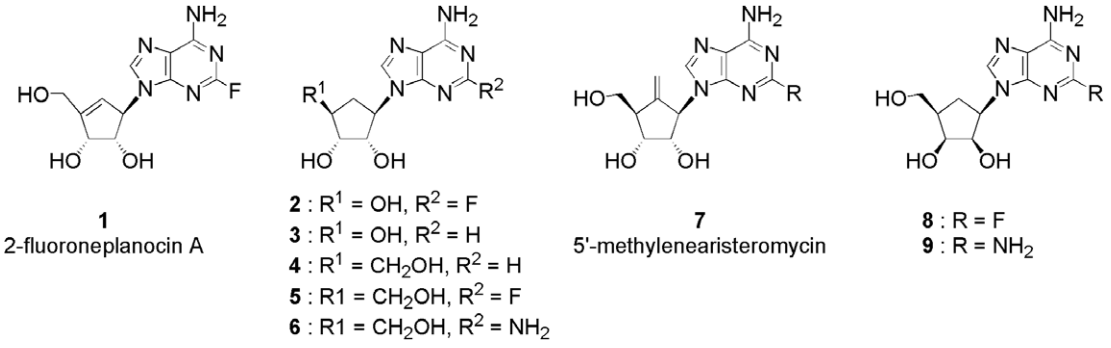


Figure 1. Various carbocyclic adenine nucleosides.

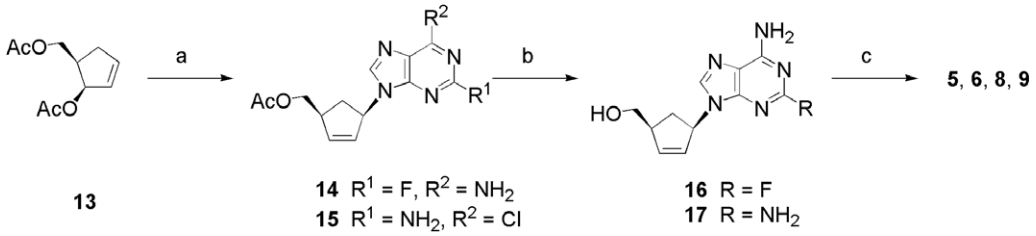
2. Chemistry

A synthetic route for the 2-modified aristeromycins and their 2′,3′ epimers is shown in Scheme 1. The cyclopentenyl purine derivatives **14** and **15** were prepared by the coupling reaction of **13**[14] with 2-fluoroadenine and 2-amino-6-chloropurine, respectively, in the presence of a palladium catalyst.[15] The treatment of **14** and **15** with methanolic ammonia yielded the de-acylated and/or 6-amidated compounds **16** and **17**, respectively. Osmium oxidation[16] of these compounds with *N*-methylmorpholine *N*-oxide (NMO) yielded 2-fluoroaristeromycin, 2-aminoaristeromycin, and their 2′,3′ epimers (**5**, **6**, **8**, and **9**).

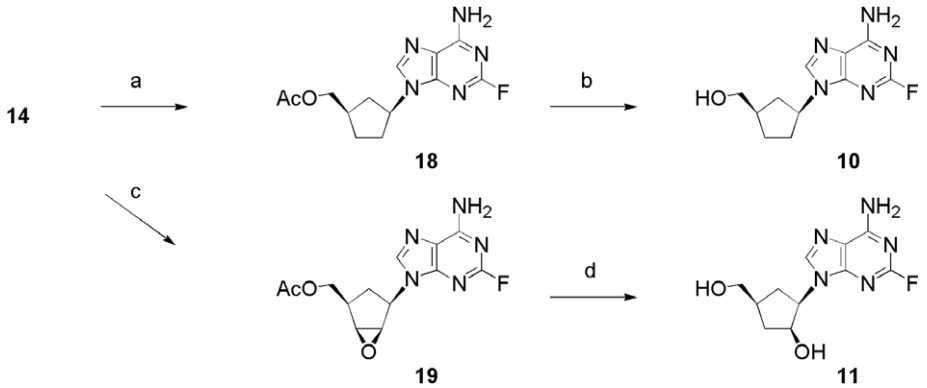
The catalytic hydrogenation of **14** afforded the reduced compound **18**; subsequent de-acetylation afforded 2′,3′-deoxy-2-fluoroaristeromycin (**10**). The 2′,3′-epoxy compound **19** was obtained by the oxidation of **14** with *m*-chloroperbenzoic acid (*m*-CPBA),[17] and the subsequent reduction using $LiAlH_4$[18,19] afforded the 2′-*epi*-3′-deoxy compound **11** (Scheme 2).

Compound **16** was oxidized by *m*-CPBA to afford 2′,3′-epoxy-2-fluoroaristeromycin (**12**, Scheme 3). Although it was possible to obtain **12** from **19** by de-acetylation, ring-opening compounds were obtained as inseparable by-products along with the desired epoxy compound.

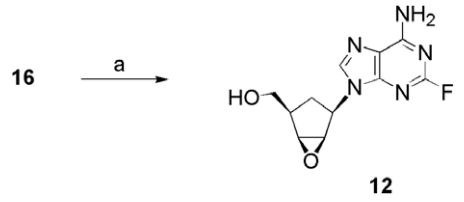
The structures of these compounds were supported by spectrum data (^{1}H NMR, ^{13}C NMR, HREIMS). The stereochemical assignments were mainly based on the differential NOE correlation of the corresponding protons.



Scheme 1. Reagents and conditions: (a) 2-fluoroadenine or 2-amino-6-chloropurine, NaH, DMSO, then $(Ph_3P)_4Pd$, Ph_3P, THF, 55 °C, 73% (for **14**) or 46% (for **15**); (b) NH_3/MeOH, 0 °C, 99% (for **16**) or 120 °C, 71% (for **17**); (c) OsO_4, NMO, THF–H_2O, rt, 45%, 52%, 35% and 40% (for **5**, **6**, **8** and **9**).



Scheme 2. Reagents and conditions: (a) H_2, Pd–C, MeOH, rt, 55%; (b) NH_3/MeOH, 0 °C, 93%; (c) *m*-CPBA, CH_2Cl_2, rt, 56%; (d) $LiAlH_4$, THF, rt, 20%.



Scheme 3. Reagents and condition: (a) *m*-CPBA, CH_2Cl_2, rt, 77%.

3. Biological assay

3.1. Inhibitory activity against SAHH

The inhibitory activities of the title compounds against HsSAHH and PfSAHH are summarized as IC_{50} values in Table 1. The 2-fluorinated compound **5** and 2-amino compound **6** showed strong inhibitory activities against PfSAHH with IC_{50} value of 1.98 and 4.51 and superior selective index 24 and 20, respectively; these values were better than those of the typical compounds we had synthesized. The other synthetic compound in this report did not show any inhibitory activity up to 500 μM.

3.2. Susceptibility to adenosine deaminase

The susceptibility of the compounds to adenosine deaminase was investigated under the previously reported condition[21] (Table 2). Compound **5** was completely resistant to adenosine deaminase unlike aristeromycin, which was rapidly deaminated within 15 min under the reaction conditions. 2-Aminoaristeromycin was slightly deaminated with a 5% conversion ratio.

Table 1. Inhibitory activities of carbocyclic nucleosides against human and *P. falciparum* SAH hydrolases

Compound	HsSAHH IC_{50} (μM)	PfSAHH IC_{50} (μM)	Selective index[d]
2	63[a]	13[a]	4.8
3	1.1[a]	3.1[a]	0.35
4	4.85	57.0	0.085
5	47.2	1.98	24
6	90.7	4.51	20
7	15.7[b]	2.1[b]	7.5[b]
8	ND[c]	ND[c]	—
9	ND[c]	ND[c]	—
10	ND[c]	ND[c]	—
11	ND[c]	ND[c]	—
12	ND[c]	ND[c]	—

[a] Ref. 11.
[b] Ref. 7c.
[c] No inhibitory activity showed at 500 μM.
[d] Selective index: mean of IC_{50}value for HsSAH/mean of IC_{50} value for PfSAHH.

Table 2. Effect of calf intestinal adenosine deaminase on 2-modified aristeromycins

Compound	15 min
4	22.4[a]
5	99.5[a]
6	95.2[a]

[a] % of remaining compound after incubation.

4. Discussion

According to the SAHH assay of compounds **5** and **6**, it is clear that the introduction of small molecular, such as a fluorine atom or a amino residue at the 2-position of adenine ring, increased the selective indexes, which was consistent with our previous work.[11] The inhibitory activity of **5** against PfSAHH was better than those of 2-fluoronoraristeromycin (**2**) because the sugar moiety structure of these aristeromycin analogs are more similar to that of adenosine. Furthermore, the deoxy- and *epi*-analogs did not show any inhibitory activity. This result indicates that 2′ and/or 3′-hydroxyl groups are essential for the inhibitions against both the SAHHs-HsSAHH and PfSAHH. The detailed modification of the sugar moiety is under the investigation.

On the other hand, 2-fluoroaristeromycin (**5**) showed the complete resistance to adenosine deaminase in comparison with that of aristeromycin. This result confirmed the previous report,[10] which stated the introduction of a halogen atom was effective in designing inhibitors showing the resistance to adenosine deaminase.

In this paper, we have demonstrated the synthesis of 2-modified aristeromycin analogs and their inhibitory activity against HsSAHH and PfSAHH. Among the reported compounds, **5** was the best selective inhibitor against PfSAHH, showing resistance to adenosine deaminase. These results will significantly contribute to the design of potent inhibitors as antimalarial agents.

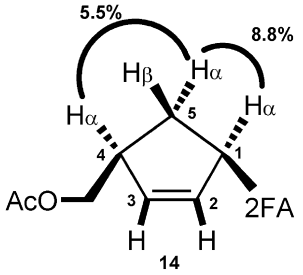
5. Experimental

5.1. General remarks

^1H and ^{13}C NMR spectra were recorded at 400 MHz on a JEOL AL-400 (operated at 400 and 100 MHz, respectively) by using $CDCl_3$ with TMS as the internal standard, DMSO-d_6, and CD_3OD. The spin multiplicities are indicated by the following symbols s (singlet), d (doublet), dd (doublet of doublet), t (triplet), ddd (doublet of doublet of doublet), q (quartet), m (multiplet), and br (broad). Coupling constants (*J*) are expressed in Hertz. The differential nuclear overhauser effect (DIFNOE) data were described; the irradiated proton (the correlated proton; % of enhancement value). Mass spectra (HREIMS) were recorded at 70 eV on Shimadzu QP 1000 A spectrometer. Reactions were monitored by thin-layer chromatography using E. MERCK silica gel 60 F_{254} glass plate. Silica gel column chromatography was carried out on Wako gel C-300. HPLC was performed using the Shimadzu HPLC 10A VP-series attached to an YMC ODS-AM column (250 mm × 20 mm) for separation or an YMC ODS-M80 column (150 mm × 8 mm) for analysis.

5.1.1. 9-[*cis*-(1′*R*,4′*S*)-4′-Acetoxymethyl-2′-cyclopenten-1′-yl]-9-*H*-2-fluoroadenine (14). A mixture of 2-fluoroadenine (385 mg, 2.5 mmol) and 60% NaH (98 mg, 2.46 mmol) in DMSO (5 mL) was stirred at rt for 0.5 h. The reaction mixture was added to a solution of

tetrakis(triphenylphosphine)palladium(0) (377 mg, 0.33 mmol), triphenylphosphine (86 mg, 0.33 mmol) and **13** (647 mg, 3.26 mmol) in THF (5 mL) under the dark condition and then stirred at 55 °C for 24 h. The reaction mixture was evaporated under reduced pressure. Chloroform was added to the residue and the insoluble matter was removed by filtration. The filtrate was washed with brine, dried (Na_2SO_4) and concentrated. Column chromatography (EtOAc/hexane, 3:1) of the residue on silica gel gave **14** (537 mg, 73%) as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (1H, s, H-8), 6.18 (1H, m, H-3′), 5.95 (1H, m, H-2′), 5.78 (2H, br s, NH_2), 5.65 (1H, m, H-1′), 4.15 (2H, m, H-6′), 3.18 (1H, m, H-4′), 2.91 (1H, m, H-5′$_\beta$), 2.07 (3H, s, CH_3CO), 1.67 (1H, m, H-5′$_\alpha$); DIFNOE: H-1′ (H-2′: 3.9%, H-5′$_\alpha$: 8.8%), H-4′ (H-3′: 1.7%, H-5′$_\alpha$: 5.5%, H-6′: 2.6%); ^{13}C NMR ($CDCl_3$, 100 MHz) 170.92, 159.12 (d, J = 210.7), 157.02 (d, J = 20.6), 151.24 (d, J = 18.9), 138.80 (d, J = 2.5), 138.07, 129.83, 118.01 (d, J = 4.1), 66.29, 59.43, 44.45, 35.17, 20.88; HREIMS calcd for $C_{13}H_{14}FN_5O_2$ 291.1132. Found: 291.1122.



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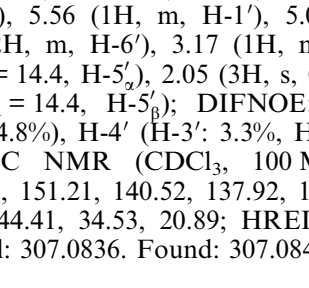
5.1.2. 9-[*cis*-(1′*R*,4′*S*)-4′-Acetoxymethyl-2′-cyclopenten-1′-yl]-9-*H*-2- amino-6-chloropurine (15). Compound **15** (671 mg, 2.18 mmol) was prepared from 2-amino-6-chloropurine (2.2 g, 13.0 mmol) and **13** (1 g, 5.05 mmol) as described from **14** in 43% yield after silica gel chromatography (EtOAc/hexane, 4:1). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.80 (1H, s, H-8), 6.13 (1H, m, H-3′), 5.88 (1H, m, H-2′), 5.56 (1H, m, H-1′), 5.08 (2H, br s, NH_2), 4.17 (2H, m, H-6′), 3.17 (1H, m, H-4′), 2.84 (1H, dt, J_{gem} = 14.4, H-5′$_\alpha$), 2.05 (3H, s, CH_3CO), 1.69 (1H, m, J_{gem} = 14.4, H-5′$_\beta$); DIFNOE: H-1′ (H-2′: 8.6%, H-5′$_\alpha$: 14.8%), H-4′ (H-3′: 3.3%, H-5′$_\alpha$: 5.3%, H-6′: 4.9%); ^{13}C NMR ($CDCl_3$, 100 MHz) 170.99, 158.92, 153.35, 151.21, 140.52, 137.92, 129.79, 125.56, 66.14, 59.46, 44.41, 34.53, 20.89; HREIMS calcd for $C_{13}H_{14}N_5O_2Cl$: 307.0836. Found: 307.0845.



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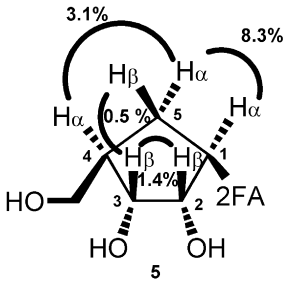
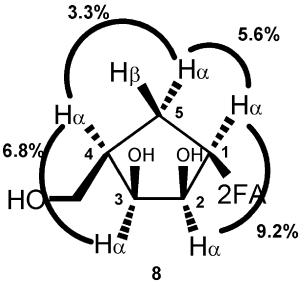
5.1.3. 9-[*cis*-(1′*R*,4′*S*)-4′-Hydroxymethyl-2′-cyclopenten-1′-yl]-9-*H*-2-fluoroadenine (16). Compound **14** (684 mg, 2.35 mmol) was treated with methanolic ammonia (50 mL, 27% w/w) at 0 °C for 36 h. The reaction mixture was evaporated under reduced pressure. Column chromatography ($CHCl_3$/MeOH, 15:1) of the residue on silica gel gave **16** (585 mg, 99%) as white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.02 (1H, s, H-8), 7.75 (2H, br s, NH_2), 6.13 (1H, m, H-3′), 5.89 (1H, m, H-2′), 5.46 (1H, m, H-1′), 4.71 (1H, t, $J_{6',OH}$ = 5.4, OH-6′), 3.45 (2H, m, H-6′), 2.87 (1H, m, H-4′), 2.65 (1H, dt, J_{gem} = 13.8, H-5′$_\alpha$), 1.62 (1H, dt, *H*-5′$_\beta$); ^{13}C NMR (DMSO-d_6, 100 MHz) 158.51 (d, J = 204.1), 157.55 (d, J = 21.4), 150.40 (d, J = 20.6), 139.24 (d, J = 2.5),138.53, 129.36, 117.30 (d, J = 4.1), 63.87, 59.24, 47.75, 34.05; HREIMS calcd for $C_{11}H_{12}N_5OF$: 249.1026. Found: 249.1030; Anal. Calcd for $C_{11}H_{12}N_5OF{\cdot}1/10H_2O$: C, 52.62; H, 4.90; N, 27.90. Found: C, 52.52; H, 4.81; N, 27.85.

5.1.4. 9-[*cis*-(1′*R*,4′*S*)-4′-Hydroxymethyl-2′-cyclopenten-1′-yl]-9-*H*-2-aminoadenine (17). Compound **15** (400 mg, 1.30 mmol) was treated with methanolic ammonia (50 mL, 27% w/w) in a sealed tube at 120 °C for 48 h. The reaction mixture was evaporated under reduced pressure. Column chromatography ($CHCl_3$/MeOH, 15:1) of the residue on silica gel gave **17** (316 mg, 99%) as white solid. ^{1}H NMR (DMSO-d_6, 100 MHz) δ 7.60 (1H, s, H-8), 6.62, 5.73 (4H, 2br s, NH_2-2, NH_2-6), 6.09 (1H, m, H-3′), 5.86 (1H, m, H-2′), 5.36 (1H, m, H-1′), 4.73 (1H, t, OH-6′), 3.43 (2H, m, H-6′), 2.85 (1H, m, H-4′), 2.57 (1H, m, J_{gem} = 13.8, H-5′$_\alpha$), 1.57 (1H, m, H-5′$_\beta$); DIFNOE: H-1′ (H-2′: 5.5%, H-5′$_\alpha$: 6.8%, H-8: 2.4%), H-4′ (H-3′: 5.5%, H-6′: 4.5%, H-5′$_\alpha$: 3.8%); ^{13}C NMR (DMSO-d_6, 100 MHz) 160.32, 156.28, 151.58, 138.13, 135.25, 130.22, 113.50, 64.27, 58.31, 47.86, 34.46; HREIMS calcd for $C_{11}H_{14}N_6O$ 246.1229. Found: 246.1224.

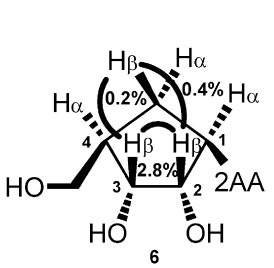
5.1.5. 9-[(1′*R*,2′*S*,3′*R*,4′*R*)-2′,3′-Dihydroxy-4′-hydroxymethyl-cyclopentan-1′-yl]-9-*H*-2-fluoroadenine (5) and 9-[(1′*R*,2′*R*,3′*S*,4′*R*)-2′,3′-dihydroxy-4′-hydroxymethyl-cyclopentan-1′-yl]-9-*H*-2-fluoroadenine (8). To a stirred solution of compound **16** (250 mg, 1.0 mmol) and *N*-methylmorpholine *N*-oxide (NMO, 353 mg, 3.0 mmol) in THF (10 mL) and H_2O (1.0 mL), 2% OsO_4solution (1.3 mL, 0.10 mmol) was added and stirred at rt for 6 h. The mixture was evaporated under reduced pressure. Column chromatography (stepwise elution $CHCl_3$/MeOH/H_2O, 5:1:0 → 3:1:0 → 5:4:1) of the residue on silica gel and preparative PLC (H_2O/MeOH, 90:10) gave **5** (128 mg, 45%) and **8** (99 mg, 35%) as white solid. For **5**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.18 (1H, s, H-8), 7.73 (2H, s, NH_2-6), 4.91 (1H, d, $J_{2',OH'}$ = 6.80, OH-2′), 4.70 (1H, t, $J_{6',OH'}$ = 5.36, OH-6′), 4.65 (1H, d, $J_{3',OH'}$ = 4.16, OH-3′), 4.58 (1H, m, H-1′), 4.25 (1H, m, H-2′), 3.80 (1H, m, H-3′), 3.43 (2H, m, H-6′), 2.20 (1H, m, H-5′$_\alpha$), 2.01 (1H, m, H-4′), 1.63 (1H, m, H-5′$_\beta$); DIFNOE: H-1′ (H-5′$_\alpha$: 8.3%), H-3′ (H-2′: 1.4%, H-5′$_\beta$: 0.4%), H-4′ (H-3′: 3.0%, H-5′$_\alpha$: 3.1%); ^{13}C NMR (DMSO-d_6, 100 MHz) 158.27 (d, J = 203.3), 157.51 (d, J = 21.4), 150.95 (d, J = 19.8),

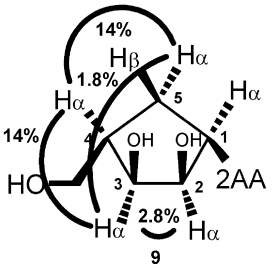
140.34, 117.56, 74.57, 71.53, 62.96, 59.15, 45.26, 29.15; HREIMS calcd for $C_{11}H_{14}FN_5O_3$ 283.1081. Found: 283.1086; Anal. Calcd for $C_{11}H_{14}FN_5O_3 \cdot 1/10H_2O$: C, 46.35; H, 5.02; N, 24.57. Found: C, 45.95; H, 4.84; N, 24.66. For **8**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.14 (1H, s, H-8), 7.70 (2H, s, NH_2-6), 5.01, 4.93 (2H, d, $J_{2',OH}$, $J_{3',OH}$ = 5.6, 3.92, OH-2′,3′), 4.80 (1H, m, H-1′), 4.48 (1H, t, $J_{6',OH}$ = 5.36, OH-6′), 4.50 (2H, s, H-2′ and 3′), 3.55 (2H, m, H-6′), 2.24 (1H, dt, $J_{1',5'\alpha}$ = 8.3, J_{gem} = 12.7, H-5′$_\alpha$), 2.05 (1H, m, H-4′), 1.81 (1H, m, H-5′$_\beta$); DIFNOE: H-1′ (H-2′: 9.2%, H-5′$_\alpha$: 5.6%), H-4′ (H-3′: 6.8%, H-5′$_\alpha$: 3.3%); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 158.45 (d, J = 204.1), 157.37 (d, J = 21.4), 151.23 (d, J = 20.6), 141.71 (d, J = 2.5), 116.45 (d, J = 4.1), 71.73 (d, J = 43.6), 60.46, 53.19, 48.58, 41.47, 32.83; HREIMS calcd for $C_{11}H_{14}FN_5O_3$ 283.1081. Found: 283.1087; Anal. Calcd for $C_{11}H_{14}FN_5O_3$: C, 46.64; H, 4.98; N, 24.72. Found: C, 46.41; H, 4.83; N, 24.80.





5.1.6. 9-[(1′*R*,2′*S*,3′*R*,4′*R*)-2′,3′-Dihydroxy-4′-hydroxymethyl-cyclopentan-1′-yl]-9-*H*-2-aminoadenine (6) and 9-[(1′*R*,2′*R*,3′*S*,4′*R*)-2′,3′-dihydroxy-4′-hydroxymethyl-cyclopentan-1′-yl]-9-*H*-2-aminoadenine (9). Compound **6** (148 mg, 0.53 mmol) and **9** (114 mg, 407 μmol) were prepared from **17** (268 mg, 1.1 mmol) as described from **16** in 48% and 37% yield, respectively, after silica gel chromatography (stepwise elution $CHCl_3$/MeOH/H_2O, 5:1:0 → 3:1:0 → 60:25:4) and preparative HPLC (H_2O/MeOH, 90:10). For **6**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.76 (1H, s, H-8), 6.59, 5.67 (4H, 2br s, 2NH_2), 4.94 (1H, d, $J_{2',OH}$ = 6.3, OH-2′), 4.69 (1H, t, $J_{6',OH}$ = 5.12, OH-6′), 4.53–4.48 (2H, m, H-1′, OH-3′), 4.22 (1H, m, H-2′), 3.79 (1H, m, H-3′), 3.45 (2H, m, H-6′), 2.18 (1H, m, H-5′$_\beta$), 1.98 (1H, m, H-4′), 1.55 (1H, m, H-5′$_\alpha$); DIFNOE: H-3′ (H-2′: 2.8%, H-4′: 6.0%), H-5′β (H-2′: 0.4%, H-3′: 0.2%, H-5′$_\alpha$: 3.8%); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 159.52, 155.73, 151.68, 135.76, 113.20, 74.33, 71.45, 62.66, 57.78, 44.90, 29.24; HREIMS calcd for $C_{11}H_{16}FN_6O_3$ 280.1284. Found: 280.1289. For **9**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.75 (1H, s, H-8), 6.59, 5.67 (4H, 2br s, 2NH_2), 5.02 (1H, d, $J_{3',OH}$ = 4.8, OH-3′), 4.91 (1H, d, $J_{2',OH}$ = 6.0, OH-2′), 4.69 (1H, m, H-1′), 4.47 (1H, t, $J_{6',OH}$ = 5.0, OH-6′), 4.04 (1H, m, H-2′), 3.99 (1H, m, H-3′), 3.63, 3.48 (2H, m, H-6′), 2.15 (1H, dt, J_{gem} = 12.4, $J_{1',5\alpha}$ = 8.0, H-5′$_\alpha$), 2.05 (1H, m, H-4′), 1.84 (1H, m, $J_{1',5'\beta}$ = 10.4, H-5′$_\beta$); DIFNOE: H-4′ (H-3′: 14%, H-5′$_\alpha$: 14%); H-3′ (H-2′: 2.8%, H-5′$_\alpha$: 1.8%); ^{13}C NMR (DMSO-d_6, 100 MHz) 160.55, 156.72, 152.64, 138.60, 113.49, 72.76, 72.39, 61.33, 53.36, 40.08, 33.09; HREIMS calcd for $C_{11}H_{16}FN_6O_3$ 280.1284. Found: 280.1291.



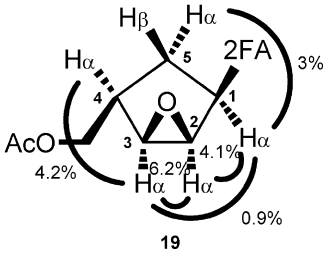


5.1.7. 9-[*cis*-(1′*R*,3′*S*)-1′-Acetoxymethyl-cyclopentan-3′-yl]-9-*H*-2-fluoroadenine (18). Compound **14** (60 mg, 206 μmol) in MeOH (20 mL) was hydrogenated in the presence of 5% Pd on carbon (50 mg) for 24 h at rt and the reaction mixture was filtered through Celite, washing with MeOH. The filtrate was concentrated, and column chromatography (EtOAc/hexane, 8:3) of the residue on silica gel gave **10** (33 mg, 55%) as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.83 (1H, s, H-8), 6.29 (2H, s, NH_2), 4.81 (1H, m, H-1′), 4.13 (2H, m, H-6′), 2.48–1.76 (7H, m, H-2′, 3′, 4′, and 5′), 2.08 (3H, s, CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz) 171.15, 161.29 (d, J = 256.8), 156.96 (d, J = 20.6), 151.57 (d, J = 19.8), 138.80 (d, J = 3.3), 118.15 (d, J = 4.1), 67.39, 55.62, 36.82, 36.11, 31.49, 26.74, 20.94; HREIMS calcd for $C_{13}H_{16}FN_5O_2$ 293.1288. Found: 293.1293.

5.1.8. 9-[*cis*-(1′*R*,3′*S*)-1′-Hydroxymethyl-cyclopentan-3′-yl]-9-*H*-2-fluoroadenine (10). Compound **10** (102 mg, 406 μmol) was prepared from **18** (128 mg, 436 μmol) as described from **16** in 93% after silica gel chromatography ($CHCl_3$/MeOH, 12:1). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.20 (1H, s, H-8), 7.74 (2H, br s, NH_2), 4.68 (1H, m, H-1′), 4.61 (1H, t, OH-6′), 3.40 (2H, m, H-6′), 2.28–1.60 (7H, m, H-2′, 3′, 4′, and 5′); ^{13}C NMR (DMSO-d_6, 100 MHz) 158.38 (d, J = 204.1), 157.55 (d, J = 21.4), 150.65 (d, J = 20.5), 139.65 (d, J = 3.3), 117.46 (d, J = 4.1), 64.73, 55.19, 48.59, 35.28, 30.92, 26.25; HREIMS calcd for $C_{11}H_{14}FN_5O$ 251.1182. Found: 251.1191.

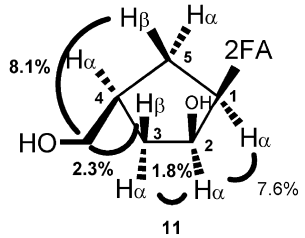
5.1.9. 9-[(1′*R*,2′*R*,3′*S*,4′*R*)-4′-Acetoxymethyl-2′,3′-epoxy-cyclopentan-1′-yl]-9-*H*-2′-fluoroadenine (19). To a solution of **14** (100 mg, 343 μmol) in CH_2Cl_2 (6 mL) was added 3-chloroperoxybenzoic acid (*m*-CPBA, 82 mg, 475 μmol), and the mixture was stirred for 18 h at rt. After completion of the reaction, the mixture was concentrated, and extracted with EtOAc. The extract was washed with sat $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. Column chromatography (EtOAc/hexane, 8:3) of residue on silica gel gave **19** (59 mg, 56%) as white crystal. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08 (1H, s, H-8), 6.03 (2H, br s, NH_2-6), 5.03 (1H, t, $J_{1',5'\alpha}$ = 10, H-1′), 4.22 (2H, m, H-6′), 3.78 (1H, s, H-2′), 3.68 (1H, s, H-3′), 2.54 (1H, m, H-4′), 2.34 (1H, m, H-5′$_\alpha$), 2.08 (3H, s, CH_3CO), 1.29 (1H, m, H-5′$_\beta$);

DIFNOE: H-1′ (H-2′: 4.1%, H-3′: 0.9%, H-5′$_\alpha$: 3.0%), H-2′ (H-1′: 5.0%, H-3′: 6.2%), H-3′ (H-1′: 0.9%, H-2′: 4.6%, H-4′: 4.2%); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 70.82, 159.23 (d, $J = 211.5$), 157.06 (d, $J = 20.6$), 151.43 (d, $J = 18.9$) 138.93 (d, $J = 3.3$), 117.35 (d, $J = 4.1$), 63.49, 57.22, 56.25, 54.14, 38.36, 28.94, 20.83; HREIMS calcd for $C_{13}H_{14}FN_5O_3$ 307.1081. Found: 307.1078.



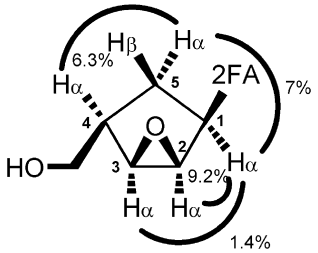
19

5.1.10. 9-[(1′*R*,2′*S*,4′*S*)-2′-Hydroxy-4′-hydroxymethyl-cyclopentan-1′-yl]-9-*H*-2-fluoroadenine (11). To a solution of **19** (50 mg, 163 μmol) in THF (5 mL) was added $LiAlH_4$ (71 mg, 1.9 mmol), and the mixture was stirred for 5 h at rt. After completion of the reaction, methanol was added at 0 °C, and the mixture was filtered through Celite. he filtrate was concentrated and preparative HPLC (MeOH/H_2O, 30:70) of residue gave **11** (10 mg, 23%) as white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 8.19 (1H, s, H-8), 4.69 (1H, m, H-1′), 4.29 (1H, m, H-2′), 3.63 (2H, m, H-6′), 2.35–2.23 (3H, m, H-3′$_\alpha$, 4′, 5′$_\alpha$), 2.14 (1H, m, H-5′$_\beta$), 1.62 (1H, m, $J_{gem} = 12.8$, H-3′$_\beta$); DIFNOE: H-2′ (H-1′: 7.6%, H-3′$_\alpha$: 1.8%), H-5′$_\beta$ (H-6′; 8.1%), H-6′ (H-5′$_\beta$: 2.4%, H-3′$_\beta$: 2.3%); ^{13}C NMR ($CDCl_3$, 100 MHz) δ158.49 (d, $J = 204.1$), 157.49 (d, $J = 21.4$), 151.20 (d, $J = 20.6$), 140.73 (d, $J = 2.5$), 116.78 (d, $J = 4.1$), 77.20, 64.35, 61.36, 53.84, 46.77, 30.34; HREIMS calcd for $C_{11}H_{14}FN_5O_2$ 267.1132. Found: 267.1112.



11

5.1.11. 9-[(1′*R*,2′*R*,3′*S*,4′*R*)-2′,3′-Epoxy-4′-hydroxy-methyl-cyclopentan-1′-yl]-9-*H*-2-fluoroadenine (12). Compound **12** (16 mg, 60 μmol) was prepared from **16** (20 mg, 80 μmol) as described from **19** in 75% after preparative HPLC (H_2O/MeOH, 50:50). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.10 (1H, s, H-8), 7.79 (2H, br s, NH_2), 4.90 (1H, t, $J_{1',5'} = 8.9$, H-1′), 4.80 (1H, t, $J_{6',OH} = 5.3$, OH-6′), 3.89 (1H, d, $J_{2',3'} = 2.9$, H-2′), 3.66 (1H, d, $J_{2',3'} = 2.9$, H-3′), 3.48 (2H, dd, $J_{4',6'} = 7.3$, H-6′), 2.32 (1H, m, H-4′), 2.13 (1H, m, $J_{gem} = 12.5$, H-5′$_\alpha$), 1.15 (1H, m, H-5′$_\beta$); DIFNOE: H-1′ (H-2′: 9.2%, H-5′$_\alpha$: 7.0%), H-2′ (H-1′: 5.1%, H-3′: 3.4%), H-3′ (H-1′: 1.4%, H-2′: 3.8%), H-4′ (H-3′: 4.2%, H-5′$_\alpha$: 6.3%, H-6′: 3.0%); ^{13}C NMR($CDCl_3$, 100 MHz) 158.68 (d, $J = 204.1$), 157.60 (d, $J = 21.4$), 150.78 (d, $J = 20.6$), 138.86 (d, $J = 2.5$), 116.94 (d, $J = 4.1$), 61.00, 56.94, 56.44, 54.37, 41.26, 27.83; HREIMS calcd for $C_{11}H_{12}FN_5O_2$ 265.0975. Found: 265.0966.



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5.2. Enzyme assay

In the synthetic direction, the enzyme assay was a modification of an earlier method.[20] The enzyme was incubated with 100 mM adenosine, 5 mM DL-homocysteine and inhibitors on 0.2 mL of 10 mM potassium phosphate, pH 7.2, buffer at 30 °C for 2 min in the standard assay system. The reaction was started by the addition of 3 mL of SAH hydrolase (human: 0.43 μg, *P. falciparum*: 0.54 μg) and terminated by the addition of 20 μL of 0.67 N HCl. The reaction mixture was kept on ice until the HPLC analysis. The mixture was analyzed for SAH by a Shimadzu HPLC system. In the synthetic reaction, one unit of SAHH was defined as the amount synthesizing 1 mmol of SAH/min at 30 °C.

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References and notes

1. Keller, B. T.; Borchardt, R. T. In *Biological Methylation and Design: Experimental and Clinical Roles of S-Adenosylmethionine*; Borchardt, R. T., Creveling, C. R., Ueland, P. M., Eds.; Humana: Clifton, NJ, 1986; p 385.
2. Wholf, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521.
3. Chiang, P. K. *Pharmacol. Ther.* **1998**, *77*, 115, and references cited therein.
4. Liu, S.; Wholfe, M. S.; Borchardt, R. T. *Antiviral Res.* **1992**, *19*, 247.
5. Wolos, J. A.; Frondorf, K. A.; Babcock, G. F.; Stripp, S. A.; Bowlin, T. L. *Cell Immunol.* **1993**, *149*, 402.
6. (a) Bitonti, A. J.; Baumann, R. J.; Jarvi, E. T.; McCarthy, J. R.; McCann, P. P. *Biochem. Pharmacol.* **1990**, *40*, 601; (b) Henderson, D. M.; Hanson, S.; Allen, T.; Wilson, K.;

Coulter-Karis, D. E., ; Grrenberg, M. L.; Hershfield, M. S.; Ulman, B. *Mol. Biochem. Parasitol.* **1992**, *53*, 169; (c) Wolos, J. A.; Frondorf, K. A.; Esser, R. E. *J. Immunol.* **1993**, *151*, 526.
7. (a) Crimmins, M. T. *Tetrahedron* **1998**, *54*, 9229, and references cited therein; (b) Kitade, Y.; Kozaki, A.; Miwa, T.; Nakanishi, M. *Tetrahedron* **2002**, *58*, 1271; (c) Takagi, C.; Sukeda, M.; Kim, H.-S.; Wataya, Y.; Yabe, S.; Kitade, Y.; Matsuda, A.; Shuto, S. *Org. Biomol. Chem.* **2005**, *3*, 1245.
8. Montgomery, J. A. *Cancer Res.* **1982**, *42*, 3911.
9. Montgomery, J. A. *Med. Res. Rev.* **1982**, *2*, 271.
10. Obara, T.; Shuto, S.; Saito, Y.; Snoeck, R.; Andarei, G.; Balzarini, J.; De Clercp, E.; Matsuda, A. *J. Med. Chem.* **1996**, *39*, 3847.
11. Kitade, Y.; Kojima, H.; Zulfiqur, F.; Kim, H.-S.; Wataya, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3963.
12. Tanaka, N.; Nakanishi, M.; Kusakabe, Y.; Shirakawa, K.; Yabe, S.; Ito, Y.; Kitade, Y.; Nakamura, K. T. *J. Mol. Biol.* **2004**, *343*, 1007.
13. Nakanishi, M.; Yabe, S.; Tanaka, N.; Ito, Y.; Nakamura, K. T.; Kitade, Y. *Mol. Biochem. Parasitol.* **2005**, *143*, 146–151.
14. MacKeith, R. A.; McCague, R.; Olivo, H. F.; Palmer, C. F.; Roberts, S. M. *J. Chem. Soc., Perkin Trans. 1* **1993**, *3*, 313.
15. Trost, B. M.; Kuo, G. –H.; Benneche, T. J. *J. Am. Chem. Soc.* **1988**, *110*, 621.
16. Poli, G. *Tetrahedron Lett.* **1989**, *30*, 7385.
17. Camps, F.; Coll, J.; Messguer, A.; Pujol, F. *J. Org. Chem.* **1982**, *47*, 5402.
18. Rickborn, B.; Lamke, W. E. *J. Org. Chem.* **1967**, *32*, 537.
19. Rickborn, B.; Quartucci, J. *J. Org. Chem.* **1984**, *29*, 3185.
20. Gomi, T.; Date, T.; Osawa, H.; Fujioka, M.; Aksamit, R. R., ; Backlund, P. S., Jr.; Cantoni, G. L. *J. Biol. Chem.* **1989**, *264*, 16138.
21. Shuto, S.; Obara, T.; Toriya, M.; Hosoya, M.; Snoeck, R.; Andarei, J. B.; De Clercp, E. *J. Med. Chem.* **1992**, *35*, 325.