Synthesis of 3-Deazaneplanocin A, a Powerful Inhibitor of S-Adenosylhomocysteine Hydrolase with Potent and Selective in Vitro and in Vivo Antiviral Activities

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The neplanocin A analogue 3-deazaneplanocin A (**2b**) has been synthesized. A direct S_N2 displacement on the cyclopentenyl mesylate **3** by the sodium salt of 6-chloro-3-deazapurine afforded the desired regioisomer **4** as the major product. After deprotection, this material was converted to 3-deazaneplanocin A in two steps. X-ray crystallographic analysis confirmed the assigned structure. Consistent with its potent inhibition of *S*-adenosylhomocysteine hydrolase, 3-deazaneplanocin A displayed excellent antiviral activity in cell culture against vesicular stomatitis, parainfluenza type 3, yellow fever, and vaccinia viruses. Antiviral activity was also displayed in vivo against vaccinia virus by using a mouse tailpox assay. The significantly lower cytotoxicity of 3-deazaneplanocin A, relative to its parent compound neplanocin A, may be due to its lack of conversion to 5′-triphosphate and *S*-adenosylmethionine metabolites.

S-Adenosylhomocysteine hydrolase (AdoHcy-ase) is a pivotal enzyme in the regulation of *S*-adenosylmethionine (AdoMet) dependent methylation reactions because its substrate, *S*-adenosylhomocysteine (AdoHcy), is a competitive inhibitor of all methyltransferases.[1] Physiologically, the reaction proceeds in the catabolic direction due to the efficient removal of adenosine (Ado) and homocysteine (Hcy) by further metabolism (i.e., deamination or phosphorylation of Ado and remethylation of Hcy back to methionine).[2] Despite the anticipated multiplicity of effects that could result from the inhibition of AdoHcy-ase by different Ado or AdoHcy analogues, the pharmacological effects of the various inhibitors studied to date appear to be rather specific.[2] Since most of the AdoHcy congeners do not enter cells efficiently, most of the interest in the area of AdoHcy-ase inhibitors has focused on the development of Ado analogues.[3]

Recently, an excellent correlation between the antiviral potency against vesicular stomatitis virus (VSV) and the inhibitory effect (K_i/K_m) on AdoHcy-ase, for a series of Ado analogues, was established.[4] The antiviral activity observed for these compounds is believed to result from the inhibition of the AdoMet-dependent methylation of the 5′-cap of viral mRNA, caused by the increased accumulation of AdoHcy inside the cell. Inhibition of this critical methylation reaction hinders translation of viral mRNA into viral proteins.[5]

One very important discovery in the search for selective AdoHcy-ase inhibitors was that the replacement of the adenine aglycon moiety in Ado by 3-deazaadenine abolished the substrate properties of the resulting compound toward both adenosine deaminase and adenosine kinase.[2a] Thus, 3-deazaadenosine interacted preferentially with AdoHcy-ase and, as expected, demonstrated significant antiviral activity.[6] Later, another important discovery indicated that replacement of the sugar moiety of Ado by a carbocyclic ring increased the affinity of the compound toward AdoHcy-ase by 2–3 orders of magnitude.[7] However, the resulting carbocyclic adenosine analogue (aristeromycin) still functioned as a substrate for both adenosine kinase and adenosine deaminase. Consequently, the compound had poor antiviral selectivity and significant cytotoxicity.[8] In 1982, Montgomery et al. reported the synthesis of a carbocyclic nucleoside containing the 3-deazaadenine moiety [3-deazaaristeromycin (1)] and found it to be very potent and more specific than 3-deazaadenosine in inhibiting AdoHcy-ase.[9] Besides having good antiviral activity against vaccinia, vesicular stomatitis, measles, parainfluenza, and reo viruses, 1 behaved as a more specific antiviral agent devoid of some of the undesirable side effects typical of other antiviral compounds that operated by the same mechanism.[10] Both 3-deazaadenosine and 3-deazaaristeromycin, however, function as alternative substrates for AdoHcy-ase and undergo further anabolism to the corresponding *S*-nucleosidylhomocysteine

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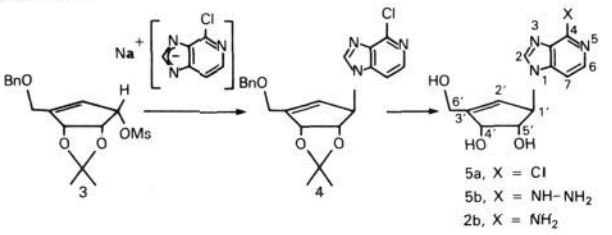
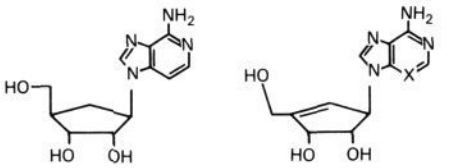
Scheme I



analogues which, in turn, are capable of interfering with other enzymes (e.g., adenosylmethionine decarboxylase).[2a,9] This anabolic conversion reduces specificity and may be responsible for some unwanted side effects.



Recently, an unsaturated, fermentation-derived carbocyclic Ado analogue, neplanocin A (**2a**), has been described by several laboratories as the most potent inhibitor of AdoHcy-ase known.[4,11] Although neplanocin A does not appear to form the corresponding *S*-nucleosidylhomocysteine metabolite, it is readily phosphorylated to the triphosphate level with the ensuing formation of the AdoMet analogue as the major metabolite.[12] High levels of this metabolite are associated with the cytotoxic effects observed with this drug.[13]

On the basis of all of these observations, we decided to prepare the corresponding cyclopentenyl analogue of 3-deazaadenosine, i.e., 3-deazaneplanocin A (**2b**), in an attempt to produce a compound with higher enzymatic specificity and reduced cellular toxicity. This compound combines the structural elements of both 3-deazaaristeromycin and neplanocin A with the expectation of optimal pharmacological properties. Preliminary accounts of the inhibition of *S*-AdoHcy-ase by **2b** have been reported by us.[13,14] This compound, when assayed with AdoHcy-ase from hamster liver, demonstrated competitive inhibition of the enzyme ($K_i = 5 \times 10^{-11}$ M) with adenosine as a substrate.[14] The K_i reported for 3-deazaaristeromycin with AdoHcy-ase from an identical source was 2×10^{-9} M.[14] In HL-60, as well as in HT-29 cells, the elevation of AdoHcy levels obtained upon treatment with 3-deazaneplanocin A was consistently superior to that achieved with equimolar concentrations of 3-deazaaristeromycin.[13,14] Also, because 3-deazaneplanocin A is neither phosphorylated nor converted to the *S*-nucleosidylhomocysteine analogue, its cytotoxicity was significantly less than that of neplanocin A.[13,14]

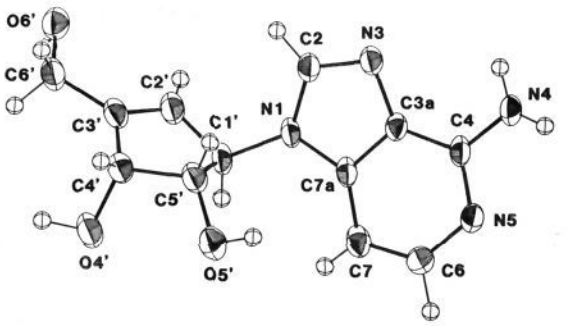


Figure 1. Conformation of 3-deazaneplanocin A. Non-hydrogen atoms are represented by thermal ellipsoids at the 50% probability level.

In the present work we wish to discuss in detail the synthesis, structure determination, and expanded biological studies of this agent, including in vitro and in vivo antiviral activities.

Chemistry

For the preparation of 3-deazaneplanocin A, we decided to utilize an approach similar to the one that we had successfully employed in a simplified synthesis of neplanocin A.[15] Thus, the cyclopentenyl mesylate **3** was reacted with the sodium salt of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine (6-chloro-3-deazapurine) to give a mixture of N-1 and N-3 isomers in 24% yield (Scheme I). The major isomer, later identified as the desired N-1 isomer (**4**), was obtained in 21% yield after purification by column chromatography. The simultaneous removal of both benzyl and isopropylidene moieties from compound **4** afforded the corresponding chloropurine **5a**, which was subsequently reacted with anhydrous hydrazine to give the intermediate hydrazino compound **5b**. This compound was immediately reduced with Raney nickel, and the resulting target compound, 3-deazaneplanocin A (**2b**), was obtained as the hydrochloride salt.

A preliminary confirmation that the N-1 nitrogen of the heterocyclic ring was linked to the C-1′ carbon of the cyclopentenyl moiety was obtained from UV spectroscopy and nuclear Overhauser effect (NOE) measurements in the proton NMR spectrum. The UV spectra of 3-deazaaristeromycin (**1**) and 3-deazaneplanocin A (**2b**, N-1 substitution) were coincident, showing λ_{max} values at 263 and 217 nm (pH 7). The N-3 isomer[16] had λ_{max} values at 285 and 210 nm. At the chloro stage, prior to hydrazinolysis, the fully deblocked N-1 (**5a**) and N-3 isomers were diagnosed by NOE measurements. Through-space interactions between purine protons and cyclopentenyl protons were examined by irradiation (0.032 W) of the aglycon protons and integration of the signals corresponding to the carbocyclic protons. An enhancement of 1.03 was considered to be experimentally significant. In the N-1 isomer, irradiation of the H-2 proton (H-8 in the purine numbering system; see Scheme I for numbering) produced an enhancement of 1.11 of the H-1′ signal. Irradiation of H-6 enhanced only H-7 (1.10), while irradiation of H-7 enhanced H-6 (1.20), H-1′ (1.10), and H-5′ (1.03). As anticipated, irradiation of either H-6 or H-7 produced no enhancement of the carbocyclic proton signals in the N-3 isomer. Definitive proof of the structure of 3-deazaneplanocin A as the N-1/β isomer was obtained from X-ray

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(16) This compound was obtained in very small amounts from the N-3 isomer of compound **4** after deprotection, reaction with hydrazine, and Raney nickel reduction.

Table I. In Vitro Antiviral Activity of 3-Deazaneplanocin

virus	strain	host cell[a]	3-deazaneplanocin A (2b) VR[b]	ID_{50}, μg/mL	SI[c]	positive control compound	VR	ID_{50} μg/mL	SI
vesicular stomatitis	Indiana	L929	4.7	0.3	>1.0	3-deazaaristeromycin	3.1	2.8	3.6
			3.6	0.07	4.6		3.2	2.6	3.8
			3.6	0.2	4.5		3.5	1.4	7.2
			4.2	0.2	2.0		3.3	0.9	3.8
parainfluenza type 3	Huebner C243	H.Ep-2	3.6	0.05	2.0	ribavirin	2.5	17.5	5.7
yellow fever	Asibi	vero	2.9	1.0	0.3	selenazole	2.4	3.3	1.0
vaccinia	Lederle CA	vero	3.1	0.3	>3.1	ara-A	3.7	2.0	16.3
			5.4	0.02	19.4		4.2	1.0	32.2
			4.5	0.03	10.0		3.6	1.8	17.9

[a] L929 are continuous-passage normal mouse fibroblasts. H.Ep-2 are continuous-passage human epidermoid carcinoma of the larynx cells. Vero are continuous-passage African green monkey kidney cells. [b] The antiviral activity of each compound is expressed as a virus rating (VR), and the potency is given as an ID_{50}. The VR is a weighted measurement of antiviral activity that takes into account both the degree of inhibition of virus-induced cytopathogenic effects and the degree of cytotoxicity produced by the test compound. USAMRIID virus rating values, calculated by the method of Sidwell and Huffman,[31] were converted to Ehrlich values[32] for consistency by multiplication by 3. The ID_{50} is the concentration of the test compound in μg/mL required to inhibit the virus-induced cytopathogenic effect by 50%. [c] Selectivity index is the minimum toxic drug concentration (μg/mL) divided by the ID_{50}.

Table II. In Vivo Antiviral Activity of 3-Deazaneplanocin (Vaccinia Tailpox Model)

parameter	experiment 1 PBS	2b (8 mg/kg)	ara-A (300 mg/kg)	experiment 2 PBS	2b (8 mg/kg)	2b (4 mg/kg)	ara-A (300 mg/kg)
no. of mice	17	20	20	19	19	20	20
median (N)[a]	28	1.0	1.5	66	0.0	1.5	1.0
range (N)	7->167	0->67	0–64	6->175	0->100	0–22	0–10
median ($\sqrt{N}$)	5.29	1.0	1.2	8.1	0.0	1.2	1.0
$p(\sqrt{N})$[b]		10^{-6}	10^{-3}		$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
% protection		74	56		84	82	88

[a] N = number of tailpox. [b] p calculated from mean N value by Student's t test.

analysis of crystalline (±)-3-deazaneplanocin A (Figure 1). Crystals of the hydrochloride salt of the pure enantiomer, obtained as described under Experimental Section, mp 168–69 °C, $[\alpha]_D^{25}$ −101.4°, proved unsuitable for X-ray analyses. However, racemic 3-deazaneplanocin A (free base, mp 229–230 °C), which was obtained under identical experimental conditions from the racemate of mesylate **3**, afforded crystals that were suitable for X-ray analysis. Racemic **3** was obtained in significant quantities during a scaleup synthesis of neplanocin A, and its synthesis has been recently described by us.[17]

X-ray Crystallography. The molecular structure of 3-deazaneplanocin A is shown in Figure 1, illustrating the β C-1′ to N-1 linkage. The deazaadenine moiety is planar within ±0.03 Å. Bond lengths and angles in this heterocycle are similar to those observed in the crystal structure of 3-deazaadenosine.[18] In both structures, the six-membered portion of the deazaadenine heterocycle is distorted slightly relative to the analogous ring in adenosine. In particular, the exocyclic angle about C-7a is increased by 6.6°, relieving any steric interactions between the proton attached to C-7 and the cyclopentene ring. This, and accompanying changes in the endocyclic angles, shifts the positions of both N-5 and the exocyclic amine nitrogen N-4 by about 0.2 Å relative to their location in adenosine. A very similar distortion is observed in 3-deazaadenosine.[18] Bond lengths and angles in the cyclopentene ring are comparable to those found in neplanocin A.[19] The cyclopentene ring is in an envelope conformation, C-5′ lying 0.451 (3) Å above the C-1′–C-2′–C-3′ plane. This is analogous to a furanose ring C-2′ endo pucker.[20] The corresponding amplitude of puckering is 29.5°, considerably less than the average of 36.6° seen for ribose moieties.[20] This suggests a more limited flexibility for the cyclopentene ring. As observed in neplanocin A, O-6′ lies approximately in the C-1′–C-2′–C-3′ plane, the value of the C-2′–C-3′–C-6′–O-6′ torsion angle being −2.4 (5)°. The C-2′–C-1′–N-1–C-7a torsion angle is 191.4 (3)°, in the anti range.[20] This conformation is also favored in 3-deazaadenosine.[18]

Antiviral Activity. In Vitro. The antiviral properties of 3-deazaadenosine are well-known. In vitro activity has been reported against Rous sarcoma virus,[6a,b] herpes simplex type 1 (HSV-1),[6c,9] SV-40,[6c] and several cytolytic RNA viruses.[6b] The saturated carbocyclic analogue 3-deazaaristeromycin (**1**) was active against several RNA viruses including vesicular stomatitis virus,[10a] and while good antivaccinia activity was observed,[10a] either low-level or no activity was found against HSV.[9,10a]

We have found 3-deazaneplanocin A (**2b**) to have excellent activity against several viral types (Table I). It is equivalent to, or more active than (based on virus rating), the positive control compounds against vesicular stomatitis, parainfluenza 3, vaccinia, and yellow fever viruses. Except for yellow fever, **2b** shows a good selectivity index (SI) values. In general, **2b** is about 10 times more potent than the standard antiviral agents used as the positive control compounds. 3-Deazaneplanocin A is inactive against human immunodeficiency virus (HIV) grown in T4+ ATH8 cells.[21]

In Vivo. On the basis of the excellent antivaccinia activity observed in vitro, 3-deazaneplanocin A (**2b**) was evaluated against this virus in vivo by using two different types of viral challenge.

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Intracranial Model. With intracranial implantation of vaccinia virus, followed by subcutaneous treatment with **2b**, animal deaths were observed at drug doses of 25 and 15 mg/kg/day. While doses of 7.5 and 3.75 mg/kg/day were not lethal, they did cause some weight loss relative to untreated control animals and did not protect the animals from the lethal effects of the virus. Ara-A, the positive control drug, was effective in this test, producing a virus rating of 1.5 at a dose of 150 mg/kg/day. It is possible that the ineffectiveness of **2b** against vaccinia virus induced encephalitis might be due to insufficient blood–brain barrier penetration.

Tailpox Model. The tailpox model[22] employs tail vein inoculation of vaccinia virus and subcutaneous drug treatment. Two separate experiments were conducted. In the first experiment (Table II), 3-deazaneplanocin A (**2b**), administered at 8 mg/kg, was compared with ara-A (positive control) at 300 mg/kg. Vehicle control mice receiving phosphate-buffered saline (PBS) developed a median 28 pox/tail. 3-Deazaneplanocin A was active and somewhat more effective than ara-A with median pox values of 1.0 and 1.5, respectively. This is equivalent to 74% protection for **2b** and 56% for ara-A, calculated according to the method of Boyle.[22] In the second experiment (Table II), 3-deazaneplanocin A was administered at two doses (8 and 4 mg/kg). PBS-treated mice developed a median of 66 pox/tail compared to only 1.0 (88% protection) with ara-A treatment. In this experiment, **2b** was approximately equivalent to ara-A with a median of 0.0 and 1.2 pox/tail at doses of 8 and 4 mg/kg (84% and 82% protection, respectively). Eleven of the nineteen mice treated with **2b**, at 8 mg/kg, had no detectable tailpox lesions while seven had less than 10 pox. One mouse had more than 100 lesions which were atypical in appearance relative to those observed in the PBS control mice.

Experimental Section

All chemical reagents were commercially available. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR was recorded on a Varian XL-200 instrument. Proton chemical shifts are expressed as δ values with reference to Me_4Si. Specific rotations were measured in a 1-dm cell with a Perkin-Elmer Model 241 polarimeter. Infrared spectra were recorded in a Perkin-Elmer Model 283 spectrometer. Positive-ion fast atom bombardment (FAB) mass spectra were obtained on a VG 7070E mass spectrometer that was equipped with a FAB ion source. The sample was dissolved in a glycerol matrix, and ionization was effected by a beam of xenon atoms derived by neutralizing xenon ions accelerated through 8.6 kV. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA. Large-scale syntheses of 4-chloro-1*H*-imidazo[4,5-*c*]pyridine and 3-deazaneplanocin were conducted at Starks Associates, Inc., and funded by NCI Contracts N01-CM-47588 and N01-CM-67926.

4-Chloro-1*H*-imidazo[4,5-*c*]pyridine.[23e-g] 4-Hydroxy-3-nitropyridine was prepared according to ref 23a and converted to 4-ethoxy-3-nitropyridine, which in turn was converted to 4-amino-3-nitropyridine by ammonium acetate.[23b,c] The product was reduced with stannous chloride in the presence of concentrated HCl to 2-chloro-3,4-diaminopyridine.[23d] Further conversion to 4-chloro-1*H*-imidazo[4,5-*c*]pyridine was carried out by using trimethyl orthoformate and a catalytic amount of formic acid. Advantages of the above overall route include ease of purification of intermediates, higher yields, and avoidance of nitramine intermediates.[23f,g] Approximately 240 g of the compound was made.

(1*S*,4*R*,5*R*)-(−)-3-(Benzyloxymethyl)-4,5-*O*-isopropylidene-2-cyclopenten-1-*O*-yl Methanesulfonate (3). This compound was prepared in nearly quantitative yield as reported previously.[17,24] This material must be stored at 0 °C under argon.

(−)-1-[(1*R*,4*R*,5*S*)-3-(Benzyloxymethyl)-4,5-*O*-isopropylidene-2-cyclopenten-1-yl]-4-chloroimidazo[4,5-*c*]pyridine (4). A suspension of 4-chloroimidazo[4,5-*c*]pyridine (11.7 g, 0.090 mol), NaH (4.34 g, of 60% oil dispersion; 0.108 mol), and dry acetonitrile (720 mL) was stirred at room temperature for 16 h. The mesylate **3** (36.1 g, 0.102 mol) was added, and the reaction mixture was heated to reflux (80 °C) for 6 h, cooled to room temperature, and then clarified by filtration. The filtrate was concentrated to dryness and extracted with ethyl acetate (200 mL). This solution was concentrated under vacuum to give a crude product as a brown residue. The resulting material was chromatographed on a 7.5 × 100 cm silica gel column eluted with ethyl acetate/hexane (2:1). Fractions containing the single N-1 isomer (less polar material by TLC) were combined and then concentrated under reduced pressure to give 7.3 g (21%) of analytically pure 4 as a yellow gum: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.24 (d, 1 H, H-6, J = 6 Hz), 7.94 (s, 1 H, H-2), 7.42 (d, 1 H, H-7, J = 6 Hz), 7.35 (m, 5 H, phenyl), 5.95 (s, 1 H, H-2′), 5.40 (s, 1 H, H-1′), 5.26 (d, 1 H, H-4′, J = 5 Hz), 4.65 (s, 2 H, OCH_2Ph), 4.55 (d, 1 H, H-5′, J = 5 Hz), 4.30 (s, 2 H, H-6′ab), 1.50 and 1.34 (s, 3 H, CH_3). Anal. ($C_{22}H_{22}ClN_3O_3$) C, H, Cl, N.

The more polar N-3 isomer was isolated after the polarity of the eluant mixture was increased by using ethyl acetate/hexane (3:1). This material obtained in ca. 3% yield was characterized only by ^{1}H NMR: δ 8.20 (d, 1 H, H-6, J = 6 Hz), 7.90 (s, 1 H, H-2), 7.63 (d, 1 H, H-7, J = 6 Hz), 7.34 (m, 5 H, phenyl), 6.21 (s, 1 H, H-2′), 5.91 (s, 1 H, H-1′), 5.23 (d, 1 H, H-4′, J = 5 Hz), 4.64 (br s, 3 H, H-5′, OCH_2Ph), 4.30 (s, 2 H, H-6′ab), 1.46 and 1.34 (s, 3 H, CH_3).

(−)-1-[(1*R*,4*R*,5*S*)-3-(Hydroxymethyl)-4,5-dihydroxy-2-cyclopenten-1-yl]-4-chloroimidazo[4,5-*c*]pyridine (5a). Compound 4 (9.10 g, 22.1 mmol) was dissolved in dry dichloromethane (1.14 L), and the solution was cooled with stirring to −78 °C under an atmosphere of argon. A solution of 1 M boron trichloride in methylene chloride (218 mL) was added dropwise during 30 min while the temperature was being maintained at ca. −75 °C. The reaction mixture was stirred at this temperature for 3.5 h, slowly diluted with methanol (235 mL), stirred at ambient temperature for an additional 15 min, and finally reduced to dryness. This material was codistilled with methanol (590 mL) and then partitioned between water (230 mL) and methylene chloride (230 mL). The organic layer was discarded, and an additional 230 mL of methylene chloride was added. Precipitation occurred from this two-phase system, and the solid formed was collected, washed with ether (50 mL), and dried to give 4.4 g (69%) of **5a** suitable for further transformation: ^{1}H NMR (200 MHz, Me_2SO-d_6/D_2O) δ 8.36 (s, 1 H, H-2), 8.10 (d, 1 H, H-6, J = 6 Hz), 7.62 (d, 1 H, H-7, J = 6 Hz), 5.84 (d, 1 H, H-2′, J < 1 Hz), 5.40 (br s, 1 H, H-1′), 4.36 (d, 1 H, H-4′, J = 5.5 Hz), 4.14 (s, 2 H, H-6′ab), 4.04 (t, 1 H, H-5′, J = 5.5 Hz).

(−)-1-[(1*R*,4*R*,5*S*)-3-(Hydroxymethyl)-4,5-dihydroxy-2-cyclopenten-1-yl]-4-aminoimidazo[4,5-*c*]pyridine Hydrochloride (3-Deazaneplanocin A Hydrochloride; 2b). A stirred solution of **5a** (3.70 g, 13.1 mmol) in anhydrous hydrazine (150 mL) under argon was heated under reflux for 1.5 h. The reaction mixture was cooled to room temperature, diluted with ethanol (75 mL), and then concentrated under vacuum to a light purple residue. This material was codistilled with ethanol (4 × 50 mL) and then dissolved in 400 mL of degassed distilled water. Raney nickel (11 g wet weight in water at pH 12) was added, and the resulting suspension was heated at reflux for 30 min and then

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filtered while hot. The nickel residue was washed by resuspension in boiling water (3 × 25 mL). The combined aqueous washings were acidified with 10% HCl (7 mL), and the solution was reduced to dryness to a white solid. This solid was codistilled with ethanol (4 × 50 mL) and then dried under vacuum to give 3.8 g (97%) of crude **2b** with an HPLC purity of >95%. An analytical sample of this material was obtained by reversed-phase chromatography on octadecylsilane-bonded silica gel (J. T. Baker disposable column, catalog no. 7020-7). A sample of 40 mg of crude material was chromatographed with distilled water as the eluant, and the product-containing fractions were monitored by UV detection at 254 nm and were combined and lyophilized to give a pale yellow solid. This solid was recrystallized from a mixture of ethanol/water to give 3-deazaneplanocin hydrochloride **2b** as a cluster of needles: mp 168–169 °C; UV (H_2O) λ_{max} 263 nm (log ϵ 4.002) and 217 nm (log ϵ 4.056); $[\alpha]^{25}_D$ −101.4° (*c* 0.105, H_2O); IR (Nujol) 3400, 3120, 2920, 2860, 2860, 1675, 1620, 1550, 1490, 1455, 1375, 1240, 1105, 990, and 780 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 8.22 (s, 1 H, H-2), 7.54 (d, 1 H, H-6, J = 7 Hz), 7.11 (d, 1 H, H-7, J = 7 Hz), 6.00 (d, 1 H, H-2′, J = 2 Hz), 5.44 (m, 1 H, H-1′), 4.54 (d, 1 H, H-4′, J = 5 Hz), 4.25 (m, 2 H, H-6′ab), 4.20 (t, 1 H, H-4′, J = 5 Hz); MS (FAB, positive mode), *m/z* (rel intensity) 263 (MH^+, 100), 135 (b + 2H, 73); high-resolution FAB MS, *m/z* 263.112 (MH^+, calcd 263.114). Anal. ($C_{12}H_{14}N_4O_3 \cdot HCl \cdot 0.33H_2O$) C, H, N.

Single-Crystal X-ray Analysis of 3-Deazaneplanocin A. Crystals of the racemic free base $C_{12}H_{14}N_4O_3 \cdot H_2O$ were obtained under the same conditions as those described in ref 17. Cell dimension and intensity data were collected from a colorless plate of approximate dimensions 0.2 × 0.02 × 0.02 mm^3 by the Molecular Structure Corp., College Station, TX. All measurements were made at −115 °C by employing a Rigaku AFC5R diffractometer with graphite-monochromated CuKα radiation from a 12-kW source. Lattice constants were obtained by least-squares refinement of the angular settings of 25 reflections in the range 87.31 < 2θ < 99.92°. These indicated a monoclinic cell with dimensions a = 12.488 (1), b = 8.839 (2), and c = 12.114 (1) Å and β = 104.922(7)°. Systematic absences $h0l, l = 2n + 1$ and $0k0, k = 2n + 1$ defined the space group as $P2_1/c$. Other crystal data are Z = 4, M_r = 280.3, V = 1292.1 Å^3, and ρ(calcd) = 1.441 g/cm^3.

Data were measured to 2θ = 120.2° by using the ω–2θ scan method with a variable scan width $\Delta\omega = (1.15 + 0.30 \tan\theta)°$ and scan rate of 16°/min. A total of 1443 unique reflections were collected, of which 1199 had $I > 1.5\sigma(I)$ and were defined as observed. Three standard reflections measured after each 150 scans showed no decline in intensity. Data were corrected for Lorentz and polarization effects, but no absorption correction was applied.

The structure was solved by direct methods employing MULTAN78.[25] An E map calculated from the set of phases with the highest combined figure of merit yielded all non-hydrogen atoms. The positions of all hydrogen atoms were then obtained from subsequent least-squares refinements and difference Fourier maps, employing only low-angle data [(sin θ)/λ < 0.4 Å^{-1}].

The structure was refined by using full-matrix least-squares techniques. The function minimized was $\sum w(\Delta F)^2$, where $\Delta F = |F_o| - |F_c|$. Weights $w = 1/\sigma_{new}^2$ were used, where $\sigma_{new}^2 = [\sigma^2 + 0.5A|F_o|^2 + 0.5B[(\sin\theta)/\lambda]^2]^{1/2}$ and $\sigma = \sigma(F_o^2)/2|F_o|$. Values of A and B were obtained by a least-squares minimization of the function $|\Delta F|^2 - \sigma_{new}^2$ for 20 separate segments in $|F_o|$ and (sin θ)/λ. Non-hydrogen atoms were refined anisotropically. Positional parameters of all hydrogen atoms were refined with isotropic temperature factors.

Final refinements converged to the values of $R = \sum||\Delta F|/\sum|F_o| = 0.052$ and $R_w = [\sum w(\Delta F)^2/\sum w|F_o|^2]^{1/2} = 0.051$ for 1199 observations m and 246 variables n. The discrepancy factor $S = [\sum w(\Delta F)^2/(m-n)]^{1/2} = 1.33$. The largest final parameter shift observed was 0.03σ and the largest peak on the final difference map 0.38e/Å^3. Atomic scattering factors for the non-hydrogen atoms were from ref 26. Scattering factors for the hydrogen atoms were those of Stewart et al.[27] The DNA system of programs[28] was used throughout. Final fractional atomic coordinates and thermal parameters are deposited.

Antiviral Evaluation. Testing was carried out at Southern Research Institute under the U.S. Army Medical Research Institute of Infectious Disease (USAMRIID) antiviral testing program (in vitro) and National Cancer Institute Purchase Order 263-MD-610174 (in vitro and in vivo). In vitro evaluation was conducted as previously described.[29]

Intracranial Challenge. Outbred, 18–20-g female Swiss mice (CD-1, Charles River Laboratories, Inc.) were randomly assigned to one of the seven treatment groups, each of which consisted of 15 mice [five uninfected (toxicity controls), 10 virus infected]. Animals were challenged intracranially with a 90% lethal dose (LD_{90}) of vaccinia virus (strain IHD). Drug treatments were administered subcutaneously once daily for 7 days, with the first treatment given on the day preceding virus challenge. Treatment groups included (1) untreated controls, (2) drug–diluent (PBS) controls, (3) ara-A (150 mg/kg/day), and (4) 3-deazaneplanocin A (25, 15, 7.5, or 3.75 mg/kg/day). Mortality was monitored for 21 days postinfection.

Tailpox Model. This in vivo vaccinia virus model was developed by Boyle et al.[22] and further refined by Joshi et al.[30] Mice inoculated in the tail vein with virus develop dermal lesions over the entire tail surface. These lesions are enumerated after fluorescence staining and are a function of viral dose, animal weight, and inoculation distance from the base of the tail.[22,30] As suggested by Boyle,[22] lesion numbers were square root transformed to produce a more normal distribution of data. The IHD strain of vaccinia virus, passed once in mouse brain and once in primary rabbit kidney cell culture, was used. Random-bred Swiss mice (CD-1, VAF+, Charles River Laboratories, Inc.), weighing 18–21 g, were inoculated via the tail vein (1 cm from the base) with 0.2 mL of a 1:40 dilution of the virus suspension. Compounds were administered subcutaneously once daily for 7 days, with the first dose given the day preceding virus challenge. The positive control drug, ara-A, was administered at 300 mg/kg/day. 3-Deazaneplanocin A was administered at 8 or 4 mg/kg/day. Drug–diluent control mice received phosphate-buffered saline (PBS, pH 7.2). Uninfected drug-treated toxicity controls were included for each treatment administered. Animals were sacrificed on the sixth day, and their tails were stained with a solution of 1% fluorescein–0.5% methylene blue in 70% methanol. Lesions were enumerated under UV light (354 nm) with the aid of a hand lens. The average number of lesions for each treatment group was calculated prior to, and following square root transformation of, the individual tailpox counts. Tailpox counts from each treatment group were statistically compared by Student's t test.

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Supplementary Material Available: Tables of fractional atomic coordinates, thermal parameters, and selected bond lengths and angles (3 pages); table of observed and calculated structure factors (8 pages). Ordering information is given on any current masthead.

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