triturated with 300 mL of hot MeOH. The mixture was filtered, and the MeOH filtrate was evaporated to give 1.03 g (30% yield) of 10: UV (AcOH) λ_{max} 248, 283 nm.

The MeOH-insoluble solid (2.46 g; UV (HOAc) λ_{max} 250, 302 nm) was retreated with BH_3 to afford another 0.9 g of 10 for a total yield of 56%.

DDQ (0.67 g, 2.93 mmol) was added to a solution of 10 (1.03 g, 2.93 mmol) in 100 mL of HOAc. The reaction mixture was stirred at room temperature for 18 h, then filtered. The filtrate was evaporated and the residue triturated with THF. The insoluble solid was collected by filtration and extracted with 200 mL of hot MeOH. The MeOH was evaporated to leave 0.62 g (61%) of 11. An analytical sample was obtained by trituration with hot DMF: UV (HOAc) λ_{max} 250, 270, 320 nm; NMR (DMSO-d_6) δ 2.00 (2 H, m, bridge CH_2), 3.05 (5 H, m, CH_2-C_5, 9-CH_2, 10-H), 3.88 (3 H, s, $COOCH_3$), 7.50 (2 H, d, 3',5'-ArH), 7.95 (2 H, d, 2',6'-ArH), 8.51 (1 H, s, 7-H); MS *m/e* 349. Anal. Calcd for $C_{19}H_{19}N_5O_2$·1.4 HCl: C, H, N, Cl.

5,10-Ethano-5,10-dideazaaminopterin Diethyl Ester (13). A suspension of 462 mg (1.32 mmol) of ester 11 in 8 mL of 2-methoxyethanol was treated with 1.36 mL (3.4 mmol) of 10% NaOH. The mixture was stirred at room temperature for 18 h; 10% NaOH (0.1 mL, 0.25 mmol) was added, and stirring was continued for another 20 h. The mixture was diluted with 5.5 mL of H_2O, acidified to pH 5 with AcOH, and stirred for 2 h. The tan precipitate was collected by filtration, washed with H_2O, and dried to leave 353 mg (80%) of the pteroic acid (12): MS *m/e* tris(trimethylsilyl) derivative, 551. This material was very insoluble in organic solvents and was not further characterized.

To a suspension of 335 mg (1 mmol) of the pteroic acid (12) in 7 mL of Me_2SO was added 0.28 mL (2 mmol) of Et_3N and 0.26 mL (2 mmol) of isobutyl chloroformate. The mixture was stirred for 1.5 h, and then 0.28 mL (2 mmol) of Et_3N and 479 mg (2 mmol) of diethyl L-glutamate hydrochloride were added. The mixture was stirred under argon for 4 h. The sequence was repeated as above with half the respective quantities of reagents, and the mixture was stirred for 18 h at room temperature. Ice water (70 mL) was added, and the tan precipitate was collected by filtration

followed by washing with H_2O. Chromatography on 25 g of silica gel with elution by $CHCl_3$–MeOH (97.5:2.5) afforded 218 mg (44%). Trituration with 2-propanol gave 160 mg (31%) of analytically pure tan solid: NMR ($CDCl_3$ + CD_3OD) δ 1.27 (6 H, m, CH_3), 1.8–2.6 (6 H, m, bridge CH_2, CH_2CH_2), 3.05 (5 H, m, CH_2-C_5, 9-CH_2, 10-H), 4.17 (4 H, m, OCH_2CH_3), 4.70 (1 H, m, CHNH), 7.30 (2 H, d, 3',5'-ArH), 7.78 (2 H, d, 2',6'-ArH), 8.42 (1 H, s, 7-H); MS *m/e* 520. Anal. Calcd for $C_{27}H_{32}N_6O_5$·H_2O: C, H, N.

5,10-Ethano-5,10-dideazaaminopterin (14). A solution of 139 mg (0.27 mmol) of diester 13 in 2 mL of 2-methoxyethanol was treated with 2 mL of 1 N NaOH. The mixture was stirred at room temperature for 4 h. The addition of 3 mL of H_2O gave complete solution. The pH was adjusted to 5 with HOAc, and the mixture was evaporated to dryness in vacuo (0.5 mm) without application of heat. The residue was treated with 4 mL of H_2O, and the product was collected to afford 91 mg (73%): UV (pH 13) λ_{max} 240·(ϵ 43 876), 343 nm (ϵ 5809); NMR (CF_3COOD) δ 2.35 (1 H, m, bridge CH_2), 2.57 (1 H, m, CH_2CH_2COOH), 2.68 (1 H, m, bridge CH_2), 2.75 (1 H, m, CH_2CH_2COOH), 2.98 (2 H, m, CH_2COOH), 3.36 (2 H, m, CH_2-C_5), 3.55 (1 H, m, C-10), 3.83 (2 H, m, C-9), 5.24 (1 H, m, CHNH), 7.62 (2 H, d, 3',5'-ArH), 8.04 (2 H, d, 2',6'-ArH), 8.87 (1 H, s, 7-H); MS *m/e* 752 (TMS$_4$), 680 (TMS$_3$), 608 (TMS$_2$). Anal. Calcd for $C_{23}H_{24}N_6O_5$·H_2O: C, H, N.

New Neplanocin Analogues. 1. Synthesis of 6'-Modified Neplanocin A Derivatives as Broad-Spectrum Antiviral Agents

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Novel neplanocin A analogues modified at the 6'-position, i.e., 6'-deoxy analogues (2, 3, 6, 9, 20), 6'-O-methylneplanocin A (15), and 6'-C-methylneplanocin A's (22a and 22b) have been synthesized and evaluated for their antiviral activity in a wide variety of DNA and RNA virus systems. These compounds showed an activity spectrum that conforms to that of *S*-adenosylhomocysteine hydrolase inhibitors. They were particularly active against pox- (vaccinia), paramyxo- (parainfluenza, measles, respiratory syncytial), arena- (Junin, Tacaribe), rhabdo- (vesicular stomatitis), reo-, and cytomegalovirus. In order of (increasing) antiviral activity, the compounds ranked as follows: 3 < 15 ~ 20 < 6 < 9 ~ 2 < 22a. Of the two diastereomeric forms of 22, only 22a was active; 22a surpassed neplanocin A both in antiviral potency and selectivity. Compound 22a appears to be a promising candidate drug for the treatment of pox-, paramyxo-, arena-, rhabdo-, reo-, and cytomegalovirus infections.

Introduction

The enzyme *S*-adenosyl-L-homocysteine hydrolase (AdoHcy hydrolase, SAH hydrolase), which is responsible for the (reversible) hydrolysis of *S*-adenosyl-L-homocysteine to adenosine (Ado) and L-homocysteine (Hcy), has been recognized as an important target for broad-spectrum antiviral agents.[1,2] AdoHcy hydrolase is a key enzyme in transmethylation reactions using *S*-adenosyl-L-methionine

(AdoMet, SAM) as the methyl donor. Such transmethylation reactions are involved in the maturation of viral mRNAs and hence play a critical role in the virus replicative cycle. Several adenosine analogues of both the acyclic and carbocyclic type are assumed to achieve their

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(1) De Clercq, E. *S*-Adenosylhomocysteine hydrolase inhibitors as broad-spectrum antiviral agents. *Biochem. Pharmacol.* 1987, *36*, 2567–2575.

(2) Wolfe, M. S.; Borchardt, R. T. *S*-Adenosyl-L-homocysteine hydrolase as a target for antiviral chemotherapy. *J. Med. Chem.* 1991, *34*, 1521–1530.

Scheme I[a]
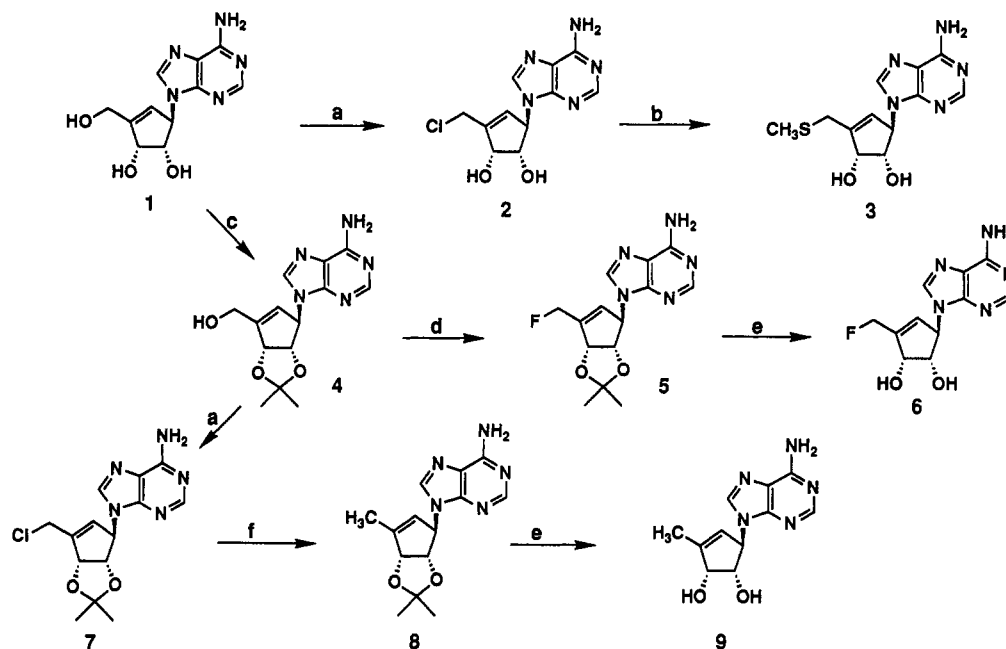
[a] Reagents: (a) Ph₃P, CCl₄, DMF; (b) NaSMe, DMF; (c) HClO₄, acetone; (d) DAST, CH₂Cl₂; (e) 50% HCOOH; (f) n-Bu₃SnH, AIBN, PhH.

broad-spectrum antiviral activity through an inhibitory effect on AdoHcy hydrolase.[1]

In fact, a close correlation has been found between the antiviral activity of a series of acyclic and carbocyclic adenosine analogues [(S)-9-(2,3-dihydroxypropyl)adenine, (RS)-3-adenin-9-yl-2-hydroxypropanoic acid (isobutyl ester), 3-deazaneplanocin A, carbocyclic 3-deazaadenosine, adenosine dialdehyde, and neplanocin A] and their inhibitory effects on AdoHcy hydrolase.[3] Moreover, within the cells, the reduction in virus yield effected by these compounds is closely correlated with increases in intracellular AdoHcy levels and elevations in AdoHcy/AdoMet ratios.[4,5]

One of the most potent AdoHcy hydrolase inhibitors is neplanocin A, and this compound also has marked activity

However, neplanocin A is not very specific as either AdoHcy hydrolase inhibitor or antiviral agent. As it can also serve as a substrate for adenosine deaminase and adenosine kinase, it may be converted to various metabolites and thus act as a "multifunctional" drug.[8] In attempts to increase the specificity of neplanocin A as an antiviral agent and/or AdoHcy hydrolase inhibitor, several neplanocin A analogues have been synthesized, i.e. 9-(hydroxyalkenyl)adenines,[9] 3-deazaneplanocin A,[10] and 9-(trans-2',trans-3'-dihydroxycyclopent-4'-enyl) derivatives of adenine (DHCA) and 3-deazaadenine (DHCDA).[11,12] As compared to neplanocin A, the compounds 3-deazaneplanocin A, DHCA, and DHCDA are indeed more specific in their antiviral action, particularly against rhabdoviruses (vesicular stomatitis) and reoviruses (rota).[13]
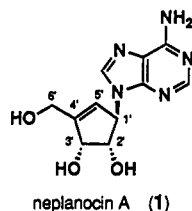
neplanocin A (1)

against a broad spectrum of RNA and DNA viruses.[6,7]

(3) Cools, M.; De Clercq, E. Correlation between the antiviral activity of acyclic and carbocyclic adenosine analogues in murine L929 cells and their inhibitory effect on L929 cell S-adenosylhomocysteine hydrolase. *Biochem. Pharmacol.* **1989**, *38*, 1061–1067.

(4) Hasobe, M.; McKee, J. G.; Borchardt, R. T. Relationship between intracellular concentration of S-adenosylhomocysteine and inhibition of vaccinia virus replication and inhibition of murine L-929 cell growth. *Antimicrob. Agents Chemother.* **1989**, *33*, 828–834.

(5) Cools, M.; De Clercq, E. Influence of S-adenosylhomocysteine hydrolase inhibitors on S-adenosylhomocysteine and S-adenosylmethionine pool levels in L929 cells. *Biochem. Pharmacol.* **1990**, *40*, 2259–2264.

(6) Borchardt, R. T.; Keller, B. T.; Patel-Thombre, U. Neplanocin A. A potent inhibitor of S-adenosylmethionine hydrolase and of vaccinia virus multiplication in mouse L-929 cells. *J. Biol. Chem.* **1984**, *259*, 4353–4358.

(7) De Clercq, E. Antiviral and antimetabolic activities of neplanocins. *Antimicrob. Agents Chemother.* **1985**, *28*, 84–89.

(8) Hasobe, M.; McKee, J. G.; Borcherding, D. R.; Keller, B. T.; Borchardt, R. T. Effects of 9-(trans-2',trans-3'-dihydroxycyclopent-4'-enyl)-adenine and -3-deazaadenine on the metabolism of S-adenosylhomocysteine in mouse L-929 cells. *Mol. Pharmacol.* **1988**, *33*, 713–720.

(9) Borcherding, D. R.; Narayanan, S.; Hasobe, M.; McKee, J. G.; Keller, B. T.; Borchardt, R. T. Potential inhibitors of S-adenosylmethionine-dependent methyltransferases. 11. Molecular dissections of neplanocin A as potential inhibitors of S-adenosylhomocysteine hydrolase. *J. Med. Chem.* **1988**, *31*, 1729–1738.

(10) Tseng, C. K. H.; Marquez, V. E.; Fuller, R. W.; Goldstein, B. M.; Haines, D. R.; McPherson, H.; Parsons, J. L.; Shannon, W. M.; Arnett, G.; Hollingshead, M.; Driscoll, J. S. Synthesis of 3-deazaneplanocin A, a powerful inhibitor of S-adenosylhomocysteine hydrolase with potent and selective in vitro and in vivo antiviral activities. *J. Med. Chem.* **1989**, *32*, 1442–1446.

(11) Hasobe, M.; McKee, J. G.; Borcherding, D. R.; Borchardt, R. T. 9-(trans-2',trans-3'-Dihydroxycyclopent-4'-enyl)-adenine and -3-deazaadenine: analogs of neplanocin A which retain potent antiviral activity but exhibit reduced cytotoxicity. *Antimicrob. Agents Chemother.* **1987**, *31*, 1849–1851.

(12) Narayanan, S. R.; Keller, B. T.; Borcherding, D. R.; Scholtz, S. A.; Borchardt, R. T. 9-(trans-2',trans-3'-dihydroxycyclopent-4'-enyl) derivatives of adenine and 3-deazaadenine: potent inhibitors of bovine liver S-adenosylhomocysteine hydrolase. *J. Med. Chem.* **1988**, *31*, 500–503.

Scheme II[a]
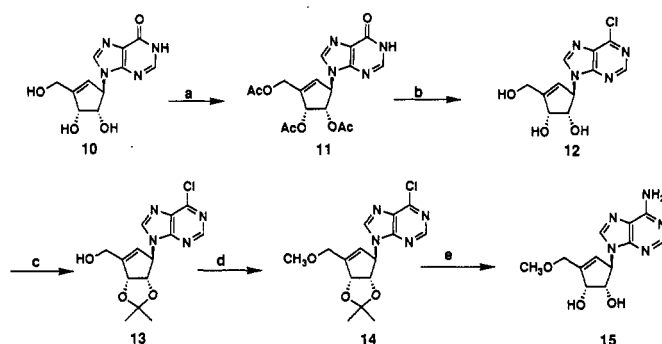
[a]Reagents: (a) Ac₂O, py; (b) (1) SOCl₂, DMF; CHCl₃, (2) NH₃, MeOH; (C) TsOH, acetone; (d) NaH, MeI, DMF; (e) (1) NH₃, MeOH, (2) 90% HCOOH.

In attempts to develop specific AdoHcy hydrolase inhibitors we designed new analogues of neplanocin A in which the 6′-hydroxymethyl group (corresponding to the 5′-hydroxymethyl group of adenosine) was modified. This group was chosen as the target for modifications because of its role in interactions with AdoHcy hydrolase, adenosine deaminase, or adenosine kinase. Two types of neplanocin A analogues were synthesized. The first type of analogues lacks the 6′-hydroxyl function (2, 3, 6, 9, 15, and 20). They cannot be phosphorylated nor are they substrate for adenosine deaminase. To the second type belong 6′-C-methylneplanocine A analogues 22a and 22b. These compounds can be considered as conformationally restricted analogues of neplanocin A. The 6′-C-methyl function may be expected to affect the interaction of these compounds with the above mentioned enzyme. In fact, the 6′-methylated derivative 22a was found to be a particularly promising candidate drug for the treatment of pox-, paramyxo-, arena-, rhabdo-, reo-, and cytomegalovirus infections.

We now report on the synthesis and antiviral activity of a new series of 6′-modified neplanocin A analogues, of which the 6′-methylated derivative (22a) emerged as a particularly promising candidate drug for the treatment of pox-, paramyxo-, arena-, rhabdo-, reo-, and cytomegalovirus infections.

Chemistry

The synthesis of the 6′-deoxyneplanocin A derivatives 2, 3, 6, and 9 is outlined in Scheme I. Neplanocin A (NPA, 1) was treated with carbon tetrachloride/triphenylphosphine system in DMF[14] to afford 6′-chloro-6′-deoxy derivative 2 in excellent yield. The reaction of 2 with sodium thiomethoxide in DMF gave 6′-methylthio derivative 3. To prepare 6′-fluoro derivative 6, the 2′,3′-cis-diol of NPA was protected by an isopropylidene group. Then fluorination at the 6′-position of acetonide 4 was achieved by treatment with (diethylamido)sulfur trifluoride (DAST) in dichloromethane to afford 6′-deoxy-6′-fluoro derivative 5. Although radical reduction of 2 with tri-n-butyltin hydride was unsuccessful, the 6′-chloro group was readily reduced when the corresponding isopropylidene-protected compound 7 was used to afford 6′-deoxy derivative 8. Deprotection of 5 and 8 was carried out with aqueous

Table I. Effects of Calf Intestinal Adenosine Deaminase on the Neplanocin A Analogues

| compd	% of total compound remaining after incubation		compd	% of total compound remaining after incubation	
	15 min	30 min		15 min	30 min
2	100	99	20	100	98
3	99	100	22a	101	100
6	100	101	22b	82	74
9	99	98	neplanocin A	5	0
15	97	98			

formic acid to afford 6 and 9, respectively.

The synthetic route for 6′-O-methylneplanocin A (15) is outlined in Scheme II. An attempt to alkylate the 6′-hydroxyl group of 4 or 17 directly with methyl iodide in the presence of a base (NaH or Et₃N) was unsuccessful. Under these conditions, the N⁶-position of the adenine moiety was preferentially methylated. The nucleophilic displacement of the chloro group of 7 at the 6′-position by methoxy anion was also unsuccessful. Therefore, we selected 6-chloropurine derivative 13 as a synthetic intermediate for 15. Transformation of 6-chloropurine to adenine can be easily achieved because of the nucleophilic lability of the 6-chloro group.[15] The 6-chloropurine congener of NPA (12) was prepared by a known procedure[16] in three steps from neplanocin D (10),[17] the minor component of the naturally occurring neplanocin family. Then 12 was converted to acetonide 13 by a standard method. The reaction of the 6′-O-sodium salt of 13, prepared from 13 and sodium hydride, with methyl iodide in DMF, afforded methyl ether 14 in 47% yield. Successive treatment of 14 with methanolic ammonia and aqueous formic acid afforded the target compound 6′-O-methylneplanocin A (15).

6′-Carbon-substituted analogues 20, 22a, and 22b were prepared as shown in Scheme III. First, the 6-amino and 2′,3′-cis-diol groups of neplanocin A were protected with a benzoyl[18] and isopropylidene group, respectively. Thus was obtained 17. Oxidation of the allyl alcohol moiety of 17 with barium manganate in 1,2-dichloroethane gave aldehyde 18, which was positive in the (2,4-dinitrophenyl)hydrazine spray test on TLC. Because of its in-

(13) De Clercq, E.; Cools, M.; Balzarini, J.; Marquez, V. E.; Borcherding, D. R.; Borchardt, R. T.; Drach, J. C.; Kitaoka, S.; Konno, T. Broad-spectrum antiviral activities of neplanocin A, 3-deazaneplanocin A, and their 5′-nor derivatives. *Antimicrob. Agents Chemother.* 1989, 33, 1291–1297.

(14) Verheyden, J. P. H.; Moffatt, J. G. Halo sugar nucleosides. III. Reaction for the chlorination and bromination of nucleoside hydroxyl groups. *J. Org. Chem.* 1972, 37, 2289–2299.

(15) (a) Fox, J. J.; Wempen, I.; Hampton, A.; Doerr, I. L. Thiation of nucleosides. I. Synthesis of 2-amino-6-mercapto-9-β-D-ribofuranosylpurine ("thioguanosine") and related purine nucleosides. *J. Am. Chem. Soc.* 1958, 80, 1669–1675. (b) Tanaka, H.; Uchida, Y.; Shinozaki, M.; Hayakawa, H.; Matsuda, A.; Miyasaka, T. A simplified synthesis of 8-substituted purine nucleosides via lithiation of 6-chloro-9-(2,3-O-isopropylidene-β-D-ribofuranosyl)purine. *Chem. Pharm. Bull.* 1983, 31, 787–790. (c) Nair, V.; Purdy, D. F. Synthetic approaches to new doubly modified nucleosides: congeners of cordycepin and 2′-deoxyadenosine. *Tetrahedron* 1991, 47, 365–382.

(16) Ikehara, M.; Uno, H.; Ishikawa, F. Study of nucleosides and nucleotides. XXIII. A versatile method for replacement of 6-hydroxyl group of purine nucleosides. *Chem. Pharm. Bull.* 1964, 12, 267–271.

(17) (a) Yaginuma, S.; Muto, N.; Tsujino, M.; Sudate, Y.; Hayashi, M.; Otani, M. Studies on neplanocin A, new antitumor antibiotic. I. Producing organism, isolation and characterization. *J. Antibiot.* 1981, 34, 359–366. (b) Hayashi, M.; Yaginuma, S.; Yoshioka, H.; Nakatsu, K. Studies of neplanocin A, new antitumor antibiotic. II. Structure determination. *J. Antibiot.* 1981, 34, 675–680. (c) Hayashi, M.; Yaginuma, S.; Muto, N.; Tsujino, M. Structures of neplanocins, new antitumor antibiotics. *Nucleic Acids Res. Symp. Ser.* 1980, 8, s65–s67.

(18) Ti, G. S.; Gaffney, B. L.; Jones, R. A. Transient protection: efficient one-flask synthesis of protected deoxynucleosides. *J. Am. Chem. Soc.* 1982, 104, 1316–1319.

Scheme III[a]
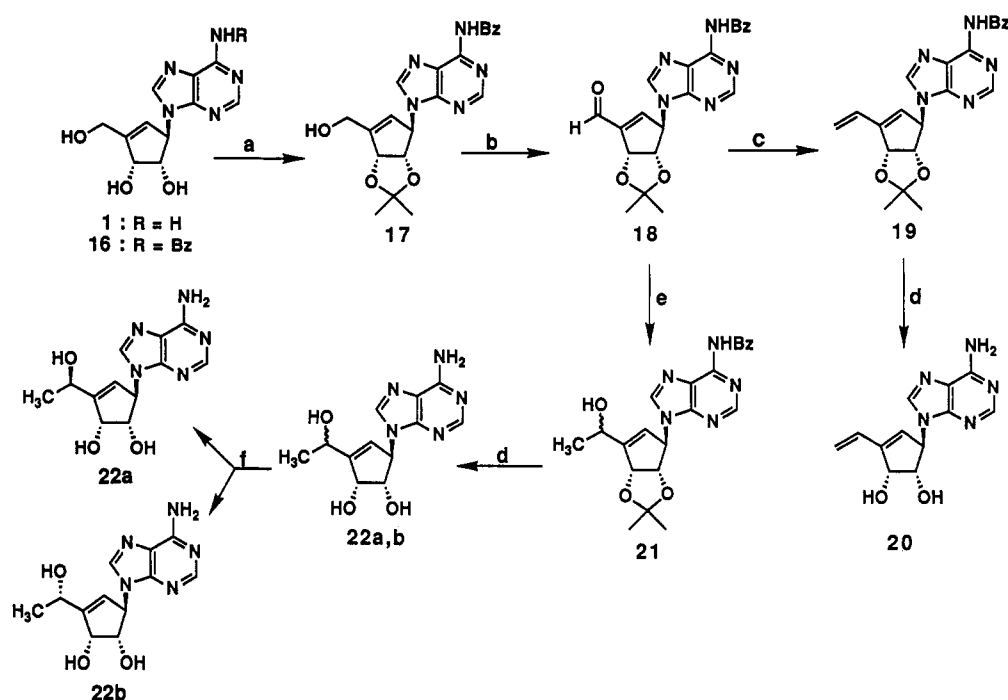
[a] Reagents: (a) $HClO_4$, acetone; (b) $BaMnO_4$, CH_2ClCH_2Cl; (c) Ph_3PCH_3Br, n-BuLi, THF; (d) (1) 90% HCOOH, (2) NH_4OH, dioxane; (e) Me_3Al, CH_2Cl_2; (f) HPLC (C_{18}).

Table II. Inhibitory Effects of the Neplanocin A Analogues on Rabbit Erythrocyte and Murine L929 Cell S-Adenosylhomocysteine Hydrolase Activity[a]

compd	rabbit erythrocyte IC_{50}, μg/mL	murine L929 K_i, μM	compd	rabbit erythrocyte IC_{50}, μg/mL	murine L929 K_i, μM
2	>10		20	>10	
3	>10		22a	0.20	0.086 ± 0.018
6	>10		22b	>10	55.7 ± 17.9
9	>10	0.15 ± 0.025	neplanocin A	0.004	
15	>10				

[a] Inhibitory effect on S-adenosylhomocysteine hydrolase was measured in the synthetic direction. [b] Data taken from ref 3.

stability this compound was used immediately without purification in the next reactions. Wittig reaction of **18** with triphenylphosphonium methylide in THF afforded 6′-methylene derivative **19** in 46% yield. Deprotection of **19** was performed by acidic hydrolysis, followed by ammonolysis to yield 6′-deoxy-6′-methylidine derivative **20**. Reaction of the α,β-unsaturated carbonyl system of **18** with trimethylaluminium in dichloromethane yielded preferentially 1,2-addition product **21** in 54% yield, and no 1,4-addition product was isolated in this reaction. It was recognized that the 6′-C-methylated product **21** was obtained as a pair of 6′-diastereomers, and the ratio was ca. 3:1 from its ^{1}H NMR spectral data. When methyllithium was used as an alkylating agent, instead of trimethylaluminium, in THF, no methylated product could be isolated. The protecting groups of **21** were removed in the usual manner to afford the diastereomeric mixture (**22a** and **22b**), from which the two individual diastereomers (**22a**, eluted early, and **22b**, eluted late)[19] were effectively separated by preparative, reverse-phase HPLC.

Results and Discussion

To be able to interfere with the AdoHcy hydrolase re-

action, the neplanocin analogues should not be prematurely deaminated by adenosine deaminase. When compounds **2, 3, 6, 9, 15, 20, 22a**, and **22b** were examined for their susceptibility to deamination by calf intestinal adenosine deaminase, they proved to be virtually resistant to deamination (with the exception of **22b**, which was partially deaminated). In contrast, neplanocin A (NPA) itself was totally deaminated by adenosine deaminase within 30 min (Table I).

Of the new neplanocin analogues, only compound **22a** showed an appreciable inhibitory effect (IC_{50} = 0.2 μg/mL) on rabbit erythrocyte AdoHcy hydrolase activity (Table II). Its diastereomeric form **22b** was not inhibitory to AdoHcy hydrolase at 10 μg/mL. K_i values were determined for the murine L929 cell AdoHcy hydrolase, and these again indicated that **22a** is a potent inhibitor of the enzyme (K_i = 0.086 μM), whereas **22b** is not (K_i = 55.7 μM) (Table II).

AdoHcy hydrolase inhibitors demonstrate a unique spectrum of antiviral activity.[1] They are particularly active against pox- (vaccinia), paramyxo- (parainfluenza, measles, respiratory syncytial), arena- (Junin, Tacaribe), rhabdo- (vesicular stomatitis), and reoviruses, but inactive against the herpesviruses HSV-1 and HSV-2, picornaviruses, togaviruses, and retroviruses (HIV-1 and HIV-2). This activity pattern was also followed by the neplanocin analogues that were subject of the present study (Tables III and IV). Of these compounds, **2, 6, 9**, and **22a** were the most active antiviral agents, and their activity was seen

(19) The 6′-configuration of **22a** and **22b** was R and S, respectively; (6′S)-6′-C-methylneplanocin A was totally synthesized from D-ribose (Shuto, S.; Obara, T., unpublished result), and was identified as **22b**. A fully detailed description of the study will be reported elsewhere.

Table III. Inhibitory Effects of Neplanocin A Analogues on Replication of DNA Viruses

virus	cell	2	3	6	9	15	20	22a	22b	NPA
						IC$_{50}$,[a] μg/mL				
HSV-1 (KOS)	PRK	>20	>400	>400	>200	>400	>200	>400	>400	>40
HSV-2 (G)	PRK	>20	>400	>400	>200	>400	>200	>200	>200	>40
HSV-1 (KOS)	ESM	b						>400	>400	20
HSV-2 (G)	ESM							>400	>400	10
TK⁻ HSV-1 (B2006)	ESM							70	>400	4
TK⁻ HSV-1 (VMW 1837)	ESM							70	>400	7
VZV (Oka)	HEL	2.5	2.5	70	20	200		21	100	8
VZV (YS)	HEL	2	2	150	2	300		65		5
TK⁻ VZV (YSR)	HEL	2	5	20	10	60		6	39	2
TK⁻ VZV (07-1)	HEL	1	2	20	4	100		38	76	5
CMV (AD-169)	HEL	2	1.5	2.5	2.5	40	18	0.06	15	0.3
CMV (Davis)	HEL	1	1	2.5	1.5	25	17	0.03	9	0.2
VV	PRK	0.7	70	2	0.7	20	20	0.1	70	0.2
VV	ESM							0.04	40	0.2
cell morphology	ESM							>400	>400	40
cell morphology	PRK	40	≥400	>400	≥400	≥400	≥400	>400	>400	40
cell growth	HEL	9	9	200	50	200	100	>200	132	16

[a] 50% inhibitory concentration, required to reduce virus-induced cytopathogenicity (HSV, VV) or virus plaque formation (VZV, CMV) or cell growth by 50%. Virus input was 100 CCID$_{50}$ (CCID$_{50}$ = infective dose for 50% of the cell cultures) in the virus-induced cytopathogenicity assays and 20 PFU (PFU = plaque forming unit) in the virus plaque formation assays. For cell morphology, the IC$_{50}$ corresponds to the lowest concentration required to cause a microscopically detectable alteration of normal cell morphology. For abbreviations, see the Experimental Section. [b] Not determined if a value is not listed.

Table IV. Inhibitory Effects of Neplanocin A Analogues on Replication of RNA Viruses

virus	cell	2	3	6	9	15	20	22a	22b	NPA
						IC$_{50}$,[a] μg/mL				
polio-1	HeLa	>40	>200	>200	>200	>200	>400	>400	>400	>40
coxs B-4	HeLa	>40	>200	>200	>200	>200	>400	>400	>400	>40
coxs B-4	Vero	>40	>200	>200	>200	>400	>200	>200	>200	>40
SV	Vero	>40	>200	7	>200	70	>200	4	150	>40
SFV	Vero	>40	>400	>200	>200	>400	>200	>200	>400	>40
reo-1	Vero	0.7	40	2	0.7	10	70	0.04	40	0.4
JV	Vero	2	40	0.4	0.4	20	8	0.4	45	0.4
TV	Vero	2	40	0.4	0.4	20	10	0.5	55	0.4
RSV	HeLa	0.8	>40	10	10	100	100	0.2	>100	0.2
PV-3	Vero	7	>200	7	20	70	40	0.1	100	0.4
measles	Vero	0.8	40	10	4	40	40	0.2	>100	0.2
influenza A	MDCK	>10	>100	>100	>100	>100	>100	>100	>100	>20
influenza B	MDCK	>10	>100	>100	>100	>100	>100	>100	>100	>20
HIV-1	MT-4	>0.06	>40	>1.5	>1.5	>40	>8	>0.03	>100	>0.006
HIV-2	MT-4	>0.06	>40	>1.5	>1.5	>40	>8	>0.03	>100	
VSV	PRK	0.7	70	0.2	0.7	20	20	0.1	70	0.2
VSV	HeLa	>40	>200	2	7	150	300	0.2	200	0.2
VSV	ESM	b						0.07	20	0.2
cell morphology	Vero	40	>400	40	40	400	400	>400	>400	10
cell morphology	PRK	40	≥400	>400	≥400	≥400	≥400	≥400	>400	40
cell morphology	HeLa	40	>400	≥200	≥400	>400	≥400	≥400	>400	20
cell morphology	ESM							>400	>400	40
cell morphology	MDCK	10	>100	>100	>100	>100	>100	>100	>100	20
RNA synthesis	Vero	0.8		5	1.7		14	0.1	36	0.21 ± 0.11
protein synthesis	Vero	5		65	4.7		47	56	>190	4.3 ± 3.2
cell growth	MT-4	0.2	60	3	2.5	45	6	0.09	>100	0.02

[a] 50% Inhibitory concentration, required to reduce virus-induced cytopathogenicity, cell growth, cellular RNA synthesis ([5-³H]uridine incorporation), or cellular DNA synthesis ([4,5-³H]leucine incorporation) by 50%. For abbreviations, see the Experimental Section. [b] Not determined if a value is not listed.

particularly with VV, PV, measles, RSV, JV, TV, VSV, and reovirus (see the Experimental Section for definitions of abbreviations). In addition, compounds 2, 3, 6, 9, and in particular 22a were also found to be active against CMV. This indicates that, as has been demonstrated with various other AdoHcy hydrolase inhibitors (Snoeck, R.; Andrei, G.; Neyts, J.; Schols, D.; Cools, M.; De Clercq, E., manuscript submitted for publication), the antiviral activity spectrum of these compounds extends to CMV. Irrespective of the choice of the virus challenge the most potent antiviral activity was noted with compound 22a (Tables III and IV).

Thus, compound 22a proved inhibitory to CMV, VV, PV, measles, RSV, JV, TV, VSV, and reovirus within the concentration range of 0.03–0.4 μg/mL while not being toxic to the host cells (PRK, ESM, HEL, Vero, HeLa) at concentrations up to 400 μg/mL (Tables III and IV). This points to a selectivity index of 1000–10000 for 22a. In comparison, neplanocin A was active against the same set of viruses within the concentration range of 0.2–1 μg/mL, but toxic to the host cells at a concentration of 10–40 μg/mL, which means a selectivity index of about 40–50.

It should be noted that although 22a was not toxic to the confluent (resting) cells that were used in the antiviral assays (except for HIV-1 and HIV-2), it was quite inhibitory (IC$_{50}$ = 0.09–0.1 μg/mL) to the growth of rapidly proliferating cells (i.e. MT-4, Vero) and RNA synthesis. This means that the specificity of 22a is apparent only if

antiviral assays are conducted with resting confluent cells. If, however, rapidly growing cells have to be used to monitor antiviral activity, as is the case for HIV, the inhibitory effect of the compound on cell growth precludes any specific action against the virus.

It is noteworthy that of the two **22** diastereomers, **22a** was very active, whereas **22b** was virtually inactive. Compound **22a** also corresponded to the diastereomeric form that was highly inhibitory to AdoHcy hydrolase (see above). The potency and selectivity of **22a** is such that it should be further pursued for its therapeutic potential as an antiviral drug. In vivo evaluation of **22a** in the appropriate models for cytomegalo-, pox-, paramyxo-, arena-, rhabdo-, and reovirus infections seem fully justified.

Although modification of neplanocin A to its 6'-methylated derivative **22a** resulted in a marked increase in the antiviral specificity of the compound, it did not change its activity spectrum. Hence, **22a** did not acquire activity against the herpesviruses HSV-1 and HSV-2, the picornaviruses, togaviruses (except for some activity against Sindbis virus), and retroviruses (HIV-1, HIV-2). This, again, proves that the antiviral activity spectrum of AdHcy hydrolase inhibitors is unique. Although their antiviral potency and selectivity can be enhanced by the appropriate chemical modifications [as shown here for **22a** and previously for 3-deazaneplanocin A and the "decapitated" analogues of neplanocin A (DHCA, DHCDA)],[10,11,13] or by the exogenous addition of L-homocysteine,[19] these manipulations do not make the AdoHcy hydrolase inhibitors work against those viruses that do not fit within their activity spectrum.

Experimental Section

Melting points were determined on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The NMR spectra were recorded with a JEOL FX-90, EX-270, or GSX-400 spectrometer with tetramethylsilane as an internal standard. Mass spectra were measured on a JEOL SX-102 spectrometer. Thin-layer chromatography was carried out on E. Merck precoated plate 60F$_{254}$. Flash chromatography was conducted with E. Merck silica gel 9385. Neplanocin A and D were prepared according to a reported method.[17]

9-[(1R,2S,3R)-4-(Chloromethyl)-2,3-dihydroxy-4-cyclopenten-1-yl]adenine (2). Triphenylphosphine (682 mg, 2.6 mmol) and carbon tetrachloride (0.29 mL, 3.0 mmol) were added to a suspension of neplanocin A (263 mg, 1.0 mmol) in 6 mL of DMF, and the mixture was stirred at room temperature. Two hours later, methanol (5 mL) was added, and the resulting mixture was stirred at room temperature for 30 min, whereafter the solvent was removed. The residue was dissolved in 2 mL of methanol to which 20 mL of chloroform was added. The precipitated solid was filtered off and purified by flash chromatography (silica gel, CHCl$_3$/MeOH, 20:1 followed by 15:1) to give 344 mg (83%) of **2** as a crystalline solid: mp > 220 °C dec; ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.19 and 8.08 (each s, each 1 H, H-2 and H-8), 6.08 (dd, 1 H, H-5', J = 3.5 and 1.5 Hz), 5.50 (m, 1 H, H-1'), 4.73 (d, 1 H, H-3', J = 5.4 Hz), 4.43 (dd, 1 H, H-2', J = 5.4 and 5.4 Hz), 4.34 (t, 2 H, H-6'). Anal. (C$_{11}$H$_{12}$N$_5$O$_2$Cl·^{1}/$_2$H$_2$O) C, H, N.

9-[(1R,2S,3R)-2,3-Dihydroxy-4-[(methylthio)methyl]-4-cyclopenten-1-yl]adenine (3). Sodium thiomethoxide (70 mg, 1.0 mmol) was added to a solution of **2** (113 mg, 0.40 mmol) in 3 mL of DMF, and the mixture was stirred at 0 °C for 1 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel, CHCl$_3$/MeOH, 15:1) to give 77 mg of **3** (66%) as a crystalline solid: mp 173 °C; ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.16 and 8.07 (each s, each 1 H, H-2 and H-8), 5.85 (m, 1 H, H-5'), 5.47 (m, 1 H, H-1'), 4.75 (d, 1 H, H-3', J = 5.9 Hz), 4.45 (dd, 1 H, H-2', J = 5.9 and 5.4 Hz), 3.33–3.29 (m, 2 H, H-6'), 2.11 (s, 3 H, SCH$_3$); MS (FAB) m/z 294 (MH$^+$). Anal. (C$_{12}$H$_{15}$N$_5$O$_2$S) C, H, N.

9-[(1R,2S,3R)-2,3-(Isopropylidenedioxy)-4-(fluoromethyl)-4-cyclopenten-1-yl]adenine (5). (Diethylamido)sulfur trifluoride (125 μL, 0.80 mmol) was added to a suspension of **4**[21]

(121 mg, 0.40 mmol) in 8 mL of dichloromethane at 0 °C, and the mixture was stirred at the same temperature. After 1 h, the mixture was removed from the ice bath, 4 mL of 0.75 N NaHCO$_3$ was added, the resulting mixture was stirred for 3 min, and then 20 mL of chloroform was added. The precipitated solid was filtered off, and the filtrate was partitioned. The organic layer was filtered through Whatman 1PS filter paper and evaporated. The residue was purified by flash chromatography (silica gel, CHCl$_3$/MeOH, 20:1) to afford 41 mg (34%) of **5** as a white powder: ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.34 (s, 1 H, H-2), 7.69 (s, 1 H, H-8), 5.89 (bs, 1 H, H-5'), 5.62 (bs, 2 H, NH$_2$), 5.61 (bs, 1 H, H-1'), 5.44 (d, 1 H, H-3', J = 5.4 Hz), 5.17 (d, 2 H, H-6', J = 46.9 Hz), 4.78 (d, 1 H, H-2', J = 5.4 Hz), 1.50 and 1.37 (each s, each 3 H, CH$_3$ × 2); MS (FAB) m/z 306 (MH$^+$). Anal. (C$_{14}$H$_{16}$N$_5$O$_2$F·^{9}/$_{10}$H$_2$O) C, H, N.

9-[(1R,2S,3R)-2,3-Dihydroxy-4-(fluoromethyl)-4-cyclopenten-1-yl]adenine (6). A solution of **5** (100 mg, 0.33 mmol) in 10 mL of 50% aqueous formic acid was stirred at room temperature for 20 h, whereafter the solvent was evaporated. Water was added to the residue and evaporated. The residue was crystallized from THF to give 31 mg of **6**. The filtrate was concentrated and purified by preparative TLC (CHCl$_3$/MeOH, 5:1) to afford another 38 mg of **6**. A total amount of 69 mg (79%) of **6** was thus obtained: mp 208–210 °C; ^{1}H NMR (400 MHz, D$_2$O-added DMSO-d$_6$) δ 8.12 and 8.11 (each s, each 1 H, H-2 and H-8), 5.96 (dd, 1 H, H-5', J = 2.9 and 1.5 Hz), 5.40 (m, 1 H, H-1'), 5.13 (ddd, 2 H, H-6', J = 46.9, 13.2 and 2.0 Hz), 4.51 (d, 1 H, H-3', J = 5.4 Hz), 4.38 (dd, 1 H, H-2', J = 5.9 and 5.4 Hz); MS (FAB) m/z 266 (MH$^+$). Anal. (C$_{11}$H$_{12}$N$_5$O$_2$F) C, H, N.

9-[(1R,2S,3R)-4-(Chloromethyl)-2,3-(isopropylidenedioxy)-4-cyclopenten-1-yl]adenine (7). A suspension of **4** (303 mg, 1.0 mmol), triphenylphosphine (341 mg, 1.3 mmol), and carbon tetrachloride (145 μL, 1.5 mmol) in 6 mL of DMF was stirred at room temperature for 20 h. Methanol (5 mL) was added, and the resulting mixture was stirred at room temperature for 30 min, whereafter the solvent was removed. The residue was purified by flash chromatography (silica gel, CHCl$_3$/MeOH, 20:1) to give 298 mg (92%) of **7** as a white solid: ^{1}H NMR (90 MHz, CDCl$_3$) δ 8.34 (s, 1 H, H-8), 7.67 (s, 1 H, H-2), 5.92 (m, 1 H, H-5'), 5.72 (b, 2 H, NH$_2$), 5.59 (bs, 1 H, H-1'), 5.49 (d, 1 H, H-3', J = 5.5 Hz), 4.76 (d, 1 H, H-2', J = 5.5 Hz), 1.48 and 1.38 (each s, each 3 H, CH$_3$ × 2); MS (FAB) m/z 322, 324 (MH$^+$). Anal. (C$_{14}$H$_{16}$N$_5$O$_2$Cl) H, N; C: calcd, 52.26; found, 52.69.

9-[(1R,2S,3R)-2,3-(Isopropylidenedioxy)-4-methyl-4-cyclopenten-1-yl]adenine (8). A mixture of **7** (540 mg, 0.17 mmol), tri-n-butyltin hydride (1.36 mL, 4.04 mmol), and 2,2'-azobis(isobutyronitrile) (16 mg, 0.10 mmol) in 30 mL of benzene was refluxed for 4 h under an argon atmosphere. The solvent was removed, and the residue was treated with hot hexane to give 456 mg (95%) of **8** as a crystalline solid: mp 206–209 °C; ^{1}H NMR (90 MHz, CDCl$_3$) δ 8.39 (s, 1 H, H-8), 7.67 (s, 1 H, H-2), 5.98 (b, 2 H, NH$_2$), 5.55 (m, 2 H, H-1' and H-5'), 5.21 (d, 1 H, H-3', J = 5.5 Hz), 4.65 (d, 1 H, H-2', J = 5.5 Hz), 1.98 (s, 3 H, 6'-CH$_3$), 1.48 and 1.37 (each s, each 3 H, OCH$_3$ × 2); MS (FAB) m/z 288 (MH$^+$). Anal. (C$_{14}$H$_{17}$N$_5$O$_2$·^{1}/$_2$H$_2$O) C, H, N.

9-[(1R,2S,3R)-2,3-Dihydroxy-4-methyl-4-cyclopenten-1-yl]adenine (9). A solution of **8** (130 mg, 0.45 mmol) in 5 mL of 50% aqueous formic acid was stirred at 50 °C for 20 h, and the solvent was then removed. Water (5 mL) was added to the residue and evaporated to dryness. The residue was purified by flash chromatography (silica gel, CHCl$_3$/MeOH, 20:1 followed by 10:1) to give 84 mg (75%) of a crystalline solid, **9**: mp 213–215 °C; ^{1}H NMR (90 MHz, CD$_3$OD) δ 8.18 and 8.06 (each s, each 1 H, H-2

(20) Fukukawa, K.; Ueda, T.; Hirano, T. Nucleosides and nucleotides. XXXXV. Facile deoxygenation of neplanocin A and nucleosides by the use of tri-n-butyltin hydride. *Chem. Pharm. Bull.* **1983**, *31*, 1842–1847.

(21) Schlols, D.; De Clercq, E.; Balzarini, J.; Baba, M.; Witvrouw, M.; Hosoya, M.; Andrei, G.; Snoeck, R.; Neyts, J.; Pauwels, R.; Nagy, M.; Györgyi-Edelényi, J.; Machovich, R.; Horváth, I.; Löw, M.; Görög, S. Sulphated polymers are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, respiratory syncytial virus, and toga-, arena- and retroviruses. *Antiviral Chem. Chemother.* **1990**, *1*, 233–240.

and H-8), 5.67 (dd, 1 H, H-5', J = 1.6 and 1.6 Hz), 5.43 (m, 1 H, H-1'), 4.50 (d, 1 H, H-3', J = 5.7 Hz), 4.34 (dd, 1 H, H-2', J = 5.7 and 5.7 Hz), 1.93 (m, 3 H, H-6'); MS (FAB) m/z 248 (MH$^+$). Anal. (C$_{11}$H$_{13}$N$_5$O$_2$) C, H, N.

9-[(1R,2S,3R)-2,3-Diacetoxy-4-(acetoxymethyl)-4-cyclopenten-1-yl]hypoxanthine (11). Acetic anhydride (4.3 mL, 45 mmol) was added to a solution of neplanocin D (10, 2.64 g, 10 mmol) in 50 mL of pyridine at 0 °C and the mixture was stirred at room temperature for 3 h. Methanol (10 mL) was added to the reaction mixture and then evaporated. The residue was partitioned between chloroform and water, and the organic layer was filtered through Whatman 1PS filter paper and evaporated. The residue was purified by flash chromatography (silica gel, CHCl$_3$/MeOH, 30:1) and crystallized from ethanol to give 2.52 g (65%) of 11: mp 213–215 °C; ^{1}H NMR (270 MHz, CDCl$_3$) δ 12.95 (b, 1 H, NH), 8.19 and 7.82 (each s, each 1 H, H-2 and H-8), 6.11 (d, 1 H, H-5', J = 1.7 Hz), 5.99 (d, 1 H, H-3', J = 5.6 Hz), 5.77 (m, 1 H, H-1'), 5.56 (dd, 1 H, H-2', J = 5.9 and 5.6 Hz), 2.14, 2.13, and 2.04 (each s, each 3 H, Ac × 3); MS (FAB) m/z 391 (MH$^+$). Anal. (C$_{17}$H$_{18}$N$_4$O$_7$) C, H, N.

6-Chloro-9-[(1R,2S,3R)-2,3-dihydroxy-4-(hydroxymethyl)-4-cyclopenten-1-yl]purine (12). Dimethylformamide (2.4 mL, 31 mmol) and thionyl chloride (4.8 mL, 62 mmol) were added to a solution of 11 (2.34 g, 6.0 mmol) in 120 mL of chloroform, and the mixture was refluxed for 4 h. The mixture was then poured into a 500 mL of stirring ice/water. The mixture was neutralized with 0.8 N NaHCO$_3$ and partitioned. The organic layer was washed twice with water, filtered through a Whatman 1PS filter paper, and evaporated to dryness. The residue was dissolved in 100 mL of saturated methanolic ammonia, and the solution was stirred at room temperature for 3 h. The solvent was removed and the residue was crystallized from ethanol to afford 1.49 g (87%) of 12; mp 194–196 °C; ^{1}H NMR (90 MHz, DMSO-d_6) δ 8.77 and 8.64 (each s, each 1 H, H-2 and H-8), 5.76 (d, 1 H, H-5', J = 2.0 Hz), 5.50 (b, 1 H, H-1'), 5.20 and 5.01 (each d, each 1 H, OH × 2), 4.87 (t, 1 H, OH), 4.51–4.11 (m, 4 H, H-2', 3', and 6'); MS (FAB) m/z 283, 285 (MH$^+$). Anal. (C$_{11}$H$_{11}$N$_4$O$_3$Cl) C, H, N.

6-Chloro-9-[(1R,2S,3R)-4-(hydroxymethyl)-2,3-(isopropylidenedioxy)-4-cyclopenten-1-yl]purine (13). A mixture of 12 (848 mg, 3.0 mmol) and p-toluenesulfonic acid monohydrate (285 mg, 1.5 mmol) in 100 mL of acetone was refluxed for 1 h. The mixture was neutralized with 0.8 N NaHCO$_3$ and cooled to room temperature. The precipitated salt was filtered off, and the filtrate was evaporated to dryness. The residue was crystallized from water to give 1.09 g (85%) of 13: mp 140–142 °C; ^{1}H NMR (90 MHz, CDCl$_3$) δ 8.76 and 8.09 (each s, each 1 H, H-2 and H-8), 5.86 (m, 1 H, H-5'), 5.68 (m, 1 H, H-1'), 5.45 (d, 1 H, H-3', J = 4.9 Hz), 4.78 (d, 1 H, H-2', J = 4.9 Hz), 4.51 (bs, 2 H, H-6'), 1.51 and 1.37 (each s, each 3 H, CH$_3$ × 2); MS (FAB) m/z 323, 325 (MH$^+$). Anal. (C$_{14}$H$_{15}$N$_4$O$_3$Cl) C, H, N.

6-Chloro-9-[(1R,2S,3R)-2,3-(isopropylidenedioxy)-4-(methoxymethyl)-4-cyclopenten-1-yl]purine (14). A mixture of 13 (323 mg, 1.0 mmol) and sodium hydride (55% dispersion in mineral oil, 57 mg, 1.3 mmol) in 10 mL of DMF was stirred at room temperature. Three minutes later, methyl iodide (620 μL, 10 mmol) was added, and the resulting mixture was stirred at room temperature for 30 min. The solvent was removed, and the residue was partitioned between chloroform and 0.5 N HCl. The organic layer was washed with saturated brine, filtered through Whatman 1PS filter paper and evaporated. The residue was purified by flash chromatography (silica gel, CHCl$_3$, followed by CHCl$_3$/MeOH, 50:1) to give 157 mg (47%) of 14 as a syrup: ^{1}H NMR (90 MHz, CDCl$_3$) δ 8.78 and 8.04 (each s, each 1 H, H-2 and H-8), 5.80 (m, 1 H, H-5'), 5.66 (m, 1 H, H-1'), 5.41 (d, 1 H, H-3', J = 5.0 Hz), 4.75 (d, 1 H, H-2', J = 5.0 Hz), 4.21 (m, 2 H, H-6'), 3.46 (s, 3 H, 6'-OCH$_3$), 1.51 and 1.38 (each s, each 3 H, isopropylidene CH$_3$ × 2); MS (FAB) m/z 337, 339 (MH$^+$). This compound was used directly for the next reaction without further purification.

9-[(1R,2S,3R)-2,3-Dihydroxy-4-(methoxymethyl)-4-cyclopenten-1-yl]adenine (15). A solution of 14 (145 mg, 0.43 mmol) in 20 mL of saturated methanolic ammonia was kept in a stainless steel tube at 100 °C for 18 h. The solvent was removed, and 20 mL of chloroform was added to the residue, then the precipitated solid was filtered off. The filtrate was evaporated

to dryness and the residue was dissolved in 3 mL of 90% aqueous formic acid and stirred at room temperature for 3 h. The solvent was removed, and the residue was purified by flash chromatography (silica gel, CHCl$_3$/MeOH, 20:1, followed by 10:1) and then crystallized from ethanol to afford 68 mg (56%) of 15: mp 201 °C; ^{1}H NMR (90 MHz, CD$_3$OD) δ 8.18 and 8.09 (each s, each 1 H, H-2 and H-8), 5.93 (dd, 1 H, H-5', J = 1.8 and 3.5 Hz), 5.57–5.43 (m, 1 H, H-1'), 4.62 (dd, 1 H, H-3', J = 5.1 and ca. 0 Hz), 4.40 (dd, 1 H, H-2', J = 5.1 and 5.7 Hz), 4.16 (d, 2 H, H-6', J = 1.8 Hz), 3.42 (s, 3 H, OCH$_3$); MS (FAB) m/z 278 (MH$^+$). Anal. (C$_{12}$H$_{15}$N$_5$O$_3$) C, H, N.

N^6-Benzoyl-9-[(1R,2S,3R)-2,3-dihydroxy-4-(hydroxymethyl)-4-cyclopenten-1-yl]adenine (16). Trimethylsilyl chloride (7.6 mL, 60 mmol) was added to a suspension of neplanocin A (1, 3.16 g, 12 mmol) in 60 mL of pyridine and stirred at room temperature. Fifteen minutes later, benzoyl chloride (7.0 mL, 60 mmol) was added, and the resulting mixture was stirred at room temperature for 2 h. The mixture was cooled in an ice bath, 24 mL of 25% NH$_4$OH was added, and the resulting mixture was stirred at room temperature for 45 min. The solvent was removed, and the residue was partitioned between ethyl acetate (60 mL) and water (180 mL). The aqueous layer was concentrated under reduced pressure to afford a white precipitate of 16. The precipitate was filtered off (2.75 g), the filtrate was evaporated, and the residue was purified by flash chromatography (silica gel, CHCl$_3$/MeOH, 20:1, followed by 15:1) to give another 0.87 g of 16. A total amount of 3.62 g (83%) of 16 was thus obtained: mp 160–162 °C (recrystallized from MeOH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 and 8.42 (each s, each 1 H, H-2 and H-8), 8.06–7.54 (m, 5 H, Bz), 5.76 (d, 1 H, H-5', J = 1.5 Hz), 5.51 (m, 1 H, H-1'), 5.22 and 5.01 (each d, each 1 H, OH × 2), 4.95 (t, 1 H, OH), 4.46 (m, 1 H, H-3'), 4.38 (m, 1 H, H-2'), 4.15 (m, 2 H, H-6'); MS (FAB) m/z 368 (MH$^+$). Anal. (C$_{18}$H$_{17}$N$_5$O$_4$·H$_2$O) C, H, N.

N^6-Benzoyl-9-[(1R,2S,3R)-2,3-(isopropylidenedioxy)-4-(hydroxymethyl)-4-cyclopenten-1-yl]adenine (17). A mixture of 16 (1.47 g, 4.0 mmol) and 1.0 mL of 70% HClO$_4$ in 100 mL of acetone was stirred at room temperature for 3 h and then neutralized with 0.75 N NaHCO$_3$. The precipitated inorganic salt was filtered off, the filtrate was evaporated, and the residue was partitioned between chloroform and brine. After drying over MgSO$_4$, the solvent was removed, and the residue was treated with diethyl ether to give 1.25 g of white crystalline solid of 17 (77%): mp 161–164 °C; ^{1}H NMR (90 MHz, CDCl$_3$) δ 8.76 and 8.31 (each s, each 1 H, H-2 and H-8), 8.10–7.42 (m, 5 H, Bz), 5.77 (bs, 1 H, H-5'), 5.61 (bs, 1 H, H-1'), 5.38 (d, 1 H, H-3', J = 5.6 Hz), 5.11 (b, 1 H, OH), 4.79 (d, 1 H, H-2', J = 5.6 Hz), 4.18 (bs, 2 H, H-6'), 1.42 and 1.30 (each s, each 3 H, CH$_3$ × 2); MS (FAB) m/z 408 (MH$^+$). Anal. (C$_{21}$H$_{21}$N$_5$O$_4$) C, H, N.

N^6-Benzoyl-9-[(1R,2S,3R)-4-formyl-2,3-(isopropylidenedioxy)-4-cyclopenten-1-yl]adenine (18). A mixture of 17 (407 mg, 1.0 mmol) and barium manganate (BaMnO$_4$, 4.0 g, 15.6 mmol) in 30 mL of 1,2-dichloroethane was refluxed for 3 h. The oxidizing agent was filtered off and washed with hot chloroform. The filtrate was evaporated to dryness under reduced pressure to give 388 mg (95%) of crude 18 as a yellow foam, which was used directly for the next reactions without further purification: ^{1}H NMR (90 MHz, D$_2$O-added CDCl$_3$) δ 9.98 (s, 1 H, 6'-CHO), 8.75 (s, 1 H, H-8), 8.09–7.47 (m, 6 H, H-2 and Bz), 6.78 (bs, 1 H, H-5') 5.83–5.78 (m, 2 H, H-1' and H-3'), 4.98 (d, 1 H, H-2', J = 6.8 Hz), 1.52 and 1.40 (each s, each 3 H, CH$_3$ × 2).

N^6-Benzoyl-9-[(1R,2S,3R)-4-ethenyl-2,3-(isopropylidenedioxy)-4-cyclopenten-1-yl]adenine (19). To a suspension of methyltriphenylphosphonium bromide (1.07 g, 3.0 mmol) in 15 mL of THF was added 1.25 mL of BuLi solution (1.6 M in hexane) under an argon atmosphere at −20 °C, and the mixture was stirred at the same temperature for 20 min. A solution of 18 (407 mg, 1.0 mmol) in 10 mL of THF was added to the mixture, and the resultant reaction mixture was stirred at −20 °C. One hour later, the mixture was removed from the cooling bath and stirred at room temperature for 15 h. Then 140 μL of acetic acid/benzene (1:9) was added, and the solvent was removed. The residue was purified by flash chromatography (silica gel, hexane/AcOEt, 1:1 followed by 1:4) to afford 178 mg (44%) of 19 as a white foam: ^{1}H NMR (400 MHz, CDCl$_3$) δ 9.08 (bs, 1 H, N^{6}H), 8.84 (s, 1 H, H-8), 8.04–7.44 (m, 5 H, Bz), 7.89 (s, 1

H, H-2), 6.60 (dd, 1 H, H-6′, J = 17.6 and 10.8 Hz), 5.80 (d, 1 H, 6′-CH=CH₂, J = 17.6 Hz), 5.78 (bs, 1 H, H-5′), 5.73 (bs, 1 H, H-1′), 5.61 (d, 1 H, H-3′, J = 5.9 Hz), 5.51 (d, 1 H, 6′-CH=CH₂, J = 10.8 Hz), 4.78 (d, 1 H, H-2′, J = 5.9 Hz), 1.50 and 1.41 (each s, each 3 H, CH₃ × 2); MS (FAB) m/z 404 (MH⁺). This compound was used directly for the next reaction without further purification.

9-[(1R,2S,3R)-4-Ethenyl-2,3-dihydroxy-4-cyclopenten-1-yl]adenine (20). A solution of 19 (121 mg, 0.30 mmol) in 3 mL of 90% aqueous formic acid was stirred for 6 h, whereafter the solvent was removed under reduced pressure. The residue was dissolved in 5 mL of water and evaporated. The resulting residue was dissolved in 4 mL of dioxane/25% NH₄OH (1:1) and stirred at room temperature for 6 h. The mixture was evaporated, and the residue was purified by flash chromatography (silica gel, CHCl₃/MeOH, 10:1, followed by 5:1) and crystallized from EtOH to give 56 mg (72%) of 20: mp 191–194 °C; ¹H NMR (400 MHz, D₂O-added DMSO-d₆) δ 8.13 and 8.11 (each s, each 1 H, H-2 and H-8), 6.47 (dd, 1 H, H-6′, J = 17.6 and 10.7 Hz), 5.92 (d, 1 H, H-5′, J = 2.0 Hz), 5.53 (d, 1 H, 6′-CH=CH₂, J = 17.6 Hz), 5.44 (d, 1 H, H-1′, J = 6.4 Hz), 5.30 (d, 1 H, 6′-CH=CH₂, J = 10.7 Hz), 4.65 (d, 1 H, H-3′, J = 5.4 Hz), 4.40 (dd, 1 H, H-2′, J = 6.4 and 5.4 Hz); MS (FAB) m/z 260 (MH⁺). Anal. (C₁₂H₁₃N₅O₂·¹/₄H₂O) C, H, N.

N⁶-Benzoyl-9-[(1R,2S,3R)-4-(1-hydroxyethyl)-2,3-(iso-propylidenedioxy)-4-cyclopenten-1-yl]adenine (21). To a solution of 18 (0.89 g, 2.2 mmol) in 30 mL of dichloromethane were added 6.0 mL of Me₃Al solution (1.38 N, in hexane) at −75 °C over a 20-min period under an argon atmosphere, and the resulting solution was stirred at the same temperature for 2 h. Then 20 mL of 1 N NH₄Cl was added, the cooling bath was removed, and 8 mL of 4 N HCl was added. The resulting mixture was partitioned, and the organic layer was washed with brine and dried over Na₂SO₄. The solvent was removed and the residue was purified by flash chromatography (silica gel, CHCl₃, followed by CHCl₃/MeOH, 80:1) to give 0.50 g (54%) of a diastereomeric mixture, 21, as a syrup: ¹H NMR (400 MHz, CDCl₃) δ 9.09 (bs), 9.06 (bs), 8.82 (s), 7.92 (s), 8.04–7.51 (m), 5.79 (bs), 5.77 (bs), 5.66 (d, J = 1.5 Hz), 5.63 (d, J = 1.5 Hz), 5.59 (d, J = 5.4 Hz), 5.51 (d, J = 5.4 Hz), 4.80 (d, J = 5.4 Hz), 4.71–4.67 (m), 1.71 (s), 1.52 (s), 1.49 (s), 1.38 (s); MS (FAB) m/z 422 (MH⁺). Anal. (C₂₂H₂₃N₅O₄·²/₃H₂O) C, H, N.

9-[(1R,2S,3R)-2,3-Dihydroxy-4-(1-hydroxyethyl)-4-cyclo-penten-1-yl]adenine (22a and 22b). Successive treatment of 21 (120 mg, 0.29 mmol) with 90% aqueous formic acid and aqueous ammonia, as described for the deprotection of compound 19, afforded the diastereomeric mixture of 22a and 22b as a white foam (52 mg, 66%), which was separated by reverse-phase HPLC (column, E. Merck Lichrosorb RP-18-5, 2.5 × 50 cm; eluate, 7% aqueous MeOH) into two diastereomerically pure compounds as crystalline solids (22a, 30 mg, eluted early; 22b, 11 mg, eluted late). 22a: analytical HPLC (column, E. Merck Superspher RP-18-4, 0.4 × 15 cm; eluate, 12% aqueous MeOH, 1.0 mL/min; temperature, 50 °C; detector, UV at 260 nm) t_R = 3.7 min; mp 211 °C; ¹H NMR (400 MHz, D₂O-added DMSO-d₆) δ 8.11 and 8.04 (each s, each 1 H, H-2 and H-8), 5.68 (bs 1 H, H-5′), 5.33 (m, 1 H, H-1′), 4.49 (d, 1 H, H-3′, J = 5.4 Hz), 4.33 (m, 1 H, H-6′), 4.22 (dd, 1 H, H-2′, J = 5.4 and 3.9 Hz), 1.25 (d, 3 H, 6′-CH(OH)CH₃, J = 6.3 Hz); MS (FAB) m/z 278 (MH⁺). Anal. (C₁₂H₁₅N₅O₃·¹/₅H₂O) C, H, N. 22b: analytical HPLC (column, E. Merck Superspher RP-18-4, 0.4 × 15 cm; eluate, 12% aqueous MeOH, 1.0 mL/min; temperature, 50 °C; detector, UV at 260 nm) t_R = 4.2 min; mp 213 °C; ¹H NMR (400 MHz, D₂O-added DMSO-d₆) δ 8.10 and 8.09 (each s, 1 H, H-2 and H-8), 5.70 (bs, 1 H, H-5′), 5.34 (d, 1 H, H-1′, J = 5.9 Hz), 4.44 (d, 1 H, H-3′, J = 5.4 Hz), 4.27–4.23 (m, 2 H, H-2′ and H-6′), 1.26 (d, 3 H, 6′-CH(OH)CH₃, J = 6.8 Hz); MS (FAB) m/z 278 (MH⁺). Anal. (C₁₂H₁₅N₅O₃·¹/₂H₂O) C, H, N.

Effect of Adenosine Deaminase. The adenosine deaminase solution, prepared from commercially available calf intestine adenosine deaminase (Boehringer Mannheim, 200 units/mL) with tris-HCl buffer (0.05 M, pH 7.2), contained 20 units/mL. Ten

microliters of the deaminase solution (0.4 unit) was added to 0.5 mL of Tris-HCl buffer (0.05 M, pH 7.2) containing 0.5 mM of the test compound, and the resulting solution was incubated at 25 °C. After 15 and 30 min, 5 μL of reaction solution was sampled and analyzed by reverse-phase HPLC (column, E. Merck Superspher RP-18-4, 0.4 × 15 cm; eluate, 10 or 15% aqueous MeOH; temperature, 50 °C; detector, UV 260 nm).

Inhibitory Effect on Rabbit Erythrocyte AdoHcy Hydrolase. Two μL of test compound solution (NPA, 0.0025, 0.01, or 0.04 μg/mL; other compounds, 0.16, 0.63, 2.5, or 10 μg/mL) and 3 μL of commercially available AdoHcy hydrolase solution (Sigma A-3291, from rabbit erythrocytes) were added to 5 μL of phosphate buffer (150 mM, pH 7.6) containing homocysteine (1 mg/mL), dithiothreitol (2 mM), EDTA (1 mM), and 2′-deoxy-coformycin (Sigma, 0.1 μg/mL). To the mixture was added 10 μL of phosphate buffer (150 mM, pH 7.6) containing 740 Bq of [8-¹⁴C]adenosine (Daiichi Pure Chemical Co.). After incubation for 2 min at 37 °C, the reaction was stopped with 8 μL of 0.6 N HCl. Twenty microliters of the resulting reaction mixture was loaded onto a silica gel TLC plate (E. Merck precoated plate 60F₂₅₄) and developed (n-BuOH/AcOH/water, 12:3:5). The [8-¹⁴C]AdoHcy synthesized was analyzed by a radiochromanizer (Aloka JTC-601).

Inhibitory Effect on Murine L929 Cell AdoHcy Hydrolase. AdoHcy hydrolase was purified from murine L929 cells to apparent homogeneity by using affinity chromatography; enzymatic activity was measured in the direction of AdoHcy synthesis as previously described.[3]

Antiviral Activity Assays. Antiviral assays (except for the anti-VZV assays) were carried out as recently described.[21] For the anti-VZV assays, see ref 22. The sources of the viruses have also been described in these previous publications. The abbreviations used for the viruses and cells are as follows: HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VZV, varicella-zoster virus; CMV, cytomegalovirus; VV, vaccinia virus; TK⁻, thymidine kinase deficient strains of HSV or VZV; SV, Sindbis virus; SFV, Semliki forest virus; JV, Junin virus; TV, Tacaribe virus; RSV, respiratory syncytial virus; PV, parainfluenza virus; VSV, vesicular stomatitis virus; HIV-1, human immuno-deficiency virus type 1; HIV-2, human immunodeficiency virus type 2; PRK, primary rabbit kidney; HEL, human embryonic lung; ESM, embryonic skin-fibroblast; MDCK, Madin–Darby canine kidney.

Cytotoxicity Assays. Cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology, inhibition of cell growth, cellular RNA synthesis, or cellular protein synthesis. The detailed methodology has been previously described.[7,21]

(22) De Clercq, E.; Holy, A.; Rosenberg, I.; Sakuma, T.; Balzarini, J.; Maudgal, P. C. A novel selective broad-spectrum anti-DNA virus agent. *Nature* 1986, *323*, 464–467.