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Synthesis of base-modified noraristeromycin derivatives and their inhibitory activity against human and *Plasmodium falciparum* recombinant *S*-adenosyl-L-homocysteine hydrolase

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Abstract—Noraristeromycin derivatives possessing 2- or 8-position modified adenine or 8-aza-7-deazaadenine were synthesized, and their inhibitory activity against human and *Plasmodium falciparum* (*P. falciparum*) recombinant SAH hydrolases was investigated. Among these noraristeromycin derivatives, 2-aminonoraristeromycin selectively showed inhibitory activity against *P. falciparum* recombinant SAH hydrolase. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

Carbocyclic nucleosides are a group of compounds structurally analogous to natural and synthetic nucleosides in which the furanose oxygen has been replaced by a methylene group. This replacement changes the furanose ring into a cyclopentane. Conformationally, the expected similarity in bond lengths and bond angles of the tetrahydrofuran and cyclopentane rings allows these analogues to behave as substrates or inhibitors of the enzymes in living cells. As a result of this similarity, many of these compounds are endowed with an interesting range of biological activities, such as antiviral,[1] antitumor,[1] antiparasitic,[2] antiarthritic[3] and immunosuppressive[3,4] effects. To our knowledge, the majority of carbocyclic nucleosides are of synthetic origin but nature has provided two of the most active compounds, neplanocin A (**1**) and aristeromycin (**2**) (Fig. 1). These compounds (**1** and **2**) are known as *S*-adenosyl-L-homocysteine (SAH) hydrolase (EC 3.3.1.1) inhibitors.

SAH is formed after the donation of the methyl group of *S*-adenosyl-L-methionine (SAM) to a methyl acceptor and is hydrolyzed to adenosine and homocysteine by SAH hydrolase physiologically. Therefore, inhibition of SAH hydrolase results in cellular accumulation of SAH, which is a potent feedback inhibitor of SAM-dependent biological methylation such as the cap-structure at the 5′-end of eukaryotic mRNA.[5,6] The eukaryotic mRNA must possess a methylated 5′-cap structure for stability against phosphatases and ribonucleases, for proper binding to ribosomes, and for the promotion of splicing. An uncapped mRNA, therefore, is much less likely to be translated into its respective protein. So SAH hydrolase has emerged as a target enzyme for the molecular design of the above chemotherapeutic agents.[4,5,7] However, when neplanocin A (**1**) and aristeromycin (**2**) act as a substrate for adenosine kinase, they show cytotoxicity. In order to overcome this disadvantage for the development of chemotherapeutic agents, chemical modifications of carbocyclic nucleosides have been carried out. One of them is compound **3** which lacks

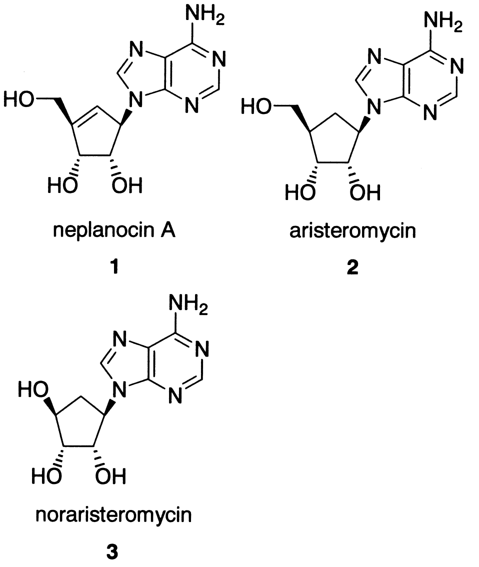

Figure 1. Structures of carbocyclic nucleosides.

Keywords: enzyme inhibitors; antivirals; nucleosides; hydrolysis; antiparasitics.

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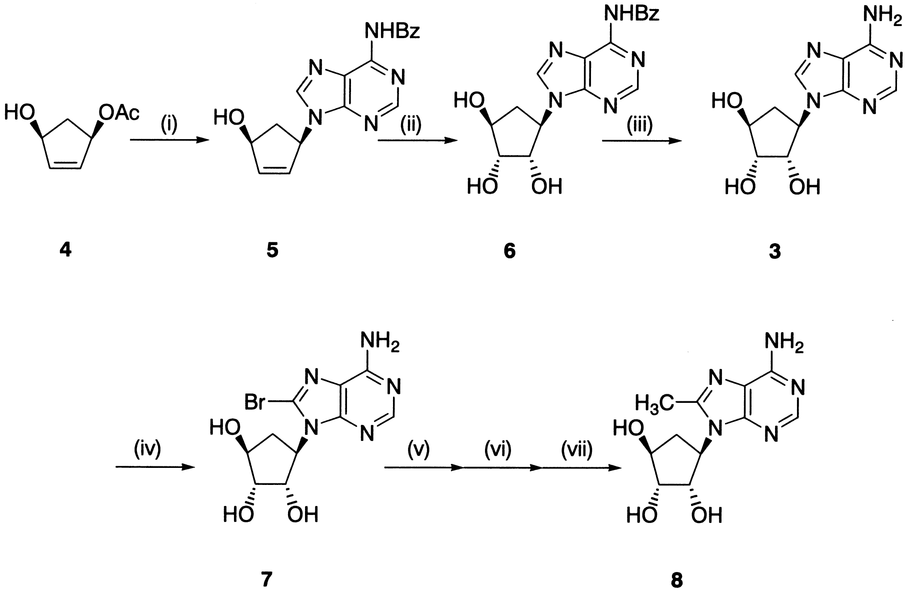
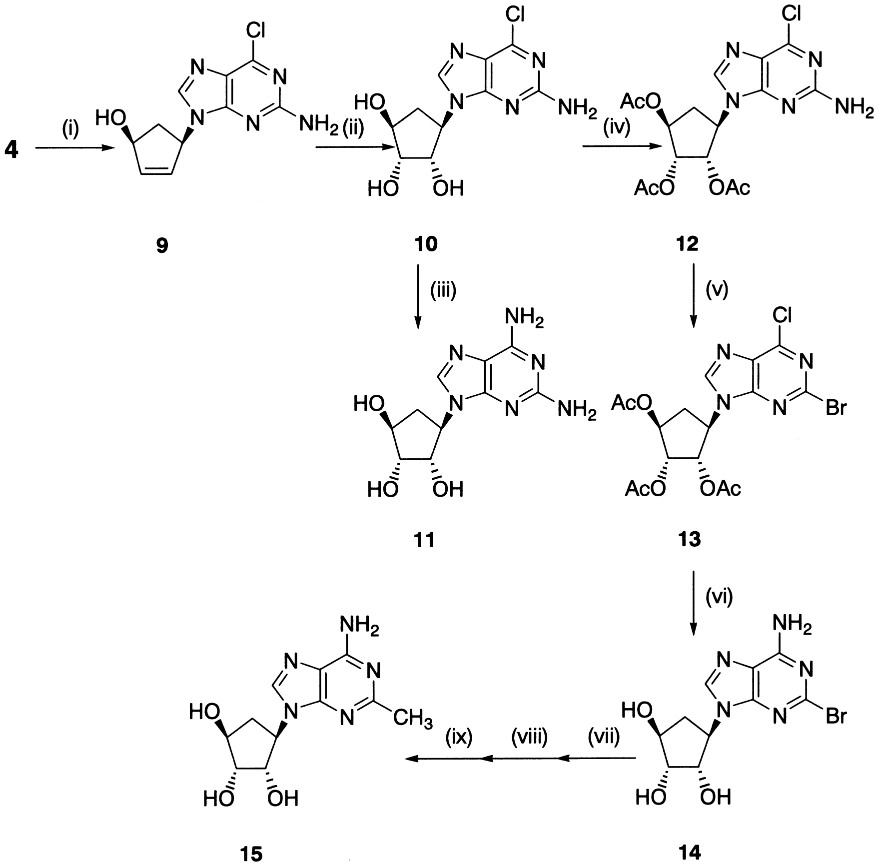
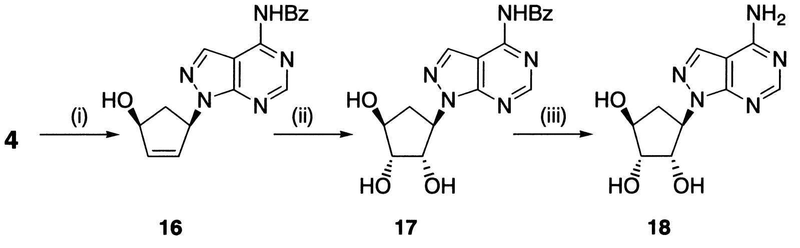


Scheme 1. *Reagents and conditions*: (i) N^6-benzoyladenine, NaH, $(Ph_3P)_4Pd$, Ph_3P, DMSO, THF, 55°C; (ii) OsO_4, NMO, THF, H_2O; (iii) NH_3–MeOH; (iv) Br_2, 0.5 M sodium acetate buffer (pH 4); (v) $(NH_4)_2SO_4$, 1,1,1,3,3,3-hexamethyldisilazane, 140°C; (vi) $Al(CH_3)_3$, $(Ph_3P)_4Pd$, THF, 90°C; (vii) 1 M ammonium chloride solution, 90°C.

the 5′-methylene unit of aristeromycin (**2**), which is called noraristeromycin (Fig. 1). In contrast to human SAH hydrolase, *Plasmodium falciparum* (*P. falciparum*) SAH hydrolase contains a 41-amino acid insert (Gly145-Lys185) inside the sequence.[8] *P. falciparum* causes malignant malaria. This difference may produce selective sensitivity against each SAH hydrolase inhibitor. Recently, we have found that IC_{50} of noraristeromycin (**3**) against human



Scheme 2. *Reagents and conditions*: (i) 2-amino-6-chloropurine, NaH, $(Ph_3P)_4Pd$, Ph_3P, DMSO, THF, 55°C; (ii) OsO_4, NMO, THF, H_2O; (iii) NH_3–MeOH, 100°C; (iv) $(CH_3CO)_2O$, pyridine, DMF; (v) $SbBr_3$, Bu^tNO_2, CH_2Br_2; (vi) NH_3–MeOH, 120°C; (vii) $(NH_4)_2SO_4$, 1,1,1,3,3,3-hexamethyldisilazane, 140°C; (viii) $Al(CH_3)_3$, $(Ph_3P)_4Pd$, THF, 90°C; (ix) 1 M ammonium chloride solution, 90°C.



Scheme 3. *Reagents and conditions*: (i) 4-benzoylaminopyrazolo[3,4-*d*]pyrimidine (N^6-benzoyl-8-aza-7-deazaadenine), NaH, $(Ph_3P)_4Pd$, Ph_3P, DMSO, THF, 55°C; (ii) OsO_4, NMO, THF, H_2O; (iii) NH_3–MeOH.

and *P. falciparum* recombinant SAH hydrolases[9] are 1.1 and 3.1 μM, respectively.

In this paper, we describe synthetic methods for the preparation of base-modified noraristeromycin derivatives and their inhibitory activities against human and *P. falciparum* recombinant SAH hydrolases.

2. Results and discussion

2.1. Chemistry

A synthetic method for the preparation of noraristeromycin[10] (**3**) and 8-modified noraristeromycin derivatives (**7** and **8**) is shown in Scheme 1. Compound **5** was prepared in 43% yield by coupling reaction of compound **4** with N^6-benzoyladenine in the presence of a palladium catalyst. Osmium oxidation of **5** gave the corresponding 2′,3′-dihydroxy derivative **6** in 70% yield. The following treatment of **6** with methanolic ammonia gave noraristeromycin (**3**) in 73% yield. Compound **3** was treated with bromine in 0.5 M sodium acetate buffer pH 4 to give 8-bromonoraristeromycin (**7**) in 73% yield. After trimethylsilyl protection of compound **7** using hexamethyldisilazane at 140°C, the following treatment with trimethylaluminium in the presence of the palladium catalyst at 90°C and subsequent deprotection by ammonium chloride solution at 90°C gave the expected 8-methylnoraristeromycin (**8**) in 55% yield.

A synthetic method for the preparation of 2-modified noraristeromycin derivatives (**11**,[11] **14** and **15**) is shown in Scheme 2. Synthesis of 2-modified noraristeromycin derivatives was carried out using compound **10**[11] as a versatile intermediate. Coupling reaction of **4** with 2-amino-6-chloropurine afforded compound **9** in 40% yield, and subsequent osmium oxidation of **9** gave the intermediate **10** in 99% yield. 2-Aminonoraristeromycin (**11**) was obtained in 62% yield by the following amination of the 6-chloro derivative **10** by methanolic ammonia. Compound **12** was obtained in 70% yield by acetylation of **10** with acetic anhydride. Treatment of **12** with *tert*-butyl nitrite in the presence of antimony(III) bromide in dibromomethane at −10°C afforded the corresponding 2-bromo derivative **13** in 68% yield. Reaction of **13** with methanolic ammonia at 120°C gave 2-bromonoraristeromycin (**14**) in 60% yield. Methylation of the 2-bromo derivative **14** was performed under the analogous conditions used for the preparation of **8** to give 2-methylnoraristeromycin (**15**) in 81% yield.

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

Compound	Human SAH hydrolase IC_{50} (μM)[a]	*P. falciparum* SAH hydrolase IC_{50} (μM)[a]	Selective index[b]
3	1.1	3.1	0.35
7	ND[c]	ND[c]	
8	590	ND[d]	
11	60	18	3.3
14	ND[c]	ND[c]	
15	430	ND[c]	
18	ND[c]	ND[c]	

[a] Values are the mean of two experiments.

[b] Selective index: human SAH hydrolase (IC_{50})/*P. falciparum* (malaria) SAH hydrolase (IC_{50}).

[c] No inhibitory activity showed at 1000 μM.

[d] Inhibitory activity at 1000 μM showed about 20%.

8-Aza-7-deazanoraristeromycin[12] (**18**) was synthesized under the analogous conditions used for the preparation of noraristeromycin (**3**) (Scheme 3). Thus, compound **16** was obtained in 50% yield by coupling reaction of compound **4** with 4-benzoylaminopyrazolo[3,4-*d*]pyrimidine (N^6-benzoyl-8-aza-7-deazaadenine) in the presence of a palladium catalyst at 55°C. Subsequent osmium oxidation gave N^6-benzoyl-8-aza-7-deazanoraristeromycin (**17**) in 59% yield. Treatment of **17** with methanolic ammonia afforded 8-aza-7-deazanoraristeromycin (**18**) in 77% yield.

The structures of these compounds (**3**, **5–18**) were supported by spectral data (^{1}H NMR, ^{13}C NMR, MS and HRMS) and microanalytical data.

2.2. Biological activity

Inhibitory activities of compounds **3**, **7**, **8**, **11**, **14**, **15** and **18** against human and *P. falciparum* recombinant SAH hydrolases[9] are summarized in Table 1. The inhibitory activity of noraristeromycin (**3**) was cited as a reference. Compounds **3**, **8**, **11** and **15** showed inhibitory activity against human recombinant SAH hydrolase with IC_{50} values of 1.1, 590, 60 and 630 μM, respectively. On the other hand, compounds **3** and **11** only showed inhibitory activity against *P. falciparum* recombinant SAH hydrolase with IC_{50} values of 3.1 and 18 μM, respectively. The selective index of compounds **3** and **11** are 0.35 and 3.3, respectively. Compound **11** showed moderate selectivity against *P. falciparum* SAH hydrolase.

2-Aminonoraristeromycin (**11**) showed moderate selectivity and the other 2-substituted noraristeromycin (**14** and **15**) did not show inhibitory activity against *P. falciparum* SAH hydrolase. Therefore, introduction of a polar group at the 2-position of noraristeromycin derivatives may account for the selectivity against *P. faluciparum* SAH hydrolase. This observation provides a clue to the development of facile chemotherapeutic agents for malaria.

3. Experimental

3.1. General

Melting points were recorded on a Yanaco Micro Medting 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 (double doublet), t (triplet), m (multiplet), br (broad). Coupling constants (J) are expressed in Hz. Mass spectra (MS and HRMS) were recorded at a 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).

3.1.1. 9-[(1′*R*,4′*S*)-4′-Hydroxy-2′-cyclopenten-1′-yl]-9-*H*-N^6-benzoyladenine (5). A mixture of N^6-benzoyladenine (870 mg, 3.6 mmol) and 60% NaH (160 mg, 4.0 mmol) in dry DMSO (5 mL) was stirred at room temperature for 0.5 h. The reaction mixture was added to a solution of tetrakis(triphenylphosphine)palladium(0) (260 mg, 0.23 mmol), triphenylphosphine (100 mg, 0.38 mmol) and (1*S*,4*R*)-*cis*-4-acetoxy-2-cyclopenten-1-ol (**4**) (516 mg, 3.6 mmol) in dry THF (5 mL) in the dark and then stirred at 55°C for 2 days. 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 evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform–methanol (70:1). Compound **5** was obtained (500 mg, yield 43%) as a solid: mp 182–183°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.22 (d, J=15.2 Hz, 1H, H-5′), 3.01 (m, 1H, H-5′), 4.88 (d, J=6.8 Hz, 1H, H-4′), 5.39 (dq, J=2.0 and 9.0 Hz, 1H, H-1′), 5.66 (br s, 1H, OH-4′), 5.85 (dd, J=2.4 and 5.6 Hz, 1H, H-2′), 6.36 (dt, 1H, J=3.6 and 5.2 Hz, H-3′), 7.48–8.01 (m, 5H, Ph), 8.05 (s, 1H, H-8), 8.72 (s, 1H, H-2), 9.07 (s, 1H, C^6–NHBz).

3.1.2. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-N^6-benzoyladenine (6). Osmium tetraoxide solution (3 mL, 15 mg/mL) was added to a solution of the compound **5** (1.00 g, 3.1 mmol) and 4-methylmorpholine *N*-oxide (0.911 g, 7.8 mmol) in THF (22.5 mL) and H_2O (2.5 mL) and stirred at room temperature for 5 h. The reaction mixture was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (30:1). Compound **6** was obtained (770 mg, yield 70%) as a solid: mp 205°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.87 (m, 1H, H-5′), 2.63 (m, 1H, H-5′), 3.79 (m, 1H, H-3′), 3.92 (m, 1H, H-4′), 4.56 (m, 1H, H-1′), 4.84 (m, 1H, H-2′), 4.92 (d, J=4.0 Hz, 1H, OH-4′), 5.07 (d, J=6.8 Hz, 1H, OH-3′), 5.20 (d, J=4.0 Hz, 1H, OH-2′), 7.53–8.05 (m, 5H, Ph), 8.52 (s, 1H, H-8), 8.71 (s, 1H, H-2); EI MS (*m/z*, relative intensity) 355 (M$^+$, 9), 216 (39), 162 (65), 136 (100), 105 (96).

3.1.3. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-adenine (noraristeromycin, 3). Compound **6** (100 mg, 0.28 mmol) was treated with methanolic ammonia (40 mL) at 120°C in a sealed tube for 12 h. The reaction mixture was evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform–methanol (10:1). Noraristeromycin (**3**) was obtained (52 mg, 73%) as a solid: mp 250°C (dec.); $[\alpha]_D^{22}=-40.7°$ (*c* 1.16, DMF); FTIR (KBr) 3341, 3229, 1663, 1493, 1192 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.81 (m, 1H, H-5′), 2.68 (m, 1H, H-5′), 3.75 (m, 1H, H-3′), 3.88 (m, 1H, H-2′), 4.49 (m, 1H, H-4′), 4.66 (q, J=4.4 Hz, 1H, H-1′), 4.85 (m, 1H, OH-4′), 4.99 (d, J=6.8 Hz, 1H, OH-3′), 5.35 (d, J=5.2 Hz, 1H, OH-2′), 7.20 (br s, 2H, C^6–NH$_2$), 8.11 (s, 1H, H-8), 8.14 (1H, s, H-2); EI MS (*m/z*, relative intensity) 251 (M$^+$, 24), 234 (10), 162 (73), 136 (100), 108 (22); HRMS (EI) Calcd for $C_{10}H_{13}N_5O_3$ 251.1018. Found 251.1010; Anal. calcd for $C_{10}H_{13}N_5O_3\cdot1/4H_2O$: C, 38.35; H, 4.48; N, 19.44. Found: C, 38.73; H, 4.24; N, 19.43.

3.1.4. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-8-bromoadenine (8-bromonoraristeromycin, 7). Noraristeromycin (**3**) (109 mg, 0.43 mmol) was suspended in 0.5 M sodium acetate buffer pH 4 (5 mL). Then Br_2 was added to the solution. The reaction mixture was stirred at room temperature for 4 h and then excess Br_2 was reduced by sodium hydrogen sulfite solution. After neutralization with NaOH, the solvent was evaporated under reduced pressure. The residue was purified by silica-gel column chromatography eluting with chloroform–methanol (10:1). Fractions were evaporated under reduced pressure and the residue was triturated with 1-propanol to give 8-bromonoraristeromycin (**7**) (104 mg, 73%) as a solid: mp 222°C (dec.); $[\alpha]_D^{22}=-9.0°$ (*c* 0.30, DMF); FTIR (KBr) 3372, 3193, 1659, 1312, 1059 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.12 (m, 1H, H-5′), 2.44 (m, 1H, H-5′), 3.75 (br s, 1H, H-3′), 3.86 (br s, 1H, H-4′), 4.68 (m, 1H, H-1′), 4.82 (m, 1H, H-2′), 4.86 (d, J=2.8 Hz, 1H, OH-3′), 5.02 (d, J=5.2 Hz, 1H, OH-2′), 5.55 (d, J=6.0 Hz, 1H, OH-3′), 7.44 (br s, 2H, C^6–NH$_2$), 8.01 (d, J=1.2 Hz, 1H, H-2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 34.00, 61.03, 72.80, 73.69, 76.55, 119.66, 127.67, 149.89, 152.01, 154.99; FAB MS (*m/z*, relative intensity) 332 (10), 330 ([M+H]$^+$, 11), 308 (9), 273 (17), 185 (100); HRMS (FAB) Calcd for $C_{10}H_{13}BrN_5O_3$ 330.0202. Found 330.0187. Anal. calcd for $C_{10}H_{12}BrN_5O_3\cdot1/2PrOH$: C, 38.35; H, 4.48; N, 19.44. Found: C, 38.73; H, 4.24; N, 19.43.

3.1.5. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-8-methyladenine (8-methylnoraristeromycin, 8). Methylation of 8-bromonoraristeromycin (**5**) was carried out by reported method.[13] A solution of 8-bromonoraristeromycin (**7**) (104 mg, 0.32 mmol) and ammonium sulfate (5 mg, 0.038 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (15 mL) was stirred at 140°C for 3.5 h. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in dry THF (7 mL). Tetrakis(triphenylphosphine)palladium(0) (5 mg, 0.0043 mmol) was added to the solution and the mixture was refluxed at 90°C for 10 min under Ar atmosphere. Trimethylaluminium (0.98 M *n*-hexane solution) (0.7 mL) was slowly dropped to the solution. The reaction mixture was stirred at 90°C for 2 h under Ar atmosphere and quenched by methanol at 0°C. The suspension was evaporated. Chloroform (5 mL) was added to the residue and washed with distilled water (5 mL). After the organic layer was dried on sodium sulfate and filtrated, the filtrate was evaporated. Methanol (10 mL) and 1 M ammonium chloride solution (5 mL) were added to the residue and the mixture was stirred at 90°C for 3.5 h. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (10:1). Fractions were evaporated under reduced pressure and the residue was triturated with 1-propanol to give 8-methylnoraristeromycin (**8**) (46 mg, 55%) as a solid: mp 250°C (dec.); $[\alpha]_D^{22}=-43.7°$ (*c* 1.04, DMF); FTIR (KBr) 3451, 3293, 1644, 1343, 1089 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.11 (m, 1H, H-5′), 2.47 (m, 1H, H-5′), 2.49 (s, 3H, CH_3), 3.71 (s, 1H, H-3′), 3.84 (m, 1H, H-4′), 4.52 (m, 1H, H-1′), 4.61 (m, 1H, H-2′), 4.85 (d, *J*=4.0 Hz, 1H, OH-3′), 4.93 (d, *J*=6.8 Hz, 1H, OH-2′), 5.80 (d, *J*=6.4 Hz, 1H, OH-4′), 7.10 (s, 2H, $\text{C}^6\text{–NH}_2$), 8.02 (s, 1H, H-2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.21, 34.18, 58.94, 74.05, 74.21, 76.70, 118.00, 149.33, 149.56, 150.81, 155.22; EI MS (*m/z*, relative intensity) 265 (M^+, 6), 204 (6), 176 (64), 149 (100), 122 (28); HRMS (EI) Calcd for $\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}_3$ 265.1175. Found 265.1182. Anal. calcd for $\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}_3\cdot3/5\text{PrOH}$: C, 51.02; H, 6.62; N, 23.24. Found: C, 51.38; H, 6.26; N, 23.28.

3.1.6. 9-[(1′*R*,4′*S*)-4′-Hydroxy-2′-cyclopenten-1′-yl]-9-*H*-2-amino-6-chloropurine (9). This compound was prepared by the analogous method for the preparation of noraristeromycin (**3**). A solution of 2-amino-6-chloropurine (368 mg, 2.2 mmol) and 60% NaH (84 mg, 2.1 mmol) in DMSO (5 mL) was stirred at room temperature for 0.5 h under Ar atmosphere. The reaction mixture was added to a solution of compound **4** (258 mg, 1.8 mmol), triphenylphosphine (84 mg, 0.32 mmol) and tetrakis(triphenylphosphine)palladium(0) (260 mg, 0.15 mmol) in dry THF in the dark and stirred at 55°C for 2 days under Ar atmosphere. 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 evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform–methanol (60:1). Fractions were evaporated under reduced pressure and the residue was triturated with 1-butanol to give compound **9** (180 mg, 40%) as a solid: mp 159–160°C; FTIR (KBr) 3206, 1636, 1570, 1408, 1200 cm^{-1}; ^{1}H NMR (400 MHz, CDCl_3) δ 2.05 (d, *J*=14.8 Hz, 1H, H-5′), 2.87 (m, 1H, H-5′), 4.77 (d, *J*=5.2 Hz, 1H, H-4′), 5.17 (d, *J*=8.8 Hz, 1H, H-1′), 5.25 (s, 2H, $\text{C}^2\text{–NH}_2$), 5.77 (s, 1H, H-2′), 6.24 (s, 1H, H-3′), 8.02 (s, 1H, H-8); EI MS (*m/z*, relative intensity) 253 (6), 251 (M^+, 20), 222 (22), 169 (94), 134 (100); Anal. calcd for $\text{C}_{10}\text{H}_{10}\text{ClN}_5\text{O}\cdot1/4\text{BuOH}$: C, 48.90; H, 4.66; N, 25.92. Found: C, 49.16; H, 4.34; N, 26.17.

3.1.7. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-2-amino-6-chloropurine[11] (10). The solution of compound **9** (33 mg, 0.13 mmol) in THF (3.6 mL) and H_2O (0.4 mL) was treated with 2% Osmium tetraoxide solution (72 μL) in the presence of 4-methylmorpholine *N*-oxide (40 mg, 0.33 mmol) and stirred at room temperature for 5 h. The reaction mixture was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (10:1). Compound **10** was obtained (38 mg, 99%) as a solid: mp 123–124°C; FTIR (KBr) 3330, 3223, 1634, 1619, 1472 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.69 (m, 1H, H-5′), 2.56 (m, 1H, H-5′), 3.73 (br s, 1H, H-3′), 3.86 (m, 1H, H-4′), 4.43 (m, 1H, H-2′), 4.62 (m, 1H, 1-H′), 4.88 (d, *J*=3.6 Hz, 1H, OH-3′), 5.03 (d, *J*=4.8 Hz, 1H, OH-2′), 5.16 (d, *J*=4.0 Hz, 1H, OH-4′), 6.86 (s, 2H, $\text{C}^2\text{–NH}_2$), 8.18 (s, 1H, H-8); EI MS (*m/z*, relative intensity) 287 (6), 285 (M^+, 18), 196 (53), 170 (100), 134 (74); Anal. calcd for $\text{C}_{10}\text{H}_{12}\text{ClN}_5\text{O}_3\cdot3/5\text{MeOH}\cdot3/5\text{H}_2\text{O}$: C, 40.32; H, 4.98; N, 22.18. Found: C, 40.49; H, 4.85; N, 22.00.

3.1.8. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-2-aminoadenine[11] (2-aminonoraristeromycin, 11). Compound **10** (21 mg, 0.075 mmol) was treated with methanolic ammonia (35 mL) and heated in a sealed tube at 100°C for 24 h. The solvent was evaporated and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (10:1). Fractions were evaporated under reduced pressure and the residue was triturated with EtOAc and ethanol to give 2-aminonoraristeromycin (**11**) (12 mg, 62%) as a solid: mp 116–118°C; $[\alpha]_D^{22}=-61.4°$ (*c* 0.22, DMF); FTIR (KBr) 3324, 3140, 1669, 1597, 1418 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.66 (br s, 1H, H-5′), 2.55 (m, 1H, H-5′), 3.72 (br s, 1H, H-3′), 3.85 (br s, 1H, H-4′), 4.42 (br s, 1H, H-2′), 4.50 (m, 1H, H-1′), 4.78 (br s, 1H, OH-3′), 5.02 (br s, 1H, OH-2′), 5.41 (br s, 1H, OH-4′), 5.81 and 6.79 (br s, each 2H, $\text{C}^2\text{–NH}_2$ and $\text{C}^6\text{–NH}_2$), 7.76 (s, 1H, H-8); ^{13}C NMR (100 MHz, DMSO-d_6) δ 37.00, 57.57, 73.64, 75.68, 77.02, 113.50, 136.47, 151.77, 156.23, 159.98; EI MS (*m/z*, relative intensity) 266 (M^+, 17), 177 (55), 150 (100), 108 (33); HRMS (EI) Calcd for $\text{C}_{10}\text{H}_{14}\text{N}_6\text{O}_3$ 266.1127. Found 266.1130. Anal. calcd for $\text{C}_{10}\text{H}_{14}\text{N}_6\text{O}_3\cdot$EtOAc$\cdot$2/3EtOH: C, 47.83; H, 6.81; N, 21.82. Found: C, 47.57; H, 7.19; N, 21.79.

3.1.9. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Triacetoxycyclopentan-1′-yl]-9-*H*-2-amino-6-chloropurine (12). The solution of compound **10** (130 mg, 0.49 mmol) in DMF (12 mL) was treated with acetic anhydride (194 μL) in the presence of pyridine (9 mL) and stirred at room temperature for 20 h. The solvent was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (50:1). Compound **12** was obtained (132 mg, 70%) as a solid: mp 60–62°C; FTIR (KBr) 1745, 1611, 1565, 1374, 1231 cm^{-1}; ^{1}H NMR (400 MHz, CDCl_3) δ 1.92 (s, 3H, OCOCH_3), 2.07 (s, 6H, OCOCH_3), 2.24 (m, 1H, H-5′), 2.85 (m, 1H, H-5′), 4.82 (m, 1H, H-3′), 5.13 (m, 1H, H-4′), 5.43–5.48 (m, 3H, H-3′ and $\text{C}^2\text{–NH}_2$), 5.73 (dd, *J*=8.0 and 5.6 Hz, 1H, H-2′), 7.77 (s, 1H, H-8); ^{13}C NMR (100 MHz, CDCl_3) δ 20.30, 20.48, 20.78, 32.59, 56.12, 73.27, 73.41, 73.50, 125.47, 140.93, 151.41, 153.48, 158.91, 169.21, 169.52, 169.70; EI MS (*m/z*, relative intensity) 413 (8), 411 (M^+, 30), 353 (38), 250 (100), 170 (98); HRMS (EI) Calcd for $\text{C}_{16}\text{H}_{18}\text{ClN}_5\text{O}_6$ 411.0946. Found 411.0935.

3.1.10. 9-[(1′,*R*2′*S*,3′*R*,4′*S*)-2′,3′,4′-Triacetoxycyclopentan-1′-yl]-9-*H*-2-bromo-6-chloropurine (13). Antimony (III) bromide (102 mg, 0.28 mmol) was added to a solution of compound **12** (78 mg, 0.189 mmol) in dibromomethane (5 mL). After cooled to −10°C, *tert*-butyl nitrite (98 μL, 0.75 mmol) was added to the solution and stirred for 5 h. After evaporation, chroloform (5 mL) was added to the residue and washed with saturated sodium hydrogen 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 chloroform–methanol (50:1). Fractions were evaporated under reduced pressure and the residue were triturated with *n*-hexane to give compound **13** (61 mg, 68%) as a solid: mp 48–49°C; FTIR (KBr) 1750, 1553, 1354, 1229, 1148 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.99, 2.17 and 2.21 (s, each 3H, $OCOCH_3$), 2.33 (m, 1H, H-5′), 3.08 (m, 1H, H-5′), 5.13 (m, 1H, H-3′), 5.20 (m, 1H, H-4′), 5.47 (dd, *J*=5.0 and 1.6 Hz, 1H, H-3′), 5.80 (dd, *J*=8.8 and 5.2 Hz, 1H, H-2′), 8.19 (s, 1H, H-8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.32, 20.56, 20.91, 33.45, 56.94, 73.30, 73.69, 74.22, 131.56, 143.03, 144.20, 151.74, 152.84, 169.08, 169.59; EI MS (*m/z*, relative intensity) 476 (3), 474 (M^+, 3), 315 (85), 259 (41), 235 (100); HRMS (EI) Calcd for $C_{16}H_{16}BrClN_4O_6$ 473.9942. Found 473.9933. Anal. calcd for $C_{16}H_{16}BrClN_4O_6\cdot1/3n$-hexane·1/3MeOH: C, 42.75; H, 4.31; N, 10.88. Found: C, 42.93, H, 4.16; N, 10.67.

3.1.11. 9-[(1′*R*,2′*S*,3′,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-2-bromoadenine (2-bromonoraristeromycin, 14). Compound **13** (127 mg, 0.28 mmol) was treated with methanolic ammonia (40 mL) and heated in sealed tube at 120°C for 8 h. The solvent was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (10:1). Fractions were evaporated under reduced pressure and the residue was triturated with EtOAc to give 2-bromonoraristeromycin (**14**) (55 mg, 60%) as a solid: mp 249°C (dec.); $[\alpha]_D^{22}=-21.7°$ (*c* 0.52, DMF); FTIR (KBr) 3343, 3218, 1663, 1597, 1310 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.70 (m, 1H, H-5′), 2.58 (m, 1H, H-5′), 3.73 (br s, 1H, H-3′), 3.88 (br s, 1H, H-4′), 4.41 (br s, 1H, H-2′), 4.62 (m, 1H, H-1′), 4.88 (s, 1H, HO-3′), 5.01 (d, *J*=6.4 Hz, 1H, OH-2′), 5.19 (s, 1H, OH-4′), 7.73 (s, 2H, C^6–NH_2), 8.17 (s, 1H, H-8); ^{13}C NMR (100 MHz, DMSO-d_6) δ 36.82, 58.09, 73.69, 75.65, 76.62, 118.36, 139.98, 143.90, 150.69, 156.58; FAB MS (*m/z*, relative intensity) 332 (1), 330 ($[M+H]^+$, 1), 277 (15), 185 (100); HRMS (FAB) Calcd for $C_{10}H_{13}BrN_5O_3$ 330.0202. Found 330.0215. Anal. calcd for $C_{10}H_{12}BrN_5O_3\cdot1/8$EtOAc: C, 36.97; H, 3.84; N, 20.53. Found: C, 36.84; H, 3.80; N, 20.78.

3.1.12. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-2-methyladenine (2-methylnoraristeromycin, 15). Methylation of 2-bromonoraristeromycin (**14**) was carried out by same procedure with methylation of 8-bromonoraristeromycin (**7**). A mixture of 2-bromonoraristeromycin (**14**) (37 mg, 0.11 mmol) and ammoium sulfate (2 mg, 0.015 mmol) in 1,1,1,3,3,3-hexamethyldisilazane (4 mL) was stirred at 140°C for 3 h. The reaction mixture was evaporated under reduced pressure and the residue was dissolved in dry THF (4 mL). Tetrakis(triphenylphosphine)palladium(0) (2 mg, 0.0017 mmol) was added to the solution and the mixture was refluxed at 90°C for 10 min under Ar atmosphere. Trimethylaluminium (0.98 M *n*-hexane solution) (224 μL) was slowly dropped to the solution. The reaction mixture was stirred at 90°C for 4 h under Ar atmosphere and quenched by methanol at 0°C. The suspension was evaporated under reduced pressure. Chloroform (3 mL) was added to the residue and washed with distilled water (3 mL). After the organic layer was dried on sodium sulfate and filtrated, the filtrate was evaporated under reduced pressure. Methanol (5 mL) and 0.2 M ammonium chloride solution (5 mL) were added to the residue and the mixture was stirred at 90°C for 3.5 h. The reaction mixture was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (10:1). Fractions were evaporated under reduced pressure and the residue was triturated with EtOAc and cyclohexane to give 2-methylnoraristeromycin (**15**) (24 mg, 81%) as a solid: mp 249–251°C; $[\alpha]_D^{22}=-25.7°$ (*c* 0.52, DMF); FTIR (KBr) 3196, 2361, 1670, 1613, 1080 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 1.76 (m, 1H, H-5′), 2.36 (s, 3H, C^2–CH_3), 2.62 (m, 1H, H-5′), 3.75 (s, 1H, H-3′), 3.88 (s, 1H, H-4′), 4.48 (s, 1H, H-2′), 4.68 (d, *J*=6.8 Hz, 1H, H-1′), 4.88 (s, 1H, OH-3′), 5.03 (d, *J*=5.6, 1H, OH-2′), 5.65 (d, *J*=4.8 Hz, 1H, OH-4′), 7.14 (s, 2H, C^6–NH_2), 8.07 (s, 1H, H-8); ^{13}C NMR (100 MHz, DMSO-d_6) δ 25.38, 36.75, 58.22, 73.77, 75.71, 76.85, 117.37, 139.50, 149.90, 155.73, 160.52; EI MS (*m/z*, relative intensity) 265 (M^+, 12), 248 (8), 176 (74), 150 (100), 108 (79); HRMS (EI) Calcd for $C_{11}H_{15}N_5O_3$ 265.1175. Found 265.1172. Anal. calcd for $C_{11}H_{15}N_5O_3\cdot$EtOAc·cyclohexane·1/4MeOH: C, 57.28; H, 8.14; N, 15.72. Found: C, 57.41; H, 7.82; N, 15.56.

3.1.13. 9-[(1′*R*,4′*S*)-4′-Hydroxy-2′-cyclopenten-1′-yl]-9-*H*-N^6-benzoylamino-8-aza-7-deazapurine (16). This compound was prepared by the analogous method for the preparation of noraristeromycin (**3**). A solution of 4-benzoylaminopyrazolo[3,4-*d*]pyrimidine (262 mg, 1.1 mmol) and 60% NaH (67 mg, 1.7 mmol) in DMSO (3 mL) was stirred at room temperature for 0.5 h under Ar atmosphere. The reaction mixture was added to a solution of compound **4** (141 mg, 0.99 mmol), triphenylphosphine (26 mg, 0.099 mmol) and tetrakis(triphenylphosphine)palladium(0) (121 mg, 0.11 mmol) in dry THF (3 mL) in the dark and stirred at 55°C for 2 days under Ar atmosphere. 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 evaporated under reduced pressure and purified by silica-gel column chromatography eluting with chloroform–methanol (70:1). Compound **16** was obtained (175 mg, 55%) as a residue: ^1H NMR (400 MHz, $CDCl_3$) δ 2.17 (d, *J*=15.2 Hz, 1H, H-5′), 2.76 (m, 1H, H-5′), 4.69 (s, 1H, H-4′), 5.46 (br s, 1H, H-1′), 5.66 (br s, 1H, OH-4′), 5.46 (m, 1H, H-2′), 6.31 (d, *J*=4.8 Hz, 1H, H-3′), 7.65–7.60 (m, 5H, Ph), 8.05 (s, 1H, H-7), 8.72 (s, 1H, H-2), 9.38 (s, 1H, C^6–NHBz); EI MS (*m/z*, relative intensity) 321 (M^+, 3), 292 (8), 240 (10), 210 (10), 105 (100).

3.1.14. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′,3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-N^6-benzoylamino-8-aza-7-deazapurine (17). The solution of compound **16** (175 mg, 0.49 mmol) in THF (1.8 mL) and H_2O (0.2 mL) was treated with 2%

Osmium tetraoxide solution (667 μL) in the presence of 4-methylmorpholine *N*-oxide (180 mg, 1.5 mmol) and stirred at room temperature for 5 h. The reaction mixture was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (30:1). Compound **17** was obtained (114 mg, 59%) as a solid: mp 96–97°C; FTIR (KBr) 3339, 1709, 1489, 1260, 1130 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.82 (m, 1H, H-5′), 2.08 (m, 1H, H-5′), 3.61 (m, 1H, H-3′), 3.92 (m, 1H, H-4′), 4.48 (m, 1H, H-2′), 4.74 (m, 1H, OH-2′), 4.88 (m, J=4.0 Hz, 1H, OH-3′), 5.08 (d, J=4.8 Hz, 1H, OH-4′), 5.19 (d, J=8.4 Hz, 1H, H-1′), 8.47–8.11 (m, 5H, Ph), 8.47 (s, 1H, H-7), 8.71 (s, 1H, H-2), 11.55 (s, 1H, C^6–NHBz); EI MS (*m*/*z*, relative intensity) 355 (M$^+$, 14), 281 (49), 266 (49), 240 (71), 105 (100); Anal. Calcd for $C_{17}H_{17}N_5O_4$: C, 57.46; H, 4.89; N, 19.48. Found: C, 57.63; H, 4.89; N, 19.48.

3.1.15. 9-[(1′*R*,2′*S*,3′*R*,4′*S*)-2′3′,4′-Trihydroxycyclopentan-1′-yl]-9-*H*-6-amino-8-aza-7-deazapurine[12] (8-aza-7-deazanoraristeromycin, 18). Compound **17** (114 mg, 0.32 mmol) was treated with methanolic ammonia (6 mL) at room temperature for 24 h. The solvent was evaporated under reduced pressure and the residue was purified by silica-gel column chromatography eluting with chloroform–methanol (10:1). Fractions were evaporated under reduced pressure and the residue was triturated with EtOAc and 1-propanol to give 8-aza-7-deazanoraristeromycin (**18**) (62 mg, 77%) as a solid: mp 210–211°C; $[\alpha]_D^{22}=-19.7°$ (*c* 0.56, DMF); FTIR (KBr) 3189, 2926, 1659, 1599, 1566 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.75 (m, 1H, H-5′), 2.43 (m, 1H, H-5′), 3.73 (br s, 1H, H-3′), 3.88 (br s, 1H, H-4′), 4.46 (br s, 1H, H-2′), 4.78 (br s, 2H, OH-2′ and OH-3′), 4.96 (m, 1H, H-1′), 5.06 (d, J=3.6 Hz, 1H, OH-4′), 7.70 (s, 2H, C^6–NH$_2$), 8.10 and 8.13 (s, each 1H, H-2 and H-7); ^{13}C NMR (100 MHz, DMSO-d_6) δ 35.88, 59.74, 73.33, 74.61, 76.92, 131.84, 153.27, 155.54, 158.02; EI MS (*m*/*z*, relative intensity) 251 (M$^+$, 17), 234 (7), 205 (45), 190 (61), 177 (100); HRMS (EI) Calcd for $C_{10}H_{13}N_5O_3$ 251.1018. Found 251.1025. Anal. calcd for $C_{10}H_{13}N_5O_3\cdot 1/2EtOAc\cdot 1/2PrOH$: C, 49.84; H, 6.51; N, 21.53. Found: C, 49.99; H, 6.74; N, 21.14.

3.2. Biological assay

In the synthetic direction, the enzyme assay was a modification of an earlier method.[14] The enzyme was incubated with 100 μM adenosine, 5 mM DL-homocysteine and inhibitors in 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 μL of SAH hydrolase (human: 0.43 μg, *P. falciparum*: 0.54 μg) and terminated by the addition of 20 μL of 0.67N HCl. The reaction mixture was kept on ice until the HPLC analysis. The mixture was analyzed for SAH by a Shimadzu HPLC system described above. In the synthetic reaction, one unit of SAH hydrolase was defined as the amount synthesizing 1 μmol of SAH/min at 30°C.

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