

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

### New Neplanocin Analogues. V. A Potent Adenosylhomocysteine Hydrolase Inhibitor Lacking Antiviral Activity. Synthesis And Antiviral Activity Of 6''-Carboxylic Acid Derivatives Of Neplanocin A

Takumi OBARA<sup>a</sup>; Satoshi SHUUTO<sup>b</sup>; Yasuyoshi SAITO<sup>a</sup>; Minoru TORIYA<sup>a</sup>; Kiyoshi OGAWA<sup>a</sup>; Satoshi YAGINUMA<sup>a</sup>; Shiro SHIGETA<sup>c</sup>; Akira MATSUDA<sup>ab</sup>

<sup>a</sup> Institute for Life Science Research, Asahi Chemical Industry Co., Ltd., Shizuoka, Japan <sup>b</sup> Faculty of Pharmaceutical Science, Hokkaido University, Sapporo, Japan <sup>c</sup> Department of Microbiology, Fukushima Medical College, Fukushima, Japan

**To cite this Article** OBARA, Takumi , SHUUTO, Satoshi , SAITO, Yasuyoshi , TORIYA, Minoru , OGAWA, Kiyoshi , YAGINUMA, Satoshi , SHIGETA, Shiro and MATSUDA, Akira(1996) 'New Neplanocin Analogues. V. A Potent Adenosylhomocysteine Hydrolase Inhibitor Lacking Antiviral Activity. Synthesis And Antiviral Activity Of 6''-Carboxylic Acid Derivatives Of Neplanocin A', *Nucleosides, Nucleotides and Nucleic Acids*, 15: 6, 1157 — 1167

**To link to this Article:** DOI: 10.1080/07328319608007384

**URL:** <http://dx.doi.org/10.1080/07328319608007384>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

**NEW NEPLANOCIN ANALOGUES. V.  
A POTENT ADENOSYLMHOCYSTEINE HYDROLASE INHIBITOR  
LACKING ANTIVIRAL ACTIVITY. SYNTHESIS AND ANTIVIRAL ACTIVITY  
OF 6'-CARBOXYLIC ACID DERIVATIVES OF NEPLANOCIN A<sup>1</sup>**

Takumi OBARA,<sup>a</sup> Satoshi SHUTO,<sup>b</sup> Yasuyoshi SAITO,<sup>a</sup> Minoru TORIYA,<sup>a</sup> Kiyoshi OGAWA,<sup>a</sup> Satoshi YAGINUMA,<sup>a</sup> Shiro SHIGETA,<sup>c</sup> and Akira MATSUDA\*,<sup>b</sup>

<sup>a</sup> *Institute for Life Science Research, Asahi Chemical Industry Co., Ltd., Mifuku, Ohito-cho, Shizuoka 410-23, Japan*

<sup>b</sup> *Faculty of Pharmaceutical Science, Hokkaido University, Kita-12, Nishi-6, Kita-ku, Sapporo 060, Japan*

<sup>c</sup> *Department of Microbiology, Fukushima Medical College, Fukushima 960-12, Japan*

**Abstract:** The 6'-carboxylic acid derivative of neplanocin A **3** was synthesized from NPA, and was converted to the corresponding methyl ester **4** and amides **5** and **6**. These were evaluated for their anti-RNA-virus activities. Of the derivatives synthesized, only **5** was active against RNA viruses within the concentration range of 0.14-4.88 µg/mL. Compounds **3** and **5** showed a potent inhibitory effect on *S*-adenosylhomocysteine (AdoHcy) hydrolase from rabbit erythrocytes. Although a close correlation between the inhibitory effect of adenosine analogues on AdoHcy hydrolase and their antiviral potency has been demonstrated, **3** did not show any anti-RNA-virus activities.

### Introduction

The enzyme *S*-adenosylhomocysteine (AdoHcy) hydrolase, which is important in the regulation of *S*-adenosylmethionine-dependent transmethylation,<sup>2</sup> has been recognized as an important target for broad-spectrum antiviral agents.<sup>3,4</sup> Such transmethylation reactions are involved in the maturation of viral mRNAs and hence play a critical role in the virus replication cycle. In fact, a close correlation has been demonstrated between the inhibitory effects of adenosine (Ado) analogues on AdoHcy hydrolase and their antiviral potency.<sup>5</sup>

Neplanocin A (**1**, NPA),<sup>6</sup> a carbocyclic nucleoside antibiotic, is the most potent AdoHcy hydrolase inhibitor,<sup>7</sup> and also has a strong antiviral activity against various RNA and DNA viruses.<sup>8</sup> However, NPA itself is cytotoxic,<sup>9</sup> and is inactivated by Ado

deaminase soon after administration.<sup>6,8,10a</sup> It has been recognized that the detrimental toxicity of NPA could be derived, for the most part, from phosphorylation of the primary hydroxyl group at the 6'-position (corresponding to the 5'-hydroxyl group of Ado) by Ado kinase and subsequent metabolism by cellular enzymes.<sup>9</sup> Consequently, to remove or reduce such side effects, much attention has been focused on chemical modification of NPA by us<sup>10</sup> and others.<sup>11</sup>

It was reported that the 3'-hydroxyl group of NPA is oxidized by NAD<sup>+</sup> bound to AdoHcy hydrolase to form an oxidized intermediate **2**, that has an enone structure, and this intermediate **2** binds tightly to the enzyme (FIG. 1).<sup>12</sup> However, the 3'-enone derivative **2** could not be isolated due to its instability.<sup>12</sup>

There is a possibility that the derivatives having an enone structure might bind tightly to the enzyme. Therefore, we designed NPA analogues having an enone system, namely 6'-carboxylic acid (**3**, 9-[(1*R*,2*S*,3*R*)-2,3-dihydroxy-4-carboxy-4-cyclopenten-1-yl]adenine) and its derivatives **4**, **5**, and **6** as potent AdoHcy hydrolase inhibitors, which could be considered as a kind of constitutional isomer of **2** (FIG. 2). Moreover, it may be possible that designed derivatives **3**, **4**, **5**, and **6** could serve as Michael acceptors of the nucleophilic functional groups of the enzyme side chains.

## Chemistry

The synthetic route for 6'-carboxylic acid **3** and its derivatives is outlined in FIG. 3. An attempt to oxidize the 6'-hydroxymethyl moiety of *N*<sup>6</sup>-benzoyl-2,3-*O*-isopropylidene-NPA (**7**) to a carboxyl group directly by KMnO<sub>4</sub> was unsuccessful. Therefore we used a stepwise oxidation of the 6'-hydroxymethyl moiety for the preparation of **3**. First, **7** was oxidized to 6'-aldehyde **8** with BaMnO<sub>4</sub>,<sup>10a</sup> and it was further oxidized by a H<sub>2</sub>NSO<sub>3</sub>H/NaClO<sub>2</sub> system to afford 6'-carboxylic acid **9** in 68% yield from **7**. Deprotection of **9** was done by successive treatment with 25% NH<sub>4</sub>OH and 1 N HCl to afford the desired carboxylic acid **3** in 86% yield. Esterification of **3** with HCl/MeOH was done to give methyl ester **4** in 42% yield.

Next, preparation of amide derivatives **5** and **6** were examined. Ammonolysis of **4** with NH<sub>3</sub>/MeOH was unsuccessful, because of concomitant formation of multiple by-products. On the other hand, in the case of a reaction of **4** with MeNH<sub>2</sub> in EtOH, an undesired Michael addition reaction on the enone system of **4** proceeded in preference to ammonolysis of the ester moiety. When the reaction was done at 0 °C for 1 h, **10a** and **10b** were obtained in 75% and 15% yields, respectively, though **10b** was obtained as a sole product in 82% yield by a prolonged reaction period. This indicated that the Michael addition reaction occurred first, followed by the amide formation. In this case, the Michael addition reaction proceeded from the α face of the cyclopentene-ring in a *cis*

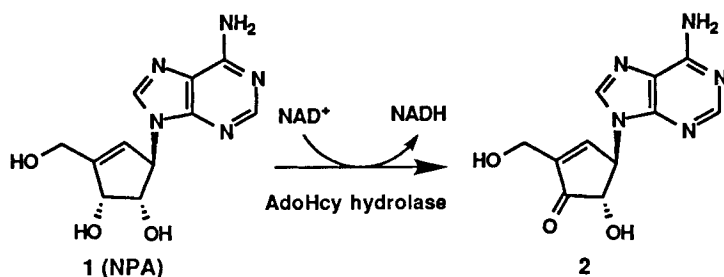


FIG. 1

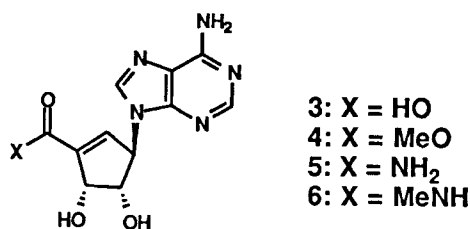
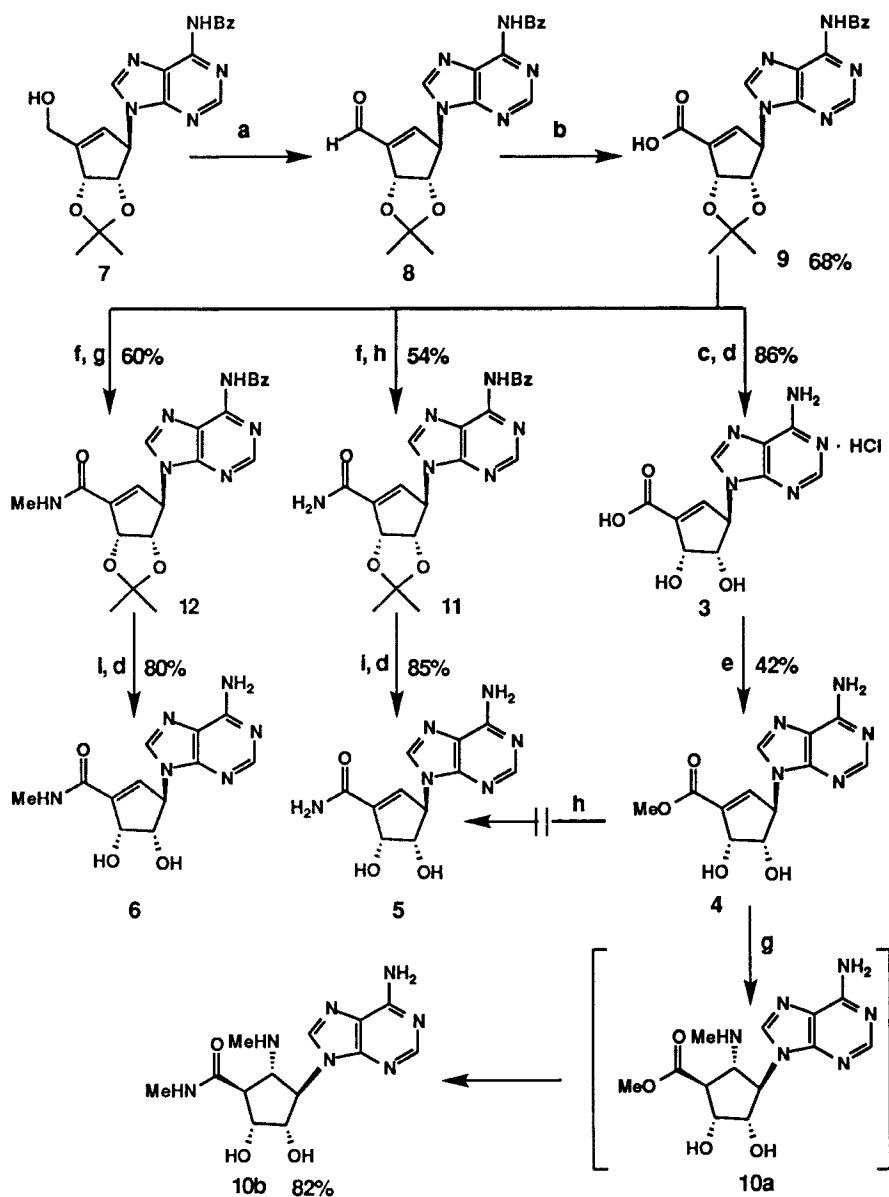


FIG. 2

manner to give **10a** initially, then it was gradually converted to its *N*-methyl-carbamoyl derivative **10b**.

Structures of the Michael adducts **10a** and **10b** were identified from the <sup>1</sup>H NMR spectra. Nuclear overhauser effect (NOE) difference experiments of **10b** done in DMSO-*d*<sub>6</sub> showed a characteristic increase of 2'-OH, 3'-OH, 1'- and 4'-proton signals when 5'-*N*-methyl protons were irradiated. Similarly, irradiation on the 2'-proton caused a characteristic increase of the 5'-proton signal as well as 8- and 3'-proton signals as illustrated in FIG. 4. These data indicated that both the 5'-MeNH group and 4'-proton were in the α face (signifying *cis* addition) of the cyclopentane ring. From a comparison of *J*<sub>4',5'</sub> values of **10a** and **10b** (6.9 Hz and 7.3 Hz, respectively), we concluded that **10a** and **10b** have the same 4',5'-stereochemistry.

This shows that methylamine selectively attacked at the 5'-position of **4** from the α face because of the steric hindrance by the adenine base, then protonation at the 4'-position occurred from the α face to minimize the steric repulsion of the product.



a)  $\text{BaMnO}_4$ ,  $\text{CHCl}_3$ ; b)  $\text{H}_2\text{NSO}_3\text{H}$ ,  $\text{NaClO}_2$ , Acetone; c) 25%  $\text{NH}_4\text{OH}$ ; d) 1 N  $\text{HCl}$ ;  
 e)  $\text{HCl}$ ,  $\text{MeOH}$ ; f)  $\text{ClCOOBu}^t$ ,  $\text{Et}_3\text{N}$ ,  $\text{DMF}$ ; g) 30%  $\text{MeNH}_2$  in  $\text{EtOH}$ ; h)  $\text{NH}_3$  gas;  
 i) reflux in  $\text{MeOH}$

Fig. 3

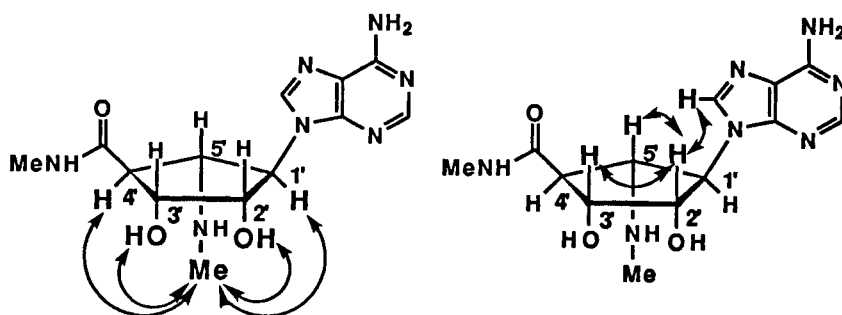
NOE interactions of **10b**.

FIG. 4

These results suggested that activation of the carboxyl moiety would be necessary to make the ammonolysis proceed prior to the Michael addition reaction. Therefore, the mixed acid-anhydride method was used. To prepare the amide derivatives **5** and **6**, 6'-carboxylic acid **9** was converted to its mixed acid-anhydride by treating it with  $\text{Et}_3\text{N}$  and  $\text{ClCOOBu}^t$  in DMF, and then it was treated with anhydrous  $\text{NH}_3$  or  $\text{MeNH}_2$  to give the corresponding amide **11** (54% yield) or *N*-methylamide **12** (60% yield), respectively. Removal of *N*-benzoyl groups of **11** and **12** with the usual  $\text{NH}_3/\text{MeOH}$  conditions were unsuccessful, probably due to the unstable enone system. However, when **11** and **12** were heated under reflux in  $\text{MeOH}$ , the *N*-benzoyl groups were deprotected to give desired **5** (85% yield) and **6** (80% yield), respectively.

### Biological activity

The susceptibilities of the derivatives to Ado deaminase from calf intestine were studied together with NPA, and the results are shown in FIG. 5. Under the conditions used, NPA itself was deaminated rapidly into a biologically inactive inosine congener, but all of the other compounds tested were completely resistant to calf intestinal Ado deaminase.

Next, we evaluated the inhibitory effects on AdoHcy hydrolase from rabbit erythrocytes and antiviral activities *in vitro*, together with cytotoxicity against Vero cells. The biological results are summarized in Table 1. None of the compounds tested except for NPA were cytotoxic to host cells (Vero) in stationary phase at a concentration up to 500  $\mu\text{g/mL}$ . Of the derivatives, only amide derivative **5** had anti-RNA-virus activities against parainfluenza-3 (PINF-3), measles, or mumps viruses within the  $\text{EC}_{50}$  range of

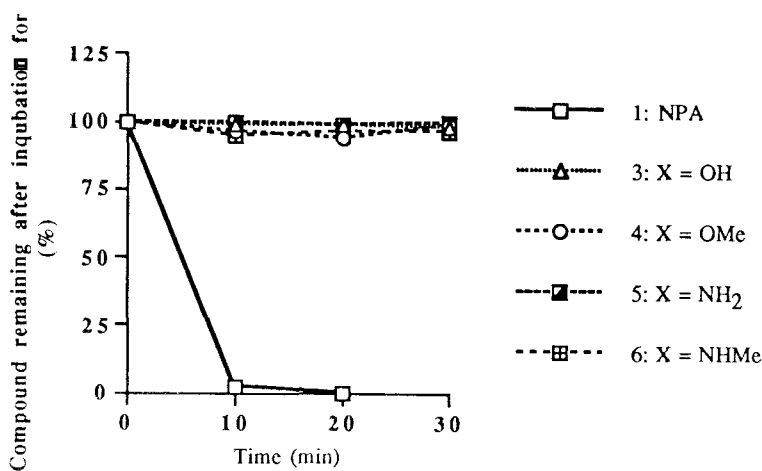


FIG. 5

Effects of calf intestinal adenosine deaminase on NPA and its derivatives.

Table 1. Antiviral activities,<sup>a</sup> cytotoxicity on Vero cells, and inhibitory effects on AdoHcy hydrolase of NPA and its derivatives

Compound (X)	EC <sub>50</sub> <sup>b</sup> (μg/mL)			IC <sub>50</sub> (μg/mL)	
	PIFV-3 (C 243)	Measles (Sugiyama)	Mumps (ECXH3)	Vero cell <sup>c</sup> (stationary)	AdoHcy hydrolase <sup>d</sup>
NPA	0.39	0.10	0.11	152 <sup>e</sup>	0.004 <sup>f</sup>
3 (OH)	>25	>25	>25	>500	0.33
4 (OMe)	>25	>25	>25	>500	>50
5 (NH <sub>2</sub> )	4.88	2.72	0.14	>500	2.56
6 (NHMe)	>25	>25	>25	>500	>50
10a	>100	ND <sup>g</sup>	ND	ND	ND
10b	>100	ND	ND	ND	ND

<sup>a</sup> Antiviral assay was done by a previously reported method.<sup>10b</sup>  
<sup>b</sup> Concentration required to inhibit virus-induced cytopathogenicity by 50%.  
<sup>c</sup> MTT method was used to measure cytotoxicity.  
<sup>d</sup> From rabbit erythrocytes; Assay was done by a previously reported method.<sup>10a</sup>  
<sup>e</sup> Data was taken by Ref. 10d. <sup>f</sup> Data was taken by Ref. 10a.  
<sup>g</sup> Not determined.

0.14–4.88  $\mu\text{g/mL}$ . 6'-Carboxylic acid derivative **3** and amide derivative **5** were potent inhibitors of AdoHcy hydrolase from rabbit erythrocytes ( $\text{IC}_{50} = 0.33 \mu\text{g/mL}$ ,  $2.56 \mu\text{g/mL}$ , respectively). Although a close correlation between the inhibitory effect of adenosine analogues on AdoHcy hydrolase and their antiviral potency has been demonstrated,<sup>4</sup> 6'-carboxylic acid derivative **3** did not show any anti-RNA-virus activities in spite of its significant inhibitory effect on AdoHcy hydrolase.

To the best of our knowledge, **3** would be the first adenosine analogue that is inactive against viruses, but inhibits AdoHcy hydrolase significantly. This may be due to its inability to pass through the cell membrane, because of its high polarity. Compound **4** did not show any inhibitory effect on AdoHcy hydrolase, in spite of having an enone structure and an ability to act as a Michael acceptor.

### Experimental section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. NMR spectra were recorded with a JEOL FX-270, or a GCX-400 spectrometer with tetramethylsilane as an internal standard. Mass spectra were measured on a JEOL SX-102 spectrometer. High-resolution mass spectra were measured on a JMX DX-303 spectrometer. Thin-layer chromatography was done on E. Merck precorted plates 60F<sub>254</sub>. Flash chromatography was conducted with E. Merck silica gel 9385. NPA were prepared according to a reported method.<sup>6</sup>

**N<sup>6</sup>-Benzoyl-9-[(1*R*,2*S*,3*R*)-2,3-*O*-isopropylidenedioxy-4-carboxy-4-cyclopenten-1-yl]adenine (9).** A mixture of **7** (1 g, 2.46 mmol) and BaMnO<sub>4</sub> (10 g, 39.00 mmol) in CHCl<sub>3</sub> (100 mL) was stirred at 65 °C for 5 h. The oxidizing agent was filtered off and the filter was washed with hot CHCl<sub>3</sub>. The filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in acetone (100 mL), and then 0.3 N H<sub>2</sub>NSO<sub>3</sub>H (17.6 mL) was added dropwise over 5 min at -30 °C. Three minutes later, 0.2 N NaClO<sub>2</sub> (14.7 mL) was added dropwise over 3 min, and the mixture was stirred at -10 °C for 30 min. To the reaction mixture, cooled water (4 °C, 100 mL) was added, and the resulting mixture was extracted with CHCl<sub>3</sub> (100 mL×3). The combined CHCl<sub>3</sub> phase was filtered through Whatman 1PS filter paper and evaporated. The residue was purified by flash chromatography (silica gel, CHCl<sub>3</sub>/MeOH, 5:1 followed by CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O, 65:25:3) to give 707 mg (68%) of **9** as a solid: <sup>1</sup>H NMR (270 MHz, CDCl<sub>3</sub>/CD<sub>3</sub>OD, 8:1)  $\delta$  8.78 and 8.10 (each s, each 1 H, H-8 and 2), 8.09 (d, 2 H, H-Ph,  $J = 6.9$  Hz), 7.62 (dd, 1 H, H-Ph,  $J = 7.3, 7.3$  Hz), 7.56 (dd, 2 H, H-Ph,  $J = 7.3, 6.9$  Hz), 6.61 (br s, 1 H, H-5'), 5.79 (br s, 1 H, H-1'), 5.72 (m, 1 H, H-3'), 4.87 (br d, 1 H, H-2',  $J = 5.3$  Hz), 1.54, 1.41 (each s, each 3 H, isopropyl-CH<sub>3</sub>); MS (FAB positive)  $m/z$  422 (MH<sup>+</sup>); HRMS (FAB positive), calcd for C<sub>21</sub>H<sub>20</sub>O<sub>5</sub>N<sub>5</sub> (MH<sup>+</sup>) 422.1464, found 422.1456.



**9-[(1R,2S,3R)-2,3-Dihydroxy-4-carboxy-4-cyclopenten-1-yl]adenine**

**hydrochloride (3).** A solution of **9** (300 mg, 0.71 mmol) in 25% NH<sub>4</sub>OH (10 mL) was stirred at 45 °C for 1 h. The solvent was removed under reduced pressure. MeOH (5 mL) was added to the residue, and the mixture was evaporated. The residue was dissolved in 1 N HCl (5 mL), and the solution was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and then H<sub>2</sub>O (5 mL) was added to the residue, and the mixture was evaporated. The residue was purified on a Dowex-1 ion-exchange resin column (1×5 cm, OH<sup>-</sup> form, H<sub>2</sub>O→50% MeOH→0.1 N HCl). The fractions containing the desired compound were evaporated to dryness. The residue was dissolved in a small amount of MeOH, then Et<sub>2</sub>O was added to give 191 mg (86%) of **3** as a white precipitate: <sup>1</sup>H NMR (270 MHz, CD<sub>3</sub>OD) δ 8.40 and 8.38 (each s, each 1 H, H-8 and 2), 6.97 (d, 1 H, H-5', *J* = 1.7 Hz), 5.75 (br d, 1 H, H-1', *J* = 5.6, 1.7 Hz), 4.84 (d, 1 H, H-3', *J* = 4.6 Hz), 4.52 (dd, 1 H, H-2', *J* = 5.6, 6.9 Hz); MS (FAB positive) *m/z* 278 (MH<sup>+</sup>); HRMS (FAB negative), calcd for C<sub>11</sub>H<sub>10</sub>O<sub>4</sub>N<sub>5</sub> (M-H)<sup>-</sup> 276.0733, found 276.0715.

**9-[(1R,2S,3R)-2,3-Dihydroxy-4-methoxycarbonyl-4-cyclopenten-1-yl]adenine**

**(4).** Acetyl chloride (300 μL) was added to MeOH (3 mL), and the solution was stirred for 30 min. To the solution, **3** (80 mg, 0.25 mmol) was added, and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and coevaporated with MeOH. To the residue, CHCl<sub>3</sub> (10 mL) was added, and the insoluble materials were collected by filtration, which was purified by flash chromatography (silica gel, CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O, 65:25:3) to give 31 mg (42%) of **4** as a solid. An analytical sample was obtained by crystallization from MeOH: mp > 207 °C (dec.); <sup>1</sup>H NMR (270 MHz, CD<sub>3</sub>OD) δ 8.07 and 8.06 (each s, each 1 H, H-8 and 2), 6.90 (d, 1 H, H-5', *J* = 1.7 Hz), 5.54 (br d, 1 H, H-1', *J* = 6.9 Hz), 4.82–4.70 (m, H-3' and MeOH), 4.46 (dd, 1 H, H-2', *J* = 6.9, 5.6 Hz), 3.72 (s, 3 H, CH<sub>3</sub>O); MS (FAB positive) *m/z* 292 (MH<sup>+</sup>). Anal. Calcd for C<sub>12</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>: C, 49.48; H, 4.50; N, 24.04. Found: C, 49.23; H, 4.30; N, 23.82.

**9-[(1R,2S,3R,4S,5S)-2,3-Dihydroxy-4-methoxycarbonyl-5-(*N*-methylamino)-4-cyclopenten-1-yl]adenine (10a) and 9-[(1R,2S,3R,4S,5S)-2,3-Dihydroxy-4-(*N*-methyl-carbamoyl)-5-(*N*-methylamino)-4-cyclopenten-1-yl]adenine (10b).** **Method A:** Compound **4** (35 mg, 0.12 mmol) was added to 30% MeNH<sub>2</sub> in EtOH (1 mL) at 0 °C, and the resulting mixture was stirred at the same temperature for 1 h. The solvent was removed under reduced pressure, and coevaporated with EtOH. The residue was purified by preparative TLC (silica gel; developed with CHCl<sub>3</sub>/MeOH/H<sub>2</sub>O, 65:25:3; extracted with CHCl<sub>3</sub>/MeOH, 2:1) to give 29 mg (75%) of **10a** and 6 mg (15%) of **10b** as solids, respectively. **Method B:** Compound **4** (11 mg, 0.04 mmol) was added to 30% MeNH<sub>2</sub> in EtOH (1.1 mL), and the mixture was stirred at room temperature for 27 h. The reaction mixture was worked up according to the procedure in method A to give 10 mg (82%) of

**10b** as a solid. Physical data for **10a**:  $^1\text{H}$  NMR (270 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.20 and 8.19 (each s, each 1 H, H-8 and 2), 4.71 (dd, 1 H, H-1',  $J = 8.9, 8.9$  Hz), 4.55 (dd, 1 H, H-2',  $J = 8.9, 5.3$  Hz), 4.38 (dd, 1 H, H-3',  $J = 5.3, 3.6$  Hz), 3.95 (dd, 1 H, H-5',  $J = 8.9, 6.9$  Hz), 3.79 (s, 3 H,  $\text{CH}_3\text{O}$ ), 2.89 (dd, 1 H, H-4',  $J = 6.9, 3.6$  Hz), 2.22 (s, 3 H, 5'- $\text{CH}_3\text{N}$ ); MS (FAB positive)  $m/z$  323 ( $\text{MH}^+$ ); HRMS (FAB positive), calcd for  $\text{C}_{13}\text{H}_{19}\text{N}_6\text{O}_4$  ( $\text{MH}^+$ ) 323.1468, found 323.1490. Physical data for **10b**:  $^1\text{H}$  NMR (270 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.32 and 8.20 (each s, each 1 H, H-8 and 2), 4.76 (dd, 1 H, H-1',  $J = 8.8, 8.3$  Hz), 4.49 (dd, 1 H, H-2',  $J = 8.3, 5.4$  Hz), 4.22 (dd, 1 H, H-3',  $J = 5.4, 4.4$  Hz), 3.83 (dd, 1 H, H-5',  $J = 8.8, 7.3$  Hz), 2.80 (s, 3 H, 6'- $\text{CH}_3\text{N}$ ), 2.73 (dd, 1 H, H-4',  $J = 7.3, 4.4$  Hz), 2.23 (s, 3 H, 5'- $\text{CH}_3\text{N}$ ); MS (FAB positive)  $m/z$  322 ( $\text{MH}^+$ ); HRMS (FAB positive), calcd for  $\text{C}_{13}\text{H}_{20}\text{N}_7\text{O}_3$  ( $\text{MH}^+$ ) 322.1627, found 322.1639.

***N*<sup>6</sup>-Benzoyl-9-[(1*R*,2*S*,3*R*)-2,3-*O*-isopropylidenedioxy-4-carbamoyl-4-cyclopenten-1-yl]adenine (**11**)**. To a mixture of **9** (150 mg, 0.36 mmol) and  $\text{Et}_3\text{N}$  (150  $\mu\text{L}$ , 1.07 mmol) in DMF (2 mL) at  $-7^\circ\text{C}$ ,  $\text{ClCOOBu}^i$  (91  $\mu\text{L}$ , 0.72 mmol) was added and the mixture was stirred for 30 min. Anhydrous  $\text{NH}_3$  gas was bubbled into the mixture for 7 min, and the resulting mixture was stirred at  $-5^\circ\text{C}$  for 2 h, then the mixture was evaporated. To the residue,  $\text{CHCl}_3$  (30 mL) and distilled water (5 mL) were added, and the mixture was partitioned. The organic layer was filtered through Whatman 1PS filter paper, and the filtrate was evaporated. The residue was purified by flash chromatography (silica gel,  $\text{CHCl}_3/\text{MeOH}$ , 40:1 $\rightarrow$ 30:1) to give 81 mg (54%) of **11** as solids. An analytical sample was obtained by crystallization from ethyl acetate/hexane: mp  $>146^\circ\text{C}$  (dec.);  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ )  $\delta$  8.11 (br s, 1 H, NH), 8.80 and 7.95 (each s, each 1 H, H-8 and 2), 8.02 (d, 2 H, Ph,  $J = 7.3$  Hz), 7.64-7.48 (m, 3 H, Ph), 6.72 (s, 1 H, H-5'), 6.61, 5.87 (each s, each 1 H,  $\text{NH}_2\text{CO}$ ), 5.71-5.69 (m, 2 H, H-1', 3'), 4.91 (d, 1 H, H-2',  $J = 5.9$  Hz), 1.53, 1.42 (each s, each 3 H, isopropyl- $\text{CH}_3$ ); MS (FAB positive)  $m/z$  421 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{21}\text{H}_{20}\text{N}_6\text{O}_4 \cdot 1/2 \text{H}_2\text{O}$ : C, 58.74; H, 4.93; N, 19.57. Found: C, 58.95; H, 5.03; N, 19.56.

**9-[(1*R*,2*S*,3*R*)-2,3-Dihydroxy-4-carbamoyl-4-cyclopenten-1-yl]adenine (**5**)**. A solution of **11** (35 mg, 0.08 mmol) in MeOH (2 mL) was heated under reflux for 52 h. After the mixture cooled to room temperature, 1 N HCl (2 mL) was added to the solution, and the mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure, a small amount of MeOH was added to the residue, and then the solvent was evaporated. MeOH (5 mL) and  $\text{Et}_3\text{N}$  (100  $\mu\text{L}$ ) was added to the residue, and the mixture was stirred for 5 min, then the resulting mixture was evaporated. The residual solid was collected by filtration and washed with  $\text{CHCl}_3$ , which was purified by flash chromatography (silica gel,  $\text{CHCl}_3/\text{MeOH}$ , 5:1 $\rightarrow$ 3:1 followed by  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ , 65:25:3) to give 20 mg (85%) of **5** as solids. An analytical sample was obtained by

crystallization from MeOH: mp 194–196 °C;  $^1\text{H}$  NMR (270 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.19 and 8.14 (each s, each 1 H, H-8 and 2), 6.77 (d, 1 H, H-5',  $J = 2.0$  Hz), 5.61 (br d, 1 H, H-1',  $J = 6.6$  Hz), 4.90 (d, 1 H, H-3',  $J = 5.6$  Hz), 4.53 (dd, 1 H, H-2',  $J = 5.6, 6.6$  Hz); MS (FAB positive)  $m/z$  277 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{11}\text{H}_{12}\text{N}_6\text{O}_3 \cdot \text{H}_2\text{O}$ : C, 44.90; H, 4.80; N, 28.56. Found: C, 45.20; H, 4.74; N, 28.36.

***N*<sup>6</sup>-Benzoyl-9-[(1*R*,2*S*,3*R*)-2,3-*O*-isopropylidenedioxy-4-(*N*-methylcarbamoyl)-4-cyclopenten-1-yl]adenine (12).** To a mixture of **9** (150 mg, 0.36 mmol) and  $\text{Et}_3\text{N}$  (150  $\mu\text{L}$ , 1.07 mmol) in DMF (1.5 mL) at 0 °C,  $\text{ClCOOBu}^i$  (93  $\mu\text{L}$ , 0.72 mmol) was added and the mixture was stirred for 10 min. 30%  $\text{MeNH}_2$  in EtOH (150  $\mu\text{L}$ ) was added to the mixture at -78 °C, and the mixture was stirred for 20 min at the same temperature. To the mixture, saturated  $\text{NH}_4\text{Cl}$  (15 mL) and ethyl acetate (30 mL) were added, and the mixture was partitioned. The organic layer was washed with water, filtered through Whatman 1PS filter paper, and evaporated. The residue was purified by preparative TLC (silica gel; developed and extracted with  $\text{CHCl}_3/\text{MeOH}$ , 10:1) to give 93 mg (60%) of **12** as solids. An analytical sample was obtained by crystallization from ethyl acetate / hexane: mp 149–150 °C;  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ )  $\delta$  9.11 (br s, 1 H, NH), 8.79 and 7.93 (each s, each 1 H, H-8 and 2), 8.02 (d, 2 H, Ph,  $J = 7.3$  Hz), 7.64–7.48 (m, 3 H, Ph), 6.68 (d, 1 H, H-5',  $J = 2.6$  Hz), 6.65 (br d, 1 H, NHCO,  $J = 5.0$  Hz), 5.69 (br d, 1 H, H-1',  $J = 2.0$  Hz), 5.66 (d, 1 H, H-3',  $J = 5.6$  Hz), 4.89 (d, 1 H, H-2',  $J = 5.6$  Hz), 2.97 (d, 3 H,  $\text{CH}_3\text{N}$ ,  $J = 5.0$  Hz), 1.52, 1.42 (each s, each 3 H, isopropyl- $\text{CH}_3$ ); MS (FAB positive)  $m/z$  435 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_4 \cdot 2/5 \text{H}_2\text{O}$ : C, 59.83; H, 5.20; N, 19.03. Found: C, 59.97; H, 4.93; N, 18.84.

**9-[(1*R*,2*S*,3*R*)-2,3-Dihydroxy-4-(*N*-methylcarbamoyl)-4-cyclopenten-1-yl]adenine (6).** A solution of **12** (53 mg, 0.12 mmol) in MeOH (10 mL) was heated under reflux for 74 h. After this cooled to room temperature, 1 N HCl (5 mL) was added to the solution, and the mixture was stirred at room temperature for 1 h. The solvent was removed under reduced pressure, and then distilled water (5 mL) was added to the residue, and the solvent was evaporated. MeOH (5 mL) and  $\text{Et}_3\text{N}$  (100  $\mu\text{L}$ ) was added to the residue, and the mixture was stirred for 5 min, and then the resulting mixture was evaporated. The residual solid was collected by filtration and washed with  $\text{CHCl}_3$ . The solid obtained was purified by flash chromatography (silica gel,  $\text{CHCl}_3/\text{MeOH}$ , 5:1→3:1 followed by  $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ , 65:25:3) to give 28 mg (80%) of **6** as solids. An analytical sample was obtained by crystallization from MeOH: mp >224 °C (dec.);  $^1\text{H}$  NMR (270 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  8.18 and 8.13 (each s, each 1 H, H-8 and 2), 6.71 (d, 1 H, H-5',  $J = 2.0$  Hz), 5.60 (br d, 1 H, H-1',  $J = 6.3$  Hz), 4.91 (d, 1 H, H-3',  $J = 5.6$  Hz), 4.52 (dd, 1 H, H-2',  $J = 5.6, 6.6$  Hz), 2.86 (s, 3 H,  $\text{CH}_3\text{N}$ ); MS (FAB)  $m/z$  278 ( $\text{MH}^+$ ). Anal. Calcd for  $\text{C}_{12}\text{H}_{14}\text{N}_6\text{O}_3 \cdot 1/2 \text{H}_2\text{O}$ : C, 48.16; H, 5.05; N, 28.08. Found: C, 48.43; H, 5.09; N, 28.20.

## REFERENCES

1. This paper constitutes part 146 of Nucleosides and Nucleotides. Part 145. Shuto, S.; Haramuishi, K.; Matsuda, A. *Tetrahedron Lett.* Submitted.
2. Ueland, P.M. *Pharmacol. Rev.* **1982**, *34*, 223-253.
3. De Clercq, E. *Biochem. Pharmacol.* **1987**, *36*, 2567-2575.
4. Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1991**, *34*, 1521-1530.
5. Cools, M.; De Clercq, E. *Biochem. Pharmacol.* **1989**, *38*, 1061-1067.
6. a) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. *J. Antibiot.* **1981**, *34*, 359-366. b) Hayashi, M.; Yaginuma, S.; Yoshioka, H.; Nakatsu, K. *J. Antibiot.* **1981**, *34*, 675-680.
7. Borchardt, R. T.; Keller, B. T.; Patel-Thombre, U. Neplanocin A. *J. Biol. Chem.* **1984**, *259*, 4353-4358.
8. De Clercq, E. *Antimicrob. Agents Chemother.* **1985**, *28*, 84-89.
9. a) Glazer, R. I.; Knode, M. C. *J. Biol. Chem.* **1984**, *259*, 12964-12969. b) Inaba, M.; Nagashima, K.; Tsukagoshi, S.; Sakurai, Y. *Cancer Res.* **1986**, *46*, 1063-1067. c) Hoshi, A.; Yoshida, M.; Iigo, M.; Tokuzen, R.; Fukukawa, K.; Ueda, T. *J. Pharmacobio-Dyn.* **1986**, *9*, 202-206.
10. a) Shuto, S.; Obara, T.; Toriya, M.; Hosoya, M.; Snoeck, R.; Andrei, G.; Balzarini, J.; De Clercq, E. *J. Med. Chem.* **1992**, *35*, 324-331. b) Shigeta, S.; Mori, S.; Baba, M.; Ito, M.; Honzumi, K.; Nakamura, K.; Oshitani, H.; Numazaki, Y.; Matsuda, A.; Obara, T.; Shuto, S.; De Clercq, E. *Antimicrob. Agents Chemother.* **1992**, *36*, 435-439. c) Shuto, S.; Obara, T.; Itoh, H.; Kosugi, Y.; Saito, Y.; Toriya, M.; Yaginuma, S.; Shigeta, S.; Matsuda, A. *Chem. Pharm. Bull.* **1994**, *42*, 1688-1690. d) Shuto, S.; Obara, T.; Kosugi, Y.; Saito, Y.; Toriya, M.; Yaginuma, S.; Shigeta, S.; Matsuda, A. *BioMed. Chem. Lett.* **1994**, *4*, 605-608.
11. For examples; a) Borcharding, D. R.; Narayanan, S.; Hasobe, M.; McKee, J. G.; Keller, B. T.; Borchardt, R. T. *J. Med. Chem.* **1988**, *31*, 1729-1738. b) Tsend, C. K. H.; Marquez, V. E.; Fuller, R. W.; Goldstein, B. M.; Arnett, G.; Hollingshead, M.; Driscoll, J. S. *J. Med. Chem.* **1989**, *32*, 1442-1446. c) Hasobe, M.; McKee, J. G.; Borcharding, D. R.; Borchardt, R. T. *Antimicrob. Agents Chemother.* **1987**, *31*, 1849-1851.
12. Paisley, S. D.; Wolfe, M. S.; Borchardt, R. T. *J. Med. Chem.* **1989**, *32*, 1415-1418.

Received December 8, 1995

Accepted January 23, 1996