New Neplanocin Analogues. 7. Synthesis and Antiviral Activity of 2-Halo Derivatives of Neplanocin A[1]

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The syntheses and the antiviral activities of 2-halo derivatives of neplanocin A (**1b**,**c**), (6′*R*)-6′-*C*-methylneplanocin A (**2b**), and dehydroxymethylneplanocin A (**3b**,**c**) are described. S_N2 reaction of the known cyclopentenyl units **12** and **13** with 2-haloadenines under basic conditions gave the protected carbocyclic nucleosides **14b**,**c** and **15b**,**c**, respectively. Starting from the cyclopentenone derivative **5**, the optically active tosyloxycyclopentene derivative **11** was prepared, which was similarly condensed with 2-fluoroadenine to give the protected (6′*R*)-6′-*C*-methyl derivative **16b**. Deprotection of these compounds afforded the target 2-halo derivatives of neplanocin A. Of these new compounds, 2-fluoroneplanocin A (**1b**) showed an antiviral potency and a spectrum that was comparable to that of neplanocin A (**1a**). It was particularly active against vaccinia virus, vesicular stomatitis virus, parainfluenza virus, reovirus, arenaviruses (Junin, Tacaribe), and human cytomegalovirus, i.e., those viruses that fall within the purview of the *S*-adenosyl-L-homocysteine hydrolase inhibitors.

Introduction

Although neplanocin A (NPA, **1a**, Chart 1), a carbocyclic nucleoside antibiotic,[2] has significant antiviral activity,[3] it is apparently cytotoxic to the host cells.[4] The mechanism of action has been extensively explored. The cytotoxic effect could be derived from, for the most part, phosphorylation of the primary hydroxyl group at its 6′-position by the adenosine kinase and subsequent metabolism by cellular enzymes.[4] The antiviral effect would be due to inhibition of the *S*-adenosylhomocysteine (AdoHcy) hydrolase, disturbing the virus mRNA maturation.[3] NPA has also been known to be rapidly deaminated by adenosine deaminase to a chemotherapeutically inactive inosine congener,[3,5,6a] reducing the therapeutic potency of NPA, especially *in vivo*. On the basis of these observations, chemical modifications of NPA have been extensively studied in developing more efficient antiviral agents.[6,7]

It has been recognized that the introduction of a halogen atom at the 2-position of adenine nucleosides allowed resistance to the adenosine deaminase;[8] for instance, arabinosyl-2-fluoroadenine and 2-chloro-2′-deoxyadenosine are potent antitumor agents resistant to adenosine deaminase.[8] 2-Halo derivatives of adenosine have also been known to inhibit AdoHcy hydrolase.[9] On the basis of these results, we designed and synthesized 2-fluoro- and 2-chloro-NPA's (**1b** and **1c**, respectively) as adenosine deaminase resistant equivalents of NPA.[10]

On the other hand, because the 5′-hydroxymethyl moiety of adenosine has an essential role in the substrate recognition by all of the above enzymes that interact with NPA, we have also modified the 6′-hydroxymethyl moiety of NPA (the 6′-position of NPA corresponds to the 5′-position of adenosine) to develop NPA derivatives that are neither phosphorylated by adenosine kinase nor deaminated by adenosine deaminase but significantly inhibit AdoHcy hydrolase.[6] Throughout our studies, we found that (6′*R*)-6′-*C*-methylneplanocin A (RMNPA, **2a**) has excellent antiviral activity against various DNA and RNA viruses and its cytotoxicity was reduced significantly when compared with that of NPA.[6a,b] In contrast with the result, the corresponding 6′-diastereomer, namely (6′*S*)-6′-*C*-methylneplanocin A, was almost biologically inactive.[6a] Borchardt and co-workers also modified the hydroxymethyl moiety of NPA and found the dehydroxymethylated derivative of NPA (DHCA, **3a**) to be another 6′-modified NPA analogue with potent antiviral activity.[7c] Accordingly, the 2-halo derivatives of RMNPA and DHCA may be of interest as potential antiviral NPA derivatives.

In this paper, we describe the syntheses and the antiviral activities of 2-fluoro and -chloro derivatives of NPA, RMNPA, and DHCA.

Results and Discussion

Chemistry. Because practical methods for introducing a substituent at the 2-position of adenine nucleosides directly were not known, we synthesized the target compounds by S_N2 reaction of the cyclopentenyl units **11**−**13** with 2-haloadenines (Scheme 1). The cyclopentenyl units **12**[11] and **13**[12] were prepared by previously reported methods. The cyclopentenyl unit **11** used in synthesizing the RMNPA derivative **2b** was derived from the optically active cyclopentenone derivative **5** (Scheme 2), which was readily prepared from D-ribose[12] and has been recognized as an efficient synthon for constructing the backbone structure of NPA.[13]

To reach **11**, we needed to introduce a 1-hydroxyethyl unit by a 1,2-addition onto the enone system. To our knowledge, efficient carbon nucleophiles that are equivalent to a 1-hydroxyethyl group are not known. There-

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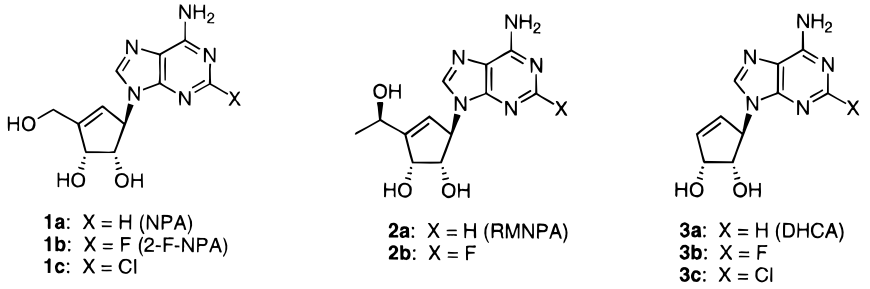
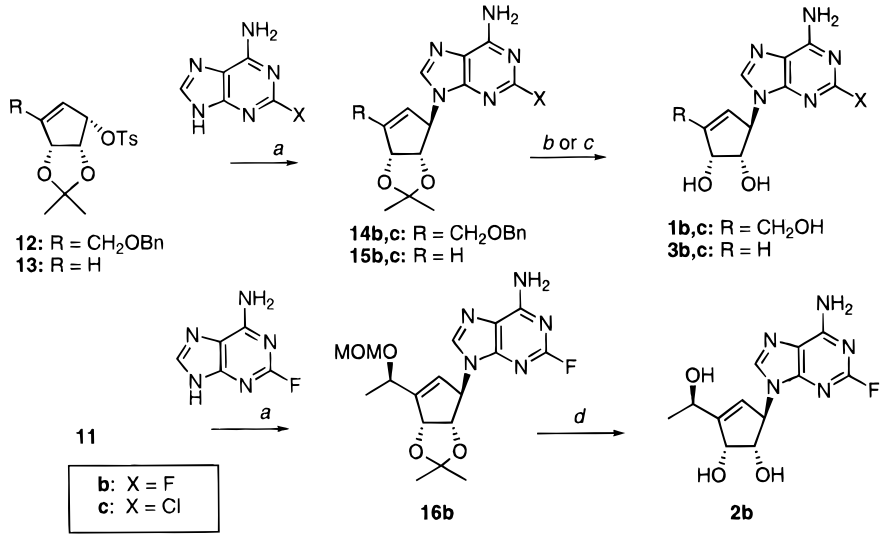
Chart 1

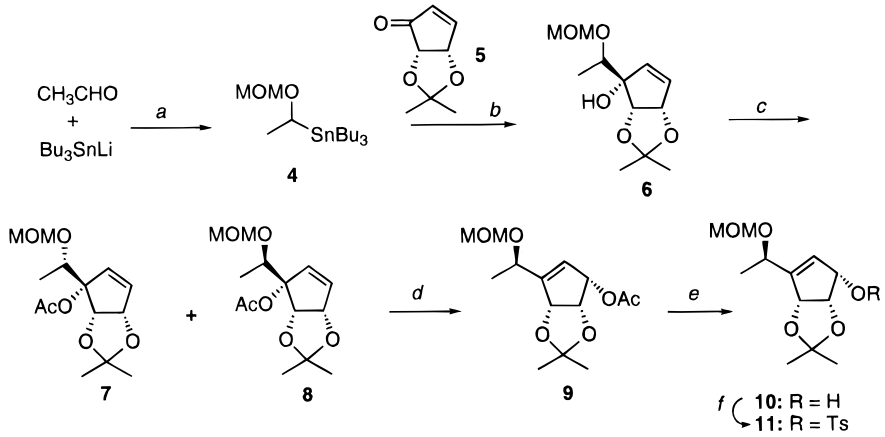


Scheme 1[a]



[a] Reagents: (a) K_2CO_3, 18-crown-6, DMF; (b) BCl_3, CH_2Cl_2; (c) 50% HCOOH, (d) HCl, MeOH.

Scheme 2[a]



[a] Reagents: (a) (1) THF, (2) MOMCl, *N*,*N*-dimethylaniline, CH_2Cl_2; (b) BuLi, THF; (c) Ac_2O, DMAP, CH_2Cl_2; (d) $PdCl_2(MeCN)_2$, benzoquinone, THF; (e) K_2CO_3, MeOH; (f) TsCl, DMAP, CH_2Cl_2.

fore, we developed a novel organotin compound (**4**) that can be readily converted *in situ* to the corresponding organolithiums by treatment with BuLi. Acetaldehyde was treated with Bu_3SnLi, prepared *in situ* from LDA and Bu_3SnH, at −78 °C in THF to give (1-hydroxyethyl)-tributyltin, which was treated then with chloromethoxymethane and *N*,*N*-dimethylaniline in dichloromethane at room temperature to afford **4** after purification by silica gel column chromatography. The cytclopentenone **5** was treated with [(1-methoxymethyloxy)ethyl]lithium, generated from **4** and BuLi, in THF at −78 °C to give the desired addition product **6** in 90% yield as a diastereomeric mixture at the α-position of the methoxymethyloxy group.[14] The tertiary-alcohol of the diastereomeric mixture **6** was acetylated with Ac_2O/DMAP in CH_2Cl_2, and the product was separated at this stage by silica gel flash chromatography into the *S*-diastereomer **7** and the *R*-diastereomer **8** in 53% and 29% yield, respectively.[15] When **8** was heated under reflux in THF with catalytic $PdCl_2(MeCN)_2$ in the presence of benzoquinone[16] for 18 h, the desired rearranged product **9** was obtained in 31% yield with a 45% recovery of **8**. Compound **9** was then converted to tosylate **11** by standard procedure.

Tosyloxycyclopentene derivative **12** was treated with 2-fluoroadenine[17] and 2-chloroadenine[18] in the presence

Table 1. Antiviral Activity and Cytotoxicity of Compounds **1a**−**c**, **2a**,**b**, and **3a**−**c** in Vero Cells

	antiviral activity, IC_{50} (μg/mL)[a]			cytotoxicity, CC_{50} (μg/mL)[b]
compound	VSV	measles	mumps	vero cells
1a (NPA)	0.25	0.10	0.11	155
1b	0.25	0.03	1.2	>200
1c	63	29	>25	>200
2a (RMNPA)	0.25	0.09	0.19	>200
2b	16	>25	7.0	>200
3a (DHCA)	1.0	0.47	1.1	>200
3b	ND[c]	>25	21	>200
3c	ND[c]	>25	>25	>200

[a] Inhibitory concentration, required to reduce virus-induced cytopathicity (VSV) or virus plaque formation (measles and mumps) by 50%. [b] 50% cytotoxic concentration, required to reduce the number of viable cells by 50%. [c] Not determined.

of K_2CO_3 and 18-crown-6 in DMF at 75 °C to give the desired 2-fluoro and 2-chloro NPA derivatives, **14b** and **14c**, in 30% and 43% yield, respectively. In the same way, the 2-fluoro derivatives **15b** and **16b** and the 2-chloro derivative **15c** were obtained by the S_N2 reactions of carbocyclic units **11** or **13** with the appropriate 2-haloadenine. Deprotection of **14b** and **14c** was done by treating them with BCl_3 in CH_2Cl_2 at room temperature to give **1b** and **1c** in 66% and 62% yield, respectively. Removal of the protecting groups of **15b**, **15c**, and **16b** was done under acidic conditions to afford the target compounds, **3b**, **3c**, and **2b**, respectively.

Biological Activity. First, the susceptibility of the compounds to adenosine deaminase was investigated. The compounds (0.5 mM) were incubated in the presence of adenosine deaminase from calf intestine (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, unlike NPA, which was rapidly deaminated to the inactive inosine congener under the reaction conditions.

Next, the compounds were evaluated for antiviral activity against vesicular stomatitis virus (VSV), mumps virus, and measles virus, together with NPA (**1a**), RMNPA (**2a**), and DHCA (**3a**) as positive controls. The results are summarized in Table 1. 2-Fluoroneplanocin A (2-F-NPA, **1b**) had significant antiviral effects with IC_{50} values of 0.25 μg/mL (VSV), 0.03 μg/mL (measles), and 1.2 μg/mL (mumps), which are comparable to those of RMNPA, DHCA, and NPA. 2-F-NPA proved not to be cytotoxic to the host cells at concentrations up to 200 μg/mL. The 2-fluoro derivative of RMNPA (**2b**) showed weak antiviral activity against VSV and mumps virus, while the 2-fluoro derivative of DHCA (**3b**) was almost inactive against these viruses. The 2-chloro derivatives **1c** and **3c** were inactive. Therefore, we decided to investigate in more detail the biological activity of 2-F-NPA (**1b**).

The inhibitory effect of 2-F-NPA on AdoHcy hydrolase was evaluated in a cell-free system with the enzyme from rabbit erythrocytes. 2-F-NPA apparently inhibited the enzyme (IC_{50} = 0.20 μg/mL), while NPA did so at an IC_{50} of 0.004 μg/mL.

As shown in Table 2, 2-F-NPA (**1b**) showed an antiviral activity spectrum that was comparable to that of NPA (**1a**); i.e., it was active against those viruses that fall within the antiviral activity spectrum that is characteristic of AdoHcy hydrolase inhibitors.[20] 2-F-NPA (**1b**), when compared with NPA, was more active against vaccinia virus (VV) and vesicular stomatitis virus (VSV), equally active as NPA against parainfluenza virus, reovirus, and arenaviruses (Junin, Tacaribe), and less active against human cytomegalovirus (CMV). Slight activity was noted with both NPA and 2-F-NPA against varicella-zoster virus (VZV) [including thymidine kinase-deficient (TK^-) VZV strains], but no activity was observed against herpes simplex virus (HSV) [including TK^- HSV-1], Coxsackie B4 virus, polio-1 virus, or Semliki forest virus. 2-F-NPA proved about 10-fold more inhibitory than NPA to the growth of HEL cells, but 10-fold less inhibitory to the growth of L1210 and FM3A cells. Both NPA and 2-F-NPA were inhibitory to the growth of CEM (human T-lymphocyte) cells at a concentration of about 1−2 μg/mL. No activity was observed with either NPA or 2-F-NPA against the human immunodeficiency virus (HIV-1, HIV-2) in CEM cells at subtoxic concentrations (data not shown).

Table 2. Comparative Antiviral and Cytostatic Effects of **1a** (NPA) and **1b** (2-F-NPA)

		IC_{50} (μg/mL)[a]	
virus	cell[b]	**1a** (NPA)	**1b** (2-F-NPA)
HSV-1 (KOS)	E_6SM	70	≥40
HSV-2 (G)	E_6SM	≥40	≥40
TK^-HSV-1 (B2006)	E_6SM	20	≥40
VV	E_6SM	0.7 (0.4−2)	0.1 (0.007−0.2)
VSV	E_6SM	7	1 (0.7−0.2)
VSV	Hela	2 (0.4−7)	0.4 (0.2−0.7)
Coxsackie B4	Hela	≥40	≥100
polio-1	Hela	≥40	≥40
parainfluenza-3	Vero	2	2 (0.7−7)
Reo-1	Vero	0.7	0.7
Sindbis	Vero	7	1
Semliki forest	Vero	≥40	≥100
Junin	Vero	0.5	0.6
Tacaribe	Vero	1.5	0.7
CMV (AD-169)	HEL	0.3	1.5
CMV (Davis)	HEL	0.4	>5
VZV(Oka, YS)	HEL	10	10
TK^-VZV (07/1, YS/R)	HEL	9	7
	E_6SM	70	40
	Hela	40	100
	Vero	40	100
	HEL	>20	2
	L1210	0.027	0.25
	FM3A	0.022	0.19
	MOLT-4	2.34	0.50
	CEM	1.12	1.75

[a] Required to reduce virus-induced cytopathicity by 50%, to cause a microscopically detectable alteration of cell morphology (E6SM, Hela, and Vero cells), or to inhibit cell growth by 50% (HEL, L1210, FM3A, MOLT-4, and CEM cells). Results originate from two or three separate experiments (range of values are indicated in parentheses where appropriate). [b] E_6SM, human embryonic skin−muscle (fibroblasts); HEL, human embryonic lung fibroblasts.

Since 2-F-NPA is completely resistant to adenosine deaminase, its concentration in cells may be kept at a relatively higher level compared with that of NPA. Therefore, 2-F-NPA was equally or even more active against various viruses when compared with NPA, while its inhibitory effect on rabbit erythrocyte AdoHcy hydrolase was weaker than that of NPA, as described above.

In conclusion, 2-F-NPA was resistant to adenosine deaminase and was particularly active against vaccinia virus, vesicular stomatitis virus, parainfluenza virus, reovirus, arenaviruses (Junin, Tacaribe), and human

cytomegalovirus, i.e., those viruses that fall within the purview of the *S*-adenosyl-L-homocysteine hydrolase inhibitors.

Experimental Section

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 -400 spectrometer with tetramethylsilane as an internal standard. Chemical shifts were 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. UV spectra were recorded with a Shimazu UV-240 spectrometer. Thin-layer chromatography was done on Merck coated plate $60F_{254}$. Silica gel chromatography and flash silica gel chromatography were done with Merck silica gel 5715 and 9385, respectively. Preparative HPLC was done with a Merck ODS column (Licrosorb RP 18−5, 20 × 250 mm).

1-[(Methoxymethyloxy)ethyl]tributyltin (4). To a solution of diisopropylamine (3.2 mL, 23 mmol) in THF (40 mL) at 0 °C was added a BuLi solution (1.6 M in hexane, 12.5 mL, 20 mmol) under an argon atmosphere. After 5 min, Bu_3SnH (5.28 mL, 20 mmol) was added to the solution and the mixture was cooled to −78 °C. A solution of acetaldehyde (1.12 mL, 20 mmol) in THF (5 mL) was added and the resulting mixture was stirred at the same temperature for 30 min. The cooling bath was removed and the reaction was quenched with saturated NH_4Cl (10 mL). To the mixture were added hexane (250 mL) and water (50 mL), and the mixture was partitioned. The organic layer was washed with water (50 mL), dried (Na_2SO_4), and evaporated under vacuum. The residue was dissolved in CH_2Cl_2 (50 mL), to which MOMCl (2.28 mL, 30 mL) and *N*,*N*-dimethylaniline (10 mL, 79 mmol) were added at 0 °C, and the mixture was stirred at room temperature for 15 h. To the resulting mixture were added hexane (200 mL) and 0.5 N HCl (100 mL), and the mixture was partitioned. The organic layer was washed with saturated $NaHCO_3$ (100 mL) and water (50 mL), dried ($MgSO_4$), and evaporated under vacuum. The residue was purified by flash chromatography (hexane/EtOAc, 25:1) to give **4** (2.78 g, 38%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) 4.54 (d, J = 6.6 Hz, 1 H), 4.66 (d, J = 6.6 Hz, 1 H), 4.08 (q, J = 7.6 Hz, 1 H), 3.34 (s, 3 H), 1.65−1.45 (m, 9 H), 1.42−1.24 (m, 6 H), 1.12−0.80 (m, 15 H).

(1*R*,2*S*,3*S*)-1-[1-(Methoxymethyloxy)ethyl]-2,3-(isopropylidenedioxy)-4-cyclopenten-1-ol (6). To a solution of **4** (2.17 g, 6.0 mmol) in THF (24 mL) at −78 °C was added dropwise a BuLi solution (1.62 M in hexane, 3.74 mL, 6.0 mmol) under an argon atmosphere. After 20 min, a solution of **5** (737 mg, 4.8 mmol) in THF (6 mL) was added to the solution and the mixture was stirred at −78 °C for 2.5 h. The cooling bath was removed and the reaction was quenched with saturated NH_4Cl (10 mL). To the mixture were added $CHCl_3$ (200 mL) and water (40 mL), and the mixture was partitioned. The organic layer was dried ($MgSO_4$) and evaporated under vacuum. The residue was purified by flash chromatography (hexane/acetone, 25:1) to give **6** (1.06 g, 90%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) 5.94 (m, 2 H), 5.79 (d, J = 5.6 Hz, 1 H), 5.77 (d, J = 5.9 Hz, 1 H), 5.03 (m, 2 H), 4.72 (d, J = 6.6 Hz, 1 H), 4.67 (d, J = 1.6 Hz, 1 H), 4.65 (d, J = 1.6 Hz, 1 H), 4.59 (d, J = 6.6 Hz, 1 H), 4.55 (d, J = 3.6 Hz, 1 H), 4.53 (d, J = 3.6 Hz, 1 H), 3.78 (q, J = 6.3 Hz, 1 H), 3.74 (q, J = 6.3 Hz, 1 H), 3.38 (s, 3 H), 3.35 (s, 3 H), 3.21 (s, 2 H), 1.46 (s, 3 H), 1.45 (s, 3 H), 1.41 (s, 6 H), 1.24 (d, J = 6.3 Hz, 3 H), 1.19 (d, J = 6.3 Hz, 3 H); FABMS m/z 245 (MH^+).

(1*R*,2*S*,3*S*)-1-[(*S*)-1-(Methoxymethyloxy)ethyl]-1-acetoxy-2,3-(isopropylidenedioxy)cyclopent-4-ene (7) and (1*R*,2*S*,3*S*)-1-[(*R*)-1-(Methoxymethyloxy)ethyl]-1-acetoxy-2,3-(isopropylidenedioxy)cyclopent-4-ene (8). A mixture of **6** (5.93 g, 24.3 mmol), Ac_2O (9.17 mL, 97.2 mmol), Et_3N (13.6 mL, 97.2 mmol), and DMAP (2.97 g, 24.3 mmol) in CH_2Cl_2 (100 mL) was stirred at room temperature for 10 days. To the mixture were added $CHCl_3$ (400 mL) and water (80 mL), and the resulting mixture was partitioned. The organic layer was dried ($MgSO_4$) and evaporated under vacuum. The residue was purified by flash chromatography (hexane/EtOAc, 6:1) to give **7** and **8**. Compound **7** (3.7 g, 53%) eluted first as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) 6.08 (m, 1 H), 5.86 (d, J = 5.9 Hz, 1 H), 5.03 (d, J = 5.0 Hz, 1 H), 4.88 (d, J = 5.0 Hz, 1 H), 4.74−4.55 (m, 3 H), 3.36 (s, 3 H), 2.06 (s, 3 H), 1.36 (s, 6 H), 1.17 (d, J = 6.3 Hz, 3 H); CIMS m/z 287 (MH^+). Compound **8** (2.0 g, 29%) eluted next as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) 5.99 (s, 2 H), 4.90 (d, J = 5.3 Hz, 1 H), 4.66 (d, J = 5.3 Hz, 1 H), 4.57 (q, J = 6.6 Hz, 1 H), 4.55 (d, J = 6.9 Hz, 1 H), 4.49 (d, J = 6.9 Hz, 1 H), 3.27 (s, 3 H), 2.01 (s, 3 H), 1.31 (s, 6 H), 0.97 (d, J = 6.6 Hz, 3 H); CIMS m/z 287 (MH^+).

(1*S*,2*R*,3*R*)-1-Acetoxy-2,3-(isopropylidenedioxy)-4-[(*R*)-1-(methoxymethyloxy)ethyl]-4-cyclopentene (9). A mixture of **8** (1.55 g, 54.0 mmol), $PdCl_2(MeCN)_2$ (71 mg, 0.27 mmol), and *p*-benzoquinone (235 mg, 2.17 mmol) in THF (50 mL) was heated under reflux under an argon atmosphere for 18 h. The mixture was cooled to room temperature and evaporated under vacuum. The residue was purified by flash chromatography (hexane/EtOAc, 5:1) to give recovered **8** (692 mg, 45%) and **9** (484 mg, 31%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) 5.70 (br s, 1 H), 5.36 (m, 1 H), 4.49 (m, 2 H), 4.65 (s, 2 H), 4.50 (q, J = 6.6 Hz, 1 H), 3.39 (s, 3 H), 2.12 (s, 3 H), 1.40−1.32 (m, 9 H); FABMS m/z 287 (MH^+).

(1*S*,2*S*,3*R*)-2,3-(Isopropylidenedioxy)-4-[(*R*)-1-(methoxymethyloxy)ethyl]-4-cyclopenten-1-ol (10). A mixture of **9** (440 mg, 1.54 mmol) and K_2CO_3 (425 mg, 3.08 mmol) in MeOH (10 mL) was stirred at room temperature for 5.5 h, and the solvent was removed under vacuum. The residue was dissolved in $CHCl_3$ (50 mL) and the solution was washed with brine (10 mL). The organic layer was filtered through a Whatman 1PS filter paper and the filtrate was evaporated under vacuum. The residue was purified by flash chromatography (hexane/EtOAc, 3:1) to give **10** (346 mg, 92%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) 5.72 (br s, 1 H), 4.95 (d, J = 5.6 Hz, 1 H), 4.77 (dd, J = 5.6, 5.6 Hz, 1 H), 4.64 (s, 2 H), 4.55 (m, 1 H), 4.46 (q, J = 6.6 Hz, 1 H), 3.38 (s, 3 H), 2.70 (d, J = 9.9 Hz, 1 H), 1.44 (s, 3 H), 1.40 (s, 3 H), 1.35 (d, J = 6.6 Hz, 3 H); FABMS m/z 245 (MH^+).

(1*S*,2*R*,3*R*)-1-[(*p*-Tolylsulfonyl)oxy]-2,3-(isopropylidenedioxy)-4-[(*R*)-1-(methoxymethyloxy)ethyl]cyclopent-4-ene (11). A mixture of **10** (626 mg, 2.57 mmol), Et_3N (1.43 mL, 10.3 mmol), and TsCl (978 mg, 5.13 mmol) in CH_2Cl_2 (15 mL) was stirred at room temperature for 2 days. To the mixture were added $CHCl_3$ (100 mL) and water (20 mL), and the resulting mixture was partitioned. The organic layer was filtered through a Whatman 1PS filter paper and evaporated under vacuum. The residue was purified by flash chromatography (hexane/EtOAc, 5:1) to give **11** (763 mg , 75%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) 7.86 (d, J = 8.6 Hz, 2 H), 7.33 (d, J = 8.3 Hz, 2 H), 5.61 (s, 1 H), 5.20 (d, J = 5.3 Hz, 1 H), 4.85 (d, J = 5.3 Hz, 1 H), 4.72 (dd, J = 5.3, 5.3 Hz, 1 H), 4.60 (d, J = 6.6 Hz, 1 H), 4.57 (d, J = 6.6 Hz, 1 H), 4.46 (q, J = 6.6 Hz, 1 H), 3.34 (s, 3 H), 2.45 (s, 3 H), 1.40−1.25 (m, 9 H); FABMS m/z 399 (MH^+).

2-Fluoro-6′-*O*-benzyl-2′,3′-*O*-isopropylideneneplanocin A (14b). A mixture of 2-fluoroadenine (427 mg, 2.79 mmol), 18-crown-6 (369 mg, 1.4 mmol), K_2CO_3 (386 mg, 2.79 mmol), and **12**[11] (600 mg, 1.4 mmol) in DMF (30 mL) was stirred for 1 h at 75 °C under an argon atmosphere. The mixture was cooled to room temperature and the solvent was removed under vacuum. To the residue was added EtOAc (50 mL) and the resulting insoluble material was filtered off. The filtrate was washed with brine, filtered through a Whatman 1PS filter paper, and evaporated under vacuum. The residue was purified by flash chromatography ($CHCl_3$/MeOH, 100:1) to give **14b** (178 mg, 30%) as a crystalline solid. An analytical sample was obtained by recrystallizing from hexane/EtOAc: mp 210−211 °C; UV (MeOH) λ_{max} 263 nm, 270 nm (shoulder); ^{1}H NMR ($CDCl_3$) 7.64 (s, 1 H), 7.36 (m, 5 H), 5.72 (br s, 2 H), 5.78 (br s, 1 H), 5.48 (m, 1 H), 5.41 (d, J = 5.9 Hz, 1 H), 4.73 (d, J = 5.9 Hz, 1 H), 4.64 (d, J = 11.7 Hz, 1 H), 4.61 (d, J = 11.7 Hz, 1 H), 4.28 (br s, 2 H), 1.36 (s, 3 H), 1.47 (s, 3 H); FABMS m/z 412 (MH^+). Anal. ($C_{21}H_{22}FN_5O_3$) C, H, N.

2-Fluoroneplanocin A (1b). To a solution of **14b** (150 mg, 0.37 mmol) in CH_2Cl_2 (15 mL) at −78 °C was added BCl_3 (1 M in hexane, 2.7 mL) dropwise under an argon atmosphere, and the mixture was stirred at the same temperature for 3 h. The reaction was quenched with MeOH (3 mL) and the resulting mixture was evaporated under vacuum. The residue was dissolved in MeOH (5 mL), and the pH of the solution was adjusted to about 10 with 7% NH_4OH. The solvent was evaporated under vacuum and the residue was purified by flash chromatography ($CHCl_3$/MeOH/25% NH_4OH, 200:10:0.5 followed by 200:40:2) to give **1b** (41 mg, 40%) as a crystalline solid. An analytical sample was obtained by further purification by HPLC (eluent, 10% MeOH in H_2O) to give **2** (23 mg, 22%): mp >245 °C (dec); UV (H_2O) λ_{max} 262 nm, 270 nm (shoulder); $[\alpha]^{24}_D$ −162 (*c* 0.10, H_2O); ^{1}H NMR (CD_3OD) 8.02 (s, 1 H), 5.88 (d, *J* = 1.5 Hz, 1 H), 5.40 (m, 1 H), 4.62 (d, *J* = 5.4 Hz, 1 H), 4.37 (dd, *J* = 5.4, 5.4 Hz, 1 H), 4.31 (br s, 2 H); FABMS *m*/*z* 282 (MH^+). Anal. ($C_{11}H_{12}FN_5O_3 \cdot {}^1/_{10}H_2O$) C, H, N.

2-Chloro-6′-*O*-benzyl-2′,3′-*O*-isopropylideneneplanocin A (14c). Compound **14c** was prepared as described above for **14b**, using 2-chloroadenine. After purification by flash chromatography ($CHCl_3$/MeOH, 100:1), **14c** was obtained (178 mg, 30%) as a crystalline solid. An analytical sample was obtained by recrystallizing from hexane/EtOAc: mp 197−198 °C; UV (MeOH) λ_{max} 265 nm; ^{1}H NMR ($CDCl_3$) 7.65 (s, 1 H), 7.35 (m, 5 H), 6.14 (br s, 2 H), 5.77 (br s, 1 H), 5.53 (m, 1 H), 5.43 (d, *J* = 5.4 Hz, 1 H), 4.73 (d, *J* = 5.4 Hz, 1 H), 4.65 (d, *J* = 11.7 Hz, 1 H), 4.61 (d, *J* = 11.7 Hz, 1 H), 4.28 (br s, 2 H), 1.34 (s, 3 H), 1.46 (s, 3 H); FABMS *m*/*z* 428 (MH^+). Anal. ($C_{11}H_{12}ClN_5O_3 \cdot {}^2/_3H_2O$) C, H, N.

2-Chloroneplanocin A (1c). To a solution of **14c** (50 mg, 0.12 mmol) in CH_2Cl_2 (5 mL) was added BCl_3 (1 M in hexane, 820 μL) dropwise at −78 °C under an argon atmosphere, and the mixture was stirred at the same temperature for 6 h. The reaction was quenched with MeOH (3 mL), and the solvent was evaporated under vacuum. The residue was dissolved in MeOH (5 mL) and the pH of the solution was adjusted to about 9 with 7% NH_4OH. The solvent was evaporated under vacuum, and the residue was purified by HPLC (eluent, 10% MeOH in H_2O) to give **1c** (18 mg, 51%) as a solid: mp 201−202 °C; UV (H_2O) λ_{max} 265 nm; $[\alpha]^{25}_D$ −132 (*c* 0.10, H_2O); ^{1}H NMR (CD_3OD) 8.04 (s, 1 H) 5.88 (d, *J* = 2.0 Hz, 1 H), 5.45 (m, 1 H), 4.63 (d, *J* = 5.4 Hz, 1 H), 4.35 (dd, *J* = 5.4, 5.4 Hz, 1 H), 4.32 (br s, 2 H); FABMS *m*/*z* 298 (MH^+). Anal. ($C_{21}H_{22}ClN_5O_3 \cdot {}^1/_4H_2O$) C, H, N.

2-Fluoro-9-[(1*R*,2*S*,3*R*)-2,3-(isopropylidenedioxy)-4-cyclopenten-1-yl]adenine (15b). Compound **15b** was prepared as described above for **14b**, except with **13**[12] instead of **12**. After purification by flash chromatography ($CHCl_3$/MeOH, 80:1), **15b** was obtained (90 mg, 22%) as a solid: ^{1}H NMR ($CDCl_3$) 7.62 (s, 1 H), 6.32 (d, *J* = 5.9 Hz, 1 H), 5.90 (m, 3 H), 5.54 (m, 2 H), 4.71 (d, *J* = 5.6 Hz, 1 H), 1.48 (s, 3 H), 1.36 (s, 3 H); FABMS *m*/*z* 292 (MH^+).

2-Chloro-9-[(1*R*,2*S*,3*R*)-2,3-(isopropylidenedioxy)-4-cyclopenten-1-yl]adenine (15c). Compound **15c** was prepared as described above for **14b**, with **13** and 2-chloroadenine. After purification by flash chromatography ($CHCl_3$/MeOH, 80:1), **15c** was obtained (213 mg, 64%) as a crystalline solid: mp 255−256 °C; ^{1}H NMR ($CDCl_3$) 7.62 (s, 1 H,), 6.32 (dd, *J* = 5.6, 1.7 Hz, 1 H), 5.90 (m, 1 H), 5.83 (br s, 2 H), 5.61 (d, *J* = 1.0 Hz, 1 H), 5.53 (dd, *J* = 5.6, 1.0 Hz, 1 H), 4.70 (d, *J* = 5.6 Hz, 1 H), 1.48 (s, 3 H), 1.37 (s, 3 H); EIMS *m*/*z* 307, 309 (M^+). Anal. ($C_{13}H_{14}ClN_5O_2$) C, H, N.

2-Fluoro-9-[(1*R*,2*S*,3*R*)-2,3-dihydroxy-4-cyclopenten-1-yl]adenine (3b). A solution of **15b** (78 mg, 0.27 mmol) in 50% HCOOH (7.6 mL) was stirred at room temperature for 3 h, and then the solvent was removed under vacuum. To the residue was added 7% NH_4OH (3 mL) and the mixture was evaporated under vacuum. The residue was purified by flash chromatography ($CHCl_3$/MeOH, 6:1) to give **3b** (55 mg, 84%) as a crystalline solid: mp >253 °C (dec); $[\alpha]^{28}_D$ −216.0 (*c* 0.2, MeOH); UV (H_2O) λ_{max} 262 nm; ^{1}H NMR ($CDCl_3$/CD_3OD, 3:1) 7.83 (s, 1 H), 6.31(ddd, *J* = 5.9, 2.9, 2.9 Hz, 1 H), 6.10 (dd, *J* = 5.9, 1.6 Hz, 1 H), 5.41 (m, 1 H), 4.75 (br d, *J* = 5.6 Hz, 1 H), 4.40−4.24 (br s, 1 H); EIMS *m*/*z* 251 (M^+). Anal. ($C_{10}H_{10}FN_5O_2 \cdot {}^1/_3H_2O$) C, H, N.

2-Chloro-9-[(1*R*,2*S*,3*R*)-2,3-dihydroxy-4-cyclopenten-1-yl]adenine (3c). Compound **3c** was prepared as described above for **3b**. After purification by flash chromatography ($CHCl_3$/MeOH, 7:1), **3c** was obtained (84 mg, 88%) as a crystalline solid: mp 233−235 °C; $[\alpha]^{28}_D$ −195.5 (*c* 0.2, MeOH); UV (H_2O) λ_{max} 265 nm; ^{1}H NMR (CD_3OD) 8.04 (s, 1 H), 6.26 (ddd, *J* = 5.9, 2.3, 2.3 Hz, 1 H), 6.09 (dd, H-5′, *J* = 5.9, 1.7 Hz, 1 H), 5.47 (m, 1 H), 4.35 (dd, *J* = 5.3, 5.3 Hz, 1 H); FABMS *m*/*z* 268, 270 (MH^+). Anal ($C_{10}H_{10}ClN_5O_2 \cdot {}^1/_3H_2O$) C, H, N.

2-Fluoro-9-[(1*S*,2*S*,3*R*)-2,3-(isopropylidenedioxy)-4-[(*R*)-1-(methoxymethyloxy)ethyl]-4-cyclopenten-1-yl]adenine (16b). Compound **16b** was prepared as described above for **14b**, with **11** instead of **12**. After purification by flash chromatography ($CHCl_3$/MeOH, 30:1), **16b** was obtained (55 mg, 29%) as a crystalline solid: mp 214−215 °C; ^{1}H NMR (270 MHz, $CDCl_3$) 7.67 (s, 1 H), 5.85 (br s, 2 H), 5.68 (br s, 1 H), 5.48 (d, *J* = 5.6 Hz, 1 H), 5.44 (m, 1 H), 4.71 (m, 3 H), 4.55 (q, *J* = 6.6 Hz, 1 H), 3.40 (s, 3 H), 1.48 (s, 3 H), 1.36 (s, 3 H), 1.47 (d, *J* = 6.6 Hz, 3 H); FABMS *m*/*z* 380 (MH^+); Anal. ($C_{17}H_{22}FN_5O_4 \cdot {}^1/_5H_2O$) C, H, N.

2-Fluoro-(6′*R*)-6′-*C*-methylneplanocin A (2b). A solution of **16b** (40 mg, 0.11 mmol) in HCl/MeOH (5 N, 2 mL) was stirred at room temperature for 1.5 h and then the solvent was evaporated under vacuum. The residue was dissolved in MeOH (2 mL), the solution was neutralized with saturated $NaHCO_3$ (1 mL), and the resulting mixture was evaporated under vacuum. EtOH (10 mL) was added to the residue, and insoluble material was filtered off. The filtrate was evaporated under vacuum. The residue was purified by HPLC (eluent, 15% MeOH in H_2O) to give **2b** (17 mg, 55%) as a crystalline solid: mp >227 °C (dec); UV (H_2O) λ_{max} 262 nm, 269 nm (shoulder); $[\alpha]^{28}_D$ −130.9 (*c* 0.097, MeOH); ^{1}H NMR (270 MHz, CD_3OD) 8.00 (s, 1 H), 5.85 (dd, *J* = 1.7, 1.7 Hz, 1 H), 5.39 (m, 1 H), 4.69 (br d, *J* = 5.3 Hz, 1 H), 4.53 (br q, *J* = 6.6 Hz, 1 H), 4.30 (t, *J* = 5.3 Hz, 1 H), 1.41 (d, *J* = 6.6 Hz, 3 H); HR-MS (FAB) calcd for $C_{12}H_{14}N_5O_3F$ 296.1159; found 296.1165. Anal. ($C_{12}H_{14}FN_5O_3 \cdot {}^4/_5H_2O$) C, H; N: calcd, 22.61; found, 21.27.

Effect of Adenosine Deaminase and Inhibitory Effect on AdoHcy Hydrolase. Assays were done according to a previously reported method.[6a]

Antiviral Assays. For the anti-VSV assay in Vero cells, the cells were inoculated into 96-well culture plates (Nunc) at a concentration of 3×10^5 cells per 0.2 mL per well and cultured for 24 h at 37 °C in 5% CO_2. Cells in the plates were infected at 50% cell culture infectious dose ($CCID_{50}$) of virus in minimum essential medium (MEM, 0.01 mL per well). Serial 4-fold dilution of the test compounds in MEM were added (0.09 mL per well) to the wells simultaneously. The infected cells were incubated at 37 °C in 5% CO_2. The degree of virus-induced cytopathic effect (CPE) and inhibitory concentration (IC_{50}) were recorded microscopically after 37 h of incubation. The assays against measles and mumps viruses in Vero cells were done according to a previously reported method.[19] The other antiviral assays were done according to established procedures.[21]

Cytotoxic and Cytostatic Assays. The cytotoxicities of the compounds were measured using the MTT assays described previously.[19] For the assays based on cell morphology, a reported procedure was used.[21] Inhibition of cell growth (cytostatic activity) was measured using an established procedure.[22]

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