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Synthesis of 3′,4′-epoxynoraristeromycin analogs for molecular labeling probe of *S*-adenosyl-L-homocysteine hydrolase

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ABSTRACT

3′,4′-Epoxynoraristeromycin analogs were designed and synthesized. Their affinities with human and *Plasmodium falciparum S*-adenosyl-L-homo-cysteine hydrolase were investigated.

1. Introduction

The cellular enzyme *S*-adenosyl-L-homocysteine (SAH) hydrolase (EC 3.3.1.1) has emerged as a target enzyme for the molecular design of anti-viral agents.[1] Inhibition of SAH hydrolase results in cellular accumulation of SAH, which is a potent product inhibitor of *S*-adenosyl-L-methionine-dependent biological methylation.[2–4] We previously reported that compounds resulting from the introduction of a small molecule to the 2-position of noraristeromycin (**1**), such as 2-fluoronoraristeromycin (**2**), increased the selective inhibition of *Plasmodium falciparum* SAH hydrolase (PfSAHH) compared with human SAHH (HsSAHH).[5] PfSAHH has additional space near the 2-position of the adenine-ring in the substrate binding pocket compared with HsSAHH.[6] Mutagenic analysis of the amino acid residue forming the additional space confirmed that inhibitor selectivity is due to the difference of only one amino acid residue.[7]

In the meantime, affinity-labeling probes were prepared for the elucidation of the molecular mechanism of SAHHs. We have reported that 9-[(2′*S*,3′*S*)-3′-formyl-2′,3′-dihydroxypropyl]adenine[8] (**FDHPA**, Fig. 1) showed ability as an affinity-labeling probe and exhibited type II inhibition against SAHH.[8] Although the potency of the compound was moderate, it was not easy to handle because of the instability of the aldehyde functional group at the 4′-position. Therefore, it is necessary to develop a useful, stable, and potent molecular probe possessing inhibitory activities against both HsSAHH and PfSAHH.

To overcome the problems, we designed novel noraristeromycin analogs possessing epoxy functional groups at the 3′,4′-positions as potential affinity-labeling probes, for the elucidation of the catalytic site of SAHH and their affinities with both SAHHs. In this paper, we described the synthesis of the title compounds and their affinities with both HsSAHH and PfSAHH.

2. Chemistry

We selected noraristeromycin (**1**) and 2-fluoronoraristeromycin (**2**) as starting materials, both of which were previously

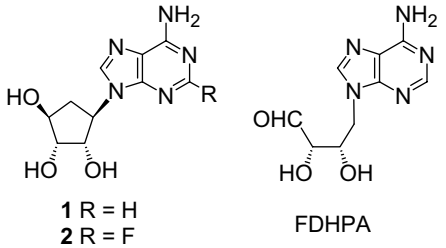

Figure 1. Structure of 2-modified noraristeromycin analogs and FDHA.

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reported.[5,9–12] Protection of the 2′ and 3′ hydroxyl groups of **1** and **2** by isopropylidene group[13] was performed by orthoformate and *p*-toluenesulfonic acid[14] to afford compounds **3** and **4**. The following mesylation by methanesulfonic chloride and bases yielded compounds **5** and **6**, which were deprotected successively to afford 4-*O*-mesyl precursors **7** and **8** via deprotection of isopropylidene group. Treatment with potassium *tert*-butoxide in DMF yielded 3′,4′-*epoxy* compounds **9** and **10** (Scheme 1). Here, the upper side of the cyclopentan-ring was defined as the α side and the lower side as the β side. We refer to these epoxy compounds **9** and **10** as α-epoxynoraristeromycin and α-epoxy-2-fluoronoraristeromycin.

In the meantime, β-epoxynoraristeromycin was synthesized to confirm and compare with the structures of these isomers. The 2′,4′-hydroxy groups of **1** were selectively protected by the TBDMS group[15] to give compound **11**. The introduction of a methanesulfonyl group (for **12**) and deprotection of the TBDMS group afforded the 3-*O*-mesyl precursor **13**. Similarly, treatment with potassium *tert*-butoxide yielded the desired β-epoxynoraristeromycin **14** (Scheme 2).

The stereochemistries of 3′,4′-α- and β-epoxynoraristeromycins were determined by NOEs of the corresponding protons. The major NOE correlations are illustrated in Figure 2. The correlation between H-3′β and H-5′β of **9** indicated that the epoxy-ring was located at the lower side (α side) of the cyclopentan-ring. Similarly, the correlations between H-1′α,H-3′α and H-4α of **14** supported the configuration of β-epoxy compound.

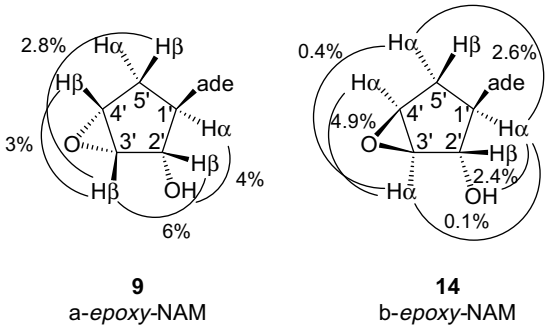
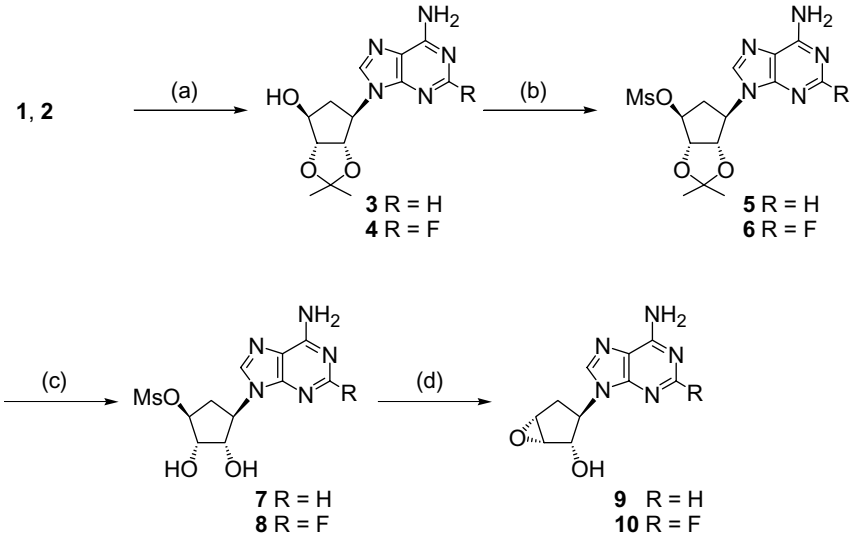
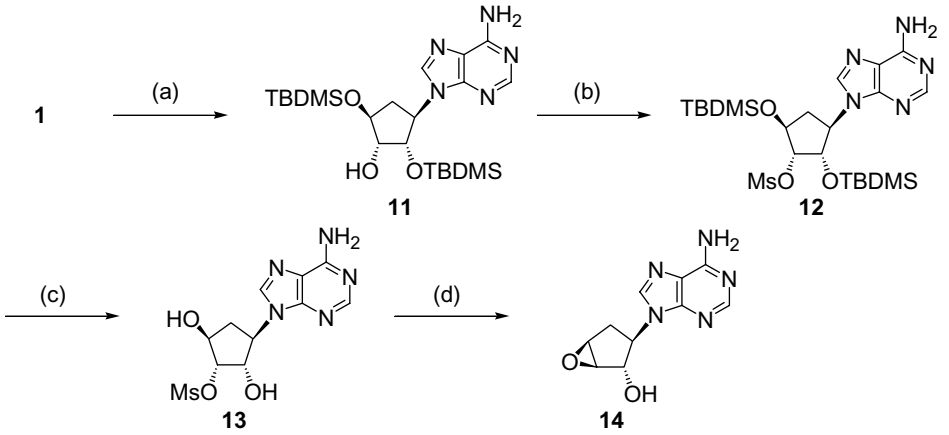


Figure 2. NOE correlations of α- and β-epoxynoraristeromycins (epoxy-NAM).



Scheme 1. Reagents and conditions: (a) $HC(OEt)_3$, *p*-toluenesulfonic acid monohydrate, acetone, 1 h, rt 88% (for **3**) and 97% (for **4**); (b) methanesulfonyl chloride, DMAP, Et_3N, CH_2Cl_2, 0 °C, 0.5 h, 79% (for **5**) and 97 % (for **6**); (c) TFA/H_2O (1:1), rt, 2 h, 99% (for **7**) and 95% (for **8**); (d) *t*-BuOK, DMF, rt 0.5 h, 79% (for **9**) and 42% (for **10**).



Scheme 2. Reagents and conditions: (a) TBDMSCl, imidazole, DMF, rt 5 h, 33%; (b) methanesulfonyl chloride, DMAP, Et_3N, CH_2Cl_2, 0 °C, 0.5 h, 86%; (c) Bu_4NF, THF, rt, 0.5 h, 86%; (d) *t*-BuOK, DMF, rt 0.5 h, 42%.

3. Biological assay

The values of K_i and k_{inact},[16] which are useful for evaluating the affinity and reactivity of an affinity-labeling reagent, are summarized in Table 1. The values of epoxy compound **9** against HsSAHH were weaker than those of previously reported FDPHA. In addition, compound **9** did not show any k_{inact} against PfSAHH. It is noteworthy that 3′,4′-epoxy-2-fluoronoraristeromycin **10** had moderate k_{inact} against HsSAHH and PfSAHH.

4. Discussion

Although the inhibitory activity of **10** against HsSAHH was equivalent to previously reported FDPHA, it showed sufficient k_{inact} against both HsSAHH and PfSAHH to act as a molecular probe, which is an advantage over FDPHA and **9**. Previously, we had reported that a small functional group (Fluorine) increased selectivity of inhibition against PfAHH compared with HsSAHH. The selective index (k_{inact} of HsSAHH/k_{inact} of PfSAHH) of **10** was 1.3, which was not an excellent value, but supported our previous report.

In this paper, we briefly described the synthesis of 3′,4′-epoxynoraristeromycin analogs and their potencies as molecular probes. Generally, epoxy functional group is more stable than aldehyde one, we can easily handle and preserve for longer time. These epoxy compounds will be used as useful and stable molecular affinity-labeling probes to clarify the molecular mechanisms of both SAHHs aiming at the molecular design of antimalarial drugs.

5. Experimental

5.1. General procedure

Melting points were recorded on a Yanaco Micro Melting Point Apparatus. Elemental analyses were carried out at the microanalytical laboratory of Gifu Pharmaceutical University. ^{1}H and ^{13}C spectra were recorded at 400 MHz on a JEOL JNM α400 (operated at 400 and 100 MHz, respectively) using $CDCl_3$ with TMS as internal standard or DMSO-d_6. The spin multiplicities are indicated by the symbols s (singlet), d (doublet), dd (doublet doublet), t (triplet), m (multiplet), and br (broad). Coupling constants (J) are expressed in Hz. The Differential Nuclear Overhauser Effect (DIFNOE) data were described; the irradiated proton (the correlated proton: % of enhancement value). Mass spectra (MS and HRMS) were recorded at 70 eV on JEOL JMS-D300 spectrometer and Shimadzu QP 1000A. Reactions were monitored by thin-layer chromatography (TLC) using MERCK silica gel 60F_{254}. Column chromatography was carried out on silica gel (Wako gel C-300).

5.1.1. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-4′-Hydroxy-2′,3′-*O*-isopropylidene-cyclopentan-1′-yl]-9-*H*-adenine (3)

To a mixture of noraristeromycin (**1**) (40.8 mg, 0.162 mmol) and *p*-toluenesulfonic acid monohydrate (37 mg) in acetone (1.4 mL) was added ethyl orthoformate (0.15 mL). The reaction mixture was stirred at room temperature for 2 h, and evaporated under reduced pressure. The resulting residue was purified by silica-gel column chromatography eluting with chloroform/methanol (30:1). Compound **3** was obtained (41.4 mg, 88%) as a white solid: mp 143–144 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.30 (1H, s, H-2), 7.87 (1H, s, H-8), 6.52 (1H, br s, OH-4′), 5.79 (2H, br s, NH_2), 4.92 (1H, d, J = 5.2 Hz, H-2′), 4.80 (1H, dt, J = 2.0 and 10.8 Hz, H-1′) 4.76 (1H, d, J = 5.2 Hz, H-3′), 4.44 (1H, d, J = 5.6 Hz, H-4′), 2.99 (1H, m, H-5′β), 2.14 (1H, m, H-5′α), 1.57,1.30 (6H, 2s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.97, 152.27, 148.50, 140.77, 119.79, 111.48, 87.59, 86.31, 75.71, 63.20, 37.91, 26.88, 24.36; HREIMS calcd for $C_{13}H_{17}N_5O_3$ 291.1331; found 291.1328.

5.1.2. 9-[(1′*R*,2′*S*,3′ *S*,4′*S*)-2′,3′-*O*-Isopropylidene-4′-methanesulfonyloxy-cyclopentan-1′-yl]-9-*H*-adenine (5)

To a mixture of compound **4** (41.4 mg, 0.142 mmol), 4-dimethylaminopyridine (17.3 mg, 0.142 mmol) and triethylamine (0.121 mL) in dichloromethane (4 mL) was added methanesulfonyl chloride (0.045 mL, 0.454 mmol). The reaction mixture was stirred at room temperature for 1 h. Chloroform (5 mL) was added to the reaction mixture and washed with saturated $NaHCO_3$. The organic layer was dried on Na_2SO_4, filtrated, and evaporated. The residue was purified by silica-gel column chromatography eluting with chloroform/methanol (30:1). Compound **5** was obtained (41.2 mg, 79%) as a white solid: mp 193–196 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.35 (1H, s, H-2), 7.85 (1H, s, H-8), 5.79 (2H, br s, NH_2), 5.15 (1H, dd, J = 2.4 and 6.4 Hz, H-2′), 5.11 (1H, m, H-4′), 4.97 (1H, dd, J = 2.4 and 6.4 Hz, H-3′), 4.91 (1H, dt, J = 2.8 and 6.8 Hz, H-1′), 3.03 (3H, s, CH_3), 2.82 (2H, m, H-5′α and β), 1.53, 1.31(6H, 2s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.21, 152.31, 142.76, 139.63, 113.32, 83.96, 83.47, 83.35, 59.89, 38.51, 35.75, 29.66, 26.67, 24.45; HREIMS calcd for $C_{14}H_{19}SN_5O_5$ 369.1107; found 369.1100.

5.1.3. 9-[(1′*R*,2′*S*,3′*S*,4′*S*)-2′,3′-Dihydroxy-4′-methanesulfonyloxy-cyclopentan-1′-yl]-9-*H*-adenine (7)

Compound **5** (41.2 mg, 0.115 mmol) was treated with TFA-H_2O (1:1, 3.2 mL) at room temperature for 2 h. The reaction mixture was evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform/methanol (10:1). Compound **7** was obtained (37.6 mg, 99%) as a white solid: mp 186 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.23 (1H, s, H-2), 8.18 (1H, s, H-8), 7.49 (2H, br s, NH_2), 4.79 (1H, m, H-4′), 4.72 (1H, m, H-3′), 4.46 (1H, m, H-2′), 4.12 (1H, m, H-1′), 3.25 (3H, s, CH_3), 2.77 (1H, m, H-5′β), 2.30 (1H, m, H-5′α); ^{13}C NMR (DMSO-d_6, 100 MHz) δ154.80, 150.62, 149.41, 140.89, 119.22, 82.85, 73.94, 73.33, 58.03, 37.81, 32.81; Anal. Calcd for $C_{11}H_{15}N_5O_5S$: C, 40.12; H, 4.59; N, 21.27; found: C, 40.12; H, 4.59; N, 21.27.

5.1.4. 9-[(1′*R*,2′*S*,3′*S*,4′*R*)-3′,4′-Epoxy-2′-hydroxy-cyclopentan-1′-yl]-9-*H*-adenine (9)

To a solution of compound **6** (17.1 mg, 0.052 mmol) in DMF (1.3 mL) was added potassium *tert*-butoxide (29 mg, 0.258 mmol). The reaction mixture was stirred at room temperature for 0.5 h, and evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform/methanol (10:1). Compound **7** was obtained (9.6 mg, 79%) as a white solid: mp 180 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.14 (1H, s, H-2), 8.10 (1H, s, H-8), 7.20 (2H, s, NH_2), 5.52 (1H, d, $J_{OH,2'}$ = 5.9 Hz,OH-2′), 4.76 (1H, t, $J_{1',2'}$ = 7.1 Hz, H-2′), 4.26 (1H, q, J = 8.8 Hz, J = 8.2 Hz, J = 8.0 Hz, H-1′), 3.63 (1H, d, $J_{3',4'}$ = 3.0 Hz, H-4′), 3.57 (1H, m, H-3′), 2.49 (1H, m, H-5′β), 2.45 (1H, m, H-5′α); DIFNOE: H-1′ (OH-2′: 4%, H-2′: 0.4%), H-3′ (H-2′: 6.0%, OH-2′: 0.7%, H-4′: 3.0%, H-5β′: 0.1%); ^{13}C NMR (DMSO-d_6, 100 MHz) δ156.08, 152.05, 147.13, 141.02, 119.52, 74.01, 56.68, 56.56, 52.22, 30.71; HREIMS calcd for $C_{10}H_{11}O_2N_5$ 233.0913;; found 233.0919.

Table 1
K_i values and k_{inact} values of epoxynoraristeromycins against HsSAHH and PfSAHH

Compound	HsSAHH		PfSAHH	
	K_i (μM)	k_{inact} (min)	K_i (μM)	k_{inact} (min)
9	12.4	0.552	[a]ND	[a]ND
10	20.3	0.133	14.3	0.099
FDPHA	[b]8.8	[b]0.09	—	—

[a] ND: no inhibitory activity.
[b] Ref. 8.

5.1.5. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-4′-Hydroxy-2′,3′-*O*-isopropylidene-cyclopentan-1′-yl]-9-*H*-2-fluoroadenine (4)

To a mixture of 2-fluoronoraristeromycin (**2**) (17 mg, 63 μmol) and *p*-toluenesulfonic acid monohydrate (15.0 mg) in acetone (0.53 mL) was added ethyl orthoformate (0.057 mL). The reaction mixture was stirred at room temperature for 1 h, evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform/methanol (30:1). Compound **10** was obtained (19 mg, 97%) as a white solid; mp 160 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94 (1H, s, H-8), 5.90 (2H, s, NH_2), 4.95 (1H, d, *J* = 4.8 Hz, OH-4′), 4.85 (1H, d, *J* = 9.2 Hz, H-1′), 4.76 (1H, d, *J* = 5.2 Hz, H-2′), 4.46 (1H, d, *J* = 5.2 Hz, H-4′), 4.39 (1H, d, *J* = 7.6 Hz, H-3′), 2.92 (1H, m, H-5′β), 2.18 (1H, d, *J* = 15.6 Hz, H-5′α), 1.52, 1.32 (6H, 2s, CH_3); ^{13}C NMR (DMSO, 100 MHz) δ 158.62 (1C, d, *J* = 202.7 Hz), 157.67 (1C, d, *J* = 20.8 Hz), 150.08 (1C, d, *J* = 19.9 Hz), 140.55 (1C, d, *J* = 2.9 Hz), 117.21 (1C, d, *J* = 3.9 Hz), 86.37, 84.48, 74.79, 59.87, 48.68, 37.12, 26.68, 24.39; HREIMS calcd for $C_{13}H_{16}FN_5O_3$ 309.1237; found 309.1228.

5.1.6. 9-[(1′*R*,2′*S*,3′*S*,4′*S*)-2′,3′-*O*-Isopropylidene-4′-methanesulfonyloxy-cyclopentan-1′-yl]-9-*H*-2-fluoroadenine (6)

To a mixture of compound **4** (19 mg, 0.061 mmol), 4-dimethylaminopyridine (8 mg, 0.061 mmol), and triethylamine (0.051 mL) in dichloromethane (2 mL) was added methanesulfonyl chloride (20 μL, 0.244 mmol). The reaction mixture was stirred at room temperature for 0.5 h. Chloroform (5 mL) was added to the reaction mixture and washed with saturated $NaHCO_3$ solution (5 mL). The organic layer was dried on Na_2SO_4, filtrated, and evaporated. The residue was purified by silica-gel column chromatography eluting with chloroform/methanol (30:1). Compound **6** was obtained (23 mg, 97%) as a white solid: mp 253–254 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (1H, s, H-8), 6.67 (2H, d, *J* = 8.0 Hz, NH_2), 5.08 (1H, dd, *J* = 2.4 and 6.4 Hz, H-1′), 5.04 (1H, dt, *J* = 2.4 and 6.0 Hz, H-2′), 4.94 (1H, d, *J* = 6.0 Hz, H-4′), 4.81 (1H, dt, *J* = 2.8 and 6.8 Hz, H-3′), 3.01 (3H, s, Mesyl), 2.80 (1H, m, H-5′β), 2.64 (1H, m, H-5′α), 1.49, 1.27 (6H, 2s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.92 (1C, d, *J* = 210.5 Hz), 157.26 (1C, d, *J* = 19.9 Hz), 151.03 (1C, d, *J* = 19.4 Hz), 139.40 (1C, d, *J* = 2.9 Hz), 117.58 (1C, d, *J* = 3.9 Hz), 83.83, 82.87, 59.76, 50.32, 38.32, 37.80, 35.86, 26.52, 24.33; HREIMS calcd for $C_{14}H_{18}FN_5O_5S$ 387.1013; found 387.1019.

5.1.7. 9-[(1′*R*,2′*S*,3′*S*,4′*S*)-2′,3′-Dihydroxy-4′-methanesulfonyloxy-cyclopentan-1′-yl]-9-*H*-2-fluoroadenine (8)

Compound **6** (15 mg, 0.039 mmol) was treated with TFA/H_2O (1:1, 4 mL) at room temperature for 2 h. The reaction mixture was evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform/methanol (10:1). Compound **8** was obtained (10 mg, 95%) as a white solid: mp 212 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.18 (1H, s, H-8), 7.78 (2H, s, NH_2), 5.51 (1H, d, *J* = 4.4 Hz, OH-2′), 5.34 (1H, d, *J* = 6.4 Hz, OH-3′), 4.77 (1H, t, *J* = 8.0 Hz, H-1′), 4.63 (1H, q, *J* = 8.8 Hz, H-2′), 4.41 (1H, q, *J* = 6.0 Hz, H-4′), 4.09 (1H, q, *J* = 4.8 Hz, H-3′), 3.25 (3H, s, Mesyl), 2.76 (1H, m, H-5′β), 2.20 (1H, m, H-5′α); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.43 (1C, d, *J* = 185.2 Hz), 157.52 (1C, d, *J* = 37.8 Hz), 150.82 (1C, d, *J* = 20.4 Hz), 140.48 (1C, d, *J* = 2.4 Hz), 117.63 (1C, d, *J* = 4.3 Hz), 82.72, 73.88, 73.31, 57.77, 37.80, 32.76; HRFABMS (positive mode) calcd for $C_{11}H_{15}FN_5O_5S$ 348.0778, found 348.0787; Anal. calcd for $C_{11}H_{14}FN_5O_5S \cdot 1/2\ H_2O$: C, 37.08; H, 4.24; N, 19.65; found: C, 37.29; H, 4.06; N, 19.51.

5.1.8. 9-[(1′*R*,2′*S*,3′*R*,5′R)-3′,4′-Epoxy-2′-hydroxy-cyclopentan-1′-yl]-9-*H*-2-fluoroadenine (10)

To a solution of compound **8**(17 mg, 48 μmol) in DMF (1.8 mL) was added potassium *tert*-butoxide (27 mg, 0.24 mmol). The reaction mixture was stirred at room temperature for 0.5 h, evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform/methanol (10:1). Compound **10** was obtained (5 mg, 42%) as a white solid: mp 230°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.16 (1H, s, H-8), 7.77 (2H, s, NH_2), 5.52 (1H, d, *J* = 6.0 Hz, OH-2′), 4.67 (1H, t, *J* = 7.2 Hz, H-2′), 4.20 (1H, q, *J* = 8.4 Hz, H-3′), 3.62 (1H, t, *J* = 2.8 Hz, H-4′), 3.57 (1H, dd, *J* = 1.6 and 3.2 Hz, H-1′), 2.38 (2H, m, H-5′α and β); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.31 (1C, d, *J* = 213.8 Hz), 157.62 (1C, d, *J* = 21.4 Hz), 150.87 (1C, d, *J* = 20.4 Hz), 141.36 (1C, d, *J* = 2.9 Hz), 117.80 (1C, d, *J* = 4.4 Hz), 74.10, 56.57, 56.46, 52.12, 30.68; HRFABMS (positive mode) calcd for $C_{10}H_{11}FN_5O_2$ 252.0897; found 252.0893.

5.1.9. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,4′-O-Di-(tert-butyldimethylsilyl)-3′-hydroxy-cyclopentan-1′-yl]-9-*H*-adenine (11)

To a mixture of noraristeromycin (**3**) (40.8 mg, 0.162 mmol) and imidazole (72 mg, 1.03 mmol) in DMF (2.3 mL) was added TBDMSCl (79 mg, 0.515 mmol). The reaction mixture was stirred at room temperature for 2 h. EtOAc (5 mL) was added to the reaction mixture and washed with saturated $NaHCO_3$ solution. The organic layer was dried on Na_2SO_4, filtrated, and evaporated. The residue was purified by silica-gel column chromatography eluting with EtOAc. Compound **11** was obtained (38 mg, 33%) solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.99 (2H, s, H-2 and H-8), 7.06 (2H, s, NH_2), 4.77 (1H, d, *J* = 4.0 Hz, OH-3′), 4.69 (1H, q, *J* = 8.4 Hz, H-1′), 4.49 (1H, t, *J* = 4.8 Hz, H-2′), 3.96 (1H, s, H-4′), 3.62 (1H, s, H-3′), 2.49 (1H, m, H-5′β), 1.88 (1H, m, H-5′α), 0.81 (9H, s, $3CH_3$), 0.54 (9H, s, $3CH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 155.99, 152.16, 149.79, 139.51, 119.00, 77.61, 76.78, 75.47, 57.89, 36.50, 25.79, 25.64, 25.43, 17.69; HRFABMS (positive mode) calcd for $C_{22}H_{42}N_5O_3\ Si_2$ 480.2826; found 480.2836.

5.1.10. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,4′-*O*-Di-(*tert*-butyldimethylsilyl)-3′-methanesulfonyloxy-cyclopentan-1′-yl]-9-*H*-adenine (12)

To a mixuture of Compound **11** (45 mg, 94 μmol), 4-dimethylaminopyridine (12 mg, 94 μmol), and triethylamine (0.08 mL) in dichloromethane (2.5 mL) was added methanesulfonyl chloride (0.02 mL, 0.188 mmol). The reaction mixture was stirred at room temperature for 0.5 h. Chloroform (5 mL) was added to the reaction mixture and washed with saturated Na_2CO_3 carbonate solution (5 mL). The organic layer was dried on sodium sulfate, filtrated, and evaporated. The residue was purified by silica-gel column chromatography eluting with EtOAc/hexane (4:1). Compound **12** was obtained (45 mg, 86%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.19 (1H, s, H-2), 7.81 (1H, s, H-8), 5.97 (2H, s, NH_2), 4.84 (1H, m, H-1′), 4.73 (1H, m, H-2′), 4.61 (1H, s, H-4′), 4.28 (1h, d, *J* = 5.2 Hz, H-3′), 2.97 (3H, s, CH_3), 2.77 (1H, m, H-5′β), 1.91 (1H, dd, *J* = 5.2 and 14.6 Hz, H-5′α), 0.810 (9H, s, $3CH_3$), 0.572 (9H, s, $3CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.66, 152.93, 150.36, 139.13, 119.66, 85.25, 72.89, 57.79, 38.67, 37.64, 29.63, 25.75, 25.62, 25.42, 17.83; HRFABMS (positive mode) calcd for $C_{23}H_{44}N_5O_5\ SSi_2$ 558.2602; found 558.2615.

5.1.11. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,4′-Dihydroxy-3′-methanesulfonyloxy-cyclopentan-1′-yl]-9-*H*-adenine (13)

To a solution of compound **12** (40 mg, 0.0717 mmol) in THF (1 mL) was added Bu_4NF (0.06 mL), and the reaction mixture was stirred at room temperature for 0.5 h. The reaction mixture was evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform/methanol (10:1). Compound **13** was obtained (14 mg, 60%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (1H, s, H-2), 8.13 (1H, s, H-8), 7.26 (2H, s, NH_2), 5.89 (1H, d, *J* = 5.2 Hz, H-1′), 5.72 (1H, d, *J* = 5.6 Hz, H-2′), 4.73-4.65 (3H, m, H-4′, OH-2′ and OH-4′), 4.17 (1H, s, H-3′), 3.23 (3H, s, CH_3), 2.63 (1H, m, H-5′β), 2.01 (1H, m, H-5′α); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.10, 152.13, 149.27, 140.13, 119.28,

86.03, 72.75, 71.45, 58.00, 38.19, 35.41; HRFABMS (positive mode) calcd for $C_{11}H_{16}N_5O_5S$ 330.0872; found 330.0865.

5.1.12. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-3′,4′-Epoxy-2′-hydroxy-cyclopentan-1′-yl]-9-*H*-adenine (14)

To a solution of compound **13**(13 mg, 0.04 mmol) in DMF (1.4 mL) was added potassium *tert*-butoxide (22 mg, 0.198 mmol). The reaction mixture was stirred at room temperature for 0.5 h, evaporated under reduced pressure, and purified by silica-gel column chromatography eluting with chloroform/methanol (10:1). Compound **14** was obtained (7 mg, 74%) as a white solid: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.13 (1H, s, H-2), 8.01 (1H, s, H-8), 7.19 (2H, s, NH), 5.86 (1H, s, OH-2′), 4.99 (1H, d, J = 9.2 Hz, H-1′), 4.18 (1H, s, H-2′), 3.78 (1H, s, H-3′), 3.54 (1H, d, J = 2.4 Hz, H-4′), 2.61 (1H, dd, J = 10 Hz, H-5′β), 2.21 (1H, d, J_{gem} = 15.6 Hz, H-5′α); DIFNOE: H-3′ (H-1′: 0.1%, H-5′α: 0.4%, H-4′: 4.9%), H-1′ (OH-2′: 2.4%, H-5′α: 2.6%); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 155.92, 152.30, 149.36, 138.57, 118.31, 75.83, 59.10, 58.81, 56.92, 32.86; HREIMS calcd for $C_{10}H_{11}N_5O_2$ 233.0913; found 233.0917.

5.2. Enzyme assay

In the synthetic direction, the enzyme assay was a modification of an earlier method.[17] 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 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. The data were analyzed by a Kitz and Wilson plot, fitting to the following equation: $k_{app} = k_{inact}$ [I]/(K_i + [I]) in this equation k_{app}, k_{inact}, K_i, and I mean pseudo-first-order rate of inactivation, maximum rate of inactivation, inhibition constant, and concentration of compound, respectively. The value of k_{app} was determined from a plot of the residual activity versus incubation time. The values of K_i and k_{inact} were obtained using a curve-fitting program, CurveExpert.

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