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Synthesis of 4′-modified noraristeromycins to clarify the effect of the 4′-hydroxyl groups for inhibitory activity against *S*-adenosyl-L-homocysteine hydrolase

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Abstract—4′-Modified noraristeromycin (NAM) analogs, 4′-sulfo-, 4′-sulfamoy, 4′-azido and 4′-amino-NAM, were systematically synthesized. The inhibitory activities of these analogs and related compounds against *Plasmodium falciparum* and human *S*-adenosyl-L-homocysteine hydrolase were investigated.

S-Adenosyl-L-homocysteine hydrolase (SAHH) is the enzyme that catalyzes the breakdown of *S*-adenosylhomocysteine (SAH) to homocysteine and adenosine.[1] SAH is a strong inhibitor of numerous methyltransferases that transfer methyl groups from *S*-adenosylmethionine to nucleic acids, proteins, lipids and other small molecules.[2] Since these methylations are required for the proliferation of viruses and tumor cells, the accumulation of SAH leads to anti-virus/anti-tumor effects.[3,4]

Various carbocyclic adenine nucleosides have been synthesized[5] to develop antimalarial reagents and their inhibitory activities against *Plasmodium falciparum*, a causative agent of human malaria, have been investigated.[6–10] We previously reported that the introduction of fluorine to the 2-position of noraristeromycin (**1**, **NAM**, Figs. 1 and 2) increased selective inhibition against *P. falciparum* SAHH (PfSAHH) compared with human SAHH (HsSAHH).[9] PfSAHH has additional space near the 2-position of the adenine-ring, in the substrate binding pocket compared with HsSAHH.[11] 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.[12] In addition, 2′ and/ or 3′ hydroxyl group modified compounds, such as deoxy,[13] dideoxy,[13] *epi*,[13] and keto,[6] did not show any or showed less inhibitory activity against both forms of SAHH. These hydroxyl groups and their configurations are essential for the inhibition of SAHH.

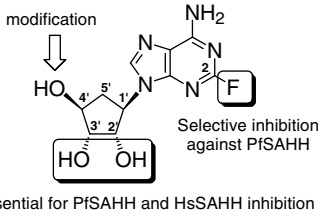

Figure 1. Structure of noraristeromycin (**NAM**) in relation to its inhibitory activity against both SAHH.

Modification of the 4′-hydroxyl group of NAM is the remaining improvement required for the development of a potent and selective inhibitor against PfSAHH. However, the interactions between NAM and the crucial amino acid in the binding site of enzyme are unclear.

Based on this information, we focused on modifying the 4′-position of NAM. In this paper, we have described

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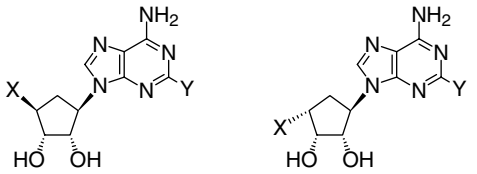


1 X = OH, Y = H
2 X = OH, Y = F
3 X = F, Y = H
4 X = OSO_3Na, Y = H
5 X = OSO_3Na, Y = NH_2
6 X = OSO_2NH_2, Y = H
7 X = OSO_2NH_2, Y = NH_2
8 X = N_3, Y = H
9 X = NH_2, Y = H

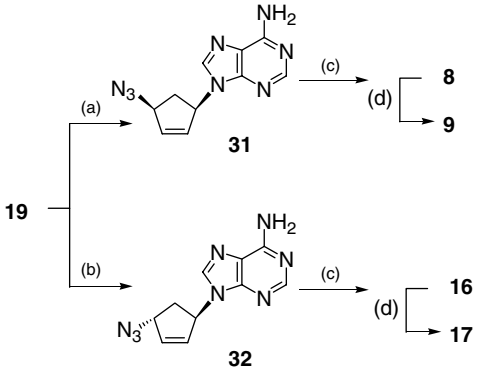
10 X = OH, Y = H
11 X = F, Y = H
12 X = OSO_3Na, Y = H
13 X = OSO_3Na, Y = NH_2
14 X = OSO_2NH_2, Y = H
15 X = OSO_2NH_2, Y = NH_2
16 X = N_3, Y = H
17 X = NH_2, Y = H
18 X = H, Y = H

Figure 2. Structures of carbocyclic adenine nucleosides: **1** (Ref. 14), **2** (Ref. 9), **3**, **11** (Ref. 10), **8**, **9** (Ref. 15), **10** (Ref. 16), **18** (Ref. 17), other compound are novel compounds.

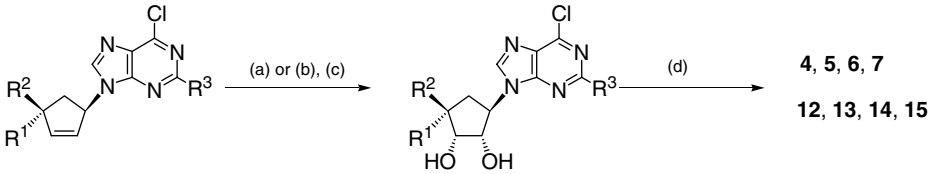
the syntheses of 4′-α- or 4′-β- sulfonyl, sulfamoyl, amino and azido NMA analogs (**4**–**9** and **12**–**17**, Fig. 2), and their inhibitory activities against PfSAHH and HsSAHH, to clarify the effect of the 4′-hydroxyl group of NAM. In addition, we have investigated and compared these effects with the inhibitory activities of the known NAM analogs (**1**–**3**, **10**, **11**, **18**, Fig. 2), which are reported by our laboratory[6–10,13] and many research groups.[14–17]

The synthetic route for 4′-sulfonated or sulfamoylated NAM is shown in Scheme 1. Compounds **19**–**22** were prepared by a coupling reaction of the cyclopentene derivative and 6-chloropurine or 2-amino-6-chloropurine using a palladium catalyst[18] for 1′,4′-*cis*- compounds **19** and **20**, or Mitsunobu conditions[19] for 1′,4′ *trans*-one **21** and **22**, as previously reported.[10] The sulfonation of **19**–**22** was performed by sulfur trioxide pyridinium complex[20] in DMF. The following oxidation using OsO_4 with *N*-methylmorpholine *N*-oxide (NMO) afforded 4′-sulfonated compounds **23**–**26**. The conversion of 6-chloro groups to an amino one yielded 4′-sulfonated NAM and their 2-amino analogs **4**,[26] **5**, **12**, and **13**. In the meantime, treatment of the starting materials **19**–**22** with NaH and sufamoyl chloride in DMF (a previously reported method[21]), afforded 4′-sulfamoylated compounds **27**–**30**. Similarly, osmium oxidation[22] afforded the corresponding 4′-sulfamoylated NAM analogs **6**,[26] **7**, **14** and **15**.

The synthetic route for 4′-azido and 4′-amino NAM analogs is shown in Scheme 2. The introduction of azido group to the 4′-β-position was performed by using NaN_3 and a palladium catalyst with retention of configuration to afford compound **31** from **19** as the reported method.[23] On the other hand, 4′-α-azido compound **32** was obtained from compound **19** with an inversion of configuration by using $N_3PO(OPh)_2$-DBU, which was developed by Shioiri et al.[24] Finally, osmium oxidation with NMO yielded 4′-β-azido-NAM **8** and α-azido-NAM **16**, followed by catalytic hydrogenation in the presence of Pd–C to afford 4′-β-amino-NAM **9** and 4′-α-amino-NAM **17**,[26] respectively. The stereochemistry of the 4′-azido, and 2′ and 3′-hydroxyl groups was de-



Scheme 2. Reagents and conditions: (a) i—NH_3/MeOH (28%, w/w) 120 °C, 18 h, ii—Ac_2O (1.1 eq), pyridine (2.2 eq), CH_2Cl_2, 0 °C, 48 h, iii—$Pd_2(dba)_3 \cdot CHCl_3$, $Ph_2P(CH_2)_3PPh_2$, THF, rt 1.5 h, then NaN_3, H_2O, rt 21 h; (b) i—NH_3/MeOH, (28%, w/w) 120 °C, 24 h, ii—$N_3PO(OPh)_2$, DBU, THF, 0 °C, 3.5 h, then rt 18 h; (c) OsO_4, NMO, THF–H_2O (10:1), rt 12 h; (d) H_2, Pd–C (5%), AcOH/MeOH (5% v/v), rt 24 h.



comp	R^1	R^2	R^3
19	H	OH	H
20	H	OH	NH_2
21	OH	H	H
22	OH	H	NH_2

comp	R^1	R^2	R^3
23	H	OSO_3Na	H
24	H	OSO_3Na	NH_2
25	OSO_3Na	H	H
26	OSO_3Na	H	NH_2
27	H	OSO_2NH_2	H
28	H	OSO_2NH_2	NH_2
29	OSO_2NH_2	H	H
30	OSO_2NH_2	H	NH_2

Scheme 1. Reagents and conditions: (a) $SO_3 \cdot$ pyridine, DMF, rt 18 h (for **23**, **24**, **25** and **26**); (b) i—NaH, DMF, rt 2 h, ii—then NH_2SO_2Cl, rt 10 h (for **27**, **28**, **29** and **30**); (c) OsO_4, NMO, THF–H_2O (10:1), rt 12 h; (d) NH_3/MeOH (28% w/w) 120 °C, 18 h.

Table 1. Inhibitory activities against HsSAHH and PfSAHH

Compound	HsSAHH IC_{50}, μM	PfSAHH IC_{50}, μM	Selective index[a]
1 (NAM)	1.1[c]	3.1[c]	0.35
2	63[c]	13[c]	4.8
3	200[d]	ND[b,d]	—
4–7 and **12–15**	ND[b]	ND[b]	—
8	117	123	0.95
9	126	76	1.7
10	79	7.6	9.6
11[d]	ND[b,d]	ND[b,d]	—
16	90	163	0.55
17	>500	>500	—
18[d]	9.0[d]	18.0[d]	0.5

[a] Selective index: mean IC_{50} value for human SAH hydrolase/mean IC_{50} value for *P. falciparum* SAH hydrolase.
[b] No inhibitory activity showed at 1000 μM.
[c] Ref. 9.
[d] Ref. 10.

fined by NOE correlations of the corresponding ring protons.[25]

The enzyme assay was a modification of an earlier method.[27] A profile of the inhibitory activities of synthetic compounds against recombinant PfSAHH and HsSAHHe is shown in Table 1. The 4′-β- and 4′-α-sulfonated and sulfamoylated NAM analogs **4–7** and **12–15** did not show any inhibitory activity against either HsSAHH or PfSAHH. The 4′-β- and 4′-α-azido NAM analogs **8** and **16** showed moderate inhibitory activities. Although 4′-β-amino NAM **9** also showed inhibitory activity similar to azido NAM, 4-α-amino NAM **17** showed a loss of activity against both SAHHs.

Surprisingly, the IC_{50} values of 4′-*epi*-NAM **10**, which possesses a hydroxyl group at the α side of carbocyclic ring, were 79 and 7.6 μM against HsSAHH and PfSAHH, respectively. The selective index was 9.6, which was better than NAM **1** and 2-fluoro-NAM **2**. The configuration of the 4′-hydroxyl group had significant effects on inhibitor selectivity between PfSAHH and HsSAHH.

We previously reported the syntheses and inhibitory activities of diverse NAM analogs against SAHHs.[6–10] The 4′-α- and 4′-β-fluoro substituents of NAM analogs **3** and **11** decreased the inhibitory activities. Furthermore, 4′-deoxy NAM **18** possessed adequate inhibitory activity against SAHHs. According to these assay results, the introduction of a functional group at the 4′-position of NAM was ineffective. However, α-configuration of the 4′-hydroxyl group considerably increased selective inhibitor activities against PfSAHH.

In conclusion, we have clarified the importance of the 4′-α-hydroxyl group of NAM for development of a potent antimalarial agent, and compared the inhibitory activities of 4′-modified NAM analogs. Further multi-modifications of NAM and the related carbocyclic adenine nucleoside are now under investigation. Additional potent analogs will be reported in due course.

Acknowledgment

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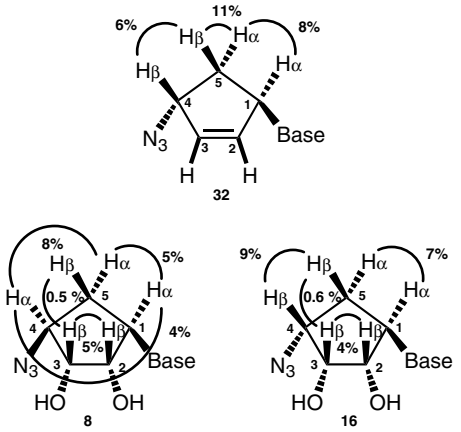
25. NOE correlations of compound **32**, **8** and **16**.



26. Selected physical dates For **4**: ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.12 (1H, s, H-2), 7.96 (1H, s, H-8), 7.16 (2H, brs, NH_2), 5.01(1H d, $J_{H,\ OH}$ = 6.8 Hz, OH-2′), 5.07(1H, d, $J_{H,\ OH}$ = 3.6 Hz, OH-3′), 4.4 (2H, m, H-2′, H-3′), 4.73 (1H, dd, J = 8.4 Hz, H-1′), 4.41 (1H, d, J = 8.4 Hz, H-4′), 2.66 (1H, m, J_{jem} = 14.8 Hz, H-5′$_\alpha$), 2.11 (1H, m, H-5′$_\beta$); HRFABMS (negative mode) m/z calcd for $C_{10}H_{12}O_6N_5S$ $[M-H]^-$: 330.05014. Found, 330.05083. For **6**: ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.13 (1H, s, H-2), 7.99 (1H, s, H-8), 4.60 (1H, dd, J = 4.8 Hz, H-3′), 4.05 (1H, d, J = 3.0 Hz, H-2′), 3.93 (1H, d, J = 4.8 Hz, H-4′), 3.47 (1H, s, H-1′), 2.80 (1H, m, J_{jem} = 14.8 Hz, H-5′$_\alpha$), 1.99 (1H, m, H-5′$_\beta$); HRFABMS (positive mode) m/z calcd for $C_{10}H_{15}N_6O_5S$ $[M + H]^+$: 331.0825. Found, 331.0804. For **17**: ^{1}H NMR (CD_3OD-d_4, 400 MHz) δ: 8.51 (1H, s, H-2), 8.35 (1H, s, H-8), 5.30 (1H, m, H-1′), 4.18 (2H, m, H-2′ and H-3′), 3.86 (1H, m, H-4′), 2.85 (1H, ddd, J_{jem} = 14.8 Hz, J = 9.6, 10.8 Hz, H-5′$_\alpha$); 2.33 (1H, ddd, J = 6.0, 10.4, H – 5′$_\beta$); HRFABMS (positive mode) m/z calcd for $C_{10}H_{15}O_2N_6$ $[M+H]^+$: 251.1256. Found, 251.1287.

27. Gomi, T.; Date, T.; Osawa, H.; Fujioka, M.; Aksamit, R. R.; Backlund, P. S., Jr.; Cantoni, G. L. *J. Biol. Chem* **1989**, *264*, 16138, Assay conditions: The enzyme was incubated with 100 μL 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 5 μL of SAHH (human: 0.43 μg, *P. falciparum*: 0.54 μg) and terminated by the addition of 20 mL 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 LC-10A VP HPLC system. In the synthetic reaction, one unit of SAH hydrolase was defined as the amount synthesizing 1 μmol of SAH/min at 30 °C.