New Neplanocin Analogues. 12. Alternative Synthesis and Antimalarial Effect of (6′R)-6′-C-Methylneplanocin A, a Potent AdoHcy Hydrolase Inhibitor[1]

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An improved method for the synthesis of (6′R)-6′-C-methylneplanocin A (RMNPA, **2**), a potent S-adenosyl-L-homocysteine (AdoHcy) hydrolase inhibitor, was developed via a chelation-controlled stereoselective addition of MeTiCl₃ to the neplanocin A 6′-aldehyde derivative **6**. Compound **2** effectively inhibited the growth of malaria parasites both in vitro and in vivo. The antimalarial EC_{50} value of **2** against *Plasmodium berghei* in mice was 1.0 mg/kg/day, which was superior to that of chloroquine (EC_{50} = 1.8 mg/kg/day).

Introduction

The increasing resistance of *Plasmodium falciparum* to currently available drugs, such as chloroquine, presents a challenge in the treatment of malaria,[2] which still remains one of the leading causes of death in the world. Consequently, new agents are urgently needed to treat the organism.

S-Adenosyl-L-homocysteine (AdoHcy) hydrolase, which is responsible for the hydrolysis of AdoHcy to adenosine (Ado) and L-homocysteine,[3] has been recognized as a new target for antimalarial agents,[4] since the parasite has a specific AdoHcy hydrolase.[4d] AdoHcy is a potent feedback inhibitor of cellular transmethylation reactions, and therefore inhibition of AdoHcy hydrolase would markedly increase the levels of AdoHcy preventing transmethylation reactions using S-adenosyl-L-methionine as the methyl donor, e.g., mRNA methylations, which are essential for parasite proliferation. In fact, several AdoHcy hydrolase inhibitors have been shown to have antimalarial effects.[4a−c]

The carbocyclic nucleoside antibiotic neplanocin A (NPA **1**),[5] one of the most potent AdoHcy hydrolase inhibitors known,[6] has a potent antiviral effect[6b] as well as a cytotoxic effect on various cells.[7] The extensively studied mechanism of action[7] of the cytotoxic effect can be attributed mainly to phosphorylation of the primary hydroxyl group at the 6′-position (the 6′-position of NPA corresponds to the 5′-position of Ado) by Ado kinase and subsequent metabolism by cellular enzymes.[7] The antiviral effect, however, is likely due to the inhibition of AdoHcy hydrolase via suppression of the virus mRNA maturation.[6a,8] NPA is also known to be rapidly deaminated by Ado deaminase to the chemotherapeutically inactive inosine congener,[9,10a] which would reduce the therapeutic potency of NPA. On the basis of these results, chemical modifications of NPA have been conducted to develop more efficient AdoHcy hydrolase inhibitors.[10,11] We previously chose the 6′-moiety of NPA as the target site for the modifications because of its

important role in interactions with these enzymes, namely, AdoHcy hydrolase, Ado deaminase, and Ado kinase.[10] We found that (6′R)-6′-C-methylneplanocin A (RMNPA, **2**) effectively inhibits AdoHcy hydrolase and thus has an efficient antiviral effect comparable to NPA, yet is significantly less cytotoxic to host cells and resistant to deamination by Ado deaminase.[10a,b] Interestingly, the corresponding 6′-diastereomer (6′S)-6′-C-methylneplanocin A is virtually biologically inactive.[10a]

On the basis of the above results, we investigated the antimalarial activity of RMNPA and found that it significantly inhibited the growth of *P. falciparum* in vitro. For an in vivo evaluation of its antimalarial effect, development of an efficient method of synthesis of RMNPA was needed. Earlier, less efficient synthesis of RMNPA had required the separation of a diastereomeric mixture of 6′-methylneplanocin A derivatives by HPLC[10a] or via an enzymatic method with Ado deaminase.[12] In this report, we describe an improved synthesis of RMNPA and its potent antimalarial activity both in vitro and in vivo.

Chemistry

First, we examined, under various conditions, the stereoselective addition of organometallic reagents, such as Me₃Al, MeLi, MeTiCl₃, MeTi(OiPr)₃, or MeMgBr to 2′,3′-O-isopropylideneneplanocin A 6′-aldehyde.[10a] However, the reactions were not stereoselective and gave the diastereomeric mixture in moderate yields (data not shown). Another 6′-aldehyde derivative **6**, having bulky *tert*-butyldimethylsilyl (TBS) groups on the 2′ and 3′-hydroxyls, was next selected as a substrate for the nucleophilic addition (Scheme 1). N⁶-Benzoylneplanocin A (**3**)[10a] was treated successively with TBSCl/imidazole in DMF and aqueous AcOH/THF to give the 2′,3′-bis-O-TBS derivative **5**. Compound **5** was oxidized with BaMnO₄[10i] in CH₂Cl₂ to yield the 6′-aldehyde **6**, the substrate for the addition of organometallics. The aldehyde **6** was stable enough to be purified by neutralized silica gel column chromatography. The reactions were performed with 3 equiv of Me₃Al, MeMgBr, MeLi, MeTiCl₃, or MeTi(Oi-Tr)₃, in CH₂Cl₂ (entries 1−5 in Table 1). Although the desired 6′R-product **7R** was not obtained as the major product in these entries, the 6′S-

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Scheme 1[a]

1: R[1] = H (NPA)
3: R[1] = Bz

4: R[2] = R[3] = TBS
5: R[2] = H, R[3] = TBS
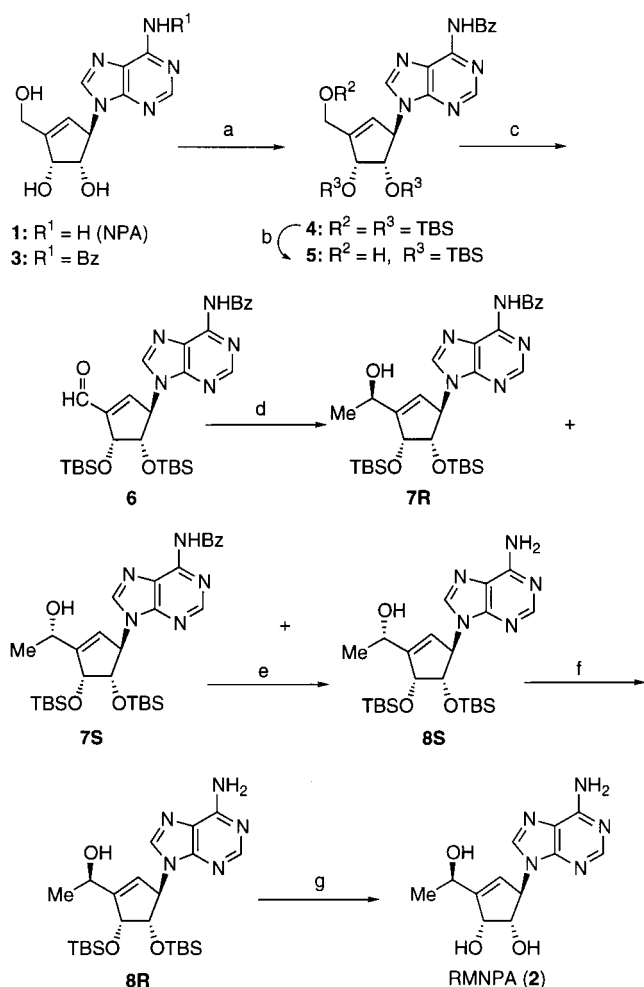
[a] Conditions: (a) TBSCl, imidazole, DMF, rt, quant; (b) AcOH, aq THF, 50 °C, 94%; (c) BaMnO$_4$, CH$_2$Cl$_2$, reflux, 63%; (d) see Table 1; (e) NH$_3$, MeOH, rt, 70%; (f) (1) DIPAD, Ph$_3$P, ClCH$_2$CO$_2$H, THF, rt, (2) NH$_3$, MeOH, rt, 90%; (g) NH$_4$F, MeOH, 74%.

Table 1. Nucleophilic Addition Reactions of Organometallic Reagents to the NPA 6′-Aldehyde Derivative **6**

entry	reagent[a]	conditions	product, yield (R:S)[b]
1	Me$_3$Al	−78 °C, 2 h	no reaction
2	MeMgBr	−78 °C, 2 h	**7**, 94% (1:1)
3	MeLi	−78 °C, 0.5 h	**7**, 88% (1:3)
4	MeTiCl$_3$	−50 °C, 0.5 h[c]	**7**, 30% (1:30<); **8**, 60% (1:30<)
5	MeTi(OiPr)$_3$	−0 °C−rt, 2 h[c]	**7**, 31% (1:3)

[a] Reactions were performed with 3 equiv of the reagent in CH$_2$Cl$_2$. [b] Isolated yield. The R/S ratio was determined by ^{1}H NMR. [c] No reaction at −78 °C.

diastereomer was produced highly selectively when **6** was treated with MeTiCl$_3$ at −50 °C. The reaction gave the 6′S-diastereomer **7S** and its N[6]-debenzoylated product **8S** in 30% and 60% yields, respectively, whereas only a trace of the corresponding diastereomers **7R** or **8R** was detected in the ^{1}H NMR. Compound **7S** was readily converted into the corresponding debenzoylated **8S** on treatment with NH$_3$/MeOH. It is well-known that addition of MeTiCl$_3$ to β-alkoxyaldehydes proceeds via a chelation controlled mechanism,[13] and therefore in this case addition from the less hindered *si*-face might occur via the chelation intermediate to produce the 6′S-product with high selectivity, as shown in Figure 1.
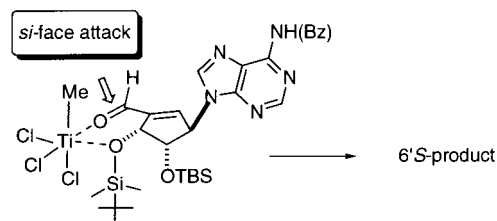
Figure 1.

Table 2. In Vitro Antimalarial Activity and Cytotoxicity of NPA, RMNPA, and Chloroquine

	IC$_{50}$ (μM)		
compound	*P. falciprum*[a]	KATO III[a] (growing)	Vero[b] (stationary)
NPA (**1**)	0.20	0.22	580
RMNPA (**2**)	0.10	1.1	>1800
chloroquine	0.018	ND[c]	ND

[a] Assay was performed by the previously reported method.[14] [b] Data were taken from ref 10a. [c] Not determined.

Successive treatment of **5** with BaMnO$_4$ in CH$_2$Cl$_2$, MeTiCl$_3$ in CH$_2$Cl$_2$, and NH$_3$/MeOH gave **8S** in 66% yield.

Inversion at the 6′-position of **8S** was examined using the Mitsunobu reaction. When **8S** was treated successively with diisopropyl azodicarboxylate (DIPAD), Ph$_3$P, and ClCH$_2$CO$_2$H in THF and with NH$_3$ in MeOH at room temperature, the desired 6′R-product **8R** was obtained in 90% yield. Removal of the silyl groups of **8R** with NH$_4$F in MeOH finally gave RMNPA (**2**).

This method, which provides RMNPA without diastereomer separation, is therefore a significant improvement over the previous methods.[10a,12]

Antimalarial Effect of RMNPA

The antimalarial activity of NPA, RMNPA, and chloroquine, a common antimalarial agent used as a positive control against *P. falciparum* (FCR-3 strain), was first evaluated in vitro using the previously reported method.[14] The results and cytotoxicity of the two compounds against human gastric carcinoma cell line (KATO III) and monkey kidney cell line (Vero) representing models of hosts are summarized in Table 2. RMNPA showed significant antimalarial activity with an IC$_{50}$ value of 0.10 μM. The potency was greater than that of the parent compound NPA (IC$_{50}$ = 0.20 μM), although this value is 5.6-fold higher than that of chloroquine. On the other hand, RMNPA was shown to exert lower cytotoxicity against proliferation of KATO III cells (IC$_{50}$ = 1.1 μM) compared with NPA (IC$_{50}$ = 0.22 μM). Furthermore, RMNPA was completely inactive in Vero cells in a stationary phase; no cytotoxic effect on the cells was observed at concentrations up to 1800 μM of RMNPA.

In vivo antimalarial activities of NPA and RNMPA against *Plasmodium berghei* (NK-65 strain) in mice were investigated according to the 4 day suppressive test,[14] and the results are shown in Table 3. The widely used antimalarial drug chloroquine was, as expected, effective in this system. It is noteworthy that RMNPA effectively inhibited the growth of *P. berghei* in mice with an ED$_{50}$ value of 1.0 mg/kg/day, which was more potent than chloroquine (ED$_{50}$ = 1.8 mg/kg/day). The parent compound NPA was less effective than RMNPA,

Table 3. In Vivo Antimalarial Activity of NPA, RMNPA, and Chloroquine in *P. berghei* in Mice[a]

| compound | untreated | dose (mg/kg/day) | | | | | | | EC_{50} (mg/kg/day)[b] |
		0.1	0.5	1.0	2	5	10	20	
NPA (**1**)									>5
% parasitemia[c]	21	20	21	21	21	12	–	–	
survival[d]	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	
RMNPA (**2**)									1.0
% parasitemia	21	21	21	10	5	2	2	–	
survival	5/5	5/5	5/5	5/5	5/5	5/5	5/5	2/5	
chloroquine									1.8
% parasitemia	21	21	20	18	7	3	2	1	
survival	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	

[a] In vivo antimalarial activity was assessed using ICR mice infected with *P. berghei* (NK 65 strain) following the protocol described previously.[14] Various concentrations of the test compounds, prepared in DMSO, were administered daily via intraperitoneal injection to groups of five mice for four consecutive days beginning on day 0. Five infected, dimethyl sulfoxide-dosed mice were used as a control. [b] The dose required to cause 50% suppression of *P. berghei* growth in mice. [c] To evaluate the antimalarial activity of the compounds, blood smears from the tails were taken and stained with Giemsa. A total of 1×10^4 erythrocytes per one thin blood film was examined under microscopy. On day 4, parasitemia of the control mice was between 18 and 22%. [d] Survival was monitored as a symptom indicative of toxicity on day 4.

having an ED_{50} of >5.0 mg/kg/day, 57% growth of *P. berghei* at 5.0 mg/kg/day, and toxic against mice at 10 mg/kg/day (survival 0/5). During administration of RMNPA (5.0 mg/kg/day), growth of the parasites was inhibited as shown in Table 3, whereas malaria parasites were still observed in circulating blood even after the 4 day suppressive test. All of the mice infected with *P. berghei* with and without RMNPA treatment died after 8−10 days of infection. However, no side effects such as diarrhea, body weight loss, or mortality were observed during the treatment with RMNPA at doses of 5.0 mg/kg/day. Therefore, mortality may not be due to toxicity caused by RMNPA but to *P. berghei* infection.

While the inhibitory effect of RMNPA against AdoHcy hydrolase is weaker than that of NPA in vitro,[10a] its antimalarial activity in vivo is superior to that of NPA. RMNAP is effective presumably because the compound is (1) completely resistant to Ado deaminase,[10a,12] keeping it at relatively higher concentrations in vitro as well as in vivo, and (2) it is less toxic to host cells, because it is not phosphorylated by Ado kinase, a possible toxic mechanism of NPA.[7]

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus (Yanagimoto, Japan) and are uncorrected. Fast atom bombardment mass spectrometry (FAB-MS) was done on a JEOL JMS-HX110 instrument at an ionizing voltage of 70 eV. The ^1H NMR spectra were recorded on a JEOL JNM-GX 270 (270 MHz) or Bruker ARX 500 (500 MHz) spectrometer with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet), or br (broad). All exchangeable protons were detected by disappearance on the addition of D_2O. TLC was done on Merck Kieselgel F_{254} precoated plates (Merck, Germany). The silica gel used for column chromatography was YMC gel 60A (70−230 mesh) (YMC Co., Ltd., Japan). Reactions were carried out under an argon atmosphere.

Practical Synthesis of (6′S)-2′,3′-Di-*O*-(*tert*-butyldimethylsilyl)-6′-methylnaplanocin A (8S). A mixture of **5** (800 mg, 1.34 mmol) and $BaMnO_4$ (3.44 g, 13.4 mmol) in CH_2Cl_2 (80 mL) was heated under reflux. After 11 h, an additional $BaMnO_4$ (3.44 g, 13.4 mmol) was added, and the reaction mixture was heated overnight. The reaction mixture was cooled to room temperature and filtered through a Celite pad, which was washed with hot $CHCl_3$. The filtrate and washings were combined and concentrated in vacuo to give crude aldehyde **6**. An Et_2O solution of MeLi (1.03 M, 4.5 mL, 4.7 mmol) was added to a solution of $TiCl_4$ (510 μL, 4.7 mmol)

in CH_2Cl_2 (15 mL) at −78 °C. The mixture was warmed to −50 °C, and the crude **6** in CH_2Cl_2 (13 mL) was added to the resulting brown solution via a cannula over 10 min. The mixture was stirred for 30 min at the same temperature. The reaction was quenched by addition of saturated aqueous NH_4Cl, and the mixture was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was dissolved in NH_3/MeOH (20 mL, saturated at 0 °C), and the mixture was kept at room temperature overnight. The solvent was removed in vacuo, and the residue was purified on a silica gel column with EtOH in $CHCl_3$ (0−8%) to give **8S** (446 mg, 66% from **5** as a white solid): MS *m/z* 505 (M$^+$); ^1H NMR (CDCl$_3$) 8.33 and 7.76 (each s, each 1 H, H-2 and 8), 5.73 (m, 1 H, H-5′), 5.57 (br s, 2 H, NH$_2$), 5.44−5.41 (m, 1 H, H-1′), 4.77 (d, 1 H, H-3′, *J* = 4.6 Hz), 4.49−4.41 (m, 2 H, H-2′and 6′), 2.73 (br s, 1 H, OH), 1.43 (d, 3 H, 6′-Me, *J* = 6.6 Hz), 0.91 and 0.74 (each s, each 9 H, *tert*-butyl), 0.13, (s, 6 H, Me x 2), −0.17, −0.56 (each s, each 3 H, Me).

(6′*R*)-2′,3′-Di-*O*-(*tert*-butyldimethylsilyl)-6′-methylnaplanocin A (8R). Diisopropyl azodicarboxylate (514 μL, 2.61 mmol) was added to a mixture of **8S** (440 mg, 0.87 mmol), Ph$_3$P (686 mg, 2.61 mmol), and ClCH$_2$CO$_2$H (247 mg, 2.61 mmol) in THF (18 mL) at 0 °C, and the mixture was stirred for 50 min at room temperature. The reaction was quenched by addition of ice, and the mixture was partitioned between AcOEt and saturated aqueous NaHCO$_3$. The organic layer was washed with H$_2$O and brine, dried (Na$_2$SO$_4$), and concentrated in vacuo. The residue in NH$_3$/MeOH (20 mL, saturated at 0 °C) was kept at room temperature overnight. The solvent was removed in vacuo, and the residue was purified on a silica gel column with EtOH in CHCl$_3$ (0−16%) to give **8R** (398 mg, 90% as a white solid). An analytical sample was crystallized from AcOEt−hexane to give white crystals: mp 211 °C; MS *m/z* 505 (M$^+$); ^1H NMR (CDCl$_3$) 8.31 and 7.72 (each s, each 1 H, H-2 and 8), 5.83 (m, 1 H, H-5′), 5.57 (br s, 2 H, NH$_2$), 5.44−5.42 (m, 1 H, H-1′), 4.58 (d, 1 H, H-3′, *J* = 5.0 Hz), 4.43−4.38 (m, 1 H, H-6′), 4.41 (t, 1 H, H-2′, *J* = 5.0 Hz), 2.64 (br s, 1 H, OH), 1.42 (d, 3 H, 6′-Me, *J* = 6.6 Hz), 0.91 and 0.76 (each s, each 9 H, *tert*-butyl), 0.12, 0.10, −0.15, −0.48 (each s, each 3 H, Me). Anal. (C$_{24}$H$_{43}$N$_5$O$_3$Si$_2$) C, H, N.

(6′*R*)-6′-Methylnaplanocin A (RMNPA, 2). A solution of **8R** (395 mg, 0.78 mmol) and NH$_4$F (303 mg, 8.4 mmol) in MeOH (15 mL) was heated for 18 h under reflux. The solvent was removed in vacuo, and the residue was purified on a silica gel column with EtOH in CHCl$_3$ (10−40%) to give **2** (160 mg, 74% as a white solid, which was crystallized from EtOH−H$_2$O): mp 210 °C (lit.[10a] 211 °C); ^1H NMR (DMSO-d_6) 8.10 and 8.01 (each s, each 1 H, H-2 and 8), 7.15 (br s, 2 H, NH$_2$), 5.66 (s, 1 H, H-5′), 5.30 (m, 1 H, H-1′), 5.10, 4.88, and 4.83 (each d, each 1 H, 2′, 3′, and 5′-OH), 4.49 (m, 1 H, H-3′), 4.43−4.32 (m, 1 H, H-6′), 4.24 (m, 1 H, H-2′), 1.24 (d, 3 H, 6′-Me, *J* = 6.6 Hz). Anal. (C$_{12}$H$_{15}$N$_5$O$_3$) C, H, N.

Supporting Information Available: Experimental Section to synthesize the compounds **4**, **5**, and **6**, and the reactions of **6** with MeTiCl₃, MeLi, and MeMgBr described in Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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