New Neplanocin Analogues. VIII. Synthesis and Biological Activity of 6′-*C*-Ethyl, -Ethenyl, and -Ethynyl Derivatives of Neplanocin A[1)]

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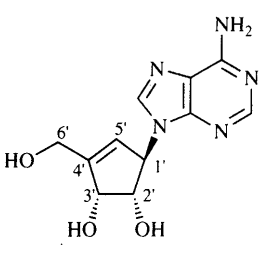
Received February 3, 1997; accepted March 10, 1997

This report describes the synthesis and antiviral effects of (6′*R*)-6′-*C*-ethynyl, -ethenyl, and -ethyl derivatives of neplanocin A (7a, 8a, and 9a, respectively) and the corresponding 6′*S*-diastereomers (7b, 8b, and 9b, respectively), as examples of 6′-*C*-substituted analogues of neplanocin A. Grignard reaction of the 6′-formyl derivative 4, which was readily prepared from neplanocin A, with ethynylmagnesium bromide gave a diastereomeric mixture of the corresponding 1,2-addition products 5a and 5b. After removal of the protecting groups, (6′*R*)- and (6′*S*)-6′-*C*-ethynylneplanocin A's (7a, 7b) were separated. The corresponding ethenyl derivatives 8a and 8b and ethyl derivatives 9a and 9b were prepared by catalytic hydrogenation of 7a and 7b, respectively. As compared to neplanocin A, the new neplanocin A derivatives were much weaker inhibitors of *S*-adenosyl-L-homocysteine hydrolase, the *R*-diastereomers being more inhibitory than the *S*-diastereomers. The decreasing order of activity was 7a > 8a > 7b > 9a > 8b > 9b. The cytotoxicity (for CEM cells) followed exactly the same order. Of these compounds, (6′*R*)-6′-*C*-ethynylneplanocin A (7a, RENPA) showed an antiviral activity spectrum that was comparable to, and an antiviral specificity that was higher than, that of neplanocin A. RENPA was particularly active against those viruses (*i.e.* vaccinia virus, vesicular stomatitis virus) that are known to be highly sensitive to AdoHcy hydrolase inhibitors.

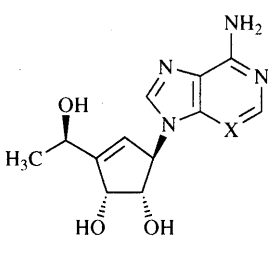
Key words *S*-adenosylhomocysteine hydrolase; neplanocin A; antiviral agent; adenosine deaminase; modified Mosher's method

In recent years, much attention has been focused on the broad-spectrum antiviral activity of *S*-adenosylhomocysteine (AdoHcy) hydrolase inhibitors.[2–4)] AdoHcy hydrolase is responsible for the hydrolysis of AdoHcy to adenosine (Ado) and L-homocysteine (Hcy),[2,3)] and is a key enzyme in transmethylation reactions using *S*-adenosyl-L-methionine (AdoMet) as a methyl donor.[2)] Because such transmethylation reactions are involved in the maturation of viral mRNAs and are critical in the virus replicative cycle, inhibitors of AdoHcy hydrolase are assumed to achieve their broad-spectrum antiviral activity by the inhibition of transmethylation reactions.[2–4)] In fact, a close correlation has been found between the antiviral activity of a series of Ado analogues and their inhibitory effects on AdoHcy hydrolase.[5)]

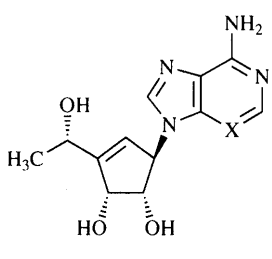
Neplanocin A (NPA, **1**),[6)] a carbocyclic nucleoside antibiotic, which is one of the most potent AdoHcy inhibitors, has a notable antiviral effect *in vitro*[7)]; however, it also has a toxic effect on the host cells.[8)] The mechanism of action of NPA has been extensively explored[8,9)]; the cytotoxic effect could be attributed mainly to phosphorylation of the primary hydroxyl group at the 6′-position by Ado kinase and subsequent metabolism by cellular enzymes,[8)] while the antiviral effect would be due to the inhibition of AdoHcy hydrolase *via* suppression of virus mRNA maturation.[9)] NPA is known to be rapidly deaminated by Ado deaminase to the chemotherapeutically inactive inosine congener,[10,11a)] which would reduce the therapeutic potency of NPA. Chemical modifications of NPA have been extensively investigated to develop efficient antiviral agents.[11,12)] We have chosen the 6′-moiety of NPA as the target site for modifications because of its important role in interactions with the above enzymes, namely, AdoHcy hydrolase, Ado deaminase, and Ado kinase. Thus, we have prepared various 6′-modified derivatives of NPA.[11a,c,e)] Throughout this study, (6′*R*)-



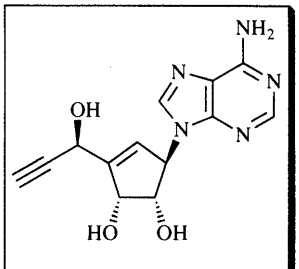
NPA (**1**)



2a (RMNPA): X = N
3a : X = CH



2b (SMNPA): X = N
3b : X = CH



7a (RENPA)

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6′-*C*-methyl-NPA (**2a**, RMNPA) and the corresponding 3-deaza derivative **3a** were found to have better antiviral effects than NPA, especially in terms of the selectivity index.[11a,c] Interestingly, the corresponding 6′-diastereomers, **2b** and **3b**, were almost inactive.[11a,c] The 6′-*C*-methyl function may restrict the conformation of the biologically important 6′-moiety of NPA and may also cause steric hindrance and thus affect the interactions between the enzymes and the NPA derivatives. Consequently, the biological activity of these compounds may depend on the configuration at the 6′-position. In fact, RMNPA and its *S*-diastereomer SMNPA are recognized differently by both AdoHcy hydrolase and Ado deaminase; RMNPA is inhibitory to AdoHcy hydrolase but SMNPA has no effect on the enzyme; on the other hand, SMNPA is deaminated by Ado deaminase, but RMNPA is virtually resistant to the deamination.[11a,h]

These results suggest that the biological effects of the 6′-*C*-substituted derivatives of NPA may depend on the nature of the substituents, as well as the configuration, at the 6′-position. Here, we describe the synthesis and biological activity of the (6′*R*)- and (6′*S*)-6′-*C*-ethynyl, -ethenyl, and -ethyl derivatives of NPA (**7a**, **7b**, **8a**, **8b**, **9a**, and **9b**, respectively).

Results and Discussion

We planned to synthesize the target compounds from the 6′-formyl derivative **4**, which was readily prepared from NPA.[11a] We have reported that the reaction of the enal system of **4** with Me_3Al in CH_2Cl_2 yielded preferentially the 1,2-addition product.[11a] However, when **4** was treated with Et_3Al instead of Me_3Al, the corresponding 1,2-addition product was obtained in only poor yield. Similarly, Grignard reactions of **4** with ethyl- or ethenylmagnesium bromide in tetrahydrofuran (THF) at −75 °C were unsuccessful. However, when **4** was treated with ethynylmagnesium bromide in THF at −75 °C, the desired 1,2-addition product **5** was obtained in 72% yield as a pair of 6′-diastereomers (**5a**, **5b**) in a ratio of about 4:1, as judged from the ^{1}H-NMR spectrum. Removal of the *N*-benzoyl group of the diastereomeric mixture of **5a** and **5b** was done with NaOMe/MeOH to give a mixture of **6a** and **6b**. Hydrolysis of the isopropylidene group of **6a** and **6b** with aqueous formic acid gave the free diastereomeric mixture of **7a** and **7b**, from which the two individual diasteromers, **7a** and **7b**, were obtained in pure form by preparative reverse-phase HPLC.

The 6′-configuration was successfully determined by use of the modified Mosher's method[13] and X-ray crystallographic analysis. From the mixture of **6a** and **6b**, the major diastereomeric **6a** was crystallized from hot MeOH in a pure form in 34% isolated yield. The 6-amino function of **6a** was protected again with a benzoyl group by the usual method to give **5a**, which was converted to the corresponding 6′-*O*-(*R*)- and 6′-*O*-(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters. From the $\Delta\delta$ ($\delta_S - \delta_R$) values of their ^{1}H-NMR spectra, as shown in Fig. 1, the 6′-configuration of the major diastereomer was determined as *R*. This result was confirmed by the X-ray

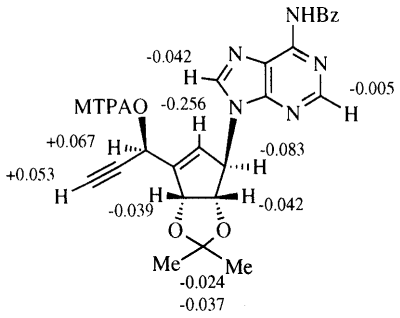


Fig. 1. $\Delta\delta$ Value (ppm) Obtained for the MPTA Esters of **6a**

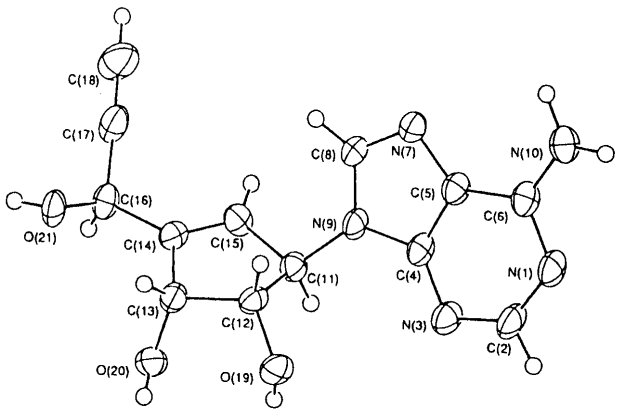
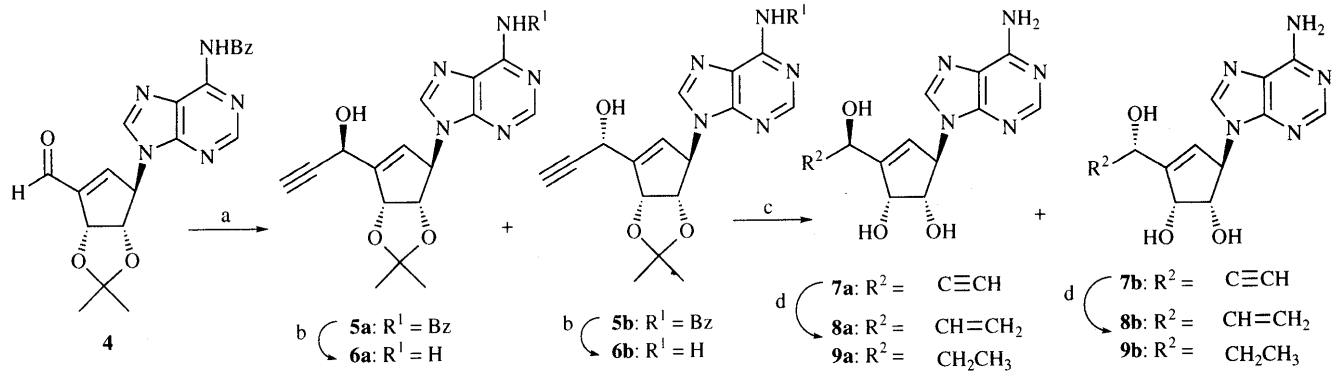


Fig. 2. X-Ray Crystallographic Structure of **7b**



Reagents: a) ethynylmagnesium bromide, THF; b) NaOMe, MeOH; c) 50 % HCOOH; d) Pd/$CaCO_3$, H_2, MeOH

Chart 1

crystallographic analysis of the 6′*S*-diastereomer **7b**, as shown in Fig. 2.

Because direct introduction of an ethyl or ethenyl group at the 6′-position of **4** by nucleophilic addition reactions with ethyl- or ethenyl organometallics was unsuccessful, we tried to prepare the 6′-ethenyl and ethyl derivatives (**8a**, **b** and **9a**, **b**, respectively) from the ethynyl derivatives **7a** and **7b** by catalytic hydrogenation. We found that the 6′-ethynyl group was hydrogenated selectively to afford into the ethenyl or the ethyl group in the presence of Lindlar catalyst without damaging the olefin moiety of the cyclopentene ring. Compound **7a** was treated with 5% $Pd/CaCO_3$ under atmospheric pressure of hydrogen at room temperature for 1 h to afford the partially hydrogenated ethenyl derivative **8a** in 84% yield. When the hydrogenation reaction was continued for a longer time (24 h) under the same conditions, the saturated (6′*R*)-6′-ethyl derivative **9a** was isolated in 74% yield. In the same way, the (6′*S*)-6′-ethenyl analogue **8b** and (6′*S*)-6′-ethyl analogue **9b** were obtained from the corresponding 6′*S*-ethynyl diastereomer **7b**.

Table 1. Inhibitory Effects of New NPA Analogues on L929 Cell AdoHcy Hydrolase Activity[a]

Compound	IC_{50} (μM)	K_i (nM)
7a	0.242±0.0025	132.8±7.2
8a	2.15±0.3	—
9a	40±12	—
7b	16±2.5	—
8b	287±27	—
9b	>500	—
1 (NPA)	0.0057±0.0015	3.4±0.3

a) The inhibitory effect was measured in the synthetic direction.

First, the new NPA analogues were examined for their susceptibility to deamination by calf intestinal adenosine deaminase. The compounds (0.5 mM) were incubated in the presence of Ado deaminase (0.8 unit/ml) at 25 °C in Tris–HCl buffer (pH 7.2). All of the newly synthesized compounds were completely resistant to deamination by the enzyme, although NPA was rapidly deaminated to the inactive inosine congener under the same reaction conditions. These results contrast with previous results for the 6′-*C*-methyl derivatives, RMNPA and SMNPA.[11a])

The compounds were next examined for their inhibitory effects on murine L929 cell AdoHcy hydrolase activity (Table 1). Inspection of the 50% inhibitory concentrations (IC_{50}) revealed two trends: (i) the 6′*R*-diastereomers were much more active than the 6′*S*-diastereomers, and (ii) their inhibitory effect decreased in the order ethynyl > ethenyl > ethyl derivatives. In order of decreasing inhibition of AdoHcy hydrolase activity, the compounds ranked as follows: **7a** > **8a** > **7b** > **9a** > **8b** > **9b**. When analyzed for their effects on the viability of the human CEM T-cells, the order of (decreasing) activity was again **7a** > **8a** > **7b** > **9a** > **8b** > **9b** (Table 2), which suggests that the inhibitory effects of the compounds on cell viability may indeed be due to their interference with AdoHcy hydrolase activity.

The compounds were also evaluated for their activity against a wide range of viruses, including herpes simplex viruses type 1 (HSV-1) and type 2 (HSV-2), thymidine kinase-deficient (TK^-) HSV-2, vaccinia virus (VV), vesicular stomatitis virus (VSV), Coxsackie B4 virus, cytomegalovirus (CMV), varicella-zoster virus (VZV), influenza A and B viruses, Sindbis virus, arenaviruses Junin

Table 2. Antiviral Activity and Cytotoxicity of New NPA Analogues in Different Cell Systems

Virus	Cell	IC_{50} (μg/ml)[a]						
		7a	**8a**	**9a**	**7b**	**8b**	**9b**	**1** (NPA)
HSV-1 (KOS)	E_6SM	>400	>200	>400	>100	>100	>200	70
HSV-2 (G)	E_6SM	>400	>200	>400	>100	>100	>200	≥40
TK-HSV-1 (B2006)	E_6SM	>400	>200	>400	>100	>100	>200	20
VV	E_6SM	0.07	0.2	7	4	70	>200	0.7
VSV	E_6SM	0.2	0.2	1	20	>100	>100	2
Coxsackie B4	HeLa	>400	>200	>400	>100	>100	>100	≥40
CMV (AD-169)	HEL	>50	>50	>50	>50	>50	>50	0.3
CMV (Davis)	HEL	>50	>50	>50	>50	>50	>50	0.4
VZV (Oka, YS)	HEL	15	>50	>50	>50	>50	>50	10
TK-ZVV (07/1, YS/R)	HEL	35	>50	>50	>59	>50	>50	9
Influenza A H_2N_2 (Japan 57)	MDCK	>50	>50	>250	>50	>250	—	—
Influenza H_2N_2 (X31)	MDCK	>50	>50	>250	>50	>250	>250	—
Influenza B (Hong Kong)	MDCK	>50	>50	>250	>50	>250	>250	—
Sindbis	Vero	>400	—	>400	>100	>100	>200	7
Junin	Vero	>50	>50	>50	>50	>50	50	0.5
Tacaribe	Vero	>50	>50	>50	>50	>50	50	1.5
HIV-1	CEM	>40	>40	>200	>40	>200	>200	—
HIV-2	CEM	>40	>40	>200	>40	>200	>200	—
Morphology	E_6SM	>400	>200	>400	>100	>100	>200	70
Morphology	HeLa	>400	>200	>400	>100	>100	>100	40
Cell growth	HEL	>50	>50	>50	>50	>50	>50	>20
Morphology	MDCK	>50	>50	>250	>50	>250	>250	—
Morphology	Vero	>50	>50	>50	>50	>50	>50	40
Cell viability	CEM	0.45	3.8	34	25	180	>200	—

a) Inhibitory concentration required to reduce virus-induced cytopathicity by 50%, to cause a microscopically detectable alteration of cell morphology, or to inhibit cell growth or cell viability by 50%. Results from 2 or 3 separate experiments.

Table 3. Cytotoxicity of Compounds **1**, **2a**, **7a** for Vero Cells. FM3A Cells and Tubercidin-Resistant FM3A (Tub^{r4}) Cells

Compound	IC_{50} (μg/ml)[a]				Relative resistance (Tub^{r4}/ wild-type)
	Vero		FM3A (wild-type)	FM3A (Tub^{r4})	
	Growing	Stationary	Growing	Growing	
1 (NPA)	0.26	152	0.0023	0.017	7.4
2a (RMNPA)	0.31	>500	0.19	0.23	1.2
7a (RENPA)	0.21	>500	0.15	0.23	1.5
Tubercidin	—	—	0.0072	>1.0	>140

a) Inhibitory concentration required to reduce the number of viable cells by 50%.

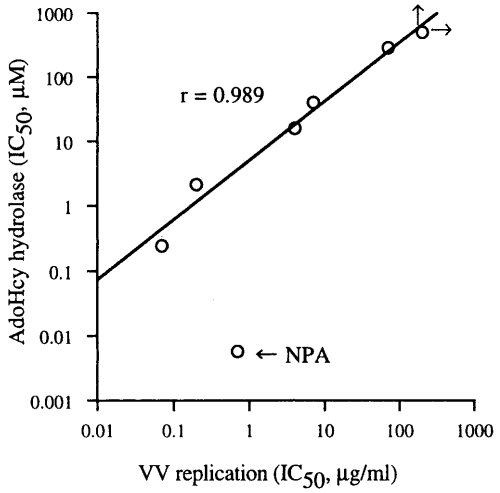


Fig. 3. Correlation between IC_{50} of New Neplanocin Analogues for L929 Cell AdoHcy Hydrolase and IC_{50} for the Replication of VV

and Tacaribe, and human immunodeficiency viruses type 1 (HIV-1) and type 2 (HIV-2). Only VV and VSV which are known to be highly sensitive to the inhibitory action of AdoHcy hydrolase inhibitors, proved susceptible to inhibition by these NPA analogues, and again, the order of decreasing activity roughly corresponded to that noted above for inhibition of AdoHcy hydrolase. Of these compounds, (6′*R*)-6′-*C*-ethynyl NPA (**7a**, RENPA) had significant antiviral effects with IC_{50} values of 0.07 μg/ml (VV) and 0.2 μg (VSV), respectively, being 10-fold more active than NPA [IC_{50} 0.7 μg/ml (VV); IC_{50} 0.2 μg/ml (VSV)].

From this series of compounds, RENPA (**7a**) appeared to be the most potent inhibitor of AdoHcy hydrolase. Although RENPA, as well as RMNPA (**2a**) showed efficient antiviral activity without cytotoxicity in stationary cells, inhibitors of AdoHcy hydrolase can inhibit cell growth.[8d,e] In fact, both RENPA and RMNPA significantly inhibited the growth of Vero cells: RENPA at an IC_{50} of 0.21 μg/ml and RMNPA at an IC_{50} of 0.31 μg/ml (Table 3). The cytotoxicity of NPA is thought to be mainly derived from the enzymatic phosphorylation of the 6′-hydroxyl group. We presumed that both RENPA and RMNPA would not be phosphorylated at the 6′-hydroxyl group in cells because the hydroxyl groups would be too sterically hindered to be phosphorylated by Ado kinase.

Therefore, we compared the effects of RMNPA, RENPA, and NPA on the growth of wild-type and tubercidin-resistant FM3A cells (FM3ATub^{r4}). In FM3A-Tub^{r4} cells, Ado kinase activity is significantly reduced.[14] The results are presented in Table 3. NPA was about 7-fold less inhibitory to the proliferation of FM3A Tub^{r4} cells than for the wild-type cells, while tubercidin was more than 100 times less cytotoxic to the Tub^{r4} cells. However, RMNPA and RENPA were equipotent against the wild-type and Tub^{r4} FM3A cells. These results argue against phosphorylation by Ado kinase as an explanation for the antiproliferative effects of RMNPA and RENPA. It is also suggested that cytotoxicity of NPA is due to its phosphorylation by Ado kinase as well as its inhibition of AdoHcy hydrolase, as reported previously,[8] though it should be mainly derived from the former.

When the IC_{50} values of the 6′-*C*-alkyl derivatives of NPA for AdoHcy hydrolase were plotted as a function of their IC_{50} for VV replication (Fig. 3), a very high correlation was found (*r* 0.989). This suggests a causal relationship between the antiviral activities of these neplanocin derivatives and their inhibitory effects on AdoHcy hydrolase. However, the result for NPA deviated from the straight line; although NPA was less active against VV when compared with RENPA (**7a**) and the corresponding 6′-ethenyl derivative **8a**, its inhibitory effect on the AdoHcy hydrolase was stronger than those of **7a** and **8a**. This result suggested that the concentration of NPA in cells may be reduced compared with that of **7a**, since NPA would be metabolized by Ado deaminase as well as Ado kinase in cells.

RENPA, as well as RMNPA, is assumed not to be phosphorylated by Ado kinase and not to be deaminated by Ado deaminase, although it significantly inhibited AdoHcy hydrolase activity. Our results suggest that both the 6′-configuration and the bulkiness around the 6′-hydroxyl group of NPA are important features for the compounds to be recognized as a substrate by all three enzymes. While RENPA and RMNPA inhibited the growth of rapidly proliferating cells, they had no effect on cells in the stationary phase. Thus, the antiviral effects noted with RENPA and RMNPA may be considered as specific, and RENPA should be further pursued for its therapeutic potential as an antiviral drug.

Experimental

Melting points were determined on a Yanagimoto MP-3 micro-melting point apparatus and are uncorrected. The NMR spectra were recorded with a JEOL EX-270 or GSX-400 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), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by the addition of D_2O. Mass spectra were measured on a JEOL JMS-D300 spectrometer. Thin-layer chromatography was carried out on Merck coated plates $60F_{254}$. Silica gel chromatography and flash silica gel chromatography were conducted with Merck Silica gel 5715 and 9385, respectively.

(6′*R*)- and (6′*S*)-N^6-Benzoyl-6′-*C*-ethynyl-2′,3′-*O*-isopropylidenenepla-nocin A (5a, 5b) A solution of ethynylmagnesium bromide (0.5 M in THF, 3.0 ml, 1.5 mmol) was added slowly to a solution of **4**[11a] (273 mg, 0.67 mmol) in THF (15 ml), at −75 °C under argon, and the resulting solution was stirred at the same temperature for 1.5 h. The mixture was warmed gradually to −20 °C and 1 N NH_4Cl (5 ml) was added. The resulting mixture was concentrated *in vacuo* (to remove THF), and then

$CHCl_3$ (15 ml) was added. The separated organic phase was dried (Na_2SO_4), evaporated, and purified by flash chromatography (silica gel; $CHCl_3$/MeOH, 80 : 1, followed by 40 : 1) to give a diastereomeric mixture of **5a** and **5b** as an oil (210 mg, 72%): FAB-MS *m/z* 432 (MH^+); ^{1}H-NMR (270 MHz, $CDCl_3$) 9.35 (br s), 8.81 (s), 8.05—7.92 (m), 7.59—7.74 (m), 6.03 (br s), 5.67 (br s), 5.56 (d, J=5.6 Hz), 5.27 (br s), 5.17 (br s), 4.80 (d, J=5.6 Hz), 2.63 (d, J=2.0 Hz), 2.63 (d, J=2.3 Hz), 1.50 (s), 1.46 (s), 1.38 (s).

(6′*R*) and (6′*S*)-6′-*C*-Ethynyl-2′,3′-*O*-isopropylideneneplanocin A (6a, 6b) A solution of NaOMe (28% in MeOH, 300 μl) was added to a solution of a mixture of **5a** and **5b** (431 mg, 1.0 mmol) in MeOH (12 ml), and the resulting solution was stirred at room temperature for 5 h. The mixture was neutralized with AcOH (1 N in benzene), and then evaporated. The residue was purified by flash chromatography (silica gel; $CHCl_3$/MeOH, 25 : 1, followed by 10 : 1) to give a diastereomeric mixture of **6a** and **6b** as a white powder (324 mg, 99%): FAB-MS *m/z* 328 (MH^+); ^{1}H-NMR ($CDCl_3$/CD_3OD; 10 : 1) 8.31 (s), 8.30 (s), 7.88 (s), 7.79 (s), 6.09 (m), 6.00 (m), 5.62 (m), 5.55 (m), 5.51 (m), 5.18 (m), 5.09 (m), 4.72 (m), 2.70 (m), 1.51 (s), 1.38 (s).

(6′*R*)- and (6′*S*)-6′-*C*-Ethynylneplanocin A (7a, 7b) A solution of the diastereomeric mixture of **6a** and **6b** (182 mg, 0.55 mmol) in aqueous formic acid (50%, 5 ml) was stirred at room temperature for 20 h. The solvent was removed *in vacuo*, and the residue was purified by flash chromatography (silica gel; $CHCl_3$/MeOH, 7 : 1, followed by 4 : 1) to give a diastereomeric mixture of **7a** and **7b** (140 mg, 88%) as a solid. This mixture was separated on a reverse-phase HPLC (column; YMC D-ODS-5, 2.5 × 25 cm; eluate, 12% aqueous MeOH) into two diastereomerically pure compounds, each as a crystalline solid (**7a**, 78 mg, eluted early; **7b**, 19 mg, eluted late). **7a**: mp 220—222 °C (recrystallized from H_2O); FAB-MS *m/z* 288 (MH^+); ^{1}H-NMR (DMSO-d_6, D_2O-added) 8.13, 8.05 (each s, each 1H, H-2, 8), 5.86 (m, 1H, H-5′), 5.36 (m, 1H, H-1′), 4.95 (dd, 1H, H-6′, J=4.8, 2.2 Hz), 4.52 (d, 1H, H-3′, J=5.5 Hz), 4.39 (dd, 1H, H-2′, J=5.5, 5.7 Hz), 3.45 (d, 1H, CH≡, J=2.2 Hz). *Anal.* Calcd for $C_{13}H_{13}N_5O_3$: C, 54.35; H, 4.56; N, 24.38. Found: C, 54.40; H, 4.83; N, 24.60. **7b**: mp >188 °C (dec., recrystallized from H_2O); FAB-MS *m/z* 288 (MH^+); ^{1}H-NMR (DMSO-d_6, D_2O-added) 8.14, 8.09 (each s, each 1H, H-2, 8), 5.95 (m, 1H, H-5′), 5.38 (m, 1H, H-1′), 4.88 (dd, 1H, H-6′, J=1.8, 2.2 Hz), 4.55 (d, 1H, H-3′, J=5.5 Hz), 4.35 (dd, 1H, H-2′, J=5.5, 5.9 Hz). 3.45 (d, 1H, CH≡, J=2.2 Hz). *Anal.* Calcd for $C_{13}H_{13}N_5O_3 \cdot 1/2H_2O$: C, 52.76; H, 4.74; N, 23.63. Found: C, 52.77; H, 4.45; N, 23.33.

Purification of 6a by Crystallization The diastereomeric mixture of **6a** and **6b** (100 mg) was treated with hot MeOH to give crystalline **6a** (34 mg) in a diastereomerically pure form: mp >240 °C; FAB-MS *m/z* 328 (MH^+); ^{1}H-NMR (DMSO-d_6) 8.14, 7.89 (each s, each 1H, H-2, 8), 7.26 (br s, 2H, NH_2), 6.06 (d, 1H, OH, J=6.6 Hz), 5.85 (br s, 1H, H-5′), 5.46 (m, 1H, H-1′), 5.45 (d, 1H, H-3′, J=5.9 Hz), 4.95 (m, 1H, H-6′), 4.78 (d, 1H, H-2′, J=5.9 Hz), 3.52 (d, 1H, CH≡, J=2.6 Hz). 1.40, 1.29 (each s, each 3H, 2 × CH_3). *Anal.* Calcd for $C_{16}H_{17}N_5O_3$: C, 58.71; H, 5.23; N, 21.39. Found: C, 58.58; H, 5.30; N, 21.48.

(6′*R*)-N^6-Benzoyl-6′-*C*-ethynyl-2′,3′-*O*-isopropylideneneplanocin A (5a) A solution of **6a** (16 mg, 0.05 mmol) in pyridine (1 ml) was treated with trimethylsilyl chloride (TMSCl) (40 μl, 0.3 mmol), and the mixture was stirred at room temperature for 30 min, BzCl (14 μl, 1.5 mmol) was added, and the whole was further stirred at room temperature for 1.5 h. Then H_2O (200 μl) was added at 0 °C, and the whole was stirred. After 5 min, 28% aqueous ammonia (200 μl) was added and the mixture was stirred at room temperature for 40 min. The resulting mixture was evaporated, and the residue was partitioned between $CHCl_3$ and brine. The organic phase was dried (Na_2SO_4), evaporated, and purified by flash chromatography (silica gel; $CHCl_3$/MeOH, 40 : 1) to give **5a** as an oil (17 mg, 76%): FAB-MS *m/z* 432 (MH^+); ^{1}H-NMR ($CDCl_3$) 9.45 (br s, 1H, N^6-H), 8.82, 7.94 (each s, each 1H, H-2, 8), 8.07—7.44 (m, 5H, Bz), 6.17 (br s, 1H, OH), 6.05 (m, 1H, H-5′), 5.68 (d, 1H, H-1′, J=1.5 Hz), 5.57 (d, 1H, H-3′, J=5.1 Hz), 5.27 (m, 1H, H-6′), 4.81 (d, 1H, H-2′, J=5.1 Hz), 2.64 (d, 1H, CH≡, J=2.5 Hz), 1.51, 1.38 (each s, each 3H, 2 × CH_3).

MTPA Esters of 6a A solution of **6a** (6.0 mg, 0.014 mmol) and 4-dimethylaminopyridine (DMAP) (9.0 mg, 0.074 mmol) in CH_3CN (0.5 ml) was treated with (+)(*R*)- or (−)(*S*)-MTPACl (10 μl, 0.029 mmol), and the resulting mixture was stirred at room temperature for 2 h. After addition of MeOH (100 μl), the mixture was evaporated, and the residue was partitioned between $CHCl_3$ and 0.5 N HCl. The organic phase was washed (brine), dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (silica gel; $CHCl_3$ followed by $CHCl_3$/MeOH, 120 : 1) to give the corresponding MTPA ester of **6a** as an oil.

(*R*)-MTPA Ester of **6a**: Yield 67%; HR-MS (FAB) Calcd for $C_{33}H_{29}F_3N_5O_6$ *m/z* 648.2070, found *m/z* 648.2073; ^{1}H-NMR ($CDCl_3$, 400 MHz) 9.03 (br s, 1H, N^6-H), 8.795 (s, 1H, H-2), 7.867 (s, 1H, H-8), 8.039—7.397 (m, 10H, Ph), 6.323 (br s, 1H, H-6′), 5.926 (br s, 1H, H-5′), 5.667 (m, 1H, H-1′), 5.608 (d, 1H, H-3′, J=5.4 Hz), 4.842 (d, 1H, H-2′, J=5.4 Hz), 3.570 (d, 3H, OMe, J=1.0 Hz), 2.710 (d, 1H, CH≡, J=2.4 Hz), 1.498, 1.390 (each s, each 3H, 2 × CH_3).

(*S*)-MTPA Ester of **6a**: Yield 56%; HR-MS (FAB) Calcd for $C_{33}H_{29}F_3N_5O_6$ *m/z* 648.2070, found *m/z* 648.2061; ^{1}H-NMR ($CDCl_3$, 400 MHz) 9.02 (br s, 1H, N^6-H), 8.790 (s, 1H, H-2), 7.825 (s, 1H, H-8), 8.038—7.398 (m, 10H, Ph), 6.390 (br s, 1H, H-6′), 5.670 (br s, 1H, H-5′), 5.584 (m, 1H, H-1′), 5.569 (d, 1H, H-3′, J=5.4 Hz), 4.800 (d, 1H, H-2′, J=5.4 Hz), 3.624 (d, 3H, OMe, J=1.0 Hz), 2.763 (d, 1H, CH≡, J=2.4 Hz), 1.461, 1.366 (each s, each 3H, 2 × CH_3).

(6′*R*)-6′-*C*-Ethenylneplanocin A (8a) A mixture of **7a** (20 mg, 0.070 mmol) and 5% $Pd/CaCO_3$ (2 mg) in MeOH (5 ml) was stirred under atmospheric pressure of hydrogen at room temperature for 1 h, and then the catalyst was filtered off. The filtrate was evaporated, and the residue was purified by reverse-phase HPLC (column; YMC D-ODS-5, 2.5 × 25 cm; eluate, 20% aqueous MeOH) to give **8a** as a crystalline solid (17 mg, 84%): mp 172—174 °C; FAB-MS *m/z* 290 (MH^+); ^{1}H-NMR (CD_3OD) 8.19, 8.05 (each s, each 1H, H-2, 8), 5.99 (ddd, 1H, H-7′, J=17.2, 10.2, 6.9 Hz), 5.96 (dd, 1H, H-5′, J=1.7, 1.7 Hz), 5.50 (m, 1H, H-1′), 5.44 (ddd, 1H, H-8′a, J=17.2, 1.3, 1.3 Hz), 5.23 (ddd, 1H, H-8′b, J=10.2, 1.3, 1.0 Hz), 4.90—4.80 (br s, CD_3OH, H-6′), 4.60 (d, 1H, H-3′, J=5.6 Hz), 4.35 (dd, 1H, H-2′, J=5.6, 5.6 Hz). *Anal.* Calcd for $C_{13}H_{15}N_5O_3 \cdot 1/5H_2O$: C, 53.31; H, 5.30; N, 23.91. Found: C, 53.27; H, 5.56; N, 24.16.

(6′*S*)-6′-*C*-Ethenylneplanocin A (8b) Compound **8b** was obtained from **7b** as a crystalline solid in 79% yield, as described above for **8a**: mp (MeOH) 211—212 °C; FAB-MS *m/z* 290 (MH^+); ^{1}H-NMR (CD_3OD) 8.09, 7.99 (each s, each 1H, H-2, 8), 5.98 (ddd, 1H, H-7′, J=17.2, 10.2, 5.9 Hz), 5.77 (dd, 1H, H-5′, J=1.7, 1.7 Hz), 5.40 (m, 1H, H-1′), 5.30 (ddd, 1H, H-8′a, J=17.2, 1.7, 1.3 Hz), 5.13 (ddd, 1H, H-8′b, J=10.2, 1.7, 1.3 Hz), 4.71 (m, 1H, H-6′), 4.62 (d, 1H, H-3′, J=5.6 Hz), 4.28 (dd, 1H, H-2′, J=5.9, 5.6 Hz). *Anal.* Calcd for $C_{13}H_{15}N_5O_3$: C, 53.97; H, 5.23; N, 24.21. Found: C, 54.09; H, 5.50; N, 24.35.

(6′*R*)-6′-*C*-Ethylneplanocin A (9a) A mixture of **7a** (10 mg, 0.035 mmol) and 5% $Pd/CaCO_3$ (3 mg) in MeOH (2 ml) was stirred under atmospheric pressure of hydrogen at room temperature for 24 h, and then the catalyst was filtered off. The filtrate was evaporated and the residue was purified by reverse-phase HPLC (column; YMC D-ODS-5, 2.5 × 25 cm; eluate, 20% aqueous MeOH) to give **9a** as a crystalline solid (8.0 mg, 79%): mp (MeOH) 210—211 °C; FAB-MS *m/z* 292 (MH^+); ^{1}H-NMR (CD_3OD) 8.09, 7.96 (each s, each 1H, H-2, 8), 5.79 (dd, 1H, H-5′, J=1.7, 1.7 Hz), 5.38 (m, 1H, H-1′), 4.58 (d, 1H, H-3′, J=5.3 Hz), 4.27 (dd, 1H, H-2′, J=5.6, 5.6 Hz), 4.23 (m, 1H, H-6′), 1.76, 1.56 (each m, each 1H, H-7′a,b), 0.92 (dd, 3H, H-8′, J=7.5, 7.3 Hz). *Anal.* Calcd for $C_{13}H_{17}N_5O_3$: C, 53.60; H, 5.88; N, 24.04. Found: C, 53.57; H, 6.01; N, 23.76.

(6′*S*)-6′-*C*-Ethylneplanocin A (9b) Compound **9b** was obtained from **8b** as a crystalline solid in 99% yield, as described above for **9a**: mp (MeOH) 229 °C; FAB-MS *m/z* 292 (MH^+); ^{1}H-NMR (CD_3OD) 8.18, 8.10 (each s, each 1H, H-2, 8), 5.90 (dd, 1H, H-5′, J=1.7, 1.3 Hz), 5.49 (m, 1H, H-1′), 4.64 (d, 1H, H-3′, J=5.3 Hz), 4.36 (dd, 1H, H-2′, J=5.9, 5.6 Hz), 4.22 (m, 1H, H-6′), 1.88, 1.66 (each m, each 1H, H-7′a,b), 1.02 (dd, 3H, H-8′, J=7.6, 7.3 Hz). *Anal.* Calcd for $C_{13}H_{17}N_5O_3$: C, 53.60; H, 5.88; N, 24.04. Found: C, 53.89; H, 6.00; N, 23.94.

X-Ray Crystallographic Data for 7b $C_{13}H_{13}N_5O_3$, M=287.28, Monoclinic, C2 (No. 5), a=23.294(6) Å, b=7.209(2) Å, c=8.201(2) Å, β=109.99(2)°, V=1294.3(6) Å^3, Z=4, D_{calcd}=1.47 g cm^{-3}. Cell parameters were determined and refined from 22 reflections in the range of 53° < 2θ < 60°. A colorless prism (0.25 × 0.25 × 0.15 mm) was mounted on a Mac Science MXC18 diffractometer with graphite-monochromated CuK_α radiation (λ=1.54178 Å). Data collection using the $\omega/2\theta$ scan technique to a maximum 2θ value of 118° gave 1137 reflections at room temperature, 1021 unique, of which 960 with $I > 3.00\sigma(I)$ were used in calculations. The intensities were corrected for the Lorentz and polarization factors, but not for the absorption or the extinction effect. The structure was solved by a direct method and refined by the full-matrix least-squares technique (Crystan-GM system[15]) as the computer program

and SIR92[16] as the structure solution method). The non-hydrogen atoms were refined anisotropically. The unweighted and weighted values (with a weighting scheme $W=\exp(20\sin^2\theta/\lambda^2)/(\sigma^2(F_o)+0.0001F_o^2)$ were 0.0335 and 0.0384, respectively. No peak above 0.42 eÅ^{-3} remained in the last Fourier-difference map.

Antiproliferative Activity Assay with FM3A Cells The cells were propagated in ES minimum medium (Nissui Pharmaceutical Co., Osaka) supplemented with 2% heat-inactivated fetal bovine serum (Gibco Lab., Grand Island, NY), in the presence of penicillin (100 U/ml) and streptomycin (100 μg/ml). Single-cell suspensions were obtained by pipetting and viable cells were counted using the trypan blue dye exclusion test. Two thousand cells in 180 μl of medium were seeded in a 96-well flat-bottomed microtest-plate (InterMed, Roskilde, Denmark), and 20 μl of drug solution of graded concentrations was simultaneously added to the wells in triplicate. The plate was incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 3 d. MTT reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, was prepared at a concentration of 2 mg/ml in Dulbecco's phosphate-buffered saline (PBS) without calcium and magnesium. On day 3, MTT reagent (25 μl) was added to each well. After further incubation for 4 h, the medium was removed by aspiration. To solubilize the resulting MTT-formazan crystals, 0.2 ml of DMSO was added to each well and thoroughly mixed by using a mechanical plate mixer. Absorbance at 540 nm (OD_{540}) was measured with an Immuno Reader NJ-2000 (InterMed Japan, Tokyo). The cell growth inhibition (%) was calculated by means of the following formula: % cell growth inhibition $=(1-T/C)\times 100$, where C is the mean OD_{540} of the control group and T is that of the treated group. The 50% inhibitory drug concentration (IC_{50} value) was determined graphically from the dose–response curve with at least 3 drug concentration points.

Effects of Adenosine Deaminase Assays were carried out according to previously reported methods.[11a]

Cytotoxicity for Vero Cells Assays were carried out according to previously reported methods.[11b]

Inhibitory Effects on AdoHcy Hydrolase Assays were carried out with the murine L929 cell-derived enzyme, as reported previously.[5]

Antiviral Activity Assays and Cytotoxicity Assays in Cells Used for the Antiviral Assays These assays were carried out according to well-established procedures.[17]

Acknowledgments We are grateful to Prof. Hideki Koyama (Yokohama City University) for his gift of wild-type and tubercidin-resistant FM3A cells. We are also grateful to Prof. Shiro Shigeta (Fukushima Medical College), and Drs. Satoshi Yaginuma and Minoru Toriya (Asahi Chemical Industry), for helpful and encouraging discussions. We also thank Anita Van Lierde, Frieda De Meyer, Anita Camps, Ann Absillis, and Ria Van Berwaer and Miette Stuyck for excellent technical assistance, and Christiane Callebaut for editorial help. This work was supported in part by grants from Biomedical Research Programme of the European Commission, the Belgian National Fonds voor Wetenschappelijk Onderzoek, and the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek.

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